THE ECOLOGY, DISTRIBUTION AND POPULATION GENETICS OF AMPHIBIANS ON HAIDA GWAII, BRITISH COLUMBIA

by

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Abstract

Amphibian populations are declining worldwide due to many threats including habitat destruction, disease, non-native species and climate change, and are in dire need of informed conservation strategies. Island populations are particularly at risk. Invasive species are a major threat to amphibians on islands, along with habitat destruction and reduced genetic diversity in island populations. Additionally, species' ranges are naturally shifting due to climate change, bringing the definition of a non-native species into flux. In this thesis, I aimed to improve the understanding of population-level dynamics and provide insights for conservation of amphibians using tools from invasion biology, experimental ecology, population genomics and conservation biology. I focussed on two amphibians of conservation concern in British Columbia (BC), Canada - the Northern Red-legged Frog (*Rana aurora;* NRLF), introduced to the Haida Gwaii Archipelago but native to southwest BC, and the Western Toad (*Anaxyrus boreas boreas,* called Hlk'yáan K̲'ust'áan in the X̲aad Kíl dialect of the Haida language (X̲aad Kíl Née; Haida Language Council), native to both areas.

I modelled the distribution of the NRLF under current and future climate, conducted a mesocosm experiment examining the effect of co-occurrence history and naïveté on competition between the Western Toad and NRLF using populations from Haida Gwaii and southwest BC, and conducted genomic analyses of diversity and divergence of 27 Western Toad (hereafter "toad") populations on Haida Gwaii and southwest BC.

Together, my research suggests that: 1) the introduced NRLF has spread since its discovery on Haida Gwaii in 2002, and will likely spread further on Haida Gwaii, especially with climate change; 2) Haida Gwaii toad tadpoles are weaker competitors with the introduced NRLF compared to southwest BC toad tadpoles, likely due to their short history of co-occurrence (but it is unknown whether competition with NRLFs affects toad population persistence); and finally, 3) Haida Gwaii toad populations are genetically distinct and less diverse compared to southwest BC toad populations. The observed toad genetic patterns could be due to a historical population bottleneck or founder effect as well as contemporary small effective population size. This thesis provides knowledge that can be used to help conserve amphibians.

Lay Summary

Amphibian populations are declining worldwide due to threats including habitat loss, diseases, non-native species and climate change. They are in dire need of informed conservation strategies. In this dissertation, I provide insights to improve conservation efforts for two amphibians of conservation concern in British Columbia (BC), Canada - the Northern Red-legged Frog, introduced to the Haida Gwaii Archipelago but native to southwest BC, and the Western Toad, called Hlk’yáan K̲’ust’áan in the X̲aad Kíl Haida language (X̲aad Kíl Née), native to both areas. My research suggests: 1) the introduced frog has spread since its introduction on Haida Gwaii, and will likely spread further, assisted by climate change; 2) compared to southwest BC toads, Haida Gwaii toad tadpoles appear weaker competitors with introduced frogs; and 3) Haida Gwaii toad populations are genetically unique and less diverse compared to southwest BC populations, suggesting they should be a higher conservation priority.

Preface

Permission to use the language of the Haida Nation

Translations and permission to use the name for toad and place names in the X̱aad Kíl dialect of the Haida language was granted by X̱aad Kíl Née, the Haida Language Council for X̱aad Kíl.

Chapter 2: The current and potential distribution of the introduced Northern Red-legged Frog (*Rana aurora*) on Haida Gwaii

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Chapter 4: Western Toads on Canada's Haida Gwaii archipelago have low genetic diverse and are strongly differentiated from southwestern British Columbia populations

Authors: R Gamlen-Greene, J Grummer, M Todd, SN Aitken

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Dedication

For my brother Oliver Johnston, who was tragically stolen from us, June 2018.

You were mine to protect,

We were meant to grow old

Chapter 1: Introduction

1.1 Rationale

Amphibians are the most threatened class of vertebrates due to drivers such as diseases, non-native or invasive species, climate change, habitat fragmentation, urbanisation, agriculture and pollution (Wake 1991, Alford and Richards 1999, Houlahan et al. 2000, Stuart et al. 2004). Over 30% of the 7296 recorded amphibian species are experiencing population declines, with 35 species extinct and 146 possibly extinct, since records began in the 1900s (IUCN 2022). These species declines are of special concern because amphibians are indicators of environmental stress and are integral to the functioning of aquatic and riparian ecosystems (Burton and Likens 1975, Blaustein 1994, Blaustein and Wake 1995, Halliday 2008, Reinhardt et al. 2013, Hocking and Babbitt 2014).

Amphibian populations on islands, and endemic island species of any taxa, are especially threatened. Species extinctions on islands account for two-thirds of all recent extinctions (Tershy et al. 2015). Many of the worlds endangered amphibian species are endemic to islands, such as the Island Forest Frog (*Platymantis insulatus*) from the Gigante Islands in the Phillipines, Richmond's Coqui (*Eleutherodactylus richmondi*) from Puerto Rico, Kirthisinghe's Rock Frog (*Nannophrys marmorata*) from Sri Lanka, Charles Darwin's Frog (*Ingerana charlesdarwini*) from the Andaman Islands in India, and the Archey's Frog (*Leiopelma archeyi*) from Aotearoa (New Zealand). Some of the main drivers of biodiversity loss on islands are invasive species, habitat destruction and climate change (Tershy et al. 2015, Russell and Kueffer 2019). Part of what makes island populations particularly vulnerable to these drivers is: smaller population sizes; lower genetic diversity; less physical space; and a lack experience with certain functional groups (Blumstein and Daniel 2005). For instance, New Zealand's fauna evolved without land mammals, apart from two species of bats (Daniel 1979), and as a result, numerous species there lack adaptations to deal with non-native predators.

To date, amphibian conservation has largely been unsuccessful due to the multitude of interacting extinction drivers facing amphibians, combined with their sensitivity to environmental conditions. Conservation actions for amphibians have included a number of strategies. For instance, there have been, and continue to be, many attempts at developing treatments for, and controlling, chytrid fungus (*Batrachochytrium dendrobatidis*); a fungal pathogen that is responsible for extensive amphibian population declines worldwide (Berger et al. 1998, Daszak et al. 2003, Skerratt et al. 2007). Additionally, other actions have included: captive breeding programs to supplement declining populations, aquatic habitat restoration, changing species designations to threatened or endangered, and trying to eliminate non-native predators (Gascon 2007, Woodhams et al. 2007, 2012).

Exacerbating and sometimes amplifying the other drivers of decline in amphibians, climate change is an urgent issue facing amphibians (López-Alcaide and Macip-Ríos 2011). Because many amphibians have limited thermal plasticity, acclimation and flexibility in phenology, many do not have the capacity to adapt to climate change (e.g., Gerick et al. 2014). Hence, assisted migration (moving species to a cooler, similar habitat, often in a poleward direction), has been proposed as a last resort to save species facing climate change (Loss et al. 2011, Lunt et al. 2013, Gallagher et al. 2015). Considering the rich history of intentional and accidental species introductions and their deleterious impacts, such relocation programs are highly controversial and must be approached with extreme caution (Ricciardi and Simberloff 2009, Hewitt et al. 2011). Additionally, the ranges of many species have already shifted in response to climate change in the absence of human assistance (Thomas 2010, Chen et al. 2011, Sunday et al. 2012), bringing into question the definition of a 'non-native' species (Parmesan 2006, Chen et al. 2011, Urban 2020). We still do not understand how such within-region movements (whether human assisted or climate-induced) will play out, both in terms of their success for species persistence, and also their potential impact on recipient ecosystems.

These issues raise numerous questions such as: **Question 1 --** how will species perform in novel environments as their ranges shift?; **Question 2** -- what effect will these range-shifting species have in recipient communities that have a similar, but not identical eco-evolutionary history?; **Question 3 --** what influences the degree of vulnerability of species to global change drivers?; and finally, **Question 4 --** how can we use this information to make conservation decisions for amphibians in a dynamic world?

In this thesis, I aim to improve understanding of population-level dynamics and provide insights for conservation using a multitude of tools from invasion biology, experimental ecology, population genomics and conservation biology. I focus on two amphibians of conservation concern in British Columbia (BC), Canada - the Northern Red-legged Frog (*Rana aurora;* NRLF), introduced to the Haida Gwaii Archipelago but native to southwest BC, and the Western Toad (*Anaxyrus boreas boreas,* called Hlk'yáan Ḵ'ust'áan in the X̱aad Kíl dialect of the Haida language (X̱aad Kíl Née; Haida Language Council), native to both areas. In Chapter 2, I model the range expansion of the introduced NRLF on Haida Gwaii under current and future climate (Q1), in Chapter 3, I use a mesocosm experiment to examine the effect of co-occurrence history and naïveté on competition between Western Toad and the NRLF (Q2, Q3), and in Chapter 4, I use genomic analyses to examine the genetic diversity and structure of 27 Western Toad populations on Haida Gwaii and southwest BC (Q4).

1.2 Invasive species: their impact and success

Invasive species are one of the leading causes of unprecedented rates of biodiversity loss worldwide, and have especially negative impacts on amphibians (Sala et al. 2000, Clavero and Garcia-Berthou 2005, Butchart et al. 2010, McGeoch et al. 2010, Pyšek and Richardson 2010). In addition, they are one of the main drivers of biodiversity loss on islands (Bellard et al. 2016, Doherty et al. 2016, Duenas et al. 2021). Invasive species have wide-ranging impacts from causing individual species extinctions, to reducing ecosystem functioning to altering community structure and assembly processes, thereby influencing long-term community stability and persistence of populations (Sanders et al. 2003, Clavero and Garcia-Berthou 2005, McGeoch et al. 2010). The number and distribution of invasive species is predicted to rise as species' ranges shift in response to climate change, human movement and habitat modifications, (Dukes and Mooney 1999, Kolar and Lodge 2001, Hellmann et al. 2008, Moran and Alexander 2014).

It is important to note here the distinction between non-native or alien, invasive, and introduced species. Non-native or alien is a species that is a recent addition and does not have a long history in the new range; invasive means the species is non-native and has the tendency to spread as well as to negatively impact humans or the environment; and an introduced species is one that humans have moved from its native range to a non-native range (Richardson et al. 2000). A species can be non-native without being invasive or introduced if it is moved there naturally and does not negatively affect the native community (Rodewald and Arcese 2016). An introduced species is always non-native but may not be invasive, making this distinction especially important for predicting, managing or assisting climate range shifts or translocations. Compared to the number of introductions, only a fraction become “invasive”. Many non-native species have a negligible effect on recipient ecosystems, and although rare, a small number of non-native species even have a positive impact on native species (Wonham et al. 2005).

Invasive species threaten amphibians through predation, competition, disease introduction and spread, and hybridization between non-native and native amphibians (Bucciarelli et al. 2014). Many studies have shown how an introduced predator can reduce amphibian abundance, for instance introduced crayfish predator decreased the abundance of native amphibian larvae (Francesco Ficetola et al. 2011). A number of studies have demonstrated non-native taxa impacting amphibian populations via competition, e.g., competition by a non-native fish decreased salamander growth and survival (Pearson and Goater 2009). However, among the many factors impacting amphibians, the effects of competition from a non-native amphibian species on resident amphibians is comparatively understudied (see section 1.3). This is in part because the effects of competitive interactions on population persistence are indirect and can be more difficult to detect (Gilbert and Levine 2013). Factors that may influence the success of non-native amphibians are discussed in the following sections, and factors that may make native island amphibian species more vulnerable to the effects of non-native species are discussed in section 1.3.

1.2.1 Amphibians as invasive species

Although many amphibians are impacted by invasive species, amphibians themselves can also be invasive. Despite amphibians requiring specific habitats for breeding and being sensitive to environmental changes because of their permeable skin (Wilbur 1987, Toledo and Jared 1993), some 40 anuran (frogs and toads) species have shown remarkable success as invaders and have been able to maintain stable populations (Kark et al. 2009, Invasive Species Specialist Group 2014). The three most pervasive amphibian invaders (who all also make the top 100 most invasive species list) are the Cane Toad *Rhinella marina*, the Caribbean Tree Frog *Eleutherodactylus coqui*, and the American Bullfrog *Lithobates catesbeianus* (Clarke et al. 2001, Lowe et al. 2004, Pearl et al. 2004, Beard et al. 2008). These species have been so successful because of their toxicity and dispersal abilities (Cane Toad), large gape (i.e., they can fit large things in their mouths relative to their body size), and generalist feeding strategy (American Bullfrog) or lack of larval life stage (Caribbean Tree Frog). Alien amphibians cause declines in the native recipient amphibian communities via mechanisms such as hybridizing with, preying upon, and poisoning and spreading chytrid fungus to native taxa as well as by competitively displacing native species from their optimum niches (Kiesecker and Blaustein 1998, Daszak et al. 2004, Walston and Mullin 2007, Ryan et al. 2009, Kraus 2015).

1.2.2 Success of non-native species

The success of a non-native species in its new environment depends on biotic and abiotic factors (Kolar and Lodge 2001, Colautti et al. 2004, Tilman 2004, Britton-Simmons and Abbott 2008, Godoy and Levine 2014). Biotic factors include fecundity, generation time, dispersal ability, predator avoidance strategies, breeding habitat flexibility, feeding strategies, phenology differences between invader and recipient community, genetic diversity of the invader (and therefore its capacity to evolve to the new environment), and the release from predators and pathogens (i.e., enemy release hypothesis). Abiotic factors include habitat availability and disturbance regime.

The capacity of a non-native species to evolve traits to match the new environment has been observed. For instance, the Cane Toad in its non-native range was able to rapidly evolve longer legs to aid dispersal (Phillips et al. 2006). Similar processes have been observed in plant invasions, whereby individuals at the invasion front had better dispersal whereas the individuals at the back had greater competitive abilities (Williams et al. 2016).

Such evolution or the release from constraints in the native range can lead to non-native species shifting or expanding their niche in the novel environment. Species may conserve, expand, shift or occupy just a subset of their niche (Weins et al. 2010, Guisan et al. 2014). Non-native species that have expanded or shifted their niche, or occupy only a subset of their potential niche ("niche unfilling") are more likely to increase their geographic distribution in the future (Guisan et al. 2014, Davies et al. 2019). Niche shifts or expansions are more common in recent introductions, small native range sizes, and lower-latitude introductions, and have been frequently observed during island invasions (Li et al. 2014, Tingley et al. 2014). Furthermore, understanding the degree of similarity between the native and non-native niche can advance understanding on the mechanisms of invasion success such as enemy release, rapid evolution, and phenotypic plasticity (Lee 2002, Sax et al. 2007, McCann et al. 2014).

It is also important to note that the pervasiveness of an invader can change over time due to frequency independent and dependent processes regulating population size. For example, it is common for invasive populations to boom and bust over time. After the initial boom, factors such as decreasing resource abundance, low genetic diversity, and disease can lead to a "bust" (Strayer et al. 2017). Additionally, there is often a lag time between when the non-native species arrives and is established and at equilibrium in the landscape (Williamson and Fitter 1996, Essl et al. 2015). It also often takes multiple introductions before species establish, sometimes due to lack of genetic diversity of the non-native species (Sakai et al. 2001, Dlugosch and Parker 2008).

Together, these factors make it difficult to predict in advance whether a species will be successful as an invader or not. However, as we build our understanding of the mechanisms and traits leading to invasiveness as well as how recipient ecosystems respond, we also increase our ability to make successful predictions. Ways to build this understanding include studying niche shifts and expansions using species distribution modelling.

1.2.3 Species distribution modelling

Species distribution models (SDMs) – or ecological niche models (ENM) – are approaches used to model habitat suitability for non-native species (Jeschke and Strayer 2008). Essentially, SDMs and ENMs correlate species presence, or presence and absence, data points in geographic space with environmental conditions. There is a plethora of different approaches and algorithms, all with their strengths and limitations. ENMs and SDMs use similar methodologies but the difference lies mainly in how they are applied; ENMs focus on understanding the conditions that allow persistence of a species, whereas SDMs focus on predicting the geographic distribution of a species (Peterson and Soberón 2012, Peterson et al. 2015). In my dissertation, I will refer to SDMs, but some parts of my research could just as easily be called ENMs. There are two main types of SDMs: correlative models and mechanistic/process-based (Peterson et al. 2015). Correlative SDMs are trained with species occurrence data in geographic space and environmental variables associated with these points, which are then used to identify potential areas of high habitat suitability (Elith et al. 2010). Mechanistic or process-based SDMs use detailed bio-physical modelling, incorporating physiology, dispersal and biotic interactions, to understand the environmental requirements that make up the fundamental niche of a species, and the processes leading to a species occupying a certain area (Kearney and Porter 2009, Dormann et al. 2012, Peterson et al. 2015).

SDMs were originally designed to model the fundamental niche – a multi-dimensional hyper-volume that represents all conditions and resources required for species persistence (Hutchinson 1957, Colwell and Rangel 2009), but in reality, SDMs model the realized niche (Pulliam 2000, Wiens and Graham 2005, Guisan et al. 2014). The realized niche is a subset of the fundamental niche and represents where the species actually occurs, and depends upon the abiotic conditions required to support positive population growth of a species, and is constrained by biotic processes such as predation, disease, competition and dispersal (Hutchinson 1957). In this dissertation, whenever I mention the niche, I am referring to this definition of the realized niche.

1.3 Naïve species' response to invasive and non-native species

Understanding what makes species, and in particular, island species, vulnerable to the effects of invasive species is a key concern for biodiversity conservation (Bellard et al. 2016, Doherty et al. 2016). Despite the large body of research focusing on invasive species and species range shifts, conservation biologists still lack the ability to predict the ecological impact of non-native species on recipient amphibians and their associated communities. In the face of unprecedented non-native species range shifts, it is crucial that we understand when amphibians may be more likely to be negatively impacted by an alien species.

The degree of impact of non-native species on the recipient ecosystem depends not only on traits of the non-native (as discussed in section 1.2.3), but also how naïve species (i.e., species with no prior exposure) respond to them. Whether naïve native species are able to co-exist (i.e., survive indefinitely – and experience population growth when in lower densities than the other species) with the non-native species can depend on many factors. For example, population size, connectivity between populations, reproductive strategy, niche breadth, generalist versus specialist feeding strategies, niche overlap with the invader, levels of genetic diversity (because variation is necessary for evolution), generation time, phenotypic plasticity (non-inherited traits that are developed in response to environments), and experience with functionally similar organisms all influence how naïve species respond to non-native species (Strauss et al. 2006).

In some cases, naïve species can evolve or adapt plastically in response to the non-native, limiting the harm of the alien in the community (Strauss et al. 2006). For instance, the saplings of red cedar (*Thuja plicata*) on Haida Gwaii, BC changed their anti-herbivore compounds in response to browsing from non-native black-tailed deer (*Odocoileus hemionus sitkensis*), experiencing less browsing as a result (Vourc'h et al. 2001). Additionally, several native molluscs have evolved thicker shells to avoid predation by non-native crabs (Vermeij 1982). However in many cases, the naïve species does not evolve or adapt plastically to the invader because individuals fail to recognise and respond appropriately to a novel enemy or competitor – this lack of appropriate response is termed "naïveté" (Diamond and Case 1986, Cox and Lima 2006). For instance, Wilson & Holway (2010) found that naïve native bees on Hawaii showed competitive naïveté as they were displaced by competitively superior invasive wasps through reduced prey availability. Naïveté has been demonstrated in many interactions, including predator-prey, plant-herbivore, and competitive interactions, and can occur for several reasons such as the lack of genetic variation or life-history traits that would enable plasticity or rapid evolution (Strauss et al. 2006, Carthey and Banks 2014).

1.3.1 Competition in amphibians

Species invasions can result in competition between non-native and native amphibians. For instance, the addition of an alien amphibian competitor *Discoglossus pictus* resulted in decreased growth and survival, and changed the phenology of two native amphibians; *Bufo calamita* and *Pelodytes punctatus* (Richter-Boix et al. 2013). Although competition between non-natives and natives does not always have negative impacts on population persistence, native anuran competitors have shown character displacement in response to non-native amphibian competitors (Blair 1974, Lemmon 2009, Pfennig and Martin 2009). Because most anuran larvae are trophic generalists, feeding on algae, detritus and macrophytes irrespective of phylogeny (Kupferberg et al. 1994, Petranka and Kennedy 1999), competition between different species of native amphibian larvae is common-place (e.g., Wilbur (1982) and Werner (1992)). Competition at the larval life stage can impact adult survival (Berven and Gill 1983, Smith 1987, Berven 1990, Pechenik 2006, Cabrera-Guzmán et al. 2013), and is the easiest life stage to study competition experimentally and is hence what I have focussed on.

To the best of my knowledge, no one has investigated whether the introduction of an alien amphibian results in strong competitive interactions with an isolated naïve populations of a native amphibian species compared to experienced populations in sympatric portions of the species' ranges. We know that amphibians are sensitive to whether their recent life history included history with an alien predator (e.g., Kiesecker and Blaustein 1997), but the question remains whether co-occurrence history influences the strength of competition and degree of naïveté towards novel competitors.

1.4 Population genetics as a tool for amphibian conservation

Understanding population-level patterns in genetic diversity and differentiation across a species' range is important for amphibian conservation (Beebee 2005, McCartney-Melstad and Shaffer 2015, Shaffer et al. 2015, Funk et al. 2018). Studying the genetics and genomics of populations has informed conservation by assessing contemporary and historic connectivity among populations, detecting population responses to natural and anthropogenic impacts, inferring demography, estimating inbreeding and effective population size, identifying traits under selection and their associated genes, and assessing the resilience of populations to climate change (Moritz 2002, Allendorf et al. 2022). The study of genetics has been used to inform conservation for the past 50 years (Soule 1980).

A number of studies have used genetic markers to investigate various drivers of biodiversity declines, such as habitat degradation. For example, Dixo et al. (2009) detected genetic changes to forest fragmentation in Brazil in the form of reduced genetic diversity of a toad (*Rhinella ornate*) – likely due to declines in population size. Funk et al. (2005) found that a frog (*Rana luteiventris*) was particularly vulnerable to habitat fragmentation in the future because of high population connectivity and dispersal. Cummins et al. (2019) used genetic data to conclude that local populations of a frog (*Pseudophryne guentheri*) were adapting in response to climate changes. Although there are ongoing challenges with translating genetic information into practice (Taylor et al. 2017, Luikart et al. 2018, Holderegger et al. 2019), genetic data has been used to change the conservation status of species', inform genetic rescue strategies, and modify management practices (Bell et al. 2019, Allendorf et al. 2022).

Conserving genetic diversity within populations and species is important for maintaining the capacity to adapt to changing conditions, and avoiding inbreeding depression and the accumulation of deleterious alleles (Hoban et al. 2020, Allendorf et al. 2022). Genetic diversity is the raw material required for evolution. Endangered populations of birds, mammals, amphibians, reptiles and fishes have been found to have lower genetic diversity than comparatively common species (Li et al. 2016). Additionally, many island populations have lower genetic diversity, due to small effective population sizes, historic population bottlenecks or founder effects, all of which reduce diversity through increased genetic drift (Frankham 1997, Allendorf et al. 2022). Furthermore, another threat facing populations is inbreeding, which can have pronounced effects on fitness, and can result in inbreeding depression (Hedrick and Kalinowski 2000, Allendorf et al. 2022); the reduced survival and fertility of highly related offspring due to the expression of deleterious recessive alleles (Allendorf et al. 2022). Inbreeding can occur either because relatives are mating (in small or large populations), or because in small populations, relatedness will increase even with random mating. Increases in the frequency of or fixation of deleterious alleles as a result of genetic drift or inbreeding can result in local population declines through mechanisms such as decreased sperm quality, female fecundity, and juvenile survival, and increased susceptibility to parasites and disease (Ralls et al. 1988, Coltman et al. 1999, Hedrick and Fredrickson 2010). The detrimental effects of inbreeding depression on population demography have been observed in many small wild and captive populations, including in amphibians (Lacy 1993, Yuan et al. 2019), and is often higher in island populations (Frankham 1998).

1.4.1 Methods in conservation genetics

In conservation genetics, genetic markers that are assumed to be selectively neutral, i.e., not affecting the fitness of individuals, are (1) used to assess population structure and the differentiation of populations in order to indirectly assess population connectivity and gene flow; and (2) used to estimate levels of genetic diversity and inbreeding within populations (Holderegger et al. 2006).

To describe population structure and the level of differentiation among populations, Wright’s F-statistics and several clustering methods are commonly used. F_{ST} is a measure of genetic differentiation between populations ranging from 0 – identical allele frequencies in two populations and no among-population variation to 1 – all loci fixed for alternate alleles in two populations. F_{ST} was originally developed by Wright (1950), but modified by Weir and Cockerham (1984) to deal with variable sample size and low levels of genetic differentiation. Several approaches are available for clustering or assigning samples into populations including Principal Components Analysis (PCA), *STRUCTURE* (Pritchard et al. 2000), and Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010). *STRUCTURE* is a Bayesian clustering method that groups genetically similar individuals together into *K* clusters that minimize departures from Hardy-Weinberg equilibrium (HWE), and estimates membership of each individual in each clusters (Pritchard et al. 2000). *STRUCTURE* has been criticized because it relies on HWE (when many populations do not meet HWE) (Evanno et al. 2005, Gilbert 2016). DAPC is a multivariate analysis that partitions the variances to among- and within-cluster components in an effort to maximize differences among clusters. It can be used to select the number of clusters (using Bayesian information criterion) or run with a pre-determined number of clusters (i.e., population or sampling location). As different approaches can produce different results, it is recommended that multiple clustering methods are used (Janes et al. 2017, Miller et al. 2020).

Standard parameters for estimating genetic diversity within populations include expected heterozygosity (H_e) and nucleotide diversity (π). H_e is calculated from allele frequencies within a population using HWE expectations, assuming random mating. Nucleotide diversity (π) measures the total diversity across the sampled genome, i.e., the number of variant nucleotide sites in relation to the total number of sites sequenced. Sequence and marker data can be used to detect alternative demographic histories (e.g., population bottlenecks) leading to current levels of genetic diversity, but these methods have high uncertainty (Peery et al. 2012, Schraiber and Akey 2015). Inspection of the site frequency spectrum (SFS), the distribution of allele frequencies across all loci, the estimation of Tajima's D, and testing of past demographic scenarios with Approximate Bayesian Computation can all shed light on the demographic history of populations (Piry et al. 1999, Beaumont et al. 2002, Peery et al. 2012, Do et al. 2014).

F_{IS}, the inbreeding coefficient, is one of Wright's F-statistics, and is a common parameter used in population genetics to estimate inbreeding level from genetic markers. It is measured as the deviation of observed heterozygosity from heterozygosity levels expected under HWE (Allendorf et al. 2022).

1.4.2 Restriction-site-associated DNA sequencing (RADseq)

The most commonly used type of genetic marker at present is single nucleotide polymorphisms (SNPs). SNPs are variations in nucleotides at a single base pair in the DNA sequence and are typically bi-allelic. They are the most abundant type of polymorphism in the genome, with one occurring approximately every 200-500bp in many wild animal populations (Brumfield et al. 2003, Morin et al. 2004). As a result of these characteristics and the consistently dropping cost, the use of SNPs in conservation studies has increased rapidly and has largely replaced microsatellites (Allendorf et al. 2022).

Hundreds of thousands or even millions of SNPs can be genotyped through methods including whole genome sequencing, exome capture, or other types of targeted sequence capture, but these methods remain too costly for most conservation genetic studies (Allendorf et al. 2022). Restriction-site-associated DNA sequencing (RADseq) offers a much less resource intensive approach by first using restriction enzymes to digest DNA into fragments, and then amplifying and sequencing these fragments (Baird et al. 2008). Genotyping-by-sequencing (GBS) is a commonly used RADseq method, developed by Elshire et al. (2011) and Davey et al. (2011). Following digestions with restriction enzymes, barcoded adapters (4-9 bp) and common adapters are ligated onto fragments to identify DNA from different individuals (Davey et al. 2011). Samples are pooled and amplified with a polymerase chain reaction (PCR). Then samples are selected for fragment size and sequenced.

Sequences from different reads (DNA fragments around 100bp long) are then aligned to either a reference genome for the study species or a closely related species, or aligned *de novo*. A reference genome generally produces better results than a *de novo* assembly as it allows the user to identify SNPS that are sequencing errors, and discard closely linked SNPs on the same chromosome (Wright et al. 2019). After alignment, SNP genotypes are called for each sample, and then filtered for quality, coverage and other criteria including read depth, missing data, maximum observed heterozygosity, minor allele count, minor allele frequency, Hardy-Weinberg Equilibrium and F_{IS} using bioinformatic pipelines (O'Leary et al. 2018, Allendorf et al. 2022). There are many different possible criteria used for filtering SNPs, and these decisions in filtering can influence the population-level inferences from these data (Shafer et al. 2017).

The downside of GBS is that there is high level of missing data, as many SNPs will not have been sequenced across all individuals in a population (Elshire et al. 2011). Common sources of missing data in GBS include: 1) low read depth; 2) loci are not present in some samples due to variation in restriction sites; and 3) DNA not being successfully digested or amplified due to poor quality or technical error. The coverage issue can be partially mitigated by decreasing the number of samples run in each sequencing lane so that there is a greater sequencing effort per sample. Another solution is to impute genotypes (i.e., replace the missing data with estimated values using statistical methods) for those loci with only small amounts of missing data (Davey et al. 2011, Chan et al. 2016). RADseq data generally includes a reasonable sample of genetic variation across the genome, but produces datasets with far fewer analysable SNPs than whole genome or exome sequencing.

1.5 Study species

The Northern Red-legged Frog (*Rana aurora*) is part of the Ranidae or "True Frog" family (class Amphibia, order Anura), native to Canada and the US. The Western Toad (*Anaxyrus boreas*) is part of the Bufonidae or "True Toads" family (class Amphibia, order Anura), native to Canada, the US and Mexico. There are two subspecies of the Western Toad; *Anaxyrus boreas halophilus,* which ranges from California to northern Mexico and western Nevada, and *Anaxyrus boreas boreas* – located in Canada, and much of the US (from Alaska to northern California, and as far west as Colorado) (COSEWIC 2012). Within the Canadian distribution, there are currently two Designatable Units of the Western Toad under the Species at Risk Act – a calling and non-calling population. The calling populations are all in Alberta, where the males possess a vocal sac, whereas the non-calling populations have no vocal sac and are in the Yukon, Northwest Territories, BC and Alberta.

The global range of the NRLF spans the Pacific coast of North America, from southwestern British Columbia (BC), Canada down to northern California in the US, and is almost entirely sympatric within the substantially larger range of the Western Toad; Figure 1.1). Both species are federally listed as species of "Special Concern" under the Species At Risk Act in Canada, due to population declines within their ranges (COSEWIC 2012, 2015). In the province of BC, the NRLF is provincially 'Blue Listed – special concern', while the Western Toad is considered 'apparently secure – Yellow Listed' (COSEWIC 2012, 2015). The NRLF is declining in its native range due to factors such as habitat destruction, chytrid fungus, invasive predators such as the American Bullfrog (*Lithobates catesbeianus*) and climate change (COSEWIC 2015).

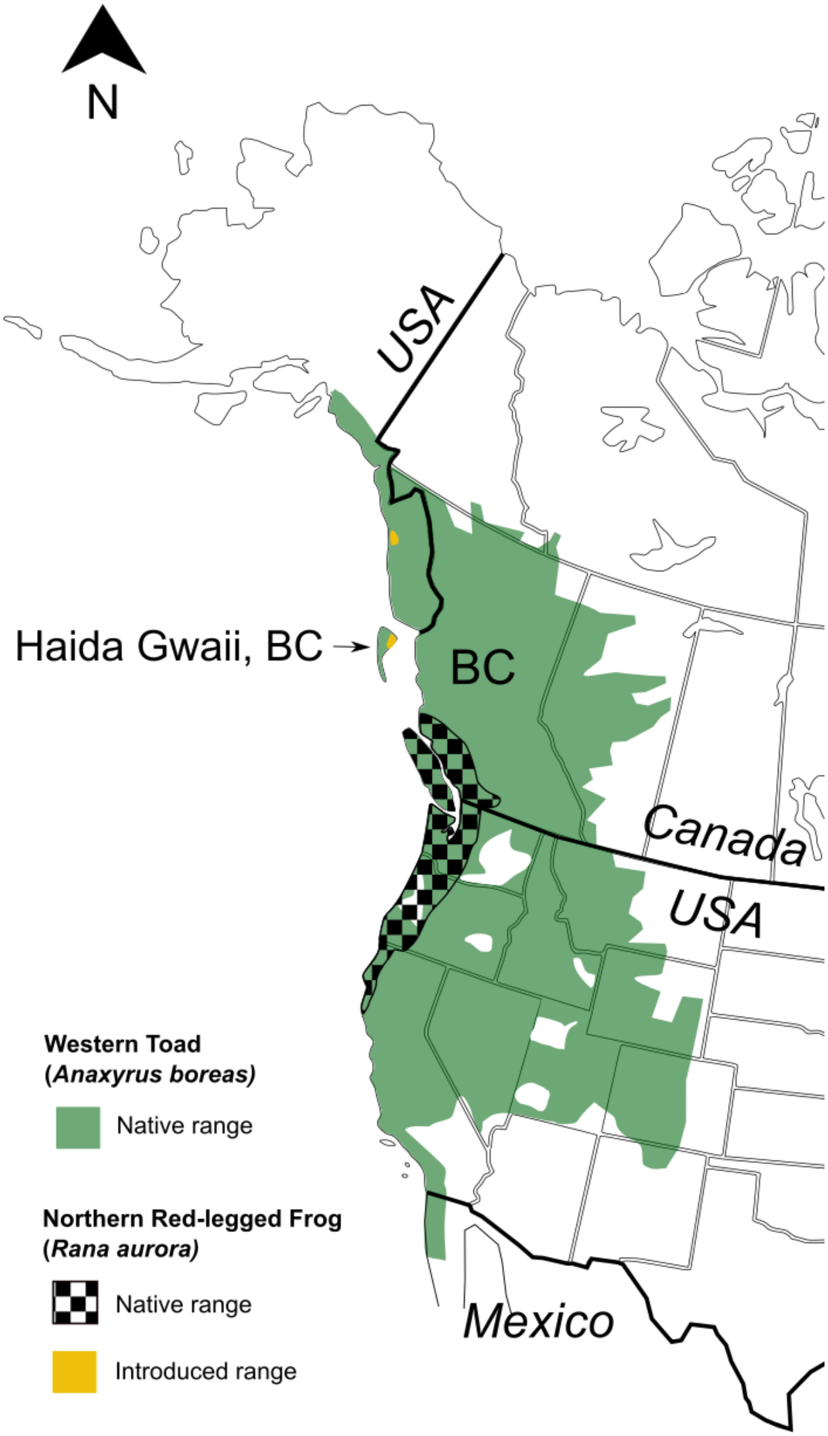


Figure 1.1. The native and introduced ranges of the Western Toad and Northern Red-legged Frog in North America. Approximate distributions, estimated from maps in the Species at Risk management plans (Environment and Climate Change Canada 2017, 2020).

The Western Toad (Figure 1.2) is declining across its range due to factors such as habitat destruction, chytrid fungus and roads (IUCN SSC Amphibian Specialist Group 2015). It is widely distributed in Western North America from Southern Alaska to Baja California and as far east as Western Alberta and Colorado (IUCN SSC Amphibian Specialist Group 2015). Local declines have been severe, with entire populations extirpated on Vancouver Island (Davis and Gregory 2003), in Colorado (Carey 1993), and in New Mexico (Jackson 2004). Western Toads are philopatric, usually returning to the site where they were born to breed (COSEWIC 2012). Sexually mature toads come together from across a large area (sites are often separated by distances up to 30km; Slough 2004) to a single breeding site, and breed *en masse* for a few weeks every spring (COSEWIC 2012). Such breeding behaviour makes for highly structured populations that are vulnerable to any threats to breeding habitat, or disruption during the short window of breeding.

Figure 1.2. An adult Western Toad on Haida Gwaii. Photo credit Roseanna Gamlen-Greene.

These two species have co-occurred at the pond scale in southwest BC since Western scientific records began in 1800 (GBIF.org 2020a, 2020b), and likely for thousands of years before, since at least the post-glacial expansion of the NRLF northward along the North American coast (Shaffer et al. 2004). Western Toad remains from 18,000 to 16,000 years ago have been recorded in karst caves on the northern tip of Vancouver Island (Harington 2011), an area thought to have acted as a glacial refugium and post-glacial recolonization source for southwest BC from ~11,000 years ago (Shafer et al. 2010).

Both species explosively breed in relatively shallow (~30-200cm) ephemeral and permanent open freshwater features, and egg masses and tadpoles of both species are often found in the same breeding habitats, though not necessarily in the same microhabitats.

Western Toads aggregate in the spring as water temperatures rise above a daytime maximum of 10°C, laying strings of tens of thousands of small eggs in warm shallow water. After hatching, the tadpoles aggregate in warm shallow water during the day. In contrast, within their southwest BC range, NRLFs breed in late winter or early spring, aggregating to oviposit large globular masses of hundreds of larger eggs in calm, comparatively deeper and cooler waters of at least 6-7°C.

Adults of both species are highly terrestrial, medium-sized (breeding adults 50-125 mm snout-vent length; Hayes, Quinn, Richter, Schuett-Hames, & Shean, 2008; Matsuda, Green, & Gregory, 2006), ground-dwelling anuran predators with similar diets. Both are long-lived (up to 12 years), relatively slow to sexual maturity (2 to 6 years, males to females), and highly fecund, suggesting they may be adapted to occasional reproductive failures (Hayes et al. 2008). Adult NRLFs (Figure 1.3) are strongly associated with moist, closed canopy forests and relatively sensitive to desiccation, as compared to adult Western Toads, which have wide thermal tolerance and resistance to desiccation, and are frequently found in open terrestrial environments.

Figure 1.3. An adult Northern Red-legged Frog (*Rana aurora*) on Haida Gwaii. Photo credit Roseanna Gamlen-Greene.

1.6 Haida Gwaii

Haida Gwaii is an archipelago off the Northwest coast of British Columbia, Canada (Figure 1.4), and is home of the Haida Nation. Haida Gwaii consists of hundreds of islands, totaling 10,180km^2, with the largest islands being Graham Island and Moresby Island. Haida Gwaii is part of the temperate coniferous rainforest biome and has a relatively mild climate for its latitude (54ºN), with cool and wet summers and winters. Paleontologists estimate that Haida Gwaii has been separated from the mainland for at least 10,000 years following the last glacial retreat and subsequent sea-level rise (McLaren et al. 2020). Parts of Haida Gwaii are thought to have acted as a glacial refugium in the last glacial maximum

(LGM) (Clague et al. 1989, Shafer et al. 2010, Barrie et al. 2014). The archipelago hosts an UNESCO world heritage site and is renowned for its unique ecosystems and numerous endemic species (Reimchen et al. 2005). It is considered a biodiversity hotspot, harbouring unique endemic mammals, fish, birds, plants, lichens and moss such as the Queen Charlotte Islands ermine (*Mustela erminea haidarum*), Haida Gwaii black bear (*Ursus americanus carlottae*), Haida Gwaii saw-whet owl (*Aegolius acadicus brooksi*), Queen Charlotte Islands violet (*Viola biflora var. carlottae*), Queen Charlotte hairy woodpecker (*Picoides villosus picoideus*), and the Northern goshawk (*Accipiter gentilis laingi*) (Osgood 1901, Moodie and Reimchen 1973, Byun et al. 1997, Withrow et al. 2014, Geraldes et al. 2019, Colella et al. 2021).

To conserve such species and their ecosystems, numerous areas of Haida Gwaii are protected with National and Provincial Parks and Conservancies – most notably the Gwaii Haanas National Park Reserve and Haida Heritage Site in the southern part of Moresby Island (hereafter "Gwaii Haanas"). These protected areas are co-managed by the Haida Nation and different levels of Canadian government. For instance, Gwaii Haanas is managed by the Archipelago Management Board which consists of an equal number of representatives from the Haida Nation and the Government of Canada. In the areas that are not protected, there are a number of small urban settlements which are mostly concentrated on Graham Island, including some small agricultural areas and many areas that have been and continue to be logged. The total human population on Haida Gwaii is less than 5000 people. Although under the current Land Use Objectives Order three quarters of the remaining forest is protected, there is much debate within the community of Haida Gwaii about whether the current rate, location, and type of trees being harvested is acceptable.

Management of forest resources on Haida Gwaii is co-governed by the Council of the Haida Nation and the provincial government of BC in the form of two structures: the Haida Gwaii Management Council and the Solutions Table.

Figure 1.4. Satellite image of Haida Gwaii, and its position along the Pacific Coast of North America. Satellite imagery from Bing Maps.

Invasive species are the top threat identified in Gwaii Haanas (Archipelago Management Board 2018). Prior introductions with particularly large negative impacts on plant and animal communities include Sitka-black tailed deer (*Odocoileus hemionus sitkensis*), native to north coastal BC and Alaska, and black rats (*Rattus rattus*), native to the Indian subcontinent (Gaston et al. 2002). The most recent vertebrate introduction was the NRLF, first recorded on Haida Gwaii in 2002, with the vector of introduction unknown (Ovaska et al. 2002). It is the second of two amphibian introductions, with the Pacific Chorus Frog (*Pseudacris regilla*; also known as the Northern Pacific Tree Frog) introduced in 1933 (Reimchen 1991). Both amphibians are native to southwestern British Columbia (BC), but were absent on Haida Gwaii prior to these introductions (Cockran and Thoms 1996).

NRLFs were first reported in 2002 on Graham Island, Haida Gwaii, Canada ~400 kilometers Northwest of the northern edge of their known range on the mainland (Ovaska et al. 2002). The NRLF was also introduced in 1982 to Chichagof Island in southeast Alaska, 500 kilometers Northwest of Haida Gwaii, where it is considered invasive (Hodge 2004, McClory and Gotthardt 2008) and anecdotal reports suggest that local Western Toad populations have since declined there (Lerum and Piehl 2007). Pauly et al. (2008) found these two extralimital NRLF populations (Haida Gwaii and Alaska) to be most closely related to haplotypes in the most northern clade of the core known range of the species, with Haida Gwaii NRLFs being most closely related to frogs from Vancouver Island and Washington state. Prior to my research, the recent range of the NRLF on Haida Gwaii since it was discovered in 2002 was unquantified.

The Western Toad, is the only indigenous amphibian on Haida Gwaii, whereas just ~600 km south on the coastal mainland there are approximately 15 native amphibian species (Green and Campbell 1984). There is concern from local people because toad sightings on Haida Gwaii have declined over the last 20 years, and it is hypothesized that these declines are due to destruction of breeding sites, invasive raccoons, climate change, or possibly, competition with introduced Northern Red-legged Frogs or Pacific Chorus Frogs (Reimchen 1991, Gaston et al. 2002, Pauly et al. 2008). However, the lack of information on the whereabouts of the toad outside of Gwaii Haanas prior to my research made it difficult to determine the status and drivers of any declines.

1.7 Thesis data chapters

My three data chapters investigate different facets of amphibian conservation, including non-native species dynamics, the vulnerability of island populations, and large-scale population patterns. In Chapter 2, I quantify the expansion rate of the Northern Red-legged Frog (NRLF) on Haida Gwaii since it was first recorded in 2002 (Question 1), investigate its potential to occupy other habitat, and compare its realized niche to its niche in its native range. To do so, I use 609 presence/absence data points of the NRLF from Haida Gwaii and the native range in British Columbia to conduct species distribution modelling, range expansion calculations and niche comparisons. In Chapter 3, I test the hypothesis that co-occurrence history between two species will influence the response to competition (Questions 2 and 3). I test this hypothesis using a tadpole mesocosm competition experiment between tadpoles of Western Toad and NRLF populations from both Haida

Gwaii and the mainland. In Chapter 4, I test the hypothesis that Haida Gwaii Western Toad populations will be genetically distinct and less diverse than in southwestern BC populations and investigate genetic patterns within Haida Gwaii Western Toad populations (Questions 3 and 4). I test this using genomic analyses of Western Toads populations from 27 locations in Haida Gwaii and southwestern BC (using hundreds of single-nucleotide polymorphisms) to characterize population structure and genetic diversity, and ultimately inform the conservation of toads in these areas (Question 4). In the final discussion chapter, I connect these results and the broader scientific themes they speak to, as well as make some management recommendations (Question 4).

Chapter 2: The current and potential distribution of the introduced Northern Red-legged Frog (*Rana aurora*) on Haida Gwaii

2.1 Introduction

Invasive species threaten ecosystems worldwide by contributing to habitat degradation, disease transmission, novel predation pressures, and biodiversity decline (Sala et al. 2000, Clavero and Garcia-Berthou 2005, Butchart et al. 2010). Such effects can be especially severe on islands harboring endemic species not equipped to co-exist with invaders (Blumstein and Daniel 2005, Kier et al. 2009, Tershy et al. 2015). As a consequence, identifying factors affecting the pace, extent, and impact of invasions on island ecosystems is a key concern for biodiversity conservation (Bellard et al. 2016, Doherty et al. 2016), particularly in insular refugia exhibiting high endemicity. Furthermore, given that the ranges of many species have already shifted in response to climate change in the absence of human assistance (Sunday et al. 2012), the definition of a 'non-native' species is in flux (Parmesan 2006, Chen et al. 2011, Urban 2020). Some ecologists have argued that introductions within the same bioregion may represent a viable conservation strategy under climate change by assisting shifts in the species range that increase its likelihood of persistence (McLachlan et al. 2007, Mueller and Hellmann 2008). Therefore, studying situations where a species is both native and non-native within the same bioregion can offer important insights into whether natural range shifts and assisted migration may offer a viable conservation solution to declining habitat suitability in a species' current native range.

The Haida Gwaii archipelago, British Columbia (BC), Canada, is a UNESCO world heritage site renowned for its unique ecosystems and numerous endemic species, due in part to its role as refugium during the last glacial maximum (~10,000 years before present) (McLaren et al. 2020). Invasive species are the top threat identified by the Gwaii Haanas National Park Reserve and Haida Heritage Site (hereafter "Gwaii Haanas"; Archipeligo Management Board 2018). Prior introductions with particularly large negative impacts on plant and animal communities include Sitka-black tailed deer (*Odocoileus hemionus sitkensis*), native to north coastal BC and Alaska, and black rats (*Rattus rattus*), native to the Indian subcontinent (Gaston et al. 2002).

The most recent vertebrate introduction was the Northern Red-legged Frog (*Rana aurora* hereafter "NRLF"), first recorded on Haida Gwaii in 2002 (Ovaska et al. 2002), and introduced from an unknown source in BC (Pauly et al. 2008). It is the second of two amphibian introductions; the Pacific Chorus Frog (*Pseudacris regilla*; also known as the Northern Pacific Tree Frog) was introduced in 1933 and also in the 1960s (Reimchen 1991). Both amphibians are native to southwestern British Columbia (BC) (Cockran and Thoms 1996). There is concern that NRLFs may influence native species assemblages on Haida Gwaii or negatively impact the Western Toad (*Anaxyrus boreas;* called Hlk'yáan K̲'ust'áan in the X̲aad Kíl dialect of the Haida language (X̲aad Kíl Née; Haida Language Council) via antagonistic interactions or as a vector for transmitting chytrid fungus (Gaston et al. 2002). The Western Toad is a species of "Special Concern" and the only indigenous amphibian on Haida Gwaii (COSEWIC 2012). A recent study found that the Western Toad on Haida Gwaii was a weaker competitor to the NRLF compared to mainland Western Toads (Chapter 3), increasing the urgency of quantifying both the current and potential future range of the NRLF on Haida Gwaii.

Although the NRLF has expanded its range on Haida Gwaii since 2002 (GBIF.org 2020b), the timing, original source, and current extent of NRLFs on Haida Gwaii is unknown

(Gaston et al. 2002, Ovaska et al. 2002, Pauly et al. 2008). Similarly, the NRLF was introduced to Chichagof Island, Alaska, in 1982, 500 kilometers northwest of Haida Gwaii, where it is classed as "Invasive" (Hodge 2004, McClory and Gotthardt 2008), and has rapidly spread (Leru and Piehl 2007). Despite its success in its non-native range, the NRLF has experienced population declines in its native range due to factors such as habitat destruction, chytrid fungus (*Batrachochytrium dendrobatidis*) and predation by the invasive American Bullfrog (*Lithobates catesbeianus*) (COSEWIC 2015). It is a species of "Special Concern" under Canada's Species at Risk Act (COSEWIC 2015), and provincially 'Yellow Listed – special concern' in BC, making it of special conservation interest to study its expansion on Haida Gwaii. Understanding the factors promoting the range expansion of NRLFs in their non-native range could both aid efforts to control them on Haida Gwaii (if necessary), whilst also informing whether northward assisted migrations may be a potentially viable conservation strategy within their native range. There are many factors that could explain the range expansion of the NRLF on Haida Gwaii, such as: dispersal rate; reproductive rate; absence of enemies from the native range e.g., the invasive American Bullfrog and chytrid fungus (Gamlen-Greene et al. unpublished data); high habitat connectivity due to a small human population (< 5000); human mediated dispersal; low elevational range across eastern Haida Gwaii; many waterbodies suitable for breeding; and many abiotic habitats within the niche of the species.

Species distribution models (SDMs) are widely-used to correlate observations with environmental conditions to identify habitat vulnerable to invasion and predict range expansions (Jeschke and Strayer 2008). SDMs typically model the realized niche (hereafter, 'niche') – which is the abiotic conditions required to support positive population growth of a species, constrained by biotic processes such as competition and dispersal (Hutchinson 1957, Pulliam 2000, Wiens and Graham 2005, Guisan et al. 2014). The ability to accurately describe the niche and predict habitat suitability for non-native species depends on two assumptions: 1) that the species has had the opportunity to disperse to all suitable habitats (distribution equilibrium) (Elith and Leathwick 2009) and 2) that the niche of a species in its native range is conserved in its non-native range (niche conservatism) (Wiens and Graham 2005). When these assumptions are violated, modeling exercises may over or under-estimate suitable habitat for a species in their non-native range (Broennimann and Guisan 2008, Elith 2017). For instance, non-native species that are currently dispersal limited (due to lack of time, rather than ability), or only occupy a subset of their native range niche are more likely to have their current distributions overpredicted, and their future potential distributions underpredicted, by only using the non-native range for prediction (Guisan et al. 2014, Davies et al. 2019). Conversely, using only native occurrences to predict the distribution of a non-native species that has expanded or shifted their niche, can result in underpredicting the species' distribution.

The niche conservatism hypothesis suggests that species' niches are conserved in space and time (Weins et al. 2010) and is frequently examined using SDMs projected onto environmental space; i.e. ecological niche models (Broennimann et al. 2012, Guisan et al. 2014, Atwater et al. 2018). There are numerous studies examining niche shifts, expansions, unfilling and niche conservatism in non-native species, but relatively few examples in amphibians. Niche shifts are more common in recent introductions, small native range sizes, and lower-latitude introductions (Li et al. 2014). Niche shifts are reported for the cane toad (*Rhinella marina*) (Tingley et al. 2014) and American bullfrog (*Lithobates catesbeianus*) (Becerra López et al. 2017). Niche conservatism versus changes to the realized niche are commonly evaluated using the following quantifications of the abiotic niche: "niche stability" (how much of non-native niche is within the native niche?), “niche expansion” (does the non-native niche contain all of the native niche habitat, and has it expanded to new environments as well?), “niche shift” (has the non-native niche shifted in environmental space compared to the native niche), and “niche unfilling” (how much of native niche is not occupied in the non-native range?). Understanding the degree of similarity between the native and non-native niche can advance understanding of the mechanisms of invasion success such as enemy release, rapid evolution, and phenotypic plasticity (Lee 2002, Sax et al. 2007, McCann et al. 2014).

Species’ distributions are also affected by climate change (Bellard et al. 2013). Gerick et al. (2014) predicted that 45% of the current native range of the NRLF in BC will be thermally limiting by 2080. One study modelled habitat suitability of Haida Gwaii for the NRLF prior to my study: Pauly et al. (2008) built a model that predicted that habitat for NRLFs on Haida Gwaii would increase in suitability with climate change. They used 72 presence observations that were mostly from the native range and low-resolution mapping ($10km^2$). Here, I updated these efforts by using: presence-absence data; 9x more observations; finer resolution climate data (Wang et al. 2012, 2016); an ensemble of 13 climate change models (Pierce et al. 2009, Wright et al. 2016, Mahony et al. 2021); and a high mapping resolution ($250m^2$), to predict habitat suitability on a scale appropriate for managing NRLFs on Haida Gwaii.

In this study, I asked: 1) How far and fast have the NRLFs expanded their range on Haida Gwaii?; 2) How does the realized niche of NRLFs on Haida Gwaii compare to the realized niche in the BC native range?; and 3) When will the NRLF colonize currently unoccupied areas of Haida Gwaii, and given climate change, what areas will be suitable if they get there? To answer these questions, I surveyed NRLFs on Haida Gwaii (n = 117), collated occurrence 492 records from the native range in BC, estimated the range expansion rate by comparing 2017 survey data to the first observations of NRLFs on Haida Gwaii in 2002, estimated realized niches using an ordination approach, and used a Random Forest model to predict present and future habitat suitability on Haida Gwaii.

2.2 Methods

2.2.1 Data preparation for all three questions

2.2.1.1 Breeding occurrence surveys

Breeding occurrences (i.e., presence or absence/non-detection of egg masses or tadpoles) were collected for the native and non-native range of the NRLF in British Columbia (BC) (n = 609 locations in total; n = 117 Haida Gwaii, n = 492 native range; Figure 2.1). Breeding occurrences, as opposed to observations at all life stages, were used because the large egg masses have been found to have a high detection probability for NRLFs (0.947, 90% CI) (Rowe et al. 2019). Adult observations have a much lower detection probability (i.e., confidence in the absences), in part due to being quite cryptic. For the native range in BC, 492 NRLF breeding occurrences were used; Vancouver Island (n = 286; Figure 2.1) and the BC mainland (n = 206). These native range observations were sourced from historic surveys from 1998-2012 (COSEWIC 2015). To describe the Haida Gwaii range (non-native range in BC), 117 NRLF breeding occurrences from Haida Gwaii were used. These consisted of: 1) a breeding survey of Graham Island in spring 2017 (54 sites), the only invaded island in the archipelago; 2) 160 additional sites that were opportunistically sampled between April-May 2017-2019; and 3) eight observations from BC Parks, Ministry of Forests, Lands and Natural Resource Operations and Rural Development and the Council of the Haida Nation from 2015-2016 (Figure 2.1). The Haida Gwaii 2017 breeding survey points were selected using a stratified random sampling using *QGIS* (v 3.16.0, 2021), by randomly selecting 54 wetlands or open waterbodies within the three main biogeoclimatic (BEC) zones represented on Graham Island from a candidate list of such sites in the BC Freshwater Atlas (BC Ministry of Forests; Figure 2.1). All survey sites were within 5km of a road (for ease of access), at least 1km apart, visited twice and searched for two hours.

2.2.1.2 Cleaning of survey data

The breeding presences and absences (n = 609 locations in total; n = 117 Haida Gwaii, n = 492 native range) were filtered down from ~800 observations to ensure they accurately reflected breeding activity. For instance, sites with non-detections (absences) were excluded if they were past peak breeding season, because Rowe et al. (2019) found that there was a lower detection probability for NRLFs later in the season. Additionally, to reduce spatial autocorrelation, I considered lakes and wetlands to harbor independent populations (Rowe et al. 2019) as long as they were separated by 200m, as that is the distance the NRLF has been documented to explore potential breeding sites in the post-thaw/pre-breeding season (Grand et al. 2017). For sites that were sampled more than once, only the most recent presence was included. Additionally, 95 absences in the Haida Gwaii dataset were removed that were both far from any presences and beyond large dispersal barriers (salt water and large mountains; Appendix A; Figure A1). This was to avoid training the models on geographic areas that were still limited by dispersal during a range expansion, as that can result in underpredicting habitat suitability (Václavík and Meentemeyer 2012).

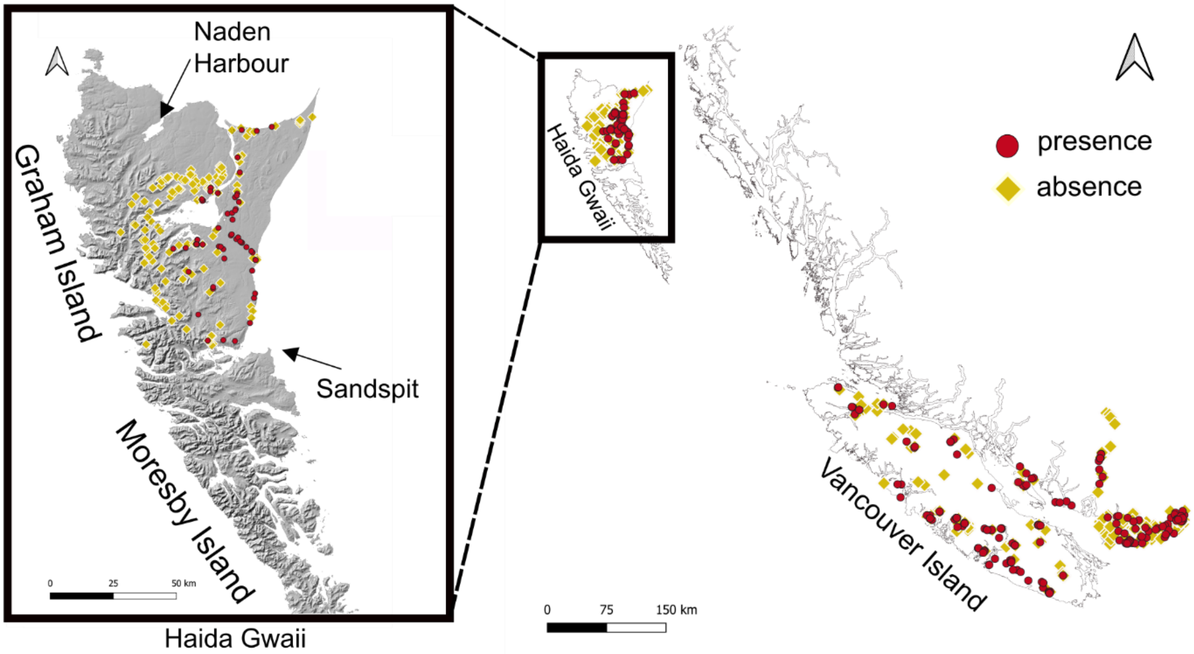


Figure 2.1. Breeding occurrences (presence/absence) of the Northern Red-legged Frog on Haida Gwaii (non-native) and southwest BC (native). 609 sites surveyed in the non-native range and in the native range in BC (n= 492). Inset shows Haida Gwaii points (n = 117); legend refers to both native range and Haida Gwaii.

2.2.1.3 Environmental variables

Fifteen publicly accessible GIS environmental layers (nine climate and six habitat variables; Table 2.1) rather than microscale habitat variables (e.g., pond depth) were used in order to model habitat suitability in areas that were not sampled. The variables were selected *a priori*, and were included because they all have been shown previously to influence the suitability of habitat for NRLF breeding (Licht 1971, Hayes et al. 2008, Gerick et al. 2014, COSEWIC 2015). All variables in the non-native (Haida Gwaii) range had analogues in the native range to: 1) determine whether a true niche shift was occurring, rather than the filling of a preadapted niche (Fitzpatrick and Hargrove 2009, Petitpierre et al. 2012), and 2) avoid predicting to environments beyond the training data (Elith and Leathwick 2009).

Table 2.1. Environmental variables used in this study. These were chosen *a priori* because they all have been shown previously to influence the suitability of habitat for Northern Red-legged Frog breeding. Rationales taken from COSEWIC (2015).

Variable	Rationale	Scale of variable	Data source
Spring min temp (Tmin_sp)	Spring temperature affects larval development	local	ClimateBC v 7.1
Spring ave temp (Tave_sp)	Spring temperature affects larval development		
Spring precipitation (PPT_sp)	Precipitation is necessary to fill ephemeral breeding habitats		
Spring degree days > 5°C (DD5_sp)	Consistent mild temperatures are necessary for breeding and larval development		
Spring number frost free days (NFFD_sp)	Frosts in spring detrimental to eggs and larvae		
Spring climate moisture index (CMI_sp)	Dry springs can reduce ephemeral breeding habitat or wet springs can make it too waterlogged		
Summer climate moisture index (CMI_sm)	Dry summers can dry out ephemeral breeding habitats or make them have fewer predators		
Summer min temp (Tmin_sp)	Spring temperature affects larval development and metamorphosis		
Summer precipitation (PPT_sm)	Summer precipitation is necessary to fill temporary breeding habitats until metamorphosis		
Distance to nearest open waterbodies	Breeding habitat	local	Ministry of forests 2002
Distance to nearest wetland	Breeding habitat		
Percent wetland	Breeding habitat	500m buffer	Ministry of forests 2002
Percent forest	Protects breeding habitat		
Stream density	Corridors for dispersal to breeding habitat		
Elevation range	High topogrpahic complexity is unsuitable breeding habitat	500m buffer	USGS one arc DEM

2.2.1.4 Habitat variables

Freshwater habitat variables were accessed from the Freshwater Atlas, and forest cover was accessed from the GeoBase Land Cover dataset in December 2020 - both published by the BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development. These consisted of polygon layers for wetlands, forest, streams and open water bodies based on 2002 habitat. The habitat polygons were converted to percentages (for wetlands, forest, open water bodies) and densities per km^2 (for streams) using buffers with multiple radii (100m, 200m, 500m, 1km and 2km) around the survey points (conducted in *R* using package *sp*; functions *st_area()* and *st_length()*, and *dplyr* package - function *summarise()*; (Pebesma and Bivand 2005, Bivand et al. 2008, Wickham et al. 2018). Buffer widths were chosen from Rose et al. (2020) and Green et al. (2020). Within each buffer, elevation was sampled using the USGS 1 arc-second resolution (~30 meters) Digital Elevation Model (DEM) (Farr and Kobrick 2000) and the zonal statistics tool in *QGIS* to calculate elevation range (the difference between the minimum and maximum elevation). To decide which buffer spatial scale to include, I followed the recommendations in Bradter et al. (2013) and ran models (model building discussed in section 2.2.4.2) with all variables, but varied buffer width and picked the model with the buffer size that explained the highest variance (out of an average of 50 simulations). The model with the 500m buffer size performed best.

2.2.1.5 Climate variables

Current and future seasonal climate variables were accessed from Climate BC version 7.1 (Wang et al. 2012, 2016, Mahony et al. 2021), which is a program that downscales PRISM gridded monthly climate normal data at 800 x 800 m resolution (Daly et al. 2008) to scale-free point locations. For the current climate, the normal period from 1991-2020 was used. For the future climate models, the CMIP6 climate projections with the SSP2-4.5 scenario for 2021-2040 and 2081-2100 were used. An ensemble of 13 general circulation models (GCM) from these climate projections created by Mahony et al. (2021) was used because an ensemble is likely to be more reliable than any single GCM projection (Pierce et al. 2009). The SSP2-4.5 assumes moderate mitigation of future greenhouse gas emissions that are approximately consistent with current economic trends and emission policies. For spring climate, I included mean and minimum temperatures, climate moisture index, number of frost-free days, degree days > 5ºC and mean precipitation. For summer climate, I included minimum temperature, climate moisture index and mean precipitation (Table 2.1).

2.2.2 How far and fast have the NRLFs expanded their range on Haida Gwaii?

2.2.2.1 Past and current range on Haida Gwaii

Presence observations of the NRLFs initial distribution (in 2002) and their 2017 distribution were used to generate past and current range extents. For the 2002 distribution, I used all ten NRLF observations from Ovaska et al. (2002) and one unpublished observation from Tom Reimchen in 2002, in a pond near Mayer Lake (T. Reimchen, unpublished data). For the 2017 range, I used all 57 presence observations from my 2017 random and non-random sampling. To compare the range of the NRLF in 2017 to 2002, the area (km^2) of the minimum convex hull (MCH) was calculated for both periods (Worton 1987, Licata et al. 2019). A minimum convex hull is the smallest polygon that contains all sites with no interval angles exceeding 180 degrees. The MCHs were generated in *QGIS* using the convex hull tool (using presence points) and clipped the hull to the coastline. I then used the *QGIS Field Calculator* tool to calculate the area (km^2) of the MCHs.

2.2.2.2 Range expansion rate on Haida Gwaii

I calculated how fast the NRLF was expanding its range on Haida Gwaii by calculating an average dispersal rate (R_{ave}) by using the square root of the area occupied in 2002 compared to 2017: R_{ave} (km/year) = ($\sqrt{}$area km^2 2017 - $\sqrt{}$area km^2 2002)/ (2017 – 2002) (year) (Skellam 1951, Gilbert and Liebhold 2010). Two straight-line dispersal rates for NRLFs were calculated using the straight-line distance from 1) the most northern NRLF observation in 2002 to the most northern point in 2017 and 2) the most western NRLF observation in 2002 to the most western point in 2017. The straight-line dispersal rate (R_s) was calculated as: R_s (km/year) = distance (km) / (2017 - 2002).

Observations near St’alaa Kun (Collison Point) were excluded from the western dispersal rate calculation because these presences are likely the result of human mediated dispersal. Collison Point is located across a very fast-flowing and deep saltwater channel that is over 500m wide and is frequently travelled across via boat (area labelled in Figure 2.2). Furthermore, all observations from around the western side of Masset Inlet (between the western-most presence and Collison Point) were absences ($n > 50$).

2.2.3 How does the realized niche of NRLFs on Haida Gwaii compare to the realized niche in the BC native range?

2.2.3.1 Haida Gwaii and native range realized niche

Abiotic realized niches in the native and non-native ranges were generated using an ordination approach developed by Warren et al. (2008), Broennimann et al. (2012), Petitpierre et al. (2012), Guisan et al. (2014) and Di Cola et al. (2017). First, background environmental conditions were generated for both ranges by creating a 250 x 250 m grid of points in *QGIS* for all Haida Gwaii (~160,000 points) and the native range (Southwestern British Columbia) (~1 million points), and environmental variables were extracted from these points. A Principal Component Analysis (PCA) was conducted to quantify the native and Haida Gwaii (non-native) realized niches using environmental variables from the grid points and presence points from Haida Gwaii and southwestern BC into the PCA (variables in Table 2.1, except per cent forest as it was unavailable for the native range) using the *ecospat* package in *R* (Di Cola et al. 2017). The first two PCs were rescaled to a resolution of 100 x 100 cells to reduce the effects of missing data and sampling bias in environmental and geographical space. Other axes were not included because the first two explained a large portion of the variation, following Broennimann et al. (2012) (Appendix A: Table A1). The PC loadings were extracted and used the *fviz_pca_var()* function of the *factoextra R* package to visualize the PC contributions (Kassambara and Mundt 2020). Finally, the native and non-native (Haida Gwaii) realized niches were generated by transforming the species occurrences into densities using a kernel smoothing function to reduce sampling bias (Broennimann et al. 2012).

2.2.3.2 Realized niche comparisons

The two niches were overlaid onto the environmental space of both native and non-native ranges and the niche metrics were calculated in the *ecospat R* package (Broennimann et al. 2012, Di Cola et al. 2017). To test if there had been a niche shift, we measured niche overlap (intersection of two niches in environmental space) using Schoener's D (0 = no overlap, 1 = total overlap) and the niche similarity test (when $p > 0.05$, non-native niche is more similar to the native niche than random) (Schoener 1974, Broennimann et al. 2012). Niche stability, expansion, and unfilling were all calculated as a proportion of environmental space and converted to percentages. Niche stability is estimated as the proportion of the non-native niche overlaps with native niche (Guisan et al. 2014). Niche expansion is estimated as the proportion of non-native niche outside of the native niche (Guisan et al. 2014). Niche unfilling is estimated as the proportion of the native niche that has not been filled by the non-native niche, within non-native environmental space (Guisan et al. 2014). I also calculated niche unfilling for a future climate scenario on Haida Gwaii (climate change scenario SSP2-4.5 2081-2100) (Mahony et al. 2021).

2.2.4 When will the NRLF colonize currently unoccupied areas of Haida Gwaii, and given climate change, what areas will be suitable if they get there?

2.2.4.1 Future range expansion on Haida Gwaii

I generated a map showing areas of Haida Gwaii the NRLF could and could not access in 2040 and 2081, assuming the constant annual range expansion rate calculated in section 2.2.2.2 (e.g., no landscape resistance or other factors). Average range expansion rate ($R_{ave} = 1.9$km/year) was used for northern Haida Gwaii and was multiplied by the number of years since the 2017 survey (2040 = 23 years; 2081= 64 years) to get the distance (d) they could disperse ($d = R_{ave}$ * (future year - 2017)). This distance was used in *QGIS* to create buffers around the 2017 minimum convex hull to generate the area the NRLF could disperse to (and hence map future range expansion). I assumed no major human assisted dispersal events, as well as creating an alternative map that assumed the NRLF would reach Moresby Island in the next few years).

2.2.4.2 Predicting habitat suitability using a Random Forest model

I used a Random Forest algorithm to model the current and future habitat suitability for the NRLF on Haida Gwaii using the *randomForest R* package (Liaw and Wiener 2002). Random Forest models have been used widely in invasion and conservation studies and SDMs (e.g., Hill et al. 2017, Sung et al. 2018, Burns et al. 2020, Zhang et al. 2020), in part because they can handle large numbers of collinear variables, and are resistant to overfitting, making them very useful for prediction (Breiman 2001, Segal 2004, Prasad et al. 2006, Sarquis et al. 2018). The Random Forest model used the covariates in Table 2.1 and presences/absences from both native and non-native ranges (n = 609), because pooling data across both non-native and native ranges has been found to improve invasive predictions (Broennimann and Guisan 2008, Fitzpatrick et al. 2008, Pili et al. 2020, Sales et al. 2021).

The Random Forest model randomly selected a subset of the full dataset to create a tree (bootstrapped data), which at every branch selected the variable that best split the data into presences and absences of the NRLF from a random subset of variables (the number of variables determined by the ‘mtry’ parameter) (Breiman 2001). After a predetermined number of iterations, the outputs were averaged to get a measure of the model fit on the testing data. The Random Forest was run as a regression with the default settings - number of trees (i.e., iterations) set to 500, and the ‘mtry’ parameter set to the number of predictors divided by three, as recommended by Breiman (2001). A variable importance ranking of the covariates (% increase in mean squared errors) was generated using the *varImp()* function in the *caret R* package (Kuhn 2008). The variable importance ranking is based on the number of times a variable was chosen to best separate presences and absences across all trees (Breiman 2001). I generated response curves for the variables using the *plotmo()* function in the *plotmo R* package (Milborrow 2018).

I assessed the accuracy of the Random Forest model (model calibration) by: 1) dividing the occurrence data into 70% training data (consisting of 70% of the Haida Gwaii data combined with all the native range data) and 30% testing data (the rest of the Haida Gwaii data); and 2) calculating the area under the receiver operating characteristic curve (AUC), Kappa coefficient and root mean square error (RMSE) (Boyce et al. 2002, Allouche et al. 2006, Fawcett 2006). The model predicting habitat suitability on Haida Gwaii performed reasonably (AUC = 0.76, Kappa = 0.52, RMSE = 0.45). As mentioned earlier, the training and testing data from Haida Gwaii did not include absences that could be due to distribution disequilibrium.

2.2.4.3 Mapping current and future suitability of habitat on Haida Gwaii

As recommended by Pili et al. (2020) and Sales et al. (2021), the model used for generating landscape predictions was trained on 100% of data from both ranges. The Haida Gwaii grid points were run through this model using the *predict()* function from the *randomForest R* package (Liaw and Wiener 2002). I then converted the georeferenced Haida Gwaii grid points with habitat suitability to a raster with a resolution of 250 x 250 m by: 1) creating a square buffer around the points with a radius of 125 m in *R*; and 2) using the rasterize tool in *QGIS* to convert the polygon layer to a raster. To generate prediction error estimates, I set *predict.all=TRUE* within the *predict()* function in the *randomForest R* package, and then calculated the standard error for every grid habitat suitability prediction for Haida Gwaii across all 500 trees using a custom function (Liaw and Wiener 2002). The average standard error was 0.04 for predictions for all time periods. To assess whether climate change would alter habitat suitability for the NRLF on Haida Gwaii in the future, I compared the mean current and future values of habitat suitability across all grid cells on Haida Gwaii (Wright et al. 2016).

2.3 Results

2.3.1 Range expansion on Haida Gwaii

The range of the NRLF on Haida Gwaii increased over 15-fold within 15 years from 2002 (97 to 1509 km^2, 2002 – 2017, respectively). This suggests a mean range expansion rate of 1.9 km/year. The area occupied by NRLFs on Haida Gwaii in 2017 represents 24% of Graham Island. The range expansion rate did not appear consistent across Graham Island, with the rate being faster in the northeast than the southwest, and the occupation of Collison Point likely reflecting human mediated dispersal. The largest straight-line distance between an observation in 2017 and the closest observation from 2002 was 38 km (Figure 2.2). This occurred in the northeast, transecting forested wetland of low topographic complexity, and represents a range expansion rate of 2.5km/year. By contrast, in the mountainous areas on the west side of Graham Island, the range expansion rate was much lower, approximately 1.1 km/year (straight-line distance of 16.6km). Additionally, there is a small, occupied area in the northwest (Collinson Point area), which is surrounded by unoccupied areas (Figure 2.2). Given that NRLFs are unlikely to naturally disperse across a large flowing body of saltwater, human transport appears the likely explanation. There is one permanent residence near Collison Point and a logging operation that has frequent traffic from areas within the core range of the NRLF on eastern Graham Island, so this explanation seems plausible.

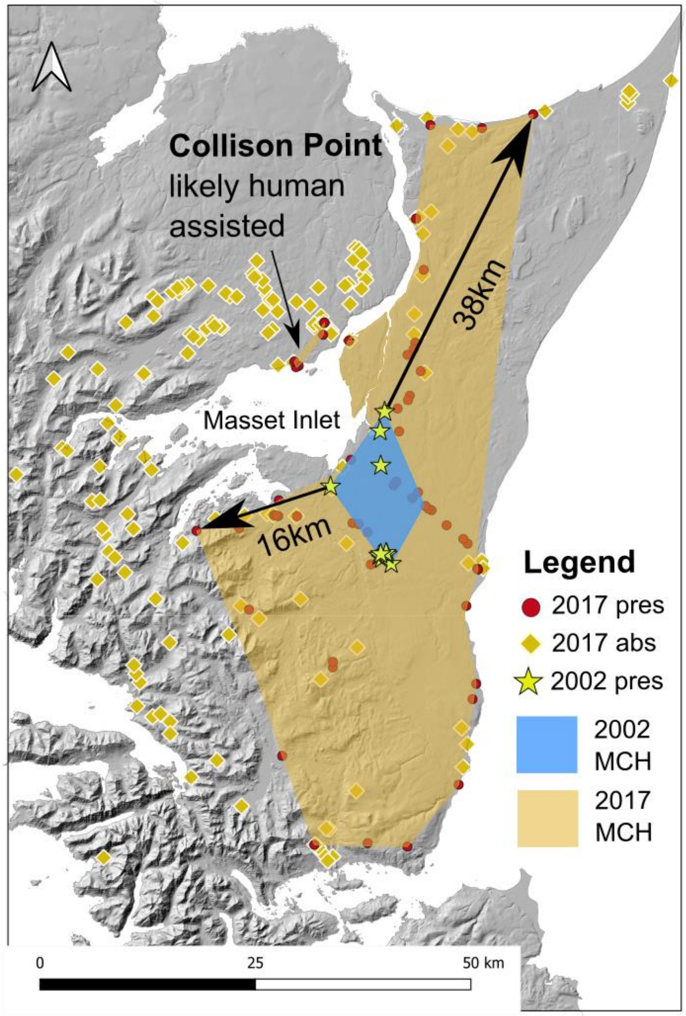


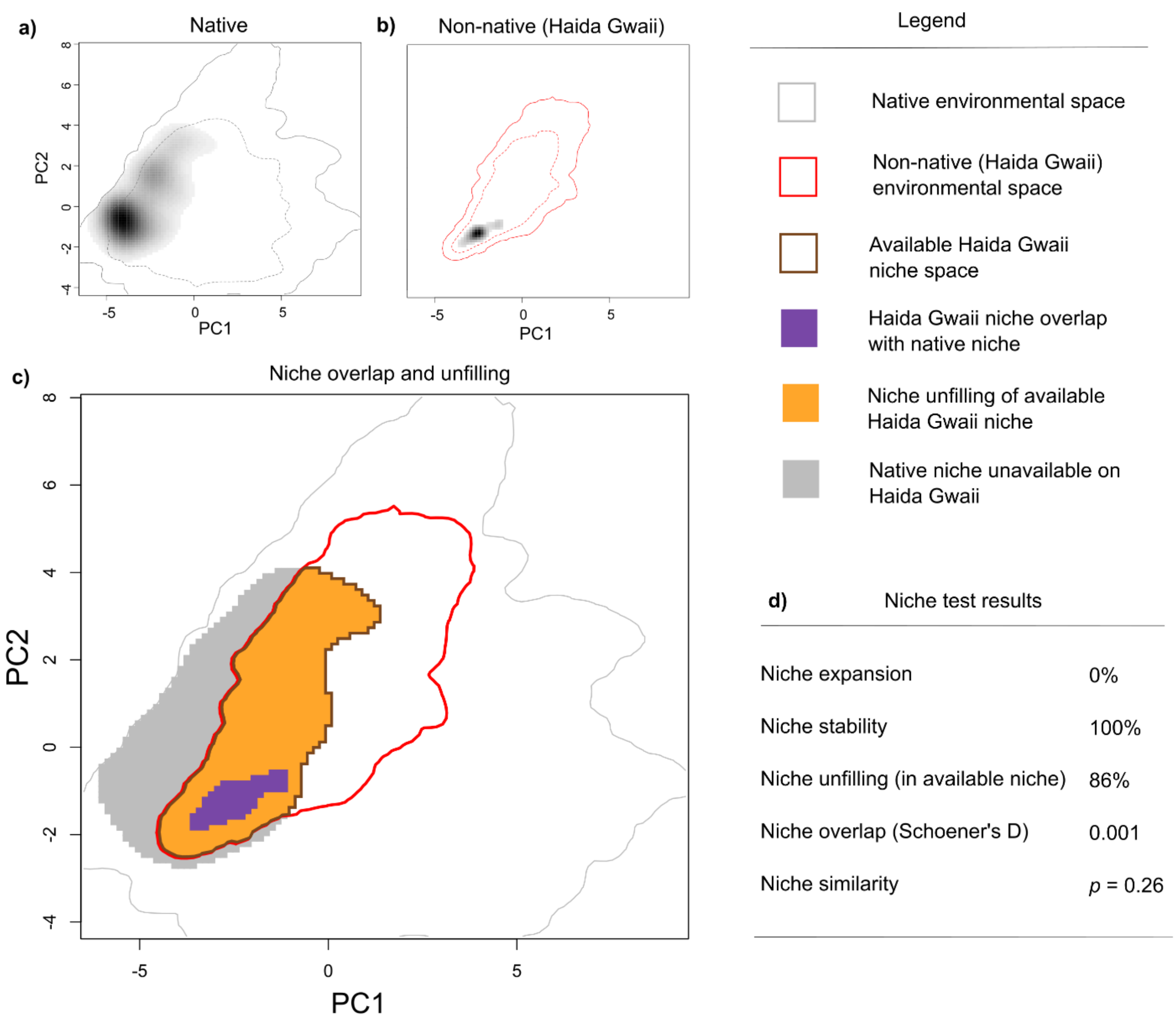
Figure 2.2. Breeding occurrences and current range of Northern Red-legged Frogs on HG in 2002 and 2017. Arrows represent the furthest and shortest distances to the range limits from the 2002 points. All features overlayed on a 1-arc digital elevation model (Farr and Kobrick 2000).

2.3.2 Realized niche comparisons

The native niche of the NRLF was conserved in the Haida Gwaii range. Additionally, there was a high degree of niche unfilling. Eighty-six per cent of the native niche on Haida Gwaii remained unfilled (Figure 2.3d) – i.e., the NRLF on Haida Gwaii only filled a small subset of the available niche (area within brown line) on Haida Gwaii (Figure 2.3c). Niche conservatism was demonstrated by complete niche stability (100%; Figure 2.3d) – i.e., all of the Haida Gwaii niche was contained within the native niche for the environmental variables included in the analysis (Figure 2.3d). The NRLF did not expand its niche on Haida Gwaii compared to its native niche (niche expansion = 0%; Figure 2.3d). There was no niche shift, despite low niche overlap (Schoener's D = 0.001; Figure 2.3d), the Haida Gwaii niche was more similar to the native niche than random, and as such, niche similarity could not be rejected: $p > 0.05$; Figure 2.3d).

In the PCA analysis, most of the variation in environmental variables was captured by PC 1 (~40%) and 2 (~24%; Appendix A; Table A1). PC1 reflected the number of frost-free days in spring, average temperature and minimum temperature, minimum summer temperature, and a strong negative loading for elevation range and distance to nearest wetland (Appendix A; Table A2; Figure A2). PC2 had a strong positive loading for spring and summer climate moisture index and precipitation.

Climate change on Haida Gwaii (2081-2100 scenario) is projected to make the non-native niche more closely resemble the native niche, as evidenced by an increase in the amount of suitable habitats that are yet to be filled (94% unfilling; Appendix A; Figure A6).



Niche expansion	0%
Niche stability	100%
Niche unfilling (in available niche)	86%
Niche overlap (Schoener's D)	0.001
Niche similarity	*p* = 0.26

Figure 2.3. Comparison of native (grey) and non-native; Haida Gwaii (red) realized niches of the Northern Red-legged Frog in environmental space. Grey shading of cells in a) and b) shows the density of the occurrences of the species by cell, solid lines show 100% of environmental space evaluated, dashed lines show 50% of environmental space. c) PCA of niche comparisons between the native range and non-native range (Haida Gwaii). Grey cells show the native niche outside of Haida Gwaii environmental space, orange cells show the unfilled niche on Haida Gwaii, purple cells show the niche overlap between Haida Gwaii and the native range, solid brown line shows available Haida Gwaii niche space (i.e., native niche within Haida Gwaii environmental space). d) Niche metric test results.

2.3.3 Potential range expansion on Haida Gwaii

Based on a constant range expansion rate, the future potential range of the NRLF on Haida Gwaii includes much of Graham Island and Moresby Island, and the extent will depend largely on whether humans facilitate NRLF dispersal. NRLFs could disperse to much of Graham Island by 2040, and all Graham Island by 2081 (Figure 2.4a). If NRLFs arrive on Moresby Island within the next few years (e.g., via humans), the range of NRLFs may include many areas of Moresby Island by 2081, including Gwaii Haanas National Park and Haida Heritage Site (Figure 2.4b).

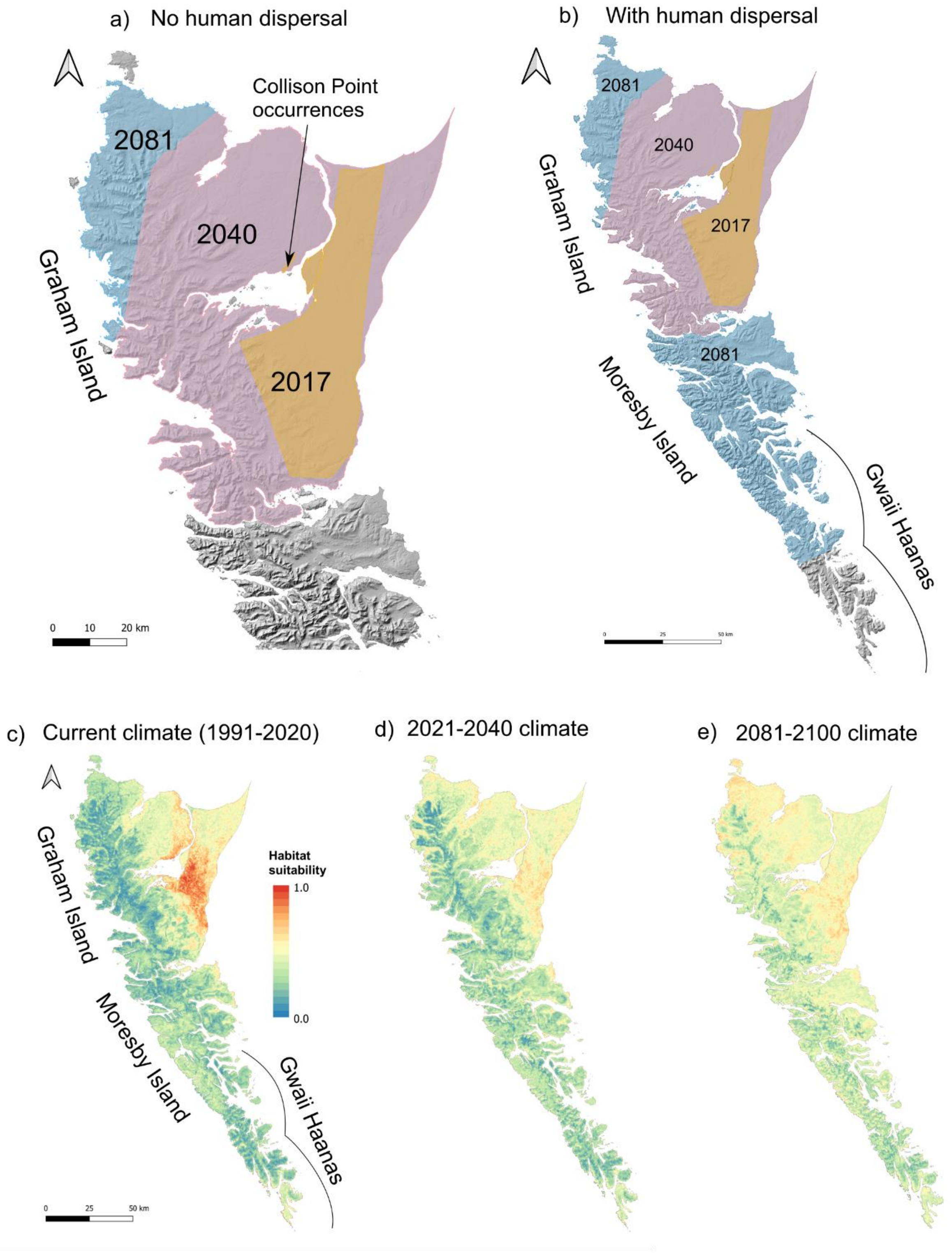


Figure 2.4. Northern Red-legged Frog a)-b) potential range based on range expansion rate c)-e) current and future habitat suitability with climate change on Haida Gwaii. a) Map showing potential range on Graham

Island based on range expansion rate but no additional human dispersal, b) potential range across Haida Gwaii based on range expansion rate with human dispersal; both a) and b) assume a constant dispersal rate of 1.9km/year; c) Habitat suitability under current conditions (normal period 1991-2020); d) Habitat suitability in 2021-2040 (ensemble model with SSP2-4.5 scenario); e) Habitat suitability in 2081-2100 (ensemble model with SSP2-4.5 scenario).

2.3.4 Current and future habitat suitability on Haida Gwaii

Under current conditions, the areas of highest habitat suitability for NRLFs on Haida are the lowlands of northeastern Graham Island (Figure 2.4c). Much of this habitat is already occupied (Figure 2.2). Under current conditions, unoccupied habitat identified as highly suitable include Sandspit and Naden Harbour (locations in Figure 2.1).

Projections using the climate ensemble for 2021-2040 (Figure 2.4d) predicted that mean suitability would increase by 7.6% on Haida Gwaii compared to current conditions (Appendix A; Figure A3). Similarly, the model using the climate ensemble for 2081-2100 (Figure 2.4e). predicted that mean suitability would increase by 29.4% on Haida Gwaii compared to current conditions (Appendix A; Figure A3). In both 2021-2040 and 2081-2100 projections, northwestern Graham Island, western Graham Island and Moresby Island became more suitable whereas central eastern Graham Island (the core of the current range) became less suitable (Figure 2.4d and e).

The summer climate moisture index was the most important variable in the Random Forest model predicting habitat suitability on Haida Gwaii, followed by seven similarly performing variables that were a mix of climate and habitat variables (Appendix A; Figure A4). Although causality is not inferred with a Random Forest (Pearl 2009), habitat suitability was predicted to increase with increased climate moisture index, precipitation, minimum spring temperature and degree days above 5ºC. Conversely, habitat suitability decreased with increasing distance to nearest wetland, stream density and elevational range (Appendix A; Figure A5). Climate change on Haida Gwaii is predicted to increase spring climate moisture index, precipitation, minimum temperature and degree days above 5ºC (Figure 2.5a-d). Climate change is also predicted to decrease summer climate moisture index on Haida Gwaii (Figure 2.5e).

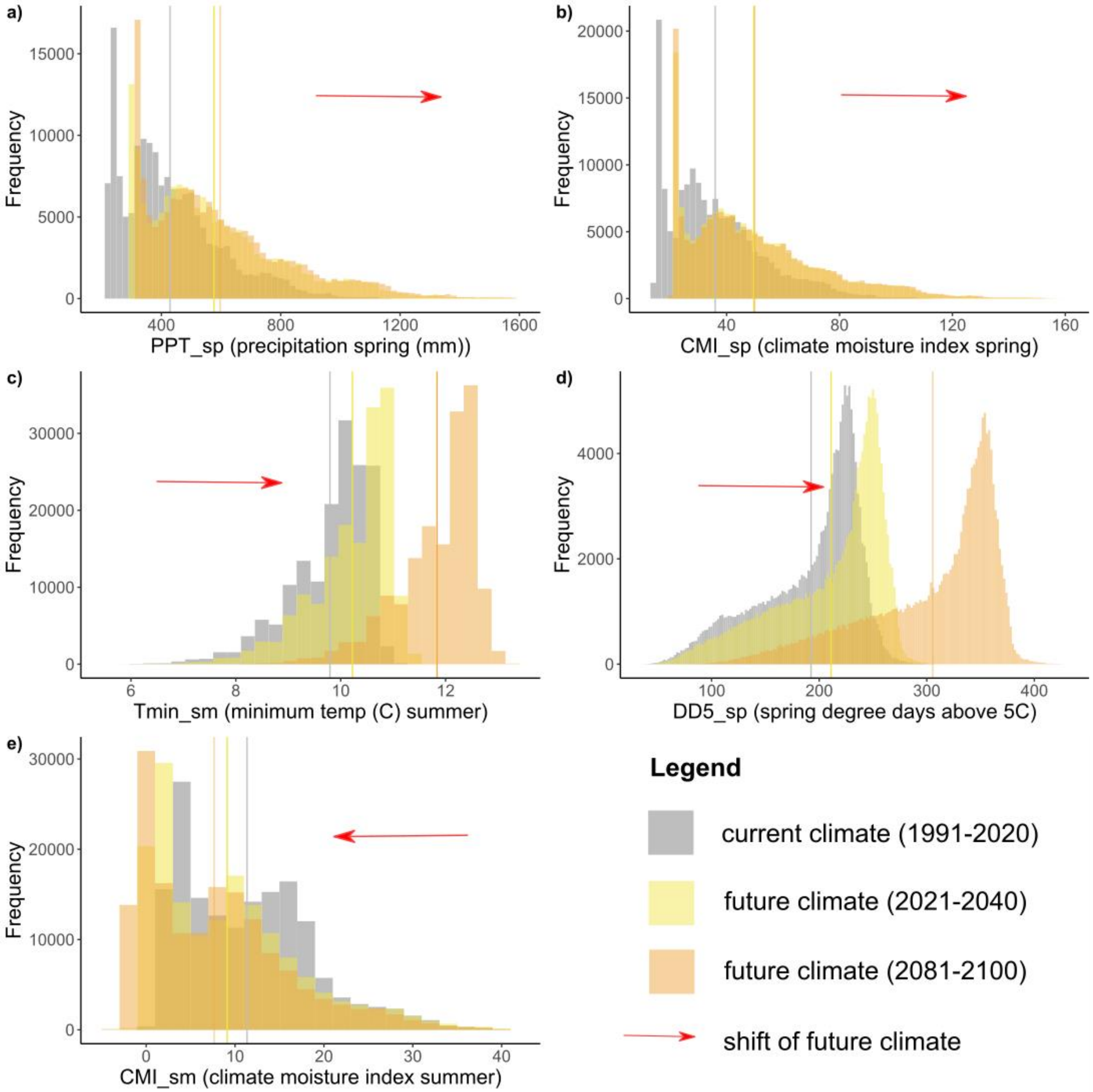


Figure 2.5. Current and future climate for Haida Gwaii for a range of variables with the means indicated as vertical lines (in the same colour as the data). a) spring precipitation; b) spring Hogg's climate moisture index; c) minimum spring temperature; d) spring degree days above 5ºC; e) summer Hogg's climate moisture index. Data from Climate BC v 7.1 (Wang et al. 2012, 2016, Mahony et al. 2021). Descriptions of the climate variables can be found in Climate BC (Wang et al. 2012).

2.4 Discussion

Motivated by reports of the concurrent spread of the Northern Red-legged Frog (NRLF) on Haida Gwaii, BC, and its decline in its native range in BC, this study aimed to provide information useful for conservation on Haida Gwaii and contribute to wider discussions of species introductions to island ecosystems and natural range shifts. I quantified the range expansion rate, realized niche, current distribution of the NRLF on Haida Gwaii, and predicted suitable habitats in the future and when they may get there. I found that NRLFs expanded their range by about 1.9 km/year and increased the size of their range 15-fold from 2002 – 2017, now occupying ~24% of Graham Island. NRLFs on Haida Gwaii showed niche conservatism with their niche in their native range and only occupied a small subset of their native niche on Haida Gwaii. This high similarity with their native range could explain in part why they have been so successful on Haida Gwaii. Additionally, the high niche unfilling indicated that their current range extent is likely limited by biotic factors or dispersal, which may just be a matter of time. Given the estimated rate of range expansion, my results suggest that NRLFs could disperse to much of Graham Island by 2040, and if assisted by humans, they will likely reach much of Moresby Island by 2100, including Gwaii Haanas. Additionally, climate change is projected to enhance habitat suitability for NRLFs on parts of Haida Gwaii, with large increases in suitability on the west coast of Graham Island and parts of Moresby Island. These areas are currently unoccupied and have significant ecological and cultural values, making them a high priority to prevent future colonization by the NRLF, if the NRLF is unwanted on Haida Gwaii.

2.4.1 Past range expansion on Haida Gwaii

NRLFs spread faster on northeastern Graham Island than mountainous areas of Graham Island. It is not surprising that range expansion was faster in northeastern Graham Island because it has very low topographic complexity compared to the western side of the island, and has relatively continuous wetland and forest habitat. These estimates are consistent with known native and non-native anuran dispersal estimates. For example, my estimates of range expansion rate are close to the range expansion rate for the introduced Pacific Chorus Frog (2 km/year) on Haida Gwaii (Reimchen 1991), and are well within estimates of other non-native amphibian range expansion rates (Urban et al. 2008). My estimates are also within the maximum known seasonal straight-line dispersal rate of the NRLF in its native range (4.8 km/year; Hayes et al. 2007). Additionally, my results suggest that humans have likely spread the NRLF to some areas in recent years.

2.4.2 Realized niche comparisons

NRLFs show niche conservatism and a high degree of niche unfilling on Haida Gwaii. The fact that much of Haida Gwaii's environmental space is within the native niche of the NRLF likely in part explains why they have successfully established on Haida Gwaii. Additionally, it is possible that a release from threats in the native range such as predators, pathogens and urbanization on Haida Gwaii could be responsible for NRLF's rapid range expansion. NRLFs on Haida Gwaii only occupy a small subset of their realized niche in their native range, with 86% of the predicted niche available remaining unfilled (niche unfilling; Guisan et al. 2014). High unfilling suggests that dispersal limitation or unidentified biotic factors are constraining the realized niche on Haida Gwaii (Simberloff 2009) which, if overcome, indicates that NRLFs have the potential to expand their niche on Haida Gwaii to match that of the native niche. It is possible that predators, pathogens or competitors may play a role in constraining the range of the NRLF on Haida Gwaii (Mack et al. 2000). On Haida Gwaii, introduced raccoons have been identified as a threat to toads (Burles et al. 2005, Johnston 2006). Whether these or other species (e.g., feral cats) influence the realized niche of NRLFs remains to be determined.

My high niche unfilling result aligns with studies of introductions to islands, but my niche conservatism result does not. My results are consistent with the high niche unfilling often observed in non-native species recently introduced to islands, in part due to dispersal limitation (Li et al. 2014). Conversely, while the niche conservatism that I observed is consistent with niche conservatism seen in many non-native species (Liu et al. 2020), it contrasts with the lack of niche conservatism seen in most amphibian introductions to islands (Liu et al. 2021). This contrast could be because environments on Haida Gwaii are similar to the native range, whereas other non-native amphibians were introduced from very different ecosystems and habitats. Additionally, a lack of niche conservatism can sometimes result from genetic changes (Sherpa et al. 2019). The NRLF has likely been on Haida Gwaii no more than thirty years, and although rapid evolution has been observed on that timescale (Otto 2018), the NRLF population on Haida Gwaii may have low genetic diversity due to a founder effect, and therefore possess low capacity to evolve (Hoban et al. 2020, Allendorf et al. 2022). Furthermore, it is unlikely there would be strong selective pressures for the NRLF on Haida Gwaii, given the biogeoclimatic similarities to the native range.

2.4.3 Current and future habitat suitability and range potential on Haida Gwaii

Based solely on their range expansion rate (and not whether habitat is suitable), NRLFs appear capable of expanding their range across much of Graham Island by 2040 (Figure 4a), and could reach Gwaii Haanas National Park Reserve and Haida Heritage Site by 2081, if Moresby Island were colonised within a decade (Figure 4b). If NRLFs disperse to all habitat that is predicted as suitable under current climate conditions on Graham Island, their range will include most of the eastern side of the island, and most of the central north of the Island. Furthermore, climate change is likely to enhance mean habitat suitability for NRLFs, which could mean that areas of the west coast of Haida Gwaii, northeast Moresby Island, and parts of Gwaii Haanas are highly suitable in the future and could likely support the NRLF should they reach there.

Habitats that increase in predicted suitability with climate change are associated with increases in precipitation and temperature on Haida Gwaii. Climate change is predicted to increase spring climate moisture index, precipitation, minimum temperature and degree days above 5ºC on Haida Gwaii (Figure 2.5a-d). The response curves of all four of these variables are positively correlated with habitat suitability (Appendix A; Figure A5), likely explaining why my results show habitat suitability increases on Haida Gwaii with climate change. However, climate change is also predicted to decrease summer climate moisture index on Haida Gwaii (Figure 2.5e), and this variable is negatively correlated with habitat suitability (Appendix A; Figure A5).

My finding of increased suitability aligns with Pauly et al. (2008), who also predicted an increase in habitat suitability for NRLFs on Haida Gwaii under climate change. However, by increasing observations 9-fold, using high resolution climate data and mapping at finer resolutions, my results refined these earlier predictions. Instead of showing a blanket increase in habitat suitability everywhere on Haida Gwaii (Pauly et al. 2008), my results show that predicted habitat suitability may increase in some areas, but reduce in others (Figure 2.4). Notably, habitat suitability will likely increase with climate change on the west coast of Graham Island (particularly around Naden Harbour) and northern Moresby Island (particularly around Sandspit), while many other areas of their current core range may become less suitable (including their current range). These more nuanced estimates of habitat suitability could aid managers planning at local scales (discussed in conservation implications).

2.4.4 Limitations and future research

Given that my predictions of range expansion do not estimate landscape resistance or population demographic factors potentially affecting dispersal rate, my predictions may therefore overestimate rate of spread, particularly in more topographically complex regions of Haida Gwaii. Conversely, the predictions could be an underestimate if NRLFs are accidentally or intentionally transported to new areas by humans, which could be a problem as tourism increases. However, given the lack of dispersal barriers and availability of suitable habitat in the currently occupied area, this estimate is likely reasonable, especially for northeast Graham Island. Placing transceivers on adult NRLFs and carrying out annual surveys at the range front would provide a more accurate prediction of when NRLFs may colonize new areas of Haida Gwaii.

Furthermore, the accuracy of my model may be inflated, as is often the case in SDM studies, due to a number of factors (Lee-Yaw et al. 2021, Santini et al. 2021). For instance, my model did not incorporate biotic interactions or local demography, even though both could influence habitat suitability and population persistence. Additionally, the habitat descriptors used here were estimated in 2002, whereas most of the surveys included were conducted after 2010.

Because significant change in land cover has taken place since 2002 on Haida Gwaii (e.g., logging) and in the native range (e.g., logging and urbanization), models based on updated habitat mapping could be used to improve model precision, and perhaps also evaluate the influence of forest harvest on the permeability or suitability of habitat for NRLFs. Hence, it is important to emphasize that the habitat suitability maps are, predictions, and may not accurately represent the future distribution of the NRLF on Haida Gwaii.

2.4.5 Conservation implications

Given the negative history of species invasions on Haida Gwaii and islands generally (Gaston et al. 2002, Russell et al. 2017), and the results of Chapter 3, the potential for NRLFs to increase in distribution with climate change on Haida Gwaii and reach significant conservation areas such as Gwaii Haanas may be concerning. Management aimed at controlling the spread of NRLFs should focus on areas predicted to be highly suitable but not yet occupied, areas with natural dispersal barriers, and areas frequented by humans. Within Graham Island, an effort could be made to stop them reaching the west coast by checking boats going to Naden Harbour, and the west coast, and if they turn up in Rennell Sound, to remove them immediately. The same could be done for boats crossing Skidegate Channel to stop colonization of Moresby Island. Education of local people and tourists, and in particular, children, who are travelling to unoccupied areas – such as Moresby Island (particularly Sandspit) and the west coast of Graham Island (particularly Naden Harbour) would be a good starting point. If NRLFs do reach Moresby Island, then a large effort should be made to remove egg masses immediately after their discovery, to prevent them establishing and potentially colonizing Gwaii Haanas. It is important to note that species often take several introductions to successfully establish, in part due to the genetic variation required to overcome small populations sizes, and also because sometimes introductions fail (Sakai et al. 2001, Dlugosch and Parker 2008). Hence, ongoing management would not be futile simply because some NRLFs reached Moresby Island.

If the NRLF is not deemed to have negative ecological or cultural impacts on Haida Gwaii, whether the NRLF should be considered an “invasive species” on Haida Gwaii or not becomes in part a question of values. Such ethical dilemmas will become more common as climate continues to change and species shift naturally (Willis and Birks 2006, Hofman and Rick 2018, Urban 2020). The cultural values of the Haida Nation, who co-manage wildlife on Haida Gwaii, will be a very important part of this decision. Additionally, Haida Gwaii is a Pleistocene refugium, and generally species introductions to these unique ecosystems are viewed negatively. Future research could investigate whether the NRLF has a negative impact on the species, ecosystems and cultural values of Haida Gwaii which, if true, would help make the course of action more obvious.

No matter the decision on how to view the introduction on Haida Gwaii, the fact that the NRLF is predicted to spread with climate change in another area of BC may give some hope for conservation in its native range. The NRLF is declining in its native range within BC (COSEWIC 2015) and Gerick et al. (2014) predicted that 45% of the current native range of the NRLF in BC will be thermally limiting by 2080. Perhaps areas of the native range in BC that are most similar to the parts of realized niche on Haida Gwaii that are predicted to increase in habitat suitability with climate change could be good candidate areas for focussed conservation actions, such as habitat restoration or invasive species removal. Additionally, areas near the northern range limit of the current range in BC could potentially be considered for recipient sites for assisted migrations. However, local research should be conducted to identify any potential negative impacts on naïve species in these areas (Chapter 3). The Haida Gwaii population of the NRLF may have conservation value in the future if NRLF populations in the native range continue to decline due to climate change or other stressors. The Haida Gwaii population could potentially be used as a source to recolonize the native range of the NRLF.

Amongst the SDM climate change literature, there are varying predictions of both increases and declines of non-native amphibians with climate change. My prediction that habitat suitability for the NRLF will increase on Haida Gwaii with climate change aligns with studies predicting increased habitat suitability with climate change for non-native amphibians (e.g., Cane Toad; Urban et al. 2007). On the other hand, my predictions contrast with others that predict declining habitat suitability with climate change (Bellard et al. 2013), such as with the American Bullfrog (Nori et al. 2011). My results align with studies demonstrating variability in climate change predictions within a single species. For instance, Ihlow et al. (2016) predicted that the invasive distribution of the African Clawed Frog (*Xenopus laevis*) would decline with climate change in some areas that get drier and colder (due to a strong lower thermal limit), and increase where precipitation increases and temperatures are milder. Many studies that predict decreasing habitat suitability with climate change compared native and non-native ranges that were extremely biogeoclimatically different. The non-native range was often located on a different continent from the native range, with completely different species assemblages and habitats. In contrast, in my study, the non-native range is within the same bioregion as the native range. As a result, the range expansion of the NRLF on Haida Gwaii and the predicted increase in habitat suitability with climate change is somewhat analogous to a natural climate-induced range shift. Therefore, the results of my study may offer some hope that poleward range shifts or assisted migrations may offer a viable conservation strategy for conserving species that are declining in their native ranges in the face of climate change and habitat destruction.

Chapter 3: Co-occurrence history affects interspecific competition between an introduced frog and island versus mainland toad populations

3.1 Introduction

Species extinctions on islands account for two-thirds of all recent extinctions (Tershy et al. 2015), and invasive species are a main driver of biodiversity loss on islands (Bellard et al. 2016, Doherty et al. 2016, Duenas et al. 2021). As a consequence, understanding what makes island ecosystems vulnerable to the effects of invasive species is a key concern for biodiversity conservation (Bellard et al. 2016, Doherty et al. 2016). Reasons for this vulnerability include a high degree of endemicity, small population sizes, lower intraspecific genetic diversity, and in some cases, naïveté of native species to the invader (Mack et al. 2000, Tershy et al. 2015, Moser et al. 2018). A species is naïve when it has no prior experience interacting with another species or functionally similar organism, and this lack of experience often translates to naïveté, which is the failure to recognise and respond appropriately to a novel enemy, pathogen or competitor (Diamond and Case 1986, Cox and Lima 2006).

Furthermore, climate induced range shifts and intentional human translocations may also bring species together in areas where they have never interacted even if they are sympatric (co-occur) elsewhere. This differs from most invasions, where native species usually have no previous co-occurrence history with the invader anywhere in their native range. Understanding when and why some populations respond naïvely to new occurrences of a species they naturally co-occur with elsewhere within their range has implications for understanding the vulnerabilities of island species to invaders, and the potential consequences of assisted migrations and climate range shifts (Urban 2020).

Naïveté has been observed in invasive ecological interactions such as plant-herbivore (e.g., Desurmont et al. 2011), competitive (e.g., Heavener et al., 2014) and predator-prey (e.g., Cox and Lima 2006, Salo et al. 2007). Competition from a novel species can result in the native species undergoing character displacement, niche shifts, and in some extreme cases, competitive exclusion. These effects may be stronger if the native species is naïve and has never encountered similar competitors (Eastwood et al. 2007, Bøhn et al. 2008, Dufour et al. 2018). For instance, Wilson & Holway (2010) found that naïve native bees on Hawaii were displaced by competitively superior invasive wasps through reduced prey availability. Conversely, there are species that have rapidly adapted to new species in their environment. For example, in response to an introduced lizard, a native lizard adopted high tree perches and within 20 generations this resulted in inherited morphological changes to the native lizard (Stuart et al. 2014).

We are still a long way from understanding all the factors that influence the degree of competitive naïveté – and hence our ability to predict the potential impacts of species range shifts on naïve communities. Research in mosquitos has shown that competitive ability can vary geographically (Leisnham et al. 2009), and some research in plants has shown that the strength of competition depends on co-occurrence history (Germain et al. 2016). The role of co-occurrence history with predators has been a key point of investigation in prey naïveté, more so than competition, since direct predation by introduced species can cause drastic and easily observed ecological effects (Sih et al. 2010). Historical exposure to non-native predators may lessen the degree of prey naïveté (e.g., Anderson and Lawler 2016, Saxon-Mills et al. 2018, Bytheway and Banks 2019, Cummins et al. 2020). While the role of co-occurrence history in determining prey naïveté is well studied in vertebrates, its role in competitive naïveté has received much less attention. This is in part because the effects of competitive interactions on population persistence are indirect and can be more difficult to detect (Gilbert and Levine 2013). To date, studies of naïveté in competing vertebrates looked exclusively at populations with no prior history with the non-native competitor (i.e., naïve populations), making it difficult to determine if the length of co-occurrence history affects competition with non-native species. Understanding the role that co-occurrence history may play in the degree of naïveté to antagonistic competitive interactions is highly relevant to anticipating the impacts of, and planning conservation responses to, climate-induced range shifts and assisted migration of species into novel suitable habitats.

Here, I asked whether a geographically isolated population of a species will be weaker competitors against a recently introduced non-native species, compared to a population that has a long history of sympatric co-occurrence with the competitor. This was motivated by the possibility that species or populations may be ill-equipped to interact with a non-native species that they have not recently co-occurred with (Cox and Lima 2006). To test this, I measured the effect of Northern Red-legged Frogs (*Rana aurora*; NRLF) competition on island and mainland populations of the Western Toad (*Anaxyrus boreas boreas*) with different co-occurrence histories. The Western Toad is the only indigenous amphibian on the remote archipelago of Haida Gwaii, British Columbia (BC). The NRLF was recently introduced to Haida Gwaii sometime in the last 25-35 years (Gaston et al. 2002, Ovaska et al. 2002), but is native to the adjacent mainland (Figure 3.1) (COSEWIC 2015) where the Western Toad is also native and the two species historically co-occur at the pond scale (COSEWIC 2012). Though only correlative, there is concern that the NRLF may have a negative impact on the toad because Western Toad sightings in some areas have declined since the NRLF and Pacific Chorus Frog (*Pseudacris regilla*; also known as the Northern Pacific Tree Frog) were introduced, and some researchers have speculated that the NRLF may have a negative impact on the toad (Reimchen 1991, Gaston et al. 2002, Pauly et al. 2008). Recent surveys and modelling suggest that introduced NRLFs now occupy ~24% of Graham Island (the largest island on Haida Gwaii) and will likely expand their distribution in the future (Chapter 2). It is of high local conservation importance to determine whether the NRLF is negatively impacting the Western Toad, which is known as Hlk'yáan K̲'ust'áan in the X̲aad Kíl dialect of the Haida language (X̲aad Kíl Née; Haida Language Council) and is highly valued by the Haida Nation.

The long co-occurrence of sympatric NRLFs and Western Toads on the mainland, the geological and eco-evolutionary history of the remote Haida Gwaii archipelago, and the very recent appearance of NRLFs in Haida Gwaii provides an ideal system in which to test the effect of co-occurrence history on competition in vertebrates. I predicted toads from Haida Gwaii with a short co-occurrence history relative to toads from the mainland, would: P1) have slower growth and development under competition with NRLFs than mainland toads; P2) be impacted by competition with NRLFs to a similar magnitude as intraspecific competition; and P3) result in greater NRLF growth and development as compared to competition with mainland toads. To estimate the effect of competition, I measured Western Toad and NRLF growth rate, weight at metamorphosis, and timing of metamorphosis using an outdoor tadpole mesocosm experiment, with tadpoles of both species from Haida Gwaii and the Lower Mainland (Figure 3.1).

3.2 Materials and methods

3.2.1 Study system and species

Haida Gwaii is an archipelago ~50-100km off the coast of northwest coast of British Columbia (BC) and has been separate from the mainland for at least ~10,000 years, since the Last Glacial Maximum (LGM) (Barrie et al. 2005). It has an extremely depauperate native vertebrate community and a long history of non-native species' introductions (Gaston et al. 2002). The Western Toad is the only indigenous amphibian on Haida Gwaii, while introduced NRLFs were first recorded on Haida Gwaii in 2002 (Gaston et al. 2002, Ovaska et al. 2002, Pauly et al. 2008). Both species are native to southwest BC, and are of "Special Concern" under Canada's Species at Risk Act, both experiencing population declines in their southwest BC range (COSEWIC 2012, 2015). These two species have co-occurred in southwest BC (Figure 3.1) since scientific records began in 1800 (GBIF.org 2020a, 2020b) and likely for thousands of years, since at least the post-glacial expansion of NRLF northward along the North American coast (Shaffer et al. 2004). Western Toads on Haida Gwaii likely have no prior experience with the NRLF before the recent introduction of NRLFs, because it is unlikely that the range of the NRLF ever extended to the same northern latitude as Haida Gwaii (Shaffer et al. 2004). In addition to the 20-30 year experience recent co-occurrence with the NRLF, Western Toad on Haida Gwaii has an additional ~60 year experience with the functionally similar (at the tadpole life stage) Pacific Chorus Frog, also native to BC, introduced to Haida Gwaii in 1933 (Reimchen 1991).

The Western Toad is distributed from Southern Alaska to Baja California and as far east as Colorado (IUCN SSC Amphibian Specialist Group 2015).Its range almost entirely encompasses the comparatively smaller range of the NRLF along the Pacific Coast of North America (Figure 3.1; GBIF.org (2020b), (2020a)). Within BC, the subspecies of these two species are *Anaxyrus boreas boreas* and *Rana aurora* and the NRLF has likely been introduced from Vancouver Island (Pauly et al. 2008). Within their range overlap, these species co-occur in syntopy (i.e., occupy same habitats at local scale), particularly in breeding habitats, as do many pond-dwelling anurans (Petranka et al. 1994). It is unknown if they compete with each other as larvae, but the NRLF has been reported to compete under experimental conditions for larval food sources with another sympatric anuran in southwest BC, the Pacific Chorus Frog (Hamilton et al. 2012).

Western Toads and NRLFs co-occur as tadpoles at the pond scale in the wild in both Haida Gwaii and southwest BC. Both species explosively breed in relatively shallow (~30-200cm) ephemeral and permanent open freshwater features, and egg masses and tadpoles of both species are often found in the same breeding habitats, though not necessarily in the same microhabitats. Ecological interactions may be moderated by spatial and temporal segregation due to differences in environmental tolerance. Western Toads aggregate in the spring as water temperatures rise above a daytime maximum of 10°C, laying strings of tens of thousands of small eggs in warm shallow water. Tadpoles hatch within a few weeks and aggregate into swarms of thousands that seek out warm shallow water during the day. In contrast, within their southwest BC range, NRLFs breed in late winter or early spring, aggregating to oviposit large globular masses of hundreds of larger eggs in calm, comparatively deeper and cooler waters of at least 6-7°C. The difference in breeding time means that NRLF tadpoles are larger than Western Toad tadpoles, possibly resulting in a competitive advantage through both larger size and priority effects (Lawler and Morin 1993). Tadpoles of both species are reported to feed on filamentous green algae; toads are also known to be detritivores, opportunistic scavengers, and at high densities, predaceous and cannibalistic (COSEWIC 2012, 2015). This study focusses on the effect of competition on growth and development during the tadpole life stage as that is when the two species' life histories are assumed to have the largest period of direct overlap in the natural environment.

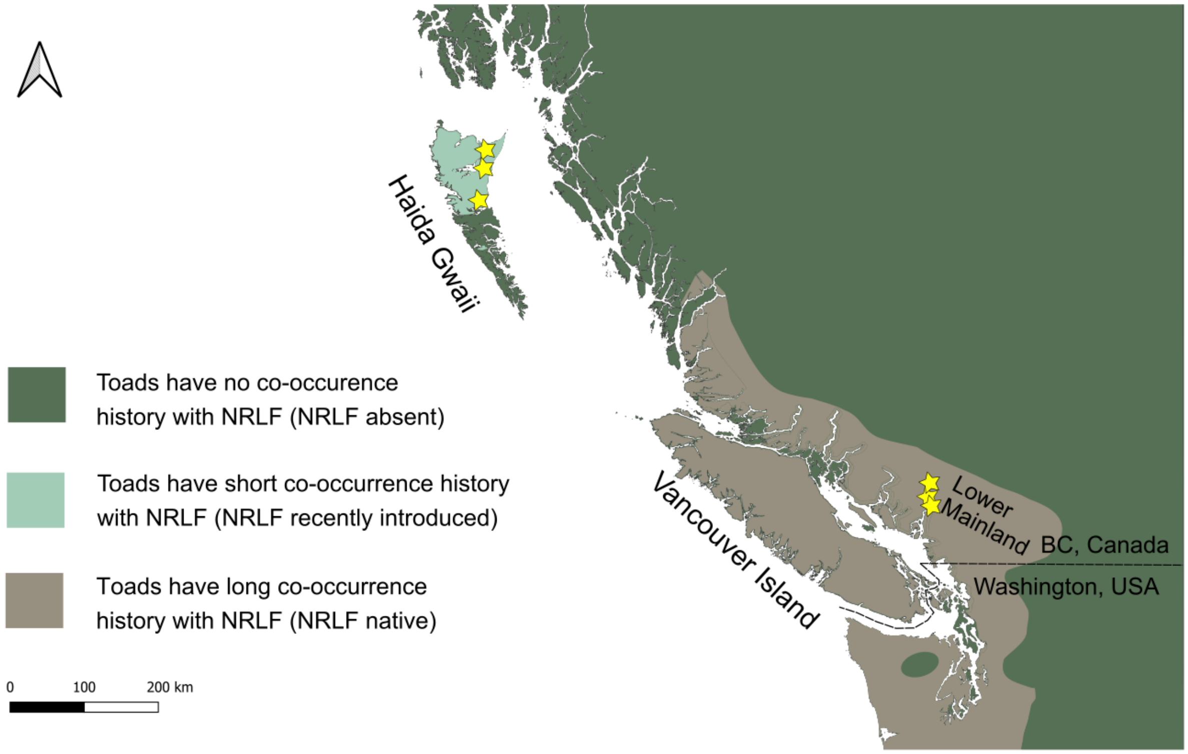


Figure 3.1. A map of Western Toad and Northern Red-legged Frog (NRLF) co-occurrence on the Pacific Coast of British Columbia, Canada and northern Washington state, USA. Sources for species distributions: (COSEWIC 2012, 2015). Stars indicate where toad populations were sourced for this study. NRLF populations were sourced from both regions.

3.2.2 Egg collection and experimental mesocosms

Western Toad and NRLF eggs were collected from the southwest mainland of British Columbia (BC), Canada, and Haida Gwaii BC, in spring 2018. On April 30th, 2018, 1250 Western Toad eggs were collected from three toad breeding sites, and 192 NRLF eggs were collected from several sites, in forested landscapes between the communities of Squamish and Whistler on the southwest mainland (~50° latitude). Large portions of the southwest coast within the range of the NRLF are highly urbanized and fragmented, so eggs were collected from breeding sites in forested landscapes more typical of those in Haida Gwaii. One breeding site was defined as a single water body where amphibians breed; eggs were collected from numerous different clutches within each breeding site. On April 30th, 2018, 192 NRLF eggs were collected from one pond on central Graham Island, Haida Gwaii, Canada at roughly 53.5° latitude by BC Parks staff. On May 17th, 2018, roughly 1250 toad eggs were collected from each of three breeding sites on Graham Island, Haida Gwaii, and flown to Vancouver by BC Parks and Ministry of Forests, Lands, and Natural Resource Operations staff. The eggs were raised to tadpoles in 1135L outdoor tanks. On the 25th of May 2018, the tadpoles were put in the outdoor experimental mesocosms, and the experiment was started. At this point, tadpoles were at Gosner stage 26 for both species (Gosner 1960). The experiment was ended after 90 days, on August 26th, 2018. Wildlife permits were obtained to conduct this sampling – see thesis preface. One female toad lays up to 16,000 eggs (COSEWIC 2012), so egg sampling of these populations should not have a substantial effect on population demographics.

The outdoor mesocosm experiment was conducted at the University of British Columbia’s South Campus Experimental Ponds Facility (Appendix B; Figure B2). Seventy-two, 1135 L Rubbermaid commercial stock tanks 160 cm (length) × 175 cm (width) × 63 cm (depth) were placed in a grid, 1 m apart (Appendix B1 and B2). To simulate the depth and temperature gradient of a pond littoral zone and account for anticipated segregated microhabitat usage between species, the tanks were placed on an incline of 9.3° (by placing cinder blocks under one end). The water was 21 cm deep at the deepest end, and 2 cm deep at the shallow end. 12.5 kg of sterilized, washed ‘Quikrete Premium Play Sand’ was spread on the bottom of each tank with 65 grams of dried poplar leaves to encourage the growth of periphyton, provide a detrital food source, and create substrate complexity. To ensure adequate algal growth, five weeks before starting the experiment, the tanks were filled with: 327 L of dechlorinated municipal water, 40 L of nearby pond water (filtered through a 250 µm sieve), and 500 ml of concentrated zooplankton collected from nine lakes in the Metro Vancouver. At the start of the experiment, before adding tadpoles, 4 L of water was systematically swapped (to adjacent tanks within the same rows, and then to tanks in opposite rows) among all tanks to ensure plankton community composition was similar. Water temperature was recorded every 30 minutes for the full duration of the experiment, with HOBO temperature loggers placed in the deepest part of each tank.

Predator fencing (plastic 625 mm^2 mesh) was placed over each tank before the experiment started which kept out large predators (such as birds), but it did not keep out predatory diving beetles. Two weeks into the experiment we noticed that tadpole densities had decreased, and we discovered that predatory diving beetle larvae were eating the tadpoles. As a result, tanks were searched and predators were removed, and remaining tadpoles were tallied. Tadpoles of comparable size and weight were added from the same original egg masses, that were reared in synchrony with but separate from the experimental tanks as replacements. Sixty per cent of the tanks were lightly affected by the diving beetles, requiring ≤12.5% of the original tadpoles replaced; 15% of the tanks were significantly affected, requiring tadpole additions of ≥50%. After the diving beetle predation incident, shade cloth (blue knitted mesh, shade factor 51% - so still let enough light in for photosynthesis; Midland Industrial Covers, British Columbia, Canada) was added over each tank to prevent diving beetles from colonising tanks, and to reduce water temperature as the summer progressed.

3.2.3 Experimental design

I used a 2 x 3 factorial design with two co-occurrence histories and three competitor treatments (Figure 3.2). This was replicated across three blocks each containing 18 tanks (along a West – East spatial gradient and a natural temperature gradient; Appendix B; Figure B2 and Figure B3). Toads were drawn from three breeding sites from each co-occurrence history/toad source region (Haida Gwaii or Lower Mainland), yielding a total of 54 tanks. Every replicate included 80 toads from a specific toad breeding site; thus treatments varied by co-occurrence history, and competition treatment; i.e., the identity and source of the additional animals sharing the mesocosm with the 80 toads. The co-occurrence history treatments were: 1) toads with a short history with the NRLF (decades – sourced from Haida Gwaii); and 2) toads with a long history with the NRLF (thousands of years – sourced from the Lower Mainland). The competition treatments were: 1) competition with toad conspecifics from one toad breeding site (160 toads/tank; n=18); 2) allometrically-scaled competition with NRLFs from Haida Gwaii (80 toads and 16 NRLFs /tank; n=18); and 3) allometrically-scaled competition with NRLFs from the Lower Mainland (80 toads and 16 NRLFs /tank; n=18). The high-density toad competition treatment still served as a control to assess the relative effect of NRLFs as competitors as opposed to just increasing biomass. Comparing *inter*specific competition to *intra*specific competition is commonly done in tadpole competition experiments (e.g., Richter-Boix et al. 2004, Haramura et al. 2022).

Initially the experiment included a low density (80 toads/tank; n = 18) toad competition treatment. However, there was extremely high toad mortality in the low density Haida Gwaii toad tanks (Appendix B: Figure B4). This was in part due to the high predation by diving beetles on many of these tanks, which was unrelated to treatment, and likely kept going even after most predators were removed two weeks (Appendix B; Figure B5). As a result, I excluded all low-density toad treatment tanks from the analysis (n = 18 tanks removed). Unfortunately, this meant that I was unable to assess the effect of increased amphibian biomass alone.

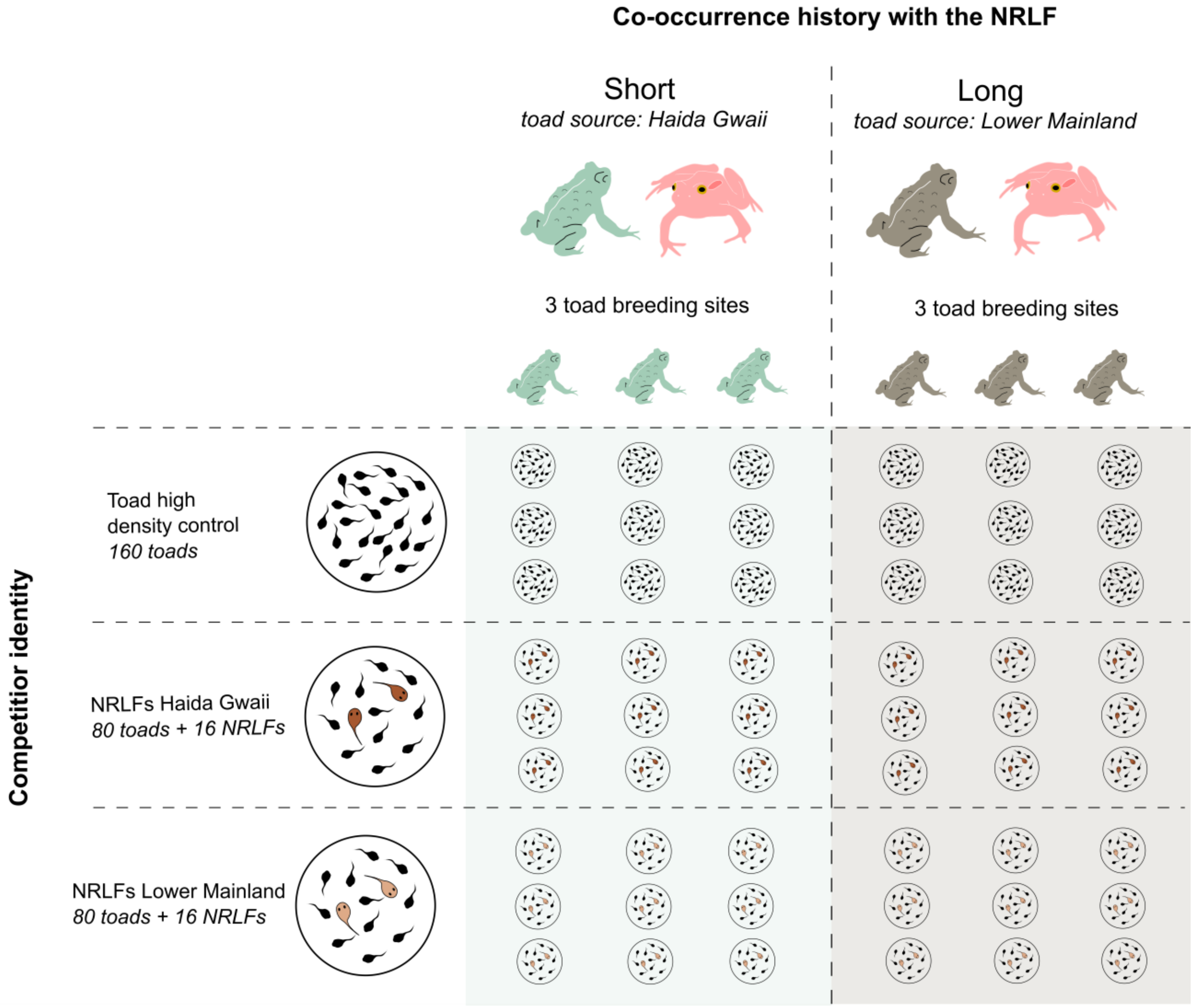


Figure 3.2. A 2 x 3 factorial experimental design with two treatments (co-occurrence history and competition). 1) The Western toad's co-occurrence history with the Northern Red-legged Frog (NRLF); short co-occurrence = decades, versus long = thousands of years; with three toad populations per co-occurrence

history, and 2) three competition treatments with three different competition identities (toads, NRLFs from Haida Gwaii, NRLFs from the Lower Mainland). Replicated three times for a total of 54 mesocosms.

The *inter*specific competition treatments between NRLFs and toads were designed to be allometrically equivalent to the high-density *intra*specific toad competition treatment (i.e., result in similar metabolic requirements and therefore likely to require a similar amount of resources) so that any effect could be attributed to NRLF presence rather than overall biomass. At the start of the experiment, NRLF tadpoles were six times heavier (0.098g) than toad tadpoles (0.018g) due to both species differences and because NRLFs hatched a month earlier than toads. Competition in amphibian experiments is often driven by resources (Alford and Harris 1988), and larger organisms eat more (Ding et al. 2015), so instead of equal numbers, a ratio of biomass was used that was allometrically scaled (Schmidt-Nielsen and Knut 1984, Savage et al. 2004). To determine the ratio of species in the *inter*specific competition treatment between NRLFs and toads, an allometric scaling coefficient of 0.75 was used to derive the numerical ratio to 1:5 to make it approximately equivalent to the high *intra*specific toad treatment. This corrected for mass-specific metabolism aimed at producing equal mass of NRLFs and toads (Schmidt-Nielsen and Knut 1984, Savage et al. 2004). It translated to 16 NRLF tadpoles and 80 toad tadpoles.

The density of NRLF tadpoles (0.05 tadpoles/L) was on the lowest end of recorded natural densities for this species (0.05-1.8 tadpoles/L) (Kiesecker et al. 2001). Experimental densities for toad tadpoles in all treatments were low relative to the usual schooling of toad tadpoles under natural conditions (pers observation). Toad tadpole densities were kept low to minimize levels of mortality observed with higher densities in mesocosm experiments (Govindarajulu 2004).

3.2.4 Responses to competition

Three commonly used measures of larval performance under competition were chosen as response variables for the strength of competition: 1) growth rate, 2) weight at metamorphosis, and 3) median time to metamorphosis. All three responses measured are known to be negatively affected by increased larval competition in amphibians; as competition between larvae increases, growth rate and weight at metamorphosis decreases, while the time to metamorphosis increases (Wilbur 1972, 1984, Morin 1983). Additionally, all three larval response variables are known to impact post-metamorphic fitness and survival (Berven and Gill 1983, Smith 1987, Berven 1990, Pechenik 2006, Cabrera-Guzmán et al. 2013). Amphibians were weighed using a G636-EJ-303 Newton 310 scale with an accuracy of 3 mg. All tadpoles were weighed at the start of the experiment and several times before metamorphosis. Other than the tadpole top-up at week two following diving beetle prediation, there were no other replacements. Once metamorphs began to emerge on day 55, metamorphs were weighed and removed from the tanks every three to four days (ten times in total) until we ended the experiment on day 90 (logistical constraints). On day 90, only 57% of all amphibians in the experiment had metamorphosed and as a result, we were unable to estimate survival to metamorphosis, as is commonly measured in amphibian experiments. My results for median time to metamorphosis were not biased by the fact that we ended the experiment early because we were interested in the relative difference in timing of metamorphosis between treatments rather than estimating the actual timing of metamorphosis.

Response variables were measured and calculated per tank (i.e., treatment replicate) for analyses. Individuals from each tank were counted and weighed (in several separate batches) to get a tank average (T) per species. **Weight at metamorphosis** (W) was calculated per tank (n=54), per weighing day(n=10), and was the average weight of metamorphs (mg) at Gosner stage 46; n = 540. **Growth rate** (R) was calculated per tank (n=54), per weighing day (n=10), and was the mass gained each day during the larval period; R (mg/day) = W (mg) / days (Richter-Boix et al. 2013); n = 540. Time to metamorphosis for every individual was defined as the time from Gosner stage 26 to Gosner stage 46 (Gosner 1960). I calculated **median time to metamorphosis** on a per tank basis for the whole experiment (i.e., a median value of all times to metamorphosis per tank); n = 54. Before running statistical analyses for toad growth rate and average weight at metamorphosis, two outlier measurements were removed (for two different weighing days) that were a result of errors during data collection.

3.2.5 Statistical analyses

To test the predictions, I used linear mixed-effects models implemented in the *lme4 R* package (v 1.1-27.1, Bates et al. 2007). The models tested the fixed effects of co-occurrence history and competition treatment and how these two variables interacted to influence the response variables. In addition, I also added several variables to control for. Block was modelled as a fixed effect, as recommended by Dixon et al. (2018) and Gelman and Hill (2006) because there were less than four blocks. Average mean water temperature was included for each tank across the duration of the experiment for all models as a fixed effect, because temperature is known influence larval development (Herreid and Kinney 1967, Harkey and Semlitsch 1988, Alvarez and Nicieza 2002). Initial average weight of an individual tadpole (per tank) was included as a fixed effect in the models of growth rate and weight at metamorphosis because these responses are known to be a function of initial body weight (Werner, 1986). I also included the tadpole additions *per capita* (relative to original number of individuals) in response to diving beetle predation in week two as a fixed effect in all models, as well as total mortality *per capita* over the course of the experiment to account for the effect on decreased competition when there were less tadpoles. Mortality *per capita* (M) was calculated by counting living individuals and subtracting that from initial numbers; $M = (N_e - N_s) / N_s$, where N_e is the number of individuals that did not die (i.e., were either removed due to metamorphosis or were remaining at the end of the experiment – in a variety of different life stages – and includes the tadpole top ups) and N_s is the total number of tadpoles at the start per tank; N = 54.

Toad breeding site was included as a random effect (random intercept) to control for within-population differences within each toad source region. For the models that included repeated measures (growth rate and weight at metamorphosis), days since the experiment began was modelled as a fixed effect, and “tank” was included as a random effect with a random intercept to account for inter-tank variability, and a random slope across days for each tank to account for repeated measures. The model structure for each toad and frog response variable can be found in Appendix B; section B.1. To meet the assumptions of normally distributed residuals and response variables, growth rate and average weight at metamorphosis were log transformed, and total toad mortality *per capita* was arcsine transformed. Distributions were checked using the *qqnorm()* and *resid()* functions in the *stats* R package (R Core Team 2021). All continuous predictor variables were scaled to allow model convergence and make the effect sizes comparable between variables. Predictor variables within each model were tested to ensure they were not correlated (except for significant interaction terms) using the *check_collinearity()* function in the *R performance* package (Lüdecke et al. 2021). There were no strong influential points driving the results for any of the models; this was checked using *cooks.distance()* in the *influence.ME R* package (Rense et al. 2012).

Statistical significance was determined using *p*-values (α threshold = 0.05), which were obtained with the *Anova()* function in the *car R* package, using the Wald's Chi-Square test (Fox 2019), with Type-II sums of squares as recommend by Langsrud (2003) and Hector et al. (2010) for models with interactions. A Wald's Chj-Square test is commonly used in both tadpole competition and common gardens experiments (e.g., Skaien and Arcese 2020, Haramura et al. 2022). If a statistically significant interaction between competition and co-occurrence history was detected, post-hoc custom contrasts were used to test the specific predictions. Post-hoc tests were used because the interaction terms alone were insufficient to test the predictions. This was because the model estimates did not compare the effect of competition treatment within a given co-occurrence history, and I was interested in how the effect of competition with NRLFs compared to the control of high *intra*specific competition with toads, within each co-occurrence history. Post-hoc analyses were conducted using the *lsmeans()* function in the *LmerTest R* package (Kunzetsova et al. 2017). To account for doing multiple contrasts, the *p* values were adjusted using the Tukey adjustment. Box and whisker plots were generated using the *ggplot2* and *ggpubr R* packages (Wickham et al. 2016, Kassambara 2020). All analyses were conducted in *R* v 4.1.1 (R Core Team 2021). *R* code from analyses is available here: https://github.com/RoseannaGG/toad_competition_experiment_thesis.

The following eight post-hoc tests were conducted to assess the three predictions evaluating the effect of co-occurrence history with NRLFs on the strength of competition. All of the following contrasts were repeated for each response measure (growth rate, weight at metamorphosis and median time to metamorphosis) – so there were six contrasts for prediction 1, 12 contrasts for prediction 2, and six contrasts for prediction 3.

Prediction 1) Naïve toads from Haida Gwaii (short co-occurrence history (SCO) with the NRLF) will be affected by competition with NRLFs more than experienced toads from the mainland (long co-occurrence history (LCO) with the NRLF). Contrasts tested were:

1) The response difference between toads from Haida Gwaii (SCO) versus the mainland (LCO) in the presence of Haida Gwaii NRLFs
2) The response difference between toads from Haida Gwaii (SCO) versus the mainland (LCO) in the presence of Mainland NRLFs

Prediction 2) Naïve toads from Haida Gwaii (SCO) will be impacted by competition with NRLFs to a similar magnitude as intraspecific competition, whereas for experienced toads from the mainland (LCO), intraspecific competition will be greater than competition with NRLFs. Contrasts tested were:

1) The response difference between toads from **Haida Gwaii** (SCO) in the presence of **Haida Gwaii** NRLF versus the high density of toads from **Haida Gwaii** (SCO)
2) The response difference between toads from **Haida Gwaii** (SCO) in the presence of **mainland** NRLF versus the high density of toads from **Haida Gwaii** (SCO)
3) The response difference between toads from **Haida Gwaii** (SCO) in the presence of **Haida Gwaii** NRLF versus the high density of toads from the **mainland** (LCO)
4) The response difference between toads from **Haida Gwaii** (SCO) in the presence of **mainland** NRLF versus the high density of toads from the **mainland** (LCO)

Prediction 3) NRLFs growing with naïve toads from Haida Gwaii (SCO) should have greater growth and development than NRLFs growing with experienced (LCO) toads from the mainland. Contrasts tested were:

5) The response difference between Haida Gwaii NRLFs under competition with toads from Haida Gwaii (SCO) versus toads from the mainland (LCO)
6) The response difference between mainland NRLFs under competition with toads from Haida Gwaii (SCO) versus the mainland (LCO)

3.3 Results

Linear mixed-effects models both for the response of the toad (Table 3.1, Appendix B; effect sizes and random effects in Table B1) and the NRLF to competition (Appendix B; Table B2; effect sizes and random effects in Table B3) found a significant interaction between co-occurrence history and competition ($p < 0.05$) for all response variables, so post hoc custom contrasts were conducted to test the three predictions (presented in the following sections).

Table 3.1. Fixed effects and variables testing for a competition by co-occurrence history interaction for each Western Toad response variable. Wald's Type-II Chi-Square test, bold text denotes statistical significance (p < 0.05). Results presented for three different larval toad response variables. Toad growth rate (mg/day) and toad weight at metamorphosis (mg) were log transformed. Median time to metamorphosis was recorded in days. Effect sizes of the fixed effects and variances of the random effects are in Appendix B (Table B1).

	Toad Response to Competition								
	Toad Growth Rate			Toad Weight at Metamorphosis			Toad Median Time to Metamorphosis		
Fixed Effects Variable or Interaction	**Chisq**	**Df**	***p* value**	**Chisq**	**Df**	***p* value**	**Chisq**	**Df**	***p* value**
Toad Co-occurrence History with the NRLF	15.48	1	**p < 0.01**	14.86	1	**p < 0.01**	0.33	1	0.568
Competition	15.17	2	**0.001**	15.10	2	**0.001**	11.01	2	**0.004**
Toad Tadpoles Top up Two Weeks per Capita Scaled	5.12	1	**0.024**	5.24	1	**0.022**	0.83	1	0.361
Average Individual Toad Tadpole Starting Weight Scaled	6.15	1	**0.013**	5.89	1	**0.015**	NA	NA	NA
Toad Mortality per Capita Scaled	17.86	1	**p < 0.01**	18.24	1	**p < 0.01**	20.45	1	**p < 0.01**
Days Since Experiment Began Scaled	2.69	1	0.101	34.24	1	**p < 0.01**	NA	NA	NA
Block	0.42	2	0.809	0.44	2	0.802	1.08	2	0.581
Mean Average Water Temperature Scaled	3.70	1	0.055	3.64	1	0.056	4.51	1	**0.034**
Toad Co-occurrence History * Competition	10.36	2	**0.006**	10.22	2	**0.006**	9.19	2	**0.010**

3.3.1 Response of toads to competition with NRLFs

Toads with a short co-occurrence history (SCO, from Haida Gwaii) with NRLFs competing with NRLFs from Haida Gwaii grew 0.36 ± 0.9 mg/day (mean ± SE) slower (20.9%; p = 0.001), emerged 26.1 ± 6.0 mg lighter (20.4%; p = 0.001) and metamorphosed 7.1 ± 3.5 days later (10.5%; p = 0.022) when compared with toads with a long co-occurrence history (LCO, from the mainland) with NRLFs (Table 3.2). When NRLFs were from the mainland, there was no significant difference in the effects of competition with toads with different co-occurrence histories with the NRLF (Table 3.2). The effect is likely due to co-occurrence history rather than toad population source as the random effect of toad breeding site explained little variation in all toad response variables (variance < 0.001; Appendix B; Table B1). Overall, these results suggest that Haida Gwaii toads with a short co-occurrence history (SCO) with NRLFs performed around 11-21% worse in competition with NRLFs than toads with a long co-occurrence history (LCO) with NRLFs on the mainland, and that the effect was strongest with NRLFs from Haida Gwaii, supporting prediction one (Figure 3.3).

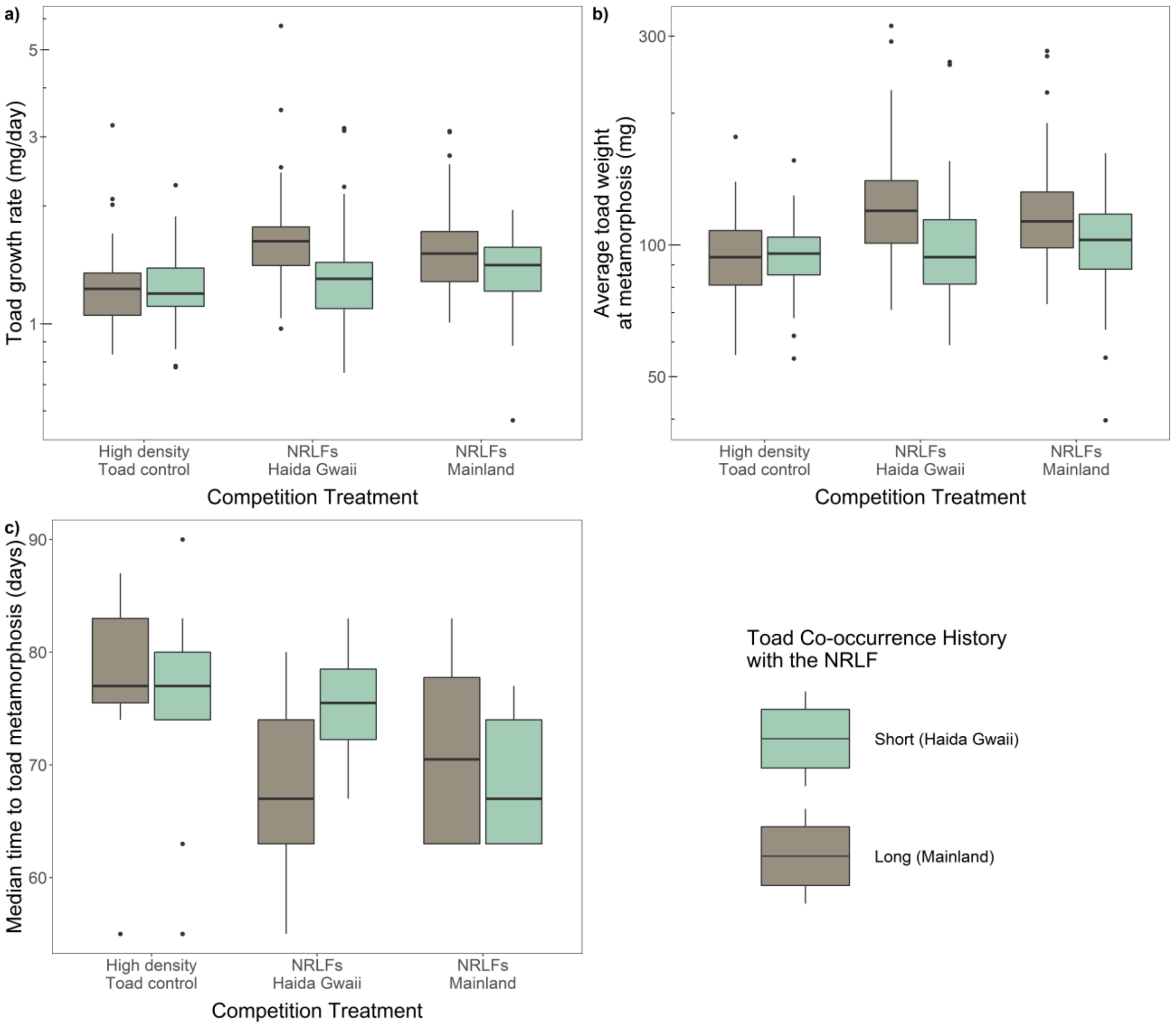


Figure 3.3. The effects of competition on Western Toad tadpoles with different co-occurrence histories with the Northern Red-legged Frog (NRLF). Results are averaged across tanks (N = 54). 3a) Toad growth rate (mg/day). 3b) Average toad weight at metamorphosis (mg). 3c) Median time (in days) to toad metamorphosis per tank (from the start of the experiment). The thick horizontal line is the median value, the upper box is the 75th percentile and the upper whisker is 1.5 × the maximum interquartile range. The lower box is the 25th percentile and the lower whisker is 1.5 × the minimum interquartile range. The outliers are values both over and under 1.5 × the 75th and 25th interquartile range, respectively.

Table 3.2. Post-hoc custom contrasts testing prediction 1 and 2 – the effect of co-occurrence history on the response of the Western Toad to competition from the NRLF. Toads with a short co-occurrence history (Haida Gwaii), long co-occurrence history (Mainland). NRLFs from both regions. Results presented for three different larval toad response variables. Toad growth rate (mg/day) and toad weight at metamorphosis (mg) were log transformed. Median time to metamorphosis was recorded in days. Statistical significance ($p < 0.05$) results in bold and have an asterisk (*). Estimates are the difference between the effect sizes from Table B1 (Appendix B).

	Contrast						
Response	Co-occurrence History with the NRLF	Competition	Estimate	SE	df	*t ratio*	*p*
Effect of toad co-occurrence history on competition with NRLF							
Toad Growth Rate	Long - Short	NRLF Haida Gwaii	0.19	0.05	21.87	4.02	**0.001** *
		NRLF Mainland	0.10	0.05	16.69	2.06	0.055
Toad Weight at Metamorphosis		NRLF Haida Gwaii	0.18	0.05	21.89	3.96	**0.001** *
		NRLF Mainland	0.10	0.05	16.70	1.99	0.063
Toad Median Time to Metamorphosis		NRLF Haida Gwaii	-8.14	3.40	39.23	-2.39	**0.022** *
		NRLF Mainland	4.12	3.35	37.62	1.23	0.226
Effect of toad co-occurrence history on competition with NRLF relative to competition with toads							
Toad Growth Rate	Short	Toad high control - NRLF Haida Gwaii	-0.01	0.04	62.05	-0.37	0.927
		Toad high control - NRLF Mainland	-0.04	0.04	65.77	-1.00	0.582
	Long	Toad high control - NRLF Haida Gwaii	-0.15	0.04	65.82	-4.06	**p < 0.01** *
		Toad high control - NRLF Mainland	-0.08	0.04	63.49	-2.28	0.066
Toad Weight at Metamorphosis	Short	Toad high control - NRLF Haida Gwaii	-0.01	0.04	62.15	-0.39	0.921
		Toad high control - NRLF Mainland	-0.04	0.04	65.72	-1.02	0.570
	Long	Toad high control - NRLF Haida Gwaii	-0.14	0.04	65.79	-4.05	**p < 0.01** *
		Toad high control - NRLF Mainland	-0.08	0.04	63.54	-2.25	0.071
Toad Median Time to Metamorphosis	Short	Toad high control - NRLF Haida Gwaii	1.98	3.33	60.87	0.60	0.823
		Toad high control - NRLF Mainland	7.05	3.22	59.81	2.19	0.081
	Long	Toad high control - NRLF Haida Gwaii	10.62	3.24	60.04	3.28	**0.005** *
		Toad high control - NRLF Mainland	3.42	3.33	61.06	1.03	0.563

3.3.2 Response of toads to competition with NRLFs versus competition with toads

The growth rate, weight at metamorphosis and time to metamorphosis of toads with a SCO with NRLFs did not different between the presence of conspecifics or the presence of NRLF competitors from either region (Table 3.2). Toads with a SCO grew 0.10 ± 0.04 mg/day (8.1%) slower, emerged 6.2 ± 3.3 mg (6.6%) lighter and emerged 0.19 ± 3.0 days later (0.26%) in the presence of conspecifics compared to NRLF competitors ($p > 0.05$ for all responses). Conversely, for toads with a LCO with NRLFs, competition from toads had a larger effect than competition from NRLFs (Table 3.2). Toads with a LCO grew slower (0.46 ± 0.09 mg/day; 36.5%; $p = 0.001$), emerged 33.0 ± 4.0 mg (34.8%; $p < 0.001$) lighter and emerged 8.6 ± 3.0 days later (11.1%; $p = 0.005$) later in the presence of conspecifics compared to NRLF competitors from Haida Gwaii (Table 3.2). Overall, these results suggest that the effect of competition from NRLFs was 11-28% greater than the effect of competition with an allometrically-scaled density of toad conspecifics when the toads came from a population with a SCO with NRLFs, which supports prediction two (Table 3.2).

3.3.3 Response of NRLFs to competition with toads

NRLFs in competition with toads with a SCO emerged 11.8 ± 2.8 days (14.2%) sooner ($p = 0.019$; Figure 3.4; Appendix B; Table B4), which supported prediction three. Though not significant, NRLFs in competition with toads with a SCO grew faster (1.7 ± 0.60 mg/day; 34.4%) and emerged heavier (70.5 ± 42.0 mg; 17.8%) than NRLFs in competition with toads with a LCO (Figure 3.4; Appendix B; Table B4). The effect is likely due to co-occurrence history rather than toad population source as the random effect of toad breeding site explained little variation in all NRLF response variables (variance = 0.008; Appendix B; Table B3).

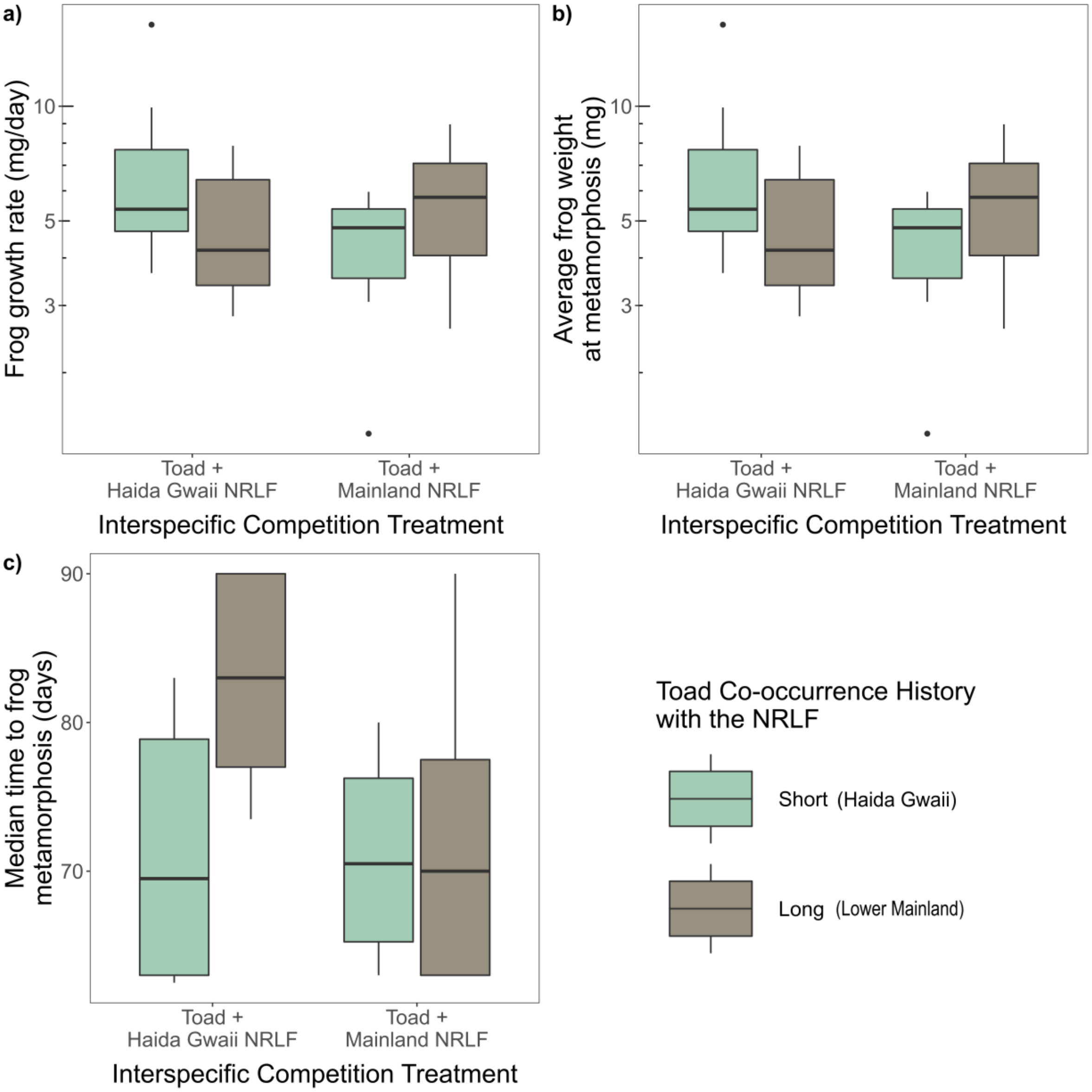


Figure 3.4. Response of Northern Red-legged Frogs (NRLFs) from Haida Gwaii and the Lower Mainland to competition with Western Toads with different co-occurrence histories with the NRLF. Results are averaged across tanks (n = 36). a) Frog growth rate (mg/degree day). b) Average frog weight at metamorphosis (mg). c)

Median time (in days) to frog metamorphosis per tank (from the start of the experiment). The thick horizontal line is the median value, the upper box is the 75th percentile and the upper whisker is 1.5 × the maximum interquartile range. The lower box is the 25th percentile and the lower whisker is 1.5 × the minimum interquartile range. The outliers are values both over and under 1.5 × the 75th and 25th interquartile range, respectively.

3.4 Discussion

This study was motivated by concern over the spread of the introduced Northern Red-legged Frog (NRLF) across Haida Gwaii (Chapter 2), observed Western Toad breeding site extirpations there, and the desire to understand the effect that range shifts will have in the future. I tested anuran larval responses to competition in an outdoor mesocosm experiment to assess the relative effects of introduced NRLF competition on a remote island population of toads. By controlling for larval density through allometric scaling between *inter*specific and high *intra*specific competition treatments, direct comparisons between these competition treatments were able to isolate the effects of competition between NRLFs and toads with different co-occurrence histories. Haida Gwaii toads (SCO) fared worse in competition with NRLFs than mainland toads (LCO), in terms of toad growth rate, weight at metamorphosis and timing of metamorphosis (Figure 3.3; Table 3.2). The strength of competition from NRLFs for Haida Gwaii toads was similar to that of competition with high densities of conspecifics, whereas toads from the mainland were more affected by competition with conspecifics than with NRLFs (Table 3.2). Additionally, NRLFs from Haida Gwaii fared better in competition with Haida Gwaii toads than with mainland toads (Figure 3.4). These results supported our prediction that Haida Gwaii toads have an inferior response to competition with NRLFs compared to mainland toads, and the results also suggest that Haida Gwaii NRLFs may have increased competitive abilities towards the Haida Gwaii toad compared to the mainland NRLF.

To my knowledge, this is the first report of different responses to *inter*specific competition among different amphibian populations of the same species with different co-occurrence histories. This effect has, however, previously been documented for amphibian predator-prey interactions (e.g., Anderson and Lawler, 2016). I did not observe any predation of toad tadpoles by frog tadpoles, or vice versa, however. NRLF tadpoles may compete with toad tadpoles either through direct exploitation of resources or through chemical or behavioural interference (Griffiths et al. 1991, Faragher and Jaeger 1998). Competition could by influenced by the size differential between tadpoles of the two species. NRLF larvae are larger than Western Toad larvae, growing up to 80mm in length immediately before metamorphosis as compared to 42mm for toads (COSEWIC 2012, 2015), with larger energetic requirements and a more imposing physical presence. Alternatively, competition may be mediated by behavioural displacement of or avoidance by toads (Faragher and Jaeger 1998). This may be facilitated by reduced feeding of Haida Gwaii toads in the presence of NRLFs from Haida Gwaii.

The inferior competitive response of Haida Gwaii toads relative to mainland toads could have several possible explanations, including the lack of plastic or genetic adaptations. Isolated island populations often have lower genetic diversity than their mainland counterparts due to the effects of genetic drift on small and founder populations (Frankham 1997), and Haida Gwaii populations of toads have lower genetic diversity than mainland toads (Chapter 4). Lower genetic diversity can indicate reduced adaptive capacity (Hoban et al. 2020, Allendorf et al. 2022). Toads on Haida Gwaii may have lower adaptive capacity, which could potentially explain the competitive naïveté observed in this experiment. The next chapter focusses on the population genetics of the Haida Gwaii toads compared to their mainland counterparts (Chapter 4). Although some species lack developmental plasticity in response to non-native competitors (e.g., Cook et al. 2013), studies have shown naïve native species can adapt or respond through plasticity to invaders in just a couple of generations (e.g., Bourke et al. 1999). For example, Pujol-Buxóet et al. (2019) showed that competition between a naïve amphibian and an invasive amphibian induced a heritable reduction in 'trophic niche space' – or resource use – of the naïve species to facilitate co-existence. It seems that, so far, Haida Gwaii toads have not adapted, but this may be a question of time. This potential could be further investigated in Haida Gwaii given the distribution of the introduced NRLF is currently restricted to Graham Island, while the Western Toad is distributed across the archipelago.

The inferior competitive response of Haida Gwaii toads to Haida Gwaii NRLFs could be due not only to naïveté of the toad, but also to greater competitive abilities of the Haida Gwaii NRLF relative to mainland NRLF. There are fewer natural enemies (e.g., no invasive American Bullfrog; *Lithobates catesbeianus,* and fewer potential amphibian competitors) for the NRLF on Haida Gwaii, and this lower competition and predation pressure on Haida Gwaii NRLFs could potentially improve adult female condition, resulting in higher total egg abundance, higher quality eggs and therefore better performance (growth and survival) in NRLF tadpoles from Haida Gwaii. Furthermore, NRLF breeding is a month later on Haida Gwaii than on the lower mainland, presumably in part because of being at a higher latitude, and water temperatures were higher (unpublished data). This could create maternal effects for NRLFs on Haida Gwaii, whereby the eggs and resulting larvae are in better condition (Watkins and Vraspir 2006). Future research could investigate potential maternal effects by comparing egg size and quality between NRLFs on Haida Gwaii and the mainland. Genetic differences between Haida Gwaii and mainland NRLF populations seem unlikely given the short duration of NRLFs on Haida Gwaii, though rapid evolution in some non-native species has been observed on this timescale (Otto 2018). I found that mainland toads do not have the same differential response to Haida Gwaii versus mainland NRLFs as Haida Gwaii toads, which may suggest that mainland toads have enough experience with the NRLF to be able to offset any superior competitive abilities the Haida Gwaii NRLF may possess. A study with more NRLF breeding sites per population and a NRLF control could be used to more carefully assess any potential differences between competitive abilities of the introduced Haida Gwaii versus native mainland NRLF populations, and assess if introduced NRLF are competitively superior.

3.4.1 Limitations and future directions

This study is unique in assessing the difference in competitive ability between multiple, geographically separated populations of an amphibian species. There are opportunities for future work to expand on my results by including populations from a wider geographic range in experimental approaches and through *in-situ* observations. The use of mesocosms, which allowed us to compare competition across populations within a controlled environment, could have increased the effects of competition relative to natural systems because of lower mortality and the simplified environment of a mesocosm experiment (Schindler 1998). I did, however, use lower densities than are often found in natural systems in order to minimize these effects. Haida Gwaii toads reared in UBC (Vancouver) mesocosms may have also been affected by the warmer climate of the southern BC experimental site compared to their native range. However, Western Toads are known to do well in warm temperatures and the effects of competition and toad co-occurrence history were larger than that of temperature in the models (Appendix B; Table B1 &

B3), suggesting that the observed effects were not driven by temperature. Future work could confirm this by conducting mesocosm experiments on Haida Gwaii as well. Additionally, developmental stage varied between toad populations from Haida Gwaii and the Lower Mainland because eggs from the two regions were collected on different dates, given ovipositing starts later is Haida Gwaii. The mainland toads were ~two weeks older when the experiment started, possibly resulting in a competitive advantage. However, the high *intra*specific competition of conspecifics depressed toad developmental response similarly for toads from different source regions suggesting that two-week growth advantage may not have outweighed the effect of co-occurrence history.

3.4.2 Conservation implications

If the inferior competitive response of Haida Gwaii Western Toads scales up from the pond (breeding site) level, my results indicate that this species could face population-level consequences on Haida Gwaii from the deleterious effects of competition on larval growth and development. Reduced tadpole growth rate can lead to greater risk from predation or desiccation, and can also influence future competitive ability (Banks and Beebee 1988, Bókony et al. 2018, Hettyey et al. 2019). Furthermore, smaller size at metamorphosis for anurans can result in poorer juvenile physiological and locomotory performance, susceptibility to parasite infections (Pough and Kamel 1984, Goater et al. 1993, Sinsch et al. 2020), and reduced survival and size at first reproduction (Berven and Gill 1983, Smith 1983, 1987, Berven 1990, Cabrera-Guzmán et al. 2013). Western Toads have synchronous metamorphosis and post-metamorphic aggregation at emergence just prior to dispersal. At this time, toadlets are particularly vulnerable to a wide range of predators, and reductions in post-metamorphic fitness may affect population stability. Emergence at a smaller size may compromise long-term survival (Berven 1990), though some studies have found that small metamorph size can be compensated by terrestrial growth in anurans (Boone 2005). If the NRLF is deemed to have population-level consequences on the Haida Gwaii Western Toad, or has other ecological or cultural impacts, a potential conservation management action could be to designate this introduced island population of NRLFs as "Invasive", which would allow them to be controlled. Indeed, the NRLF is currently managed as an invasive species in the only other non-native population of this species, on Chichagof Island in southeast Alaska (Hodge 2004, McClory and Gotthardt 2008). I recommend further field study of the three amphibian species on Haida Gwaii to determine if the NRLF or Pacific Chorus Frog are having population-level impacts on Western Toad population persistence, and if the NRLF is impacting the unique ecosystems of Haida Gwaii in other ways.

The magnitude of any negative population-level effects on the Haida Gwaii toad population from NRLF larval competition would increase as both the relative abundance of NRLFs to toads and overall density of NRLFs at toad breeding sites increases. Tadpole competition is highly dependent on tadpole density (Dash and Hota 1980, Griffiths 1991), and resource availability (Alford and Harris 1988). If resources are scarce, due to factors such as small water body size or poor productivity, competitive effects may be stronger. A number of the toad breeding sites on Haida Gwaii are in peat bogs, which are nutrient poor ecosystems, so competition from NRLFs may be more intense in these areas. Given the results of the experiment, if there is more than one NRLF tadpole for every five toad tadpoles (the ratio used in the experiment), NRLFs may have a large effect on toad larval growth and development if resources are scarce relative to tadpole densities. One potential management action on Haida

Gwaii would be to remove NRLF egg masses from toad breeding sites, especially at toad breeding sites that have a small volume of water relative to the number of amphibians or are low productivity sites.

Aside from NRLF competition, there are a number of potential threats to the Western Toad on Haida Gwaii that could explain the observed declines in toad sightings, other than competition with NRLFs at the tadpole life stage. These include chytrid fugus, other non-native competitors and invasive predators, habitat destruction and climate change. The Pacific Chorus Frog, also introduced to Haida Gwaii, may also compete with Haida Gwaii toads, as posited by Reimchen (1991). It is also possible the adult life stage of both introduced frogs are impacting adult toads through providing subsidies to predators, thereby increasing predator densities and increasing predation on toads, or whether adult frogs eat toad eggs or tadpoles – both of which hypotheses remain untested. Both the NRLF and Pacific Chorus Frog could potentially have brought chytrid fungus (*Batrachochytrium dendrobatidis*) to the Western Toad population on Haida Gwaii; however, my recent swabbing surveys suggest it is not currently there (unpublished data 2019). Breeding aggregation behaviour and strong philopatry to aquatic breeding habitats make toad populations highly vulnerable to threats at their breeding sites. Raccoons (*Procyon lotor*) were introduced to Haida Gwaii in the 1940s and are effective toad predators (Gaston et al. 2002, Johnston 2006, COSEWIC 2012). It is also unknown whether feral cats eat toads. Given the weak competitive response of toads to NRLFs, it may be worth investigating whether Haida Gwaii toads have inferior responses to these other non-native species, and if so, investigate options to remove these species from toad breeding sites.

Beyond local conservation implications, our results illustrate that competitive naivete may play a role in the response of native species populations to introductions of species which naturally co-occur elsewhere within the range of the native species, and this could have important implications for assisted migration, translocations and natural species range shifts. It is difficult to acquire sufficient knowledge of a species and its interactions that is required to accurately assess risks involved in translocating it to a new environment, and proposals to date often assume that interactions in one part of the range will be replicated elsewhere (Davidson and Simkanin 2008). My results show that we cannot assume that the ecological relationships between sympatric species within their natural ranges will translate to novel locations, and environments where those same species have no history of co-occurrence. This contributes to the wider debate on whether some within-range translocations or natural climate induced range shifts will be positive or negative for recipient ecosystems (Urban 2020). We recommend that when planning for assisted migrations, researchers should assess the adaptive capacity of functionally similar species in host environments regardless of sympatric co-occurrence history elsewhere.

Chapter 4: Western Toads on Canada's Haida Gwaii archipelago have low genetic diversity and are strongly differentiated from southwestern British Columbia populations

4.1 Introduction

Amphibians are severely endangered globally, with over 30% of species facing extinction (Wake and Vredenburg 2008). Amphibians are declining due to habitat loss and fragmentation, climate change, disease, non-native species and UV-B radiation (Beebee and Griffiths 2005, Hof et al. 2011). Many of these drivers act together to reduce population size, genetic diversity (Frankham 1996) and thereby threaten the persistence of populations by compromising fecundity, survival, competitive ability, adaptive capacity, and increasing inbreeding (Keller and Waller 2002, Allentoft and O'Brien 2010, Chen et al. 2012). Habitat fragmentation can decrease genetic diversity by reducing gene flow and effective population size (Johansson et al. 2007). To slow or reverse amphibian declines through management interventions, it is informative to understand population genetic structure and diversity across the landscape.

Haida Gwaii, an archipelago in British Columbia (BC), has been separated from mainland North America since a connecting continental land bridge was submerged approximately 10,000 calendar years ago as glaciers melted following the last glacial maximum (LGM; McLaren et al. 2020). Haida Gwaii's history of geographic isolation combined with its relatively mild climate for its latitude (54ºN) has led to the evolution of a plethora of endemic taxa (Reimchen et al. 2005). It is considered a biodiversity hotspot, harbouring unique mammals, fish, birds, plants, lichens and moss such as the Queen Charlotte Islands Ermine (*Mustela* *erminea haidarum*), Haida Gwaii Black Bear (*Ursus americanus carlottae*), Queen Charlotte Islands violet (*Viola biflora var. carlottae*), and the Northern Goshawk (*Accipiter gentilis laingi*) (Osgood 1901, Byun et al. 1997, Withrow et al. 2014, Geraldes et al. 2019, Colella et al. 2021).

The Western Toad (*Anaxyrus boreas boreas*, called Hlk'yáan K̲'ust'áan in the X̲aad Kíl dialect of the Haida language (X̲aad Kíl Née; Haida Language Council)), is the only indigenous amphibian on Haida Gwaii and has not previously been the subject of population genetic analyses. There is concern that some local toad populations are reported to have been extirpated in the last few decades. Additionally, a recent experiment (Chapter 3) found that Haida Gwaii toad populations had weaker competitive abilities in the presence of introduced Northern Red-legged Frogs (*Rana aurora*) than toads from the sympatric range on the southern BC mainland. This study is motivated by a desire to better understand the population structure of the Western Toad in coastal BC, and to provide land managers on Haida Gwaii with information that could be used to help inform the conservation management of the toad.

The Western Toad is widely distributed in western North America – from southern Alaska to Baja California and as far east as Colorado (IUCN SSC Amphibian Specialist Group 2015). There are two subspecies, *Anaxyrus boreas halophilus* and *Anaxyrus boreas boreas*. The latter is the only subspecies in Canada, and is a species of conservation concern in BC and the US (IUCN SSC Amphibian Specialist Group 2015), listed as a species of "Special Concern" in the Canadian Species at Risk Act (SARA). However, within BC it is 'Yellow Listed - apparently secure'. There are currently two Designatable Units under SARA within Canada – the calling and non-calling population – and the Western Toad on Haida Gwaii is part of the non-calling population. The Western Toad has experienced local declines in many parts of its range. For example, entire populations have been extirpated on Vancouver Island (Davis and Gregory

2003), in Colorado (Carey 1993) and New Mexico (Jackson 2004). Western Toads are highly philopatric (loyal) to their breeding sites (COSEWIC 2012). Breeding sites are often separated by distances up to 30km (Slough 2004) and sexually mature toads come together from across a large area and to a single breeding site, and breed *en masse* for a few weeks every spring (COSEWIC 2012). Such breeding behaviour can make for highly structured populations that are vulnerable to threats to the breeding site habitat.

Only a handful of studies have investigated the population genetics of Western Toads and most of the work has been on southern and eastern US populations; of these, few have used high-resolution genomic methods. One recent study using mitochondrial DNA markers found no genetic structure in northern Idaho and northwest Washington toad populations (Lucid et al. 2021). By contrast, in a much smaller study area, Myers (2020) used SNPs and found genetic structuring in Western Toad populations in Snoqualmie Pass, Washington State. Additionally, Moore et al. (2011) found moderate structuring in southeast Alaska populations. Goebel et al. (2009) used mitochondrial DNA to identify three main clades of Western Toads across their whole range – northwestern, southwestern and eastern clades. However, there were only two samples from British Columbia, and none from Haida Gwaii, an archipelago off the coast of northern coastal BC.

In this study, I addressed three specific questions regarding the evolutionary history of Western Toads on Haida Gwaii in relation to their most closely related populations:

1. *How genetically divergent are Haida Gwaii populations from southwestern BC populations?*
2. *Are levels of genetic diversity and inbreeding different in Haida Gwaii populations compared to southwestern BC populations?*

3. *What is the level of genetic diversity of and divergence among breeding sites on Haida Gwaii?*

To address these questions, I sampled toads from across Haida Gwaii, Vancouver Island, and Lower Mainland BC populations, and generated a set of genome-wide SNPs using Restriction site Associated DNA Sequencing (RADseq). These data were analyzed to determine patterns of diversity within and divergence among Western Toad populations.

4.2 Materials and methods

4.2.1 Field sampling

Western Toad (*Anaxyrus boreas boreas*) larvae (i.e., tadpoles) were sampled from 27 breeding sites across coastal British Columbia, Canada (Figure 4.1). Thirteen breeding sites were from Haida Gwaii, and 14 were from southwestern BC. The southwestern BC sites included seven sites from the lower mainland, six from Vancouver Island and one from Lasqueti Island, between Vancouver Island and the lower mainland. At each breeding site, 25-30 larvae were sampled between May 2018 and July 2019 which resulted in a total of 805 samples. Larvae were sampled from different areas within each waterbody to reduce the chance that samples were from the same egg mass (and therefore siblings). Larvae samples were kept in vials of 100% ethanol and were stored in a cooler bin with ice packs in the field, and a -20°C freezer once they reached the lab. The three management regions for the full dataset were: Haida Gwaii, Vancouver Island (which included Lasqueti Island) and the Lower Mainland, because these correspond to BC government management units. The two management regions within Haida Gwaii were: 1) the National Park, “Gwaii Haanas National Park Reserve and Haida Heritage Site” (hereafter referred to as “Gwaii Haanas”); 2) and everything north of that (referred to here as “Northern

Haida Gwaii”). Wildlife permits were obtained to conduct this sampling – see thesis preface for details.

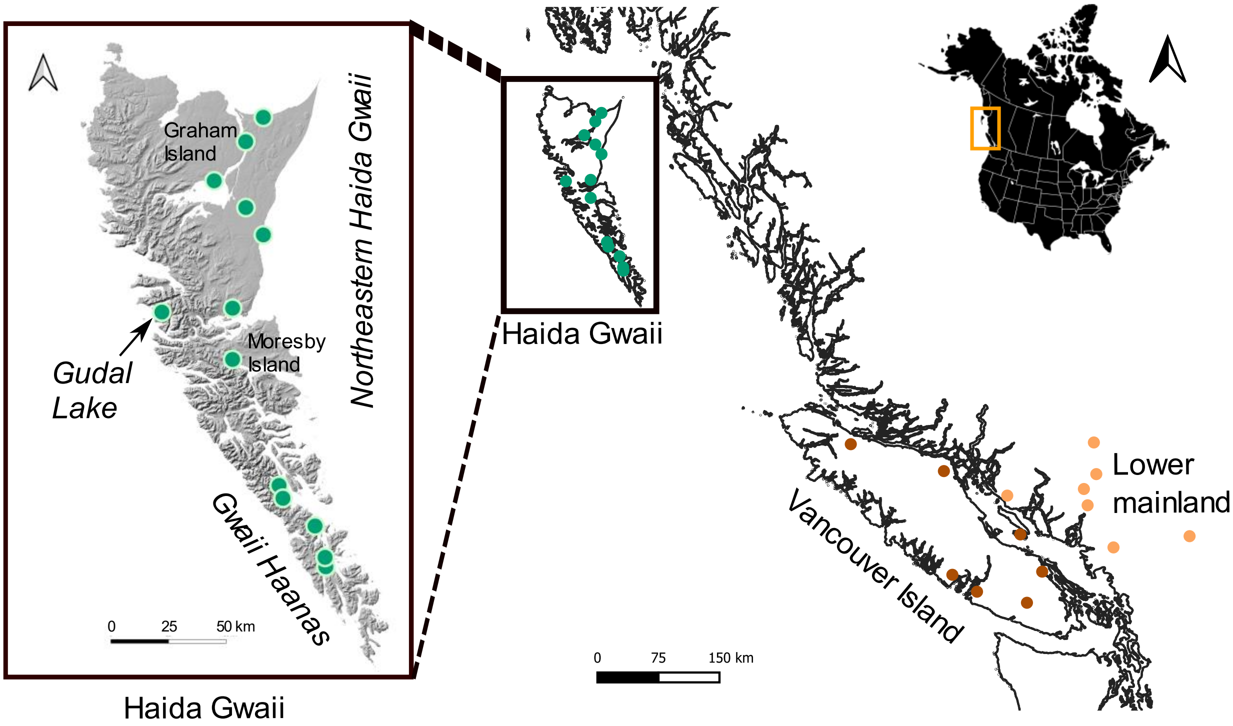


Figure 4.1. Sampling locations of the 27 toad breeding sites used in this study. Thirteen on Haida Gwaii (five of which are in Gwaii Haanas), seven on the Lower Mainland, six on Vancouver Island and one on Lasqueti Island.

4.2.2 DNA extraction and sequencing

DNA was extracted in the Hamelin Genomics and Forest Health Lab at UBC from 10-20 mg of tadpole tail tissue using a MagMAX™ DNA Multi-Sample Ultra 2.0 Kit and KingFisher Duo Prime (Thermo Fisher Scientific, Waltham, MA, USA). Genomic DNA quantity and quality was tested using the NanoDrop 1000 spectrophotometer and the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA quality was assessed via 0.7% agarose gel electrophoresis.

RADseq, a reduced representation genome sequencing method, and specifically genotyping-by-sequencing (GBS) (Elshire et al. 2011), was used to generate a dataset of genome-wide SNPs (single nucleotide polymorphisms). Samples were sent to the Plateforme d'Analyses Génomiques of the Institut de Biologie Intégrative et des Systèmes (PAG-IBIS, Université Laval, Quebec, Canada) for GBS library preparation. The PstI/MspI double digest procedure first described in Poland et al. (2012) was used with the following modifications: the adapters were modified to include plate indexes as described in Colston-Nepali et al. (2019) and a BluePippin (Sage Biosciences, Beverly, MA, USA) size selection step prior to the final PCR was added as described in Abed et al. (2019) with times settings to elute between 56 to 65 minutes. Nine plate-indexed pools of GBS libraries, each containing up to 96 in-line barcoded samples were prepared. Each library was quantified using a fluorometric method and the profile was checked on a Bioanalyzer high sensitivity DNA chip (Agilent, Santa Clara, CA, USA). The libraries were sent for sequencing at the Génome Québec Innovation Centre (McGill University) on an Illumina NovaSeq 6000 S4 lane with 2 x 100 cycles (paired-end mode). The libraries were normalized and pooled and then denatured in 0.05N NaOH and neutralized using HT1 buffer. The pool was loaded at 225pM on an Illumina NovaSeq 6000 S4 PE100 lane using Xp protocol as per the manufacturer's recommendations. The run was performed for 2 x 100 cycles (paired-end mode). A phiX library was used as a control and mixed with libraries at 10% level. Base calling was performed with RTA v3. The program *bcl2fastq2* v2.20 was then used to demultiplex samples by plate barcode, trim the Illumina adapters, and generate raw fastq reads (Illumina 2019).

4.2.3 Cleaning, filtering, and calling SNP genotypes

Sequencing of all 805 individuals resulted in over 2 billion reads. I used *fastqc* (v 0.11.9 ; Andrews 2017) to check for sequencing errors and found the only issue was that the Mspl restriction cut site on the reverse reads had poor per base sequence quality. *Trimmomatic* (v 0.39 Bolger et al. 2014) was used to trim the Mspl cutsite from the reserve reads which resulted in 98 bp fragments for the reverse read (the forward reads were 93 bp long). Samples were demultiplexed into individuals using the *STACKS* (v 2.53, Rochette and Catchen 2017, Rochette et al. 2019), *process_radtags* module (settings: --inline_null, --renz_1 sbfI, --quality, --rescue, --barcode_dist_1, s = 20, w = 0.25) to remove the barcode and discard reads that had a PHRED quality <20 in a sliding window of 25 bases or with more than one mismatch in the barcode or cutsite (Rochette et al. 2019). This resulted in a small fraction of reads being discarded from each plate (3.1% to 3.6%).

I aligned my reads to three amphibian reference genomes (*Bufo bufo, Bufo gargarizans*, and *Rhinella marina;* Edwards et al. 2018, Lu et al. 2021, Streicher and of Life 2021), but fewer than 35% of all reads mapped to these reference genomes with mapping quality over 20, so I proceeded with *de novo* assembly. I ran the *STACKS de novo* pipeline to call the SNPs in two sets – once for all samples (*n* = 805) and another time for just the Haida Gwaii samples (*n* = 385). I used *STACKS* with settings n = 3 (number of mismatches allowed between sample loci when building the catalog), m = 3 (minimum depth of coverage required to create a stack), and M = 3 (maximum distance in nucleotides allowed between stacks) and allowed for 10% of an alignment to contain gaps (--min-gapped 0.9). I chose these parameter values after testing different parameter values of n and M as per the recommended method in Rochette and Catchen (2017).

The *STACKS de novo* pipeline generated 1,872,270 loci for the full dataset with a mean depth of 7 reads per locus, and 1,088,195 SNPs for the Haida Gwaii dataset with a mean depth of 7.2 reads per locus. As per the recommendation of Rochette et al. (2019), I did not remove PCR duplicates because I used a GBS protocol – which produces identical length inserts, and removing PCR replicates only works for variable length inserts.

STACKS populations module was used to filter reads and generate an ordered vcf file. Loci with a maximum observed heterozygosity (H_o) > 0.6 across all populations were removed as these were likely the result of paralogs rather than allelic variation (Geraldes et al. 2019). Loci with a minor allele frequency (MAF) of 0.01 or less were also removed (Gallego-García et al. 2019). Only loci that were genotyped in all 27 populations, and genotyped in ≥ 60% of individuals were retained. SNPs were filtered to only include the first SNP per read because analyzing multiple SNPs within the same locus can influence statistical power (Morin et al. 2009). This left 4,697 SNPs for the full dataset and 4,553 SNPs for the Haida Gwaii dataset. To calculate nucleotide diversity (π), the "write single SNP" filter (and all additional filters below) was not applied to ensure the dataset had both variant and invariant loci. The dataset used to calculate π had 805 individuals and 174,424 loci for the full dataset and 385 individuals and 163,906 loci for Haida Gwaii.

Next, using *vcftools* (v 0.1.14, Danecek et al. 2011), I removed SNPs that: (1) had insertion/deletion (indel) polymorphisms; (2) were not biallelic; (4) had a genotype and site quality less than 20; or (3) had less than 3x coverage (min-meanDP 3). This left 2,392 SNPs for the full dataset and 3,124 SNPs for the Haida Gwaii dataset.

Individuals with more than 30% missing data were removed using *vcftools*. At this point, the full dataset consisted of 371 individuals and 2,392 SNPs, the Haida Gwaii dataset contained

164 individuals and 3,124 SNPs. All breeding sites had between 5 and 25 samples remaining except for the site on Lasqueti Island, which had no samples left.

Although numerous studies advocate for removing siblings (e.g., O'Connell et al. 2019), if not done correctly, removing siblings can do more harm than good (Waples and Anderson 2017). The presence of siblings was tested using the KING method in the *SNPRelate R* package within the *SambaR* package, (Manichaikul et al. 2010, Zheng et al. 2012, de Jong et al. 2021). The analysis identified all individuals per pond as siblings – which is extremely unlikely. This result could possibly be due to the high levels of missing data observed per individual. Therefore, all analyses were performed on datasets that included potential siblings but plan to filter for siblings when I re-assemble the data once the Western Toad reference genome is available (C. Funk, pers. Comm.).

SNPs that did not meet Hardy-Weinberg expectations (HWE) ($p < 0.01$) were removed using the *seppop* and *hw.test* functions from the *R* packages *adegenet* and *pegas* (Jombart 2008, Paradis 2010). HWE was tested within each breeding site and SNPs were removed that were out of HWE in at least two thirds of breeding sites. This resulted in 1,211 SNPs for the full dataset and 2,893 SNPs for Haida Gwaii dataset.

SNPs that had outlier estimates of inbreeding (average F_{IS}) were removed, i.e., less than -0.1 and greater than 0.1 per locus, per pond (Shafer et al. 2017), using a custom R script that extracted F_{IS} values from the *basic.stats* function from the *hierfstat R* package (Goudet 2005). The distribution of F_{IS} values before and after filtering can be seen in the Appendix C (Figure C1). The final filtered dataset included 371 individuals and 732 SNPs for the full dataset, and 164 individuals and 1,644 SNPs for the Haida Gwaii dataset.

4.2.4 Analyses

4.2.4.1 Genetic differentiation

I assessed genetic differentiation between Haida Gwaii and southwestern BC in addition to genetic differentiation within Haida Gwaii through pairwise F_{ST} estimation and a variety of population clustering analyses including a principal components analysis - PCA, *STRUCTURE*, and a discriminant analysis of principal components (DAPC). I used a variety of methods to investigate differentiation as they have different assumptions and limitations. For example, *STRUCTURE* is sensitive to the MAF filter while DAPC and PCA are not (Linck and Battey 2019).

To visually assess the genetic patterns across the sampled range of the Western Toad, I ran a PCA using the *glPca* function from the *adegenet R* package (Jombart 2008): 1) for the full dataset; and 2) just within Haida Gwaii. My *a priori* number of populations for the full dataset was three: Haida Gwaii, Vancouver Island and the Lower Mainland. The *a priori* number of populations within Haida Gwaii was two: Gwaii Haanas (the National Park and Haida Heritage Site), and northern Haida Gwaii.

I ran a *STRUCTURE* analysis – a Bayesian clustering method to investigate individual population assignment (Pritchard et al. 2000), 1) for the full dataset, and also 2) just within Haida Gwaii. I converted the filtered genind file to *STRUCTURE* format using the *genind2structure* conversion in R (Clark 2017), then ran *K* values (number of clusters/populations) 1 to 13 for the full dataset, and also *K* 1 to 13 for Haida Gwaii (maximum sites), with 5 replicates of each *K* value. I picked an upper *K* limit of 13 for the full dataset as a compromise between the maximum possible total number of sites sampled (27) and computational time. Each replicate had a burn-in period of 75,000 generations, followed by 250,000 iterations. I used Structure Harvester to determine the optimal *K* value with the Evanno method (Evanno et al. 2005, Earl 2012). The Evanno method cannot identify less than two populations (Evanno et al. 2005, Janes et al. 2017), however. I used *CLUMPAK* to average across independent replicates and create membership plots of the optimal *K* value and a number of other *K* values (Kopelman et al. 2015).

A DAPC with k-means clustering and model selection was also conducted to test for the number of populations (Jombart et al. 2010). I used the *dapc*, *find.clusters* and *scatter* functions in the *adegenet R* package (Jombart 2008). To choose the number of principal components for DAPC, I used the *optim.a.score* function. From this, the Bayesian Information Criterion (*BIC*) was plotted against the number of clusters to determine the optimal number of clusters.

F_{ST} among regions, and among breeding sites within regions, was calculated with the Weir and Cockerham (1984) method, using the *hierfstat R* package (Goudet 2005). The *pairwise.WCfst* function was used for the among cluster pairwise weighted F_{ST}, and the *wc* function was used to calculate within cluster $F_{ST.}$ The statistical significance of the pairwise F_{ST} estimates was calculated with the *stamppFst* function from the *StAMPP R* package (Pembleton et al. 2013). The distribution of (non-weighted) pairwise F_{ST} estimates per locus between Haida Gwaii and southwest BC was plotted using the *hierfstat R* package. However, given the strong neutral population structure, relatively low saturation of the genome with markers, and relatively high level of missing data, these data were not considered suitable for testing for local adaptation, e.g., through an F_{ST} outlier test (Lowry et al. 2016).

For the full dataset (27 breeding sites), a site frequency spectrum (SFS) was generated for each of the three management regions to assess whether there may have been a population bottleneck or founder effect on Haida Gwaii. The SFS was generated using the *maf* function in the *vcfR* package (Knaus and Grünwald 2017), after splitting the VCF file into three regions.

To test whether isolation-by-distance (IBD) was a possible explanation for genetic divergence between southwest BC and Haida Gwaii, and breeding sites within Haida Gwaii, a Mantel test was conducted using the *mantel.randtest* function from the *R adegenet* package (Jombart 2008). I took the natural log of the distance (in kilometers) between the 13 breeding sites on Haida Gwaii as calculated by the *earth.dist* function in the *R fossil* package (Vavrek and Vavrek 2020), and calculated genetic differentiation between the same sites as (F_{ST} / 1- F_{ST}) (Rousset 1997, Diniz-Filho et al. 2013). The *p* value of the Mantel test provides an indication of the existence of isolation by distance (i.e., genetic differentiation and geographic distance are correlated).

4.2.4.2 Genetic diversity and inbreeding

I assessed whether levels of genetic diversity and inbreeding differed between Haida Gwaii populations and those in southwestern BC, and also whether genetic diversity varied spatially across Haida Gwaii. Nucleotide diversity (π), the number of variant sites out of the total number of sites, was calculated in the *STACKS populations* module across all assembled reads (Hohenlohe et al. 2010). Expected heterozygosity was calculated using allele frequencies generated with the *summary* function in the *adegenet R* package (Jombart 2008). The inbreeding coefficient (F_{IS}) was calculated using the *basic.stats* function in the *hierfstat R* package (Goudet 2005). I tested for significant differences for all the above analyses by calculating 95% confidence intervals (mean ± 1.96*(stdev/√n)); values were considered significantly different if the confidence intervals did not overlap.

4.3 Results

4.3.1 Genetic differentiation between Haida Gwaii and southwestern BC

Western Toads on Haida Gwaii are strongly differentiated from southwestern BC populations. This result was consistent across the PCA, *STRUCTURE*, and F_{ST} analyses. In the PCA (Figure 4.2a), the first principal component (PC1) explained 22.1% of the variation and clearly separated Haida Gwaii from the two other regions. PC2 explained just 1.5% of the variation and did not separate Haida Gwaii from the other regions, but somewhat separated Vancouver Island populations from the Lower Mainland. PC3 and 4 explained substantially less variation (2.6%) (Appendix C; Figure C2).

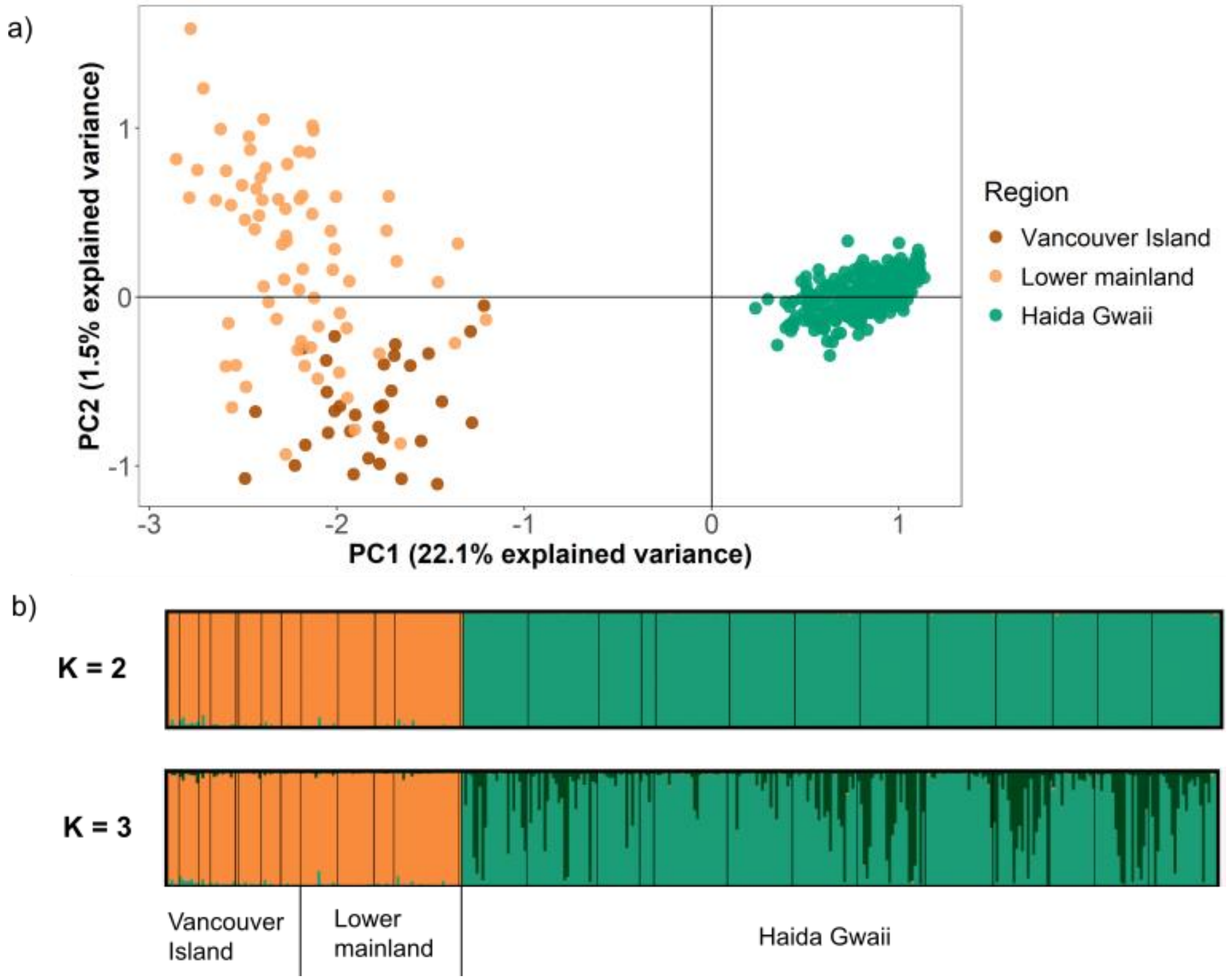


Figure 4.2. Population structure of Haida Gwaii, Vancouver Island and Lower Mainland toads. a) Plot of first two dimensions of a PCA and b) membership plot from the STRUCTURE analysis with K = 2 & 3, where each colored vertical bar represents an individual and black vertical lines separate breeding sites.

The *STRUCTURE* population assignment plot and the Evanno method both suggested the most probable number of genetic clusters to be $K = 2$ (Appendix C; Figure C3a). For the *STRUCTURE* plot of $K = 2$, all toads on Haida Gwaii showed 100% membership in the Haida Gwaii cluster, while some toads in the Lower Mainland and Vancouver Island had small amounts of membership in the Haida Gwaii cluster (Figure 4.2b). For the $K = 3$ *STRUCTURE* plot, the same general pattern as $K = 2$ remained but numerous individuals on Haida Gwaii had membership to a second cluster, and some toads in the Lower Mainland and Vancouver Island had small amounts of membership to this additional cluster. Additionally, the *BIC* from the DAPC also suggested $K = 2$ to be the most probable number of genetic clusters (Appendix C; Figure C3b).

The pairwise F_{ST} estimate between the $K = 2$ clusters (Haida Gwaii vs southwestern BC) was very high (0.290) ($p < 0.05$), indicating strong genetic divergence. When considering the three management regions ($K = 3$), pairwise F_{ST} estimates were highest between sites on Haida Gwaii and the Lower Mainland (0.307), followed closely by Haida Gwaii vs Vancouver Island (0.306), but an order of magnitude lower for Vancouver Island compared to the Lower Mainland (0.029) ($p < 0.05$; Table C1 shows all pairwise comparisons).

F_{ST} estimates among breeding sites within the $K = 2$ clusters were highest for southwestern BC (0.046) and somewhat lower for Haida Gwaii (0.035). When comparing F_{ST} estimates among breeding sites for the three management regions, Lower Mainland (0.043) sites had the highest pairwise F_{ST} estimates, followed by Haida Gwaii (0.036), and finally Vancouver Island (0.018). The distribution of pairwise F_{ST} per locus between southwest BC and Haida Gwaii was left skewed, but also showed some high outliers (Appendix C; Figure C4).

The distribution of allele frequencies (SFS) across all loci shows the Haida Gwaii population has more monomorphic loci and rare alleles compared to lower mainland and Vancouver Island populations (Figure 4.3). This pattern is consistent with a population bottleneck or founder effect on Haida Gwaii. There appeared to be more fixed SNPs in the Vancouver Island populations compared to the lower mainland, but that may partially be an artefact of lower coverage of the Vancouver Island populations during sequencing. When testing for isolation-by-distance with the full dataset, the Mantel test revealed a significant positive correlation between genetic and geographic distance across all sites in Haida Gwaii and southwestern BC (R = 0.83, p = 0.01; Appendix C; Figure C5), but this was expected given the large distances between regions.

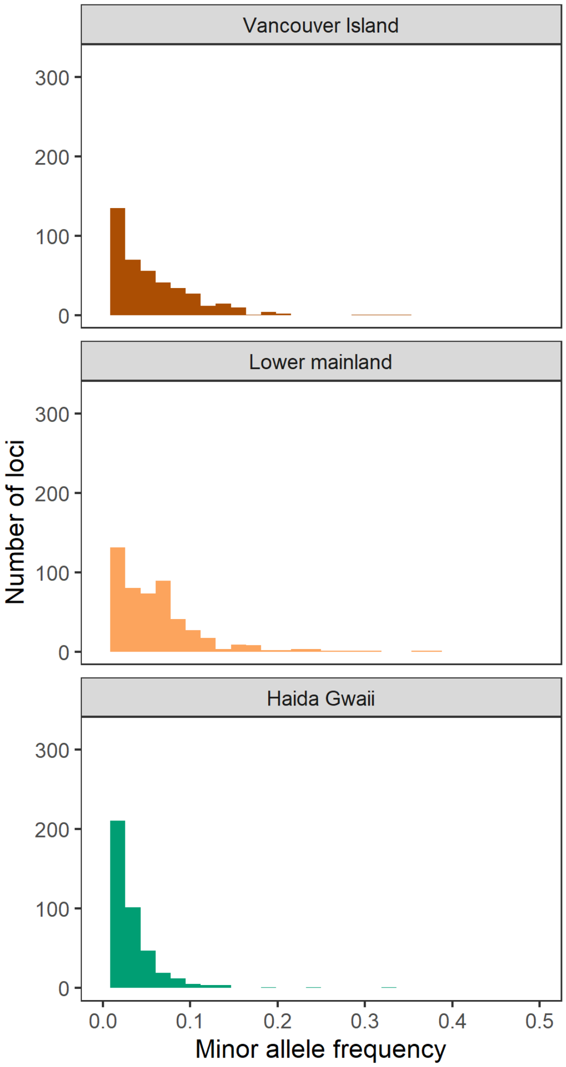


Figure 4.3. Site frequency spectrum (distribution of allele frequencies) for the Western toad, for three management regions in southwest BC. Number of loci with a given frequency of minor alleles.

4.3.2 Genetic diversity and inbreeding

Genetic diversity was considerably lower on Haida Gwaii than in southwestern BC populations for all parameters estimated (i.e., using $K = 2$). Nucleotide diversity (π) was higher in the southwestern BC cluster (π_{mean} ± st. dev = 0.00070 ± 0.00003) and than Haida Gwaii (0.00040 ± 0.00002). When considering the three management regions, Haida Gwaii was significantly lower than the other two regions. There was no significant difference between Vancouver Island and the Lower Mainland, although Vancouver Island had slightly lower nucleotide diversity (overlapping CIs; Figure 4.4a). Expected heterozygosity (H_e) across all SNPs passing filters was highest in the Lower Mainland (H_e mean ± SE = 0.075 ± 0.003), followed by Vancouver Island (0.060 ± 0.003) and lowest on Haida Gwaii (0.037 ± 0.002; Figure 4.4b). There was no difference in inbreeding (F_{IS}) estimates among the three regions (Figure 4.4c).

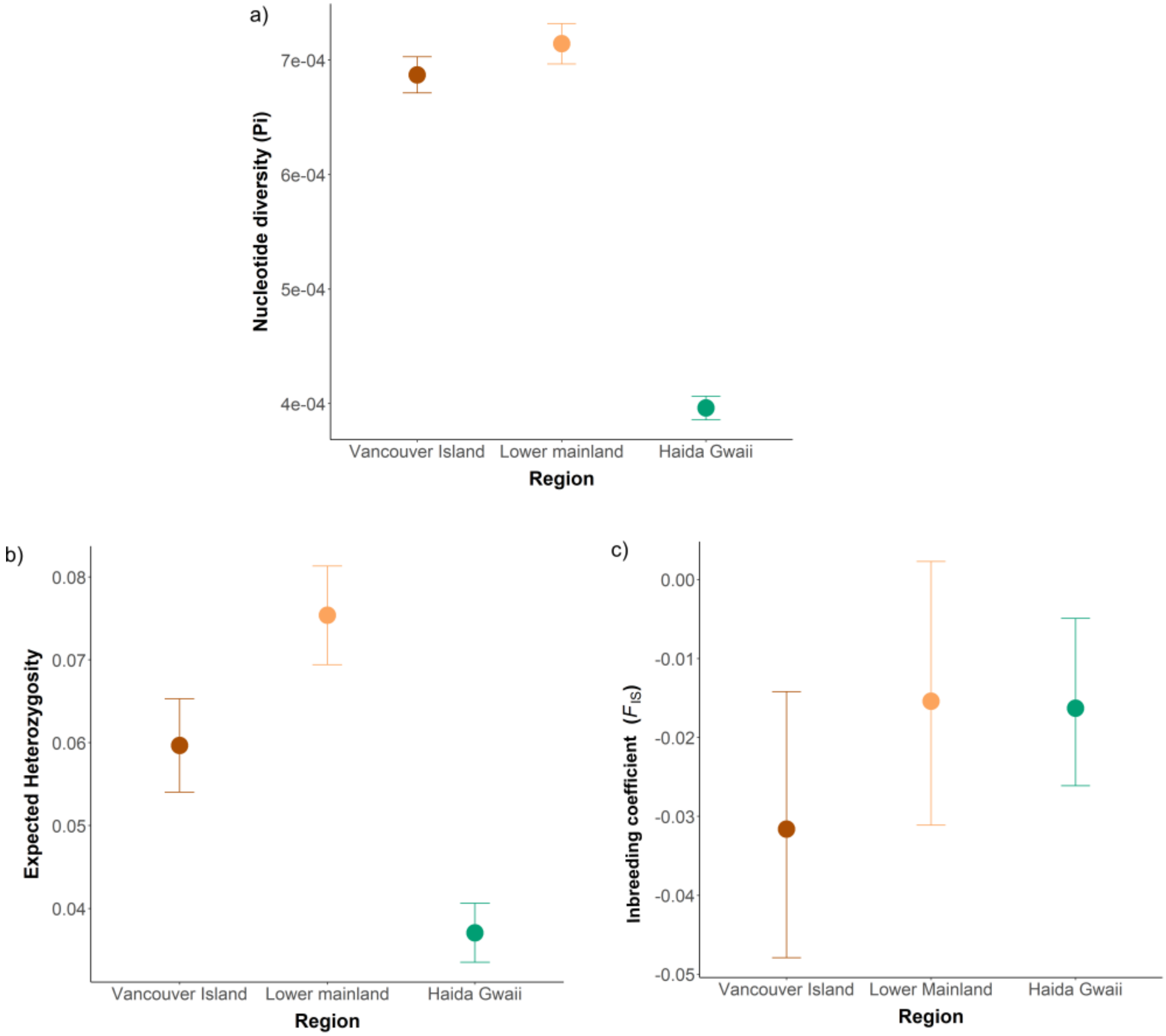


Figure 4.4. Genetic diversity of Haida Gwaii versus Vancouver Island and the Lower Mainland toad populations. a) Nucleotide diversity (Pi), b) expected heterozygosity (H_e), and c) inbreeding coefficient (F_{IS}) – all with mean and 95% confidence intervals.

4.3.3 Population structure and genetic diversity within Haida Gwaii

The PCA of samples from within Haida Gwaii showed three clusters: Gwaii Haanas (the National Park at the southern end of the archipelago), northeastern Haida Gwaii, and Gudal Lake – a single site on the northwest coast of Graham Island, and the only sampled breeding site on the west coast of Haida Gwaii (Figure 4.1). PC1 explained 8.3% of the variation and separated Gwaii Haanas samples from northern Haida Gwaii individuals (Figure 4.5a); PC2 explained 3.6% of the variation and separated Gudal Lake from the rest of Haida Gwaii. PC3 and 4 explained substantially less variation (4.6%) (Appendix C; Figure C6).

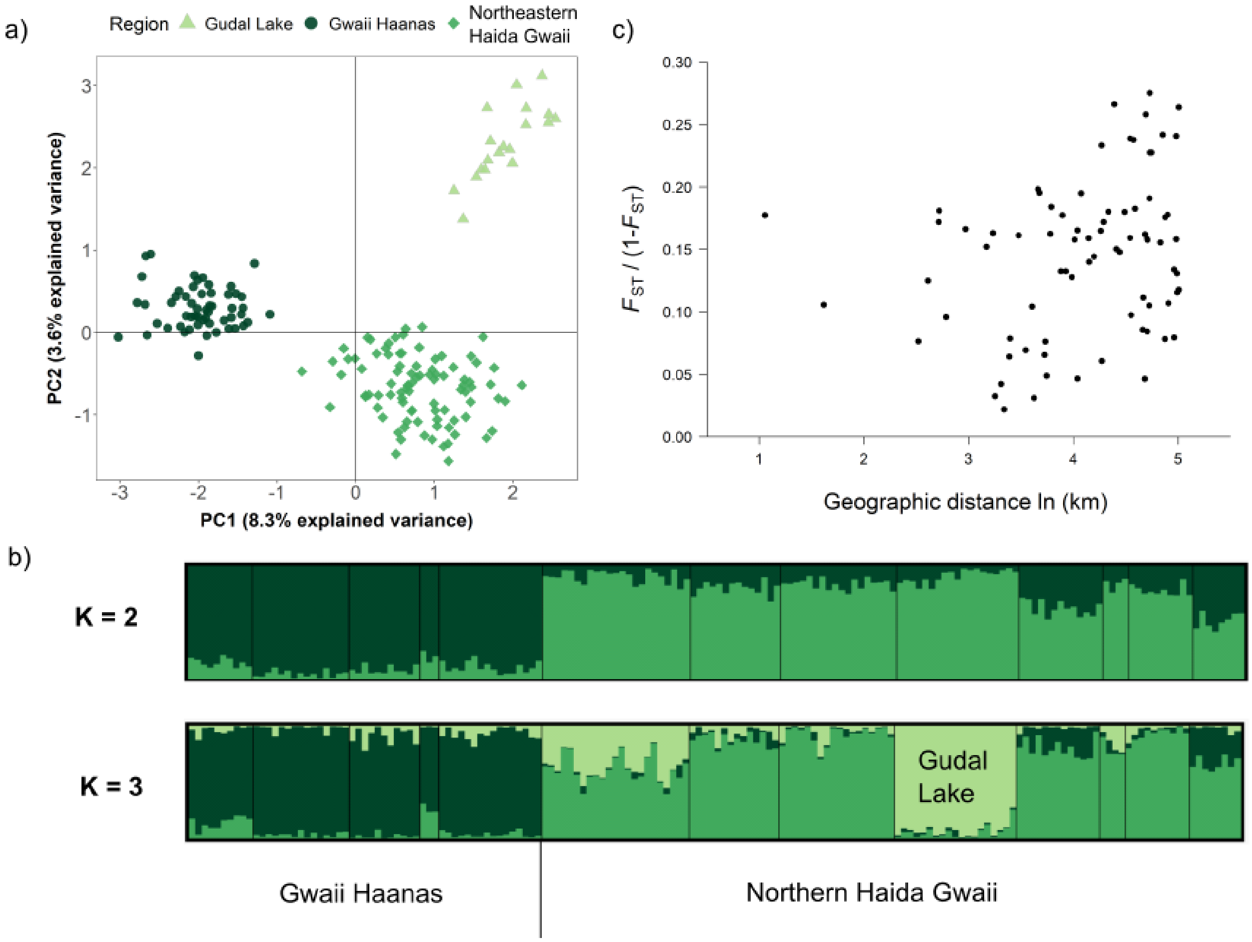


Figure 4.5. Haida Gwaii population structure. a) Plot of first two dimensions of a PCA for *K* = 3 and b) membership plot from the *STRUCTURE* analysis with *K* = 2 and 3, where each colored vertical bar represents an individual and black vertical lines separate breeding sites. c) isolation by distance plot.

The *STRUCTURE* analysis using the Evanno method suggested the most probable number of genetic clusters within Haida Gwaii to be $K = 2$ (Appendix C; Figure C7a). However, the *STRUCTURE* population assignment plots showed support for both $K = 2$ and $K = 3$. For $K = 2$, all Gwaii Haanas samples had > 80% membership to the Gwaii Haanas cluster, and most northern Haida Gwaii samples had > 80% membership to the northern Haida Gwaii cluster (Figure 4.5b). For $K = 3$, all the individuals from Gudal Lake (northwest coastal site) had > 80% membership in a third cluster (Figure 4.5b). The *BIC* from the DAPC supported the uncertainty between the number of clusters seen in the *STRUCTURE* membership plots, selecting both $K = 2$ and $K = 3$ as the most probable number of clusters (Appendix C; Figure C7).

Pairwise F_{ST} for $K = 2$ clusters indicated moderate differentiation between the Gwaii Haanas and northern Haida Gwaii clusters (including northeast and northwest) ($F_{ST} = 0.089$; $p < 0.05$). When considering $K = 3$ clusters, pairwise F_{ST} estimates were highest between sites from Gwaii Haanas and Gudal Lake (0.179), followed by Gudal Lake versus northeastern Haida Gwaii (0.0946), and then Gwaii Haanas versus northeastern Haida Gwaii (0.0868) ($p < 0.05$).

Genetic differentiation among breeding sites within Haida Gwaii was variable. F_{ST} ranged from 0.021 to 0.216 ($p < 0.05$ except between Porque Lake and Dead Toad Lake, two sites that are geographically close $p = 0.11$; Appendix C; Table C2). Gudal Lake had the highest average pairwise F_{ST} value of all breeding sites (average = 0.171, max = 0.216; Appendix C; Table C2). F_{ST} estimates among breeding sites within each $K = 2$ cluster were higher for northern Haida Gwaii (0.101), indicating lower population connectivity than Gwaii Haanas breeding sites (0.047). When comparing sites separated by similar distances, pairwise F_{ST} estimates were always higher for northern Haida Gwaii breeding sites. F_{ST} estimates among breeding sites within each $K = 3$ cluster were not calculated as Gudal Lake only had one breeding site.

The Mantel test revealed a weak but significant correlation between genetic and geographic distance within Haida Gwaii (R = 0.26, $p = 0.018$; Figure 4.5c), indicating some of the genetic differentiation among populations may be due to isolation-by-distance.

For the two management regions on Haida Gwaii, I found no difference in genetic diversity or inbreeding between populations from Gwaii Haanas versus northern Haida Gwaii as evidenced by overlapping confidence intervals for all analyses ($\pi = 0.0022$ to 0.0025; Appendix C; Figure C8). Gudal Lake had similar diversity to northeastern Haida Gwaii and Gwaii Haanas ($\pi \pm$ st. dev $= 0.022 \pm 0.00006$). There was no pattern in either nucleotide diversity or expected heterozygosity (Appendix C; Figure C8). Inbreeding (F_{IS}) was slightly higher for northern Haida Gwaii (F_{IS} mean ± SE = 0.0464 ± 0.0124) than Gwaii Haanas (-0.0071 ± 0.0121; Appendix C; Figure C8).

4.4 Discussion

Motivated by local population extirpations and the lower competitive abilities of Haida Gwaii toads compared to their southwestern BC counterparts (Chapter 3), I performed genomic analyses of 27 Western Toad populations from these regions to characterize population structure and genetic diversity to ultimately inform the conservation of toads in these areas. I found that Haida Gwaii populations were less genetically diverse and were highly differentiated from populations in southwestern BC. To aid conservation of toads on Haida Gwaii, I suggest protecting breeding habitat, controlling invasive predators around breeding sites, maintaining habitat connectivity between breeding sites, and potentially recognizing the Haida Gwaii toad as a distinct population segment, if the Haida Nation desires.

4.4.1 Genetic diversity and differentiation between Haida Gwaii and southwestern BC

Population clustering and F_{ST} analyses showed that Haida Gwaii populations are genetically distinct from southwestern BC populations. In comparison, Vancouver Island and Lower Mainland populations are much less differentiated. The pairwise F_{ST} estimate (0.29) between Haida Gwaii and southwestern BC is relatively high, and is similar to levels of differentiation often seen between subspecies using SNP data (e.g., Fredrickson et al. 2015, Colella et al. 2018, Winker 2021). The F_{ST} is higher than the only other study that calculated pairwise F_{ST} between populations of a species on and off Haida Gwaii using SNP data. For the Northern Goshawk, the weighted pairwise F_{ST} between Haida Gwaii and Vancouver Island was 0.079, and weighted pairwise F_{ST} between Haida Gwaii and coastal BC was 0.093 (Geraldes et al. 2019). Of the endemic taxa on Haida Gwaii, most are differentiated from their mainland relatives at the level of subspecies, while some are even considered different species (Reimchen et al. 2005).

If past populations on Haida Gwaii were smaller and less connected than those on the mainland, genetic drift may explain the high population divergence and lower genetic diversity (Allendorf et al. 2013). Haida Gwaii’s small land area and large distance from the mainland may also be a factor - genetic drift acting on island populations can be particularly strong; leading to higher genetic divergence and lower diversity. Although Vancouver Island is also insular, it is three times larger than Haida Gwaii, and geographically closer to the mainland (50-144km vs 3-19km), with many islands as potential stepping stones to the mainland, potentially resulting in multiple founding events, which would reduce the impact of genetic drift. Alternatively, toad populations on Haida Gwaii may have lower genetic diversity because a small number of toads founded the original Haida Gwaii populations, or because of a population bottleneck that occurred at some point, for example, if toads were present on the island during the LGM. Both these processes would reduce the effective population size, which could result in the genetic patterns observed in this study. It is also possible both processes could have occurred at different times.

My study revealed that Haida Gwaii populations are strikingly less genetically diverse than either Vancouver Island and Lower Mainland populations – consistent both with expectations for small island populations (Frankham 1997, 1998), and nucleotide diversity levels seen in amphibian populations in a historically glaciated landscape (Chiocchio et al. 2021). Additionally, there is a higher frequency of rare alleles and fixed loci in Haida Gwaii populations compared to both Vancouver Island and Lower Mainland populations. Together, these results of low genetic diversity, high genetic differentiation and more rare alleles suggest that Haida Gwaii populations have been isolated for a long time, and that toads on Haida Gwaii may have experienced a population bottleneck or founder effect, resulting in a small effective population size, strong genetic drift, and ultimately a loss of genetic variation. Genetic diversity (nucleotide diversity, expected heterozygosity) is similarly low across all Haida Gwaii populations, which suggests the founder effect or bottleneck occurred long ago (Nei et al. 1975, Allendorf et al. 2022).

Several studies have tested for founder or bottleneck effects on different species on Haida Gwaii. In one such study, Reimchen et al. (2013) detected the signature of a founder effect in threespined stickleback (*Gasterosteus aculeatus*), and in another study, two vascular plants (*Senecio newcombei* and *Saxifraga taylori*) showed levels of diversity consistent with bottleneck or founder effect (Goertzen 1996). Conversely, the signature of either effect was not detected in several birds (Pruett et al. 2013), or kelp (Brooks 2020), likely due to the high dispersal ability of these species.

It is unknown when toads arrived on Haida Gwaii, but toads likely colonized Haida Gwaii prior to the submersion of the land bridge given that they are unlikely to disperse through ~ 100km of seawater and appear to be highly genetically differentiated currently. Toads could have arrived via the Hecate land bridge in the Holocene, as a number of other species likely did (Reimchen et al. 2005). The land bridge across Hecate Straight existed between ~11,700 and 10,000 years before present (Hetherington et al. 2004, McLaren et al. 2020), so colonization by toads could have conceivably occurred during this time, as the land bridge is thought to have been a mosaic of forest and open freshwater and marine marsh habitat (Fedje and Josenhans 2000, Lacourse et al. 2003). Alternatively, toads may have arrived on Haida Gwaii before the LGM and persisted in unglaciated areas. There is evidence that parts of Haida Gwaii were likely unglaciated refugia during the LGM (Clague et al. 1989, Shafer et al. 2010, Pruett et al. 2013, Barrie et al. 2014), so toad populations may have survived through the LGM on Haida Gwaii. It is unknown whether there were coastal toad populations at Haida Gwaii latitudes prior to the LGM, but Western Toad remains from 18,000 to 16,000 years ago have been recorded in karst caves on the northern tip of Vancouver Island (Harington 2011), so it is conceivable that toads could have also reached Haida Gwaii prior to this time. Additionally, Haida Gwaii has been posited to be a source of post-glacial recolonization of the continental Pacific Northwest via the Hecate land bridge for some species, such as the black bear (*Ursus americanus*) (Reimchen et al. 2005).

Additionally, although this study was not designed to evaluate whether Haida Gwaii toads are locally adapted to environments of the archipelago, it is possible the few individual loci with high F_{ST} values in Figure C4 (Appendix C) are associated with loci involved in local adaptation. There are examples of local adaptation in other species on Haida Gwaii; for instance, phenotypic variation in sticklebacks is attributed to natural selection due to the unique habitats on Haida Gwaii (Moodie and Reimchen 1973, Reimchen et al. 2005). Future study using genotype-environment association approaches on larger numbers of SNPs distributed across the genome or phenotypes from reciprocal transplant experiments would be needed to understand whether Haida Gwaii toads are locally adapted.

Although there were lower levels of genetic diversity in Haida Gwaii compared to southwestern BC, inbreeding levels did not differ among regions. The slightly negative but similar F_{IS} estimates for toads in all regions may indicate a slight tendency for disassortative mating (when individuals chose mates that are more phenotypically dissimilar than expected by chance). Disassortative mating has been previously documented in a small number of amphibians (*Ambystoma*; Bos et al. 2009), as well as other taxa (Watt et al. 1986, Houtman and Falls 1994, Day and Gilburn 1997, Hedrick et al. 2016).

4.4.2 Population structure within Haida Gwaii

Haida Gwaii toad breeding sites on Haida Gwaii can be grouped into two or three populations (based on PCA, *STRUCTURE* and DAPC results for $K = 2$ and $K = 3$). Isolation-by-distance is a driver, but the genetic structure could also reflect saltwater and topographic barriers, glaciation history, and genetic drift. Northern Haida Gwaii populations are more strongly structured than Gwaii Haanas populations (higher within-region F_{ST}), even when controlling for geographic distance. Higher differentiation among northern Haida Gwaii populations may have resulted from lower population sizes or less gene flow among them, perhaps due to recolonization from multiple glacial refugia. Research suggests there was little to no ice cover on the west coast of Haida Gwaii during the last glaciation (Barrie et al. 2014). The strong differentiation of the

Gudal Lake population on the west coast of Graham Island, despite proximity to several other breeding sites, may be evidence for it being descended from a different glacial refugium. Alternatively, the high differentiation could be due to topographic barriers preventing gene flow. The Gudal Lake site is isolated from other toad breeding sites included in this analysis by mountains over 1000m high and rugged areas are known to reduce amphibian dispersal (Funk et al. 1999). Furthermore, it is possible the high differentiation of Gudal Lake from all other breeding sites could be due to accidentally sampling siblings or relatives. Genotyping additional samples from the west coast and conducting habitat suitability modelling would help differentiate among these hypotheses.

4.4.3 Limitations and future research

A caveat of my study is that I did not have northern populations on the mainland adjacent to Haida Gwaii. Planned genotyping of these northern populations will allow us to tease apart whether the genetic differentiation of Haida Gwaii populations is associated more with environment or merely distance. Sampling these populations by the BC Ministry of Forests, Lands, and Natural Resource Operations was delayed by the COVID19 pandemic, and I plan to include northern mainland populations in analyses later in 2022. Additionally, when I have these extra samples, I will run Approximate Bayesian Computation to test alternative demographic scenarios to better understand the history of the toad population on Haida Gwaii versus the mainland.

Another caveat of my study is that I had a high level of missing genetic data – both at the SNP- and individual-level – as is often the case with GBS datasets (Elshire et al. 2011, Lowry et al. 2016). This problem was exacerbated by having relatively low coverage per individual sample on the sequencing lane. Low coverage was likely exacerbated by the relatively large genome size of the Western Toad. In future analyses, the number of samples per lane should be reduced to get greater coverage per sample. However, it is unlikely that the level of missing data that passed the filters has changed the patterns of diversity and differentiation presented here. I repeated many analyses using different filtering criteria (the less stringent resulting in more SNPs and individuals retained) and my results were robust across these changes.

4.4.4 Conservation implications

If Haida Gwaii populations are strongly genetically differentiated from north coast populations (as they are from southwestern BC populations), then if the Haida Nation desires, I suggest treating Western Toads on Haida Gwaii as a Designatable Unit under Canada's Species At Risk Act, equivalent to an evolutionarily significant unit (Moritz 1994), or even a new subspecies. Given the relatively high level of observed differentiation and physical isolation, they may meet the criteria for describing a third subspecies (Winker 2021). Anecdotal observations suggest that they are morphologically similar to their southwest BC relatives, though this has not been quantified. Additional genetic data, including mitochondrial DNA sequencing, and whole genome sequencing of a small number of mainland and island individuals could also be considered. Making Haida Gwaii toad populations a separate Designatable Unit, would more accurately reflect the unique evolutionary history of this lineage, and provide land managers with more tools to protect and conserve the Western Toad on Haida Gwaii. Recognition of Haida Gwaii toads as a new Designatable Unit under the Species at Risk Act could allow their conservation status (currently "Special Concern") to be tracked independently of the other non-calling Western Toad populations in BC over time. Additionally, it may be prudent to reconsider the provincial Conservation Status Ranking in BC, perhaps changing it from ‘Yellow Listed - apparently secure’ to ‘Blue Listed - special concern’. These federal and provincial changes in conservation status could result in increased protection of toad breeding sites, and perhaps increase funding for research and conservation actions such as monitoring and invasive predator control.

Although there is no evidence to support an intervention like genetic rescue for Haida Gwaii toads at present, my results suggest that translocations of toads from any of the southwestern BC populations to Haida Gwaii for genetic rescue or restoration would not be advisable because this could potentially lead to outbreeding depression (Frankham et al. 2011). Haida Gwaii populations may have developed local adaptations to the Haida Gwaii environment or coadapted gene complexes that could be disrupted if they interbred with southwestern populations. Further research will be done to investigate if north coast populations are closely related to Haida Gwaii populations.

Low genetic diversity of Haida Gwaii populations may be cause for concern. Reductions in genetic diversity can reduce fitness and the potential of populations to adapt to future environmental changes (Frankham 2005, Bouzat 2010, DeWoody et al. 2021). However, whether this low diversity has fitness or adaptation implications for toads on Haida Gwaii remains to be seen. Low diversity may result in Haida Gwaii populations being more vulnerable to diseases (such as chytrid fungus), having a reduced ability to adapt to climate change, or lacking adaptations to avoid invasive predators. The weaker competitive response of Western Toad tadpoles from Haida Gwaii (versus southwestern BC toad tadpoles) in a controlled experiment with introduced Northern Red-legged Frogs (Chapter 3), may be in part indicative of lower overall fitness due to lower genetic diversity. Haida Gwaii populations may also be declining due to predation by invasive raccoons (*Procyon lotor*) (Burles et al. 2005, Johnston 2006), and this may have impacted demography or genetic diversity. As Haida Gwaii populations are less diverse, raccoons (and other possible invasive predators - e.g., feral cats) could have a disproportionate effect on toads by reducing genetic diversity further. A potential management action could be removing invasive predators from around toad breeding sites.

Given that Haida Gwaii consists of many islands, habitat connectivity is expected to be lower, making toad populations more vulnerable to habitat destruction from logging or urban development. If one population experiences a large demographic decline, it is unlikely there will be enough migration to replace the reduction in population size and genetic diversity, because toads are typically highly philopatric (loyal) to their breeding sites. Given the high differentiation among breeding sites, this makes any movement of individuals and genes between sites especially important to maintain. I posit that the protection of toad breeding habitat, monitoring of breeding sites, invasive predator control around breeding sites and the maintenance of any existing migration corridors among breeding sites will be crucial to maintain healthy Western Toad populations on Haida Gwaii. This research provides more evidence for the uniqueness of Haida Gwaii’s fauna and flora, and the importance of the archipelago for conservation.

Chapter 5: Conclusion

5.1 Summary of findings

Amphibian populations are declining worldwide due to many different threats such as habitat destruction, disease, non-native species and climate change, and are in dire need of informed conservation strategies. Island populations are particularly at risk. Invasive species are a major threat to amphibians on islands, along with factors such as habitat destruction and reduced genetic diversity in island populations. Additionally, species' ranges are naturally shifting due to climate change, bringing the definition of a non-native species into flux. To conserve amphibians, an increased understanding of population-level dynamics is necessary, using a diversity of methods.

In the introduction, I posed four broad research questions: **Question 1 --** how will species perform in novel environments as their ranges shift?; **Question 2** -- what effect will these range-shifting species have in recipient communities that have a similar, but not identical eco-evolutionary history?; **Question 3 --** what influences the degree of vulnerability of species to global change drivers?; and finally, **Question 4 --** how can we use this information to make conservation decisions for amphibians in a dynamic world? Although I do not give definitive answers to these broad questions – as they are part of much larger lines of research – in this chapter, I discuss how my findings advance knowledge in these areas.

I focussed on two amphibians of conservation concern in British Columbia (BC), Canada - the Northern Red-legged Frog (*Rana aurora;* NRLF), introduced to the Haida Gwaii Archipelago but native to southwest BC, and the Western Toad (*Anaxyrus boreas boreas,* called Hlk'yáan K̲'ust'áan in the X̲aad Kíl dialect of the Haida language (X̲aad Kíl Née; Haida

Language Council); hereafter “toad”), native to both areas. In Chapter 2, I modelled the range expansion of the introduced NRLF under current and future climate on Haida Gwaii (Q1), in Chapter 3, I conducted a mesocosm experiment to examine the effect of co-occurrence history and naïveté on competition between Western Toad and the NRLF (Q2, Q3), and in Chapter 4, I conducted genomic analyses to examine the genetic diversity and structure of 27 Western Toad (hereafter “toad”) populations on Haida Gwaii and southwest BC (Q4).

Together, my research suggests that: 1) the introduced NRLF has expanded its range since its discovery in 2002, and will likely continue to spread on Haida Gwaii, especially with climate change; 2) at the tadpole stage, Western Toads from Haida Gwaii were weaker competitors with introduced NRLF tadpoles compared to Western Toads tadpoles from southwestern BC (but it is unknown whether competition with NRLFs affect toad population persistence); and finally, 3) toad populations on Haida Gwaii are genetically less diverse and strongly differentiated from southwestern BC populations. The implications and possible explanations for these results are discussed in depth in the sections below, but I briefly present some possible explanations here. The reason the NRLFs have spread on Haida Gwaii could be in part because much of Haida Gwaii is within the native ecological niche of the species. Haida Gwaii Western Toad tadpoles may respond worse in competition towards the non-native NRLF tadpoles compared to toads on the mainland due to behavioral or dietary differences stemming from their short co-occurrence history with the NRLF or functionally similar competitors. This could be due in part to genetic differences observed in Chapter 4. The genetic patterns seen in Western Toads could be due to a combination of a historical population bottleneck or founder effect as well as contemporary small effective population size.

My research has wider implications for the fields of amphibian conservation, including non-native species dynamics, the vulnerability of island populations, and large-scale population patterns. A major strength of my thesis is that my study is one of only a handful of studies that examine a species introduction within the same bioregion. Additionally, the use of three different and complementary methods – species distribution modelling, experimental mesocosms and population genomics – greatly strengthened my research. The broader implications, limitations, conservation applications, and future research directions of my thesis are discussed herein.

5.2 The Northern Red-legged Frog has expanded its range on Haida Gwaii and will likely spread further

The NRLF is introduced on Haida Gwaii and spreading, but declining in southwest British Columbia where it is native (Gaston et al. 2002, COSEWIC 2015). I addressed three questions in this chapter to help quantify the current and future range of the NRLF on Haida Gwaii: 1) How far and at what rate have the NRLFs spread on Haida Gwaii? 2) How does the Haida Gwaii realized niche of NRLFs compare to the realized native range niche? 3) What areas of Haida Gwaii are NRLFs likely to occupy in the future?

I used species distribution modelling (SDM), a simple range expansion rate calculation, and niche comparisons to test these questions. I found that NRLFs have expanded their range 15-fold since 2002, and now occupy ~24% of Graham Island (the main island on Haida Gwaii). These dispersal estimates are within known annual movement estimates for the NRLF in its native range (4.8km/year; Hayes et al. 2007), and are close to the dispersal estimate from the other introduced amphibian species on Haida Gwaii (2km/year; Reimchen 1991). I also found that the environmental conditions of the Haida Gwaii realized niche of the NRLF are a subset of the conditions in the native range realized niche, suggesting the current niche is limited on Haida Gwaii, potentially by dispersal (or biotic factors), which may be overcome with time. Additionally, the predictions from my model suggest that the NRLF could potentially occupy many other areas of Haida Gwaii, and that habitat suitability on Haida Gwaii will increase with climate change.

Chapter 2 contributes to the growing body of knowledge that many introduced species conserve their niche in their non-native range. Niche conservatism makes it easier to predict habitat suitability in the introduced range of a species. I also highlight the utility of including a dispersal analysis alongside a species distribution model. It allows habitat suitability models to be grounded in species ecology, and gives a more informed estimate of when suitable habitat could be reached. This is especially important to consider in the face of climate change, when habitats are becoming more or less suitable.

Chapter 2 also highlighted the importance of conducting species distribution models at finer scales. I compared my map of predictions of habitat suitability with climate change to predictions from a previous, lower resolution model. While the lower resolution approach picked up the overall trend of increasing habitat suitability with climate change, the poor spatial resolution meant that nuances were missed. My higher resolution predictions revealed that climate change may not act heterogeneously on habitat suitability. For instance, some areas of Haida Gwaii may increase in habitat suitability for the NRLF with climate change, while others may decrease. This finding has significant implications for species management generally (both native and non-native). Many conservation actions are targeted at the local scale, so this research highlights the importance of obtaining finer resolution habitat variables when they are available.

My findings from Chapter 2 also hint at the potential for some species to do well when migrating naturally or due to translocations within the same bioregion (Question 1), but raises the issue of the ethics of such translocations (discussed in conservation implications).

5.3 Co-occurrence history affects interspecific competition between an introduced frog and island versus mainland toad populations

Haida Gwaii residents are concerned because some toad populations have recently been extirpated, and biologists have wondered if the NRLF introduction is partly to blame. Furthermore, Chapter 2 showed that the NRLF will likely increase its distribution across Haida Gwaii. In this study, I tested the hypothesis that within the same species, historically isolated (allopatric) populations with only a short co-occurrence history will be more impacted by competition with non-native potential competitors, compared to sympatric populations that have a long history of co-occurrence with the competitor (i.e., in areas where both species are native). I did this by conducting a tadpole mesocosm competition experiment using toad and NRLF tadpoles from both Haida Gwaii (short co-occurrence history) and the mainland (long co-occurrence history).

NRLFs decreased the growth rate, weight at metamorphosis, and delayed the timing of metamorphosis of toads from Haida Gwaii compared to southwest BC, suggesting Haida Gwaii toads were competitively inferior compared to mainland toads, and that this was driven by a short co-occurrence history with the NRLF. We don't, however, know, how their interaction plays out *in situ*, and this is an area for future research. Additionally, NRLFs were slightly better competitors under competition with the Haida Gwaii toads compared to mainland toads, perhaps suggesting that they possessed plastic adaptations that advantaged them when in the presence of naïve island toads. This could potentially put Haida Gwaii toads at an additional disadvantage; not only are they poor at competing with NRLFs, but NRLFs are extra good at competing with them. Further research could investigate the behaviour and population genetics of NRLFs from Haida Gwaii compared to their native range.

Predicting how native species will respond to invasive species, and how the strength of species interactions vary across space and time is an active area of research, and this study suggests that we need to consider the co-occurrence history of competitors. The finding of that competition strength varies with co-occurrence history is significant to the field of ecology and conservation biology and has implications for conservation programs considering assisted gene flow for climate change adaptation (Aitken and Whitlock 2013) or translocations for genetic rescue of populations (Whiteley et al. 2015) (Questions 2 and 3).

While I did not test whether the populations are able to co-exist (in terms of testing whether populations can maintain a positive growth rate despite perturbations), my research raises further concerns in the face of shifting climate and assisted migration programs. Will these shifting species negatively impact naïve species? Or is the effect I observed because island species are more vulnerable, making Haida Gwaii populations especially defenceless – or will my results scale up to non-island species?

5.4 Western Toads on Canada's Haida Gwaii archipelago have low genetic diversity and are strongly differentiated from southwestern British Columbia populations

I compared levels of genetic diversity and differentiation between Haida Gwaii and southwestern BC toad populations and evaluated how closely related a number of populations are on Haida Gwaii. I found that Haida Gwaii toad populations were considerably less genetically diverse and were highly differentiated from populations in southwestern BC. This indicates the Haida Gwaii populations have experienced a smaller effective population size, likely the result of a population bottleneck or founder effect, and likely have been separated from southwestern BC populations for at least ~10,000 years, since the Last Glacial Maximum (LGM) (Barrie et al. 2005).

Chapter 4 contributes to the knowledge that island populations are less diverse and often highly differentiated from their mainland counterparts. Combined with the experimental results from Chapter 3, it could also suggest that island populations may be genetically vulnerable to the effects of non-native competitors (Question 3).

5.5 Limitations

5.5.1 Species distribution modelling

A major limitation of this research was that I undoubtedly did not include all the variables that are important for this species' persistence (Mod et al. 2016). This is a constant challenge with SDMs and why we talk about SDMs modelling the realized niche rather than the fundamental niche (Pulliam 2000, Wiens and Graham 2005, Elith and Leathwick 2009, Guisan et al. 2014). For instance, the Random Forest model did not incorporate biotic interactions or local demography, even though both could be influential for habitat suitability. Additionally, the habitat descriptors used here were estimated in 2002, whereas most of the surveys included were conducted after 2010. Because significant change in land cover has taken place since forest harvest on Haida Gwaii and in the native range since 2002, models based on updated habitat mapping could be used to improve model precision and perhaps also evaluate the influence of forest harvest on the permeability or suitability of habitat for NRLFs.

Furthermore, the accuracy of my model may be inflated, as is often the case in SDM studies (Lee-Yaw et al. 2021, Santini et al. 2021). Hence it is important to emphasize that my habitat suitability maps are predictions, and may not accurately represent the future distribution of the NRLF on Haida Gwaii. Furthermore, the climate change predictions are based on the SSP2-4.5 emissions schemes scenario, and so if the world does not conform to this scenario, the predictions may be inaccurate. This limitation can be over-come by running further scenarios and using updated models as climate predictions change.

5.5.2 Competition experiment

A major limitation of the experiment is that it did not include Western Toad populations that had never been exposed to the NRLF (e.g., populations on Moresby Island). However, even if an effort had been made to collect populations from Moresby Island (at great cost!), those populations could have been exposed to the Pacific Chorus Frog, which may impact their response to the NRLF. Furthermore, the temporary nature of how frogs use breeding habitats annually could also make it difficult to be sure that frogs had never occupied this habitat at the time of sampling. Additionally, a study with more NRLF breeding sites per population and a NRLF control could be used to more carefully assess any potential differences between competitive abilities of the NRLF populations, and to determine if they are competitively superior, or if the differential response is solely due to naïveté of the Haida Gwaii toad.

It is unknown if the results of my mesocosm experiment scale up to the pond or lake scale, or if they would result in population-level demographic impacts. The advantage of mesocosms is that they allow the simplification of ecosystems to test drivers that would normally be too hard to tease apart in nature. However, there are many known pitfalls to using mesocosms such as: lower mortality than in natural systems, lack of other potentially mediating species, and less physical space and habitat complexity than in nature (Schindler 1998). As a result, mesocosm studies are known to artificially inflate the interactions beyond what would be observed naturally (Schindler 1998). I tried to mediate some of these effects by creating a shallow to deep depth gradient, and scaling the ratio of NRLFs to toads allometrically (rather than using equal numbers, as many competition studies have done). Additionally, I only used three toad breeding sites and one NRLF breeding site from each region, and was constrained with the number of replicates of each treatment in the experiment, which reduces the power to detect effects. Furthermore, the mesocosm experiment was conducted in the climate of the mainland toads, therefore, the inferior competitive response of toads from Haida Gwaii could be partly due to reduced acclimation or local adaptation of some populations to the experimental environment, though this seems unlikely given the effect size of temperature in the models.

Due to my experimental design, I was also limited in my power to compare the competitive abilities of the NRLF between source regions. A study with more NRLF breeding sites per population and a NRLF control without toads is needed to more carefully assess any potential differences between competitive abilities of the NRLF populations.

5.5.3 Genetic analyses

A limitation of the population genetic analyses was the high level of missing data for individual toads and for individual loci, which resulted in a low number of SNPs for subsequent analyses compared to other population genomics studies. While the amount of data was adequate for classic analyses of population diversity and differentiation, this low number of SNPs made it hard to determine drivers of population differences, for example, historic demographic events and neutral genetic structure versus local adaptation. Fewer SNPs also made it harder to compare within population differences such as inbreeding.

Additionally, it would have been ideal to have Western Toad populations from northern coastal BC and more populations from Vancouver Island, but sampling of these areas was delayed by the COVID-19 pandemic. Future analyses will include Western Toad populations from the North Coast of BC to determine if the observed genetic differentiation is just due to isolation by distance, or due to some other effect (such as climate adaption). It would have been useful to compare differentiation patterns within Vancouver Island to within Haida Gwaii to see if the patterns I observed were unique to Haida Gwaii or reflect patterns seen on other islands of coastal BC. However, this study only used seven populations from Vancouver Island, which combined with especially high levels of missing SNP data for Vancouver Island, made it unadvisable to investigate within-island patterns for Vancouver Island. Ongoing research in collaboration with BC Ministry of Forests, Lands and Natural Resource Operations will include seven additional populations from Vancouver Island, as well as some from northern coastal BC.

Another limitation to the genetic analyses was that I used *de novo* assembly to call my SNPs, because an adequate reference genome was not available at the time of analysis. Reference based assembly is better than *de novo* assembly because it allows the identification (and removal) of SNPS that are sequencing errors and “linked SNPs” that are close to each other on each chromosome (Wright et al. 2019). A draft Western Toad reference genome sequence has been produced (Chris Funk, Colorado State University, pers. comm.) and future analyses of these data will align sequences from these and additional samples to this reference when it is available.

5.6 Conservation management implications

5.6.1 Northern Red-legged Frog

Climate induced range shifts are blurring the lines between non-native and native species (Urban 2020). Given the NRLF is a species of conservation concern in Canada and the US, where it is native, its potential to be classed as an invasive species on Haida Gwaii in the future (as it has in its non-native range in Alaska) may raise ethical issues given it is declining in its native range. An argument could be made for treating the introduction on Haida Gwaii as positive for conservation, if the NRLF does not negatively impact native species, ecosystems or cultural values.

Whether the NRLF is classed as an invasives species on Haida Gwaii is in part a question of values. The cultural values of the Haida Nation, who co-manage wildlife on Haida Gwaii, will be a very important part of this decision. Additionally, Haida Gwaii is a Pleistocene Refugium, and generally introductions to these unique ecosystems are viewed negatively. Future research should investigate whether the NRLF has a negative impact on the species, ecosystems and cultural values of Haida Gwaii which, if true, would help make the course of action more obvious.

The results from my competition experiment suggest that the NRLF may have negative effects on the native Western Toad, but it is not known if these effects have population-level implications for conservation (Chapter 3). Given that my results show potential for unfavorable impacts on the native toad, and it has been classed invasive in southeast Alaska, perhaps managers should err on the side of caution, potentially protecting Haida Gwaii toad populations, at the expense of the NRLF. It could be advisable to at least slow or prevent the expansion of NRLFs to new islands on Haida Gwaii, such as Moresby Island. The results from my species distribution models suggest that certain areas of Haida Gwaii will likely be more suitable than others in the future with climate change (Chapter 2). There are still many significant barriers to dispersal that the NRLF will have to overcome to reach these areas, so it could be advisable to focus eradication efforts near these large natural dispersal barriers (such as salt, water and mountains). In particular, the two currently unoccupied areas - the west coast of Haida Gwaii and northern Moresby Island, the gateway to Gwaii Haanas, both show potential to increase in habitat suitability for the NRLF with climate change.

Gerick et al. (2014) predicted that 45% of the current native range of the NRLF in BC will be thermally limiting by 2080. No matter the decision on how to view the introduction on Haida Gwaii, the fact that the NRLF is predicted to spread with climate change in another area of BC may give some hope for native range conservation in the northern range. Perhaps areas of the native range in BC that are most similar to the parts of realized niche on Haida Gwaii that are predicted to increase in habitat suitability with climate change could be good candidate areas for focused conservation actions, such as habitat restoration and invasive species removal. Additionally, areas near the northern range limit of the current range in BC could potentially be considered for recipient sites for assisted or natural migrations. However, local research should be conducted to identify any potential negative impacts on naïve species in these areas (Chapter 3). The Haida Gwaii population of the NRLF may have conservation value in the future if NRLF populations in the native range continue to decline due to climate change or other stressors. The Haida Gwaii population could potentially be used as a source to recolonize the native range of the NRLF.

5.6.2 Western Toad (Hlk’yáan K̲’ust’áan) populations on Haida Gwaii

Like the NRLF, the Western Toad is a species of conservation concern in Canada and the US. Local people on Haida Gwaii are concerned because there have been fewer toad sightings in the past few decades. There are numerous possible explanations for Western Toad population declines on Haida Gwaii, including the NRLF (via several direct and indirect mechanisms discussed below), invasive predatory raccoons and feral cats, destruction of breeding sites, climate change, and low genetic diversity.

Results from Chapter 3 suggest that the NRLF may pose a threat to the toad via competition at the larval stage. Although decreases in growth rate, weight and timing of the metamorphosis have been known to affect juvenile survival, it is unknown if the negative effects of NRLFs result in population-level impacts. The magnitude of any negative population-level effects on the Haida Gwaii toad population from NRLF larval competition would increase as both the relative abundance of NRLFs to toads and overall density of NRLFs at toad breeding sites increases. This could mean that smaller ponds or low productivity sites that are toad breeding sites could be especially vulnerable to competition from the NRLF at the larval stage. One potential management action on Haida Gwaii would be to remove NRLF egg masses from toad breeding sites, especially at toad breeding sites that have a small volume of water relative to the number of amphibians or are low productivity sites. Future research could investigate the interaction between the two species at a pond scale to see if NRLFs reduce population growth rate of toads. Additionally, research could investigate whether NRLFs are having indirect impacts on the toads, for instance, through providing subsidies to predators, thereby increasing predator densities and increasing predation on toads, or whether adult NRLFs eat toad eggs or tadpoles. It is possible that Pacific Chorus Frogs also have negative impacts on the toads through similar mechanisms, as hypothesized by Reimchen (1991), so future research could include this species as well.

Chytrid fungus (*Batrachochytrium dendrobatidis*) is a cause of amphibian declines worldwide (Berger et al. 1998, Daszak et al. 2003, Skerratt et al. 2007). Researchers have also wondered if the NRLF may have spread chytrid fungus to Western Toad populations on Haida Gwaii. However, to date chytrid fungus has not been observed on Haida Gwaii, but testing has been limited to a single round of 30 individuals in 2018 (unpublished data). It is also possible that chytrid fungus went through toad populations some years ago, and toad populations are still recovering. Future monitoring should test higher numbers of amphibians on Haida Gwaii for chytrid fungus on a regular basis.

There are numerous other potential reasons that could explain Western Toad population declines on Haida Gwaii that are unrelated to the NRLF. For instance, during my research, I learned that invasive raccoons target toad breeding sites on Haida Gwaii for predation (Burles et al. 2005, Johnston 2006). There is one lake on Haida Gwaii called “Dead Toad Lake” because of the sheer density of dead toad skins observed following a raccoon attack (the raccoons presumably skin the toads to avoid the bufotoxin) (Tom Reimchen, personal communication 2017). While predation of Western Toads by raccoons has been observed outside of Haida Gwaii, it is unknown whether the volume of predation occurring is much larger than predation within the native range of raccoons. Perhaps Haida Gwaii Western Toads do not possess behavioural adaptations to avoid raccoons? The ability to adapt to new threats, including novel predators, can be hampered by low genetic diversity (Willi et al. 2006, Allendorf et al. 2022). Additionally, deleterious alleles are known to accumulate in small, isolated populations with high levels of genetic drift (Willi et al. 2006, Allendorf et al. 2022). Perhaps the low genetic diversity I observed in Haida Gwaii toads (Chapter 4) is a contributing factor in their presumed vulnerability to that predator. Furthermore, given that direct mortality of sexually mature adults is the worst threat to toad population numbers (COSEWIC 2012), it could be advisable to control invasive raccoons and any other invasive predators around toad breeding sites on Haida Gwaii.

Since Western Toads are philopatric (loyal) to their breeding sites, monitoring and protecting toad breeding sites is crucial for conservation. Given that the location of many Western Toad breeding sites in the most human populated parts of Haida Gwaii were unknown until recently, it is possible that some of the recent population declines may be due to the unintentional destruction of toad breeding sites. During three years of field work and extensive bog tromping, I located nine toad breeding sites on Haida Gwaii that were previously unrecorded in recent memory (Appendix D), making the total number of known toad breeding sites on Haida Gwaii at least 16. Six of the previously known toad breeding sites are in Gwaii Haanas National Park and Haida Heritage Site, and have been monitored every year or two for the last ten years. I recommend annual monitoring of toad breeding sites on Graham Island to monitor population level trends, particularly of easily accessible breeding sites, and am currently working with local conservation managers to start citizen science monitoring of toad breeding sites (www.haidagwaiitoads.weebly.com). I have included an Appendix with the location and satellite imagery of all toad breeding sites on Haida Gwaii that I visited (Appendix D).

Climate change combined with their philopatry could also be a driver of local toad population extinctions on Haida Gwaii. Toads sometimes breed in habitats that are shallow or temporary, and some of these may have dried up naturally over the years. Such climate change-related effects have been observed on Vancouver Island, where a breeding site dried up completely, but toads still went there and attempted to breed (Melissa Todd, personal communication 2021).

Given the concern about Western Toad population persistence on Haida Gwaii, their high genetic differentiation from southwestern BC toads and low genetic diversity (Chapter 4), along with their isolation from all other toad populations, if the Haida Nation desires, Haida Gwaii Western Toads could be evaluated for consideration as a separate Designatable Unit under the Species at Risk Act legislation in Canada, or even a new subspecies. They are currently part of the "non-calling population" Designatable Unit. Anecdotal observations suggest that they are morphologically similar to their southwest BC relatives, though this has not been quantified. Additional genetic data, including mitochondrial DNA sequencing, and whole genome sequencing of a small number of mainland and island individuals could also be considered. Recognition of Haida Gwaii toads as a Designatable Unit under the Species at Risk Act could allow their conservation status (currently "Special Concern") to be tracked independently of the other non-calling Western Toad populations in BC over time. Additionally, it may be prudent to reconsider the provincial Conservation Status Ranking in BC, perhaps changing it from 'Yellow Listed - apparently secure' to 'Blue Listed - special concern'. These federal and provincial changes in conservation status could result in increased protection of toad breeding sites, and perhaps increase funding for research and conservation actions such as monitoring and invasive predator control. Given the involvement of the local Haida Gwaii community in toad monitoring, and the interest from the Haida Nation and BC provincial government in protecting this species and their breeding sites, I am optimistic for the conservation of the Western Toad on Haida Gwaii.

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Appendices

Appendix A Supplemental materials for Chapter 2

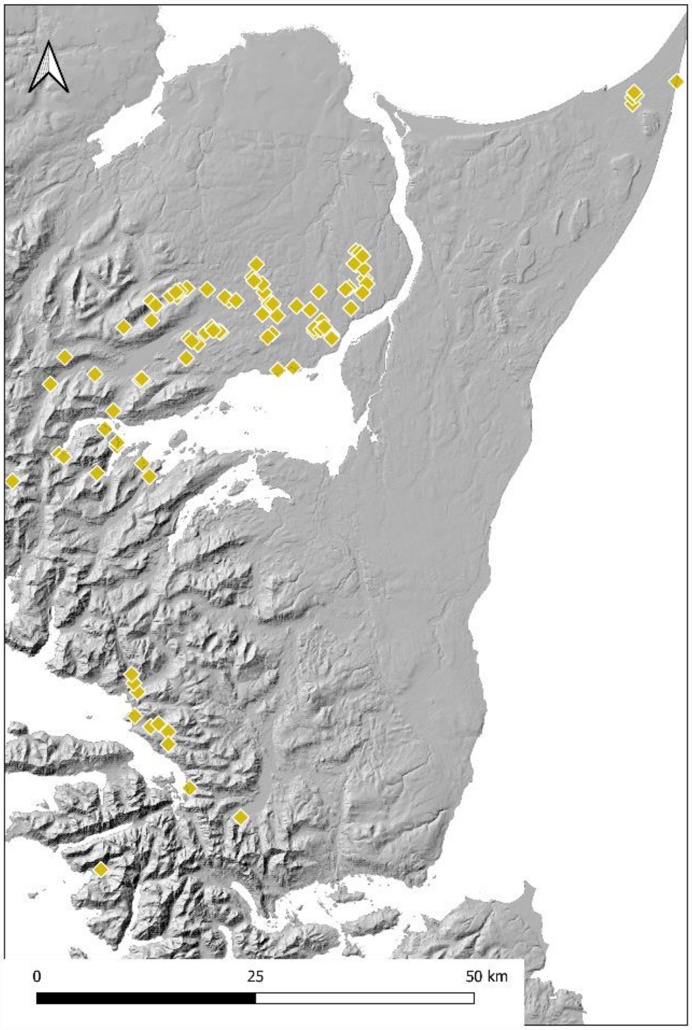


Figure A1. Map of central Graham Island, Haida Gwaii, showing the absences that were removed (n=95) from the Random Forest model. These were removed because they were outside the dispersal range from 2002 observations (based on dispersal rate) or beyond a large dispersal barrier (e.g., high mountains in southwest Graham Island). Points overlayed on a 1-arc digital elevation model (Farr and Kobrick 2000).

Table A1. Eigenvalues and variance explained for all PC axes.

PC axis	Eigenvalue	Variance %	Cumulative variance %
PC1	5.59	39.95	39.95
PC2	3.34	23.85	63.80
PC3	1.15	8.25	72.05
PC4	1.07	7.65	79.71
PC5	0.94	6.75	86.45
PC6	0.72	5.16	91.61
PC7	0.57	4.08	95.68
PC8	0.44	3.17	98.85
PC9	0.09	0.67	99.52
PC10	0.05	0.33	99.85
PC11	0.01	0.10	99.95
PC12	0.01	0.04	99.99
PC13	0.00	0.01	100.00
PC14	0.00	0.00	100.00

Table A2. Loadings for the first four PC axes. High positive loadings for PC1 and 2 are highlighted in yellow. Variable acronyms are presented, full names can be found in Climate BC manual (Wang et al. 2012).

Variable	PC1	PC2	PC3	PC4
PPT_sp	-0.105	0.495	0.015	-0.030
PPT_sm	-0.149	0.474	-0.062	-0.034
DD5_sp	0.397	0.071	0.116	-0.094
NFFD_sp	0.393	0.180	0.039	-0.023
Tave_sp	0.400	0.147	0.099	0.025
Tmin_sp	0.394	0.180	0.048	0.000
Tmin_sm	0.390	0.180	0.070	0.005
CMI_sp	-0.157	0.475	-0.012	-0.033
CMI_sm	-0.216	0.409	-0.145	-0.081
distance_nearest_lake_m	-0.009	-0.026	0.623	-0.144
distance_nearest_wetland_m	-0.214	-0.018	0.308	-0.234
pctwetland_buf500m	0.079	-0.045	-0.586	0.153
streamdens_buf500m	0.025	0.074	0.171	0.880
elevationrange_buf500m	-0.252	0.063	0.295	0.326

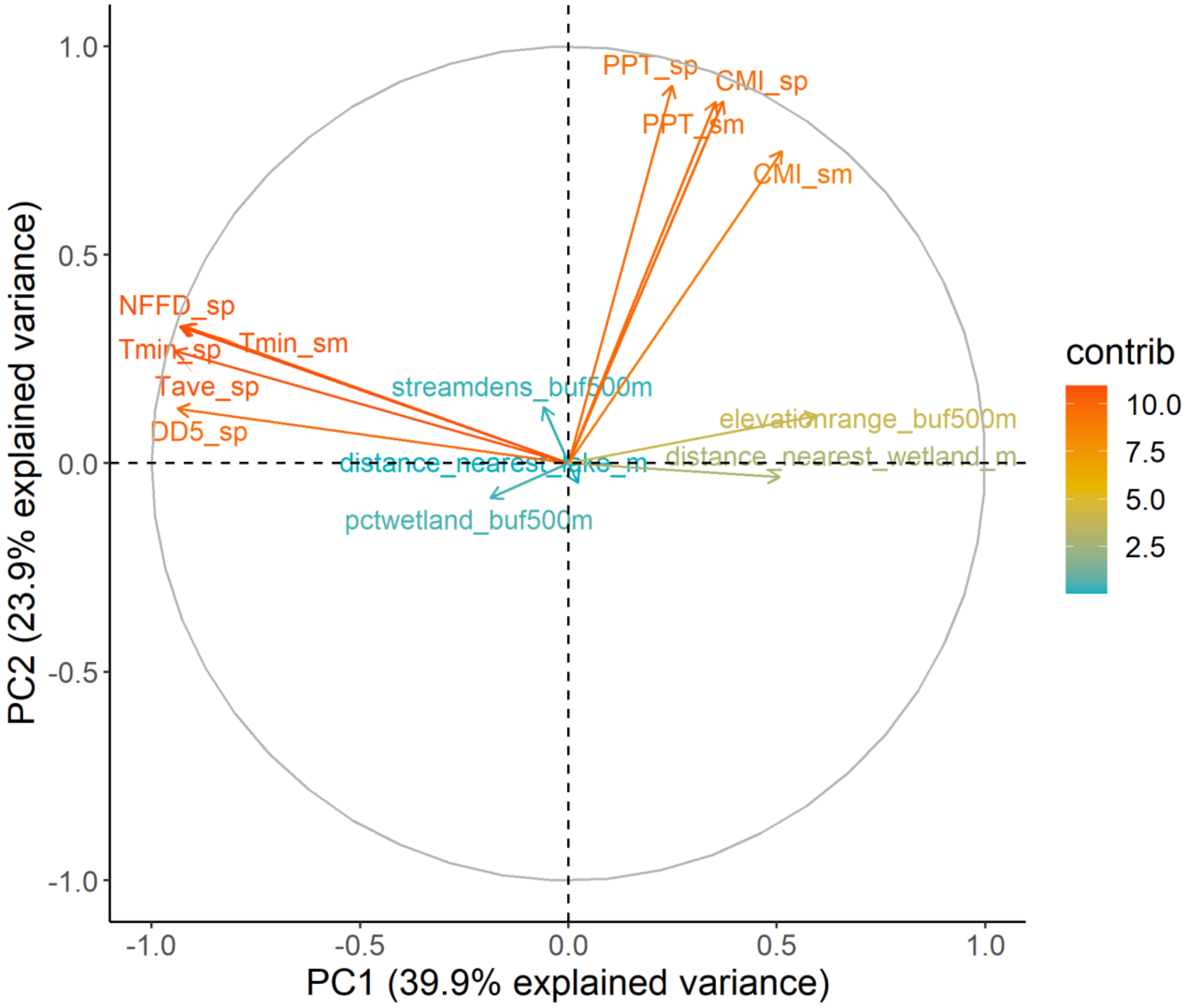


Figure A2. Plot of the contributions of each variable to PC1 and 2 axes. Acronyms for the climate and other environmental variables are presented, full names can be found in Table 2.1 and descriptions are available in the Climate BC manual (Wang et al. 2012).

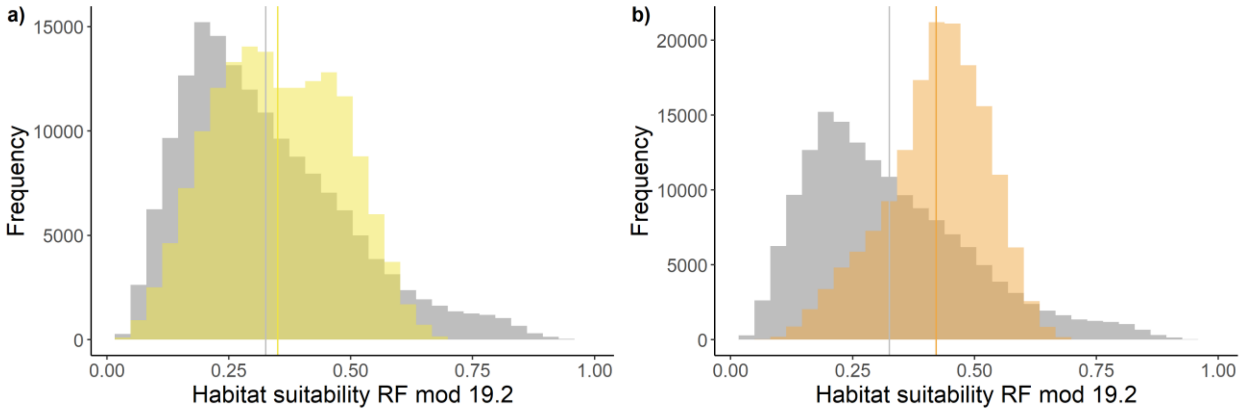


Figure A3. Histogram and mean (vertical lines) of habitat suitability across Haida Gwaii. Generated from the Random Forest model output comparing current conditions (grey) to future a) 2021-2040 ensemble SSP2-4.5 (yellow) yellow and b) 2081-2100 ensemble SPP2-4.5 (orange).

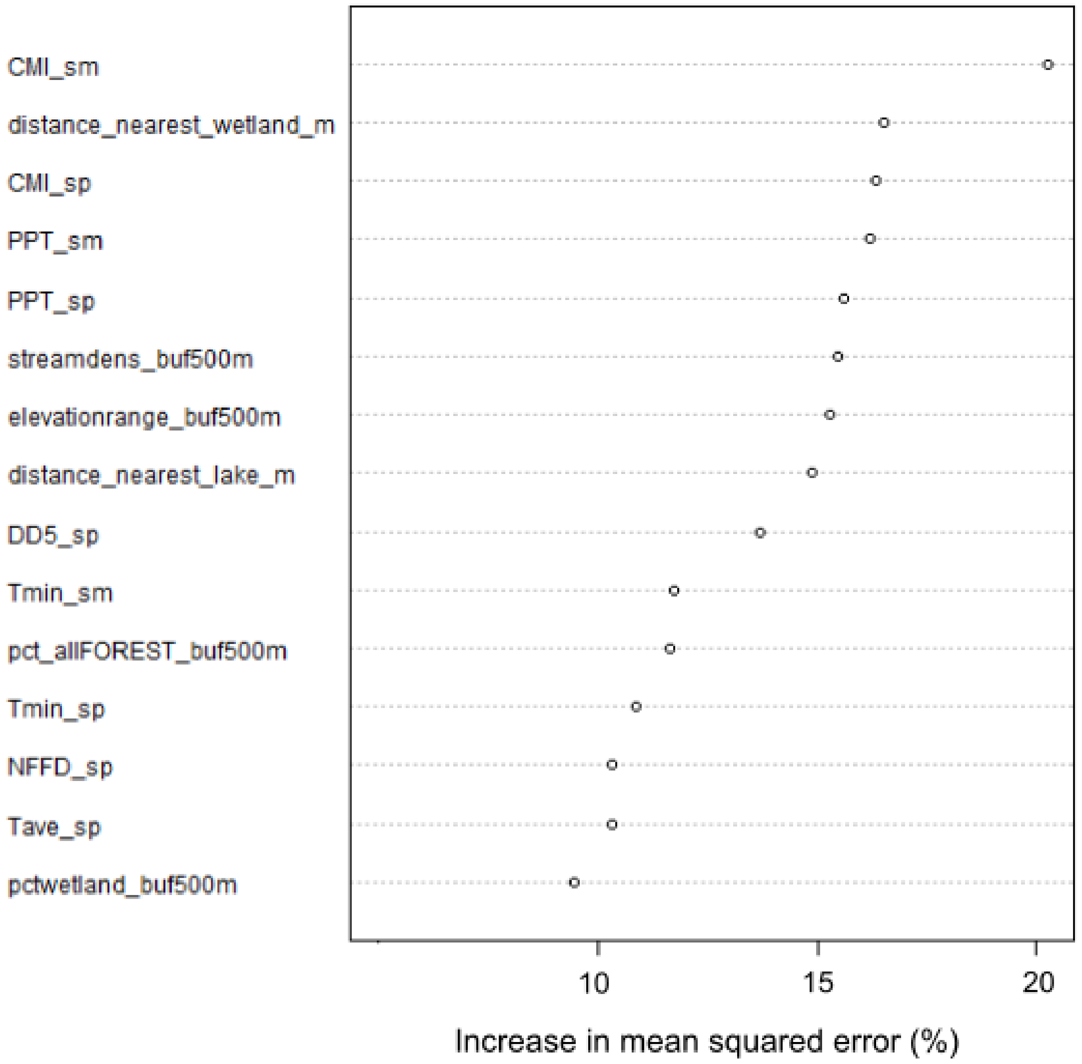


Figure A4. Increase in mean squared error for the variables in the Random Forest model with both Haida Gwaii and native range data (n=609). Higher increase in mean squared error = higher importance. Full variable names are in Table 2.1 and descriptions of the climate variables can be found in Climate BC (Wang et al. 2012).

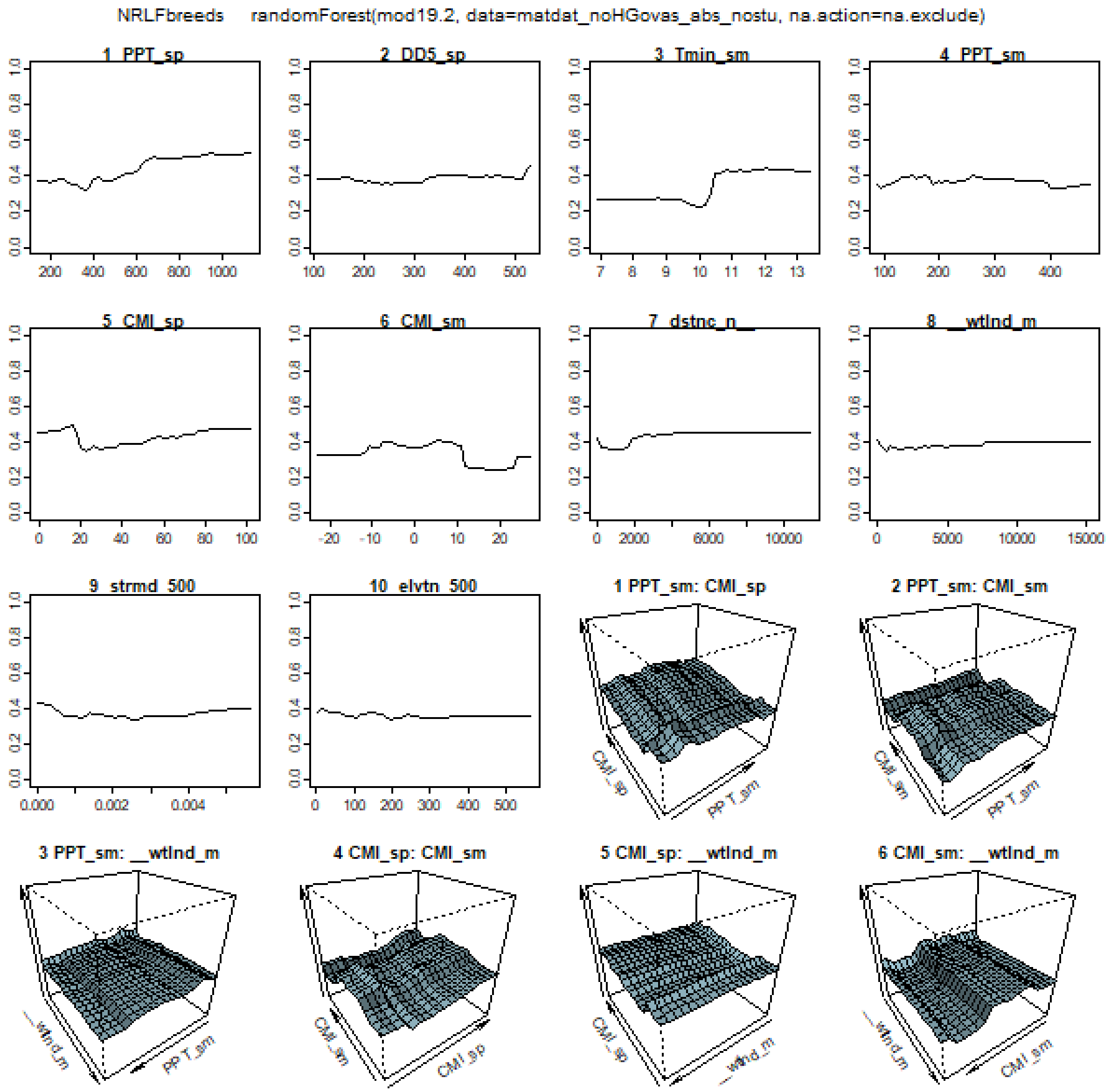


Figure A5. Response curves for the Random Forest model, using both native range and Haida Gwaii data (n=609). Full names can be found in Table 2.1 and descriptions of the climate variables can be found in Climate BC (Wang et al. 2012). "distnc_n" = distance nearest water body, "wtland_m" = distance nearest wetland, "strmd_500" = stream density (km2) in 500m buffer, "elvtn 500" = elevation range (m) in 500m buffer.

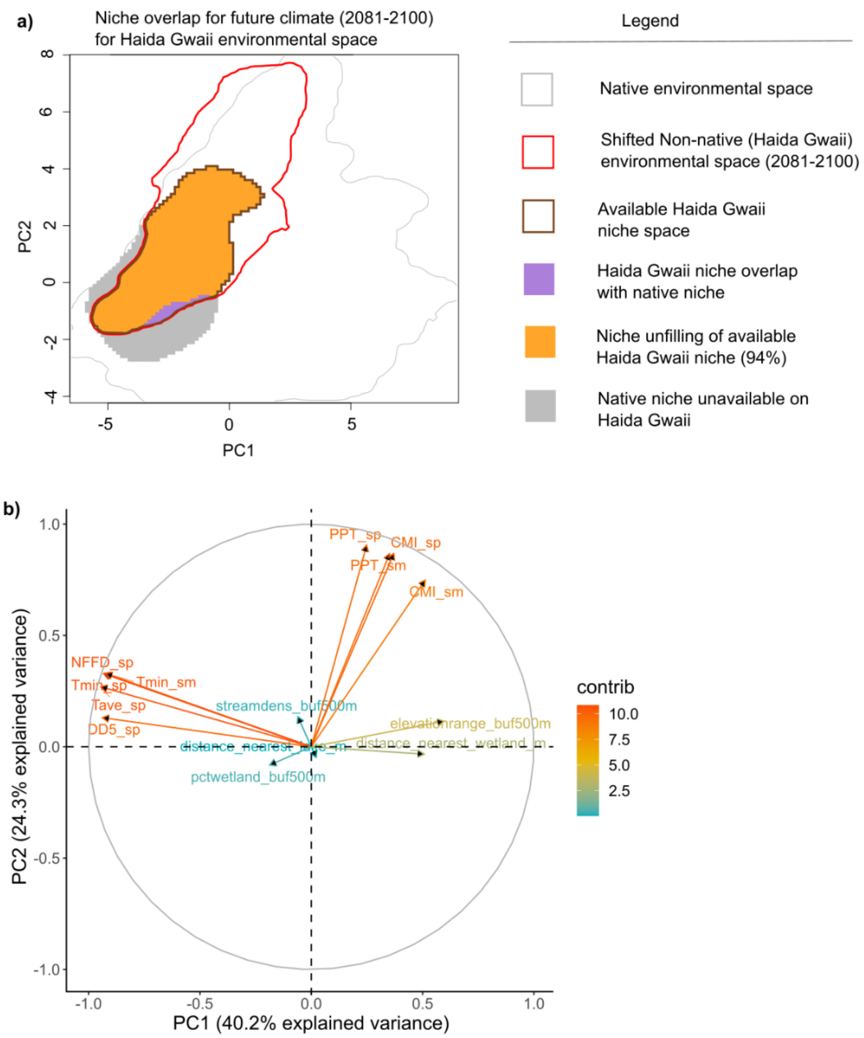


Figure A6. a) PCA of niche comparisons between the current climate of the native range and shifted future climate 2081-2100 (climate from ensemble model with SPP2-4.5 scenario) for the non-native range (Haida Gwaii). Grey cells show the native niche outside of Haida Gwaii environmental space, yellow cells show the unfilled niche on Haida Gwaii (of available niche), purple cells show the niche overlap between Haida Gwaii and the native range, solid brown line shows available Haida Gwaii niche space (i.e., native niche within Haida Gwaii environmental space). Niche unfilling (94%) result from *ecospat R* package (Di Cola et al. 2017). b) Plot of the contributions of each variable to the PC1 and 2 axes. Acronyms for environmental variables are presented, full names can be found in Table 2.1 and descriptions in the Climate BC manual (Wang et al. 2012).

Appendix B Supplemental materials for Chapter 3

B.1 Model structures for Chapter 3

As per the *lme4 R* package, “1 | random effect” denotes a random intercept and “1 + x | random effect” denotes a random intercept and slope (Bates et al. 2007).

Toad responses

1) *Toad weight at metamorphosis*

Average weight of juvenile toads at metamorphosis (mg) ~

Toad Co-occurrence History*Competitor Identity + Toad Mortality per Capita Scaled + Toad Tadpoles Top up Two Weeks per Capita Scaled + Days Since Experiment Began Scaled + Average Individual Toad Tadpole Starting Weight Scaled + Block + Mean Average Water Temperature Scaled + (1+ Days Since Experiment Began Scaled | *Tank*) + (1 | *Toad Breeding Site*)

2) *Toad growth rate*

$\log_{10}$ toad growth rate (mg/day) ~

Toad Co-occurrence History*Competitor Identity + Toad Mortality per Capita Scaled + Toad Tadpoles Top up Two Weeks per Capita Scaled + Days Since Experiment Began Scaled + Average Individual Toad Tadpole Starting Weight Scaled + Block + Mean Average Water Temperature Scaled + (1+ Days Since Experiment Began Scaled | *Tank*) + (1 | *Toad Breeding Site*)

3) *Toad median time to metamorphosis*

Median time to toad metamorphosis (days) ~

Toad Co-occurrence History*Competitor Identity + Toad Tadpoles Top up Two Weeks per Capita Scaled + Toad Mortality per Capita Scaled + Block + Mean Average Water Temperature Scaled + (1 | *Toad Breeding Site*)

Northern Red-legged Frog (NRLF) responses

1) *NRLF weight at metamorphosis*

Average weight of juvenile NRLFs at metamorphosis (mg) ~

Toad Co-occurrence History* NRLF Source + NRLF Tadpoles Top up Two Weeks per Capita Scaled + NRLF Mortality per Capita +Average Individual NRLF Tadpole Starting Weight Scaled + Days + Block + Mean Average Water Temperature Scaled + (1 | *Toad Breeding Site*) + (1 + Days | *Tank*)

2) *NRLF growth rate*

$\log_{10}$ NRLF growth rate (mg/day) ~

Toad Co-occurrence History* NRLF Source + NRLF Tadpoles Top up Two Weeks per Capita Scaled + NRLF Mortality per Capita +Average Individual NRLF Tadpole Starting Weight Scaled + Days + Block + Mean Average Water Temperature Scaled + (1 | *Toad Breeding Site*) + (1 + Days | *Tank*)

3) NRLF median time to metamorphosis

Median time to toad metamorphosis (days) ~ Toad Co-occurrence History* NRLF Source + NRLF Tadpoles Top up Two Weeks per Capita Scaled + NRLF Mortality per Capita + Block + Mean Average Water Temperature Scaled + (1 | *Toad Breeding Site*)

B.2 Additional figures and tables for Chapter 3

Figure B1. Mesocosm set up (before placing netting and shade cloth over tanks).

Figure B2. Satellite image of the experimental ponds, taken in July 2018 (Source: Google Earth). The ponds used in this experiment are bounded by the thick purple line. The three blocks are labeled with pink numbers.

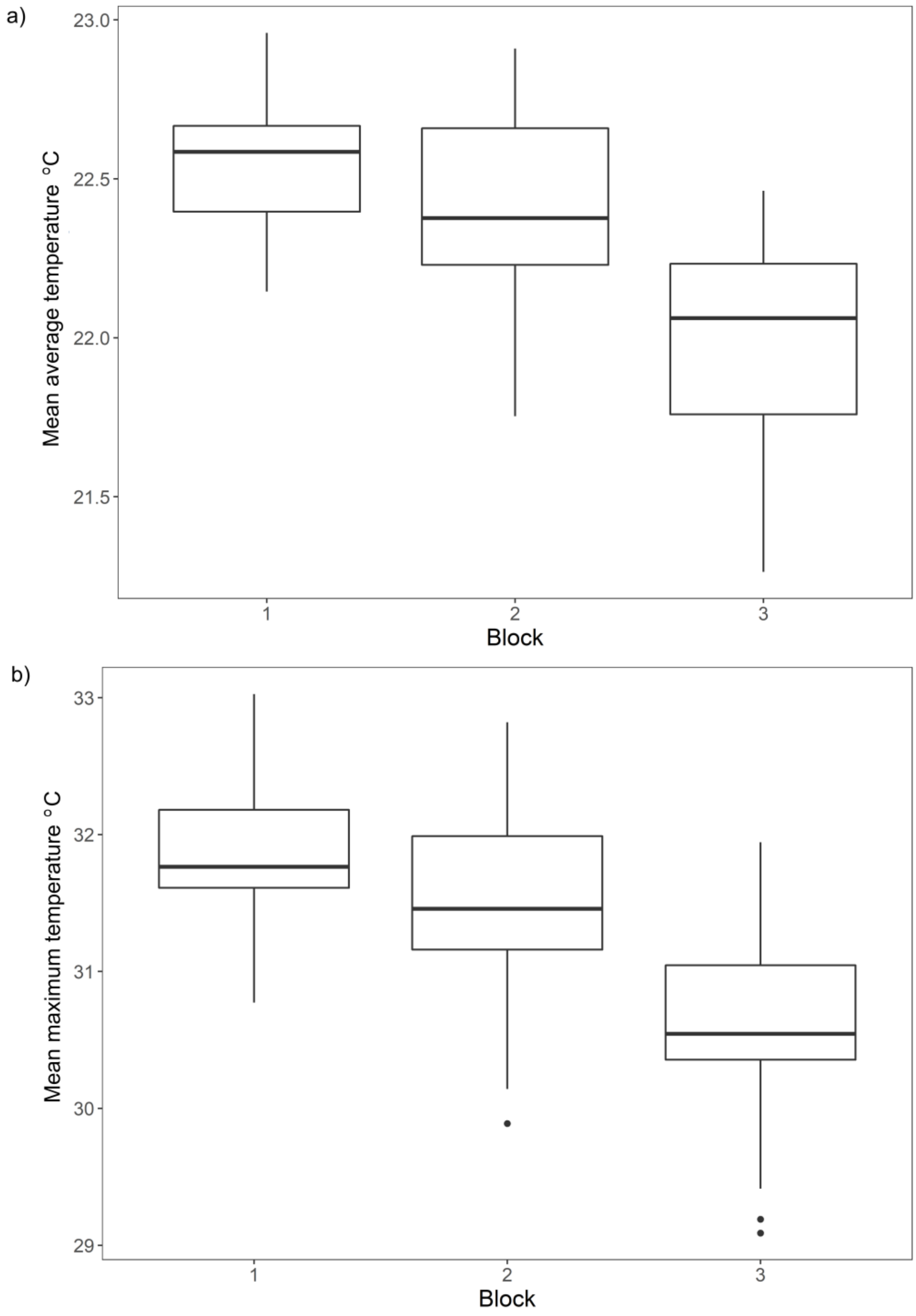


Figure B3. Temperature per block, a) mean average temperature, b) mean maximum temperature.

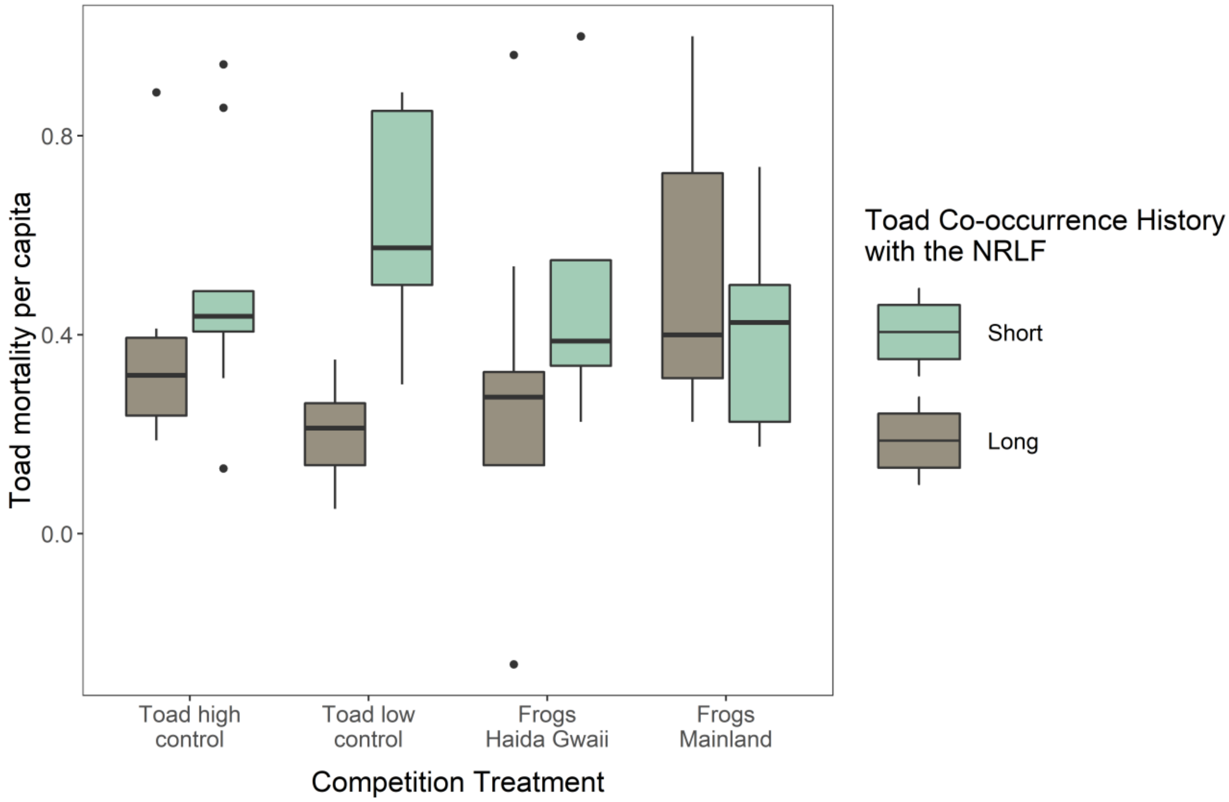


Figure B4. Toad mortality *per capita* under different competition treatments, toads with a short co-occurrence history with NRLFs (Haida Gwaii) compared to toads with a long co-occurrence history (Lower Mainland). The high-density toad treatment had 160 toads, low-density toad treatment had 80 toads and toad-NRLF treatments had 80 toads and 16 NRLFs. Results are averaged across tanks (n = 72).

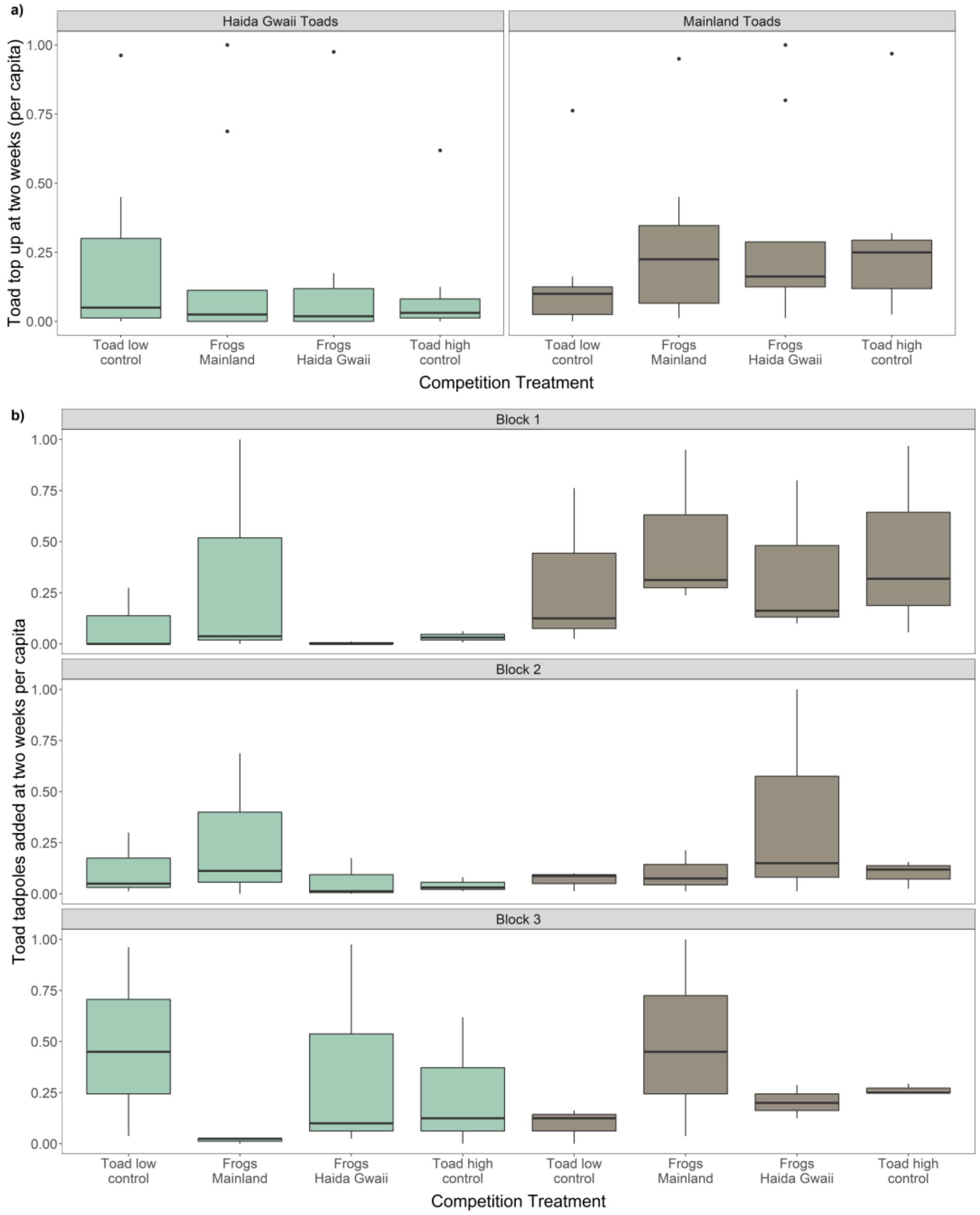


Figure B5. Toad tadpoles added at two weeks. a) for the four competition treatments and two toad sources (Western Toads from Haida Gwaii and the mainland), b) per block. Haida Gwaii toads = short co-occurrence history with the NRLF, Mainland Toads = Long co-occurrence history with the NRLF. Toad low control = low density (80 toads/tank), Toad high control (160 toads/tank). Block position in Figure B2 (Appendix B).

Table B1. Effect sizes +/- SE for the fixed effects (beta coefficients), and the variance +/- SD for the random effects, for the models of the response of the Western Toad to competition. Results presented for three different larval toad response variables. Toad growth rate (mg/day) and weight at metamorphosis (mg) were log transformed. Median time to metamorphosis is in days.

	Toad Response to Competition					
	Toad Growth Rate		Toad Weight at Metamorphosis		Toad Median Time to Metamorphosis	
Fixed Effects Variable or Interaction	**Effect size**	**SE**	**Effect size**	**SE**	**Effect size**	**SE**
(Intercept)	-0.12	0.06	1.63	0.06	94.90	5.36
Toad Co-occurrence History: Short	-0.06	0.04	-0.05	0.04	-0.49	2.98
Competition: NRLF HG	0.15	0.03	0.14	0.03	-10.62	2.87
Competition: NRLF ML	0.08	0.03	0.08	0.03	-3.42	2.94
Toad Tadpoles Top up Two Weeks per Capita Scaled	0.07	0.03	0.07	0.03	-2.74	3.00
Average Individual Toad Tadpole Starting Weight Scaled	0.15	0.06	0.14	0.06	NA	NA
Toad Mortality per Capita Scaled	0.26	0.06	0.26	0.06	-22.34	4.94
Days Since Experiment Began Scaled	-0.05	0.03	0.16	0.03	NA	NA
Block2	0.00	0.02	0.00	0.02	1.99	2.14
Block3	-0.02	0.03	-0.02	0.03	2.58	2.92
Mean Average Water Temperature Scaled	0.11	0.06	0.10	0.05	-11.07	5.21
Toad Co-occurrence History: Short * Competition: NRLF HG	-0.13	0.04	-0.13	0.04	8.63	4.06
Toad Co-occurrence History: Short * Competition: NRLF ML	-0.05	0.04	-0.04	0.04	-3.63	4.13
Random Effects Variable	**Variance**	**SD**	**Variance**	**SD**	**Variance**	**SD**
Tank Intercept	0.010	0.098	0.009	0.096	NA	NA
Slope across Days per Tank	0.029	0.170	0.028	0.167	NA	NA
Toad Breeding Site Intercept	0.000	0.000	0.000	0.000	0.000	0.000
Residual	0.004	0.067	0.004	0.066	35.700	5.975

Table B2. Fixed effects and variables testing for a competition by co-occurrence history interaction for each Northern Red-legged Frog response variable. Wald's Type-II Chi-Square test, bold text denotes statistical significance ($p < 0.05$). Results presented for three different larval toad response variables. NRLF growth rate (mg/day) and weight at metamorphosis (mg) were log transformed. Median time to metamorphosis was recorded in days. Effect sizes of the fixed effects and variances of the random effects are in Appendix B (Table B3).

	NRLF Response to Competition								
	NRLF Growth Rate			NRLF Weight at Metamorphosis			NRLF Median Time to Metamorphosis		
Fixed Effects Variables	Chisq	df	p	Chisq	df	p	Chisq	df	p
NRLF Source	1.47	1.00	0.225	1.41	1.00	0.235	4.22	1.00	**0.040**
Toad Co-occurrence History	0.20	1.00	0.656	0.24	1.00	0.627	3.33	1.00	0.068
NRLF Source * Toad Co-occurrence History	15.99	1.00	**< 0.001**	17.12	1.00	**< 0.001**	9.99	1.00	**0.002**
Average Water Temperature	0.00	1.00	0.986	0.00	1.00	0.986	0.02	1.00	0.902
Initial Toad Weight	1.85	1.00	0.174	1.82	1.00	0.177	NA	NA	NA
NRLF Tadpole Additions at Two Weeks per capita	14.31	1.00	**< 0.001**	14.81	1.00	**< 0.001**	0.02	1.00	0.889
NRLF Mortality per capita	4.02	1.00	**0.045**	4.22	1.00	**0.040**	9.56	1.00	**0.002**
Days since experiment began	8.08	1.00	**0.004**	1.81	1.00	0.179	NA	NA	NA
Block	6.54	2.00	**0.038**	7.08	2.00	**0.029**	1.16	2.00	0.560

Table B3. Effect sizes +/- SE for the fixed effects (beta coefficients), and the variance +/- SD for the random effects, for the model of the response of the Northern Red-legged Frog to competition. Results presented for three different larval toad response variables. NRLF growth rate and weight at metamorphosis were log transformed. Median time to metamorphosis is in days.

	NRLF Response to Competition					
	NRLF Growth Rate		NRLF Weight at Metamorphosis		NRLF Median Time to Metamorphosis	
Fixed Effects Variable or Interaction	**Effect size**	**SE**	**Effect size**	**SE**	**Effect size**	**SE**
(Intercept)	1.23	0.17	2.86	2.14	72.99	130.33
Toad Co-occurrence History: Short	-0.22	0.10	-0.22	0.10	8.70	5.54
Competition: NRLF HG	-0.25	0.07	-0.25	0.07	14.94	4.06
NRLF Tadpoles Top up Two Weeks per Capita Scaled	0.06	0.02	0.35	0.09	-0.06	0.44
Average Individual NRLF Tadpole Starting Weight Scaled	-0.05	0.03	-2.95	2.19	NA	NA
NRLF Mortality per Capita	-0.50	0.25	-0.50	0.24	52.71	17.05
Mean Average Water Temperature Scaled	0.00	0.04	0.00	0.10	-0.70	5.71
Days Since Experiment Began	0.00	0.00	0.00	0.00	NA	NA
Block2	0.08	0.06	0.08	0.06	-3.36	3.90
Block3	-0.08	0.08	-0.08	0.08	0.01	5.10
Toad Co-occurrence History: Short * Competition: NRLF HG	0.34	0.09	0.34	0.08	-20.09	6.36
Random Effects Variable	**Variance**	**SD**	**Variance**	**SD**	**Variance**	**SD**
Tank Intercept	0.066	0.257	0.058	0.242	NA	NA
Slope across Days per Tank	0.000	0.003	0.000	0.003	NA	NA
Toad Breeding Site Intercept	0.008	0.089	0.008	0.088	0.000	0.000
Residual	0.006	0.075	0.005	0.074	44.690	6.685

Table B4. Post-hoc results for prediction 3 - the effect of co-occurrence history on the response of Northern Red-legged Frogs (NRLFs) from Haida Gwaii and the Lower Mainland to competition with Western Toads. Results presented for three different larval NRLF response variables. NRLF growth rate (mg/day) and weight at metamorphosis (mg) were log transformed. Median time to metamorphosis was recorded in days. Statistical significance ($p < 0.05$) results in bold and have an asterisk (*). Estimates are the difference between the effect sizes from Table B3 (Appendix B).

	Contrast							
Response	NRLF source	Toad Co-occurrence History	estimate	SE	df	*t ratio*	*p*	
NRLF Growth Rate	Haida Gwaii	Short - Long	0.12	0.12	13.78	1.00	0.333	
	Mainland		-0.22	0.16	24.47	-1.41	0.170	
NRLF Weight at Metamorphosis	Haida Gwaii	Short - Long	0.12	0.12	13.73	1.04	0.315	
	Mainland		-0.22	0.16	24.33	-1.43	0.164	
NRLF Median Time to Metamorphosis	Haida Gwaii	Short - Long	-11.73	4.55	17.19	-2.58	**0.019**	*
	Mainland		6.56	7.09	24.58	0.93	0.363	

Appendix C Supplementary materials for Chapter 4

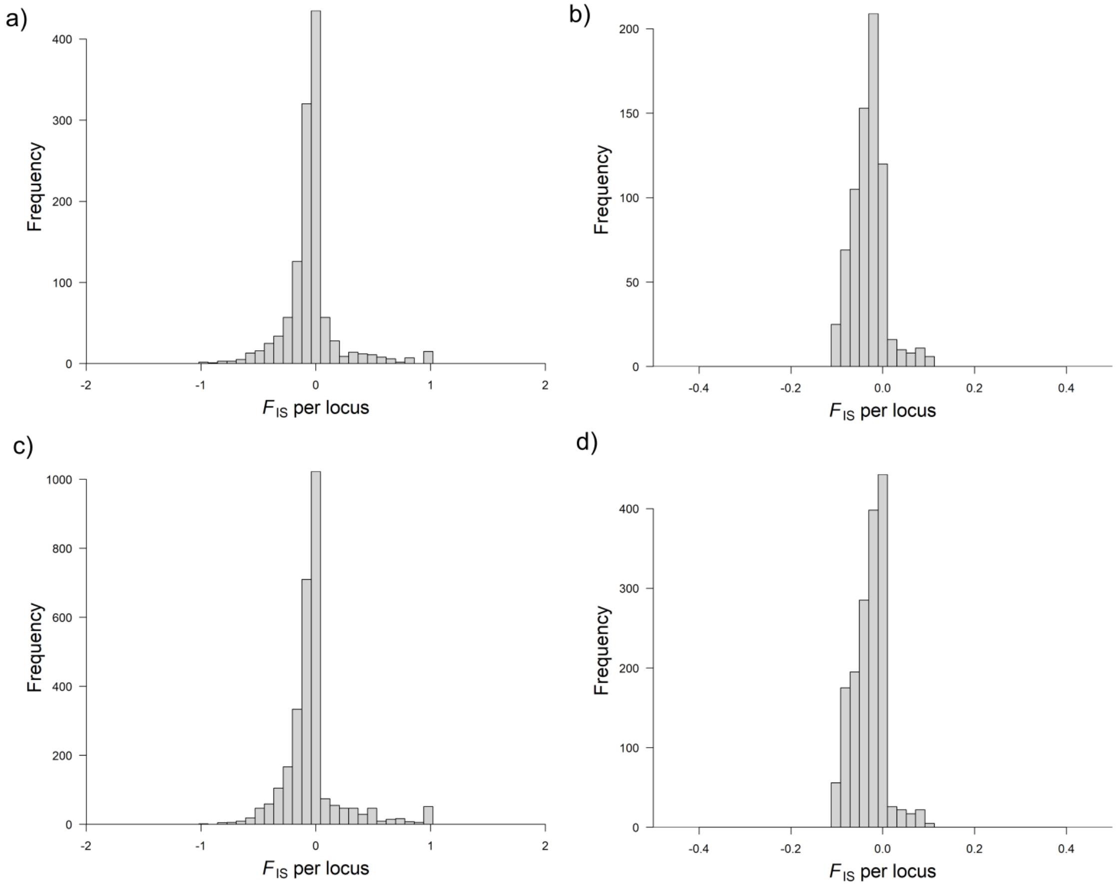


Figure C1. Distribution of F_{IS} per locus before and after F_{IS} filtering. a) and b) full dataset, c) and d) Haida Gwaii dataset.

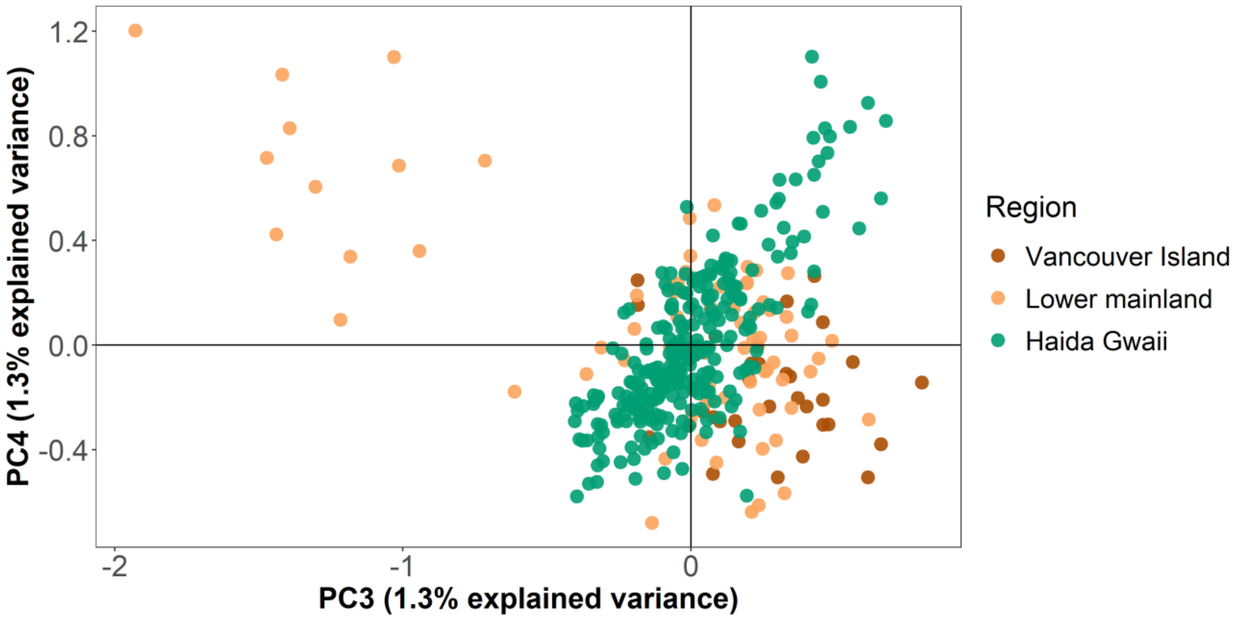


Figure C2. Full dataset PCA with PC3 and PC4 axes.

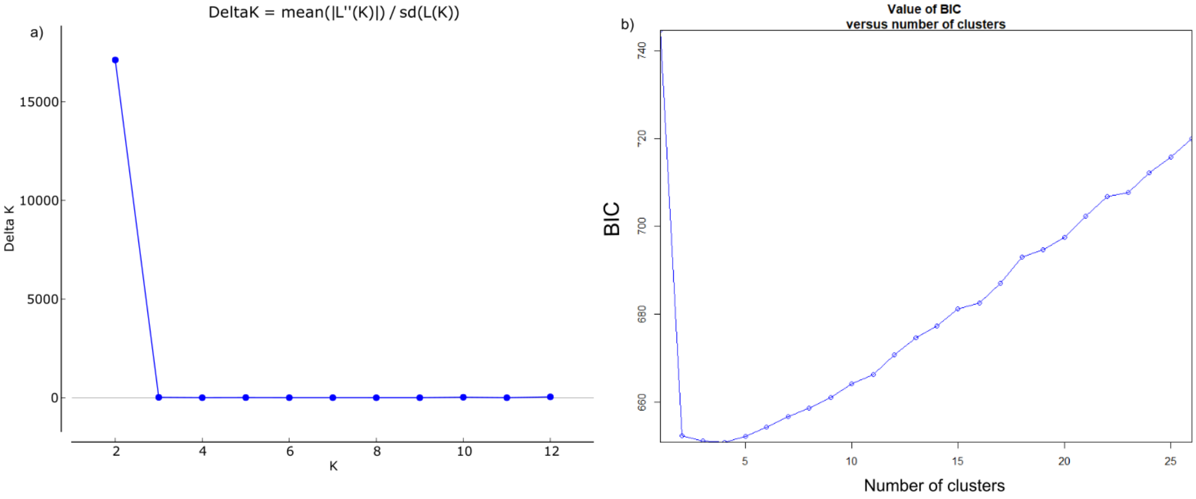


Figure C3. Full dataset a) *STRUCTURE* Evanno method optimal *K* value output from Structure Harvester, b) BIC plot from DAPC.

Table C1. Table of pairwise F_{ST} estimates per breeding site for the full dataset. Breeding site codes (first three characters of every name) are as follows: Haida Gwaii = R06, Vancouver Island = R01, lower mainland = R02.

	R01-CR-CE	R01-NI-CX	R01-SI-CA	R01-SI-FR	R01-SI-MC	R01-SI-SJ	R02-CW-K	R02-CW-M	R02-SC-IN	R02-SS-CR	R02-SS-FA	R02-SS-LC	R02-SS-LT	R06-GH-D	R06-GH-G	R06-GH-LL	R06-GH-PC	R06-GH-PT	R06-GI-CK	R06-GI-CN	R06-GI-EV	R06-GI-GL	R06-GI-LV	R06-GI-MY	R06-GI-RR	R06-MI-M
R01-CR-CE	NA	-0.00311	0.00432	0.005811	0.007406	-0.024	0.038589	0.088614	0.000263	0.035513	0.023939	0.01142	0.065868	0.330004	0.355002	0.367335	0.358343	0.354809	0.350994	0.344309	0.322887	0.351189	0.324539	0.306877	0.32868	0.324429
R01-NI-CX	-0.00311	NA	0.028762	0.038531	0.024681	0.028347	0.077587	0.115503	0.040225	0.073963	0.058609	0.046856	0.10848	0.29307	0.319375	0.32474	0.297555	0.317794	0.319886	0.304937	0.289004	0.320535	0.289512	0.284363	0.299188	0.295338
R01-SI-CA	0.00432	0.028762	NA	0.011515	-0.0321	-0.00332	0.066251	0.095093	0.035417	0.060845	0.042893	0.035727	0.04065	0.347619	0.369552	0.362086	0.345444	0.366052	0.359863	0.35864	0.332779	0.369321	0.337836	0.329547	0.340716	0.343698
R01-SI-FR	0.005811	0.038531	0.011515	NA	0.032813	0.022598	0.081701	0.117223	0.038694	0.06646	0.055271	0.056821	0.112285	0.322919	0.337176	0.335327	0.308281	0.343	0.34238	0.337787	0.319397	0.339469	0.307381	0.311903	0.317941	0.316747
R01-SI-MC	0.007406	0.024681	-0.0321	0.032813	NA	0.007054	-0.00037	0.045845	0.028157	0.025088	-0.02001	-0.00467	0	0.38027	0.405914	0.390385	0.449037	0.406645	0.396124	0.396506	0.367714	0.402884	0.364679	0.36355	0.369569	0.373664
R01-SI-SJ	-0.024	0.028347	-0.00332	0.022598	0.007054	NA	0.059465	0.092859	0.021012	0.063451	0.051058	0.044537	0.070584	0.315293	0.339145	0.334423	0.294281	0.333232	0.329095	0.326551	0.309256	0.333225	0.310369	0.297087	0.313107	0.309588
R02-CW-K	0.038589	0.077587	0.066251	0.081701	-0.00037	0.059465	NA	0.020672	0.059414	0.054364	0.043878	0.051979	0.028176	0.357424	0.378973	0.363062	0.322001	0.374284	0.37243	0.366176	0.350991	0.370368	0.352843	0.334029	0.355374	0.352569
R02-CW-M	0.088614	0.115503	0.095093	0.117223	0.045845	0.092859	0.020672	NA	0.085053	0.090824	0.083617	0.064133	0.109982	0.369199	0.388892	0.378124	0.348024	0.386355	0.3858	0.379898	0.363375	0.38789	0.359872	0.346795	0.365388	0.360255
R02-SC-IN	0.000263	0.040225	0.035417	0.038694	0.028157	0.021012	0.059414	0.085053	NA	0.051596	0.038062	0.032216	0.047658	0.305659	0.32429	0.316069	0.268759	0.323898	0.320463	0.310195	0.299118	0.316322	0.295896	0.282204	0.298684	0.301
R02-SS-CR	0.035513	0.073963	0.060845	0.06646	0.025088	0.063451	0.054364	0.090824	0.051596	NA	0.027295	0.029652	0.001321	0.338001	0.354533	0.325643	0.283282	0.354753	0.351788	0.346132	0.336711	0.349333	0.33056	0.315274	0.336005	0.335451
R02-SS-FA	0.023939	0.058609	0.042893	0.055271	-0.02001	0.051058	0.043878	0.083617	0.038062	0.027295	NA	0.014532	-0.00273	0.332703	0.349195	0.33342	0.294409	0.347809	0.341075	0.343581	0.32888	0.343326	0.324995	0.312382	0.327799	0.32641
R02-SS-LC	0.01142	0.046856	0.035727	0.056821	-0.00467	0.044537	0.051979	0.064133	0.032216	0.029652	0.014532	NA	-0.03173	0.268509	0.282815	0.266509	0.219531	0.278469	0.278001	0.268407	0.266068	0.274572	0.267547	0.242252	0.268617	0.268607
R02-SS-LT	0.065868	0.10848	0.04065	0.112285	0	0.070584	0.028176	0.109982	0.047658	0.001321	-0.00273	-0.03173	NA	0.405428	0.429586	0.418846	0.469694	0.434725	0.42446	0.427296	0.397221	0.430067	0.394928	0.388315	0.399845	0.402136
R06-GH-D	0.330004	0.29307	0.347619	0.322919	0.38027	0.315293	0.357424	0.369199	0.305659	0.338001	0.332703	0.268509	0.405428	NA	0.014223	0.059195	0.00949	0.022407	0.045513	0.021475	0.024804	0.039817	0.031602	0.044544	0.045995	0.025539
R06-GH-G	0.355002	0.319375	0.369552	0.337176	0.405914	0.339145	0.378973	0.388892	0.32429	0.354533	0.349195	0.282815	0.429586	0.014223	NA	0.038401	0.037355	0.012286	0.054397	0.033634	0.033762	0.0523	0.033832	0.060544	0.050718	0.036314
R06-GH-LL	0.367335	0.32474	0.362086	0.335327	0.390385	0.334423	0.363062	0.378124	0.316069	0.325643	0.33342	0.266509	0.418846	0.059195	0.038401	NA	0.067934	0.044466	0.069251	0.074946	0.082879	0.07185	0.068188	0.09173	0.086593	0.078118
R06-GH-PC	0.358343	0.297555	0.345444	0.308281	0.449037	0.294281	0.322001	0.348024	0.268759	0.283282	0.294409	0.219531	0.469694	0.00949	0.037355	0.067934	NA	0.031831	0.061562	0.047092	0.04425	0.055205	0.053124	0.063513	0.051579	0.042875
R06-GH-PT	0.354809	0.317794	0.366052	0.343	0.406645	0.333232	0.374284	0.386355	0.323898	0.354753	0.347809	0.278469	0.434725	0.022407	0.012286	0.044466	0.031831	NA	0.039692	0.018573	0.038957	0.030871	0.047328	0.051392	0.053492	0.041478
R06-GI-CK	0.350994	0.319886	0.359863	0.34238	0.396124	0.329095	0.37243	0.3858	0.320463	0.351788	0.341075	0.278001	0.42446	0.045513	0.054397	0.069251	0.061562	0.039692	NA	0.030773	0.045076	0.025714	0.062304	0.036542	0.056561	0.054177
R06-GI-CN	0.344309	0.304937	0.35864	0.337787	0.396506	0.326551	0.366176	0.379898	0.310195	0.346132	0.343581	0.268407	0.427296	0.021475	0.033634	0.074946	0.047092	0.018573	0.030773	NA	0.010522	0.033197	0.032618	0.023635	0.031887	0.025383
R06-GI-EV	0.322887	0.289004	0.332779	0.319397	0.367714	0.309256	0.350991	0.363375	0.299118	0.336711	0.32888	0.266068	0.397221	0.024804	0.033762	0.082879	0.04425	0.038957	0.045076	0.010522	NA	0.051337	0.018282	0.037188	0.014788	0.013508
R06-GI-GL	0.351189	0.320535	0.369321	0.339469	0.402884	0.333225	0.370368	0.38789	0.316322	0.349333	0.343326	0.274572	0.430067	0.039817	0.0523	0.07185	0.055205	0.030871	0.025714	0.033197	0.051337	NA	0.053896	0.051386	0.050271	0.051438
R06-GI-LV	0.324539	0.289512	0.337836	0.307381	0.364679	0.310369	0.352843	0.359872	0.295896	0.33056	0.324995	0.267547	0.394928	0.031602	0.033832	0.068188	0.053124	0.047328	0.062304	0.032618	0.018282	0.053896	NA	0.047507	0.010353	0.011187
R06-GI-MY	0.306877	0.284363	0.329547	0.311903	0.36355	0.297087	0.334029	0.346795	0.282204	0.315274	0.312382	0.242252	0.388315	0.044544	0.060544	0.09173	0.063513	0.051392	0.036542	0.023635	0.037188	0.051386	0.047507	NA	0.044676	0.036806
R06-GI-RR	0.32868	0.299188	0.340716	0.317941	0.369569	0.313107	0.355374	0.365388	0.298684	0.336005	0.327799	0.268617	0.399845	0.045995	0.050718	0.086593	0.051579	0.053492	0.056561	0.031887	0.014788	0.050271	0.010353	0.044676	NA	0.019444
R06-MI-M	0.324429	0.295338	0.343698	0.316747	0.373664	0.309588	0.352569	0.360255	0.301	0.335451	0.32641	0.268607	0.402136	0.025539	0.036314	0.078118	0.042875	0.041478	0.054177	0.025383	0.013508	0.051438	0.011187	0.036806	0.019444	NA

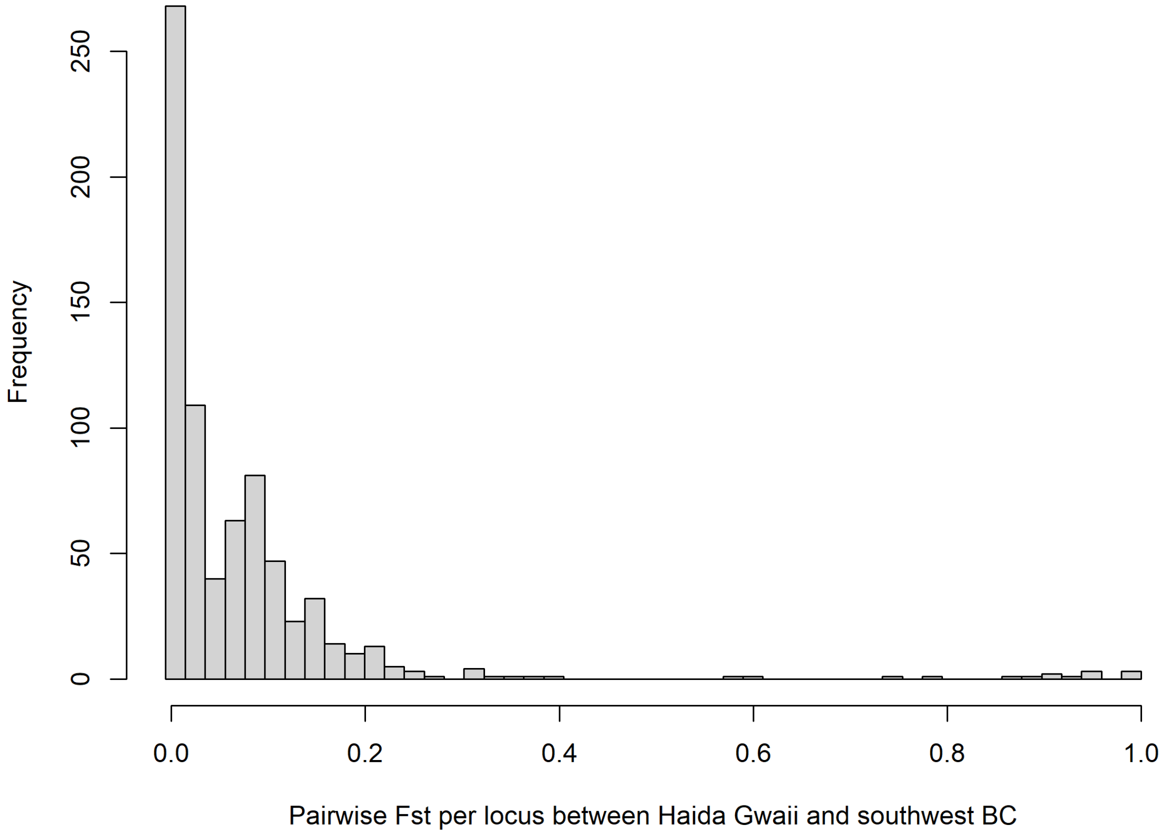


Figure C4. Distribution of (non-weighted) pairwise F_{ST} per locus for Haida Gwaii versus southwest BC.

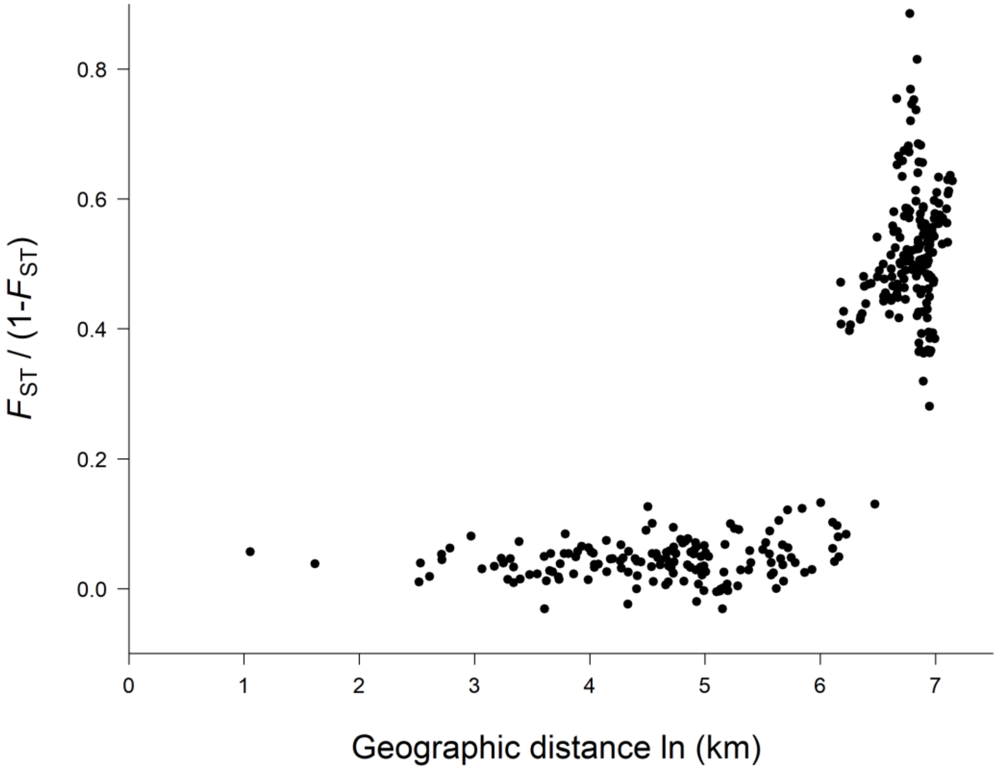


Figure C5. Isolation by distance plot for the full dataset; Mantel test results: R = 0.83, *p* = 0.001.

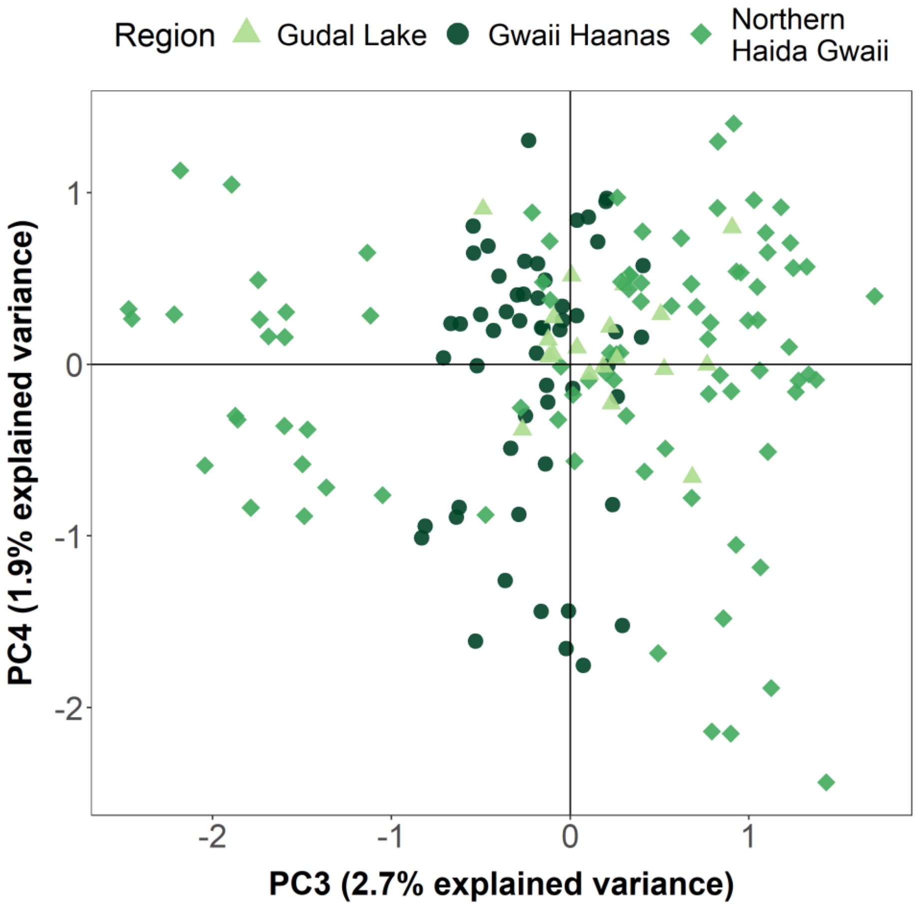


Figure C6. PCA of Haida Gwaii SNPs with PC3 and PC4 axes.

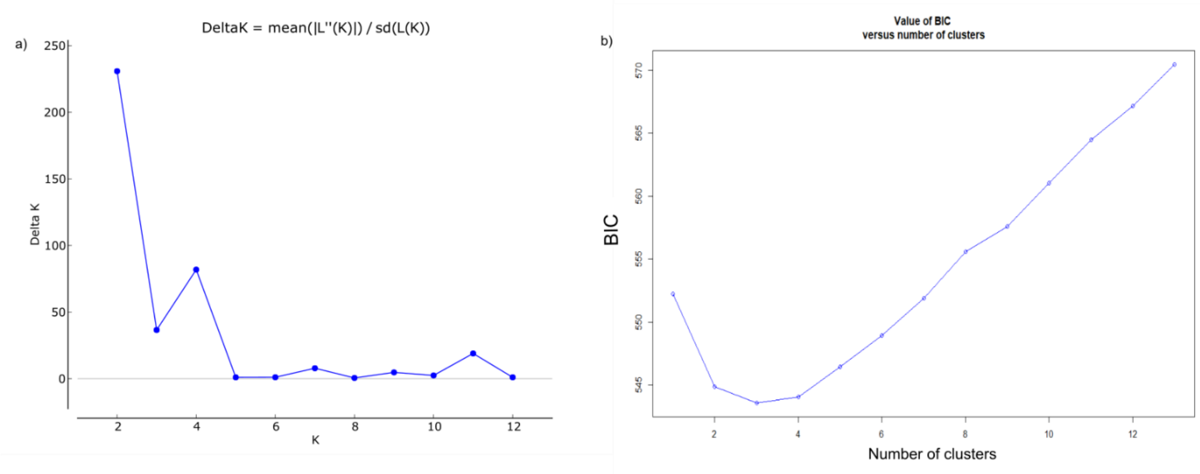


Figure C7. Haida Gwaii a) *STRUCTURE* Evanno method optimal *K* value output from Structure Harvester, b) BIC plot from DAPC.

Table C2. Table of pairwise F_{ST} values per breeding site for Haida Gwaii analysis.

	R06-GH-D	R06-GH-G	R06-GH-LL	R06-GH-P	R06-GH-PT	R06-GI-CK	R06-GI-CN	R06-GI-EV	R06-GI-GL	R06-GI-LV	R06-GI-MY	R06-GI-RR	R06-MI-M
R06-GH-D	NA	0.070764	0.087503	0.021405	0.064771	0.146754	0.138889	0.152651	0.215891	0.136638	0.210175	0.154364	0.163373
R06-GH-G	0.070764	NA	0.031472	0.046542	0.030076	0.15066	0.132067	0.139468	0.208756	0.11558	0.185388	0.150972	0.141833
R06-GH-LL	0.087503	0.031472	NA	0.060277	0.040585	0.137205	0.142528	0.152545	0.194638	0.134672	0.192744	0.160322	0.155469
R06-GH-P	0.021405	0.046542	0.060277	NA	0.044536	0.116963	0.117036	0.126015	0.205097	0.100132	0.189094	0.137251	0.153269
R06-GH-PT	0.064771	0.030076	0.040585	0.044536	NA	0.12881	0.111039	0.136105	0.193988	0.118097	0.18546	0.149424	0.14151
R06-GI-CK	0.146754	0.15066	0.137205	0.116963	0.12881	NA	0.057168	0.078991	0.105442	0.103552	0.077772	0.096607	0.13628
R06-GI-CN	0.138889	0.132067	0.142528	0.117036	0.111039	0.057168	NA	0.044354	0.136619	0.07355	0.095151	0.072616	0.122834
R06-GI-EV	0.152651	0.139468	0.152545	0.126015	0.136105	0.078991	0.044354	NA	0.139669	0.061494	0.095645	0.072931	0.113159
R06-GI-GL	0.215891	0.208756	0.194638	0.205097	0.193988	0.105442	0.136619	0.139669	NA	0.150617	0.16541	0.146725	0.192105
R06-GI-LV	0.136638	0.11558	0.134672	0.100132	0.118097	0.103552	0.07355	0.061494	0.150617	NA	0.094438	0.071054	0.088724
R06-GI-MY	0.210175	0.185388	0.192744	0.189094	0.18546	0.077772	0.095151	0.095645	0.16541	0.094438	NA	0.140164	0.163131
R06-GI-RR	0.154364	0.150972	0.160322	0.137251	0.149424	0.096607	0.072616	0.072931	0.146725	0.071054	0.140164	NA	0.130667
R06-MI-M	0.163373	0.141833	0.155469	0.153269	0.14151	0.13628	0.122834	0.113159	0.192105	0.088724	0.163131	0.130667	NA

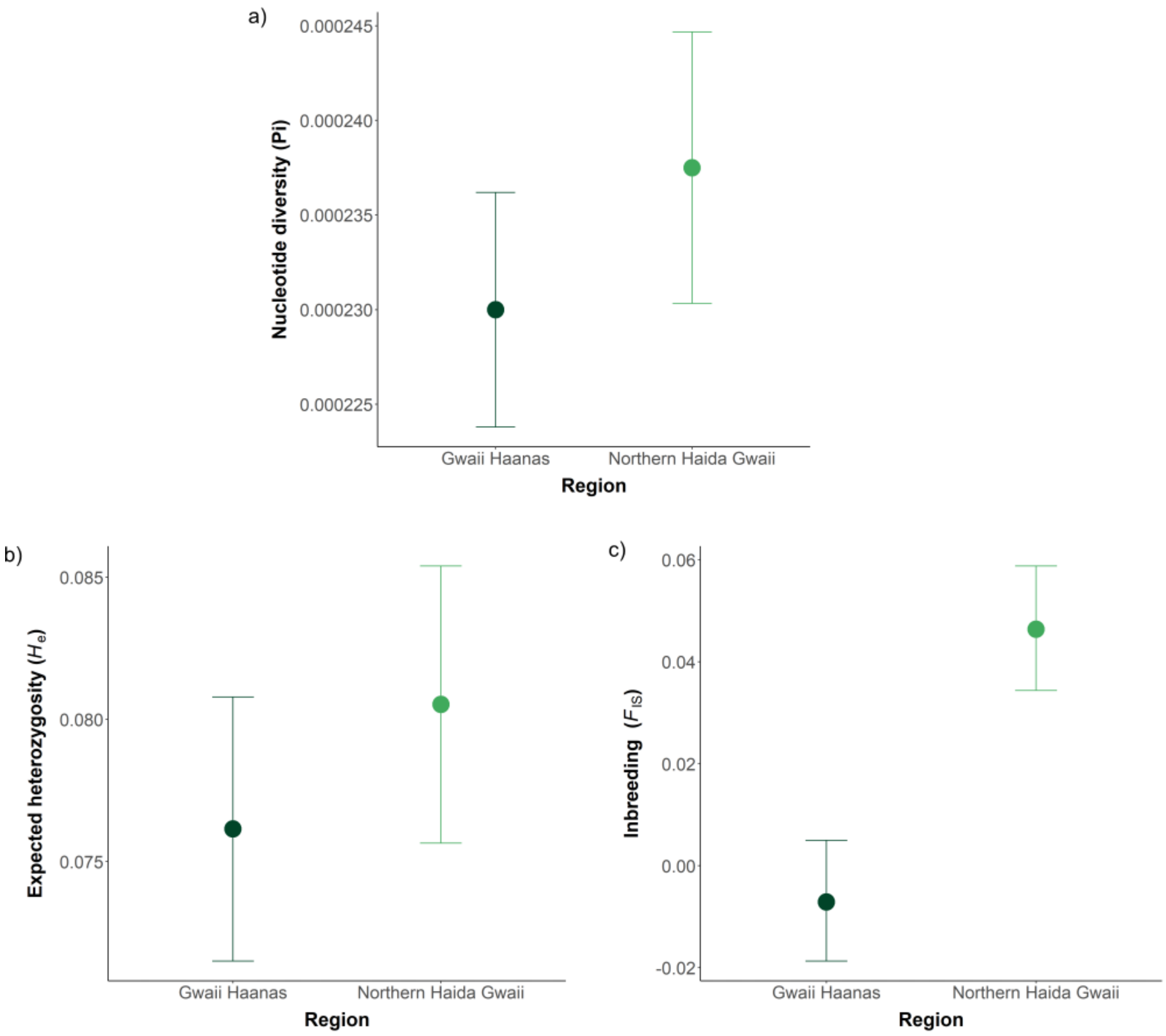


Figure C8. Genetic diversity of Haida Gwaii toad populations – for Gwaii Haanas versus northern Haida Gwaii. a) Nucleotide diversity (Pi), b) expected heterozygosity (H_e), and c) inbreeding coefficient (F_{IS}) – all with mean and 95% confidence intervals.

Appendix D Location of Western Toad breeding sites on Haida Gwaii visited by Roseanna Gamlen-Greene

D1. Coordinates and map.

D2. Satellite images showing where the toad tadpoles have been found previously.

More information on the toad breeding sites can be found on this website, including a link to an interactive google map: www.haidagwaiitoads.weebly.com

Please cite this document as:

Gamlen-Greene, R. 2022. The ecology, distribution and population genetics of amphibians on Haida Gwaii, British Columbia. PhD thesis. University of British Columbia

D.1 Coordinates and map of Western Toad breeding sites on Haida Gwaii visited by Roseanna Gamlen-Greene between 2017-2019

Table D1. Coordinates of Western Toad breeding sites on Haida Gwaii visited or confirmed by Roseanna Gamlen-Greene between 2017-2019. Latitude and longitude in WGS84. There are undoubtedly numerous other toad breeding sites on Haida Gwaii that are not on this list that have just not been located or confirmed. N.B. Site names are not place names – they are just what RGG used to keep track of sites in the absence of knowledge of the Haida names for these places.

Figure name	Unofficial site name	Location	Latitude	Longitude	Surveyed by RGG
Figure D2	Chown Slough	Graham Island, Haida Gwaii	54.02273	-131.99868	2018
Figure D3	Collison Point Lake	Graham Island, Haida Gwaii	53.827371	-132.405762	2018
Figure D4	Tsunami Evacuation Pond	Graham Island, Haida Gwaii	53.922716	-132.100963	2018 & 2019
Figure D5	Lavoie Pond	Graham Island, Haida Gwaii	53.760617	-132.285490	2017 & 2019
Figure D6	Mayer Lake	Graham Island, Haida Gwaii	53.667772	-132.062526	2017 - 2019
Figure D7	Richardson's Ranch Pond	Graham Island, Haida Gwaii	53.567762	-131.933165	2017 - 2019
Figure D8	Chinukundl Pond	Graham Island, Haida Gwaii	53.273937	-132.092496	2017 - 2019
Figure D9	Gudal Lake	Graham Island, Haida Gwaii	53.234311	-132.548151	2018
Figure D10	Mt Moresby Adventure Camp Bog	Moresby Island, Haida Gwaii	53.075464	-132.064207	2018
Figure D11	Dead Toad Lake	Gwaii Haanas, Haida Gwaii	52.554094	-131.667113	2019
Figure D12	George Wetland	Gwaii Haanas, Haida Gwaii	52.301540	-131.360460	2019
Figure D13	Lutea Lake	Gwaii Haanas, Haida Gwaii	52.337840	-131.369798	2019
Figure D14	Poque Lake	Gwaii Haanas, Haida Gwaii	52.602547	-131.698715	2019
Figure D15	Post Lake	Gwaii Haanas, Haida Gwaii	52.456404	-131.448986	2019
NA	Cape Fife - Kumara Lake	Graham Island, Haida Gwaii	54.088747,	-131.675565	2019
NA	Echo Harbour	Gwaii Haanas, Haida Gwaii	52.695905	-131.768369	2019

Figure D1. Western Toad breeding sites on Haida Gwaii visited by Roseanna Gamlen-Greene between 2017-2019. Latitude and longitude in Table D1.

D.2 Satellite images of Western Toad breeding sites on Haida Gwaii visited by Roseanna Gamlen-Greene between 2017-2019

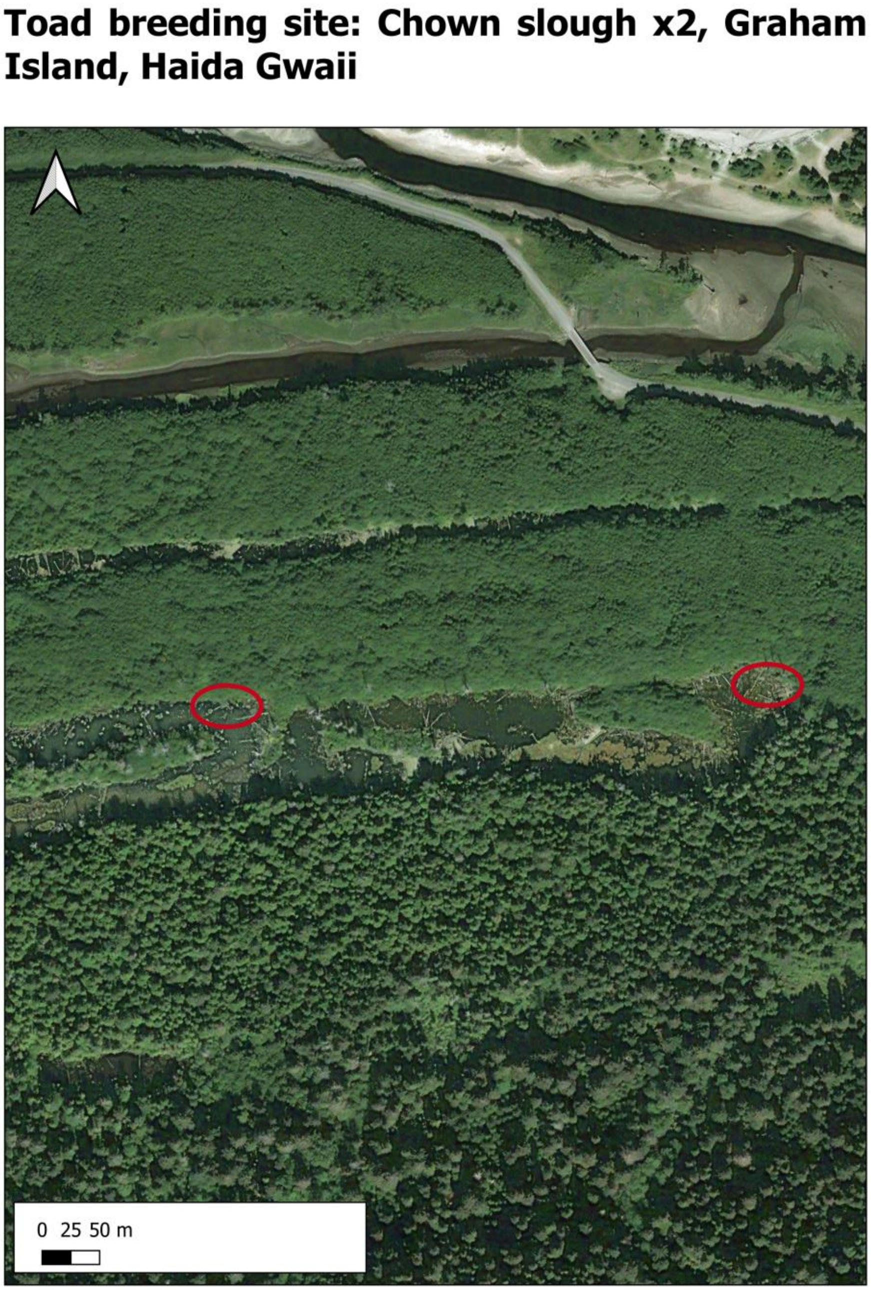


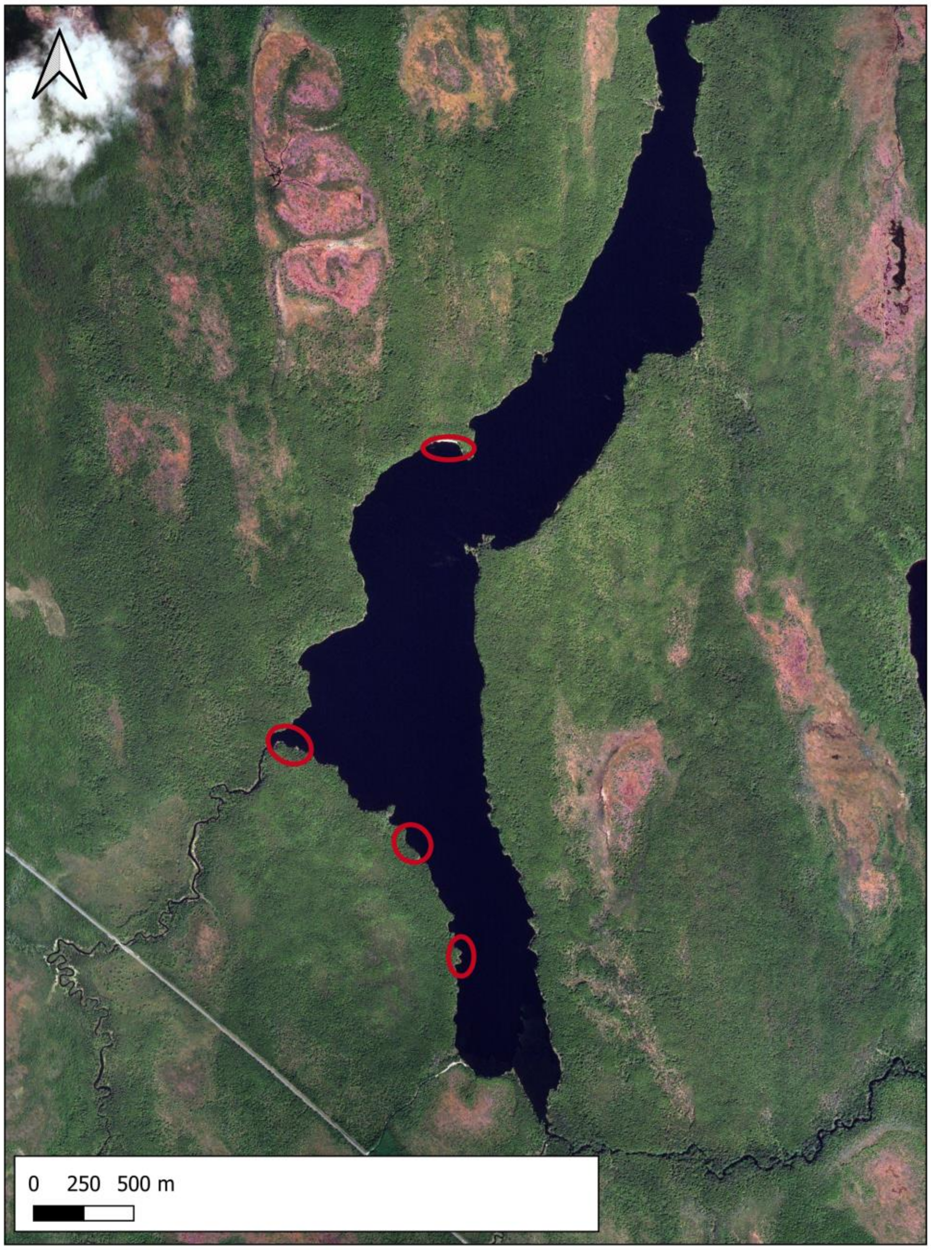
Figure D2. Location of schooling Western Toad tadpoles in the Chown Slough, Graham Island, Haida Gwaii, in 2019. Latitude and longitude in Table D1.

Toad breeding site: Collison Point Lake, Graham Island, Haida Gwaii

Figure D3. Location of schooling Western Toad tadpoles at a Lake near Collison Point, Graham Island, Haida Gwaii, in 2017. Latitude and longitude in Table D1.

Figure D4. Location of schooling Western Toad tadpoles at a pond at the Tsunamic Evacuation site, Graham Island, Haida Gwaii, in 2017. Latitude and longitude in Table D1.

Figure D5. Location of schooling Western Toad tadpoles at a pond on the Lavoie's property at Sewall, Graham Island, Haida Gwaii, in 2017. Latitude and longitude in Table D1.



Toad breeding site: Mayer Lake, Graham Island, Haida Gwaii

Figure D6. Location of schooling Western Toad tadpoles at Mayer, Lake, Graham Island, Haida Gwaii, in 2017. Latitude and longitude in Table D1.

Figure D7. Location of schooling Western Toad tadpoles at a pond on Richardson's Ranch, Graham Island, Haida Gwaii, in 2017. Latitude and longitude in Table D1.

Figure D8. Location of schooling Western Toad tadpoles at a pond off Chinukundl Road, Graham Island, Haida Gwaii, in 2018. Latitude and longitude in Table D1.

Figure D9. Location of schooling Western Toad tadpoles at Gudal Lake, Graham Island, Haida Gwaii, in 2019. Latitude and longitude in Table D1.

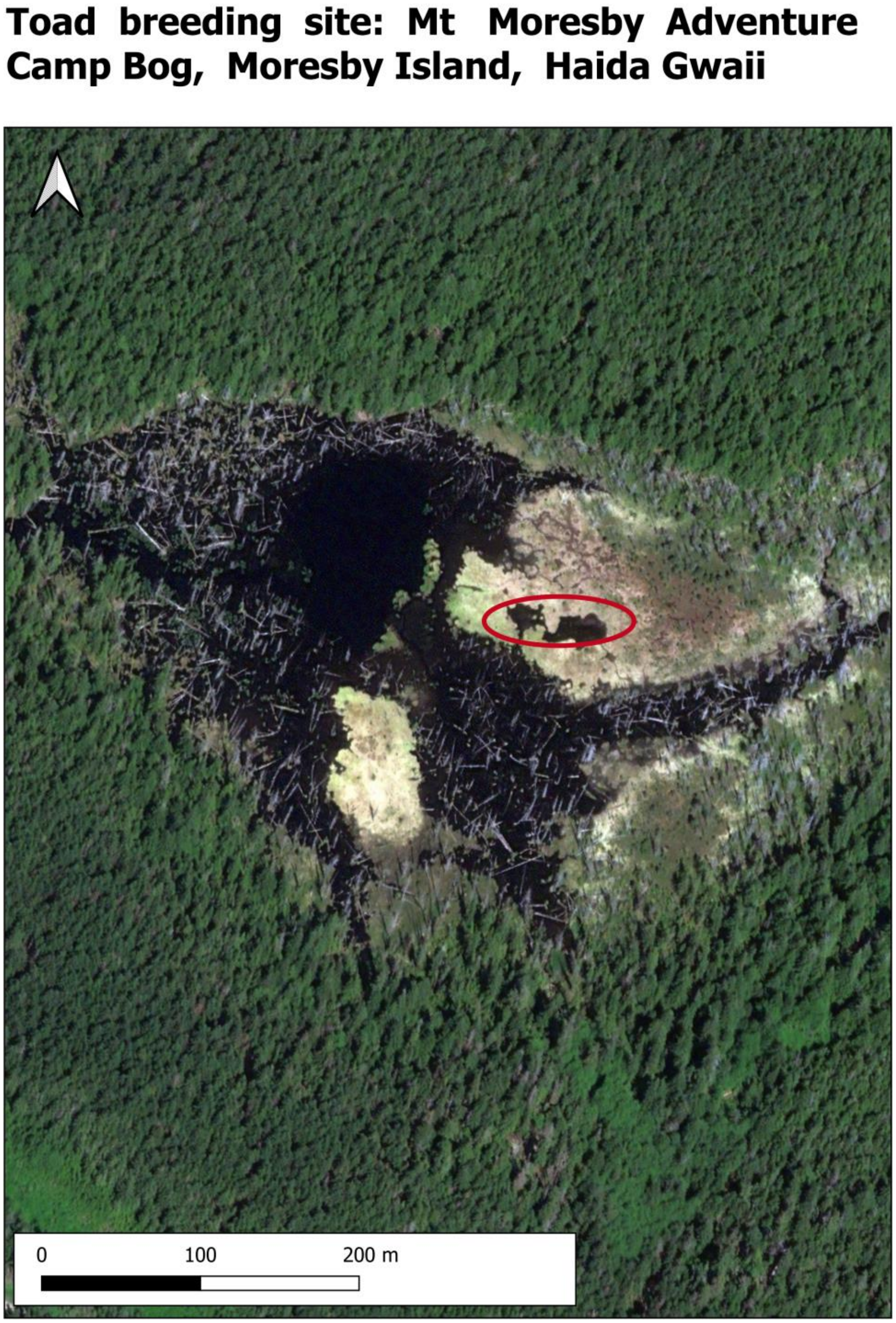


Figure D10. Location of schooling Western Toad tadpoles at a bog near Mt Moresby Adventure Camp, Moresby Island, Haida Gwaii, in 2018. Latitude and longitude in Table D1.

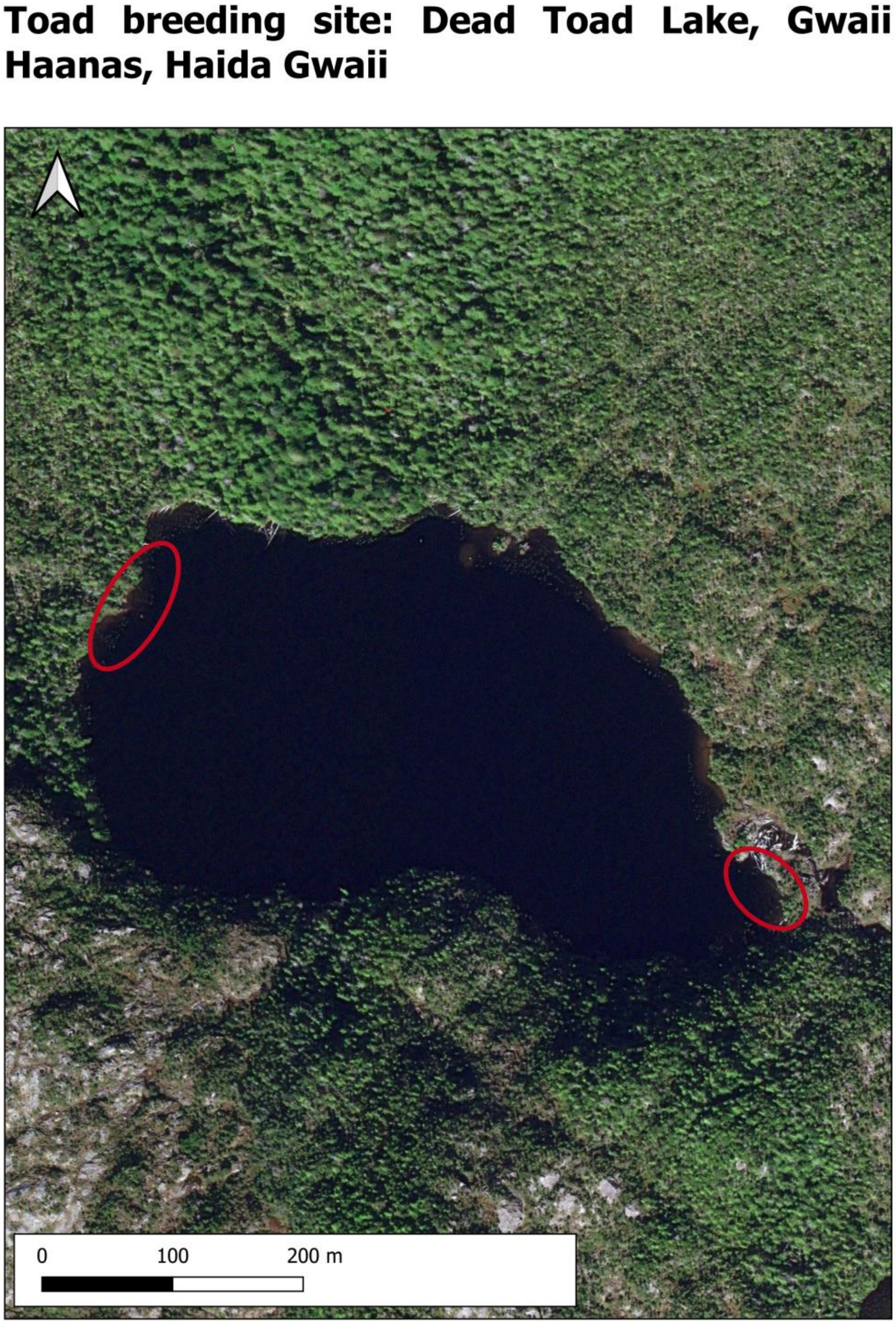


Figure D11. Location of schooling Western Toad tadpoles at Dead Toad Lake, Gwaii Haanas, Haida Gwaii, in 2019. Latitude and longitude in Table D1.

Toad breeding site: George Wetland, Gwaii Haanas, Haida Gwaii

Figure D12. Location of schooling Western Toad tadpoles at George Wetland, Gwaii Haanas, Haida Gwaii in 2019. Latitude and longitude in Table D1.


Toad breeding site: Lutea Lake, Gwaii Haanas, Haida Gwaii

Figure D13. Location of schooling Western Toad tadpoles at Lutea Lake, Gwaii Haanas, Haida Gwaii, in 2019. Latitude and longitude in Table D1.

Figure D14. Location of schooling Western Toad tadpoles at Poque Lake, Gwaii, Haanas, Haida Gwaii, in 2019. Latitude and longitude in Table D1.

Figure D15. Location of schooling Western Toad tadpoles at Post Lake, Gwaii, Haanas, Haida Gwaii, in 2019. Latitude and longitude in Table D1.