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WILD RAINBOW TROUT RECOVERY EFFORTS IN COLORADO: PROJECT UPDATES

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The Colorado Division of Wildlife has experimented with a whirling disease resistant strain of rainbow trout (known as Hofer or GR strain) and their crosses in a variety of management and research applications (Schisler et al. 2006). Better growth, higher return-to-creel and angler satisfaction, and lower parasite load has been observed with the GR strain and its crosses in put-and-take rainbow trout fisheries. The state is moving toward using these crosses in most put-and-take or put-grow-and-take locations where *Myxobolus cerebralis* is present.

Reestablishment of naturally reproducing populations in coldwater streams and rivers is also a high priority for the State of Colorado. In the past, many of these waters were managed without reliance on stocking. However, since the introduction of *M. cerebralis*, rainbow trout populations have been heavily augmented with fingerling trout stocking to maintain a rainbow trout component in these fisheries. Resistant rainbow trout are currently being used to improve resistance to whirling disease in existing stocks of rainbow trout such as the Colorado River rainbow trout (CRR) through crossbreeding. The GR-CRR cross fish have been stocked in multiple different locations in Colorado, including the Gunnison, Colorado, Yampa, South Platte, Poudre, Fryingpan and Rio Grande rivers. Success of stocking efforts has been monitored in some locations, such as the Colorado and Gunnison Rivers, by stocking fish with batch marks to identify fish by year stocked and strain of fish. The objective of these stocking events is to evaluate the growth, survival,

reproduction, and infection severity of the experimental fish, and potentially establish self-sustaining rainbow trout populations.

Annual fish population estimates are typically conducted in locations of interest using mark-recapture electrofishing. Marked fish from the experimental stocking events, which are found during the population estimates, are used to gauge growth and survival. Reproduction is monitored with multiple-pass fry removal estimates as well as spot-shocking in areas with abundant fry habitat. In addition, tissue samples have been taken from rainbow trout fry in recent years and analyzed with amplified fragment length polymorphisms (AFLP) (Vos et al. 1995) to determine if genetic markers associated with the resistant strains are present in these fry.

Survival of stocked fish has been variable, depending on stocking location, size of stocked fish, and variety of cross. Stocking fish that are too large to be preyed upon by resident brown trout seems to greatly improve survival. In every trial where pure CRR and GR-CRR crosses have been stocked together, survival in the crossed varieties was equal to, or greater than, their pure CRR counterparts. The crossed varieties have successfully reproduced in the Gunnison River, where natural recruitment also appears to have occurred during 2008. Evidence for limited reproduction is present in the Colorado River, and increased reproductive success is anticipated in 2009 as more GR-CRR cross fish become sexually mature.

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DISTRIBUTION, OCCURRENCE AND RELATIVE ABUNDANCE OF *TUBIFEX TUBIFEX* LINEAGES IN COLORADO: RESULTS OF A 6-YEAR ASSESSMENT

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Three sub-species of cutthroat trout are native to Colorado. Greenback cutthroat trout *Oncorhynchus clarki stomias* are native to the South Platte and Arkansas River drainages. Rio Grande cutthroat trout *O. clarki virginalis* are native to the Rio Grande basin. Colorado River cutthroat trout *O. c. pleuriticus* are native to the Colorado, Dolores, Gunnison, San Juan, White and Yampa River basins. First and second order streams at higher elevations (≥ 2744 m or 9,000 feet) are the habitats where these fish tend to occur.

During the 1990s, all three sub-species were held in sentinel fish cages and exposed to ambient levels of *Myxobolus cerebralis (Mc)* in the Colorado River. Brown trout *Salmo trutta,* brook trout *Salvelinus fontinalis,* and rainbow trout *O. mykiss* were concurrently exposed with the cutthroat trout. All of the exposed fish were young-of-the year fry, 1-2 months post-swim-up. The exposure period ranged from 12 to 18 months. The objectives of these tests were to 1) assess the relative differences in vulnerability to *Mc* infection, and 2) determine the level of mortality among the species. These exposures demonstrated that Colorado's native cutthroat trout sub-species were highly vulnerable to infection by *Myxobolus cerebralis* and experienced heavy mortality. One-month old Colorado River cutthroat trout suffered 85% high mortality 98 days after initial exposure. Even when first exposure occurred at 8 to 9 weeks post-swim-up, all three cutthroat trout sub-species experienced mortality rates ≥ that occurring among concurrently exposed rainbow trout fry of a younger age and smaller size (Thompson et al. 1999).

Until recently little was known about the spread of *M. cerebralis* into higher elevation habitats capable of supporting Colorado's native cutthroat trout. A 6-year study was initiated in 2003 to determine if the *Mc* parasite was spreading into these habitats. The objectives of the study were as follows:

1. Collect population density and biomass data on salmonid populations in higher elevation streams that either support native cutthroat trout or are capable of supporting them.

2. Sample young-of-the-year (YOY) and yearling trout from each study stream and test them by polymerase chain reaction (PCR) and pepsin-trypsin digest (PTD) for evidence of *M. cerebralis* infection.

3. Collect aquatic oligochaetes to determine the presence or absence of *Tubifex tubifex* in the study streams.

4. Test two 50-worm samples of aquatic oligochaetes with haired chaetae by qPCR to determine the relative abundance of DNA in each sample specific for the various lineages (I, III, V and VI) of *T. tubifex* using genetic markers identified by Beauchamp et al. 2001.

Beauchamp et al. (2001) identified genetic markers of mitochondrial 16S rDNA specific for 4 lineages of *T. tubifex* that are known to occur in Colorado. These markers were used in conjunction with a 4-probe multiplex qPCR technique to determine the relative percentage of mitochondrial 16S rDNA specific for *T. tubifex* belonging to lineages I, III, V and VI. All collection sites were marked by GPS so that the statewide distribution of each lineage of *T. tubifex* could be determined. Worms from each sample were sorted, counted and separated into haired and non-haired groups. This process continued until two aliquots of 50 haired worms were identified or all oligochaetes had been sorted from the sample. The 50 worm aliquots were preserved in 70% ethanol in 50 mL test tubes for DNA extraction and qPCR testing to determine the percentage of mitochondrial 16S rDNA specific to each lineage.

Over the 6-year study, 537 sites were visited. There were 161 sites where no attempt was made to collect sediment samples. Sediment samples at collected at 353 sites. There were 63 samples that contained no aquatic oligochaetes. There were 103 samples that contained tubificid worms with haired chaetae but there

was no amplification of mitochondrial 16S rDNA specific for 4 lineages of *T. tubifex* that are known to occur in Colorado. Subsequent qPCR testing of a random subset of these samples revealed that most contained DNA specific for other tubifid worms, usually either *Limnodrilus hoffmeisteri* and/or *Ilyodrilus templetoni*. There were 212 collection sites where qPCR testing resulted in amplification of mitochondrial 16S rDNA specific for one or more of the 4 lineages of *T. tubifex* known to occur in Colorado. Mitochondrial 16S rDNA specific to lineage III *T. tubifex* was detected in qPCR samples from 189 of those 212 sites. Mitochondrial 16S rDNA specific to lineage I *T. tubifex* was detected in samples from36 sites. DNA specific to lineage V *T. tubifex* was detected in samples at 22 sites. DNA specific to lineage VI *T. tubifex* was detected in samples from 82 sites. Among the 212 sites where mitochondrial 16S rDNA specific to *T. tubifex* was detected, only DNA specific to lineage III *T. tubifex* was found in 111 samples.

Mitochondrial 16S rDNA specific for *T. tubifex* was found in samples at elevations ranging from 4,500 feet to 12,834 feet above MSL. Stratification of the entire data set into 1,000 foot elevation zones revealed that DNA specific to lineage III *T. tubifex* was detected more often within every elevation stratum ≥ 6,000 feet than any other *T. tubifex* lineage. It is noteworthy that in two lakes located more than 12,000 feet above MSL, lineage-III *T. tubifex* were present and cutthroat trout in both lakes were infected with *M. cerebralis.*

All of the foregoing indicates that among the 4 lineages of *T. tubifex* known to occur in Colorado, the lineage III strain is the most widely distributed. Laboratory studies have repeatedly demonstrated that lineage-III *T. tubifex* are highly susceptible to infection by *M. cerebralis* (Beauchamp et al. 2002, 2005; Kerans et al. 2004, 2005). Our sampling demonstrated that lineage III *T. tubifex* occur in lake and stream habitats that support Colorado's native cutthroat trout. In numerous areas where cutthroat trout were present, mitochondrial 16S rDNA specific to lineage III *T. tubifex* was the only DNA that amplified with the 4-probe multiplex qPCR testing process. In those areas where lineage III *T. tubifex* oligochaetes are sympatric with cutthroat trout, there would be a significant risk to the health and survival of Colorado's native cutthroat trout if *M. cerebralis* was enzootic as well.

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Risk Assessment: Introduction and Establishment of Myxobolus cerebralis in the Deschutes River Basin, Oregon, USA

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A plan for the reestablishment of anadromous salmonid stocks into waters above the Pelton Round Butte Hydroelectric Project (PRB) located on the Deschutes River, Oregon has been under development since 1996. The PRB complex, starting at river kilometer (Rkm) 103, blocks the further upstream migration of anadromous salmonids into upper river basin waters. Part of the process for evaluating the passage plan was the development of a Fish Health Management Program. The program is, in part, designed to minimize and communicate the magnitude of risk associated with passing serious fish pathogens upstream of the PRB until evaluation of the fish passage effort confirms that the reestablishment of anadromous fish species can be successful. With the goal of minimizing the risk of passing serious fish pathogens along with evaluating the magnitude of risk, several potential pathogens of concern were identified. At the forefront of concern is the fish pathogen *Myxobolus cerebralis* which is responsible for whirling disease in salmonids. Annual, or at least periodic, introduction of *M. cerebralis* into the lower DRB has occurred since at least 1984 as a result of infected stray adult salmonids spawning and dying there. These are primarily hatchery summer steelhead (*Oncorhynchus mykiss*), and to a lesser extent spring Chinook salmon (*O. tshawytscha*), from the Snake River system.

Because of its' potential to cause significant mortality, the risk associated with *M. cerebralis* establishment in the DRB needed to be addressed. The risk of the parasite being transmitted and established upstream of the PRB with the renewal of fish passage at the PRB, and the potential for *M. cerebralis* to affect survival of reintroduced anadromous fish species required investigation. To evaluate these potential risks, we conducted multi-year studies (1998-2007) that informed a risk-assessment model. Tailored specifically for *M. cerebralis*, this model was used to identify key characteristics that will influence pathways of *M. cerebralis* introduction, the potential for establishment, and the consequences of establishment. The three steps involved in the risk assessment model are the release, exposure and consequence assessments. Using this model we evaluated the probability of introduction and establishment of *M. cerebralis* in the lower Deschutes River Basin (DRB) as well as in waters above the PRB with future passage of anadromous fish.

Benthic sediment surveys conducted between 1998-2007 demonstrated *T. tubifex* had a patchy distribution and low relative abundance. Mitochondrial 16S rDNA gene analysis revealed two lineages of *T. tubifex,* III and VI, occurring both above and below the PRB. Laboratory susceptibility studies conducted to characterize differences in total numbers of triactinomyxons produced and infection prevalence between selected *T. tubifex* populations revealed that production varied among exposed groups and was proportional to the number of lineage III worms present. Taking into consideration the factors presented in this study, not all areas of the Deschutes River basin can be classified as having the same likelihood for establishment and the potential impact the parasite poses will not be identical from one location to another. It is clear, however, that *M. cerebralis* could become established above the PRB if infected fish carry the myxospore into these waters. Concurrent sentinel studies confirmed that the parasite life cycle was established in Trout Creek, a tributary of the lower DRB. How this finding will affect the reintroduction plan is unknown at this time; however, it means that DR stocks, whether marked or unmarked, cannot be assumed negative for the parasite.

Update on Status of Whirling Disease in Maryland

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Whirling disease sampling in Maryland found no new infected locations in 2009. Sentinel sampling using fry trout was conducted in areas where the parasite had been found or in potentially exposed areas. Positive areas remaining include: the Mettiki facility and Sand Run; North Branch of the Potomac River in the vicinity of the dismantled Jennings Randolph Net Pens facility, and the pond and pond discharge at Bear Creek hatchery.

The clean-up of the Bear Creek facility is on-going. The wastewater management pond containing spores was excavated and the contaminated spoil was deposited in a deep strip mine pit and mixed with highly caustic flyash. After the material hardened, it was buried under fill. Eventually, the entire site will be filled and reclaimed, but the spore spoil will be buried at a depth greater than one hundred feet.

Old disintegrating raceways at Bear Creek were cleaned and refaced with an epoxy material to remove habitat for *Tubifex*. Sentinel testing through the newly renovated raceways found no evidence of the parasite using PCR testing. However, the water that pools behind the dam at the old stormwater management pond continues to be positive, although parasite loading has been significantly reduced. Even though discharge water was bypassing the pond, one or two low yield springs wetted the remaining spores to allow the life cycle to continue. Staff was directed to cut all flow through the facility and a new discharge site for hatchery outfall was identified. (This was the old discharge point prior to the wastewater management pond.) Plans are underway to construct the new discharge line and install a new treatment facility. Unfortunately, the renovated pond walls have disintegrated in the absence of water and will have to be re-done or staff will have to use four newer, undamaged raceways. Staff is working with indoor culture of fingerlings using well water until full facility renovation is completed. Future plans call for disinfection and filtration units on the hatchery intake.

A new biosecurity plan is now in place, and import and fish health standards are being upgraded to prevent the introduction of pathogens to State waters.

Wilderness and Whirling Disease: An Emerging Threat To Cutthroat Trout Recovery?

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Whirling disease (WD) caused by the metazoan parasite *Myxobolus cerebralis (Mc)* can have devastating effects on some species of trout and salmon. Two hosts, a susceptible salmonid fish and a particular lineage of the aquatic oligochaete *Tubifex tubifex,* must be present for the *Mc* parasite to become established in an aquatic ecosystem (Wolf and Markiw 1984). Studies in the 1990s demonstrated that WD was the primary factor leading to the demise of numerous wild rainbow trout populations in Colorado (Nehring and Thompson 2001; Nehring 2006) and Montana (Vincent 1996). It was first detected in Yellowstone cutthroat (YC) trout (*Oncorhynchus clarki bouvieri)* in Yellowstone Lake, Yellowstone National Park, in 1998. By 2005, NPS fisheries personnel reported that this species in the lake and the Yellowstone River downstream is in peril (Koel et al. 2005, 2006). In Pelican Creek, Yellowstone Lake's second largest tributary, the spawning run of YC trout numbered almost 30,000 in 1981. *Mc* infection prevalence among YC trout fry held in sentinel fish cages in Pelican Creek for 10 days in 2000 and 2001 was 100% and was considered severe. Netting surveys from 2002 to 2004 near the mouth of Pelican Creek indicated that the spawning population in this tributary was essentially lost (Koel et al. 2005).

Three sub-species of cutthroat trout are native to Colorado; Rio Grande cutthroat trout *O. c. virginalis,* greenback cutthroat trout *O. clarki stomias,* and Colorado River cutthroat trout *O. c. pleuriticus*. During the 1990s, all three sub-species were held in sentinel fish cages and exposed to ambient levels of the *Mc* parasite in the Colorado River. Brown trout *Salmo trutta,* brook trout *Salvelinus fontinalis,* and rainbow trout *O. mykiss* were concurrently exposed with the cutthroat trout. All of the exposed fish were young-of-the year fry, 1-2 months post-swim-up. The exposure period ranged from 12 to 18 months. The objectives of these tests were to 1) assess the relative differences in vulnerability to *Mc* infection, and 2) determine the level of mortality among the species resulting from continuous exposure at a young age. Colorado's native cutthroat trout sub-species, exposed at the fry-fingerling stage, were highly vulnerable to infection and experienced heavy mortality. One-month old Colorado River cutthroat trout suffered 85% mortality after 98 days of continuous exposure. Even when initial exposure occurred at 8 to 9 weeks post-swim-up, all three cutthroat trout sub-species experienced mortality rates ≥ that experienced among concurrently exposed rainbow trout fry of a younger age and smaller size (Thompson et al. 1999).

Like YC trout, all three sub-species of Colorado's cutthroat trout are highly susceptible to *Mc* infection. Establishment of *M. cerebralis* in aquatic ecosystems connected to and/or capable of supporting cutthroat trout could put recovery programs and the survival of these fishes in jeopardy. However, at the end of the 20th century, little was known about the spread of *M. cerebralis* into Colorado's higher elevation (≥ 9,000 feet) aquatic habitats capable of supporting native cutthroat trout. In 2003, a 6-year study was initiated to determine if the *Mc* parasite was spreading into these habitats. Study objectives were three-fold:

1. Collect young-of-the-year (YOY) and yearling trout from lake and stream habitats capable of supporting cutthroat trout and test them for evidence of *M. cerebralis* infection.

2. Collect population density and biomass data on salmonid populations in higher elevation streams that either support native cutthroat trout or are capable of supporting them.

3. Collect aquatic oligochaetes across a wide range of lakes and streams to determine the relative abundance, distribution and elevation range of the lineages of *Tubifex tubifex* and assess the risk of establishing the *Mc* parasite were exposure through stocking or other means to occur.

Data from the study are summarized in Tables 1 and 2. Taken together these results do not support the hypothesis that there is an elevation barrier in Colorado above which lineage III *T. tubifex* cannot occur, nor an elevation barrier that would prohibit the establishment of *M. cerebralis* if the susceptible salmonid and oligochaete hosts are sympatric in the ecosystem. *Mc*-susceptible lineage III *T. tubifex* DNA was detected in 45.9% of the oligochaete samples screened by PCR and/or PTD (Table 1). Forty percent of the fish collections screened by PCR and/or PTD were positive for *Mc* infection (Table 2) At the 6 samples sites above 11,000 feet elevation where evidence of *Mc* infections in salmonids was detected, PCR screening of oligochaetes from those same lakes indicated DNA of lineage III *T. tubifex* was present in every sample. Prevalence of infection of infection was ≥ 50% in cutthroat trout collected from two lakes at elevations of 11,755 feet and 12,054 feet. Overt clinical signs of whirling disease were easily visible in most fish.

Gill netting data from the lake above 12,000 feet suggests that few (if any) cutthroat trout fingerling stocked in the lake since 2003 have survived. Only 4 cutthroat trout were caught during 2 successive nights of gill netting. The smallest was 14.8 inches and the others were all 16.9 inches long. Trout infected with *Myxobolus cerebralis* have been collected from 11 streams and 6 lakes in 6 separate wilderness areas of Colorado. Infections among the trout in 10 of the 17 aquatic ecosystems in the wilderness areas are severe enough to be causing population level impacts.

Table 1. Frequency of occurrence of various lineages of *Tubifex tubifex* stratified by 1,000 foot elevation increments in Colorado (2003 – 2008).

Elevation Strata(ft.)	**Number of Sites where each Lineage of *Tubifex tubifex* was present**						
	Lineage I	**Lineage III**	**Lineage V**	**Lineage VI**	**No Lineage**	**Elevation Stratum**	
						Total	**% No Lineage**
5,000 – 6,000	0	3	0	4	0	7	0
6,001 – 7,000	6	28	3	15	3	55	5.50%
7,001 – 8,000	1	29	2	7	6	45	13.3%
8,001 – 9,000	8	46	5	16	19	94	20.2%
9,001- 10,000	3	33	2	7	25	70	35.7%
10,001- 11,000	1	17	1	2	14	35	40.0%
11,001 – 12,000	0	4	0	3	24	31	77.4%
12,001 – 13,000	0	2	0	2	12	16	75.0%
Total	19	162	13	56	103	353	29.1%
Percent/Lineage	5.4%	45.9%	3.7%	15.9%	29.1%	100%	

Table 2. Numbers and percentages of fish collections testing negative and positive for *Myxobolus cerebralis* (*Mc)* infection stratified by 1,000 foot elevation increments in Colorado (2003 – 2008).

Elevation Strata (ft.)	*Mc* Negative Samples		*Mc* Positive Samples	
	Number	Percent	Number	Percent
5,000 – 6,000	1	11.11	8	88.89
6,001 – 7,000	13	40.63	19	59.37
7,001 – 8,000	32	60.38	21	39.62
8,001 – 9,000	57	58.16	41	41.84
9,001- 10,000	57	55.34	46	44.66
10,001- 11,000	47	73.44	17	26.56
11,001 – 12,000	25	86.21	4	13.79
12,001 – 13,000	13	86.67	2	13.33
Total	245	60.79	158	39.21

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Whitefish Population Declines in Montana

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There are indications that some mountain whitefish (*Prosopium williamsoni*) populations are showing signs of severe declines in number, although the extent, cause and degree of severity of these declines are unknown. Actual population estimates are rare with most of the data coming in the form of either trend or antidotal. There is limited data on some streams such as the Madison River, Blackfoot River drainage and Mission Creek, a tributary to the Flathead River, on the Salish-Kootenai lands. Probably the best data comes from long term gill netting data on Hebgen reservoir. Population trend data from bottom set gill nets have been extracted beginning in 1971. Data includes average number per net set, plus size and age structure. Mountain whitefish population appeared to be very stable from 1971 through 2000 where average numbers per net were 16.4. Beginning in 2001, there was a downward trend in the numbers per net set averaging 8.4 for the 2004-07 period. It appears that recruitment of young whitefish into the adult population is inadequate to maintain the pre-2001population. From 1971through 2001, approximately 20% of the whitefish were three years and younger (less than 13.0 inches in length), but since 2000 that percentage has continually declined and by 2007 made up less than 5% of the whitefish netted. With the decline in total numbers, the existing mountain whitefish are living to older ages and the average size in the nets has steadily increased. Whitefish larger than 19.0 inches which were rare (less than 2% of the catch) prior to 2001 comprised over 12% of the catch by 2007. The cause of this decline is unknown with possible factors being whirling disease, water flows, water temperatures or some other unknown factor. Population data from the Blackfoot River drainage and the upper Madison River show the same lack of younger age groups although there is no long term data for comparisons. Mission Creek located on the Salish-Kootenai tribal lands has had an ongoing study monitoring the out migration of wild rainbow trout and mountain whitefish. This and several other tributaries to the Flathead River are thought to be important source of recruitment for populations in the Flathead River below Flathead Lake. Evaluation of the out migration of wild rainbow trout and mountain whitefish using rotary fry traps has shown a steady decline in wild rainbow trout and mountain whitefish fry moving to the Flathead River since the early 2000's. Mission Creek and some other tributaries to the Flathead River located on the Salish-Kootenai lands have experienced the introduction and escalation of *Myxobolus cerebralis* infections. The exact date of introduction is unknown, but sentinel cage exposures from 2003 through 2007 have measured average infection levels as high as 4.97 in Mission Creek. The tribal fisheries biologist believes that the decline in rainbow trout out migrating is due to the high whirling disease infections. The decline in mountain whitefish numbers could also be related to the disease. In one year over 20% of the out migrant mountain whitefish showed severe caudal deformities.

Where do we go from here? What are the data needs? There is the need for the most basic of data: 1) life history data; 2) population data; 3) habitat requirements; 4) water temperature requirements; 5) stream flows requirements, 6) impacts of various water pollutants and 7) disease issues.

Life History- research to determine 1) when and where spawning occurs; 2) habitat needs for fry rearing; 3) extent and timing of movement of adults and sub adults; and 4) habitat needs for both adults and fry.

Population data – 1) establishment of long term trend or mark-recapture population data in a many streams as possible; and 2) gathering age structure data to determine what is a typical age and size structure of a stable population versus declining population.

Habitat needs- research to determine 1) what are the habitat needs adult whitefish need at various times of the year; and 2) what are the habitat needs for spawning and rearing of fry in rearing areas and 3) habitat needs once fry leave their rearing areas.

Water temperatures – determine the optimum water temperature ranged for all stages of their life history.

Stream flow requirements- determine the minimum flow needs for spawning, hatching, rearing, sub adult and adults.

Water quality issues – determine what water quality factors may limit the success of mountain whitefish in all stages of their life history.

Disease issues – determine if whirling disease can be or is a problem for survival of young mountain whitefish fry; 2) if whirling disease is a negative population issue, at what size and age does Myxobolus cerebralis become a survival issue; 3) need for additional controlled testing of whitefish vulnerability to the whirling disease parasite.

Dealing with Invasive Species in Maryland

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Whirling disease was the first invasive agent brought to the attention of Inland Fisheries in 1995. In 2002, snakeheads were found at a state pond and invasives have been occurring with increasing frequency.

Snakeheads (*Channa argus*) were originally found in a pond in Crofton and were removed by poisoning the pond after lengthy deliberations. Following this event, the federal and state governments enacted legislation banning the possession of snakeheads. This action caused some hobbyists to release their fish into the wild. Snakeheads are found in the tidal Potomac River below Great Falls in the DC area and are also now found in the Anacostia River and Mattawoman Creek in Maryland and in Dogue and Hunting Creeks in Virginia.

Other invasive species that have been found since 2002 include the rusty crayfish (*Orconectes rusticus*), didymo (*Didymosphenia geminata*), Chinese mitten crab (*Eriocheir sinensis*), Blue catfish (*Ictalurus furcatus*), flathead catfish (*Pylodictis olivaris*), and zebra mussels (*Dreissena polymorpha*). Special regulations have been enacted regarding the rusty crayfish and both catfish species, although all of these species except didymo and disease agents are covered by regulations that restrict at a minimum transport of the species and at a maximum prohibition of possession.

Maryland has an Invasive Species Matrix Team (ISMT) within the Department of Natural Resources that addresses all invasive issues and is working toward a proactive approach to prevent the introduction of invaders. To that end, the ISMT and Fisheries Service are actively trying to educate the public on the dangers of these species on aquatic habitats.