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The Ichthyogram

VOLUME 14, NUMBER 2

JULY 2003

Sand Filtration for Removal of *Myxobolus cerebralis* triactinomyxons: Effects of Extended Backflushing or Diversion of Return Flow

INSIDE THIS ISSUE:

Previous tests at the Fisheries Experiment Station have indicated that sand filtration holds some promise for removal of the triactinomyxon stage of *Myxobolus cerebralis* from hatchery water supplies (Ichthyogram 13 (3)) if sand of # 180 um diameter was used. A single spore in one of the sand filter treatment fish indicated that the system still needed some tweaking to maintain uninfected stocks. Backflushing was suspected as a variable that could be a weak point in the process.

A laboratory experiment was conducted in which three treatments were compared to positive and negative controls: 1) *extended backflush*, in which the duration of backflushing was increased to theoretically flush out more triactinomyxons, 2) *diverted return flow*, in which water that was headed for the tanks with fish, immediately after backflushing, was diverted for 5 min to "re-seat" the sand in the filter, and 3) *slow sand filtration*, in which water was passed through a larger filter at a slower rate and no backflushing was conducted (see Figure 1.). The first two treatments and the controls were conducted using the same recycle system described in the previous article. The slow sand filtration treatment was conducted using large plastic garbage cans that had the same depth of sand used in the other treatments (18 cm). The mortar sand was placed over a bed of pea gravel. Water was recycled with a pump from the tanks with the fish back to the top of each filter. A float valve maintained the water level in the filter.

Figure 1. Slow sand filtration unit

Each treatment had 3 replicate tanks with twenty rainbow trout fry of the Sand Creek strain stocked into each. Fish had been on feed for four and a half weeks when they were initially exposed (20 January 2003). The head boxes for each of the treatments (except negative controls) were spiked with 13,000 triactinomyxons per treatment (217 per fish) thrice weekly until the experiment was concluded on 05 May 2003. Backflushes were conducted once a week initially and up to twice a week near the end of the experiment. The backflush was collected into a container, which was subsequently poured through a 180 Fm sieve for each treatment to determine if there had been any sand loss during the backflush. Three times daily the fish were fed an amount of feed they would consume in roughly 1 minute without wastage. This ration was limiting, however it allowed for average specific growth rates of 2.3 (%/day). Water quality was measured weekly to assure that the biofilters were functioning properly and that adequate dissolved oxygen was available. Temperatures during the study ranged from 14.9 to 16.6E C for all but the slow sand filters which ranged from 13.1 to 15.7E C.

At the end of the experiment, fish were harvested individually, removing the head with a saw and placing the head in a separate wire-closure plastic bag. The saw was cleaned with a brush and disinfected between uses using a 50% bleach solution. In addition, a fresh bleach solution was

(Continued on page 4)

Myxobolus Cerebralis Found in Hobble Creek, Spanish Fork River

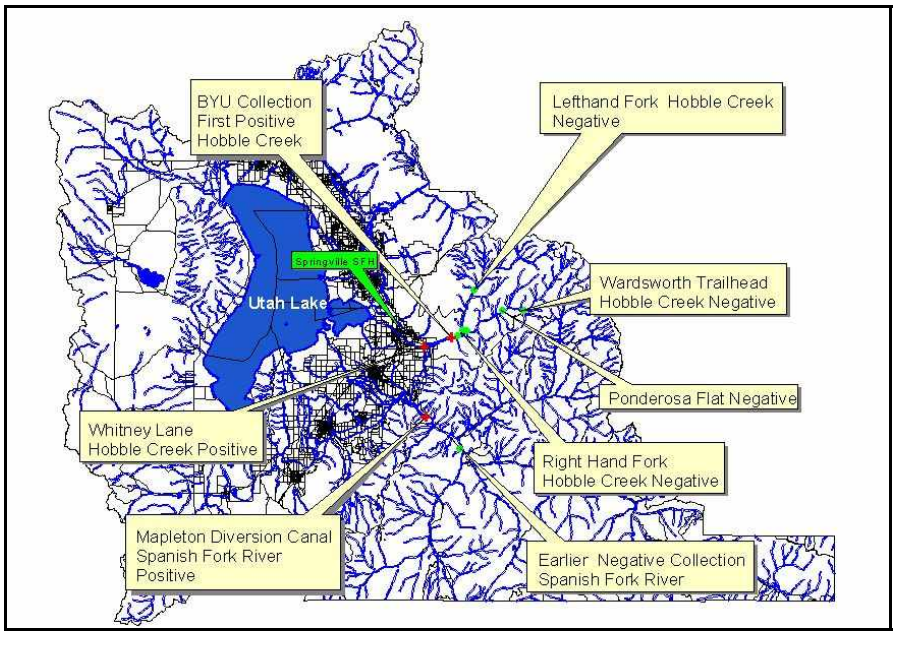
The technical services team at the Fisheries Experiment Station, in cooperation with the Central region's aquatic biologists have discovered the presence of *Myxobolus cerebralis* (MC*)*, the parasite that causes whirling disease, in trout from Hobble Creek and the Spanish Fork River. The significance of these findings is that both of these drainages are in close proximity to the Springville State Hatchery . Biosecurity precautions have been escalated in order to prevent the spread of the parasite into hatchery fish.

The finding was made as part of the State of Utah's whirling disease survey intended to improve our understanding of the distribution of the parasite. The first samples tested were obtained by biology students at Brigham Young University whom had permission to extend their classroom work into a field. In the Fall of 2002, they electroshocked sixty brown trout from Hobble Creek near the golf course, and the samples were tested using polymerase chain reaction (PCR) techniques. Several of the trout were found to contain DNA from the MC parasite. Division of Wildlife biologists conducted more extensive sampling on Hobble Creek the following spring. Six sites were selected on the creek (see map), extending from the upper reaches of the left and right hand forks downstream to the city of Springville. Once again, sixty fish were assayed with single round PCR, and one Brown Trout from the down creek sampling site near Whitney Lane was found to be infected. The confirmatory finding was reported to the Fish Health Policy Board as well as DWR biologists and culturists. A meeting was held to address the finding, and ensure that all precautions were being taken to avoid further spread of the disease. Efforts are also being made to determine the risk of spread to native cutthroat populations existing in the headwaters of Hobble Creek.

The source of the infection is not known; however, Hobble Creek is readily accessible by anglers, and it is possible the parasite was transferred from a nearby contaminated site. Another possibility is that the parasite traveled from an upstream drainage without a vector. Biologists sampled fish from the Spanish Fork River, which has a diversion maintained by the Strawberry Water Users Association to supply water to Hobble Creek. Once again, a representative sample of six fish were collected and tested with PCR. The prevalence of infected fish in the sampling was considerably higher (7 out of 60 fish) in this river system, and so it is suspected that the infection originated from somewhere upstream. Histopathology was also used to confirm the finding and also help determine the chronicity of the outbreak. Pathology results are still pending.

Additional survey collections of trout will be made on other river systems in the Spanish Fork drainage (i.e. Diamond Fork and Sixth Waters). The risk of the disease spreading to Strawberry Reservoir via Diamond Fork River is fairly limited, since the East portal out of the reservoir is an adequate fish barrier. However, anglers should be careful and thoroughly clean off all mud and dry all fishing equipment after fishing these drainages and don't transfer fish carcasses from these contaminated waters.

Patrick Goddard



Whirling Disease Resistant Trout Project Gets Underway

The Fisheries Experiment Station, in cooperation with Richard Vincent of Montana, Fish, Wildlife and Parks, and with Utah State University researchers Mark Miller and Karen Mock, submitted a proposal that was recently funded by the Whirling Disease Initiative. This is federal funding managed by the Whirling Disease Steering Committee via the Montana Water Center at Montana State University. The aim of the study is to find molecular markers for resistance in Fish Lake-DeSmet rainbow trout (RTFD). Preliminary research by Dick Vincent indicated that RTFD from Ennis National Fish Hatchery broodstock displayed some resistance to whirling disease. The resistance was indicated by the very low to no scores for the histological samples from fish exposed to *Myxobolus cerebralis* triactinomyxons in controlled dosage exposures. Some field exposed fish have also shown a similar pattern of resistance, with about half with high scores and half with scores of 0 or 1 on the McConnell-Baldwin scale. The test was repeated with RTFD from Egan State Fish Hatchery in Utah, but the results were typical of other susceptible rainbow trout strains. However, the fish for that study were size selected for smaller fish, so only the runts of the lot were tested, possibly biasing the outcome.

Unfortunately, the work on identifying the molecular markers associated with resistance was to begin with the Egan SFH fish. Since no resistance was observed in these, the molecular marker portion of the work will be delayed.

In the meantime, we have initiated a few additional exposure tests. Dick Vincent was supplied with additional RTFD from Egan (not size selected) that have been exposed to triactinomyxons along with RTFD from the Ennis stock. Additional Ennis RTFD from a brood replacement lot being thinned have been shipped to Utah State University where an additional exposure test is underway. Fish there are being exposed to either a single acute exposure of 1000 triactinomyxons per fish for 2 hours (positive control), or to chronic exposures of 100 or 200 triactinomyxons per fish over the course of 10 exposures.

Results from these tests should indicate whether the Utah stocks carry the disease resistance genes. The results should also provide more information on how widespread the resistance may be among the broodstock at Ennis. Possibly only a few fish carry the resistance genes, making the hunt for a resistant rainbow trout much more difficult. Closer screening of the progeny of individual females and males at Ennis might be necessary to track it down. However, if the resistance is a trait of several of the Ennis and Egan RTFD, molecular markers identified via exposures studies could then identify the broodstock with the resistance. These could be selectively bred to produce a whirling-disease resistant rainbow trout.
Eric Wagner

New Faces at FES

The Fisheries Experiment Station would like to welcome the new June sucker seasonal worker **Becca Ogden**. Becca is from Pocatello, Idaho, and has attended college in South Dakota where she also played soccer, Utah State University where she majored in Wildlife Management and will be attending the College of Veterinary Medicine at Washington State University this fall. Becca plays on the women's rugby team at USU and will be playing for the Pacific Northwest's select side team for a second year in a row. Becca enjoys hunting, especially archery, and fishing. She also enjoys dancing under the right conditions and playing with her dog Jack.

(Continued from page 1)
used for each treatment. Sampling was done in the following order to minimize cross contamination: negative controls first, then treatments, and positive controls last.

Table 1. Comparison of mean (n = 3) PCR and PTD results with positive and negative controls for rainbow trout fry downstream of sand filters that were backflushed with 10 gal of water (extended backflush), slow sand filters, or for which the filtered water was diverted from the fish for the first 5 min after backflushing was completed. The mean myxospore burden in each head is given (derived from positive fish only from the PTD test).

Treatment	PTD prevalence (%)	PCR prevalence (%)	Myxospores/head
Diverted backflush	0.0	0.0	0
Extended backflush	2.9	0.0	333
Slow sand filter	0.0	1.6	0
Positive control	100.0	100.0	79,651
Negative control	0.0,	0.0	0

Samples were prepared according to the pepsin-trypsin digest method (Thoesen 1994). The product of the pepsin-trypsin digest was diluted to 2 mL with phosphate buffered saline. Aliquots of 0.5 mL were transferred from each sample to each of two different microcentrifuge tubes. One set of tubes were sent for PCR analysis by Pisces Molecular Inc. using the heat shock primer for *Myxobolus cerebralis*. The other set of samples was sent to Utah State University researcher Carey Wicks for testing using the ELISA technique with antibodies developed there. Her work is still ongoing. The remaining fraction was examined by transferring a portion of the vortexed sample to a hemocytometer. All 9 large squares of both sides of the hemocytometer were counted.

Results are summarized in Table 1. The one treatment absent of infection, with the exception of the negative controls, was the diverted backflush treatment. This result supports the hypothesis that residual TAMs may be loose within a filter after a backflush event, and before the sand bed is reseated. Several infected fish were found to be positive within one replicate aquarium of the extended backflush treatment, although the infection was very light. What is curious about this result is the lack of positive supportive data from the PCR results. Because each fish was analyzed by PCR, PTD, and an aliquot of sample was sent to USU for ELISA research, the quantity of sample for each assay was small and it is possible the myxospores found positive in the PTD samples comprised the entire amount of spores and none were found via PCR. It is also possible that there was cross-contamination of the few samples after the samples were split. One positive sample was also found from one fish within one replicate of the slow sand filter treatment, indicating possible deficiencies within that filter design with respect to TAM removal. The correlation between spores per head and the PCR code was weak (r = 0.185).

Overall, despite infected fish within two of the three treatments, the information gathered from this study is very valuable. The clean results from the diverted backflush treatment indicate the need to follow a specific protocol when backflushing a TAM-sandfilter. They also show that where infected fish were found in the previous study, within a similar treatment, altering the protocol resulted in clean fish. The results also indicate, once again, that sand filtration is a viable, relatively inexpensive means of cleaning up TAM-infected water sources.

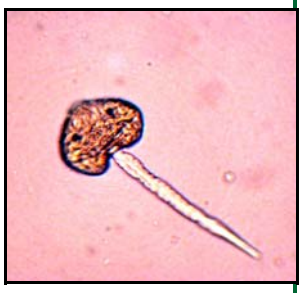
Literature cited

Thoesen, J.C. 1994. Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. Fish Health Section of the American Fisheries Society, Bethesda, Maryland.

Ronney Arndt and Eric Wagner

Centrocestus Parasite Discovered at Second Location in Utah

As previously reported *Ichthyogram* (Volume 14, #1), the parasite *Centrocestus formanosus* was recently discovered in speckled dace found at Gandy Warm Springs in western Utah. The parasite can cause substantial gill damage, and is associated with the exotic and invasive warm water snail, *Melanoides tuberculata.*



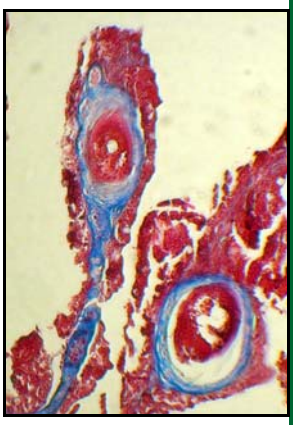


Figure Legends:
Above: Melanoides tuberculata, first intermediate host of the parasite
Top, right: cecaria of centrocestus released from snails
Right: pathology sections of Gambusia from Goshen showing parasitic cysts in blue

Following up on another reported finding of *Melanoides* at Goshen Warm Springs by Dr. Mark Vinson at Utah State University, UDWR biologists took histopathology samples from *Gambusia* living at the Goshen springs and have confirmed extensive infection of that species with the parasite.

Snails collected at that location have been taken to an aquarium at the Fisheries Experiment Station. Water samples from the aquarium have shown large numbers of cecaria (infective stage for fish) of the parasite released from the snails.

Plans are underway to examine other warm spring locations throughout the state to determine the extent of this parasite.

Chris Wilson

Renovation at FES, Let the Fun to Begin!

Empty raceways, drying vegetation, construction trailers and backhoes awaited the beginning of construction of new raceways at FES. They will replace old crumbling raceways and significantly increase the fish production potential.

In the meantime, FES biologists salvaged escapees from the raceways and stocked over 200 jumbo sized catchables to the delight of young anglers at First Dam on the Logan River

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