The Ichthyogram

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Comparison of Susceptibility of Five Cutthroat Trout Strains to *Myxobolus cerebralis* Infection

This study was an effort to discover any innate differences in susceptibility to whirling disease among the strains of cutthroat trout available in the state of Utah for fisheries management. Several susceptibility tests were conducted, and the last two of which are the focus of this article. In the first of these, survival between infected and non-infected cutthroat trout were compared in a saltwater stress challenge test in addition to prevalence testing. In the second experiment, infection prevalence was compared among five cutthroat strains exposed to 1000 tams/fish at 5 and 10 weeks of age.

Methods

Test 1 **C** Three strains of cutthroat trout (Bear Lake Bonneville, BL; southern Bonneville, BV; and Yellowstone, YL) were exposed to 1000 tams per fish for 2 h in 8.0 L of water. Tams were donated by researcher Don Roberts, Utah State University, Logan. The BL were exposed 53 d after initial feeding and BV and YL were exposed 46 d after initial feeding. These were done separately due to the lack of sufficient tams and the different dates of hatching for the strains. After the 2-h exposure, the fish were transferred to plastic tanks, 30 fish per tank. For each of the three strains, two tanks held exposed fish and two additional tanks held controls that had not been exposed to tams. Automatic feeders dispensed commercial pelleted feed once a day. Mortalities were recorded during maintenance visits (3 times/week). The week prior to the salt challenge tests, fish were fin clipped to differentiate among exposure treatments and strains.

The three strains were challenged with salt solutions in a static 24-h test. On 16-17 November 1998, BL were used for the first challenge test (55 d incubation) and on 3-4 December 1998, YL and BV were used (60 d incubation). Salt solutions were made by dissolving uniodized rock salt in 170 L of spring-pond water in each of two barrels. Two additional barrels were used as freshwater controls. All barrels were supplied with supplemental oxygen via airstones during the test. Dissolved oxygen, pH, salinity, conductivity, total alkalinity, and temperature were monitored during the test using standard methods. Fish were transferred into the barrels such that the population from each rearing tank was split, half (14-16 fish) going into a

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salt treatment, half into a control treatment. Total mortality was summarized after 24 h and percentages were arc-sine transformed for comparison with a t-test. Mortalities and survivors alike were saved for diagnostic testing using the single-round polymerase chain reaction assay (PCR; Epp and Wood 1998). Results were scored into one of five categories based on the intensity of the banding pattern (negative, -; weak positive, w+; positive, +; strong positive, ++; or +++, very strong positive).

Test 2**C** Five strains of cutthroat trout were evaluated in this test: BL, BV, YL, Snake River fine-spotted (SN), and Colorado River cutthroat trout (CR). An extensive effort was made to get eggs from each strain at about the same time to insure equal ages and sizes of fish at the time of exposure. Fish from each strain were exposed to 1000 tams/fish in 8.0 L of hatchery well water for 2 h. Fish were then dip-netted into 114 L tanks, 30 fish per tank. Each strain had two tanks and were fed by automatic feeders once a day. Unexposed controls were adipose fin clipped and 15 fish were put into the same tanks as the exposed fish. Tanks were cleaned and mortalities counted three times a week. Water for the tanks was supplied by a pond fed by a spring. Temperatures ranged from 10 to 14EC during the 5 week incubation period for the 5-week-old group and from 5 to 14EC for the 10-week-old group.

After 5 weeks in the tanks, 25 exposed fish and 5 controls were sampled from each of the two replicates for each time period. Whole heads or whole fry were sampled and stored individually, disinfecting the saw between individuals with 100% chlorine bleach solution and a clean paper towel. The samples were kept on ice and later frozen at -45EC until they were shipped for analysis. Prevalence was determined by single-round PCR (Epp and Wood 1998). Total length of each fish was also measured at the time of harvest.

Results

Test 1**C** The prevalence of *M. cerebralis* among the strains exposed to the parasite varied significantly ($P < 0.001$) in chi-square tests with the data separated into the five categories of infection (Figure 1) or collapsed into tables of positive versus negative. By either method, BL had a significantly lower prevalence (78.5%, $n = 65$) than BV (100%, $n = 41$) or YL (97.8%, $n = 45$), which did not differ from each other. Ordinary least-squares linear regression between fish length and the results of the PCR test indicated no significant correlation between the two for all three strains (r^2 # 0.010).

Table 1. Comparison of survival between exposed (1000 tams/fish) and unexposed cutthroat trout of three strains (Bear Lake Bonneville, BL; southern Bonneville, BV; and Yellowstone, YL) when challenged with hypersalinity 8 weeks post-exposure.

	Salinity	Percent survival	
Strain	**mS/cm**	**Unexposed % (N)**	**Exposed % (N)**
BL	34.5 ± 0.4	0 (32)	0 (30)
	0.6 ± 0.0	100 (33)	100 (31)
BV	32.7 ± 0.6	95 (21)	95 (22)
	0.8 ± 0.0	100 (22)	100 (22)
YL	32.7 ± 0.6	100 (22)	100 (21)
	0.8 ± 0.0	100 (25)	100 (22)

At 33.9-34.9 mS/cm conductivity, 100% of the BL died regardless of whether they were infected with *M. cerebralis* or not (Table 1). At 32.1-33.4 mS/cm, there was some partial mortality of BV in both exposed and unexposed groups (9-10%), but no mortality among YL groups (Table 1). No significant differences in mortality

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were found between exposed and non-exposed groups nor between the salinity and freshwater treatments. Control fish all survived in both tests. Mortality rates in the rearing tanks prior to the salt challenge did not differ among the three strains nor between infected and non-infected fish ($P > 0.05$, two-way ANOVA of arc-sine transformed data).

Test 2**C** At 5 weeks of age, exposure to 1000 tams/fish resulted in significant differences in infection prevalence among the five cutthroat strains (chi-square $P < 0.001$, replicates combined). BL had a significantly lower prevalence (54%) than the other four strains (94-98%) of cutthroat trout. This result was also the case for the fish exposed at 10 weeks of age; BL infection prevalence (82%) was significantly lower (chi-square test, $P < 0.001$) than that of the other four strains (94-100%). The severity of infection as determined by the PCR assay rating supported the prevalence results. Fewer BL were classified as ›strong positive‹ or ›very strong positive‹ than the other strains at both 5 and 10 weeks old at exposure (Figure 1 and 2). There was no significant difference in total length between positive and negative fish for a given strain in either age group.

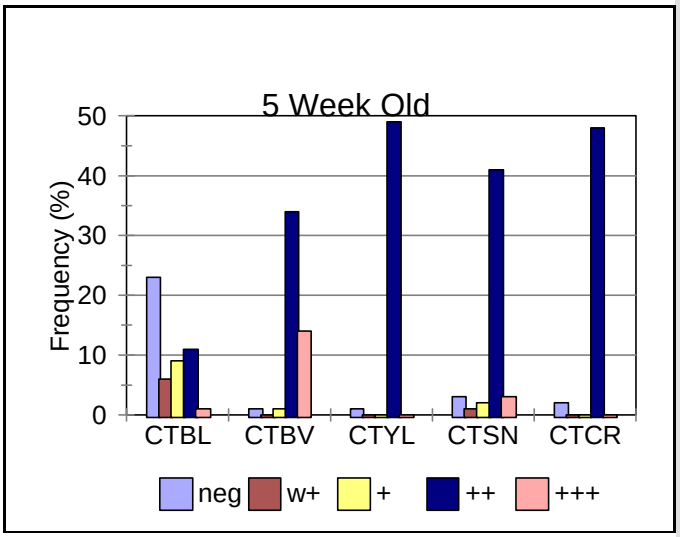

Figure 1. Comparison of the frequency distribution (%) of infection severity among three strains of cutthroat trout (Bear Lake Bonneville, BL; southern Bonneville, BV; and Yellowstone, YL) exposed to *M. cerebralis.* Results were categorized during single-round PCR analysis as either negative (-), weakly positive (w+), positive (+), strong positive (++), or very strong positive (+++).

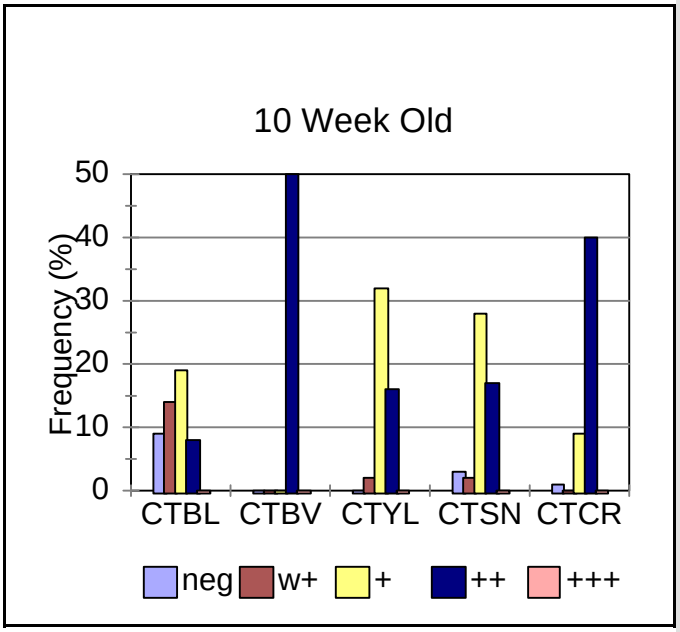

Figure 2. Comparison of the number of fish in each category of single-round PCR results for five strains of cutthroat trout: Bear Lake Bonneville, BL; southern Bonneville, BV; Yellowstone YL; Snake River fine-spotted, SN; and Colorado River, CR. PCR results are classified as negative (empty bar), weak positive (red), positive (yellow), strong positive (blue), or very strong positive (pink).

Discussion

The prevalence of *M. cerebralis* was significantly lower in the Bear Lake strain of Bonneville cutthroat trout than in the Yellowstone, Colorado River, southern Bonneville, or Snake River cutthroat trout in both tests and at two different ages. The severity of the infection was also reduced in BL as measured by the single-round PCR assay severity rating. The reason for the difference is unknown. The parasite was not discovered in Utah until 1991 and is not currently found in any of the waters in which cutthroat trout are used for broodstock.

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This precludes any natural selection for resistance to *M. cerebralis* per se. If differences in total length were responsible, one would expect that the YL to have similar infection rates to BL in the 5-week-old group and have lower rates in the 10-week-old group than BV, SN, and CR. There was considerable overlap in total lengths among the strains in both age groups and infection was not correlated with size. The Bear Lake strain is endemic to Bear Lake, Idaho-Utah, an oligotrophic lake in which the cutthroat trout rely heavily on other fish for prey. This strain has evolved to gain length as quickly as possible to switch over from a planktivorous diet to a piscivorous one (Neilson and Lentsch 1988) . Perhaps some physiological relationship between growth rate and immunological competence is involved. Evidence for this phenomenon has been noted for rainbow trout fry which were protected by immunization only if they were immunized at the time of or after the transition to a faster rate of weight gain (Tatner 1996).

The salt water challenge indicated no difference between infected and non-infected YL and BV in their osmoregulatory ability and susceptibility to stress based upon infection with *M. cerebralis*. The level of salinity chosen for the first test with BL proved lethal under the conditions in the barrels; However, in previous laboratory tests (*Ichthyogram* 7(4)), BL juveniles experienced only 60% and 80% mortality at even higher salinities of 36.0 and 38.0 mS/cm (21 to 22 ppt). Using swimming endurance as a stress challenge at 17 weeks post-exposure, Ryce et al. (1999) evaluated the effects of fish age and parasite dose; time to fatigue of rainbow trout was correlated with infection dose and severity of infection. In this study, the salt challenge was conducted at 7 weeks post-exposure. Different post-infection timing of the salt stress challenge may provide different results as the infection progresses within the fish. Further tests over a range of fish ages, post-infection times, and infection severity are needed to better characterize when infected fish are most susceptible to stressors.

In summary, Bear Lake Bonneville cutthroat trout was less susceptible to infection than four other cutthroat trout strains when exposed to a single high dose. However, fish were still infected at a high rate. Whether or not these lab tests apply to fish in the wild, where fish experience a chronic dose of tams, fluctuating temperatures, and other environmental variables, remains to be seen.

Literature Cited

- Epp, J., and J. Wood. 1998. Whirling Disease Symposium: Research in Progress. p.173-176, Whirling Disease Foundation, Bozeman, Montana.
- Nielson, B. R., and L. Lentsch. 1988. Am. Fish. Soc. Symp. 4:128-133.
- Ryce, E. K., et al. 1999. Whirling Disease Symposium 5: 41-44, Whirling Disease Foundation, Bozeman, Montana.
- Tatner, M. F. 1996. Pages 255-287 *in* G. Iwama and T. Nakanishi, eds. The Fish Immune System. Academic Press, New York.

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Effects of Two Diets on Deformities in June Sucker

The June sucker *(Chasmisties liorus)* is a midwater planktivore endemic to Utah Lake. It was federally listed as an endangered species in April of 1986. As part of the recovery plan, the Utah Division of Wildlife Resources (UDWR) has been artificially spawning June sucker from the wild run in the Provo River to raise for future broodstock. In 1991 construction of a rearing facility was completed at the Fisheries Experiment Station with the goal of rearing June suckers for both research needs and broodstock.

Initially, diets for the suckers were essentially trout diets and similar nutritional needs were assumed. When the sucker culture program began, the fish were fed an expensive diet (BioKyowa Inc., Chesterfield, Missouri, which imports their feed from Japan). At swim-up, when the fry have absorbed the yolk sac and begun to feed, the larval fish were fed BioKyowa fry feed type A, 250 ì m size. The manufacturer suggests the A type feed is a substitute for rotifers. The cost of the A type feed was $53/lb. The June suckers remained on the type A feed until they were large enough to switch to the smallest type C diet, a 700 ì m feed pellet. Type C was developed to substitute for minced fish and shell fish. The cost of the C type feed was $7.27 /lb. As the fish from the first few year classes grew large enough for a 1 mm feed, other feeds were tried in an effort to reduce feed costs.

Early Feed Trials

In 1992, under Brandt Gutermuth=s direction, Tim Miles and Dwight Aplanalp started a 7 month feed comparison at FES. They compared three mixtures: Biodiet moist formula mixed 50% with Silvercup, 100% Silvercup, and BioKyowa mixed 50% with Silvercup. Comparisons were made on the basis of feed conversion, feed cost/pound of growth, and length increase. Results of this experiment are shown in Table I.

Table 1. Comparison of feed conversion, cost per pound of fish weight gain, and growth rate among June suckers fed 4 diet mixtures.

	BioKyowa/ Silvercup	Biodiet/ Silvercup	BioKyowa/ Silvercup	Silvercup
Initial size (Fish/lb)	181	177	33.1	19.2
Feed conversion ratio	1.49	1.57	1.03	1.30
Daily length increase (inches)	.004	.004	.009	.008
Feed cost/lb gain	$4.29	$4.34	$2.97	$0.42

By mid 1993, most of the lots were being fed 100% Silvercup trout food, presumably to save money. After approximately one and a half years on Silvercup, many of the brood fish developed severe deformities in spine, fins and opercles. It is possible that many of these deformities were due to a nutritional deficiency and/

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or genetics. When it was noticed that the number of deformities in the June Sucker were increasing, vitamin supplements were added to the feed; vitamin C was added without success. In 1997 the fish were switched back to the BioKyowa diet to prevent further deformities.

Biodiet vs BioKyowa

In May of 1998, Doug Routledge and Roger Mellenthin started a feed comparison between Biodiet Dry formula and BioKyowa. This study compared the change in the deformity index between fish fed 100% BioKyowa and fish fed 100% Biodiet Dry. The fish used in this study were already 45 - 91g when the study began. To establish baseline deformity data, all of the fish from four lots were evaluated using the deformity index in May of 1998. Thereafter, two lots were fed Biodiet and two BioKyowa. After 5 and 12 months on the diets, 25% of the fish from each treatment were sampled without replacement.

The deformity index was developed by Ron Goede (see *Ichthyogram*, Volume 6 #3) and is a simple composite index summing binomial nominal variables, A0" being not deformed and A1" is deformed. The categories for deformities are: vertebral, mandibular, cranial, opercular, and fins; each category is scored 0 or 1, for a total possible individual score of zero to five.

Four investigators evaluated deformities. Due to inconsistencies in the way deformities were measured in the fins, they were thrown out of the final evaluation. The results showed an overall decrease in deformities in fish fed either diet, but the decrease in the fish fed BioKyowa was significantly ($P<.001$, Mann-Whitney rank sum test) greater (Table 2). Better nutrition and higher mortality of deformed fish are some possible reasons for the better recovery rate in the fish fed BioKyowa. Some deformities continued to increase. A drop in the number of opercular deformities dominated the changes in the deformity index. The investigators noted that eroded nares were common but not measured in the deformity index. Feed conversion rates are listed in Table 3 however they are not adjusted for moisture content. Biodiet is a moist feed as opposed to the Biokyowa. We would like to duplicate this study with larval fish in the future. This would allow us to see how the feed affects development.

Table 3. Specific growth rate, yearly mortality rate, conversion factor, and cost per kilogram weight gain.

	SGR	YMR	conversion	cost/kg gain
JS1	0.286	6.7%	1.74	$27.84
JS5	0.251	4.9%	1.94	$31.15
JS3	0.356	0.6%	2.35	$12.04
JS4	0.234	4.9%	2.47	$12.61

Specific Growth Rate (SGR) = 100 * [ln (final weight g) - ln (initial weight g)] / days
Yearly Mortality Rate (YMR) = (total mortality / days) / average population
Conversion = weight of food fed / weight gain
Cost per kg gain = total cost of feed / total kg gain

Table 2. Deformity index of June suckers in each of 4 lots (JS1, JS3, JS4, JS5) before and 5 and 12 months after feeding either BioKyowa or Biodiet.

	JS1 BioKyowa			JS3 Biodiet		
Date	05/06/98	10/28/98	05/05/99	04/28/98	10/28/98	05/05/99
Sample size	475	77	35	880	523	260
Weight (g)	43.97	84.55	124.73	49.83	104.6	168.51
Length (mm)	163.09	203.77	232.74	170.01	213.04	246.96
Vertebral (%)	42.54	45.45	42.11	2.61	3.25	4.23
Mandibular (%)	2.76	5.19	5.26	7.38	14.53	20
Cranial (%)	0	0	0	0.34	0.38	1.54
Opercular (%)	75.69	74.03	63.16	92.28	83.75	74.62
Fin (%)	84.53	97.4	92.11	94.67	73.04	96.54
Deformity index	1.65	2.22	2.2	2.01	1.75	1.97
DI as a percent	33	44.4	44	40.2	35.4	39.4
DI Without fins	1.26	1.26	1.2	1.06	0.99	1.01
PWF	25.2	25.2	24	21.2	19.8	20.2
	JS5 BioKyowa			**JS4 Bio Diet**		
Date	05/06/98	10/28/98	05/05/99	04/30/98	10/27/98	05/05/99
Sample size	475	236	117	355	173	169
Weight (g)	99.01	179.04	246.95	95.09	175.1	225.84
length (mm)	209.85	253.72	285.85	209.86	265.64	277.47
Vertebral (%)	0	0	0	1.69	4.62	11.24
Mandibular (%)	1.05	1.27	3.42	5.35	3.47	4.73
Cranial (%)	1.05	0	0	1.41	2.31	1.18
Opercular (%)	78.95	35.17	18.8	49.58	52.02	23.67
Fin (%)	84.42	96.61	91.45	99.72	98.84	98.82
Deformity index	1.65	1.75	1.14	1.67	1.61	1.4
DI as a percent	33	35	22.8	33.4	32.2	28
DI Without fins	0.81	0.36	0.22	0.67	0.62	0.41
PWF	16.2	7.2	4.4	13.4	12.4	8.2

(%): The total number of deformities in the sample divided by the number of fish sampled
Deformity index: Average number of deformities per fish
DI as a percent: percent of possible deformities
DI without fins: Average number of deformities per fish excluding fin deformities
PWF: percent of possible deformities excluding fin deformities

Roger Mellenthin

Effect of Calcium Ion Concentration of Heat Bath on Triploidy Rates

The July issue of the *Ichthyogram* (vol. 10 issue 2) reported on our recent research that involved the combination of heat shocks with various chemical solutions (methylxanthines and MS-222). The average eye-up for this study (with the exception of the controls) was 12% but five of the six treatments exhibited triploidy greater than 83% and up to 100%. These results were encouraging although we were concerned with the low eye-up. As a result a follow-up study was conducted this past November which involved exposing eggs to various ionic concentrations of water in combination with a heat shock.

The idea behind shocking eggs at a giving time post-fertilization is that by subjecting the fertilized egg to a shock, the second meiotic process is inhibited, the second polar body is retained (an extra set of chromosomes), and you end up with a triploid egg. Before eggs are fertilized they undergo two meiotic divisions. Prior to fertilization they are arrested in the metaphase stage of the second meiotic division. Upon fertilization, the sperm then revives the eggs development by initiating a cascade of calcium release from cellular stores. With sperm activation of the egg, meiosis can be completed, the chromosomes decondense to form a haploid nucleus, and the second polar body is expelled. When this process proceeds normally you get a haploid female gamete being joined by a haploid male gamete which results in a diploid zygote.

When the sperm activates the egg as discussed above, the Ca^{++} released diminishes over a short time (10-20 min). But research by others into fertilization and cellular division has indicated that the presence of Ca^{++} at unnatural levels after the cascade, may play a role in the inactivation of chromosome condensation, a step the egg must go through to expel the second polar body. By exposing the eggs to low or no Ca^{++} during the normal heat shock, the goal was to achieve a higher percentage of triploids than produced by the normal heat shock treatment by itself. For this study the fertilized eggs were exposed to heat shock in combination with deionized water or water that contained 1 mg/L Ca^{++} ions.

This past April (1999), eggs and milt were collected from three year old rainbow trout of the Fish Lake DeSmet strain at the Egan Hatchery. Eggs were placed into the following treatments 1) a control: ambient hatchery water (8.2 EC), 2) 7.9EC deionized water, 3) heated deionized water (27.9 EC), and 4) heated hatchery water that contained 1 mg/L Ca^{++}. For each of three replicates, eggs were stripped from five females and milt was collected from four males. A 1% salt solution was used as a fertilization medium and after two minutes the eggs were washed to await the treatments which occurred at 20 minutes post fertilization. At this point the eggs were divided into four roughly equal groups (2,500 eggs each). This procedure was carried out three times to accommodate the four treatments (control included) run in triplicate. All shocks occurred in plastic coolers that were fitted with recirculating heat pumps. Each lot of eggs was placed into a mosquito netting bag which was lowered into a perforated aluminum tray that sat about one cm off the bottom of the cooler. The shock duration was 20 minutes after which the eggs were immediately removed and placed into Heath-type incubators.

Once the eggs had eyed, they were mechanically bumped, sorted, and then transported to the FES and placed into incubators until they hatched. Eye-up, hatch, and crippling

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percentages were all calculated during this process. After the eggs had hatched the fry were placed into small flow-through troughs where they were grown to a size large enough to obtain an adequate blood sample for analysis. The blood samples were processed and ploidy determined by flow cytometry as mentioned in previous articles.

There were significant differences between treatments for all variables measured during this trial. Eye-up was 81% and 85% for the control and deionized groups respectively and 7% and 32% for the DI/heat and 1mg/L Ca^{++}/heat treatments respectively. The percent hatch was also highest for the control and deionized eggs compared to the DI/heat eggs; the Ca^{++}/heat eggs had an intermediate hatch of 57%. The type of treatment also had a significant effect on the percentage of crippled fish produced. The control, deionized water, and DI/heat eggs all had a similar crippling percentage (0.2% " 0.1) compared to 1.5% for the Ca^{++}/heat treatment.

Table 1. Percent eye-up, hatch, crippling and triploid created by exposing rainbow trout eggs to solutions of varying ionic concentrations at 20 minutes post fertilization. A common letter following means within a column indicates no significant difference.
* N = number of samples run to determine ploidy

Treatment	% eye-up	% hatch	% cripple	% 3N	(N)
control	81.3 a	96.4 a	0.12 a	0 a	49
deionized	84.9 a	96.7 a	0.16 ab	0 a	60
DI/heat	7.0 b	19.8 b	0.29 ab	70.7 b	58
1 mg/L Ca^{++}/heat	31.8 b	56.7 ab	1.53 b	98.3 b	60
P =	0.002	0.003	0.036	0.006	

No triploids were produced in the control and deionized water groups, but relatively high numbers were produced in the Ca^{++}/heat and DI/heat (71%) groups. However, this latter number may be artificially high or low depending on your perspective. For one replicate, 2 of 17 fish assayed were triploid (12%). For the second replicate, 20 fish of 20 sampled were triploid (100%); the third replicate also had 100% triploid, but that number was based on a single survivor. The percentage of triploids produced for the 1 mg/L Ca^{++}/heat treatment is more convincing. For each of two replicates, 20 of 20 fish sampled were confirmed triploid, and for the third, 19 of 20 were triploid.

This study did indicate that a combination of heat shock and 1 mg/L calcium in solution was a good way of obtaining a high number of triploid rainbow trout. Although the eye-up and hatch, 32% and 57% respectively, were low for the Ca^{++}/heat treatment, that level of egg survival may be acceptable if it means a consistent source of sterile rainbows. Whether the calcium was the determining element in the treatment, or if the combination of it and heat were, can=t be determined because a straight heat treatment was not run as a comparison as part of the study. Further tests examining the effects of varying levels of hardness are recommended.

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