THE DISTRIBUTION OF COPEPODS IN THE SHUBENACADIE RIVER ESTUARY RELATIVE TO THE DIET OF LARVAL STRIPED BASS (*MORONE SAXATILIS*)

by

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DEDICATION

I dedicate this thesis to my Dad, Ronald Findlay, expert bass and salmon angler. Thank you for inspiring me to continue my studies in the field of aquatic sciences and fisheries. Thank you for instilling your love of fish upon me and teaching me to appreciate life under water.

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ABSTRACT

The distribution of both copepods and larval striped bass were quantified in the upper estuary (25-40 river kilometer) from May to August 2016 and 2017. Examining the distributions of larvae and potential prey relative to time of year and salinity was important to test the Match-Mismatch Hypothesis. The abundance of first-feeding stage striped bass (6mm total length, TL) peaked at 463/m^3 May 29-June 4th, 2016, and 595/m^3 June 12-18th, 2017, in 1-10ppt salinity. Larvae were broadly distributed from tidal freshwater to 25.0ppt. The larvae were 'gape limited' and failed to feed until late June due to the absence of prey of suitable size. Once feeding commenced, the initial prey was a small adult harpacticoid (0.8-1.0mm TL) of the family Ectinosomatidae, genera possibly *Halectinosoma* or *Pseudobradya* based on DNA and morphological analysis. Daily mean abundance of this copepod was 209-281/m^3 in salinities of 1.1-2.0ppt in June 2017. Once larvae started growing, larger prey included copepods (*Coullana canadensis* and *Pseudodiaptomus pelagicus*) and mesoplankton (mysids and amphipods).

LIST OF ABBREVIATIONS USED

bp	Base Pair
BW	Body Weight
°C	Degrees Celsius
cm	Centimeter
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CTD	Conductivity, Temperature, Depth Probe
DFO	Department of Fisheries and Oceans
DNA	Deoxyribonucleic Acid
dph	Days Post Hatch
ETM	Estuarine Turbidity Maximum
FL	Fork Length
g	Gravitational Force
g	Gram
Gov't	Government
GPS	Global Positioning System
h	Hour
IND/m^3	Individuals Per Cubic Meter of Water Filtered
kg	Kilogram
km/h	Velocity, Kilometer Per Hour
L	Liter
m^3	Volume, Cubic Meter
m	Meter
mg/mL	Milligram Per Milliliter
mg/L	Milligram Per Liter
mL	Milliliter
mm	Millimeter
NOAA	National Oceanic and Atmospheric Administration
NTU	Turbidity, Nephelometric Turbidity Unit
PCR	Polymerase Chain Reaction
ppt	Parts Per Thousand
rkm	River Kilometers
SD	Standard Deviation
SE	Standard Error
SEM	Scanning Electron Microscopy
sp.	Species
TAE	Tris-Acetate-EDTA Buffer
TL	Total Length
™	Trademark
µL	Microliter
µm	Micrometer
US	United States
UV	Ultraviolet
v	Volts

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"The important thing is not to stop questioning. Curiosity has its own reason for existing"
-Albert Einstein

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Thank you to my supervisory committee members, Dr. Janine Gray and Dr. Stephanie Collins, for their support throughout the journey of my project. Thank you for your guidance, insight, and sharing your passion for research. Thank you to Juan Manriquez for the technical support of graciously assisting with my data sorting and statistical analysis instruction. Thank you to Mr. Bill Stone for always sharing your knowledge of fish and the river with me. Thank you for sharing your stories and teaching me how to build a boat. Molecular work was conducted in the Atlantic Research Centre for Agricultural Genomics under the supervision and instruction of Dr. Nasif Sarowar. Thank you to Dr. Sarowar for the hands-on teaching of DNA extraction, results interpretation, sequencing analysis, and sharing your research experiences. Thank you to Ms. Tanya Muggeridge for the DNA sequencing and support. Thank you to my river partners Hayden Breau and Carolyn Martin. Thank you for turning rough days on the river to some of my fondest and funniest memories. Thank you, Carolyn, for helping to fuel my curiosity of the estuary and always providing snacks. Thank you, Hayden, for the copepod image analysis and length measurements.

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CHAPTER 1: INTRODUCTION

Striped bass (*Morone saxatilis*) is an anadromous species native to the eastern seaboard of Canada and the United States, ranging from North Carolina (US) to the Gulf of St. Lawrence and Miramichi River in Canada (Rulifson and Dadswell, 1995; Cooper et al., 1998). In Atlantic Canada, two genetically discrete populations of striped bass exist: one in the Bay of Fundy that spawns in the Shubenacadie-Stewiacke River Estuary, the other in the Miramichi River (Wirgin et al., 1993). The Bay of Fundy population is designated as endangered by COSEWIC (2015) because the Shubenacadie-Stewiacke River Estuary is the sole confirmed successful spawning and nursery habitat. Historically, the Annapolis River and Saint John River contributed to the nursery habitat of the Bay of Fundy population, but there has been no evidence of successful spawning and recruitment in both rivers since the 1970's (Douglas et al., 2003; Andrews et al., 2017). However, a recent publication identified some Saint John River stock genetically different from the Shubenacadie-Stewiacke stock (LeBlanc et al., 2018). The Shubenacadie-Stewiacke River striped bass population is the only existing population across the species' range that spawns in a tidal bore estuary, furthering the uniqueness of this nursery habitat (Rulifson and Tull, 1999). The number of adults gathering in the Shubenacadie-Stewiacke Rivers for spawning has increased greatly following strong recruitment of the 1999 year-class (Bradford et al., 2015). Survival and growth during the egg and larval stages are important factors dictating recruitment success among striped bass populations (Rutherford and Houde, 1995). The early life history of this population was published recently and included data on gut contents and growth of larval and early juvenile stages, but no information on the identity of the copepod prey (Duston et al., 2018). This thesis focuses on the copepod population in the Shubenacadie River Estuary and the availability of specific species as prey for striped bass larvae at the critical first-feeding stage.

Copepods and cladocerans are essential food for first feeding striped bass and their abundance is important for first feeding success (Shideler and Houde, 2014). First feeding may be defined as the stage at which a fish larva is physically or anatomically capable of active feeding (Miller et al., 1988). *Eurytemora affinis* and *Bosmina longirostris* are two dominant prey items of first feeding striped bass in the nursery habitat of upper Chesapeake Bay while *E. affinis* is the main prey item in the Miramichi River Estuary (Beaven and Mihurksy, 1980; Robichaud-LeBlanc et al., 1997;

Shideler and Houde, 2014). The gape, or mouth size, of planktivorous larval fish is a morphological characteristic which limits the maximum prey size that a fish can swallow whole (Wong and Ward, 1981; Schael et al., 1991; DeVries et al., 1998). The gape of first feeding striped bass relative to body size of potential prey items has not received extensive consideration in the literature. The identity and distribution of copepods and the food of first feeding larvae in this macrotidal nursery habitat has not been verified. From a preliminary assessment of gut contents of first feeding larvae, MacInnis (2012) suggested the dominant prey item was *E. affinis*. More detailed work, beginning in 2013 using a 250µm mesh plankton, concluded *E. affinis* was very rare, a contrast to other striped bass nursery habitats. The unpublished data from 2013 onwards on copepod distributions in the Shubenacadie River Estuary was incorporated into this thesis, together with new data collected in 2016 and 2017.

Understanding predator-prey relationships of larval fish is key to understanding recruitment success. The "Match-Mismatch" hypothesis, first proposed by Hjort (1914), is a cornerstone of fisheries science, and is considered to be an important factor in the recruitment dynamics of marine pelagic species (Cushing, 1990). Match-mismatch relationships are also evident in estuarine ecosystems, often in association with salinity as an important factor affecting the distribution of zooplankton prey and predators (Houde, 2008). A match in the timing and location of spawning and larval production with the spring zooplankton bloom (larval prey) is critical for recruitment success (Cushing, 1990; Houde, 2008). In Chesapeake Bay, strong year-classes have been associated with the first feeding stage striped bass larvae occurring during the spring zooplankton blooms both of which are influenced substantially by environmental factors, particularly freshwater run-off and temperature (Houde, 2008).

The current high abundance of striped bass larvae in the Shubenacadie River Estuary (2-1000/m^3; Duston et al., 2018), offers a great opportunity to investigate their feeding and distribution patterns relative to their prey. In Chesapeake Bay, by contrast, the abundance of striped bass larvae is very low, <1/m^3, making data collection difficult (Martino and Houde, 2010). A higher abundance allows for a greater sample size to investigate. The objective of my thesis was to describe the temporal and spatial distributions of striped bass larvae and their potential copepod prey from first feeding around late June to their transition into juveniles in August (30mm TL). Their distribution

is largely dictated by the salinity gradient driven by the tidal cycle (Duston et al., 2018). The abundance of each copepod species relative to salinity and body size relative to the gape of striped bass larvae were important parameters measured.

The project was part of the pre-operational Estuarial Environmental Monitoring required of Alton Natural Gas Storage LP to develop caverns in salt deposits, to store natural gas. Since 2008, funding from Alton Gas to Dr. J. Duston has supported valuable monitoring and research on the estuary allowing the study of inter-annual variations in environmental conditions and zooplankton. My contributions to the project began in 2015 as an undergraduate summer student employee. Copepod data collected from 2013 to 2017 and larval data from 2016 to 2017 is presented here. Chapter 2 reviews the state of knowledge of the early life history of striped bass in North America, develops the rationale for the research, and outlines the objectives of the study. Chapter 3 describes the Materials and Methods. Chapter 4 presents and discusses the copepod distributions relative to time of year and salinity. Chapter 5 presents and discusses the spawning and larval distributions in the estuary as well as the gut contents and gape limitations. This thesis concludes with recommendations for future research.

CHAPTER 2: STATE OF KNOWLEDGE AND RESEARCH RATIONALE

2.1 Physical Characteristics of the Stewiacke-Shubenacadie River Estuary

The main study site of the project was located at 25 kilometers from the mouth of the Shubenacadie River (25rkm; Figure 1). This location is the site of the Alton Natural Gas mixing station and planned brine effluent outfall. The watershed of the Shubenacadie-Stewiacke River is the largest in Nova Scotia with an area of about 2800 km^2 (Bailey, 1981; Mudroch et al., 1987). The tide extends up to 64 km of the Shubenacadie River and 16 km of the Stewiacke River (Mudroch et al., 1987). The exchange ratio of water in Cobequid Bay is extremely high with 76-94% replaced each tidal cycle (Bousfield and Leim, 1958). An estimated $50x10^6$ m^3 of water is exchanged per tidal cycle at the mouth of the Shubenacadie and $5x10^6$ m^3 at the main study site at 25rkm (Martec, 2007). The Stewiacke River is the main tributary of the Shubenacadie River and their confluence is 27.5rkm from the estuary mouth (Figure 1). The Shubenacadie River is approximately 110km long, it flows north from Shubenacadie Grand Lake, discharging into Cobequid Bay in the Minas Basin of the Inner Bay of Fundy (Figure 1; Mudroch et al., 1987). At its mouth, the Shubenacadie River has a tidal range on average of 11m resulting in a water column that is fully mixed and highly turbid (Bousfield and Leim, 1958; Dalrymple et al., 1990). At the main study site, the estuary is considered macro-tidal as the tidal range is between 4-6m (Dyer, 2000).

The Shubenacadie River over the tidal cycle exhibits significant changes in salinity and depth, typical characteristics of tidal bore rivers (Lynch 1982; Martec, 2007). The tidal bore is created when the fast rise in tidal elevation meets resistance from the ebb flowing water exiting the river (Lynch, 1982; Martec, 2007). The water of Cobequid Bay and Shubenacadie River is extremely turbid with no light below 1.5m, even on a sunny day (Bousfield and Leim, 1958; Duston and Astatkie, 2012). The highest turbidity with Secchi visibility was 5.0-15.0cm during the study from May 23-June 15, 1994 (Rulifson and Tull, 1999). The turbidity at the main study site can reach 103 Nephelometric Turbidity Units (NTU), or 580mg/L of suspended solids, a Secchi depth of about 3cm (Duston et al., 2018). The turbidity of Cobequid Bay can exceed 200mg/L and the Secchi visibility can range from 22.0 to 110.0cm over various tidal cycles (Stone, 1985). The tidal bore induces strong turbulent mixing, both vertically and horizontally, and significant sediment

transport (Martec, 2007). In comparison to the Shubenacadie, the Miramichi River Estuary Secchi depths were 3m in the tidal river and Inner Bay and reached 7m in the adjacent Gulf of St. Lawrence. The total suspended sediment ranged between 5 and 70mg/L (Locke and Courtenay, 1996).

The tide of the inner Cobequid Bay and the Shubenacadie-Stewiacke River Estuary is asymmetric, with a flood tide of shorter duration than the ebb tide (Dalrymple, 1977; Rulifson and Tull, 1999). At the main study site, the duration of the ebb and flood tide are about 10.5 and 1.5 hours, respectively (Martec, 2007; MacInnis, 2012). The timing of the tidal bore and the tidal cycle is independent of the large freshwater flows from the watershed, but the velocity of the flood tide and extent of saltwater intrusion are greatly reduced when freshwater runoff is high (Martec, 2007; Duston et al., 2018). The shallow water of the upper estuary is generally warmer in summer and cooler in winter when compared to the deeper water of the Minas Basin (Dalrymple et al., 1990). The temperature of the estuary experiences extreme seasonal variations ranging from 20°C in summer to -0.5°C from April to December when ice is present (Dalrymple et al., 1990).

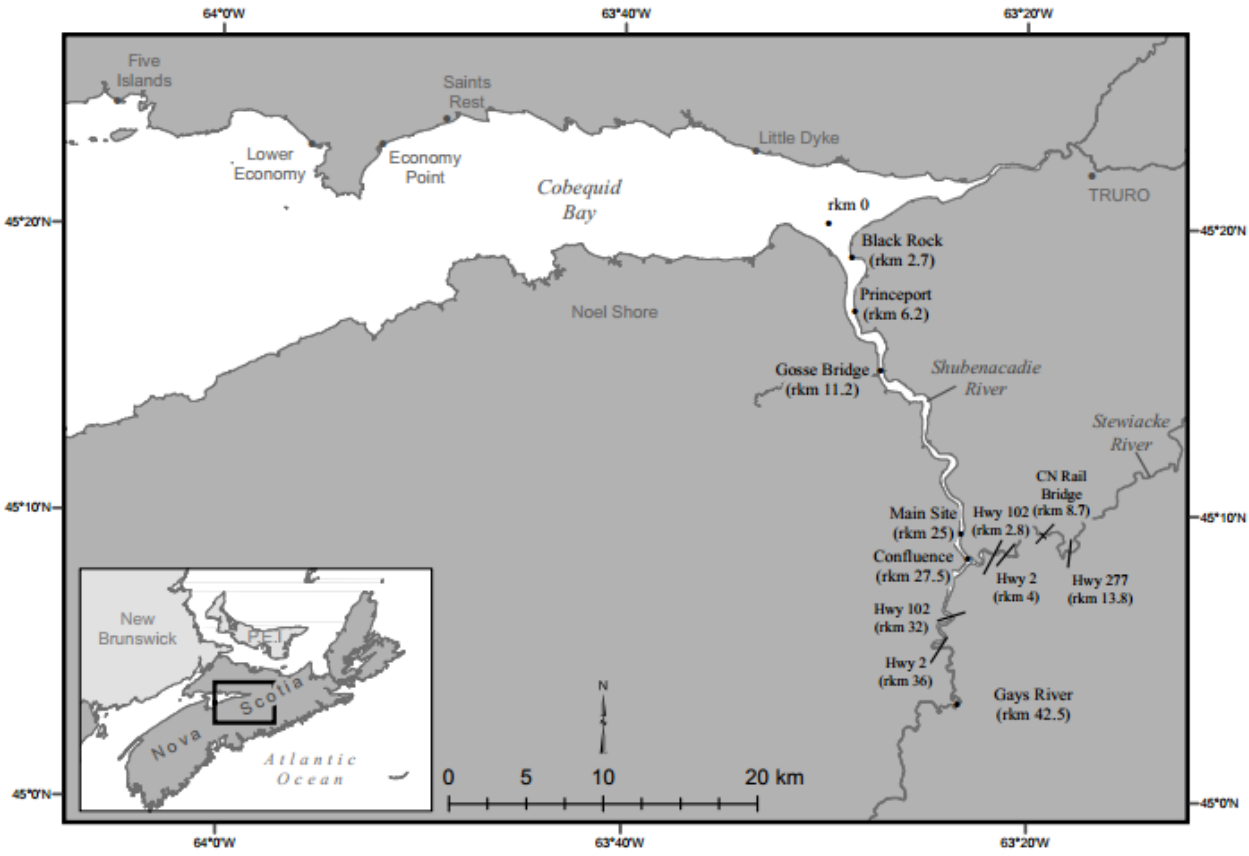

Figure 1. Map of the Shubenacadie-Stewiacke River Estuary showing the study area extending from 25.0rkm to upwards of 41.0rkm of the Shubenacadie branch (source: Duston et al., 2018).

The incoming tide of Cobequid Bay creates a tidal bore that begins at the estuary mouth. The mean flood tide velocity between Gosse Bridge and the confluence of the Stewiacke and Shubenacadie Rivers was estimated at 7.4 km/h (Rulifson and Tull, 1999). At the main study site, the mean water velocity reached a maximum of about 7km/h on the flood tide and 4km/h on the ebb tide (Figure 2). The Shubenacadie River Estuary is classified as a fully mixed estuary, or vertically homogeneous and not stratified, because the tidal range is large relative to water depth and the turbulence created by the velocity mixes the water column completely (Dyer, 2000; Martec, 2007).

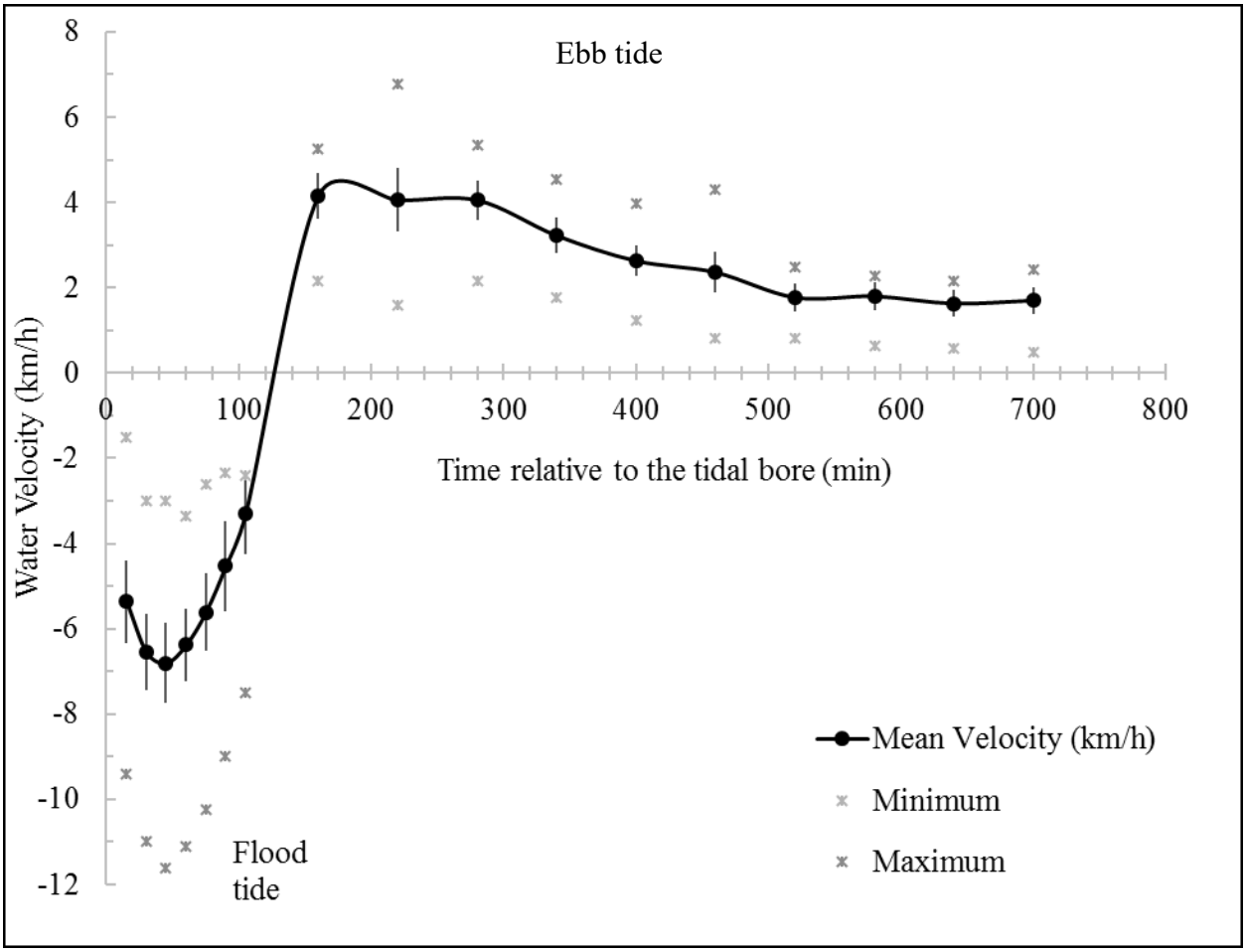

Figure 2. Mean water velocity (±SD; km/h) at the main study site (25.0rkm) relative to the arrival of the tidal bore. Data compiled from a total of 51 flood tides and 18 ebb tides from May to August in both 2012 and 2014 (source: Duston et al., unpubl. data).

The Shubenacadie-Stewiacke River Estuary undergoes large changes in salinity with each tide (Figure 3). Salinity intrusion is beyond 36.0rkm when freshwater run-off is low, reaching 2ppt during the day tide, and 5ppt on the larger night tide (Figure 3 upper panel). When freshwater run-off is high, by comparison, saltwater intrusion is limited to about 25rkm, and then only briefly, around high tide (Figure 3 lower panel). The estuary temperature changes greatly with time of year (0 to 26ºC) but not greatly over a tidal cycle. The temperature may drop by about 2ºC during the flood tide in the spring when the seawater from the Inner Bay of Fundy entering the estuary is colder than the tidal freshwater of the estuary. The temperature may also drop a couple of degrees over the flood tide in the fall months (Duston et al., 2018).

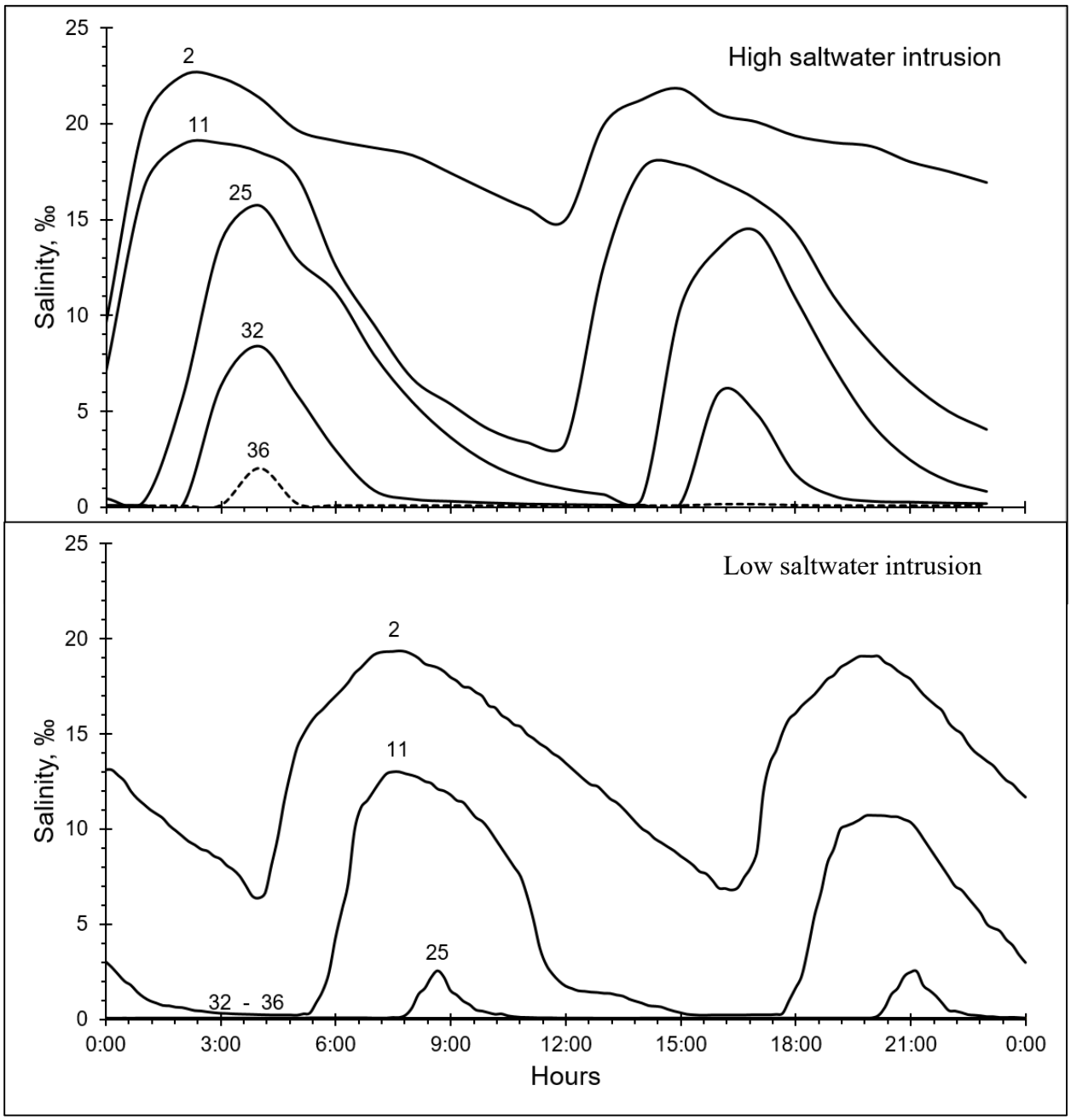

Figure 3. Top Panel: High saltwater intrusion in the Shubenacadie River Estuary on June 3, 2015, recorded with CTD loggers. Salinity (parts per thousand, ‰) was measured at: Maitland (mouth of river) 2rkm, Gosse Bridge 11rkm, main study site 25rkm, 102 Highway Bridge 32rkm, and Shubenacadie Village Bridge 36rkm. **Lower Panel:** Low saltwater intrusion in the Shubenacadie River Estuary on June 24, 2015 following heavy rain (source: Duston et al. 2018).

The tides of the Bay of Fundy, and thus the Shubenacadie River Estuary, are influenced by the distance between the moon and earth and the positions of the moon, sun, and earth relative to each other (Desplanque and Mossman, 1998). Strong tides occur during the full month each month when the moon is closest to earth and has a stronger gravitational pull. The position of the moon, sun, and earth relative to each other result in "neap" and "spring" tides. The neap and spring tides represent variations in tide height (Desplanque and Mossman, 1998). Twice a month during the full moon and new moon, the moon, earth, and sun are almost aligned and the gravitational pull from the sun also acts upon the tides and causes high tides to be higher and low tides to be lower (Gov. of Canada, 2017; NOAA, 2018). These are known as spring tides. Neap tides occur quarterly when the moon, earth, and sun form right angles and cause the high tides to be lower and low tides to be higher (Gov. of Canada, 2017; NOAA, 2018). Extreme high tides can be achieved in the Bay of Fundy when the full moon coincides with a spring tide. When the moon is furthest away from earth, by contrast, even a spring tide can be reduced (Gov. of Canada, 2017).

2.2 Striped Bass (*Morone saxatilis*) Taxonomy and Geographic Distribution

Striped bass is an anadromous perciform (spiny-finned) of the Moronidae family that typically spawns in fresh water at the head of the tide, then return to coastal waters to feed (Rulifson and Dadswell, 1995; Williams et al., 2012). Many fish from the Shubenacadie-Stewiacke River stock descend from Grand Lake in spring to spawn in the Stewiacke River and continue to descend, reaching Cobequid Bay to feed (Rulifson and Dadswell, 1995). The juvenile stage of the Shubenacadie River stock is fully euryhaline; survival and growth is independent of salinity between 0 and 30ppt at 16-28°C (Duston et al., 2004). The striped bass is a close relative of the European sea bass (*Dicentrarchus labrax*), and the anadromous life history may have evolved from coastal spawning characteristics of *D. labrax* (Williams et al., 2012).

The two self-sustaining populations of striped bass in Canada recognized by DFO are genetically discrete. The genotype frequencies of mitochondrial DNA of striped bass sampled from the Miramichi and Tabusintac Rivers of the Gulf of St. Lawrence are indistinguishable from one another, but they differed from striped bass of the Shubenacadie River (Wirgin et al., 1993). All Canadian stocks of striped bass are genetically discrete from US stocks, the most important being

the Chesapeake Bay stock. The striped bass population of the Shubenacadie River is reproductively separated from the Gulf of St. Lawrence River stock (Wirgin et al., 1993). There has been no evidence of viable spawning and recruitment within the last thirty years from the Annapolis and Saint John Rivers (Douglas et al., 2003; Andrews et al., 2017). The Committee on the Status of Endangered Wildlife (COSEWIC) initially designated the Bay of Fundy striped bass population as 'threatened' in 2004 and 'endangered' in 2012 primarily because the Shubenacadie-Stewiacke River Estuary is the sole spawning and nursery habitat (COSEWIC, 2015). The recent discovery of a Saint John River stock that is genetically discrete from the Shubenacadie river stock will demand that the Department of Fisheries and Oceans (DFO) reconsider the Bay of Fundy designatable unit (LeBlanc et al. 2018).

Elsewhere along the eastern seaboard, Chesapeake Bay (Maryland and Virginia, US), Hudson River (New York, US), and the Delaware River (Maryland, US) are important spawning and nursery habitats for striped bass (Hartman and Margraf, 2003). Anadromous striped bass are found as far south as North Carolina in the Albemarle Sound (Cooper et al., 1998). Chesapeake Bay and the Hudson River are vertically stratified and possess an Estuarine Turbidity Maximum (ETM), unlike the fully mixed Shubenacadie estuary. An ETM is a zone within an estuary that possesses an increased concentration of suspended particles and is associated with the up-stream limit of salinity intrusion. High concentrations of zooplankton are also associated with ETMs, making them ideal habitat for larval fish (Roman et al., 2001).

2.3 Striped Bass Life History

Adult striped bass are an important predator in coastal and estuarine ecosystems. The timing of spawning of striped bass varies with latitude, and generally occurs during local spring from April to June along the Atlantic coast (Merriman, 1941). The spawning season of the striped bass population in Chesapeake Bay, the species' mid-range, begins mid-April and lasts up to six weeks (Setzler-Hamilton et al., 1981). In Nova Scotia, spawning occurs mostly in the Stewiacke River approximately 3-5 km upstream of the confluence of the Shubenacadie and Stewiacke Rivers above the limit of saltwater intrusion (Rulifson and Dadswell, 1995; Rulifson and Tull, 1999). In the Shubenacadie-Stewiacke River Estuary, the onset of spawning occurs once an average daily

water temperature of 12ºC is exceeded. To estimate the occurrence of major spawning events in the Shubenacadie-Stewiacke River, 11 to 20-degree days above 12.0ºC is a useful indicator (Duston et al., 2018). For both populations of striped bass in Canada, spawning begins late May and lasts for approximately 3-4 weeks (Robichaud-LeBlanc et al., 1996; Rulifson and Tull, 1999; Duston et al., 2018). Canadian striped bass can live over 25 years and can exceed 1.0m TL (DFO, 2006). A specimen caught in the Shubenacadie River on May 10, 2010, was 1.08m TL and weighed 16.27kg. It was tagged on October 3, 1985, by Dr. R. Rulifson and was estimated to be 27-28 years old (T. Avery, pers. comm. to Dr. J. Duston, 2013).

Striped bass females are iteroparous and highly fecund. The fecundity of the Stewiacke-Shubenacadie River population ranged between 41,000 and 2.1 million eggs for females between 44.9 and 0.91m TL caught in 1994 (Paramore, 1998). Among females caught in 2011, the mean fork length, body weight, fecundity, and age were 0.815m, 6.68kg, 905,254 eggs, and 9.5 years, respectively (MacInnis, 2012). The fecundity of female striped bass from the Roanoke River-Albemarle Sound (North Carolina, US) increased by about 100,000 to 200,000 eggs with each year of growth (Olsen and Rulifson, 1992). The average fecundity of age 3 and 16-year-old striped bass was 181,000 and 5,000,000 eggs, respectively. Female striped bass produce about 50,000-83,000 eggs per kilogram of total body weight (Olsen and Rulifson, 1992; Douglas et al., 2006). Age at first maturity among female striped bass is 4-6 years of age and among males at 3-4 years at 0.5 and 0.32m FL (Merriman, 1941; Bradford et al., 2012, 2015). Striped bass broadcast their eggs and milt into the water column simultaneously near the surface (Douglas and Chaput, 2001).

Striped bass eggs are semi-buoyant, distributed throughout the water column by the turbulent estuary current. Their mean (SD) diameter in the Stewiacke River is 3.7 (0.10) mm after fertilization with a surface to volume ratio of 1.6:1 (Bergey et al., 2003). Eggs collected from the Stewiacke-Shubenacadie River Estuary are similar in diameter to eggs from other high physical energy watersheds including the Miramichi River, Roanoke River (Virginia, US), and Dan River (North Carolina, US; Bergey et al., 2003). Eggs hatch 2-3 days after fertilization and transition to the first feeding stage by 5-10 dph (Table 1; Mansueti, 1958; Bayless, 1972; Secor and Houde, 1995; Robichaud-LeBlanc et al., 1998). Striped bass eggs normally hatch within 30 hours at 21.7-22.2ºC and 70-74 hours at 14.4-15.6ºC (Merriman, 1941). Striped bass eggs of the Stewiacke-

Shubenacadie River Estuary generally hatch within 2-4 days and the emergent yolk-sac larvae are about 3.0mm TL (MacInnis, 2012). Larvae are restricted to brackish water in the Shubenacadie River Estuary and are absent from tidal freshwater (Duston et al., 2018). Most eggs were found in salinities of 6.0-7.9ppt while most larvae were collected in salinities of 10.0-11.9ppt (Rulifson and Tull, 1999). The highest density of eggs in the estuary ranged between 1.0-5.0ppt and was lowest in tidal freshwater and above 10.0ppt (Duston et al., 2018).

Striped bass larvae hatch before the mouth becomes functional and eye pigmentation (Table 1; Mansueti, 1958). They rely on their endogenous reserves (yolk and oil globule) to provide structural materials for development and sustaining its growth until they capture their first prey (Eldridge et al., 1981; Rogers and Westin, 1981). At 5mm TL, larvae are 2-5 days old with partial yolk sac absorption (Table 1; Mansueti, 1958). Larvae may reach 6.0mm TL in 7 days after fertilization and can begin to feed actively (Eldridge et al., 1981). The transition from endogenous to exogenous nutrition is defined by the switch from absorption of the yolk and partial oil globule to actively feeding. The endogenous energy source in an embryo consists of both yolk and oil and only an oil globule in a feeding larva. Starved larvae retain their oil globules longer than fed larvae whereas fed larvae quickly mobilize the oil globule energy as they develop (Eldridge et al., 1981).

Table 1. Striped bass early life stage synonymized nomenclature with Hardy (1978) as standard description. Shubenacadie River Estuary stock descriptions in comparison to Hardy (1978).

Hardy, 1978	Shubenacadie River Estuary- K. Findlay	Miramichi River- Robichaud-LeBlanc et al., 1996	Beaven and Mihurksy, 1980	Mansueti, 1958
Yolk-sac larvae 3.2-7.4mm TL	Newly Hatched Yolk-Sac Larvae (Non-Feeding) 3.5-4.9mm TL	Early Yolk-Sac Larvae	Yolk-Sac Larvae 5.0-7.6mm TL	Figure 17 Pro-Larvae Hatching 2.9-3.7mm TL
Yolk-sac larvae 5.0-5.8mm TL	Non-Feeding Larvae 5.0-5.9mm TL	Late Yolk-Sac Larvae	Yolk-Sac Larvae 5.0-7.6mm TL	Figure 18 5.3mm TL Figure 19 5.5mm TL Larvae 5.0mm TL 2-5 dph
Larvae 5.8-6.3mm TL	First Feeding Larvae 6.0mm TL	Late Yolk-Sac Larvae and Post Yolk-Sac Larvae	Yolk-Sac Larvae 5.0-7.6mm TL	Figure 21 6.3mm TL
Larvae 6.3-11.4mm TL	Feeding Stage Larvae 6.0-8.9mm TL Critical first feeding stage	Post Yolk-Sac Larvae	Finfold Larvae 6.5-9.5mm TL	7.5mm TL 10-15 dph 10.0mm TL 20-30 dph
Larvae 12.0-16.0mm TL	Feeding Stage Larvae 9.0-15.0mm TL		Post-Finfold Larvae	15.0mm TL 30-40 dph
Larvae or Early Juvenile 29.0-46.0mm TL	Transitioning to Juvenile Stage 15.1-30.0mm TL	Juveniles	9.4-15.3mm TL	20.0mm TL 50-70 dph 30.0mm TL 70-100 dph

Larvae can reach 7.5mm TL by 10 to 15 days post hatch, at which stage the yolk sac may be fully absorbed and the pectoral fins developing (Table 1; Mansueti, 1958). In the Shubenacadie River Estuary, the critical first feeding stage occurs from 6.0 to 7.0mm TL (Table 1). Larvae transition to the juvenile stage at about 20-25mm TL, defined by developing fins and spines, pigmentation, and well-developed mouths. Fins are fully developed by about 30mm TL and the horizontal stripes

appear around 50mm (Table 1; Mansueti, 1958). Mid-summer migration of striped bass juveniles occurs from the estuary into Cobequid Bay of the Inner Bay of Fundy (Duston et al., 2018). Striped bass are caught less frequently in plankton net tows in the main channel of the estuary as the summer progresses (MacInnis, 2012). Age-0 juvenile striped bass remain in Cobequid Bay throughout the summer growing months while larger bass may move to the Minas Basin and some remain in the estuary (Rulifson et al., 2008; Duston et al., 2018). Elsewhere, striped bass typically spend the first 1-2 years of life in the fresh or brackish waters of their native rivers (Merriman, 1941; Robichaud-LeBlanc et al., 1998). Over the winter, a proportion of the striped bass population reside in Shubenacadie Grand Lake, located at the head of the Shubenacadie River (Rulifson and Dadswell, 1995), but some have been recorded in the Minas Basin (Keyser et al., 2016).

2.4 Predator-Prey Relationships: Striped Bass Larvae and Prey

2.4.1 Copepod Biology and the Match-Mismatch Hypothesis

The zooplankton and ichthyoplankton (striped bass eggs and larvae) in the Shubenacadie estuarine ecosystem form a food web that has not been documented in the literature. Describing the food web will lead to insight of the factors influencing the survival and growth of striped bass early life stages and ultimately recruitment and population dynamics. The early life stage of striped bass are planktivorous and consume zooplankton, mostly copepods. Zooplankton contribute to the food web as primary consumers while mesoplankton, mysids and amphipods, are secondary consumers. Copepods are the numerically dominant prey item in the guts of larval striped bass 5-10mm TL in the Shubenacadie River Estuary (MacInnis, 2012). *Eurytemora affinis*, a calanoid copepod, was suggested by MacInnis (2012) to be the main prey, perhaps on the basis it is an important prey for first feeding striped bass in both Chesapeake Bay and the Miramichi River Estuary (Robichaud-LeBlanc et al., 1997; North and Houde, 2006; Campfield and Houde, 2011). Subsequent research following MacInnis (2012) established that *E. affinis* was very rare in the Shubenacadie River Estuary. The correct identification of the copepod species consumed by striped bass larvae at first feeding in the Shubenacadie River Estuary, and their distribution relative to salinity and time of year was the principal objective of this thesis.

Variation in the abundance of key copepod species may affect other trophic levels in an estuarine ecosystem since the abundance of fish larvae is related to changes in copepod abundance and location in an estuary (Devreker et al., 2010). Copepods are a sub-class within the Class Crustacea and are generally 0.2-5.0mm TL with a chitinous exoskeleton surrounding a segmented body (Dole-Olivier et al., 2000; Williamson and Reid, 2009). Ten Orders of copepods exist worldwide with Calanoida, Cyclopoida, and Harpacticoida the three most important (Dole-Olivier et al., 2000; Williamson and Reid, 2009). Calanoids are typically pelagic, cycloids mainly benthic with some planktonic species, and harpacticoids are benthic (Roff, 1978; Williamson and Reid, 2009). Some species are epibenthic with a pelagic naupliar stage in the upper layer of water and a benthic adult stage at the bottom of the water column. Eggs are fertilized by the attachment of the spermatophore by the male to the copulatory pore of the female (Dole-Oliver et al., 2000). The number of eggs per brood is dependent on species and correlates with body size with total fecundity of 500-1000 eggs (Allan, 1976). Fertilized copepod eggs hatch into the larval stage known as the nauplius (Allan, 1976). The twelve post-embryonic developmental stages of the copepod life cycle are divided into two groups. The first six stages are called nauplii and are designated N1-N6 and the last six are called copepodids, or copepodites, CI-CVI. The last stage, CVI, is the adult stage (Czaika, 1982).

Copepods have varying numbers of pairs of appendages according to the developmental stage (Czaika, 1982). In adults, a major articulation separates the anterior portion of the body, the prosome, from the posterior portion, the urosome (Dole-Olivier et al., 2000; Williamson and Reid, 2009). Pelagic copepods propel themselves through water with their first antennae by paddle-like thrusts of their thoracic limbs and quickly flicking their abdomen (Allan, 1976). Copepods are more capable of escaping predators and selective feeding compared to rotifers, cladocerans, and other zooplankton species (Allan, 1976). Copepods can consume prey ranging in size from 5 to 200μm (Gasparini and Castel, 1997). They feed either through filtration or raptorially by grasping particles. Calanoid copepods primarily feed through filtration of small particles and raptorial on larger particles while cyclopoid copepods are solely raptorial feeders (Allan, 1976). Harpacticoid copepods typically feed on the surfaces of sinking particles (Kiorboe, 2010).

Larval striped bass encounter spatial and temporal variation in prey abundance that affects their survival, feeding success, and growth (Rutherford and Houde, 1995; Chick and Van Den Avyle, 2000). The Match-Mismatch Hypothesis was extended to include the development of larvae up to the metamorphosis to the juvenile, or under-yearling stage (Cushing, 1990). The original Match-Mismatch Hypothesis proposed that the recruitment level was established during the period from hatching to first feeding. A match in the timing of fish spawning and larval production with the spring zooplankton bloom is critical for recruitment success (Cushing, 1990). The Match-Mismatch Hypothesis refined Hjort's "Critical Period" Hypothesis by including the degree of temporal overlap between the spring zooplankton blooms and larval fish growth as a critical determinant of recruitment strength (Cushing, 1990; Houde, 2008). The alignment in time and space of larval fish production and the spring zooplankton bloom can have a large influence on recruitment strength and survival. The temporal and spatial alignment of striped bass larvae and zooplankton in the Shubenacadie River Estuary has previously not been investigated.

The Critical Period Hypothesis proposes the fate of fish year classes is determined in the early larval stages after yolk-sac absorption when larvae need to find sufficient quantities of prey (Houde, 2008). The distribution of zooplankton prey relative to time of year and salinity and first feeding striped bass larvae must align. In both the Match-Mismatch and Critical Period hypotheses, higher density of prey leads to improved encounter rates between fish larvae and prey species (Houde, 2008; Martino and Houde, 2010). Improved encounter rates can lead to increased growth and survival of larval fish (Houde, 2008; Martino and Houde, 2010). If a shortage of prey occurs, it is likely to result in a reduction of growth or condition of the predator species and reduced survival. A decrease in prey availability can be expressed as a reduction in growth of the predator or a switch in diet to a less preferred prey species (Hartman and Margraf, 2003). In stratified estuaries that serve as nursery habitat for striped bass, such as Chesapeake Bay, the distributions of feeding larvae and zooplankton prey, mainly *Eurytemora affinis* and *Bosmina longirostris*, are located up-estuary of the salt front and/or within the Estuarine Turbidity maximum (ETM; Campfield and Houde, 2011). The Shubenacadie River Estuary, by contrast, is fully mixed with no ETM. Hence, the main objective was to determine the distribution patterns and abundance of potential prey relative to first feeding larval striped bass.

The gape, or mouth size, of larval planktivorous fish restricts the maximum prey size ingested (Seifert, 1972; Schael et al., 1991; DeVries et al., 1998). The cleithrum orifice, the smallest internal dimension, which opens into the esophagus is assumed to dictate the maximum ingestible prey size for a larval fish at maximum gape (Arts and Evan, 1987). Gape size typically increases linearly with total body length (DeVries et al., 1998). To be a potential prey item of larval yellow perch (*Perca flavescens*), the organism must be small enough for the larvae to ingest and slow enough for it to capture (Seifert, 1972).

2.4.2 Plankton of the Inner Bay of Fundy and Shubenacadie River

Published descriptions of the plankton species inhabiting the Shubenacadie River Estuary is sparse. Much of the information is based upon the plankton species of the near-by Cornwallis River which flows to the Minas Basin (Brown, 1984). In the Shubenacadie River, plankton data has been collected since 2013 through biological monitoring as part of the Alton Natural Gas Project. The sampling, sorting, identification, and enumeration of the plankton community structure and ichthyoplankton of the Shubenacadie River Estuary was the responsibility of G. MacInnis up to and including the 2012 sampling season (MacInnis 2012). Thereafter, the plankton have received greater attention, as evidenced by the use of increasingly smaller mesh on the capture gear. A 500µm plankton net mesh size was used in the Alton Gas survey from 2008 to 2012 inclusive, primarily to catch striped bass eggs and larvae. In 2013, the mesh size was reduced to 250µm to include surveying of potential prey species of striped bass larvae, without getting plugged by the high turbidity at the main study site. The data of this thesis incorporates the copepod abundances and distributions from 2013 to 2017 of *Acartia tonsa*, *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus* (Table 2) and in 2017 of a new harpacticoid.

Table 2. Taxonomic classification of copepod species captured in the Shubenacadie River Estuary from 2013-2017, not including the previously undescribed harpacticoid species. Kingdom Animalia, Phylum Arthropoda, Class Maxillopoda, and Sub-Class Copepoda (Dole-Olivier et al., 2000; Walter and Boxshall, 2018; Webref 1, 2018).

	Accepted Species Name:			
Taxonomy:	***Acartia tonsa*** (Dana, 1849)	***Coullana canadensis*** (Willey, 1923)	***Diacyclops bicuspidatus*** (Claus, 1857)	***Pseudodiaptomus pelagicus*** (Herrick, 1884)
Order:	Calanoida	Harpacticoida	Cyclopoida	Calanoida
Family:	Acartiidae	Canuellidae	Cyclopidae	Pseudodiaptomidae
Genus:	*Acartia*	*Coullana*	*Diacyclops*	*Pseudodiaptomus*
Species:	*tonsa*	*canadensis*	*bicuspidatus*	*pelagicus*
Original description:	*Acartia tonsa* (Dana, 1849)	*Canuella canadensis* (Willey, 1923)	*Acanthocyclops bicuspidatus* (Claus, 1857)	*Pseudodiaptomus pelagicus* (Herrick, 1884)
Synonymized names:	Not applicable	*Canuella elongata* (Wilson, 1932) *Scottolana canadensis* (Coull, 1972)	*Cyclops bicuspidatus* (Claus, 1857), *forbesi* (Herrick, 1895), *minnilus* (Forbes, 1893), *roseus* (Daday, 1885), *serratus* (Forbes, 1882), *subterraneus* (Pratz, 1866); *Megacyclops bicuspidatus* (Claus, 1857)	*Pseudodiaptomus americanus* (Wright, 1937) *Pseudodiaptomus coronatus* (Williams, 1906)

Zooplankton in the mouth of the Inner Bay of Fundy and Minas Channel that were considered as immigrants from the Gulf of Maine included: *Calanus finmarchicus*, *Sagitta elegans*, *Pseudocalanus minutus*, *Oithona similis*, and *Parafavella gigantea* (Jermolajev, 1958). Zooplankton species present in the Inner Bay of Fundy and Minas Channel included: *Acartia tonsa*, *Eurytemora herdmani*, *Pseudodiaptomus coronatus*, *Centropages hamatus* (Jermolajev, 1958). *Canuella canadensis* was restricted to the estuary of the Shubenacadie River (Jermolajev, 1958). Zooplankton densities in Cobequid Bay were relatively low in 1983 (Stone, 1985). *Acartia* and *Pseudodiaptomus* sp. were dominant zooplankton species from June to August while the mysid, *Neomysis americana*, was the dominant mesoplankton species. *Acartia* and *Pseudodiaptomus* sp. are common calanoid copepods in brackish, turbid waters of the Inner Bay

of Fundy (Stone, 1985). Other copepod species in Cobequid Bay included *Labidocera aestiva*, *Eurytemora* sp., and *Centropages hamatus* but were only occasionally present (Stone, 1985). *P. pelagicus* and *A. tonsa* were also reported by Bromley (1976) as present in the Minas Basin and Minas Channel and in Passamaquoddy Bay by Roff (1978).

Zooplankton diversity is generally low in highly turbid water (Jermolajev, 1958; Daborn and Pennachetti, 1979). Low densities of zooplankton were reported in the Inner Bay of Fundy and Minas Channel by Jermolajev (1958). The production of phytoplankton, a food source for zooplankton, is very low in the Bay of Fundy since the light necessary for plant growth does not penetrate deeply into the water column due to the high turbidity (Gran and Braarud, 1935). The vertical tidal mixing of the Bay of Fundy decreases the length of time for plants to remain sufficiently near the surface to obtain enough light for growth (Gran and Braarud, 1935; Jermolajev, 1958). It was speculated that zooplankton obtain nutrients from the organic matter associated with suspended sediments (Daborn and Pennachetti,1979). Maximum rates of benthic primary production by microalgae in Cumberland and Minas Basin of the Inner Bay of Fundy occurred during the early and late summer months (Hargrave et al., 1983). The concentration of chlorophyll *a* in surface sediments and the benthic primary production was lower in Minas Basin when compared to Cumberland Basin. The coarser grained deposits in Minas Basin were indicative of extensive sediment transport (Hargrave et al., 1983). The uptake of the isotope 14C was utilized to measure the phytoplankton production since high turbidity severely limited the depth of light penetration in these locations (Hargrave et al., 1983). In comparison, the ETM of the Gironde Estuary with high turbidity, had low a chlorophyll *a* rate while seaward in the estuary with lower suspended particles, had higher primary production (Irigoien and Castel, 1997). High turbidity is associated with low primary production (Irigoien and Castel, 1997).

Summarizing each copepod in alphabetical order, *Acartia (Acanthacartia) tonsa* (Dana, 1849) is a marine and estuarine calanoid copepod species and is globally distributed ranging from Australia, North Atlantic including the Gulf of St. Lawrence, and California (Figure 4; Wilson, 1932; Roff, 1978; David et al., 2005). *Acartia tonsa* is considered a summer calanoid copepod species with the peak abundance occurring from August to October in the UK (Wilson, 1932; Chinnery and Williams, 2004). It was reported in the highly turbid and macrotidal Cornwallis River Estuary of

the Bay of Fundy (Brown 1984). *Acartia tonsa* was a seasonally numerous species present among *Eurytemora affinis*, *Eurytemora herdmani*, *Pseudodiaptomus pelagicus*, and *Ectinosoma curticorne* and was the second most frequent species (Brown, 1984). *Acartia tonsa* and *Pseudodiaptomus pelagicus* replaced *E. herdmani* as the dominant species during the summer months in the Cornwallis River Estuary (Brown, 1984). *Acartia tonsa* reached densities of 2314/m^3 and 1816/m^3 in mid-June and early July, respectively, in Cobequid Bay (Stone, 1985).

Figure 4. Adult calanoid copepod *Acartia tonsa* (Dana, 1849) captured in the Shubenacadie River Estuary in 2014 (Image: Dalhousie Faculty of Agriculture, Aquaculture Centre). Mean total length 1.0mm.

Coullana canadensis is an epibenthic harpacticoid copepod that is widely distributed in estuarine brackish waters along the Eastern seaboard (Figure 5, 6; Coull, 1972; Lonsdale, 1981a; Lonsdale and Levinton, 1986). *Coullana canadensis* is an inadvertent pelagic species in the Shubenacadie River Estuary as the turbulence from the tidal bore suspends the species in the water column. It was first discovered by Willey (1923) from samples collected by Leim in 1919 from the Shubenacadie River Estuary near Shubenacadie Village (36.0rkm). *Coullana canadensis* was originally named as *Canuella canadensis* by Willey (1923) and independently by Wilson (1932) as *Canuella elongata* from samples from the upper Chesapeake Bay, Maryland (Table 1; Coull, 1972). Lang (1948) determined that *C. canadensis* and *C. elongata* were synonymous. *Canuella canadensis* was re-named *Scottolana canadensis* and finally as *Coullana canadensis* (Table 1; Coull, 1972).

Salinities of less than 10ppt favour the population growth of *C. canadensis* (Lonsdale, 1981b). The Pamlico River (North Carolina), Delaware Bay (New Jersey), Patuxent River, Chesapeake Bay, and the Shubenacadie River Estuary all are habitats of *C. canadensis* (Coull, 1972; Harris, 1977; Lonsdale, 1981a). *C. canadensis* has been collected from as far south as Tampa, Florida (Lonsdale and Levinton, 1986). The life cycle of *C. canadensis* is characterized by the naupliar development in the upper surface layers of the water column followed by adults descending to or near the benthic sediments (Lonsdale, 1981a, b; Lonsdale et al., 1988). Female *C. canadensis* have paired external eggs sacs and eggs that take 3-4 days to hatch at 20ºC (Lonsdale and Levinton, 1985). The population size of *C. canadensis* increases from late winter to early summer (Willey, 1923; Lonsdale, 1981a). Development rate of *C. canadensis,* collected from Biddeford, Maine, in 10.0ppt, was fastest at 15.0ºC when compared to 20.0 and 25.0ºC (Lonsdale and Levinton, 1986). *C. canadensis* population size in Chesapeake Bay were highest in April and May (Lonsdale, 1981b; Lonsdale and Levinton, 1989).

Figure 5. Adult female gravid *Coullana canadensis* (Willey, 1923) harpacticoid copepod captured in the Shubenacadie River Estuary in 2014 (Image: Dalhousie Faculty of Agriculture, Aquaculture Centre). Mean total length 2.0mm.

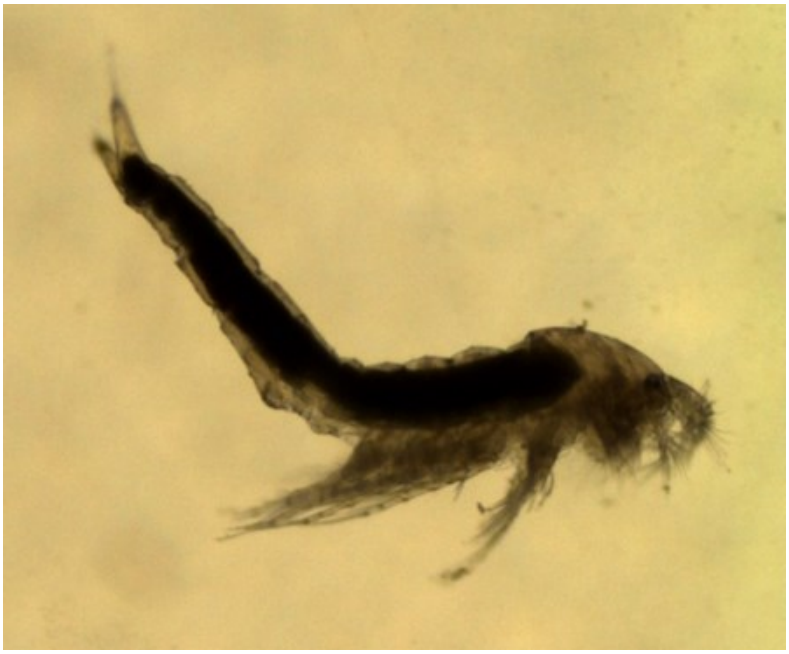

Figure 6. Adult *Coullana canadensis* (Willey, 1923) harpacticoid copepod captured in the Shubenacadie River Estuary in 2014 (Image: Dalhousie Faculty of Agriculture, Aquaculture Centre). Mean total length 2.0mm.

Diacyclops bicuspidatus (Claud, 1857; Figure 7) is a cyclopoid copepod is generally benthic and has been found in freshwater eutrophic lakes in South Germany and ground water in Finland (Maier, 1990; Maier, 1998; Sarkka et al., 1998). Cyclopoid copepods have also been recorded in the Great Lakes and other large lakes in North America (Czaika, 1982; Maier, 1990). Its presence in the Shubenacadie River is the first report of *D. bicuspidatus* in an estuary.

Figure 7. Adult female gravid *Diacyclops bicuspidatus* (Claus, 1857) cyclopoid copepod captured in the Shubenacadie River Estuary in 2014 (Image: Dalhousie Faculty of Agriculture, Aquaculture Centre). Mean total length 3.1mm.

Figure 8. Adult *Diacyclops bicuspidatus* (Claus, 1857) cyclopoid copepod captured in the Shubenacadie River Estuary in 2014 (Image: Dalhousie Faculty of Agriculture, Aquaculture Centre). Mean total length 3.1mm.

Pseudodiaptomus pelagicus (Herrick, 1884) is a globally distributed calanoid copepod that is predominantly estuarine, but also found in shallow coastal waters (Figure 9; Roff, 1978; Walter, 1989). *Pseudodiaptomus* species are semi-benthic calanoid copepods since the adults are generally substrate oriented and the nauplii and copepodites are pelagic (Walter, 1989; Ohs et al., 2010). *Pseudodiaptomus pelagicus* was originally described by Herrick (1884) and is synonymous with *Pseudodiaptomus coronatus,* Williams (1906; Table 2). *Pseudodiaptomus coronatus* was described by Williams (1906) from samples captured from Narragansett Bay, Rhode Island. The accepted name is *Pseudodiaptomus pelagicus* (Table 2). *P. pelagicus* was recorded in the Miramichi River by Willey (1923; Table 2). *Pseudodiaptomus* species predominantly inhabit estuaries (Grice, 1969). *P. pelagicus* differs from other copepod species as it only undergoes five naupliar stages instead of six (Jacobs, 1961; Grice, 1969). *Pseudodiaptomus pelagicus* was abundant in late July in Cobequid Bay reaching a density of 472/m^3 (Stone, 1985). *P. pelagicus* was also identified in the Cornwallis River Estuary of the Bay of Fundy as a dominant species during the summer months in the turbid estuary (Brown, 1984).

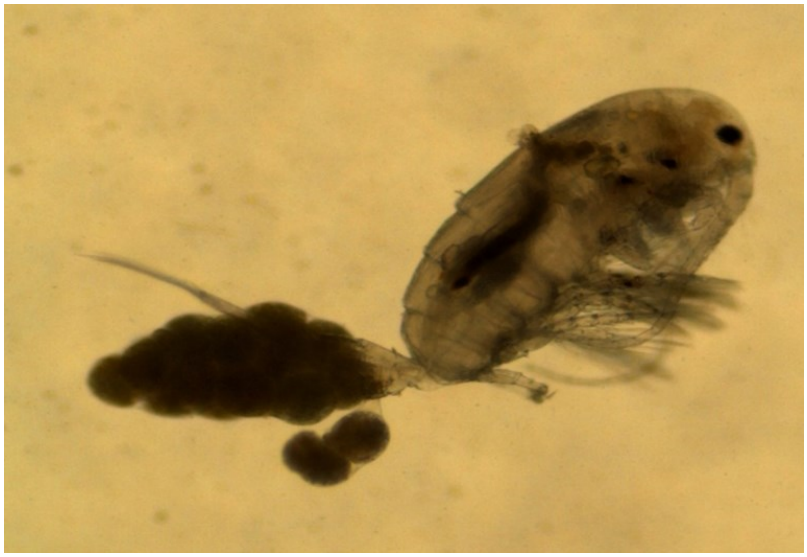

Figure 9. Adult female gravid *Pseudodiaptomus pelagicus* (Herrick, 1884) calanoid copepod captured in the Shubenacadie River Estuary in 2014 (Image: Dalhousie Faculty of Agriculture, Aquaculture Centre). Mean total length 2.1mm.

Figure 10. Adult *Pseudodiaptomus pelagicus* (Herrick, 1884) calanoid copepod captured in the Shubenacadie River Estuary in 2014 (Image: Dalhousie Faculty of Agriculture, Aquaculture Centre). Mean total length 1.9mm.

The most frequently occurring copepods of the Cornwallis River Estuary of the Bay of Fundy between 1980 and 1982 were *Eurytemora affinis*, *Acartia tonsa*, *Pseudodiaptomus pelagicus*, *Temera longicornis*, *Centropages* sp., *Pseudocalanus* sp., *Temera discaudatus*, and *Labidocera aestiva* (Brown, 1984). *P. pelagicus* and *A. tonsa* dominated the zooplankton structure during the summer months. *Eurytemora herdmani* is also an abundant zooplankton species present in the Cornwallis River in the summer months especially at the entrance to the Southern Bight (Daborn and Pennachetti, 1979). The physical characteristics of the Cornwallis River Estuary are similar to the Shubenacadie River Estuary. The water column is almost completely mixed in terms of temperature and salinity and the salt front moves up and down the estuary each tidal cycle. The estuary can reach 22.0ºC in August and the salinity ranges from tidal fresh water to 28.0ppt (Brown, 1984). The light penetration of the river is restricted by the high suspended sediment concentration with light penetrating to 1.0m (Daborn and Pennachetti, 1979).

Striped bass larvae develop quickly to the first-feeding stage in the Shubenacadie River Estuary, but do not begin to feed and grow until late June (MacInnis, 2012; Duston et al., 2018). The multi-year data presented in Duston et al., (2018) demonstrate this a consistent phenomenon. I hypothesized that the high incidence of empty guts of striped bass larvae in the Shubenacadie River

Estuary was due to a lack of suitable sized zooplankton prey. The incidence of empty guts has been recorded as high as 64% in larvae 5-7mm TL (Duston et al., 2018). Copepods serve as prey for early feeding striped bass along the Eastern seaboard with the calanoid copepod *Eurytemora affinis* documented as the most dominant prey item along with the cladoceran *Bosmina longirostris*. The abundance of *A. tonsa*, *C. canadensis*, and *P. pelagicus* in the Shubenacadie River Estuary, Cobequid Bay, and the Minas Basin of the Inner Bay of Fundy has been documented to some extent (Willey, 1923; Jermolajev, 1958; Roff, 1978; Stone, 1985). However, the distribution of these potential prey items and striped bass larvae relative to salinity and season have not been investigated. Using data collected from 2013-2015 by summer students under the direction of Dr. J. Duston, and data I collected 2016-17, this thesis describes the temporal (time of year) and spatial (salinity) distribution of copepods relative to the diet of first feeding stage striped bass in the Shubenacadie River Estuary.

2.5 Summary of Thesis Objectives

The objectives to the study were to:

1. Describe the copepod prey of striped bass from first feeding (TL 6.0-9.0mm) in late-June through to August by analysis of gut contents.

2. Test the match-mismatch hypothesis by describing the distribution of striped bass larvae and their gape relative to the distribution of potential prey.

3. Verify the identity of copepods using DNA analysis, in particular, the principal prey of first feeding striped bass larvae.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Area: Temperature and Salinity of the Shubenacadie River Estuary

Conductivity, temperature and depth (CTD) was recorded between May and October at 5-minute intervals using a logger (CTD-Diver®; Van Essen Instruments) deployed at the main study site at Alton (25.0rkm; Figure 11, 13) and up-estuary at the Shubenacadie Village Bridge (36.0rkm; Figure 12, 14).

The mean daily water temperature and salinity at the main study site, 25.0rkm, on April 27, 2016, was 9.2ºC and 6.1ppt (Figure 11). The temperature rose to a mean of 16.0ºC by the end of May. The highest mean temperature was 22.3ºC on July 1, 2016. The temperature decreased slightly to a mean of 17.6ºC on July 11, 2016 (Figure 11). The salinity dropped in early June due to the freshwater run-off from the high precipitation near the end of May. The salinity in 2016 was much higher than average. The logger was beached after July 11, 2016 (Figure 11).

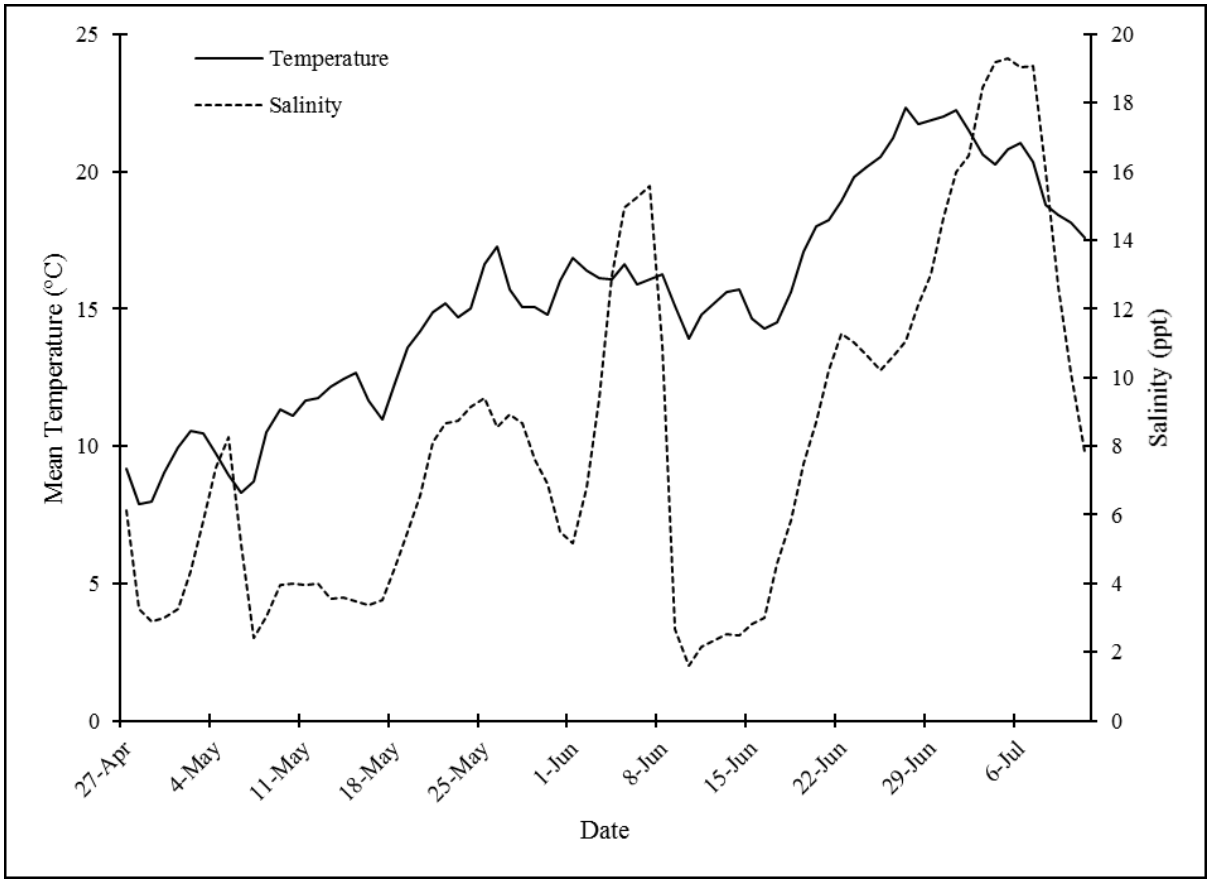

Figure 11. Daily mean temperature (ºC) and salinity (ppt) in the Shubenacadie River Estuary at the main study site (25.0rkm) from April 27 to July 11, 2016.

The mean daily water temperature and salinity at 36.0rkm on May 2, 2016, was 11.2ºC and 0.1ppt (Figure 12). The mean temperature decreased to 8.5ºC on May 6, 2016. The temperature rose to a mean of 15.9ºC by the end of May. The highest mean temperature was 25.5ºC on July 29, 2016. The highest mean salinity was 14.0ppt on August 31, 2016. The monthly peaks in salinity were due to the spring tides on the new moon. The mean temperature decreased to 20.6ºC on September 5, 2016 (Figure 12).

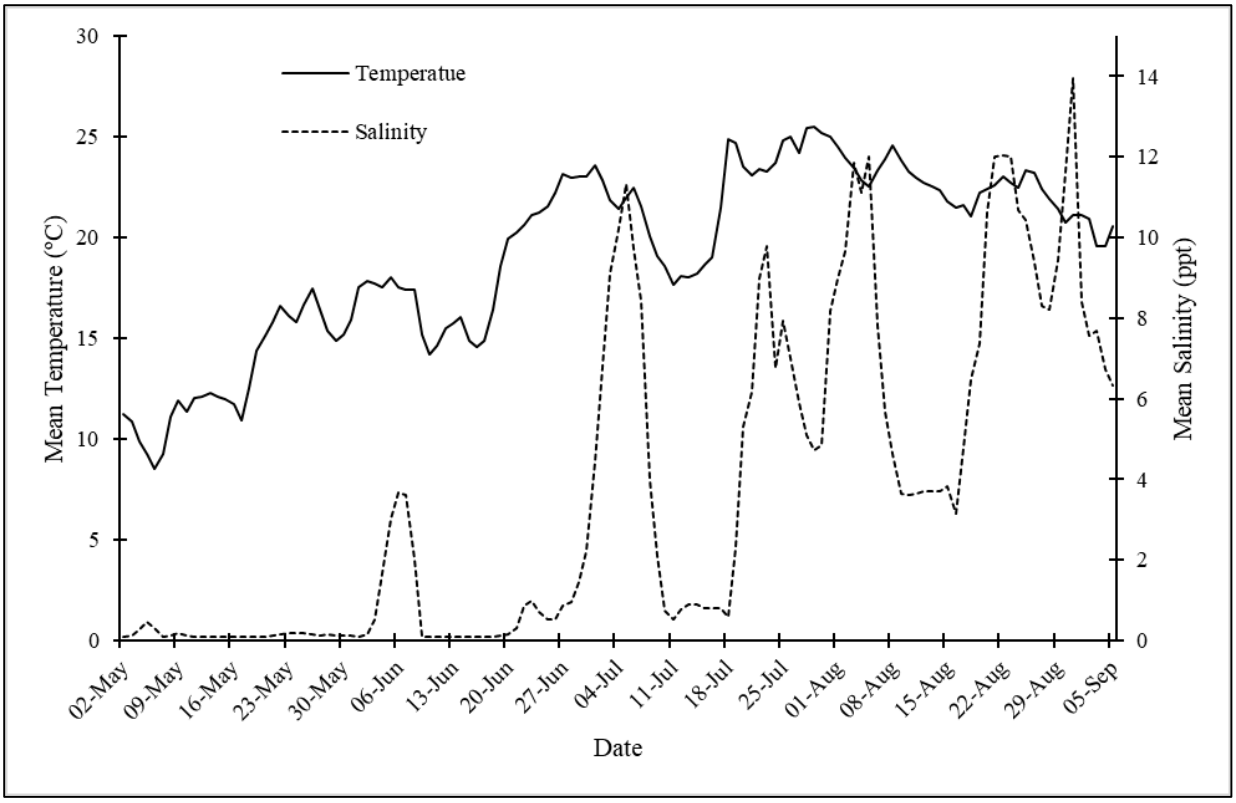

Figure 12. Daily mean temperature (ºC) and salinity (ppt) in the Shubenacadie River Estuary at Shubenacadie Village Bridge (36.0rkm) from May 2 until September 5, 2016.

In 2017, the temperatures were similar to 2016 but the salinity was lower. The daily mean water temperature and salinity at 25.0rkm on May 3, 2017, was 10.7ºC and 2.3ppt (Figure 13). The temperature rose to a mean of 13.7ºC by the end of May. The highest mean temperature was 24.3ºC on August 5, 2017. The highest mean salinity was 18.8 ppt on July 25, 2017 (Figure 13). The high precipitation in early July attributed to the freshwater run-off and low salinity in the estuary with a total rainfall for July of 145.0mm (Gov. of Canada, 2018).

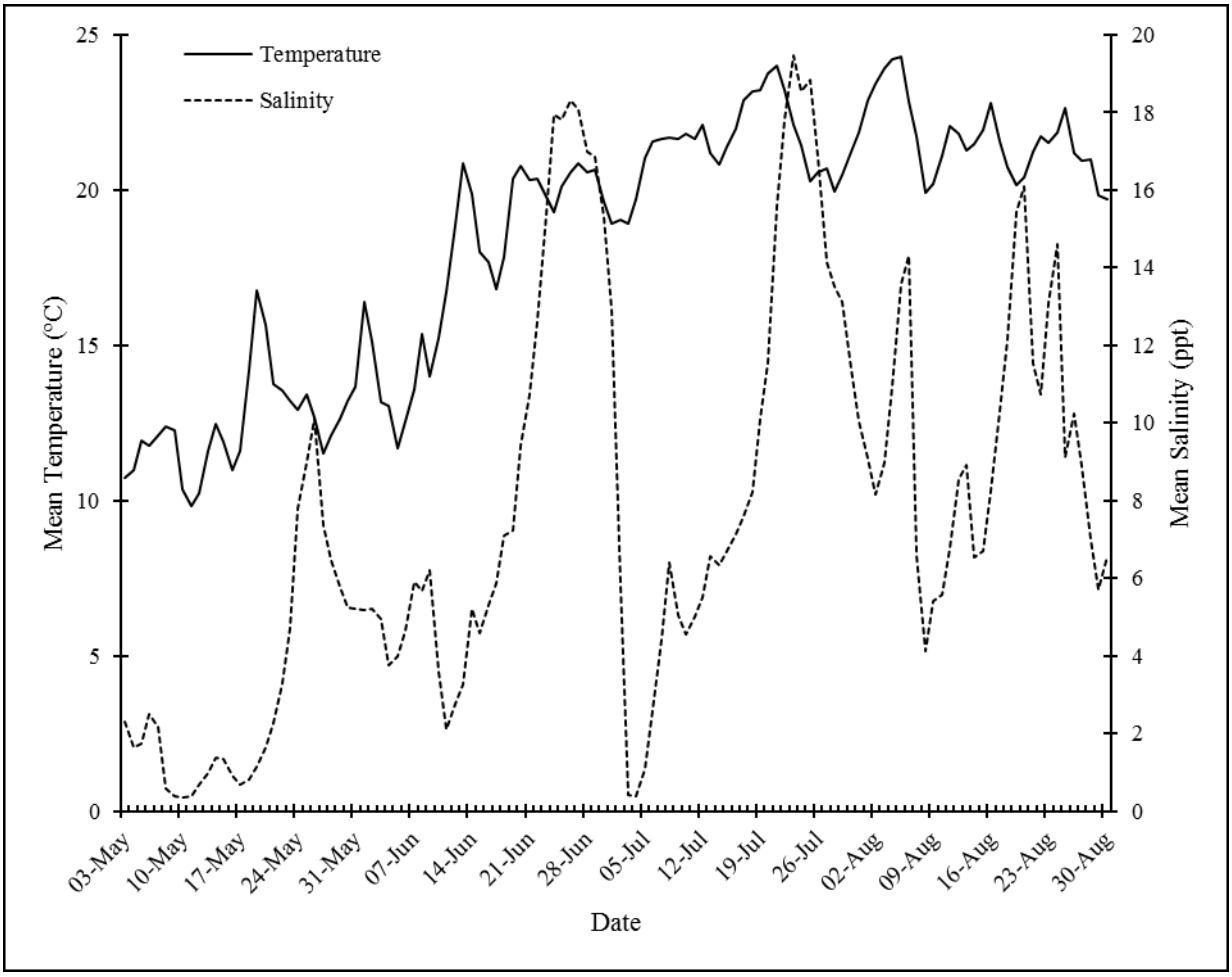

Figure 13. Daily mean temperature (ºC) and salinity (ppt) in the Shubenacadie River Estuary at the main study site (25.0rkm) from May 3 until August 30, 2017.

The daily mean water temperature and salinity on May 2, 2017, was 8.5ºC and 0.1ppt (Figure 14). The temperature rose to a mean of 15.1ºC by the end of May. The highest mean temperature was 25.8ºC on July 22, 2017. The highest mean salinity was 8.8ppt on August 20, 2017 (Figure 14). The monthly peaks in salinity were due to the large tides caused by the new moon.

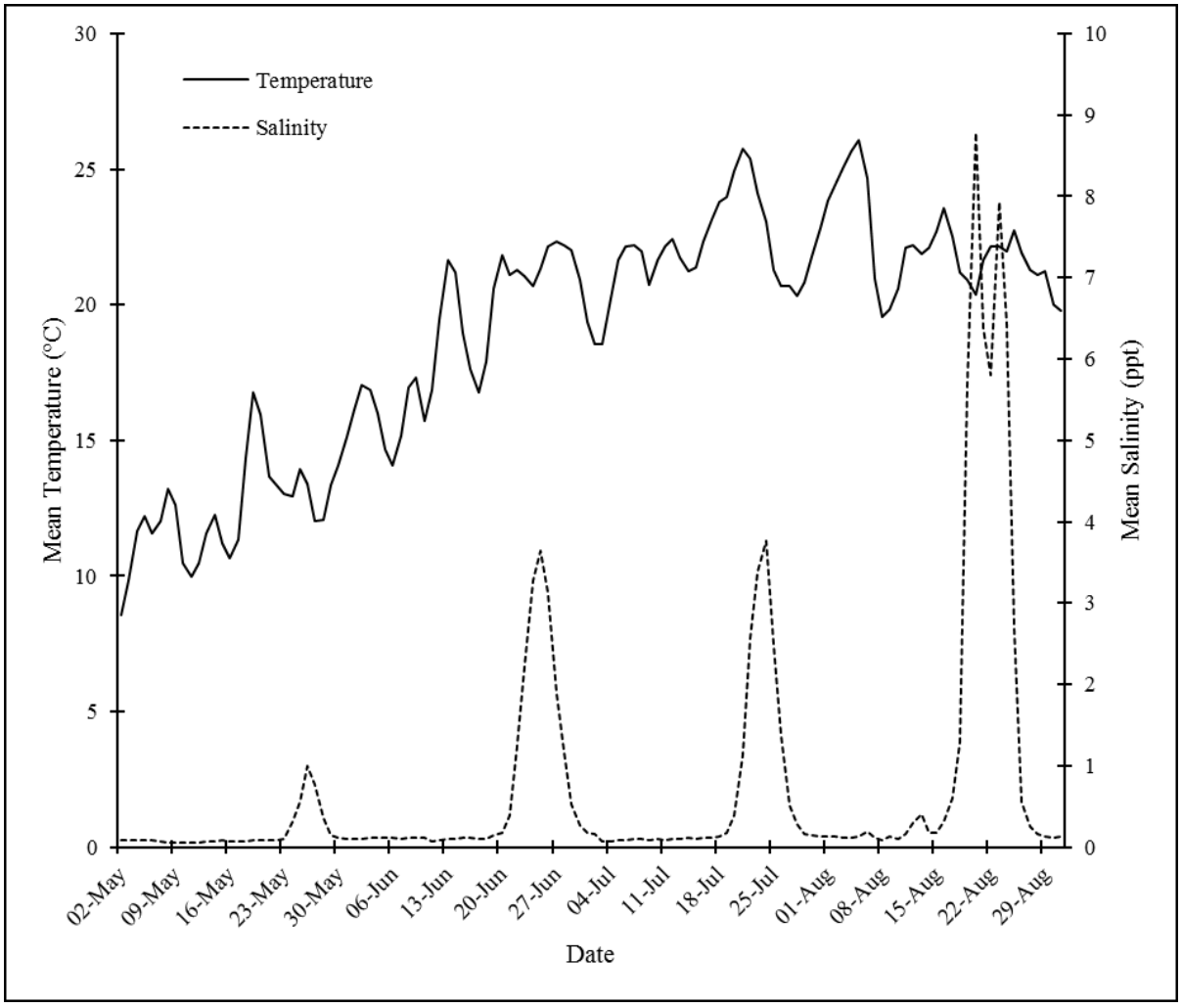

Figure 14. Daily mean temperature (ºC) and salinity (ppt) in the Shubenacadie River Estuary at Shubenacadie Village Bridge (36.0rkm) from May 2 until August 31, 2017.

Air temperature and precipitation data was obtained from the Halifax International Airport weather station (Gov. of Canada, 2018). In 2016, the monthly mean air temperature for May, June, July, and August was 10.7, 14.4, 19.5, and 19.6°C, respectively (Gov. of Canada, 2018). The highest monthly maximum temperature for May, June, July, and August was 26.3, 26.9, 30.0, and 29.0°C, respectively. The lowest monthly minimum air temperature for May, June, July, and August was 0.0, 1.8, 10.6, and 10.4°C, respectively. The total monthly precipitation for May, June, July, and August was 100.1, 72.5, 73.4, and 44.5mm, respectively (Gov. of Canada, 2018). As the summer progressed, the water in the upper estuary became more saline as the freshwater run-off decreased and the air and water temperature increased.

In 2017, the monthly mean air temperature for May, June, July, and August was 10.2, 15.7, 18.6, and18.8°C, respectively (Gov. of Canada, 2018). The highest monthly maximum temperature for May, June, July, and August was 29.8, 30.2, 29.3, and 30.1°C, respectively. The lowest monthly minimum temperature for May, June, July, and August was1.6, 4.2, 9.3, and 8.9, respectively. The total monthly precipitation for May, June, July, and August was 156.0, 69.3, 145.0, and 93.7mm, respectively (Gov. of Canada, 2018). Similar to 2016, as the summer progressed, the water in the upper estuary became more saline as the freshwater run-off decreased and the air and water temperature increased. June 2017 was warmer and drier than June 2016 during peak striped bass spawning and the emergence of striped bass larvae in the estuary.

3.2 Plankton Net Sampling: Distribution Relative to Time of Year and Salinity

Plankton net tows were conducted in the main channel of the estuary to estimate the zooplankton abundance (individuals per m^3 water filtered; IND/m^3). The sampling enabled the quantification of the abundance of striped bass eggs and larvae, the gut contents from first-feeding larvae to juveniles, the distributions of invertebrate plankton relative to salinity and time of year, and to determine food available for the first feeding striped bass. The work included the monitoring requirements defined in the document 'Alton Natural Gas Storage Estuarial Environmental Monitoring and Toxicity Testing' (2015). Copepod species distribution and abundance (IND/m^3) data from 2013 to 2015 were compiled by undergraduate summer research students under the direction of Dr. J. Duston, and data from 2016 and 2017 was compiled by the author.

To quantify the temporal (time of year) distribution of zooplankton and striped bass larvae (ichthyoplankton) through the flood tide, plankton net tows were conducted at the main study site, a fixed location at 25.0rkm, from 2013 to 2017 from May until August. Samples were collected from the top 1.0m of the water column in the main channel facing the current using a standard conical plankton net with a 250µm mesh and 0.5m diameter mouth and 3:1 length to mouth ratio (Aquatic Research Instruments, Idaho). Tows were completed behind a 3.7m long wooden, flat-bottomed scow equipped with a 4-horsepower outboard motor (Yamaha four-stroke, short shaft). Water temperature (°C), time (hh:mm), and salinity (ppt) were recorded for each tow using a

handheld meter (model YSI Pro Plus). The plankton net was fitted with a calibrated flowmeter (model number 2030R6; General Oceanics, Florida) to estimate the volume of water (m^3) filtered during each tow. Approximately $5m^3$ of water was filtered per 30-second tow. At the main study site, two tows were completed within the last thirty minutes of the ebb tide, then a single tow every ten minutes through the flood tide until high slack was reached (approximately 90 minutes).

The samples were removed from the plankton net screened collection cup, rinsed with river water, and immediately transferred to a 2L plastic container (Ropak, Springhill, Nova Scotia) and labelled. The samples were fixed with 10% buffered formalin (Fisher Scientific) coloured pale pink with Rose Bengal dye (Sigma Chemicals) by adding 5 drops. The formalin utilized was 10% but when mixed with the river water became diluted to about 5%. The fixed plankton samples were transported back to the laboratory for sorting, identification, and enumeration of species. The Rose Bengal dye stained the zooplankton pink to facilitate their sorting from the excessive detritus and help with identification of morphological features.

To determine the distribution of ichthyoplankton and zooplankton relative to salinity, plankton net tows were conducted both at a fixed location (25.0rkm, main site) through the flood tide, and also up-estuary beginning at the salt-front at high tide on the Shubenacadie River which ranged from 25 to 44rkm depending on the magnitude of the tide and freshwater run-off. Access to the upper estuary was achieved using a second boat docked at 28rkm on the Shubenacadie River. Navigating the confluence of the Stewiacke-Shubenacadie Rivers was too dangerous due to hazardous mud flats.

To determine the spatial distribution of ichthyoplankton and zooplankton up-estuary of the main site, plankton net tows were conducted from May to August 2013 to 2017 between 28 and to 44rkm. Duplicate 30-second plankton net tows were conducted utilizing the same conical plankton net (250µm mesh size). Sampling started at the salt front (0.15ppt) at high day time tide (high slack), then proceeded down estuary on the early ebb tide at intervals of approximately 1.0rkm ending at 28rkm docking site. The samples were collected from the top 1.0m of the water column in the main channel of the upper estuary facing the current. The location of each replicate tow at

each site was recorded by a handheld global positioning system device (Garmin model GPSMAP® 62s).

To catch the smaller potential prey items, in 2016 exploratory sampling was conducted using a small plankton net (20cm diameter) with an 80µm mesh. The material collected was preserved in 90% ethanol for DNA analysis and included a small harpacticoid also found in the gut of striped bass. In 2017, to catch the small harpacticoid more efficiently, a Bongo net with a 150µm mesh was purchased and first used June 26 (Aquatic Research Instruments, Idaho). It had a 3:1 length (120cm) to mouth (30cm) ratio and a screened collection cup (9cm diameter) and yielded two duplicate samples. It could not be used for sampling during the flood tide at the main site because of plugging due to the high quantity of suspended sediment but worked well up-estuary.

3.3 Sample Sorting and Enumeration

Identification and enumeration of species was typically completed within two days of collection. The contents of each sample container (100-250mL) was emptied into a 5L plastic graduated beaker, diluted with tap water to either 1500, 2000, 2500, or 3000mL depending on the degree of detritus and turbidity. A silica air stone was bubbled vigorously to re-suspend and homogenize the material. Three sub-samples, each 30mL, were taken using a 50mL glass beaker. Each sub-sample was poured into a large petri dish (14.5cm diameter, partitioned into quarters) then examined under a dissecting microscope (Leica EZ4), and the ichthyoplankton and zooplankton were identified and counted.

Striped bass eggs and larvae were identified by developmental stage using the guide by Bayless (1972). Striped bass were described either as new eggs, 24-hr eggs, close to hatch eggs, non-feeding larvae, feeding stage larvae, or juveniles. Juveniles were defined as young of the year (age-0) that had a general adult appearance with differentiated soft and spiny fins (Robichaud-LeBlanc et al., 1996). Horizontal stripes along the flanks developed around 4-5cm TL. The gut was teased apart using fine-tipped forceps (Dumont #5; Fine Science Tools, Vancouver) to allow the contents to be counted and identified. Gape was estimated by inserting fine forceps into the mouth to open

the jaws and measured by eye with a standard ruler to the nearest 0.5mm with aid of a dissecting scope.

3.4 Copepod DNA Extraction, PCR, and Sequencing

To identify the species of copepod visible in the gut of first-feeding larval striped bass, specimens of the same species captured in the water column were initially stored in 70% ethanol prior to DNA extraction. Initially, a DNeasy® Blood and Tissue Kit (Qiagen) was utilized to extract DNA from a total of 180 specimens in December 2016. This method proved inadequate; the DNA extracted was of poor quality for conventional PCR to be carried out and yielded inconclusive results. The protocols from Easton and Thistle (2014) using 6% InstaGene™ Matrix were followed for the DNA Extraction Method 5. 6% InstaGene™ Matrix was ordered from Bio-Rad Laboratories and included iProof™ High-Fidelity buffer and iProof™ High-Fidelity DNA polymerase. The InstaGene™ Matrix was mixed at room temperature for 60 seconds on a stirring hot plate. 10.0µl was pipetted into a sterile 1.5mL microcentrifuge tube. The copepods were transferred to the tube of InstaGene™ Matrix and submerged. The tube was incubated in a heating block at 56°C for 30 minutes. The tube was vortexed for 10 seconds and shook by hand to ensure that the copepods were submerged. The tube was placed back into the heating block overnight. The following morning, the tube was vortexed for 10 seconds and shook by hand to ensure the copepods were submerged. The tube was placed in the heating block for 8 minutes at 100°C followed by an additional vortex (10 seconds). The tube was centrifuged (11,200rcf) for 2 minutes. The supernatant was transferred to a new sterile 1.5mL microcentrifuge tube using a pipette.

3.4.1 PCR Amplification

DNase-RNase free water was added to obtain 100µM stock concentration of both the forward primer (18SFHI) and reverse primer (18SR329; Integrated DNA Technologies, Illinois, US; Easton et al., 2010). Forward primer: FHI (5'GTGCATGGCCGTTCTTAGTTG-'3'). Reverse primer: R329 (5'TAATGATCCTTCCGCAGGTTCACCTACGG-3'). The stock was diluted to a 10µM working solution before they were used in the PCR reactions. The targeted region was the gene that coded for the 18S component of the ribosome.

A Master Mix was prepared for five reactions (Table 3). Master Mix reagents consisted of nuclease free water, buffer, magnesium chloride ($MgCl_2$), deoxyribonucleotide triphosphate (dNTPs), forward primer (FHI), reverse primer (R329), and the DNA sample (Table 3). Reactions included: 1) DNA supernatant from InstaGene™ Matrix, 2-4) DNA previously extracted from three copepods, and 5) one negative control (nuclease free water). The Master Mix total volume was 250µl.

In the sterile 200µl PCR tubes, 45.0µl of the Master Mix (Table 3) and 5.0µl of each reaction were pipetted. The PCR protocol consisted of three cycles: 1) 95°C for 3 minutes, 2) 45 cycles each having 95°C for 30 seconds, 57°C for 1 minute (annealing temperature), 72°C for 45 seconds (extension temperature) and 3) 72°C for 5 minutes and 4°C for 5 minutes to cool down.

Table 3. PCR Master Mix prepared for five PCR reactions for the DNA extraction using InstaGene™ Matrix. The total volume was 250µl.

Reagent	**1 Reaction (µl)**	**5 Reactions (µl)**
Nuclease Free Water	23.5	117.5
Buffer	10.0	50.0
$MgCl_2$	0.0	0.0
dNTPs	1.0	5.0
Forward Primer	5.0	25.0
Reverse Primer	5.0	25.0
Polymerase	0.5	2.5
DNA	5.0	25.0
Total Volume:	50.0	250.0

3.4.2 Gel Electrophoresis and Clean-Up

A 50mL, 1% agarose gel was prepared by adding 0.5g of powdered agarose (Invitrogen UltraPure™ Agarose) to 50mL Tris-Acetate-EDTA (TAE) buffer and heated for 1 minute in a microwave until the mixture boiled. Ethidium bromide (2.0µl of 10mg/mL) was added, then the mixture was cooled for 10 minutes, then poured into a gel tray with a comb to create wells for the PCR assay products. The gel electrophoresis tank (Electrophoresis Labnet Power Station 300) was filled with TAE buffer then the gel tray was submerged into the tank after setting and removing the comb. 3.0µl of 100bp Gene Ruler (Fisher Scientific) DNA Ladder and 10.0µl of each of the six PCR products were pipetted into the gel wells. The gel was run at 120V for 50 minutes.

To detect the DNA bands, the gel was removed from the TAE buffer and visualized under UV light in a BioRad Transilluminator GelDoc™ to detect DNA bands (Figure 15). The DNA band was excised from lane 1 by placing the gel under UV light and cutting the band with a scalpel (Figure 15). A Wizard® SV Gel and PCR Clean-Up System kit (Promega) was utilized to extract and recover the amplicon band from the agarose gel. The extracted amplicon band from the gel was placed in aa separate microcentrifuge tube, weighed (0.001gm), and equal parts of binding solution provided with the kit was added to the tube. The gel and binding solution in the tube was heated to dissolve the gel. Sample was transferred to column containing a mini-column inserted into a collection tube both of which were provided with the kit, incubated at room temperature for 5 minutes then centrifuged (16,000g) for 1 minute. 700µl of wash solution provided with the kit was added to the tube and centrifuged (16,000g) for 1 minute and the flow through liquid was discarded. 500µl of wash solution was added to the tube again and centrifuged (16,000g) for 5 minutes; flow through decanted. The sample was further centrifuged (16,000g) again for 1 minute then the mini-column was placed into new 1.5mL sterile microcentrifuge tubes. 10.0µl of nuclease free water was added to the tube and incubated at room temperature for 1 minute followed by an additional centrifuge (16,000g) for 1 minute. 5.0µl was added to the column again and centrifuged for 2 minutes. The concentration of amplicon from the supernatant obtained from the gel clean-up system was measured using the NanoDrop™ Spectrophotometer (Thermo Scientific™).

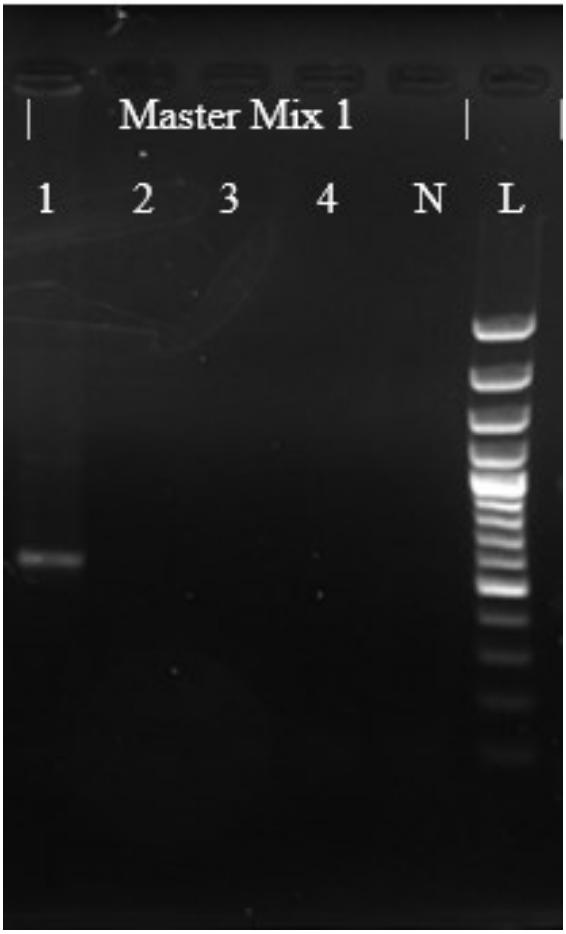

Figure 15. Gel image of the PCR products from the DNA extraction showing bands in **lane 1** of the Ectinosomatidae copepod species. Gel was run using 1% TAE agarose gel at 120V for 50 minutes. **L** DNA Ladder; **1-4** copepod DNA; **N** negative control of nuclease free water.

3.4.3 DNA Sequencing

Sample was prepared and sent for Sanger nucleotide sequencing at the Atlantic Centre for Agricultural Genomics, Dalhousie Faculty of Agriculture. The DNA sequencing result was edited using the BioEdit Sequence Alignment Editor 7.0.5 (1997-2005) Software. The forward and reverse sequences were edited by trimming the nucleotides of the sequence starting at the 3' end. The complimentary reverse sequence was aligned with the forward sequence by running a ClustalW to create a Consensus Sequence. The Consensus Sequence was assembled and edited from the sequenced PCR products (Easton and Thistle, 2016). The Consensus Sequence was copied and pasted into GenBank under a Nucleotide Blast search to search for the most closely

related copepod species. The information obtained from GenBank was utilized for the phylogenetic analysis of the Ectinosomatidae copepod species.

3.5 Gut Content Analysis

Gut contents of each striped bass larva were examined. The transparent bodies allowed gut contents to be counted and identified without dissection up to a body size of about 25 mm TL. The stomachs of striped bass larger than 25 mm TL were dissected from the body cavity using a scalpel. Gut contents were examined under a dissecting microscope (Leica EZ4) and counted were classified by taxa: (1) copepods identified to species, (2) mysid (*Neomysis americana*), (3) amphipods, (4) digested material was recorded as present or absent, but could not be quantified. Among the digesta, mysids could be identified by the presence of paired eye stalks and amphipods by their segmented exoskeleton.

The mean number prey in the gut per larva were categorized according to larvae body size: 6.0-6.9, 7.0-7.9, 8.0-8.9, 9.0-15.0, 15.1-20.0, and 20.1-25.0mm TL. The abundance of zooplankton in the water column was also compared with the gut contents of larvae from the same tow. The relative abundance was calculated to compare the gut content items to the zooplankton in the water column in the same sample tows. The mean daily relative abundance of potential zooplankton prey in the water column was calculated based on samples taken between June 6 and July 28, 2016 when larval striped bass were present in the estuary. The relative abundance was calculated as the density (IND/m^3) of zooplankton prey taxa species in the water column for each tow as a percentage of the sum of densities over all tows within a single flood or ebb tide.

3.6 Copepod Image Analysis

The digital imaging analysis of copepods was conducted to determine the body size of each species. Images were captured using a Motic Stereo Microscope (Model #SMZ-140-143-LED), fitted with a 2-megapixel Motic camera (Model # DCM2.2). Motic Image Plus 2.0 software was utilized, and the images were captured in 1680x1050 resolution with a 1 mm scalebar present. The scalebar was used to calibrate further image analysis software. The image analysis and associated

statistical analysis was conducted using Image Pro 7 software by Hayden Breau, M.Sc. Candidate, Dalhousie Faculty of Agriculture (2017) as part of a graduate module supervised by Dr. D. Barrett. Differences in mean body length between copepod species was compared by one-way ANOVA analysis of variance. Tukey's Honest Significant Difference and Tamhane test were utilized to determine which species differed significantly in length. These tests were utilized due to the unequal sample size. Additionally, egg-bearing, or gravid, copepods were compared to non-gravid specimens of the same species using an Independent Samples t-test.

Scanning Electron Microscopy (SEM) and imaging of the harpacticoid that was the initial copepod prey of striped bass larvae was completed at the Scientific Imaging Suite (SIS), Department of Biology, Dalhousie University, under the supervision of Dr. Ping Li. Ten adult stage copepods were diffusely coated with gold palladium. Five copepods were placed on a copper coated stab and the other five on a carbon coated stab. The diffuse gold plating ran at a current of 30mA for 130 seconds to obtain a thickness of 10nm in a Leica EM ACE200 Vacuum Coater. Since biological specimens are non-conductive, the gold palladium served as a conductive metal coating to produce electrons for imaging.

3.7 Statistical Methods

3.7.1 Copepod Abundance Relative to Salinity and Time of Year (2013 to 2017)

The objective of the statistical analysis was to quantify the relationship between the abundance of copepods in relation to both salinity and time of year. The plankton net tows (250µm) yielded the abundance of zooplankton per cubic meter (m^3) of water filtered. The unpublished abundance data for each species of copepod collected from 2013 to 2017 from all ebb and flood tides was pooled then sorted into categories by week (Table 4) and by salinity. Prior to pooling the data, a quality control check was conducted. All plankton net tows with less than $1/m^3$ of water filtered were deleted since the low volume of water filtered indicated a malfunction of the flowmeter or the net was plugged with sediment. For each species (*A. tonsa*, *C. canadensis*, *D. bicuspidatus*, *P. pelagicus*), using the entire dataset as a reference, tows with an abundance of $0/m^3$ were deleted when either the salinity was outside the species range or time periods of the year when the species

was normally absent from the water column. In addition, any weeks or salinity categories with only one data point were excluded. Week 34, August 21-27, was excluded from all species distributions and all sampling years (2013-2017) since no sampling was conducted. For *Coullana canadensis*, week 18 and 31 were missing. The mean abundance was calculated of the two replicate tows conducted at each site up-estuary. The mean abundance (IND/m^3) of each species was calculated within each week for each of nine salinity categories: 0.00-0.15, 0.16-1.0, 1.1-2.0, 2.1-5.0, 5.1-10.0, 10.1-15.0, 15.1-20.0, 20.1-25.0, and 25.1-30.0ppt. The categories were the same used to describe the distribution of striped bass larvae (Duston et al. 2018).

The experimental unit was a plankton net tow and the response variable was the abundance of each species (IND/m^3). Weekly and salinity categories with more than ten data values were subjected to a random selection procedure using Minitab. The sub-sampling randomly selected ten values from each category for analysis. Sub-sampling was necessary because some categories had a high number of values that overly increased the sensitivity of the one-way ANOVA analysis due to the increased range of error and therefore would not abide by the normality parameters. The number of data values for each category for each species ranged from four to ten. The mean abundance of each species between weeks (salinities pooled), and between salinity categories (weeks pooled) were compared using a one-way ANOVA analysis of variance following appropriate transformation to meet normal distribution and constant variance assumptions on the error terms (Montgomery, 2012). The logarithmic transformation was utilized for all species for the abundance per week and per salinity group. The Anderson-Darling normality test and Tukey's Pairwise comparison was utilized (Minitab 18).

The abundance of the harpacticoid was analyzed using samples collected using both the 150 and 250µm plankton net. The mean actual abundance was calculated per week and per salinity category in 2017 and the data was subjected to the same quality control parameters as the other copepods. The logarithmic transformation was utilized prior to the one-way ANOVA analysis of variance.

Table 4. Weeks of the year and corresponding dates utilized to describe the seasonal distribution of both zooplankton and striped bass larvae mean abundance (IND/m^3).

Week of the Year	Date
18	May 1- May 7
19	May 8- May 14
20	May 15- May 21
21	May 22- May 28
22	May 29- June 4
23	June 5- June 11
24	June 12- June 18
25	June 19- June 25
26	June 26- July 2
27	July 3- July 9
28	July 10- July 16
29	July 17- July 23
30	July 24- July 30
31	July 31- August 6
32	August 7- August 13
33	August 14- August 20
34	August 21- August 27
35	August 28- September 3

3.7.2 2016 and 2017 Striped Bass Egg and Larvae Abundance Relative to Salinity and Time of Year

The plankton net tows (250µm) yielded the abundance of striped bass eggs, non-feeding, and feeding stage larvae per cubic meter (m^3) of water filtered. The data was collected in 2016 and 2017 from both ebb and flood tides and was separated into abundance by both week and salinity category. The mean daily abundance of eggs/m^3 in both 2016 and 2017 was calculated from all plankton net tows conducted each sampling day. The egg and larvae data are reported separately for 2016 and 2017. The mean abundance (IND/m^3) of non-feeding larvae was calculated per week and per salinity category from May until the end of June 2016 and 2017. The mean abundance (IND/m^3) of feeding stage larvae was calculated per week and per salinity category from May until July 2016 and 2017.

The egg and larval data were subjected to the same quality control criteria as the copepod data described above in Section 3.8.1. The mean abundance of non-feeding and feeding stage larvae per week and per salinity category were compared using a one-way ANOVA analysis of variance

following appropriate transformation to meet normal distribution and constant variance assumptions on the error terms (Montgomery, 2012). The logarithmic transformation was utilized. The Anderson-Darling normality test and Tukey's Pairwise comparison was utilized. A non-parametric analysis, Mood's Median Test, was used to compare the seasonal changes the median temporal abundance of non-feeding larvae in 2016 since the data did not conform to the normality parameters.

CHAPTER 4: COPEPOD DIVERSITY, ABUNDANCE AND DISTRIBUTION RELATIVE TO TIME OF YEAR AND SALINITY

4.1 INTRODUCTION

The predator-prey relationship of zooplankton (copepods) and striped bass larvae in the Shubenacadie River Estuary has not been well documented in the literature beyond the scope of MacInnis (2012) and Duston et al. (2018). Zooplankton are the numerically dominant prey item of first feeding striped bass larvae in the Shubenacadie River estuarine ecosystem (MacInnis, 2012). *Eurytemora affinis* and *Bosmina longirostris* are two dominant prey items of first feeding striped bass in the nursery habitat of upper Chesapeake Bay and *E. affinis* is the main prey item in the Miramichi River Estuary (Beaven and Mihurksy, 1980; Robichaud-LeBlanc et al., 1997; Shideler and Houde, 2014). In the ongoing study, by contrast, *E. affinis* is rare in the Shubenacadie River Estuary. Identification of the copepod species consumed by striped bass larvae at first feeding in the Shubenacadie River Estuary, and their distribution relative to salinity and time of year, was the principal objective of this study. Additionally, the comparison of copepod body length to the gape, or mouth size, of striped bass larvae was important for feeding success.

The overlap in the distribution of copepods and first feeding larval fish relative to time of year and salinity is important for the recruitment and population dynamics of fish stocks (Cushing, 1990; Houde, 2008). Only one paper describes the plankton species inhabiting the Shubenacadie River Estuary (Jermolajev 1958). Zooplankton species present in the Inner Bay of Fundy and Minas Channel include *Acartia tonsa*, *Eurytemora herdmani*, *Pseudodiaptomus coronatus*, *Centropages hamatus* while *Canuella canadensis* was restricted to the estuary of the Shubenacadie River (Jermolajev, 1958). From 2013 to 2017, the copepods *Acartia tonsa*, *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus* were captured in the Shubenacadie River Estuary and identified. *Acartia tonsa* were not present in the water column at the same time as year of first feeding striped bass, however, *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus* were present.

4.2 METHODS SUMMARY

Plankton net tows were conducted in the main channel of the estuary to estimate the zooplankton abundance (individuals per m^3 water filtered; IND/m^3). To quantify the temporal (time of year) distribution of zooplankton through the flood tide, plankton net tows were conducted at the main study site, a fixed location at 25.0rkm from May until August 2013 to 2017. To determine the distribution zooplankton relative to salinity (spatial), plankton net tows were conducted both at a fixed location (25.0rkm, main site) through the flood tide, and also up-estuary beginning at the salt-front at high tide on the Shubenacadie River which ranged from 25 to 44rkm. Samples were collected from the top 1.0m of the water column. For each species (*A. tonsa*, *C. canadensis*, *D. bicuspidatus*, *P. pelagicus*), The mean abundance of each species between weeks (salinities pooled), and between salinity categories (weeks pooled) were compared using a one-way ANOVA analysis of variance following appropriate transformation to meet normal distribution and constant variance assumptions on the error terms (Montgomery, 2012). To identify the first feeding prey species of copepod, DNA extraction and sequencing was conducted (see section 3.4). Copepod species were imaged using a Motic Stereo Microscope to measure their body length and Scanning Electron Microscopy to identify anatomical structures (see section 3.6).

4.3 RESULTS: COPEPOD DIVERSITY

4.3.1 Chronology of Copepod Identification

In July 2013, three copepod species were sent for identification to Dr. Gerard Pohle at the Atlantic Reference Centre, Huntsman Marine Science Centre (Saint Andrews, New Brunswick; Table 5). A fourth species was identified as *Pseudodiaptomus pelagicus* using the key by Gerber (2000). DNA analysis in 2018 led by Dr. Nasif Sarowar (Dalhousie Faculty of Agriculture), called into question the species identification according to Dr. Pohle (Table 5). In 2019, Jackie Spry from Spry Techbiological, an experienced contract researcher, verified the identity of each species based on morphology (Table 5).

The sampling, sorting, identification, and enumeration of the plankton samples from the estuary was completed by undergraduate summer students C. Martin and T. MacDonald in 2014 and C. Martin and I in 2015. The calanoid copepod *Pseudodiaptomus pelagicus* was also identified in the plankton net samples and the cladoceran *Daphnia sp*. was identified by MacInnis (2012). Mesoplankton species of the estuarine ecosystem were identified as mysids (*Neomysis americana*), amphipods (*Calliopinus sp.*), and sand shrimp (*Crangon septemspinosa*) by MacInnis (2012). A larger mysid typically found in high salinity within the lower portion of the estuary near Gosse Bridge (11rkm) was identified as *Mysis stenolepis* by C. Martin in 2016 using a taxonomic key by Johnson and Allen (2012).

Table 5. Chronology of the assessment of copepod identity from the Shubenacadie River Estuary.

Species	**2013** Dr. Gerard Pohle By Morphology	**2018** Dr. Nasif Sarowar By DNA Analysis	**2019** Jackie Spry By Morphology
Acartia tonsa	Identified	*Pseudodiaptomus euryhalinus* copepodite	Confirmed *A. tonsa*
Coullana canadensis	Identified	Confirmed	Confirmed *C. canadensis*
Diacyclops bicuspidatus	Identified	*Eurytemora affinis*	Confirmed *D. bicuspidatus*
Pseudodiaptomus pelagicus		*Pseudodiaptomus euryhalinus*	Confirmed *P. pelagicus*

4.3.2 Copepod Body Size

Acartia tonsa, Coullana canadensis, Diacyclops bicuspidatus, Ectinosomatidae sp., and *Pseudodiaptomus pelagicus* were captured in the Shubenacadie River Estuary. *Acartia tonsa* are approximately 1.0mm TL and gravid specimens were not captured in plankton net tows. The mean total lengths of adult copepods, gravid and non-gravid, captured in the 250µm plankton net mesh size were analyzed and compared (Tables 6, 7). There was no significant difference in length between *C. canadensis*, *D. bicuspidatus*, and *P. pelagicus* but they were significantly larger than the Ectinosomatidae copepod ($P<0.05$; Table 6). The mean length *P. pelagicus* and *C. canadensis* was 2.0 and 2.1mm TL, respectively, and were not significantly different in length (Table 6). The mean length of the Ectinosomatidae copepod was 1.2mm TL and was significantly different in length from the other three potential zooplankton prey of striped bass larvae (Table 6).

The mean length of gravid *P. pelagicus* and Ectinosomatidae copepods were significantly different from the corresponding non-gravid adult stage (Table 7). Only a single *Diacyclops bicuspidatus* specimen was detected with eggs, therefore, no comparison of the means existed (Table 7). The mean length of gravid *C. canadensis* was not significantly different from non-gravid *C. canadensis* specimens (Table 7).

Table 6. Mean total length (mm) of copepods captured in the Shubenacadie River Estuary in 2017. Means that do not share a letter are significantly different. Copepods were digitally imaged and analyzed using a Motic Stereo Microscope with a 2-megapixel Motic camera (H. Breau, 2017). Means that do not share a letter are significantly different ($P<0.05$).

Species	**Mean Length (mm)**
Pseudodiaptomus pelagicus	2.0 (a)
Diacyclops bicuspidatus	2.2 (a)
Coullana canadensis	2.1 (a)
Ectinosomatidae sp.	1.2 (b)

Table 7. Mean total length (mm) of gravid versus non-gravid copepods captured in the Shubenacadie River Estuary in 2017. Means that do not share a letter are significantly different. Copepods were digitally imaged and analyzed using a Motic Stereo Microscope with a 2-megapixel Motic camera (H. Breau, 2017). Means that do not share a letter are significantly different ($P<0.05$).

Species	**Mean Length (mm) Gravid**	**Mean Length (mm) Non-Gravid**
Pseudodiaptomus pelagicus	2.1 (a)	1.9 (b)
Diacyclops bicuspidatus	3.1	2.2
Coullana canadensis	2.0 (c)	2.1 (c)
Ectinosomatidae sp.	1.2 (d)	1.0 (e)

4.3.3 Ectinosomatidae Copepod Morphology

The morphological structures of the swimming appendages of the Ectinosomatidae copepod were identified and utilized to compare to morphological keys to identify the species. The basis, coxa, and endopod were identified (Figure 16).

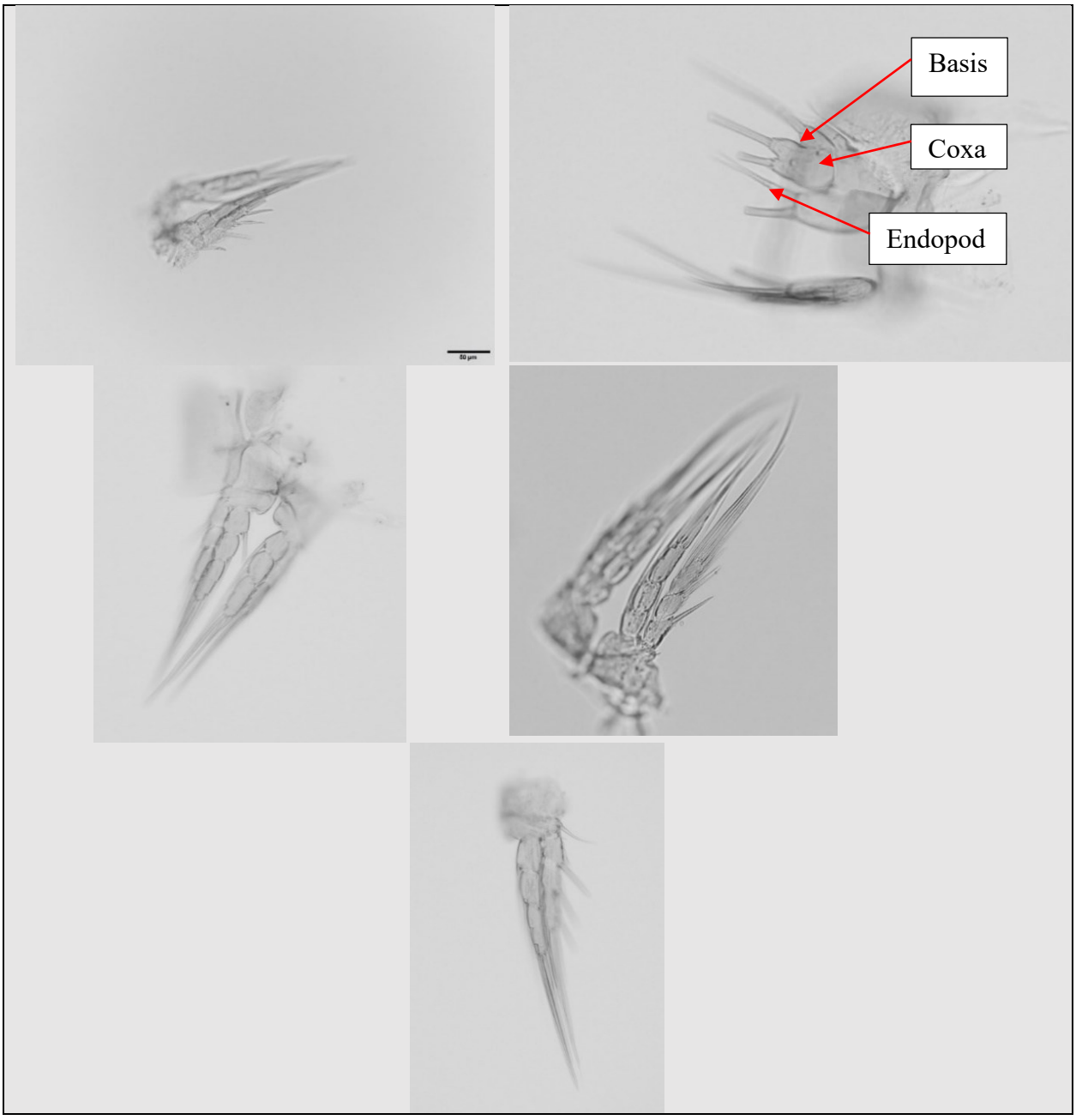

Figure 16. Dissection images of the swimming appendages of the Ectinosomatidae harpacticoid copepod species. Dissection completed by Rebecca Milne at the Atlantic Reference Centre, Huntsman Marine Science Centre in St. Andrews, New Brunswick.

The anal operculum and caudal ramus of the urosome of the Ectinosomatidae copepod were identified (Figure 17).

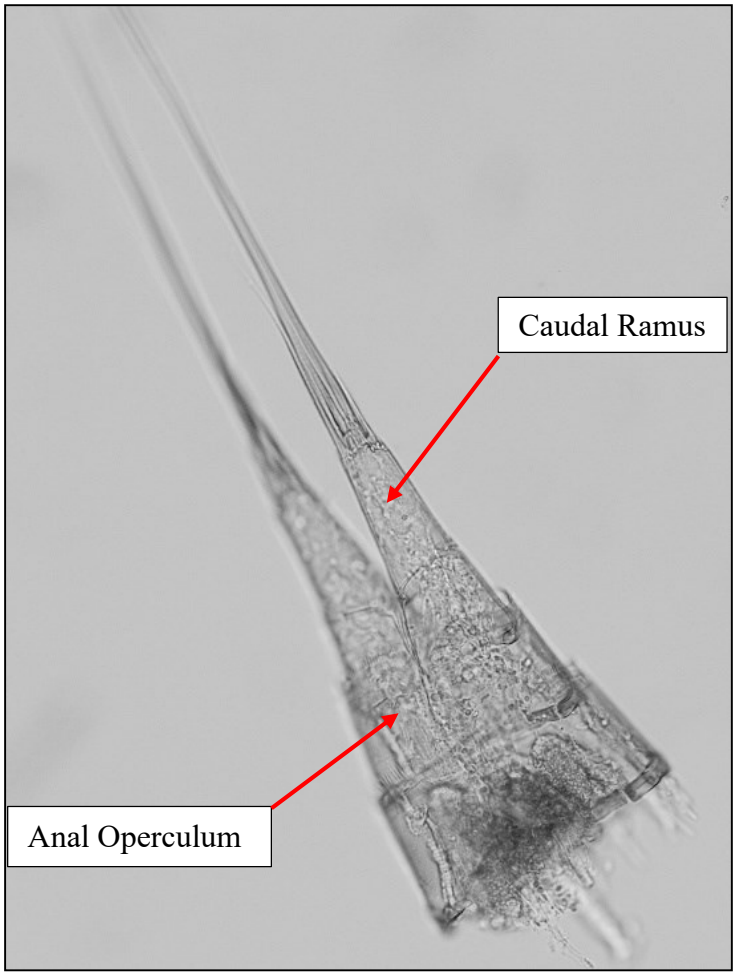

Figure 17. Dissection image of the urosome of the Ectinosomatidae harpacticoid copepod species. Dissection completed by Rebecca Milne at the Atlantic Reference Centre, Huntsman Marine Science Centre in St. Andrews, New Brunswick.

The morphological structures of the head of the Ectinosomatidae copepod were identified (Figure 18). The rostrum, antenna, and cephalic shield are outlined (Figure 18).

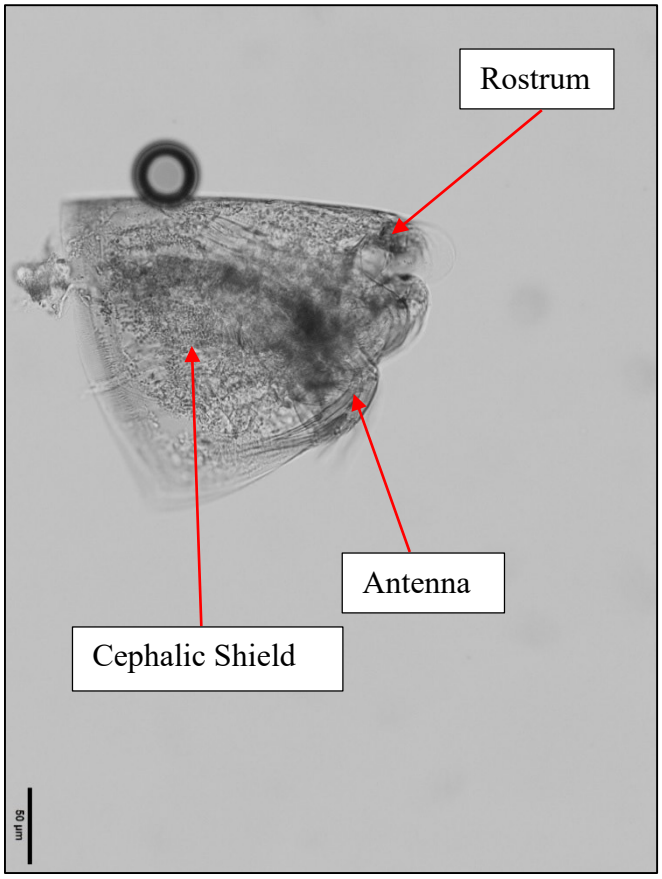

Figure 18. Dissection images of the head of the Ectinosomatidae harpacticoid copepod species. Dissection completed by Rebecca Milne at the Atlantic Reference Centre, Huntsman Marine Science Centre in St. Andrews, New Brunswick.

4.3.4 Scanning Electron Microscopy (SEM) Images of the Ectinosomatidae Copepod

The ectinosomatid body is uniformly brown pigmented. The ectinosomatid's body is cylindrical and is typically the same breadth throughout its length (Wells, 2007). The greatest breadth of the body is found at the distal edge of the cephalic shield (Figure 19). The cephalic shield is the dorsal carapace that covers the anterior section of the body (Wells, 2007). The urosome tapers towards the posterior end of the body (Roff, 1978; Wells, 2007). The metasome bears the second to fourth pereiopod appendages (P2-P4; Wells, 2007). The maxilliped, or the fourth appendages of the mouthparts, is the first thoracic appendage. P1-P4 are the second to fifth thoracic appendages, commonly known as the "swimming appendages". P-5 is the sixth thoracic appendage and is modified as a secondary sexual structure (Wells, 2007). Approximately ten somites, or segments, are visible (Figure 19).

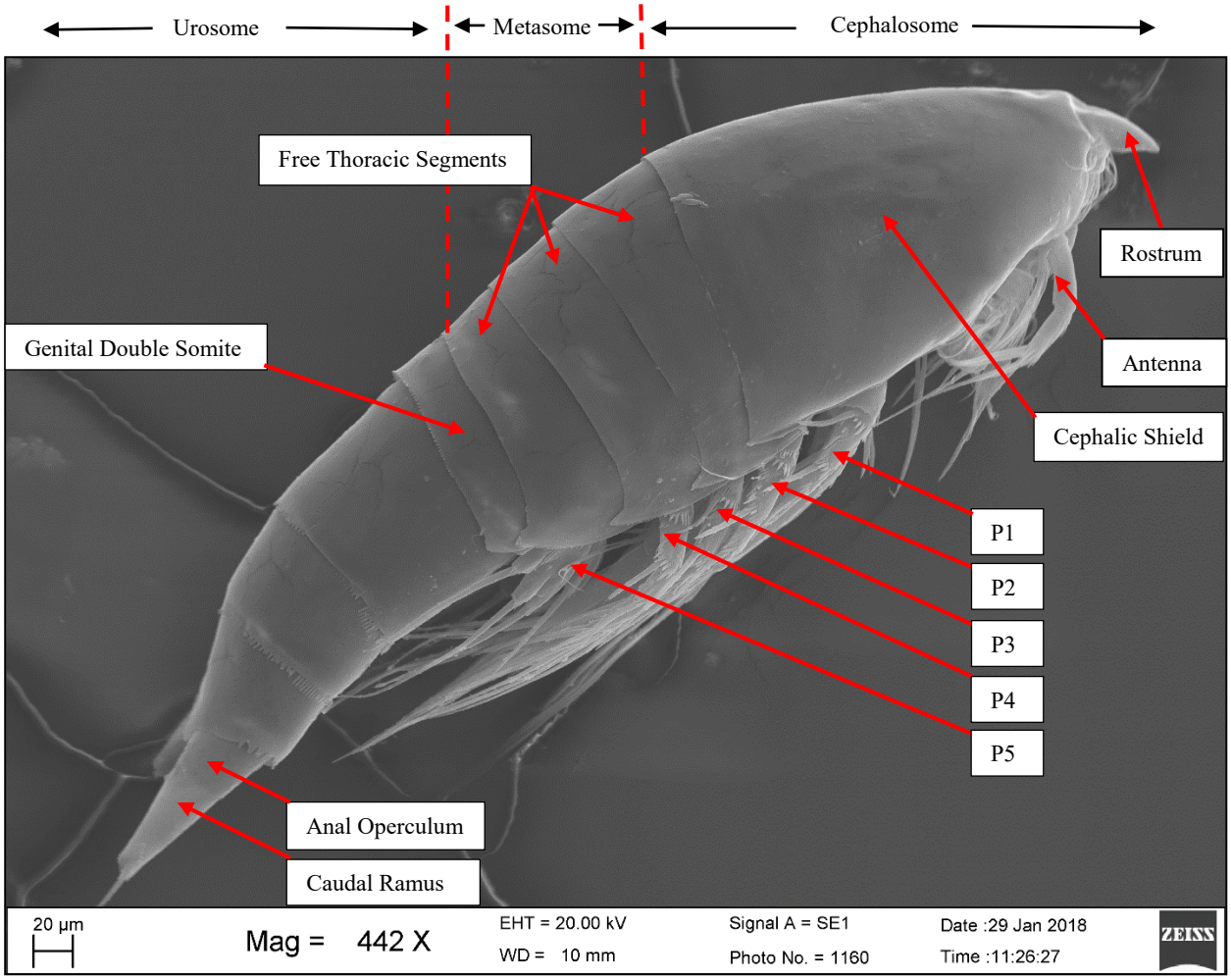

Figure 19. Lateral view of the Ectinosomatidae harpacticoid copepod species using Scanning Electron Microscopy (P= pereiopod appendages).

The genital double somite bears the external genitalia (Wells, 2007). The genital double somite bears the egg sac of the ectinosomatid female (Figure 20).

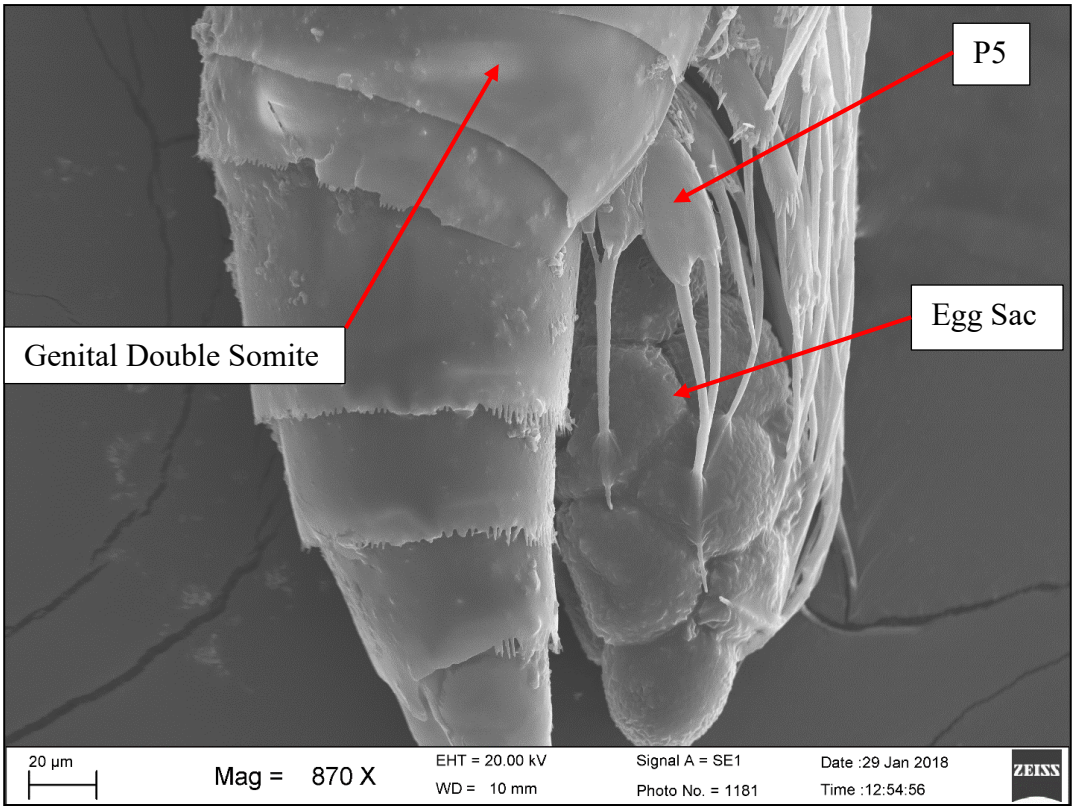

Figure 20. Swimming legs and egg sac of the Ectinosomatidae harpacticoid copepod species obtained by Scanning Electron Microscopy (P= pereiopod appendage).

The endopod is described as the inner ramus or medial branch of a biramous, or paired, appendage (Figure 21; Roff, 1978; Wells, 2007). The exopod is the outer ramus or lateral branch of the appendage (Roff, 1978). The basis bears the protopod, or the endopod and exopod. Setae, or spines, are slender and hollow tube-like structures that are associated with nerve tissue and form the armature of the body (Figure 21; Wells, 2007).

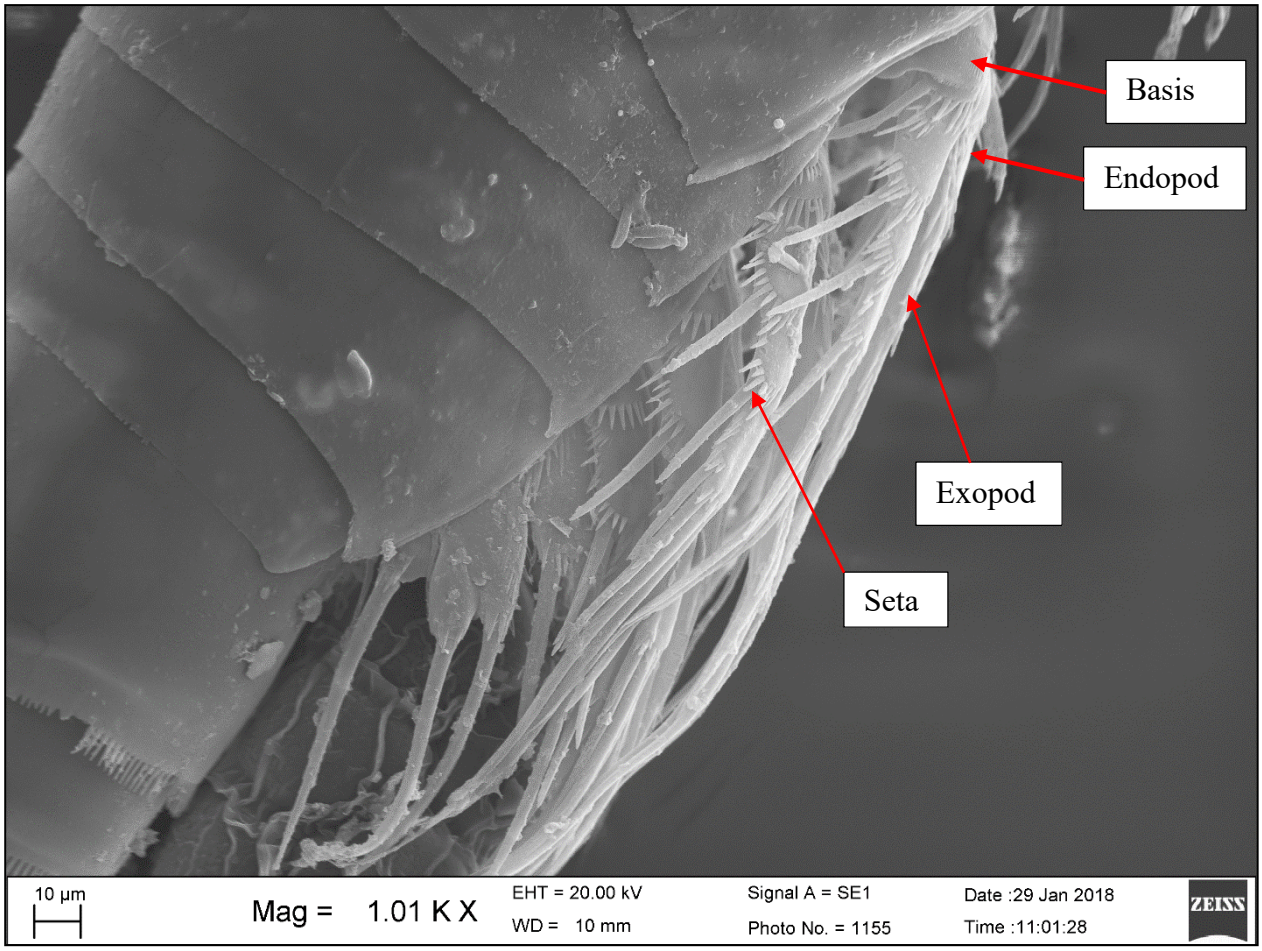

Figure 21. Swimming legs and egg sac of the Ectinosomatidae harpacticoid copepod species obtained by Scanning Electron Microscopy.

The aesthetasc is a long tubular and very flexible sensory filament of the antennule (Figure 22; Wells, 2007). The cephalothorax consists of the cephalosome and the second thoracic somite. The second thoracic somite bears the P1, or first set of swimming appendages. The rostrum is a prominent extension of the cephalic shield (Figure 22; Roff, 1978; Wells, 2007).

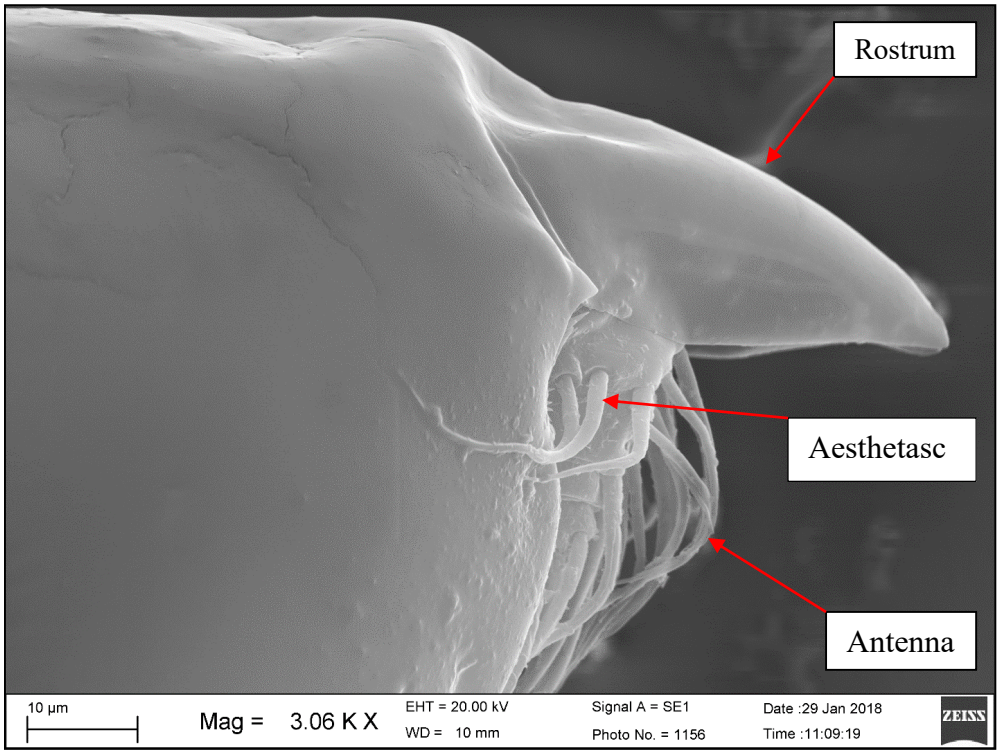

Figure 22. Cephalothorax of the Ectinosomatidae harpacticoid copepod species obtained by Scanning Electron Microscopy.

The “mouthparts” of the ectinosomatid consist of four appendages (Figure 23). The appendages include the mandible, maxillule, maxilla, and maxilliped (Wells, 2007).

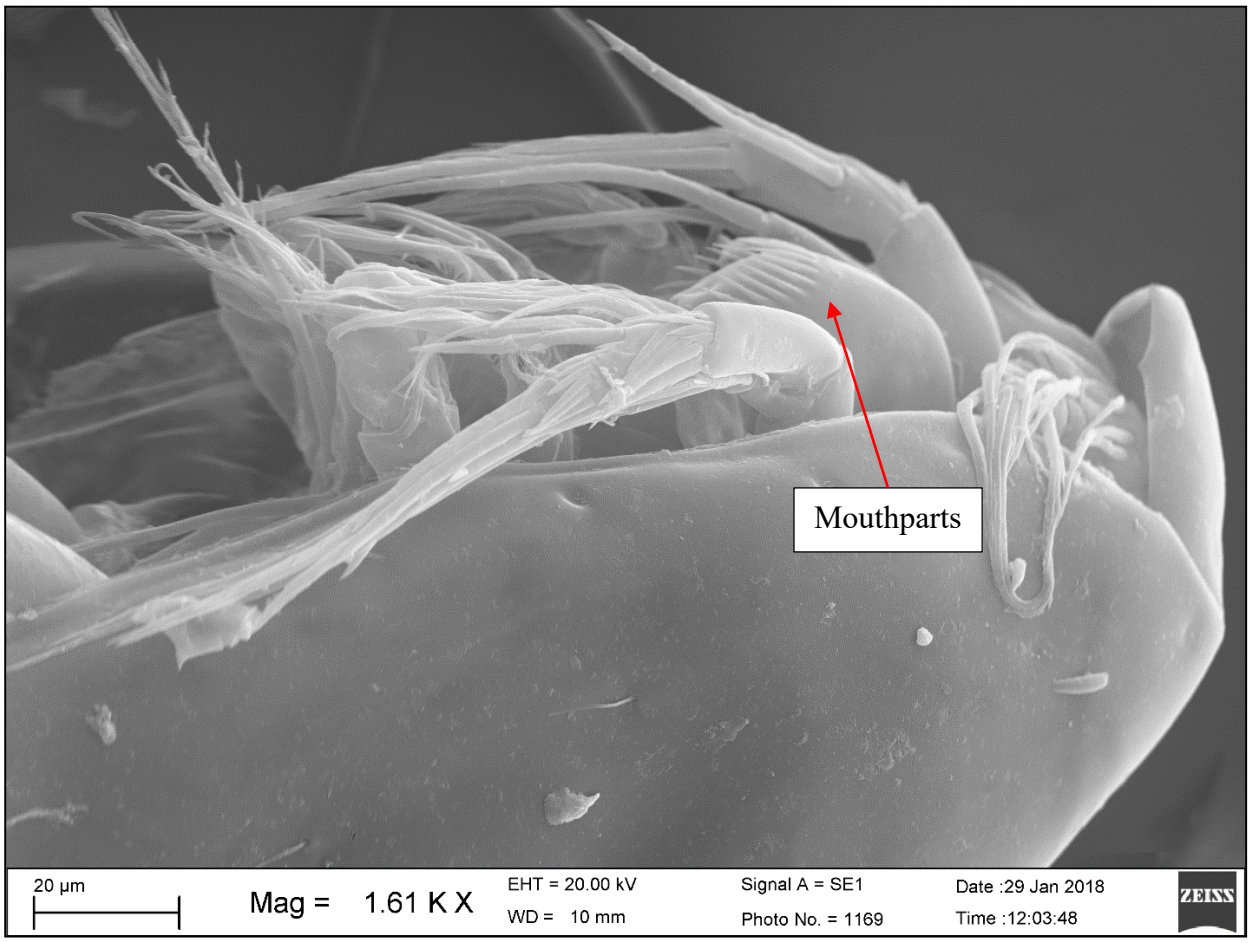

Figure 23. Latero-ventral Image obtained of the ventral view of the cephalothorax, antenna, and mouthparts (mandible, maxillule, maxilla, and maxilliped) of the Ectinosomatidae harpacticoid copepod species obtained by Scanning Electron Microscopy.

The caudal ramus is described as the terminal structure of the anal somite, or the last body segment (Roff, 1978; Wells, 2007). The caudal ramus is a paired structure, thus referred to as the caudal rami (Figure 24).

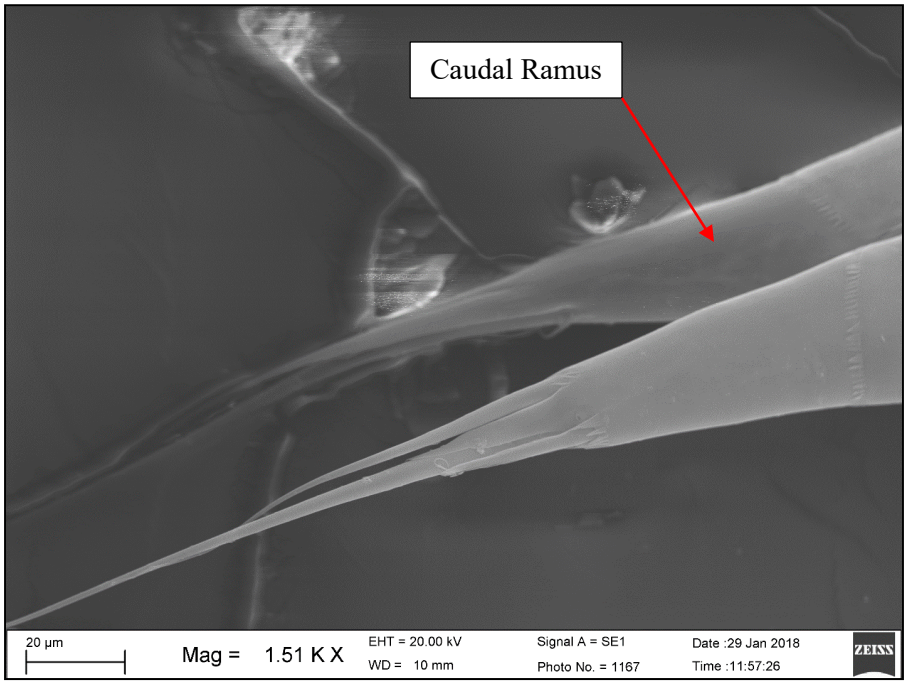

Figure 24. Urosome of the Ectinosomatidae harpacticoid copepod species obtained by Scanning Electron Microscopy.

4.4 Results: Copepod Distributions Relative to Time of Year

The abundance of *A. tonsa* in the Shubenacadie River was dependent on time of year (P<0.001; Figure 25). The highest temporal abundance (±SE; IND/m^3) of *A. tonsa* was 24286/m^3 (±664.0) during the week of August 28-September 3 (Figure 25). The presence of *A. tonsa* in the estuary from May to July 17th-23rd was very low and ranged from 5 to 35/m^3 (Figure 25).

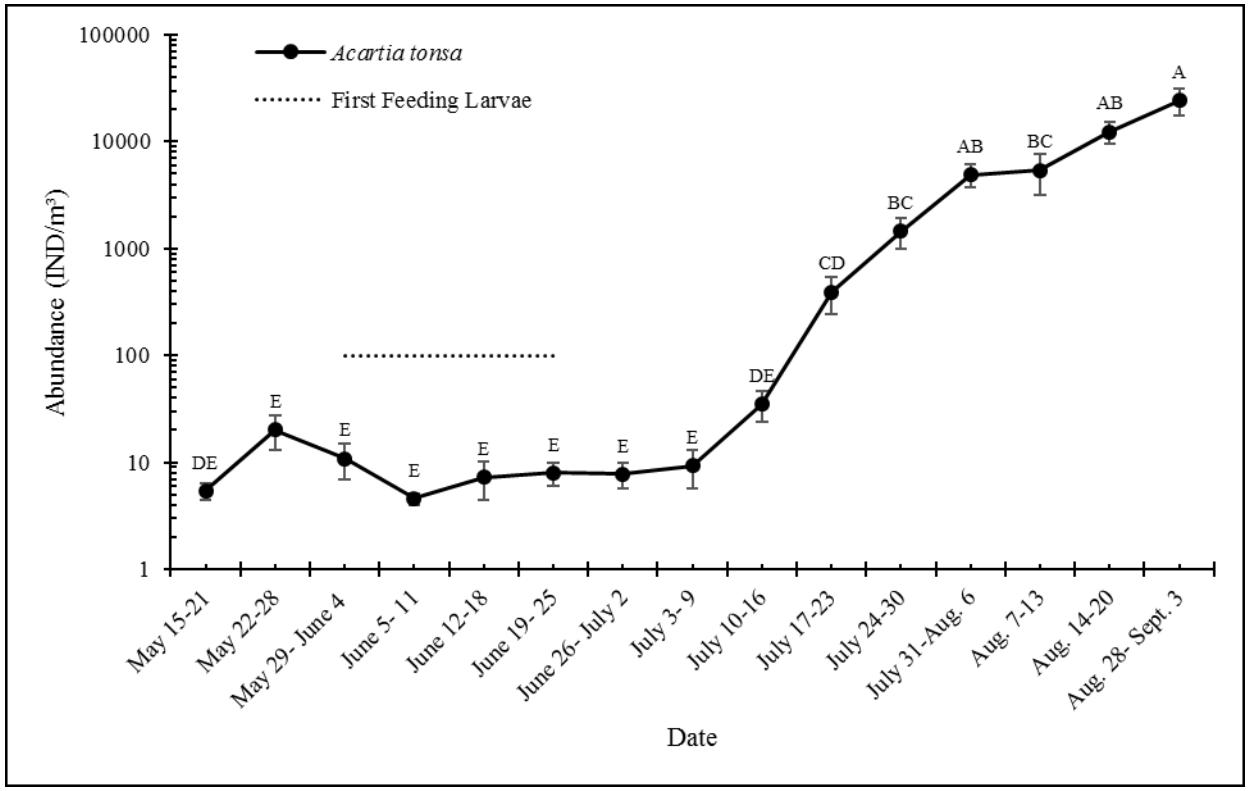

Figure 25. Mean weekly abundance (±SE; IND/m^3) of *Acartia tonsa* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. Means that do not share a letter are significantly different (P<0.05). Dotted line represents the presence of first feeding striped bass larvae in the estuary in 2016 and 2017. Logarithmic y-axis.

The abundance of *Coullana canadensis* in the Shubenacadie River was dependent on time of year (P<0.001; Figure 26). *C. canadensis* was rare in May with less than 5/m^3 (IND/m^3). Abundance significantly increased during the first week of June (Figure 26). The highest temporal abundances occurred from mid-June to mid-July. The highest mean temporal abundance (±SE) of *C. canadensis* was 106/m^3 (±53.8) during the week of July 17-23 (Figure 26). The highest temporal

abundance was not significantly different (P=0.234) from the abundance of June 26-July 2 but was significantly different from June 12-18 (Figure 26). The abundance of *C. canadensis* fluctuated multiple times between June and August ranging from 5/m^3 to 13/m^3 (Figure 26). The abundance of *C. canadensis* was independent of time of year from June 19 to August 20 (Figure 26).

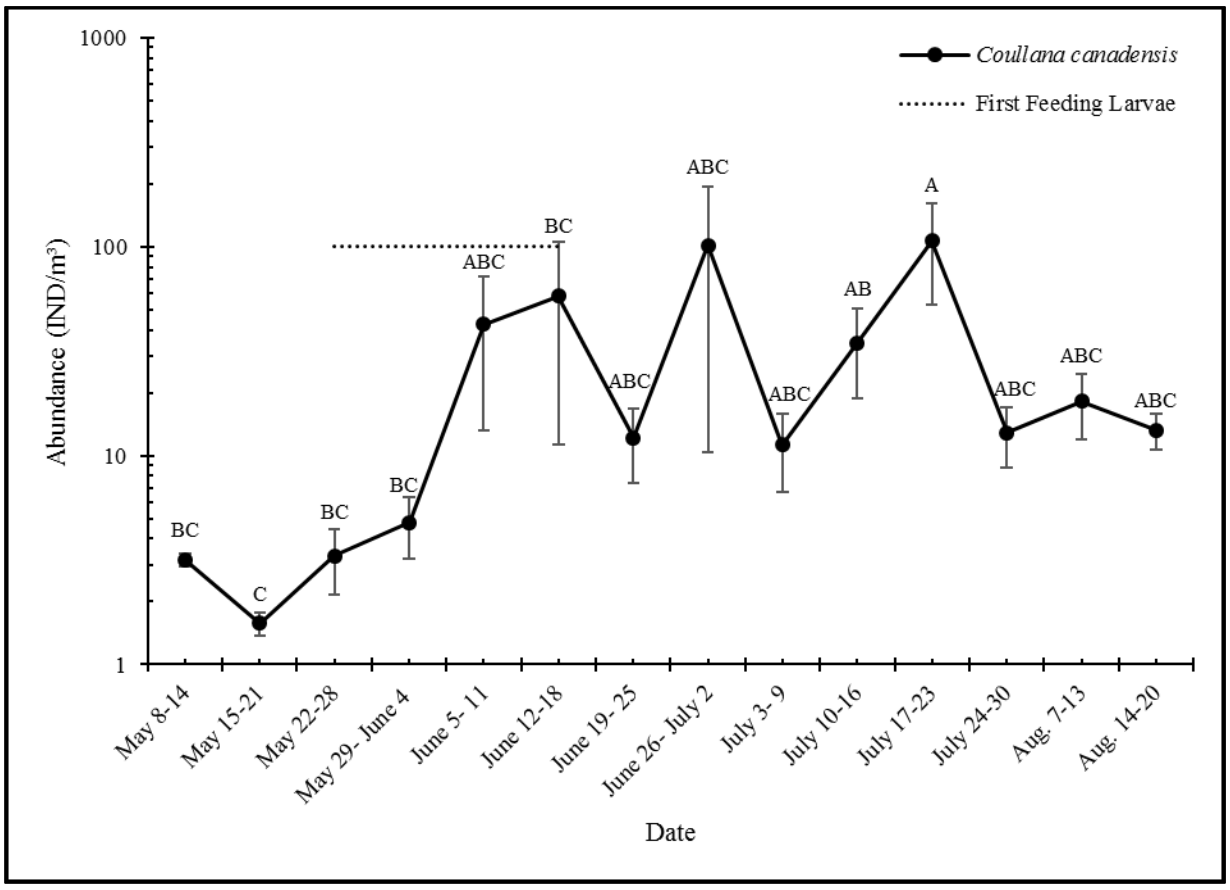

Figure 26. Mean weekly abundance (±SE; IND/m^3) of *Coullana canadensis* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. Means that do not share a letter are significantly different (P<0.05). Dotted line represents the presence of first feeding striped bass larvae in the estuary in 2016 and 2017. Logarithmic y-axis.

The abundance of *Diacyclops bicuspidatus* in the Shubenacadie River was dependent on time of year (P=0.023; Figure 27). *D. bicuspidatus* was not recorded in 2016. The highest mean abundance (±SE; IND/m^3) was 12/m^3 (±4.2) during the week of June 5-11 (Figure 27). *Diacyclops*

bicuspidatus was mainly captured up-estuary of the confluence of the Stewiacke and Shubenacadie Rivers.

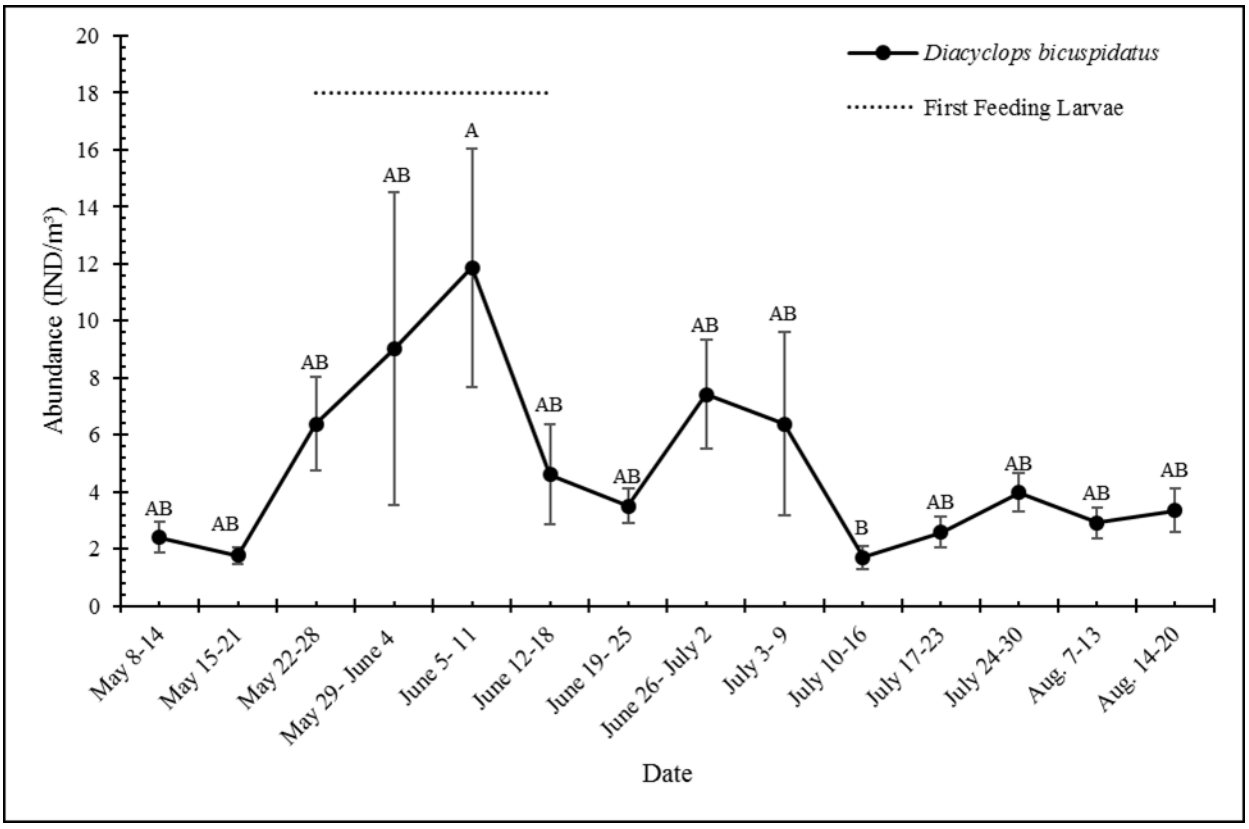

Figure 27. Mean weekly abundance (±SE; IND/m^3) of *Diacyclops bicuspidatus* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. *D. bicuspidatus* was not recorded in 2016. Means that do not share a letter are significantly different (P<0.05). Dotted line represents the presence of first feeding striped bass larvae in the estuary in 2016 and 2017.

The abundance of the ectinosomatid was independent of time year of time relative to both the 150 and 250µm plankton net mesh sizes (P>0.05). The ectinosomatid was absent in the 250µm plankton net from the beginning of sampling season in late May until late June in 2017 (Figure 28). The peak abundance of the ectinosomatid occurred the week of June 26-July 2, 2017 (Figure 28). Using the 250µm mesh net, the mean peak weekly abundance (IND/m^3) was 214/m^3 (±105.0) June 26-July 2, 2017 (Figure 28). A low number of plankton net tows (<100) yielded ectinosomatids across the 2017 sampling season.

In 2017, using the 150µm plankton net mesh from the last week of June onwards, the mean peak weekly abundance (±SE; IND/m^3) of the Ectinosomatidae copepod was 281/m^3 (±177.0) during the week of June 26-July 2, 2017 (Figure 28). The weeks of July 3-9, July 24-30, and August 7-13 were excluded as the 150µm net could not be used because the net plugged due to high turbidity. The catch rate of ectinosomatids was higher utilizing the 150µm plankton net mesh than the 250µm mesh. A low number of plankton net tows yielded ectinosomatids across all weeks of study in 2017 utilizing the 150µm net. The large standard error indicated that the distribution of the species was heterogenous (Figure 28).

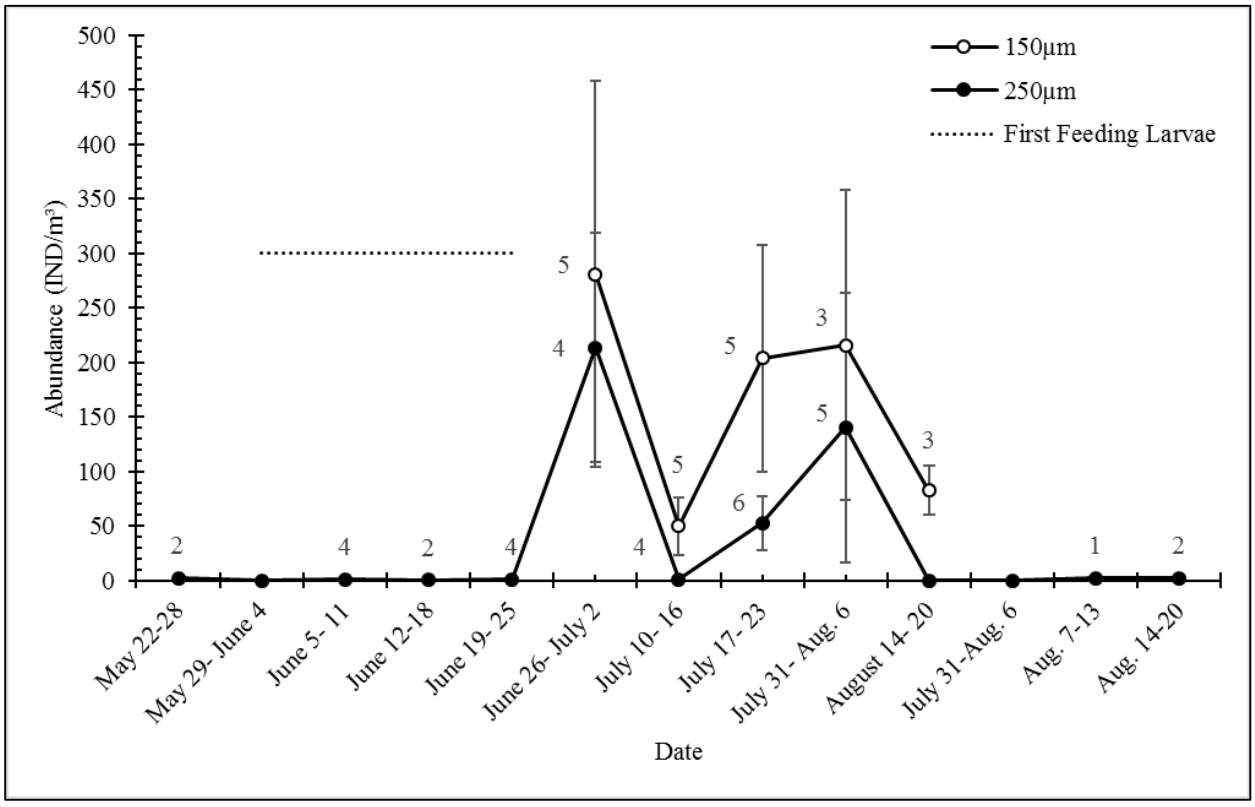

Figure 28. Mean (±SE) weekly abundance (IND/m^3) of the Ectinosomatidae harpacticoid copepod captured in the Shubenacadie River Estuary from June to August 2017. Tows were completed with the 150 and 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides. Data labels represent the number of tows during each week. Dotted line represents the presence of first feeding striped bass larvae in the estuary in 2016 and 2017.

The abundance of *Pseudodiaptomus pelagicus* in the Shubenacadie River was dependent on time of year ($P<0.001$; Figure 29). *P. pelagicus* presented a consistent temporal distribution as it was

detected in the water column across all sampling weeks from May to August (Figure 29). The abundance of *P. pelagicus* in the Shubenacadie River Estuary increased progressively from May to September. The highest mean temporal abundance (±SE; IND/m^3) was 1061/m^3 (±371.0) during the week of August 14-20 (Figure 29). However, the highest abundance was not significantly different from the peak of July 10-16. The highest mean temporal abundance was significantly different from May 1-21, May 29-June 4, and June 19-July 9 as they do not share the letter 'A' (Figure 29).

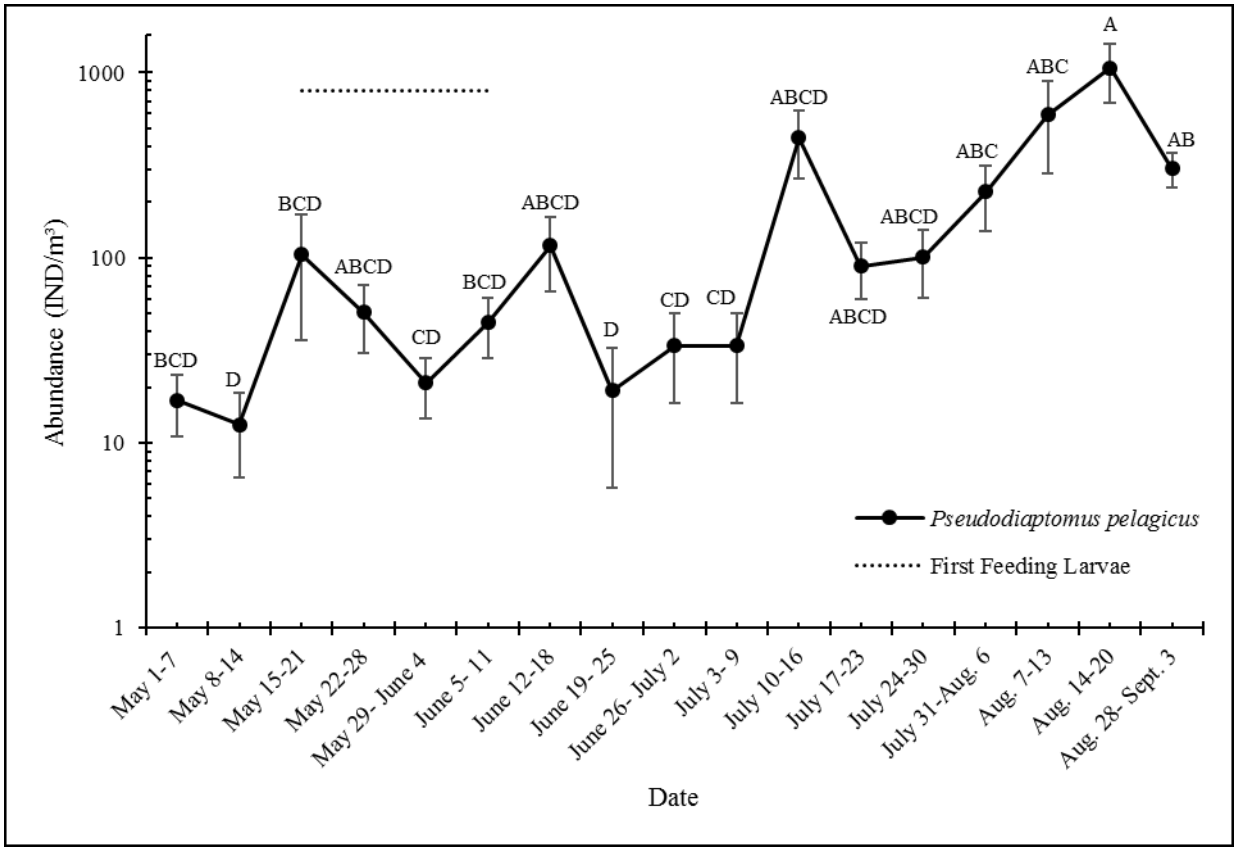

Figure 29. Mean weekly abundance (±SE; IND/m^3) of *Pseudodiaptomus pelagicus* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. Means that do not share a letter are significantly different ($P<0.05$). Dotted line represents the presence of first feeding striped bass larvae in the estuary in 2016 and 2017. Logarithmic y-axis.

4.5 Results: Copepod Distributions Relative to Salinity

The distribution of *Acartia tonsa* in the Shubenacadie River Estuary was dependent on salinity ($P<0.001$; Figure 30). The highest mean abundance (±SE; IND/m^3) relative to salinity was 11702/m³ (±2139.0) at 25.1-30.0ppt, however, there was no significant difference between 15.1 and 30.0ppt (Figure 30). As the summer progressed, the abundance of *A. tonsa* increased as the salinity increased in the upper estuary (Figure 12, 14). Low abundances of *A. tonsa* were evident in May and June when the salinity was lowest in the estuary.

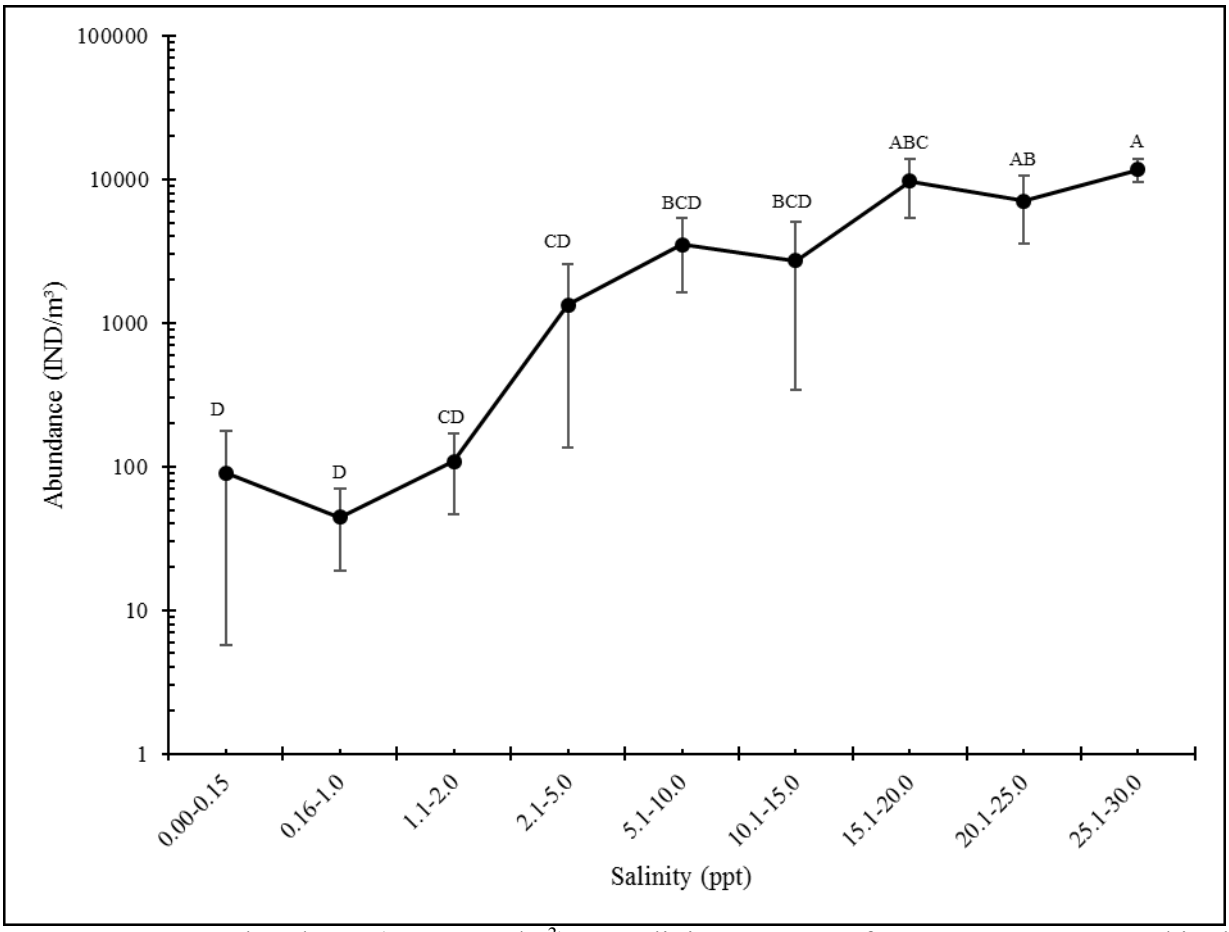

Figure 30. Mean abundance (±SE; IND/m^3) per salinity category of *Acartia tonsa* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. Means that do not share a letter are significantly different ($P<0.05$). Logarithmic y-axis.

The mean abundance (±SE; IND/m^3) of *C. canadensis* relative to salinity at 1.1-2.0ppt was 70/m^3 (±45.9; Figure 31). The abundance of *C. canadensis* was independent of salinity as the highest abundance at 1.1-1.2ppt was not significantly different from the abundance of each other salinity interval (P=0.270; Figure 31).

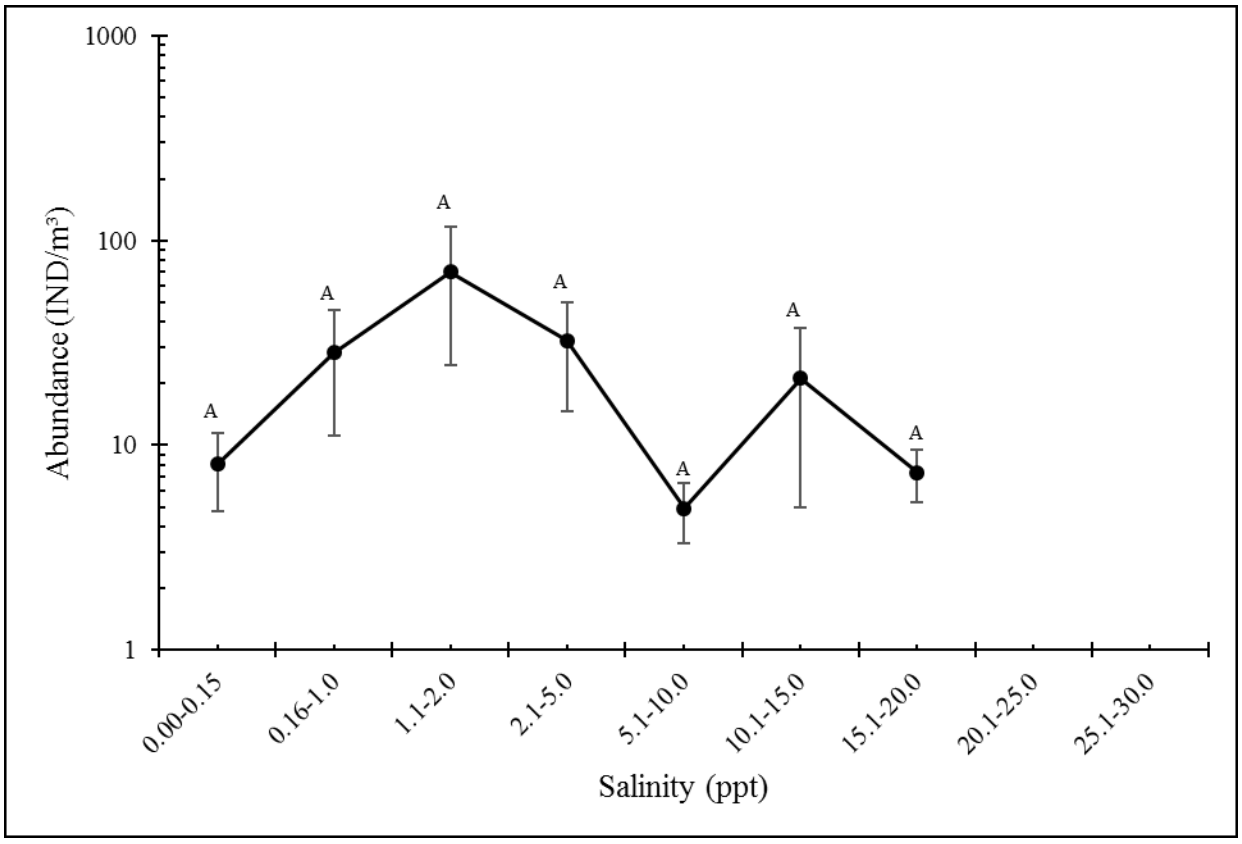

Figure 31. Mean abundance (±SE; IND/m^3) per salinity category of *Coullana canadensis* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. Means that do not share a letter are significantly different (P<0.05). Logarithmic y-axis.

Abundance of *Diacyclops bicuspidatus* increased with salinity reaching 27/m^3 (±7.0) at 15.1-20.0ppt (Figure 32). The abundance of *D. bicuspidatus* was dependent on salinity (P<0.05; Figure 32). The abundance of *D. bicuspidatus* was significantly different at 15.1-20.0ppt when compared to the abundance from 0 to 15.0ppt as indicated by the letter groupings (Figure 32).

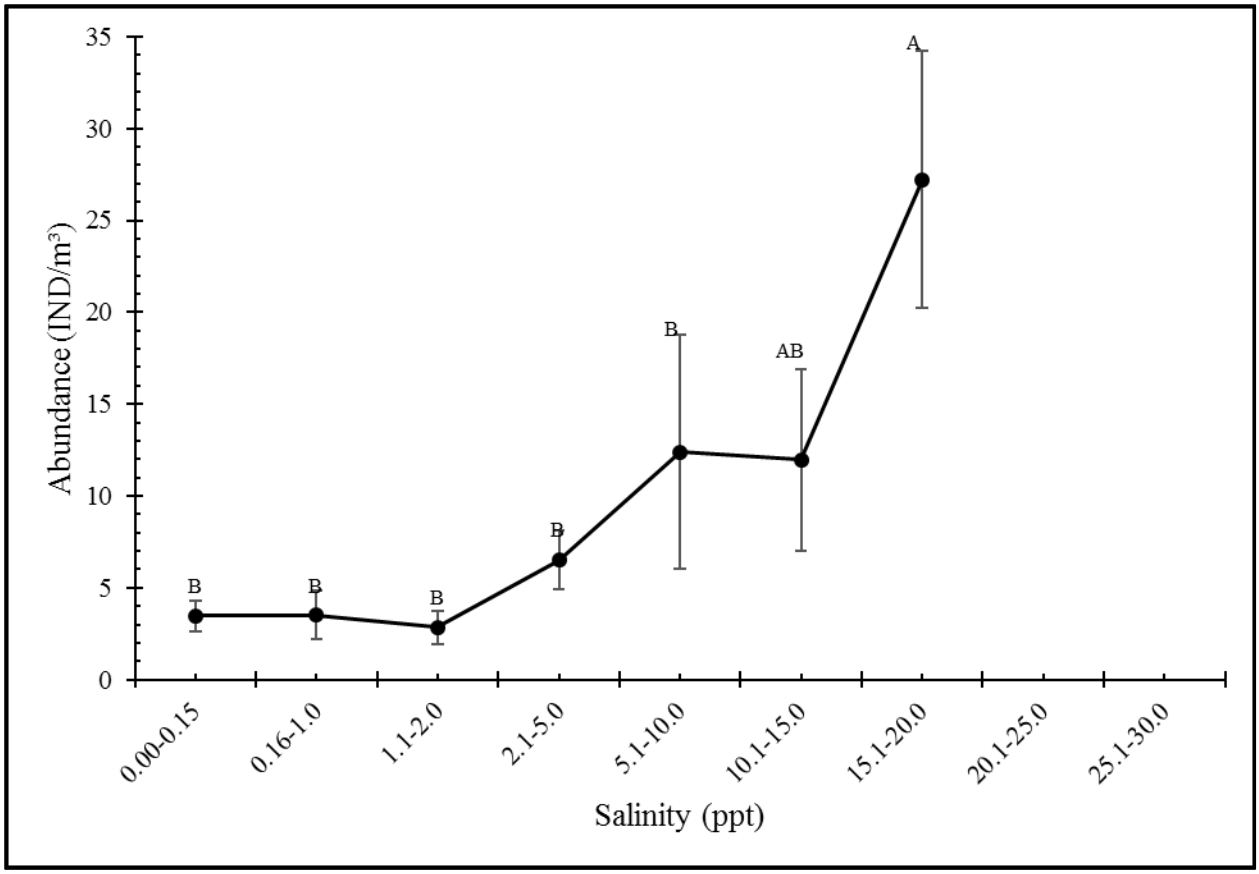

Figure 32. Mean abundance (±SE; IND/m^3) per salinity category of *Diacyclops bicuspidatus* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. Means that do not share a letter are significantly different ($P<0.05$).

The distribution of ectinosomatids in the Shubenacadie River Estuary suggested that salinity may have some influence on the distribution, but the differences were not statistically significant due to the large variation (Figure 33). The mean abundance per salinity category (±SE; IND/m^3) was 209/m^3 (±119.0) in 1.1-2.0ppt (Figure 33). The ectinosomatid was distributed in brackish water from 0.15 to 10ppt, with both 150 and 250µm mesh nets yielding similar distribution and abundance patterns (Figure 33). Using the 150µm mesh net by comparison, the highest mean abundance (±SE; IND/m^3) was similar 250/m^3 (±128.0) in 1.1-2.0ppt (Figure 33).

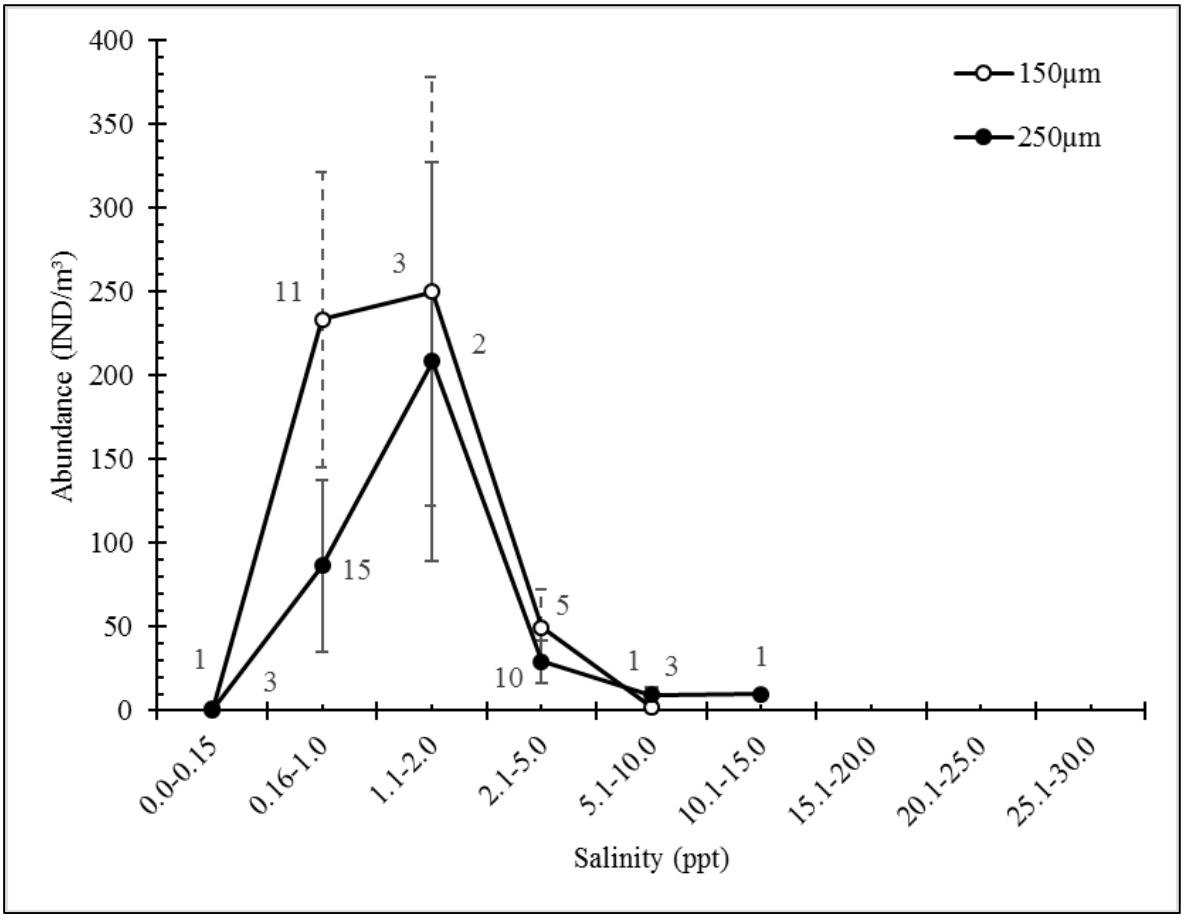

Figure 33. Mean (±SE) abundance (IND/m^3) per salinity category of the Ectinosomatidae harpacticoid copepod captured in the Shubenacadie River Estuary from June to August 2017. Tows were completed with the 150 and 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides. Data labels show the number of tows.

Pseudodiaptomus pelagicus was detected in the water column across all salinity categories from 0.00 to 30.0ppt (Figure 34). The distribution of *P. pelagicus* was dependent on salinity in the Shubenacadie River Estuary (P<0.001). The highest abundance (±SE; IND/m^3) relative to salinity was 993/m³ (±422.0) at 20.1-25.0ppt (Figure 34). The abundance increased significantly from 0.00 to 10.0ppt (Figure 34).

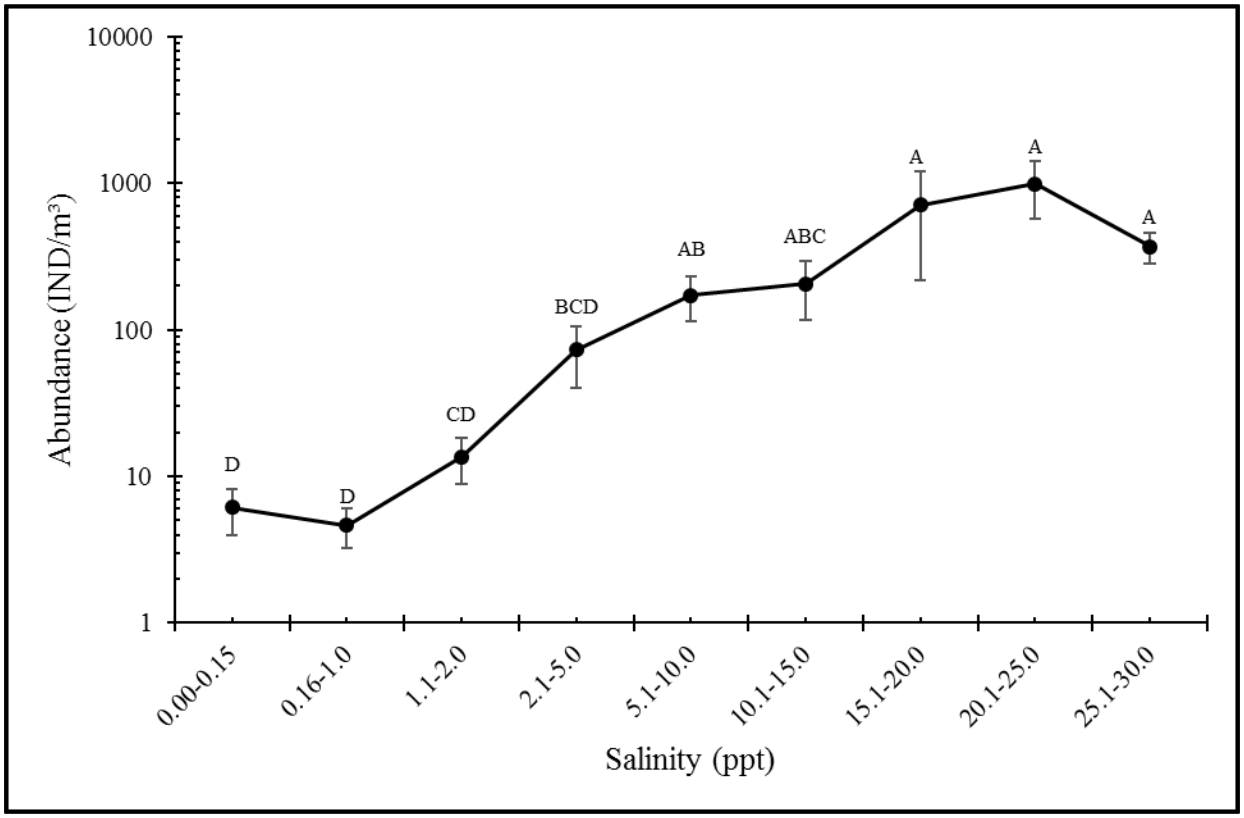

Figure 34. Mean abundance (±SE; IND/m^3) per salinity category of *Pseudodiaptomus pelagicus* captured in the Shubenacadie River Estuary from May to August 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 20.8-41.0rkm on the ebb and flood tides. Means that do not share a letter are significantly different ($P<0.05$). Logarithmic y-axis.

4.6 Discussion

4.6.1 Copepod Diversity: Identification Methods

All copepod species were captured in the top 1.0m of the water column in the Shubenacadie River Estuary despite some species considered benthic or epibenthic. *Coullana canadensis* is a species that is typically benthic, however, due to the high turbulence and mixing of the estuary, they were captured in the water column. Three copepods were sent for identification to Dr. Pohle at the Atlantic Reference Centre (Table 5). Dr. Pohle, using morphological keys, identified *Acartia tonsa* (calanoid), *Diacyclops bicuspidatus* (cyclopoid), and *Coullana canadensis* (harpacticoid; Table 5; G. Pohle pers. comm., 2013). A fourth species was identified as *Pseudodiaptomus pelagicus* (calanoid) using Gerber (2000) key.

Dr. Nasif Sarowar called into question the species identification according to Dr. Pohle (Table 5). Dr. Sarowar conducted DNA analysis in 2018 to verify the identification of *Acartia tonsa*, *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus*. The DNA sequencing results were compared to DNA sequences in GenBank, the nucleotide sequence database. The taxon identified as the calanoid copepod *Acartia tonsa* is possibly a copepodite stage of a species belonging to the genus *Pseudodiaptomus*. The taxonomic identity at the genus and species level was uncertain according to the GenBank results. The morphological identification of *Pseudodiaptomus pelagicus* and *Acartia tonsa* differed from the DNA sequencing results. In GenBank, there is only information for one species of the genus P*seudodiaptomus*, *Pseudodiaptomus euryhalinus*. Therefore, *P. pelagicus* and *A. tonsa* were identified as *Pseudodiaptomus euryhalinus*. The DNA analysis also placed the *Diacyclops bicuspidatus* (cyclopoid) specimens in close relation to *Eurytemora affinis* or *Eurytemora carolleeae* (calanoid). The morphological identification and DNA sequencing of *Coullana canadensis* aligned. In 2019, Jackie Spry from Dalhousie University verified the identity of each species based on morphology and expelled the uncertainties of identification (Table 5). There is great variation in identifying and verifying copepods of apparently the same species between genetic and morphological analysis (Sukhikh et al., 2016). A combination of morphological and genetic features can be utilized to correctly identify copepod species (Sukhikh et al., 2016). However, a lack of

collaboration between morphological taxonomists and DNA specialists restricts the correct identification of copepod species.

4.6.2 Copepod Body Length

The Shubenacadie River ectinosomatid had a mean length of 1.2mm (Table 6). Copepod species from the family Ectinosomatidae ranged in size from 0.45 to 0.95mm TL in Woods Hole, Massachusetts (Wilson, 1932). The body sizes of *Ectinosoma normani*, *Ectinosoma curticorne*, and *Ectinosoma elongatum* (Wilson, 1932) were smaller than the body size of the ectinosomatid collected in the Shubenacadie River Estuary. *Coullana canadensis* had a mean body length of 2.1mm (Table 6). Female *Coullana canadensis* from Pamlico River, North Carolina, were recorded as 1.2 to 1.3mm TL while males were smaller (Coull, 1972). A mature male *C. canadensis* was recorded as 1.1mm TL (Coull, 1972). The body size of gravid *C. canadensis* was not significantly different from the body size of non-gravid specimens (Table 7).

The mean body size of *Pseudodiaptomus pelagicus* in the Shubenacadie River was 2.0mm (Table 6). *Pseudodiaptomus pelagicus* females ranged in size from 1.30-1.57mm TL and males 0.92-1.13mm TL (Walter, 1989). Female *P. pelagicus* were also recorded as 1.5mm TL and males as 1.2mm TL from Rhode Island (Williams, 1906). Adult male *P. pelagicus* of the Bay of Fundy ranged from 1.0-1.2mm and females 1.2-1.5mm (Roff, 1978). *P. pelagicus* collected from the Shubenacadie River Estuary were physically larger than specimens recorded in the literature. Marine calanoid copepods tend to reach larger body sizes at maturity along geographical gradients with deceasing temperature (Leinaas et al., 2016). Arctic populations of calanoid copepods were significantly longer than southern populations (Leinaas et al., 2016). The larger body size of *P. pelagicus* may be an adaptation to the turbulent and high velocity water of the Shubenacadie River Estuary. Shubenacadie River *Acartia tonsa* were approximately 1.0mm in length. Female *Acartia tonsa* range in length from 1.3-1.5mm and males 1.0-1.3mm (Roff, 1978). Female *A. tonsa* range in length from 1.3-1.5mm and males 1.0-1.3mm (Roff, 1978). *A. tonsa* collected from the estuary corresponded with the body size stated in published literature.

4.6.3 Ectinosomatidae DNA Sequencing and Identification

In 2016 and 2017, a previously undescribed small, adult stage harpacticoid copepod was identified as the main prey item in the gut of first feeding striped bass larvae (Chapter 5). Due to its small size, the harpacticoid was not captured in the 250µm plankton net mesh in 2016. A 150µm plankton net mesh was introduced in late June 2017, to try and capture the small harpacticoid copepod. The smaller mesh easily plugged in the high turbidity at the main site but worked satisfactorily further up-estuary where there was less suspended sediment. A plankton mesh size which is 75% of a copepod carapace width will catch 95% of specimens of that size present in the water (Nichols and Thompson, 1991). This may explain why it was difficult to capture the ectinosomatids in a mesh size of 250µm. The carapace width was not measured by H. Breau (2017). However, from Figure 19, the carapace width can be estimated as 140µm, less than half the mesh size of the 250µm plankton mesh size.

Identifying the species using morphological identification keys was difficult. In July 2017, preserved samples were sent for identification to R. Milne at the Atlantic Reference Centre, Huntsman Marine Science Centre, in St. Andrews, New Brunswick. She concluded that the copepod belongs to the family Ectinosomatidae, possibly from the genera *Halectinosoma* sp. or *Pseudobradya* sp. The taxonomic key checklist utilized for harpacticoid identification was followed by Seifried's (2003) hierarchy (Wells, 2007). The anatomical structures of the ectinosomatid were evaluated, measured, and compared to other descriptions to establish phylogenetic relationships (Seifried, 2003). This species of harpacticoid copepod is an ecologically important component of the estuarine ecosystem of the Shubenacadie River as it was the sole prey item of first feeding stage striped bass larvae in 2016 and 2017 (Chapter 5). The family Ectinosomatidae was established by Sars (1904).

Description:

Kingdom Animalia
Phylum Arthropoda
Class Maxillopoda, Sub-Class Copepoda
Order Harpacticoida
Family Ectinosomatidae
Halectinosoma nov. sp. or *Pseudobradya* sp. nov. (Figures 16-24)

The state of knowledge of the taxonomy of this family of harpacticoids is rather poorly defined based on classical morphological analysis. Hence, we turned to DNA analysis. Ectinosomatids are a notoriously difficult group of copepods to identify, even for harpacticoid specialists (Wells, 2007; Suarez-Morales and Fuentes-Reines, 2015; Sciberras et al., 2018). Genera of the family Ectinosomatidae including *Pseudobradya*, *Halectinosoma*, *Bradya*, and *Ectinosoma*, need to be revised due to the lack of material, unverifiable descriptions, and errors in identification (Wells, 2007; Suarez-Morales and Fuentes-Reines, 2015). It is highly likely that many species of these genera have been assigned to the wrong genus (Wells, 2007). There are additional species of the family Ectinosomatidae that remain unnamed (Sciberras et al., 2018). Basic morphological structures are not described for many species of the genus *Halectinosoma* (Wells, 2007). The genus *Pseudobradya* most closely resembles those species of the genus *Halectinosoma* (Karanovic and Pesce, 2001; Wells, 2007). The images captured of the Shubenacadie ectinosomatid are vitally important for taxonomists to identify and compare the anatomical structures to other published descriptions and taxonomic keys (Figures 16-24). Without the images of the bodily structures of this previously undescribed species, it would be difficult to compare to published drawings and descriptions. Subtle differences can separate species in the family Ectinosomatidae such as the swimming leg armature of a new species of the genus *Halectinosoma* discovered in brackish water of an Argentinian estuary (Sciberras et al., 2018). Images and descriptions are important and can be utilized to update keys of the genera of the family Ectinosomatidae as well as comparing species of the genus from distant geographic locations (Sciberras et al., 2018).

In the Shubenacadie River Estuary, the ectinosomatid is pelagic as it was caught in the plankton net in the top 1.0m of the water column, and it was found in the guts of striped bass larvae which are accepted as being pelagic. By contrast, in the published literature there is only evidence of two ectinosomatids that are pelagic as they are generally a marine benthic copepod family (Seifried and Durbaum, 2000; Chertoprud et al., 2018). The copepodites of the ectinosomatid *Microsetella rosea* were captured in the surface water along the western Magellan coast of Chile (Canete et al., 2016). *Microsetella norvegica* is also considered a marine pelagic ectinosomatid (Uye et al., 2012). Approximately 0.5% of harpacticoids inhabit the pelagic zone of the ocean (Uye et al., 2012; Canete et al., 2016). There is no published knowledge of ectinosomatids from the genera *Pseudobradya* and *Halectinosoma* that are described as pelagic. It seems reasonable to conclude

the turbulent water currents in the Shubenacadie River Estuary elevate the ectinosomatid into the water column together with the detritus and particulates that resulting in the very high turbidity. The results indicate the unique physical characteristics of the Shubenacadie River has created a nursery habitat for striped bass that is dependent on a harpacticoid that is the only copepod small enough to be ingested by first-feeding stage larvae (Chapter 5).

Ectinosomatids differ from other harpacticoid copepods as they possess the ability to glide through sediment surfaces, dive quickly into sediment, and have highly flexible bodies (Seifried and Durbaum, 2000). Seven species of the family Ectinosomatidae are silt-burrowing and have well developed swimming appendages (Chertoprud et al., 2014). Species of the genus *Halectinosoma* are often dominant members of the harpacticoid copepod assemblage of marine sediments (Clement and Moore, 2007; Sciberras et al., 2018). The sediment of the riverbed changes throughout the Shubenacadie River Estuary. At the main study site, 25.0rkm, the river experiences a tidal range of 4-6m (Dyer, 2000). The riverbed is scoured and rocky bottomed and assumed to be caused by the large tidal amplitude, velocity, and turbulence. In the upper estuary above the confluence of the Shubenacadie and Stewiacke Rivers, the riverbed increases in muddy sediment. Similar to the Cobequid Bay and Salmon River Estuary, the Shubenacadie River Estuary increases in muddy sediments headward and sandy, coarse sediments and mudflats seaward (Dalrymple et al., 1990). The harpacticoids, mainly ectinosomatids, of the Laptev Sea in the Artic were higher in abundance with mud 3-4cm in depth when compared to a site with only 1cm of mud (Chertoprud et al., 2018). The effect of the changing riverbed sediment throughout the Shubenacadie River Estuary upon the habitat of the ectinosomatids is unknown. However, it is speculated that an increase in rocky and sandy sediment may reduce the abundance of ectinosomatids as mud is cited as the preferred sediment. This may also explain the higher abundance of ectinosomatids captured in the upper estuary as they were captured less frequently at 25.0rkm. The presence or absence of bottom silt in the Shubenacadie River Estuary may be important for defining the optimum habitat of first feeding striped bass since the bottom silt may affect the abundance of their main prey.

The ectinosomatid of the Shubenacadie River Estuary most closely resemble other species from the genus *Halectinosoma* based on their morphology. The Shubenacadie ectinosomatid resembles *Halectinosoma arangureni* sp. nov. from Columbia (Suarez-Morales and Fuentes-Reines, 2015).

The images obtained from Scanning Electron Microscopy of the Shubenacadie ectinosomatid (Figures 19-24) can be compared to *H. arangureni* sp. nov. in Figure 1 of Suarez-Morales and Fuentes-Reines (2015). *H. arangureni* sp. nov possesses an elongated urosome and caudal rami when compared to the Shubenacadie ectinosomatid but are otherwise noticeably similar in body shape (Suarez-Morales and Fuentes-Reines, 2015). The genus *Halectinosoma* is the largest of the family Ectinosomatidae and contains over sixty species (Karanovic and Pesce, 2001; Sciberras et al., 2018). It is difficult to identify members of *Halectinosoma* to the species level since many descriptions are inadequate which prevents an accurate identification (Sciberras et al., 2018). In comparison to the Shubenacadie River Estuary ectinosomatid, some species of the genus *Halectinosoma* are described as possessing coloured patches but are generally dark grey, brown, or brown-yellow (Wells, 2007). Shubenacadie ectinosomatids are browner or brown-yellow in color with no patches. *Rangabradya indica,* however, is described as less than 1.0mm TL and the color of the preserved sample as yellowish, similar in body length to the species reported here. The genus *Rangabradya* belongs to the same evolutionary line as *Halectinosoma* and *Pseudobradya* (Karanovic and Pesce, 2001). The family Ectinosomatidae have been recorded in Woods Hole, Massachusetts (Wilson, 1932). Their body length ranged from 0.45 to 0.95mm TL and being the widest near the center of the body (Wilson 1932), similar to specimens the ectinosomatids in the Shubenacadie River Estuary. The diet of harpacticoids includes detritus while ectinosomatids have been recorded to consume diatoms, ciliates, detritus, and bacteria (Seifried and Durbaum, 2000). The diet of the Shubenacadie ectinosomatid has not been investigated, and beyond the scope of this study. Carnivory was exemplified by two ectinosomatids including *Ectinosoma carnivora* and *Ectinosoma melaniceps* that consumed ostracods (Seifried and Durbaum, 2000).

For identification, DNA extractions were conducted to obtain an adequate concentration of DNA from the ectinosomatids to use for DNA sequencing. Both molecular and morphological approaches were used in the present study to identify the Ectinosomatidae copepod (Easton and Thistle, 2016). The morphological portion of the approach included the identification of the family at the Atlantic Reference Centre. The goal to isolate and recover DNA from the microscopic copepods was successful as the PCR enabled the amplification of the targeted 18S rRNA gene template. A major challenge was faced when the DNA sequence obtained from the Ectinosomatidae copepods did not yield any matches from Nucleotide Blast searches in GenBank.

The DNA sequences obtained from the samples of the 180 combined copepods collected in 2016 and the individual copepods collected in 2017 were identical. The DNA analysis conclusions emphasized the primitive state of copepod data in Gen Bank. The lack of 18S rRNA matches from the search in GenBank indicated that the species is a previously undescribed harpacticoid copepod species.

4.6.4 Copepod Distributions Relative to Time of Year

Acartia tonsa was the predominant copepod species in the Shubenacadie River Estuary reaching a peak abundance (IND/m^3) of 24286/m^3 at the end of August (Figure 25). In the Cobequid Bay of the Inner Bay of Fundy, adult *Acartia* sp. and copepodites I-IV were the predominant calanoid copepod species in the summer of 1983 (Stone, 1985). *Acartia* sp. reached densities of 2314/m^3 and 1816/m^3 mid-June and early July, respectively (Stone, 1985). The mean abundance of *A. tonsa* remained under 100/m^3 in the Shubenacadie River Estuary until July 10-16 while higher densities were recorded in Cobequid Bay during the same time period where the salinity was approximately 20ppt. Temperature was considered one of the most important factors that affected the growth rate of *A. tonsa* (Chinnery and Williams, 2004). The highest percent hatch rate (85.4%) and naupliar survival (72.9%) occurred at 20°C when compared to 13.1% and 0%, respectfully, at 5°C (Chinnery and Williams, 2004). The reported peaks in June and July in Cobequid Bay by Stone (1985) perhaps would have been greater abundances if the bay was as warm as the estuary in late summer (20-26°C).

The abundance of the harpacticoid copepod *Coullana canadensis* was dependent on time of year (Figure 26). The abundance of *C. canadensis* increased significantly between May and June (Figure 26). From the weeks of May 8 to June 25, the mean density of *C. canadensis* ranged from 2-58/m^3 in the estuary. The abundance of *C. canadensis* increased as the summer progressed and corresponded with an increase in water temperature in the estuary from May to August from 8.5°C to nearly 26°C (Figures 11-14). The abundance of *C. canadensis* in the Shubenacadie River Estuary was consistently lower than other estuaries. In Chesapeake Bay, the abundance of *C. canadensis* was highest in April and May at 8000/m^3 (Lonsdale, 1981b; Lonsdale and Levinton, 1989). Similarly, the highest densities of *C. canadensis* in the Shubenacadie River Estuary

occurred in spring and early summer (Figure 26). The density of *C. canadensis* increased above 10ºC and declined at 20ºC (Lonsdale, 1981b). The difference between the abundance of *C. canadensis* in Chesapeake Bay was 100-fold greater than the peak abundance in the Shubenacadie River Estuary.

Adult stage *Acartia tonsa* preyed on the nauplii of *C. canadensis* and *Oithona colcarva,* in Chesapeake Bay (Lonsdale, 1981b). This was a major factor that regulated the densities of *C. canadensis* in Chesapeake Bay from late spring to fall. The decline in early May from 8000/m^3 to less than 2000/m^3 in late June coincided with the rise of *A. tonsa* adults (Lonsdale, 1981b). In the Shubenacadie River Estuary a temporal and spatial match occurred between first feeding striped bass larvae and *C. canadensis*, their peak abundances relative to time of year and salinity aligned. Unfortunately, the body size of *C. canadensis* was too large for first feeding striped bass larvae to consume. In addition, I speculate that the rise in *A. tonsa* during the week of July 24 could have affected the abundance of *C. canadensis* as they may have fallen prey to *A. tonsa* in the Shubenacadie River Estuary.

From the weeks of May 8 to June 25, the mean density of *D. bicuspidatus* ranged from 2-12/m^3 in the estuary (Figure 27). The mean abundance of *Diacyclops thomasi* in the St. Lawrence River was 0.23/l or 0.00023/m^3 near Massena, NY, and Cornwall, ON, at a mean temperature of 20.0-23.0°C in freshwater (Thorp and Casper, 2003). The mean abundance of the nauplii of cyclopoid and calanoid copepods was recorded as 8.02/l or 0.008/m^3 (Thorp and Casper, 2003). *D. bicuspidatus* was collected from the Gronne Lake in South Germany that has a maximum depth of 4.5m (Maier, 1990). The embryonic developmental time of *D. bicuspidatus* in Germany was strongly temperature dependent and ranged from 13.8 days to 1.8 days at 2.0ºC and 25.0ºC, respectively. *D. bicuspidatus* exhibited a pelagic stage from February to June with the presence of reproductive females from December to May (Maier, 1998). *D. bicuspidatus* was pelagic in the water column up to 13ºC and entered diapause at the C-IV stage during the summer (Maier, 1990). No adults were detected in the pelagic stage, only in or near the muddy sediment with densities (IND/m^3) ranging from 31,000-46,000/m^3. The peak abundance of copepodites and adults occurred between April to June 1986 (Maier, 1990). *D. bicuspidatus* was captured in the top 1.0m

of the water column but were predominantly captured in the upper estuary of the Shubenacadie River Estuary from May until August.

Published data of the seasonal distributions of the family Ectinosomatidae is sparse. The mean water temperature of the Shubenacadie River Estuary at 25.0 and 36.0rkm increased from May to August in 2016 and 2017 from 8.5° to almost 26°C (Figures 11-14). Correspondingly, the ectinosomatids were present in the estuary from the end of June to August when the highest water temperatures occurred at 25.0 and 36.0rkm (Figure 28). The catch rate of ectinosomatids was more successful utilizing the 150µm plankton net when compared to the 250µm mesh size. The ectinosomatid *Microsetella norvegica* of a sub-Arctic fjord in Greenland experienced inter-annual variation in seasonal distribution (Arendt et al., 2013). *M. norvegica* dominated the plankton community biomass from July to August and were captured in using a 45µm mesh size in the fjord of a depth of 250m (Arendt et al., 2013). The peak abundance of *M. norvegica* occurred in July similar to the Shubenacadie ectinosomatid which peaked the week of June 26-July 2 (Figure 28). There are considerable differences between the habitats of a sub-Arctic fjord and the Shubenacadie River Estuary as well as the interactions between other zooplankton species.

The peak abundance of *Pseudodiaptomus pelagicus* in the Shubenacadie River Estuary occurred from August 14-20 (Figure 29). From the weeks of May 1 to July 30, the mean density (IND/m^3) of *P. pelagicus* ranged from 17-101/m^3. The abundance pattern of *P. pelagicus* in the Shubenacadie River Estuary resembled the pattern in other habitats where the peak densities occurred mid to late summer. *P. pelagicus* was the second most dominant calanoid copepod in Cobequid Bay in 1983 reaching 472/m^3 in late July (Stone, 1985). *P. pelagicus* was also the second most dominant copepod species next to *A. tonsa* in the Shubenacadie River Estuary. *P. pelagicus* reached a maximum density of 472/m^3 in late July in Cobequid Bay (Stone, 1985). In the Navesink River Estuary of the New York Bight, the abundance of *P. pelagicus* was 758/m^3 from May 1972 to June 1973 (Knatz, 1978). The mean abundance of *P. pelagicus* in the Navesink River Estuary was 200/m^3 from mid-June to early August and was present in the water column until September (Knatz, 1978). The water temperature was 31.9°C on July 19 but decreased seaward when compared to the shallow waters at the upper samples station. The mean salinity of the Navesink River Estuary ranged from 13ppt in May to 23ppt in August (Knatz, 1978). A similar pattern

occurred in the Shubenacadie River Estuary with temperature and salinity increasing as the summer months progressed (Figures 11-14) and *P. pelagicus* abundance peaking during the late summer months.

Pseudodiaptomus pelagicus was reared at the Marine Institute of the University of Georgia in natural tidal water of 20.0-25.0ppt and 18.0 to 22.0ºC (Jacobs, 1961). The average time of naupliar development measured from egg hatching to molting into the first copepodite stage was 6.2±0.3 days at a mean temperature of 20.4ºC. The completion of one generation at 20.0ºC took approximately 25 days (Jacobs, 1961). The temperature of the Shubenacadie River Estuary increased to over 20°C in late June to the end of the sampling season in August in 2016 and 2017 (Figures 11-14). The increase in temperature in the estuary associated with the increase in abundance of *P. pelagicus* in early July (Figure 29). The survival and maturation of cultured *P. pelagicus* from south Florida waters was optimal at 24.0-30.0ºC, 80.8-98.7% (Rhyne et al., 2009). Generation time was 12.8±0.4 days at 24.0ºC and 8.0±0.0 days at 34.0ºC. (Rhyne et al., 2009).

4.6.5 Copepod Distributions Relative to Salinity

Acartia tonsa in the Shubenacadie River Estuary were concentrated between 25 and 30ppt (Figure 30). From the weeks of May 15 to July 16, the mean density of *A. tonsa* ranged from 5-35/m^3 in the estuary (Figure 25). The abundance pattern of *A. tonsa* in the Shubenacadie River Estuary resembled the pattern in other estuarine environments where they were found in higher salinities during the summer months. The salinity increased in the estuary as temperature increased (Figures 11-14). The mean abundance of *A. tonsa* in the Navesink River Estuary of the New York Bight was 17,114/m^3 from May 1972 to June 1973 (Knatz, 1978). The peak abundance achieved by *A. tonsa* in the Navesink River Estuary was 150,000/m^3 in late June with a mean salinity of about 16ppt (Knatz, 1978). The peak abundance of *A. tonsa* in the Gironde Estuary, France, was 1400/m^3 in September increasing from 0.0/m^3 in winter and spring to less than 400/m^3 (David et al., 2005). Their combined abundances of *A. tonsa* and *E. affinis* in Chesapeake Bay ranged from less than 1000/m^3 in winter to over 200,000 nauplii/m^3 in late summer-early fall (Elliott and Tang, 2011). The abundance of *A. tonsa* in the Shubenacadie River Estuary, Chesapeake Bay, and the Gironde Estuary peaked in late summer, August-September. In the sub-estuaries of the Waquoit Bay system

MA, *A. tonsa* was dominant in salinities of 20.0-30.0ppt by June and comprised more than 80% of the calanoid copepod community. The abundance of *A. tons*a exceeded *E. affinis* by August in the estuarine system and increased significantly with salinity in Waquoit Bay (Lawrence et al., 2004).

Salinity was a major factor that affected the abundance of *A. tonsa* in the Shubenacadie River Estuary from between May and August (Figure 30). In the Gironde Estuary, the peak abundances of *A. tonsa* correlated with the highest summer salinities (10-14ppt) and were present seaward of the ETM (David et al., 2005). The seasonal variability of *A. tonsa* in the Gironde Estuary was significantly and positively correlated to salinity (David et al., 2005). The salinity and temperature increased from May to August in the Shubenacadie River Estuary and corresponded with the rise in the density of *A. tonsa* (Figures 11-14). In the summer months, freshwater run-off decreases in the Shubenacadie River Estuary with the seawater intruding further into the reaches of the estuary. *A. tonsa* from Southampton Water (UK) survived longer at 30.0ppt at 17.0°C when compared to lower salinities at the same temperature (Lance, 1963). *A. tonsa* from Southampton Water developed most rapidly at higher temperatures and high salinity with the lowest mean number of days for the first copepodite stage to appear was 7.7 days (±1.15 days) at 20°C and 33.3ppt (Chinnery and Williams, 2004). The mean percent survival increased from 20.9±8.80% to 72.9±25.30% at salinities of 15.5 and 33.3ppt, respectively (Chinnery and Williams, 2004). It was concluded that *A. tonsa* possesses a strong relationship with temperature and salinity and the peak abundance occurs in late summer when temperature and salinity are highest in estuaries.

The abundance of *Coullana canadensis* in the Shubenacadie was independent of salinity between 0 and 20.0ppt, comparable to other rivers (Figure 31). The distribution of *C. canadensis* relative to salinity in the Shubenacadie River Estuary was comparable to other rivers. *C. canadensis* was collected in 1.0-12.0ppt water in Pamlico River (North Carolina) and 5.0-15.0ppt in Dividing Creek, Delaware Bay (New Jersey; Coull, 1972). Harpacticoid copepods are predominantly benthic-dwelling in estuarine and marine sediments (Harris, 1977; Stringer et al., 2012). Harpacticoid copepods which inhabit the surface layer of sediment frequently enter the water column where they can fall prey to invertebrate and juvenile fish (Chertoprud et al., 2014). The naupliar development of *C. canadensis* occurred in the upper layers of the water column in

Chesapeake Bay and after metamorphosis to the Copepodite I-II stage, they descended were epibenthic as adults (Lonsdale, 1981a). *C. canadensis* may not have been available to first feeding striped bass larvae in Chesapeake Bay due to the epibenthic life history of adults, however, the high turbulence of the Shubenacadie River Estuary not only re-suspends the sediment causing high turbidity but forced adult stage *C. canadensis* up in the water column. The high turbulence allowed *C. canadensis* to be caught in the plankton net in the top 1.0m of the water column.

Diacyclops bicuspidatus is mainly a freshwater species of copepod, however, the abundance ranged from 3-27/m^3 from the lowest salinity category of 0.00-0.15ppt to 15.1-20.0ppt in the Shubenacadie River Estuary (Figure 32; Maier, 1990). *D. bicuspidatus* was not recorded in the estuary in 2016. The 2016 sampling season was a record year for high salinity within the estuary and may have surpassed the salinity tolerance of the species (Figure 11, 12). The abundance of *D. bicuspidatus* increased as salinity increased. The literature clearly indicates that this species is a freshwater species only (Maier, 1990). *D. bicuspidatus* may have been washed down-estuary from tidal freshwater by the strong currents.

Published knowledge of the family Ectinosomatidae is primarily focused on the taxonomic descriptions and location of discovery of species. Ectinosomatids are described in the literature as predominantly benthic in marine sediments (Seifried and Durbaum, 2000; Chertoprud et al., 2018; Sciberras et al., 2018). An increase in the number of recognized ectinosomatids has increased over the last few decades and are being discovered in other habitats besides marine sediments (Clement and Moore, 1995; Sciberras et al., 2018). Species from genera recorded in brackish and freshwater systems include *Rangabradya*, *Ectinosoma* Boeck, *Microstella*, *Pseudobradya*, *Pseudectinosoma*, *Halectinosoma*, and *Arenosetella* (Defaye and Dussart, 2011). Species of the genus *Pseudectinosoma* of the family Ectinosomatidae are predominantly freshwater (Karanovic and Pesce, 2001). *Rangabradya indica* was discovered in fresh ground water in India and was initially thought to belong to the genus *Halectinosoma*. It has been described in a new genus which no other species from the family Ectinosomatidae can be placed into with confidence (Karanovic and Pesce, 2001). The highest abundance of the Shubenacadie ectinosomatid was captured with the 150 and 250µm plankton mesh sizes in brackish water of 1.1-2.0ppt (Figure 33). Species of the genera *Pseudectinosoma* and *Rangabradya* have not been recorded in salinity. The Shubenacadie

ectinosomatid was distributed in less saline water similar to other genera of the family Ectinosomatidae such as *Pseudectinosoma* and *Rangabradya*. The ectinosomatid was distributed from 0 to 15.0ppt with high abundance between 0.16-2.0ppt (Figure 33). Copepodites of the ectinosomatid *Microsetella rosea*, in contrast, were captured in 26-33ppt in southern Chile with densities of 1000-10000IND/5 min drag (50µm mesh) at a temperature of 6.5-8.5°C (Canete et al., 2016). Species within the genus *Microsetella* have relatively strong swimming abilities and a unique anatomy including an elongated body and long caudal setae that delay sinking velocity (Canete et al., 2016). Future studies of the Shubenacadie ectinosomatid should assess the swimming ability and the possible swimming features of the species that serve as valuable adaptations to the macrotidal habitat. The Shubenacadie ectinosomatids exhibited large variation in distribution as seen in the large standard errors (Figure 33). Similarly, the *M. rosea* distribution pattern varied up to four orders of magnitude between sampling sites and was classified as clumped in the Magellan Region along the Chilean coast (Canete et al., 2016).

The distribution of *Pseudodiaptomus pelagicus* in the Shubenacadie River Estuary was dependent on salinity (Figure 34). Salinity significantly affected the population composition and developmental stages of cultured *P. pelagicus* in a laboratory setting (Ohs et al., 2010). The greatest mean abundance of each copepod stage, nauplii, copepodite, and gravid females, occurred at 25.0ppt and the mean number of adults was highest at 15.0ppt. The optimal salinity range for *P. pelagicus* in the lab based on survival rate and nauplii production was 15.0-25.0ppt (Ohs et al., 2010). The peak density of *P. pelagicus* in the Shubenacadie River Estuary coincided with the optimal cultured salinity range of 15.0-25.0ppt. *Pseudodiaptomus pelagicus* appears to be well adapted to the high energy and turbid water of the Shubenacadie River Estuary. *Pseudodiaptomus* species are tolerant to heavy aeration and the presence of sediment and suspended organics (Ohs et al., 2010). *P. pelagicus* was present in the highly turbulent and turbid waters of Cobequid Bay and the Minas Basin (Jermolajev, 1958; Stone, 1985).

4.7 Conclusion

Copepods identified in the Shubenacadie River Estuary included the calanoids *Acartia tonsa* and *Pseudodiaptomus pelagicus*, the cyclopoid *Diacyclops bicuspidatus*, and the harpacticoid *Coullana canadensis*. The copepods' identity was verified by Dr. G. Pohle in 2013 and Jackie Spry in 2019. A previously undescribed harpacticoid copepod species was discovered as the dominant prey item of first feeding striped bass larvae in the Shubenacadie River Estuary. It was identified from the family Ectinosomatidae, a difficult family of copepods to identify and a lack of published literature exists. The Shubenacadie ectinosomatid was significantly smaller in body length than *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus*. *P. pelagicus* was larger than reported specimens from other estuaries. The distributions of copepods varied according to time of year and salinity in the estuary. Peak densities of *Acartia tonsa* and *Pseudodiaptomus pelagicus* occurred in late August in high salinity. Ectinosomatids and *Coullana canadensis* were located in lower salinity and peaked in early summer. The distributions of *Acartia tonsa*, *Coullana canadensis*, and *Pseudodiaptomus pelagicus* were comparative to the published literature. Future research includes verifying the identification by sending ectinosomatid specimens to harpacticoid specialists as well as cataloging all known information pertaining to the morphology, DNA sequencing, and characterization of its habitat within the Shubenacadie River Estuary. Descriptions of the females and males would further aid in the morphological taxonomic description.

CHAPTER 5: STRIPED BASS SPAWNING, LARVAE ABUNDANCE, DISTRIBUTION, AND GUT CONTENTS

5.1 INTRODUCTION

The Bay of Fundy population of striped bass is designated as endangered by COSEWIC (2015) since the Shubenacadie-Stewiacke River Estuary is the sole confirmed successful spawning and nursery habitat. The survival and growth during the egg and larval stages are important factors affecting recruitment success (Rutherford and Houde, 1995). The 2016 spawning data of striped bass in the Shubenacadie River Estuary has been published in Duston et al. (2018). The 2016 spawning data is also presented here independently but the 2017 data has not been previously published. Striped bass eggs of the Shubenacadie-Stewiacke River Estuary typically hatch within 2-4 days (MacInnis, 2012). The emergent yolk-sac larvae rely on their yolk and oil globule for energy until they capture their first prey (Eldridge et al., 1981). The identification of the availability of specific species as prey for striped bass larvae at the critical first-feeding stage was the main focus of this study. Larvae require prey small enough to ingest after yolk-sac absorption, mouth gape being the limiting factor. The gut contents of first feeding larvae provide insight into the feeding habits. The gape size of first feeding striped bass relative to body size of potential prey items has not been reported in the published literature.

5.2 METHODS SUMMARY

Plankton net tows were conducted to estimate the abundance of striped bass larvae relative to time of year and salinity. Tows were conducted at the main study site, a fixed location at 25.0rkm, from 2013 to 2017 from May until August to quantify the temporal (time of year) distribution (Chapter 3). Additionally, plankton net tows were conducted both at a fixed location (25.0rkm, main site) through the flood tide, and also up-estuary beginning at the salt-front at high tide to determine the distribution relative to salinity. The distributions of striped bass larvae relative to time of year and salinity were compared to the distributions of copepods in the Shubenacadie River Estuary. The gut contents of each striped bass larva were examined and each prey item was classified by taxa. The relative abundance was calculated to compare the prey items in the gut to the prey species present in the water column. Copepods were measured to determine the body length and compared

to the gape size of striped bass larvae. The mean abundance of larvae was calculated per week and salinity category. The abundances were compared using a one-way ANOVA analysis of variance following appropriate transformation to meet normal distribution and constant variance assumptions on the error terms (Montgomery, 2012).

5.3 Results: Striped Bass Spawning

In 2016, striped bass eggs were first detected on May 20 with a mean abundance (eggs/m^3) of 11/m^3 at 13.9ºC (Figure 35). Peak mean daily abundance occurred May 23 with 1842/m^3 at 14.8ºC and 9.0ppt. The second highest mean abundance occurred May 25 with 184/m^3 at 18.6ºC and 4.2ppt on the flood tide at 25.0rkm. Eggs were last detected in the estuary June 28, 2016, at a density of 1/m^3 at 21.7ºC and 3.6ppt. Eggs only exceeded 100 eggs/m^3 on May 23 and 25, 2016 (Figure 35).

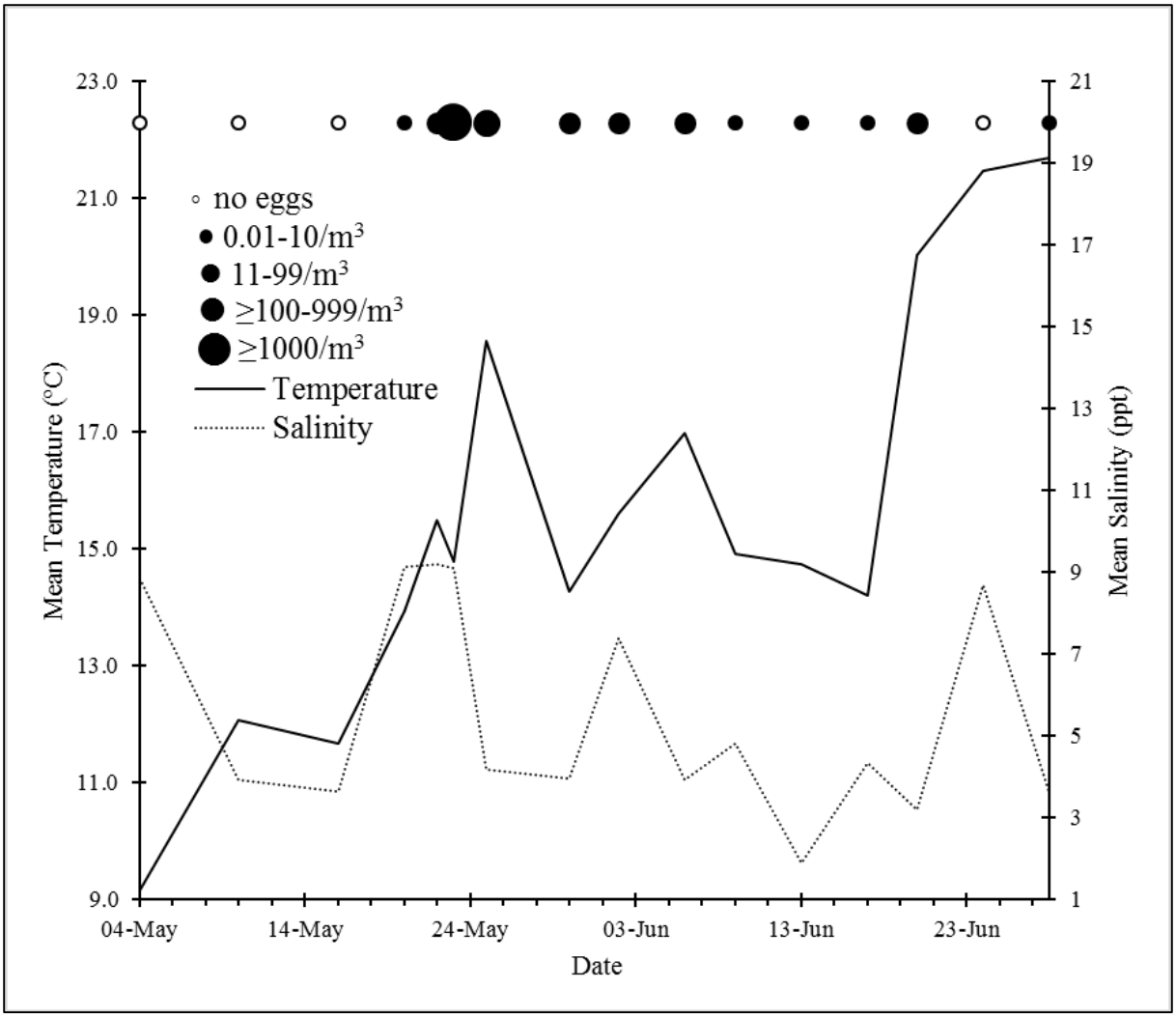

Figure 35. Timing of spawning of striped bass as judged by presence of eggs in the Shubenacadie River Estuary between 25 and 40rkm in 2016. Data pooled from ebb and flood tides. Daily mean temperature (ºC). The black circles indicate the abundance of eggs per m^3.

In 2017, the first largest spawning episode occurred approximately one week later than 2016 and the density of eggs (eggs/m^3) was 73% greater. The largest spawning in 2017 occurred at a higher temperature (°C) and lower salinity than 2016. The mean daily abundance of greater than 100 eggs/m^3 occurred more frequently in 2017 than 2016. In 2017, striped bass eggs were first detected May 15 with a mean abundance (eggs/m^3) of 2/m^3 at 12.0ºC (Figure 36), five days earlier than 2016. Highest mean abundance occurred June 2 with 2523/m^3 at 6.7ppt and 15.4ºC. The second highest mean abundance occurred May 22 with 487/m^3 at 1.8ºC and 3.7ppt. Eggs were last detected June 19, 2017, at a density of 1/m^3 at 20.0ºC. Eggs surpassed a density of greater than 100 eggs/m^3

in 2017 on May 22, June 9, and June 10 at densities of 487, 219, and 182/m^3, respectively (Figure 36).

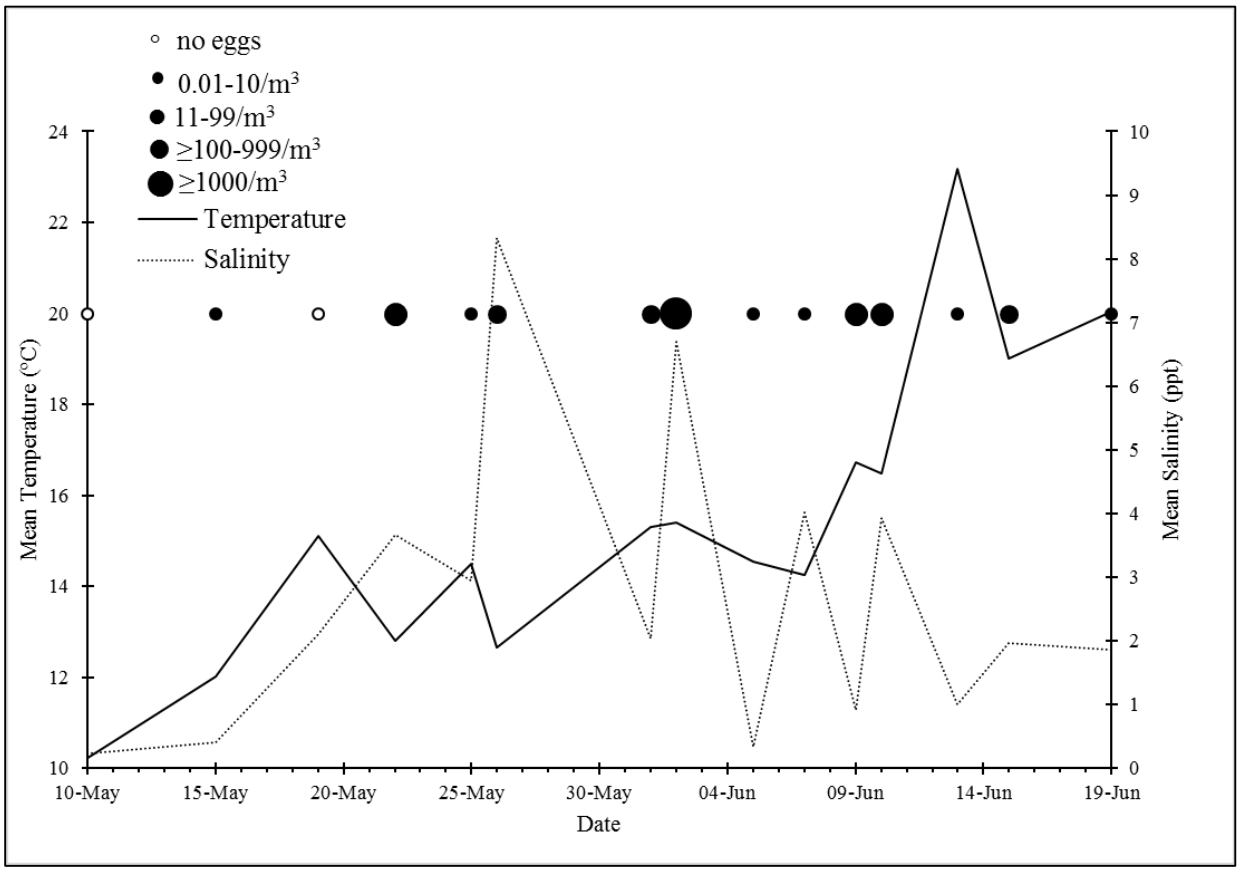

Figure 36. 2017 timing of spawning of striped bass as judged by presence of eggs in the Shubenacadie River Estuary between 25 and 40rkm. Data pooled from ebb and flood tides. Daily mean temperature (ºC). The black circles indicate the abundance of eggs per m^3 of water filtered.

5.4 Results: Distribution of Striped Bass Larvae Relative to Time of Year

Non-feeding stage larvae were detected in 2016 from May 22 to the end of June and in 2017 from May 25 to June 23 (Figure 37). The abundance of non-feeding larvae was dependent on time of year in 2016 (P=0.016) and 2017 (P=0.005) decreasing significantly through June (Figure 37). Their total body length in both years ranged from 4.5 to 6.0mm. Peak mean weekly abundance (IND/m^3) in 2016 was 1113/m^3 from May 22-28 (Figure 37). In 2017, by comparison, peak mean weekly abundance occurred in the same week, May 22-28, but was almost 20-fold lower, 58/m^3 from May 22-28 (Figure 37). In 2016, the mean abundance decreased from May 22-28 to May 29-

June 4 from about 1100/m^3 to 300/m^3, but the difference was not statistically significant. In 2016 and 2017, the peak mean abundance of non-feeding larvae occurred during the same period as peak spawning (Figure 37). The highest mean abundances relative to time of year of non-feeding larvae occurred during the same week in 2016 and 2017.

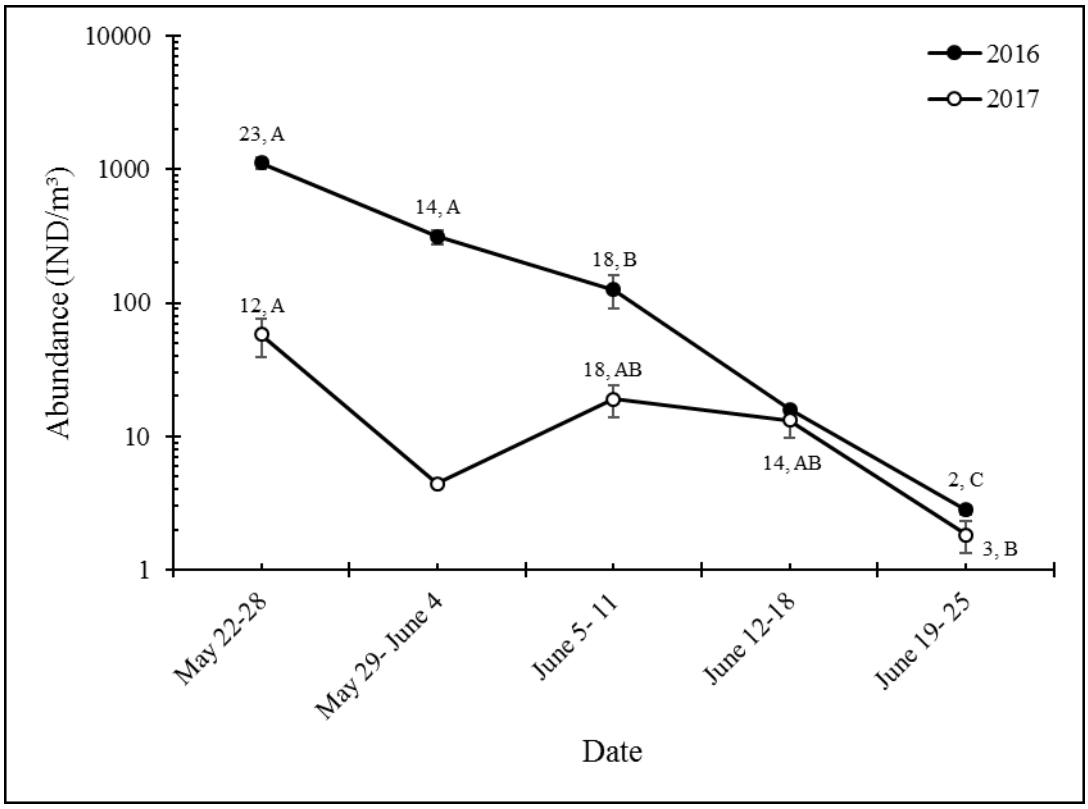

Figure 37. Mean (±SE) weekly abundance (IND/m^3) of non-feeding striped bass larvae (4.5-6mm TL) in the Shubenacadie River Estuary from May to June 2016 and 2017. Data are pooled from ebb and flood tides. Within each year, means sharing the same letter are not significantly different ($P<0.05$). Data labels indicate the number of tows conducted during each week. Logarithmic scale for y-axis.

In 2016, feeding stage larvae were detected in the estuary from May 30 to July 15 and the abundance was dependent on time of year ($P<0.01$; Figure 38). Through May 29-June 4 to June 19-25, the mean weekly abundance (IND/m^3) ranged from 180 to 460/m^3, but the differences were not significant (Figure 38). The mean weekly abundance of feeding stage larvae in July were significantly lower than June. In 2017, feeding stage larvae were detected in the estuary from June 1 to July 20 and the abundance was independent on time of year ($P<0.05$; Figure 38). The highest mean weekly abundance (IND/m^3) was 595/m^3 from June 12-18 (Figure 38). The highest mean

abundance in 2017 relative to time of year of feeding stage striped bass larvae was higher than in 2016 and occurred nearly two weeks later than 2016. However, the second highest abundance of feeding stage larvae in 2016 did occur during the week of June 12-18. The largest larvae captured in the plankton net samples was less than 30.0mm TL. Larvae begin to transition to the juvenile stage at this body size (Table 1).

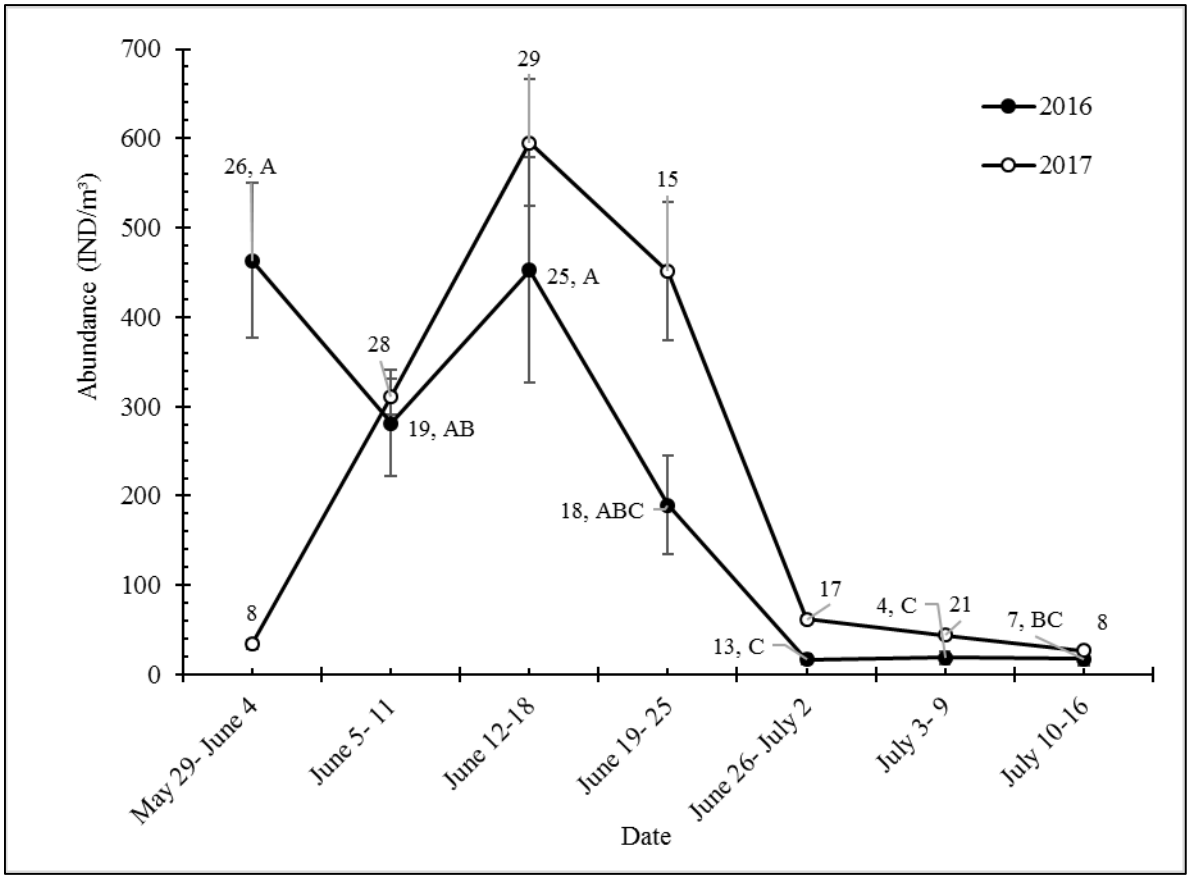

Figure 38. Mean (±SE) weekly abundance (IND/m^3) of feeding stage striped bass larvae (6-25mm TL) in the Shubenacadie River Estuary from May to July in 2016 and 2017. Data pooled from ebb and flood tides. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm. Means that do not share a letter are significantly different (P>0.01). Data labels represent the number of tows conducted during each week.

5.5 Results: Distribution of Striped Bass Larvae Relative to Salinity

In 2016, high abundance (IND/m^3) of non-feeding stage larvae in the Shubenacadie River Estuary was reported as 867/m^3 in 10.1-15.0ppt (Figure 39). In 2017, the high abundance was 48/m^3 in

15.1-20.0ppt (Figure 39). The mean abundance (IND/m^3) of non-feeding stage larvae was independent of salinity both in 2016 (P=0.441) and 2017 (P=0.800; Figure 39). The mean abundance of 47/m^3 in the category of 2.1-5.0ppt in 2017 was similar to the mean abundance in 1.1-20.0ppt (Figure 39). In 2016, the estuary was very salty with low freshwater run-off (Figure 11, 12). The salinity intrusion was too far up-estuary to sample in tidal freshwater.

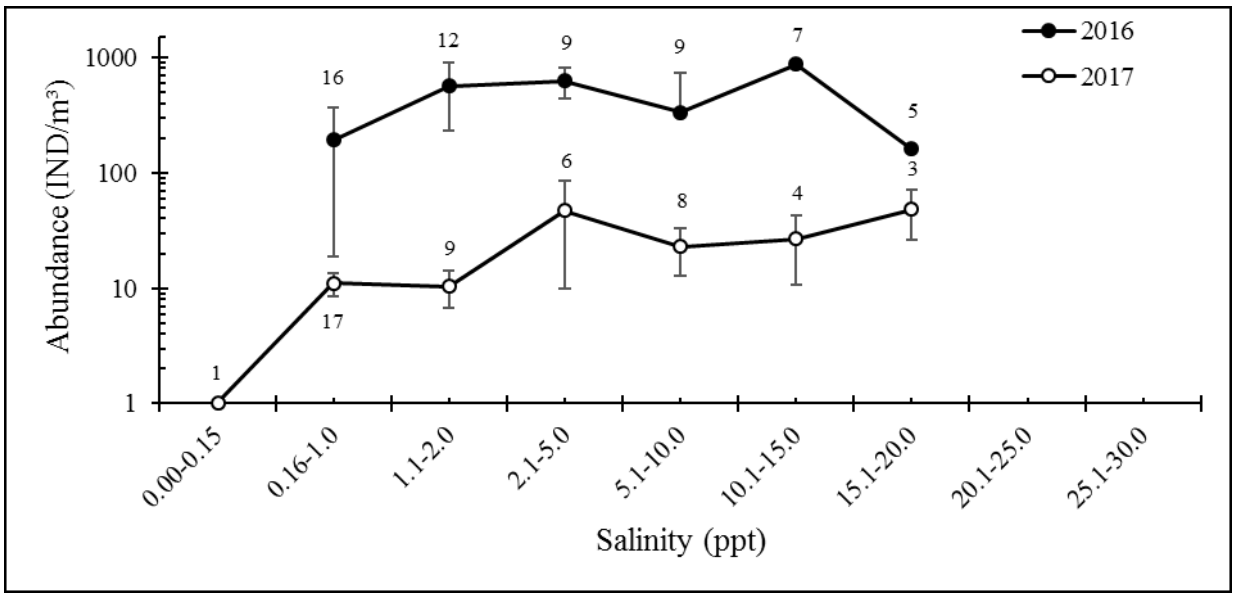

Figure 39. Mean (±SE) abundance (IND/m^3) per salinity category of non-feeding striped bass larvae (4.5-6.0mm TL) from May to June 2016 and 2017 in the Shubenacadie River Estuary. Data pooled from ebb and flood tides (250µm mesh size) from 25.0-41.0rkm. Data labels indicate the number of tows. Logarithmic scale for y-axis.

The mean abundance (IND/m^3) of feeding stage larvae was independent of salinity in 2016 (P=0.604) and 2017 (P=0.298; Figure 40). In 2016, the mean abundance (IND/m^3) ranged from 352/m^3 in 1.1-2.0ppt to 321/m^3 in 5.1-10.0ppt but the differences were not significant (Figure 40). In 2017, the mean abundance ranged from 157/m^3 in 2.1-5.0ppt to 162/m^3 in 5.1-10.0ppt (Figure 40).

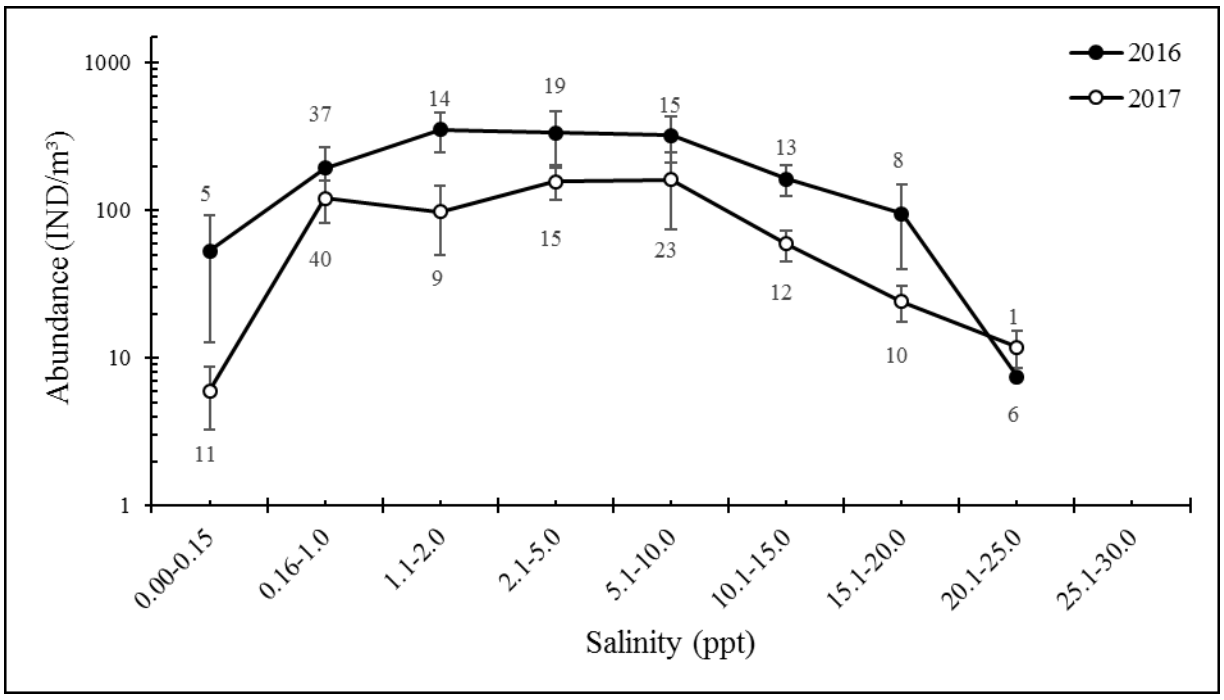

Figure 40. Mean (±SE) abundance (IND/m^3) per salinity category of feeding stage striped bass larvae (6-25mm TL) in 2016 and 2017 (250µm mesh size) in the Shubenacadie River Estuary. Data pooled from ebb and flood tides from 25.0-41.0rkm. Data labels represent the number of tows. Logarithmic scale for y-axis.

5.6 Results: Transition from Endogenous to Exogenous Nutrition Relative to Gape and Body Length

The estimated gape size of larval striped bass in 2016 and 2017 increased in proportion to total body length from 0.5mm at 6.0mm from to about 4.0mm at 30.0mm TL (Figure 41). The mean gape size from 7.0-7.9, 8.0-8.9, 9.0-15.0, 15.1-25.0, and 25.1-30.0mm TL was 0.7, 1.0, 2.0, 3.3, and 4.2mm, respectively. The large variability in the gape estimates was a consequence of the extreme difficulty manipulating the larvae lower jaw. First feeding stage larvae at 6.0mm TL with a gape of 1 .0mm or smaller, were capable of capturing prey items no larger than 1.0mm (Figure 41). At 9.0mm TL, the maximum prey size that larvae could consume was 2.0mm (Figure 41).

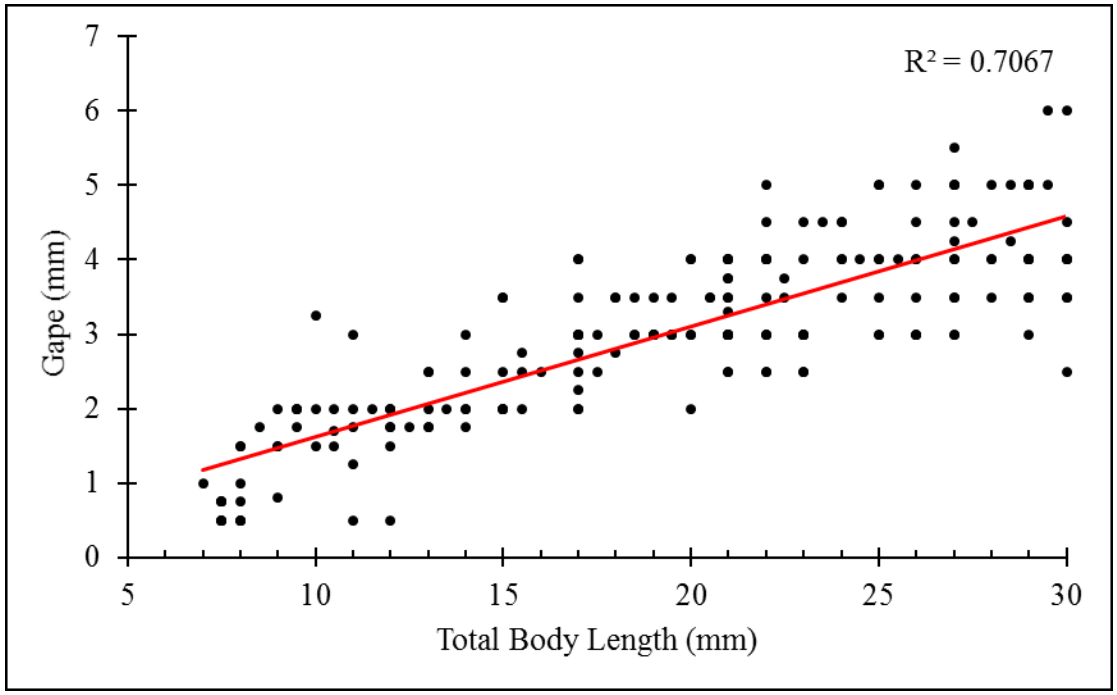

Figure 41. Gape size (mm) of larval striped bass relative to body length (mm) in the Shubenacadie River Estuary in 2016 and 2017 from May to July. Equation of regression line: y=0.1485x+0.1338 with an R^2 value of 0.7607. N= 217.

Unequivocal evidence of the onset of the transition from endogenous to exogenous nutrition of striped bass larvae in the Shubenacadie River Estuary was a) presence of food in gut and b) by the increase in body length above 7mm TL. The first indicator of the transition was the growth of larvae in the estuary beginning in July (Figure 42). The second indicator of the transition was the

presence of prey items in the guts of larvae as presented in the next section. The total body length of striped bass larvae in 2016 and 2017 remained at 7mm from the beginning of June until near the end of June (Figure 42). Larvae began to grow at the end June and early July across both year classes (Figure 42).

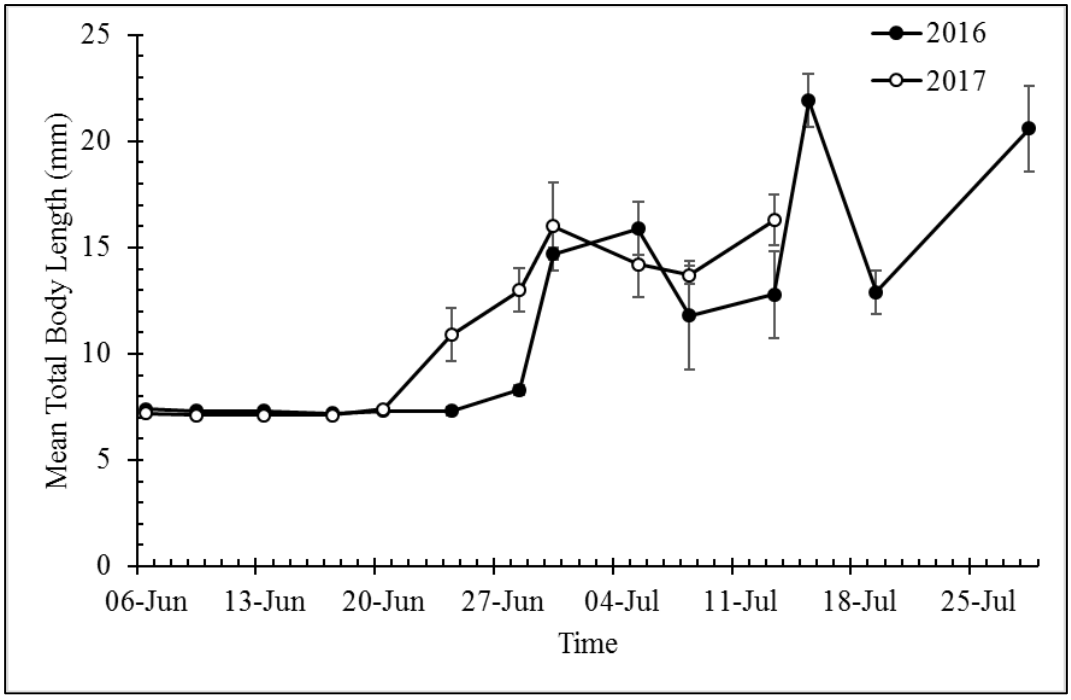

Figure 42. Total body length (mm; ±SE) of feeding stage striped bass in the Shubenacadie River Estuary captured in plankton from June to end of July in 2016 and 2017.

5.7 Results: Gut Contents of First Feeding Striped Bass Larvae

Striped bass larvae transitioned to the first feeding stage at approximately 6.0-6.9mm TL with eyes fully pigmented and mouth fully developed with a gape of less than 1mm (Table 8, Figure 41). In 2016, the only identifiable prey item of first feeding striped bass was an adult stage Ectinosomatidae harpacticoid copepod (Table 8). All larvae ingesting this prey item were captured from 25.0-41.0rkm on the ebb and flood tides. The Ectinosomatidae copepod was the dominant prey item among larvae ranging in body size categories from 6.0 to 15.0mm TL (Table 8). The critical body size at which exogenous feeding commenced was 6.0 to 6.9mm TL. The mean number of prey per larval gut increased as body increased from 0.3 to 3.2prey/gut from 6.0 to

15.1mm TL, respectively (Table 8). The mean number of Ectinosomatidae copepods per gut ranged from 1.0 to 1.8/gut among larvae 6.0-9.0mm TL (Table 8). The maximum number of ectinosomatids per gut was 6 in a larva 8.0mm TL on June 6. Larvae prior to June 6 had a higher incidence of empty guts. Other copepod species in the gut could not be identified due to their decomposition.

Table 8. Mean (±SE) number of prey taxa per body size category (mm TL) of feeding stage striped bass larvae June 6 to July 28, 2016, captured in the Shubenacadie River Estuary from 25.0-41.0rkm on the ebb and flood tides (250µm plankton net). N is the number of larvae examined per size category. A total of 408 larvae were examined.

	Size Class (TL in mm)					
	6.0-6.9	**7.0-7.9**	**8.0-8.9**	**9.0-15.0**	**15.1-25.0**	
Prey Taxa N	12	276	57	45	18	**Mean**
Ectinosomatidae	1.0 (0.25)	1.5 (0.05)	2.0 (0.17)	1.8 (0.62)	0	1.26
Digested	0.3 (0.13)	0.1 (0.02)	0.1 (0.04)	0.4 (0.08)	0.3 (0.11)	0.24
Amphipod	0	0	0	0.2 (0.12)	0	0.04
Other Copepods	0	0	0	1.3 (0.56)	3.2 (0)	0.90
Mysid	0	0	0	0	1.7 (0)	0.34
% Empty	25.0	8.7	8.8	37.8	27.8	21.6
Capture date	June 6-20	June 6-28	June 6-July 5	July 8-28	July 8-28	

In 2017, the dominant prey item of striped bass larvae ranging from 6.0 to 15.0mm TL was the same Ectinosomatidae copepod as 2016 (Table 9). The mean number of ectinosomatids per gut was similar per body size category from 6.0 to 15.0mm TL than 2016 (Table 9). The mean number of prey items per gut increased from 1.1 to 2.2/gut from 6.0 to 15.1mm TL, respectively (Table 9). The mean number of prey items per gut was lower in 2017 than 2016. The mean number of Ectinosomatidae copepods per gut ranged from 0.1 to 1.6/gut among the striped bass larvae from 6.0 to 25.0mm TL (Table 9). In 2017, the maximum number of ectinosomatids per gut was 11 in the gut of a larvae 7.3mm TL on June 15. The mean number of mysids, *Neomysis americana*, per larval gut was higher in 2017 with mysids entering the diet earlier at 7.0-7.9mm TL (Table 9). Mysids did not enter the diet of larvae in 2016 until the category of 15.1-25.0mm TL (Table 8). The mysids consumed were juvenile stage. Juveniles are defined as age-0 fish that have a general adult appearance with differentiated soft and spiny fins (See Section 3.1). Prey items were not identified in the gut of larvae until June 13, 2017 with zero empty guts recorded from 6.0 to 8.9mm TL (Table 9).

Table 9. 2017 mean (±SE) number of prey taxa per body size category (mm TL) of first feeding stage striped bass larvae June 13 to July 20, 2017, captured in the Shubenacadie River Estuary from 20.8-41.0rkm on the ebb and flood tides (150 and 250µm plankton nets). N is the number of larvae examined per size category. 284 larvae were examined for gut contents in total.

		Size Class (TL in mm)					
		6.0-6.9	**7.0-7.9**	**8.0-8.9**	**9.0-15.0**	**15.1-25.0**	
Prey Taxa	**N**	31	162	11	51	29	**Mean**
Ectinosomatidae		1.1 (0.12)	1.5 (0.10)	1.6 (0.59)	1.3 (0.27)	0.1 (0)	1.12
Digested		0	0	0.1 (0)	0.1 (0.05)	0.2 (0)	0.08
Amphipod		0	0	0	0.1 (0.05)	0.2 (0.12)	0.06
Other Copepods		0	0	0	0	0	0
Mysid		0	0.1 (0.02)	0.1 (0)	0.3 (0.09)	2.2 (0.55)	0.54
% Empty		0	0	0	11.7	0	2.3
Captured		June 13-23	June 13-July 6	June 13-July 6	June 30-July 20	June 30-July 20	

One ectinosomatid was present in the gut of a first feeding striped bass larvae, 7.0mm TL (Figure 43). The brown pigmented body of the copepod was clearly visible through the transparent body and gut of the striped bass larvae. The oil globule was still prominent at this stage indicating the utilization of both endogenous and exogenous nutrition (Figure 43). The inset shows the adult stage ectinosomatid present in the gut of the first feeding striped bass larva (Figure 43). The cephalosome, metasome, urosome, pereiopod "swimming" appendages, and caudal rami of the ectinosomatid are distinguishable (Figure 43).

Figure 43. First feeding striped bass larvae (7.0mm TL) caught in the Shubenacadie River Estuary with a 250µm plankton net mesh size. One Ectinosomatidae harpacticoid copepod visible in the gut indicated by red arrow. Inset: adult stage ectinosomatid (1.0mm TL) that was present in the gut. Images captured by Leica EZ4 dissecting microscope and Leica AirLab App.

The relative abundance of gut content species was compared to the zooplankton from the same plankton net tow (Table 8). In 2016, the Ectinosomatidae copepod was the only prey item consumed of larvae 6.0 to 8.9mm TL from June 6 to July 5 (Table 8). For larvae 6.0-6.9, 7.0-7.9, 8.0-8.9, and 9.0-15.0mm TL, the Ectinosomatidae copepod ranged from 67-100%, 87-100%, 77-100%, and 33-50% of the relative abundance of prey items in the gut, respectively (Table 10). Other gut contents included digested and unidentifiable particles, larger copepods, amphipods, striped bass larvae, unidentifiable eggs, and mysids (Table 10). Other copepod species were not identifiable until July 19, 2016 (Table 10). The Ectinosomatidae copepod was not captured in the 250µm plankton net mesh in 2016 from June 6 to July 28 (Table 11). Other copepod species including *Pseudodiaptomus pelagicus*, *Coullana canadensis*, and *Acartia tonsa* were abundant in the water column but were not identified in gut contents from June 6 to July 15 (Table 10). In 2017, *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus* were identified in the gut contents; whereas they were absent in 2016 (Table 12). In 2017, prey was first identified in gut contents on June 13, 2017 (Table 12).

Table 10. Gut contents of feeding stage striped bass larvae expressed as the mean daily relative abundance (%) from June 6 to July 28, 2016. N is the number of larvae analyzed per size category (total= 408).

Date	Total body length (mm)	N	Ectinoso-matidae	Mysid	Amphipod	Other Copepods	Digested	Empty gut
June 6	6.0-6.9	1	100	0	0	0	0	0
	7.0-7.9	14	93	0	0	0	7	0
	8.0-8.9	4	100	0	0	0	0	0
June 9	7.0-7.9	31	87	0	0	0	13	0
	8.0-8.9	5	80	0	0	0	20	0
June 13	6.0-6.9	6	67	0	0	0	33	0
	7.0-7.9	70	89	0	0	0	11	0
	8.0-8.9	1	77	0	0	0	23	0
June 17	6.0-6.9	3	67	0	0	0	33	0
	7.0-7.9	44	89	0	0	0	11	0
	8.0-8.9	5	80	0	0	0	20	0
June 20	6.0-6.9	2	100	0	0	0	0	0
	7.0-7.9	97	95	0	0	0	5	0
	8.0-8.9	15	100	0	0	0	0	0
	9.0-15.0	3	33	0	0	0	67	0
June 24	7.0-7.9	7	86	0	0	0	14	0
	8.0-8.9	1	100	0	0	0	0	0
June 28	7.0-7.9	11	91	0	0	0	0	9
	8.0-8.9	8	100	0	0	0	0	0
	9.0-15.0	6	50	0	0	0	33	17
June 30	9.0-15.0	3	67	0	0	0	33	0
July 5	7.0-7.9	1	100	0	0	0	0	0
	8.0-8.9	3	100	0	0	0	0	0
	9.0-15.0	2	50	0	0	0	50	0
July 8	7.0-7.9	1	0	0	0	0	0	100
	9.0-15.0	3	0	0	0	0	0	100
	20.1-25.0	1	0	0	0	0	0	100
July 13	9.0-15.0	8	0	0	25	0	0	75
July 15	15.1-20.0	5	0	0	0	0	0	100
	20.1-25.0	4	0	0	0	0	0	100
July 19	9.0-15.0	15	0	0	1	46	53	0
	15.1-20.0	1	0	0	0	100	0	0
July 28	9.0-15.0	5	0	0	0	0	60	40
	15.1-20.0	5	0	20	0	0	80	0
	20.1-25.0	2	0	0	0	0	50	50

Table 11. Mean daily relative abundance (%) of zooplankton present in the water column based on 250µm mesh plankton net tows taken concurrently with feeding stage striped bass larvae from June 6 to July 28, 2016. N is equal to the number of tows with 180 analyzed in total.

Date	N	*Pseudodiaptomus pelagicus*	*Coullana canadensis*	*Acartia tonsa*
June 6	22	43	55	2
June 9	9	67	33	0
June 13	20	24	76	0
June 17	11	33	67	0
June 20	20	17	83	0
June 24	8	78	17	5
June 28	17	47	52	1
June 30	9	56	0	0
July 5	11	0	64	0
July 08	2	86	14	0
July 13	7	84	6	10
July 15	10	67	11	22
July19	16	30	47	23
July 28	18	7	36	57

The mean daily relative abundance of the Ectinosomatidae copepod in the gut contents and in the water column coincided (Table 12, 13). For larvae 6.0-6.9, 7.0-7.9, 8.0-8.9, and 9.0-15.0mm TL, the Ectinosomatidae copepod ranged from 82-100%, 75-100%, 50-100%, and 33-100% relative abundance of prey in gut, respectively (Table 12). Other gut contents included digested and unidentifiable particles, larger copepods, amphipods, striped bass larvae, unidentifiable eggs, and mysids (Table 12). In 2017, 150µm and 250µm plankton net tows were compared since the 250µm mesh size in 2016 yielded no Ectinosomatidae copepods. *Acartia tonsa, Coullana canadensis, Diacyclops bicuspidatus,* and *Pseudodiaptomus pelagicus* were abundant in the water column but were rarely consumed by larvae throughout June and July (Table 12). These other copepod species were only consumed until June 19, 2017 (Table 12).

Table 12. Gut contents of 284 feeding stage striped bass larvae expressed as mean daily relative abundance (%) from June 13 to July 20, 2017. N is the number of larvae analyzed per size category.

Date	Total length (mm)	N	Ectino.	Juvenile Mysid	Amphi-pod	Daphnia	Striped bass	Digested	No gut contents	*Pseudo.*	Unkno-wn eggs	*Coullan.*	*Diacycl.*
June 13	6.0-6.9	3	100	0	0	0	0	0	0	0	0	0	0
	7.0-7.9	6	75	17	0	0	0	0	0	8	0	0	0
	8.0-8.9	1	100	0	0	0	0	0	0	0	0	0	0
June 15	6.0-6.9	14	100	0	0	0	0	0	0	0	0	0	0
	7.0-7.9	84	95	2	0	1	0	0	0	0	2	0	0
	8.0-8.9	2	75	0	0	0	25	0	0	0	0	0	0
June 19	6.0-6.9	11	82	0	0	9	0	0	0	0	9	0	0
	7.0-7.9	52	94	0	0	0	0	0	0	0	2	0	4
	8.0-8.9	4	75	0	0	0	0	0	0	0	0	25	0
June 23	6.0-6.9	3	100	0	0	0	0	0	0	0	0	0	0
	7.0-7.9	12	100	0	0	0	0	0	0	0	0	0	0
	8.0-8.9	1	100	0	0	0	0	0	0	0	0	0	0
June 26	7.0-7.9	5	0	60	20	0	0	20	0	0	0	0	0
June 30	7.0-7.9	1	100	0	0	0	0	0	0	0	0	0	0
	8.0-8.9	2	50	50	0	0	0	0	0	0	0	0	0
	9.0-15.0	4	100	0	0	0	0	0	0	0	0	0	0
	15.1-20.0	2	0	0	0	0	0	100	0	0	0	0	0
July 6	7.0-7.9	1	100	0	0	0	0	0	0	0	0	0	0
	8.0-8.9	1	0	0	0	0	0	100	0	0	0	0	0
	9.0-15.0	14	82	4	0	0	0	14	0	0	0	0	0
	15.1-20.0	5	0	40	0	0	40	20	0	0	0	0	0
	20.1-25.0	4	0	63	0	0	38	0	0	0	0	0	0
July 7	9.0-15.0	6	33	50	0	0	0	17	0	0	0	0	0
	20.1-25.0	5	15	85	0	0	0	0	0	0	0	0	0
July 13	9.0-15.0	11	64	9	9	0	0	18	0	0	0	0	0
	15.1-20.0	2	0	50	0	0	0	50	0	0	0	0	0
	20.1-25.0	1	0	100	0	0	0	0	0	0	0	0	0
July 19	9.0-15.0	6	0	0	50	0	0	33	17	0	0	0	0
	15.1-20.0	1	0	0	0	0	0	100	0	0	0	0	0
July 20	9.0-15.0	10	0	30	10	0	0	0	60	0	0	0	0
	15.1-20.0	7	0	57	29	0	0	14	0	0	0	0	0
	20.1-25.0	2	0	50	50	0	0	0	0	0	0	0	0

Table 13. Mean daily relative abundance (%) of zooplankton present in the water column based on samples taken concurrently with feeding stage striped bass larvae from June 13 to July 20, 2017. N is equal to the number of tows with 199 analyzed in total.

Date	**N**	***Acartia tonsa***	***Diacyclops bicuspidatus***	***Pseudodiaptomus pelagicus***	***Coullana canadensis***	**Ectinoso-matidae**
June 13	6	0	35	0	45	20
June 15	22	0	0	98	0	2
June 19	14	1	34	41	16	9
June 23	12	52	0	48	0	0
June 26	35	54	0	44	2	0
June 30	21	6	8	22	46	18
July 6	27	21	10	18	48	3
July 7	6	24	11	34	24	7
July 13	15	0	2	24	50	24
July 19	18	21	2	15	44	18
July 20	23	72	0	27	1	0

5.8 Discussion: Striped Bass Spawning

The spawning of striped bass in the Shubenacadie-Stewiacke River Estuary in late May to late June corresponded with previously published data of the timing of spawning in the estuary (Rulifson and Dadswell, 1995; Rulifson and Tull, 1999; Duston et al., 2018). Eggs were detected until the end of June in 2016 and 2017 (Figure 35, 36). The episodic spawning of striped bass in Chesapeake Bay, the mid-range of the species population along the Eastern Seaboard, persists for up to six weeks (Setzler-Hamilton et al., 1981). Similarly, the episodic spawning in the Shubenacadie-Stewiacke River Estuary persisted approximately six weeks (Figure 35, 36). The first large spawning occurred in the Shubenacadie between May 16 and June 1 in 7 of 9 years between 2008 and 2016 (Duston et al., 2018). Spawning occurred after an accumulation of 11-20 degree-days above 12°C (Duston et al., 2018). In 2016, eggs were first detected May 20 at 13.9°C while eggs were first detected in 2017 earlier on May 15 and at a lower temperature of 12.0°C. In 2017, the largest spawning occurred at a higher temperature and a lower salinity than 2016 with a greater egg density of 73%. The first large spawning episode in 2017 occurred at 15.4°C.

The high abundances (eggs/m^3) of striped bass eggs in 2016 and 2017 in the Shubenacadie River Estuary greatly surpassed the recorded abundances of the stratified nursery habitats of the Eastern Seaboard and was similar to all years studied 2008 onwards (Duston et al., 2018). Egg densities were recorded at 0.7/m^3 in the Miramichi River (Robichaud-LeBlanc et al., 1996) and 0.1-12.0/m^3 in four major estuaries in the US (Setzler-Hamilton et al., 1994; Van den Avyle and Maynard, 1994; Bilkovic et al., 2002). The high abundances of striped bass eggs in the Shubenacadie River in 2016 and 2017 were of a similar order of magnitude to the recorded densities of 1562/m^3 on June 1, 2008, and 17,729/m^3 on May 17, 2012 (Duston et al., 2018). The highest recorded spawning episode occurred on May 17, 2012 and was estimated as 14.3×10^9 eggs through the ebb tide in the main channel of the river (Duston et al., 2018). The highest abundances of eggs in the Shubenacadie River Estuary occurred between 1.0 and 5.0 ppt and was lowest in tidal freshwater (≤0.15ppt) and above 10.0ppt (Duston et al., 2018). In 2016 and 2017, the highest abundances of eggs occurred at 9.0 and 6.7ppt, respectively (Figure 35, 36). In contrast, in the Miramichi River Estuary and Chesapeake Bay, eggs densities were highest in tidal freshwater of less than 0.15ppt (Setzler-Hamilton et al., 1981; Robichaud-LeBlanc et al., 1996; Secor et al., 2017).

5.9 Discussion: Distribution of Striped Bass Larvae

The peak abundance of non-feeding larvae of both Canadian populations occurred later in the spring than the Potomac Estuary, the second largest tributary of Chesapeake Bay. Spawning began in the Potomac Estuary during the second week of April and peaked at the end of the month (Setzler-Hamilton et al., 1981). Non-feeding stage larvae were detected in the Shubenacadie River Estuary in 2016 and 2017 during the last week of May until the end of June (Figure 37). Non-feeding larvae were captured in the plankton net in 2016 two days after eggs were first detected in the estuary. Eggs hatch approximately 2-3 days after fertilization and transition to the first feeding stage by 5-10 dph (Mansueti, 1958; Bayless, 1972; Secor and Houde, 1995; Robichaud-LeBlanc et al., 1998; Douglas and Chaput, 2001). The greatest spawning in 2016 and 2017 occurred at 14.8 and 15.4°C and eggs can hatch within 70-74 hours at 14.4-15.6°C (Merriman, 1941). In 2016, the greatest spawn (Figure 37) might be directly responsible for the peak in non-feeding stage larvae as the temperature in the upper estuary ranged between approximately 15 and 18°C (Figure 12). In the Miramichi River Estuary, early yolk-sac larvae were collected from May 31 to June 2 in 1992 with a peak abundance of 0.61/m^3 two days after the peak egg density (Robichaud-LeBlanc et al., 1996). The peak abundance of late yolk-sac larvae was 0.86/m^3 and full absorption of the yolk-sac was not evident before June 14, 1992 (Robichaud-LeBlanc et al., 1996). In the Potomac Estuary the highest density (IND/m^3) of yolk-sac larvae was 8.9/m^3 during the first week of May in 1977 (Setzler-Hamilton et al., 1981). Similarly, in the Shubenacadie, the mean peak abundance of non-feeding stage larvae during the week of May 22 to 28 in 2016 and 2017 reaching 1113/m^3 and 58/m^3, respectively.

The nursery habitat of non-feeding stage larvae in Chesapeake Bay differed from the Canadian populations of the Shubenacadie and Miramichi Rivers (Setzler-Hamilton et al., 1981; Shideler and Houde, 2014). The highest abundances of non-feeding stage larvae in the Shubenacadie and Miramichi River Estuaries occurred at higher salinities than the larvae present in the stratified estuaries of Chesapeake Bay. Non-feeding stage larvae are anatomically incapable of feeding due to the lack of mouth development and rely on the endogenous nutrition of the yolk sac and oil globule (Eldridge et al., 1981; Rogers and Westin, 1981). Early yolk-sac larvae were present in fresh water with low densities at of 1.0-5.0ppt in the Miramichi River Estuary (Robichaud-LeBlanc

et al., 1996). In the Miramichi, late yolk-sac larvae were present over a wider range of salinities from 1.0-10.0ppt and only 29% were present in full fresh water (Robichaud-LeBlanc et al., 1996). Non-feeding stage larvae in the Shubenacadie were captured from tidal freshwater to 20.0ppt in 2016 and 2017 (Figure 39). The presence of larvae in tidal freshwater is unusual. Larvae were highly concentrated between 1 and 10ppt and decreased significantly below and above this range in the Shubenacadie River between 2008 and 2018 (Duston et al., 2018). In the Miramichi, larvae in June with a mean TL of 5.7mm were present from 0.0-5.0ppt where the initial fresh and saltwater mixing occurred (Robichaud-LeBlanc et al., 1998). In the Patuxent, by contrast, yolk-sac larvae occurred most frequently up-estuary of the salt front in tidal freshwater of less than 2.0ppt in the Patuxent River in Chesapeake Bay in 2000-2001 (Campfield and Houde, 2011).

The nursery habitat of striped bass larvae in the Shubenacadie River Estuary differs markedly from other major nursery habitats along the Eastern Seaboard. The distribution of striped bass larvae in Chesapeake Bay and the Miramichi River Estuary were primarily located up-estuary in freshwater above the salt wedge (Robichaud-LeBlanc et al., 1996, 1998; Campfield and Houde, 2011). The salt front of 0.15ppt in the Shubenacadie River Estuary acts as a physiological boundary for striped bass eggs and larvae (Duston et al., 2018). The nursery habitat of 0.16-15.0ppt moved up and down each tidal cycle over several kilometers within the estuary (Duston et al., 2018). The nursery habitat in the Shubenacadie River Estuary was a salinity distribution of 0.16-25.0ppt in 2016 and 2017 rather than a fixed location.

The peak magnitude and timing of feeding stage larvae in the Shubenacadie River Estuary in 2016 and 2017 were similar to previously recorded years. The peak mean abundance (IND/m^3) of larvae from 2010 to 2011 ranged from 200-500/m^3 on the ebb tide at 25.0rkm (MacInnis, 2012). The peak abundances of larvae occurred between May 22 and June 19 in multiple years of study and reached 1010/m^3 in 2012 (Duston et al., 2018). In the Miramichi River Estuary, the peak abundance (IND/m^3) of post yolk-sac larvae was recorded as 0.17/m^3 in 1992 (Robichaud-LeBlanc et al., 1996). In the early 1990s, the population of the Miramichi River Estuary was very low but has since rebounded and deserves a re-assessment of the abundance of early life stages. The abundance of feeding stage larvae in the Shubenacadie River Estuary greatly exceeded those in the Miramichi River Estuary in 1992. The peak density of eggs in 2016, 1842/m^3, resulted in a peak abundance

of 463/m^3 feeding stage larvae. The peak density of eggs in 2017, 2523/m^3, resulted in a peak abundance of 595/m^3 feeding stage larvae. The peak abundances of larvae could not be assigned to specific cohorts because of multiple spawning episodes and mixed together as they were passively transported by the tide throughout the estuary (Duston et al., 2018). Two to four large spawning episodes typically occur within each year with several smaller events in the Shubenacadie River Estuary (Duston et al., 2018).

The 2016 and 2017 cohorts of striped bass experienced an approximate 4-fold decrease in abundance within the estuary within the first month post-hatch. Striped bass eggs may be subject to advection into Cobequid Bay and experience exposure to potential predation, become stranded on mud flats, and exposed to high salinity (Duston et al., 2018). It is difficult to determine if the decrease in the abundance of larvae is due to mortality or dispersal from the main channel of the river. However, if the decrease in abundance was associated with an increase in body size and appearance of suitable prey, then dispersal is a more realistic explanation than mortality.

In the Miramichi River Estuary, feeding stage larvae were collected from June 14 to 25, 1992, and zero larvae were captured after June 25 in the plankton net with a mesh size of 500µm (Robichaud-LeBlanc et al., 1996; 1998). Similarly, in the Shubenacadie, larvae at the same developmental stage were not captured in the main channel with the plankton net after the end of June to mid-July in 2016 and 2017 (Figure 37, 38). No juvenile stage striped bass were collected in plankton net samples in the Miramichi or in the Shubenacadie River Estuary (Robichaud-LeBlanc et al., 1996, 1998). The largest larvae collected in the plankton net samples was 9.4mm TL in the Miramichi (Robichaud-LeBlanc et al., 1998). I speculate larvae greater than 30.0mm are transitioning into juveniles and have better swimming ability that were capable of dispersing from the main channel of the Shubenacadie River Estuary.

The abundance of feeding stage larvae was independent of salinity in 2016 and 2017 and were present in tidal freshwater to 25.0ppt (Figure 40). Suitable salinities for larvae in the Shubenacadie River Estuary ranged from 0.15 to 15.0ppt (Duston et al., 2018). In the Miramichi River Estuary, by contrast, post yolk-sac larvae were collected up-stream of the edge of the salt wedge with 94.3% present in fresh water (Robichaud-LeBlanc et al., 1996). In July, the striped bass population, with

a mean TL of 25.6mm, were captured in the middle of Miramichi River Estuary in 11.0-15.0ppt with the majority still present in fresh water in the upper estuary (Robichaud-LeBlanc et al., 1998). In the Patuxent River of Chesapeake Bay, the majority of feeding stage striped bass larvae and small juveniles with a TL of less than 75mm were located within or up-estuary of the salt front, 0.2-1.0 ppt, in 2000-2001 (Campfield and Houde, 2011). No striped bass eggs or larvae were collected in water with salinities greater than 1.0ppt in 1988-1989 in the Pamunkey River, Virginia (McGovern and Olney, 1996). The Miramichi, Patuxent, and Pamunkey Rivers are classified as stratified estuaries unlike the Shubenacadie.

Striped bass larvae from the Shubenacadie-Stewiacke Rivers exhibited survival at high salinities during laboratory testing trials (Cook et al., 2010). The highest survival of larvae ten days post hatch (dph) of 90% or more occurred at 10.0ppt at 21 and 26ºC. Larvae reared from 10-17dph survived across salinities of 1.0-35.0ppt at temperatures of 16 and 21ºC. Larvae reared from 10-17dph survived from 1.0 to 30.0ppt at 26ºC (Cook et al., 2010). Feeding stage larvae were not present in the water column after the salinity category of 20.1-25.0ppt in 2016 (Duston et al., 2018). Duston et al (2018) suggested that mysids, *Neomysis americana*, consumed much of the copepod prey needed by striped bass less than 10.0mm TL during the early feeding stages in the Shubenacadie River Estuary. It was speculated that mysids out-compete striped bass larvae for zooplankton prey at salinities greater than 20.0ppt. I speculate that the salinity tolerance, or distribution relative to salinity, and the predator-prey interaction of striped bass larvae, zooplankton, and mysids overlapped in the higher salinity of the estuary. Mysids may indirectly reduce the quality of striped bass larvae habitat by consuming too many copepods. The predation of copepods by mysids exceeded the predation of larval fish upon copepods in Lake Ontario during the spring and summer months (Gal et al., 2006). Mysids were historically introduced to lake ecosystems as prey for larval fish, however, the mysids affected the zooplankton species composition and decreased the growth rates of zooplanktivorous fish species (Gal et al., 2006). The deprivation of copepod prey for feeding stage larvae in the Shubenacadie River Estuary in higher salinities may have contributed to the predation of ectinosomatids by mysids. Mysids change from being a possible threat of first feeding striped bass larvae in the Shubenacadie River Estuary to a prey item of larvae. Juvenile mysids were consumed by larvae 15.1-25.0mm in 2016 (Table 8) and 7.0-25.0mm in 2017 (Table 9).

5.10 Discussion: Identification of Potential Zooplankton Prey Item Species and Gut Contents of Larvae

5.10.1 The Transition of Striped Bass Larvae from Endogenous to Exogenous Nutrition

The transition of striped bass larvae in the Shubenacadie River Estuary from endogenous to exogenous nutrition was indicated by the presence of food in the gut or by body size exceeding 6.0mm TL. Based on the criteria, feeding in 2016 and 2017 commenced June 6 and June 13, respectively (Table 8, 9). The transition from endogenous to exogenous feeding of striped bass larvae in the Miramichi River Estuary correlated spatially and temporally with a peak in the abundance of prey taxa as the temperature within the estuary increased (Robichaud-LeBlanc et al., 1997). Striped bass larvae at 6.0mm TL in the Shubenacadie River Estuary in 2016 and 2017 were characterized by fully pigmented eyes and developed mouths. Larvae were anatomically capable of feeding at 6.0 to 7.0mm TL. First feeding larvae were reported at 5.0mm TL in the Shubenacadie River Estuary as described by Duston et al. (2018). However, larvae analyzed in 2016 and 2017 did not begin to feed until 6.0mm TL. The smallest striped larvae with food in their guts were 5.0mm TL in the Potomac Estuary of Chesapeake Bay while larvae under 5.0mm appeared incapable of consuming prey due to poorly developed intestinal tracts and mouth parts (Beaven and Mihursky, 1980). From approximately 3.5mm TL after hatching to 5.9mm TL, non-feeding stage larvae transitioned from the yolk-sac stage to the feeding stage in the Shubenacadie River Estuary. Eighty-nine percent of larvae under 5.0mm TL had empty stomachs in the Miramichi River Estuary (Robichaud-LeBlanc et al., 1997). Similarly, striped bass larvae ranging from 3.5-5.9mm TL in the Shubenacadie River Estuary were incapable of consuming prey due to the lack of developed mouth parts.

The oil globule was still present in some larvae at 6.0mm TL in the Shubenacadie River Estuary in 2016 and 2017 but the yolk-sac was fully absorbed. Likewise, feeding larvae from the Nanticoke River, Maryland, stock did not completely absorb their yolk until 8.0mm TL (Rogers and Westin, 1981). Striped bass larvae rely on the yolk-sac reserves to provide structural materials for development and sustaining its growth after it hatches until it captures its first prey (Rogers and Westin, 1981). The total yolk absorption typically coincides with the onset of first feeding

(Eldridge et al., 1981). Starved striped bass larvae retain the oil globule longer than fed larvae who use the oil energy for feeding and development (Eldridge et al., 1981).

Larvae remained under 9.0mm TL for nearly four weeks after hatching in 2016 and 2017 (Table 8, 9; Figure 42). The total body length of striped bass larvae in the Shubenacadie River Estuary in 2016 and 2017 were measured throughout June and July (Figure 42). Larvae in the estuary developed into the first feeding stage within about five days at 16 to 18°C (Duston et al., 2018). Larvae typically do not grow for up to four weeks post yolk-sac absorption in May-June and the incidence of empty guts is high in the Shubenacadie River Estuary (MacInnis, 2012). The prevalence of empty guts of striped bass larvae in the Shubenacadie River Estuary was hypothesized to be correlated with a lack of suitable sized zooplankton prey. In 2016, larvae 6.0-6.9mm TL persisted in the estuary until June 20 while larvae 7.0-8.9mm TL were present until the end of June (Table 8). In 2017, larvae 6.0-6.9mm TL were present in the estuary until June 23 and larvae 7.0-8.9mm TL were present until July 6 (Table 9). Unfed striped bass larvae from the Nanticoke River stock survived two and a half weeks after hatching at 24.0ºC and upwards of four weeks at 15.0ºC (Rogers and Westin, 1981). The longer striped bass larvae remain small in the estuary, the longer they remain vulnerable and susceptible to predation. Larval fish must find suitable prey after yolk-sac absorption or risk high mortality (Houde, 2008). Striped bass larvae who remained under 9.0mm TL in the Shubenacadie River Estuary may experience slower rates of ossification. Striped bass larvae who were exposed to prolonged periods of starvation experienced slower rates of ossification with no change in cartilage (Eldridge et al., 1981). Striped bass larvae can survive starved for 22 days at 24°C and 32 days at 15°C (Rogers and Westin, 1981). Starved larvae may also experience deterioration and disintegration of cells in the tissues of the digestive tract (Eldridge et al., 1981). Striped bass larvae can continue to grow and develop after exposure to a lack of zooplankton prey after yolk-sac absorption. The oil globule can be conserved when prey is not abundant (Eldridge et al., 1981; 1982; Rogers and Westin, 1981). Larvae can continue to develop and grow once they are exposed to food and are very resistant to food deprivation as demonstrated by long survival times when starved (Eldridge et al., 1981; Rogers and Westin, 1981).

5.10.2 Striped Bass Larval Gape Limitation

There is no published literature describing the gape size of larval striped bass or the likelihood of gape limitation. Striped bass larvae in the Shubenacadie River Estuary can be considered gape-limited zooplanktivores making them reliant on a single species of small harpacticoid until reaching about 9.0mm TL in 2016 and 2017. At this point, a shift in the diet occurred with larger prey items, larger copepods, amphipods and mysids, becoming more abundant in the gut contents (Table 8, 9). In the Miramichi, by comparison, striped bass larvae switched from smaller, immature calanoid copepods to adults after attaining 8.0mm TL two weeks after the onset of active feeding (Robichaud-LeBlanc et al., 1997).

Gape-limited larval predators are initially restricted to small zooplankton prey such as white crappies and gizzard shad (Wong and Ward, 1972; DeVries et al., 1998). The maximum prey size of larval white crappies (*Pomoxis annularis*) ranging from 8.0 to 10.0mm TL was typically less than 0.75mm TL. The mean prey size of larval white crappies also increased with body length. Gizzard shad (*Dorosoma cepedianum*) were restricted by their gape size to larger body lengths of 12.0-17.0mm TL in comparison to white crappies who were restricted until 10.0mm TL (DeVries et al., 1998). Larval striped bass and white crappies were gape-limited until roughly the same body length while all three species were classified as zooplankton predators who swallow prey whole. The gape size of yellow perch (*Perca flavescens*) limited the size of *Daphnia pulicana* that could be consumed until larvae reached 18.0mm TL (Wong and Ward, 1972). The zooplankton abundances may have influenced the gape limitation of striped bass larvae and prey size selection in the Shubenacadie River Estuary since the abundant copepod species were out of the range of the maximum gape size at first feeding.

The data presented here establish the Ectinosomatidae copepod was the only prey item that matched the gape size of first feeding striped bass in the Shubenacadie River Estuary that was suitable for larvae to ingest. The gape size of striped bass larvae limited the maximum prey size that they could consume with ectinosomatids as the only appropriate sized prey item (1.0-1.2mmTL). Larvae at 7.0mm TL in the Shubenacadie River Estuary with a mean gape size of approximately 1.0mm would be incapable of consuming an adult mysid with a carapace length of

4.0-5.0mm. Data from the Miramichi River Estuary and Chesapeake Bay support the general idea that striped bass larvae are non-selective, opportunistic omnivores and will consume whatever they can capture. However, published literature from the Miramichi River and Chesapeake Bay Estuaries failed to consider the gape size of larvae and body sizes of their planktonic prey. *Eurytemora affinis* and *Bosmina longirostris* are important prey in other striped bass nursery habitats such as the Miramichi and Chesapeake Bay because they are small enough to be ingested. *Eurytemora affinis* is approximately 1.2mm TL (Torke, 2001) and *Bosmina longirostris* is less than 0.5mm TL (Zaret and Kerfoot, 1980). *E. affinis* and *B. longirostris* are absent from the Shubenacadie River Estuary. Daphnia sp., cladoceran, was the largest sized food organism found in first feeding striped bass guts in the Potomac Estuary of Chesapeake Bay measuring 1.7mm (Beaven and Mihurksy, 1980). The body size range of daphnids were comparable to the Ectinosomatidae copepod present in the Shubenacadie River Estuary. Other copepods present in the Shubenacadie in June, *Coullana canadensis, Diacyclops bicuspidatus,* and *Pseudodiaptomus pelagicus* appear to be too large to be ingested by first feeding striped bass larvae.

5.10.3 Relative Abundance of Copepods in the Gut Contents Versus Water Column

In 2016, the relative abundance of the Ectinosomatidae copepod in the gut was 67-100% from larvae 6.0-8.9mm TL (Table 10). In 2017, the relative abundance of the Ectinosomatidae copepod was 50-100% from larvae 6.0-8.9mm TL (Table 12). The most abundant prey item of larval striped bass in the Potomac Estuary of Chesapeake Bay were copepods, rotifers, and cladocerans (Beaven and Mihurksy, 1980). Adult and copepodite *E. affinis* and cyclopoid species occurred most frequently in the guts with 40% copepods (mostly *E. affinis*), 26% cladocerans almost exclusively *Bosmina longirostris*, 33% rotifers mainly *Brachionus calyciflorus* (Beaven and Mihurksy, 1980). *A. tonsa*, *C. canadensis*, and *P. pelagicus* were relatively abundant copepods in the water column in 2016 and 2017. The relative abundance of copepods in the water column did not match with the species abundance in the gut. In contrast, the relative abundance of copepods in late summer matched in time with the number of copepods per gut in yellow perch in Oneida Lake, New York (Hansen and Wahl, 1981).

The dominant prey item across all body size categories from 6.0 to 15.0mm TL was the Ectinosomatidae copepod. Ectinosomatids were consumed by larvae with gape sizes larger than 1.0mm who were capable of consuming larger copepods. Larval white crappies consumed larger prey as gape increased but continued to select prey below their prey limitation when larger prey was available (DeVries et al., 1998). Larvae may consume prey smaller than the maximum gape size and may select smaller prey than those also captured in the same plankton net tow (Hansen and Wahl, 1981; Michaletz et al., 1987). Daphnids present in the guts of yellow perch were significantly smaller than the daphnids present in the plankton net samples (Hansen and Wahl, 1981). In the Shubenacadie, *A. tonsa*, *C. canadensis*, and *P. pelagicus* were abundant in the water column at the same location and time as larvae with ectinosomatids in the gut but did not necessarily mean that they were prey of larvae or that larvae could capture and consume them whole.

5.10.4 Relationship Between the Ectinosomatidae Harpacticoid Copepod and Striped Bass Larvae

The Ectinosomatidae harpacticoid copepod species was the dominant prey item of feeding striped bass larvae at first feeding in June and July in the Shubenacadie River Estuary in 2016 and 2017. No previous studies have reported a harpacticoid species as a principal initial prey item of first feeding stage striped bass larvae. I first detected the harpacticoid species in the guts of first feeding striped bass in 2016, whereas other students in past years did not record it. Between 2014 and 2016 it was assumed to be a naupliar stage of *C. canadensis* (Carolyn Martin, pers. comm. June 2016). Gravid ectinosomatid specimens were captured in 2017. MacInnis (2012) suggested *E. affinis* was the prey, possibly influenced by its ubiquity in other striped bass nursery habitats. This suggestion has proved incorrect. *E. affinis* was not detected in the gut of larvae either by me, nor by surveys from 2013 onwards.

Striped bass larvae in other major nursery habitats along the Eastern Seaboard predominantly consumed calanoid copepods and cladocerans at first feeding. In the Miramichi River Estuary, striped bass larvae less than 15.0mm TL consumed immature and adult copepods, primarily *Eurytemora* species, while larvae 15.0-25.0mm TL consumed adult calanoid copepods (Robichaud-LeBlanc et al., 1997). Ninety-two percent of gut contents of larvae 8.0-10.0mm TL

were calanoid copepodites, mainly *E. affinis* in the Upper Chesapeake Bay in 1998-1999 (North and Houde, 2006). Adult stage *E. affinis* and *Acartia tonsa* were the dominant prey items of striped bass larvae in the Patuxent River of Chesapeake Bay in 2000-2001 down estuary of the salt front in higher salinity (Campfield and Houde, 2011). *Bosmina longirostris* was the primary prey item of larvae 9.0-11.0mm TL in the salt front and up-estuary in freshwater (Campfield and Houde, 2011). The primary prey of feeding stage striped bass larvae (6.0-9.0mm TL) of the Upper Chesapeake Bay in 2007-2008 was *E. affinis* and *B. longirostris* (Shideler and Houde, 2014). Larvae amongst these nursery habitats were never recorded as consuming a harpacticoid copepod species at first feeding much less as a harpacticoid as the dominant prey item.

In Chesapeake Bay, the mean number of prey per larvae increased with respect to total body length and 0.51prey/mm and 0.35prey/mm in 2007 and 2008, respectively (Shideler and Houde, 2014). In other words, the mean number of prey per gut in a 7.0mm TL larvae could range from 2.5 to 3.6/gut. Similarly, the mean number of prey items per gut increased as body length increased in the Shubenacadie River Estuary from 1.0 to 3.2/gut in 2016 (Table 8). Other minor prey items of feeding larvae in the Upper Chesapeake Bay included *Acartia* copepod, detrital particles, unattached copepod eggs, and unidentifiable material (Shideler and Houde, 2014). *Acartia tonsa* was dismissed as a potential prey item of first feeding stage striped bass larvae in the Shubenacadie River Estuary as they are not abundant in the water column until August (Figure 31). *B. longirostris* was mostly absent in the stomach contents of larvae down estuary of the salt front in higher salinities in Chesapeake Bay as it is predominantly a freshwater species. The highest incidence of feeding occurred within (66%) and up-estuary (65%) of the salt front (Shideler and Houde, 2014). The eutrophic waters of the upper estuary of Chesapeake Bay are highly productive and provide adequate nutrients to support the growth and development of larval striped bass. Whereas the tidal freshwater of the Shubenacadie River Estuary is oligotrophic, and the abundance of zooplankton is very low compared to brackish water in the estuary (Mudroch et al., 1987). Striped bass larvae were absent in tidal freshwater in the Shubenacadie River Estuary (Duston et al., 2018).

The absence of *Eurytemora affinis* in the water column of the Shubenacadie River Estuary and as a prey item of feeding stage striped bass larvae contrasts to all other striped bass nursery habitats along the Eastern Seaboard. I speculate the absence of *E. affinis* in the Shubenacadie River Estuary

is correlated with a lack of an estuarine turbidity maximum (ETM) region and that the environmental conditions of the macrotidal estuary are unsuitable. *E. affinis* populations reach maximum abundance in ETMs of Chesapeake Bay (Lloyd et al., 2013). The Shubenacadie River Estuary lacks an ETM region due to the tidal dynamics created by the tidal bore. The salt front in Chesapeake Bay is associated with the ETM and entraps suspended particles such as sediment and zooplankton, in the upper portion of the bay (Roman et al., 2001). The entrapped particles also included detritus, protozoa, and phytoplankton, which served as food for zooplankton. *E. affinis* was concentrated and dominated the ETM of Chesapeake Bay as they thrived on the high suspended particles in low salinity. The maximum concentration of zooplankton in the Chesapeake Bay ETM exceeded 200 copepods/L (Roman et al., 2001). Abiotic factors including predation, advection, and temperature are suggested to limit the populations of *E. affinis* in Chesapeake Bay (Lloyd et al., 2013). The egg production started to decrease when temperatures exceeded 18°C and may explain the switch of copepod abundance structure from *E. affinis* to *A. tonsa* in the summer (Lloyd et al., 2013).

Eurytemora affinis is also a dominant zooplankton species in the Gironde Estuary of France (David et al., 2005). The Gironde Estuary is one of the most turbid estuaries in Europe and concentrations have reached 1g/L at the upper limit of the salinity intrusion in the estuary (Sautour and Castel, 1995; David et al., 2005). *E. affinis* was present in salinities of 0.0-6.0ppt in the Gironde Estuary and became entrapped in the ETM region of the Gironde Estuary similar to E. *affinis* in Chesapeake Bay (Sautour and Castel, 1995; Gasparini and Castel, 1997; David et al., 2005). It appears unlikely that turbidity is the factor affecting the low abundance of *E. affinis* in the Shubenacadie River Estuary. The absence of an ETM in the Shubenacadie River Estuary is a consequence of the macrotidal high velocity and completely mixed water column. These conditions may be an unsuitable environment for *E. affinis* since it typically thrives in the oligohaline zone close to an ETM and perhaps it is the large changes in salinity that it cannot tolerate.

5.10.5 Match-Mismatch of Copepods and First Feeding Striped Bass Larvae

The dominant copepod species of the estuary, *Acartia tonsa*, *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus*, and striped bass larvae experienced matches and mismatches in distributions relative to time of year and salinity as well as body size of copepods to gape size of larvae. In order to have successful feeding, growth, and survival, the distributions of feeding stage striped bass larvae and copepod prey must align in abundance relative to time of year and salinity in the estuary. Therefore, larvae need food at the correct time of year and salinity distribution in the Shubenacadie River Estuary. To test the Match-Mismatch Hypothesis, the peak abundance of each copepod species was compared to the abundance of larvae relative to time of year and salinity. The body size of the copepods was also compared to the gape size of larvae to determine if they could possibly be consumed at first feeding.

A temporal mismatch occurred between *Acartia tonsa, Pseudodiaptomus pelagicus,* and first feeding striped bass larvae in the Shubenacadie River Estuary. *A. tonsa* was of suitable size for consumption at first feeding reaching body lengths of approximately 1.0mm. However, the peak abundance of *A. tonsa* in the estuary occurred from August 28-September 3 (Figure 25) while the peak abundance of first feeding striped bass larvae occurred from May 30 to June 25 in 2016 and 2017. The peak abundance of *P. pelagicus* in the Shubenacadie River Estuary also occurred in late summer from August 14-20 (Figure 29). A temporal match occurred between *Coullana canadensis*, *Diacyclops bicuspidatus*, and first feeding striped bass larvae. The peak abundance of *C. canadensis* in the estuary occurred from July 17-23 while the peak abundance of first feeding striped bass larvae occurred from May 30 to June 25 in 2016 and 2017. However, the second highest peak of *C. canadensis* ($101/m^3$) and occurred June 26-July 2 and overlapped in time with striped bass larvae in the estuary (Figure 26). The peak abundance of *D. bicuspidatus* in the estuary occurred from June 5-11 (Figure 27).

The abundance of striped bass larvae in the Shubenacadie River Estuary peaked in salinities of 1.1 to 10.0ppt in 2016 and 2017 (Figure 40). Mismatches of peak abundances relative to salinity occurred between striped bass larvae and copepods. The peak abundance of *Acartia tonsa* in the Shubenacadie was concentrated between 25 and 30ppt (Figure 30). A spatial mismatch in

distribution occurred between *A. tonsa* and first feeding striped bass larvae in Chesapeake Bay as *A. tonsa* resided in higher salinities whereas larvae were present in the ETM above the salt wedge (Campfield and Houde, 2011). The peak abundance of *A. tonsa* in the Patuxent River of Chesapeake Bay was located down-estuary of the salt front at approximately 10ppt (Campfield and Houde, 2011). A temporal and spatial mismatch occurred between first feeding striped bass larvae and *A. tonsa* because the densities did not align according to time of year or salinity. During May 30 and June 25 when the highest density of first feeding larvae was present, the salinity of the estuary at 25.0rkm did not exceed 10ppt and at 36.0rkm did not exceed 5ppt until the end of June in 2016 and 2017 (Figures 11-14). The peak abundance of *Pseudodiaptomus pelagicus* occurred in 20.1-25.0ppt (Figure 34). From 1.1-10.0ppt, *P. pelagicus* ranged from 5-173/m^3 and the densities from 0.00-5.0ppt were significantly lower than the densities at 5.1-30.0ppt (Figure 34). The abundance of *C. canadensis* ranged from 5-70/m^3 in 1.1-10.0ppt (Figure 31) while *Diacyclops bicuspidatus* ranged from 3-12/m^3 (Figure 32).

The gape size of striped bass larvae at first feeding from 6.0 to 9.0mm TL was indicative to the size of prey that could be consumed. The body width of the copepods was not measured during this study. The gape size of first feeding larvae 6.0-6.9mm and 7.0-7.9mm was less than 1.0mm. The gape size of larvae 8.0-8.9mm ranged from <1.0mm to 1.0mm (Figure 41). A mismatch occurred between the body size of *Coullana canadensis*, *Diacyclops bicuspidatus*, and *Pseudodiaptomus pelagicus* relative to the gape size of first feeding larvae. *Coullana canadensis* was considered a potential prey item of first feeding striped bass greater than 8.0mm TL. *C. canadensis* was only recorded in the gut of one larva 8.0-8.9mm TL on June 19, 2017 (Table 12), and larger copepods were recorded in the guts of larvae 9.0-20.0mm in 2016 on July 19 (Table 12). In 2016, *P. pelagicus* was only recorded in the guts of larvae 7.0-7.9mm TL on June 13 (Table 10). In 2017, larger copepods were recorded in the guts of larvae 9.0-20.0mm TL on July 19 (Table 12). The mean body lengths of *C. canadensis, D. bicuspidatus, and P. pelagicus* exceeded the gape size of striped bass larvae in the Shubenacadie River Estuary (Table 6, 7).

Only gravid and non-gravid Ectinosomatidae copepods were of suitable size for ingestion by striped bass larvae ranging from 6.0 to 8.9mm TL, the most critical early life stage. The gape size of larvae 9.0-15.0mm and 15.1-25.0mm ranged from 1.5-2.5mm and 2.0-4.5mm, respectively. *P.*

pelagicus and *C. canadensis* were of appropriate size for larvae 9.0-25.0mm TL. The timing of the increase in abundance of ectinosomatids matched the onset of feeding and growth of striped bass larvae in mid-June to early July in 2017 (Figure 28). This is an important discovery. The abundance of the ectinosomatid aligned in time with the presence of larvae in the estuary during the month of June into July. However, larvae were distributed over a broad range of salinities from 0.0-25.0ppt (Figure 40). Therefore, the abundance of the ectinosomatid only aligned in salinity with striped bass larvae from 0.0-15.0ppt (Figure 33). The gape size of larval striped bass at first feeding, copepod abundance, copepod and larvae distribution matches relative to time of year and salinity, and the copepod body lengths all contributed to the success of first feeding in the Shubenacadie River Estuary.

Larval gape size must match the body size of their copepod prey for successful consumption. Larvae must also match in time of year and salinity profile of potential prey. Mismatches amongst these factors may have detrimental effects upon the growth, development, and recruitment of larval striped bass in the estuary. A spatial mismatch of prey and larvae may lead to negative consequences for development and survival of larvae (Martino and Houde, 2010). Studies of US populations of striped bass suggested that the year-class abundance and recruitment strength was determined mainly during the early larval stages (Goodyear et al., 1985; Secor and Houde, 1995). The growth of striped bass young-of-the-year (age-0) is vitally important for over-winter survival and is dependent on the feeding success. The spawning of striped bass in the Shubenacadie River Estuary in late May to early June coincided with spawning in the Miramichi River Estuary (Robichaud-LeBlanc et al., 1996). Spawning of the two remaining Canadian populations is approximately one month later than spawning in the center of the species' range along the Eastern seaboard, namely Chesapeake Bay (Robichaud-LeBlanc et al., 1996). The period which age-0 striped bass actively feed is shorter in duration and occurred from June to September/October in the Miramichi and Shubenacadie River Estuaries (Robichaud-LeBlanc et al., 1997). A large size attained prior to over-wintering is desirable to survive the harsh winter conditions of these northern estuaries (Robichaud-LeBlanc et al., 1996, 1998).

5.11 Conclusion

The density of striped bass eggs in 2016 and 2017 greatly exceeded the recorded densities of stratified nursery habitats of the Eastern Seaboard. The transition from endogenous to exogenous nutrition was identified by the presence of prey in the gut and by an increase in growth, or body length. The abundance of larvae in 2016 and 2017 experienced a decrease from egg to the first feeding stage. The Ectinosomatidae copepod species was identified as the dominant prey item of first feeding striped bass larvae. The relative abundance of prey items in the gut compared to the water column revealed that even if potential prey is present at the same time and location as larvae, does not mean they are of appropriate size for larvae to consume. Gape limitation was determined to be a limiting factor of the survival and growth of striped bass larvae in the Shubenacadie River Estuary. Striped bass larvae survive at higher salinities in the Shubenacadie River Estuary than other populations including the Miramichi River Estuary and Chesapeake Bay estuaries. To be a potential prey of striped bass larvae, zooplankton must be smaller in body size than the larval gape size and the distributions relative to time of year and salinity must align. The conditions to be a potential prey abide by the Match-Mismatch Hypothesis.

CHAPTER 6: CONCLUSION

The zooplankton structure of the Shubenacadie River Estuary and the prey of first feeding striped bass differed markedly from the stratified estuaries that serve as spawning and nursery habitats along the Eastern seaboard, specifically the Miramichi River and Chesapeake Bay. The success of first feeding larvae was dependent on the distributions of zooplankton relative to time of year and salinity as well as prey body size as they were gape limited. The distributions of potential prey species relative to time of year and salinity affected the feeding success of early life stage striped bass. The potential prey species included *Acartia tonsa*, *Coullana canadensis*, *Diacyclops* bicuspidatus, Ectinosomatidae sp., and *Pseudodiaptomus pelagicus*. First feeding striped bass and the potential prey species experienced matches and mismatches in distributions in the Shubenacadie River Estuary. First feeding striped bass were gape limited and the body sizes of the dominant potential prey surpassed the maximum gape size of larvae 6.0-9.0mm TL and were too large to consume.

The copepod diversity of the Shubenacadie River Estuary contrasted with stratified coastal striped bass nursery habitats along the Eastern seaboard where the calanoid copepod *Eurytemora affinis* and the cladoceran *Bosmina longirostris* were the main first feeding prey. The dominant prey item of first feeding larvae in the Shubenacadie River Estuary was an adult stage harpacticoid copepod species from the Family Ectinosomatidae. The DNA sequencing of the 18S gene of the Ectinosomatidae copepod indicated that it was a potentially new species of harpacticoid copepod. The discovery of the Ectinosomatidae adult stage harpacticoid copepod species shed new light on the feeding ecology of early life stage striped bass larvae in the Shubenacadie River Estuary. The presence of first feeding striped bass matched temporally and spatially with the distributions of the ectinosomatids. The distributions of first feeding striped bass and the ectinosomatids overlapped in time of year and salinity. The body size of the ectinosomatids matched the gape size of first feeding striped bass larvae and was significantly smaller than the dominant copepod species inhabiting the estuary.

Possible reasons the ectinosomatids as the sole, dominant prey included: 1) food present in estuary but too large to consume, 2) food of appropriate size was present but distributions relative to time

of year and salinity differed from larvae, or 3) restricted until approximately 9.0mm TL by gape size. Long-term studying of the feeding ecology and trophic interactions of striped bass early life stages relative to zooplankton in the Shubenacadie River Estuary is valuable for evaluating the survival and recruitment of the striped bass population. In order to successfully capture prey, grow, and survive, larvae must align in time space with potential prey and the body size of prey must not exceed the gape size of larvae. In order to survive the dynamic environment of the Shubenacadie River Estuary, striped bass larvae must find suitable prey to consume during the early life stages or risk starvation.

Future research recommendations include investigating the life cycle of the critically important Ectinosomatidae harpacticoid copepod and the onset of spring zooplankton blooms relative to the distribution of first feeding striped bass larvae. The diet and survival of copepods within the estuary deserves more attention. To determine the diet composition of copepods, the concentrations of suspended sediments, chlorophyll a, and phytoplankton need to be measured throughout the estuary. Future research also includes verifying the taxonomic identify of copepods inhabiting the Shubenacadie River Estuary by comparing the morphological identification and DNA sequencing results.

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APPENDIX A: RAW DATA

Table 14. Mean weekly abundance (IND/m^3) of *Acartia tonsa* captured in the Shubenacadie River Estuary from May to November 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides.

Week	2013	2014	2015	2016	2017
May 1-7					
May 8-14					
May 15-21			6		
May 22-28			33	21	15
May 29- June 4			11	12	3
June 5- 11		3	5	3	4
June 12-18	1	0	7	0	0
June 19- 25	0	0	6	6	6
June 26- July 2	0	0	10	11	7
July 3- 9	0	0	8	0	3
July 10-16	74	156	26	139	3
July 17-23	0	364	0	676	474
July 24-30	151	3,131	1,149	2,793	0
July 31-Aug. 6	65	0	0	6,901	3,663
Aug. 7-13	4,075	7,296	8,015	14,797	2,959
Aug. 14-20	8,776	0	2,135	0	9,717
Aug. 21-27	0	0		0	
Aug. 28- Sept. 3	22,649	26,352		30,286	
Sept. 4-10	0	0		0	
Sept. 11-17	2,390	12,101		4,548	
Sept. 18-24	665	0			
Sept. 25- Oct. 1	189	383			
Oct. 2-8	367	0			
Oct. 9-15	0	677			
Oct. 16-22	210	1,435			
Oct. 23-29	0	0			
Oct. 30-Nov. 5	74	0			
Nov. 6-12		19			

Table 15. Mean weekly abundance (IND/m^3) of *Coullana canadensis* captured in the Shubenacadie River Estuary from May to October 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides.

Week	2013	2014	2015	2016	2017
May 1-7				4	
May 8-14			3	4	2
May 15-21			2	2	2
May 22-28			5	4	3
May 29- June 4			4	6	0
June 5- 11		25	3	44	3
June 12-18	1	174	2	4	3
June 19- 25	10	24	3	41	1
June 26- July 2	55	28	7	21	225
July 3- 9	9	6	20	55	6
July 10-16	8	20	20	3	88
July 17-23	0	21	0	10	103
July 24-30	9	0	12	34	0
July 31-Aug. 6	0	0	0		0
Aug. 7-13	8	11	12		1
Aug. 14-20	7	0	17		8
Aug. 21-27		0			
Aug. 28- Sept. 3		0			
Sept. 4-10		0			
Sept. 11-17		0			
Sept. 18-24		0			
Sept. 25- Oct. 1		9			
Oct. 2-8		0			
Oct. 9-15		0			
Oct. 16-22		38			
Oct. 23-29					
Oct. 30-Nov. 5					
Nov. 6-12					

Table 16. Mean weekly abundance (IND/m^3) of *Pseudodiaptomus pelagicus* captured in the Shubenacadie River Estuary from May to November 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides.

Week	2013	2014	2015	2016	2017
May 1-7				19	
May 8-14			9	5	2
May 15-21		172	13	37	3
May 22-28		67	31	33	16
May 29- June 4	24	82	31	40	20
June 5- 11	13	116	17	4	9
June 12-18	3	175	8	3	10
June 19- 25	14	311	5	5	4
June 26- July 2	64	65	8	24	7
July 3- 9	112	159	4	11	6
July 10-16	529	628	4	204	6
July 17-23	0	599	0	95	54
July 24-30	123	200	28	156	0
July 31-Aug. 6	74	0	0	358	186
Aug. 7-13	295	584	50	280	40
Aug. 14-20	863	0	10	0	112
Aug. 21-27	0	0		0	
Aug. 28- Sept. 3	537	257		142	
Sept. 4-10	0	0		0	
Sept. 11-17	32	624		51	
Sept. 18-24	16	0			
Sept. 25- Oct. 1	52	26			
Oct. 2-8	16	0			
Oct. 9-15	0	70			
Oct. 16-22	12	722			
Oct. 23-29	0	0			
Oct. 30-Nov. 5	189	0			
Nov. 6-12		98			

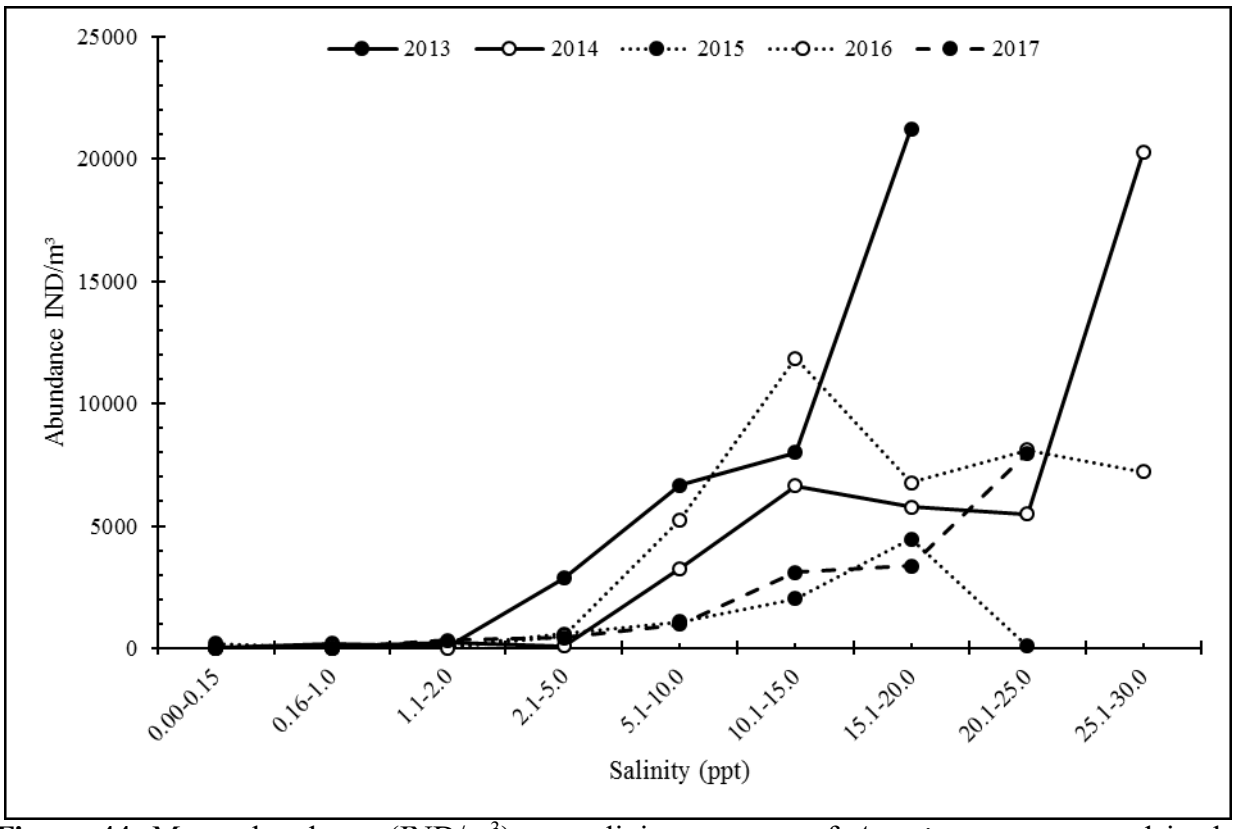

Figure 44. Mean abundance (IND/m^3) per salinity category of *Acartia tonsa* captured in the Shubenacadie River Estuary from May to November 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides.

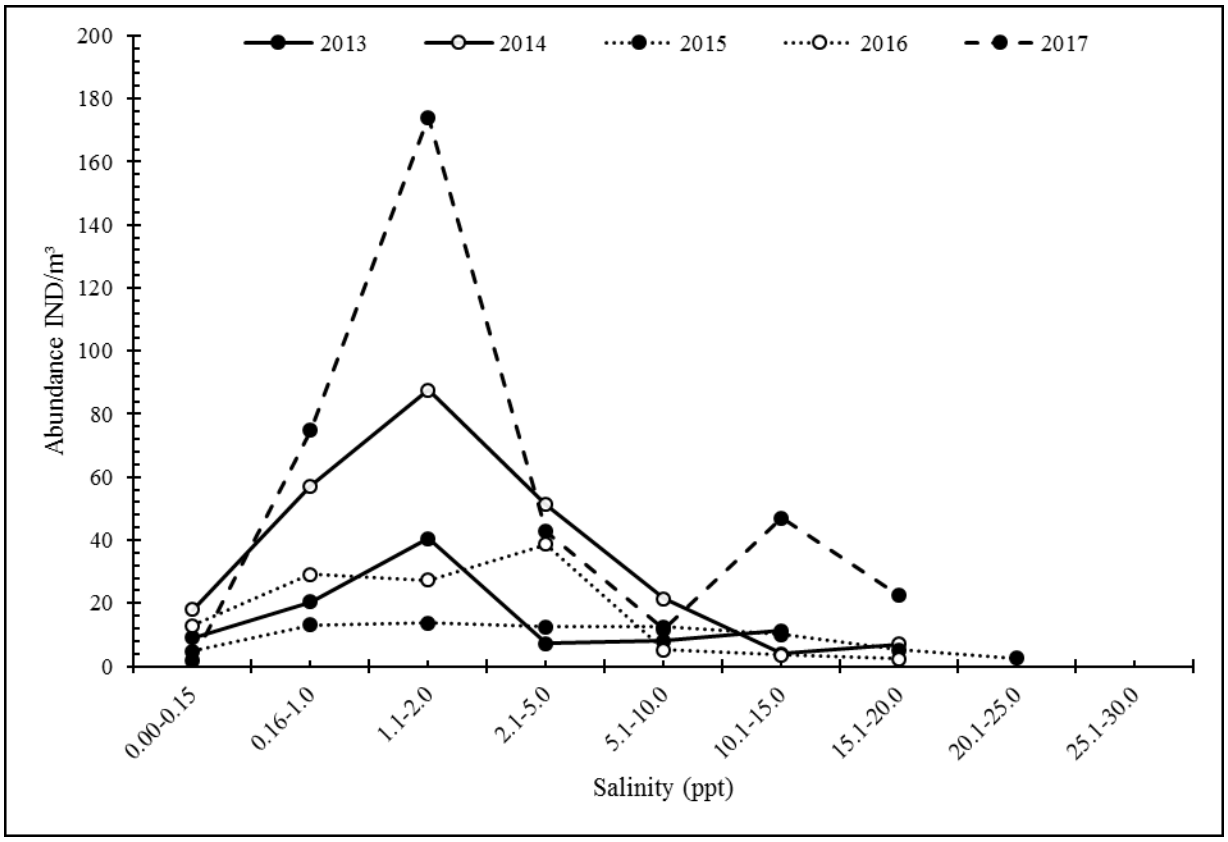

Figure 45. Mean abundance (IND/m^3) per salinity category of *Coullana canadensis* captured in the Shubenacadie River Estuary from May to October 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides.

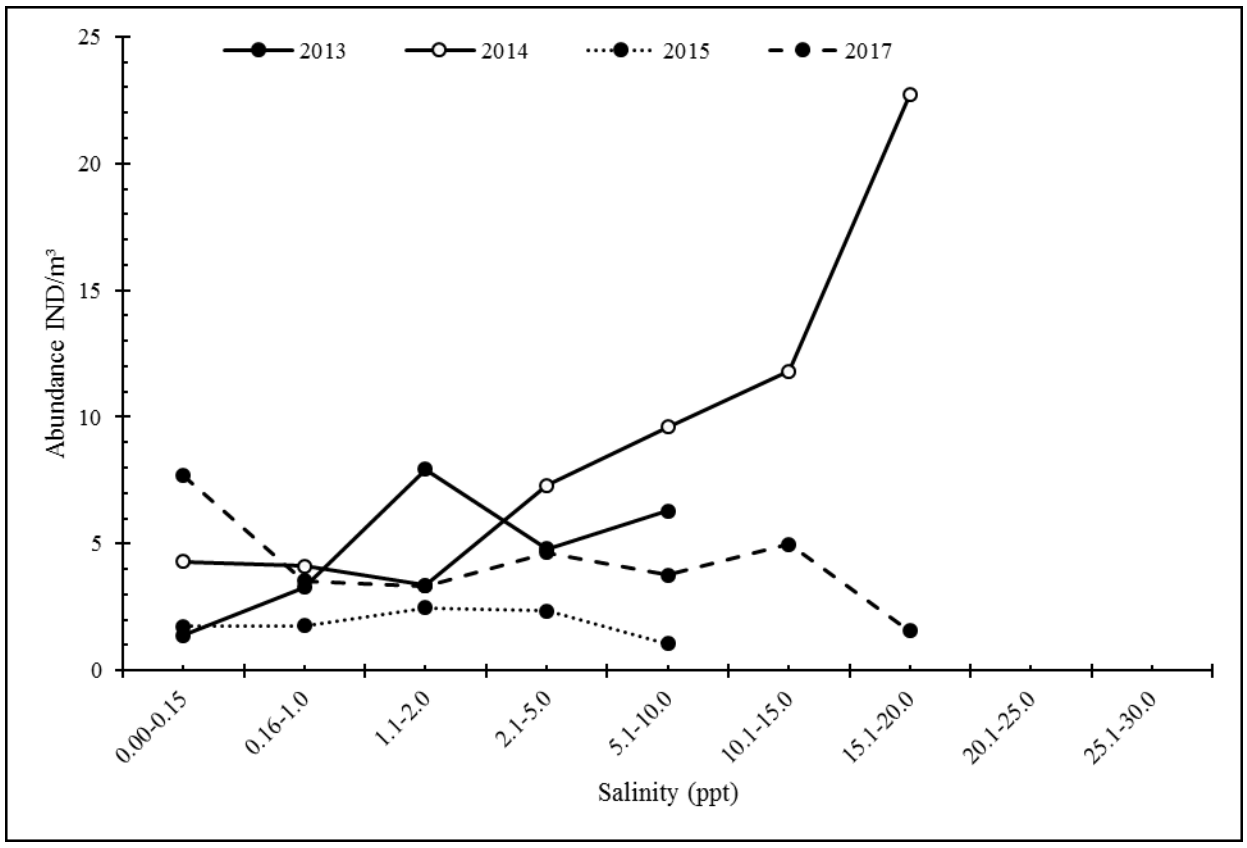

Figure 46. Mean abundance (IND/m^3) per salinity category of *Diacyclops bicuspidatus* captured in the Shubenacadie River Estuary from May to October 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides.

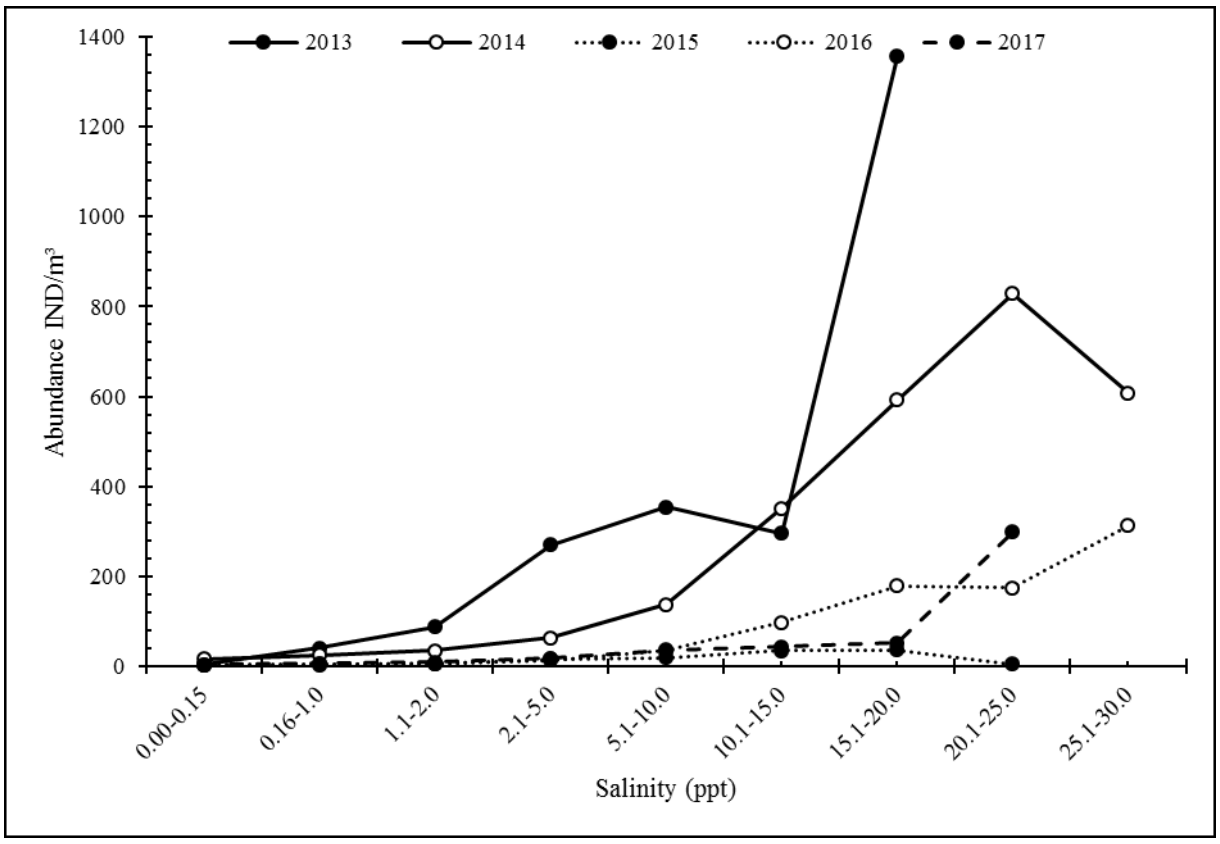

Figure 47. Mean abundance (IND/m^3) per salinity category of *Pseudodiaptomus pelagicus* captured in the Shubenacadie River Estuary from May to November 2013 to 2017. Tows were completed with the 250µm plankton net mesh size from 25.0-41.0rkm on the ebb and flood tides.

APPENDIX B: PHYLOGENETIC ANALYSIS

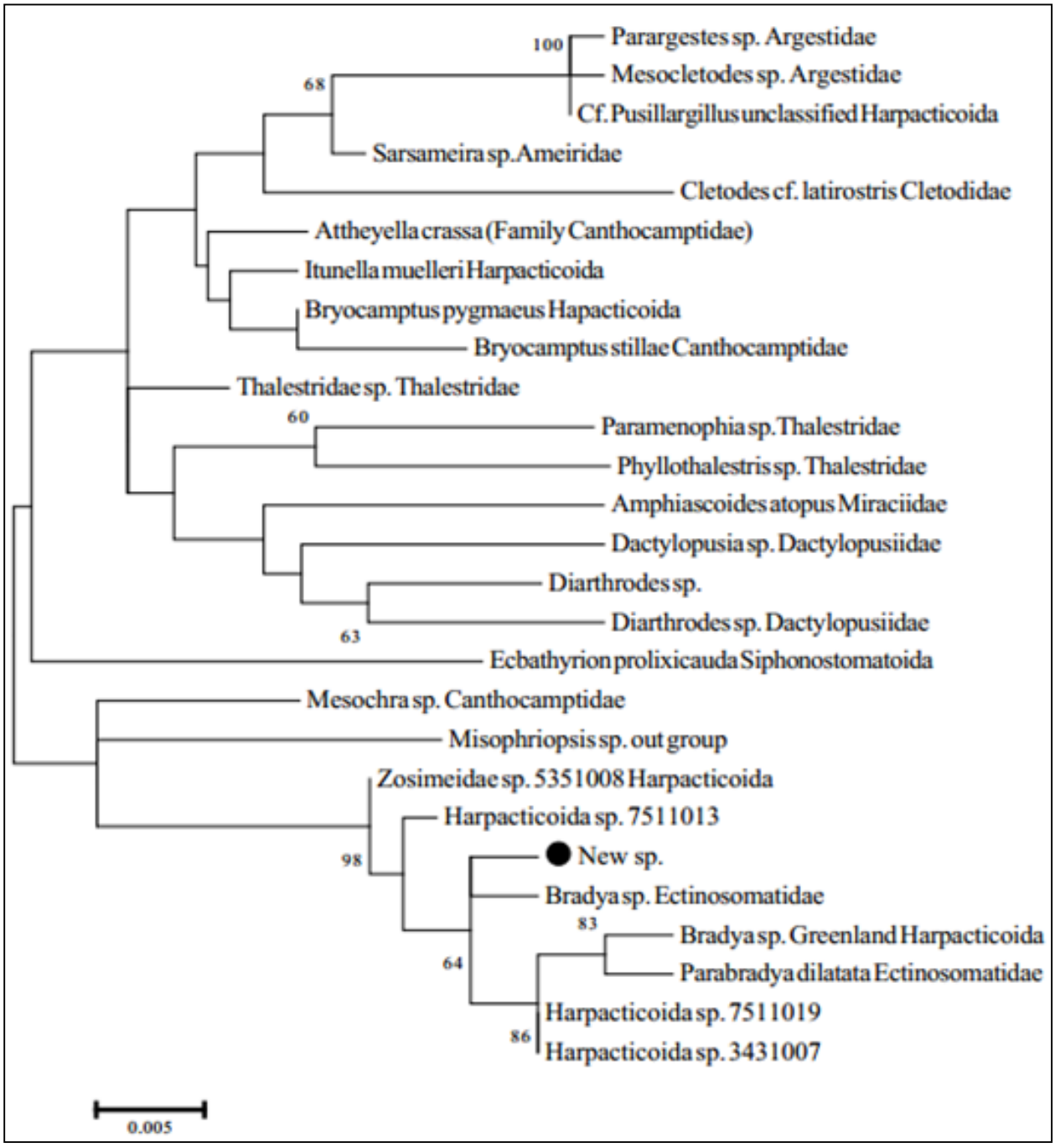

Figure 48. Phylogenetic tree of the 18S rRNA gene sequence with the inclusion of the adult stage Ectinosomatidae copepod species captured in the Shubenacadie River Estuary in 2016 and 2017. Phylogenetic tree created by Dr. Nasif Sarowar, 2018.

APPENDIX C: 150µM RAW DATA

Table 17. Mean weekly abundance (IND/m^3) of the copepod species and striped bass larvae in the Shubenacadie River Estuary from June to August 2017. Tows were completed with the 150µm plankton net mesh size from 27.0-41.0rkm on the ebb tide in the upper estuary. Bongo net was first implemented June 30, 2017. One-way ANOVA statistical analysis of variance, Anderson-Darling test for normality, and Tukey Pairwise comparison was utilized. Mood's Median Test (non-parametric) was used for *Acartia tonsa*. Means that do not share a letter are significantly different.

Week	*Acartia tonsa*	*Diacyclops bicuspidatus*	*Pseudodiaptomus pelagicus*	*Coullana canadensis*	Ectinosomatidae Harpacticoid	Feeding Larvae
18						
19						
20						
21						
22						
23						
24						
25						
26	5 (a)	9 (b)	29 (cd)	300 (e)	207 (f)	50 (g)
27	0	0	0	0	0	0
28	0	4 (b)	19 (d)	65 (e)	39 (f)	29 (g)
29	645 (a)	23 (b)	201 (c)	259 (e)	181 (f)	
30	0	0	0	0	0	
31	15 (a)	4 (b)	17	665 (e)	216 (f)	
32	0	0	0	0	0	
33	3325 (a)	10 (b)	61 (cd)	352 (e)	84 (f)	
34						
35						
36						
37						
38						
39						
40						
41						
42						
43						

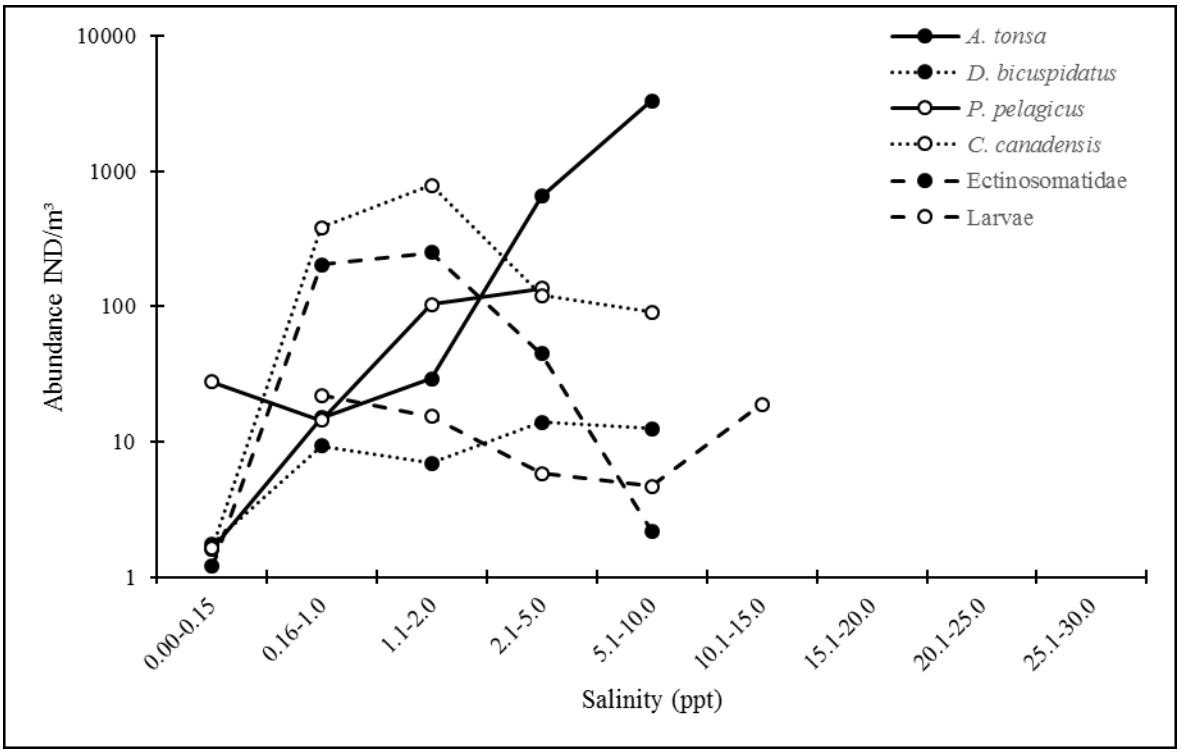

Figure 49. Mean abundance (IND/m^3) of potential zooplankton prey relative to salinity compared to feeding striped bass larvae in 2017 (150µm) in the Shubenacadie River Estuary. Logarithmic scale for y-axis.