THESIS

GILL LICE *SALMINCOLA CALIFORINESIS*: IMPLICATIONS TO COLORADO FISHERIES MANAGEMENT

Submitted by

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ABSTRACT

GILL LICE *SALMINCOLA CALIFORNIENSIS*: IMPLICATIONS TO COLORADO FISHERIES MANAGEMENT

Gill lice, *Salmincola* spp., are external parasitic copepods that target many different species of fish across the northern hemisphere. Gill lice limit oxygen exchange through gill filaments on which they are attached and negatively impact fish behavior, immune system function, growth, temperature tolerance, sexual maturation, fecundity, and survival. In Colorado, gill lice were first documented in the early 1900s but were not considered a threat to fish populations until recently. In the early 2000s, gill lice were documented in a Colorado reservoir that subsequently experienced a significant decline in kokanee salmon (*Oncorhynchus nerka*) populations. This population decline was attributed to gill lice infection coupled with unfavorable environmental conditions.

Colorado Parks and Wildlife became concerned by the apparent effects of gill lice on this population and determined that research was warranted to identify and characterize the potential threat that gill lice pose to the state's fisheries. The first priority of my research was to identify which species of gill lice were present in the state and, therefore, identify which host species of fish in the state may be at risk of gill lice infection. In addition to species identification, mapping the current extent of gill lice infection in the state was prioritized. The statewide sampling efforts identified one species of gill lice, *Salmincola californiensis*, which infects Pacific Salmonids of the *Oncorhynchus* genus.

Rocky Mountain Cutthroat Trout *(Oncorhynchus virginalis)* have been documented as susceptible to S. californiensis; however, no infections were found during statewide sampling efforts. The absence of gill lice infections in Rocky Mountain Cutthroat Trout in the state prompted a susceptibility experiment. I conducted research to investigate gill lice infection and mortality rates between a known gill lice host, Rainbow Trout *Oncorhynchus mykiss,* and the Rio Grande Cutthroat Trout (*Oncorhynchus virginalis virginalis*) subspecies of Rocky Mountain Cutthroat Trout. In this experiment, I also examined four distinct habitat types to determine whether habitat influenced infection or mortality in either species. I used a generalized linear mixed-effect model (glmer) in R (version 1.3.1073) to analyze these relationships. Estimated marginal means were used to generate pairwise comparisons for the probability of infection and survival between the two species. I determined that Rio Grande Cutthroat Trout are susceptible to *S. californiensis* infection and were infected at similar rates to those of Rainbow Trout. I also found that habitat may influence infection rates for both species. Unfortunately, no inferences of infection-causing mortality were possible due to the difficulty in identifying gill lice on decomposed or missing carcasses.

The results of this study provide a baseline for gill lice research in the State of Colorado and help to inform fisheries managers of the potential risks associated with *S. californiensis* infections.

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CHAPTER 1

LIFE HISTORY OF GILL LICE, *SALMINCOLA CALIFORNIENSIS*

Life History Introduction

Gill lice are parasitic copepods belonging to the family Lernaepodidae that contains seven freshwater genera and are found throughout the northern hemisphere in a circumpolar distribution (Kabata 1969; Piasecki et al. 2004). The genus *Salmincola*, commonly known as the gill maggot or gill louse, is comprised of 15 species that are primarily parasites of salmonid fishes (Kabata 1969). Some species of gill lice have very specific host species, while others are more generalized and can infect multiple species of fish (Table 1.1) (Kabata 1969).

As described by Kabata and Cousens (1973), gill lice life history is very complex, consisting of many developmental stages (Figure 1.1). Eggs develop in paired egg sacs produced by the adult female, where they become pigmented before the egg sac ruptures. Osmotic uptake of water causes the eggs to swell and burst, at which point the nauplius emerges and molts into the copepodid stage. The copepodid stage is the free-swimming stage of the life cycle, at which time the juvenile copepodid actively seeks a host fish. After hatching, the copepodid spends approximately 30 minutes motionless and, during this period, can sink up to 2.2 meters in the water column (Monzyk et al. 2015). The copepodid then moves through the water using two pairs of swimming legs in search of a host and exhibits burst-like swimming movements. The movement of copepodids is characterized by multiple bursts of swimming followed by short periods in which swimming activity slows and the copepodid starts to sink. The copepodid must attach to a host fish within a few days to survive (Kabata and Cousens 1973). Swimming activity of copepodids has been shown to increase in response to external stimuli, such as shock waves in the water or shadows passing overhead (Poulin et al. 1990; Vigil et al. 2015).

Presumably, these stimuli indicate the presence of a potential fish host and increase the likelihood that the copepodid encounters it (Poulin et al. 1990). The length of copepodid survival prior to attachment to a host is temperature-dependent, with individuals surviving longer at lower temperatures (Vigil et al. 2015). Copepodid survival for *S. californiensis* is 1 day to 13 days, during which time attachment to a suitable host must occur for louse survival (Vigil et al. 2015).

When a host fish is encountered, the copepodid uses a prehensile hook of the second antenna to penetrate the epidermis of the fish. The claws of the second maxillae attach in a similar manner to complete the initial attachment. Once contact with a host has been established, the copepodid moves along the fish to find a suitable attachment point that provides solid subdermal support. At this primary attachment site, the copepodid uses the claws of the maxillipeds to excavate a cavity in the tissue of the host. A natural cement substance is then excreted by the copepodid to fix the terminal plug of the frontal filament into the excavated area. This is the first stage of primary attachment, and in some cases, the development does not progress any further. It is unknown why some individuals cease development at this point, but it has been suggested that it may be due to a poor attachment point or to damage to the organism caused by the attachment process (Kabata and Cousens 1973).

Once the copepodid has safely anchored to the primary attachment point, it begins molting through a series of five chalimi stages (Kabata and Cousens 1973; Murphy et al. 2020). During each of these molts, the organism experiences small morphological changes, concurrent with detachment and reattachment of the frontal filament. During the fifth and final chalimus stage, the male does not reattach its frontal filament and instead searches for a female to fertilize (Kabata and Cousens 1973), while the female louse breaks the frontal filament and searches for a final attachment site. When a final attachment site has been chosen, the female louse begins to excavate a new cavity in the tissue of the host fish. A cement substance is then used to attach the bulla structure to the newly excavated cavity. At this point in the life cycle, the female louse is permanently attached to the host fish, and egg sac development begins.

Male gill lice can fertilize females before a female has fully matured (Kabata and Cousens 1973). Once a female louse has been located, fertilization starts with the male attaching itself to the genital process of the female. The male uses a thin terminal tube to enter the vaginal orifice of the female and a spermatophore is inserted into the genital duct of the female. The male then seals the vaginal orifice with a cement substance to complete the process. The vaginal opening is sealed for life, and no further fertilization is possible (Kabata and Cousens 1973). After a female has reached adulthood and chosen its final attachment point, it takes approximately 28-32 days for the egg sacs to fully develop and hatch (Kabata and Cousens 1973).

TABLES

Table 1.1 Table showing gill lice species and associated host fish species

***Salmincola* species**	**Hosts**
salmoneus	Atlantic Salmon
carpionis	Arctic Char, Dolly Varden, Brook Trout
californiensis	Rainbow Trout, Cutthroat Trout, Kokanee, Chinook Salmon, Cherry Salmon
thymalii	Grayling, Arctic Char, Whitefish
coregonorum	Whitefish
edwardsii	Arctic Char, Dolly Varden, Brook Trout
siscowet	Lake Trout
lotae	Burbot
cottidarum	Sculpin
stellatus	Sakhalin Taimen
nordmanni	Beloribitsa
estensus	Whitefish, Least Cisco, Lake Whitefish, Cisco
jacuticus	Whitefish
extumescens	Whitefish, Arctic Cisco, Cisco

FIGURES

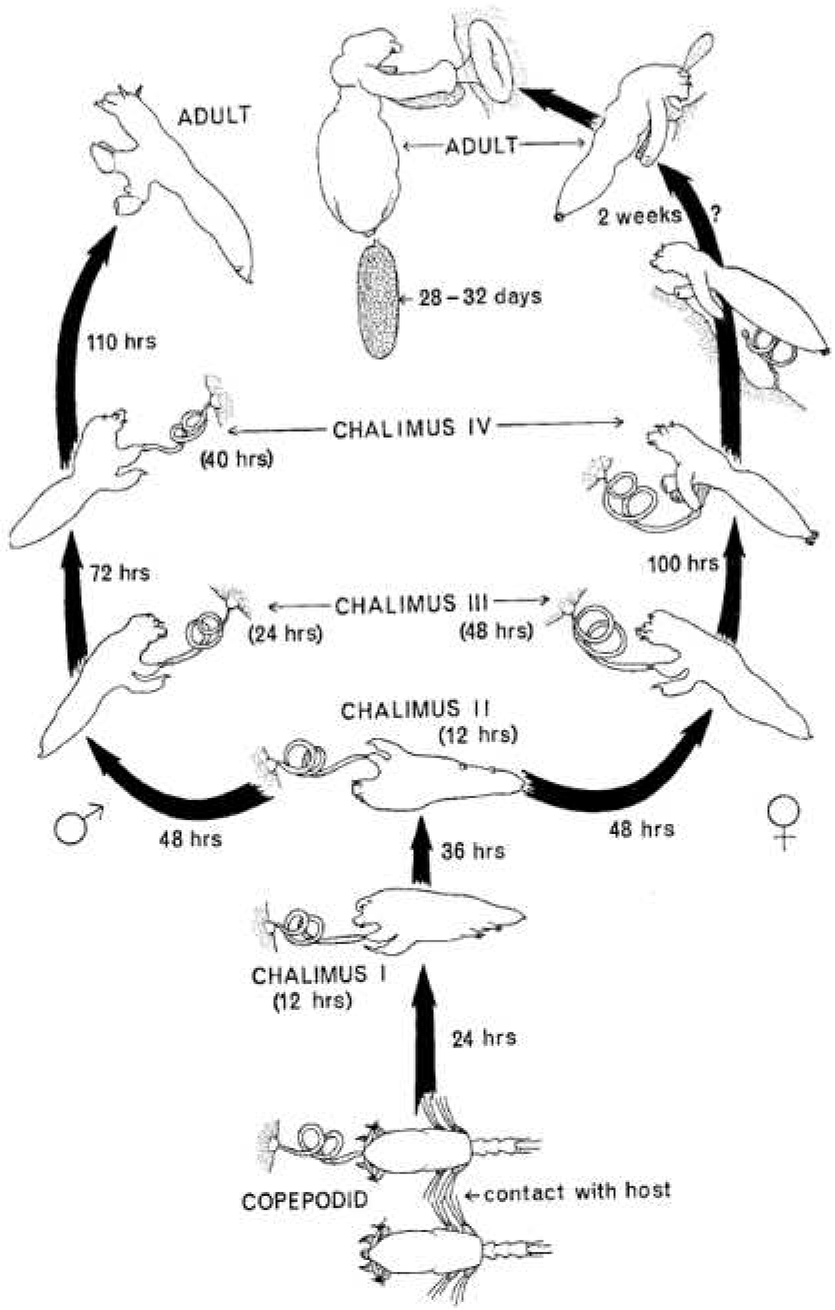

Figure 1.1 *Salmincola californiensis* life cycle diagram from Kabata and Cousens 1973. (Times in parenthesis represent duration of life stage, times without parenthesis represent time from contact with host fish).

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CHAPTER 2

HISTORY AND CURRENT STATUS OF GILL LICE, *SALMINCOLA CALIFORNIENSIS* IN COLORADO

Introduction

Many studies have shown that gill lice can have negative effects on fish in aquaculture settings (Gall 1972; Vaughn and Coble 1975; Sutherland and Wittrock 1985; Modin and Veek 2002). Historically, gill lice infections in the wild have been considered a minimal threat to fish populations (Black 1982; Black et al. 1983; Bowen and Stedman 1990; Amundsen et al. 1997). However, more recently, gill lice infections in natural systems have been implicated in causing negative effects on wild fish populations (Hargis et al. 2014; Lepak et al. 2021). Negative effects associated with gill lice infection include reduced oxygen uptake, growth, and fecundity as well as delayed sexual maturation, decreased resistance to other stressors, and mortality (Poulin et al. 1991; Chigbu 2001; Brandt and Stone 2003).

Gill lice infections in the wild are typically characterized by low prevalence and infection intensity and have minimal impacts on fish populations (Amundsen et al. 1997; Modin and Veek 2002; Piasecki et al. 2004). However, experimental and anecdotal observations by biologists and anglers across the country suggest that gill lice have much more detrimental effects on wild fish populations than originally thought (Mitro 2016; Vigil et al. 2015; Ruiz et al. 2017; Mitro and Griffin 2018) and, in recent years, many studies have investigated the potential effects of gill lice infections on wild fish populations (Hargis et al. 2014; Mitro 2016; Ruiz et al. 2017; Mitro and Griffin 2018; Lepak et al. 2021). It appears that gill lice infections in the wild are increasing and spreading into more waters, causing concern for fisheries managers across the country. Concern about gill lice infections in Colorado specifically prompted a desire to understand the distribution and prevalence of gill lice in Colorado's lakes and streams.

Gill lice have been documented in Colorado since the early 1900s, with the earliest reports of infections in non-native Rainbow Trout *Oncorhynchus mykiss*, and Chinook Salmon *Oncorhynchus tshawytscha* (Wilson 1909; Vigil et al. 2015). Gill lice were of little concern to state fish managers until the early 1990s, when reports of gill lice infections in Colorado started to increase (Walker 1995). In 1994, the Poudre Canyon State Trout Rearing Facility was the first reported state hatchery to be infected with gill lice (Walker 1995). Although there were growing concerns about gill lice infections in Colorado at the time, they were still considered a minimal threat to wild fish populations. Gill lice have now been documented in more waterbodies in Colorado and are likely spread through natural fish migration and human stocking activities (Prokosch et al. 2025).

In 2007, kokanee *Oncorhynchus nerka* (landlocked Sockeye Salmon) in Elevenmile Reservoir (Park County, Colorado) experienced a significant population decline after becoming infected with gill lice (Vigil et al. 2015). The kokanee population in Elevenmile Reservoir dropped to levels that were unable to support historic spawning operations. Kokanee populations in other Colorado waters also experienced declines concurrent with gill lice infection (Jon Ewert and Jeff Spohn, Colorado Parks and Wildlife, personal communication, 2019). These troubling population declines of kokanee, as well as additional reports of gill lice infection in Colorado, increased concern about gill lice interactions with wild fish populations in the state.

Relatively little was known about gill lice in Colorado prior to these population declines. Past reports released by Colorado Parks and Wildlife (CPW) have suggested as many as three different species of gill lice occur in the state (Walker 1995). Additionally, the species of fish susceptible to gill lice infection were unknown, as well as the distribution of the parasite in the state. My research was undertaken to help inform the State of Colorado of the status and potential effects of gill lice on state fisheries. My objectives were to identify the species of gill lice found in Colorado, the species of fish infected throughout the state, and the distribution of gill lice infections throughout Colorado.

Methods

Fishes in the coregonidae and salmonidae families are most susceptible to gill lice infections, therefore sampling efforts in Colorado were focused on detecting gill lice from fish in these families. Species sampled included Brook Trout *Salvelinus fontinalis*, Brown Trout *Salmo trutta*, cutbows (Cutthroat Trout *Oncorhynchus clarkii* × Rainbow Trout *O. mykiss*), Cutthroat Trout *Oncorhynchus clarkii*, Grayling *Thymallus thymallus*, kokanee, Lake Trout *Salvelinus namaycush*, Mountain Whitefish *Prosopium williamsoni*, Rainbow Trout, and splake (Brook Trout *Salvelinus fontinalis* × Lake Trout *S. namaycush*).

Fishes from both lentic and lotic systems in Colorado were surveyed using a variety of sampling techniques (Table 2.1). Sampling efforts were completed by Colorado State University (CSU) staff as well as CPW Aquatic Biologists. In lentic waters, experimental gill nets, vertical gill nets, boat electrofishing, and hook and line sampling were used to collect fish for inspection and gill nets were the most common sampling gear utilized (Table 2.1). In lotic systems, most fish sampled were captured using electrofishing backpacks or rafts (Table 2.1). For all fish sampled, data were collected on the number of lice on each fish, the species of fish, and location sampled. Waters were classified as positive if gill lice were detected on any species during sampling (Table 2.1). Prevalence and mean intensity of the gill lice infection were calculated for each positive water. Prevalence is defined as the number of individuals of a host species infected with gill lice divided by number of hosts examined (Margolis et al. 1982). The mean intensity of infection is defined as the mean number of individual gill lice per infected host in the sample (Margolis et al. 1982).

Utilizing preserved specimens from a previous Cutthroat Trout study allowed for a more efficient inspection of 49 different Cutthroat Trout populations (Bestgen et al. 2013). Examining the preserved specimens allowed me to inspect 744 fish across all sub-species of Cutthroat Trout found in the state without traveling to each individual site. Freshly obtained fish infected with gill lice were preserved using the same method as Bestgen et al. 2013 to determine that preservation did not affect gill lice attachment. The complete contents of the preservation jars were inspected to ensure no gill lice were present in the sample.

Random samples of gill lice were collected from multiple host species and waters for gill lice species identification. Gill lice were collected by removing the structure to which the louse was attached (e.g. gill arch) and preserving it in 95% ethanol. All specimens collected were shipped to the Aquatic Health Laboratory at CPW for species identification. Species identification was completed by mounting the buccal region on a microscope slide with a drop of distilled water and a cover slip. Gill lice specimens were identified to species using characteristics of the second antenna as described by Kabata (1988). Proper identification was accomplished by inspecting the exopod and endopod of the second antenna using a light microscope and 400x magnification.

Results

A total of 115 waters were surveyed consisting of 30 lotic habitats, 36 lentic habitats, and 49 Cutthroat Trout specific lotic habitats. No lice were observed on fish in the Cutthroat Trout specific habitats. Among the remaining waters surveyed, 38 (57.6%) were determined positive for gill lice infections. Positive waters consisted of 60% lentic habitats and 55.6% lotic habitats (Table 2.1.).

Only one species of gill louse was identified in our samples from Colorado, *Salmincola californiensis*. Gill lice infection in Colorado was confirmed on most pacific salmonids *Oncorhynchus* spp. in the state, including cutbows, kokanee, and Rainbow Trout. No gill lice were observed on Brook Trout, Brown Trout, Grayling, Lake Trout, Mountain Whitefish, or splake at any location. Gill lice were not detected in our inspections of 49 conservation populations of Cutthroat Trout surveyed across the state by Bestgen et al. 2013.

The prevalence of gill lice infections for cutbows, kokanee, and Rainbow Trout were 20%, 45%, and 49%, respectively (Figure 2.2). The mean intensities of infections were 4.9 lice/fish for cutbows, 4.7 lice/fish for kokanee, and 5.5 lice/fish for Rainbow Trout (Figure 2.2).

Discussion

My study indicated that the only gill louse species in Colorado was *Salmincola californiensis*, and was common and widespread in lakes, reservoirs, and streams throughout the state. In Colorado, three fish host species were infected by *S. californiensis;* cutbows, kokanee, and Rainbow Trout and are all in the genus *Oncorhynchus*, which are known to be susceptible to this gill louse (Kabata 1969; Vigil et al. 2015). Rainbow Trout and kokanee typically had the highest prevalence (45%) in infected populations, compared to cutbows (20%). The infection intensity was highest in Rainbow Trout at 5.5 lice per infected fish.

The current widespread distribution of gill lice and the potential for them to spread combined with the documented negative effects on fish populations (Chigbu 2001; Barndt and Stone 2003; Kamerath 2009; Gunn et al. 2012) raises concern that gill lice could pose a serious threat to Colorado salmonid fisheries. Population declines of kokanee following infection with gill lice in some reservoirs have raised concern about the persistence of kokanee in the state. Kokanee are propagated using wild brood stock at Blue Mesa, Nighthorse, Wolford, Granby, McPhee, and Navajo Reservoirs in Colorado, as well as a captive broodstock at Roaring Judy Fish Hatchery. Two other reservoirs, Williams Fork and Elevenmile, which historically supported broodstock operations, have experienced population declines due to gill lice infestations. The observed kokanee declines resulted in a decision to discontinue all spawning efforts in those reservoirs. Deleterious effects of gill lice on kokanee populations are cause for concern because of the potential for more brood stocks to experience population declines, thereby threatening the persistence of kokanee in Colorado.

Rainbow Trout are one of the most sought-after sportfish for anglers in the State of Colorado, however many Rainbow Trout populations in the state declined significantly after the establishment of whirling disease (Nehring and Walker 1996). The gill lice prevalence and infection intensity are high for Rainbow trout; however, the effect of the parasite on Rainbow Trout population persistence is unknown. Longstanding efforts to understand and manage the impacts of whirling disease and reestablish Rainbow Trout in Colorado are now complicated by the emerging threat of *S. californiensis*.

I did not find gill lice on any Cutthroat Trout in our samples. Cutthroat Trout belong to the genus of Pacific Salmonids *Oncorhynchus*, and are susceptible to *S. californiensis* infection (Kabata 1969; Heckmann and Ching 1987; Modin and Veek 2002; Barndt and Stone 2003; Murphy et al. 2020). We suspect that the lack of gill lice infection on Cutthroat Trout in Colorado was due to their conservation status. All my samples came from established conservation populations that are protected from non-native sportfish invasion by stream barriers. The exclusion of fish immigration into these populations is likely the reason gill lice were not detected on any of the Cutthroat Trout we sampled. My data showed that cutbow hybrids were infected with gill lice, indicating that potential exists for pure Cutthroat Trout species to be infected as well. Despite the lack of gill lice detection, managers should remain concerned about the potential for gill lice invasion through fish movements across the state. All three species of Cutthroat Trout in Colorado are of conservation concern. Currently, the Greenback Cutthroat Trout *O. v. stomias* is federally listed under the Endangered Species Act as a Threatened Species. Two other subspecies of Cutthroat Trout native to Colorado, the Colorado River Cutthroat Trout *O. v. pleuriticus*, and the Rio Grande Cutthroat Trout *O. v. virginalis*, are listed as State Species of Special Concern. Although our wild fish survey did not detect gill lice on Cutthroat Trout, it is likely that they are susceptible, and the parasite could still pose a threat to conservation populations of Cutthroat Trout.

Gill lice pose a serious threat to both native and sportfish fisheries in the State of Colorado. Kokanee and Rainbow Trout are both popular sportfish species targeted by anglers. Kokanee are one of the most sought-after sportfish species in Colorado, historically generating about 30 million dollars in revenue for the state annually (Johnson and Martinez 2000; Martinez et al. 2009). Non-native kokanee also have little interaction with native fishes and are prey for other sportfish, such as Lake Trout, making them an ideal species to provide recreational angling opportunities (Martinez and Wiltzius 1995; Johnson and Martinez 2000; Martinez et al. 2009; Hargis et al. 2014; Pate et al. 2014). The re-establishment of Rainbow Trout fisheries are have been the focus of Colorado Parks and Wildlife after significant declines in the early 1990's due to whirling disease (*Myxobolus cerebralis)* (Fetherman et al. 2011; Avila et al. 2018). Gill lice pose another threat to Rainbow Trout reestablishment. Gill lice may also pose a threat to native Cutthroat Trout conservation efforts in Colorado. The confirmation of gill lice infection on cutbows, kokanee, and Rainbow Trout allows managers to focus mitigation efforts on these species. The spread of gill lice is concerning because of the potential effects on both sportfish and native fish populations. My research sets the foundation for continued monitoring and studies of gill lice impacts on fisheries in Colorado.

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TABLES

Table 2.1. List of all waters sampled, gear used, and species sampled, status of gill lice infection, and species infected during wild fish surveys, excluding Cutthroat specific habitats. (Species listed by 3-letter code: BRK- Brook Trout, LOC- Brown Trout, Cut-Cutthroat, GRY- Greyling, KOK-kokanee, MAC-Lake Trout, MWF-Mountain Whitefish, RBT-Rainbow Trout, RXN-Cuttbow, SPL-splake.)

Water Body (County)	Sampling Technique	Fish Species	Gill lice	Species infected
Beaver Creek (Larimer)	Electrofishing Backpack	BRK, LOC	Negative	
Big Thompson River (Larimer)	Bank Electrofishing	RBT	Positive	RBT
Blue Mesa Reservoir (Gunnison)	Gillnets	KOK, LOC, MAC, RBT	Positive	KOK, RBT
Blue River (Grand)	Bank Electrofishing	LOC, RBT, RXN	Positive	RBT, RXN
Blue River (Summit)	Bank Electrofishing	LOC, RBT, RXN	Positive	RBT, RXN
Canyon Creek (Garfield)	Electrofishing Backpack	MWF	Negative	MWF
Chambers Lake (Larimer)	Gillnets, Hook and Line	MAC, RBT	Positive	RBT
Cheeseman Reservoir (Douglas)	Gillnets	KOK, RBT	Positive	KOK, RBT
Clear Creek Reservoir (Chaffee)	Gillnets	CUT, KOK, RBT	Positive	KOK, RBT
Cochetopa Creek (Saguache)	Electrofishing Backpack	RBT	Negative	
Colorado River (Eagle)	Bank Electrofishing	MWF, RBT	Positive	
Colorado River (Garfield)	Bank Electrofishing	RBT	Positive	RBT
Colorado River (Grand)	Bank Electrofishing	RBT	Positive	RBT
Conejos River (Conejos)	Raft Electrofishing	LOC, RBT	Positive	RBT
Continental Reservoir (Hinsdale)	Gillnets, Hook and Line	RBT	Positive	RBT
Cottonwood Lake (Chaffee)	Gillnets	RBT	Positive	RBT
Dowdy Lake (Larimer)	Hook and Line	RBT	Negative	RBT
Eagle River (Eagle)	Bank Electrofishing	LOC, RBT	Negative	
Elevenmile Reservoir (Park)	Gillnets	CUT, KOK, LOC, RBT, RXN	Positive	
Elkhorn Creek (Larimer)	Electrofishing Backpack	LOC, RBT	Negative	CUT, KOK, RBT, RXN
Flatiron Reservoir (Larimer)	Gillnets	RBT	Positive	
Fryingpan River (Eagle)	Bank Electrofishing	RBT	Positive	RBT
Grand Lake (Grand)	Gillnets	MAC, RBT	Positive	RBT
Green Mtn Reservoir (Summit)	Gillnets	KOK, LOC, MAC, SPL	Positive	RBT
Grizzly Creek (Garfield)	Electrofishing Backpack	MWF	Negative	KOK
Gross Reservoir (Boulder)	Gillnets	RBT	Positive	
Gunnison River (Gunnison)	Raft Electrofishing	KOK, LOC, RBT	Positive	RBT
Horsetooth Reservoir (Larimer)	Gillnets	RBT	Positive	KOK, RBT
Island Lake (Delta)	Gillnets	BRK, RBT, SPL	Positive	RBT
Joe Wright Reservoir (Larimer)	Gillnets, Hook and Line	GRY, RXN	Negative	RBT
Killpecker Creek (Larimer)	Electrofishing Backpack	BRK	Negative	

Table 2.1. (Continued) List of all waters sampled, gear used, and species sampled, status of gill lice infection, and species infected during wild fish surveys excluding Cutthroat specific habitats. (Species listed by 3 letter code: BRK- Brook Trout, LOC- Brown Trout, Cut-Cutthroat, KOK-kokanee, MAC-Lake Trout, MWF-Mountain Whitefish, RBT-Rainbow Trout, RXN- Cutbow, SPL-splake.)

Water Body (County)	Sampling Technique	Fish Species	Gill lice	Species infected
Lake Agnes (Grand)	Hook and Line	LOC, RBT	Negative	RBT
Lake Catamount (Routt)	Trapnets	RBT	Positive	RBT
Lake Fork of Gunnison (Gunnison)	Raft Electrofishing	LOC, RBT	Positive	RBT
Lake Granby (Grand)	Gillnets	KOK, LOC, MAC, RBT	Negative	
Lake Nighthorse (La Plata)	Gillnets	KOK, RBT	Negative	
Middle Fork South Platte (Park)	Hook and Line	LOC	Negative	
Mt. Elbert Fore Bay (Lake)	Gillnets	CUT, LOC, MAC, RBT	Negative	
North Fork Poudre (Larimer)	Electrofishing Backpack	RBT	Positive	RBT
North Fork S. Platte (Jefferson)	Bank Electrofishing	RBT	Positive	RBT
Parvin Lake (Larimer)	Boat Electrofishing	BRK, LOC, RBT, RXN, SPL	Positive	RBT, RXN
Piceance Creek (Rio Blanco)	Electrofishing Backpack	RBT	Negative	
Piedra River (Archuleta)	Electrofishing Backpack	RBT	Negative	
Pinewood Reservoir (Larimer)	Gillnets	RBT	Positive	RBT
Poudre River (Larimer)	Bank Electrofishing	RBT	Negative	
Rio Blanco (Archuleta)	Electrofishing Backpack	RBT	Positive	RBT
Rio Grande River (Mineral)	Raft Electrofishing	LOC, RBT	Positive	RBT
Rio Grande River (Rio Grande)	Bank Electrofishing	LOC, RBT	Positive	RBT
Roaring Fork River (Garfield)	Bank Electrofishing	RBT	Positive	RBT
Salt Creek (Garfield)	Electrofishing Backpack	RBT	Negative	
Sevenmile Creek (Larimer)	Electrofishing Backpack	BRK	Negative	
South Platte River (Jefferson)	Bank Electrofishing	LOC, RBT	Positive	RBT
Spinney Mountain Reservoir (Park)	Gillnets	RBT, RXN	Positive	RBT, RXN
Stagecoach Reservoir (Routt)	Gillnets	RBT	Positive	RBT
Taylor Reservoir (Gunnison)	Gillnets	LOC, MAC, RBT	Negative	
Tomichi Creek (Gunnison)	Electrofishing Backpack	RBT	Negative	
Trappers Lake (Garfield)	Gillnets, Hook and Line	BRK, CUT	Negative	
Turquoise Lake (Lake)	Gillnets	BRK, LOC, MAC, RBT	Negative	
Twin Lakes (Lake)	Gillnets	LOC, MAC, RBT	Negative	
West Lake (Larimer)	Gillnets, Hook and Line	RBT	Negative	
White River (Rio Blanco)	Raft Electrofishing	MWF, RBT	Positive	RBT
Williams Fork Reservoir (Grand)	Gillnets	KOK, RBT	Positive	KOK, RBT
Willow Creek (La Plata)	Electrofishing Backpack	RBT	Negative	
Wolford Mtn. Reservoir (Grand)	Gillnets	RBT	Negative	
Yampa River (Routt)	Bank Electrofishing	RBT	Positive	RBT

FIGURES

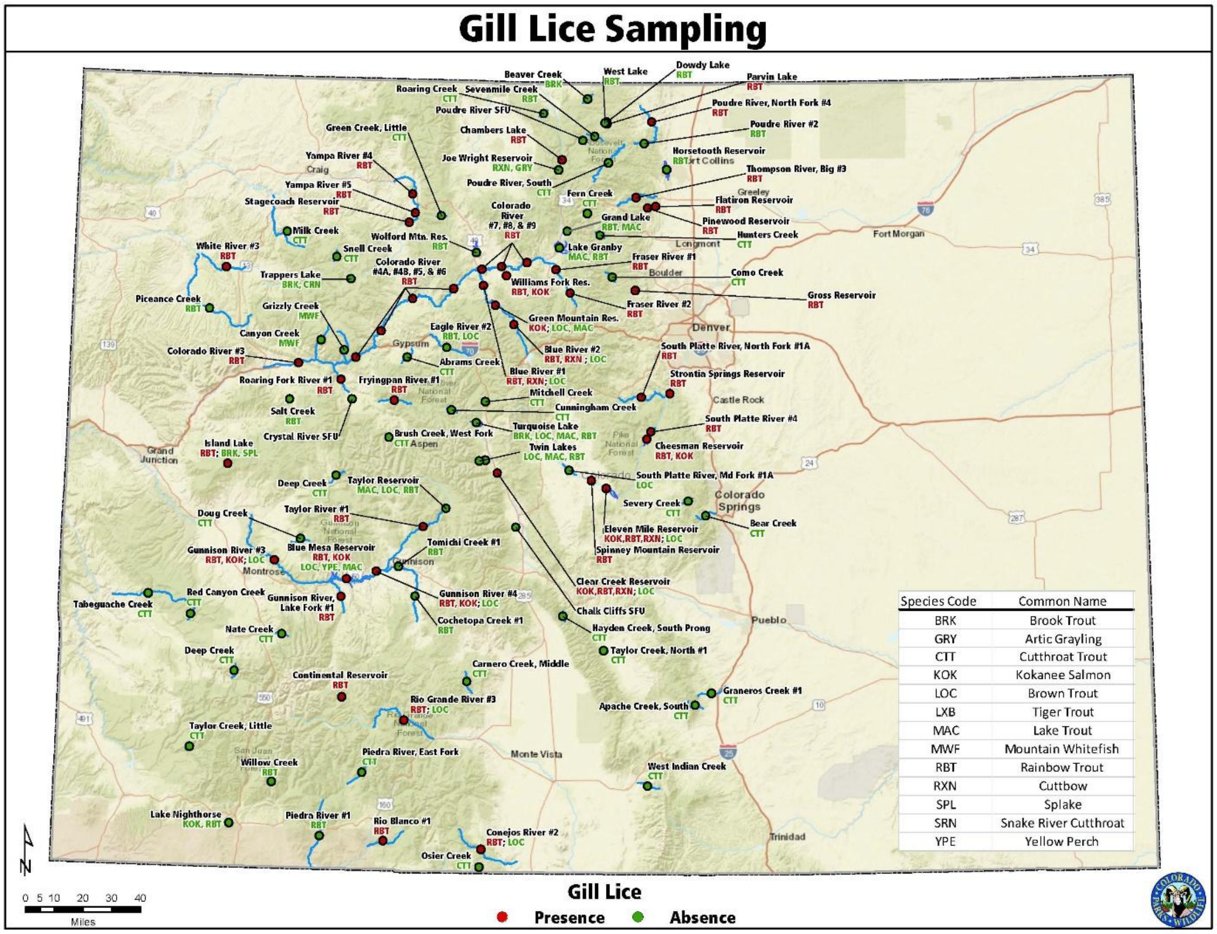

Figure 2.1. Map of waters surveyed for gill lice infection 2013-2016. (Species listed as 3 letter codes: KOK-kokanee, LOC-Brown Trout, MAC-Lake Trout, MWF-Mountain Whitefish, RBT-Rainbow Trout, RXN-Cutbows, CTT-Cutthroat, SPL-Splake.)

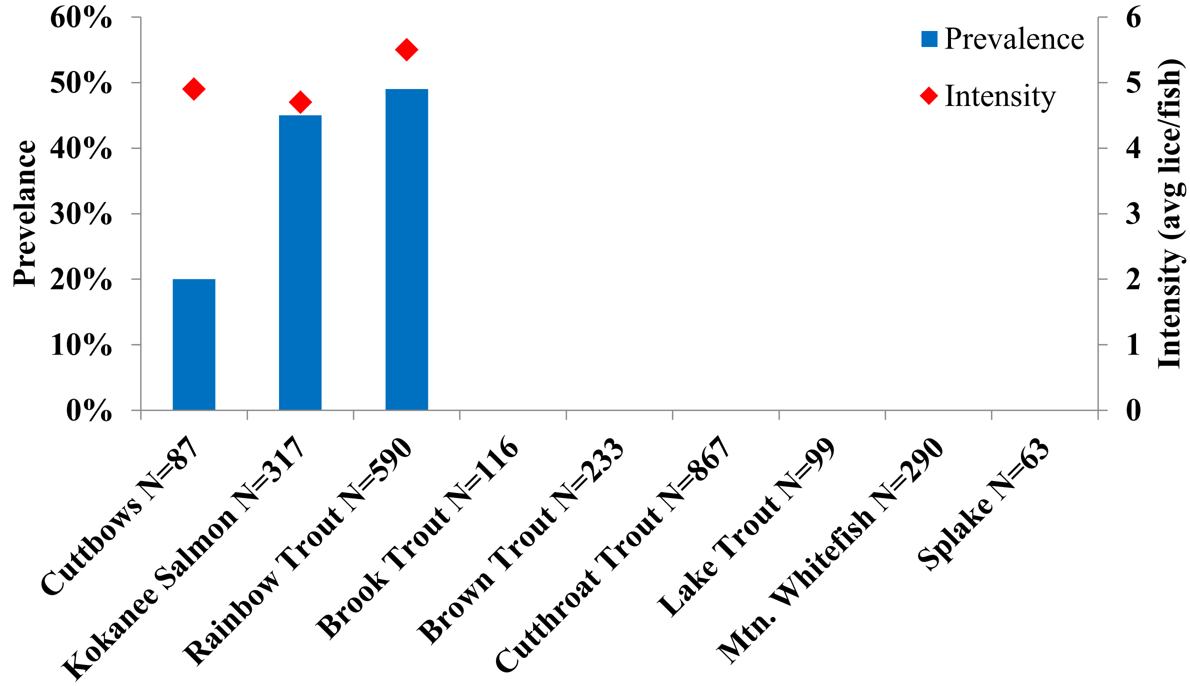

Figure 2.2. Prevalence and Intensity of gill lice infections by host species.

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CHAPTER 3

SUSCEPTIBILITY OF GILL LICE, *SALMINCOLA CALIFORNIENSIS* INFECTION BETWEEN TWO PACIFIC SALMONIDS ACROSS FOUR HABITAT TYPES

Introduction

Rocky Mountain Cutthroat Trout (*Oncorhynchus virginalis*) are native to the western United States and are important both for recreation and conservation. Many species have special conservation status, particularly in Colorado, and there are three extant sub-species of Cutthroat Trout native to the State of Colorado. The Greenback Cutthroat Trout (*O. v.* stomias) is federally listed as Threatened (Metcalf et al. 2012; Rogers et al. 2018), and the remaining two sub-species are the Colorado River Cutthroat Trout (*O. v. pleuriticus*) and the Rio Grande Cutthroat Trout (*O. v. virginalis*), both listed in Colorado as "State Species of Special Concern" (Colorado Parks and Wildlife, State Wildlife Action Plan, 2015). Due to their conservation status, managers are concerned about potential threats to these populations, including hybridization, climate change, non-native invasion, and disease.

One potential threat to Cutthroat Trout are gill lice (*Salmincola californiensis*), which infect several *Oncorhynchus* species, including Rocky Mountain Cutthroat Trout (Kabata 1969; Heckmann and Ching 1987; Modin and Veek 2002; Barndt and Stone 2003; Murphy et al. 2020). *Salmincola californiensis* have adversely impacted several fisheries in Colorado (Hargis et al. 2014; Vigil et al. 2015; Lepak et al. 2021); however, *S. californiensis* have not been found on Cutthroat Trout in the state. In Colorado, 867 individual Cutthroat Trout were collected from 49 distinct populations and preserved during a previous study (Bestgen et al. 2013) and comprised all three extant sub-species in Colorado. These fish were later inspected for *S. californiensis* infection and found to be negative for the presence of *S. californiensis*. The reason for the lack of *S. californiensis* infections in Colorado is currently unknown but one potential explanation is that current populations of Cutthroat Trout are protected by fish migration barriers (McGrath and Lewis 2007; Metcalf et al. 2012; Bestgen et al. 2013), thereby limiting the movement of other salmonids and their parasites (i.e., gill lice). These waters are also not stocked to protect the conservation of these native species, further limiting fish movement into these systems. Another possible explanation is the resistance of Rocky Mountain Cutthroat Trout to infection by gill lice. I found that no *S. californiensis* were found on Rocky Mountain Cutthroat Trout inspected in Colorado (Chapter 2), and that Rocky Mountain Cutthroat Trout hybrids had a lower mean infection prevalence (20%) than other salmonid species (45%) (Chapter 2). Both observations led me to hypothesize that Rocky Mountain Cutthroat Trout in Colorado might be resistant to the parasite.

The goal of my experiment was to evaluate if Rocky Mountain Cutthroat Trout were resistant to infection by *Salmincola californiensis* and to understand if gill lice represent a significant threat to Rocky Mountain Cutthroat Trout conservation. Rio Grande Cutthroat Trout were available from the Colorado Parks and Wildlife hatchery system, and we utilized them for my study. They are the southernmost sub-species of Rocky Mountain Cutthroat Trout native to the Rio Grande (Budy et al. 2019, Rogers et al. 2018) and have been geographically isolated from the pacific coast Cutthroat Trout (*Oncorhynchus clarkii*) that are known to be susceptible to infection (Kabata 1969; Murphy et al. 2020).

Methods

Experimental design

To determine if Rio Grande Cutthroat Trout had any resistance to *S. californiensis* infection, I designed a caging experiment to compare a species known to be susceptible to gill lice, Rainbow Trout, to Rio Grande Cutthroat Trout. My study was conducted at the Parvin Lake Colorado Parks and Wildlife (CPW) research station in Colorado and was selected as the experimental site due to an established *S. californiensis* infection on Rainbow Trout *Oncorhynchus mykiss* (George Schisler, CPW personal communication 2015). Parvin Lake is a 27.4 ha waterbody with a maximum depth of 11 meters and is located approximately 72 km northwest of Fort Collins, Colorado at an elevation of 2479 meters.

I caged Rio Grande Cutthroat Trout and Rainbow Trout in four distinct habitat types, two lentic habitats (littoral and pelagic) and two lotic habitats (the inlet and outlet streams) to evaluate if habitat played a role in the infection or survival of experimental fish. Fish of both species ranged in size from 140 mm to 220 mm. Therefore, the experimental design consisted of 8 treatments (2 species by 4 habitats), and these were replicated 5 times across 3 habitat types (inlet, littoral, and pelagic). The outlet habitats were replicated 3 times due to limited habitat availability in Parvin Lake. Littoral cages were placed along the shoreline of the lake at depths between 1 and 2 meters. The pelagic cages were suspended at a depth of 4 meters in locations where the lake depth was greater than 9 meters. Inlet cages were placed along the inlet channel entering the lake at depths less than 1 meter. Outlet cages could not be placed in the stream due to low water levels and were placed at the mouth of the outlet in depths less than 1 meter. Ten fish of the appropriate species were placed in each 0.216m^3 galvanized steel cages with 1.27 cm mesh. Cages were randomly placed in pairs in all four habitat types. One cage of each pair was randomly assigned to Rainbow Trout and the other to Rio Grande Cutthroat Trout. Cages were equipped with automatic feeders. All cages were cleaned, and feeders were changed weekly for the duration of the experiment.

Fish in each cage were inspected monthly from July to November 2016 for the presence of adult *S. californiensis*. All external structures, including fins, internal mouthparts, external mouthparts, operculum, and gills were inspected by two trained technicians during monthly inspections. Gill lice prevalence was calculated by cage and is equal to the proportion of infected fish to non-infected fish during the monthly inspections.

Analyses

I used a generalized linear mixed-effect model (glmer) in R (version 1.3.1073) to analyze the relationships between species and habitat on the proportion of infected fish and host survival. Both infection and survival are binary responses, allowing me to fit a mixed logistic regression analysis to the data. The proportion of infected fish by species, in each cage and habitat type, was calculated and analyzed using a generalized linear mixed-effect model to examine the influence of fixed effects (Species*Habitat*Month) on the prevalence of *S. californiensis* infection. To account for repeat observations on cages over time, cage was included as a random effect.

For the survival analysis, cages were grouped by habitat type (inlet, littoral, outlet, and pelagic). Monthly survival was calculated for each habitat type by subtracting the total number of live fish from the previous month's total. We used a generalized linear model with estimated marginal means pairwise comparisons to examine the influence of species and habitat on survival in the presence of *S. californiensis*. Survival could not be directly attributed to *S. californiensis* because mortalities were usually decomposed or absent during inspections.

Model results are presented as predicted probabilities (of infection or survival) and odds ratios. These calculations were done using the emmeans (estimated marginal means) package. I made three general comparisons; first, I examined the proportion of infected individuals by species and survival by habitat type to determine if one species was more susceptible to *S. californiensis* infection than the other. My estimated marginal means analysis shows the probability of infection and survival for species at each habitat. I tested if habitat influenced infection and survival rates with species as the predictor. The pairwise comparison results produced the probability of infection for each species at all habitats. The contrast results of the response type produced log odds ratios for habitat comparisons with species as the predictor. Therefore, I was able to determine whether individuals in one habitat had a greater infection or survival rate than others for a particular species. P-values were adjusted using the Tukey method to compare four habitat estimates. Third, the estimated marginal means were used to assess overall species survival rate without habitat as a predictor. Species survival, averaged across all habitats, represents the probability of survival for both species for a particular month. Then, contrasts were calculated on the log odds ratio scale to determine whether survival differed by species.

Results

My study demonstrated that Rio Grande Cutthroat Trout are susceptible to *S. californiensis* infection, with prevalence rates similar to those of Rainbow Trout. I also found that habitat type influences the prevalence of *S. californiensis* infection and mortality of both host species. Initial mortality rates of Rio Grande Cutthroat Trout were high and significantly higher than those of Rainbow Trout.

Infection Results between Species

The prevalence of fish infected at the end of the experiment did not differ significantly between species in any of the four habitat types (Figure 3.1). When species were compared with a fixed habitat pairwise comparison model, we saw fluctuating monthly odds ratios, but no significant differences in the prevalence of gill lice infection between the two host species (Table 3.1). Large standard errors and odds ratios are observed at the littoral cages for the months of August, October, and November as well as the outlet cages during the month of November. The high variability can be attributed to differences in the number of fish remaining and the proportion that were infected in the cages during these times (Table 3.1).

Infection Results across Habitats

My analyses suggest that habitat influences initial *S. californiensis* infection rates. The proportion of fish infected in July are significantly higher in the outlet compared to all three other habitats (Table 3.2). When compared to the inlet, the outlet showed greater likelihood of *S. californiensis* infection for Rainbow Trout (odds ratio 0.02, P=0.003), and a greater likelihood of *S. californiensis* infection for Rio Grande Cutthroat Trout (odds ratio 0.04, p=0.001) (Table 3.2). When compared to the littoral zone, the outlet had a greater likelihood of *S. californiensis* infection for Rainbow Trout (odds ratio 0.16, p=0.006) and no significant difference for Rio Grande Cutthroat Trout (Table 3.2). When comparing the outlet to the pelagic zone, I saw an greater likelihood of *S. californiensis* infection for Rainbow Trout (odds ratio 13.13, p=0.001) and a greater likelihood of *S. californiensis* infection rate for Rio Grande Cutthroat Trout (odds ratio 3.8, p=0.044).

For the second month of exposure (August), habitat comparisons were not significant for Rio Grande Cutthroat Trout, however, the proportion of fish infected in the outlet remained higher for Rainbow Trout. I saw a greater likelihood of *S. californiensis* infection for Rainbow Trout in the outlet, when compared to the inlet (odds ratio 0, p=0.003). I saw the same odds ratio when comparing the Rainbow Trout outlet cages to the littoral cages (odds ratio 0, p=0.03) (Table 3.2). Lastly, I observed a greater likelihood of Rainbow Trout being infected by *S. californiensis* in the outlet cages when compared to the pelagic cages (odds ratio 5.0, p=0.045). The lack of differences among habitats in Rio Grande Cutthroat Trout is likely due to high mortality, leaving fewer individuals to be exposed to *S. californiensis* (Figure 3.2). For the remaining duration of the experiment, I did not observe habitat having significant influence on *S. californiensis* infection rates for Rainbow Trout or Rio Grande Cutthroat Trout.

<u>Survival Results between Species</u>

Very little mortality of either host species was experienced during July and mortality between species was not significant (Table 3.3). However, survival of Rainbow Trout was significantly higher in August, September, and October, having 9.35, 8.44, and 9.35 times higher survival rates, respectively, than Rio Grande Cutthroat Trout (p=<.0001) (Table 3.3). Few Rio Grande Cutthroat survived until the end of the experiment in November (Figure 3.2), and the low sample number does not allow for significant differences to be seen (p=0.98).

<u>Habitat Specific Survival Results</u>

Survival was generally highest in the inlet habitat for both species, which did not differ between species in this habitat during any month (Figure 3.4). There was no significant difference in survival between the two species in July in the outlet habitat. However, Rainbow Trout survival was higher in the outlet habitat than that of Rio Grande Cutthroat Trout survival for the rest of the experiment (Figure 3.3). The same trend was also observed in the littoral habitat, with no difference in survival observed for the month of June, and higher survival of

Rainbow Trout for the remaining duration of the experiment (Figure 3.3). Survival was lowest for both species in the pelagic habitat, yet significant differences in survival were still observed for the months of August, September, and October (Figure 3.3). No Rio Grande Cutthroat Trout survived past the month of October in the pelagic habitat, so no comparison was possible for November.

Discussion

My experiment demonstrated that Rio Grande Cutthroat Trout are susceptible to *S. californiensis* infection, suggesting that these gill lice may pose a threat to native Rio Grande Cutthroat Trout conservation in the State of Colorado. Infection of Rio Grande Cutthroat Trout also suggests that other Cutthroat Trout species might be at risk and should be assessed for susceptibility. Infection prevalence was similar for Rainbow Trout and Rio Grande Cutthroat Trout in every habitat for the duration of the experiment, suggesting that Rio Grande Cutthroat Trout are susceptible to gill lice at a level similar to that seen in Rainbow Trout. Rio Grande Cutthroat Trout experienced lower survival than Rainbow Trout across all four habitat types; however, multiple factors may have contributed to higher mortality and may not be related to gill lice infection.

Infection prevalence differed across habitats, and the outlet had the highest prevalence for both species after one month of exposure. My outlet cages were placed in the lake at the mouth of the outlet, rather than directly in the stream, because the stream was too shallow to accommodate them. The higher initial prevalence in the outlet cages may be due to increased contact rates with copepodids, due to copepodids being entrained in water exiting the lake. The pelagic and littoral cages had similar prevalence at the end of the study as the outlet cages. This suggests that gill lice in Parvin Lake were likely being transported to the outlet cages by the directional flow of water exiting the lake, causing the higher initial infection prevalence.

The inlet cages also had a high prevalence at the end of the experiment, but the pattern differed from the outlet cages. The inlet cage prevalence was initially low but gradually increased over time. The incoming water from the inlet of Parvin Lake does not have a source of *S. californiensis,* and therefore, initial exposure to juvenile gill lice copepodids was potentially lower than the exposure rate in the outlet or other littoral cages. The lowest initial prevalence and the gradual increase in infection observed in the inlet over time may be explained by a potential lower infection risk in streams (Monzyk et al., 2015). Fish movement in Parvin Lake might also explain this pattern. Infected fish moving upstream allows copepodids to hatch and infect fish within the inlet habitat (Prokosch et al 2025).

The Rio Grande Cutthroat Trout cages in the pelagic littoral habitats experienced extremely high mortality. Only eight of the initial 48 Rio Grande Cutthroat Trout survived after just one month of exposure (Table 3.1). By October, only one Rio Grande Cutthroat Trout was alive in the pelagic habitat treatment, and only four Rio Grande Cutthroat Trout were surviving in the littoral habitat by August. Due to the absence or decomposition of the carcasses, mortality could not be attributed to gill lice. However, the similarity in mortality across habitats suggests that it is due to factors unrelated to habitat type. Rainbow Trout cages in the pelagic and littoral habitats showed a gradual increase in gill lice prevalence over time.

After one month of exposure, the survival of Rio Grande Cutthroat Trout and Rainbow Trout was similar across all four habitats. By August and continuing into September and October, we saw higher numbers of Rainbow Trout than Rio Grande Cutthroat Trout in every habitat except for the inlet. Survival rates of the two species did not differ in the inlet at any time during the experiment. Survival in the inlet was influenced by fresh, flowing water to all caged fish for the duration of the experiment. The low survival rates of Rio Grande Cutthroat Trout cannot be attributed to *S. californiensis* infection; other unmeasured stressors, such as water temperature, whirling disease exposure, and cage cleaning, may have influenced survival. Other studies investigating different stressors have also shown higher survival rates in Rainbow Trout than in Cutthroat Trout (Bear et al. 2007, Thompson et al. 1999, DuBey et al. 2007). In November, no significant differences in survival were observed between the two species. However, the number of Rio Grande Cutthroat Trout that had survived until November was extremely low and likely confounds the comparison.

My research shows that gill lice infect Rio Grande Cutthroat Trout and may pose a mortality risk. Rio Grande Cutthroat Trout are identified by Colorado Parks and Wildlife in their State Wildlife Action Plan (2015) as a Species of Greatest Conservation Need. Given their conservation status and susceptibility to gill lice, state fisheries managers should be aware of where Rio Grande Cutthroat Trout populations occur and work to reduce the likelihood of gill lice introduction into those systems. Additionally, given the conservation status of Cutthroat Trout in the State of Colorado, more research should be directed toward understanding the susceptibility of other Cutthroat Trout species. Given recent declines in fish populations attributed to gill lice in Colorado, more research is warranted to determine how these infections may impact the state's fisheries resources.

TABLES

Table 3.1. Infection odds ratios of Rainbow Trout and Rio Grande Cutthroat Trout caged at multiple habitat types in Parvin Lake, 2016. Estimated marginal means were used for pairwise comparisons of habitat type on species infection probability (df, infinite; $P \leq 0.05$)

Month	Habitat	RBT n	RGN n	odds.ratio	SE	z ratio	p value
July	Inlet	47	47	0.48	0.60	-0.59	0.55
	Littoral	50	34	0.33	0.19	-1.94	0.07
	Outlet	30	30	0.88	0.45	-0.26	0.80
	Pelagic	48	48	0.25	0.18	-1.98	0.06
August	Inlet	47	36	1.00	0.00	-0.87	0.39
	Littoral	42	8	1.37E+13	3.68E+19	0.00	1.00
	Outlet	20	8	1.00	1.00	0.24	0.81
	Pelagic	32	8	2.00	2.00	0.41	0.68
September	Inlet	38	30	0.53	0.27	-1.23	0.22
	Littoral	31	4	0.56	0.70	-0.47	0.64
	Outlet	20	6	2.69	3.21	0.83	0.41
	Pelagic	23	4	0.44	0.65	-0.56	0.56
October	Inlet	37	30	2.00	1.00	0.82	0.41
	Littoral	31	4	5.92E+16	1.99E+24	0.00	1.00
	Outlet	13	2	1.00	1.00	-0.31	0.76
	Pelagic	16	1	0.00	0.00	0.00	1.00
November	Inlet	37	30	2.00	1.00	1.26	0.21
	Littoral	31	3	9.21E=19	3.57E+27	0.00	1.00
	Outlet	13	2	3.04E+46	1.44E+54	0.00	1.00
	Pelagic	10	0	NA	NA	NA	NA

Table 3.2. *S. californiensis* infection odd ratios of Rainbow Trout and Rio Grande Cutthroat Trout that were placed into suspended cages at multiple habitats in Parvin Lake, July, and August 2016. Habitats are denoted by I, L, O, P (Inlet, Littoral, Outlet, Pelagic). Significant differences are noted by the use of *.

Month	Species	Habitat Contrast	odds.ratio	SE	z ratio	p value
July	RBT					
		I/L	0.15	0.17	-1.71	0.320
		I/O*	0.02	0.03	-3.48	0.003
		I/P	0.31	0.37	-0.99	0.754
		L/O*	0.16	0.09	-3.27	0.006
		L/P	2.05	1.51	0.97	0.767
		O/P*	13.13	9.18	368.00	0.001
	RGN					
		I/L*	0.10	0.09	-2.78	0.028
		I/O*	0.04	0.04	-3.87	0.001
		I/P	0.17	0.13	-2.24	0.113
		L/O	0.42	0.22	-1.67	0.340
		L/P	1.58	0.82	0.89	0.811
		O/P*	3.80	1.94	2.62	0.044
August	RBT					
		I/L	1.00	0.00	-0.85	0.831
		I/O*	0.00	0.00	-3.43	0.003
		I/P	1.00	0.00	-0.76	0.871
		L/O*	0.00	0.00	-2.76	0.030
		L/P	1.00	1.00	0.00	1.000
		O/P*	5.00	3.00	2.61	0.045
	RGN					
		I/L	1.40E+13	3.78E+19	0.00	1.000
		I/O	0.00	0.00	-1.73	0.310
		I/P	2.00	2.00	0.46	0.968
		L/O	0.41	0.25	0.00	1.000
		L/P	0.00	0.00	0.00	1.000
		O/P	7.00	9.00	1.52	0.427

Table 3.3. Survival odds ratios for Rainbow Trout (RBT), and Rio Grande Cutthroat Trout (RGN) across all four habitat types.

Contrast	Month	odds.ratio	SE	z ratio	p value
RBT/RGN	July	195.00	5.54E+05	0.00	1.00
	August	9.35	3.58	5.85	<.0001
	September	8.44	2.60	6.93	<.0001
	October	9.35	3.58	5.85	<.0001
	November	409.00	1.24E+05	0.02	0.98

Table 3.4. Survival odd ratios of adult Rainbow Trout and Rio Grande Cutthroat Trout that were placed into cages at multiple habitats in Parvin Lake, 2016. * denote significant p-value.

Month	Habitat	RBT n	RGN n	odds.ratio	SE	z ratio	p value
July	Inlet	47	47	2.00	3.00	0.575	0.5653
	Littoral	50	34	7.12E+08	3.88E+12	0.004	0.9993
	Outlet	30	30	1.00	9.95E+03	0.000	1
	Pelagic	48	48	1.00	1.00	0.000	1
August	Inlet	47	36	2.11	0.93	1.701	0.0890
	Littoral*	42	8	14.68	8.84	4.461	<0.0001
	Outlet*	20	8	10.71	8.77	2.893	0.0038
	Pelagic*	32	8	23.06	24.32	2.975	0.0029
September	Inlet	38	30	2.11	0.93	1.701	0.089
	Littoral*	31	4	14.68	8.84	4.461	<0.0001
	Outlet*	20	6	8.00	4.79	3.474	0.0005
	Pelagic*	23	4	20.44	15.85	3.891	0.001
October	Inlet	37	30	2.11	0.93	1.701	0.089
	Littoral	31	4	14.68	8.84	4.461	<0.0001
	Outlet	13	2	10.71	8.77	2.893	0.0038
	Pelagic	16	1	23.06	24.32	2.975	0.0029
November	Inlet	37	30	2.00	1.00	1.895	0.0581
	Littoral*	31	3	20.00	13.00	4.499	<0.0001
	Outlet*	13	2	11.00	9.00	2.893	0.0038
	Pelagic	10	0	5.67E+07	6.89E+10	0.015	0.9983

Table 3.5. Survival odd ratios of Rainbow Trout and Rio Grande Cutthroat Trout that were placed into cages at multiple habitats in Parvin Lake, 2016. Habitats are denoted by I, L, O, P (Inlet, Littoral, Outlet, Pelagic). * denotes significant p-value.

Month	Species	Contrast	odds.ratio	SE	z ratio	p value
July	RBT					
		I/L	0.00	0.00	0.00	1.00
		I/O	0.00	0.00	0.00	1.00
		I/P	2.00	3.00	0.58	0.94
		L/O	1.00	8900.00	0.00	1.00
		L/P	1.68E+08	9.16E+11	0.00	1.00
		O/P	1.68E+08	1.18E+12	0.00	1.00
	RGN					
		I/L	4.00	4.00	1.71	0.32
		I/O	0.00	0.00	0.00	1.00
		I/P	1.00	1.00	0.00	1.00
		L/O	0.00	0.00	0.00	1.00
		L/P	0.00	0.00	-1.71	0.32
		O/P	1.68E+08	1.18E+12	0.00	1.00
August	RBT					
		I/L	1.94	0.86	1.50	0.44
		I/O*	4.14	2.05	2.87	0.02
		I/P*	6.73	3.02	4.25	0.0001
		L/O	2.13	1.00	1.61	0.37
		L/P*	3.47	1.46	2.96	0.02
		O/P	1.62	0.78	1.02	0.74
	RGN					
		I/L*	13.50	8.11	4.33	0.0001
		I/O*	21.00	16.52	3.87	0.001
		I/P*	73.50	77.22	4.09	0.0003
		L/O	1.56	1.40	0.49	0.96
		L/P	5.44	6.20	1.49	0.45
		O/P	3.50	4.37	1.00	0.75
September	RBT					
		I/L	1.94	0.86	1.50	0.44
		I/O	1.58	0.81	0.90	0.81
		I/P*	3.72	1.62	3.01	0.01
		L/O	0.82	0.40	-0.42	0.98
		L/P	1.92	0.78	1.60	0.38
		O/P	2.35	1.13	1.78	0.28

Table 3.5. (Continued.) Survival odd ratios of Rainbow Trout and Rio Grande Cutthroat Trout that were placed into cages at multiple habitats in Parvin Lake, 2016. Habitats are denoted by I, L, O, P (Inlet, Littoral, Outlet, Pelagic). * denotes significant p-value.

Month	Species	Contrast	odds.ratio	SE	z ratio	p value
September	RGN					
		I/L*	13.50	8.11	4.33	0.0001
		I/O*	6.00	3.24	3.32	0.01
		I/P*	36.00	27.98	4.61	<0.0001
		L/O	0.44	0.31	-1.16	0.65
		L/P	2.67	2.38	1.10	0.69
		O/P	6.00	5.12	2.10	0.15
October	RBT					
		I/L	1.94	0.86	1.50	0.44
		I/O*	4.14	2.05	2.87	0.02
		I/P*	6.73	3.02	4.25	0.0001
		L/O	2.13	1.00	1.61	0.37
		L/P*	3.47	1.46	2.96	0.02
		O/P	1.62	0.78	1.02	0.74
	RGN					
		I/L*	13.50	8.11	4.33	0.0001
		I/O*	21.00	16.52	3.87	0.001
		I/P*	73.50	77.22	4.09	0.0003
		L/O	1.56	1.40	0.49	0.96
		L/P	5.44	6.20	1.49	0.45
		O/P	3.50	4.37	1.00	0.75
November	RBT					
		I/L	2.00	1.00	1.50	0.44
		I/O*	4.00	2.00	2.87	0.02
		I/P*	1.10	5.00	5.09	<0.0001
		L/O	2.00	1.00	1.61	0.37
		L/P*	6.00	3.00	3.91	0.00
		O/P	3.00	1.00	1.99	0.19
	RGN					
		I/L*	17.00	11.00	4.26	0.0001
		I/O*	19.00	15.00	3.77	0.001
		I/P	2.77E+08	3.37E+11	0.02	1.00
		L/O	1.00	1.00	0.13	1.00
		L/P	1.63E+07	1.98E+10	0.01	1.00
		O/P	1.44E+07	1.74E+10	0.01	1.00

FIGURES

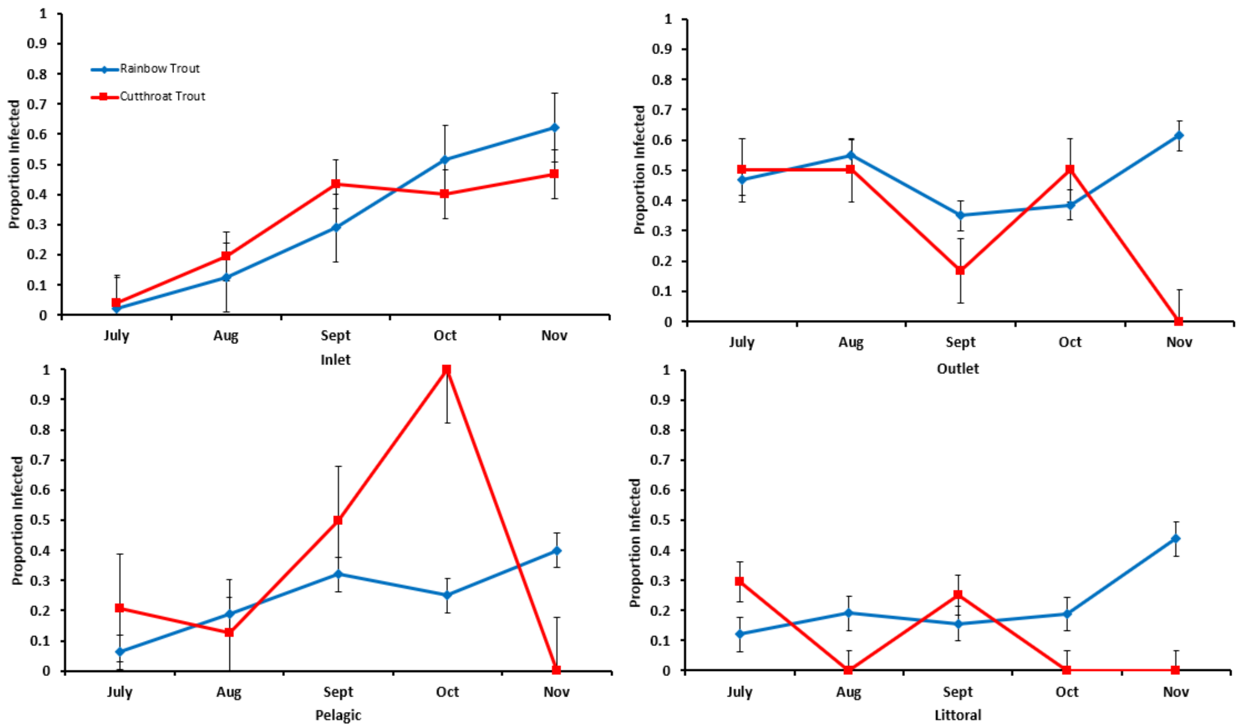

Figure 3.1. Infection rate of Rainbow Trout and Rio Grande Cutthroat Trout by habitat type, with 95% confidence intervals.

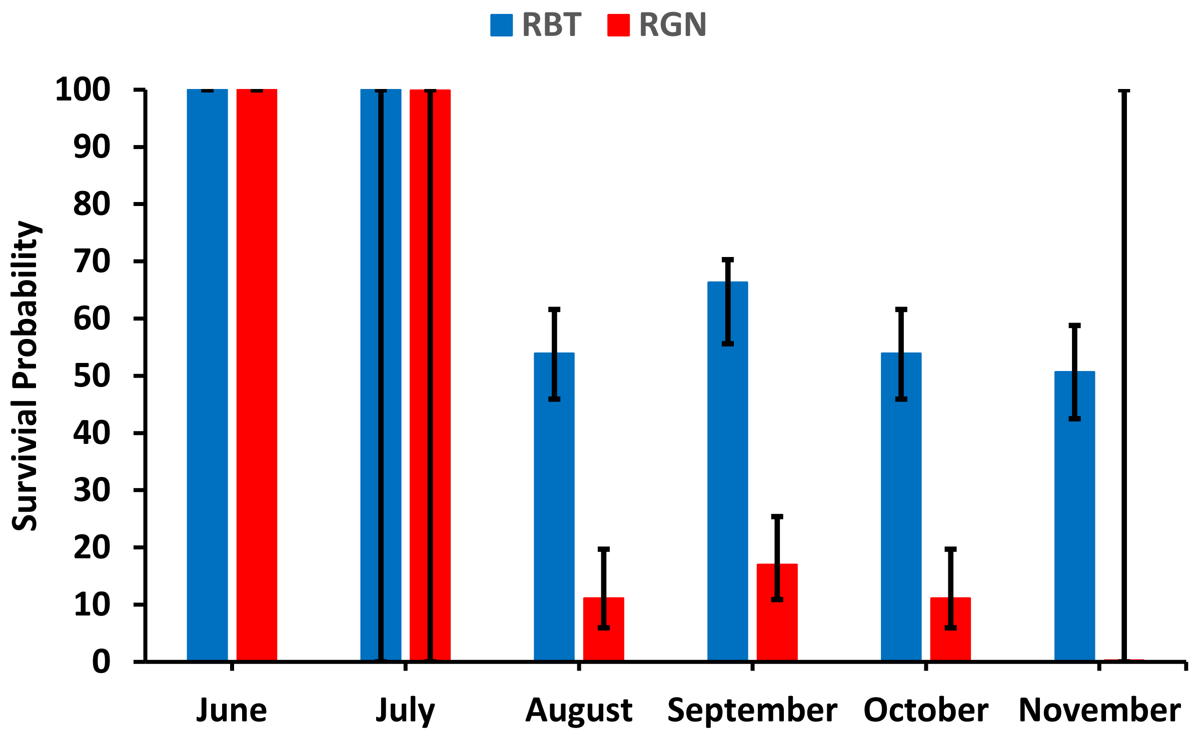

Figure 3.2: Estimated marginal means pairwise comparison of Rainbow Trout (RBT) and Rio Grande Cutthroat Trout (RGN) survival probability overall.

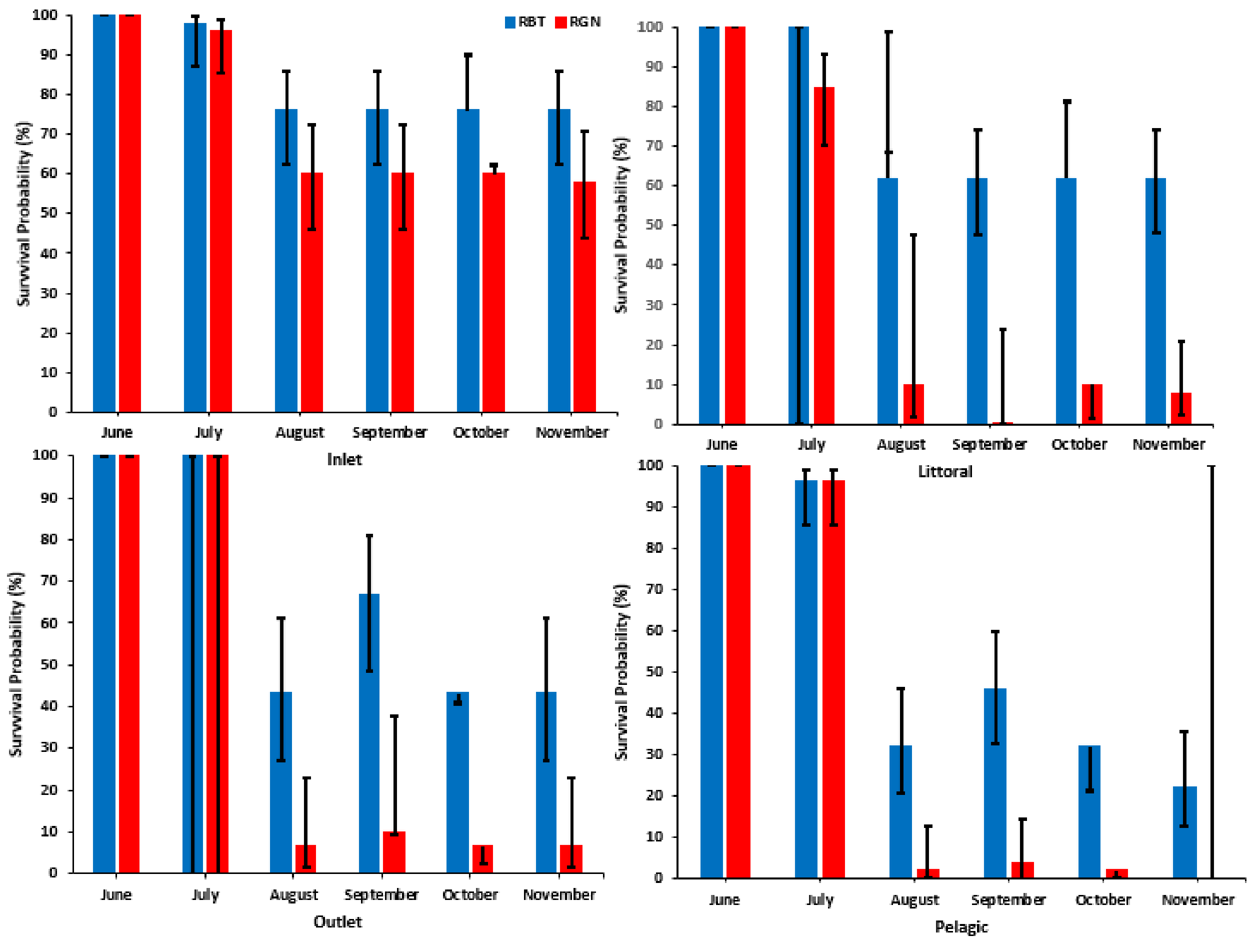

Figure 3.3: Survival probability of Rainbow Trout (RBT) and Rio Grande Cutthroat Trout (RGN) at individual habitats from June to November.

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