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Introduction

Following discovery of whirling disease (WD) in the Madison River in December, 1994, additional waters of concern were identified by Montana Fish, Wildlife, and Parks (FWP) fisheries biologists. These were identified as waters that should be tested for the presence of WD, but FWP lacked the manpower resources to test. Knowledge of the current statewide distribution of WD is a fundamental step in understanding this disease. It provides a starting point to monitor the spread and learn how the disease affects individual fish as well as entire fish populations. A cooperative project was initiated between FWP and the Montana Cooperative Fishery Research Unit at Montana State University-Bozeman, to meet this need. A three person testing crew was assembled by the Montana Cooperative Fishery Research Unit with funding from FWP to sample these waters.

This report presents the results of the supplemental WD sampling project and does not include results from samples collected by FWP regional fisheries personnel. The sum total of all collections statewide is currently being summarized by FWP personnel in Bozeman, and should be available in data base format in the near future. The data base will include more detailed information on the our collections, as well as a complete summary of the current status of all testing conducted statewide.

Methods

FWP provided the sampling crew with a list of streams to sample. When no specific sample site was identified on a given body of water, we selected our sampling sites with consideration for convenience. Convenience was given some priority due to the large number of streams to be sampled and the wide geographic area to be covered. We used the downstream most road crossing when possible. If there were no road crossings, we sampled at the point nearest a road. These criteria were often modified due to private property considerations. Road crossings were selected because they provide good access for sampling and are the most likely points of illegal introduction, if they were to occur. Illegal introduction of infected fish is one possible vector for the disease. We often selected the downstream most road crossing because when only one sample is being collected, the downstream sections would be the most appropriate areas to sample for two main reasons. The lower reaches of rivers and streams are often more degraded than the headwaters, and the intermediate host, *Tubifex tubifex* , flourishes in areas where the aquatic invertebrate community is depressed (Klemm 1985). In addition, if drift is important in spreading the disease, which it intuitively should be, downstream areas are the most appropriate areas to take isolated point samples.

Prior to entering a site, all equipment to be used was disinfected. Household bleach in a 1.5:10 ratio with water was used as the disinfective agent. This provided a 0.79% solution of sodium hypochlorite. This exceeds the recommended 0.525% sodium hypochlorite solution necessary to destroy *M. cerebralis* spores (B.

MacConnell, US Fish and Wildlife Service, personal communication). The gear and equipment was laid out on the ground away from the body of water to be sampled, then sprayed down with the bleach solution using a one gallon chemical sprayer. A minimum 10 minute contact time was allowed. Water was then bailed up from the body of water to be sampled and the equipment rinsed. After sampling was completed on a given stream, the equipment was rinsed with water from the same stream to remove mud or aquatic vegetation. The equipment was then placed in plastic garbage bags to move to the next site where the disinfection process was repeated. Equipment was dried between bodies of water when possible.

Fish were collected primarily using a Smith-Root Model 15-C backpack electrofishing unit. Gill-netting and boat-mounted mobile electrofishing were used when necessary. The target sample was 60, 4 to 12 month old salmonids based on recommendations by Thoesen (1994). An additional 10 fish were collected where possible and archived. The target species varied with stream, but rainbow trout *Oncorhynchus mykiss* were preferred as their susceptibility is well documented.

Sixty whole heads were collected and frozen on site using dry ice. The heads were then shipped to the Washington State University Animal Disease Diagnostic Laboratory at Pullman, Washington where they were processed using the digestion method described by Thoesen (1994). Histology was used to confirm any positives by the digestion method.

The additional 10 fish were processed by removing the heads immediately posterior to the dorsal fin and bisecting the heads along the vertical axis. One half of each head was fixed in Davidson's solution (Kent 1992) for 72 hours, then

transferred to isopropyl alcohol. The other half of each head was frozen on site with dry ice and later transferred to an ultra-cold freezer and stored at -80^{0}C. These samples were considered archive samples to be used as needed. When fewer fish were collected than desired, a sub-sample, usually comprising 5-10% of the entire sample, was archived as described above.

Beginning in 1996, kidney and spleen samples were collected from sacrificed fish when time permitted according to the methods described by Thoesen (1994). These samples were used to test for the presence of *Renibacterium salmoninarum* (Rs, the causative agent of bacterial kidney disease), the infectious pancreatic necrosis virus (IPNV), and the viral hemorrhagic septicemia virus (VHSV). The USFWS Fish Health Laboratory at Fort Morgan, Colorado processed the majority of the bacteriological and viral samples. The direct flourescent antibody test (Bullock et al. 1980) was used to test for the presence of Rs. Virological examination was conducted using cell culture assay. This consists of the assay of kidney and spleen tissue on cell cultures of epithelioma papillosum cyprini (EPC) and rainbow trout gonad (RTG-2). Kidney impressions were also taken to identify any additional pathogens. Beth MacConnell at the USFWS Bozeman Fish Technology Center will analyze these samples.

Results

The WD testing crew sampled a total of 125 waters during 1995 and 1996 and documented the presence of WD in 23 of these (Table 1).

Table 1. Number of waters sampled by WD testing crew in FWP adminstrative regions during 1995 and 1996.

FWP Region	No. Waters Tested	No. of Positives for WD
1	22	0
2	1	1
3	69	19
4	30	3
5	3	0

Between August and September 1996, samples were collected from 22 waters in FWP administrative region one to test for the presence of WD (Appendix A). All of these streams tested negative. Seventeen of these waters also tested negative for additional fish pathogens (Appendix B).

We sampled only one stream in FWP administrative region two during 1996 for the presence of WD (Appendix D). This stream, the East Fork of Rock Creek, tested positive.

Sixty-nine waters were tested for the presence of WD in FWP administrative region three during 1995 and 1996 (Appendix E). Nineteen tested positive. Six waters were also tested for the presence of additional fish pathogens and all tested negative (Appendix F).

Thirty waters were tested for the presence of WD in FWP administrative

region four during 1996 (Appendix G). Of these, three tested positive. Twelve waters were also tested for the presence of additional fish pathogens and all tested negative (Appendix H).

Three waters were tested for the presence of WD in FWP administrative region five during 1995 and 1996 (Appendix I). All of these tested negative.

Discussion

The WD testing crew documented the presence of WD in 23 waters, bringing the total number of WD positive waters in Montana to 45 (Appendix J). Findings demonstrate that WD is more widespread in Montana than anticipated. The samples collected are point samples and do not constitute a statistically valid sample for stating that a water is negative for WD. The most appropriate way of interpreting negative sites is that WD has not been detected to date.

The same approach should be taken when interpreting the results from the testing for additional fish pathogens. Although all of the results were negative, these tests should be viewed as an attempt to identify positive waters and not to certify negative ones. Sample size should also be taken into consideration when drawing conclusions about these data.

We currently have a good picture of WD's present distribution in Montana. This current distribution map will be valuable in designing investigations into how WD affects wild trout at the individual and population levels under a variety of habitat conditions. This map also provides a starting point for studies to investigate methods for control of the disease within rivers and streams.

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Appendicies

Table 6. Waters within FWP administrative region 3 tested for the presence of WD by the WD testing crew during 1995 and 1996.

Stream	Drainage	Location Lat./Lon.(D,M,S)[a]	Species collected[b]	n	Results
Alder Gulch Ck.	Beaverhead	45,23,46 N 111,54,11 W	rb	35	negative
Beaver Ck.	Missouri	46,30,43 N 111,38,29 W	rb	62	negative
			eb	9	negative
Beaverhead R.	Beaverhead	45,07,45 N 112,44,30 W	ll	57	positive
		45,00,19 N 112,50,59 W	cot	40	negative
			ling	11	negative
Big Pipestone Ck.	Jefferson	45,51,42 N 112,05,46 W	ll	16	negative
Big Hole R.	Big Hole	45,21,38 N 113,26,27 W	eb	70	negative
Big Sheep Ck.	Beaverhead	44,39,14 N 112,46,29 W	ll	59	positive
Big Springs Spawning Channel	Missouri	46,06,26 N 111,23,46 W	rb	59	negative
Birch Ck.	Beaverhead	44,34,00 N 112,37,15 W	rb	20	negative
Birch Ck. Reservior	Beaverhead	44,35,55 N 112,36,46 W	rb	10	positive
Blacktail Deer Ck.	Beaverhead	45,02,46 N 112,32,54 W	eb	8	positive
			mwf	2	negative
		44,54,21 N 112,21,24 W	eb	34	positive
			rb	19	positive
Black Sand Spring Ck.	Madison	44,39,57 N 111,10,6 W	rb	68	negative
			ll	9	negative
Blaine Spring Ck.	Madison	46,18,00 N 111,47,29 W	rb	23	positive

Table 6. Continued.

Stream	Drainage	Location Lat./Lon.(D,M,S)[a]	Species collected[b]	n	Results
Boulder R.	Jefferson	45,52,16 N 111,56,28 W	ll	63	positive
			rb	1	positive
		46,10,38 N 112,01,54 W	ll	15	negative
			eb	2	positive
			rb	1	negative
Canyon Pond	Beaverhead	44,34,33 N 112,47,54 W	ll	2	positive
			rb x wct	1	negative
			rb	1	negative
Cherry Ck.	Madison	45,37,11 N 111,32,52 W	rb	52	positive
			ll	12	negative
			wf	2	negative
		45,33,36 N 111,26,35 W	eb	30	negative
			rb	6	archived
Cherry Ck. Pond	Madison	45,35,12 N 111,29,17 W	yct	16	negative
Confederate Ck.	Missouri	46,29,10 N 111,31,03 W	rb	70	negative
			wf	2	archived
			ll	2	archived
Cougar Ck.	Madison	44,46,12 N 111,06,46 W	rb	54	negative
			ll	16	negative
Crow Ck.	Missouri	46,15,13 N 111,40,36 W	rb	57	negative
			eb	2	negative
Deep Ck.	Missouri	46,17,47 N 111,27,47 W	rb	59	negative
			ll	11	negative

Table 6. Continued.

Stream	Drainage	Location Lat./Lon.(D,M,S)[a]	Species collected[b]	n	Results
Dry Ck.	Missouri	46,09,08 N 111,26,49 W	rb	70	negative
Duck Ck.	Madison	44,47,57 N 111,19,01 W	rb	57	negative
			ll	11	negative
East Gallatin R.	Gallatin	45,50,19 N 111,09,38 W	rb	42	negative
			ll	2	negative
		45,53,29 N 111,20,07 W	ll	11	negative
			eb	1	negative
			mwf	6	suspect
		45,44,21 N 111,04,09 W	rb	9	negative
			ll	2	negative
			mwf	3	negative
East Fk. Hyalite Ck.	Gallatin	45,28,29 N 110,57,20 W	yct	60	negative
			eb	10	negative
Elk Springs Ck.	Beaverhead	44,39,02 N 111,39,54 W	N/S[c]		
			cot	55	negative
			wsu	5	negative
Emerald Pond	Beaverhead	44,33,30 N 112,37,00 W	rb	15	negative
Gallatin R.	Gallatin	45,53,07 N 111,25,57 W	ll	8	negative
			rb	3	negative
		45,49,47 N 111,16,17 W	ll	61	negative
		45,49,38 N 111,16,18 W	ll	10	negative
			eb	3	negative

Table 6. Continued.

Stream	Drainage	Location Lat./Lon.(D,M,S)[a]	Species collected[b]	n	Results
			wf	12	negative
Grasshopper Ck.	Beaverhead	45,06,24 N 112,48,00 W	ll	10	negative
		45,14,02 N 113,04,45 W	ll	45	positive
			rb	3	positive
			eb	2	negative
Grayling Ck.	Madison	44,47,46 N 111,09,12 W	rb	70	negative
Halfway Ck.	Jefferson	45,59,09 N 112,18,25 W	eb	56	negative
			wct	1	negative
Hebgen Lake	Madison	44,47,09 N 111,13,30 W	rb	~~0~~ 43	negative
			ll	~~12~~30	negative
Hell's Canyon Ck.	Jefferson	45,37,00 N 112,21,20 W	rb	51	positive
			ll	25	positive
Horse Prairie Ck.	Beaverhead	44,58,32 N 112,55,17 W	rb	6	negative
			ll	6	negative
		45,01,56 N 113,13,07 W	eb	19	positive
			ll	11	negative
			mwf	3	negative
			cot	10	negative
			wsu	4	negative
			lndc	4	negative
Hyalite Ck.	Gallatin	45,33,49 N 111,04,18 W	rb	79	negative
Jack Ck.	Madison	45,22,28 N 111,41,22 W	ll	64	positive
			rb	6	positive

Table 6. Continued.

Stream	Drainage	Location Lat./Lon.(D,M,S)[a]	Species collected[b]	n	Results
Jefferson R.	Jefferson	45,54,09 N 111,35,50 W	mwf	10	negative
Lima Gravel Pit Pond	Beaverhead	44,34,00 N 112,37,15 W	rb	10	negative
Little Sheep Ck.	Beaverhead	44,36,31 N 112,38,45 W	eb	23	negative
Long Ck.	Beaverhead	44,41,53 N 112,05,36 W	eb	9	negative
			cot	20	negative
MacDonald Pond	Beaverhead	44,39,04 N 111,39,55 W	yct	8	negative
Magpie Ck.	Missouri	46,38,50 N 111,40,20 W	rb	70	negative
Marsh Ck.	Missouri	46,09,14 N 111,30,51 W	rb	52	negative
			ll	7	negative
Moore Ck.	Missouri	45,20,12 N 111,44,28 W	ll	25	positive
			rb	1	negative
Muskrat Ck.	Jefferson	46,17,53 N 112,02,00 W	eb	66	negative
			wct	1	negative
North Fk. Big Hole R.	Big Hole	45,41,50 N 113,27,45 W	eb	41	negative
O'Dell Ck.	Beaverhead	44,35,34 N 111,47,24 W	eb	8	negative
			wct	2	negative
O'dell Ck.	Madison	45,20,28 N 111,43,08 W	ll	67	positive
			rb	3	positive
Poindexter Slough	Beaverhead	45,11,00 N 112,40,42 W	cot	32	inconclusive
Rocky Ck.	Gallatin	45,39,34 N 110,58,02 W	rb	57	negative
			ll	13	negative

Table 6. Continued.

Stream	Drainage	Location Lat./Lon.(D,M,S)[a]	Species collected[b]	n	Results
Ruby R.	Beaverhead	45,15,00 N 112,06,30 W	cot	60	inconclusive
		45,02,07 N 111,58,57 W	rb	61	negative
Sixteenmile Ck.	Missouri	46,06,25 N 111,23,46 W	ll	10	suspect
		46,08,10 N 111,18,53 W	ll	2	negative
			mwf	1	negative
		46,06,31 N 111,10,05 W	rb	2	negative
			ll	1	negative
			mcot	20	negative
			lndc	10	negative
		46,16,00 N 110,41,57 W	eb	11	negative
			wsu	12	negative
			mcot	9	negative
Sourdough Ck.	Gallatin	45,38,00 N 111,01,50 W	rb	55	negative
			eb	8	negative
			ll	7	negative
South Boulder R.	Jefferson	45,48,20 N 111,55,40 W	ll	19	positive
			rb	9	positive
			eb	1	positive
South Fk. Madison R.	Madison	44,43,08 N 111,13,22 W	rb	64	negative
			ll	6	archived
Taylor's Fk. R.	Gallatin	45,04,40 N 111,13,00 W	rb	13	negative
			ll	1	negative

Table 6. Continued.

Stream	Drainage	Location Lat./Lon.(D,M,S)[a]	Species collected[b]	n	Results
Unnamed slough of Red Rock R.	Beaverhead	44,51,49 N 112,47,43 W	rb	34	positive
			ll	36	positive
Wade Lake Spawning Channel	Madison	44,48,13 N 111,33,40 W	rb	76	negative
West Fk. Gallatin R.	Gallatin	45,15,58 N 111,15,25 W	rb	70	negative
Whitetail Ck.	Jefferson	45,54,03 N 112,06,43 W	ll	81	positive
Willow Ck.	Jefferson	45,39,39 N 111,53,23 W	rb	60	positive
		45,44,50 N 111,39,24 W	rb	69	negative
			ll	5	negative
Willow Springs	Jefferson	45,45,01 N 112,09,31 W	rb	54	negative
			ll	15	positive

[a] Location in latitude/longitude in degrees, minutes, and seconds.
[b] See Appendix C for species codes.
[c] N/S No salmonids captured.

Appendix F-Additional pathogen test results for FWP administrative region 3.

Table 7. Waters within FWP administrative region 3 tested for additional fish pathogens by WD testing crew during 1996.

Water	Location Lat./Lon. (D,M,S)[d]	Species collected[e]	n	Rs[a] (+/-)	IPNV[b] (+/-)	VHSV[c] (+/-)	Kidney impressions
Beaverhead R.	45,07,45 N 112,44,30 W	ll	47	-			pending
Halfway Ck.	45,59,09 N 112,18,25 W	eb	56	-	-	-	
		wct	1	-	-	-	
Muskrat Ck.	46,17,53 N 112,02,00 W	eb	60	-	-	-	
North Fk. Big Hole R.	45,41,50 N 113,27,45 W	eb	10	-			pending
Porcupine Ck.	47,51,44 N 113,52,48 W	eb	26	-			
Taylor's Fk. R.	45,04,40 N 111,13,00 W	rb	12	-			pending

[a] Renibacterium salmoninarum
[b] Infectious Pancreatic Necrosis Virus
[c] Viral Hemorrhagic Septicemia Virus
[d] Location in latitude/longitude in degrees, minutes, and seconds.
[e] See Appendix C for species codes.

Appendix G-WD test results for FWP administrative region 4.

Table 8. Waters within FWP administrative region 4 tested for the presence of WD by the WD testing crew during 1996 .

Stream	Drainage	Location Lat./Lon. (D,M,S)[a]	Species collected[b]	n	Results
Badger Ck.	Marias	48,19,45 N 112,58,30 W	rb	16	negative
			eb	7	negative
			cot	20	negative
Beaver Ck.	Missouri	46,47,54 N 111,51,01 W	rb	60	negative
			ll	5	negative
			eb	3	negative
Belt Ck.	Missouri	47,22,01 N 110,54,16 W	ll	57	negative
			rb	12	negative
Big Otter Ck.	Missouri	47,17,59 N 110,49,48 W	ll	3	negative
			cot	20	negative
Big Spring Ck.	Missouri	46,59,59 N 109,26,27 W	rb	34	negative
Big Birch Ck.	Missouri	46,32,45 N 111,05,30 W	eb	58	negative
			ll	7	negative
			rb	5	negative
Birch Ck.	Marias	48,15,10 N 112,37,33 W	rb	4	negative
			eb	4	negative
			cot	14	negative
			mtsu	4	negative
			wsu	2	negative
Camas Ck.	Missouri	46,41,17 N 111,11,20 W	eb	54	negative
			ll	6	negative
Cottonwood Ck.	Musselshell	46,20,20 N 110,24,00 W	eb	54	negative
			ll	11	negative

Table 8. Continued.

Stream	Drainage	Location Lat./Lon. (D,M,S)[a]	Species collected[b]	n	Results
			rb	6	negative
Cut Bank Ck.	Marias	48,29,40 N 112,15,45 W	N/S[c]		
			Indc	10	archived
			mcot	6	archived
Dearborn R.	Missouri	47,12,17 N 112,06,05 W	rb	67	negative
			ll	4	negative
Highwood Ck.	Missouri	47,34,56 N 110,48,20 W	eb	33	negative
			ll	30	negative
Hound Ck.	Missouri	47,12,31 N 111,24,48 W	ll	20	positive
			rb	3	negative
Kennedy Ck.	St. Mary's	48,51,20 N 113,32,00 W	eb	35	negative
			wct x rb	1	negative
Little Prickly Pear Ck.	Missouri	47,00,15 N 112,04,45 W	rb	61	positive
			rb	30	pending
			rb	30	pending
		46,59,35 N 112,04,37 W	rb	62	positive
			ll	1	negative
Logging Ck.	Missouri	47,05,40 N 111,00,30 W	eb	27	negative
			rb	15	negative
Marias R.	Marias	48,29,01 N 112,13,34 W	N/S		
Middle Fk. Judith R.	Missouri	46,57,23 N 110,15,26 W	eb	18	negative
			rb	5	negative
			wct	1	negative

Table 8. Continued.

Stream	Drainage	Location Lat./Lon. (D,M,S)[a]	Species collected[b]	n	Results
Missouri R.	Missouri	47,02,00 N 112,00,30 W	rb	90	positive
North Fk. Musselshell R.	Musselshell	46,34,04 N 110,31,21 W	ll	31	negative
			eb	13	negative
			rb	4	negative
North Fk. Smith R.	Missouri	46,34,18 N 110,51,08 W	eb	61	negative
			rb	1	negative
Silver Ck.	Missouri	46,41,03 N 111,59,43 W	ll	60	negative
Smith R.	Missouri	47,21,17 N 111,26,13 W	ll	12	negative
			rb	3	negative
South Fork Judith R.	Missouri	46,51,32 N 110,17,25 W	eb	17	negative
			rb	6	negative
			mwf	5	negative
Sun R.	Sun	47,37,10 N 112,41,37 W	rb	29	negative
			eb	6	negative
Tenderfoot Ck.	Missouri	46,57,11 N 111,09,49 W	rb	57	negative
			eb	2	negative
			ll	1	negative
Tenmile Ck.	Missouri	46,36,10 N 112,05,19 W	ll	30	negative
			eb	10	negative
			rb	3	negative
Teton R.	Marias	47,52,17 N 112,27,52 W	eb	55	negative
			ll	2	negative

Table 8. Continued.

Stream	Drainage	Location Lat./Lon. (D,M,S)[a]	Species collected[b]	n	Results
Trout Ck.	Missouri	46,43,45 N 111,44,13 W	rb	50	negative
			ll	14	negative
Two-medicine R.	Marias	48,28,12 N 113,14,03 W	eb	11	negative

[a] Location in latitude/longitude in degrees, minutes, and seconds.
[b] See Appendix C for species codes.
[c] N/S No salmonids captured

Appendix H-Additional pathogen test results for FWP administrative region 4.

Table 9. Waters within FWP administrative region 4 tested for additional fish pathogens by the WD testing crew during 1996.

Water	Location Lat./Lon. (D,M,S)[d]	Species collected[e]	n	Rs[a] (+/-)	IPNV[b] (+/-)	VHSV[c] (+/-)	Kidney impressions
Beaver Ck.	46,47,54 N 111,51,01 W	rb	60	-	-	-	
Belt Ck.	47,22,01 N 110,54,16 W	ll	57	-	-	-	
		rb	3	-	-	-	
Camas Ck.	46,41,17 N 111,11,20 W	eb	54	-	-	-	
		ll	6	-	-	-	
Dearborn R.	47,12,17 N 112,06,05 W	rb	60	-	-	-	
Highwood Ck.	47,34,56 N 110,48,20 W	eb	33	-	-	-	
		ll	28	-	-	-	
Kennedy Ck.	48,51,20 N 113,32,00 W	eb	25	-			pending
North Fk. Smith R.	46,34,18 N 110,51,08 W	eb	60	-	-	-	
Silver Ck.	46,41,03 N 111,59,43 W	ll	60	-	-	-	
South Fk. Judith R.	46,51,32 N 110,17,25 W	eb	17	-	-	-	
		rb	6	-	-	-	

Table 11. Continued.

Water	Drainage	Species tested positive[b]
O'dell Ck.[a]	Madison	ll, rb
Poindexter Slough	Beaverhead	ll
Racetrack Ck.	Clark Fork	eb, ll
Red Rock R. Springs	Beaverhead	eb, ll
Red Rock Ck.	Beaverhead	eb, rb x yct
Rock Ck.	Clark Fork	eb, ll, rb, rb x wct
Ruby R.	Beaverhead	ll
Ruby reservoir	Beaverhead	rb
South Boulder R.[a]	Jefferson	eb, ll, rb
Stuart Mill Ck.	Clark Fork	eb
Swan R.	Flathead	rb
Unnamed slough of Red Rock R.[a]	Beaverhead	rb, ll
Warm Spring Ck.	Clark Fork	ll
West Fork Madison R.	Madison	ll, rb
Whitetail Ck.[a]	Jefferson	ll
Willow Ck. (above res.)	Jefferson	rb
Willow Springs Ck.[a]	Jefferson	ll

[a] Collection by supplemental WD testing crew.
[b] See Appendix C for species codes.