Geographic distribution and aspects of the parasite/host relationships of the invasive swim bladder parasite *Anguillicoloides crassus* infecting American eel (*Anguilla rostrata*) in mainland Nova Scotia and New Brunswick

By

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Abstract

Geographic distribution and aspects of the parasite/host relationships of the invasive swim bladder parasite *Anguillicoloides crassus* infecting American eel (*Anguilla rostrata*) in mainland Nova Scotia and New Brunswick

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Between 2008-2013, 1,981 eels were collected from 174 localities throughout mainland Nova Scotia and New Brunswick and necropsied for the swim bladder nematode *Anguillicoloides crassus*. Overall prevalence of *A. crassus* was 4 % with a mean intensity of 3.8 ± 8 SD (1-63 parasites). The Southern Uplands, Gulf of St. Lawrence and the Bay of Fundy regions were all identified as having rivers with eels infected with the nematode. The prevalence within the Bay of Fundy region was 7.4 % (40/539) with infected eels found in the Saint John River and Shubenacadie River. The Southern Uplands had a prevalence of 2.4 % (34/1395) with a focus of infection in the Mersey and Medway Rivers. Finally, the Gulf region had a prevalence of 4 % (2/47), with a single site West River, Antigonish having the 2 infected fish. Condition factor, HSI, and SSI did not correlate with eel length and weight. Infected fish were significantly longer and heavier than non infected eels sampled. Information obtained on the distribution of the parasite in the present study is joined with previously published surveys, revealing the parasite to be in the St. John River and throughout Cape Breton with isolated localities in Mersey, Medway, St, Mary`s and Salmon (Halifax Co.) River. Further work is needed to monitor the spread of *A. crassus* and to understand how eels are adapting or not, to this invasive parasite in Atlantic Canada.

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Introduction

American eel, *Anguilla rostrata,* is a common species found in both freshwater and marine ecosystems of Atlantic Canada (Scott & Crossman 1973). It has an extensive distribution along the coastal northwest Atlantic Ocean, including southwestern Greenland, southward along the eastern coast of North America, including inland areas of the St. Lawrence, Great Lakes, and along the coastal United States, southward toward the northern region of South America (Scott & Crossman 1973, Tesch 1977). The latitudinal range spans from 5 degrees to 60 degrees North (Bertin 1956) covering a range of approximately 30,000 km of coastline. American eel is thought to occupy one of the largest ranges of habitats of any fish in the world (Helfman et al. 1987).

American eel was initially thought to be an obligate catadromous species, where it spent its life in freshwater and only entered the sea once sexual maturity was reached (Tesch 1977). Catadromy was indeed demonstrated to be facultative, in that the species does not need to live specifically in freshwater before migrating to sea (Tzeng et al. 2000, Tsukamoto & Arai 2001). Japanese eel (*Anguilla japonica*) was the first anguillid species studied for inter-habitat movement during the yellow eel phase by microchemistry analysis of trace elements in otoliths (ear bones) (Tsukamoto & Arai 2001), which identified the Japanese eel as facultatively catadromous moving between salt and freshwater habitats. American eel was then also found to be facultatively catadromous; there can be resident eels in freshwater or marine habitats, and also inter-habitat transients (Lamson et al. 2006 & 2009, Jessop et al. 2008).

Eels are largely nocturnal foragers (Tesch 2003). They can burrow laterally through muddy substrates, typically for distances < 1 meter (Tomie et al. 2013). During the winter months, eels become sluggish with the onset of cooler temperatures, and typically burrow into the mud of lakes and rivers, as they enter a state of torpor (temporary hibernation) at temperatures around 5ºC (Scott & Scott 1988, Tesch 2003). Eels are opportunistic foragers meaning they will feed on dead, dying or living organisms including aquatic insects, crustaceans, molluscs, amphibians, and small fishes including smaller eels (Tesch 2003, Russell & Campbell 2011).

American eel is a panmictic semelparous species, meaning they comprise a single population wherein adults spawn in the Sargasso Sea (in the western Atlantic Ocean, east of Bahamas and south of Bermuda) only once and die. Typically, eels begin to descend from lakes and rivers in the fall in Atlantic Canada, with spawning in the Sargasso Sea beginning in late winter lasting into early spring (February – June) (Schmidt 1922, Vladykov 1964).

Larval eels are known as leptocephali, a delicate, ribbon-like, transparent larva (Scott & Scott 1988). The larva is pelagic. They are transported by Gulf Stream from the Sargasso Sea for seven to twelve months (Helfman et al. 1987, McCleave et al. 1987, Tesch 2003). During this oceanic phase, the leptocephali are presumed to metamorphose into glass eels nearing the continental shelf, and then continue on to coastal estuaries (early spring in the Maritimes region of Atlantic Canada)(Tesch 2003). Once they enter estuaries, pigmentation begins and the glass eels become more eel-like in appearance (Bertin 1956). Known now as elvers, they become more robust, with a smoother, cylindrical shape and elongated form, ranging in size from 70-90 mm (Jessop 1987, Scott

& Scott 1988). As elvers grow into yellow phase eels, skin thickens, and turns into an olive-brown color on the dorsal side, with pale yellow to a light brown pigmentation on the ventral side. The yellow eel phase, which can last up to forty years, is the principal stage for feeding, growth and sexual differentiation (Jessop 1987). The final stage in eel development is the silver eel phase, occurring once sexual maturity beings and when the eels are preparing to migrate to the spawning grounds of the Sargasso Sea. During the silver phase, eels become darker on their dorsal side, taking on a white to iridescent coloring on the ventral side, as well as undergoing morphological changes such as thickening of the body wall, shrinking of the stomach, and enlargement of gonads, eyes, and pectoral fins (Tesch 1977, Scott & Scott 1988). Males mature at between 5-13 years and lengths of 30-50 cm, while females are found to be larger and older than silver males. Females mature from 5-40 years at lengths of 50-100 cm (Scott & Crossman 1973, Helfman et al. 1987, Jessop 1987).

In 2006, the Committee on the Status of Endangered Wildlife in Canada designated American eel as a species of “Special Concern” (COSEWIC 2006), for the freshwater and coastal habitats of Quebec, Newfoundland and Labrador, Gulf, Maritimes and Ontario regions. Most recently, the COSEWIC species designation has been reviewed and the fish has been re-assessed as "Threatened" (COSEWIC 2012). The threatened designation identifies that American eel will reach the endangered status if nothing is done to reverse the decline in the populations in Canadian waters and beyond.

American eel indices of abundance have declined by approximately 99 % throughout the upper St. Lawrence and Great Lakes Basin area, since the 1970`s (Castonguay et al. 1994, COSEWIC 2006). Based on the rapid and yet unexplained decline in recruitment of juvenile eel to the Lake Ontario area, the potential for further declines in the whole Canadian eel population should be noted (COSEWIC 2006).

American eel is a biologically mysterious species with a long life span, but much of the life cycle being poorly understood. The eel has an extensive migration, semelparous reproduction with death after spawning, and a perilous drift up the east coast of North America before metamorphosing when finally reaching their preferred habitat either freshwater and/or brackish environments. It is important to understand factors that could interfere with the eels' survival and reproduction. These factors include chemical pollution, commercial fishing, parasite introductions and diseases, anthropogenic habitat modifications (e.g. hydro-electric facilities), climate change, and stocking uncertainty (Castonguay et al. 1994, Haro, et al. 2000, COSEWIC 2006, MacGregor et al. 2008).

Appearance of the exotic parasite *Anguillicoloides crassus* (Kuwahara, et al. 1974, Moravec & Taraschewski, 1988) (Nematoda, Dracunculoidea, Anguillicolida), which causes major pathology to the swim bladder of American eel, is a major concern for it has spread rapidly over the last decade along the northeast coast of North America (Johnson et al. 1995, Fries et al. 1996, Barse & Secor 1999, Barse et al. 2001, Moser 2001, Aieta & Oliveira 2009, and Rockwell et al. 2009). The parasite is native to Asia where it infects the Japanese eel (*Anguilla japonica)* with little or no observable effect on the host eel (Moravec & Tarashewski 1988, Han et al. 2008).

Anguillicoloides crassus males and females copulate in the swim bladder of the eel (DeCharleroy et al. 1990). The fertilized eggs develop in the female reproductive system. The eggs pass through the pneumatic duct to digestive tract, where they can hatchand be expelled into the water column as an L2 (second stage) or as eggs that subsequently hatch. The free-living L2 larvae will attach to substrate and imitate prey to attract predation, fromcopepods, gastropods and ostracods (Moravec & Skorikova 1998) that serve as intermediate hosts. The larvae moult in the intermediate hosts and reach the infective third stage after 10 -12 days (Thomas & Ollevier 1989). When these infected intermediate hosts are consumed by fish or amphibians, known as paratenic host, the L3 larvae survive. When the host eel consumes an intermediate or paratenic host L3 larvae reach the swim bladder wall by passing through the intestinal wall and body cavity of the eel (Haenen et al. 1989). Nematodes moult into the L4 stage after 2 -3 weeks in the bladder, feeding on the blood and moult again before becoming sexually mature (De Charleroy et al. 1990).

All life stages of *A. crassus* survive in both fresh water and marine environments in laboratory and natural settings (Kirk et al. 2000), although it generally favors freshwater with an 80-day, 100% survival compared to eight-day, 100% survival rate in seawater at 10 °C (Kirk et al. 2000). The nematode can tolerate water temperatures of 4 to 20°C and exhibit rapid development with increasing temperatures (Knopf et al. 1998, Kirk et al. 2000). Its ability to adapt to different salinities and temperatures, coupled with the high reproductive rate of females and their rapid two to four month life cycle has certainly fueled its rapid spread over the last three decades both in Europe (De Charleroy et al. 1990, Kennedy et al. 1990, Moravec et al. 1994, Kirk et al. 2000) and in North America.

Anguillicoloides crassus infections affect different eel species in different ways. Studies in Japan indicate that *A. crassus* does not typically cause serious pathology in

Japanese eel (*Anguilla japonica*) (Egusa 1992). Studies of *A. crassus* infections in European eels suggested that the parasite was more harmful to the new hosts than what has been shown for the Japanese eel. European eels reared in Japan were found to be more susceptible to infection than the native Japanese eels (Moravec & Taraschewski 1988, Knopf et al. 2006). Infected European eels presented swollen abdomens, loss of appetite, thickened swim bladders and emaciation (Egusa 1992, Nagasawa et al. 1994). Ooi et al. (1996) observed in Taiwanese rearing facilities that among cultured American eels some were found infected with the parasite and had hemorrhaging, rupturing of the swim bladder, anemia and possible mortality.

Over the last three decades the parasite has spread to both European and North American eels as a result of initial accidental introductions (Kirk 2003). The first reported infection among European eel was from Germany in 1982, traced back to imported Japanese eel in a rearing facility (Peters & Hartmann 1986, Koie 1991). The parasite quickly spread through wild and farmed European eel, taking about one decade to infect most areas of Europe (Szekely et al. 1991, Moravec 1992, Evans & Matthews 1999).

In 1994, the first record of *A. crassus* in wild American eel was found at a Texas aquaculture facility, which had acquired elvers from a supplier on the U.S. east coast (Johnson et al. 1995). Eel from several Texas rivers and a river in South Carolina had no swim bladder parasites. The first wild American eel found to be infected with *A. crassus* was from South Carolina (Fries et al. 1996). Since the parasite has expanded its range along the North American east coast from South Carolina to Chesapeake Bay, the Hudson River, Rhode Island, Massachusetts, and Maine, USA (Fries et al. 1996, Barse & Secor 1999, Morrison & Secor 2003, and Aieta & Oliveira 2009). *Anguillicoloides crassus* has been found in Canada in the eastern provinces of Nova Scotia and New Brunswick (Aieta & Oliveira 2009, Rockwell et al. 2009, Wall 2011 and Denny 2013).

The first records (Aeita & Oliveira 2009, Rockwell et al. 2009) of *A. crassus* in the Maritime Provinces of Canada indicate *A.crassus* reached our region sometime between 1999 and 2006, but the current geographical distribution of the parasite in mainland Nova Scotia and New Brunswick, the extent of its distribution within individual locations, and the parasite/host relationships of growth, condition, and age are all poorly understood. Wall (2011) reported that *A. crassus* is present in American eel throughout Cape Breton Island at high prevalence's and intensities, raising concerns about the nematode's status in the rest of Atlantic Canada.

The present thesis extends and updates knowledge of *A. crassus* distribution in watersheds of New Brunswick and mainland Nova Scotia. The overall objective is to document the distribution of this nematode in a broad regional context and to examine the basics of the parasite/host relationship. The data collected from 2008 and 2009 have been previously published and used to support a national assessment for the Recovery Potential of American eel (Campbell et al. 2013).

Materials & Methods

Eel collection

During the summers and falls of 2008, 2009, 2012 and 2013 a total of 1,981 eels were collected primarily through electrofishing, rotary screw trap, beach seining, weirs, eel pots and downstream bypass facilities for *Anguillicoloides crassus* presence (Figure 1). Overall, eels were collected from 174 sites throughout 63 separate drainages from the provinces of Nova Scotia and New Brunswick (Tables 1-4, Figure 2). The majority of eels captured for this study were from freshwater habitats, with a small sample from brackish waters (Tables 1-4). Distance to sea measurements (km) were collected by using the measuring tool in Google Earth, by starting at the site coordinates where eels were collected and extending to the mouth of each river where it entered the Atlantic Ocean or the Gulf of St. Lawrence.

Eels were a bycatch of a juvenile salmonid electrofishing survey undertaken by the Department of Fisheries and Oceans (DFO), Dartmouth, Nova Scotia, Population Ecology Division. Backpack electrofishers (Smith & Root LR -24) were operated by certified DFO technicians. For each site sampled, up to 35 eels were retained and euthanized with an overdose of a clove oil (Eugenol) - water mix (Kennedy 2007), and placed on ice until transferred to freezer storage (-15^0 C) at the Bedford Institute of Oceanography.

To complement the DFO electrofishing survey samples, additional eels were obtained from a variety of sources. In May of 2008, a rotary screw trap was installed on Little Southwest Miramichi River, which supplied 40 eels. During the summer of 2009, there were various methods used including directed electrofishing, beach seining, downstream bypass facilities, and eel pots. Eel were also retained from a DFO young-of-the-year striped bass *(Morone saxatilis)* beach seine survey of the tidal Shubenacadie - Stewiacke River, Cobequid Bay and Minas Basin (July-September 2009). During the fall of 2009, eels were collected from downstream bypass facilities installed in hydroelectric dams located at Morgan's Falls, LaHave River, (May 2009) and Ruth Falls, East River, Sheet Harbour (September - October, 2009). Finally, a collection of eels was obtained from commercial eel pot catches during the fall of 2009 from the tidal portions of the Stewiacke River, inner Bay of Fundy, Nova Scotia.

The Medway River, in the Southern Uplands was sampled initially as part of the juvenile salmonid electrofishing survey preformed by DFO in 2008, and then sampled up river (Wildcat River joins the Medway River) in the fall of 2012 and 2013 by a commercial fisher using eel weirs, Louis Walmboldt (Table 3).

Eel necropsies

Specimens were processed within one year of capture. Each eel was measured for total length (cm) and total weight (± 0.1 g). It should be noted that freezing specimens leads to a reduction in total length and weight, of 1.2 -3.0 % and 1.9 % respectively (Morrison & Secor 2003, Machut & Limburg 2008). Total lengths and weights reported in this document have not been adjusted for the reduction since all eels were processed frozen. An incision was made on the ventral side of each eel, from just below the gills (i.e. the isthmus) to the anal vent. The visceral cavity was then macroscopically inspected for any parasites (removed/preserved if present), abnormalities, and/or hemorrhages. Stomach contents were examined at a macroscopic level and the contents identified when possible. Spleen and liver were removed and weighed separately (± 0.01 g). The swim bladder was removed, macroscopically inspected for *A. crassus* and other parasites, and any eel pathology noted (Figure 1). Any parasites present within the swim bladder were removed and stored in vials containing 95% ethanol (C_2H_5OH) until identification could be confirmed. Finally, otoliths were removed through an incision made from the tip of the snout to the middle of the brain case, and stored cleaned and dry in vials.

Data analysis

For analysis purposes the study area was broken into three sub-areas; 1) Bay of Fundy (BoF) - From the Maine-New Brunswick border around to Digby Nova Scotia; 2) the Southern Uplands (SU) - from Digby along the southern coast of Nova Scotia to the Canso Causeway (draining into the Atlantic); and 3) the Gulf of St. Lawrence (Gulf) - from the western side of the Canso Causeway to the New Brunswick-Québec border (Figure 2).

Anguillicoloides crassus infections were calculated using prevalence and mean intensity measures (Bush et al. 1997). Prevalence is the percentage of fish in a sample that are infected with at least one parasite. Mean intensity is the mean number of parasites per infected fish for a sample (Bush et al. 1997). Fulton`s condition (K) factor, which is an indicator of fish health, was calculated using the weight of the fish (g) (100)/ length of the fish $(cm)^3$) (Bagenal & Tesch, 1978, Moyle & Cech 2004). Mean hepatosomatic index (HSI = (liver weight (g)/total body weight (g))*100) and mean splenosomatic index (SSI = (spleen weight (g)/total body weight (g))*100) were calculated (Oliveira et al. 2005). Data were tabulated in Microsoft Excel and analyses were conducted using Excel and Minitab 16 applications.

Linear regressions were performed to explore the potential effects of predictor variables (length, weight, distance to sea, HSI, SSI and K), and prevalence and intensity. Variables were tested for normality using the Anderson Darling (AD) test. The intensity variable was not normally distributed (AD = 13.5, p-value < 0.005) directing the use of nonparametric Mann-Whitney (M-W) test to compare infected and non-infected HSI, SSI.

Results

A total of 1,981 eels from 174 localities in 63 separate drainages in Nova Scotia and New Brunswick was necropsied for presence of *A. crassus*. Eels ranged in length from 13.5 cm to 92.7 cm, with weights ranging from 0.2 g to 2.2 kg (Tables 1-4).

Overall prevalence of *A. crassus* was 4 % (76 of 1,981 eels) with a mean intensity of 4 ± 8 (SD) (range 1-63 parasites/fish). Eels of all sizes were infected, ranging from 13.5 cm to 81.3 cm in length (Figure 3) and 3.7 to 984.1g in weight. The smallest infected eel was 13.5 cm long, weighed 3.7 g, and was from the Saint John River tributary known as Coal Creek (site 3), located 92.2 km from the Bay of Fundy (BoF). This small eel had a single worm found in the swim bladder. The largest infected eel was from the Medway River (Wildcat site), located 39.7 km from the Atlantic Ocean; it measured 81.3 cm long, and had 6 adult parasites. The eel with the highest intensity of infection (63 parasites) was 74.7 cm long from the Medway River. Details of the infections of *A. crassus* by geographic region are presented in Tables 1 - 4.

Anguillicoloides crassus was present in the BoF region, with the highest prevalence and intensity of infection occurring in the Saint John River, from the Nerepris River northward to the Keswick and Nashwaak rivers (Table 1). Prevalence of infection did not change significantly with distance (km) from the BoF ($r = 0.208$, $p = 0.440$). Intensity was patchy in distribution with no significant correlation with distance from the BoF ($r = -0.092$, $p = 0.736$) (Figure 4).

The only other site in the BoF region to have infected eels was the tidal portion (brackish) of the Shubenacadie/Stewiacke River, Nova Scotia, where prevalence was 18 % (2 of 11) and mean intensity of 1 (Table 1). Of the 66 sites sampled in the BoF region, prevalence was 7 % (40 of 539) with a mean intensity of 2.5 ± 3.95 (SD) (ranging from 1-23) (Table 1).

The Southern Uplands (SU) region was extensively sampled during the years 2008, 2009 with specific site sampling on the Medway River during 2012, and 2013 for a total of 1,395 necropsied eels. From the 2008 - 2009 collections, prevalence was low at 0.3 % (4 of 1,350) and mean intensity of 1.5 ± 0.58 (SD) (Table 2), with one infected eel found from the St. Mary`s River, Salmon River (Lawrencetown), Medway, and Mersey Rivers. In 2012 - 2013, the additional collection of 45 eels from the Medway River upstream at the junction of the Wildcat River included 30 infected eels which raised the prevalence of infection for the SU region in the combined sample to 2.2 % (30 of 1,395 with a mean intensity to 6.1 ± 11.4 (SD) (Table 3). Prevalence at the Medway Wildcat site, increased from 58 % in 2012 to 76 % in 2013, but this was not significant statistically.

The Gulf Region had the lowest sample size, with only 47 eels necropsied (Table 4). Prevalence was 4 % (2 of 47) with a mean intensity of 2 ± 1.4 (SD) (range 1-3 parasites). These two infected eels were the only fish sampled from the West River, Antigonish County, 18 km upstream from the Northumberland Strait. These infected eels were 15.7 cm and 79 cm in length with corresponding weights of 4.8 g and 946.1 g. None of the 40 eels examined from the Miramichi were found to be infected with *A. crassus* (Table 4).

<u>Parasite host relationships and diet</u>

Necropsy data for the 1,981 eels were examined for insights into parasite/host relationships. Intensity of infection was weakly, but significantly correlated with increased length and weight ($r = 0.335$ and 0.251, respectively) ($p < 0.050$) (Figure 6, 7).

Condition factor (K) did not vary significantly with intensity (r = 0.105, p = 0.419) (Figure 8), but did vary between infected (mean K= 0.175) and non-infected (mean K = 0.162).

Interestingly, infected eels sampled were significantly longer (mean non-infected = 27.4 cm ± 10.2 (SD), mean infected = 40.4 cm ± 17 (SD) (t-value = -6.42, p-value = 0.001, DF = 72)) and heavier than non-infected eels sampled, (mean non-infected = 49 g ± 101 (SD) mean infected = 157g ± 214 (SD) (t-value = -3.99, p-value 0.001, DF = 61)) with both, two sampled t-tests showing a significant difference between the groups (Table 6, Figure 9). Length-weight growth relationships were compared and plotted as log transformed graphs of infected (r^2 = 0.9892) versus non-infected (r^2 = 0.9393) eels and were found not to be significantly different (Figure 10).

Mean HSI was significantly higher in infected eels 1.378 ± 0.322 (SD) than in uninfected eels (1.220 ± 1.196 (SD) (M-W p < 0.001, U-value = 36042, n= 1932, Median non-infected = 1.111, Median infected = 1.369). The mean SSI for non-infected eels was 0.327 ± 0.602 (SD) and for infected eels was 0.264 ± 0.173 (SD), with a weak difference between them (Mann Whitney p = 0.031, U-value = 69899, n =1924, Median non infected = 0.272, Median infected = 0.265). There was no significant correlation found between intensity and HSI (r = -0.173, p = 0.208; Figure 11), nor between intensity and SSI (r = 0.169, p = 0.218; Figure 12).

Examined stomachs contained primarily invertebrates, notably mayfly larva and caddisfly larva, and also unidentified aquatic insects, crabs (legs) and salmon fry, and unidentifiablel fishes). One eel sampled in 2009 from the Nashwaaksis River, NB, contained a larval Northern Two-lined Salamander (*Eurycea bislineata)* (specimen ID NBM9142) (Russell & Campbell, 2011).

Discussion

The results from this broad survey revealed that *A. crassus* occurs in 3 isolated pockets (Medway/Mersey Rivers, Salmon (Hfx Co.) River, St. Mary`s River) in the Southern Uplands of mainland Nova Scotia, in a single site (West River; site WstR 1) within the Gulf region, throughout the Saint John River up to Keswick River and at a site in the Shubenacadie River in the Bay of Fundy region. Prevalence from the Medway River obtained in 2008, 2012, and 2013 reached 76 % indicating that the parasite is well established at this site and, along with the adjacent Mersey River, represents a previously unrecognized focus of infection along the south coast of Nova Scotia. Parasite surveys of American eel from various Nova Scotia watersheds in the mid-1990s, that included the Mersey River, revealed no infections of *A. crassus* within the Southern Uplands (Cone et al. 1993, Marcogliese & Cone 1996, Barker et al. 1996) indicating that this parasite is relatively new (15 years or less) to Nova Scotia. The first reports of *A. crassus* on eels in Nova Scotia were from the Bras d'Or Lakes, Sydney Harbour, Mira River, Margaree Harbour, and Lochabere Lake in NS and in Silver Lake and the Saint John River, NB during 2006 and 2007 summer sampling (Aieta & Oliveira 2009, Rockwell et al. 2009). The survey by Wall (2011) of eels in Cape Breton rivers and estuaries found *A. crassus* present at 25 of the 30 sites sampled during the summers of 2008 and 2009. Prevalence varied among Cape Breton Island sampling locations, ranging from 0 to 100 % and maximum intensities of 7.3 ± 6.6 (SD), with an overall prevalence of 42 % and a mean intensity of 4.6 ±5.6 (SD). The only areas free of infection were in Northern Cape Breton (although sample sizes were low from those areas). Most recently, the Unama'ki Institute of Natural Resources (UINR) collected eels from the Bras d'Or Lakes, Cape Breton, and found a prevalence of 46 %, with a mean intensity of 9 ± 11.0 (SD) (Denny et al. 2013), complementing the extensive distribution in Cape Breton reported by Wall (2011) (Figure 12).

The present study shows that *A. crassus* is widespread throughout the Saint John River with further sites of infection found in the nearby Keswick River. Prevalence and intensities are comparable to that reported for eels in Cape Breton and thus represent another important focus of infection in the Maritimes. The BoF region was extensively sampled throughout the Saint John River (SJR) and neighbouring rivers in NB (Big Salmon and St Croix Rivers) and five rivers (Gaspereau, Annapolis, Bear, Stewiacke, and Shubenacadie Rivers) from NS. The Big Salmon and St. Croix rivers did not have infected eels during the 2009 sampling.

Prevalence and intensity of infection in eels at sites throughout the Saint John River did not vary significantly with distance from the sea, but there was an unexplained spike in intensity at a site 50 km from sea. The lack of a consistent relationship with distance from sea likely reflects the speed with which the parasite adapts to new environments, with eel movement throughout resident streams surviving to distribute the parasite locally within a system (Hedger et al. 2010).

In the Southern Uplands of NS, overall prevalence in 2008 to 2013 was 2.4 % (34 of 1,395) and reflects the large number of rivers with eels free of infection. Three isolated pockets of infected eel were found in this region; the Mersey/Medway area, Salmon River (Lawrencetown) and in the upper reaches of the St Mary`s River. Suggesting that 3 apparently independent colonization's by the parasite have occurred along this stretch of coast. .

In Europe, infection rates increased from 10 to 50 % within one year (Belpaire et al. 1989, Koops & Hartmann 1989), with the possibility to increase to 100 % prevalence over one year (Kennedy & Fitch, 1990; Haenen et al. 1994). Interestingly, intensities have been shown to stabilize among European eels once the parasite established (Haenen et al. 1994). With the present study being the first extensive survey of American eel throughout southern New Brunswick and mainland Nova Scotia (primarily in freshwater river habitats) it provides baseline information for future studies of the potential, if not expected, spread of the parasite throughout the region.

One might assume the patchiness of the parasite throughout the Southern Uplands of Nova Scotia likely represents the early stages of local range expansion and eventual consolidation in the region.

The Gulf region of NS and NB had few eels necropsied because few or no eels were caught at sites sampled. The only infected site was, West River, Antigonish Co., which had 100 % infection in the 2 eels collected by the Nova Scotia Department of Fisheries and Aquaculture. At this point, we do not have enough data to say for certain whether the West River represents another important regional focus of infection, as the site has not been revisited for eel collections.

This parasite is thought to be a successful invader of coastal environments because of its short life cycle of less than two months under ideal (i.e. 20 °C) conditions (De Charleroy et al. 1990) with parasite development being temperature dependent. (Ashworth & Kennedy 1999). All eels used in the present study were collected from July to October, with most river waters with temperatures ranging from 10 - 25°C, ideal for parasite survival, at least during the summer months in the Maritimes region.

An important reason for parasite establishment in our region must be the variety of potential paratenic and intermediate hosts for *A. crassus* that are available for it to complete its lifecycle. In North America, the paratenic hosts of *A. crassus* has yet to be identified, but hosts documented in Europe include a diverse community of aquatic invertebrates, i.e. snails and insect larvae (Moravec & Skorikova 1998), aquatic vertebrates, i.e. frogs, and newt tadpoles (Moravec & Skorikova 1998), and fish including trout, perch and sunfish (van Banning & Haenen 1990, Thomas & Ollevier 1992, Moravec & Konecy 1994). Because eels are generalist feeders (Helfman et al. 1987), the potential for infection from a variety of hosts is vast (Barak & Mason 1992). In Atlantic Canada, many of these types of species could be potential hosts, but further research on the hosts involved is needed.

The parasite presence at isolated sites in mainland New Brunswick and Nova Scotia could be linked to shipping traffic and ballast water as mentioned by previous studies (Hayward et al. 2001, Rockwell et al. 2009, Wall 2011, Denny et al. 2013). Cargo shipments passing through the Port of Saint John exceeded 30 million tonnes in a single year for the first time in 2010 (Saint John Port Authority, 2011); the Bras d'Or Lakes also have commercial shipping traffic for gypsum mined in the region. The cargo and recreational shipping activity include vessels from all over the world, so it makes for an easy mode of introduction for invasive aquatic parasites via either transport of parasite eggs/larvae, intermediate or paratenic hosts or adult eels. How the parasite ended up infecting distant populations of eels along the Southern Uplands remains a mystery, but could have involved activity of smaller vessels or accidental distribution of hosts in gear.

The present study found that infected eels were on average longer and heavier than the non-infected eels. It is possible that eels that have the parasite are easier to capture because of limited movement and available energy . As seen in Figure 10, the growth (log length vs log weight) of infected and non-infected eels were similar and are similar to that observed by Wall (2011) and Denny et al. (2013) in Cape Breton eels. One explanation for the lack of correlation is the acute nature of the pathology and the quickness by which the parasite appears to spread in the wild. It follows that if growth is not affected then it is not surprising to observe no significant correlation between condition factor and intensity.

When examining the HSI and SSI values from this study, we found that infected fish had higher values or larger spleen and livers than non infected eels. Lefebvre (2004) found a significant increase in spleen size in European eel, associated with *A. crassuss* infections suggesting that the enlargement was an adaptive host response of both hematolical (blood) and immunological functions triggering the red blood cells to respond to the parasite feeding on the eel blood and the compromise of immune cells. While Lefebvre et al (2001) & Moller et al., (1991), did not find any significant differences between uninfected and infected liver masses the present study did find that the liver was heavier than compared to non infected eels. This leads us to believe that *A. crassus* infection in the eel could have an impact on energy storage in the liver, but further work is needed to test this. As mentioned before, much of the work done with *A crassus* and its health impacts and effects have been performed on European eels and little is known on American eels.

The concern in our region with American eel populations (Castonguay et al. 1994, Haro et al. 2000) is that they are being watched more closely due to the COSEWIC status up-listing from "Species of Concern" to a "Threatened" species. Palstra et al. (2007) found that swimming performance was severely impaired in silver European eels either infected with *A. crassus* or having previous swim bladders damaged from earlier infections. Damaged swim bladders may lessen the chances of the fish making the long trip to the Sargasso Sea for reproduction (Kirk et al. 2000).

The study, while paired with the previously documented presence of *A. crassus* infection throughout sites within NS and NB (Aieta & Oliveira 2009, Rockwell et al. 2009, Wall 2011, and Denny et al. 2013), will be used as a tool to provide a better understanding of the distribution of *A. crassus* infecting American eel within local watersheds. It will be important to monitor the prevalence and effect the parasite has on the eels as this invasive species inevitably becomes more established, as eel are an important ecological and economic resource, as well as an important part of the cultural traditional the Maritime Provinces' aboriginal peoples. In addition to providing parasite distribution information, this study also provided age data that could serve as an important reference set to fully understanding future impacts of the parasite on age structure of the eel population in the region.

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Table 1: American eel (*Anguilla rostrata*) collection sites and necropsy data from the Bay of Fundy (BoF) region of Nova Scotia and New Brunswick.

Year	Province	Drainage	SiteID	Lat (d'd)	Long (d'd)	Distance to sea (km)	No of eels processed	No of eels infected	Prevalence (%)	Intensity (±SD)
2009	NB	Saint John River	ColCr 1	46.17491	-65.6892	102.3	3	2	66.7	1.5 (±0.7)
2009	NB	Saint John River	BelCr 1	45.67493	-65.8095	49.3	12	5	41.7	8 (± 9)
2009	NB	Saint John River	ColCr 3	46.11877	-65.8106	92.2	16	8	50.0	1.87 (± 1.36)
2009	NB	Saint John River	Kenb 1	45.8515	-65.5649	75.9	5	2	40.0	1
2009	NB	Saint John River	Nerpis 2	45.4152	-66.3156	8.1	3	1	33.3	3
2009	NB	Saint John River	Ormct 2	45.67816	-66.6885	4.9	4	1	25.0	1
2009	NB	Saint John River	Brp 1	45.96552	-66.3249	98.6	21	3	20.0	1
2009	NS	Stewiacke River	StewR 2	45.13785	-63.3717	1.8	11	2	18.2	1
2009	NB	Saint John River	Ormt R 1	45.80197	-66.6241	56.7	31	5	16.1	3.2 (± 4.9)
2009	NB	Saint John River	Ormct 5	45.48081	-66.4259	19.9	7	1	14.3	2
2009	NB	Saint John River	Keswk 3	46.09011	-66.9366	100.4	8	1	12.5	2
2009	NB	Saint John River	Nerpis 3	45.39925	-66.3068	6.3	8	1	12.5	1
2009	NB	Saint John River	Ormct 3	45.57261	-66.5787	35.2	30	3	10.0	1.33 (± 0.57)
2009	NB	Salmon River	SlmCk 1	46.20628	-65.9128	100.3	11	1	9.1	1
2009	NB	Saint John River	Nhwksi 2	46.0273	-66.6992	85.3	26	2	7.7	1
2009	NB	Saint John River	Ormt R 2	45.86828	-66.7012	68.9	28	2	7.1	1
2008	NS	Annapolis River	SU107	44.9584	-64.9029	34.5	15	0		
2008	NS	Annapolis River	SU108	44.82186	-65.3094	38.6	9	0		
2008	NS	Annapolis River	SU109	44.77214	-65.4037	21.6	9	0		
2008	NS	Annapolis River	SU110	45.00222	-64.8212	102.9	7	0		
2008	NS	Bear River	SU3B	44.56782	-65.637	1.2	4	0		
2008	NS	Belliveau River	SU5A	44.37395	-66.0594	3.8	10	0		
2009	NB	Big Salmon River	Bsr Ab 1	45.59874	-65.3154	21.1	1	0		
2009	NB	Big Salmon River	Bsr Cr 1	45.55477	-65.3223	17.2	5	0		
2009	NB	Big Salmon River	Bsr Mb 1	45.5008	-65.3693	9.5	2	0		
2009	NB	Big Salmon River	Bsr Sd 1	45.58388	-65.3108	20.2	6	0		
2009	NB	Big Salmon River	Bsr Wl 1	45.61473	-65.3211	22.7	1	0		

Year	Province	Drainage	SiteID	Lat (d'd)	Long (d'd)	Distance to sea (km)	No of eels processed	No of eels infected	Prevalence (%)	Intensity (±SD)
2009	NB	Digdeguash	Digash 1	45.40083	-67.1593	22.781	1	0		
2009	NB	Digdeguash	Digash 2	45.32167	-67.0762	15.815	1	0		
2009	NB	Saint John River	NewRv 1	45.14558	-66.5477	1.265	2	0		
2009	NB	Saint John River	NewRv 2	45.18239	-66.5474	5.722	1	0		
2009	NB	Saint John River	Nonan 1	45.97499	-66.4461	73.121	5	0		
2009	NB	Saint John River	BelCr 2	45.692	-65.7824	52.554	5	0		
2009	NB	Saint John River	ColCr 2	46.21197	-65.698	106.731	1	0		
2009	NB	Saint John River	Dunbr 1	46.16591	-66.6892	99.434	1	0		
2009	NB	Saint John River	Dunbr 2	46.13053	-66.6675	95.278	5	0		
2009	NB	Saint John River	Dunbr 3	46.136	-66.7119	96.692	8	0		
2009	NB	Saint John River	Dunbr 4	46.15598	-66.6992	99.3648	8	0		
2009	NB	Saint John River	Dunbr 5	46.15325	-66.6414	97.155	4	0		
2009	NB	Saint John River	Keswk 1	46.13962	-67.0336	109.438	8	0		
2009	NB	Saint John River	Keswk 2	46.10815	-67.0202	105.18	7	0		
2009	NB	Saint John River	Keswk 4	46.07254	-66.8645	95.467	4	0		
2009	NB	Saint John River	Macqc 1	46.04996	-67.0938	104.709	1	0		
2009	NB	Saint John River	Nakwc 1	46.04498	-67.239	110.885	1	0		
2009	NB	Saint John River	Nshwk 5	46.26892	-66.6659	148.9	16	0		
2009	NB	Saint John River	Nshwk 2	46.26707	-66.6406	110.47	12	0		
2009	NB	Saint John River	Nshwk 3	46.19827	-66.6627	103.426	14	0		
2009	NB	Saint John River	Nshwk 1	46.17666	-66.4636	94.853	10	0		
2009	NB	Saint John River	Nhwksi 1	46.08059	-66.7683	93.299	13	0		
2009	NB	Saint John River	Nerpis 1	45.45482	-66.3401	13.027	2	0		
2009	NB	Saint John River	Ormct 1	45.70632	-66.6039	47.348	8	0		
2009	NB	Saint John River	Ormct 4	45.50246	-66.4763	24.001	8	0		
2009	NB	Saint John River	Ormt R 3	45.88188	-66.7563	70.25	18	0		
2009	NB	Saint John River	Ormt R 4	45.79089	-66.727	60.34	2	0		
2009	NB	Saint John River	Pkok 1	45.83476	-67.0731	83.23	1	0		

Year	Province	Drainage	SiteID	Lat (d'd)	Long (d'd)	Distance to sea (km)	No of eels processed	No of eels infected	Prevalence (%)	Intensity (±SD)
2009	NS	Salmon River (Truro)	SalTru 2	45.35087	-63.1	19.45	1	0		
2009	NS	Salmon River (Truro)	SalTru 1	45.36	-63.2719	0.904	14	0		
2009	NS	Shubenacadie River	ShubR 1	45.10703	-63.3939	5.65	5	0		
2009	NS	Shubenacadie River	ShubR 3	44.93143	-63.537	42	15	0		
2009	NB	St. Croix River (Estuary)	DenSt 1	45.25758	-67.275	7.24	3	0		
2009	NB	St. Croix River (Estuary)	DenSt 2	45.23958	-67.2691	6.87	13	0		
2009	NB	St. Croix River (Estuary)	DenSt 3	45.23883	-67.2873	8.4	1	0		
2009	NS	Stewiacke River	StewR 1	45.2817	-62.9253	68.243	1	0		
2009	NB	Waweig	Wawe 1	45.24317	-67.1379	4.183	6	0		
2009	NB	Waweig	Wawe 2	45.23989	-67.1351	0.434	11	0		
2009	NB	Waweig	Wawe 3	45.26081	-67.1426	5.792	9	0		
							539	40		

Table 2: American eel (*Anguilla rostrata*) collection sites and necropsy data from the Southern Uplands (SU) region of Nova Scotia.

Year	Province	Drainage	SiteID	Lat (d'd)	Long (d'd)	Distance to sea (km)	No of eels processed	No of eels infected	Prevalence (%)	Intensity (±SD)
2008	NS	Mersey River	SU19D	44.0934	-64.8462	11.82	4	1	25.0	1
2008	NS	St. Mary's River	STMR859.4	45.2677	-62.3260	47.19	18	1	5.6	2
2008	NS	Salmon River (Lawrencetown)	SU36A	44.6925	-63.3792	0.20	20	1	5.0	2
2008	NS	Medway River	Medw109	44.1723	-64.6532	5.61	31	1	3.2	2
2012	NS	Medway River	Wildcat	44.3579	-64.9374	39.67	24	14	58.3	10.4 (±17)
2013	NS	Medway River	Wildcat	44.3579	-64.9374	39.67	21	16	76.2	5.3 (±4.2)
2008	NS	Annis River	SU9A	43.9404	-65.9946	19.43	36	0		
2008	NS	Annis River	SU9B	43.9519	-65.9942	20.11	35	0		
2008	NS	Blacks Brook	SU102	43.7744	-65.3228	0.78	8	0		
2008	NS	Chegoggin River	SU106	43.8687	-66.1481	11.63	4	0		
2008	NS	Clyde River	SU13B	43.7830	-65.5308	24.56	20	0		
2008	NS	East River (Lockeport)	SU16A	43.7455	-65.1436	0.61	4	0		
2008	NS	East River (Chester)	SU27A	44.5967	-64.1647	1.37	7	0		
2008	NS	East River (Chester)	SU27B	44.6486	-64.1428	9.67	14	0		
2008	NS	East River (Tantallon)	SU31A	44.6861	-63.8703	2.05	11	0		
2009	NS	East River (Sheet Harbour)	NSPI 1	44.9552	-62.4981	2.83	16	0		
2008	NS	Ecum Secum River	SU54A	45.0803	-62.2451	17.68	14	0		
2008	NS	Ecum Secum River	SU54B	45.0133	-62.1689	5.72	8	0		
2008	NS	Ecum Secum River	SU54C	45.0087	-62.1751	4.94	2	0		
2008	NS	Ecum Secum River	SU54D	45.1056	-62.2214	21.33	10	0		
2008	NS	Gaspereau Brook	SU56A	45.0318	-61.9979	0.20	16	0		
2008	NS	Gegogan Brook	SU57A	45.0825	-61.9987	2.34	32	0		
2008	NS	Gold River	Gold002	44.7390	-64.4486	27.68	1	0		
2008	NS	Gold River	Gold005	44.6793	-64.4569	20.56	3	0		
2008	NS	Gold River	Gold015	44.6016	-64.4226	9.76	4	0		
2008	NS	Gold River	Gold018	44.7384	-64.4495	27.56	1	0		
2008	NS	Granite Village Brook	SU103	43.8692	-64.9772	0.20	4	0		

Year	Province	Drainage	SiteID	Lat (d'd)	Long (d'd)	Distance to sea (km)	No of eels processed	No of eels infected	Prevalence (%)	Intensity (±SD)
2008	NS	Halfway Brook	Hafb003	44.9043	-62.4533	4.75	13	0		
2008	NS	Indian River	SU30A	44.6953	-63.8903	0.38	2	0		
2008	NS	Ingram River	SU29A	44.6989	-63.9728	3.52	19	0		
2008	NS	Ingram River	SU29B	44.6883	-63.9636	2.25	17	0		
2008	NS	Ingram River	SU29D	44.7534	-63.9754	13.00	8	0		
2008	NS	Jordan River	SU15B	43.8836	-65.2341	11.28	11	0		
2009	NS	LaHave River	MF 1	44.5346	-64.7134	42.74	14	0		
2008	NS	Little West River	SU46A	44.9014	-62.5606	4.06	13	0		
2008	NS	Little West River	SU46B	44.90777	-62.5447	0.76	21	0		
2008	NS	Martin's River	SU24A	44.4892	-64.3411	0.52	12	0		
2008	NS	Martin's River	SU24B	44.4901	-64.3548	2.31	39	0		
2008	NS	Medway River	Medw108	44.4060	-64.9861	54.17	6	0		
2008	NS	Mersey River	SU19C	44.0794	-64.8083	8.14	36	0		
2008	NS	Mosher River (NS)	SU52A	44.9914	-62.2864	4.10	26	0		
2008	NS	Mosher River (NS)	SU52D	45.0252	-62.2822	7.95	2	0		
2008	NS	Mushamush River	SU23A	44.4731	-64.4371	7.57	17	0		
2008	NS	Mushamush River	SU23B	44.5096	-64.5339	18.14	4	0		
2008	NS	Mushamush River	SU23D	44.5312	-64.4973	19.19	5	0		
2008	NS	Musquodoboit River	SU40A	45.0689	-63.1045	65.54	1	0		
2008	NS	Nine Mile River	SU32A	44.6543	-63.7317	15.99	6	0		
2008	NS	Petite River	SU21C	44.2493	-64.4758	3.76	22	0		
2008	NS	Pumey Brook	SU100	43.7819	-65.2575	0.25	13	0		
2008	NS	Quoddy River	SU51A	44.9763	-62.3429	6.78	24	0		
2008	NS	Quoddy River	SU51B	44.9608	-62.3430	4.69	10	0		
2008	NS	Quoddy River	SU51C	44.9327	-62.3472	0.76	33	0		
2008	NS	Quoddy River	SU51D	44.9465	-62.3555	2.82	9	0		
2008	NS	Rodney Brook	SU101	43.7622	-65.2572	0.47	10	0		
2008	NS	Roseway River	SU112B	43.9839	-65.4326	30.09	10	0		

Year	Province	Drainage	SiteID	Lat (d'd)	Long (d'd)	Distance to sea (km)	No of eels processed	No of eels infected	Prevalence (%)	Intensity (±SD)
2008	NS	Annapolis, Round Hill River	SU2B	44.7379	-65.4061	5.98	5	0		
2008	NS	Sable River	SU17A	43.8519	-65.0743	1.08	10	0		
2008	NS	Sable River	SU17B	43.8436	-65.0656	2.38	15	0		
2008	NS	Salmon River (Digby)	SU8A	44.0578	-66.1384	3.27	35	0		
2008	NS	Salmon River (Digby)	SU8B	44.0509	-66.0954	10.65	23	0		
2008	NS	Salmon River (Digby)	SU8C	44.0981	-66.0713	13.53	12	0		
2008	NS	Salmon River (Musq.)	SU41A	44.83361	-63.0467	9.76	1	0		
2008	NS	Salmon River (Musq.)	SU41B	44.8639	-63.0935	6.07	2	0		
2008	NS	Salmon River (Musq.)	SU41C	44.8711	-63.1077	6.27	5	0		
2009	NS	Salmon River (Lake Echo)	SU36A	44.6925	-63.3792	0.20	11	0		
2008	NS	Salmon River (Lake Major)	SU35A	44.6822	-63.4531	5.28	6	0		
2008	NS	Salmon River (Lake Major)	SU35B	44.6958	-63.4550	1.89	17	0		
2008	NS	Salmon River (Lake Major)	SU35C	44.7019	-63.4606	2.96	12	0		
2008	NS	Salmon River (Lawrencetown)	SU36B	44.6950	-63.3828	0.59	17	0		
2008	NS	Salmon River (Lawrencetown)	SU36C	44.7417	-63.3857	6.86	41	0		
2008	NS	Salmon River (Port Dufferin)	SU50A	44.9461	-62.3983	4.37	2	0		
2008	NS	Ship Harbour River	SU42B	44.8639	-62.9417	13.12	20	0		
2008	NS	Smith Brook	SU53A	44.9695	-62.2096	0.92	8	0		
2009	NS	St. Mary's River	STMR002	45.2719	-62.2464	38.65	10	0		
2009	NS	St. Mary's River	STMR8510.8	45.4302	-62.3126	68.16	11	0		
2009	NS	St. Mary's River	STMR859.4	45.2682	-62.3246	47.19	11	0		
2009	NS	St. Mary's River	STMR858.1	45.2728	-62.3632	51.15	1	0		
2009	NS	St. Mary's River	STMR853.2	45.2727	-62.0971	22.41	2	0		
2009	NS	St. Mary's River	STMR924	45.2801	-62.4060	55.34	13	0		
2009	NS	St. Mary's River	STMR928	45.2976	-62.6665	86.89	18	0		
2009	NS	St. Mary's River	STMR001	45.4155	-62.0340	39.17	5	0		
2009	NS	St. Mary's River	STMR923	45.3216	-62.0821	35.86	9	0		
2008	NS	St. Mary's River	STMR854.2	45.2707	-62.0173	23.61	2	0		

Year	Province	Drainage	SiteID	Lat (d'd)	Long (d'd)	Distance to sea (km)	No of eels processed	No of eels infected	Prevalence (%)	Intensity (±SD)
2008	NS	St. Mary's River	STMR854.4	45.2721	-62.0179	23.44	6	0		
2008	NS	St. Mary's River	STMR863.1	45.3410	-62.1077	39.55	12	0		
2008	NS	St. Mary's River	STMR923	45.3216	-62.0821	35.86	11	0		
2008	NS	St. Mary's River	STMR924	45.2801	-62.4058	55.34	26	0		
2008	NS	St. Mary's River	STMR925.1+2	45.2794	-62.2881	43.61	5	0		
2008	NS	St. Mary's River	STMR928	45.2982	-62.6659	86.89	8	0		
2008	NS	Tangier River	SU43A	44.9667	-62.8203	26.81	6	0		
2008	NS	Tangier River	SU43B	44.94694	-62.7850	23.08	36	0		
2008	NS	Tangier River	SU43C	44.8143	-62.7133	1.03	21	0		
2008	NS	Tidney River	SU18A	43.8725	-65.0272	6.96	5	0		
2008	NS	Tusket River	SU10A	43.9254	-65.9568	8.95	43	0		
2008	NS	Tusket River	SU10D	44.1313	-65.9270	42.43	4	0		
2008	NS	Tusket River	SU10E	44.1133	-65.9161	39.82	13	0		
2008	NS	Tusket River	SU10F	44.1144	-65.8453	43.88	17	0		
2008	NS	Tusket River	SU10G	44.1161	-65.6600	65.88	7	0		
2008	NS	Tusket River	SU10H	44.0805	-65.8203	40.03	2	0		
2008	NS	West Brook	SU37A	44.8089	-63.3855	0.33	20	0		
2008	NS	West River (Porter's Lake)	SU38A	44.8206	-63.3750	2.18	12	0		
2008	NS	West River (Porter's Lake)	SU38B	44.8218	-63.3733	2.42	16	0		
2008	NS	West River (Sheet Harbour)	WsR SH 1	45.0538	-62.8006	31.83	20	0		
2008	NS	West Taylor Bay	SU45A	44.8436	-62.6047	0.36	2	0		
2008	NS	West Taylor Bay	SU45B	44.8467	-62.6228	0.17	8	0		
							1395	34		

Table 3: American eel (*Anguilla rostrata*) collection sites and necropsy data from the Medway River, Nova Scotia during the years 2008, 2012, and 2013.

Year	Drainage	Site	Lat (D'd) N	Long (D'd) W	Distance to sea (km)	No. of eels processed	Average length (cm)	Average weight (g)	Average (k)	No. of eels infected (%)	Intensity (±SD)
2008	Medway	Medw108	44.4060	64.9861	48.2	6	33.6	71.4	0.1637	0	
2008	Medway	Medw109	44.1723	64.6532	5.6	31	28.7	43.9	0.1649	1 (3)	2
2012	Medway	Wildcat	44.3579	64.9374	39.7	24	69.0	na	na	14 (58)	10.4 (± 17)
2013	Medway	Wildcat	44.3579	64.9374	39.7	21	50.5	308	0.1833	16 (76)	5.3 (± 4.2)

Table 4: American eel (*Anguilla rostrata*) collection sites and necropsy data from the Gulf of Saint Lawrence (Gulf) region of Nova Scotia and New Brunswick.

Prov.	**Drainage**	**Latdd**	**Longdd**	**Dist. to sea (km)**	**No. of eels processed**	**No. of eels infected**	**Prevalence (%)**	**Intensity (± SD)**
NS	West River (Antigo.)	45.55449	-62.0885	17.65	2	2	100.0	2 (± 1.414)
NB	Miramichi	46.9577	-65.8608	31.53	40	0	0	
NS	Bailey`s Brook	45.6921	-62.2698	1.80	5	0	0	
					47	2		

Table 5: The breakdown of American eel (*Anguilla rostrata*) infected with *Anguillicoloides crassus* from the 3 major areas; Bay of Fundy, Southern Uplands and the Gulf of St. Lawrence from Nova Scotia and New Brunswick during the 2008-2013 collections.

Area	No. of sites sampled	No. of eels processed	No. of eels infected	Prevalence (%)	Intensity (± SD)
Bay of Fundy (BoF)	68	539	40	7.42	2 ± 3.95
Southern Uplands (SU)	103	1395	34	2.44	6 ± 11.34
Gulf of St. Lawrence (Gulf)	3	47	2	4.25	2 ± 1.41
Total	174	1981	76	3.8	4 ± 8.01

Table 6: Comparison of various variables (average length, weight, HSI, & SSI values) of infected versus non-infected American eel (*Anguilla rostrata*).

Variables	**Non-infected**	**Infected**
Number of fish (n)	1905	76
Average length (cm)	27.3	42.9
Average weight (g)	48.9	157.4
Condition factor (K)	0.162	0.175
Average liver weight (g)	0.59	1.22
Hepatosomatic Index (HSI)	1.232	1.369
Average spleen weight (g)	0.120	0.251
Spleen Somatic Index (SSI)	0.334	0.309

Figure 1: Image of an American eel (*Anguilla rostrata*) swim bladder necropsy found infected with the round worm parasite, *Anguillicoloides crassus*. This was collected from an infected eel captured in Belleisle Creek, New Brunswick, which had 23 adult parasites in the swim bladder.


September 8, 2009
Bellisle Creek
Springfield
DC09 087
12 American Green Eels
mm 1cm 2 3 4 5 6 7 8 9 10

Figure 2: Map of regional drainages and collection sites indicating infected and non-infected American eel (*Anguilla rostrata*) with *Anguillicoloides crassus* in mainland Nova Scotia and New Brunswick (Southern Uplands, Gulf of St. Lawrence, and the Bay of Fundy regions) from 2008-2013.

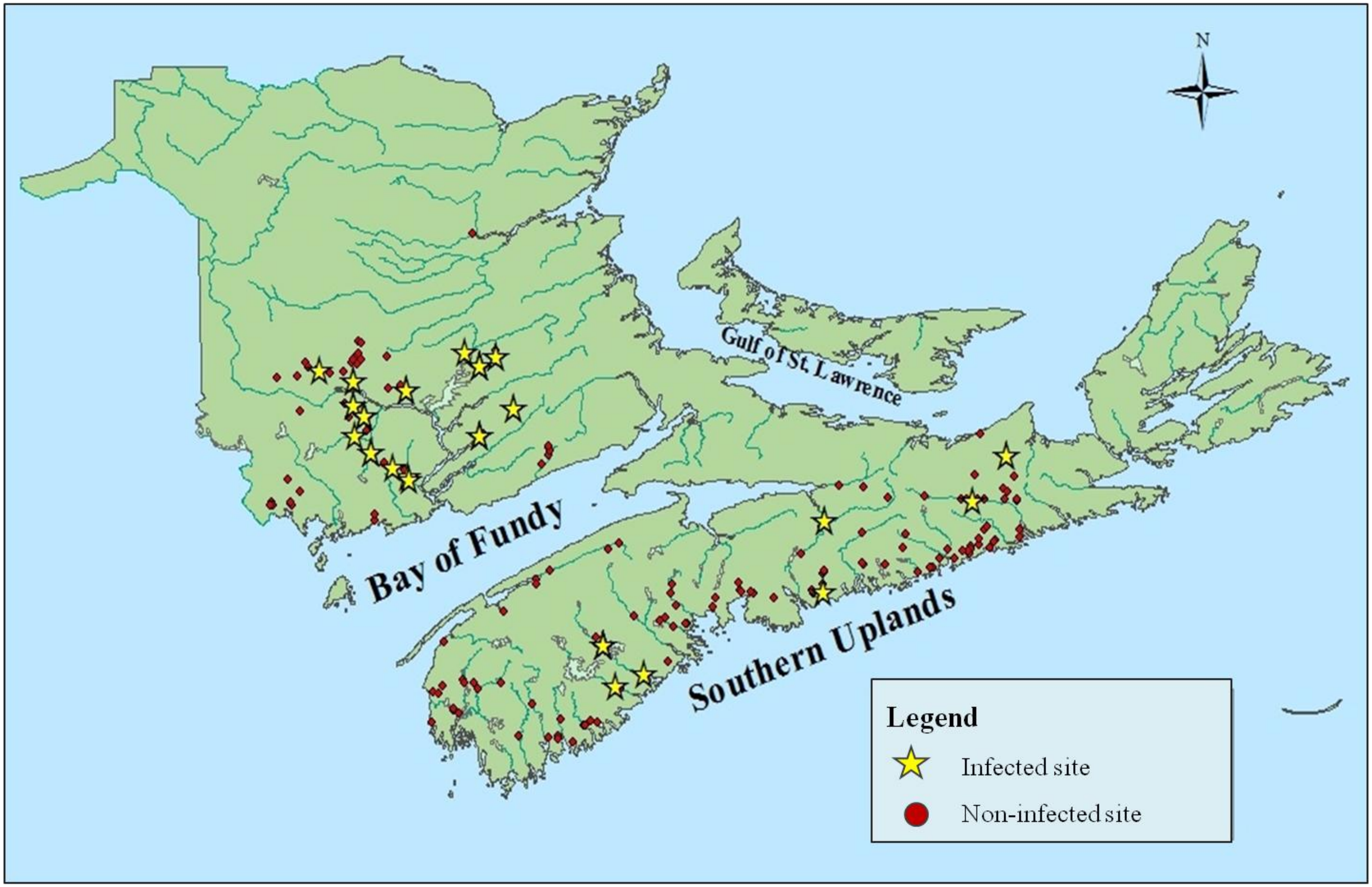

N
Gulf of St. Lawrence
Bay of Fundy
Southern Uplands
Legend
Infected site
Non-infected site

Figure 3: Length (cm) frequency distribution of infected (red) and non-infected (blue) American eel (*Anguilla rostrata)* with the swim bladder parasite *Anguillicoloides crassus* and including all eel processed from the 2008-2013 collections.

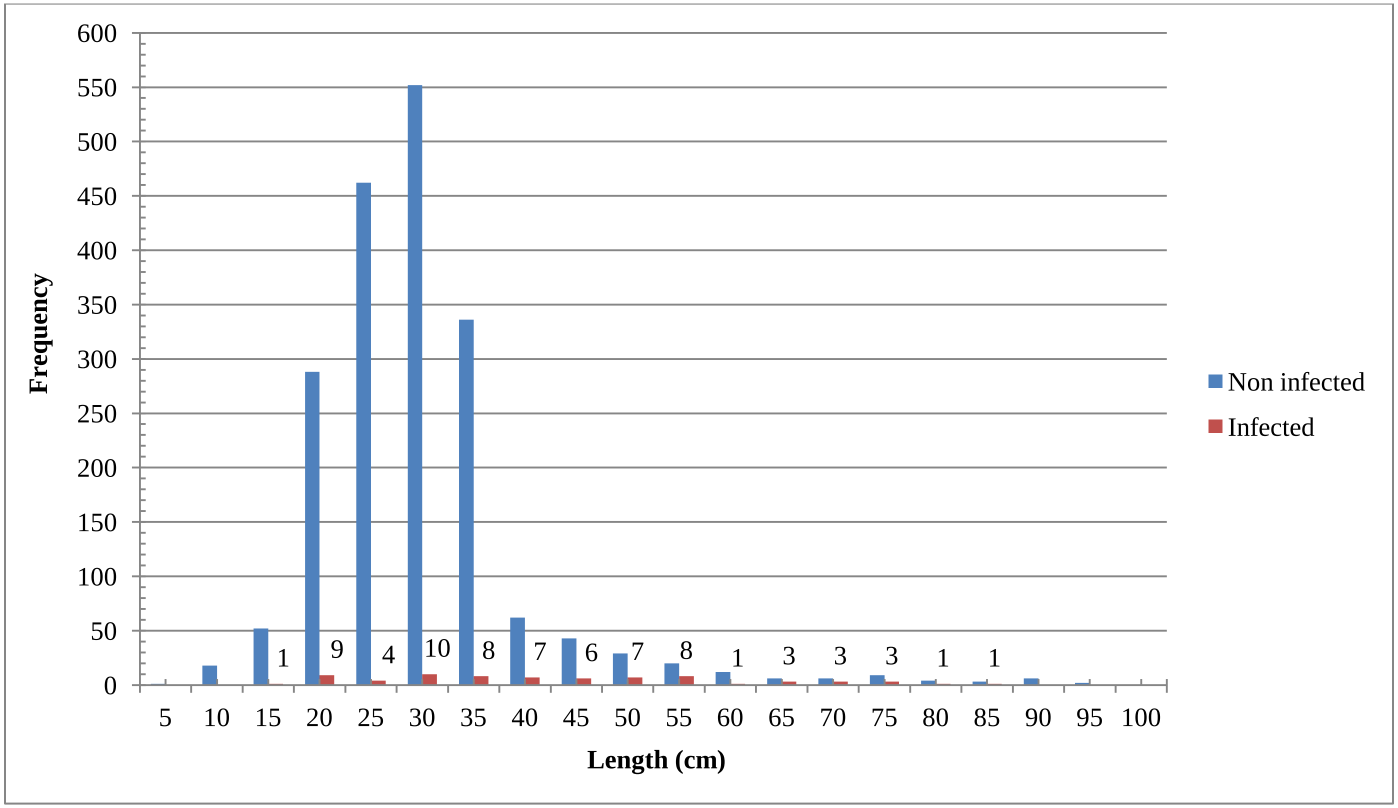
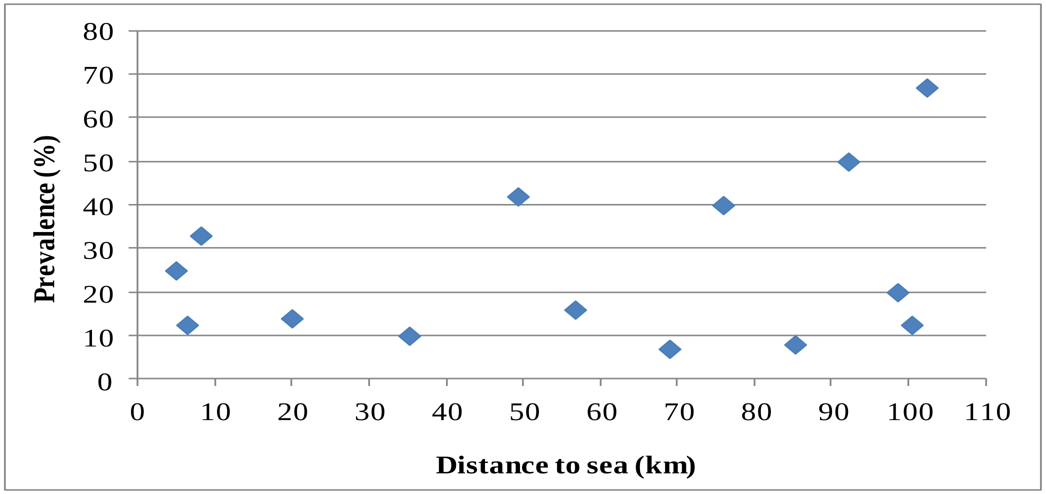
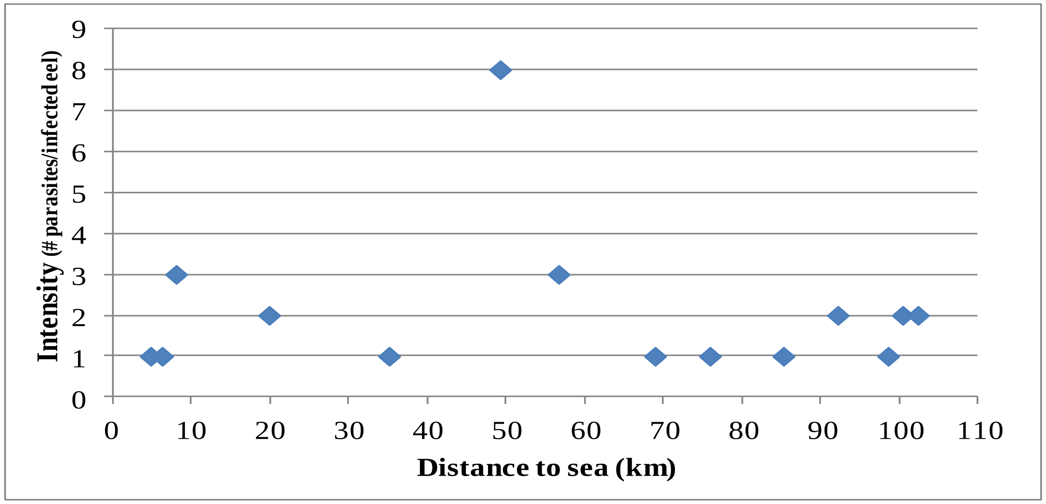

Frequency
600
550
500
450
400
350
300
250
200
150
100
50
0
1
9
4
10
8
7
6
7
8
1
3
3
3
1
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
Length (cm)
Non infected
Infected

Figure 4: Scatter plot of prevalence (r^2 = 0.0789, r = 0.208, p-value = 0.440) and intensity (r^2 = 0.0065, r = - 0.092, p-value = 0.736) of *A. crassus* versus distance (km) up the Saint John River, New Brunswick.

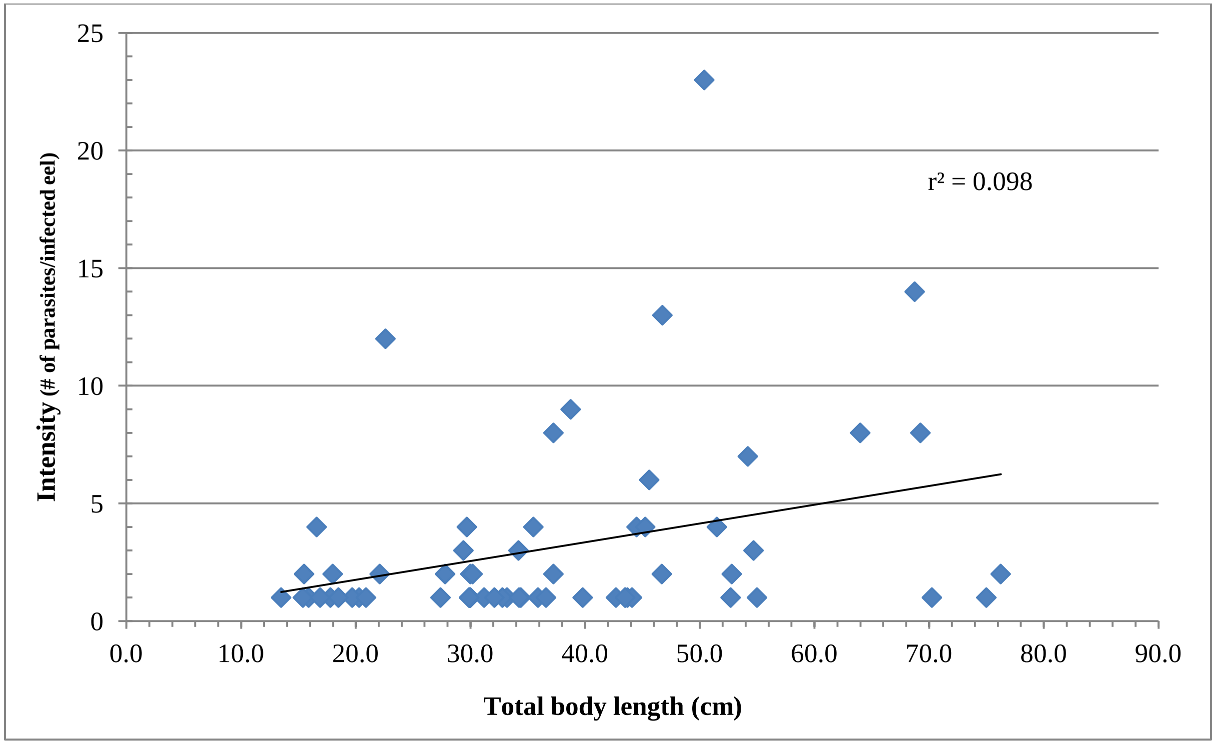

Prevalence (%)
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110
Distance to sea (km)

a.


Intensity (# parasites/infected eel)
9
8
7
6
5
4
3
2
1
0
0 10 20 30 40 50 60 70 80 90 100 110
Distance to sea (km)

b.

Figure 5: Scatter plot of the relationship between intensity (number of parasites/per infected eel) and total length (cm) of American eel (*Anguilla rostrata*). A correlation analysis was used between intensity and eel length (r = 0.313, p-value = 0.013).



25
20
15
10
5
0
Intensity (# of parasites/infected eel)
r² = 0.098
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
Total body length (cm)

Figure 6: Scatter plot of the relationship between intensity (number of parasites/per infected eel) and total weight (g) of American eel (*Anguilla rostrata*). A correlation analysis was preformed with intensity and eel weight (r = 0.251, p-value = 0.049).

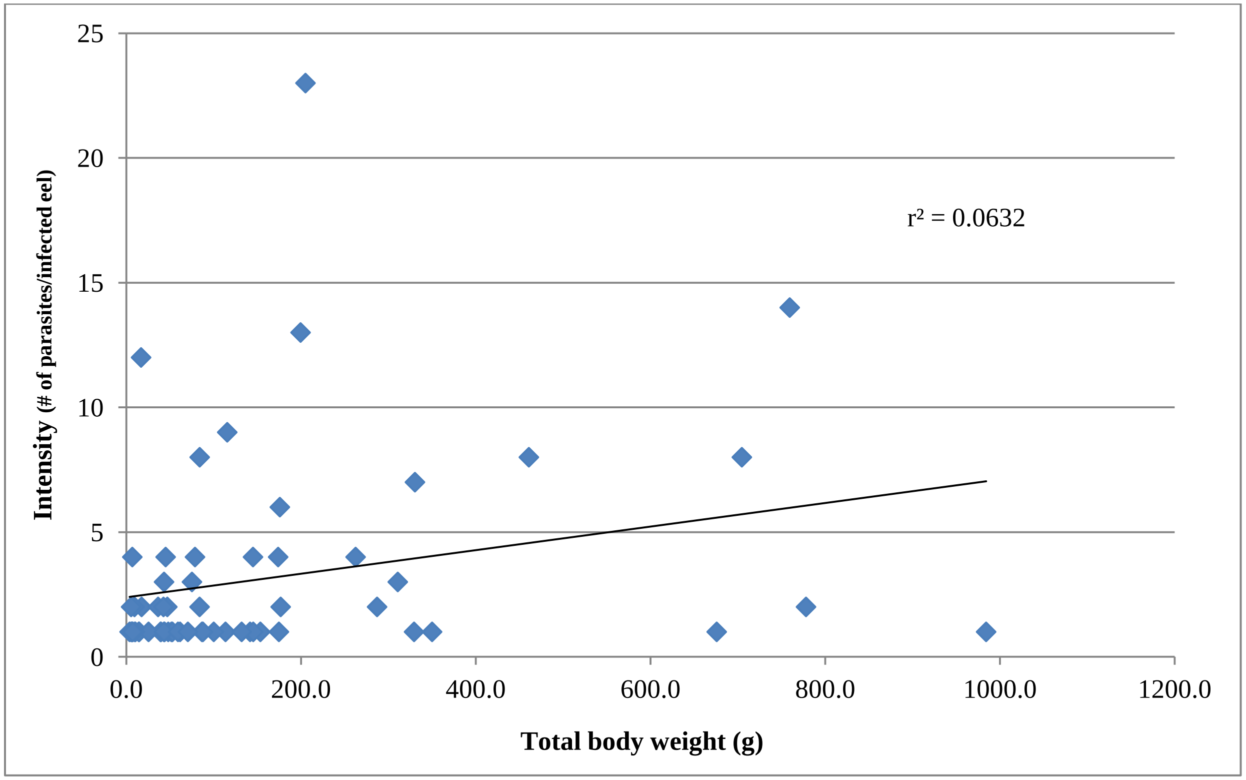
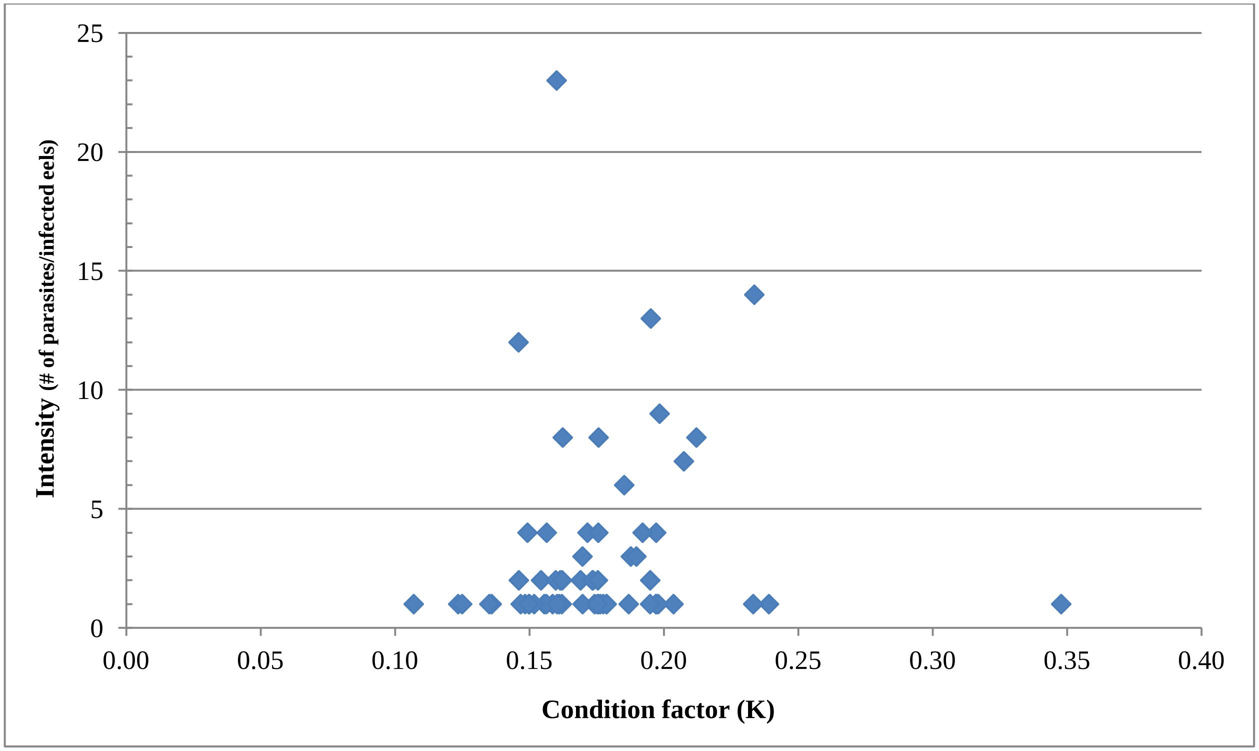
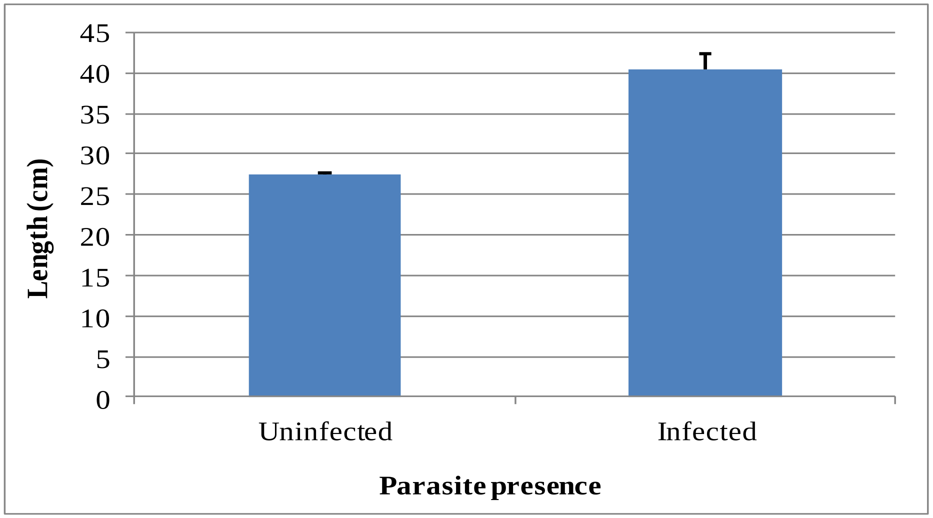

25
20
15
10
5
0
r² = 0.0632
0.0
200.0
400.0
600.0
800.0
1000.0
1200.0
Total body weight (g)
Intensity (# of parasites/infected eel)

Figure 7: Scatter plot of the relationship between intensity (number of parasites/per infected eel) and condition factor (K) of American eel (*Anguilla rostrata*). Linear trend line (r^2 = 0.011) and correlation (r = 0.105, p-value = 0.419).

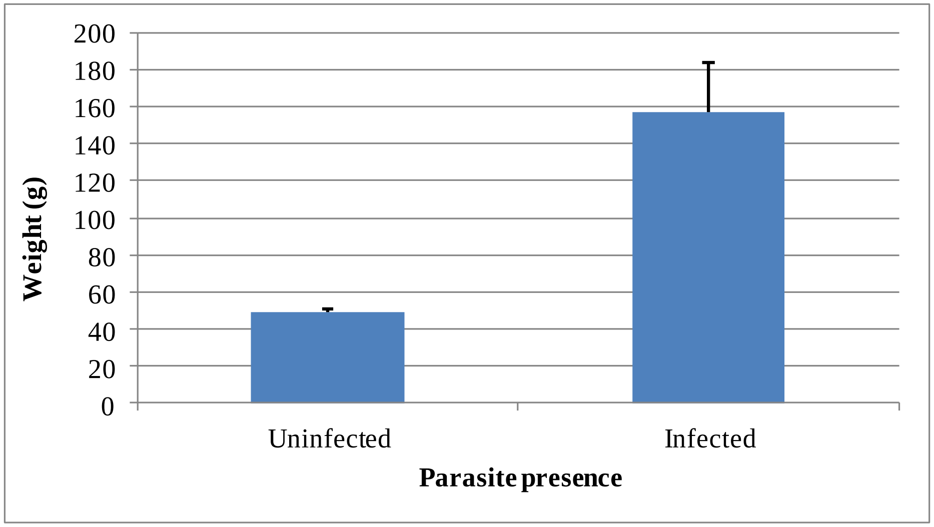


25
20
15
10
5
0
Intensity (# of parasites/infected eels)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
Condition factor (K)

Figure 8: Graph of collected American eel (*Anguilla rostrata*) (a) total length (cm) and (b) total weight (g) comparing uninfected and infected with mean values and standard error bars.

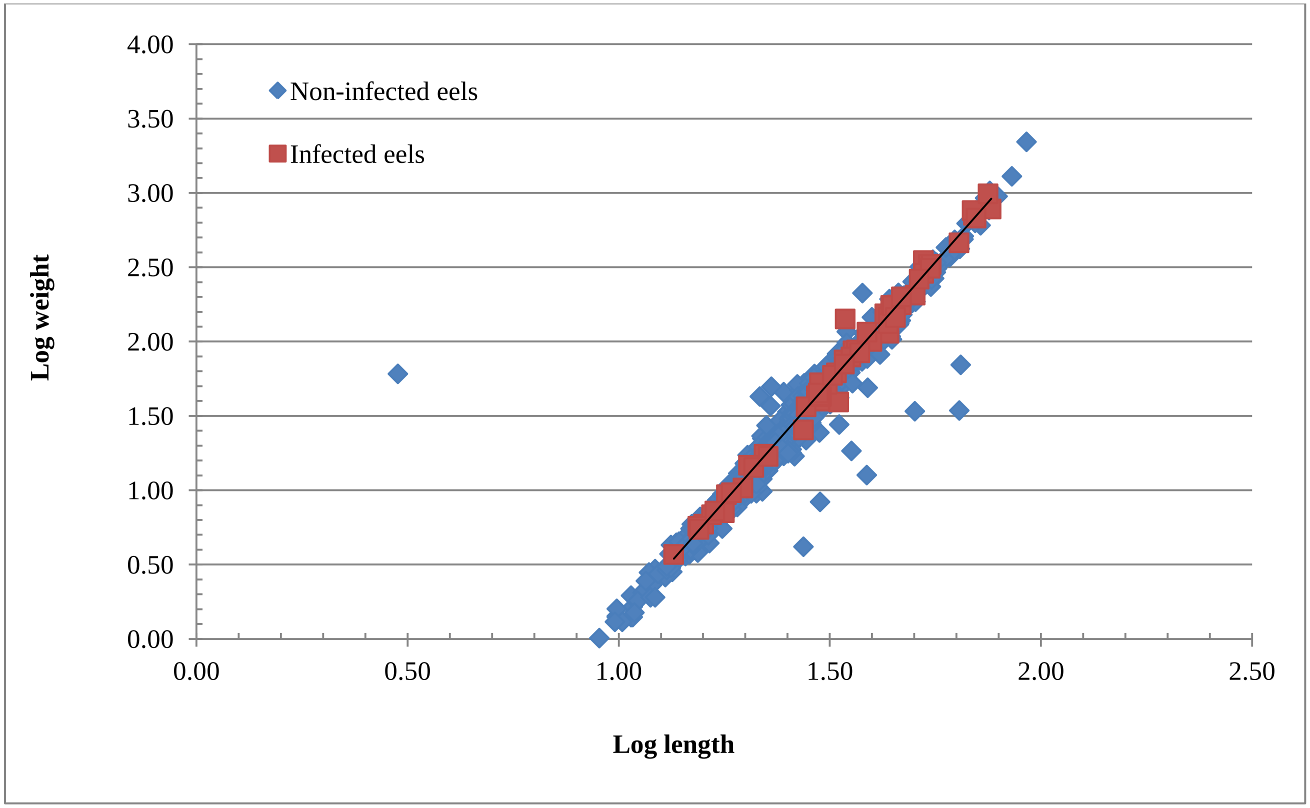

Length (cm)
45
40
35
30
25
20
15
10
5
0
Uninfected
Infected
Parasite presence

a.


Weight (g)
200
180
160
140
120
100
80
60
40
20
0
Uninfected
Infected
Parasite presence

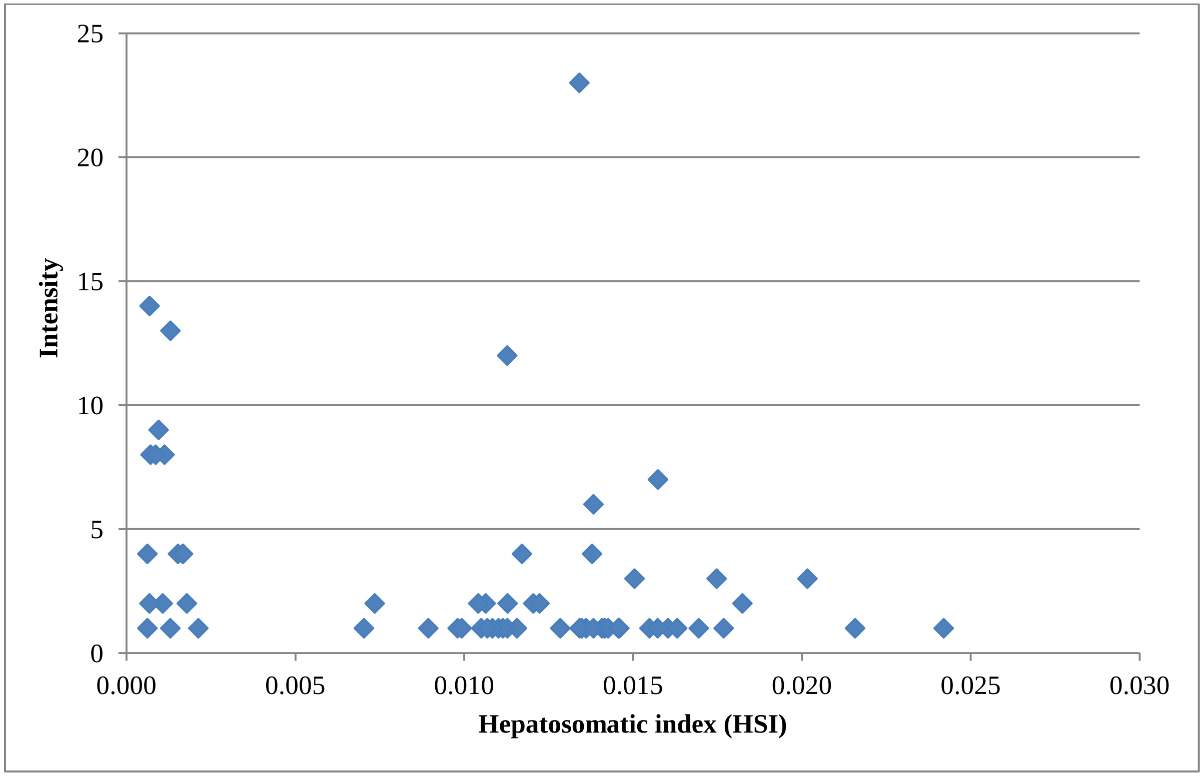
b.

Figure 9: Length (cm) and weight (g) of infected and non-infected American eel (*Anguilla rostrata*). A linear trend line (infected r^2 = 0.9892 and non-infected r^2 = 0.9393) was added to the graph.



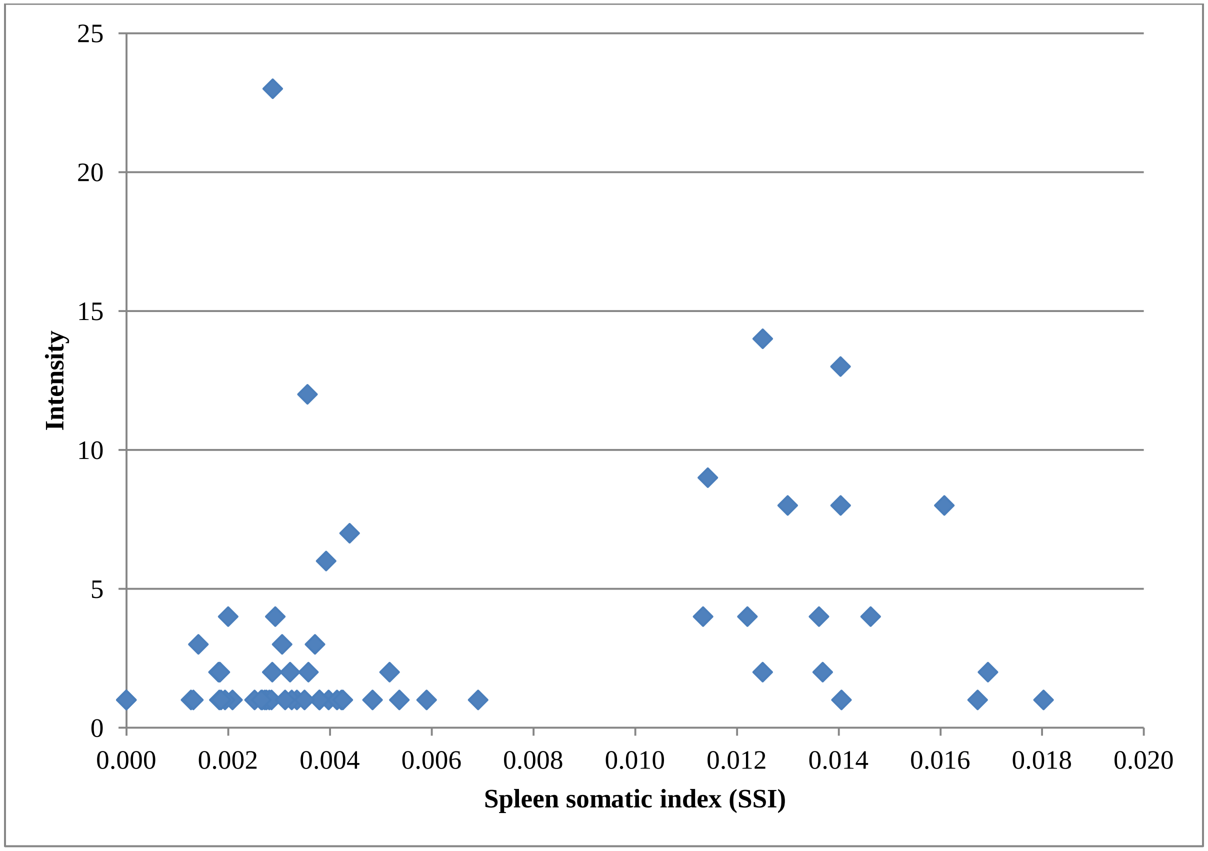
Non-infected eels
Infected eels
Log weight
Log length
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.00
0.50
1.00
1.50
2.00
2.50

Figure 10: Hepatosomatic index (HSI = liver weight (g)/eel total weight (g)) versus the number of *Anguillicoloides crassus* parasites found in the infected American eels (*Anguilla rostrata*). A correlation comparing intensity of the parasite infection versus HSI was preformed (r = -0.173, p = 0.208).



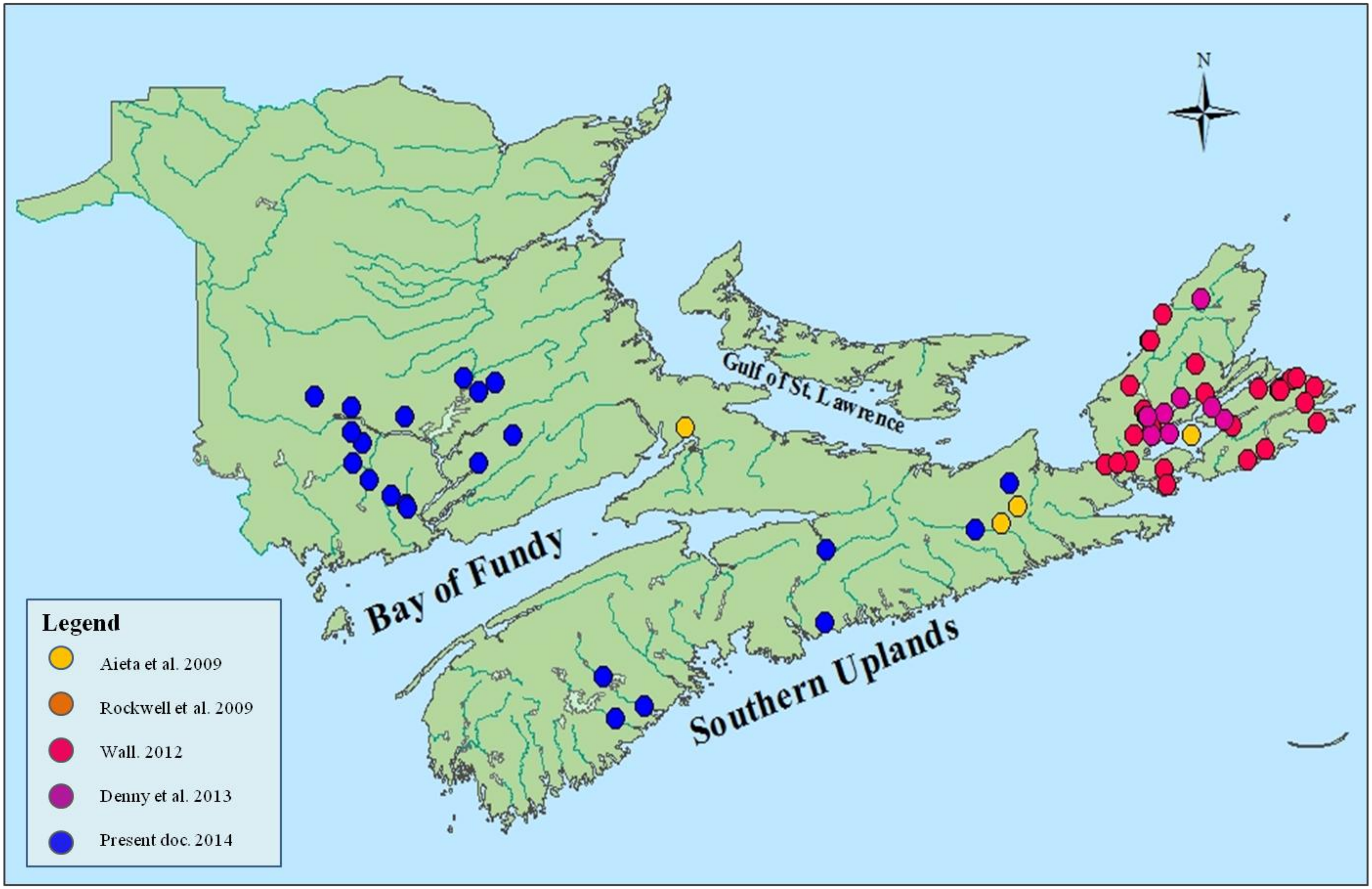
25
20
15
10
5
0
Intensity
0.000
0.005
0.010
0.015
0.020
0.025
0.030
Hepatosomatic index (HSI)

Figure 11: Spleen somatic index (SSI = spleen weight (g)/eel total weight (g)) versus the number of *Anguillicoloides crassus* parasites found in the infected American eels (*Anguilla rostrata*). A correlation was preformed comparing intensity of the parasite infection versus the SSI ($r = 0.169$, $p = 0.218$).



25
20
15
10
5
0
Intensity
0.000
0.002
0.004
0.006
0.008
0.010
0.012
0.014
0.016
0.018
0.020
Spleen somatic index (SSI)

Figure 12: Nova Scotia and New Brunswick watersheds where the parasite *Anguillicoloides crassus* has been documented to infect American eels (*Anguilla rostrata*) between 2006 - 2013.


N
Gulf of St. Lawrence
Bay of Fundy
Southern Uplands
Legend
Aieta et al. 2009
Rockwell et al. 2009
Wall. 2012
Denny et al. 2013
Present doc. 2014

Appendix 1

In order to get an age of an American eel we use otoliths (ear stones, calcium deposits) instead of their embedded scales (Bouillon 1985, Bertin 1956). Liew (1974) after comparing known eels with otolith ages, concluded that otoliths can be used with confidence in age determination. Although it should be noted that Facey and LaBar (1981) mentioned that, when using otoliths to determine eel age there can be differences among researchers when interpreting annual rings and false annulis.

With this basic eel otolith information in mind and researching the background of what people had done previously I was directed by a committee member to the International Council for the Exploration of the Sea (ICES): Workshop on Age Reading of European and American Eel (WKAREA). These documents describe how to prepare the otoliths using the cut and burn technique and how to estimate the age of each otolith.

Methods

Preparing the otoliths using the cut and burn technique

The cut and burn technique as described in ICES otolith workshop manual was used to prepare otoliths for ageing and guidelines on how to age the American eel otoliths(ICES 2009, 2011). The right sagittal otolith was used for ageing, unless it crumbled, cracked, or over-burned in the process, in which case the left otolith was used. The otolith was placed with edges facing up (bowl or boat-like) on a glass slide and secured to the slide with clear tape. The otolith was then cut in half with a scalpel blade, through the nucleus or as close to the middle as possible, at the narrowest part of the otolith.

For burning, one half of the right otolith was placed on a scalpel blade and positioned in the middle of an open flame. A propane Bunsen burner or torch was used instead of an ethanol flame because propane has an intense heat that enables quicker charring, resulting in more prominent annular rings. The time in the flame varied, depending on the size of the otolith. After 8-20 seconds in the flame, the otolith was cooled for a few seconds and was then set in a small dab of silicone on a clean microscope slide. Forceps were used with a dab of silicone on the end in order to hold the otolith while sliding it into position with the cut side facing the surface of the slide. A dissecting microscope was used when manipulating the otolith position in the silicone to ensure no air bubbles obscured the plane of view. Once the otolith was in position and the silicone had set, an extra dab of silicone was added to the otolith to ensure it was sealed for archival purposes. Digital images were obtained of the otoliths using a Zeiss Axioskop dissecting microscope with AxioVision AC 4.5 image software. The digital images of the otoliths were saved and then the chosen otoliths were sent to the agers, in order to assist in determining eel ages.

Learning how to age an eel otolith

By following the ICES guide for ageing European and American eel otoliths I was able to gain confidence in the process (ICES, 2009, 2011). In the guide, there were previously aged otoliths that were useful to have as reference tools to compare to, when ageing.

Various methods of quality control were performed to make sure I was understanding the ageing methodology and interpretation.

1. I aged 10 digital images of the cut and burnt otoliths and then images were sent for ageing to an experienced DFO eel ager (Noella McDonald). Our ages were compared and any discrepancies were discussed and resolved.

2. Next, 20 random left otoliths from 10 NS watersheds were sent to N. McDonald to process. She used the grind, polish and stain technique on those otoliths and then we both performed age readings. Again, ages were compared and differences in readings were discussed when there were different ages..

3. Finally, a set of 15 otolith images from the cut and burn, were sent to individuals with eel ageing experience: BM Jessop (Canada), J Casselman (Canada), KM Jones (CBU) and R Poole (Ireland) in order to gather additional age information for comparison purposes (Table 6, Figures 13-27).

Once the images had been sent to the agers and the eel ages (comments) returned, the ages were compared to my ages and then compared to the other agers to see the differences in ages even with experienced agers (Table 7).

After seeing how close the initial ages were compared to the experienced agers, it was time to start ageing the rest of the otoliths for the project. 19 watersheds were chosen, and then 10 fish per watershed were processed and ages were estimated. In the end, 174 otoliths were confidently aged, while the rest that had been processed ended up either burning up too quickly, crumbling after the burn, or were just not able to readable.

Results

From the subsample (n= 174) of eel otoliths aged using the cut and burn technique to prepare the otoliths, the range of ages found in Nova Scotia and New Brunswick watersheds were between 3 to 18 years old (Table 8). A weak relationship was found between age and length when correlated (Pearson correlation = 0.142, p = 0.063) (Figure 28). When looking at age versus weight correlation there was no significant relationships (Pearson correlation = 0.111, p = 0.148) (Figure 29).

Discussion

This portion of the project did not fit into the initial parasite work, so the appendix was created. The otoliths were chosen by way of watersheds first, rather than just ageing all the infected fish (which were at low number compared to all the eels sampled). The otolith ageing part of the project did take up a large portion of time; from learning how to properly execute the cut and burn technique on eel otoliths, to then being able to provide ages confidently after working closely with Noella McDonald.

What was found from the 174 freshwater eel otoliths aged (D Campbell) was that there was no relationship between age and weight and a very low relationship between age and length. It would have been ideal to have all the fish that had been infected with *Anguillicoloides crassus* aged, but time was not available. This will be something that could be looked at in the future as most otoliths were collected from the 1,981 eels collected. Ageing is an important component when trying to understand populations, which is why this was added as an appendix to show the skill that was gained though it was not able to be incorporated into the parasite document. If more fish could have been aged and watersheds components looked into such as watershed size, water quality, and habitat structure this could provide more insight on eel and how they grow in Nova Scotia and New Brunswick various habitats as well as more information on *Anguillicoloides crassus* in North America`s more northern waters.

This appendix will serve as a guide to preparing and comparing American eel otoliths for ageing using the cut and burn technique.

Table 7: Summary of otolith age estimates (n=15) from various experts in the field, images of cut and burnt otoliths were sent to experienced otolith agers Noella McDonald (NM), Katherine Martha Jones (KMJ), Brian Jessop (BJ), and Russell Poole (RP).(DC = Dollie Campbell) (Figures 13-27).

Province	Drainage	Image ID	Total lgt (cm)	Total wgt (g)	Age (DC)	Age (NM)	Age (KMJ)	Age (BJ)	Age (RP)	Average of ages	Comments
NS	Annapolis	DC080517	32.4	54.33	17	18	14			16	
NS	Annapolis	0255FRAR	29.1	37.5	8	9	8	8	10	9	
NS	Annis	0464ANAR	23.1	16.1	14	12	9	10	14	12	
NS	Annis	0488ANAR	23.9	18.79	9		6		16	10	Hard to read
NS	Ecum Secum	DC080134	33.9	83.5	12	13	13	14	13	13	Hard to read
NS	Ecum Secum	DC080154	25.9	24.5	7	6	6	6	6	6	
NS	Ingram	DC080528	28.5	48.29	11	11	11			11	
NS	Ingram	DC080592	20.8	11.64	8	7	6			7	
NS	Medway	DC080640	31.7	51.59	8	8	9			8	
NS	Mersey	0337MEAR	37.8	211.4	14	11	11	11	19	13	
NS	Salmon (Digby)	DC080426	30.7	51.15	9		13			11	Hard to read
NS	Salmon (Ltown)	DC080558	31	54.49	6	7	7			7	
NS	St. Mary`s River	DC080005	34.6	75.06	13	11	10	10	15	11.8	
NS	St. Mary`s River	DC090588	29.3	44.54	13	14	9			12	
NS	Tusket	DC080408	40.2	112.81	12	12	13		13	13	

Table 8: American eel (*Anguilla rostrata*) age estimated data (by Dollie Campbell) from a selection of 19 watersheds from the 2008-2009 electrofishing collections from Nova Scotia and New Brunswick.

River	Site ID	Latitude (D.d) N	Longitude (D.d) W	Distance to sea (km)	Length (cm)	Weight (g)	No. of parasites	Est. ages
St. Croix	Wag 1	45.2399	67.1351	0.43	34	75.57	0	8
St. Croix	Wag 1	45.2399	67.1351	0.43	31.6	60.79	0	11
St. Croix	Wag 1	45.2399	67.1351	0.43	33.6	72.06	0	6
St. Croix	Wag 1	45.2399	67.1351	0.43	32.8	66.49	0	6
St. Croix	Wag 1	45.2399	67.1351	0.43	31.4	60.49	0	9
St. Croix	Wag 1	45.2399	67.1351	0.43	31.8	66.04	0	10
St. Croix	Wag 1	45.2399	67.1351	0.43	32.6	57.78	0	7
St. Croix	Wag 1	45.2399	67.1351	0.43	26.4	29.74	0	8
St. Croix	Wag 1	45.2399	67.1351	0.43	26.9	31.09	0	10
St. Croix	Wag 1	45.2399	67.1351	0.43	25	26.26	0	9
Rodney Brook	SU101	43.7622	65.2572	0.47	32.4	54.33	0	17
Rodney Brook	SU101	43.7622	65.2572	0.47	29.4	47.00	0	15
Rodney Brook	SU101	43.7622	65.2572	0.47	29.0	37.78	0	12
Rodney Brook	SU101	43.7622	65.2572	0.47	28.7	38.09	0	9
Rodney Brook	SU101	43.7622	65.2572	0.47	29.7	38.19	0	16
Rodney Brook	SU101	43.7622	65.2572	0.47	29.5	41.27	0	15
Rodney Brook	SU101	43.7622	65.2572	0.47	25.0	20.60	0	10
Rodney Brook	SU101	43.7622	65.2572	0.47	22.6	18.06	0	10
Tangier River	SU43C	44.8143	62.7133	1.03	22.6	20.40	0	11
Tangier River	SU43C	44.8143	62.7133	1.03	21.3	14.88	0	7
Salmon River (Lawrencetown)	SU35B	44.6958	63.4550	1.89	29.0	43.30	0	5
Salmon River (Lawrencetown)	SU35B	44.6958	63.4550	1.89	31.0	54.49	0	6
Salmon River (Lawrencetown)	SU35B	44.6958	63.4550	1.89	26.5	35.91	0	9
Salmon River (Lawrencetown)	SU35B	44.6958	63.4550	1.89	23.6	24.30	0	5
Salmon River (Lawrencetown)	SU35B	44.6958	63.4550	1.89	31.0	38.25	0	4
Salmon River (Lawrencetown)	SU35B	44.6958	63.4550	1.89	20.1	11.35	0	6
Salmon River (Lawrencetown)	SU35B	44.6958	-63.4550	1.89	25.0	22.45	0	3
Salmon River (Lawrencetown)	SU35B	44.6958	-63.4550	1.89	29.7	44.24	0	3

River	Site ID	Latitude (D.d) N	Longitude (D.d) W	Distance to sea (km)	Length (cm)	Weight (g)	No. of parasites	Est. ages
Ingram River	SU29B	44.6883	63.9636	2.25	30.3	50.57	0	11
Ingram River	SU29B	44.6883	63.9636	2.25	28.4	46.14	0	14
Medway River	Medw109	44.1723	64.6532	5.61	33.2	71.81	0	10
Medway River	Medw109	44.1723	64.6532	5.61	33.8	63.84	0	6
Medway River	Medw109	44.1723	64.6532	5.61	32	56.73	0	8
Medway River	Medw109	44.1723	64.6532	5.61	29.5	40.85	0	12
Medway River	Medw109	44.1723	64.6532	5.61	25.2	31.03	0	12
Medway River	Medw109	44.1723	64.6532	5.61	27.1	30.13	0	10
Medway River	Medw109	44.1723	64.6532	5.61	25.6	22.07	0	10
Medway River	Medw109	44.1723	64.6532	5.61	23.6	21.96	0	8
Ecum Secum	SU54B	45.0133	62.1689	5.72	33.1	65.40	0	12
Ecum Secum	SU54B	45.0133	62.1689	5.72	25.3	25.60	0	14
Ecum Secum	SU54B	45.0133	62.1689	5.72	33.9	83.50	0	12
Mersey River	SU19C	44.0794	64.8083	8.14	28.1	48.88	0	10
Mersey River	SU19C	44.0794	64.8083	8.14	28.9	45.97	0	9
Mersey River	SU19C	44.0794	64.8083	8.14	29.4	47.82	0	10
Mersey River	SU19C	44.0794	64.8083	8.14	26.5	39.63	0	8
Mersey River	SU19C	44.0794	64.8083	8.14	25.7	30.60	0	8
Mersey River	SU19C	44.0794	64.8083	8.14	20.7	15.78	0	8
Mersey River	SU19C	44.0794	64.8083	8.14	23.3	19.36	0	8
Mersey River	SU19C	44.0794	64.8083	8.14	21.6	17.22	0	7
Mersey River	SU19C	44.0794	64.8083	8.14	21.6	17.06	0	7
Mersey River	SU19C	44.0794	64.8083	8.14	21.0	14.85	0	7
Mersey River	SU19C	44.0794	64.8083	8.14	21.9	22.22	0	8
Mersey River	SU19C	44.0794	64.8083	8.14	21.2	16.25	0	8
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	24.6	30.12	0	4
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	30.7	51.15	0	8
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	28.8	43.65	0	6
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	27.0	33.67	0	9

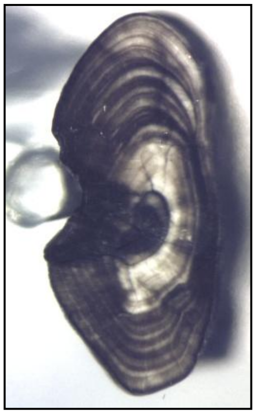
River	Site ID	Latitude (D.d) N	Longitude (D.d) W	Distance to sea (km)	Length (cm)	Weight (g)	No. of parasites	Est. ages
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	26.0	24.66	0	9
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	23.7	20.89	0	7
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	22.8	20.54	0	8
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	21.8	17.05	0	6
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	22.9	18.00	0	7
Salmon River (Digby)	SU8B	44.0509	66.0954	10.65	23.1	19.45	0	4
Mersey River	SU19D	44.0934	64.8462	11.82	34.4	141.60	1	6
Mersey River	SU19D	44.0934	64.8462	11.82	34.7	116.30	0	9
Ingram River	SU29D	44.7534	63.9754	13.00	27.7	33.50	0	13
Ingram River	SU29D	44.7534	63.9754	13.00	29.4	44.71	0	16
Ingram River	SU29D	44.7534	63.9754	13.00	27.4	29.04	0	10
Ingram River	SU29D	44.7534	63.9754	13.00	30.2	42.87	0	16
Ingram River	SU29D	44.7534	63.9754	13.00	25.6	28.68	0	13
Ingram River	SU29D	44.7534	63.9754	13.00	26.3	23.55	0	11
Ingram River	SU29D	44.7534	63.9754	13.00	24.5	19.48	0	10
Ingram River	SU29D	44.7534	63.9754	13.00	20.8	11.64	0	7
Ecum Secum	SU54A	45.0803	62.2451	17.68	20.2	12.90	0	7
Annis River	SU9B	43.9519	65.9942	20.11	25.2	20.60	0	13
Annis River	SU9B	43.9519	65.9942	20.11	29.6	47.80	0	13
Annis River	SU9B	43.9519	65.9942	20.11	27	30.20	0	14
Annis River	SU9B	43.9519	65.9942	20.11	23.1	16.10	0	12
Annis River	SU9B	43.9519	65.9942	20.11	21.4	13.00	0	15
Annis River	SU9B	43.9519	65.9942	20.11	22.6	13.50	0	12
Annis River	SU9B	43.9519	65.9942	20.11	23	49.60	0	17
Annis River	SU9B	43.9519	65.9942	20.11	21.5	12.30	0	11
Annis River	SU9B	43.9519	65.9942	20.11	23.9	18.80	0	9
Ecum Secum	SU54D	45.1056	62.2214	21.33	25.9	24.50	0	7
Ecum Secum	SU54D	45.1056	62.2214	21.33	27.9	33.86	0	12
Ecum Secum	SU54D	45.1056	62.2214	21.33	25.9	24.50	0	10

River	Site ID	Latitude (D.d) N	Longitude (D.d) W	Distance to sea (km)	Length (cm)	Weight (g)	No. of parasites	Est. ages
Ecum Secum	SU54D	45.1056	62.2214	21.33	26.5	21.81	0	18
Tangier River	SU43A	44.9667	62.8203	26.81	28.7	27.38	0	10
Tangier River	SU43A	44.9667	62.8203	26.81	26.1	27.81	0	10
Tangier River	SU43A	44.9667	62.8203	26.81	20.7	13.77	0	5
St. Mary`s River	STMR854.2	45.2707	62.0173	31.33	24.3	23.74	0	5
St. Mary`s River	STMR854.4	45.2721	62.0179	31.50	33.2	56.00	0	6
St. Mary`s River	STMR854.4	45.2721	62.0179	31.50	24.7	23.22	0	6
St. Mary`s River	STMR854.4	45.2721	62.0179	31.50	25.4	24.70	0	9
St. Mary`s River	STMR854.4	45.2721	62.0179	31.50	24.5	19.79	0	5
St. Mary`s River	STMR854.4	45.2721	62.0179	31.50	23.4	15.44	0	7
Annapolis River	SU107	44.9584	64.9029	34.50	33.6	64.50	0	10
Annapolis River	SU107	44.9584	64.9029	34.50	29.9	36.80	0	8
Annapolis River	SU107	44.9584	64.9029	34.50	33.5	61.20	0	8
St. Mary`s River	STMR923	45.3216	62.0821	35.86	34.6	75.06	0	11
St. Mary`s River	STMR923	45.3216	62.0821	35.86	22.5	17.02	0	6
St. Mary`s River	STMR923	45.3216	62.0821	35.86	23.0	17.07	0	8
St. Mary`s River	STMR923	45.3216	62.0821	35.86	22.8	16.82	0	7
Tusket River	SU10E	44.1133	65.9161	39.82	30.3	41.52	0	7
Tusket River	SU10E	44.1133	65.9161	39.82	30.7	45.50	0	7
Tusket River	SU10E	44.1133	65.9161	39.82	33.8	72.05	0	8
Tusket River	SU10E	44.1133	65.9161	39.82	32.1	51.71	0	7
Tusket River	SU10E	44.1133	65.9161	39.82	30.9	43.00	0	7
Tusket River	SU10E	44.1133	65.9161	39.82	26.0	28.14	0	4
Tusket River	SU10E	44.1133	65.9161	39.82	30.1	42.02	0	8
Tusket River	SU10E	44.1133	65.9161	39.82	28.4	40.58	0	9
Tusket River	SU10E	44.1133	65.9161	39.82	25.3	30.03	0	5
Saint John River	Ormct 1	45.7063	66.6039	47.35	27.8	45.66	0	9
Medway River	Medw108	44.4060	64.9861	48.20	31.7	51.59	0	8
Medway River	Medw108	44.4060	64.9861	48.20	29.9	43.04	0	7

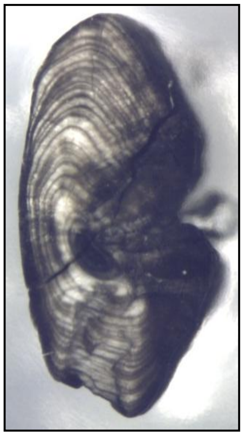
River	Site ID	Latitude (D.d) N	Longitude (D.d) W	Distance to sea (km)	Length (cm)	Weight (g)	No. of parasites	Est. ages
Medway River	Medw108	44.4060	64.9861	48.20	27.8	30.66	0	8
Saint John River	BelCr 1	45.6749	65.8095	49.26	31.4	52.32	0	9
Saint John River	BelCr 1	45.6749	65.8095	49.26	29.4	43.14	3	8
Saint John River	BelCr 1	45.6749	65.8095	49.26	30.3	45.47	0	7
Saint John River	BelCr 1	45.6749	65.8095	49.26	29.9	39.67	1	7
Saint John River	BelCr 1	45.6749	65.8095	49.26	26.2	32.88	0	8
Saint John River	BelCr 2	45.6920	65.7824	52.55	32.9	67.74	0	10
Saint John River	BelCr 2	45.6920	65.7824	52.55	34.1	60.66	0	9
Saint John River	BelCr 2	45.6920	65.7824	52.55	28.6	33.59	0	6
Saint John River	BelCr 2	45.6920	65.7824	52.55	27.6	35.63	0	6
St Mary's River	STMR867.2	45.4408	62.3242	61.68	27.4	33.82	0	7
St Mary's River	STMR867.2	45.4408	62.3242	61.68	29.3	44.54	0	13
St Mary's River	STMR867.2	45.4408	62.3242	61.68	30.9	44.72	0	6
St Mary's River	STMR867.2	45.4408	62.3242	61.68	30	48.35	0	5
St Mary's River	STMR867.2	45.4408	62.3242	61.68	30.8	48.04	0	6
St Mary's River	STMR867.2	45.4408	62.3242	61.68	26.9	34.22	0	7
St Mary's River	STMR 8510-8	45.4302	62.3126	68.16	26.1	27.44	0	6
St Mary's River	STMR 8510-8	45.4302	62.3126	68.16	20.8	12.23	0	3
Saint John River	Ormt R 2	45.8683	66.7012	68.94	34	72.81	0	8
Saint John River	Ormt R 2	45.8683	66.7012	68.94	33.4	69.01	0	11
Saint John River	Ormt R 2	45.8683	66.7012	68.94	32.3	74.76	0	6
Saint John River	Ormt R 2	45.8683	66.7012	68.94	34.3	85.63	0	7
Saint John River	Ormt R 2	45.8683	66.7012	68.94	31.9	65.4	0	9
Saint John River	Ormt R 2	45.8683	66.7012	68.94	32.8	51.07	0	9
Saint John River	Ormt R 2	45.8683	66.7012	68.94	30.5	52.81	0	10
Saint John River	Kenb 1	45.8515	65.5649	75.91	33.5	52.7	0	4
Saint John River	Kenb 1	45.8515	65.5649	75.91	26.4	26.57	0	6
Saint John River	Kenb 1	45.8515	65.5649	75.91	24.6	21.85	0	4
Saint John River	Nhwksi 1	46.0806	66.7683	93.30	33.7	70.5	0	11

River	Site ID	Easting (D.d)	Northing (D.d)	Distance to sea (km)	Length (cm)	Weight (g)	No. of parasites	Est. ages
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	31.6	62.77	0	8
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	32.7	64.08	0	10
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	31.2	45.95	0	8
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	28.3	31.55	0	9
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	26.4	25.82	0	7
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	23.3	18.18	0	8
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	21.6	15.63	0	4
Saint John River	Nhwksi 1	46.0806	-66.7683	93.30	20.9	13.9	0	7
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	32.6	68.59	0	10
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	30.9	49.62	0	10
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	31.1	52.73	0	8
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	29.5	36.11	0	8
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	28.7	36.43	0	9
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	25	20.19	0	9
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	26.3	29.69	0	8
Saint John River	Nshwk 1	46.1767	-66.4636	94.85	20.5	12.29	0	6
Saint John River	Keswk 2	46.1082	-67.0202	105.18	29.8	44.83	0	8
Saint John River	Keswk 2	46.1082	-67.0202	105.18	31	54.1	0	9
Saint John River	Keswk 2	46.1082	-67.0202	105.18	33	64.48	0	11
Saint John River	Keswk 2	46.1082	-67.0202	105.18	27.5	35.16	0	8
Saint John River	Keswk 2	46.1082	-67.0202	105.18	28	38.1	0	9
Saint John River	Keswk 1	46.1396	-67.0336	109.44	33.9	70.19	0	11
Saint John River	Keswk 1	46.1396	-67.0336	109.44	30.7	37.36	0	9
Saint John River	Keswk 1	46.1396	-67.0336	109.44	29.4	41.79	0	8
Saint John River	Keswk 1	46.1396	-67.0336	109.44	28.8	37.39	0	8
Saint John River	Keswk 1	46.1396	-67.0336	109.44	26.1	26.69	0	9
Saint John River	Keswk 1	46.1396	-67.0336	109.44	22.6	17.69	0	9

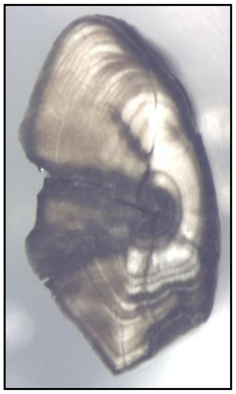
Figures



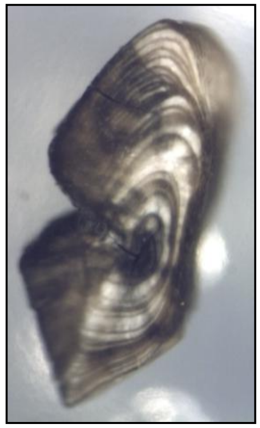
Figures 13: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID 0255FRAR (Annapolis River), total length = 29.1 cm, and weight = 37.5 g. Estimated age is 8 years.



Figures 14: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080517 (Annapolis River), total length = 34.2 cm, and weight = 54.3 g. Estimated age is 17 years.



Figures 15: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID 0488ANAR (Annis River), total length = 23.9 cm and weight = 18.8 g. Estimated age is 9 years.



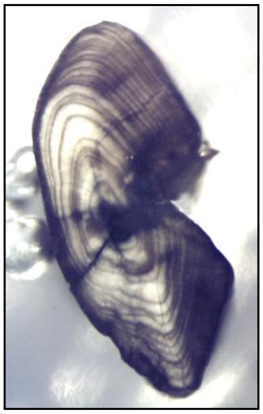
Figures 16: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID 0464ANAR (Annis River), total length = 23.1 cm, and weight = 16.1 g. Estimated age is 14 years.

Figures 17: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080134 (Ecum Secum River), total length = 33.9 cm and weight = 83.5 g. Estimated age is 13 years.

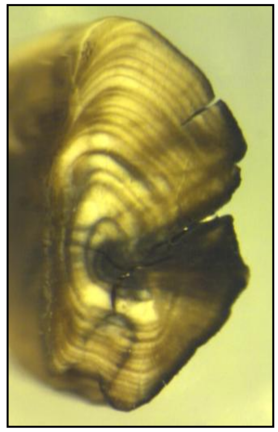
Figures 18: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080154 (Ecum Secum River), total length = 25.9 cm and weight = 24.5 g. Estimated age is 6 years.

Figures 19: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080528 (Ingram River), total length = 28.5 cm, and weight = 48.3 g. Estimated age is 11 years.

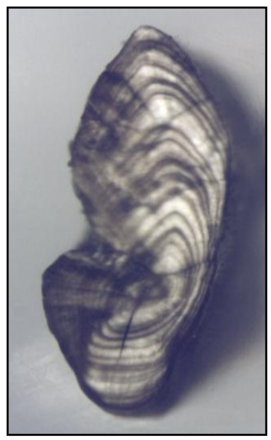
Figures 20: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080592 (Ingram River), total length = 20.8 cm, and weight = 11.6 g. Estimated age is 7 years.



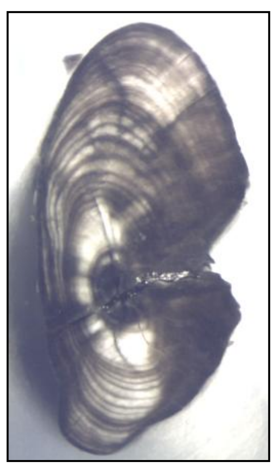
Figures 21: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080005 (St. Mary`s River), total length = 34.6 cm, and weight = 75.1 g. Estimated age is 11 years.



Figures 22: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC090588 (St. Mary`s River), total length = 29.3 cm, and weight = 44.5 g. Estimated age is 12 years.



Figures 23: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080408 (Tusket River), total length = 40.2 cm, and weight = 112.8 g. Estimated age is 12 years.



Figures 24: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080426 (Salmon River - Digby), total length = 30.7 cm, and weight = 51.2 g. Estimated age is 11 years.

Figures 25: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080558 (Salmon River - Lawrencetown), total length = 31.0 cm, and weight = 54.5 g. Estimated age is 7 years.

Figures 26: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID DC080640 (Medway River), total length = 31.7 cm, and weight = 51.6 g. Estimated age is 8 years.

Figures 27: American eel otoliths images using the cut and burn technique, that were sent to experienced eel otolith agers. Otolith ID 0337MEAR (Mersey River), total length = 37.8 cm and weight = 211.4 g. Estimated age is 11 years.

Figure 28: Scatter plot representing selected American eel (*Anguilla rostrata*) age estimates ranged through the 20 cm to 35 cm range (n=174). A correlation (r = 0.142, p-value = 0.063) was preformed comparing the estimated ages and the total lengths.

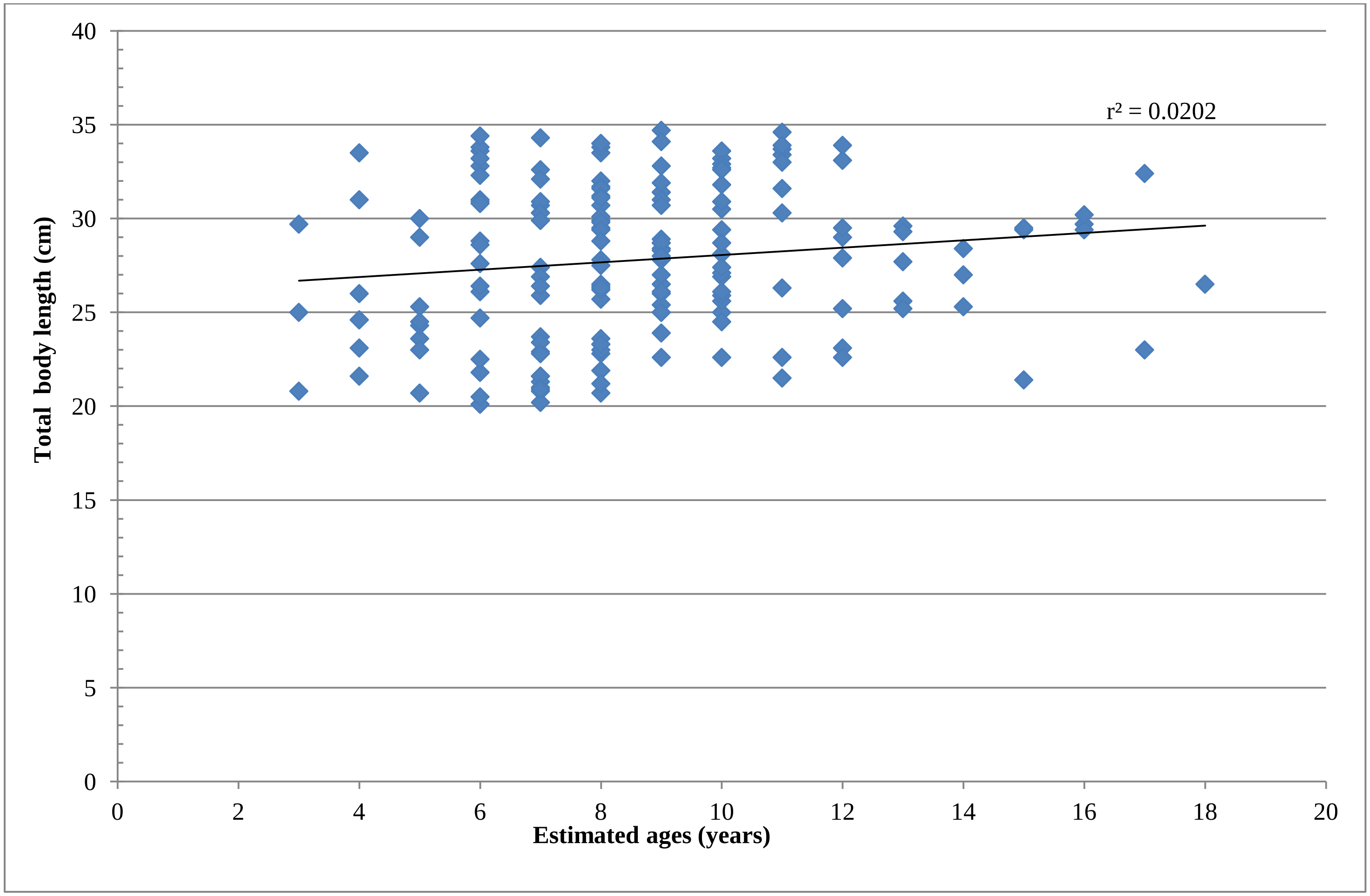
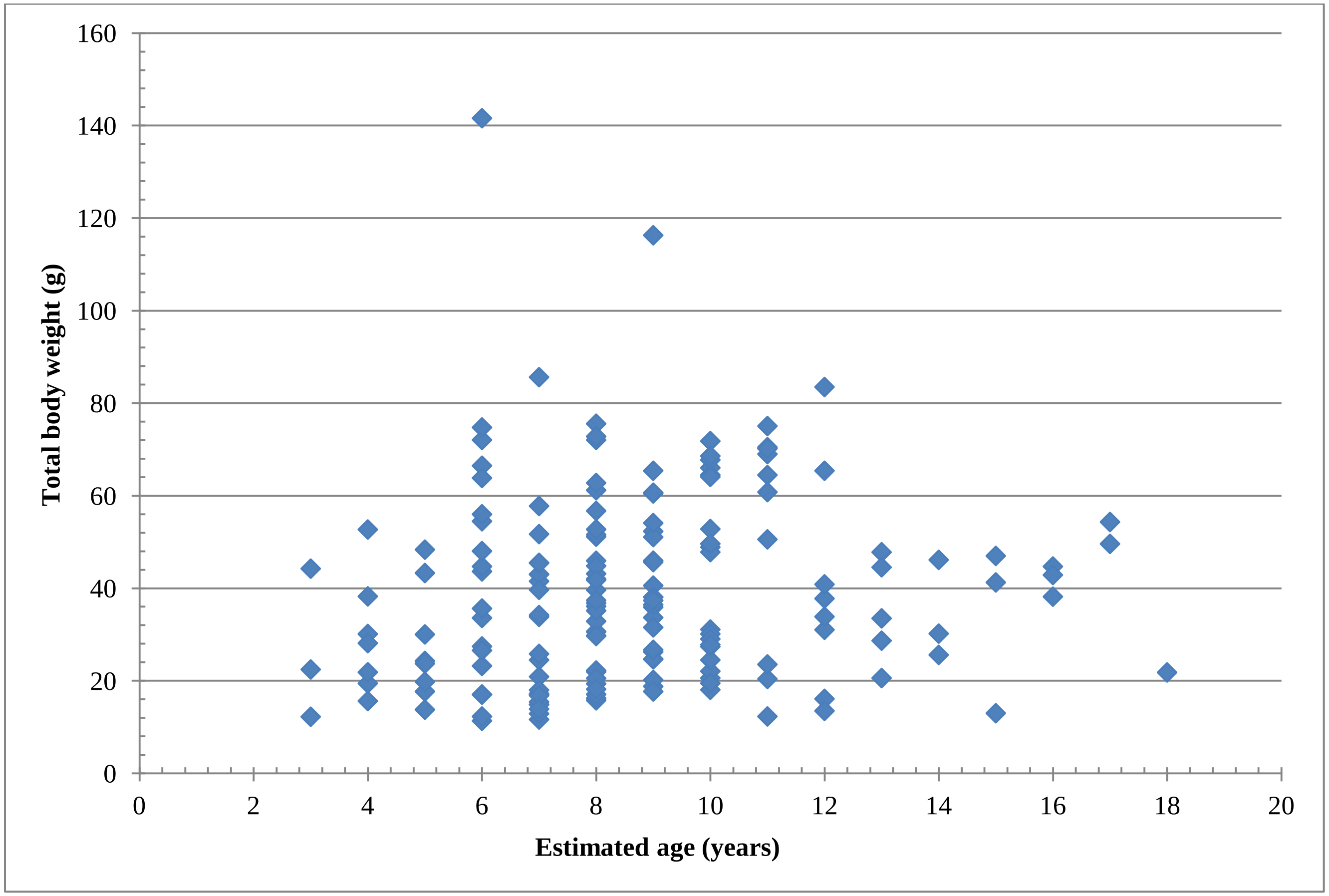

r² = 0.0202
Total body length (cm)
Estimated ages (years)
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10
12
14
16
18
20

Figure 29: Scatter plot representing selected American eel (*Anguilla rostrata*) age estimates (n=174) ranging in weight from 5 g to 142 g. Linear trend line was added ($r^2 = 0.012$) and a correlation ($r = 0.111$, p-value = 0.148) was used comparing estimated eel age and the eel weights.



160
140
120
100
80
60
40
20
0
Total body weight (g)
0
2
4
6
8
10
12
14
16
18
20
Estimated age (years)