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Infection of bluefish (*Pomatomus saltatrix*) ovaries by the dracunculoid nematode, *Philometra saltatrix*: prevalence, intensity, and effect on reproductive potential

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Abstract of the Thesis

Infection of bluefish (*Pomatomus saltatrix*) ovaries by the dracunculoid nematode, *Philometra saltatrix*: prevalence, intensity, and effect on reproductive potential

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In order to better understand the recruitment dynamics of bluefish along the east coast of the U.S., I investigated the prevalence and intensity of an ovarian nematode parasite, *Philometra saltatrix* (Dracunculoidea), and its effect on bluefish reproductive potential. Necropsies were performed on both spawning adults (when infections are most intense) and age-0 fish to determine the life-cycle of the worm, its pathogenesis, and the prevalence and intensity of infection in various life-stages of the bluefish population. Standard histological examinations of ovaries were conducted to evaluate pathologies associated with the infection. The effects of these pathologies were quantified by scaling the ovarian damage, developmental stage of the ovaries, oocyte size, and fecundity as they relate to the prevalence and intensity of infection. I hypothesized that high levels of *Philometra* infection in ovaries result in reduced fecundity and are a contributing factor in the inter-annual recruitment variability of bluefish. Results indicated that when using oocyte size as an indicator of egg condition, non-infected fish were on average 6.1 % larger than oocytes from infected fish. Additionally, non-infected fish had relative fecundity that on average was 35.8 % higher than infected fish.

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Acknowledgments

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Introduction

In 2002 and 2003, a dracunculoid nematode, *Philometra saltatrix* Ramachandran 1973, was found in the ovaries of adult bluefish (*Pomatomus saltatrix*) collected from New York waters (Clarke *et al*. 2006). The nematode was also found occasionally in the pericardium of juvenile fish. This species was first described from specimens collected in Connecticut waters in 1965, and until a redescription by Moravec and Genc (2004), and Clarke *et al*. (2006), there has been only one reference to it in the published literature, an abstract describing the occurrence of philometrid worms in the pericardial sac of juvenile bluefish (Cheung *et al*. 1984). Helminths of the family Philometridae (superfamily Dracunculoidea) are often parasites of the body cavity and tissues of many marine and freshwater tropical and sub-tropical fishes worldwide (Moravec *et al.* 2003). Philometrids are viviparous and descriptions generally exist only for females, since males and larvae are near microscopic in size.

Studies of the effects of philometrid infection on fish ovaries are rare, and at best, merely suggest that ovarian infection can cause a decrease in fecundity. Ramachandran (1975) briefly described necrosis in the ovaries of striped mullet (*Mugil* cephalus) due to *Philometra cephalus*, and Oliva *et al*. (1992) proposed that philometrid infection in Chilean sea bass (*Paralabrax humeralis*) reduces the effective volume of the ovaries, thereby resulting in reduced fecundity. Moravec *et al*. (1997), Hesp *et al*. (2002), and Moravec *et al*. (2002) mentioned ovarian damage due to *Philometra* infection in various reef-fishes but made no attempt at assessing or quantifying this damage in terms of its potential for reducing fecundity. Since none of the above studies present any quantitative

data, the extent to which ovarian philometrid infection affects fecundity in bluefish or other fishes is unknown.

Bluefish have long been one of the most important recreational fish species along the Atlantic coast of the U.S., with recreational landings between 1974-2001 averaging 22,000 metric tons (MT) per year (Lazar and Gibson 2002). Since the 1980s, however, bluefish stock abundance has declined sharply. In 1986 the recreational bluefish catch along the east coast was approximately 42,000 MT while in 2000 the catch was 4,500 MT, a decline of over 90% (Lazar and Gibson 2002). In recent years catches have made moderate gains with recreational catches in 2002 and 2003 of 5,300 MT and 6,100 MT, respectively (NMFS 2004). Further gains were made in 2004 with catches totaling 7,176 MT and in 2005 totaling 8,662 MT (NMFS 2007).

Reasons for the decline in bluefish abundance, as well as for its recent rebound, are unknown, but proposed causes include overfishing, natural fluctuations in abundance, a shift in habitat use to offshore waters, interspecific interactions with striped bass, and subsequent years of low recruitment (Conover and Munch 2002). With the exception of fairly strong year classes in 1988 and 1989, recruitment since the mid-1980s has declined (Munch and Conover 2000, ASMFC 2002). This suggests that the reduction of the bluefish stock can be attributed to poor recruitment (Conover *et al*. 2003).

Bluefish are unusual in that they have a bimodal temporal and spatial pattern in spawning and recruitment, with distinct spawning episodes occurring in the South Atlantic Bight (SAB: from Cape Canaveral, Florida to Cape Hatteras, North Carolina) from March-May, and in the Middle Atlantic Bight (MAB: from Cape Hatteras to Cape Cod, Massachusetts) from June-August (McBride and Conover 1991, McBride *et al*.

1993). Larvae are hatched in the SAB along the edge of the Gulf Stream and most are advected northward to the MAB, while others remain in the SAB and eventually recruit to nearshore waters (Kendall and Walford 1979, Hare and Cowen 1996). After about 60 days, juveniles recruit to estuarine and surf-zone waters in the MAB (Kendall and Walford 1979). At this time the fish are generally about 40-60 mm in length and begin to shift from a zooplanktivorous diet to a piscivorous one (Marks and Conover 1993, Juanes and Conover 1995). The summer spawning event occurs in continental shelf waters of the MAB and peaks in late July. Juveniles of that cohort usually appear in coastal waters by mid-August (McBride and Conover 1991). In autumn both the spring and summer-spawned cohorts migrate to outer-shelf waters of the SAB where they spend the winter (Munch and Conover 2000).

Recruitment of each of these multiple cohorts to the population varies from year to year and from decade to decade. In the 1980's, when stock biomass was at its maximum (ASMFC 2002), spring-spawned young-of-the-year (YOY) fish were much more abundant than summer-spawned YOY, whereas in recent years, summer-spawned fish have predominated (Munch and Conover 2000, Conover *et al*. 2003, Taylor *et al*. 2006). Recent studies, however, show that this dominant summer-spawned cohort contributes much less to the adult population than the spring-spawned cohort (Conover *et al*. 2003, Scharf *et al*. 2006). Clearly, variation in recruitment is dependent upon the proportional contribution of each of these multiple cohorts to the adult population.

Like most marine fishes, bluefish recruitment is highly variable and can be attributed to a variety of biotic and abiotic factors including diet and growth (McBride and Conover 1991, Juanes and Conover 1995, Buckel *et al*. 1999), physical

oceanographic processes (Juanes and Conover 1995, Hare and Cowen 1996, Munch and Conover 2000), and habitat use (Able *et al*. 2003). With the singular exception of Marks *et al*. (1996), the effects of parasitism on bluefish recruitment have not been examined. This is the first study that attempts to evaluate and quantify *Philometra*-induced ovarian damage in a fish, thus enabling an estimation of the proportional loss in fecundity and its effect on recruitment to the adult population. Since *Philometra saltatrix* had not been observed for decades, its sudden reappearance is all the more striking given that a causative relationship could exist between its recurrence and the low bluefish recruitment that has taken place over the past 15-20 years.

The purpose of this study was to examine the prevalence and intensity of *P. saltatrix* in adult bluefish, how infection affects bluefish fecundity, and to determine the onset of infection, pathogenesis, and effect on fitness in YOY bluefish. I hypothesized that philometrid infection results in reduced fecundity through direct pathological effects on the ovary and that bluefish acquire these infections as juveniles. Furthermore, this infection may negatively affect YOY fitness through pericardial damage. These factors may substantially contribute to the inter-annual recruitment variability of bluefish.

SPECIFIC OBJECTIVES

1. To track the prevalence and intensity of *Philometra* infection in adult bluefish across multiple years in the South and Middle Atlantic Bights, and to assess their effect on ovarian development and pathogenesis.
2. To estimate any reduction in relative fecundity caused by *Philometra* infection.

3. To determine the size and age at which infection first occurs in YOY fish, and to track the tissue migration of the worm as it develops within its host.

Methods

ADULT BLUEFISH

Adult bluefish were obtained from commercial gill-netters and occasionally from commercial trawlers located in Wanchese, NC and Hampton Bays, NY. North Carolina fish were caught approximately 80 km offshore, east of Oregon Inlet, and New York fish were caught approximately 1-6 km offshore within approximately 35 km of Shinnecock Inlet (Figure 1). Sampling periods were determined by the availability of fish to commercial fisherman in each locale. Sampling in NY waters occurred in 2004 from late May to late October (167 females, 127 males), and in 2005 from mid-June to late September (123 females). North Carolina waters were sampled in 2005 from late March to mid-May (84 females).

Standard necropsies were performed on fresh fish that had been kept on ice for less than 24 hours. Fork length, weight, and gonad weight measurements were obtained. The number of live female *Philometra* and the tissue type in which found were recorded, and the intensity of dead *Philometra* per past-infected tissue type were ranked on a scale of 0-3, with zero indicating the absence of dead worms, and three indicating severe past infection. This method of quantifying the presence of dead worms was used because the worms were almost always encapsulated in layers of fibrotic tissue which made counting impossible. For most specimens only female worms were counted because males and larvae are near microscopic in size. The ovaries of 12 female fish, however, were thoroughly searched with the aid of a dissecting microscope. The gonadosomatic index (GSI) was calculated with the following formula: $GSI = (gonad\ weight \diagup (body\ weight -$

gonad weight)) ×*100*. Initially, male fish were examined along with females but once it became evident that *Philometra* were not infecting male gonads, only female specimens were examined.

Standard histopathological methods (Luna 1968) were used to evaluate damage to ovaries. Specifically, tissue biopsies were taken from the center portion of one of the ovary lobes and placed in histocassettes, dehydrated through a series of increasing alcohol concentrations, cleared in xylene, and then embedded in paraffin. Five µm sections were transversely cut, stained with haematoxylin and eosin, and then mounted on glass slides. Sections were examined on a light microscope at a magnification of 100× and three images per section were taken using a Spot Insight digital camera (Diagnostic Instruments, Inc.). Locations were selected by rolling a six-sided die and moving the microscope stage the corresponding number of fields-of-view in one direction from the edge of the image. The stage was then moved in a perpendicular direction corresponding to a second roll of the die. The first movement was along the long axis of the slide, and the second movement was along the short axis. Directions of movement (*i.e.* left, right, forward, backward) were alternated for each sequence of movements. All images were analyzed using ImagePro Plus software (Media Cybernetics, Inc.). A total of 113 fish (339 images) were analyzed histologically.

Using the identification methods and terminology of Blazer (2002), and Wallace and Selman (1981), tissue pathologies were documented for each image. Postovulatory follicles were nearly identical in appearance to atretic follicles so the two were not distinguished from one another. Each lesion type was scored for extent (focal, multi-focal, coalescing, and diffuse) and severity (mild, moderate, severe). A matrix was

constructed that allowed each lesion to be assigned a score of tissue damage ranging from 0 for no lesion to 6 for a diffuse, severe lesion (Table 1). Scores for each lesion were summed to provide an overall score for each section and these three scores (one from each photo) were averaged to yield an overall score for each fish. To ensure accuracy, this procedure was repeated for half the sections. Since the coefficient of variation of the pathology scores differed by less that 10% the results were considered accurate and repeatable. This score was the primary means by which tissue damage was measured.

A modified version of the methods of Corriero *et al*. (2003) was used to identify and distinguish six different stages of oocyte maturity. The average oocyte diameter for each developmental stage was used as an indicator of egg quality with relatively larger oocytes presumably of higher quality than smaller ones. These measurements were made with ImagePro by averaging two measurements along perpendicular axes passing through the approximate center of each oocyte. To prevent measuring oocytes that were sectioned obliquely and thus are not representative of typical oocyte circumferences, only oocytes that were clearly sectioned through the nucleus were measured and counted. Similarly, only oocytes that were entirely located within the field-of-view were measured or counted.

As a proxy for potential fecundity, developmental stage-specific oocyte counts per field-of-view were used. To account for temporal changes in oocyte maturity, only specimens captured in New York in July (the height of the spawning season) were used. Counts from each of the three images per section were averaged to yield an average stage-specific count per fish. These counts were used to compare relative potential fecundity in non-infected versus infected fish.

YOUNG-OF-THE-YEAR BLUEFISH

YOY bluefish were obtained periodically from when they first appeared in NY waters in spring, until they migrated southward in autumn. Specimens were obtained from shore-zone, estuarine, and inner-shelf waters of southern Long Island, NY by means of beach seining, surface trawling, and angling. In 2004 sampling took place from early June to mid-October (n = 275), and in 2005 from early July to mid-October (n = 259). Necropsies were performed to gauge the prevalence, intensity, onset, and tissue location of infection. The vast majority (n = 385) of specimens were fresh and kept on ice for less than 12 hours prior to dissection but some fish had been previously preserved in 10 % buffered formalin (n = 149). For fresh specimens, the fork length and weight of each fish, number of worms discovered, and the tissue type in which found were recorded, and the intensity of dead, melanin-encapsulated *Philometra* per past-infected tissue type was ranked on a scale of 0-3, with zero indicating the absence of dead worms, and three indicating severe past infection. For preserved fish, specimen weight, and intensities of encapsulated dead worms were not recorded.

Specimens under approximately 150 mm in length were dissected under a dissecting scope in a large Petri dish so that the microscopic *Philometra* males and larvae could be detected. For larger fish, all internal organs were removed and then placed under a dissecting scope for examination. Body cavities of the eviscerated fish were searched with the aid of a hand-held lens.

DATA ANALYSIS

All statistical analyses were performed with Minitab statistical software. Results were considered significant if $P<0.05$. Unless stated otherwise, data presented in the text are in the format Mean ± Standard Error of the Mean. Two-sample *t*-tests were used to determine differences in stage-specific oocyte diameters between non-infected and infected fish. Pathology scores were not distributed normally and were non-transformable due to the large number of zero values, so Mann-Whitney tests were used to assess differences between non-infected and infected fish. Developmental stage-specific oocyte counts used as a proxy for potential fecundity were also non-normally distributed, so Mann-Whitney tests were again used to evaluate differences between non-infected and infected fish. To adjust for ties in the data, an adjusted *W*-statistic was used to test hypotheses in all Mann-Whitney tests and these adjusted values are reported in the text (Sokal and Rohlf, 1995).

Results

DESCRIPTION AND TISSUE LOCATION OF WORMS IN ADULT FISH

Live *Philometra saltatrix* were found in the ovaries of adult bluefish ranging in size from 285-780 mm (forklength). The majority of live worms found in ovarian tissue were relatively large (7-110 mm long and approximately 20-300 μm wide) gravid (with larvae) or sub-gravid (with eggs) females often filled with host erythrocytes (Figure 2). Live male and larval worms were also found and the maximum lengths of males and larvae were approximately 450 μm and 250 μm, respectively (Figure 3). One female fish out of the 374 that were examined had live *Philometra* present within the pericardial cavity. All other live worms were found in ovarian tissue. Of the 51 adult male bluefish that were thoroughly examined, none was found to be infected with live *P. saltatrix*.

PREVALENCE AND INTENSITY IN OVARIES OF ADULT FISH

In 2004 the prevalence of live female *P. saltatrix* in ovaries was highest in July when 39 % of sampled fish were infected with live worms (Figure 4). In 2005 live *Philometra* prevalence peaked in August at 26 %. When pooled weekly for the same five week period spanning July and August of both years, maximum prevalence occurred at the height of the New York spawning season during the last week in July at 58 % and 33 % for 2004 and 2005, respectively (Figure 5). In North Carolina waters, prevalence of infection also peaked at the height of the spawning season, but at much lower values than those encountered in New York waters. The maximum monthly prevalence peaked in April with only 6.25 % of fish containing live worms (Figure 4). Peaks in prevalence in

both New York and North Carolina were followed by gradual decreases over a one-to-two-month period until no live worms were found (Figure 4). In June and July of 2004, single lobes from the ovaries of 6 fish obtained in each month were searched with the aid of a dissecting scope to locate male and larval worms. In both months, three of the six lobes contained live males and/or larvae.

Dead worms encapsulated in melanized fibrotic tissue were present in the ovaries of 54.1 % of all bluefish and were found during all sampling periods (Figure 4). These dead worms were of varying sizes and presumably different sexes and spawning conditions (*i.e*. mature, sub-gravid, gravid, *etc*.). Often these dead worms took the form of large fibrotic masses multi-layered with melanin (Figure 6). Due to the relatively large size of these fibrotic masses (approximately 3-80 mm in length and/or width, and weighing as much as 1.24 g) they apparently represent *Philometra* that had already released their larvae and subsequently died. Occasionally, remains of eggs or larval worms were found within them. These fibrotic masses tended to be located in the posterior end of the ovarian lobes.

Dead *Philometra* were present within the pericardial cavity (often within the membrane surrounding the *bulbus arteriosus*) in 21.4 % of all specimens including 19.0 % of females and 39.2 % of males. Of all adult specimens of either sex, 75.5 % had either live or dead *P. saltatrix* present.

Intensity of infection, measured as the average number of live female worms per fish reached its maximum in New York in July 2004 at 2.39 ± 0.70, and in North Carolina in March 2005 at 0.15 ± 0.11 (Figure 7). As with prevalence of infection, there was considerable interannual variation with maximum intensity in 2005 occurring in

August at 0.82 ± 0.36 female worms/fish. Intensity followed a similar pattern to prevalence, gradually reaching a maximum at or very near the height of both the spring and summer bluefish spawning seasons, followed by a gradual decline. There were no temporal patterns in intensity with regard to dead encapsulated worms found in ovarian tissue.

Since the methods of determining parasite intensity differed for live worms (which were counted) and dead worms (which were scored), the two criteria were examined separately. There were no correlations found between fish length and either current or past infection intensity, nor was there a correlation between fish weight and intensity of past infection. However, there was a positive correlation between fish weight and intensity of live worms ($R = 0.120$, $P = 0.045$). There was no correlation between GSI and intensity of dead *Philometra*, but there was a positive one found between GSI and live *Philometra* intensity ($R = 0.495$, $P = 0.000$). *P*-values were adjusted via the Bonferroni method to account for a lack of independence (Sokal and Rohlf, 1995).

DESCRIPTION AND TISSUE LOCATION OF WORMS IN YOY FISH

Necropsies were performed on a total of 534 spring and summer-spawned YOY bluefish in 2004 ($n = 275$) and 2005 ($n = 259$) captured from the south shore of Long Island, New York. Specimens ranged in size from 28-203 mm (forklength), and the smallest infected fish measured 59 mm (forklength). Live female *Philometra* were found in 5.6 % of all specimens: 6.9 % of 2004-captured fish, and 4.2 % of 2005-captured fish. Microscopic searches for larval and male worms were conducted 156 times and yielded just one live male worm (found in the body cavity of a spring-spawned fish). All

infections were in the pericardial cavity (again often within the membrane surrounding the *bulbus arteriosus*) except for four fish in which worms were found in the body cavity (Figure 8). Of these four fish, one contained a mature female and a male worm; another contained a sub-gravid female, while the other two each contained a single immature female worm. All live worms found in the pericardial sac of YOY bluefish were adult females. Five fish contained sub-gravid worms, and 3 fish contained gravid worms. These particular specimens were captured in August and September of both 2004 and 2005. It must be noted, however, that the spawning condition of the vast majority of *Philometra* was not recorded, so it is possible that sub-gravid and gravid female worms were present in other months as well.

PREVALENCE AND INTENSITY IN YOY FISH

Monthly prevalence of live worms infecting the pericardial sac in 2004 was 0 % in June (n = 27), climbed to 7.44 % in August (n = 121), and then to 7.55 % in September (n = 160, Figure 9). Dead worms within the pericardial sac were found throughout the sampling period. In 2005, no worms either live or dead, were found in July and August. *Philometra* first appeared in YOY samples in September with 6.25 % containing live worms, and 3.75 % containing exclusively dead worms. In October of both 2004 and 2005 only two YOY bluefish were captured so they were not included in Figure 9. It is worth noting, however, that all four of these fish were infected with live *Philometra,* two of which were sub-gravid.

It was possible to distinguish the spring-spawned (SAB) cohort from the summer-spawned (MAB) cohort for most of the specimens since two distinct size classes were

readily apparent. Prevalence of live *Philometra* was 4.5 % in spring-spawned YOY (*n* = 378), and 8.3 % in summer-spawned YOY (*n* = 156). Prevalence of dead *Philometra* in the spring-spawned cohort was 6.1 %, while in the younger summer-spawned cohort no dead worms were found.

Intensity of infection followed a very similar pattern to that of prevalence with peaks in September of each year at an average of 0.11 ± 0.058 live worms per fish in 2004 and 0.07 ± 0.022 live worms per fish in 2005 (Figure 10). No correlations were found between fish length and intensity of dead worms, or weight and intensity of live worms. Positive correlations did exist between fish length and intensity of live worms ($R = 0.251$, $P = 0.003$), and between fish weight and intensity of dead worms ($R = 0.318$, $P = 0.000$). *P*-values were adjusted via the Bonferroni method.

HISTOPATHOLOGY

Healthy, non-philometrid infected ovarian tissue was comprised of densely-packed oocytes of various developmental stages and little or no connective tissue (Figure 11A). The following pathologies were documented in the ovaries of bluefish infected with *P. saltatrix*: melanomacrophage centers, macrophage infiltrate, interstitial granulomatous inflammation, lymphocytic infiltrate, pre-necrotic pyknosis (a pre-necrotic change), connective tissue hyperplasia, interstitial hemorrhage, fibrosis, and atresia/postovulatory follicles (Figures 11 & 12).

HISTOLOGICAL OBSERVATIONS OF OOCYTE DEVELOPMENT

Since bluefish ovaries develop asynchronously, it was necessary to quantify the relative contribution of each oocyte developmental stage. A modified version of the method used by Corriero *et al*. (2003) was used to identify six distinct stages of maturity (Figure 13). The *Perinucleolar stage* (Stage 1) is characterized by strong basophily in the ooplasm, a high nucleus: cytoplasm ratio, and the presence of numerous small nucleoli in the nucleus. The *Lipid stage* (Stage 2) has small lipid droplets and weak basophily in the ooplasm, as well as a thin *zona radiata*. The *Early vittellogenesis* stage (Stage 3) is characterized by the appearance of small eosinophillic yolk globules, *cortical alveoli*, and increasing thickness of the *zona radiata*. Oocytes in the *Late vittellogenesis* stage (Stage 4) show a substantial increase in the size and number of yolk globules and a thickening of the *zona radiata*. The *Migratory nucleus* stage (Stage 5) is characterized by the movement of the nucleus towards the animal pole, and early coalescence of yolk and lipid granules. The final stage observed, the *Hydrated stage* (Stage 6) is distinguished by the increased coalescence of yolk and lipid granules, and detachment of the follicular cell layer. Oocytes at each successive developmental stage were on average substantially larger than the previous one (Table 2).

COMPARISONS BETWEEN NON-INFECTED AND INFECTED FISH

Using oocyte size as an indicator of egg condition, non-infected fish for stage 1-3 oocytes were on average 6.1 ± 0.80 % larger than oocytes from infected fish (two-sample *t*-tests: Stage 1 = 5911 DF, $T = 6.82$, $P = 0.000$; Stage 2 = 2990 DF, $T = 7.21$, $P = 0.000$;

Stage 3 = 385 DF, $T = 2.20$, $P = 0.028$). Figure 14 and Table 3 detail results and *t*-tests for each oocyte developmental stage.

Pathology scores of ovary damage in infected fish were significantly higher in fish containing only dead worms (Mann-Whitney test: $W = 24419.5$, $P = 0.0286$), but not in regards to fish infected only with live worms or to the presence/absence in the strict sense (*i.e*. both live and dead worms, Table 4).

Frequencies of stage-specific oocytes per field of view were used as a proxy for relative potential fecundity. To account for seasonal changes in development that alter the proportional contribution of the various stages, only data from July of both years (the peak of the MAB spawning season) were used. Non-infected fish were on average 35.8 % more fecund in regards to Stage 1 and Stage 2 oocytes than infected fish (Table 5 and Figure 15). There were 25.0% more Stage1 (*Perinucleolar stage*) oocytes, and 46.7 % more Stage 2 (*Lipid stage*) oocytes in non-infected fish than in infected fish (Mann-Whitney tests: $W = 1762.0$, $P = 0.0431$; and $W = 1981.5$, $P = 0.0006$, respectively). Results for Stage 3 (*Early vittellogenesis stage*) and Stage 4 (*Late vittellogenesis stage*) oocytes were non-significant, and complete data for Stage 5 (*Migratory nucleus stage*) and Stage 6 (*Hydrated stage*) oocytes were not available since all ovaries with these stages present ($n = 3$ and $n = 14$, respectively) were either infected or showed signs of past infection.

Seasonal changes in GSI between non-infected and infected fish did not show a discernible pattern when pooled my month, but infected fish did have higher average GSI values than non-infected fish for all months except in October, 2004 (Figure 16). The

percent difference ranged from a low of 13.2 % in August 2004, to a high of 82.7 % in June 2004. The mean percent difference for all months was 34.9 %.

Discussion

PHILOMETRA SALTATRIX LIFE HISTORY

In 2004 and 2005, adult bluefish along the Atlantic coast of the U.S. were infected with *Philometra saltatrix*, but at rates considerably lower than those observed in 2002 and 2003 by Clarke *et al*. (2006). In 2004 monthly prevalence of live worms was highest in July at 39 % whereas in 2005 monthly prevalence peaked in August at approximately 26 %. This is considerably lower than in July of 2002 and 2003 when monthly prevalence peaked at 79 % and 45 %, respectively (Figure 4). As in 2002-2003, however, the highest prevalence of *P. saltatrix* in adult bluefish occurred at the height of the MAB spawning season which had previously been determined to occur in late July and early August (McBride and Conover 1991, McBride *et al*. 1993). This suggests that the parasite's spawning coincides with that of its host. Since live *Philometra* prevalence sharply declined once the spawning season subsided, it is likely that larvae are expelled along with bluefish eggs during the act of spawning and female worms subsequently die. This method of larval dispersion is known to occur in other philometrid species such as *Philonema oncorhynchi* and *Philonema agubernaculum* and is presumed to be regulated by host hormonal processes (Anderson 1992).

The first intermediate hosts in all known philometrid life-cycles are cyclopoid copepods, (Moravec 1980, 2004). The general pattern in nematodes of the family Philometridae is that helminths develop in the haemocoel of the copepod through two larval stages before reaching the infective third stage. Once copepods are ingested by a planktivorous fish, the third-stage *Philometra* migrate to suitable tissue (often the

kidneys, or the serosa of the swimbladder) and ultimately develop into fifth-stage (adult) males and females (Anderson 1992). Following fertilization, the females, and in some cases the males (depending on the species) migrate to the definitive tissue location, grow prodigiously and become gravid (Moravec *et al*. 1995). Development can be quite rapid, as has been shown by Molnar and Fernando (1975) who experimentally infected copepods with 1st stage *Philometra cylindracea* larvae. After ten days the copepods were fed to their definitive piscine host, yellow perch (*Perca flavescens*), and within a week 4th stage larvae were found in the fish's body cavity. Development has also been shown to be temperature-dependent. For instance, Platzer and Adams (1967) demonstrated empirically that *Philonema oncorhynchi* developed into 3rd stage larvae in copepod hosts after only 17 days at 12 ° C, while it took 70 days at 8° C.

It is possible that bluefish, while still zooplanktivorous, first acquire *P. saltatrix* by feeding on infected copepods. It is also possible that bluefish acquire the parasite once they have become piscivorous through either a paratenic or a second intermediate fish host (Moravec and Dyková 1978). The fact that the smallest infected bluefish specimen was 59 mm (forklength), and certainly piscivorous, supports the latter scenario. Fish as small as 28 mm were examined and, had *Philometra* been mainly acquired directly from copepods, then these fish, being exclusively planktivorous, would likely have shown signs of infection. Also, the positive relationships found between adult fish weight and parasite intensity, along with similar relationships found between YOY body size (weight and length) and parasite intensity favor the likelihood of this particular mode of transmission.

In some *Philometra* spp. worms live within the pericardial cavity of juvenile fish until intercepting some type of hormonal cue signaling maturation, upon which they migrate to the ovaries to complete their life-cycle (Bashirullah and Adams 1983, Hesp *et al*. 2002). *Philometa saltatrix* likely follow a similar life-cycle even though gravid worms were occasionally observed in YOY (albeit in the pericardial sac). These YOY infections along with infections of adult male fish are most likely anomalous, and result in the death of the worms prior to being able to distribute their larvae. There are other possibilities, however, since both tissue migration and modes of transmission of parasitic helminths can be considerably plastic (Read and Skorping 1995). For instance, Poulin (2003) and Poulin and Lefebvre (2006) showed that various trematodes are capable of accelerating their development and reaching maturity while still in their crustacean intermediate hosts. Normally these worms reach maturity only following a chemical cue from their definitive fish host. Levsen and Jakobsen (2002) observed the spirurid nematode *Camallanus cotti* switch from heteroxeny (transmission through one or more intermediate hosts) to monoxeny (direct transmission) when the parasite's copepod intermediate host was absent. In several *Philometra* spp. larvae are distributed by means of gravid females migrating to an area of their fish host that is exposed to the outside environment (*e.g.* gills, anus, or just under the skin) where they break open to release larvae or possibly become vulnerable to predation from a potential intermediate or paratenic host (Anderson 1992). Given, however, that *P. saltatrix* mature in ovaries and that dead post-spawned worms are often found there, the likely explanation is that *P. saltatrix* completes its life-cycle by expelling larvae along with the eggs of its host. It is quite possible however, that other modes of transmission and patterns of tissue migration

do exist and represent distinct phenotypes that provide the nematode a means of increasing its fitness through bet-hedging.

In cases where both live and dead worms are present in either the ovaries of adult fish or in the pericardium of adults or YOY, it is unclear whether they represent the same infection or different infections. The likely explanations differ for female fish and for male and YOY fish. Figure 17 illustrates the proposed life-cycle of *P. saltatrix* in its bluefish host. In female fish, initial infection occurs during the bluefish spawning season and subsequent larval stages reside in various host tissue (*e.g.* the pericardium) before migrating to the ovaries following some sort of chemical cue from the host. Once in the ovaries the helminths mature and complete their life-cycle by releasing their larvae at the same time the host spawns. In male and YOY bluefish, dead worms probably represent an entirely different infection from any current one. Worms in these fish may reach maturity, but excluding the existence of any bet-hedging phenotypes, they are not able to successfully distribute their offspring. This proposed life-cycle represents the most parsimonious explanation of the data, and accounts for the sudden emergence of mature *Philometra* at the beginning of the spawning season in both YOY and in the ovaries of mature fish. The observed patterns in seasonal prevalence support this. In 2004, spring-spawned YOY captured in June contained no live worms yet 22.2 % contained dead worms (Figure 9). Live worms first appeared in July, and monthly prevalence increased until September of that year when 7.5 % of sampled YOY contained live worms, and 15.1 % contained either live or dead worms. The increasing prevalence following the MAB spawning season can only mean that the YOY are acquiring the parasite through predation upon an intermediate host. The lag time between peak bluefish spawning

activity and increases in prevalence is due to the development time of the nematode within the copepod host and any piscine intermediates. This is a different pattern of prevalence than the one seen in ovaries where the timing of peak bluefish spawning activity and peak prevalence are almost identical (Figures 4 and 5). There is no lag time because larval female worms presumably are already in the 4th stage of development, and once they detect a hormonal cue from their host indicating maturation, they simply migrate to ovarian tissue to grow and reproduce.

EFFECTS ON BLUEFISH REPRODUCTIVE POTENTIAL

The spawning condition of female bluefish as represented by GSI did not show any temporal patterns in regards to non-infected versus infected fish, but in all months except for October 2004, infected fish had higher GSI than non-infected fish (Figure 16). This difference is most likely not a functional one, but a pathological one. The greater proportional mass of infected ovaries probably does not represent increased gonadal maturation. The increase is mass is instead due to edema, hemorrhaging, and perhaps the effects of other pathologies.

Studies of the effects of *Philometra* on fish ovaries are uncommon, as are those addressing parasitism of fish gonads in general. Heins and Baker (2003) found that mean egg size in threespine stickleback (*Gasterosteus aculeatus*) was smaller in fish infected with the cestode *Schistocephalus solidus* than in non-infected fish. Oliva *et al*. (1992) suggested that philometrid infection resulted in reduced fecundity in Chilean sea bass (*Paralabrax humeralis*) because the effective volume of the ovaries was reduced. Necrosis was described in the ovaries of striped mullet (*Mugil cephalus*) due to infection

by *Philometra cephalus* (Ramachandran 1975). Atrophy in the testes and ovaries of New Zealand snapper (*Chrysophrys auratus*) due to infection by *Philometra* sp. was described by Hine and Anderson (1981). Similarly, Moravec *et al*. (1997), Hesp *et al*. (2002), and Moravec *et al*. (2002) discussed *Philometra*-induced ovary damage in various reef-fishes but made no attempt at assessing the level of damage.

In this study, results indicated that histological damage due to *P. saltatrix* infection of bluefish was considerable. Stage 1 through Stage 3 oocytes from non-infected fish were 6.1 % larger than those from *Philometra*-infected fish suggesting that the condition of oocytes was adversely affected by the presence of the parasite. Albeit from a non-ovarian dwelling parasite, for comparison Heins and Baker (2003) found that ovum size in threespine stickleback was reduced by 7.5 % to 32.1 % in nine different populations infected with the cestode *Schistocephalus solidus*.

Pathology scores representing ovary damage in non-infected versus infected fish differed significantly only with respect to the presence of dead worms, and not live ones. Pathologies surrounding dead masses of worms tended to be more severe than those associated with live worms, often with the latter consisting only of mild to moderate hemorrhaging. Since masses of dead worms were often relatively large, the effective volume of infected ovaries was reduced (Figure 6). It is also possible that these large fibrotic masses blocked the oviduct and prevented the expulsion of ripe ova from the lumen of the ovary. The fact that most of these masses were located in the posterior, oviduct-end of the ovary supports this.

Since *Philometra* feed on blood, intense infections can have serious detrimental effects on the host by means of nutrient theft. No more is this evident than in infections

of YOY where the large size of female worms allow for a sizeable portion of the host's blood supply to be appropriated by the parasite. In several instances, blood-engorged worms occupied as much volume within the pericardial sac as did the heart itself (Figure 8A). Precluding the existence of any bet-hedging phenotypes, it is possible that anomalous infections of the pericardium are not only a dead end in terms of reproduction for *Philometra*, but also result in the death of the host.

Frequencies of oocytes per field of view were used as a measure of relative potential fecundity. At the peak of the New York spawning season, non-infected fish were on average 35.8 % more fecund in regards to *Perinucleolar Stage* and *Lipid Stage* oocytes than infected fish (Figure 15). It must be noted that these results do not represent either true fecundity or true batch fecundity. In batch-spawners such as bluefish only hydrated oocytes are actually spawned, so the latter is a much more useful measure of reproductive output. Unfortunately, a measure of batch fecundity was not possible since very few hydrated oocytes were encountered, and those that were happened to all be from *Philometra*-infected fish making useful comparisons impossible. A reduction, however, in the number of available oocytes undoubtedly has an effect on potential fecundity, and this is what is demonstrated here. This potential reduction in fecundity is sizeable and could have a significant population-wise effect, especially coupled with the smaller size (*i.e.* lower condition factor) of oocytes from *Philometra*-infected fish.

SPRING-SPAWNED *VERSUS* SUMMER-SPAWNED COHORTS

An important finding of this study is that prevalence and intensity of infection in specimens from North Carolina waters were much lower than in specimens from New

York waters. As in MAB specimens, prevalence of infection peaked at the height of the spawning season, which has been determined to occur in April in the SAB (Nyman and Conover 1988). As in the MAB, this is evidence of *P. saltatrix* employing the mode of transmission whereby larvae are expelled along with host eggs. It can be speculated that the large differences in prevalence and intensity between the MAB and SAB likely have to do with geographical/environmental factors. Temperature has already been mentioned in its role in philometrid larval development, and it undoubtedly plays an important role in bluefish larval development as well (Kendall and Walford 1979, Munch and Conover 2000). Since prevalence and intensity differ greatly between the SAB and the MAB, it follows that there should be discernible differences in the patterns of infection between the spring-spawned cohort and the summer-spawned cohort. This was indeed the case with the summer-spawned cohort having infection prevalence 84.4 % higher than the spring-spawned cohort.

Much of the reason for variability in the recruitment of bluefish is believed to be related to differing contributions of the spring-spawned and summer-spawned cohort in both recruitment to the juvenile stage, and to the adult population (Conover *et al*. 2003, Scharf *et al*. 2006). In recent years, the summer-spawned cohort has been dominant yet contributes little to the adult population. Reasons for this are unknown but possible explanations include differential advection and mortality of eggs and larvae (Hare and Cowen 1996), different nursery habitats for the two cohorts (Able *et al*. 2003), and size-dependant overwintering mortality (Munch and Conover 2000, Wiedenmann and Essington 2006). Given the fact that *P. saltatrix* is much more likely to infect summer-spawned fish than spring-spawned fish, it is reasonable to suspect that the summer-

spawned cohort is failing to recruit to the adult population in part due to *Philometra*-induced mortality.

It has been show that the spring-spawned and summer-spawned cohorts each utilize different prey items based upon the timing of the particular cohort's arrival to nursery habitats. Spring-spawned fish recruit to nearshore environments well before many other piscine species and thus are able to utilize the later arrivals as prey items (Juanes and Conover 1995). Conversely, summer-spawned bluefish arrive to nursery grounds late in the summer when suitably sized prey items are limited mostly to bay anchovies (*Anchoa mitchilli*) which become their primary food source (Juanes and Conover 1995, Buckel et *al*. 1999). Since bay anchovies are endemic to the Atlantic coast of the U.S. from Maine to Florida and exclusively planktivorous, they would make ideal intermediate fish hosts for *P. saltatrix*.

CONCLUSION

Philometra infection of bluefish could be a significant contributing factor to the inter-annual recruitment variability of bluefish. Since 2002 prevalence of the parasite in bluefish ovaries has been gradually decreasing, while over the same time period, bluefish abundance as evidenced by catch data from the recreational fishery has increased (NMFS 2004, 2007). The gradual reduction in prevalence observed since 2002 indicates that the abundance of *Philometra saltatrix* varies considerably over time. Prior to its rediscovery in 2002, there had been only two references in the literature of *Philometra saltatrix* occurring in MAB waters; the original description of the species (Ramachandran 1973) discovered in Long Island Sound in 1965, and the abstract by Cheung *et al*. in 1984

describing its occurrence in YOY fish. Notably, *P. saltatrix* were not found during routine GSI studies of New York bluefish throughout the 1980s (Conover personal observation, *quoted in* Clarke *et al*. 2006). Considering this, it is quite feasible that the abundance of the parasite fluctuates dramatically over decadal time scales.

This was the first study to evaluate and quantify *Philometra*-induced ovarian damage in a fish, thereby enabling a direct estimation of the proportional loss in fecundity. Future studies on the effects of helminth parasites on ovaries should focus on further elucidation of the relationship between infection and fecundity. A useful complement to this study would be one in which either volumetric or gravimetric methods of assessing fecundity (such as those described in Adlerstein and Dorn 1998, or in West 1990) are used instead of the histological method used here. Other beneficial work would include experimental infections of copepods and bluefish along with an analysis of geographical differences between species compositions and abundances of both copepods and potential intermediate or paratenic piscine hosts.

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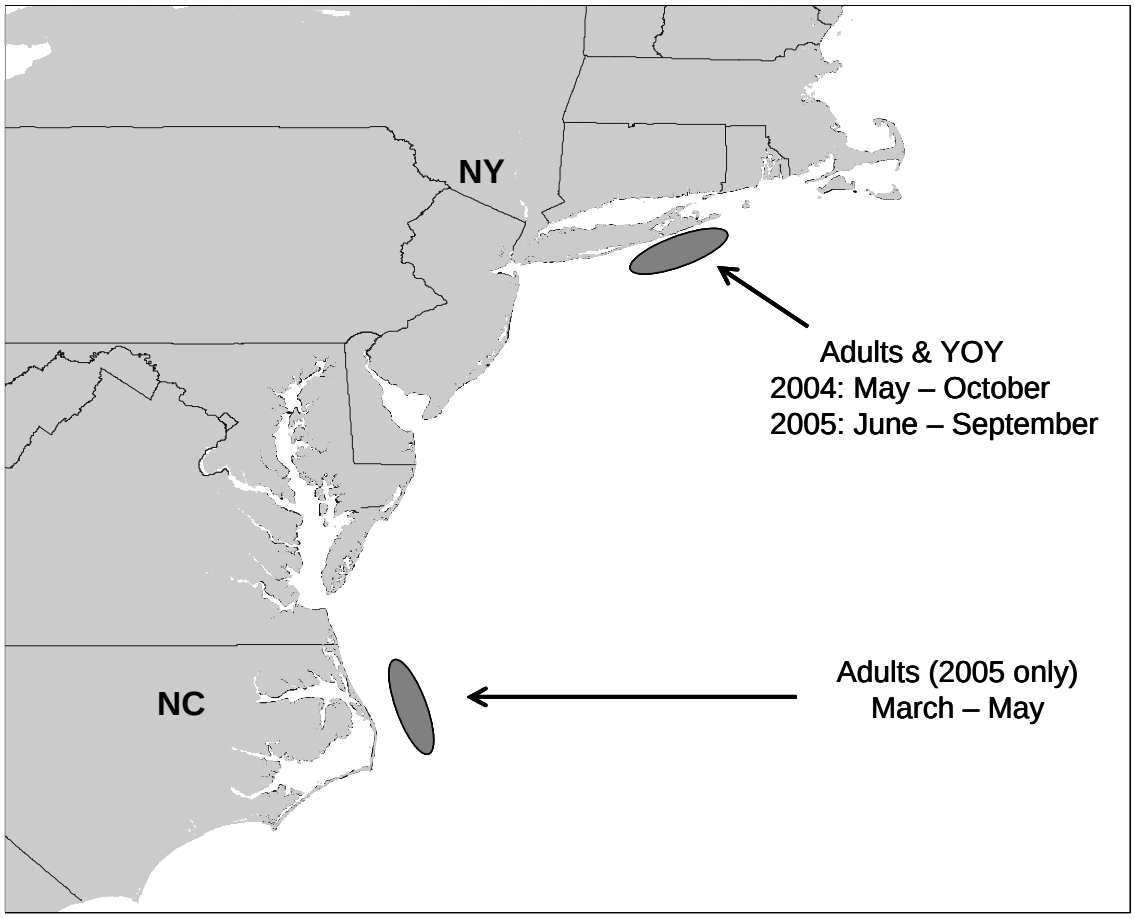

Figure 1. Map of sampling sites in 2004-2005 off Long Island, NY and the Outer Banks of North Carolina.

Table 1. Scoring method used to assess ovarian tissue damage.

Severity	Extent				
	None	Focal	Multifocal	Coalescing	Diffuse
None	0	0	0	0	0
Mild	0	1	2	3	4
Moderate	0	2	3	4	5
Severe	0	3	4	5	6

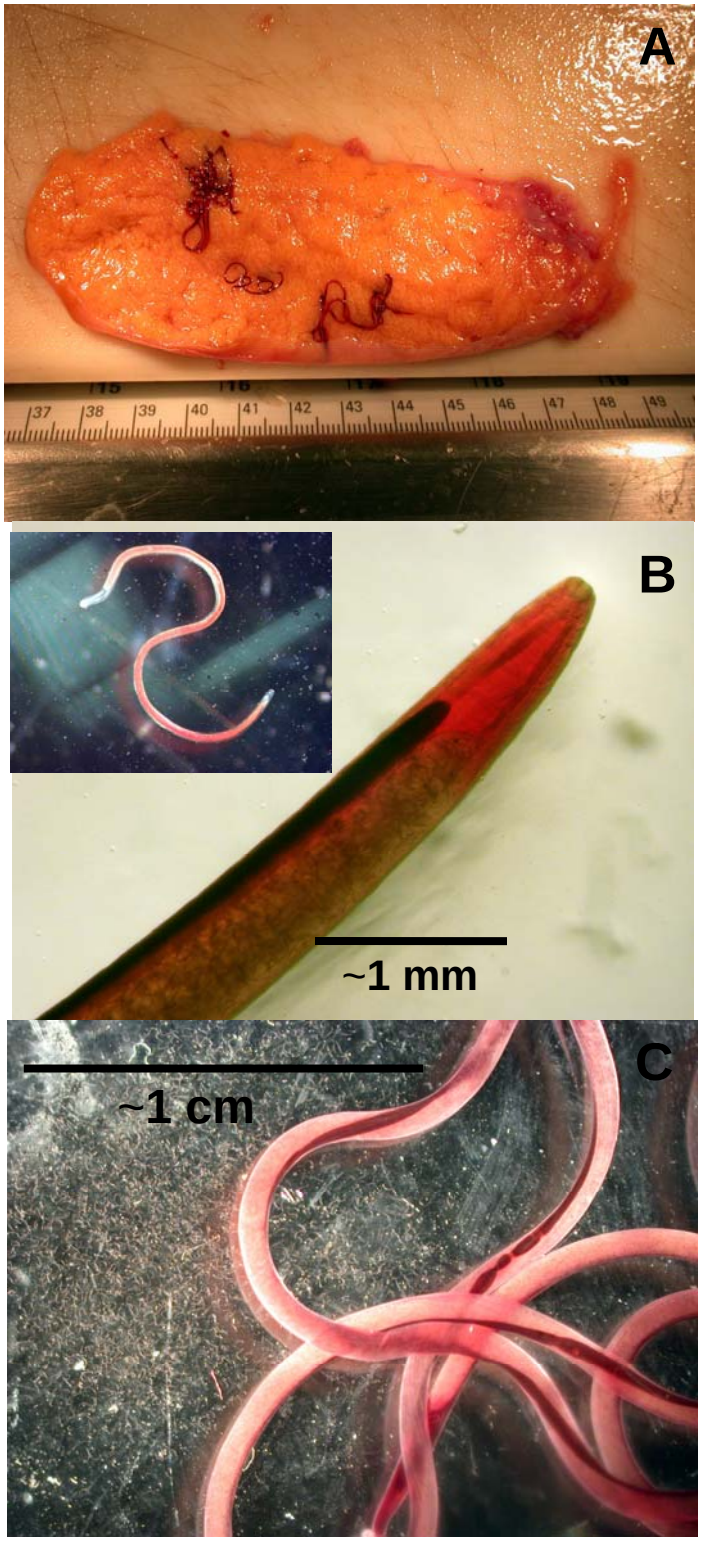

Figure 2. A) Female *Philometra saltatrix* in ripe ovary of bluefish. B) Gravid female *P. saltatrix*. C) Gravid female *P. saltatrix* expelling larvae through broken cuticle surface.

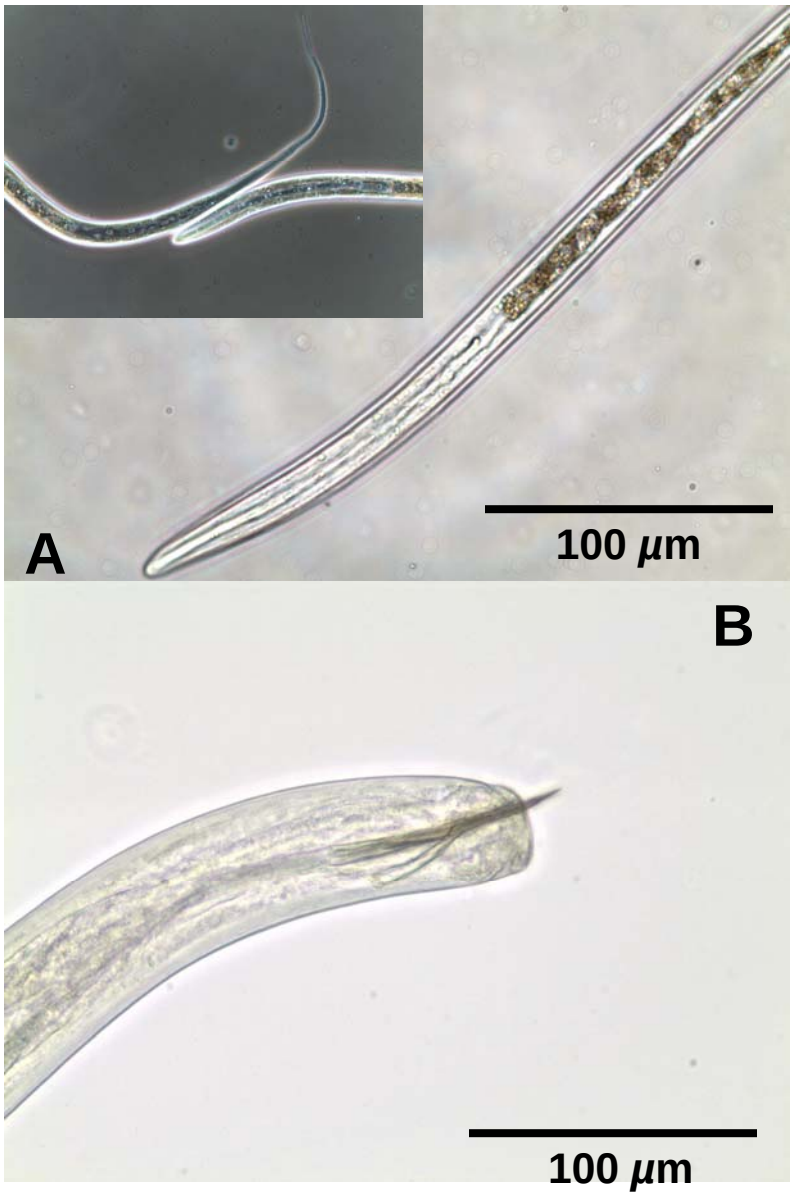

Figure 3. A) Larval *Philometra saltatrix*. B) Posterior end of adult male *Philometra saltatrix* showing spicule.

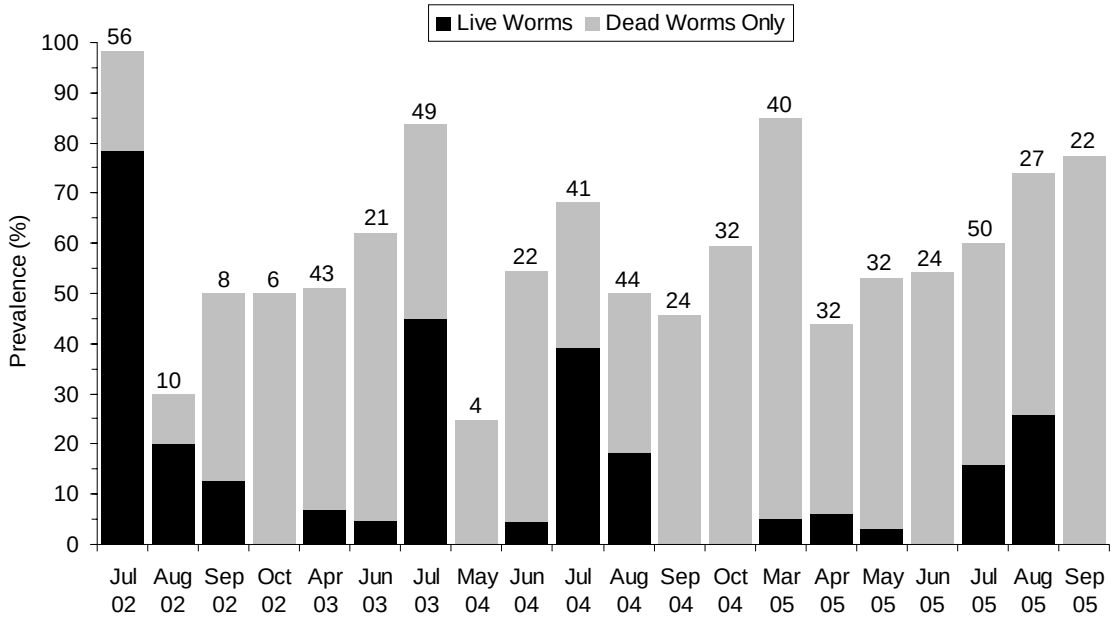

Figure 4. Monthly prevalence of live and dead *Philometra saltatrix* in the ovaries of bluefish (*Pomatomus saltatrix*). Data from 2002 and 2003 taken from Clarke *et al.* 2006 and from unpublished data provided by L.M. Clarke. All fish were captured from New York waters except for April 2003, and March-May 2005 specimens which were captured in North Carolina waters. Numbers indicate sample sizes.

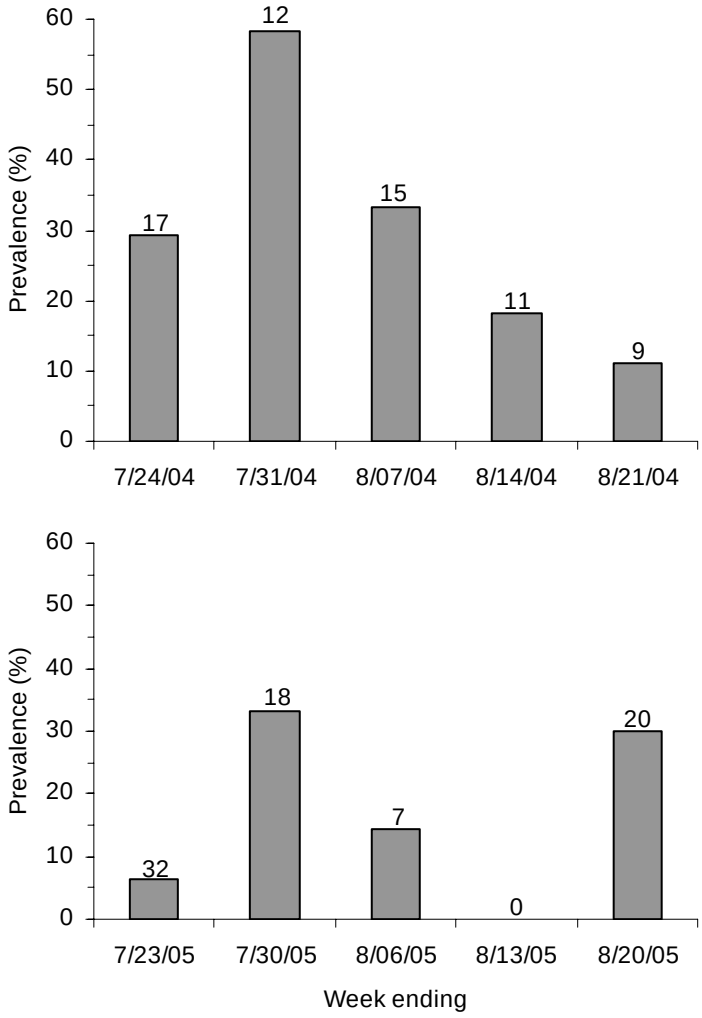

Figure 5. Weekly prevalence of live *Philometra saltatrix* in the ovaries of bluefish (*Pomatomus saltatrix*) captured off southern Long Island, NY. Numbers indicate sample sizes.

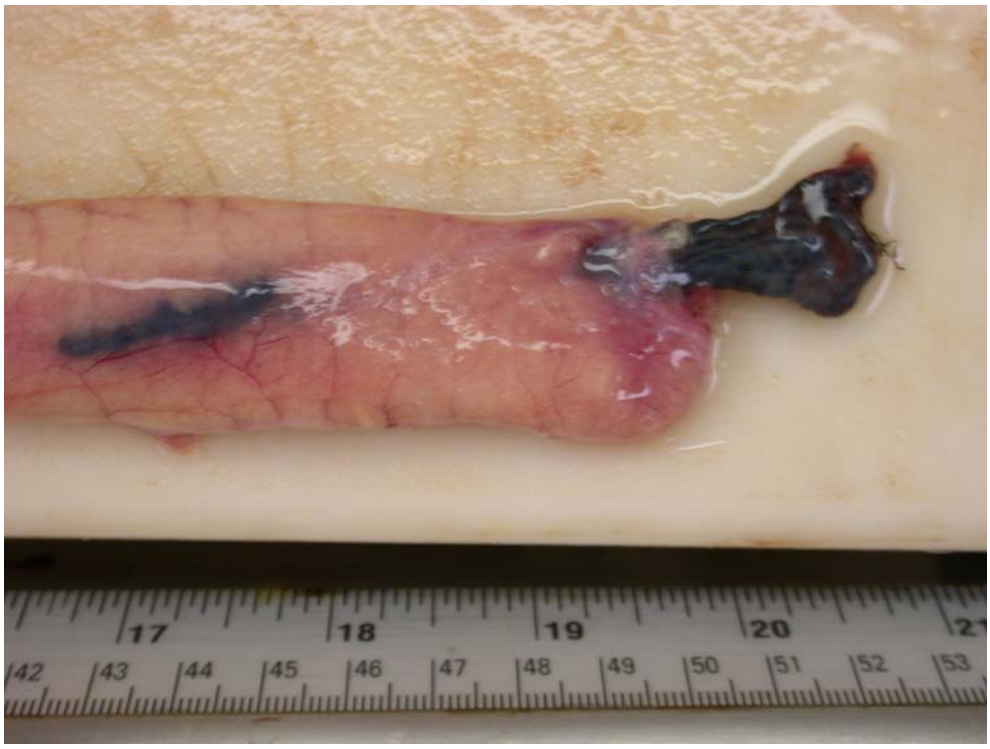

Figure 6. Bluefish ovary with melanized fibrotic masses of dead *Philometra saltatrix*.

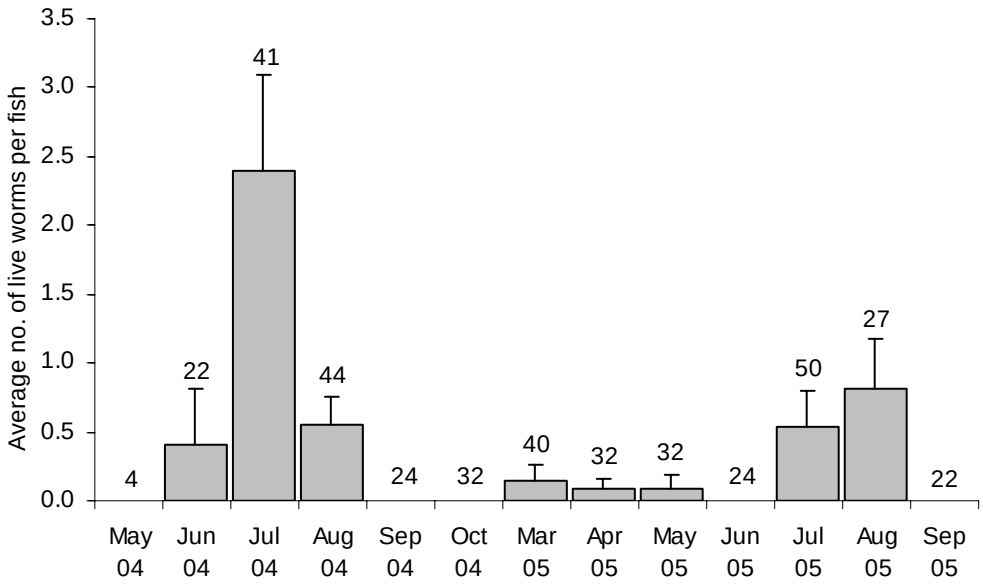

Figure 7. Average monthly intensity of live *Philometra saltatrix* in the ovaries of bluefish (*Pomatomus saltatrix*). All fish were captured from New York waters except for March-May 2005 specimens which were captured in North Carolina waters. Numbers indicate sample sizes, and error bars represent the standard error of the mean.

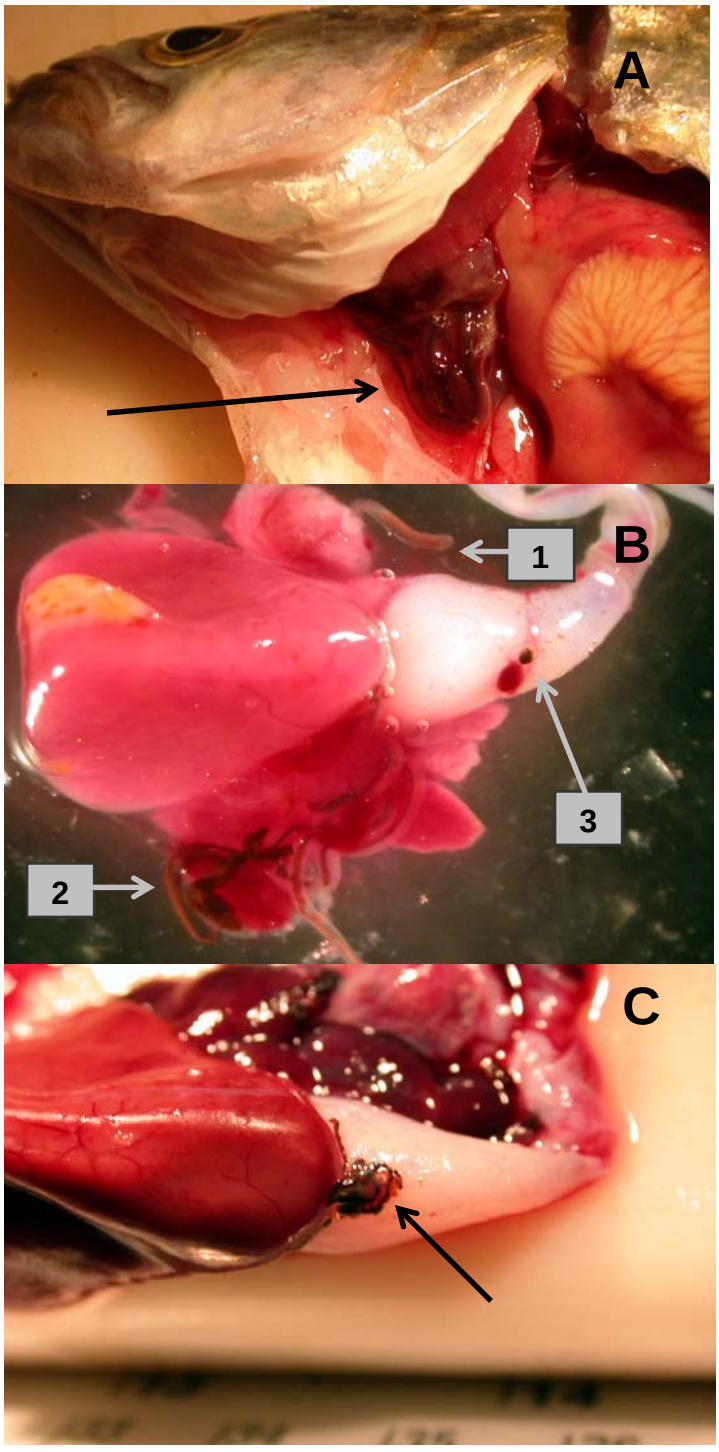

Figure 8. A) Live *Philometra saltatrix* infecting pericardial sac of YOY bluefish. B) Live and dead *P. saltatrix* in heart of YOY bluefish. 1 = live adult worm, 2 = dead adult worm, 3 = dead immature or male worm C) Dead *P. saltatrix* within membrane covering bulbus arteriosus of YOY bluefish.

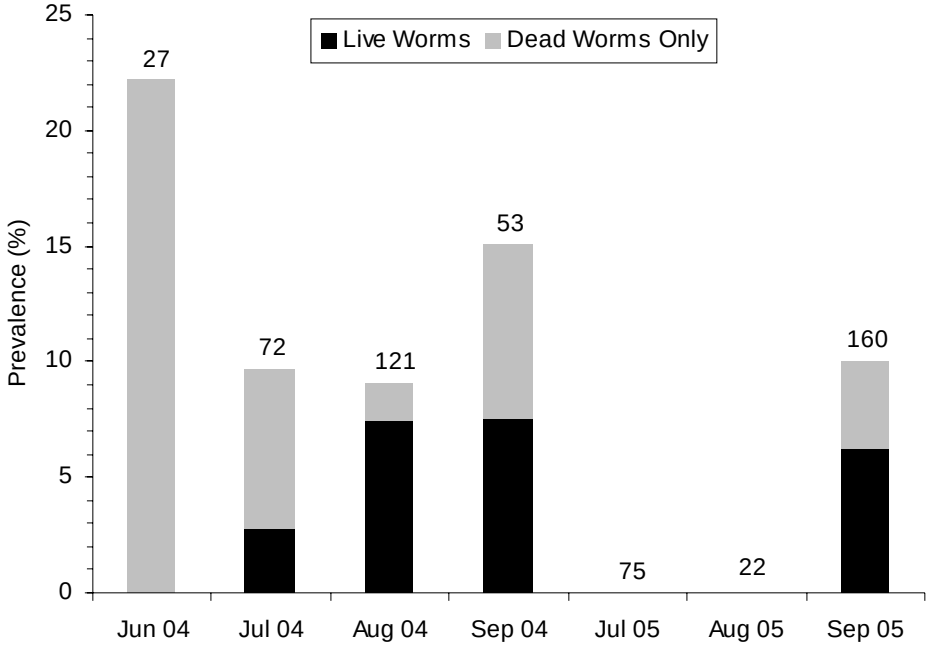

Figure 9. Monthly prevalence of live and dead *Philometra saltatrix* in the pericardial cavity of young-of-the-year bluefish (*Pomatomus saltatrix*) captured off southern Long Island, NY. In both October 2004 and October 2005 two additional fish not represented in figure were examined. All four fish were infected with live worms only. Numbers indicate sample sizes.

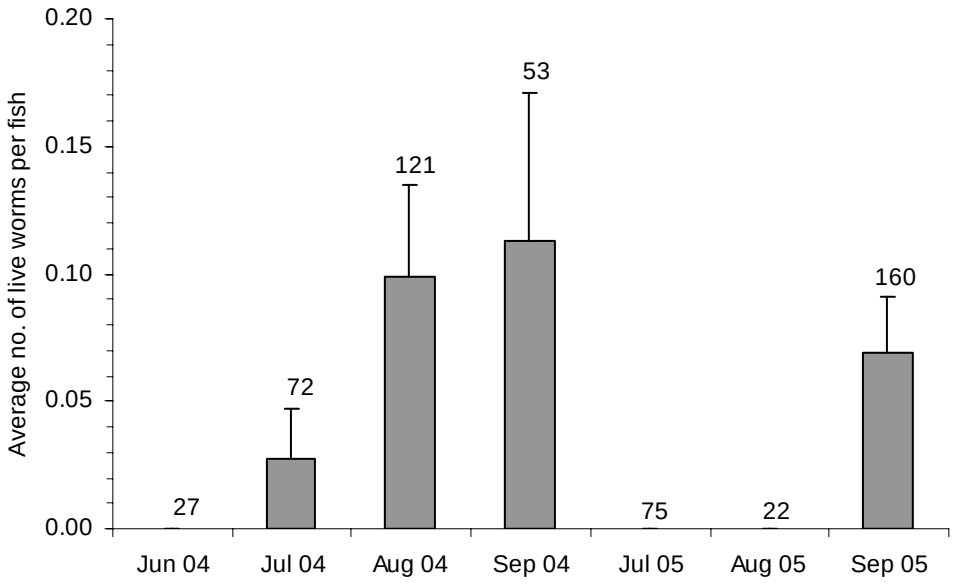

Figure 10. Average monthly intensity of live *Philometra saltatrix* in the pericardial cavity of young-of-the-year bluefish (*Pomatomus saltatrix*) captured off southern Long Island, NY. Numbers indicate sample sizes, and error bars represent the standard error of the mean.

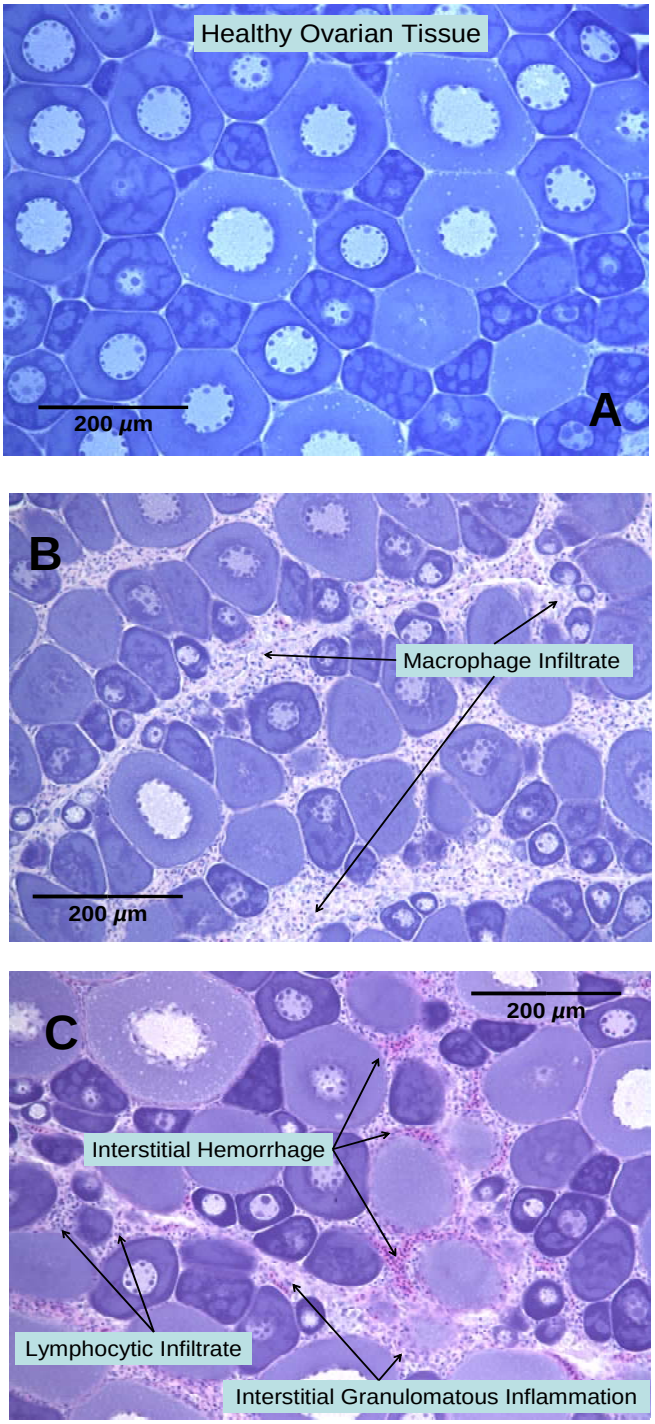

Figure 11. A) Healthy ovarian tissue from non-*Philometra*-infected bluefish. B) Ovarian tissue from bluefish infected with *P. saltatrix* showing macrophage infiltrate. C) Ovarian tissue from bluefish infected with *P. saltatrix* showing interstitial hemorrhage, lymphocytic infiltrate, and interstitial granulomatous inflammation.

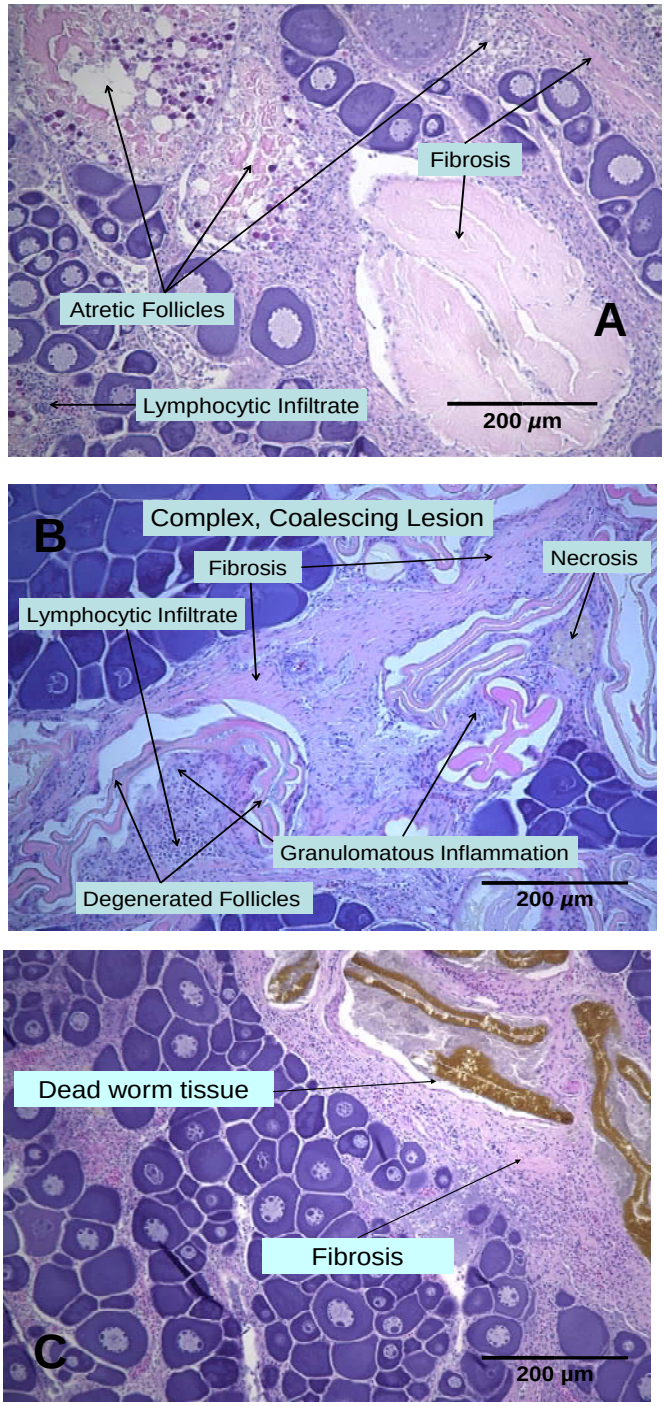

Figure 12. Ovarian tissue from bluefish infected with *Philometra saltatrix* showing: A) atretic follicles, fibrosis, and lymphocytic infiltrate; B) a complex coalescing lesion with degenerated follicles, characteristics of fibrosis, necrosis, lymphocytic infiltrate, and granulomatous inflammation; C) extensive fibrosis surrounding dead *Philometra* tissue.

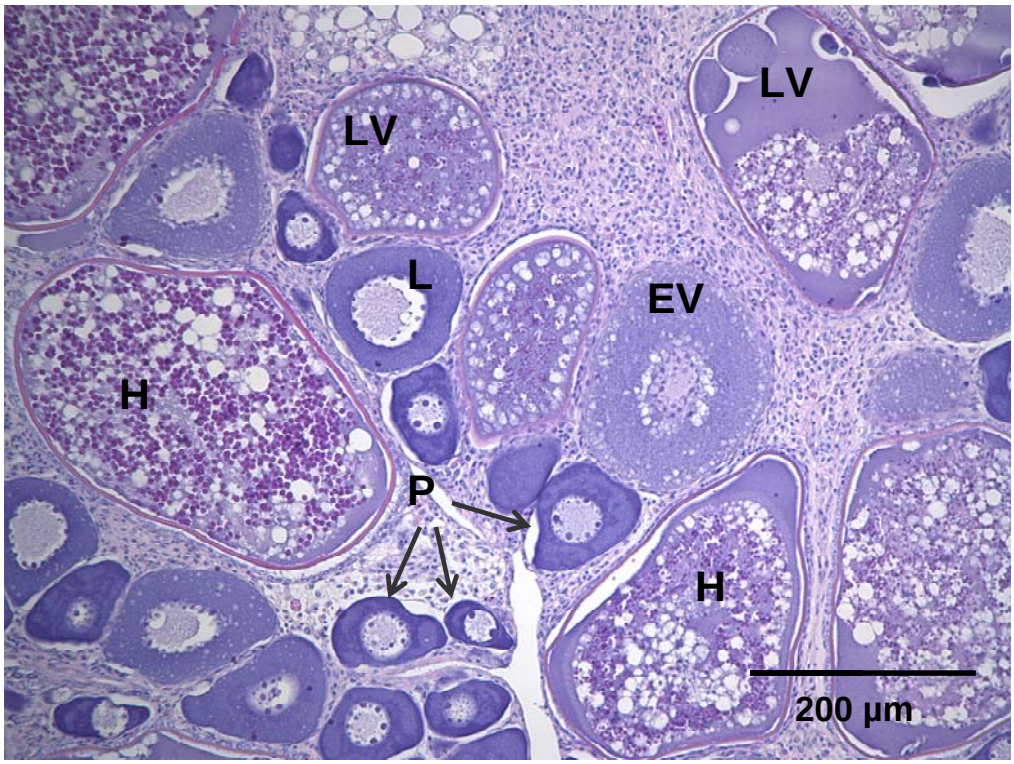

Figure 13. Bluefish ovary showing oocyte developmental stages. P = Perinucleolar stage, L = Lipid stage, EV = Early vitellogenesis stage, LV = Late vittellogenesis stage, H = Hydrated stage

Table 2. Oocyte cell diameters at each developmental stage.

Developmental stage	Abbr.	Diameter (μm)	Average ± std. dev.(μm)
Perinucleolar (stage 1)	P	10-180	65 ± 30
Lipid (stage 2)	L	20-260	122 ± 33
Early vittellogenesis (stage 3)	EV	90-310	184 ± 43
Late vittellogenesis (stage 4)	LV	150-460	262 ± 77
Migratory nucleus (stage 5)	M	240-320	283 ± 32
Hydrated (stage 6)	H	280-620	425 ± 77

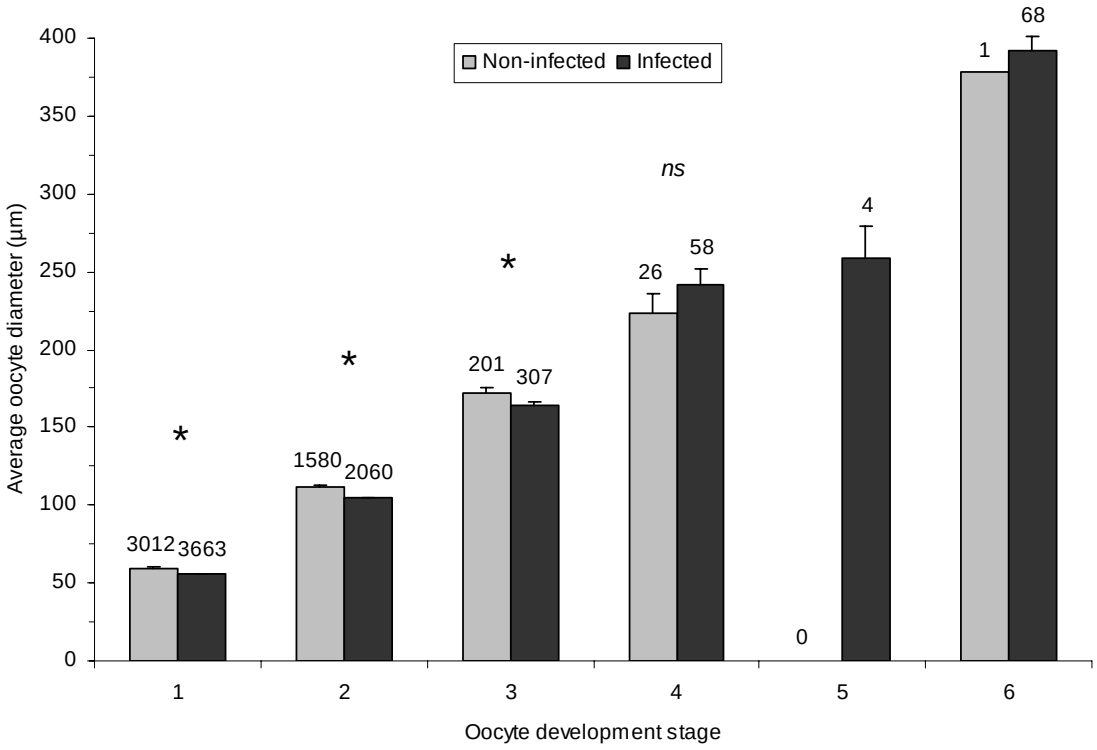

Figure 14. Average oocyte size per developmental stage in bluefish (*Pomatomus saltatrix*) infected and non-infected by *Philometra saltatrix*. Numbers indicate sample sizes, and error bars represent the standard error of the mean. Asterisks indicate significant differences resulting from two-sample *t*-tests, and *ns* indicates non-significant differences. 1 = Perinucleolar stage, 2 = Lipid stage, 3 = Early vittellogenesis stage, 4 = Late vittellogenesis stage, 5 = Migratory nucleus stage, 6 = Hydrated stage.

Table 3. Results of two-sample *t*-tests on average stage-specific oocyte diameter of non-infected versus infected bluefish.

Two-Sample *t*-Test and CI: stage 1 non-infected versus stage 1 infected

```
                     N      Mean      StDev     SE Mean
non-infected        3012     59.8      27.6      0.50
infected            3663     55.4      23.4      0.39
Difference = mu (0) - mu (1)
Estimate for difference:  4.331
95% CI for difference: (3.086, 5.575)
t-Test of difference = 0 (vs not =): T-Value = 6.82  P-Value = 0.000  DF = 5911
```

Two-Sample *t*-Test and CI: stage 2 non-infected versus stage 2 infected

```
                     N       Mean     StDev      SE Mean
non-infected        1580    111.5      32.0      0.80
infected            2060    104.4      25.9      0.57
Difference = mu (0) - mu (1)
Estimate for difference:  7.111
95% CI for difference: (5.177, 9.046)
t-Test of difference = 0 (vs not =): T-Value = 7.21  P-Value = 0.000  DF = 2990
```

Two-Sample *t*-Test and CI: stage 3 non-infected versus stage 3 infected

```
                     N       Mean     StDev      SE Mean
non-infected         201    172.2      41.0      2.9
infected             307    164.4      35.7      2.0
Difference = mu (0) - mu (1)
Estimate for difference:  7.80
95% CI for difference: (0.84, 14.76)
t-Test of difference = 0 (vs not =): T-Value = 2.20  P-Value = 0.028  DF = 385
```

Two-Sample *t*-Test and CI: stage 4 non-infected versus stage 4 infected

```
                     N      Mean     StDev      SE Mean
non-infected         26     23.5      64.1      13
infected             58    242.1      72.8       9.6
Difference = mu (0) - mu (1)
Estimate for difference:  -18.6
95% CI for difference: (-50.3, 13.1)
t-Test of difference = 0 (vs not =): T-Value = -1.18  P-Value = 0.244  DF = 54
```

Table 4. Results of Mann-Whitney tests on total pathology scores

Mann-Whitney Test and CI: Pathology scores versus presence/absence of live or dead *Philometra* in ovary [by micrograph]

```
absent       N = 135     Median = 0.0000
present      N = 204     Median = 1.0000
Point estimate for ETA1-ETA2 is -0.0000
95.0 Percent CI for ETA1-ETA2 is (-0.0000,0.0000)
W = 21986.5
Test of ETA1 = ETA2  vs  ETA1 < ETA2 is significant at 0.1378
The test is significant at 0.1207 (adjusted for ties)
Cannot reject at alpha = 0.05
```

Mann-Whitney Test and CI: Pathology scores versus presence/absence of dead *Philometra* in ovary [by micrograph]

```
absent      N = 153     Median =  0.0000
present     N = 186     Median =  1.0000
Point estimate for ETA1-ETA2 is  0.0000
95.0 Percent CI for ETA1-ETA2 is (-0.0003,-0.0002)
W = 24419.5
Test of ETA1 = ETA2  vs  ETA1 < ETA2 is significant at 0.0383
The test is significant at 0.0286 (adjusted for ties)
```

Mann-Whitney Test and CI: Pathology scores versus presence/absence of live *Philometra* in ovary [by micrograph]

```
absent      N = 294     Median =  0.000
present     N =  45     Median =  1.000
Point estimate for ETA1-ETA2 is   -0.000
95.0 Percent CI for ETA1-ETA2 is (-0.000,-0.000)
W = 49863.5
Test of ETA1 = ETA2  vs  ETA1 < ETA2 is significant at 0.4249
The test is significant at 0.4193 (adjusted for ties)
Cannot reject at alpha = 0.05
```

Table 5. Results of Mann-Whitney tests on stage-specific oocyte frequency per image as a function of presence/absence of *Philometra*

Mann-Whitney Test and CI: stage 1 non-infected versus stage 1 infected

```
stage 1 non-infected     N =  27     Median =  30.000
stage 1 infected         N =  84     Median =  24.000
Point estimate for ETA1-ETA2 is  6.000
95.0 Percent CI for ETA1-ETA2 is (-1.000,13.000)
W = 1762.0
Test of ETA1 = ETA2  vs  ETA1 > ETA2 is significant at 0.0432
The test is significant at 0.0431 (adjusted for ties)
```

Mann-Whitney Test and CI: stage 2 non-infected versus stage 2 infected

```
stage 2 non-infected     N =  27     Median =   22.000
stage 2 infected         N =  84     Median =   15.500
Point estimate for ETA1-ETA2 is   7.000
95.0 Percent CI for ETA1-ETA2 is (2.999,10.000)
W = 1981.5
Test of ETA1 = ETA2  vs  ETA1 > ETA2 is significant at 0.0006
The test is significant at 0.0006 (adjusted for ties)
```

Mann-Whitney Test and CI: stage 3 non-infected versus stage 3 infected

```
stage 3 non-infected     N =  27     Median =    0.000
stage 3 infected         N =  84     Median =    0.000
Point estimate for ETA1-ETA2 is  0.000
95.0 Percent CI for ETA1-ETA2 is (-0.000,0.000)
W = 1462.0
Test of ETA1 = ETA2  vs  ETA1 > ETA2
Cannot reject since W is < 1512.0
```

Mann-Whitney Test and CI: stage 4 non-infected versus stage 4 infected

```
stage 4 non-infected     N =  27     Median =     0.000
stage 4 infected          N =  84     Median =     0.000
Point estimate for ETA1-ETA2 is  0.000
95.0 Percent CI for ETA1-ETA2 is (-0.000,0.000)
W = 1466.0
Test of ETA1 = ETA2  vs  ETA1 > ETA2
Cannot reject since W is < 1512.0
```

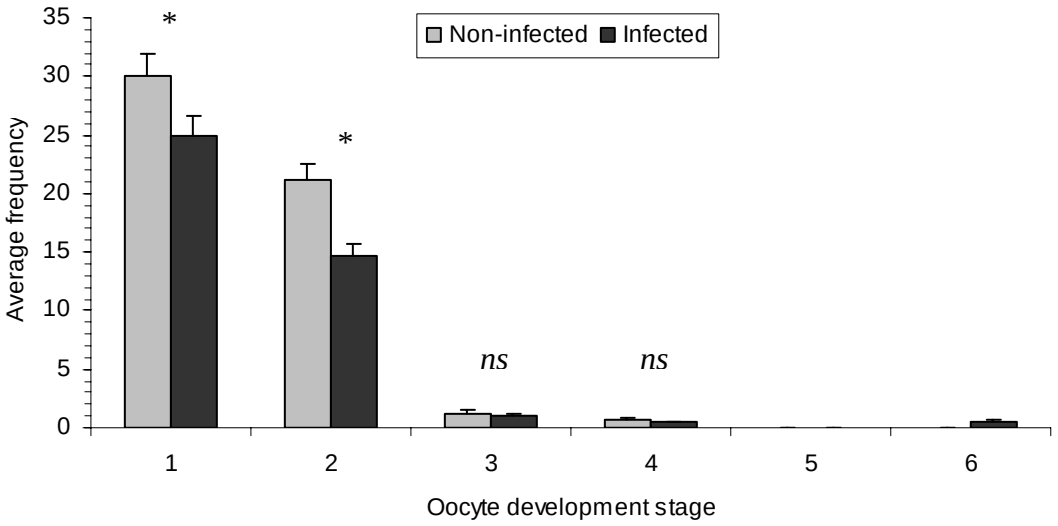

Figure 15. Average frequency of oocyte developmental stages in bluefish (*Pomatomus saltatrix*) infected and non-infected by *Philometra saltatrix*. Data are combined from July (the height of spawning season in NY) of 2004 and 2005. Sample sizes of images from non-infected and infected fish are 27 and 84, respectively. Error bars represent the standard error of the mean. Asterisks indicate significant differences resulting from Mann-Whitney tests and *ns* indicates non-significant differences. 1 = Perinucleolar stage, 2 = Lipid stage, 3 = Early vittellogenesis stage, 4 = Late vittellogenesis stage, 5 = Migratory nucleus stage, 6 = Hydrated stage.

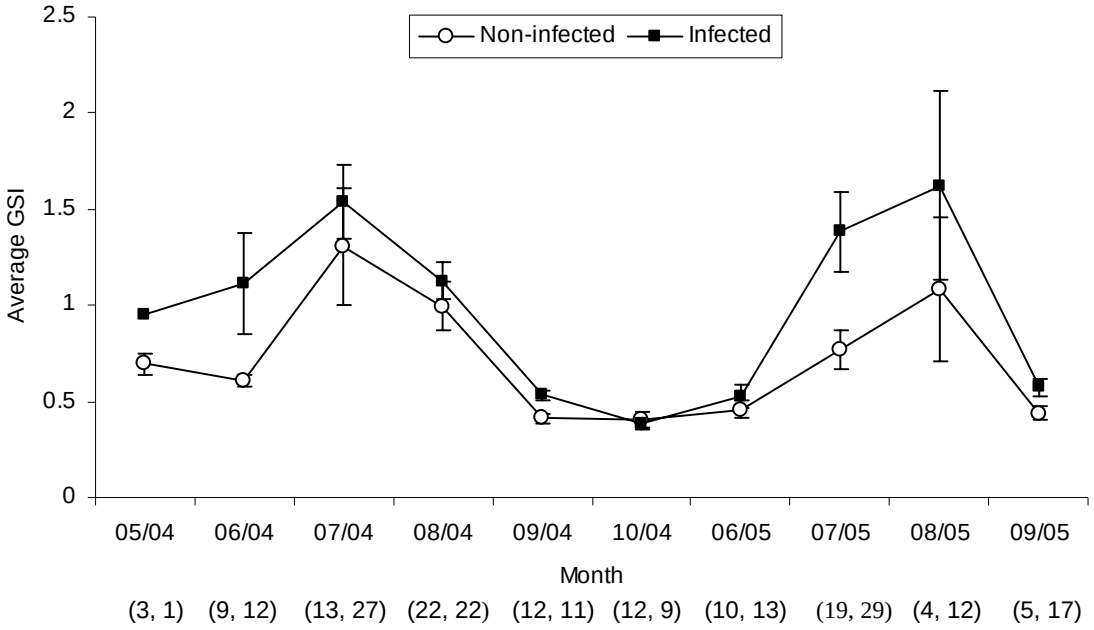

Figure 16. Changes in gonadosomatic index (GSI) of female bluefish (*Pomatomus saltatrix*) infected or non-infected with *Philometra saltatrix*. Data points indicate mean values, and error bars indicate SEM. Sample sizes of non-infected, and infected specimens, respectively are shown in brackets.

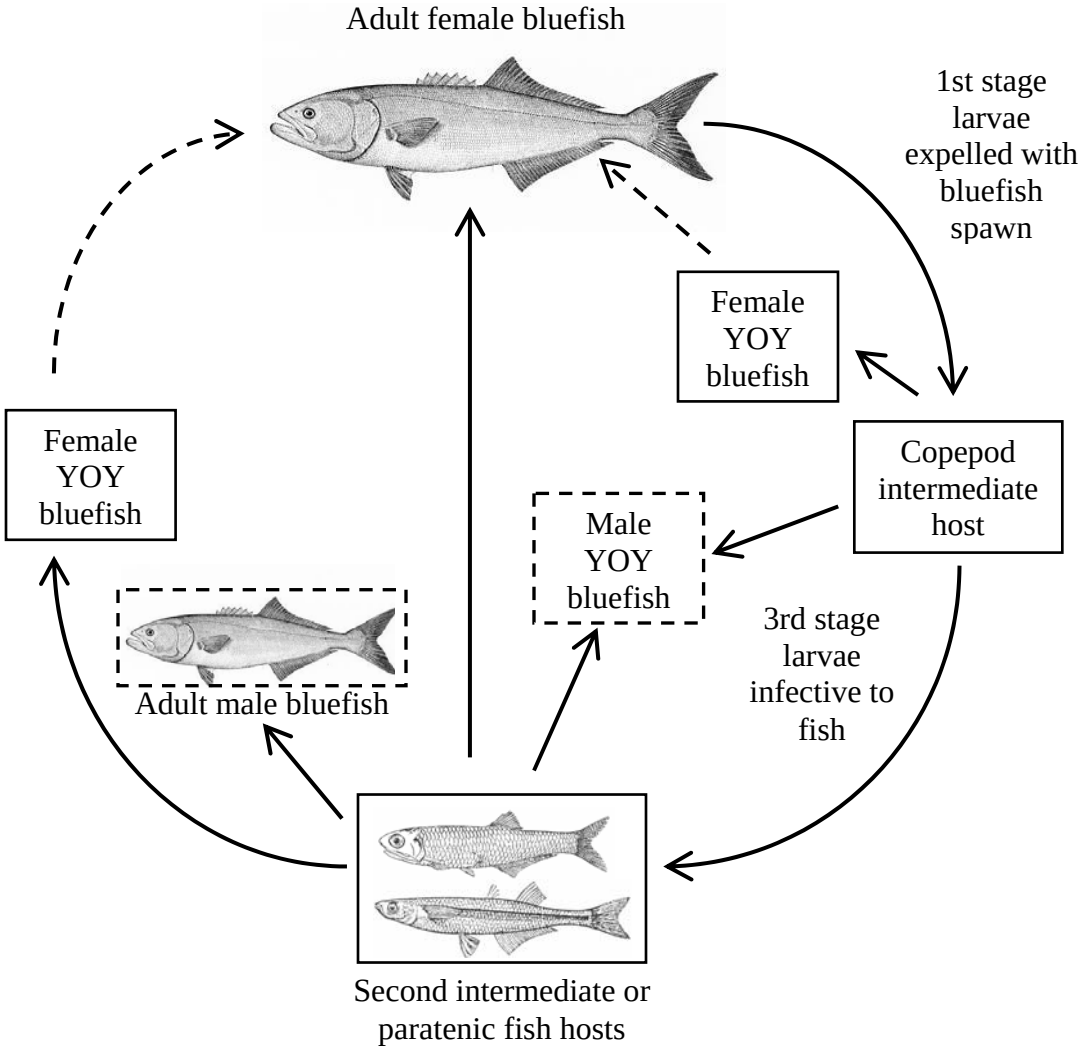

Figure 17. Proposed life-cycle of *Philometra saltatrix*. Solid arrows represent trophic relationships. Dashed arrows represent growth stages. Dashed boxes indicate non-completion of *P. saltatrix* life-cycle.