Ascorbic Acid Diet Test and use of Water Hardening Solution

By Wes Orr and John B. Shrable

Ascorbic Acid Diet Test - 10/89

For many years, post spawning mortality of rainbow trout has resulted in losses of 25 to 90 percent of male fish and from 2 to 25 percent of female fish. The loss is characterized by frayed fins, loss of external mucous as evidenced by feel, and then the appearance of circular rings of fungus which grow to cover the fish in just a few days. As the integrity of the skin is compromised by fungal invasion, internal osmotic balance is impossible and mortality can be severe. Therefore, we wanted to find out whether or not Vitamin C as a feed additive would affect post-spawning mortality. In 1989 a diet test was conducted comparing mortality, growth, and egg production of Erwin broodstock fed 100 ppm (AI) Vitamin C versus 500 ppm (AI) Vitamin C in their diet. The GR-6 formulas were manufactured on 04/19/89 by Murray Elevators. The AaPP (Ascorbic acid polyphosphate) was donated by Rangens.

During April 1989, 5,480 two-year old Erwin Rainbow Trout were equally divided in six raceways. Three raceways were fed feed containing 100 ppm Vitamin C and three were fed the diet with 500 ppm Vitamin C. Prior to spawning, the feeding rate was based on a hatchery constant of 13.0 and a length increase of 0.70"/month. After spawning began the fish were fed a reduced amount by eye but each group received the same amount each day. The test was conducted for 156 days beginning 04/24/89 and ending 09/27/89. The fish were sample counted 04/24/89, 06/26/89 and 09/27/89. Spawning began 06/26/89 and ended 09/15/89. Results of the test are shown below:

	100 PPM Vitamin C	500 PPM Vitamin C
Number Start 04/24	2740	2740
Average Weight lbs. 04/24	3.61	3.53
Average Weight lbs. 06/26	4.12	4.04
Average Weight lbs. 09/27	4.18	4.18
156 Days total mortality	125	128
Average % eyed	76.33	75.75
Average Number eyed eggs/# of female	792	765
% Total Mortality	4.56	4.67

There was no significant difference between the two groups in mortality, growth rate and egg production. External appearance of both groups of fish was good throughout the test and mortality was very low. Most of the mortality was due to handling and anesthetizing during sorting and spawning. Post spawning mortality, particularly among male fish did not occur during this test and was still not evident one month after the end of the spawning season. Average total mortality was only 4.60% compared to 30 and 90 percent in prior years. Average percent eyeup was about 6.0% higher and eyed eggs per female was 10% higher than in prior years. Since there was no control group, it is not possible to determine whether the Vitamin C was responsible for these improved results. However, the stabilized form of Vitamin

C, even at 100 ppm, may be more beneficial than other forms of Vitamin C which break down rapidly in manufacturing, handling and storage.

Ascorbic Acid Diet Test - 10/90

In 1989 a diet test was initiated to determine whether Vitamin C as a feed additive would affect post spawning mortality of Erwin strain rainbow trout. In 1990 a similar test was conducted, but this time two year old Erwin strain rbt were divided into 3 groups. One group received the GR-6 broodstock diet and the other two groups received a GR-6 formulation with 20ppm and 100ppm Vitamin C derived from AaPP(Ascorbic acid Polyphosphate). Test results are shown below.

	Controls	20 PPM	100 PPM
Number Start April 1	1680	830	830
Pounds Each @ Start	3.00	3.30	3.20
Pounds Each @ End	3.70	4.20	3.80
Pounds Feed Fed	4482	2367	2326
Total Mortality (183 days)	100	43	44
Percent Mortality	5.95	5.18	5.30
Percent Eggs Eyed	78.43	81.27	76.49
Eggs/# of Female	990	914	939
Percent of Females Spawned	77.57	80.24	81.70

The test was initiated on April 1, 3 months before spawning, and terminated September 30 at the end of spawning. For the first 3 months all fish were fed by demand feeders . Feed rate was based on a growth rate of 0.7 inches per month and a hatchery constant of 13. During the last three months all fish were fed by eye. About 75% of the mortality in all groups occurred during spawning and was attributed to handling. Only two or three fish in each group ever developed the classic fungusing patterns experienced in prior years. There were no significant differences in fish survival between test groups. Addition of vitamin C to the diet did not appear to affect female maturation, but males in the 100 ppm vitamin C group matured slower than males in the other two groups. After the 6th week of spawning only 50% of males in the 100 ppm group were flowing milt, as compared to 80% in the other 2 groups. This may have influenced the eyeup percentage in the 100 ppm group.

Effects of Vitamin C on Egg Fertilization- 8/89

At Ennis, rainbow trout eggs are routinely fertilized in a 0.75% saline solution. Based on information received at the 1988 Northwest Fish Culture Conference, we wanted to test the effects of adding Vitamin C to the fertilization medium. On August 30, 1989, the eggs from 15 7-WvEw females were pooled and sperm was pooled from 10 7-WvEw males. The eggs were divided into ten pans containing the solutions to be tested in duplicate. Each pan of eggs were fertilized immediately, water hardened, and incubated until they were eyed. AaPP (Ascorbic acid polyphosphate 10.5% AI)was used for this test. Results are shown below:

Egg Fertilization Medium	% Eyed Group A	% Eyed Group B	% Eyed Average
0.75% Saline	68.0	72.3	70.1
0.75% Saline + 25 ppm AI Vitamin C	73.9	72.5	73.1
0.75% Saline + 50 ppm AI Vitamin C	70.8	69.2	70.0

0.75% Saline + 75 ppm AI Vitamin C	73.4	72.0	72.7
0.75% Saline +100 ppm AI Vitamin C	75.5	71.6	73.5

Conclusion: The results are unclear. The variation between groups A and B is more than the variation between levels of Vitamin C. The addition of 25 to 100 ppm Vitamin C did not adversely effect egg survival.

Water Hardening Eggs in a Solution with Vitamin C 9/89

At Ennis, rainbow trout eggs are routinely water hardened in a solution containing 75 ppm AI of Iodophore. Based on information received at the 1988 Northwest Fish Culture Conference, we wanted to test the effects of adding Vitamin C to the water hardening solution.

On September 13, 1989, a group of 26 females were spawned into a 0.75% saline solution and the eggs were fertilized with pooled milt from a similar number of males. After rinsing, approximately one-half of the eggs were poured into a bucket containing 75 ppm Betadine and the other half was poured into a bucket containing 75 ppm Betadine plus 50 ppm Vitamin C. The eggs were hardened for one hour.

On the same day, another group of 47 females were spawned and fertilized in the same manner. One half of these eggs were water hardened for one hour in 75 ppm Betadine and the other half were water hardened in 75 ppm Betadine plus 100 ppm Vitamin C.

The test was repeated September 20, 1989, when 37 Erwin X Arlee females were spawned and fertilized in the same manner. They were divided into three groups and water hardened in 75 ppm Betadine, 75 ppm Betadine with 50 ppm Vitamin C, and 75 ppm Betadine with 100 ppm Vitamin C for one hour. Results are shown below:

Date	Water Hardening Medium	% Eyed
09/13/89	Betadine Solution	74.91
	Betadine Solution plus 50 ppm (AI) Vitamin C	76.93
09/13/89	Betadine Solution	87.63
	Betadine Solution plus 100 ppm (AI) Vitamin C	85.67
09/20/89	Betadine Solution	81.85
	Betadine Solution plus 50 ppm (AI) Vitamin C	82.06
	Betadine Solution plus 100 ppm (AI) Vitamin C	82.52

Conclusion: Addition of 50-100 ppm Vit.C did not affect egg survival.

Saline vs. Water as Fertilization Medium: 5 groups totaling 1,400,000 eggs. One half fertilized with water and one half fertilized with saline. Average with water = 73% eyeup. Average with saline = 84% eyeup.

12/2/89 Testing 10 Fertilization Mediums

Dr. Halver in Washington showed that fertilizing salmonid eggs in water yielded an 85% eyeup, but adding 50 ppm ascorbic acid polyphosphate increased eyeup to 96 percent, 500 ppm yielded a 90 percent eyeup, and 1000 ppm dropped eyeup percentage to 43.

To test the effect of Vitamin C on fertilization of rainbow trout eggs at Ennis, we designed a comparison test of 10 spawning mediums. Green

eggs from sixteen two year old Arlee strain rainbow trout were pooled and mixed. One cup of eggs was measured into each of 30 buckets containing the 10 solutions in triplicate. The eggs in each bucket were fertilized immediately using pooled sperm from 12 two year old Arlee males. The eggs were rinsed, water hardened for 30 minutes in water, then placed into five Heath incubator trays subdivided into six compartments per tray. The location of each sample in the tray was determined by random drawing. The eggs were incubated for 15 days, shocked, picked, and the percent eyeup calculated. Results using 3 replicates are shown below:

	A	B	C	Aug.
Water	73.91	72.50	54.02	66.81
0.75% Saline Solution	67.39	74.71	68.37	70.15
50 PPM Vitamin C/Water	67.57	66.67	75.95	70.06
100 PPM Vitamin C/Water	60.97	59.75	66.67	62.46
250 PPM Vitamin C/Water	53.66	76.47	46.51	58.88
500 PPM Vitamin C/Water	40.22	62.02	39.77	47.33
50 PPM Vitamin C/0.75% Saline	53.85	53.33	50.00	52.39
100 PPM Vitamin C/0.75% Saline	72.83	41.17	79.01	64.34
250 PPM Vitamin C/0.75% Saline	71.43	77.27	8.09	58.93
500 PPM Vitamin C/0.75% Saline	12.08	2.00	2.00	5.36

Survival of Eggs Water Hardened in Different Solutions 12/14/90

In Washington, Vitamin C had profound affects on survival of eggs fertilized in a solution with Vitamin C. Water yielded an 85% eyeup, but adding 50 ppm ascorbic acid polyphosphate increased eyeup to 96 percent, 500 ppm yielded a 90 percent eyeup, and 1000 ppm dropped eyeup percentage to 43. To test the effect of Vitamin C during water hardening, we designed a test comparing four different water hardening solutions in triplicate. Eggs from eight two year old Arlee strain rainbow trout were pooled together and fertilized in a 0.75% saline solution with pooled sperm from six two year old Arlee males. After three minutes, one cup of eggs was placed into each of the four solutions being tested, in triplicate. After water hardening, the eggs were placed into 2 Heath trays subdivided into six compartments each. Location of egg batches in each tray was determined by random drawing. After 15 days the eggs from each compartment were shocked, picked, and the percent of survival calculated. Results using 3 replicates are shown below:

	A	B	C	Aug
Water	76.72	76.73	77.61	77.02
50 PPM Vitamin C/Water	77.50	75.47	75.98	76.32
250 PPM Vitamin C/Water	75.62	76.44	78.05	76.70
500 PPM Vitamin C/Water	75.00	75.61	75.76	75.45

Survival of eggs water hardened in Iodophores

There is evidence that rainbow trout eggs are sensitive to Iodophores during the water hardening process. We decided to compare the percent survival of eggs water hardened in 75 PPM Betadine versus plain water.

Eggs from ten two year old Arlee females were pooled and fertilized with pooled sperm from eight two year old Arlee males. After fertilization, one-half of the eggs were placed in water and the other half was placed in a 75 PPM Betadine solution. After water hardening, the eggs were placed in two Heath trays for incubating. After 15 days, the eggs were shocked, picked, and the percent survival calculated. The percent survival for eggs water hardened in water was 80.05 and for eggs water hardened in Betadine was 79.04.

Time Test-Saline medium. 02/24/88. Precollected sperm from 40 males in 4 containers and pooled into 1 container immediately before using. Spawned 24 females and pooled the eggs. Set up 12 buckets with 0.75% saline. Added 1 cup of eggs to each bucket with saline solution. Added sperm to first two buckets immediately and then at intervals of 3, 6, 9, 12, and 15 minutes after adding eggs to the saline solution.

Results

Time in Minutes	% Eyeup A	B	Average % Eyeup	% Change From 0
0	50.5	47.8	49.15	0
3	54.5	55.1	54.80	+11.50
6	59.9	49.9	54.90	+11.70
9	44.4	50.8	47.60	-3.15
12	50.5	55.2	52.85	+7.53
15	53.5	55.0	54.25	+10.38

Conclusion: Eggs are viable in 0.75% saline solution for at least 6 minutes and maybe 15 minutes

Time Test in Duplicate

How long can unfertilized eggs be held in saline before losing the ability to be fertilized? Unfertilized eggs were held in 0.75% saline solution for 1 to 10 minutes before fertilization.

Results: Time	Average eyeup
0 minutes	58 percent
1	67
2	70
3	65
4	61
5	65
6	69
7	61
10	38

Time Test with 0.75% saline and water: 04/22/87

20 Females 20 Females

Time(minutes	% eyeup in Saline	and Water
1	61	71
2	75	75
3	66	18
4	23	27
5	74	6
6	70	2
7	56	0
8	44	0
9	44	0
10	22	10
12	76	31
15	20	2

Saline Versus Water As Fertilization Medium

Using the last spawn of the year, 60 females in 6 fish pools. Pooled eggs split and 1/2 fertilized with water the other half with saline. Results:Saline - 53.1% Water-37.8% eyeup Kamloop strain: 0.75% saline=73.5%. Water=27.6% eyeup

Fertilizing With Diluent (from France)

Erwin Strain RBT Diluent % eyeup = 78% (229,000 eggs)
Water = 79% (221,000 eggs)
Erwin Strain RBT Diluent % eyeup = 63% (125,000 eggs)
Water = 40% (86,000 eggs)

Saline Time Test.

02/24/88 using 6mcd males and females Eggs held in 0.75% Saline Precollected sperm from 40 males in 4 containers and pooled into 1 container immediately before using. Spawned 24 females and pooled the eggs. Set up 12 buckets with 0.75% saline. Added 1 cup of eggs to each bucket with saline solution. Added sperm to first two buckets immediately and then at intervals of 3, 6, 9, 12, and 15 minutes after adding eggs to the saline solution.

Results

Time in Minutes	% Eyeup A	% Eyeup B	Average % Eyeup	% Change From 0
0	50.5	47.8	49.15	0
3	54.5	55.1	54.80	+11.50
6	59.9	49.9	54.90	+11.70
9	44.4	50.8	47.60	-3.15
12	50.5	55.2	52.85	+7.53
15	53.5	55.0	54.25	+10.38

Conclusion: Eggs are viable in 0.75% saline solution for at least 6 minutes and maybe 15 minutes

Saline water versus plain water

Females spawned in 5 fish pools and eggs split approximately in half

Date	Water	Saline
08/13	eggs - 212,718	232,046
	eyeup-74.1	85
08/22	eggs - 334,683	321,480
	eyeup-74.5	82
09/05	eggs - 110,340	105,450
	eyeup-80.5	87.6
09/12	eggs- 55,802	71,812
	eyeup-62.6	85.6
09/19	eggs - 36,315	36,073
	eyeup-73.2	78.5
Average eyeup =	72.98	83.74

Difference=10.76%

Spawning - Wet versus Dry 03/29/84

Spawned into 1% saline solution in ml					Spawned into dry pan in ml				
	Good	Bad	Total	% eyeup		Good	Bad	Total	% eyeup
1	210	25	235	89	1	0	100	100	0
2	25	185	210	12	2	0	380	380	0
3	290	65	355	82	3	12	40	52	23
4	230	105	335	69	4	120	180	300	40
5	230	160	390	59	5	40	175	215	19
6	220	70	290	76	6	30	135	165	18
Avg	1205	610	1815	66.4	Avg	202	1010	1212	16.7
Eliminate Female No 2					Eliminate Female No. 1 & 2				
Avg	1180		1605	73.5	Avg	202	530	732	27.6

Therefore, the difference between spawning wet in a 1% saline solution (66.4%) and spawning dry into a pan (27.6%) was 46%!

Purpose: How long eggs can be held in 0.75% saline before fertilizing.

Test Date: 03/16/88 using 5-FCan males and females
Spawned 27 5-FCan females and pooled the 80,000 total eggs.
Precollected sperm from 30 5-FCan males and pooled sperm.
Set up 14 green buckets with 0.75% saline solution. Eggs held in the solution for 0, 3, 6, 9, 12, 15, and 18 minutes before sperm was added.

Average %		% Eyeup		
Change from 0	Minutes	A	B	Average
0	0	54.32	51.43	52.88
+0.68	3	52.90	53.57	53.24

-8.11	6	46.84	50.33	48.59
-6.11	9	47.98	51.32	49.65
-1.72	12	52.78	51.15	51.97
-12.67	15	51.93	40.43	46.18
-12.14	18	47.73	45.18	46.46

Conclusion: 15 minutes in saline does not affect fertilization rate.

Purpose: To determine how long sperm may be held in 0.75% saline before attempting fertilization without reducing eyeup.
Test Date: 03/16/88 Spawned 27 5-FCan females and pooled 80,000 eggs. Precollected and pooled sperm from 30 5-FCan males. Set up 16 green buckets with 0.75% saline solution. Added sperm to the saline solution. Added one cup of eggs to each bucket at time intervals in seconds as follows: 0,15,30,45,60,75,90,120 (two buckets each time).

Ave. % Change from 0	Seconds	A %eyeup	B	Average % eyeup
0	0	49.38	53.33	51.36
+6.81	15	55.17	54.55	54.36
-74.06	30	21.05	5.59	13.32
-50.80	45	36.67	13.87	25.27
-55.96	60	5.71	39.52	22.62
-76.97	75	21.52	2.13	11.83
-42.70	90	42.19	16.67	29.43
-97.61	120	1.72	0.74	1.23

Conclusion: Get highest % of fertilization before sperm motility ceases. It appears that sperm can fertilize eggs after motility ceases.

Effects of Vitamin C on Egg Fertilization 08/08/89

At Ennis, rainbow trout eggs are routinely fertilized in a 0.75 percent saline solution. Based on information received at the 1988 Northwest Fish Culture Conference, we wanted to test the effects of adding Vitamin C to the fertilization medium. On August 30, 1989, the eggs from 15 7-WvEw females were pooled and sperm was pooled from 10 7-WvEw males. The eggs were divided into ten pans containing the solutions to be tested in duplicate. Each pan of eggs was fertilized immediately, water hardened, and incubated until they were eyed.
Rangens donated AaPP (Ascorbic acid polyphosphate 10.5% AI) for this test. Results are shown below:

Egg Fertilization Medium	% Eyed Group A	% Eyed Group B	% Eyed Average
0.75% saline	68.0	72.3	70.1
0.75% saline + 25 ppm AI Vitamin C	73.9	72.5	73.1
0.75% saline + 50 ppm AI Vitamin C	70.8	69.2	70.0
0.75% saline + 75 ppm AI Vitamin C	73.4	72.0	72.7
0.75% saline +100 ppm AI Vitamin C	75.5	71.6	73.5

The results are unclear. The variation between Group A and B is more than the variation between levels of Vitamin C.

09/89 Water Hardening Eggs in a Solution with Vitamin C

At Ennis, rainbow trout eggs are routinely water hardened in a solution containing 75 ppm AI of an Iodophore. Based on information received at the

1988 Northwest Fish Culture Conference, we wanted to test the effects of adding Vitamin C to the water hardening solution. On September 13, 1989, a group of 26 females were spawned into a 0.75% saline solution and the eggs were fertilized with pooled milt from a similar number of males. After rinsing, approximately one-half of the eggs were poured into a bucket containing 75 ppm Betadine and the other half was poured into a bucket containing 75 ppm Betadine plus 50 ppm Vitamin C. The eggs were water hardened for one hour. On the same day, another group of 47 females were spawned and fertilized in the same manner. One half of these eggs were water hardened for one hour in 75 ppm Betadine and the other half was water hardened in 75 ppm Betadine plus 100 ppm Vitamin C. The test was repeated September 20, 1989, when 37 Erwin X Arlee females were spawned and fertilized in the same manner. They were divided into three groups and water hardened in 75 ppm Betadine, 75 ppm Betadine with 50 ppm Vitamin C, and 75 ppm Betadine with 100 ppm Vitamin C for one hour. Results are shown below.

Water Hardening Medium	% Eyed
Betadine Solution	74.91
Betadine Solution plus 50 ppm (AI) Vitamin C	76.93
Betadine Solution	87.63
Betadine Solution plus 100 ppm (AI) Vitamin C	85.67
Betadine Solution	81.85
Betadine Solution plus 50 ppm (AI) Vitamin C	82.06
Betadine Solution plus 100 ppm (AI) Vitamin C	82.52

Disinfection of eggs with betadine

Standard treatment before shipping is 100ppm for 10 minutes. Eggs were treated with 20,25,30,40,50,60,75,100,150 and 200ppm betadine for 1 minute and 10 minutes. Eggs are treated in the egg trays and are drained but not rinsed. At levels from 20ppm to 150ppm there was no mortality after 48 hours in the shipping case. At 200ppm there was egg mortality after 24 hours in the shipping box. TSA slants were inoculated from all treatments and bacteria was grown from all treatments, yes, even the 200ppm treatment for 1 minute, and the 100ppm treatments for 10 minutes!!

Eggs were disinfected in 100ppm betadine for 1 minute, drained and packed in shipping boxes. Ice used to cool and moisten the eggs was frozen with various concentrations of betadine in it. Results showed that ice with 60ppm betadine frozen in it caused 10% mortality (in the center of the tray). Mortality increased as the concentration in the ice increased. TSA slants of this egg fluid also yielded bacterial growth.

Moral of the story: EGGS SURFACE DISINFECTED WITH RECOMMENDED LEVELS OF BETADINE CANNOT BE CONSIDERED BACTERIA FREE.....THE NUMBERS OF BACTERIA WERE REDUCED FROM MILLIONS TO TENS OF THOUSANDS. IS THAT GOOD ENOUGH??????????????????????????

Effect of siphoning eggs within 2 hours of fertilization.
On 1/20/88, 1/3 of a jar of eggs was siphoned into a bucket and then poured into another incubation jar about 1 1/2 to 2 hours after fertilization. The first jar had been filled about 1 hour after fertilization. Several eggs turned white shortly after siphoning.

	Siphoned	Not Siphoned
Liters Good	5.85	15.2
Liters Bad	1.80	3.0
Total	7.65	18.4
% Eyeup	76.47	83.7

Results: 7.23% more loss due to siphon 2 hours after fertilization.

Rinsed Eggs Versus Unrinsed Eggs - RBT
broken Eggs: rinsed = 46.9% eyeup unrinsed = 31.1% eyeup
Bloody Eggs: rinsed = 53.9% eyeup unrinsed = 51.0% eyeup
Control = 194 females = 67% average eyeup

How often do we need to sort and spawn at 54 degrees F.?

Days Fish Were Held Before Spawning	No. Fish	% Eyeup
2	34	79
5	42	80
7	29	72

Water Hardening Eggs
Water Hardened 1 hour = eyeup 77%
one half hour = eyeup 78%
Water Hardened in saline = 70%
in water = 70%

Effect of Shipping Green Eggs.
On 1/27/88 the following were shipped to Garrison:
2.5 liters of green eggs were packed in two gallons of water in a plastic bag with ice around it. They were shipped Federal Express and were opened 24 hours later. The temperature was 33 degrees F. All the eggs were dead.
2.5 liters of green eggs were packed in a regular egg box with towels and 2 ice trays. They were shipped Federal Express and were opened 24 hours later. The temperature was 36 degrees F. All the eggs were dead.

The following eggs were shipped to Leadville:
5 liters of green eggs were packed in 2 trays with 2 trays of ice. They were shipped by Federal Express and were opened 24 hours later. The temperature was 41 degrees F. and all the eggs were dead. Green eggs shipped with dry method do not survive in transit commercially, however, they have been shipped successfully by car when care is taken not to shock them.

Effect of transporting eggs in water on the road versus not moving them from the hatchery within 24 hours after fertilization.
On 2/3/88 2 boxes were packed with eggs in water in plastic bags inside shipping cases. One was transported to Ennis and back and one kept at the hatchery. They were opened at 11:00 a.m. on 02/04/88. The temperature of both boxes was 42 degrees F. By visual exam it appeared that twice as many white eggs were visible in the transported box versus the one not moved.

Effect of Handling Eggs at 24 and 48 Hours After Fertilization.
On 2/3/88 110 6-LMNE females were spawned with usual procedures filling two 5 gallon buckets. The buckets were brought into the hatchery and a hose placed in the bottom of each bucket running about 1 gpm. One bucket was poured into the hatching jar after 24 hours and the other after 48 hours. No eggs turned white due to handling in either case. % eyeup = 62%

Effect of Transporting Fertilized Eggs in Regular Shipping Cases Within 24 Hours of Fertilization.
On 2/3/88 48 5-LMNE females were spawned with usual procedures at 2:00 p.m. The eggs were hardened 1 to 1 1/2 hours then two boxes were packed in the usual manner of shipping eyed eggs (one tray of eggs per box - 4 dippers). One box was kept at the hatchery and one box was transported to Wes's house and back to the hatchery the next morning. Both boxes were opened at 11:00 a.m. on 02/04/88.
Results: Box #1 transported in usual manner:
Temperature = 42F, Many of the eggs appeared to have broken yolk membranes. Eggs tempered then poured in hatching jar
Box #2 not transported:
Temperature 38F no apparent mortality when tempered in water
Eggs tempered then poured in hatching jars

Sperm motility in water versus saline.

On 3/15/88 Sperm was collected from 5-FCan males and pooled, then examined under a microscope at various time intervals. Motility was timed until all motion ceased.

Solution	Results: Seconds Motility Lasts	Average
Water	18, 22, 21, 20, 24, 25	21.67
2.0% saline	No motion (motion when water was added)	0
1.75% saline	No motion (motion when water was added)	0
1.50% saline	No motion (motion when water was added)	0
1.25% saline	17, 22, 27, 23, 15, 22	21.00
1.00% saline	19, 22, 19, 23	20.75
0.75% saline	34, 28, 24, 24, 28, 25	27.17
0.50% saline	24, 26, 26, 26, 30	26.4

Conclusion: 0.75% saline is optimum for sperm motility. At Ennis sperm is not activated in saline solutions of 1.50% or stronger.

Comparing Sperm motility in water vs saline vs ovarian fluid.

On 2/9/88 sperm was collected from 6-LMNE RBT, pooled, and placed under the microscope. Motility was timed after solution was added to the slide. Motility was timed until motion was drastically reduced.

Seconds until Motion Almost Ceases	Average
Water - 12, 15, 12, 13, 15, 12, 13	13.14
0.50% saline- 18, 22, 20, 28, 24, 22, 22	22.29
0.60% saline- 22, 20, 17, 18	19.25
0.70% saline- 18, 20, 20, 20, 18	19.20
0.80% saline- 18, 18	18.00
0.90% saline- 16, 22, 18	18.70
1.00% saline- 21, 20, 20, 18, 21, 13, 17, 17	18.40
Ovarian Fluid - 13, 18, 18, 17, 18, 23, 18, 18	17.88

Conclusion: Sperm motility time is longer in saline and ovarian fluid compared to water.

Compare water and saline as a fertilization medium on RBT eggs.

On 2/11/88 spawned 62 6-LMNE females and pooled the eggs. Precollected sperm from 30 6-LMNE males in 3 freezer containers then pooled into one container immediately before fertilizing the eggs. One cup of eggs was placed in each of 18 buckets with the various solutions being tested in duplicate. Pooled sperm was added immediately

% Eyeup Average	% Change A	% Change B	% Eyeup A and B	% Eyeup from water
Water	60.87	68.97	65.38	0
0.50% Saline	83.33	80.52	81.67	+24.92
0.75% Saline	87.10	83.33	85.25	+30.39
1.00% Saline	80.65	82.14	81.36	+24.44
1.25% Saline	76.67	82.78	79.73	+21.95
1.50% Saline	79.41	83.33	81.43	+24.55
1.75% Saline	72.73	74.29	73.53	+12.47
2.00% Saline	72.73	80.00	76.19	+16.53
2.50% Saline	34.60	40.00	37.50	-42.64

Conclusion: 0.5 to 1.0% saline performed best.

Length of time sperm is viable in water. Pool eggs from 62 6 females and sperm from 30 males. Fertilize pooled eggs at different time intervals

Minutes	% Eyed	% change from 1 minute
1	68.90	0
3	65.87	-4.40
5	44.44	-35.43
7	41.74	-39.42

Compare eyeup of RBT eggs held in 54 degrees water for 0,5,10, and 15 minutes before fertilization.

Spawned 27 5-FCan females on 3/16/88 and pooled the 80,000 total eggs. Precollected sperm from 30 5-FCan males and pooled sperm.
Set up 8 green buckets with water. Added 1 cup of eggs to each bucket. Added sperm at intervals of 0, 5, 10, and 15 minutes.

Average % Change from 0	Minutes	% Eyeup A	% Eyeup B	Average
0	0	45.66	42.86	44.26
-34.84	5	29.41	28.27	28.84
-71.69	10	13.41	11.64	12.53
-97.02	15	1.05	1.59	1.32

Conclusion: Eggs in water for 5 minutes or longer before fertilization exhibited reduced survival.

Test for value of stripping females a second time.

11/84 Arlee Strain 07/85 Erwin Strain

There is value in restripping spawned out females a second time to improve egg quality the next year by eliminating abnormal egg development, bloody eggs, and egg shells. In the past we have restripped all fish after spawning season is over, and found many of the fish to be too hard to strip without damage to the fish. It appears that by restripping 5 or 6 days after initial spawn, most of the eggs can be removed easily and rapidly. Are these eggs worth saving or is it a waste of time? 11/06/84 restripped 135 females spawned 6 days earlier. 11/13 restripped 174 females, and on 11/20 restripped 178 females. On 07/85 145 females were restripped after 6 days. Results follow:

No. spawned	Total Eggs	Eyed Eggs	% Eyeup	No.Eggs/Female	No.Eyed Eggs/Female
135	40,541	25,027	61.7	300	185
174	46,100	11,593	25.2	265	67
178	68,000	17,850	26.2	382	100
145	56,275	32,949	58.5	388	227-Erwin

Conclusion: A significant number of eggs remain in the fish after air spawning. In many cases at least these eggs are not free from the ovarian tissue at the initial spawning (determined by dissection).
After 5 or 6 days, the eggs can be easily hand stripped and are usually accompanied by large volumes of ovarian fluid (or water?).
The eggs are of poor quality (over-ripe or water hardened), take up incubator space, and expend formalin and the manpower to pick the eggs. In the strains tested at least, we feel the eggs received are not worth the effort expended. However, it has helped us focus on the fact that we need to somehow do a

better job of stripping the fish even with air spawning.

LHRHa Test 08/18/87
Used: LHRHa 20 micrograms/kgbw RBT

07/20/87 170 ripe females
07/27/87 230 ripe females
08/03/87 230 ripe females
08/10/87 235 ripe females
08/17/87 212 ripe females
08/18/87 465 females injected with 10 micrograms/kgbw
08/25/87 106 ripe females (21%)
09/01/87 84 ripe females (18%)
09/03/87 314 females injected a second time with 20/kgbw
09/09/87 130 ripe females (41%)
09/16/87 37 ripe females (12%)
Injected fish appeared to be more susceptible to fungus

LHRHa Test 08/08/88
Used: 10 micrograms/kg body weight RBT
5 mg/100 ml of saline (.6ml/3kg)
07/20/88 A&B 48 spawned
07/27/88 A&B 83 spawned
08/03/88 A&B 108 spawned
08/10/88 A&B 123 spawned
08/08/88 RW-A 675 injected
RW-B 307 controls
08/17/88 RW-A 282 spawned (42%)
RW-B 49 spawned (16%)
08/24/88 RW-A 18 spawned (05%)
RW-B 46 spawned (46%)
08/31/88 A&B combined 57 spawned
09/07/88 58 spawned
09/14/88 29 spawned
Injected fish appeared to be more susceptible to fungus

LHRHa Test on Two-Year Old Erwin Brood (7-WVEW)
07/10/89 - 225 females from the 100 ppm vitamin C group and 225 females from the 500 ppm vitamin C group were injected with 5 micrograms of LHRHa/pound of fish to induce maturation. 50 controls were separated from each group. Because of space restrictions, all fish were put back into their respective groups on 07/24.

	100 PPM Group		500 PPM Group	
Date	Injected	Controls	Injected	Controls
07/19 No. Ripe	112	13	101	10
07/19 % Ripe	49.8	26.0	44.9	20.0
07/24 No. Ripe	22	8	7	9
07/24 % Ripe	9.8	16.0	3.1	18.0
Total % ripe after 14 Days	59.6	42.0	48.0	38.0

Percent eyeup for the week before injection averaged 79.3. Eyeup for the eggs from the 07/19 egg take averaged 72.7.

Restripping: Fish should be restripped to remove eggs that were not removed during the first spawning. Broodstock held over to spawn the next year without restripping may exhibit abnormal egg development, bloody eggs, eggs shells, plugs, deteriorated ovaries and even mortality. Even after air spawning an average of 250 eggs remain in our larger fish. These eggs can be saved and fertilized or discarded, but they should probably be removed! Test this theory.

Spawn 205 pairs 1 on 1 and hold separate to eyeup (Arlee strain RBT)

No. Fish	% Eyeup
57	98-100
64	94-97
33	90-93
17	85-89
7	80-84
11	70-79
3	60-69
1	50-59
0	20-49
12	less than 20

Summary: 83% over 85% eyeup; 6% with less than 20% eyeup; 59% over 94% eyeup. Obviously a few fish with less than 20% can reduce average eyeup. Problem is not a few mediocre fish but a few fish with little or no success.

Feeding Rate Test With Arlee Strain RBT 1982

Different feed rates:	RW 21-22	RW 23-24
No Fish	1,600	1,600
Conversion	1.76	1.76
Hatchery Constant	13.8	11.0
Actual growth/month	.82"	.66"
% eyeup	85	89
Egg Size / l	16,782	16,795
% blanks	4.4	3.0
Eyed Eggs/Female Spawned	2,784	2,699
Feed Cost/1000 Eyed Eggs (cents)	.555	.476

Difference is 7.9 cents / 1000 eyed eggs or 79 dollars / million eyed eggs.

Metal Colander versus net colander for dry method of spawning

Metal = 77.9% eyeup
Net = 82.4% eyeup
Diff. = 4.5%

Motility: 3 of 4 males with watery sperm exhibited no motility when water was added to the sperm.