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Rates of swim bladder parasite infection and PIT tag retention in upstream migrant American eels of the upper Potomac River drainage

Jennifer L. Zimmerman
West Virginia University

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Rates of Swim Bladder Parasite Infection and PIT Tag Retention in Upstream Migrant
American Eels of the Upper Potomac River Drainage

Jennifer L. Zimmerman

Thesis Submitted to the
Davis College of Agriculture, Forestry, and Consumer Sciences
in partial fulfillment of the requirements
for the degree of

Master of Science
in
Wildlife and Fisheries Resources

Stuart A. Welsh, Ph.D., Chair
J. Todd Petty, Ph.D.
Kenneth Oliveira, Ph.D.

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Abstract

Rates of Swim bladder Parasite Infection and PIT Tag Retention in Upstream Migrant American Eels of the Upper Potomac River Drainage

Jennifer L. Zimmerman

This thesis involved two components: identification of the parasite *Anguillicola crassus* in American eels, *Anguilla rostrata* and PIT tag retention in eels in a laboratory setting. The recent spread of the swim bladder nematode parasite, *Anguillicola crassus*, in American eels has caused concern among biologists and fishery managers. A total of 244 yellow-phase American eels were collected at the Millville Dam eel ladder on the Shenandoah River, WV. Swim bladders were removed and examined for the presence of the nematode parasite. The number of parasites in each eel was recorded, and prevalence, intensity, and mean intensity were calculated. A swim bladder degenerative index (SDI) was also used on a subsample of 50 eels to document previous infections and the health state of the swim bladder. Prevalence of the parasite was 2%, and both intensity and mean intensity were 1. Based on the SDI, 38% of the eels showed signs of a previous infection of *A. crassus*. None of the eels from the Shenandoah River had severely degraded swim bladders, 38% had moderately damaged swim bladders, and 62% had healthy swim bladders. This is the first study that confirms the presence of *A. crassus* in the Potomac River watershed and the first to document the parasite in West Virginia. For the second part of the thesis, retention rates of passive integrated transponders (PIT tags) were compared among three tagging locations of small (205-370 mm) American eels: the dorsal musculature behind the head, the dorsal musculature near the dorsal fin origin, and the abdominal cavity. Eighteen American eels from the Shenandoah River, WV were PIT tagged in the three locations and tag retention was measured for a total of 9 weeks. Tag retention was highest in the dorsal musculature (100%) and in the abdominal cavity (100%), and lowest behind the head (88%). These results are consistent with previous literature. This research was a pilot study to determine PIT tag placement for a study of upstream migration in American eels.

Dedication

To my husband, Mike, and son, Kelvin, for all the love and joy that they have brought to my life, and to my mom and sister for their continual support and help in making me who I am today

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Chapter 1: Literature Review

This thesis includes three chapters. The first chapter is a review of American eel ecology, and consists of information on general ecology, upstream migration, age and growth patterns, dams and eel ladders, and the parasite *Anguillicola crassus*. The second chapter is on the swim bladder nematode parasite *A. crassus* in American eels utilizing the eel ladder on the Shenandoah River, WV, and includes a swim bladder health index and an ageing component. The final chapter is a laboratory study on PIT tag retention in small American eels.

General Ecology

The American eel, *Anguilla rostrata*, ranges from Greenland to Venezuela and colonizes a wide variety of habitat types, including lakes, streams, and estuaries (Facey and Van den Avyle 1987). In the United States, American eels are found in Atlantic coastal drainages, and the Mississippi watershed as far inland as Minnesota (Facey and Van den Avyle 1987; Tesch 2003). The range of the American eel has been documented as 5° to 60° N (Bertin 1956), and covers approximately 30,000 km of coastline (50 CFR 17). This widely-distributed species is thought to inhabit the broadest array of habitats of any fish in the world (Helfman et al. 1987).

American eels were once an important food source to Native Americans and early European settlers due to their high nutritional value (Casselman 2003). Today, American eels still represent a valuable resource and support a large commercial fishery on the Atlantic coast (ASMFC 2007). Landings of yellow and silver eels totaled 738,657 pounds in 2006, with New Jersey, Delaware, and Maryland accounting for 69% of the catch (ASMFC 2007). Additionally, eels are commonly used as bait for larger game fish, like the striped bass (ASMFC 2007). American eels are also preyed upon by a variety of fishes, mammals and birds, including striped bass, mink, raccoon, and bald eagles (Sinha and Jones 1967; Seymour 1974; Thompson et al. 2005). As they can contribute up to

25% of the biomass in individual systems, they may be a very important part of the food web (Smith and Saunders 1955; Ogden 1970). However, their importance to other species is not well documented in the literature (ASMFC 2000).

The life cycle of the American eel is considered to be facultative catadromous. Eels spawn in the marine waters of the Sargasso Sea, and after an oceanic larval period, migrate into estuaries and rivers where they feed and mature (ASMFC 2000). The term “facultative” is used because some eels may remain in saltwater until the onset of their downstream spawning migration without ever entering freshwater (Morrison et al. 2003; Lamson et al. 2006). Migrations between saltwater and freshwater have also been documented (Jessop et al. 2002). Upon reaching maturity, eels migrate downstream to the Sargasso Sea, where they spawn and die (Facey and Van den Avyle 1987). Scientists propose that spawning occurs on the warm side of thermal fronts during the winter and spring (McCleave and Kleckner 1985; McCleave et al. 1987), however, no direct spawning of American eels has been observed, and spawning locations have only been inferred by the collection of larvae (Kleckner et al. 1983; Kleckner and McCleave 1985).

American eels undergo several phases throughout their life cycle. The first life stage is the larval eel, termed leptocephali. Leptocephali passively drift in the upper 250 m of Sargasso Sea for about a year and metamorphose into glass eels over the continental shelf (Castonguay and McCleave 1987). Glass eels use selective tidal transport to begin migration into estuaries, where they become pigmented and enter the elver life stage (McCleave and Wippelhauser 1987). Elvers stall at the freshwater saltwater interface before continuing to migrate upstream (Sorensen and Bianchini 1986).

The next phase is the yellow eel, which may last from 7 to 30 years. This life stage, characterized by a lack of sexual maturity, includes growth and maturation (Helfman et al. 1987). Yellow eels either remain in saltwater systems or migrate upstream into freshwater where they feed and grow to maturity before migrating downstream (Morrison et al. 2003; Lamson et al. 2006). During or before downstream migration, eels become sexually mature adults, or silver eels, and undergo a number of physiological changes to

equip themselves for the oceanic portion of their migration (reviewed in Facey and Van den Avyle 1987). Silver eels are thought to be semelparous; spawning once in the Sargasso Sea before dying (ASMFC 2000).

Upstream Migration by Yellow-Phase Eels

Upstream migrations of yellow eels in freshwater occur in the spring and fall from March to October (Richkus and Whalen 1999). Movements are thought to occur primarily at night, although migration may occasionally occur during the day (McGrath et al. 2003; Verdon et al. 2003). Verdon et al (2003) found that American eels were most active at 21:00 hours, peaked at 1:00 hours, and declined at dawn on the Richelieu River, Quebec Canada. Similarly, McGrath et al. (2003) reported American eel activity primarily between 19:30 and 5:30 hours at an eel ladder on the St. Lawrence River, New York.

Environmental influences on yellow eel upstream migration include temperature, river flow, rain events, and lunar cycle (Winn et al. 1975; Cairns and Hooley 2003; Hildebrand 2005). Hildebrand (2005) concluded that peaks in eels using an eel ladder at Millville Dam on the Shenandoah River, West Virginia coincided with low light lunar phases. Additionally, migrations were also associated with increased river flow (Hildebrand 2005). However, Cairns and Hooley (2003) found that although American eel catches decreased sharply in Prince Edward Island bays and estuaries during the full moon, lunar cycles were not a trigger for migration. Verdon et al. (2003) also found no correlation between eel catches and temperature, river flow, or lunar cycle despite the fact that most peaks in migration occurred with increased temperature.

Age and Growth

Growth rates of eels vary depending on latitude, with eels reaching larger sizes in the northern portion of their range than in the south (Helfman et al. 1987). Eels in the north also remain in freshwater systems for longer periods of time before migrating back to the Sargasso Sea (Helfman et al. 1987). Eels migrate from the north at 12 to 23 years of age,

while eels migrate from the south at 6 to 7 years of age (Gray and Andrews 1971; Hansen and Eversole 1984). Males mature and migrate out of freshwater systems at younger ages than females, while females favor a long, slow growth that results in increased size and fecundity (Helfman et al. 1987).

In Georgia, Helfman et al. (1984) found that eels in freshwater were larger and older than estuarine eels, with lengths ranging from 344 to 486 mm and an average age of 6.2 years. In the estuaries, eels were on average 4.6 years old (Helfman et al. 1984). Hansen and Eversole (1984) found that in the brackish portion of the Cooper River, South Carolina, lengths of American eels ranged from 260 mm to 687 mm, with the majority of eels ranging from 300 to 640 mm long. Ages ranged from 1 to 12 years, with a mean of 4.3 years. Most of the eels were older than 2 and younger than 7, suggesting that eels in this habitat migrate after ages 6 to 7. Michener and Eversole (1983) found similar results for eels from the Charleston Harbor, South Carolina. Eels in their study were an average of 4.4 years old and ranged from 213 to 719 mm in length. Females were also found to be larger than males in each age class and the youngest eels were found in saline waters (Michener and Eversole 1983).

Rulifson et al. (2004) documented the ages of American eels in Lake Mattamuskeet, adjacent creeks and canals, and in northwestern Pamlico Sound, North Carolina. They found that eels ranged from 280 mm to 580 mm TL and had an average age of 4.8 years. Eels located in the lake were larger and older than eels from the sound and canal areas (Rulifson et al. 2004). In the Chesapeake Bay, the majority of American eels ranged from 110 to 560 mm in size and most were ages 2 to 6 (Owens and Geer 2003). The age range in their study was 1 to 18 (Owens and Geer 2003).

Morrison and Secor (2003) reported that American eels in brackish water portions of the Hudson River were approximately 8 years old, while eels in freshwater portions were approximately 17 years old. In New Jersey streams, eels ranged from 3 to 19 years, and were on average 10 years old (Ogden 1970). American eels in the tidal creek system in Great Sippewisset Marsh, Massachusetts averaged 306 mm (Ford and Mercer 1986).

Length for both brackish and freshwater eels was about 457 mm. In the freshwater Lake Champlain, Vermont, American eels were an average of 15.9 years old and ranged from 430 to 900 mm in length (Facey and LaBar 1981). Gray and Andrews (1971) documented that American eels in brackish waters in Newfoundland migrated back to sea at ages 12 to 13 and were a mean size of 690 mm.

Most of the studies on age and growth have taken place in estuaries, at the saltwater interface, or on tidally dominated coastal rivers. Few studies have focused on eels in inland waters (Goodwin and Angermeier 2003). Hildebrand (2005) found that eels in the Shenandoah River ranging from 214 to 550 mm TL were 3 to 10 years of age. This estimate is lower than other reported ages for American eels in the mid-latitude range (Goodwin and Angermeier 2003; Owens and Geer 2003). Goodwin and Angermeier (2003) also studied American eels in the Shenandoah River. They found that eels were a mean length of 767 mm and were on average 11 years of age, which is a higher estimate than most eels in the mid-latitude range and older than other age estimates for eels within the same watershed (Hildebrand 2005).

The differences in the age and size composition between the two studies may possibly be attributed to capture methods. Hildebrand (2005) captured upstream migrant eels using the Millville eel ladder, whereas Goodwin and Angermeier (2003) captured eels using boat electroshocking. Eels of Goodwin and Angermeier (2003) may have been larger yellow eels with an established home range (Parker 1995; Hammond 2003), as Hammond (2003) found that large American eels (500-800 mm TL) in the Shenandoah River established home ranges during the summer.

Threats to Migrating Eels

Trends in capture data indicate that American eel population are declining (Castonguay et al. 1994 a,b; Richkus and Whalen 1999; Haro et al. 2000; Richkus and Whalen 2000; Casselman 2005). Casselman (2005) reported that during the 31-day peak migration period, the mean number of eels passing through the Moser Saunders Hydroelectric Dam

at Cornwall, Ontario, decreased from over 27,000 eels to 274 eels per day in 2004 (Casselman 2005). Recruitment at the ladder had dwindled to 0.2% of its level since the early 1980s (COSEWIC 2006). Landings in the United States have also fallen from a high of 1.8 million pounds in 1985 to a low of 641,000 pounds in 2002 (ASMFC 2007).

Concern about the decline of eels has increased worldwide. Reports of diminished catches prompted the ASMFC to petition the American eel as an endangered or threatened species under the Endangered Species Act. NOAA Fisheries and U.S. Fish & Wildlife Service underwent a 12-month status review to determine whether American eels should be protected under the Endangered Species Act (50 CFR 17). The findings of the status review indicated that listing the American eel as a threatened or endangered species was not warranted at this time. This decision was based on the elasticity of the eel's habitat use, their ability to live the duration of their lives in saltwater, their wide distribution, and the fact that their numbers still remain in the millions. Furthermore, there is still ample historic habitat available, recruitment trends appear to be stable, and the factors affecting eels do not threaten them at a population level (50 CFR 17 2007).

Factors implemented in the population decline of American eel include fishing pressure and habitat modification, including obstruction to migration, oceanic changes, and chemical contamination (Castonguay et al. 1994 a,b). Recently, the nematode parasite, *Anguillacola crassus*, has also been implemented as a potential threat to the eel population (Haro et al. 2000). Only a few studies have documented the presence of this nematode, and its spread throughout the Atlantic coastal watershed remains largely unknown (Barse and Secor 1999; Barse et al. 2001).

Impacts of Dams

Like many other fish species, American eels are affected by dams (Wiley et al. 2004; McCleave 2001; Haro and Castro-Santos 2000; Richkus and Whalen 2000). Impacts on downstream and upstream migrants include blockage of prime upstream habitat (McCleave 2001; Verdon et al. 2003; Verreault et al. 2004) and direct mortality by

turbines to downstream migrants (McCleave 2001). Busch et al. (1998) used Geographic Information Systems (GIS) to assess the amount of available American eel habitat. They found that 15,115 dams on Atlantic coastal tributaries block upstream and downstream migration routes, and that these dams restricted 556,801 km to 90,755 km of American eel stream habitat; a total of 84% of all available habitat. The North Atlantic (Maine to Connecticut) had the greatest loss of habitat (91%) with a reduction in stream access from 111,482 km to 10,349 km. The Mid-Atlantic (New York through Virginia) had an 88% loss (199,312 km to 24,534 km) and the South Atlantic (North Carolina to Florida) had a 77% reduction in stream habitat (246,007 km to 55,872 km).

In the St. Lawrence watershed, Verreault et al. (2004) identified 151 hydrodams greater than 2.5 m equipped with turbines and 8,260 dams greater than 2.5 m without turbines. They estimate that these dams block 12,140 km² of suitable eel habitat in the St. Lawrence watershed, and that this loss of habitat represents a loss of 836,545 fecund females.

For yellow eels migrating upstream, barriers to migration may also significantly reduce recruitment into the upper watersheds, which may ultimately reduce the numbers of female eels migrating from upstream habitats to the Sargasso Sea (Verdon et al. 2003). Krueger and Oliveira (1999) suggest that phenotypic sex in American eels may be determined by environmental factors such as population density, and crowding is thought to produce males (Oliveira 1999; Oliveira et al. 2001). Reports of American eels congregating below barriers lacking fish passageways (Liew 1982; Wiley et al. 2004) could potentially result in imbalanced sex ratios dominated by males (Richkus and Whalen 1999; Haro et al. 2000). As American eels are a panmictic (randomly breeding) species, imbalanced sex ratios could impact the entire population. Other consequences of being detained below barriers include increased risk of predation, increased spread of disease, and increased competition for food (Haro et al. 2000).

As a mitigation measure, hydroelectric facilities and fishery management agencies have installed eel ladders on some hydropower dams to help pass American eels into the upper

portions of watersheds. These passageways have proven an effective method to assist in the conservation of this species (Liew 1982; Laffaille et al. 2005). However, most river blockages do not contain any provisions for migrating fish, and those dams that do have fish passageways are not always evaluated for their effectiveness (Knights and White 1998; Verreault et al. 2004).

Anguillicola crassus

The swimbladder nematode parasite, *Anguillicola crassus*, is native to East Asia and is commonly found in the Japanese eel, *Anguilla japonica* (Moravec 1992), where it appears to do little harm (Nagawasa et al. 1994). The parasite is one of five helminthes found to infect eel swim bladders. The other species include *A. globiceps* from Eastern Asia, the South African species, *A. papernai*, the Australian parasite, *A. australiensis*, and *A. novaezelandiae* from New Zealand (Moravec and Taraschewski 1988). *A. crassus* is described as having a dark, plump, fusiform body that tapers on both ends and a rounded head end. Important taxonomic identifiers include a well sclerotized buccal cavity with an anterior rim that contains 22 large circumeral teeth (Moravec and Taraschewski 1988).

In the 1980's the parasite was accidentally introduced to the European eel, *Anguilla anguilla*, by an expanding eel commerce and has become widespread throughout most of Europe (Køie 1991). Since then, *A. crassus* has been documented in most European countries and in North Africa, where its dissemination closely resembles the full geographic range of the European eel (Lefebvre and Crivelli 2004). In some cases, the infection rate has been documented close to 100% (Kennedy and Fitch 1990; Höglund and Thomas 1992; Nagasawa et al. 1994). In 1995, the first occurrence of *A. crassus* was identified in wild American eels in Winyah Bay, South Carolina (Fries et al. 1996) and has since been found in most of the states along the Atlantic seaboard, including Maine, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Delaware (John Clark, Delaware Department of Natural Resources and Environmental Control, personal communication), Maryland, Virginia, North Carolina, South Carolina, and Florida (Barse and Secor 1999; Barse et al. 2001; Moser et al. 2001; Geer 2003; Morrison and Secor

2003; Rulifson et al. 2004; Sokolowski and Dove 2006; Machut and Limburg 2008; Aieta and Oliveira In Prep). Recently, the parasite has expanded into Canada and has been identified in New Brunswick and Nova Scotia (Aieta and Oliveira In Prep).

The life cycle of the parasite relies on predator/prey interactions (Kirk 2003) and can be completed in as little as two months at 20°C (De Charleroy et al. 1990). American eels become infected by the parasite when they consume an intermediate or paratenic host (De Charleroy et al. 1990; Thomas and Ollevier 1992). A paratenic host is a host species not required for completion of the parasite's life cycle; this host just passes the parasite to the definitive host (American eel). However, the obligate intermediate host is required for the completion of the parasite's development (Kirk 2003).

While no host species have been identified in North America, 37 species of marine, estuarine, and freshwater fish all serve as paratenic hosts in European eels (Höglund and Thomas 1992; Thomas and Ollevier 1992; Reimer et al. 1994; Kirk 2003). In addition, snails, amphibians, and insect larvae may also be potential paratenic hosts (Moravec 1996; Moravec and Škoríková 1998). Intermediate hosts have been identified in Europe as small crustaceans like ostracods and copepods (Thomas and Ollevier 1989; Moravec and Konecny 1994). No paratenic host species have been documented for Japanese eels (Nagawasa et al. 1994).

Once inside the eel's swim bladder, the parasite copulates and the eggs are expelled into water with the feces. Hatching into the L₂ larvae occurs either during or after the passage through the eel's digestive tract. After entering the water, the L₂ larvae attach to the substrate headfirst and undulate to stimulate predation by an intermediate host. Within the host, the larvae molt into the L₃ infective stage, and eels consuming the intermediate host become infected (De Charleroy et al. 1990). Infection by an intermediate host can occur as early as the glass eel phase, as these eels are likely to feed on small crustaceans (Kennedy and Fitch 1990).

The infected intermediate host can also be consumed by a paratenic host. In these host species, the L₃ larvae remain alive, but do not complete their lifecycle until the eel consumes the paratenic host. Once in the eel's body, the L₃ larvae pass through the intestinal wall and the body cavity to reach the swim bladder. After a couple of weeks, the parasite molts into the L₄ stage where they consume blood and continue to grow. Molting occurs once more and the sexually mature adults begin reproduction (De Charleroy et al. 1990).

Few studies have documented the presence of *A. crassus* in American eels. In the Southeast, Moser et al. (2001) found high levels of infestation in North Carolina. They collected 1,111 eels from various rivers and estuaries and found that 52% of the eels were infected with the parasite. Prevalence rates ranged from 26% to 100% and mean intensity was 3.9 nematodes per eel (Moser et al. 2001). Rulifson et al. (2004) found that 35% of the eels in coastal lakes of Pamlico Sound, North Carolina were infected with the parasite, and that sexually undifferentiated eels had higher incidences of the parasite. Geer (2003) examined 126 eels in Virginia tributaries and found an infection rate of 61% in York River, a 44% infection rate in the Rappahannock River and a 27% infection rate in the James River.

Barse and Secor (1999) examined the swim bladders of 329 American eels in the Chesapeake Bay and 150 eels in the Hudson River, New York. They found that the prevalence was 10% to 29% in the Chesapeake Bay and 0% to 12% in the Hudson River. However, the authors state that these infection rates were an underestimate, as they only identified nematodes living in the swim bladder lumen, and did not count the juveniles stages of the parasite, which live in the swim bladder wall (Barse and Secor 1999).

Recent research in New York has shown increased infestations of the parasite as compared to earlier studies. Machut and Limburg (2008) found that prevalence averaged 39% in tributaries of the Hudson River and that eels contained an average intensity of 2.4 nematodes per eel. Sokolowski and Dove (2006) examined the pathology of American eels from the Carlls River, Babylon, New York, and found that 100% of the eels showed

evidence of previous or ongoing infections of the parasite. In the Northern United States, Aieta and Oliveira (In Prep) found that in Rhode Island the infection rate ranged from 28 to 69%. In Massachusetts, prevalence ranged from 7 to 76%, and in Maine, the percentage of eels infected by *A. crassus* was 0 to 65%.

The parasite *A. crassus* exhibits many characteristics that makes it a successful invader (Kennedy and Fitch 1990; Kirk 2003; Kennedy 2007). It has a life cycle that can be completed in a short time period (De Charleroy et al. 1990), high reproductive potential (Kennedy and Fitch 1990), the ability to survive a wide range of environmental conditions in a host species (Kennedy and Fitch 1990; Kirk et al. 2000a,b), and it can occupy a vast array of host species (Kennedy and Fitch 1990; Kirk 2003). It also can infect eels of all sizes (Kennedy and Fitch 1990). Furthermore, the parasite has invaded an empty niche. Only one other nematode, *Daniconema anguillae*, is found in the eel's swim bladder, and unlike *A. crassus*, this parasite is usually found in the serous cover and only occurs in low numbers (Kirk 2003).

In Europe, the rapid dissemination of the parasite has been attributed to the introduction of eels into watersheds for stocking and human consumption, the natural movements of the eels, and regurgitation by piscivorous birds, like cormorants (Wlasow et al. 1998; Kirk 2003). Urbanization and introductions through ballast waters and bait buckets may also play a factor in the distribution of the parasite (Machut and Limburg 2008; Aieta and Oliveira In Prep). Recent research shows that urbanization may increase the eel's susceptibility to the parasite, and trends of elevated infection rates in urbanized areas have been found in eels from the Hudson River (Machut and Limburg 2008). Introductions via ballast water at commercial ports may further increase the distribution of the parasite (Aieta and Oliveira In Prep). Aieta and Oliveira (In Prep) found that in the northeast, prevalence and intensity were highest at sites located near major shipping ports.

The only limitations to the spread of *A. crassus* are thought to be host availability and temperature (Thomas and Ollevier 1993; Knopf et al. 1998; Kirk 2003). Knopf et al.

(1998) found increased mortality, decreased growth, and decreased reproduction in adult worms held at 4°C for four months. Therefore, the authors concluded that the parasite would be unable to colonize the most northerly parts of the American eel's range (Knopf et al. 1998). However, Aieta and Oliveira (In Prep) located the parasite as far north as Cape Breton Island, Nova Scotia, Canada, and documented well established parasite populations in eels in Penobscot River, Maine, where temperatures are often below 4°C for four months a year. Thus, indicating that temperature may not halt the invasion of the parasite as suggested by other studies (Aieta and Oliveira In Prep).

Dams may also slow the spread of *A. crassus* by blocking the movement and distribution of host species (Machut and Limburg 2008). As many fish host species are unable to circumvent natural and manmade barriers, the spread of the parasite is dependent on American eel upstream migrations, and could take a long time to reach currently healthy systems (Machut and Limburg 2008). Once established within a particular watershed, eels exhibit home ranging behavior (Parker 1995), which may decrease the likelihood of spreading the parasite to other systems (Aieta and Oliveira In Prep).

Many studies have suggested that the nematode parasite may be detrimental to *Anguilla* eels and propose that the parasite could ultimately impact reproduction (Sprengel and Lüchtenberg 1991; Molnár 1994; Barse and Secor 1999; Palstra et al. 2007a). Mass mortality has been reported in the European eel (Molnár et al. 1991) and in American eels from eel farms in Taiwan (Ooi et al. 1996). Other documented effects include enlarged abdomens, swim bladder rupture, dilation of the blood vessels in the swim bladder, thickened swim bladder wall, skin ulcers in the abdomen, a red and swollen anus, increased stress response to hypoxia, damage to the gas glands, increase in spleen mass, and secondary bacterial infections (van Banning and Haenen 1990, Molnár et al. 1993; Molnár 1994; Würtz et al. 1996; Nimeth et al. 2000; Würtz and Taraschewski 2000; Crean et al. 2003; Lefebvre et al. 2004; Gollock et al. 2005; Sokolowski and Dove 2006).

The parasite may also impact the swimming ability of eels, which could impair the eel's ability to complete their migration and make them more susceptible to predation and

capture in both fisheries and cooling water intakes (Sprengel and Luchtenberg 1991; Kirk 2003; Kennedy 2007; Palstra et al. 2007a,b). A study by Sprengel and Luchtenberg (1991) compared maximum swimming speeds of European eels infected with *A. crassus* with uninfected eels in a laboratory setting. They demonstrated that the presence of *A. crassus* in European eels had an impact on swimming speed. Results of their study showed the presence of the nematode parasite in the swim bladder of the eel reduced swimming speeds in eels with more than 10 nematodes. Based on this, the authors speculate that eels infected with nematodes would be unlikely to reach the spawning grounds in the Sargasso Sea. Furthermore, they suggest that fish with a decreased ability to swim would not be as effective in competing for food and would be more likely to be affected by anthropogenic impacts (Sprengel and Luchtenberg 1991).

More recently, Palstra et al. (2007a) found that infected silver European eels and silver eels with damaged swim bladders showed a reduced swimming performance, a loss of endurance, and were less efficient swimmers. Results of their swimming fitness tests found that silver eels had higher oxygen consumption, lower optimum swim speeds, and higher cost of transport at optimum swim speeds. They state that infected eels and eels with heavily damaged swim bladders would need to spend at least 20% more of their energy reserves on migration, leaving less fat for egg production. The reduced swimming performance could also cause an increase in the duration of the migration causing delays in reaching the spawning grounds. The authors conclude that the parasite is a serious threat to reproductive success in European eels and speculate that the parasite may have played a role in the collapse of the eel population. Palstra et al. (2007b) may also have observed migration failure in silver European eels. During laboratory migration simulations, six eels showed problems swimming and did not reach the 1000 km border, indicating an inability to migrate the distance needed to reach the Sargasso Sea. Half of these eels were infected with the swim bladder parasite and 83% of them had heavily damaged swim bladders, suggesting a decreased swimming performance due to the parasite.

These findings contrast with Munderle et al. (2004), who found that the parasite did not affect the amount of the time the eels were able to withstand a current. Eels used by Palstra et al. (2007a), however, were much larger, in a different developmental stage, and exposed to swimming trials of a longer duration. One could assume that impacts of the swim bladder parasite are similar for European and American eels. Preferably, however, additional research should be conducted on the impacts of *A. crassus* to American eels.

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Chapter 2: Swim Bladder Nematode Parasite *Anguillicola crassus*

Introduction

The American eel, *Anguilla rostrata*, is a commercially important fish species along the east coast of the United States, particularly in the mid-Atlantic region (ASMFC 2007). Data from commercial fish landings, upstream passage counts, and seine, trawl, and electrofishing surveys indicate that American eel populations are in decline throughout their range (Haro et al. 2000; Casselman 2003). As a result, concern about eel species has increased worldwide (ASMFC 2007, Haro et al. 2000, Casselman 2005, COSEWIC 2006). Habitat modification, fishing pressure, pollution, and oceanic changes have all been cited as potentially playing a role in the decline of the American eel (Castonguay et al. 1994 a,b). In addition, the recently identified invasive swim bladder nematode parasite, *Anguillicola crassus*, has also been implemented as another possible contributing factor to the decline (Haro et al. 2000).

The nematode, *A. crassus*, is native to Asia and is commonly found in Japanese eels, where it appears to cause little harm (Nagawasa et al. 1994). The parasite's short life cycle, coupled with its high fecundity, ability to tolerate a wide range of salinities, and use multiple paratenic hosts to complete its lifecycle, makes it a highly successful invasive colonizer (Kennedy and Fitch 1990; Kirk 2003; Kennedy 2007). It was introduced to Europe via eel commerce in the early 1980's and has since spread rapidly throughout the continent. In many European watersheds, infestation levels are close to 100% (Kennedy and Fitch 1990; Höglund and Thomas 1992; Nagasawa et al. 1994). To date, it has been found in every European country and in North Africa (Lefebvre and Crivelli 2004).

The first documented case of *A. crassus* in the United States occurred the mid-1990s in a Texas aquaculture facility and in wild eels in Winyah Bay, South Carolina (Fries 1996). Since then, it has been detected in almost all of the states along the Atlantic seaboard,

including Maine, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Delaware (John Clark, Delaware Department of Natural Resources and Environmental Control, personal communication), Maryland, Virginia, North Carolina, South Carolina, and Florida (Barse and Secor 1999; Barse et al. 2001; Moser et al. 2001; Geer 2003; Morrison and Secor 2003; Rulifson et al. 2004; Sokolowski and Dove 2006; Machut and Limburg 2008; Aieta and Oliveira In Prep). Reported infection rates in the United States range from 0 to 100% (Sokolowski and Dove 2006; Aieta and Oliveira In Prep). The parasite was also recently identified in New Brunswick and Nova Scotia, Canada, with the northernmost part of its range being documented as Cape Breton Island, Nova Scotia (Aieta and Oliveira In Prep).

The parasite is detrimental to the eel's swim bladder and reported effects of the nematode include enlarged abdomens, swim bladder rupture, dilation of the blood vessels in the swim bladder, thickened swim bladder wall, skin ulcers in the abdomen, a red and swollen anus, secondary bacterial infections, decreased swimming ability, and mass mortality (van Banning and Haenen 1990, Molnár et al. 1991; Molnár et al. 1993; Molnár 1994; Ooi et al. 1996; Würtz et al. 1996; Nimeth et al. 2000; Würtz and Taraschewski 2000; Crean et al. 2003; Lefebvre et al. 2004; Gollock et al. 2005a; Sokolowski and Dove 2006). Recent research by Palstra et al. (2007a,b) also indicates that eels severely infected by *A. crassus* are unlikely to reach the spawning grounds. A decrease in recruitment may occur if fewer eels are arriving at the spawning grounds, and ultimately, this could lead to a decrease in the eel population (Barse and Secor 1999; Palstra et al. 2007).

The majority of research on *A. crassus* in American eels has focused on classic parasite identification, including prevalence, intensity, and mean intensity to determine parasite infection rates (Lefebvre et al. 2002; Sokolowski and Dove 2006). However, studies on European eels have shown that these methods may underestimate the impact of *A. crassus* on eels and do not provide an accurate indication of the severity of the infection (Lefebvre et al. 2002; Lefebvre and Crivelli 2004). This is because previous infections by the parasite may pathologically render the swim bladder unsuitable for re-infection,

and therefore, the absence of parasites can actually represent a more severe stage of infection (Molnár et al. 1993). Numerous other studies have documented a large number of eels containing no helminthes, but having thickened and damaged swim bladders (van Willigen and Dekker 1989; van Banning and Haenen 1990; Thomas and Ollevier 1992; Molnár et al. 1993), and van Banning and Haenen (1990) concluded that eels with thickened swim bladder walls could no longer support parasites.

Thus, the pathological changes to the swim bladder as identified by the health state of the organ itself may provide a better indication of parasite pressure than just the presence of worms alone (Lefebvre et al. 2002). Pathological changes caused by the parasite include a thickened and opaque swim bladder caused by fibrosis (van Banning and Haenen 1990; Molnar et al. 1993), as well as a swollen and enlarged abdomen that may contain a black mass of decaying worms and a cloudy fluid containing eggs and second-stage juveniles (van Banning and Haenen 1990; Kirk 2003). Furthermore, the pneumatic duct may become dilated and inflamed by worms blocking its opening (Kirk et al. 2000), and eels may have bacterial lesions on the abdomen and a red and swollen anus (Crean et al. 2003). In addition to thickened swim bladders, eels with previous infections may also have pigment accumulation (Molnar et al. 1995), as well as a lumen containing the black slugde of dead worms, digested blood. Debris from the swim bladder wall may also contain encapsulated adults and juveniles (Kirk 2003). In the most severe cases, the lumen collapses and the swim bladder adheres to the surrounding organs (van Banning and Haenen 1990).

Many authors have recognized the importance of quantifying damage to the infected organ instead of studying the infective agent alone (Csaba et al. 1993 and Hartmann 1994, reviewed in Lefebvre et al. 2002; Beregi et al. 1998) and have recommended using histopathological and pathological changes to the swim bladder as an alternative to classic identification methods in eels (Lefebvre et al. 2002; Sokolowski and Dove 2006). One such method was developed by Lefebvre et al. (2002), and involves the use of three criteria, including the transparency and opacity of the swim bladder wall, presence of pigmentation and exudates, and thickness of the swim bladder wall. This macroscopic

measure of the health state of the swim bladder allows for easy quantification of present and previous infections while taking into account the effect of both the larval and adult stages (Lefebvre et al. 2002).

The objectives of this study include documenting the presence of the nematode in eels from the Shenandoah River, West Virginia and determining the prevalence, mean intensity, and intensity of the infection. In addition, the severity of the *A. crassus* infection was measured utilizing the swim bladder degenerative index developed by Lefebvre et al. (2002). Ages of eels with current and previous infections were determined and correlations between age and length to parasite infection were analyzed.

Methods

Study Site

The Shenandoah River lies mostly in Virginia, with a small portion in West Virginia, and has a catchment size of approximately 7,870 km². The drainage lies mostly within the Valley and Ridge physiographic province, although some tributaries flow out of the Blue Ridge province (Jenkins and Burkhead 1994). The river is made up of a North Fork and the South Fork; both of which flow for over 250 river kilometers (rkm) before converging at Front Royal, Virginia to form the Shenandoah River. The Shenandoah River joins with the Potomac River at Harper's Ferry, West Virginia, and ultimately drains into the Chesapeake Bay (Figure 2-1).

The Millville hydroelectric dam and eel ladder where the eels were collected is owned and operated by Allegheny Power, and is located on the lower Shenandoah River (Figure 2-1). The dam is 7.24 km from Harper's Ferry, West Virginia and 249 rkm from the mouth of the Potomac River at the head of tide. This low head dam is 700 m wide, has a 5 m head, and has a generating capacity of approximately 1.8 MW. The Millville dam eel ladder extends 11 m on the western end of the dam, and consists of a covered metal sluice that slopes 50° (Appendix A). American eels are attracted to the eel ladder by an attractant flow located adjacent to the base of the eel ladder (Appendix A). A pool

created by flashboards on the face of the dam allows eels to distinguish between the spillway and the attractant flows. Movements through the eel ladder are facilitated by three rows of vertically placed PVC pipes situated in a “peg board” pattern that act as a substrate for the eels (Appendix A). The eels pass through the dam via a pipe at the top of the ladder and enter into the collection tank (Appendix A). The tank contains a 2.13 m long (60.96 cm diameter with 6.35 mm) mesh net to constrain the upstream migrants (Appendix A). Nets were checked weekly during the summer to early fall 2006 to 2008.

Parasite Identification

A total of 242 yellow-phase upstream migrant American eels were examined for the swim bladder nematode parasite, *Anguillicola crassus*. Eels were captured in the holding tank of the Millville eel ladder during September and October 2006 (n=143), August through October 2007 (n=49), and June 2008 (n=50). After collection, the eels were euthanized with an overdose of clove oil (Eugenol) and were frozen until further swim bladder analysis. Eel total length (mm) was measured, and swim bladders were examined for the presence of the pre-adult and adult parasites. The number of parasites in each eel was recorded, and prevalence, intensity, and mean intensity were calculated. Prevalence was defined as the percent of infected eels, intensity was the total number of nematodes per infected host, and mean intensity was the average number of nematodes per infected hosts in a sample of hosts (Bush et al. 1997). Confidence intervals for the prevalence data were generated with SAS version 9.1 using the profile likelihood method. Positive identification of the parasite was made based on the descriptions by Moravec and Taraschewski (1988).

Swim Bladder Health Index

A subsample of 50 eels was examined for macroscopic damage inflicted to the swim bladder by the parasite. A swim bladder degenerative index (SDI) was employed based on methods by Lefebvre et al. (2002). Three criteria were considered:

- 1). Transparency and opacity of the swim bladder wall

- 2). Presence of pigmentation and exudates, including dead worms, erythrocytes, decaying swim bladder tissue, eggs, and L₂ *A. crassus*.
- 3). Thickness of the swim bladder wall, measured using stacked 1 mm glass slides (Molnár et al. 1994).

Scores of 0 to 2 were assigned to each category with increasing swim bladder degradation. For the transparency of the swim bladder, a score of 0 indicated a normal transparent swim bladder, a 1 indicated intermediate opacity, and a 2 meant that the swim bladder was completely opaque. For the second category, a score of 0 indicated that neither exudate or pigment were present. If either exudate or pigment were found, a score of 1 was assigned, and a 2 was given when both exudates and pigments were present. Eels with swim bladders < 1 mm were given a score of 0 for swim bladder thickness, while eels with swim bladders > 3 mm were assigned a score of 2. These eels had little lumen space remaining. Eels with swim bladder thickness ranging from 1 to 3 mm were given a 1. The final cumulative index ranged from 0 to 6, with a value of 0 corresponding to a healthy intact swim bladder, 1 to 3 being a moderately damaged swim bladder, and 4 to 6 being a severely damaged swim bladder.

Ageing

American eels with current or previous infections were aged following methods developed by Secor et al. (1992) and Oliveira (1996). Paired sagittal otoliths were removed from the eels and were embedded in epoxy resin. A transverse cut was made using a diamond wafering saw (Buehler Isomet 1000, Germany). Once cut, the sections were placed on a slide with thermoplastic crystalbond glue, ground, and polished. The slides were etched and stained using 2% EDTA and 5% toluidine blue. Ages were estimated by counting the number of annual rings along multiple axes, and ages were tripled checked. An additional year was added to the age estimate to account for the larval salt water phase. A regression analysis analyzed the relationship between SDI scores and eel ages.

Results

Upstream migrants collected at the Millville dam eel ladder ranged from 200 to 527 mm TL (mean = 351.5 ± 3.8 mm SE). Eels with current and previous infections (n=24) ranged from 255 mm to 473 mm TL (mean = 341 ± 10.9 mm SE). Those eels showing signs of infection or containing worms ranged from 4 to 11 years of age, with a mean age of 6.9 ± 1.79 SE (n=15). *A. crassus* was the only parasite found in the American eel's swim bladder. Prevalence of the parasite was 2.0% (lower CI = 0.007 and upper CI = 0.044), and both intensity and mean intensity were 1 (Table 2-1). Results from the swim bladder degenerative index showed a much higher parasite pressure than the classic predictors, with 38% of the eels showing signs of a previous infection of *A. crassus*. While none of the eels from the Shenandoah River had severely degraded swim bladders, 38% had moderately damaged swim bladders, and 62% had healthy swim bladders (Figures 2-2 and 2-3). The eel age to SDI score regression revealed an R^2 value of 0.06 for 21 eels (Figure 2-4).

Discussion

American eels from the Shenandoah River showed symptoms of previous and ongoing infections of anguillicolosis. This is the first report of *A. crassus* being documented in an inland state, as well as within the upper portions of the Potomac River watershed. Previous reports in the upper and middle Chesapeake Bay indicate higher levels of infections than the 2.0% found in this study (Barse and Secor 1999; Barse et al. 2001; Geer 2003). In Virginia, Geer (2003) found a 61% infestation in the York River, 44% in the Rappahannock River, and 27% in the James River. Barse et al. (2001) reported prevalence rates of 13 to 82%, with higher levels being found within freshwater sites of the upper Chesapeake Bay. The site located closest to the mouth of the Potomac River

(St. Jeromes Creek) had infection rates of 40% for adult parasites and 57.1% for juveniles (Barse et al. 2001).

The dissemination of the parasite has been documented as being extremely rapid in most watersheds (Kennedy and Fitch 1990; Kirk 2003; Kennedy 2007). Barse et al. (2001) reported that in some Chesapeake Bay sites, prevalence doubled during the two year duration of their study. Therefore, it was hypothesized that infection rates would have increased throughout the duration of this study. However, the presence of the parasites only occurred during the first year of the study in 2006, and infection rates and intensities were much lower than those found in lower reaches of the watershed. This phenomenon was also seen by Machut and Limburg (2008) who found that eels from tributaries of the Hudson River, New York, had much lower parasite infestation rates than in larger river systems. Sampling in the Shenandoah River occurred during the summer when the parasite is thought to be most active (Kennedy and Fitch 1990; Knopf et al. 1998), so if it were present, it should have been located during the three year study. However, it is possible that prevalence may vary seasonally and more research is needed in this area.

Potential reasons for lower prevalence in the Shenandoah River as compared to other Chesapeake Bay sites may include the distance upstream of the Millville eel ladder and length of time for eels to migrate upstream, as well as the presence of dams on the Potomac River. Finally, previously infected eels may have developed an unsuitable environment for further infection. American eels can live in freshwater for up to 30 years (Helfman et al. 1987), and larger and older eels are primarily found upstream in freshwater reaches (Helfman et al. 1987; Morrison et al. 2003). Migrations upstream can take years and the Millville dam eel ladder is located 249 rkm upstream from the head of tide, so the parasite's spread into these areas may be slow. However, age estimates of 42 eels showed that upstream migrants from the Shenandoah River ranged from 4 to 11 years of age ($\mu = 6.7 \pm 0.29$ SE; Table 2-2), indicating that they could potentially have been infected by the parasite since it was first documented in the Chesapeake Bay in 1997 (Barse and Secor 1999). These ages are consistent with Hildebrand (2005), who

found that upstream migrants at the Millville dam eel ladder ranged from 3 to 9 years of age.

Physical barriers may also have slowed the spread of the parasite into the Shenandoah River. A total of two dams and one natural falls (Great Falls) exist on the lower Potomac River, and may slow the spread of the parasite. Machut and Limburg (2008) found that the presence of physical barriers reduced *A. crassus* infection upstream in the Hudson River, New York, and suggest that barriers may play the most important role in determining the distribution of the parasite. American eels become infected by the parasite when consuming an infected paratenic or intermediate host species. Paratenic host species have been identified in European eels as being numerous species of marine, estuarine, and freshwater fish, as well as snails, amphibians and insect larvae (reviewed in Kennedy 2007). Most of these small host species would be unable to circumvent dams, thus upstream migrant eels are primarily responsible for the invasion of the parasite (Machut and Limburg 2008). Machut and Limburg (2008) conclude that while parasite invasion is not halted by physical barriers, it could delay the parasite's arrival into upper portions of watersheds for many years.

Another reason that prevalence may be low in the Shenandoah River is that eels with previously damaged swim bladders may harbor few nematode parasites. Lefebvre et al. (2002) found a non-linear relationship between swim bladder damage and the presence of live worms, and eels could contain parasites, but show no signs of swim bladder damage. In their study, 92% of the eels showed pathological signs of infection, while only 47% contained worms. Lefebvre and Crivelli (2004) had similar results; infection rates were lower among eels with severely damaged swim bladders. This is because it may take multiple infections events for pathological changes to occur in the swim bladder (van Banning and Haenen 1990; Molnar et al. 1994; Wurtz and Tarachewski 2000). In their laboratory experiment, Wurtz and Tarachewski (2000) showed that eels infected with up to 40 larvae showed no serious alterations of the swim bladder, and that infections of up to 20 larvae for a period of 20 weeks did not cause a thickening of the swim bladder wall.

Eels from the Shenandoah River showed signs of having undergone previous infections of the parasite. While only a total of 2.0% of the eels contained actual parasites within their swim bladder lumen, the use of the swim bladder degenerative index on the subsample of 50 eels indicated that 38% of the eels had undergone previous infections (Figure 2-2). These eels contained macroscopic damage to the swim bladder including opacity of the swim bladder, black pigment, and the remnants of dead worms. None of the eels in this study contained thickened swim bladder walls (Figure 2-3). This was also described by Molnár et al. (1994), who noted a smoke like opacity of the swim bladder, minor haemorrhages, parasite nodules, and the presence of pigment in some eels that had thin-walled swim bladders. Sokolowski and Dove (2008) found similar pathological results for eels from the Carlls River, Babylon, New York. All of the eels from their study showed previous and ongoing infections of *A. crassus* (Sokolowski and Dove 2008).

Based on the results of the swim bladder degenerative index, those eels that exhibited signs of infection only had moderate damage to their swim bladder and none of the eels had a severe infection (Figure 2-2 and 2-3). It is possible that eels in this watershed are not severely infected, or those with a more severe infection incurred mortality further downstream. Mass mortality caused by the infection in conjunction with other environmental factors has been reported in both farmed and wild eels (Molnar et al. 1991, 1994; Nagasawa et al. 1994). Additionally, previous research has documented that infected eels are less tolerant to high temperatures and hypoxia (Gollock et al. 2005a,b); conditions that frequently occur in the Chesapeake Bay (Anon. 2003). These eels may also be more likely to be caught in fisheries or may be more susceptible to predation (Gollock et al. 2004; Kennedy 2007). Furthermore, eels may have the ability to restore some function of their swim bladders, and even those with intensively infected swim bladders may undergo regeneration (Molnár et al. 1994). These eels have a swim bladder with slight opacity (the result of fibrosis), brownish or black pigmentation left behind at the site of former hemorrhages, and calcified parasitic nodules (Molnar et al. 1994).

Lebeuvre et al. (2002) stated that eels with moderately damaged swim bladders may not contain worms because of an insufficient food supply due to destruction of the swim bladder capillary system on which the worms feed. Also, they may develop an unsuitable habitat for larval development or develop an immune response against the parasite (Lefebvre et al. 2002). Eels with severely affected swim bladder may not contain worms because there is no room remaining in the lumen for the parasite (Lefebvre et al. 2002). This did not appear to be an issue with eels from the Shenandoah River, as none of the eels contained thickened swim bladders.

The health state of the eel's swim bladder is important because damage to the organ by *A. crassus* may impact eel populations by hindering the spawning migration to the Sargasso Sea. The Sargasso Sea is the only place that the eels spawn, so it is possible that the altered ability to migrate could cause a decrease in recruitment (Barse and Secor 1999). The eel's migration to the Sargasso Sea involves long distances at very deep depths, and the eels undergo vertical migrations at night (reviewed in Kirk et al. 2000). As a result, a properly functioning swim bladder is necessary to complete migration (Kirk et al. 2000). Studies by Palstra et al. (2007 a,b) have shown that eels with damaged swim bladders may not be able to complete migrations. However, their study was on the European eel, which has to travel further distances to the spawning grounds than American eels. Furthermore, the parasite may cause a drain on the eel's energy resources during their migrations, as they do not feed during this time and therefore cannot compensate for losses by increased feeding (Aieta and Oliveira In Prep). This can lead to less fat reserves for egg production (Palstra et al. 2007a). Eels may also endure direct stress of the entire duration of its migration (Kennedy 2007) because the parasite can survive for several months in seawater in a host species (Kirk et al. 2000). Additional studies determining the impact of *A. crassus* on American eel populations are needed.

In conclusion, American eels from the Shenandoah River, West Virginia showed signs of both previous and current infections of *A. crassus*, and continued monitoring of eels in this watershed is warranted. The prevalence and intensity of the parasite in eels from the Shenandoah River is much lower than in many other localities reported in the literature

including sites located in the Chesapeake Bay (Barse and Secor 1999; Barse et al. 2001; Moser et al 2001; Geer 2003; Morrison and Secor 2003; Rulifson et al. 2004; Sokolowski and Dove 2006; Machut and Limburg 2008; Aieta and Oliveira In Prep). This may be due to a combination of factors, including the distance upstream of the eel ladder and the presence of natural or manmade barriers on the Potomac River blocking the host species that serve as vectors of the parasite and consequently slowing the spread of the parasite.

Eels may also lose their anguillicolosis infection during upstream migrations. These previously infected eels are either unable to become reinfected due to damage to their swim bladders or there may be no infected host species present to reinfect them. To date, no host species have been identified in the United States, and this information is pertinent in understanding the dissemination of the parasite. Furthermore, the presence of ports (Aieta and Oliveira In Prep) and increased urbanization (Machut and Limburg 2008) have also been associated with trends of increased parasite infections. These factors may also contribute to the distribution of the parasite and should be explored.

As multiple infection events may render the swim bladder unsuitable for habitation by the parasite, the pathological effects of the parasite on the swim bladder may be more important than the actual presence of the parasite itself. Therefore, the use of the swim bladder degenerative index developed by Lefebvre et al. (2002) may more accurately characterize the infection by taking into account the history of the infection by the parasite, as well as measuring both adult and larval impacts. This method should be utilized in conjunction with classic parasite identification when determining the impact of *A. crassus* on American eels.

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Table 2-1: Summary of prevalence and intensity of American eels collected from the Millville eel ladder on the Shenandoah River, WV.

Year	Eels Collected n	Length (mm $\pm$ SE)	Infected Eels N	Prevalence	Intensity	Mean Intensity
2006	143	375.9 $\pm$ 49.1	5	3.4%	1	1
2007	49	315.6 $\pm$ 57.6	0	0%	0	0
2008	50	315.6 $\pm$ 46.2	0	0%	0	0
Total	242	351.5 $\pm$ 58.8	5	2.0%	1	1

Table 2-2: Summary of ages and lengths of a subsample of 42 American eels from the Shenandoah River. Of the 42 eels, 15 were those that either contained parasites in their swim bladder or eels that showed signs of a previous infection.

All Eels				Infected Eels				Non-Infected Eels			
Age	N	Mean length (TL mm)	SE	Age	N	Mean length (TL mm)	SE	Age	N	Mean length (TL mm)	SE
4	5	298.6	22.6	4	1	279	-	4	4	303.5	28.5
5	7	317.1	16.0	5	4	302.8	19.8	5	3	336.3	26.3
6	6	351.2	21.3	6	2	352	28	6	4	350.8	31.7
7	13	326	13.3	7	3	287.3	2.7	7	10	337.6	15.5
8	4	408.8	22.4	8	2	397.5	23.5	8	2	420	33.2
9	2	363.5	86.5	9	0	-	-	9	2	363.5	61.2
10	4	353.5	23.0	10	2	314	6	10	2	393	5
11	1	420	-	11	1	420	-	11	0	-	-

Figure 2-1: Location of the eel ladder at Millville Dam on the Shenandoah River, WV.

Figure 2-2: Frequency distribution of the swim bladder degenerative index (SDI). The SDI was used to determine the impact of *A. crassus* on the swim bladder of upstream migrant American eels captured at the Millville eel ladder on the Shenandoah River. A value of 0 corresponds to a healthy intact swim bladder, 1 to 3 is a moderately damaged swim bladder, and 4 to 6 is a severely damaged swim bladder.

Figure 2-3: Frequency distribution of the swim bladder degenerative index criteria including the transparency of the swim bladder, the presence of pigmentation and exudates, and the thickness of the swim bladder. The SDI was used to determine the impact of *A. crassus* on the swim bladder of upstream migrant American eels captured at the Millville eel ladder on the Shenandoah River. Scores ranged from 0 to 6, with a 0 indicating no pathological signs of infection were observed and 6 denoting extreme abnormalities.

Figure 2-1: Age to Swim bladder degenerative index score relationships of American eels from the Millville Dam eel ladder, Shenandoah River, WV.

Chapter 3: Pit Tag Retention in Small (205-370 mm) American Eels

Abstract

Passive integrated transponders (PIT tags) are commonly used in ecological studies of aquatic and terrestrial animals. Researchers have used PIT tags in American eels (*Anguilla rostrata*) to study growth rates, home range size, and migration. The placement of PIT tags within the body of American eels differs among studies, including insertion in the dorsal musculature behind the head, the dorsal musculature near the dorsal fin origin, and the abdominal cavity. Retention rates of PIT tags for American eels are reported in the literature, but researchers have not previously compared and reported tag retention rates among different tagging locations. The objective of this study was to compare retention rates of PIT tags placed in the dorsal musculature behind the head, the dorsal musculature near the dorsal fin origin, and the abdominal cavity of American eels. Eighteen American eels were collected during fall 2006 from the Millville dam eel ladder on the lower Shenandoah River, West Virginia. Each eel was PIT tagged in three locations and tag retention was monitored for 9 weeks. Tag retention in the dorsal musculature near the dorsal fin origin (100%) and the abdominal cavity (100%) were higher than the retention rate of tags placed behind the head (88%). Our results, consistent with previous literature, support an overall high retention rate of PIT tags in American eels. Previous studies have primarily focused on large eels and our results document high tag retention rates in small eels (205-370 mm total length).

Introduction

Passive integrated transponders (PIT tags) are small microchips with coiled antennas enclosed in biocompatible glass. These tags are typically injected into the body cavity or muscle tissue of an animal. Researchers have used PIT tags on many taxa, such as bats (*Eptesicus fuscus*; Neubaum et al. 2005), crayfish (*Pacifastacus leniusculus*; Bubbs et al. 2002), ferrets (*Mustela putorius furo* and *Mustela nigripes*; Fagerstone and Johns 1987), frogs (*Rana muscosa*; Matthews 2003), king crabs (*Paralithodes camtschaticus*; Donaldson et al. 1992), penguins (*Pygoscelis adeliae*; Ballard et al. 2001), quail (*Colinus virginianus*; Carver et al. 1999), rattlesnakes (*Sistrurus miliarius*; Jemison et al. 1995), seals (*Mirounga leonine*; Galimberti et al. 2000), sea turtles (*Chelonia mydas* and *Caretta*

caretta; Broderick and Godley 1999), and sea urchins (*Strongylocentrotus franciscanus*; Shelton et al. 2006). These “passive” tags remain dormant in the body cavity or muscle tissue until activated by a handheld or automated reader. The reader sends a low frequency signal to the microchip, which activates the tag, and retrieves an individual alphanumeric sequence code. These sequence codes are unique to each tag, thus allowing researchers to identify individual animals (Prentice et al. 1990a).

In fisheries, PIT tags were first used for mark-recapture studies of coho and Chinook salmon (*Oncorhynchus kisutch* and *Oncorhynchus tshawytsch*; Prentice and Park 1984). Since this initial study, PIT tags have gained extensive use in fisheries research, including studies of arctic grayling (*Thymallus arcticus*; Buzby and Deegan 1999), black rockfish (*Sebastes melanops*; Parker and Rankin 2003), creek chub (*Semotilus atromaculatus*; Smithson and Johnston 1999), damselfish (*Pomacentrus amboinensis*; McCormick and Smith 2004), lampreys (*Petromyzon marinus*; Quintella et al. 2005), red drum (*Sciaenops ocellatus*; Jenkins and Smith 1990), sculpin (*Cottus bairdii*; Ruetz et al. 2006), striped bass (*Morone saxatilis*; Jenkins and Smith 1990), sturgeon (*Acipenser oxyrinchus desotoi*; Clugston 1996), and sunfish (*Lepomis cyanellus* and *Lepomis megalotis*; Smithson and Johnston 1999). Researchers have also used PIT tags to study movements of fishes associated with fishways and dams, as PIT tag readers can be set up directly where fish pass, allowing for scanning of individual fish (Prentice et al. 1990b; Burke and Jepson 2006).

PIT tags have many advantages to other methods of mark-recapture in fisheries, including fin clipping, branding, use of dyes and pigments, and coded wire tags (Harvey and Campbell 1989; Jenkins and Smith 1990; Prentice et al. 1990b). These other tags suffer from faded identification numbers, unrecognizable marks, tag loss, or the need to sacrifice the study animal to obtain information (Prentice and Park 1984; McFarlane et al. 1990; Gibbons and Andrews 2004). Handling time of tagged animals is reduced with PIT tags relative to other forms of tagging (Prentice et al. 1990b). Further, PIT tags are internal and often permanent (except for tag loss), have little influence on animal behaviors, and allow for identification of individuals (Prentice and Park 1984; Jenkins

and Smith 1990; Prentice et al. 1990a). Also, PIT tags often have high tag detection rates (95-100%), as well as reader accuracy near 100% (Prentice et al. 1990b; McCutcheon et al. 1994).

Despite the overall high success rate of PIT tags, tag retention rates differ among species and the area of tag placement. Tag loss ranges from 0% in largemouth bass (*Micropterus salmoides*; Harvey and Campbell 1989) to 99.4% in paddlefish (*Polyodon spathula*; Onders et al. 2001). Most studies on bony fish, however, report tag loss rates of less than 5% (Prentice and Park 1984; Moore 1992; Ombredane et al. 1998; Das Mahaptra et al. 2001; Dare 2003). In American eel (*Anguilla rostrata*) studies, PIT tag retention rates range from 89% to 100% (Table 3-1; Morrison and Secor 2003; Verdon and Desrochers 2003; Verdon et al. 2003; Thomas 2006). Tag loss can result from incorrect tagging techniques, rejection of the tag by the animal's body, or migration of the tag within the body (Gibbons and Andrews 2004). Tag loss is a serious problem for mark-recapture studies (McFarlane et al. 1990). For example, recaptured animals (with lost tags) counted as newly-tagged animals bias the ratio of recaptured animals to tagged animals (Arnason and Mills 1981; Stobo and Horne 1994; Schwarz and Stobo 1999), although biases associated with tag loss are correctable with estimates of retention rates.

PIT tag retention is influenced, in part, by tag placement (Elbin and Burger 1994; Gibbons and Andrews 2004). Gibbons and Andrews (2004) state that “the success of tagging technique is determined almost solely by placement of the tag”, and recommend that researchers perform pilot tag retention studies before using PIT tags. Studies of PIT tag retention in American eel have not compared different tagging locations. Our study objective was to quantify retention rates of three PIT tag placements in American eels. This research was a pilot study to determine PIT tag placement for a study of upstream migration in American eels, but results will also apply to other PIT tag studies of American eels.

Materials and Methods

Collection and Laboratory Setting

Eighteen American eels were collected in October 2006 from the Millville Dam eel ladder on the lower Shenandoah River, West Virginia. Eels were collected in a 4.76 mm mesh net attached to the upstream end of the eel ladder. Once in the laboratory, eels were held in a 380 L recirculation-system holding tank with constant water flow, and fed minnows and crayfish. Water temperatures within the tank ranged from 13.4°C – 18.8°C over the duration of the study. The eels were acclimated to this tank setting for two months prior to the start of the study.

PIT Tagging Methods

Before insertion of PIT tags, eels were anesthetized with a clove oil solution (10 L H₂O, 1.2 ml clove oil, and 12 ml of ethanol) following methods of Anderson et al. (1997). Prior to tagging, eels remained in the clove oil solution for approximately 5 minutes. Each eel was tagged in three separate locations with PIT tags (Destron-Fearing TX1411L, size 12.50 mm X 2.07 mm) using a modified syringe with a 12 gauge needle. Tagging locations included the dorsal musculature near the dorsal fin origin, the abdominal cavity, and the dorsal musculature behind the head (Figure 3-1 and Appendix B). Eel lengths and corresponding individual PIT tag numbers were recorded. The tagging wounds were left unclosed to heal naturally. After being tagged, the eels were placed in an aerated recovery tank for approximately 20 minutes before being returned to the holding tank.

PIT tag retention was checked weekly for a period of 5 weeks and then bi-weekly for 4 additional weeks, for a total of 9 weeks. To check tag retention, eels were removed from the tank and anesthetized (as described above). A handheld PIT tag reader (Destron-Fearing, Mini Portable Reader 2, Part # 800-0249-01) was used to check each eel for all three PIT tags. After tag checks, eels were placed in a recovery tank for 20 minutes

before return to the holding tank. Shed tags were located in the substrate of the holding tank following the study. Confidence intervals for tag retention data were generated with SAS version 9.1 using the profile likelihood method.

Results

Eels ranged in length from 205 to 370 mm, with a mean length of 311.7 ± 39.0 SE mm (Table 3-2). Tag retention was relatively high in all three tagging locations. Retention rates were highest (100%) in the dorsal musculature near the dorsal fin origin and in the abdominal cavity (lower CI = 0.885). Lower retention rates (88%) for the dorsal musculature near the head resulted from two lost tags within the first week of the study (lower CI = 0.695 and upper CI = 0.985) (Table 3-2). Both shed tags were located in the tank, confirming tag loss and not tag or scanner malfunctions.

Discussion

PIT tag retention in this study was 100% for American eels tagged in the dorsal musculature and in the body cavity, and 88% for eels tagged behind the head. These results are similar to those published in the literature (Table 3-1). In a 6-month laboratory study, Thomas (2006) also found 100% retention for American eels tagged in the dorsal musculature. Conversely, Verdon et al. (2003) reported a lower retention rate for eels tagged in the dorsal musculature, with a success rate of 93.9%. Morrison and Secor (2003) also found lower retention rates (89%) for eels tagged in the abdominal cavity relative to our study.

Estimates of PIT tag retention rates from laboratory studies may overestimate retention rates from natural environments. Lower retention rates reported by Verdon et al. (2003) and Morrison and Secor (2003) were from eels in riverine environments, and eels within rivers may have a higher likelihood of tag loss than those in captivity (Thomas 2006). The range of water temperatures ($13.4^{\circ}\text{C} - 18.8^{\circ}\text{C}$) during this study may have reduced

eel activity. Temperature triggers activity in eels, and temperatures $\geq 20^{\circ}\text{C}$ are associated with peak increases in eel activity (Knights and White 1998; Verdon and Desrochers 2003; Verdon et al. 2003). Eels that are migrating upstream, like those reported by Verdon et al. (2003), may be more likely to lose tags than more sedentary eels in laboratory holding tanks.

Thomas et al. (2006) suggested that retention rates may be influenced by eel size. Thomas et al. (2006) tagged eels ≥ 500 mm; larger than mean eel lengths from other PIT tag studies (Table 3-1). Verdon and Desrochers (2003) found higher retention rates (98%) for eels tagged behind the head, as compared to this study (88%). The larger mean length (464.8 mm) of the eels tagged by Verdon and Desrochers (2003) relative to our study (311.6 mm) may explain differences in retention rates. The low mean length of the eels in our study (311.7 ± 39 mm SE), however, indicates that high tag retention is possible in small eels.

Loss of PIT tags may result from incorrect tagging technique or rejection of the tag by the animal's body. Also, migration of a tag within an animal's body, and tag or scanner malfunctions mimic tag loss, because undetected tags are assumed lost. Most tag losses result from improper implantation of the tag (Gibbons and Andrews 2004). Dare (2003) attributed inexperience of the tagging crew to high tag loss in spring Chinook salmon (*Oncorhynchus tshawytscha*), because tag loss decreased with an increase in experience of the tagging crew (Dare 2003). Tag migration may increase with the size of the tagged animal (Gibbons and Andrews 2004). Camper and Dixon (1988) reported tag migration in 44% of the kingsnakes (*Lampropeltis getulus holbrooki*) and 71% of collared lizards (*Crotaphytus c. collaris*) with abdominally-placed PIT tags. Prentice and Park (1984) reported migration of abdominally-placed PIT tags in 5% of juvenile fall Chinook salmon (*Oncorhynchus tshawytscha*). Tag migration, however, is unlikely to influence tag detection in American eels, because tag readers can easily scan the entire body of the eel.

Tag loss in our study probably resulted from either improper implantation or tag rejection. The two tags shed during the first week of this study likely exited through the

tagging wound. Feldheim et al. (2002) found that lemon sharks (*Negaprion brevirostris*) shed PIT tags through unclosed wounds a few days after tagging. Prentice and Park (1984) also reported shed tags (through the tagging wound) in coho and Chinook salmon (*Oncorhynchus kisutch* and *Oncorhynchus tshawytscha*) within a few days after tag placement.

Our results support two body locations for PIT tag placement in American eels; the abdominal cavity and the dorsal musculature near the dorsal fin origin. Tags inserted into the abdominal cavity, however, may increase risk of mortality in American eels owing to the proximity of the tagging syringe needle to internal organs, but we did not experience tagging mortality. Our study was relatively short-term (9-weeks) and longer-term studies are needed to further understand differential rates of retention among tagging locations. Additionally, laboratory-based studies restrict eel movements and provide a relatively benign environment, hence, our laboratory results may not allow inference to tag-retention rates of American eels within riverine habitats.

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Table 3-1: Synthesis of PIT tag retention rates from American eel studies.

Study	Location of Study	Duration	Eel Length (mm)	Tag Location	Tag Retention
Thomas (2006)	Laboratory	6 months	≥ 500	Dorsal musculature	100%
Morrison and Secor (2003)	Hudson River, NY	2 months	Mean = 457	Visceral cavity	89%
Verdon and Desrochers (2003)	St. Lawrence River, NY	1998-1999	Mean = 471.7 (1998) Mean = 468.7 (1999)	Behind the head	98%
Verdon et al. (2003)	Richelieu River, Quebec	1997-1999	Mean = 379.7	Dorsal Musculature	93.9%

Table 3-2: PIT tag retention in small American eels (205 – 370 mm total length) during a 9 week laboratory study (P = tag present, H = head tag shed, D = dorsal tag shed, and A = abdominal tag shed).

Length (mm)	Week								
	1	2	3	4	5	6	7	8	9
205	P	P	P	P	P	P	P	P	P
270	H	-	-	-	-	-	-	-	-
275	P	P	P	P	P	P	P	P	P
280	P	P	P	P	P	P	P	P	P
295	P	P	P	P	P	P	P	P	P
300	P	P	P	P	P	P	P	P	P
300	P	P	P	P	P	P	P	P	P
305	P	P	P	P	P	P	P	P	P
310	P	P	P	P	P	P	P	P	P
310	P	P	P	P	P	P	P	P	P
330	P	P	P	P	P	P	P	P	P
340	H	-	-	-	-	-	-	-	-
340	P	P	P	P	P	P	P	P	P
340	P	P	P	P	P	P	P	P	P
345	P	P	P	P	P	P	P	P	P
345	P	P	P	P	P	P	P	P	P
350	P	P	P	P	P	P	P	P	P
370	P	P	P	P	P	P	P	P	P

Figure 3-1: PIT tag location for American eels including 1) dorsal musculature behind the head, 2) dorsal musculature near the dorsal fin origin, and 3) the abdominal cavity.

Appendix A: Pictures of the Millville Dam Eel Ladder

Millville Dam eel ladder on the Shenandoah River (designed by Milieu, Inc. Canada). Photo by J.L. Zimmerman

Design specifications of the Millville Dam eel ladder (A) flashboard placement and attraction flow, (B) internal design, (C) source of facilitation flow, and (D) upstream collection tank, which contains the mesh nets. Photos from Hildebrand (2005).

Mesh net used to collect eels in the holding tank. Photo by J.L. Zimmerman.

Appendix B: PIT Tagging Pictures

Pit tagging an American eel in the dorsal fin origin. Photo by J.L. Zimmerman

Close-up of an American eel being PIT tagged in the dorsal fin origin. Photo by J.L. Zimmerman

Appendix C: Parasite, Length, and Ageing Data

Length	Nematode Present	Intensity	Age	Swim Bladder Transparency	Swim Bladder Size	Exudate/ Pigment	SDI Score
200	NO	0	-	-	-	-	-
215	NO	0	-	-	-	-	-
224	NO	0	-	-	-	-	-
231	NO	0	-	-	-	-	-
233	NO	0	-	-	-	-	-
243	NO	0	-	-	-	-	-
244	NO	0	4	0	0	0	0
250	NO	0	-	-	-	-	-
253	NO	0	-	-	-	-	-
254	NO	0	-	-	-	-	-
255	NO	0	5	0	0	2	2
256	NO	0	-	-	-	-	-
256	NO	0	-	-	-	-	-
258	NO	0	-	0	0	0	0
259	NO	0	-	0	0	0	0
265	NO	0	-	-	-	-	-
266	NO	0	-	0	0	0	0
269	NO	0	-	0	0	0	0
269	NO	0	-	0	0	0	0
276	NO	0	-	-	-	-	-
277	NO	0	9	-	-	-	-
278	NO	0	6	0	0	0	0
279	NO	0	-	-	-	-	-
279	NO	0	4	0	0	1	1
280	NO	0	-	-	-	-	-
280	NO	0	-	0	0	0	0
282	NO	0	7	-	-	-	-
282	NO	0	-	-	-	-	-
282	NO	0	7	0	0	2	2
284	NO	0	-	-	-	-	-
285	NO	0	7	0	0	0	0
285	NO	0	-	0	0	0	0
285	NO	0	-	0	0	0	0

Length	Nematode Present	Intensity	Age	Swim Bladder Transparency	Swim Bladder Size	Exudate/ Pigment	SDI Score
286	NO	0	-	-	-	-	-
286	NO	0	-	0	0	0	0
288	NO	0	-	-	-	-	-
289	NO	0	5	-	-	-	-
289	NO	0	5	1	0	0	1
290	NO	0	4	-	-	-	-
290	NO	0	-	-	-	-	-
290	NO	0	7	1	0	1	2
290	NO	0	7	0	0	1	1
291	NO	0	-	0	0	0	0
293	NO	0	-	-	-	-	-
294	NO	0	-	0	0	0	0
295	NO	0	-	-	-	-	-
295	NO	0	-	-	-	-	-
296	NO	0	-	0	0	0	0
299	NO	0	4	0	0	0	0
299	NO	0	7	0	0	0	0
299	NO	0	-	0	0	0	0
300	NO	0	-	-	-	-	-
301	NO	0	-	-	-	-	-
301	NO	0	-	0	0	0	0
302	NO	0	-	0	0	0	0
302	NO	0	-	0	0	2	2
303	NO	0	-	-	-	-	-
303	NO	0	-	-	-	-	-
304	NO	0	7	0	0	0	0
308	NO	0	10	1	0	1	2
309	NO	0	-	-	-	-	-
309	NO	0	-	-	-	-	-
310	NO	0	-	-	-	-	-
310	NO	0	-	-	-	-	-
310	NO	0	-	-	-	-	-
310	NO	0	-	-	-	-	-
311	NO	0	-	-	-	-	-
311	NO	0	7	0	0	0	0
316	NO	0	-	0	0	0	0

Length	Nematode Present	Intensity	Age	Swim Bladder Transparency	Swim Bladder Size	Exudate/ Pigment	SDI Score
317	NO	0	-	-	-	-	-
320	NO	0	-	-	-	-	-
320	NO	0	-	-	-	-	-
320	NO	0	-	-	-	-	-
320	NO	0	5	1	0	1	2
320	NO	0	10	1	0	1	2
324	NO	0	-	-	-	-	-
324	NO	0	6	0	0	1	1
325	NO	0	-	-	-	-	-
326	NO	0	-	-	-	-	-
326	NO	0	-	-	-	-	-
326	NO	0	-	-	-	-	-
326	NO	0	-	1	0	1	2
328	NO	0	-	0	0	0	0
329	NO	0	-	-	-	-	-
330	NO	0	6	-	-	-	-
330	NO	0	-	0	0	0	0
331	NO	0	7	-	-	-	-
331	NO	0	-	-	-	-	-
331	NO	0	-	-	-	-	-
334	NO	0	-	-	-	-	-
335	NO	0	-	-	-	-	-
335	NO	0	-	-	-	-	-
335	NO	0	-	-	-	-	-
336	YES	1	-	-	-	-	-
338	NO	0	-	-	-	-	-
338	NO	0	-	-	-	-	-
339	NO	0	-	-	-	-	-
339	NO	0	-	-	-	-	-
339	NO	0	-	-	-	-	-
340	NO	0	5	-	-	-	-
340	NO	0	-	-	-	-	-
340	NO	0	-	-	-	-	-
340	NO	0	-	-	-	-	-
340	NO	0	-	-	-	-	-
341	NO	0	-	-	-	-	-

Length	Nematode Present	Intensity	Age	Swim Bladder Transparency	Swim Bladder Size	Exudate/ Pigment	SDI Score
342	NO	0	-	-	-	-	-
342	NO	0	-	-	-	-	-
343	NO	0	-	-	-	-	-
343	NO	0	-	-	-	-	-
344	NO	0	-	0	0	1	1
346	NO	0	-	-	-	-	-
346	NO	0	-	-	-	-	-
347	NO	0	-	-	-	-	-
347	NO	0	-	-	-	-	-
347	NO	0	5	0	0	1	1
348	NO	0	-	-	-	-	-
349	NO	0	-	-	-	-	-
349	NO	0	-	0	0	1	1
350	NO	0	-	-	-	-	-
350	NO	0	-	0	0	0	0
351	NO	0	-	-	-	-	-
351	NO	0	-	-	-	-	-
352	NO	0	-	-	-	-	-
352	NO	0	-	-	-	-	-
354	NO	0	-	-	-	-	-
355	NO	0	-	-	-	-	-
355	NO	0	-	-	-	-	-
355	NO	0	-	-	-	-	-
356	NO	0	-	-	-	-	-
356	NO	0	-	-	-	-	-
358	NO	0	-	-	-	-	-
360	NO	0	-	-	-	-	-
360	NO	0	-	-	-	-	-
361	NO	0	-	-	-	-	-
361	NO	0	-	-	-	-	-
361	NO	0	-	-	-	-	-
362	NO	0	-	-	-	-	-
362	NO	0	-	-	-	-	-
362	NO	0	-	-	-	-	-
362	NO	0	-	0	0	0	0
364	NO	0	-	-	-	-	-

Length	Nematode Present	Intensity	Age	Swim Bladder Transparency	Swim Bladder Size	Exudate/ Pigment	SDI Score
365	NO	0	-	-	-	-	-
366	NO	0	6	-	-	-	-
366	NO	0	-	-	-	-	-
366	NO	0	-	-	-	-	-
368	NO	0	-	-	-	-	-
368	NO	0	-	-	-	-	-
368	NO	0	-	-	-	-	-
368	NO	0	-	-	-	-	-
369	NO	0	-	-	-	-	-
369	NO	0	-	-	-	-	-
370	NO	0	7	-	-	-	-
370	NO	0	-	-	-	-	-
370	NO	0	-	-	-	-	-
370	NO	0	-	-	-	-	-
371	NO	0	-	-	-	-	-
373	NO	0	8	-	-	-	-
373	NO	0	-	-	-	-	-
373	NO	0	-	-	-	-	-
374	NO	0	-	-	-	-	-
374	NO	0	8	1	0	0	1
375	NO	0	-	-	-	-	-
375	NO	0	-	-	-	-	-
375	NO	0	-	-	-	-	-
376	NO	0	-	-	-	-	-
378	NO	0	-	-	-	-	-
378	NO	0	-	-	-	-	-
379	NO	0	-	-	-	-	-
379	NO	0	-	-	-	-	-
379	NO	0	-	-	-	-	-
379	NO	0	-	1	0	1	2
380	NO	0	5	-	-	-	-
380	NO	0	7	-	-	-	-
380	NO	0	-	-	-	-	-
380	NO	0	6	0	0	1	1
381	NO	0	4	-	-	-	-
383	NO	0	-	-	-	-	-

Length	Nematode Present	Intensity	Age	Swim Bladder Transparency	Swim Bladder Size	Exudate/ Pigment	SDI Score
386	YES	1	-	-	-	-	-
387	NO	0	-	-	-	-	-
388	NO	0	10	-	-	-	-
389	NO	0	-	-	-	-	-
390	NO	0	-	-	-	-	-
390	NO	0	-	-	-	-	-
390	NO	0	-	-	-	-	-
390	NO	0	-	0	0	0	0
390	NO	0	-	0	0	1	1
391	NO	0	-	-	-	-	-
391	NO	0	-	-	-	-	-
392	NO	0	-	-	-	-	-
392	NO	0	-	-	-	-	-
394	NO	0	-	-	-	-	-
394	NO	0	-	-	-	-	-
394	NO	0	-	-	-	-	-
395	NO	0	-	-	-	-	-
396	NO	0	-	-	-	-	-
398	NO	0	10	-	-	-	-
400	NO	0	-	-	-	-	-
400	NO	0	-	-	-	-	-
402	NO	0	-	-	-	-	-
402	NO	0	-	-	-	-	-
404	NO	0	-	-	-	-	-
405	NO	0	-	-	-	-	-
405	NO	0	-	0	0	0	0
406	NO	0	7	-	-	-	-
407	NO	0	-	-	-	-	-
407	NO	0	-	-	-	-	-
408	NO	0	7	-	-	-	-
408	NO	0	-	-	-	-	-
408	NO	0	-	-	-	-	-
413	NO	0	-	-	-	-	-
415	NO	0	-	-	-	-	-
418	NO	0	-	0	0	0	0
419	NO	0	-	-	-	-	-

Length	Nematode Present	Intensity	Age	Swim Bladder Transparency	Swim Bladder Size	Exudate/ Pigment	SDI Score
420	YES	1	11	-	-	-	-
421	YES	1	8	-	-	-	-
422	NO	0	-	-	-	-	-
423	NO	0	-	-	-	-	-
425	NO	0	-	-	-	-	-
429	NO	0	6	-	-	-	-
429	NO	0	-	-	-	-	-
434	NO	0	-	-	-	-	-
438	NO	0	-	-	-	-	-
439	NO	0	-	-	-	-	-
440	NO	0	-	-	-	-	-
440	NO	0	-	-	-	-	-
440	NO	0	-	-	-	-	-
442	NO	0	-	-	-	-	-
443	NO	0	-	-	-	-	-
450	NO	0	9	-	-	-	-
450	NO	0	-	-	-	-	-
450	NO	0	-	-	-	-	-
452	NO	0	-	-	-	-	-
467	NO	0	8	0	0	0	0
471	NO	0	-	-	-	-	-
473	YES	1	-	-	-	-	-
475	NO	0	-	-	-	-	-
480	NO	0	-	-	-	-	-
482	NO	0	-	-	-	-	-
485	NO	0	-	-	-	-	-
486	NO	0	-	-	-	-	-
518	NO	0	-	-	-	-	-
527	NO	0	-	-	-	-	-