

Abstract—Examination of 203 adult bluefish (*Pomatomus saltatrix*) from Long Island, New York, in 2002 and 2003 and 66 from the Outer Banks, North Carolina, in 2003 revealed the presence of dracunculoid nematodes (*Philometra saltatrix*) in the ovaries of female fish. Percent prevalence reached 88% in July and then decreased after the peak of the spawning season. Bluefish contained up to 100 parasites per fish. Infection was associated with a range of disorders, including hemorrhage, inflammation, edema, pre-necrotic and necrotic changes, and follicular atresia, that may prevent proper development of oocytes and probably affect bluefish fecundity. Historical occurrences, life cycle, and geographical distribution of this nematode remain largely unknown, but may play important roles in recruitment processes of bluefish.

Prevalence, intensity, and effect of a nematode (*Philometra saltatrix*) in the ovaries of bluefish (*Pomatomus saltatrix*)

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Factors influencing recruitment variability in marine fishes are often complex and poorly understood. Slight variations in mortality rates, growth rates, and stage durations in the early life stages can result in tenfold or greater fluctuations in abundance (Houde, 1987). Recruitment variation appears to be driven by a combination of factors, such as environmental and oceanographic processes (Munch and Conover, 2000), diet (Friedland et al., 1988; Marks and Conover, 1993; Juanes and Conover, 1995), growth and development (McBride and Conover, 1991; Hare and Cowen, 1997) and habitat use (Able et al., 2003). The importance of parasitism and disease has, however, seldom been considered.

In the Northwest Atlantic, the bluefish (*Pomatomus saltatrix*) is distributed from Florida to the Gulf of Maine and is both commercially and recreationally important. This highly migratory species has at least two distinct spawning seasons. The first occurs in the spring, from March to May, south of Cape Hatteras, North Carolina (NC) (Kendall and Walford, 1979; Collins and Stender, 1987) and

the second occurs off the coast of New York (NY) from late June to August (Norcross et al., 1974; Sherman et al., 1984), peaking in July (Chiarella and Conover, 1990). Ichthyoplankton surveys have indicated that a third spawning event occurs south of Cape Hatteras, NC, in the autumn, but juveniles spawned during this time frame have rarely been captured (Collins and Stender, 1987).

During the collection of bluefish ovaries for another study, the nematode *Philometra saltatrix* Ramachandran, 1973 was detected in the ovaries of adult bluefish. Previous studies of *Philometra* spp. in other host species have indicated that their presence can have a negative effect on fecundity (Oliva et al., 1992; Hesp et al., 2002), implying that a more complete understanding of parasites may be important to understanding reproductive success. Although factors such as female size and condition are often considered in determining reproductive success, the role of parasitism is rarely investigated (Marshall et al., 1998; Marteinsdottir and Begg, 2002). The potential effect of this nematode on the reproductive

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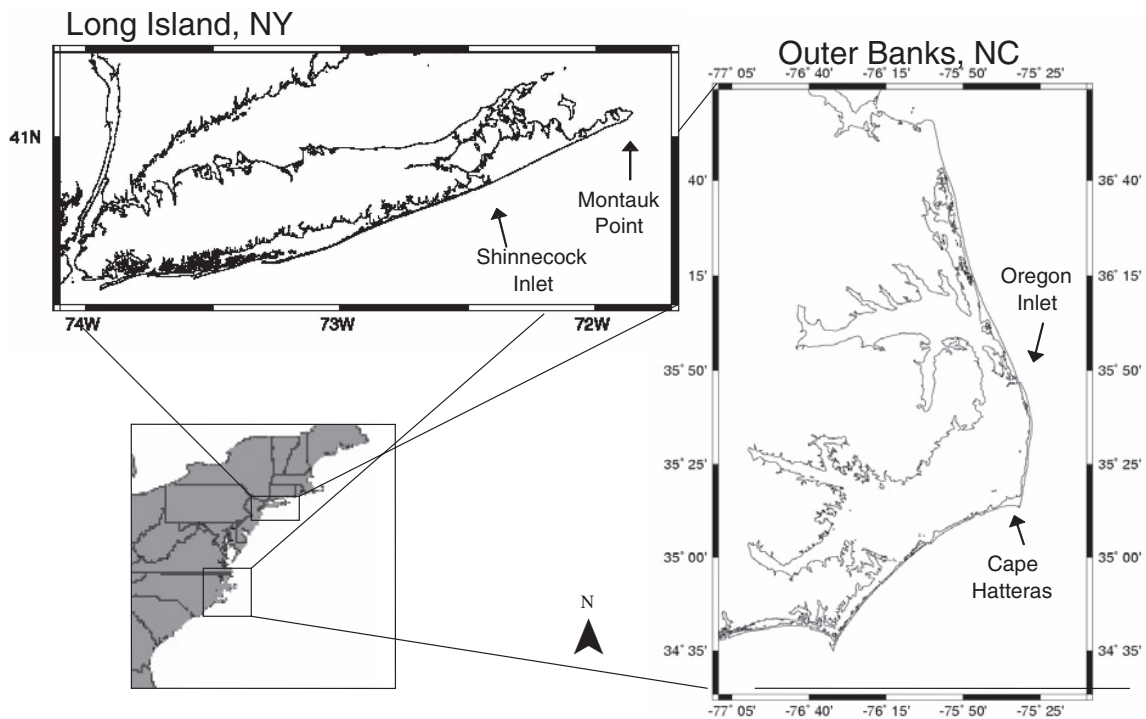


Figure 1

Map of the sampling area, Long Island, New York, and Outer Banks, North Carolina, where adult bluefish (*Pomatomus saltatrix*) were collected from commercial gill-netters, trawlers, and seafood markets in 2002–03 for examination of infestation by the nematode *Philometra saltatrix*.

potential and early life history success of bluefish is unknown. No information other than location of occurrence (Ramachandran, 1973) and a brief abstract describing the presence of philometrids in the heart of juvenile bluefish is available (Cheung et al.¹). The purpose of this study is to investigate the prevalence, intensity, and effect of *Philometra saltatrix* in the ovaries of bluefish.

Materials and methods

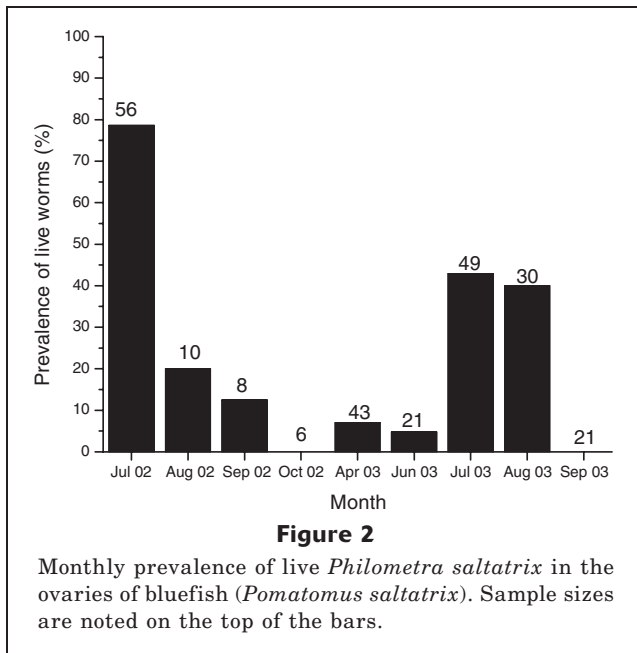
Adult bluefish were collected from commercial gill-netters, trawlers, and seafood markets on Long Island, NY, and the Outer Banks, NC (Fig. 1). In NY, fish were caught off the southern coast of Long Island from Shinnecock Inlet to Montauk Point, approximately 1–15 km offshore. In NC, fish were caught approximately 1–40 km off the coast, and the majority of fish were caught 30–40 km east of Oregon Inlet.

Sampling dates were determined by the availability of fish through the local fishermen. In 2002, bluefish were sampled from mid-July through early October off the southern coast of Long Island, NY (80 females, 108 males). In 2003, fish were collected in NC in April (43 females, 21 males) and in NY from the end of June through September (123 females, 42 males).

Fork length (FL), fish weight, gonad weight, prevalence of both live and dead worms, total worm weight, and gonadosomatic indices (GSI) were recorded for each fish. Worms were often intertwined making it difficult to count the number of worms in each ovary; therefore, total worm weight per ovary was used as a proxy for intensity. Representative samples were fixed in a solution of 95% glacial acid and 5% formalin for identification. Initially, examinations of both male and female fish were conducted, but after preliminary evidence showed that nematodes were not present in the gonads of male fish, future examinations were restricted to female fish.

Haphazardly selected ovaries were preserved in 10% formalin and processed according to standard histological methods (Luna, 1968) to investigate pathologies associated with the parasite. Transverse sections were cut from the same region in the center of each ovary. These were examined under a light microscope and images were captured with a Spot Insight digital CCD and processed with ImagePro Plus software (Media Cybernetics, Silver Spring, MD).

¹ Cheung, P. J., R. F. Nigrelli, and G. D. Ruggieri. 1984. *Philometra saltatrix* infecting the heart of the 0-class bluefish, *Pomatomus saltatrix* (L.), from the New York coast. In S. F. Snieszko commemoration fish disease workshop, p. 27. Joint Workshop of Fish Health Section, AFS, and Midwest Disease Group, Little Rock, AR.



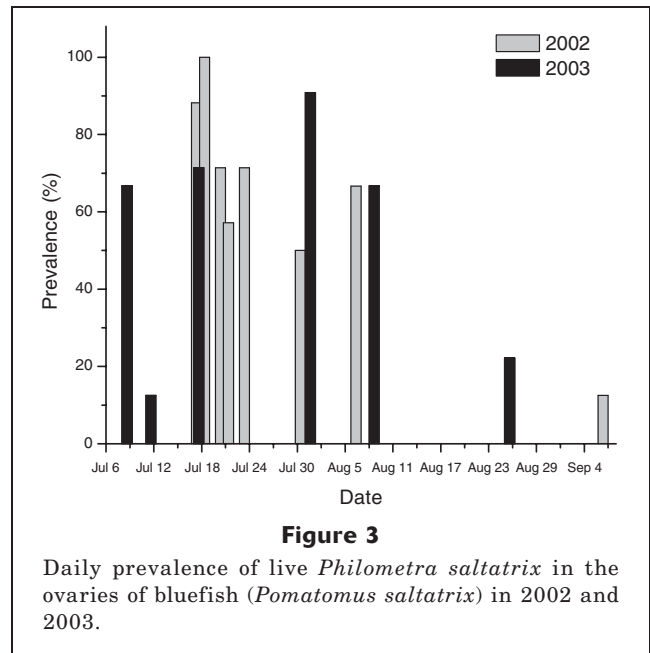
Results

Description and location of worm

Philometra saltatrix was identified in the gonads of female fish ranging in size from 363 to 815 mm (FL) in both NC and NY samples. The majority of worms found in the ovaries were gravid females. Gravid female worms were visible macroscopically and most often visible even before the initiation of ovary dissection. Female worms reached a maximum of 150 mm in length and approximately 300 μ m in width. Dead worms were present in all months sampled and were encapsulated by several melanised layers of fibrotic tissue. Approximately 20 juvenile male and female worms were identified in the ovary of a female fish in July 2003. These immature worms reached a maximum length of 2 mm. It should be noted, however, that not all fish were examined microscopically; therefore, it is possible that other male and juvenile worms were present in other hosts but not detected.

Prevalence and intensity

Bluefish in the western North Atlantic represent a single genetic stock (Graves et al., 1992), allowing us to combine data from New York and North Carolina to examine seasonal trends. Pooled by month of capture, the prevalence of infection varied seasonally during the two sampling years. In NC, prevalence of live nematodes was 7% in April, the spring spawning season (Fig. 2). Prevalence of live worms in NY in June was 4.8% and reached a maximum of 79% (2002) and 42% (2003) in July during the summer spawning season (Fig. 2). It is important to note that sampling in 2002 did not begin

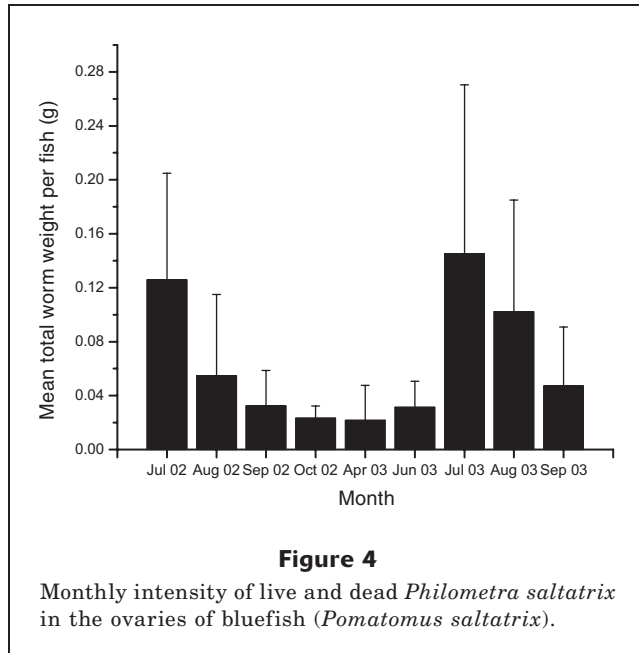


until mid-July but was conducted for the entire month in 2003. If only the second half of July is examined for both years, the prevalence is 79% and 83% for 2002 and 2003, respectively. This peak in prevalence was followed by a slow decrease in live worms until late August and early September after which time live worms were no longer detected.

Examination of prevalence by sampling day showed greater evidence of interannual variation. In the middle of July 2002, 100% of bluefish examined were infected with live *Philometra saltatrix*, whereas in 2003 the highest percentage of fish sampled that were infected was 91% (Fig. 3). Furthermore, in 2002 prevalence peaked in the middle of July, whereas in 2003 prevalence was highest at the end of the month. Additionally, live worms were detected until the end of August in 2002 but were detected two weeks later in 2003.

Intensity of parasite infection, as indicated by total live and dead worm weight per fish, reached a maximum 0.145 g (mean of 0.081 g \pm 0.1367) and was highest during the month of July in both years of the study (Fig. 4). Patterns of intensity were similar to those of prevalence; intensity was highest during the summer spawning season and then later decreased. One bluefish caught just south of Cape Hatteras, NC, in the beginning of May 2003 had the highest intensity observed with over 100 nematodes present (Fig. 5, A and B). This fish was not included in the data analysis because it was the only fish examined during that month, but this extreme instance of infection sets the upper bound on the level of observed intensity.

Binary logistic regression was used to test whether the presence or absence of the nematode was related to fork length ($P=0.096$), fish weight ($P=0.292$), or GSI ($P=0.783$), but no significant relationships were found.



However, a significant positive correlation was found between intensity and fish weight ($R = 0.169$, $P = 0.000$), intensity and fork length ($R = 0.221$, $P = 0.003$), and intensity and GSI ($R = 0.262$, $P = 0.000$). The Bonferroni method was applied to account for the multiple comparisons. Adjusted P -values are given.

Observations in adult males and YOY

Although not the target of this study, we also detected the presence of *Philometra saltatrix* in the pericardial cavity of one adult male bluefish collected in NC and three adult males in NY. Fish size ranged from 411 to 429 mm FL. Worms were not detected in the gonads of male fish.

Additionally, we detected worms in the pericardial cavity of three young of the year (YOY) bluefish caught in the Long Island Sound. Fish ranged in size from 140 to 185 FL.

Histopathology

Worms found in the ovary were surrounded by oocytes at various stages of development. The guts of these worms were most often filled with host erythrocytes. A diversity of pathological responses was noted, and significant variability was found among host individuals (Fig. 5). In many cases, infection was associated with interstitial hemorrhage in the connective tissues of the ovary. Occasionally, hemorrhage into the lumen of the ovary was also observed, but it was not clear whether this was caused by feeding activities of the worm or from tissue damage by other means. In many cases, moderate lymphocytic infiltration was observed in ovarian connective tissues. Some specimens showed marked edema in

the perifollicular spaces. Prenecrotic changes, including pyknosis and cellular swelling, were observed in connective tissue cells, and necrosis of both connective tissue and oocytes (atresia) was observed in several instances. Granulomatous inflammation and fibrosis occurred in association with dead worms and, occasionally, atretic follicles. Fibrotic capsules surrounding dead worms were polylaminar and melanised and appeared to represent female worms that had expelled larvae and had subsequently died. Encapsulated worms that still contained larvae were also observed occasionally.

Discussion

Bluefish along the east coast of the United States appear to be heavily infected with the ovarian nematode *Philometra saltatrix*. Although many studies provide descriptions of various philometrid species, very few provide information about the prevalence, intensity, or effect of these nematodes. Ramachandran (1973) described the presence of several female and male worms in a single ovary but provided no information on pathological changes associated with infection.

Intensity and prevalence of *Philometra saltatrix* cycle seasonally and appear to be synchronous with the bluefish spawning cycle. Although live worms were detected from April to October, the levels of infection were higher in July than in any other month sampled and this timing is coincident with the peak of the summer spawning season off NY (Chiarella and Conover, 1990). The life cycle of *Philometra saltatrix* is unknown and, therefore, it is not clear whether initial infection coincides with the spawning season or if nematodes are acquired at an earlier time and reside in some other host tissue site before migrating to the ovaries during the spawning season, perhaps stimulated by hormonal cues from the host. Prevalence and intensity were much lower during the spring spawning season south of Cape Hatteras, NC. It is difficult to determine whether this was due to geographical differences or the limited sampling that month; it is possible that we missed the peak spawning period in 2003. The rapid decline in both prevalence and intensity after the summer spawning period indicates that *Philometra saltatrix* are released or migrate out of the host fish synchronously with the release of fish eggs. We speculate that the reproduction cycle of the nematode is closely matched to that of the host bluefish.

Other studies have reported that infection occurs after first host maturity and that female nematodes have only been observed in the gonads of females (Oliva et al., 1992; Hesp et al., 2002). Although not the focus of this study, the pericardial cavity was also found to be infected by *Philometra saltatrix* in adult males in NC and NY and in YOY bluefish in NY waters, in contradiction to these other findings. It is not clear if these YOY infections represent separate infections or if they are the same infections observed in adult females. Reviewing the reported lifecycle of other spirurid nematodes

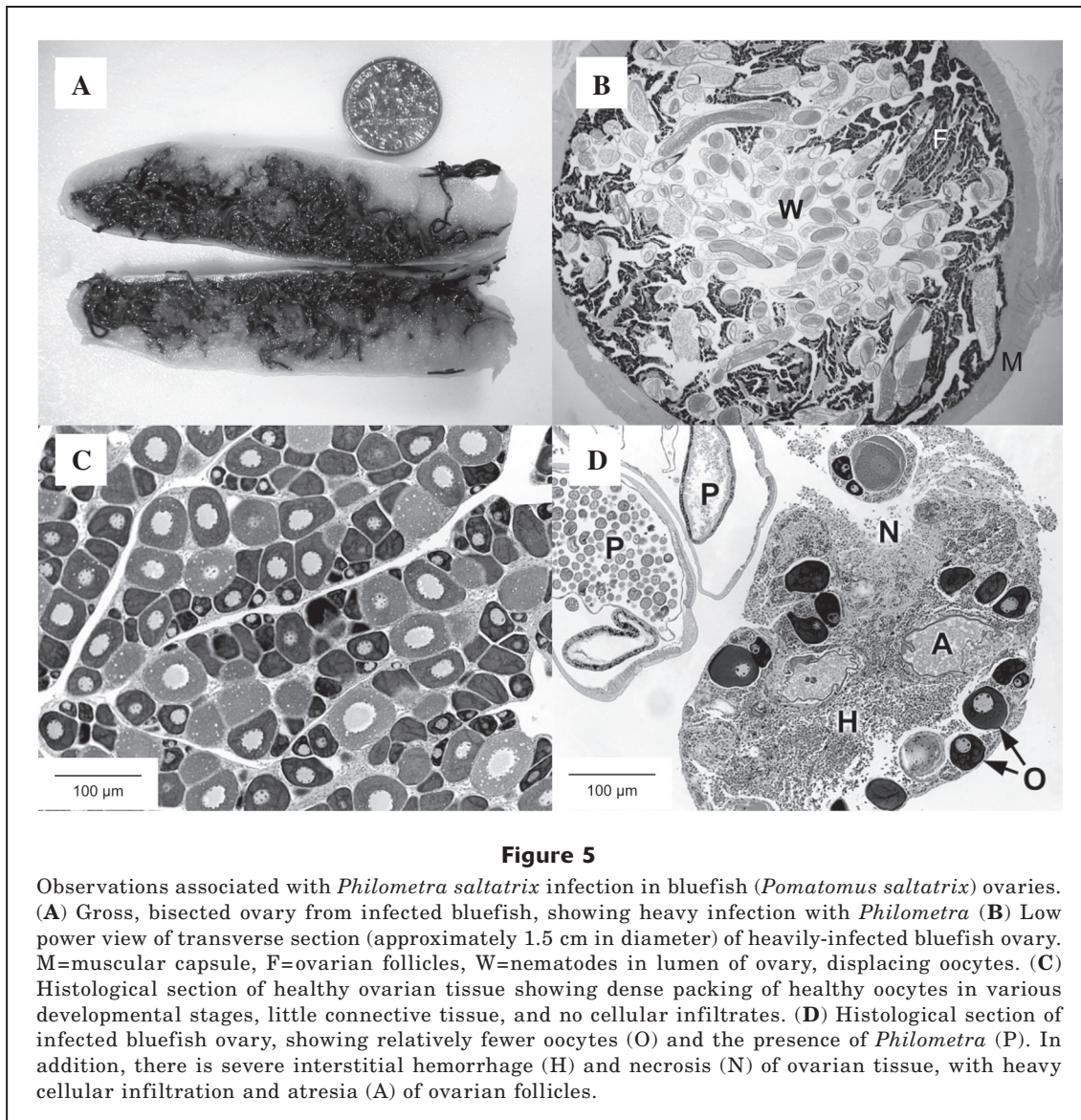


Figure 5

Observations associated with *Philometra saltatrix* infection in bluefish (*Pomatomus saltatrix*) ovaries. (A) Gross, bisected ovary from infected bluefish, showing heavy infection with *Philometra* (B) Low power view of transverse section (approximately 1.5 cm in diameter) of heavily-infected bluefish ovary. M=muscular capsule, F=ovarian follicles, W=nematodes in lumen of ovary, displacing oocytes. (C) Histological section of healthy ovarian tissue showing dense packing of healthy oocytes in various developmental stages, little connective tissue, and no cellular infiltrates. (D) Histological section of infected bluefish ovary, showing relatively fewer oocytes (O) and the presence of *Philometra* (P). In addition, there is severe interstitial hemorrhage (H) and necrosis (N) of ovarian tissue, with heavy cellular infiltration and atresia (A) of ovarian follicles.

and *Philometra* spp. (Williams and Jones, 1994), we speculate that nematode larvae are released at spawning time, pass through the copepod intermediate stage, and a second perhaps paratenic intermediate host (or perhaps pass through only one intermediate host), and infect YOY bluefish. It is important to note that most YOY bluefish larger than approximately 40 mm TL are piscivorous (Marks and Conover 1993); thus, it is possible that the infected YOY bluefish observed in this study were infected through a second intermediate host. The existence of a second intermediate fish host is supported by the observed positive relationship between host body size and parasite intensity. After initial infection, these worms reside in non-ovarian sites such as the pericardial cavity (as we observed), where they may remain quiescent until maturation of the host. At that time, worms may migrate through the tissues

to the ovary for spawning and the completion of their life-cycle. This proposed life history would explain the ontogenetic and seasonal patterns of worm distribution that we observed and explain the rapid appearance of well-developed worms in the ovary at the onset of the spawning season.

The prevalence and intensity of infections we observed in bluefish were significantly higher than those reported in most other fish species. For example, intensity of infection in *Glaucosoma hebraicum* by *Philometra lateolabracis* ranged from 1 to 7 nematodes (mean of 2 nematodes) (Hesp et al., 2002), whereas prevalence of *Philometra margolisi* in the gonads of red grouper, *Epinephelus morio*, ranged from 14 to 28% depending on locality (Moravec et al., 1995) and prevalence of *Philometra lateolabracis* in *Parupeneus indicus* was 5.3% (intensity of 1–2 nematodes) (Moravec et al., 1988).

Although most studies indicate the occurrence of these nematodes to be lower than in bluefish, in a later study Moravec et al. (1997) reported prevalence of *Philometra margolisi* in the gonads of *Epinephelus morio* to be as high as 88% with an intensity of up to 84 nematodes. Additionally, although most studies report the absence of male worms (Hesp et al., 2002), we found up to 20 males in the ovary of one female fish. The high percentage of adult female bluefish that are infected may indicate that *Philometra saltatrix* are significantly affecting the reproductive success of bluefish.

Despite some studies showing deleterious effects of other parasites on fish ovaries (see for example, Adlerstein and Dorn, 1998), studies on the effects of philometrids on fish ovaries remain scarce. Indeed, in a recent review of histopathological assessments of gonadal tissue in wild fishes, Blazer (2002) made no mention of *Philometra* spp., despite the fact that it is probably the most common adult helminth observed in fish gonads. Annigeri (1962) described damage to ovaries of *Otolithus argenteus* by an unidentified philometrid, and Ramachandran (1975) mentioned necrosis in the ovaries of *Mugil cephalus* caused by *Philometra cephalus*. The infection of testes of pink snapper *Pagrus auratus* by *Philometra lateolabracis* resulted in partial or extensive atrophy of those gonads (Hine and Anderson 1981). Oliva et al. (1992) alluded briefly to lower fecundity in *Paralabrax humeralis* as a result of infection with *Philometra* but provided no data in support of this assertion. Hesp et al. (2002) studied the dynamics and effects of *P. lateolabracis* in the gonads of the Australian predatory marine fish *Glaucosoma hebraicum*, but did not observe significant pathological abnormalities associated with infection.

Histopathological changes associated with philometrid infection in bluefish were significant. The hemorrhaging, edema, inflammation, atresia, and necrosis observed most likely reduce oocyte number and quality, leading to lower fecundity. The presence of erythrocytes in the guts of nematodes indicates that the parasites were feeding on the blood of the host; this diversion of nutrients to the parasite may exacerbate the impacts of the worm on ovarian tissue. Furthermore, intense infections can reduce the effective volume of the ovary and thus lead to lower fecundity (Fig. 5, A and B).

The high prevalence, intensity, and pathological damage observed could be an important factor in recruitment variation in bluefish. Although modest interannual differences in prevalence and timing of infection were observed in this study (Fig. 4), *Philometra saltatrix* was not observed by researchers conducting studies of the GSI of bluefish from NY waters in the 1980s (Chiarella and Conover, 1990; Conover, personal observ.). Hence, it is possible that the abundance of *Philometra* may fluctuate greatly over long time scales. The reason for the current prevalence of the worm and its effect on bluefish recruitment is unknown. Future studies of this host-parasite system should seek to account for temporal variations of this kind.

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