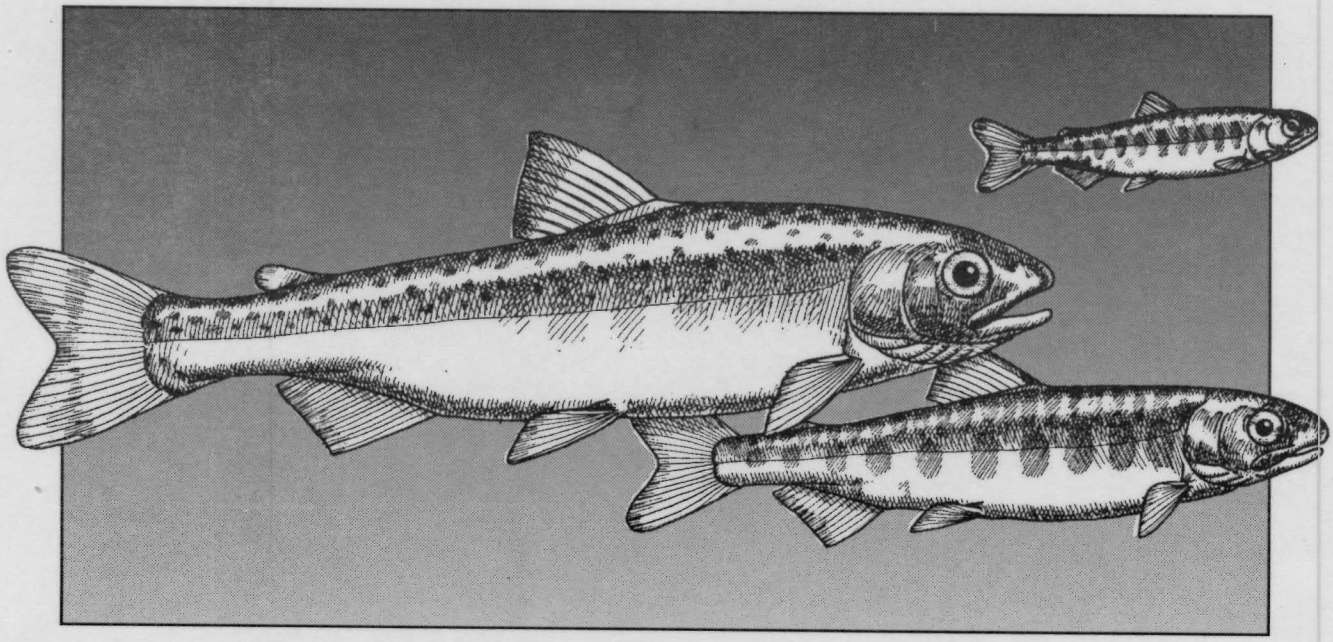
44th Annual Northwest FISH CULTURE CONFERENCE

Proceedings



December 7 - 9, 1993

Spokane, Washington

PROCEEDINGS

OF THE

FORTY-FOURTH ANNUAL

NORTHWEST FISH CULTURE CONFERENCE

SPOKANE, WASHINGTON
DECEMBER 7-9, 1993

Henry E. Forner, Jr.
Fisheries and Federal Aid
U.S. Fish and Wildlife Service

THE NORTHWEST FISH CULTURE CONFERENCE

The Northwest Fish culture Conference is an annual informal meeting by and between fish culturists for the exchange of information and ideas about all aspects of fish culture. These conferences are hosted on a rotating basis by the various fisheries agencies and entities of the Northwest. At the conferences, progress reports of management practices and problems, new developments, and research studies are presented. Both within the meeting and outside the formal meeting setting, active discussion, constructive criticism, and personal contacts are not only encouraged but actively cultivated. All persons interested in or associated with fish husbandry are invited to attend and to actively participate. The subject matter is limited to topics that have direct application to fish culture.

This **PROCEEDINGS** contain abstracts and or talks presented at the conference. They are unedited, contain progress reports of uncompleted programs, and, as such, **SHOULD NOT BE CONSIDERED A FORMAL, PEER-REVIEWED PUBLICATION.**

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Ed Crateau, U.S. Fish and Wildlife Service

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Bryan Kenworthy, U.S. Fish and Wildlife Service

FISH CULTURE AT THE CROSS-ROADS

Bill Shake, U.S. Fish and Wildlife Service
Conference Keynote Speaker

On behalf of the U.S. Fish and Wildlife Service, and Marv Plenert our Regional Director, I would like to welcome you to the 44th annual Northwest Fish Culture Conference. I know Ed Forner and his conference committee have been working hard to make this a productive and enjoyable meeting. For 44 years the Northwest Fish Culture Conference has provided the opportunity for fishery managers to discuss valuable ideas and information. I am personally a strong supporter of this conference.

I believe that it may be more important today to share information and improve communication. Unless you have spent the last couple of years on Mars, you know that northwest fisheries management, including fish culture, is at the crossroads. Warning flags are flying: stocks of salmon are listed in the Sacramento and Snake Rivers; the National Marine Fisheries Service has petitions for many more stocks of salmon and steelhead; the U.S. Fish and Wildlife Service has been petitioned to list bull trout, and the American Fisheries Society has identified 214 stocks at risk.

Why the increase in concern for these stocks? Their numbers are dangerously low.

Why are the numbers low? Most people put the blame on the 4 H's: Hydropower and irrigation systems; Habitat; Harvest; and, yes, Hatcheries.

How can hatcheries, which have been constructed to mitigate for fish and fish habitat loss, and to rebuild and enhance fisheries, be a part of the problem? There are a number of reasons. Abundant hatchery fish may have resulted in over-harvest of naturally spawning stocks. Abundant hatchery fish in streams may out-compete less aggressive naturally spawned fish, or may cause a "pied piper" effect on natural fish when they smolt, pulling them downstream. Hatchery fish stocked in streams, or that stray into streams, may not be of the same or closely related genetic stock, which can reduce the genetic variability of natural stocks. Hatchery fish may spread diseases to wild stocks.

Truth or Fiction? Probably both, but I do not believe that hatcheries are the primary reason for declines in northwest salmon and steelhead stocks. However, if hatchery programs are to continue, they must be part of the solution to the problem. I believe the next five years will be some of the most challenging we have faced. We are cosponsoring a comprehensive environmental review of the Columbia River hatchery system. To my knowledge, this has never been done on a comprehensive basis. Most assessments have been done on an individual hatchery basis.

The purpose of this review is to evaluate the total hatchery program, determine impacts on wild stocks, and make recommendations on future program direction. This is not a review to justify status quo. Our management paradigms are changing and our hatchery program must change with them. The Endangered Species Act will change the way we manage our hatcheries. We currently do Section 7 consultations on hatchery operations in the Snake and Columbia basins. We are required by law to demonstrate that our operations will not jeopardize the continued existence of listed Snake River stocks. Some of the changes that have occurred are: changes in release times to minimize interactions with wild stocks; and a reduction, and in some cases, elimination of using non-hatchery origin broodstock to maximize their opportunity to spawn naturally. Hatchery stocks may be a part of listed populations and used to supplement and reseed habitat. Hatcheries may hold and rear three adult broodstocks to provide an emergency effort to save the gene pool and the stock.

Hatcheries have been criticized because of the different standards and policies used to provide direction. In the Columbia Basin we have an Integrated Hatchery Operations Team which is charged with setting performance standards, and reaching agreements on fish health policies, genetics policies, ecological interactions and regional coordination of production.

As I stated earlier, I am not convinced that hatcheries are a major part of the problem for declines in fish stocks. I am convinced that hatcheries are a valuable tool for fisheries management in the 21st century; but we must be willing to change with the times. I hope you enjoy the conference, and thank you for the opportunity to talk with you.

Effects of Single Male Matings and Multiple Male Matings on Egg Survival in Erwin Strain Rainbow Trout

December 1993

Wes Orr, Bernie Shrable, Dan Brown
Ennis National Fish Hatchery, Montana

Introduction:

At Ennis NFH, MT paired matings (one female x one male) of rainbow trout to create future broodstocks have historically exhibited an 8 to 10 percent reduction in survival to the eyed stage as compared to multiple male matings. The purpose of this investigation was to determine what causes this problem.

Methods:

Milt was collected from 20 Erwin strain males into 20 individual test tubes. One ml of milt was removed from each tube and pooled into a separate container. To ensure viability any milt that was thick or watery or abnormal in appearance was discarded. The eggs from 20 Erwin strain females were air spawned into individual pans containing equal volumes of 0.75% saline solution. Only eggs that appeared viable were used in the test. The eggs from each container were equally divided into 2 containers, making a total of 40 containers, 2 from each female.

One container of eggs from each female was fertilized with 1 ml of precollected milt from 1 male. The other container of eggs from each female was fertilized with 1 ml of milt from the container of pooled milt representing 20 males.

After fertilization, eggs from each of the 40 containers were rinsed, and then water hardened in a 75 mg/l iodophor solution for 30 minutes. After water hardening, one-half of the eggs from each container were individually incubated in 1 liter upwelling jars. The remaining eggs in the 20 - 1 female x 1 male containers were pooled, mixed and then split into 4 replicates and incubated in 1 liter upwelling jars. Eggs remaining in each of the 20 - 1 female x 20 male matings were also pooled and divided into 4 - 1 liter upwelling incubators. All incubators were treated daily with 1200 mg/L of formalin for 15 minutes to prevent fungus. All eggs were mechanically shocked on day 14.

Each batch of eggs was picked mechanically, and an electronic egg picker was used to count normal eyed eggs, white eggs, dim eyed eggs, and blank eggs. A dim eyed egg is defined as an eyed egg which has very small eyes and dim eye pigmentation. A blank egg is defined as any post shock translucent egg with no visible cellular development.

Results:

In this test it appears that reduced eyeup can be attributed to a few individuals in the population. We can also surmise that even experienced spawn takers cannot always tell whether gametes are viable or not.

Statistically the study was broken into 2 parts; The first dealing with 20 single male treatments and 20 pooled male treatments, and the second part dealing with 4 replicates of single male and 4 replicates of pooled male treatments. In the first part there was a slight effect of female and male on percent eyeup, probably due to the wide variation between treatments. Also, there was a strong effect of female but no effect of male on blank eggs, and no effect of male or female on dim eyed eggs. In the second part of the study the effect of female was designed out and statistics confirmed there was a strong effect of male on the percent survival to the eyed stage.

Discussion:

If spawntakers could ascertain 100% of the time which eggs or sperm were not viable, the risk of losing genetic material would be greatly reduced. The problem of an occasional bad male can be overcome by using 2 males to fertilize one females eggs. If there are not enough males in the population, pool 2 males to fertilize the eggs of 2 females. Similarly, mating 2 females with 2 males insures that the gametes from 2 males is not lost because of 1 bad female. If future broodstock are derived from a parent population of several hundred fish, losing genetic material from a few individuals may not be important. However, when dealing with small populations, which is usually the case with threatened or endangered species, the loss of genetic material from a few individuals becomes paramount.

TABLE I

POOLED MILT
VS
SINGLE MALE MATINGS

Female No.	Percent Eyeup Single Male	Percent Eyeup Pooled Males	Number Dim Eyed Single Male	Number Dim Eyed Pooled Males	Number Blank Single Male	Number Blank Pooled Males
1	5.4	5.4	1	12	819	811
2	95.4	97.6	7	1	0	2
3	96.4	97.2	2	1	2	1
4	97.3	96.3	3	0	0	4
5	78.5	78.1	0	5	1	15
6	98.6	97.4	1	0	1	1
7	93.1	96.9	6	1	40	3
8	85.8	80.9	1	2	7	10
9	94.8	92.9	1	1	15	11
10	96.9	51.6	0	0	6	3
11	95.7	95.5	0	0	5	2
12	94.8	93.9	1	3	2	5
13	45.9	43.2	0	0	230	50
14	89.8	93.1	3	2	39	26
15	99.0	98.0	0	1	1	7
16	78.1	80.1	5	2	11	18
17	11.1	96.6	0	1	1	1
18	1.2	69.9	0	0	0	80
19	90.5	92.6	7	26	8	2
20	70.4	76.2	0	6	215	202
AVERAGE	75.9	81.7				

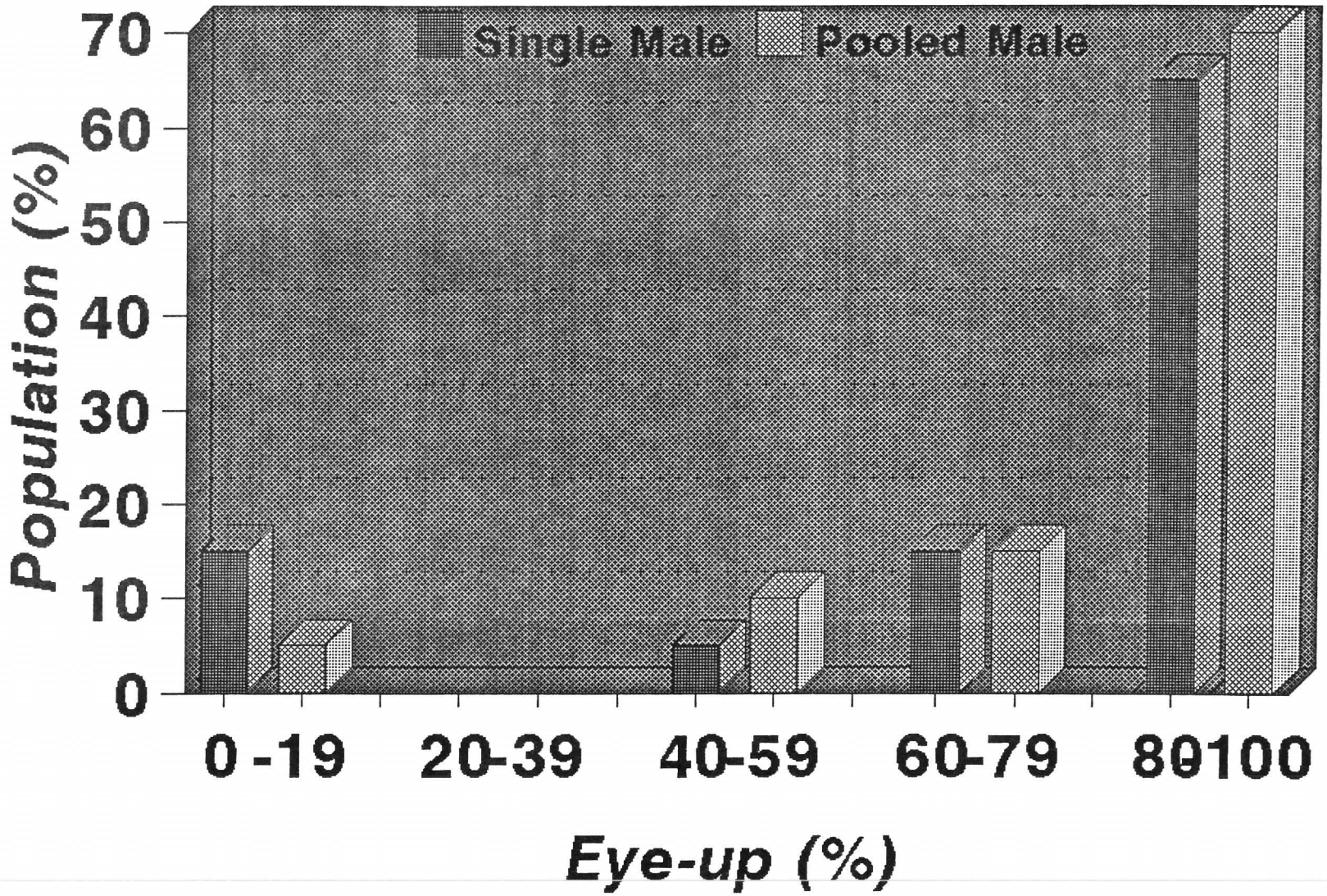
TABLE 2

POOLED MILT
VS
SINGLE MALE MATINGS

SINGLE MALE MATINGS	PERCENT EYEUP	PERCENT DIM EYED	PERCENT BLANK
R1	67.8	2	7
R2	75.7	3	10
R3	74.6	2	12
R4	72.9	3	12
AVERAGE	72.8		

POOLED MALE MATINGS	PERCENT EYEUP	PERCENT DIM EYED	PERCENT BLANK
R1-P	83.2	2	7
R2-P	81.7	3	8
R3-P	82.9	1	7
R4-P	80.2	2	8
AVERAGE	82.0		

Survival from Pooled and Single Male Matings



THE EFFECT OF DIET ON DORSAL FIN EROSION IN STEELHEAD TROUT.

Bill Lellis

U.S. Fish & Wildlife Service
Hagerman Field Station
3059-F NFH Road
Hagerman, ID 83332

Rick Barrows

U.S. Fish & Wildlife Service
Bozeman Fish Technology Center
4050 Bridger Canyon Road
Bozeman, MT 59715

Erosion of dorsal fins is a common problem among hatchery-reared steelhead trout *(Oncorhynchus mykiss)*. Numerous factors have been correlated with the severity of deterioration, including diet, stocking density, water temperature and quality, feeding rate and method, sunlight, culture conditions, and environmental contaminants. Whether these factors are the direct cause of fin erosion, or whether they antagonize a pre-existing condition is unknown.

Diets commonly fed to steelhead trout typically contain fish meal as the principle source of animal protein. Commercial fish meals are generally manufactured from mature fish, and may thus contain significant amounts of vertebrate steroid hormones. This is contrary to the wild situation, in which juvenile steelhead would feed primarily on various invertebrates during their freshwater phase of life. The objective of this study was to determine if diet type (vertebrate vs. invertebrate) is correlated with the severity of dorsal fin erosion in steelhead, and if so, if an exogenous steroid hormone (testosterone) is somehow responsible.

Twelve 35 l tanks supplied with 6 l/min 15°C water were each stocked with 250 steelhead fry. Triplicate tanks of fish were fed one of four diets from first-feeding, including: (1) a fish meal-based commercial trout diet (FISH MEAL 0); (2) the fish meal diet with 30 ppm added testosterone (FISH MEAL +); (3) a crustacean meal-based experimental diet (KRILL MEAL 0), and; (4) the krill meal diet with 30 ppm added testosterone (KRILL MEAL +). Fish were fed at a rate to maintain equal growth among all treatments, consistent with growth of fish from the same lot cultured at the Hagerman National Fish Hatchery. Fish were transferred to larger tanks (560 l) at week 18 of the trial when mean density index reached 0.4.

Percent dorsal fin index (DFI, measured as: mean dorsal fin height * 100/total fish length) was greater ($P<0.01$) among fish fed the krill meal diet than for fish fed the fish meal diet at both 12 and 20 weeks of the experiment (Fig. 1). Added testosterone decreased DFI among fish fed the krill meal diet, but not among fish fed the fish meal diet. Addition of testosterone to either diet decreased ($P<0.01$) critical thermal maxima (CTM), which is a measure of resistance to an environmental temperature stress (Fig. 2). As of week 20, average fish weight was 25 g among all treatments. This study will continue until fish reach a stocking size of 100 g (4.5/lb).

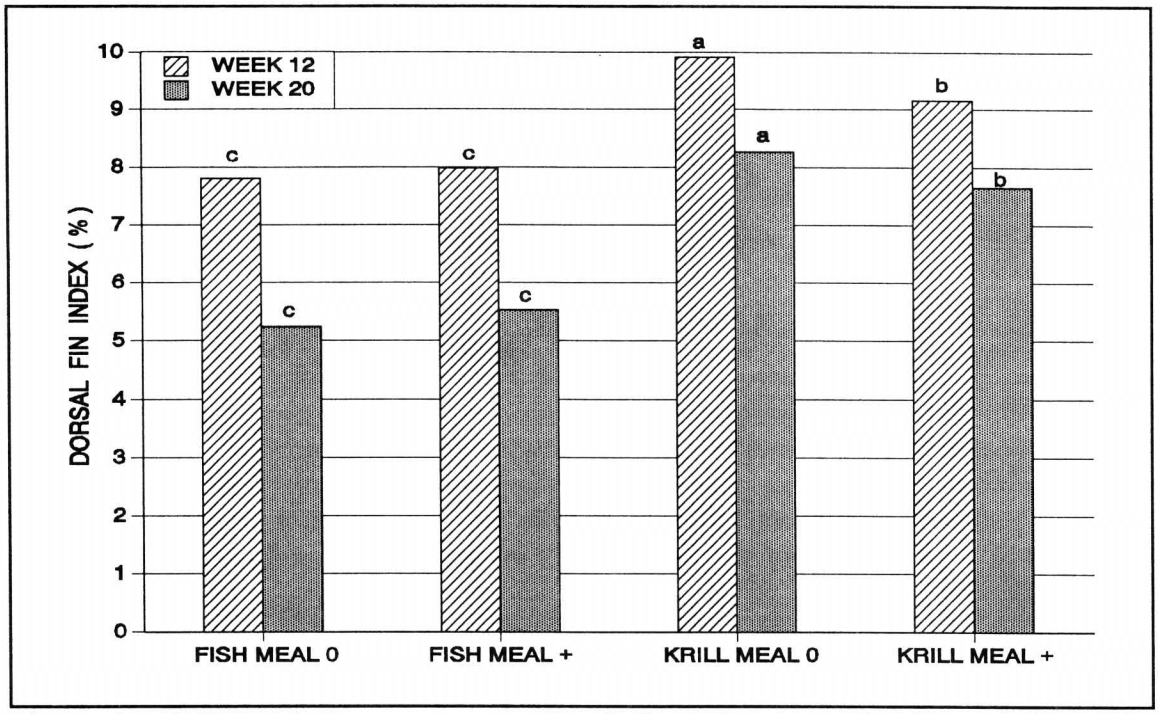


Figure 1. Effect of diet on percent dorsal fin index at weeks 12 and 20. Diet designation 0/+ indicate absence/presence of added testosterone. Superscripts on bars indicate differences ($P < 0.01$) between diets within each sampling period.

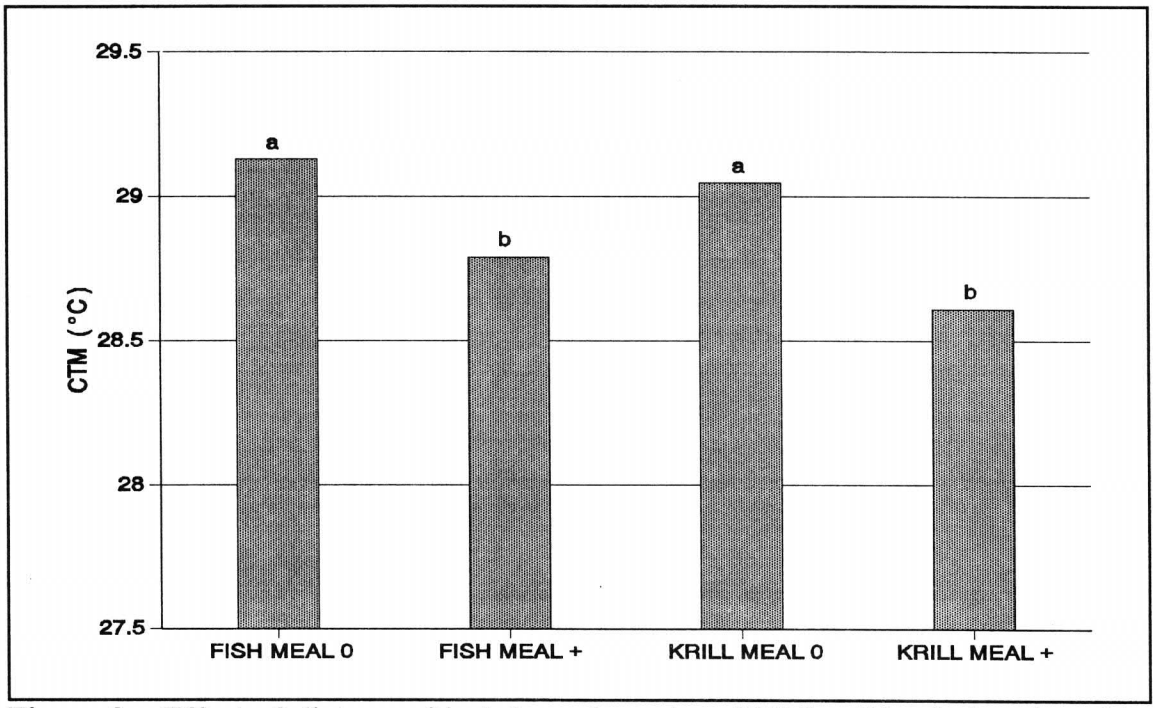


Figure 2. Effect of diet on critical thermal maxima (CTM). Diet designations 0/+ indicate absence/presence of added testosterone. Superscripts on bars indicate differences ($P < 0.01$) between diets.

A COMPARISON OF TRIPLOID AND DIPLOID RAINBOW TROUT

Mike Fallon
Fort Richardson Fish Hatchery
P.O. Box 5267
Fort Richardson, Alaska 99505

INTRODUCTION:

Due to recent concern over preservation of "wild rainbow stocks" our facility began producing and evaluating genetically manipulated sterile rainbow trout or "triploids". Subsequent projects included production of all-female rainbows as well as all-female triploids. Their survival, growth, and performance compared to the normal diploid-mixed sex rainbows was closely monitored for three years.

MATERIALS & METHODS:

Rainbow trout used in this comparison were originally from the Swanson River stock which is located on the Kenai Peninsula in southcentral Alaska. The comparison of growth data between normal and genetically manipulated rainbow trout occurred at Fort Richardson Fish Hatchery which operates under the State of Alaska Department of Fish & Game, Sport Fish Division. It is located about 7 miles from downtown Anchorage, Alaska. The hatchery is one of the oldest in the state and was built in 1957 in cooperation with the U.S. Army. Its location is adjacent to the military power plant. The facility utilizes a heat exchanger to extract heat produced from the power plant providing rearing for trout and salmon at temperaturesof 9 to 15 degrees celsius. In addition a variable 5 hp. pump is located in line with the heat exchanger and is operated via a computer to maintain temperature thus minimizing power and cost. The hatchery operates year round on 2500 gpm of well water. The hatchery program includes 2 million rainbow fingerling released @ 1 gram; 250,000 rainbow catchables @ 100g.; 300,000 coho smolt @ 20g.; 400,000 King salmon smolt @ 15g.; and 160,000 catchable Kings released at 100g. into land-locked lakes for local ice fishing. The rainbow catchable program is achieved using 10 3'x 30' concrete indoor raceways and 5 8'x 80' concrete outdoor raceways. The indoor raceways are arranged in a single pass system utilizing oxygen contactors. The entire rainbow catchable program begins with the indoor raceways and upon reaching the maximum loading capacity of (1 lb/cubic foot/inch) the raceways are split and or transferred to the outdoor raceways. Heath trays are used for incubation and are arranged in half stacks. About 25% of the catchable program is retained indoors while the balance is distributed to the outdoor raceways. To simulate daylight the indoor raceways use

fluorescent lighting operated via a lutron timer with a duration of up to an 18 hour day. Feeding is accomplished using North Star Loudon II feeders set on a timer. The outdoor raceways are hand fed and supplemented with Babington demand feeders. The outdoor raceways are arranged in a 4 pass system and also use oxygen contactors. For the three years of growth comparisons, the Rainbow trout were fed exclusively BioProducts BioDiet and BioDry. All groups were fed daily rations based on computer projected growth rates. Periodic sampling was done to monitor feed schedules to minimize waste and improve conversions.
The procedure for producing triploids is critical and involves placing eggs 20 minutes after fertilization into a bath at 26 degrees celsius for 20 minutes. This heat shocking procedure was developed from previous trials in which varying the time and or temperature during the immersion greatly affects the survival rate (Olito 90). Establishing the all-female rainbow population involves irradiating sperm with ultraviolet light and crossing with normal diploid females. Though motility is not affected, the genetic material of the sperm however is destroyed causing the fertilized egg to possess all female inheritance. At emergence a small population of these gynogenetic female fish are fed testosterone for 500 TU's after hatching. The hormone treatment results in a future brood population which is referred to as "double X" males. This procedure ensures the second generation of all-females (Olito 91).

RESULTS & DISCUSSION:
The first year class of genetically manipulated rainbows were the 1990 rainbow triploids. Only a relatively small lot of 40,000 (2 trays) were developed, hatched, and reared along side the normal diploid mix sex rainbow catchables. The fertilization rates (Figure 1) for all year classes, for that matter, were virtually the same with the exception of the 1990 diploid mixed sex. The low percentage was due to the standard operating procedures using only water for sperm activation during spawning. When saline was used as the diluent a dramatic increase in fertilization rates was observed (Olito 88). Survival to eyed (Fig. 2) was also indicative of the positive use of saline. Ponding to fingerling survival (Figure 3) did reveal an unexplained reduction in both year classes of 91 and 92 all-female triploids. Prior to completion of the fingerling program the catchables are selected. Diploids and triploids were inventoried and transferred into the section of the indoor raceways designated for the catchable program. Initially, each of the raceways was loaded at about 40,000 (.5 lb/cf/inch). Comparisons of growth and conversions began at this time (Figure 4). Growth proceeded evenly with only a difference toward the later time of rearing just prior to release. Despite anticipation the triploids generally performed and looked the same as their normal chromosome rivals. At first, a wide range of sizes seemed to have occurred amongst the triploids. However, upon individual sampling no significant difference existed. The triploids did display a sensitivity to changes in temperature. Occasionally, power plant effluent temperatures result in a 3 to

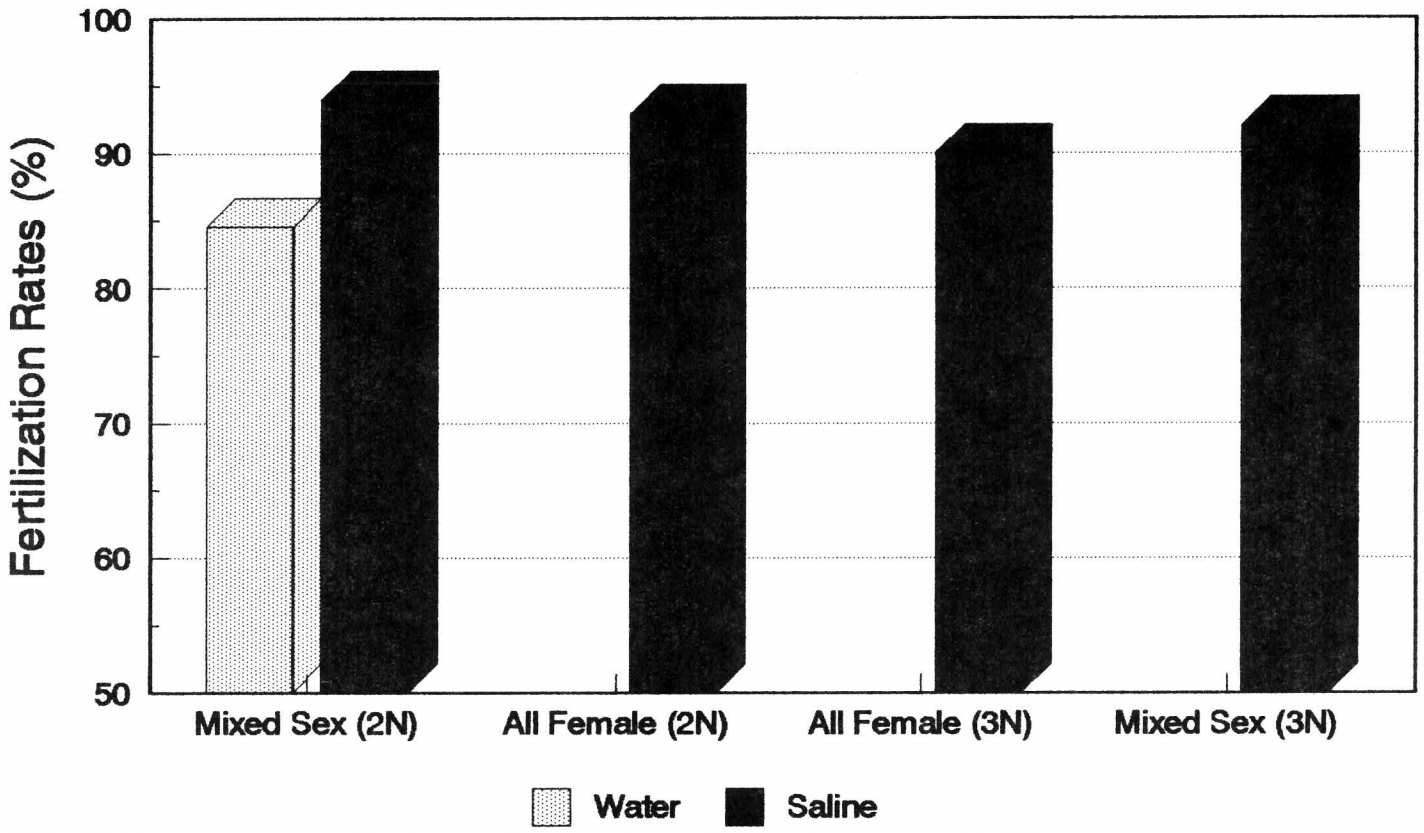


Figure 1. Fertilization rates of mixed-sex and all-female populations, diploid and triploid fish, averaged over three years.

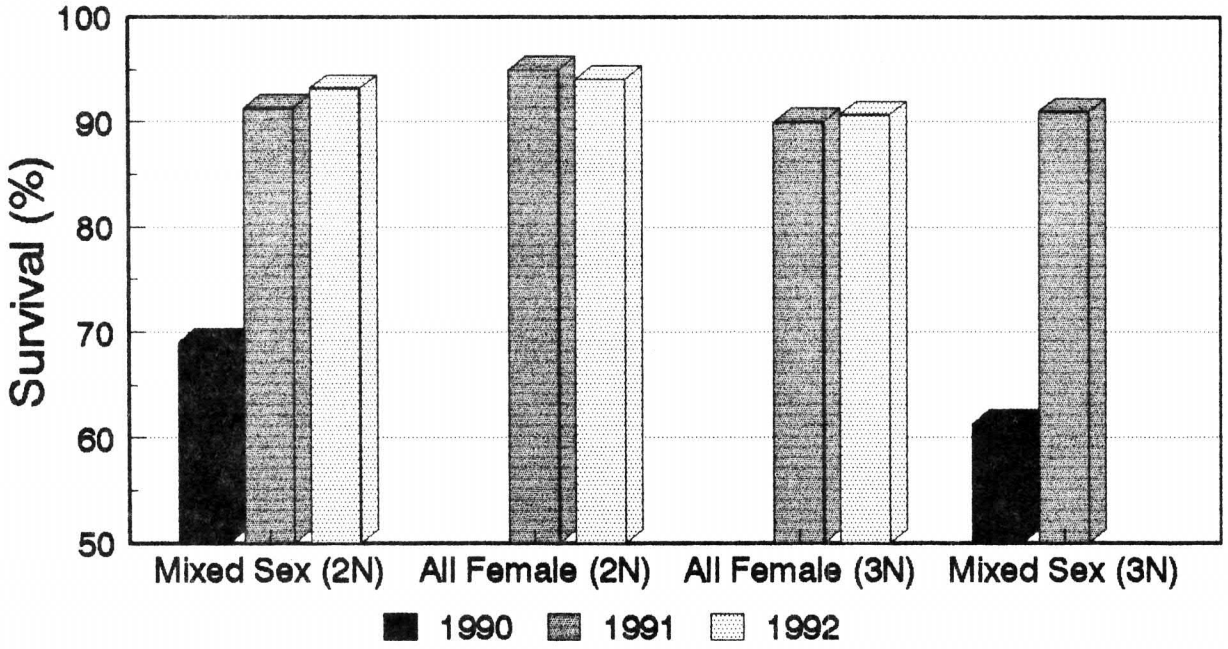


Figure 2. Survival from green egg to eyed egg stage.

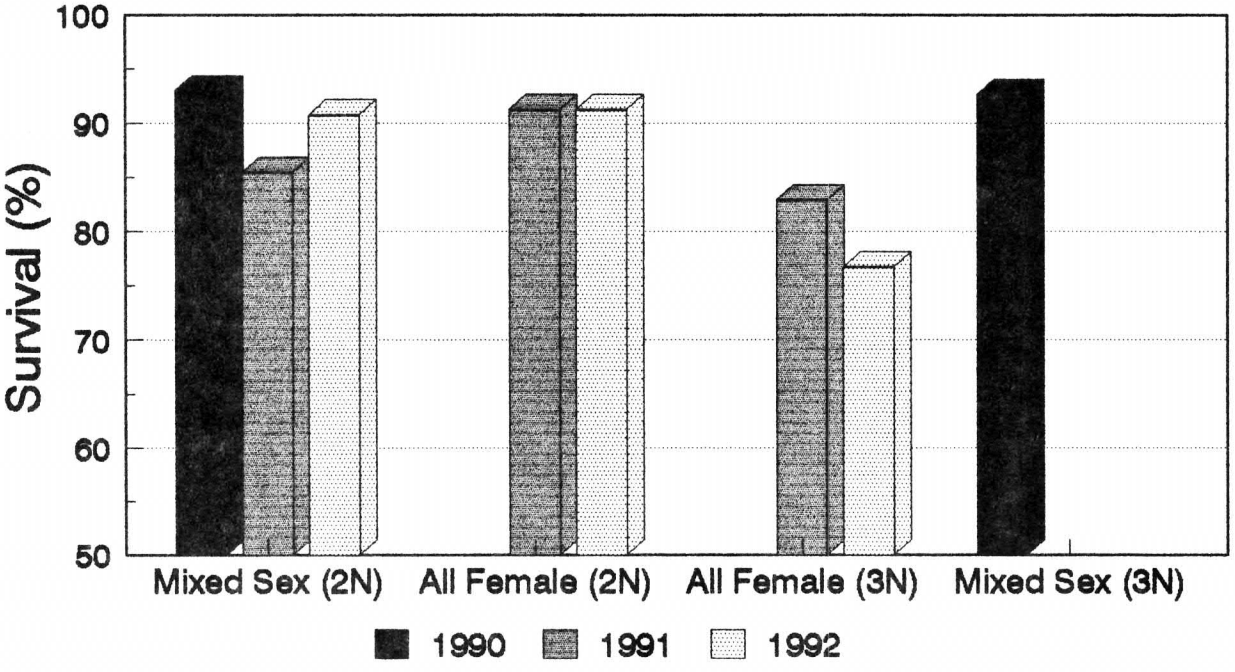


Figure 3. Survival from ponding to fingerling. Fingerling are 1g.

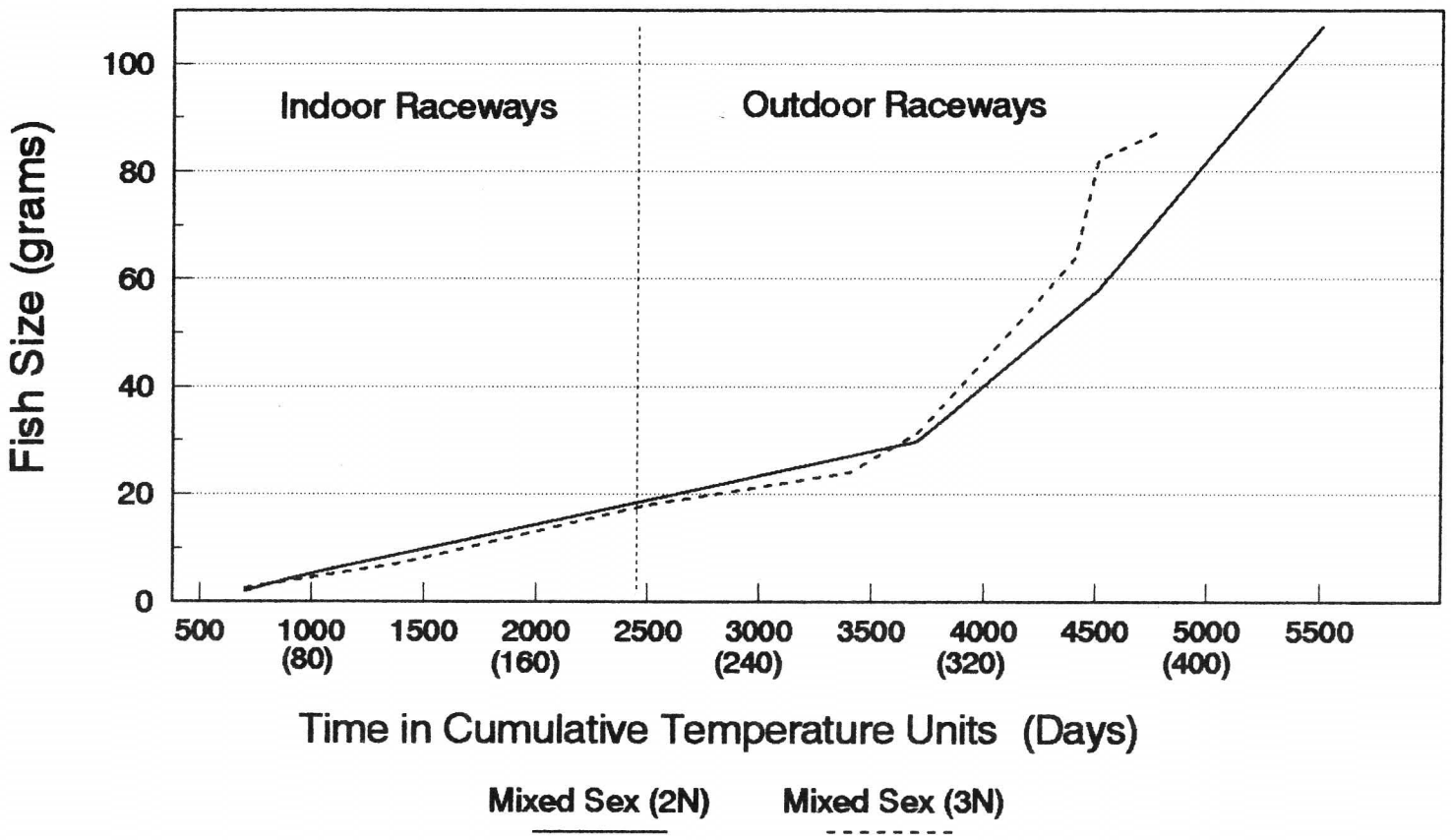


Figure 4. Rainbow trout diploid and triploid growth in 1990.

4 degree C decline on weekends. Though expected feeding activity and requirements reduce, the triploids appeared to take a longer time to return to their previous activity (Unterburg 90). There also appeared to be a difference in the "Snap factor". The triploids were less aggressive to the feed presented than the normal diploids so vigorously demonstrate. Though the triploid population represented only 20% of the catchable program the compensation growth during the last few weeks of rearing was notable. Daily growth of both diploid and triploid was in excess of 1g/day, 30 days prior to release.

The second year of rearing (1991) brought yet another oddity for comparison. The all-female (AF-2N) and all-female triploids (AFT-3N) were produced and included in the catchable program. Of the 234,000 catchables 16% were AFT-3N, 44% AF-2N and 40% diploid mixed sex (MS-2N). Comparisons in rearing (Figures 5 & 6) show a relatively close association in growth between the normal MS-2N diploids and the AF-2N population. Growth in the AFT-3N, however, was minimal and resulted primarily from their experience with indoor rearing. Both the AF-2N and AFT-3N demonstrated what appears to be a sensitiviy to the shorter photo period experienced in Dec.- Feb. resulting in a lag period of growth. Despite a low conversion (Figure 9) the AFT-3N growth resulted in requiring 30 more days to reach the release goal size.

The third and final year again involved the normal diploid mixed sex, all-female , and all-female triploids. The rearing logistics for this year, however, located all the normal diploids (20% of the total catchable program) to one outdoor raceway and the AF-2N (38%) and AF-3N (42%) were distributed relatively equally to the indoor and remaining 4 outdoor raceways. A lag period in growth occurred again (Figure 8) when the portion of AF-2N and AFT-3N populations were moved to the outside raceways. However, growth compensation was observed again, enabling the production goal size to be reached only 10 days later than the MS-2N groups. An important aspect in this comparison was the feed conversions. As previously mentioned, the indoor raceways (Figure 9) are on the timed loudon II feeders and tend to show a higher conversion with a range between groups of 1.3 - 1.7. The low value for 91AFT-3N, however, was due to its short rearing time indoors before transfer to outdoor raceways. The outdoor conversions (Fig.10), which involve the Babington demand feeders, did indicate a lower range of conversions. Considering the sensitivity to temperature and possibly photo period, the resulting off feed duration explains in part the relatively high conversion of 92AFT-3n group.

Some other interesting observations of triploids have recently been reported after release from the hatchery. In comparing survival and growth of one gram diploids and triploids in several lakes, the diploids survived twice as much as the triploids to age 1 (Brock,Hanson,Havens & McBride 94). In addition, the previously noted sensitivity to temperature was observed during release into another unrelated lake system. Several thousand

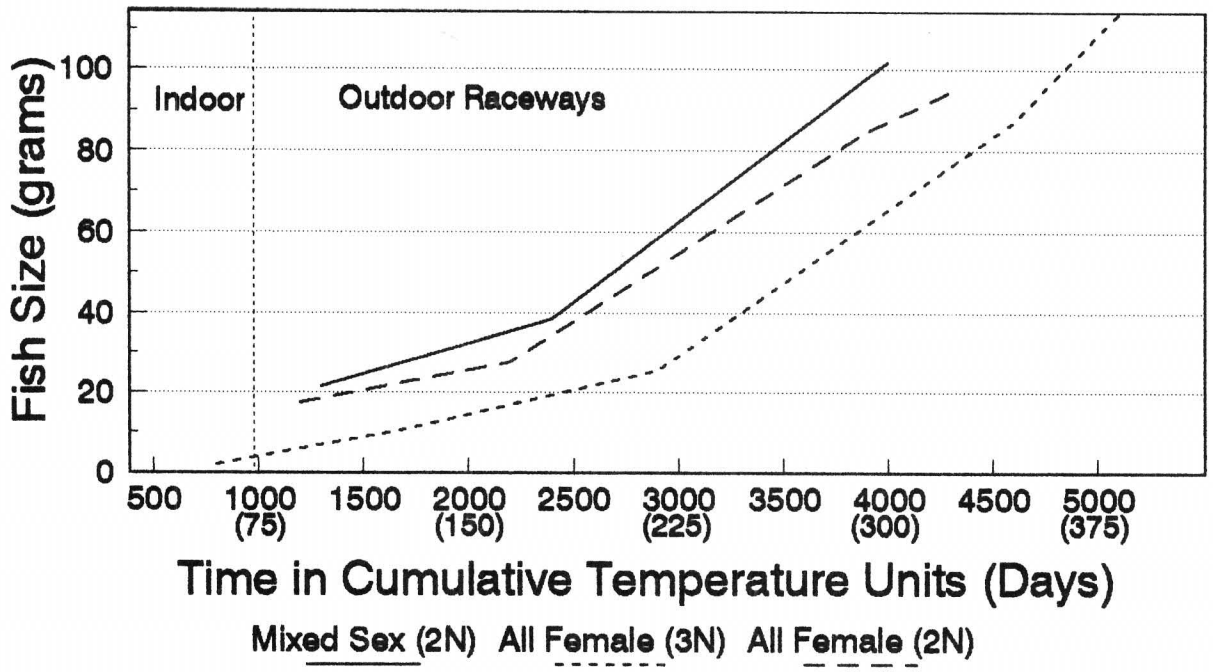


Figure 5. Rainbow trout all-female and mixed-sex growth in 1991.

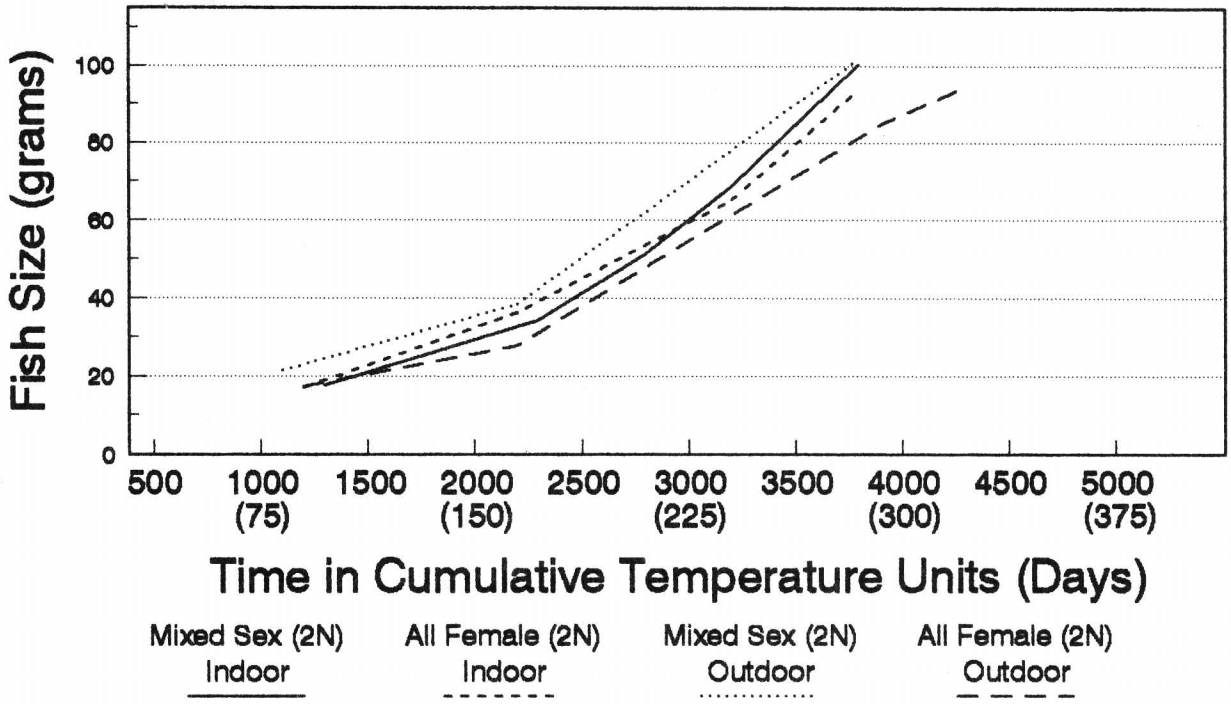


Figure 6. Rainbow trout growth in 1991, indoor and outdoor rearing.

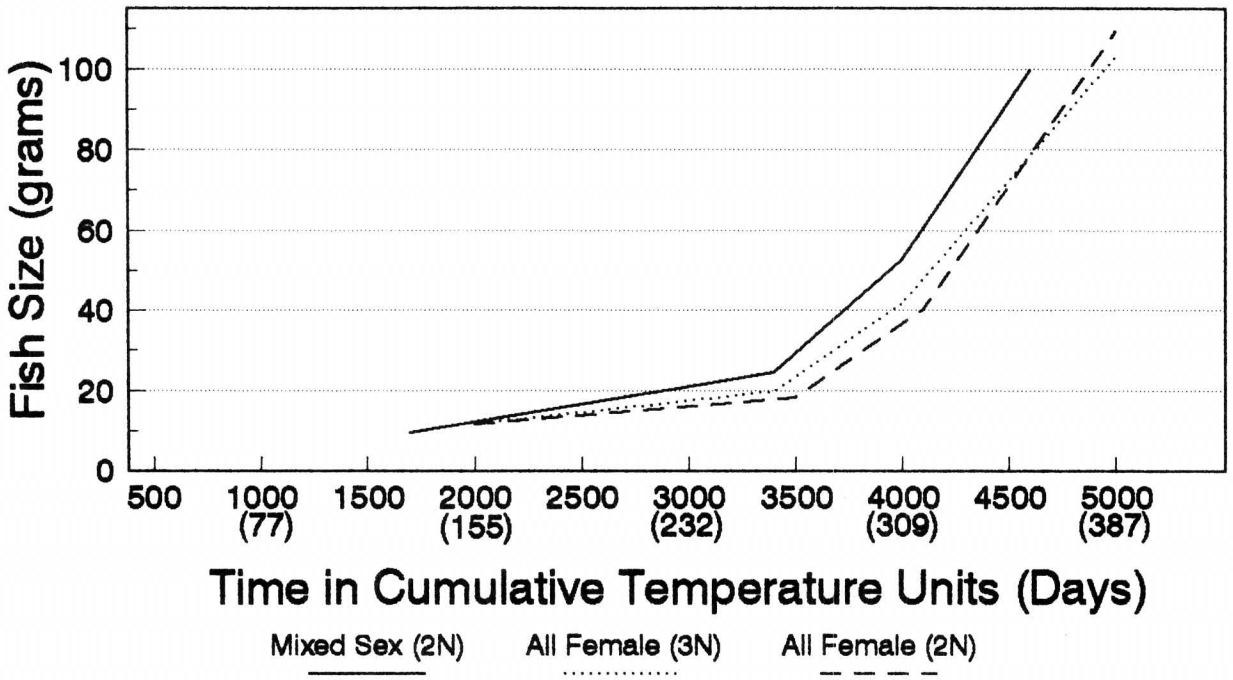


Figure 7. Rainbow trout mixed-sex and all-female growth in 1992.

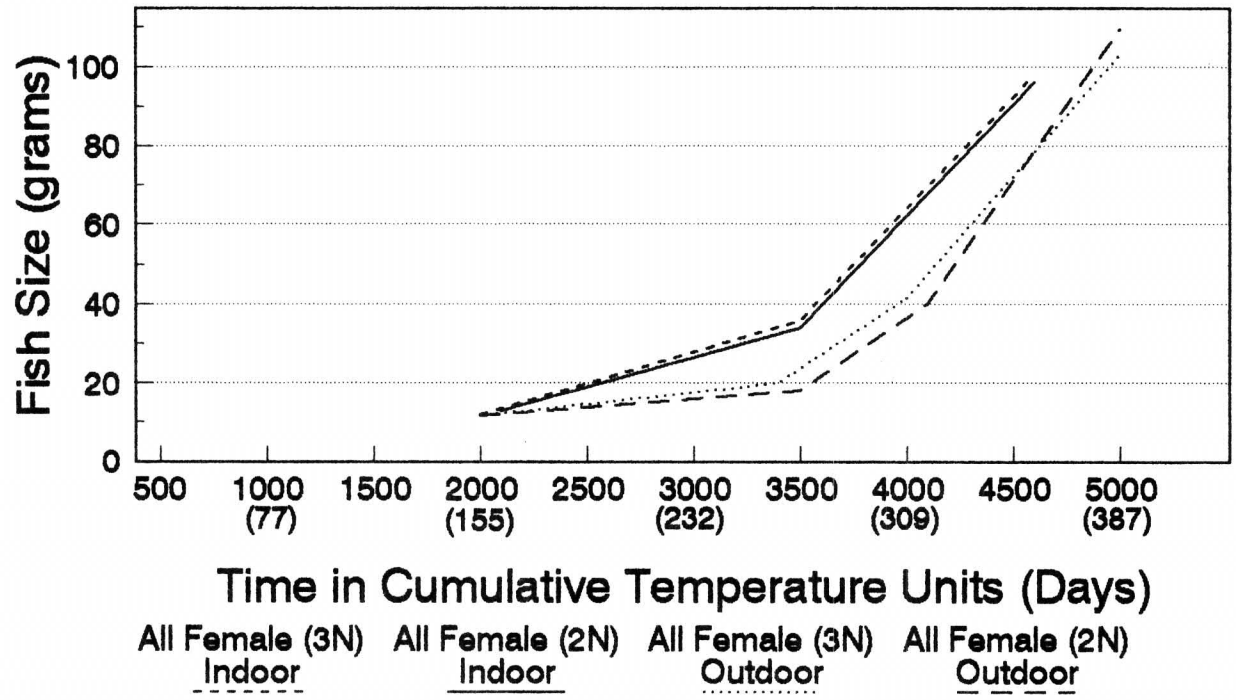


Figure 8. Rainbow trout growth in 1992, indoor and outdoor rearing.

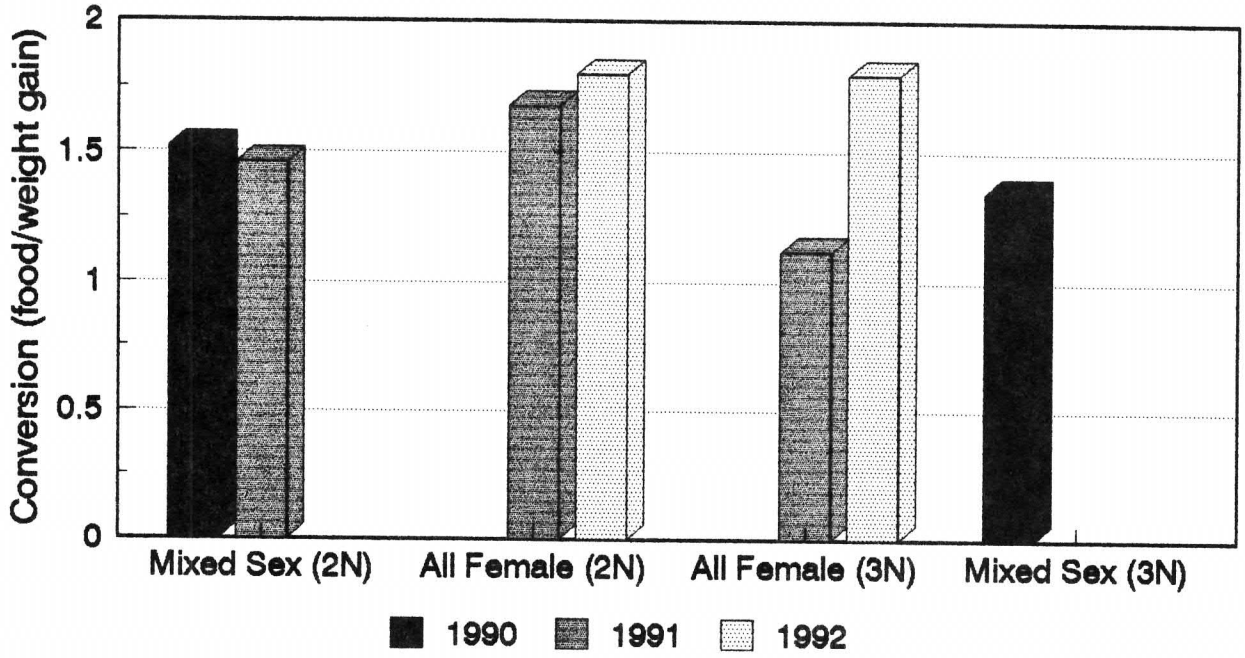


Figure 9. Feed conversions in indoor raceways.

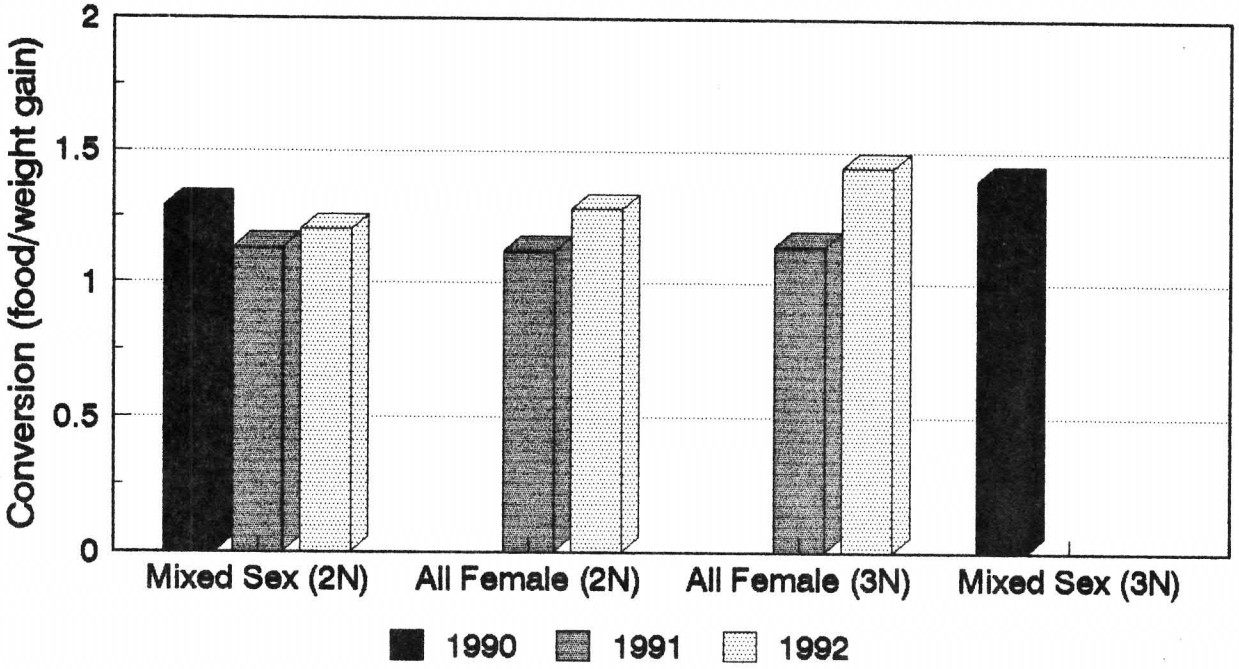


Figure 10. Feed conversions in outdoor raceways.

diploid and triploid fingerlings of equal numbers were released into a lake during an unusually hot summer which brought lake surface temperatures to at least 20 degrees C. The immediate overwhelming loss of the triploids indicated their limit to acclimate to temperature stress (Havens 93).

Despite the expected rearing performance suggested in the literature (Simon 93, Thorgaard et al), our hatchery experience in rearing the all female and triploid rainbow combinations fell below the normal diploid mixed sex. From a production standpoint, though, rearing rainbow triploids and all female populations for release is still quite within the hatchery program and management plan. Performance in the wild and future evaluations may, however, dictate the hatchery program. Hatchery projects and studies in the future may investigate further the temperature and possible photo period sensitivity that has been observed. The results may suggest an adjustment or difference in optimum rearing conditions and temperature (SET) for normal rainbow trout.

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GOING TOTALLY METRIC

G. W. Klontz
Department of Fish and Wildlife Resources
University of Idaho
Moscow, Idaho 83843

A few years back at a meeting such as this I made a presentation on this subject and did not make many converts. Now, here it is again. First off, I am not going to attempt to convert anyone from their tried and true beliefs in using English units in fish culture. That must be decided by the individual.

First off, let's look at some comparisons between the English and metric systems. What measurements are we using now in fish culture?

Fish measurements:	length in mm
	weight in number per pound
	total pond weight in pounds
	condition factor using inches and pounds
Pond measurements:	dimensions in feet
	volume in cubic feet or gallons
Water measurements:	cubic feet per second
	gallons per minute
	temperature in F
	dissolved oxygen in mg/l
Feed measurements:	pounds per day
	50 lb bags per day
Treatment methods:	mg/l in water
	parts per million in water
	grams per 100 lb of fish

The foregoing list is not complete by a long shot. Fish culturists, the world over, are unique individualists. There are about as many ways of doing a single task as there are fish culturists - particularly those who are in my age group.

Before I get too deep into this, I would like to point out that there are but two things which must be changed on a fish hatchery for the entire system to go totally metric. Think of it, just two - and one of them does not cost a cent! One is replacing all the English unit weighing devices - scales or balances or whatever they are called - with metric weighing devices, preferably battery-powered digital affairs rather than the spring-operated ones. Now for the "kicker", the cheapest and yet the hardest thing to change is the mind-set. The fish culturists must want to change and must think metric - not think metric and translate it into English. It is like being bilingual or even moreso. Such a person thinks in the language and does not mental translations into his/her native

language. The main reason for this is that a lot is lost in translation back and forth. The same is true for converting from English units to metric units and back again. Statisticians call this "rounding error". I call it "rubbish" and "nonsense". It's rounding error, alright - error of the grossest form.

The best "proof of the pudding" I can think of for going totally metric is the sensitivity and the simplicity. At this point I must ask the "Doubting Thomas's" to sit back and enjoy. There will be plenty of holes for you to put your fingers into.

Fact: 1.0 gram is 1/454th of a pound.
1.0 ounce is 28.3 grams
1000 grams are 1 kilogram
1.0 kilogram is 2.2 pounds

Question: Which weighing system provides the greatest sensitivity and accuracy? It seems that fish can be weighed individually or collectively to +/- 0.1 g but only, perhaps +/- 0.25 ounce (+/- 7 g). One concern frequently stated by fish culturists is not being able to know +/- 15-20% what is in the pond. I think using a weighing method which provides the best sensitivity would reduce this concern.

Next, let's take a look at administering chemicals in the water and antibacterials in the feed. The recommended dosages for the majority of water-administered chemicals for treating diseases are either as mg/l or parts per million (ppm). But a few are as a dilution; e.g., formalin is administered at 1:4,000 - 1:6,000. The conversion of mg/l to whatever English unit value has been responsible for the deaths of many fish. So, what's the big deal about not going metric? If the ponds were measured in meters and the volume multiplied by 1,000, the result would be liters. If the water inflow in cfs were to be divided by 28.32, the result would be liters per second (lps). Now, the dosage calculation is quite simple.

On administering antibacterials in the feed, the process is not quite so complicated - but it is, nonetheless, complicated. Fish are fed so many grams per hundred pounds of biomass. The correct dose must be calculated as feed fed, which is so many pounds per hundredweight of fish. Why not keep it all in the same units and simplify the process, plus making it less expensive and more effective.

In summary, please give what I have said some thought. I speak from my own experience that going totally metric fish culture has made things much less difficult and stressful. It did take some time, though. As I said earlier, it is like speaking another language. So long as one thinks in his/her native tongue and speaks the other language, they will never master the nuances of the new language. They will continue to be frustrated switching from one to the other. So, I think the place to begin is to be convinced that it can be done and then do it.

THE IMPACT OF THE METRIC SYSTEM ON FISH FARMING

AUTHOR: UNKNOWN

I WAS JUST GETTING WELL-STARTED IN MY FISH FARMING VENTURE WHEN THE DEPARTMENT OF WEIGHTS AND MEASURES CHANGED THE OUNCES AND POUND UNITS TO GRAMS AND KILOGRAMS. MY FISH PRODUCTION FELL BETTER THAN 50%. BUT I WAS BUYING ONLY HALF AS MUCH FEED, WHICH IS DOUBLE THE PRICE!!

THEN, THE WEATHER BUREAU CHANGED THE RAINFALL MEASUREMENT FROM INCHES TO MILLIMETERS AND THERE HAS NOT BEEN AN INCH OF RAIN SINCE. THE SPRING FLOWS SEEM ABOUT THE SAME THOUGH, IN AS MUCH AS GPM HAS BEEN REPLACED WITH LPM, ABOUT 1/4TH THE WATER AMOUNT.

SO, THEN WHAT DID THE WEATHER BUREAU DO TO TOP THEIR LAST CHANGE? THEY CHANGED THE TEMPERATURE UNITS FROM FAHRENHEIT TO CENTIGRADE. NOW THE WATER TEMPERATURE HAS DROPPED 20 DEGREES - IT'S NO WONDER MY FISH AREN'T GROWING AS THEY USED TO.

NOW, AS IF THAT WERE NOT ENOUGH, THE DEPARTMENT OF AGRICULTURE CHANGED THE LAND MEASUREMENT UNITS FROM ACRES TO HECTARES AND NOW I HAVE ABOUT HALF THE LAND I DID BEFORE.

THAT WAS THE LAST STRAW! I DECIDED TO SELL OUT. I HAD JUST PUT UP THE FOR SALE SIGNS WHEN THE DEPARTMENT OF HIGHWAYS CHANGED THE MEASUREMENT UNITS FROM MILES TO KILOMETERS. NOW I AM TOO FAR OUT OF TOWN FOR ANYONE TO BUY MY FARM.

NOW, I ASK YOU, IS THIS WHAT THE BUREAUCRATS CALL "PROGRESS"??

Evaluation of four low-phosphorous feeds fed to rainbow trout in raceways

Ron Zitzow and Charlie Smith
Bozeman Fish Technology Center
4050 Bridger Canyon Road
Bozeman, MT 59715
ph. (406) 587-9265

ABSTRACT- Four low-phosphorous feeds and a standard trout grower feed were fed to rainbow trout fingerlings in raceways at two National Fish Hatcheries. Performance of fingerlings (i.e. survival, growth, condition factor, Goede's Fish Health Assessment, and feed conversion) was compared to evaluate the feeds. At Creston NFH, no differences in performance was observed between fingerlings fed Silvercup Trout Grower (STG; growth rate was 0.012-in/day) and USFWS open-formula T2M.55P (T2M; 0.012-in/day) low-phosphorous feeds. However, fingerlings fed Silvercup Low-Phosphorous feed grew slower (0.008-in/day) and fingerlings fed BioDry 500 low-phosphorous feed grew faster (0.015-in/day) than those fed STG. There were no differences in performance of fingerlings fed STG, T2M, and Rangen's low-phosphorous feeds at Hotchkiss NFH. Compared to raceways fed Silvercup Trout Grower, calculated, daily total-phosphorous discharge was reduced by 30.2 % (at Hotchkiss NFH) to 33.7 % (at Creston NFH) from raceways fed T2M.55P feed. Calculated, daily total-phosphorous discharge was reduced by 70.1 percent in raceways fed Silvercup low-phosphorous feed, however, it would take 33.3 % more time to rear fingerlings to targeted size. Calculated, daily total-phosphorous discharge would actually increase by 37.5 % and 106.1 %, respectively, from raceways fed Rangen's and Biodry 500 low-phosphorous feeds.

Adult Survival, Size and Time of Return for Age One and Age Two Winter Steelhead Smolts released from Cole Rivers Hatchery, Oregon

Michael D. Evenson
Oregon Department of Fish and Wildlife
Cole River Hatchery
Trail, Oregon 97541

Introduction

Investigations into the life history of steelhead trout indicate that parr-smolt transformation and subsequent seaward migration occurs in the spring after juveniles have attained a minimum length of about 18 cm (Buchanan 1980) In Oregon, naturally produced steelhead commonly smolt following 1-3 years of freshwater residence. To maximize efficient use of pond space and manpower, most Oregon hatcheries structure their rearing program so as to produce steelhead smolts after one year of hatchery residence. Fish culture practices that are frequently employed to achieve this goal include: a) selection of brood stock for early spawning time; b) grading out of slower growing fish; c) intense feeding practices; and d) the manipulation of water temperature to accelerate egg and fry incubation in order to extend the rearing period and produce optimum growing conditions.

The production of yearling winter steelhead smolts at Cole Rivers Hatchery is limited by the late migration and spawning time of the stocks reared at the hatchery. In addition, growth rates are restricted during the winter months by cold water temperatures. In response to these constraints, managers utilize both one-year and two-year rearing strategies for production of winter steelhead smolts. This report describes return rates, size of returning adults and timing of returns to the adult collection facilities for groups of Rogue and Applegate stocks of winter steelhead released as one-year and two-year old smolts from 1982 to 1991.

Methods

Adult Rogue stock winter steelhead from the 1980-1981 broods, and 1983-90 broods returned to Cole Rivers Hatchery (located at river km 254 on the Rogue River, Oregon). Applegate stock winter steelhead that composed the 1985-90 broods returned and were captured at the adult trap below Applegate Dam (located at km 75 on the Applegate River,

Oregon). Adults from both stocks were trapped from February through May and were spawned from March through May.

Yearling smolts were produced primarily from the fastest growing juveniles and from eggs spawned on the earliest dates. Two-year smolts were generally reared from slower growing juveniles and eggs spawned at later times. Heated water was used to accelerate the incubation rate on eggs designated for release as yearling smolts; and to synchronize development stages for eggs taken on different dates. Ponds used for production of one-year smolts were supplied with warmer water from the surface of Lost Creek Reservoir, while ponds used for the rearing of two-year old smolts were supplied with cooler water drawn from the Rogue River.

Steelhead were reared in 30.5 m x 6.1 m x 1.2 m raceways on various types of commercially available feeds. Juveniles programmed for release as yearling smolts were reared on more expensive high protein diets (Oregon Moist Pellet-formulas 2 and 4, and Biomoist Grower), while juveniles programmed for release as two-year smolts generally received less expensive feeds. Juvenile steelhead were graded by size two or three times during their hatchery rearing period using a Morton adjustable grader (Morton 1956).

Fish were marked with distinct fin marks by clipping one or more fins and/or one maxillary bone before release. For most years, complimentary fin clips were used to prevent differential mortalities from fin loss (Shetter, 1951; Wales, 1947). Four to six weeks prior to release of two-year old smolts, precocious males and fish measuring less than about 16 cm in fork length (projected to be less than 18 cm in fork length at release) were removed by hand sorting to eliminate fish with little expectancy of smolting (Buchanan, 1979; Ward and Slaney 1990). Precocials and grade-outs from this final hand-sorting of two-year old smolts comprised about 12-20% of the population.

Rogue stock winter steelhead were released from the raceway ponds into a 1 m wide smolt release channel which empties into the river. For Applegate stock winter steelhead, two-year smolts were transported by truck and released below Applegate Dam in early to mid-April (except for 1987, which were released in late April and early May), while yearling smolts were transported and released in late April to early May.

Sizes at release varied between smolt age and year of release with one-year old smolts usually released at a larger size than two-year old smolts. For Rogue stock, mean size at release for yearling smolts ranged from 10.4 to 14.1 fish/kg, while two-year old smolts ranged from 7.7 to 10.6 fish/kg (Table 1). For the Applegate stock, mean size at

release for yearling smolts ranged from 10.8 to 12.3 fish/kg, while two-year old smolts ranged from 9.5 to 10.6 fish/kg (Table 2).

Adults were captured and examined when they returned to Cole Rivers Hatchery and the Applegate adult collection pond. Sex, fin mark and fork lengths were recorded for each fish.

Results

For Rogue winter steelhead average return rates for all years were higher for two-year smolts (1.08%) than for yearlings (0.79%). However, average yields for all years were similar for both yearling (9.58 adults/100 kg smolts) and two-year old smolt (9.93 adults/100 kg smolts) release groups (Table 1). Considerable variation was observed in survival rates and yields between years with no consistent advantage associated with smolt age.

Applegate winter steelhead released as two-year old smolts returned at higher average rates and produced greater average yields than those released as yearlings (Table 2). Average return rate and average yield for all groups of Applegate stock two-year old smolts were 1.7 and 1.5 times greater, respectively, than for yearling smolts. In two of the five years of observations, return rates and yields were similar for both one-year and two-year old smolt release groups.

Mean length of returning adults for both stocks tended to be larger for males and females that were released as two-year old smolts (Table 3 and 4). These differences were most consistent for adults returning two years after release. Combined mean lengths for all females from Rogue and Applegate stocks returning after two years at sea were 1.9 cm and 2.2 cm greater, respectively, for adults released as two-year old smolts as compared to adults released as yearling smolts. Combined mean lengths for Rogue and Applegate stock males returning after two years at sea and released as two-year old smolts were 2.8 cm and 3.2 cm greater, respectively, than adults originating from yearling releases. For adults returning after three years at sea, mean lengths for Rogue and Applegate stock females and Rogue stock males were similar. However, returning Applegate stock males released as two-year old smolts were 5.0 cm larger than males that were released as yearlings.

Time of return for adults released as smolts at different ages showed considerable variation and inconsistency between years. There was a tendency for adults originating from two-year old smolts to return later than those released as yearling smolts. This trend was most pronounced for the

Table 1. Summary of release and return data for Rogue winter steelhead released as one and two-year old smolts into the Rogue River. Return data is through 1993.

Release year, smolt age	Release dates	Release size, Fish/kg	Number released,	Fin mark	Return after each ocean year					% return	Adults/100 kg smolts
					1	2	3	4	Total		
1982											
One-year	5/6/82	14.1	19,940	ADRPLM	0	176	75	2	253	1.27	17.89
Two-year	4/26/82	8.8	22,631	ADLPRM	6	292	27	0	325	1.44	12.64
1985											
One-year	5/3/85	14.1	20,000	LV	4	78	23	0	105	0.53	7.40
Two-year	4/25-5/1/85	10.6	20,000	ADLP	4	227	42	3	276	1.38	14.63
1986											
One-year	5/6/86	11.9	17,719	ADLV	1	218	116	1	336	1.90	22.57
Two-year	5/7/86	10.1	19,526	ADRV	1	256	38	0	295	1.51	15.26
1987											
One-year	4/30/87	10.8	21,152	LVRM	0	206	46	1	253	1.20	12.92
Two-year	4/29/87	8.8	20,608	LVLM	3	185	32	2	222	1.08	9.48
1988											
One-year	5/3-13/88	11.0	20,344	ADLP	0	71	41	2	114	0.56	6.16
Two-year	4/26/88	7.7	20,345	LP	5	385	22	2	414	2.03	15.67
1989											
One-year	5/10-11/89	10.4	20,735	ADRV	0	80	13	0	93	0.45	4.66
Two-year	4/25/89	10.1	20,126	ADLV	0	81	12	0	93	0.46	4.67
1990											
One-year	5/4/90	11.7	20,260	ADLP	0	24	20		44	0.22	2.54
Two-year	4/27/90	9.0	20,027	ADRP	0	74	21		95	0.47	4.27
1991											
One-year	5/6-7/91	10.4	20,000	ADRV	0	48			48	0.24	2.50
Two-year	4/26/91	10.6	20,109	ADLV	1	52			53	0.26	2.79
Average all years											
One-year										0.79	9.58
Two-year										1.08	9.93

Table 2. Summary of release and return data for Applegate winter steelhead released as one and two-year old smolts into the Applegate River. Return data is through 1993.

Release year, smolt age	Release dates	Release size, Fish/kg	Number released,	Fin mark	Return after each ocean year 1	2	3	4	Total	% return	Adults/100 kg smolts
1987											
One-year	5/4-14/87	11.0	75,334	RVRM	0	691	51	0	742	0.98	10.86
Two-year	4/23-5/6/87	9.7	136,001	RV	1	1,957	126	1	2,085	1.53	14.87
1988											
One-year	4/27-5/11/88	11.0	19,764	ADRP	0	63	10	0	73	0.37	4.07
Two-year	4/12-15/88	9.7	20,247	RP	6	124	13	0	143	0.71	6.85
1989											
One-year	4/26-27/89	12.1	19,990	ADRPRM	0	34	3	0	37	0.19	2.24
Two-year	4/5-14/89	10.6	19,994	ADRPLM	1	34	5	0	40	0.20	2.12
1990											
One-year	5/4-14/90	12.3	20,043	ADRVLM	0	5	4		9	0.04	0.55
Two-year	4/10-19/90	9.5	19,982	ADRVRM	1	57	13		71	0.36	3.37
1991											
One-year	5/8-15/91	10.8	19,673	ADRPLM	0	30			30	0.15	1.65
Two-year	4/10-16/91	10.6	19,997	ADRVRM	0	27			27	0.14	1.43
Average all years											
One-year										0.35	3.87
Two-year										0.59	5.73

Table 3. Mean fork length in centimeters for Rogue one-year and two-year old winter steelhead smolts returning after two and three years at sea. Numbers of fish are given in parentheses.

Ocean year:	2				3			
Release year,	Females		Males		Females		Males	
smolt age	(N)	Length ± 95% C.I.	(N)	Length ± 95% C.I.	(N)	Length ± 95% C.I.	(N)	Length ± 95% C.I.
1982								
One-year	(79)	59.3 ± 0.6	(97)	61.9 ± 0.8	(54)	70.1 ± 0.8	(22)	73.1 ± 2.4
Two-year	(143)	62.6 ± 0.6	(149)	64.1 ± 0.8	(20)	69.0 ± 1.8	(7)	69.8 ± 3.1
1985								
One-year	(45)	61.1 ± 1.4	(33)	60.3 ± 2.6	(15)	66.7 ± 2.6	(8)	71.9 ± 6.8
Two-year	(116)	61.6 ± 0.8	(111)	63.5 ± 1.2	(22)	68.2 ± 2.0	(21)	73.6 ± 1.9
1986								
One-year	(86)	60.1 ± 0.8	(131)	60.5 ± 0.9	(72)	70.7 ± 0.8	(44)	74.1 ± 1.5
Two-year	(131)	60.6 ± 0.6	(126)	62.2 ± 0.8	(24)	68.5 ± 1.8	(14)	71.5 ± 2.5
1987								
One-year	(93)	60.1 ± 1.0	(113)	61.6 ± 1.1	(18)	66.9 ± 2.2	(28)	72.0 ± 1.7
Two-year	(93)	60.2 ± 0.9	(92)	62.4 ± 1.2	(19)	68.0 ± 1.8	(13)	71.0 ± 2.6
1988								
One-year	(23)	60.5 ± 2.3	(48)	62.4 ± 1.6	(19)	64.7 ± 2.2	(22)	67.0 ± 2.7
Two-year	(220)	63.5 ± 0.5	(165)	66.5 ± 0.7	(15)	69.1 ± 2.0	(7)	72.6 ± 7.0
1989								
One-year	(38)	60.3 ± 1.4	(42)	60.6 ± 1.6	(10)	66.8 ± 3.7	(3)	71.2 ± 11.2
Two-year	(33)	61.4 ± 1.0	(48)	61.7 ± 1.5	(11)	68.0 ± 2.3	(1)	70.5 ± -
1990								
One-year	(7)	61.4 ± 3.6	(16)	58.9 ± 2.9	(12)	67.2 ± 1.9	(8)	63.2 ± 4.0
Two-year	(39)	62.5 ± 1.0	(35)	60.6 ± 1.8	(13)	63.9 ± 1.9	(8)	70.5 ± 2.5
1991								
One-year	(13)	57.2 ± 3.0	(35)	56.1 ± 1.4	-	- -	-	- -
Two-year	(26)	59.3 ± 1.7	(26)	61.4 ± 1.9	-	- -	-	- -
Average all years								
One-year	(384)	60.0 ± 0.4	(515)	60.8 ± 0.5	(200)	68.9 ± 0.6	(135)	71.5 ± 1.0
Two-year	(801)	61.9 ± 0.3	(752)	63.6 ± 0.4	(124)	68.0 ± 0.7	(71)	71.8 ± 1.0

Table 4. Mean fork length in centimeters for Applegate one-year and two-year winter steelhead smolts returning after two and three years at sea. Numbers of fish are given in parentheses.

Release year, smolt age	Ocean years: 2 Females (N)	Length ± 95% C.I.	Males (N)	Length ± 95% C.I.	3 Females (N)	Length ± 95% C.I.	Males (N)	Length ± 95% C.I.
1987								
One-year	(373)	55.4 ± 0.5	(318)	56.6 ± 0.6	(31)	65.0 ± 1.6	(20)	63.9 ± 2.8
Two-year	(1,015)	57.4 ± 0.3	(942)	59.1 ± 0.4	(74)	65.7 ± 1.0	(45)	68.3 ± 1.1
1988								
One-year	(9)	54.4 ± 3.8	(54)	54.4 ± 1.9	(5)	60.9 ± 5.6	(5)	60.7 ± 2.7
Two-year	(55)	58.3 ± 1.2	(69)	61.8 ± 1.3	(5)	63.3 ± 7.2	(8)	70.2 ± 3.3
1989								
One-year	(11)	56.8 ± 1.4	(23)	55.3 ± 1.5	(1)	64.5 ± -	(2)	61.0 ± 44.4
Two-year	(11)	56.8 ± 2.0	(23)	59.2 ± 2.3	(2)	65.0 ± 31.8	(3)	63.8 ± 10.0
1990								
One-year	(2)	57.5 ± 38.1	(3)	56.5 ± 2.5	(1)	66.5 ± -	(3)	66.8 ± 9.4
Two-year	(31)	59.6 ± 1.4	(26)	61.4 ± 2.0	(10)	65.3 ± 2.7	(3)	70.2 ± 14.6
1991								
One-year	(15)	53.1 ± 1.3	(12)	51.5 ± 2.0	-	- -	-	- -
Two-year	(14)	60.6 ± 2.3	(16)	58.2 ± 3.0	-	- -	-	- -
Combined all years								
One-year	(410)	55.3 ± 0.4	(410)	56.1 ± 0.5	(38)	64.5 ± 1.5	(30)	63.5 ± 2.0
Two-year	(1,126)	57.5 ± 0.3	(1,076)	59.3 ± 0.4	(91)	65.5 ± 0.9	(59)	68.5 ± 1.0

Applegate stock returning after two years at sea (Tables 5). Much of the variability observed in return timing may be because smolts released at different ages originated from two different brood years and not from common parents.

Discussion

The results from this study support the viability of a two-year rearing regime as an alternative for production of steelhead smolts. Return rates and yields observed for two-year smolts were comparable to those of yearling smolts for the Rogue stock and were generally better for the Applegate stock.

The differences in survival rates and yields observed for the Applegate stock may be influenced by release practices and downstream migration conditions that exist on the Applegate River. The most favorable downstream migration conditions on the Applegate River are thought to occur early in the spring when water flows are generally higher and fewer irrigation diversions are in operation. To take advantage of these conditions, two-year old smolts were usually transported and released in the Applegate River 2-4 weeks before the release of yearlings. Yearlings were retained until late April to early May to allow achievement of a larger release size. The benefits of improved survival normally associated with releasing smolts at a larger size may be off-set by higher mortality during seaward migration, because of less favorable river conditions. In contrast, Rogue stock steelhead were released in late April to early May and probably encountered more favorable downstream migration conditions.

The differences observed in the size of returning adults produced by yearling and two-year old smolts may be explained by either (a) differences in size of smolts at release or (b) differences in the frequency of life history types produced by each rearing strategy. Scale samples were collected only from adults returning from 1982 Rogue stock release groups. Analysis of these scales suggests that both the above factors may be involved. For fish returning after two years at sea, mean lengths of adults that were released as two-year old smolts and exhibited the most common adult life history type were 1.9 cm and 1.4 cm greater for females and males, respectively, as compared to adults released as yearlings smolts. In addition, the smaller and dominant half-pounder life history type (Everest 1973, Evenson et al. 1992) was most frequent (92%) among adults produced from yearling smolts as compared to those produced from two-year old smolts (75%).

Table 5. Percentage of adult Rogue and Applegate winter steelhead captured after April 15 from groups released as one and two-year old smolts. Total numbers returning are in parentheses.

Release year, smolt age	Rogue stock Years at sea				Applegate stock Years at sea			
	2		3		2		3	
1982								
One-year	(176)	48.9	(75)	9.3	-	-	-	-
Two-year	(285)	35.8	(27)	0.0	-	-	-	-
1985								
One-year	(78)	5.1	(23)	8.7	-	-	-	-
Two-year	(227)	23.8	(42)	14.3	-	-	-	-
1986								
One-year	(218)	22.5	(116)	20.7	-	-	-	-
Two-year	(256)	5.0	(38)	5.3	-	-	-	-
1987								
One-year	(206)	41.7	(46)	17.4	(691)	21.7	(51)	23.5
Two-year	(185)	37.3	(32)	18.8	(1,957)	20.6	(126)	19.0
1988								
One-year	(71)	18.3	(41)	36.6	(63)	3.2	(10)	70.0
Two-year	(385)	46.8	(22)	40.9	(123)	38.2	(13)	30.8
1989								
One-year	(80)	37.5	(13)	7.7	(34)	61.8	(3)	33.0
Two-year	(81)	39.5	(12)	16.6	(34)	85.3	(5)	60.0
1990								
One-year	(23)	17.4	(20)	10.0	(5)	20.0	(4)	25.0
Two-year	(74)	23.0	(21)	23.8	(57)	43.9	(13)	46.2
1991								
One-year	(48)	56.2	-	-	(27)	29.6	-	-
Two-year	(52)	71.2	-	-	(30)	43.3	-	-
Average all years								
One-year		31.0		15.8		27.3		37.9
Two-year		35.3		17.1		46.3		39.0

The variation observed in time of return to the collection facilities may indicate some advantage of releasing smolts of different ages. Release of only yearling smolts may tend to reduce variation in run timing and restrict the duration of dependent fisheries, since hatchery practices often tend to select against later arriving and later spawning adults in order to achieve production goals. In contrast, production strategies that include release of both one-year old and two-year old smolts may avoid these selective pressures. Smolts released as yearling and two-year olds include the genetic variation from two brood years and may tend to extend run time and reduce between year variation in run timing.

Fixed conditions at some hatcheries and the late spawning characteristics of some steelhead stocks can substantially hinder achievement of a sufficient release size to produce parr-smolt transformation in a one year rearing period. Managers may choose to either release juveniles at a smaller size, employ intensive grading and brood stock selection practices to advance spawning time, or raise juveniles for an additional year to achieve a larger release size.

Releasing juvenile steelhead at a small size is likely to result in reduced survival rates; and increase potential for adverse impacts on wild populations. Many juveniles may fail to migrate to the ocean because they are too small to undergo parr-smolt transformation. Juveniles that fail to migrate are subject to at least one additional year of exposure to harsh mortality factors and poor feeding conditions in freshwater. In addition, residual juveniles may adversely interact with wild fish through competition for food and space, and predation.

Employment of intensive brood stock selection and grading practices tend to reduce genetic variability, which may adversely impact stock vitality, or the ability of stocks to adapt to change. Studies suggest that when placed in the wild, the potential for survival is substantially reduced for the progeny of hatchery fish which have been selected for characteristics designed to accommodate artificial propagation (Reisenbichler and McIntyre 1977).

With the increased concern for wild fish and the genetic vitality of hatchery stocks, practices that involve intensive selection for particular traits are becoming less acceptable as viable approaches to solving fish culture problems.

In view of the above concerns, production of steelhead smolts utilizing a two-year rearing regime is receiving greater attention; especially with the increased interest in culturing later spawning wild stocks. The results reported

in this study provide encouragement for the consideration of this approach as a viable fish culture alternative.

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WINTER-RUN CHINOOK SALMON PROPAGATION AT COLEMAN NATIONAL FISH HATCHERY

Presented at the 44th Annual Northwest Fish Culture Conference
Spokane, Washington, December 7-9, 1993

John Rueth
Untied States Fish and Wildlife Service
Coleman National Fish Hatchery
Anderson, California 96007

Numbers of winter-run chinook salmon (*Ocorhynchus tschawytscha*) returning to the Sacramento River have been in steady decline over the past two decades. As a result of this decline the National Marine Fisheries Service listed the winter-run chinook salmon as threatened under the emergency listing procedures of the Endangered Species Act in August of 1989 and the winter-run chinook salmon were formally placed on the list of threatened species in November of 1990.

Propagation of winter-run chinook salmon at Coleman was initially attempted in 1958, and nine other times between 1958 and 1985 with little or no success. Most problems were attributed to high water temperatures, often exceeding 70 degrees fahrenheit. These temperatures resulted in high mortalities of both adults and incubating eggs. Attempts were also made to hold adults in fish traps located in the Sacramento river, where water temperatures were cooler. In one such attempt 102,000 eggs were taken, but only 10,250 fish survived to release.

In May of 1988 several State and Federal agencies, including the Fish and Wildlife Service entered a cooperative agreement to develop and initiate a artificial propagation program for winter-run chinook salmon at Coleman National Fish Hatchery.

In the first two years of the program only minimal success was achieved. Of the 67 adults captured only 4,489 fish where released, and adult pre-spawning mortality exceeded 85 percent. The main problem in both years was inadequate holding facilities for adults and elevated water temperatures. The major problem was the existing large adult holding ponds. These ponds were not designed to hold such small numbers of fish (under 50 adults), and did not allow for close observation of the adults. Also, almost constant water chiller malfunctions with the existing system for the adult holding ponds and hatchery building, that often sent water temperatures above 60 °F.

In 1991, major modifications of the existing winter-run chinook salmon program began. The first change was the addition of a twenty foot diameter circular fiberglass adult holding tank, surrounded with canvas to completely isolate the fish. To reduce adult holding time, an accelerated photoperiod lighting system was also installed. This lighting system consisted of three full spectrum lights controlled by a 24 hour timer, that allowed for light levels to be adjusted to simulate natural lighting conditions. The last major change was the installation of an granulated activated carbon filter, which allowed for the use malachite green (INAD permit 2573) to control fungus. These improvement and several others resulted in a ten fold increases in production from 1990 to 1991.

One of the problems encountered in the brood year 1991 efforts, was the difficulty in getting small (100 to 3,000 fish) family groups to start feeding in the large troughs or tanks. Because Winter-run chinook salmon are a wild stock they tend to be very skittish and shied away from noises or movements. This resulted in many of the smaller groups of fish never starting on feed and being combined to form larger ones. This combining of family groups resulted in a possible loss of genetic identity from the proposed broodstock program.

To solve this problem with the brood year 1992 fish, a small enclosure was constructed inside the existing hatchery building to hold ten small circular tanks. Each tank was 30 inches in diameter and held 10.25 cubic feet of water. The water delivery system is capable of providing: ambient, single pass chilled, and double pass chilled water. A timed lighting system was also installed, that allow for adjustable light levels and extended feeding times. The use of Zeilger 12 hour belt feeders allowed for the fish to remain undistributed once the feeders were filled. The use of these tanks and feeders resulted in 22 individual family groups (some as small as 200 fish), and increased production three fold over 1991, to a high of 28,000 fish. With these excellent results, another enclosure with ten tanks was built for this year.

These are just some of the modifications that were required to successfully raise winter-run chinook salmon at Coleman NFH. However, some addition modification are still needed. One of the last problems to overcome has been temperature spikes caused by water chiller malfunctions and power failures. These spikes have resulted in water temperature increases of up to 12 °F in just minutes, causing severe coagulated yolk problems within specific family groups. Hopefully, in the near future, these last major problems will be corrected and production numbers will continue to increase.

TROUT PRODUCTION IN MEXICO

George W. Klontz
Department of Fish and Wildlife Resources
University of Idaho

When Americans are asked about their opinions of fish farming around the world, Mexico hardly ever is mentioned. Yet, Mexico is a leading producer of farmed fish and shellfish. In 1990, it ranked 14th in worldwide production. It ranks 5th in the production of carp and 2nd in the production of channel catfish and tilapia. However, its trout production is not ranked.

The aquaculture community in Mexico is divided into three sectors: public (state and federal), private (commercial entrepreneurs), and social (farmers). Aquaculture activities are quite restricted to sectors. For example, shrimp farming cannot be done by persons in the private sector - it can be done only by persons in the social sector.

The production of rainbow trout for conservation (fingerling production) and the commercial market is a relatively new aquaculture activity in Mexico. Nonetheless, there are more than 200 commercial trout farms and 10-15 conservation facilities. Virtually all the facilities lie in the highlands along a line drawn between Veracruz on the east and Guadalajara on the west. The annual production in the foodfish sector is 700-800 metric tonnes (mt). As in the U.S., acquiring total production data is very difficult.

The rainbow trout production community consists of "typical" trout farms; i.e., intensive raceway production producing 25-100 mt annually, and small family-owned farms producing 1-5 mt annually. There are 6 "typical" farms and >150 family-owned farms.

The fingerlings produced in the public sector are given to the farmers in social sector to raise, harvest and sell on the open market. Some fingerlings are sold in the social sector by the private sector because the demand for fingerlings cannot be met by the public sector.

Rainbow trout as a tablefish is marketed through all levels of the Mexican culture - from the upper class to the lowest of the low. This is the only country known in which this fish is utilised by the entire spectrum of the population. All the production is marketed domestically as dressed (eviscerated, head-on), dressed and boned (head-off), and smoked, boned fillets. The primary point of sales is the restaurant. Very few fish are sold in the retail market.

Finally, according to the major producers and market analysts, the annual trout production will increase slowly in the foreseeable future. As more product types are successfully market tested, production will increase.

ABSTRACT

TITLE: Management Techniques used to operate remote satellite stations.

AUTHORS: Jerry McGehee and John Rankin
Idaho Fish and Game, Clearwater Hatchery
Ahsahka, Idaho

A slide presentation was given showing the Clearwater Hatchery and three satellites. Subjects presented were:

1) ADMINISTRATIVE TRAINING
2) EQUIPMENT MAINTENANCE TRAINING
3) VEHICLE SAFETY AND OPERATION TRAINING
4) LONG RANGE AND LONG TERM PLANNING
5) EMPLOYEE MOTIVATION

AN ALTERNATIVE SITE FOR INTRAPERITONEAL INJECTIONS

by A. Doug Munson and A. Kent Hauck

Abstract

Curently, IDFG is utilizing an intraperitoneal injection to deliver erythromycin to brood chinook salmon. This route seems to deliver the antibiotic without leaking, appears to be less stressful, and the fish is returned to the water in a minimum of time.

Although the intraperitoneal injection method is not a new practice in aquaculture, practitioners in the salmonid culture industry more frequently use either a dorsal sinus (subcutaneous) or a traditional IP location (off midline of abdomen) for injecting antibiotics. Use of IP during air spawning of salmonids is an exception (Leititz and Lewis, 1980).

The midline IP injection site (spleen injury) and dorsal sinus techniques (leakage and stress) have long been concerns in the industry; however, with this location , an antibiotic or other agent can be injected swiftly with minimal chance of injury to the fish, without leakage, and into an area where the drug is well absorbed.

To reduce injury to the spleen or other vital organs, fish are injected directly posterior to the pelvic fin. At this location the needle is inserted anteriorly at 45 degree angle to to the surface of the fish. Because the fish is lying belly-up and head lower than tail, the distance between viscera and needle is increased, further reducing injury to internal organs, associated stress, trauma and mortality. In addition, injections can be done

quickly (there are no scales or dense muscle to slow the process), the site is readily located, and handling stress and time out-of-water are reduced considerably. Because the leakage of the drug is reduced, the fish has more drug available to absorb. Reduction of egg viability is negligible and might be resolved by using a one inch needle on all but the largest fish. As with any intraperitoneal injection, care must be taken while handling the fish and not inserting the needle too far and damaging the ovary (Herwig, 1979).

Currently, all antibiotic injections at Idaho Department of Fish and Game Hatcheries are utilizing this route. Clear Springs Foods at Buhl, Idaho, has begun to utilize this technique in their day-to-day operations (Personal Communications with Scott LaPatra).

This technique provides enhanced delivery and improved survival to salmonids (or any other fish) and should be seriously considered whenever injections are administered.

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ENTEROCYTOZOON SALMONIS, AN INTRANUCLEAR MICROSPORIDIAN IN LITTLE KERN GOLDEN TROUT, **ONCORHYNCHUS AQUABONITA WHITEI**

JUDY URRUTIA
Kern River Fish Hatchery
P.O. Box X
Kernville, California 93238

The Kern River Fish Hatchery was built in 1928 and is located on the Kern River in the Sequoia National Forest in California at an elevation of 2800 feet. The water source is the Kern River with temperatures ranging from 33F during the winter to 73F in the summer. The twenty miles of river above the hatchery is planted with catchable rainbow trout for a put and take program. The hatchery has been involved in the Little Kern Golden Trout Restoration project since 1983.

The Little Kern Golden Trout Restoration's goal is to restore the Little Kern golden trout, **Oncorhynchus aquabonita whitei**, a threatened species, to its historical population levels and range. The designated Critical Habitat area includes all the Little Kern River drainage watershed to one mile down stream of Trout Meadow Creek. The genetic integrity of the GT-LK has been threatened by hybridization with rainbow trout which were introduced into the drainage in the late 19th century. Other threats include habitat deterioration and overharvest by angling. Original efforts to transplant pure populations to chemically treated streams were not effective.

An artificial propagation program was started at the Kern River Hatchery. Fish from pure population were transported to the hatchery during the summer of 1982 where the different strains were held in separate troughs. Eggs were taken in the spring and hatched in well water. The fry were held in well water for six months and then switched to river water in the fall when the river water temperature drops below the well temperature. The well water ranges from 60F to 68F. Two hundred fish from each year class are held for broodstock. No grading or selection is done.

Each summer as the water temperatures have risen the yearling LKGT have experienced severe losses due to columnaris and fungus. Since 1985 an intranuclear microsporidian was suspected of being present but was not confirmed by microscopic examination.

In 1992 we experienced heavy losses in our three strains of LKGT yearling broodstock. The loss occurred from mid June when water temperatures reached 59F to mid August with a peak in July when water temperature reached 68F. All three strains showed signs of popeyes, blotting, darkening and then death. Internal examination showed signs of hemorrhaging in the fat, yellow mucus in the intestine, swollen lower kidneys and spleen. Most fish

showed signs of columnaris and fungus. Losses were attributed to the later infections.

In 1993 larger numbers of fish were held to be planted as yearlings. Due to abnormal weather conditions and heavy snow chemical treatments were delayed in the high country until July. Water temperatures reached 64F by mid July and 66F by the first of August.

Due to size of the fish and space available the LKGT-Soda Spring Creek-92, 3 fish per pound, were placed in the outside ponds with older Broodstock. The LKGT-Dead Man Creek-92 and LKGT-Wet Meadow Creek-92 strains, 12 fish per pound, were held in fiberglass deep troughs in the hatchery building. All fish were on river water.

In mid July we started experiencing large losses in the LKGT-Deadman strain. Same symptoms as the year before. A few fish this year developed bent heads and swam on their sides before death. Large losses occurred after feeding or treatment for fungus. The LKGT-Wet Meadow strain in the trough next to them showed no signs of disease.

First of August the GTLK-Dead Man losses declined and losses in both the GTLK-Wet Meadow strain and the GTLK-Soda Spring strain in the outside pond began. The GTLK-Soda Spring strain showed no sign of columnaris. Most fish had hemorrhaging in the fat, yellow mucus, swollen abdomens, swollen spleens and lower kidneys.

Pathology found spores on wet mount microscopic examination in early August from samples from gills and skin. Tissues from the kidney and spleen were taken to UC Davis for examination by Ron Hedricks. Spores in all stages were also found.

The **ENTEROCYTOZOON SALMONIS** was first observed in pen reared chinook salmon at a rearing site in the Puget Sound in Washington in 1987. Since then the parasite has been reported in California, Idaho, Canada and France. The host species range from salt water chinook salmon to fresh water chinook salmon, rainbow, golden and brook trout.

Transmission is assumed to be by oral ingestion of spores in the water. Experiments performed by Dr. Ron Hedrick, at the University of Calif. Davis, by feeding infected kidney tissue to recipient fish have resulted in death 65 days after the first feeding. Cohabitation experiments also resulted in infection of recipient fish.

The main cell types infected are the hematopoietic cells in the spleen, kidney and blood leukocytes which results in anemia and leukemic-like conditions. The infected cells circulate in the blood, invade and multiply in many tissues. With lower resistance secondary infections such as columnaris and fungus invade the body causing high mortality rates.

The characteristic development of Enterocytozoon salmonis are unique among the microsporida. The meronts, the earliest stages that have been observed, were rounded or elongated uninucleate cells found in the host cell nucleus. Sporonts were found in a budding position with respect to the host cell nucleus. The number of sporonts dividing from the sporoblasts dependence on the size of the host cell. Each sporoblast contains 4-5 coils of the polar tube, a single large vacuole, an anchoring system and sporoplasm. Spores contain a polar tube with 5-6 turns that is attached at the anterior end of the spore to the anchoring disc. Spores are released into the nucleus and dispersed following rupture of the nuclear membrane.

There are only two other known similar microsporidian in the genus **Enterocytozoon.** Both have been found in humans with AIDS. **Enterocytozoon bieneusi** which is found in the intestinal mucosa and **Microsporidium sp.** which has been found in the cornea.

At this time there is no approved treatment for **Enterocytozoon salmonis.** Experiments with Fumagillin DCH at UC Davis are proving to be effect in experimental infections.

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Spatial and Temporal Distribution of Fungus in a ReUse Hatchery System

Paul R. Waterstrat DVM, Ph.D.
U.S.F.W.S.
Abernathy Salmon Cultural Technology Center
1440 Abernathy Road
Longview WA, 986320
206-425-6072

Introduction

Fungal infection is a widespread problem in the hatchery rearing of a wide variety of fish species. The management of fungal infections has traditionally relied upon prophylactic or therapeutic administration of chemicals such as malachite green, formalin or potassium permanganate. The withdrawal of malachite green and the increasing scrutiny of formalin by regulatory agencies has prompted the screening of a wide variety of less hazardous compounds as antifungal agents. Efforts in developing and obtaining regulatory approval for new antifungals for use in fish have been centered at the U.S. Fish and Wildlife, National Fisheries Research Center, Lacross WI (NFLX) which has established a laboratory meeting the regulatory standards for Good Laboratory Practices (GLP) for registration of antifungal agents. Abernathy Salmon Cultural Technology Center (ASCTC) has cooperated with NFLX in their effort to screen, develop and obtain regulatory approval for new antifungals. In addition to the cooperative work with NFLX, Abernathy SCTC has initiated an effort to examine the the pathogen-host-environment interactions associated with fungal infections.

Currently, only limited information exists with respect to the host-pathogen interactions involved in the development of fungal infections in fish. Current literature indicates that aquatic fungi appear to be ubiquitous in water supplies serving fish hatcheries. The taxonomy of the fungi affecting fish and fish eggs, however, appears troublesome. The original description of

Saprolegnia sp by Coker (1932) appears erroneous and untenable (Scott and O'Bier 1962). Revision of the systematics surrounding fungi affecting fish has been conducted by Scott and O'Bier (1962) and Seymour (1970). Of 64 fungal isolates from fish and fish eggs in 14 states examined by Scott and O'Bier (1962), the majority, 41 of 64, were from the genus Saprolegnia. Since speciation of oomycete fungi is generally based on the morphology of sexual stages and requires the often troublesome induction of sexual stages, most fish pathologists lump external fungal infections of fish and eggs into the "Saprolegnia". While potentially dangerous, lumping fish fungal infections into the catch-all taxon "Saprolegnia" appears warranted by a variety of investigations indicating that most external fungal infections of salmonids and salmonid eggs fall into closely related taxa considered to be a *Saprolegnia declina* - *S. parasitica complex* (Willoughby, 1978, 1985; Pickering and Willoughby 1982). Members of the Saprolegniaceae also exhibit a fair degree of cross reaction with polyclonal sera raised to *S. parasitica* , ATCC 52719 (Bullis, Noga and Levi 1990).

The life cycle of Saprolegnia is characterized by a diploid vegetative stage with relatively large diameter aseptate hyphae. Sexual reproduction occurs; meiosis within the antheridia produces male gametes while the female gametes, zoogonia, are produced within the oogonium. Among the Saprolegnia, multiple oospores are produced within each oogonium. No flagellate gametes are produced, male nuclei move into the aoogonium from the antheridia where they fertilize the eggs. Following fertilization, the diploid zygotes develop into oospores which germinate and develop through mitotic divisions into the vegetative or mycelial stage. Hyphae from the mycelium elaborate zoosporogonia which liberate primary zoospores. The primary zoospores are apically biflagellate and undergo a brief period of weak motility prior to encystment. The primary zoospore cysts eventually release a laterally a biflagellate secondary zoospore capable of a lengthy and vigorous motility. The secondary

zoospore is believed to be responsible for initiating fungal infections (Willoughby andRoberts 1992). Parasitic fungi (*S. parasitica*) can apparently be distinguished from other water-born fungi by the presence of long, hooked hairs on the secondary zoospore and the low nutrient levels required for germination (Pickering, Willoughby and McGrory 1979, Willoughby and Roberts 1992). Fungal invasion of egg masses appears to be initiated by the infection of dead eggs with fungal zoospores and the subsequent spread of fungal hyphae to live eggs. Zoospores are not considered to infect live eggs or undamaged fish tissue; the susceptibility of dead eggs to zoospores appears related to nutrient loss from the dead or debilitated eggs. Fungal zoospores and hyphae respond chemotactically to amino acids released by damaged eggs (Smith, Armstrong and Rimmer 1984). Once infection has been established, fungal hyphae can infect both dead and live eggs (Smith, Armstrong, Springate and Barker 1985). Fungal infections adversely affect hatching success by limiting oxygen availability to the eggs, by damaging andweakening the egg membranes and by penetrating and invading the yolk (Smith et al. 1985, Gajduesek and Rubcov 1985).

In an examination of bacterial flora of stream incubated eggs, Bell, Hoskin and Hodgkiss (1971) have restated Snieszko's (1964) contention that an understanding of host/pathogen/ environment ecosystem is essential to management of the disease processes affecting fish eggs. Application and dissection of the host/pathogen/ environment paradigm with respect to fungal infections appears particularily appropriate to the development of effective management strategies. What host and environmental factors do fungi exploit ? Are there opportunities in management of fungal infections related to environment parameters such as water pretreatment, filtration or the design in rearing facilities and equipment ? Can host and environmental factors be modified by therapeutants or management to prevent or block fungal infection ? An improved understanding the interactions of fungus with the host and environment will hopefully allow

improvements in the efficiency of prophylactic and therapeutic measures and the development of hatchery management practices to minimize fungal infections and its impact on hatchery production.

Work completed during the current year was directed at the development of an assay procedure to detect fungus within hatchery water supplies, the monitoring of hatchery water during the production production cycle, preliminary studies of Saprolegnia life history and epidemiology and the evaluation of hydrogen peroxide, formalin and salt as antifungal agents.

Materials and Methods

Sampling Plan:

Three separate 250 ml water samples were taken from each of five sample sites within the Abernathy SCTC hatchery system at two week intervals over the production period of 4 January to 14 May 1993. Sample Sites were as follows:

Creek water: Single- pass Abernathy Creek Water at raceway:
Inlet Outlet
Reuse: 90% recycle system at biofilter
Inlet Outlet
Well water: Wellwater following KMnO4 treatment and Sand filtration Inlet

Fungal Assay Procedures:

An assay system was developed for monitoring the presence of fungus in hatchery water supplies using modifications of the procedure of Celio and Padget (1989). From each of the three 250 ml samples, a 20 ml aliquot was added to each of 4 petri

dishes previously supplemented with 400 μl of Peptone- Yeast-Glucose Broth supplemented with chloramphenicol (PYG-CA media). Two of the petri dishes were administered 200 μl of a 1:100 dilution of pimaricin (Sigma Chemical Co. St. Louis, MO) to block the development of septate fungi (P+ plates) while the remaining 2 plates received 200 μl of sterile distilled water (P- plates). After the addition of the the 20 ml aliquot, the petri dish was swirled, sprinkled with 0.9 g hydroxyethylcellulose and swirled a second time to solidify the media. For each 250 ml water sample, the procedure yielded duplicate P+ and P- plates. Duplicate positive and negative control plates were tested at each sample period. Each sampling location and period was represented by triplicate sets of P+ and P- plates. The inoculated petri dishes were incubated at room temperature, observed for growth at 24, 48 and 72 hr and the number of colonies determined. The effectiveness of pimaricin in blocking development of septate fungi was monitored periodically through microscopic examination of wet mount preparations of individual colonies from P+ plates.

Growth and Life History Studies :

Selected colonies from P+ plates were subcultured by placing sterile, boiled hemp seed in the petri dish and transferring the seeded hemp to cornmeal agar supplemented with chloramphenicol (CMA-CA). The production of zoospores and sexual stages were induced by transferring hemp seed colonies to a media consisting of 14 ml of 0.22 μm filter-sterilized ASCTC wellwater, 6 ml of 0.22 μm filter-sterilized distilled water, 0.7 mg chloramphenicol and 0.05 mg pimaricin. Morphological measurements and growth rates were determined from selected colonies taken from P+ petri dishes and subcultured on cornmeal agar. Growth was measured as the change in the radius (mm) of the fungal colony per hour.

Results

Evaluation of Fungal Assay Procedures

The PYG-CA media readily supported the growth of both septate and aseptate fungi. Differential counts of ten 20 ml PYG-CA supplemented water samples yielded a total of 110 septate fungal isolates and 16 aseptate isolates. Pimaricin was effective in selecting for aseptate fungi, no isolates of septate fungi and were observed on pimaricin treated samples. Growth of aseptate fungal colonies on PYG-CA media was first observed at 24 hrs, the plates appeared suitable for counting by 48 hrs of incubation. At 72 hr of incubation, many of the fungal colonies became confluent and made the counting of individual colonies difficult. A comparison of the number of fungal colonies observed on P - and P+ plates at 48 and 72 hr is presented in (Figure 1). Among the P+ plates, there was no significant difference in the number of fungal colonies between 48 hr and 72 hr. The number of colonies observed on P - plate however was significantly greater at 72 hr ($p = 0.0001$), reflecting slower growth among septate fungi. Similar results were observed when results were summarized over sample locations and sampling periods. (Figure 2).

Morphological characteristics of aseptate fungi on cornmeal agar are presented in Table 1a. Typically, the aseptate fungi exhibited robust hyphae. Zoospongia when induced by subculture of hemp seed in water exhibited morphology associated with Saprolegnia. The induction of sexual stages used in the speciation of Saprolegnia was only occasionally observed and then only after prolonged culture in water (> 1 month). Because of the difficulties in obtaining the sexual stages necessary for the speciation of the fungal isolates, isolates observed on P- plates were categorized as "Saprolegnia". Growth of fungal isolates measured as the change in radius (mm/hr) is presented in Table 1b. Aseptate fungi exhibited relatively rapid growth (0.778 mm/hr); P+ plates generally achieved confluence 72 hr following inoculation.

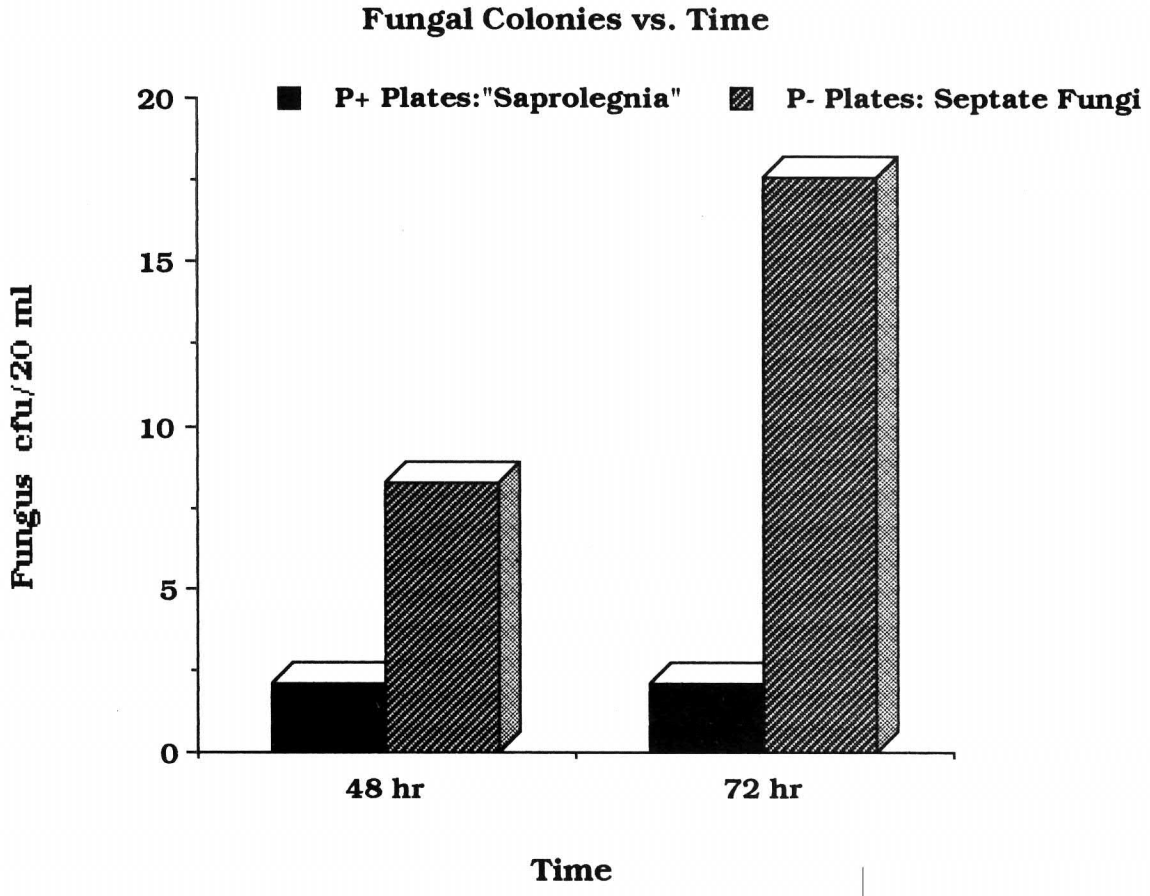


Figure 1. Mean number of fungal colonies observed on P+ and P- plates at 48 and 72 hours incubation at room temperature. P- plates represent 20 ml water samples supplemented with PYG-CA media; P+ plates are treated with pimaricin to suppress growth of septate fungi (Celio and Padget 1989) .

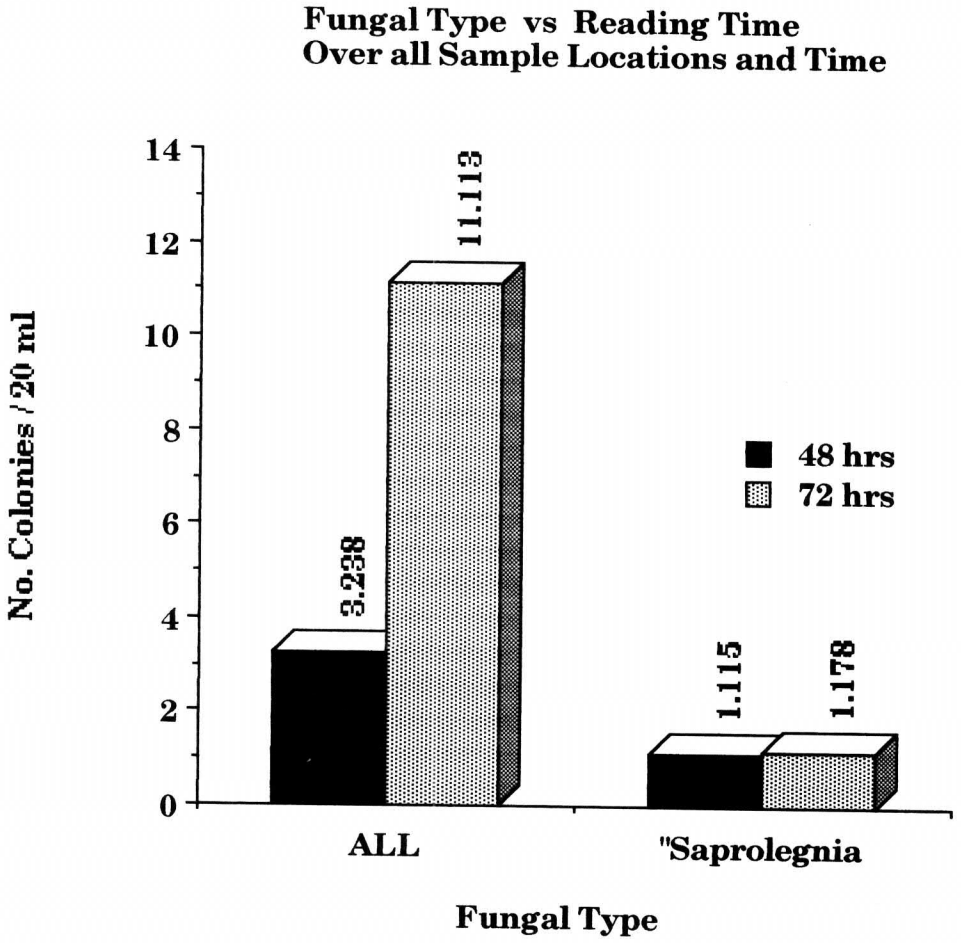


Figure 2. Mean number of fungal colonies observed at 48 and 72 hours incubation summarized over all sampling locations and sample periods from 4 January to 14 May 1993. The means are from 20 ml water samples supplemented with PYG-CA media according to modifications of the procedure of Celio and Padget (1989). " Saprolegnia" represents values from PYG-CA media supplemented with pimaricin.

Table 1. Observations of morphology and growth rate of representative fungal isolates on cornmeal agar (CMA) or on hemp seed in water. 1a) Morphological measurements of fungal structure and hyphal width was determined from CMA colonies, measurements of oogonia and oocytes were determined form hemp seed colonies in water media. 1b) Measurement of growth was determined by change in colony radius (mm/hr) on corn meal agar (CMA) and CMA supplemented with penicillin/streptomycin (CMA-PS).

1a. Morphological measurements (μm)

	Hyphal width	OOgonial Diameter	Oocyte Width
Mean	8.72	53.83	30.38
S.D.	2.66	4.94	4.77
S.E.	1.33	2.02	3.88
Min	6.75	47.30	27.00
Max	12.38	58.50	33.75

1b. Growth (mm/hr)

	Media	
	CMA	CMA-PS
Mean:	0.778	0.614
S.D.	0.041	0.370
S.E.	0.021	0.021

Temporal and Spatial Distribution of Fungi at Abernathy SCTC.

Statistically significant differences were observed in the distribution of "Saprolegnia" in the Abernathy SCTC water system over the course of the five month hatchery production cycle (Figure 3). Fungi were present in relatively low densities in the single pass creek water system (0.025 colonies/20 ml water); no aseptate fungi ("Saprolegnia") were isolated from the well water. Densities of "Saprolegnia" were significantly greater in the reuse water system and varied with time during the production cycle. The dramatic reduction in fungal density observed during the 1 March 1993 sampling period occurred following a partial release of fish from the reuse system which reduced fish biomass (10,011 lbs to 3,046 lbs) and flow index (1.243 to 0.379). Following the decrease in fungal density associated with the fish release, fungal density in the reuse system exhibited a steady increase in density until the final release of fish. While not statistically significant, a trend of greater fungal density in water from the raceway outlets was also observed (Figure 4.)

Effects of Environmental Factors on Fungal Density

Hatchery production records were used to investigate environmental factors influencing fungal density. A relationship was observed when fungal density was plotted against water temperature in the hatchery system (Figure 5). Since fungal density was low at temperatures below 8 °C , mean fungal densities in water at temperatures above and below 8 °C were compared. Significantly greater fungal densities ($p = 0.0001$) were observed above 8 °C than at water temperatures below 8 °C (Figure 6). A preliminary laboratory study was also initiated to further examine the effect of temperature on fungal growth. Nine hemp seed cultures grown from a single isolate of *Saprolegnia* sp. were transferred to the center of cornmeal agar plates to yield triplicate plates for incubation temperatures of 22 °C, 26

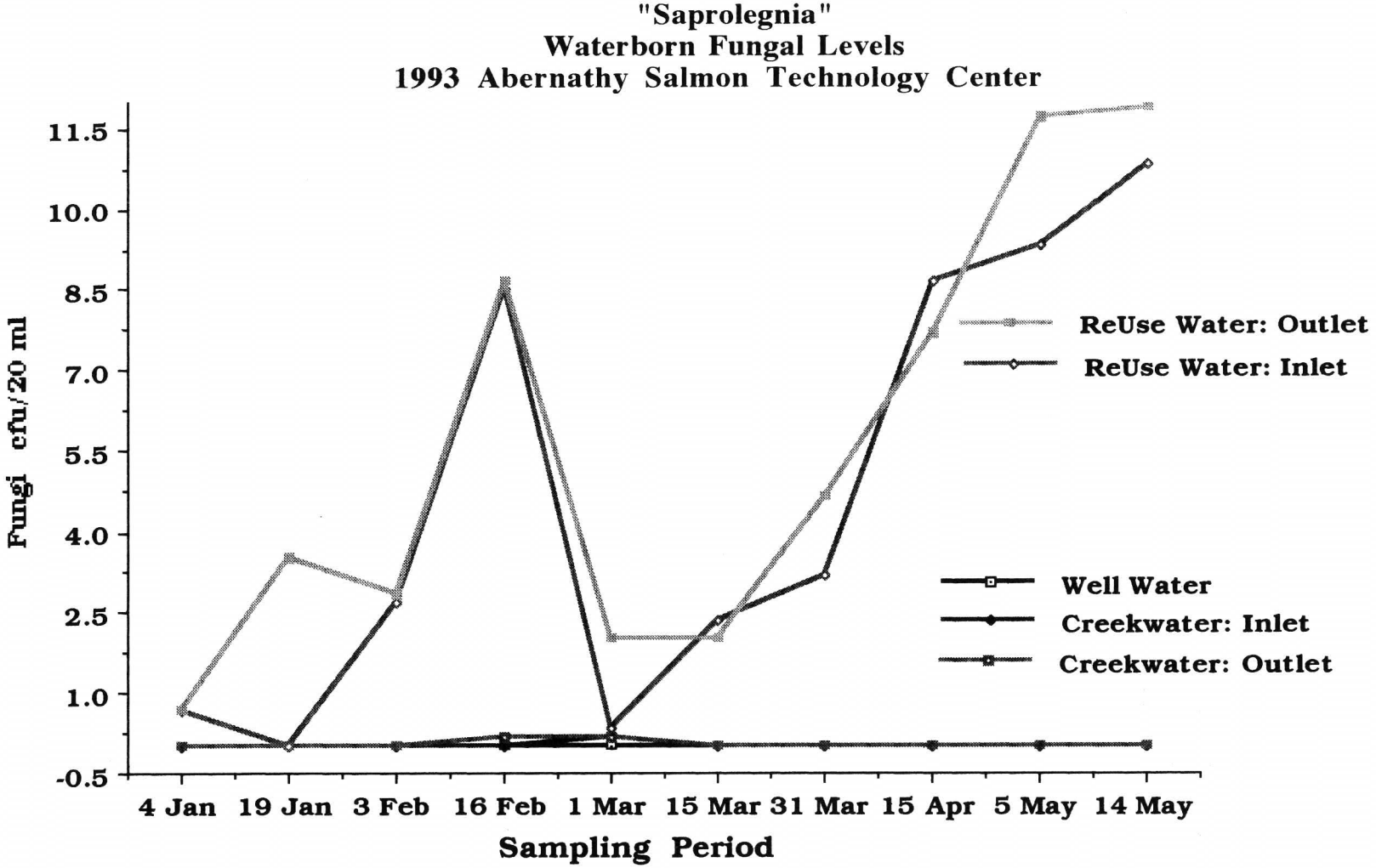


Figure 3. Distribution of "Saprolegnia" at Abernathy Salmon Cultural Technology Center during a production rearing cycle of fall chinook salmon. Values represent the mean of 3 replicate water samples. The value for each replicate represents the mean number of colony forming units observed in duplicate 20 ml aliquotes cultured PYG-CA media supplemented with pimaricin according to procedures of Celio and Padget (1989).

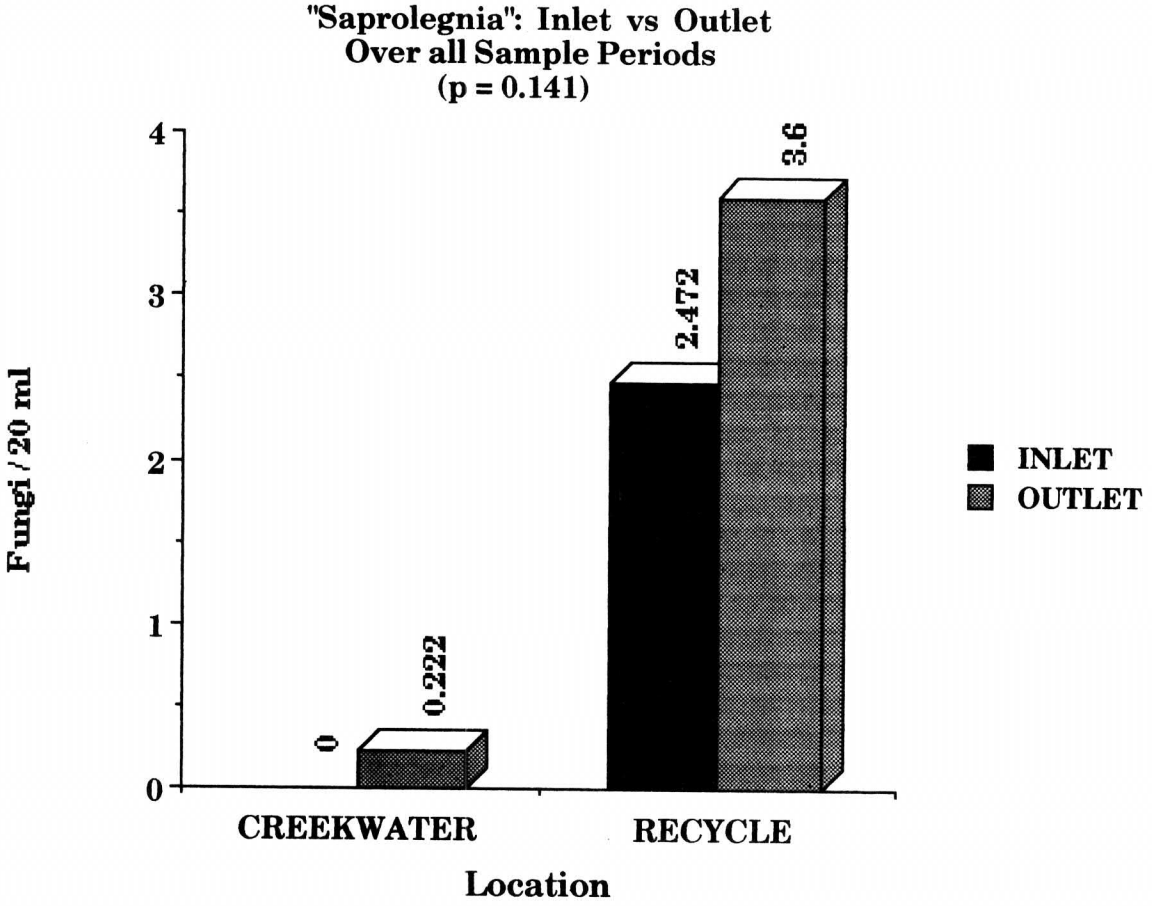
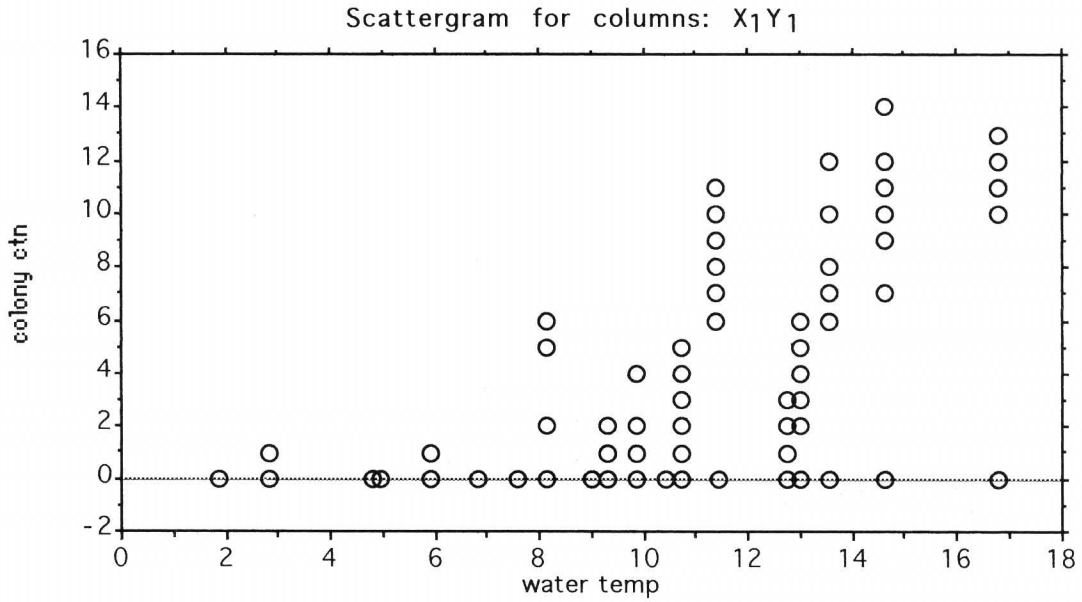


Figure 4. Density of "Saprolegnia" in water sampled at raceway inlets and outlets over all sample periods from 4 January to 14 May 1993. Values represent the mean of 20 ml water samples supplemented with PYG-CA media treated with pimaricin after procedures of Celio and Padget (1989).



Temperature ^{o}C

Figure 5. Fungal density versus water temperature. Fungal density represents the number of fungal colonies isolated from pimaricin treated 20 ml water samples supplemented with PYG-CA media . The data represent isolations over all sampling sites and sampling periods.

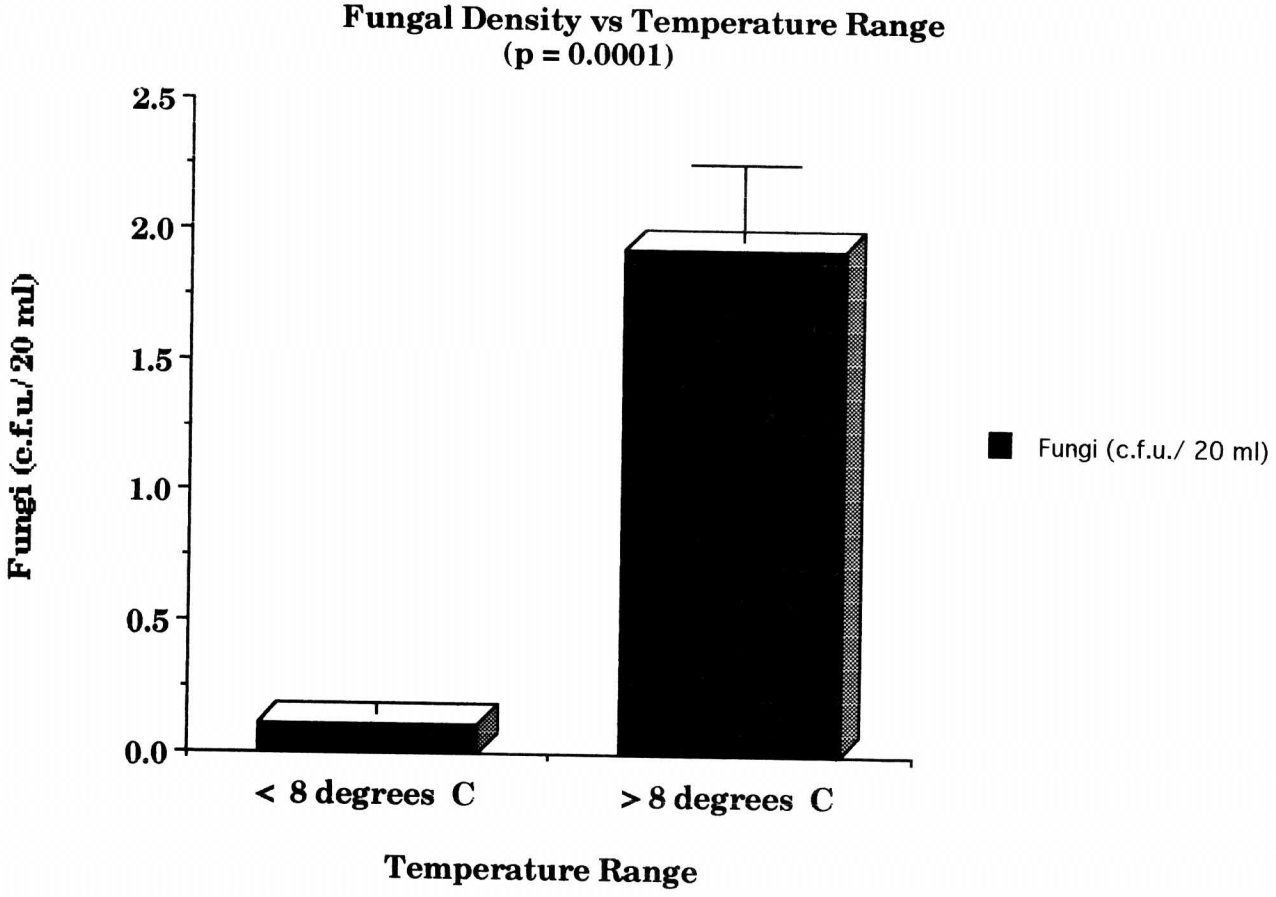


Figure 6. Mean density of aseptate fungal, "Saprolegnia", isolated at water temperatures above and below 8 ^{o}C. Fungal density represents the mean number of fungal colonies isolated from pimaricin treated 20 ml water samples supplemented with PYG-CA media. The results were significant at a level of $p = 0.0001$.

°C and 5.4°C. Results of the growth study indicated a significant reduction in growth of *Saprolegnia* sp. at 5.4 °C (Figure7).

The effect of fish biomass was examined by plotting the number of fungal isolates against fish biomass. The number of fungi generally increased with fish biomass (Figure 6). To further investigate the effects of environmental variables on the density of *Saprolegnia* sp in the Abernathy SCTC system, data from the fungal survey was tested in a linear model incorporating sampling location, historical flow rates in Abernathy Creek, fish biomass (fish weight) and water temperature. Preliminary results form the model indicated that water temperature and fish biomass appeared to exert the greatest influence on fungal density as measured by partial F values (Table 2). Since temperature, fish biomass and fish feeding rate all increase during the production cycle further separation of the effects of environmental variable may be difficult.

Conclusions

The assay system provided a relatively straight forward method to determine fungal density in hatchery system. The survey of the Abernathy SCTC water systems revealed undetectable levels of "Saprolegnia" in the well water supplying the hatchery system. Results of the present survey are reflected by production records from egg rearing which indicate fungal infections of less than 2%. The density of "Saprolegnia" in the reuse water system increased with time during the production cycle and approached densities of 600/liter. The observation thatimilar increases of fish biomass in single pass creekwater did not produce similiar increases in fungal density provides some indication that reuse water or biofilter may serve as a reservoir for fungal infection. The inability to detect "Saprolegnia" in well water may be either an absence of fungi in well water supplying Abernathy SCTC or a reflection of the sensitivity of the fungal assay system. Work during the upcoming year will

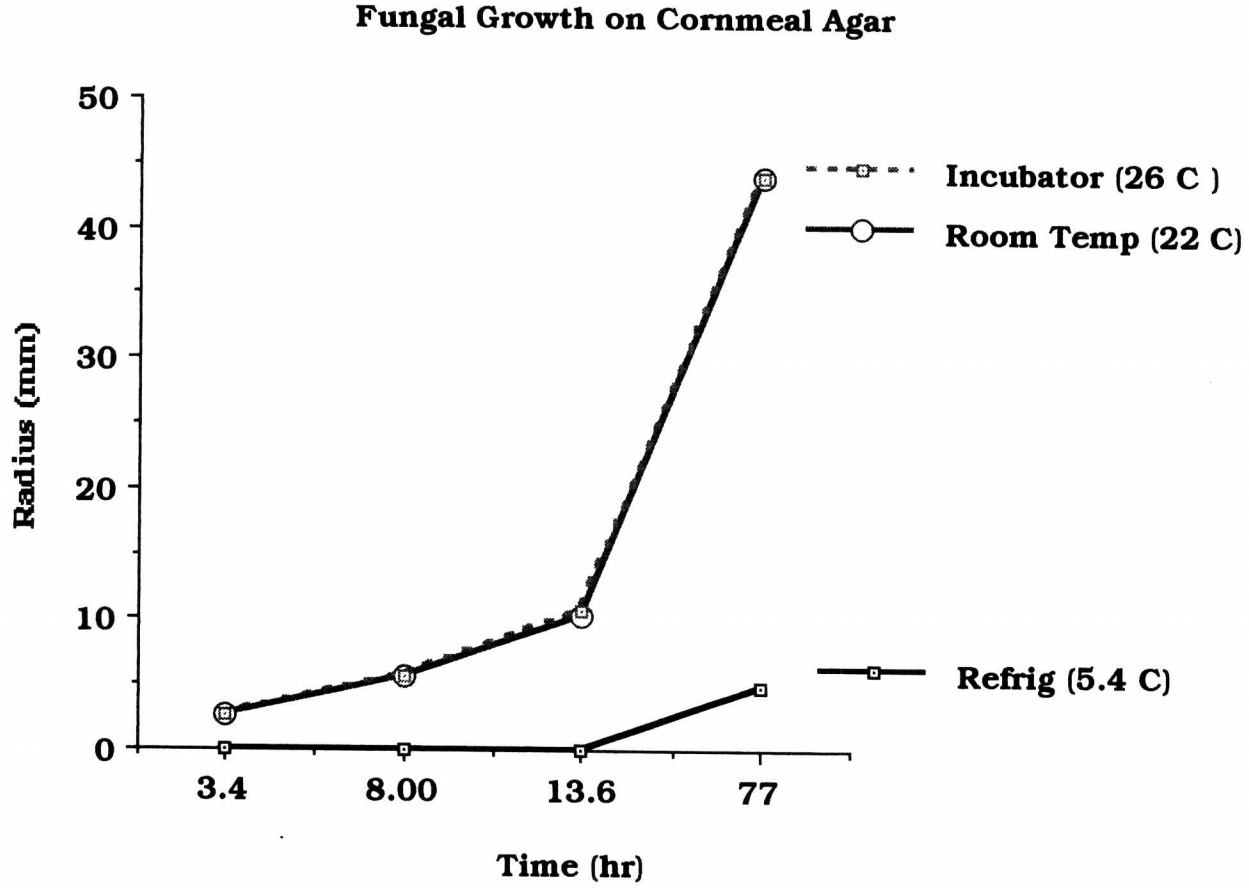


Figure 7. Growth of Saprolegnia sp. at 22 °C, 26 °C and 5.4 °C. Growth was measured by plotting mean colony radius against incubation time. Triplicate cornmeal agar plates for each temperature were inoculated with hemp seed cultures taken from a single Saprolegnia isolate. Plates were incubated in the dark at either room temperature (mean temperature = 22 °C), in a incubator (mean temperature = 26 °C) or in a standard refrigerator (mean temperature = 5.4 °C).

Table 2. Regression analysis of the density of Saprolegnia sp (colony ctn) with the environmental parameters sampling location, historical water flow in Abernathy Creek (hist. flow cfs) water temperature and fish biomass (fish wgt lbs).

Multiple Regression Y_1:colony ctn 4 X variables

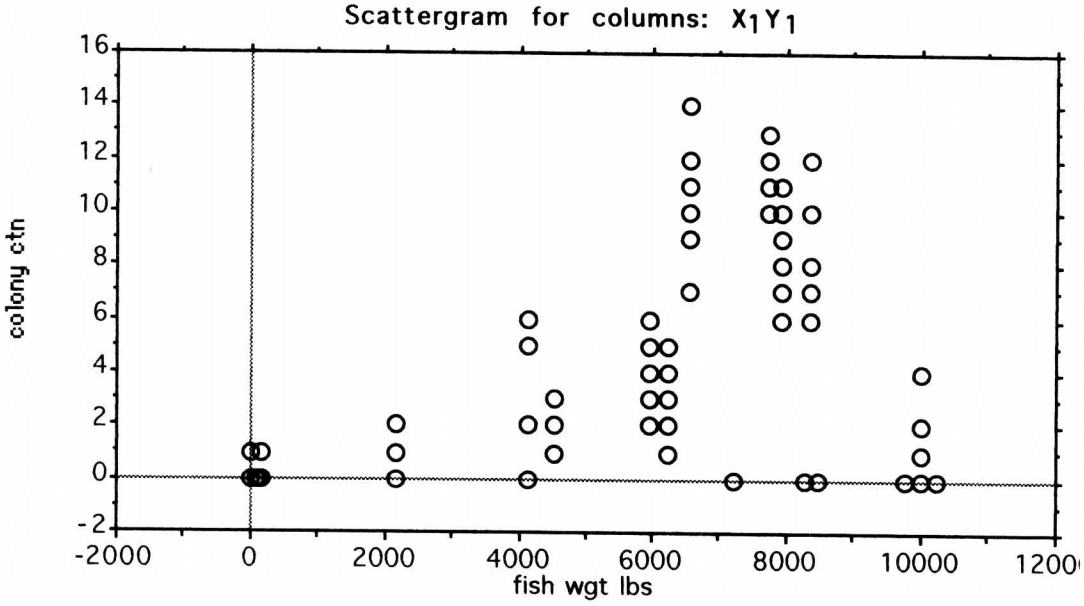
Beta Coefficient Table

Parameter:	Value:	Std. Err.:	Std. Value:	t-Value:	Probability:
INTERCEPT	-3.181				
location	.709	.228	.205	3.111	.0021
hist. flow cfs	-.01	.003	-.17	2.882	.0043
water temp	.708	.085	.703	8.379	.0001
fish wgt lbs	-2.970E-4	5.837E-5	-.277	5.088	.0001

Multiple Regression Y_1:colony ctn 4 X variables

Confidence Intervals and Partial F Table

Parameter:	95% Lower:	95% Upper:	90% Lower:	90% Upper:	Partial F:
INTERCEPT					
location	.26	1.158	.332	1.085	9.677
hist. flow cfs	-.016	-.003	-.015	-.004	8.305
water temp	.542	.875	.568	.848	70.202
fish wgt lbs	-4.120E-4	-1.820E-4	-3.934E-4	-2.006E-4	25.891



Fish Weight (pounds)

Figure 8. Fungal density (colony ctn) versus fish biomass (fish wgt lbs). Fungal density represents the number of fungal colonies isolated from pimaricin treated 20 ml water samples supplemented with PYG-CA media (Celio and Padget 1989). The data represent isolations over all sampling sites and sampling periods.

examine the limits of assay sensitivity and measures to improve sensitivity. Induction of the sexual stages necessary for the speciation of *Saprolegnia* was difficult, time consuming and often unattainable. Improvements in procedures to speciate *Saprolegnia* are essential to a greater understanding of the environmental and cultural practices precipitating fungal infections. Research directed at rapid or more effective diagnostic procedure such as immunological or genetic procedures should be initiated.

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Toxicity of Erythromycin to Juvenile Spring Chinook Salmon

J. C. Faler and C. M. Moffitt
Department of Fish and Wildlife Resources
University of Idaho
Moscow, ID 83843

Abstract -Rations containing erythromycin thiocyanate were fed to replicate groups of yearling spring chinook salmon *Oncorhynchus tshawytscha*, at two temperatures, to determine the toxic effects. Eight diets were formulated to achieve target dosages of 0, 100, 200, and 300 mg erythromycin/kg body weight when fed at 1 or 1.5% body weight at 10 and 14 °C, respectively. Fish were fed rations containing erythromycin for 84 d, followed by 56 d of rations without erythromycin. Fish were sampled at 28 d intervals throughout the trial. Rejection of feed (resulting in failure to achieve actual target dosage), tetany response to a 30 sec net-stress test, and decrease in hematocrit occurred in fish fed erythromycin rations for 28 d. Livers with jaundiced discoloration occurred in fish fed a target dosage of 300 mg erythromycin/kg body weight for 28 d at 10 °C, and fish fed target dosages of 100, 200, and 300 mg erythromycin/kg body weight for 56 d at 10 and 14 °C. Consumption of feed increased, tetany response decreased, and hematocrit levels returned to normal values after 28 d of rations without erythromycin. Liver discoloration was still evident in some fish from all three drug dosages after 56 d of rations without erythromycin, at both 10 and 14 °C.

Introduction

Erythromycin is used experimentally in the prevention and treatment of bacterial kidney disease, caused by *Renibacterium salmoninarum* , in salmonids . Erythromycin is not yet registered with the U.S. Food and Drug Administration (FDA), and can be used only with Investigational New Animal Drug (INAD) permits (Moffitt et al. 1992).

To register erythromycin for use in salmonids, the FDA requires testing to define the safety limits of the drug. In order to define a window of safety, FDA suggests testing dosages of up to five times the optimal level and three times the optimal duration. Previous dose titration tests (Moffitt 1991) and other studies (Wolf and Dunbar 1959; Austin 1985) indicated 100 mg erythromycin/kg body weight may be an optimal dose. Optimal duration of therapy may be 28 d, confirmed in recently completed studies in our labs (Moffitt et al. 1992). Erythromycin feed is unpalatable (Schreck and Moffitt 1987) and even at daily dosages of 100 mg/kg fish reject rations and do not achieve the target dosage (Piper 1961; Moffitt et al. 1992). FDA therefore concurred with us in testing to

only three times the optimal dosage.

We observed the toxic effects of feeding erythromycin thiocyanate at target daily dosages of 100, 200, and 300 mg/kg to juvenile spring chinook salmon *Oncorhynchus tshawytscha* for 84 d, at 10 and 14 °C. We also collected data from hatcheries participating in field trials under INAD 4333 on the toxic effects of feeding erythromycin.

Methods

Laboratory Tests

Yearling spring chinook salmon from Leavenworth National Fish Hatchery were transported to the University of Idaho in January, 1993, for the study. Upon arrival at the University, fish were distributed randomly to twenty, 340 L circular tanks and redistributed one week later to all 26 tanks, approximately 100 fish per tank. Fish were fed BioDiet grower 3-mm pellets (Bioproducts, Warrenton, Oregon) twice daily for 5 weeks before the start of medicated rations. After 3 weeks of acclimation, 10 fish were sampled from each tank to develop a profile of the health of fish at the start of trials. Each group of 10 fish was tested for response to handling stress. Fish were removed by net from the tank to a square bucket. All ten fish were netted out of the bucket at the same time, and held in the air for 30 sec, before returning to the bucket. Fish were then observed for 5 min for any signs of adverse reaction (toxicity) including arched back and stiff spinal column, abnormal swimming behavior, discoloration or bruising, and death. Fish were then anesthetized with tricane methane sulfonate, weighed (0.1g) and measured for total length (mm), assessed visually for external condition, and the first three fish preserved for histology. Blood was collected from a severed caudal artery from each of the remaining seven fish from each tank. We determined percent red blood cells (hematocrit) and froze the separated plasma fraction for later assay. Each fish was inspected visually for internal condition, the liver weighed (0.0001g), and kidneys removed and frozen at -80 °C for ELISA testing of soluble antigen to *Renibacterium salmoninarum* .

Using the data on length and weights of fish examined before tests began, we kept only 70 fish in each tank between 115 and 150 mm. Water temperatures were adjusted so that half the tanks were maintained at 10 or 14 °C. Fish were acclimated to these temperatures for 2 weeks, and fed at 1 and 1.5% body weight, respectively. Drug treatments were then assigned randomly to tanks within temperature groups: three replicate tanks for each target drug dosage of 100, 200, and 300 mg/kg, two replicate tanks for fish administered rations without erythromycin at target percent body weight, and two replicate tanks for fish administered rations without erythromycin at reduced (50% or less) levels. We used a reduced ration control to distinguish between effects caused by erythromycin, and those caused by rejection of feed (starvation).

We began feeding experimental rations 10 - 12 March, starting one of the three replicate drug treatment tanks of fish each day. The two replicates of each control treatment were started on the first and third days. We formulated rations for these trials at Bioproducts in Warrenton, Oregon (Table 1), using the proposed new carrier produced by Sanofi Animal Health (Overland Park, KS). If fish fed erythromycin rations

consumed all of their daily rations in each temperature, they would attain dosages of 100, 200, and 300 mg/kg. All diets including control diets were formulated with equivalent amounts of carrier so that the feeds were nutritionally comparable. We used the amount of carrier necessary for target dosages of 100 mg erythromycin/kg body weight. The additional erythromycin thiocyanate in the rations of higher dosage was added without carrier directly to the rations.

Table 1. Percent body weight fed, target daily dosage of erythromycin, percent erythromycin in feed, and amount of premix used to achieve target dosage. Rations without erythromycin ([a]) were formulated with wheat germ carrier without erythromycin.

		Erythromycin		
Water temp. (°C)	% Body wt. fed daily	Target daily dosage (mg/kg)	Percent in feed	Percent premix in feed
14	1.5	100	0.67	3.3
		200	1.34	3.3
		300	2.00	3.3
		0	0.00	3.3[a]
10	1.0	100	1.00	5.0
		200	2.00	5.0
		300	3.00	5.0
		0	0.00	5.0[a]

Ten fish per tank were sampled on 7 - 9 April, 5 - 7 May, and 2 - 4 June, after 28, 56, and 84 d of continuous feeding of rations with erythromycin. Parameters examined at sampling were similar to those used before the trial began, with the first, fourth, and eighth fish preserved for histology. Liver and kidneys of remaining fish were removed and frozen individually (-20 °C) for future analysis of erythromycin.

After 84 d of erythromycin rations, all fish were fed rations of BioDiet Grower 3-mm pellets without erythromycin, for 56 d. Fish previously fed rations without erythromycin at reduced levels were fed full rations for the final 56 d. Fish were sampled during this time on 30 June - 2 July, and 28 - 30 July, after 28 and 56 d of rations without erythromycin. Sampling procedures were the same as those outlined for sampling during drug treatment. At the end sampling, all fish remaining in each tank were killed.

Field Tests

In 1993, participants at 52 hatcheries in AK, WA, OR, ID, and CA, conducted 143 tests on fish fed rations containing erythromycin thiocyanate, and reported the results to the University of Idaho. Field tests were similar to lab tests except the 30 sec net-stress was omitted. Field tests required the removal of 10 fish at a time from the treated raceway

or pond to a bucket of water for 5 min of observation, repeated up to 6 times. Recorded observations were the same as those performed in the laboratory: arched back and stiff spinal column, abnormal swimming behavior, discoloration or bruising, death. Participants also reported the species of fish tested, percentage body weight fed, percent erythromycin thiocyanate used, and length and dates of drug treatment.

Results

Laboratory Tests

The percentage of fish observed with a toxic response following the net-stress test increased from less than 1% before start of erythromycin rations, to 50% of fish administered target rations of 100 mg/kg, and 67 - 77% of fish administered target rations of 200 or 300 mg/kg for 28 d at both 10 and 14 °C (Figure 1). A decrease in toxic response was observed after 56 d of erythromycin rations, and response was maintained at comparable levels after 84 d in all but one of the drug treatments. Fish administered a target ration of 300 mg/kg at 10 °C maintained high levels (73-76%) of toxic response after 28, 56, and 84 d of erythromycin rations. Upon resumption of normal rations without erythromycin, the toxic response of fish from all drug treatments dropped to near levels recorded in control fish.

Percent red blood cells (hematocrit) decreased during the period of administration of erythromycin in all test groups except fish fed 100 mg erythromycin/kg at 14 °C. Upon resumption of normal rations without erythromycin, hematocrit values in samples of fish in each drug dosage group increased to normal values (Table 2).

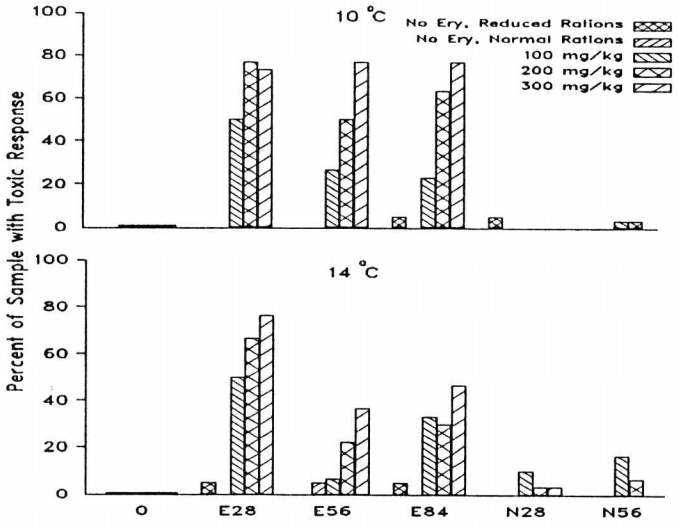

Figure 1. Percent of fish exhibiting toxic response in standardized 30 sec net-stress toxicity tests conducted at intervals throughout the trials. Fish were sampled from each tank before feeding of erythromycin rations began (0), during the administration of erythromycin (E28, E56, E84), and at two, 28 d intervals after cessation of erythromycin when fish were administered normal rations (N28, N56).

Table 2. Summary of average percent packed red blood cells (hematocrit) in fish sampled from tanks held at 10° and 14 °C, by dosage group and sampling interval 1993. Fish were sampled from tanks before feeding of erythromycin began (pretrial), following 28, 56, and 84 d of administration, and then following 28 and 56 d of normal rations.

Sample after days during or post treatment	Normal rations		Reduced rations		Erythromycin dosage mg/kg 100		200		300	
	Temperature °C									
	10	14	10	14	10	14	10	14	10	14
Pretrial	36.9	35.2	37.8	39.6	37.7	35.7	35.3	36.5	35.0	35.5
28	40.6	46.4	40.7	48.7	28.3	37.9	28.6	36.1	28.6	32.5
56	46.1	42.7	46.6	46.6	20.9	32.7	25.0	31.5	24.7	24.7
84	48.6	43.2	50.5	46.9	21.5	37.3	18.3	30.6	22.8	24.2
28 Post	49.6	47.7	45.4	43.6	38.5	44.7	35.4	41.0	34.7	38.7
56 Post	43.6	42.9	43.1	42.7	46.1	41.9	37.6	38.2	36.7	39.3

At each sampling interval, we evaluated the condition of livers in the ten fish sampled from each tank using the Goede index (Goede and Barton 1990). Livers that were dark reddish-brown to light tan were characterized as healthy, pale to white as pale, large patchy discolorations as general discoloration, and discreet hemorrhages or discolorations as focal discoloration. Two codes were added for use in fish fed erythromycin rations: yellow/jaundiced for livers that were tinged yellow to very distinct yellow throughout, and green for livers that were tinged green to very dark green throughout the liver. Ten to 20% of fish fed rations with erythromycin and held at 10 °C had visible yellow or green livers at the end of the 84 d erythromycin treatment period (Figure 2).

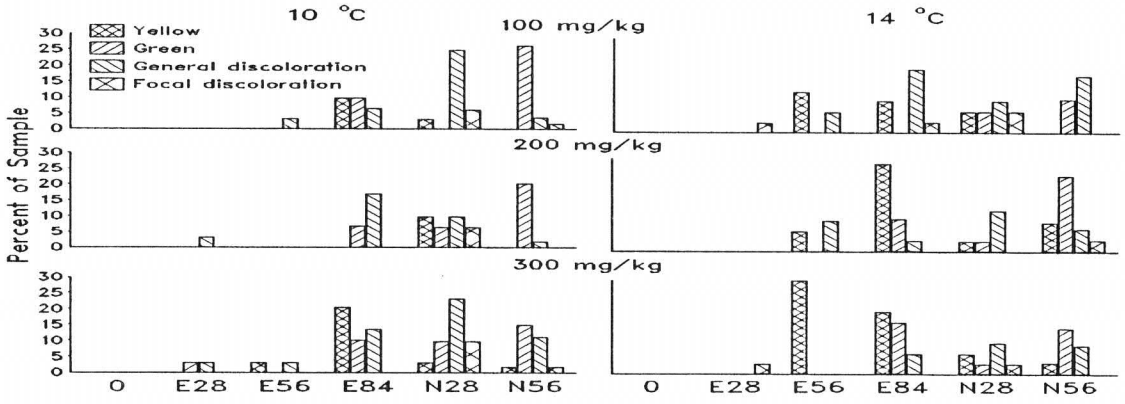

Figure 2. Percent of fish with abnormal livers by sampling interval. Fish were administered erythromycin rations at one of three target dosages for 84 d. Fish were sampled from each tank at 28 d intervals during administration of erythromycin (E28, E56, E84), and after cessation of erythromycin when fish were administered normal rations (N28, N56).

Less than 5% of fish administered the highest dosage at 10 °C had yellow or green livers at the earlier sampling periods, 28 and 56 d after administration of erythromycin rations. A portion of fish from all three drug dosage groups had yellow or green livers after 28 and 56 d of rations without erythromycin. Fish fed erythromycin rations at 14 °C had visible yellow livers in samples examined after 56 and 84 d of continuous feeding (Figure 2). Fish with yellow and green livers were still observed in samples removed from these groups 28 and 56 d after the erythromycin therapy ceased. Livers of fish administered rations without erythromycin had less than 6% general or focal discoloration observed toward the end of the rearing period (Figure 3), with 1/36 observed with a slightly yellow liver among fish sampled from groups fed reduced or normal rations.

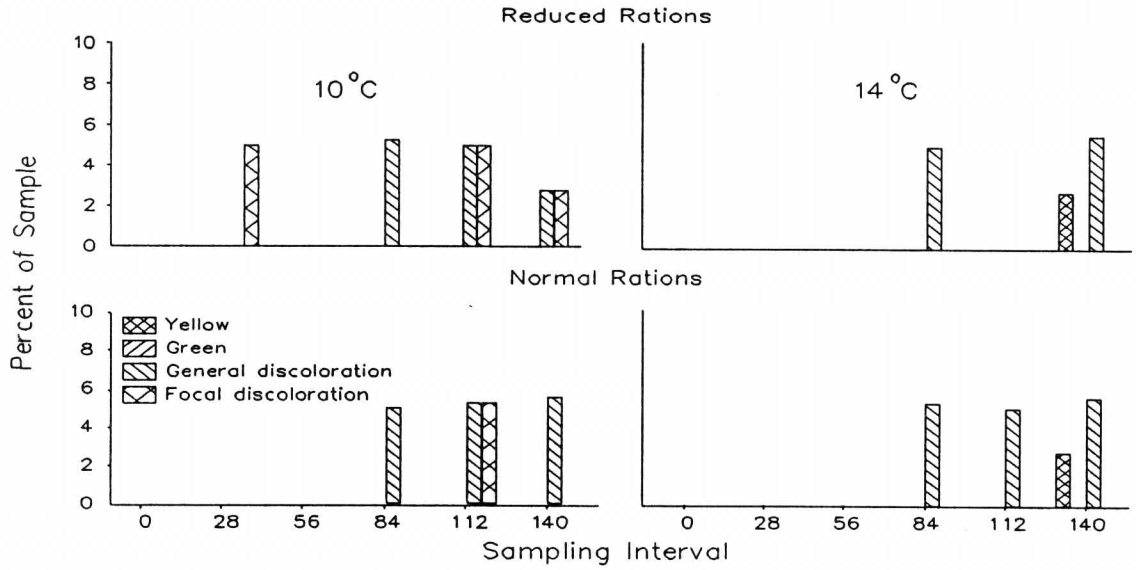

Figure 3. Percent of fish with abnormal livers by sampling interval. Fish were administered rations without erythromycin at 1 or 1.5% body weight (normal rations) for trials at 10 and 14 °C, respectively. For the first 84 d of the trial, additional groups of fish were administered rations without erythromycin at approximately 0.5 and 0.75% body weight (reduced rations).

Fish held at 10 and 14 °C and administered rations with erythromycin rejected portions of their feed throughout the therapy. Fish administered target rations of 100, 200, and 300 mg erythromycin/kg body weight consumed an average of 79 - 89%, 56 - 58%, and 44 - 48% of their rations respectively during the initial 28 d of the trial (Figure 4). This pattern of feed consumption continued through the 84 d of feeding rations with erythromycin. When the fish were returned to rations without erythromycin, fish in all treatment groups increased their consumption to levels of fish fed diets without erythromycin.

Field Tests

Toxicity was reported in 34 tests at 20 sites in ID, OR, and WA (Table 3). By correlating number of fish/lb with toxicity, we found that larger fish exhibited increased severity of response (Figure 5a). A higher percentage of tests show a toxic response in the early months of the year and in the fall months, with increased severity of response within a test occurring in the fall (Figure 5b).

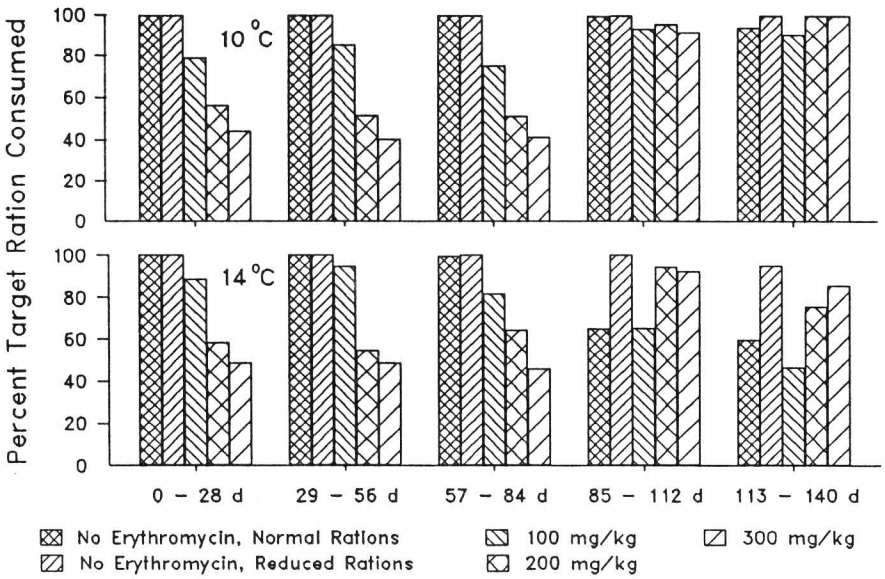

Figure 4. Consumption of rations summarized by 28 d intervals over the 140 d trial. Target rations were calculated based on 1.0 and 1.5% body weight for the trials at 10 and 14 °C, respectively.

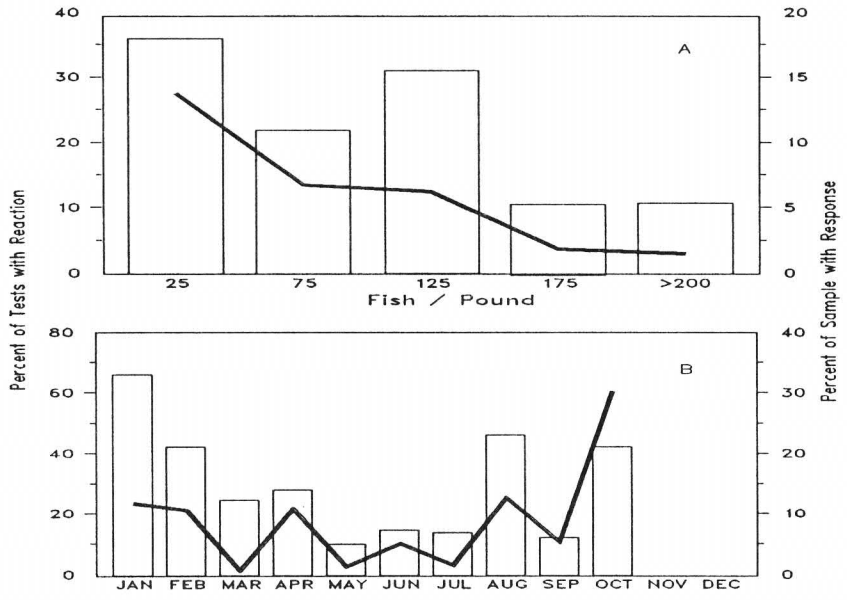

Figure 5.a. Percent of all field trials showing toxicity by fish size (bars). Percent of fish in each sample with the toxic response is represented by solid line (right-hand scale).
b. Percent of all trials showing toxicity by time of year (bars). Tests were conducted January - October, 1993. The percent of fish in the sample showing a toxic reaction is shown by right hand scale, solid line.

Table 3. Summary of hatchery sites that reported tetany or bruising of fish following standardized toxicity testing after feeding erythromycin rations. Fish species listed are: SprCk = spring chinook salmon; SumCk = Summer chinook salmon; coho = coho salmon; Fallck = fall chinook. Percent carrier is expressed as percentage Gallimycin 50P or [a]the proposed new carrier, Aquamycin.

State	Hatchery name	Fish species	#Fish/lb	Percent body weight fed	Percent carrier	Water temp (°F)	Date	Percent toxicity
ID	Clearwater	SprCk	70	2.4	4.5[a]	50	23 Jul	2
	Kooskia (USFWS)	SprCk	145	2.2	2.25[a]	47	11 May	6
OR	Salmon River	coho	217	2.7	4.58	55	30 May	2
		coho	175	2.3	4.58	55	30 May	2
	Umatilla	SprCk	10	1	4.5	52	23 Feb	2
		SprCk	44	3	4.5	53	23 Apr	25
	Willamette	SprCk	70	2.2	2.25[a]	54	03 Aug	8
WA	Little White Salmon (USFWS)	SprCk	66	1.9	2.25[a]	46	28 Jun	3
	Deschutes Complex	FallCk	19	0.8	9	46	08 Mar	3
	Dungeness	FallCk	226	2	4.5	47	23 Jun	28
	Eastbank	SumCk	150	2	4.5	48	29 Apr	13
		SumCk		2	4.5		03 Aug	7
		SumCk	180	2	4.5	49	04 Aug	32
	Hood Canal	SprCk	34	2	4.5	48	20 Aug	2
	Hupp Springs	SprCk	26	1	4.5[a]	48	07 Oct	18
	Kalama Falls	SprCk	235	2	4.5	44	16 Apr	2
	Klickitat	SprCk	41	1	9	56	16 Sep	42
	Lewis River	SprCk	9	0.8	9	42	12 Feb	25
		SprCk	7.5	0.7	9	42	13 Feb	47
		SprCk	57	1.7	4.5	53	25 Jul	17
		SprCk	54	1.7	4.5	54	01 Aug	63
		SprCk	20	1.7	4.5	57	10 Oct	98
		SprCk	20	1.7	4.5	57	10 Oct	95
	Lower Kalama	SprCk	33	2	4.5	60	30 Aug	2
	Nemah	coho	36	1	9	34	11 Jan	2
		FallCk	134	2	4.5	53	16 Jun	8
		FallCk	50	2	4.5	60	23 Aug	75
	Skagit	SprCk	138	2	4.5	44	30 Apr	37
		SumCk	130	2	4.5	48	10 May	35
	Skykomish	SumCk	70	2	2.25[a]	52	16 Jun	88
	Soleduck	SprCk	320	2	4.5	46	14 Mar	2
		coho	14	2	9	45	10 Apr	67
		coho	14	2	9	45	24 Apr	67
	Speelyai	SprCk	11	0.7	9	46	31 Jan	33

Discussion

Maximum response to a net-stress toxicity test in lab trials occurred after 28 d of erythromycin rations. Tests after 56 and 84 d of erythromycin rations yielded similar (fish held at 10 °C) or lower (fish held at 14 °C) values. The decrease in toxic response (except in those fish fed 300 mg/kg at 10 °C) may indicate adaptation of the fish to the drug, perhaps as a result of increased metabolism of the drug. Laboratory results of toxic response were similar to those of field tests. The yearling salmon used in the laboratory study were at 15 fish/lb after 28 d of rations of 100 mg erythromycin/kg body weight. In 6 net-stress toxicity tests (3 replicates at 2 temperatures) of these yearlings, an average of 50% of the fish in each test showed evidence of toxic response (tetany and/or bruising). In another study not discussed in this paper (unpublished data, Faler and Moffitt), fry at 197 fish/lb that had been fed rations containing 100 mg erythromycin/kg body weight for 28 d had no toxic response in any of three tests. These results follow the relationship found in hatchery field trials of fish weight vs toxic response in Figure 5a. The juvenile fish in our laboratory exhibited a higher response than those reported in the field trials most likely due to the added 30 sec net-stress test.

Erythromycin is concentrated in the liver (Moffitt and Schreck 1988) and other organs, and excreted into the bile as an active drug; some of the drug is N-demethylated in the liver microsomal system (Periti et al. 1989; Guarino and Lech 1986). Previous studies have demonstrated cellular damage to the kidney, but not to the liver of fish administered erythromycin rations (Piper 1961; Warren 1963; Hicks and Geraci 1984). However, excessive amounts of erythromycin and/or increased duration of administration of the drug might lead to liver damage. Liver discoloration was evident in this study in the form of yellow or green livers in fish administered erythromycin rations: after 28 d of erythromycin rations (fish fed 300 mg/kg at 10 °C), and extending to 56 d after fish were fed normal rations without erythromycin (fish fed 100, 200, or 300 mg/kg at both 10 and 14 °C). We believe the yellow livers may be due to an excess of bilirubin (Endo et al. 1992), and accumulation of bile pigments in the bile ducts; and the green livers may reflect severe levels.

Schreck and Moffitt (1987) showed increased rejection of feed with increasing dosages of erythromycin. We also saw increased rejection of feed with increasing dosage, at both 10 and 14 °C. Rejection of feed occurred in fish during the first 28 d of drug treatment, and continued at similar levels throughout the 84 d trial. When the fish were fed rations without erythromycin, fish in all treatment groups increased their consumption of feed to levels similar to fish fed diets without erythromycin. However, fish held at 14 °C and administered normal rations and or fed rations containing daily dosages of 100 mg erythromycin/kg body weight did not increase consumption at the end of the trial. These two groups of fish had consumed more earlier in the trial and were larger than the fish in the other treatment groups, making the 1.5% body weight feeding rate inappropriate for them.

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Erythromycin and Oxytetracycline Injections in Adult Chinook Salmon Challenged with *Renibacterium salmoninarum*

A. H. Haukenes and C. M. Moffitt
Department of Fish an Wildlife Resources
University of Idaho
Moscow, Idaho 83843

Abstract -Adult chinook salmon were challenged with *Renibacterium salmoninarum* (Rs) and treated with 1 - 3 injections of erythromycin at dosages of 10, 20, and 40 mg/kg. We compared percent survival, quantity of circulating Rs antigen determined by ELISA, and number of Rs cells found in coelomic fluid in erythromycin treated fish with Rs challenged and non-challenged fish injected with no erythromycin. In response to an episode of furunculosis all surviving fish, including those receiving no erythromycin, were treated with 3 injections of oxytetracycline (12 mg/kg, 12 mg/kg, and 6 mg/kg). Percent survival of Rs challenged fish treated with erythromycin and oxytetracycline was similar to survival of fish not challenged and was significantly higher than the survival of Rs challenged fish receiving oxytetracycline only. The quantity of circulating antigen in Rs challenged fish treated with both erythromycin and oxytetracycline was similar to antigen levels in non-challenged fish and was significantly lower than levels in Rs challenged fish treated with oxytetracycline only. In Rs challenged female fish treated with erythromycin the number Rs cells/mL in the coelomic fluid was higher (90 - 368 x 10^3) than in female fish not challenged (2 x 10^3).

Introduction

Injection of erythromycin into pre-spawning salmon has shown to be effective in controlling mortality from bacterial kidney disease (Groman and Klontz 1983). Deposition of erythromycin in the eggs of the female and retention in the yolk of the developing embryo may decrease the vertical transmission of *Renibacterium salmoninarum* (Bullock and Leek 1986, Evelyn et al. 1986). Studies of juvenile salmon fed erythromycin prior to an acute challenge of *R. salmoninarum* (Rs) have shown erythromycin effective as prophylactic against bacterial kidney disease (Moffitt and Bjornn 1992). To gain FDA approval of erythromycin as an injectable drug, controlled dose titration experiments must be completed in maturing salmon to demonstrate the efficacy of injecting erythromycin in fish challenged with Rs. The objectives of this study were: to show the efficacy of erythromycin injections to control pre-spawning mortality due to bacterial kidney disease; to illustrate any differences in survival or other fish health indices between erythromycin dosages of 10, 20, or 40 mg/kg applied 1, 2, or 3 times before spawning; and to evaluate erythromycin as a control for vertical transmission of Rs, the causative agent for BKD.

Methods

Adult spring chinook salmon were transported from Cowlitz Salmon Hatchery, Salkum, Washington, to the University of Idaho Aquaculture Laboratory in June, 1991 and

distributed among 12, 3.6 m, circular tanks supplied with 1900 L/m water flow. To determine fish weights, collect baseline information on fish health, and inject fish with a Rs challenge, we crowded the fish in the tank, anesthetized the fish with tricaine (50 mg/L), and assigned each fish to one of eleven treatment groups. We then weighed and tagged each fish with jaw tags coded with a unique number and color coded cable ties around the caudal peduncle. A 2 - 3 mL blood sample was drawn from the caudal artery and the plasma portion saved for later ELISA assay of Rs antigen using Kirkegard and Perry reagents (Pascho et al. 1991). After the blood sample was taken, depending on fish size, 2 or 3 mL of a 3 x 10^9 cells/mL suspension of Rs in sterile PBS-peptone, prepared as in Moffitt (1992), was injected into the peritoneal cavity. Two fish in each tank were not challenged with Rs but were injected with 2 or 3 mL of sterile PBS-peptone as a placebo challenge.

The antibiotic used in dose titration studies was Erythro-200 (Sanofi Animal Health, Inc., Overland Park, KS). Fish receiving carrier were injected with a solution of PEG-400, ethyl acetate, and ethyl alcohol, resembling the drug carrier in Erythro-200. We assigned two fish in each tank to one of nine erythromycin treatments that included all combinations of erythromycin dosage (10, 20, or 40 mg/kg) and frequency of injection (1, 2, or 3). An additional two fish per tank were challenged with Rs and not injected with erythromycin and two fish per tank received a placebo challenge and received no erythromycin treatment. On 24 June, four days following the bacterial challenge we began injections. The fish in each tank were crowded, anesthetized, a blood sample taken as before, and the appropriate volume of Erythro-200 or carrier was injected into the peritoneal cavity. This process was repeated twice at 24 d intervals. We injected drug carrier as a substitute for erythromycin in fish receiving 1 of 2 injections. Throughout the study, fish were observed at least twice a day and dead fish were removed and examined for signs of disease.

Acute mortality of fish attributed to furunculosis, caused by *Aeromonas salmonicida*, within the first 15 d after the Rs challenge prompted the injection, on 10 July, of all experimental fish with 12 mg/kg of oxytetracycline (Pfizer, Inc. Lee's Summit, MD) into the dorsal sinus. All surviving fish in the study were injected again with oxytetracycline two more times when the fish were sampled and injected with erythromycin or carrier. Dosages of oxytetracycline at these dates were 12 mg/kg and 6 mg/kg, respectively.

After the third injection, starting on 30 August, all fish were crowded and anesthetized as before and inspected weekly to determine sexual maturity. A blood sample was removed from all fish surviving to the first spawning for analysis of Rs antigen by ELISA assay. The prevalence of Rs (cells/mL) found in the ovarian fluid was measured using Membrane Filter FAT (Elliot and Barila 1987). Daily percent survival for each of the treatments was calculated for each of the treatment groups. Differences between treatments in: arcsine transformed percent survival to the first spawn date; the ELISA optical density values; and the number of Rs cells/mL coelomic fluid; were determined by ANOVA using general linear model procedures. In all analyses the level of

significance was set at $P \leq 0.05$.

Results

Rapid mortality following the initiation of the experiment was most severe in Rs challenged fish not injected with erythromycin (Figure 1). The pattern of mortality and clinical symptoms of large external lesions and reddened viscera were followed by isolation of *A. salmonicida* from the kidneys of these fish.

The first injection of oxytetracycline immediately halted mortality from furunculosis in both groups of fish that received no erythromycin (Figure 1) but continued oxytetracycline injections in the Rs challenged fish were less effective than in the fish injected with a placebo challenge. For all erythromycin treated groups the first injection of oxytetracycline decreased mortality similar to fish injected with the placebo challenge (Figure 2).

Percent survival of fish challenged with Rs and injected with erythromycin was significantly higher than survival of Rs challenged fish not administered erythromycin in all treatment groups except for fish injected twice with 20 mg/kg (Table 1). The overall mean survival of fish challenged with Rs and treated with erythromycin (62%) was similar to the survival of non-challenged fish (67%) and higher than fish challenge with live Rs and injected with oxytetracycline only (13%).

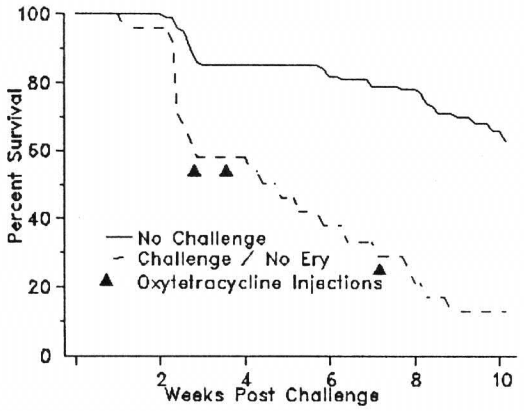

Figure 1. -Percent survival of adult chinook salmon not injected with erythromycin and challenged with a sterile placebo or live Rs. Timing of injections of oxytetracycline administered to all fish during dose titration tests, 1991.

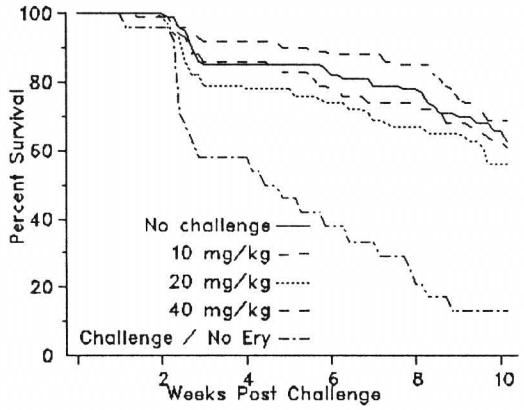

Figure 2. -Percent survival of adult chinook salmon summarized by dosage of erythromycin. Percent survival of dosage groups are pooled across 1, 2, or 3 injections.

Table 1. Percent survival of fish by treatment group at the time of first spawning, summarized by dosage of erythromycin and frequency of injection. Letters in parenthesis (A, B, C) next to percentages correspond with significantly different groupings ($P \leq 0.05$) by ANOVA.

Erythromycin dosage (mg/kg)	Frequency of injection: One	Two	Three	Mean percent survival
R. salmoninarum challenged				
10	63 (AB)	60 (AB)	63 (AB)	61
20	50 (AB)	42 (BC)	75 (A)	56
40	75 (AB)	58 (AB)	69 (A)	69
Means	63	53	71	62
0 (carrier only)				13 (C)
No challenge				
0 (carrier only)				67 (AB)

On the date of bacterial challenge, and at the first injection, all fish had similar levels of circulating Rs antigen determined by ELISA assay (Figure 3). On the second injection/sampling, 27 d after Rs challenge, the levels of circulating antigen were significantly lower in fish challenged with Rs and injected with erythromycin than levels in Rs challenged fish not injected with erythromycin. Circulating antigen in Rs challenged fish not injected with erythromycin remained significantly higher than antigen in erythromycin treated fish and in fish injected with a placebo challenge at the third injection/sampling and at the first spawning.

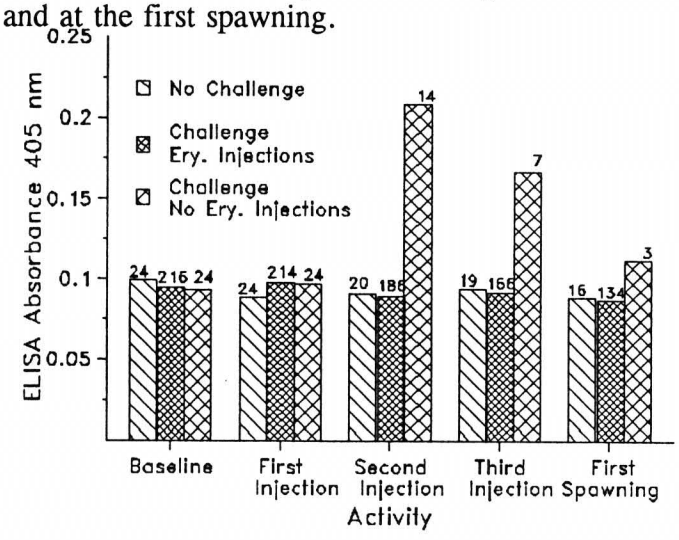

Figure 3. -Mean optical density determined by ELISA of soluble Rs antigen in the plasma of adult chinook salmon sampled before Rs challenge, on each injection date, and at the first spawning date grouped by treatments. The number above each bar represents the number of fish sampled.

The number of bacteria found in the coelomic fluid of spawning female salmon was higher than in female salmon not challenged: 90 - 368 x 10^3 vs 2 x 10^3. All female fish challenged with Rs and injected with oxytetracycline only died before the first spawning date (Table 2).

Table 2. -Average number of Rs cells/mL x 10^3 found in samples of coelomic fluid of female adult chinook salmon surviving to spawn determined by florescent antibody microscopy. No females challenged with Rs and not injected with erythromycin survived to spawning. Numbers in parenthesis indicate number of fish sampled.

Erythromycin Dosage (mg/kg)	Frequency of Injection		
	One	Two	Three
10	368 (3)	227 (3)	297 (4)
20	340 (6)	279 (3)	120 (5)
40	90 (3)	249 (2)	157 (5)
0 (no Rs challenge)	-	-	2 (10)

Discussion

Before the first injection of oxytetracycline, the mortality attributed to furunculosis in fish challenged with Rs was most severe in the fish not injected with erythromycin, indicating erythromycin's limited protection against *A. salmonicida*. Bacterial kidney disease may diminish the host response to other pathogens or erythromycin could act as an immunostimulant (Fraschini et al. 1986), but by controlling bacterial kidney disease in acutely challenged adult chinook salmon, mortality from furunculosis was reduced. Among fish administered erythromycin more fish injected twice with 20 mg/kg died during the tests and percent survival was not significantly different from the Rs challenged fish not injected with erythromycin. The efficacy that erythromycin had in controlling *A. salmonicida* may not be a quantitative response dependent upon erythromycin dosage but rather act as a non-specific protection against other fish pathogens. This may account for the higher survival in fish receiving smaller dosages of erythromycin. Injecting Rs challenged fish with oxytetracycline only was not successful in keeping any female fish alive to spawning.

Injections of erythromycin in salmon challenged with live Rs controlled mortality attributable to bacterial kidney disease. Average percent survival to first spawning of Rs challenged fish injected with erythromycin was 62% while percent survival of challenged fish not injected with erythromycin was 13% Circulating Rs antigen was also lower in Rs challenged fish treated with erythromycin than in challenged fish injected with no

erythromycin. Percent survival and quantity of circulating antigen in Rs challenged fish injected with erythromycin were similar to survival and levels of circulating antigen in fish injected with a placebo challenge. The relative ranking of survival of fish within treatment groups injected with erythromycin may have been altered by furunculosis.

Although mortality was controlled and ELISA profiles of circulating antigen were similar between erythromycin treated fish and the non-challenged fish, the coelomic fluid of female salmon that were challenged and injected with erythromycin had a significantly higher number of Rs cells/mL than coelomic fluid in fish not challenged. The residence time of erythromycin in the eggs and developing progeny of fish injected with erythromycin is well documented (Moffitt 1990, Moffitt et al. 1993). We continued to rear progeny from five of the adult treatment groups described in this study for two years after spawning at the University of Idaho to evaluate what effect erythromycin injections have on controlling vertical transmission. Throughout rearing, juvenile fish were sampled and kidney tissue assayed for the presence of Rs by ELISA. While Rs may have been present in kidneys of some of the fish sampled during the two years of rearing, bacterial kidney disease was not expressed in any of the progeny from these Rs challenged and erythromycin injected adult salmon.

The Rs challenge in adult salmon carrying *A. salmonicida* resulted in mortality showing symptoms unrelated to bacterial kidney disease. Since returning adult spring chinook salmon can carry a variety of disease causing organisms or are exposed to other pathogens during the holding period before spawning erythromycin dosages of 10 - 40 mg/kg injected 1 - 3 times reduced losses to diseases other than BKD.

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GILL DISEASES: THEIR NATURE, PREVENTION AND CONTROL

George W. Klontz, M.S., D.V.M.
Professor of Aquaculture
Department of Fish and Wildlife Resources
University of Idaho
Moscow, Idaho 83843

I. INTRODUCTION

The gill diseases of fish under confined; i.e., farm ponds or raceways, and free-living conditions are very complex and, in many cases, not well understood from epidemiological and etiological standpoints. This group of diseases, in the opinion of many, is one of the major production-limiting factors in farmed fishes (Klontz, 1973, 1974; Snieszko, 1974). Subclinical episodes are often difficult to detect due to their insidious onset. Clinical episodes are frequently dramatic in terms of the mortality involved, which has a rapid onset and often an exponential daily increase.

In a 1972-3 survey of the annual infectious and noninfectious disease mortality in public and private salmonid hatcheries in Idaho, the data indicate that 75-80% of the total annual mortality involved one or more of the gill diseases (Klontz, 1973). Recent personal communications with fish health management specialists in the Pacific Northwest states provide generalizations suggesting that the 1972 pattern is still present. In economic terms, the annual cost of gill diseases in salmonid aquaculture systems probably exceeds 10% of the production costs. In the public and private sectors in Idaho alone this cost would be approximately $3.25 million.

One of the major cost-incurring factors in this group of diseases is the chemotherapeutic regimens, which could best be described as "categorical." That is, fish with the clinical signs of rapid, shallow respiratory movements, grossly enlarged gill tissues, and incomplete opercular closure are "treated" with one of the many medicaments added to the pond water. The results have ranged from "rewarding" to "well, we guessed wrong." the latter cases could have been prevented, perhaps, by elucidating the nature of the problem prior to treatment.

The purpose of this presentation is to provide information about the broad nature of gill diseases in fish. It is not intended as a review of each and every reported gill disease.

II. NATURE OF GILL DISEASES OF FISH

The known causal factors in gill diseases of confined and free-living fishes are myriad, and can be grouped into those which are (1) infectious or are (2) noninfectious. Each group can be further subdivided into those factors which are primary and those which are secondary (Tables 1-3).

The primary causal factors are those which have a direct effect on the gill tissues and/or processes. Included in this group are the nonsystemic myxobacteria, eubacteria and fungi, the external protozoans and monogenetic trematodes, the water-borne chemical and physical agents, and the genetic and nutritional factors.

The secondary causal factors are those which affect the gill tissues and processes indirectly. Included in this group are the systemic bacterial and viral pathogens, the systemic protozoans and digenetic trematodes, the environmental chemical and physical stressors and certain metastatic neoplastic processes. These factors, in most cases, exert their pathogenic capabilities throughout the body with the gill involvement being just a part of the somatic process.

The major factors predisposing fish to subclinical and/or clinical episodes of gill diseases are (1) the stress response; (2) age; (3) environmental conditions favoring the proliferation of infectious agents; i.e., myxobacteria, eubacteria, protozoa and fungi. The three factors are virtually always involved in clinical episodes.

The stress response, particularly to chronic stressors such as high population densities, has been shown to create pathological changes in the gill tissues which are conducive to the involvement of secondary infectious and/or noninfectious factors (Klontz, et al, 1985).

The susceptibility to noninfectious and infectious gill disease-inducing factors decreases with age. The highest incidence of gill diseases occurs in the sac fry stage during which the most common malady is sestonosis with subsequent myxobacterial and protozoal involvements. This condition is typified by the accumulation of particulate materials and fungal elements on the buccal aspect of the gill rakers. Death is usually by suffocation. Because of its nature, sestonosis is virtually untreatable. Strict cleanliness is the only sure method of prophylaxis.

The bacterial gill diseases are quite common in populations of fishes from the fingerling stage through the mid-juvenile stage (Snieszko, 1981). This group of maladies is most frequently a sequel to a noninfectious process, environmental gill disease (EGD) (Klontz et al, 1985). The EGD syndrome is considered to be, first, stress-mediated and, second, environmentally-mediated. By itself; i.e., uncomplicated by pathogens or noninfectious factors, it is more a debilitating process than it is lethal. This aspect is, perhaps, what makes

Table 1: Primary (direct) infectious causes of gill diseases of fish in aquaculture systems.

Etiological Agent	
Bacteria	Protozoa - systemic
Myxobacteria	Plistophora
Pseudomonas sp.	Henneguya
Flavobacterium sp.	
	Monogenetic trematodes
Fungi	
	Dactylogyrus
Saprolegnia sp.	Gyrodactylus
Branchiomyces sp.	Cleidodiscus
Dermocystidium sp.	Monocoelium
	Urocleidus
Protozoa - external	Diplozoon
	Mazocraeoides
Trichodina	
Chilodon	Trematodes - digenetic
Scyphidia	
Epistylis	Clinostomum
Amphileptus	Sanguinicola
Oodinium	Nanophyetus
Trichophrya	
Glossatella	
Bodomonas	
Tripartiella	
Costia	
Henneguya	
Crustacea	
Ergasilus	
Lernaea	
Achtheres	
Salmincola	

Table 2: Primary (direct) noninfectious causes of gill diseases of fish in aquaculture systems.

Etiological Agent	
Physical factors	**Neoplasia**
Silting	Metastatic malignancies
Chemical flocculation or precipitation	
	Nutritional factors
Gentic factors	Vitamin C deficiency
	Pantothenic acid deficiency
Malformations of gill arches, filaments or lamellae	Vitamin E deficiency
	Nicotinic acid deficiency
	Vitamin A excess
	Starvation
	Vitamin B complex deficiency
Chemical factors	**Idiopathic factors**
pH [<6.0 - >8.0]	Intralamellar congestion
Ammonia (NH_3)	
Chemotherapeutants	
Copper	
Thiourea	
DDT	
Aflatoxins	
Detergents	
Herbicides	
Pesticides	
Nitrogen supersaturation	

Table 3: Secondary infectious causes of gill diseases of fish in aquaculture systems.

Etiological Agent	
Bacteria	Protozoa
Renibacterium	Cryptobia
Aeromonas salmonicida	Trypanosoma
Aeromonas hydrophila	Ceratomyxa
Yersinia ruckeri	Myxobolus
Vibrio anguillarum	Plistophora
Viruses	Trematodes
Infectious hematopoietic necrosis virus	Sanguinicola (Cardicola)
Hemorrhagic septicemia virus	Clinostomum
	Nanophyetus
	Cestodes
	Proteocephalus

it such an economically significant disease process. There is no specific recommended treatment regimen largely because the causal factors are often quite obscure, if evident at all.

III. PATHOLOGICAL CHANGES IN Gill DISEASES OF FISH

A. Noninfectious causal factors: The gross and microscopic pathological changes in gill tissues, especially the lamellae, are dose:response dependent. The inflammatory process ensuring from the initial exposure begins with lamellar epithelial hypertrophy in which there is focal-to-generalized involvement of the squamous epithelial cells. If the irritant exposure is short-lived, the hypertrophic condition subsides within a few days, usually without notice.

If the irritant exposure continues, the hypertrophic condition is "joined" by lamellar epithelial-capillary endothelial separation (ECS). In this lesion the squamous epithelium becomes separated from the underlying capillary endothelium and the resultant space is filled with a serous exudate. At this point in the process, especially if there is generalized involvement, the fish could be exhibiting clinical gill disease, particularly during or immediately following physical handling. This condition can be readily seen in wet mounts of gill tissues following a 1-hour exposure to certain water-administered chemotherapeutants such as formalin.

In the subacute inflammatory process,the lamellar hypertrophy is replaced with epithelial hyperplasia of, first, the lamella epithelium and then the filamental epithelium. The hyperplastic response can be terminal; i.e., involving only the distal portions of the lamellae, or it can involve the entire lamellae. In the case of nutritional gill disease (NGD) the hyperplastic response typically begins at the interlamellar filamental space and progresses distal to - but not beyond - the lamellar tips. This condition, over a period of 1-2 weeks, gradually worsens to become interlamellar hyperplastic occlusion, in which the interlamellar spaces are completely obliterated. At this point the fish is often visibly distressed. Gill tissues frequently protrude from the opercular cavity and there is incomplete closure of the opercula. Grossly, the excised gill is quite characteristic with filamental separation due to the increased mucus production and the entrapped aquatic particulates. Many fish pathologists regard this stage in the process as the point where secondary involvement by bacterial and protozoan pathogens occurs (Bullock, 1972). In contrast, many reports of myxobacterial gills disease include the conclusion that the myxobacterium was the exciting agent in this condition (Eller, 1975).

With proper chemotherapy and management practices the foregoing responses can be reversed. The repair process in the more severe cases requires 2-3 weeks, provided there are no further insults to the physiological respiratory process.

In recent years there has been considerable interest in a pathological process called "gill necrosis" (GN) (Snieszko, 1976; Musselius, 1981; Bekesi et al, 1981). The syndrome occurs predominately in farm-raised carp and is thought to be the result of chronic exposure to "autointoxication by ammonia under the influence of pH" (Snieszko, 1976); however, by most it is considered to be an idiopathic process (Musselius, 1981).

Another idiopathic process affecting the gill lamellae both anatomically and physiologically is intralamellar engorgement. This condition has been reported as lamellar telangiectasis and as lamellar aneurysm (Roberts, 1978). The lesion is quite characteristic and involves only isolated lamellae. The process apparently begins with the piller cell losing its function and the lemellum becoming engorged with blood to several times its usual size. At this point the lesion is quite susceptible to being physically ruptured. Without rupture there is, within a few days, thrombus formation followed by typical clot organization, fibrosis and resorption. Under optimum environmental conditions, the entire process from onset to complete resorption can require 30-50 days (Klontz, unpublished).

B. Infectious causal factors: The pathological changes associated with direct and indirect infectious causal factors are often quite confusing for the diagnostician. In the directly associated causal factors the pathogen usually occupied an opportunistic role. That is, it "set up housekeeping" on gill tissues already affected by some moderately long-standing noninfectious process.

Following the establishment of the host-parasite relationship a series of degenerative changes ensure within a matter of days. Among the degenerative changes are (1) hemorrhage; (2) necrosis; and (3) death due to anoxia or to critical loss of osmoregulatory function. This series of changes is most commonly seen in the bacterial gill disease (BGD) complex in which both ubiquitous aquatic myxobacteria and eubacteria are involved.

The changes associated with the parasitic protozoa and monogenetic trematodes are frequently less dramatic unless there are other noninfectious environmental factors operating. In the case of chronic Trichodina infection, during the winter months there is often a characteristic host response to the organism "hibernating" on the gill lamellae and/or filaments.

In systemic bacterial diseases such as furunculosis, enteric redmouth disease, vibriosis, bacterial hemorrhagic septicemia and

edwardsiellosis there is often secondary, nonspecific involvement of the gill tissues. The main lesion in these cases is frank hemorrhage and necrosis with secondary fungal involvement.

Nonspecific necrotic changes in the gill tissues are often attributable to secondary involvement with Saprolegnia, a ubiquitous aquatic phycomycete. On the other hand, in the case of European gill rot or fungal gill rot, the causative organisms, Branchiomyces sanguinis and B. demigrans are intravascularly invasive, which causes an infarctive necrosis of gill lamellae. The terminal lesion has been termed "gangrenous branchitis," for which there is no therapeutic regimen (Roberts, 1978).

One of the more infrequently observed, but nonetheless puzzling, lesions is the granuloma. This lesion appears sporadically and may be a benign neoplasm or of bacterial (Renibacterium salmoninarum or Mycobacterium sp..) origin. Similar lesions are protozoan sporocysts; e.g., Plistophora salmonis or developing metacercaria; e.g., Sanguinicola klamathensis. When these organisms emerge from the lamellae there can be a transient blood-loss anemia, which under certain circumstance can reduce the resistance of the host to secondary bacterial infections.

IV. PREVENTION AND CONTROL OF Gill DISEASES OF FISH

There are several approaches to preventing and controlling gill disease episodes in confined fish populations. The first is avoidance of the conditions which are conducive to the occurrence of subclinical and clinical episodes. This is best accomplished by (1) maintaining the fish within the environmental "no-effect" limits with respect to settleable and suspended solids, ammonia-nitrogen, dissolved oxygen and population density (Klontz 1991) and by (2) routine examination of gill wet mounts, production data and feed conversion ratios.

The "no-effect" limits of environmental factors have been established for some species of salmonids under certain, albeit limited, conditions (Wedemeyer and Wood). The best suggestion is for the aquaculturist to establish the unique limits for his/her facility. To accomplish this one must establish a protocol for measuring the environmental parameters and their effects on fish growth and gill tissues. This process should begin with sac fry and continue throughout the production cycle. One caveat is that the process is time-consuming and often frustrating, but always rewarding in the long term.

During the process of establishing the specific "no-effect" limits of environmental parameters, wet mounts of gill tissues should be examined on a regular basis. Fish should be taken from the "healthy" and the "unhealthy" or "sickly" portions of the populations. The fish are prepared for examination by killing them with a sharp blow to the head and exanguination by severing

either the causal peduncle or the spinal cord immediately posterior to the base of the skill. An entire gill arch is removed and placed into 10% neutral buffered formalin for no longer than 1 minute. It is either examined in toto (small fish only) or a few of the filaments are removed with scissors, mounted in pH 7.2 phosphate-buffered normal saline and examined using the 10X and 100X objectives. Paraffin-imbedded sections of the lamellae and filaments may be prepared but there are very time-consuming and expensive and not any more illustrative than are wet mount preparation, which can be done on site very rapidly.

If and when a clinical episode of gill disease occurs, then an accurate diagnosis must be made prior to initiating any therapeutic regimen. The sequence of changes occurring in the gill tissues is the best indicator of the nature of the causal factors involved. Lesions such as hypertrophy, ECS, and occlusive hyperplasia all suggest basic physiological upsets which may be reflected by alterations in other systems. The presence of bacteria and the so-called gill parasites often is a reflection of an underlying environmental problem, most common of which is "poor housekeeping." At this juncture, it might be apropos to present an oft-quoted saying by Frederick Fish, a fish pathologist of the 1930's, to wit: "In fish culture, cleanliness is not next to godliness - it supersedes it." (Fish, 1939).

Once the problem is defined; i.e., the major causal factors identified, the next step is to "re-balance" the system. This is best accomplished by, first, withholding feed for 3-4 days, if the fish are of sufficient size to permit this. This will (1) reduce the oxygen demand of the fish; (2) reduce the ammonia-nitrogen generation by the fish and (3) reduce the fecal and uneaten solids in the system. Second, administer sufficient salt (as granulated NaCl) to the system to obtain a 1-2% solution. This will (1) reduce the blood ammonia-nitrogen levels; (2) stimulate mucus secretion; and (3) have an astringent effect on the gill tissues. Third, reduce the population density to approximately one-half the oxygen-related carrying capacity of the system. This should be accomplished without unduly stressing the fish.

If, in addition to the environmental factors being corrected, there are infectious agents involved, the following treatment regimen has been effective. First, select a candidate chemotherapeutant which can be administered by water exposure. Second, conduct a bioassay using at least two and preferably three levels of the candidate chemotherapeutant administered in the fashion to be used for the affected population. The fish to be tested for efficacy and tolerance to the chemotherapeutant should be obtained from the clinically affected and "healthy" portions of the affected population. At the end of the bioassay period - usually an hour for pond conditions, the treated fish should be examined for efficacy. If the target organisms were killed or removed from the gills, the treatment was effective

and safe, if the test fish survived. These fish should remain under separate conditions for 12-18 hours following the bioassay to detect any delayed effect.

SUMMARY

As was implied at the outset of this presentation, gill diseases of fish in aquaculture systems have been dealt with in a somewhat haphazard fashion. Many aquaculturists regard them as a nuisance and apply all manners of medicaments to prevent their occurrence. This approach, although sound in some respects, has had its shortcomings; namely, the problem was identified but inadequately defined. As a result, the factors - intrinsic and extrinsic - involved were not corrected. For some strange reason the term "disease" in the vocabulary of the majority of aquaculturists and, to some extent, the practicing fish pathologists, denotes only those conditions involving a microbial pathogen. The noninfectious elements of a clinical episode are virtually ignored, thus being left to exert their respective influences another day. To illustrate this point, all one must do is to examine the textbooks of fish diseases and see that the causative agents of infectious diseases are described in what could best be defined as "loving terms." This quite understandable since the authors are, for the most part, well-known and highly respected microbiologists. Perhaps a case should be made to increase our understanding of the epidemiological facets of disease episodes. This aspect of medicine has a proven track record in human and veterinary medicine.

As this article is intended to be informative about a particular group of fish-associated maladies and not a wailing stone for perceived shortcomings in our professional acumen, it seems appropriate to close with a favorite quotation: "Believe what is seen - do not see what is believed." (Anon.)

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ABSTRACT

VITAMIN C INTAKE FOR STRESS AND DISEASE CONDITIONS

John E. Halver
School of Fisheries HF-15, University of Washington, Seattle, WA 98195

L- ascorbic acid (Vitamin C_1) is the major cellular reducing compound in animal tissues. It's major role seems to be in keeping metalo-enzymes in their active reduced state for hydroxylation reactions of protein synthesis, hormone production, detoxification reactions, stress-trauma and wound repair. Specific studies have shown growth requirements vary with species, size, and environment, and reports often are based upon type of C compound used in diet manufacture. Other studies have shown growth requirements need to be increased 6 to 10 fold to potentiate resistance to bacterial or viral diseases. Rainbow trout appear to have one of the highest growth needs, with other salmonids tested requiring less amounts, and catfish or carp lower still. Unfortunately, specific growth/size/species studies have not been completed on chinook, sockeye, pink, chum, or cherry salmon, tilapia, marine fish or other candidates for effective aquaculture.

Several new derivatives of ascorbic acid are now available for diet formulations. Each has improved manufacturing and storage stability, and each has limitations that affect utilization by the fish. Most of these new compounds involve stabilization of the ene-lactone ring by substitution of an electron-dense molecule at the labile 2-position of the ene-diol ring of ascorbic acid. Feed manufacturing and storage stability of these derivatives are: C2S--C2TP--C2MP--C2G--C6P--CP--DHC--C. Other forms on the market have intermediate stability from adjuncts with gelatin, sugars, gums, and silicate. Therefore, the effective vitamin C intake must be determined with respect to the form and level of C invested in the diet manufactured, stored, and used.

Ingested L-ascorbic acid equivalents for growth and maximum health can be estimated as follows in terms of milligrams per kilogram dry diet:

	No Scurvy	Max Growth	Max Health
Rainbow Trout	40-50	100	300-1000
Coho Salmon	25-30	50	200-1000
Atlantic Salmon	20-25	50	200- 800
Channel Catfish	15-20	30	100-2000
Common Carp	10-15	30	100- 300
Chinook Salmon	R	R	R
Sockeye Salmon	R	R	R
Other Salmonids	R	R	R
Other Fishes	R	R	R

A Multiple Marking Strategy Using Oxytetracycline

Ronald A. Snyder

Montana Department of Fish Wildlife & Parks
Jocko River Trout Hatchery
Arlee, MT 59821

Abstract.- Oxytetracycline is routinely used by fish culturists as a food additive to produce bands in the calcified tissues of fishes that fluoresce under ultraviolet light. TM-50 from Phizer Inc. added at 10% of the diet by weight produces a single internal logical batch mark. Six groups of Arlee Rainbow Trout each received a group specific series of oxytetracycline laced diets in an attempt to produce six distinct marks. The series consisted of two to four ten day treatments. All six groups were administered the initial treatment or band one. All bands were applied during the same time interval. Bands were separated by a mean standard growth interval of one inch. Actual unmarked growth between bands equaled approximately 0.7" and the band width represented 0.3" of growth. The absence or presence of any one band was predetermined. The initial treatment was administered when the combined mean population length was 2.5 in. The last band was applied when the mean population length was 5.5 in. Six distinct fluorescing sets of bands,"marks", were acheived. Use of a single mark would increase the number of available marks to seven. All groups received a mark specific fin clip post band four application. A small lot comprised of all six groups was held captive at the facility. The majority of the fish were released into several ponds. Both the captive and released populations were sampled annually for two years post band four application. Mark retention was seen in all fish sampled that exhibited a fin clip. All marks corresponded to their respective fin clip. Overall band fluorescence remained excellent.

RETURN OF THE SALMON

WENATCHEE RIVER SALMON FESTIVAL

1993 Accomplishment Report

Authors:

Corky Broaddus, Information and Educational Specialist
Ron Marvin, USFS Recreation Planner

Located in North Central Washington, 470 miles upstream from the Pacific Ocean, the Wenatchee River is a last remaining treasure in the Columbia River Basin. Where else can one still find viable, natural runs of spring chinook salmon, summer chinook salmon and sockeye salmon. The recent problems surrounding the spotted owl and Snake River salmon runs highlight the growing demands upon our natural resources. Demands placed upon natural resources and land management agencies will continue to increase with the pressure of a growing human population. At the same time, with an increasingly urban population, the public's basic understanding of the natural world and environmental issues is diminishing.

The USDA Forest Service, Wenatchee National Forest and the USDI Fish and Wildlife Service, Leavenworth National Fish Hatchery recognize a responsibility to provide leadership in natural resource management. The Wenatchee River Salmon Festival was conceived as a mechanism to educate the public about the fish and other riparian resources. Primary goals for the festival are to increase awareness and appreciation for the unique value of the local aquatic resources, provide educational activities which focus on students and families, and highlight the cultural significance of the salmon to the people of the Northwest. Secondary objectives are to promote interest in outdoor recreation and expand visitor activities for the local tourism economy.

Location

Leavenworth, Washington - approximately 25 miles west of Wenatchee and 120 miles east of Seattle - has evolved into a destination tourist center. This Bavarian theme town at the eastern edge of the Cascade Mountains attracts over 1.3 million visitors annually from all over the world but primarily the Pacific Northwest and British Columbia. The Leavenworth area and Chelan County, Washington are also rapidly growing as people seek to escape the pressures of metropolitan life and enjoy the Central Washington weather. Beside being a destination tourist area, Leavenworth is only 2 1/2 hours from downtown Seattle and thus receives a large number of weekend visitors who come not only to visit the town but also participate in a multitude of year-round, outdoor activities in the adjacent Wenatchee National Forest.

The Leavenworth National Fish Hatchery Complex, located near the national forest boundary just two miles from town, is the largest federal salmon production facility in Washington state. Fish Hatchery and Wenatchee National Forest personnel have long been partners in natural resource management. That long-standing partnership and working relationship were instrumental to the success of the salmon festival. With over 160 acres, the Hatchery grounds provide a prime site for a special event of this caliber.

EXECUTIVE SUMMARY

The 1993 "Return of the Salmon, Wenatchee River Salmon Festival", sponsored by the Wenatchee National Forest and the Leavenworth National Fish Hatchery was held October 7-10, 1993. Fifty other government agencies, businesses, organizations and private individuals along with 165 volunteers contributed to the event. The objectives of this annual event are to provide natural resource educational activities focusing on kids and families, highlight the cultural significance of salmon to the Northwest, promote interest in outdoor recreation, raise scholarship money for students pursuing higher education in natural resource management, promote interest and support for an aquatic education and research facility, the Na-sik-elt River Discovery Center, at the Leavenworth National Fish Hatchery and expand visitor activities for the local tourist economy.

The 1993 event held at the Leavenworth National Fish Hatchery drew over 11,000 visitors including up to 2000 students from local schools who attended the special "Opening School Day" on October 7th. Partners and sponsors contributed over $50,000 in support of this event. The Chelan County PUD, a continuing major contributor to the festival, produced a 1994 calendar featuring the artwork of the poster contest finalists. In addition enough money was raised to offer two $1,500 scholarships in the spring of 1994. Activities included a teacher workshop to introduce a newly developed salmon festival curricula for pre and post school activities, Native American cultural performances, educational exhibits and demonstrations provided by environmental groups, outdoor recreation companies and schools. Another new entry to this year's festival was the salmon maze, an adventure for young and old to discover the many salmon predators. "The Web of Life" activity featured a costume parade of insects, frogs, mushrooms, eagles, bats and other assorted flora and fauna as part of an activity to teach concepts of food chains and food webs.

Planning

Early in 1991, members of the Leavenworth and Lake Wenatchee Ranger Districts and the Leavenworth Fish Hatchery met to establish a core committee to plan and implement the event. Critical to the eventual success of the project was the ability of personnel from the two agencies to work together as one, regardless of which uniform a person wore and the support of the line officers in both agencies. A core team from the Forest and Hatchery was established to plan and implement the project. Corky Broaddus, then Public Affairs Specialist at the Lake Wenatchee Ranger District, was assigned the task of Festival Coordinator. She has continued in this role to the present time. Members of the support planning team includes: District Rangers, Hatchery Managers, Public Affairs Specialists, Environmental Educators and Interpreters, Recreation Planner, Fish and Wildlife Biologists, Native American program coordinator and representatives from the Leavenworth Chamber of Commerce. Eventually, the festival would involve all the hatchery staff, a wide range of Forest Service employees from fire crews to the Forest Supervisor and local community volunteers.

To stay consistent with the overall festival objectives it was decided that not only must the activities be educational and fun but that they also remain non-political and non-commercial.

Other features that evolved during the planning and implementation of the first two salmon festivals include:

> a scholarship fund to support students pursuing higher education in natural resources

> assist local schools with their environmental education programs with special activities such as the ENVIROTHON, Kids in the Creek, Kids Day for Conservation and other ongoing educational events

> to raise interest and support for establishing an aquatic education and research center at the hatchery, the Na-sik-elt River Discovery Program

> a pre- and post- salmon festival school curricula

> student and teacher workshops

> and facilitating development of an environmental educators coalition for the Wenatchee Valley area.

Pre-Festival Activities

The October dates were selected for this event because they coincide with the peak of the wild summer chinook salmon spawning in the Wenatchee River. The timing of the festival was complemented by colorful autumn foliage, beautiful fall weather and schools in session. Based upon these dates, time-lines were established for the implementation of pre-festival activities such as promotion and marketing, logistics, purchases and facility arrangements.

Working with the Icicle Valley Chapter of Trout Unlimited, a bank account was established with the help of Central Washington Bank in which people and/or corporations can donate to the Salmon Festival "spawnsorship" fund. Annually, Dan O'Connor, the Forest's graphic artist designs a logo for the festival. This logo is used on garments which are sole to raise funds to support the educational activities of the Salmon Festival. A "Booster Club" program was developed for interested individuals and companies to complement the other fundraising efforts for two $1500 scholarships. Trout Unlimited's tax-exempt status allows a tax deduction for any such donation. Businesses and individuals receive special recognition in festival promotions.

School Program

Wenatchee Valley schools kick off the festival in the spring of each year with the annual poster contest. To start the Festival emphasis on environmental education, this contest is held in the third grade level. Forest Service and Fish and Wildlife interpreters and educators present a short, interactive program highlighting salmon and their environment. In 1993 there were 26 classroom presentations given to an estimated 2,000 students. Students are challenged to design a poster with the festival theme, "Return of the Salmon." The winning poster is selected by a team of judges and developed into the promotional poster seen throughout the northwest. Over 500 entries were received in 1993. The winning poster created by Winton School third grader, Tasha Cabe, featured a jumping, headphone attired salmon with the slogan "Instinctively Cool...Musical Scales". An awards ceremony is held annually in May at the "Kid's Day for Conservation" in Wenatchee, recognizing the work of all entrants. Five finalists are awarded a $50 savings bond and the winner is presented a $200 savings bond. The 1993 poster was printed courtesy of festival sponsors Alcoa, City of Leavenworth and Hooked on Toys and a twelve-month 1994 full color calendar was designed and printed by the Chelan County P.U.D.

In addition to the poster, a broad spectrum of media opportunities were developed to make the residents throughout the northwest aware of the festival. This included: articles printed free of charge by Sunset Magazine, AAA Motorist, the Cascade Loop Tour brochure; radio advertisements by local radio station sponsor and statewide public service announcements; a 10 minute video production was produced by both agencies and Gateway Productions, and shown to a variety of audiences to share the festival story. Even a request from Sesame Street Magazine came to the festival media coordinator.

Other promotional publications include a rack brochure printed courtesy of Luhr-Jensen, Inc. and Schedule of Events printed by Chelan County Public Utility District. A number of feature stories appeared in such regional newspapers such as Seattle Post Intelligencer, Oregonian, Wenatchee World and Spokane Review. Just prior to the event, the Leavenworth Echo included a special salmon festival insert with their weekly publication. Articles not only highlighted the festival but shared information about the salmon resource. The Leavenworth Chamber of Commerce also provides support to the promotional campaign. The Chamber's Executive Director offers guidance in identifying and selecting media to pursue. The Chamber assists in distributing festival rack cards and other publications through their distribution network and incorporating festival information in their publications.

Festival Activities

A primary goal for the 1993 festival and continuing onto the 1994 event is to strengthen the quality and diversity of the natural resource education activities of the Salmon Festival. This year a major step in accomplishing this goal was achieved with the development of a school curricula to enhance and build upon the learning outcomes of the Salmon Festival. The guide begins in the classroom with interactive, hands-on lesson plans that easily integrate into a teacher's busy schedule and aid teachers in meeting Washington state's Environmental Education mandate (WAC #105-50). Through this pre-work, students anticipate and prepare for the Salmon Festival. After students return to the classroom they reinforce and demonstrate their understanding of particular Salmon Festival themes through follow-up lessons. This curricula serves as the guidebook for festival activities. Teacher orientation workshops will be held annually to familiarize teachers with the curricula and its implementation process.

Festival activities include a handicapped-accessible interpretive nature trail. This one-mile long, self-guiding trail with numbered stops keyed to a brochure, offers people an opportunity to learn about aquatic and riparian habitat. A self-guided auto tour up the scenic Tumwater Canyon also allows visitors the chance to see the summer chinook spawning in the Wenatchee River. A Native American encampment was set up by tribal members of the Yakima Indian Nation and Colville Confederated Tribes. Elder women prepared salmon using the traditional methods of baking, arts and craft demonstrations were given and a horse parade with Indian riders took place each week-end day of the festival. Special cultural performances of drumming and dancing were also presented to festival visitors.

Environmental education and interest in outdoor recreation objectives were emphasized by displays and exhibits at the festival. Any company or group who had an environmental education or outdoor recreation message linked to aquatic resources were invited to participate as long as the display was educational, non-political and no sales were allowed. Environmental education displays have included a "Conservation Trail" by the Wenatchee National Forest, an interactive computer-based salmon questionaire was designed by the Chelan County P.U.D.play, which the Chelan County PUD designed specifically for the festival, exhibits by Alcoa, Bonneville Power Administration (who also contributed $5,000 to the festival), U.S. Fish and Wildlife Service, Ecological Services wetland exhibit, Washington Department of Fisheries, NOAA, the local Audubon chapter, and the Fish Hatchery sponsored a display from the Seattle Aquarium. The Chelan County Sheriff also have shared their display which includes a search and rescue boat, helicopter, and their DARE exhibit which connected with the "Hooked on Fishing Not on Drugs" theme. These displays, which included aquariums and hands-on activities, were enjoyed by young and old alike. Fish Hatchery tours were also conducted.

Displays with more of an outdoor recreation theme have included Worden Lures/Yakima Bait Co., Luhr.Jensen, Clearwater Anglers, the Wenatchee River Flyfishers, and Trout Unlimited. The Flyfishers and Yakima Bait also put on fishing clinics.

A popular cultural feature of the festival is the unique presentation of world reknown storyteller, Susan Strauss. Susan has shared her stories with audiences at each of the three festivals. Ms. Strauss comes from Bend, Oregon, and specializes in Native American stories. Her performance specifically prepared for the festival, "Coyote meets the Wild Salmon and other stories of the Columbia River" is truly a high point of the festival weekend.

Guided by the 1993 Festival school curricula, all hands-on activities were big hits of the festival. Gyotaku, (gyo=fish and taku=rub of print),the Japanese art of fish printing is among the most popular activities of the festival. Visitors are offered an opportunity to paint and print on rice or newspaper Wenatchee River sockeye and Columbia River shad, allowing people to learn about the anatomy and physiology of the fish in a fun and artistic way. Another activity is the "salmon tent," a colorful inflated, salmon-shaped, 50 feet long by 10 wide and 8 feet high nylon tent. Children enter the salmon tent where volunteer interpreters and storytellers tell local Native American stories about the Columbia River, covering both the environmental education and cultural objectives of the festival. Next door to the salmon tent in 1993 was held the "Web of Life" activity. This is a new addition to the festival. Colorful custom-designed costumes of such critters as bears, mosquitoes, eagles in addition to mushrooms and frogs represent plants and animals found in the salmon's community. Through interactive simulations, children (students) will learn how we are all connected in the "Web of Life". Learning the concept of food chains, building on to understand food webs and then expanding to discover food pyramids provide the focus of this wonderful addition.

The AMAZING SALMON MAZE , another new festival feature, was designed by the US-FWS and built by volunteers and hatchery personnnel. The MAZE offers visitors a chance to experience the many challenges of aquatic life and migration when they discover themselves as a salmon, hatching and struggling to survive and complete the salmon's aMAZEing life cycle. Artistic paintings of common natural salmon predators greet maze walkers at each of the dead ends. This 40' x 40' maze captured the enjoyment of several thousand people at the festival and also received front page full color coverage on the Wenatchee World newspaper.

Another activity which deserves mention is a Volksmarch sponsored by Bavarian Volkssport Association and financed by Valley Tractor and Equipment, Inc., the local John Deere dealer. Volksmarching has become a popular non-competitive walking activity and fits well with the outdoor recreation and family activity themes the festival strives to reach. This 10K walk took place at Lake Wenatchee with its trail winding along the upper Wenatchee River.

For answering all those "fishy" questions visitors to the festival ask, the development of the new "What's My Line?" aqua booth fit the bill. Adult-size costumes of Frank and Frances Fish, the fish of the national forest, were filled by fish biologist volunteers and located inside a 10' x 10' aqua booth. Young people are given the opportunity to ask Frank or Frances a question.

Labor and materials were donated by a variety of construction businesses for the completion of a handicapped accessible underwater fish viewing aquarium located just across from the main hatchery building. Hatchery tours provided festival visitors with an in-depth look into fish culture. The more than 2.1

million spring chinook salmon eggs the hatchery raises were reaching their "eye-up" stage and was a fascination to hatchery trout takers.

Finally, people enjoyed a variety of foods and entertainment (to provide those very basic "creature comforts" necessary for an event of this type) the festival program included. Food, festival T-shirts, hats and lapel pins are sold annually at the festival and help tremendously with raising money for the scholarships and other educational activity expenses. Non-profit groups, including Trout Unlimited, Kiwanis, Trout Unlimited (with yummy barbequed salmon) and others, had food booths and donate 15% of their gross receipts to the festival account. Live entertainment by a variety of musicians and a full sound system filled the four days with folk music, bluegrass, Indian drumming and dancing and jazz. A local quintet even sang contemporary songs about rivers, fish and western living.

Accomplishments

The first annual "Return of the Salmon, Wenatchee River Salmon Festival," hosted by the USDA Forest Service, Wenatchee National Forest, and the U.S. Fish and Wildlife Service, Leavenworth National Fish Hatchery, and sponsored by 29 corporate, agency, and individual partners was held at the Leavenworth National Fish Hatchery on October 10-13, 1991. Over 8,000 people attended the four-day event, not including the 450 Volksmarch participants. The 1992 Salmon Festival, held October 8-11, 1992, greeted 10,000 visitors, over 2,000 of that in scheduled school children tours, and was supported by 35 partners. This third annual festival hosted almost 12,000 festival visitors, fifty plus "spawnsors", raised over $50,000 of contributions, provided teacher workshops to thirty teachers, scheduled activities for students from 12 different north central Washington schools and had 165 volunteers join in the festival four-day operation between October 7-10.

Another positive aspect of the festival continues to be the unique teamwork and cooperation exhibited by the Forest Service and Fish and Wildlife Service. Over 100 Forest Service and Fish and Wildlife Service personnel worked side by side during the event. The cooperative relationship between the two agencies will be extremely valuable in meeting future challenges in natural resource management. The help of all volunteers including high school students from four area high schools, a "senior" softball group, who helped with parking, to members of a Minnesota tour group who came back on Sunday to help with Gyotaku was outstanding.

The three-year old festival is judged to be a great success. Annually, enough money has been received so that two $1,500 scholarships will be awarded to students pursuing a career in natural resource management. Not only has attendance continued to rise,but comments from people at the festival and community at large are very positive. People especially liked the relaxed, non-commercial, educational theme. People's awareness and appreciation of the local resources also seem to be enhanced.

It appears the festival has been also successful in achieving a secondary goal of assisting the local tourism economy, as evidenced by the 44% of the out-of-area festival visitors who came specifically for this event. Of these out-of-area visitors who spent the night, 66% stayed in local lodging

facilities and another 31% stayed in private campgrounds, these figures came from visitor surveys taken on the festival grounds during the weekend.

Planning for the 1994 festival began immediately after the 1993 event. It is hoped to expand activities but also maintain the low-pressure relaxed atmosphere. The core committee feels the festival will be enhanced by adding high quality educational activities (yet to be developed) designed for families and expanding upon the curricula and its components for guiding the festival agenda.instruction. It is also hoped that the interpretive trail at the Fish Hatchery will be completed and made fully accessible. Work has begun on planning the NaSikelt River Discovery Center, an aquatic education,research and visitor center and forming a non-profit cooperation to oversee these activities. To accomplish all these activities even more marketing efforts will be made developing sponsorship support.

1993 "Spawnsors"

Chelan County P.U.D.
Bonneville Power Administration
Leavenworth Chamber of Commerce
Valley Tractor and Equipment, Inc.
Alcoa
Skipper's Seafood and Chowder House
Luhr-Jensen
City of Leavenworth
Solideck
Black Swan
Gateway Productions
Yakima Indian Nation
Central Washington Bank
Colville Confederated Tribes

1993 Booster Club Members

Chinook $250

Cascade School District
Lake Wenatchee Recreation Club
The Evergreen Inn
Pacific Northwest Power Planning Council
The Leavenworth Echo

Coho $100

Obertal Investments,Inc
C & D Orchards
The Gingerbread Factory
Hotel Pension Anna
Robert S. Smith,DDS
Jay and Alyne Fortgang
Timberwood Construction,Inc.
Leavenworth Food Giant

Fingerling $25

Autumn Pon Bed and Brkfst
Broaddus Ck. Construction
Ron and Sue Marvin
Leavenworth Village Inn
Jim and Connie Rae Leo
Allyssa Jumars Family

Sockeye $50

Images and Sounds
Wedge Mountain Inn
Bob and Karen Zylman
The Taffy Shop and the The Cuckoo Shop
Home Fires Bakery
Howard and Kay Somers
Icicle River RV Park
Kahler Glen Golf Course & Condominiums
Run of the River Bed and Breakfast
Paul and Liz Rawlins

The following list features the individual Salmon Festival coordinators and their contact numbers. For further information on any aspect of the festival, the festival director may be reached at the:

Corky Broaddus
Leavenworth National Fish Hatchery
P.O. Box 549
Leavenworth, WA. 98826
(509) 548-6662
Hatchery Complex Manager Greg Pratschner

Ron Marvin
Ass't Director
Leavenworth Ranger District
600 Sherbourne
Leavenworth,WA 98826
(509) 548-7641
District Ranger Becki Heath

Activity leaders are assigned to run each activity and schedule volunteer help. Some of these people are full time employees of both the Forest Service and Fish & Wildlife Service. The following shares those names and phone numbers of the activity leaders that are currently employed by either agency. For specific information on the activity, contact:

Gyotaku	Judy DeLaVergne, FS	(509) 763-3103
Salmon Tent	Barbara Fish,FS	(509) 782-1413
Web of Life	Susan Thomas.FS	(509) 782-1413
Salmon Maze	Laura Penington,FWS	(509) 548-6662
School Curricula and field trip scheduling	Susan Thomas,FS	(509) 782-1413
Food Booths	Corky Broaddus,FWS	(509) 548-6662
Volksmarch	Fran Taber,volunteer	(509) 782-1630
Interp.Trail	Bill Thorson,FWS	(509) 548-7641
Auto Tour	Corky Broaddus,FWS	(509) 548-6662
Displays & Exhibits	Susan Thomas,FS	(509) 782-1413
What's My Line?	Ron Marvin,FS	(509) 548-7641
Entertainment	Corky Broaddus,FWS	(509) 548-6662
Media	Chris Rader,FS	(509) 763-3103

Marketing	Laura Penington	(509) 548-6662
Logistics	Corky Broaddus	(509) 548-6662
	Ron Marvin	(509) 782-1413
Schools	Susan Thomas	(509) 782-1413
	Rachel Little	(509) 548-6662
Native American Coord.	Jim Baugh	(509) 674-4411
Poster Contest	Barbara Fish	(509) 782-1413

California Aerial Fingerling Stocking Program

Anna Kastner
San Joaquin Hatchery
Friant, California

Progressive history of aerial planting. Slide presentation of the process of aerial planting of fingerling Rainbow Trout (Oncorhynchus mykiss), Eastern Brook Trout (Salvelinus fontinalis), some Golden Trout (Oncorhynchus aquabonita), and some Kokanee Salmon (Oncorhynchus nerka) on high altitude lakes in the Sierra Nevada range. This annual stocking program insures quality fishing for anglers that hike or pack into the beautiful wilderness areas of the rugged Sierra Nevada range.

WHITE RIVER SPRING CHINOOK PROGRAM

Dennis D. Moore and Richard Johnson
Muckleshoot Indian Tribe
Auburn, Washington

Part I.

Spring-run chinook salmon once populated several river systems in Puget Sound, Washington. The White River spring chinook stock is the last viable population in Southern Puget Sound (Fig. 1). The present day White River is a primary tributary of the Puyallup River which empties into Puget Sound at Tacoma, Washington. Historically the White was a major river in the Duwamish River system flowing into the Sound 40 miles north of Tacoma at Seattle. The vast Duwamish watershed drained over 1500 square miles with the Cedar, Black, Green and White Rivers as tributaries (Fig. 2). Right after the turn of the century, the Cedar was diverted into Lake Washington and out through the Ballard Locks, drying up the Black River. The majority of the Green was piped to Tacoma for domestic and industrial uses, and the White was diverted south to meet the Puyallup River at the town of Sumner (Fig. 3).

White River spring chinook returning to a trap on the White at river mile 24 numbered over 5,000 in the 1940's. By 1985, the run dwindled to zero fish returning (Fig. 4). This decline is attributed to to over-fishing, industrial and urban development, a power generation project, lower river habitat alterations, flood control projects, and logging practices. In Tacoma, the White-Puyallup estuary is covered with an industrial complex. Up-river, Puget Sound Power and Light Company dammed the river and diverted the majority of flows into power turbines (Fig. 5). The once meandering White and Puyallup Rivers are now diked, mined for gravel, and resemble open topped pipelines. Above Puget Power's diversion dam at river mile 29 is the Mud Mountain Dam, an Army Corps of Engineers flood control facility. Anadromous fish blockage mitigation calls for the Corps to trap up-river bound fish at Puget Power's dam, then truck them to a point beyond Mud Mountain.

Above Mud Mountain Dam, the habitat is mixed. Some is still being logged, but much of it is recovering from poor logging practices. Habitat recovery programs are being conducted by the U.S. Forest Service, and the Forest Service and Muckleshoot and Puyallup Tribes are building acclimation ponds as a tool for the restoration program.

Part II.

The White River Hatchery was built by Puget Sound Power and Light Company (Puget Power) as part of the 1986 settlement agreement between the Muckleshoot Indian Tribe and Puget Power. Located near the the town of Buckley, Washington, the hatchery went into operation in 1989 with the sole purpose of reviving the nearly extinct spring chinook stock in the White River. The first stage of hatchery construction called for a 5 CFS pumped ground water supply provided by five submersible pumps. The full designed capacity of 10 CFS was achieved in the spring of 1993 with the completion of the surface water intake system which uses three vertical turbine pumps to draw water directly from the White River about a quarter mile upstream of the hatchery. The river water is passed through dual centrifugal vortex separators which remove large sediment before the water is fed into the headtank and then to the fish rearing ponds. Well water is oxygenated as it drops down through packed columns into an adjacent headtank.

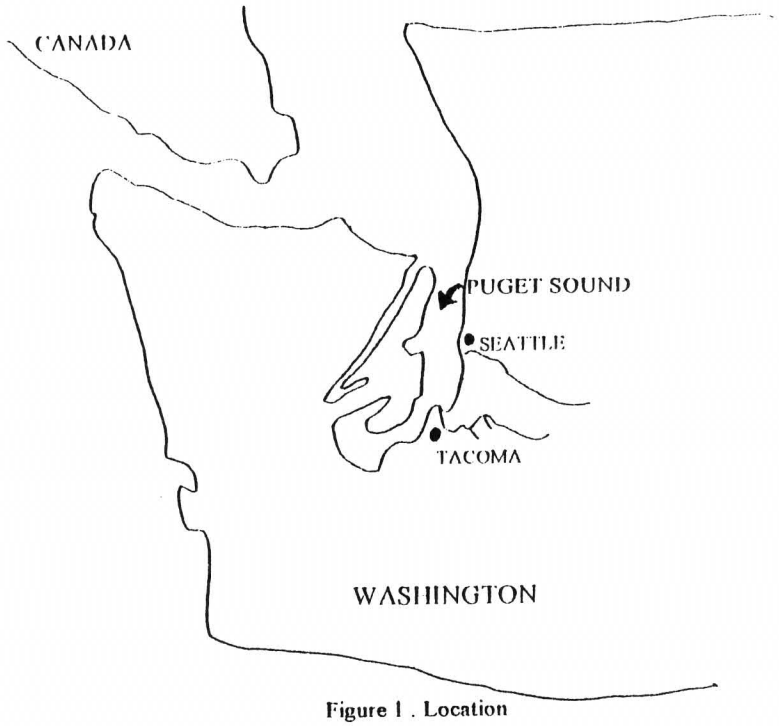


Figure 1 . Location

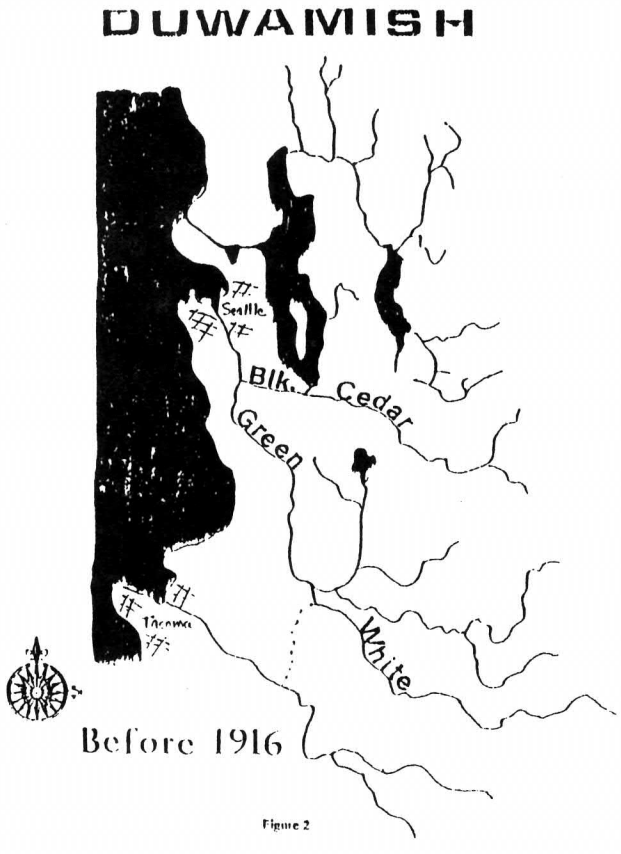
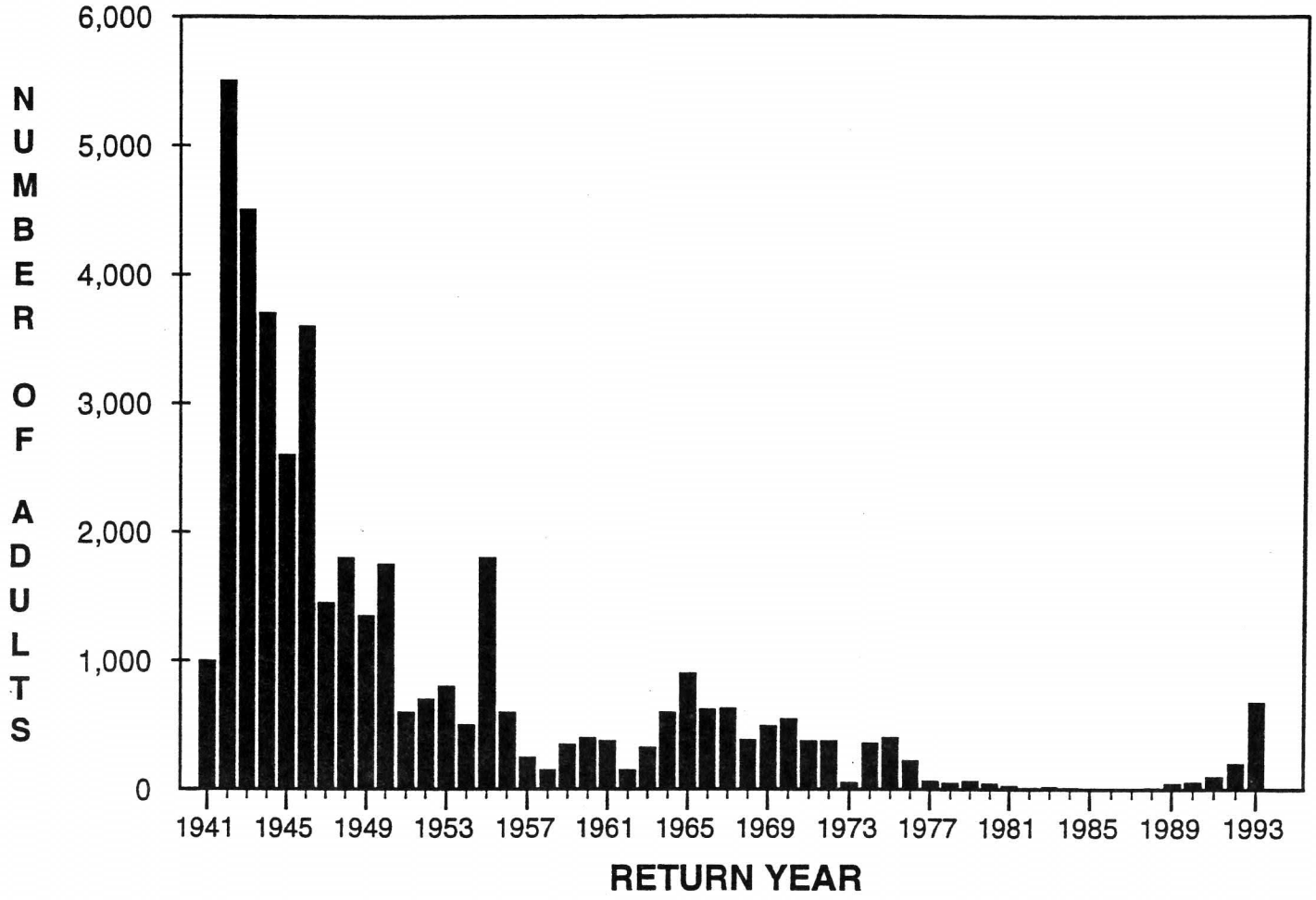


Figure 2

Figure 3



Number of adult White River spring chinook salmon returning to the Buckley trap.

Figure 4.

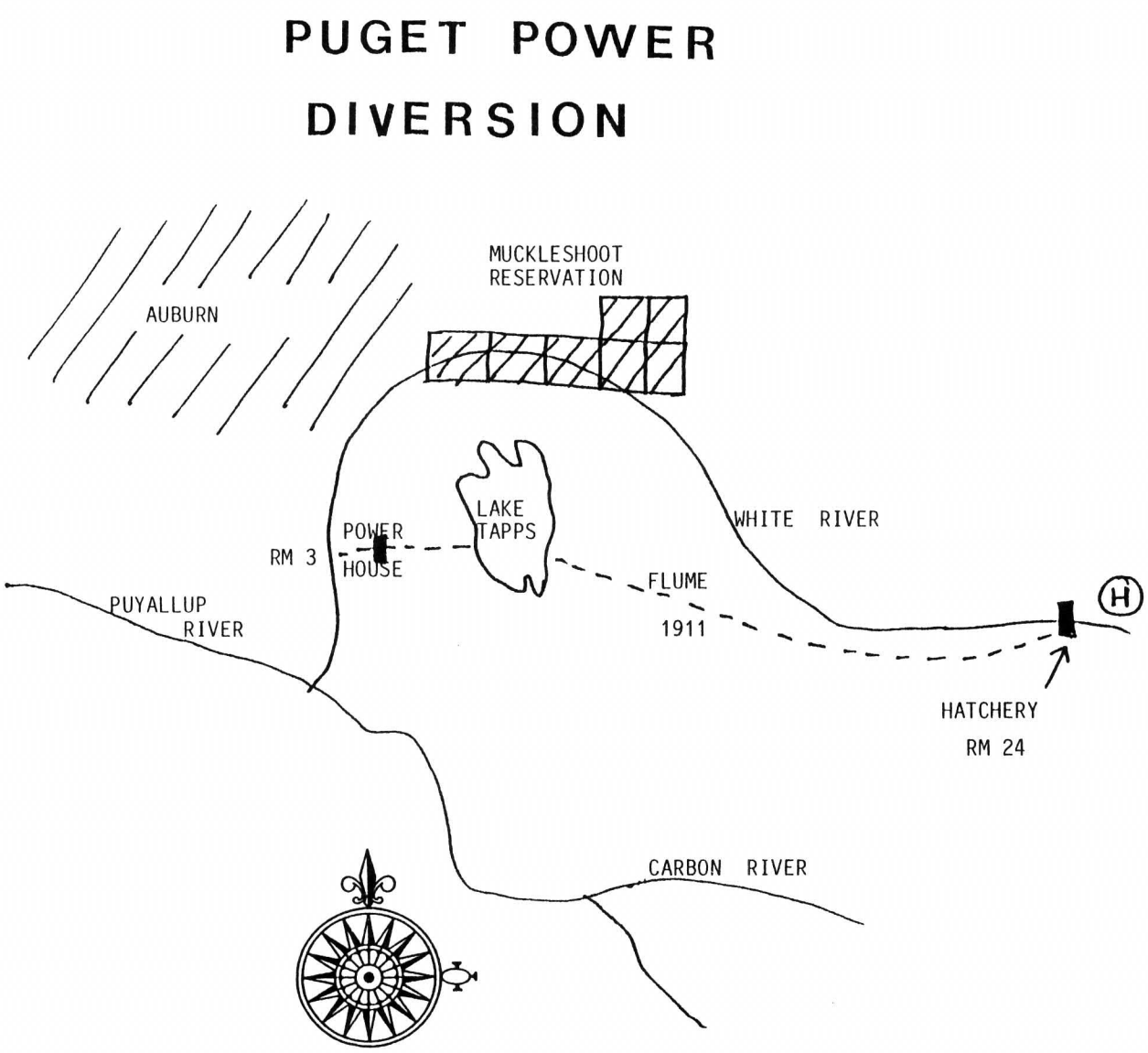

PUGET POWER
DIVERSION
MUCKLESHOOT
RESERVATION
AUBURN
LAKE
TAPPS
WHITE RIVER
POWER
HOUSE
RM 3
FLUME
1911
PUYALLUP
RIVER
HATCHERY
RM 24
CARBON RIVER

Figure 5.

The combination of surfacewater and groundwater systems allows flexibility in holding different age classes of fish throughout the year. The hatchery building consists of the incubation room with 192 Heath Tray Incubators (24 - 8 tray stacks), start tank room with 16 - 3' X 12' fiberglass tanks, walk-in freezer, dry storage, garage, laboratory, offices and a crew room. Outdoor ponds include 4 - 8' X 95' concrete raceways and 1 - 52' X 95' concrete rearing pond. A covered spawning shed is adjacent to the rearing pond. Adult fish capture occurs at the hatchery trap as well as the trap and haul facility operated by the Army Corps of Engineers directly across the river. All wild chinook and any other anadromous species that enter the traps are released upstream, above Mud Mountain Dam, in the White River. Hatchery fish are identified by an adipose fin clip and coded wire tag detection.

The White River Hatchery is operated by the Muckleshoot Tribe's Fish Enhancement Division. Full time hatchery staff members include a fish biologist, hatchery manager, and two fish culturists. The White River Spring Chinook restoration program is a multi-agency, cooperative effort, involving Tribal, State, and Federal agencies. Operational and technical support is provided by the Northwest Indian Fisheries Commission. The hatchery has produced over 1.8 million juvenile spring chinook to date and several acclimation ponds have been built in the upper White River Watershed to hold spring chinook juveniles for imprinting. The first pond, on Huckleberry Creek, went into operation in April 1993 and was renovated by the Corps of Engineers and Washington Dept. of Fisheries during the summer of 1993.

Adult returns to the hatchery in 1992 (170 - 3 yr olds) and 1993 (210 - 3 and 4 yr olds) reversed the trend of declining spawners that had been occurring for decades. The spring chinook return at White River Hatchery is augmented by a salt water captive brood program in south Puget Sound and the Minter Creek Hatchery/ Hupp Springs facility (Washington Department of Fisheries) which has established a spring chinook run from White River stock. The captive broodstock are transported from the marine net pens at Squaxin Island to the receiving hatcheries for ripening in late August or early September. Adults spawned at White River Hatchery are 100% health screened for Bacterial Kidney Disease (BKD) and viruses such as IHN, IPN, and VHS.

Additions and improvements are being made continuously at the hatchery. In conjunction with the recent surface water intake construction, a computerized Hatchery Management System (HMS) was installed which upgraded the original equipment. The new version has a programmable logic controller (PLC) as the main operating component. All control and monitoring functions are performed by the PLC which is connected to the operator stations where hatchery staff can access the system. The main operator station in the hatchery building is a 486 computer with a Windows driven software package that provides graphical representations of the hatchery complex. The computer program allows for display, printing, storage, and trending of operational information collected by the system. The HMS also features two Panel View display stations for remote operation and monitoring at the generator buildiing and the river pumphouse building. Control of all well pumps and river pumps is possible at any of the operator stations. An automatic dialer monitors inputs from the HMS and initiates a predetermined call up routine when an alarm condition occurs after normal hours. Future expansion of the hatchery will include the addition of four raceways as well as a possible fourth river pump.

A REVIEW OF THE EFFECTS OF REARING DENSITY ON SURVIVAL TO ADULTHOOD FOR PACIFIC SALMON

R. D. Ewing
and
S. K. Ewing

Biotech Research and Consulting, Inc.
2340 SE Ryan St.
Corvallis, OR 97333
503-752-8259

ABSTRACT

Rearing density is a major concern in the rearing of Pacific salmon. Since 1975, there have been 20 brood years of coho salmon and 15 brood years of chinook salmon used in density experiments to determine optimal rearing densities. Results from these experiments were compiled and standardized to determine if significant relationships occurred between rearing density and percent survival to adulthood or number of adults produced per rearing pond.

We performed three types of analyses on the results from the experiments: analysis of variance, if the experiments were replicated; regression analysis if the experimental conditions were not replicated but there were more than four groups; graphic analysis if the experimental groups were not replicated and were less than four.

Results indicated that with coho salmon there was a positive relationship between rearing density and adults per pond for 9 of 20 brood years. Only 7 of 20 brood years showed a negative relationship between rearing density and percent survival to adulthood. On the other hand, chinook salmon showed a negative relationship between rearing density and percent survival in 14 of 15 brood years. The relationship between rearing density and adults per pond was not as good. There was a negative relationship on 4 brood years, a positive relationship on 4 brood years, and no apparent relationship on 7 brood years of the 15 brood years of chinook salmon released.

Analysis of the five year rearing density study at Elk River Hatchery, Oregon, indicated that fish at higher rearing densities survived at lower rates in proportion to ocean conditions. This suggests that the stresses imposed by the rearing density show up mostly during harsh years when the percent survival is reduced. On very good years, the differences between medium and high densities would probably not be significant.

These results suggest some major differences between coho and chinook salmon in their response to rearing density. Coho should be reared as dense as possible because the number of adults that return will be proportional to the rearing density. Chinook salmon should be reared at as low densities as possible because the number of fish that return seems to remain relatively independent of how many are reared.

We suggest that hatchery personnel performing density experiments consider the following suggestions:

1) Run the experimental ponds in duplicate. This permits a much more powerful analysis of variance to be used for analysis.

2) Separate the groups widely in density. There are many things influencing survival so a wide spread in rearing density is ncessary to assure significant differences of the final results.

3) Run the density experiment for as long as possible. Large numbers of brood years may provide some insights into the relationship between ocean conditions and survival after hatchery stresses.

4) Keep the experiment as simple as possible. These are very complex experiments. While it is tempting to piggy-back another experiment on top of the density experiment, too often the complexity overwhelms the results and conclusions from both are lost.

5) Keep good records on flows, fish numbers, fish sizes, mortalities, and pond sizes. These are the bases on which the interpretation of the results rely.

An Evaluation of Timing on Post Release Performance of Spring Chinook Salmon

Ray Jones
U.S. Fish and Wildlife Service
P.O. Box 18
Ahsahka, ID 83520
208-476-7242

ABSTRACT

In 1992, the Dworshak/Kooskia Hatchery Evaluation Team designed a pilot study to evaluate release time for spring chinook salmon smolts at Dworshak NFH. Theoretically, later releases should be more fully smolted, should migrate faster, and realize higher survival. We released three groups of spring chinook salmon smolts on April 8, April 22, and May 6, 1992. Smolt development was examined by measuring gill ATPase activity on a bi-weekly schedule from March 1 to release. Migration time and survival was examined by PIT-tagging about 1500 smolts in each release. Two coded-wire tag groups of 60,000 fish per release group were released to evaluate adult returns. We found that ATPase activity did not vary significantly between release groups over time or between release dates. We did observe that later release groups migrated significantly faster and had significantly higher survival rates than earlier release groups. However, because of increased flow in Lower Granite pool during late April and early May, we cannot attribute differences in migration time and-survival just to smolt development, since increased flow must have been an important variable. The study was successful in that: 1) We obtained useful data on release time, migration time, and survival, 2) We identified several components of the study that need to be modified and additional data that needs to be collected, and 3) the need to conduct multi-year evaluation studies in order to account for year to year variation in such factors as flow was reinforced.

TITLE: THE PERFORMANCE OF HATCHERY-REARED SUMMER STEELHEAD (*Oncorhynchus mykiss*) SMOLTS: ACCLIMATED AND DIRECT STREAM RELEASE COMPARISONS.

M.W. Flesher. Oregon Department of Fish and Wildlife, Research and Development, 1410 L Avenue, 211 Inlow Hall, EOSC, LaGrande, OR 97850

Abstract: Acclimation of smolts prior to release is often thought to enhance smolt-to-adult survival and increase homing accuracy, although little quantitative evidence is available to support these hypotheses. We compared juvenile migration timing and survival to Lower Granite Dam, and adult catch and escapement between acclimated and direct stream released groups of summer steelhead (*Oncorhynchus mykiss*). Wallowa stock steelhead were released during mid- to late-April from Wallowa Hatchery (1986-89 broods) and from the Big Canyon Facility (1990-92 broods) while Imnaha stock steelhead were released at the Little Sheep Creek Facility (1990-92 broods). For most broods and stocks, mean migration rates of juveniles to Lower Granite Dam were slightly reduced for the acclimated groups; however, the peak arrival time of both groups tended to be two to four weeks after release. Juvenile survival indexes at the dam ranged from 10.0-53.2% for acclimated and 7.1-40.2% for direct stream released groups. Smolt-to-adult survival ranged from 0.09-1.08% for acclimated and 0.05-0.62% for direct stream releases. For the 1986-88 broods released at Wallowa Hatchery, catch to escapement ratios (used as an index of homing accuracy) were 2.8, 3.7 and 4.8 for acclimated and 6.9, 3.2 and 3.7 for direct stream releases, respectively. Although we found little difference between groups in juvenile migration performance, and no consistent trend in homing accuracy, the data suggest that acclimation seems to enhance smolt-to-adult survival.

Abstract, 44th Annual Northwest Fish Culture Conference

Title: An evaluation of the success of supplementing Little Sheep Creek summer steelhead (*Oncorhynchus mykiss*) with hatchery-reared smolts.

Rhine T. Messmer; Oregon Department of Fish and Wildlife, Research and Development, 211 Inlow Hall, Eastern Oregon State College, La Grande, Oregon 97850.

The Imnaha River summer steelhead hatchery supplementation program was initiated in 1982 under the Lower Snake River Compensation Plan. The management objectives for this hatchery program are to enhance natural production in Little Sheep Creek and restore and maintain recreational and tribal fisheries in the Imnaha River basin, while maintaining life history and genetic characteristics of the endemic population. To determine the success of this program we are: comparing aspects of life history and genetic profiles of hatchery reared and naturally produced juveniles; assessing survival to adult and progeny-to-parent ratios for hatchery fish; monitoring the success of the recreational fishery; and assessing natural adult returns to Little Sheep Creek. Wild fish were used for broodstock from 1982-1984, wild and hatchery fish have been used for broodstock since 1985. Ocean residence time and run-timing into Little Sheep Creek has been similar for hatchery and naturally produced adults. Progeny-to-parent ratios for hatchery fish were equal to or below 1.0 for the 1982 and 1983 brood years; however, ratios were greater than 2.0 for the 1984-1988 brood years. The supplementation program has been partially successful in that it has substantially increased total escapement to the Imnaha Basin and Little Sheep Creek. Recreational fisheries have been successfully restored and sustained with hatchery fish since fisheries reopened in 1986. However, the supplementation program will not be totally successful until increases in natural escapement to Little Sheep Creek can be sustained without altering the life history and genetic characteristics of the endemic population.

Integrated Hatchery Operations Team Pros and Cons

Tom Sheldrake
U.S. Fish and Wildlife Service
Columbia River Coordinator
9317 Hwy 99, Suite A
Vancouver, WA 98665
206-696-7888

Like Ole I am always fearful that when we talk about improving hatchery operations we will see our words used against us in tomorrow's headlines, however I truly believe that IHOT will help us do our jobs and actually decrease the amount of hatchery bashing in the future.

The Integrated Hatchery Operations Team (IHOT) is a multi-agency group called for by the Northwest Power Planning Council (Council) to develop new basin wide policies and standards on the operation and maintenance of all current and future Columbia River Basin anadromous fish hatcheries.

For many years people from outside as well as some within the agencies have been trying to put together a group that would create the products that you are now seeing from IHOT. Unless you work with hatchery information all the time it does take some interpretation to know what is going on in the basin. An example being the often asked question. How many fish were released into the river last year? This information is readily available in annual reports and other working documents, but it appears in different formats. Unless you understood the difference between brood year, fiscal year, calendar year, etc. the same question might get you different answers, sometimes even from the same agency.

In the past when agencies were asked to put together another basin wide group to look at hatchery operations they cooperated to some extent, but no matter how beneficial this appeared the task was not one of high priority, and most attempts ended with a compilation of existing documents that did not satisfy those that wanted all the information summarized in one source. In 1992 the Councils "strategy for Salmon" identified the Bonneville Power Administration as the source of funds to accomplish this type of work and IHOT was created.

IHOT is a diverse group with representation from all the state, tribal and federal fisheries co-managers in the basin as well as the Bonneville Power Administration, the Chelan County PUD, The Corps of Engineers, The Northwest Power Planning Council and the Pacific Northwest Utilities Coordinating Committee.

Partly because of this diversity it took us several meetings to establish limits to our task. We have limited ourselves to only looking at hatchery operations and not the purpose or use of

hatchery fish or other fishery management issues. This is important because there are those that believed that the hatchery system controlled management decisions, or who believed they could use this process to reprogram the use of hatchery fish.

Early in this process the agencies settled on a format, and compiled descriptions of their existing hatchery operations in this common format. You can now look at all the hatcheries in the basin without converting data.

The team gathered all the existing policies affecting hatchery operations, and analyzed them to find areas of commonality or gaps.

Sub-committees were created to work on the five policies requested in the councils program. The purpose of these policies is to provide regional guidelines by which all anadromous fish hatcheries in the columbia basin will be operated. They will be adopted by the fisheries co-managers, and will be used to provide guidance to operate hatcheries in the most efficient and biologically sound manner.

The five policies are Fish Health, Genetics, Ecological Interactions, Performance Standards and Coordination.

The Fish Health policy is modeled after the Pacific Northwest Fish Health Protection Committees model program that most of you are familiar with. The Genetics policy is based on protocols we have all been using to prevent genetic selection. The Ecological Interaction Policy is based on common sense and our existing stocking and release guidelines. The Performance Standards Policy sets down in one place the rearing limitations that have and are still evolving as the science of our profession. Parameters like flow, density, temperature and water chemistry that effect fish quality. It also sets standards and measures to be used in an audit that I will talk more about later. The Coordination Policy is based on existing cooperation among the agencies, but should make it even easier for us to share facilities and manpower, to communicate, and to resolve technical issues. It will also facilitate our ability to report our activities, especially to those outside our community.

As you can see There is not much new or earthshaking in these policies. They are new statements of existing policy or principals that we have been operating under for a long time. The goals are all motherhood and apple pie, and confirm ecologically sound fishery management ideals. It is the standards and performance measures that will be of most interest to hatchery managers.

The performance standards are intended to provide a point of reference against which to monitor change, and units of measure to define change. I won,t go into the many different standards we have in the policies. Many of them are based on research and

knowledge of long standing such as recommended spawning, incubation, and rearing temperatures for different species.

We were conservative in our setting of standards in areas where we have little knowledge, and those areas gave us the most difficulty. An example would be in genetics where some experts recommended electrophoresis or DNA typing and elaborate spawning protocol, and others claimed our technology is not good enough to measure even the most basic change in survival. Since each hatchery program is different we tried to take a common sense approach, allow for different standards, and require that each hatchery have a genetics plan developed with the assistance of a geneticist.

I used a story to illustrate this risk assessment approach to the Policy Group. I grew up in Chetek, Wisconsin a town with a population of 300 people. I was related to many of them so when I started dating I was warned to check out the lineage of any girl I started to get serious about. Then we moved to Chicago where I had even more relatives, but the chances of me dating an unknown cousin were so small that I quit asking. Our spawning standards reflect this kind of risk assessment and allow for modification based on population size, purpose, or need to mimic a known natural trait. An example being we require a 1:1 male female spawning ratio unless a hatchery takes .5 million eggs per day when they may go to as high as 1:3. If you are wondering when a couple in my family get divorced we still consider them brother and sister?

The performance measures range from record keeping to show that the hatchery did not exceed a density level, or that your fish were within a certain size range of the goal set for that program, to attendance at meetings or updating plans yearly.

The Councils Strategy calls for independent audits of hatchery performance in consultation with the IHOT. We are in the process of finalizing the criteria and performance measures the auditors will use. The audit measures are included in the draft policy that IHOT will have ready for agency review this month, and I expect the audits will start this year. The policies will be available to the public through the Councils public review process and I predict that the agency approval will take place within one year.

The future of the team is uncertain. The Councils Strategy calls for some involvement in the future, and the agencies see IHOT as a useful forum, replacing some existing groups that are not specific to legal or treaty requirements. The products the IHOT can produce are unique and of use in the management of the basin fishery resources, but priority for funding and manpower will determine it's continued existence.

Comparison of Two Methods of Oxygen Supplementation for Enhancing Water Quality in Fish Culture

by

Speros K. Doulos

Anthony J. Garland

John R. Marshall

Mark D. White

U.S. Fish and Wildlife Service

Erwin National Fish Hatchery

1715 Johnson City Hwy.

Erwin, TN 37650

Abstract

In this study, we compared sealed column oxygen supplementation to a pipeline manifold oxygenation system at gas-to-liquid ratios of 0.7, 1.5, and 2.2%. The sealed columns receiving oxygen at a gas-to-liquid ratio of 2.2% increased raceway dissolved oxygen by 73.5% (149.3% oxygen saturation) while decreasing nitrogen saturation and total dissolved gas to 93.1 and 105.0% respectively. Oxygen added at a gas-to-liquid ratio of 2.2% through the pipeline manifold increased raceway dissolved oxygen by 53.7% (134.6% oxygen saturation) and reduced nitrogen saturation and total dissolved gas to 99.0 and 106.1% respectively. Sealed column oxygen supplementation was most efficient, having an oxygen transfer efficiency of 82.3% when a 0.7% gas-to-liquid ratio was utilized. Oxygen added through the pipeline manifold resulted in a 54.1 - 62.7% variation from mean column effluent dissolved oxygen. The pipeline manifold also caused column water flow fluctuations that varied by as much as 21%. We recommend oxygen injection directly into sealed columns for adding supplemental oxygen to fish-rearing water when compared to a pipeline manifold system.

Introduction

In recent years, several methods of injecting pure oxygen into fish-rearing water have been developed. The use of pure oxygen for fish culture can increase fish production (Kindschi et al. 1991) and reduce nitrogen saturation (Marking 1987). Some oxygen supplementation techniques contribute to excessively high total gas pressures that can adversely affect fish health (Doulos and Kindschi 1990). The sealed column, however, has proven to be one of the most effective devices for adding pure oxygen to fish rearing water (Dwyer et al. 1991).

The spring water supply at Erwin National Fish Hatchery (NFH), TN consistently produces water with dissolved oxygen (DO) at less than 90% saturation. The combination of limited spring water flow, serial reuse, and pumped reuse results in dangerously low DO levels throughout the hatchery. In addition, nitrogen saturation levels regularly exceed 120%.

An oxygen supplementation system was installed to increase DO and reduce nitrogen saturation where water first enters the facility. This area has the capability of using two different methods of oxygen supplementation. Pure oxygen can be added directly to two sealed columns or injected into the main pump line through a pipeline manifold. This study was performed to evaluate the effectiveness of these two methods of oxygen supplementation.

Methods and Materials

Erwin National Fish Hatchery is located in northeast Tennessee and produces rainbow trout (Oncorhynchus mykiss) broodstock as part of the U.S. Fish and Wildlife Service National Broodstock Program. The hatchery has two water sources. A main spring produces 1,044 gal/min that uses gravity flow to supply rearing water to the upper production raceways. An additional 358 gal/min of spring water are collected and pumped to the upper raceways. The water sources are mixed and supply rearing water to the entire hatchery (Figure 1). Water temperature is a constant 55^{0}F, with only slight deviations that result from the effect of ambient air temperature.

Liquid oxygen, stored in a 1,500-gal vertical bulk tank, is used for adding supplemental oxygen to fish-rearing water at Erwin NFH. Spring water leaches through a rock substrate and is collected in a concrete collection box. A 5 hp pump is used to pump 358 gal/min of water through 200 ft of 4-in-diameter pipeline to two upper production raceways. Oxygen is added to the pumped small spring water supply (Figure 1). This pipeline terminates with two 12-in diameter sealed columns (Figure 2). The oxygenated effluent from these columns mixes with the main spring supply (no supplemental oxygen added) at the head end of the upper production raceways. Oxygen can be injected directly into both sealed columns and a main pipeline manifold.

The sealed columns are 5 ft high and contain 3 ft of 1 1/2-in-diameter Koch ring media (Figure 2). Oxygen flowmeters (Victor Model No. MOF15-4A) were installed on both columns to control the oxygen flow rate. The effluent ends of the columns have no restrictions and are submersed 1 ft below the raceway water level.

The pipeline oxygen injection site consists of a 2-in-diameter stainless steel manifold with three 3/8-in-diameter, deflector wide angle spray nozzles capable of delivering 0.4 ft^3/min gas at 30 psi (Figure 3). This in-line injection system was used as it was easily adapted to the existing water line and requires little maintenance. The manifold is installed within water bypass piping that allows water to be diverted from the main 4-in-diameter pipeline. Pipeline control valves are regulated so that the entire volume of pumped water can be diverted through the bypass piping when oxygen supplementation is desired. A 1-in-diameter stainless steel oxygen supply line is connected to the manifold. Supplemental oxygen is added through the manifold spray nozzles and is mixed with the pumped water in 190 ft of pipeline before exiting the sealed columns. An oxygen flowmeter (Key Instruments Model No. FR2A18-BVBN) having a gas flow range of 0.4 - 3.5 ft^3/min was installed adjacent to the manifold to control the oxygen flow rate.

This study was designed to compare efficiencies of the two oxygen injection locations at various gas-to-liquid ratios. Gas-to-liquid ratios of 0.7, 1.5, and 2.2% were used to evaluate the effectiveness of the sealed columns and pipeline manifold for supplementing rearing fish-rearing water with oxygen. Each sealed column was used to add supplemental oxygen to half of the pumped spring water volume. Oxygen

Figure 1.

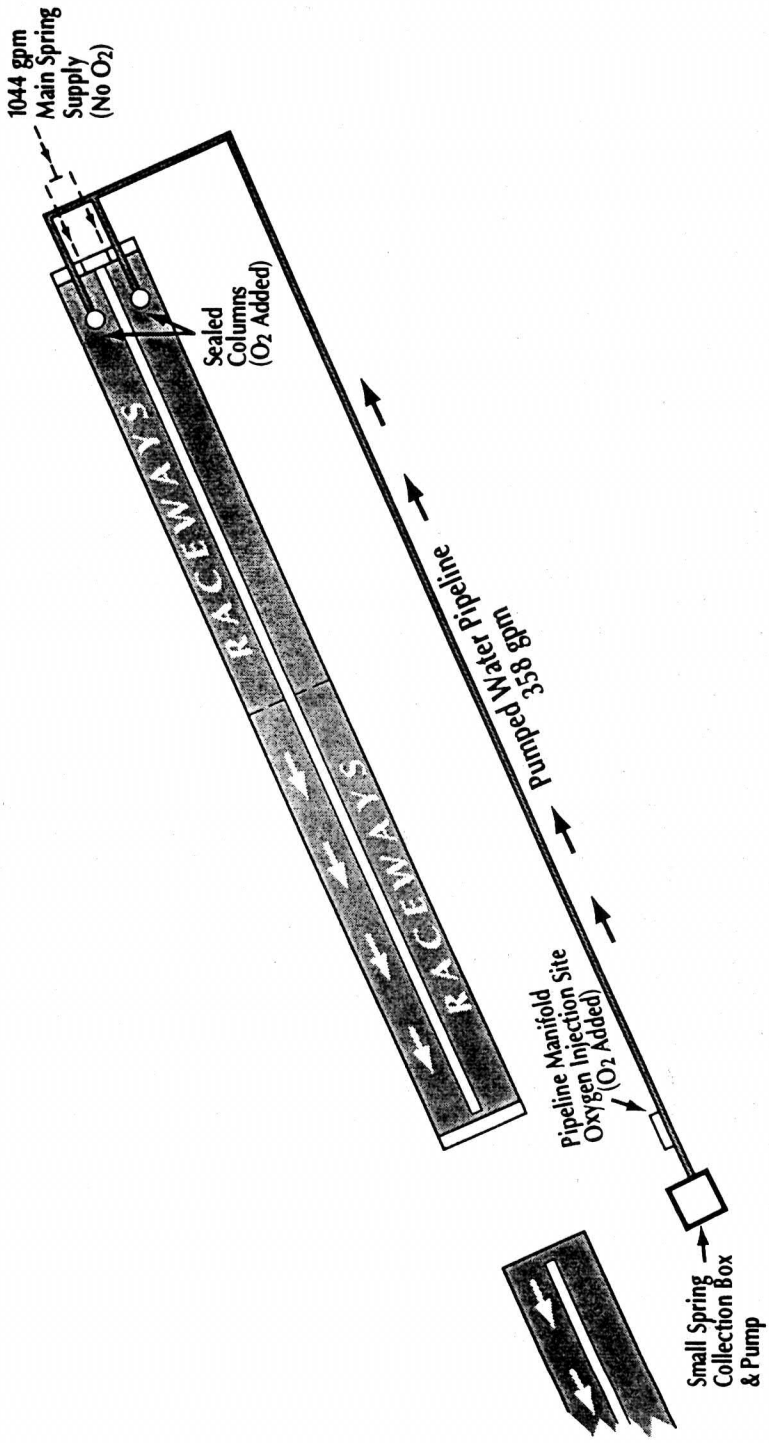

1044 gpm
Main Spring
Supply
(No O_2)
Sealed
Columns
(O_2 Added)
RACEWAYS
RACEWAYS
Pumped Water Pipeline
358 gpm
Pipeline Manifold
Oxygen Injection Site
(O_2 Added)
Small Spring
Collection Box
& Pump

Figure 2.

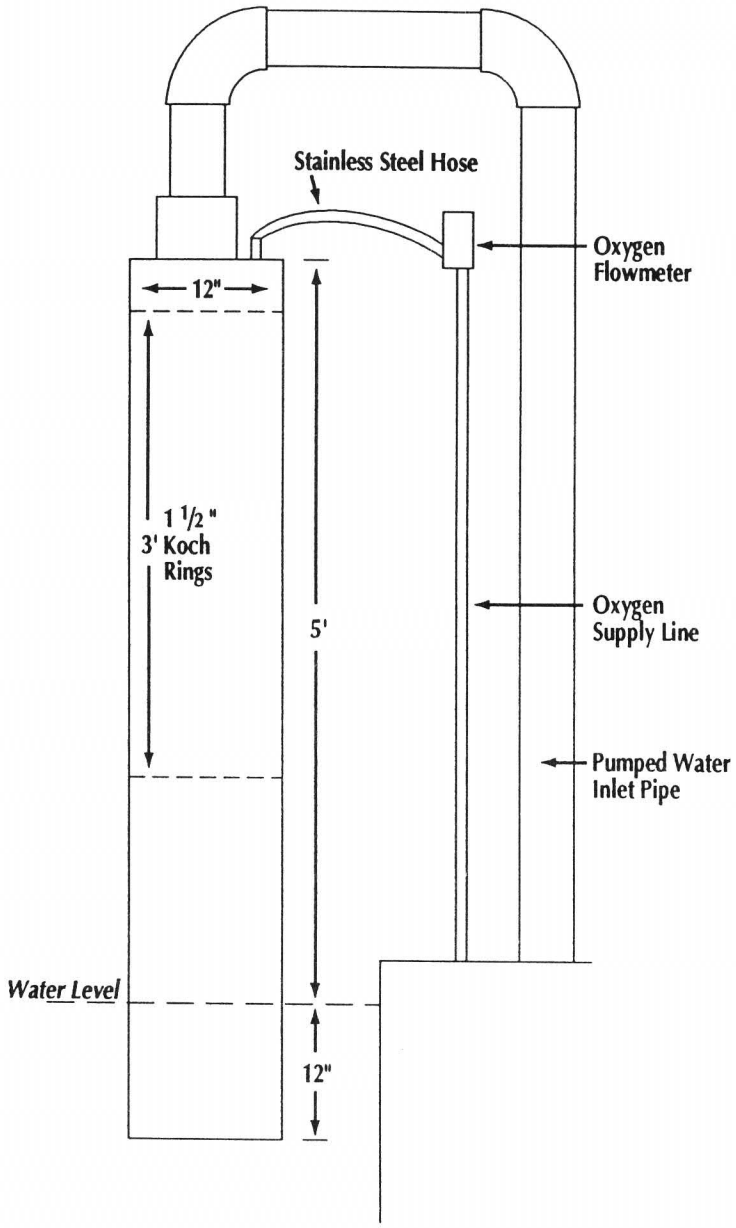
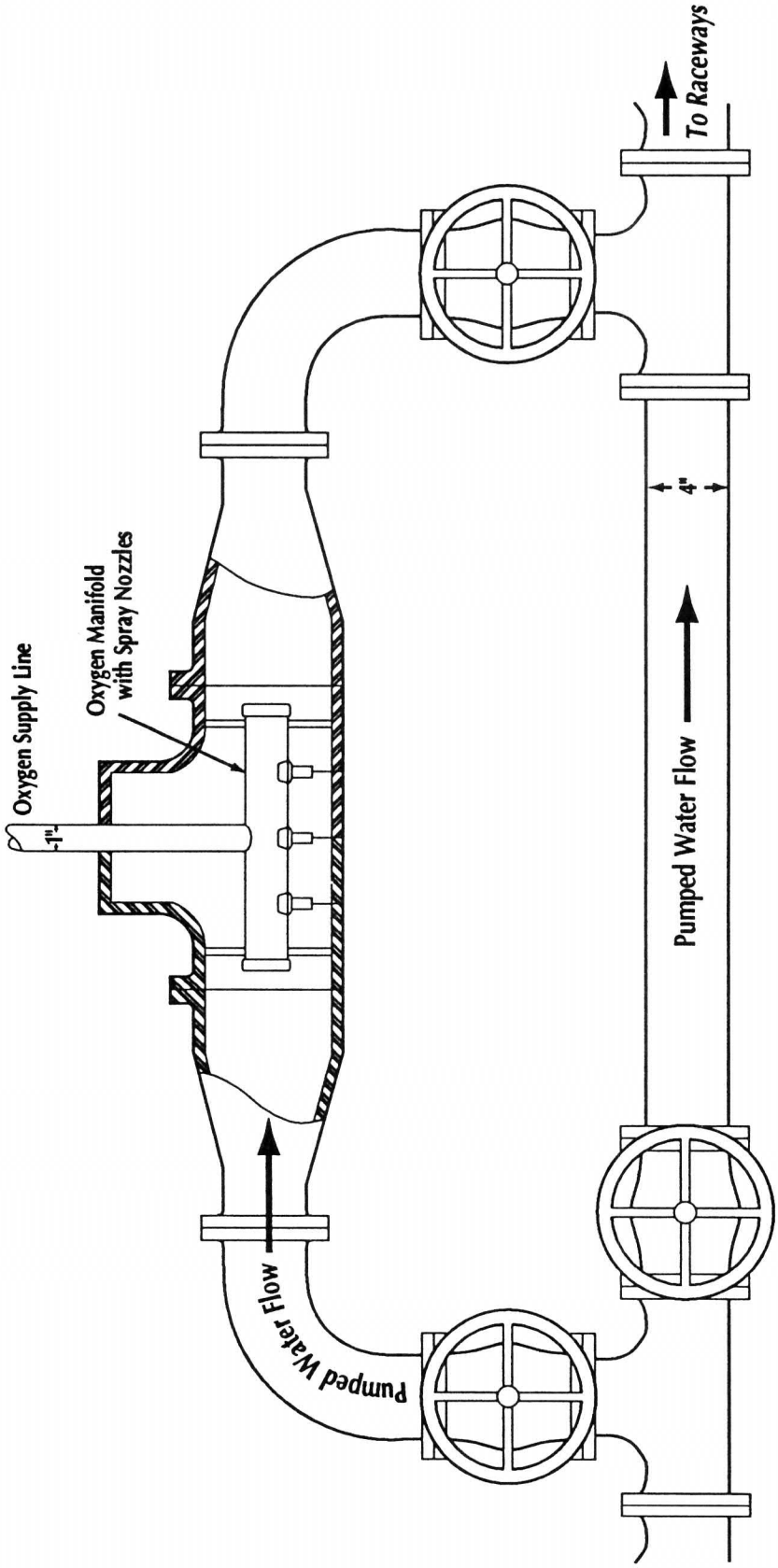

Stainless Steel Hose
Oxygen Flowmeter
12"
1 1/2 " 3' Koch Rings
5'
Oxygen Supply Line
Pumped Water Inlet Pipe
Water Level
12"

Figure 3.


To Raceways
4"
Pumped Water Flow
Oxygen Manifold
with Spray Nozzles
Oxygen Supply Line
-1"
Pumped Water Flow

flow rates were doubled when using the pipeline manifold. The entire pumped water flow (358 gal/min) received supplemental oxygen at this location. Oxygenated pumped water was then divided equally between the two sealed columns. Water quality data were collected for 5 d at each oxygen injection site and for each gas-to-liquid ratio. Duration of the study was 6 weeks.

Water quality data were collected once per day during the early morning hours. Dissolved oxygen, nitrogen saturation, and total dissolved gas (TDG) were measured in the main spring supply standpipes at the head of the upper raceways to obtain water quality data prior to the addition of supplemental oxygen. Water quality was also measured 25 ft from the head end of each raceway to determine the effects of oxygen supplementation after mixing with the pumped spring water oxygenated effluent. Dissolved oxygen and temperature were measured using a YSI Model 50B DO meter. Nitrogen saturation and TDG were determined using a Model ES-2 Weiss saturometer.

Oxygen transfer efficiencies were determined once for each oxygen injection location and for each oxygen flow rate A 10-gal bucket was used to collect the oxygenated column effluent. Dissolved oxygen was measured using the Winkler titration method (APHA 1985). At the same time, DO was measured in the small spring concrete collection box. Pumped spring water flows were measured by recording the time taken to fill a 10-gal bucket. These data were used to calculate oxygen transfer efficiency (%) with the equation:

$$\frac{\text{Water Flow (gal/min)} \times 0.134\ \text{ft}^3/\text{gal} \times \text{DO Increase (ppm)}}{1{,}428\ \text{ppm}\ O_2 \times \text{Pure}\ O_2\ \text{Added (ft}^3/\text{min)}} \times 100$$

The effluent DO was measured in both columns and used to estimate the variability in performance between the two columns.

Results and Discussion

Data collected from the main spring water supply standpipes (no oxygen supplementation) were consistent throughout the study. Incoming DO averaged 8.61 ppm (range 8.42 - 8.73 ppm). Mean oxygen saturation for the spring water receiving no supplemental oxygen was 86.3%. Mean nitrogen saturation was 121.2% (range 120.5 - 121.6%), and average TDG was 113.7% (range 113.3 - 113.9%). These data are consistent with baseline water quality data collected during the previous year (1991-92).

The sealed column oxygen injection site provided higher raceway DO, lower nitrogen saturation, and lower TDG compared with the pipeline manifold site (Table 1). These data were obtained from the mixing of 358 gal/min of oxygenated pumped water effluent with 1,044 gal/min of main spring water that received no supplemental oxygen. The highest DO levels were achieved using sealed column oxygen supplementation at a

Table 1. Mean water quality data collected at Erwin National Fish Hatchery for unoxygenated spring water and following the addition of supplemental oxygen at gas-to-liquid ratios of 0.7, 1.5, and 2.2% using sealed columns and a pipeline manifold oxygen injection system.

Variable	Main Spring	Sealed Column 0.7	Sealed Column 1.5	Sealed Column 2.2	Pipeline Manifold 0.7	Pipeline Manifold 1.5	Pipeline Manifold 2.2
Dissolved oxygen (ppm)	8.6	11.6	13.4	14.9	11.2	12.2	13.4
Oxygen saturation (%)	86.3	115.9	133.4	149.3	112.2	121.9	134.6
Nitrogen saturation (%)	121.2	102.3	97.4	93.1	103.5	102.9	99.0
Total dissolved gas (%)	113.7	104.9	104.9	105.0	105.2	106.8	106.1
Oxygen transfer efficiency (%)		82.3	62.6	49.0	67.1	46.7	38.9
Variation in column effluent DO (%)		1.7	1.5	2.7	54.1	62.7	59.9

Figure 1. Upper production raceways at Erwin National Fish Hatchery showing location of oxygen supply lines, pumped water piping, and main spring water line. Oxygen is added at the pipeline manifold site and sealed columns.

Figure 2. Sealed column at Erwin National Fish Hatchery showing column dimensions, water inlet pipe, and oxygen supply line.

Figure 3. The pipeline manifold oxygen injection system at Erwin National Fish Hatchery. Water bypass piping and control valves allow water to be diverted from the main pipeline when oxygen supplementation is desired.

gas-to-liquid ratio of 2.2%, which resulted in a 73.5% increase in raceway DO (6.33 ppm) and increased oxygen saturation to 149.3%. This method of oxygen supplementation reduced nitrogen to 93.1% and TDG to 105.0%. By comparison, pipeline manifold oxygen supplementation at a gas-to-liquid ratio of 2.2% resulted in a 53.7% increase in raceway DO (4.83 ppm) and increased oxygen saturation to 134.6%. Nitrogen saturation was reduced to 99.0% and TDG to 106.1%.

The greatest oxygen transfer efficiency was achieved when adding oxygen to the sealed columns at a gas-to-liquid ratio of 0.7%. Oxygen supplementation using the sealed columns was consistently more efficient than adding oxygen through the pipeline manifold. Oxygen transfer efficiency was reduced as the gas-to-liquid ratio was increased for both methods. This finding is consistent with data reported by Colt et al. (1993) and Dwyer et al. (1991).

Dissolved oxygen measurements taken directly from the sealed column effluent showed extreme variation (Table 1). Percent variation from mean column effluent DO was far greater when supplemental oxygen was added through the pipeline manifold. The addition of oxygen directly into the sealed columns resulted in a 1.5 - 2.7% variation in column effluent DO. Oxygen supplementation through the pipeline manifold resulted in a 54.1 - 62.7% variation in column effluent DO. Column water flows were checked following the collection of effluent DO data. Column water flows were initially set at equal flow rates (179 gal/min through each column). While minor variations in water flow occurred when adding oxygen directly to the sealed columns, pipeline manifold oxygen injection resulted in a 21% variation in column water flow. Colt and Watten (1988) describe bubble formation when injecting pure oxygen into air-saturated water. These bubbles typically form at elbows, pipe transitions, and other areas of low pressure or high turbulence. Oxygenated water within the pumped water line flows through five elbows and several pipe transitions. It appears that gas due to bubble formation collected near the top of the pipeline, reducing the cross-sectional area of the pipe and ultimately changing water flow. Resulting variations in column effluent DO were impossible to control when adding supplemental oxygen through the pipeline manifold.

Acknowledgments

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RENOVATION OF THE GLENWOOD HATCHERY
UTAH DIVISION OF WILDLIFE RESOURCES
GLENWOOD, UTAH

by

PAUL A. HARMER
UTAH DIVISION OF WILDLIFE RESOURCES
GLENWOOD HATCHERY
GLENWOOD, UTAH 84730

The Glenwood hatchery was built in 1921, the third State Hatchery in Utah. Located 150 miles south of Salt Lake City on a bench above the town of Glenwood at an elevation of 5400 ft.. The hatchery is fed by two springs with a total flow of 9 cfs., oxygen 8.3 ppm, a temperature of 14.4° C.(57° F.), and a hardness of 103 ppm.

The Hatchery remained unchanged until 1975 when a water reuse system was built, engineered by UMA of Portland, Ore. and built by Valley Builders of Gunnison, Ut..

Although this reuse system was mechanically sound, by 1978 it became clear it was biologically unsound. By July 1979 the system was basically shut down due to the failure of the bio-filters. Since 1979 the hatchery produced approximately 60 -70,000 lbs. of fish per year, 25,000 lbs. in the reuse system and the remainder in the old dirt system.

Considerable effort went into trying to find a solution to the reuse problem. The Fisheries Experimental Station in Logan, Ut. under Ron Goede was directed to study the problem and try to find a solution. These investigations included 1) Replacing the stryofoam balls with expanded shale for filter media; 2) Remodel a filter with a air scrubber system to clean the shale in place of the water scrubber; 3) Binoxide generators were purchased and the gas was pumped into the water to oxidize the Nitrite to Nitrate and 4) ATEC Inc. of Riverton, Wyoming demonstrated a moving bed ion exchange system for Clinoptilolite using heat for the recovery and regeneration of the zeolite.

Although the ATEC system removed the Ammonia the Division was not prepared financially to start a $5,000,000 hatchery renovation project with far less information available on the ATEC process than the Division had on the reuse system.

It is believed by involved personnel that a logical sequence was followed to find a solution to the reuse problem to no avail. Decided to look at the feasibility of building a flow through system. To begin the process we visited three hatcheries that were under construction at the time. 1) the Tonto Hatchery at Tonto ,Az. 2) The Pueblo Hatchery at Pueblo Colo. and 3) The Park View Hatchery at Chama, N.M..

We then proceeded to design a hatchery around the 9 cfs of water with 18 new concrete raceways 8' x 80' x 3.5', designed with oxygen injection possibilities, oxygen being the limiting factor in the design of the hatchery and with several injection systems in use in Utah. Raceways designed to make three uses of the water. Production in the new system could reach 200,000 lbs. per year.

Before presenting the plans to the state building board, we took care of some preliminary work, including; 1) Visit with Glenwood Town Board to present plans and to get permission to relocate their culinary water line; 2) relocate the culinary water line; 3) Relocate the telephone and power lines; 4) Build a water control structure from the south spring; 5) cover the south spring; 6) replace water line from south spring; 7) prepare site by removing trees, raceways and septic tank; 8) install new septic tank for the residences and hatchery building.

With these preliminaries completed and money earmarked for the project we then met with the Division of Facilities Construction and Maintenance. With all the prior planning the project was accepted and construction began in April 1991. Engineering was done by CH$_2$M Hill and construction by Herm Hughs and Sons of South Salt Lake City with a bid of $1,032,900.00. Some of the features of the new hatchery include 1) Low Head Oxygen injection units (LHOs) from Zeigler Brothers, Inc.in the second and third pass raceway series; 2) a sludge collection system for removal of settleable solids; 3) adjustable weirs at the tail end of each raceway; 4) a 5 ft. drop between series for O_2 injection; 5) a truck disinfection pad for use before contaminated trucks are allowed on the hatchery grounds; 6) a 6ft. chain link fence enclosing the hatchery with an electronically controlled gate for privacy and people control; 7) landscaped grounds and 8) asphalted roadways.

In summary, we identified a need, looked for solutions to the reuse problem, did the necessary paper work to check feasibility for a flow through system, visited other hatcheries under construction, sent plans to our other hatcheries for critique, held planning meetings, prepared site, contacted DFCM, hired an engineering firm, and are now operating a very efficient flow through hatchery.

ADULT HOLDING AND SPAWNING FACILITY
LITTLE WHITE SALMON/WILLARD NFH COMPLEX
1896-1993

D.L. Free II
Fishery Biologist
U.S. Fish and Wildlife Service
Little White Salmon/Willard NFH Complex

Abstract

The Little White Salmon NFH was authorized in 1887 for the purpose of maintaining salmon runs on the Columbia River. The hatchery was placed in operation in 1896. Fall chinook salmon (Onchorhynchus tshawytscha) production was the major thrust of the rearing program at the Little White Salmon NFH. Present production responsibilities at the Little White Salmon/Willard NFH Complex are 5.4 million upriver bright fall chinook salmon (O.tshawytscha), 1.5 million spring chinook salmon (O. tshawytscha), and 2.5 million coho salmon (O. kisutch).

After 98 years of operation, The Little White Salmon/Willard NFH Complex has endeavored to improve spawning operations by maximizing worker efficiency, decreasing adult pre-spawn mortality, and promoting public visibility. Improved production resulted from the enhancement of these factors. The present adult holding and spawning facility at the Little White Salmon/Willard NFH Complex has accomplished its preconstruction goal of improving spawning operations.

A comparison of the rearing performance of chinook salmon (*Oncorhynchus tshawytscha*) reared from Michigan and Oregon systems.

Michael C. Hayes, Richard A. Carmichael, Shannon M. Focher, and Mary Louise Keefe.

The Umatilla Hatchery was approved by the Northwest Power Planning Council in 1986 and hatchery construction was completed in December 1991. Umatilla Hatchery serves as the foundation for restoring spring and fall chinook salmon in the Umatilla Basin and is expected to contribute substantially to adult doubling goals in the Columbia Basin. Measure 703 of the NWPPC basin program amended the original authorization for the hatchery and specified evaluation of the Michigan type rearing system using oxygen supplementation to achieve production goals. The Michigan rearing system was selected for Umatilla Hatchery because of the potential to increase smolt production with a limited well water supply and because it provided an opportunity to compare Michigan rearing with the standard Oregon method. Results of testing both systems will have system-wide application in the Columbia Basin.

To evaluate the performance of chinook salmon we are monitoring water quality, growth, smolt condition, migration performance, and smolt-to-adult survival. Preliminary data suggests that rearing chinook salmon in Michigan ponds produces smolts similar in size and performance to those reared in Oregon ponds. However, cortisol data and food conversion comparisons indicate that Michigan reared chinook salmon may be subject to increased stress. Data on smolt-to-adult survival, is yet unavailable and will provide the most crucial information for evaluating the success of the hatchery program. In the future we will continue to evaluate the Michigan and Oregon systems, participate in planning and coordination activities in the Umatilla Basin, and initiate studies for spring chinook and summer steelhead as water availability increases.

EVALUATION OF THE ABSORPTION EFFICIENCY OF THE LOW HEAD OXYGENATION SYSTEM

Eric J. Wagner, Thomas Bosakowski, & Scott A. Miller

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

ABSTRACT

The "Low Head Oxygenation System" or LHO™ is a device recently patented for injection of oxygen or other gases into liquids, relying on serial reuse of oxygen through a series of chambers or stages. The device is especially suited for applications where the low hydraulic head limits the use of other oxygen injection devices. The LHO has recently been used to supersaturate water with oxygen for increased production of fish. In this study, the absorption efficiency of the LHO and nitrogen gas supersaturation concentrations were evaluated at five different oxygen gas to liquid ratios (G/L) ranging from 0.10 to 0.83% (0.40 to 3.20 g O_2/min). The mean absorption efficiency of the LHOs ranged from 67.3 to 90.6%, peaking at a G/L of 0.20% (0.79 g O_2/min). This oxygen flow corresponded to a mean dissolved oxygen concentration of 12 to 13 mg/l entering the raceway. Absorption efficiency decreased as oxygen flows increased. Nitrogen gas saturation was inversely proportional to oxygen flow, and did not fall below 100% saturation until oxygen flows exceeded a G/L of 0.64% (2.50 g/min).

INTRODUCTION

Oxygen is often the limiting factor in the culture of aquatic organisms (Willoughby, 1968; Liao, 1971; Piper *et al.*, 1986). Therefore to increase production, an increase in the amount of dissolved oxygen (DO) is required in most situations. Many of the hatchery facilities in Utah have a limited amount of hydraulic head, and a demand for greater production of fish (Creer, 1989).

Supersaturation of incoming water by injection of pure oxygen has been achieved recently by using various devices such as the U-tube aerator (Speece and Orosco, 1970), downflow bubble contact aerator (Speece *et al.*, 1971), packed columns (Hackney and Colt, 1982; Watten and Boyd, 1989), and pressurized packed columns (Schutte, 1988; Klar and Parker, 1990). Watten (1989) took the packed column design one step further by connecting several of them side by side. This created a device that reduced the amount of hydraulic head required to oxygenate water, eliminating the need for pumping water and thus reducing the risk of system failure. The device is currently marketed as the "Low Head Oxygenation System" or LHO™, and is in use at several Utah hatcheries. This project evaluated the oxygen absorption efficiency of the LHO and its effectiveness in reducing nitrogen gas supersaturation at five different oxygen flows.

METHODS

Absorption efficiency and nitrogen gas supersaturation concentrations were evaluated at five different oxygen gas-to-liquid ratios (G/L) ranging from 0.10 to 0.83% (0.40 to 3.20 g O_2/min). Oxygen flows were determined by measuring the change in weight of the oxygen tank and dividing by the amount of time the tank was supplying oxygen. The length of time for each test varied (372 to 2498 min), being longer at lower oxygen flows. This maximized the change in weight of the oxygen tank, reducing experimental error in the weight measurement. The precision of the digital scale used was ± 45 g, which would result in only a slight variation (±1%) in the absorption efficiency. Each mass flow value was converted to a volumetric gas-to-liquid ratio by using the specific volume of oxygen under standard conditions (Colt and Watten, 1988; Dwyer and Peterson, 1993).

Absorption efficiency (AE) was calculated using the formula (Watten and Boyd 1990),

$$AE = \frac{Q_L (DO_{out} - DO_i)\, 10^{-3}}{M_{O_2}}$$

where DO_{out} is the mean dissolved oxygen of water exiting the eight LHO chambers in mg/l,

DO_i is the dissolved oxygen of the water entering the LHO,

Q_L is the water flow in liters/min,

10^{-3} is the conversion from mg to g, and

M_{O2} is the mass flow of oxygen to the unit in g/min.

Gas saturation measurements were made with a saturometer (Sweeney Aquamatic) at the time of the oxygen determinations, with the probe located in the center of the raceway about a meter downstream of the LHO where the water had mixed sufficiently to get a representative reading. Nitrogen gas saturation values were calculated by a computer program (Cook and Canton, 1988) using barometric pressure readings taken from the saturometer. Temperature (17.2 ± 0.1°C), DO (7.0 ± 0.1 mg/l), and water flow (291 l/min) of the hatchery well water were constant during the study. The total gas saturation of the well water was 107.9% (6.78 kPa above the barometric pressure), nitrogen gas saturation was 116.3%, and barometric pressure during the study ranged from 85.0 to 86.2 kPa.

Four raceways 1.22 m wide x 11.58 m long x 0.57 m deep, with commercial LHO units (Zeigler Brothers Inc.), were supplied with oxygen from a single compressed oxygen tank. During the tests, rainbow trout fingerlings, Oncorhynchus mykiss, were present in the raceways at densities ranging from 23.4 to 98.0 kg/raceway. The oxygen was delivered to each LHO unit by splitting the flow in half at one manifold and again at another manifold downstream of the first. The manifold fittings were four times larger than the supply line to insure equal amounts of gas to each line. Downstream of the first manifold, a mass-flow controller (Omega

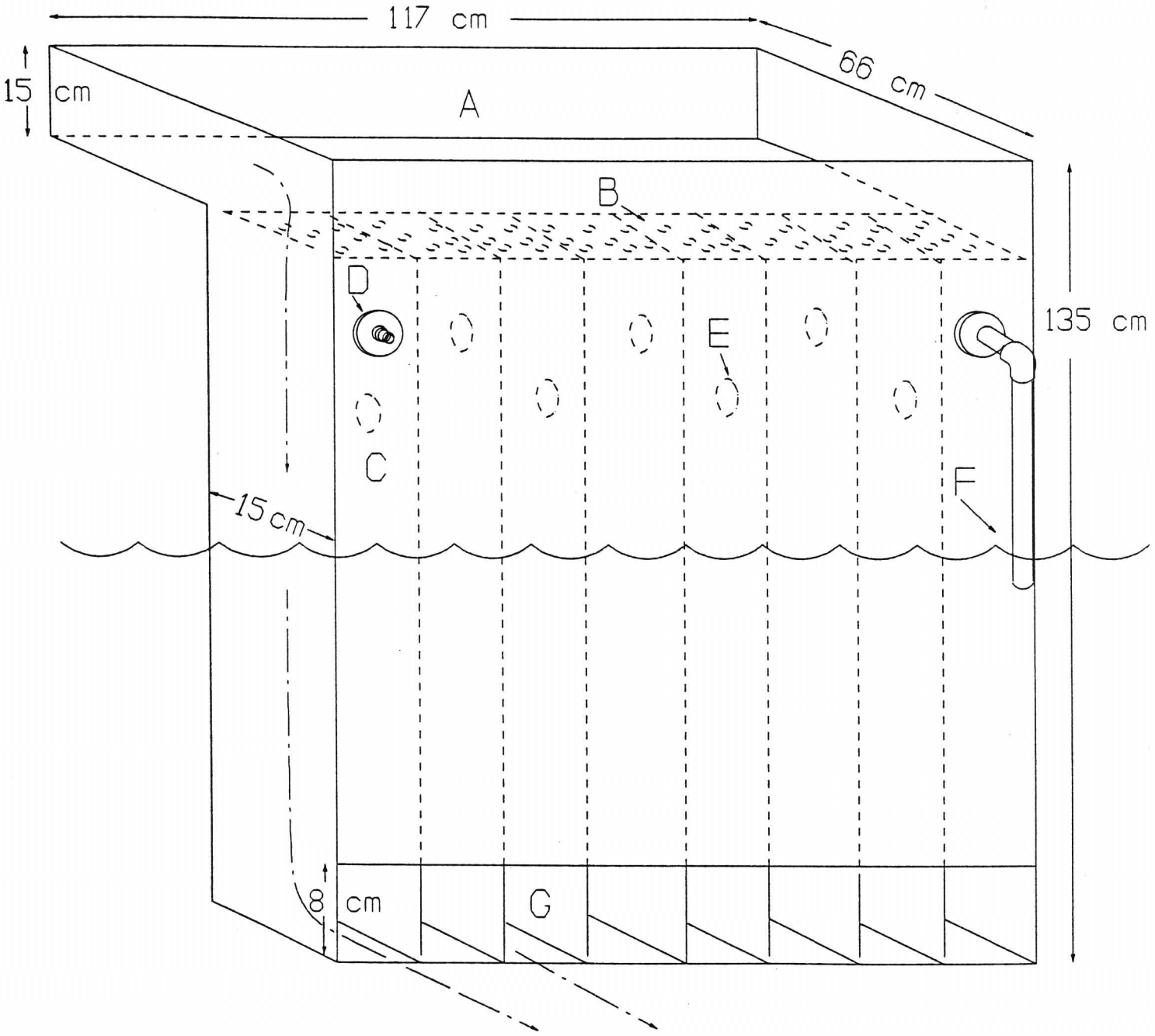
Engineering) measured the oxygen flow to the next manifold. Rotameters (Victor, 7 l/min capacity) were used to control the flow of oxygen to each raceway. Uniform oxygen pressure was controlled in the inflow line by a regulator on the liquid oxygen tank.

The open trough at the top of the LHO (Figure 1) spread the inflowing water over a horizontal perforated distribution plate. The water jetting through the holes (9 mm diameter) was oxygenated as oxygen flowed perpendicularly through a single hole in each of eight stages or compartments. The oxygen concentration was highest in the first stage and dropped en-route to the last, where the remaining gas is vented. The outlet of the off-gas pipe was kept just below the water surface to provide enough back pressure to maintain a 10 cm depth of water above the distribution plate. The distance from the distribution plate to the water surface of the raceway was 60 cm.

Dissolved oxygen (DO) concentrations were measured with a digital oxygen meter (YSI Inc.) calibrated with replicate Winkler tests (APHA *et al*. 1989). Oxygen concentrations of water leaving the LHO were made by placing the probe within each stage, on the floor of the raceway. The mechanical stirrer supplied with the oxygen probe was used during each measurement. The meter was given time to equilibrate, then an average of the maximum and minimum DO values observed (nearest hundredth) was recorded (rounding to the nearest tenth). The mean DO of water exiting the eight stages (chambers) of each LHO was used as the DO

Figure 1. Diagram of the Low Head Oxygen Injection unit. Water flows into a collection trough (A), through a perforated distribution plate (B), and is oxygenated in the chambers (C) as oxygen flows from the inlet hose barb (D), through the holes between chambers (E), to the off-gas pipe (F) where excess gas is bubbled off underwater. Water exits at the bottom of the unit (G).

Figure 2. Mean (± SD) oxygen absorption efficiency and nitrogen gas saturation of three Low Head Oxygen Injection units at five different oxygen flows.

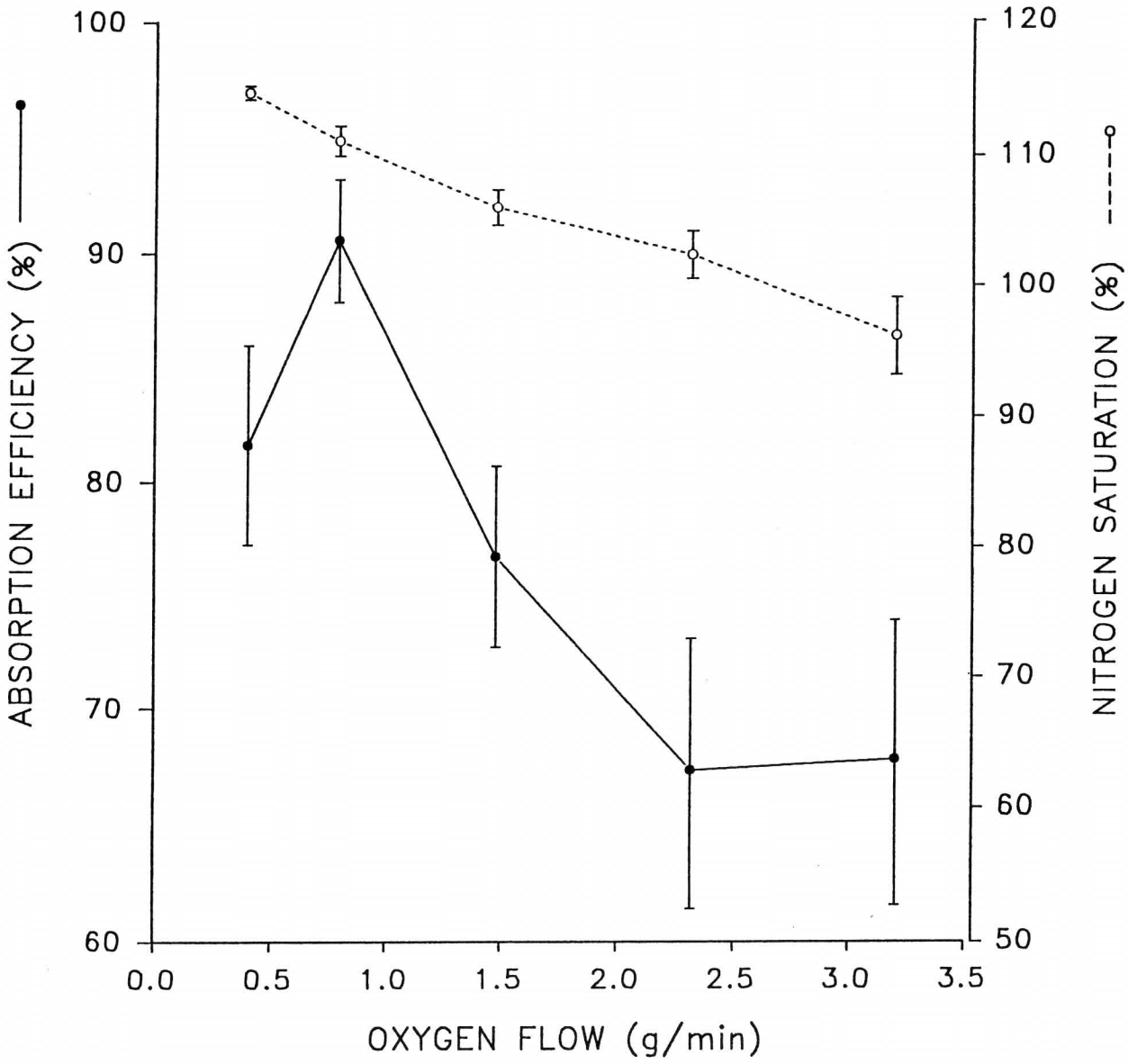

117 cm
66 cm
15 cm
A
B
D
E
C
F
135 cm
15 cm
8 cm
G

concentration leaving the LHO. DO of the incoming water was measured in the trough of the LHO. DO readings were taken several times during the testing of each oxygen flow rate to determine if there were any daily fluctuations. No significant fluctuations were observed.

Preliminary tests were conducted from 12 to 18 November 1992 to compare DO concentrations at the tail end of the raceway with the mean DO leaving the LHO. Readings were taken at five different oxygen flow rates in fishless raceways, freshly scrubbed and disinfected with sodium hypochlorite.

RESULTS AND DISCUSSION

The mean absorption efficiency of the LHOs ranged from 67.3 to 90.6%, peaking at a G/L of 0.20% (0.79 g O_2/min), and decreased as oxygen flows increased or decreased from this flow (Figure 2). For a pressurized column, Schutte (1988) reported absorption efficiencies ranging from 32 to 93%, depending upon oxygen flow and chamber pressure. In Shutte's (1988) study, the absorption efficiency increased with the amount of oxygen added at a given pressure. The rate of oxygen flow in that study was lower than the oxygen flow where peak efficiency was noted in this study, corresponding with the increase in absorption efficiency from G/L 0.10 to 0.20% (0.40 to 0.79 g/min). The absorption efficiency in another study of a pressurized column


100
90
80
70
60
120
110
100
90
80
70
60
50
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
ABSORPTION EFFICIENCY (%)
NITROGEN SATURATION (%)
OXYGEN FLOW (g/min)

(Klar and Parker, 1990) peaked at 82.8% at a G/L of 7.7% (2.87 g/min) and a pressure of 5.8 kPa. However, the peak absorption efficiency occurred at lower oxygen flows as the pressure dropped (Klar and Parker, 1990). The pressure of the LHO chambers was controlled by the off-gas pipe depth (hydrostatic pressure), resulting in gauge pressures of 0.20 to 0.49 kPa. At these low pressures, the peak absorption efficiency of this study and of Klar and Parker's (1990) study occur at similar oxygen flows. The absorption efficiency predicted by Watten and Boyd (1990) for a 10 stage LHO at a G/L of 0.8% was 85%, based on a column height of 0.60 m. At that gas-liquid ratio in this study, the absorption efficiency was 68%. The difference may be related to a number of factors: the number of stages (10 versus 8 in this study), which increase the absorption efficiency (Watten and Boyd, 1990); lower barometric pressures in this study; and cooler temperatures in this study. Oxygen absorption efficiency for LHO units evaluated by Dwyer and Peterson (1993) peaked at 91% at a G/L of 0.12, which was similar to the peak efficiency observed in this study. However, the peak occurred at a slightly higher oxygen flow in this study. Despite the different field conditions and LHO dimensions, the range of absorption efficiencies in Dwyer and Peterson's (1993) study (65.2 to 91.1%) was similar to the range in this study, where similar G/L ratios were tested.

One of the LHO units in this study was a different design than that of Figure 1, differing only in the size of the trough.

In this case, the trough was deeper (38 cm) at the back, forming a stilling basin before the water flowed up and over the distribution plate. The absorption efficiency of this LHO was 22% lower than the mean of the other three units at a G/L of 0.10% (0.400 g O_2/min); at higher oxygen flows, this LHO was 7 to 11% less (Table 1). Since there was only one of these units, no statistical comparison with the other design could be made. However, the data from this LHO was not included in Figure 2. The reason for the discrepancy is not clear, since the distribution plate, number of stages, width, and height were identical to the other units. Fish density was not a factor since the density of fish in this raceway was identical to that supplied by LHO unit 1 where a peak absorption efficiency of 91.7 was observed.

Nitrogen gas saturation was inversely proportional to oxygen flow, and did not fall below 100% saturation until oxygen flows exceeded a G/L of 0.64% (2.5 g/min; Figure 2). Nitrogen gas supersaturation can cause gas bubble disease, characterized by lesions in the blood (emboli) or tissues (emphysema; Bouck, 1980). This non-infectious disease may be chronic at low levels of supersaturation, causing pathologies such as blindness (Stroud *et al.*, 1975), or cause significant mortality at high levels of supersaturation (Harvey, 1975). When total gas saturation values are above 100%, higher oxygen-to-nitrogen ratios significantly reduce mortality (Nebeker *et al.*, 1976). Total gas saturation values in this study varied little, ranging only from 108.45 to

110.33 (Table 1).

Measurement of oxygen flows at the tail end of a fishless raceway were similar to measurements derived from the means of the eight stages (Table 2). This was an indication that loss of DO to the atmosphere as it flows down the raceway was negligible and that the baffle mean was an accurate measure of DO leaving the LHO units.

Results of this study indicated that if oxygen flows are high enough, LHO units can be used to successfully increase oxygen concentrations for greater fish production, as well as decreasing the nitrogen gas supersaturation to benign levels. Other oxygen injection devices with absorption efficiencies in the same range include the pressurized packed columns (95-100%), U-tube with off-gas recycling (60-90%) and aeration cones (80-90%; Colt and Watten, 1988). However, the LHO unit is especially applicable in situations where there is limited hydraulic head. As in some of the other oxygen injection methods, the lack of moving parts or the need for pumps also eliminates power costs and reduces maintenance.

ACKNOWLEDGEMENTS

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Table 1. Oxygen absorption efficiency (AE) and gas saturation levels (TG=total gas, N=nitrogen) from four LHO units under different oxygen flow rates (G/L=gas-to-liquid ratio). The dissolved oxygen concentration (DO) of the water supply was 7.0±0.1 mg/l.

LHO Unit	G/L (%)	O_2 Flow (g/min)	AE (%)	TG (%)	N_2 (%)	ratio O_2/N_2	final DO (mg/l)
1	0.10	0.40	85.98	109.56	115.13	0.78	8.09
	0.20	0.79	91.70	109.37	110.92	0.94	9.51
	0.38	1.48	72.58	109.53	107.56	1.09	10.75
	0.60	2.32	66.65	110.29	103.69	1.31	12.39
	0.83	3.20	60.63	110.33	99.52	1.53	13.89
2	0.10	0.40	77.28	109.15	114.84	0.77	8.01
	0.20	0.79	87.50	110.24	112.32	0.92	9.42
	0.38	1.48	77.01	108.55	105.74	1.13	10.95
	0.60	2.32	61.75	108.89	103.19	1.27	11.94
	0.83	3.20	71.45	109.17	94.63	1.74	15.06
3[a]	0.10	0.40	59.16	109.50	115.86	0.74	7.81
	0.20	0.79	71.22	109.34	112.47	0.87	8.96
	0.38	1.48	61.63	109.27	108.88	1.02	10.18
	0.60	2.32	56.60	110.21	105.82	1.21	11.62
	0.83	3.20	60.70	109.56	98.64	1.54	13.84
4	0.10	0.40	81.54	108.90	114.17	0.79	8.13
	0.20	0.79	92.53	108.90	110.13	0.95	9.58
	0.38	1.48	80.56	108.45	104.87	1.17	11.20
	0.60	2.32	73.39	109.04	100.34	1.42	12.99
	0.83	3.20	71.17	108.78	94.22	1.74	15.02

[a]Unit 3 featured a deeper trough to the rear of the distribution plate of the LHO.

Table 2. Comparison of the mean dissolved oxygen (DO) concentrations leaving the low-head oxygen injection unit and DO leaving the tail end of a fishless raceway at five different oxygen flows.

LHO Unit	LHO DO (mg/l)	tail DO (mg/l)
1	6.7	6.8
	9.2	9.4
	12.0	11.7
	14.1	14.3
	14.9	15.0
2	6.6	6.7
	9.1	9.4
	12.1	12.2
	14.3	14.2
	15.2	15.3
3	6.6	6.7
	9.0	9.2
	12.1	12.0
	14.3	14.4
	15.1	15.0
4	6.8	7.0
	9.0	9.4
	12.4	12.2
	14.2	13.8
	15.0	15.0

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Cowlitz Salmon Hatchery Fish Electroanesthesia System

Mark G. LaRiviere
Tacoma Public Utilities, Light Division
Tacoma, WA 98411

BACKGROUND

The Cowlitz Salmon Hatchery (CSH) barrier dam is currently the upstream limit for unassisted anadromous salmonid fish migration in the Cowlitz River, Washington. Adult salmonid fish returning to the barrier dam are attracted into the mouth of a fish ladder and ascend into a holding pool that leads to the existing separator facilities. The CSH experiences returns of thousands of adult salmon and steelhead every year.

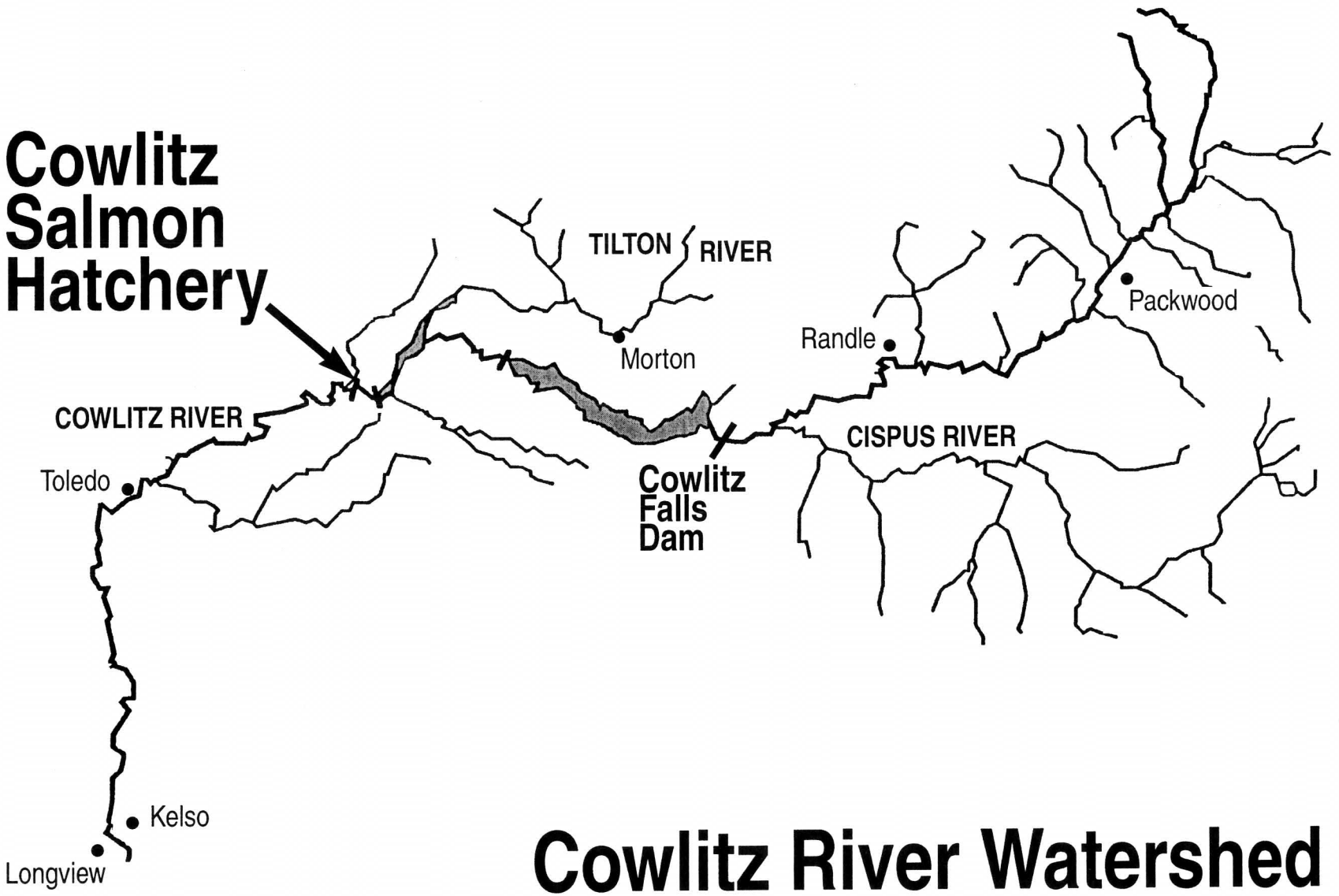
Fish returning to the separator originate from three primary sources: the CSH, the Cowlitz Trout Hatchery, or from natural production upstream of Mayfield Dam on the Cowlitz River.

A continuing need exists for a safe and efficient manner of handling and manually sorting the returns for differentiation by species, sex, tag, or stock characteristic. Manual sorting is used to separate hatchery and naturally produced fish to facilitate transportation of those fish within the Cowlitz River system. In addition, the separation system is used for handling adult fish needed at the Cowlitz River Salmon and Trout hatcheries.

During 1992 and 1993, Tacoma Public Utilities, Light Division (TPU) purchased, installed and tested an electroanesthesia system for handling and sorting adult salmonid fish at the CSH. This system replaced an older anesthesia tank and supplanted the usage of marginally safe and/or inefficient anesthesia agents such as Methane tricainesulfonate (MS 222) and carbon dioxide. The electroanesthesia system is flexible enough to use on a range of fish species and sizes under varying water quality conditions, and is operationally simple and safe. Handling and sorting rates of up to 2,000 fish per day are anticipated at the CSH.

DESCRIPTION

The electroanesthesia system at the CSH was installed in November 1992 and initial tests were conducted with adult coho salmon. Electroanesthesia is now the sole anesthetic in use at the CSH separator.



Cowlitz River Watershed

TPU purchased a Coffelt Electroanesthesia System 91, Model No. EA-7 (serial no. 921188). This new unit, installed by Coffelt Manufacturing, Inc. of Flagstaff, Arizona, uses patented complex pulsed system circuitry. This circuitry is designed to quickly and effectively anesthetize a wide variety of fish species and sizes, without injury.

The system consists of:

* a mobile control center for the electronic components
* a 10' x 2' x 3' rugged fiberglass tank
* power cables
* foot switches
*a fresh water flow-through design and
* removable cathode and anode arrays in the tank.

The electroanesthesia system replaced a galvanized steel tank and the chemical anesthetic agents. The new fiberglass tank was installed in the same location at the separator, in an unused circular tank with a false wooden bottom. Change over was facilitated by the minimal system requirements of 110 volt AC power and 50 gallons per minute (gpm) of fresh water.

Safety features include an audible warning beeper, foot switches that must be depressed simultaneously to operate, a full power delay timer and an auxiliary outlet for external visual or audible warning alarms. Hatchery and project personnel operating the system were trained by Coffelt personnel in safety and operations.

OPERATION

The fish enter the existing separator facilities by jumping or being crowded over a false weir at the head of the pool. The fish fall into a slide and travel down a padded flume where an operator can make a visual identification and open and close pneumatically powered gates to guide the fish to the new tank. A maximum of 30 steelhead or 50 coho are diverted into the electroanesthesia tank.

After a short time to allow the fish to settle and orient, the operator activates the electroanesthesia system. Electroanesthesia system settings now utilized are: coho salmon - 500 V for 6 - 10 seconds, fall chinook jacks - 500 V for 3 seconds, steelhead - 500 V for 3 seconds and spring chinook adults and jacks - 500 V for 2 seconds. Fish can be handled immediately after shocking and recovery is complete in 10 minutes or less.

EVALUATION

Coho salmon were subjected to a 500 V, 10 to 20 second output during November and December, 1992. A total of 381 treatment and 381 control fish were differentially marked and held with other coho broodstock in a hatchery pond. Some injuries were noted immediately on the treatment fish, and adults swimming erratically or floating upside down after 3 days were considered moralities for the purposes of this study.

Control fish had a **5.0 % mortality rate** and the test fish had an **11.3 % mortality rate** at the end of the test in March 1993. Test fish mortality rates were comparable to overall mortality rates for adult coho salmon at the CSH since 1987. External pigment markings, spinal injuries and internal hemorrhaging were attributed to varying voltage/timing combinations used in the initial tests. Autopsies and x-ray examinations indicate injuries were primarily associated with the spinal cord - a compressed disc on one coho adult and hemorrhaging into the musculature along the backbone in fillets of surplus fish.

Injury rates were reduced for coho salmon once salt was added to the water to increase conductivity and reduce the duration of voltage applied. A rule of thumb now used in the electroanesthesia unit operation at the CSH is 2 handfuls of rock salt added to each tank of water. Since water exchange is continuous in the tank, the salt is usually added in the morning and the afternoon during a full day of operating the unit.

Use of Chilled Recirculated Water for Delaying Development of Rainbow Trout Eggs

by

Speros K. Doulos

Anthony J. Garland

U.S. Fish and Wildlife Service

Erwin National Fish Hatchery

1715 Johnson City Hwy.

Erwin, TN 37650

Abstract

The use of chilled recirculated water (with no addition of fresh water) was evaluated for incubating and delaying development of rainbow trout (*Onchorhynchus mykiss*) eggs. Chilled recirculated water at a temperature of 44.8^{0}F was higher in dissolved oxygen, and lower in nitrogen saturation and total dissolved gas than unchilled spring water at a mean temperature of 55.8^{0}F. Eggs incubated in chilled recirculated water had a 71.3% survival rate to the eyed stage and an 84.3% survival rate to hatch. Eggs incubated in unchilled water had a 70.1% survival rate to the eyed stage and an 86.0% survival rate to hatch. The use of a one-time 25 ppm formalin treatment in the chilled recirculating incubation system effectively controlled fungal infection, and resulted in a 1639-fold decrease in the amount of chemical required for fungus control. Use of chilled water nearly doubled the amount of time required for eggs to develop to the eyed stage. Electrical power demand was minimal when using chilled recirculated incubation water for delaying the development of rainbow trout eggs. Electric power costs for the time that the chiller was operational were $4.91 higher than normal.

Introduction

The rate of development of salmonid eggs is dependent upon water temperature. Cooler water temperatures retard embryonic development (Leitritz and Lewis 1980). As a result, egg incubation periods can be extended much longer than normal when a chilled water supply is used. This extends the availability of eyed eggs throughout the spawning season. Timing of egg shipments and fry hatching is critical at some production facilities. Hatchery managers plan production programs to avoid poor water quality, to meet growth projections, and to assure that adequate rearing space is available. Cooling broodstock hatchery incubation water is one method of producing eggs to meet the needs of hatchery production programs.

The use of chillers has been the most popular method for cooling incubation water. Chilling egg incubation water requires an excessive amount of electricity, resulting in higher operational costs. Electrical demand is compounded by the traditional flow-through chiller system where water is chilled, used for incubation, and then wasted. Several attempts have been made to reduce the costs of chilling incubation water by incubating eggs in a refrigerated moist environment (Reiser and White 1981) and holding adult brood fish in controlled light conditions to delay ovulation (W. Orr, U.S. Fish and Wildlife Service, personal communication).

In this study, a system was tested to economically chill egg incubation water at the Erwin (Tennessee) National Fish Hatchery (NFH). Data was collected to determine the effectiveness of using chilled recirculated incubation water to delay the development of rainbow trout (RBT) eggs. Both water quality and egg development were monitored for eggs incubated

with chilled recirculated water. A formalin treatment regime was also evaluated to control fungus in a recirculating incubation system.

Methods and Materials

Erwin NFH is a RBT broodstock hatchery that is operated as part of the U.S. Fish and Wildlife Service National Broodstock Program. Incubation water at Erwin NFH originates from a spring at a constant temperature of 55^{0}F with slight deviations due to the effect of ambient air temperatures. Eggs from six strains of RBT are incubated to the eyed stage and then shipped to hatcheries and research facilities throughout the United States. Approximately 15 million eyed eggs are shipped annually.

On September 16, 1993, 156 female Erwin-Arlee backcross (EED) strain fish were spawned and fertilized with sperm from 156 male EED strain fish. The freshly fertilized eggs were water hardened in iodophor (Argentyne) for 30 minutes, and then transferred to a trough for water hardening in fresh water for an additional 2 hours. The pooled eggs were then enumerated using the displacement method (Piper et al. 1982).

A recirculating chiller system was constructed to cool water temperatures and minimize electrical demand. The chilled incubation system consisted of four, 12-inch diameter Eagar egg jars that were located in a 21-ft X 2.5-ft X 2.5-ft concrete rearing tank. The jars were plumbed to a PVC header that extended from a fresh water supply line used to initially fill the system. Egg jar effluent water was collected in a 4-inch diameter pipeline that emptied into a 35-gallon plastic basin. Effluent water passed through a mesh bag containing activated charcoal prior to entering the collection basin. Incubation water was recirculated through the system using a 1/10 hp magnetic drive pump. Maximum pump flow was 11 gpm. Pumped incubation water then entered a 115 volt, 1/4 hp chiller (Aquatic Ecosystems Model AE-3) before re-entering the PVC header and egg jars. All exposed piping was double-wrapped with pipe insulation, and the egg jars and collection basin were covered with 2-inches of sheet styrofoam insulation.

The chilled incubation system was initially filled with spring water at an ambient temperature of 55.9^{0}F. The magnetic pump drive was started to circulate water through the chiller. The fresh water supply valve was closed after 15 minutes of pump operation. Incubation water was then recirculated without adding fresh water. Water control valves were adjusted to provide a flow of 2 gpm to each egg jar.

A total of 244,800 fertilized green eggs were measured by displacement into three egg jars within the chilled incubation system (81,600 eggs/jar). The chiller setpoint water temperature was adjusted to 44^{0}F. An additional 244,800 eggs were measured into three Eagar egg jars (81,600 eggs/jar) that were plumbed to a fresh water supply line in a separate concrete tank. The eggs in the control group were incubated using fresh spring water at an

ambient temperature of±55^{0}F. Control group egg jar water flows were set at 2 gpm.

Control group eggs were treated 15 minutes daily with 1667 ppm formalin to control fungus. This group also received a weekly 75 ppm iodophor flush to control soft-egg disease. Eggs in the chilled incubation system were given a one-time 25 ppm formalin treatment to control fungus. Formalin was added one day post-spawning and allowed to recirculate during the entire incubation period. Chilled eggs were not treated with iodophor to control soft-egg disease.

Incubation water temperature and dissolved oxygen (DO) from both groups were measured daily using a YSI Model 50-B DO meter. In addition, nitrogen saturation, total dissolved gas (TDG), and pH were measured three times per week. Nitrogen saturation and TDG were measured using a Weiss saturometer (ECO Enterprises Model ES-2), and pH was measured using a Hanna Instruments (Model HI9023) pH meter.

Chiller water temperature adjustments were made when eggs in the chilled group reached the eyed stage. The chiller setpoint temperature was gradually increased over a period of 3-days to reach ambient spring water temperatures. This required a 4^{0}F increase each day and was accomplished by increasing the chiller temperature 2^{0}F every 12 hours.

Eggs from both groups were shocked at the eyed stage to allow for the removal of unfertile eggs. Shocking was achieved by dropping the eggs 3-feet into fresh spring water at ambient temperature. Shocked eggs from individual jars were kept separate and placed into Eagar egg jars that received unchilled incubation water. Following shocking, all eggs were treated 15 minutes daily with 1667 ppm formalin for fungus control. Two days following shocking, the eggs were picked to remove unfertile eggs using a Jensorter egg picker (Model JH). Eggs in both the chilled and control group jars were picked separately. Following picking, eggs in each jar were enumerated by displacement to determine survival to the eyed stage (eye-up). A 100-egg sample was collected randomly from each jar and placed on hatching screens wedged in a nursery tank that received fresh spring water at ambient temperature. Eggs were allowed to hatch to determine percent hatch for both groups. Remaining eggs were combined and shipped to other hatcheries.

Results and Discussion

There was extreme variation in the measured water quality parameters for the unchilled and chilled egg groups (Table 1). Mean water temperature for the unchilled group was 55.8^{0}F compared to a mean temperature of 44.8^{0}F for chilled water. Data indicates that water quality was enhanced when incubation water was chilled.

Table 1. Mean water quality parameters, egg survival, and total amount of formalin required to control fungus, for rainbow trout eggs incubated in chilled recirculated water and unchilled fresh spring water.

	Unchilled	Chilled
Temperature (^{0}F)	55.8	44.8
Dissolved Oxygen (ppm)	7.8	10.6
pH	7.76	7.61
Total Dissolved Gas (%)	105.7	98.7
Nitrogen Saturation (%)	113.0	100.2
Eye-up (%)	70.1	71.3
*Hatch (%)	86.0	84.3
**Formalin (oz)	327.7	0.2

*Random sample of 100 eggs from each jar (300 total).

**Unchilled eggs treated with 1667 ppm formalin, 15 minutes daily. Chilled eggs treated once with 25 ppm formalin.

Table 2. Number of days post-spawn and daily temperature units required for the development of eggs incubated in chilled recirculated water and unchilled fresh spring water. One daily temperature unit (DTU) equals 1^{0}F above freezing (32^{0}F) for a 24 hour period.

	Unchilled		Chilled	
Stage/Event	Days	DTU	Days	DTU
Weak Eye	12	287	24	303
Strong Eye/Shock	15	358	29	381
*Shipment/Placed on Hatching Screens	18	429	**32	453
Begin Hatch	23	547	38	595
Hatching Complete	25	595	40	642

*All eggs shipped except for a random sample of 300 eggs (100 eggs from each jar) for incubation to hatch.

**Chilled egg group placed in 56^{0}F spring water to simulate incubation conditions following shipment.

Chilled incubation water had a higher DO level and was lower in nitrogen saturation and TDG than unchilled water. Mean DO for incubation water at ambient temperature was 7.8 ppm (78.8% saturation). Mean DO for chilled incubation water was 10.6 ppm (93.3% saturation). Nitrogen saturation was 113.0% for unchilled incubation water, compared to 100.2% for chilled incubation water. The spring water supply at Erwin NFH is characterized by nitrogen saturation levels that can exceed 120%.

Egg survival was unaffected when eggs were incubated in chilled recirculated water (Table 1). Survival to the eyed stage was 70.1% for the unchilled group compared to 71.3% for eggs incubated in chilled recirculated water. Following shocking, no fungus was evident in either egg group. Historical data at Erwin NFH reveals that a 15 minute daily treatment of formalin at a treatment concentration of 1667 ppm effectively controls fungus. The recirculation of 25 ppm formalin in the chilled egg group also effectively controlled fungus and had no harmful effect on egg survival to hatch. A total of 86.0% of the unchilled eggs hatched compared to an 84.3% hatch for the chilled group. A total of 327.7 oz of formalin were required for daily treatments at a concentration of 1667 ppm (unchilled water). Only 0.2 oz formalin were required for the one-time 25 ppm treatment in the chilled group. Incubation water pH was slightly lower in the chilled recirculating incubation system due to recirculation of the acidic formalin. The addition of formalin to the chilled recirculated incubation water resulted in a 1639-fold decrease in the amount of chemical required to control fungus on incubating RBT eggs.

Eggs incubated in chilled recirculated water developed to the eyed-stage (strong eye) in 29 days (Table 2). Eggs incubated at ambient temperature (mean 55.8^{0}F) developed a strong eye in 15 days. Use of chilled water at a mean temperature of 44.8^{0}F nearly doubled the amount of time required for eggs to develop to the eyed stage. In addition, the hatchery electric bill that included the time of chiller operation was $4.91 higher than normal. This confirms the economy of using chilled recirculated water for incubation of RBT eggs.

Data in Table 2 also includes cumulative daily temperature units (DTU) for various stages of egg development. One DTU equals 1^{0}F above freezing (32^{0}F) for a 24 hour period. Eggs incubated in the warmer unchilled water developed at a faster rate than eggs incubated in chilled water.

It is possible that the incubation of RBT eggs at Erwin NFH can be delayed even longer if colder water temperatures are used for incubation. For this study, we chose to use a temperature of $\pm$44^{0}F so as not to bias our data with lower threshold temperature induced mortality. Burrows and Combs (1957) determined that the lower threshold temperature for developing chinook salmon eggs was 42.5^{0}F. Leitritz and Lewis (1980) also indicate that RBT and chinook salmon eggs cannot be incubated in water below 42^{0}F without excessive loss. Nevertheless, eggs could be incubated at temperatures above 42.5^{0}F until development of the 128-cell or early blastula stage, and then chilled to achieve much cooler temperatures

without any significant mortality (Combs 1965). This would further delay egg development and allow greater flexibility in the timing of egg shipments to other facilities.

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OXYGEN CAN BEAT THE SUMMER TIME BLUES

Jim Byrne
Washington Department of Wildlife

INTRODUCTION

Beaver Creek Hatchery raises steelhead and sea-run cutthroat trout and must contend with warm Elochoman River temperatures up to 22°C (72°F) during the summer months. These high temperatures limit the amount of dissolved oxygen present in the water, reducing oxygen available for fish. Piper et al.(1982) indicated oxygen levels should be a minimum of 5 ppm. In summer, Beaver Creek's dissolved oxygen level drops below this minimal value in the lower portions of the raceways.

This lack of dissolved oxygen stresses fish and impacts health and growth. To increase oxygen, two methods of supplementation were investigated during the summers of 1992 and 1993.

Dwyer, Colt and Owsley (1991) demonstrated the effectiveness of oxygen injection into sealed packed columns in increasing oxygen levels. Valentine and Leppink (1990) documented the benefits of side stream oxygen injection in various Utah fish hatcheries to increase production. Edsall and Smith (1991) reported increased growth in rainbow trout fed with demand feeders using supplemental oxygen. Dwyer and Peterson (1993) evaluated a low head oxygen generator at a Montana hatchery. Beaver Creek also requires a low head application.

METHODS

Oxygen was supplemented by direct oxygen injection and through the use of packed columns. Rearing water was pumped from the Elochoman River and gravity flowed into the raceways. Head varied from 9 to 14 inches depending on dam board height.

Using a design developed by the United States Fish & Wildlife Service's Abernathy Salmon Technology Center, hatchery staff constructed an oxygen chamber from a discarded 55 gal (220 l) formalin barrel. In 1992, one chamber and in 1993, two oxygen chambers per raceway were evaluated. Oxygen was metered from a 4500 cu. ft. liquid oxygen cylinder. Operating pressure was 50 PSIG at the flowmeter inlet. Oxygen was pushed from the gas portion of the liquid oxygen cylinder by the pressure generated through evaporation from a liquid to gas. Chambers were positioned at the head end of the raceway. The use of a relief valve to vent off displaced nitrogen and excess oxygen gas is critical to proper chamber operation. Liquid oxygen was not delivered to the treatment raceways.

Oxygen was monitored with an Orion model 820 dissolved oxygen meter. A target level of 6.0 (ppm) at the raceway tail screen was chosen as the acceptable minimum oxygen level.

A 6 ft packed column (6 in diameter PVC pipe containing Koch rings) was installed in a second raceway. One fourth, (≈ 60 GPM) of the raceway flow was pumped by a 1 HP centrifugal pump into the top of the column and allowed to trickle down through the column. This method relied on ambient oxygen present in air for oxygen recharge. In 1993, due to the enhanced performance of the packed column the prior year, two raceways were monitored using this technique. An additional raceway, with no modifications, served as a control.

Daily records of water temperatures, dissolved oxygen, mortality, rations, and medications were recorded. Dissolved oxygen and temperature readings were taken at mid-depth at the raceway head and tail screen at 0700, 1500 and 2200 hours. The D.O. meter was calibrated daily.

Biweekly sampling was conducted by taking samples of a minimum of 100 fish per raceway. Fish were anesthetized in tricaine methane sulfonate (MS-222) and monitored for individual fork length (mm), weight (g) and condition factors (K-factor). Dorsal fin erosion was monitored according to the scale used previously at Beaver Creek. Flow and Density indices were calculated at biweekly intervals :

Flow index = raceway biomass/[fish length (in) * inflow (GPM)]

Density index = biomass (lbs)/[fish length (in) * volume (cu ft)]

Biweekly health monitoring of treatment and control fish was performed by Larry Durham, a Department of Wildlife Fish Health Specialist. Gill conditions and parasite levels were noted and assigned an overall ranking. In 1993, skin parasites and hematocrit were included.

At each sampling, mean length, weight, dorsal fin erosion, K-factor and health parameters were compared using Analysis of Variance. The Student Neuman-Keuls test was used to determine which populations, if any, showed statistical significance. Percentage of dorsal fin erosion, and flow and density indices were compared to ideal values. The experiment ceased when D.O. levels rose above the safe levels (6 PPM at tail screen) for a sustained period in late September.

After an initial period of feed manipulation to bring all treatment groups into a statistically similar length and weight, fish were fed to satiation using Babbington demand feeders. Feed was replenished in 50 lb increments. Fish received similar diets.

In 1992, hatchery winter steelhead from a single spawn were split (approximately 35,000 fish each,) into three production raceways (≈2,000 cu ft, 250-320 GPM). In 1993, experiment fish were from differing spawns and similar sized fish were utilized in the experiment at ≈39,000 per raceway.

In 1992 and 1993, fish were treated with formalin through station wide drips. In 1993, medicated feeds were administered to some treatment raceways. Water chemistry was monitored with a Hach Fish Farmer test kit.

RESULTS

Water flows changed over time depending on river flows, pump operation and debris buildup on valve screens. In 1992, flows dropped throughout the study period. In 1993, flows were greater and increased as the study progressed until declining near completion. Raceways flows varied by as much as 50 GPM over time.

At higher delivery levels, oxygen transfer becomes less efficient (Table 1). The oxygen chamber could add up to 11.6 ppm at maximum oxygen flow, but at reduced (33%) efficiency. The packed column added about 0.5 ppm oxygen.

Table 1. Oxygen Transfer Efficiencies for individual oxygen chambers.

Liters per minute	Cubic feet per hour	Oxygen in chamber ppm	Increase in oxygen ppm	% Transfer Efficiency
1	2.12	14.9	4.5	89.93
2	4.24	16.9	6.5	64.51
3	6.36	18.7	8.3	54.92
4	8.48	19.4	9.3	46.16
5	10.59	20.6	10.2	40.50
6	12.71	21.1	10.7	35.40
7	14.83	22.0	11.6	32.90

Water temperature increased throughout the day reaching a maximum during late evening. Water temperatures lagged behind air temperature, remaining warm into the evening (Figure 1), even after air temperature had declined. Oxygen levels fell, throughout the day and reached their lowest levels near midnight and early morning. At daylight, oxygen level increased as photosynthesis began. Oxygen levels dropped dramatically after pond cleaning, and remained at reduced levels for nearly an hour as raceways refilled and full circulation was re-established. Mean temperature and oxygen values during both study periods are presented in Figure 2.

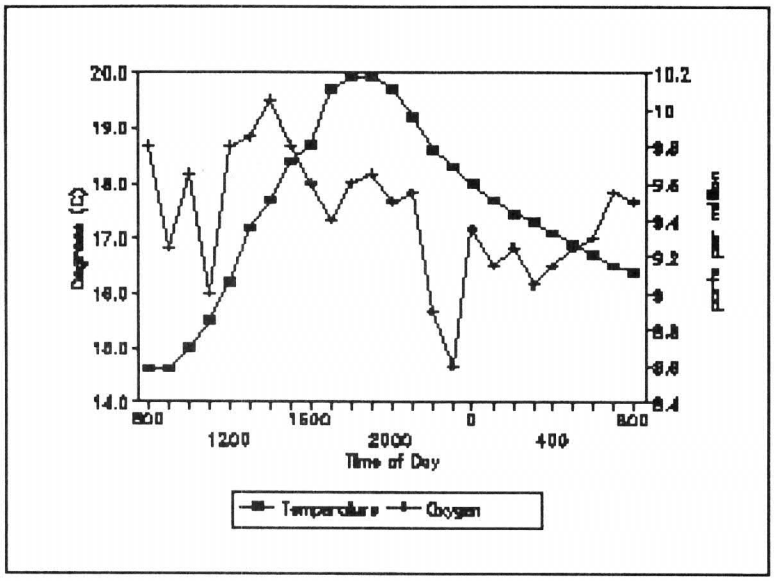

Figure 1. Typical Beaver Creek daily oxygen and temperature fluctuations.

The combination of water temperature and biomass did not reach a point of oxygen deprivation until early August, when daily monitoring of oxygen levels commenced. Prior to this point, feed amounts were manipulated to make all treatments of uniform size and weight.

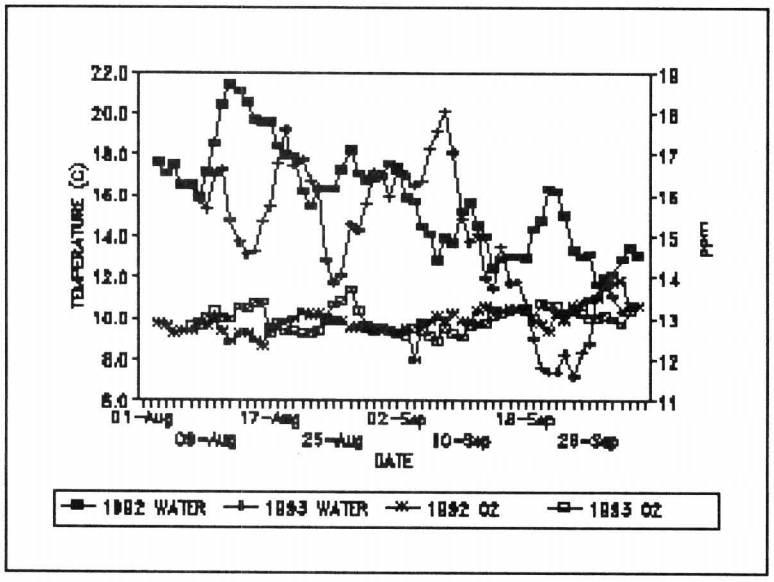

Figure 2. Beaver Creek mean temperature and oxygen values throughout oxygen supplementation experiments.

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Treatment growth patterns are presented in Tables 2 and 3.

Table 2. 1992 Treatment Mean Lengths and Weights.

DATE	Fork Length mm.			Weight g.		
	CONTROL	COLUMN	OXYGEN	CONTROL	COLUMN	OXYGEN
8/07	106.94	105.68	105.38	15.99	15.94	15.01
8/21	119.62	119.20	118.52	23.80	24.25	22.45
9/04	127.43	129.60	126.37	25.50	28.54*	25.83
9/18	133.77	137.06	134.44	29.61	32.38*	28.64
10/02	139.11	144.10	141.90	31.68	36.25*	34.57

* Significant $p<0.05$

In early September, the column group became longer and statistically significantly ($p<0.05$) heavier than other treatments and remained so throughout the study.

Table 3. 1992 Treatment Mean K-Factor and Mean Dorsal Fin Erosion.

DATE	K-Factor			Dorsal Fin Erosion		
	CONTROL	COLUMN	OXYGEN	CONTROL	COLUMN	OXYGEN
8/07	1.27	1.30*	1.25	3.54	3.40	3.69
8/21	1.36*	1.41*	1.32*	3.17	3.11	3.35
9/04	1.19*	1.27*	1.24*	3.18	3.28	3.45*
9/18	1.20*	1.23*	1.15*	3.36	3.27	3.32
10/02	1.15*	1.18	1.18	3.20	3.24	3.20

* Significant $p<0.05$

By early August, the column K-Factor was significantly greater than others. By mid-August all treatment K-Factors were significantly different, until the final sampling when the control became significantly less. The oxygen group showed significantly greater dorsal fin erosion in early September, but there were no differences at the study's end.

The Fish Health Specialist examined ten fish from each treatment biweekly. He rated gill condition on a scale of 1 (poor) to 4 (superior) and checked gills for parasites and bacteria (Table 4).

Feed conversion, flow, and density indices are presented in Table 5 and were maximum at the experiment's termination. Piper et al. (1982) suggested a flow index of 1.5 as a guide for trout and salmon. This figure was exceeded by the packed column on August 21 by direct oxygen on September 4, and by the control on September 18.

Table 4. Mean gill ranking and mean number of Ichthyopthirus multifilis present on gill per ten fish in Beaver Creek oxygen supplementation in 1992.

	Gill Ranking			# Ich present		
DATE	CONTROL	COLUMN	OXYGEN	CONTROL	COLUMN	OXYGEN
8/21	2.30	2.61	2.11	0.00	0.00	0.00
9/04	3.25	3.10	2.95	0.00	0.10	0.00
9/18	3.15	3.25	3.40	0.00	0.00	0.00
10/02	3.35?	2.75?	3.10+	0.00	0.00	0.00

* Significant $p<0.05$ + bacterial gill disease outbreak
? ANOVA indicates significant difference, but Newman-Keuls test unable to determine different treatment, probably different.

Gill rankings:
1 Severe hyperplasia/hypertrophy, very little lamellae surface area.
2 Obvious hyperplasia/hypertrophy, reduced lamellae surface area.
3 Slight hyperplasia/hypertrophy, good lamellae surface area.
4 No hyperplasia/hypertrophy, good lamellae surface area.

Only one of the treatment densities approached the recommended density index of 0.25 for steelhead (Klontz et al. 1979). At termination, the control was 0.22, the column was 0.25 and direct oxygen was 0.24. The control had the best overall feed conversion at 1.08. It was followed by the packed column at 1.11 and direct oxygen at 1.13 (Table 5).

Table 5. 1992 Feed conversion and flow and density indices.

	CONTROL			COLUMN			OXYGEN		
Date	FL.	DN.	CONV.	FL.	DN.	CONV.	FL.	DN.	CONV.
8/07	1.12	0.15	0.98	1.13	0.15	1.16	1.07	0.14	1.17
8/21	1.49	0.20	0.90	1.54	0.20	0.90	1.42	0.19	0.94
9/04	1.49	0.20	3.13	1.65	0.22	1.27	1.53	0.20	1.38
9/18	1.65	0.22	0.63	1.76	0.23	1.02	1.58	0.21	1.46
10/02	1.70	0.22	1.24	1.88	0.25	1.33	1.81	0.24	0.98
OVERALL			1.08			1.11			1.13

Water chemistry stayed fairly consistent throughout the study. PH would drop 0.5-0.8 units from the raceway head to tailscreen, hardness remained constant, ammonia nitrogen would increase 0.5-0.8 ppm, and carbon increased ≈ 5 ppm. Since the

Hach kit uses a colormetric means to determine pH, hardness, carbon dioxide and ammonia nitrogen; precision is limited. All ponds exhibited similar pH drops and increases in carbon dioxide and ammonia nitrogen. It would have been desirable to have the ability to measure CO_2 levels with greater accuracy.

During the experiment three oxygen cylinders were used costing $188.85. Three months tank rental charges were also assessed at $96.84. Total oxygen cost including tax and delivery was $285.69. The cost of powering a 1 horsepower centrifugal pump was estimated at $0.72 per day or $43.92 for the duration of the experiment.

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The summer temperature regime was quite different from the previous year (Figure 2). The summer of 1992 was extremely hot and dry. In contrast, the summer of 1993 was initially cool and wet. Oxygen decrease due to temperature was not as great. However, the numbers of fish per raceway were increased from ≈ 35,000 to ≈ 39,000, which increased the oxygen demand.

For a ten day period, August 31 to September 9, electricity could not be provided to packed column pumps due to a defective circuit breaker in the raceway area electric circuit. Unfortunately, this coincided with some of the warmest weather during the study period.

Fish in the oxygen treatment, were larger than other raceways due to differing spawns. These fish were placed on reduced ration, to allow other treatments to catch up. By the second sampling, the control and one of the packed columns surpassed the oxygen group (Table 6 and 7) in length and weight. Overall, fish were ≈ 10 mm shorter and 4 g less than the previous year's fish at the same point in time. Fish used the prior year came from a single spawn and were more homogeneous.

Table 6. 1993 Treatment Mean Lengths and Weights.

	Fork Length mm.				Weight g.			
date	CONTROL	COLUMN 1	COLUMN 2	OXYGEN	CONTROL	COLUMN 1	COLUMN 2	OXYGEN
8/06	95.6	96.0	95.4	99.7*	11.22	11.03	10.93	11.71
8/20	109.2	109.0	105.1	107.7	16.05	16.82?	14.58	14.54
9/3	116.7	117.3	113.9	126.5*	19.82	20.47	18.74	24.93*
9/15	122.1	118.1	119.2	134.0*	21.46	21.13	20.33	31.04*
9/30	133.0	128.7	128.4	142.8*	28.37	26.30	26.13	37.19*

* denotes statistical significance $p<0.05$

? ANOVA indicates significant difference, but Newman-Keuls test unable to determine different treatment, probably different.

By the third sample date, fish of the oxygen group were statistically longer and heavier than other groups and this continued until the end of the study. The oxygen group was about one third heavier than other groups.

Initially, the oxygen group was the leanest (lowest K-Factor) of all groups, but by the fourth and fifth samples it became statistically significantly plumper than other groups.

Dorsal fin erosion, constantly improved in the oxygen group. It produced some of the best looking fish on station.

Table 7. Treatment K-Factors and Dorsal Fin Erosion.

	K-Factor				Dorsal Fin Erosion			
date	CONTROL	COLUMN 1	COLUMN 2	OXYGEN	CONTROL	COLUMN 1	COLUMN 2	OXYGEN
8/06	1.26*	1.20	1.20	1.14*	3.47?	3.25?	3.39	3.41
8/20	1.21	1.25*	1.21	1.13*	3.56?	3.29	3.42	3.37
9/3	1.21	1.21	1.20	1.21	3.21	3.19	3.28	3.25
9/15	1.15	1.20*	1.15	1.24*	3.24	3.21	3.41?	3.09?
9/30	1.17	1.17	1.18	1.23*	3.29	3.03*	3.39	2.85*

* denotes statistical significance $p<0.05$
? ANOVA indicates significant difference, but Newman-Keuls test unable to determine different treatment, probably different.

Health inspections are reported in Tables 8 and 9. Sampling was more comprehensive than in the previous year with skin parasites and hematocrit additionally monitored.

Table 8. Mean number of Ichthyopthirus multifilis present on gill and skin scrape per ten fish sample in 1993.

	Skin				Gill			
date	CONTROL	COLUMN 1	COLUMN 2	OXYGEN	CONTROL	COLUMN 1	COLUMN 2	OXYGEN
8/06	0.7	0.2	0.2	3.5	0.6	0.0	0.0	1.9*
8/20	0.0	0.2	0.1	0.1	0.0	0.2	0.0	0.1
9/3	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.1
9/15	0.0	0.6	0.1	0.1	0.0	0.0	0.1	0.1
9/30	0.5	0.0	0.2	0.0	0.0	0.0	0.1	0.1

* denotes statistical significance $p<0.05$

The oxygen group initially suffered from an outbreak of the parasite Ichthyopthirus multifilis or Ich. All raceways on station were treated by formalin drips, and the numbers of Ich present was drastically reduced in the oxygen group by study's end.

Table 9. 1993 Mean gill ranking and hematocrit per ten fish sample.

	Overall gill ranking				Hematocrit			
date	CONTROL	COLUMN 1	COLUMN 2	OXYGEN	CONTROL	COLUMN 1	COLUMN 2	OXYGEN
8/06	2.6	2.4	2.6	3.25*	36.0?	40.5?	39.0	37.5
8/20	2.7	2.8	3.0	2.7	36.9	36.2	39.7?	44.9?
9/3	2.9*	1.8	2.0	3.0*	36.7	39.4	40.3	39.9
9/15	2.7	2.7	2.5?	3.3?	41.3	43.6	45.7	43.9
9/30	2.5	2.6	2.8	3.1	47.2*	41.1	36.7*	43.7

* denotes statistical significance $p<0.05$
? ANOVA indicates significant difference, but Newman-Keuls test unable to determine different treatment, probably different.

The oxygen group had the highest overall gill ranking, although it was not statistically significant ($p=0.075$).

Mortality was greater than last year for the same period. In 1992, controls had 310 morts, the packed column had 263 and the oxygen had 236. This year, the control had 1237, the columns had 1193 and 1059, and the oxygen group had only 634 morts. Oxygen mortality was 51% of controls and 57% of the column average mortality.

Epsom salt was administered to all raceways except the oxygen group in August. Controls received ormetoprin sulfadimethoxine (ROMET) in their feed in August. Packed column pond 1 received epsom salt and column pond 2 received oxytetracycline (TM) in feed during September. There are extra costs associated with these medicated feeds.

Flows and Density indices are presented in Table 10. Both indices increased as the study progressed. The density index reached and exceeded the recommended index of 0.25 for steelhead only in the oxygen group. Flow indices exceeded the conventional optimum of 1.5 for all groups on the final sampling, however the oxygen group exceeded this value by the third sample.

Table 10. 1993 Flow and density indices.

Date	Control Flow	Control Density	Column 1 Flow	Column 1 Density	Column 2 Flow	Column 2 Density	Oxygen Flow	Oxygen Density
08/06	1.18	0.13	1.12	0.13	1.08	0.13	1.08	0.13
08/20	1.28	0.16	1.41	0.17	1.24	0.15	1.19	0.15
09/03	1.49	0.19	1.57	0.19	1.40	0.18	1.70	0.21
09/15	1.40	0.19	1.44	0.20	1.30	0.19	1.81	0.25
09/30	1.79	0.23	1.74	0.22	1.66	0.22	2.23	0.28

Feed conversion was much better for the oxygen group, 0.75 vs 1.00 for the control and 1.2 and 0.98 for the packed columns. Overall, the oxygen group ate the second greatest amount of feed, but cost per pound of gain was nine to thirteen cents less than other treatments. Treatments other than oxygen received medicated feeds. The varying prices of regular and medicated feeds are combined in the one cost per lb of feed figure for each treatment in Table 15.

Table 15. 1993 feed, gain and feed cost parameters.

	Control	Column 1	Column 2	Oxygen
lbs of feed used	1560	1660	1350	1628
lbs fish increase	1566	1383	1373	2182
Overall feed conversion	1.00	1.20	0.98	0.75
feed cost	$541.75	$502.94	$433.11	$496.51
feed cost per lb	$0.35	$0.30	$0.32	$0.30
feed cost per lb of fish produced	$0.32	$0.36	$0.32	$0.23

Water chemistry duplicated the prior year. One 4500 cu ft liquid oxygen cylinder was used throughout the experiment. One year earlier, three cylinders were used. Oxygen cost $62.95 and there were 2 monthly tank rentals of $32.28. Total oxygen cost was $127.51, which was a cost of $0.058 per pound of fish gain.

The cost of powering a 1 HP pump was estimated at $1.27 per day. This was a 55 cent increase over the prior year. Total power costs per packed column were estimated at $58.42.

DISCUSSION

In 1992, at the loadings and densities used, with one

chamber; there was no benefit to oxygen injection. The packed column led to the best growth and greatest biomass. Extra growth was gained at an cost of $0.72 per day. Column fish were longer and significantly heavier (1.7 g) than other groups. Whether this small 1.7 gram weight difference means much in fish quality is questionable.

Controls had the best feed conversion followed by the packed column and injected oxygen. These differences were negligible and did not have a great effect on feed cost.

There were no real improvements in fish health with oxygen addition, nor real health problems without it. Perhaps fish were not stressed enough to affect health. Raceways were stocked to a typical hatchery loading, but density indices approached the accepted standard of 0.25 for steelhead. Anticipated improvements in gill condition were not forthcoming. Overall bacteria and parasite loads were masked by hatchery wide prophylactic treatments. Mean gill ranking was best in the control group and surprisingly worse in the oxygen supplemented group. No group showed excessive amounts of bacteria or parasites.

It is uncertain why the packed column performed as well as it did. The packed column added about 0.50 ppm oxygen to the raceway.

The oxygen supplied fish appeared to have a greater "vitality" according to hatchery staff. They appeared to be more lively than other groups.

In 1992, using one chamber, there were inefficiencies in oxygen delivery. At higher oxygen flows transfer efficiency decreased markedly. To improve efficiency, two oxygen chambers were used in the second study. Since initially, the packed column appeared to give somewhat better growth (1.7 g heavier and 2.2 mm longer), two raceways using packed columns were evaluated.

The use of two oxygen chambers made a great difference in efficiency, and ease of operation of the oxygen system. Because two cylinders were operating, the flow per cylinder was reduced approximately by half, allowing a doubling of transfer efficiency. The flowmeter required less adjustment. There were less swings in oxygen demand and it was easier to maintain the target level of 6.0 ppm.

One third less oxygen was used than previously. Although summer temperatures were cooler, increasing oxygen solubility somewhat, the greatest reason for less consumption was increased transfer efficiencies and reduced gas wastage.

Oxygen fish grew significantly longer, heavier and plumper than other groups. Column groups averaged 17.35 fish per lb (26 g), the control was 16.0 per pound(28 g), but the oxygen group

was 12.2 fish per pound (37 g), nearly a third larger than other groups. Although initially having the lowest K-Factor, the oxygen group reached a higher K-Factor than other treatments. It is assumed that this will reduce during winter and onset of smolting. WDW smolt release criteria call for winter steelhead smolts to be less than 1.0.

Dorsal fin erosion was much reduced in the oxygen group. Beaver Creek is notorious for eroded dorsals. Fin condition in the oxygen group was significantly better than other groups, and readily visible to the eye. Fin condition of column 1 was also significantly better than other treatments, but less than the oxygen group.

The primary focus of the study was to see if oxygen improved fish health during the warm conditions of summer. Apparently, the therapeutic value of oxygen was substantial. Initially the oxygen pond had significantly higher levels of Ich on gills and the highest level of Ich on the skin, in comparison to other groups. With station wide formalin drips, this was brought under control. Ich was not seen in skin samples of oxygen fish at the experiment's end, and was present in low levels on gills. The oxygen group also scored highest in Larry Durham's overall gill rating, $p=0.075$, approaching the 0.05 level of significance. Hematocrit varied widely within normal ranges. No correlation between hematocrit or any treatment was apparent. The oxygen pond was the only treatment not to receive the more expensive medicated feed.

Column pond 1, consumed the most feed, followed by the oxygen group, the control and the column 2. The oxygen treatment had superior feed conversion with a 0.75. It was followed by column raceway 26 at 0.98, the control at 1.00 and the second column at 1.20. Oxygen fish were much better able to utilize their feed, grew an extra 500 to 700 lbs over other treatments, and did it at a reduced cost per pound of fish gain. It cost 23 cents for the oxygen group, 32 cents for the control and column raceway 26, and 36 cents for column raceway 22 to grow a pound of fish. Oxygen saved nine to thirteen cents over other groups. The cost of medicated fish feed is included in these prices. The oxygen pond did not require medicated feed.

The oxygen treatment had the highest flow and density indices (Piper et al. 1982). An additional 4,000 fish were added in 1993, to increase the density, but only the oxygen group exceeded the recommended index of 0.25 for steelhead. Piper et al. (1982) suggested an optimum flow index of 1.5 as a guide for trout and salmon. The oxygen group exceeded this figure by early September, and all others reached it by the last sample period. The unusually wet summer increased flows over last year and helped reduce flow indices.

The total cost for oxygen which included one cylinder and tank rental came to $127.51 or $0.058 for each pound of fish

produced. This was offset by the nine cent savings in feed cost per pound of fish produced by using oxygen. One third the amount of oxygen was utilized this year.

Unlike the prior year, no real benefits were obtained by using the packed columns. Fish did not grow as well as controls and health aspects were only slightly better. It cost $58.42 cents to power each pump over the study period

Again, the oxygen supplied fish appeared to have a greater "vitality" according to hatchery staff. They appeared to be more lively than other groups and remained deeper in the water column. They dispersed wider in the raceway, particularly utilizing the head end of the raceway.

In summary, using two chambers, the growth and health of direct oxygen supplemented fish were superior to packed columns and controls. Dorsal fin erosion decreased, feed conversion was superior and the feed costs to rear a pound of fish were reduced.

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Effect of Contactor Height and Oxygen Flow Rate on Transfer Effeciency of Oxygen With and Without the Use of Substrate

Irvin R. Brock
State of Alaska, Department of Fish and Game
Kimleigh Clayton Brown
North Pacific Rim, Inc./ Alaska Fisheries Consultants

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Introduction

The injection of pure O2 into sealed columns (oxygen contactors) is a technique which is effective in decreasing the partial pressure of dissolved N2 and increasing the partial pressure of O2 in water (Dwyer, et al. 1991 Westers et al. 1987; Westers 1989). By doing so, water sources which are supersaturated with N2 may be made acceptable for fish culture (Owsley 1981; Speece 1981; Westers et al. 1987; Westers 1989) Additionally, a greater biomass of fish may be produced in water supersaturated with O2, thus making fish culture facilities more efficient (Colt and Watten 1988; Dwyer, et al. 1991;)

A general description of an oxygen contactor is given is Dwyer, et al., 1991. In that study, a contactor with a diameter of 24" was used. Column lengths of 3-ft and 5-ft were compared. Another study, at Dworshak National Fish Hatchery (NFH) compared column lengths of 5-ft and 7-ft. Westers (1989) reported that column lengths less than 12-in (30 cm) proved to be economically inefficient with respect to variable costs such as O2 and electrical power.

The studies noted above were performed with a view toward the culture of fish in raceways at established hatcheries. However, conditions in Alaska are often much more restrictive with respect to the availability and quality of water, the choice of hatchery sites, the cost of construction, access to supplies, and the cost of power. New hatchery facilities are desired at locations such as schools and villages where the quantity and quality of water and cost of power and construction make fish culture with present techniques both physically and fiscally impractical. Existing facilities may also require a greater production of fish with the existing water supply, but do not have funds to materially alter the buildings or to pump the water for serial re-use.

Consequently, contactor lengths of two to 2.5 m are impractical, because they cannot be readily and economically incorporated into most existing incubation buildings, the extra space needed to accommodate them in future buildings is often too expensive to construct, and the cost of pumping water that high to provide for serial re-use is prohibitive.

This study is designed to examine the absorption efficiency of O2 over a wide range of contactor lengths, water flows, and gas/liquid ratios, with and without substrate. The results will provide data necessary for the design and incorporation of oxygen contactors in both new and existing facilities.

Materials and Methods

Variables and formulas utilized in this study are given in Table 1. The formulas were taken from American Fisheries Society Special Publication No.14, Computation of Dissolved Gas Concentrations in Water as Functions of Temperature, Salinity and Pressure.

Table 1. Variables and formulas used in oxygen contactor tests.

Variables

BP = Barometric Pressure (mm Hg)
O2 = Oxygen Concentration (mg/l)
O2P = Oxygen Pressure (mm Hg)
N2A = Nitrogen plus Argon (mm Hg)
BO2 = Bunson Coefficient for Oxygen (from table)
WVP = Water Vapor Pressure (from table)
TDG = Total Dissolved Gas Pressure (mm Hg)
WF = Water Flow (lpm)
DO1 = Dissolved Oxygen into Contactor
DO2 = Dissolved Oxygen after Contactor
O2F = Oxygen Flow into Contactor
O2% = Percent Oxygen Saturation (%)
N2A% = Percent Nitrogen plus Argon saturation (%)
TDG% = Percent Total Dissolved Gas Saturation (%)
OTE = Oxygen Transfer Efficiency (%)

Formulas

1. To convert oxygen from mg/l to mm pressure:

$$O2P - O2 * 0.5318/BO2$$

2. Percent oxygen saturation:

$$O2\% = (O2P * 100)/((BP-WP) * 0.20946)$$

3. Oxygen transfer efficiency:

$$OTE = WF * (DO2 - DO1) * 100/(O2F * 1430)$$

4. Total gas saturation:

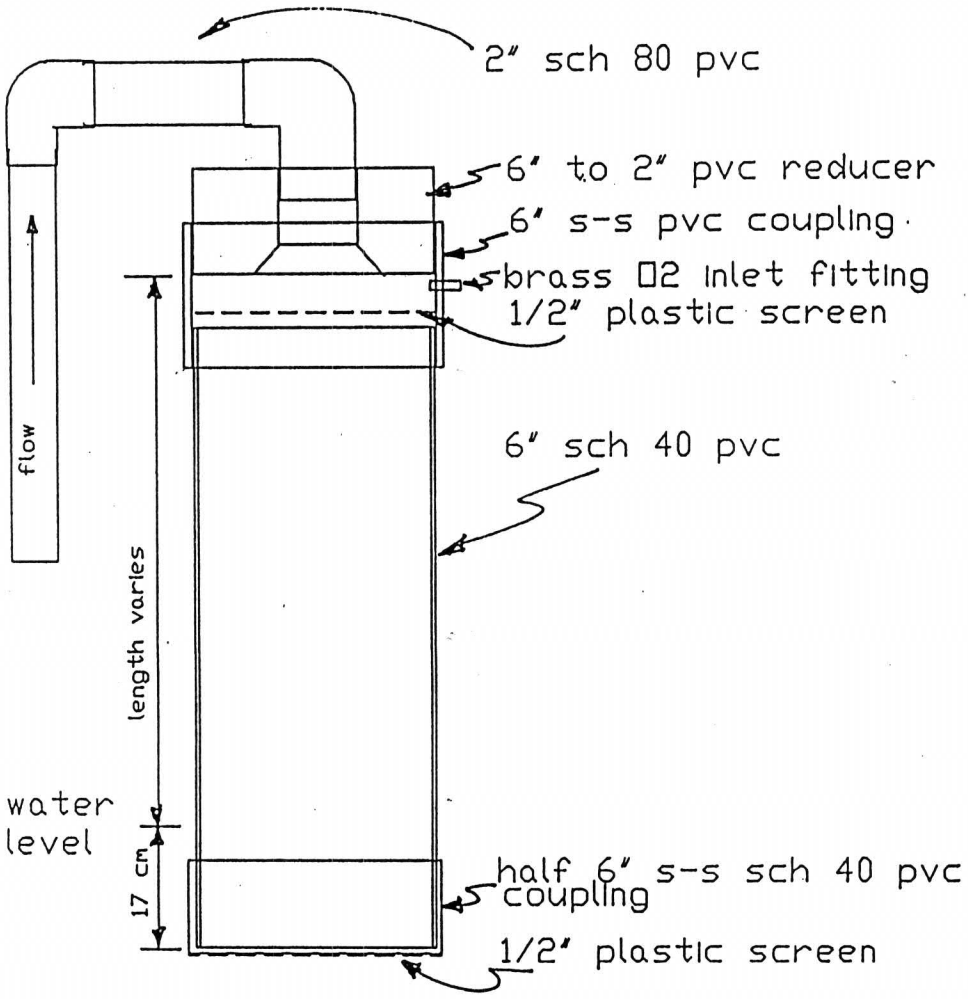
$$TDG\% = TDG * 100/BP$$

Oxygen transfer efficiency (OTE), it should be noted, is given in the percentage of available oxygen transferred, and does not have a component for efficiency with respect to expended energy.

The oxygen contactor was constructed as illustrated in Figure 1. The bottom was submerged to a depth of 17 cm in a 50 - gal (189 l) fiberglass tub with an overflow which provided for a constant depth of water during testing. The effective contactor length was the distance from the top of the inside cavity to the water level on the outside of the contactor while water was flowing.

A plastic screen was placed 6 cm below the top of the cavity to prevent substrate from reaching the top and interfering with the inflow of either water or oxygen. When substrate was used, it extended from this screen to the screen on the bottom of the

Figure 1. Diagram of oxygen contactor.



column. Intalox saddles (Norton Chemical Co.) were used as substrate.

Water was supplied from an untreated (unheated) source in the Broodstock Development Center, and was pumped to the contactor with a 1-hp submersible pump. Water flow was regulated immediately downstream of the pump with a submerged ball valve and entered the contactor through an open discharge. The rate of flow was determined by timing the rise of water between two calibrated marks (94.5 l and 189 l) in the fiberglass tub.

Commercial O2 supplied in standard 100 lb. pressure tanks was used. It was routed through a pressure-reducing regulator, an initial flow meter and then through an electronic in-line flowmeter with an accuracy of .01 l/min.

The effective contactor length was decreased in 15 cm intervals from a maximum length of 170 cm to a minimum length of 20 cm. At each interval, environmental data including incoming DO, water temperature and flow, and barometric pressure were taken and recorded. Periodically, TDG in both the incoming and effluent water was determined and recorded.

Three different O2 flows were tested at each length interval: 2.65 l/min; 1.80 l/min; and 1.0 l/min. After each change in O2 flow the contactor was operated for a minimum of 15 min to allow the system to stabilize. Then three water samples were obtained from the effluent water in the fiberglass tub and tested for DO content and reported in mg/l. The water flow was held at a nominal 189 l/min for all tests.

For each length interval, the percentage of O2 saturation and the percentage of O2 transfer was calculated. When TDG data was taken, the percentage of TDG saturation and percentage of N+Ar saturation was also calculated.

RESULTS

In tests run with no substrate, the transfer of O2 (mg/l, percentage saturation) generally increased with contactor length and with the flow of O2 into the contactor (Figures 2, 3,). Transfer efficiency, however, decreased with increased flow of O2, but displayed the same tendency to increase with the effective length of the contactor (Figure 4). The slope of the graphs remained relatively constant for each flow until the effective length of the contactor was reduced from 35 cm to 20 cm, when a large relative decrease in the transfer of O2 occurred.

When substrate was added to the contactor, the trends of the results were generally similar to those of the first treatment (Figures 5, 6, 7). An important difference occurred, however, when the effective length was reduced from 35 cm to 20 cm. The relative decrease in O2 transfer was not nearly as great as was observed in the treatment above.

A comparison of both treatments (substrate, no substrate) for each flow of O2 with respect to mg/l and transfer efficiency reveals that, for most of the effective contactor lengths, the graphs are very similar in shape, show the same basic trend, and often overlap. As noted above, however, there is a relatively great difference between treatments for both mg/l and transfer efficiency

Figure 2.

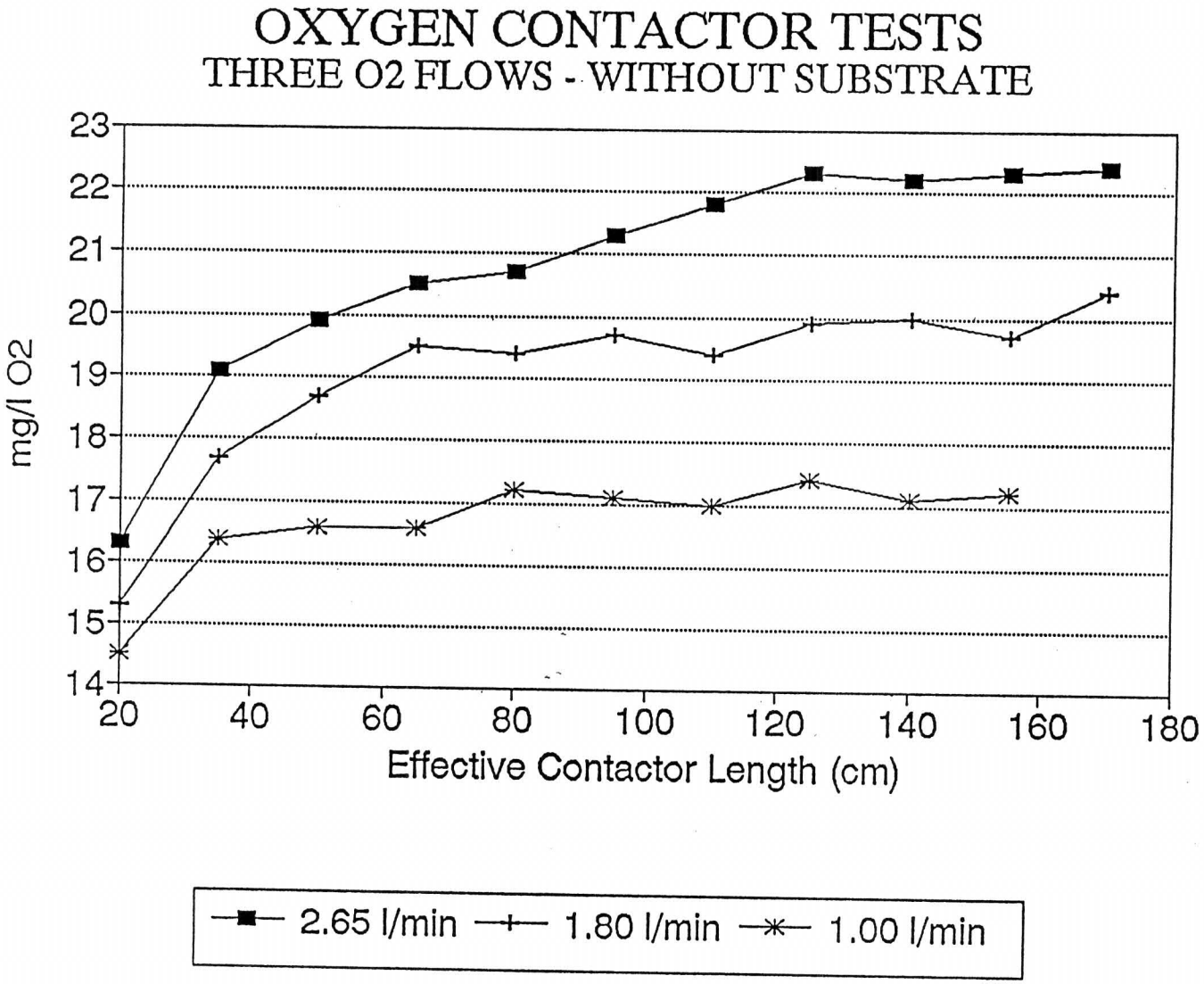

OXYGEN CONTACTOR TESTS
THREE O2 FLOWS - WITHOUT SUBSTRATE
mg/l O2
23
22
21
20
19
18
17
16
15
14
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
2.65 l/min
1.80 l/min
1.00 l/min

Figure 3.

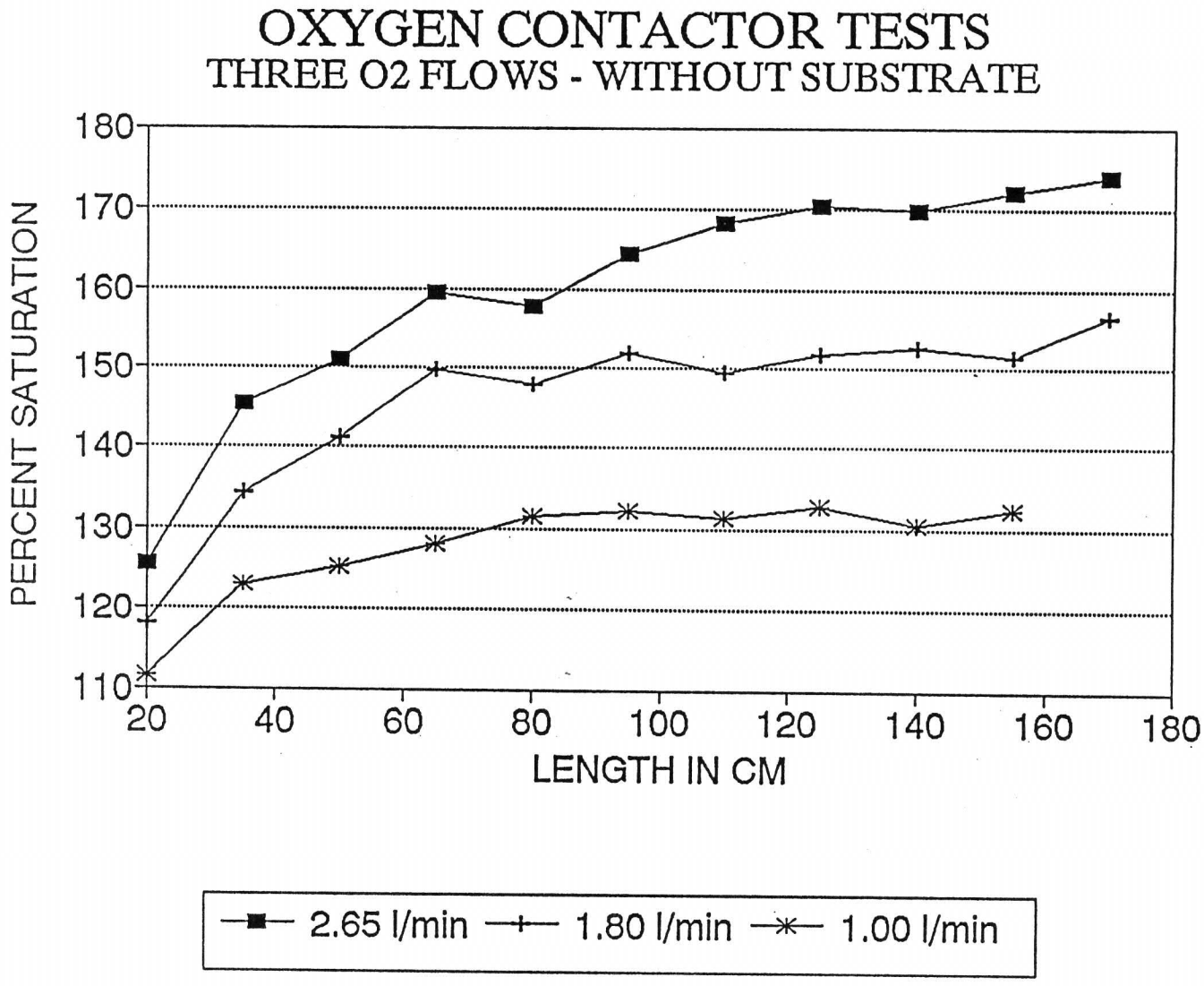

OXYGEN CONTACTOR TESTS
THREE O2 FLOWS - WITHOUT SUBSTRATE
PERCENT SATURATION
180
170
160
150
140
130
120
110
20
40
60
80
100
120
140
160
180
LENGTH IN CM
2.65 l/min
1.80 l/min
1.00 l/min

Figure 4.

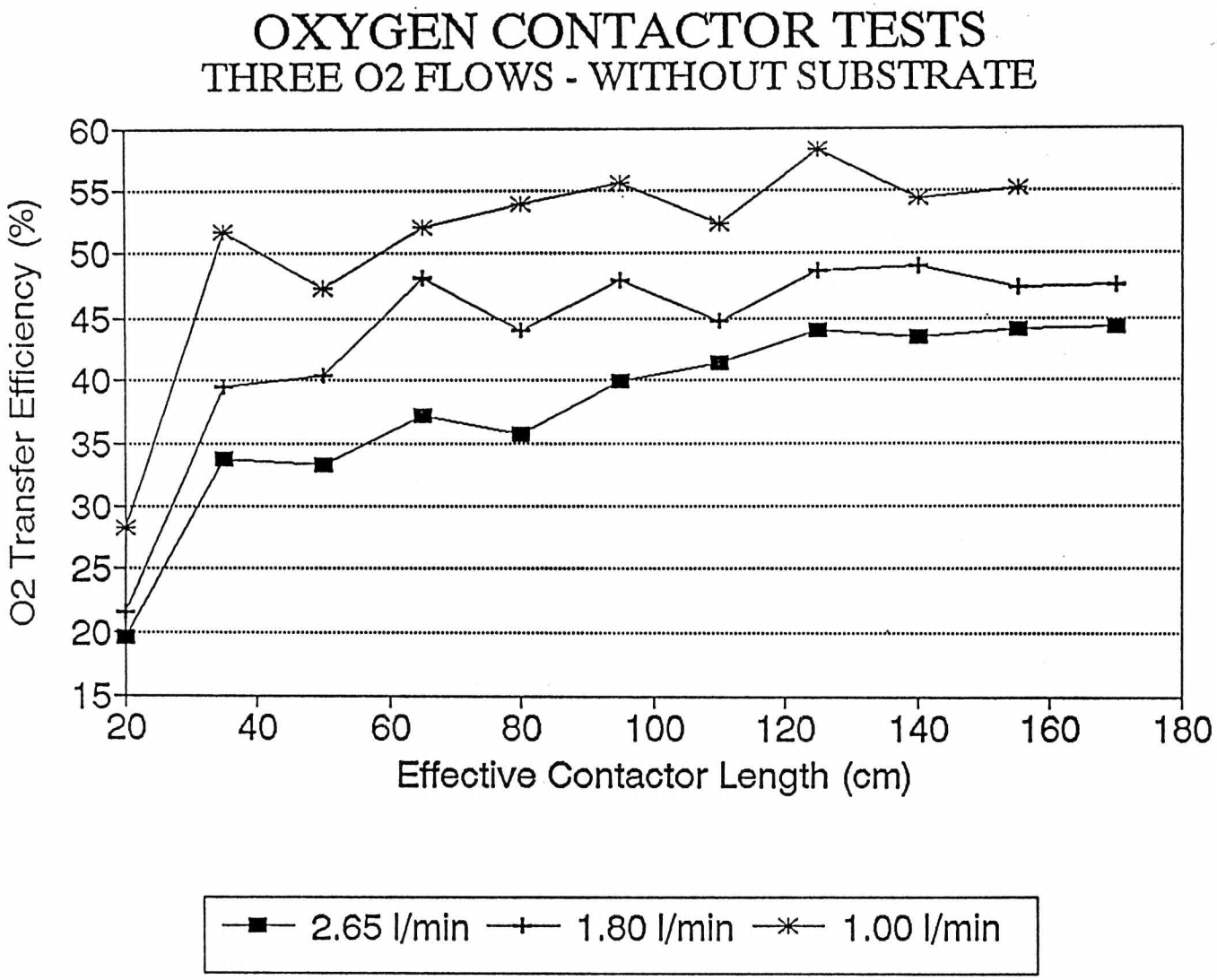

OXYGEN CONTACTOR TESTS
THREE O2 FLOWS - WITHOUT SUBSTRATE
O2 Transfer Efficiency (%)
60
55
50
45
40
35
30
25
20
15
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
2.65 l/min
1.80 l/min
1.00 l/min

Figure 5.

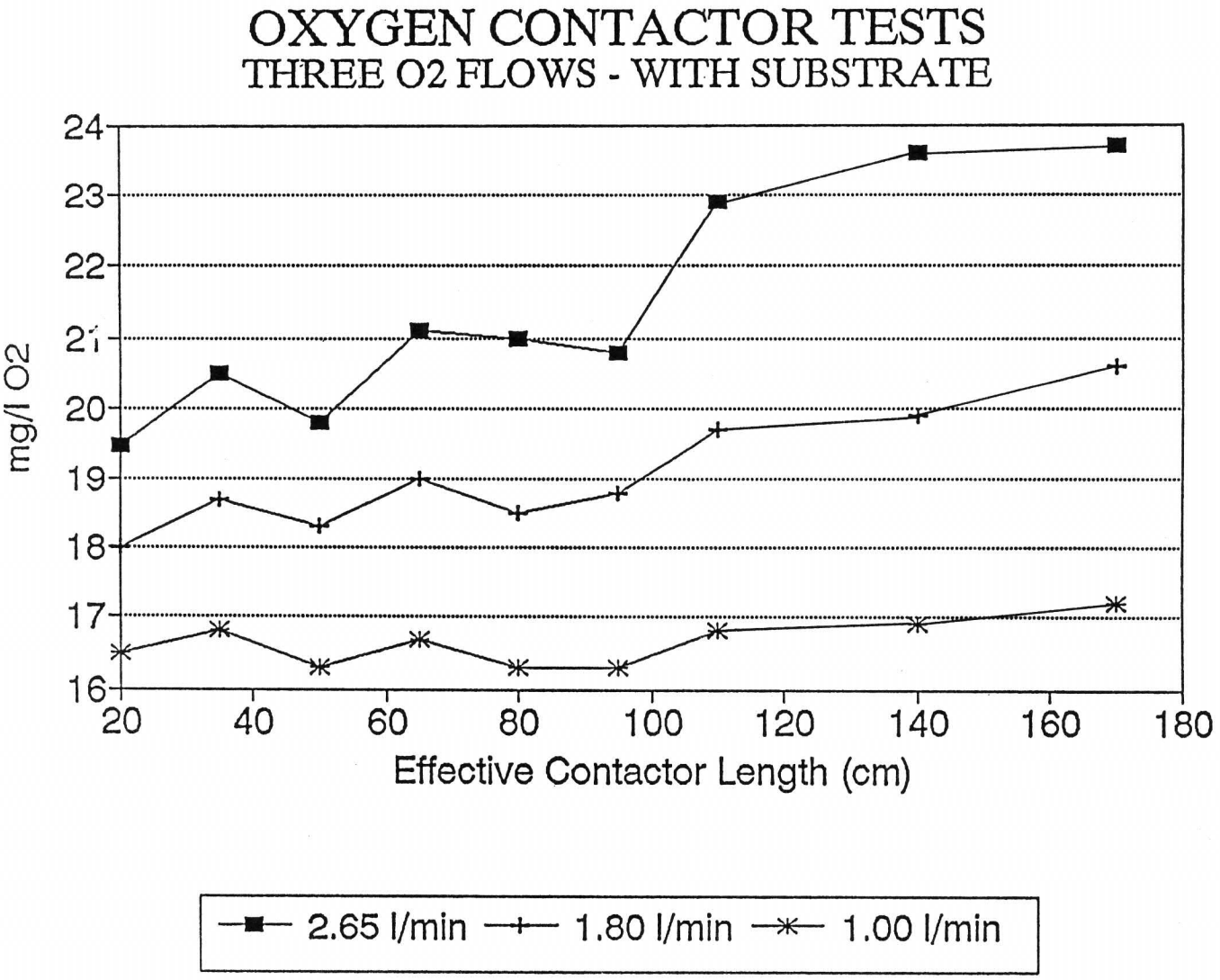

OXYGEN CONTACTOR TESTS
THREE O2 FLOWS - WITH SUBSTRATE
mg/l O2
24
23
22
21
20
19
18
17
16
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
2.65 l/min
1.80 l/min
1.00 l/min

Figure 6.

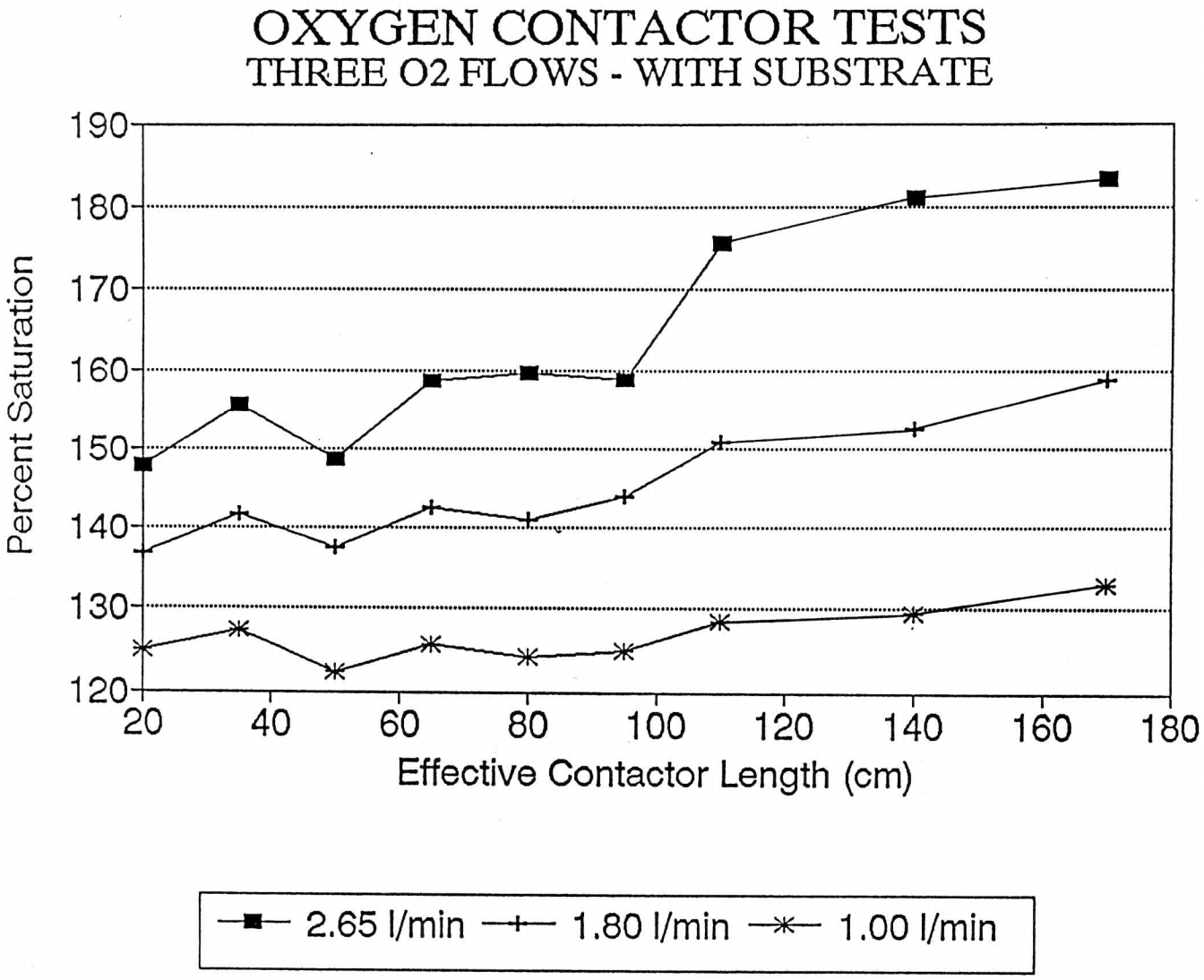
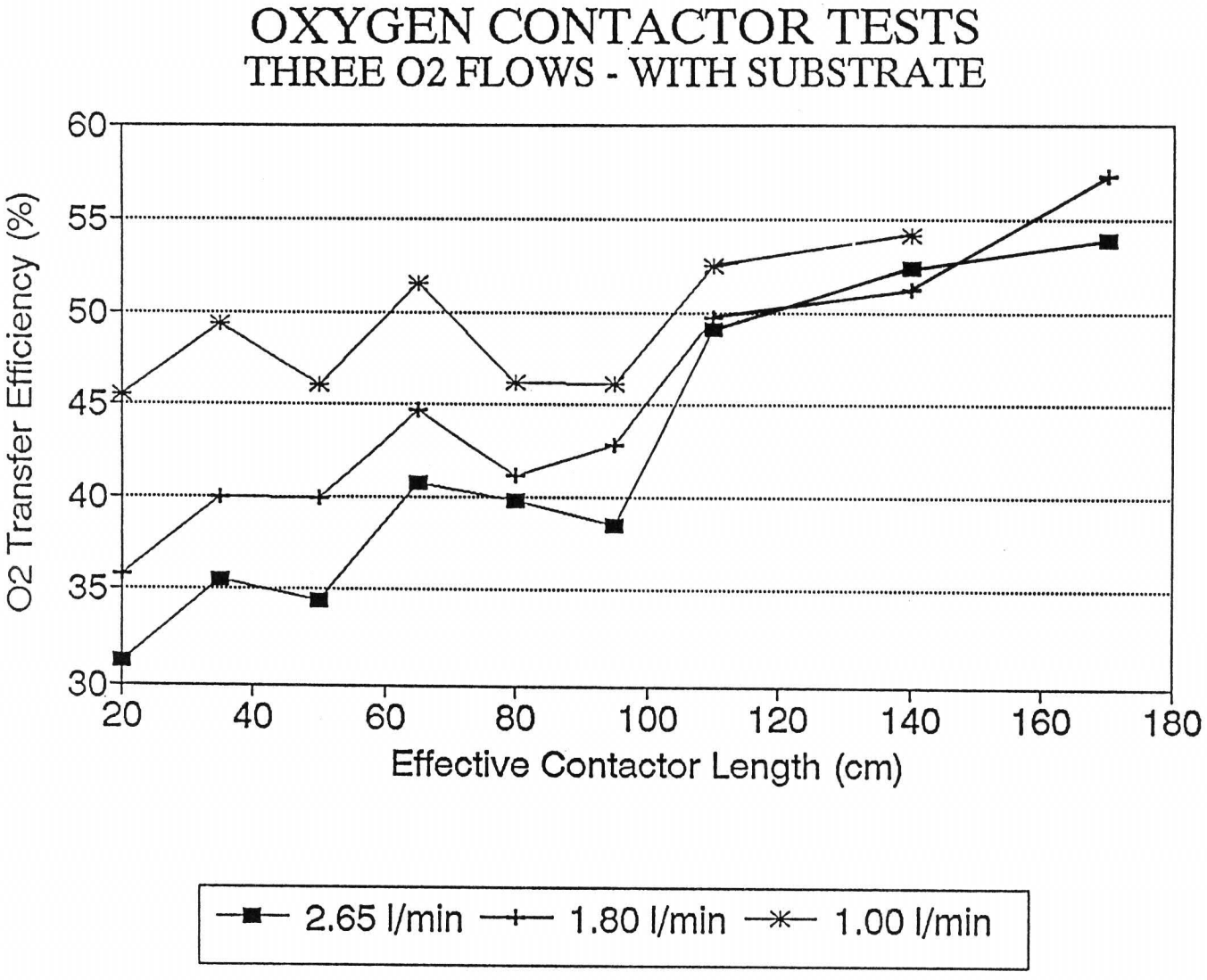

OXYGEN CONTACTOR TESTS
THREE O2 FLOWS - WITH SUBSTRATE
Percent Saturation
190
180
170
160
150
140
130
120
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
2.65 l/min
1.80 l/min
1.00 l/min

Figure 7.


OXYGEN CONTACTOR TESTS
THREE O2 FLOWS - WITH SUBSTRATE
O2 Transfer Efficiency (%)
60
55
50
45
40
35
30
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
2.65 l/min
1.80 l/min
1.00 l/min

when the effective length of the contactor decreases from 35 cm to 20 cm, with the efficiency much higher for the substrate treatment.

DISCUSSION

It is apparent, based on this study, that O2 contactors do not need to be several feet tall to be useful. In fact, units with only 20 cm effective length can raise the DO content of water significantly using any of the O2 flows tested and at a water flow of 1 l/cm2 of contactor cross section. This will allow designers of hatcheries to significantly decrease the cost of construction by utilizing buildings with a smaller footprint and water supply facilities of lesser capacity. Additionally, because serial uses of the water is made possible by the supersaturated O2, further pumping will either be eliminated or the costs reduced by not having to pump the water against several feet of head to re-oxygenate it in tall contactors.

The presence of substrate in the contactor appears to result in little, or at best, a marginal advantage over an empty contactor with respect to the efficiency of O2 transfer over most of the range of contactor lengths (Figures 8 - 13). Because substrate often serves as a matrix for the growth or buildup of algae and other organic material, which often results in a severely restricted flow of water through the contactor, it does not appear to be advisable to use a filled contactor unless the hatchery operator needs the additional efficiency that might be obtained at longer column lengths and high flows of O2 (Figures 12, 13). An exception to this, of course, is the very short, 20 - 35 cm, contactors in which efficiency increases dramatically with substrate.

It is very clear that the most efficient transfer of O2 at any given effective contactor length, with or without substrate, occurs with the lesser flows of O2 (Figures 3, 6). However, the amount of O2 transferred is considerably less (Figures 2, 5) resulting in lower DO concentrations, thus less O2 available for fish at a given flow of water. The trade-off of efficiency of transfer and the amount of O2 available for the fish is one which will have to be considered by the hatchery designer and operator. The cost of oxygen production and pumping, the availability of water, the water distribution scheme, etc. all will enter into the decision-making process when a contactor process is considered or implemented.

The various tables and figures contain the basic information necessary for individuals to design an O2 contactor system which will supply treated water up to 185 % saturation.

Figure 8.

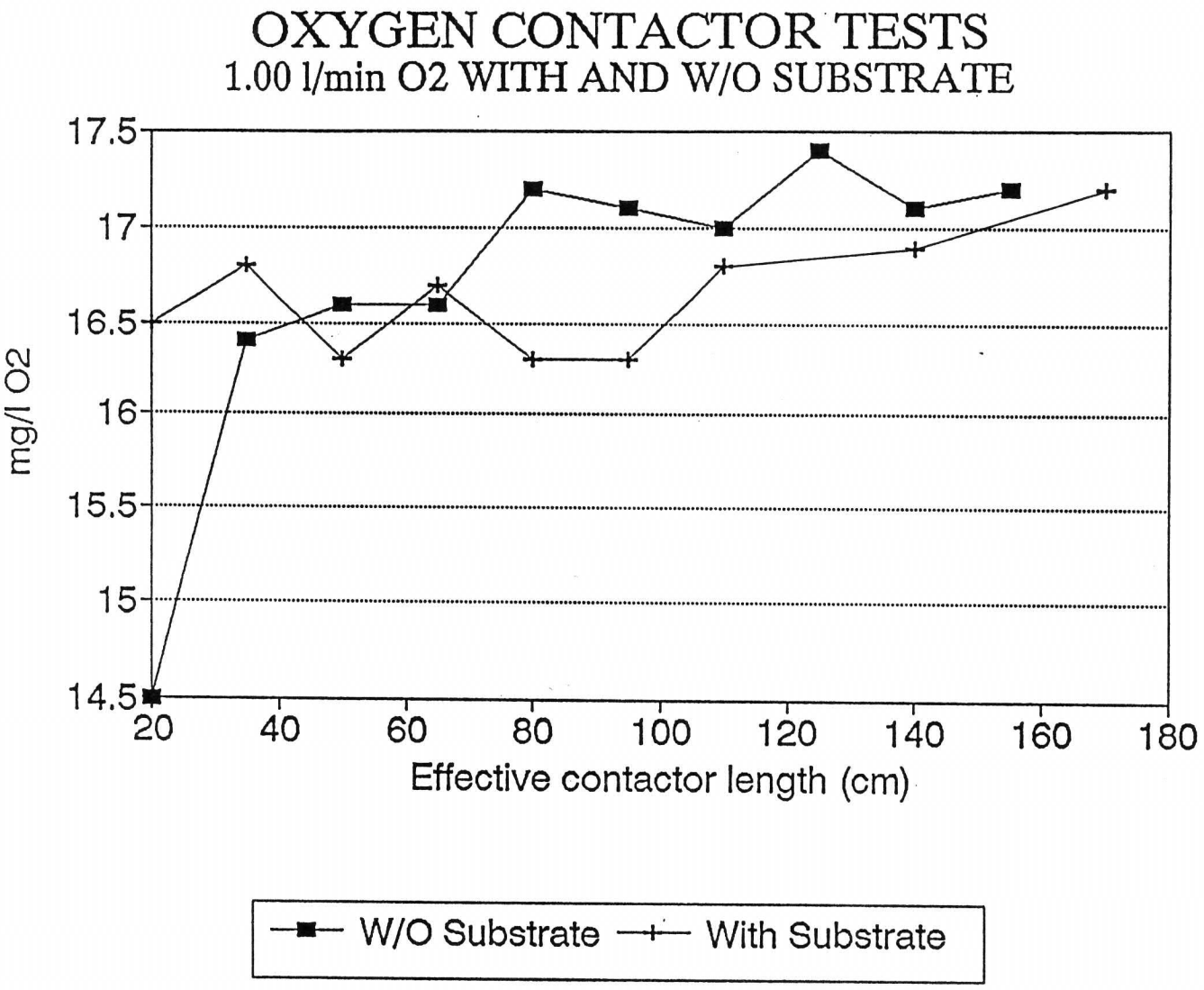

OXYGEN CONTACTOR TESTS
1.00 l/min O2 WITH AND W/O SUBSTRATE
17.5
17
16.5
16
15.5
15
14.5
mg/l O2
20
40
60
80
100
120
140
160
180
Effective contactor length (cm)
W/O Substrate
With Substrate

Figure 9.

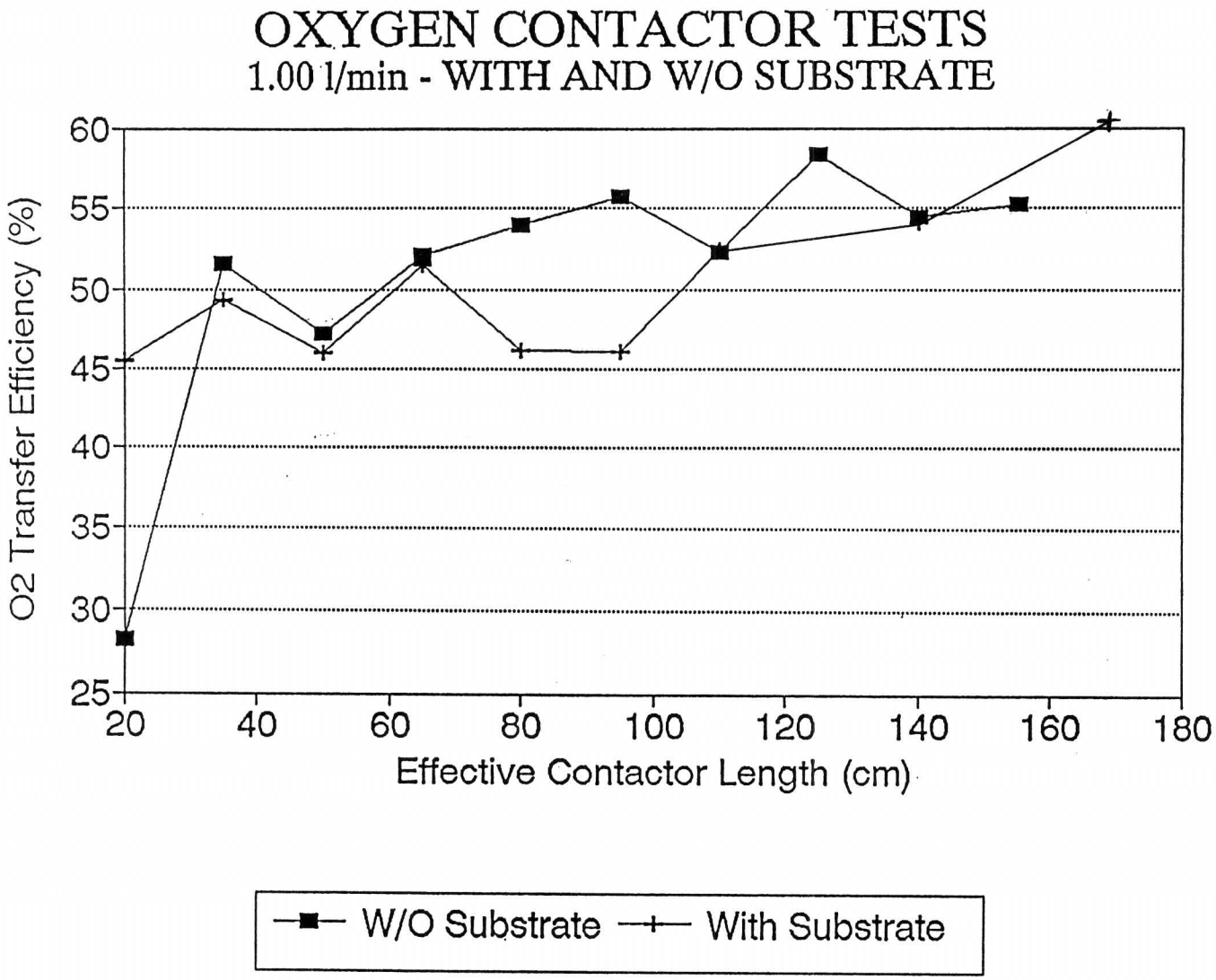

OXYGEN CONTACTOR TESTS
1.00 l/min - WITH AND W/O SUBSTRATE
O2 Transfer Efficiency (%)
60
55
50
45
40
35
30
25
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
W/O Substrate
With Substrate

Figure 10.

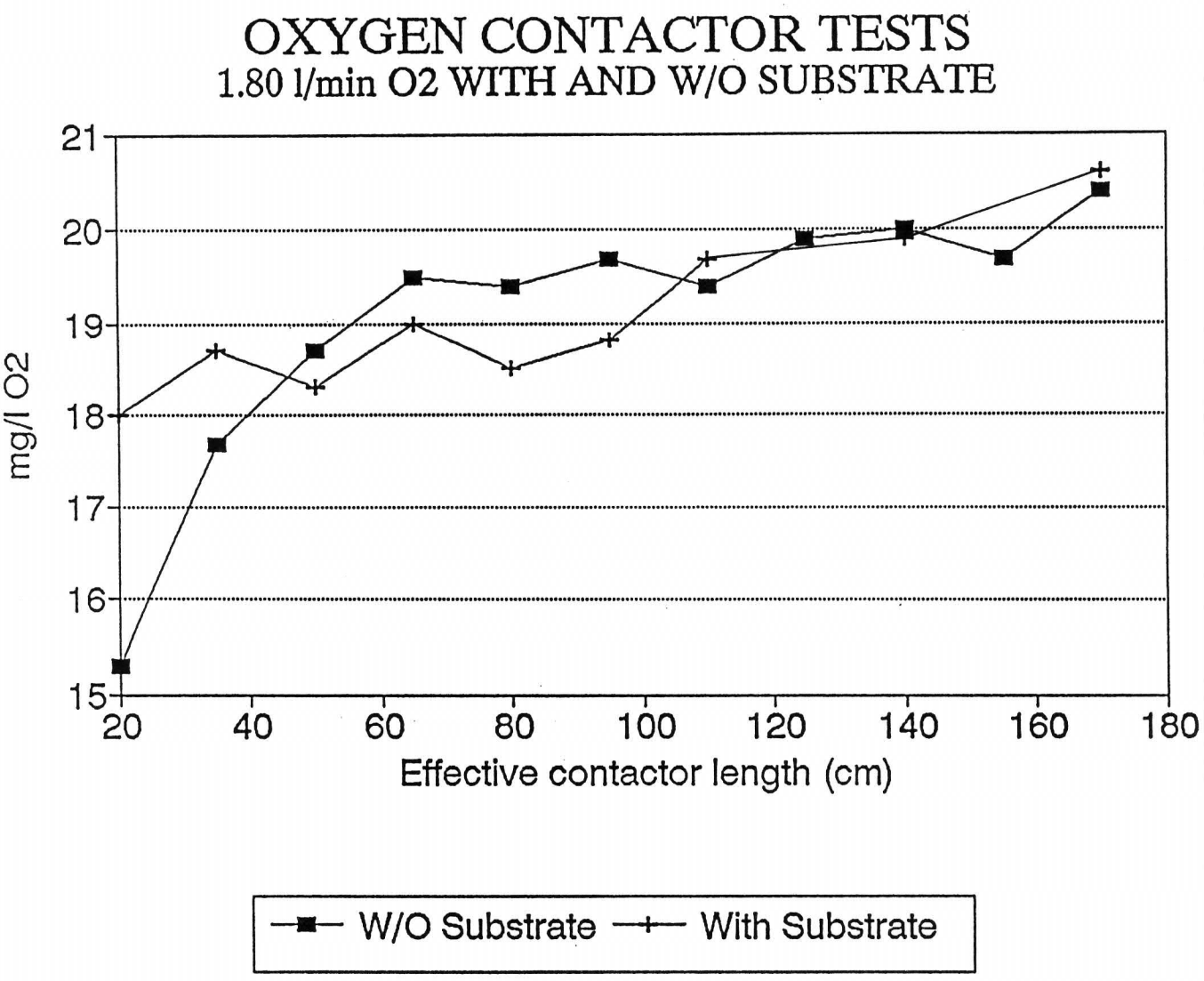

OXYGEN CONTACTOR TESTS
1.80 l/min O2 WITH AND W/O SUBSTRATE
mg/l O2
21
20
19
18
17
16
15
20
40
60
80
100
120
140
160
180
Effective contactor length (cm)
W/O Substrate
With Substrate

Figure 11.

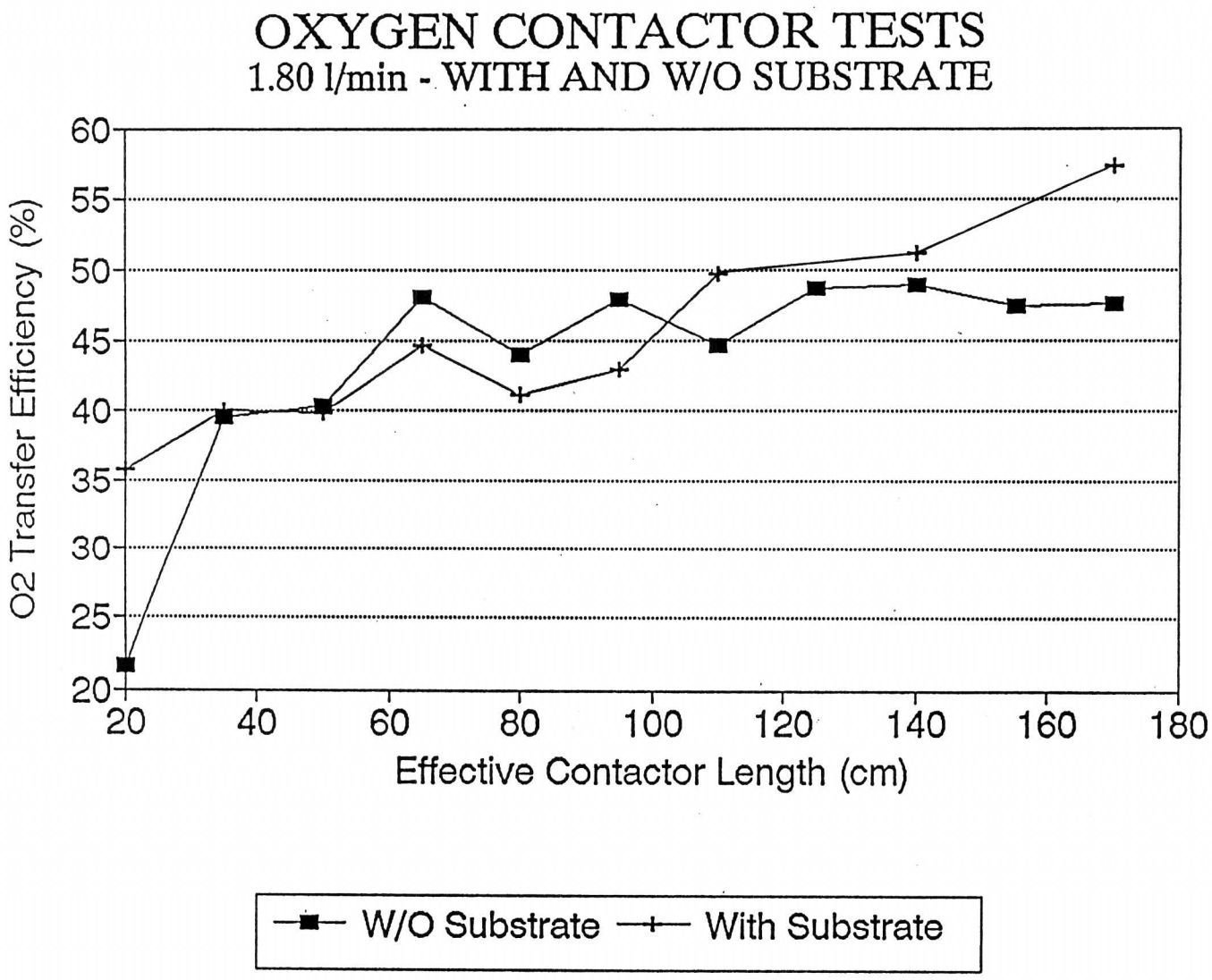

OXYGEN CONTACTOR TESTS
1.80 l/min - WITH AND W/O SUBSTRATE
O2 Transfer Efficiency (%)
60
55
50
45
40
35
30
25
20
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
W/O Substrate
With Substrate

Figure 12.

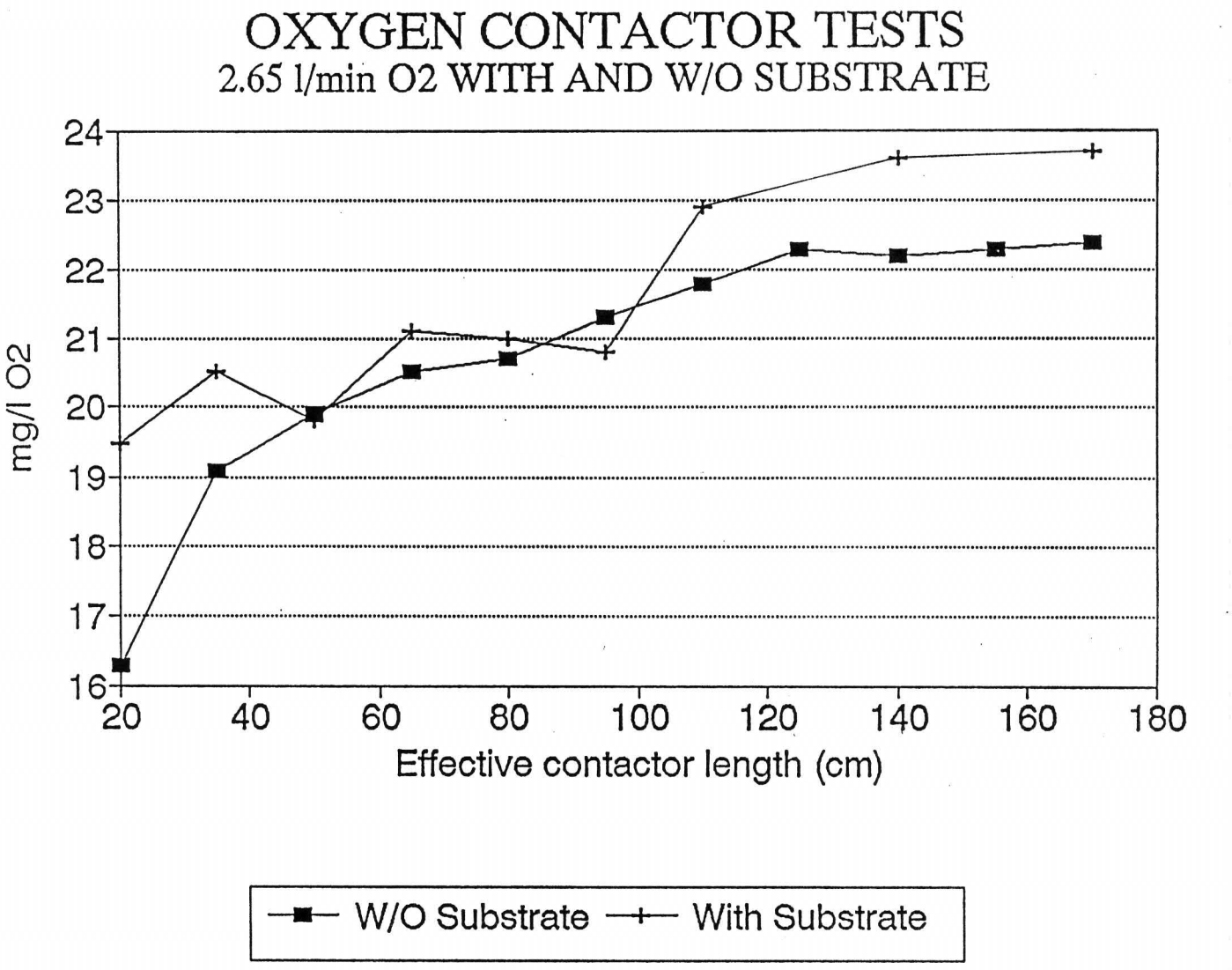
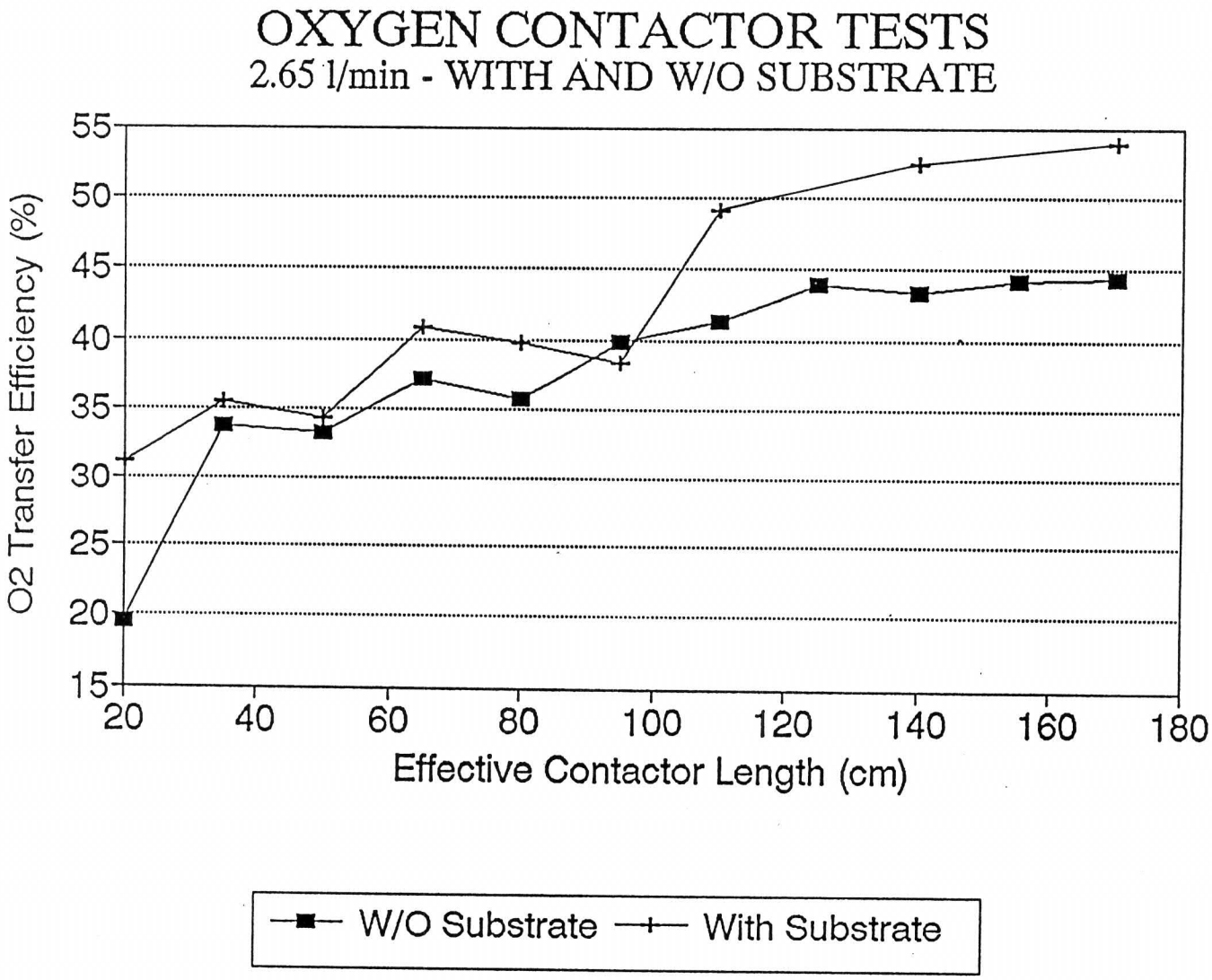

OXYGEN CONTACTOR TESTS
2.65 l/min O2 WITH AND W/O SUBSTRATE
mg/l O2
24
23
22
21
20
19
18
17
16
20
40
60
80
100
120
140
160
180
Effective contactor length (cm)
W/O Substrate
With Substrate

Figure 13


OXYGEN CONTACTOR TESTS
2.65 l/min - WITH AND W/O SUBSTRATE
O2 Transfer Efficiency (%)
55
50
45
40
35
30
25
20
15
20
40
60
80
100
120
140
160
180
Effective Contactor Length (cm)
W/O Substrate
With Substrate

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Spokane, Washington
December 7-9, 1993

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33. Mark LaRiviere, Tacoma PU, (206) 591-9767
34. Speros Doulos, USFWS, Erwin, TN, (615) 743-4712
35. Jim Byrne, Washington DW, (206) 887-3076
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Argent Chemical	Salmon Production Management & Allocation book	Jim Parsons Brian Zimmerman
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Cyrus O'Learys	2 Free Appetizers	John Rankin
Dan Davies	Spring Chinook Salmon fishing trip for one (Icicle River).	Paul Harmer Clyde Parke
Dan Davies & Craig Carson	Spring Chinook Salmon fishing trip for one (Icicle River).	Steven Roberts
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DONATION BY	DONATION	RECEIVER
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General Store	Spinning Rod & Reel	Loren Jensen Tom Rogers
Hecla Mining Company	1oz. Silver Coin	Tran Wayne Bill Doerr Jim Hutches Sharon Vendshus Don Parker Amy Kishpaugh
Hecla Mining Company	1oz. Silver Coin	Richard Berry Butch Don Johnson Mike Haynes Terry Jones Bob Adams Dennis Dahrens Stan Woody Steven Roberts Jodi Stroklund Don Edsall Bob Jones Debra Eddy Paul Abbott
Icicle Valley Chapter of Trout Unlimited	Coffee Mug	Tran Wayne Bill Doerr Michael Pena Don Parker Richard Berry Butch Cobb Don Johnson Mike Haynes Bob Adams

DONATION BY	DONATION	RECEIVER
Icicle Valley Chapter of Trout Ulimited (Cont.)	Coffee Mug	Dennis Dahrens Jodi Stroklund Debra Eddy Paul Abbott Don Edsall
Jensorter	Far-Side Calendar	Charlie Smith
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Salmon Festival	Pin	Don Parker Todd Ols Anna Kastner Judy Urrutia Richard Berry Art Brown Leonard Garcia Gerald Cross Donna Tiffan Paul Waterstrat Douglas W. Maxey Sharon Vendshus Stan Woody
Sandy Building Co . (Sandy Or.)	Manzella Wool Gloves	Dan Free
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Spokane Convention Board	Peanut Brittle	Roy Douglas

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Warren Water Broom	Pond Brush	David Rogers
Western Out-Doors	1yr Subscription	Paul Waterstrat
Wide World Of Golf	Summer Golf Hat	Mike Paiya
Wright & McGill Co.	Fishing Rod	Marge Park

GRAND PRIZE WINNER OF THE VCR & TV COMBO: JIM GRAYBILL

The following donated towards the GRAND PRIZE - VCR & TV COMBO:

Aurum Aquaculture L.T.D	Doreen Yates	Common Sensing
Electronic Data Solution	Engineered Products (T.R. Gregg)	Globel Feed
Hatchery Operations Consultants	Northwest Marine Tech Keith Jefferts	PRA Manufacturing
Rangen INC.	Reiff Manufacturing	

44th Annual Northwest Fish Culture Conference
Tradeshow participants

Company Name and Address	Contact Person
Aquatic Eco-Systems, Inc. 2056 Apopka Blvd. Apopka, FL 32703	
Aquionics, Inc. 8121 Portsmouth Dr. Darien, IL 60561	Mike Blake
Argent Laboratories 8702 152nd Ave NE Redmond, WA 98502	Elliot Lieberman 206-885-3777
Aurum Aquaculture Ltd. 7080 River Rd. Unit 128 Richmond, BC V6X1X5	Dean Trembley
Bioproducts P.O. Box 429 Warrenton, OR 97146	Russ Farmer 503-861-2256
Christensen Networks 4074 Sucua Dr. Ferndale, WA 98248	Scott Christiansen 206-671-8060
Common Sensing P.O. Box 130 Clark Fork, ID 83811-1541	Brian D'Aoust 208-266-1541
Electronic Data Solution P.O. Box 31 Jerome, ID 83338	Marcie 208-324-8009
Engineered Products P.O. Box 10 Philomath, OR 97370	T.R. Gregg 503-929-3225
Global Feed Consortium 1504 Fairview Ave Bellingham, WA 98226	Don Merryman

Company Name and Address	Contact Person
Harper Brush Distributing 18009 25th St Court East Sumner, WA 98390	Ken Taylor
Ideal Horizons P.O. Box 1707 Rutland, VT 05701-1707	Jesse Rodriguez
Industrial Plastics 740 S. 28th St. Washougal, WA 98671	Jim Ochs 800-634-5082
J.L Eagar, Inc. P.O. Box 476 North Salt Lake, UT 84054	Amy Eagar 800-423-6249
Jensorter, Inc. 20225 Harvest Lane Bend, OR 97701	Gregg Jensen 503-389-3591
Lummi Fisheries Supply, Inc. 851 Coho Way Bellingham, WA 98225	Terry Crump 206-734-3336
MariSource P.O. Box 9037 Tacoma, WA 98409	Lisa Larson 206-475-5772
Micrologix International, Ltd. 6761 Kirkpatric Crescent Victoria, BC V8X3X1	Jeremy Hackett
Moore-Clark P.O. Box M LaConner, WA 98257	Scott McKnight 206-466-3131
Mt Hood Community College 3634 NE 17th St. Apt. E18 Gresham, OR 97030	Jerry Neill
Nelson and Sons, Inc. P.O. Box 57428 Murray, UT 84157-0428	Chris Nelson 800-521-9092

Company Name and Address	Contact Person
PRA Manufacturing, Ltd. P.O. Box 774, Station A Nanaimo, BC V9R5M2	Wayne Gorrie 604-754-4844
Point Four Systems, Inc. 2702 Clarke St. Port Moody, BC V3H121	Tjarda Barratt 604-936-9936
Rangen, Inc. P.O. Box 706 Buhl, ID 83316	Karen Winkle 208-543-6569
Reiff Manufacturing Rt. 4, Box 183 Walla Walla, WA 99362	Steve Reiff 509-525-1081
Smith-Root, Inc. 14014 NE Salmon Creek Ave Vancouver, WA 98686	Barbara
The Mallory Company 1814 Baker Way Kelso, WA 98626	Ramona Ainslie 800-426-6830
VMG Industries 2175 Meadows Court Grand Junction, CO 81503	Bruce Marshall 303-242-8623
Warren Water Broom Rt. 4 Box 543C Astoria, OR 97103	Dell Warren 503-458-6694

ANNUAL NORTHWEST FISH CULTURE CONFERENCES
HISTORICAL RECORD

Year	Location	Host Agency	Chairman
1950	Portland, Oregon	U..S Fish and Wildlife Service	Perry, T.
1951	Wenatchee, Washington	U.S. Fish and Wildlife Service	Burrows, R.
1952	Seattle, Washington	Washington Dept. of Fisheries	Ellis, B.
1953	Portland, Oregon	Oregon Fish Commission	Cleaver, F.
1954	Seattle, Washington	U.S. Fish and Wildlife Service	Rucker, R.
1955	Portland, Oregon	Oregon Game Commission	Rayner, J.
1956	Seattle, Washington	Washington Dept. of Game	Millenbach, C.
1957	Portland, Oregon	U.S. Fish and Wildlife Service	Johnson, H.
1958	Seattle, Washington	Washington Dept. of Fisheries	Ellis, R.
1959	Portland, Oregon	Oregon Fish Commission	Jeffries, E.
1960	Olympia, Washington	Washington Dept. of Game	Johansen, J.
1961	Portland, Oregon	Washington Dept. of Fisheries	Jensen, C.
1962	Longview, Washington	U.S. Fish and Wildlife Service	Burrows, R.
1963	Olympia, Washington	Washington Dept. of Fisheries	Ellis, B.
1964	Corvallis, Oregon	Oregon State University	Fryer, J.
1965	Portland, Oregon	U.S. Fish and Wildlife Service	Halver, J.
1966	Portland, Oregon	Oregon Fish Commission	Hublou, H.
1967	Seattle, Washington	University of Washington	Donaldson, L.
1968	Boise, Idaho	Idaho Fish and Game	Cuplin, P.
1969	Olympia, Washington	Washington Dept. of Game	Johansen, J.
1970	Portland, Oregon	Oregon Game Commission	Jensen, C.
1971	Portland, Oregon	U.S. Fish and Wildlife Service	Smith, M.
1972	Seattle-Tacoma, WA	Washington Dept. of Fisheries	Noble, R.
1973	Wemme, Oregon	Oregon Fish Commission	Jeffries, E.
1974	Seattle, Washington	University of Washington	Salo-Brannon
1975	Newport, Oregon	Oregon State University	Donaldson, J.
1976	Twin Falls, Idaho	University of Idaho	Klontz, B.
1977	Olympia, Washington	Washington Dept. of Game	Morrow, J.
1978	Vancouver, Washington	U.S. Fish and Wildlife Service	Leith, D.
1979	Portland, Oregon	Oregon Dept. of Fish and Wildlife	Jeffries, E.
1980	Courtenay, B.C.	Fisheries and Oceans	Sandercock, K.
1981	Tumwater, Washington	Washington Dept. of Fisheries	Ashcraft, W.
1982	Portland, Oregon	National Marine Fisheries Service	Wold, E.
1983	Moscow, Idaho	Idaho Dept. of Fish and Game and Univerity of Idaho	Parrish, E. & Klontz, B.
1984	Kennewick, Washington	Wahsington Dept. of Game	Gearheard, J.
1985	Tacoma, Washington	U.S. Fish and Wildlife Service	Forner, E.
1986	Springfield, Oregon	Oregon Dept. of Fish and Wildlife	Bauer, J.
1987	Fife, Washington	Washington Dept. of Fisheries	Ashcraft, W.
1988	Richmond, B.C.	B.C. Ministery of Environment	Peterson, D.
1989	Glenendon Beach, OR	National Marine Fisheries Service	Smith R.Z.
1990	Boise, Idaho	Idaho Dept. of Fish and Game	Hutchinson, B.
1991	Redding, California	California Dept. of Fish and Game	Hashagen, K.
1992	Wenatchee, Washington	Washington Dept. of Wildlife and Alaska Dept. of Fish and Game	Kerwin, J. & Brock, I.
1993	Spokane, Washington	U.S. Fish and Wildlife Service	Forner, E.