North American Journal of Aquaculture 66:261–270, 2004

Rapid and Slow Sand Filtration Techniques and Their Efficacy at Filtering Triactinomyxons of *Myxobolus cerebralis* from Contaminated Water

RONNEY E. ARNDT* AND ERIC J. WAGNER

Utah Division of Wildlife Resources, Fisheries Experiment Station, 1465 West 200 North, Logan, Utah 84321, USA

Abstract.—Slow and rapid sand filtration methods were tested for their ability to remove triactinomyxon actinospores (Tams), the waterborne infective stage of the salmonid parasite *Myxobolus cerebralis*, from contaminated water. Within the rapid sand filtration treatments, two backflush protocols were tested. The first consisted of extended backflush duration, and the second consisted of diverted flow past the aquaria with fish for 5 min after backflushing. A slow sand filter treatment served as a nonbackflushed control to the two rapid sand filters and also as its own unique filtration technique. Negative and positive controls were run simultaneously and served both slow and rapid sand filters. The sand used consisted only of particles greater than 180 μm (diameter). Triactinomyxon actinospores were regularly introduced to the fish-rearing systems above the sand filters. After 60 d, clinical signs of whirling behavior and black tails were seen among the positive controls. A polymerase chain reaction (PCR) assay for *M. cerebralis* conducted at the study's conclusion indicated no infection among the negative controls and both of the rapid sand filter treatments. In the slow sand filter treatment 1.6% of all fish were infected, whereas 98% of the positive controls were infected. Portions of the same tissue samples used for the PCR analysis were also assayed according to the pepsin–trypsin digest (PTD) test. Within the rapid sand filters, 2.9% of fish within the long back flush treatment were infected, as were 100% of the positive controls. The diverted backflush, slow sand filter, and negative controls were all negative according to the PTD test. These results demonstrate that the backflush technique is important in the proper function of rapid sand filters used to remove Tams and that both rapid and slow sand filtration could be viable options in treating hatchery water supplies that are contaminated with whirling disease.

With sand filtration, water passes through a layer of sand and via mechanical and biological processes removes particles such as organic debris, bacteria, and viruses. There are two distinct types of sand filtration: slow sand filtration and rapid sand filtration. Slow sand filtration (SSF) is characterized by gravity-fed flows in the range of 1 to 8 $m^3 \cdot m^{-2} \cdot d^{-1}$ (volume per filter surface area per unit time) and is further characterized by a biologically active zone of the sand bed called the *schmutzdecke* (Haarhoff and Cleasby 1991), a German word for dirt blanket. The biological zone must be removed or harrowed regularly as the development of organisms and the entrapment of organic materials impedes water flow as the zone matures. So SSF involves mechanical filtration by restriction of particle movement through the sand bed and biological filtration of organics by the organisms living in and adjacent to the *schmutzdecke*.

Slow-filtration technology has been used for almost two centuries to treat municipal water supplies. As a technology for cleaning water for culinary use, sand filtration had fallen out of favor during the mid-20th century due to the availability of newer, higher technologies. However, the simplicity of design and lower costs of setup and maintenance has made sand filtration, especially SSF, a very viable option of water filtration, and new construction of sand filters for municipal water systems has experienced a renaissance over the past 20 years (Collins 1998). Slow sand filters have been built to accommodate flows over 15,000 L/min (Letterman and Cullen 1985).

Rapid sand filtration (RSF) operates at much higher rates, 100–475 $m^3 \cdot m^{-2} \cdot d^{-1}$, either via pumping or adequate head pressure (Droste 1997). Due to higher flows, rapid sand filters accumulate more debris over a shorter period leading to frequent backflushes. To remove the debris from the filter bed, a volume of water is flushed in the opposite direction of normal water flow. This fluidizes the sand bed and dislodges trapped particles. The backflush process may be necessary 1–3 times daily depending on the incoming water quality. Commercial RSFs are available for industrial systems that can accommodate flows of up to 3,700 L/min, although site-built systems can accom-

* Corresponding author: ronneyarndt@utah.gov

Received January 20, 2004; accepted May 16, 2004

modate significantly higher rates. For both RSF and SSF, filtration efficacy is determined by variables including incoming organic load, hydraulic loading rate, sand size composition, water temperature, and frequency of backflushing (Al-Ani et al. 1985; Wheaton 1985).

The salmonid parasite *Myxobolus cerebralis*, the causative agent of whirling disease, has spread across the western USA, and during the past decade it has entrenched itself in many watersheds of Utah (Bartholomew and Reno 2002). Due to proximity to infected wild sites, two Utah state hatcheries have become contaminated with the pathogen. In both situations, the hatchery water source became contaminated with the Triactinomyxon actinospores (Tams) of *M. cerebralis*, the fish-infective stage of the parasite. Grappling-hook-shaped, Tams and are generally 146 μm long and, when their processes are folded together, they may only be 12 μm wide (El-Matbouli and Hoffmann 1998). Mesh filters of 20 μm NITEX mesh, although not 100% efficient, are routinely used in the field (Thompson and Nehring 2000) and the laboratory to capture Tams.

Sand filters have been shown to be highly effective at removing potential pathogenic particles from water supplies. Using seasoned SSF, Schuler et al. (1991) removed from contaminated water 99.9% of experimentally added cysts of *Giardia* and *Cryptosporidium*, ranging from 1 to 25 μm, as well as coliform bacteria. Bellamy et al. (1985a) accomplished 100% removal of *Giardia* cysts using sands with effective sizes of 130, 280, and 620 μm. Effective size is defined as the size range at which only 10% of smaller particles remain in a quantity of sand (Wheaton 1985). Slow sand filters are also effective in removing viruses from water supplies (Hendricks and Bellamy 1991).

Rapid sand filtration has also been 99% effective in removing *Giardia* cysts when hydraulic loading rates were kept at 5–19 $m^3 \cdot m^{-2} \cdot d^{-1}$ (Al-Ani et al. 1985). In laboratory and field studies, Arndt and Wagner (2003) demonstrated the efficacy of a hatchery-scale RSF system for reducing and eliminating the incidence of whirling disease infection in rainbow trout *Oncorhynchus mykiss*. When the effective sand size was kept at or below 180 μm, infection could be contained or greatly reduced; when the effective size was increased to 300 μm, a significant portion of fish became infected (Arndt and Wagner 2003).

From those initial tests we felt confident that whirling disease–contaminated hatchery water could be filtered through sand filters and be safely used for production fish culture. However follow-up tests were necessary to improve the efficiency of Tam removal to 100%. Results from the above-mentioned experiment indicated that after over 5 months of exposure, a single fish from three replicates was infected within the filter treatment that contained sand with an effective size of 180 μm (Arndt and Wagner 2003). From those results we theorized the filter's shortcomings were either sand bed depth or the backflushing protocol. The sand bed depth used from our initial work was only 10 cm. Rapid sand filters generally contain a sand bed 0.75–1.0 m deep (Droste 1997), and even experimental filters used successfully in the past to remove *Giardia* contained 76 cm of sand (Al-Ani et al. 1985).

The backflush protocol from our initial work may have also been flawed. In theory, if the sand bed is not completely flushed, Tams may only be partially liberated, and when restored to normal flow-through operation, the Tams may be able to pass through the filter before it has reseated. We addressed that theory in this current research by revising the back-flush protocol from the initial work in the form of two treatments, which either, (1) increase the duration of the backflush event, or (2) divert filtered water away from fish for a period after backflushing. As a further test of the backflush protocol we included a third sand filter treatment, an SSF. The slow sand would test (1) whether or not slow sand filtration was an effective method for Tams removal, and (2) because SSFs are not backflushed, whether or not our backflush protocol was suspect based on the presence or absence of infection within the SSF. To rectify the above-mentioned deficiencies in sand bed depth, all three sand filters contained sand beds that were 18 cm deep.

Methods

Sand filters and fish culture systems.—A single batch of masonry sand purchased from a local landscape supply dealer was mechanically sifted so that all grain sizes smaller than 180 μm were removed. This sand (≤180 μm) was subsequently used for all RSFs and SSFs. The rapid filters used were identical to those described by Arndt and Wagner (2003) and essentially consisted of a 61-cm length of 15-cm (inside diameter) polyvinyl chloride sewer pipe. Water entered the pipe through the top, flowing through the sand and support media beds and then through a collection manifold, where it was discharged to the fish holding tanks. The support media used was a mixture of

aquarium and pea-sized gravel, which had an average major axis of 7.2 mm and a minor axis of 5.2 mm. The depth of the support media was 10.6 cm, and the sand bed depth was 17.8 cm.

At the conclusion of the study, 75-mL subsamples of sand were removed at the filter's surface, at 9 cm below the surface (midway down), and at the zone of the support media. This material was then sifted to determine the size composition of sand at varying depths within the filters. This procedure was only done on one filter each from both of the RSF treatments. After this, all sand from rapid filters was removed, dried, sifted through 12 sieves ranging from 2 mm to less than 150 μm, and recharacterized for effective size and uniformity coefficient (i.e., the amount of size variation within a batch of sand, a larger coefficient indicating less uniformity in size). When 60% (by weight) of remaining sand has a smaller diameter, the uniformity coefficient of the sand batch is that percentage divided by the 10% effective size value.

The SSFs contained identical depths of sand, but because of the necessity for a larger water collection manifold, the support media depth was increased to 17.8 cm, compared with 10.6 cm for the RSFs. The SSFs were constructed from 121-L commercial garbage cans. As with the RSFs, water entered the filters from their top, flowed through the sand and support media bed, was collected via the collection manifold, and was then discharged to the rearing tanks (Figure 1). Water from the rearing tank was cycled back up to the filter by a pump rated for 780 L/h at no head. A float valve maintained the water level above the sand bed in the filter; water depth was generally 25 cm from surface to sand bed. The rearing tanks were 68-L plastic storage boxes, and water volume was kept at 54 L. The slow filter systems did not contain a biofilter because we believed the sand bed would adequately serve that function. All filter treatments were based on water recirculating systems. The RSF systems contained designated biofilters, and fish were reared in 113-L glass aquaria (Arndt and Wagner 2003).

The backflushing equipment used and the general procedures followed were the same as previously used (Arndt and Wagner 2003). From weeks 1–11, rapid sand filters were backflushed on a weekly basis, and beginning week 12 through the end of the study, filters were backflushed twice weekly. The extra back flushing was initiated when it appeared the filters were backing up because of accumulated matter. For the first backflush treatment (long backflush), we pumped 40 L of well water through the sand filter, opposite the direction of normal water flow, and out a discharge line. The full pressure of the pump was used to completely fluidize the sand filter bed, but as soon as the bed was fluidized, the flow was cut back, using a valve, so that the sand was not washed from the filter. For the second back flush treatment (diverted back flush), we pumped 20 L of well water through each replicate filter, and then flow through the filter and to the aquaria was diverted for 5 min, after which normal flow was reinitiated.

During each backflush the flushed water from each filter was captured and filtered through a 125-μm sieve, the retentate was quantified volumetrically, and the material was replaced in the filter. Backflush duration was also recorded to calculate flow rates. For the diverted backflush treatment, the amount of bypass volume was also recorded. After backflushing, all tanks were measured for water inflow, and large differences among tanks within and among treatments were adjusted.

The test fish used were the Sand Creek strain of rainbow trout obtained from Utah's Egan broodstock hatchery (Bicknell, Utah) as eyed eggs. The eggs were hatched at the Fisheries Experiment Station (FES, Logan, Utah), and when the fish reached 0.53 g (about 4 weeks after first feeding), they were stocked into the experimental aquaria at a density of 20 fish/tank. The fish were fed a commercial trout diet (Silver Cup, Nelson and Sons, Inc., Murray, Utah) to apparent satiation three times per day. Mortalities were noted on a daily basis, and total fish length and weights were recorded for all fish at the end of the study. Debris was siphoned from the tanks approximately thrice weekly. This was usually incorporated into the water exchange process that consisted of removing approximately 10% of a treatment's water (about 19 L for RSFs and 9 L for the SSFs) and replacing it with fresh well water.

To avoid the elevated ammonia levels experienced in the previous trial (Arndt and Wagner 2003), recirculation systems were started 25 d before adding fish. The systems were spiked with aged water from another recirculation system to introduce beneficial bacteria and then an ammonium chloride solution was added regularly until ammonia was processed through to safe levels of nitrite. Because of the reliance of the entire test on the proper functioning of the water recirculation systems, water quality analysis was conducted on a weekly basis, plus more frequent spot checks of ammonia. Alkalinity, hardness, oxygen, pH, am-

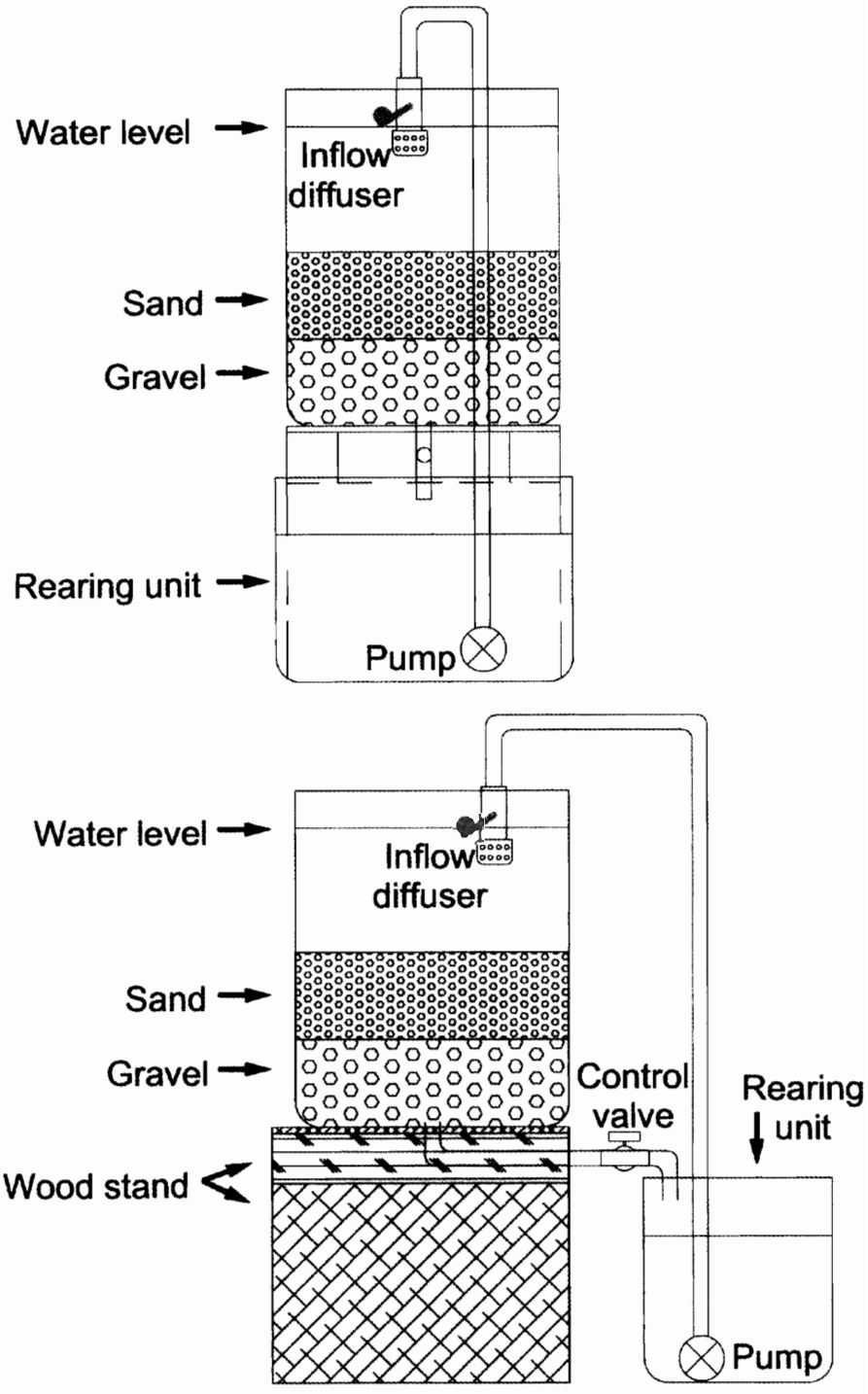

FIGURE 1.—Front and side views of an individual slow sand filter.

monia, and nitrite were measured using a commercial testing kit (Hach Company, Loveland, Colorado) and temperature by an electronic thermometer (model 309, Corning Inc., Corning, New York).

Fish exposures and disease diagnosis.—Over a 14-week period fish were exposed to a thrice-weekly dose of 4,333 Tams/tank (4,333 Tams/filter), but on several occasions exposures were skipped. The actual average dose for the course of the study was 4,333 Tams/tank multiplied by 36 exposures, resulting in a cumulative dose of 155,988 Tams/tank. For the exposures Tams were placed into a 3.8-L chicken water feeder with 2 L of well water, and the diluted Tams were then discharged into a treatment's head box over a 44-min (SD = 13) period. Processing Tams from worm collections was conducted according to methods outlined by Arndt et al. (2002). Various worm cultures were used for Tam production and they contained a mixed collection of oligochaete species, which had been fed myxospores of *M. cerebralis* to induce infection. Past worm cultures used have consisted of 16% *Tubifex tubifex*, 9% *Quistodrilus multisetosus*, 8% *Limnodrilus hoffmeistri*, and 64% immature, unidentified worms.

During enumeration all Tams viewed microscopically were classified as either viable or nonviable. For a Tam to be viable it had to have intact polar capsules and tightly compacted spore bodies with no loose sporoplasms. Those Tams with fired polar capsules, those that lacked sporoplasms or had loosely packed sporoplasms within the style, or those Tams that had damaged or missing caudal processes were termed nonviable. This qualification step was found to closely compare with vital staining techniques (Arndt and Wagner 2003).

For disease diagnosis, 2 weeks after the final Tam exposures and 16 weeks from the start, all fish were euthanatized, their lengths and weights were measured, and a deformity index designed to quantify cranial and skeletal deformities was conducted. For each treatment, we recorded typical clinical signs of whirling disease (Lorz and Amandi 1994), such as vertebral (lordosis, scoliosis, or kyphosis), mandibular (misaligned or shortened), cranial (sloped head), or opercular deformities; fish without deformities were categorized as normal. Fish heads were then removed immediately posterior to the operculum and placed individually into separate plastic bags. The scalpels used were brushed and disinfected with a 50% chlorox solution (2.6% sodium hypochlorite) between each fish, and a clean paper towel served as the cutting surface for each head.

The fish heads were then processed using the pepsin–trypsin digest method (PTD; Markiw and Wolf 1974). After digestion, the spore preparation homogenate was divided, with part being used for continued PTD methodology and part for polymerase chain reaction (PCR) analysis. The PCR homogenate samples were then frozen and sent to an independent laboratory (Pisces-Molecular LLC) for assaying of *M. cerebralis* via PCR. The PCR assay was a single-round test (Baldwin and Myklebust 2002) using the heat shock protein gene (*Hsp70*) as the region targeted, as opposed to the 18S rRNA gene. Epp, Wood, and Mitton, who have determined it to be sensitive and specific to *M. cerebralis*, presented this modified assay at the 8th Annual Whirling Disease Symposium in 2002 (J. Epp, Pisces-Molecular, personal communication). To quantify the degree of infection, the PCR results were scored according to Schisler et al. (2001), based on the intensity of the DNA banding pattern. The categories used were: negative (−), weakly positive (w+), positive (+), strongly positive (++), and very strongly positive (+++).

Statistical analysis.—For all statistical tests we set $\alpha = 0.05$. Percentage data were arcsine-transformed and analyzed by one-way analysis of variance (ANOVA) with multiple comparisons via Tukey's test. For analysis of deformities, the frequency of presence or absence data were compared with the frequency of normal scores using chi-square analysis. The total percent of fish with deformities in a group was arcsine-transformed and analyzed by one-way ANOVA with multiple comparisons via the Tukey's test. Data obtained from the PCR and PTD assays were not analyzed in depth statistically because the study was designed, not to determine to what degree sand filters reduced whirling disease, but instead whether they could prevent it. However, percent prevalence data defined as positive or negative for infection by the PTD assay were cursorily analyzed by chi-square analysis.

Results

Sand Filter and Fish Culture Systems

At the study's conclusion the sand within the RSFs had an average uniformity coefficient of 2.3 (range = 2.0–2.4), and an effective size of 180 μm (range = 0.15–0.25). The 75-mL subsamples sifted to determine size distribution within the two filters revealed finer sand was positioned near the

TABLE 1.—Summary of water quality characteristics measured over the course of a study ($N = 15$) testing two water filtration systems for removing triactinomyxons of *Myxobolus cerebralis*. Where individual tank values were measured, values were averaged for each tank within a treatment's water recirculation system and then averaged across systems.

Variable	Rapid sand filters		Slow sand filters	
	Average	Range	Average	Range
Temperature (°C)	15.9	14.8–16.8	14.4	12.9–15.8
Dissolved oxygen (mg/L)	7.9	7.0–8.5	6.7	5.0–7.0
Alkalinity (mg/L $CaCO_3$)	211.1	188.1–239.4	206.4	171.0–239.4
pH	8.1	7.9–8.2	8.0	7.6–8.1
Nitrite-nitrogen (mg/L)	0.02	0.00–0.05	0.03	0.00–0.50
Total ammonia nitrogen (mg/L)	0.4	0.2–0.5	0.36	0.2–0.5
Un-ionized ammonia (mg/L)	0.0129	0.0123–0.0135	0.0092	0.0089–0.0092

filter's surface and coarser sand near the bottom. Greater than 90% of sand in the range of 180–355 μm was found in the top fraction, and roughly 80% of sand in the range of 300–1,000 μm was found in the mid and bottom sections. No sand characterization was conducted on the SSFs.

Adjustments conducted after the regular backflush events maintained flow rates through rapid filters at nearly uniform levels. The average filter flow through the RSFs was 1.9 L/min (SD = 0.1) and that into both negative and positive control was 2.0 L/min (±0.1). Per filter surface area, this translates to 6.8 $m^3 \cdot m^{-2} \cdot h^{-1}$ for the RSFs and 7.21 $m^3 \cdot m^{-2} \cdot h^{-1}$ for the positive controls. Because the SSFs were not backflushed and the *schmutzdecke* grew, flow decreased over the course of the study. The filter flow for all three SSFs averaged 3.3 L/min (SD, 0.8; 1.1 $m^3 \cdot m^{-2} \cdot h^{-1}$) at week 1, was 2.9 L/min (SD, 0.4; 1.0 $m^3 \cdot m^{-2} \cdot h^{-1}$) at week 9, and was 1.8 L/min (SD, 0.4; 0.6 $m^3 \cdot m^{-2} \cdot h^{-1}$) at week 14.

Despite the differences in the two RSF treatments with respect to backflush volume (37.8 L versus 18.9 L), there were only slight differences in backflush flow rate. Backflush flow was 10.5 L/min (SD, 0.4; 37.4 $m^3 \cdot m^{-2} \cdot h^{-1}$) for the long backflush treatment, and 10.6 L/min (SD, 0.0; 37.9 $m^3 \cdot m^{-2} \cdot h^{-1}$) for the diverted treatment. The quantity of water collected during the 5-min diversion period following the backflush for the diverted back flush treatment averaged 14.1 L (±1.1). The quantity of sand collected from the backflushed water varied between filters and between the two RSF treatments. The average volume recovered from the long back flush treatment filters was 2.4 mL (SD, 2.0; 48 mL total) and the volume recovered from the diverted back flush treatment filters was 5.7 mL (SD, 9.0; 108 mL total). The between-treatment differences were not significant however ($P = 0.331$).

All water quality variables measured during the course of the study were adequate for trout culture (Piper et al. 1992). Un-ionized ammonia for the rapid sand filter systems and the two control systems averaged 0.013 mg/L, which is right at the acceptable upper limit of 0.013 for continuous exposure, as outlined by Piper et al. (1992). Un-ionized ammonia, which averaged 0.009 mg/L, never approached the critical level within the SSF systems. However, early in the study, oxygen concentrations within the SSF systems dropped to 5.0 mg/L on occasion (Table 1), so the outflow from the filter to the fish rearing tank was modified to provide more fall and this served to raise the oxygen levels.

During the study fish from all filter treatments and controls experienced 98% or better survival. The diverted backflush, SSF treatments, and the positive controls had 2% cumulative mortality, compared with 1% for the long backflush treatment and 0% for the negative controls. These differences were not significant. Although the fish were loosely fed on an ad libitum basis, they nonetheless grew reasonably well. From an initial average weight of 0.5 g, fish in the long backflush grew to 5.8 g, to 6.7 g for the diverted backflush, to 6.0 g for the SSF, to 6.8 g for negative controls, and to 6.5 g for positive controls. Because fish growth was not an integral part of this study, direct statistical comparisons were not made.

Fish Exposures and Disease Diagnosis

Whirling behavior among test fish was only observed within the positive controls and only then when fish were being fed or disturbed with tank maintenance. By week 14, one fish within one of the positive control tanks exhibited a black caudle peduncle and tail. The deformity index data indicated that positive control fish had a significantly higher occurrence ($P = 0.008$) of deformities (29%) than did the treatments or negative controls. The negative controls contained no deformities,

TABLE 2.—Summary of infection percentages of *Myxobolus cerebralis* for rainbow trout reared in systems supplied with water filtered to remove triactinomyxons via the following sand filter regimens: (1) extended backflush, (2) diverted backflush, (3) slow sand filtration, (4) negative control, and (5) positive control. Individual samples were split and assayed by either polymerase chain reaction (PCR) or pepsin–trypsin digest (PTD). Percent positive for *M. cerebralis* infection was calculated by averaging the percent prevalence within a given treatment replicate; *N* is the number of fish examined via PCR or PTD.

Treatment	Positive via PCR		Positive via PTD	
	Percent	*N*	Percent	*N*
Long backflush	0.0	69	2.9[a]	68
Diverted backflush	0.0	59	0.0	59
Slow sand filter	1.6[b]	63	0.0	63
Negative control	0.0	59	0.0	60
Positive control	98.3	59	100.0	58

[a] Two positive fish within one replicate.
[b] One positive fish within one replicate.

the long backflush treatment had 3% of the fish deformed, the diverted backflush had 5%, and the SSF had 2%.

The results generated by the PCR assay indicated that fish from the negative controls, diverted backflush, and long backflush treatment group were negative for *M. cerebralis* infection; however, 1.6% ($N = 63$) were positive in the SSF group. This infection was limited to one fish within one replicate (Table 2). The positive controls exhibited a high level of infection; 98.3% ($N = 59$) were infected, all of which were scored as being very strongly positive (+++). The single infected fish in the SSF was scored simply positive (+).

The PTD assay reiterated the level of infection among the positive controls; 100% were infected (Table 2) and the average spore count per head was 79,651 (SD, 63,406). Within the long backflush treatment two fish within one replicate were found to contain spores (2.9% of fish in the treatment); these two fish had an average spore count of 333 spores per head. With the PTD assay, no infected fish were found in the negative controls or the diverted backflush or SSF treatments.

Discussion

This test demonstrated that both rapid and slow filtration are effective means of filtering whirling disease–contaminated aquacultural water supplies. With the appropriate backflush protocol, RSFs were 100% effective in preventing infection of fish with whirling disease, and SSFs were capable of keeping 98% of the fish free from infection. During the study over 150,000 Tams had been introduced into water flowing to each treatment filter.

Although SSF treatment was not 100% effective at preventing infection, only one fish within one replicate became infected. There may several reasons to explain this infection. One explanation may be the aging of the filter's *schmutzdecke* and our failure to remove it. From the start of the trial to its conclusion, there was a steady decline in flow rate through the SSFs. This decrease in flow is characteristic of an aged *schmutzdecke* in need of revitalization (i.e., scraping or the removal of the top several centimeters of sand). In a survey of operational SSFs in several northeastern U.S. states, Letterman and Cullen (1985) found that filters required scraping on average five times a year or every 10 weeks. This, along with our loss of flow indicates our filters were past due for scraping. From the onset of the study it was not our intention to scrap the *scmutzdecke.* We believed that by not disturbing the sand beds, they would better serve as nonbackflushed controls compared with our two backflush treatments.

Because flow through the filters was restricted by an over-aged *schmutzdecke,* it is possible that one or several infiltration channels were formed, which allowed particles, including Tams, to more readily bypass the sand bed and to reach the fish. With an over-aged *schmutzdecke* head loss occurs and the sand bed compresses; as the bed compresses, fractures develop in the bed that might allow particle infiltration further into the filter bed (Baylis et al. 1971). No fractures were observed in our filters, but it is possible this is what happened with the one filter that allowed Tam passage and fish infection.

For our trial we seeded the SSFs with water from an active water recirculation system and aged the sand biofilter until it could process the ammonia excreted by fish. So it could be argued that the SSFs were seasoned by the time fish were added

and the Tam exposures were begun. Although the proper aging of SSFs is important for dissolved organic removal, for larger particles it appears that un-aged filters may be similarly able to filter out larger particles, probably because of the mechanical action of the sand. Bellamy et al. (1985b), found only a very slight difference in filtering efficiency to remove *Giardia* cysts between filters containing new sand and support gravel compared with filters with mature sand and gravel. This agrees with our results that showed only slight infection within the SSF treatment as assayed by the PCR test and none by the PTD test. Because *M. cerebralis* was found by PCR and not PTD, the infection probably occurred towards the end of the trial. The PCR test can confirm infection that has occurred within days, but because the PTD test is based on the presence of spores and sporogenesis takes 2–3 months to occur, the PTD test can only confirm an infection event that happened several months previously (Andree et al. 1998). The PCR technique is generally considered to be more sensitive than PTD and is capable of detecting very light infections, but it also has produced false positives (Schisler et al. 2001). Because of the positive PCR results and negative PTD findings, we do not believe the ripening or lack of filter aging in our tests contributed to Tam passage.

The Tam infiltration and subsequent fish infection within our SSF treatment may have also been a result of inadequate design. The sand bed depth of SSFs is generally 60–120 cm, and the support gravel bed is 30–50 cm (Bellamy et al. 1985b). The sand bed of our filter was only 17.8 cm and the support gravel was 17.8 cm. When Bellamy et al. (1985a) reduced the sand bed depth of their experimental filter from 1.0 m to 0.5 m, they found that coliform removal decreased from 97% to 95%. Our sand bed depth was less than 25% of the recommended depth, and it is very possible that, combined with particle infiltration from channelization due to an over-aged *schmutzdecke,* the Tams had begun to pass through the filters.

Concerning the rapid sand filtration portion of this study, it appears that the postbackflush diversions of filter outflow away from the fish are necessary until the filter completely reseats and any possible Tam contamination is washed out. Based on the infection results from our previous sand filter work, several theories were tested in this study with respect to improving rapid sand filtration. The first theory would be our original backflush duration was too short and did not allow for complete removal of Tams trapped in the sand bed (Arndt and Wagner 2003), which subsequently passed through the filter and infected fish. This theory has been supported in research presented at the 6th (2000) and 8th (2002) Annual Whirling Disease Symposia by Barrows and his coworkers, where they determined that filter columns packed with 200-mm diameter glass beads experienced incomplete Tam removal after backflushing when the backflush rate was to low or the duration too short (F. T. Barrows, U.S. Fish and Wildlife Service, personal communication). Our research did not totally support those findings in that our long backflush treatment was the one treatment that exhibited infection.

The infection results did support the second of our theories concerning proper backflush protocol—that is, that backflushing removes Tams from the sand bed but residual viable Tams may be found in the support gravel or plumbing leading from the filter to the fish. Diverting water from the fish immediately following the backflush allows any remaining Tams to be safely discharged from the system. Because we found no sign of *M. cerebralis* infection either by PTD or PCR, we are confident that rapid sand filtration with a modified backflush protocol that includes water diversion, is an effective means of removing Tams from contaminated hatchery water supplies.

The results from the PTD and PCR assays of the RSF tests present some conflicting results. Because the two fish within the one replicate of the long backflush treatment were positive by PTD, one would have to assume the infection occurred several months previous to diagnosis. The conflicting results are the lack of the PCR test to verify the results found by PTD. In past comparisons of PTD and PCR, the PCR assay has been found to be more sensitive at finding light infections months after the initial exposure (Andree et al. 1998), but one has to wonder why the positive PTD result was not confirmed by the PCR assay. Because the homogenate from each fish was divided into aliquots for PTD, PCR, and ongoing enzyme-linked immunosorbent assay research, the quantity for each sample was limited, and it is possible the positive myxospores found in the PTD samples composed the entire quantity of spores (none were found via PCR). There is also the possibility of cross-contamination, although extreme care was taken to avoid contamination during the sampling of fish and when performing the pepsin-trypsin digests. Equipment used to enumerate spores from head tissues, including the working area, were cleaned and disinfected between treatment repli-

cates. Even with these precautions cross-contamination may have occurred. The spores found within the long back flush treatment fish were found immediately after slides from one of the positive controls had been examined.

The SSFs and RSFs demonstrated that this is a technology that can be adopted by the aquaculture industry as a means of cleaning contaminated water. Both of these designs do have their limitations when scaled up to the production level. Because SSFs process water very slowly, they require a large surface area to handle production-scale flows. If the flow rates we used for our test were used as a basis, and a volume of 2.4 million L/d (or 1 ft^3/s) was to be treated, an SSF of 17 × 17 m would be required. State hatcheries in Utah require several times that amount of water and as a result would require several filters of those dimensions. Unless a hatchery has adequate surplus land, the SSF may not be a viable option. Also, if the hatchery were located in climates where there is a risk of the filter freezing, a structure would be required to cover the filter. For production-scale aquaculture, the RSF may be more feasible option. Using the flow rates we used previously and a treatment volume of 2.4 million L/d, an RSF would have a radius of two meters. Rapid sand filtration involves a higher level of technology involving more complex plumbing systems and higher maintenance requirements, but we believe our research has demonstrated SSF is a technology ripe for use in the effort to eliminate whirling disease from hatcheries.

Acknowledgments

The authors thank David Latremioulle for assisting in the maintenance of the recirculating and filtration systems. Thanks to Patrick Goddard, David Thompson, and Jared Zirenberg for helping process the fish tissues. Anna Marie Miller's help in guiding us through the pepsin-trypsin digests was invaluable. We also appreciate John Wood and Janet Epp at Pisces-Molecular for PCR analysis. Martha Arndt's help with drafting of filter schematics was very much appreciated. This research was funded by the Federal Aid in Sport Fish Restoration program, project F-96-R, and the Utah Division of Wildlife Resources.

References

Al-Ani, M., J. M. McElroy, C. P. Hibler, and D. W. Hendricks. 1985. Filtration of *Giardia* cysts and other substances, volume 3. Rapid-rate filtration. U.S. Environmental Protection Agency, Report 600/S2-85/027, Washington, D.C.

Andree, K. B., E. MacConnell, and R. P. Hedrick. 1998. A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 34:145–154.

Arndt, R. E., and E. J. Wagner. 2003. Filtering *Myxobolus cerebralis* triactinomyxons from contaminated water using rapid sand filtration. Aquacultural Engineering 29:77–91.

Arndt, R. E., E. J. Wagner, Q. Cannon, and M. Smith. 2002. Triactinomyxon production as related to rearing substrate and diel light cycle. Pages 87–91 *in* J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

Baldwin, T. J., and K. A. Myklebust. 2002. Validation of a single-round polymerase chain reaction assay for identification of *Myxobolus cerebralis* myxospores. Diseases of Aquatic Organisms 49:185–190.

Bartholomew, J. L. and P. W. Reno. 2002. The history and dissemination of whirling disease. Pages 3–24 *in* J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

Baylis, J. R., O. Gullans, and H. E. Hudson, Jr. 1971. Filtration. Pages 245–281 *in* H. B. Crawford and D. N. Fischel, editors. Water quality and treatment, 3rd edition. McGraw-Hill, New York.

Bellamy, W. D., G. P. Silverman, and D. W. Hendricks. 1985a. Filtration of *Giardia* cysts and other substances, volume 2. Slow sand filtration. U.S. Environmental Protection Agency, Report 600/S2-85/026, Washington, D.C.

Bellamy, W. D., G. P. Silverman, D. W. Hendricks, and G. S. Logsdon. 1985b. Removing *Giardia* cysts with slow sand filtration. Journal of the American Water Works Association 77:52–60.

Collins, M. R. 1998. Experiences introducing "new" technology: slow sand filtration. Pages 213–224 *in* J. A. Cotruvo, G. F. Craun, and N. Hearn, editors. Providing safe drinking water in small systems: technology, operations, and economics. Proceedings of the 1st International Symposium on Safe Drinking Water in Small Systems: technology, operations, and economics. CRC Press, Boca Raton, Florida.

Droste, R. L. 1997. The theory and practice of water and wastewater treatment. Wiley, New York.

El-Matbouli, M., and R. W. Hoffmann. 1998. Light and electron microscopic studies on the chronological development of *Myxobolus cerebralis* to the actinosporean stage in *Tubifex tubifex*. International Journal for Parasitology 28:195–217.

Haarhoff, J., and J. L. Cleasby. 1991. Biological and physical mechanisms in slow sand filtration. Pages 19–68 *in* G. S. Logsdon, editor. Slow sand filtration, American Society of Civil Engineers, New York.

Hendricks, D. W., and W. D. Bellamy. 1991. Microor-

ganism removal by slow sand filtration. Pages 101–121 *in* G. S. Logsdon, editor. Slow sand filtration. American Society of Civil Engineers, New York.

Letterman, R. D., and T. R. Cullen, Jr. 1985. Slow sand filter maintenance: costs and effects on water quality. U.S. Environmental Protection Agency, Report 600/S2-85/056, Washington, D.C.

Lorz, H. V., and A. Amandi. 1994. VI. Whirling disease of salmonids. Pages 1–7 *in* J. C. Thoesen, editor. Suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 4th edition. American Fisheries Society, Fish Health Section, Bethesda, Maryland.

Markiw, M. E., and K. Wolf. 1974. *Myxosoma cerebralis*: isolation and concentration from fish skeletal elements—sequential enzymatic digestions and purification by differential centrifugation. Journal of the Fisheries Research Board of Canada 31:15–20.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1992. Fish hatchery management. U.S. Fish and Wildlife Service, Washington, D.C.

Schisler, G. J., E. P. Bergersen, P. G. Walker, J. Wood, and J. K. Epp. 2001. Comparison of single-round polymerase chain reaction (PCR) and pepsin–trypsin digest (PTD) methods for detection of *Myxobolus cerebralis*. Diseases of Aquatic Organisms 45: 109–114.

Schuler, P. F., M. M. Ghosh, and P. Gopalan. 1991. Slow sand and diatomaceous earth filtration of cysts and other particulates. Water Research 25:995–1005.

Thompson, K. G., and R. B. Nehring. 2000. A simple technique used to filter and quantify the actinospore of *Myxobolus cerebralis* and determine its seasonal abundance in the Colorado River. Journal of Aquatic Animal Health 12:316–323.

Wheaton, F. W. 1985. Aquacultural engineering. Robert F. Krieger Publishing Company, Malabar, Florida.