WHIRLING DISEASE LABORATORY STUDIES REPORT

Lab Studies

Species susceptibility
Fish Size vs Infection Rate
Impact on Growth
Montana Fish, Wildlife and Parks

Project 3860

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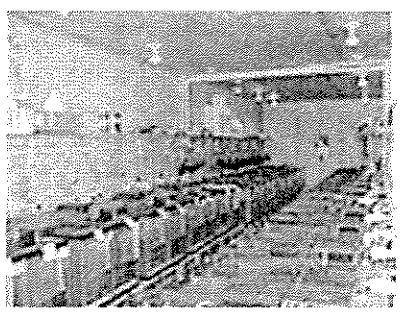

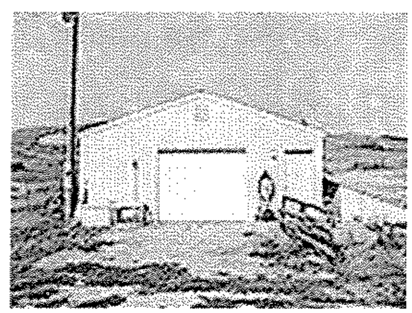

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Whirling Disease Studies Part I - Factors Effecting Fish Infection Rates

General Introduction

Whirling disease research in Montana during 1999 was focused in two main areas: **Part I** - Controlled laboratory experiments at the Pony WD laboratory trying to determine relative susceptibility of various species and strains of species to infections by the parasite Myxobolus cerebralis. **Part II** - Field sentinel cage studies attempting to determine impacts of whirling disease on wild trout populations in some of Montana=s high quality wild trout fisheries.

The Field Studies were as follows: 1) Continued survey of Montana=s cool water fisheries for the presence and intensity of *Myxobolus cerebralis* infections and 2) more detailed whirling disease research on certain high quality wild trout streams emphasizing spatial and seasonal changes in infection intensities. Streams included under the special research projects are as follows: Big Blackfoot River and tributaries, Madison River and tributaries, Little Prickley Pear Creek/mid-Missouri River system, Rock Creek of the Clark Fork River and Willow Creek, a tributary to the Jefferson River.

Part Ia – Relative susceptibility of various salmonid species and strains of species to whirling disease infections

Background

In order to determine the level of risk various wild populations of salmonids in Montana have to whirling disease infections the following information is essential: 1) presence or absence of the alternate host *Tubifex tubifex* in each wild salmonid stream; 2) relative abundance of *T. Tubifex* in each stream; 3) seasonal water temperature profiles; 4) species or strain of salmonid present in each stream 5) the relationship of water flow rate and whirling disease infection intensity and 6) when salmonids at the critical age and size are present in each stream. Each of these factors plays a role in whether a particular wild salmonid population will suffer population level damage. This report will focus on two of these factors: relative susceptibility to Myxobolus cerebralis infections by species and strains and the relationship of age and size of fish to infection intensity. Sentinel cage work in Montana from 1996-98 has already shown a wide variability in whirling disease infection intensities from stream to stream and year to year (Vincent, 2000).

The relative susceptibility of various species or strains of salmonids to infection by the parasite *M. cerebralis* is governed by a number of factors including genetics, age and size of the fish at the time of exposure, water temperature, and dosage. Most of the existing whirling disease infected waters in Montana contain a mixture of rainbow and brown trout with a small contingent of native wild Westslope or Yellowstone cutthroat trout (*O. clarki lewsi* or *O. clarki bouvier).* But there are many western cold water stream's which have significant populations of the native salmonids (Westslope cutthroat trout, Yellowstone cutthroat trout and bull trout – *Salvelinus confluentus*). Certainly many of these wild native trout streams have the right conditions to have viable whirling disease infections, thus determining their relative susceptibility to the parasite is very important. Comparisons between *M. cerebralis* infected brown and rainbow trout where they coexist in a particular Montana wild trout stream show that rainbow trout not only exhibited a much higher lesion severity and rate of infection than brown trout, but the parasite tended to concentrate in the cranial cartilage in rainbow trout versus gill arches in the brown trout (Baldwin et al. 2000). In controlled lab exposures, Hedrick et al. (1999) found rainbow trout were much more susceptible to whirling disease infections when compared to either Westslope cutthroat trout (*Oncorhynchus clarki lewisi*) or Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*). In contrast, lake trout (*Salvelinus namaycush*) and arctic grayling (*Thymallus arcticus*) were found to experience a very low disease severity. The age of fish when exposed to various dosages of triactinomyxon appeared to regulate the rate and intensity of the whirling disease infection in rainbow trout (Ryce et al. personal communication). Water temperatures also were found to regulate the infection intensity in rainbow trout during field exposures in the Madison River, Missouri River, Willow Creek, and Little Prickley Pear Creek, (Vincent, unpublished data). In these studies, field exposures of rainbow trout showed the highest infection intensities at water temperatures between 12-16^0 C with infection intensities declining rapidly as mean daily water temperatures decreased or increased from these optimum water temperatures.

Species susceptibility studies in Montana.

Methods

From 1997 through 1999, a total of 18 strains, species or subspecies have been tested to determine relative species susceptibility either at the Pony Whirling Disease Laboratory or at the Willow Creek field station (Vincent, 2001). The laboratory susceptibility experiments were utilized various dosage levels of triactinomyxon (100 to 4000 per fish) in 7.6 liters (two gallons) of water for two hours and then placed in 114 liter (30 gallon) aquariums and held for 90 days at 10 C. Test fish were usually 60-70 days old and approximately 35-55 mm in length. At 90 days of age, fish were sacrificed and sent to the Washington State University Animal Diagnostic Laboratory at Pullman, WA., where histology was using on the cranial cartilage with the degree

Table 1. Grading scheme used to quantify M. cerebralis lesion severity.

Grade	Description
0	No abnormalities noted
1	Discrete, rare (usually single), small foci of cartilage degeneration with intralesional *M. cerebralis* myxospores /generative stages present. Any associated leukocyte infiltrates are small.
2	Single, locally extensive focus or several smaller foci (usually 2) of cartilage degeneration/necrosis with intralesional *M. cerebralis* myxospores /generative stages. Lytic foci typically surrounded and/or infiltrated by few to moderate numbers of leukocytes.
3	Multiple foci of cartilage degeneration/necrosis (usually 3 or 4) with intralesional M. cerebralis myxospores / generative stages. Moderate numbers of leukocytes typically associated with lytic cartilage.
4	Multifocal (usually > 4 sites) to coalescing, often locally extensive areas of cartilage degeneration /necrosis with intralesional *M. cerebralis* myxospores/ generative stages. Moderate to large numbers of leukocytes typically border and/or infiltrate degenerate cartilage.
5	Multifocal (6 or more) to coalescing areas of cartilage necrosis, with locally extensive destruction in at least one focus (preferably more) are present and have intralesional *M. cerebralis* myxospores/generative stages. Moderate to large numbers of leukocytes typically border and/or infiltrate necrotic cartilage.

of parasitic damage was given a score from 0 – 5 based upon the degree of cartilage damage and cell inflammation. Table 1 shows the histological scoring systems parameters which were developed by Elizabeth MacConnell of the U.S. Fish and Wildlife Service, Bozeman, MT and Dr. Tom Baldwin of Washington State University. All histological scores represent average infection intensity from the total exposed fish in each test lot. The field experiments were done at the Willow Creek Field Station where sentinel cages were placed into Willow Creek with 50 test fish and exposed to stream water for a period of 123 days from early June through early September, 1997. The fish were then removed from the sentinel cages, sacrificed and sent to the Washington State University Animal diagnostic Lab for histological analysis.

Results

Laboratory exposures. Each salmonid strain or species tested was placed in one of six categories based on the degree of histological damage occurring when exposed to 1000 triactinomyxon per fish. The categories ranged from very low (average histology scores ranging from 0 - 0.99 to the very high category with had average scores ranging from 3.71 to 5.00 (Table 2). Using this system, with the exception of the DeSmet rainbow trout strain, rainbow trout were the most susceptible salmonid

Table 2. Relationship of relative susceptibility categories based on average histological scores when test fish are exposed to 1000 triactinomyxon/fish.

Av Grade of Infection	Relative Susceptibility
0.00 – 1.00	Very low
1.00 – 1.99	Low
2.00 – 2.49	Moderate
2.50 – 2.99	Moderately high
3.00 – 3.70	High
3.71 – 5.00	Very High

tested (Table 3). Even at the very low dosages of 100 triactinomyxon per fish, all of the rainbow trout strains tested, except DeSmet, showed moderately high to very high susceptibility to this parasite. Brook trout also fell into the very high susceptible group with mean infection intensities of 3.76 at the 1000 triactinomyxon per fish dosage. DeSmet rainbow trout, kokanee salmon and all the cutthroat trout subspecies tested were placed in the moderate group, but when exposed to very low triactinomyxon dosages (100 per fish) they dropped into the very low to low categories. Using the 1000 triactinomyxon dosage as standard, both bull trout and brown trout were placed in the low to very low categories, even when exposed to 4000 triactinomyxon per fish they stayed in to low category (Table 4). Arctic grayling exposed in Willow Creek for 123 days showed no sign of Myxobolus cerebralis infections.

Further evaluation of bull trout's reaction to *M. cerebralis* infections was done because serious deformities were noted in the caudal areas of the young bull trout exposed to 1000 and 4000 triactinomyxon per bull trout. The routine histological examination of these young bull trout was done on the cranial area, but due to the severe caudal deformities noted from the exterior the caudal peduncle, histology was also done on this portion of the bull trout. Table 4 shows that while cranial infection intensities were in the very low to low categories damage to the caudal vertebrae was severe. The degree of cranial infection found even at the very high triactinomyxon dosages (4000/fish) should not cause significant mortality to individuals or cause measurable population losses, but the severity of parasitic infection in the caudal area causing bent tails may compromise survival. Further studies need to be done in this area to determine with impacts the caudal infections may have on a population of young-of-the-year bull trout and could it significantly reduce recruitments levels to the adult population.

Table 3. Comparison of relative susceptibility to M. cerebralis infections using average infection intensities from histological grading of cranial cartilage in all salmonids tested. Species, subspecies or strains were ranked on the basis of average severity of infection with the 1000 triactinomyxon per fish dosage. All fish tested were approximately 60 days of age and exposed in 10 C water for two hours. Histological scoring scale 0 – 5 (Vincent 2001).

Species, Strain or Subspecies	Number of Triactinomyxon/fish 100 (n)	1000 (n)
Very High Susceptibility		
Eagle Lake Rainbow Trout	3.08(70)	3.94(64)
Finger Lake Rainbow Trout	2.14(76)	3.73(78)
Firehole River Rainbow Trout	3.96(93)	4.29(47)
Madison River Rainbow Trout	2.58(67)	3.78(67)
Missouri River Rainbow Trout	2.70(69)	4.00(64)
Randolph Rainbow Trout	1.33(97)	3.70(95)
Av of six rainbow trout strains	**2.68 (472)**	**3.90 (415)**
Brook trout	2.75 (98)	3.76 (93)
High Susceptibility		
Arlee rainbow trout	No data	3.63 (100)
Erwin rainbow trout	2.80 (50)	3.04 (50)
Moderate Susceptibility		
Kokanee salmon	0.50 (65)	2.53 (93)
DeSmet rainbow trout	0.86 (147)	2.24 (186)
Yellowstone cutthroat trout	0.86 (146)	2.21 (148)
Snake River cutthroat trout	1.39 (138)	1.93 (94)
Westslope cutthroat trout	0.59 (131)	1.41 (139)
Low Susceptibility		
Bull trout	0.31 (66)	1.12 (33)
Chinook salmon[2]	0.00 (90)	0.22 (95)
Brown trout		0.06 [1]
Arctic grayling		0.00 [1]

1 based on 123 days of exposure to Willow Creek water during the June – August period, 1997.

Field Exposures . Filed exposures were done on three strains of rainbow trout (DeSmet, Eagle Lake and Deschutes); two subspecies of cutthroat trout (Westslope and Yellowstone); kokanee salmon, brown trout and arctic grayling (Table 5). With all salmonid species that were tested under both laboratory and field conditions, the average infection intensity was greater with the stream exposure than the moderate (1000 triactinomyxon per fish) exposures suggesting that this stream had triactinomyxon dosages greater than found with the moderate lab exposure dosage.

Table 4. Comparison of M. cerebralis infections in cranial and caudal cartilage tissue of young-of-the-year bull trout exposed to various dosages of triactinomyxon at 72 days of age.

	Number of 0	**triactinomxyon 100**	**Per fish in each 1000**	**Exposure 4000**
Average Cranial Infection Intensity	0.00	0.30	1.13	1.58
Average Number of Caudal Lesions per Fish Caused *by M. cerebralis*	0	2.50	3.87	7.65
Percent with Severe Caudal Deformities "bent tail"	0	9.2	75.0	100.0

Table 5. Comparison of relative infection rates of certain salmonids exposed to 123 days of Willow Creek water for the June – mid September period in 1997.

Species or strains	Av Infection Intensity 1000 tams/fish	Willow Creek
Dechutes rainbow trout	No data	3.54
DeSmet rainbow trout	2.24	3.12
Eagle Lake rainbow trout	3.94	4.80
Westslope cutthroat trout	1.41	3.19
Yellowstone cutthroat trout	2.21	3.91
Kokanee Salmon	2.53	2.86
Brown trout	No data	0.06
Arctic grayling	No data	0.00

Discussion

The variability in the degree of susceptibility to *M. cerebralis* infections between strains or between species of salmonids influences the risk level for developing a population threatening whirling disease infection for any particular cold water wild salmonid stream. Since most strains of rainbow trout tested tend to fall in the high to very high susceptibility category, wild rainbow trout fisheries have the highest risk to develop severe cases of whirling disease to the point where significant population declines occur. Based solely on relative susceptibility to infection by *M. cerebralis*, wild brook trout populations are also at high risk. Wild populations of DeSmet rainbow trout, Yellowstone cutthroat trout and Westslope cutthroat trout are at the next risk level to develop severe cases of whirling disease. Finally brown trout, bull trout and arctic grayling have the least risk to develop population threatening whirling disease. Obviously, there is more factors involved in disease severity than salmonid species present. A good example is the DeSmet strain of rainbow trout which appears to be only moderately susceptible to the parasite, but if the level of infection (density of triactinomyxon) is increased as shown in Willow Creek they can develop a higher level of infection (2.24 at 1000 triactinomyxon/fish to 3.12 in the Willow Creek stream environment). Yellowstone cutthroat trout, Westslope cutthroat trout and kokanee all experienced more severe infections in Willow Creek than they did exposed to 1000 triactinomyxon under laboratory conditions. Each salmonid species or strain probably has a dosage curve in which when the concentration of triactinomyxon is increased the level of infection will increase. In the case of most rainbow trout, this curve shape is steep and by the time the concentration is greater than 100 spores per fish is exceeded they develop infections severe enough to cause extreme mortality. DeSmet rainbow trout, cutthroat trout and kokanee may have much more moderate curve where high triactinomyxon doses are required to cause severe infections. It is also very important to determine at what point in the life history the young fish is exposed to the infective spore, as size and age at exposure and water temperature determine susceptibility. Further work needs to be done to develop a dosage curve for important salmonids found in the moderate susceptibility category. This plus the knowledge of stream water temperatures, life history, and alternate host densities will allow the determination of risk to these wild salmonids to losses due to whirling disease infection.

Part Ib - Relationship of Fish Size and Susceptibility to WD Infection

Background and Objectives

The relative susceptibility of individual species or strains of salmonids to *Myxobolus cerebralis* infections is based on genetic variations between these various species and strains, but other variables such as age, size and pre exposure to triactinomyxon can alter the level of the infection. Ryce et. al.(1999) examined the impact of age on relative susceptibility of rainbow trout to the parasite under various dosages of triactinomyxon. Preliminary results show that the older the rainbow trout was at first exposure to a given triactinomyxon dosage, the lower the infection intensity and rate. By nine weeks of age, there was no visible external signs of the disease, such as black tail, skeletal deformities. It is uncertain at this time whether age or size or a combination of both is the primary factor for this reduced infection intensity. If size is the primary factor and not age in causing this increased resistance to the parasite, this is an important consideration for wild salmonid populations. Even though age and size are somewhat connected, food supply and water temperature may be a more important factors than time in determining size. If there is a critical size a fish has to obtain to receive adequate resistance to the parasite, some wild populations of rainbow or cutthroat trout might be highly vulnerable to *M. cerebralis* much longer than nine weeks. This is particularly true for wild cutthroat trout populations which exist in colder less productive streams during their early life history and then migrate into warmer more productive streams where exposure to high doses of triactinomyxons are possible while they are still relatively small in size, but older than nine weeks of age. Several experiments were set up at the Pony WD lab to examine the relative susceptibility of rainbow and bull trout that were of the same age, but different sizes.

Methods

Two different methods were used to obtain trout of equal age, but different sizes. The first method used lots of rainbow trout of the same age, which were then sorted into two to three different size groups. The second method separated rainbow trout with a common hatching date into separate aquariums and were raised under water temperatures ranging from 7C (45F) to 14C (57F). Trout in these tanks were all fed the same amount of food to insure trout were kept nutritionally healthy. Using rainbow trout from each method, experiments were set up to compare M. cerebralis infection intensities in fish of the same age but different sizes. Each test lot was exposed to a known concentration of the infective spore for a two hour period in 7.6 liters (2 gallons) of water and then rinsed with fresh water and placed in 114 liter (30 gallon) aquariums and kept for 90 days in 10 C (50F) water. At the end of the 90 day period the fish were sacrificed and sent to the Washington State University Animal Diagnostic Lab at Pullman, WA with they cranial cartilage samples were given a histology score (same as in species susceptibility studies described in Section Ia). A total of five experiments were conducted to determine the relationship between size of fish at exposure and the relative infection intensity. Experiments 1 – 4 utilized young-of-the-year Arlee and Erwin strain rainbow trout which have shown similar levels of susceptibility to the parasite (Table 3). Experiment five utilized older DeSmet strain rainbow trout which have been shown to be moderately susceptible to the parasite. Three different triactinomyxon dosages were utilized in experiment one (50, 200 and 1000 per fish) versus 2000 per fish in the remaining four experiments.

Experiment 1 - A total of 204 Erwin strain rainbow trout obtained from the Ennis National Fish Hatchery at 60 days of age were sorted into two groups based on size. The average size of the two groups was 31.5 mm (1.23 inches) and 54.5 mm (2.13 inches). Both groups were then divided into three groups of 34 fish each and exposed to either 50 tams/fish, 200 tams/fish or 1000 tams/fish.

Experiment 2 - A total of 150 Arlee strain rainbow trout obtained from the Ennis National Fish Hatchery at 44 days of age were divided into three groups of 50 fish each and placed in three separate aquariums being supplied by three different water temperatures. Water temperature in the three tanks averaged: tank 1- 6.7 C (44 F); tank 2 - 11.1 mm (52 F); and tank 3 - 14.2 C (57.5 F). The fish were then held in the three tanks for 30 days at which time they were measured and each group exposed to 2000 tam/fish.

Experiment 3 - A total of 150 Arlee strain rainbow trout obtained from the Ennis National Fish Hatchery at 44 days of age were divided into three groups of 50 fish each and placed in three separate aquariums supplied by water of three different temperatures. Water temperature in the three tanks averaged: tank 1- 6.7 C (44 F); tank 2 - 11.1 C (52 F); and tank 3 - 14.2 C (57.5 F). The fish were then held in the three tanks for 44 days at which time they were measured and each group exposed to 2000 tam/fish.

Experiment 4 - A total of 300 Erwin strain rainbow trout obtained from the Ennis National Fish Hatchery at 70 day of age were divided into three groups of 100 fish and placed in three separate aquariums supplied by water of three different temperatures. The water in tank one averaged 7.2 C (45 F); in tank two 11.1 C (52 F) and 14.4 C (58 F) in tank three. The rainbow trout were then held for 30 days at which time they were measured and then exposed to 2000 tams/fish.

Results

Experiment 1 - Comparisons of histology scores between two size groups of the same age (60 days) under the 50 and 200 triactinomyxon/fish dosages, show the fish in the smaller size lot had a statistically higher infection intensity than the fish in the larger size groups (Table 6). P values were >0.001 for both groups. At the 1000 tams/fish exposure rate, no practical differences were found between the smaller rainbow trout (31.5 mm) and the larger sized rainbow trout (54.5 mm), as infection rates were near the maximum possible (4.00) with only one fish in the smaller size group having an infection intensity less than 4.

Table 6. Comparison of average infection rates of *Myxobolus cerebralis* between two different size groups of 60 day old Erwin strain rainbow trout exposed to 50, 200 and 1000 triactinomyxon per fish. Histology scale (0 - 4).

No. of triactinomyxon per fish	Small average size Group 31.5 mm (1.24 inches) Av. Infection Intensity Sample size in ( ).	Large average size Group 54.5 mm (2.15 inches) Av. Infection Intensity
50 per fish	3.60 (20)	2.41 (34)
200 per fish	4.00 (28)	3.33 (33)
1000 per fish	4.00 (27)	3.88 (33)

Experiment 2 - Comparison of average histology infection intensities between the three size groups of 74 day old Arlee rainbow trout showed the larger the fish at triactinomyxon exposure the lower the average infection intensity (Table 7). Using the Student T Test, the differences in infection rate between each lot was significant (P> 0.001). Exposures of these young Arlee strain rainbow trout to 2000 tams/fish at 74 days of age showed lethal infection levels at all three sizes.

Table 7. Comparison of average infection rates and intensities of *Myxobolus cerebralis* between three different size groups of 74 day old Arlee rainbow trout exposed to 2000 tams/fish. Histology scale used was (0 - 5).

Average size Sample size in parenthesis	Average Infection Intensity	Average Infection Rate
32.3 mm -1.26 in (34)	4.56	100%
38.5 mm – 1.50 in (46)	4.26	100%
43.1 mm – 1.68 in (49)	3.88	100%

Experiment 3 – Table 8 shows the comparison of infection intensities between two size groups of 87 day old Arlee strain rainbow trout with the larger size group being a combined lot of fish from both the medium and warmer water lots of fish. The medium and warm lots of fish were the same average size. The smaller sized Arlee rainbow trout had infection intensities more than double found in the larger size group. Differences were significant (P>0.001).

Table 8. Comparison of average in infection rates and intensities of *Myxobolus cerebralis* between the two size groups of 87 day old Arlee rainbow trout exposed to 2000 tams/fish. Histology scale used was (0 - 5).

Average size Sample size in parenthesis	Average Infection Intensity	Average Infection Rate
43.3 mm – 1.69 in (43)	4.58	100%
61.1 mm – 2.39 in (172)	2.13	79.1%

Experiment 4 – - Using 102 day old Erwin strain rainbow trout sorted into three size groups and exposed to 2000 triactinomyxon per fish, average infection intensity decreased as average size at exposure increased (Table 9 A linear regression plot for the six exposure lots show a significant decrease as size at exposure increased (R^2= 0.875, 95%).

Table 9. Comparison of three different size groups of Erwin strain rainbow trout which were 102 days of age when exposed to 2000 tams/fish. Histology scale used was (0 - 5). Sample size in parenthesis.

Size Group	Av. Infection Intensity		
	Lot 1	Lot 2	Average
53.5 mm – 2.09 in	4.08 (48)	4.20 (49)	4.14
59.9 mm – 2.34 in	3.67 (49)	3.24 (49)	3.46
61.2 mm – 2.39 in	3.00 (48)	3.02 (46)	3.01

Summary

Since the Erwin and Arlee rainbow trout strains have very similar susceptibility to the parasite, *Myxobolus cerebralis*, test fish from experiments 2 through 4 were used to determine the effect of average size of fish at exposure and its infection intensity at 90 days post exposure (Figure 1). This polynomial regression shows that there was no meaningful decline in infection intensity until the average size reached approximately 53mm (2.10 inches) in length and at the 2000

triactinomyxon per fish exposure level infection intensities were at lethal levels up to 60 mm (2.34 inches) in length in this experiment. A similar comparison between infection intensity and average size at exposure showed a small (not significant) decline with increasing age. This decline may be more tied to increasing size with age than age itself ($R^2 = 0.0795$, 14 df, 95%).

Part Ic - Impacts of *Myxobolus cerebralis* Infections on Trout Growth

Background and Objectives

As shown in section Ia and Ib, both strain or species of salmonid and size of fish at exposure to the triactinomyxon have impact on the relative susceptibility to the parasite. Since severe whirling disease infections can cause increased fish mortality, it is extremely likely that these same parasitic infections may also cause some losses in the trout=s growth rate. Survival of wild trout relies on many factors with size and condition being extremely important, especially during their first winter when food supplies are limited and the water is too cold for any significant growth or weight maintenance. Survival rates for this first winter period is dependent on their physical condition going into the winter period. They must put on most the their growth prior to the winter cooling which generally begins in mid to late October. If young-of-the-year wild trout are too small or in poor physical condition at this time their chances to survive the winter period decreases significantly. Part Ic will attempt to examine the impact of M. cerebralis on rainbow and bull trout young-of-the-year.

Methods

Experiment 1. A total of 133 sixty day old Erwin rainbow trout which averaged 31.5 mm in length were divided into four lots with each lot receiving a different concentration of tams. The tam dosage for each lot was as follows: Lot I - 0 tams/fish; Lot II - 60 tams/fish; Lot III - 230 tams/fish and Lot IV - 1200 tams/fish. After the initial exposure, each lot of rainbow trout were measured every 20 days and then at 120 days of age all the rainbow trout survivors were sacrificed and send to Washington State University to determine the level of disease severity through histology scoring.

Experiment 2. A total of 400 Erwin strain rainbow trout at 60 days of age were separated into four lots as follows and exposed to the following concentrations of tams/fish: Lot 1 - Control - 0; Lot 2 - 300; Lot 3 - 1000 and Lot 4 - 3000. The initial average length of each lot of fish was 27.5 mm with each lot being measured every 20 days until the experiment was terminated at 120 days. At the end of the experiment the fish were sacrificed and sent to the Washington State Histology Lab for analysis.

Experiment 3. A total of 360 bull trout at 72 days of age were separated into seven lots of 60 fish each and exposed to various dosages of tams. The exposure rate by lot of bull trout are as follows: Lot 1 - 0 tams; Lots 2 and 3 - 100 tams/fish; Lots 4 and 5 - 1000 tams/fish and Lots 6 and 7 - 4000 tams/fish. The initial average length at the time of exposure was 22.6 mm for each lot. After the initial exposure, the lots were held for 90 days and then measured and sent to the Washington State University Histology Lab at Pullman for histology.

Results

Experiment 1. When young-of-the-year Erwin rainbow trout were exposed to three difference dosages of triactinomyxon growth rates began to slow in all exposure levels by day 20 post exposure and experiment all lots of fish exposed to *Myxobolus cerebralis* triactinomyxon showed a lower growth rate than the control non exposed lot by day 20 post exposure (Table 10). The greatest difference in length between those fish exposed to tams and the control group occurred at day 60 post exposure averaging 18.2% less in length. No noticeable difference was noted in growth between the three exposed groups. By day 120 post exposure, the triactinomyxon exposed lots had caught up with the unexposed lots. This equalization of average size between the exposed lots and the one unexposed lot may reflect some size limitations of the holding tanks or a recovery from the stress of the early infection by the parasite. Little difference was noted between the lots, but little difference was noted between infection intensities.

Table 10. Comparison of growth rates between four lots of Erwin rainbow trout over a 140 day period following an exposure to four different dosages of *Myxobolus cerebralis* tams. Initial length of test fish was 31.5 mm. Histology scores using the 0 - 4 scale.

Triactinomyxon Dosage/ fish	Av. Infection	20 days	40 days	60 days	80 days	100 days	120 days
0	0.00	44.8	55.8	70.4	76.8	84.7	96.9
60	3.60	42.0	48.9	57.3	69.6	82.2	100.1
200	4.00	39.7	49.7	57.2	68.6	78.6	92.9
1200	4.00	41.0	49.7	58.9	69.6	78.6	88.6

Experiment 2. Twenty days after the initial tam exposure there was only a small 11% decline in growth rates for the exposed Erwin rainbow trout when compared with the control group (Table 11). By day 60, these differences were small and not significant with 3000 tam/fish

Table 11. Comparison of infection intensity rates and the rate of growth for 60 day old Erwin rainbow trout exposed to various dosages of *Myxobolus cerebralis* tams. Histology scale was 0 - 5.

Tam Dosage	Av Infection Grade	Initial size mm	20 days	60 days	120 days	Mortality rate in tanks
0 tams/fish	0.00	27.5	40.7	55.3	71.4	2%
300tams/fish	4.46	27.5	38.9	54.5	73.5	20%
1000 tams/fish	4.97	27.5	40.2	52.5	74.8	24%
3000 tams/fish	4.74	27.5	38.9	58.1	75.8	30%

group actual showing a higher growth rate than the control group. At the completion of the experiment, there were no significant differences in average size between the control group and the three tam exposed groups. Average infection rates were very similar between the three groups exposed to tams (4.46 to 4.74). The only unusual factor is this experiment was than significant amounts of young rainbow trout died in the tanks during the experiment. The rate of mortality increased as triactinomyxon dosage increased from zero to 3000 triactinomyxon per fish. Mortality rates were very low in the control tank (one fish during the 120 period) very 30% in the high dosage tank.

Experiment 3. As the dosage level of triactinomyxon per fish increased from 100 to 4000 per fish growth rates of the young-of-the-year bull trout decreased (Table 12). At 90 days post exposure, the 100 triactinomyxon per fish exposure showed no difference in average length from the control group, but the two higher triactinomyxon dosages (1000 and 4000/fish) showed a 14.8% and 31.3% decline in average length when compared to the control group. Mortality in the holding tanks during the 90 day period was high, 24% in the control tank and an average of 48% for all exposed bull trout..

Table 12. Comparison of infection intensity rates and the rate of growth for 72 day old bull (average length -22.6 mm) exposed to various dosages of *Myxobolus cerebralis* tams. Histology included cranial average cranial histology scores on the 0 - 5 scale and histology of the caudal area shown as the number of infected loci.

Triactinomyxon dosage per fish	Av Cranial histology score	Av Caudal histology score Av loci/slide	Av length at 90 days post exposure (mm)
Control Group 0	0.00	0.00	55.5
100	0.33	2.50	57.2
1000	1.12	3.87	50.6
4000	1.59	7.65	45.2

Summary

To separate experiments were conducted to determine the impact of M. cerebralis infections and growth rate of young-of-the-year Erwin strain rainbow trout. In the first experiment, growth rates of the three *M. cerebralis* infected lots averaged 31.6% slower growth rates than the control group with little difference between the three exposure levels. After day 60 post exposure, the three M. cerebralis infected groups began to increased their growth rates and by day 120 little difference in average size was noted between control and the three exposure groups. The second experiment showed in difference in growth rates between the control and exposure groups. With bull trout, growth rates declined in the two higher triactinomyxon exposure groups with no

noticeable recovery in average size by day 90. Since two of the three experiments did show exposure to M. cerebralis triactinomyxon impacted early growth rates of these young trout under laboratory conditions, it is likely that exposure to the parasite and subsequent infection puts some stress on these young trout which is reflected by a lowering of the rate of growth. One could assume that the early infection stress which impacts growth rates 0 – 60 days post exposure to the parasite spore could have greater impact on wild young-of-the-year wild trout than aquarium held fish, since young wild trout must maintain position in stream current, seek out food and avoid predation where aquarium held fish do not have to maintain themselves in a stream current, are supplied daily with food and have no predators. If growth is impacted during the early infection stages of the parasite, one could expect increased mortality of these young fish. Further studies on growth rate impacts probably are warranted.

General Discussion of Salmonid Susceptibility in Relation to Species/strain, size and growth and the Impact on Wild Populations of Salmonids

The presence of the parasite *Myxobolus cerebralis* (whirling disease) can cause severe declines in wild salmonid populations, as shown in the upper Madison River where the number of wild rainbow trout declined 70-90% following the introduction of whirling disease parasite (Vincent, 1998). Similarly in Colorado, numerous trout streams have experienced similar large declines in wild rainbow trout numbers following the introduction of this parasite (Walker and Nehring, 1995). But, the mere presence of the infective parasite does not always result in salmonid population declines, even susceptible species such as rainbow trout. In the Madison river, wild brown trout showed no population declines during the same period that wild rainbow trout were showing severe losses. There are many cases in Montana and other states where the parasite is present and no measurable negative impacts on the wild trout populations are noted. Population level impacts generally do not occur until infection severity reaches some critical level. Using the histological score measurement of disease intensity, cranial cartilage damage has to reach the 2.50 – 2.70 level in young-of-the-year rainbow trout before population level damage occurs. The bottom line is that the susceptible size and/or age of young-of-the-year trout have to be exposed to sufficient triactinomyxon to cause a severe infection. As shown in Table 3, each salmonid strain or species may require different dosages of triactinomyxon to cause similar disease severities. There are three primary factors which appear to control infection intensities in wild salmonids are: 1) the number of triactinomyxon which attach to each fish 2) the relative susceptibility of individual strains or species to the parasite; and 3) whether the life history of salmonids allow contact with sufficient quantities of triactinomyxon during periods when the young-of-the-year are at susceptible sizes or ages.

Risk Factors

Each wild salmonid population has a different level of susceptibility to infection by the parasite *Myxobolus cerebralis.* Some wild populations suffer severe population losses when infected by this parasite while others show moderate or benign infections. In order to determine, the relative risk a particular salmonid population has to whirling disease, certain risk factors must be considered and then evaluated. **The known risks factors are as follows: I – the relative susceptibility of the salmonid species present in the stream reach; II – the number of infective spores (triactinomyxon and myxospores) available to infect both parasite hosts (fish and Tubifex worm); III – the fishes life history at this site.** *This discussion is speculative in some areas with the need for additional research.* **The following risk factors can be used to determine whether a particular wild salmonid population in a given stream reach will suffer severe negative impacts from whirling disease infections, have a benign infection or no detectable infection.**

I. The relative susceptibility of the salmonid species resident in the stream reach.

A. **<u>The Strain Or Species of Salmonid Present.</u> Each salmonid species or strain may have a different level of susceptibility to any given dosage of triactinomyxon. Experiments showing differences in resistance (Table 3) found rainbow trout to be the most susceptible salmonid species followed by brook trout, cutthroat trout, bull trout, mountain whitefish, brown trout and arctic grayling.** ***Additional research should be done to determine the dose of triactinomyxon it takes to cause different infection intensity by species and/or strain (dose response curve).***

B. **<u>Age of Fish Present When Triactinomyxon are the Most Common</u>. Preliminary studies have shown that age of fish when exposed to the triactinomyxon changes the susceptibility and severity of the disease and for rainbow trout they become considerably more resistant after 9 weeks of age (Ryce, et.al. 1999).** ***More research needs to be done in this area with each species or strain of interest being tested, since this age threshold may vary with species or strain.***

C. **<u>Size of Fish Present When Triactinomyxon are the Most Common</u>. Preliminary studies (Tables 6 – 9) indicate that the relative susceptibility of a species of salmonid may more relate to fishes size than age. There may be a maximum size for each salmonid species, when they become resistant to severe infections by the parasite and if first or high exposures occur at this size, negative population level impacts may not occur.** ***More research needs to be done in this area with each species or strain of interest, since the size threshold for high resistance to the parasite may vary with fish species.***

II. The number of infective triactinomyxon and myxospores available to infect both the fish and worm hosts.

A. **<u>Absence of Either the Fish or Worm Host (*Tubifex tubifex*).</u> This would prevent the establishment of the parasite, since the life history could not be completed.** ***Research would be useful to determine why alternate host Tubifex tubifex is not present in the stream reach.***

B. **<u>The Population Density of *T. Tubifex*.</u> There is some data suggesting that there is a relationship between Tubifex densities and the severity of the parasitic infections in susceptible salmonids (Krueger et. al., 2000). The relationship may not be entirely linear as there may be some threshold value of worms necessary to create a severe infection in the existing salmonid population and increases in** worm numbers become this point may not worsen the disease. Current research in wild worm populations show a very low infection rate in worms (1-5%), but percent infected may increase if worm numbers are low._ *More research needed.*

C. Water Temperatures can Affect Infection Intensities in Wild Rainbow Trout Populations. Sentinel cage exposure studies in the Madison River, Missouri River, Little Prickley Pear Creek and Willow Creek shows increases in infection intensity as daily mean water temperatures increase from 0 to 15 C, but further increases in the daily mean water temperature shows a steady decline in infection intensity until 20 C is reached when infection intensities are near zero (Vincent, 1998). While speculative, this relationship is probably the result of increased triactinomyxon production as water temperatures increase until some threshold water temperature is reached which is followed by decreasing triactinomyxon production with continued increases in water temperature. There is also a relationship between the rainbow trout's susceptibility to the triactinomxyon and mean water temperature. As water temperature increases from 5 to 20 C it takes fewer triactinomyxon to cause the same infection intensity (Vincent unpublished data). *Although the impact of changing water temperatures and infection rates in rainbow trout has had considerable research, the relationship of triactinomyxon production in wild trout streams and water temperature changes needs more research.*

D. Water Flow Volumes May Affect Infection Intensities in Wild Rainbow Trout Populations. New Research is beginning to show that water flow rates may change the rate of infection in wild rainbow trout populations. Previous research has shown that the severity of infection is related to the number of triactinomyxon the young fish are exposed (Table 3). Any factor which alters the triactinomyxon concentrations and either decreases or increases the chance of a triactinomyxon attaching to a susceptible fish would change the rate of infection. Assuming the number of triactinomxyon produced by any wild Tubifex worm populations is relatively constant for any given water temperature and year, then changes in flow rate should change the number of triactinomyxon per unit volume of water. As the number of triactinomyxon per unit volume of water decreases, the chance of any fish being "hit" by a triactinomyxon should decrease. If this is true increases in volume of flow for any given water temperature should decrease infection intensities and decreases in flow volume should increase infection intensities. If research confirms this possibility, than young salmonids hatching and emerging during periods of higher water flows should experience lower infection intensities or conversely there infection intensity would increase if flow volumes decreases during this period. *Research in ongoing in this area.*

E. The Number of Myxospores Available to Infect the Tubifex Worm Colonies May Regulate Number of Triactinomyxon Being Produced. There is undoubtedly some relationship between the number of myxospores produced by the fish host, their availability to the Tubifex worm colonies and the number of worms infected by the parasite. In theory, a particular worm colony could have an insufficient amount of myxospores available to adequately infect the worm

colony to cause a severe infection in the resident salmonid population. This could occur if salmonids are very low in number, or infected spawning aged salmonids do not frequent a particular area of a stream. It is unknown how limiting myxospores supplies may be in some streams or stream reaches. *Research needs to be done in this area.*

III. Fish Life History Type May Impact Severity of Whirling Disease.

A. <u>Type I. – Young-of-the-year salmonids hatch and emerge from redds in reaches of streams which have active whirling disease infections.</u>

1. **In this scenario, the relative survival of young salmonids is based on the conditions present when they hatch and emerge. If tam concentrations are high due to either optimum water temperatures for triactinomyxon production and/or low water conditions, then infection intensities would be high. If they hatch and emerge either during colder or warmer water periods and/or high water conditions, their infection intensities would be lower.**
2. **Example 1 - *<u>Rainbow trout</u>* have a wide range of spawning periods which is probably regulated by both its genetics and water temperature present in the stream system. Rainbow trout are a highly susceptible species to whirling disease and in order for them to avoid severe infections of whirling disease in a highly infected reach of stream, they must utilize a hatching and emerging period to coincide with either high water conditions or low water temperatures or both.**
3. **Example 2 – *<u>Brook trout</u>* another highly susceptible salmonid species which spawns during the late fall (October – November) may avoid serious infection intensities since the hatching and emerging period is very early in the spring when water temperatures are generally below those where triactinomyxon production is high or the relative susceptibility to the triactinomyxon is low, thus avoiding serious infections when they are at the most vulnerable size and age.**
4. **Example 3 – *<u>Brown trout</u>* which is a highly resistant salmonid species, also uses a fall spawning period with early hatching and emerging. Since brown trout have a natural high resistance to whirling disease infections and young hatch and emerge during seasons of the year when triactinomyxon production is low and the fish are less susceptible to the triactinomyxon because of low water temperatures, it is rare to find moderate or high infection rates in this species in wild populations.**

B. <u>Type II - Young-of-the-year salmonids hatch and emerge in tributary streams where *M. cerebralis* infections are less severe or do not exist.</u> Young salmonids then migrate to the primary stream which have more severe infections of whirling disease. In this scenario, the relative survival of young salmonids in the face of whirling disease is based more on the size and age of emigrating young salmonids when they reach the more highly infected reaches of the parent stream.

1. **Example 1 – Young trout hatch in a low or non whirling disease infected tributary and migrate immediately upon emerging to a highly infected main stem stream and because they young salmonids are either too small or young to obtained any significant resistance they become severely infected with whirling disease.**
2. **Example 2 - Young susceptible salmonids hatch in low or non whirling disease tributaries and remain in these low or non infected sites until they reach ages or sizes which allow them to be sufficiently resistant to infection by the parasite resulting in low infection intensities when they reach and inhabit the higher infected main stem sites.**

C. ***More research or information is essential to fully understand the life history of a particular wild population of salmonids. Information necessary is: time of spawning, hatching, and emergence; water temperatures at hatching; infection rates at hatching and emergence using sentinel cage exposures ; age and size when they emigrate; and infection intensity potential in main stem stream when young arrive.***

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