Prevalence and intensity of *Salmincola edwardsii* on brook trout, *Salvelinus fontinalis*, in the Western Brook system of Gros Morne National Park, Newfoundland and Labrador, Canada©

by

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Abstract

Brook trout (*Salvelinus fontinalis*) is a salmonid species commonly found in rivers and estuarine systems in Newfoundland and Labrador. It is one of the most recreationally angled salmonids in the province. Brook trout health and reproductive fitness may be influenced by parasite load (both endo and ecto parasites) therefore, a baseline study was conducted on parasitic abundance on the *S. fontinalis* species. Brook trout from the Western Brook and Deer Arm Brook systems in Gros Morne National Park, Newfoundland were sampled to determine prevalence and intensity of ectoparasitism with respect to fish size. *Salmincola edwardsii* was the only ectoparasite found on *S. fontinalis*. Parasite intensity in the Western Brook system was approximately1.65 parasites per infected fish, with a range of 1-12. Prevalence ranged from 45-50% of fish infected from estuarine to freshwater conditions. Data were collected by excising the heads, tails, and fins of brook trout (n=52 from estuary and n=10 from fresh water) after determining fish fork length and weight. Fish were caught by recreational fisherman. Samples from Deer Arm Brook were excluded from the study due to improper sample preservation. Weak correlations were found using a Pearson Product-Moment correlation between log transformed ectoparasite abundance and fish fork length ($r=0.425$, $r=0.261$) and weight ($r=0.577$, $r=0.198$) in Western Brook Pond and Western Brook estuary, respectively. These results were compared to other studies conducted on abundance and distribution patterns of *S. edwardsii* on brook trout to provide baseline knowledge of parasite loads on brook trout in western Newfoundland. Comparative study sites included systems within Newfoundland and Labrador, as well as other Canadian provinces.

Introduction

Salvelinus fontinalis (Mitchill, 1814) is the common brook trout found in rivers of Newfoundland and Labrador. From the family Salmonidae, brook trout (also referred to as brook charr) are native to streams of eastern North America. They range from eastern Canada, from Newfoundland to the western side of the Hudson Bay, to the southern Atlantic including the Great Lakes and Mississippi River basins (Froese and Pauly, 2009). Two life history strategies exist within this species: 1) the fish spends its entire life in fresh water and 2) the fish migrates from freshwater to the sea (for varying periods of time) and back to the freshwater to spawn (anadromous). These varying migratory patterns are adaptations to optimize the most beneficial feeding and spawning strategies. Sea trout generally stay within eighty kilometres of their natal river (Crisp, 2000). These anadromous brook trout can return to their natal river at any time (as early as the spring) and await the next spawning period in the fall. They often spend a brief period in the estuarine environment (where fresh and salt water meet) to acclimatize to fresh water conditions and to await suitable water flow conditions to continue their migration upstream (Crisp, 2000).

Brook trout are an important environmental resource serving as an indicator of sound management and conservation of fluvial resources (Crisp, 2000). Brook trout are also one of the most recreationally fished species in the province and provide economic value within local communities (Sutton, 2000). Newfoundland has the highest participation rate of resident anglers in Canada (DFO, 2000). Additionally, recreational fishing contributes approximately $2.5 billion in direct expenditures by anglers annually in Canada.

With an increase in encroachment on spawning, nursery, and rearing habitats by industry and recreational anglers, it is important to have an overview of the general biology and ecological role that brook trout have in their environment (Dietrich, 2002). Population size and fitness can be maintained by monitoring brook trout in their natural and human impacted environments. This will increase our understanding of the impacts that various stressors and types of environments can have on brook trout populations. Stressors can include high water temperatures, predation, pollution, parasitism, etc. Focus on abundance of parasites and their behavioural characteristics on wild populations of brook trout will be examined in this study.

Parasites can be classified as endo or ecto-parasitic. Endoparasites live in cells, tissues, organs, and blood of a host organism. Ectoparasites live on the body surfaces of their host (Lawrence, 2005), specifically fins, gill filaments, mouth, and skin surfaces in the case of fish hosts (See Table 1) Parasites occur on virtually all wild populations of salmonids, with fish organs and tissues providing niche habitats for continued existence and procreation of a multitude of parasitic species (Willers, 1981). Parasitic species are highly specialized for survival in their respective hosts, some thriving in a variety of hosts, while others being host specific. This study focuses specifically on the parasitic abundance of the copepod ectoparasite, *Salmincola edwardsii* (Olsson, 1869) on *S. fontinalis*. Hosts for *Salmincola edwardsii* include both salmonids and freshwater whitefish from the genus *Coregonus*. Fish from the family Coregonidae were previously included in the family Salmonidae. Fish commonly infected with *Salmincola edwardsii* include: Arctic char (*Salvelinus alpinus*), brook trout (*S. fontinalis*), Dolly Varden trout (*S. malma*), and the inconnu (*Stenodus leucichthys*) (Kabata, 1992).

There are seventeen known species of *Salmincola* (gill lice), almost all parasites on salmonids and coregonid fishes (Kabata, 1992). In natural environmental conditions *S. edwardsii* are rarely present in sufficient numbers to cause serious injuries to hosts (Froese and Pauly, 2009). However, when fully established (like most parasitic copepods) they are difficult to control (e.g. in an aquaculture setting). This is primarily because of the sclerotized exoskeleton of the adult, which is very resistant to chemical pesticide solutions (Cheng, 1973). Human influences such as the use of trout hatcheries may increase the opportunity for free-swimming immature stages of *S. edwardsii* to find suitable hosts.

Increased abundance of *Salmincola* can impact the overall fitness of brook trout populations. The influence of *Salmincola californiensis* (a close relative to *S. edwardsii*) on fish egg production was monitored over a four year period at the Mt. Shasta trout hatchery, California. Fish egg abundance was measured two years before parasite removal from the hatchery and two years after. Both age II and age III brood fish eggs were examined. During the period before parasite removal, the average number of eggs per female between age II and age III brood fish was similar. After parasite removal, egg production increased from age II to age III brood fish, suggesting parasitism reduces the fishes' ability to produce eggs (Gall et al., 1972). As the fish grow larger, hatcheries could potentially lead to increases in infection intensity (mean number of parasites per infected fish), prevalence (percentage of fish infected), and overall decreased fitness for *Salvelinus* populations in the wild (Black, 1982). High prevalence and intensity can have adverse effects on the health and fitness of brook trout. *Salmincola* can cause swelling around the attachment site of their bulla with heavy parasite loads, reducing the feeding activity of the host fish and consequently causing a decrease in body weight (Nagasawa et al., 1998).

When considering parasite abundance on its fish host, size-related factors must be considered. These include: surface area of the fish, ventilation volume by the fish (i.e. gill size), and probability of parasites contacting the skin. These factors contribute to the prevalence and intensity of infection on the host fish. In a study by Poulin et al. (1991) size related factors of fish had a greater influence on parasite abundance than fish behavioural patterns. Therefore, in this study fish length and weight with respect to parasite abundance were examined for any correlations.

Table 1. Common parasites of brook trout (*Salvelinus fontinalis*) and their external or internal location (from Barnham, 2008).

Parasite	**Phylum**	**Location**
Fluke	Platyhelminthes	Monogenetic-almost exclusively external. Digenetic-internal with salmonids serving as both final and intermediate host
Tapeworm	Cestoda	The intestinal tract
Roundworm	Nematoda	Infesting an organ or tissue
Thorny-headed worm	Acanthocephala	The intestinal tract
Leech	Annelid	External on host
Copepod	Arthropoda	External on host

Life Cycle of Brook Trout

Brook trout, both the anadromous and estuarine form, start their upstream migration in the fall. The migration upstream is often performed in a series of stages, finding refuge in 'holding pools'. These 'holding pools' provide shelter from sunlight and predators and are located in deep pools, behind boulders, or around undercut banks and fallen trees (Crisp,2000). Four stimuli responsible for upstream migration, suggested by Crisp (2000), include: physiological readiness to spawn, temperature and dissolved oxygen concentrations, time of day (brook trout typically migrate during periods of darkness), and river flow. However, limitations to geographical areas conclude that these observations are strictly empirical and are dependent on the sampling methods favoured by each researcher. Obstructions can also hinder migration. Non-anadromous brook trout reside in freshwater ponds, spawning in their natal stream or pond shores where groundwater seepage occurs (Anions, 1994).

The life cycle of brook trout starts in freshwater rivers, where the egg develops into an alevin after fertilization (Figure 1). Although salmonids differ with respect to the time of year which they spawn, it is determined mainly by day length. During the spawning cycle, the female chooses a site with clean flowing water and suitably sized gravel and deposits the eggs after excavating a pit or redd. To examine the pit she crouches into it and lowers her anal fin, testing the water flow. If the site is not suitable, she will abandon the pit and choose a more appropriate site (Crisp, 2000). After a suitable redd is excavated, the female will deposit the eggs and the male will subsequently release the sperm. The redd is usually attended by a

dominant male, protecting the eggs from other males seeking to take part in the spawning. The eggs develop in the gravel to produce alevins which subsist upon their yolk sacs and emerge when the egg sac is almost exhausted, at this point becoming a fry. The fry then leave the redd to mature and become known as parr or 'young of the year'. These parr may reside in their native river or lake to mature or go to sea (anadromous) when temperatures in the river start to rise, typically occurring in late May to early June. Parr are known to adopt feeding areas which they will defend from other salmonids. Brook trout that go to sea are commonly referred to a smolts and return to their natal river to spawn after varying number of years at sea. The preparation of departure downstream to sea is known as smoltification. During this time the salmonid parr acquire a suite of physiological, morphological, biochemical, and behavioural changes.

Brook trout that are non-anadromous are identifiable by colourful markings with parr marks inter-dotted with red spots. They have diurnal spawning from October- November by age three in suitable streams or pond shores where groundwater or seepage occurs (Anions, 1994). Anadromous brook trout are often a silvery colour with no parr marks or red spots. They typically attain a much larger size due to greater food availability in the sea. If sea-run trout remain in fresh water, their colouration becomes like non-anadromous trout ('mud trout'). Anadromous brook trout first go to sea at varying ages in late spring, reaching sexual maturity by four years of age. They return to spawn in fall and overwinter in fresh water. Brook trout stop feeding on their return to fresh water due to the sudden absence of larger food and physiological readjustment (Anions, 1994). The estuarine form of brook trout inhabits estuaries and river mouths. Their growth rate is intermediate between the two other forms (Anions, 1994).

Brook trout are susceptible to parasitism by *Salmincola edwardsii* once they become young of the year. However, the infection intensity is found to increase once the salmonid

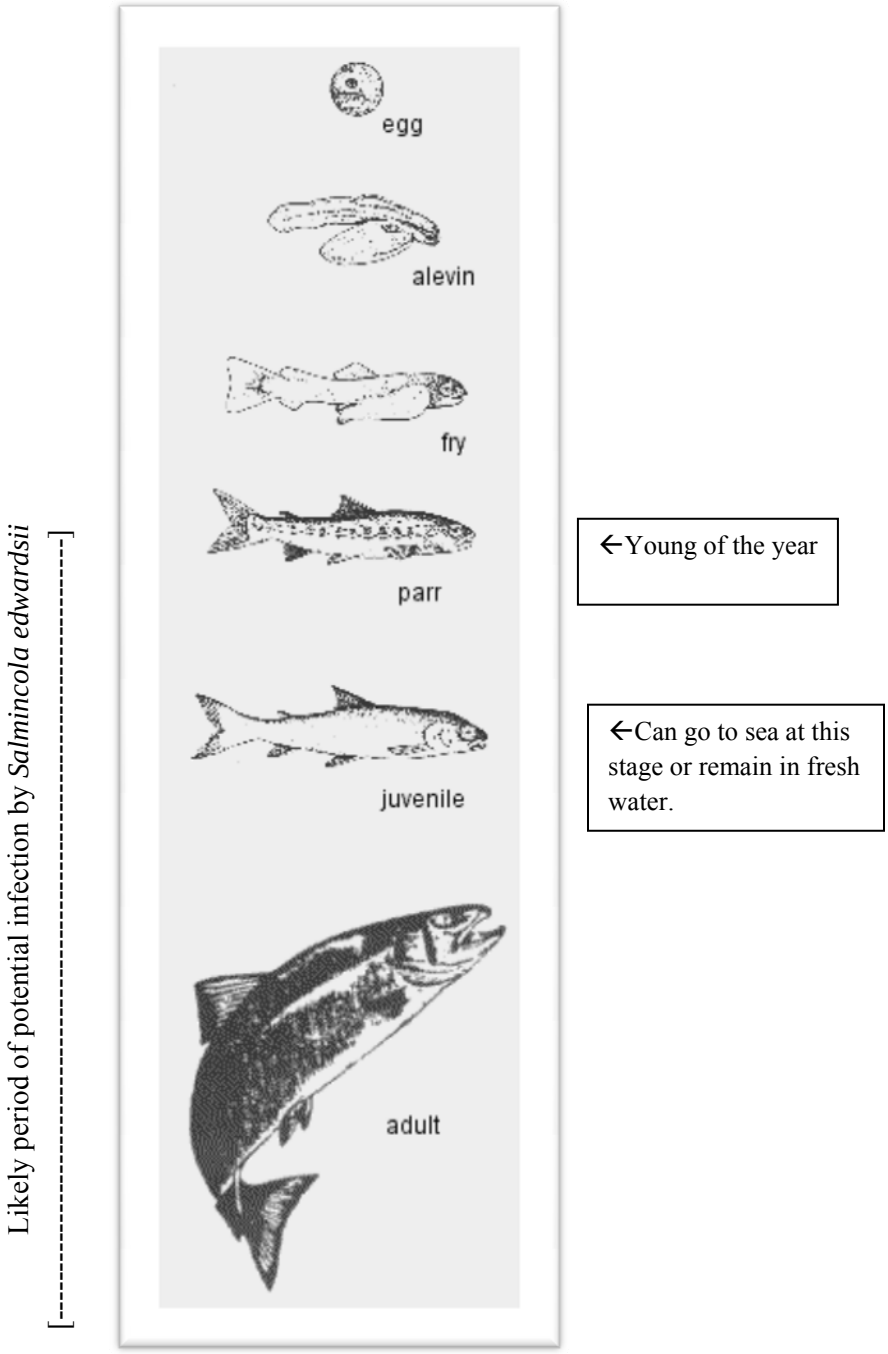


Figure1. The life cycle of brook trout (from brook trout haven)

reaches its juvenile stage (Muzzall, 2007). In natural host populations, the number of *S. edwardsii* per fish of similar size can range from zero to 50 or more (Poulin et al., 1991).

Morphology and Life Cycle of *Salmincola edwardsii*

Salmincola edwardsii (Phylum Arthropoda, subphylum Crustacea, family *Lernaeopodidae* (Wilson, 1915), is commonly known as 'gill lice' or 'gill maggot'. It is mainly found in cold, northern climates and exhibits a Holarctic distribution. Although the species is strictly fresh water, it can survive in a marine environment for a short time during its host's seaward migration (Kabata, 1992). They are known to infect fish of the genus *Salvelinus* and are a permanent ectoparasite, attaching to a single host throughout their entire lifecycle. They are commonly found on the surface of the body including gills, fins, and in the mouth of salmonids. Their external form has become so modified as a result of their adaptation to exoparasitism that they are hardly recognizable as copepods (Cheng, 1973). The body of *S. edwardsii* is composed of the cephalothorax and trunk, with no traces of trunk segmentation and thus distinct from free-living planktonic copepods (Kabata, 1969). The female and male exhibit sexual dimorphism, the male being reduced in size. The species is identifiable by the unusual prominence of process four on the endopod of its second antennae. *S. edwardsii* are distinguishable from other subspecies by the anatomical characteristics of the maxilliped and second antennae. *Salmincola edwardsii* are polymorphic, differing in size from region to region (Kabata, 1992). Even under low magnification its rami show very definite characteristics including inflation of the spiny pads of the endopod and sympod ; their

spines being long, slender, and prominent (Kabata, 1969). *Salmincola edwardsii* female adults are found attached to the distal tips of the host's gill filaments (Figure 2).

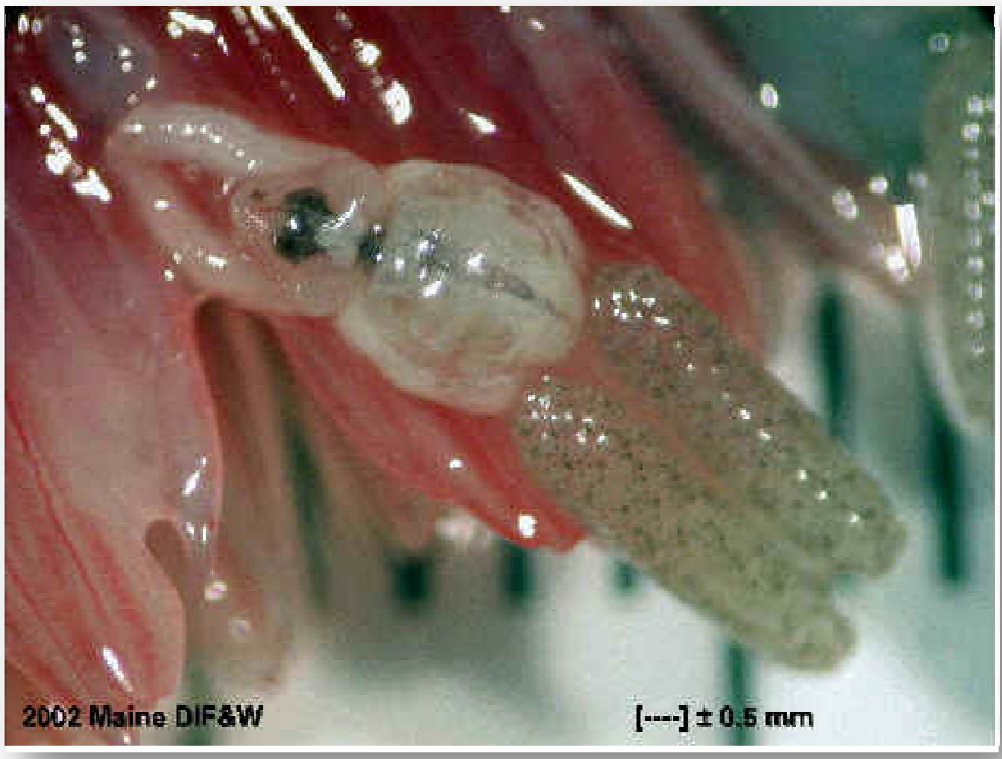


Figure 2. Mature female of *Salmincola edwardsii* with egg sacs, attached to distal tip of trout gill filament (Danner, 2000).

They typically feed on blood and haematin residues (Harris-Linton, 2001). Upon attachment to the host, the female continues to grow and is fertilized by the attachment of a male to her genital area. Following fertilization males are free-living and can fertilize other females (Kamerath et al., 2009).

S. edwardsii is an obligate aquatic species, with four larval (chalimus stages) and a fifth adult stage. Mature females have two identical egg sacs attached to the genital segment of the thorax. These sacs rupture as soon as the egg hatches into small, active larvae, known as the copepodid (Cheng, 1973). The nauplii stages typically seen in planktonic copepods are passed within the eggshell and the first copepodid is a free-swimming plankter for a brief period before attaching to a fish host (Pennak, 1978).Water temperature significantly affects the hatching time of eggs, with warmer waters (8-20°C) accelerating hatching time. During their lifecycle, the sexes of early larvae are indeterminant (Figure 3). Hatching out of the egg, the copepodid attaches to a fish host and undergoes a series of larval development or chalimus stages until reaching the adult form (Poulin et al., 1991). The length of these larval stages is variable depending on the time it takes the parasite to find a host and a suitable attachment site (Harris-Linton, 2001). Modifications of form still occur after attachment to host (Cheng, 1973). Chalimus stage I (copepodid stage) typically requires between 0.5-1.5 days of development. This chalimus stage is free-living and non-feeding, where most of their time is spent in the bottom of the water column before attachment to a host. Chalimus stage II occurs between days 1-2.5 and chalimus stage III occurs between days 2-4, with sex becoming determinable at the later part of this stage. Male chalimus stage IV occurs 2.5-5 days after

hatching from the egg. Adult males are formed between 3-8 days after hatching whereas females take anywhere between 4-20 days. Female chalimus stage III occurs 3.5-5 days after hatching and stage IV between 4-20 days. The male can mate with a female of chalimus stage IV of the same generation or with unfertilized females of other generations. The difference in maturation time allows adult males to breed with females of different generations, reducing unfavourable effects of inbreeding (Harris-Linton, 2001).

As an adult, the female makes a depression in the host tissue at the attachment site by scraping with the claws on its maxillipeds. It is not uncommon for the parasite to excavate several depressions before selecting a suitable attachment site (Kamerath et al., 2009). Upon attachment, the female is permanently anchored to its host's flesh by a bulb-like structure called the bulla (Figure 4). The bulla is non-living and formed from head and maxillary gland secretions. The maxillae, which are very large in proportion to the rest of the body, are used as a grasping structure and fused to the bulla (Figure 4). The abdomen is absent or vestigial in the adult female (Harris- Linton, 2001). After initial contact with a fish host, the female lifecycle is completed within 28-30 days (Kamerath et al., 2009).The male and female adults are approximately 718.6 μm and 2055.8 μm in length, respectively (Harris-Linton, 2001).

Salmincola edwardsii copepodids respond to stimuli produced by the movement of brook trout; this includes moving shadows and disturbances in the surrounding water. Upon stimulation *S. edwardsii* parasites will greatly increase their swimming or host-locating activity, leading to a potentially greater encounter rate with a host (Poulin et al., 1991).

Description of Study Site

A baseline study of ectoparasite abundance of *S. edwardsii* on *S. fontinalis* was undertaken at Western Brook Pond, Western Brook (its outlet tributary), and Deer Arm Brook. These sampling sites are located in Gros Morne National Park, Newfoundland and Labrador. Western Brook Pond is located in the northern part of Gros Morne National Park at 49° 44'N and 57° 46' W (Figure 5). Western Brook Pond is classified as ultra-oligotrophic, the basin being primarily composed of igneous rock, with relatively thin soil (Kerekes, 1978). The lake receives drainage from more than twenty streams with the majority of the streams cascading down from the highland plateau. The waters of these streams are very low in calcium with a pH of 5.5. However, turbulent mixing with the input from the largest inflow, Stag Lake (which has drainage off sedimentary rocks causing higher pH and calcium levels) helps maintain a circumneutral pH in the lake (Kerekes, 1978). Western Brook Pond has a total surface area of 23 km², volume of 1.65 km^3 , maximum depth of 165 m (mean depth of 72.5 m), total length of 42.5 km, residence time of 15.4 years, and catchment area of 171 km^2 (Kerekes, 1978). Western Brook River is the only stream which drains Western Brook Pond. It is approximately 4.83 km long travelling across low lying flatlands until emptying into the Gulf of St. Lawrence via a small estuarine system. The entire watershed of the river is within the boundaries of Gros Morne National Park (Kerekes, 1978). It hosts both landlocked and anadromous brook trout.

Deer Arm Brook system is located at 49°33'N and 57°50'W (Figure 6). Deer Arm Brook empties into Deer Arm, an estuary in the East arm of Bonne Bay. Deer Arm is

approximately 15.9 km long, with three main water bodies draining into it. These include: Eastern Arm Pond, Ten Mile Pond, and Half Moon Pond. Most of Deer Arm's watershed is located within the boundaries of Gros Morne National Park (McCue, 2006).

Estuarine and freshwater fish were examined together and then separately to determine possible relationships between parasite abundance and water type/flow. An estuary can be defined as a partially enclosed coastal body of water, having an open connection with the ocean, where freshwater from inland is mixed with saltwater from the sea. The water in an estuary usually has a salinity range of 0.5-30 ppt (NOAA). Fresh water is defined as naturally occurring water on the earth's surface present in bogs, ponds, lakes, rivers, and underground aquifers. It is characterized by having a low concentration of dissolved salts and other dissolved solids, with salinity less than 0.5 ppt (NOAA).

Data were collected during spring and summer 2009 from both Western Brook and Deer Arm Brook systems. Due to the sampling period coinciding with the migration of brook trout to sea, it is believed that brook trout caught in the estuary were likely anadromous and migrating to sea, while brook trout caught in Western Brook Pond were resident (M. Caputo, pers.com).

This project was conducted in association with a master's student research project (M. Caputo, Dept. of Biology, MUN) tracking migratory patterns of brook trout. It involved MUN's Ocean Science Centre, Parks Canada, and the Community Research and Recovery Alliance (CURRA). CURRA is a university and community group involved in research initiatives along western Newfoundland that seek to develop strategies to recover fish stocks and fishery communities.

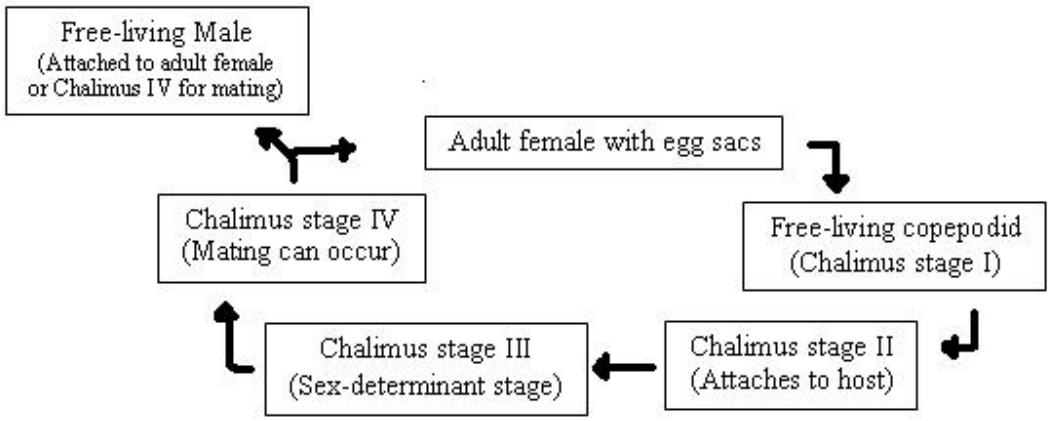


Figure 3. Lifecycle of *Salmincola edwardsii*. Juvenile copepodid stage and adult male are free-living; all other stages are attached to fish host.

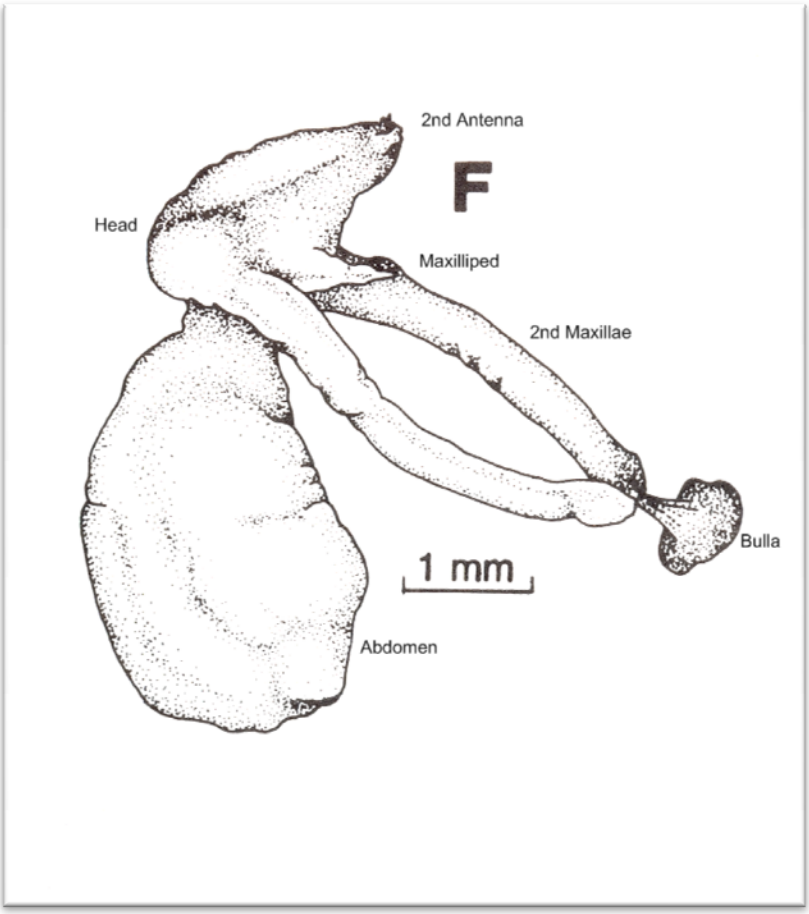


Figure 4. Mature female, *Salmincola edwardsii,* without egg sacs (Kabata, 1992).

Figure 5. Western Brook Pond and associated Western Brook (outlined in yellow) and estuary in Gros Morne National Park, Newfoundland and Labrador, Canada (M. Caputo, pers.com).

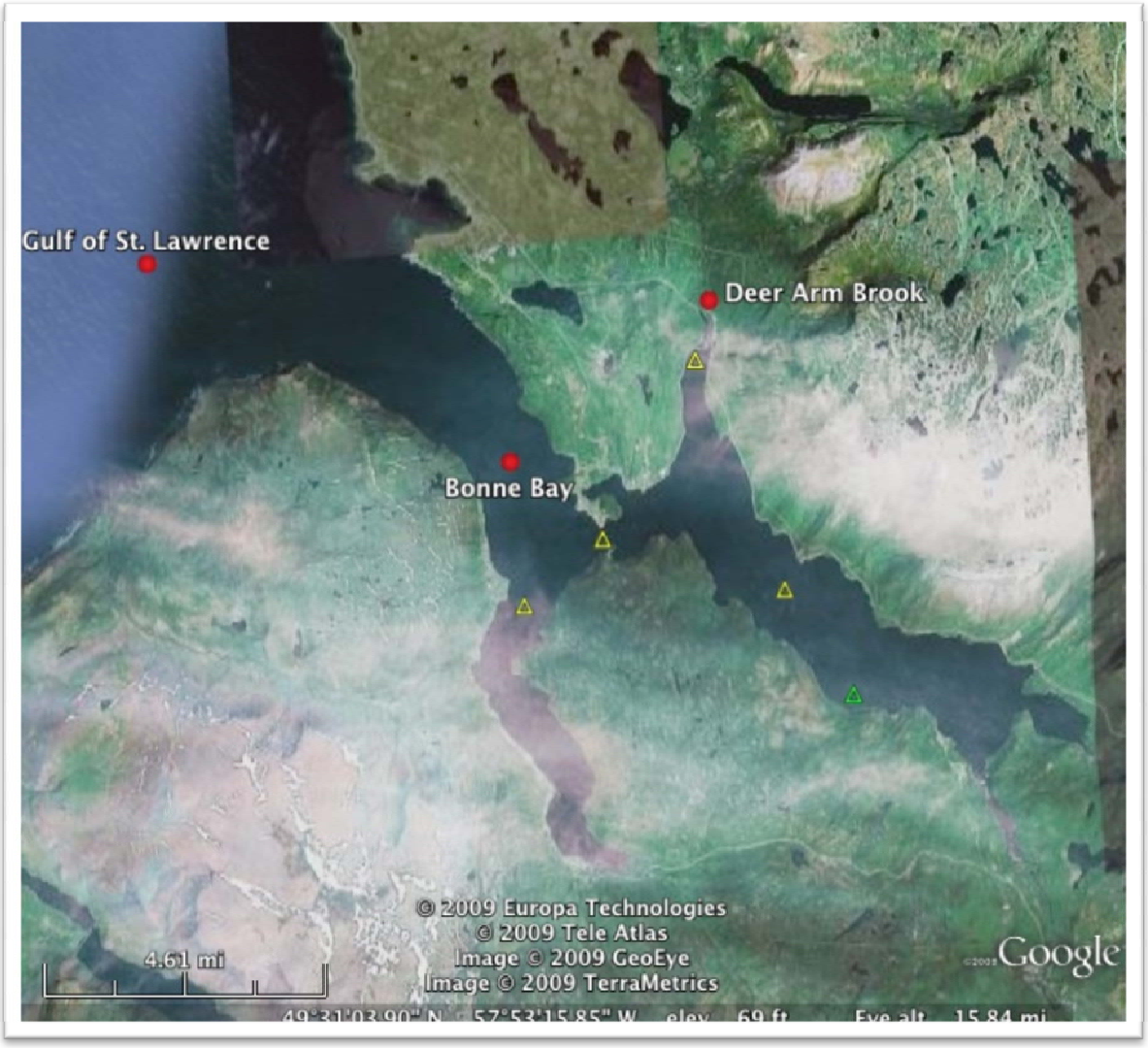


Figure 6. Deer Arm Brook located in Gros Morne Park, Newfoundland, Canada (M. Caputo, pers.com).

Materials and Methods

Data

Brook trout were caught in both the estuary (n=50) and fresh water (n=10) of Western Brook from May 28, 2009- June 24, 2009. Fish sampled in Western Brook estuary were caught by recreational anglers; we obtained all fish on the day of the catch. Fish sampled from Western Brook Pond were caught by our research team upon approval by Parks Canada. The prohibition of fishing in Western Brook Pond (regulated by Parks Canada) due to threatened population sizes only allowed for ten fish to be sampled. Fish sampled from Deer Arm Brook were caught by recreational anglers in the estuary of Bonne Bay. Fish heads from Deer Arm were saved by fisherman and picked up at their residence. No length and weight data were collected for these fish. Unfortunately fish obtained from Deer Arm Brook did not remain intact for further analysis due to improper storage of$. These specimens were therefore discarded and not used in this study.

Each fish was measured for weight (g) using a Scout Pro balance, and fork length (mm) was obtained using a fish measuring board. Fish heads with complete sets of gills were collected for otolith analysis and subsequent analysis for ectoparasite abundance. Tails and fins were also collected at Western Brook estuary, stored in polyethylene bags (with the heads), and put in an iced cooler until transferred to a freezer in the laboratory. Each bag was labelled and included only one fish.

Fish were examined using a compound microscope at 40x magnification for visible ectoparasites. The copepod parasites found were identified using the key of Pennak (1978)

and Kabata (1992). Determinations of body length and sex were obtained for a subsample of the specimens. Ectoparasites were fixed in 10% formalin for twenty-four hours, then preserved in 70% ethanol, and stored in glass vials. *S. edwardsii* abundances on gills, fins, and tail were compared to determine aggregation patterns.

Water chemistry parameters were measured on both Western Brook and Deer Arm Brook from May 24th to June 28th. Temperature, conductivity, and salinity were measured weekly using a YSI-30 metre. Each parameter was measured at 25 cm intervals from the surface to bottom. The pH was also measured using a pHep metre by HANNA. However, due to suspicion of inaccurate readings for both metres, the data were not included in the results. Temperature was used as a comparison to other studies, but not used for analysis in this study.

Throughout the data analysis, determinations were made of:

1. Number of ectoparasites on each fish
2. Percent of fish infected with ectoparasites (i.e. prevalence)
3. Mean number of ectoparasites per infected fish (i.e. intensity)
4. Ectoparasite length
5. Distribution of ectoparasites on host
6. Fish size

Statistical Analysis

Statistical analyses were carried out using Minitab (Minitab 12.2, Minitab Inc 1998). Fish fork length, weight, and parasite abundance were first analyzed using the Kolmogorov-Smirnov Test to determine the statistical distribution of the data and check for normality. Therefore, for statistical analysis, all infection data (number if parasites per host) were

normalized with $\log_{10}$ (x+1) transformation. Data from the fresh water and estuary environments were then analyzed separately, again with parasite abundance $\log_{10}$ transformed to normalize the data. A frequency distribution was also conducted to examine any aggregation patterns of *S. edwardsii*.

Using the Pearson product-moment correlation, the relationship between $\log_{10}$ transformed parasite abundance and fish weight was tested. Combined data from the Western Brook system was analyzed, followed by the fresh water and estuary environments separately. The same statistic was performed for the $\log_{10}$ transformed parasite abundance and fish fork length (results were considered significant with a $\alpha \geq 0.05$).

Data were again analyzed for correlations using the Pearson product-moment test, omitting fish without parasites. The strength of the correlations was obtained using Martin and Bateson (1993) as a reference. The Pearson product-moment test was chosen because it is typically used to examine whether two variables vary together, while not implying a cause and effect relationship. It is also reasonably robust when there is a departure from normality (Martin & Bateson, 1993). A Mann-Whitney U Test was then performed to test fish weight and fish length relationships between freshwater and estuarine locations. This test was chosen because it does not make assumptions about homogeneity of variances or normal distributions (Dytham, 2003).

Results

Parasite load in the Western Brook system shows an intensity of 1.7 in Western Brook estuary (WBE) and 1.6 in Western Brook Pond (WBP) (Table 2). These results are low compared to other lakes in Newfoundland (Headwater Pond; Cone and Ryan, 1983), Labrador (Smallwood Reservoir; Chinniah and Threlfall, 1978), and Algonquin Park, Ontario (Dickson Lake; Black, 1982). Mean prevalence (45% in WBE, 50% in WBP) was slightly higher than in the Smallwood Reservoir and Headwater Pond, but lower than in Dickson Lake (Table 2). The fish fork length (mean 247 in WBE and 347 in WBP) and weight (mean 201 in WBE and 648 in WBP) were within the range of other studies, therefore allowing for further comparisons.

A frequency distribution was generated to compare the number of parasites per host, showing that eighty percent of brook trout sampled harboured 0-2 parasites, while five percent of brook trout harboured a larger aggregation of parasites (11-12 parasites per fish; Figure 7). These results were comparable to that of Poulin et al. (1991), who conducted laboratory trials on brook trout and found *Salmincola* to be aggregated on their fish hosts, with few fish carrying greater parasites loads and the majority having less than three ectoparasites per fish.

The attachment location of each parasite on the fish was observed. The total percentage of parasites found on the gills was 86.7% for Western Brook Pond and 100% for Western Brook estuary. In both systems, the gills appear to be the more preferred site of attachment (Table 3). Preference of attachment site with respect to length classes of brook

trout was previously examined by Black (1982) at Dickson Lake, Algonquin Provincial Park. Findings indicate that intensity of attachment on the gills increases with fish length.

A subsample of *S.edwardsii* taken from the gills, tail, and fins of brook trout were measured for total body length (excluding egg sacs), with all specimens found to be over 3000 µm. Based on length criteria for sex determination (Harris-Linton, 2001) and that females are the only sex to form a permanent attachment to the host, all parasites in the subsample were determined to be adult female (Figure 8).

Table 2. Comparison of intensity (# parasites per infected fish), Prevalence (% of fish infected), length, and weight of Brook trout (*Salvelinus fontinalis*) infected by *Salmincola edwardsii*.

	Intensity (range)	Prevalence	# fish sampled	Fish Length (mm)		Fish Weight (g)	
				mean	range	Mean	Range
WBP estuary	1.7 (1-12)	45	52	247	196-397	201	43-497
WBP freshwater	1.6 (1-11)	50	10	347	286-421	648	203-1024
Smallwood Reservoir	5 (1-31)	37	68	380	115-600	1011	15-2268
Headwater Pond	2.4(1-8)	37	25		151->200		
Dickson Lake	8.1 (1-61)	71	100		200-500		

(Smallwood Reservior: Chinniah and Threlfall (1978), Headwater Pond: Cone and Ryan (1983), Dickson Lake: Black (1982))

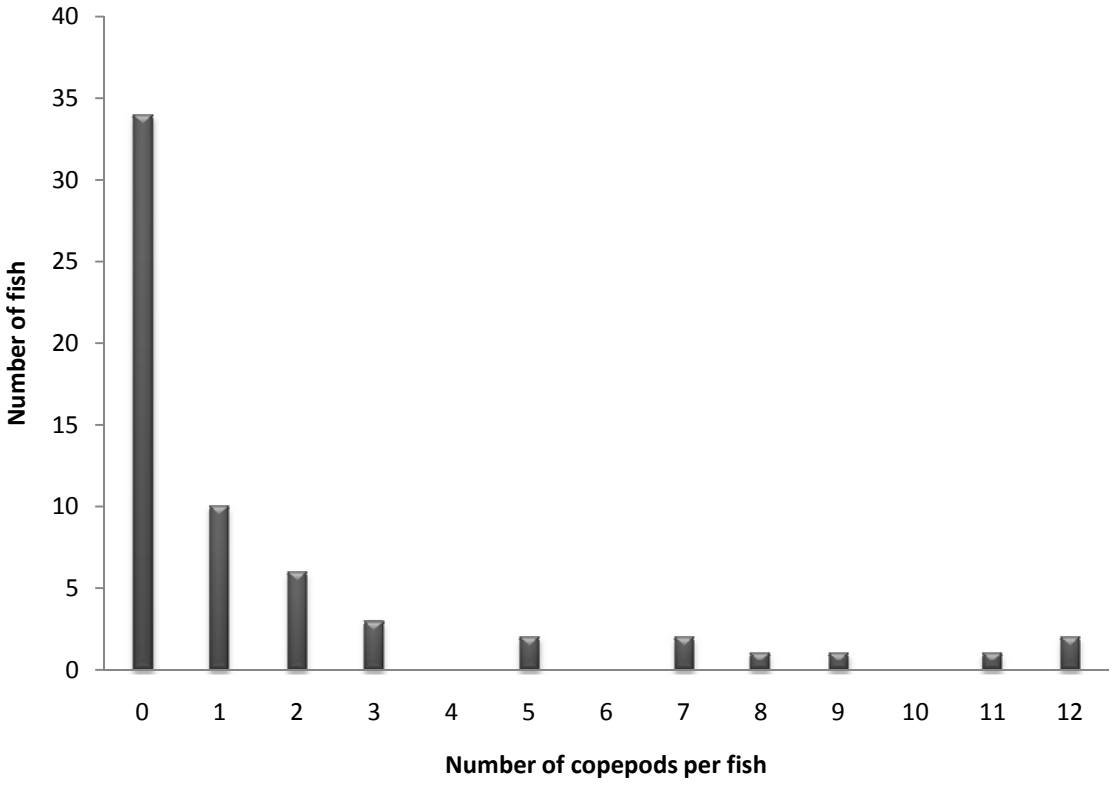


Figure 7. Frequency distribution of the number of *S. edwardsii* parasites, per host among *S. fontinalis* in the Western Brook system (n=62).

Table 3. Parasite distribution with respect to location found on the host expressed in terms of percent of total parasites.

	Western Brook All Data	Western Brook Pond	Western Brook Estuary
Gills	99.98%	86.7%	100%
Tail and Fins	0.02%	13.3%	0%

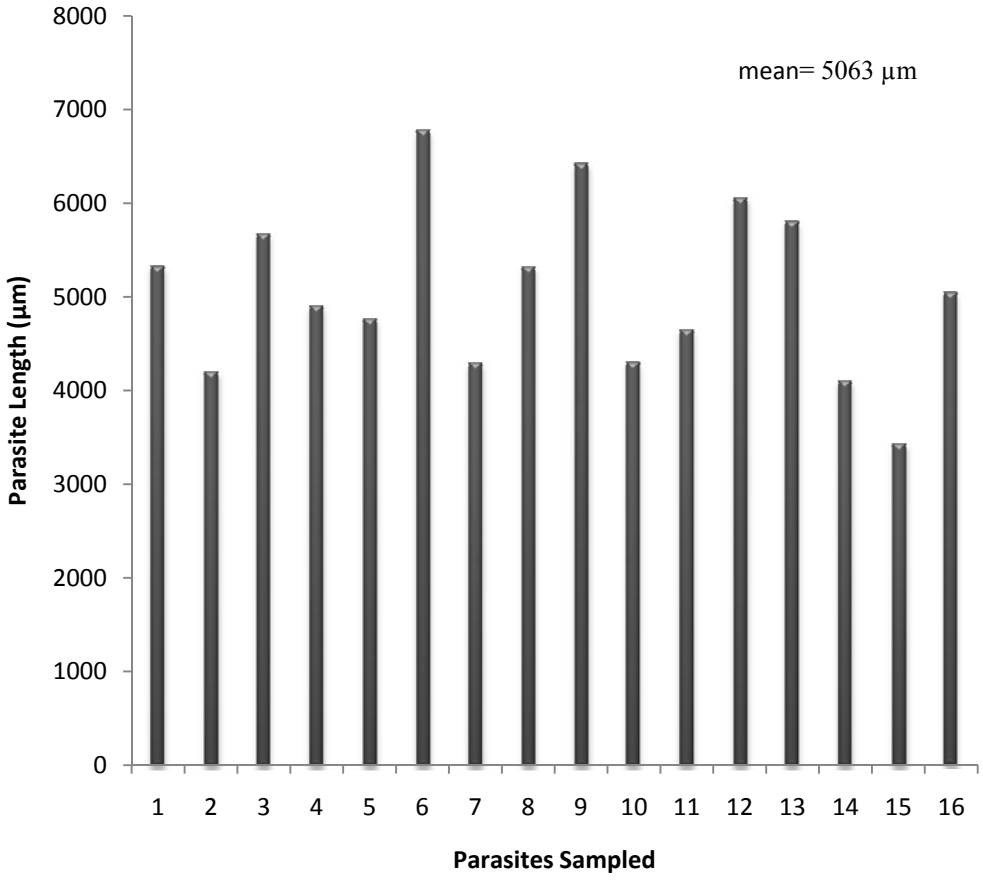


Figure 8. Total body length measurements from subsample of sixteen *S. edwardsii* obtained from the fins, gills, and tail of brook trout in Western Brook, Gros Morne National Park.

Based on the K-S tests, the fish length data showed a normal distribution (KS= 0.089, p=0.150), while fish weight and parasite abundance did not. Parasite abundance was successfully transformed using a $\log_{10}$ transformation (p-value of 0.015 was obtained, see Appendix I). However, log transformation did not improve normality for the fish weight data. Therefore, the weight data were not transformed, in order to facilitate comparisons with other studies (Poulin et al. 1991) that used non-transformed weight data.

Fish in the fresh water were slightly larger than in the estuary: fish length ranged from 286 to 421 mm in Western Brook Pond and from 196 to 397 mm in Western Brook estuary. Weight ranged from 203 to 1024 g in the Western Brook Pond compared to 43 to 497 g in Western Brook estuary.

Correlation analyses show that there are only weak relationships between ectoparasite abundance and fish size. The samples from the Western Brook system as a whole show do not show a correlation between ectoparasite abundance and fish weight (p=0.203, r=0.164) and ectoparasite abundance and fish fork length (p=0.114, r=0.202). The samples taken from the Western Brook estuary show a very small correlation with ectoparasite abundance and fish fork length (p=0.061, r=0.261) and no correlation with ectoparasite abundance and fish weight (p=0.158, r=0.198). The samples taken from Western Brook Pond show a small correlation between ectoparasite abundance and fish weight (p=0.081, r=0.577) and no correlation between ectoparasite abundance and fish length (p=0.221, r=0.425). However, this may be attributed to the smaller sample size (n=10). Overall, the results do not show strong relationships between ectoparasite abundance and fish fork length and ectoparasite abundance and fish weight (See Figure 9). When fish without parasites were omitted from the data, there was no increase in the strength of the correlations (See Appendix I).

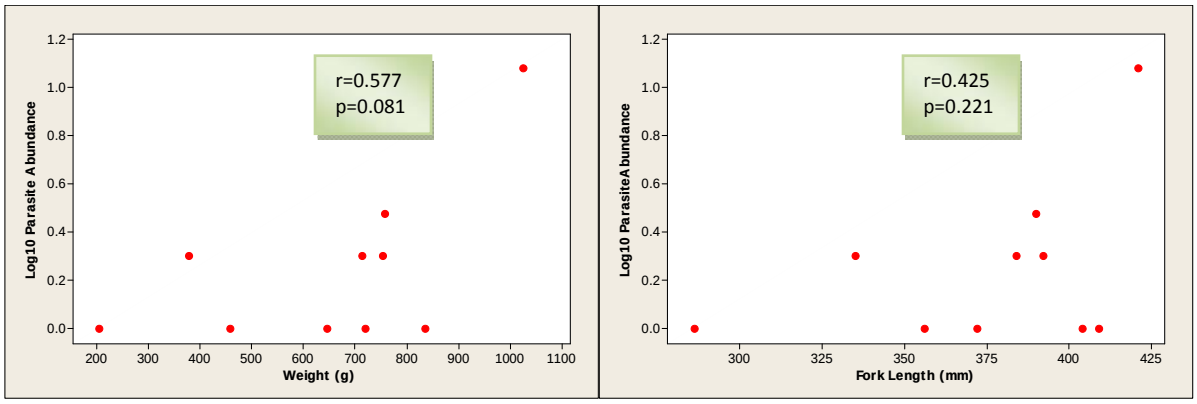
The Mann-Whitney U Test obtained values of $p<0.05$ in both tests, therefore concluding that both fish length and fish fork length do not come from the same distribution or have the same median values in the estuarine and freshwater system (See Table 4).

Surface water temperatures showed similar patterns at both Deer Arm and Western Brook, with much similarity between estuary and freshwater locations. Temperatures were low until early June (<10°C) then increased to 20+°C by June 30, remaining approximately 20°C thereafter (Figure 10).

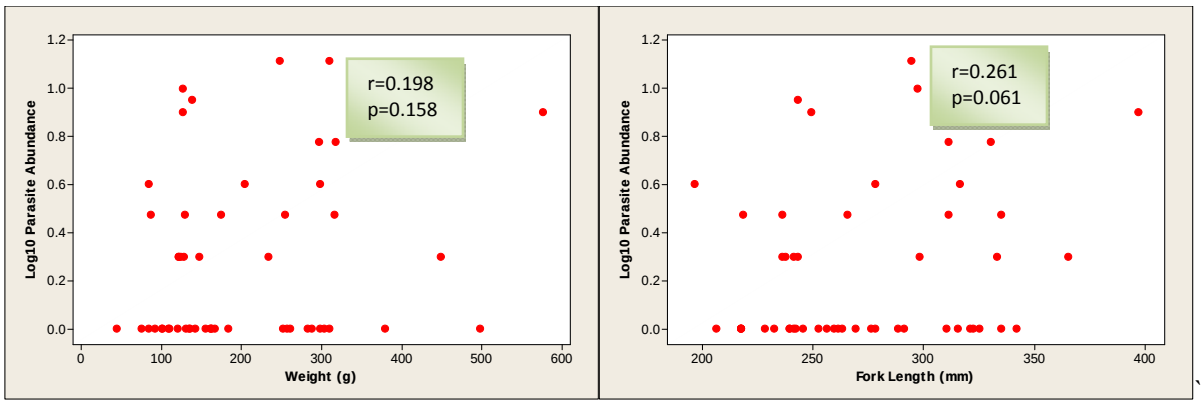
Table 4. Results from a Mann-Whitney U Test, comparing freshwater and estuarine data from Western Brook.

	N	Median	W	P-value (adjusted for ties)
weight estuary	52	160.9	1405.0	0.0000
weight freshwater	10	715.5		
length estuary	52	264.0	1409.0	0.0000
length freshwater	10	387.0		

A)Western brook Pond



B)Western Brook estuary



C)Western Brook Pond and estuary combined

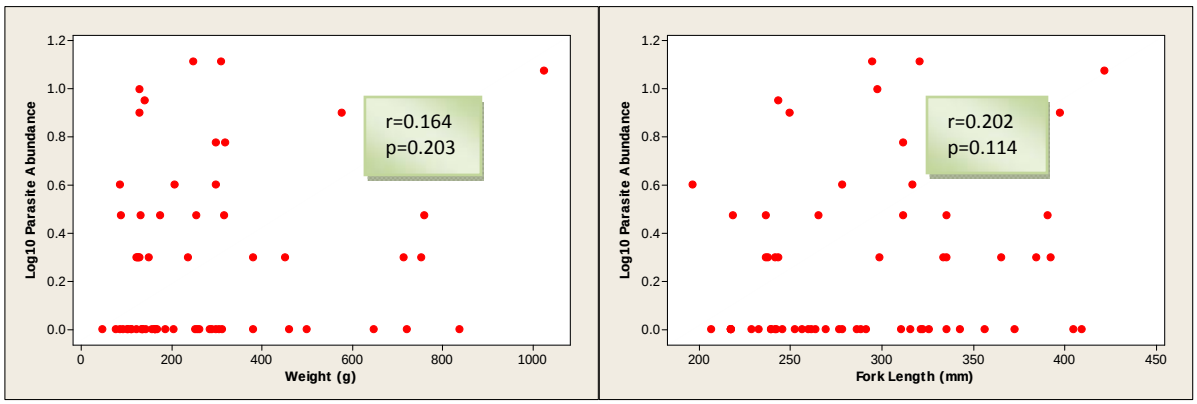


Figure 9. Pearson product-moment correlation of $\log_{10}$ parasite abundance versus weight (g) and fork length (mm) in Western Brook Pond, Western Brook estuary and both systems combined.

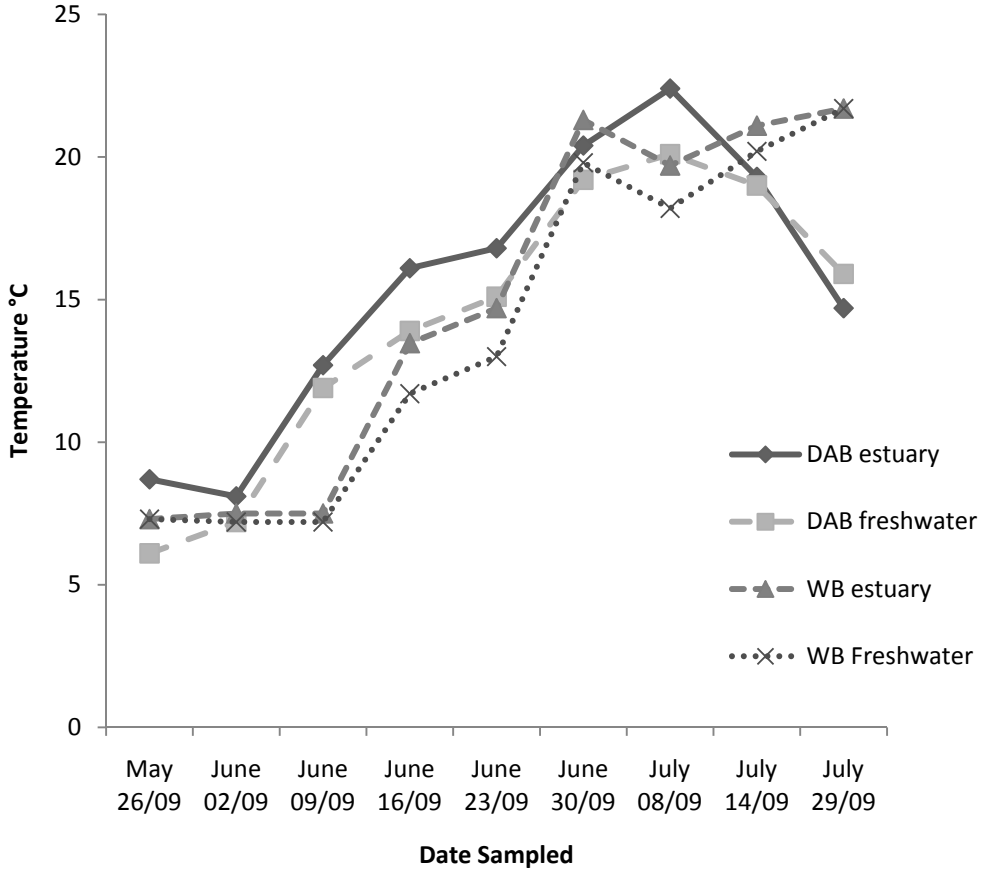


Figure 10. Surface water temperature in Western Brook, Gros Morne National Park during the study period collected from freshwater and estuarine location.

Discussion

Examination of prevalence and intensity of *S. edwardsii* parasitism on brook trout in the Western Brook system revealed results similar to that of other studies conducted in Newfoundland and Labrador (e.g. Headwater Pond, Smallwood Reservoir) and other Canadian provinces (e.g. Dickson Lake, Ontario and Moisie River, Quebec). While 45-50% of the sampled fish in the Western Brook system harboured parasites, the intensity of infection was low (between 1.6 and 1.7 parasites per infected fish). Large aggregations were predominant on a small proportion of the fish sampled, with eighty-five percent of fish hosting three or fewer *Salmincola*. A comparable study by Cone and Ryan (1983) examining population sizes of metazoan parasite of brook trout in a small Newfoundland lake (Headwater Pond) found *S. edwardsii* to be most abundant in larger fish (>150 mm). Similar to the Western Brook system, the majority of the parasites were harboured by a small portion of the population. In this pond, intensity of infection was 2.4 and prevalence was 37%. Headwater Pond provides a good comparative study with Western Brook Pond because both lakes are postglacial with a circumneutral pH. Another study conducted by Chinniah and Threlfall (1978) examined brook trout parasitism in the Smallwood Reservoir, Labrador, revealing similar results to those of Western Brook and Headwater Pond (i.e. a few hosts harbouring most parasites). In the Smallwood Reservoir, *S. edwardsii* had a prevalence of 37%, an intensity of 5, and a range of 1-31 parasites per host. The Smallwood Reservoir is an oligotrophic water body with a pH ranging between 6.0 and 7.0.

Fish sampled from the estuarine and freshwater environment of Western Brook did not differ noticeably in terms of intensity of infection (Table 2). The prevalence was slightly higher for brook trout in freshwater conditions. Angling prohibition in Western Brook Pond is

likely a contributing factor to the mean weight and length of brook trout in the pond being greater than in the estuarine environment. Increased fish size likely produces a greater stimulus (e.g. larger shadows and water movement) which provokes increased responses from *S. edwardsii* copepodids. Examination of parasite abundance of *S. edwardsii* with respect to location of its host within the riverine environment was carried out by Black et al. (1983). The study sampled the Moisie River system in Quebec, finding prevalence higher on brook trout that were migrating to sea and remaining in the estuary throughout June and July, compared to those returning to spawn in the autumn. The estuary and river is more frequented by brook trout due to increased food availability, therefore increased prevalence of *S. edwardsii* may be a result of adaptations to optimize the most optimal location of potential contact with a host. Although *Salmincola* predominantly reside in fresh water, they can survive considerable periods of time in the saline environments of the estuary and sea because of their sclerotized exoskeleton in the adult form (Cheng, 1973).

Other mechanisms, such as habitat selection by salmonids, have also been studied to examine the extent of their influence on parasite acquisition. Poulin et al. (1991) monitored both physiological (e.g. activity level) and behavioural mechanisms (e.g. microhabitat selection) of *S. fontinalis* to determine if they caused aggregated distributions of *S. edwardsii*. Correlation analysis of fish weight with parasite abundance produced r-values of 0.596 in trial one and 0.650 in trial two of the study ($p<0.01$ in both cases). Correlation analysis of behavioural mechanisms with parasite abundance produced r-values of 0.204 in trial one and -0.335 in trial two of the study. These values were not statistically significant. The results suggest that fish size (i.e. physiological characteristic) plays a greater role than behavioural mechanisms in distribution of *S. edwardsii* on its host. Fish sampled in this study were of a

different size range than those sampled in Western Brook (9.4-123 g vs. 43-1024 g, respectively). This may explain the different correlation results observed between the two studies.

Attachment site of *S. edwardsii* may vary with fish size. Among brook trout sampled from WBE and WBP, *S. edwardsii* was most commonly found on the gills of fish from 196 mm to 421 mm in length. A similar study by Poulin et al. (1991) found 74.4% of copepods attached to the gills of their host and 19.1% on or at the base of the pectoral fins (the remainder were on the body or another fin). Fish ranged from 104 mm to 227 mm in length. The predominance of attachment on the gills was also found by Black (1982), examining the gills of *Salvelinus fontinalis* as an attachment site for *S. edwardsii* at Dickson Lake, Algonquin Provincial Park. Infection intensity was similar for male and female fish, with copepods primarily attached to the distal tips of gill filaments. Fish ranged from 200 mm to 500 mm in length.

Black et al. (1983) in an experiment of abundance and distribution of *Salmincola edwardsii* on anadromous brook trout on the Moisie River system in Quebec, found *S. edwardsii* to be attached mainly to the dorsal (52%) and adipose fins (31%) of brook trout. Fish sampled were small, ranging from 80-200 mm in length. This preferential fin attachment site was also seen at Dickson Lake where no adult copepods were recovered from the gills of salmon fry (32-58 mm long) and rarely from the gills of juveniles (102-270 mm long). These distributions suggest a decrease in parasite aggregation on the gills with smaller host sizes. The high proportion of parasites on the fins of small salmonids (<200 mm) can be attributed to the reduced surface area of the gills, decreasing the parasites probability of finding a suitable substrate for attachment (Black, 1982). The gills are also one of the major attachment

sites of *Salmincola californiensis*, a close relative to *S. edwardsii* (Kamerath et al., 2009). With both species parasitic to salmonids, changes in attachment site have been found to occur in both species with increasing host length (Black, 1982).

Correlations examined for WBP and WBE with respect to host length, weight, and parasite abundance did not reveal results consistent with the findings of other studies. All correlations for both freshwater and estuarine locations had p-values greater than 0.05. This suggests that a relationship between parasite abundance and fish size may exist, however it is not statistically significant. Results obtained at Dickson Lake (Black, 1982), where analysis of possible relationships between ectoparasite abundance (on the gills of brook trout) and fish length was examined, did however show significant relationships. Blacks' methods involved dividing brook trout into length classes to see if a trend existed. With infection considered similar for male and female brook trout, an increase in parasite prevalence and intensity was positively related to host length. Cone and Ryan (1983) in Headwater Pond, also divided fish into length classes for analysis of parasitism and found small fish (<151 mm) did not acquire any parasites. Medium sized fish (151-200 mm) harboured 35% of total parasites found, and large fish (>200 mm) harboured 65% of the parasites found.

Larger fish are typically in contact with a greater volume of water than smaller fish due to increased gill and body size. The potential for the host fish to acquire ectoparasites is positively related to the amount of contact between water and the potential attachment site. Therefore, the volume of water in contact with the host increases with host length and weight. Increased host length also influences *S. edwardsii's* response to visual and mechanical cues. The free-living parasite will increase its swimming activity when stimulated. Stimuli include

moving shadows and increased disturbance in the surrounding water (Poulin et al., 1991). Larger fish cause increased disturbance, thus increased stimuli for *S. edwardsii*.

Temperature was monitored during the study on a weekly basis for the fresh water and estuarine sites (Figure 11). However, correlations could not be examined between temperature and parasite abundance due to the inconsistency of fish sampling times (a majority of the samples were collected within a few days). Nonetheless, this association has been shown to be relevant to the duration of swimming activity and survival of *S.edwardsii* (Conley and Curtis, 1992) as well as the resistance of brook trout to high temperatures (Vaughan and Coble, 1975). Conley and Curtis (1992) found that an optimal time frame exists within which *S. edwardsii* should acquire a host. Experimentation determined that 8°C was the most optimal temperature for parasite acquisition in their experiment with sampling stations of 8, 12, 16, and 20°C (Conley and Curtis, 1992). Data from this study conclude that copepods are capable of swimming for an extended period of time, especially at lower temperatures.

The tolerance of brook trout to higher temperatures is also affected by parasitism. In a study by Vaughan and Coble (1975) brook trout infected with *S. edwardsii* had a lower resistance to high temperatures than brook trout not infected with parasites. In the study, brook trout with parasites reached 50% mortality after approximately 27.4 hours at 25°C. Brook trout without parasites did not reach 50% mortality during the experiment.

Conclusion

Salmincola edwardsii found on brook trout populations in western Newfoundland were of comparable abundances to other studies. Therefore, knowledge of current prevalence and intensity ranges for this parasitic species provides an important baseline study for future research. Although *Salmincola edwardsii* is considered non-pathogenic to humans, infected fish populations typically suffer reduced fitness and reproductive output. This will ultimately reduce population sizes and thus the production and economic output of the fishery (Kamerath et al. 2009). These consequences are important concerns for recreational anglers, residents of Newfoundland and Labrador, and overall fisheries management. The only stage of *S. edwardsii* known to be susceptible to chemical treatment is the copepodid stage. Therefore, knowing temperature influences on development can allow for treatment to be scheduled on a routine basis in aquaculture settings (Conley and Curtis, 1992).

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Appendix I

Table A1. Statistical test for normal distribution using the Kolmogorov-Smirnov Test for Western Brook Pond, Western Brook estuary, and both sites data combined ($p>0.05$).

	Western Brook All Data		Western Brook Pond		Western Brook estuary	
	P-value	KS-value	P-value	KS-value	P-value	KS-value
Parasite Abundance	<0.010	0.150	<0.010	0.353	<0.010	0.232
Log_{10} Parasite Abundance	>0.150	0.094	>0.150	0.150	>0.150	0.095
Fish Fork Length	0.089	0.150	>0.150	0.189	0.050	0.123
Fish Weight	0.010	0.211	0.010	0.211	<0.010	0.182
Log Fish Weight	0.070	0.108			>0.150	0.104

Table A2. Statistical test for normal distribution using the Kolmogorov-Smirnov Test for Western Brook Pond, Western Brook estuary, and both sites data combined with hosts harbouring no parasites not included ($p>0.05$).

	Western Brook All Data		Western Brook Pond		Western Brook estuary	
	P-value	KS-value	P-value	KS-value	P-value	KS-value
Parasite Abundance	<0.010	0.233	<0.010	0.408	<0.010	0.215
Log_{10} Parasite Abundance	>0.150	0.124	0.094	0.317	>0.150	0.108
Fish Fork Length	>0.150	0.131	>0.150	0.295	>0.150	0.151
Fish Weight	<0.010	0.236	>0.150	0.278	0.032	0.195
Log Fish Weight	0.113	0.148			0.095	0.167

Table A.3. Pearson-product moment correlation of log_{10} parasite abundance versus weight (g) and fork length (mm) in Western Brook Pond, Western Brook estuary, and both systems combined excluding hosts harbouring no parasites.

	Western Brook All Data		Western Brook Pond		Western Brook estuary	
	p-value	r-value	p-value	r-value	p-value	r-value
Log_{10} Parasite Abundance and weight	0.604	0.103	0.129	0.769	0.264	0.243
Log_{10} Parasite Abundance and log_{10} weight	0.603	0.103			0.243	0.254
Log_{10} Parasite Abundance and length	0.579	0.110	0.186	0.703	0.323	0.216

Table A3. Temperature readings for freshwater and estuarine environments of Deer Brook and Western Brook from May to July 2009 in Gros Morne National Park, Newfoundland.

Date	temp °C			
	DAB estuary	DAB fw	WB estuary	WB fw
May 26/09	8.7	6.1	7.3	7.3
June 2/09	8.1	7.2	7.5	7.2
June 9.09	12.7	11.9	7.5	7.2
June 16/09	16.1	13.9	13.47	11.7
June 23/09	16.8	15.1	14.7	13
June 30/09	20.4	19.2	21.3	19.8
July 8/09	22.4	20.1	19.7	18.2
July 14/09	19.3	19	21.1	20.2
July 29/09	14.7	15.9	21.7	21.7

Table A4. pH reading for freshwater and estuarine environments in Deer Brook and Western Brook from May to July 2009 in Gros Morne National Park, Newfoundland.

Date	pH			
	DAB estuary	DAB fw	WB estuary	WB fw
May 26/09	7.68	7.45	7.97	8.52
June 02/09	7.98	7.54	8.16	7.95
June 09/09	7.64	7.84	7.93	7.79
June 16/09	8.02	7.75	7.83	7.92
June 23/09	8.02	8.09	8.15	8.11
June 30/09	8.24		8.51	8
July 08/09	8.75	8.35	8.57	8.21
July 14/09	8.41	8.07	8.2	8.21
July 29/09	7.95	7.61	8.94	8.25

Table A5. Length measurements (µm) for female sub-sample of *Salmincola edwardsii* in Western Brook system, Gros Morne National Park, Newfoundland (Totals do not include egg sacs).

Sample #	Bulla	Body	2nd Maxillae	Eggs	Total
1	761.6	2832.2	1737.4	1332.8	5331.2
2	404.6	2427.6	1356.6		4188.8
3	380.8	3498.6	1785	1666	5664.4
4	523.6	2927.4	1451.8		4902.8
5	476	2761.2	1523.2		4760.4
6	1190	3332	2261	2856	6783
7	285.6	2499	1499.4		4284
8	428.4	3355.8	1523.2	2332.4	5307.4
9	476	4093.6	1856.4	3046.4	6426
10	428.4	2546.6	1332.8		4307.8
11		3498.6	1142.4	2665.6	4641
12	404.6	3094	2546.6	2023	6045.2
13	666.4	3332	1808.8		5807.2
14	523.6	2380	1190		4093.6
15	428.4	1904	1094.8		3427.2
16	476	2975	1594.6	1285.2	5045.6