

Host, seasonal and habitat influences on incidence of *Lernaeenicus radiatus* (Copepoda: Pennellidae) in the mid-Atlantic Bight

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ABSTRACT: *Lernaeenicus radiatus* is a pennellid copepod with a 2-host life cycle that exhibits high host-specificity to their first host, black sea bass *Centropristis striata*. This parasite was prevalent in the gills of black sea bass juveniles and adults along the coast of New Jersey, USA, April to December 2019. Parasite incidence was high in the summer and fall in near-shore areas and dropped significantly in fish from deep waters further off-shore in December. Heavy infections of *L. radiatus* occurred in gills of adult black sea bass inhabiting reef-associated structures, in which parasite incidence rate was 2–3.7 times higher than in non-structure habitat. Less host-specificity occurred in second hosts which support female metamorphosis. In total, 7 fish species were confirmed as second hosts, with the most common being Atlantic menhaden *Brevoortia tyrannus* and bay anchovy *Anchoa mitchilli*. Incidence of *L. radiatus* depends on host abundance and habitats that support interactions of the preferred fish hosts, which may explain the heavy infections in reef habitats. The *L. radiatus* anchor process in metamorphosed females was highly polymorphic, depending on tissue tropism. Parasite length varied considerably, with neck and trunk measurements of *L. radiatus* from adult menhaden being 2–4 times larger than those from smaller host species. Mitochondrial cytochrome oxidase *c* subunit I (COI) sequences demonstrated all parasites to be *L. radiatus*, with sequence divergence limited to 0.3%. These findings show that morphology of the metamorphosed females has poor taxonomic value, and polymorphisms instead are related to attachment site and host characteristics.

KEY WORDS: *Lernaeenicus radiatus* · Pennellidae · Reef habitat · Black sea bass · Atlantic menhaden

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1. INTRODUCTION

Fish parasites are a natural component of the aquatic biota, and their presence can be a sign of a healthy and biodiverse ecosystem. Though some parasites may have little impact on fish health, others can have negative physiological consequences to their fish hosts. For example, the pennellid copepod *Lernaeocera branchialis* was estimated to cause reductions in the wild gadoid fishery around Scotland

of over 1000 t yr⁻¹ from lost body condition (Kabata 1970) and has resulted in mortality and poor weight gain in farmed Atlantic cod (Khan et al. 1990). Overall, impacts of infectious pathogens, including parasites, are difficult to estimate in wild marine fish populations. Morbidity and chronic mortality are rarely documented due to the large size of marine systems; thus pathogen surveillance programs can provide insights into disease ecology of marine species. Two important marine fish species in the northwest At-

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lantic, the black sea bass *Centropristis striata* and the Atlantic menhaden *Brevoortia tyrannus*, are known hosts in the life cycle of the pennellid copepod *Lernaeenicus radiatus* (Shields 1977, Voorhees & Schwartz 1979), though little information is known about the ecology of this parasite in the region.

Black sea bass and Atlantic menhaden are common in the mid-Atlantic Bight, an area in eastern USA delineated by Cape Cod, Massachusetts, and the Georges Bank to the north, and Cape Hatteras, North Carolina, to the south (Able & Fahay 2010). Black sea bass are protogynous hermaphrodites that spend spring and summers in near-shore environments and overwinter off-shore over the continental shelf (Provost et al. 2017). They account for an important recreational and commercial fishery throughout the northwest Atlantic (ASMFC 2019). Black sea bass is also a target species for marine aquaculture due to their ease of adapting to intensive aquaculture methods and their high marketability (Watanabe 2011). Atlantic menhaden are migratory fish that overwinter in the southeast and move northward in spring and summer (Nicholson 1978). They are a vital species to marine ecosystems; juvenile fish are filter-feeders that can impact water quality by reducing eutrophication (Gottlieb 1998) and adults are a primary forage fish for marine mammals, birds and other commercially important fish species such as the striped bass (Walter & Austin 2003, Glass & Watts 2009). Atlantic menhaden also support the largest commercial fishery in the eastern USA for use as fish meal, fish oil and bait in other fisheries (SEDAR 2015). Knowledge of infectious diseases in these species is important for understanding and identifying risk factors that can influence their ecology, contributing to population fluctuations in the wild and potential risks for aquaculture of marine species.

Black sea bass is a known first host in the life cycle of *L. radiatus* in the mid-Atlantic Bight (Shields 1977), with infections causing gill pathology resulting in reduced gill surface area (Lovy & Friend 2020). With little knowledge on the extent of *L. radiatus* infections in wild black sea bass, this study set out to determine the incidence of *L. radiatus* between various locations and seasons from juvenile and adult black sea bass populations off the coast of New Jersey. With much of the bottom habitat off the coast of New Jersey being sandy and devoid of structure, an artificial reef program exists to enhance fisheries opportunities and recreational diving. The artificial reef program in the state provides additional structural habitat in 13 federally permitted reef sites to support fish biomass, biodiversity and increased fish-

ing opportunities, particularly for species that prefer rocky habitat such as black sea bass (Resciniti et al. 2009). The diversity and abundance of parasites, particularly ones that require multiple hosts in their life cycle, is linked to biodiverse ecosystems such as reef habitats (Cribb et al. 1994, Hudson et al. 2006, Laferty et al. 2008). We compared incidence rates of *L. radiatus* in black sea bass from artificial reefs and non-structure-related habitat to see if differences existed between these habitat types.

Following maturation and sexual reproduction, female pennellid copepods undergo metamorphosis into considerably larger egg-bearing parasites (Lester & Hayward 2006). The developmental process has been shown to occur within a single host, as reported for *Lernaeenicus sprattae* (Schram 1979) and *L. ramosus* (Izawa 2019), or 2 different fish hosts, as reported for *Lernaeocera branchialis* (reviewed by Brooker et al. 2007). Unlike other described *Lernaeenicus* spp., *L. radiatus* has a 2 host life cycle (Shields 1977, Overstreet 1978), more similar to *Lernaeocera* spp. In fact, *L. radiatus* has been shown to be phylogenetically more closely related to *L. branchialis* rather than other *Lernaeenicus* spp. (Lovy & Friend 2020). In the mid-Atlantic Bight, the black sea bass has been shown to be the first host for *L. radiatus* development, whereas in more southern ranges the rock sea bass *Centropristis philadelphica* has been reported as a primary first host (Overstreet 1978). Black sea bass is in fact the definitive host since sexual reproduction occurs in this species, though since a second host is required for female metamorphosis, we refer to them as the first host herein. Parasites that utilize multiple hosts in their life cycle depend on the interaction of these species throughout their habitat. Changes influencing habitat biodiversity or population densities may cause changes in the abundance of these parasites. To understand the ecological factors involved with transmission of *L. radiatus*, it is critical to identify its host range. To date, only black sea bass have been identified as a first host for the parasites in the mid-Atlantic Bight. Herein, other marine fish species found in similar habitats were examined to explore the host-range of *L. radiatus* and to understand risk factors related to transmission of this parasite.

Upon metamorphosis, female *L. radiatus* undergo dramatic changes from a typical microscopic copepod in the gill to a relatively large mesoparasite that embeds its cephalothorax in the muscle of the fish host, while the neck, trunk and egg sacs occur externally (Wilson 1917). *Lernaeenicus* is a species-rich genus from which, out of necessity, species have been de-

scribed based on morphology of metamorphosed females (Wilson 1917, Hogans 2018, Walter & Boxshall 2018). The morphology of metamorphosed females can be highly polymorphic, making it a poor species marker (Castro-Romero et al. 2016); thus it is possible that some species have been defined erroneously. Genetic sequences of the mitochondrial cytochrome *c* oxidase gene (COI), used for DNA barcoding, have helped to better define species boundaries within copepods and to overcome the high phenotypic plasticity in these species (Richter et al. 2015, Castro-Romero et al. 2016). To understand transmission risks of *L. radiatus*, it is critical to document the host species that support female metamorphosis, since these will be the host species responsible for amplification of the parasite. To date, metamorphosed female forms of *L. radiatus* have been well documented in at least 16 different marine fish species, including common species in the mid-Atlantic Bight such as Atlantic menhaden, blueback herring *Alosa aestivalis*, bay anchovy *Anchoa mitchilli*, Atlantic croaker *Micropogonias undulates*, scup *Stenotomus chrysops*, striped killifish *Fundulus majalis* and mummichog *F. heteroclitus* (Wilson 1917, Voorhees & Schwartz 1979, Hogans 2018, Walter & Boxshall 2018). In the present study, metamorphosed female forms of the parasite were collected from a variety of species whose habitat overlapped with that of the black sea bass. Parasite morphology and genetics were assessed to link the 2 life stages of *L. radiatus* from various hosts, as well as to document polymorphisms within the metamorphosed females of *L. radiatus*.

2. MATERIALS AND METHODS

2.1. Juvenile black sea bass collection and sampling

Collection of juvenile black sea bass occurred from April–November 2019 from 3 sampling locations: Great Bay (n = 209), Barnegat Bay (n = 34) and Little Egg Harbor (n = 29) on the coast of New Jersey, USA (Fig. 1). Fish collection was done by ichthyoplankton sampling, wire-mesh traps and otter trawl. Ichthyoplankton samples were collected weekly from Great Bay estuary with a 1 mm mesh circular plankton net with a 1 m diameter (see Able et al. 2017 for additional details). Un-baited Gee wire mesh traps were deployed in the Rutgers University Marine Field Station (RUMFS) boat basin and sampled twice weekly (see Able et al. 2017). Otter trawl samples were collected via three 120 s otter trawl tows using a 4.9 m

headrope, 19 mm mesh wings and 6.3 mm mesh cod-end liner at habitat-specific locations throughout the salinity gradient (see Nickerson et al. 2018). Samples collected from April and May were considered spring samples, June through August were grouped as summer samples and September and October were fall samples. Water temperatures and dissolved oxygen for all sites by season were, respectively, 10–15°C and 8–9 mg l⁻¹ in spring, 20–25°C and 6–7.5 mg l⁻¹ in summer and 15–20°C and 6–8.25 mg l⁻¹ in fall.

Throughout each month of collection, juvenile fish were maintained alive and transferred to RUMFS wet lab holding tanks with flow-through ambient seawater and supplemental aeration. Water temperature in the holding tanks was about 18.6 ± 2.6°C throughout the study. Average salinity was 28.13 ± 1.82 ppt. Fish were offered feed daily and held for several days up to 3 wk until transfer to the Pequest Fish Health Lab for analysis. During extended holding it was possible for *Lernaenicus radiatus* to drop off the gills of held fish. Shields (1977) reported high water temperatures, between 27 and 30°C, to be crit-

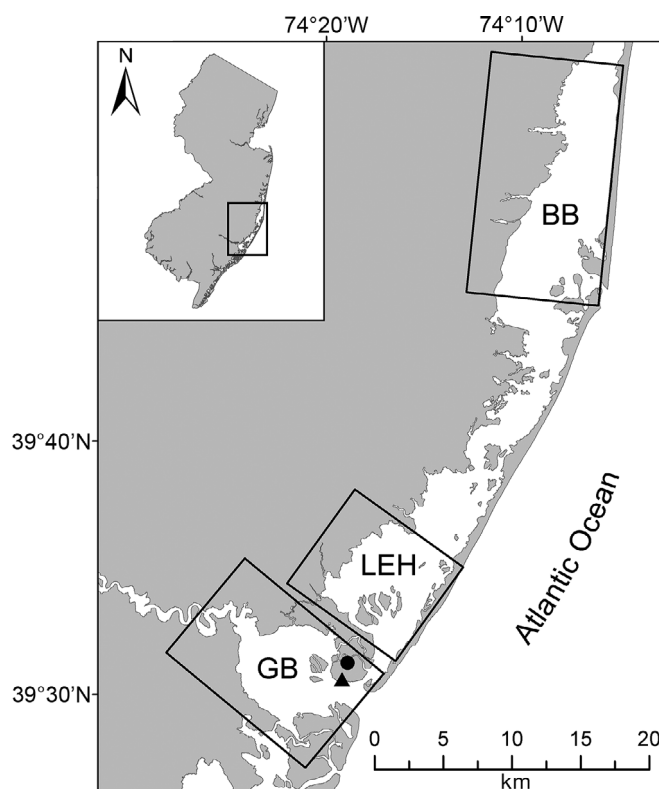


Fig. 1. Three sampling locations for juvenile black sea bass *Centropomus striata* (GB: Great Bay; LEH: Little Egg Harbor; BB: Barnegat Bay) along coastal New Jersey, USA. Wire mesh traps were set at the Rutgers University Marine Field Station (triangle); ichthyoplankton sampling occurred at Little Sheepshead Creek bridge (circle)

ical for the development (4–5 d) of the parasite in the gills of black sea bass. The cooler holding temperatures would considerably delay parasite development; thus significant losses of parasites were not likely. Holding tanks were provided with unfiltered seawater from Great Bay; thus the possibility existed that free-swimming *L. radiatus* stages could enter the system during fish holding and infect them prior to examination. The water supply for the tanks was from the same site where fish collection occurred in Great Bay, which had the lowest incidence rate of the parasite throughout the study. With minimal flow rates and a lack of chemical cues for copepods at the inlet pipe, this was not believed to be a substantial incoming source of parasites in held fish.

Within 24 h of transfer, fish were euthanized with an overdose of tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate. Fish were measured for total length (TL) (see Table 1) and all 8 gill arches were dissected from each individual and observed using a Zeiss Stemi-2000 stereomicroscope (Carl Zeiss). As the parasite only infects gills of black sea bass, total counts from all gill arches were made to determine the number of *L. radiatus* in each fish. Fresh gills with large numbers of *L. radiatus* were fixed in 10% neutral buffered formalin and routinely processed for histology.

2.2. Adult black sea bass collection and sampling

Adult black sea bass were collected between June and November 2019 in order to compare parasite incidence in the summer and fall seasons. Fish were also collected from 2 different surveys: the ocean trawl survey and the artificial reef survey, representing 2 different types of marine habitat. The ocean trawl survey is part of an ongoing ocean stock assessment project, and fish are collected from sites with sandy bottom and little to no bottom structure. Fish collection was done by using a 30 m trawl net with a 6.35 mm mesh nylon cod-end liner and 1.5 m steel trawl doors. Each tow was about 1 nautical mile at 3 knots (20 min tow). Fish were collected from various points within the ocean trawl survey zone off the coast of New Jersey (Fig. 2). In summer, between June and August, surface water temperature was between 20 and 24°C and bottom water temperature was between 10 and 20°C. The bottom dissolved oxygen ranged from 4.8 to 7 mg l⁻¹. In the fall, surface and bottom water temperatures were between 14 and 16°C, with dissolved oxygen at the bottom measuring around 7.8 mg l⁻¹.

Black sea bass collected from the artificial reef survey were taken from 2 artificial reefs off the coast of New Jersey: Sea Girt Reef, with a depth of 20 m, and Little Egg Harbor Reef, with a depth of 15 to 18 m (Fig. 2). The artificial reefs were composed of dense materials, including old ships, barges, tanks, concrete and steel demolition debris and dredge rock, intentionally placed there to support habitat for fish, shellfish and crustaceans. Fish collection from artificial reefs was done using 22 un-baited pot traps that were deployed on each of the reefs and hauled within 7 d of deployment. Bottom water temperatures in summer were between 11.7 and 13.7°C.

To compare these inshore sites to deeper offshore sites, 20 adult black sea bass were collected from a charter fishing boat about 70 miles (~113 km) offshore of Barnegat Bay from a depth of 80 m on 21 December 2019. The fish were frozen until analysis. Lastly, black sea bass gills, either frozen or fixed in 96% ethanol, were provided from the coasts of Delaware, Virginia and Florida (Fig. 2) from depths of 26, 85 and 43 m, respectively.

Fish were sampled within 24 h of collection or were frozen within 24 h and subsequently sampled after fully thawing. Mean fish TL was recorded for each fish (see Table 2). From 60 fish distributed throughout the survey, all 4 gill arches from both sides of the fish were dissected and prepared for counting *L. radiatus* on the gills. For counts, each gill arch was scraped to remove the copepods, and they were dispersed on a Petri dish in a thin layer diluted with seawater for counting. Total counts were obtained for each of the gill arches to understand the distribution of the parasites within the gills. The counts of the 2 outermost gills from the left side were estimated to make up 29.939% of total copepod numbers for each fish. For the remaining fish, this factor was used to estimate the total number of copepods per fish based on counts from those 2 gill arches.

Select gills from fresh fish were fixed in 10% neutral buffered formalin and routinely processed for histology. Genetic samples were taken by aseptically removing the copepods and either fixing them in 96% ethanol or freezing them at -80°C.

2.3. Screening other fish species for *L. radiatus*

To determine host preferences for *L. radiatus*, other fish species were collected from the above-described surveys along the coast of New Jersey between June 2019 and January 2020 (see Table 3). Smaller bodied fish captured from the Great Bay estuary with evi-

dence of *L. radiatus* infection were immediately fixed whole in 96% ethanol for subsequent examination. Additionally, adult Atlantic menhaden were collected in cooperation with the commercial pound-net fishery from various sites off the coast of New Jersey (Fig. 2) and frozen until examination. All fish were externally examined for metamorphosed female *L. radiatus* and all gill arches were dissected from each fish and examined using a stereomicroscope to screen for developing or mature *L. radiatus*.

Metamorphosed female *L. radiatus* were carefully dissected from the muscle and/or bone of affected fish to document their morphology. The anchor process was examined and photographed using a stereomicroscope equipped with a ProgRes Gryphax Arktur CMOS digital camera (Jenoptik AG). Measurements of metamorphosed females included TL of the parasite including the egg sacs, and separate measurements of the neck and trunk. Measurements were done grossly and with the aid of a stereoscope.

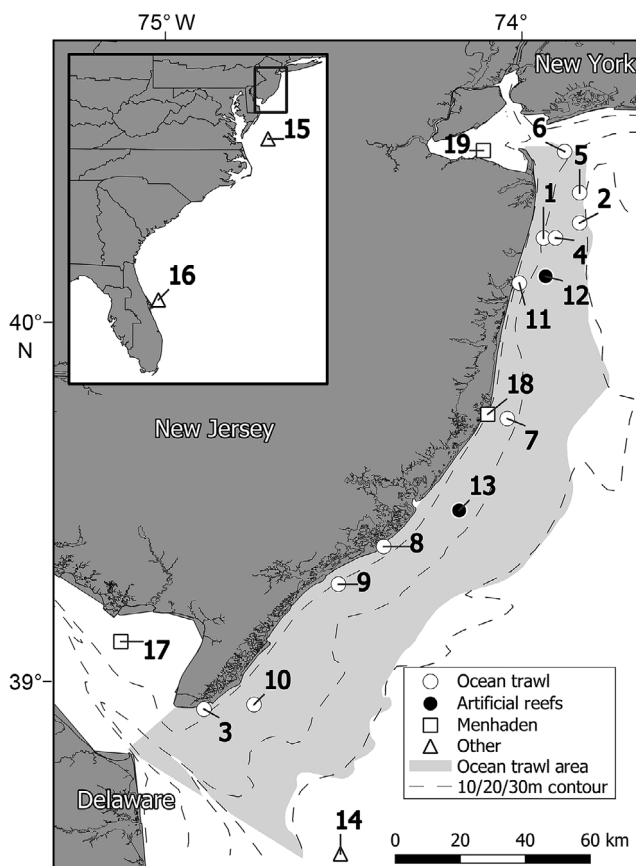


Fig. 2. Sampling locations for adult black sea bass *Centropristis striata* from the ocean trawl survey, artificial reef survey and other surveys. Adult Atlantic menhaden *Brevoortia tyrannus* were collected from 3 different sites. Site locations correspond to biological data provided in Table 2

Genetic samples from individual parasites were taken using an aseptic tissue sample as further described in Section 2.5.

2.4. Statistical analysis and relative condition factor

Data were organized using Microsoft Excel before being imported into Stata 12.1. Juvenile black sea bass data were initially evaluated for associations, confounding and model fit. The outcome, counts of *L. radiatus*, was found to fit the Poisson model based on results from the goodness-of-fit test. Predictor variables found to be associated with the outcome and therefore included in the model were location, season and TL.

Adult black sea bass data were initially evaluated for various associations, confounding and model fit. The outcome, counts of *L. radiatus*, was again found to fit the Poisson model based on results from goodness-of-fit testing. Predictor variables included in the model were the same as the juvenile black seabass data, with the addition of condition factor. The differences between seasons and locations with respect to parasite counts were evaluated in both juvenile and adult black sea bass.

Adult black sea bass condition was compared using relative condition factor (K_n) as described by Le Cren (1951) and Blackwell et al. (2000). TL and weight were logarithmically transformed to generate a 1:1 linear slope, from which the equation $Y = \beta_0 + \beta_1 X_1$ was developed to predict the log relative weight (Y), where β_0 = constant or intercept, β_1 = coefficient for log TL, and $X_1 = \log TL$. The inverse log then was used to obtain a relative weight for each fish according to TL. K_n was calculated as $K_n = W/W'$, where W = observed weight and W' = relative weight; see Table 2 for these results. K_n was calculated for Atlantic menhaden as described above, except TL and weight were not logarithmically transformed. Evaluation of the impact of *L. radiatus* totals on K_n was done using a linear regression analysis.

2.5. Genetic analysis of *L. radiatus* from various host species and locations

To better understand the relationship of the *L. radiatus* stages from the gills of black sea bass and fully metamorphosed female stages from the muscle of a variety of fish species, genetic samples were taken to represent both stages of development. Genetic samples from gills of black sea bass involved

taking a single whole parasite; for samples from metamorphosed females, either a piece of the trunk or egg sacs were collected. Additionally, to understand if genetic differences existed in parasites from black sea bass from different locations, representative samples were taken off the coast of New Jersey, Delaware and Virginia ($n = 15$). This included 6 individual copepod samples from New Jersey collected from Sea Girt Reef, 5 from Delaware fish and 4 from Virginia. Metamorphosed female samples were all collected from the coast of New Jersey and included 9 samples from Atlantic menhaden, 10 samples from bay anchovy *Anchoa mitchilli*, 3 samples from naked goby *Gobiosoma bosc*, 3 from silver perch *Bairdiella chrysoura*, 3 from summer flounder *Paralichthys dentatus*, 1 sample from mummichog *Fundulus heteroclitus* and 1 sample from Atlantic herring *Clupea harengus*. In total, 45 samples were fixed in 96% ethanol and maintained at -20°C until further processing.

DNA was extracted by first lysing in a solution of 180 μl buffer ATL (Qiagen) and 20 μl Proteinase K by bead beating for 2 min at 20 Hz and incubating for 2 h at 56°C . For metamorphosed female samples, the incubation time was doubled. DNA was purified using the DNeasy blood and tissue kit, either manually or automated on a QIAcube (Qiagen) according to manufacturer's instructions for extracting DNA from animal tissues. A 710 bp fragment of the COI gene was amplified by PCR and sequenced in both directions using the forward primer LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G), and reverse primer HCO2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA), described by Folmer et al. (1994). Reactions were performed in 50 μl volumes containing 3 μl of template DNA, 1 \times PCR buffer, 1.5 mM MgCl_2 , 0.2 mM each deoxynucleoside triphosphate (dNTP), 0.4 μM of each primer and 5 U Platinum *Taq* DNA Polymerase (Invitrogen) and topped up with molecular grade water. Amplification was done on a Veriti thermocycler (Applied Biosystems) with initial denaturation at 94°C for 5 min, followed by 38 cycles of 94°C for 45 s, 48°C for 45 s and 72°C for 80 s, followed by final extension at 72°C for 7 min. Products were sequenced using the amplification primers. Sequences were assembled using BioEdit (version 7.2.5) and queried in the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool Nucleotide (BLASTn) database to compare identities to other known species.

The COI sequences were compared to each other to determine sequence divergence using a median-joining network (Bandelt et al. 1999). Two network maps were generated using Population Analysis with Reticulate Trees (PopART; version 1.7; [http://popart.](http://popart.otago.ac.nz)

[otago.ac.nz](http://popart.otago.ac.nz)) to determine patterns of sequence divergence by host species and by location of sampling.

3. RESULTS

3.1. *Lernaeenicus radiatus* in juvenile black sea bass populations

L. radiatus development to maturity occurred only in the gill tissue of black sea bass. The developing copepods in gills were uniform in size irrespective of host size; thus, pathology related to a single copepod affected a larger relative proportion of the gill in small fish compared to larger fish (Fig. 3A,B). In young-of-the-year (YOY) fish, a single copepod caused lamellar fusion affecting an entire gill filament and a neighboring filament (Fig. 3B), whereas in larger fish a single copepod would impact a smaller relative portion of a gill filament.

L. radiatus was present in the Great Bay estuary during the earliest sampling point in April and remained present throughout the duration of sampling, with prevalence in fish ranging from 34 to 49% (Table 1), but parasite incidence increased in gill samples the later that they were collected in the year (Fig. 4). The incidence rate of gill *L. radiatus* in the summer was 1.84 times higher than in spring ($p < 0.001$), 1.16 times higher in fall than in summer ($p < 0.001$) and 1.59 times higher in fall than in spring ($p < 0.001$). In Barnegat Bay, prevalence of infection dropped in the fall compared to summer, though a similar number of copepods occurred with fewer fish having a higher infection intensity (Table 1). There was no difference in *L. radiatus* incidence in Barnegat Bay between summer and fall ($p = 0.182$). In Little Egg Harbor the incidence rate of gill *L. radiatus* was 2.11 times higher in the summer than in fall ($p < 0.001$) (Fig. 4).

An association existed between fish TL and incidence of *L. radiatus* in gills (Fig. 5). Overall, for every unit increase in TL there was a 1.33 factor increase in the gill *L. radiatus* totals ($p < 0.001$) when considering all sites and seasons. Since TLs of fish were variable between sites (Table 1), TL could be a factor attributing to differences between sites. When controlling for fish TL and seasons in the model, a significant difference in incidence rates of gill *L. radiatus* existed between sites. Overall, Little Egg Harbor had the highest incidence rate of *L. radiatus*, being 4.04 times higher ($p < 0.001$) than Great Bay and 2.71 times higher ($p < 0.001$) than Barnegat Bay. The incidence rate at Barnegat Bay was 1.48 times higher ($p < 0.001$) than in Great Bay.

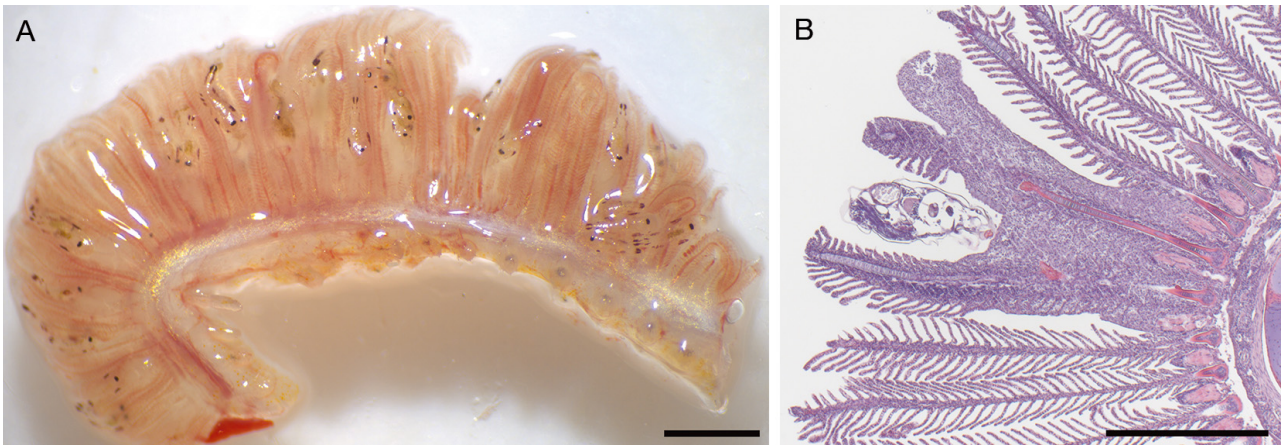


Fig. 3. *Lernaeenicus radiatus* infecting the gills of juvenile black sea bass *Centropristis striata*. (A) Entire gill arch with pale gills and hyperplasia adjacent to attached parasites (scale bar: 1 mm); (B) histology showing a single parasite causing lamellar fusion affecting 2 adjacent gill filaments (scale bar: 0.5 mm)

3.2. *L. radiatus* in adult black sea bass populations

Similar to juvenile fish, developing and mature *L. radiatus* were found only in gill tissue in adult black sea bass. Distribution of *L. radiatus* in gills of black sea bass was relatively even and occurred in all gill arches. The 2 outermost gill arches on both sides had the largest numbers of parasites with fewer parasites in the 3rd and 4th gill arches, which are the innermost gills and are smaller in relation to the outer 2 gill arches. Based on counts of 60 fish, the percent distribution of parasites in the gills from the left (L) and right (R) sides was: L1 = 14.8; L2 = 14.1; L3 = 10.7; L4 = 9.3; R1 = 15.6; R2 = 14.3; R3 = 11.8; R4 = 9.4.

Gill infections with *L. radiatus* occurred in every adult black sea bass (n = 244) collected from the artificial reefs and the ocean trawl survey, but lower incidence occurred in fish from depths of 80 m (Table 2). Reference samples collected south of New Jersey detected *L. radiatus* in the gills, though with less frequency. Off the coast of Delaware (Site 14 on Fig. 2), all fish (n = 8) were infected with a mean of 100 ± 46 copepods per fish. Black sea bass collected off the coast of Virginia (Site 15 on Fig. 2) showed a considerable drop in infection; 5 out of 18 fish were infected and infections ranged from 3 to 10 gill copepods per fish. Gill copepods were not detected in black sea bass collected off the coast of Florida (n = 10; Site 16 on Fig. 2) from 43 m depth.

Differences in the incidence rates of *L. radiatus* in gills of black sea bass collected off the coast of New Jersey varied with habitat (Fig. 6). Most notably, some fish collected from the 2 artificial reefs were heavily infected (>5000 copepods per fish), whereas this did not occur in fish collected from any of the

Table 1. Mean (±SD) total lengths (TL) of juvenile black sea bass *Centropristis striata* with *Lernaeenicus radiatus* infection, collected during different seasons from 3 different sites. Prevalence (no. and %) and total number of *L. radiatus* individuals from each season and site are provided

Season	TL	Prevalence No. %	Total <i>L. radiatus</i>
Great Bay			
Spring	65 ± 12	39/86 45	288
Summer	76 ± 28	17/50 34	449
Fall	72 ± 29	36/73 49	1416
Barnegat Bay			
Summer	113 ± 43	17/19 89	1536
Fall	122 ± 45	5/15 33	1372
Little Egg Harbor			
Summer	88 ± 35	16/16 100	2263
Fall	64 ± 28	7/13 54	425

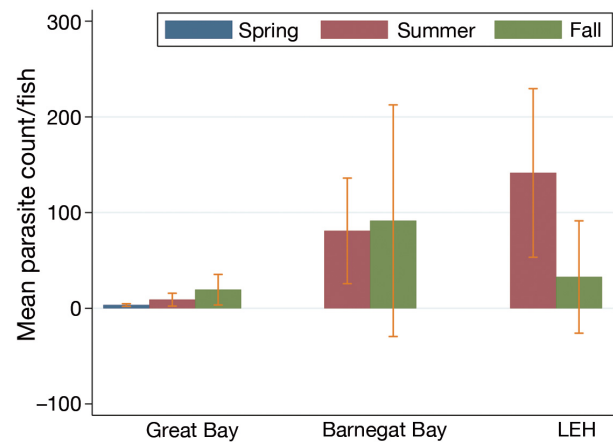


Fig. 4. Mean (±SE) *Lernaeenicus radiatus* counts in gills of juvenile black sea bass *Centropristis striata*, from 3 sampling locations (LEH: Little Egg Harbor)

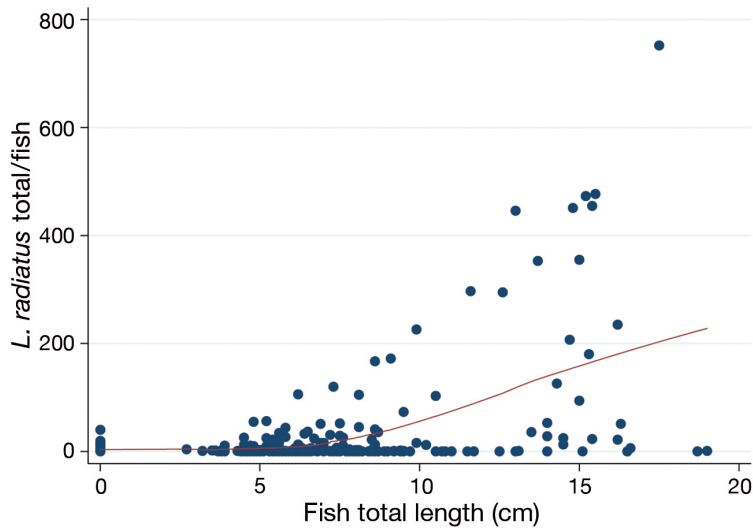


Fig. 5. Juvenile black sea bass *Centropristis striata* total length versus number of *Lernaenicus radiatus* in the gills of each fish

ocean trawl survey sites. Of all the fish collected from the artificial reefs ($n = 130$), 14 had heavy infections and 6 of these were hyper-infected, defined as having over 10 000 copepods per fish. The fish with the heaviest infection was estimated to have 22 235 copepods in the gills. The hyper-infected fish had attached copepods covering the gill tissue (Fig. 7A,B) which severely altered the surface of the gill (see infected gill in Fig. 7C compared to normal gill in Fig. 7D). The gill had diffuse lamellar fusion, causing reduction of gill lamellar surface area, and branchitis associated with attachment of the copepods to the gill filament cartilage (Fig. 7C).

Parasite incidence increased with fish TL (Fig. 8). For every 1 cm increase in TL the number of parasites increased by 1.099 ($p < 0.001$). There was also an association of parasite incidence and sex, which was expected since black sea bass are protogynous hermaphrodites and males had greater TL than females. Males had an incidence rate 1.5 times higher than females ($p < 0.001$) and 7.11 times higher than fish with undeveloped gonads ($p < 0.001$). Overall, when combining the seasons and controlling for fish TL, significant differences in the incidence rate of *L. radiatus* occurred in gills of black sea bass between sites. Little Egg Harbor reef had the highest parasite incidence rate, which was 1.19 times higher

($p < 0.001$) than Sea Girt Reef and 2.42 times higher ($p < 0.001$) than the ocean trawl survey. Sea Girt Reef had a parasite incidence rate 2.04 times higher ($p < 0.001$) than the ocean trawl survey. In the summer only, Little Egg Harbor Reef had a parasite incidence rate 1.76 times higher than Sea Girt Reef ($p < 0.001$) and 2.82 times higher than the ocean trawl survey ($p < 0.001$). Sea Girt Reef had a parasite incidence rate 1.60 times higher than the ocean trawl survey. In the fall only, Little Egg Harbor Reef had a parasite incidence rate 2.97 times higher than the ocean trawl survey ($p < 0.001$). Sea Girt Reef had a parasite incidence rate 1.25 times higher than Little Egg Harbor Reef ($p < 0.001$) and 3.71 times higher than the ocean trawl survey ($p < 0.001$).

3.3. K_n and *L. radiatus* in black sea bass gills

K_n was significantly lower in the summer than in the fall ($p < 0.001$) for all sites (Fig. 9). There was no difference in K_n between sites within either summer or fall. Within each site and season, there was no association between K_n and *L. radiatus* incidence. Similarly, when the 2 reef sites were combined, there was no association between K_n and *L. radiatus* in the summer ($p = 0.384$) or the fall ($p = 0.138$) (Fig. 10).

Table 2. Adult black sea bass *Centropristis striata* collection sites (Site no. corresponds to locations in Fig. 2) and dates (all in 2019), with mean (\pm SD) fish total length (TL), relative condition factor (K_n), numbers of *Lernaenicus radiatus* in gills per fish and total numbers of *L. radiatus* from gills of all fish for each site. *: site not on the map located 100 km offshore of Barnegat Bay, NJ, at a depth of 80 m; ND: not determined

Site no.	Collection date	Sample size	TL (cm)	K_n	<i>L. radiatus</i> fish ⁻¹	Total <i>L. radiatus</i>
1, 2	29–30 Jun	40	28 \pm 3.45	1.08 \pm 0.1	937 \pm 530	37 462
3	16 Aug	15	14 \pm 0.94	0.95 \pm 0.05	323 \pm 142	4842
4, 5, 6	19 Aug	36	25.7 \pm 5.71	0.93 \pm 0.1	1047 \pm 678	37 704
7, 8, 9	4–5 Nov	14	17.4 \pm 1.4	1.0 \pm 0.1	326 \pm 92	4557
10, 11	9–11 Nov	4	15.6 \pm 1.01	1.12 \pm 0.1	169 \pm 84	677
12	24 Jul	31	29.7 \pm 4.0	0.95 \pm 0.1	1975 \pm 1405	61 229
13	18 Jul	30	26.9 \pm 3.3	0.98 \pm 0.1	2779 \pm 3312	83 366
12	23 Oct–20 Nov	29	25.1 \pm 4.5	1.04 \pm 0.2	3303 \pm 3437	89 188
13	20 Oct–27 Nov	42	24.4 \pm 6.4	1.05 \pm 0.1	2697 \pm 4208	113 254
14	25 Jun	8	26.5 \pm 3.5	1.01 \pm 0.03	100 \pm 46	802
15	12 Jul	18	29.4 \pm 2.1	1.0 \pm 0.02	1 \pm 3	23
16	17 Aug	10	25.6 \pm 4	ND	0	0
*	21 Dec	20	47.3 \pm 5.6	ND	27 \pm 32	539

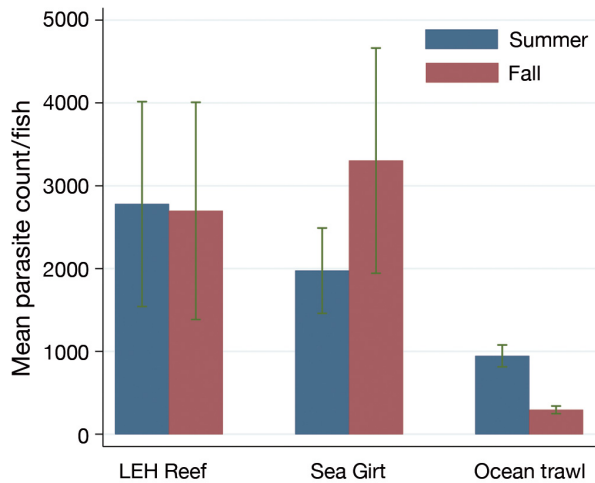


Fig. 6. Mean (\pm SE) parasite counts in gills of adult black sea bass *Centropristis striata* relative to 3 sampling locations: artificial reefs Little Egg Harbor (LEH) and Sea Girt, and non-reef associated habitat from the ocean trawl

3.4. Other fish species screened for *L. radiatus*

Screening of 18 other estuarine and marine fish species (Table 3) with habitat overlapping that of black sea bass failed to detect another fish host that supported development of *L. radiatus* in the gills. Of the 521 fish examined, no gill *L. radiatus* stages like those seen in black sea bass were observed. A single sum-

mer flounder that was collected on 23 October 2019 from Sea Girt Reef, where incidence of *L. radiatus* in black sea bass gills was high, had a total of 47 *L. radiatus* copepodid stages. Microscopic examination revealed that all copepodids occurred only in the gill mucus layer with no attachment to the gill tissue. The absence of attached and other developmental stages suggest that the parasite was unable to infect this host.

3.5. Female metamorphosis of *L. radiatus* in various hosts

Metamorphosis of females rarely occurred in black sea bass. Of 277 adult black sea bass that all had gill infections with *L. radiatus*, metamorphosis of females never occurred. From a total of 273 juvenile black sea bass, 6 that were collected from Barnegat Bay and Great Bay from 29 August to 9 September 2019 had various stages of female metamorphosis (Fig. 11) within the operculum, fins and rarely near the eye. Early settlement of the copepods on skin included pre-metamorphic females with elongated trunk segments, which were encapsulated by host epithelial hyperplasia (Fig. 11A). This was followed by the parasite cephalothorax embedding into the host tissue with metamorphosis associated with enlargement, reddish pigmentation and increased rigidity of the

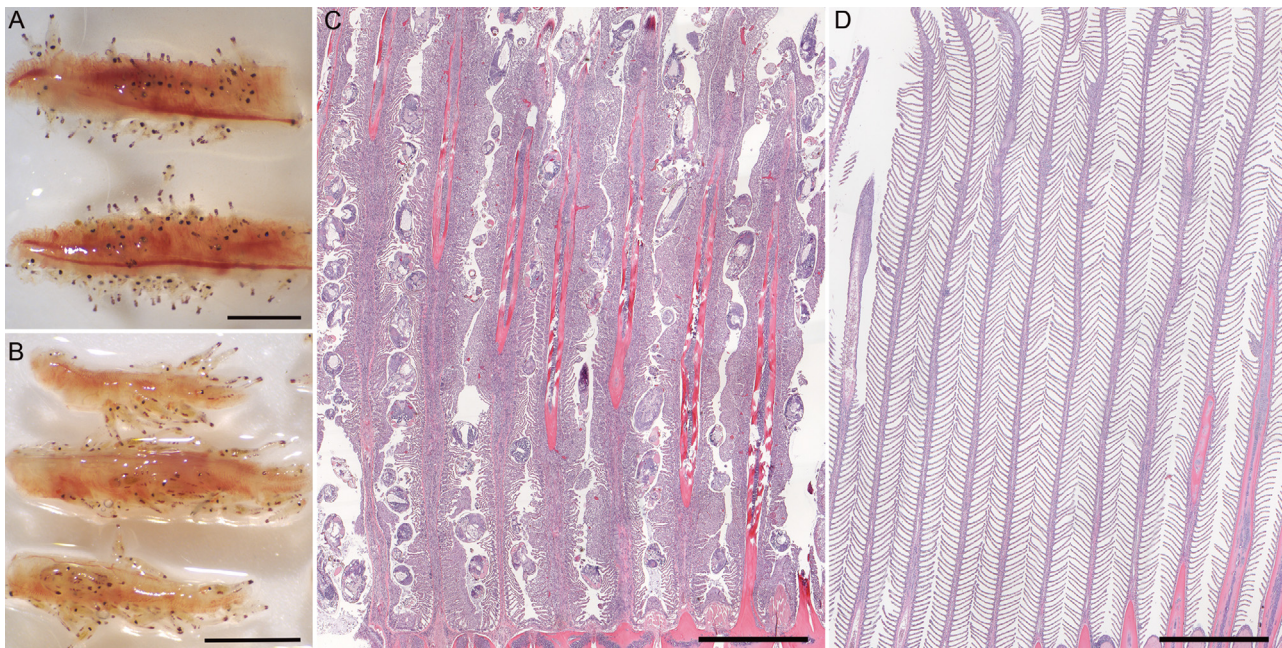


Fig. 7. Gills of adult black sea bass *Centropristis striata* (A–C) hyper-infected with *Lernaeenicus radiatus* compared to (D) normal uninfected gills. (A,B) Dissected gill filaments of adult fish containing predominantly early chalimus stages (A; scale bar: 1 mm) and mature male and female stages of *L. radiatus* (B; scale bar: 2 mm); (C) histology of hyper-infected gill showing lamellar fusion and reduced gill surface area compared to (D) normal gill (scale bars: 1 mm)

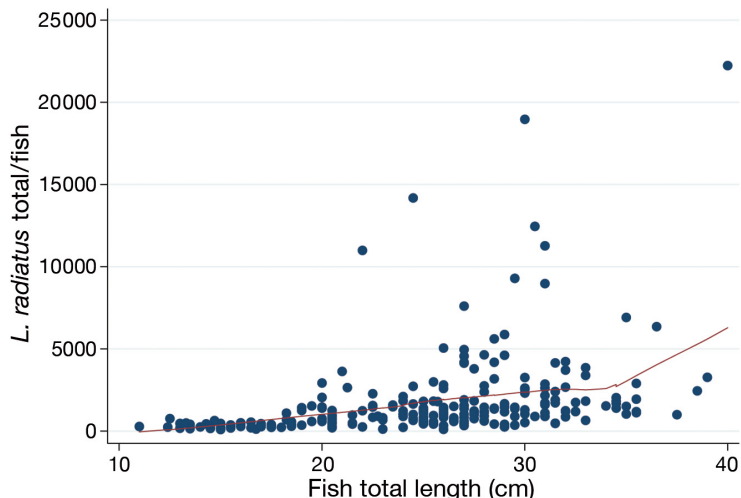


Fig. 8. Adult black sea bass *Centropristis striata* total length versus numbers of *Lernaenicus radiatus* in the gills

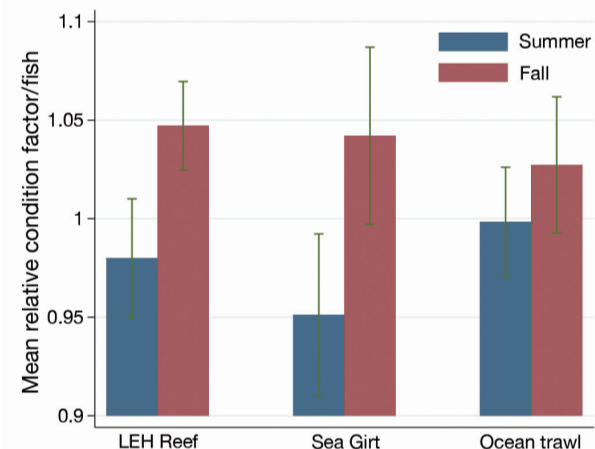


Fig. 9. Mean (\pm SE) relative condition factor of adult black sea bass *Centropristis striata* from 3 different sampling locations in the summer and fall (LEH: Little Egg Harbor)

body (Fig. 11B). Stages of metamorphosis in black sea bass included the beginning development of the horns in the anchor process (Fig. 11C). Metamorphosed females with egg sacs were not found in any black sea bass we examined.

A commonly infected host supporting female metamorphosis was the Atlantic menhaden *Brevoortia tyrannus*. Infection occurred mainly in the muscle and was associated with circular, well circumscribed ulcers penetrating the skin (Fig. 12A). The trunk and egg strands occurred externally to the fish (Fig. 12A), while a large portion of the neck, anchor process and cephalothorax were embedded in the musculature. Less frequently, metamorphosed females occurred on the fins, particularly around the dorsal fin. Prevalence and infection intensity of metamorphosed female *L. radiatus* in adult Atlantic menhaden were highest in the Barnegat Bay area and lower in Raritan and Delaware Bays (Table 4). Parasite incidence rate in Barnegat Bay fish was 9.13 times higher than that in Delaware Bay ($p = 0.001$) and 7.21 times higher than in Raritan Bay ($p < 0.001$). Barnegat Bay fish had up to 19 metamorphosed females per fish. There was an association of parasite infection and K_n in fish collected from the Barnegat Bay; for every increase in *L. radiatus* there was a decrease in K_n by 0.011 ($p = 0.029$). There was no significant effect of parasite intensity on K_n in Atlantic menhaden from Delaware Bay ($p = 0.250$) or Raritan Bay ($p = 0.996$) and there were no significant differences in K_n among any of the 3 sites ($p = 0.427-0.859$).

Full metamorphosis of *L. radiatus* females was confirmed in 6 other host species from the Great Bay

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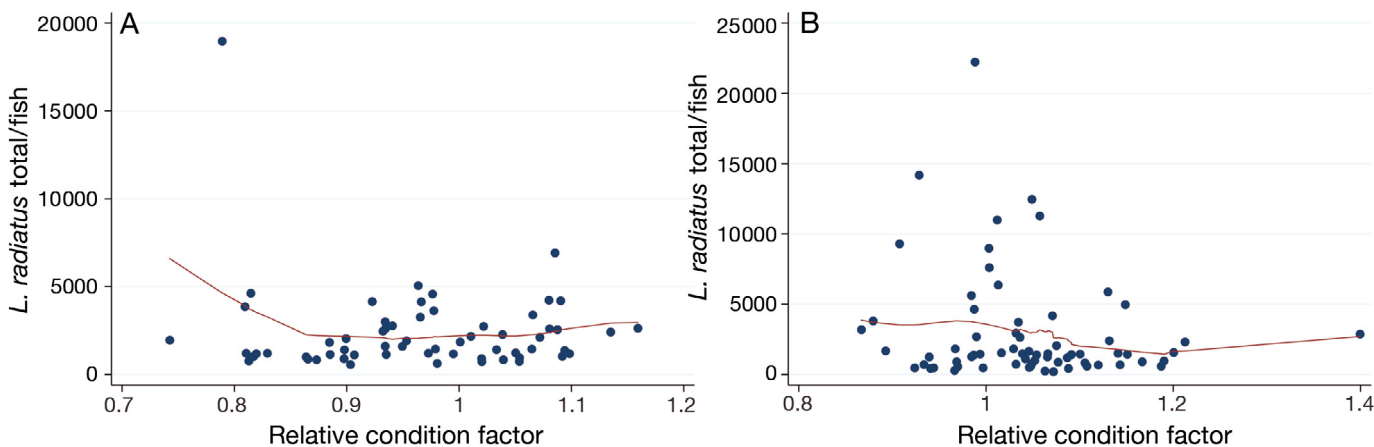


Fig. 10. Relationship between relative condition factor and *Lernaenicus radiatus* incidence rate in gills of black sea bass *Centropristis striata* for the combined reefs, in (A) summer and (B) fall

Table 3. Fish species, with sample sizes (n), collected from marine and estuarine habitats in coastal New Jersey that were examined for developmental and mature stages of *Lernaeenicus radiatus* in gill and metamorphosed female (MF) stages occurring in the muscle and/or fins. Total numbers of metamorphosed females from each species are reported

Species	Common name	<i>L. radiatus</i>	
		n	MF
<i>Brevoortia tyrannus</i>	Atlantic menhaden	76	129
<i>Anchoa mitchilli</i>	Bay anchovy	111	16
<i>Bairdiella chrysoura</i>	Silver perch	3	4
<i>Gobiosoma bosc</i>	Naked goby	3	4
<i>Paralichthys dentatus</i>	Summer flounder	7	4
<i>Fundulus heteroclitus</i>	Mummichog	1	1
<i>Clupea harengus</i>	Atlantic herring	1	1
<i>Urophycis regia</i>	Spotted hake	143	0
<i>Stenotomus chrysops</i>	Scup	22	0
<i>Merlangius merlangus</i>	Whiting	24	0
<i>Urophycis chuss</i>	Red hake	9	0
<i>Lagodon rhomboides</i>	Pinfish	3	0
<i>Tautoga onitis</i>	Tautog	14	0
<i>Prionotus carolinus</i>	Sea robin	89	0
<i>Pollachius virens</i>	Pollock	3	0
<i>Cynoscion regalis</i>	Weakfish	5	0
<i>Menticirrhus saxatilis</i>	Northern kingfish	2	0
<i>Alosa pseudoharengus</i>	Alewife	5	0

survey area including bay anchovy *Anchoa mitchilli*, Atlantic herring *Clupea harengus*, summer flounder *Paralichthys dentatus*, naked goby *Gobiosoma bosc* (Fig. 12B); silver perch *Bairdiella chrysoura* (Fig. 12C); and mummichog *Fundulus heteroclitus*. In Atlantic herring, infection caused a large ulceration with dark margins (Fig. 12A inset), similar to that seen in Atlantic menhaden. Some of the host fish body sizes were considerably smaller than those of adult menhaden (Table 5). In the smaller hosts, the parasites were well affixed to bony structures, and infection in the fins was not observed. In the head region, the parasite attached to either bones within the head or the gill arch (Fig. 12C). When the parasite was found attached to the body of a smaller host fish, the head of the parasite attached to the fish's spine.

3.6. Polymorphisms in metamorphosed female *L. radiatus*

Metamorphosed female *L. radiatus* from adult Atlantic menhaden were considerably larger than those from bay anchovy, silver perch, naked goby and mummichog, which were also smaller hosts (Table 5). This size variation between *L. radiatus* occurred most in the neck region, though mean measurements of the abdomen were also considerably larger in those from Atlantic menhaden than the other species (Table 5). The anchor process of *L. radiatus* also showed high variability. Within Atlantic menhaden, the anchor process had 4–9 well developed horns that radiated from the anterior copepod body (Fig. 13A–D). In bay anchovy the anchor process in some parasites was made up of 2–3 horns that were much shorter than those from Atlantic menhaden. Some of the parasites from bay anchovy did not have any horns, and the anterior portion of the parasite was more robust and developed a strong adhesion to cartilage and/or bone with a plate-like lamellae in areas of attachment (Fig. 13E). Similar variations of horn structures were evident in silver perch, naked goby and mummichog. In general, with attachment to bony and cartilaginous structures, there were 2–3 short horns or no apparent horns with a hammer-like shape with strong adhesion to the bone. The prominent horns, as seen in

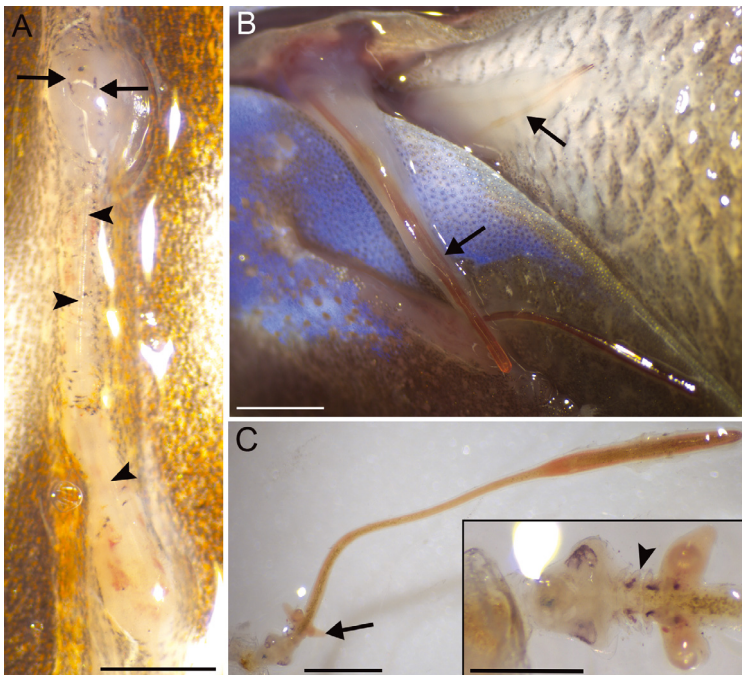


Fig. 11. Stages of *Lernaeenicus radiatus* female metamorphosis in juvenile black sea bass *Centropristis striata*. (A) Newly settled female on a fin with epithelial hyperplasia encapsulating the cephalothorax (arrows) and the elongated trunk segment (arrowheads) of the parasite (scale bar: 1 mm); (B) several females in the process of metamorphosis (arrows) around the operculum (scale bar: 2 mm); (C) a removed female *L. radiatus* in early stages of metamorphosis showing newly developed horns (arrow) and swimming legs (arrowhead) (scale bars: 1 mm; inset: 0.5 mm)

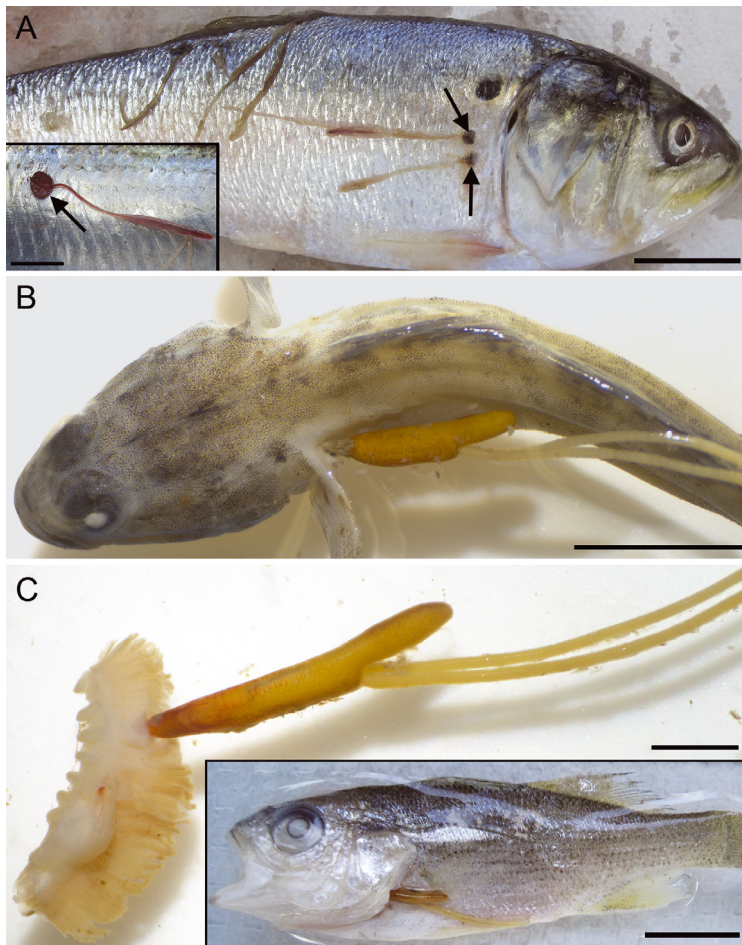


Fig. 12. Metamorphosed female *Lernaenicus radiatus* on (A) Atlantic menhaden *Brevoortia tyrannus*, with dark ulcers (arrows) where the parasite is embedded in the skin (scale bar: 3 cm); in inset, notice the large ulceration (arrow) associated with infection in Atlantic herring *Clupea harengus*; (B) naked goby *Gobiosoma bosc* with parasite attached to the side (scale bar: 5 mm); (C) silver perch *Bairdiella chrysoura* with parasite under the operculum (inset, scale bar: 1 cm); dissected gill showing *L. radiatus* embedded in the cartilage of the gill arch (scale bar: 2 mm)

Table 4. Mean (\pm SD) total length (TL, mm) and relative condition (K_n) of Atlantic menhaden *Brevoortia tyrannus* from 3 different bays that correspond to the map in Fig. 2 (Site no.). The prevalence of fish infected with metamorphosed female *Lernaenicus radiatus* in the muscle is shown as well as the total number of metamorphosed females from all fish at that site (Total)

Site no.	TL	K_n	Prevalence No.	%	Total
Raritan Bay					
19	253 \pm 09	1.0 \pm 0.10	10/38	26	17
Barnegat Bay					
18	310 \pm 16	1.0 \pm 0.04	22/24	92	105
Delaware Bay					
17	279 \pm 21	1.0 \pm 0.05	5/12	42	7

adult Atlantic menhaden, were embedded in the soft muscle and not associated with bone. One parasite in silver perch that was embedded in muscle had 5 horns, which was similar to observations from *L. radiatus* in Atlantic menhaden. The trunk segment in mummichog, silver perch and naked goby had pigment cells, though this was not always apparent in these species and did not occur in *L. radiatus* from bay anchovy and Atlantic menhaden.

3.7. DNA sequences of the mitochondrial COI

All 45 COI sequences, representing developing copepods from the gills of black sea bass and metamorphosed female forms from the muscle of other host species described above were confirmed to be *L. radiatus*. In total, 23 of the sequences were identical and made up a main sequence type; this representative sequence was deposited in GenBank under accession number MN989956. There were 20 unique sequence types that were limited to 1 or 2 nucleotide changes (0.3% sequence divergence) from the main sequence type; these were deposited in GenBank under accession numbers MN989957–MN989973, MT-260203 and MT260204. Identical sequence types occurred in samples from black sea bass gills and metamorphosed females from all hosts except for mummichog, which only had a single representative sample.

Mutations occurred throughout the sequence length, with the most frequent mutations occurring between nucleotide sites 478–568, accounting for 37.5% of all mutations. The most common mutation types were between cytosine (C) and thymine (T) (46%), with C–T and T–C mutations accounting for 29 and 17% of the mutations, respectively. This was closely followed by mutations between guanine (G) and adenine (A) (42%), with A–G and G–A mutations accounting for 29 and 13%, respectively. Less frequent mutations included T–A, which occurred in 2 samples, and A–C, occurring in a single sample. One mutation, C–T at nucleotide site 488, occurred in 3 different samples: 2 *L. radiatus* samples from bay anchovy and a single sample from

Table 5. Mean (\pm SD) measurements (in mm) of host total length (TL) and fully metamorphosed female *Lernaenicus radiatus* from 7 different host species. Measurements of *L. radiatus* include total length (Total) including egg sacs, and body measurements separated into trunk and neck. All measurements include sample size (n)

Host	Host TL	<i>L. radiatus</i>		
		Total	Trunk	Neck
Menhaden	300 \pm 23 (n = 20)	57.6 \pm 6.7 (n = 23)	13 \pm 1.8 (n = 8)	23.6 \pm 5.4 (n = 6)
Bay anchovy	54.6 \pm 6.7 (n = 10)	21.9 \pm 3.5 (n = 10)	7.5 \pm 1.8 (n = 10)	6.6 \pm 1.7 (n = 10)
Silver perch	59.3 \pm 2.5 (n = 3)	20.3 \pm 3.2 (n = 3)	8.1 \pm 0.9 (n = 3)	2.3 \pm 0.1 (n = 3)
Naked goby	38.3 \pm 7.2 (n = 3)	16 \pm 3.6 (n = 3)	5.3 \pm 1.2 (n = 3)	2.5 \pm 0.5 (n = 3)
Summer flounder	247 (n = 1)	38.9 \pm 9.1 (n = 4)	12.5 \pm 1.7 (n = 4)	9.8 \pm 3.8 (n = 4)
Atlantic herring	187 (n = 1)	61.5 (n = 1)	17 (n = 1)	26 (n = 1)
Mummichog	55 (n = 1)	17 (n = 1)	7.1 (n = 1)	4.9 (n = 1)

Atlantic menhaden. Another C–T mutation at site 373 occurred in 2 *L. radiatus* samples: a black sea bass from Virginia and a bay anchovy from New Jersey. Median joining networks showing sequence divergence by host species and by sampling location did not show any sequence types associated with a particular species or location (Fig. 14), indicating a mixed population.

4. DISCUSSION

The high prevalence and heavy gill infections of *Lernaenicus radiatus* in black sea bass demonstrate the importance of this host species for the development of this copepod in the mid-Atlantic Bight. Despite screening 18 other species found in the same environment, including those that support female

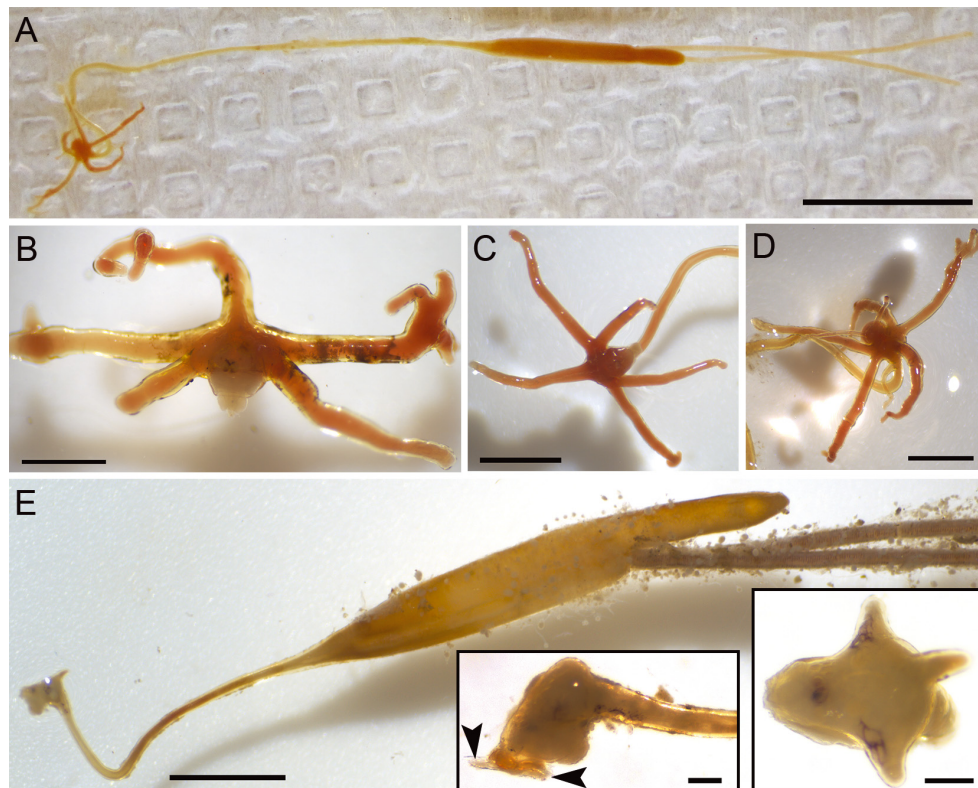


Fig. 13. Polymorphisms of *Lernaenicus radiatus* from (A–D) Atlantic menhaden *Brevoortia tyrannus* and (E) bay anchovy *Anchoa mitchilli*. (A) Full female showing the well-developed anchor process, long neck, trunk and egg sacs (scale bar: 1 cm); (B–D) variations of the well-developed and branching horns in the anchor process that anchors the parasite into the muscle of the fish (scale bars: 1 mm in [B]; 2 mm in [C,D]); (E) metamorphosed female with horns absent, shorter neck, trunk and egg sacs (scale bar: 2 mm); insets show details of anchor region with smaller or lacking horns and plate-like lamellae (arrowheads) that attach to bone or cartilage of the host (scale bars: 200 μ m)

metamorphosis, no attached gill *L. radiatus* were found. This is supported by tank infections conducted by Shields (1977), which showed female metamorphosis of *L. radiatus* occurred in *Fundulus heteroclitus* only when black sea bass were present to support development of the parasite. Interestingly, copepodid stages of *L. radiatus* occurred in gill mucus of an adult summer flounder collected from one of the offshore reef sites, demonstrating that the parasite can seek out other hosts but is unable to attach and infect them. A narrow host range allows 'specialist' parasites to develop specific adaptations to their particular host, whereas 'generalist' parasites trade off specializations to infect a broader host range (Poulin 1998). Gill infections with *L. radiatus* rely on an attachment process that penetrates the gill tissue and adheres to the gill filament cartilage (Lovy & Friend 2020). Perhaps this more complex form of attachment with deep penetration into gill tissue requires more elaborate specializations, explaining the specificity towards black sea bass. Parasites that are host specialists typically occur in high numbers within a narrow host range, whereas in generalists there are lower parasite numbers spread over a wider host range (Poulin 1998). The high prevalence and heavy infections of *L. radiatus* in gills of black sea bass further support the theory of *L. radiatus* being a 'specialist' parasite in its first host.

The number of copepods developing in gills of adult black sea bass was massive, particularly in the artificial reef sites, and is similar to that observed for a closely related parasite, *Lernaeocera branchialis*.

Lumpfish *Cyclopterus lumpus* are the primary first host supporting development of *L. branchialis* in the gills, and observations of over 10 000 parasites per fish were reported in waters off Newfoundland, Canada (Templeman et al. 1976). Requiring a second host in the life cycle reduces the chances of successful development, since this relies on finding a new host. Heavy gill infections may be necessary to ensure enough females successfully find a second host to perpetuate the life cycle. Host specialization within species can vary based on geography and ecological factors. *L. branchialis* is found on both sides of the Atlantic Ocean, with North American populations mainly specific to gills of lumpfish, whereas European populations are specific to flounder *Platichthys flesus* (Whitfield et al. 1988). Despite the differences in host preference, these geographically separate populations are known to be the same species, supported by identical morphology (Kabata 1961) and genetics (Lovy & Friend 2020). Similarly, southern ranging *L. radiatus* have been shown to utilize a different but closely related host, the rock sea bass *Centropristis philadelphica* (Overstreet 1978); thus, clearly host availability and other ecological factors can influence the primary first host species. Indeed, *L. radiatus* was not observed in black sea bass sampled off the coast of Florida from this study, and perhaps this is an indication that they are not a suitable host in this region—though it must be noted that only a limited number of samples were available from Florida ($n = 10$). *L. radiatus* has been reported in black sea bass gills as far south as North Carolina,

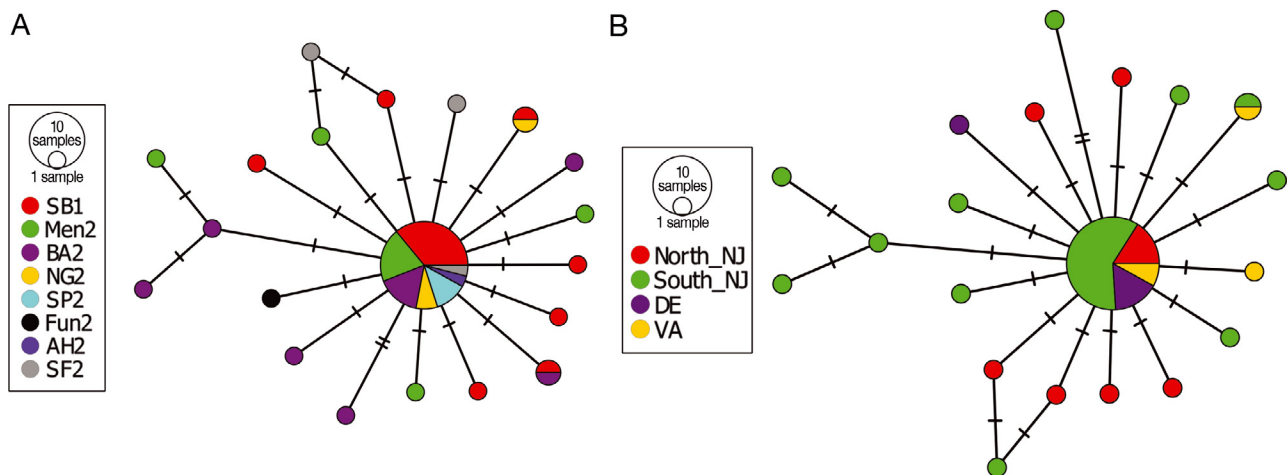


Fig. 14. Nucleotide sequence divergence networks showing unique sequence types and nucleotide differences, denoted by number of dashes (-). (A) Sequence types by species showing gill stages in black sea bass *Centropristis striata* (SB1) and metamorphosed female forms from Atlantic menhaden *Brevoortia tyrannus* (Men2), bay anchovy *Anchoa mitchilli* (BA2), naked goby *Gobiosoma bosc* (NG2), silver perch *Bairdiella chrysoura* (SP2), mummichog *Fundulus heteroclitus* (Fun2), Atlantic herring *Clupea harengus* (AH2) and summer flounder *Paralichthys dentatus* (SF2). (B) Sequence types by location of sampling: northern and southern New Jersey (NJ), Delaware (DE) and Virginia (VA)

although in this occurrence the parasite was described as *Lernaeocera centropristi* (Pearse 1947), which is now known to be conspecific to *L. radiatus* (Lovy & Friend 2020).

L. radiatus in black sea bass gills herein did not show a clear seasonal cycle for development off the coast of New Jersey, though heavy infections occurred in both the summer and fall. Parasites were found from the earliest sampling points in April from YOY fish in estuaries through to the last sampling of adult fish in November. The high variability of developmental stages of *L. radiatus* in the gills from April–November indicated that transmission and development was ongoing throughout these seasons. This was similar to the seasonality described for *L. branchialis* off the coast of Newfoundland, Canada, with heaviest infections occurring in July and August and most late-stage parasites occurring at the end of the summer (Templeman et al. 1976), and for *Lernaeocera lusci*, which had peak transmission of stages to the final host between June and September (Van Damme et al. 1996). The present study showed that black sea bass from near-shore environments were more heavily infected with *L. radiatus* than those at deeper, off-shore locations. This pattern mirrors that of *L. branchialis* in lumpfish collected from Newfoundland, which were also found mainly in near-shore sites (Templeman et al. 1976). Heavy infections in summer and fall support the fact that the transmission cycle occurs in this period, which depends on the interaction of both host species. This is the season when both Atlantic menhaden and black sea bass are present in near-shore environments in this region (Able & Fahay 2010).

In both YOY and adult black sea bass populations from the present study, there was an association of fish TL and incidence of *L. radiatus* in the gills. For YOY fish, this may be explained by time spent in the environment, which increases chances for *L. radiatus* infection. In YOY black sea bass of the Great Bay estuary, TL is a good indication of age. Fish hatched during the summer range in size from 2 to 11 cm TL by October of that year; little growth occurs through the winter and by midsummer the following season when fish are about 1 yr old, they range in size from 8 to 21 cm TL (Able & Fahay 2010). YOY fish stay in estuaries and move offshore to the continental shelf to overwinter (Able et al. 1995). The association of age and increased incidence of *L. radiatus* cannot explain the relationship between TL and *L. radiatus* in adult fish, since all fish are older than the expected developmental cycle of *L. radiatus*. Instead, this may be related to available surface area of gill and other

transmission factors that favor larger fish. Positive associations between fish TL and gill ectoparasite abundance have been described and were attributed to increased gill surface area for parasites to colonize, increased water flow over gills and larger fish having higher physical and chemical cues increasing their attractiveness to parasites, all improving the likelihood of infection by ectoparasites in larger fish (Lo et al. 1998, Heuch et al. 2007, Brooker et al. 2013).

Notably, the highest incidence of *L. radiatus* in gills of adult black sea bass occurred from the 2 artificial reefs, with non-reef-associated areas having considerably lighter infections. This difference is most likely explained by the life cycle preferences of *L. radiatus* and the success of the reefs in maintaining higher biodiversity. In fact, rich parasite diversity is considered a sign of a healthy ecosystem that supports biodiversity (Hudson et al. 2006, Lafferty 2012). Parasites with complex life cycles rely on biodiversity, high host densities and species interactions to complete their life cycle. For example, when heavily fished reefs were compared to 'pristine' un-fished reefs, the pristine reefs supported higher parasite diversity, abundance and higher parasite prevalence rates, particularly for parasites that required multiple-host life cycles (Lafferty et al. 2008). With *L. radiatus* having a 2 host life cycle, abundance of the parasite can be related to 2 major host factors: (1) having abundant black sea bass to support parasite development and sexual reproduction and (2) having abundant second hosts with habitat overlapping that of black sea bass. These host factors for transmission are more supported in a reef habitat, which attracts biodiversity and enhances species interactions. Black sea bass are known to inhabit artificial reefs in high numbers, and schools of baitfish, such as Atlantic menhaden, have been shown to school around the artificial reefs (Resciniti et al. 2009). Considering this observation, perhaps artificial reefs support closer interactions of black sea bass and Atlantic menhaden, which could explain the higher incidence of *L. radiatus* in these habitats. Understanding the ecological consequences of *L. radiatus* in their respective hosts from these areas may be more difficult.

Heavy and hyper-infections in the gill, as defined in this study, cause considerable pathologic changes resulting in decreased gill surface area. As previously discussed by Lovy & Friend (2020), fish may be able to compensate for this reduction in good environmental conditions, but their ability to handle environmental stressors would likely be compromised. K_n is a measure of a fish's body condition relative to length (Le Cren 1951) and can be an indication of

poor condition related to parasitism. In wild fish, condition factors are also known to be impacted by spawning condition and season (Mello & Rose 2005). In the present study, we found that K_n of black sea bass was considerably better in the fall than in early summer, which is not surprising since fish are at their highest condition prior to winter. This study did not find a link between parasite infection and K_n in black sea bass, though a decline in K_n was associated with metamorphosed female *L. radiatus* in Atlantic menhaden from Barnegat Bay, where infections were heavy. Metamorphosed females feed on blood (Overstreet 1978) and cause large ulcers, described herein, that would likely cause osmoregulatory issues. The association of K_n and *L. radiatus* in Barnegat Bay Atlantic menhaden should be cautiously interpreted, since K_n was similar in these fish as in other sites that had fewer to no parasites.

Female metamorphosis of *L. radiatus* is required for egg production; thus this stage of the parasite is required for amplification and transmission to black sea bass. Contrary to the high host specificity to black sea bass during development and sexual reproduction in the mid-Atlantic Bight, there appears to be less host specificity for female metamorphosis. Prior reports have identified metamorphosed females in at least 16 different species of marine fish (Wilson 1917, Voorhees & Schwartz 1979, Hogans 2018, Walter & Boxshall 2018). We documented metamorphosed females in 7 species, of which 4 had not been previously reported (summer flounder *Paralichthys dentatus*, silver perch *Bairdiella chrysoura*, Atlantic herring *Clupea harengus* and naked goby *Gobiosoma bosc*). Undoubtedly, the lack of host specificity for female metamorphosis would improve the chances of finding a suitable second host. Despite a lack of host specificity, Atlantic menhaden were a preferred host, based on the common detection in this species in this region. Heaviest infections in menhaden occurred around Barnegat Bay, which was the site with closest proximity to the artificial reefs from which heavy gill infections in black sea bass occurred. Bay anchovy was a second host in which infections were common. Host preferences for these 2 species were previously reported in fish from North Carolina and are thought to be related to the delicate deciduous scales of these hosts, making penetration by the parasite easier (Voorhees & Schwartz 1979). It was rare to observe black sea bass with metamorphosed females, suggesting that the 2 host life cycle is the most common life cycle route for this parasite. Only several juvenile black sea bass were observed with metamorphosed female *L. radiatus*,

whereas this never occurred in adult fish in the artificial reefs that had the highest numbers of the parasite. The rare occurrence of female metamorphosis in black sea bass may be related to immunity resulting from previously high parasite levels in the gill, or perhaps to less suitable attachment sites on this host species which has robust scales.

L. radiatus incidence is linked to availability of their preferred hosts, and Atlantic menhaden and black sea bass populations are currently relatively abundant in the region. Indications suggest that Atlantic menhaden populations have seen a recent resurgence, based on more restrictive catch rates set for the species. Starting in 2013, an amendment capped the Atlantic menhaden fishery to better protect the populations; the resulting higher biomass in subsequent years resulted in total allowable catch quotas to be increased (ASMFC 2017). For black sea bass, after depressed spawning stock biomass and recruitment being below the target value since at least 1989, in 2009 the black sea bass fishery was declared rebuilt and has since seen a substantial increase in spawning stock biomass (NEFSC 2017, ASMFC 2019). We have no reference for previous incidence of *L. radiatus* in these marine species in this region, as this was the first study to document prevalence and incidence rates of this parasite. Nonetheless, perhaps the high numbers of *L. radiatus* is related to a resurgence of these preferred hosts. The high parasite incidence could be a positive sign related to increasing biomass of these 2 important species in the region.

Mitochondrial COI sequences are informative markers for copepod species, with divergence rates of 0 to 4% within species and 9 to 25% between species (Raupach et al. 2015, Castro-Romero et al. 2016). The COI sequences were critical for linking life stages of *L. radiatus* in this study, thus supporting parasite transmission from black sea bass gills to female metamorphosis in the second hosts. Identical COI sequences linked the gill stages of *L. radiatus* from black sea bass to the metamorphosed females from various species. Intraspecific divergence of the COI in copepods is useful for understanding differences in ecologically distinct and geographically isolated populations (Bucklin et al. 2003). We found that sequence divergence was particularly low for *L. radiatus*. Though 20 unique sequence types were identified in the 45 samples, divergence rates of only up to 0.3% (2 nucleotide substitutions) occurred. Considering the relatively low fidelity of *Taq* polymerase used in this study, it is possible that some of the nucleotide substitutions were the result of error during PCR

amplification. The error rate for *Taq* polymerase is reported to be $4.3 \times 10^{-5} \pm 1.8$ mutations bp^{-1} per template duplication (McInerney et al. 2014). Further, the majority of errors related to *Taq* polymerase are A–G and T–C transitions, followed by G–A and C–T transitions (McInerney et al. 2014), which correspond to the majority of mutations detected in our sequences. Thus, it is likely that some mutations were related to the error rate in the polymerase, although some mutations that occurred in multiple samples are most likely real. For example, the C–T mutation at nucleotide site 488 occurred in 3 independent samples and the C–T mutation from site 373 occurred in 2 independent samples. The small sequence divergence did not indicate any geographic or ecologically isolated populations in this study. Gill stages of *L. radiatus* had identical sequences from New Jersey, Delaware and Virginia, suggesting that these populations mix. This is not unexpected, as one of the preferred second hosts for *L. radiatus* is the Atlantic menhaden, which is highly migratory (Able & Fahay 2010). Atlantic menhaden are considered a single population that overwinters in the southeast and migrates northward in the spring and summer (Nicholson 1978). Considering the wide host range of *L. radiatus* and its preference for a different first host species in the southern part of the range (Overstreet 1978), it would be important to examine the COI sequences from a wider geographic range to better understand ecological and geographical differences in *L. radiatus*. To this end, parasites can be informative as biological tags to distinguish stocks of marine fish (Mackenzie & Abaunza 2005), and perhaps *L. radiatus* may help us to understand black sea bass stocks and their interactions with other fish species. Considering the low nucleotide divergence rates of this gene in *L. radiatus*, it will be important to utilize higher fidelity *Taq* polymerase for detecting unique nucleotide sequences and how they relate to geography and host range.

Importantly, female metamorphosis of *L. radiatus* from various hosts showed remarkable morphological differences in both size and anchor structure. Pennellid copepod species have a high degree of polymorphisms in metamorphosed females (Castro-Romero et al. 2016), thus making this a poor marker with which to distinguish species. In the present study, we noted that metamorphosed females from smaller bodied hosts were considerably smaller than those found in adult menhaden, which were larger hosts. In fact, it was common to see trunk measurements nearly double and neck measurements sometimes 10× larger in *L. radiatus* recovered from adult

Atlantic menhaden compared to those of all the other smaller-bodied species. Further, attachment differences occurred in adult Atlantic menhaden and the smaller-bodied hosts. Attachment in small-bodied hosts was almost always to hard tissues, including cartilage and bone, and horns in the anchor process were not apparent. The absence of horns was most likely related to this different attachment strategy, forming an adhesion to cartilage and bone. In small-bodied hosts that lack robust musculature for attachment, this unique attachment to bone and muscle is likely necessary for secure attachment. In adult Atlantic menhaden that have more robust musculature, the parasite may attach directly in the muscle using radiating horns to anchor them in position in the muscle. This attachment in the muscle also explains the size difference, since in Atlantic menhaden the parasite develops a long neck to deeply anchor itself into the muscle, whereas in small-bodied hosts the parasite does not need to penetrate very deep before finding bone and cartilage for attachment.

Morphological differences in the anchor process and size of *L. radiatus* metamorphosed females had previously been interpreted as species-level differences. Three species of *Lernaeenicus* have been described from the northwest Atlantic coast: *L. radiatus*, *L. affixus* and *L. longiventris* (Hogans 2018). Hogans (2018) commented on some similarities between *L. radiatus* and *L. affixus* and noted the main differences were size, lack of horns in the anchor process and presence of lamellae that attached to bone. The metamorphosed female forms of *L. radiatus* from small-bodied hosts found herein are consistent with the description of *L. affixus* (Hogans 2018). Based on our COI sequences, these clearly different morphologic forms were confirmed to be genetically identical. This strongly suggests that *L. affixus* is synonymous with *L. radiatus*; this possibility was proposed by Hogans (2018) when discussing the species differences. Confirming that these are the same species is important in understanding host transmission factors, since different species would likely use different hosts. Metamorphosed females of *L. affixus* were primarily reported from the tomcod *Microgadus tomcod*, but also recorded in mummichog *F. heteroclitus* (as also seen herein), white perch *Morone americana* and the glut herring *Alosa aestivalis* (Wilson 1917). We were unable to confirm identities of *L. longiventris*, which use different host species and are reportedly a much larger parasite, with an anchor process that is hammer- or T-shaped. We did show that size cannot be used as a reliable morphological feature for speciation and that size is

likely linked to host body size and the type of tissue affected. Further, the metamorphosed females described here had a hammer-shaped anchor process (see Fig. 12E) similar to previous descriptions of *L. longiventris* (Hogans 2018). Considering these findings of morphologic plasticity in *L. radiatus* related to host size and tissue type used for attachment, it is important that any morphological data of metamorphosed females be supplemented with COI sequences to confirm species.

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