Life cycle and population dynamics of the marine ectoparasite *Dichelesthium oblongum* (Copepoda: Dichelesthiidae) on Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*)

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Abstract of the Thesis

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Dichelesthium oblongum belongs to the family Dichelesthiidae, within the Suborder Siphonostomatoida, subclass Copepoda (Kabata 1979; Huys and Boxshall 1991). A description of the life cycle and the morphology of its developmental stages is described and compared with that of other organisms within this family and suborder. As expected within the Dichelesthiidae, *Dichelesthium oblongum*, life cycle appears to consist of nine stages, from nauplius 1 to adult. Sexes are easily distinguished from the 1^{st} copepodid stage on the host through adulthood. General trends include the development of the lateral process on the endopod of the second leg on the male, the drastic elongation of the genital complex of the female, and an increase in size of all appendages in both the male and females through maturation.

Atlantic sturgeon are an ancient fish which are parasitized by the marine ectoparasite *Dichelesthium oblongum*. These parasites have been observed on sturgeon in marine habitats in Europe and on the east coast of North America. *D. oblongum* were sampled from Atlantic sturgeon along the Atlantic coasts of New York, Connecticut and Delaware from 2007-2010.

The life stages of individual parasites were determined. A high infection pressure for this parasite in the late summer/early autumn was observed for Atlantic sturgeon, and based on abundance, prevalence and staging data the Jones Beach sampling area appeared to have the highest infection pressure, potentially even acting as a major source of infection. Prevalence and abundance of *D. oblongum* was higher along the South shore of New York than Delaware, despite larger fish caught in the latter location, yet this may be a function of temporal differences between the sampling. Using this information, a generation time for *D.oblongum* was estimated. This information will be useful in determining a epidemiological model for this host-parasite relationship.

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Glossary

Abdomen: in crustaceans is the part of the body which is below/behind the thorax

Aliform: wing-like extensions

Brachiform: resembling the form of an arm

Brachium: the lower part of the arm

Cephalothorax: fused head (cephalon) and first thoracic segment

Copepodid: postnaupliar developmental stage of copepods

Denticles: small tooth-like structures

Digitiform: finger-like

Endopod/ exopod: the lobes of the leg originating from the sympod, one being closer to the center of the body (endo) and one being more lateral (exo)

Genital complex: the area of the body, anterior to the abdomen, which contains the genital pores

Labium: Bottom lip of copepod mouth

Labrum: top lip of copepod mouth

Lacertus: the upper part of the “arm” appendage

Mandible: paired appendages which aid in feeding

Maxilliped: most anterior appendage on the cephalothorax, it functions as a prehensile limb, and probably aids in feeding

Myxal: a swelling, near the midlength of the corpus in copepods, which is usually armed with denticles

Naupliar Larvae: First developmental stage of crustaceans typically unsegmented with three appendages 1st antenna, 2nd antenna, and mandible

Papillae: small nipple shaped projection

Pyriform: Pear-shaped

Refractile: able to refract light

Seta(e): thin, hair-like structures often associated with sensory organs

Siphonostome: the order Siphonostomatoida which is characterized by a cone shaped mouth

Strigil: a curved structure in the mouth which has fine sharp teeth, used for feeding

Subchelate: See A, two segmented with one, terminal segment (subechela) which can bend towards the basal stationary segment (corpus)

Subquadrangular: four-sided with four ill-defined corners

Sympod: part of the leg which is attached to the body and gives rise to the endo- and exopods

Thoracic segment: a segment of the thorax….the middle segment of a crustacean body plan with a head, thorax and abdomen

Uniramous/biramous: having one/two “branch(es)”

Uropod: flattened lateral appendage of the last abdominal segment of a crustacean

1st Antenna(e)/2nd Antenna(e): one of the paired, flexible, segmented sensory appendages on the head of an insect. In *Dichelesthium oblongum* the second antenna is used for attachment to the host

1st Maxilla: a paired moving appendage situated behind the mandible, part of the feeding complex, no specific function has been attributed to it yet (Kabata 1979)

2nd Maxilla: a paired appendage, which is not part of the feeding apparatus, can be prehensile and aid in movement on the host

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CHAPTER 1: INTRODUCTION

Host-Parasite Background

Atlantic sturgeon

Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) are large anadromous fish found along the east coast of North America from the St. Lawrence River in Canada to the St. Johns River in Florida. Atlantic Sturgeon spend up to six years in their natal rivers before migrating to the open ocean where they remain for long periods of their lifetime. They return to freshwater to spawn. They are susceptible to overfishing due to their slow growth rate and late maturation time (8-17 years), as well as large spawning aggregations (ASSRT, 2007; Dovel and Berggren 1983). Sturgeon populations have been declining since the late 1800s from overfishing. A coastwide fishing moratorium on Atlantic Sturgeon was enacted in 1998, due to their diminishing populations (ASSRT 2007). The largest known population of Atlantic sturgeon is found in the Hudson River in New York, yet is also in decline, more evidence that the population is steady at low population sizes(Dovel and Berggren 1983). Recently, aggregations of juvenile Atlantic sturgeon were discovered near the mouth of the Hudson River and the New York Bight (Sandy Hook, NJ, and Rockaway Beaches, NY) in Spring and Fall (Dunton *et al.* submitted, Dunton *et* *al.* In Press). These sturgeon have an extremely high prevalence of infection with the ectoparasitic copepod *Dichelesthium oblongum* (>90%). Open lesions and heavy infections of *D. oblongum* in the gill chamber of juvenile sturgeon, and proximity of residence to sewage outfalls were associated with multiple indicators of stress (osmoregulatory impairment and depressed lymphocyte numbers, etc.) (Fast *et al.*, 2009; Fast, *et al. in press*). Most *D. oblongum* found on Atlantic sturgeon were adult females and located within the gill chamber (>80%; Fast *et al.*, 2009; Fast, *et al. in press*). As adult stages of parasitic copepods are often more pathogenic than intermediate stages and gills are the main osmoregulatory organ in fish, it is suspected that this infection is causing physiological impairment in affected Atlantic sturgeon. Extensive damage to the gills may negatively affect a sturgeon's ability to osmoregulate when migrating from marine to fresh water (i.e. spawning), causing increased stress on the fish, possibly further reducing host fitness (Fast *et al.* 2009).

Parasitic Copepods

The subclass Copepoda has over 10,000 described species (Huys and Boxshall 1991), with more than 2000 considered parasitic (Cressey 1983). In the transition from free-living to attached parasitic, very distinctive morphologies have developed evolved in copepods. All parasitic copepods have developed appendages for attachment to the host, and specialized mouths, to penetrate the skin and eat the tissue of the host. The siphonostome is the defining characteristic oral cone of the suborder Siphonostomatoida (Thorell 1859). This taxa is composed of 40 families made up of 1550 species, most of which are parasitic on fish (Huys and Boxshall 1991). These parasitic copepods are dependent on their hosts, there for they have evolved complementary biology and life cycles.

All species of the suborder Crustacea have naupliar larvae, including parasitic copepods. The number of naupliar stages can vary between parasitic copepod species, along with the number of juvenile attached stages (Ohtsuka *et al.* 2009). Following moults through the naupliar life stages, Caligid copepods possess a free-living copepodid stage which allows them to actively seek out their host species followed by moulting on the host and producing a frontal filament for permanent attachment to the host (Johnson and Albright 1991). Also many parasitic copepods attach to different areas of the host, and have adapted to their specific microniches (Tang and Newbound 2004). Ectoparsitic copepods which are found on the body of fish hosts are typically flat (Kabata 1979), such as caligids, while gill parasite morphology is not as restrained by water flow dynamics as evidenced by their different body shapes (Kabata 1979). Egg production and generation time can also vary greatly between species and is highly sensitive to salinity and temperature (Ohtsuka *et al.* 2009). Generation times range from a few hours between life stages to weeks or longer (Ohtsuka *et al.* 2009). Also egg production is highly variable within caligids ranging from just a few eggs per egg string to hundreds (Ohtsuka *et al.* 2009).

Dichelesthium oblongum is a marine ectoparasite which has only been found to infect both European and North American sturgeon. Little is known about this siphonostomatoid copepod parasite, though a few descriptions of adult and the initial infective stages exist (Kabata 1979; Kabata and Khodorevsky 1977). While closely related copepods, like the well studied *Lepeophtheirus salmonis*, are known to have serious ecological implications for their hosts (Krkosek *et al.* 2007; Brooks and Jones 2008; Wagner *et al.* 2008), the relationship between *D. oblongum* and Atlantic sturgeon remains unclear. It is thought that *D. oblongum* infects juvenile

Atlantic sturgeon as they are migrating from a freshwater to marine habitat, however the length of these associations, whether they are maintained at different salinities, or whether they affect host behavior are unknown, but important questions that need answering if a comprehensive approach to Atlantic sturgeon conservation is to be achieved. Furthermore, modeling the ecological interactions of marine ectoparasites on anadromous fish has been highly contentious and only just begun, requiring significant investigation to determine the effects these pathogens can have on estuarine fisheries (Krkosek *et al.* 2007; Brooks and Jones 2008).

Main Objective:

Identify life history constraints of *D. oblongum* infection in Atlantic sturgeon populations

Sub-Objectives:

a. **What is the complete the life cycle of D. oblongum?** Siphonostomatoid copepods like *D. oblongum* generally have an infective copepodid stage followed by several attached copepod/chalimus stages prior to maturation to adults. However, currently only the initial infective stage and the adult stages have been described for *D. oblongum.* Archived *D. oblongum* stages collected from Atlantic sturgeon (2007-2008) will be identified to their individual stages using light microscopy. These samples will be supplemented with fresh samples collected from Atlantic sturgeon from New York, Connecticut and Delaware sturgeon sampling programs.

b. **What is the generation time for D. oblongum within the NY Bight?**_ Archived and fresh *D. oblongum* samples collected throughout the year (Spring-Autumn) will be used to estimate generation time for this parasite based on abundance of individual stages on sturgeon over time (at particular temperature and salinity). Live samples will also be brought back to Marine Animal Disease Laboratory at Stony Brook University and hatched to infective stages.

c. **Do new infections establish themselves throughout the year or occur once?** Presence and abundance of infective and other juvenile *D. oblongum* stages from the samples collected will be used to estimate (when, where, etc.) the major pulses of infection exist.

d. **What is the production of infective stages of D. oblongum for the NY Bight?** The presence, abundance and development of eggs from *D. oblongum* females over time will be determined from already archived and newly acquired samples collected as above.

Broader Impacts

Only a handful of siphonostomes have had their complete life cycles described, out of the 1550 species which make up the suborder. In the extensively researched Caligidae family only 15 of the more than 450 species have complete life cycles described (Boxshall 2008). There is no life cycle described for the three species in the family Dichelesthiidae. Understanding the life cycles and ecology of parasitic copepods is important, considering caligid copepods are known to have large economic and ecological impacts on farmed fish species. High mortality rates in some rare cases, extensive pathology and substantial costs to the industry of farmed salmon have been attributed to the copepod *L. salmonis* (Johnson *et al.* 2004). Knowledge of the *L. salmonis* life cycle has been used to implement preventative measures on salmon farms, to lower mortality. Life cycles of parasites could also be to implement conservation measures for endangered or threatened host species, such as the Atlantic sturgeon.

Overall I want to determine the life history relationship between *D.oblongum* and Atlantic sturgeon in the hopes of developing an infection model in the future and determining potential negative effects on local Atlantic sturgeon populations (stress, fitness, habitat usage, etc.). An infection model of this sort would also be applicable to other anadromous species encountering strictly marine pathogens, such as salmonid smolts encountering sea lice or infectious salmon anemia virus.

CHAPTER 2: FIRST DESCRIPTION OF THE JUVENILE DEVELOPMENTAL STAGES AND LIFE CYCLE OF *DICHELESTHIUM OBLONGUM* (COPEPODA: DICHELESTHIIDAE) PARASITIC ON ATLANTIC STURGEON (*ACIPENSER OXYRINCHUS OXYRINCHUS)*

Introduction

Copepods are a morphologically diverse group of organisms. The order Siphonostomatoida has over 1550 species, of those, approximately 1050 are parasites of fish the other 500 are parasites of invertebrate hosts (Huys and Boxshall 1991). *Dichelesthium oblongum* is a marine ectoparasitic copepod which infects *Acipenserid* hosts, including Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*). Little is known about this parasite, as only the adult and first copepodid stages have been described (Kabata and Khodorevsky 1977; Kabata 1979). Based on Kabata (1979) and Cabral *et al*. (1984) we expect the life cycle to contain two naupliar stages, an infective copepodid stage, fixed/parasitic copepodid stages I-V, and an adult stage. Within the Dichelesthiidae family, only one description of juvenile stages exists and that is for early and late juvenile stages of *Anthosoma crassum* (Benz *et al.* 2002) a parasite of short fin mako sharks

(*Isurus oxyrinchus*) along with other pelagic shark species (Kabata 1979; Benz 1994). While little may be known about the Dicheleshiidae family, other families of Siphonostomatoida, such as the Caligidae are known to be serious pathogens of wild and farmed fish (Kabata 1979; Krkosek *et al.* 2007). The Caligid copepod *Lepeophtheirus salmonis,* for instance, is known to cause considerable pathology and mortality in host salmonids and at the center of the controversy between wild and farmed fish interactions (Krkosek *et al.* 2007; Brooks and Jones 2008; Wagner *et al.* 2008).

Although previously described as a gill parasite (Kabata 1979). *D. oblongum* settlement sites are found mainly in the gill chamber, though a significant percentage 10-15% can be found on the body (Fast *et al.* 2009; Fast *et al. In press*). Gross lesions (>10 mm) on Atlantic sturgeon have been observed associated with attachment and feeding of juvenile *D. oblongum* stages on the body (Fast *et al*., 2009). Furthermore, infections of highest gravid female abundance (>6 per fish) have been found in sturgeon exhibiting signs of osmoregulatory stress and collected from sites close to sewage outfalls (Fast *et al*., 2009). The Convention on International Trade of Endangered Species and the National Oceanic and Atmospheric Administration list the Atlantic sturgeon as a species of concern, developing a better understanding of the host-parasite relationship between them and *D. oblongum* may have important conservation implications (ASSRT 2007).

Due to the paucity of information on this family within the Suborder Siphonostomatoida, and the lack of a life cycle description for *D. oblongum*, this study will describe the morphology of developmental stages and compare them with descriptions of closest available parasitic copepod species. The terminology basically follows that of Kabata (1979). In this paper two naupliar stages are described, as well as the first descriptions of copepodid stages I-V for both males and females, on the host. The life cycle of this primitive parasite could also be important for understanding early host-parasite relationships.

Materials and Methods

Fish sampling and parasite collection

Juvenile Atlantic sturgeon were captured by bottom trawling using an 80' otter trawl aboard the RV "Seawolf" within juvenile/immature ocean habitat outside of Jones Beach, NY, Rockaway, NY, and Sandy Hook, NJ. Tows were conducted for 10-20 minutes, to minimize damage and/or stress related impacts on the fish. Five collection trips were conducted in October 2007, November 2007, September 2008, November 2008, and May, 2010. Upon capture, all sturgeon were measured for total and fork length, and weight, ectoparasites were collected (10% neutral-buffered formalin), and blood samples were taken.

On May 19, 2010, egg strings were detached from seven of the collected gravid females. The egg strings were kept in seawater at ~13ºC during transport and then kept in aerated seawater at 15ºC in the lab. The egg strings were monitored daily for two weeks. Nauplius larvae were collected and observed daily upon first appearance. Naupliar II stages were first observed five days after collection, by nine days after collection, most nauplii had died.

Photo processing and staging

Forty-eight individuals of representative sizes were used to identify the different juvenile life stages. Each specimen was labeled and observed using a depression slide and a Nikon Eclipse inverse light microscope. Photographs were taken using an Insight QE camera and Image pro digital imaging software. The entire animal was photographed, both dorsal and ventral sides. The images were taken in sequence in varying degrees of focus. The freeware CombineZP was used to create one frame, with all planes/ parts of the organism in focus. Once the images were stacked, they were further processed using Adobe Photoshop software. By taking overlapping pictures, I was able to use Photoshop to overlap the images and blend them together, making a complete organism. I changed the opacity of the images to align the images correctly. The images were arranged to develop composite images of the ventral and dorsal sides of the specimen. Using the lasso tool, I was able to remove excess background from the pictures. I also used the pen tool in photoshop to trace the images and make outlines of the various stages. Once the images were processed, they were analyzed to determine difference between life stages based on mean length (mm ± SEM), development of appendages and segmentation. Intermolt specimens were also useful in identifying key features between molting stages for both males and females.

Results

Description of *Dichelesthium oblongum*

Nauplius 1

(Length = *0.38±0.005mm, and n=7*)

Naupliar larvae are universal in the subphyla Crustacea (Brooks 2005; Suarez-Morales *et al.* 2007; Izawa 2009). The naupliar larvae (Figures 2.2 and 2.3) of *D. oblongum* closely resemble the larvae of many other parasitic copepod larvae (Johnson and Albright 1991; Suarez-Morales et al. 2007; Izawa 2009; Ohtsuka *et al.* 2009; Okada *et al.* 2009). There are two naupliar stages, which are differentiated by body shape and inner segmentation, while the appendages show no visible changes between stages. The best images and measurements of this stage were obtained from developmentally complete but unhatched eggs. No live Nauplius 1 larvae were observed. The first nauplius has an elliptical body shape. The body has a pigmented area (most likely egg stores, yolk) and two posterior balancers, which extend laterally from the body (Johnson and Albright 1991). The 1st antenna is uniramous and two segmented, both are equal in length. The basal segment is armed with a short spine and the distal segment has two long and one shorter thin apical seta. The 2nd antenna is biramous with a long slender sympod. The exopod is indistinctly five segmented, decreasing in size distally. The basal segment is unarmed and slender with no setae. Segments two through four have one long, thin seta. Segment five has two long, thin setae. The endopod of the 2nd antenna is indistinctly two segmented with the basal segment long, slender and lacking armature. The distal segment has two long slender setae. The mandible is biramous, with a long slender sympod. The endopod is indistinctly three segmented, decreasing in size distally. Segment one and two lack setae, segment three has two long thin apical setae. The exopod is indistinctly five segmented, decreasing in size distally. The basal segment is lacking armature, whereas segments one through four each have one long, thin seta. Segment five has two long, thin apical setae.

Nauplius 2

(L = *0.45±0.005mm, and n=2*)

The appendages of the second nauplius stage (Figure 2.3) remain unchanged from the first nauplius, however the body increases in length and becomes slightly more slender. In some individuals, presumably later stage nauplius 2, copepodid segmentation is visible beneath the integument, and the egg stores are reduced.

Infective Copepodid

It is expected that *Dichelesthium oblongum* have only one planktonic copepodid life stage as seen in many other closely related Siphonostomatoid families (Benz *et al*., 2002; Ohtsuka *et al*., 2009). We were unable to successfully observe the molt from nauplii II to this stage in the laboratory; however, the authors did observe the presence of a preformed copepodid under the exoskeleton of several nauplii 2 larvae intermolts. We therefore, refer the reader to the description of this life stage by Kabata and Khodorevsky, (1979). This appears to be the first planktonic life stage that actively seeks a host. Presumably, as with other parasitic copepods, this stage is an obligate parasite and will expire within a short time without finding a host.

Parasitic Copepodid I

This stage is the first in which sexual dimorphism occurs, and is the first molt that occurs on the host.

Male

(L = *1.3±0.08mm, and n=2*)

Cephalothorax comprises 2/3 of the total length, with distinct indentions 1/5 of the way down from the anterior edge (Figure 2.5A). Three free thoracic segments and an indistinctly segmented abdomen/genital complex comprise 1/3 of the total length. The 1st antenna is long, slender and indistinctly five segmented, segments increase in length distally. Segment five has several apical setae of varying lengths. Segment one has a short setae near the margin with segment two. Segments two through four lack armature. The 2nd antenna has a thick base, decreasing in girth from the base to the end and is retractile. The distal end of the 2nd antenna has a well defined claw. In the myxal area of the 2nd antenna there is a cluster of small denticulations atop a raised area. The siphonostome/oral cone or mouth tube is similar to the adult, described by Kabata (1979). The mandible was unable to be seen using light microscopy. The 1st maxilla is biramous, with a slender endopod surmounted by two long thin apical setae. The exopod is short, with a rounded end and three apical setae; two short, and one much longer. The 2nd maxilla is brachiform, the lacertus robust lacking armature and comprising half of the total length. The brachium has a smooth apical digitiform process and two rows of large denticles, extending from a subapical point. Below the digitiform process, there are two peg-like projections as described by Kabata (1979). The maxilliped and has a slender corpus and is smooth, with no points and a well defined claw. Legs are longer than thoracic segments, and third leg is much shorter than in the adult, barely visible beneath the second leg. The endopod and exopod of the first and second leg are shorter relative to the setae, as is the third leg. There are some denticles on the exopods of the first and second legs, but the endopods are smooth. There are two uropods, with three strong, short apical setae on each.

Female

(L = *2.5mm, n=1*)

The female is very similar to the male, except the segmentation is not as well defined (Figure 2.4A). The only specimen collected, was damaged and thus it is difficult to fully describe the entire individual. Most of the appendages were preserved, and were similar to those found on the male.

Parasitic Copepodid II

Male

(L = *2.3mm, n=1*)

The cephalopthorax has a well defined dorsal shield suboval (Figure 2.5B). There are well defined indentations ¼ of the length of the cephalothorax, from the anterior end. The cephalothorax constitutes about ½ of the entire length of the body. The second leg-bearing segment is roughly 3 times as broad as it is long and rectangular, devoid of lateral processes. The free thoracic segments decrease in width from the anterior to the posterior end. All leg-bearing segments are roughly equal in length. The genital complex is the same width as the previous segment and twice as long as the previous segment. The abdomen is fairly circular in shape and at the posterior end there are two uropods with three short, pointed setae. The 1st maxilla is biramous. The endopod is long and slender with a curved tip, surmounted by two long and slender setae which are fused at the base, comprising approximately half of the total length. The exopod is short, with a curved end surmounted by three setae, of two types. The medial seta are longer and thicker than the lateral seta. Total length of the endopod and setae is equal to the length of the mouth tube. The 2nd maxilla is brachiform with the upper lacertus comprising half the entire length. The lacertus is broader than the brachium, and is smooth with no armature or setae. The brachium is opposable and has two subapical processes facing outward. The brachium also has an apical digitiform process with denitcals extending from a subapical point to the apex on both sides of the process. Below the digitiform process, there is another process covered in a cluster of long, thin setae. The maxilliped has a prominent subchelate corpus oval in shape, it has two short, points in the myxal area, the corpus is not as broad as in the adult described by Kabata (1979). There is a well delimited claw and no other armature. The siphonostome mouth tube is long and slender, with fringing setae. Small strigil are visible, behind labrum and labium (Kabata 1979). Similar to adult described by Kabata (1979). The 1st antenna has six segments decreasing in circumference from the base to the apex. The two most apical are longer than the previous segments unlike the adult appendage described by Kabata (1979), in which all segments, were of equal length. There is a cluster of several setae at the apical end of the 1st antenna. There is also a laterally oriented seta on the apical segment. As in the description by Kabata (1979) there are two setae on segment 1 and 2 with 3-5 lacking armature. The 2nd antenna has a well defined claw at the tip which is retractable. In most specimens, it is bent, but can be longer, when fully extended. There is a growth in the myxal area which is covered in small denticulations and no other armature is observed.

1st Swimming leg: The sympod is broader than it is long. The exopod is small and suboval and has two long slender setae and small denticles lining the edge of the exopod. At the base of the exopod there is a pointed, robust seta/outgrowth. The sympod also has a seta near the base of the exopod, extending laterally outward. The endopod is longer than it is broad and suboval. The outer edge of the endopod is lined with denticles, but there are no clusters of denticles as described in Kabata (1979). It has five/six setae, with the most lateral, being farther comparatively from the rest of the setae. The setae decrease in length from medial to lateral.

2ndSwimming leg: The sympod is broader than it is long, and is larger than the sympod of the first swimming leg, with the same basic shape. Laterally, near the base of the endopod, there is a thin short seta. The endopod is larger than that of the first leg, but is also suboval with two setae and denticles lining the outer edge. The exopod has five short setae, and denticles along the outer edge.

3rd leg: It is a flipper like structure and has four papillae. Three are thick and short, with the fourth, most lateral being thinner. The surface is smooth and lacking denticles.

Female

(L=*3.3±0.1, and n=18*)

The cephalothorax is approximately 50% of the total length (Figure 2.4B). Similar to the male, there are four free thoracic segments and abdomen. The segments are not as well defined as in the male. The first free thoracic segment is broader than it is long, expanding from anterior to the posterior end. It is longer than the first thoracic segment in the male. The second free thoracic segment is less broad than the first thoracic segment, and less well defined. The third free thoracic segment is even more indistinct, and has rounded lateral borders. The fourth free thoracic segment/ genital complex tapers from the anterior end to the posterior end. The abdomen is somewhat circular with two uropods similar to the male, on the posterior end. The appendages are overall very similar to the male, but the endopod of the second leg is lacking denticles and is broader along the medial margin than the endopod of the second leg found in the male.

Parasitic Copepodid III

Male

(L=*4.0±0.11mm, and n=11*)

The dorsal segments are well defined, and shorter than in the previous stage (Figure 2.5C). The cephalothorax still makes up between 50-60% of the total length. Segments are still much broader than they are long. Most appendages have not changed. The segmentation of the 1st antenna is more defined. The 2nd antenna has a more defined process in the myxal area, with less denticulation, becoming more of a projection, than a raised area. The corpus of the maxilliped is also much broader than in the previous stage, and the points in the myxal area are more defined. There is also increased denticulation on the lateral margins of the endopods and exopods of the first and second legs. The endopod of the second leg has broadened and is beginning to develop the lateral projection described in the adult by Kabata (1979). The third leg has also greatly increased in breadth. There is a relative reduction of the length of the uropods' setae.

Female

(L=*5.2±0.27mm, and n=18*)

The cephalothorax is approximately 50% of the total length, and somewhat less circular than the previous stage (Figure 2.4C). The dorsal segmentation becomes more defined. The genital complex is two segmented. The first and second free thoracic segment are much broader than long. The segments decrease in breadth but not in length from the anterior to the posterior. There is little change of the appendages. The third leg lengthens and broadens. There is also an increase in the size and number of subapical denticles on the maxilla, and the peg-like processes are reduced.

Parasitic Copepodid IV

Male

(L=5.1± *0.28mm, and n=14*)

The cephalothorax comprises only 40% of the total length (Figure 2.5D). The first thoracic segment increases in breadth from the anterior margin to the posterior margin. The thoracic segments increase in length, but remain broader than they are long. The abdomen becomes more defined. The appendages change minimally from the last stage. The subapical denticles on the 2nd maxilla increase in number and size, and the peg-like processes are reduced. The endopod of the second leg broadens, and the lateral projection becomes more prominent. The third leg is slightly broader than in the previous stage.

Female

(L=*5.4±0.37mm, and n=17*)

The cephalothorax is 40% of the total length (Figure 2.4D). The total length has increased over all. The first and second segments retain a similar shape to the previous stage, however, the third and fourth thoracic segments are less defined and the genital complex/fourth segment is somewhat extended compared to the male. Also the abdomen is less defined. The uropods have reduced setae. Similar appendage development changes as seen in the male. The endopod of the second leg doesn’t change from the previous stage; no development of a lateral projection is seen. The third leg also does broaden as it does in the male, it remains fairly slender. The denticulation of the first and second legs increases.

Parasitic Copepodid V

Male

(L=*7.5±0.29mm, and n=7*)

The cephalothorax is 25-30% of total length and more pyriform (Figure 2.5E). Dorsal segments have elongated, but not broadened. Lateral denticulation is present on the lateral edges of the cephalothorax and the first free thoracic segment. The projection in the myxal area of the 2^{nd} antenna is very well defined and prominent, and the denticulation is absent. The claw of the maxilliped is nearly equal in length to the corpus. The brachium of the 2^{nd} maxilla is slender and slightly longer than lacertus. The lateral projection on the endopod of the second leg is now fully developed. The third leg is very broad with some minor denticulation. There are overlapping genital flaps at the margin between the genital complex and the abdomen.

Female

(L=*8.94±0.54mm, and n=10*)

The cephalothorax is about 40% of the total length (Figure 2.4E). The first, second and third dorsal segments are broad and very short. The first thoracic segment is beginning to develop lateral digitiform processes. The fourth thoracic segment is slightly longer than the preceding segments. The genital complex is three times the length of each of the other segments. It is well defined, and subquadrangular, with corners at posterior lateral margin. The abdomen is circular and well defined, and slightly separated from the genital complex. The uropods have elongated, and the setae are smaller relative to the uropod, than in the previous stage. The 2^{nd} antenna has a well defined process in the myxal area, no denticulation. The peg-like processes on the 2^{nd} maxilla are reduced. There is denticulation on the sympods of both the first and second leg. Minute papillae are dispersed on each of the free thoracic segments' lateral edges as well as the genital complex. The endopod of the second leg is very broad with many prominent denticles along the lateral edge. The third leg has minor papillae throughout the ventral surface.

Adult

Male

(L=*8.8±0.68mm, and n=186*)

The cephalothorax is about 30%, as described in Kabata (1979), yet general body shape of the adult male (Figure 2.8), differed from the original description. The 3rd free thoracic segment is broader than the adult male described by Kabata (1979), and the 1st free thoracic segment has smaller, or less prominent lateral processes. The male is smaller than the female, and lacks the elongated genital complex, as well as the digitiform lateral processes found on the female. The appendages on the adult are very similar to the late juvenile stages, and are generally larger (Figure 2.5F). The growth in the myxal area of the 2nd antennae is now devoid of the denticulation, seen in earlier stages, and the lateral process on the endopod of the 2nd leg is now fully formed.

Female

(L=*12.3±0.08mm, and n=470*)

Drastic elongation of genital complex and thoracic segments 2, 3 and 4 (Figure 2.4F). This is seen in more mature adult females, but less mature adult females have some cuticular folding throughout the body, allowing for elongation throughout the entire body (Figure 2.9). The first thoracic segment has digitiform lateral processes and is much broader than it is long. The Cephalothorax is about 30% of the total length and more pyriform. The process in the myxal area is now bulb-shaped, lacking denticulation and the 2nd antenna is much thicker. The adult appendages are very similar to previous stage. The third leg is longer. Points on maxilliped are very prominent. Small papillae scattered over ventral surface of all free thoracic segments, genital complex and abdomen. In adult females, spermatophores were observed with crossed tubules,not yet been described in Dichelesthiidae (Figure 2.10).

Discussion

In the literature there are descriptions of adult stages of *D. oblongum* and the only other two species in the family Dichelesthiidae, *A. crassum* and the extinct *K. pattersoni*, but information on larval or juvenile stages is lacking. Only a couple developmental stages have been described for the sister taxa, *Anthosoma crassum* (Benz et al. 2002). In Benz *et al*. (2002), only 1 intermediate male and 1 intermediate female stages were described. Benz *et al* (2002) used the related species *Lernanthropus kroyeri* from the family Lernanthropidae, for comparisons as the life cycle of this species was described by Cabral *et al.* (1984). Unfortunately, Benz *et al*., (2002) misreferenced or mistranslated (French publication) Cabral *et al*. (1984) stating there were 10 stages including 6 copepodid stages and an adult stage in this parasite. Yet Cabral *et al*. (1984) describe only 9 life stages; 2 nauplii, one free or planktonic copepodid, 4 'fixed' copepodids, one pre-adult and an adult stage. As articulated by Ohtsuka *et al*. (2009), even in the more well-studied genera such as the caligids there appears to be inconsistency with numbering of stages, in this case however due to misidentification. Ohtsuka *et al*. (2009) suggest the revisitation of certain fixed stages in which molts may not have occurred in *Lepeophtheirus spp.* which is the only example in the entire Copepoda where the number of stages in copepodid phase development exceeds five before the adult. Based on this standard within the sub-phyla we expect *D. oblongum* to have 9 life stages with *molt*s interspersed in between each stage (Figure 2.1). This paper described two naupliar stages and parasitic copepodid stages I-V, with supplemental descriptions of the adults of *D. oblongum.* The adults and planktonic infective copepodid stages have already been described by Kabata (1979) and Kabata and Khodorevsky (1977), respectively.

Morphologically, *D. oblongum* is very similar to *A. crassum* and *Kabatarina. pattersoni,* with similar mouth parts, characteristic of siphonostomatoida, and a prominent dorsal shield, but is lacking the elytra-like structures found on those species (Cressey and Boxshall 1989; Benz et al. 2002). *K. pattersonii* was found fossilized in the gill of *Cladocyclus gardneri* and is thought to have been a gill and buccal cavity parasite, like *A. crassum* in Galoid sharks (Kabata 1979; Benz 1994; Benz et al. 2002). While *D. oblongum* is primarily a gill parasite, a significant number of juvenile and adult male stages have been found on the fins and lateral surfaces of its host the Atlantic sturgeon (Fast et al. 2009). The elytra-like structures on *A. crassum* are thought to be used for protection from the pressure of the tissues from the host shark, since it burrows into the tissue. Since *D. oblongum* has a more superficial attachment, the need for elytra-like structures is reduced. The digitiform processes on the second thoracic segment of the female may be remnants of elytra-like structures from ancestor species, such as *K. pattersoni*. This extinct species of the family Dichelesthiidae has morphological features of both freshwater and marine ectoparasites and may represent one of the first species to begin to parasitize marine fishes (Cressey and Boxshall 1989).

Throughout the development process the dorsal segmentation became more defined in both males and females (Kabata 1979, Figures 4 & 5) and the myxal area of the 2nd antenna increased in size and prominence (Figure 2.6 A, B, C). As noted by Boxshall (1974) and

Ohtsuka *et al*. (2009) copepods metamorphose from free-living copepods through parasitic life stages to an adult. Within this process there is an initial regression (lost or less prominent limbs, less segmentation, etc.) and then a progression of stronger more prominent limbs and body segmentation (Boxshall 2004; Ohtsuka et al. 2009). As seen in the first copepodid stages I and II, there is a loss of dorsal segmentation, especially within the female stages, compared to that in Kabata and Khodorevsky (1979), which then progresses to more defined segmentation from copepodid III-adulthood.

The progression of the myxal area of the 2nd antennae through toward adulthood can also be seen in the *A. crassum* individuals described by Benz *et al*. (2002) and *Lernanthropus spp*. However, *Anthosoma crassum,* as well as *Lernanthropus spp*. in general have a much smaller process on the 2nd antenna than *D. oblongum.* Differences exist in the interaction these species have with their hosts, as mentioned above, which may explain the morphological differences between them, for instance *A. crassum* burrows into the host tissue, using its 2nd antenna, while infections by *D. oblongum* being more superficial, increase the need for a strong adhesion to the host by the 2nd antenna. In early parasitic stages of *D. oblongum* the myxal area of the 2nd antennae has denticulation, and it is lost later in development. The denticulation could aid in the initial attachment on the host, and possibly minimize movement on the host, until a more favorable area is found for permanent attachment (Figure 2.6 A, B, C).

Anthosoma crassum, *D. oblongum,* and *L. kroyeri*, juveniles are all described to attach without the formation of a frontal filament (Cabral *et al.* 1984; Benz et al. 2002; Fast et al. 2009). This was confirmed throughout all life stages of *D. oblongum* described here. Frontal filaments are common features of several families, including Caligidae, within the fish-parasitic lineage of siphonostomatoid copepods (Ohtsuka et al. 2009). All three of the species in Dichelesthiidae have large, prominent 2nd antenna, which are the primary appendage used to attach to the host, with a secondary point of attachment being the 2nd maxilla (Figure 2.6 D, E, F). This 2nd antenna structure could have been adapted for soft tissue attachment. Placoid scales on sharks are thought to be difficult for attachment from some caligid copepods (Tang and Newbound 2004) and some caligids parasitize the eyes and gills of sharks, instead of the body (Benz 1994; Deets 1994; Benz et al. 2002; Tang and Newbound 2004), even though caligids are found on the body on other host species (Kabata 1979; Huys and Boxshall 1991). Sturgeon have bony scutes and ganoid scales, which could make it difficult for attachment along the body, though a small percentage *D. oblongum* juveniles and adult males, not females, have been found there (Fast et al. 2009). Compared to *D. oblongum* which has lost its 4th, 5th and 6th swimming legs, caligids are much more mobile as adults on the host, and flat, reducing drag and allowing the parasite to move easily along the fish without being removed by water flow. The 2nd antenna attachment can be fairly substantial, reducing the ability of *D. oblongum* to move across the surface of the host. Also, the large size of *D. oblongum* probably increases drag along the body of the fish, leading to a reduction in the infections outside of the gill, since the water flow could remove the parasite prior to firm attachment on the body. The posterior of the gill cavity, where most *D. oblongum* are found is relatively protected from high water flow.

An overall developmental trend of *D. oblongum* males was the formation of the lateral process on the endopod of the second leg (Figure 2.7). It is unknown why there is modification of the 2nd leg, it could be for aid in the mating process, possibly allowing mobility of the male to find a mate. This development could also aid in attachment to the host, explaining why there have been adult males found outside of the gill cavity, but not adult or late juvenile females (Fast et al. 2009; Fast et al. *In press*). It is interesting to note that in closely related species in the family Eudactylinidae the maxilliped is used for grasping the female during copulation and is sexually dimorphic (Kabata 1979; Deets 1994). This is not observed in Dichelesthiidae, and as such the projection on the 2nd leg could aid in copulation as well. Genital flaps over the abdomen and a broadening of the third leg also occurred. Genital flaps, cover the genital openings on males and may aid in sperm competition or be associated with mate guarding as seen in other parasitic copepods (Ritchie *et al.* 1996). The broadening third leg could be from the close relationship to species of parasites with elytra-like structures. In *A. crassum* all three pairs of swimming legs have been modified to aliform plates, very broad and with small refractile depressions (Kabata 1979).

The segmentation of the female exhibits drastic changes throughout the later copepodid stages (Figures 2.4 and 2.1). The abdomen and genital complex become more distinct and the genital complex elongates to approximately 30% of the total length. In adult female cuticular folding was observed throughout the entire body (Figure 2.9). In caligid parasites, some extra cuticular folding has been observed to increase the length of the genital complex during elongation (Smith and Whitfield 1988; Johnson and Albright 1991), but not throughout the entire body. Genital complex elongation could allow room for more egg development and increase reproductive capacity. In general with larger females, there is an increase in the egg string length and the number of eggs produced (Chapter 3). Also in mature adult females, deposited spermatophores were observed, with crossed spermatophore tubules (Figure 2.10). Crossing of spermatophore tubules is very characteristic within this Order and has been described in the

Caligidae and Asterocheridae families, but to the author's knowledge not before in the Dichelesthiidae. Medial migration of the copulatory pores is also seen in the Bariaka-like state and considered ancestral (Huys and Boxshall 1991), confirmed further by its presence here in the Dichelesthiidae family, a parasite which retains several ancestral features.

Between the genders, there is more denticulation and papillae on the female than the male. This increase on the female may increase adhesion to the host, increasing the time of attachment. The abdomen and genital complex in the female became greatly elongated in later stages as compared to the male, presumably to allow for egg development. Also the digitiform processes of the first and second free thoracic segment became more prominent and well defined in the female, while the shape of these segments in the male did not change much throughout the stages.

There were several trends observed throughout development in male and female *D. oblongum*, which included morphological changes throughout the life cycle. In males and females, the process in the myxal area of the 2nd antenna became more prominent and lost denticulation, the 3rd leg broadened and all appendages increase in size. In males, the development of a lateral process on the endopod of the 2nd leg occurred, and in females, there is a drastic elongation of the genital complex. Our analysis suggests that the *D. oblongum* life cycle consists of 2 nauplii stages, one free-living parasitic copepodid stage, 5 juvenile attached copepodid stages and one adult stage (9 life stages in total). This life history exhibits sexual dimorphism as early as the first attached copepodid stage, similar to *Lernanthropus kroyeri* (Cabral et al. 1984). Future studies will include electron and confocal microscopy of future collection specimens as well as controlled in-lab studies to confirm the delineation of stages.

Controlled lab studies will also allow determination of degree-day ranges for specific developmental stages and temperature and salinity optima for *D. oblongum* planktonic and parasitic stages. Answering these life history questions will be the first step in a better understanding of this primitive host—parasite relationship.

Figure Legends:

Figure 2.1: Life Cycle of *Dichelesthium oblongum*: 2 free living naupliar stages, 1 infective copepodid stage, 5 parasitic copepodid stages, and the parasitic adult stage

Figure 2.2: First Naupliar Larvae of *D. oblongum* from lab cultures of egg strings

Figure 2.3: Second Naulpiar Larvae of *D. oblongum* from lab cultures of egg strings

Figure 2.4: Female life stages of *Dichelsthium oblongum* parasitic on Atlantic sturgeon. A) Copepodid 1, B) Copepodid 2, C) Copepodid 3, D) Copepodid 4, E) Copepodid 5, F) Adult

Figure 2.5: Male life stages of *Dichelsthium oblongum* parasitic on Atlantic sturgeon. A) Copepodid 1, B) Copepodid 2, C) Copepodid 3, D) Copepodid 4, E) Copepodid 5, F) Adult

Figure 2.6: Morphological changes to the 2nd antenna (A, B, C) and 2nd maxilla (D, E, F, G) throughout the life cycle of *D. oblongum* A) Male copepodid 2; B) Female copepodid 4; C) Adult Male; D) Male copepodid 2 E) Male copepodid 3; F) Male copepodid 4; G) adult male

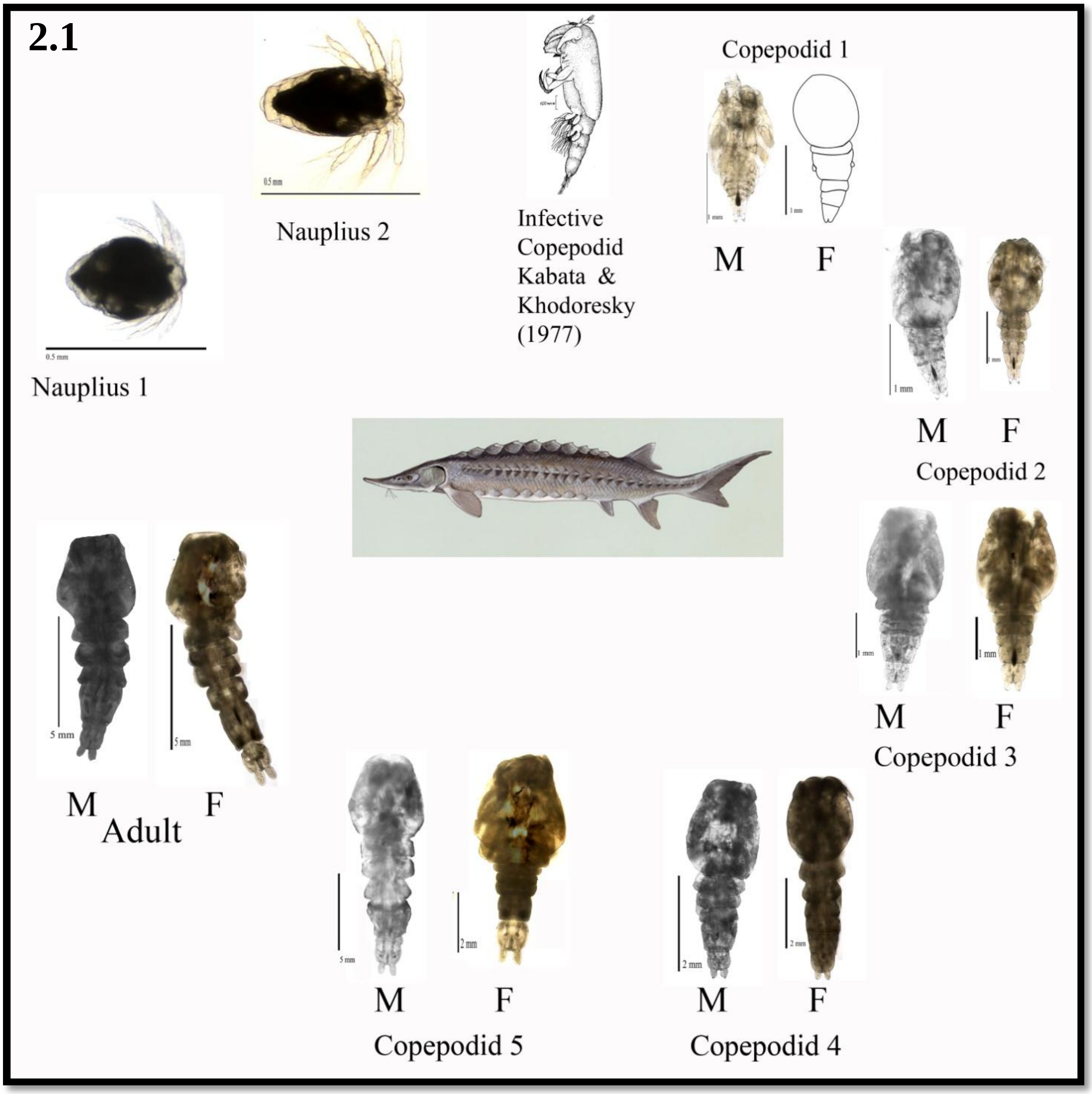
Figure 2.7: Swimming leg morphology changes throughout the life cycle of *D. oblongum* in Females (A, B, C, D) and Males (E, F, G, H) in 1st Legs (A, E) and 2nd Legs (B, C, D, F, G, H) A. Female copepodid; 2 B. Same; C. Female copepodid 3; D. Female copepodid 5; E. Male copepodid 1; F. Male copepodid 3; G. Male copepodid 4; H. Adult Male

Figure 2.8: Segment morphology of A) adult male from archived samples and B) adult male described by Kabata 1979.

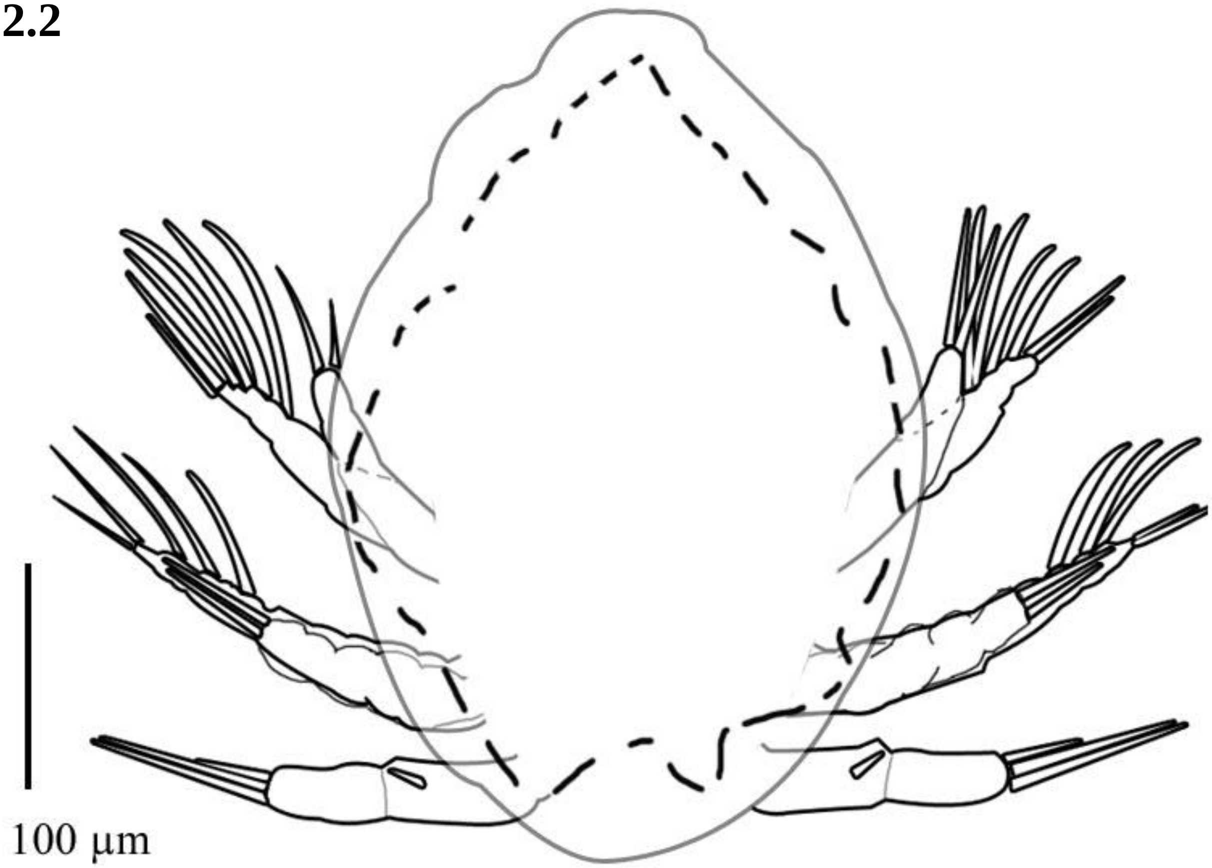
Figure 2.9: Two adult females of *Dichelesthium oblongum*, one is younger (A) displaying cuticular folding, there is no cuticular folding in the older female (B)

Figure 2.10: An attached spermatophore from a male on the abdomen of an adult female *Dichelesthium oblongum.* * Note the crossing tubules.

2.1


Nauplius 2
0.5 mm
Infective
Copepodid
Kabata &
Khodoresky
(1977)
Copepodid 1
M
F
1 mm
Nauplius 1
0.5 mm
M
F
Copepodid 2
1 mm
M
F
Copepodid 3
1 mm
M
Adult
F
5 mm
M
F
Copepodid 5
5 mm
2 mm
M
F
Copepodid 4
2 mm

2.2



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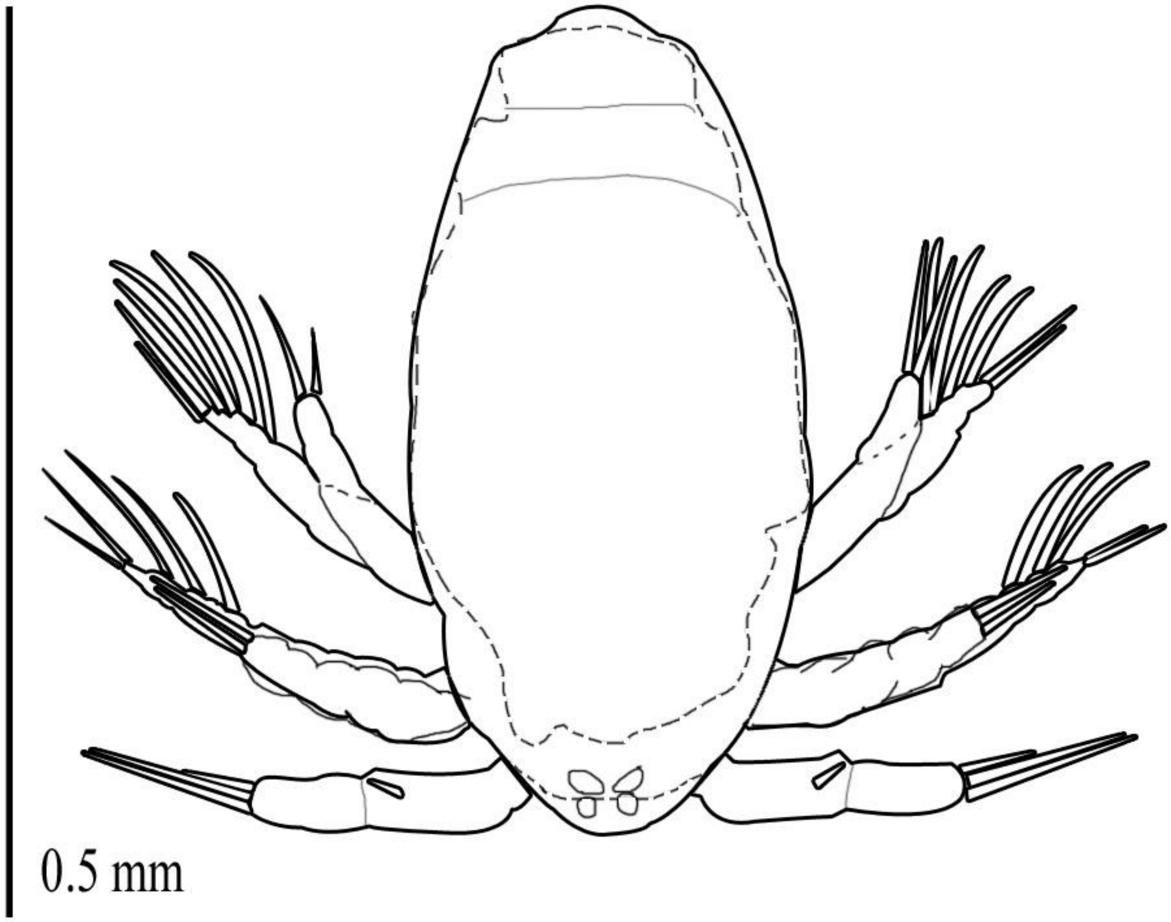

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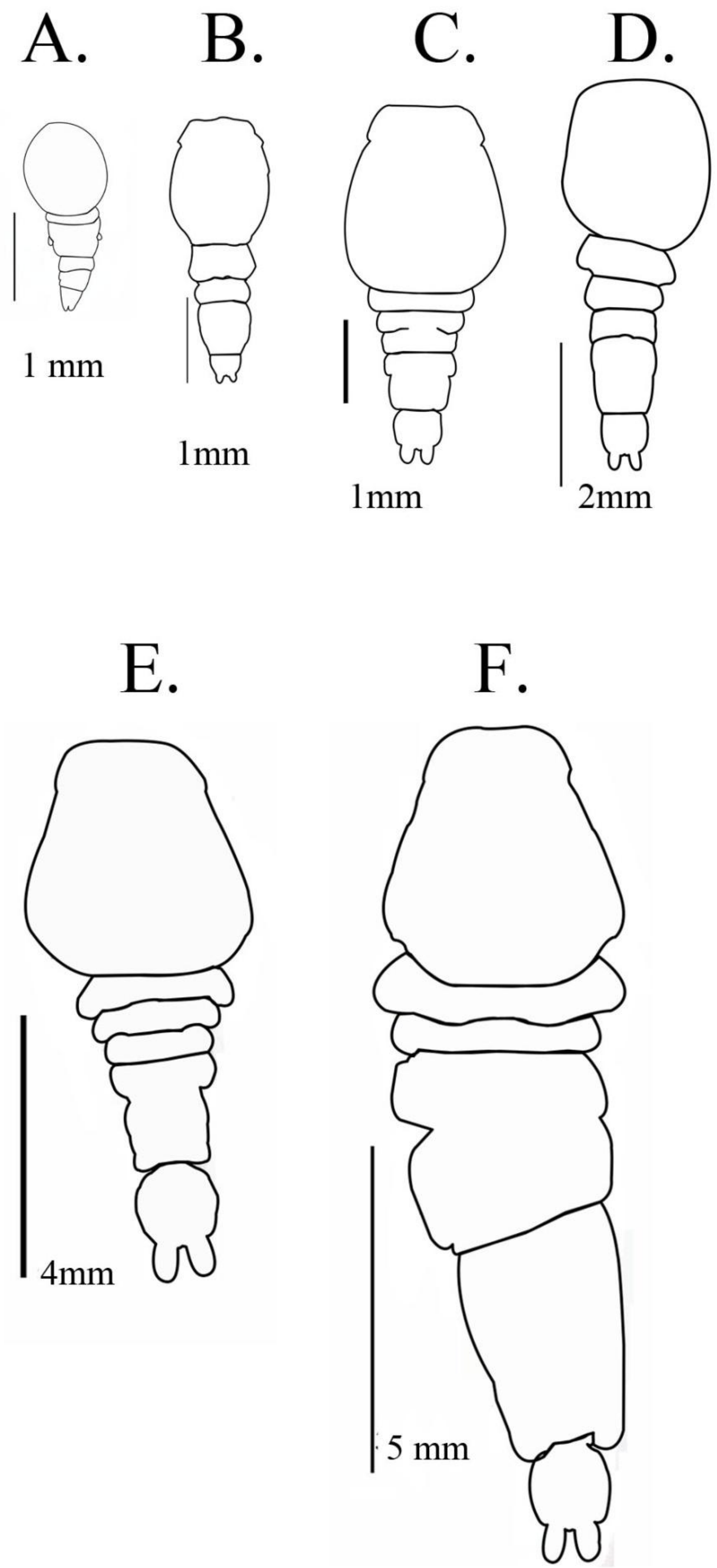
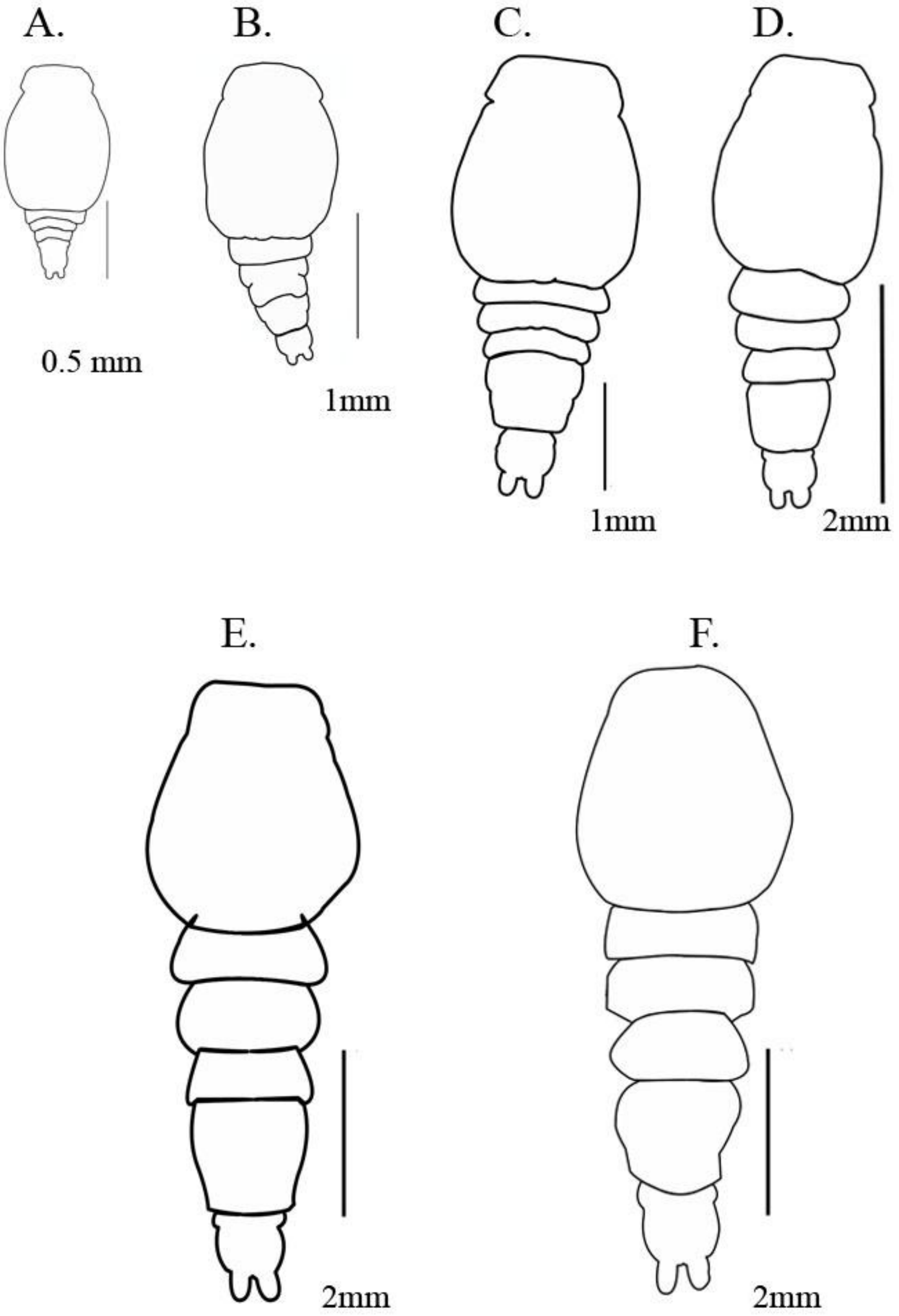
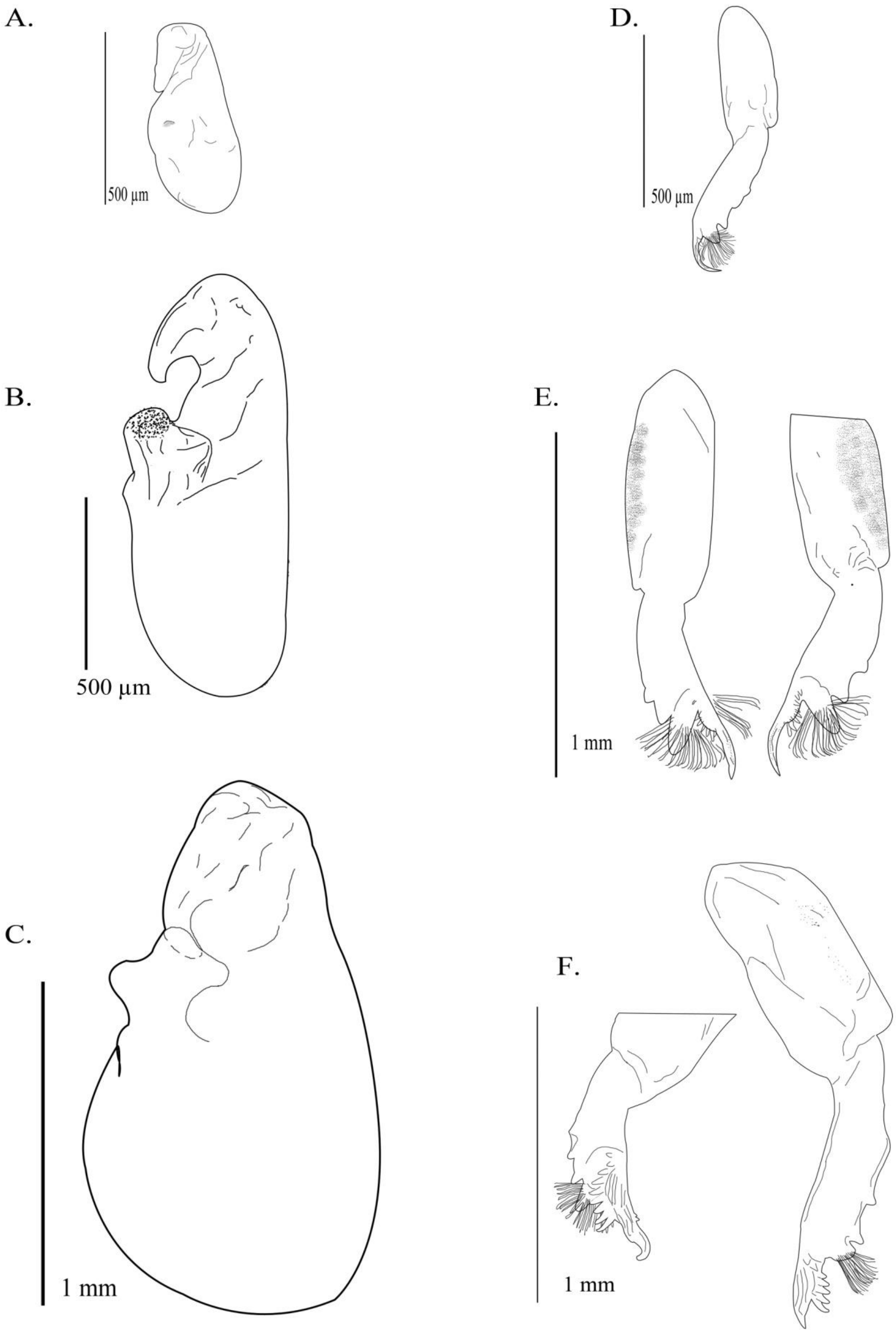


2.5



2.6



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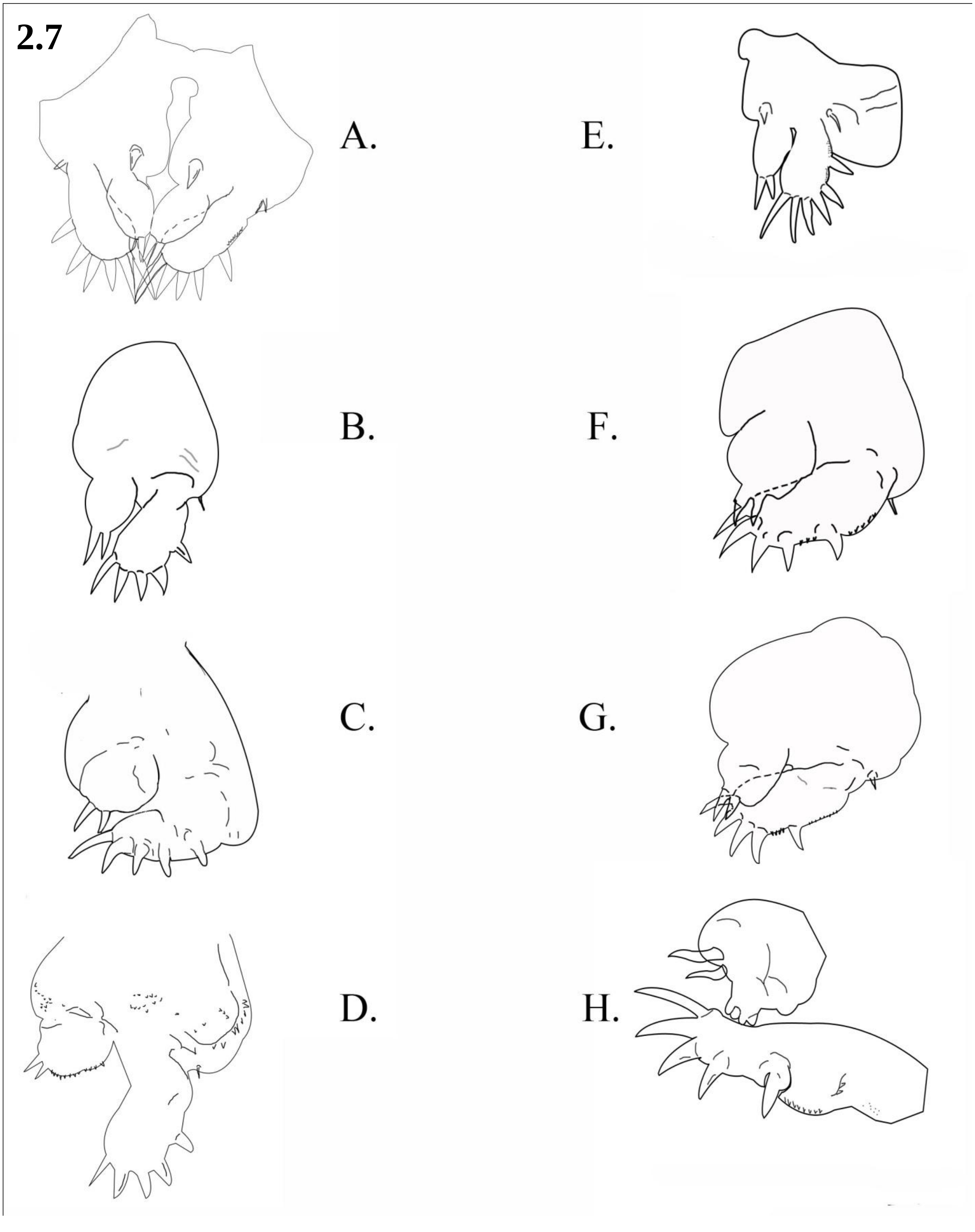
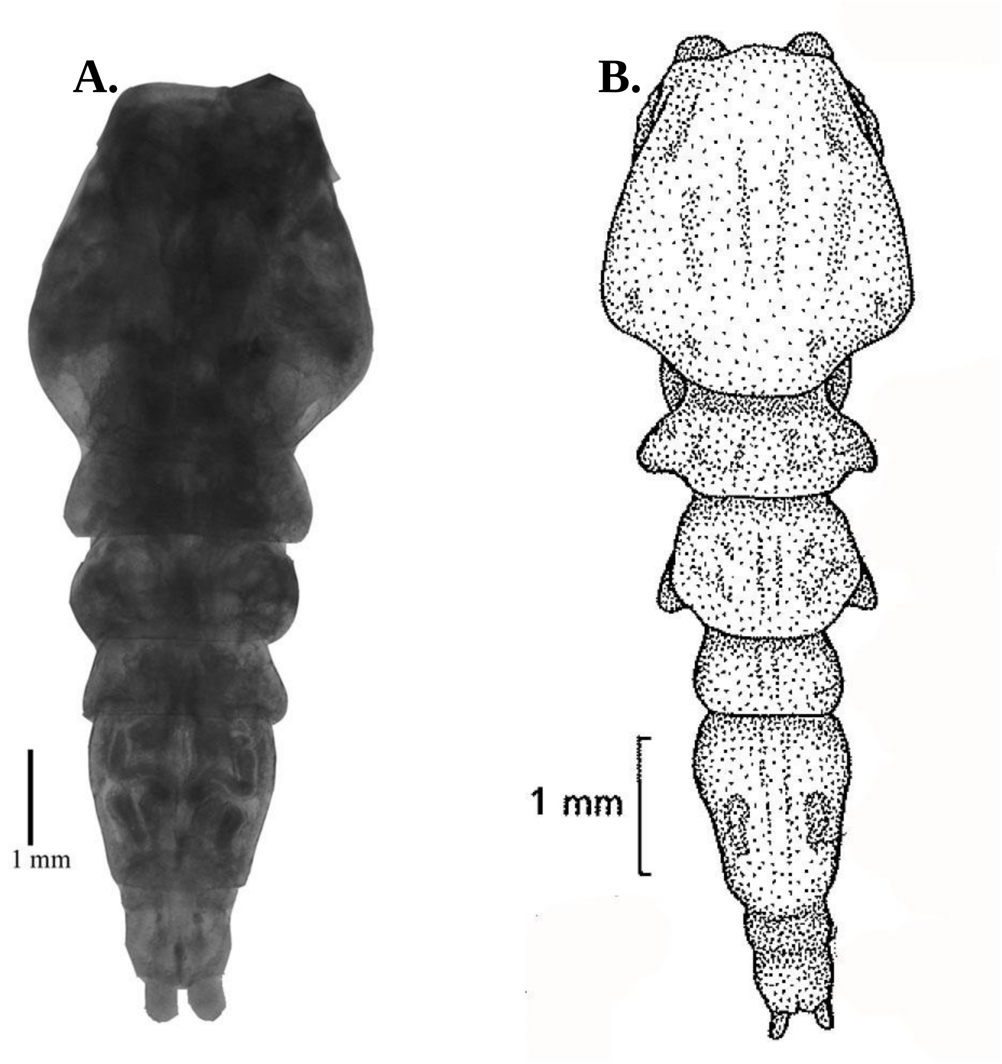
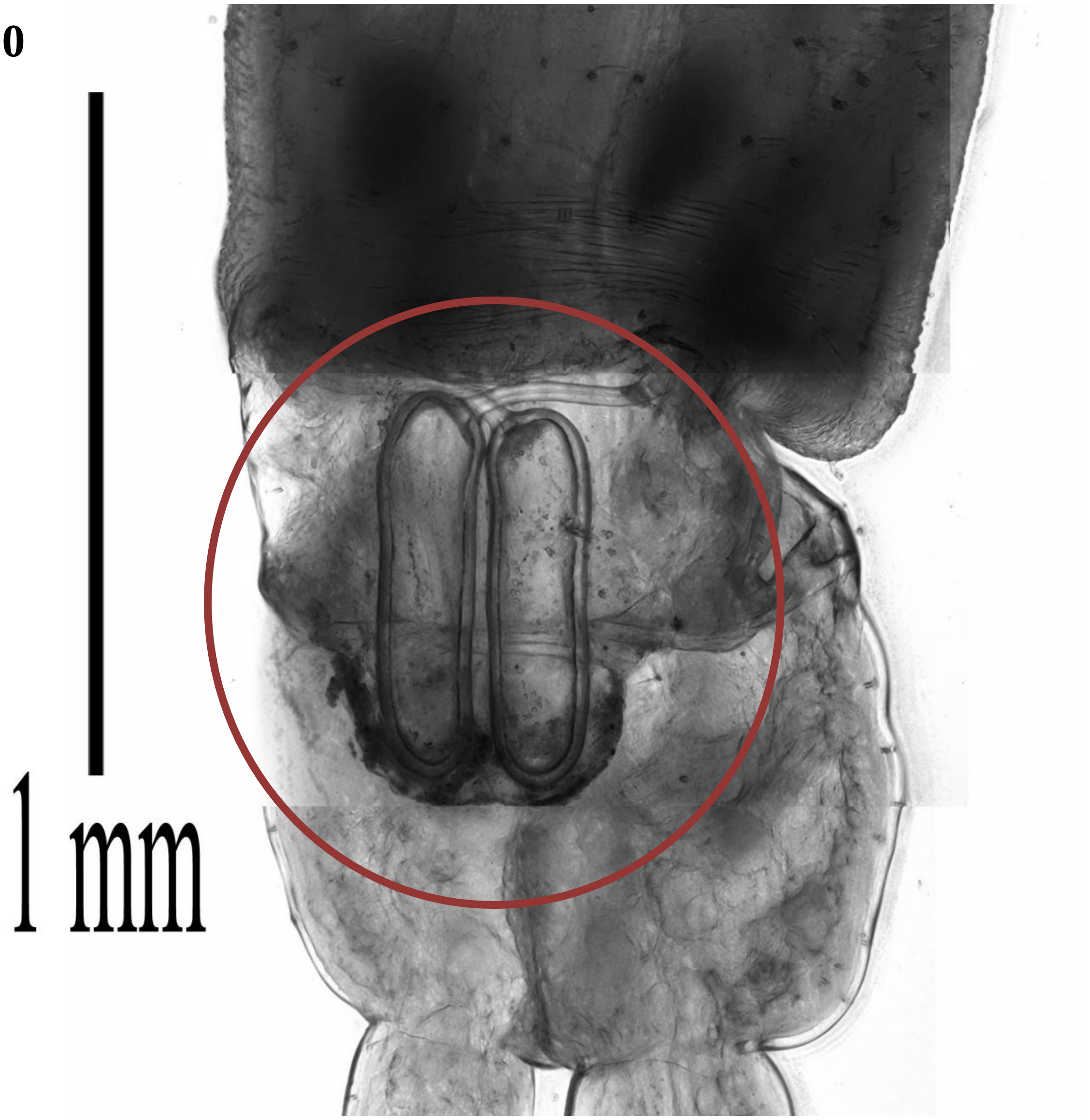


2.8




2.9
A.
B.
5 mm
5 mm

2.10



CHAPTER 3: TEMPORAL AND SPATIAL DISTRIBUTION OF *DICHELESTHIUM OBLONGUM* STAGES IN ATLANTIC STURGEON (*ACIPENSER OXYRINCHUS OXYRINCHUS*) POPULATIONS

Introduction

Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) are large anadromous fish. Young sturgeon spend up to the first 6 years of their lives in their natal rivers, as juveniles they begin their marine migrant stage between 7-16 years, until they mature and return to freshwater to spawn. Atlantic sturgeon are long-lived and late maturing, making them susceptible to overfishing and environmental changes. Populations have been declining since the 1800s, resulting in a recent fishing moratorium enacted in 1998 (ASSRT 2007(Dovel and Berggren 1983). Currently the largest population of Atlantic Sturgeon is found in the Hudson River in New York. However despite extensive mixing in coastal waters, the most recent studies on genetic diversity of Atlantic sturgeon suggest between 7 and 10 different populations exist along the East coast of North America (King et al., 2001; Wirgin et al., 2002; Waldman et al., 2002). These populations are in decline, mostly from by-catch mortality and habitat degradation (ASSRT 2007). Another historically large population is found in the Delaware River, the population in this area is at historical lows, reportedly decreasing form 5,600 to 1,000 in a four year period (ASSRT 2007). Recently it was found the Chesapeake Bay population had genetic contributions from of Hudson River, Delaware River and Chesapeake Bay sturgeon (King 2001). The Chesapeake Bay and its tributaries along with the Delaware River have been identified as spawning rivers, though little is known about the spawning populations of the Delaware (ASSRT 2007). Another smaller population of Sturgeon is found in the Connecticut River, though due to the Holyoke dam, it is unknown whether this site is still a big spawning river (ASSRT 2007). Spawning migrations occur in the spring and early summer (ASSRT 2007) and is when most sturgeon mortalities in rivers have been reported. The southern feeding aggregations of Hudson River juveniles have been observed, during the summer and autumn along the south shore of Long Island, NY, as they begin the marine migrant stage (Dunton *et al. In Press*). Individuals sampled from these areas are heavily infected with the copepod parasite *Dichelesthium oblongum* (Fast *et al.* 2009).

Dichelesthium oblongum is an ectoparasite of Acipenserids, from the Order Siphonostomatoida. Siphonostomatoids include the Salmon louse *Lepeophtheirus salmonis* which is an ecologically important and economically detrimental parasite (Krkosek *et al.* 2007; Wagner *et al.* 2008). Siphonostomatoids infect many species of fish including sharks along with teleosts (Kabata 1979). They have free-living larvae and attach to host, during juvenile and adult stages. The life cycle of *D.oblongum* (Chapter 2), is similar to that of closely related species, with 2 naupliar stages, 5 attached copepodid stages and an adult stage. A review of *D. oblongum* by Fast, et. al (2009) described increased infection pressure in the autumn, and a crude estimation of generation time of 4-6 weeks, based on gravid female data. With further investigation, we can develop accurate generation time estimates and begin to fully understand the ecology of *D. oblongum* and how and when the sturgeon pick up the infections. In this chapter the ecology and population structure of *D. oblongum* in the New York Bight will be described, using information from freshly collected and archived samples. Using the life cycle described in chapter 2, I will describe how the numbers and proportions of stages change over spatially and temporally. This information is the first step in developing an infection model for this host parasite interaction. Since the Atlantic sturgeon is a near threatened species according to Convention on International Trade of Endangered Species (CITES), with a declining population, elucidating the host-parasite ecological interactions is important for the conservation of this species.

Materials and Methods

Fish Sampling

Juvenile Atlantic sturgeon were captured by bottom trawling using an 80' otter trawl aboard the RV "Seawolf" within juvenile/immature coastal habitat outside of Jones Beach, NY, Rockaway, NY, and Sandy Hook, NJ. Collection trips were conducted during summer of 2010, consisting of several days. Archived samples were also used from trawls which were conducted on the following dates: October 16, 2007; November 15, 2007; September 15, 2008; November 21, 2008; and May 19, 2010, samples were collected from Sturgeon along the south shore of Long Island. Samples were also collected from the mouth of the Connecticut River in September and October, 2009 and the Atlantic Coast of Delaware in April 2010 using gill nets by Drs. Tom Savoy and Dewayne Fox, respectively, during their gill net survey 2009-2010. These were used to supplement data at different times of the year and under different salinity/temperature conditions. All parasites were removed and stored in 10% buffered formalin, or 70% ethanol.

Staging and Quantification

Each individual copepod was measured along with all of the egg strings collected with gravid females. The sex and life stage of each parasite was established using a Nikon Eclipse inverted microscope and Image Pro Digital Imaging. Early stages of *D. oblongum* were distinguished by segmentation, females are less distinctively segmented than males (Kabata 1979) and the cephalothorax of the male is larger in proportion to the body, than seen in the female, in copepodid 2 and later, the formation of the lateral process in the male is the distinguishing character. Stages are distinguished by limb development, segmentation changes and overall size. The stages were identified based on the life cycle described in Chapter 2. In addition to staging information, egg strings were used to determine egg production.

Statistical analysis

Spatial and temporal comparisons between *D. oblongum* infections, female size, eggstring size, and egg number were made using 2-way ANOVA. The effect of size on Delaware and New York sturgeon infections was tested using an ANCOVA to compare regression line slopes. These statistical analyses were carried out using SigmaStat software package (Systat software, Inc.).

Results

New York

Prevalence of *D. oblongum* in the New York sampling areas is between 65% and 100% (October 2007, 63%; November 2007, 81%; September 2008, 100%; November 2008, 86%). The parasites are mainly found in the gills of Atlantic sturgeon but, some are found along the body and in or around the mouth (Figure 1). Most (68%) of the individuals found outside of the gill are juvenile stages, with some adult males but no adult females. Over time, in New York the sex ratios in the population remain fairly steady, with females making up the majority, about 70% (Figure 2). This is consistent with previous work (Fast et al. 2009; Fast *et al. In press*).

The most common life stage of *D. oblongum* observed on Atlantic sturgeon were adults, specifically female adults. A larger percentage of adults was found during October and November than in September. This trend is evident in both males and females (Figure 3). Most juvenile stages appear in early autumn and progress in later autumn to later developmental stages, without the observation of apparent new infections. Similarly, in September there are more infections found outside of the gills (Figure 1). The collection on May 19, 2010, was our only spring sample from the South Shore of Long Island, unfortunately, we had a very small sample size (n=9). From this sample we observed a prevalence of ~ 60% and an abundance of 16 total *D. oblongum*. These were all made up of gravid female and a few adult male stages, without the presence of juveniles.

All *Dichelesthium oblongum* fecundity measurements (egg string size, eggs/gravid female, etc.) increased in November of 2007 and 2008 in the South Shore samples, though not all significantly (Figures 4 and 5). The egg production by *D. oblongum* females averaged between 900-1400 eggs/ host throughout the autumn, and a maximum of 3825 eggs were produced on one host. From 71-87% of the adult females were found with egg strings, averaged 96-113 eggs/egg string during the autumn sampling along the South shore (Figure 4 and 5). The maximum egg string length recorded was 27 mm containing 317 eggs, and 605 eggs over both pairs for that female.

Aside from temporal differences in stage development, there were some differences in the population compositions of *D. oblongum* spatially as well. Samples were also collected in and around the Connecticut River. The use of gill nets and presence of stronger freshwater inputs within this sample set (i.e. site salinities were not always recorded), makes direct comparisons with the South Shore data difficult. Only 10 sturgeon were collected over the September 2009 and October 2009 sampling periods. There was a 60% prevalence of *D. oblongum* with low parasite burdens, between 1 and 4 parasites per fish. There were two juvenile stages collected, a copepodid 4 female and a copepodid 5 male. The rest of the parasites (n=16) were adults, including five gravid females. The egg strings of the gravid females on the Connecticut fish (*L=7.25±0.94mm, n=10*) were on average shorter than those collected in New York (*L=10.8±0.29, n=557).*

Delaware

The sampling in Delaware was also conducted using a gill net as well. The Delaware sampling took place during the month of April 2010. There was a much lower prevalence (44%) as compared to the New York samples (>80%). Fewer juvenile stages were also observed in the Delaware samples, only a few stages, nine stage 5 copepodids were present. Though the infected Delaware fish were larger (183±4.2cm, n=55) than those caught in New York (117±3.6cm, n=66) they did not have a significant difference in the number of total *D. oblongum* or adult female *D. oblongum* found on the individuals, compared to those in New York (Figures 6 and 7; ANCOVA, P>0.05). The egg strings of the gravid females on the Delaware fish were significantly larger (*L=20.0±0.41mm, n=176)* (ANOVA, p<0.05) than those collected in New York (*L=10.8±0.29, n=557)*. The size of adult females from Delaware fish were not significantly different from the NY fish (*L=12.06±0.18mm, n=72)*.

Discussion

When looking at both the New York and Delaware collections, a general trend of increased adult stages (particularly females) and decreased juvenile stages from early (September) to late (November) Fall, was observed. Furthermore, very few juvenile stages were observed in the spring (April-May). This would suggest the peak infection pressure from infective copepodid stages is most likely in the summer and early autumn months, which would agree with both wild and farmed data for Atlantic salmon infected with *Lepeophtheirus salmonis* and *Caligus elongatus* (Revie *et al.* 2002; Tucker *et al.*

2002; Heuch *et al.* 2003; Lees *et al.* 2008). A build-up of adult stages late in the year is a feature of the natural life cycle of *L. salmonis* on sea trout (*Salmo trutta*) and occurs because the adult stages live longer than the other stages of the parasite life cycle, and adult longevity is inversely correlated with temperature (Schram *et al.* 1998; Nordhagen *et al.* 2000). Accumulation of adult stages, particularly females, over winter may be part of the reproductive strategy of *D. oblongum* and other copepod parasites, such as *L. salmonis* (Schram et al. 1998; Heuch et al. 2003). For *L. salmonis* it is well described that copepodid (initial infective stage) fitness is reduced during the winter and decreased establishment of these copepodids occurs at 7°C vs. 12°C (Pike and Wadsworth 2000; Tucker *et al.* 2000). Therefore, development and release of eggs is delayed during this time, only to have increased likelihood of successful infection in spring and summer when water temperatures increase. The longer egg strings associated with smaller gravid females in the Delaware spring collection, would also support this occurring in *D. oblongum*. However, until we are able to obtain significant numbers of samples in June-August, and over winter, these will remain untested hypotheses for *D. oblongum* infections of Atlantic sturgeon.

Generation time estimates:

Fast et al., (*in press*) crudely estimated the generation time of *D. oblongum* to be around 4-6 wks. Since all fish at Rockaways and Jones Beaches were caught in 8-10 M depth, and Sandy Hook 16-18 M depth, we can estimate the degree day (dd) age per life stage using mean surface water temperatures (Sept. 20.5°C, October 16°C and November 12.5°C) along the South Shore of Long Island (Station ALSN6 - Ambrose Light, NY).

Making an assumption that there are no new infections in Atlantic sturgeon after the beginning of September, based on that being the only month with the Copepodid I life stage in our collection, in 2007, it took 30 days at approximately 14.3^{o}C [mean Oct/Nov] (427 dd) for male *D.oblongum* to proceed 1.5-2 life stages (i.e. copepodid III to copepodid IV/V), which would be around 213 dd per life stage between 12.5-16^{o}C. In 2008, it took 57 days at approximately 16.3^{o}C [mean Sept/Oct/Nov] (929 dd) for male *D.oblongum* to proceed 2-3 life stages (i.e. copepodid I/III to copepodid IV/adult), which would be around 310 dd per life stage between 12.5-20.5^{o}C. Female *D. oblongum*, also appear to develop 2-3 life stages over this 1-2 month period. These rather rudimentary estimates, on a limited survey are much longer than that observed for Caligid copepods. This may be an adapted condition for this primitive parasite. Salmon are known to return to their natal rivers every other year, insuring areas of aggregation, relatively frequently. Atlantic sturgeon are known to experience a marine migrant stage from 7 to 16 years, before reaching sexual maturity, little is known about this life stage of the sturgeon. A longer life cycle, would be advantageous to *D. oblongum,* with lower metabolic costs, during a time when transmission is unlikely.

In comparison, the Northern Atlantic parasitic copepod species we know the most about, *L. salmonis,* could be estimated at around 50 dd per life stage at 9-11^{o}C on Atlantic salmon (Johnson, 1993). For *L. salmonis*, slower development has been reported on more resistant (*Oncorhynchus spp*.) hosts (Johnson and Albright 1992; Johnson 1993) and within the host gills compared to body surfaces (Johnson and Albright 1992), though the latter was not confirmed by Johnson (1993). Although *D. oblongum* is generally found in the gills of its host, and Atlantic sturgeon may not be as susceptible a host for *D. oblongum* as Atlantic salmon are for *L. salmonis*, we will need a much more complete data set before we can suggest a 4-6 times longer development in *D. oblongum* than *L. salmonis*.

Spatial Diffferences

There were several differences between the sturgeon and parasite populations. Again, due to the time of year (spring) and sampling method (gill net) used in the Delaware collection, direct comparisons with New York data will not tell the full story. If *D. oblongum* adult females overwinter on their hosts at low densities, similar to that observed in *L. salmonis*, prevalence and abundance would be expected to be lower in Delaware than in the South Shore data. The sturgeon caught in Delaware were bigger than fish caught in New York, and although their prevalences were lower their parasite burden was relatively the same (Figures 6 and 7) ,Tucker et al. (2002) found that smaller hosts (40 g) were more susceptible to laboratory infections with *L. salmonis* than larger hosts (170-600 g). Epidemiological data from farm infections of Atlantic salmon also support this (Heuch *et al.* 2002). Targeted sampling in both areas using the same gear and over the same temporal scales are a must to determine if larger sturgeon or those from different river systems are more/less susceptible to *D. oblongum* infection.

Future Work

A force-of-infection model could be used to further understand host-parasite interactions. Using the equation $\pi(t) = 1 - e^{(-\lambda t)}$, where $\pi(t)$ is cumulative incidence (proportion of positive individuals) at time t (days post-infection), force of infection (λ) can be determined. Through the acquisition of more temporal and spatial data the development of a model of this type could be important for our understanding of the host-parasite relationship and possibly for conservation efforts for Atlantic sturgeon. Other important aspects of this model would include, understanding the hydrology of the sampling sites. The hydrology would be a factor in initial establishment of the parasites on the host, i.e. the infective copepodid stage finding a host. Also the migration and aggregation patterns of Atlantic sturgeon would be important to understand. With current transmitter tagging experiments and receiver arrays throughout the NY/NJ aggregation areas, our understanding of host habitat usage and therefore environmental conditions and host density patterns encountered by the parasite, will drastically improve our understanding of this interaction. The changing salinities, temperatures and pollution they swim through during their marine migrant and adult stages could negatively affect the parasite and the host. There is little known about the marine migrant stage of Atlantic sturgeon. If there are few aggregations during this time, *D. oblongum* may not be able to infect new individuals. Sturgeon may only pick up new infections in coastal waters, near known aggregation sites. *L. salmonis* is known to have a range throughout the Pacific Ocean (Costello 2006), due to transmission by their hosts, it is unknown if this could be possible for *D. oblongum*.

Future work should include more sampling at more frequent intervals along the South shore of Long Island. Some sampling should be done near the mouth of the Hudson and in other sturgeon rivers, to determine if *D. oblongum* can survive in freshwater and how the lower salinity might affect it. Also infection studies in a lab are important for determining further information about life stages and generation times. Tank experiments could control salinity and temperature for development, and determine the length of time each life stage lasts. All of this information would improve the model and understanding of the host-parasite ecology.

Figure Legend

Figure 3.1: Percentage of the *D. oblongum* population found on the body, gills, and mouth of Atlantic Sturgeon collected from the south shore of Long Island, NY in 2007 and 2008

Figure 3.2: Percentage of Males and Females in the *D. oblongum* populations collected from Atlantic Sturgeon along the south shore of Long Island, NY in 2007 and 2008

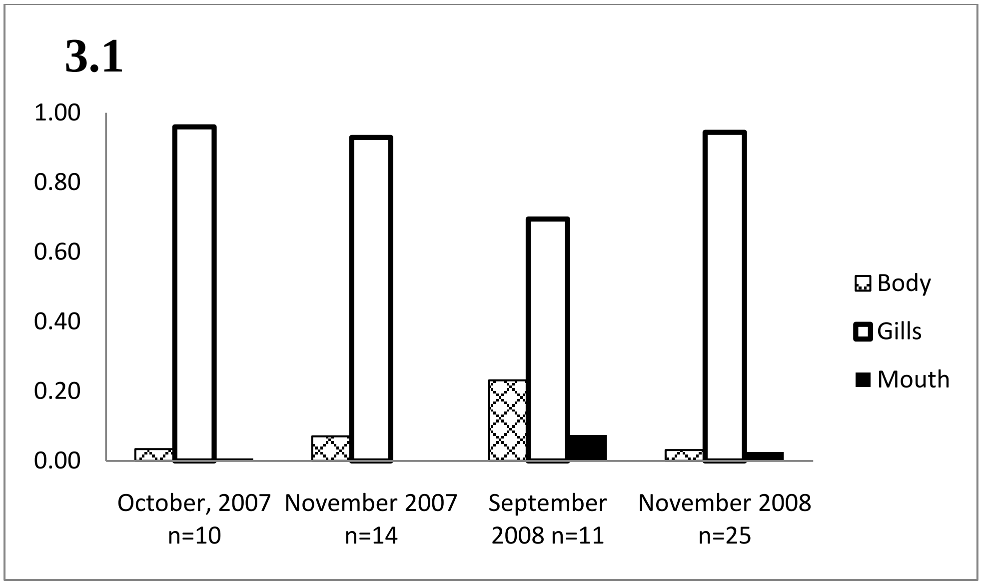
Figure 3.3: Percentage of different life stages of the *D. oblongum* (a) males, and (b) females collected from Atlantic Sturgeon along the south shore of Long Island, NY in 2007 and 2008

Figure 3.4: Temporal distribution of eggs/egg string (ES) and eggs/gravid female (GF) collected from Oct. 2007-black bar; Nov. 2007-grey bar; Sept. 2008-hashed bar; Nov. 2008-white bar. *denotes significant differences over time

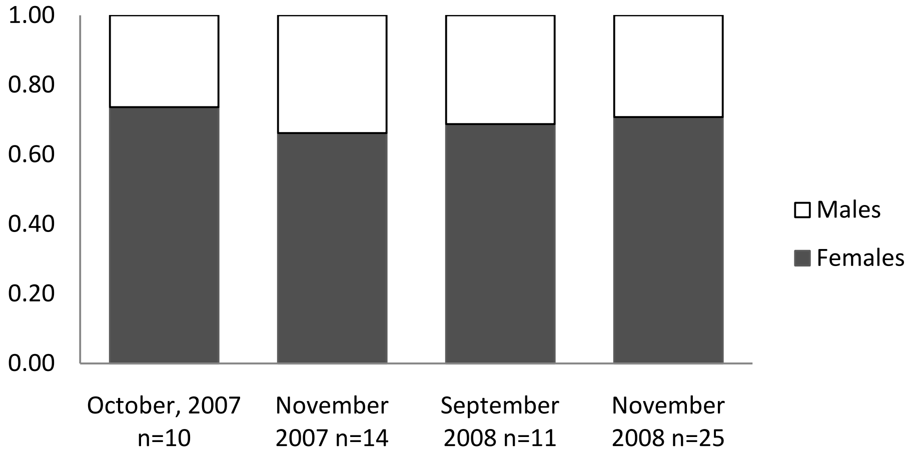
Figure 3.5: Temporal distribution of gravid female (GF) size (mmx10), egg string (ES) size (mmx10) and eggs/egg string size (ESS-mmx10) [i.e proxy for egg size/batch quality] collected from Oct. 2007-black bar; Nov. 2007-grey bar; Sept. 2008-hashed bar; Nov. 2008-white bar. *denotes significant differences over time

Figure 3.6: Number of individuals of *D. oblongum* on each infected Atlantic Sturgeon as related to the mass (kg) (a) and tail length (cm) (b) of each sturgeon collected from sites in New York and Delaware

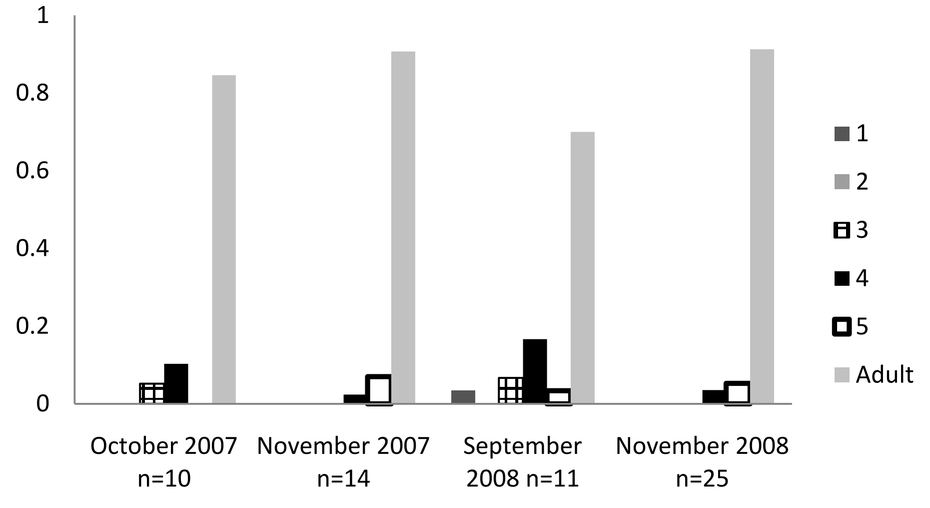
Figure 3.7: Number of gravid females of *D. oblongum* on each infected Atlantic Sturgeon as related to the mass (kg)(a) and tail length (cm) (b) of each sturgeon collected from sites in New York and Delaware


3.1
1.00
0.80
0.60
0.40
0.20
0.00
October, 2007 n=10
November 2007 n=14
September 2008 n=11
November 2008 n=25
Body
Gills
Mouth

3.2

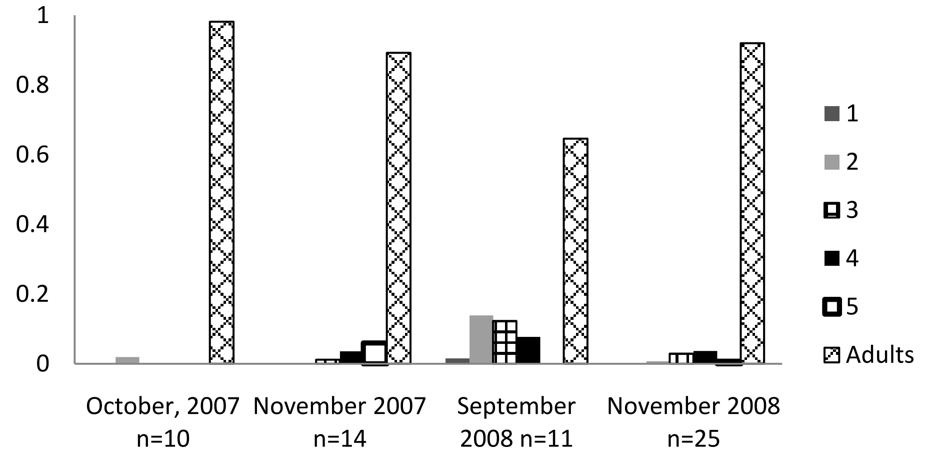

1.00
0.80
0.60
0.40
0.20
0.00
October, 2007 n=10
November 2007 n=14
September 2008 n=11
November 2008 n=25
☐ Males
■ Females

3.3a



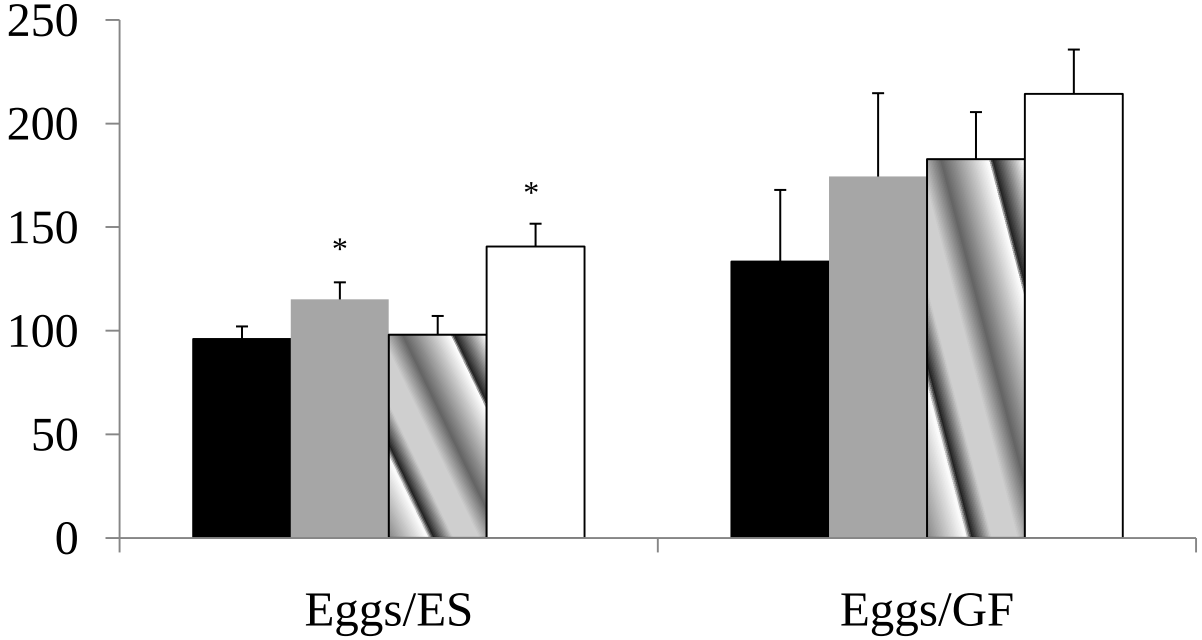
1
0.8
0.6
0.4
0.2
0
October 2007 n=10
November 2007 n=14
September 2008 n=11
November 2008 n=25
1
2
3
4
5
Adult

3.3b

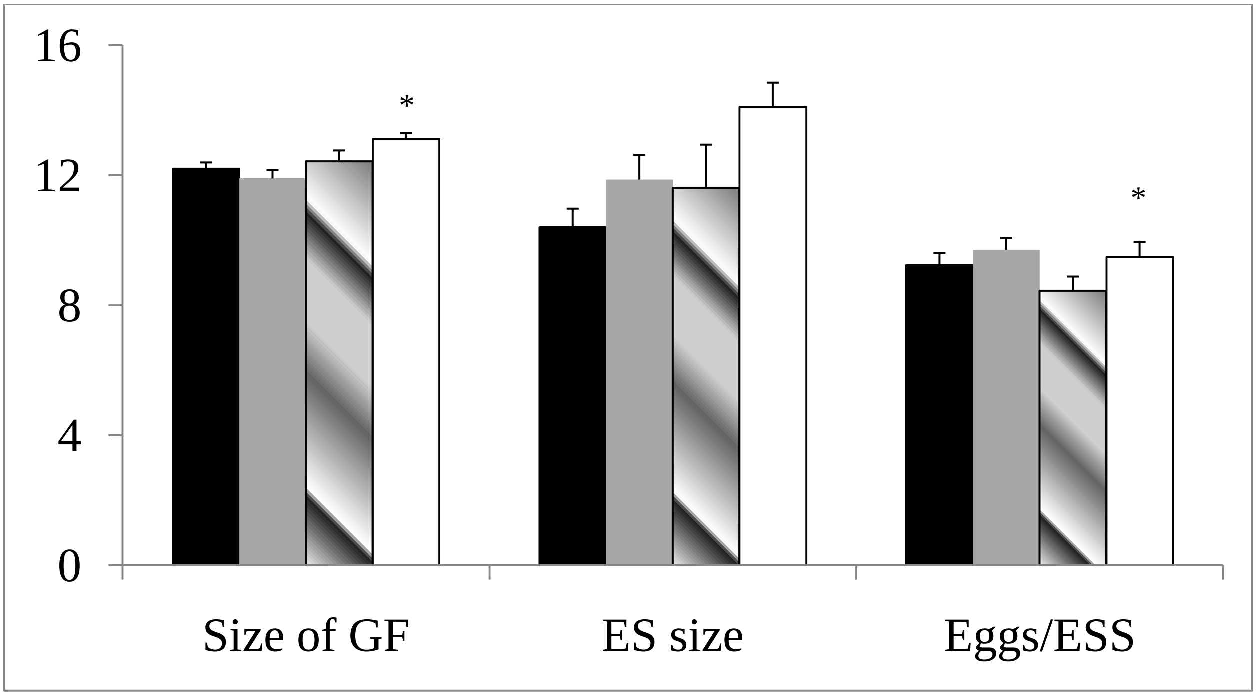


1
0.8
0.6
0.4
0.2
0
October, 2007 n=10
November 2007 n=14
September 2008 n=11
November 2008 n=25
1
2
3
4
5
Adults

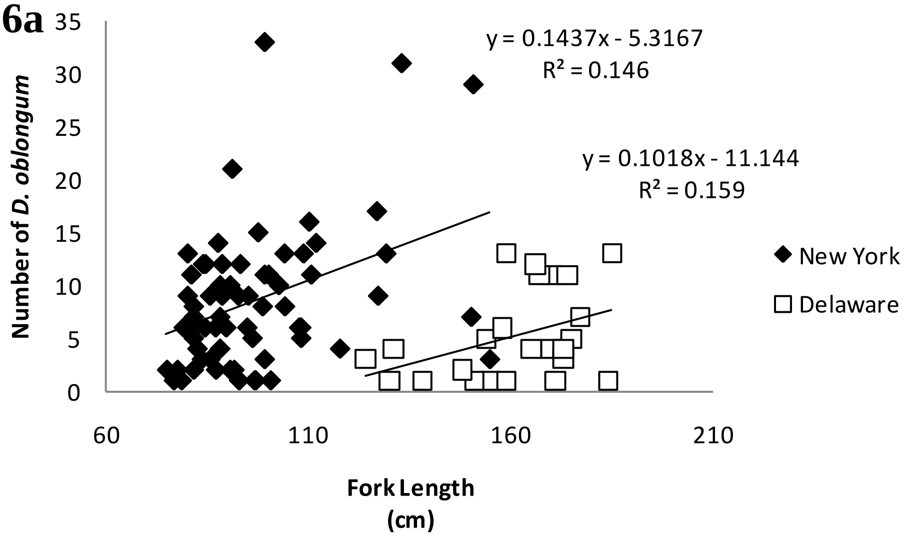
3.4



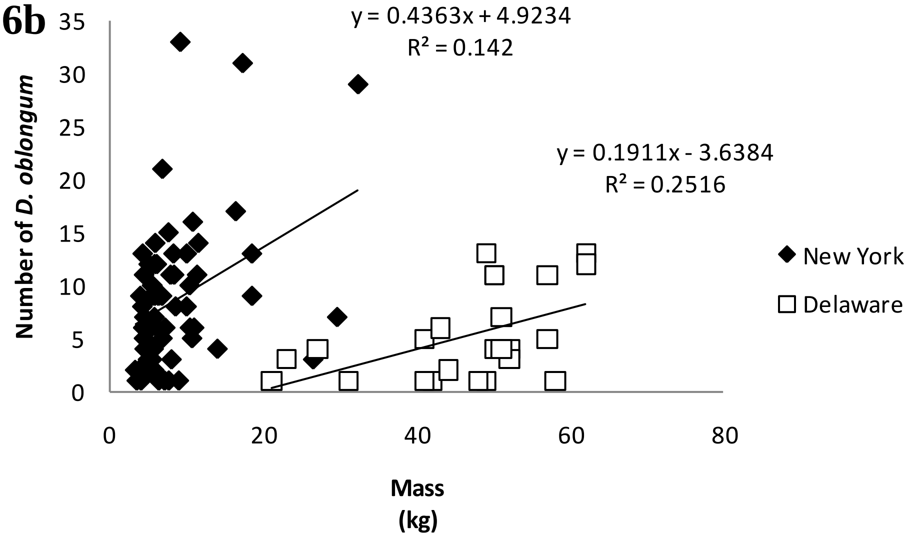
3.5



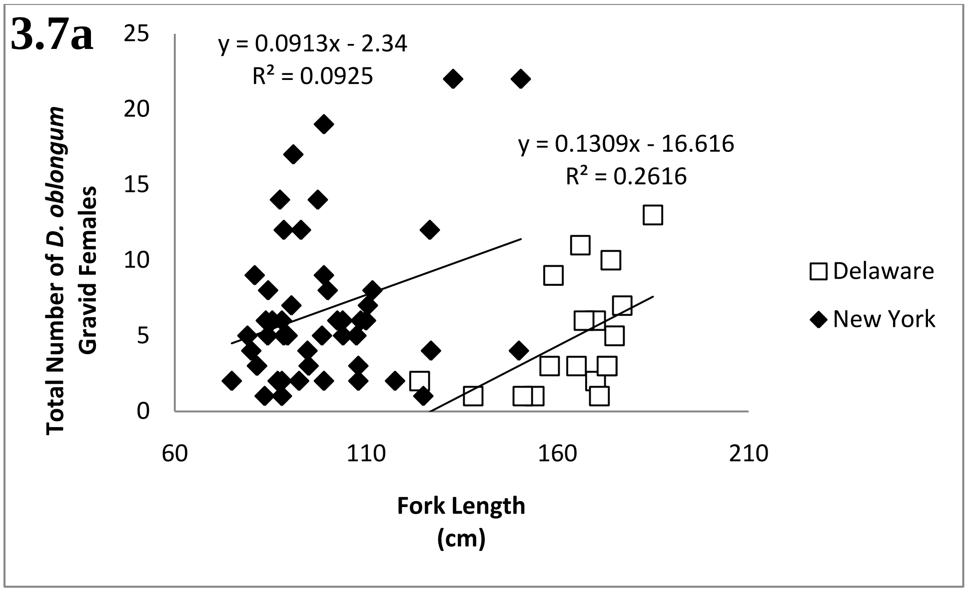
3.6a


y = 0.1437x - 5.3167
R² = 0.146
y = 0.1018x - 11.144
R² = 0.159
Number of *D. oblongum*
35
30
25
20
15
10
5
0
60
110
160
210
Fork Length
(cm)
◆ New York
☐ Delaware

3.6b

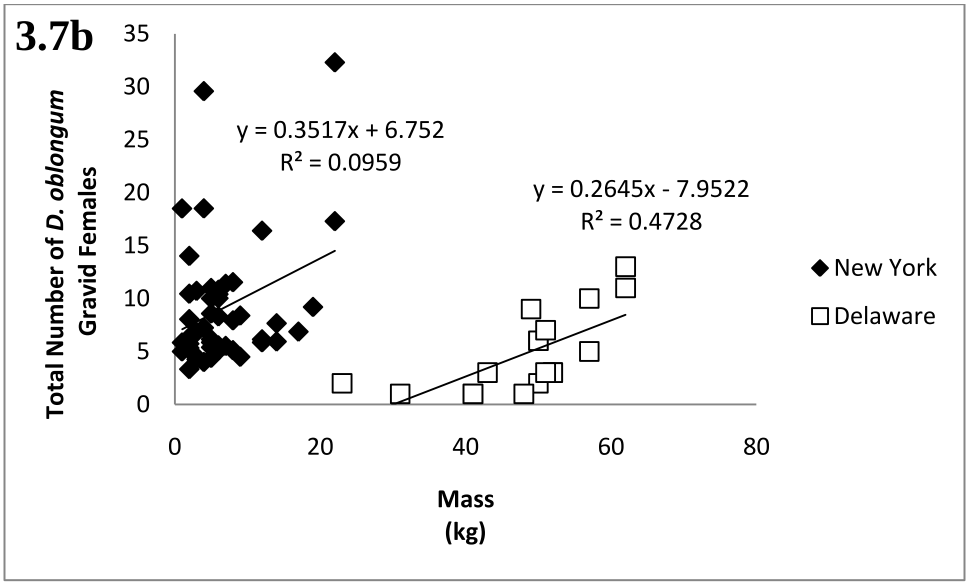

y = 0.4363x + 4.9234
R² = 0.142
y = 0.1911x - 3.6384
R² = 0.2516
Number of *D. oblongum*
35
30
25
20
15
10
5
0
0
20
40
60
80
Mass
(kg)
◆ New York
☐ Delaware

3.7a



y = 0.0913x - 2.34
R² = 0.0925
y = 0.1309x - 16.616
R² = 0.2616
Total Number of D. oblongum Gravid Females
25
20
15
10
5
0
60
110
160
210
Fork Length (cm)
Delaware
New York

3.7b



y = 0.3517x + 6.752
R² = 0.0959
y = 0.2645x - 7.9522
R² = 0.4728
Total Number of D. oblongum Gravid Females
35
30
25
20
15
10
5
0
0
20
40
60
80
Mass (kg)
New York
Delaware

CONCLUSIONS

Summary

The goals of this project were to describe the complete life cycle of *Dichelesthium oblongum* and use archived samples to understand the ecology and biology of the host parasite relationship between *D. oblongum* and Atlantic sturgeon in the New York Bight.

What is the complete the life cycle of D. oblongum?

Through the process of this study a complete life cycle was determined for the primitive siphonostome *Dichelesthium oblongum.* It has a total of 9 life stages: 2 naupliar stages, an infective copepodid stage, 5 attached parasitic copepodid stages and an attached adult stage, as seen in *Lernanthropus kroyeri* (Cabral *et al.* 1984). Prior to this paper, only the adult stage and infective copepodid stage of *D. oblongum* had been described (Kabata and Khodorevsky 1977; Kabata 1979). Some other interesting trends were seen in *D. oblongum* morphology specifically, the development of the myxal area on the 2nd antennae, the drastic body elongation seen in the female, and the development of a lateral process on the endopod of the second leg. Also, previously undescribed aspects of Dichelesthiidae biology are documented in this study. The adult males in the archived samples differed slightly from the adult male described by Kabata (1979). This shows that there is some morphological diversity within the species. Cuticular folding in the immature adult female was also observed, this allows for elongation without molting, cuticular folding is also seen in other Siphonostomatoid parasites. Spermatophores were seen on adult females, displaying crossed tubules, this is thought to be an ancestral characteristic further confirming that Dichelesthiidae is a primitive family and may have evolved earlier, considering its host is an ancient fish.

What is the generation time for D. oblongum within the NY Bight?

Using all of the archived samples which included all attached stages of *D. oblongum* generation time was able to be estimated. Because we found younger individuals in September, and a reduction in juvenile stages in October and November, we were able to estimate a generation time of 213 dd per life stage for the male and 310 dd per life stage in the female. This is much longer than what is seen in other caligid copepods.

Do new infections establish themselves throughout the year or occur once?

Based on the life stages collected during the fall of 2007 and 2008, at least over a three month period, there seems to be only one infection pulse. This occurs during the late summer and early fall, this was the time when there was the highest number of juvenile stages. Also Jones Beach seems to be a location of high infection rates, and is most likely a source of parasitic infections. If Jones Beach acts as a hotspot for infections, the development of an infection source model similar to that developed by Krkosek *et al.* for salmon farms could be used to identify potential infection spread to other aggregation areas. (Johnson *et al.* 2004; Krkosek *et al.* 2007; Krkosek *et al.* 2008).

What is the production of infective stages of D. oblongum for the NY Bight?

A majority of the parasites collected from Atlantic sturgeon were gravid females. The measures of increased from summer to fall over the two sampling years. The egg string length seems to increase from September to November, possibly due to the maturing of immature females, and more time allowed for more egg production. The number of eggs per mm of egg string stays constant both spatially and temporally. Samples collected in the spring, in Delaware had significantly longer egg strings than those collected in NY, this could be a reproductive strategy, like the one employed by *L. salmonis*, in which the attached egg string from the fall are kept through the winter, until the warms, resulting in longer egg strings in the Spring (Tucker *et al.* 2000). Also, even though the fish samples in Delaware were much larger than those sampled along the south shore of Long Island, there was not an increase in parasite abundance, as has been observed in other siphonostomes (Tucker *et al.* 2002).

Based on our results *D. oblongum* appears to have similar ecological traits to other siphonostome parasites, including the well studied *L. salmonis.* More extensive data needs to be collected, to further elucidate the host-parasite relationship between *D. oblongum* and Atlantic sturgeon. Information regarding the infective copepodid would be important for the development of an epidemiological model. Knowing the movement patterns of Atlantic Sturgeon within and between aggregation areas, would be useful in identifying sites of disease transmission. The hydrographic data would be useful in identifying and understanding how the infective copepodid is transported throughout those sites. Also the hydrology in coastal areas would affect the salinity and temperature, affecting the growth rate of the parasite. Another interesting factor in this particular host-parasite relationship is anthropogenic input. At Jones beach there are two large sewage outflows. This site also had the highest proportion of juvenile *D. oblongum* infections. Previously these sewage inputs, were attributed to high stress levels in juvenile Atlantic sturgeon (Fast *et al.* 2009; Fast *et al. In press*), possibly increasing the susceptibility of the sturgeon.

Further investigation of the host parasite relationship could help identify evolutionary characteristics of siphonostomes. *D. oblongum* is known to be a primitive parasite, and acipinserids evolved over 200 million years ago. Obtaining a better understanding of life cycles of sturgeon and *D.oblongum*, will help determine basic parasitic strategies for successful settlement on the host and could also be useful in determining common host strategies for fighting off ectoparasitic infection. Specifically, for Acipenserids, understanding the potential negative impacts from their most prevalent pathogens and how they interact with environmental conditions, habitats and stressors, could be incorporated into mortality estimates and management strategies for this already threatened group.

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