The Ichthyogram

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Cleaning Up Whirling Disease With Sand Filtration

Sand filters have been used for centuries as a means of preparing water for culinary use and as a way to clean up wastewater. Sand filters can mechanically trap particles that are too large to fit between the individual grains of sand that comprise the filter. They can also aid in the breakdown of nutrients and chemicals by the action of bacteria, which inhabit the sand. With the infection of the Midway Hatchery we became interested in using the mechanical filtering properties of sand filters to remove *Myxobolus cerebralis* triactinomyxons (TAMs) from incoming waters rendering the water safe for fish production. Previous work (Icthyogram Vol 12, Issue 3-4, Dec 2001) indicated that sand particles of 300 ɥm and bed depth of 2 cm, or 425 ɥm and bed depth of 4 cm effectively removed TAMs.

The results from these tests were used to guide further testing. A sand filter experiment was conducted in aquaria using a recycle/biofilter system, beginning November 13, 2001. Four treatments were 1) negative control, 2) positive control, 3) 4 inches of sand of >180 ɥm, and 4) 4 inches of sand of >300 ɥm in diameter. Each treatment had its own biofilter, headbox, and 3 aquaria. Initially, 30 rainbow trout were added to each aquarium. Triactinomyxons (13,000) were added to each headbox three times weekly (except for negative controls). Preliminary samples (up to 10 fish per tank) were taken December 18th to thin the aquaria fish and test for infection. The PCR (polymerase chain reaction using heat shock protein primers) results after 1 month of exposure indicated that negative controls and the >180 ɥm treatment group were negative for *M. cerebralis,* whereas 3 of 30 were positive in the >300 ɥm group and 15 of 21 fish were infected in the positive control group.

On 29 April 2002 the 179 remaining fish were harvested, their heads split, and one half processed by the pepsin-trypsin digest method (PTD), and the other by PCR. The PCR data indicated that all 54 fish from the >180 ɥm treatment were negative, while in the >300 ɥm treatment, 49% were infected with *M. cerebralis* (4 of 47 were weakly positive, 12 were strong positives, and 3 were very strongly positive). All 29 of the positive control fish were infected (5 positive, 17 strong positive, and 7 very strong positive) and all 49 of the negative control fish were negative. The PTD results closely matched those of the PCR tests, but there were a few discrepancies. By PTD, there was a single spore (1,111 spores per head) found for one fish of 54 in the >180 ɥm treatment. In the >300 ɥm treatment

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group, 25 of 47 were infected (53%) with an average of 14,222 ± 23,612 spores per head. All 29 positive-control fish were heavily infected, averaging 2.41 ± 1.48 million spores per head. As for the PCR data, no infected fish were found in the negative control fish. The data indicated that a sand filter, with a sand bed 10 cm deep, and using sand 180 μm and larger diameter, was effective in preventing triactinomyxons from reaching susceptible fish. The data also suggested that the PCR using heat shock protein primers was capable of identifying infected fish with a similar sensitivity to the PTD method.

While this study was ongoing at the Fisheries Experiment Station (FES), Chuck Bobo and Tom St. John of Utah's Midway State Fish Hatchery conducted a separate test in the hatchery building there. At Midway, a commercially produced filter (Baker Hydro, 36"dia.) was fitted to Main Spring, which is contaminated with *M. cerebralis*. The bottom of the filter was filled with 18 cm of pea gravel and topped with 17 cm of sand (>180 μm). The filter was gravity fed from the spring and was run at approximately 57 L/min (15 gpm), and was back-flushed thrice weekly at a rate of 51 L/min (13.5 gpm). The test fish (rainbow trout) were reared from eyed eggs in the filtered water since hatching November 5th, 2001. Control fish were reared in spring water without filtration.

Samples taken after 4 months (March 13, 2002) were split and analyzed by either PCR or the PTD methods. Samples analyzed by single-round PCR and PTD indicated that the sand filter was able to prevent infection (0 of 20 fish infected). For the positive controls, one fish was weakly positive by PCR. One fish was also positive by PTD (4 spores total on 2 sides of hemacytometer), although it was a different individual from that identified by PCR.

The remaining fish were held until August 12th to see if the irrigation season increased the exposure levels and prevalence rates in the control fish. Irrigation water from the Provo River, infected with whirling disease, has been shown to infiltrate into the Midway spring complex. These final fish were prepared similarly as discussed above by splitting each head sagitally and analyzing one half by PCR (using the heat shock primer) and the other by PTD. For the PTD assay, the whole wetted surface area of both sides of the hemocytometer was examined. If any spores were present, the large grids were used to estimate spore numbers.

The PCR results indicated a significant reduction in prevalence rates using sand filtration. Within the control fish, which had been reared in unfiltered spring water for 269 days, 28 of 60 (47%) fish were infected. Of these 28 fish, 1 was weakly positive, 5 were positive, 11 strongly positive, and 11 very strongly positive. Within the group of fish that had been in filtered water for the same length of time, 3 of 60 (5%) were classified as infected, with 1 positive, and 2 strongly positive. The PTD results differed significantly from the PCR results. For the fish from sand-filtered water, no spores were observed in any of the PTD samples. For control fish, only 1 suspicious spore was observed in a single sample which was PCR-negative. PTD was clearly much less sensitive for detection of *M. cerebralis* in this case. Likely, the infected control fish had not had time to develop spores.

When looking at the control fish at Midway, the results from the March sample indicated that TAMs were present in Main Spring at a low level from winter through spring, but the much higher prevalence of infection revealed in the August sample suggested a higher concentration of TAMs in the spring water during the summer. These TAMs may be transported to the spring from infected irrigation water, via underground channels, or it is possible there are infected *Tubifex tubifex* worms within the spring system that are producing TAMs seasonally.

In both the FES and Midway tests, the sand beds consisting of grains >180 µm were effective at filtering TAMs out of infected water for some time. At Midway, treatment fish were disease free for four months and at the FES, >180 µm treatment fish were disease free for at least one month, and at the conclusion of that study, only one fish had become infected. These results indicate that sand filters are capable of removing TAMs from infected water, but at some point, filtering efficiency is lost, and TAMs pass through. It is possible that the back-flush process is the weak link. When the media bed is fluidized during a back-flush, trapped TAMs should be freed from the filter and discharged from the rearing system. Perhaps some TAMs are only partially liberated from the filter bed by the time back-flushing is complete. When normal flow is restored through the filter, these TAMs may be able to pass through the filter before the sand bed has completely seated itself after being fluidized. Other potential unknowns are the effects of water volume (i.e., does more flow push the tams through the media?), time (sand loss over time?; size changes over time? what is the best replacement schedule?) and bacterial biofilms that develop on the sand grains over time (greater trapping efficiency? reduced flows?).

Hopefully these shortcomings may be overcome, because rapid sand filtration appears to be an inexpensive, low maintenance alternative to costly, maintenance intensive, and potentially user harmful alternatives such U.V. and ozonation techniques. The sand filters designed for the FES trial allowed for a reasonable flow (0.7 gpm, 2.8 l/min, 8.6 m/h) and fell within the range that defines rapid sand filters (4-20 m/h). The commercial filter used at Midway was rated for flows up to 141 gpm (543 L/min), but the actual flow of 15 gpm (57 L/min) through a filter bed of 7.1 ft^2 resulted in a rating of 5.2 m/h. The "Catch-22" of the sand filter's application to TAM removal is that a deeper sand bed of smaller sand may be needed to affect 100% TAM removal, however such a design necessitates a loss of head pressure. By determining the appropriate depth and size of sand, it seems probable that sand filters could be scaled up to accommodate the flows necessary to feed a large fish hatchery.
Ronney Arndt and Eric Wagner

Least Chub Return to FES

The Fisheries Experiment Station is once again a site where least chub, *Iotichthys phlegethontis,* can be found. The least chub is a "sensitive species" found in the Bonneville Basin and are very small with a short life span. FES received 75 fish in April 2002 from the Mona Springs complex after fish health inspections were conducted on other species found at the springs. Upon arrival the fish were quarantined in the June sucker compound and prophylactically treated for any possible internal and external parasites before being transferred to a raceway in the main hatchery in June. Habitat was provided in the raceway where reproduction took place over the summer. In October, least chub from Antelope Island (originally from Mona Springs) were transferred to FES and are currently in quarantine. Additional groups will be arriving in the next few weeks. Least chub are being transferred to FES to establish a refuge population; habitat degradation and competition with other species have resulted in a decline in their numbers and distribution. Research and culture were performed on this species at the Station in the early 1970's by Marianne Crawford and others.
Eriek Hansen

New Places and New Faces…

The revolving door continues to turn with the Technical Services team of the Fisheries Experiment Station. Biologist **Mary George** decided to move back to the mountains of Colorado, and is currently pursuing other medical interests. We wish Mary the best.

A search was undertaken to fill Mary's shoes, and it was decided that the best person for the job would be **Anna Marie Miller**. Anna has been working as a Technician in the lab, and has done a wonderful job taking over the responsibilities of running the parasitology and bacteriology services. She also has interests in research, and expanding on the histopathology services performed by the team. Anna completed a graduate degree in Wildlife and Fisheries from Texas A&M University, and can still be reached at (435) 752-1066 extension 20.

Anna's technician position was filled by Utah native **Jared Zierenberg**. Jared went to high school in Springville, Utah, and completed his Bachelors of Science in Fisheries and Wildlife at Utah State University. His work experience is extensive; having worked as a Hotshot Firefighter for the Logan Ranger District, a Forestry Technician for the Spanish Fork Ranger District and most recently as a Lead Biological Technician for the same district. He has been helping on several projects focused on monitoring threatened and endangered fish and wildlife species of the Uinta National forest. After spending most of his work hours in the field, Jared is looking forward to picking fish heads in the laboratory so he can spend more evenings with his wife Crystal and their newly born daughter. When he is not changing diapers, Jared enjoys many of the outdoor activities relished by his new colleagues, like hunting, fishing and hiking. We are looking forward to Jared's enthusiasm about the natural resources. Jared can be reached at (435) 752-1066 extension 13.

Best wishes to these employees in their new career positions.

Patrick Goddard

Diatomaceous Earth As A Potential Control Agent For *Tubifex tubifex*: the Intermediate Host of *Myxobolus cerebralis*

Myxolobus cerebalis is the causative agent of whirling disease in salmonids. Whirling disease is the condition that results from thousands of *Myxolobus cerebalis* spores attacking nervous tissue and cartilage of afflicted salmonids. The *Myxolobus cerebalis* parasite has two hosts, the common sludge worm *Tubifex tubifex* and salmonids. The parasite requires both hosts to complete its lifecycle successfully. *Tubifex* worms generally are found wherever there is wet mud present. This study was undertaken to determine the efficacy of diatomaceous earth as a means of eliminating or significantly reducing the number of *Tubifex* worms in a given system.

Diatomaceous earth is comprised primarily of ground up diatoms, which have a substantial amount of silicon dioxide as a structural component of their exoskeletons. Microscopically, diatoms have thousands of hard spicule like projections that lend an abrasive nature to the texture of diatomaceous earth. This abrasive property has been exploited by gardeners, botanists, veterinarians, and various agricultural interests among others to control insects and various internal and external parasites of horses and livestock. The main goal of this study was to ascertain whether the abrasive nature of diatomaceous earth would lend itself to an application for eliminating tubifex worms from a system.

Five different treatments were used, with three replicates for each with ninety worms each at the trial's outset. The trial was comprised of the following treatments: (i) 0% diatomaceous earth: 100% soil, (ii) 25% diatomaceous earth: 75% soil, (iii) 50% diatomaceous earth: 50% soil, (iv) 75% diatomaceous earth: 25% soil, and (v) 100% diatomaceous earth: 0% soil. One-liter beakers were used to house each treatment. Each treatment contained the appropriate ratios of diatomaceous earth to sediment to achieve 100 grams of substrate in each beaker. The remaining volume required to reach a volume of one liter for each treatment was attained with hatchery well water. The treatment that resulted in the lowest survival for *Tubifex* worms was found to be the 75% diatomaceous earth: 25% soil (Table 1). The 100% diatomaceous earth treatment was not much more effective for controlling *Tubifex* worms than the 50% and 25% diatomaceous earth treatments. These trends are highlighted in the table below.

Table 1 Average survival of *Tubifex* worms at four different proportions of diatomaceous earth to Soil. Survival means that are significantly different are denoted by different letters.

Treatment	Average Survival (%)	Standard Deviation
0% Diatomaceous earth: 100% Soil	81 b	2.3
25% Diatomaceous earth: 75% Soil	72 b	16.6
50% Diatomaceous earth: 50% Soil	71 b	6.1
75% Diatomaceous earth: 25% Soil	42 a	10.1
100% Diatomaceous earth: 0% Soil	66 b	12.4

In this study, garden grade diatomaceous earth was used. This grade has a textural consistency similar to baking flour. Coarser grades of diatomaceous earth have been used in pool filtration systems. Testing of the efficacy of coarser grades of diatomaceous earth in the control of tubifex worms is a further avenue to be pursued. Overall, this initial study indicates that garden grade diatomaceous earth has minimal potential as a mechanism of control for tubifex worms.

David Latremouille

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