UTAH DIVISION OF WILDLIFE RESOURCES
FISHERIES EXPERIMENT STATION
1465 WEST 200 NORTH
LOGAN, UT 84321

The Ichthyogram

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Comparison of Hatchery Performance Between a Whirling-Disease Resistant Strain and Tensleep Strain of Rainbow Trout

Introduction

Salmonid species vary substantially with respect to both susceptibility and pathology when challenged with *Myxobolus cerebralis* (O'Grodnick 1979). Even within a species, strain differences in susceptibility have been demonstrated (Densmore et al. 2001). Rainbow trout are generally accepted to be one of the most susceptible species to *M. cerebralis* infection, yet are one of the most popular sport fish. A resistant rainbow trout strain would provide the sport fish benefits that anglers are accustomed to, while reducing myxospore concentrations in the environment and potentially reducing mortality and deformity among naturally infected fry of susceptible salmonids.

Photo courtesy of Colorado Division of Wildlife

One promising development is the discovery of rainbow trout strains resistant to the parasite. One strain, the Hofer, originated from Germany, where the fish has been exposed to the parasite since it was imported from Colorado in the late 1800s. Through efforts spearheaded by Ron Hedrick at U.C. Davis, the Hofer was imported back into the U.S. via California. It was later sent to Colorado where George Shisler and others with the Colorado Division of Wildlife have begun a broodstock program to develop a resistant strain of rainbow trout. Research by Hedrick et al (2003) has shown that the Hofer strain is resistant to infection, with spore loads 10- to 100-fold less than susceptible strains. The Hofer strain has been domesticated and adapted for commercial aquaculture, so it grows very well in the hatchery with little fright response. Good growth is a desirable attribute, but more 'survival savvy' is desirable for a fish that is stocked into waters where it must fend for itself. Fortunately, parallel research with another strain of rainbow trout, the Harrison Lake (HL) strain, has unveiled another whirling disease resistant stock that is derived from wild, lake-adapted stock (Wagner et al. 2006). Harrison Lake was stocked with fish from Lake DeSmet, WY. HL fish exposed to the parasite had significantly reduced spore loads and a high percentage of fish still uninfected after exposure. However, not every fish is resistant, so selection for resistance is needed in this strain. Unreplicated observations by one of the authors (Routledge) indicate that HL have slower growth than our Utah strains.

So, based on what we know about each strain's strengths and weaknesses, we decided that a cross between the two would be desirable. This would incorporate the wild aspects of the HL and the growth and superior resistance attributes of the Hofer. The objective of the current project is to compare both the field and hatchery performance of the whirling-disease resistant cross with a traditional rainbow trout strain in Utah, the TenSleep strain (TS). The hatchery portion is presented below.

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Methods

We obtained two separate batches of eyed eggs in December 2005 derived from a backcross of the Hofer x HL (F1) with Hofer females. The Fisheries Experiment Station received 13,000 eggs in the first batch and 31,680 in the second, for a total of 44,680 eyed eggs of the Hofer-Harrison cross (HH). The first batch was split among three incubation trays and the second batch among 6 trays. The number of dead eggs removed between receipt of the eggs and hatching was divided by the initial number of eyed eggs to derive a percent hatch value for each tray. The same approach was used for the TS eggs. Fry were incubated in troughs until they were transferred to outdoor raceways on 24 February 2006 to initiate the study. A total of 10,000 fish with an average weight of 1.24 g (both strains) were put into each of six concrete plug-flow raceways (1.22 m x 11.58 m x 0.57 m deep).

Fish were fed a commercial pellet (Silvercup) 3-4 times per day and the study was concluded after 90 days. While differences in growth occurred, the percentage of body weight fed was kept constant for each raceway. During the study each raceway received flows of 114-167 L/min and density index values did not exceed 0.25. Samples of fish were collected monthly from each raceway to estimate total biomass and project feed needs. T-tests were used to compare hatchery performance variables between strains.

The study fish were stocked into two reservoirs on 24-26 May 2006. Both have a recent history of *Myxobolus cerebralis* infection. A total of 15,105 TS and 16,012 HH were stocked into Hyrum Reservoir; 11,479 TS and 11,805 HH were stocked into Porcupine Reservoir. Porcupine Reservoir is mesotrophic with self-reproducing populations of kokanee, cutthroat and brown trout and desert sucker. Hyrum Reservoir has self-reproducing populations of brown trout, Utah sucker, and yellow perch. Rainbow trout are stocked annually in both reservoirs.

A baseline sample of fish was taken from each reservoir prior to stocking in May 2006. Heads from rainbow trout and kokanee *Oncorhynchus kisutch* were assayed individually for *M. cerebralis* using the pepsin-trypsin digest method. A hemocytometer was used to count myxospores in two samples per head. Deformities, if any, were also noted. Myxospore counts were calculated using only positive samples.

Results and Discussion

The percent hatch for the HH was 93.4% for the first lot and averaged 95.3% for the second lot, which was similar to that for the TS lot (91.0%). After 90 days, the mortality rate in the raceways did not significantly differ between strains (Table 1; $P = 0.110$). However, the feed conversion efficiency was significantly better for HH (0.97) than for TS (1.14; $P = 0.008$). Final mean weight was also significantly higher for the HH than TS ($P = 0.001$; Table 1).

Table 1. Comparison of the hatchery performance of the Harrison-Hofer strain (HH) with the TenSleep (TS) strain of rainbow trout. Means (± SD, *n* = 3) that are significantly different between strains are indicated with an asterisk (*).

	TS	HH
Mean final weight (g)	**13.2 ± 0.33**	**16.5 ± 0.52***
Feed conversion rate (g feed/g biomass)	**1.14 ± 0.05**	**0.97 ± 0.03***
Cumulative mortality (%)	**8.5 ± 3.06**	**4.5 ± 1.56**

Results indicated that the HH strain hatchery performance was superior to the TS strain of rainbow trout. Field survival and performance evaluations are ongoing. If those results are as promising as the hatchery data, the HH shows great potential as a tool in the effort to control whirling disease.

Acknowledgments

We gratefully acknowledge the efforts of George Schisler, Phil Schler, and Eric Hughes, from the Colorado Division of Wildlife, who provided the Harrison-Hofer rainbow trout eggs for the study. We thank Egan Hatchery staff for the TenSleep strain eggs. Thanks also to Roger Mellenthin and Jared Smith for help maintaining the fish.

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Eric J. Wagner, Ronney Arndt, and M. Douglas Routledge

New Faces at FES

We are pleased to announce our latest addition to the FES staff. **Cristi Swan** has joined the Technical Services staff (fish health), replacing Chris Heck who joined the crew at the USU Veterinary Diagnostic Laboratory.

Cristi earned both her B.S. and M.S. degrees from the University of Idaho in Fisheries Resources. Her master's work focused on the identification of a localized mucosal immune response in rainbow trout and purification of IgM and other proteins from serum and mucus of rainbow trout. Prior to her studies in Idaho, Cristi attended Green River Community College and earned A.S. degrees in Water Quality, Park Management and Forestry. A native of Montana, Cristi has also lived in Idaho and Washington and looks forward to getting to know Utah's people, history, and landscape.

Cristi's main duties will be the management of bacteriology services, and she will use her considerable expertise in the ongoing development of a vaccine against coldwater disease in our state hatchery system.

FES June Suckers Love New Recirculating Aquaculture Facility

The June sucker (*Chasmistes liorus)* is an endangered species endemic to Utah Lake, Utah. In the past, the Fisheries Experiment Station raised June sucker in 60 – 65 ^{o}F water. Due to a lack of warm water in our facility, the fish grew at a rate of 3"/year and the water temperature was a constant stressor on the June sucker and it increased the possibility of disease outbreaks in the hatchery.

A new 42' x 120' recirculation facility, was completed in July 2006 at FES to allow us to culture the fish in 73-75^{o}F water and get better growth rates and increased survival. Our goal is to rear at least 33,000 fish to 8" annually (5900) pounds and then stock them into Utah Lake and refuge sites such as the Rosebud Ponds, Red Butte Reservoir, Mona Reservoir and Camp Creek Reservoir.

The recirculation facility is divided into two areas. The early rearing portion of the facility is designed to raise the June sucker to 2-3 inches before being placed into the grow out portion of the facility where the fish will be raised to 8-9 inches. To aid in disease control, each area is a separate recirculation system from the other.

On August 25, 2006 we placed some 2005 progeny fish at 4.74" into 73^{o}F water in the grow out portion of the facility. A portion of this group remained in the 65^{o}F water, which is what the fish were raised on since initial feeding in June 2005. After being in the 73^{o}F water for 97 days the fish grew to be 7.32 inches. This is a 2.58 inch growth increase and a daily growth of .0266 inches. In November 2005, these fish grew .97 inches, which is a daily growth of .032 inches. In comparison, over the same 97 day period, the fish that remained on the 65^{o}F water were only 6.01 inches and this is a daily growth of only .012 inches. Just an observation, but when condition factors were taken on these fish the fins on the fish in the 73^{o}F water looked very good, while those fish on the 65^{o}F water had a high percentage of frayed and eroded fins.

Table 1. Condition Factor on fish as of 2/23/2007

Treatment	Condition Factor
Fish moved to 73F water	.0003527
Fish remaining on 65F water	.0003485

Table 2. Other comparisons: Condition factors were taken on the 2005 June sucker when they were placed into the grow out portion of the facility.

Tank Number	C-Factor before going to 73F water	C-Factor February 2007
G11	.0003519	.0003842
G6	.0003519	.0003931

Table 3. Daily growth comparisons from various groups

Daily growth (inches)	Water Temperature (^{o}F)	Comments
.011	65	Average of all 2005 progeny fish kept on 65F water
.0235	73	Average of all 2005 fish after moving to 73F water
.022	73	Average of all 2006 progeny fish placed into 73F water

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Conclusion:

For the short period of time that we have been raising fish in the recirculating aquaculture facility, it is obvious that the fish do much better in the 73°F water. The overall health and condition of the fish are much improved and disease issues have been all but eliminated so far. If the growth rates continue, we should expect to get the fish to 8 inches in 12-13 months compared to 24 months at 65° F water. It looks like recirculating aquaculture facilities are very promising. To help in recovery efforts, more recirculating aquaculture facilities are being considered to allow us to introduce healthier fish back into Utah Lake and other waters.

Figure 1. Above is a photo showing the improved condition factor and growth on a typical fish being raised in 73F water in the recirculation facility compared to 65F water in our flow through system. The photo shows two fish from the same lot with the larger one placed into the 73F water on August 25, 2006. This photo was taken on 2/21/2007.

Doug Routledge

Comparison of Adhesions and Antibody Titers in Rainbow Trout Injected with a Subunit Vaccine for*Flavobacterium psychrophilum* Mixed with One of Three Adjuvants

Several attempts have been made at producing an efficacious vaccine against the bacterium *Flavobacterium psychrophilum* (syn.- *Cytophaga psychrophila*, *Flexibacter psychrophilus*), the etiological agent of coldwater disease and 'rainbow trout fry syndrome'. These studies generally demonstrated improved survival using whole cells mixed with either Freund's Complete Adjuvant (FCA; Holt 1987; LaFrentz et al. 2002), Montanidae ISA 763A or squalene adjuvants (Rahman et al. 2000), mineral oil (Madetoja et al. 2006) or no adjuvant (Obach and Laurencin 1991). Results of these tests indicated that use of adjuvants improved survival and intraperitoneal (i. p.) injections were preferable to bath vaccination. Several studies have shown that use of immunogenic fractions provide better protection than whole cell vaccines (Rahman et al. 2002; Salati 1988; Gudmundsdottir and Magnadottir 1997). The fraction that appears to be most useful are the larger molecular weight fractions (>20 kD, Velji et al. 1992; 70-100 kD, LaFrentz, et al. 2004).

Based on the studies noted above, a subunit vaccine using an adjuvant and immunogenic fractions injected i.p. was pursued for fish health management in Utah. The objective of this study was to compare adhesions and specific antibody titer response among rainbow trout injected with one of three different adjuvants mixed with a subunit vaccine. One concern was the possibility of adhesions in injected broodstock that could compromise gamete production (Midtlyng et al. 1996) as well as possible toxicity effects. So, in separate preliminary tests we evaluated the toxicity and safety of the vaccine.

Methods

A subunit vaccine consisting of particles >50kD was prepared using the methodology of Rahman et al. (2002) and Filip et al. (1973). A virulent isolate of *F. psychrophilum* from southern Idaho was used for the vaccine. Three adjuvants combined with a subunit vaccine were compared to a phosphate buffered saline (PBS) control: (1) Freund's incomplete adjuvant (FIA, Sigma Chemical Co., F 5506), (2) squalene (Sigma Chemical Co., S3626), and (3) mineral oil mixture (90% mineral oil, 9% Span®20, and 1% Tween®80). Rainbow trout were injected i.p. with 100 uL of the mix (10 ug of vaccine). Fluorescent elastomer tags were used to identify fish from the four treatments. Mean weight and length of the fish at the start of the study averaged 42.5 g and 157.8 mm, respectively.

For specific antibody titer ELISA assays (LaFrentz et al. 2002), baseline blood sera samples were collected prior to injection with the vaccine on 11 April 2006. Additional blood samples were collected from the caudal vasculature of 16 fish per treatment at 6, 12, and 24 weeks. Adhesions were assessed by necropsy at 12 and 24 weeks after injection using the Speilberg score that ranges from 0 (no visible lesions) to 6 (vicera unremovable without damage to fillet)(Midtlyng et al. 1996). From each treatment, 20 to 22 fish were necropsied, carefully making the incision around the visceral cavity to facilitate examination of the interface between the ventral portion of the cavity and the internal organs.

Results and Discussion

There were significant differences in the adhesion scores among the four treatments, though scores were generally low overall. In the 12 week sample, there were significantly more fish with a score of 1 in the FIA treatment than in the other three treatments ($P < 0.001$), which did not differ from one another and had more '0' scores (Figure 1). In the 24 week sample, fish in the FIA group were similarly significantly higher ($P < 0.001$) in adhesion score than PBS controls or in mineral oil-injected fish. Twenty percent of squalene-injected fish had a score of '2', whereas none of the other treatments had fish that exceeded a score of '1' (Figure 1). The frequency distribution was significantly different between the squalene treatment and each of the other treatments.
In the squalene treatment, 8 of 20 fish had a blackish material like a thin tissue layer with melanin present. A

smaller percentage of fish in the mineral oil treatment (14.3%) also had a similar response. Fish in the FIA treatment (17 of 22) had white globules near the spleen or in the adipose tissue near the intestine. Examination of this material by light microscopy indicated it was not a granuloma, nor a growth from any particular organ; it appeared to be oily and amorphous and was likely unmetabolized vaccine-adjuvant. The mineral oil mixture was more stable than the others (less tendency to separate) and less expensive.

The antibody titer assay results indicated that there was an antibody response to vaccination that differed significantly among adjuvant treatments in certain sampling times (Table 1). For example, after six weeks there was a significantly higher antibody response among fish in the FIA and mineral oil treatments than control fish injected with PBS ($P = 0.024$); titers for fish injected with squalene+vaccine did not significantly differ from any of the other treatments. At 12 weeks, there was no significant difference among any of the treatments ($P = 0.291$). At 24 weeks, the fish in the FIA+vaccine treatment had significantly higher antibody titers ($P = 0.009$) than the other three treatments, which did not significantly differ from each other.

The results indicated that all the adjuvants tested were not producing significant adhesions that would be of concern for broodstock or for aquaculture. The specific antibody titer assay results indicated that FIA and mineral oil produced better antibody responses than squalene and either would be recommended for future vaccines.

Table 1. Comparison of specific antibody titer optical density (OD) measurements from sera of rainbow trout injected with phosphate-buffered saline (PBS control) or a sub-unit vaccine against *Flavobacterium psychrophilum* mixed with one of three types of adjuvants. Means (± SE) within a sampling time (weeks after injection) that are not significantly different are followed by a common letter.

	Optical Density			
Weeks	FIA	Mineral Oil	Squalene	PBS
0	0.176 ± 0.016	0.176 ± 0.016	0.176 ± 0.016	0.176 ± 0.016
6	0.326 ± 0.044 b	0.366 ± 0.050 b	0.271 ± 0.046 ab	0.201 ± 0.029 a
12	0.283 ± 0.042 a	0.200 ± 0.035 a	0.219 ± 0.034 a	0.176 ± 0.029 a
24	0.310 ± 0.038 b	0.185 ± 0.015 a	0.190 ± 0.023 a	0.206 ± 0.028 a

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Eric J. Wagner, Chris Heck, and Ronney Arndt

Myxobolus cerebralis Detected in the Duchesne River

Fish pathologists at Utah's Fisheries Experiment Station have recently discovered the whirling disease parasite, *Myxobolus cerebralis*, in trout collected from the upper Duchesne River in the Uinta Mountains. This finding follows a recent detection of the parasite in Rock Creek, which is a tributary to the Duchesne River near the town of Tabiona.

To better identify the parasite's distribution in the area, fish were collected from the Duchesne River above the confluence with Rock Creek on November 21, 2006 and examined at Utah's fish diagnostic laboratory using polymerase chain reaction (PCR) methods. Results from this analysis confirmed that trout from this location were infected with the parasite.

The parasite's presence in this region is concerning as the Duchesne River flows east along the base of the Unita Mountains where it converges with a number of the state's important fisheries including the Strawberry River and its connected reservoirs. Also, a genetically pure strain of Colorado Cutthroat Trout, which are used for the state's native trout supplementation program, can be found in the upper West Fork of the Duchesne River.

In light of these concerns, additional samples were collected from the West Fork and the upper main-stem of the Duchesne River on November 21, 2006 and likewise examined using PCR methods. Results from this analysis confirmed that trout on the upper main-stem were also infected while those on the West Fork remain pathogen free.

Since the parasite can become dispersed with the migration of infected fish and the movement of flowing water, the establishment of *Myxobolus cerebralis* in the West Fork of the Duchesne seems inevitable unless efforts to prevent its spread are considered. Managers currently believe the absence of infected fish in the West Fork is a direct result of a water diversion dam located on the upper portion of this tributary. The diversion normally functions to move water from the West Fork to Currant Creek and Strawberry reservoirs, but the structure also serves to deter the upstream migration of infected fish.

Even though the diversion is currently offering some protection to native fish populations found on the upper West Fork of the Duchesne, this system could also provide a route for the parasite's dispersion to several of the state's important reservoirs. As a result, regional biologists are working to install an additional barrier to further restrict the movement of infected fish into these critical areas.

How the parasite became established at these locations is unknown, but angler use, avian vectors and in-stream diversions may all be factors in its increasing dissemination throughout Utah. Although eradicating *Myxobolus cerebralis* from infected waters is unlikely, efforts through the state's whirling disease survey continue to provide valuable information, which is used to limit the parasites dispersion into uninfected areas.
Wade Cavender

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

Phone:435-752-1066
Fax: 435-752-6977
Email: chriswilson@utah.gov

The Ichthyogram is a quarterly publication of the Fisheries Experiment Station, Utah Division of Wildlife Resources, Logan Utah 84321.
Editor:
Chris Wilson (chriswilson@utah.gov)
Contributors:
Doug Routledge (dougroutledge@utah.gov)
Eric Wagner (ericwagner@utah.gov)
Wade Cavender (wadecavender@utah.gov
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