

IMPACT OF THE PARASITIC NEMATODE *ANGUILLICOLOIDES CRASSUS* ON
AMERICAN EELS (*ANGUILLA ROSTRATA*) IN CHESAPEAKE BAY

A Thesis

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PREFACE

Chapter 1 will be submitted for publication in *Canadian Journal of Fisheries and Aquatic Sciences*. Chapter 2 will be submitted for publication in *Diseases of Aquatic Organisms*. Both chapters are formatted for publication by the specifications of each journal.

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ABSTRACT

American eels are infected by the introduced parasitic nematode, *Anguillicoloides crassus*, which can cause significant damage to their swimbladders. Despite the high prevalence and severe damage caused by *A. crassus*, the population level effects on American eels are not well understood. The prevalence and swimbladder damage in young glass eels and elvers are relatively unstudied, despite the potential for this parasite to cause tissue damage. Additionally, the effects of environmental, temporal, and spatial variables have been debated in previous studies without consensus. Also, the potential for eels to recover from infection and tissue damage has been speculated but not definitively shown. Therefore this Master's thesis sought to answer these questions through field and laboratory studies. Glass and elvers stage American eels were collected during the spring and summer of 2015 and dissected to enumerate infection intensity and estimate swimbladder condition using the swimbladder degenerative index (SDI). Data were then combined with a larger dataset for yellow eels and analyzed using zero-inflated and ordinal logistic regressions to determine the effects of season, site, and eel length on infection intensity and swimbladder condition. The relationship between infection intensity and swimbladder condition was evaluated. This dataset was then used to investigate if the force-of-infection (i.e. rate of uninfected eels becoming infected) varied by host age and if there was evidence of disease associated mortality. To investigate recovery from parasite-induced swimbladder damage, 270 wild caught (presumably infected), individually tagged yellow eels were held in a freshwater recirculating system and fed a parasite-free diet for six months. Each month eels were x-rayed, weighted, and measured. The length and area of the swimbladder of each individually tagged eel were measured on x-ray images for temporal comparisons. At the end of the experiment, all eels were euthanized and dissected to determine infection intensity, SDI, and dissected swimbladder length. The trends of average monthly length ratio index (LRI; length of swimbladder to eel total length) and area of swimbladders were determined, as well as the relationships to SDI and infection intensity. Our field study showed that glass eels have a very low prevalence compared to elvers and yellow eels. Infection intensities of elvers and yellow eels varied by season and site and increased with total length. Swimbladder damage also varied by season and increased with total length. Infection intensity and swimbladder damage were non-linearly related. Force-of-infection was highest for age 2 eels and also varied by season, with the highest values in the winter and lowest in the early spring. Parasite-associated mortality was observed, with infected eels having an annual survival rate of 0.76 that of uninfected eels. Results from our x-ray experiment showed that LRI and area increased slightly through time. SDI also increased slightly over the course of the experiment, and SDI, LRI, and swimbladder area all were in agreement of improvement in swimbladder condition, however full recovery was not observed. In conclusion, the health of American eels in the Chesapeake Bay is adversely impacted by *A. crassus*, though that effect varies by season, system, length of the eel and whether infection level is being measured by infection intensity or swimbladder condition. Also American eels may have the ability to recover from *A. crassus* infection, but more work is needed to determine if this occurs in the wild.

THE IMPACT OF THE PARASITIC NEMATODE *ANGUILLICOLOIDES CRASSUS* ON
CHESAPEAKE BAY AMERICAN EELS (*ANGUILLA ROSTRATA*)

GENERAL INTRODUCTION

American eel fishery

The American eel (*Anguilla rostrata*) is an economically important, yet data-poor species managed by the Atlantic States Marine Fisheries Commission (ASMFC). American eels are targeted throughout its range, but commercial fishing for this species is especially vital in the Chesapeake Bay region, given that landings comprise approximately 60% of the annual U.S. catch (ASMFC 2012). The export demand for American eels to Asia for aquaculture production has increased the value of this fishery in the U.S. in recent years. In 2010, the total value of live and frozen eel export was estimated at \$6M and has ranged between \$3M and \$7M from 2000 to 2010 (ASMFC 2012). The recreational fishery for American eel is also important due to its use as bait in other fisheries such as striped bass and cobia. Although American eels have supported fisheries of varying intensities beginning with Native Americans, the stock has been largely unregulated until concerns about abundances in the mid-1990s prompted development of an interstate Fishery Management Plan (FMP) under the auspices of the ASMFC. Total U.S. landings peaked in the 1970s to mid-1980s at 2.5 to 3.6 million pounds, but then declined to 1.6 million pounds in the late 1980s and since have remained low (ASMFC 2012). Catches dropped so drastically that the American eel has twice been petitioned to be listed under the Federal Endangered Species Act (ESA). Yet both subsequent species status reviews (2007 and 2015) by U.S. Fish and Wildlife Service (USFWS) found that the U.S. American eel stock does not need protection given its wide distribution and local abundances, and because sources of mortality do not threaten the overall species (Shepard 2015). Conversely, the American eel species as a whole was classified as endangered by the International Union for the Conservation of Nature (IUCN) in 2014 due to the finding that silver eel escapement had decreased by around 50% over the past

three decades, as have yellow eel recruitment and their population (Jacoby et al. 2014). The IUCN finding was in agreement with USFWS that the American eel species was not at immediate risk of extinction due to its wide geographic range, but concern was expressed that the population may be outside of safe biological limits (Jacoby et al. 2014). The most recent stock assessment by ASMFC in 2012 determined the stock was “depleted” and at or near historic lows, but the lack of data for this species prevented a definitive conclusion as to the factor(s) driving the decline in abundance. Many hypotheses have been proposed to explain the species’ decline including overfishing, pollution, changing climate, altered habitats and food webs, and parasites and disease (Castonguay et al. 1994; Haro et al. 2000). However, the very complex and somewhat unique life history of American eels presents challenges to studying its population dynamics. Given the economic importance of the American eel and the general lack of population level data, development of a sustainable FMP necessitates a considerable amount of additional research.

Anguillicoloides crassus

My project addresses one of the proposed hypotheses for the observed decline of the American eel population, namely, the impact of infection by an introduced parasitic nematode to the American eel population. This topic was listed as an area of high priority for future research in the ASFMC 2012 stock assessment, as well as a threat in the 2014 IUCN listing. Infection by *Anguillicoloides crassus* is endemic in Japanese eel (*Anguilla japonica*) in eastern Asia, but does not cause significant harm or notable negative population level impacts (Sokolowski and Dove 2006). In contrast, *A. crassus* has been shown to be pathogenic to the American eel (Sokolowski and Dove 2006) and the European eel (*Anguilla anguilla*; Nagasawa et al. 1994). The

emergence, rapid spread, high prevalence, and pathogenicity of *A. crassus* have been linked to declines in European eel populations and in Asian aquaculture facilities holding American and European eels (Barse et al. 2001; Ooi et al. 1996). For the American eel in the U.S., the parasite was first discovered in 1995 in a Texas aquaculture facility and was first noted in the wild in South Carolina that same year (Fries et al. 1996). As such it is believed to be an exotic parasite introduced to the USA in the 1990's. Since its emergence, the distribution of *A. crassus* has expanded rapidly and can now be found in eel populations in the Gulf of Mexico northward to Nova Scotia (Rockwell et al. 2009; Lefebvre et al. 2012). *A. crassus* was first detected in Chesapeake Bay eels in 1997 (Barse and Secor 1999) and currently can be found in all major tributaries with prevalences as high as 90% (T. Tuckey, personal communication).

The rapid spread of *A. crassus* throughout the range of the American eel is similar to what was documented in Europe, where the parasites biology has been studied in more detail (as reviewed in Lefebvre and Crivelli 2004, Kirk 2003; Lefebvre et al 2012). There are several reasons for the rapid spread of *A. crassus* including high reproductive potential (5×10^5 eggs per female), a relatively simple life cycle, and the ability to utilize a range of intermediate and paratenic host species (Kennedy and Fitch 1990). Additionally, eggs and free-living larvae are hardy and can remain infective for extended periods under different environmental conditions (Kennedy and Fitch 1990). Also, *A. crassus* can infect eels from glass to silver phases (Kennedy and Fitch 1990). The wide geographic distribution of American eels and the parasite's ability to survive and reproduce within diverse environmental conditions has also contributed to the spread of the nematode (Kennedy and Fitch 1990).

Eel and Parasite Life Histories

The interconnectedness of the eel and parasite life cycles are important to understanding the impact of *A. crassus* on the American eel population (Figure 1). The life of an American eel begins in the Sargasso Sea as an egg, which hatches into the larval stage called the leptocephalus (ASMFC 2012). These leaf-like eel larvae are carried by the Gulf Stream and metamorphose into the next stage, the glass eel, which is able to actively swim towards the coast (ASMFC 2012). Once in coastal waters along the entire east coast of North America, these young eels are susceptible to infection by *A. crassus* through the consumption of crustacean zooplankton such as copepods and ostracods, the intermediate hosts of the parasite (De Charelory et al. 1990). Some glass eels may continue migrating further inland to fresh water, while others remain in the brackish coastal waters (ASMFC 2012). As the glass eels eat and grow, they become pigmented and enter their next phase known as elvers (ASMFC 2012). These young eels also feed on the zooplankton intermediate host, but as they continue to grow and enter their next stage, the yellow eel, their diet broadens and they consume a wide range of organisms such as other fish, snails, worms, insect larvae, and amphibians (ASMFC 2012). All these organisms, which also can feed on infected zooplankton, are paratenic hosts of *A. crassus*, where the nematode is able to survive, but does not grow or molt (Thomas and Ollevier 1992). Once an infected prey item is consumed by an eel, *A. crassus* moves from the gut of the eel, through its body cavity, to its swimbladder wall, where it grows (Haenen et al. 1989). It continues to mature within the swimbladder wall and enters the lumen to become a sexually dimorphic adult, with females being much larger than males (De Charleroy et al. 1990). The nematodes feed on the blood flowing through the swimbladder wall (Würtz and Taraschewski 2000). After reproduction, the adult nematodes die within the swimbladder where they degrade or are forced out through the pneumatic duct (De

Charleroy et al. 1990). The fertilized *A. crassus* eggs are released within the swimbladder and exit the swimbladder through the pneumatic duct into the eel's digestive tract, and are expelled into the water with the feces (De Charleroy et al. 1990). After hatching, the parasite larvae attach themselves to substrate and wiggle to attract the crustacean zooplankton intermediate host (De Charleroy et al. 1990). In lab settings, *A. crassus* is able to complete its life cycle in under 2 months (De Charleroy et al. 1990). This cycle continues throughout the life of the eel until the eels reach sexually maturity at anywhere from 4 to 40+ years and undergo many physiological transformations to prepare them for oceanic migration (ASMFC 2012). Once these changes are complete, the eels have now entered the silver eel phase; they cease eating and exit the inland and coastal waters to begin their long migration back to the Sargasso Sea, where they will spawn and die (ASMFC 2012). During the lifespan of an eel, a great deal of damage can accumulate within their swimbladder due to many repeated infections.

The majority of the research conducted on the American and European eels has focused on yellow phase individuals. Yet, younger eel stages, glass and elvers, can also be infected both experimentally and naturally (Nimeth et al. 2000). Additionally, swimbladder damage occurs in these young eels, thereby impairing this organ similarly to older eels (Nimeth et al. 2000). A recent study in South Carolina surveyed younger eels and found that 8% of glass eels and about 60% of elvers were infected with *A. crassus* (Hein et al. 2015). Only the most pigmented glass eels (pigment stage 7 based on Haro and Krueger (1988)) were found to have adult worms (Hein et al. 2015). Interestingly, no elvers (n=30) from Maryland in 1998 were found to be infected (Barse et al. 2001). More research is needed on younger eels to determine when infection first begins and how it affects these younger stages.

Physiological damage from *A. crassus*

The histopathology associated with swimbladder damage due to *A. crassus* has been studied in both the American and European eels, with similar findings. The swimbladder wall is made up of (starting with the luminal surface) the mucosa comprised of a simple cuboidal epithelium, lamina propria, muscular mucosa (muscle tissue), submucosa (loose connective tissue), serosa (dense connective tissue), and blood vessels (Wurtz and Taraschewski 2000; Figure 2). When an eel is infected with *A. crassus*, the normally smooth mucosa becomes papillose in appearance in conjunction with hyperplasia (cellular proliferation) of the lamina propria, muscle tissue, and submucosa (Sokolowski and Dove 2006; Figure 2). Also there is edema (swelling) of the mucosa and muscle tissue, as well as dilated blood vessels (Sokolowski and Dove 2006). Damage is due to the migration of L3 and L4 larvae through the swimbladder walls, adult nematodes feeding on the swimbladder blood, and the degradation of dead adults (Sokolowski and Dove 2006). Eels can mount a humoral immune response with production of specific antibodies to *A. crassus*, granulocytes formed around L3 and L4 larvae, and fibrosis of the swimbladder wall (Buchmann et al. 1991; Molnar et al. 1993; Van Banning and Haenen 1990). External damage to the eel is also possible, manifested as reddening or lesions around the anus and/or a swollen abdomen (Moser et al. 2001; Ooi et al. 1996). Repeated and heavy infections can result in extensive damage to the swimbladder such that the wall of the organ thickens and becomes opaque, the volume of the lumen is reduced, and the pneumatic duct becomes blocked (Van Banning and Haenen 1990; Kirk 2003; Kirk et al. 2000a). The swimbladder can accumulate so much pathology that it collapses (Molnar et al. 1995) and laboratory experiments have shown that the damage can affect swimming, ability to hold oxygen, and function as a hydrostatic organ (Wurtz et al. 1996; Kobayashi et al. 1990).

Disease progression

Despite our understanding of the pathology of anguillicolosis, its progression timeline is relatively unknown due to most observations being made at a single point in time during dissections. Additionally, the ability for an eel to recover from infection has been speculated but not definitively shown. An innovative way to understand this degenerative process has been used on European eels with good success—analyzing the radiographic images produced by an x-ray machine (Beregi et al. 1998; Szekely et al. 2004; Szekely et al. 2005; Palastra et al. 2007; Frisch et al. 2015). This technique is possible due to the radiotransparency of the air filled swimbladder, allowing the organ to appear as a dark area on the radiograph in contrast to the white of the other internal structures. This method was first developed by Beregi et al. (1998), and it was found to be useful for assessing the presence of nematodes, air content of the swimbladder, and the thickness of the wall indirectly by interpreting changes in swimbladder shape. They found that healthy/normal swimbladders were equal in length to 15 intervertebral spaces, and a decrease in length relative to vertebrae indicated a more heavily infected bladder. This finding is similar to other work that shows that swimbladder length decreases with increased damage (Lefebvre et al. 2011).

To interpret radiographic images, Beregi et al. (1998) used four grade levels ranging from healthy/normal to severe damage (i.e. no air content of the swimbladder), with each grade corresponding to a visual image and dissection parameters. Interestingly, grade 2 was identified as a swimbladder that was in recovery and showed regeneration after a previous infection. According to the authors, such a swimbladder was dilated in its middle third and tapered at the ends, with a non-homogeneous x-ray shadow due to areas of inflammation. This type of

swimbladder also has an air filled pneumatic duct, which is normally empty, in addition to small nematodes visible in the outer ends or within the pneumatic duct. When a grade 2 swimbladder is dissected, it is still filled with air, its wall is relatively thin and only slightly opaque, and usually contains a few small nematodes. Yet, there is no definitive proof, such as previous images of the swimbladder in a worse condition or histological data to support that this description signifies a recovering swimbladder. The subsequent grades describe increasing severity.

This radiodiagnostic technique was used in a monitoring study conducted by Szekely et al. (2005) to determine how the swimbladder condition of infected European eels changed over time when reinfection was not possible. The condition of the swimbladder was based on the extent of the radiographic shadow and whether any worms were visible. Findings indicated that 55% of the eels had a worse swimbladder condition at the end of the three months, 37% did not have a change in condition, 7% had a variable condition (i.e. got worse then better or vice versa), and 1% (1 eel) had improvement (Szekely et al. 2005). Yet the previous studies using x-ray images are more qualitative than quantitative, necessitating the use of qualitative metrics such as swimbladder length and area obtained from the images to track swimbladder condition.

Epidemiology modeling of *A. crassus* in American eels

In wild eel populations, there is concern about disease-associated reductions in overall fitness, mortality, and fertility due to *A. crassus* (ASMFC 2012). Additionally, if the infection is compromising migrations to the spawning grounds in the Sargasso Sea, then population level impacts such as lower recruitment and population declines could result. Despite the abundance of research in the European system, there is currently no data to determine if the patterns observed

there are consistent in North America, and as such, specific research must be conducted on the American system to accurately determine infection-associated impacts.

Disease modeling is a powerful tool to determine how the infection metrics and disease parameters of individual eels translate to population level effects. This technique, in concert with traditional stock assessment analyses, is not common, but nonetheless has been shown to be useful. For example, force-of-infection modeling and growth analyses of striped bass (*Morone saxatilis*) exhibiting a bacterial infection called mycobacteriosis found that force-of-infection varied by age and disease-associated mortality was occurring such that the annual survival of infected striped bass was 0.69 that of uninfected striped bass (Gauthier et al. 2008; Latour et al. 2012). This research has provided insights into disease-associated impacts that have stimulated awareness and interest within the management community.

Very little infection modeling has been conducted for the European or American eel despite its potential usefulness in stock assessments and management decisions. Additionally, published results often contradict each other. In North Carolina, infection intensity and arcsine transformed prevalence was found to increase with eel size, but was not affected by month of collection (Moser et al. 2001). Conversely, Fenske et al. (2010) found infection intensity and prevalence decreased with increasing eel size class for animals in Chesapeake Bay, but did not find an association between prevalence or swimbladder condition and female eel growth rate or age. Additionally, they found lower infection intensity and swimbladder condition in the fall compared to the summer (Fenske et al. 2010). Hein et al. (2014) did not find eel total length to be a significant predictor of parasite prevalence, intensity, or abundance in South Carolina eels, but did find significant variation among sites. Prevalence of *A. crassus* in glass eels and elvers varied significantly based on an interaction with total length and month; in March to July, prevalence

increased with total length, but prevalence was lower and less dependent on total length in August to December (Hein et al. 2015). More robust models such those modeling zero-inflation, the ordinal aspect of swimbladder damage, and the onset of disease may clarify the contradictory conclusions found in the published literature.

The majority of the published analyses on American eels have focused mainly on prevalence and mean infection intensity of *A. crassus* (Fenske et al. 2010; Aieta and Oliveria 2009; Hein et al. 2014), and factors affecting infection intensity and swimbladder condition have not yet been widely explored. This discrepancy can be problematic because prevalence and mean intensity illustrate the infection level at a single point in time whereas swimbladder condition shows damage accumulated over time. In addition, the number of nematodes within a swimbladder does not necessarily linearly correlate to the current swimbladder condition (Lefebvre et al. 2013). Finally, the ultimate question of whether *A. crassus* causes infection-associated mortality is inconclusive and requires robust epidemiology modeling to better discern if disease-associated mortality is occurring (Lefebvre et al. 2013). Although these recent studies have provided valuable information, additional research is needed to more comprehensively assess the impact of *A. crassus* on all American eel stages using more comprehensive modeling techniques.

Objectives

1. Quantify prevalence, infection intensity, and swimbladder damage resulting from *Anguillicoloides crassus* infections in glass eels, elvers, and yellow American eels in the Chesapeake Bay.

2. Determine if factors such as length, season, and capture site are associated with *A. crassus* parasite load and swimbladder damage.
3. Determine how swimbladder condition of yellow eels changes over time when reinfection is blocked.
4. Determine if swimbladder recovery from infection can occur.
5. Apply a force-of-infection model using infection data from glass eels, elvers, and yellow American eels to investigate population level impacts of *A. crassus* and presence of parasite-associated natural mortality.

NOTE: Objectives 1, 2, and 5 are addressed in the first chapter and objectives 3 and 4 are addressed in the second chapter.

References

- Atlantic States Marine Fisheries Commission. 2012. American Eel Benchmark Stock Assessment for Peer Review. Stock Assessment Report No. 12-01.
- Aieta, A.E., and Oliveira, K. 2009. Distribution, prevalence, and intensity of the swimbladder parasite *Anguillicola crassus* in New England and eastern Canada. *Dis. Aquat. Organ.* 84: 229–35. doi:10.3354/dao02049.
- Barse, A.M., Mcguire, S.A., Vinoses, M.A., Eierman, L.E., and Weeder, J.A. 2001. The Swimbladder Nematode *Anguillicola crassus* in American Eels (*Anguilla rostrata*) from Middle and Upper Regions of Chesapeake Bay. *J. Parasitol.* 87(6): 1366–1370. doi:10.1645/0022-3395(2001)087[1366:TSNACI]2.0.CO;2.
- Barse, A.M., and Secor, D.H. 1999. An Exotic Nematode Parasite of the American Eel. *Fisheries* 24(2): 6–10. doi:10.1577/1548-8446(1999)024.
- Beregi, A., Molnár, K., Békési, L., and Székely, C. 1998. Radiodiagnostic method for studying swimbladder inflammation caused by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Dis. Aquat. Organ.* 34: 155–160. doi:10.3354/dao034155.
- Buchmann, K., Pedersen, L.O., and Glamann, J. 1991. Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Dis. Aquat. Organ.* 12: 55–57. doi:10.3354/dao012055.
- Castonguay, M., Hodson, P.V., Couillard, C.M., Eckersley, M.J., Dutil, J.-D., and Verreault, G. 1994. Why is recruitment of the american eel, *Anguilla rostrata*, declining in the St. Lawrence River and Gulf? *Can. J. Fish. Aquat. Sci.* 51(2): 479–488. doi:10.1139/f94-050.
- De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C., and Ollevier, F. 1990. The life cycle of *Anguillicola crassus*. *Dis. Aquat. Organ.* 8: 77–84. doi:10.3354/dao008077.
- Fenske, K.H., Secor, D.H., and Wilberg, M.J. 2010. Demographics and Parasitism of American Eels in the Chesapeake Bay, USA. *Trans. Am. Fish. Soc.* 139(6): 1699–1710. doi:10.1577/T09-206.1.
- Fries, L.T., Williams, D.J., and Johnson, S.K. 1996. Notes : Occurrence of *Anguillicola crassus*, an Exotic Parasitic Swim Bladder Nematode of Eels, in the Southeastern United States. *Trans. Am. Fish. Soc.* 125(5): 794–797. doi:10.1577/1548-8659(1996)125<0794:NOOCAE>2.3.CO;2.
- Frisch, K., Davie, A., Schwarz, T., and Turnbull, J. 2015. Comparative imaging of European eels (*Anguilla anguilla*) for the evaluation of swimbladder nematode (*Anguillicoloides crassus*) infestation. *J. Fish Dis.* 39: 635–647. doi:10.1111/jfd.12383.

- Gauthier, D.T., Latour, R.J., Heisey, D.M., Bonzek, C.F., Gartland, J., Burge, E.J., and Vogelbein, W.K. 2008. Mycobacteriosis-associated mortality in wild striped bass (*Morone saxatilis*) from Chesapeake Bay, U.S.A. *Ecol. Appl.* 18(7): 1718–11727. doi:10.1890/07-2083.1.
- Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C., and Ollevier, F. 1989. Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Dis. Aquat. Organ.* 7: 97–101. doi:10.3354/dao007097.
- Haro, A., Richkus, W., Whalen, K., Hoar, A., Busch, W.-D., Lary, S., Brush, T., and Dixon, D. 2000. Population Decline of the American Eel: Implications for Research and Management. *Fisheries* 25(9): 7–16. Taylor & Francis Group. doi:10.1577/1548-8446(2000)025<0007:PDOTAE>2.0.CO;2.
- Haro, A.J., and Krueger, W.H. 1988. Pigmentation, size, and migration of elvers (*Anguilla rostrata* (Lesueur)) in a coastal Rhode Island stream. *Can. J. Zool.* 66: 2528–2533. doi:10.1139/z88-375.
- Hein, J.L., Arnott, S.A., Roumillat, W.A., Allen, D.M., and de Buron, I. 2014. Invasive swimbladder parasite *Anguillicoloides crassus*: infection status 15 years after discovery in wild populations of American eel *Anguilla rostrata*. *Dis. Aquat. Organ.* 107: 199–209. doi:10.3354/dao02686.
- Hein, J.L., Buron, I. De, Roumillat, W.A., Post, W.C., Hazel, A.P., and Arnott, S.A. 2015. Infection of newly recruited American eels (*Anguilla rostrata*) by the invasive swimbladder parasite *Anguillicoloides crassus* in a US Atlantic tidal creek. *ICES J. Mar. Sci.*: 1–8. doi:10.1093/icesjms/fsv097.
- Jacoby, D., Casselman, J., DeLucia, M., Hammerson, G.A., and Gollock, M. 2014. *Anguilla rostrata*. The IUCN Red List of Threatened Species 2014: e.T191108A72965914.
- Kennedy, C.R., and Fitch, D.J. 1990. Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *J. Fish Biol.* 36: 117–131. doi:10.1111/j.1095-8649.1990.tb05588.x.
- Kirk, R.S. 2003. The impact of *Anguillicola crassus* on European eels. *Fish. Manag. Ecol.* 10: 385–394. doi:10.1111/j.1365-2400.2003.00355.x.
- Kirk, R.S., Lewis, J.W., and Kennedy, C.R. 2000. Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in in seawater eels. *Parasitology* 120: 289–295.
- Kobayashi, H., Pelster, B., and Scheid, P. 1990. CO₂ back-diffusion in the rete aids O₂ secretion in the swimbladder of the eel. *Respir. Physiol.* 79(3): 231–242. doi:10.1016/0034-5687(90)90129-M.

- Latour, R.J., Gauthier, D.T., Gartland, J., Bonzek, C.F., McNamee, K.A., and Vogelbein, W.K. 2012. Impacts of mycobacteriosis on the growth of striped bass (*Morone saxatilis*) in Chesapeake Bay. *Can. J. Fish. Aquat. Sci.* 69(2): 247–258. doi:10.1139/F2011-158.
- Lefebvre, F., Fazio, G., Mounaix, B., and Crivelli, A.J. 2013. Is the continental life of the European eel *Anguilla anguilla* affected by the parasitic invader *Anguillicoloides crassus*? *Proc. R. Soc. B* 280: 20122916. doi:10.1098/rspb.2012.2916.
- Lefebvre, F., Fazio, G., Palstra, A.P., Székely, C., and Crivelli, A.J. 2011. An evaluation of indices of gross pathology associated with the nematode *Anguillicoloides crassus* in eels. *J. Fish Dis.* 34: 31–45. doi:10.1111/j.1365-2761.2010.01207.x.
- Lefebvre, F., Wielgoss, S., Nagasawa, K., and Moravec, F. 2012. On the origin of *Anguillicoloides crassus*, the invasive nematode of anguillid eels. *Aquat. Invasions* 7(4): 443–453. doi:10.3391/ai.2012.7.4.001.
- Lefebvre, F.S., and Crivelli, A.J. 2004. Anguillicolosis: dynamics of the infection over two decades. *Dis. Aquat. Organ.* 62: 227–232. doi:10.3354/dao062227.
- Molnár, K., Baska, F., Csaba, G., Glavits, R., and Székely, C. 1993. Pathological and histopathological studies of the swimbladder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Dis. Aquat. Organ.* 15: 41–50. doi:10.3354/dao015041.
- Molnár, K., Szokolczai, J., and Vetési, F. 1995. Histological changes in the swimbladder wall of eels due to abnormal location of adult and second stage larvae of *Anguillicola crassus*. *Acta Vet. Hung.* 43(1): 125–137.
- Moser, M.L., Patrick, W.S., and Crutchfield, J.U. 2001. Infection of American Eels, *Anguilla rostrata*, by an Introduced Nematode Parasite, *Anguillicola crassus*, in North Carolina. *Copeia* 3: 848–853. doi:10.1643/0045-8511(2001)001[0848:IOAEAR]2.0.CO;2.
- Nagasawa, K., Kim, Y.-G., and Hirose, H. 1994. *Anguillicola crassus* and *A. globiceps* (Nematoda: Dracunculoidea) parasitic in the swimbladder of eels (*Anguilla japonica* and *A. anguilla*) in East Asia: A review. *Folia Parasitol. (Praha)*. 41: 127–137.
- Nimeth, K., Zwerger, P., Würtz, J., Salvenmoser, W., and Pelster, B. 2000. Infection of the glass-eel swimbladder with the nematode *Anguillicola crassus*. *Parasitology* 121: 75–83.
- Ooi, H.K., Wang, W.S., Chang, H.Y., Wu, C.H., Lin, C.C., and Hsieh, M.T. 1996. An epizootic of Anguillicolosis in cultured American eels in Taiwan. *J. Aquat. Anim. Health* 8(2): 163–166. doi:10.1577/1548-8667(1996)008<0163:AEOAIC>2.3.CO;2.
- Palstra, A.P., Heppener, D.F.M., van Ginneken, V.J.T., Székely, C., and van den Thillart, G.E.E.J.M. 2007. Swimming performance of silver eels is severely impaired by the swimbladder parasite *Anguillicola crassus*. *J. Exp. Mar. Bio. Ecol.* 352: 244–256. doi:10.1016/j.jembe.2007.08.003.

- R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rockwell, L.S., Jones, K.M.M., and Cone, D.K. 2009. First Record of *Anguillicoloides crassus* (Nematoda) in American Eels (*Anguilla rostrata*) in Canadian Estuaries, Cape Breton, Nova Scotia. *J. Parasitol.* 95(2): 483–486. doi:10.1645/GE-1739.1.
- Shepard, S.L. 2015. American eel biological species report. U.S. Fish and Wildlife Service. Hadley, MA. Xii + 120 pages.
- Sokolowski, M.S., and Dove, A.D.M. 2006. Histopathological Examination of Wild American Eels Infected with *Anguillicola crassus*. *J. Aquat. Anim. Health* 18(4): 257–262. doi:10.1577/H06-009.1.
- Székely, C., Molnár, K., Müller, T., Szabó, A., Romvári, R., Hancz, C., and Bercsényi, M. 2004. Comparative study of X-ray computerised tomography and conventional X-ray methods in diagnosis of swimbladder infection in eels caused by *Anguillicola crassus*. *Dis. Aquat. Organ.* 58: 157–164. doi:10.3354/dao058157.
- Székely, C., Molnár, K., and Rácz, O.Z. 2005. Radiodiagnostic method for studying the dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection and pathological status of the swimbladder in Lake Balaton eels. *Dis. Aquat. Organ.* 64: 53–61. doi:10.3354/dao064053.
- Thomas, K., and Ollevier, F. 1992. Paratenic hosts of the swimbladder nematode *Anguillicola crassus*. *Dis. Aquat. Organ.* 13: 165–174.
- Van Banning, P. and Haenen, O.L.M. 1990. Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*. In *Pathology in marine science*. Edited by F.O. Perkins and T.C. Cheng. Academic Press, New York, NY. pp. 317–330.
- Würtz, J., and Taraschewski, H. 2000. Histopathological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. *Dis. Aquat. Organ.* 39: 121–134. doi:10.3354/dao039121.
- Würtz, J., Taraschewski, H., and Pelster, B. 1996. Changes in gas composition in the swimbladder of the European eel (*Anguilla anguilla*) infected with *Anguillicola crassus* (Nematoda). *Parasitology* 112: 233–238. doi:10.1017/S003118200008481X.

Figures

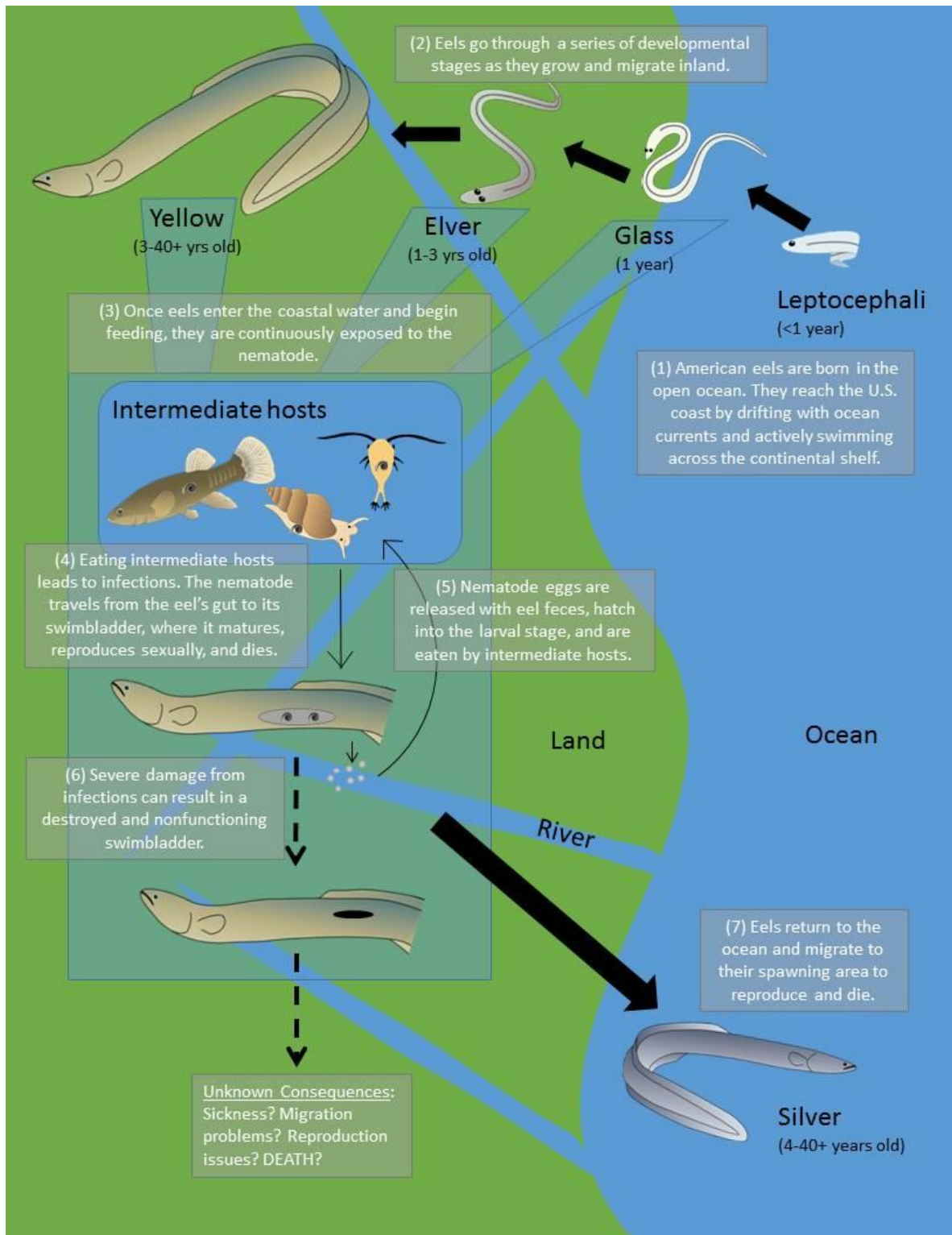


Figure 1. Combined life cycles of American eel (*Anguilla rostrata*) and the parasitic nematode *Anguillicoloides crassus*. Eel images from Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

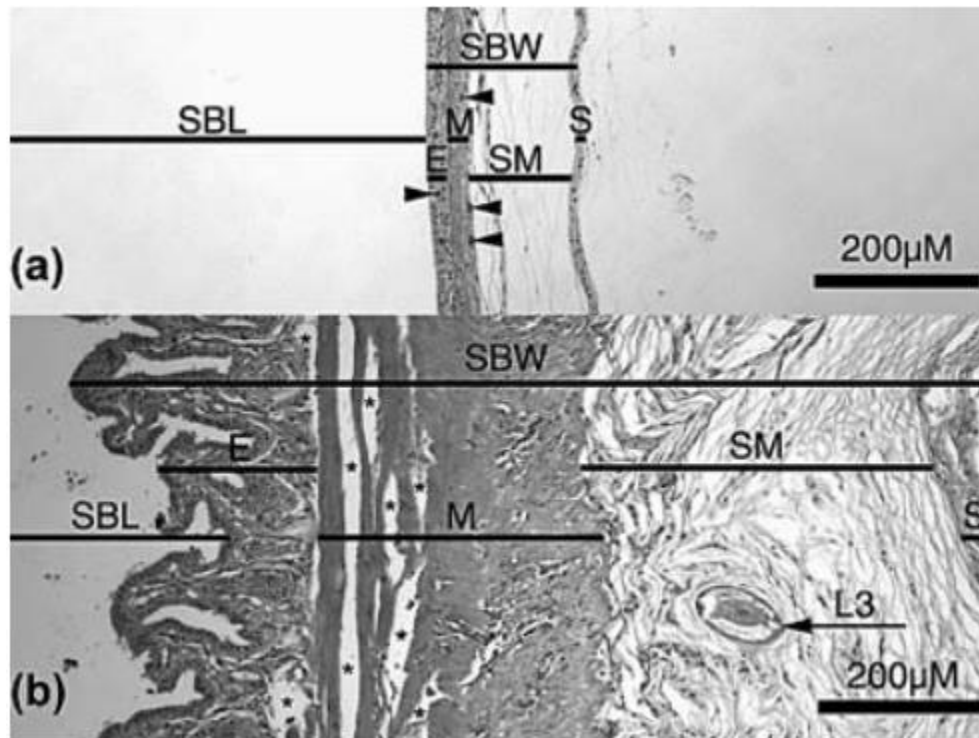


Figure 2. (a) Section of a swim bladder of an uninfected American eel collected from the Hudson River, New York. The normal swim bladder wall (SBW) consists of the mucosa (E), simple cuboidal epithelial cells and lamina propria; the muscularis mucosa (M), muscle tissue; the submucosa (SM), loose connective tissue; serosa (S), dense connective tissue; and normal blood vessels (arrowheads). The swim bladder lumen (SBL) is also indicated. (b) A section of an *Anguillicoloides crassus*-infected swim bladder is shown from an American eel collected in the Carlls River, New York. Papillose mucosal layer (E); edema (*) of the mucosal layer and muscularis mucosa (M); migrating *A. crassus* L3 larva (arrow); and hyperplasia in the lamina propria, muscularis mucosa (M), submucosa (SM), and serosa (S) are indicated. Hematoxylin and eosin stain was used. (from Sokolowski and Dove 2006).

Chapter 1

Temporal, spatial, and biological variation of nematode infection in American eels

ABSTRACT

American eels (*Anguilla rostrata*) are infected by an introduced parasitic nematode *Anguillicoloides crassus*, which can cause severe swimbladder damage. We investigated the disease dynamics of *A. crassus* to better understand its effects on the American eel. Nematode count and swimbladder damage were quantified in glass eels, elvers, and yellow eels from the lower Chesapeake Bay and related to season of capture, river system, and total length of the eel. Age-variant force of infection and disease-associated mortality were determined by using a compartmental disease model. Results showed that glass eels have very low infection prevalence and severity compared to elvers and yellow eels. Infection intensity and swimbladder damage were found to be non-linearly related. Additionally, infection intensity varied by season, river, and eel length, whereas swimbladder damage varied by season and eel length. Force of infection based on presence of swimbladder damage peaked at age 2 and disease positive eels had an estimated annual survival rate of 0.76 compared to disease negative eels. Our study illustrated the impact *A. crassus* has on American eels in the tributaries of the lower Chesapeake Bay.

INTRODUCTION

The American eel (*Anguilla rostrata*) is an economically and ecologically important, yet relatively data-poor, species distributed along the entire Atlantic coast of the United States and throughout the Gulf of Mexico (ASMFC 2012). The American eel population has been declining for the past several decades and is currently considered depleted and at a historically low level of abundance according to the most recent stock assessment by the Atlantic States Marine Fisheries Commission (ASMFC 2012). Several hypotheses have been proposed to explain the species' decline such as overfishing, pollution, changing climate, altered habitats and food webs, parasites, and emergent disease (Castonguay et al. 1994; Haro et al. 2000). One such proposed hypothesis is the impact of the introduced parasitic nematode, *Anguillicoloides crassus*, which can cause severe damage to the swimbladders of American eels.

Infection by *Anguillicoloides crassus* is endemic in Japanese eel (*Anguilla japonica*) in Asia, but significant harm or notable negative population level impacts have not been observed (Sokolowski and Dove 2006). In contrast, the emergence, rapid spread, high prevalence, and pathogenicity of *A. crassus* have been linked to declines in wild European eel (*Anguilla anguilla*) populations and in European eel aquaculture facilities in Asia (Barse et al. 2001; Ooi et al. 1996). Within the American eel population, the parasite was first discovered in 1995 in a Texas aquaculture facility and was first noted in wild animals in South Carolina that same year (Fries et al. 1996). Since its discovery, the distribution of *A. crassus* has expanded rapidly and can now be found in eels in the Gulf of Mexico northward to Nova Scotia (Rockwell et al. 2009).

Eels become infected by consuming intermediate hosts such as copepods and oystercods or by ingesting paratenic hosts (i.e. non-essential intermediate hosts) such as fishes, amphibians,

snails, or insect larvae (Thomas and Ollevier 1992; Moravec 1996; Moravec and Skorikova 1998). Once inside the eel, the parasite moves from the gut through the body cavity, eventually residing in the swimbladder where it matures, sexually reproduces, and then dies and decays or is forced out through the pneumatic duct (Haenen et al. 1989; De Charleroy et al. 1990). Damage to the eel occurs as a result of larval nematode migration through the swimbladder wall, feeding on blood by adults, and inflammation and degradation of dead adults within the lumen (Sokolowski and Dove 2006). Damage clinically manifests as increased opacity, thickening, and pigmentation of the swimbladder wall (Lefevbre et al. 2011). Tissue damage by *A. crassus* can be so severe that it results in complete degradation and loss of function of the swimbladder (Molnar et al. 1995; Wurtz et al. 1996; Kobayashi et al. 1990). Overall nematode impacts on American eel health and population dynamics are not well understood, in part due to the complex relationship between infection intensity and swimbladder condition (Lefevbre et al. 2013).

Previous studies addressing the impacts of *A. crassus* have mainly focused on parasite prevalence and mean intensity (Fenske et al. 2010; Aieta and Oliveria 2009; Hein et al. 2014), but these metrics may give an inaccurate or incomplete picture of the multifaceted epidemiology. Complications with trophic transmission, *A. crassus* dying within the swimbladder and decaying or being cleared, and the possibility that a highly damaged swimbladder may not serve as a suitable habitat for *A. crassus* create potential discrepancies between parasite load and swimbladder damage when describing infection (De Charleroy et al. 1990; van Banning and Haenen 1990). Additionally, previous analyses have focused mainly on yellow eels and do not include the younger stages of glass eels and elvers. Yet these eel stages have the ability to become infected (Hein et al. 2015; De Charelory et al. 1990) and may play an important role in the epidemiology of the parasite, necessitating further investigation. Finally, whether *A. crassus*

causes infection-associated mortality is unknown (Lefebvre et al. 2013) limiting our understanding of the epidemiology and population level impacts of *A. crassus* on American eels.

The objectives of our study were to (1) quantify prevalence, incidence, and intensity of *Anguillicoloides crassus* in glass eels, elvers, and yellow American eels in the Chesapeake Bay; (2) determine how infection intensity and swimbladder damage are related and associated with site, eel size, and season; (3) model *A. crassus* force of infection in Chesapeake Bay and determine if the parasite causes disease-associated mortality; and (4) investigate if covariates such as location and seasonality of capture play a role in shaping transmission and mortality.

METHODS

Field collections

Glass and elver stage American eels were collected from six sites within the lower Chesapeake Bay from March through June 2015 using Irish eel ramps (Figure 1). The traps were placed in areas where there was runoff of freshwater and a dam that impeded the eels' upstream movements. The six sites were on the James River (Wareham's Pond), York River (Bracken's Pond and Wormley Pond), Rappahannock River (Kamp's Millpond), and Potomac River (Clark's Millpond and Gardy's Millpond). Sampling was conducted from late March to late June 2015 in collaboration with young-of-year glass eel monitoring mandated by the ASMFC (ASMFC 2012). Traps were checked a minimum of two days per week, with increasing frequency depending on the strength of the glass eel ingress. On the first sampling day of each

week, a maximum of 30 glass eels and 20 elvers were collected if possible, followed by up to 10 glass and 5 elvers each subsequent sampling day depending on availability of eels. This sampling technique was designed to collect enough eels to detect the nematode at low prevalence while minimizing potential sampling biases such as autocorrelation with prevalence and swimbladder damage within a catch. Differentiation between glass and elver eel stages was determined based on pigmentation stage (Haro and Kruger 1988), with fully pigmented eels being categorized as elvers, and the non-fully pigmented eels as glass eels.

Yellow stage American eels were collected from 2013 to 2015 by the VIMS Seine Survey and Trawl Survey (Tuckey and Fabrizio 2013) in addition to the Virginia Department of Game and Inland Fisheries Electrofishing Survey. The Trawl and Seine Surveys sampled eels in primarily brackish water sites whereas the Electrofishing Survey sampled eels in mainly freshwater locations. All yellow eels were collected within the James, York, Rappahannock, and Potomac River systems.

Laboratory processing

Weight (± 0.001 g) and length (± 0.01 mm) of glass and elver eels were measured before freezing for storage and after thawing prior to dissection for yellow eels. For all eels, the swimbladder was removed after thawing and opened to enumerate adult *A. crassus* in the swimbladder lumen. Counts of larval *A. crassus* in the swimbladder wall were quantified for glass eels under a dissecting scope, after placing the swimbladder between two glass slides. Only adult *A. crassus* parasites were recorded for elver and yellow eels. A macroscopic quantification of the condition of the swimbladder was conducted using the Swimbladder Degenerative Index (SDI; Lefevbre et al. 2002). This index consists of three categories—opacity, thickness, and

pigmentation/exudate and each of these categories receives a ranking from 0 (healthy, normal condition) to 2 (severe condition). The three categories are added together to generate a final SDI score ranging from 0 to 6.

Elver and yellow eel sagittal otoliths were extracted and processed for age determination (Michaud et al. 1988, Cieri and McCleave 2000, and Morrison and Secor 2003). Otoliths were mounted on a glass slide with CrystalBond™ and sanded down on the frontal plane until the core (age-0 ring) is visible. The otolith was then flipped and the opposite end was sanded down until the otolith was transparent and annuli were easily visible. Annuli were tabulated using a microscope with transmitted light. Each otolith was read by two readers and those specimens with annuli counts that differed were read again. Final age assignments were based on consensus among both readers. Protocols for sampling and euthanizing eels were approved by the College of William & Mary's Institutional Animal Care and Use Committee.

Statistical analyses

Prevalence (percent infected), mean abundance (average number of nematodes across all eels surveyed), and mean intensity (average number of nematodes per infected individual eel) were calculated for all stages and river systems using adult *A. crassus* counts (Bush et al. 1997). Final models for all analyses were selected as the lowest Akaike's Information Criterion value (AIC; Burnham and Anderson 2002).

Infection intensity

Presence/absence of *A. crassus* larval and adult stages combined in relation to glass eel total length (TL, continuous) was investigated using a binomial general linear model with a logit link function. Glass eels were excluded from subsequent analyses due to low infection levels.

Preliminary analyses indicated that infection intensity data were zero-inflated using Vuong's non-nested test ($p < 0.0001$) and therefore zero-inflated negative binomial regressions were used to explore the effects of covariates on infection intensity and the probability of a false zero (Zuur et al. 2012). The covariates included river system (categorical, James, York, Rappahannock, Potomac), season (categorical created by assigning the date of capture into the four seasons based on the solstices and equinoxes of that year), SDI (categorical), and TL. Multiple model parameterizations were considered that reflected different combinations of covariates for the count and false zero model components. Partial predictions from the most empirically supported model were generated using marginal means (Searle et al. 1980).

SDI

The swimbladder degenerative index (SDI) is an ordered categorical response variable, requiring a specific regression framework to capture the sequential nature of the data. Ordinal logistic regressions meet this requirement by modelling the probability of being in a certain level (i.e. SDI score) or higher and utilize the proportional odds assumption (Agresti 2010). This assumption assumes that the relationship (i.e. coefficient) between all pairs of levels within a covariate is the same. In other words, the probability of having a lower SDI score compared to any greater score is the same within a given level of a covariate, thereby allowing one coefficient per given level of a covariate or one coefficient for a continuous variable. This assumption can

be relaxed by utilizing partial proportional odds, which allows coefficients to vary with thresholds of SDI scores (i.e. different coefficients for each transition between the categories of the response variable) such that:

$$\text{logit}(P(Y_i \leq j)) = \theta_j - \beta_1 X_{1i} - \beta_{2j} X_{2i}. \quad (1)$$

The cumulative logit $\text{logit}(P(Y_i \leq j))$ represents the probability of an eel having a j^{th} SDI score or less for the i th observation. The parameter θ_j is the intercept for the j th cumulative logit, also known as the threshold parameter. Covariate X_{1i} follows the proportional odds assumption such that there is only one β coefficient, whereas covariate X_{2i} follows partial proportional odds such that there is a different β coefficient for every level of j (Christensen 2015). Odds ratios ($Y \geq j$) are obtained through $\exp(\beta)$, and indicate the odds ratio of a swimbladder having a SDI score j or above for a given covariate (Christensen 2015).

An ordinal logistic regression with partial proportional odds was used to explore the effects of covariates river system, TL, season, parasite load (continuous), and catch ID (continuous) on the proportional odds of having attained greater than or equal to the j^{th} level of swimbladder damage. To aid convergence and model interpretation, SDI scores were condensed into three ordered levels (low: 0-1, moderate: 2-3, severe: 4-6, such that $j = 0, 1, 2$). The proportional odds assumption was evaluated by fitting multiple models with factors as partial proportional odds. Catch ID was modeled as a random effect. Odds ratios were calculated by applying the exponential function to estimated coefficients. Partial predictions from the most empirically supported model were generated using marginal means (Searle et al. 1980).

Epidemiology

To determine the probability that an uninfected eel becomes infected, termed force-of-infection (FOI), and evaluate the potential presence of infection-associated mortality, we applied a three-state irreversible disease model (see Heisey et al. 2006 for full details). The model is designed to provide estimates of key epidemiological parameters from cross-sectional, binary prevalence-at-age data. Disease-positive eels were those that had a swimbladder with an SDI score ≥ 3 or contained adult *A. crassus*. The model assumes no vertical transmission (i.e. transmission from mother to offspring) or recovery. The model structure can accommodate parameterizations that allow force-of-infection to be either age-invariant or age-dependent, and an additive disease-associated mortality parameter can be estimated (i.e., the additional mortality rate experienced by disease-positive individuals relative to the background mortality rate of disease-negative individuals). Age-dependent FOI was modeled using the Weibull, Pareto, Gompertz, and log-logistic functions to identify the appropriate functional shape. The effects of covariates month, season, and river system (all categorical) on force-of-infection were investigated using log-linear models such that:

$$\lambda(t) = \lambda_0(t)e^{X\beta}. \quad (2)$$

The age-dependent FOI $\lambda(t)$ is dependent on the baseline FOI λ_0 , the design matrix X , and the vector of parameters associated with the covariates β (Heisey et al. 2006). The covariate month was redefined to represent two-month time periods (six levels) starting in January/February. Because the model is unable to handle age-0 individuals due to the no vertical transmission assumption, one year was added to all ages. Given that eels have been alive for at least one year once they reach the elver or yellow stage (ASMFC 2012), this adjustment has biological

relevancy. Due to low sample size of older eels, a plus group was defined such that ages ranged from zero to 11+.

All statistical analyses were performed using the R software package (R Core Team 2014). The ‘pscl’ package was accessed for fitting zero-inflated GLMs (Zeileis et al. 2008) and the ‘ordinal’ package (Christensen 2015) was accessed for fitting ordinal logistic regression models. Results are presented as the mean or estimate \pm standard error.

RESULTS

***A. crassus* infection and disease in glass eels**

A total of 1480 glass eels were sampled from all six sites ranging in total length from 47.3 to 77.5 mm (mean: 57.6 mm \pm 0.103). For glass eels, combining adult and larval nematodes, overall prevalence was 3.2%, mean nematode abundance per eel was 0.047 \pm 0.009 (range: 0-10), and mean infection intensity was 1.46 \pm 0.195. Only glass eels collected in the Potomac and Rappahannock rivers were infected (Table 1). Glass eels had higher infection levels of the larval stage of *A. crassus* (prevalence: 2.5%, mean abundance: 0.039 \pm 0.009, mean intensity: 1.57 \pm 0.25, range: 0-10) compared to the parasite adult stage (prevalence: 0.8%, mean abundance: 0.008 \pm 0.002, mean intensity: 1 \pm 0, range: 0-1). Prevalence of larval and adult nematodes combined increased with the length of glass eels ($p < 0.001$, binomial GLM, Figure 2). Furthermore, only the more advanced pigment stages (4-6) of glass eels were found to be infected with larval and adult *A. crassus*, and only pigment stages 5 and 6 showed any

swimbladder damage. Overall, minimal swimbladder damage was found with only seven glass eels having scores greater than 0 (mean: 1.14 ± 0.143). Due to the very low infection rates in glass eels, they were excluded from subsequent analysis.

A. *crassus* infection and disease in elver and yellow eels

Across all six sampling sites, a total of 814 elvers were sampled and total length ranged from 49.0 to 238.0 mm (mean: $113.8 \text{ mm} \pm 1.02$). Adult nematode prevalence was 62.0%, mean abundance per eel was 1.51 ± 0.061 , mean intensity per infected eel was 2.44 ± 0.072 , and average SDI was 1.62 ± 0.055 . When summarized by river system, James River elvers showed the highest prevalence (66.7%), average abundance (1.83 ± 0.13), mean intensity (2.73 ± 0.13), and average SDI (1.82 ± 0.11); however the difference in infection and disease levels between sites was small (Table 1). There was substantial variation between individual elvers such that infection intensity ranged from 0 to 10 nematodes and the full range (0-6) of SDI was observed.

A total of 973 yellow eels were sampled across all four river systems and all three surveys. Total length of these individuals ranged from 60 to 700 mm (mean: $285.9 \text{ mm} \pm 3.71$). Nematode prevalence was 46%, mean abundance per eel was 1.35 ± 0.079 , mean intensity per infected eel was 2.92 ± 0.136 , and average SDI was 2.44 ± 0.055 . Yellow eels from the Potomac River showed the highest prevalence (55.2%), whereas those from the Rappahannock River exhibited the highest mean abundance (1.53 ± 0.13) and mean intensity (3.09 ± 0.22), and those from the James River displayed the highest average SDI (2.3 ± 0.15); however the differences between sites was again small (Table 1). As with elvers, there was substantial variation among individuals in infection and disease, such that infection intensity in yellow eels ranged from 0 to 28 nematodes and the full range (0-6) of SDI was observed.

Infection intensity

The zero-inflated negative binomial model that received the most empirical support contained covariates river system, season, TL, and SDI for both the zero-inflated and count components of the model (Supplemental Table 1). This model was closely followed ($\Delta\text{AIC} < 2$) by a model without SDI in the zero-inflated component. Given that there were significant differences in the several levels of SDI when compared to the baseline of 0 in the full model (Supplemental Table 1), the results from the full model were chosen to be reported.

When comparing $\log(\text{infection intensity})$ (Supplemental Table 2), the negative binomial component of model, the Potomac River had significantly lower $\log(\text{infection intensity})$ compared to the James River (-0.452 ± 0.112 , $p < 0.001$), but there was no significant difference between the $\log(\text{infection intensity})$ of the Rappahannock and York rivers compared to the James River ($p > 0.05$, Figure 3). Seasonally, spring had a significantly higher $\log(\text{infection intensity})$ compared to fall (0.294 ± 0.130 , $p < 0.05$), but there was no significant difference between the $\log(\text{infection intensity})$ for summer and winter ($p > 0.05$) compared to fall (Figure 4). For every unit increase in TL, there was a significant increase in the $\log(\text{infection intensity})$ ($1.37 \times 10^{-3} \pm 4.42 \times 10^{-4}$, $p < 0.01$). For SDI, the $\log(\text{infection intensity})$ was significantly higher for all scores compared to 0 (0.279 ± 0.124 , 0.512 ± 0.116 , 0.295 ± 0.131 , 0.502 ± 0.156 , 0.387 ± 0.194 , 0.500 ± 0.238 for SDI 1-6 respectively, $p < 0.05$, Figure 3).

Swimbladder condition

The ordinal logistic regression model with the most empirical support included covariates TL as a proportional odds factor, season and infection intensity as partial proportional odds, and

catch ID as a random effect (Supplemental Tables 3 and 4). However, the effect of the random factor was relatively minimal, with only a few catches driving the effect (Supplemental Figure 1), but resulted in a significant drop in AIC ($\Delta\text{AIC} = 128.7$). For a one unit increase in TL, the odds of moving from a low swimbladder condition to moderate or severe (or low or moderate to severe) are multiplied by 1.004 ($p < 0.001$), meaning the longer an eel, the more likely it has a more damaged swimbladder. Infection intensity did not follow the proportional odds assumption such that the odds of moving from a low swimbladder condition to moderate or severe is multiplied by 0.832 ($p < 0.001$) for every unit increase in infection intensity whereas the odds of moving from a low or moderate swimbladder condition to severe is multiplied by 0.914 ($p < 0.01$). In other words, the more parasites an eel has, the less likely it has a healthy swimbladder, and that effect is greater for low compared to moderate or severe than it is for low or moderate compared to severe (Figure 4). Season also did not follow the proportional odds assumption such that the effect sizes for each threshold of swimbladder damage were not the same for each season relative to the baseline of fall. Only the odds of having a swimbladder condition severe vs. low or moderate in the summer is significantly different from the baseline of fall (0.442, $p < 0.01$). Comparing the predicted probabilities, the probability of having a healthy swimbladder was similar among all seasons (0.333 ± 0.039 , 0.315 ± 0.044 , 0.229 ± 0.110 , 0.359 ± 0.024 for summer to spring respectively), but the probability for a moderately damaged swimbladder was highest in winter and lowest in summer (0.709 ± 0.108 and 0.451 ± 0.042 respectively), whereas summer had the highest probability of finding a severely damaged swimbladder and winter had the lowest (0.216 ± 0.031 and 0.062 ± 0.038 , respectively). Overall, eels have a higher predicted probability of having a moderately damaged swimbladder compared

to a low or severely damaged swimbladder (0.309 ± 0.054 , 0.566 ± 0.053 , 0.125 ± 0.024 for low, moderate, and severe respectively; Figure 4).

Force of infection and disease associated mortality

A total of 64 elvers and 661 yellow eels were included in the force of infection analysis, ranging in age from 0 to 15 years (age range of 0 to 11+ used in analysis due to small sample size of older individuals). Prevalence of swimbladder damage increased drastically from age 0 to 1 and then slightly with increasing age, whereas prevalence of *A. crassus* infection was highest in the younger and older eels but lower in the middle ages (Figure 5). For prevalence of swimbladder damage, age-dependent models received more support than age-invariant models (Table 2). The model with the most empirical support was the log-logistic with month pairs and the mortality term, though two other models were within 2 AIC units and several others were within 3 units (Table 2). The unit hazard ratios (i.e. proportional difference in force of infection) for month pairs relative to the baseline November/December were (-0.026, -0.793, -0.294, -0.430, -0.486 (January/February, March/April, May/June, July/August, September/October, respectively). Force of infection peaked in all month pairs at age 2 and then decreased with subsequent ages (Figure 6). The disease-associated mortality term was estimated as 0.277 (95% CI: 0.0845-0.507) and the annual survival ratio of a diseased eel relative to a non-diseased eel is $e^{-(-0.277)}$ (Heisey et al. 2006) or 0.76 (95% CI: 0.602-0.919).

Prevalence of *A. crassus* parasites was not found to be a suitable definition of disease to be analyzed by the force-of-infection model. Because *A. crassus* can die within the swimbladder and degrade (which are not counted in infection intensity) or be cleared out of the swimbladder

through the pneumatic duct, parasite prevalence does not meet the no-recovery assumption of the force of infection model.

DISCUSSION

Our study illustrates the complex dynamics of infection by *A. crassus* over different developmental stages and environmental factors experienced by American eels. Overall, glass eels exhibit a much lower prevalence and severity of infection than elvers or yellow eels. These lower infection rates are due to less time in the estuary and therefore less exposure to *A. crassus*. Larval nematodes were found in glass eels, differing from the findings of Hein et al. (2015), where only adult nematodes were found in glass eels. This difference could be due to sampling location, since glass eels for our study were caught at downstream sites where they were most likely first exposed to *A. crassus*, and eels collected in the South Carolina study were caught further upstream allowing the larval *A. crassus* to develop into adults (Hein et al. 2015). Yet prevalence, mean intensity, and mean abundance values were similar between the two studies for glass eels (Hein et al. 2015). Prevalence was also similar between the Hein et al. (2015) study and our findings for elvers, but our study showed slightly lower average intensity and mean abundance of *A. crassus*, which could be due to natural variation among sampling sites. Glass eels and elvers are rarely considered when studying *A. crassus*, but high elver infection levels warrant inclusion in this and future studies. The infection severity in yellow eels were similar to those seen in the elvers, except for a lower nematode prevalence, but still within the range of previous studies of eels from Chesapeake Bay (Barse and Secor 1999; Barse et al. 2001; Fenske et al. 2010).

The analyses of infection intensity and swimbladder damage showed that these two different definitions of *A. crassus* infection are non-linearly related, meaning that an increase in infection intensity does not necessarily correlate to more swimbladder damage and vice versa. For example, the estimated average infection intensity was highest for the highest SDI score of 6 and lowest for the lowest SDI score of 0, but the remaining intermediate values did not increase linearly. Also as infection intensity increases, the probability of having a low, moderate, or severely damaged swimbladder becomes indistinguishable. Additionally, for lower infection intensity, the probabilities of having a low or severely damaged swimbladder are very similar. Therefore as infection intensity increases swimbladder damage does not necessarily follow the same increase. A nonlinear relationship was also found by several studies on European eels (Lefebvre et al. 2002; Lefebvre et al. 2013), but is not well documented for American eels.

The nonlinear relationship between parasite count and swimbladder damage may be caused by various aspects of the complex relationship between *A. crassus* and American eels. Nematodes can die within the swimbladder and degrade or be cleared out, but leave behind damage. Also, there may be a lag between nematode presence and damage accumulation such that multiple infections may occur before damage accrues (Van Banning and Haenen 1990; Molnar et al 1993; Wurtz and Tarachewski 2000). Additionally, density dependence among *A. crassus* exists such that more adult nematodes within the lumen can arrest further movement of larval nematodes into the lumen (Ashworth and Kennedy 1999). Furthermore, as a swimbladder becomes more damaged, it becomes a less suitable habitat for nematodes (Van Banning and Haenen 1990; Molnar et al. 1993). Therefore a swimbladder can be in poor condition but it may have no nematodes within it or it can be healthy and harbor many parasites. Lefebvre et al. (2002 and 2013) suggested that the health state of the swimbladder may be a better indicator of overall

infection than number of living nematodes. Nematode count represents parasite pressure at a single point in time, whereas swimbladder damage shows past and present damage, thereby giving a more comprehensive indication of severity of infection.

Infection intensity and swimbladder damage were both significantly affected by season and total length. Mean infection intensity was slightly higher in the spring and slightly lower in the winter and severe swimbladder damage had the highest probability in the summer and lowest in winter. The difference in timing of high nematode count and swimbladder damage could be due to the lag time between acquiring parasites and accumulating damage. Infection intensity and swimbladder damage both increased with increasing eel length. The effects of season and eel length on parasite presence are not well agreed upon in the literature for studies on American eels, with some studies finding significant effects and others not detecting effects of these factors (Hein et al. 2014; Moser et al. 2001; Fenske et al. 2010; Hein et al. 2015; Morrison and Secor 2003; Machut and Limburg 2008). The discrepancies between studies could be due to other conflicting factors such as intermediate host availability and the life cycle dynamics of *A. crassus*, both areas where more research needs to be focused. The timing of when *A. crassus* becomes more abundant in the environment is unknown, but could provide valuable information on the dynamics of infection. At lower temperatures, the reproductive cycle of *A. crassus* slows (Kim et al. 1989; Nagasawa et al. 1994; Knopf et al. 1998), and therefore may reduce the infection intensity and swimbladder damage in the colder months. Also, because *A. crassus* is trophically transferred and bigger eels are presumably consuming more, larger eels are likely more exposed to *A. crassus* and therefore may accumulate more nematodes and swimbladder damage. Additionally, no previous analyses took into account zero inflation of the nematode

count data or the ordinal nature of swimbladder damage, which may change interpretation of the effects.

River system was found to have a minimal effect on infection intensity with more variation within a site than among sites, but there was no effect on swimbladder damage. Most previous studies in various regions of eastern North America have also found variation in infection metrics among sampling sites (Hein et al. 2014; Morrison and Secor 2003; Fenske et al. 2010; Machut and Limburg 2008; Aieta and Oliveira 2009). Our classification of river system was broad and included the full range of the rivers, from brackish to fresh. Higher salinity has been shown to have a negative effect on *A. crassus* infection (Kirk et al. 2000; Lefebvre and Crivelli 2012), but we were limited by our available dataset and could not investigate this covariate because direct measures of salinity synoptically collected with eel samples were not available. Additionally, different locations could have different availabilities of intermediate hosts and could vary with other stressors such as temperature and oxygen level, all of which could impact *A. crassus* transmission and infection levels in eels (De Charleroy et al. 1989; Kennedy and Fitch 1990; Molnar et al. 1991; Molnar 1993).

Force of infection for prevalence of swimbladder damage peaked at age 2 and subsequently decreased with age, meaning most eels become infected shortly after entering the coastal waters. The highest force of infection was found in November/December and January/February and the lowest in March/April. This pattern could be due to eels acquiring more nematodes in the spring and summer when they are feeding more, as shown in the infection intensity analysis. Then, because of the potential lag between infection and swimbladder condition, accumulation of swimbladder damage is be highest in the winter months as uninfected eels show signs of swimbladder damage. These results are consistent with the results of the

previous analyses showing infection intensity is highest in the spring and summer and moderate swimbladder damage is highest in the winter. Eels may not accumulate much more damage over the winter because they are not feeding (Kennedy and Fitch 1990) and therefore not becoming re-infected, which may explain the lower force-of-infection in March/April. But then, as the eels start feeding again, the force-of-infection rises throughout the subsequent months.

The significant mortality term estimated in the force-of-infection model indicates that there is lower annual survival of eels with swimbladder damage compared to those with very low or no damage. Previous studies have shown that higher *A. crassus* infection levels affect the ability of eels to swim, tolerate hypoxic conditions or high temperatures, avoid hydraulic dams, and avoid predators and fishing pressure (Molnar et al. 1991; Molnar 1993; Gollock et al. 2005; Lefebvre and Crivelli 2007), creating potential sources of elevated mortality. Because the model is not able to differentiate between mortality and recovery, more research is needed to determine if recovery could also be occurring. The ability of the swimbladder to recover from infection is speculated but not definitely shown to occur (Molnàr et al. 1994; Szèkely et al. 2005; Lefebvre et al. 2012). Additionally, due to the widespread availability of *A. crassus* intermediate and paratenic hosts and the lack of acquired immunity (Knopf 2006), eels may be constantly exposed to the nematode and never have the opportunity or ability to fully recover, although partial healing of the swimbladder could be possible.

Clearance of individual nematodes from the swimbladder through either decay or forced exit through the pneumatic duct would result in fewer nematodes within the swimbladder and would represent recovery by the definition of the force of infection model. Yet, the relationship between parasite load and swimbladder damage is complex and fewer parasites does not necessarily mean a less damaged organ. *A. crassus* prevalence was determined to not be a

suitable definition of disease for the force-of-infection model and therefore was not used in the force-of-infection model.

In conclusion, the infection dynamics of *A. crassus* in American eels are very complex. Parasite load and swimbladder damage, though related, illustrate different components of infection; nematode count shows parasite pressure at a given point in time, whereas swimbladder damage likely represents a more comprehensive indicator of overall infection and its negative impacts over time. Additionally, we have shown that *A. crassus* infection may contribute to American eel mortality and therefore may require consideration in future American eel stock assessments. Understanding the relationship between these two components would be improved with additional studies on disease progression and recovery. A better understanding of the timeline of the lifecycle of *A. crassus* would allow us to determine if fluctuations in infection intensity and swimbladder damage are due to parasite availability or mortality. These fluctuations could also be better informed with information regarding the lag between nematode infection and swimbladder damage. We intend for this work to motivate more consideration of the differences and relationship between nematode count and swimbladder damage and further investigation into parasite induced host mortality by *A. crassus*.

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REFERENCES

- Agresti, A. 2010. Analysis of ordinal categorical data. John Wiley & Sons, Hoboken, NJ.
- Aieta, A.E., and Oliveira, K. 2009. Distribution, prevalence, and intensity of the swimbladder parasite *Anguillicola crassus* in New England and eastern Canada. *Dis. Aquat. Organ.* 84: 229–35. doi:10.3354/dao02049.
- Ashworth, S.T., and Kennedy, C.R. 1999. Density-dependent effects on *Anguillicola crassus* (Nematoda) within its European eel definitive host. *Parasitology* 118(3): 289–296.
- Atlantic States Marine Fisheries Commission. 2012. American Eel Benchmark Stock Assessment for Peer Review. Stock Assessment Report No. 12-01.
- Barse, A.M., McGuire, S.A., Vinoses, M.A., Eierman, L.E., and Weeder, J.A. 2001. The Swimbladder Nematode *Anguillicola crassus* in American Eels (*Anguilla rostrata*) from Middle and Upper Regions of Chesapeake Bay. *J. Parasitol.* 87(6): 1366–1370. doi:10.1645/0022-3395(2001)087[1366:TSNACI]2.0.CO;2.
- Barse, A.M., and Secor, D.H. 1999. An Exotic Nematode Parasite of the American Eel. *Fisheries* 24(2): 6–10. doi:10.1577/1548-8446(1999)024.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* 83(4): 575–583. doi:10.2307/3284227.
- Burnham, K.P., and Anderson D.R. 2002. Model selection and interference: a practical information-theoretic approach, Springer, New York, NY.
- Castonguay, M., Hodson, P.V., Couillard, C.M., Eckersley, M.J., Dutil, J.-D., and Verreault, G. 1994. Why is recruitment of the american eel, *Anguilla rostrata*, declining in the St. Lawrence River and Gulf? *Can. J. Fish. Aquat. Sci.* 51(2): 479–488. doi:10.1139/f94-050.
- De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C., and Ollevier, F. 1990. The life cycle of *Anguillicola crassus*. *Dis. Aquat. Organ.* 8: 77–84. doi:10.3354/dao008077.
- De Charleroy, D., Thomas, K., Belpaire, C., and Ollevier, F. 1989. The viability of the free living larvae of *Anguillicola crassus*. *J. Appl. Ichthyol.* 5: 154–156. doi:10.1111/j.1439-0426.1989.tb00487.x.
- Christensen, R.H.B. 2015. A tutorial on fitting cumulative link models with the ordinal package [online]. Available from https://cran.r-project.org/web/packages/ordinal/vignettes/clm_tutorial.pdf [accessed 14 August 2017].

- Cieri, M.D., and McCleave, J.D. 2000. Discrepancies between otoliths of larvae and juveniles of the American eel: is something fishy happening at metamorphosis? *J. Fish Biol.* 57: 1189–1198. doi:10.1006/jfbi.2000.1381.
- Fenske, K.H., Secor, D.H., and Wilberg, M.J. 2010. Demographics and Parasitism of American Eels in the Chesapeake Bay, USA. *Trans. Am. Fish. Soc.* 139(6): 1699–1710. doi:10.1577/T09-206.1.
- Fries, L.T., Williams, D.J., and Johnson, S.K. 1996. Notes : Occurrence of *Anguillicola crassus*, an Exotic Parasitic Swim Bladder Nematode of Eels, in the Southeastern United States. *Trans. Am. Fish. Soc.* 125(5): 794–797. doi:10.1577/1548-8659(1996)125<0794:NOOCAE>2.3.CO;2.
- Gollock, M.J., Kennedy, C.R., and Brown, J.A. 2005. European eels, *Anguilla anguilla* (L.), infected with *Anguillicola crassus* exhibit a more pronounced stress response to severe hypoxia than uninfected eels. *J. Fish Dis.* 28: 429–436. doi:10.1111/j.1365-2761.2005.00649.x.
- Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C., and Ollevier, F. 1989. Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Dis. Aquat. Organ.* 7: 97–101. doi:10.3354/dao007097.
- Haro, A., Richkus, W., Whalen, K., Hoar, A., Busch, W.-D., Lary, S., Brush, T., and Dixon, D. 2000. Population Decline of the American Eel: Implications for Research and Management. *Fisheries* 25(9): 7–16. Taylor & Francis Group. doi:10.1577/1548-8446(2000)025<0007:PDOTAE>2.0.CO;2.
- Haro, A.J., and Krueger, W.H. 1988. Pigmentation, size, and migration of elvers (*Anguilla rostrata* (Lesueur)) in a coastal Rhode Island stream. *Can. J. Zool.* 66: 2528–2533. doi:10.1139/z88-375.
- Hein, J.L., Arnott, S.A., Roumillat, W.A., Allen, D.M., and de Buron, I. 2014. Invasive swimbladder parasite *Anguillicoloides crassus*: infection status 15 years after discovery in wild populations of American eel *Anguilla rostrata*. *Dis. Aquat. Organ.* 107: 199–209. doi:10.3354/dao02686.
- Hein, J.L., Buron, I. De, Roumillat, W.A., Post, W.C., Hazel, A.P., and Arnott, S.A. 2015. Infection of newly recruited American eels (*Anguilla rostrata*) by the invasive swimbladder parasite *Anguillicoloides crassus* in a US Atlantic tidal creek. *ICES J. Mar. Sci.*: 1–8. doi:10.1093/icesjms/fsv097.
- Heisey, D.M., Joly, D.O., and Messier, F. 2006. The fitting of general force-of-infection models to wildlife disease prevalence data. *Ecology* 87(9): 2356–2365. doi:10.1890/0012-9658(2006)87[2356:TFOGFM]2.0.CO;2.

- Kennedy, C.R., and Fitch, D.J. 1990. Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *J. Fish Biol.* 36: 117–131. doi:10.1111/j.1095-8649.1990.tb05588.x.
- Kim, Y.-G., Kim, E.-B., Kim, J.-Y., and Chun, S.-K. 1989. Studies on a nematode, *Anguillicola crassa* parasitic in the air bladder of the eel. *J. fish Pathol.* 2(1): 1–18.
- Kirk, R.S., Kennedy, C.R., and Lewis, J.W. 2000. Effect of salinity on hatching, survival and infectivity of *Anguillicola crassus* (Nematoda: Dracunculoidea) larvae. *Dis. Aquat. Organ.* 40: 211–218. doi:10.3354/dao040211.
- Knopf, K. 2006. The swimbladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla* and the Japanese eel *Anguilla japonica*: differences in susceptibility and immunity between a recently colonized host and the original host. *J. Helminthol.* 80: 129–136. doi:10.1079/JOH2006353.
- Knopf, K., Naser, K., van der Heijden, M.H., and Taraschewski, H. 2000. Humoral immune response of European eel *Anguilla anguilla* experimentally infected with *Anguillicola crassus*. *Dis. Aquat. Organ.* 42(1): 61–9. doi:10.3354/dao042061.
- Knopf, K., Würtz, J., Sures, B., and Taraschewski, H. 1998. Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Dis. Aquat. Organ.* 33: 143–149. doi:10.3354/dao033143.
- Kobayashi, H., Pelster, B., and Scheid, P. 1990. CO₂ back-diffusion in the rete aids O₂ secretion in the swimbladder of the eel. *Respir. Physiol.* 79(3): 231–242. doi:10.1016/0034-5687(90)90129-M.
- Lefebvre, F., Contournet, P., and Crivelli, A.J. 2002. The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology* 124: 457–463. doi:10.1017/S0031182001001378.
- Lefebvre, F., Contournet, P., and Crivelli, A.J. 2007. Interaction between the severity of the infection by the nematode *Anguillicola crassus* and the tolerance to hypoxia in the European eel *Anguilla anguilla*. *Acta Parasitol.* 52(2): 171–175. doi:10.2478/s11686-007-0013-4.
- Lefebvre, F., and Crivelli, A.J. 2012. Salinity effects on anguillicolosis in Atlantic eels: A natural tool for disease control. *Mar. Ecol. Prog. Ser.* 471: 193–202. doi:10.3354/meps10032.
- Lefebvre, F., Fazio, G., Mounaix, B., and Crivelli, A.J. 2013. Is the continental life of the European eel *Anguilla anguilla* affected by the parasitic invader *Anguillicoloides crassus*? *Proc. R. Soc. B* 280: 20122916. doi:10.1098/rspb.2012.2916.

- Lefebvre, F., Fazio, G., Palstra, A.P., Székely, C., and Crivelli, A.J. 2011. An evaluation of indices of gross pathology associated with the nematode *Anguillicoloides crassus* in eels. *J. Fish Dis.* 34: 31–45. doi:10.1111/j.1365-2761.2010.01207.x.
- Machut, L.S., and Limburg, K.E. 2008. *Anguillicola crassus* infection in *Anguilla rostrata* from small tributaries of the Hudson River watershed, New York, USA. *Dis. Aquat. Organ.* 79: 37–45. doi:10.3354/dao01901.
- Michaud, M., Dutil, J.-D., and Dodson, J.J. 1988. Determination of the age of young American eels, in fresh water, based on otolith surface area and microstructure. *J. Fish Biol.* 32: 179–189. doi:10.1111/j.1095-8649.1988.tb05351.x.
- Molnár, K. 1993. Effects of decreased oxygen content on eels (*Anguilla anguilla*) infected by *Anguillicola crassus* (Nematoda: Dracunculoidae). *Acta Vet. Hungarica* 43(3–4): 349–360.
- Molnár, K., Baska, F., Csaba, G., Glavits, R., and Szekely, C. 1993. Pathological and histopathological studies of the swimbladder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda : Dracunculoidea). *Dis. Aquat. Organ.* 15: 41–50. doi:10.3354/dao015041.
- Molnár, K., Szakolczai, J., and Vetési, F. 1995. Histological changes in the swimbladder wall of eels due to abnormal location of adult and second stage larvae of *Anguillicola crassus*. *Acta Vet. Hung.* 43(1): 125–137.
- Molnár, K., Székely, C., and Baska, F. 1991. Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bull. Eur. Assoc. Fish Pathol.* 11(6): 211–212.
- Molnár, K., Székely, C., and Perényi, M. 1994. Dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection in eels of Lake Balaton, Hungary. *Folia Parasitol. (Praha)*. 41: 193–202. doi:10.1017/CBO9781107415324.004.
- Moravec, F. 1996. Aquatic invertebrates (snails) as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea) and the role of paratenic hosts in the life cycle of this parasite. *Dis. Aquat. Org.* 27: 237–239. doi:10.3354/dao027237.
- Moravec, F., and Skoríková, B. 1998. Amphibians and larvae of aquatic insects as new paratenic hosts of *Anguillicola crassus* (Nematoda : Dracunculoidea), a swimbladder parasite of eels. 34(1996): 217–222. doi:10.3354/dao034217.
- Morrison, W.E., and Secor, D.H. 2003. Demographic attributes of yellow-phase American eels (*Anguilla rostrata*) in the Hudson River estuary. *Can. J. Fish. Aquat. Sci.* 60: 1487–1501. doi:10.1139/f03-129.
- Moser, M.L., Patrick, W.S., and Jr., J.U.C. 2001. Infection of American Eels, *Anguilla rostrata*, by an Introduced Nematode Parasite, *Anguillicola crassus*, in North Carolina. Available

from [http://www.asihcopeiaonline.org/doi/abs/10.1643/0045-8511\(2001\)001%5B0848:IOAEAR%5D2.0.CO%3B2](http://www.asihcopeiaonline.org/doi/abs/10.1643/0045-8511(2001)001%5B0848:IOAEAR%5D2.0.CO%3B2) [accessed 22 September 2014].

- Nagasawa, K., Kim, Y.-G., and Hirose, H. 1994. *Anguillicola crassus* and *A.globiceps* (Nematoda: Dracunculoidea) parasitic in the swimbladder of eels (*Anguilla japonica* and *A.anguilla*) in East Asia: A review. *Folia Parasitol. (Praha)*. 41: 127–137.
- Ooi, H.K., Wang, W.S., Chang, H.Y., Wu, C.H., Lin, C.C., and Hsieh, M.T. 1996. An epizootic of Anguillicolosis in cultured American eels in Taiwan. *J. Aquat. Anim. Health* 8(2): 163–166. doi:10.1577/1548-8667(1996)008<0163:AEOAIC>2.3.CO;2.
- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rockwell, L.S., Jones, K.M.M., and Cone, D.K. 2009. First Record of *Anguillicoloides crassus* (Nematoda) in American Eels (*Anguilla rostrata*) in Canadian Estuaries, Cape Breton, Nova Scotia. *J. Parasitol.* 95(2): 483–486. doi:10.1645/GE-1739.1.
- Searle, S.R., Speed, F.M., and Milliken, G.A. 1980. Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. *Am. Stat.* 34(4): 216–221. doi:10.1080/00031305.1980.10483031.
- Sokolowski, M.S., and Dove, A.D.M. 2006. Histopathological Examination of Wild American Eels Infected with *Anguillicola crassus*. *J. Aquat. Anim. Health* 18(4): 257–262. doi:10.1577/H06-009.1.
- Székely, C., Molnár, K., and Rácz, O.Z. 2005. Radiodiagnostic method for studying the dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection and pathological status of the swimbladder in Lake Balaton eels. *Dis. Aquat. Organ.* 64: 53–61. doi:10.3354/dao064053.
- Thomas, K., and Ollevier, F. 1992. Population biology of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Dis. Aquat. Organ.* 14: 163–170.
- Tuckey, T.D., and Fabrizio, M.C. 2013. Influence of Survey Design on Fish Assemblages : Implications from a Study in Chesapeake Bay Tributaries. *Trans. Am. Fish. Soc.* 142(4): 957–973. doi:10.1080/00028487.2013.788555.
- Van Banning, P. and Haenen, O.L.M. 1990. Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*. In *Pathology in marine science. Edited by F.O. Perkins and T.C. Cheng.* Academic Press, New York, NY. pp. 317–330.
- Wang, C.H., and Tzeng, W.N. 1998. Interpretation of geographic variation in size of American eel *Anguilla rostrata* elvers on the Atlantic coast of North America using their life history and otolith ageing. *Mar. Ecol. Prog. Ser.* 168: 35–43. doi:10.3354/meps168035.

- Würtz, J., and Taraschewski, H. 2000. Histopathological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. *Dis. Aquat. Organ.* 39: 121–134. doi:10.3354/dao039121.
- Würtz, J., Taraschewski, H., and Pelster, B. 1996. Changes in gas composition in the swimbladder of the European eel (*Anguilla anguilla*) infected with *Anguillicola crassus* (Nematoda). *Parasitology* 112: 233–238. doi:10.1017/S003118200008481X.
- Zeileis, A., Kleiber, C., and Jackman S. 2008. Regression Models for Count Data in R. *Journal of Statistical Software.* 27:8.
- Zuur, A.F., Saveliev, A.A., and Ieno, E.N. 2012. *Zero Inflated Models and Generalized Linear Mixed Models.* Highland Statistics Limited.

TABLES

Table 1. Prevalence (%), average abundance (SE; range), and mean intensity (SE) of *Anguillicoloides crassus* in American eels by river system and eel stage.

Location	Stage	N	Prevalence (%)	Abundance	Intensity	SDI
Potomac	Glass	115	5.2	0.05 (0.02; 0-1)	1 (0)	0.02 (0.01)
	Elver	265	59.6	1.26 (0.09; 0-10)	2.11 (0.11)	1.81 (0.1)
	Yellow	29	55.2	1.21 (0.24; 0-4)	2.19 (0.23)	1.97 (0.38)
	Total	409	44	0.91 (0.07; 0-10)	2.08 (0.1)	1.28 (0.08)
Rappahannock	Glass	248	2.4	0.02 (0.01; 0-1)	1 (0)	0.01 (0.01)
	Elver	105	53.3	1.13 (0.15; 0-9)	2.13 (0.2)	1.1 (0.17)
	Yellow	378	49.7	1.53 (0.13; 0-21)	3.09 (0.22)	1.03 (0.15)
	Total	731	34.2	0.96 (0.08; 0-21)	2.82 (0.17)	1.43 (0.06)
York	Glass	774	0	0 (0)	0 (0)	0.002 (0.002)
	Elver	213	64.8	1.72 (0.13; 0-10)	2.66 (0.15)	1.52 (0.1)
	Yellow	153	45.1	1.23 (0.23; 0-28)	2.77 (0.45)	2.85 (0.15)
	Total	1110	18.6	0.5 (0.05; 0-28)	2.7 (0.18)	0.67 (0.04)
James	Glass	371	0	0 (0)	0 (0)	0.01 (0.003)
	Elver	195	66.7	1.83 (0.13; 0-7)	2.73 (0.13)	1.82 (0.11)
	Yellow	412	43	1.24 (0.11; 0-18)	2.88 (0.19)	2.3 (0.08)
	Total	607	50.6	1.43 (0.08; 0-18)	2.82 (0.12)	1.32 (0.05)

Table 2. Assessment of force of infection model fits for presence of swimbladder damage ($SDI \geq 2$) in American eels based on Akaike's information criterion.

Infection Hazard	ΔAIC					
	Null	μ	Season	Season, μ	System	System, μ
constant	40.6	7	35.1	11	40.3	6.5
Weibull	13.8	9	18.6	12.8	12	8.4
Pareto	9.2	7.5	13.9	11.8	8.5	7.9
Gompertz	5.3	7	10.4	11.6	5.5	7.5
log-logistic	11.2	3	15.7	6.4	10.4	5.5

Infection Hazard	ΔAIC					
	Season, System	Season, System, μ	month pairs	month pairs, μ	month pairs, system	month pairs, system, μ
constant	35.1	10.8	27.8	3.4	31.8	6.3
Weibull	17	12.5	10.2	5.3	11.9	8.2
Pareto	13.4	12.4	6.1	4.7	8.5	7.9
Gompertz	12.5	10.7	3.1	4.7	5.9	7.9
log-logistic	15.2	9.4	6.3	0	9.3	4.8

FIGURES

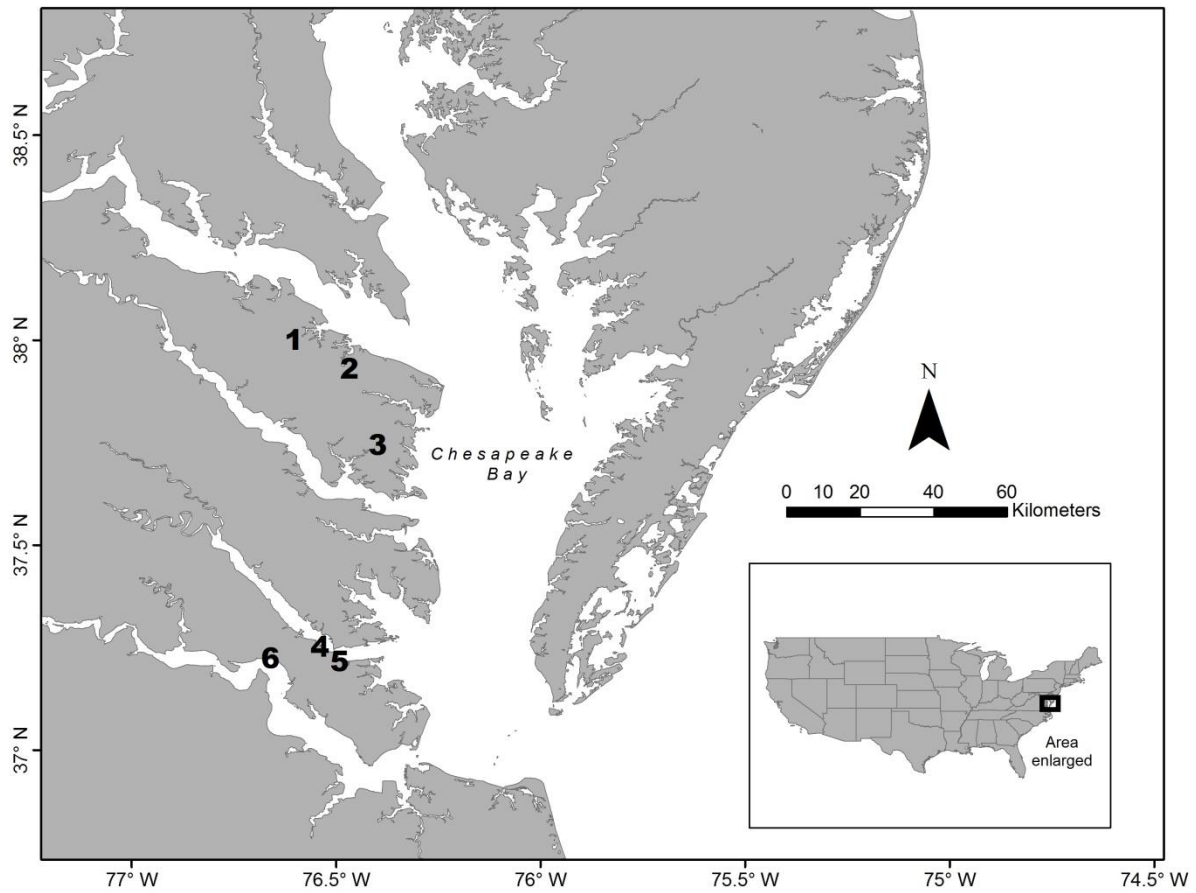


Figure 1. Map of collection sites for glass and elver American eels from lower Chesapeake Bay, USA. (1) Gardy's Millpond, (2) Clark's Millpond, (3) Kamp's Millpond, (4) Bracken's Pond, (5) Wormley Pond, (6) Wareham's Pond.

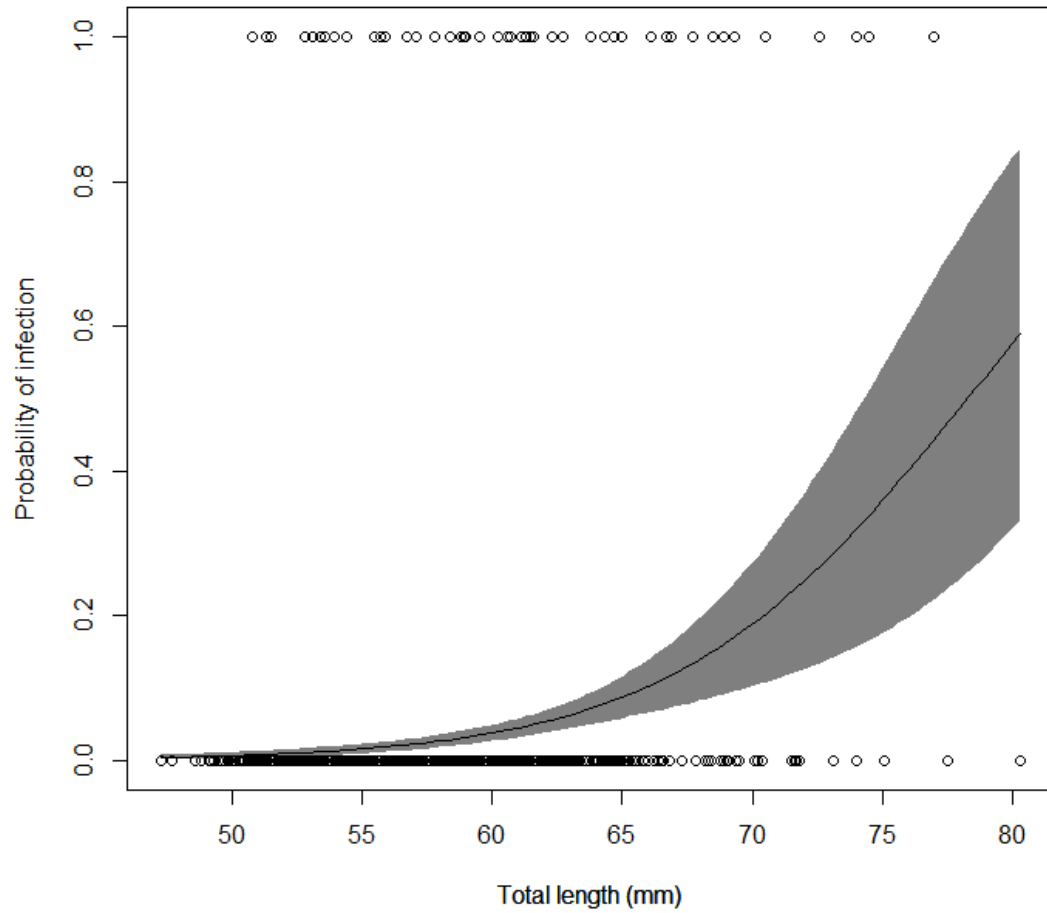


Figure 2. Probability of infection with larval and adult *Anguillicoloides crassus* by total length (mm) for glass eels. Black line represents binomial model results with 95% CI (grey shaded area). Open circles are individual eel observations.

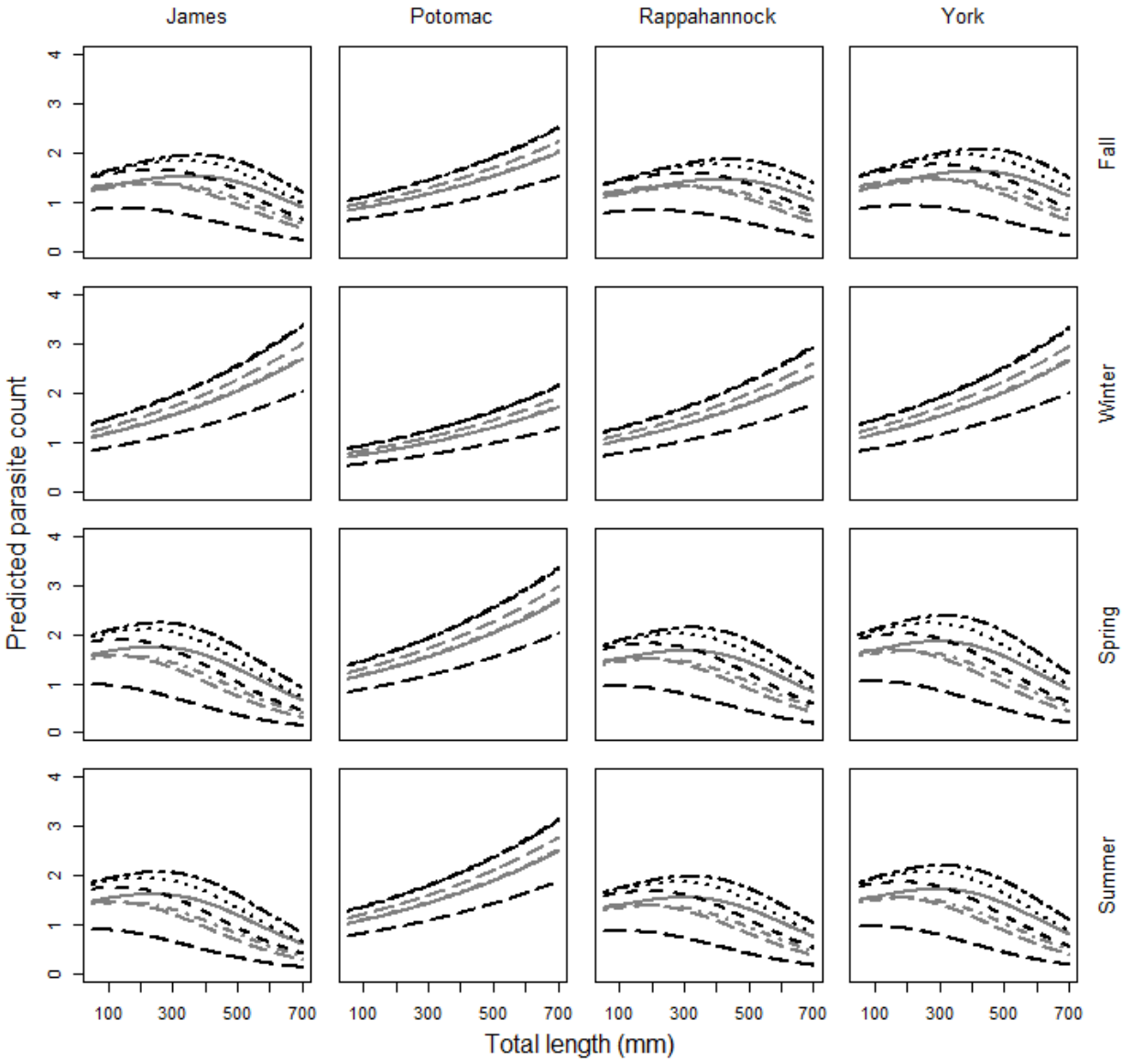


Figure 3. Predicted *Anguillicoloides crassus* parasite count for elver and yellow American eels for season of capture, system, total length (mm), and swimbladder degenerative index (SDI) total score. Results are from a zero-inflated negative binomial model. Individual lines represent SDI scores: 0 = ————, 1 = - - - - - , 2 = - - - - - , 3 = - . - . - , 4 = , 5 = - - - - - , 6 = - . - . - .

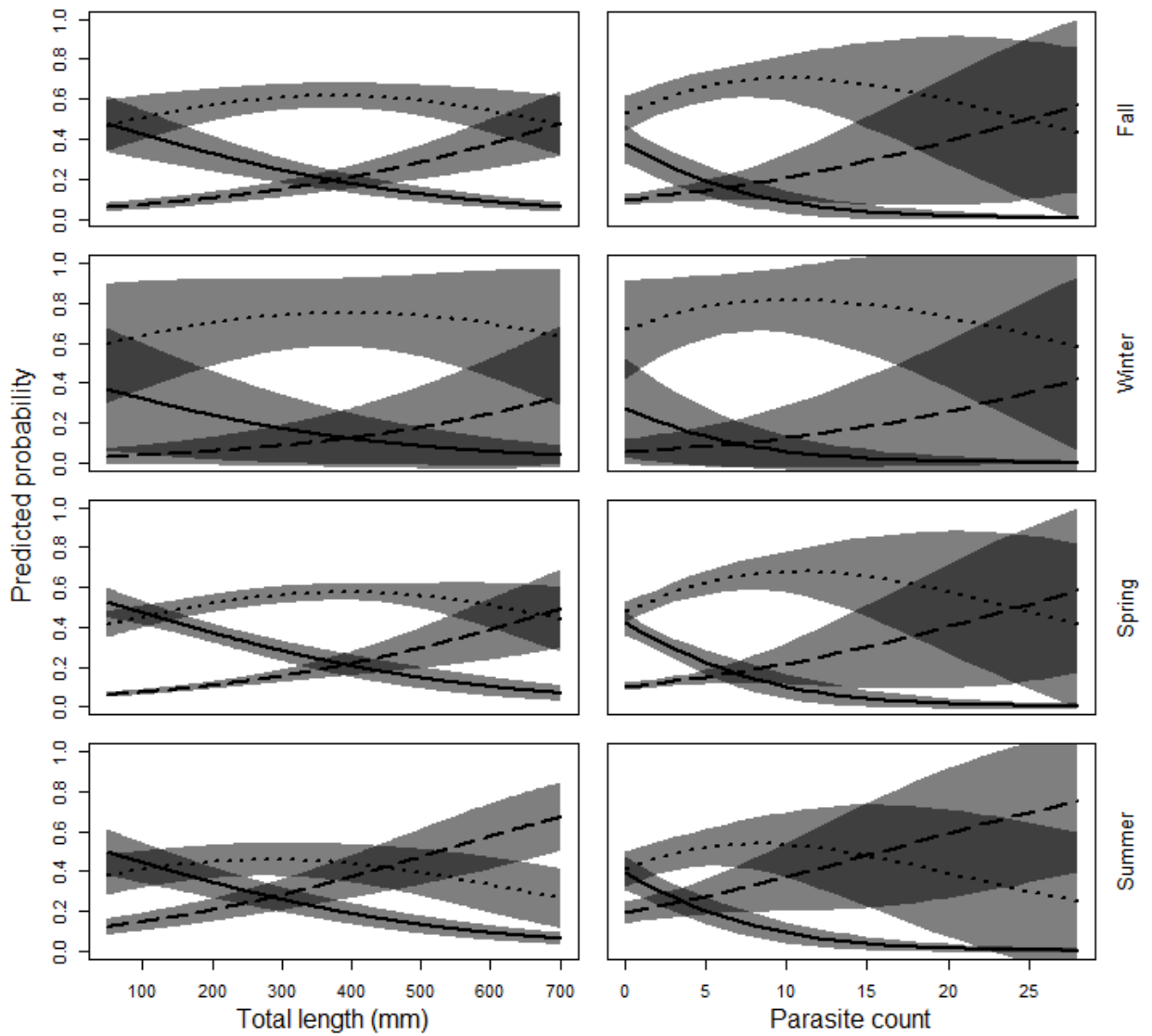


Figure 4. Predicted probability of elver and yellow eels being in a swimbladder condition category (low = SDI 0-1, — ; moderate = SDI 2-3, ; severe = SDI 4-6, - - -) by season of capture, total length (left panel), and *A. crassus* parasite count (right panel). Total length is held constant at its mean in right panel and parasite count is held constant at its mean in the left panel. Results are from the ordinal logistic regression with catch ID as a random effect.

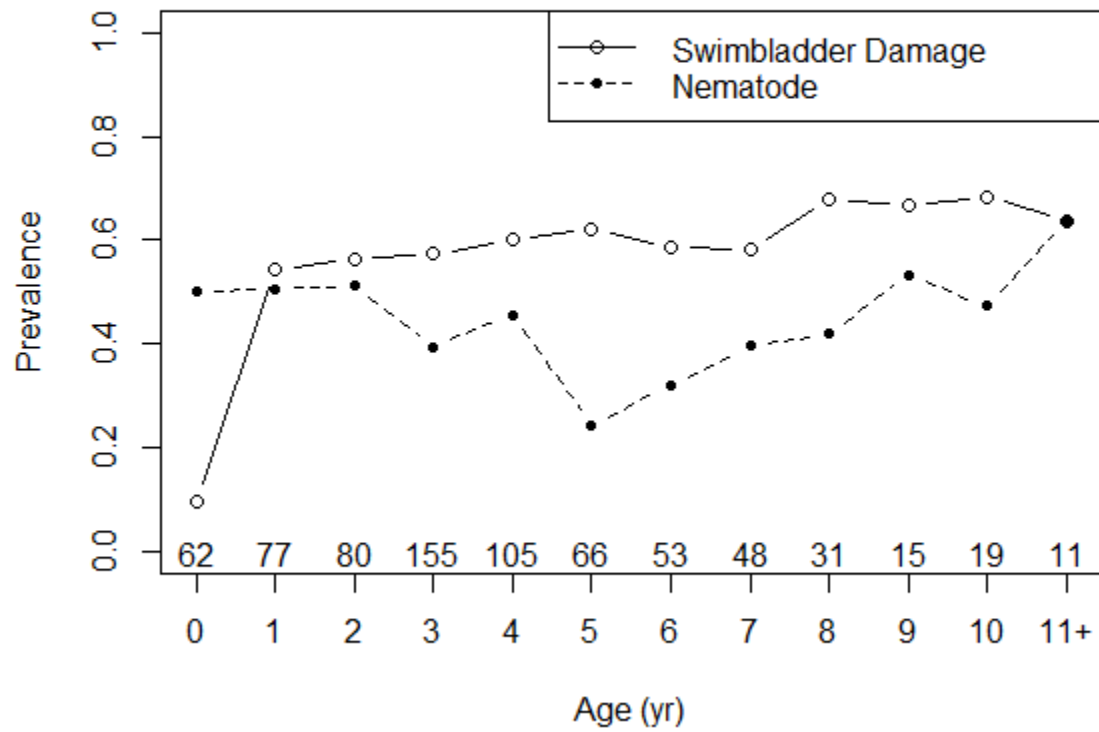


Figure 5. Prevalence of swimbladder damage ($SDI \geq 3$; open circles with solid line) and *A. crassus* (closed circle with dashed line) presence by age of elver and yellow American eels. Numbers above x-axis indicate sample size in each age group.

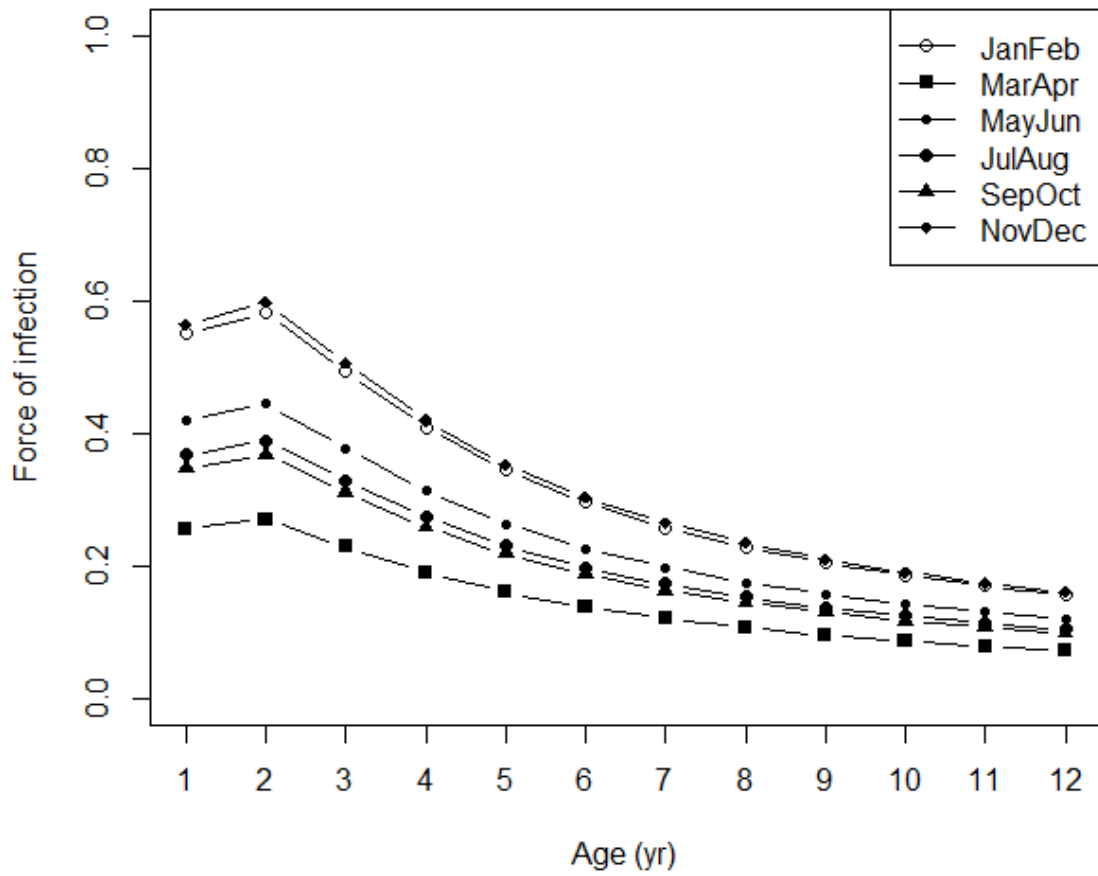


Figure 6. Force-of-infection of swimbladder damage by age for elver and yellow American eels by month pairs from best fitting force-of-infection model (log-logistic, month pair, mortality).

SUPPLEMENTAL MATERIAL

TABLES

Suppl. Table 1. Model parameterizations used to test the effect of covariates on count of *Anguillicoloides crassus* in American eels. Zero-inflated distributions have two parts binomial and count denoted by “|”. df = degrees of freedom.

Model Number and Distribution	Covariates	df	2log(L)	AIC	ΔAIC
1 (Poisson)	River system, season, TL, SDI	14	-6539.0	6567.0	1180.2
2 (Negative Binomial)	River system, season, TL, SDI	15	-5411.4	5441.4	54.6
3 (Zero-inflated Poisson)	River system, season, TL, SDI River system, season, TL, SDI	28	-5619.3	5675.3	288.5
4 (Zero-inflated negative binomial)	River system, season, TL, SDI River system, season, TL, SDI	29	-5328	5386.8	0
5 (Zero-inflated negative binomial)	River system, season, TL, SDI River system, season, TL	23	-5342	5388.6	1.8
6 (Zero-inflated negative binomial)	River system, season, TL River system, season, TL, SDI	23	-5350	5396.9	10.1
7 (Zero-inflated negative binomial)	River system, season, TL River system, season, TL	17	-5604	5637.5	250.7

Suppl. Table 2. Model components and estimates from the most empirically supported model describing the effects of covariates on count of *Anguillicoloides crassus* in American eels. Bolded p-values indicate significance at the $\alpha < 0.05$ level.

Model Component	Parameter	Level	Estimate \pm Standard Error	p-value
Zero-inflated	River system	James	Baseline	N/A
		Potomac	-16.8 \pm 0.002	0.994
		Rappahannock	-0.423 \pm 0.295	0.147
		York	-0.357 \pm 0.288	0.216
	Season	Fall	Baseline	N/A
		Winter	-12.5 \pm 225	0.996
		Spring	0.724 \pm 0.358	0.043
		Summer	0.746 \pm 0.375	0.046
	Total length		0.0064 \pm 0.001	<0.001
	SDI	0	Baseline	N/A
		1	-1.36 \pm 0.470	0.004
		2	-0.656 \pm 0.620	0.040
		3	-0.758 \pm 0.359	0.035
		4	-1.19 \pm 0.503	0.017
		5	-0.402 \pm 0.464	0.386
		6	-1.48 \pm 1.11	0.182
	Count (negative binomial)	River system	James	Baseline
Potomac			-0.452 \pm 0.112	<0.001
Rappahannock			-0.135 \pm 0.103	0.188
York			-0.015 \pm 0.103	0.884
Season		Fall	Baseline	N/A
		Winter	-0.149 \pm 0.316	0.639
		Spring	0.294 \pm 0.130	0.024
		Summer	0.224 \pm 0.138	0.106
Total length			0.0014 \pm 4 x 10 ⁻⁴	0.002
SDI		0	Baseline	N/A
		1	0.279 \pm 0.124	0.025
		2	0.512 \pm 0.116	<0.001
		3	0.295 \pm 0.131	0.024
		4	0.502 \pm 0.156	0.001
		5	0.387 \pm 0.194	0.046
		6	0.500 \pm 0.238	0.036
Log(theta)			0.334 \pm 0.139	0.016

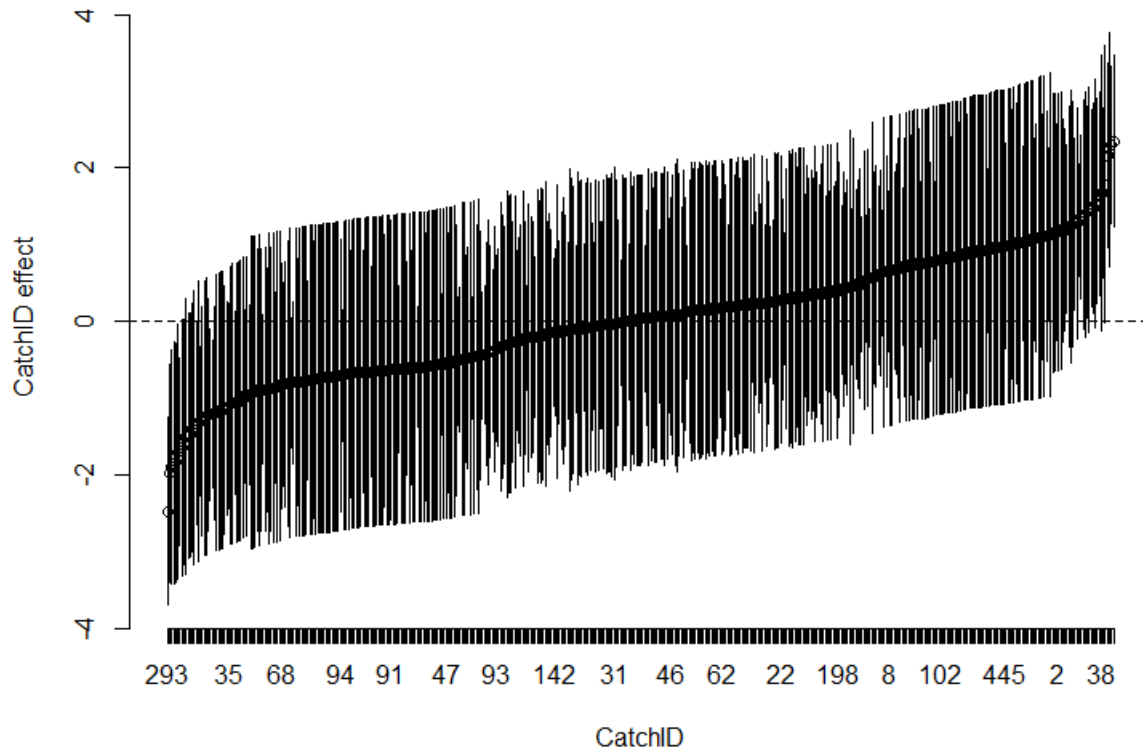
Suppl. Table 3. Model parameterizations used to test the effect of covariates on swimbladder damage from *Anguillicoloides crassus* in American eels. Partial proportional odds covariates follow “|” except for catchID which is a random factor. df = degrees of freedom.

Model Number and Distribution	Covariates	df	2log(L)	AIC	ΔAIC
1 (proportional odds)	River system, season, TL, parasite count	10	-3351	3371.4	147
2 (proportional odds)	River system, season, TL	9	-3379.4	3397.4	173
3 (partial proportional odds)	TL, river system season, parasite count	14	-3327.2	3355.3	130.9
4 (partial proportional odds)	TL season, parasite count	11	-3331.1	3353.1	128.7
5 (proportional odds, random effect)	River system, season, TL, parasite count, catchID	11	-3219.7	3241.7	17.3
6 (proportional odds, random effect)	Season, TL, parasite count, catchID	8	-3221.5	3237.5	13.1
7 (proportional odds, random effect)	River system, season, TL, catchID	10	-3249.8	3269.8	45.4
8 (partial proportional odds, random effect)	River system, TL season, parasite count, catchID	15	-3198.8	3228.8	4.4
9 (partial proportional odds, random effect)	TL season, parasite count, catchID	12	-3200.4	3224.4	0

Suppl. Table 4. Model components and estimates from the most empirically supported model describing the effects of covariates on swimbladder damage from *Anguillicoloides crassus* in American eels. Random effect estimate is the variance \pm standard deviation. Bolded p-values indicate significance at the $\alpha < 0.05$ level.

Parameter type	Parameter	Level	Estimate \pm SE	p-value
Proportional odds	Total Length		0.0041 \pm 0.001	<0.0001
Partial proportional odds	Season	0 1 fall	Baseline	
		1 2 fall	Baseline	
		0 1 winter	-0.436 \pm 0.908	0.316
		1 2 winter	0.603 \pm 0.969	0.267
		0 1 spring	0.200 \pm 0.251	0.212
		1 2 spring	-0.059 \pm 0.266	0.412
		0 1 summer	0.084 \pm 0.281	0.299
		1 2 summer	-0.816 \pm 0.293	0.003
			Parasite count	0 1
		1 2	-0.090 \pm 0.031	0.002
Random effect	Catch ID		1.423 \pm 1.194	

FIGURES



Suppl. Figure 1. Effect of catch cohort (Catch ID) as a random effect given by conditional modes with 95% confidence intervals based on the conditional variance from the ordinal logistic regression modeling the probability of being in a swimbladder condition category. Effect is ordered by degree of effect. Confidence intervals of individual observations that intersect with 0 have no overall effect.

Chapter 2

Progression of swimbladder damage by *Anguillicoloides crassus* in yellow stage American eels
(*Anguilla rostrata*)

ABSTRACT

The invasive parasitic nematode *Anguillicoloides crassus* can cause severe damage to the swimbladders of American eels (*Anguilla rostrata*), but the progression of this damage and the potential for recovery is unknown. To further study the disease progression by *A. crassus* in American eels, we used x-ray imaging to repeatedly monitor the length and area of 200 wild-caught and wild-infected eels over six months, under conditions where reinfection was not possible. A subset of eels was sampled monthly for dissection to compare with x-ray images and all remaining eels were sampled at the end of the study. Eel swimbladders were dissected, measured, and swimbladder condition was assessed using the Swimbladder Degenerative Index (SDI). Swimbladder length and eel total length was used to calculate the Length Ratio Index (LRI). Results showed that the LRI and swimbladder area increased over the course of the experiment, when calculated by x-ray or dissection. LRI, SDI, and area were all in agreement regarding infection status, but nematode count did not correlate with these metrics. Our study suggests that American eels may have the potential to heal damage caused by *A. crassus* and that LRI and x-ray imaging are useful, non-lethal tools to study *A. crassus* infection.

INTRODUCTION

Anguillicoloides crassus is a parasitic nematode of Anguillid eel swimbladders that endemically infects the Japanese eel (*Anguilla japonica*) in Asia, but does not cause significant harm or notable negative population level impacts (Sokolowski and Dove 2006). In contrast, *A. crassus* has been linked to declines in European eel populations (*Anguilla Anguilla*) and in Asian aquaculture facilities housing American eels (*Anguilla rostrata*; Barse et al. 2001; Ooi et al. 1996). Within the American eel population, the parasite was first discovered in 1995 in a Texas aquaculture facility and was first noted in the wild in South Carolina that same year (Fries et al. 1996). Since its discovery, the distribution of *A. crassus* has expanded rapidly and can now be found in eels in the Gulf of Mexico northward to Nova Scotia (Rockwell et al. 2009).

Eels become infected by eating intermediate hosts such as copepods and ostracods (Hirose et al. 1976; De Charleroy et al. 1990; Moravec et al. 1994) or paratenic hosts such as fish, amphibians, snails, and insect larvae containing the larval stage of *A. crassus* (Thomas and Ollevier 1992; Moravec and Konecny 1994; Moravec 1996; Moravec and Skorikova 1998). Once inside eels, the parasite moves from the gut into the swimbladder, where it matures (Haenen et al. 1989; De Charleroy et al. 1990). Damage occurs as a result of larval migration through the swimbladder wall, feeding on swimbladder blood by adults, and decay of dead adults within the lumen (Sokolowski and Dove 2006). Damage manifests as increased opacity, thickening, and pigmentation of the swimbladder wall (Lefevbre et al. 2011). Complete degradation and loss of function of the swimbladder can result from infection by *A. crassus* and laboratory experiments have shown that the damage can affect swimming, ability to hold gases, and use of the swimbladder as a hydrostatic organ (Molnar et al. 1995; Wurtz et al. 1996; Kobayashi et al. 1990).

The progression of damage by *A. crassus* is relatively unknown due to most observations being made at a single point in time through dissections after an eel has been euthanized. How long swimbladder damage takes to accrue and if it can be reversed is unknown, yet this information is very useful in determining the infection dynamics of *A. crassus* as well as the long-term disease impacts on eels. Previous studies have shown seasonal variation in disease levels, and this variation could be partially due availability of the parasite or from mortality of the nematode or eel host, (Knopf et al. 1998, Székely et al. 2009). Variation in swimbladder condition could also be due to healing of the organ particularly during the winter when eels cease feeding and are not exposed to *A. crassus* (Kennedy and Fitch 1990).

The ability for American eels to recover from infection has been speculated but not definitively shown (Lefebvre et al. 2012, Beregi et al. 1998). Beregi et al. (1998) suggested that infected swimbladders that have a dilated middle and tapered ends is indicative of recovery and regeneration, but this description was made without repeatedly monitoring swimbladders through time. An innovative way to understand the degenerative and recovery process has been used on European eels (*Anguilla Anguilla*)—analyzing the radiographic images produced by an x-ray machine (Beregi et al. 1998; Szekely et al. 2004; Szekely et al. 2005; Palastra et al. 2007; Frisch et al. 2015). This technique is possible due to the radiotransparency of the gas filled swimbladder, allowing the organ to appear as a dark area on the radiograph in contrast to the white of the other internal structures. X-ray imagery allows for the calculation of the Length Ratio Index (LRI; Palstra et al. 2007, Lefebvre et al. 2011), which was introduced as a quantitative metric for swimbladder damage to be utilized in conjunction with non-lethal observation techniques, but its utility has not been well tested on either European or American eels.

When x-ray studies have been conducted on European eels, they have been relatively short in duration and used qualitative rather than quantitative metrics. As a result, they are able to accurately quantify the full extent of swimbladder degradation or recovery. Therefore the objectives of this study were to use x-ray imaging to (1) quantitatively determine how swimbladder condition of yellow American eels changes over time when no reinfections are possible, (2) determine if swimbladder recovery from infection can occur, and (3) compare swimbladder damage as seen on x-ray images to swimbladder damage determined through dissection.

METHODS

Experimental setup

Two-hundred and seventy yellow American eels were obtained from a commercial eel distributor (Chesapeake Star Seafood in White Stone, VA, USA). All eels were caught by commercial eelers in lower Chesapeake Bay tributaries during late summer 2016. Eels were transported on ice from the distributor to a freshwater recirculating system located at the Virginia Institute of Marine Science Seawater Research Lab. Eels were initially treated using a formalin bath for 30 minutes per day for two days to remove common ectoparasites. After the treatment, eels were divided evenly among four circular 100 gallon tanks. Water temperature was maintained between 19 and 20°C for the duration of the experiment. Eels were fed a maintenance diet of African night crawlers (*Eudrilus eugeniae*) 2-3 times per week. Due to the use of pathogen free UV irradiated fresh well water, the indirect transmission of the parasite, and land

based food source, reinfection of the eels with *A. crassus* from intermediate or paratenic hosts was unlikely, if not impossible.

Due to the high prevalence reported from the Chesapeake Bay (Fenske et al. 2010, Barse et al. 2001), the eels were assumed to be already infected with *A. crassus*. To check this assumption and determine initial infection levels, 49 of the eels were sacrificed a week after arrival using MS-222, and then were subsequently x-rayed and frozen until dissection. The remaining 220 eels were acclimatized for two more weeks in the system and then tagged with 8.4 mm passive integrated transponder (PIT) tags (Biomark[®] MiniHPT8) in their dorsal musculature at the anterior of the dorsal fin to individually track eels throughout the experiment. Two days after tagging, eels were x-rayed using the methods of Beregi et al. (1998, 2001) and Szekely et al. (2004). Eels were anesthetized in an ice bath and then placed on the x-ray table and a 10 second exposure digital x-ray was taken. A recovery tank was used to bring the internal temperature of the eels slowly back to the system temperature. Subsequent x-rays occurred every four weeks for a total of six x-ray examinations. Two days after each x-ray, eels were weighed (\pm 0.1 g) and measured (\pm 1 mm). An ice bath was again used for anesthesia and a recovery tank was used to re-acclimate the eels to the system temperature. After each weighing and measuring, 10 randomly chosen eels (n=40 total for experiment) were sacrificed with MS-222 and frozen until dissection to compare x-ray results with dissection measurements. Any eels that died during the experiment were also frozen until dissection (n=64).

At 16 weeks (after month 4 and before month 5 sampling), eels were treated with an antibiotic (Baytril[®], active ingredient: enrofloxacin) for an infection causing skin ulceration and mortality. Preliminary diagnostics through veterinarian consultation, plating on thiosulfate-citrate-bile salts-sucrose (TCBS) agar and trypticase soy agar (TSA), and 16S sequencing,

indicated that cause of disease was likely bacterial, and potentially a *Vibrio* species, although the diagnostic results were not definitive. As such, the general antibiotic Batryil was chosen for treatment. The treatment consisted of an eight hour bath for five days with an antibiotic concentration of 5 mg/L. The mortality rate rapidly decreased to zero after treatment. At the conclusion of the experiment, all remaining eels were euthanized using MS-222 and frozen until dissection.

X-ray image analysis and dissections

X-ray images were stored digitally and analyzed using ImageJ to determine the length (± 0.1 mm) and area (± 0.1 mm²) of the radiographic shadow of the swimbladder. The length ratio index (LRI; Palstra et al. 2007) was calculated by dividing the swimbladder length by eel total length. This index utilizes the observation of a decrease in length of the swimbladder with increasing damage due to infection, with a smaller index indicative of a more damaged swimbladder (Palstra et al. 2007).

All eels were dissected and their swimbladders were removed for examination. The length of the dissected swimbladder was measured using a ruler to the nearest millimeter and the condition was recorded using the Swimbladder Degenerative Index (SDI) created by Lefevbre et al (2002). This index consists of three categories—opacity, thickness, and pigmentation/exudate and each of these categories receives a ranking from 0 (healthy, normal condition) to 2 (severe condition). The three categories are added together to generate a final SDI score ranging from 0 to 6. *A. crassus* adults in the swimbladder lumen were also enumerated at the time of dissection by opening the swimbladder and counting the number of nematodes macroscopically.

Statistical analyses

The change in LRI over the course of the experiment (month as categorical factor) was analyzed using a beta regression approach with repeated measures and an AR1 correlation structure with the GLIMMIX (generalized linear mixed models) procedure in SAS (Statistical Analysis System [SAS] version 9.3; SAS Institute, Cary, North Carolina). To determine if the eels grew throughout the experiment, a Welch two sample t-test was used to compare initial length of eels to their final length. The differences in swimbladder area among months was analyzed using a linear mixed effects model with repeated measures and an AR1 correlation structure (function `lme()` from the ‘nlme’ package in R, Pinheiro et al. 2017) with month as a categorical factor and PIT tag ID (i.e. individual eel ID) as a random effect. A generalized linear model with a beta regression (function `betareg()` from the ‘betareg’ package in R, Cribari-Neto and Zeileis 2010) was used to investigate how SDI (categorical), swimbladder length measurement method (i.e. x-ray image or dissection), and their interaction related to final (i.e. at time of death) LRI. A linear regression model (function `lm()` from the ‘stats’ package in base R, R Core Team 2017) was used to determine how SDI (categorical) related to final swimbladder area. A multivariate analysis of variance (MANOVA, function `manova()` from the ‘stats’ package in base R, R Core Team 2017) was used to determine how SDI and infection intensity varied by month. Maximal models were used without model simplification. All results are presented with standard errors (SE) unless otherwise noted.

RESULTS

Initial infection status of eels

The 49 eels initially sacrificed at the start of the study ranged in length from 293 to 638 mm (mean=435 mm) and weighed between 40.2 and 540 g (mean=157.4 g). The overall prevalence of adult *A. crassus* was 24.2% with a mean abundance of 0.632 ± 0.229 nematodes per eel and a mean intensity of 2.58 ± 0.69 nematodes per infected eel. The average SDI score was 1.53, the average LRI was 50.9 ± 2.74 , and the average swimbladder area was 335.5 ± 31.66 mm², calculated by dissection

Swimbladder progression through time as measured by x-ray

One hundred and thirteen eels survived to the end of the experiment. Causes of mortality (excluding the 40 purposefully sacrificed for dissection comparison throughout the experiment) included escapement out of the system (n=13) and bacterial infection (n=51). Eels that survived until at least the second month were used in the analysis.

The LRI of eels varied significantly over the course of the experiment, with an increasing trend of the LRI (Figure 1). LRIs recorded in months one through three were significantly smaller than month six ($p < 0.0001$) whereas there was no significant difference between months four and five compared to month six ($p > 0.1$, Table 1). The length of eels did not significantly change over the six months of the experiment ($F = 0.226$, $df = 954$, $p > 0.1$, Figure 2), indicating that the change in the LRI resulted from a changing swimbladder length rather than eel length.

Swimbladder area also varied significantly over the course of the experiment, with an increasing trend (Figure 3). Swimbladder areas for months 4 to 6 were significantly larger

($p < 0.0001$) than the swimbladder area recorded in month 1 ($p < 0.0001$), but there was no significant difference between month 1 and months 2 and 3 ($p > 0.1$, Table 2).

Association between swimbladder damage metrics

The final (i.e. at time of death) LRI using swimbladder length measured from x-ray images and the LRI using dissected swimbladder length were not significantly different from each other ($p > 0.1$, Table 3). Among SDI scores, LRI was significantly smaller for SDI scores 2 through 5 compared to the baseline of 0 ($p < 0.05$) with LRI decreasing with an increased SDI score, illustrating the agreement between the two metrics for determining swimbladder condition (Figure 4). Additionally, there were no significant differences for any interaction terms ($p > 0.1$), indicating similar relationships with SDI for LRI measurement by X-ray or dissection. The final swimbladder areas as measured from x-ray images showed a similar trend to LRI such that swimbladders with a SDI score of 5 and 6 combined had significantly smaller areas than swimbladders that had a score of 0 ($p < 0.01$, Table 4, Figure 5), illustrating agreement between the different methods for determining swimbladder condition.

Monthly variation in SDI and nematode count

SDI score varied significantly by month ($F = 3.17$, $p < 0.05$) but nematode count did not ($F = 2.2$, $p = 0.06$). The only significant difference in SDI scores among months was between months three and six ($p < 0.05$). The highest SDI was found in month 3 (2.08 ± 0.271) and the lowest was in month 6 (1.21 ± 0.109 , Figure 6).

DISCUSSION

Swimbladder length and area increased over the course of the experiment, suggesting that the swimbladders of American eels may be able to heal from *A. crassus*-induced damage. The decreasing trends of the LRI and SDI and the increasing trend of swimbladder area all show agreement of improved condition of swimbladders over the course of the experiment, illustrating that eels may have the ability to recover from damage due to *A. crassus* infection. A similar study done by Székely et al. (2005) found that, after three months, the majority of eels either were in a worse or similar condition and only 1% of the eels showed improvement. A major difference between this study and ours was their use of qualitative definitions of swimbladder condition as opposed to the quantitative LRI used in our study. A more quantitative approach allowed us to conduct more robust statistical analyses and reduced the potential for subjectivity when using a qualitative rubric. Additionally, the shorter experimental time of Székely et al. (2005) may not have allowed sufficient time for swimbladders to recover.

The metrics of infection studied—LRI, SDI, and swimbladder area—all were in agreement when indicating disease level. The lower SDI scores correlated with a higher LRI, regardless of how LRI was measured, showing that the degradation of the swimbladder is inversely correlated to LRI. Previous work suggests that as the swimbladder becomes more infected and damaged, the walls thicken and the swimbladder shrinks lengthwise, leading to a smaller LRI (Lefebvre et al. 2011, Palstra et al. 2007). Swimbladder area as seen on an x-ray image, though not as widely studied, could also indicate degree of damage by showing differing sizes of the lumen which holds essential gasses for functioning of the swimbladder. Swimbladder area was also related to SDI, given that eels with higher SDI scores had smaller swimbladder areas, further showing the damage from infection by *A. crassus*.

Infection levels can also be measured by presence and intensity of the number of *A. crassus* nematodes within the swimbladder. American eels used in our study had lower prevalence and intensity of *A. crassus* compared to previous studies (Hein et al. 2015, Fenske et al. 2010, Morrison and Secor 2003). Additionally, lower average SDI in our eels compared to previous studies was also noted. Possible reasons for these differences could be due to annual, seasonal, or spatial variation in infection levels (Fenske et al. 2010, Moser et al. 2001). Using eels with lower initial infection levels could have affected our ability to discern differences in monthly variation among eels because the swimbladders were already in reasonable condition and therefore did not have as much damage to recover from. Yet differences among months were detected, meaning that we may have been under-estimating recovery potential, and more substantial recovery could have been seen if the eels had higher initial infection levels and also if the study had been run for a longer time period. More research is needed on the dynamics of higher infection levels, specifically severe infections, to determine if there is a point of no return where the swimbladder becomes so damaged that the eel is not able to recover.

The unidentified bacterial infection that occurred early in the experiment may have altered the ability of the eels to devote energy and resources to fighting their *A. crassus* infection or, alternatively, infection with *A. crassus* could have prevented the eels from combating the bacterial infection (Sures et al. 2001, Muñoz et al. 2015). Despite this difficulty, the signal of recovery observed in this study, beginning in month 4, occurred before treatment began, indicating that any changes in these metrics were not the result of the treatment, and that eels had enough resources to at least partially combat infection. Furthermore, the antibiotic given specifically targets bacteria and is believed to have no effect on parasitic nematodes such as *A.*

crassus (van der Hoeven et al. 2008), which can be effectively treated but with different medications (Geets et al. 1992).

Eel length did not change throughout the course of the experiment likely due to inadequate feeding or other stressors. Previous experimental studies did not feed eels for extended periods of time or fed a maintenance diet and did not reported any deleterious effects (Székely et al. 2005, Kirk et al. 2002, Gillis 1998, Geets et al. 1992). Additionally, during the winter, eels are believed to largely cease feeding (Kennedy and Fitch 1990) indicating that eels can survive on limited food resources for extended periods of time. A better experimental diet may have allowed eels to devote more energy and resources to fighting *A. crassus* infection; yet an increase in swimbladder length and area was still observed, indicating that more recovery could have been observed if a higher calorie diet was utilized.

This study was the first to utilize the LRI in conjunction with a non-lethal monitoring technique to study the progression of infection from *A. crassus* in American eels. Despite challenges such as low levels of initial infection, bacterial infection, and inadequate feeding, our data suggest that partial recovery was occurring. This trend was also observed for SDI data collected during dissection, illustrating an agreement of metrics regarding the ability for swimbladders to heal from *A. crassus* infection. It should be noted that extent of recovery observed in this study was relatively small and the biological significance is currently unknown. Furthermore, whether eels have the ability to fully recover from infection and are able to do so in the wild is still unknown and requires further research. Additionally, the necessity of the swimbladder during the continental phase of the eel should be further studied, given that eels live in relatively shallow water and have a small home range (Morrison and Secor 2003) and therefore may not require the a swimbladder while in shallow estuarine or fresh water systems.

Yet when eels migrate back to the Sargasso Sea, they undergo diel vertical migration (Aarestrup et al. 2009) and would rely on a functioning swimbladder. If eels are able to recover from infection, even partially, as suggested by our study, then during their long migration when no reinfection is possible, infected eels may have a better chance of reaching their spawning grounds. Whether this recovery can occur during migration while many more stressors and energetic costs, such as constant swimming and reproductive development occur requires a great deal more research.

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REFERENCES

- Aarestrup, K., Økland, F., Hansen, M.M., Righton, D., Gargan, P., Castonguay, M., Bernatchez, L., Howey, P., Sparholt, H., Pedersen, M.I., and McKinley, R.S. 2009. Oceanic spawning migration of the european eel (*Anguilla anguilla*). *Science* 325: 1660. doi:10.1126/science.1178120.
- Barse, A.M., Mcguire, S.A., Vinoses, M.A., Eierman, L.E., and Weeder, J.A. 2001. The Swimbladder Nematode *Anguillicola crassus* in American Eels (*Anguilla rostrata*) from Middle and Upper Regions of Chesapeake Bay. *J. Parasitol.* 87(6): 1366–1370. doi:10.1645/0022-3395(2001)087[1366:TSNACI]2.0.CO;2.
- Beregi, A., Molnár, K., Békési, L., and Székely, C. 1998. Radiodiagnostic method for studying swimbladder inflammation caused by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Dis. Aquat. Organ.* 34: 155–160. doi:10.3354/dao034155.
- Beregi, A., Szekely, C., Bekesi, L., Szabo, J., Molnar, V., and Molnar, K. 2001. Radiodiagnostic examination of the swimbladder of some fish species. *Acta Vet. Hung.* 49(1): 87–98. doi:10.1556/004.49.2001.1.11.
- De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C., and Ollevier, F. 1990. The life cycle of *Anguillicola crassus*. *Dis. Aquat. Organ.* 8: 77–84. doi:10.3354/dao008077.
- Fenske, K.H., Secor, D.H., and Wilberg, M.J. 2010. Demographics and Parasitism of American Eels in the Chesapeake Bay, USA. *Trans. Am. Fish. Soc.* 139(6): 1699–1710. doi:10.1577/T09-206.1.
- Francisco Cribari-Neto, Achim Zeileis (2010). Beta Regression in R. *Journal of Statistical Software* 34(2), 1-24. URL <http://www.jstatsoft.org/v34/i02/>.
- Fries, L.T., Williams, D.J., and Johnson, S.K. 1996. Notes : Occurrence of *Anguillicola crassus*, an Exotic Parasitic Swim Bladder Nematode of Eels, in the Southeastern United States. *Trans. Am. Fish. Soc.* 125(5): 794–797. doi:10.1577/1548-8659(1996)125<0794:NOOCAE>2.3.CO;2.
- Frisch, K., Davie, A., Schwarz, T., and Turnbull, J.F. 2015. Comparative imaging of European eels (*Anguilla anguilla*) for the evaluation of swimbladder nematode (*Anguillicoloides crassus*) infestation. *J. Fish Dis.* doi:10.1111/jfd.12383.
- Geets, A., Liewes, E.W., and Ollevier, F. 1992. Efficacy of some anthelmintics against the swimbladder nematode *Anguillicola crassus* of eel *Anguilla anguilla* under saltwater conditions. *Dis. Aquat. Organ.* 13: 123–128. doi:10.3354/dao013123.
- Gillis, G.B. 1998. Environmental effects on undulatory locomotion in the American eel *Anguilla rostrata*: kinematics in water and on land. *J. Exp. Biol.* 201: 949–961. Available from

<http://jeb.biologists.org/content/201/7/949.abstract%5Cnpapers3://publication/uuid/67ED0B1A-8E1C-4732-AEBA-581C07D176A5>.

- Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C., and Ollevier, F. 1989. Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Dis. Aquat. Organ.* 7: 97–101. doi:10.3354/dao007097.
- Hein, J.L., Buron, I. De, Roumillat, W.A., Post, W.C., Hazel, A.P., and Arnott, S.A. 2015. Infection of newly recruited American eels (*Anguilla rostrata*) by the invasive swimbladder parasite *Anguillicoloides crassus* in a US Atlantic tidal creek. *ICES J. Mar. Sci.*: 1–8. doi:10.1093/icesjms/fsv097.
- Hirose, H., and Sekino, T. 1976. Notes on egg deposition, larval migration and intermediate host of the nematode *Anguillicola*.
- van der Hoeven, R., Betrabet, G., and Forst, S. 2008. Characterization of the gut bacterial community in *Manduca sexta* and effect of antibiotics on bacterial diversity and nematode reproduction. *FEMS Microb. Lett.* 286: 249–256. Academic Press,. doi:10.1111/j.1574-6968.2008.01277.x.
- Kennedy, C.R., and Fitch, D.J. 1990. Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *J. Fish Biol.* 36: 117–131. doi:10.1111/j.1095-8649.1990.tb05588.x.
- Kirk, R.S., Morritt, D., Lewis, J.W., and Kennedy, C.R. 2002. The osmotic relationship of the swimbladder nematode *Anguillicola crassus* with seawater eels. *Parasitology* 124(Pt 3): 339–47. Available from <http://www.ncbi.nlm.nih.gov/pubmed/11922435>.
- Knopf, K., Würtz, J., Sures, B., and Taraschewski, H. 1998. Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Dis. Aquat. Organ.* 33: 143–149. doi:10.3354/dao033143.
- Kobayashi, H., Pelster, B., and Scheid, P. 1990. CO₂ back-diffusion in the rete aids O₂ secretion in the swimbladder of the eel. *Respir. Physiol.* 79(3): 231–242. doi:10.1016/0034-5687(90)90129-M.
- Lefebvre, F., Contournet, P., and Crivelli, A.J. 2002. The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology* 124: 457–463. doi:10.1017/S0031182001001378.
- Lefebvre, F., Fazio, G., Palstra, A.P., Székely, C., and Crivelli, A.J. 2011. An evaluation of indices of gross pathology associated with the nematode *Anguillicoloides crassus* in eels. *J. Fish Dis.* 34: 31–45. doi:10.1111/j.1365-2761.2010.01207.x.

- Lefebvre, F., Wielgoss, S., Nagasawa, K., and Moravec, F. 2012. On the origin of *Anguillicoloides crassus*, the invasive nematode of anguillid eels. *Aquat. Invasions* 7(4): 443–453. doi:10.3391/ai.2012.7.4.001.
- Molnár, K., Szakolczai, J., and Vetési, F. 1995. Histological changes in the swimbladder wall of eels due to abnormal location of adult and second stage larvae of *Anguillicola crassus*. *Acta Vet. Hung.* 43(1): 125–137.
- Moravec, F. 1996. Aquatic invertebrates (snails) as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea) and the role of paratenic hosts in the life cycle of this parasite. *Dis. Aquat. Org.* 27: 237–239. doi:10.3354/dao027237.
- Moravec, F., Di Cave, D., Orecchia, P., and Paggi, L. 1994. Present occurrence of *Anguillicola novaezelandiae* (Nematoda: Dracunculoidea) in Europe and its development in the intermediate host. *Folia Parasitol. (Praha)*. 41: 203–208.
- Moravec, F., and Konecny, R. 1994. Some new data on the intermediate and paratenic hosts of the nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 (Dracunculoidea), a swimbladder parasite of eels. *Folia Parasitol. (Praha)*. 41: 65–70. Available from isi:A1994NN69700012.
- Moravec, F., and Skoríková, B. 1998. Amphibians and larvae of aquatic insects as new paratenic hosts of *Anguillicola crassus* (Nematoda : Dracunculoidea), a swimbladder parasite of eels. *34(1996): 217–222.* doi:10.3354/dao034217.
- Morrison, W.E., and Secor, D.H. 2003. Demographic attributes of yellow-phase American eels (*Anguilla rostrata*) in the Hudson River estuary. *Can. J. Fish. Aquat. Sci.* 60: 1487–1501. doi:10.1139/f03-129.
- Moser, M.L., Patrick, W.S., and Crutchfield, J.U. 2001. Infection of American Eels, *Anguilla rostrata*, by an Introduced Nematode Parasite, *Anguillicola crassus*, in North Carolina. *Copeia* 3: 848–853. doi:10.1643/0045-8511(2001)001[0848:IOAEAR]2.0.CO;2.
- Muñoz, P., Peñalver, J., Ruiz de Ybañez, R., and Garcia, J. 2015. Influence of adult *Anguillicoloides crassus* load in European eels swimbladder on macrophage response. *Fish Shellfish Immunol.* 42: 221–224. doi:10.1016/j.fsi.2014.11.011.
- Ooi, H.K., Wang, W.S., Chang, H.Y., Wu, C.H., Lin, C.C., and Hsieh, M.T. 1996. An epizootic of Anguillicolosis in cultured American eels in Taiwan. *J. Aquat. Anim. Health* 8(2): 163–166. doi:10.1577/1548-8667(1996)008<0163:AEOAIC>2.3.CO;2.
- Palstra, A.P., Heppener, D.F.M., van Ginneken, V.J.T., Székely, C., and van den Thillart, G.E.E.J.M. 2007. Swimming performance of silver eels is severely impaired by the swimbladder parasite *Anguillicola crassus*. *J. Exp. Mar. Bio. Ecol.* 352: 244–256. doi:10.1016/j.jembe.2007.08.003.

- Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2017). *_nlme: Linear and Nonlinear Mixed Effects Models_*. R package version 3.1-131, <URL: <https://CRAN.R-project.org/package=nlme>>.
- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rockwell, L.S., Jones, K.M.M., and Cone, D.K. 2009. First Record of *Anguillicoloides crassus* (Nematoda) in American Eels (*Anguilla rostrata*) in Canadian Estuaries, Cape Breton, Nova Scotia. *J. Parasitol.* 95(2): 483–486. doi:10.1645/GE-1739.1.
- Sokolowski, M.S., and Dove, A.D.M. 2006. Histopathological Examination of Wild American Eels Infected with *Anguillicola crassus*. *J. Aquat. Anim. Health* 18(4): 257–262. doi:10.1577/H06-009.1.
- Sures, B., Knopf, K., and Kloas, W. 2001. Induction of stress by the swimbladder nematode *Anguillicola crassus* in European eels, *Anguilla anguilla*, after repeated experimental infection. *Parasitology* 123: 179–184. doi:10.1017/S003118200100823X.
- Székely, C., Molnár, K., Müller, T., Szabó, A., Romvári, R., Hancz, C., and Bercsényi, M. 2004. Comparative study of X-ray computerised tomography and conventional X-ray methods in diagnosis of swimbladder infection in eels caused by *Anguillicola crassus*. *Dis. Aquat. Organ.* 58: 157–164. doi:10.3354/dao058157.
- Székely, C., Molnár, K., and Rácz, O.Z. 2005. Radiodiagnostic method for studying the dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection and pathological status of the swimbladder in Lake Balaton eels. *Dis. Aquat. Organ.* 64: 53–61. doi:10.3354/dao064053.
- Székely, C., Palstra, A., Molnár, K., and Thillart, G. Van Den. 2009. Impact of the Swim-Bladder Parasite on the Health and Performance of European Eels. In *Spawning Migration of the European Eel*. Springer, Netherlands. pp. 199–225.
- Thomas, K., and Ollevier, F. 1992. Paratenic hosts of the swimbladder nematode *Anguillicola crassus*. *Dis. Aquat. Organ.* 13: 165–174.
- Würtz, J., Taraschewski, H., and Pelster, B. 1996. Changes in gas composition in the swimbladder of the European eel (*Anguilla anguilla*) infected with *Anguillicola crassus* (Nematoda). *Parasitology* 112: 233–238. doi:10.1017/S003118200008481X.

TABLES

Table 1. Covariate types, covariate estimates (\pm standard errors), and p-values for a beta regression with random effects for the length ratio index (LRI) of American eel swimbladders infected with the parasitic nematode *Anguillicoloides crassus* by month of experiment.

Covariate type	Covariate	Level	Estimate \pm SE	<i>df</i>	<i>t</i>	p-value
	Intercept		-1.76 \pm 0.024	307.3	-74.26	< 0.0001
Fixed	Month	1	-0.083 \pm 0.019	735.3	-4.42	< 0.0001
		2	-0.077 \pm 0.019	735.2	-4.13	< 0.0001
		3	-0.079 \pm 0.019	724.9	-4.19	< 0.0001
		4	0.026 \pm 0.019	695.9	1.38	0.17
		5	0.012 \pm 0.018	537.7	0.67	0.561
		6	Baseline			
Random	PIT ID		0.068 \pm 0.009			

Table 2. Covariate types, covariate estimates (\pm standard errors), degrees of freedom (*df*), t-values, and p-values for a linear mixed-effects model for the swimbladder area of American eel swimbladders infected with the parasitic nematode *Anguillicoloides crassus* by month of experiment. The random effect estimate gives the intercept and variance.

Covariate type	Covariate	Level	Estimate \pm SE	<i>df</i>	<i>t</i>	p-value
	Intercept		532.6 \pm 18.5	752	28.8	<0.0001
Fixed	Month	1	0			
		2	9.29 \pm 9.36	752	0.99	0.32
		3	7.34 \pm 11.5	752	0.64	0.52
		4	125.8 \pm 12.7	752	9.90	<0.0001
		5	104.3 \pm 13.7	752	7.62	<0.0001
		6	101.4	752	14.3	<0.0001
Random	PIT ID		240.1 (166.5)			

Table 3. Covariate estimates (\pm standard errors), z -values, and p -values for a beta regression model for the comparison of length ratio index (LRI) values among swimbladder degenerative index (SDI) scores and how swimbladder length was measured (type = x-ray image or dissection).

Covariate	Level	Estimate \pm SE	z	p -value
Intercept		-1.72 \pm 0.04	-44.6	<0.0001
type	x-ray dissection	0.049 \pm 0.05 baseline	0.91	0.36
SDI	0	baseline		
	1	-0.10 \pm 0.05	-1.9	0.061
	2	-0.25 \pm 0.06	-4.1	<0.0001
	3	-0.31 \pm 0.06	-4.6	<0.0001
	4	-0.29 \pm 0.10	-2.8	<0.01
	5	-0.76 \pm 0.18	-4.2	<0.0001
type x SDI	x-ray x SDI 0	Baseline		
	x-ray x SDI 1	0.03 \pm 0.07	0.40	0.69
	x-ray x SDI 2	0.09 \pm 0.08	1.1	0.27
	x-ray x SDI 3	0.02 \pm 0.10	0.2	0.83
	x-ray x SDI 4	0.02 \pm 0.15	-0.1	0.90
	x-ray x SDI 5	0.19 \pm 0.28	0.7	0.49

Table 4. Covariate estimates (\pm standard errors), t -values, and p-values for a linear model for the comparison of swimbladder area among swimbladder degenerative index (SDI) scores.

Covariate	Level	Estimate \pm SE	t	p-value
Intercept		584.4 \pm 35.6	16.4	<0.0001
SDI	0	baseline		
	1	37.9 \pm 46.7	0.8	0.42
	2	1.64 \pm 53.8	0.03	0.98
	3	-115.9 \pm 59.1	-2.0	0.051
	4	-132.5 \pm 89.0	-1.5	0.14
	5	-430.2 \pm 131.3	-3.3	0.001

FIGURES

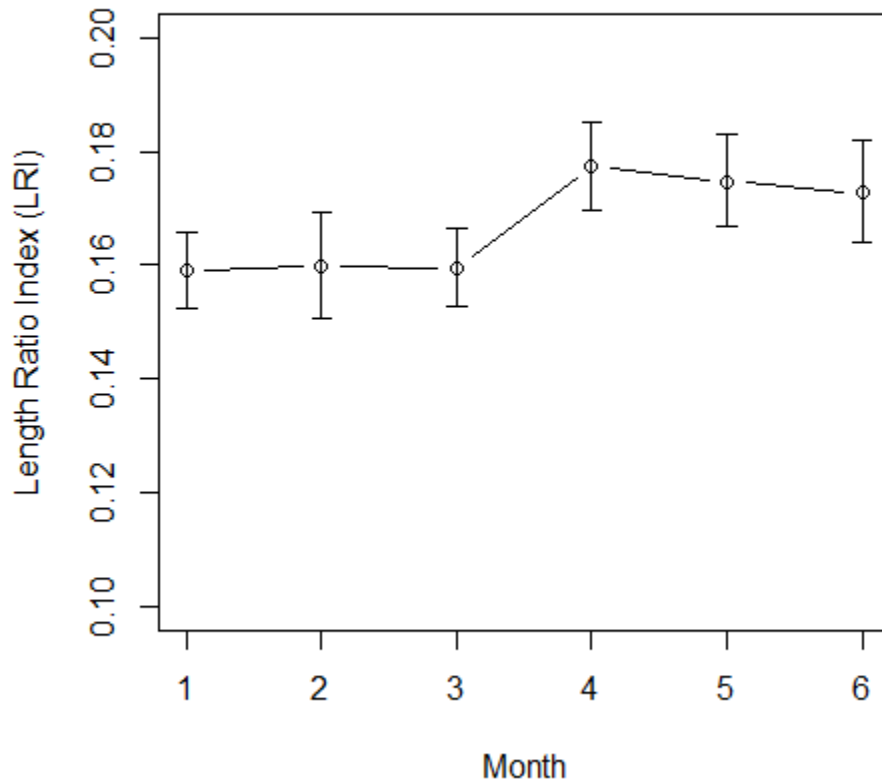


Figure 1. Change in the average Length Ratio Index (LRI), calculated using x-ray images, in American eels infected with the parasitic nematode *Anguillicoloides crassus* over 6 months. Results are from a beta regression with random effects. Error bars are 95% confidence intervals.

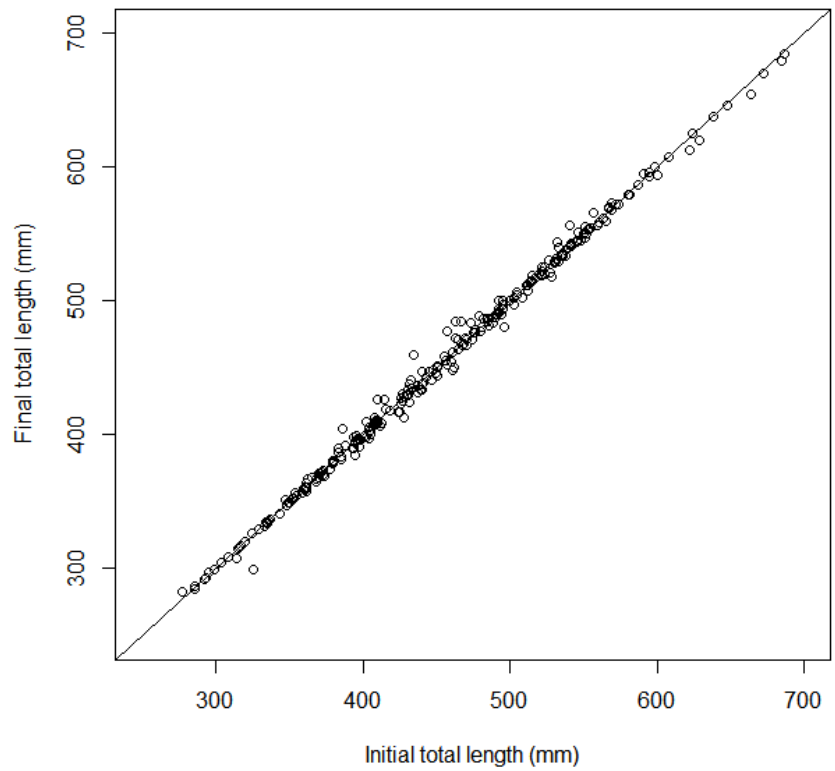


Figure 2. Initial total length (mm) at the beginning of the experiment compared to final total length (mm) at time of death or end of experiment of American eels infected with the parasitic nematode *Anguillicoloides crassus*. Open circles are observations of individual eels and solid line is a 1:1 line.

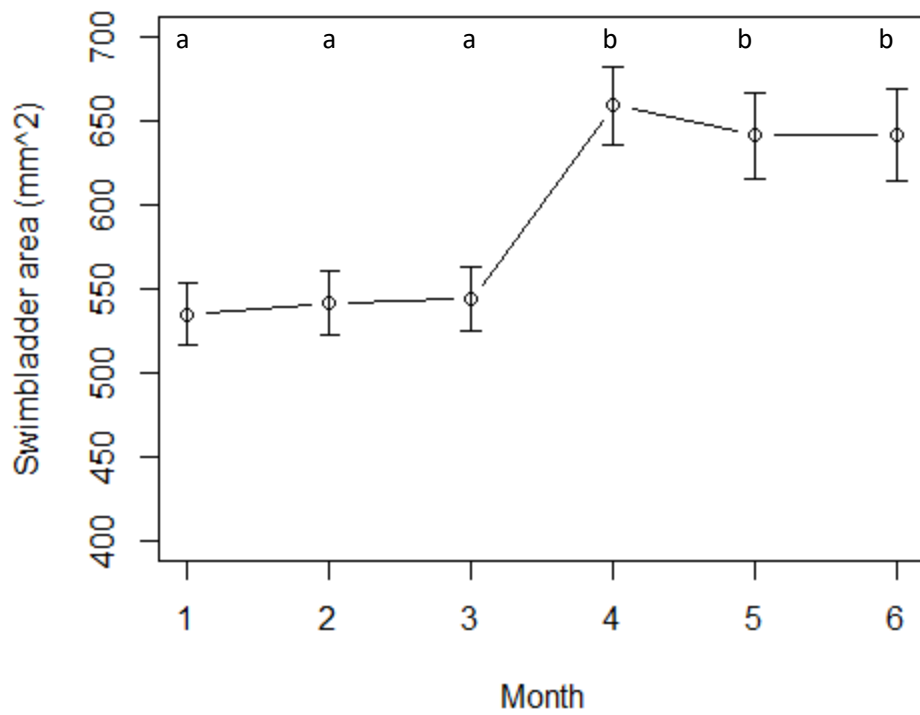


Figure 3. Average swimbladder area (mm²), measured using x-ray images, for American eels infected with the parasitic nematode *Anguillicoloides crassus* over 6 months. Error bars are standard error. Bars with the same lower case letter are not significantly different ($p > 0.05$).

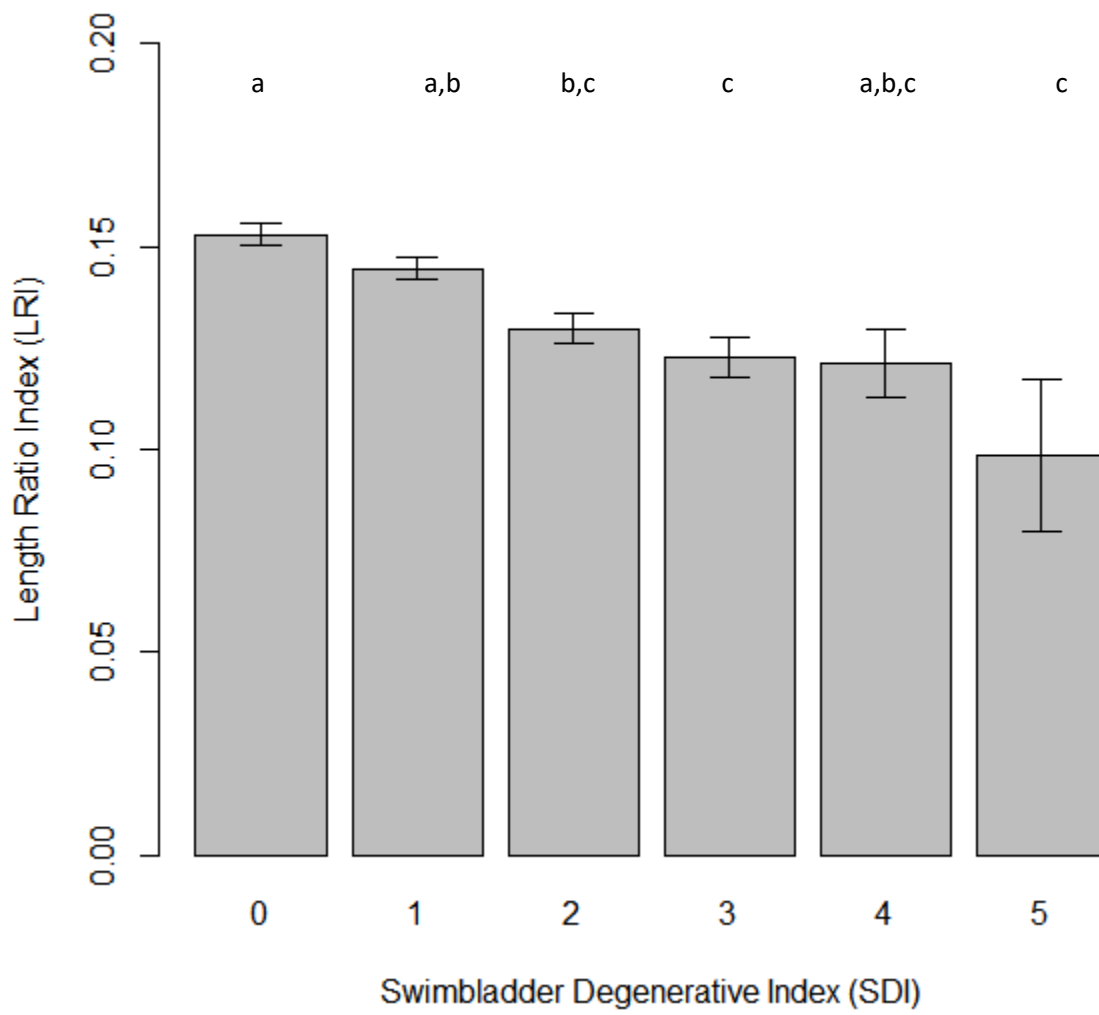


Figure 4. Comparison of final (i.e. at time of death) Length Ratio Index (LRI) to swimbladder degenerative index (SDI). LRI is averaged across swimbladder length measurements from both x-ray images and dissections. SDI score 5 represents scores 5 and 6 combined due to small sample size. Error bars are standard error. Bars with the same lower case letter are not significantly different ($p > 0.05$).

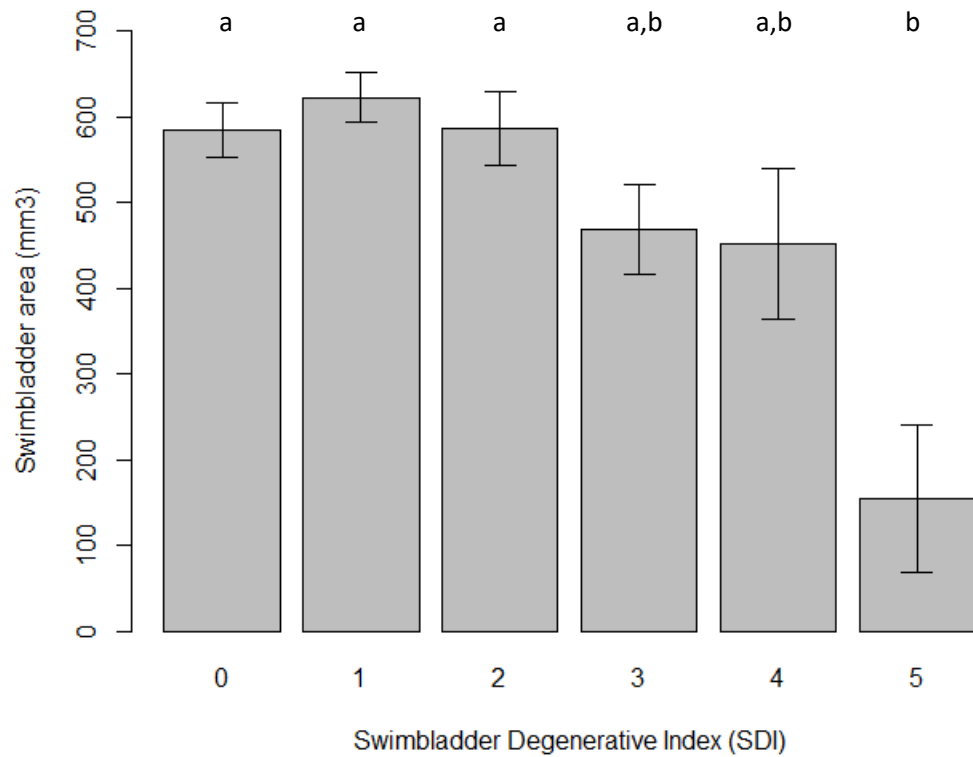


Figure 5. Average final swimbladder area (mm²) across swimbladder degenerative index (SDI) scores. Error bars are standard error. SDI 5 represents scores 5 and 6 combined due to small sample size. Bars with the same lower case letter are not significantly different ($p>0.05$).

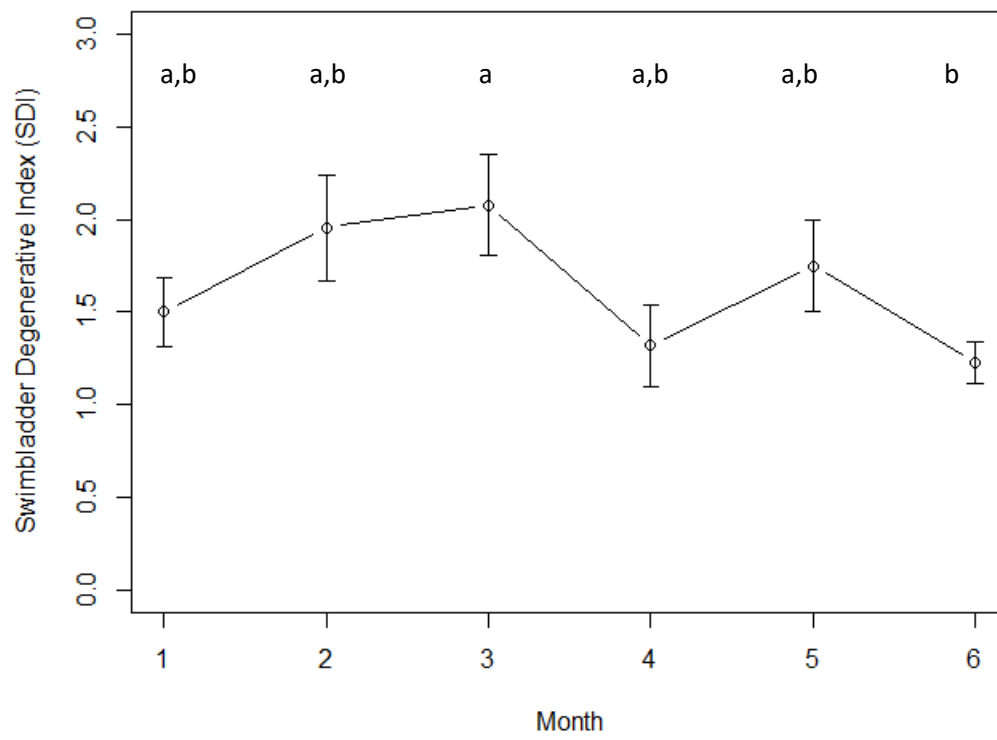


Figure 6. Average swimbladder degenerative index (SDI) score by month with standard error bars. Bars with the same lower case letter are not significantly different ($p>0.05$).

VITA

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