

# The Ichthyogram

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## Influence of Raceway Coating on Fin Erosion

Fin erosion can be a common problem in modern, large-scale aquaculture. Trout stocked from a hatchery with eroded fins may not be as desirable to anglers as wild trout and may also have impaired survival. The exact cause of fin erosion is not known but it may derive from aggression between fish, nutritional imbalances in feeds, or environmental factors inherent to the hatchery setting including rearing densities and abrasions from rough concrete raceway walls. This study was designed to address the impact concrete walled raceways have on the fin condition of rainbow trout (*Onchorhynchus mykiss*).

Rainbow trout of the Sand Creek strain were stocked into six different raceways at approximately three weeks post first feeding (1 g/fish). The fish were stocked at densities of 1,200 fish per raceway. The concrete raceways were identical and had the dimensions of: width = 0.9 m, depth = 0.6 m, and length = 7.7 m. The walls and floors of three of the six raceways were coated with a resin that smoothed the surface of the concrete walls. The resin (Silmar® isophtalic resin) was produced by Border Industrial Inc.,

Troy, Idaho, and was a proprietary formulation composed in part of polyester, silicon dioxide, and styrene.

The flow indices [weight of fish/(raceway volume x fish length)] used for the study ranged from 0.3 at the beginning to 0.7 by the end of the 176 day study. Density indices [weight of fish/(water inflow x fish length)] ranged from 0.2 to 0.4. The density was adjusted by moving the tail screen further down the raceway length whenever the density index was at or near 0.4. Water was supplied to the raceways by a well which had the following properties: temperature = 13° C, oxygen = 6.4 mg/l, alkalinity = 248 mg/l, hardness = 219 mg/l, pH = 7.2. Supplemental oxygen was supplied to the well water via liquid oxygen through low head oxygenators. This raised the oxygen content at the raceway head to 8.0 ± 0.7 mg/l.

The fish were hand fed a commercial trout formulation (Silver Cup, Nelson's and Sons, Murray, Utah). At the start of the study the daily feed ration was 4.2% of the fish body weight, and by the end

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of the study this ration had dropped to 2.0%. Fish were inventoried monthly for weight gain, and fin measurements were made at the start of the study and after three months into the study they were made monthly. Fin measurements were used to calculate relative fin index values. At the end of the study necropsies were performed on ten fish from each raceway (30 total per treatment) according to the Health Condition Profile (HCP) methodology.

After the first month of the study, the control fish were significantly larger (3.1 g/fish) than the test fish (2.9 g/fish;  $P = 0.03$ ). However, by the end of the study no differences in fish weights were found. Control fish grew from 1.0 to 56.2 g/fish and test fish grew from 1.0 to 53.6 g/fish. No significant differences were found with respect to specific growth rate, which averaged 1.7% for both groups, or feed conversion ratio, which averaged 1.1. Percent mortality was slightly higher for the test group, 2.1%, compared to 1.9% for the control fish, although this difference was not significant.

Relative fin index calculations made from the fin measurements revealed no consistent trend in comparisons between groups, although some significant differences were found. By day 73 of the study, anal, left pectoral, and right pectoral fin indices were significantly better for the control fish compared to the test fish (Table 1). Fin lengths of both ventral fins were better for the control fish on day 108, and by day 134 caudal and right ventral fins measured significantly better for the control fish than the test. Caudal and right pectoral fins were significantly longer for the control fish on day 162, which was the final measurement. Fin index scores from the initial measurements taken, day 1, were

taken from a common lot of fish before they were stocked out into their respective raceways (Table 1).

The final fin index scores calculated according to the HCP methodology were lower for the control fish, 0.6, compared to 0.8 for the test fish, but this difference was not significant. The HCP system ranks fins from 0-2, with 0 = no erosion and 2 = active erosion with hemorrhaging. All other indices measured according to the HCP were within normal ranges for rainbow trout and none of them were significantly different between treatments with the exception of the bile score. The control fish had a bile score of 0.5 and the test group had a score of 1.1. A zero bile score indicates an empty gall bladder or recent feeding, and a higher score, 1-2, indicates no or little recent feeding.

Fish growth and health were not negatively impacted by coating raceways with the resin. The test fish did lag behind in monthly growth and were significantly smaller for the first month, but final weights were the same. It is possible the resin leached out sub-lethal amounts of chemical initially that had an impact on growth. The raceways were coated in early fall and were thought to have had ample time to dry and cure before the study started (mid winter). Ultimately the resin had no negative impact on growth. Health condition profile measurements indicated fish from both groups were healthy and that the resin had no adverse impact on fish health.

The one significant finding from the HCP was the bile index. The control fish had a low bile score indicating recent feeding while the test fish had a higher score indicating little or no recent feeding. No good answer can be given to explain this result because all six raceways went

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without feed the day the HCP's were conducted, so bile scores all should have been elevated. One possible explanation could be the presence of more algae and moss and resulting invertebrates on the walls of the control raceways compared to the test raceways. Although raceways were cleaned once to twice a week, hatchery personnel noticed the resin coated raceways maintained a lower level of plant growth compared to the controls. It is possible the control fish were getting a few snacks off of the raceway walls.

Fin conditions measured by both the HCP and the relative fin index methodologies indicated consistently better fins among the control fish compared to the test fish.

The HCP fin scores were not significantly different however and may have been due to random chance. Values obtained from the relative fin index gave a better idea of what was happening to the fins over time, but there was not a constant trend with respect to type of fin that was in better shape. Despite these inconsistencies, the fact remains that on any of the four sampling dates (not including day 1), any fin found to be significantly longer, was found in the control group. This implies that uncoated concrete raceways may be more beneficial to fin condition than resin coated ones, or that the resin coating may have in some way negatively impacted fin quality. In this study, with the raceways

Table 1. Comparisons of relative fin length<sup>1</sup> (% of total length) of rainbow trout raised in untreated raceways (control), or resin coated raceways (coated). Fin abbreviations are: DOR = dorsal, CAD = caudal, ANL = anal, LVT = left ventral, RVT = right ventral, LPC = left pectoral, and RPC = right pectoral. Mean values within a given sampling date that are significantly different ( $P \leq 0.05$ ) are marked by an asterisk.

<sup>1</sup> Relative fin index = (fin length/total length x 100)

	DOR	CAD	ANL	LVT	RVT	LPC	RPC
Day 1 (combined)	9.3	13.7	10.1	9.2	9.2	10.5	10.7
Day 73							
control	5.9	12.9	9.5*	9.7	9.7	10.7*	10.5*
coated	5.3	12.6	9.2	9.8	9.8	10.2	9.9
Day 108							
control	4.4	11.9	8.8	9.5*	9.5*	9.7	9.6
coated	4.2	12.0	8.9	9.1	9.1	9.3	9.2
Day 134							
control	3.3	11.1*	8.0	8.7	8.8*	8.6	8.7
coated	3.9	10.7	8.0	8.5	8.4	8.2	8.0
Day 162							
control	4.2	11.4*	8.5	9.0	9.0	8.5	8.3*
coated	4.2	11.1	8.2	8.8	8.8	7.9	7.0

## TAM Vital Staining: Effect of Sodium Hypochlorite on Viability

Sodium hypochlorite, better known as Chlorox® (5.25% solution) bleach, is widely used as a disinfectant. Previous research has investigated the effect of this chemical on the viability of the myxospore stage of *Myxobolus cerebralis*, but with mixed results. Hoffman and Putz (1969) reported that 24-hour exposure to 1600 ppm chlorine (as sodium hypochlorite) was effective in "causing distortion and probable death of spores"; 200 ppm chlorine gave variable results. Hoffman and Hoffman (1972) reported that 400 ppm chlorine killed 36-90% of the myxospores treated in-vitro for 2-4 days. Hoffman and O'Grodnick (1977) treated a 2 cm layer of infected mud in aquaria with 1200 ppm chlorine (calcium hypochlorite) for 18 h; when fish from these aquaria were sampled 5 months later, the fish were infected. These researchers also treated water that had been mixed with contaminated muds at 10, 20, 50, and 250 ppm chlorine for 30 min; control fish were infected, but no infection was found in fish from any of the treatment groups.

Most of the research on chlorine treatment occurred before the complete life cycle was known (Markiw and Wolf 1983). Prior to this, the actinosporean stage which infects the fish was thought to be strictly a parasite of worms. *Triactinomyxon* (TAM) is the name for the infective actinospore stage for *M. cerebralis*. The effect of chlorine on TAMs has only been examined indirectly. For example, a hatchery water supply treated with 0.5 ppm for 2 h once a week reduced infection by 73% in one group and 63% in another group of trout (Markiw 1992).

It is not known if TAMs or worms were affected by the treatment. Similarly, prophylactic treatment with chlorine gas (300 ppm) prevented the recurrence of the

disease in a Pennsylvania hatchery (Hoffman and Dunbar 1961).

This article describes results of tests with chlorine (as sodium hypochlorite) and its effect on TAM viability as assessed by vital staining. The aim of the research was to determine what concentrations of sodium hypochlorite were necessary to kill 100% of TAMs in a short time. The results could then be applied to disinfection protocols to prevent the spread of the disease or eliminate it entirely from a location.

For each test, equal volumes of a TAM stock solution and sodium hypochlorite were mixed and left at room temperature for either 1 or 10 min. After mixing with TAMs, the resulting chemical concentrations ranged from 0.025% to 0.5% bleach solution or 2.6 to 262 ppm actual chlorine as sodium hypochlorite. The TAMs were generously provided by Don Roberts and Jarod Austin of the Utah State University Biology Dept. After the test time had elapsed, the solution was filtered through a 10 um mesh screen and rinsed with 20 mL of hatchery well water. As the last of the rinse water filtered through, this water was harvested with a pipette and 100 ul put on each of 1 to 3 slides. Then 50 ul of propidium iodide (52 mg/l stock solution thawed shortly before staining) and 50 ul of fluorescein diacetate (100 ul of 5 mg/ml stock solution added to 8.3 ml hatchery well water) were added to the slide. The slides were coverslipped and kept in a dark chamber in the refrigerator for at least 45 min prior to examination. Using blue light of an epifluorescent microscope, the spore body of the TAM fluoresced either red or green. Some TAMs had both colors present. The dead organisms stain red with propidium iodide and live organisms stain green, as enzymes in living organisms release fluorescein. This process was

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Table 1. Mean percentage of viable, non-viable, and possibly viable of triactinomyxon stage of *Myxobolus cerebralis* treated with various concentrations of sodium hypochlorite for either 1 or 10 min. Means within a duration and column that are not significantly different share a common letter ( $P \geq 0.05$ ).

Duration	Sodium hypochlorite (ppm)	Percent viable	Percent non-viable	Percent possibly viable
1 min	0	78.0 a	10.8 a	11.2 a
	26	2.0 b	88.9 b	8.8 a
	131	0.0 b	100.0 c	0.0 b
10 min	0	69.8 a	16.8 a	13.4 a
	2.6	0.5 b	76.3 a	23.2 a
	13	0.0 b	100.0 b	0.0 b
	26	0.0 b	97.6 b	2.4 b
	262	0.0 b	100.0 b	0.0 b

repeated 2 to 3 times for each treatment.

The viability of control TAMs was compared by date (one-way ANOVA of arcsin transformed data) and no significant differences were observed. These were pooled for each duration for comparison with the other concentrations tested (one-way ANOVA or arcsin transformed data). Data for each duration was tested separately.

Test results (Table 1) indicated that concentrations of chlorine greater 13 ppm were effective in killing TAMs in a 10 min treatment. However, a small percentage of possibly viable TAMs were found at 26 ppm. The difference between this and the 13 ppm results may be due to different bottles of bleach that were used for the two tests. For disinfection purposes, the TAMs staining both red and green should be considered as potentially viable until further

testing indicates otherwise. Conversely, for infection purposes these should be considered non-viable.

For 1 min treatments, higher concentrations were necessary to kill 100%. Therefore for quick disinfection, concentrations of about 130 ppm or greater are recommended when targeting the infective stage. This is about a 0.25% solution of bleach or about 2.5 ml of bleach per liter (9.5 ml/gal). A higher concentration would likely be needed against the myxosporean stage of the parasite .

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## Whirling Disease in Utah: New Findings in Upper Weber River Drainage

As part of an ongoing survey for the presence of *Myxobolus cerebralis* (MC), the whirling disease parasite in the state of Utah, samples were collected in Beaver creek, a tributary to the Weber river near the town of Kamas. Previously, MC had been discovered in fish from a private pond in Smith-Morehouse canyon as early as 1993, in the Weber river near the town of Morgan and at Rockport reservoir. Unconfirmed findings of MC have also been made in fish in the Provo river at its junction with a canal connecting it to the Weber river, and at other sites upstream. Since this canal is directly connected to Beaver creek, biologists were concerned about a possible transfer of the parasite via a water route.

Rainbow, cutthroat and brown trout were sampled at a site approximately two miles downstream of the Kamas state hatchery, which is currently closed for renovations. The hatchery utilizes only spring water and has been free of the parasite since testing first began in 1987. Of the fish sampled in the stream, only cutthroat trout tested positive. Although rainbow trout are considered more susceptible to the parasite, they had originated from the hatchery and likely had not been in the stream long enough for detectable levels of spores to be formed. The presence of MC was confirmed by the use of histopathology .

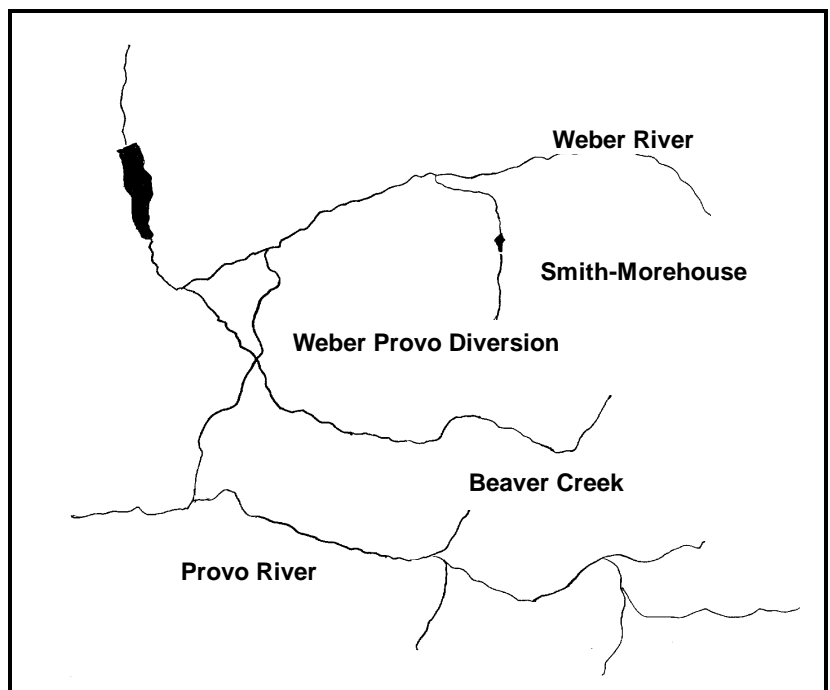
Subsequent testing in November failed to show the parasite in Beaver creek closer to the hatchery, or at a site upstream of the hatchery within the National Forest.

In addition, no spores were discovered in feral fish from Willow Springs creek, a nearby tributary. Collaborative testing by the state Department of Agriculture has failed to show any spores at a nearby commercial fee-fishing operation.

By contrast, sampling in the Weber river has shown the presence of spores in numerous pools of cutthroat and brook trout sampled above and below the confluence with Smith Morehouse creek. Histopathologic testing to confirm the parasite as MC is underway. If confirmed, this will be the highest site of infection in the Weber river drainage.

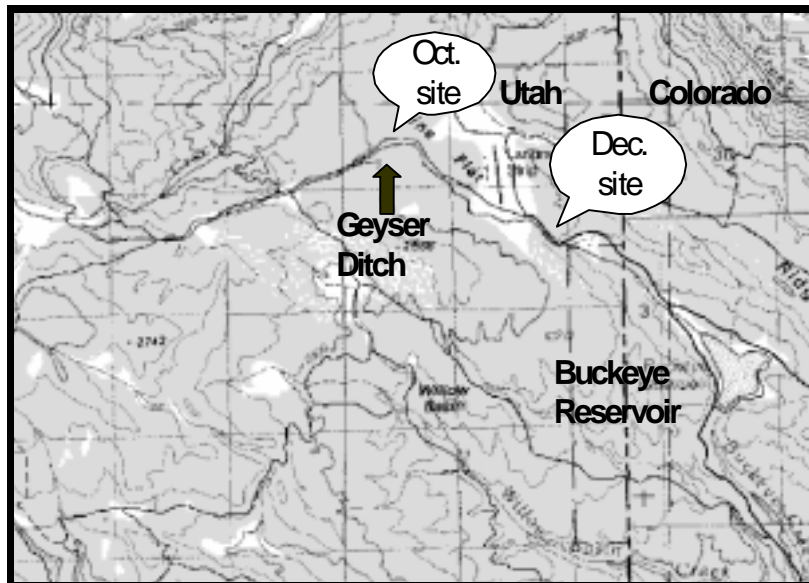
Sampling has been curtailed this year due to formation of ice in these streams. Expanded sampling higher in the drainage is being planned for 1999.

Plans are underway to add additional security measures such as bird covers to protect the Kamas Hatchery against contamination of the parasite.



## Whirling Disease in Utah: First Discovery in Southeast Region

Utah Division of Wildlife Resources (UDWR) fish pathologists have confirmed the presence of the whirling disease parasite in cutthroat trout from Geyser Ditch, a small stream in the LaSal Mountains in San Juan county which flows eastward from Utah into Colorado. This marks the first discovery of the parasite in the Southeastern management region of the state.



Geyser Ditch is a manmade canal where most of the water from Geyser Creek is diverted. Geyser Creek was sampled and found negative for the parasite. Samples were first collected from Geyser Ditch on October 2, 1998 to determine the genetic purity of wild cutthroat which inhabit the watershed. Regional fisheries manager Louis Berg noted the size distribution of the cutthroat trout population changed dramatically. In 1996, 49% of the fish were <150 mm, in 1998 only 5% of the fish were <150 mm. No deformities were noted in fish during 1996, however in 1998 about 75% of the fish had short opercles..

Laboratory analysis of these samples at the Fisheries Experiment Station in Logan showed the presence of low numbers of spores suggestive of the parasite, but the normal confirmatory process of microscopic examination of stained tissue preparations (histopathology) failed to identify the spores as *Myxobolus cerebralis* (MC), the whirling disease parasite. Warm weather made it possible for biologists to resample the waters in early December 1998 at a point downstream from the original site, closer to the Colorado/Utah boundary. At that time, 3/31 adult fish showed observable cranial or opercular deformities. Laboratory studies showed a

significantly higher number of spores at this second site and histopathologic examinations have confirmed the spores as MC.

Geyser Creek/Ditch is managed as a wild cutthroat stream in Utah, with no additional stocking. However a short distance away in Colorado, the water from the ditch is impounded in Buckeye Reservoir, which is stocked with rainbow trout. Communications with Colorado Division of Wildlife biologists confirm that fish positive for MC were stocked by that state into Buckeye Reservoir as recently as 1995, raising the possibility that the infection has spread upstream and over the border into wild fish in Utah. The finding of more heavily infected fish and deformities closer to the border is consistent with this theory.

Utah's newly created Fish Health Board has been notified of this finding as part of the pathogen notification policy. UDWR's emergency response team has no immediate plans for management changes to this wild trout stream, other than to survey other streams in the area and monitor the cutthroat population of the Geyser Creek drainage for future impacts of the infection.

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