

CONSERVATION ECOLOGY OF A UNIQUE POPULATION OF LAKE CHUB (CYPRINIDAE:
COUESIUS PLUMBEUS): POPULATION SIZE, MOVEMENT ECOLOGY, HABITAT USE AND
POTENTIAL INTERACTIONS WITH THE EXOTIC CHERRY SHRIMP (*NEOCARIDINA DAVIDI*
var. *RED*)

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the thesis entitled:

CONSERVATION ECOLOGY OF A UNIQUE POPULATION OF LAKE CHUB (CYPRINIDAE: *COUESIUS PLUMBEUS*): POPULATION SIZE, MOVEMENT ECOLOGY, HABITAT USE AND POTENTIAL INTERACTIONS WITH THE EXOTIC CHERRY SHRIMP (*NEOCARIDINA DAVIDI* var. *RED*)

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the degree of Master of Science
in Zoology

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Abstract

This research investigated a population of lake chub (*Couesius plumbeus*) inhabiting a geothermal spring complex south of the community of Atlin, in the far northwest of British Columbia. These lake chub live in thermal isolation in 13-26°C water year round, while the only neighbouring water body, glacier-fed Atlin Lake, remains below 6°C year round and has no known population of lake chub. Through mark-recapture sampling I estimated the population size of this isolated and physiologically distinct population to be on the order of 1,000 to 2,000 mature individuals. I measured its area of occupancy as 3,602m². As such, the population is small in both habitat extent and population size, as well as being physically isolated from other populations of this widespread species. Tagging of fish in individual sections of the warm springs showed a general lack of movement among different sites. Size-frequency histograms for individual sites and points in time illustrated the presence of distinct cohorts suggesting lifespans of three to four years, with outliers living a year or two longer. I also studied the invasive cherry shrimp (*Neocaridina davidi* var. *red*), performing experiments to observe survivorship and behaviour under different temperature regimes. I found no significant decrease in mortality in adults when water was cooled to 5°C, but a significant reduction in their ability to react to stimuli below 15°C. I also observed 100% mortality in juveniles at water temperatures of 10°C or lower within two days. Based on these data, barring future adaptation to considerably colder conditions, is unlikely that cherry shrimp will be able to expand their range further into the Yukon River basin. Overall, my study provides a baseline of understanding about the population and life history of an isolated warm-springs population of lake chub, and provides observations of the early stages and probable outcomes of an exotic species invading an isolated location.

Lay Summary

The purpose of this research was to determine the size of a small, distinct and isolated population of lake chub living in the Atlin Warm Springs. By better understanding this fish, I can contribute to our understanding of how to study and conserve species that have significant parts of their overall diversity living in isolation and at low population sizes. This will help to preserve genetic diversity into the future. A secondary purpose of this research was to answer a direct question: 'are the cherry shrimp that were recently introduced to the Atlin Warm Springs a threat to the Yukon River?' Through laboratory experimentation, I demonstrated that their intolerance of low temperatures makes further short-term spread unlikely.

Preface

This research site and species were brought to my attention by Dr. Eric B. Taylor, who has conducted past research with the same species and location. Tagging, measuring and counting of lake chub (*Couesius plumbeus*) was conducted on-site while thermal experimentation on cherry shrimp (*Neocaridina davidi* var. *red*) took place at the UBC Zoology Aquatic Facilities. Sampling was permitted by the BC Fish and Wildlife Branch (Permit #SM16-228574). Field research was led by myself, with the assistance of family and friends. Lab experimentation was conducted by myself and undergraduate student Wonbin Choi, who used the data collected in Chapter 3 for his Directed Studies course. Our apparatus was assisted and supervised by Patrick Tamkee and Eric Lotto of the UBC Zoology Aquatic Research Facility.

Observations at the field site have led to a publication that I co-authored with Brian Slough in the Canadian Field-Naturalist: 'The observed decline of Western Toads (*Anaxyrus boreas*) over several decades at a novel winter breeding site' (Slough and deBruyn, 2018). I performed field observations, created a map of the warm springs and helped to edit the written content of said paper. A variation of the warm springs map which exists in Chapters 1, 2 and 3 of this thesis has been published in the Canadian Field-Naturalist. All other materials, written content and images are novel to this thesis.

Unless explicitly noted otherwise, I was the lead investigator for all research projects involved in this thesis, with development feedback provided by my committee: Eric B. Taylor, Chris Harley and Jordan Rosenfeld.

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I also must give my thanks to my committee members, Jordan Rosenfeld and Chris Harley. Both provided great assistance in helping me to refine and focus my research plan. Jordan's suggestion that the lake chub may move between different regions to up and downregulate their metabolism according to the varying amounts of food resources present in different seasons was a driving force behind the intensification of my research in the second field season. Chris led me along several useful paths of inquiry, including the digestive observations I performed in the field on the lake chub, and encouraged me to incorporate a laboratory component to my work, which in part led to my research into the invasive capabilities of cherry shrimp that comprises much of Chapter 3.

On that note, I'd like to thank my undergraduate collaborator and directed studies student Wonbin Choi for his help in conducting our daily research on the thermal tolerance of cherry shrimp, as well as Patrick Tamkee and Eric Lotto, who provided space in the Zoology Aquatic Research Facility and helped us to realize our general research plan as a proper experimental apparatus.

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Chapter 1 Conservation of Freshwater Fishes with Particular Reference to the Lake Chub (*Couesius plumbeus*)

1.1 Conservation of Freshwater Fishes

Fresh water comprises less than a single percent of the world's surface water, but contains a disproportionate six percent of the world's aquatic biodiversity (Dudgeon et al. 2006). Humans interact very closely with fresh water, and we have drawn ever more heavily from freshwater resources as history has progressed. Existing on the very front lines of human interaction with the Earth's ecosystems, freshwater fishes are thus disproportionately affected by our actions (Dextrase & Mandrak 2004). Deleterious human activity can take the forms of organic and inorganic pollution (eutrophication, metal toxicity, and sedimentation, for example), water extraction, channeling, damming and other structural modifications, as well as direct alterations to local biota, either by overexploiting commercially relevant species or by introducing exotic species. These can all contribute to habitat alteration, degradation and outright destruction, and threaten the function and continuation of freshwater ecosystems (Dextrase & Mandrak 2004; Dudgeon et al. 2006; Grooten & Almond 2018).

Due to their geography and division into distinct drainage basins, freshwater ecosystems tend to develop high distinctiveness from one another (Taylor 2004; Carrara et al. 2013; Thomaz et al. 2016). Their physical structures and the frequent occurrence of natural barriers makes dispersal relatively difficult between different freshwater basins and individual streams, and may tend to create high beta diversity, the distinctiveness between individual ecosystems (Angermeier & Winston 1999; Taylor 2004). Conversely, marine ecosystems are relatively more open to dispersal and migration and thus

have a trend towards greater homogeneity (Ward et al. 1994; Sheaves & Johnson 2008).

Thus, not only are freshwater ecosystems generally more distinctive and varied than their open-water marine counterparts across small spatial scales, they are also more directly exposed to human activities. This combination of uniqueness and vulnerability illustrates the importance of efforts to understand freshwater fishes and their ecosystems, so that we might recognize and appropriately address the issues that they face.

1.2 Conservation Below the Species Level

Conservation that focuses exclusively on the species level runs the risk of missing out on the distinctness of the various populations that can exist within a species, particularly one that is geographically widespread (Taylor et al. 2013, Grooten & Almond 2018). Designating an abundant and cosmopolitan species with a single status risks denying protection to distinct subunits of the species. It may be the case that significant portions of a given species' overall biodiversity can be contained in small and isolated populations with limited ranges. In order to address the issue of subspecific conservation units, COSEWIC, the Committee on the Status of Endangered Wildlife in Canada, makes use of 'designatable units' (DUs), which are discrete and evolutionarily significant populations or population groups (COSEWIC 2018a). A designatable unit must be a recognized variety that is genetically, physiologically or geographically distinctive, and is most typically applied when several of these attributes distinguish the population(s). For example, COSEWIC recognizes 14 DUs of caribou (*Rangifer tarandus*) and two DUs of the showy goldenrod (*Solidago speciosa*). Twenty-four DUs of sockeye salmon (*Oncorhynchus nerka*) are recognized in British Columbia's Fraser River alone (Government of Canada 2018b).

By recognizing and understanding designatable units, and taking them into account when

devising conservation policy, one can prioritize areas and populations where conservation efforts will be more effective in preserving biodiversity and distinctiveness. If conservation approaches are limited to the species level, however, large amounts of intraspecific biodiversity may be lost due to a lack of fine-scale understanding of the constituent components that make up the complex of populations that is a species. A lack of 'biocomplexity' within species can have serious negative consequences for species persistence across variable environments, particularly if those species are impacted by human activities (Hilborn et al. 2003).

It is with this in mind that I address the species around which this thesis is centred: *Couesius plumbeus*, more commonly known as the lake chub.

1.3 The Lake Chub

The lake chub, *Couesius plumbeus*, is a medium-sized cyprinid fish (Figure 1.1) that is endemic to North America. It is found in rivers and streams where it is an opportunistic, generally insectivorous fish that reaches a maximum size of about 200 mm, first matures at 3-4 years of age, and rarely lives beyond 5 years, with extreme outliers living to age 7 (Scott & Crossman 1973; Mansfeld 2004). *C. plumbeus* is considered a significant forage fish supporting populations of larger fishes across its range (Scott & Crossman 1973).

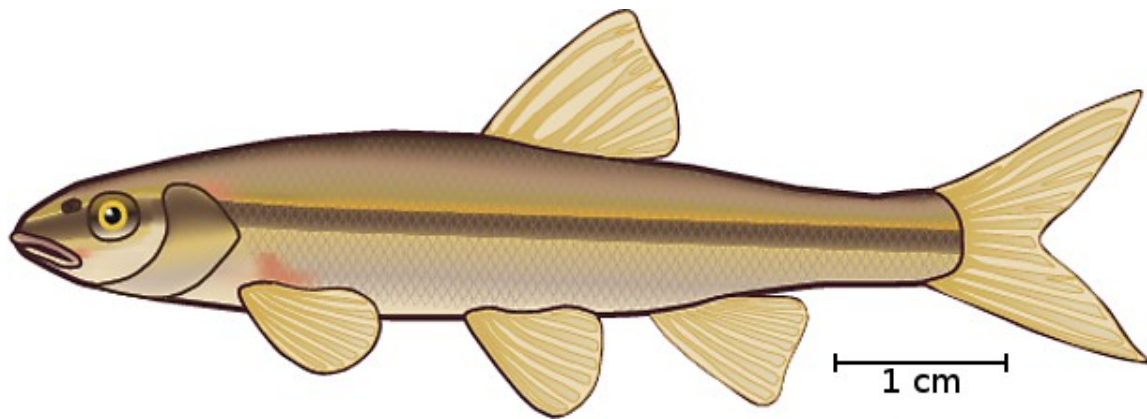


Figure 1.1 Lake chub (*Couesius plumbeus*), lateral view (illustration by A. deBruyn).

1.3.1 Lake Chub Range

C. plumbeus is one of the most widely distributed freshwater fishes in North America. It is present from central Alaska in the west to Labrador in the east, with a northern range near the tree line, reaching a northern extreme in the Mackenzie River Delta (Figure 1.2). The southern boundary of its range roughly follows the Canada-US border. *C. plumbeus* exists in a patchy distribution through New England as far south as the upper Delaware River, and in the drainage basins of the Great Lakes. The species is absent from a region of southern Saskatchewan and Manitoba, and its southernmost populations are isolated relicts in the Rocky Mountains of Colorado. West of the Continental Divide, *C. plumbeus* is present in the upper branches of many Pacific-draining rivers, such as the Yukon, Stikine and Fraser rivers, but is absent from coastal regions except in a small portion of southeastern Alaska. The present distribution and diversity of lake chub is a product of recent glaciations, which temporarily limited the species to several major refugia in Beringia, the Pacific coast, the Mississippi/Missouri system, and the Atlantic coast (Taylor et al. 2013).

Across such a vast range, the lake chub not surprisingly exists in a variety of habitats ranging from large and small rivers to innumerable lakes. One of the most unusual and rare habitats for lake

chub, and for Canadian freshwater fishes in general, are the so called 'hot' or 'warm' springs (more generally referred to as 'thermal springs') found in northwestern British Columbia (Figure 1.2). These habitats are known for their sustained warmer water temperatures compared to the surrounding waterscape, high pH and hardness, and for their unusual biological characteristics such as a more temperate biota than their surrounding environs (Waring & Blankenship 1965; Souther & Halstead 1969 as cited in Danks & Downes 1997; Darveau et al. 2012). Owing to the diverse habitats occupied by lake chub, ranges derived from distinct glacial refugia, and the evidence of differences in thermal performance of lake chub from thermal springs relative to fish from non-thermal spring aquatic habitats (Darveau et al. 2012), the existence of 12 DUs of lake chub in Canada has been proposed, with the Atlin Warm Springs population considered a DU separate from all others (Taylor et al. 2013).



Figure 1.2 Range of the lake chub *Couesius plumbeus*, shaded in grey (Unpublished COSEWIC Status Report 2004). The general locations of thermal springs populated by *Couesius plumbeus* in northwestern British Columbia are circled: the Atlin Warm Springs is the western circle and the Liard River and Deer River Hot Springs are represented by the circle to the east.

Small, diverse and abundant, with a broad range stretching to all three coasts of the North American continent, *C. plumbeus* is an excellent species with which to address questions of conservation of freshwater fishes. Lake chub are regarded as a species of Least Concern globally by the International Union for the Conservation of Nature (NatureServe 2013). Although COSEWIC once regarded Canadian populations as being data deficient (2004, unpublished data), the latest assessment in November 2018 has recognized two DUs of thermal springs lake chub as Threatened (COSEWIC 2018b).

1.4 The Atlin Warm Springs

1.4.1 Warm Springs Site

Atlin Lake is the largest natural lake in British Columbia, with a small portion extending north into the Yukon Territory. It exists in the northwestern corner of the province, on the inland edge of the Coast Mountains (Figure 1.3). The lake is fed in large part by the Llewellyn Glacier, an interior-draining portion of the Juneau Icefield, which keeps its temperatures between 0-5°C throughout the year, though isolated and shallow bays can reach peak temperatures of 12°C (Gilbert et al. 2006). Atlin Lake is part of the Southern Lakes system, a collection of lakes in southern Yukon and northwestern British Columbia including Atlin, Tagish, Tutshi and Bennett lakes. Together these lakes form the headwaters of the Yukon River, which at 3200 kilometres is the third longest river in North America, after the Mississippi/Missouri and the Mackenzie rivers, with the seventh largest drainage basin (Benke & Cushing 2011). Each of these lakes are long and slender in shape, filling the large valleys that have been formed as a result of the glacial action which defines the topography of much of the region. The Yukon River ultimately drains northwestwards through central Alaska before entering the Bering Sea of

the North Pacific Ocean. The Atlin Warm Springs (Figure 1.3, 1.4) are approximately 23 kilometres south-southeast of the town of Atlin, British Columbia and 200 km south of Whitehorse, Yukon. The springs are located at coordinates 59° 24' 13.98" N, 133° 34' 31.21", and are accessible year-round by a gravel road. The springs are drained by a series of warm streams and wetlands, before flowing out into Warm Bay in Atlin Lake, approximately 850 metres downstream (Figure 1.3, 1.4). The Atlin Warm Springs complex is composed of two separate parts: a western portion which exists on a private homestead, and an eastern portion which is more frequently accessed by the public. The former is a single unbranching stream which is used to heat and water greenhouses. It is ditched in several areas, and lacks any large wetlands in its vicinity. The latter is a branching complex of streams, divided by several tufa (limestone precipitate, also sometimes referred to as travertine) cascades, featuring several significant marshy wetlands. Both geothermal areas drain independently into Warm Bay in Atlin Lake, with their outlets separated by a 290 metre-stretch of glacial water. As such, it is unlikely that there is any movement of warm-adapted aquatic organisms between the two areas of the warm springs.

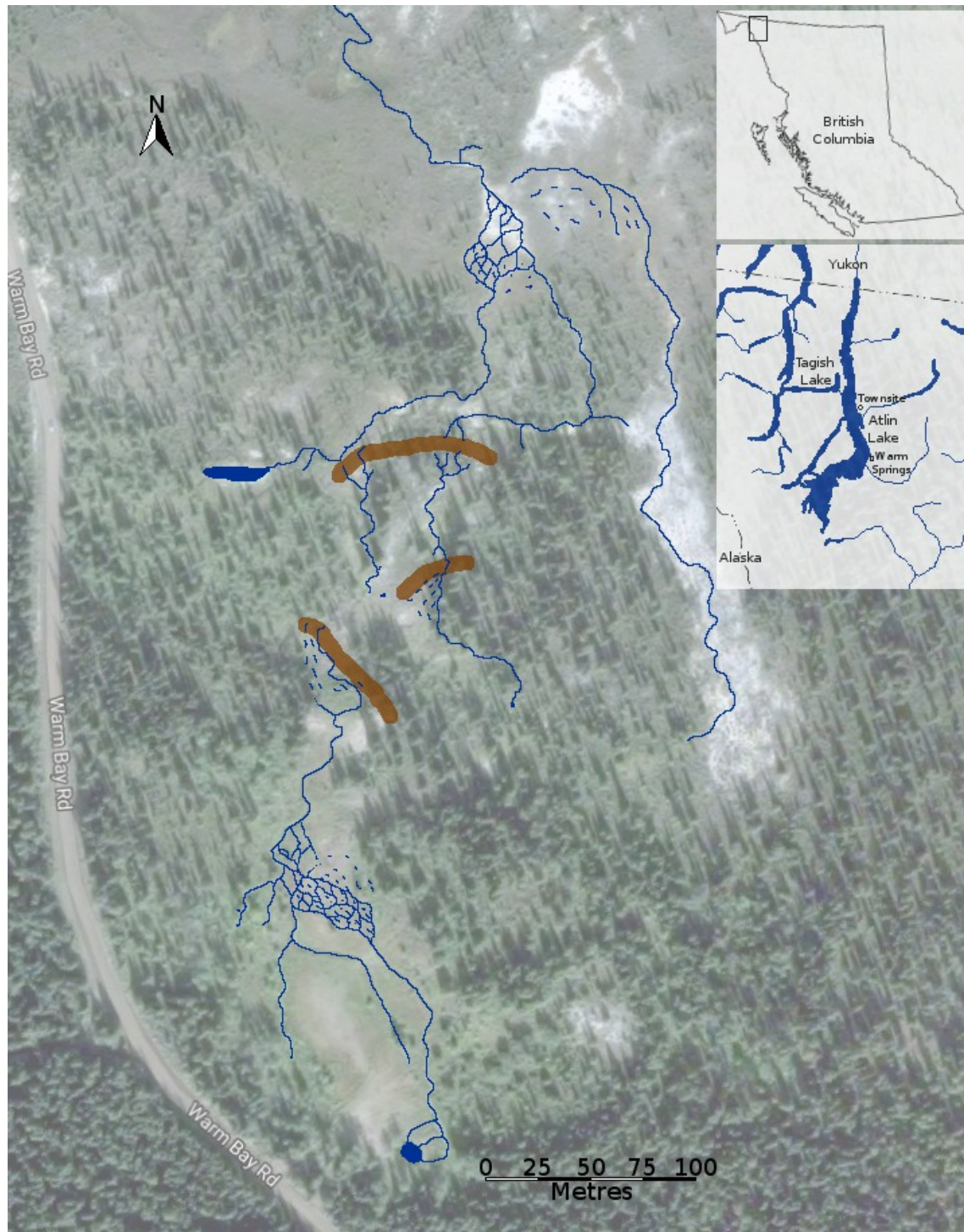


Figure 1.3. Atlin Warm Springs (eastern portion) map. Thin blue lines indicate water, while broad brown lines indicate natural barriers and abrupt changes in elevation which obstruct movement and disrupt surface flow. The top right insets show the position of the warm springs in relation to Atlin Lake and the position of the study area in northwestern British Columbia. The pond at lower left is shown in Figure 1.4. Satellite Imagery ©2018 DigitalGlobe; Map Data ©2018 Google.

Both sites are populated by *C. plumbeus*, and both have a prominent invasive species present. These are goldfish (*Carassius auratus*) in the western portion, and cherry shrimp (*Neocaridina davidi* var. *red*) in the eastern portion. The research conducted during the completion of this thesis took place in the eastern portion, unless explicitly stated otherwise.

1.4.2 Geology of the Atlin Warm Springs

The southern Yukon and far northwestern British Columbia in the vicinity of Atlin Lake is a tectonically-active region located between two volcanic fields, Surprise Lake and Llangorse, which are typically grouped together as the Atlin Volcanic Field (Edwards et al. 2003). On a larger scale, this area is part of the Northern Cordilleran volcanic province, which stretches from central British Columbia to the Yukon-Alaska border near Dawson City, Yukon, and is also considered to be part of the Coast Range volcanic arc (Edwards et al. 2003). It is not presently known whether it is proximity to volcanism, or simple mantle heating of groundwater that generates the warmth of these springs. As of 2018, the upper pool maintains a consistent 26°C throughout the year, with not more than a 3°C change from day to night (K. Frazer, personal communication; see Appendix Table A1 and Figure A3). Downstream sections maintain a minimum water temperature of 11-15°C year round despite surrounding air temperatures that average between -5 to -15°C between November and March and may plunge to -40°C and lower (Figure 1.4; see Appendix Table A1 and Figure A3; Climate-Data 2018).



Figure 1.4. Pond at the head of the east Atlin Warm Springs complex during summer (left, August 2008) and winter (right, January 2009). The pond is about 10 metres wide by 1 metre deep. Photos by E. Taylor (summer) and M. Connor (winter).

The Atlin Warm Springs maintain a temperature that remains higher than the average ambient temperatures year-round. Only record breaking air temperatures, such as 30.6°C recorded on June 3, 1936, exceed the peak temperature in the warm springs, and the 30 year climate normals recorded from 1981 to 2010 provide a daily maximum air temperature in July of 19.0°C (Climate-Data 2018; Government of Canada 2018a). As such, this region's water temperature is driven largely by geothermal factors, rather than atmospheric conditions. While the water in the upper regions of the springs have small daily swings of roughly two degrees, they remain largely steady across the year in the upper regions. Further away from the warm springs, ambient air temperature plays a larger role, and the waters grow cooler in the winter.

1.4.3 Ecology of the Atlin Warm Springs

The area surrounding the warm springs is typical of the Canadian boreal forest, populated by a low-diversity forest of white spruce (*Picea glauca*), black spruce (*Picea mariana*), lodgepole pine (*Pinus*

contorta) and subalpine fir (*Abies labioscarpa*). Deciduous trees and large shrubs present include balsam poplar (*Populus balsamifera*), trembling aspen (*Populus tremuloides*) and a variety of willows (*Salix* spp.). Mammals present include ungulates such as moose (*Alces alces*) and caribou (*Rangifer tarandus*), rodents such as voles (*Microtus* spp.), American red squirrels (*Tamiasciurus hudsonicus*), deer mice (*Peromyscus* spp.), jumping mice (*Zapus* spp.), porcupines (*Erethizon dorsatum*), and others. Mammalian predators include black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*), grey wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), lynx (*Lynx canadensis*) and a variety of mustelids.

A large variety of birds also make use of the site. The most prominent of the year-round occupants are black-capped chickadees (*Poecile atricapillus*) and common ravens (*Corvus corax*), although many more small passerines and raptors are known to be present at the site.

Of some further interest are the amphibians of the warm springs. The accounts of Atlin's inhabitants, as well as the work of Slough & Mennell (2006) indicate that the warm springs contained, until recently, a population of western toads (*Bufo boreas*) that were inferred to be adapted to the warm springs given their distinct reproductive timing relative to cold-water populations nearby (Slough & deBruyn 2018). Additional amphibians native to the area are the wood frog (*Rana sylvatica*) and Columbia spotted frog (*Rana luteiventris*). My first observations of the site in late 2015 revealed no amphibians, but recently the presence of *B. boreas* tadpoles in the warm springs complex was documented by Slough & deBruyn (2018). As discussed by Slough & deBruyn (2018), the re-appearance of the western toad is likely a result of recolonization by individuals from a nearby population rather than from resurgence of a remnant warm springs' population.

The pools and streams feature an abundance of fairy shrimp (Anostraca), leeches (Hirudinea), freshwater snails (Gastropoda) and aquatic beetles (Coleoptera). During the course of my research, a

population of cherry shrimp (*Neocaridina davidi* var. *red*), native to the island of Taiwan, was introduced in late 2015 and has multiplied rapidly, invading the warmest regions of the springs (see Chapter 3).

The lake chub, *Couesius plumbeus*, is the only native fish present in the portion of the warm springs studied, although the western spring which lies outside of this study has had goldfish (*Carassius auratus*) introduced to it in the last ten years.

Outside of humans, mammals have had relatively little impact on the warm springs. Moose hoofprints and droppings have been found in the soft earth and mud of the lower stream and wetland, and an unidentified bear (either the grizzly *Ursus arctos horribilis* or the American black bear *Ursus americanus*) was responsible for pulling out many traps in one incident, but it left most of the trapped fish uneaten, suggesting that they do not regularly prey on the small fish that inhabit the warm springs. Similarly, no fishing birds have been directly observed in the area, and there are no obvious signs that *C. plumbeus* is subject to significant predation.

The Atlin Warm Springs have been known to First Nations for thousands of years, and has been long used as a place of healing and comfort by the First Nations of the area, including Athapaskan-speaking peoples and inland Tlingit (McClellan 1953; Emmons et al. 1991). Since the arrival of Europeans in the region in the late 19th century, the area has been host to placer mines, although none of this has taken place in the direct watershed of the warm springs. Today, the upper reaches of the warm springs are frequented by the public, who have used manpower and excavation equipment to dam the walls of the upper pool and dig out the floor, with the intention of making the pool deeper and more suited for swimming and human use (A. deBruyn, personal observation). Lake chub are absent from this pool, and it is not known if they were present in the past, prior to human modification of the site.

The streams flowing from the warm springs pool are much less heavily trafficked, although a

few signs of previous trapping and human use in the area can be found, including rusted pieces of metal mesh and decaying foot bridges. It is in these lower streams that the Atlin Warm Springs lake chub are found.

In summary, the natural and human-influenced history of the lake chub makes it a fish of significant conservation concern. Its biogeography and occupancy of rare and distinctive habitats in thermal springs suggests that *C. plumbeus* has a rich biodiversity below the species level and that humans may have significantly impacted its biology and probability of persistence via the introduction of exotic species.

1.5 Thesis Objectives

With this background in mind, my thesis has the broad goal of informing the conservation ecology of this species by conducting inventory and basic ecological research to better understand the population size, habitat use and movements of lake chub, and to investigate interactions between lake chub and the exotic cherry shrimp. In Chapter 2, I conducted a series of mark-and recapture studies to estimate population size, map habitat use and investigate movement between different parts of the eastern warm springs complex. In Chapter 3 I studied aspects of the thermal biology of the cherry shrimp to assess the potential for the spread of this exotic species outside of the warm springs complex. As COSEWIC has recently conducted an assessment of thermal springs lake chub in Canada, my research should assist subsequent recovery efforts and re-assessments by providing baseline demographic information and assessing the potential for the cherry shrimp to spread beyond the confines of the warm springs.

Chapter 2 Population Size, Movement, and Habitat

Occupancy of Lake Chub (*Couesius plumbeus*) in the Atlin Warm Springs

2.1 Introduction

Conservation biology is concerned with the maintenance and protection of species, natural spaces, resources and ecosystems. Recognizing that humanity has a large and consistently growing impact on the natural environment around us, conservation is the set of practices that allows us to protect nature from this threat, for various ethical or pragmatic motivations. Conservation takes various different forms. It can involve limiting human contact with natural spaces, or managing that contact so that natural processes are able to proceed largely unhindered by human presence (Hunter & Gibbs 2006). Abell (2002) characterized the circumstances of freshwater biodiversity as a crisis, both for its significance as a global conservation priority and for its relative lack of attention. The conservation of freshwater organisms faces several challenges distinct from conservation of their terrestrial and marine counterparts, and is in some ways comparable to the difficulties of island conservation (Simberloff & Abele 1976; Biber 2002). Due to the relatively tiny area covered by fresh water, freshwater organisms' ranges are often highly constrained. This greatly limits their potential habitat extent, and also presents them with a problem of reduced connectivity (Fagan et al. 2002; Gido et al. 2016). While marine species are generally considered to exhibit greater connectivity among habitats (Waters et al. 2000) and most terrestrial species can spread overland, at least within their native ranges, freshwater fishes have fewer opportunities to disperse beyond their native ranges (Nislow et al. 2011). Poor connectivity

between potential habitats can prevent a species from dispersing to an area to which it would otherwise be well-suited. This issue affects fish particularly severely (Shurin et al. 2009), as many other freshwater organisms have superior means of dispersal, such as the winged adult life stages of freshwater insects, the terrestrial locomotion of amphibians, or the resilient eggs of crustaceans. This combination of factors leaves freshwater fishes prone to having limited ranges that are slow to expand, leading to correspondingly small populations that are vulnerable to stochastic events.

A key aspect of conservation of freshwater fishes is the assessment of the current state of populations and the species as a whole (Akçakaya & Sjögren-Gulve, 2000). Status assessments of individual species and the apportionment of efforts to protect and recover the many species that could be at risk are facilitated by the application of an objective set of criteria to assess characteristics of species that are central to their persistence across the landscape. Basic population demographics such as size, growth and decline rates, the geographic extent of occurrence, the actual area of occupancy, and connectivity among populations are examples of key traits that strongly influence species persistence (Jones & Diamond 1976; Purvis et al. 2000).

The International Union for the Conservation of Nature (IUCN) has developed a series of quantitative criteria for such traits that they use, alone or in combination with each other, to assess species in terms of their global conservation status as Extinct, Extinct in the Wild, Critically Endangered, Endangered, Vulnerable, Threatened, Near Threatened, Least Concern, or Data Deficient on a global basis (Mace et al. 2008). Within a Canadian context, The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has adopted a modified version of IUCN criteria to assess the conservation status of Canadian species, using the categories Extinct (X), Extirpated (XT), Endangered (E), Threatened (T), Special Concern (SC), Not at Risk (NAR), and Data Deficient (DD) (COSEWIC 2018a). COSEWIC uses two 'D' criteria to recognize very small or restricted total

Canadian populations, D1 and D2. Under D1, the 'Endangered' category is applied if the total number of mature individuals is fewer than 250, and 'Threatened' if the number is fewer than 1,000 individuals. The D2 criterion triggers a 'Threatened' status if any index of the area of occupancy is $\leq 20 \text{ km}^2$, or if the total number of locations is ≤ 5 . These criteria all act on the principle, supported by abundant empirical evidence, that small and geographically-restricted populations are highly susceptible to human impacts or stochastic environmental factors (Mace et al. 2008).

2.1.1 Lake Chub in the Atlin Warm Springs

The *C. plumbeus* of thermal springs represent a microcosm of several issues in freshwater fish conservation. They have an uncertain population size, low connectivity with other populations, and a severely limited habitat extent. They are part of a widespread polytypic species, and several subpopulations have independently adapted to live in warm, geothermal waters. Three of these exist in the Liard River drainage basin in northeastern British Columbia, in the Liard and Deer River Hot Springs, and in the Atlin Warm Springs of the Yukon River system (Darveau et al. 2012). Given the very small geographic range of Atlin Warm Springs lake chub (see Chapter 1) and its proximity to potential human impacts (a gravel road, recreation site, and a small number of residences), this thermal spring population may be at great risk. Concordantly, COSEWIC has recently assessed both the Liard and Atlin thermal springs lake chub as Threatened (COSEWIC 2018b) based largely on the small ranges of these populations. Despite this assessment, there are no estimates of population size, and habitat size is estimated by overlaying 2x2 km grids over maps of the springs. This greatly overestimates the area occupied by including surrounding terrestrial habitat. Given these limitations in current knowledge, the overall goal of this part of the field study was to provide the first estimates of population size, habitat area, and basic population structure (in terms of movements between areas and

size distributions) of *C. plumbeus* in the Atlin Warm Springs. By obtaining this information, the larger goal was to help inform the current and future conservation assessments on warm/hot springs lake chub in Canada. Early on, it was decided that mark-recapture trapping, coupled with basic physical measurements, would be an effective way of making these estimates, and it was with this in mind that I first visited the site in the fall of 2015.

2.2 Methods

2.2.1 Study Site and General Methods

A general description of the Atlin Warm Springs and surrounding environs was provided in Chapter 1. To conduct a general survey of the area, gauge its size and layout, and determine the extent of the range of the *C. plumbeus*, I conducted a series of walking surveys in October and December 2015. Each survey involved the recording of GPS coordinates and qualitative observations of the local environment. Based on this survey, I produced a map of the area and divided the warm springs into three contiguous regions (Figure 2.1).

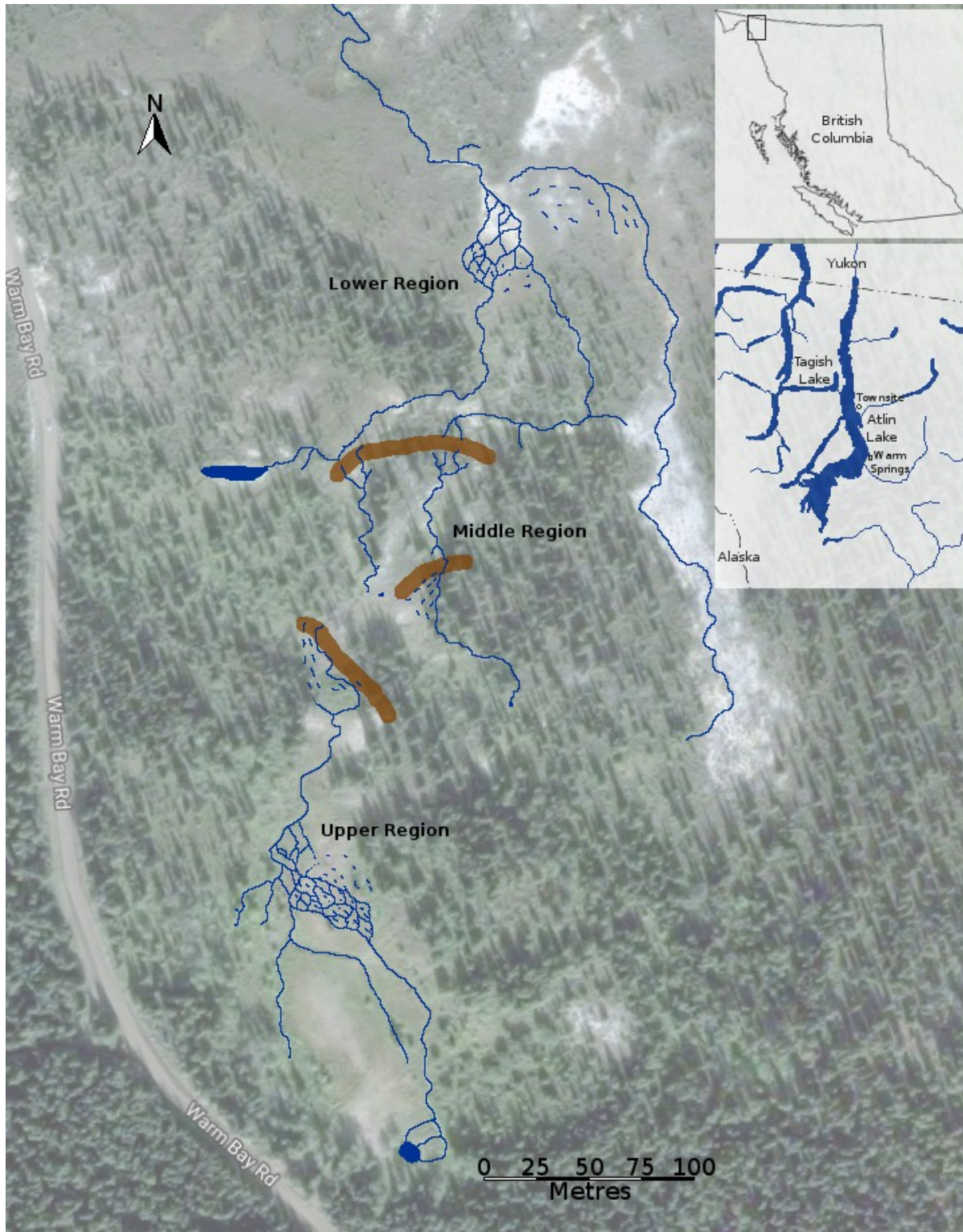


Figure 2.1 Atlin Warm Springs (eastern portion) map. Thin blue lines indicate water, while broad brown lines indicate natural barriers and abrupt changes in elevation which obstruct movement and disrupt surface flow. The three broad areas referred to in the study are labelled as the Lower, Middle and Upper Regions. The top right insets show the position of the warm springs in relation to Atlin Lake and the position of the study area in northwestern British Columbia. Satellite Imagery ©2018 DigitalGlobe; Map Data ©2018 Google.

Each area was separated by structures that I refer to as cascades, previously described by others as 'tufa barrages' (Profe et al. 2016). Each cascade is a series of calcified steps composed of tufa limestone, forming a series of terraced pools (Figure 2.2). Steady flow proceeds through narrow waterfalls or underground between these pools. There was no visible connection between the different regions, suggesting that they formed natural barriers which impeded *C. plumbeus*' ability to disperse.



Figure 2.2 Image of a cascade, separating regions of open water in the warm springs complex. Tufa has precipitated over mosses to form hanging pools. This image shows approximately 6 metres of elevation change, the entire cascade contains between 11 and 13 metres of elevation difference between the middle and lower regions.

The area immediately downstream from the warm springs pool, from which the geothermal water emerged from the ground, was named the 'Upper Region' (Figure 2.3). This region is comprised of several streams which emerge hot from the ground or out of pools, and pass through an open marsh before coalescing into a single stream, which then reaches the first cascade.

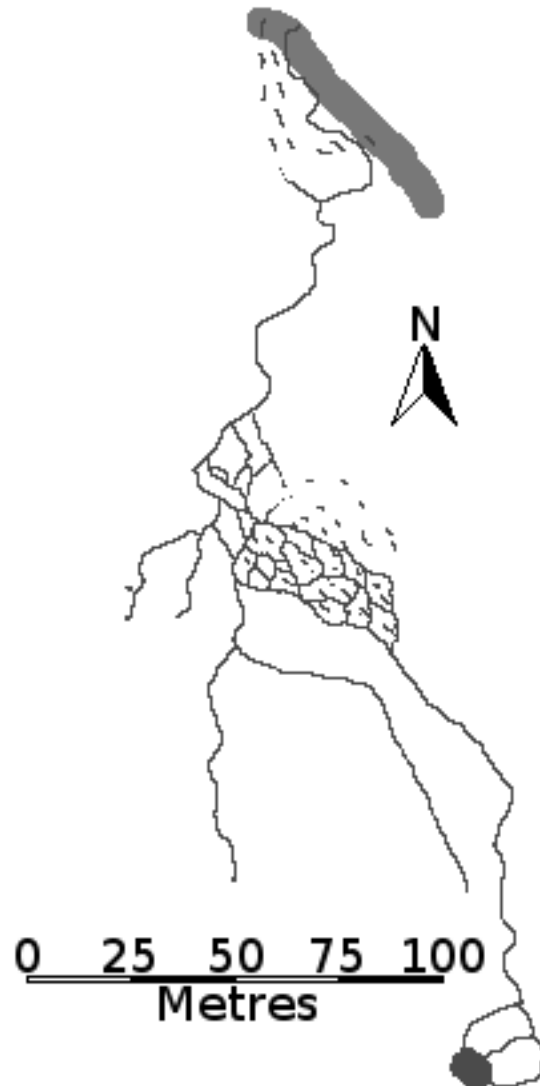


Figure 2.3 The Upper Region of the Atlin Warm Springs complex, stretching from the upper pool (circular area near the bottom right) to the first cascade. Fine lines represent surface water, thick shaded areas represent cascades.

The 'Middle Region' (Figure 2.4) was initially comprised of two connected streams separated from the upper region by 20 metres of tufa-encrusted moss and dry land. Between the 2016 and 2017 field seasons the connecting stream went dry, leaving the area as the two disconnected streams portrayed in the map. These streams in this region are narrow and shallow, with a few exceptions in the upper reaches of the eastern stream, and their surroundings are heavily forested.

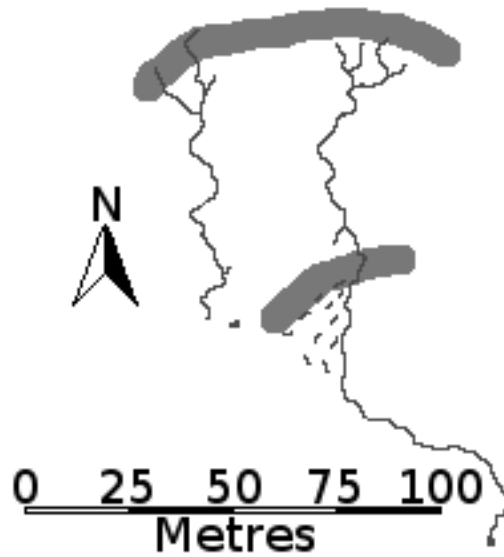


Figure 2.4 The Middle Region of the Atlin Warm Springs complex, stretching from the east pool down to the second cascade. Fine lines represent surface water, thick shaded areas represent cascades.

The final and largest area studied was called the 'Lower Region' (Figure 2.5), which includes the widest, deepest and coolest parts of the stream. Its two main stems both begin in forested areas: the eastern branch begins below the second cascade, while the western branch begins at the western pond, or 'toad pond', a site of western toad (*Anaxyrus boreas*) reproduction (see Slough & deBruyn 2018). As these branches proceed downstream, they drain into an open marshland, which ultimately coalesces into a single stream which drains through a culvert under Warm Bay Road into Warm Bay, in Atlin Lake.

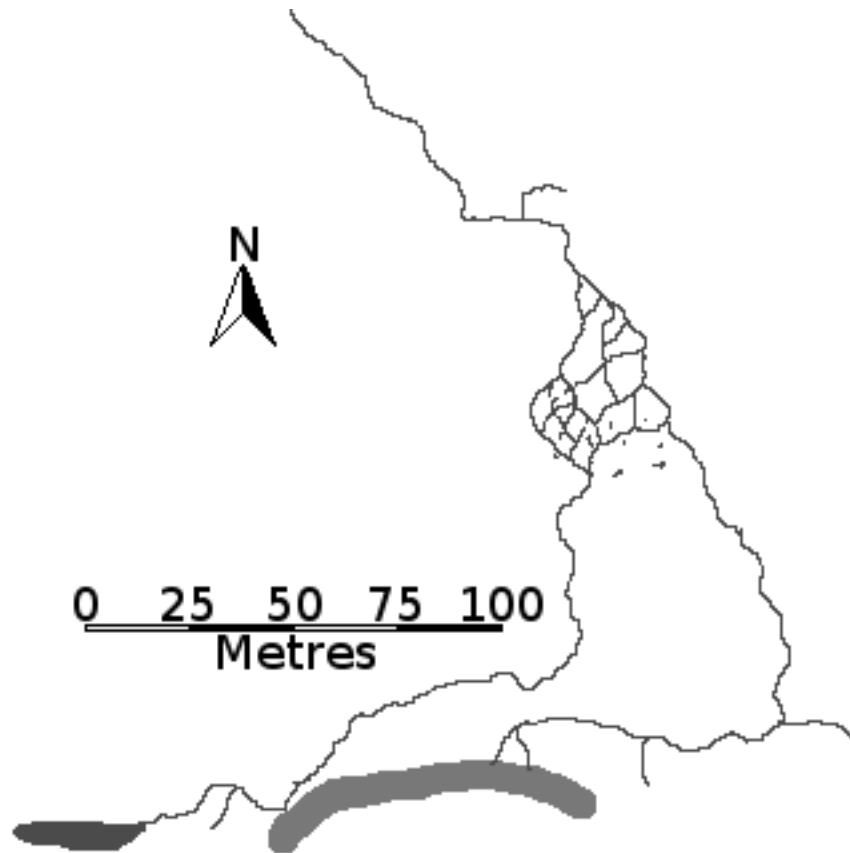


Figure 2.5 The Lower Region, stretching from the toad pond (oblong area at the bottom left) and second cascade to the downstream extent of *C. plumbeus*' range. Fine lines represent surface water, thick shaded areas represent cascades. Warm Bay of Atlin Lake lies 300 meters north-northwest of the confluence of the two streams.

The streams in this area are highly dynamic, as was illustrated by the rerouting of the middle region's streams in the winter of 2016/17. This is likely attributable to the soft, marshy ground, which is composed largely of spongy moss. Falling logs, seasonal freezing and human or animal presence can easily reroute streams.

Based on the natural barriers between the three regions, I established trapping sites in each of them to conduct a mark-recapture study.

2.2.2 Trapping, Tagging and Measuring Techniques

Between October 2015 and February 2018 I made 22 sampling visits to the Atlin Warm Springs, as well as two non-sampling exploratory visits. These site visits took place across the calendar for a 29 month period; 16 of the sampling events took place in the summer months between May and August, six visits also took place in September, October, January and February (Appendix Figure A1).

Owing to the small extent and delicate nature of stream habitats and aquatic vegetation, seining and its associated human trampling was deemed potentially too destructive. Consequently, to collect fish I placed galvanized 6mm mesh minnow traps with 2.5 cm diameter entrances parallel to the flow of water and with their entrances fully submerged, to ensure that they were easily accessible to any fish in the area. Each trap was baited with cheese and attached by a string to a stake, marked with flagging tape for easy recovery. The traps reached saturation within half an hour, and leaving them in for longer did not yield more fish. Additionally, I found that the traps accumulated many fish regardless of whether or not they were baited. The minnow traps were most effective on fish longer than 3 cm. Those smaller than that length could easily swim through the circular apertures on either end, or even squeeze through the mesh. At the end of the trapping period, I removed the fish from their traps and placed them into a 10 L pail with a bubbler to provide oxygen. I used Visible Implant Elastomer (VIE) tags, produced by Northwest Marine Technologies, to tag the fish following the manufacturer's instructions. I mixed proportions of coloured liquid elastomer with a curing agent, then transferred the mixture into an injection syringe. Each of the three regions was given its own colour code to be used for tagging: red for the upper region, yellow for the middle region and green for the lower region.

Prior to tagging, I produced an anaesthetic solution by dissolving 0.5 grams of the anaesthetic and muscle relaxant tricaine mesylate (tricaine methanesulphonate/TMS/MS-222) in 5 L of stream water stored in a second pail, creating a solution of approximately 0.38 mM concentration. This

rendered the fish immobile within one to two minutes. This allowed them to be tagged, measured (to the nearest 0.5 mm) and weighed (to the nearest 0.1 g) while still allowing them to recover quickly when placed back into a third pail filled with fresh stream water. I injected the tags in a vertical orientation on the left side of their caudal peduncles (Figure 2.6), leaving them visible from the side and through their paler lower skin, but obscured when looking from above. Any fish that failed to recover from this procedure were preserved in an ethanol solution.

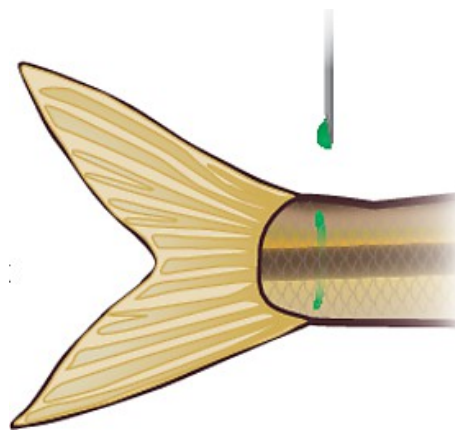


Figure 2.6 Appearance of green VIE tag in caudal peduncle of *C. plumbeus*.

I also conducted dip-net sampling at select sites, in order to capture and measure juveniles who were not captured in the minnow traps. Based on these exploratory mark and recapture efforts between the fall of 2015 and the spring of 2016, I selected three sites for subsequent trapping during the summer of 2016 based on relatively high occurrences of *C. plumbeus*, sufficient depth, and ease of access (Figure 2.7).

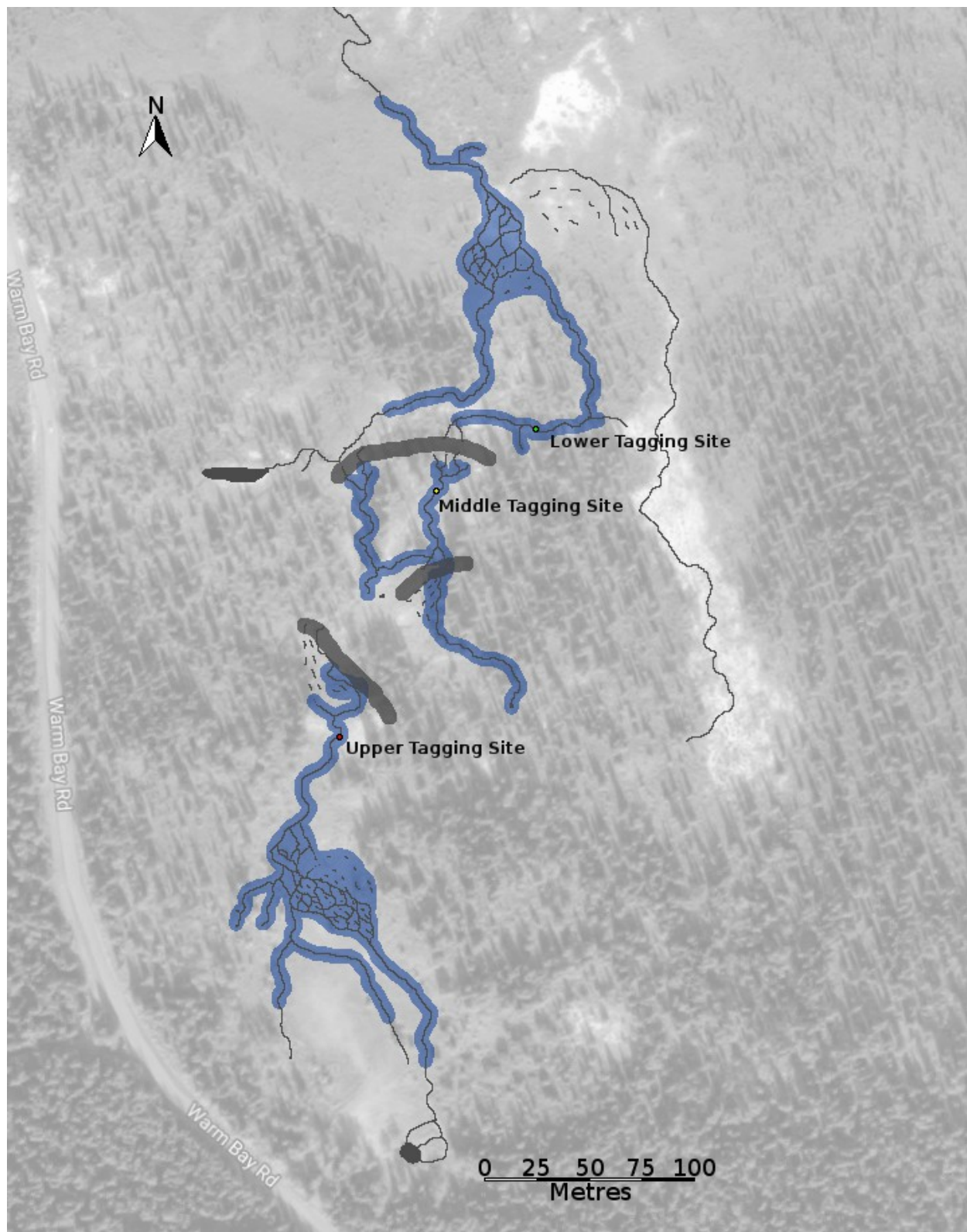


Figure 2.7. Map of 2016 primary trapping sites. Blue highlights the stream areas in which *C. plumbeus* was observed.

The discovery of the lack of movement of the *C. plumbeus* between the different regions in 2016 (see Section 2.3, Results) led me to modify my survey methods. I adopted a more spatially intensive trapping regimen in 2017. I established twenty-six sites, in comparison to 2016's three sites, where a mark and recapture survey was performed (Figure 2.8). Two new tagging colours, orange and blue, were used in traps A-K in the upper region and L-S in the middle region, to distinguish them from the previous year's red and yellow tags. Additionally, seven new tagging colours were used (brown, white, purple, fluorescent red, yellow, black and pink), each unique to a single site in the lower region (T-Z), to assess degrees of mobility between tagging sites. With the few movements observed between sites in 2016, I hoped that this spatially intensified sampling would present the entire population with the opportunity to be sampled. Continued trapping over the fall and winter would illuminate any seasonal movements between the regions. Under this new regimen, most traps were separated by a gap of between 25 and 50 metres, except for the more widely spaced sites on the periphery of each region.

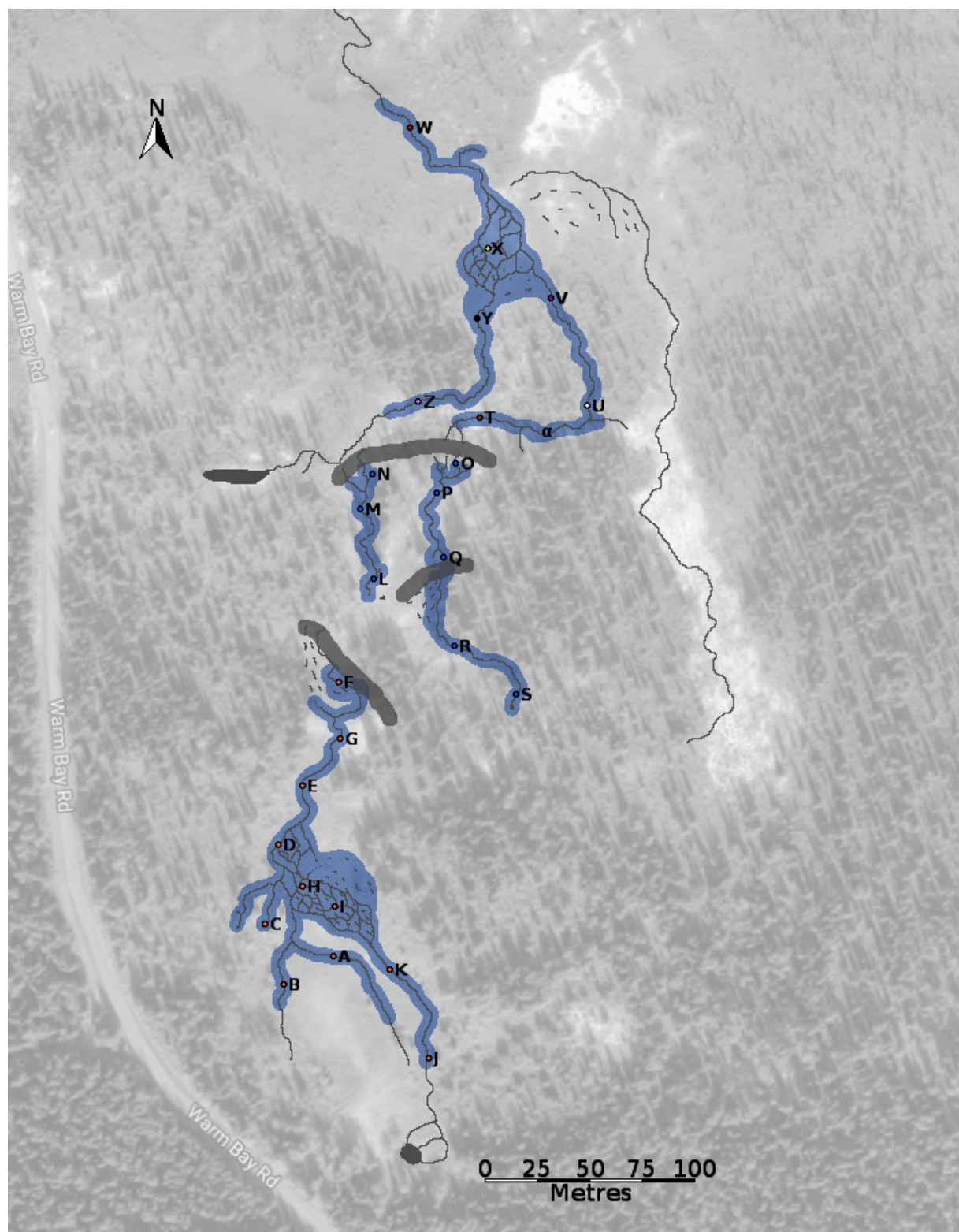


Figure 2.8. Map of 2017 trapping sites. Blue highlights the streams in which *C. plumbeus* were observed to be present. Dots at each site are coded by the colour of tag used at each location. Fine lines represent surface water, thick transparent lines represent cascades.

I conducted recapturing at each tagging site six times for the lower and middle regions and seven times for the upper region in 2016. Owing to more intensive trapping within regions in 2017, I conducted recapture trapping twice each for the lower and middle regions and three times for the upper region. In both years marking occurred in June and recaptures occurred at least 14 days later between late June and August. In addition to my research in the warm springs, I also trapped the surrounding lakes and creeks to get a better sense of the local distribution of lake chub which was unknown. These sites included Palmer Lake (a 3 kilometre long lake 2 kilometres north-northeast of the warm springs), Como Lake (an 800 metre long lake 22 km north of the warm springs, on the north side of Atlin), and The Grotto (a larger stream 2.5 km south-southeast of the warm springs), as well as Atlin Lake proper.

2.2.3 Area of Occupancy

To obtain an estimate of area of occupancy of the warm springs complex by *C. plumbeus*, I first delimited the maximum upstream and downstream sites of occurrence of *C. plumbeus* using a combination of information from my walking surveys and trapping efforts. I walked along every single branch of the stream in each of the three regions, and visually surveyed for the presence of *C. plumbeus*, taking point counts each time they were observed. I divided up each stream into segments, starting a new segment each time there was a significant change in depth, width, or stream direction. The shortest segments were about three metres long while the longest were thirty, but most were close to the mean size of ten metres in length. For each segment, I measured the depth and width with a tape measure and recorded the values on my map, and took point counts of any visible fish. I determined the segment lengths with the aid of my satellite map: I ran transect tapes between several known landmarks visible from the satellite image and used those values to generate a ratio between pixels on the image and metres in the field (laying transect tape along every meter and bend of each stream would have

been a very arduous task). These data allowed me to measure the length and area of each segment of inhabited streams, as well as providing a secondary source of information regarding the relative populations of different parts of the warm springs complex.

2.2.4 Statistical Methods

To generate population size estimates, I used the Lincoln-Petersen estimate with Chapman's adjustment (Krebs 1989). To calculate a Lincoln-Petersen estimate I employed a formula that incorporates the Chapman (1951) correction for small samples size (e.g., when recaptures of marked fish were 0), where \hat{N}_c = estimated population size, M = number of fish marked during the first survey, C = number of fish captured during the second survey, and R = the number of fish captured during the second survey that bore marks.

Equation 1:

$$\hat{N}_c = \frac{(M + 1)(C + 1)}{R + 1} - 1$$

I repeated the recapture trapping from two to seven times, with each recapture session treated as a new mark-recapture estimate. I calculated 95% confidence intervals for Lincoln-Petersen-Chapman estimates using the procedure of Chapman (1951).

All fish trapped were weighed and measured as described in Section 2.2.2. To compare the means of length, weight, and condition factor from fish captured across the three regions over time, I used a two-way analysis of variance (with region and month as the main factors) with PAST 3 (Hammer et al. 2001). I conducted a least square linear regression on the relationships between the log (base 10) transformations of these data using PAST 3. To estimate the number of age classes in samples collected with regions at the same time, I used the mixture analysis tool in PAST 3 to find the

number of modes that best fit the length-frequency distributions (Hammer et al. 2001). The mixture analysis uses a maximum-likelihood approach to estimate the parameters (mean, standard deviation and proportion) of two or more normal distributions of size data from a pooled sample of fish lengths. The model with the lowest Akaike Information Criterion was regarded as the most appropriate model for each distribution, and the number of modes present in that model was interpreted as the number of age classes present in the population (Beamish & Potter 1975).

2.3 Results

2.3.1 General Observations on Sampled *Couesius plumbeus*

Judging from their visible gut contents and feces, the Atlin Warm Springs *C. plumbeus* have diets typical of their species, preying on small invertebrates, including snails, insects, and crustaceans, including the invasive cherry shrimp (*Neocaridina davidi* var. *red*, see Chapter 3). I observed no cases of predation by birds or fishing mammals, leaving *C. plumbeus* as the apparent top predators in their small ecosystem.

I observed the expression of milt and eggs by a minority of fish in June, while also noticing many adults with 'deflated' bodies and the presence of many tiny fry with lengths less than 1 cm, far too small to effectively catch with minnow traps or even nets. I also made several qualitative observations about the behaviour of *C. plumbeus*. They are schooling fish, and only rarely observed on their own. Different size categories mingle with each other in these schools. They are skittish in response to noise and movement, and react by darting away when shadows pass over them. They frequently took shelter underneath floating plant matter, and are often seen darting in clusters from one area of cover to another. They are effective jumpers, being able to leap over barriers of at least 15 centimetres (A.

deBruyn, personal observation) with only 10 centimetres of water to build up speed, and are very active, rarely remaining motionless.

2.3.2 2016 Population Surveys

During my initial observations of the various stream sections, large numbers of lake chub were observed, particularly in the lower region. I regularly observed large schools of *C. plumbeus* which appeared to have fish numbering in the hundreds (Figure 2.9).



Figure 2.9 *C. plumbeus* schooling together in a typical stream in the Atlin Warm Springs complex. Twenty-four individuals, the largest of whom are approximately 8 cm long, are visible in this photo, and schools of over one hundred individuals were commonly sighted.

During the summer of 2016, 86 fish were tagged across the three regions (Table 2.1). A total of

130 fish were caught during resampling efforts between June and August, 15 of which had been tagged. Population estimates were made for each region across several time periods (Table 2.1). These ranged from a low of 24 in the middle region (June) to a high of 707 (August) in the upper region. In total, the estimated population sizes ranged from 342 in the spring to 1,179 in late summer. Over all sampling periods, the middle region produced the lowest estimates (Table 2.1).

Date	Region	N ₁	N ₂	N _m	Estimated population size	95% CI
June 13, 2016	Lower	17	-	-	-	-
	Middle	4	-	-	-	-
	Upper	65	-	-	-	-
June 19, 2016	Lower	-	10	2	241	171 - 311
	Middle	-	4	0	24	0 - 52
	Upper	-	12	2	77	16 - 138
July 4, 2016	Lower	-	16	4	223	78 - 368
	Middle	-	16	0	84	0 - 186
	Upper	-	6	0	125	0 - 282
August 8, 2016	Lower		46	7	388	271 - 605
	Middle		16	0	84	0 - 186
	Upper		5	0	707	574 - 840

Table 2.1 Results of mark and recapture surveys for Atlin Warm Springs across three months in 2016. N₁ = Number of fish captured and marked at time 1, N₂ = number of fish captured at time 2, N_m = number of fish captured at time 2 that bore marks, - = not applicable. Population size was estimated using the Lincoln-Peterson-Chapman estimator with 95% confidence intervals calculated using Chapman's (1951) procedure.

2.3.3 2017 Population Surveys

From my first summer sampling on June, 9 2017 until as late as July 3, 2017 I observed *C. plumbeus* which expressed milt or eggs when massaged. All gravid females were longer than 5.9 cm and weighed more than 2.4 grams. Milt-producing males were no smaller than 5.1 cm and 1.7 grams. On June 17 2017, I observed many fry around Trap F, near the downstream end of the upper region, several of whom were little over a centimetre in length. Based on qualitative dip net sampling, fry congregate in

slow-flowing pools outside of the main stream. The larger and more open streams are populated predominately by larger lake chub.

Trapping success varied markedly across all 26 sites. Four sites, E in the upper region, S in the middle region, and T and Z in the lower region, accounted for 55% of all captures (see Appendix Table A2). Several of the least populated sites were in the peripheral streams of the upper region. For example, sites A, B and C were all on shallow, plant-choked streams not fed directly by the surface flow from the source pool, all in the open meadow that defines the upper region. While fish were occasionally observed at Site B, none were ever trapped there (this was the only site in which a fish was never caught; at least one was captured at all other sites).

On August 29 2017, walking point counts recorded 529 individuals in the lower region, 382 in the middle region, but only 51 in the upper region, for a total of 962 individuals. Over the 2017 summer field season, I marked a total of 261 fish and during recapturing collected 761 fish, of which 158 were recaptures (Table 2.2). Population estimates in the three regions across five resampling efforts ranged from a low of 151 in the middle region (mid-July) to 1,499 in the upper region (late August). Summing the average estimates for each region calculated across all time periods yielded an estimate of 1,664 individuals.

Date	Region	N ₁	N ₂	N _m	Estimated population size	95% CI
June 18, 2017	Lower	94	-	-	-	-
	Middle	67	-	-	-	-
	Upper	100	-	-	-	-
July 3, 2017	Lower	-	-	-	-	-
	Middle	-	-	-	-	-
	Upper	-	75	5	1,267	383 – 2,151
July 19, 2017	Lower	-	-	-	-	-
	Middle	-	68	30	151	123 - 179
	Upper	-	65	12	508	283 - 733
July 30, 2017	Lower	-	-	-	-	-
	Middle	-	134	34	263	210 - 316
	Upper	-	-	-	-	-
August 30, 2017	Lower	-	110	32	320	247 - 393
	Middle	-	-	-	-	-
	Upper	-	149	9	1,499	676 – 2,322
September 3, 2017	Lower	-	160	36	412	322 - 502
	Middle	-	-	-	-	-
	Upper	-	-	-	-	-

Table 2.2 Results of mark and recapture surveys for Atlin Warms Springs *C. plumbeus* across three months in 2017. N₁ = Number of fish captured and marked at time 1, N₂ = number of fish captured at time 2, N_m = number of fish captured at time 2 that bore marks, - = not applicable. Population size was estimated using the Lincoln-Peterson-Chapman estimator with 95% confidence intervals calculated using Chapman's (1951) procedure.

2.3.4 Movement Between Sites

The use of unique colours for each sampling site in the lower region allowed me to detect the movement of individuals between each tagging site (Figure 2.10). I captured two fish bearing tags from site α (the 2016 lower tagging site) at site T, while three fish bearing tags from site T were found at site α . Limited three-way movement was observed between sites X, Y and Z, all on the western branch of the lower region's two populated forks. Two individuals tagged at site Y and one from site X were recovered at Z. A single lake chub tagged at site X was recovered at Y, and one tagged at Z was

captured at X. Additionally, my 2016 sampling had shown one single movement between regions, as one green-tagged lake chub from site α in the lower region had been recaptured in the yellow-tagged middle region, at the location referred to in my second year sampling as 'Site P'. This fish may have leaped and flopped across the land gap, or navigated an unknown tunnel. All recaptures took place either two or three weeks after initial tagging, except for the capture of the 2016-tagged individuals from site α in 2017.

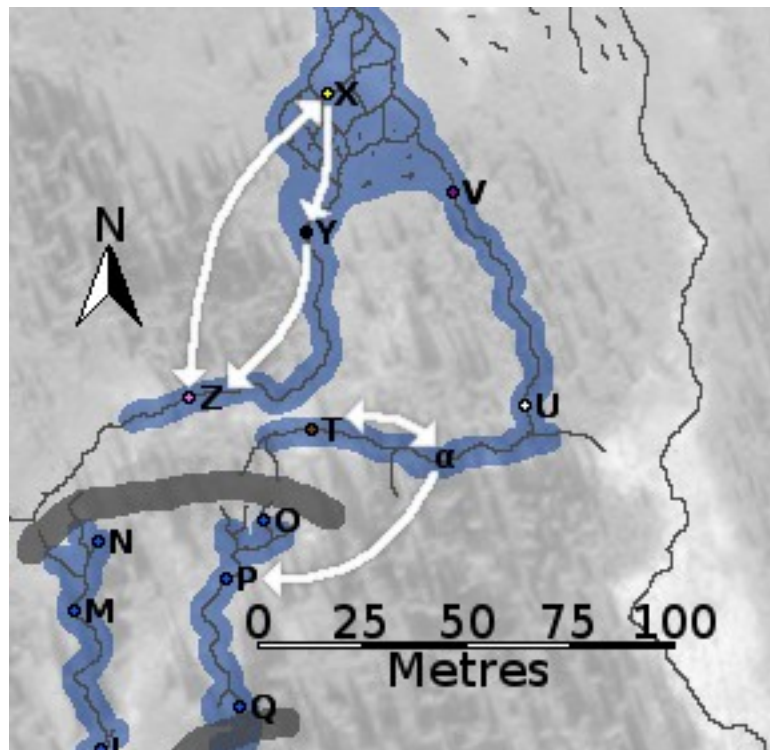


Figure 2.10 Map of movements of tagged *C. plumbeus* in the summers of 2016 and 2017 between sampling sites in the middle and lower regions. White arrows indicate confirmed directions movement during two to three week periods.

The longest confirmed movement was between sites X and Z, barely exceeding 100 metres. Of the 192 marked lake chub that were recaptured with marks, only 10 moved more than 25 metres away from their point of tagging (Figure 2.11).

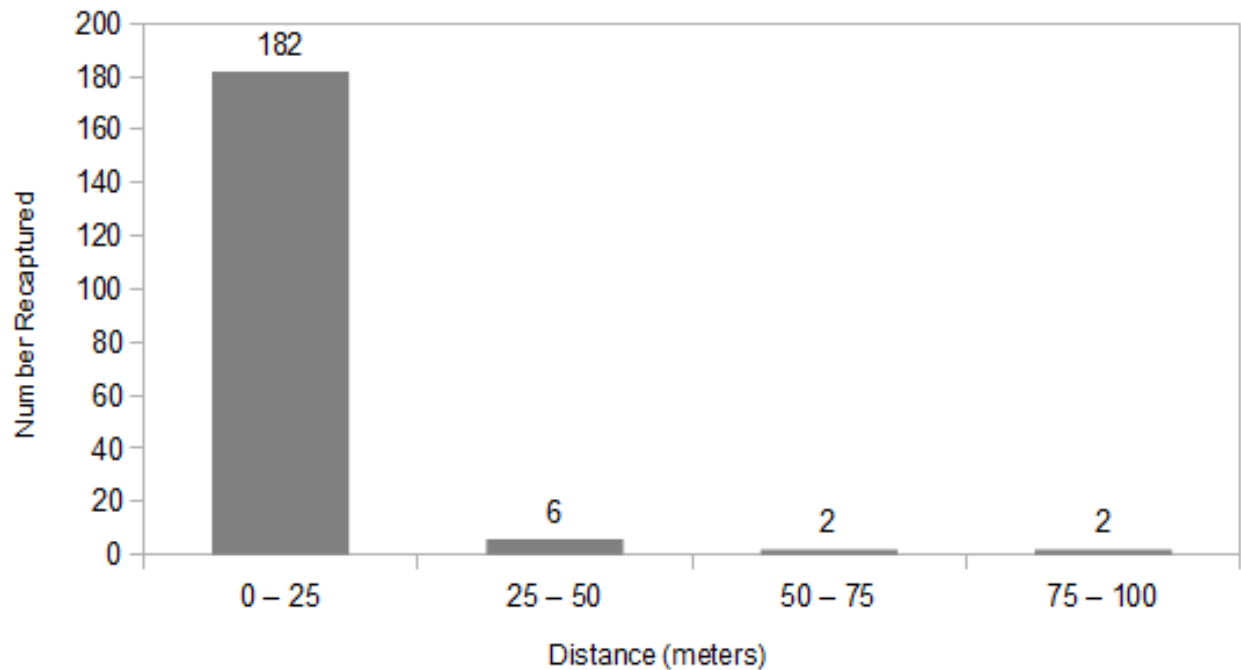


Figure 2.11. Distance traveled between tagging site and recapture site of the 192 *C. plumbeus* recaptured in the summers of 2016 and 2017.

Further sampling on January 3 and February 25, 2018 provided the opportunity to detect seasonal movement between the regions. Due to the practical difficulties of winter sampling, such as limited daylight and freezing temperatures, only sites T and Z were visited. Out of 18 marked recaptures, 16 were found at their original tagging site, while two individuals had moved to site T, from W and V respectively (Figure 2.12). The movement from W to T is the longest confirmed movement of a lake chub in the Atlin Warm Springs, approximately 220 metres.

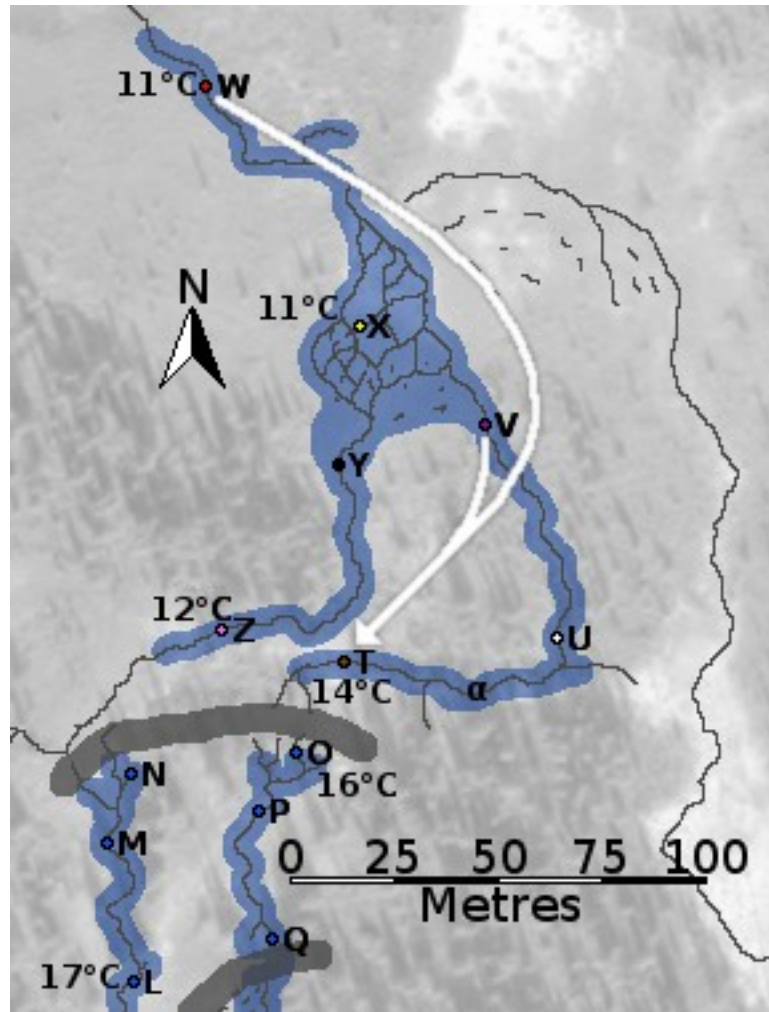


Figure 2.12 Map of water temperatures and movements of tagged *C. plumbeus* in the winter of 2017-2018 between sampling sites in the middle and lower regions. White arrows indicate confirmed movements in the 6 to 7 month period between summer tagging and winter recapture.

Only three fish tagged in 2016 were found in 2017, and all were large. At trap T, one was 7.9 cm long at 6.1 g and another was 6.6 cm and 3.8 g, while one at trap α was 9.5 cm and 10.2 g. This puts all fish marked in 2016 and recaptured in 2017 at well above the 80th percentile in terms of size.

I trapped lakes surrounding the Atlin Warm Springs for a total of 28 trap hours per site (4 traps and 7 hours each per site). This trapping effort recovered no populations of *C. plumbeus* in Palmer Lake, Como Lake, Atlin Lake, or The Grotto.

2.3.5 Area of Occupancy

The limits of the distribution of the Atlin Warm Springs *C. plumbeus* are formed by the natural physical and thermal barriers of the location (Figure 2.8). Observations of fish were very infrequent in the furthest upstream reaches of the upper region, being absent from the beginnings of the streams containing sites A and B, as well as the upper pool of the warm springs themselves. They were present throughout the rest of the upper and middle region, except the lowest pools of the cascades separating each region (likely due to their limited volumes), and throughout the eastern branch of the lower region. This branch was devoid of plant and animal life and formed thick mineral crusts around its edges, suggesting that it was highly alkaline, although its pH was not measured. In the western branch, lake chub were absent upstream of the point where warm water from the middle region heats up the water draining out of the cool western pool ('toad pool'). This pool is roughly 10°C in the summer and freezes in the winter; its temperature appears to be determined by atmospheric rather than geothermal forces. Lake chub appeared to be much less common in the lower wetland, with no fish being trapped or observed beyond the bend some 50 meters downstream from site W, the lowest trapping location.

The area of occupancy (AO) was estimated as 3,602m², or 0.004km². The upper region's AO was 1,983m², the middle's was 398m², while the lower region's area was 1,222m². The small size of the middle region was more pronounced in terms of area than in terms of volume because both the upper and lower regions have sizeable areas of very shallow wetland, which contributed heavily to the area of occupancy estimate (see Appendix Table A3).

2.3.6 Size and Weight of Atlin Warm Springs *Couesius plumbeus*

The sizes of *C. plumbeus* appeared to differ substantially among the sections with the largest fish being in the lower section and the smallest in the upper section (Figure 2.13). Within-region trends were

observed as well: dip netting in the shallow marshes and side ponds in both the upper and lower regions revealed the presence of juveniles and individuals at or below 2 cm in length, which were not observed elsewhere. While the middle region appears to lack these 'nursery ponds', I caught many individuals below 3 cm in length, suggesting either juvenile migration or an unobserved spawning location in the region.

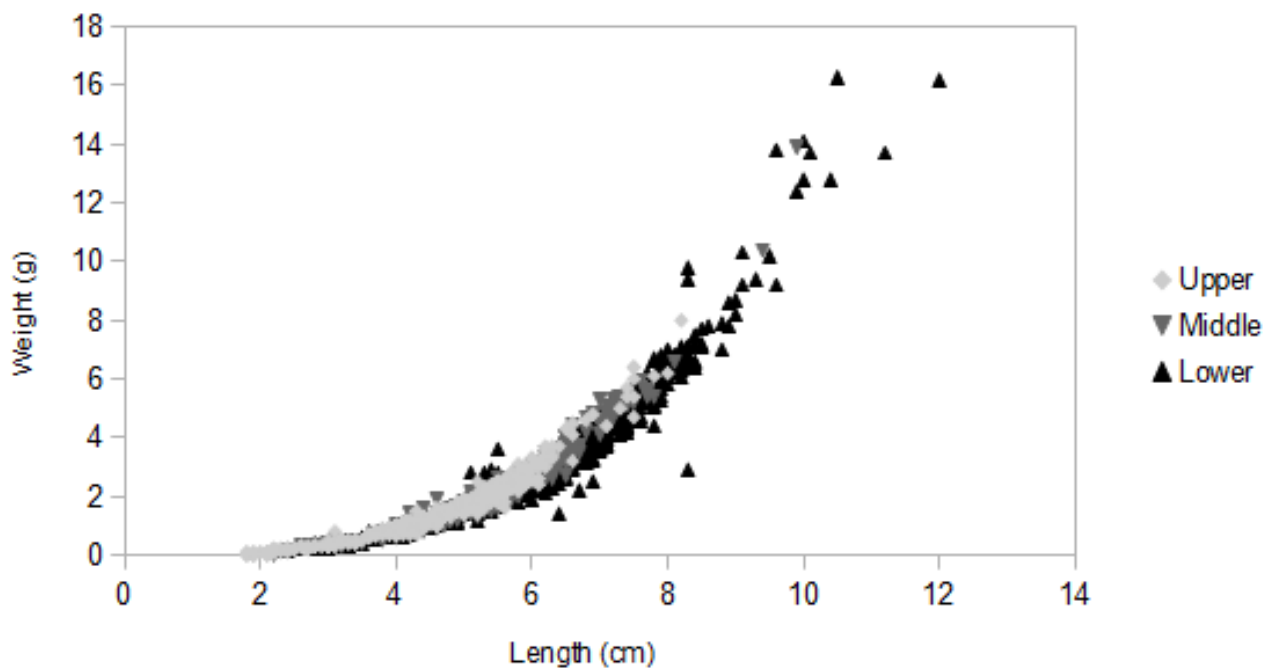


Figure 2.13 *Couesius plumbeus* length plotted against weight across multiple re-samplings from 2016 to 2018, Lower N = 1,099, Middle N = 526, Upper N = 484.

These data were linearized by a log-log regression, yielding an overall regression of log weight = $6.74(\log \text{ fork length}) - 4.27$, $r^2 = 0.974$, $P < 0.001$. A two factor analysis of variance detected significant effects on fork length of region, month, as well as an interaction between these two main factors (all $P < 0.001$). Fish in the lower region were consistently the largest, followed usually by fish in the middle, then upper region, but the magnitude of differences differed across months from June

through January (Fig. 2.14).

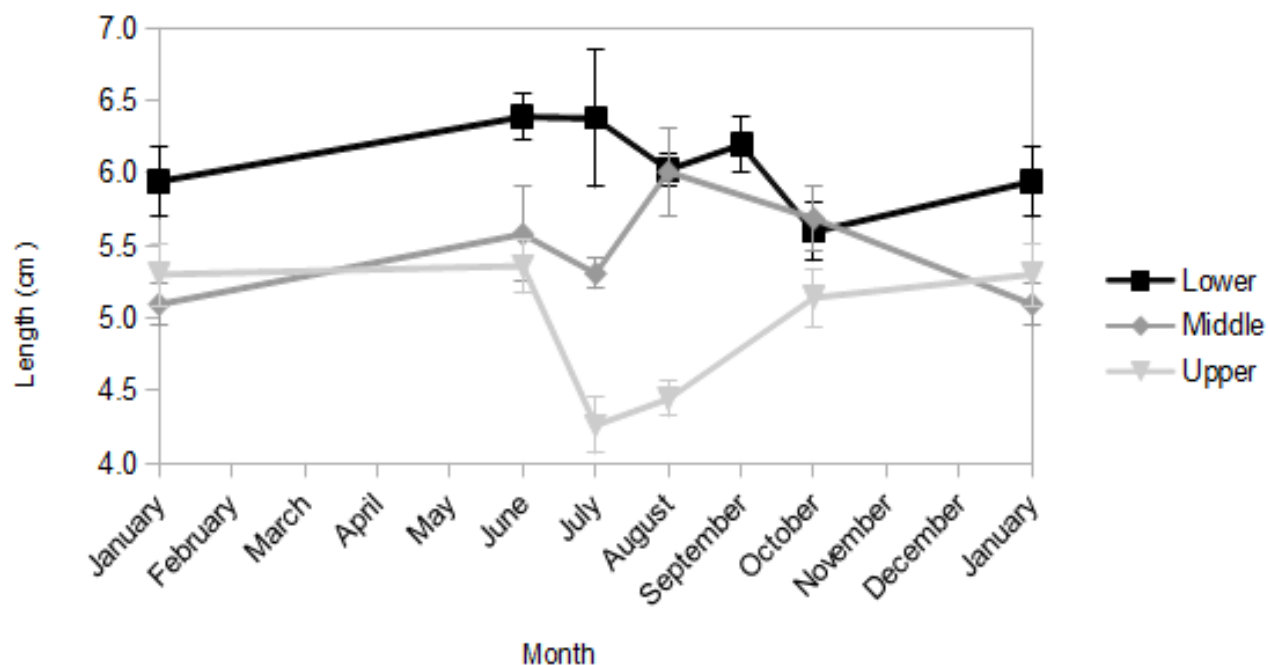


Figure 2.14. Mean fork length of *C. plumbeus* sampled from three regions of the Atlin Warm Springs complex between June 2016 and January 2018. Vertical bars indicate 95% confidence intervals.

In 2016, 30 out of 295 fish captured in the lower region were tagged with green dye (~10%). Consequently, I would have expected 34 out of the 336 fish captured in 2017 in the lower region to be green tagged, assuming no mortality, tag loss or dispersal out of the lower region. Given that only three were recaptured in 2017, my data suggests that between 2016 and 2017 there was a 90% turnover in the population of *C. plumbeus* that were large enough to be tagged.

Several times periods had sufficient sample sizes ($N \sim 30$) to examine the size-frequency distribution of lake chub across the three regions (see Appendix Figure A2). Most of these distributions supported the existence of between two and four size classes of lake chub (Table 2.3).

Date and region	N	Best supported number of size modes	Δ AIC (next most supported number of size classes)	Mean fork lengths under best model, cm
June 2016, Lower	73	3	10.6 (2)	5.8, 7.0, 10.8
June 2017, Lower	121	3	11.4 (2)	5.2, 6.7, 9.8
June 2017, Upper	77	4	3.2 (3)	2.4, 4.9, 6.4, 7.6
October 2016, Lower	119	4	0.8 (3)	4.2, 5.8, 6.5, 8.3
July 2017, Middle	135	4	5.0 (3)	3.1, 5.0, 6.3, 7.2
July 2017, Upper	65	2	2.2 (3)	4.1, 5.1
August 2017, Lower	243	6	1.6 (5)	4.3, 5.7, 6.1, 7.1, 8.0, 9.2
August 2017, Upper	149	2	2.4 (3)	4.2, 5.0
January 2018, Upper	29	2	1.8 (1)	4.0, 5.4
January 2018, Middle	138	5	0.2 (3)	4.1, 5.1, 5.8, 6.6 7.1
January 2018, Lower	28	3	3.3 (2)	4.5, 6.2, 7.6

Table 2.3 Results of Mixture analyses on size (fork length) frequency distributions of *C. plumbeus* sampled in three regions of the Atlin Warm Springs complex (see also Appendix Figure A2).

2.4 Discussion

2.4.1 Population Size Estimates

My study provides the first estimates of population size and area of occupancy, two parameters critical to conservation assessment for a unique population of lake chub in Canada. Based on the research conducted, I would tentatively place the population in the general range of 1,000 to 2,000 individuals. This estimate, however, is subject to a number of uncertainties. First, based on my initial visual observations, I was expecting a population comfortably in the range of multiple thousands. This estimate was based on routinely seeing schools containing hundreds of fish, in all three regions of the warm springs. I was confronted by an apparent disagreement between visual estimates and calculated

population estimates. My suspicion that certain assumptions of my mark-recapture sampling technique were being broken was confirmed later in the summer, when I moved my upper, middle and lower trapping sites to new locations, each within the same region, and my recapture rates dropped. If the population was dispersing freely throughout the region, I would have expected comparable catch levels across sites after moving my trapping site a hundred meters along the stream. The Atlin Warm Springs *C. plumbeus* had been observed to be quite active, and I thus assumed that they would be able to disperse throughout a whole region within a one to two week period, which was the minimum time between my marking and recapturing. As such, I had deemed it appropriate to place just one trap to represent each region. In spite of their quick movement and high activity levels, however, my tagging data suggested that individual *C. plumbeus* remained within the same general locations for weeks and likely months on end which violated a key assumption of mark and recapture studies. Thus, my 2016 estimate was not an estimate of the entire Atlin Warm Springs population, but rather a measurement of the population of the immediate environs of the three sampling sites, but this still provided an important insight into some of the peculiarities of the population I was studying.

In terms of other critical assumptions of the mark and recapture method I used, I am comfortable with the warm springs being a closed population as there are no nearby populations from which lake chub could immigrate into Atlin Warm Springs. During my 2017 tagging, I observed no mortality or tag loss in marked fish that I held before releasing (2016 saw the loss of 8 fish during tagging). Tag retention has been observed over a period of 3 months in lake chub held in the laboratory (K. Frazer, UBC Department of Zoology, personal communication), and tag retention over one year has been reported in some fishes (Walsh and Winkleman 2004; Simon and Dörner 2011), while Silver et al. state that subdermal tags can remain detectable for 1-2 years (2009). There exists the potential that the fish that I sampled were non-random samples because the population did not fully mix between

samplings. I addressed this in 2017 by placing traps in close proximity to one another, many within 25 metres of each other, thus giving the entirety of the population the opportunity to be captured. I did not conduct a test to confirm that tagged fish became more or less likely to be captured again, but cannot surmise a likely reason that tagging would cause either 'trap-happy' or 'trap-shy' behaviour. Finally, the tags were quite visible when viewed directly from the side, or through pale ventral skin, while appearing merely as a small coloured dot (if at all) on a dark green-brown background when viewed from above. This makes them highly detectable for humans, and probably does not increase their detectability by potential terrestrial or aerial predators. As such, natural mortality and tag failure are the only likely ways for tagged individuals to be removed from the population.

Given the apparent high site fidelity that I observed in lake chub, the change in methodology between 2016 and 2017 had several impacts on the data. In 2016, limited recaptures of marked individuals led to very high uncertainty, particularly in the upper region. In 2017, however, the much more thorough and widespread trapping regimen ensured that a large number of individuals could be marked in all regions and resulted in what I suspect are more reasonable estimates and a larger mean estimate (1,664) compared to 2016 (1,179). My walking surveys were associated with several practical shortcomings. For example, a swiftly-moving school is difficult to count, I could not be sure that I spotted every single fish that I walked past, I could have double-counted fish, and there are many areas where detectability was reduced. The warm springs have many areas where there are undercut banks in the stream, overhanging moss and other vegetation, and underground tunnels. *C. plumbeus* exhibited a sensitivity to changes in light, fleeing or changing their behaviour when a shadow moved over them, which may have reduced the number that I counted by driving more of them under cover. Detectability also varied significantly between regions: the bright, warm and exposed upper region was choked with *Nasturtium* sp., a floral invasive, which severely impeded views under the surface. This may account

for the low visual count attested in the upper region.

Even with these difficulties, my walking survey suggested that my mark-and-recapture population estimates are too low. For instance, if my visual estimate of 529 fish in the lower region in 2017 is accurate, then I saw almost all of the estimated population of the region in a single walk. This would suggest a near complete lack of fish hiding or going unobserved when I walked by and took point counts, or it could reflect that my point-counts were able to detect the younger and smaller fish who were not reliably caught in my minnow traps. Alternatively, it may suggest that my mark and recapture estimates are too low, perhaps due to a systematic bias towards low values. If fish once trapped become more likely to be trapped again, this could inflate my recapture rates and depress my overall population estimate. Perhaps even my intensified trapping regimen for 2017 was insufficient to account for the apparent strong site fidelity of Atlin Warm Springs *C. plumbeus*.

My walking survey count for the middle region were up to 2.5 times my mark and recapture point estimate for 2017. This is an even more dramatic illustration of the phenomenon observed in the lower region, which I interpret in the same way. In contrast, I am strongly inclined to discount my point-counts in the upper region, due to that site's major visibility issues, which occluded most of the known *C. plumbeus* habitat.

My data and experience suggest that it is appropriate to use a 1,000 – 2,000 population size estimate for fish greater than about ≥ 3 cm fork length. This estimate should be interpreted cautiously because of the absence of juvenile fish from my sampling, and because I lack information about juvenile mortality and adult reproductive rates. Consequently, I do not know for certain what portion of the population is juvenile. In summary, the total population has the potential to be significantly larger, potentially into the low mid-thousands.

2.4.2 Movement Between Sites

As my trapping method could only inconsistently capture individuals under 3 cm in length, information on movements reflects only the behaviour of larger lake chub, and misses patterns that exist in juveniles. With that said, my observations suggest that juveniles appear to remain in their sheltered, peripheral pools for their first several months, so it is unlikely that they undergo major migrations. Ninety-five percent of all recaptures were taken at the very same site, indicating a high degree of site fidelity. In spite of their ability to swim from one site to another in a matter of seconds or minutes, the overwhelming majority of recaptures remained within a narrow range over the course of months of experimentation.

A limited number of winter observations indicated longer-ranged movement upstream than was ever observed during the summer. Given that I sampled only the sites T and Z (the uppermost inhabited sites in the lower region) in the winter, it was only possible for me to detect fish that remained in their original locations, or came from further downstream. It is evident from my data that lake chub in the warm springs complex do not move very much from one site to another. I observed no major seasonal migration up and down the stream; not a single marked upper fish appeared in the middle region, nor did a marked middle fish appear in the lower region. One remarkable exception to this general pattern, however, is the observation that one fish marked at site α in the lower region was recaptured at site P in the middle region. This required passing through the tufa cascade of about 10 m elevation change largely in the absence of any surface flow. Other freshwater fishes may have partially subterranean habitats which enable dispersal (Niemiller & Poulson 2010), which is a plausible explanation for how an Atlin Warm Springs lake chub was able to move in such a manner. In different geographic contexts such as interconnected lakes and streams of similar water temperatures, lake chub have been observed to migrate distances of up to 3 km for spawning (Brown et al. 1970; Fuiman & Baker 1981).

Notwithstanding the aforementioned exception, my data suggest that it is reasonable to treat the Atlin Warm Springs lake chub as a complex of at least three semi-independent subpopulations. Further experimentation might see fit to conduct genetic analyses from individuals collected from different areas to determine the degree of relatedness among individuals and detect long and short-term movements between areas (Manel et al. 2003; Rozenfeld et al. 2008).

2.4.3 Growth Rate, Age and Life Cycle

Given that I first observed gravid females, milt-expressing males, some adults that appeared to have already spawned, and some small fry (<10 mm) in early June, I suggest that the spawning period lasts from at least late May until July (when I observed the last gamete-expressing fish). This agrees with observations in other regions in non-thermal springs habitats (Brown et al. 1970; McPhail 2007). Given the more consistently warm water temperature of the Atlin Warm Springs complex, it is possible that the lake chub could spawn more than once annually, although thorough observations during the early spring period when this may happen have not yet taken place. Additionally, the strong pulses visible in my size-frequency histograms strongly imply a single annual reproductive period. These size-frequency histograms suggest several conclusions about the age structure of trapped fish. First, the Mixture analysis and visual inspection suggested the existence of at least three size classes, and sometimes four to six. If these different size classes represent distinct age classes, then it suggests that lake chub in the warm spring likely live to up to five or six years of age, but that perhaps the majority live for 3-4 years. Given that I could not sample fish <3 cm in length using minnow trapping, there are likely one to two younger age classes as well. Again, these inferences agree with what is known of lake chub from other regions that suggest a maximum age span of 5-7 years (Brown et al. 1970; Scott & Crossman 1973; McPhail 2007). With an accelerated metabolism in the warm water of the springs, Atlin Warm Springs

chub lifespan would probably be proportionately reduced (Gillooly et al. 2001). Second, given the observed infrequency of movement between sites, the site-specific histograms derived from different time periods can generally be assumed to represent the same population at two different points in time. Inspection of size-frequency histograms for the lower region between October 2016 and June 2017 suggests that the lower of the two modes changed from 4.1 - 4.2 cm to 5.3 - 5.4 cm, while the higher changed from 5.9 - 6.0 cm to 6.7 - 6.8 cm. If these each represent a cohort of a consistent population, which the movement data suggest is the case, then this further suggests a mean growth of adult lake chub between 0.7 and 1.3 cm over the 8-month period between October and June, with the growth rate appearing larger at smaller sizes. Fish shorter than 4.3 cm were absent (0% of the population) from the June measurements, and there was a rightward skew towards larger fish in this data set, with 14 fish (12% of the population) longer than 7.5 cm. By comparison, there were 23 fish (19% of the population) shorter than 4.3 cm in October, with only 3 (3%) exceeding 7.5 cm. Fish smaller than 3.2 centimetres in length are absent as these data came entirely from minnow traps, which do not sample smaller fish effectively, and include no dip-netting. This implies, therefore, that over the summer fish grow to become susceptible to minnow trapping and likely represent one to two cohorts of age 0+ (born in early 2016) and 1+ (born in 2015). Based on sexual maturity occurring around 5 cm in fork length, and a mean length at one year being between 4.9 and 5.8 cm, I would infer that between 50% and 75% of the trappable population are sexually mature- critically, this proportion appears to be highest in the May-June mating season.

Unfortunately, I was unable to apply multiple colour codes to be able to individually identify a large number of fish. At Site W, however, I was able to apply rare-coloured tags to three individuals, each of which was easily distinguishable by size (4.5 cm and 1.1 g, 6.6 cm and 3.6 g, and 7.4 cm and 4.7 g). I recaptured one fish with a Site W tag during each of my two return visits to this site. This fish

grew in length from 7.4 cm to 7.6 cm over two weeks and from 4.7 g to 5.2 g, representing a growth rate of 0.015 cm/day and 0.038 g/day, a 0.78% daily increase in body weight. Growing at this rate, if it were sustained throughout the year, would result in 5.5 centimetres of growth annually, which is 3-4 times greater than suggested by the histogram analysis. Consequently, this comparison indeed suggests that reduced food availability between October and April, due to reduced photosynthesis among primary producers, slows growth for a large part of the year, and this would produce annual growth rates more in line with those suggested by the histograms, roughly 1.5 to 2 cm/year.

My data also suggest that there is rapid turnover of the adult population; there was a disproportionate absence of green-tagged individuals (2016 lower region tags) from the 2017 sampling season at the same site. This is not explained by the possibility of large scale tag-loss because after successfully lasting for two or more weeks, the typical time gap between marking and recapturing, subdermal tags remain detectable for 1-2 years (Silver et al. 2009). Mass emigration was also unlikely to be the cause of this loss, due to the extreme lack of movement between regions and the absence of disproportionately large numbers of green-tagged *C. plumbeus* outside of site α .

2.4.4 Conservation implications

The most direct applicability of my data to conservation assessment is their application to the various criteria that COSEWIC uses. These criteria employ population declines and causes of those declines (Criterion A), geographic distribution, population fluctuations, and declines in habitat quality (Criterion B), small and declining populations (Criterion C), small population size, a small number of populations or area of occupancy (Criterion D), or quantitative analyses such as population viability analysis (Criterion E; COSEWIC 2018a). There has not been a sufficiently long period of observation or previous estimates of population sizes of mature individuals to test for a decline in the total number of

mature individuals (Criteria A or C), but my data now allow for more effective monitoring of potential future changes. Local observational accounts preceding my own survey, however, do not suggest a decline over the recent past (S. Badhwar, personal communication; Taku River Tlingit First Nation, personal communication).

Regarding Criterion B, the area of occurrence of the Atlin Warm Springs is only 3,602 m² and this population of *C. plumbeus* is known to live only in two sites: the publicly-accessible Atlin Warm Springs and the separate portion of the warm springs which exists several hundred metres to the west which also contains exotic goldfish (*Carassius auratus*). Therefore, the Atlin Warm Springs chub meet several subcriteria of Criterion B under Endangered (extent of occurrence, index of area of occupancy, number of locations), but there is no evidence of extreme population size fluctuations, extreme fragmentation, or declines in habitat quality needed to invoke the necessary full suite of criteria. Criterion C involves small and declining numbers of mature individuals. The population size of mature *C. plumbeus* in the Atlin Warm Springs may be below 1,500, according to my mark-recapture study. While this estimate does not include fish < 3 cm in size, it does include many fish that are probably not yet mature. Consequently, the population size of mature lake chub in the Atlin Warm Springs is almost certainly below the COSEWIC criterion C1 threshold of 10,000 for Threatened and likely below the threshold of 2,500 for Endangered. Further, if the three regions of the warm spring can be said to be distinct subpopulations, my data suggest that no subpopulation likely contains more than 1,000 mature individuals. This means that the population meets part of the C2 criterion for being designated as Threatened. However, because there is a lack of evidence for any continuing declines in population size, or for extreme fluctuations in population size, the Atlin Warm Springs lake chub does not meet the C1 or C2 criteria. Finally, criterion D addresses a very small or restricted total Canadian population. While the population size is estimated to be perhaps larger than 1,000 mature individuals, failing to

meet criterion D1 (Endangered), the area of occupancy (Criterion D2) is well below the 20 km² threshold for Threatened (i.e., less than a square kilometre). COSEWIC defines 'location' as being a distinct area where a single plausible threatening event can rapidly affect the entire population. Given the small and interconnected nature of the three regions of the Atlin Warm Springs, they likely constitute three at most, and likely only one location. This falls below the alternative threshold for Threatened of ≤ 5 locations under the D2 criterion. Consequently, the Atlin Warm Springs lake chub clearly meet the D2 criterion for Threatened as their extremely limited distribution renders the population vulnerable to human activities and stochastic events which could extirpate or endanger them in a very short period of time (COSEWIC 2018a). There is potential geological instability at the site, and the upper pool is accessible by road and regularly visited by both locals and tourists. There are placer mines in both directions on warm bay road (though none in the springs' drainage area), which bring additional traffic, heavy equipment and large vehicles close to the stream. Coupled with the presence of two exotic species, the Atlin Warm Springs lake chub is highly susceptible to the kinds of stochastic environmental catastrophes that Criterion D (Threatened) addresses.

Efforts to create a model of the population to apply Criterion E are stymied due to lack of data regarding reproductive parameters and juvenile mortality. Finally, COSEWIC's guidelines allow for the potential for the rescue effect to temper conservation assessments. The rescue effect, however, is not applicable to the Atlin Warm Springs lake chub. Sampling of plausible lake chub habitat in the lakes and streams south of Atlin which surround the warm springs failed to detect other *C. plumbeus* populations. Further, the nearest thermal springs lake chub are two populations in the Liard River drainage basin, almost 430 km to the east and across the Continental Divide and they are considered to be a distinct designatable unit by COSEWIC. Under COSEWIC guidelines one DU cannot be rescued by another DU from the same taxon because each DU is, by definition, a distinct 'wildlife species'

under the federal *Species at Risk Act*.

2.4.5 Conclusion

The population of *C. plumbeus* in the Atlin Warm Springs has an estimated population roughly between 1,000 and 2,000 mature individuals, although there is cause to believe that the population may be marginally higher. *C. plumbeus* were found in a variety of habitats throughout their range, from reedy shallows and isolated side ponds, to fast-flowing streams, waterfalls, and pools nearly half a metre deep. These side pools and shallow, marshy areas were observed to be heavily used by juvenile lake chub and may serve as critical nursery areas. A significant positive trend in size was observed when moving downstream. This could result from different growth potential among regions or the movement of fish downstream as they age. All current evidence, however, suggests that this is not a product of such movement; not a single tagged fish was recaptured in a region downstream from where it was tagged. The lack of movement among different regions, and even different individual sites, is one of the major discoveries of this study. This provides valuable insight into the behaviour of the Atlin Warm Springs *C. plumbeus* and provides a useful baseline to inform future conservation efforts and research. While this provides many opportunities and advantages for future research, it also highlights the difficulties in studying this population. Mark-recapture sampling is based around the idea of tagging a sample of the population and allowing it to randomly distribute itself before recapturing, to gain an estimate of the population. As the location of a fish, over time, is now understood to be anything but random, it becomes necessary to place many traps to accurately assess the population. This nullifies one of the major benefits of mark-recapture, namely that it is less labour-intensive than attempting to trap the entirety of the population being studied.

The data that I collected, such as the estimated population, the isolation and the small size of the

inhabited region support the assessment of *Couesius plumbeus* in the Atlin Warm Springs as Threatened under the D2 criterion. Further, COSEWIC procedures allow for modifications of assessment criteria based on the biology of the species in question, and acknowledges that species with low dispersal have an elevated risk of extinction. The thermal isolation of the Atlin Warm Springs lake chub leaves them with essentially no options for dispersal to other appropriately warm bodies of water. Such consequences of such isolation may be exacerbated by the introduction of exotic species, a phenomenon investigated in Chapter 3.

Chapter 3 An Investigation of Abiotic Limits to Expansion in an Exotic Species: Invasive Cherry Shrimp in the Atlin Warm Springs

3.1 Introduction

3.1.1 Invasive Species as a Threat to Native Biodiversity

Exotic species that expand their ranges and pose a threat and challenge to ecosystem conservation worldwide are conventionally referred to as 'invasive' (Hoddle 2004). Human migrations across the world have frequently brought other organisms along with them, often with disruptive results. The degree to which they are a major contributor to the extinctions of individual species remains debated (Gurevitch & Padilla 2004; Clavero & García-Berthou 2005), but the connection between invasions and extinctions is nonetheless well-attested (Miller et al. 1989). Over the last several centuries of human history, the increasing interconnectedness of the world has opened up many new opportunities for species to gain footholds in areas far beyond the range of their natural dispersal abilities. In the most heavily human-impacted parts of North America, invasive species are the second major cause of extinction among freshwater fishes, after physical habitat alteration and ahead of pollution, hybridization, and overharvesting (Miller et al. 1989; Dextrase & Madrak 2006).

Introduced species present novel challenges for native species assemblages, because native species have not had the opportunity to evolve innate behaviours or responses to deal with these new arrivals. This naïveté can be expressed in a variety of ways. Introduced predators can have ruinous impacts on their native prey, which may lack adaptations to defend against novel predation behaviour

(Sih et al. 2010). This is well-documented with the extinctions of many island endemics, particularly with ground-nesting or flightless birds where rodents or cats have been introduced (Biber 2002). Native predators are vulnerable for reciprocal reasons, as their prey species are better able to recognize and evade native predators than invasives (Polo-Cavia et al. 2010). These trends are even more pronounced when the invasive species have no close relatives or analogues in their new environments (Strauss et al. 2006).

Some introduced species, both plant and animal, may have no predators and thus grow in abundance to overwhelm their competitors (Lee 2002). Introduced parasites and diseases can be devastating to populations with no immunity or resistance to them, as evidenced by the decimation of Indigenous New World humans by Old World diseases, or by the ongoing chytridiomycosis epizootic, initially carried by the African clawed frog *Xenopus laevis* and now affecting amphibians worldwide (Skerratt et al. 2007).

Biologically, invasive species represent a reduction of global beta biodiversity (the distinctiveness between different local ecosystems) and contribute towards the homogenization of the world's ecosystems (McKinney et al. 1999; Taylor 2004; Clavero & García-Berthou 2005). Successful invasive species must, by definition, possess competitive advantages over native species, and can thus contribute to their extirpation, and subsequent reductions in alpha (local total) diversity, and by extension gamma (global total) diversity.

Invasive species also present a major economic concern. By disrupting local ecosystems, invasive species may reduce a site's ability to provide ecosystem services (Pimentel et al. 2005; Charles & Dukes 2008; Pejchar & Mooney 2009). Invasives may prey on or outcompete economically important species, or may simply prove to be disruptive to infrastructure. For instance, the Ponto-Caspian native zebra mussel (*Dreissena polymorpha*) has invaded the North American Great Lakes

after being introduced from shipping ballast water (Ricciardi & MacIsaac 2000). By clogging water pipes with their rapid growth, zebra mussels have forced major water users, such as water treatment and power stations, to expend hundreds of millions of United States dollars (USD) in repairs and countermeasures, between 1989 and 2005 (Connelly et al. 2007). Other, more inclusive estimates have posited costs as high as one billion USD annually (Pimentel et al. 2005), although this value is contested (Connelly et al. 2007). Beyond these economic threats, these invasive mussels are also strong competitors, crowding out, starving and smothering native mussels (Ricciardi et al. 1997) and structurally transforming their ecosystems (Strayer and Malcolm 2007).

There can also be positive or nuanced impacts from introduced species. Rogers and Chown (2014) demonstrated that structure forming plants, such as invasive *Acacia* in South Africa's Western Cape, increase species richness and functional diversity in native avian communities. Exotic species have the potential to occupy niches that are not contested by other local organisms, thus forming a net contribution and adding complexity to their new, novel ecosystems (Hobbs et al. 2006).

Canada is a large country possessed of a great deal of freshwater habitat, and perhaps unsurprisingly is also subject to many invasions of species into fresh water. Sea lampreys (*Petromyzon marinus*) and alewives (*Alosa pseudoharengus*) are two well-known invasive vertebrates that have used human-constructed canals to invade previously inaccessible regions such as the Great Lakes, and their arrivals added additional stresses to a system already suffering heavily from pollution and overfishing (Christie 1974). Invasive species are considered a significant threat, second only to habitat loss and degradation, to Canada's freshwater fish biodiversity (Dextrase & Mandrak 2006).

In British Columbia, a variety of introduced freshwater fishes can be found (Taylor 2004, 2010; Nowosad & Taylor 2012). Largemouth bass (*Micropterus salmoides*) are predators of smaller fish and their fry, as well as the bearers of exotic parasites. Yellow perch (*Perca flavescens*) are invasive in

Vancouver Island and the Thompson-Nicola region, common carp (*Cyprinus carpio*) are invasive in the Fraser River and other basins, while northern pike (*Esox lucius*) have been introduced into the Columbia River watershed, posing a serious threat to Kokanee salmon (*Onchorhynchus nerka*), and to efforts at the re-establishment of salmon runs throughout the Columbia (Rutz et al. 2018). Various crustaceans, such as the rusty crayfish (*Oronectes rusticus*) and the American signal crayfish (*Pacifastacus leniusculus*) have established themselves in non-native water bodies, with the latter being responsible for a significant ecological disruption that led to the transformation of a threespine stickleback (*Gasterosteus aculeatus*) benthic/limnetic species pair into a hybrid swarm (Taylor et al. 2006).

In general, isolated and low-diversity systems may enjoy reduced susceptibility to invasion due to their isolation, but are particularly vulnerable to the impacts of invasion when they do occur (MacDougall 2013). Frequently, these vulnerable systems are small islands isolated by large stretches of water, but in the case of Atlin Warm Springs water temperature is an important factor that strongly separates this small environment from the glacially-fed Atlin Lake, which lies 750 metres downstream (Figure 1.3), and the Yukon River system as a whole. With relatively warm temperate conditions amidst subarctic boreal surroundings, the warm springs are host to several organisms that live in conditions that are only duplicated in a small number of similarly-isolated areas in British Columbia, separated by hundreds of kilometres. It is because of this that the arrival of the exotic cherry shrimp (*Neocaridina davidi* var. *red*) in the Atlin Warm Springs is of such interest and concern.

3.1.2 Introduction to the Cherry Shrimp

Neocaridina davidi var. *red*, commonly referred to as cherry shrimp, are freshwater shrimp belonging to the family Atyidae (Figure 3.1). While native to Taiwan, they have spread across the world in the

ornamental pet trade, being very resilient to the rigors of transportation, and proving capable of reproducing in captivity with little effort from aquarium-keepers (Pantaleão et al. 2015). Both of these factors contribute to *N. davidi*'s capacity for invasiveness. *Neocaridina davidi* and its congeners have been recorded as invasive in Hawai'i (Ray 2005), Germany (Chucholl 2016), Turkey (Turkmen & Karadal, 2012), and Japan (Saito et al. 2016; Mitsugi et al. 2017), giving ample precedent for their new invasion in northwestern Canada.



Figure 3.1 Cherry shrimp (*Neocaridina davidi* var. *red*) collected from Atlin Warm Springs on August 1, 2016. On the left is a dorsal view of a male, on the right is a lateral view of a gravid female. Both are approximately 20mm in length.

N. davidi grow to approximately two centimetres long, and have a variety of dull shades of crimson colouration, although a wild-breeding population will tend to revert back to a more drab, wild type appearance. They typically feed on meiobenthos, algae and biofilms, and spend most of their time holding onto plants or resting in the bottoms of streams and pools, although they are quite capable of swimming rapidly through the water when prompted (Weber & Traunspurger 2016).

3.1.3 Discovery and Presence in the Warm Springs

In December of 2015, a population of cherry shrimp was discovered in the Atlin Warm Springs (A. deBruyn, personal observation; Figure 3.2). Photographic and observational records from October 2015 and earlier in 2008 (Taylor, personal communication) failed to indicate the presence of this species, thus placing their likely date of introduction into the two month period between October and December 2015.

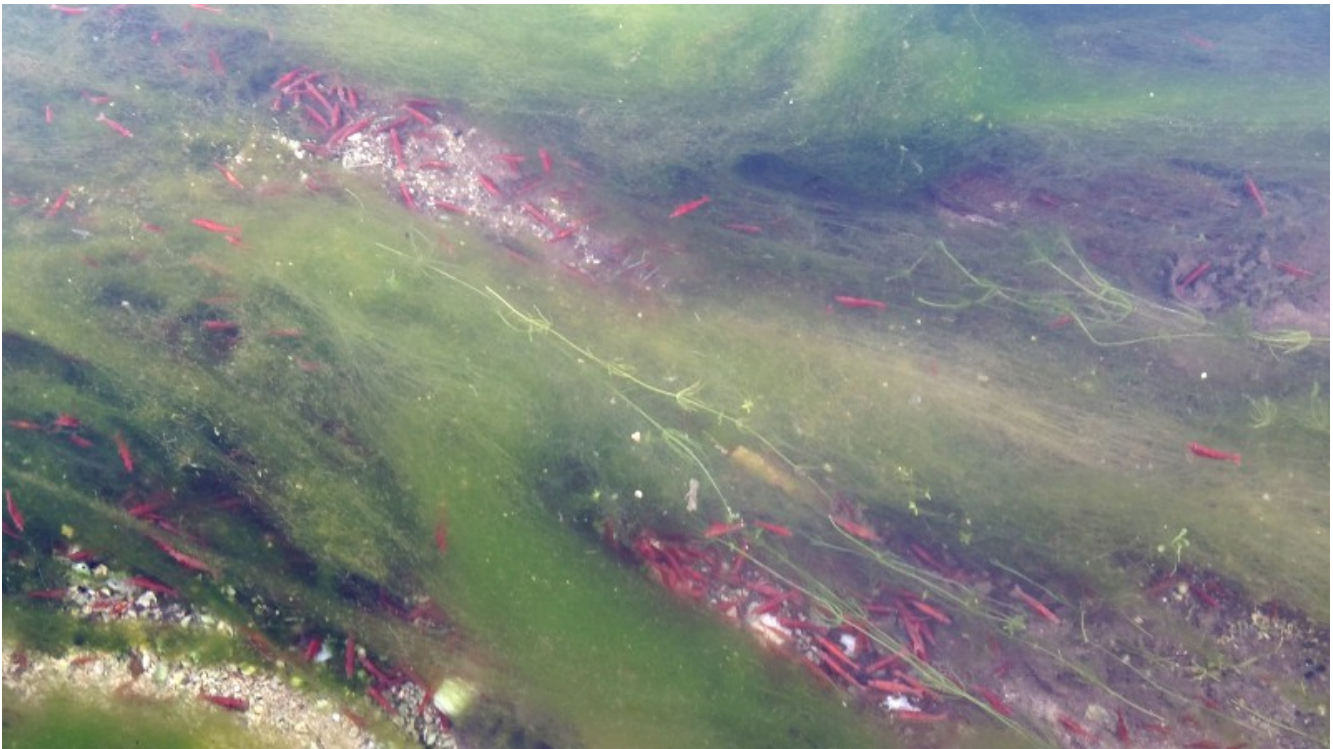


Figure 3.2 *N. davidi* var. *red* clustering in the algae and stream bottom of the Atlin Warm Springs complex, May 20, 2016.

Our current understanding is that the *N. davidi* arrived through the contents of a tropical aquarium being released into the waters of the warm springs. One piece of evidence for this is the discovery of brightly-coloured blue and green treated aquarium gravel in the upper reaches of the warm springs complex, immediately downstream from the upper pool (A. deBruyn, personal observation).

This was close to the centre of the area that had been populated by the *N. davidi* at the time of their discovery. Additional support for this means of introduction comes from the residents living nearest to the warm springs (S. Badhwar, personal communication), and the fact that *N. davidi* were available for purchase at the nearest pet store in Whitehorse, Yukon. After a conversation with the proprietor, they opted to cease stocking *N. davidi*, in hopes of preventing further invasions. Unfortunately, by March of 2019 this store was once again selling cherry shrimp, renewing the risks to other thermal springs in the Yukon, Alaska and northern British Columbia. Releases of red *N. davidi* from the aquarium trade have been reported several times in Europe and Hawai'i (Ray 2005; Jabłońska et al. 2018).

Mykles & Hui (2015) suggested that *Neocaridina* shrimp can comfortably tolerate water temperatures up to 30°C and pH between 6.5 and 8, with optima between 22°C and 25°C, and between 7.0 and 7.5 pH. These values suggest that the conditions in the upper regions of the Atlin Warm Springs, which have a slightly basic pH range between 7.5 and 8.0, and have temperatures between 21°C and 27°C, are close to optimal for *N. davidi* survival and reproduction (Panteleão et al. 2015).

Since their arrival in the Atlin Warm Springs, the *N. davidi* have expanded their range steadily downstream and increased in observed abundance (A. deBruyn, personal observation). Generation times in the genus *Neocaridina* are short; juveniles can reach reproductive size and age in 3 months (Panteleão et al. 2017). Once sexually mature, females can reproduce at a rapid rate, producing clutches of 20 to 30 eggs that will hatch in 2 to 3 weeks, and being able to produce new eggs almost immediately after hatching the previous ones (Panteleão et al. 2017). As the Atlin Warm Springs maintain near-optimal abiotic conditions for the cherry shrimp year-round, a single breeding pair has the capacity to grow to a seven figure population within a year.

While no methodical survey of the cherry shrimp population has yet taken place, estimates generated by combining observed shrimp densities with the size of the area known to be occupied by

these recent invaders suggests that the population has grown into the hundreds of thousands. This number is consistent with geometric growth based on the work of Panteleão et al. (2017). Three years since their first observation, the cherry shrimp appear to be firmly entrenched in the upper warm springs (Figure 3.3).

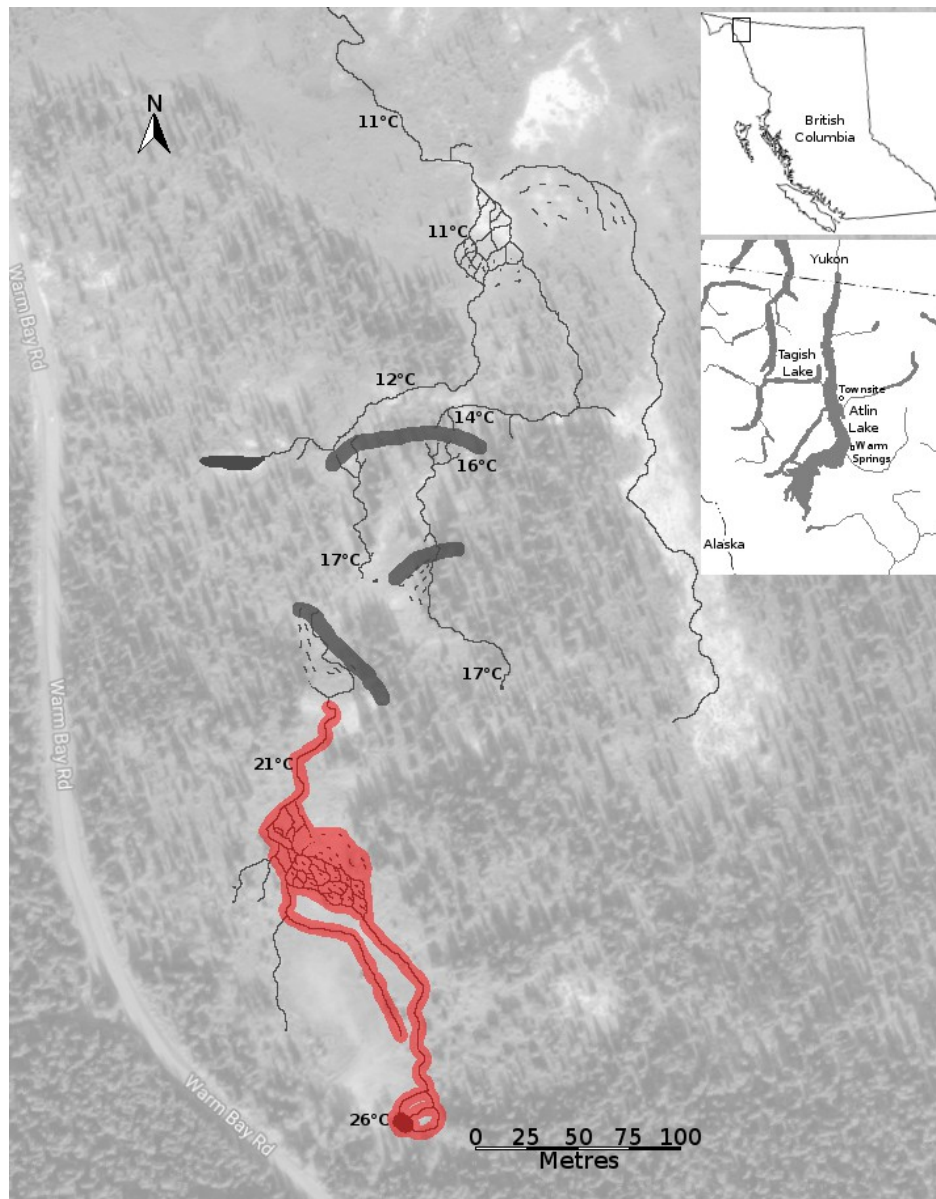


Figure 3.3 *N. davidi* var. red range, shown in red, and wintertime water temperatures in the Atlin Warm Springs, recorded January 3, 2018. Thin lines indicate water, while broad grey lines indicate natural barriers and abrupt changes in elevation which obstruct movement and disrupt surface flow. Insets show the position of the warm spring relative to Atlin Lake, and position of the study area in northwestern British Columbia. Satellite Imagery ©2018 DigitalGlobe ; Map Data ©2018 Google.

At the time of writing, *N. davidi* has failed to expand further downstream than the uppermost, warmest region, where temperatures do not drop below 20°C. Further downstream, the temperatures steadily dwindle as the source of warm water becomes more distant. The outlet of the Atlin Warm Springs is approximately 750 metres downstream from the source pool, where the stream discharges through a culvert into Warm Bay in Atlin Lake. While the Atlin Warm Springs themselves exhibit mild water temperatures year-round, Atlin Lake is the coldest body of water in the Yukon River system. Atlin Lake is fed directly by the Llewellyn Glacier, part of the Juneau Icefield, and reliably freezes for several months each winter (A. deBruyn, personal observation). Atlin Lake is British Columbia's largest natural lake, at roughly 791 square kilometres in area, with water temperatures in the main body of the lake ranging between 0-5°C throughout the year, although isolated and shallow bays can have surface temperatures as warm as 12°C (Gilbert et al. 2006). Warm Bay is, as its name suggests, the warmest part of the lake, and the only part that remains open in the winter (A. deBruyn, personal observation). While the upper springs are comparable to the temperate conditions to which these shrimp are native, Atlin Lake is well below their known tolerated range (Panteleão et al. 2017), and any direct introduction to the lake would likely fail due to the difference in temperatures. Due to the gentle gradient provided by the warm springs, with steady changes in temperature spread across hundreds of metres of stream, however, the potential for acclimation to colder temperatures exists, providing *N. davidi* with a mechanism to further expand their range. There is a possibility that this temperature gradient provides a region where selection for increased cold tolerance could take place (Lee 2002), or possibly facilitate the development of plasticity in *N. davidi*'s thermal tolerance range. Rapid adaptation is an oft-noted feature of emerging invasive species (Prentis et al. 2008) (Sakai et al. 2001), making research during this early phase of the *N. davidi* invasion particularly relevant.

The question of the ultimate limits to *N. davidi*'s downstream expansion is thus of great concern

to biodiversity managers of the Yukon and British Columbia governments, and to the Taku River Tlingit First Nation. If the cherry shrimp were able to enter and persist within Atlin Lake, they could impact its ecology and potentially the ecology of the entire 833,000 km² Yukon River drainage basin. Atlin Lake is a major part of the southern lakes district, a complex of long, thin post-glacial lakes that stand at the headwaters of the main branch of the Yukon River. This concern motivated my investigation into the responses of this population of cherry shrimp to temperatures at the lower extreme of their tolerance range. Consequently, the goal of this chapter was to conduct a manipulative experiment to test the degree to which the cherry shrimp could tolerate cold and declining water temperatures. I designed an experiment that would measure the *N. davidi*'s behaviour and survivability in a slowly cooling environment, starting off near their optimal temperature, and ending up with conditions comparable to those found in Atlin Lake.

3.2 Methods

3.2.1 Collection and Transportation of Cherry Shrimp

I sampled 100 *N. davidi* from the source pool at the south end of the upper region of the Atlin Warm Spring using handheld aquarium nets. These samples were then placed into a plastic container containing food (Nutrafin Max *Spirulina* algae tablets), plants from the warm springs and an air bubbler. To minimize the chances of accidentally providing a vector for their expansion beyond the first cascade, I made sure to not step in *N. davidi*-inhabited water before stepping into uninvaded areas and disinfected my equipment after using it in the upper region. After being driven back to Whitehorse, Yukon, the shrimp were placed into a small plastic aquarium, where they were stored for several days before being sorted into 50 mL falcon tubes and transported to a 5 litre aquarium in Vancouver. Prior to the experiment, they were kept in aerated, dechlorinated water at a temperature of 20°C and fed half of

a Nutrafin Max *Spirulina* tablet daily.

3.2.2 Experimental Apparatus and Procedure

I built four floating tanks, 30 centimetres by 16 centimetres in dimension, and filled them to a depth of 10cm with fresh water. Each tank was a simple plastic box with semicircular segments of foam attached with waterproof epoxy to each side for stability and buoyancy. I furnished each tank with an artificial plant for habitat enrichment, structure and shelter. Each tank container held 20 adult-sized *N. davidi*, which were fed one quarter of a *Spirulina* tablet per tank weekly. Each tank was given a daily water change and a weekly cleaning to maintain water quality and provide oxygenation.

These tanks were placed into a series of recirculating temperature baths, maintained at a constant water temperature of 26.5°C by 150 watt aquarium heaters. One tank was left at a steady temperature of 26.5°C to serve as a control, and the other three were to be steadily lowered in temperature. By restricting the flow of warmed recirculating water into the system and turning down the heaters, I was able to vary the temperature from its elevated starting point of 26.5°C down to the room's ambient air temperature, which was typically between 16°C and 17°C. The next steps of cooling were conducted by steadily introducing more of the facility's cold water supply into the temperature baths, chilling them down to 9°C. Subsequently, a flow-through water chiller was applied to the system, which was able to lower temperatures down to 4°C. The average daily change in temperature was -0.5°C over the course of a six week experimental period.

On each weekday between March 16 and April 27, 2017, at a time between 10 AM and 4 PM, each tank was observed. The number of cherry shrimp remaining alive was determined with a visual count and confirmed by a photograph, in case the shrimp were mobile. The population of each tank was subjected to behavioural observation, over 5 minutes during each tank inspection, where the cherry

shrimp were scored based on their activity, and on their responsiveness to a touch stimulus. These observations were quantified by a scoring system that summarized activity by numbered categories. A '3' indicated visible movement (twitching, feeding, crawling or swimming) in 67-100% of the surviving population, a '2' indicated movement in 34-66% of the population, a '1' indicated movement in 1-33% of the population, and a '0' indicated no visible movement. For responsiveness, the categories were as follows: a '3' indicated swift reactions to a metal probe, with rapid movement near the limit of their observed capabilities before being touched, a '2' indicated sluggish or slow reactions, a '1' indicated responsiveness only after physical contact, and a '0' indicated no response even when physically prodded with a metal probe. When cherry shrimp in the same tank displayed multiple levels of responsiveness, an average score was applied based on all observations.

3.2.3 Statistical Analyses

In order to determine the effect of temperature on cherry shrimp mortality, I subtracted the number of cherry shrimp alive in each experimental tank from the number alive in the control tank at the end of each temperature treatment period. Under the null hypothesis of no effect of declining temperature, I expected the mean of these comparisons to be 0 and tested this expectation with a one sample *t*-test. This comparison was made at two different time points, once at the end of the period during which the temperature was between 9°C and 15°C, and again after the temperature was held between 5°C and 4°C. The behavioural observations were made across multiple time periods so for each treatment period, I used the value for each experimental tank averaged across all observations (i.e., $N = 3$ each for the 9-15°C and $\leq 5^\circ\text{C}$ experimental treatments). I used one sample *t*-tests to assess the significance of the difference in activity and responsiveness scores in the cooled tanks compared to the mean value observed in the control tank.

3.3 Results

3.3.1 Experimental Temperature

There were several practical difficulties that were encountered regarding water temperature control. Due to constraints of laboratory layout, the three tanks had to be cooled in a separate system from the control. Difficulties in getting this system to its proper temperature led to the three manipulated treatments spending the first ten days at temperatures between 16°C and 18°C, rather than at 26.5°C. After the tanks were raised up to their proper temperature and allowed to acclimate for one week, I began lowering the temperature at a rate of roughly 1°C per day. This proceeded until the temperature reached 9°C, at which point it became difficult to lower the temperature further. Bags of ice melted away before they could be replaced the following day, and thus provided an inconsistent method of cooling. The refrigeration coil proved insufficient to cool the water of the main reservoir in the recirculation system, causing the water temperature to repeatedly rise back to near-room temperature. To resolve this, a flow-through water chiller was installed, which allowed for the temperature to be lowered beyond 9°C, and down to 4°C, to allow for the completion of the experiment. Consequently, rather than having a smooth decline in temperature, the temperature plateaued at several points, then dropped at speeds roughly twice the value that had been initially intended, 0.5°C per day (Figure 3.4). Additionally, the failure of ice-cooling caused the temperatures to rise from below 10°C to nearly 15°C for a week, late in the experiment.

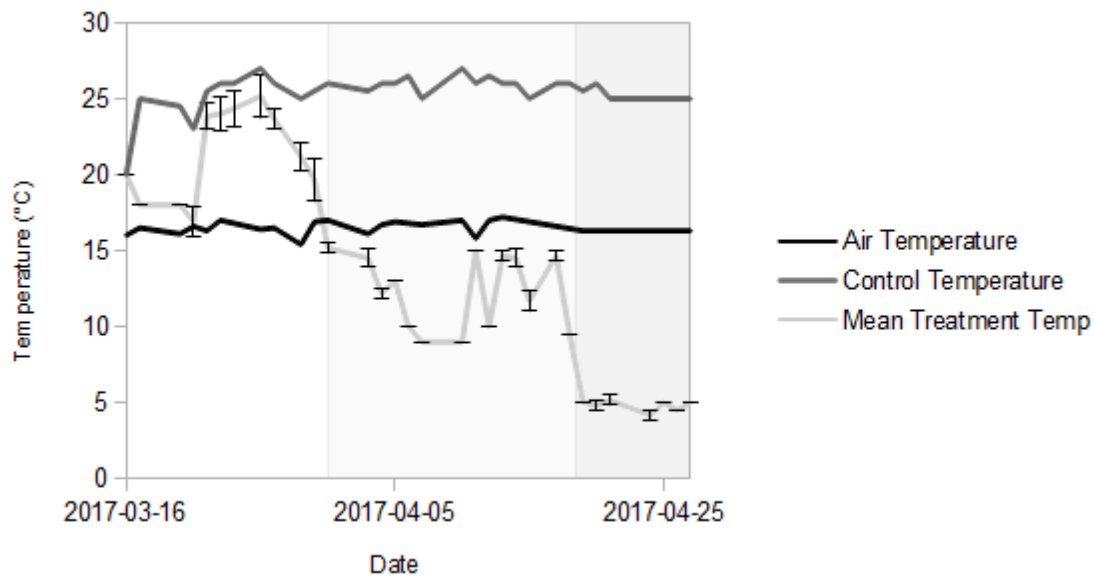


Figure 3.4 Temperatures of control and manipulated experimental tanks over time, compared to ambient air temperature, highlighting three distinct periods of time: the start of the experiment (unshaded), the 'mild period' between 15°C and 9°C (lighter grey), and the 'cold period' below 5°C (darker grey). The treatment tank temperatures are the means ($\pm 95\%$ confidence intervals) across the three tanks.

3.3.2 Survivorship

Mortalities remained low (less than an average of one individual per container) during the acclimation period, both in the cooling tanks and the control tank. Once temperatures dropped below 9°C the number of *N. davidi* surviving began to decline sharply (Figure 3.5). Even after spending a week at 4°C, nearly as cold as Atlin Lake's summer conditions, however, no container had lost more than 40% of its starting population, and mortality evened off.

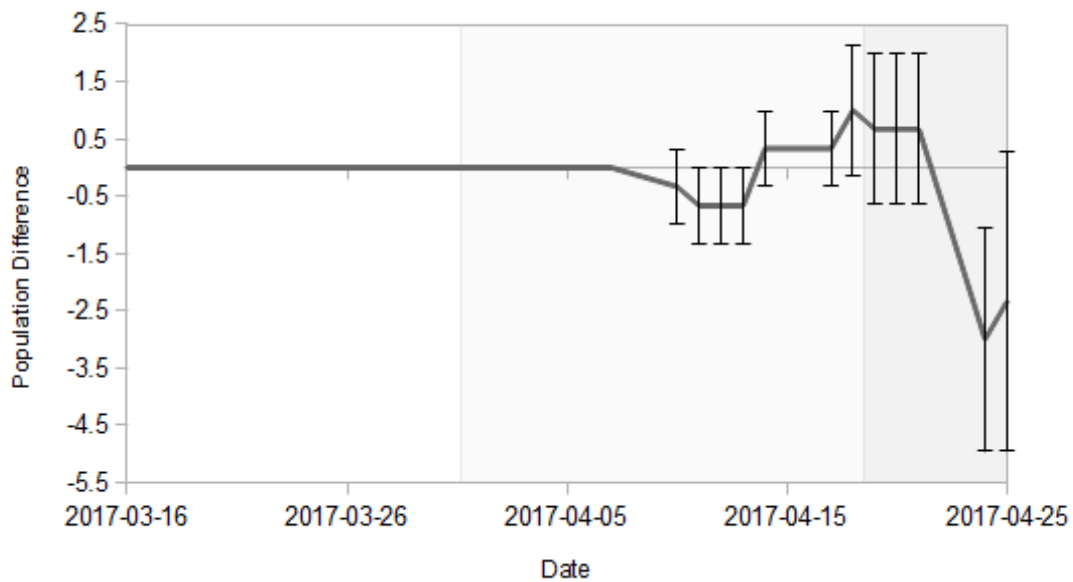


Figure 3.5 Difference in survivorship of *N. davidi* var. *red* during cooling experiment. Shown are the mean ($\pm 95\%$ confidence intervals) of the differences between survival in the treatment tanks and the control tank. No shading indicates the acclimation time period of the experiment, light grey shading indicates the time period when water temperatures were lowered from 15°C to 9°C and, and darker grey shading indicates the period when water temperatures were lowered to below 5°C. Each control (N = 1) and replicate tank (N = 3) was initially stocked with 20 adult shrimp.

Through the cooling experiment, I observed a trend of increasing mortality at temperatures below 5°C (Figure 3.5). There was no difference in the number of cherry shrimp alive at the end of the 9°C and 15°C treatment relative to the control (both means of 19.6), but there was an average 1.1 (SD = 1.5) more shrimp dead in the treatment tanks than in the control tank when the water temperature was below 5°C ($t = -1.3$, $df = 2$, $P = 0.32$).

3.3.3 Behaviour

Overwhelmingly, *N. davidi* were motionless during my observations both in the control tank and during the 15-9°C treatment (both means ≈ 0.0). During the $< 5^\circ\text{C}$ treatment period, mean activity scores were higher in the control than in the treatments, 0.86 vs. 0.0, but as there was zero variance observed

among the treatment tanks, I could not test this statistically.

Qualitatively, while little difference in responsiveness was observed between 26.5 and 15°C, beyond a slight reduction in 'skittishness', where *N. davidi* would become less prone to flee from movement or vibrations, below 15°C the cherry shrimp exhibited less and less movement and responsiveness to stimuli, ultimately becoming torpid to the point of near-immobility at 4°C. Water temperature had a significant impact on the ability of cherry shrimp to respond to stimuli. *N. davidi* maintained high responsiveness scores throughout the experiment in the warm control, which remained between 23°C and 27°C (Figure 3.5). Cherry shrimp in the treatment tanks, however, began to decline in responsiveness score once temperatures fell below 15°C and responsiveness was significantly lower than the control value (Figure 3.8, 1.3 vs 2.9, $df = 2$, $t = -40.9$, $P = 0.0006$). During the $< 5^\circ\text{C}$ treatment, the mean responsiveness score across the treatment tanks was lower than the control value (0.64 vs. 3.0, $t = -33.0$, $df = 2$, $P = 0.0009$).

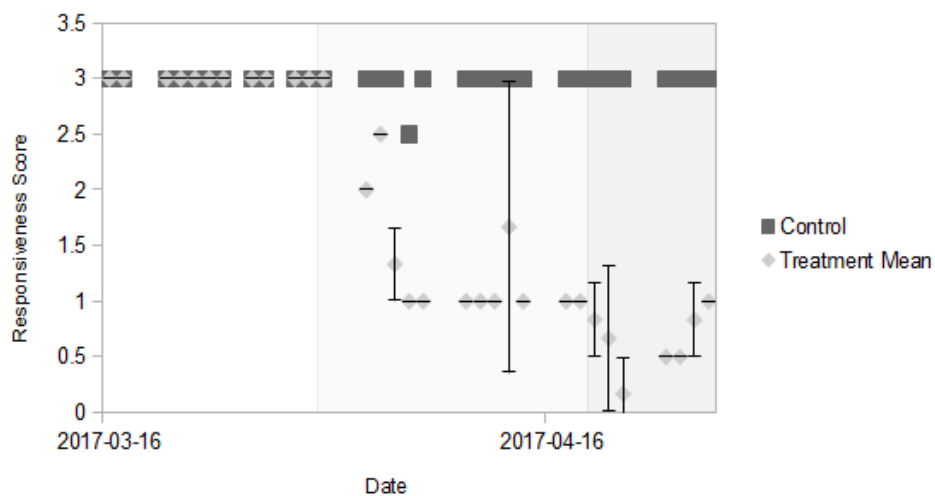


Figure 3.6 Change in responsiveness score of *N. davidi* var. *red* during temperature experiment. Error bars represent 95% confidence intervals of the responsiveness scores of the three replicates. No shading indicates the acclimation time period of the experiment, light grey shading indicates temperatures between 9°C and 15°C, and darker grey shading indicates temperatures below 5°C. Each control and replicate tank contained 20 adult shrimp. Some points have 0 confidence interval as each value was the same at that time period across the three tanks.

3.3.4 Juvenile Development, Growth, and Survivorship

Unexpectedly, female cherry shrimp gave birth to young during the experiment. Throughout the observation period, juvenile *N. davidi* exhibited linear growth (Figure 3.6). Once the temperatures of the treatments were reduced below 15°C, however, the juveniles began experiencing elevated mortality. In many cases, due to their small size, it was very difficult to find their bodies amidst the biological debris in the containers, but in several instances I found dead, but intact bodies. No juveniles survived for more than 2 days at 10°C or lower (Table 3.1).

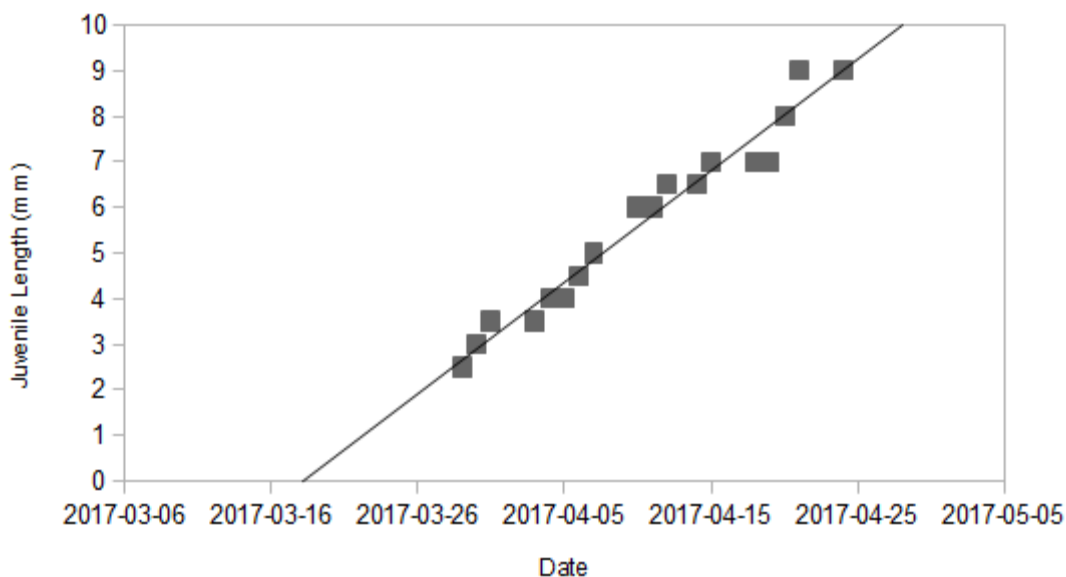


Figure 3.7 Maximum length, in millimetres, of juvenile *N. davidi* var. *red* during the experiment, when water temperatures were > 5°C. Grey line indicates linear best fit.

Date	Control	Exp.	Exp.	Exp.	Control	Exp.	Exp.	Exp.
	Tank	Tank 1	Tank 2	Tank 3	Tank	Tank 1	Tank 2	Tank3
03/27/17	26.0	24.0	24.0	23.0	NP	NP	NP	NP
03/29/17	25.0	21.0	22.0	20.5	+	+	NP	NP
03/30/17	25.5	19.0	18.5	21.0	+	+	+	NP
03/31/17	26.0	15.0	15.0	15.5	+	+	+	NP
04/03/17	25.5	14.0	14.5	15.0	+	+	+	NP
04/04/17	26.0	12.0	12.0	12.5	+	+	+	+
04/05/17	26.0	13.0	13.0	13.0	+	+	+	+
04/06/17	26.5	10.0	10.0	10.0	+	+	-	-
04/07/17	25.0	9.0	9.0	9.0	+	-	-	-

Table 3.1 Water temperature (°C) and *Neocaridina davidi* var. *red* juvenile presence in temperature experiment. The 'NP' means that no juvenile *N. davidi* were present, a '+' indicates that juveniles were present and alive, a '-' means that no juveniles were present, but none were alive.

3.4 Discussion

3.4.1 Thermal Tolerance

My results indicated that adult *Neocaridina davidi* did not die en masse from a week of extreme cold temperatures, nor do they die from a month-long exposure to temperatures well below their range of preference (Mykles & Hui 2015). When water temperature dropped below 5°C, however, roughly one third of the populations perished. This may indicate a certain degree of variation in the cold-hardiness of the shrimp, and suggests that the adults may be able to survive in conditions as cold as those found in many shallow shoreline portions of Atlin Lake.

In order to successfully invade, however, survival alone is not enough. My qualitative observations demonstrated that *N. davidi* became steadily less responsive at low temperatures, losing their caridoid escape reaction response (colloquially known as 'lobstering') to sudden sources of stimulus, such as the vibrations from a dropped metal probe and becoming more tolerant of being touched by the same probe. Below 10°C, these cherry shrimp moved only sluggishly when actively

pushed around by a probe, if at all. For context, native fairy shrimp (order Anostraca) remain active and responsive even in 4°C water (A. deBruyn, personal observation). Even if a group of adult cherry shrimp were able to survive in sub-10°C water, such a population would likely be moribund due to a lack of local reproduction, serving only as a population sink reinforced by the immigration of more adults who were able to develop in warmer waters. In the state of torpor caused by these temperatures, it seems very unlikely that *N. davidi* would be able to effectively graze, evade predators, or reproduce. Furthermore, my results indicate that juveniles have a measurably lower tolerance to near-freezing water temperatures, presenting another limiting life stage for *Neocaridina davidi*. Interestingly, there was an observed rebound in responsiveness score after the initial die-offs below 5°C. This may reflect plasticity or individual acclimatization to cooler conditions, or it could reflect a diversity of physiological tolerances, wherein the individual *N. davidi* that had been responsible for lowering the score died off, while the higher-scoring individuals remained alive, thus increasing the treatment mean.

Macroinvertebrates native to subarctic aquatic habitats show a limited ability to physiologically tolerate freezing or near-freezing water temperatures (Irons et al. 1993). Their typical response to such conditions is to migrate to less severe habitats, although some species can survive freezing. An investigation of individual variation in cold tolerance, its possible genetic basis, and the influence of acclimation temperature, time, and rate of temperature change in cherry shrimp to cold tolerance could further explore this possibility (Aarset 1982).

3.4.2 Further Invasion Threat

There remains the possibility that *N. davidi* may be able to adapt to the colder conditions outside of the springs. The Atlin Warm Springs provide an excellent natural temperature gradient, akin to a thermal estuary, a region where adaptation to colder conditions could potentially take place, with no single

extreme jump of temperature required. As there is a survival advantage to be gained from expanding their range into colder waters, *N. davidi* could undergo natural selection for cold-tolerant individuals, or for those with greater developmental plasticity in terms of cold-hardiness. Given the low generation time and fecundity of the cherry shrimp, the Atlin Warm Springs population may have the potential to evolve rapidly to overcome this barrier. It would be wise to continue to test the thermal tolerance of the lower-ranging *N. davidi*, to determine if there are any changes in their ability to withstand cold temperatures over time.

Because Atlin Lake is consistently the coldest body of water in the Yukon River system, with its main body remaining between 0 and 5°C throughout the year (Gilbert et al. 2006), it is a significant thermal barrier to *N. davidi* expansion. Its inhospitality prevents any dispersing cherry shrimp from spreading downriver to other parts of the Southern Lakes region, and to the greater Yukon River basin. Should such a 'thermal wall' be breached, *N. davidi* would be able to gain access to other warmer bodies of water in the system, other tributaries of the Yukon. I have been unable to find specific data on the retention time of Atlin Lake, but other lakes of comparable size and structure (long, thin and post-glacial, over 100 km in length) tend to have minimum retention times of one to three years. It is an outside possibility that a dislodged adult could drift across the lake, and given the burgeoning population of the warm springs cherry shrimp, there could be many opportunities for such an improbable dispersal event to occur. As this would have to happen over a single summer, however, such an event remains unlikely.

I would consider the *N. davidi* invasion to be contained to the waters of the Atlin Warm Springs, at least in the short term and in terms of natural spread. The threat of human-caused further invasions remains possible. The *N. davidi* in the warm springs themselves show no signs of declining or disappearing, beyond a possible seasonal variation, perhaps driven by changes in sunlight and

subsequently reduced availability of food. My laboratory experimentation has, however, demonstrated that the conditions in the only body of water directly connected to the warm springs, Atlin Lake, are inimical to the survival and propagation of the invasive species. Thus, their presence is likely to remain limited to a region of a few hundred metres of warm ponds and streams for the foreseeable future, with *N. davidi* isolated from any other possibly habitable waters in the region.

Further expansion of *N. davidi* by human-mediated invasions remains a distinct possibility, though actions have been taken to minimize the risks. When informed of what had happened, the proprietor of the sole aquarium store in Whitehorse initially opted to cease selling *N. davidi*. Unfortunately, this self-imposed embargo was broken between January and March 2019. This illustrates the strength of our fascination with exotic species, and the challenge of halting the human-mediated spread of exotic species, even when they are known to be locally invasive. There are still accessible and potentially vulnerable sites in the region. The Liard River Hot Springs are a large complex of thermal springs and intervening stream habitats located in the Liard River system in north-central British Columbia. The hot springs complex has a maximum water temperature of about 52°C, but its extensive system of ponds and streams contain many potential habitats with temperatures well within the optimal range of *N. davidi* (Darveau et al. 2012). The springs complex is located right off the Alaska Highway a moderate day's drive about 500 km southeast of Whitehorse and is the focus of a provincial park that receives thousands of visitors year round (BC Parks 2018). Liard Hot Springs contains another designatable unit of *Couesius plumbeus* and it is certainly possible that if cherry shrimp could be introduced to the Atlin Warm Springs, the threat of their introduction to the Liard Hot Springs, or other similarly highly accessible thermal springs in Yukon, is very real.

3.4.3 Proactive Conservation and Eradication Options

Due to the soft substrate, branched and heavily vegetated nature of the Atlin Warm Springs complex, physical removal of the cherry shrimp is not a feasible option. A general anti-arthropod agent, or chemical purge of the springs would inflict serious damage on many other parts of the native invertebrate community, which would be counterproductive. Biocontrol, however, is an option. While introducing a predator of *Neocaridina davidi* var. *red* runs the risk of simply adding one more invasive species to the Atlin Warm Springs, introducing a virulent virus which affects caridean shrimp, such as white spot syndrome virus (*Whispovirus*), could be a potential tool for removing the cherry shrimp (Wang et al. 2011). White spot syndrome virus does originate from the same area, Taiwan, as the cherry shrimp. *Whispovirus* is, however, known to also affect other crustaceans (Lo et al. 1996), which might cause it to become a vastly more problematic invasive species than *Neocaridina davidi* var. *red*. Until such time as a biocontrol agent specific only to cherry shrimp is identified, it may be preferable to avoid taking actions to actively remove this invasion.

Assuming that the cherry shrimp remains in the warm spring at least in the short term, what are the potential impacts of this species on the unique population of lake chub? Given that I observed cherry shrimp in the diets of at least some lake chub, it is conceivable that the cherry shrimp could benefit the lake chub by providing an abundant food resource. Indeed, Schoolmann & Arndt (2018) reported intense predation both by native and exotic species on exotic cherry shrimp in a German stream. Conversely, Weber & Traunspurger (2016) documented significant predation by cherry shrimp on meiofaunal assemblages in experimental populations and suggested that they may disrupt the transfer of energy from microbial communities to macroinvertebrates and fishes. To date, however, there is no evidence of deleterious impacts of exotic cherry shrimp on freshwater ecosystems in nature, but there are few direct studies of its potential impacts (Jabłońska et al. 2018).

The impacts of the introduction of *N. davidi* to the Atlin Warm Springs remain uncertain. I recommend continued field observation of the limits of their expansion downstream, to address the possibility of their natural range growth. A survey of the invertebrate communities in the presently uninvaded regions should also be conducted posthaste, to develop a picture of the biota of the pre-invasion warm springs. Their impact on the *C. plumbeus* of the warm springs also bears investigation. While they are a prey species for adult *C. plumbeus*, they could be harmful in many other ways: damaging egg-laying or feeding habitat, preying on lake chub eggs and newly-hatched fry, outcompeting other prey species, or simply disrupting nutrient flows upon which *C. plumbeus* rely. Close observation of the degree of overlap between cherry shrimp and juvenile lake chub habitat could shed light on these interactions, helping to establish an understanding of how these species will interact in this developing novel ecosystem.

Chapter 4 General Discussion

4.1 Conserving Atlin Warm Springs Lake Chub

Notwithstanding the fact that *Couesius plumbeus* is one of Canada's most widespread freshwater fishes, the species has been little studied, especially over the past 40 years. Attention on this North American endemic in Canada has increased recently owing to an appreciation that some populations reside in rare aquatic habitats, such as thermal springs, and that these populations show traits that appear to be adaptations to these distinctive habitats (Darveau et al. 2012). My research conducted at the Atlin Warm Springs has considerable relevance to those interested in conservation science and supports the recent assessment by COSEWIC of the Atlin Warm Springs lake chub as Threatened. Not only is this site relevant for the study of thermal physiology, and how species can adapt to live under different temperature regimes, it is also an excellent natural laboratory, hosting small populations that are thermally and physically isolated from similar nearby environments. The geothermal nature of these warm springs probably isolates them from the most direct impacts of climate change, and will likely remain largely disconnected from atmospheric temperatures for many years to come. The site has also experienced an apparently successful introduction of a temperate exotic species.

My study generated mean estimates of 1,169 lake chub in 2016 and 1,664 fish in 2017, although uncertainty in the estimate due to variation in the results of repeated surveys suggests that a broader range, such as 1,000 to 2,000 individuals, would be appropriate to use. All of these estimates exclude juveniles that were too small to trap. My qualitative observations and visual surveys lead me to believe that my quantitative estimates are low. It is improbable that I observed almost the entirety of the population during the single visual census that I conducted given the number of overhanging banks, vegetated areas, subterranean streams and other hiding places that exist in the Atlin Warm Springs. My

repeated mark-recapture estimates, however, conducted in two consecutive years both produced values in this general range.

Future experiments conducted in this manner would be advised to use the Schnabel method for generating population estimates, using repeated tagging events in order to better take advantage of the ability to repeatedly resample a site over time. Lincoln-Petersen estimates with Chapman correction, which I used throughout the study, are best suited to single recaptures, and could have produced similar results with reduced experimental effort. Nonetheless, repeated recaptures after single tagging events still enabled me to establish a probable range of expected values for future monitoring, indicating the variability of results one can expect from repeating a population survey at this site, or others like it.

The acquisition of further data will allow for population viability analysis (PVA) with Vortex software (Lacy & Pollak 2018). Such an analysis would allow us to quantify the short term extinction risk in the population, for purposes of setting conservation priorities and determining the immediacy of any threats to the Atlin Warm Springs lake hub. At present, such analysis is difficult due to a dearth of information about reproduction in the warm springs. Extant research about *C. plumbeus* reproduction offers a broad range of fecundity estimates. Bruce & Parsons (1976) reported a mean of 914 eggs, ranging between 214 and 1,540, from a population in Labrador. Brown et al. (1970) observed a higher range from 650-2,500 from a Saskatchewan population which had a larger mean size. Isaak et al. (2003) also supported the dependence of fecundity on size, though none of these papers state a numerical relationship between size and egg production, leaving the best way to model reproduction in the Atlin Warm Springs unclear.

Juvenile survivorship is another important factor to consider when estimating juvenile recruitment. Due to my trapping methods favouring the capture of larger fish, the difficulty of tagging juveniles (they are mechanically challenging to tag and such tags, even if retained, will be difficult to

find on a fully grown adult), this important segment of the population is poorly understood, compounding uncertainties in efforts to model the population.

A final hurdle to overcome before a PVA can be calculated is to determine the carrying capacity of the Atlin Warm Springs. While I have identified several of the organisms that this population of lake chub eats, the amount of prey needed to support their growth and survival remains unknown. As such, the potential carrying capacity of the area, and how close the current population is to that value, remain unknown.

While PVA is not usable at present due to these uncertainties, it may become a useful conservation tool in the future. Conducting springtime observations in April and May would clear up many unknowns regarding reproduction, including what portion of females are reproductive, how many eggs are produced by each female, the hatching and survival rate of these eggs into juvenile and young adult life stages. Additional dip-net studies, focused specifically on juveniles, could contribute to this while also elucidating growth rates and juvenile mortality rates.

Based on my mark-recapture research in 2016 and particularly 2017, it appears that only a small minority of fish move away from their sites of tagging. The lake chub's lack of movement in this environment was one of the major discoveries of this study. I observed no evidence of downstream movement of lake chub. In fact the only inter-region movement detected was in the upstream direction (see Chapter 2). Such restricted movement in fish is not unprecedented, having been observed in multiple species by Gerking (1959). Rather than functioning as a single population, the *C. plumbeus* in the Atlin Warm Springs seem to behave like a collection of semi-isolated, neighbouring populations- perhaps best fitting a metapopulation model. This raises interesting questions that may be pursued in the future: are there distinctions in the behaviour of lake chub in the different regions? Are the different sites genetically distinguishable from one another? How long has this lack of movement existed in the

warm springs, and what factors constrain movement? At any rate, it will be important for any future studies of the population, genetics and behaviour of this population to take into consideration this very low rate of movement between different sites, and the possible causes of this phenomenon. If it is driven by specialization to particular temperature regimes, for instance, it could make the local extirpation of lake chub from a single region into a major loss of local functional diversity and slow down the potential recolonization from neighbouring regions. Alternatively, if this trend is driven by behaviours such as territoriality, recolonization could potentially take place much faster, and disasters befalling a single region of the warm springs would have a less severe effect on the health and long term survival prospects of the population as a whole.

I would infer from the long-term presence of lake chub in the Atlin Warm Springs, which have been present at least as long as living memory in the region (Taku River Tlingit First Nation, personal communication) that the population appears to be at a low risk of extinction from internal factors. The small area of occupancy and low population size, however, renders the warm springs lake chub vulnerable to stochastic catastrophes, both natural and man-made. Humans have had significant physical impacts on the springs, using heavy equipment to deepen the upper pool (S. Badhwar, personal communication), and recreational use runs the risk of contaminating the site with sun screen and insect repellent. Additionally, active placer mining takes place in nearby sites also accessed by Warm Bay Road (Mihalynuk et al. 2001), creating a small potential for chemical contamination and mechanical damage. However, a more fundamental threat is largely outside of human hands. It is conceivable that an earthquake could disrupt the flow of geothermal water to the springs, causing a drop in temperature (Bird & Lamontagne 2015), disrupting the source of heat that distinguishes this small and isolated ecosystem. For these vulnerabilities, I believe that the recent assessment classification as Threatened by COSEWIC is appropriate.

4.2 Threats from Cherry Shrimp in the Atlin Warm Springs

Cherry shrimp (*Neocaridina davidi* var. *red*) have been present in the Atlin Warm Springs since late 2015. Since their initial invasion, they have attracted both local and national media attention (Rudyk 2016). In the three years since their initial appearance, they have spread across the upper region. At present, they occupy down to the first cascade, but have not yet been detected in the middle or lower regions. The locations in which they are present all have temperatures above 20°C. It remains unknown if they have failed to spread further due to the physical barriers between the upper and middle regions, or due to a lack of ability to thrive in the cooler middle region.

In the areas that they have invaded, cherry shrimp have formed dense blankets over the stream bed, often with hundreds of individuals per square metre. They may be responsible for the reduction of wintertime algae presence due to their grazing. Their impacts on the warm springs ecosystem, particularly its flora and invertebrate fauna, remain unstudied. It is unclear whether the presence of cherry shrimp enriches or threatens biodiversity at the Atlin Warm Springs, and the absence of a prior in-depth survey of the site's invertebrates makes answering this question difficult.

Between 2017 and 2018, the population density, based on observational study of the streams, fell during the winter periods. I speculate that, in their rapid population growth, they may have exhausted the local food resources and undergone a population decline due to a lack of nourishment. Cherry shrimp may have become an important part of the diet of the lake chub native to the warm springs. Due to this, even if they are reducing the populations of other local stream invertebrates, it is possible that there is no net negative impact of the cherry shrimp on the lake chub's access to food.

My studies into cherry shrimp survivorship and behaviour with reducing temperatures suggest that it is unlikely that the cherry shrimp could spread from the Atlin Warm Springs into Atlin Lake. Atlin Lake's conditions, typically 0-5°C year-round (Gilbert et al. 2006), lie outside the limits of their

current physical tolerance. The lake's warmest summer temperatures, even in shorelines and shallows, are too cold for cherry shrimp to do anything but rest in a state of torpor. The conditions of Atlin Lake are even more inhospitable for juvenile cherry shrimp, which my study suggests die when their environmental temperature drops to below 10°C. This may be due to limited energy reserves of the smaller-bodied juveniles.

Broadly speaking, cherry shrimp are ill-suited to conditions below 15°C; their responsiveness declined precipitously below this value. Even though adults may survive through periods of very low water temperature, they are motionless and unresponsive in these conditions, while native shrimp of order *Anostraca* remain motile and responsive in water close to the freezing point. This suggests that in sufficiently cold water, cherry shrimp are unable to evade predators, feed, reproduce, or meet any prerequisite of survival, giving them a profound competitive disadvantage against their native competitors. Alternatively, due to their short generation time, and the fact that the streams draining the warm springs cool off slowly, forming a smooth gradient, the warm springs could present a site for evolution and acclimatization, where subsequent generations of cherry shrimp could move steadily further downstream.

It would be prudent to continue to test the thermal limits of the furthest-downstream cherry shrimp in the Atlin Warm Springs, lest the tolerances of the population change, but for now and the immediate future I am confident in concluding that the cherry shrimp will remain within the confines of the Atlin Warm Springs. Even if they are accidentally transported to another nearby body of water, there are no suitable environments in their immediate environs that could plausibly host a breeding population, based on my experimental data.

4.3 Implications for Understanding Warm Springs Lake Chub Life History

Despite being one of the most widespread cyprinid fishes, the life history of the lake chub is not well understood (Scott & Crossman 1973; McPhail 2007). Although my study was not designed to examine life-history *per se*, the intensive trapping and size-frequency data allowed an opportunity to make some reasonable inferences about their life history. Specifically, the higher frequency of large fish and the absence of smaller fish in June samples, combined with observed reproduction of adults as small as 5 centimetres in length and the presence of newborn fry around the same time allowed me to piece together a probable life history for the Atlin Warm Springs lake chub.

Adults mate in May and June, laying eggs in slow-flowing side pools which hatch in 8-10 days (Stasiak 2006). This seasonal mating is what creates the size-frequency pulses that are visible on the histograms, as there are no new *C. plumbeus* being born at other times of the year. These juveniles are too small to be captured in the summer, but by the late fall and early winter (4-5 months) their cohort has grown to a modal size a little over 4 cm in length, large enough to be caught in minnow traps. This is supported by the abundant presence of fish of such sizes in October 2016, and the absence of small fish in June 2017.

Upon reaching one year of age, this cohort has survived its first winter and reached a modal size of approximately 5.5 cm. Based on the observed expression of gametes compared to the sizes of measured *C. plumbeus*, the bulk of these individuals have reached sexual maturity and will reproduce for the first time. Over the course of their second summer, this cohort grows to a size of around 6 cm.

During their second winter and spring, the cohort reaches a modal size of 7 cm. It is almost certain that all individuals are sexually mature at this point, and based on their size are likely to be responsible for the bulk of juveniles that will be born this year.

Beyond this point, at two years old, the interpretation of these size patterns becomes more

difficult. Variation in growth rate have widened the pulse, although the October 2016 data suggest that many *C. plumbeus* die off after their second year, giving a common lifespan of about 30 months. The existence of a long tail, and a few startlingly large individuals beyond 8 cm in length, suggests that a minority of individuals live to three, four or more years in age. Assuming that it was one of the faster growers (perhaps growing 2.5 cm each year, after reaching sexual maturity), the 12 cm, 16.2 g fish captured at Site W on September 3, 2017, may have been 4 or 5 years old.

Based on a late spring breeding season, most surviving juveniles probably reach sexual maturity around one year of age, experiencing high juvenile attrition, but upon reaching adulthood will likely live through two breeding seasons. Beyond 24 months, attrition is high, with the majority of the adult cohort dying in each of the coming years. The existence of a few very large-sized fish suggests that a small minority of the population will continue to grow and breed throughout their lives, enjoying a maximum lifespan of around five years. As cold-water populations of lake chub are noted to have lifespans of three to five years, with outliers living as long as seven (Scott & Crossman 1973; Mansfield 2004), these values are plausible. Given the negative relationship of moderate temperature increases and longevity (Jonsson et al. 1991; Conti 2008) it is to be expected that warm-water populations reduced life spans owing to their accelerated metabolism.

4.3 Future Research at the Atlin Warm Springs

With the completion of this stage of research, a solid background for future research into the lake chub (*Couesius plumbeus*) and cherry shrimp (*Neocaridina davidi* var. *red*) has been established. Detailed information now exists for the size metrics and population of lake chub at the Atlin Warm Springs. Basic facts such as where lake chub can be found, how little they move between sites, and the relationships between areas and sizes, are now well-attested. Information about life history, including

reproductive timings and likely lifespan, are also now reported, albeit to a lesser degree of confidence. Additional research to solidify our knowledge of these topics would be useful.

Cherry shrimp have invaded and established themselves, but do not appear to pose an immediate threat to expand outside of the thermal environment of the warm springs. Their ability to invade temperate water is demonstrated once again, while their inability to thrive and reproduce in colder water has been quantitatively demonstrated.

As with many research projects, several new avenues of study have also been clarified and illuminated. For population research, to take advantage of the multiple site visits I conducted, a series of recaptures with re-tagging (as opposed to a single tagging event) could allow for the use of the Schnabel method, which would allow me to generate a more accurate and confident population estimate, as well as identify violations of my mark-recapture assumptions, with little additional expenditure of time and effort. More broadly, this research had little focus on the interspecies interactions taking place at the experimental site. An ecosystems ecology approach could yield interesting results. Such research might either demonstrate how the warm springs functions as a 'natural laboratory', partially isolated from outside influences, or demonstrate that is much more intimately tied to the surrounding forest and lake than I have previously suggested. The invasive goldfish, and the eastern springs on the other side of the Atlin Warm Springs road, are another potential stepping-off point for future research. The population and status of lake chub in this area remain unknown, and their apparent coexistence with goldfish presents another opportunity to study the impacts of invasive species on native populations.

It is very likely that the year-round warm water present in the Atlin Warm Springs has caused various different thermal adaptations by the local stream invertebrates (Ward & Stanford 1982). Given the recent glacial history of northwestern Canada, it is even more probable that the denizens of the

Atlin Warm Springs bear the marks of recent adaptation, rather than existing merely as relicts from a warmer period. There are likely many unique evolutionary stories hidden in the genomes and physiologies of each of these warm springs residents.

For future research involving cherry shrimp, for field studies I would recommend mapping the extent of their spread and the temperature of the waters they inhabit. Due to practical difficulties with tagging small and relatively short-lived invertebrates, an effective technique of population estimation may come from calculating density and streambed area, which can be done rather easily with quadrat plots and the stream area survey that I conducted.

The Atlin Warm Springs provide a natural window of insight into numerous topical ecological phenomena: adaptation to warmer thermal regimes, behavioural responses to varying habitats and temperatures, and invasion by exotic species, to name a few. This study of lake chub in the Atlin Warm Springs serves as a foundation upon which future research at this site can be built, and a baseline against which future surveys can be measured. It is the first detailed snapshot of this small, isolated population that will in future years continue to generate insight into the structure and strategies of other populations, whether they be in isolated desert streams, thermal springs throughout the world, or insular habitats of any kind, each hosting their own distinct pockets of ecological and evolutionary diversity.

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Appendix

Figure A1. Number of sampling visits in each month between June 2016 and February 2018.

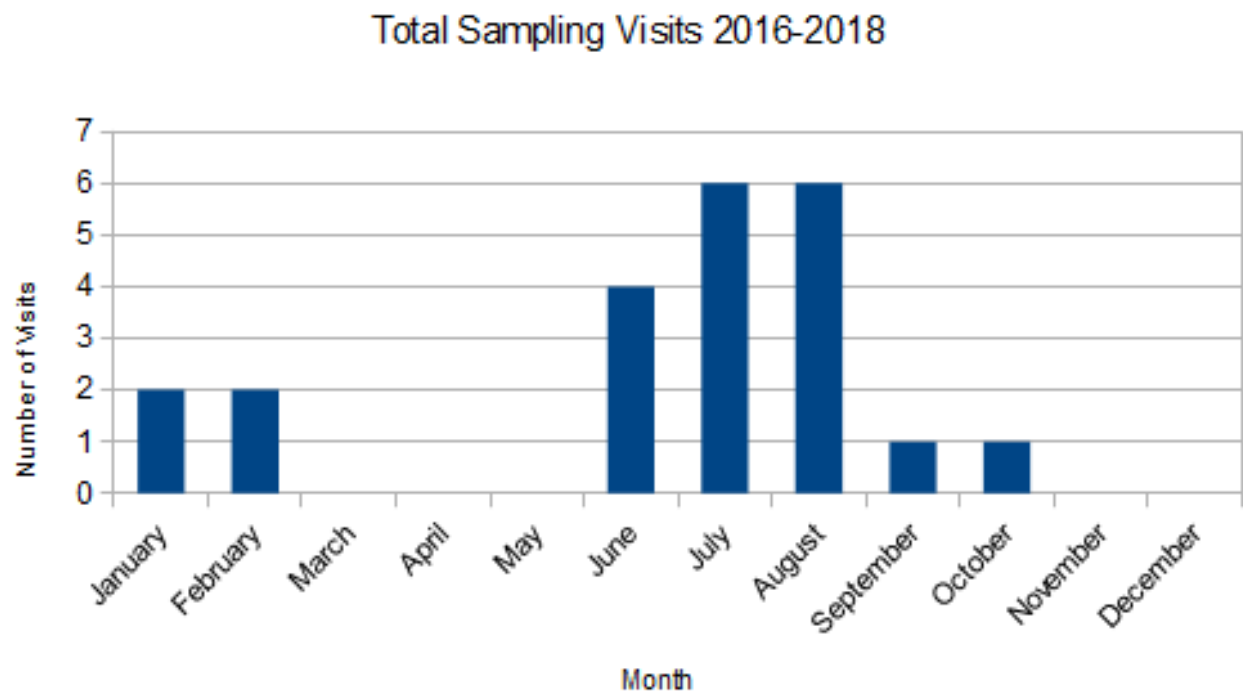


Figure A2. Size (fork length, cm) frequency histograms for lake chub (*Couesius plumbeus*) captured in three regions (Lower, Middle, Upper) of the Atlin Warm Springs complex between June 2016 and January 2018. Curves show the distributions of size classes inferred from maximum-likelihood Mixture analyses.

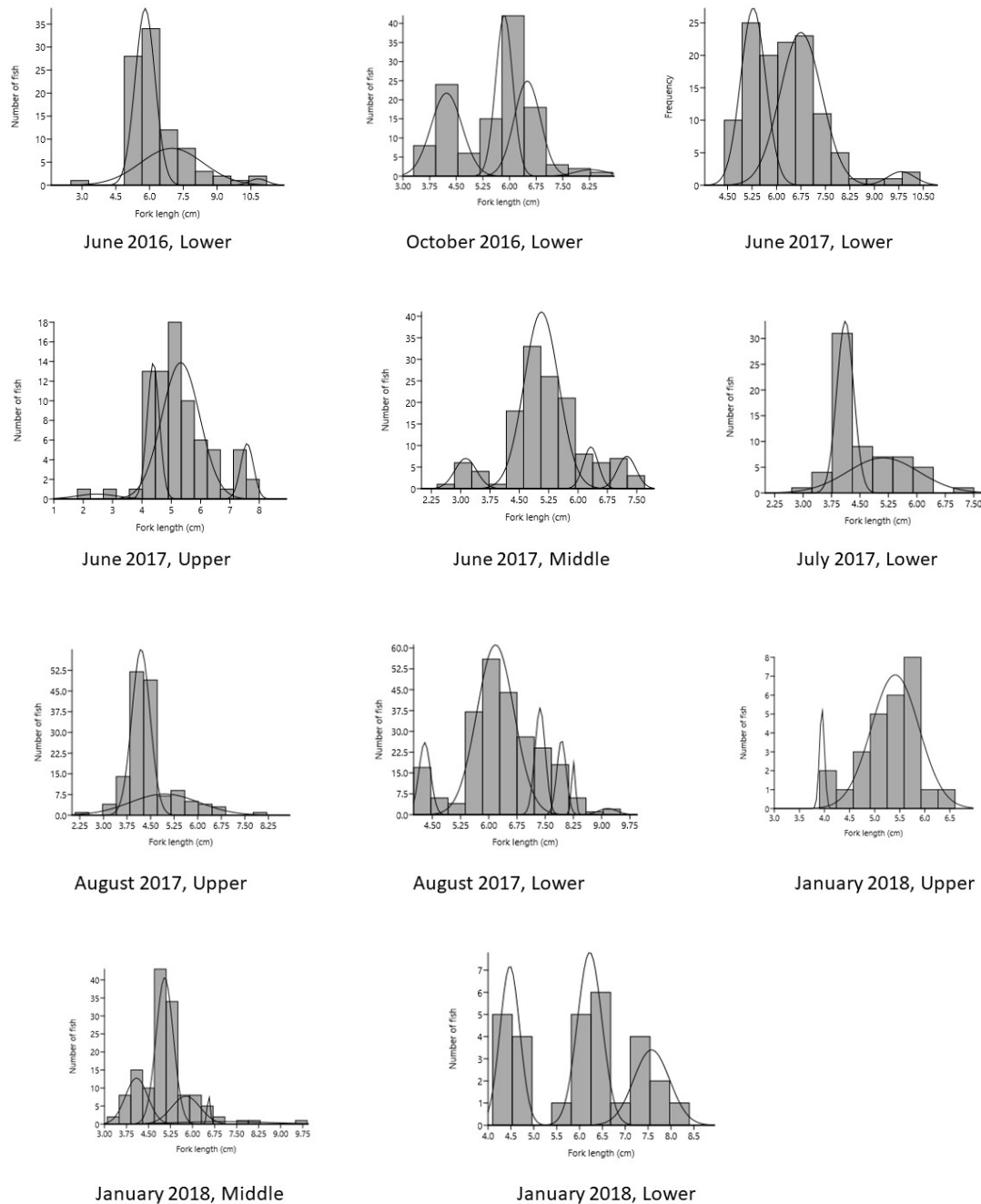


Figure A3. Water temperature at Site E of the Upper Region and air temperature in the meadow near the source of the Atlin Warm Springs from June 15 to August 31 2016.

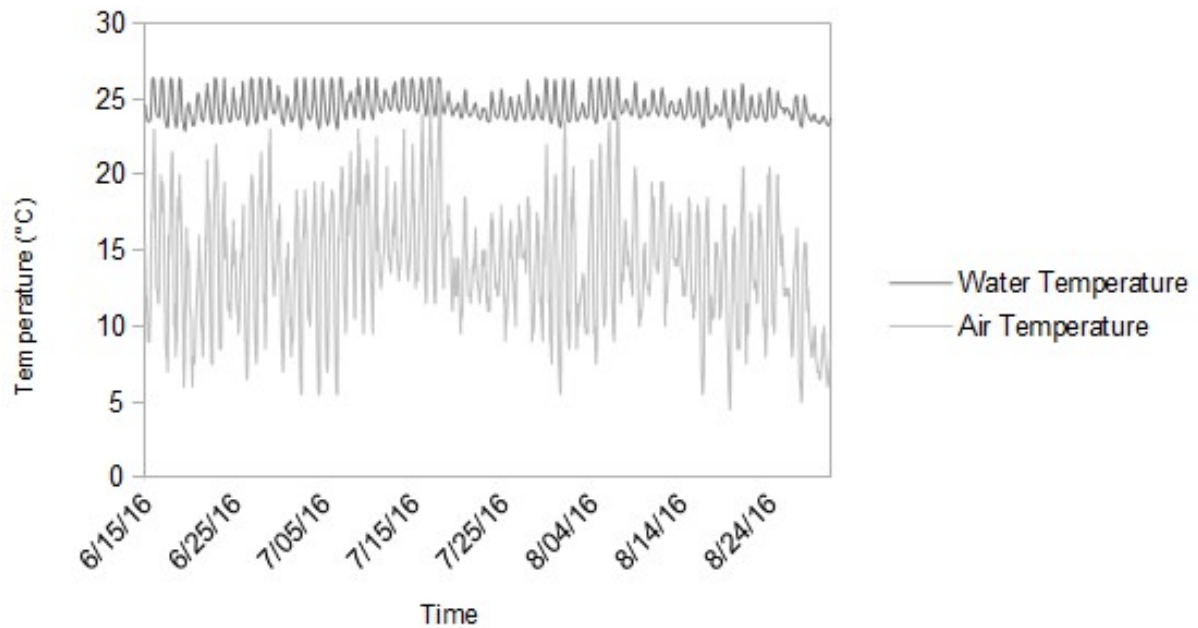


Table A1. Temperature data

Date	Time	Region	Site	Water Temp (°C)	Air Temp (°C)	Notes
2015-12-30		Upper	Top Pool	27	-2	
2015-12-30		Upper	J	26	-2	
2015-12-30		Upper	K Upstream	26	-2	
2015-12-30		Upper	K	25	-2	
2015-12-30		Upper	A	25	-2	
2015-12-30		Upper	B	25	-2	
2015-12-30		Upper	B Upstream	28	-2	
2015-12-30		Upper	A Downstream	14	-2	
2015-12-30		Upper	C	26	-2	
2015-12-30		Upper	D	22	-2	
2015-12-30		Upper	C Upper Tributary	22	-2	
2015-12-30		Upper	C Lower Tributary	23	-2	
2015-12-30		Upper	E	22	-2	
2015-12-30		Upper	F	21	-2	Top of Upper Cascade
2015-12-30		Upper	F Downstream	17	-2	Bottom of Upper Cascade
2015-12-30		Middle	L	17	-2	Start of Western Middle Stream
2015-12-30		Middle	L Isolated Pool	7	-2	No Surface Connection
2015-12-30		Middle	R Side Pool	13	-2	
2015-12-30		Middle	P	19	-2	
2015-12-30		Middle	O	17	-2	
2015-12-30		Lower	T	15	-2	
2015-12-30		Lower	Z	13	-2	
2015-12-30		Lower	Z Upstream	14	-2	Below Western Middle Stream
2015-12-30		Lower	Y	13	-2	
2015-12-30		Warm Bay	Outlet	4	-2	
2015-12-30		Warm Bay	Lake	0	-2	
2016-06-13	1401	Middle	P	22		
2016-06-13	1407	Lower	α	19.5		
2016-06-13	1415	Upper	E	25		
2016-07-04	1458	Upper	E	26	16	
2016-07-04	1509	Middle	P	24	16	
2016-07-04	1515	Lower	α	20	16	
2016-07-18	1352	Middle	S	22		'Southeast Point'
2016-07-18	1403	Lower	Z Upstream	24.8		'Northwest Point'
2016-07-18	1408	Lower	V	22.6		'Low Point'
2016-08-01	1354	Middle	S	21	21.5	'Southeast Point'
2016-08-01	1408	Lower	Z Upstream	22	21.5	'Northwest Point'
2016-08-01	1418	Lower	V	20	21.5	'Low Point'
2016-08-22	1503	Upper	E	25.5	20.5	
2016-08-22	1511	Middle	P	22	20.5	
2016-08-22	1517	Lower	α	20	20.5	
2016-08-30	1513	Lower	α	20		
2016-08-30	1520	Lower	X	14		'Bottom Wetland'
2016-10-10	1339	Upper	E	21		
2016-10-10	1347	Middle	P	20		
2016-10-10	1353	Lower	α	19.2		
2017-01-02	1202	Upper	E	20		
2017-01-02	1218	Lower	α	15		
2017-01-02		Upper	Top Pool	24		
2017-02-26	1058	Upper	E	18		
2017-02-26	1108	Middle	P	16		
2017-02-26	1113	Lower	α	14		
2017-02-26		Upper	Top Pool	26		
2017-06-10	1245	Upper	E	26		
2017-06-10	1255	Middle	P	22		
2017-06-10	1308	Lower	α	19		
2017-06-18	1330	Upper	A	26		
2017-06-18	1345	Upper	B	26		
2017-06-18	1352	Upper	C	27		
2017-06-18	1356	Upper	D	25.5		
2017-06-18	1445	Upper	G	26.5		
2017-06-18		Upper	E	25.5		
2017-06-18		Upper	F	26		
2017-06-18		Upper	H	27.5		
2017-06-18		Upper	I	27		
2017-06-18		Upper	J	27		
2017-06-18		Upper	K	29		

Date	Time	Region	Site	Water Temp (°C)	Air Temp (°C)	Notes
2017-07-03	1325	Upper	A	26		
2017-07-03	1355	Upper	D	24		
2017-07-03	1415	Upper	E	23		
2017-07-03	1440	Upper	F	24		
2017-07-03	1645	Upper	C	25		
2017-07-03	1650	Upper	B	26		
2017-07-03		Upper	G	24		
2017-07-16	1224	Middle	L	22		
2017-07-16	1238	Middle	M	22		
2017-07-16	1244	Middle	N	22		
2017-07-16	1254	Middle	O	20		
2017-07-16	1257	Middle	P	20.5		
2017-07-16	1301	Middle	Q	20.5		
2017-07-16	1307	Middle	R	20		
2017-07-16	1325	Middle	S	20		
2017-08-21	1402	Lower	T	20		
2017-08-21	1517	Lower	V	19.5		
2017-08-21	1539	Lower	W	19.5		
2017-08-21	1552	Lower	X	20		
2017-08-21	1627	Lower	Y	20		
2017-08-21	1642	Lower	Z	20		
2017-08-29	1517	Lower	T	20		
2017-08-29	1600	Lower	α	20		
2017-08-29	1635	Lower	U	20		
2017-08-29	1645	Lower	V	19		
2017-08-29	1654	Lower	W	19		
2017-08-29	1719	Lower	X	20		
2017-08-29	1740	Lower	Y	20		
2017-08-29	1751	Lower	Z	20		
2017-08-31	1514	Upper	D	25		
2017-08-31	1534	Upper	E	25		
2017-08-31	1653	Upper	F	24		
2017-08-31	1708	Upper	G	24		
2017-08-31	1730	Upper	H	24		
2017-08-31	1751	Upper	J	26		
2017-08-31		Upper	A	25		
2017-08-31		Upper	B	26		
2017-08-31		Upper	C	25		
2017-08-31		Upper	I	24		
2017-08-31		Upper	K	25		
2017-09-03	1214	Lower	T	20		
2017-09-03	1241	Lower	α	20		
2017-09-03	1305	Lower	U	19		
2017-09-03	1326	Lower	W	19		
2017-09-03	1351	Lower	X	20		
2017-09-03	1416	Lower	Y	20		
2017-09-03	1432	Lower	Z	20		
2017-09-03	1541	Lower	V	19		
2018-01-03	1245	Upper	E	21		
2018-01-03	1320	Middle	L	17		
2018-01-03	1340	Middle	S	17		
2018-01-03	1455	Middle	O	16		
2018-01-03	1525	Lower	T	14		
2018-01-03	1548	Lower	Z	12		
2018-01-03	1640	Lower	Toad Pond	9		
2018-01-03	1705	Warm Bay	Outlet	4.5		
2018-01-03		Lower	X	11		
2018-01-03		Lower	W	11		
2018-02-25	1447	Middle	S	16.3		Used Higher-Precision Thermometer
2018-02-25	1610	Middle	O	12.3		Used Higher-Precision Thermometer
2018-02-25	1622	Lower	T	13.3		Used Higher-Precision Thermometer
2018-02-25	1700	Lower	Z	11.8		Used Higher-Precision Thermometer

Table A2. Mean number of fish captured at each site per sampling in summer 2017.

Warm Spring Section	Site	N trapping events	Mean catch (+SD)
Upper	A	3	1.0 (1.0)
Upper	B	3	0.0 (N/A)
Upper	C	3	0.3 (0.6)
Upper	D	4	6.0 (4.2)
Upper	E	12	18.8 (27.9)
Upper	F	4	9.8 (11.3)
Upper	G	3	8.7 (5.8)
Upper	H	4	12.3 (3.9)
Upper	I	3	0.3 (0.6)
Upper	J	4	11.0 (6.1)
Upper	K	3	13.7 (21.1)
Middle	L	4	10.5 (6.9)
Middle	M	3	3.0 (2.0)
Middle	N	3	0.3 (0.6)
Middle	O	4	12.8 (3.8)
Middle	P	10	6.4 (5.4)
Middle	Q	3	2.7 (2.5)
Middle	R	3	7.7 (9.9)
Middle	S	6	50.7 (34.8)
Lower	T	4	31.0 (15.4)
Lower	U	3	6.3 (6.8)
Lower	V	3	2.7 (2.9)

Lower	W	3	10.0 (6.2)
Lower	X	3	17.3 (5.9)
Lower	Y	3	3.7 (2.1)
Lower	Z	4	24.8 (25.1)
Lower	α	10	53.5 (40.3)

Table A3. Stream area and volume measurements for the Atlin Warm Spring complex. Volume is recorded in cubic metres, and area in square meters. Measured areas that are outside of the observed range of *Couesius plumbeus* have their area and volume occupied left blank, for ease of calculation.

Region	Stream	Width (m)	Depth (m)	Length (m)	Volume Occupied	Area Occupied	Notes
Upper	Branch 1 (J+K)	2	0.15	15.6			No Fish Above
Upper	Branch 1 (J+K)	1.8	0.3	14.0	7.5	25.2	
Upper	Branch 1 (J+K)	1.3	0.2	13.4	3.5	17.5	
Upper	Branch 1 (J+K)	4.7	0.1	7.9	3.7	36.9	
Upper	Branch 1 (J+K)	2.2	0.25	7.4	4.1	16.4	
Upper	Branch 1 (J+K)	1.3	0.25	8.9	2.9	11.6	
Upper	Branch 1 (J+K)	0.9	0.2	16.1	2.9	14.5	
Upper	Branch 1 (J+K)	2.1	0.1	9.8	2.1	20.7	
Upper	Branch 2 (A)	1.2	0.3	22.0	7.9	26.4	
Upper	Branch 2 (A)	1.6	0.3	19.1	9.2	30.5	
Upper	Branch 2 (A)	0.6	0.4	13.5	3.2	8.1	
Upper	Branch 2 (A)	2.7	0.4	11.7	12.6	31.6	
Upper	Branch 2 (A)	1.4	0.1	17.0	2.4	23.9	
Upper	Branch 2+3 (A+B)	0.8	0.2	14.9	2.4	11.9	
Upper	Branch 3 (B)	0.7	0.05	16.0			No Fish Above
Upper	Branch 3 (B)	2.7	0.1	12.7	3.4	34.2	
Upper	Branch 3 (B)	1.1	0.15	7.8	1.3	8.6	
Upper	Branch 3 (B)	0.7	0.1	6.7	0.5	4.7	
Upper	Branch 3 (B)	0.2	0.1	13.4	0.3	2.7	
Upper	Branch 1+2+3 Above Wetland	0.6	0.15	14.0	1.3	8.4	
Upper	Branch 4 (C)	2.7	0.2	7.4	4.0	20.1	
Upper	Branch 4 (C)	1.5	0.15	13.7	3.1	20.5	
Upper	Branch 5	0.9	0.05	16.7	0.8	15.0	
Upper	Branch 5	0.6	0.1	14.2	0.9	8.5	
Upper	Upper Wetland (H+I)	25.4	0.1	56.0	142.3	1423.4	
Upper	Combined Stream (D+E+G)	0.6	0.1	8.8	0.5	5.3	
Upper	Combined Stream (D+E+G)	0.9	0.15	12.2	1.6	11.0	
Upper	Combined Stream (D+E+G)	0.9	0.15	12.0	1.6	10.8	
Upper	Combined Stream (D+E+G)	1.1	0.2	10.0	2.2	11.0	
Upper	Combined Stream (D+E+G)	1	0.2	14.5	2.9	14.5	
Upper	Combined Stream (D+E+G)	2.1	0.2	11.2	4.7	23.4	
Upper	Combined Stream (D+E+G)	0.7	0.15	9.8	1.0	6.8	
Upper	Combined Stream (D+E+G)	0.9	0.15	19.8	2.7	17.8	
Upper	Combined Stream (D+E+G)	2.1	0.2	15.4	6.5	32.4	
Upper	Combined Stream (D+E+G)	2.2	0.2	6.8	3.0	15.0	
Upper	Step Pool (F)	0.9	0.2	1.8	0.3	1.6	
Upper	Step Pool (F)	2.4	0.2	4.8	2.3	11.5	
		Sum			251.6	1982.5	

Region	Stream	Width (m)	Depth (m)	Length (m)	Volume Occupied	Area Occupied	Notes
Middle	Stream 1 (L+M+N)	2	0.45	4.4	4.0	8.9	
Middle	Stream 1 (L+M+N)	3.2	0.25	5.9	4.8	19.0	
Middle	Stream 1 (L+M+N)	1.8	0.25	5.0	2.2	9.0	
Middle	Stream 1 (L+M+N)	1.6	0.25	4.4	1.8	7.0	
Middle	Stream 1 (L+M+N)	1.7	0.2	3.0	1.0	5.1	
Middle	Stream 1 (L+M+N)	1	0.1	4.1	0.4	4.1	
Middle	Stream 1 (L+M+N)	1.2	0.1	7.2	0.9	8.6	
Middle	Stream 1 (L+M+N)	1.6	0.1	6.8	1.1	10.9	
Middle	Stream 1 (L+M+N)	1.5	0.1	4.4	0.7	6.7	
Middle	Stream 1 (L+M+N)	1.5	0.1	7.7	1.2	11.5	
Middle	Stream 1 (L+M+N)	0.5	0.1	7.0	0.4	3.5	
Middle	Stream 1 (L+M+N)	3.8	0.25	4.4	4.2	16.6	
Middle	Stream 1 (L+M+N)	3.7	0.3	4.8	5.3	17.8	
Middle	Stream 1 (L+M+N)	1.2	0.15	9.3	1.7	11.2	
Middle	Stream 1 (L+M+N)	0.9	0.1	8.5	0.8	7.7	
Middle	Stream 1 (L+M+N)	0.8	0.1	5.6	0.4	4.5	
Middle	Stream 1 (L+M+N)	1.3	0.1	6.1	0.8	7.9	
Middle	Stream 1 (L+M+N)	1.2	0.2	7.2	1.7	8.6	
Middle	Stream 1 (L+M+N)	1.1	0.15	14.6	2.4	16.0	
Middle	Stream 1 (L+M+N)	1.5	0.2	16.6	5.0	24.8	
Middle	Branch 1 (L+M+N) Distributary	0.5	0.1	11.0	0.5	5.5	
Middle	Branch 1 (L+M+N) Distributary	0.5	0.1	10.4	0.5	5.2	
Middle	Branch 1 (L+M+N) Distributary	0.5	0.1	16.2	0.8	8.1	
Middle	Branch 1 (L+M+N) Distributary	0.5	0.1	13.7	0.7	6.9	
Middle	Branch 2 (O+P+Q+R+S)	1.7	0.25	7.1	3.0	12.0	
Middle	Branch 2 (O+P+Q+R+S)	2.3	0.2	6.2	2.8	14.2	
Middle	Branch 2 (O+P+Q+R+S)	1.4	0.15	7.5	1.6	10.5	
Middle	Branch 2 (O+P+Q+R+S)	1.2	0.1	5.8	0.7	6.9	
Middle	Branch 2 (O+P+Q+R+S)	1.9	0.1	6.4	1.2	12.2	
Middle	Branch 2 (O+P+Q+R+S)	1.4	0.1	9.4	1.3	13.1	
Middle	Branch 2 (O+P+Q+R+S)	0.8	0.1	13.3	1.1	10.6	
Middle	Branch 2 (O+P+Q+R+S) East	1	0.1	7.6	0.8	7.6	
Middle	Branch 2 (O+P+Q+R+S) East	4.5	0.2	11.4	10.3	51.3	
Middle	Branch 2 (O+P+Q+R+S) West	0.5	0.05	6.7	0.2	3.3	
Middle	Branch 2 (O+P+Q+R+S) West	2.4	0.3	8.8	6.4	21.2	
Sum					72.4	398.1	

Region	Stream	Width (m)	Depth (m)	Length (m)	Volume Occupied	Area Occupied	Notes
Lower	Stream East (T+α+U+V+W)	3.6	0.25	9.24	8.3	33.3	
Lower	Stream East (T+α+U+V+W)	1.6	0.1	12.12	1.9	19.4	
Lower	Stream East (T+α+U+V+W)	1.8	0.15	8.76	2.4	15.8	
Lower	Stream East (T+α+U+V+W)	1.2	0.1	5.22	0.6	6.3	
Lower	Stream East Side Pool	1.8	0.15	11.22	3.0	20.2	
Lower	Stream East (T+α+U+V+W)	3.6	0.25	18.18	16.4	65.4	
Lower	Stream East (T+α+U+V+W)	4	0.15	11.22	6.7	44.9	
Lower	Stream East (T+α+U+V+W)	0.75	0.15	7.92	0.9	5.9	
Lower	Stream East (T+α+U+V+W)	0.7	0.1	11.1	0.8	7.8	
Lower	Stream East (T+α+U+V+W)	1.6	0.15	10.2	2.4	16.3	
Lower	Stream East (T+α+U+V+W)	1	0.2	6.24	1.2	6.2	
Lower	Stream East (T+α+U+V+W)	0.9	0.1	5.7	0.5	5.1	
Lower	Stream East (T+α+U+V+W)	1	0.1	7.56	0.8	7.6	
Lower	Stream East (T+α+U+V+W)	1.8	0.1	12	2.2	21.6	
Lower	Stream East (T+α+U+V+W)	0.9	0.1	14.58	1.3	13.1	
Lower	Stream East (T+α+U+V+W)	1.2	0.15	29.04	5.2	34.8	
Lower	Stream East (T+α+U+V+W)	1	0.1	20.76	2.1	20.8	
Lower	Stream East (T+α+U+V+W)	1	0.15	11.04	1.7	11.0	
Lower	Stream East (T+α+U+V+W)	1	0.2	12.6	2.5	12.6	
Lower	Stream East (T+α+U+V+W)	0.8	0.2	9.18	1.5	7.3	
Lower	Stream East (T+α+U+V+W)	1	0.4	15.48	6.2	15.5	Bridge and Wate
Lower	Stream East (T+α+U+V+W)	0.6	0.2	15.54	1.9	9.3	
Lower	Stream East (T+α+U+V+W)	0.5	0.4	21			No Fish Below
Lower	Stream East (T+α+U+V+W)	0.6	0.2	19.68			No Fish
Lower	Stream East (T+α+U+V+W)	0.7	0.25	12.84			No Fish
Lower	Lower Wetland (X)	29	0.1	24.48	71.0	709.9	
Lower	Stream West (Z+Y)	1.6	0.1	14.52			No Fish
Lower	Stream West (Z+Y)	1.4	0.1	9.78			No Fish
Lower	Stream West (Z+Y)	1.4	0.1	18.06	2.5	25.3	
Lower	Stream West (Z+Y)	2	0.15	14.7	4.4	29.4	
Lower	Stream West (Z+Y)	1.6	0.05	7.56	0.6	12.1	
Lower	Stream West (Z+Y)	1	0.05	7.44	0.4	7.4	
Lower	Stream West (Z+Y)	0.8	0.5	11.64	4.7	9.3	
Lower	Stream West (Z+Y)	0.6	0.1	12.96	0.8	7.8	
Lower	Stream West (Z+Y)	0.6	0.05	6.72	0.2	4.0	
Lower	Stream West (Z+Y)	0.4	0.1	4.98	0.2	2.0	
Lower	Stream West (Z+Y)	0.6	0.1	4.86	0.3	2.9	
Lower	Stream West (Z+Y)	0.8	0.1	4.68	0.4	3.7	
Lower	Stream West (Z+Y)	0.6	0.1	5.4	0.3	3.2	
Lower	Stream West (Z+Y)	0.8	0.1	5.28	0.4	4.2	
		Sum			156.6	1221.7	