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EVALUATION OF FEED REGIMES FOR REARING JUNE SUCKER (*Chasmistes liorus*)

Introduction

The June sucker (*Chasmistes liorus*) is an endangered fish species endemic to Utah Lake, Utah. A recovery program has been implemented with a goal of propagating June sucker for stocking into Utah Lake. The development of proper culture techniques is needed prior to the construction of a native species warm water hatchery necessary to meet recovery goals (Routledge, 2001).

This study is second in a series of studies to establish a diet for use in culturing June sucker. The first feed study began with fish nine months old and continued until fish were one year old. The results from the first study identified three diets for potential use for rearing June sucker: Razorback, Bio Vita and Bio Flake (Hansen, 2002).

Methods

The study began at initial feeding (swim up) and ran for 236 days. Fish used in the study were from one lot of eggs collected from the Provo River. The study consisted of five feed regime treatments with three replicates per treatment (Table 1). Treatment 1 was fed brine shrimp, and the Bio Flake diet, manufactured by Bio Oregon. Treatment 2 was fed rotifers, Zeigler AP 100 and Z+ larval diets and Finfish Meal. Due to problems with the availability of Zeigler AP100, the Zeigler diets Z+ and Finfish Meal were substituted. Treatment 3 was fed brine shrimp and the "Willow Beach" diet used for razorback sucker, and Bio Diet, which replaced Silvercup due to results from the first feed study. Treatment 4 was fed brine shrimp and the Razorback diet formulated by the Bozeman Fish Technology Center and manufactured by Nelson & Sons. Treatment 5 was fed brine shrimp and the Bio Vita diet manufactured by Bio Oregon. Brine shrimp were decapsulated, and fed for twenty-eight days; at day fifteen, brine shrimp were supplemented with the treatment feed. Rotifers were fed for fifty days, where upon feeding ended due to population crash; at day fifteen, rotifers were supplemented with Zeigler AP 100 larval diet. During the study, parameters (flow, density and percent body weight fed) were kept consistent relative to the number of fish. Due to low numbers of fish in treatments 3 and 5 after 146 days into the study, densities were kept consistent between replicates within the treatment. Due to high gas saturation levels, degassing columns were installed to reduce the total gas saturation; this did not appear to affect the different treatments.

In order to evaluate diet effects on fish health, the health condition profile (HCP; Goede and Barton 1990), Deformity Index, Skin Lesion Index, and Fin Deformity Index were used to compare the replicates and treatments upon completion of the study. Due to small fish size not all variables in the HCP were quantified. The HCP variables quantified in each replicate include: length,

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Table 1. The sequence of diets used by treatment for June sucker

	Study Days						
Treatment	0-14	15-28	29-49	50-100	101-142	143-149	150-236
1	Brine Shrimp	Brine Shrimp/ Bio Flake	Bio Flake	Bio Flake	Bio Flake	Bio Flake	Bio Flake
2	Rotifers	Rotifers/ AP 100	Rotifers/ AP 100	AP 100	Z+	Z+	FinFish Meal
3	Brine Shrimp	Brine Shrimp/ Willow Beach	Willow Beach	Willow Beach	Willow Beach	Willow Beach/Bio Diet	Bio Diet
4	Brine Shrimp	Brine Shrimp/ Razorback	Razorback	Razorback	Razorback	Razorback	Razorback
5	Brine Shrimp	Brine Shrimp/ Bio Vita	Bio Vita	Bio Vita	Bio Vita	Bio Vita	Bio Vita

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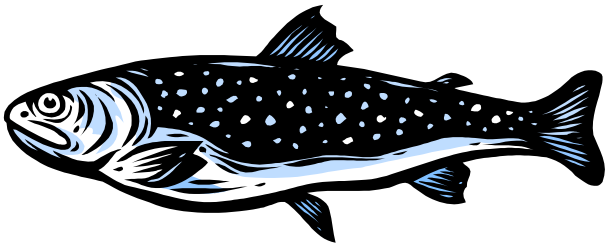
Lake Trout Triploidy Induction: Heat Shock Results for the 2002 Experiment

Bear Lake, Utah-Idaho, is home for four species found nowhere else in the world. These include two whitefish species, the Bonneville cisco, and the Bear Lake sculpin. Lake trout were introduced into Bear Lake in 1911 and sporadically after that, supplementing natural reproduction. Due to the native species present, and the responsibility of the fish and wildlife agencies to sustain their existence, demands by anglers for more lake trout must be addressed cautiously. Ideally, an agency could stock sterile lake trout giving it stricter control of reproduction and lake trout numbers and predation. One way of controlling reproduction is to use sterile triploids. This approach has worked for other species such as the rainbow trout, but lake trout have not been tested as extensively for triploid production techniques. Triploids have 3 sets of chromosomes instead of two (diploid). Our test last year indicated that triploidy induction rates were variable, but encouraging (100% in one experiment, 60-87% triploid in another; *Ichthyogram* 13(2)). However, fry survival was unacceptably low, typically less than half that of controls.

In an effort to reduce mortality associated with the heat shock process, we conducted a subsequent experiment on 21 October 2002 in which the duration of the heat shock treatments was reduced. This was done in cooperation with Joe Kofskay and Dwight Alplanalp, Idaho Fish and Game, and Ed Stege, Superintendent at the Saratoga National Fish Hatchery. The treatments were 1) 29.4 C at 18 min after fertilization for 7 min, 2) 28.0 C at 10 min after fertilization for 7 min, 3) 28.0 C at 15 min after fertilization, 4) 29.4 C at 18 min after fertilization for 5 min, and 5) untreated controls handled the same, but left at the hatchery well water temperature (9 C). Treatment 1 was a repetition of last year's test to compare results from one year to the next. Each treatment had three replicates.

For heat shocking, the three replicates consisted of three separate lots of lake trout (Lewis Lake strain) eggs from 5 8-year-old females and fertilized by 5-6 males. Milt was pooled into a plastic bag before adding it to the eggs. For fertilization of each lot, the eggs were divided into 3 buckets and a proportionate amount of milt was added to each. Water was then added to initiate fertilization. The eggs were rinsed with fresh water after 1-2 min, then left to water harden until it was time for the heat shock. Using a net and small containers, the lot was divided into 5 equivalent groups of eggs, one for each treatment. For the heat shock treatments, temperature was monitored at the start and end of each exposure. Heat shocks were



conducted in coolers which had been modified to recirculate water using a heat pump. Within the cooler, a perforated aluminum tray on short legs permitted water flow under the eggs. Mosquito netting was used on the tray to ease handling of the eggs after heat treatment. The eggs were transferred to Heath egg incubation trays where the eggs were treated with a 30 min bath of 100 ppm povidone iodine. The tray location in the three stacks was randomized to avoid any bias in survival based on the stack or tray, replicate, or treatment order.

After the fish had been reared in individual circular tanks at Idaho Fish and Game's Grace Hatchery for a few months, a blood sample was taken from the caudal vein after fry were anesthetized and the caudal peduncle was severed. The blood was mixed in Alsevere's anticoagulant solution in microcentrifuge tubes and kept on ice-water. The blood was shipped to Washington State University for analysis of ploidy by flow cytometry. The blood was sampled twice due to many unreadable samples in the first attempt. A sample size of 20 per replicate in the experimental lots was not always achievable due to mortality prior to sampling.

Two additional egg groups with larger number of eggs were heat shocked to provide more sterile lake trout for stocking. The first was from Saratoga Hatchery where the experiment was conducted, and the second from Egan Hatchery, Bicknell, UT. These were not part of the experiment discussed above, but were strictly for production purposes. For those heat shocks, the protocol in Treatment 1 above was followed. Blood was sampled from these two lots at the same time as the experimental groups.

Results

The experimental results were similar to that for 2001 in that survival of the heat shocked groups was unacceptably poor. Survival to eyeup in the heat shocked groups ranged from 40-48%, compared to 65% in controls (Table 1). There was no significant difference among treatment groups in eyeup survival. Survival in Replicate 3 was

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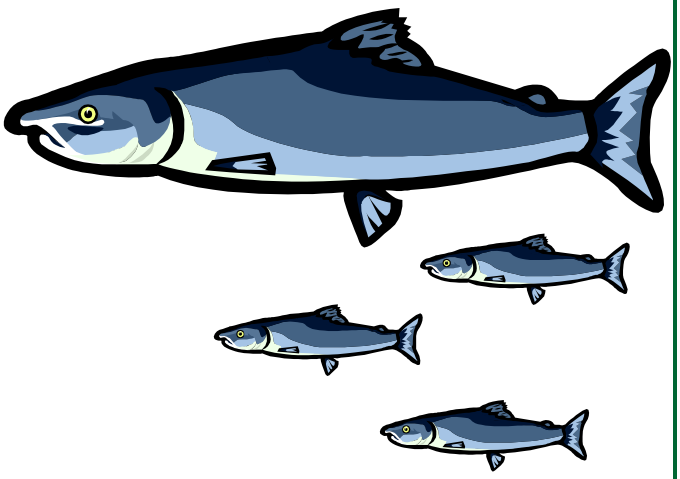
Mixed-Sized Rainbow Trout Culture As a Means of Improving Fin Condition

Fin erosion is common among rainbow trout reared in large-scale aquaculture facilities. Fish with eroded or missing fins may be undesirable to anglers, and may have impaired survival (Nicola and Cordone 1973). A number of causes for poor fin condition have been documented including aggression (Abbott and Dill 1985), rearing densities (Winfree et al. 1998), nutritional imbalances (Kindschi et al. 1991; Lellis and Barrow 1997), and conditions inherent to a hatchery (Bosakowski and Wagner 1994). Adams et al. (2000) demonstrated when a few older (larger) Atlantic salmon parr were reared with groups of 0+ parr, aggression among the younger fish was reduced, and growth rates were improved. The purpose of this project was to determine if rainbow trout reared in raceways containing 1,3, or 6 larger fish per 100 smaller fish experienced different levels of aggression, and further, improved fin condition and better growth.



Into each of twelve raceways, approximately 800 juvenile rainbow trout (1.2 g, 4.8 cm) of the Ten Sleep strain were stocked. Three raceways served as controls and contained only small fish. For the 1:100 treatment, eight larger (8.0 g, 9.0 cm) rainbow trout (Sand Creek Strain) were stocked with the smaller fish. For the 3:100 treatment 24 larger fish were stocked with the smaller ones, and for the 6:100 treatment, 47 larger fish were stocked. Growth of the fish was monitored monthly via weight inventory, and fin measurements were also made monthly to determine fin condition. During the study attempts were made to quantify aggression levels by video observations, but it was impossible to distinguish large from small fish with the equipment at hand. At the conclusion of the study final fin measurements were made on 60 fish/treatment, and necropsies were performed on 10 fish/treatment. A complete water quality profile was also conducted at the study's conclusion.

Throughout the majority of the study, the presence of large fish did not significantly alter fish growth, however there was a trend towards better growth among the treatments compared to the control fish. An anomaly in final weights did occur by the end of the trial however. Weight data collected during the final fin measurements and necropsies indicated fish from each of the treatment groups had gained significantly more weight ($P < 0.01$) than the control fish. By the end of the 6-month study the average final weight for the three treatments was 71.8 g/fish compared to 60.1 g/fish for the controls. The large fish grew to 156.0 " 2.7 g/fish. This is opposite of the growth effects found from the monthly inventory data which indicated no significant differences in final fish weights between any treatments. Condition factor and feed conversion ratio were unaffected by treatment, with condition factor averaging 1.13 " 0.02, and feed conversion 0.86 " 0.02 averaged across all fish. By the end of the study it became increasingly difficult to separate out the larger fish from the smaller while conducting monthly inventories. Over the course of the study, an average of 11 large fish were recovered from 1:100 raceways where 8 were expected, eighteen were recovered from 1:300 raceways where 24 were expected, and 28 were recovered from 6:100 raceways where 47 were expected. Mortalities cannot account for these discrepancies, so it appears we were inefficient at sorting large from small, or some larger fish retarded their growth.

Significant differences between treatments with respect to fin condition were found. At the end of April, six weeks into the study, all treatment fish had significantly better dorsal fins than the controls ($P < 0.01$), however at the same time, caudal fins were better for the control fish compared with fish from the 6:100 treatment. Measurements made at the end of May and June revealed no significant differences. July measurements indicated that caudal, anal, and right pelvic fins were better for 6:100 treatment fish compared to the controls, while the other two treatments had intermediate fins. The final measurements (Aug) indicated the anal and right pectoral were better for 6:100 treatment fish compared to the controls, while the 1:100, and 3:100 treatments had intermediate fins (Figure 1). While these differences were significant, no trend was found which indicated a strong relationship between the presence of large fish, and fin condition among the smaller fish.

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Whirling Disease Found in the Northern Uintas…

The technical services section at the Fisheries Experiment Station in cooperation with biologists from the Northeastern Region have discovered the presence of the parasite that causes whirling disease in fish from Burnt Fork River in the Northern Uinta Mountains. Brook and cutthroat trout sampled this winter from several locations along the lower reaches of the river were found to be infected with *Myxobolus cerebralis*. This finding represents the first reported case of whirling disease in this region.

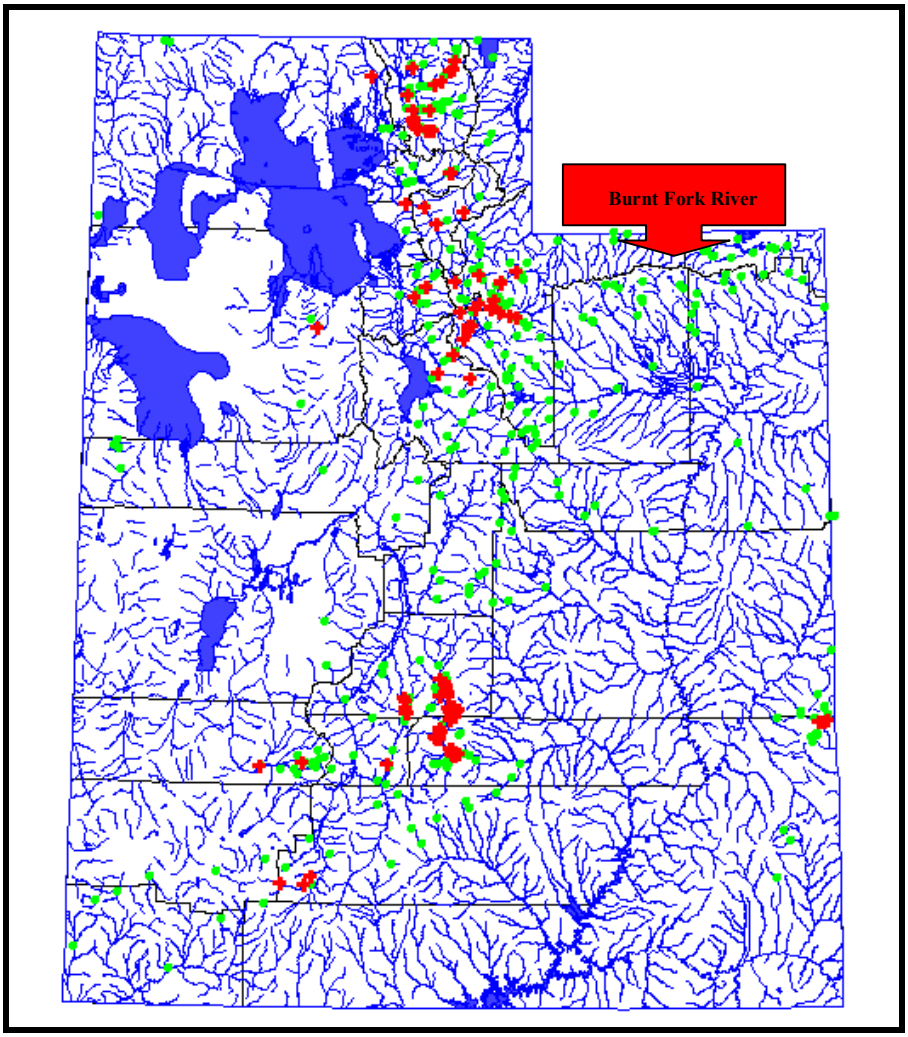


Figure 1. Map of Utah, showing locations of sample sites for Myxobolus cerebralis. Green dots represent negative findings, red crosses show positive locations.

The Burnt Fork drainage is located just west of Sheep Creek, which flows into Flaming Gorge Reservoir. Kirk Mullins and Garn Birchell, Wildlife Biologists, had hoped to use cutthroat trout collected from the Burnt Fork River as a wild broodstock source for systems in the northern Uinta Mountains to aid in the recovery of native cutthroat trout in those locales.

Fish were first collected from the river in December of 2002 in order to begin the process of obtaining fish health approval for this new site. The samples were processed at the Fisheries Experiment Station in January of this year and spores with morphologies consistent with a *Myxobolus* parasite were detected using the pepsin–trypsin digest methodologies in 16 of the 23 cutthroat trout and 3 of the 6 brook trout. The presumptive results were somewhat complicated by the observation of spores slightly larger than the reference range for *M. cerebralis*; however positive results of polymerase chain reaction tests (PCR) conducted by two outside laboratories confirmed the finding. Additional samples were collected during a snowstorm in the end of February, and four of the six cutthroat processed were found to be positive by PTD. Parallel samples were submitted for histopathology, and two half-head samples were found to have areas of cartilage lysis with *M. cerebralis* generative stages present. One histology sample also had a granulomatous lesion in the dorsal calvarium but no definitive parasite life stages. Fish health specialists observed gross cranial lesions in several electroshocked fish that were also indicative of a whirling disease infection.

Obviously, fish health approval was denied, no fish will be moved to or from this site, and all the appropriate officials from Utah have been notified. Given this discovery is the first finding of MC in the northern Uinta drainage, and since the river flows north into Wyoming, biologists from that state have been notified of the report. Plans are underway to broaden the WD survey efforts in the region, in order to get a handle on the extent of this outbreak. Furthermore, efforts are being made to determine the source of the infection. Biologists as well as anglers should be made aware of this news, and take precautions not to spread the disease any further.

Patrick Goddard

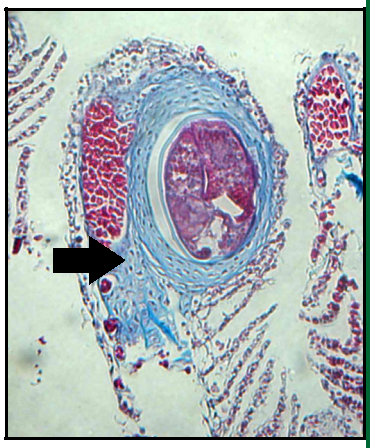
Another New Exotic Fish Pathogen Comes to Utah

Pathologists at the Fisheries Experiment Station have confirmed the presence of a new exotic parasite in speckled dace at Gandy Warm Springs in far western Utah. The parasite, *Centrocestus formanosus*, is a digenetic trematode which has been described in fish from Florida, Arkansas, Texas and other southern states in recent years. The parasite is thought to have been introduced via aquarium fish.

The exotic snail, *Melanoides tuberculata,* intermediate host to several parasites

The parasite undergoes a complex life cycle, and fish serve as a secondary intermediate host. Another exotic nuisance species, the red-rimmed snail (*Melanoides tuberculata*) serves as the first intermediate host. The final hosts are fish-eating aquatic birds such as herons

The Melanoides species is only found in warm water environs and was described at a number of warm water springs in the mid 90's by Dr. Mark Vinson of Utah State University. Fish from the spring were sampled in January 2003 after the finding of Melanoides was discovered.



Histopathology section of gills from speckled dace showing encysted metacecaria of *Centrocestus formanosus*

The cecariae of the parasite can infect a number of fish species and the metacecariae can cause significant gill pathology. At least one recorded incidence of a significant fish population decline has been reported in Texas. This finding raises concern for native species in Utah which may survive in similar springs throughout the west desert.

Pathologists plan to sample fish from other warm water spring sites in Utah where the Melanoides snail has been reported to determine if the fish parasite is present there as well. *Chris Wilson*

Triploid Production of Rainbow Trout: 2002 Cohort Results

Production of triploid rainbow trout continued in this spawning season. Nearly all of the rainbow trout of the Sand Creek (RTSC) and a portion of the Ten Sleep (RTTS) strain were heat shocked this year. The recipe for the heat shock was the same as last year: 20 min of 26-27 C at 20 min after fertilization. Data on the hatchery survival is still being summarized, but initial reports indicate that egg and fry mortality has been higher than expected. Some of the production lots were randomly selected for sampling to determine the percentage of triploidy achieved using flow cytometry. Blood was collected from at least 30 fish per lot and kept cold in anti-coagulant solution. Some of the samples were not readable, so they were not included in the percentage calculations. The lab at Washington State University conducted the ploidy analysis of the fresh samples.

Results indicate that this year's cohort had a high percentage of triploids. Of 253 fish sampled, all but 3 fish were triploid (98.8%). Table 1 summarizes the data for the lots tested. Control lots were all diploid. Kudos to the crew at the Egan State Fish Hatchery for their efforts and to the others that were involved with triploid production.

Table 1. Percent triploidy of production rainbow trout lots heat shocked at 26-27 C for 20 min at 20 min after fertilization in the fall of 2002.

Hatchery	Strain and spawn date	Triploidy (%)	Sample size
Loa	RTSC, 9-04-02	98.3	60
Kamas	RTSC, 10-01-02	96.4	28
Fisheries Exp. Sta.	RTSC, 10-01-02	100	30
Fountain Green	RTSC, 10-15-02	100	16
Glenwood	RTSC, 10-29-02	96.5	29
Fisheries Exp. Sta.	RTSC, 10-29-02	100	30
Glenwood	RTSC, 11-12-02	100	30
Fisheries Exp. Sta.	RTTS, 12-10-02	100	30

Eric Wagner

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weight, condition factor (Ktl), eye condition, fin erosion, and opercle shortening. The Deformity Index classifies fish as normal or as an anomaly: vertebral, mandibular, cranial, opercular, fin, rakers, and other. The Skin Lesion Index classifies fish as normal or as an anomaly: red lesion, open lesion, fungus, loss of scales, tumor/ neoplasm, and other. The Fin Deformity Index classifies fish fins as normal or as an anomaly: pectoral, pelvic, anal, ventral, caudal, adipose, dorsal, and other. Additional variables were quantified, (hemorrhaging, the presence of "black spots", crippling and mortality), that were not specifically addressed in the HCP or indices. An external examination of the fish was used to verify if hemorrhaging was present or absent. The formation of "black spots", two bilateral darkened areas on the sides anterior and posterior to the dorsal fin, were an abnormality found in fish, which eventually developed vertebral deformities resulting in the fish being immobilized. These types of cripples were culled after 188 days and included as mortalities. Daily records on mortality in the replicates were recorded.

Table 2. Comparison of hatchery performance of June sucker fed five feed regimes. Matching subscripts among treatment means depict no significant difference between treatments for a given variable.

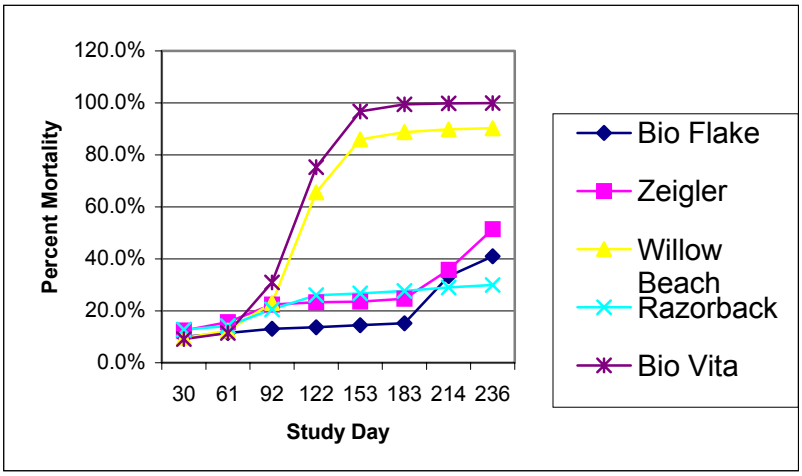
Treatment	1	2	3	4	5
Diet	*Bio Flake*	Zeigler	Willow Beach	Razorback	Bio Vita
Mortality (%)	41.1_{yx}	51.5_{y}	90.3_{z}	30.1_{x}	99.9_{z}
S.D.	0.055	0.078	0.035	0.1289	0.00075
Crippling (%)	18.3_{z}	10.7_{z}	0.4_{yx}	0.3_{y}	0.0_{x}
S.D.	0.059	0.027	0.0053	0.0025	0.0000
Length (TL)	42.7_{z}	33.1_{y}	34.3_{y}	44.4_{z}	------
S.D.	5.0273	6.1075	5.4159	6.0329	------
Weight	0.69_{z}	0.27_{x}	0.33_{y}	0.76_{z}	------
S.D.	0.6925	0.2733	0.3315	0.7640	------
Condition Factor (K) 10^5	0.8347_{z}	0.6239_{x}	0.7543_{y}	0.8207_{z}	------
S.D.	0.1170	0.1242	0.0890	0.0940	------

The data was analyzed using SPSS. The Bio Vita feed regime treatment was not included in statistical analysis for HCP and indices, due to low survival. A twenty fish sample from each replicate of four treatments was used to quantify most variables. The percent mortality and crippling was calculated for each replicate, providing for a total of three samples per treatment. The mean weight, percent mortality and crippling were log transformed to normalize the data. Analysis of variance (ANOVA) was used to test for significant differences in percent crippling, percent mortality, mean length, mean weight, and mean condition factor. Post hoc tests using the least significant differences method was used to compare between treatments for the variables with a significant difference. Chi squared tests using maximum likelihood ratios were used to analyze the eye, opercle, fin deformity index, and hemorrhaging variables. Variables with a significant difference were subsequently analyzed in paired treatments with maximum likelihood ratios. The level of significance 0.05 was used for all tests. There was no variation in the fin erosion and the skin lesion indices, so no statistics were required.

Results

By the end of the study there were significant differences in fish performance between feed regime treatments. Crippling, mortality, length, weight, and condition factor differed significantly among feed regime treatments (Table 2). The percent mortality ranged from 30.1% to 99.9%. Mortality was significantly higher in the Bio Vita and Willow Beach feed regimes than in the other three treatments and began to increase after the feeding of brine shrimp had ended. The percent mortality in the Bio Flake feed regime was not significantly different than the Zeigler or Razorback regimes, but mortality in the Razorback feed regime was significantly lower than the Zeigler feed regime (Figure 1). The percent crippling ranged from 0.0% to 18.3% with the Bio Flake and Zeigler feed regimes having a significantly higher rate of occurrence than the other feed regimes. The amount of crippling in the Willow Beach feed regime was not significantly different from the Razorback and Bio Vita feed regimes, but the Razorback feed regime had a significantly higher occurrence of

Figure 1. The comparison of cumulative mortality in feed study treatments.



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crippling than the Bio Vita regime where no crippling was observed. The mean total length ranged from 33.1 to 44.4 mm with significantly longer fish in the Razorback and Bio Flake feed regimes. The weights ranged from 0.27 to 0.76 g. Fish were significantly heavier in the Razorback and Bio Flake feed regimes, and fish were significantly lighter in the Zeigler feed regime. The mean condition factor ranged from 0.6239 to 0.8347 with significantly larger fish in the Razorback and Bio Flake feed regimes and the significantly smaller fish found in the Zeigler feed regime.

Table 3. Comparison of the percentage of hemorrhaging, eye anomalies, opercle shortening and fin deformities. Matching subscripts among treatment percentages depict no significant difference between treatments for a given variable.

Treatment	1	2	3	4	5
Diet	*Bio Flake*	Zeigler	Willow Beach	Razorback	Bio Vita
Eye anomalies	$0.0\%_{y}$	$5.0\%_{z}$	$6.7\%_{z}$	$0.0\%_{y}$	------
Opercle shortening	$20.0\%_{y}$	$55.0\%_{z}$	$40.0\%_{z}$	$53.3\%_{z}$	------
Hemorrhaging	$6.7\%_{y}$	$23.3\%_{z}$	$1.7\%_{y}$	$5.0\%_{y}$	------
Fin deformities	$3.3\%_{yx}$	$0.0\%_{x}$	$21.7\%_{z}$	$5.0\%_{y}$	------

Using chi squared test maximum likelihood ratios, significant differences were found in the eye condition, opercle shortening, hemorrhaging, and fin deformity index variables (Table 3). The percent of eye anomalies ranged from 0.0% to 6.7% with the Willow Beach and Zeigler feed regimes having a significantly higher occurrence than the Bio Flake and Razorback feed regimes wherein no anomalies occurred. The percent occurrence of shortened opercles ranged from 20.0% to 55.0% with a significantly higher occurrence in the Zeigler, Razorback and Willow Beach feed regimes than in the Bio Flake feed regime. The percent occurrence of hemorrhaging ranged from 1.7% to 23.3%, with the Zeigler feed regime exhibiting a significantly higher occurrence than the Bio Flake, Razorback and Willow Beach feed regimes. The percent of fin deformities occurring ranged from 0.0% to 21.7% with a significantly higher occurrence in the Willow Beach feed regime. The Bio Flake feed regime was not significantly different than the Razorback and Zeigler feed regimes, but the Razorback feed regime had a significantly higher occurrence than the Zeigler feed regime where no fin deformities were observed.

Conclusions

The Razorback feed regime was determined to be the best diet for June sucker in comparison to the other four feed regimes in this study. Though fish fed this regime had a significantly higher occurrence of opercle shortening than the Bio Flake feed regime, it is not as detrimental of a problem at this time as the crippling levels found in the Bio Flake diet. The Bio Flake feed regime was found to be better than the other three diets. Fish in the Bio Flake feed regime experienced a significantly higher percent crippling than three of the other four feed regimes. The crippling did not begin occurring until 126 days into the study. The type of crippling experienced was preceded by the appearance of two bilateral black spots occurring in the same area on all fish in which this abnormality was found. The fish with these spots went on to develop the spinal deformities lordosis and scoliosis to the severity of immobilization. All fish were from the same lot, so the problem appears to be feed related rather than genetic. Further research needs to be conducted to determine if this diet is lacking in something required at certain point in development and would be sufficient during other stages of June sucker development.

The Zeigler feed regime did not appear to be appropriate for June sucker due to poor fish condition and growth, but certain aspects of the regime should be evaluated with further research. The rotifer population was never at a production level to meet fish requirements. The Willow Beach feed regime was not a sufficient regime in the study due to high mortality in addition to poor fish condition and growth. The Bio Diet feed needs further research due to the fish condition prior to switching feeds. The Bio Vita feed regime was the most inferior diet due to the high level of mortality. In the first feed study Bio Vita was a good diet. However in study #1, fish started on study feeds at nine months old and this study started at initial feeding. Bio Vita is likely a sufficient diet at certain developmental stages of June sucker.

Future research for diets used with June sucker should focus around the Razorback and brine shrimp feeds. The Zeigler larval diets should be evaluated with the use of brine shrimp. Bio Diet needs to be evaluated with feeds which have been successful before fish reach a size that Bio Diet can be fed. Rotifers still have the potential use for rearing June sucker after more work is done to produce larger numbers for feeding.

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Eriek Hansen

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Table 1. Mean (± SD, *n* = 3) survival to the eyed egg stage and percent triploidy of lake trout exposed to thermal shock under five different treatment regimes

Treatment temperature (C)	Duration (min)	Initiation time after fertilization	Eyeup (%)	Mortality from eyed egg to 3/5/03	Triploid (%)
29.4	7	18	39.6 ± 18.4	91.1 ± 8.4	55.8 ± 41.2
29.4	5	18	48.2 ± 19.4	89.0 ± 9.6	47.0 ± 45.9
28.0	7	10	43.8 ± 19.5	90.8 ± 8.5	68.9 ± 49.3
28.0	7	15	45.2 ± 15.9	87.3 ± 11.3	46.4 ± 21.4
9.0	7	Not applicable	65.0 ± 18.6	53.7 ± 18.4	0.0 ± 0.0

generally lower across all treatments, indicating poor egg quality to start with in at least one female for that lot (control eyeup was only 44.6%). Among the treatment groups, the reduction in heat shock duration from 10 to 7 min appeared to have improved survival compared to last year (eyeup of 19-34% among heat shocked groups), although this could not be statistically evaluated due to lot differences. For the treatment that was repeated (1), mean eyeup of 39.6% was similar to last year (33.6%). Mortality from eyed egg to March 5, 2003 was high, averaging 53.7% among controls and from 87 to 91% among treatment groups (Table 1). This survival was not significantly different among treatments ($p = 0.123$; one-way ANOVA).

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The percent triploidy rate did not significantly differ among treatments. Variation in the rate was quite high, with lower triploidy rates for replicate one than the other two. Since the temperatures and treatment variables were on target, the variation must be related to other factors such as genetics of the lot tested. For treatment 1, the triploidy rate this year (55.8 %) was similar to last year (60.0 %). The shorter heat shock durations may have helped survival, but did not appear to help with the triploidy rate. These were generally lower than the rates achieved last year (60 to 100%). Regarding the time at which the heat shock is applied, no difference in triploidy rates was noted for treatments falling between 10 and 18 min.

In the two additional production lots treated at 29.4 C, the Lewis Lake strain fish had only 5.1% triploidy ($n = 39$) and the Jenny Lake strain fish (Egan Hatchery) had 53.3% triploidy ($n = 45$). There were problems maintaining temperature in the Lewis Lake lot due to the volume of eggs relative to the amount of heated water, so the low triploidy rate was not totally unexpected. Also, the eggs were stacked in aluminum baskets several cm deep, rather than in monolayers, inhibiting water movement around the eggs.

Based on the two years of data, it appears that perhaps the shorter initiation time may result in a higher triploidy rate. Also, temperatures of 28 to 29.4 C appear to give comparable results, but 27 C does not induce as high a triploidy rate. Mortality rates are still very high in the heat shocked groups, so future heat shock studies don't appear feasible given the temperatures at which high rates of triploidy are possible. Recent work with Arctic char (*Salvelinus alpinus*) has indicated that pressure treatments may be the way to proceed (Eric Johnson, Rochester WA, personal communication). Using 9500 psi for 5 min at 300 degree(C)-minutes after fertilization provided nearly 100% triploid char with nearly identical survival to diploids.

Eric Wagner

Ernie Dean Retires…

After a career with the Division of Natural Resources that spanned over 30 years, Ernest Dean, biologist/virologist at the Fisheries Experiment Station, retired at the beginning of this year. Ernie was instrumental in the development and upkeep of the tools required to ensure the fish stocked throughout Utah did not harvest viruses that could have deleterious effects on existing populations. He also was involved in the collections and spawning of fish and made many friends along the way. Ernie is already enjoying his retirement days... giving him more time to spend with his family and duck decoys. A retirement party is being coordinated by Don Bone, at the Egan State Fish Hatchery, and he should be contacted for the details.

(Continued from page 3)

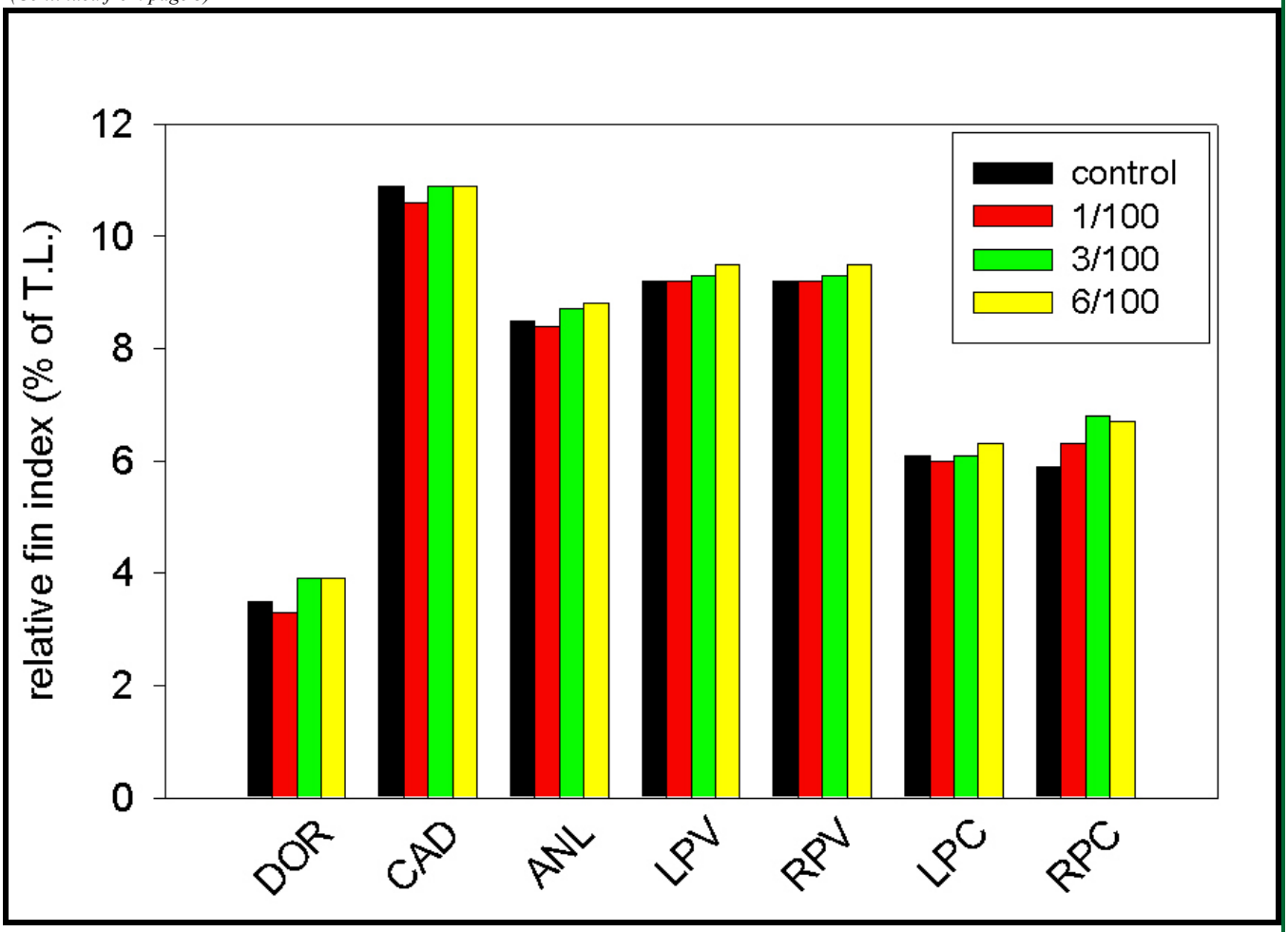

Figure 1. Final fin index values for rainbow trout reared with larger conspecifics. DOR = dorsal, CAD = caudal, ANL = anal, LPV = left pelvic, RPV = right pelvic, LPC = left pectoral, and RPC = right pectoral.

This study did demonstrate that it was possible to improve fin condition and growth among juvenile rainbow trout by co-culturing them with larger conspecifics. Follow-up work may include using higher ratios of large:small fish or the use of even larger-sized fish. It would also be of interest to analyze the effects of using larger fish of other trout species to serve as "hall monitor".

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Ronney Arndt, Eric Wagner, Doug Routledge, and Roger Mellenthin

VALIDITY OF CORE AND WEDGE SAMPLING METHODS FOR THE DETECTION OF *MYXOBOLUS CEREBRALIS* IN BROWN TROUT

Introduction

All fish that are moved from one waterway to another or stocked out of a hatchery must be certified as disease free. Fisheries biologists follow a standard set of procedures delineated by American Fisheries Society to determine if fish harbor prohibited diseases or parasites such as whirling disease, bacterial kidney disease or one of several viruses. The Standard Procedures for Aquatic Animal Health Inspections states that when collecting samples for the detection of *Myxobolus cerebralis*, the parasite that causes whirling disease, a wedge or core sample can be taken in larger fish where size makes collection and processing of the entire head impractical (American Fisheries Society Blue Book, 5th Edition, 2002). The distribution of spore stages in the cartilage of salmonid fishes varies within and among species (Markiw and Wolf 1974). It is possible that this collection protocol is not adequate to detect low numbers of spores of *M. cerebralis* in fish skeletal tissues. This study was performed to evaluate the validity of core and wedge sampling methods for the detection of spores in brown trout, *Salmo trutta*. Additionally, we have compared the ability to detect spores using the pepsin-trypsin digest (PTD) procedures versus the DNA polymerase chain reaction confirmatory diagnostic procedure.

Biologist Anna Miller displays her recent poster showing sampling method data which won "Best Poster" award at the Bonneville AFS meeting in March 2003

Methods

The Beaver River just west of Beaver, Utah was chosen as the study area because it has a known history of *M. cerebralis* infection (Wilson 1997). Four 100 m sections of the river were electrofished on October 22-23, 2002. Ninety-two brown trout greater than 220 mm were collected and randomly assigned to a treatment group: core, wedge or whole head processing. Core, wedge and whole head samples were collected and processed with the pepsin-trypsin digest (PTD) using methods in the American Fisheries Society Bluebook (2002). Samples were examined for *M. cerebralis* spores on a hemacytometer at 200x magnification. Identification of myxospores with the appropriate size and morphology (8-10 µm, rounded, two polar capsules) resulted in a sample designation of positive. If a spore was observed off the hemacytometer grid, the sample was considered positive but number of spores per sample was not calculated.

DNA-Polymerase Chain Reaction (PCR) analysis was conducted on PTD suspensions pooled for individual fish, using a performed a single-round PCR amplification for the presence of the *M. cerebralis* Hsp70 gene segment. For core and wedge sampled fish, 0.3 ml of the PTD suspension from the core or wedge sample, gill arches, and the rest of the head were pooled. For the whole head treatment 0.5 ml of the PTD suspensions for the gill arches and the rest of the head were pooled and sent for PCR analysis (Baldwin and Mykelbust 2002).

Results

PCR and PTD results were concurrent in 78 (84.8%) of the 92 fish tested. PCR identified 36 (39.1%) of the brown trout as *M. cerebralis* positive, whereas PTD identified 44 (44.6%) as positive. Eleven fish were identified as positive with the PTD method but negative with the PCR method; 3 fish were identified as positive with PCR but negative with the PTD method. Two of the PTD positive, PCR negative fish had myxospores slightly larger in size (11-12 µm), suggesting that these fish could be infected with a different species of myxospore such as *M. neurobius*. The 3 fish that were identified as positive with PCR but negative with the PTD method could be due to a relatively new *M. cerebralis* infection with immature spores not detectable by PTD or a low *M. cerebralis* infection that is not readily detectable by PTD.

Analysis of spore counts in *M. cerebralis* fish suggests that very few spores are incorporated into core or wedge sample tissue and the majority of the spores were distributed in the gill arches or the rest of the head (Table 1). The high standard deviations indicate that spores were not equally distributed in the skeletal elements among individual fish. Additionally, when the spore counts of the PTD for the core or wedge sample, gill arches and rest of head of individual fish were pooled, a positive correlation was found between PTD spore counts and PCR code: -, weak +, +, ++ and +++ (Least squares linear regression, $R = 0.454$, $p < 0.001$). When PTD of the whole head and PCR methodologies were compared, no significant differences were detected (McNemar's Test, $p = 0.057$).

Table 1: Number of fish identified as *M. cerebralis* positive with PTD by treatment and tissue sampled (n_1 = number of fish per treatment). Mean number of spores (±S.D.) and mean occurrence of spores (±S.D.) per tissue in the 41 fish with quantifiable spore counts by treatment (n_2 = number of fish identified as *M. cerebralis* positive with PTD by analyzing the entire head).

Treatment	Tissue Sampled	PTD		Mean number of spores per tissue sample (±S.D.)	Mean occurrence of spores per tissue sample (±S.D.)
		+	-		
Core (n_1=31)	Core	2	29	911.1 ± 2717.5 (n_2= 10)	2.65% ± 6.41 (n_2= 10)
	Rest of Head	6	25	5738.3 ± 7946.8 (n_2= 10)	43.30 ± 43.23 (n_2= 10)
	Gill Arches	8	21	12600.6 ± 18655.6 (n_2= 10)	54.05 ± 43.12 (n_2= 10)
	Entire head: core + gill arches + rest of head	10	19	13015.5 ± 14800.2 (n_2= 10)	100% ± 0.0 (n_2= 10)
Wedge (n_1=30)	Wedge	3	27	158.7 ± 476.1 (n_2= 14)	0.84% ± 2.58 (n_2= 14)
	Rest of Head	5	25	4415.5 ± 10539.5 (n_2= 14)	24.9% ± 41.4 (n_2= 14)
	Gill Arches	12	18	8441.3 ± 9732.1 (n_2= 14)	74.3 ± 41.0 (n_2= 14)
	Entire head: Wedge + gill arches + rest of head	14	16	13015.5 ±14800.2 (n_2= 14)	100% ± 0.0 (n_2= 14)
Whole Head (n_1=31)	Head – gill arches	12	19	6724.8 ± 8735.1 (n_2= 17)	59.69% ± 45.06 (n_2= 17)
	Gill Arches	11	20	5661.1 ± 16331.6 (n_2= 17)	40.31 ± 45.06 (n_2= 17)
	Entire head: head + gill arches	17	14	12385.9 ± 23786.8 (n_2= 17)	100% ± 0.0 (n_2= 17)
All Treatments (n_1=92)	Gill Arches	34	58	8303.0 ± 14945.5 (n_2= 41)	55.26% ± 44.70 (n_2= 41)
	Entire head: core or wedge + gill arches + rest of head	44	48	14275.1 ± 19765.1 (n_2= 41)	100% ± 0.0 (n_2= 41)

The number of *M. cerebralis* positive fish was equally distributed between core, wedge and whole head samples (Pearson chi-square, $p = 0.194$). When PTD sampling methodologies were compared, significant differences were found between processing the entire head and core or wedge samples (McNemar's Test, $p < 0.001$) and the entire head versus core or wedge sample and gill arches combined (McNemar's Test, $p = 0.002$).

Conclusion

Analysis of spore counts in *M. cerebralis* positive brown trout suggests that very few spores are incorporated into core or wedge sample tissue and the majority of the spores were distributed in the gill arches or the rest of the head. The high standard deviations indicate that spores were not equally distributed in the skeletal elements between individual fish. The high degree of variability in spore distribution in individual fish heads suggests that just processing a small parts of the head, such as a core and gill arch sample, with PTD may further reduce the ability to detect spores if that small sample is split in half as the Blue Book suggests. The significant differences found between PTD sampling methods indicates that a core or wedge sample collected with the gill arches is ineffective for detecting *M. cerebralis* infections in brown trout. Since the distribution of myxospores in the cartilage of salmonid fishes varies among species, the validity of core or wedge sampling should be evaluated on a species by species basis. The American Fisheries Society should consider these findings when they revise The Standard Procedures for Aquatic Animal Health Inspections.

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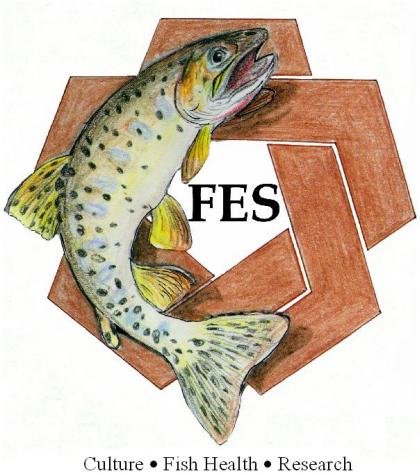

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