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The Spatial and Temporal Distribution of *Tetracapsuloides bryosalmonae* in the Beaverhead River of Southwest Montana

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Abstract

The purpose of this study is to use environmental DNA (eDNA) analysis to determine the spatial and temporal distribution of *Tetracapsuloides bryosalmonae* in the Beaverhead River drainage of southwest Montana. *Tetracapsuloides bryosalmonae* is a microscopic parasite that causes Proliferative Kidney Disease (PKD) in salmonid fish. An outbreak of PKD in the Yellowstone River in summer of 2016 resulted in a significant kill of mountain whitefish (*Prosopium williamsoni*); however, the exact reason for this outbreak remains unknown. The leading hypothesis is that it was due to environmental conditions. To determine the effects of environmental conditions on parasite distribution, researchers have started to examine the spatial and temporal distribution of *T. bryosalmonae* in the Yellowstone River and other freestone rivers with no history of PKD outbreaks. We thought it would be useful to compare the parasite distributions from these freestone rivers to a tailwater fishery exhibiting different environmental conditions. The Beaverhead River was a suitable tailwater candidate. Beginning in June 2019, we collected monthly eDNA samples from four locations on the Beaverhead River starting just below Clark Canyon dam and moving progressively farther downstream. In addition, we sampled two locations on Clark Canyon Reservoir. These samples were screened by PCR using primers specific for *T. bryosalmonae*. PCR results indicated that *T. bryosalmonae* was present in the Beaverhead River from June through September. We detected the strongest PCR signals in the river just below the dam and the parasite was barely detectable at the site farthest from the dam. The two locations on Clark Canyon Reservoir exhibited the strongest overall signals and positive river samples were always accompanied by positive reservoir samples. To our knowledge, this is the first report of *T. bryosalmonae* in the Beaverhead River system and suggests that Clark Canyon Reservoir may be the source of the parasite.

Keywords: Proliferative Kidney Disease, Salmonids, Myxozoan Parasites

1. Introduction

Tetracapsuloides bryosalmonae is the myxozoan parasite that causes proliferative kidney disease (PKD) in salmonid fish[1]. PKD is characterized by an autoimmune response to the reproduction of *T. bryosalmonae* in the kidney of salmonids. This leads to the proliferation and damage of kidney tissue that can be fatal to the fish host. In the summer of 2016, the Yellowstone River in Montana experienced a severe outbreak of PKD which killed a significant number of mountain whitefish (*Prosopium williamsoni*). Although a previous PKD outbreak had been reported in a remote Montana reservoir[2], this was the first documented outbreak of the disease in one of the state's rivers. Fearing the outbreak of PKD on the Yellowstone River was due to a novel invasion of *T. bryosalmonae*, managers closed 186 miles of the river to all recreational activities for several weeks to prevent any potential spread of the parasite. Due to the large portion of revenue created by recreation on the Yellowstone River; Park County, Montana experienced negative economic impacts during the closure[3].

T. bryosalmonae has a complex life cycle that alternates between salmonid fish and freshwater bryozoans[4]. Bryozoans are a phylum of suspension-feeding colonial invertebrates that inhabit a variety of freshwater systems (i.e. rivers, lakes, ponds) and live on submerged roots, branches, and rocks[5]. The life cycle begins when *T. bryosalmonae* spores with two polar capsules are released in the urine of an infected fish and infect the bryozoan host (Figure 1). It is likely that bryozoans ingest the spores while filter feeding. The infection begins as a covert infection in which the parasite is reproducing in the bryozoan host as a single cellular unit. The infection is considered to be overt with the development of multi-cellular spore sacs that can be observed using light microscopy. Cycling between covert and overt infections is known to occur in the bryozoan host.

During overt infections bryozoans release spores with four polar capsules that are infective to salmonid fish. Infection of salmonids is initiated when *T. bryosalmonae* penetrates the gills with extrusible filaments contained within the polar capsules. The infection further develops when *T. bryosalmonae* enters the kidneys. This is where reproduction and subsequent development of spores with two polar capsules takes place. Spores from the salmonid host are then released through the urine and are again infective to bryozoans. In laboratory settings, the development of PKD in the salmonid host is dependent on temperature[6]. Although *T. bryosalmonae* is capable of infecting and causing PKD in all salmonids, thus far only brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) have been shown to produce viable spores capable of infecting bryozoan hosts[7,8]. There are five genera of bryozoans that can be experimentally infected with *T. bryosalmonae*, but two (*Fredericella* and *Plumatella*) are commonly found in association with disease outbreaks[4]. Very little is known about bryozoan species present in Montana or which species may serve as hosts for *T. bryosalmonae* in Montana rivers.

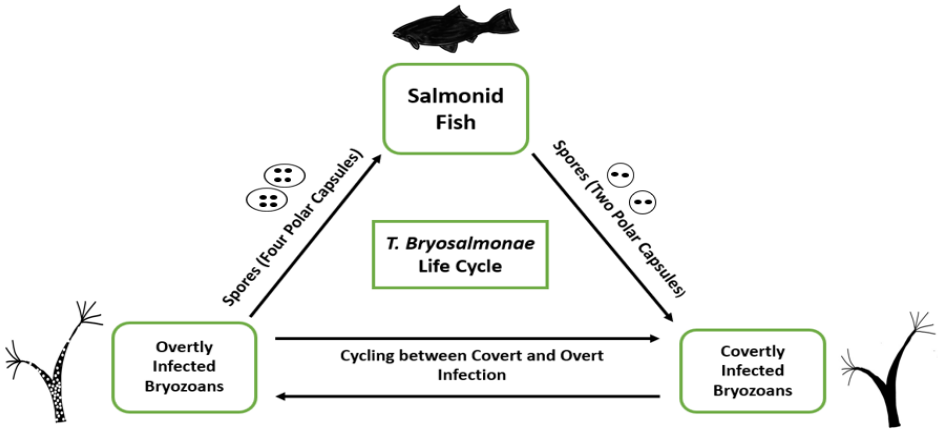

Figure 1. Life cycle diagram of *Tetracapsuloides bryosalmonae*.

As mentioned above, management initially thought the 2016 outbreak of PKD in the Yellowstone River was due to a novel invasion of *T. bryosalmonae*; however, PCR analysis of preserved fish kidney samples collected from the Yellowstone River in 2012 showed that the parasite was present prior to the outbreak[9]. This led to the hypothesis that the outbreak was due to stressful environmental conditions such as low flows and high water temperatures. Fish samples from other freestone rivers with environmental conditions similar, or even worse, than those seen on the Yellowstone River were also collected in 2016. The analysis of these samples showed that the parasite was also present in other rivers in Montana, yet no fish kills were observed. This suggests that environmental conditions alone were not responsible for the 2016 disease outbreak[9].

Due to the economic importance of recreational fishing in Montana, researchers from state and federal agencies are continuing to monitor the distribution of the parasite on the Yellowstone and other Montana rivers using a variety of techniques including environmental DNA analysis. Environmental DNA analysis, commonly referred to as eDNA analysis, has become an increasingly common way of detecting both freshwater and marine aquatic organisms and documenting their distribution [10, 11]. More specifically, the use of eDNA has been successful in detecting many groups of aquatic parasites [11]. Recently, both conventional and quantitative PCR primers have been developed for the detection and quantification of *T. bryosalmonae* DNA in eDNA samples [12, 13]. The efforts by state and federal agencies to Monitor the parasite have been primarily focused on freestone rivers (i.e., rivers not impeded by dams) in the Greater Yellowstone Area. In contrast, there have been very few efforts to determine if the parasite is present in Montana's tailwater fisheries (i.e., waters immediately downstream of a dam with controlled flows). The Bighorn

River below Yellowtail Dam, the Missouri River below Holter Dam and the Beaverhead River below Clark Canyon Dam are world-renowned trout fisheries and economically important resources for nearby communities.

In 2017, one sample was taken from the Bighorn River and one sample was taken from the Missouri River and both of these samples tested positive. No efforts have been made to determine if *T. bryosalmonae* is present in the Beaverhead River or Clark Canyon Reservoir. In addition, there have been no attempts to determine the spatial and temporal distribution of *T. bryosalmonae* in a tailwater fishery. The purpose of this study was to use eDNA analysis to look for the presence of *T. bryosalmonae* in the Beaverhead River and Clark Canyon Reservoir of southwest Montana and describe the parasite's spatial and temporal distribution in the tailwater section below Clark Canyon Dam. Due to the widespread distribution of *T. bryosalmonae* in Montana rivers, we expected the parasite to be present in the Beaverhead River. However, we had no expectations regarding the spatial or temporal distribution of *T. bryosalmonae* within the river, nor the presence or distribution of the parasite in Clark Canyon Reservoir.

2. Methodology

2.1 Study Area and Sample Locations

The Beaverhead River is located in southwest Montana near the city of Dillon. It starts at Clark Canyon Dam and flows northeast for approximately 80 miles before joining with the Big Hole River to form the Jefferson River. The first 16 miles of the Beaverhead river, from Clark Canyon Dam to the Barrett's diversion Dam, is one of Montana's premier tailwater trout fisheries and served as our study area. Six sample locations were chosen within this area (Figure 2). Two sample sites were on Clark Canyon Reservoir near the dam. One site was located on the west side of the dam (site CCW) and the other was located on the east side of the dam (site CCE). Four sites were located along the Beaverhead River below the spillway of Clark Canyon Dam. Site A was located immediately below the spillway. Site B was located approximately 1.8 river miles downstream from site A. Site C was located approximately 7.3 river miles downstream from site A and site D was located just above the Barrett's diversion dam approximately 15.5 river miles downstream from the spillway of Clark Canyon Dam. The only significant tributary of the Beaverhead River in our study area is Grasshopper Creek. The confluence of the Beaverhead River and Grasshopper creek is between sites C and D, approximately 11.8 river miles downstream from Site A.

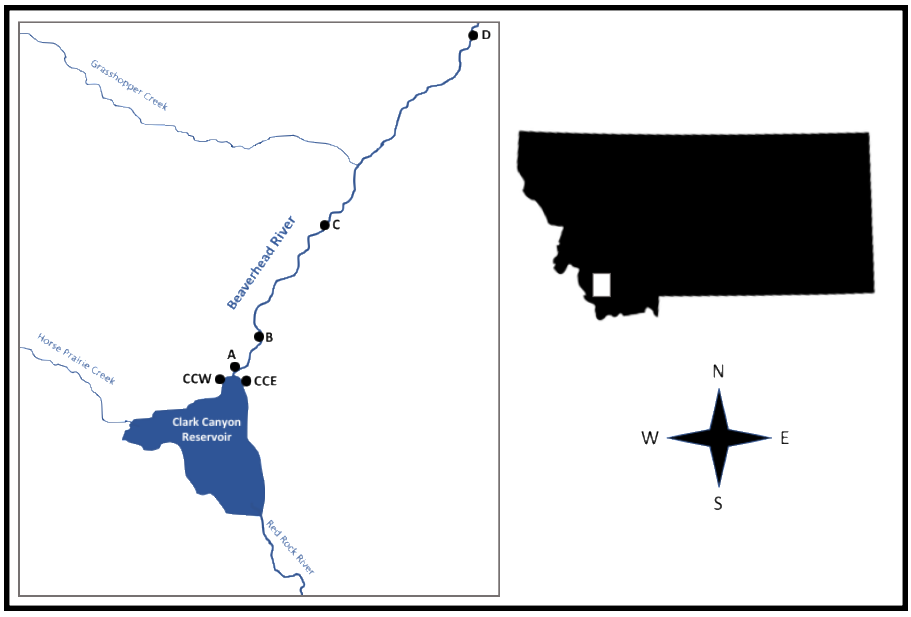

Figure 2. Map of showing relative location of the six sample sites on Clark Canyon and the Beaverhead river.

2.2 Sample Collection and DNA Extraction

Monthly samples were collected from all six sites in the study area (Figure 2) from June through November of 2019. In December 2019 Clark Canyon Reservoir was frozen. Therefore, only the Beaverhead River sites (i.e., A, B, C and D) were sampled. Two-liter water samples were collected from each site in sterile Whirlpak bags. The samples were vacuum filtered through Whatman glass fiber filters (1.5 mM) on site. The filters were then immediately placed in 1.5 ml microfuge tubes containing 360 ml Qiagen buffer ATL and 40 ml of proteinase K using sterile forceps. After a 24 hour incubation at 56 °C, 200 ml of the lysate was transferred to a fresh 1.5 ml microfuge tube and the DNA was extracted using the Qiagen DNAeasy Blood and Tissue kit (Qiagen.com) according to the manufacture's protocol. Extracted DNA samples served as template DNA for PCR analysis.

2.3 PCR Analysis

PCR reactions took place with primers PKX-18s-1266f and PKX-18s-1426r which are specific for *T. bryosalmonae* 18S rDNA[13.] The DNA was amplified in 50-ml reactions containing 0.5 mM of each primer, 400 mM of each dNTP, 1.5 mM MgCl2, 2.5 units of Taq DNA polymerase (New England Biolabs) and 5-ml of Standard Taq Buffer and 1-ml of eDNA sample as template. PCR inhibitors are often present in eDNA samples[14]. To avoid the effects of these inhibitors, a dilution series of each eDNA sample was made (i.e., 1:10, 1:100, 1:1000, 1:10000) and 4 additional PCR reactions using 1-ml of eDNA dilution as template was conducted. Positive control reactions contained 1-ml of *T. bryosalmonae* DNA as template. Negative control reactions, in which template DNA was substituted with 1-ml of water, were run periodically to ensure that positive results were not due to contaminated reagents or equipment. The thermal cycler was programmed for a 5-min hot start at 95 °C, then 35 cycles of 1 min at 94 °C, 1 min at 54 °C, and 1 min at 72 °C. This was followed by a 5-min post amplification step at 72 °C. The PCR products (10-ml from each reaction) were loaded into 2% submarine agarose gels containing 0.5 mg/ml ethidium bromide and subjected to electrophoresis for 40 min at 100 V. The DNA bands were visualized by transillumination with ultraviolet light (254 nm). The presence of *T. bryosalmonae* 18S rDNA gives rise to a 160 bp product.

3. Results

T. bryosalmonae DNA was detected at all sample sites from June through October 2019 (Table 1). In November 2019, *T. bryosalmonae* DNA was only detected at the two sites on Clark Canyon Reservoir (CCW and CCE) and sites A and B, the two sites on the Beaverhead River closest to Clark Canyon Dam (Table 1). In December 2019, Clark Canyon Reservoir was frozen over so only the Beaverhead River sites (i.e. A, B, C and D) were sampled and *T. bryosalmonae* DNA was only detected at site A (Table 1). During the period from June through October, when *T. bryosalmonae* DNA was detected at all sample sites, we obtained strong PCR signals from the two sites on Clark Canyon Reservoir (CCW and CCE) and sites A and B, the two sites on the Beaverhead River closest to Clark Canyon Dam; however, we obtained very weak PCR signals (i.e., the 160 bp band indicative of *T. bryosalmonae* DNA was barely visible) from sites C and D. The results from August show this trend (Figure 3). The only exception to this observed trend occurred during June 2019 when we obtained a very strong PCR signal from the site D sample (Table 1, Figure 4).

Table 1. Results from PCR analysis. (+) indicates a positive result (*T. bryosalmonae* DNA detected). (–) indicates a negative result. (±) indicates a weak positive result. (N.D.) sampling not done.

Site	June	July	Aug	Sept.	Oct.	Nov.	Dec.
CCW	+	+	+	+	+	+	**N.D.**
CCE	+	+	+	+	+	+	**N.D.**
A	+	+	+	+	+	+	±
B	+	+	+	+	+	+	–
C	±	±	±	±	±	–	–
D	+	±	±	±	±	–	–

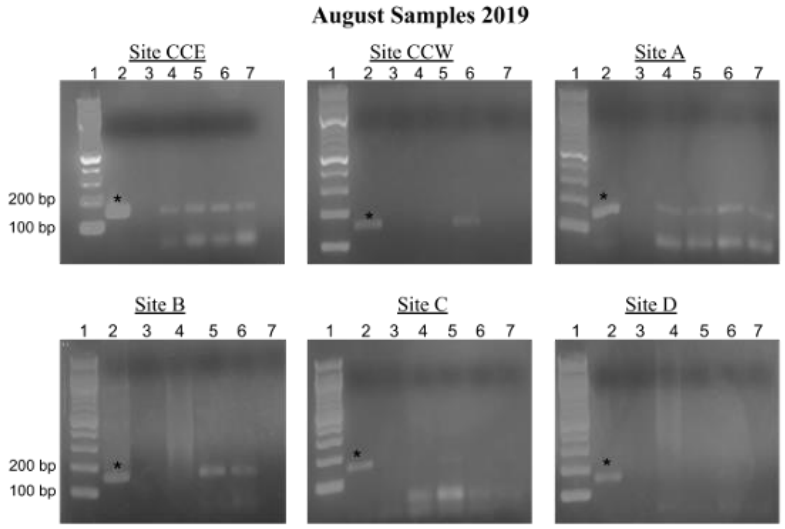

Figure 3**.** 2% agarose gels showing the results of PCR from eDNA samples taken in August of 2019. Lane 1- 100 bp ladder, Lane 2- positive control, Lane 3- undiluted eDNA sample, Lane 4- 1:10 dilution, Lane 5- 1:100 dilution, Lane 6- 1:1000 dilution, Lane 7- 1:10000 dilution. The presence of *T. bryosalmonae* 18s rDNA gives rise to a 160 bp product. Positive control band is indicated by an *.

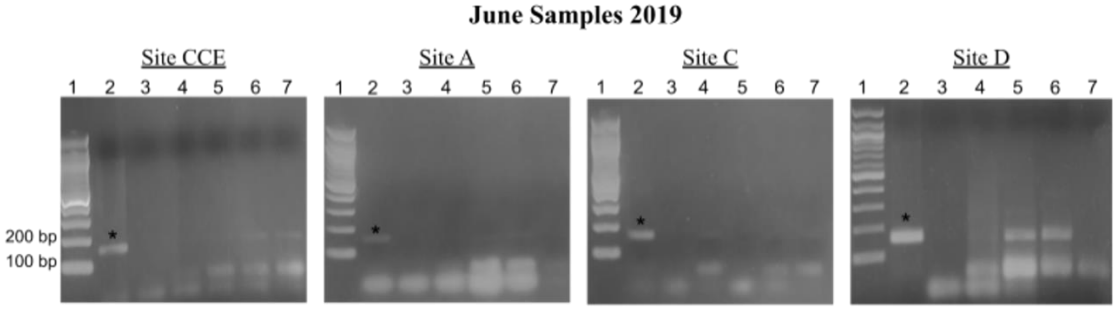

Figure 4. 2% agarose gels showing the results of PCR from eDNA samples taken from sites CCE, A, C and D in June of 2019. Lane 1- 100 bp ladder, Lane 2- positive control, Lane 3- undiluted eDNA sample, Lane 4- 1:10 dilution, Lane 5- 1:100 dilution, Lane 6- 1:1000 dilution, Lane 7- 1:10000 dilution. The presence of *T. bryosalmonae* 18s rDNA gives rise to a 160 bp product. Positive control band is indicated by an *.

4. Discussion

To our knowledge, this is the first report of *T. bryosalmonae* in the Beaverhead River and Clark Canyon Reservoir. We obtained strong PCR signals from Clark Canyon Reservoir (i.e., sites CCW and CCE) from June through November. We also obtained strong PCR signals in the Beaverhead River near the spillway of Clark Canyon Reservoir (i.e., sites A and B) during this same period. However, at sites farther downstream from the Dam (i.e., sites C and D) the signal was either very weak (i.e., the 160 bp band indicative of *T. bryosalmonae* DNA was barely visible) or not detected. These results suggest that Clark Canyon Reservoir may be the source of the T. bryosalmonae DNA detected in the Beaverhead River. The only exception to this trend was observed in June 2019 when we obtained a very strong PCR signal from the site D. Site D is the only site downstream of the confluence of Grasshopper Creek, the only significant tributary of the Beaverhead River in our study area. At the time the June sample was taken, Grasshopper Creek was discharging high levels of water into the Beaverhead River, relative to its normal flows, due to melting snow. Therefore, it is possible that Grasshopper Creek was the source of T. *bryosalmonae* that gave rise to the strong PCR signal detected at site D in June.

The presence of parasite DNA is not indicative of diseased fish, fish mortality, or viable *T. bryosalmonae* spores. Therefore, the results of this study cannot be used to assess the threat of a potential PKD outbreak on the Beaverhead River. However, these results raise some interesting possibilities regarding the spatial distribution of the parasite in this drainage (i.e., that Clark Canyon Reservoir and Grasshopper Creek may be potential sources of *T. bryosalmonae*). Obviously, this warrants further study of the two tributaries that flow into the reservoir (Horse Prairie Creek and the Red Rock River) as well as Grasshopper Creek. As such, these results will be extremely useful in the design of a long term study that will attempt to more accurately describe the temporal and spatial distribution of the parasite in this drainage and form correlations between environmental conditions and these distributions. In addition, this long-term study will attempt to gain insight into bryozoan species present in the Beaverhead River system and which species are serving as hosts for *T. bryosalmonae*. Ultimately, this information may help fisheries managers reduce the potential for PKD outbreaks in our wild trout populations.

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