Incidence of *Salmincola californiensis* in Westslope Cutthroat Trout in Relation to Brook Trout Density in the Lolo Creek Drainage

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Table of Contents

List of Tables

List of Figures

Abstract

The objective of my study was to discover if the density of invasive Brook trout had an effect on the incidence of native Westslope Cutthroat trout infected by *Salmincola californiensis*. The study was conducted under a United States Fish and Wildlife collection permit while electro fishing in the Lolo Creek drainage in northwestern Montana. Streams in the Lolo Creek drainage were sampled and trout densities and infection incidence were quantified. A linear regression test was performed to test for significant associations. A significant negative correlation was observed between the Brook trout density and the incidence of *Salmincola californiensis* infection in Westslope Cutthroat. Brook trout are effective at reducing the amount of *Saslmincola californiensis* infected Westslope Cutthroat trout. My study showed an invasive species aiding a native species.

Introduction

Species of pacific salmon and trout are parasitized by the copepod *Salmincola californiensis* (Kabata and Cousens 1973). *Salmincola californiensis* (SC) is common in the Western USA and infects many species including Westslope Cutthroat Trout (WCT) *Oncorhynchus clarkii lewisi* (Modin and Veek 2002). Traditionally the distribution of SC was limited to the streams that connected to the Pacific Ocean in the Western part of the United States, but more recently fish infected with SC have been discovered outside of these waterways, including several lakes (Kamerath et al. 2009).

The six-stage life cycle of SC usually involves one host individual and can be fatal to the host (Kabata and Cousens 1973). The attachment of SC can only be accomplished with mechanical damage to the host tissues of the fish (Kabata and Cousens 1977). When the copepod attaches to the fish both macroscopic and microscopic mechanical injuries affect gill, tissue, muscle, and even bone (Kabata and Cousens 1977). External surfaces can become damaged in numerous ways: damage to the integument causing breaches in protection, forming lesions in the integument, the parasite can exert pressure on adjacent tissues resulting in their modification, and indirect damage can occur by causing the fish to use defensive movements to prevent further damage and causing the fish to expend more energy (Kabata and Cousens 1977). The extent of the damage depends on the numbers and sizes of the parasite present (Kabata and Cousens 1977). The injuries that result from the parasite may be seen as no more than irritants or can be fatal (Kabata and Cousens 1977). Heavy infestations of SC may affect oxygen uptake, reduce fecundity, slow growth, and delay sexual maturation of host fish (Kabata and Cousens 1977).

In female SC, a burrowing phenomenon is observed where she will typically burrow until a suitable hard tissue is found to implant her bulla. The bulla is an anchoring organ that allows the parasite to embed itself into the tissues of the fish while also allowing the parasite to select its attachment site (Kabata and Cousens 1972). One end of the bulla is permanently fused while the other end allows the parasite to move within the radius of the cylinder's length (Kabata and Cousens 1972). This movement allows the parasite to select the best location to anchor to the surface of the fish in which it is the most likely to remain attached. In smaller fish with softer tissues this can be lethal because she will keep burrowing and create a larger wound; anchoring to softer tissues reduces the likely hood of remaining attached (Kabata and Cousens 1977).

Most parasite attachment occurs on and around the fins and gills but parasite attachment also occurs on body surfaces including the branchial and buccal cavities of the hosts (Kabata and Cousens 1977). The attachment site is size related, shifting from body surfaces and fins in smaller fish into the gills of larger fish (Kabata and Cousens 1977). In most fork length fish between 10.2 and 27 cm the copepods are found on the pectoral and pelvic fins (Kabata and Cousens 1977).

In laboratory trials, brook trout, *Salvelinus fontinalis*, (BT) held in experimental aquaria upstream of rainbow trout effectively removed copepod larvae from the water and substantially reduced parasite infestation in the rainbow trout by at least 89% (Modin and Veek 2002). BT reduce the number SC by ingesting juveniles before they have a chance to attach to hosts. If juveniles do not attach to a host within two days they will die. (Kabata and Cousens 1973.) SC infected rainbow Trout were not found for more than two years in the hatchery after BT ponds filtered SC infested water running into the hatchery (Modin and Veek 2002). When BT were placed in holding ponds in the hatchery upstream of Rainbow Trout, no Rainbow Trout under 2 lbs were detected to have a macroscopic parasite for three years (Modin and Veek 2002).

Adult female copepods are the only SC visible to the naked eye; males die after mating and do not develop into a large enough adult copepod to generally be seen (Modin and Veek 2002). Low numbers of copepod larvae escaping through water inhabited by BT may not be numerous enough for adult males to successfully locate and mate with females (Modin and Veek 2002).

The US Fish and Wildlife Service allocates thousands of dollars each year protecting the native WCT habitat from the invasive brook trout. Brook trout out compete WCT for resources and habitat (Young 1995). However, by occupying the same stream as WCT the brook trout may be lowering the incidence of SC seen in WCT. Anglers in other states such as California have complained about so-called grubby fish (trout infected with SC). Keeping infection rates of SC down is important for both the health and esthetic appeal of the trout population.

If significant numbers of BT are in the same vicinity as WCT they may lower the incidence of SC infecting WCT. In the Lolo Creek drainage in Western Montana, WCT are infected with *SC.* Lolo Creek drainage also has significant BT populations that live among populations of WCT. The purpose of my study was to test whether the density of BT influenced the infection rate of SC on neighboring WCT.

I expect that in streams where BT density is high, SC infection rate in WCT should be lower because BT are likely eating the juvenile SC.

Methods

The technique of electro shocking was used to sample creeks in the Lolo Creek Drainage. The Lolo Creek drainage is located in the Lolo National Forest approximately 40 miles Southwest of Missoula, MT or approximately 5 miles South of Lolo Hot Springs. Streams varied in size, flow, temperature, and were separated by several miles in some cases. All streams sampled eventually drain into Lolo Creek (Fig. 1).

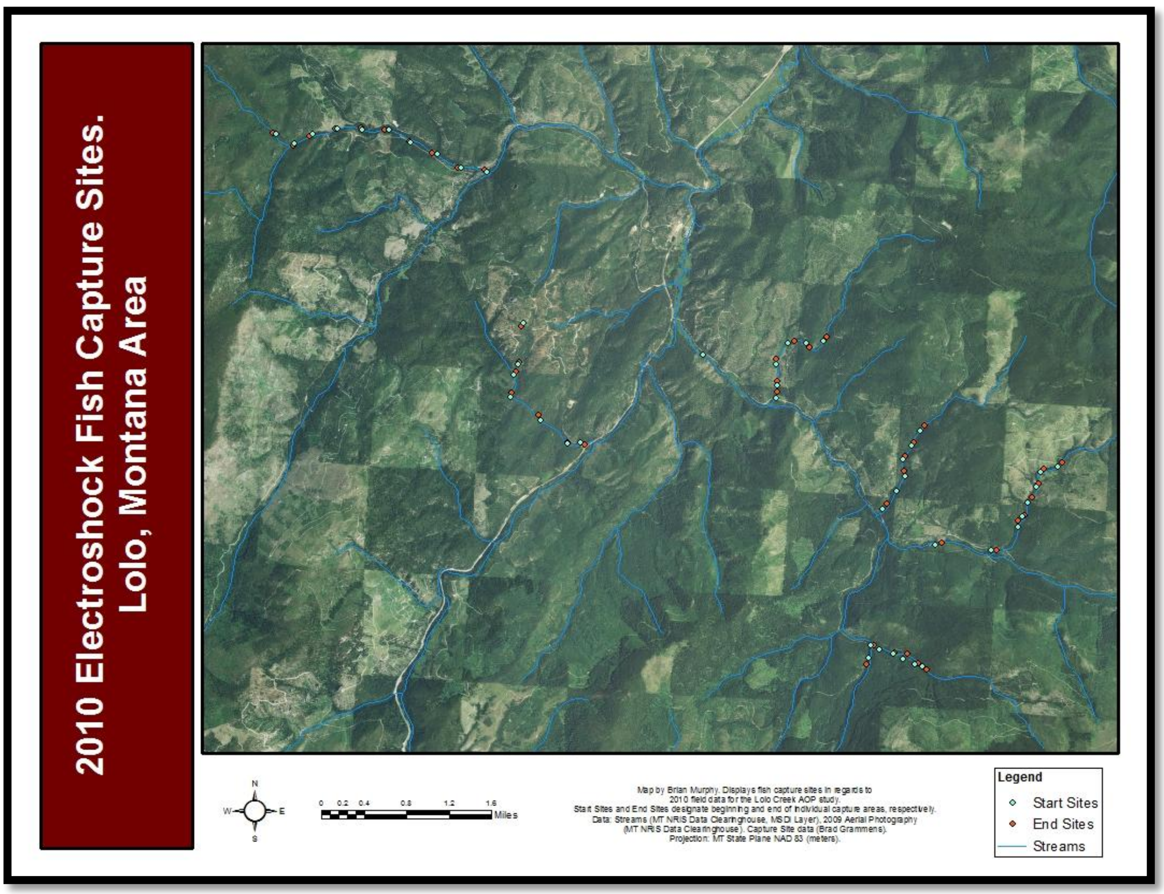

Figure 1. Map of Lolo Creek Drainage and Capture Sites

A three man team was generally used with one person wearing the Smith Roots shocker backpack and moving the anode and cathode while the other two people were netting and handling fish. The team would start at the downstream end of the randomly selected sample area and wade upstream in an attempt not to disturb fish. Fishing downstream allows fish to sense something is in the stream from soot being kicked up. Fish spooked from the team traveling upstream may still hide and burrow underneath the bank or debris; many times burrowed or hidden fish were shocked and stunned, but were unable to be netted. Fish may also sense the electrical field and hide. When these unnettable fish are stunned they may simply get caught in the debris and get stuck or swept under the bank either being out view or out of reach. Hidden fish can receive too much electrical shock from the shocker being left powered from not seeing the fish which can easily burn or prove fatal to fish if their longitudinal muscles contract too hard causing spinal injury (Dwyer et al. 2001). Fifty and 100 meter stretches were randomly selected to be sampled prior to entering the field. Sample sites were found using a combination of GPS coordinates, previously placed flags, and always using a measuring tape to measure the actual length of the stream. Widths of the actual stream were also taken every 10 meters in order to estimate the surface area of the stream.

Block nets were occasionally placed on the lower and upper portions of the sections that were sampled in order to ensure no fish escaped as well as to calculate capture efficiency. The stretches of stream that block nets were used were electro shocked three times in order to calculate capture efficiency. Capture efficiency was not calculated for this particular study, but was used in the initial study of WCT and BT barrier movement study.

Fish were placed in buckets temporarily along the bank as the team worked upstream so there would be less chance of spilling the bucket and reducing the strain on the fish. After the sample area had been thoroughly electro fished, the fish analysis began immediately. All fish were placed in a holding bucket of water until they were ready to be analyzed. Then, fish were placed into a bucket mixed with approximately 10g of Finquel per gallon of water in order to anesthetize them. Finquel is added to water in different concentration levels depending on the size and type of the fish being studied. Trout were placed in the bucket separately or very few at a time. The fish would begin to roll ventral side up within 3 to 5 minutes; the loss of equilibrium indicated that the fish were ready to be handled. If the trout rolled earlier than this, the mixture was too strong and was diluted. If the trout took longer 3 to 5 minutes to roll the mixture was strengthened. If the fish were left in the Finquel mixture too long this proved fatal especially when water temperatures and or air temperatures were too warm. If air and or water temperatures were too warm the Finquel mixture was diluted to put less strain on the fish.

The trout were immediately measured, weighed, and inspected for SC. Infected WCT trout were quantified as having the parasite visibly attached on a surface or as not having SC. This was used to calculate a proportion of infected WTC. The trout were then batch marked with a hypodermic syringe filled with different colored paint for different capture sights and were injected under their lower mandible skin in order to mark them for future recapture. The trout received a small incision on their ventral side and a PIT (Passive Integrated Transmitter) tag was inserted. The fish were then allowed to recover and were released. If fish were not recovering in a normal allotted time they were resuscitated by moving them back and forth through fresh water in order to get more oxygen into their gills to aid in their recovery. On certain occasions trout would be kept in holding nets overnight placed in deep pools in the stream to check survival rate as well as to make sure that PIT tags did not fall out of the fish.

Results

The data suggest BT density has an influence on the SC infection rate in WCT. (Table 1). A negative correlation was observed between the BT density and the infection rate of SC in WCT (Table 2 and Figure 2).

Table 1. Data for Analysis

Streams	Number of Cutthroat Trout	Number of Infected Cutthroat Trout	Percentage of Infected Cutthroat	Estimated Stream Surface area (m^2)	Number of Brook Trout per meter sampled
Sugar Bear Creek	11	8	72.73	868.5	0.02
N Fk Granite Cr	26	24	92.31	1817.5	0.01
Stream 521	87	32	36.78	1105.4	0.07
Stream 523	39	4	10.26	976.2	0.06
E Fk Lolo Cr	74	40	54.05	1798.2	0.04
Lost Park Cr	31	24	77.42	312	0.02
Sally Basin Cr	10	4	40	490	0.02

Table 2. Statistics for Linear Regression Test

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	90.81	11.73	7.74	0.001
X Variable 1	-1093.98	303.41	-3.61	0.02

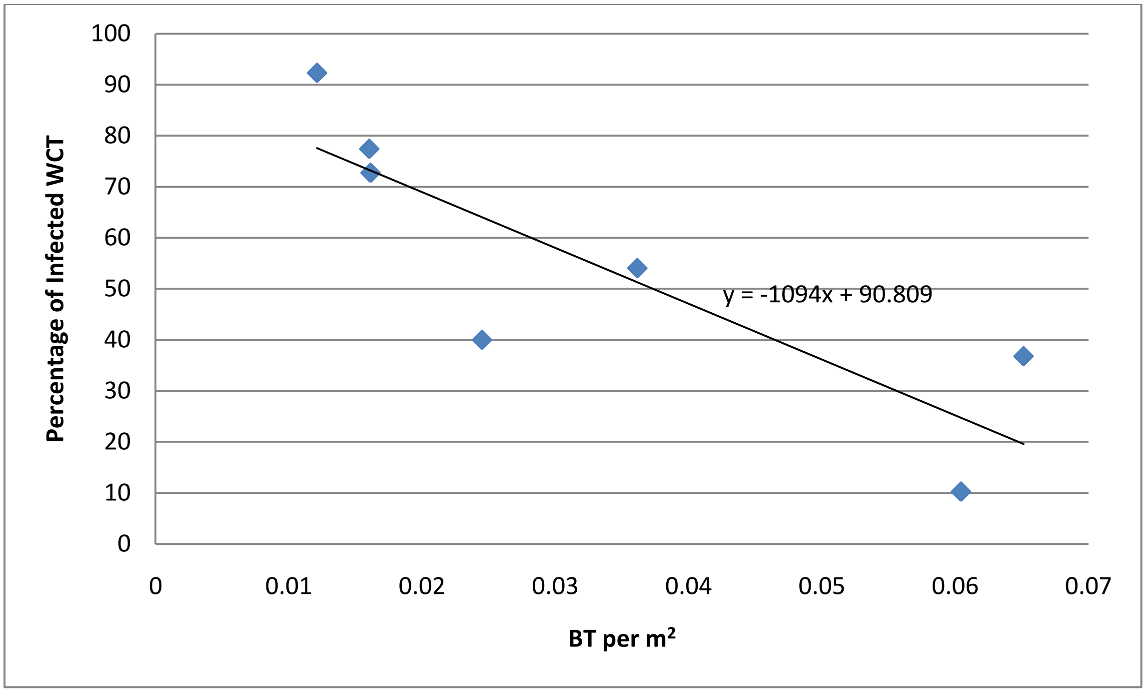

Figure 2. The relationship between infected WCT and concentration of BT

Discussion

A negative correlation was observed between the density of BT and the occurrence of the parasite SC in native WCT. The calculated p-value of 0.02 suggested a strong negative correlation between the percentage of infected WCT and the density of BT. When BT were in the same vicinity as WCT the infection rates were lower with higher concentrations of BT. -Thus, BT trout appear to reduce the infection rates of SC in WCT.

The strong correlation suggests that BT reduce the numbers of visible SC copepods. Female SC copepods get large enough to be seen by the naked eye and are usually the parasites seen on the infected fish. BT reduce the numbers of SC by ingesting the juvenile copepods and not allowing them to attach to a host fish. It is difficult to know if SC specimens other than the juvenile copepods are being ingested by the BT as well. By eliminating juvenile copepods the BT do not allow the juveniles to mature and attach to host fish. In this way BT effectively reduce the population of visible and possibly all forms of SC.

In the Lolo Creek drainage most of the WCT sampled were smaller fish. SC is a health concern to WCT, especially to the younger smaller trout that have more soft tissue. SC burrow into this soft tissue. This can be more of an issue in smaller fish because the parasite has more of a chance of burrowing into tissues that are important to fish survival (Kabata and Cousens 177). Also in smaller fish the parasite takes more nutrients per unit of fish causing more stress than the same parasite attached to a larger fish. In many cases SC has been noted in destroying gill tissue. Although none of the fish sampled had an excessive amount of parasites, the parasites could have a negative impact on the overall health of the fish. The infected trout's gills were not extensively searched, but SC infection was not noted on the gills. None of the fish captured had life threatening levels of SC. In the Lolo Creek Drainage the parasites appeared to be more of a nuisance than a serious health threat.

My study suggests that BT are effective at reducing the number of SC infected populations of WCT. Because WCT are a native Montana species, they are being protected against invasive species such as BT that out compete them. Even though reducing the number of SC aids WCT, at the low levels of infection seen in the Lolo Creek Drainage BT likely inhibit WCT more through competition for food and habitat rather than aiding them through parasite load reduction (Young 1995). BT hatch earlier in the spring than do WCT giving them a possible size advantage over the WCT. Because BT are born earlier in the season, this may also give them an advantage in not getting infected by SC because of the changing flows and distribution of trout at that time.

My data were collected in one season during the months of July and August. Stream temperatures and flows vary dramatically throughout the year. SC could have a greater or lesser impact during different seasons. The initial study was being conducted to discover movements of BT and WCT through replaced culverts as well as stream road crossing. BT and WCT are not always able to move through culverts at the same time limiting one species to a particular zone of the river (Peterson et al. 2008). BT at other research sites tended to travel upstream in the summer (Peterson and Fausch 2003). This probable increase in BT in the summer months may have had an impact on the incidence of BT at the time of collections. Many of the culverts on the smaller streams were impassable at the time of sampling for any species of trout because of the gap from the culvert down to the stream at the downstream side of the culvert. Also in different river flows the current is too strong for trout to move up through culverts because the water is channeled into small diameter culverts that increase the current speed. Culverts have no barriers in them to break the current to give trout pockets of slower current in which to rest. At certain flows, the parasite may be limited because of the movement of infected WCT. Water temperature as well as current, and environment may also impact the incidence of SC on WCT.

Future research may be centered on BT densities upstream of SC infected WCT. Sampling could be conducted downstream of high density populations of BT. In the Modin and Veek (2002) study, BT were held upstream in order to lower incidence of SC. Sampling could be conducted at different distances from WCT to see how close populations of BT need to be in order to reduce the numbers of SC infected fish. Possibly a single isolated population of BT could keep a population of WCT downstream free of SC. Fish isolation barriers have been considered to separate native populations of fish from invasive species of trout, but barriers have the potential to exacerbate problems of isolating native populations to smaller habitats (Peterson et al. 2008). It would be useful to test if an isolated population of BT upstream of WCT would clean the downstream side of this isolated population of BT of SC. Larger water systems as well as streams in different types of environments could be sampled to discover if this type of relationship is exhibited in larger water systems with more fish and if different environments have an effect. Different types of stream beds with different amounts of vegetation would also be sampled to see if these have effects as well. Sampling would also be conducted throughout different seasons in the year to observe at what points the parasite is the most prevalent. Regardless, my study suggests BT may be effective at reducing parasite load in native WCT in Western Montana.

Works Cited

Barndt, S., Stone, J. (2003). Infestation of *salmincola californiensis* (Copepoda: Lernaeopodidae) in wild coho salmon, steelhead, and coastal cutthroat trout juveniles in a small columbia river tributary. *Transactions of the American Fisheries Society,* 132, 1027-1032.

Kabata, Z., Couesens, B., (1972). The structure of the attachment organ of Lernaeopodidae (Crustacea: Copepoda). *Fisheries Research Board of Canada Pacific Biological Station,* 29(7), 1015-1023.

Kabata, Z., Couesens, B. (1973). Life cycle of *Salmincola californiensis*. *Journal Fisheries Research Board of Canada,* 30(7), 881-903.

Kabata, Z., Couesens, B., (1977). Host-Parasite relationships between sockeye salmon, Oncorhynchus nerka, and Salmincola californiensis (Copepoda: Lernaeopodidae). *Fisheries Research Board of Canada Pacific Biological Station,* 34, 191-202.

Kamerath, M., Allen, B.C., Chandral, S. (2009). First documentation of *Salmincola Californiensis* in Lake Tahoe, CA-NV, USA. *Western North American Naturalist*, 69(2), 257-259.

Modin, J.C., Veek, T.M., (2002). Biological control of the parasitic copepod *Salmincola californiensis* in a commercial trout hatchery on the Lower Merced River, California. *North American Journal of Aquaculture,*64, 122-128.

Peterson, P. Douglas, Fausch, D. Kurt, (2003). Upstream movement by nonnative brook trout (*Salvelinus fontalis*) promotes invasion of native cutthroat trout (*Oncorhynchus clarki*) habitat. *Canadian Journal & Aquatic Sciences,* 60, 1502-1516.

Petterson, D.P., Rienman, B.E., Dunham, J.B., Fausch, K. D., Young, M.K., (2008). Analysis of trade-offs between threats of invasion by nonnative brook trout (*Salvelinus fontinalis*) and intentional isolation for native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). *Canadian Journal & Aquatic Sciences,* 65, 557-573.

Dwyer, W.P, Shepard, B.B., White, R.B., Effect of Backpack Electroshock on Westslope Cutthroat Trout Injury and Growth 110 and 250 Days Posttreatment. *North American Journal of Fisheries Management,* 21, 646-650.

Young, M.K. (Editor). (1995). Conservation assessment for inland cutthroat trout. Rocky Mountain Forest and Range Experimental Station, U.S. Department of Agriculture Forest Service, General Technical Report RM-GTR-226, Fort Collins, Co.