# POTENTIAL IMPACT OF INVASIVE ANURAN SPECIES IN WESTERN NEWFOUNDLAND

by

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# ABSTRACT

Successful dispersal and establishment of invasive anurans (frogs and toads) may be influenced by competitive exclusion and/or niche differentiation with competing species. I investigated the dispersal of anurans in western Newfoundland using anuran calling surveys and pond-edge visual encounter surveys. The Mink Frog, *Lithobates septentrionalis*, had dispersed ~50 km northeast from the original (2001) discovery location and ~34 km southwest; displaying spatial separation from Green Frogs, *Lithobates clamitans*, at landscape and local scales. Visual encounter surveys did not reveal any correlation between adult Mink Frogs and odonate competitors. Additionally, I assessed the impact of varying tadpole densities on removal of epilithic periphyton by providing epilithon covered substrates for American Toad, *Anaxyrus americanus*, tadpoles raised in laboratory or field enclosures. Higher tadpole densities resulted in smaller tadpoles that removed more periphyton from substrates. As anuran population ranges expand, there may be effects on ecological resources for vertebrate and invertebrate competitors.

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# 1. Introduction and Overview

## 1.1. Invasive Species

Non-indigenous species enter new ecosystems and establish viable populations either naturally or through human-assisted migration. While the majority of non-indigenous species fail to persist or may have no major effect following introduction, the term *invasive* is normally used to indicate circumstances where there is a noticeable negative effect of a non-indigenous species in the novel ecosystem (Kraus 2008). Introduced species are becoming a frequent consequence of human-mediated linkages across small to large spatial scales around the globe and, regardless of terminology, the introduction of any non-indigenous species may have either a positive (enhancing ecosystem biodiversity) or negative (causing biodiversity loss, as is more often the case) effect on the target ecosystem (Knight et al. 2005, Colautti et al. 2006, Paolucci et al. 2013). Although not all invasive species create negative ecological effects, invasive species have been identified as a substantial threat to ecosystem biodiversity (Kolar and Lodge 2001, Catford et al. 2012, Simberloff et al. 2012), particularly for insular ecosystems which are often characterized by simpler trophic webs (Chapuis 1995) and lack either coevolved predators or strong competitive influences (Stone et al. 1994).

Successful invasion is a three stage process whereby the species is first transported to a new location where it may establish a viable population from which individuals subsequently disperse to other similar habitats (Shigesada and Kawasaki 1997, Mack et al. 2000, Sakai et al. 2001). The transportation or dispersal of non-indigenous species across a landscape is dependent on factors such as species perceptual range, capacity to identify suitable habitat, organism size, motility, life history traits, behavioural characteristics (Zollner & Lima 1997, Sakai et al. 2001; Baldwin et al. 2006,) as well as the level of landscape connectivity (Stevens et al. 2006). Anthropogenic activities also contribute to the accidental/intentional introduction of non-indigenous species through human-mediated species transportation or sufficient disturbance of ecosystems to facilitate trans-boundary movement of non-indigenous species (Kolar & Lodge 2001; Chytrý et al. 2008).

While most individuals of these non-indigenous species will perish during dispersal (Mack et al. 2000), Kolar & Lodge (2001) estimated that more than 10% survive this phase and establish populations in a new location at some distance from the original point of introduction. Aspects which contribute to the establishment of a non-indigenous species include the nature of introduction (natural dispersal or human mediated introductions), the invasability of the habitat and characteristics of the invader such as propagule pressure, population age structure and abundance (Sakai et al. 2001, Catford et al. 2012, Kohler et al. 2012). Successful non-indigenous species also are more likely to be generalists, exhibiting wide habitat preferences and either utilizing untapped resources or out-competing native/established species for shared resources (Marvier et al., 2004). Once established, non-indigenous species may begin exploiting niche opportunities (Shea and Chesson 2002) which contribute to greater fitness, increased population size, and an expanded range. The most worrisome stage of the invasion process to managers responsible for ecological integrity is that associated with the post-establishment dispersal of viable invasive populations (Sakai et al. 2001, Shea and Chesson 2002). Dispersal can be enhanced or inhibited by abiotic and biotic conditions that combine to influence the availability of habitat suitable for breeding, as well as through the provision of movement corridors that link habitats, or landscape barriers that block dispersal (Blomquist and Hunter Jr 2009, Li et al. 2009). The abundance of predators and interactions with competitive resident species are biotic factors which also influence invader dispersal rate. The introduction and continued expansion of the Cane Toad (*Rhinella marina*, formerly *Bufo marinus*; Pramuk 2006, Frost et al. 2008) in Australia is an example of an introduced species of anuran (frogs and toads) that has affected both resident vertebrate and invertebrate species (Crossland et al. 2009, Shine 2010). In places where the reproductive timing of the native Ornate Burrowing Frog, *Opisthodon ornatus* followed that of the Cane Toad, the size and survival of the Ornate Burrowing Frog tadpoles has been negatively impacted by competitive interactions with Cane Toad tadpoles (Crossland et al. 2009). Native predators, such as *Hoplocephalus* and *Acanthophis* snakes, are susceptible to Cane Toad toxins (Phillips et al. 2003) which act through changes to the locomotion and survival of the snakes as influenced by the proportion of Cane Toads in their diet.

## 1.2. **Anuran Ecological Effects**

Anurans with dimorphic life stages have the capacity to act as links between trophic levels (Whiles et al. 2006). At the larval stage, tadpoles of many species consume periphyton (a biofilm mixture of bacteria, algae, protozoa and detritus) attached to substrates in freshwater systems. Hence, tadpoles can act as transitory consumers that regulate periphyton productivity and facilitate energy transfer to higher tertiary trophic levels when preyed upon or when they metamorphose (Ranvestel et al. 2004, Hopkins 2007) or are consumed by terrestrial or semi-aquatic predators. Consumption of periphyton biomass can potentially limit food resources for conspecifics and other herbivores. Tadpoles exhibit growth plasticity or variation in time to, and size at, metamorphosis in response to environmental stressors such as high conspecific density (Brockelman 1969, Semlitsch and Caldwell 1982), pond drying (Crump 1989), and temperature and food quality (Alvarez and Nicieza 2002). In several studies high conspecific density has increased tadpole development rate (Cohen and Alford 1993, Hensley 1993, Kehr et al. 2014), leading to metamorphosis at an earlier Gosner stage (Gosner 1960) or slowed growth rate (change in size), resulting in metamorphosis at smaller sizes. Similar results have been noted for growth after tadpoles metamorphosed into adults (Dodd 2010, Wells 2010).

For adult frogs and toads, competitive interactions may also arise between conspecifics and congeners for foraging and breeding habitats in ecosystems with limited resources. The effects of competition can extend to other taxa, for example between Anura and Odonata (dragonflies and damselflies) species which live in similar habitats (Werner et al. 1995, Hammond 2007) and exhibit similar diets that include, for example, small invertebrates from the Order Diptera (Werner et al. 1995, Saha et al. 2012). Similar anuran and odonate larval habitat requirements as well as adult interspecific competition for food resources, will directly and indirectly affect populations within communities through trophic cascading, particularly where predators metamorphose (Knight et al. 2005). The extent of competition between species depends on the level of overlap for spatial and/or resource requirements (Hairston 1980, Shea and Chesson 2002). Variation in species’ resource use contributes to competitive interactions between sympatric invaders and established species (da Silva Lima et al. 2014) which consequently influences species' distributions (Connell 1983). Assessing the co-occurrence of species and availability of limiting resources (Hairston 1980) provides the opportunity to infer potential competitive interference between species.

### 1.3. **Invasive Anurans in Newfoundland**

Class Amphibia (which includes frogs, toads, salamanders and caecilians) is considered to be one of the most endangered taxa globally; populations of amphibians are declining or going extinct at alarming rates due to habitat destruction, climate change, disease and overexploitation (Stuart 2004, Sodhi et al. 2008, Alford 2011, Blaustein et al. 2011). Up to 43% of amphibians are threatened with extinction (Vredenburg et al. 2010, Barnosky et al. 2011) while close to 30% of anuran species (frogs and toads) are considered threatened (Sodhi et al. 2008). In Canada, at least 20 amphibian species are at risk of extinction or extirpation (Lesbarrères et al. 2014). While countries worldwide are experiencing declines in anuran populations and species richness, some North American species in the genera *Anaxyrus* and *Lithobates* are listed as “Least Concern” by the IUCN (2013a, 2013b, 2013c) because of their wide spread distribution, diverse habitat tolerance, low probability of rapid population decline, and continued range expansion.

There are no native anurans on insular Newfoundland (Maunder 1983). The most probable theory for the absence of anurans is the salt water isolation of Newfoundland which acts as a barrier to the natural dispersal of anuran species from mainland Canada. Salt water affects osmoregulation in anurans and only a few species can tolerate saline or brackish conditions for temporary periods (Freda and Dunson, 1984; Pierce et al., 1984; Wells, 2010). Human mediated dispersal across such a barrier is therefore the only means of anuran introductions to insular Newfoundland and indeed six anuran species have been introduced (Buckle 1971, Maunder 1997, Warkentin et al. 2003): the Western Chorus Frog (*Pseudacris triseriata*), Northern Leopard Frog (*Lithobates pipiens*), Wood Frog (*Lithobates sylvaticus*), Green Frog (*Lithobates clamitans*), Eastern American Toad (*Anaxyrus americanus americanus*) and the Mink Frog (*Lithobates septentrionalis*). The Green Frog was likely first introduced to the eastern parts of insular Newfoundland circa 1850 (Maret 1867, Johansen 1926, Maunder 1983). The Wood Frog and American Toad populations originated from locations in southern Ontario, Canada and were successfully translocated during the early 1960s to the Corner Brook area by James Buckle (Buckle 1971). The Mink Frog was first identified in the vicinity of Corner Brook in 2001 but its origins are unknown (Powell 2002, Warkentin et al. 2003). Although additional translocations to the Northern Peninsula occurred in the late 1970s, both the Northern Leopard Frog and Western Chorus Frog failed to establish populations and appear to have been extirpated since neither has been detected during numerous surveys since 1989 (Maunder 1997, Campbell et al. 2004, Stapleton 2011).

Despite general declines of amphibians globally, populations of some anurans introduced to western Newfoundland over the past 50 years are expanding their range and population density (Maunder 1997, Campbell et al. 2004). Anecdotal evidence suggests increasing ranges for Mink Frog and American Toad in the Humber Valley - including Corner Brook, the purported initial site of introduction – but there have been no indications of expanding Green Frog populations. As with the introductions of the Cane Toad to Australia or the Cuban Tree Frog *Osteopilus septentrionalis* to Florida (Smith 2005, Crossland et al. 2009, Shine 2010), the resource exploitation patterns of the Mink

Frog likely have enhanced its distribution since initial identification on the island in 2001. Surveys conducted along the west coast of Newfoundland (from the Northern Peninsular to the Codroy Valley in the southwest) identified the Mink Frog in ponds between Stephenville and Corner Brook (Powell 2002, Warkentin et al. 2003, Stapleton 2011). It seems unlikely that temperature would be a limiting factor for distribution of Mink Frogs since the Mink Frog is endemic to places with colder climates such as Labrador (Maunder 1997, Desroches et al. 2006). The Green Frog, a closely related species to the Mink Frog (Shirose and Brooks 1995), predominantly inhabits warmer climates (Conant and Collins 1998) but is widely distributed across insular Newfoundland (Maunder 1983, 1997), with the exception of the Northern Peninsula which Maunder (1983) suggested would be the case due to climatic conditions. A knowledge gap exists regarding the current distribution of anurans in western Newfoundland, the factors which influence their dispersal and the effects dispersing populations have on ecosystem resources and structure.

Based on previous surveys (Powell 2002, Stapleton 2011), American Toads are the one of the most abundant and widely distributed anuran species in western Newfoundland. Stapleton (2011) identified the species at twenty-seven of thirty monitoring locations between Stephenville and Deer Lake. Unlike Mink and Green Frogs, the American Toad has expanded its range to the Northern Peninsula since its introduction (Maunder 1983, Stapleton 2011). Although Hecnar and M’Closkey (1996) Hecnar 1997 and Sanzo (2005) did not find that water chemistry was a useful indicator of species richness or abundance, Campbell et al. (2004) and Stapleton (2011) identified dissolved oxygen concentration as the main predictor of toad dispersal in western

Newfoundland. The species has high fecundity, being capable of breeding in temporary and permanent aquatic systems (Conant and Collins 1998).

## 1.4. Thesis Objectives and Outline

My aim was to document changes to the distributions of anurans (specifically Mink Frog, Green Frog and American Toad) in areas around Corner Brook, and then to evaluate two aspects of the potential ecological impact of these species on the ponds they occupy. First, having identified the current range of the recently introduced Mink Frog, I assessed the habitat and water quality factors which may potentially be affecting the species' distribution. These data were collected during the peak of the 2014 Mink Frog breeding season, June 27 - July 6 (Stapleton, 2011), using established protocols for manual calling surveys, the North American Amphibian Monitoring Protocol (NAAMP). The manual calling surveys, coupled with visual encounter surveys, provided relative abundance and distribution data for the Mink Frog and the closely related Green Frog, the results of which are presented in Chapter 2. Visual encounter surveys also included the recording of odonate species diversity and abundance information, to determine if populations of these potentially competing insects might exhibit habitat use patterns correlated with adult frog abundance. These data are reported in the Appendix. The Mink Frog and Green Frog were selected as target species for this research as they are closely related (Shirose and Brooks 1995; Conant and Collins 1998) and have similar breeding periods, from late June to early July (Stapleton 2011), in western Newfoundland. This timing permitted the simultaneous determination of relative abundances and comparison of the most recent introduction, the Mink Frog (Warkentin et al. 2003), with that of the oldest invasive anuran, the Green Frog (Maunder 1983).

The second aspect of my project was to determine the level of impact that varying densities of invasive anurans have on the invaded ecosystem during their larval stage of development. Effects on freshwater habitats could be stronger in the Corner Brook region when compared to other parts of American Toad distribution on the island as population densities of toads will likely be greater in this region given the prolonged time since initial colonization (approximately 50 years). American Toad tadpoles are aquatic herbivores that feed largely on algae by scraping surfaces (Kupferberg 1997, Altig et al. 2007) thereby having the potential to affect epilithic biomass accumulation and nutrient cycling (Seale 1980, Pryor 2003). The impact of tadpoles on the ecosystem can be measured through the removal of algae from toad breeding habitat. I evaluated the effect of introduced anurans on epilithic biomass accumulation in ponds by providing epilithon-covered substrates upon which different controlled densities of American Toad tadpoles could feed. These tadpoles were taken from local sources and raised in laboratory or field enclosures at varying densities relative to that of local populations. American Toad tadpoles were well-suited to these experiments due to the early availability of the larvae and the ease with which the species can be identified relative to the other species in western Newfoundland. The results of these experiments are presented in Chapter 3. American Toads and Mink Frogs do not breed at the same time (toads usually breed earlier in the spring); nonetheless survey data collected during the 2014 Mink Frog breeding season also allowed for a rough estimate of American Toad distribution in the region, as presented in Chapter 4, which also summarizes the major findings from my research study.

## 1.5. **Co-authorship Statement**

This study was conducted independently but with input from my supervisor, Dr. Ian Warkentin, and other members of my supervisory committee, Dr. Christine Campbell and Dr. Robert Scott. With their guidance I designed the research experiments and surveys. Data collection was aided by two field/laboratory assistants, Jasmine Pinksen and Lindsay Batt. Data analyses were performed with advice from Dr. Warkentin and Dr. Scott. The thesis is structured in a manuscript format with two chapters (2 and 3) representing individual projects within the study; these chapters have been submitted to journals. I wrote the manuscripts and made revisions based on comments and advice from the co-authors, Dr. Warkentin, Dr. Campbell and Dr. Scott. The first manuscript (Chapter 2), "Initial dispersal and habitat use of newly introduced Mink Frogs in western Newfoundland, Canada" was submitted as a manuscript to *Copeia* in January 2016. The second manuscript (Chapter 3), "Impact of Invasive Eastern American Toad Tadpole Populations on Pond Epilithon in Western Newfoundland" was submitted to *Food Webs* in December 2015. Due to the submission to different journals, there are differences in formatting between the chapters, and with the remainder of the thesis. The results of the third aspect of the research, a measure of competition between adult Mink Frogs and adult Odonata (dragonflies and damselflies) were not submitted as a manuscript but recorded in the appendices of this thesis.

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# 2. Initial Dispersal and Habitat Use of Newly Introduced Mink Frogs in Western Newfoundland, Canada

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**Running Head**

Mink Frog Dispersal in Newfoundland

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## 2.1. **Abstract**

Insular Newfoundland has no native amphibians. While global amphibian populations are declining at alarming rates, populations of introduced anurans (frogs and toads) continue to expand in western Newfoundland, Canada. However we expected the establishment and dispersal of the most recently introduced species, Mink Frog (*Lithobates septentrionalis*), to be influenced by competitive exclusion and/or niche differentiation with the previously introduced and ecologically similar Green Frog (*Lithobates clamitans*). We used a combination of anuran calling surveys and pond-edge surveys to assess the relative regional distribution, local habitat use, and ongoing dispersal for these two species in western Newfoundland. The recently established Mink Frog has dispersed ~3.8 km/year northeast from the original (2001) discovery location and ~2.6 km/year southwest; binary logistic and co-occurrence analyses revealed that this population displayed an unexpected spatial separation from long-established Green Frog populations, at landscape and local scales. This niche differentiation appears to be exacerbated by the additional influence of pH on species presence; acidic environments negatively affect Mink Frog presence while favouring Green Frogs.

## 2.2. **Introduction**

Anurans (frogs and toads) often excel as invasive species due to their r-selective life history, growth plasticity, and ability to tolerate a range of environmental conditions (Sakai et al., 2001; Shine, 2010). As ectotherms and generalists that occupy various trophic niches, anurans can quickly become important components of terrestrial and aquatic ecosystems (Shea and Chesson, 2002; Hopkins, 2007). As such, they are present in nearly every habitat except oceans and locations with either extremely cold climates or extremely dry conditions (Wells, 2010) making them one of the most ecologically diverse and widely dispersed Orders among vertebrates. The range of environments occupied by even a single species may be extensive; the American Bullfrog (*Lithobates catesbeianus*) can be found inhabiting tropical coastal wetlands as well as temperate freshwater systems (Conant and Collins, 1998). But basic habitat requirements remain vital determinants of establishment success and population expansion by any invasive species. For those species with aquatic larval stages that require suitable ephemeral/permanent systems, breeding sites are one such resource that affects their distribution and abundance (Gómez-Rodríguez et al., 2009).

Habitat fragmentation and reduced landscape connectivity, caused by geographic barriers such as mountains, deserts and rivers, further limit opportunities for anuran dispersal (Li et al., 2009). While generally tolerant of a range of environmental conditions, anurans do exhibit species-specific responses to human-mediated habitat alterations such as habitat fragmentation caused by road construction and clear-cut logging (Blomquist and Hunter Jr, 2009; Deguise and Richardson, 2009). Mazerolle & Desroches (2005) noted that dispersal of the Green Frog (*Lithobates clamitans*) and the Northern Leopard Frog (*Lithobates pipiens*) was restricted in disturbed areas. By contrast, Western Toads (*Anayxyrus boreas*) utilize roads for dispersal and are unaffected by clear-cuts (Deguise and Richardson, 2009). Some introduced anuran species, such as those with high dispersal ability and motility can spread quickly across fragmented landscapes (Sakai et al., 2001). Introduced species may also interact with and exhibit an advantage over native species, exploiting niche opportunities (Shea and Chesson, 2002) either by utilizing untapped resources or out-competing native and other previously established invasive species for shared resources (Marvier et al., 2004). These competitive abilities contribute to greater success in establishment, as well as enhancing fitness and increasing population size for some newly arrived invasive species. The classic example of this is the widespread and ongoing dispersal of the Cane Toad (*Rhinella marinus*) across Australia, a species which has out-competed or affected native species such as the Ornate Burrowing Frog (*Lymnodynastes ornatum*) as well as many other invertebrate and vertebrate predators (Urban et al., 2007; Shine, 2010). However, the extent of competition between any two species depends on the level of overlap in spatial and resource requirements (Hairston, 1980; Shea and Chesson, 2002). Habitat selection is therefore important to the distribution and competitive interactions among species.

Habitats, such as some in Canada's eastern boreal zone, may offer greater resistance to invaders due to the extreme weather conditions, acidic soils and low productivity of the ecosystem and so might be expected to support fewer successful invasive species (Langor et al., 2014). Within this boreal zone, the island of Newfoundland was originally devoid of anurans (Maunder, 1983) but has seen the introduction of six species with populations of four species currently extant (Warkentin et al., 2003; Stapleton, 2011): the Eastern American Toad (*Anaxyrus americanus americanus,* formerly *Bufo americanus americanus*) and three species of the *Lithobates* (formerly *Rana*). Of the four, the Green Frog is the longest established species, having been introduced to the eastern portion of the island as early as the 1860s (Cameron and Tomlinson, 1962) and having extended its range to western Newfoundland by the 1960s (Maunder, 1997). The Mink Frog (*Lithobates septentrionalis*) was first identified in insular Newfoundland in 2001 and is the most recently introduced (Warkentin et al., 2003). Besides the Eastern American Toad, the Wood Frog (*Lithobates sylvaticus*) is the other extant anuran. Introductions of Northern Leopard Frog and Western Chorus Frog (*Pseudacris triseriata*) were unsuccessful (Maunder, 1997; Warkentin et al., 2003).

We found no reports assessing the influence of one introduced anuran on another introduced anuran, and efforts to understand the impact of non-indigenous anurans in western Newfoundland have been limited. Here we attempted to identify the primary factors influencing the dispersal and population expansion of one introduced species in the context of another introduced anuran. Specifically we i) examined the establishment and dispersal of Mink Frogs away from their initial areas of introduction through areas where Green Frogs are already established, ii) assessed habitat use by both species and iii) identified predictors of occurrence and co-occurrence along with natural and anthropogenic factors known to influence anuran presence.

## 2.3. **Materials and Methods**

### 2.3.1. Study Species

Mink and Green Frogs have similar habitat requirements: both are primarily aquatic, occupying and remaining close to permanent freshwater systems such as ponds and lakes throughout their life span (Martof, 1953; Hedeen, 1986; Shirose and Brooks, 1995). However, Mink Frogs are adapted to cold-water ecosystems (Desroches et al., 2006; Popescu and Gibbs, 2009). Hedeen (1986) suggested that water temperatures >20°C limited the southern distribution of the species; warmer waters did not supply adequate oxygen to the submerged spherical egg mass of Mink Frogs. The northern edge of the Mink Frog range largely coincides with the northern extent of the boreal forest in regions of eastern Canada from Labrador to Manitoba (Hedeen, 1986; Desroches et al., 2006) and extends south to approximately the 43rd parallel N, incorporating the northern tier of states of the United States from Minnesota through Maine (Hedeen, 1986; Popescu and Gibbs, 2009; Green et al., 2014). Mink Frog microhabitat selection suggests a general reliance on floating or emergent vegetation in deeper water (Stewart and Sandison, 1972; Shirose and Brooks, 1995).

The northern extent of the Green Frog range also includes Atlantic Canada and southern portions of Quebec as far north as the mouth of the St. Lawrence River, and across eastern Canada to the northern shores of Lake Superior and the Ontario-Manitoba border (Conant and Collins, 1998). The species has also been introduced to British Columbia in western North America (IUCN, 2016). In contrast to the Mink Frog, the Green Frog's range extends southwards to the Gulf of Mexico from eastern Texas across to northern Florida (Hamilton, 1948; Gibbs et al., 2007). Green Frogs are able to tolerate warmer climates (Conant and Collins, 1998) and more acidic environments (Wells, 2010). The egg masses of Green Frogs are often found along the water's surface (Parmelee et al., 2002), allowing for more oxygenation. In contrast to Mink Frogs, Green Frogs primarily occupy grassy or shrubby open pond shoreline with emergent vegetation such as Ericaceous spp. (Courtois et al., 1995; Shirose and Brooks, 1995).

### 2.3.2. Study Sites

The majority of intentional anuran introductions documented for insular Newfoundland were in the vicinity of Corner Brook in the western part of the island

(Buckle, 1971; Maunder, 1997; Warkentin et al., 2003), therefore we focused on this region. We sampled ponds west of the Long Range Mountains, between Deer Lake and Stephenville which are located approximately 50 km northeast and southwest of Corner Brook, respectively (see Fig. 2-1). The aquatic ecosystems of this region are characterized primarily by oligotrophic ponds and streams (Campbell, 2002). The terrestrial landscape of the study area (approximately 6 to 183 m above sea level) features extensive boreal conifer forests dominated by Balsam Fir, *Abies balsamea* (Thompson et al., 2003). The southern portion of the study area (approximately 12 to 49 m above sea level), around Stephenville, possesses more acidic *Sphagnum* bogs than found in the north.

### 2.3.3. Sampling Anuran Distribution

Initial site selection was based on replicating data collection protocols from prior studies (Campbell et al., 2004; Stapleton, 2011), but augmenting this set of study sites to increase the sampling intensity (total of 40 locations chosen for surveys) and provide more detailed information on species' distributions. Anuran species can be identified by the calls of the breeding males and surveyed using nighttime manual calling survey (MCS) protocols developed for the North American Amphibian Monitoring Program, USGS Patuxent Wildlife Research Center [https://www.pwrc.usgs.gov/naamp/]. Our survey period and sites were chosen to coincide with local breeding periods for the two target species. Calling surveys were conducted during two periods, including June 18 - 24 and June 29 - July 1, 2014 with each site being visited twice. Surveys were standardized with respect to time and factors that affect frog calling behaviour/call recognition by the observer, such as air temperature (°C), wind speed (kph), noise index, precipitation and %

cloud cover. MCS provided confirmation of Mink and/or Green Frog presence and an estimate of abundance (Anuran Calling Index).

In addition, (following Crump and Scott Jr, 1994), daytime visual encounter surveys (VES) were conducted at 77 sites between the hours of 10 a.m. and 3 p.m. from July 9 - 31, 2014. Sixteen of these sites were from the nighttime MCS; due to their ease of access and proximity to the southern leading edge of the population dispersal, the remainder were unique to this dataset (see Fig. 2-2). VES were run to i) provide better data on local spatial utilization of pond systems by each species, ii) identify the leading edge of Mink Frog population dispersal more precisely, and iii) provide an additional measure of relative abundance. The VES protocol included walking 30 m of shoreline where emergent vegetation was prominent and noting the frog species seen within 2 m, either side of the pond margin, as well as those individuals greater than 2 m away from the shoreline on land and in the water. The presence and relative abundance of both Mink and Green Frogs encountered were recorded and expressed as number of frogs/m.

### 2.3.4. Habitat Classification

For both MCS (Fig. 2-1) and VES (Fig. 2-2), we recorded general habitat characteristics (Table I) and physiochemical water-quality parameters known to influence the distribution of anuran species. These included variables that aid dispersal and habitat connectivity, such as beaver dams which create movement corridors (Anderson et al., 2014) and the presence of roads (Deguise and Richardson, 2009). Courtois et al. (1995) identified favourable microhabitat for Mink Frogs within ponds, such as emergent vegetation. Mean air temperature was determined using alcohol-in-glass thermometers accurate to 0.5 °C. Wind speed (m/s) was measured using a digital handheld anemometer. Water temperature (TP), dissolved oxygen (DO) and conductivity (COND) were measured using YSI Model 30 & 55 meters (YSI 1700/1725 Brannum Lane, Yellow Springs, Ohio 45387-1107, USA). The acidity/alkalinity of the pond was measured using a pHep Model HI 98107 pH meter (HANNA Instruments, 584 Park East Drive, Woonsocket, RI 02895, United States). Each meter was calibrated on a daily basis prior to use in the field.

### 2.3.5. Statistical Analyses

Data from the manual calling surveys and visual encounter surveys were analyzed separately. Correlation was used to identify significant associations between habitat characteristics and anuran presence. The presence of anuran species was also assessed using binomial logistic regression (logit transformation) against water quality and habitat classifications; the residuals were assessed for normality and homogeneity of variance. Kruskal-Wallis non-parametric tests followed by post-hoc procedures were conducted to identify differences in the water quality of those ponds that had only Mink Frogs, those with Green Frogs only, those that contained both species and those in which neither species were present. The correlation matrix was examined for significant associations based on variable pairings with correlation values > 0.5 being considered to be collinear (Dormann et al., 2013). Test statistics with $\alpha \leq 0.05$ were considered statistically significant. Analyses were performed using the statistical package R version 3.1.1 (R Foundation for Statistical Computing). The co-occurrence of Mink Frogs and Green Frogs, was quantified as a checkerboard or C-score (Stone and Roberts, 1990; Gotelli, 2000), and assessed against a random null model using EcoSim Version 7.0 (Gotelli and Entsminger, 2001). Nearest neighbour analysis using ESRI ArcGIS (Version 10.2) was also used to assess the spatial distribution and clustering of known Mink Frog populations across the study area.

## 2.4. **Results**

Through manual calling surveys (MCS), we identified Mink Frogs at 19 of 40 sites with the majority of male Mink Frog choruses heard within 15 km of Corner Brook. Based on these locations, Mink Frogs range expanded from the 2001 point of original discovery at a rate of 3.8 km/year northeast along the Humber River and Deer Lake to the town of Deer Lake. The south-western leading edge of the dispersal was approximately 34 km from the original discovery location, a dispersal rate of 2.6 km/year. Mink Frogs were also identified north of the Humber River in the town of Hughes Brook (dispersal rate of 1.1 km/year) and in Humber Village as well as Gillams on the northern shore of the Bay of Islands (Fig. 2-1). Mink Frogs were first noticed outside of Corner Brook in a man-made pond at the Humber Village site in 2011 (Andrew May, personal communication, 30 June 2015). The Humber Village site is ~16 km from the original discovery site.

The results of the VES were similar to those obtained by the MCS (Mink Frogs present at 42 of 77 sites surveyed) and indicated that the highest densities of Mink Frogs were present in the vicinity of Corner Brook (Fig. 2-2). Nearest neighbour analysis of MCS data indicated that Mink Frogs were widely dispersed throughout the study area (z score = 4.1841, $p < 0.001$; < 1% chance the dispersal pattern was random). However, the

VES was more effective in identifying the southern leading edge of the population expansion of the Mink Frog as being Moose Pond ~34 km from the initial discovery location (Fig. 2-2). Using VES techniques we identified Mink Frogs in Deer Lake to the north-east (similar to the MCS findings), but also found Mink Frogs in Mount Moriah to the west. Both MCS and VES failed to identify any Mink Frogs in the southern portions of the study area, near Stephenville, which contrasts with the presence of Mink Frogs at a single location there during the 2011 surveys (Stapleton 2011).

Green Frogs were identified at 19 of 40 MCS monitoring sites, primarily in the vicinity of Stephenville, the southern portion of the research area (Fig 2-1). Green Frogs were also heard in the vicinity of Pynn's Brook and near Hughes Brook, on the northern shore of the Bay of Islands. There was little overlap of Green Frog and Mink Frog populations (Fig. 2-1), and at sites where both species occurred in the same pond, they appeared to occupy opposite ends of the water body based on both MCS and VES results. Both species were simultaneously identified at only 5 of 105 locations in total using MCS and VES methods.

The assessment of patterns of co-occurrence of the Mink Frog-Green Frog species pair revealed a C-score of 225. When tested against a random null model of independent species presence (10,000 simulations), the observed C-score of 225 was well within the highest 5% of the simulated values ($p = 0.002$), criteria which Beaudrot et al. (2013) suggest are indicative of a checkerboard distribution by two species which is not likely due to chance alone.

The model for the binary logistic regression of Mink Frog Presence against water quality parameters was:

$$logit(MF) = e^{\beta_0+\beta_1 DO+\beta_2 TEMP+\beta_3 COND+\beta_4 pH} + \varepsilon,$$

where MF is the probability of Mink Frog Presence (dichotomous data: 0/1, absence/presence), $\beta_0$ = the overall mean, DO = dissolved oxygen (mg/L), TEMP = water temperature (°C), COND = conductivity in (μS/cm), ε = binomial error. The binary logistic model for geographic predictors was:

$$logit(MF) = e^{\beta_0+\beta_1 LAT+\beta_2 LON\ +\beta_3 TER+\beta_4 ELEV} + \varepsilon,$$

where LAT = latitude, LON = longitude and TER = terrain, ELEV = elevation, (sloping/undulating or flat). The binary logistic equation for other habitat predictors (Table I) was:

$$logit(MF) = e^{\beta_0+\beta_1 HAB+\beta_2 ATTZ+\beta_3 WB+\beta_4 RS+\beta_5 RD+\beta_6 VEG+\beta_7 BV)} + \varepsilon,$$

where $\beta_0$ = the overall mean, HAB = dominant habitat type, ATTZ = aquatic terrestrial transition zone, WB = water body type, RS = human residence (100 m range), RD = road (100 m range), VEG = emergent vegetation, TER = terrain and BV = beaver dam. All three binary logistic models were also replicated for the analysis of Green Frog presence.

Further inspection of binary logistic regression analyses indicated that Mink Frog presence potentially was influenced by pH (z value = 1.787, p = 0.074; significant – avoidance of Type II error), while all other predictor variables were not significant (p-values exceeding 0.210). When pH was included as the only predictor variable in a univariate analysis, Mink Frog presence was clearly associated with increasing pH values (z value = 2.584, p = 0.010). There was no influence of dissolved oxygen (z value = -0.729, p = 0.466), conductivity (z value = 0.537, p = 0.591) or water temperature (z value -1.237, p = 0.216), when all water quality variables were modelled together as predictors of Mink Frog presence. Green Frog presence was related to elevation (z value = -2.215, p = 0.028), temperature (z value = 2.163, p = 0.031) and water conductivity (z value = -2.154, p = 0.032).

The pH of ponds in the southern regions of the study area, near Stephenville, were generally more acidic than those in the vicinity and north of Corner Brook (Fig. 2-3). Sites of Mink and Green Frog co-occurrence were located north-east of Corner Brook, varied in pond size and had an average pH of 7.13 ± 0.50 SD (Table II). For all ponds where they occurred, the density of Mink Frogs observed within 2 m of the shoreline (0.167 frogs/m) was significantly greater ($t = 6.08$, $p < 0.001$) than those observed >2 m away (0.043 frogs/m). In ponds where the Green Frog was present, the density 0.018 frogs/m within 2 m of the shoreline was also significantly greater ($t = 2.421$, $p = 0.021$) than beyond 2 m (0.005 frogs/m).

Notably, pond pH (Fig.2-4) differed significantly among habitats in which only Mink Frogs were found, in comparison with those where only Green Frogs were present, those where both species were found or ponds in which neither species was present (Kruskal-Wallis non-parametric tests; chi-squared = 16.697, df = 3, p = 0.001). Post hoc comparisons identified pairwise pH differences between Green Frog only-Mink Frog only ponds (calculated observed difference between pH average ranks = 15.878; calculated critical difference = 10.895) as well as between Green Frog only-neither species ponds (calculated observed difference between pH average ranks = 14.450, calculated critical difference = 14.162).

There were also significant differences in the DO concentrations recorded in 2014 among the ponds occupied by Mink Frogs, Green Frogs, both species or neither species

(Kruskal-Wallis non-parametric tests; chi-squared = 11.043, df = 3, p = 0.012). Post- hoc tests however failed to conclude which pond types differed statistically. Note, for comparison of the Green Frog-only and Mink Frog-only occupied ponds, calculated observed difference between average ranks was 10.567 while the calculated critical difference was 10.984.

## 2.5. **Discussion**

Mink Frogs are clearly in the dispersal stage of the invasion process, having established a successful breeding population at the point of introduction and dispersed at least 50 km along two aquatic corridors, populating the intervening available freshwater habitats. From its location of first identification in 2001 at a pond on the outskirts of Corner Brook (Warkentin et al., 2003), the Mink Frog dispersed 1.0 km/year to the northeast and 6.5 km/year to the south over the next 10 years (Stapleton, 2011). Our manual calling surveys and visual encounter surveys confirmed that the Mink Frogs were continuing to disperse and had extended their range further from the point of initial discovery in Corner Brook at rates which suggest faster expansion to the northeast at 3.8 km/year. Movement through the Humber River-Deer Lake corridor was particularly rapid, having covered an apparent 48 km during 3 years from 2011 to 2014. No Mink Frogs were identified at 20 monitoring sites in the Stephenville area during our 2014 surveys. It is possible that Mink Frogs identified in Stephenville during 2011 were there due to human assisted transportation and by 2014 had been locally extirpated. The next furthest south that Stapleton (2011) identified Mink Frogs was at Blue Pond which is 19 km southwest from the point of origin. In 2014 however, we identified Mink Frogs at

Moose Pond (Fig. 2-2.), suggesting further movement of the main southern boundary of Mink Frog distribution by about 15 km southwest. Mink Frogs were also reported in the Cook's Brook drainage at the time of initial discovery on the island (Warkentin et al., 2003) and during our current surveys were detected in a pond near Mount Moriah which is 6 km northwest of the original discovery point in this drainage at Little Cook's Pond (Fig. 2-2).

In contrast to widespread Mink Frog dispersal, MCS and VES in 2014 identified the existence of Green Frog at just one locale near Stephenville and at Pynn's Brook (Fig. 2-1). During 2001 (Powell, 2002) and 2011 (Stapleton, 2011), Green Frogs were identified using MCS at six sites around Stephenville and at only one site in Corner Brook (Stapleton, 2011). Amphibian calling indices using MCS have confirmed the presence and abundance of Mink Frog and Green Frog populations in other Canadian Provinces, such as New Brunswick (Green et al., 2014) and Ontario (Sanzo, 2005). Gilhen et al. (1984) also identified both species in Nova Scotia. However there were no reports of Mink Frog density using visual encounter surveys with which to compare our values.

Habitat variables assessed were not adequate predictors of Mink Frog presence and did not appear to influence the dispersal patterns observed. Water chemistry in general is not always useful in predicting anuran species richness and abundance (Hecnar and M'Closkey, 1996; Hecnar, 1997; Sanzo, 2005). Studies by Campbell et al. (2004) and Stapleton (2011) however identified dissolved oxygen concentration as the main predictor of toad dispersal in western Newfoundland. Dissolved oxygen, thought to be a limiting factor for Mink Frog breeding (Hedeen, 1986; Gibbs and Breisch, 2001; Desroches et al., 2006; Popescu and Gibbs, 2009), had no detectable influence on Mink Frog dispersal in western Newfoundland. However, observed water temperature and dissolved oxygen values did not vary widely within the relatively small geographic region we monitored, with oxygen levels apparently not sufficiently low enough to affect the survival of eggs and larvae. It was therefore not surprising that dissolved oxygen was not identified as a significant predictor for Mink Frog presence.

The co-occurrence analyses confirmed that Mink Frogs and Green Frogs were currently segregated. Mink Frogs were primarily found in ponds with circumneutral pH. Green Frogs were mainly found in the acidic bog ponds in the lowland southern portion of the study region (Fig. 2-4), which consisted mostly of *Sphagnum* bogs and less limestone bedrock than in the northern areas (Burzynski, 2011). *Sphagnum* bogs aid in creating acidic pond water through the exchange of $H^+$ ions for $Ca^{2+}$ ions (Clymo and Hayward, 1982; Dodd, 2010). The apparent influence of pH on Mink Frog dispersal may indicate a physiological relationship between distribution and intolerance to acidic environments over extended periods, particularly as it influences survival and development of eggs. Freda and Dunson (1986) reported similar findings in their study on Fowler's Toads (*Anaxyrus fowleri*) where the species was generally absent from acidic environments. Low pH can affect osmoregulation in some anuran species, for example, exposure to breeding ponds with $pH < 4$ decreases total body sodium by at least 50% in Northern Leopard Frog (Freda and Dunson, 1984; Pierce et al., 1984; Wells, 2010). However, species such as the Green Frog are more tolerant of low pH, retaining more sodium ions than either Leopard Frogs or Bullfrogs (Wells, 2010). Therefore Green Frogs are capable of surviving in acidic bogs which may not be suitable for other, closely related species.

Many frog species exhibit syntopic occurrence with congeners through resource partitioning (Gorman and Haas, 2011). For example, the Florida Bog Frog (*Lithobates okaloosae*) and a subspecies of the Green Frog (*Lithobates clamitans clamitans*) have coexisted within their ranges through variation in microhabitat selection. Green Frogs and Mink Frogs are also closely related, highly territorial, exhibiting sympatric population overlap in other parts of its range (Shirose and Brooks, 1995; Bevier et al., 2006). They have similar feeding strategies and habitats, living in permanent ponds with emergent vegetation and prolonged breeding periods during summer. Unlike Shirose and Brooks (1995), we found that Mink and Green Frogs used similar pond margin (< 2 m from shoreline) habitat. Because ponds in western Newfoundland are oligotrophic (Campbell, 2002) and have limited floating aquatic or emergent vegetation, it may be that Mink Frogs are occupying what little preferred habitat is available to them at the margins of ponds, and consequently must either co-exist with Green Frogs, or maintain spatial separation. During our 2014 MCS, we heard Mink Frogs and Green Frogs at different ends of the same ponds with no evident habitat differences, suggesting at least some spatial separation of breeding sites.

In northern Ontario, Canada there are historic and/or recent sightings for both Mink and Green Frogs that extend into the boreal forest (ORAA, 2016a; 2016b). This contrasts with known occurrences of the Green Frog in Newfoundland. Maunder (1983) noted that the Green Frog did not appear within forested habitat in Newfoundland’s boreal zone and was unlikely to do so or to establish viable populations on the Northern Peninsula of Newfoundland. Thus far this prediction has held true. The Northern Peninsula generally has higher pH than other regions of western Newfoundland (Stapleton, 2011) and there is no evidence that Green Frogs can tolerate the climatic conditions of the Northern Peninsula. We identified Green Frog existence in predominantly lowland acidic bogs. For Mink Frogs, pond habitat pH appears to be the predominant limiting factor influencing their dispersal patterns. As such, we expect that the Mink Frog population in western Newfoundland will continue the trend of expanding towards suitable northern and north-eastern habitats where pH in aquatic habitat is higher (Powell, 2002; Stapleton, 2011). In areas where Mink and Green Frog populations co-exist, there is the potential to limit the intensity of competition through spatial segregation within ponds.

## 2.6. Acknowledgements

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## 2.8. **Figures**

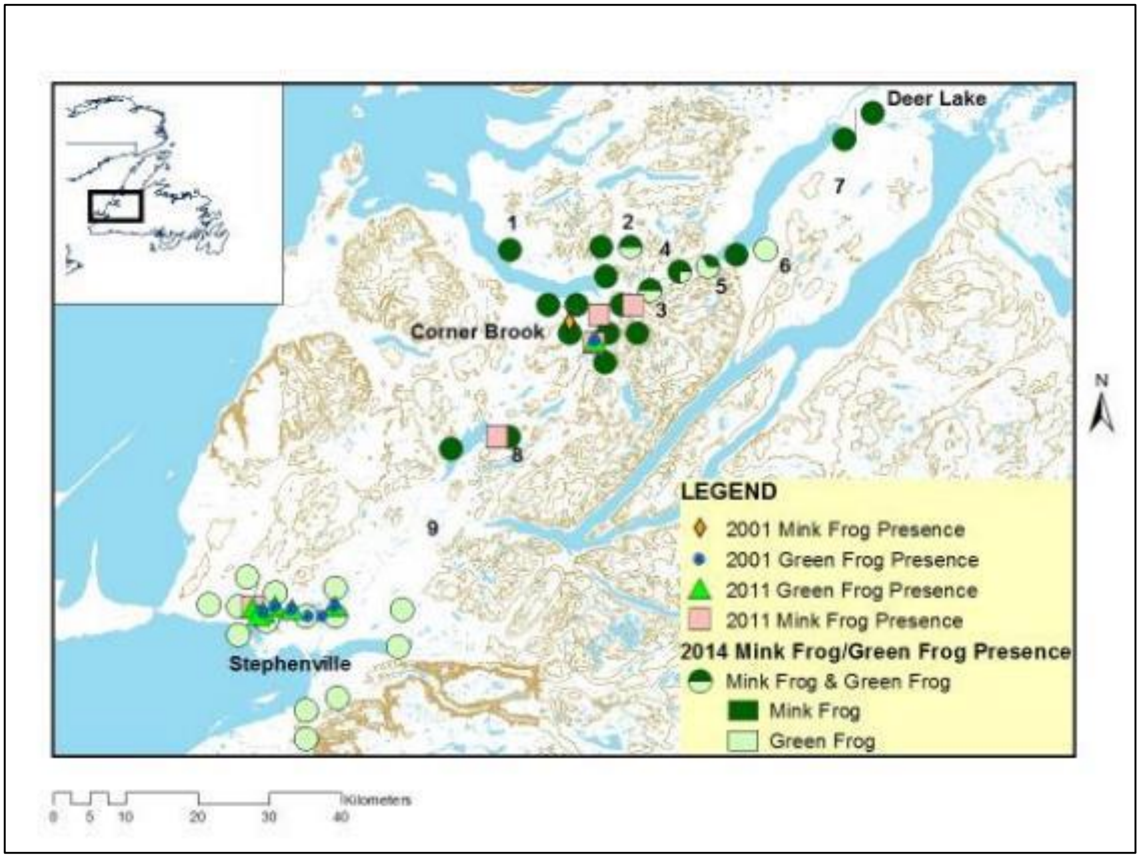

Fig. 2-1. Distribution of Mink Frogs and Green Frogs based on Manual Calling Surveys [NAAMP Protocol]. Surveys conducted at 40 sites between June 18 - July 1, 2014. Sites: 1 – Gillams, 2 – Town of Hughes Brook, 3 – Steady Brook 4 – Humber Village, 5 - Rapid Pond, 6 – Pasadena Beach 7 – Pynn’s Brook, 8 – Blue Pond and 9 – Moose Pond.

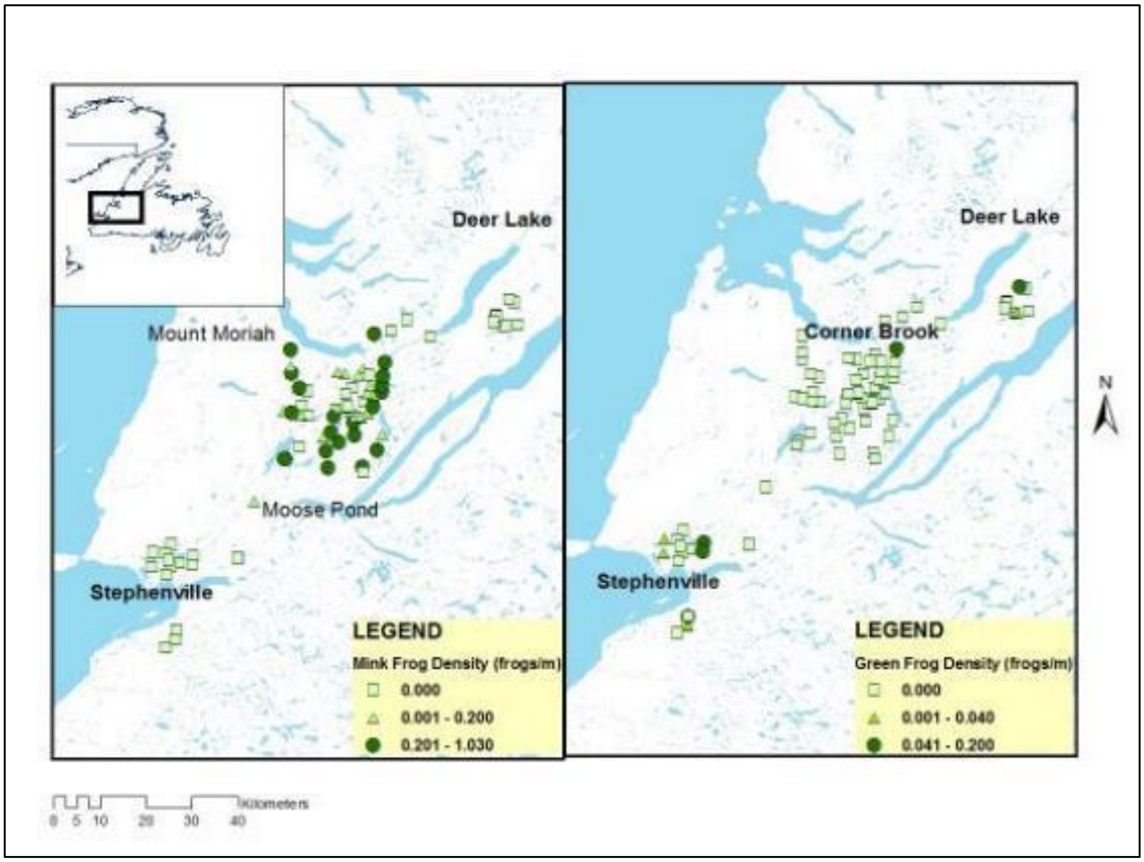

Fig. 2-2. Population densities (frogs/m) of Mink Frog and Green Frog observed during visual encounter surveys of pond shorelines in western Newfoundland. Surveys were conducted at 77 sites between July 9 - 31, 2014.

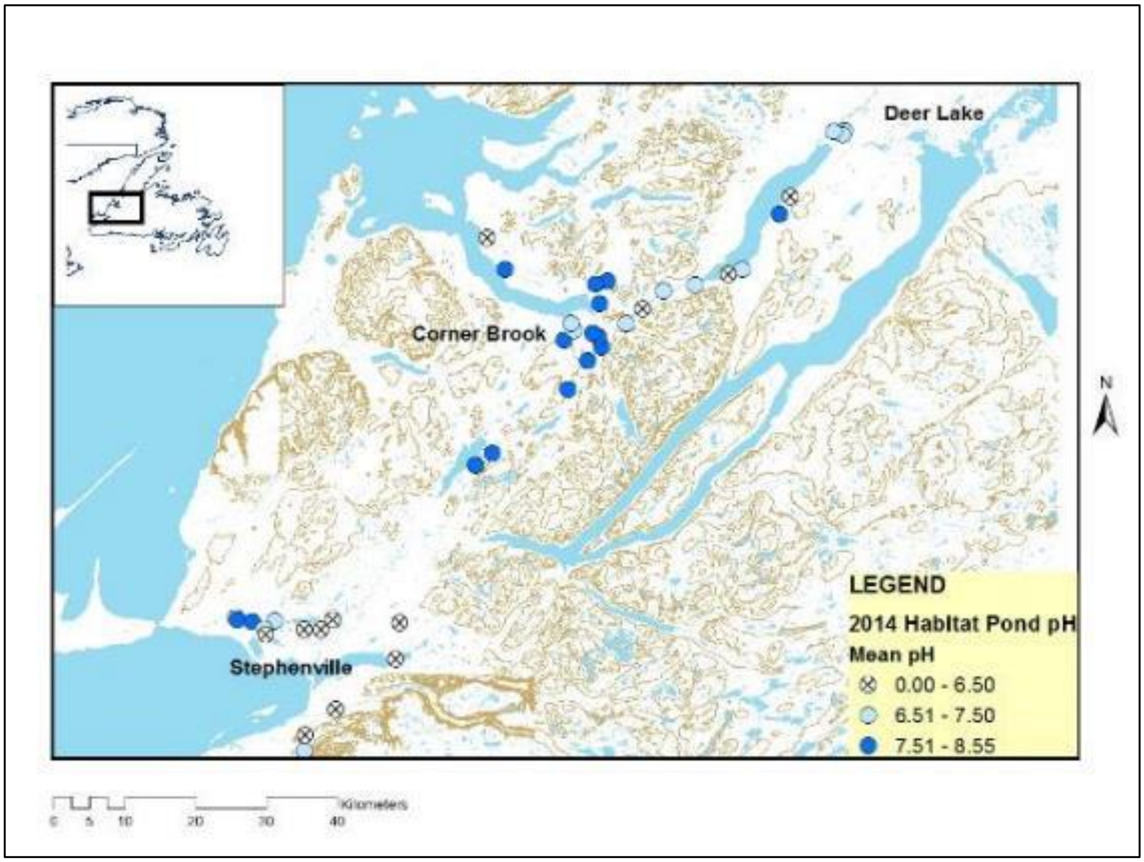

Fig. 2-3. Pond pH for sites surveyed during Manual Calling Survey in 2014 in western Newfoundland. Surveys conducted at 40 sites between June 18 - July 1, 2014.

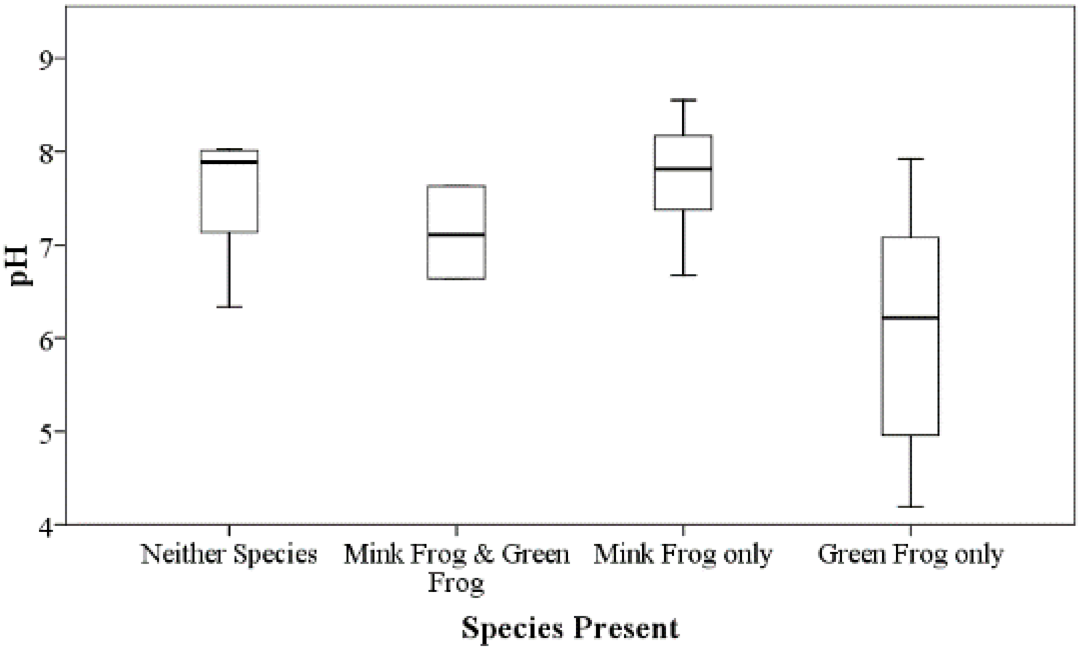

Fig. 2-4. Pond pH for sites occupied by Green Frogs and Mink Frogs during manual calling surveys between June 18 - July 1, 2014. The black horizontal lines represent the median values, the boxes represent the 1st and 3rd quartiles while the error bars represent the minimum and maximum values excluding outliers.

## 2.9. **Tables**

### 2.9.1. Table 2-1. Habitat Variables Recorded at Monitoring Sites

| Abbreviation | Variable | Values |
|---|---|---|
| HAB | Dominant habitat type | pasture, fen, forest, other (within 100 m of wetland edge) |
| ATTZ | Aquatic Terrestrial Transition Zone (substrate at aquatic transition) | sand, rocks, trees, grass, other |
| WB | Water Body Type | Ditch, lake, pond-large, pond-medium, pond-small, fen, marsh, other |
| TE | General Terrain (100 m range) | Flat, Undulating/Sloping |
| ELEV | Elevation (m) | Value Recorded |
| PM | Permanence | Permanent/Temporary |
| RS | Human residence (100 m range) | Presence/ Absence |
| RD | Road (100 m range) | Presence/ Absence |
| VEG | Emergent Vegetation | 0%, 1 – 10%, 11-25%, 26-50%, 50+% |
| BEV | Beaver Dam | Presence/ Absence |

2.9.2. Table 2-2. Water Quality Data – Manual Calling Surveys (2014). Average values obtained during 4-week study.

| Species Identified | pH | DO (mg/L) | TP (˚C) | COND (µS/cm) |
|---|---|---|---|---|
| Neither | 7.54 ± 0.68 | 8.83 ± 1.42 | 17.30 ± 2.94 | 262.48 ± 198.81 |
| Mink Frog only | 7.73 ± 0.56 | 9.19 ± 2.07 | 18.68 ± 4.02 | 289.09 ± 149.47 |
| Both Mink Frog & Green Frog | 7.13 ± 0.50 | 7.06 ± 0.89 | 19.10 ± 2.71 | 101.93 ± 56.97 |
| Green Frog only | 6.13 ± 1.16 | 7.62± 1.01 | 23.07 ± 3.01 | 116.08 ±103.99 |

# 3. Impact of Invasive Eastern American Toad Tadpole Populations on Pond Epilithon in Western Newfoundland

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## 3.1. Abstract

The effect of invasive species on ecosystem resources is often more pronounced for islands. For insular Newfoundland which was originally devoid of anurans, tadpoles of introduced American Toads, *Anaxyrus americanus*, may be reshaping aquatic ecosystem structure by modifying epilithon biomass accumulation. To evaluate the effect of this introduced species on biomass accumulation in ponds, we provided epilithon-covered substrates for American Toad tadpoles taken from local sources and raised in laboratory or field enclosures at varying densities relative to that of local populations. Larger population densities resulted in smaller tadpoles that removed more epilithon biomass from substrates. However, the short term effect on organic resources appears to be influenced primarily by the presence of anurans rather than by population density. With greater dispersal of anurans in Newfoundland, greater reduction of pond epilithic layers may result in changes to freshwater community structure.

**Keywords**

tadpoles, epilithon, growth, density dependence

## 3.2. **Introduction**

Understanding changes in community dynamics of epilithic biofilm layers is important to assessing the trophic shifts that can occur in freshwater ecosystems. The biofilm growing on or attached to substrates in these ecosystems is a mixture of bacteria, algae, protozoa, extracellular exudates and detritus that comprise the periphyton. The epilithon component refers specifically to periphyton that is attached to submerged rocks (Azim et al. 2005, Kohler et al. 2012), of which the algal element is particularly important in the littoral zones of oligotrophic systems because it represents the majority of primary production occurring there (Loeb et al. 1983). As periphyton is a food resource for a wide range of vertebrate and invertebrate aquatic organisms, it consequently plays a vital role in the community dynamics of such systems. But equally, primary consumers such as aquatic insect larvae and anuran tadpoles can regulate rates of primary productivity, decomposition and nutrient cycling in epilithic communities (Mokany 2007, Bellmore et al. 2014) through both the removal of periphyton and the associated increase in the heterogeneity of periphyton distribution. Periphyton growth is also influenced by nutrient supply (Kupferberg 1997, Bellmore et al. 2014), light (Gjerløv and Richardson 2010), and hydrology (Kohler et al. 2012). Periphyton is therefore subject to both top-down and bottom-up biomass control with the top-down effects of grazing often exceeding that of bottom-up influences (Hillebrand et al. 2009). Continuous removal of epilithic biofilm can lead to changes in the biomass of algal periphyton components such as diatoms, chlorophytes and cyanophytes (Kupferberg 1997), and decrease the availability of food resources for grazers such as snails (Class Gastropoda), mayfly nymphs and caddisfly larvae (Class Insecta) and tadpoles (Class Amphibia).

Trophic shifts across food webs can result from grazing pressure on periphyton; the magnitude of which will vary depending on habitat type, hydrology, the productivity of the system, the morphology of the grazer, and whether the primary consumer is an invasive or introduced species. The extent to which invasive species drive ecological change is particularly important for insular ecosystems (Paolucci et al. 2013), where altered competition and changes in habitat structure and quality can lead to greater biological effects (Knight et al. 2005, Colautti et al. 2006). Insular ecosystems are typically characterized by simpler trophic food webs (Chapuis 1995) and often lack either the coevolved predators of invasive species or strong competitive influences (Stone et al. 1994). As a consequence, such ecosystems are particularly sensitive to invasion, with the outcome frequently being a reduction in local biodiversity (Courchamp et al. 2003). The introduction of anurans to Florida, for example the Cane Toad, *Rhinella marina,* and Cuban Tree Frog, *Osteopilus septentrionalis,* to Florida has affected the survival and larval development of native anuran species such as the Southern Toad, *Anaxyrus terrestris,* and the American Green Tree Frog, *Hyla cinerea* (Smith 2005). Crossland (2009) also found that, depending on the timing of emergence, introduced Cane Toads in Australia can affect the survival of the Ornate Burrowing Frog, *Platyplectrum ornatum,* through larval competition when both are tadpoles or through adult Cane Toad predation of tadpole and adult Ornate Burrowing Frogs. However, the effect of invasive anurans is not limited to competitive interactions with native species at the same or similar trophic levels. Wider community effects include facilitating shifts in algal periphyton structure (Kupferberg 1997), affecting invertebrate species abundance through predation, as well as spatial or resource competition (Feminella and Hawkins 1995, Mokany and Shine 2002), and the potential introduction of pathogens and diseases to the ecosystem, which are then transmitted to species at varying trophic levels (Mokany and Shine 2002, Shine 2010).

Algal periphyton is a basal food resource low in protein and high in carbohydrates, commonly identified in the gut contents of anuran larvae such as American Toad, *Anaxyrus americanus* (Baffico and Ubeda 2006, Altig et al. 2007). Herbivorous tadpole populations at high conspecific densities and competition for food resources require greater periphyton ingestion rates to increase protein intake so that the minimum growth size for metamorphosis can be achieved. An increase in conspecific competition or reduction in resource quality or quantity can increase developmental rate (change in stage; Gosner 1960) or slow growth rate (change in size), resulting in metamorphosis at smaller sizes. Monitoring tadpole growth and ingestion rates enables quantification of the effect of conspecific density on algal food resources.

For insular Newfoundland, all extant anuran species including the American Toad, Wood Frog (*Lithobates sylvaticus*), Green Frog (*Lithobates clamitans*) and Mink Frog (*Lithobates septentrionalis*) were introduced (Buckle 1971, Maunder 1997, Warkentin et al. 2003). American Toads in particular have been successful in establishing and expanding their populations from the initial sites of introduction in western Newfoundland (Powell 2002, Stapleton 2011). Amphibians have the capacity to influence ecosystem structure in both terrestrial and aquatic environments because of their high fecundity and dimorphic life stages (Whiles et al. 2006). While all of these effects have the potential to influence ecosystem function, we focus here on examining the effect of introduced tadpoles on epilithon biomass. We examined the growth of American Toad tadpoles in field and laboratory settings at varying densities to quantify the density-dependent ecological effects of anuran populations on basal food resources in pond communities in western Newfoundland, Canada. We report on the i) short-term removal of epilithon biomass, through the assessment of ash free dry mass and ii) overall tadpole growth represented by weight (mg) and length (mm). We expected that at higher population densities, tadpoles would have a slower growth rate but also collectively remove more biomass from the grazing substrates provided. If this is so, then in our oligotrophic system where resources may be limited, it is likely that these introduced anuran populations are having community-wide impacts.

## 3.3. **Methods**

### 3.3.1. Study Sites and Experimental Setup

The 4-week study occurred during June and July of 2014 and included a field site near Corner Brook in western Newfoundland, Canada (Muskrat Pond; 432618m E, 5410993m N, UTM 21) which was paired with a laboratory component. Tadpole enclosures were constructed from 12-L plastic buckets which, in the field setting, had two windows (12 cm x 16 cm) cut into opposite sides that were covered with 1.0 mm mesh (Kupferberg 1997) to allow for water flow through the bucket; these buckets were placed in suitable shoreline habitat where water depths were ~15 cm. For the laboratory experiment, 12-L plastic buckets were filled to ~15 cm deep (~2.5 L) with water taken from a nearby water body (Tippings Pond; 435325m E, 5420322m N, UTM 21). Water in the latter buckets was aerated via air stones and maintained at ~20°C through control of room temperature (*Anaxyrus* feeding behaviour is maximized at ~20°C; Wells 2010). Laboratory enclosures were placed near windows to allow for natural light exposure.

Approximately 80% of the bucket water volume was exchanged each week with water from the same source pond. Prior to experimental set up, unglazed ceramic tiles (15 cm x 15 cm) were submerged in Tippings Pond and Muskrat Pond to allow for natural accumulation of epilithon. After 5 weeks, the tiles were retrieved, macroinvertebrates were removed and the tiles randomly placed in each enclosure to provide food resources for American Toad tadpoles. Tiles from Muskrat Pond were used in enclosures at that site while those from Tippings Pond were placed in laboratory enclosures. To provide a supplementary food source for tadpoles, epilithon covered rocks with an exposed surface area ~76 cm$^2$ were taken from Tippings Pond and added to the laboratory and field enclosures during week 2 (Rock 1) and week 3 (Rock 2) of the experiment.

Experimental stocking densities of tadpoles were determined based on observed natural densities at Muskrat Pond and nearby Abel Pond (430713m E, 5412561m N, UTM 21; where a second field experimental site failed due to stormy conditions). We used 2500 cm$^2$ quadrats to assess the naturally occurring densities of free-swimming feeding tadpoles (following Dodd 2010) at stage 25 of development (Gosner 1960); ~10 mm in total length (TL). Field enclosures in Muskrat Pond were stocked on 12 June 2014 and maintained in seven replicates of three density treatments: natural densities of 13 tadpoles per enclosure (1x), double natural densities of 26 tadpoles (2x) and control densities of 0 tadpoles. Tadpoles collected on 13 June 2014 from Abel Pond were used to stock laboratory buckets in seven replicates of four population treatments: naturally occurring densities of 12 tadpoles per enclosure (1x), 24 tadpoles (2x), 6 tadpoles (0.5x) and 0 tadpoles [control]. Two 1x treatments in the laboratory were abandoned after week 2 due to the death of all tadpoles in those enclosures and measurements omitted from analyses. At both sites, if the population numbers fell below 80% of the original density, the enclosures were restocked from original source ponds with tadpoles of appropriately the same age and size. All tadpoles were handled in accordance with protocols approved by the Institutional Animal Care Committee - Memorial University of Newfoundland.

### 3.3.2. Growth Rate Assessment

Growth rate of tadpoles was estimated by averaging weekly length (mm) and weight (mg) measurements across all individuals per enclosure. For the 4-week study tadpoles were weighed, following the protocol of Browne and Antwis (2009) and photographed. Length measurements (total length (TL), body length (BL) [snout-to-tail-base] and approximate dorsal surface area) were estimated using ImageJ programme (Davis et al. 2008). Water quality parameters (conductivity, pH, dissolved oxygen and temperature) known to affect tadpole growth and development (Wells 2010) were also measured weekly for both lab and field enclosures (Table 3-1).

### 3.3.3. Periphyton Removal

The ash free dry mass (AFDM) method (APHA 1995) was used to assess the periphyton remaining after 4 weeks. Tiles and rocks were collected at the end of the experiment and stored separately by bucket in Ziploc bags at 4°C until processed. The entire exposed surface of each tile was scraped (area of 225 $cm^2$) with a brush and all materials rinsed into a beaker with the sample volume made up to 100 ml with distilled water and stored at 4°C until analysis. Our approach was modified for assessing the remaining population of epilithon present on rocks using the “Top Rock Scrape” method

(Moulton et al. 2002). For rocks, only a section of the top (exposed) surface of the rocks containing the periphyton was scraped, with the area scraped then estimated using techniques described by Moulton et al. (2002). For both substrate types, subsamples of 5-10 ml were filtered onto pre-ashed and weighed glass microfiber filters (dia 25 mm). AFDM was then obtained by ashing and reweighing filters (Ranvestel et al. 2004, Mallory and Richardson 2005). Calculations for AFDM were conducted as described by APHA (1995).

### 3.3.4. Statistical Analysis

All response variables were square root transformed to meet assumptions of linearity. Average outliers that would significantly affect linear models were removed using the Bonferroni p-value method. One way Analysis of Variance (ANOVA) with Tukey’s HSD post hoc tests applied for >2 factor levels, were used to identify overall differences for AFDM samples remaining on substrates after exposure to varying population densities. Differences in tadpole growth were assessed using Analysis of Covariance (ANCOVA); identifying the effects of treatment (population density) and week (Time) as covariates and week*treatment interactive effects (Cohen and Alford 1993). Cumulative degrees days (CDD) of larval development above a standard base temperature ($T_0$) of 5˚C (Chezik et al. 2013) was also assessed. All analyses were conducted using the statistical package R version 3.1.1 (R Foundation for Statistical Computing); significance was determined at $\alpha = 0.05$.

## 3.4. **Results**

### 3.4.1. Growth Rate Analysis

The average survival rates of tadpoles raised at Muskrat Field Pond were 87.7% (1x) and 87.8% (2x) while in the laboratory tadpoles survival rates were 89.1% (0.5x), 87.3% (1x) and 86.4% (2x). Tadpoles raised at lower population densities in field enclosures attained greater mean weight than those raised at higher population densities (Fig. 3-1) and there was a steady increase in weight over time for both treatment levels. At the Muskrat field site, both treatment ($F_{1,66} = 16.085$, $p < 0.001$) and week ($F_{1,66} = 57.624$, $p < 0.001$) were significant factors, and there was no interactive effect ($F_{1,66} = 1.613$, $p = 0.209$). Tadpoles raised in the field experienced wider temperature ranges than those maintained at approx. 19.8˚C in the laboratory (Table 3-1). Field-raised tadpoles increased in size faster than those maintained at approximately similar densities in the laboratory; mean increase in weight was greater for field 1x tadpoles (5.7 mg/wk ± 16.3 SD) than for laboratory 1x tadpoles (3.2 mg/wk ± 4.8 SD) (Table 3-2). While attaining smaller sizes, laboratory tadpoles progressed through Gosner developmental stages (Gosner 1960) more rapidly than individuals in the field. Analysis of the effect of treatment regression slopes on weight of laboratory tadpoles, controlling for week, revealed a significant week*treatment interaction term (ANCOVA; $F_{2,86} = 7.254$, $p < 0.001$). The regression slopes for natural (1x) and twice natural (2x) densities crossed during Week 1 of the study, thus the response variable depended on treatment as well as time and suggested different growth rates among treatments; those in the 2x treatment ultimately had the lowest mass and those in the 0.5x treatment had the highest.

At Muskrat Pond, tadpoles raised at lower (1x) population densities also had higher growth rates in total length (0.8 mm/wk ± 0.9 SD) than those raised at higher (2x) population densities (0.5 mm/wk ± 0.9 SD) (Table 3-2). Analysis of field tadpole total length showed that the interactive term week*treatment was significant ($F_{1, 66} = 15.572$, $p < 0.001$), indicating that the growth rates were different within treatment level. Analyses of body length revealed similar results and a significant interactive term ($F_{1, 66} = 18.008$, $p < 0.001$).

In the laboratory, increases in length per week were greater for tadpoles at 0.5x densities (0.8 mm/wk ± 0.9) than for 1x (0.4 mm/wk ± 0.9) or 2x (0.4 mm/wk ± 0.7) densities (Table 3-2). The regression lines for total length of laboratory tadpoles also crossed, reflecting differences in growth rates. Analysis revealed a significant interaction term week*treatment (ANCOVA; $F_{2,86} = 10.477$, $p<0.001$). Consequently, the effect of the main treatment variable (density) could not be interpreted. The week*treatment interaction for body length response in laboratory tadpoles was not statistically significant ($F_{2,85} = 2.921$, $p = 0.059$). However, effects of both week ($F_{2,85} = 113.151$, $p < 0.001$) and treatment ($F_{1,85} = 11.462$, $p < 0.001$) were significant. Rapid increases in total length and body length of laboratory tadpoles appeared to slow after the second measurement; that is, lower mm/week increases were observed (Fig. 3-2 and 3-3). Total length continued to exhibit a gradual increase (Fig. 3-2) while body length levelled off (Fig. 3-3); indicating growth of the tail. Tadpoles from field sites had accelerated growth during the last weeks of the study.

Mean ratio of body length to total length for field density treatments varied from 0.437 (1x) to 0.441 (2x) while laboratory ratios were 0.444 (0.5x), 0.443(1x) and

0.441(2x). Dorsal area increase mirrored increases in weight of the tadpoles but there were significant week*treatment interactions for both the laboratory (ANCOVA; $F_{2,83}$ = 13.010, p < 0.001) and field samples (ANCOVA; $F_{1,56}$ = 8.035, p = 0.006). Growth measurements were assessed in relation to time and expressed in experimental weeks. Cumulative degrees days (CDD) of larval development above a standard base temperature ($T_0$) of 5˚C (Chezik et al. 2013) was also assessed as an explanatory variable but yielded similar results to week as the predictor variable, and thus was not included in the results presented.

### 3.4.2. Periphyton Removal

The presence and varying densities of American Toad tadpoles resulted in a notable contrast between values in AFDM for treatment versus control enclosures at field sites after 4 weeks (Fig. 3-4). Tiles taken from Muskrat Pond that were exposed to 1x and 2x tadpole population densities had significantly less periphyton remaining compared to the control (0x) tiles ($F_{2,15}$ = 34.65, p < 0.001), but were not different from each other in the amount of AFDM (p=0.817). There were no differences in AFDM among the treatments for Rock#1 ($F_{2,17}$ = 2.939, p= 0.0801) or Rock#2 ($F_{2,17}$ = 0.646, p = 0.536) which had been placed in the Muskrat enclosures. By contrast, substrate grazing by laboratory tadpoles (Fig. 3-5.) revealed no differences among treatments: Tile ($F_{3,\ 21}$ = 0.46, p = 0.713), Rock #1 ($F_{3,\ 21}$ = 2.064, p = 0.136) and Rock # 2 ($F_{3,\ 19}$ = 3.003, p = 0.0561).

## 3.5. **Discussion**

### 3.5.1. Density-Dependent Effects on Tadpole Growth

Although the substrate AFDM remaining did not differ with increased tadpole density, tadpole growth patterns were similar to those found in other studies (Browne et al. 2003, Kehr et al. 2014) in that tadpoles raised at lower densities (0.5x/1x) had higher growth rates than those raised at higher densities (2x). Semlitsch and Caldwell (1982) noted in laboratory experiments on Eastern Spadefoot Toad, *Scaphiopus holbrookii*, that tadpole body size was negatively affected by tadpole density. More recently, Kehr et al. (2014) indicated that in addition to the noted density dependent effects on growth, faster tadpole development of the Red-spotted Argentina Frog, *Argenteohyla siemersi pederseni* was also influenced by combinations of low density and low water volume.

Our study showed that American Toad tadpoles raised in field enclosures also had higher growth rates for mass, body length and total length than those raised in the laboratory at similar densities. A constant ratio of body length to total length seen in the growth rate of tadpoles is reflective of the linear relationship between snout-vent-length [synonymous with BL measured in this study] and tadpole tail length (di Cerbo and Biancardi 2010); as snout vent length increases, so does tail length. The body length to total length ratio in our study was approximately 0.44 for both treatments with field and laboratory settings. Increase in both the body length and tail length of laboratory tadpoles in each treatment appeared to plateau after the second series of measurements. In contrast, field tadpoles were still in the exponential tail-growth phase until the end of the experiment.

While the water for the laboratory enclosures came from natural sources and was replaced on a regular basis, the enclosures in the field would have been exposed to more natural light thereby facilitating continued growth of the phototrophic content of the epilithon on tile and rock substrates to provide more food resources for tadpoles. Limited food resources in the laboratory enclosures were reflected in the slowing of tadpole growth rate over the duration of the experiment. This finding was not unexpected. Feminella and Hawkins (1995) also reported loss of periphyton biomass reducing the growth rate of periphyton grazers. In our study, despite epilithon visually present on the tile substrate, tadpoles generally began foraging immediately on supplementary rocks when the rocks were added to enclosures. As well, upon the occasional injury/death of a tadpole, conspecifics were observed feeding on the remains. Saprophagous or carnivorous behaviour by tadpoles has been observed in previous studies (Semlitsch and Caldwell 1982, Smith 2005). Such feeding behaviour can reduce the density-dependent effects on growth and survival (Alvarez and Nicieza 2002) particularly if food resources are limiting.

Anuran embryonic development and growth is temperature dependent (Morrison and Hero 2003, Wells 2010) with the transition between Gosner stages (Gosner 1960) delayed at lower temperatures. This growth plasticity itself is also often influenced by biotic factors, such as conspecific density (Semlitsch and Caldwell 1982, Dodd 2010, Kehr et al. 2014) as well as by abiotic factors such as temperature (Alvarez and Nicieza 2002, Wells 2010), pH (Relyea 2006), and nutrient availability (Kupferberg 1997). For example, Berven and Gill (1983) found in field experiments that Wood Frog tadpoles taken from cooler, higher elevations of Virginia, USA grew more slowly but transformed at larger sizes than individuals taken from populations in warmer, lower-elevation coastal areas of Maryland. Interestingly, they also noted in laboratory experiments that populations from colder Canadian lowland climates were less sensitive to temperature than those from Virginia or Maryland. Individuals from the northern populations had shorter larval periods and metamorphosed at smaller sizes, an example of adaptation to shortened growing seasons influencing development. In our study, American Toad tadpoles raised at relatively similar densities but generally colder temperatures in the field grew larger than those in the laboratory. This could be the result of the combined influences of temperature variation, along with food availability and quality (Alvarez and Nicieza 2002).

### 3.5.2. Periphyton Biomass Removal by American Toad Tadpoles

Tadpoles are primarily herbivorous (Kupferberg 1997, Ranvestel et al. 2004, Mokany 2007) potentially exerting top-down biotic control of pond epilithon by depleting biomass. The difference in AFDM among treatments and control samples at the Muskrat Pond field site indicates that American Toad tadpoles do remove measureable amounts of epilithon biomass from aquatic systems in western Newfoundland. Ponds in western Newfoundland are typically classified as oligotrophic (Campbell 2002) with high oxygen levels and low algal biomass. Aquatic, particularly oligotrophic, systems may vulnerable to top down grazing pressure, and further affected by additional grazing pressure from resident and invasive species (McQueen et al. 1989, Brown 2012). However, the similar levels of epilithon removal by tadpole populations at 1x and 2x natural pond density suggests that the impact on the pond ecosystem is likely due more to the presence and/or timing of emergence of tadpoles (Mokany 2007), rather than by the specific tadpole densities tested. In our study, natural stocking densities for enclosures were 12 tadpoles (equivalent to 182 tadpoles $m^{-2}$) in the laboratory and 13 tadpoles (equivalent to 197 tadpoles $m^{-2}$) in field enclosures. Comparable studies using stocking densities at 25 tadpoles $m^{-2}$ (Kupferberg 1997) and densities ranging from 10 to 86 tadpoles $m^{-2}$ (Mallory and Richardson 2005) demonstrated similar negative linear relationships between tadpole density and AFDM on substrates.

The general effect of tadpole presence and density on periphyton biomass is not clear-cut. Although some anuran species have the expected negative effect on epilithon biomass (Ranvestel et al. 2004, Altig et al. 2007), others appear to promote the long term growth of algae through bioturbation - a feeding activity that disturbs/removes inorganic content and alters nutrient cycling to favour algal populations. Osborne and McLachlan (1985) found that bioturbation by Plain Grass Frog, *Ptychadera anchietae*, tadpoles in vernal pools and the presence of tadpole remains promoted the long-term growth of periphyton by contributing to the nutrient cycling within the sediments inhabited by the algae. In contrast, Kiffeney and Richardson (2001) found that tadpoles of the Coastal Tailed-Frog, *Ascaphus truei*, had no effect on periphyton biomass, although they did appear to limit the abundance of small invertebrates. It is therefore difficult to predict the effect of a given species in an ecosystem since the periphyton-herbivore dynamic is not only influenced by consumer species but also by stream hydrology (Kohler et al. 2012) and nutrient flux (Hillebrand 2002, Mallory and Richardson 2005, Bellmore et al. 2014).

### 3.5.3. Conclusion

Our findings suggest that invasive anurans can reduce periphyton biomass in ponds of western Newfoundland. American Toad tadpoles in 1x and 2x density treatments had different growth rates but similar negative effects on periphyton biomass. The continuous removal of this food resource by benthic grazers, such as tadpoles, affects algal periphyton abundance and availability. An increase in anuran populations will likely have both short-term and long-term effects on food resources. Variations in food resource levels may subsequently lead to further trophic shifts in consumer species abundances and richness within the ecological community. A more complete measure of the long-term impact will be gained through continued monitoring of resources and the dispersal of breeding anurans.

## 3.6. Acknowledgements

Special thanks to Jasmine Pinksen and Lindsay Batt for their invaluable assistance in collecting the data for this project. Funding to support this research was provided by the Wildlife Division, Government of Newfoundland and Labrador and the Natural Sciences and Engineering Research Council of Canada.

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## 3.8. **Figures**

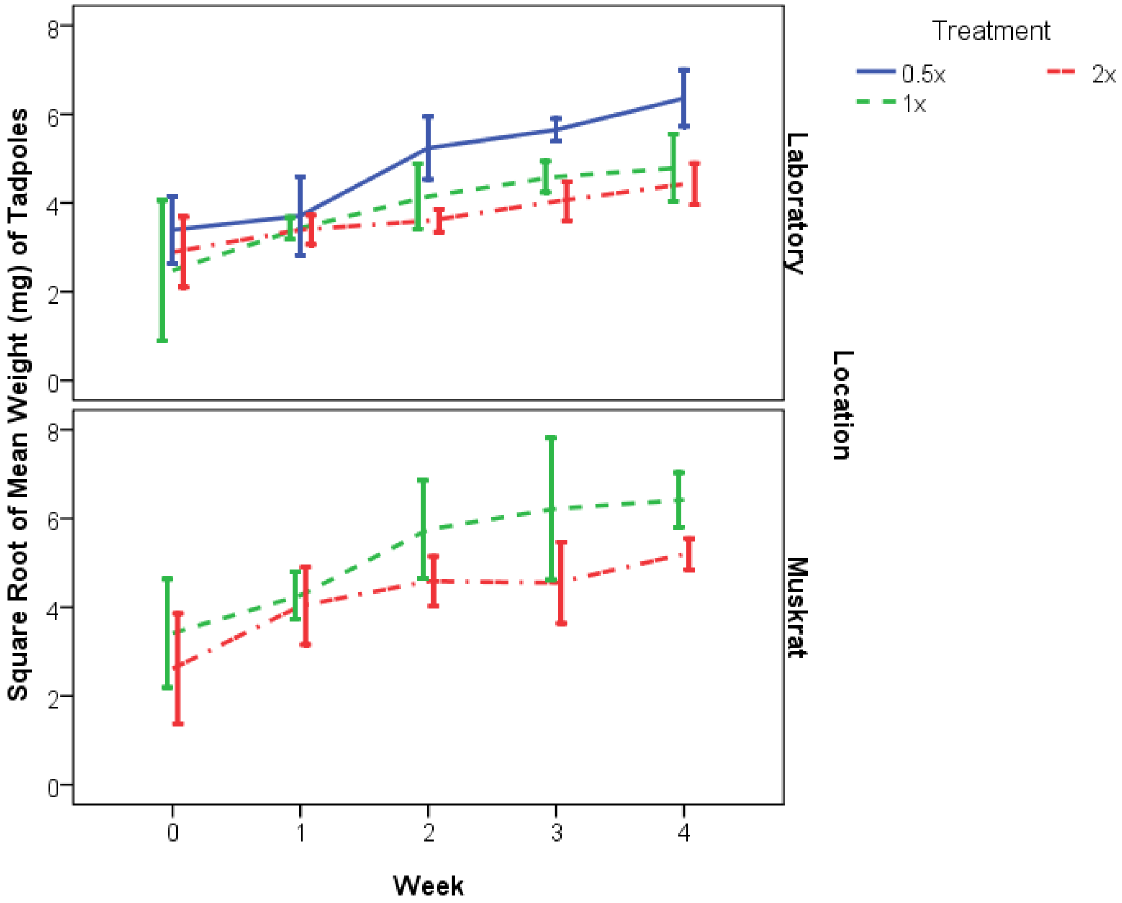

Fig. 3-1. Square root of mean weight (mg) of tadpoles raised in laboratory and field enclosures at one-half (0.5x), naturally occurring densities (1x) and two times (2x) naturally occurring densities. There were seven replicates of each density treatment. Error bars represent 95% confidence intervals.

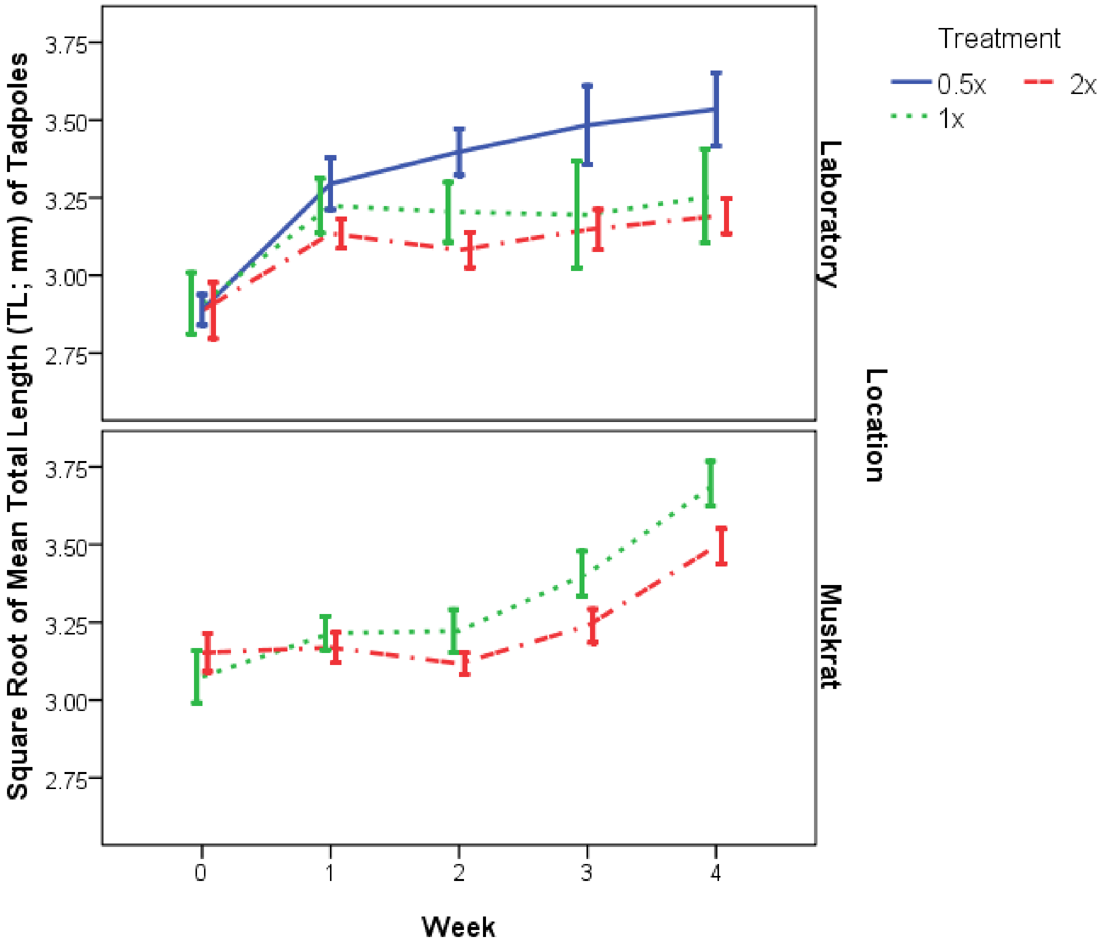

Fig. 3-2. Square root of mean total length (TL; mm) of tadpoles in laboratory and field enclosures at one-half (0.5x), naturally occurring densities (1x) and two times (2x) naturally occurring densities. There were seven replicates of each density treatment. Error bars represent 95% confidence intervals.

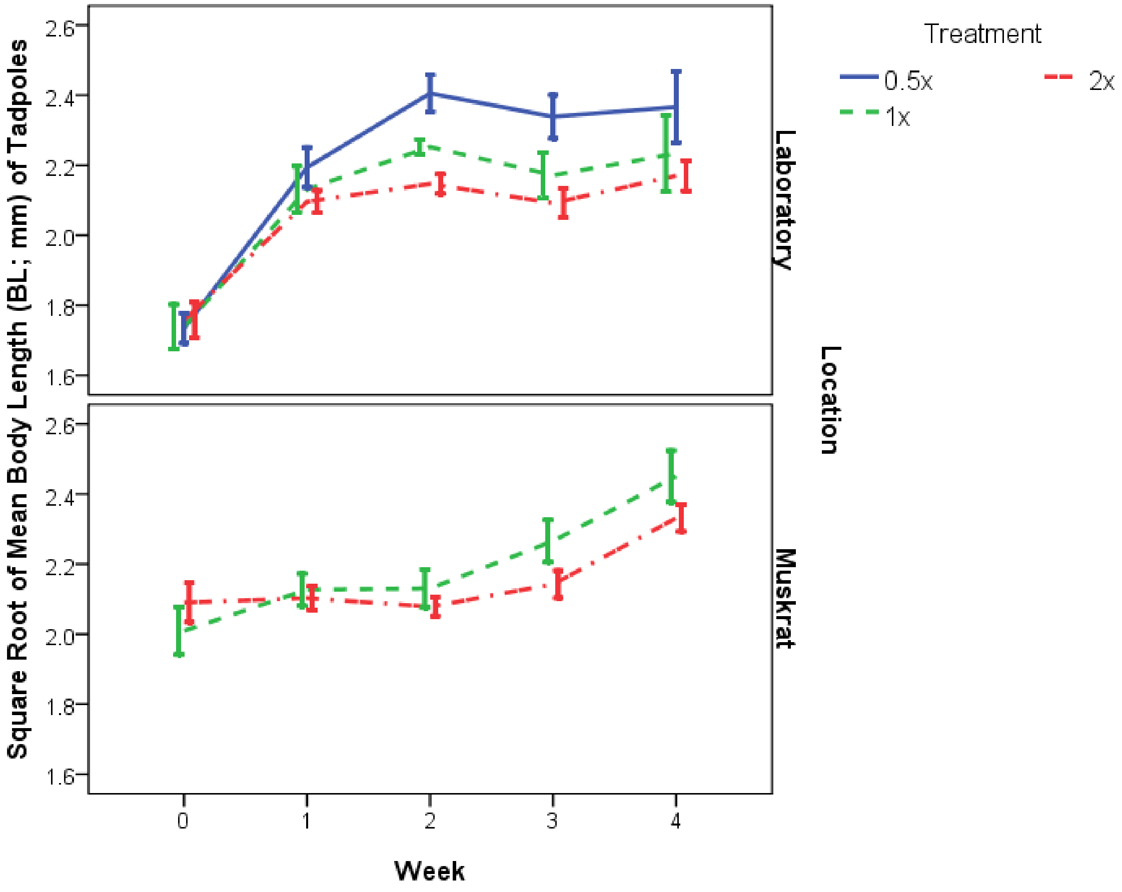

Fig. 3-3. Square root of mean body length (BL; mm) of tadpoles in laboratory and field enclosures at one-half (0.5x), naturally occurring densities (1x) and two times (2x) naturally occurring densities. There were seven replicates of each density treatment. Error bars represent 95% confidence intervals.

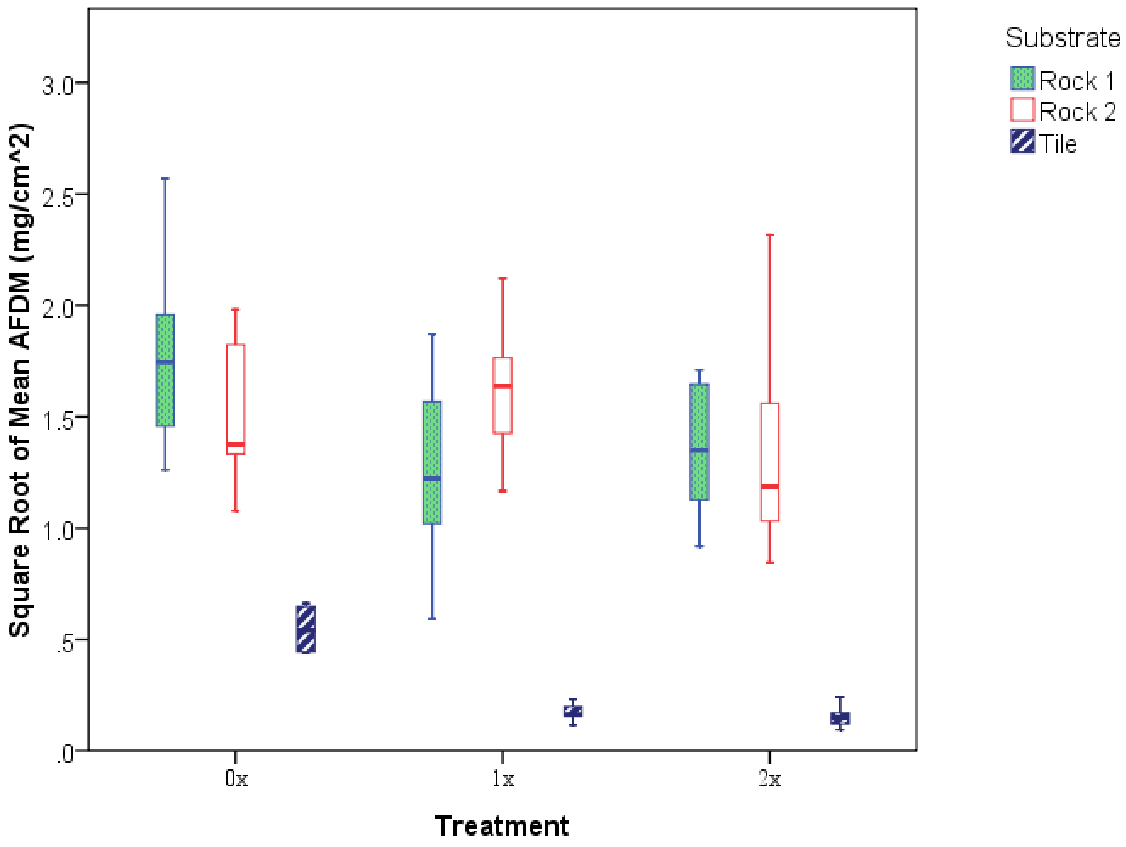

Fig. 3-4. Square root of mean ash free dry mass (AFDM; mg) of epilithon covered substrates exposed to no tadpoles (0x), naturally occurring densities (1x) and two times (2x) naturally occurring densities at the Muskrat Pond field site. There were seven replicates of each density treatment. Error bars represent 95% confidence intervals. The horizontal lines of the box plots represent the median values, the boxes represent the 1st and 3rd quartiles while the error bars represent the minimum and maximum values excluding outliers.

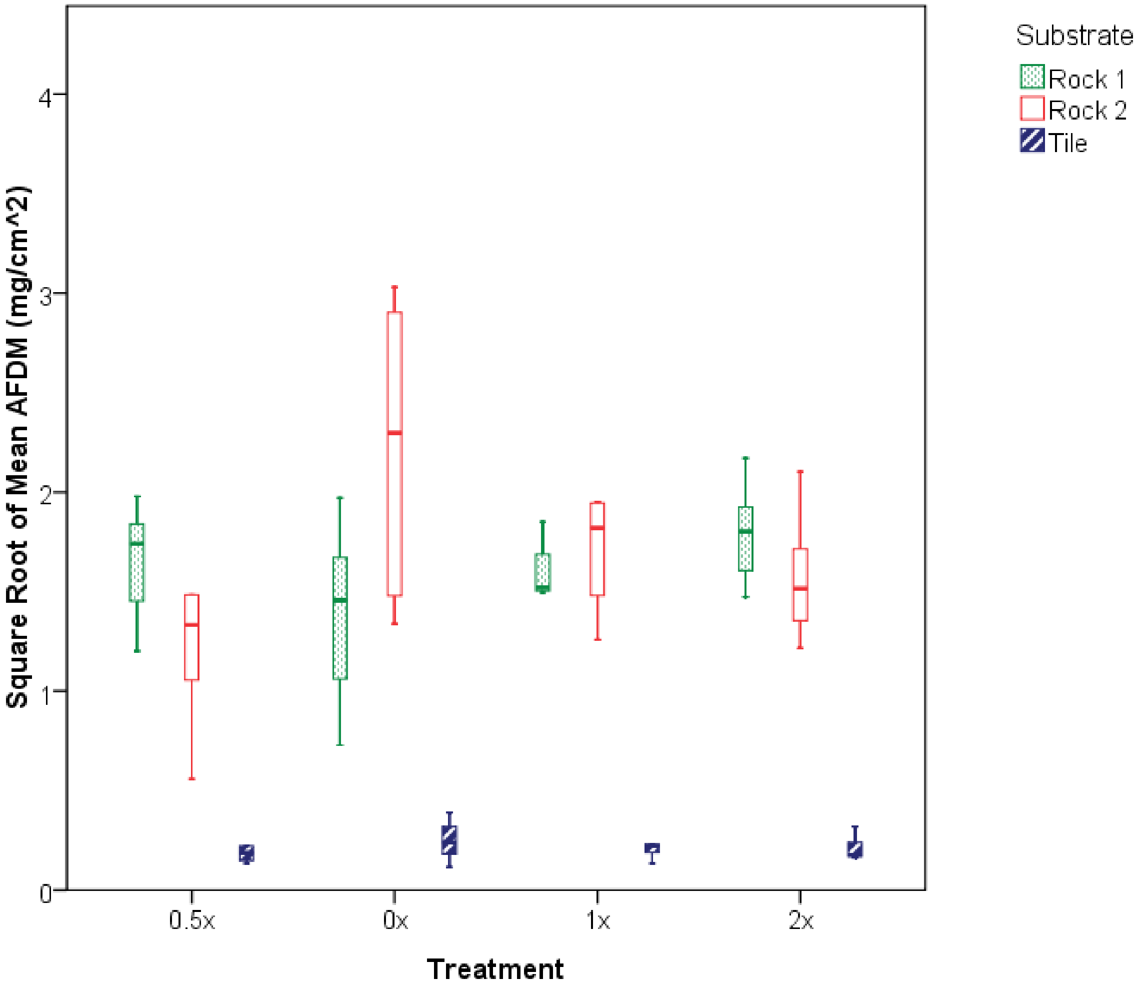

Fig. 3-5. Square root of mean ash free dry mass (AFDM; mg) of epilithon covered substrates exposed to no tadpoles (0x), one-half (0.5x), naturally occurring densities (1x) and two times (2x) naturally occurring densities in the laboratory. There were seven replicates of each density treatment. Error bars represent 95% confidence intervals.

## 3.9. Tables

Table 3-1. Weekly Water Quality - pH, Dissolved Oxygen (mg/l), Conductivity (µS/cm) and Water Temperature (°C) for Water in Laboratory and Field (Muskrat) Enclosures. Measurements were taken during the four week study.

| Location | pH | Conductivity (µS/cm) | Dissolved Oxygen (mg/l) | Temperature (°C) |
|---|---|---|---|---|
| **Muskrat** | | | | |
| Range | 7.57 – 7.92 | 120.90 – 142.0 | 5.92 – 9.45 | 17.85 – 22.30 |
| Average | 7.75 | 130.3 | 8.12 | 19.8^ |
| **Laboratory** (sourced from Tippings Pond) | | | | |
| Range | 8.15 – 8.24 | 235.0 – 256.5 | 9.40 – 9.80 | 12.4 – 20.3 |
| Average | 8.20 | 249.5 | 9.58 | 17.2* |

* Temperature readings taken upon collection of pond water. Average room temperature in laboratory was 19.6°C.

^Temperatures measured at ~10:00. Maximum and minimum field temperatures during the study were 31.6°C and 1.7°C, respectively (Environment Canada 2014).

Table 3-2. Average Growth Rate (weight ± SD and length ± SD) of Tadpoles Raised for Four Weeks in the Laboratory and at Muskrat Pond. There were seven replicates of each density treatment.

| Growth Response | Treatment | Laboratory | Muskrat |
|---|---|---|---|
| **Weight (mg/wk)** | | | |
| | 0.5x | 5.5 ± 9.4 | - |
| | 1x | 3.2 ± 4.8 | 5.7 ± 16.3 |
| | 2x | 2.2 ± 3.7 | 3. 7 ± 8.1 |
| **Total Length (mm/wk)** | | | |
| | 0.5x | 0.8 ± 0.9 | - |
| | 1x | 0.4 ± 0.9 | 0.8 ± 0.9 |
| | 2x | 0.4 ± 0.7 | 0.5 ± 0.9 |

## 4. **Summary**

### 4.1. **Anuran Distribution and Ecological Impact**

After introduction and establishment, dispersal remains the third and arguably, for wildlife managers, the most troublesome stage in the invasion of a non-indigenous species (Sakai et al. 2001, Shea and Chesson 2002). The effects on the species richness, relative abundance of species and ecological functioning of the invaded community become important and long-term, particularly for insular systems (Knight et al. 2005, Colautti et al. 2006). For insular Newfoundland, there is much evidence indicating continued dispersal of introduced anurans. Equally, the extant introduced anurans, the Mink Frog, Green Frog, Wood Frog and American Toad all have dimorphic life stages and consequently their presence can impact both aquatic and terrestrial systems (Whiles et al. 2006).

My research has uncovered an interesting and unexpected dispersal pattern in the newly introduced Mink Frog population. Since 2001, when the species was identified at two sites (Powell 2002, Warkentin et al. 2003), the Mink Frog range has expanded with chemical aspects of the aquatic environment clearly a prominent influencing force. But in contrast to what previous research might suggest, the distribution of the species has been influenced primarily by habitat pH rather than dissolved oxygen. During 2011 manual calling surveys, Mink Frogs were detected at five sites extending primarily southwards from their point of original detection on the edge of Corner Brook (Stapleton 2011). Three years later, the population has continued to spread throughout the aquatic habitats of western Newfoundland, predominantly in a north-eastern direction. In 2014, the species was identified at 19 sites using similar monitoring locations and survey protocols to the 2011 study. Visual encounter surveys confirmed the southern leading edge of the dispersing Mink Frog population in the vicinity of Moose Pond, north of Stephenville (Fig. 2-2). By contrast, Green Frog distribution has remained static in the region to the south of Corner Brook, primarily inhabiting lowland acidic *Sphagnum* bog ponds in Stephenville. As well, Green Frogs now appear to be occupying more habitat to the northeast along the Humber River valley, occurring at three sites during my surveys where they had not previously been detected. However, there was little overlap between the Mink and Green Frogs in terms of occupying the same water body (only at five locations based on both MCS and VES methods) and at those sites the two species appeared to occupy opposite ends of the water body based on visual observations and detection of calling activity. Green Frog presence was best predicted by temperature and water conductivity. However, the probability of finding the Green Frog also decreased with increasing alkalinity (Fig. 4-1). By contrast, Mink Frog presence was associated with increasing alkalinity (Fig. 4-2), which highlights potential physiological differences between the species with implications for both potential competition and resource utilization.

*Sphagnum* bogs aid in creating acidic pond water through the exchange of $H^+$ ions for $Ca^{2+}$ ions (Clymo and Hayward 1982, Dodd 2010). This acidity affects the $H^+$ transfer and osmoregulation in frogs resulting in low survival rates (Freda 1986, Wells 2010). However, Green Frogs are among those species which can tolerate acidic environments (<4 pH; Freda et al. 1991). Mink Frogs are intolerant of such acidity and thus the initial dispersal of the species has been northeast towards circumneutral habitats. Conversely, elevated pH (8.0-10.4) and temperature can also be toxic to anurans (Boyer and Grue

1995). The absence of the Green Frog from the Northern Peninsula where elevated pH habitats occur could be due to this factor (Powell 2002, Stapleton 2011). Maunder (1983) also speculated on the absence of the Green Frog from the Northern Peninsula, suggesting that climate may be influential in limiting their northern dispersal on the island. Several studies have cited the varying effects of water chemistry on anuran distribution (Freda and Dunson 1986, Freda et al. 1991, Campbell et al. 2004, Sanzo 2005). However, my results contrast with similar studies in mainland Canada, where studies on anuran species richness and distribution did not identify pH as the most influential variable in Mink Frog species presence (Hecnar 1997, Sanzo 2005). Hecnar and M'Closkey (1996) noted that in southwestern Ontario, water chemistry was generally a weak indicator of amphibian presence and species richness.

No other habitat characteristic suitably predicted the presence of the Mink Frog. Studies by Powell (2002) and Stapleton (2011) in western Newfoundland similarly did not identify landscape geographical variables nor habitat variables as significant predictors of Mink Frogs in Newfoundland. It can therefore be inferred that the species assessed, Mink Frog, has the capacity to invade various habitats on the Island of Newfoundland through tolerance to a range of environmental conditions.

The second implication of the distribution patterns identified is the potential for competition between Mink and Green Frogs. In other regions where there could be resource overlap between Mink and Green Frogs, there has been reported syntopic existence, the co-occurrence of species without interference of each other. Green Frogs principally occupy pond margins with emergent vegetation while Mink Frogs occupy microhabitat with deeper water and floating vegetation (Stewart and Sandison 1972, Courtois et al. 1995, Shirose and Brooks 1995). However, aquatic habitats in western Newfoundland tend to be oligotrophic (Campbell 2002), with low nutrient levels potentially leading to less floating aquatic vegetation. As expected from the absence of floating vegetation, and confirmed through the visual encounter surveys, Mink Frogs selected microhabitat similar to that of Green Frogs. There is the potential for competitive interactions between these two species in habitats where they co-exist since they are closely related and have similar habitat and food resource requirements. (Shirose and Brooks 1995, Bevier et al. 2006). Thus far ponds where they co-occur have been few, as confirmed by C-score statistics, and the species have exhibited at least some spatial separation of breeding habitat as determined during visual and auditory surveys.

The American Toad population is increasingly well distributed across the landscape in western Newfoundland, based on a number of surveys (Campbell et al. 2004, Stapleton 2011, this research; Fig. 4-3). Using manual calling surveys, toads were identified at 17 of 40 sites, inhabiting ponds that ranged from acidic bogs near Stephenville to more circumneutral habitats in Deer Lake and on the north shore of the Humber Arm (Fig. 4-3). The widespread distribution of the adults and larvae potentially affect food resources in these aquatic systems. I used American Toad tadpoles to assess the impact of tadpole density on periphyton removal, but given the aquatic nature of all four species of introduced anurans on the island, there is the potential for all of them to influence ecosystem structure and function where they occur. In the density-dependent study using American Toad tadpoles, I found that higher density populations resulted in tadpoles that were smaller in mass, dorsal area and total length, but also higher density populations also led to the removal of more periphyton from both laboratory and field aquatic systems.

The display of plasticity in growth rates of tadpoles that I observed has been documented in several studies (Cohen and Alford 1993, Browne et al. 2003, Kehr et al. 2014) and has been proven to be a response to environmental and biotic stressors (Semlitsch and Caldwell 1982, Alvarez and Nicieza 2002, Dodd 2010). The most revealing statistics from the tadpole field research were: i) a significant difference in epilithon biomass remaining on the tile substrates exposed to no tadpoles (control) versus those exposed to 1x and 2x natural tadpole population density treatments, and ii) the lack of differences between 1x and 2x treatments in epilithon biomass remaining on tile substrates. These findings suggest that the tadpole populations could have a negative effect through the removal of periphyton which is a food resource for many other aquatic invertebrate and vertebrate larvae such as snails (Class Gastropoda), mayfly nymphs and caddisfly larvae (Class Insecta) including other anuran species (Class Amphibia). Additionally, the absence of any difference in epilithon biomass between 1x and 2x treatments suggests that the effects of those densities are similar and that the ecosystem may already be at carrying capacity at natural densities. This is worrisome within insular oligotrophic aquatic systems, such as we have in western Newfoundland, because top-down grazing effects of tadpoles in low productivity systems can be influential in decreasing the accumulation of epilithon biomass (McQueen et al. 1986). The increasing dispersal of r-selected anuran species and the subsequent continued removal of epilithic resources at rates faster than they are replenished, could affect species abundance, triggering trophic shifts in the food web, through competitive interactions and reduction of limiting resources (Feminella et al. 1995, Kupferberg 1997, Mokany 2007).

This study has been an important initial assessment of the potential impacts that the current distribution of anurans and their larvae are having on aquatic systems of western Newfoundland. Also uncovered were a myriad of questions regarding the conservation of insular ecosystems. Areas for further research include: what are the competitive interactions in areas of overlap between Mink Frogs and Green Frogs, why do Green Frogs appear to be avoiding waterbodies with high pH, what are the long-term effects of these anurans on periphyton resources, and what potential changes are occurring in the anuran and invertebrate populations in response to variations in resources. The effect that climate change will have on Mink and Green Frog populations is also particularly interesting considering that the Green Frog's range appears currently to be restricted by colder temperatures on the Northern Peninsula, and the Mink Frog's range could continue to expand northwards to colder habitats with greater dissolved oxygen concentrations. I expect that the Mink Frog population will, through natural and human-assisted means, continue to disperse in a generally northward direction. Newfoundland therefore represents the ideal location to study the Mink Frog, the potential competitive interactions between *Lithobates* species occupying similar microhabitats, as well as the impending effects of climate change on the dispersal of anuran species.

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## 4.3. **Figures**

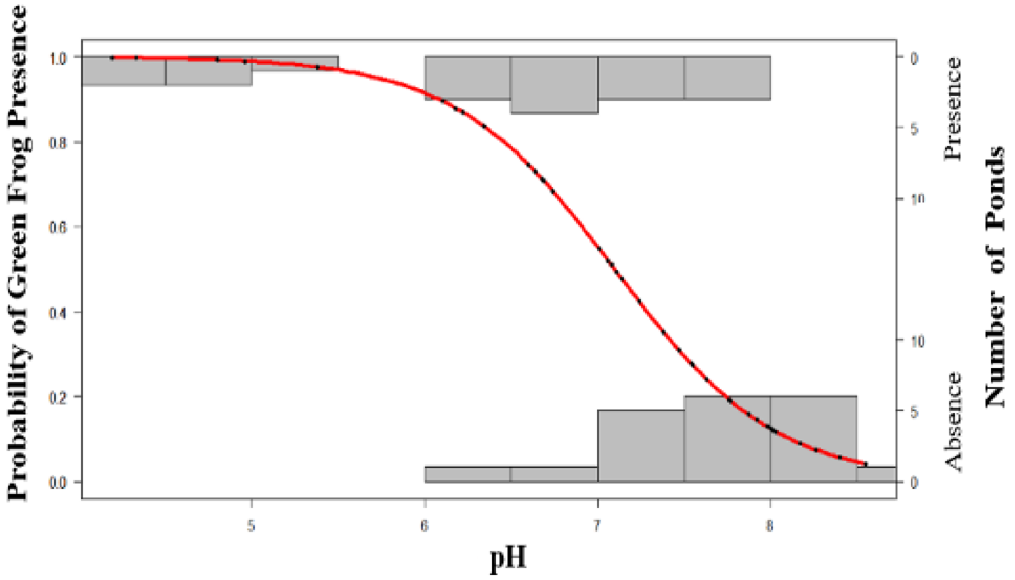

Fig. 4-1. Probability of Green Frog Absence or Presence in relation to pond pH. Surveys conducted at 40 ponds between June 18 - July 1, 2014 in western Newfoundland.

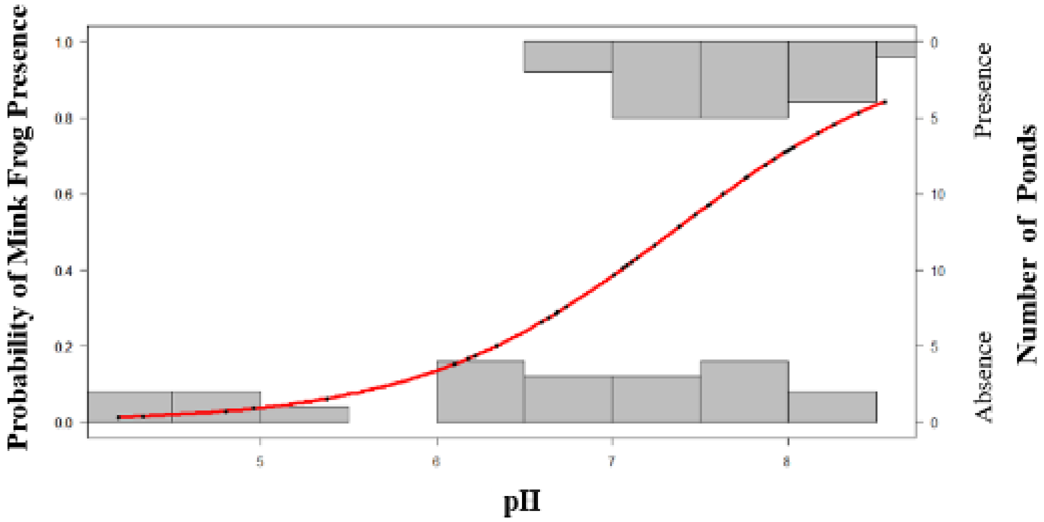

Fig. 4-2. Probability of Mink Frog Absence or Presence in relation to pond pH. Surveys conducted at 40 ponds between June 18 - July 1, 2014 in western Newfoundland.

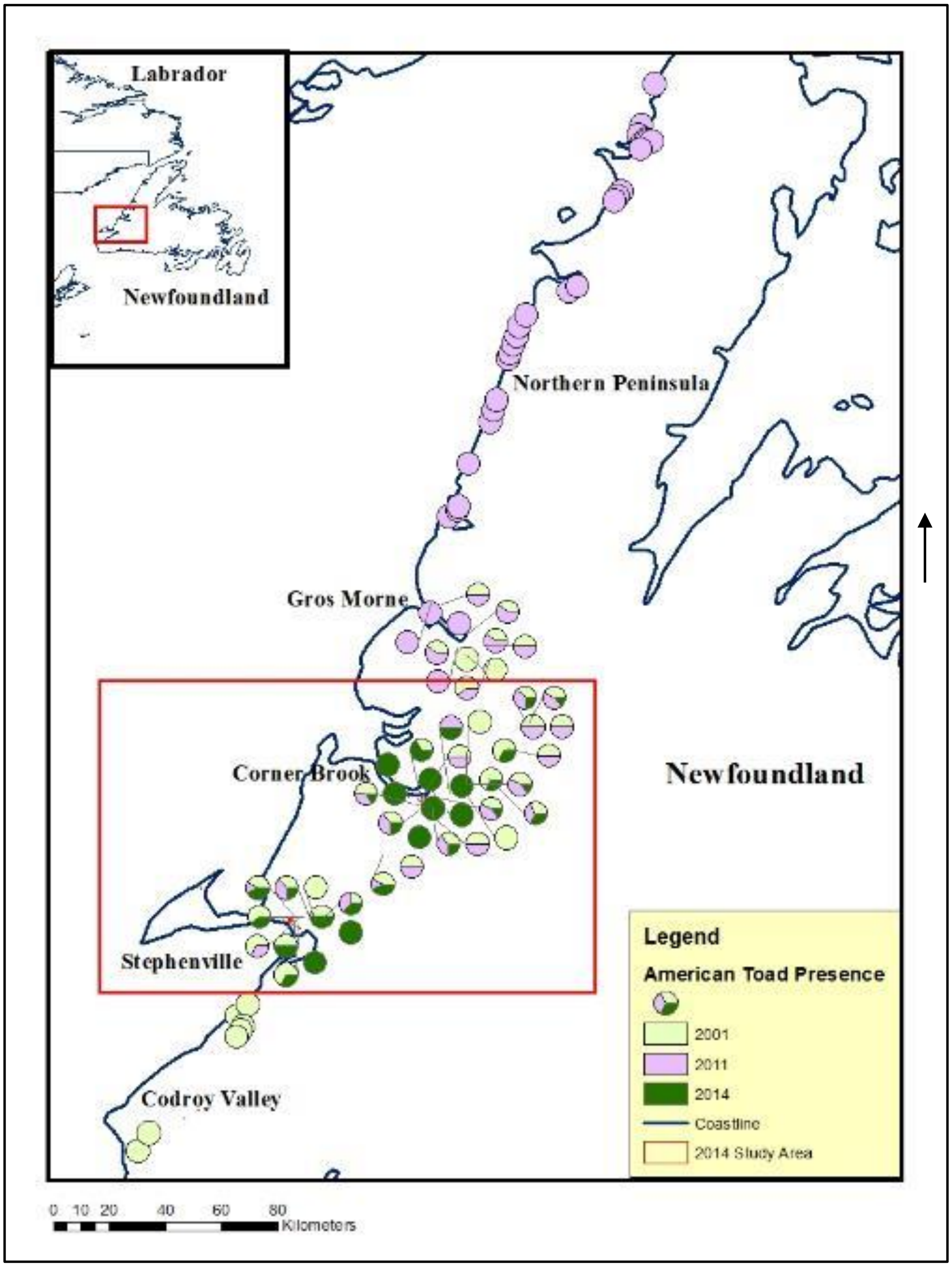

Fig. 4-3. American Toad Distribution in western Newfoundland. Data obtained from manual calling surveys conducted by Powell (2002), Stapleton (2011) and from this 2014 project.

# 5. Appendices

## 5.1. **Appendix I – Anuran-Odonate Surveys**

### 5.1.1. **Introduction**

For competition to occur between two species, they must co-exist and there must be an overlap of limiting spatial and/or food resource requirements (Connell 1980, Hairston 1980, Shea and Chesson 2002). Anurans and odonates have similar larval habitat requirements and exhibit adult interspecific competition for food resources such as small invertebrates from the Order Diptera (Werner et al. 1995, Saha et al. 2012). Knight et al. (2005) suggested that strong interspecific competition can result in trophic cascades of prey populations. The manipulation and monitoring of predator and prey population fluctuations is therefore an ideal method for examining the effects of competition for limiting resources (Hairston 1980, Connell 1983). However it is prudent to first determine if there is sufficient co-occurrence of competing species which could possibly lead to competing interactions. This project was therefore designed to first identify if adult dragonflies and damselflies significantly co-exist with adult Mink Frogs.

Mink Frogs generally occupy the microhabitat of floating or emergent vegetation of permanent freshwater ponds (Shirose and Brooks 1995). In Newfoundland where the aquatic system is predominantly oligotrophic (Campbell 2002) the species occurs along the margins of ponds (this study). Odonates are also reliant on freshwater systems for larval development and survival (Kalkman 2008). Some factors affecting the distribution of odonate species include salinity, water pH, pond openness, habitat type and fish presence (Kadoya et al. 2004, Knight et al. 2005, Oppel 2005, Kalkman et al. 2008, Rychla et al. 2011).

### 5.1.2. **Methods**

Daytime visual encounter surveys (VES) (Crump, Scott Jr 1994) were conducted at 77 sites between July 9 - 31 July 2014. The VES protocol included walking 30 m of shoreline where emergent vegetation was prominent and noting any frog species seen within 2 m, either side of the pond margin, as well as those individuals greater than 2 m away from the shoreline on land and in the water. The presence and relative abundance of dragonflies and damselflies observed along this transect were also recorded. Where possible the dragonflies and damselflies seen were identified to genus level. Factors known to affect the distribution and detection of both taxa were included in the data collection. VES were conducted between 10 a.m. and 3 p.m. each day. Mean air temperature was determined using alcohol-in-glass thermometers accurate to 0.5 °C. Wind speed (m/s) was measured using a digital handheld anemometer. Water temperature (TP), dissolved oxygen (DO) and conductivity (COND) were measured using YSI Model 30 & 55 meters (YSI 1700/1725 Brannum Lane, Yellow Springs, Ohio 45387-1107, USA). The acidity/alkalinity of ponds was measured using a pHep Model HI 98107 pH meter (HANNA Instruments, 584 Park East Drive, Woonsocket, RI 02895, United States). Each meter was calibrated on a daily basis prior to its use for field sampling.

Graphs of dragonfly abundance versus Mink Frog abundance, and also damselfly abundance versus Mink Frog abundance were created. The correlation matrix of data collected was examined for significant associations based on variable pairings, with correlation values > 0.5 being considered to be collinear (Dormann et al. 2013). Test statistics with $\alpha \leq 0.05$ were considered statistically significant.

### 5.1.3. **Results**

Only the results at the broad levels of the damselfly (*Nehalenia sp. Ischnura sp., Lestes sp.*, and Bluets) and dragonfly (*Aeshna sp.*, *Cordulia sp.*, *Leucorrhinia sp.*, *Libellula sp.*, *Stomatochlora sp.*, and *Sympetrum sp*.) were analyzed in the context of Mink Frog census data. Examination of visual encounter survey data indicated that as Mink Frog density (number of frogs per m of transect) increased, there was a general decrease in damselfly (Fig. 5-1) and dragonfly densities (Fig. 5-2). However, there was no significant correlation between the density of Mink Frog with either group of odonates (Table 5-1), and only Mink Frog presence was associated with damselfly density. Damselfly density and dragonfly density were both weakly negatively correlated with Mink Frog Density: $r = -0.182$ and $r = -0.113$) respectively.

### 5.1.4. **Discussion**

There was no evidence to suggest that the damselflies and dragonflies significantly co-occurred with Mink Frogs. There is a notable difference between the foraging tactics employed by the Mink Frogs and odonates. Mink Frogs are generalist predators that may employ a “sit-and-wait” tactic in hunting prey (Kramek 1976). However adult odonates are winged active predators which will tend to have a wider range for foraging. The amount of overlap in the foraging habitat may therefore be too limited to be detectable.

The weak negative correlations between Mink Frogs density and odonate density could be explained by intraguild predation. During the larval stages, odonate larvae are carnivorous and predators of tadpoles (Semlitsch 1990). As such, odonates potentially influence tadpole abundance, the number of tadpoles that metamorphose and thus adult frog abundances. It is also quite likely that intraguild predation exists between adult frogs and adult odonates (Werner et al. 1995). So while there may also be limited competitive interactions for food resources, the effects of predator avoidance may also have be prevalent.

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### 5.1.6. **Figures**

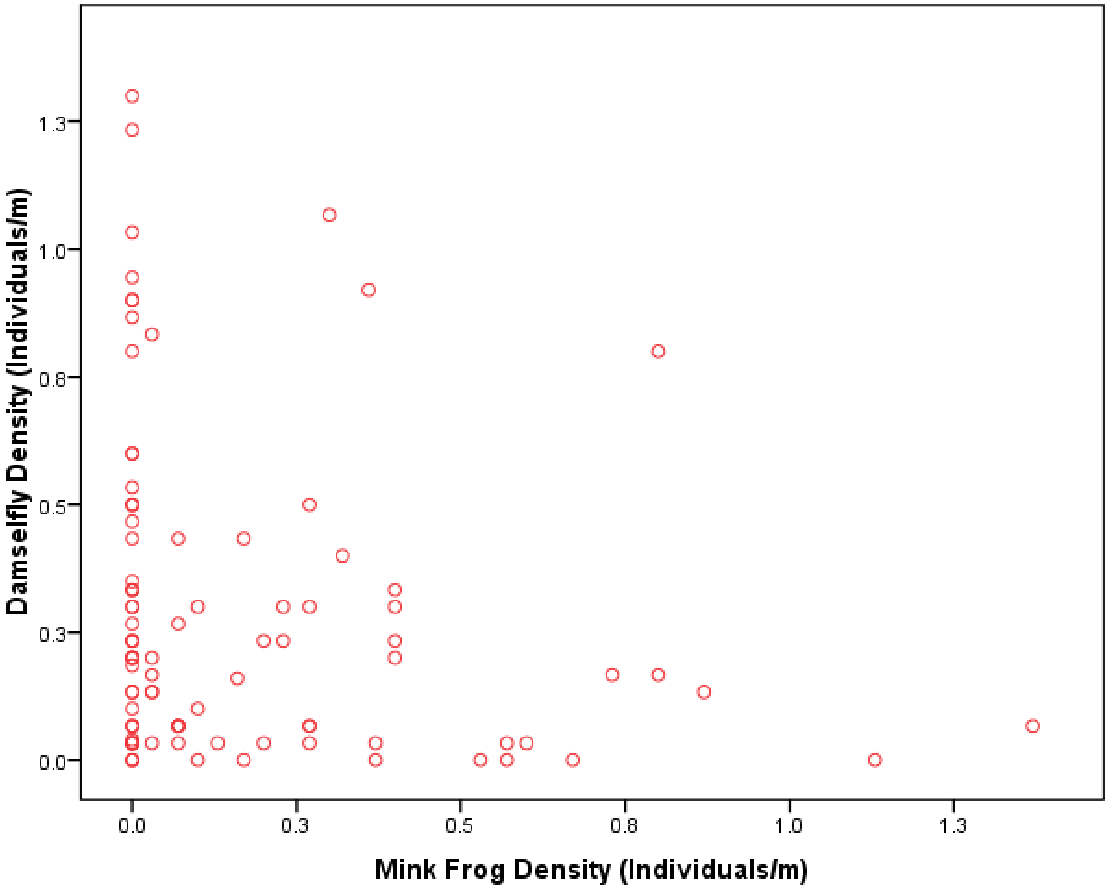

Fig. 5-1. Scatterplot of Mink Frog and Damselfly Co-occurrence in 77 Ponds in western Newfoundland. Visual Encounter Surveys were conducted between the hours of 10 a.m. and 3 pm between July 9 - 31, 2014. p value = 0.077 at α = 0.05.

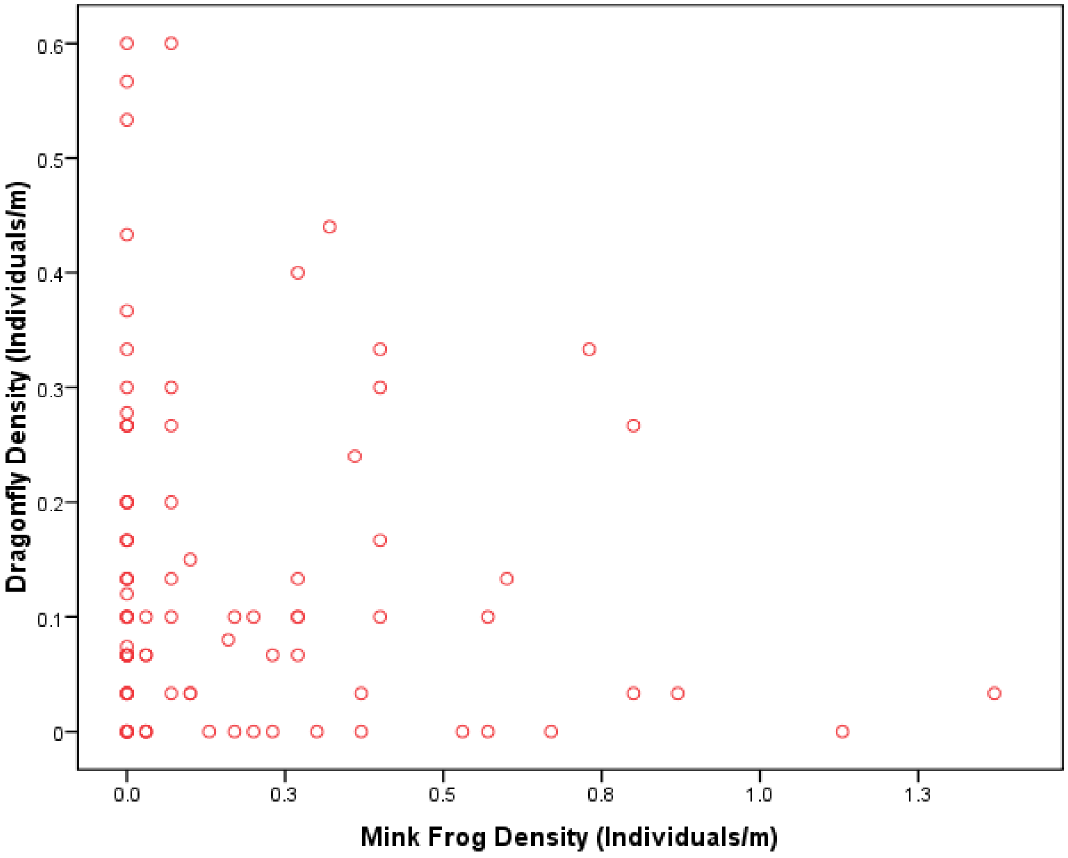

Fig. 5-2. Scatterplot of Dragonfly and Mink Frog Co-occurrence in 77 Ponds in western Newfoundland. Visual Encounter Surveys were conducted between the hours of 10 a.m. and 3 pm between July 9 - 31, 2014. p value = 0.275 at α = 0.05.

### 5.1.7. Table

Table 5.1-1. Indicating the Correlation Coefficients (r) for the Mink Frog and Odonate Abundance Variables

| | Longitude (UTM E, m) | Latitude (UTM N, m) | Mink Frog Density (Individuals/m) | Mink Frog Presence | Dragonfly Density (Individuals/m) | Damselfly Density (Individuals/m) | Emergent Vegetation in Transect |
|---|---|---|---|---|---|---|---|
| Longitude (UTM E, m) | 1 | 0.887** | 0.104 | 0.105 | -0.145 | 0.356** | -.343** |
| Latitude (UTM N, m) | 0.887** | 1 | 0.148 | 0.236* | -0.136 | 0.281** | -.329** |
| Mink Frog Density (Individuals/m) | 0.104 | 0.148 | 1 | 0.619** | -0.113 | -0.182 | -0.017 |
| Mink Frog Presence | 0.105 | 0.236* | 0.619** | 1 | -0.124 | -0.304** | -0.146 |
| Dragonfly Density (Individuals/m) | -.145 | -.136 | -0.113 | -.124 | 1 | 0.090 | 0.356** |
| Damselfly Density (Individuals/m) | 0.356** | 0.281** | -0.182 | -.304** | .090 | 1 | 0.031 |
| Emergent Vegetation in Transect | -0.343** | -0.329** | -0.017 | -0.146 | .356** | 0.031 | 1 |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Correlation coefficients reveal a significant association between Mink Frog Presence and a decrease in Damselfly Density (Individuals/m).

## 5.2. Appendix II - Tables

Table 5.2-1. Habitat Variables Assessed

| Abbreviation | Variable | Values |
|---|---|---|
| HAB | Dominant habitat type (within 100 m of wetland edge) | pasture, fen, forest, other |
| ATTZ | Aquatic Terrestrial Transition Zone (substrate at aquatic transition) | sand, rocks, trees, grass, other |
| WB | Water Body Type | Ditch, lake, pond-large, pond-medium, pond-small, fen, marsh, other |
| TE | General Terrain (100 m range) | Flat, Undulating/Sloping |
| ELEV | Elevation (m) | Value Recorded |
| PM | Permanence | Permanent/Temporary |
| RS | Human residence (100 m range) | Presence/ Absence |
| RD | Road (100 m range) | Presence/ Absence |
| VEG | Emergent Vegetation | 0%, 1 – 10%, 11-25%, 26-50%, 50+% |
| BEV | Beaver Dam | Presence/ Absence |

Table 5.2-2. Monitoring Sites and Habitat Variables Recorded conducted between June 18 and July 1, 2014 in Ponds in Western Newfoundland.

| **Date** | **Location** | **Latitude (UTM N, m)** | **Longitude (UTM E, m)** | **HAB** | **ATTZ** | **WB** | **TER** | **ELEV** | **PM** | **RS** | **RD** | **VEG** | **BEV** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18-Jun-14 | Humber Village | 5426810 | 444377 | forest | grass | pond-small | flat | 122 | YES | YES | YES | 26-50% | NO |
| 18-Jun-14 | Sewage Lagoon | 5448953 | 469652 | fen | trees-grass | stream | flat | 79 | YES | NO | YES | 26-50% | NO |
| 18-Jun-14 | South Brook | 5429169 | 453468 | forest | trees | pond-large | sloping | 89 | YES | YES | YES | 11-25% | NO |
| 18-Jun-14 | Steady Brook | 5424260 | 441448 | fen | grass | fen | flat | 27 | YES | YES | YES | 50-75% | NO |
| 23-Jun-14 | Abel | 5412794 | 430819 | forest | trees-grass | pond-large | flat | 1025 | YES | NO | NO | 11-25% | NO |
| 23-Jun-14 | Bottom of Dump Hill | 5424934 | 435345 | fen | trees-grass | pond-small | flat | 45 | YES | NO | YES | 11-25% | YES |
| 23-Jun-14 | Gillams | 5429819 | 421956 | forest | trees-grass | stream | flat | 29 | YES | NO | NO | 11-25% | NO |
| 23-Jun-14 | Hughes Brook | 5427688 | 434666 | fen | trees | pond-small | sloping | 39 | YES | YES | YES | 11-25% | NO |
| 23-Jun-14 | Parson's Pond | 5418847 | 435588 | forest | trees | pond-large | sloping | 816 | YES | NO | YES | 0% | NO |
| 23-Jun-14 | Tippings Lake | 5420282 | 435181 | forest | trees | pond-medium | flat | 849 | YES | NO | YES | 1-10% | NO |
| 29-Jun-14 | 800m from 461 | 5378858 | 395847 | fen | grass | pond-large | flat | 116 | YES | NO | YES | 1-10% | YES |
| 29-Jun-14 | Black Duck Siding | 5380252 | 397604 | forest | trees-grass | pond-large | flat | 123 | YES | NO | YES | 1-10% | NO |
| 29-Jun-14 | CONA Pond | 5380017 | 386115 | forest | trees-grass | pond-large | undulating | 166 | YES | YES | YES | 1-10% | YES |
| 29-Jun-14 | First Pond | 5374663 | 406423 | forest | trees-grass | pond-large | flat | 119 | YES | NO | YES | 26-50% | NO |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29-Jun-14 | Goose Pond | 5363962 | 393608 | forest | trees-grass | pond-large | flat | 374 | YES | NO | YES | 1-10% | NO |
| 29-Jun-14 | JNC 460/490 | 5379018 | 387766 | forest | trees-grass | pond-large | flat | 100 | YES | NO | YES | 26-50% | NO |
| 29-Jun-14 | JNC 461 West | 5378852 | 393588 | fen | trees-grass | pond-large | flat | 143 | YES | NO | YES | 11-25% | YES |
| 29-Jun-14 | Long Gull Pond | 5380509 | 383738 | fen | grass | pond-small | flat | 149 | YES | NO | YES | 1-10% | NO |
| 29-Jun-14 | Ned's Pond | 5380229 | 384085 | forest | trees-grass | pond-large | undulating | 123 | YES | YES | YES | 11-25% | NO |
| 29-Jun-14 | Noels Pond | 5380174 | 389292 | forest | trees-grass | pond-large | undulating | 95 | YES | YES | YES | 11-25% | YES |
| 29-Jun-14 | Stephenville Warning Lights | 5378172 | 388067 | fen | trees-grass | pond-large | flat | 113 | YES | NO | YES | 1-10% | YES |
| 29-Jun-14 | TCH 1 | 5379799 | 406976 | fen | grass | pond-large | flat | 441 | YES | NO | NO | 26-50% | NO |
| 29-Jun-14 | TCH 2 | 5367575 | 397941 | forest | trees-grass | pond-large | undulating | 78 | YES | NO | YES | 1-10% | NO |
| 29-Jun-14 | TCH 3 | 5361785 | 393497 | fen | trees-grass | pond-medium | flat | 350 | YES | NO | YES | 11-25% | NO |
| 30-Jun-14 | Deer Lake Airport | 5449532 | 469932 | other | grass | stream | flat | 26 | YES | NO | YES | 26-50% | NO |
| 30-Jun-14 | Insectarium | 5449317 | 468441 | fen | grass | pond-small | flat | 38 | YES | NO | NO | 50-75% | YES |
| 30-Jun-14 | Marble Mtn. Pond | 5422221 | 439052 | other | grass | pond-small | flat | 40 | YES | YES | YES | 11-25% | NO |
| 30-Jun-14 | McIvers | 5434233 | 419380 | fen | trees-grass | stream | flat | 146 | YES | YES | YES | 50-75% | NO |
| 30-Jun-14 | Pasadena Beach | 5429802 | 455416 | other | other | stream | sloping | 10 | YES | NO | NO | 0% | YES |
| 30-Jun-14 | Pynn's Brook | 5437567 | 460714 | other | grass | pond-small | sloping | 139 | YES | NO | NO | 50-75% | NO |
| 30-Jun-14 | Rapid Pond | 5427550 | 448790 | forest | trees-grass | pond-large | sloping | 67 | YES | NO | YES | 11-25% | NO |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30-Jun-14 | Town of Hughes Brook | 5428179 | 436408 | forest | trees | pond-large | flat | 211 | YES | NO | YES | 11-25% | YES |
| 1-Jul-14 | Blue Pond | 5403856 | 420184 | forest | rocks | pond-medium | undulating | 625 | YES | YES | YES | 1-10% | YES |
| 1-Jul-14 | CB Ring Road | 5419768 | 430287 | fen | grass | pond-small | flat | 797 | YES | NO | YES | 50-75% | NO |
| 1-Jul-14 | Dozer Pond 2 | 5402132 | 417709 | forest | trees-grass | pond-medium | sloping | 654 | YES | NO | YES | 26-50% | NO |
| 1-Jul-14 | Ducks unlim. Marsh | 5422206 | 431196 | fen | grass | pond-small | flat | 46 | YES | NO | NO | 50-75% | NO |
| 1-Jul-14 | Link Pond | 5420838 | 434357 | forest | trees-grass | pond-medium | sloping | 786 | YES | YES | YES | 11-25% | NO |
| 1-Jul-14 | Near Watson's Pond | 5416829 | 433578 | forest | trees-grass | pond-small | sloping | 902 | YES | YES | YES | 1-10% | NO |
| 1-Jul-14 | Pinchgut | 5402132 | 417709 | other | rocks | lake | flat | 655 | YES | YES | YES | 0% | NO |
| 1-Jul-14 | SWGC Fen | 5421195 | 431619 | fen | trees-grass | ditch | sloping | 290 | YES | YES | YES | 26-50% | NO |

Table 5.2-3. Amphibian Calling Index (NAAMP Protocol)

| **Index and Code Definitions Amphibian Calling Index** | |
|---|---|
| 0 | None Calling |
| 1 | Individuals can be counted; there is space between calls |
| 2 | Calls of individuals can be distinguished but there is some overlapping of calls |
| 3 | Full chorus, calls are constant, continuous and overlapping |

Table 5.2-4. Beaufort Wind Codes

| **Beaufort Wind Codes** | |
|---|---|
| 0 | Calm (<1mph) ( < 1.6 kph) Smoke rises vertically |
| 1 | Light Air (1-3 mph) (6 - 4.8 kph) smoke drifts, weather vane inactive |
| 2 | Light Breeze (4-7 mph) (6.4 – 11.3 kph) leaves rustle, can feel wind on face |
| 3 | Gentle Breeze (8-12 mph) (12.9 – 19.3 kph) leaves and twigs move around, small flags extend |
| 4* | Moderate Breeze (13-18 mph) (20.9 – 28.9 kph) moves thin branches, raises loose papers * Do not conduct survey at Level 4, unless in Great Plains |
| 5** | Fresh Breeze (19 mph or greater) (30.6 kph) small trees begin to sway ** Do not conduct survey at Level 5 in ALL REGIONS |

Table 5.2-5. Sky Codes

| **Sky Codes (numbers 3 and 6 are not used)** | |
|---|---|
| 0 | Few clouds |
| 1 | Partly cloudy (scattered) or variable sky |
| 2 | Cloudy or overcast |
| 4 | Fog or smoke |
| 5 | Drizzle or light rain (not affecting hearing ability) |
| 7 | Snow |
| 8* | Showers (is affecting hearing ability). *Do not conduct survey. |

Table 5.2-6. Noise Index

| Noise Index* | | |
|---|---|---|
| **Massachusetts Noise Index** | **Yes/No System** | **Definition** |
| 0 | No | No appreciable effect (e.g. owl calling) |
| 1 | No | Slightly affecting sampling (e.g. distant traffic, dog barking, one car passing) |
| 2 | Yes | Moderately affecting sampling (e.g. nearby traffic, 2-5 cars passing) |
| 3 | Yes | Seriously affecting sampling (e.g. continuous traffic nearby, 6-10 cars passing) |
| 4 | Yes | Profoundly affecting sampling (e.g. continuous traffic passing, construction noise) |

Table 5.2-7. Manual Calling Survey Results Conducted between June 18 - July 1, 2014 in Ponds in Western Newfoundland.

| **Date** | **Location** | **Time (24hr)** | **Green Frog ACI** | **Mink Frog ACI** | **American Toad ACI** | **Wood Frog ACI** | **Dissolved Oxygen (mg/L)** | **Dissolved Oxygen Temp. (°C)** | **Conductivity (µS/cm)** | **Temperature (°C)** | **pH** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 18-Jun-14 | Humber Village | 1:44 | 1 | 3 | 2 | 0 | 7.47 | 15.7 | 38.2 | 16.0 | 6.64 |
| 18-Jun-14 | Sewage Lagoon | 23:00 | 0 | 1 | 1 | 0 | 6.19 | 18.4 | 266.9 | 18.4 | 6.68 |
| 18-Jun-14 | South Brook | 1:06 | 0 | 1 | 1 | 0 | 11.15 | 15.4 | 242.0 | 15.4 | NA |
| 18-Jun-14 | Steady Brook | 2:02 | 3 | 3 | 3 | 0 | NA | NA | NA | NA | NA |
| 23-Jun-14 | Abel | 23:00 | 0 | 2 | 2 | 0 | 8.03 | 24.4 | 142.4 | 24.4 | 7.76 |
| 23-Jun-14 | Bottom of Dump Hill | 1:13 | 0 | 1 | 1 | 0 | 9.70 | 14.2 | 135.6 | 14.1 | 7.87 |
| 23-Jun-14 | Gillams | 2:04 | 0 | 2 | 2 | 0 | 9.81 | 14.2 | 148.7 | 14.0 | 7.55 |
| 23-Jun-14 | Hughes Brook | 1:40 | 0 | 1 | 0 | 0 | 14.47 | 17.2 | 320.5 | 17.5 | 8.03 |
| 23-Jun-14 | Parson's Pond | 23:28 | 0 | 1 | 1 | 0 | 10.38 | 16.0 | 149.5 | 15.8 | 8.01 |
| 23-Jun-14 | Tippings Lake | 23:48 | 0 | 1 | 2 | 0 | 8.64 | 13.6 | 143.3 | 13.5 | 8.40 |
| 29-Jun-14 | 800m from 461 | 23:43 | 3 | 0 | 1 | 0 | 8.00 | 24.4 | 36.9 | 24.4 | 4.19 |
| 29-Jun-14 | Black Duck Siding | 23:30 | 2 | 0 | 1 | 0 | 8.56 | 25.4 | 31.8 | 25.4 | 4.33 |
| 29-Jun-14 | CONA Pond | 1:03 | 2 | 0 | 0 | 0 | 8.78 | 23.1 | 279.7 | 23.2 | 7.92 |
| 29-Jun-14 | First Pond | 22:51 | 3 | 0 | 0 | 0 | 7.53 | 21.0 | 140.0 | 20.9 | 6.22 |
| 29-Jun-14 | Goose Pond | 22:17 | 2 | 0 | 0 | 0 | 5.69 | 23.6 | 45.1 | 23.6 | 5.38 |
| 29-Jun-14 | JNC 460/490 | 0:49 | 3 | 0 | 0 | 0 | 7.51 | 26.6 | 84.5 | 25.6 | 7.24 |
| 29-Jun-14 | JNC 461 West | 23:55 | 3 | 0 | 0 | 0 | 6.75 | 25.8 | 68.6 | 25.9 | 6.18 |

|  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 29-Jun-14 | Long Gull Pond | 0:08 | 3 | 0 | 1 | 0 | 7.52 | 24.3 | 412.2 | 24.4 | 6.74 |
| 29-Jun-14 | Ned's Pond | 1:19 | 3 | 0 | 0 | 0 | 7.25 | 24.7 | 168.3 | 24.4 | 7.54 |
| 29-Jun-14 | Noels Pond | 0:21 | 3 | 0 | 0 | 0 | 7.49 | 24.0 | 83.1 | 24.1 | 6.60 |
| 29-Jun-14 | Stephenville Warning Lights | 0:36 | 3 | 0 | 0 | 0 | 8.61 | 20.7 | 50.1 | 20.7 | 4.96 |
| 29-Jun-14 | TCH 1 | 23:04 | 3 | 0 | 0 | 0 | 5.70 | 22.7 | 67.8 | 22.9 | 6.10 |
| 29-Jun-14 | TCH 2 | 22:31 | 2 | 0 | 0 | 0 | 7.63 | 25.4 | 60.9 | 25.5 | 4.80 |
| 29-Jun-14 | TCH 3 | 22:05 | 3 | 0 | 0 | 0 | 9.11 | 20.7 | 84.2 | 20.8 | 6.69 |
| 30-Jun-14 | Deer Lake Airport | 22:00 | 0 | 1 | 0 | 0 | 8.26 | 14.2 | 167.0 | 14.2 | 7.38 |
| 30-Jun-14 | Insectarium | 22:32 | 0 | 0 | 1 | 0 | 6.32 | 20.1 | 299.0 | 20.1 | 7.14 |
| 30-Jun-14 | Marble Mtn. Pond | 0:55 | 0 | 2 | 0 | 0 | 8.52 | 21.2 | 325.1 | 21.1 | 7.47 |
| 30-Jun-14 | McIvers | 2:22 | 0 | 0 | 0 | 0 | 8.42 | 16.7 | 63.5 | 16.6 | 6.34 |
| 30-Jun-14 | Pasadena Beach | 23:32 | 2 | 0 | 3 | 0 | 8.14 | 14.2 | 128.0 | 14.2 | 7.08 |
| 30-Jun-14 | Pynn's Brook | 23:10 | 0 | 0 | 1 | 0 | 9.28 | 14.2 | 285.0 | 14.2 | 7.77 |
| 30-Jun-14 | Rapid Pond | 0:10 | 2 | 1 | 0 | 0 | 7.67 | 21.0 | 119.7 | 21.0 | 7.11 |
| 30-Jun-14 | Town of Hughes Brook | 1:30 | 1 | 1 | 0 | 0 | 6.03 | 19.8 | 147.9 | 20.3 | 7.63 |
| 1-Jul-14 | Blue Pond | 22:07 | 0 | 2 | 0 | 1 | 8.39 | 20.6 | 371.1 | 21.2 | 8.17 |
| 1-Jul-14 | CB Ring Road | 0:16 | 0 | 2 | 0 | 0 | 9.78 | 23.8 | 422.0 | 23.1 | 8.26 |
| 1-Jul-14 | Dozer Pond 2 | 21:51 | 0 | 1 | 0 | 0 | 8.37 | 23.2 | 706.0 | 23.2 | 7.98 |
| 1-Jul-14 | Ducks unlim. Marsh | 23:58 | 0 | 1 | 0 | 0 | 6.25 | 23.1 | 362.6 | 22.4 | 7.06 |
| 1-Jul-14 | Link Pond | 23:41 | 0 | 3 | 1 | 0 | 11.50 | 22.4 | 331.6 | 22.4 | 8.55 |

|  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-Jul-14 | Near Watson's Pond | 23:03 | 0 | 0 | 1 | 0 | 8.78 | 21.7 | 625.0 | 21.7 | 8.00 |
| 1-Jul-14 | Pinchgut | 22:20 | 0 | 0 | 0 | 0 | 9.82 | 15.6 | 152.9 | 15.4 | 8.03 |
| 1-Jul-14 | SWGC Fen | 0:30 | 0 | 1 | 0 | 0 | 8.79 | 15.3 | 251.6 | 15.3 | 7.01 |

Table 5.2-8. Atmospheric Data Collected during Manual Calling Surveys conducted between June 18 - July 1, 2014 in Ponds in Western Newfoundland.

| **Date** | **Location** | **Time (24hr)** | **Sky Code** | **Wind Speed (kph)** | **Wind Code** | **Noise Index** | **Air Temp. (°C)** |
|---|---|---|---|---|---|---|---|
| 18-Jun-14 | Humber Village | 1:44 | 5 | 0.9 | 0 | 0 | 17 |
| 18-Jun-14 | Sewage Lagoon | 23:00 | 2 | 0.6 | 0 | 3 | 19 |
| 18-Jun-14 | South Brook | 1:06 | 2 | 1.0 | 0 | 3 | 16 |
| 18-Jun-14 | Steady Brook | 2:02 | 5 | 0.0 | 0 | 2 | 17 |
| 23-Jun-14 | Abel | 23:00 | 0 | 2.0 | 1 | 2 | 14 |
| 23-Jun-14 | Bottom of Dump Hill | 1:13 | 0 | 2.6 | 1 | 1 | 12 |
| 23-Jun-14 | Gillams | 2:04 | 0 | 1.5 | 0 | 0 | 14 |
| 23-Jun-14 | Hughes Brook | 1:40 | 0 | 0.0 | 0 | 1 | 14 |
| 23-Jun-14 | Parson's Pond | 23:28 | 0 | 0.4 | 0 | 3 | 14 |
| 23-Jun-14 | Tippings Lake | 23:48 | 0 | 1.8 | 1 | 2 | 12 |
| 29-Jun-14 | 800m from 461 | 23:43 | 0 | 1.4 | 0 | 0 | 14 |
| 29-Jun-14 | Black Duck Siding | 23:30 | 0 | 0.8 | 0 | 2 | 15 |
| 29-Jun-14 | CONA Pond | 1:03 | 0 | 0.6 | 0 | 0 | 16 |
| 29-Jun-14 | First Pond | 22:51 | 1 | 0.4 | 0 | 1 | 18 |
| 29-Jun-14 | Goose Pond | 22:17 | 1 | 3.7 | 1 | 1 | 20 |
| 29-Jun-14 | JNC 460/490 | 0:49 | 0 | 1.3 | 0 | 0 | 14 |
| 29-Jun-14 | JNC 461 West | 23:55 | 0 | 0.5 | 0 | 1 | 16 |
| 29-Jun-14 | Long Gull Pond | 0:08 | 0 | 0.8 | 0 | 1 | 15 |
| 29-Jun-14 | Ned's Pond | 1:19 | 0 | 1.1 | 0 | 0 | 12 |
| 29-Jun-14 | Noels Pond | 0:21 | 0 | 1.5 | 0 | 0 | 14 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 29-Jun-14 | Stephenville Warning Lights | 0:36 | 0 | 0.4 | 0 | 0 | 16 |
| 29-Jun-14 | TCH 1 | 23:04 | 1 | 0.0 | 0 | 1 | 16 |
| 29-Jun-14 | TCH 2 | 22:31 | 1 | 2.0 | 1 | 3 | 19 |
| 29-Jun-14 | TCH 3 | 22:05 | 1 | 2.9 | 1 | 2 | 19 |
| 30-Jun-14 | Deer Lake Airport | 22:00 | 2 | 0.0 | 0 | 2 | 22 |
| 30-Jun-14 | Insectarium | 22:32 | 2 | 0.0 | 0 | 2 | 23 |
| 30-Jun-14 | Marble Mtn. Pond | 0:55 | 2 | 1.8 | 1 | 2 | 20 |
| 30-Jun-14 | McIvers | 2:22 | 2 | 4.7 | 1 | 0 | 20 |
| 30-Jun-14 | Pasadena Beach | 23:32 | 2 | 0.0 | 0 | 1 | 18 |
| 30-Jun-14 | Pynn's Brook | 23:10 | 2 | 1.8 | 1 | 3 | 19 |
| 30-Jun-14 | Rapid Pond | 0:10 | 2 | 0.9 | 0 | 1 | 23 |
| 30-Jun-14 | Town of hughes brook | 1:30 | 2 | 0.4 | 0 | 0 | 19 |
| 1-Jul-14 | Blue Pond | 22:07 | 1 | 2.4 | 1 | 1 | 21 |
| 1-Jul-14 | CB Ring Road | 0:16 | 2 | 6.5 | 2 | 2 | 20 |
| 1-Jul-14 | Dozer Pond 2 | 21:51 | 1 | 9.0 | 2 | 2 | 21 |
| 1-Jul-14 | Ducks unlim. Marsh | 23:58 | 2 | 3.4 | 1 | 1 | 20 |
| 1-Jul-14 | Link Pond | 23:41 | 2 | 6.4 | 2 | 3 | 20 |
| 1-Jul-14 | Near Watson's Pond | 23:03 | 2 | 6.8 | 2 | 3 | 20 |
| 1-Jul-14 | Pinchgut | 22:20 | 2 | 5.0 | 2 | 1 | 21 |
| 1-Jul-14 | SWGC Fen | 0:30 | 2 | 0.6 | 0 | 1 | 20 |

Table 5.2-9. Visual Encounter Surveys for Mink Frogs, Green Frogs, Damselflies and Dragonflies. 95 Surveys were conducted along the Pond Margins of 77 Ponds in Western Newfoundland between July 9 - 31, 2014.

| **Transect ID** | **Date** | **Region** | **Longitude (UTM 21 E, m)** | **Latitude (UTM21 N, m)** | **Total MF Density (Frogs/m)** | **Total GF Density (Frogs/m)** | **Damselfly Density (Individuals/m)** | **Dragonfly Density (Individuals/m)** |
|---|---|---|---|---|---|---|---|---|
| 1 | 12-Jul-14 | Stephenville | 394316 | 5378760 | 0.000 | 0.033 | 0.200 | 0.067 |
| 2 | 12-Jul-14 | Stephenville | 394316 | 5378760 | 0.000 | 0.067 | 0.033 | 0.133 |
| 3 | 12-Jul-14 | Stephenville | 394316 | 5378760 | 0.000 | 0.000 | 0.033 | 0.000 |
| 4 | 12-Jul-14 | Stephenville | 394444 | 5378694 | 0.000 | 0.100 | 0.100 | 0.033 |
| 5 | 12-Jul-14 | Stephenville | 393709 | 5378791 | 0.000 | 0.000 | 0.300 | 0.033 |
| 6 | 12-Jul-14 | Stephenville | 393709 | 5378791 | 0.000 | 0.033 | 0.233 | 0.067 |
| 7 | 12-Jul-14 | Stephenville | 393845 | 5378886 | 0.000 | 0.000 | 0.233 | 0.533 |
| 8 | 12-Jul-14 | Stephenville | 393845 | 5378861 | 0.000 | 0.000 | 0.133 | 0.200 |
| 9 | 12-Jul-14 | Stephenville | 393596 | 5378831 | 0.000 | 0.000 | 0.267 | 0.433 |
| 10 | 12-Jul-14 | Stephenville | 393596 | 5378831 | 0.000 | 0.033 | 0.233 | 0.600 |
| 11 | 12-Jul-14 | Stephenville | 393497 | 5361785 | 0.000 | 0.000 | 0.200 | 0.333 |
| 12 | 12-Jul-14 | Stephenville | 393497 | 5361785 | 0.000 | 0.067 | 0.033 | 0.567 |
| 13 | 12-Jul-14 | Stephenville | 406983 | 5379639 | 0.000 | 0.037 | 0.185 | 0.074 |
| 14 | 12-Jul-14 | Stephenville | 407073 | 5379542 | 0.000 | 0.000 | 0.467 | 0.133 |
| 15 | 12-Jul-14 | Stephenville | 407073 | 5379542 | 0.000 | 0.000 | 0.533 | 0.100 |
| 16 | 12-Jul-14 | Stephenville | 406966 | 5379516 | 0.000 | 0.000 | 0.300 | 0.067 |
| 17 | 12-Jul-14 | Stephenville | 391324 | 5360214 | 0.000 | 0.000 | 0.350 | 0.000 |
| 18 | 12-Jul-14 | Stephenville | 391324 | 5360214 | 0.000 | 0.000 | 0.000 | 0.200 |
| 19 | 12-Jul-14 | Stephenville | 393667 | 5364039 | 0.000 | 0.267 | 0.500 | 0.267 |
| 20 | 12-Jul-14 | Stephenville | 393667 | 5364039 | 0.000 | 0.000 | 0.433 | 0.167 |
| 21 | 14-Jul-14 | Pynn's Brook | 462778 | 5431789 | 0.000 | 0.000 | 0.600 | 0.167 |
| 22 | 14-Jul-14 | Pynn's Brook | 462778 | 5431759 | 0.000 | 0.000 | 0.333 | 0.300 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 23 | 14-Jul-14 | Pynn's Brook | 464667 | 5429893 | 0.000 | 0.000 | 0.600 | 0.067 |
| 24 | 14-Jul-14 | Pynn's Brook | 467712 | 5430170 | 0.000 | 0.000 | 0.500 | 0.100 |
| 25 | 14-Jul-14 | Pynn's Brook | 465218 | 5429983 | 0.000 | 0.033 | 0.333 | 0.100 |
| 26 | 14-Jul-14 | Pynn's Brook | 463042 | 5431189 | 0.000 | 0.067 | 0.900 | 0.067 |
| 27 | 14-Jul-14 | Pynn's Brook | 462883 | 5431989 | 0.000 | 0.000 | 0.500 | 0.067 |
| 28 | 14-Jul-14 | Pynn's Brook | 463116 | 5432330 | 0.000 | 0.000 | 0.800 | 0.033 |
| 29 | 14-Jul-14 | Pynn's Brook | 463086 | 5432215 | 0.000 | 0.000 | 0.067 | 0.000 |
| 30 | 15-Jul-14 | Pynn's Brook | 467021 | 5435074 | 0.000 | 0.000 | 1.033 | 0.267 |
| 31 | 15-Jul-14 | Pynn's Brook | 467021 | 5435074 | 0.000 | 0.000 | 0.867 | 0.033 |
| 32 | 15-Jul-14 | Pynn's Brook | 465797 | 5435686 | 0.000 | 0.056 | 0.944 | 0.278 |
| 33 | 15-Jul-14 | Pynn's Brook | 462510 | 5430912 | 0.000 | 0.000 | 1.300 | 0.367 |
| 34 | 15-Jul-14 | Little Rapids | 448780 | 5427546 | 0.000 | 0.000 | 0.900 | 0.267 |
| 35 | 18-Jul-14 | Corner Brook | 434453 | 5413512 | 0.000 | 0.000 | 0.200 | 0.067 |
| 36 | 18-Jul-14 | Corner Brook | 432626 | 5410994 | 0.133 | 0.000 | 0.033 | 0.000 |
| 37 | 18-Jul-14 | Corner Brook | 432626 | 5410994 | 0.267 | 0.000 | 0.500 | 0.100 |
| 38 | 18-Jul-14 | Corner Brook | 432626 | 5410994 | 0.033 | 0.000 | 0.133 | 0.000 |
| 39 | 18-Jul-14 | Corner Brook | 432084 | 5409033 | 0.533 | 0.000 | 0.000 | 0.000 |
| 40 | 18-Jul-14 | Corner Brook | 432084 | 5409033 | 0.800 | 0.000 | 0.800 | 0.033 |
| 41 | 18-Jul-14 | Corner Brook | 432873 | 5410134 | 0.000 | 0.000 | 0.200 | 0.067 |
| 42 | 21-Jul-14 | Corner Brook | 435162 | 5415454 | 0.100 | 0.000 | 0.000 | 0.033 |
| 43 | 21-Jul-14 | Corner Brook | 435162 | 5415454 | 0.033 | 0.000 | 0.167 | 0.067 |
| 44 | 21-Jul-14 | Corner Brook | 435309 | 5415494 | 0.733 | 0.000 | 0.167 | 0.333 |
| 45 | 21-Jul-14 | Corner Brook | 435224 | 5411034 | 0.200 | 0.000 | 0.033 | 0.000 |
| 46 | 21-Jul-14 | Corner Brook | 435224 | 5411034 | 0.100 | 0.000 | 0.100 | 0.033 |
| 47 | 22-Jul-14 | Corner Brook | 433612 | 5410161 | 0.067 | 0.000 | 0.067 | 0.033 |
| 48 | 21-Jul-14 | Corner Brook | 433750 | 5417199 | 0.233 | 0.000 | 0.233 | 0.000 |
| 49 | 21-Jul-14 | Corner Brook | 433576 | 5417001 | 1.367 | 0.000 | 0.067 | 0.033 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 50 | 21-Jul-14 | Corner Brook | 435668 | 5418856 | 0.600 | 0.000 | 0.033 | 0.133 |
| 51 | 22-Jul-14 | Corner Brook | 433689 | 5410447 | 0.033 | 0.000 | 0.033 | 0.100 |
| 52 | 22-Jul-14 | Corner Brook | 432279 | 5409120 | 0.800 | 0.000 | 0.167 | 0.267 |
| 53 | 22-Jul-14 | Corner Brook | 431587 | 5411382 | 0.567 | 0.000 | 0.033 | 0.000 |
| 54 | 22-Jul-14 | Corner Brook | 431558 | 5411448 | 0.033 | 0.000 | 0.200 | 0.000 |
| 55 | 22-Jul-14 | Corner Brook | 431169 | 5408844 | 0.400 | 0.000 | 0.300 | 0.333 |
| 56 | 22-Jul-14 | Corner Brook | 434370 | 5420882 | 0.067 | 0.000 | 0.267 | 0.267 |
| 57 | 23-Jul-14 | Corner Brook | 430346 | 5412370 | 0.067 | 0.000 | 0.067 | 0.300 |
| 58 | 23-Jul-14 | Corner Brook | 428345 | 5411895 | 0.000 | 0.000 | 0.067 | 0.100 |
| 59 | 23-Jul-14 | Corner Brook | 422361 | 5410465 | 0.000 | 0.000 | 0.033 | 0.067 |
| 60 | 23-Jul-14 | Corner Brook | 420636 | 5410723 | 0.067 | 0.000 | 0.033 | 0.600 |
| 61 | 23-Jul-14 | Corner Brook | 420884 | 5412606 | 0.000 | 0.000 | 0.333 | 0.167 |
| 62 | 23-Jul-14 | Corner Brook | 422173 | 5415946 | 0.000 | 0.000 | 1.233 | 0.033 |
| 63 | 26-Jul-14 | Corner Brook | 430309 | 5419793 | 0.267 | 0.000 | 0.067 | 0.100 |
| 64 | 26-Jul-14 | Corner Brook | 428803 | 5419966 | 0.100 | 0.000 | 0.300 | 0.150 |
| 65 | 26-Jul-14 | Corner Brook | 428662 | 5419936 | 0.267 | 0.000 | 0.067 | 0.400 |
| 66 | 26-Jul-14 | Corner Brook | 434219 | 5417758 | 0.067 | 0.000 | 0.433 | 0.200 |
| 67 | 26-Jul-14 | Corner Brook | 430840 | 5412127 | 0.320 | 0.000 | 0.400 | 0.440 |
| 68 | 26-Jul-14 | Corner Brook | 430571 | 5411710 | 0.400 | 0.000 | 0.200 | 0.300 |
| 69 | 26-Jul-14 | Corner Brook | 425542 | 5405828 | 0.160 | 0.000 | 0.160 | 0.080 |
| 70 | 26-Jul-14 | Corner Brook | 426092 | 5402944 | 0.300 | 0.000 | 1.067 | 0.000 |
| 71 | 26-Jul-14 | Corner Brook | 426577 | 5399298 | 0.567 | 0.000 | 0.000 | 0.100 |
| 72 | 26-Jul-14 | Corner Brook | 420307 | 5403786 | 0.000 | 0.000 | 0.000 | 0.000 |
| 73 | 26-Jul-14 | Corner Brook | 417721 | 5402061 | 0.233 | 0.000 | 0.300 | 0.067 |
| 74 | 27-Jul-14 | Corner Brook | 417246 | 5401123 | 0.267 | 0.000 | 0.300 | 0.067 |
| 75 | 27-Jul-14 | Corner Brook | 417242 | 5401103 | 0.667 | 0.000 | 0.000 | 0.000 |
| 76 | 27-Jul-14 | Corner Brook | 410543 | 5391914 | 0.033 | 0.000 | 0.133 | 0.000 |
| 77 | 28-Jul-14 | Corner Brook | 431821 | 5416643 | 0.000 | 0.000 | 0.033 | 0.133 |

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|---|---|---|---|---|---|---|---|---|
| 78 | 28-Jul-14 | Corner Brook | 430742 | 5412586 | 0.033 | 0.000 | 0.833 | 0.067 |
| 79 | 28-Jul-14 | Corner Brook | 416960 | 5411599 | 0.067 | 0.000 | 0.067 | 0.100 |
| 80 | 30-Jul-14 | Corner Brook | 438247 | 5406421 | 0.167 | 0.000 | 0.000 | 0.100 |
| 81 | 30-Jul-14 | Corner Brook | 437351 | 5403129 | 1.133 | 0.000 | 0.000 | 0.000 |
| 82 | 30-Jul-14 | Corner Brook | 433983 | 5399393 | 0.367 | 0.000 | 0.033 | 0.000 |
| 83 | 30-Jul-14 | Corner Brook | 434333 | 5398173 | 0.000 | 0.000 | 0.067 | 0.000 |
| 84 | 30-Jul-14 | Corner Brook | 418625 | 5411203 | 0.267 | 0.000 | 0.033 | 0.133 |
| 85 | 30-Jul-14 | Corner Brook | 428634 | 5412236 | 0.200 | 0.000 | 0.233 | 0.100 |
| 86 | 30-Jul-14 | Corner Brook | 433754 | 5417014 | 0.000 | 0.000 | 0.040 | 0.120 |
| 87 | 30-Jul-14 | Corner Brook | 434160 | 5417573 | 0.067 | 0.000 | 0.067 | 0.133 |
| 88 | 31-Jul-14 | Corner Brook | 436591 | 5428197 | 0.360 | 0.000 | 0.920 | 0.240 |
| 89 | 31-Jul-14 | Corner Brook | 443626 | 5431189 | 0.000 | 0.000 | 0.000 | 0.200 |
| 90 | 31-Jul-14 | Corner Brook | 440341 | 5429032 | 0.000 | 0.000 | 0.133 | 0.000 |
| 91 | 31-Jul-14 | Corner Brook | 438999 | 5422167 | 0.867 | 0.067 | 0.133 | 0.033 |
| 92 | 1-Aug-14 | Corner Brook | 420466 | 5416508 | 0.367 | 0.000 | 0.000 | 0.033 |
| 93 | 1-Aug-14 | Corner Brook | 418574 | 5419682 | 0.400 | 0.000 | 0.333 | 0.100 |
| 94 | 1-Aug-14 | Corner Brook | 418479 | 5421174 | 0.167 | 0.000 | 0.433 | 0.000 |
| 95 | 1-Aug-14 | Corner Brook | 418441 | 5424856 | 0.400 | 0.000 | 0.233 | 0.167 |