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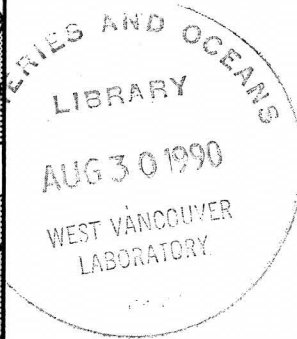
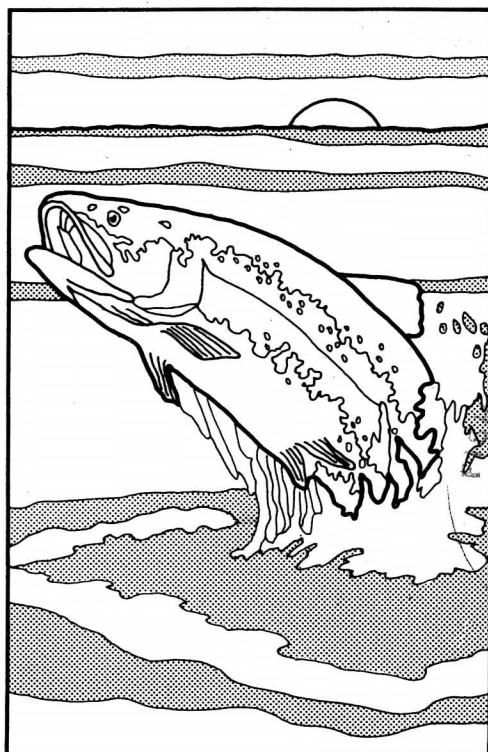


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PROCEEDINGS OF THE

39th ANNUAL

NORTHWEST FISH CULTURE CONFERENCE



Richmond, B.C.

December 6, 7, 8, 1988

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Ministry of Environment

RECREATIONAL FISHERIES BRANCH

FISH CULTURE SECTION

# PROV. FISH HEALTH LAB

PROCEEDINGS  
of the  
Thirty-Ninth Annual  
NORTHWEST FISH CULTURE CONFERENCE

December 6-8, 1988  
Richmond, British Columbia



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## THE NORTHWEST FISH CULTURE CONFERENCE

Northwest Fish Culture Workshops are informal meetings for exchange of information and ideas concerning all areas of fish culture. Current progress reports of management practices and problems, new developments, and research studies are presented. Active discussion and constructive criticism are encouraged and furnish highlights of the conference. All persons interested in or associated with fish husbandry are invited to attend and to participate. Subject material is limited to topics that have direct application to fish culture.

The PROCEEDINGS contain unedited briefs or oral reports presented at each conference. Much of the material concerns progress of uncompleted studies or reports. THESE INFORMAL RECORDS ARE NOT TO BE INTERPRETED OR QUOTED AS A PUBLICATION.

## PREFACE

The 39th Annual Northwest Fish Culture Conference was held at the Richmond Inn, Richmond, British Columbia from noon December 6 through noon December 8, 1988. This was the first time the Provincial Recreational Fisheries Branch hosted the NWFCC. There were 361 conference registrants from British Columbia, Washington, Oregon, Idaho, Alaska, Montana, Colorado, Utah, California, Wyoming and Alberta.

Dr. David W. Narver, Director, Recreational Fisheries Branch, in giving the Keynote Address emphasized not only the quality of the angling experience but also the growth of sport fishing as a recreational pursuit and important contributor to the economy.

As Executive Director for the Conference Organizing Committee, I very much appreciated the dedication of Fish Culture staff in hosting what I hope attendees felt was a successful conference. In particular, I wish to especially thank Don Peterson (Finance and Facilities) and Peter Brown (Program), who in addition to their named functions also co-chaired the conference, also special thanks to Laird Siemens for Registration, Ray Billings for Exhibitors, Nick Basok and Rory Smith for Prizes, Greg Ralfs and Doug Crawley for Audio-Visual, Darren Greiner for Mementos and to all the convenors who organized the speaker panels and kept the program on time. Other personnel of the Recreational Fisheries Branch assisting in organizing the Conference were Larry Wells for the graphics, Eileen Currier and June Lum for word processing and Robin Sebastian for secretarial and other help during the planning period. The loyal support of David W. Narver, Director, and Harvey Andrusak, Manager of Fish Culture was very much appreciated.

Some 18 exhibitors or vendors displayed their fish culture equipment and technology at the Conference and their participation was most appreciated. Also, since it often goes unsaid, the organizers wish to recognize the feed companies which hosted the after-hour fellowship gathering and all the companies who so generously donated prizes for the conference.

Thanks to all who presented papers at this year's Conference. The 1989 meeting will be hosted by the National Marine Fisheries Service in Oregon.

R.A. Hugh Sparrow  
Executive Director  
NWFCC Organizing Committee

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A no abstract presented

NORTHWEST FISH CULTURE CONFERENCE  
RICHMOND INN  
DECEMBER 6-8, 1988

DAVID W. NARVER  
DIRECTOR, FISHERIES BRANCH  
MINISTRY OF ENVIRONMENT

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I appreciate the opportunity to again welcome all of you to the 39th Annual Northwest Fish Culture Conference. I am sorry that the Honourable Bruce Strachan, Minister of Environment, was unable to personally welcome all of you from out-of-Province. The Premier called an unexpected Cabinet meeting. I know that Mr. Strachan was looking forward to visiting with you and sharing some of his ideas, particularly about sustainable development within a sustainable environment.

This concept of sustainability comes from the United Nations' Brundtland World Commission on Economic Development and was refined by the Canadian Environment Ministers' Task Force on Environment and Economy.

With the present alarum about dioxins, Greenhouse Effect and shrinking ozone layer, it is clear that today's challenge is to ensure sustainability of the environment in the face of the increased demands placed upon it by multiple resource use and economic development. I believe that you in the fish culture field have a major role in this sustainable environment concept.

This was the subject that Mr. Strachan wanted to discuss with you. However, I now have the dual role of welcoming and keynoting, and I have chosen a different theme, a theme in keeping with the wild fish culture display of our agency out in the hall.

But first, I sincerely hope that each of you will enjoy your short stay in British Columbia and the Vancouver area. I know that you will have a successful conference. The staff of our Fisheries

Program has worked hard and enthusiastically to make this Conference the best ever. In fact, I am pleased with how many staff people are participating in the program. I would like to recognize the person who is singularly responsible for taking on this commitment for our agency and delivering a well-packaged conference. Hugh Sparrow is our immediate past Head of Fish Culture Section and recently retired after over 30 years in the Branch. Please stand up, Hugh.

During my nearly 30 years in fisheries, I have always been impressed with the dedication of agency and private sector fisheries workers. But in virtually every agency I have seen, it is the fish culturists who really epitomize enthusiasm, zeal and competence. In fact, it is this undying commitment to more and higher quality fish that occasionally causes frictions between the stock managers and the fish culturists in every agency. Well...never in our agency, but probably in yours.

Every time I visit one of our hatcheries, I get that evangelistic tour of the facility. But the superintendent always has some new project out back to show me. It is never budgeted, but it is always going to be a world beater. It reminds me of my cousin who raises chickens for the fryer market down near Castle Rock, Washington. Last time I visited the ranch, he just had to show me his new project which was rearing three legged chickens. He had them running all over the place. I said, "That is a really spectacular advance. How do they taste?" He said, "I don't know. I've never caught one."

Of course, I have never seen anything like that in our hatcheries, but I think I have seen analogies in the back of some of the Federal salmon hatcheries.

I would like to know where you are from. Washington? Oregon? Idaho? Alaska? California? Montana? Wyoming? Alberta? British Columbia? Other? How many of you are here supported by your employer?



In our organization, as with all of yours I am sure, fish culture is a critical component of our overall fisheries program. I am very proud of our fish culture program. I would like to tell you a little about it, and I guess "rub it in a little" in that we have some opportunities here in British Columbia that most of you no longer have.

To give you an idea of our modest size, we annually liberate about 14 million fish, mainly rainbow, cutthroat and kokanee, in over 1,000 lakes and about 50 streams. We operate four major hatcheries, five small rearing facilities, and have up to 30 egg taking stations. In addition, about 1.5 million steelhead and searun cutthroat smolts are reared cooperatively in Federal Department of Fisheries and Oceans salmon facilities.

By policy, we have our steelhead streams classified in three categories: hatchery, augmented and wild. The few hatchery streams are those deemed to have little or no remaining productive capacity relative to fishing demand. Dump streams. The augmented streams are those in which our management objective is to maintain a major wild component in the run as well as to increase the total stock. For those streams, brood stock is only wild (unmarked) fish, and only hatchery fish can be killed in the fishery. The majority of steelhead streams are classified as wild with no fish culture enhancement. With few exceptions, brood stock collection is by angling which we have found to be by far the most effective. Again by policy, all stocks are kept separate and fish are released only back into streams of parental origin.

We are equally conservative in our nonanadromous program which comprises, by far, the largest part. We maintain a small stock of domestic rainbows for our urban catchable program. These fish are reared in net pens by inmates in a minimum security prison camp. I might add that the cost of these fish is very low! Otherwise, all eggs, except for some limited special stocks for streams and large

lakes, are taken from essentially wild adults of some 30 different stocks depending on regional requests and availability. Overall, we are 99% dependent on eggs from fish reared in the wild.

The angler catch of freshwater gamefish in British Columbia is around 9 million with about 30% being of hatchery origin, mainly in the southern third of the Province. Clearly, while fish culture is vital to our Program, we still have healthy wild stocks as well.

I would guess that most of you are engaged in rearing fish that are at least partially planned for the recreational angler. This is one of my biases, for obvious reasons, and I would like to dwell on it for a few minutes.

The whole philosophy of freshwater sport fishing for trout is changing in North America and that certainly includes British Columbia. The change is in favour of quality of the angling experience, with reduced kill and catch and release being widely embraced.

For example, in our 1985 survey of resident and nonresident freshwater anglers in British Columbia, we learned that the success of a day's fishing was primarily measured by the quality of environment encountered. Major factors for enjoyment were natural beauty, quality of the water, access to wilderness areas, weather conditions, and lack of crowding. In fact, fish were not even mentioned until the seventh most important factor. Species, size and wild fish were more important than the number of fish caught or catching fish for food, which ranked 10th and 11th respectively. The day of sport fishing to fill the freezer is rapidly disappearing--even in rural British Columbia.

Too often, sport fishing has been dismissed by politicians, bureaucrats and the public in general, as fun and games--a secondary consideration to resource development and land use planning. In fact, even today in British Columbia, fish are not legal users of water, and there is very little licenced water for fish.

The truth is that sport fishing throughout North America is a major recreational activity with exceptional economic impact. Sport fishing is one of the fastest growing participation sports. In highly industrialized Michigan, sport fishing is now the most important economic activity in the State. Let me walk you through some other examples.

In the fresh and salt waters of North America in 1985, 65 million anglers spent 30 billion dollars to fish 1.1 billion days. (REPEAT). This angling effort of 1.1 billion days of fishing can be compared with 365 million paid attendance at all spectator sports (from baseball and football to wrestling and auto racing) at all levels of competition in the United States in 1985. In other words, North Americans spend 3 times as many days fishing as attending paid sporting events. So much for couch potatoes! One in every 4 people over the age of 6 in North America went fishing in 1985.

Moving to the fresh and salt waters of Canada, in 1985, nearly 7 million anglers spent 2.6 billion dollars to fish nearly 84 million days. In fact, the 1985 market value of sport fishing equipment used solely for angling was 8.6 billion dollars. Let me put these expenditure values in context by telling you that the average 1981-85 payment to commercial fishermen for landed fish and shellfish in Canada was 870 million dollars (landed value). Further, the total payments to processing plants for fish and shellfish was 1.9 billion dollars (wholesale value). If the retail value is double the wholesale value, then the expenditures for sport fishing in 1985 were only a third less than the retail value of fish purchases.

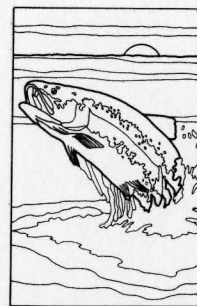
In the fresh and salt waters of British Columbia in 1985, 680,000 anglers spent 382 million dollars fishing 8 million days to catch 16.5 million fish. By anyone's reckoning, this is major social activity and big business.

Another way of looking at these British Columbia values is by the government revenues generated from sport fishing. In 1985, 36 million dollars in Provincial revenues and 47 million dollars in federal revenues resulted from sport fishing in British Columbia. These gross revenues are estimated from licence sales, sales tax and import tax.

It seems clear to me, with this value and the continued increase in demand for sport fishing, coupled with the rapidly growing aquaculture field, that the future of fish culture as a technology and as an area of career employment is bright.

I appreciate the opportunity to share some thoughts with you. I intend to be here into the evening to renew some old associations. Again, I want to wish all of you a successful and valuable conference as well as a pleasant visit for those of you from out of Province.

## Genetics/Broodstocks



Development of an All Female Rainbow Trout Stock  
in British Columbia Lakes

Eric Parkinson and Kanji Tsumura  
B.C. Recreational Fisheries Branch

An all-female of rainbow trout stock was developed as a partial solution to the problem of precocious maturation in hatchery-reared male rainbow trout. The major problems in developing our all-female stock were discriminating between XX and XY males in the first generation and confirming the ripeness of the XX males that do not have gonoducts. Absence of a gonoduct can be used as a distinguishing character in choosing XX males in the first generation but, since not all XX males lack a gonoduct, males with gonoducts do not necessarily have to be discarded in further generations. Maturation in males without functional gonoducts can only be examined by killing them and checking sperm motility. The simplest method of ensuring that males are ready to spawn before killing them is to rear both males and females in a common environment and to use females as indicators of male maturation timing. If eggs from wild females are used, this means that the XX males have to be outplanted into broodstock lakes. Differences between a conventional egg-take and an all-female egg-take include the determination of male maturation solely by external characteristics, the surgical removal of the testes and the crushing rather than squeezing of the testes in order to extract milt. Although we sieved the crushed gonad, extraneous material produced in the crushing process does not appear to interfere with fertilization. Use of the all-female stock will be focused on productive, heavily used lakes where harvest rates are high and the problem of precociousness is particularly serious.

A PRELIMINARY REPORT ON THE PRODUCTION OF  
TRIPLOID AND ALL FEMALE RAINBOW TROUT

by

Carmen Olito and Irvin R. Brock

Alaska Department of Fish and Game  
Division of Fisheries Rehabilitation,  
Enhancement, and Development (FRED)  
Broodstock Development Center  
P.O. Box 5-337  
Fort Richardson, Alaska 99505

SUMMARY

High mortality, slower growth rates, and poor flesh quality are detrimental effects of sexual maturation in two year old rainbow trout (Salmo gairdneri) males. These fish place an economic burden on hatcheries and make the production of a triploid or an all female population desirable.

Sterile triploids are desirable for some rainbow trout stocking programs because they are relatively easy to produce after procedures are established. The simplest method of creating sterile rainbows is through heat shocking fertilized eggs to induce triploidy. This experiment was designed to find the heat shock parameters for Swanson River strain rainbow trout held at the Broodstock Development Center. Fertilized eggs were

exposed to two heat shock temperatures (27C and 29C). Test lots were placed in the heat bath at 5 and 10 minutes post fertilization for durations of 5 and 10 minutes to determine the most effective way of creating triploids. The procedure was duplicated for each heat shock temperature. The most successful treatments were 10 minute exposures to 29C water 10 minutes post-fertilization which created 50-60% triploids.

An all female population would overcome some of the problems precocious males create in rainbow trout broodstocks. The first step in creating this population is to eliminate genetic males from the spawning population and reverse the sex in genetic females. The next step is to fertilize eggs with sperm from sex reversed females.

Males can be eliminated either directly by removing their genetic contribution at spawning or indirectly by weeding out genetic males over generations. Both these approaches were begun last spring. We fed three groups of rainbow trout 17-alpha-methyltestosterone from emergence until 300, 500, and 700 temperature units were accumulated. Groups fed hormone for 500 and 700 temperature units were all male. The groups fed for only 300 temperature units were 1% female, 65% male, and 34% hermaphrodites.



MANAGEMENT OF MONTANA DEPARTMENT OF FISH, WILDLIFE & PARKS  
WESTSLOPE CUTTHROAT TROUT BROOD STOCK

Mark A. Sweeney  
Fish Culturist  
Washoe Park Trout Hatchery  
Anaconda, MT 59711

Recently the State of Montana has been involved in the development of a new westslope cutthroat trout (Oncorhynchus clarki lewisi) hatchery brood stock. I would first like to introduce you to this fish and share the course of events that persuaded us to develop a new brood stock and relocate them to a different hatchery.

The westslope was described in 1853-55 by the Pacific Railway surveys as inhabiting all waters in the mountainous regions of western Montana. This includes all tributaries of the upper Missouri river on the east side of the continental divide. The present distribution is quite restricted and many streams have only small relic populations in the extreme headwaters. It is now believed that this unique subspecies is on the brink of extinction. Genetically pure populations are currently found in only 25 streams with a total of 290.3 stream km or 1.1% of their original range (Licknes, 1984). Reasons for this drastic decline is the loss of habitat from hydroelectric dams, improper logging practices, over harvesting of fish, competition with exotic species and hybridization with rainbow (Oncorhynchus mykiss) and the Yellowstone cutthroat trout (Oncorhynchus clarki bouvieri). This drastic decline of the

(Oncorhynchus clarki bouvieri). This drastic decline of the westslope cutthroat range has brought them to be classified in Montana as a species of special concern.

At the turn of the century biologist and hatchery personnel did not distinguish between the two subspecies of cutthroat and they were planted, along with rainbow trout, throughout the range of the westslope. In some hatcheries the Yellowstone and westslope cutthroat were purposely crossed and their offspring planted all over Montana. Because these species evolved independently they have no innate isolating mechanisms which might allow them to coexist in the wild without crossing.

Montana has been involved in the propagation of cutthroat since the late 1800's. At this time the fish were mostly maintained in brood lakes and were usually of the Yellowstone variety or cutthroat hybrids. The progeny, as stated earlier, were indiscriminately planted throughout the state which only further helped to contaminate the species.

The first attempt at maintaining a pure strain westslope cutthroat hatchery brood stock was in 1965 at the Jocko River Trout Hatchery in Arlee, Mt. This brood stock was derived from fertilized eggs taken from Hungry Horse creek, a tributary to the South Fork Flathead River. The number of adult fish actually contributing gametes was probably less than 50 fish. (Huston 1985). This small number of founding parents is what would eventually lead to a change in gene frequencies and a loss in genetic variation in this original brood stock. There

this egg take, which may have been a result of the low genetic variability from this population.

Various attempts were made since 1967 to increase the gene pool of the Arlee westslope brood stock by adding fertilized wild eggs or half wild eggs. In these few attempts the number of fish contributing was always less than 50 and the wild fish usually came from the Hungry Horse Creek population. Morphological changes in this brood included deformed fins, irregular scale numbers and poor fertilization and egg quality.

The University of Montana Population Genetics Lab has shown that individual westslope cutthroat populations have relatively low amounts of genetic variability. Much of the genetic variation that is present in the taxon is genetic differences between populations. So basing a hatchery brood stock on any single population, as was the case with the Arlee westslope, would severely limit diversity of that brood stock. In addition to the founding population size, much of the decline in gene frequencies can be attributed to past management practices and hatchery selection.

This original (Arlee) brood stock was relocated to Murray Springs Fish Hatchery in 1978. Murray Springs was constructed to mitigate fishery losses in the Kootenai River caused by Libby Dam. This was the westslope cutthroat brood station until 1984.

In 1983 Montana decided to develop a new brood stock based on as many individually pure populations as possible and phase out the original brood stock.

out the original brood stock.

In the spring of 1983 3,000 fish were collected from twelve tributaries to the South Fork Flathead river above Hungry Horse dam. Again in 1984 3,400 fish were collected from these same twelve creeks plus two tributaries to the Lower Clark Fork river. These fish were tested to be genetically pure by the University of Montana Population Genetics Lab, using starch gel electrophoresis analysis. Before the fish entered the hatchery system they had to be certified as being disease free. At the time of collection they ranged in size from 100mm to 177mm and were age 2 to 4 years. At no time during the collection were fish within this size range rejected from brood stock recruitment.

Another problem associated with this species was the poor development of eggs, low fertility and high mortality of young fish. Researchers at the Fish Cultural Development Center in Bozeman, Montana found that the westslope needs a fluctuating water temperature in order for the female to produce viable eggs. Their data showed that the egg quality of the westslope cutthroat was greatly affected by water temperature and that holding females in creek water at reduced temperatures significantly increased egg quality and viability. This convinced us to move the new brood stock to Washoe Park Trout Hatchery in Anaconda, Montana. This station is supplied with both spring and creek water and is located within the westslope natural range on the upper Clark Fork River.

first time in the spring 1985 with 608 females and 670 males contributing gametes. They spawned again in 1986 when eggs were taken from 605 females. The 1984 collection year class were first spawned in 1987, when 941 females from both populations contributed. In 1988 the total number of females spawned was 699. The low number of spawning fish is due to alternate year spawning and a high post spawning mortality. The number of parent fish is still high enough to ensure an accurate reflection of the genetic composition of the wild population.

In the wild, these fish enter streams to spawn when minimum daily temperature is around 4.4 C. This seems to be holding true in the hatchery brood also, with spawning season generally running from the first week in May to July.

During spawning season the fish are sorted for ripeness once a week and are generally stripped that same day. Sperm from ten males is pre-collected and pooled into vials prior to spawning and is used to fertilize the eggs pooled from ten females.

During spawning, no fish are selected for any reason except for ripeness. Spawning is at random with each fish having an equal chance of mating with any other fish. All the fertilized eggs are then pooled and put away in Heath incubators until the eyed stage, around 26 days at 10 C. At eye up the dead eggs are removed and 1% of the original egg take is taken out at random for contribution to the future brood lot.

These fish are raised separately from the production fish

and are kept in spring water for up to 3 years to maximize growth. They are first stripped at age three and their eggs are used for production only. This is also the case with the four year old brood. Future brood eggs will taken from five year old females only.

We will also monitor each generation for any genetic changes and make adjustments as necessary in our breeding program. With this breeding scheme it is our hopes to return to the wild only every two to three generation to upgrade the genetic integrity of our hatchery based westslope cutthroat brood stock.

Adult Returns from a Two and Three Salt  
Winter Steelhead Cross

Jack Tipping  
Washington Department of Wildlife

Summary

To determine if the selective spawning of large winter steelhead in the 1970s at the Cowlitz Trout Hatchery had resulted in the apparent increased size in the 1980s, an experiment was conducted in 1985 where large 3s males and females were crossed and smaller 2s males and females were crossed. Juveniles were reared similarly and adults were recovered as 2s and 3s in 1986-88 at the Cowlitz Barrier Dam. A total of 57.1% of the recovered 2s progeny were recovered as 2s while 42.9% were recovered as 3s. Only 20.6% of the 3s progeny were recovered as 2s and 79.4% as 3s. For both males and females in both years, 3s progeny were significantly longer than 2s progeny. There was no significant difference in return rates between groups, suggesting possibly more 3s fish should be utilized in some broodstocks so a single year class is not depended upon to provide a fishery.

**EFFECTS OF SMOLT QUALITY ON MARINE SURVIVAL**  
**OF NET-PEN CULTURED STEELHEAD**

Pat A Slaney  
B.C. Ministry of Environment

Two weeks prior to release, net-pen cultured steelhead smolts were graded into two groups according to the incidence of scale loss and fin abrasion. Each group was differentially clipped (maxillary), sub-sampled for size and for incidence of abrasion, and then released near the mouth of the Keogh River. Returns of adult steelhead were examined two and three years later.

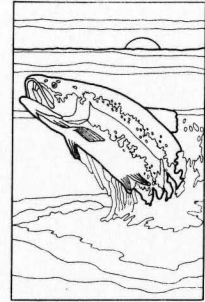
The two groups, 7,000 "high quality" and 10,000 "low quality" smolts, were identical in mean size (60 g) and similar in standard deviation (22 and 19 g). Based on indices of scale loss and fin abrasion, damage was much less in the "high quality" group, with the exception of pelvic fins. For example, 40 % of smolts in the "low quality" group had >5 % scale loss and 11 % had >10 % loss, in contrast with 0 % with >5 % loss in the "high quality" group.

Returns of adults were significantly different; 7.2 % and 9.2 % in the "low" and "high" quality groups respectively, with only about half the returns of 3-ocean fish in the former group. Studies of wild Keogh steelhead indicate that smaller younger smolts return at a greater frequency as 3-ocean fish, which could explain this difference because smaller smolts should be less resistant to effects of scale loss and fin abrasion. Further replication is needed to confirm this result, but it suggests that fish culture practices that reduce surface damage can significantly improve marine survival.



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## System Overviews



## OVERVIEW OF THE B.C. SALMON FARMING INDUSTRY

BY  
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Thank you very much Grant. It is indeed a pleasure to be able to share some information with you this afternoon regarding the salmon farming industry in British Columbia.

The growth of our industry has been talked about, written about (not always truthfully however), and generally hashed over ad nauseum. I'm sure most of you are familiar with the story: from 10 to 125 farms in four years; a young new provincial industry providing a whole raft of economic benefits; from 107 tons of production in 1984 to 6,500 this year and so on. I do not want to bore you with repetition. However, what I would like to do is to provide you with an overview of what's happening right now and why we in the industry, feel salmon farming has turned the corner.

The rapidity with which the industry has developed has involved some major growing pains. Near the top of this list is was our lack of experience. We thought we could import European technology, add Beautiful British Columbia water, and tah dah....instant success. However, as many of our farmers have discovered, the formula is slightly more complicated. We found, through expensive trial and error, that Pacific species could not be stocked at densities

anywhere near those of the Atlantics. As a result, our stocking density is between 4-6 kg/cubic meter. Compare this to 20 kg or more with the Atlantics.

Seed stock has also been a problem. Up until this year, we have been dependent on wild stocks. Chinook stocks being severely limited, millions of coho were grown. To get around the problem of early maturity the practice of neutering them was almost universally adopted. It soon became evident however, that neutered coho did not grow as quickly and the stocks used tended to have a wide disparity in development. At harvest, the size distribution in a pen commonly ranged from two to seven pounds. This resulted in lower than expected yields and higher processing costs.

Plankton blooms have taken their toll. While we now know a lot more about these phenomenon, a number of our farms are sited in a plankton belt and they will continue to be vulnerable to this natural hazard.

The lack of infrastructure development also proved to be a frustration. Transportation, equipment manufacture, fish health expertise, and quality feed manufacturing all developed much more slowly than the industry itself. Consequently, a lot of the materials and services available were somewhat less than prime quality.

Well, so much for what has been. Where are we now? 1988 appears to be a year of transition. Generally, the member farms of the BCSFA report dramatically higher survival and growth rates. Programs are in place to lead to future improvements. Even a year ago, mortalities were commonly in the 40%-50% range. Today, industry estimates are under 20% and going down. Many of our farmers report smolts introduced to marine sites this spring are up to 100% bigger than the same time last year.

One of the prime factors in this change is the high degree of expertise acquired by the farmers. High quality technical workshops sponsored by the Association, training programs at Colleges and Universities and the farmers willingness to share their experiences have all contributed to industry knowledge.

This year, the industry is for the first time self-sufficient in domestic egg supply. We will generate in excess of 50 million eggs supplied from domestic Atlantic stocks. The two prime factors in this situation have been the Broodstock Development Program, instituted in 1985 with financial assistance from the Federal government and the mono-sex milt program.

What this means for the future is that nearly all the smolts introduced into the sea next spring will be first generation

domestic Chinook and predominately female, resulting in better performance and substantially reduced jacking. To improve genetic diversity, we now have stocks from over 20 different streams in the Province in a strain selection and family study program.

With the construction of three new feed mills in the past year, the quality of nutrition has substantially improved, contributing to better growth and survivals.

The fish health infrastructure has also developed. We now have veterinarians with expertise in the industry; vaccines that work and support companies in the fish health field, with knowledgeable personnel.

Additionally, our Quality Assurance Program, developed to distinguish our product as of consistently high quality in a very competitive market place, will enable us to positively promote in the U.S., Canada and Japan, "Fresh Ocean Farmed Salmon from British Columbia".

So what challenges do we still have to face? Financing, particularly working capital, remains difficult to obtain. However, with the success being experienced by a good number of farms, we expect to see an improvement in this area.

The 1988 harvest of 6500 tonnes will generate 65 million in revenues this year and should generate 125 million in revenue to the B.C. economy in 1989 as we produce 12,500 tonnes of fish.

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Stimulation and coordination of research and development will continue to command much of our energy. The formation of the Aquaculture R&D Council has provided a major impetus for this. A by-product of this activity has been an application for the formation of a Network of Centres of Excellence. If successful, this initiative will provide over 12 million in Federal funds for Aquaculture R&D over the next four years.

Public relations will continue to be a challenge. We must, as an Industry, continue to maintain the consumer's confidence that ours is a healthy superior quality product, containing no undesirable residues, and that it is worth the premium price it commands. We must ensure we do not unwittingly provide our critics with ammunition. We can accomplish this through adherence to our Code of Practice and further public education and information programs.

From a marketing perspective, with increased competition from the Norwegian markets, our Industry must be prepared to commit substantial funds to marketing in the future, or face price erosion and reduction of profit margins.

So as 1988 comes to a close, the industry celebrates the fourth year with pride in its' achievements and confidence in its' growing success.

Thank you so much Ladies & Gentlemen, may you all enjoy the next 2 days of your conference.

## Inland Egg Collection Stations of B.C.

### - An Overview -

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D.L. Greiner  
Fish Culture Section  
Recreational Fisheries Branch  
B.C. Ministry of Environment

The five major British Columbia Trout Hatcheries do not have spawning runs returning to, or even close to, existing hatchery facilities. In addition, the policy of the B.C. Fish Culture Section for inland egg collection includes:

- Wild parent stock emphasis.
- Disease-free egg sources.
- Egg collection from lakes that require little or no restocking.
- Enhancement of fish stocks to lakes of similar size and/or habitat to the egg donor lake.

Fish species dealt with include - native rainbow trout, brook trout, Yellowstone cutthroat trout (Westslope), kokanee salmon, and on a limited basis lake trout.

In order to meet annual fish production goals, egg collections must occur at a number of field stations each year. Most field stations are permanent or semi-permanent sites, with a number of sites reserved as back-ups, and/or exploratory stations. Sites are usually equipped with a cabin or trailer for Fish Culture Staff; however, tents, campers and motels have also been used. The Fish Culture Section's capture and holding method is the typical lead/fence/trap familiar to most fish culturists. At some stations however, trap-nets and tooth entanglement are used.

Eggs collected from inland field stations supply five Provincial Hatcheries:

Fraser Valley Trout Hatchery in Abbotsford,  
Kootenay Trout Hatchery in Wardner,  
Summerland Trout Hatchery in Summerland,  
Vancouver Island Hatchery in Duncan,  
Loon Creek Hatchery near Clinton/Cache Creek



Native rainbow trout account for the majority of egg collection effort, with about 10.5 million eggs collected from seven different locations throughout the province.

Tunkwa Lake near the town of Logan Lake produces 1-2 million eggs annually at traps on two outlet creeks. Eggs are shipped unfertilized by truck to Fraser Valley Trout Hatchery, where they are fertilized upon arrival. This is an early May egg collection station.

Dragon Lake near Quesnel is also an early May station producing 1-2 million eggs annually at one inlet trap. Fertilized/water-hardened eggs are shipped either to Summerland or Loon Creek Hatcheries for incubation.

Premier Lake near Skookumchuck is a mid-May egg collection station producing 2-3 million eggs each year. Fertilized/water-hardened eggs are shipped to Kootenay Trout Hatchery.

Pennask Lake near Peachland is an early-mid June egg station producing 1-2 million eggs annually, which are incubated and reared at Summerland Trout Hatchery.

Beaver Lake near Kelowna has in past years produced 1-2 million eggs annually. Due to competition from coarse fish species now present in Beaver Lake, this station is only used when other stations fail to meet egg quotas. Fertilized/water-hardened eggs are shipped to Summerland Hatchery via truck to be incubated and reared.

Other rainbow trout egg stations that have been run in previous years, and still held as back-ups include: Badger, Knouff, Roche, Taweel, Bear, Salmon, Oyama, Trap and Boula Lakes.

Kokanee salmon egg collections occur at Meadow Creek in the West Kootenays near Kaslo, and at Mission Creek near Kelowna in the Okanagan Valley. Meadow Creek has a mid to late September run of kokanee which supplies 5-6 million eggs each year. Eggs are shipped via aircraft or trucks as green eggs or eyed eggs to Loon Creek Hatchery and Skaha Hatchery (a contract facility monitored by Summerland Trout Hatchery near OK Falls on Skaha Lake).

Other kokanee egg collection sites used in previous years include Kikomun and Norbury Creeks in the East Kootenays, as well as the Upper Chilliwack River in the lower mainland.

Brook trout eggs are currently collected exclusively at Aylmer Lake near Chase. This is a mid October run producing 1-2 million eggs annually from mostly two year old spawners. Trap nets are utilized at this station to capture brood fish.

Yellowstone (Westslope) cutthroat eggs are collected at Connor Lake in the Elk Valley of the East Kootenays. A fly-in only station; this site is operated in June and produces approximately 500,000 eggs from fish captured in wire mesh traps.

Lake trout eggs are collected at Cunningham Lake near Vanderhoof and are currently being raised at Loon Creek Hatchery on an experimental basis. There are no require numbers for lake trout egg collections yet; however, an increasing recreational demand in this part of the province requires hatchery input if historic levels are to be maintained in the future.

In addition to the traditional egg collection sites, the Provincial Fish Culture Section runs a number of exploratory egg collection sites throughout the province each year. These sites are operated to gather the following information:

- Acquire new egg sources and maintain genetic integrity.
- Supplement present egg numbers and to investigate possible early or late run stocks to use as back-ups and aid in rearing strategies.
- To satisfy the three year minimum viral and bacterial pathological sampling period required before introduction of new stocks into Provincial Hatchery facilities.

Exploratory sites include:

Rainbow trout - Lorin, Tisdall and Leighton Lakes.

Brook trout - Fortress Lake in the Columbia Icefields near Golden and Whitetail Lake near Canal Flats.

Kokanee salmon - Horse Lake near 100 Mile House and McLeese Lake near Williams Lake.

There are a number of obvious problems with operating so many individual and unique egg collection sites throughout the province, in both remote and non-remote areas. But the Recreational Fisheries Branch, Fish Culture Section believes the benefits outweigh

the problems and contribute to a healthy egg collection program that meets the policies for provincial egg collection, restocking and enhancement.

## AQUACULTURE IN SOUTH AFRICA

G. W. (Bill) Klontz

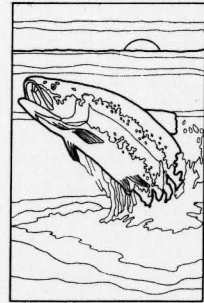
Department of Fish and Wildlife Resources  
University of Idaho

The aquaculture community in the Republic of South Africa consists of both public and private sectors. In many respects the organizational structure is strikingly similar to that in either the U.S. or Canada; i.e., there are public and private sectors. The provincial and national agencies are conservation oriented. The private sector consists of producing the following for the marketplace: finfish (rainbow trout, catfish and ornamentals), oysters (Pacific and European), mussels, crocodiles and waterblommetjies (an edible aquatic plant).

The problems facing both the public and private sectors are being dealt with quite systematically. The resolution process is based upon not making the mistakes occurring under similar circumstances by their counterparts in the Northern Hemisphere. Thus, in many respects, this community is nearly up to date, and, in some instances, ahead of the times. For example, the aquaculture community has created a National Aquaculture Steering Committee which advises the many federal and provincial ministries on the needs of the community. This committee grew out of a need to find a "home" in the central governmental structure for aquaculture. Alas, the central bureaucracy in South Africa is just as complex as it is in the U.S. when it comes to dealing with aquatic organisms, so this committee has its work cut out for it. The task is being made much easier by the privatisation of provincial fish hatcheries. This removes much of the perceived "competitiveness" between public and private sectors we sense in our aquaculture community.

This presentation focuses on the breadth and depth of the South African aquacultural community. It also points out some methods by which we may solve some of our heretofore unsolvable problems; e.g., vertical transmission of IPN virus and the quarantine of imported ova.

## Food and Feeding



A COMPARISON OF FOUR FEEDS FOR REARING SWANSON  
RIVER STRAIN RAINBOW TROUT

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by

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ABSTRACT

There was an abnormally high rate of mortality of Swanson River strain rainbow trout (Salmo gairdneri) at Fort Richardson Hatchery in 1986. Poor nutrition may have been associated with the mortality. In 1987, four different fish feeds were fed to fry from first feeding until they had grown to about 2.0 g: Alaska Dry Pellet, BioDiet, BioDiet containing 1.5% liver additive, and Oregon Moist Pellet. There was no significant difference in average weight of the surviving fish in the four groups. The group of fish being fed Oregon Moist Pellet (OMP) suffered significantly greater mortality (23.3%). Degenerative liver lesions (hypertrophic hepatocytes) were associated with the excessive mortality in the OMP fed fish. Mortalities of fish fed the other three feed types were not significantly different from one another and were in an acceptable range (1.84% to 2.64%).

# FEEDING STRATEGIES FOR COHO SALMON USING DEMAND FEEDERS

By

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With increasingly tighter budgets and more emphasis put on quality of fish produced at Washington Department of Fisheries hatcheries, more refined feeding practices are of increased importance.

We conducted a study to assess the relationship of feeding rate and method of feeding coho salmon on growth , food conversions and size variability. Coho fingerlings (50-70 fpp) were stocked into four raceways (40,500 per raceway). Two raceways were fed a ration of 1.8 % body weight/day by hand or with demand feeders. The other two raceways were fed the same 7 day ration in 3 days ; either by hand or with demand feeders. Growth , food conversions and size variation were better in fish fed by hand rather than with demand feeders. Fish fed everyday by hand performed the best. Fish fed everyday with demand feeders had intermediate growth rates, the poorest food conversions and the greatest amount of size variation. Fish fed everyday had higher growth rates and less size variation than fish fed for three days, but the latter had lower food conversions. Mortality rates were equal among treatments. Even though differences occurred among treatments all groups performed within reasonable limits for production scale hatcheries.

EFFECTS OF FEEDING FREQUENCY ON  
NET-PEN CULTURE OF STEELHEAD SMOLTS

Robert W. Land  
B.C. Recreational Fisheries Branch

Effects of feeding regime on the production of steelhead trout smolts were assessed within net-pens (3.7m x 4.9m x 4.9m) at Oconnor Lake from mid-September until late April. Three feeding regimes were evaluated; (1) spray type automatic feeder set at at 0.5 hr, (2) spray type automatic feeder at 4 hr, and demand feeders (4 per net-pen). Fish numbers and densities and were 7500 to 8000 fish per pen and 0.09 fish/l respectively.

In the spring, smolts were slightly larger in mean length in the demand fed group, but all were similar in mean weight. Demand fed fish had a lower incidence of scale loss and fin abrasion at the smolt stage. Mean condition factor tended to be lower among the demand fed smolts, similar to the decline in spring measured in wild smolts. Length and weight distributions of the auto-fed groups were skewed to smaller fish, whereas demand fed smolts were skewed to larger smolts. Based on size and marine survival relationships (eg. Royal 1972) the demand fed group was predicted to return 30% better than the 0.5 and 4 hr fed groups, with additional gains to the former because of less surface damage.



## VITAMERS C ASSAY TECHNIQUES FOR FEEDS AND FISH TISSUES

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Vitamin C is a dietary requirement for most fish species, and diets deficient in this nutrient inhibit growth, maturation, and resistance to disease or other stressors. The family of vitamers C include many forms with varying biological efficiency when incorporated into the diets or present in major metabolic fish tissues such as blood, muscle or liver. Assay for total ascorbate moieties is relatively simple and was formerly based upon (1) extraction from feed or tissue with acidic medium; (2) protection of labile ascorbic acid and dehydroascorbic acid with metaphosphoric acid; (3) controlled oxidation of ascorbic acid to dehydroascorbic acid; (4) reacting the diketone form with a chromogen; and (5) reading the final product at specific wave length in a spectrophotometer. This indirect method gives reasonable estimates of lightly bound tissue L-ascorbic acid and dehydroascorbic acid, but does not measure tightly bound tissue ascorbate moieties or transitory ascorbate intermediates. L-ascorbyl-2-sulfate is not extracted or hydrolyzed, and addition of L-ascorbyl-2-phosphates to feeds is only partially measured under the standard Roe-Kuether method. More recently, an improved method was developed to estimate total ascorbate by hot acid extraction and comparison to cold acid extraction of ascorbic acid with bound ascorbate (mostly ascorbate-2-sulfate) calculated by difference. Other more recent methods involve measurement of the reducing potential of L-ascorbic acid in extracted and protected solutions. This method yields more reproducible estimates of extracted ascorbate but the accuracy is dependent upon thoroughness of extraction and completeness of hydrolysis of the extracted solute.

A more direct and simple method for determination of vitamers C in feeds and fish tissues has recently been developed. Liver or feed was homogenized in cold distilled water, homogenate was microwave denatured for one minute, material was cooled in an ice bath, homogenate was rehomogenized, then cleared by centrifugation, the pellet was resuspended and microwaved for one minute, the homogenate was cooled and cleared again by centrifugation, the supernatants were combined and filtered through an acrodisc (0.45 $\mu$ m), filtrate was brought to volume and was ready

for HPLC analysis. A Spectro Physics, Inc. Model SP8000 instrument was used with tandem columns of C<sub>18</sub> connected to a 254nm UV detector. The cold mobile phase was 0.1M sodium acetate containing 200mg of EDTA and 0.17ml of n-octylamine per liter. An isocratic elution flow rate of 1.5ml/min was made at 2500psi past the 254nm UV detector.

Reference standards of crystalline L-ascorbic acid(C<sub>1</sub>), L-ascorbyl-2-sulfate(C<sub>2</sub>), and L-ascorbyl-2-triphosphate(C<sub>3</sub>) gave reproducible curves and quantitative separation. C<sub>1</sub> appeared at 4-5 minutes, C<sub>2</sub> at 8-9 minutes, and C<sub>3</sub> at 20-21 minutes under these experimental conditions. Recovery of standards exceeded 98% when assays were conducted promptly. Feed and tissue extracts gave recovery exceeding 90% of added reference standards but disclosed several other peaks in the ascorbate ranges. Isomers of C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub> as well as hydrolysis moieties from C<sub>3</sub> (probably L-ascorbyl-2-diphosphate and L-ascorbyl-2-monophosphate) were detected. Hydrolysis of C<sub>3</sub> to C<sub>1</sub> with phosphates was simple and similar hydrolysis of C<sub>2</sub> to C<sub>1</sub> could be measured.

The practical value of this reported method will enable the fish nutritionist to assay in one extraction for the family of vitamers C present in feed or tissue, and the assay will read the actual chemical compounds present rather than rely on estimates of vitamin C from indirect methods.

Diets for the Intensive Production  
of Montana Arctic Grayling

By

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Abstract

A trial was conducted to evaluate the effect of feeding one of five diets for 77 days on the performance of Montana Arctic grayling (Thymallus arcticus). There were two parts to the study; an initial feeding period of 14 days, and a 63 day growth period. After the first period, grayling fed the BioKyowa diet had better weight gain, survival, total length and feed conversion ( $P < 0.05$ ) than fish fed the other diets. Survival ranged from only 6.3 to 26.0 % in tanks fed Silvercup, Biodiet, Soft-moist and SD-9. Survival averaged 80.8 % in tanks fed the BioKyowa diet. After the 63 day growth period, grayling fed the BioKyowa diet had better weight gain, total length and feed conversion ( $P < 0.05$ ). Fish fed BioKyowa, Biodiet or SD-9 had survival rates from 79.1 to 88.8 %. The cost of feed per kg of fish produced with the BioKyowa diet was \$18.90; much higher than the other diets tested (\$0.84 to 3.17 per kg of fish produced). Arctic grayling culturists need to determine if growth and survival are more important than feed cost with the intensive propagation of this species.

## INTRODUCTION

In recent years the numbers of Arctic grayling (Thymallus arcticus) in Montana have decreased. They presently only occupy a small part of their original range (Brown 1943, Nelson 1954, Vincent 1962). This is due in part to the introduction of brown (Salmo trutta) and rainbow (Salmo gairdneri) trout which prey on grayling eggs and fry (Likness and Gould 1987). Other reasons for the decline of grayling populations in Montana are livestock overgrazing which contributes to siltation, and dewatering streams for irrigation which increase water temperatures (Vincent 1962, Nelson 1954).

The stocking of newly hatched grayling fry has proven very effective at increasing fish numbers in certain Alaskan lakes (Van Wyhe 1963). In Alaskan rivers, however, stocking fry has been unsuccessful (Kalb and Peckham 1975). Stocking of hatchery reared fingerlings, however, have been very effective and contribute a large portion of grayling to the creel (Ridder 1980).

Peckham (1978) noted that after being reared in hatchery ponds for 3 months, grayling survival was only 5 to 34 %. Armstrong (1986) indicated that the future of grayling in some areas may depend on supplemental stocking. He also mentioned that there is considerable lack of information on the culture of this species.

In this present trial the effects of five starter diets on the performance of Montana Arctic grayling reared

intensively for 77 days, was determined. A new diet, BioKyowa, was evaluated. To our knowledge this diet has not been tested previously with coldwater fish.

#### MATERIALS AND METHODS

Arctic grayling gametes were collected from adults sampled by electrofishing Red Rock Creek within the Red Rock Lakes National Wildlife Refuge, Montana. Eggs were fertilized and then incubated at the Bozeman, Montana, Fish Technology Center. At the start of feeding (swim-up) 300 fry were counted into each of fifteen 95 L fiberglass circular tanks. The remainder of the fry (54,000) were placed in rectangular production tanks. Single-pass spring water (temperature, 15<sup>o</sup> C; dissolved oxygen, 8.5 mg/L; pH, 8.0; total hardness, 190 mg/L) was supplied to each tank at a rate of 6 L/min (3.8 exchanges/hr).

This study was conducted during 2 different periods totaling 77 days. The initial starting period was for 14 days and the growth period for 63 days. At the end of the starting period survival was low in most tanks. Consequently, fish from each tank were counted, weighed and then replaced with 300 fish from the production tanks which had been fed the BioKyowa<sup>1</sup> diet. These production fish had good survival and allowed for the continuation of the growth period.

Four commercially developed diets and one open-formula

<sup>1</sup>References to trade names or manufacturers do not imply Government endorsement of commercial products.

diet were each fed to triplicate lots of fish during both periods. The four commercial diets tested were Biodiet (Bioproducts, Incorporated, Warrenton, Oregon); BioKyowa (BioKyowa, Incorporated, Chesterfield, Missouri); Soft-moist (Rangen, Incorporated, Buhl, Idaho) and Silvercup Salmon feed (Murray Elevators, Murray, Utah). The U. S. Fish and Wildlife Service open-formula diet SD-9 (starter, No. 1's and 2's), GR6-30 (No. 3's) and GR7-30 (No. 4's) was donated by the McNenney, South Dakota, State Fish Hatchery. Production fish were fed the BioKyowa diet during the 14 day initial feeding period. All diets and fish tissues were subjected to proximate analysis according to methods recommended by the Association of Official Analytical Chemists (AOAC 1984).

Sweeney automatic feeders (model AF-6) dispensed feed every 10 min, 24 h/d, at a hatchery constant of 45.72 as described by Buterbaugh and Willoughby (1967) on a percent dry matter basis. All fish from each tank were weighed and counted every 2 weeks and feeding rates adjusted weekly. Feed sizes were adjusted according to feed manufacturer recommendations. Tanks were cleaned by siphoning and mortalities recorded daily. Condition factors (K) and proximate analysis of fish tissue were determined for 50 fish from each tank at the end of the trial.

Two weeks before the end of the study, fish in all tanks were diagnosed as having an infestation of the gill parasite Costia sp. A 167 ppm formalin treatment was administered over a 1 hour period each of 5 weeks which

eliminated this malady.

The means of data collected were analyzed using the analysis of variance procedure of the Statistical Analysis System (SAS 1982). Differences between treatment means were determined using the Waller-Duncan Multiple Range test.

## RESULTS AND DISCUSSION

### 14 Day Starter Period

Grayling fed the BioKyowa diet during the initial feeding period of 14 days outperformed fish fed the other four diets tested (Table 1). Fish fed BioKyowa had greater weight gain, survival and total length ( $P < 0.05$ ). Feed conversion was also better due to the low survival of fish fed Silvercup, Biodiet, Soft-moist and SD-9, creating a loss in total weight gained. Survival ranged from only 6.3 to 26.0 % in these tanks, and there was no difference in weight gain, total length or feed conversion.

### 63 Day Growth Period

After the 63 day growth period, grayling fed BioKyowa had greater weight gain, total length and feed conversion ( $P < 0.05$ ) than those fed the other four diets (Table 2). Fish fed the Soft-moist diet had the lowest weight gain (2.66 g/fish) while those fed Silvercup had the lowest survival rate (56.7 %). BioKyowa, Biodiet and SD-9 fed fish had similar survival rates (79.1-88.8 %). Parks et al. (1986) recorded survival rates of from 44.4 to 70 % with grayling reared intensively for 32 days and fed the Oregon Moist

Pellet II starter mash with a double vitamin pack.

Grayling fed the BioKyowa diet along with those fed the Soft-moist diet had the least amount of total length variation ( $P < 0.05$ ). Fish fed Silvercup, Biodiet and SD-9 had the greatest total length variation (Table 2).

The BioKyowa diet provided an excellent feed conversion of 0.84 which was best of the five study diets (Table 3). Soft-moist yielded the poorest feed conversion of 2.17. The remaining diets provided feed conversions of from 1.10 to 1.32. Soft-moist also produced the poorest condition factor (K).

Parks et al. (1986) noted increased survival when stocking tanks at a density of  $0.194 \text{ kg/m}^3$ . Stocking fish at higher densities resulted in lower survival rates. The stocking density at the beginning of this study was  $.78 \text{ kg/m}^3$ . Cannibalism was observed but did not seem to be diet or density related.

The most inexpensive diets were Silvercup and SD-9 (Table 3). BioKyowa was by far the most expensive, costing \$18.90 to produce a kg of fish. The next most expensive feed was Soft-moist, which cost \$3.17 to produce a kg of fish.

There was an effect of diet ( $P < 0.05$ ) on the percentage of body protein, fat and moisture but not ash of grayling fed the different diets (Table 4). Grayling fed BioKyowa gained more weight than the fish fed the other diets, but the percentage of body fat and protein was not different from the fish fed Silvercup. The body fat content of the



BioKyowa-fed fish was not different from fish fed Biodiet, but the protein content was greater ( $P < 0.05$ ). The SD-9 fed fish had the lowest body fat content.

The additional weight gained by fish fed BioKyowa was not the result of additional body fat deposition, indicating efficient utilization of the feed. Body fat content of fish fed either Soft-moist or SD-9 was different than that of fish fed the other diets. Weight gain of the fish fed Soft-moist or SD-9 was also much less than that of fish fed the other diets. Feeding either Biodiet, BioKyowa or Silvercup resulted in grayling with good body protein and fat levels.

#### CONCLUSION

We recommend feeding BioKyowa for the first 14 days of initial feeding to achieve acceptable survival rates and weight gain. After this, if greater weight gain is needed BioKyowa should be fed up to day 77. However, if survival is most important either Biodiet, BioKyowa or SD-9 can be fed. Assuming cost is the limiting factor, Silvercup or SD-9 can be fed after feeding BioKyowa for the first 14 days.

The cost of the BioKyowa diet may appear to be prohibitive especially if used on a large production scale. However, in some circumstances the survival and large body size of the fish may be the most important factor. This is the situation with the culture of Montana Arctic grayling. Survival and weight gain for the reestablishment or restoration of this species is most important at this time.

## ACKNOWLEDGMENTS

We thank Randy Elliott, Elizabeth MacConnell, Blake Norsworthy, Jan Pisano, Charlie Smith and Art Viola for their technical assistance, along with the McNenny, South Dakota, State Fish Hatchery for supplying the open-formula feeds. We are also grateful to the commercial feed mills for supplying their products for testing and to the Creston Fish and Wildlife Center for the collection of gametes.

Table 1. Performance of Montana Arctic grayling fed one of five starter diets for the 14 day starter period. All values are means (N = 3). Within each column, values followed by the same letter are not significantly different ( $P > 0.05$ ).

<u>14 Day Starter Period</u>				
Diet	Weight Gain <sup>1</sup> (g/fish)	Survival (%)	Total Length <sup>2</sup> (mm)	Feed Conversion
BioKyowa	.12 a	80.8 a	28.3 a	1.28 a
Silvercup	.01 b	6.3 c	18.2 b	0 b
Biodiet	.01 b	26.0 b	19.1 b	0 b
Soft-moist	.02 b	22.6 b	18.8 b	0 b
SD-9	.01 b	12.4 bc	18.1 b	0 b

Table 2. The effect of diet on weight gain, survival and total length of Montana Arctic grayling during the 63 day growth period.

<u>63 Day Growth Period</u>				
Diet	Weight Gain <sup>3</sup> (g/fish)	Survival (%)	Total Length <sup>4</sup> (mm)	Length Range (sd)
BioKyowa	6.97 a	85.9 ab	94.3 a	6.4 a
Silvercup	4.27 b	56.7 c	79.3 b	11.6 c
Biodiet	3.89 bc	88.8 a	77.8 b	9.7 bc
Soft-moist	2.66 d	70.4 bc	71.0 c	7.9 ab
SD-9	3.64 c	79.1 ab	71.9 c	11.0 bc

Table 3. The effect of diet on feed conversion, condition factor and cost of producing Montana Arctic grayling.

Diet	<u>63 Day Growth Period</u>		<u>After 77 Days</u>	
	Feed Conversion	<sup>5</sup> K	Cost/kg of feed (\$US) <sup>7</sup>	Cost/kg of fish produced (\$US)
BioKyowa	0.84 a	0.773 a	22.51	18.90 a
Silvercup	1.10 b	0.742 a	0.77	0.84 d
Biodiet	1.32 b	0.727 a	1.56	2.05 c
Soft-moist	2.17 c	0.658 b	1.48	3.17 b
SD-9	1.13 b	0.763 a	0.90	1.01 d

<sup>1</sup>  
Initial average weight = .026 g

<sup>2</sup>  
Average of 10 fish per tank (30 per diet)

<sup>3</sup>  
Initial average weight = .070 g

<sup>4</sup>  
Average of 50 fish per tank (150 per diet)

<sup>5</sup>  
(Weight of feed fed)(90.9/% dry matter of feed)/(Weight gained by fish)

<sup>6</sup>  
Condition factor K =  $\frac{\text{average weight (g)} \times 10^5}{\text{average total length (mm)}^3}$

<sup>7</sup>  
Based on price of feed purchased at feed mill, 1988; does not include shipping charges.

Table 4. Proximate analysis of trial diets and Montana Arctic grayling tissue (in parentheses) after the completion of the study. All values are mean percentages (N = 3). Within each column, values followed by the same letter are not significantly different ( $P > 0.05$ ).

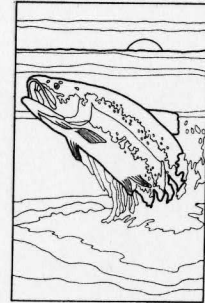
Diet				
(Fish Tissue)	Protein	Fat	Moisture	Ash
BioKyowa	58.1	16.4	5.0	12.7
(fish)	(14.03) a	(8.53) b	(73.9) ab	(2.43) a
Silvercup	50.3	15.8	8.2	9.0
(fish)	(13.93) a	(8.67) b	(73.6) b	(2.60) a
Biodiet	43.8	15.0	20.3	8.7
(fish)	(13.07) b	(8.83) b	(74.4) ab	(2.37) a
Soft-moist	46.8	21.0	16.2	8.4
(fish)	(12.83) b	(10.13) a	(73.3) b	(2.33) a
SD-9	45.7	14.3	8.0	9.9
(fish)	(13.67) a	(7.83) c	(74.9) a	(2.57) a

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## Rearing Life Stages





## WORKING WITH VERTICAL INCUBATION SYSTEMS

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Vertical incubation systems have been in use for over one hundred years. Originally the incubators were made entirely of wood. As stainless steel and aluminum became available the incubation systems improved in quality and efficiency.

The popular eight and sixteen tray vertical incubation system (VIS) currently seen in many hatcheries throughout North America was designed and developed in the late 1950's and has remained virtually unchanged for twenty five years. The current VIS is primarily used for salmon and trout culture with some application in muskelunge and northern pike culture in the Great Lakes region of Canada and the United States.

Two years ago the first of three major changes to the current VIS was made. All of the fiberglass reinforced plastic components were converted to vacuum formed and injection molded parts. The change in material increased durability but compromised interchangeability. The second change was the addition of a front discharge kit. The front discharge kit will keep the water from flowing into the next tray providing the user with an isolation incubation option. When the front discharge kit is used with the bottem water tray of each eight tray unit it can be used for recirculating egg treatments, water conservation, and/or energy conservation. The third change is the wider range of screen

mesh sizes that have been made available. This extends the range of VIS application from what was almost exclusively salmon and trout culture to the cultivation of species ranging from, and not limited to sturgeon and arctic char to the seed production of oysters, manila clams, and scallops.

The change in materials required designing thicker wall sections into the VIS components. This causes some interference when trying to fit the newer plastic parts with the older fiberglass parts.

The VIS is made up of four basic components, the cabinet, water tray, egg tray, and egg tray lid. The most frequent replacement item on the older brittle fiberglass incubators is the egg tray lid. The current plastic egg tray lid can be modified to fit the fiberglass egg tray. There are two protrusions on the back edge of the egg tray lid. Simply remove these with a knife or block file and the plastic lid will fit on the fiberglass egg tray. The modified plastic egg tray lid can still be used on the plastic egg tray if two round head #10x1/4" stainless steel sheet metal screws are tapped into the back of the egg basket. The screw heads will function as the egg tray lid retainer.

The first version of the egg tray lid is very difficult to remove and install even with the water tray pulled almost completely out of the incubator cabinet. This is due to the sharpness of the radii on the back corners of the egg tray lid and the full channel section on the underside of the

back of the lid. To ease the operation of the lid remove just enough material from the outside corners of the radii with a sander or block plane to clear the egg tray retainers in the water tray. (the fiberglass water tray has plastic pins and the plastic water tray has #10 sheet metal screws with plastic spacers) . If too much material is removed the lid could warp excessively. Removing a small amount of material from the inside leg on the back of the lid will ease operation. To reduce the chance of warpage no more than 4mm (3/16") should be removed. Latch pin operation can be eased adding a spacer under the latch pin retainer. This modification is performed by carefully removing the upset end (on the bottom side of the egg tray lid). Just remove the end of the rivet; do not try to drill through, drilling through will melt the plastic and enlarge the hole; and punch the rivet out of the hole. Place a small plastic washer under the latch pin retainer and fasten the assembly together with a #10x3/4" stainless steel or plastic bolt and nut. This will allow the latch pin to ride up over the glue bead on the egg tray lid.

The plastic egg basket can be adapted to the fiberglass tray. This is accomplished by extending the egg tray retainers found on the back of the fiberglass water tray. By removing the original plastic keeper pins and replacing them with two #10x1/4" stainless steel or plastic bolts and two 1/8 plastic spacers to a position 4mm(3/16") lower than the original retainer pins. One version of the egg tray has a lip

molded over the latch pin opening. This will need to be removed so that the egg tray will fit down into the water tray. The current model of the egg basket does not have this lip.

The plastic incubator trays can be used in the original fiberglass cabinets but do not move smoothly. Removing the plastic buttens from the fiberglass side panels will ease the binding but will lead to excessive wear.

The function of the water tray can be improved with several modifications. The most common complaint is leaky watertray plugs. When the rubber plug ages it loses some of its flexibility. By applying some petroleum jelly to the plug some of its flexibility can be restored. Replacement plugs, clean out rods and water tray plug grommets may be ordered from the manufacturer.

The water trays have an annoying tendency to drip water off the front of the trays and onto the floor. Many times this is the result of water not falling cleanly out of the spillways. Adding some small bumps or a notch across the underside of the water channel, about 4mm-9mm(3/16"-3/8") from the spillway, the water will drip into the water receiver end of the water tray below. The latest version of the water tray has this feature molded in.

When the VIS cabinet is set up the entire unit is tilted towards the front. This is to prevent air bubbles from being trapped under the screen. Air bubbles will block the flow of

water causing the eggs to die directly above the bubble. When the cabinet is tipped forward some of the waterflow capability is diminished. One of the ways to separate the air from the water is to float a 10mm-12mm block of coarse filter foam in the water receiver end of the water tray. The foam should cover the entire water receiver surface and can be fastened with two small screws or a plastic clamp to the back of the water tray. This will allow the cabinet to be leveled providing greater water flow capability. For more information on product service, workshops, and new product please contact Jay Rideout at Rideout Pacific.

EFFECTS OF BAFFLES FOR EARLY REARING OF  
SUMMER CHINOOK SALMON  
Thomas S. Frew

During the winter of 1987-88 a study was undertaken at McCall Chinook Salmon hatchery to study the effects of baffles on the early rearing of chinook salmon fry.

The baffles consist of solid sheets of aluminum that are inserted into a hatchery vat perpendicular to the water flow through the vat. The baffles are held a small distance from the bottom of the vat to create an area of high water velocity flow. When used in series, the settleable solids are moved the complete length of the vat and accumulate behind the tail screen.

The baffles are installed as suggested by Boerson and Westers, 1986. We found the preciseness that is eluded to in the formula in this paper is not necessary, but is an excellent starting point for baffle installation. The distance the baffle is held off the bottom of the vat is dependent upon the density of the fry in the vat and the horizontal velocity of the water column.

We monitored growth rates, mortality rates, feed conversion, and final length at the end of the experimental period. There was little difference in the total length at ponding with all groups falling within a 1.4 mm range (51.41mm to 52.86mm). The fry with the baffles were heavier at ponding than the normal fish by approximately 6%, due to a corresponding increased conversion rate. Mortality rates were very close in value. The greatest benefit was to the culturist, the time required to maintain the vats was cut by 60%.

## CARRYING CAPACITIES IN SINGLE AND MULTIPLE USE SYSTEMS

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### INTRODUCTION

Three carrying capacities of rearing units which must be defined are: (1) life support; (2) density; and (3) ammonia. Each must be considered as an individual criterion, with the lowest carrying capacity value being the determinant for the rearing unit. It should be noted that the fish will (should) not suffer irreparable harm if one or more of the carrying capacity values are exceeded - but their performance; i.e., growth rate and appearance, will be compromised to a measurable degree.

The oxygen carrying capacity of a raceway pond may be estimated using one of several models. The most convenient and reliable model for single-pass, open water systems is Piper's Flow Index method. It should be pointed out that this model is not applicable to circular or other styles of circulating ponds.

$$Wcap = Fls * Rw$$

Where: Wcap = biomass (lb or kg) of fish per unit  
of body length (in. or cm) per pond

Fls = life support index - (Flow Index  
table)

Rw = water inflow (gpm or lps)

The foregoing model provides the user with an index of oxygen-supported carrying capacity for each rearing unit. The index (lb of fish per pond per inch of body length or kg per pond per cm of body length) should be recorded for the particular pond. It represents the maximum life support loading for the pond.

The density carrying capacity of a pond is based upon the spatial requirements for the fish in the system. The model is:

$$Wden = Pvol * Dfac$$

Where: Wden = biomass (lb or kg) of fish per unit  
of body length (in. or cm) per pond

Pvol = pond volume (cu. ft. or cu. m.)

Dfac = density index (lb or kg fish per cu. ft or cu. m of rearing space per in. or cm of body length)

#### Density Indices for salmonids:

	lb/in./cu.ft.	kg/cm/cu.m
RBT-Shasta	0.5	3.0
RBT-Kamloop	0.5	3.0
Steelhead	0.25	1.5
Chinook salmon	0.3	1.8
Coho salmon	0.4	2.4
Atlantic salmon	0.3	1.8

The density carrying capacity, like the oxygen carrying capacity, is recorded for each rearing unit on the facility.

The ammonia, as total ammonia-nitrogen, carrying capacity is based upon the Median Tolerance Limit - Chronic (TLMc) of free-ammonia by salmonids. The values for non-salmonid species have not been determined. The currently accepted TLMc value for salmonids is 0.03 mg/l free-ammonia. This value is obtained from the total ammonia-nitrogen value, which can be either measured or determined from one of the several models with difficulty and frustration. Next, the pH and water temperature-based percent free-ammonia portion of the total ammonia value must be obtained. Thus, this carrying capacity is determined usually after the fact during the process of producing fish. It should be noted, however, that in most instances if the water retention time of the pond is less than 2 hours, there should be no problems incurred by the accumulation of free-ammonia.

#### POND LOADING (SINGLE-PASS SYSTEMS)

In many of today's finfish production facilities, fish are stocked into ponds often quite arbitrarily - sort of by the "seat of the pants". These fish are fed daily and, perhaps, inventoried for growth at biweekly or monthly intervals. When the pond "looks about overloaded" the population is reduced either by grading off a selected size or randomly transferring a portion of the population to a vacant pond or to one not having so many fish. The practice brings to mind an age-old administrative philosophy: "Which way did they go? I must hurry after them for I am their leader." The fish culturist should be "leading" the fish and not trying to "catch up". Growth, as will be seen, is a quantifiable entity, thus, it is quite predictable, given the circumstances.

An alternative to the foregoing scenerio is a practice which has come to be known as "loading the pond for take-out". This means that the pond is loaded to have a certain



biomass by a specified date - provided no "disasters" occur. The process is quite simple, and it goes like this:

1. Establish the date on which the pond population is to be reduced by grading or by random transfer of fish.
2. Determine the number of growing days between the date of pond stocking (Day-1) and the date of population reduction (Day-X).
3. Calculate the temperature-based daily growth rate of the fish from Day-1 through Day-X. The following model may be used for this:

$$dLt = dLset * (1 - (dT * 0.0825))$$

Where: dLt = the daily increase in length (in. or mm) at the expected water temperature.

dLset = the daily increase in length at S.E.T.

dT = the difference between the expected water temperature and the S.E.T.

4. Apply the daily length increase data in a day-by-day fashion throughout the period between stocking and reduction. Most mid-priced calculators; e.g., TI-55, can handle this process quite easily. This will determine the length of the fish on Day-X.
5. From an appropriate weight-length table determine the weight (either no./lb or g/fish) of the fish on Day-X
6. Determine the allowable biomass (either lb./in or kg/cm) of the fish on Day-X. Multiply this number by the weight (either no./lb or no./kg) to determine the ending head-count.
7. Using that head-count plus the anticipated mortality (use 0.03% per day for "routine" mortality) determine the number of fish to be stocked into the pond on Day-1.
8. Divide the head-count by the number of fish per lb or kg on hand to be stocked into the pond. This designates the lb or kg of fish of the size on hand to be stocked.

## POND LOADING (MULTIPLE-PASS SYSTEMS)

Ponds, particularly raceway ponds, arranged for serial passage of water have been one of the more serious constraints to successful fish health management. In systems utilizing 3-5 serial water uses the status of health in succeeding populations often gets progressively worse. This condition is influenced by at least three major factors within the system: (1) the pond loadings; (2) the successive accumulation of waste products (fecal material and ammonia) and uneaten feed; (3) the successive depletion of dissolved oxygen. Thus, any successful production in situations such as this must take these three constraints into account.

First, the anticipated dissolved oxygen depletion must be defined. By way of example, the system to be modeled is a series of five raceways (3 m \* 30 m \* 1 m). Between each series is a 91 cm fall, which serves to recharge the water with dissolved oxygen. The water inflow to the first-use pond is 50 lps and the dissolved oxygen content of the water at 95% saturation is 10.0 mg/l. Thus, there is a total of 1,800,000 mg dissolved oxygen entering the pond per hour. From our studies, there should not be more than a 30% depletion of total dissolved oxygen in the first use. Thus, the water exiting the first-use pond should be 65% of saturation. With the fall from the first use pond into the second use pond there will be an oxygen recharge to 80% of saturation (8 mg/l). The water leaving the second use pond should have a partial pressure of oxygen (pO<sub>2</sub>) of not less than 90 mm Hg or about 60% of saturation (6 mg/l). With the fall between the second use and the third use, the oxygen saturation of the water entering the third use pond will be 77% (7.7 mg/l). Again, as with the second use pond, the water exiting the third use pond should not be less than 60% of oxygen saturation. Now the system has "stabilized" with respect to oxygen utilization. The fourth and successive passages of water will be the same.

To recap, there will be 4 mg/l D.O. available in the first use; 2.0 mg/l D.O. available in the second use; and ca. 2.0 mg/l available in the subsequent use in the system. Expanding that oxygen availability per hour in each pond, the first pond will have 720,000 mg; the second pond will have 360,000 mg; the third and subsequent ponds will have ca. 300,000 mg.

The next step is to determine the oxygen utilization (mg/hr) by fish of a specified size. This can be accomplished using the following model:

$$\text{Log } Y = -0.84753166 + 0.84281471 * \text{Log } X + (0.03733078 * T)$$

Where: Y = the oxygen requirement (mg/hr) of fish  
(size X) at temperature T (oC)

X = average weight of fish (g)

T = water temperature (oC)

With the oxygen requirement of the fish known, this value divided into the amount of oxygen available in each pond generates the permissible head-count. The head-count divided by the number of fish per kg generates the permissible biomass.

To use this concept as a predictive "tool", the date on which the pond population is to be reduced (Day-X) and the size of the fish on that date are established. The permissible head-count for that size fish plus the anticipated mortality occurring between Day-1 and Day-X generate the head-count for stocking the pond. The biomass to be stocked is derived by dividing the beginning head-count by the number of fish per kg on Day-1.

Given the complexities of the foregoing, there is an easier way, although it is less precise, to accomplish the same act. For the first use pond, reduce the Life Support Index by 25% and by 30% for each succeeding use. This method can have some attendant respiratory problems; e.g., environmental gill disease (EGD), but the fish will not die unless the situation gets completely out of control.

# Evaluation of Transfers of Fall Chinook Salmon to Saltwater Net Pens

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## Introduction

Since its inception in 1975, Fox Island Net Pens has been plagued with high transfer losses of its fall chinook. The Washington department of Fisheries facility is located in Carr Inlet in southern Puget Sound. The mortality of these chinook usually occurs within the first four days following entry into saltwater. From 1975-1983, this loss averaged 27% with a range of from 90% to 5%.

Since the Fall of 1984, attempts were made to reduce this loss by evaluating the effects of four variables: 1) saltwater tempering of fish prior to transfer versus shipment of untempered fish, 2) two different transfer methods, 3) increasing the no-feed period from two days to three days before transfer, and 4) the effects of fish size on survival.

Applying lessons learned from those tests, the loss declined to an average of 4.3% with a range from 6.2% to 1.5%.

## Methods and Materials

Two definitions considered here may be helpful in explaining this report. They are as follows: 1) transfer loss-only that fish loss occurring within the first four days immediately following transfer to saltwater which is not caused by mechanical breakdown,

pathogens, or predation, and 2) tempering--the incremental addition of saltwater to a freshwater holding area seven to ten days prior to transfer of fish to saltwater.

The design of these tests was developed using the aforementioned variables: method of transfer, saltwater tempering versus no tempering, fish size, and two-day fasts versus three-day fasts prior to transfer.

The method of transfer comparison consisted of a net pen barge (Fox Island Net Barge), powered by an outboard motor, with a capacity of 450 cubic feet. The other transfer method was a converted landing craft (Squaxin Tank Barge) with two holding tanks having capacities of 290 cubic feet and 240 cubic feet respectively. A power take-off system pumps approximately 150 gallons of saltwater per minute into the two tanks.

Saltwater tempering was accomplished by saltwater entry into a freshwater holding area according to the following schedule: day one--4 to 5 parts-per-thousand (ppt); day two--6 ppt; day three--7 to 8 ppt; day four--9 to 10 ppt; day five--12 to 13 ppt; day six--16 ppt; and day seven--19 ppt.

For fasting trials, fish were starved three days prior to transfer in 1987 and 1988 rather than two days as in 1985 and in 1986.

To see the effects of fish size, fish of varying lengths were transferred from 1985 through 1988, and observations were made of fish size/survival relationships.

In 1984, a series of tests was set up as follows (Figure 1): Test #1--Fish were tempered in saltwater and transferred by Squaxin Tank Barge; Test #2--Fish were tempered in saltwater and

transferred by Fox Island Net Barge; Test #3--Untempered fish were moved directly from freshwater to saltwater by Squaxin Tank Barge; and Test #4--Untempered fish were moved directly to saltwater by Fox Island Net Barge. In each of these four tests, fish size was uniform at 28 grams/fish.

### Results

Mortality rates of these four tests in 1984 ranged from 0.6% to 37.6% (Figure 2). The lowest mortality (0.6%) resulted from using saltwater tempering and the Squaxin Tank Barge. The highest loss (37.6%) resulted from moving untempered fish with the Fox Island Net Barge. The mortality rate for Test #2 was 21.9% using saltwater tempering and the Fox Island Net Barge; the loss for Test #3 of 28.0% resulted from moving untempered fish in the Squaxin Tank Barge.

From 1985-1988 fish were moved using saltwater tempering and the Squaxin Tank Barge (the most effective combination noted in earlier tests). The average loss for fish in the two day fast group (1985-1986) was 5.9% (Figure 3). The average loss during the three-day fast group (1987-1988) was 2.9%.

In both fast groups, the larger fish (25-27 grams) had lower loss (5.5% and 1.5%) than the smaller fish (21-22 grams) that had losses of 6.2% and 4.3% respectively.

### Conclusions

1. Saltwater tempering when combined with use of the Squaxin Tank Barge reduced loss significantly.
2. There was loss reduction when larger fish of 25-27 grams were transferred.
3. Increasing the no-feed period from two days to three days prior

to transfer further reduced loss (Figure 4).

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**Figure 1. Descriptions of Tests 1-4 Fox Island Net Pens**

















		TEST			
		1	2	3	4
WATER SOURCE	SALTWATER TEMPERED				
	100% FRESHWATER				
TRANSFER METHOD	SQUAXIN TANK BARGE				
	FOX ISLAND NET BARGE				



Figure 2. Transfer Loss ( Tests 1-4 ) Fox Island Net Pens

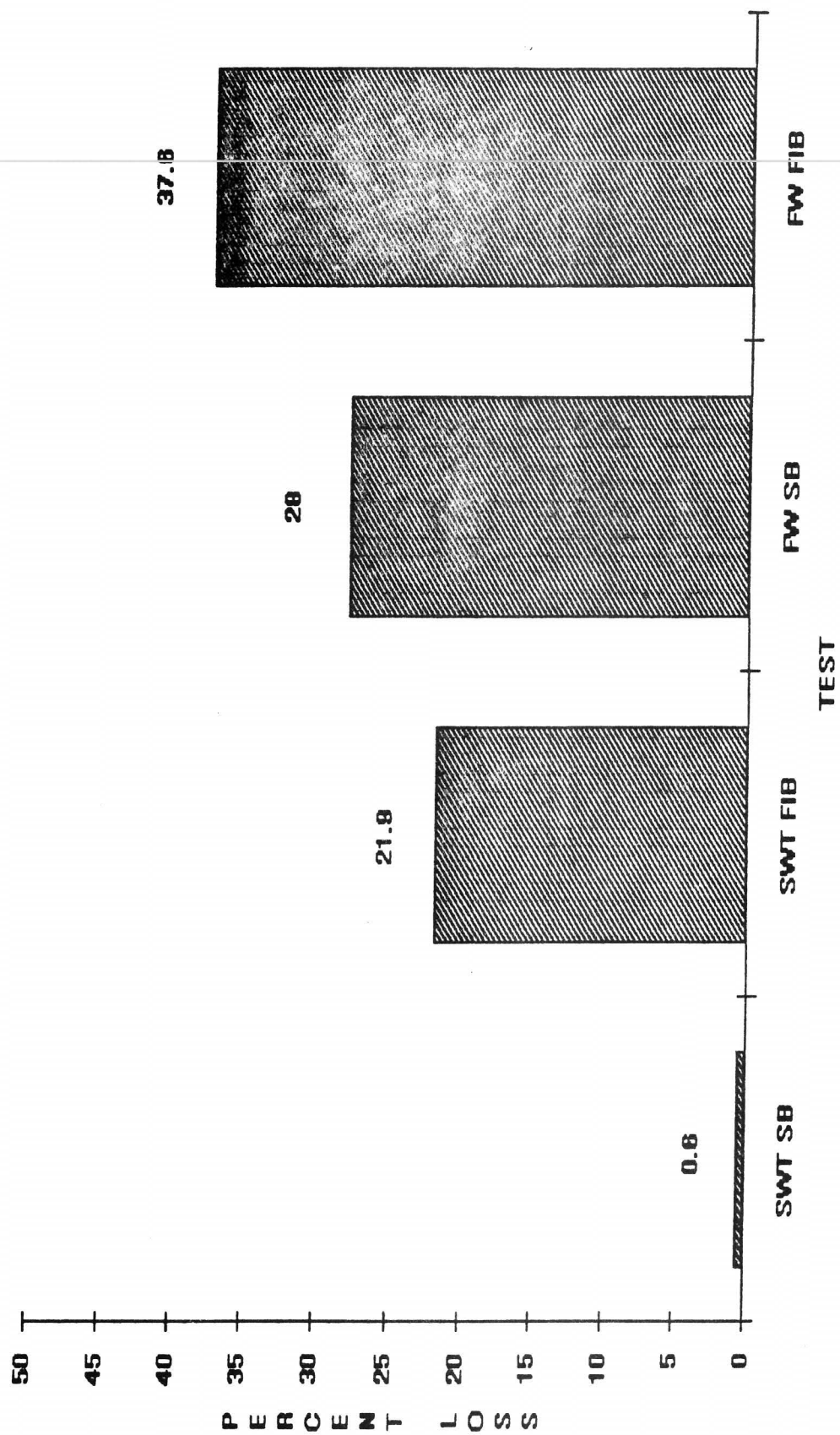
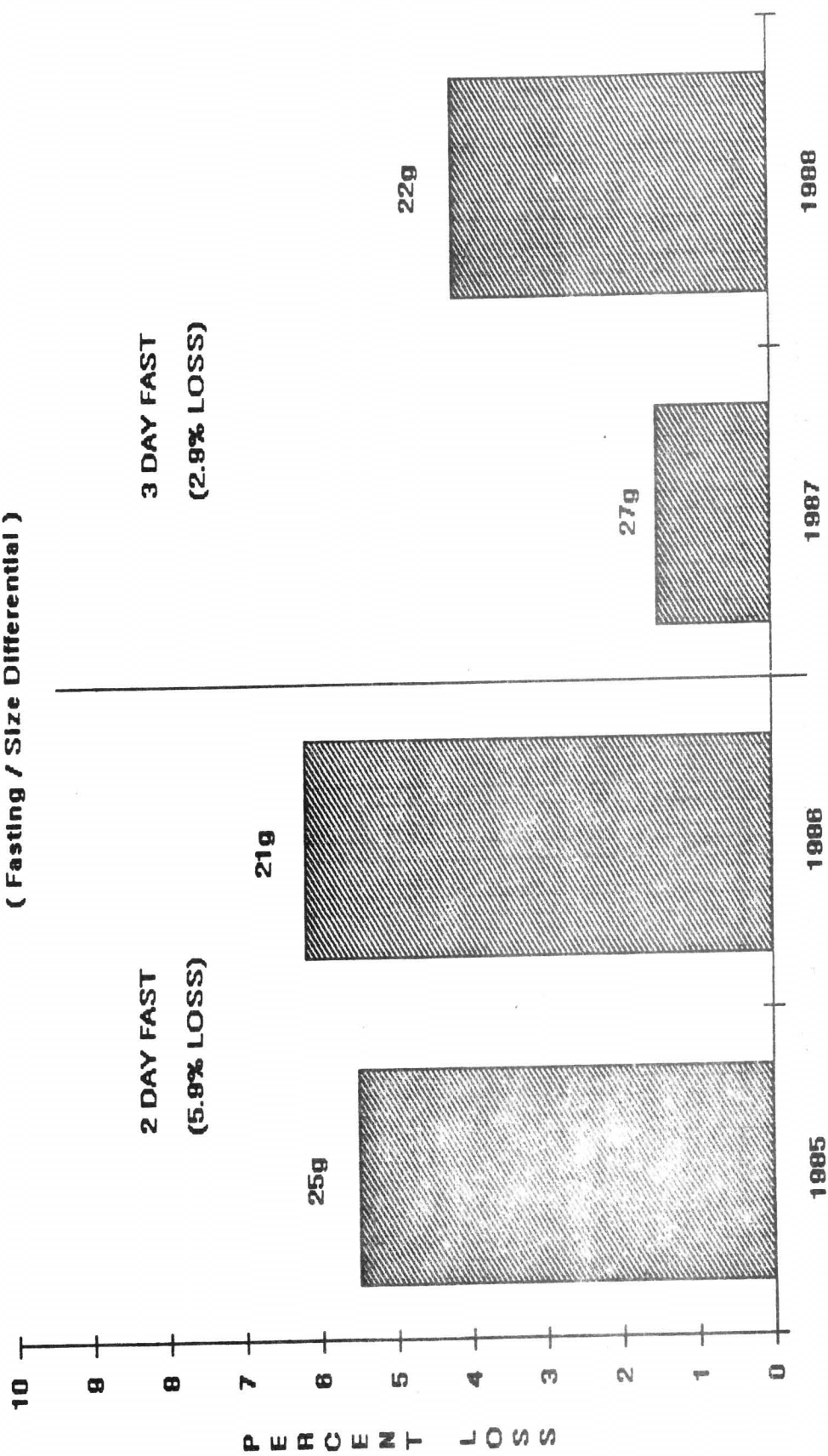
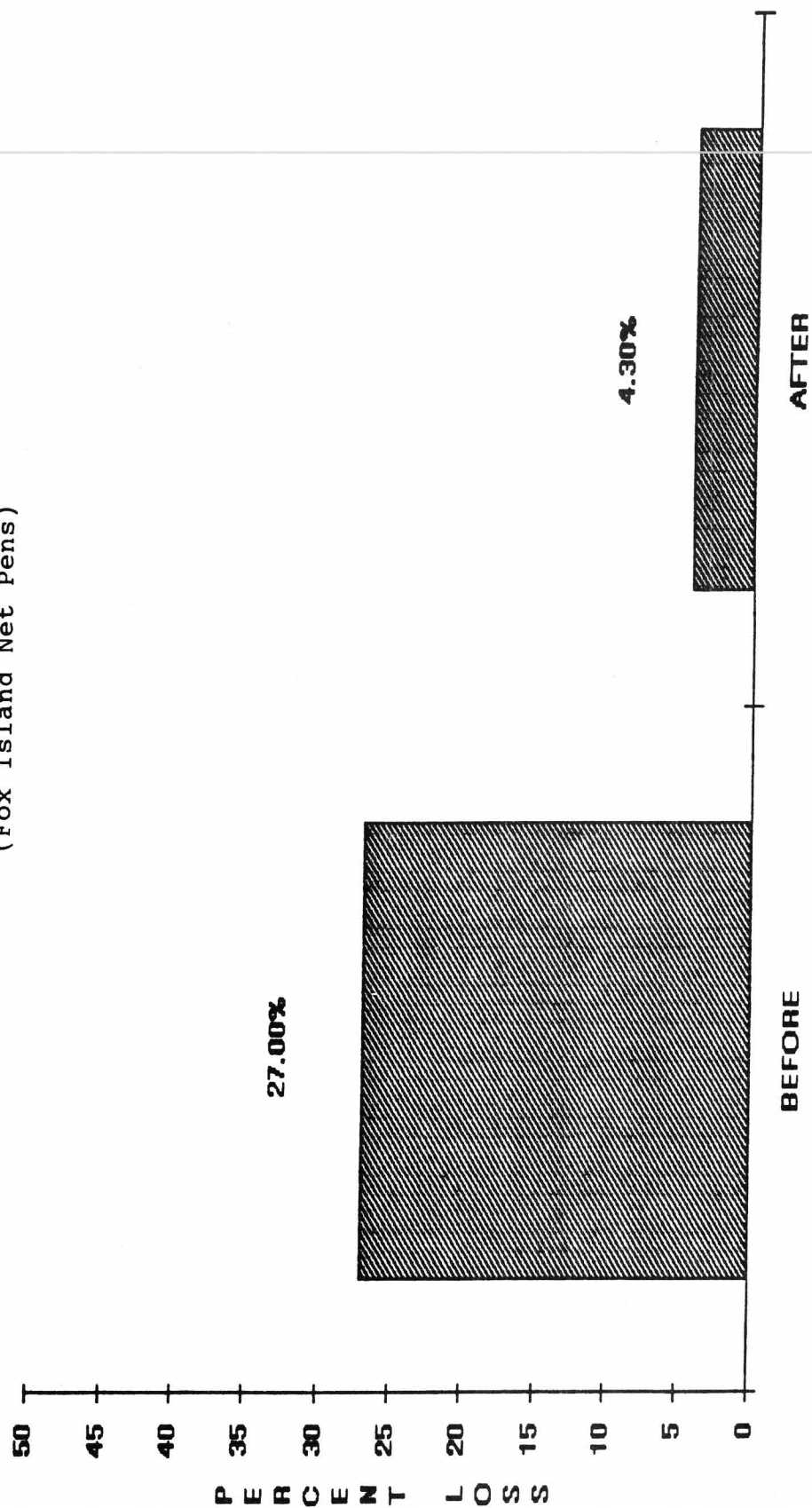


Figure 3. Transfer Loss ( 1985-1988 ) Fox Island Net Pens  
( Fasting / Size Differential )



**Figure 4. Transfer Loss Before and After Tests**  
 (Fox Island Net Pens)



# **Wet Vacuum Fish Pump Technology**

Ted Palmer  
Innovac Technology (U.S.A.), Inc.

Wet vacuum fish pumping was originally developed for off-loading fishing vessels dockside at processing plants. Before long, commercial fishermen took them one step further, . . . to sea. For example, in the highly competitive short herring season, fish pumps are used to rapidly empty purse seine nets of herring into waiting tenders and packers.

On large processing ships, the pumps are part of a "central vacuum system" for moving fish. This central vacuum concept has been applied in hatcheries.

Boat mounted pumps have ushered into the commercial fishing industry a damage-free product delivered faster. However, faster isn't necessarily the focus of today's coastal fisheries. As portions of this industry shift to include fish farming and ranching, terms like "reduced cumulative stress" and "O<sub>2</sub> depletion" are used commonly by the live hauler, who in his not too distant past may have been a packer. Many processing plants now prefer to buy live fish. After all, the meat packing industry buys live animals!

The advent of wet vacuum pumps delivering live fish to a stunning bleeding process has coincided with the international demand for freshness, particularly in the sushi market. Up and down the coast, pen culturists have adopted fish transfer systems into their everyday operation.

The U.S. Fish and Wildlife Service will be using a boat for fish stocking on the Great Lakes. A deck mounted wet vacuum pump will pump fish out of tanks below deck into the lake. A comprehensive study of the pump was thus required.

Upon request from the Region III Fisheries Division of the U.S.F.W.S., the Bozeman Montana Fish Technology Center was asked to evaluate the Model

2410 Transvac (6 inch) Aquaculture Pump. The test took place at the Center in December of 1987. The purpose of the test was to approximate the stress of planting.

5500 (160 lbs.) of 4.2 inch Lake Trout were pumped 3 times to simulate pumping fish from a raceway into a distribution tank, from the tank into a boat, and finally from a boat into the lake.

Initially fish were crowded to the lower end of a raceway and pumped into a crowded area of an adjacent raceway. Fish were held there at approximately 40 lbs./ft.<sup>3</sup> for 4 hours. They were then pumped from this area back into a similar sized space in the original raceway, simulating the boat hold. Fish were held for an additional 4 hours, then pumped above the crowder into the raceway, simulating pumping into the lake.

Only 8 fish were lost during this test, and it appeared these were crushed by improper placement of the intake nozzle on the end of the hose. Approximately 100 fish were examined for evidence of descaling and little to none was found when compared with a similar number of fish from the control group. Mortality was recorded over a 20 day period, and no difference was noted between pumped and unpumped fish.

For additional testing efficacy, 2 other species (Arlee Rainbow Trout and McBride Cutthroat) comprising 3 groups, were each pumped once. The size range of these 5,280 trout varied from 4 inches to 12 inches. Five fish died during the following 20 days, and it was doubtful if this was due to pumping. Pumped and unpumped fish were examined for evidence of descaling and, again, little to none was found.

The U.S.F.W.S. Vessel "Togue" is being refitted this Winter (88/89) as a live hauler. Next Spring Lake Trout fingerlings will be pumped to a depth of 32 feet, . . . 3 ft. off an ideal spawning reef. It is hoped this will give rise to

better imprinting. A remote camera-equipped submarine will be used for documentation.

In June of 1988, another U.S.F.W.S. evaluation of a Transvac Pump took place at the Ennis National Fish Hatchery in Montana. In less than 3 hrs. actual pumping time, the 8 in. Model 3310 pump moved about 17,000 Rainbow Trout weighing 21,000 lbs., from 8 raceways into 10 other raceways. The weight of the fish ranged from 0.5 lbs. to 10 lbs. each. The four strains of rainbow trout; McConaughy, Eagle Lake, Erwin and Arlee involved in the test, handled the vacuum system equally well. There was no post mortality and no visible scale loss.

It should be mentioned here that no matter how well designed a fish pump is, the potential for stress on the fish is generally improper crowding technique. It's been found that those most familiar with the fish on a daily basis are best at crowding those same fish to a pump. Keeping the fish in a comfortable cushion of water, just before and during pumping, is a key lesson. Likewise, attention should be given to the discharge end of the hose, the outflow of fish and water providing that same cushion.

As a fish pump is often the common link from site to site, the possibility of disease conveyance can be reduced by disinfection. Placing the nozzle and the discharge end of hose in a common disinfection "bath" and running the pump for 10 mins. works effectively.

The third and very challenging area of fish pumping, is fish passage-ways at dams. Government relicensing requirements for dam operation have become more environmentally stringent. Standards now seem to use fish as a benchmark of water quality, . . . and of course, the public wants cleaner water, bigger fish and more of them.

Retrofitting older dams with fish ladders is difficult. Recently a fish

pump was installed in New England on the face of a dam in lieu of building a fish ladder. The pump's power source and controls are located remotely. Several pick up collection weirs, designed by Lakeside Engineering of Mirror Lake, N.H., placed strategically along the tailrace, will tie into the main suction line. The pump will then transfer fish to a sorting station for separation of desired and undesired species, . . . for upriver planting or downriver return.

Fish pumps, either as a permanent structure at a dam, or simply used as a tool for sampling fish populations (validating pop. models) can now provide a workable adjunct to fish passage-ways at dams.

Recently a video-imaging inventory system built by Pisces Computing, Ltd. has been tested in tandem with fish pumps manufactured by Innovac Technology. This inventory system also provides a bio-mass measurement of the fish for weight and size distribution. Obtained data is screen or LCD displayed in real time, or by computer printout. For actual review of fish passage, storage on video tape is possible. Information on this system used in conjunction with fish pumps is forthcoming.

Currently in the planning stages, is a study with the National Marine Fisheries Services to pump PIT (Passive Integrated Transponder) tagged fish through a wet vacuum pump. Tagged fish will be decoded by a tunnel detector system on the discharge side of the pump, and of course, be released live.

Now that fish can be pumped fast, virtually stress-free, and in the near future inspected and debriefed all at the same time, . . . what's left? Fish waste and sediment. This can be pumped too.

Maintaining quality of source, rearing, and effluent water at hatcheries is crucial to the higher densities fish are being raised in today. The "firehose"



technique of flushing offal and inorganic sediment through effluent ponds into a watershed is no longer an option.

Depending on the design of a fish pump, some can pump solids in suspension as well. For example, the State of Minnesota has a system that will go into operation next spring (89). It will use a continual flow wet vacuum pump for cleaning raceways and effluent ponds of fish waste, sediment, and aquatic plants. The "saved" material will then be shunted through a buried 4 in. PVC manifold hundreds of feet to a retention pond. After settling, it will be repumped into a dumptruck, for eventual use as a fertilizing agent.

In Michigan, sand from source water hatchery ponds has been removed with wet vacuum fish pumps. In other areas drilling mud, cranberries, and pickles have been pumped as well.

A successful fish transfer system is generally site-specific, consequently advance research is paramount. While I have referred to wet vacuum technology, other types of fish pumps in the area should be addressed. These other systems use impellers, an airlift technology and an archimedes screw. In the research wing of Innovac Technology, headway is being made on continual flow "tankless" pumps.

Likewise, ongoing research on fish attraction and fish nozzle design calls for combined input from fisheries biologists and those involved in the manufacturing of fish transfer equipment.

Hopefully as our efforts continue to dovetail, the fish will be the ultimate winner.

Ted Palmer 12/7/88



## A FRESH LOOK AT EGG DEVELOPMENT

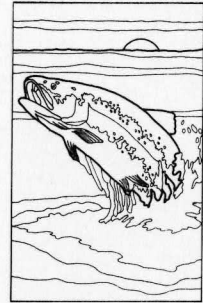
John Riger  
Colorado Division of Wildlife  
Crystal River Broodfish Hatchery  
2957 Highway 133  
Carbondale, CO 81623

Presented by:  
Peter Brown  
B.C. Recreational Fisheries Branch

A series of slides was shown which illustrated various stages of egg development from prefertilization to hatched alevins. A description of the technique that was used to take the photographs was also given and is outlined as follows:

- a close-up lens was constructed, starting with a Canon 50mm Macro lens with the automatic mode by-passed, i.e. it became a manual lens.
- this unit was taped onto a piece of PVC pipe; 12" for low magnification, 18-24" for higher magnification. This pipe/lens unit was taped onto an Olympus camera body. The tube had to be connected at about a 5 degree angle to the camera body. All joints were carefully wrapped to prevent any light intrusion.
- this unit was mounted on a tripod.
- 75 watt 'hot lights' were set up for lighting.
- eggs at various stages of development were placed in a 50% solution of formalin and water (i.e. 1:1) for one week.
- after removal from the solution, the shell was carefully removed.
- the egg was set on a microscope slide, placed in a petri dish, then flooded with water. A black felt background was used for contrast.
- the egg was then focused in with the aperture wide open for perfect focussing. After focussing, the aperture was closed to F32.
- all lights in the room were turned off, the shutter was opened and the camera was left for several seconds to ensure stillness.
- the lights were then turned on until the proper exposure was made. This was automatically done by the camera. Generally exposures were 15-30 seconds in length.

## Fish Health



CHILLS (Chinook Lateral Line Syndrome)  
at Lyons Ferry Hatchery, Washington

by

Patrick F. Chapman  
Department of Fisheries

Few people currently working in aquaculture or fish health were working in the field in the early 1960's and consequently CHILLS (Chinook Lateral Line Syndrome) is not a fish disease most of us are familiar with. During 1963, CHILLS was a disease seen in most chinook hatcheries in Washington and resulted in significant mortality. Hatchery personnel and fish health specialists became intimately familiar with the disease that year, but CHILLS wasn't noted in subsequent years and so it has become known as "ancient history" to current fishery workers.

Little published information exists about CHILLS. The original description of the disease is in a paper by Parisot, Yasutake and Klontz (1965) on virus diseases of western salmonids and the only other known reference to CHILLS is a half-page description in Diseases of Pacific Salmon (Wood, 1968). Subsequent editions of this book also contain a section on CHILLS.

According to these references, CHILLS was first seen in fall chinook salmon at Washington Department of Fisheries' (WDF) Skagit Hatchery in 1962. In 1963, chinook in all but one WDF hatchery and in eight U.S. Fish and Wildlife Service hatcheries experienced CHILLS which was characterized by hemorrhaging of the muscle in the area of the lateral line, pale gills and mortality elevated to as great as 2% per day. Intensive investigation failed to identify a definite cause of CHILLS, although its occurrence

correlated well with the feeding of raw salmon viscera to fish that eventually were diagnosed with the disease. A virus was isolated from affected fish as well as from raw viscera used in the diet, but laboratory challenges could not show that it caused CHILLS. The disappearance of the disease in years following 1963 coincided with the elimination of raw salmon viscera from the diet.

To the surprise of everyone, CHILLS reappeared in 1985 when it was diagnosed by Jim Wood in fall chinook yearlings at Lyons Ferry Hatchery in February. Lyons Ferry Hatchery had only recently been constructed on the banks of the Snake River approximately 10 miles downstream from Little Goose Dam in southeastern Washington. Supplied with near constant 12°C well water, the hatchery rears spring and fall chinook salmon, steelhead and rainbow trout and is jointly operated by Washington Department of Fisheries and Washington Department of Wildlife.

Fall chinook with CHILLS at Lyons Ferry in 1985 were larger than those having CHILLS in 1963 and ranged between 23 and 45 grams (10-20/lb). Signs of the disease were similar to those seen during the 1963 cases, but additional signs also were noted, including fluid filled stomachs, enlarged spleens and hemorrhage and congestion of the heart. Red blood cells from fish affected with CHILLS often were observed to contain cytoplasmic inclusions (a condition currently termed erythrocytic inclusion body syndrome, or EIBS). Mortality was not severe and peaked in individual ponds at approximately 0.35% weekly. Elevated mortality occurred for approximately 2 months, resulting in total mortality of about 1.3%

Histological examination of tissues from fish with CHILLS showed

hemorrhage, congestion and degeneration of lateral or "red" muscle with "white" skeletal muscle only mildly affected. Similar pathology was observed in the myocardium.

A number of people became involved that year in the investigation of CHILLS in an effort to determine the cause including Jim Wood and Kathy Hopper (WDF), John Morrison and Charlie Smith (U.S. Fish and Wildlife Service) and Jim Winton (Oregon State University). The case was particularly baffling in light of the fact that raw salmon viscera, which had been implicated in the 1963 cases, had not been used since that year and fish cultural practices at Lyons Ferry were similar to those used at other WDF hatcheries which were not experiencing CHILLS.

Bacterial pathogens could not be isolated, although bacteria were observed in blood films of some fish with CHILLS. Viral assays on routine fish cell lines of several tissues from fish with CHILLS were negative. Examination by electron microscopy of three fish with CHILLS, however, revealed a packet of virus-like particles in the heart tissue of one fish. Its significance was unclear, however.

Transmission studies were inconclusive. Jim Winton injected kidney homogenate taken from fish with CHILLS into fall chinook fingerlings and held these fish for 21 days. Injected fish developed EIBS and viral particles were observed in red blood cells from these fish, but CHILLS was never observed. Unfortunately, blood from fish affected with CHILLS was not examined by electron microscopy, so it can only be assumed that virus was present.

CHILLS reportedly was present again in fall chinook yearlings at Lyons Ferry in February, 1986 but the case was not investigated beyond

noting its occurrence.

CHILLS was not seen again at Lyons Ferry Hatchery until September, 1988 when it was diagnosed in fall chinook yearling ranging between 13 and 18 grams (25-35/lb). Previously described signs were observed in affected fish, but pale, yellowish livers were also noted. Hematocrits and serum protein values of CHILLS affected fish were always less than 10% and 2.3 g/dL, respectively. Furthermore, it was observed that 1) the occurrence of CHILLS in mortalities from a particular pond always followed the diagnosis of EIBS in fish from that pond by three weeks, 2) following the diagnosis of EIBS in fish from a pond, average hematocrit for that population declined to a low of 24% at which time cytoplasmic inclusions were no longer observed and hematocrits recovered to normal, 3) fish with CHILLS always contained at least some red blood cells with cytoplasmic inclusions, 4) CHILLS could be found in mortalities from a pond for only three weeks, 5) CHILLS was only observed in dead or dying fish and never from randomly selected "healthy" fish, 6) once EIBS could no longer be found in fish from the pond, CHILLS also could no longer be found in mortalities from that pond.

Mortality to CHILLS in 1988 has been similar to that seen in 1985, peaking in the most severely affected ponds at 0.45% weekly. At the time of this writing, some ponds are still experiencing loss of fish with CHILLS, but total mortality will be approximately 1.5% for the whole hatchery. Elevated mortality lasted as long as 12 weeks in some ponds but only 4 weeks in others.

The cause of CHILLS has yet to be determined. Again in 1988, bacterial and viral assays were negative and the only factor that appeared

to correlate with the occurrence of CHILLS was the presence of EIBS. However, other hatchery populations of chinook salmon have been observed to have EIBS but not CHILLS.

Ongoing investigations of CHILLS include further electron microscopy of tissues and blood, cell culture assays in untested cell lines and transmission studies.

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Aknowledgement: Current fish health monitoring at Lyons Ferry Hatchery is funded by Bonneville Power Administration's Augumented Fish Health Monitoring Program. BPA Contract DE-A179-86 BP3461.

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## **IHN VIRUS AT DWORSHAK NATIONAL FISH HATCHERY - PAST AND FUTURE -**

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### **INTRODUCTION**

Dworshak National Fish Hatchery is located below Dworshak Dam on the North Fork of the Clearwater River in north central Idaho. Water for the hatchery is pumped from the river. Anadromous brood fish, both steelhead trout and spring chinook salmon, are found in the vicinity of the hatchery water intake. The river supply is suspect for carrying Infectious Hematopoietic Necrosis (IHN); a viral disease that is causing devastating losses to steelhead production in the hatchery.

Dworshak's steelhead program has consistently experienced high losses to IHN virus since first discovered in young steelhead in 1982. Yearly losses have ranged from 25 to 98 percent, totaling 15 million fish from 37 million eggs taken. Another 8.6 million eggs, from positive IHN parents, have been destroyed. Dworshak, alone, has initially started 18 million steelhead fry in nursery tanks and taken a loss of 11.8 million over a seven year period, or 65 percent of the total. Kooskia National Fish Hatchery, operating as a complex with Dworshak, returned 15.8 million fingerling steelhead during this same period from eyed eggs received earlier from Dworshak production. This move to manage around IHN, begun in 1982, became a routine operating procedure in succeeding years resulting from Kooskia's successful rearing on well water in absence of IHN. However, of this 15.8 million fingerling returned at 250 per pound size, 3.25 million were later lost to IHN virus after being exposed to Dworshak's river supply.

### **HISTORY**

#### Brood Year 1982

IHN virus confirmed for the first time in returning adult steelhead. A total of 3.8 million fry was started with a 48 percent loss in production in June through August from IHN. Kooskia hatchery received 1.2 million eyed eggs. These fish were returned as 2-inch fingerling to supplement losses at Dworshak; no virus problems at Kooskia. Early fry (800 per pound) returned as a test group from Kooskia suffered high losses; at 300 per pound survival was excellent. Losses in Dworshak's nursery production highly evident in young steelhead 1.5 to 2.0 inches in length.

#### Brood Year 1983

Broodstock culling was initiated on a limited basis sampling only females. Samples showed an 80 to 90 percent positive incidence. Early collection of 150 adult steelhead (November/December) indicated 100 percent incidence of IHN virus from extended holding until fish were spawned in late January.



Some 370,000 eggs were destroyed from positive IHN parents. The hatchery recorded a 98 percent loss (3.3 million) in steelhead hatched and early reared. Heavy losses were in the range of 1.5 to 2.0 inches. Kooskia successfully reared 2.2 million young steelhead for return to Dworshak. Losses in this group were only 15 percent to the virus at Dworshak.

Also, in 1983, rainbow trout production experienced high IHN losses.

- small fry, 1 1/2" to 2 1/2" = 100%
- large fingerlings, 3" to 4" = 30%
- yearlings, 7" to 13" = 10%

Rainbow production was destined for release in Dworshak Reservoir (hatchery's water supply). Distribution was cancelled and rainbow in succeeding years were furnished from elsewhere.

#### Brood Year 1984

Full-scale broodstock sampling of all males and females for IHN initiated. Thirty-seven percent of steelhead eggs or 3.7 million were culled as IHN positive and destroyed. Extensive use of iodine as a disinfectant throughout the spawning procedures was initiated and established sanitation practices were followed. Dworshak's production incurred a 68 percent or 2.6 million fish loss. Kooskia received 2.6 million eyed eggs and returned 2-inch fish to Dworshak where they were successfully reared to smolt size.

#### Brood Year 1985

Losses to IHN virus were 65 percent or 1.2 million. Kooskia's program continued to perform well upon return as young steelhead from eyed eggs earlier received from Dworshak; only 10 percent loss to the virus at Dworshak. Initial ozone testing was 100 percent successful with control fish suffering high losses to IHN; test groups with no mortality.

#### Brood Year 1986

Broodstock culling procedures and water hardening of eggs in 100 mg/l iodophore treatment continued to be followed. Individual colanders for incubation expanded. Dworshak's eyed egg production was reduced to less than 1 million. Kooskia's program was increased to 3.4 million eggs (based upon success in an IHN-free water supply) for a later return as fingerlings to Dworshak to meet a planned 2.3 million steelhead smolt release.

Dworshak's nursery rearing experienced reduced losses (15%) to IHN virus. Kooskia's returning fingerlings into Dworshak's river water supply have a loss of 35 percent (1.1 million fish) at a size ranging from 2.5 to 3.5 inches.

#### Brood Year 1987

Incidence of IHN virus in steelhead adults sampled showed only 6 percent; a considerable drop from previous years of 15 to 25 percent. Dworshak's early rearing program again was reduced to 1.2 million. Only 10 percent

were lost to the virus. Kooskia received 3.7 million eyed eggs and upon return to Dworshak, at 2-inch size (250/lb), lost 1.1 million (30 percent) to IHN in the river water supply. Six weeks (40 to 44 days) after transfer into Dworshak, losses appeared in the Kooskia group.

#### Brood Year 1988

Incidence of IHN virus in all brood fish sampled was again low, only 4.5 percent. Dworshak began production with 3.6 million feeding fry on station. Seventy-five percent (75%) or 2.6 million fingerling were lost to the virus as of October 1, 1988 (4 to 5 month production period) leaving only 900,000 fish on station. Kooskia hatchery returned 500,000 fingerling in mid-summer to Dworshak and recorded a 12 percent or 50,000 loss in fish at 3.0 to 3.5 inches. Sixty-five percent of Dworshak's production was lost in nursery rearing; another 10 percent to the disease after outside ponding. Five of 128 nursery tanks holding young steelhead escaped the deadly virus---no explanation as to why these fish were immune to the virus.

Despite high losses since 1982 in steelhead production from IHN virus, yearly smolt release in 1983 through 1987 were maintained at mitigation levels averaging 2.3 million fish weighing 400,000 pounds. Spring 1989 smolt releases, however, will be reduced by 1 million fish as a result of IHN losses in Dworshak's Brood Year 1988 production.

#### OBSERVATIONS

High losses in steelhead production at Dworshak when fish are reared on a river water supply without treatment.

No IHN virus found at Kooskia in 7 years of receiving negative IHN eyed eggs from Dworshak for rearing to 2-inches and return.

Expect a 25 to 35 percent loss in young steelhead returned from Kooskia to Dworshak in late spring. Losses are reduced the longer fish are held at Kooskia before returning.

Losses in fingerling steelhead returned at 250 per pound from Kooskia's well water to Dworshak's river water supply experience IHN losses 6 weeks after exposure at 54°F.

No relationship seen between varied fish loadings in degree of IHN incidence.

Survivors of an IHN epizootic do not experience further losses to the virus.

Extended holding of steelhead broodstock, beyond 4 weeks, increases IHN incidence.

Fewer numbers of steelhead started at Dworshak--less percent IHN mortality.

No losses to IHN in spring chinook production reared on station with steelhead trout.

Late arriving brood fish show increased incidence of IHN virus---75 to 100 percent.

Cross contamination with equipment, moribund fish, and water, to a "clean" tank has not expressed IHN.

Healthy steelhead from nursery rearing tanks, held in floating baskets in Burrows (circular) outside ponds showing high IHN losses, did not contact the virus.

Nursery tanks of steelhead at various locations in building, having same egg take, water supply, treatment and handling, escape the virus while similar groups, side-by-side in rearing, are destroyed.

Incidence of IHN virus in adult broodstock lower in recent years (5%) when compared to a much higher incidence in earlier years.

Ozone is an effective treatment on a single-pass water flow.

Steelhead between 1.5 and 2.0 inches are highly susceptible to IHN virus in Dworshak's untreated river supply.

Destroying steelhead eggs from positive IHN parents not shown to be effective at Dworshak.

What works well in the laboratory may not be true on a production basis.

#### METHODS OF CONTROL

##### Broodstock Culling

The practice of culling IHN and destroying infected steelhead eggs began at Dworshak in 1983 to attempt to reduce vertical transmission of IHN through the hatchery's water supply. Culling procedures are performed by first tagging individual adults with numbered stainless steel pins. This facilitates laboratory personnel in identifying resulting egg lots as to IHN disposition for culling. Females are spawned individually with one male to one female crosses, whenever possible, and fish health samples collected. Sampling consists of examination of spleens from all males and ovarian fluid from females with exception of Brood Year 1983 when only females were sampled. Egg lots are water hardened in iodophore (100 mg/l) for one hour and incubated individually in numbered colanders supplied with single-pass raw water. After approximately 8 days, viral cell culture analysis of fish tissue samples are received and IHN+ egg lots are destroyed. Aseptic procedures are followed during the entire operation to prevent possible cross contamination between adults and egg lots.

Table 1 shows the historic summary of IHN virus incidence in sampled adult steelhead and, also, total egg numbers destroyed. As seen, IHN incidence increases in later egg takes. The high incidence in 1983 and 1985 early egg takes involved adults held in Dworshak's holding facilities for an extended period of time.

Extended holding of adult steelhead shows a direct relationship to IHN incidence as seen in 1986. Adults entering the hatchery in excess of spawning requirements were held for a University of Idaho research project. IHN sampling revealed a nearly eight-fold increase in virus prevalence of adults held for one to nearly four weeks. Holding adults for even short periods of time apparently increases the potential for spreading IHN.

#### Vaccination

One field trial conducted by Oregon State University (Jo-Ann Leong) involved use of a subunit vaccine. The trial, initiated May 26, 1988 at Kooskia, vaccinated by immersion 200,000 steelhead fingerlings. Controls (200,000) were segregated and received no treatment. Test fish were returned to Dworshak on June 23 and held in eight nursery tanks (4 vaccinated, 4 control at 5,000 each). Artificial challenges were made on July 7 involving one set of control and vaccinated tanks by adding moribund fish from a nursery tank experiencing an IHN epizootic together with an addition of 100 ml of virus or 200 pfu/ml final concentration. Tank mortality was recorded daily until movement to outside Burrows ponds on September 3. Percent mortality ranged from 1-4 percent; not characteristic of epizootic conditions.

In 1987, researchers from Seattle National Fisheries Research Center (SNFRC), assisted by hatchery personnel, used a newly developed killed (formalin) whole virus vaccine to immunize juvenile steelhead resident in Dworshak's ozone treated water supplied to nursery tanks. Administering of the vaccine was performed in two methods: (1) interperitoneal injection, and immersion in hyperosmotic salt solution containing killed virus. Siblings used as controls were (1) injected with saline only, (2) immersed in saline only, or (3) handled but not injected or immersed. Isolation of all individual groups was accomplished in ozone treated tanks until August 14 then moved to outside ponds. Analysis for IHN incidence for all groups was negative. Efforts to infect test fish, by placing subsamples of 100 fish from each group in floating baskets suspended in ponds with an active IHN epizootic, were unsuccessful.

#### Ozone Treatment

The U.S. Army Corps of Engineers purchased a package treatment system consisting of 4.75 pounds per day generator, air prep and contractor from U.S. Ozonair, San Francisco, California. A pilot study in 1984 was set up using four 6-foot diameter circular tanks for fish rearing. One stack of incubator trays was plumbed to receive ozonated water. A 1200 gallon detention tank was located on the building roof for gravity flow of the ozonated water to the fish and eggs. Packed columns were installed prior to the fish and eggs. Total flow in the system was 90 gallon per minute.

Each fish rearing tank received 20 gallons per minute and the incubator stack was set for 10 gallons per minute. The study was terminated after four months of mechanical failures in the ozone equipment. The only results were (1) packed columns were shown effective in stripping ozone from the water, and (2) rainbow trout could tolerate higher levels of ozone in the water than had been reported. Fish appeared to do well in water with 0.02 to 0.03 ppm ozone.

A joint study in 1985 by the Corps of Engineers, Idaho Department of Fish and Game (IDFG) and U.S. Fish and Wildlife Service (USFWS) was initiated to find a safe, reliable water sterilization system for controlling IHN. Ozone was selected based on work done at SNFRC. James M. Montgomery, consulting engineers, was awarded a contract to design and install a 600 gpm ozone system. The study was set for six months and was to preclude design of the new IDFG Clearwater hatchery. Two distinct water treatments were tested in the study. The ozone treated system received 60 gpm and the untreated control received 600 gpm. Both the control and the ozone study groups were operated on single-pass operation. Ozone generators were supplied by Emery Industries, Inc. Each generator supplied 10 pounds per day of ozone using a liquid oxygen system. A liquid oxygen system was used during the study in place of compressors. The ozonated water from the contactor was deoxygenated using packed columns, located at the pump sump. The packed columns removed between 75-90 percent of the residual ozone. Once through the packed columns, water was pumped to two existing bioreactors for detention time. The pump sump and bioreactors were part of the existing nursery reuse system. Each bioreactor offered a 36 minute detention period for the ozonated water. Test results were very positive despite numerous power outages and mechanical problems. Eleven of the 14 control tanks were destroyed due to the IHN virus. None of the 14 ozone tanks were affected.

In 1986, an exact replica of the 1985 study was noted. Compressors were used for the air supply to the ozone generators instead of liquid oxygen; liquid oxygen being quite costly. After a week of operation, the ozone generators started losing dielectrics. It was discovered that the air dryers for the compressors were not sized properly and were allowing moisture into the ozone generators. One month after initial start up, the study was terminated because of equipment problems. The dielectrics and the air dryers were later replaced and tested with good results.

In 1987, a repeat of the 1985 study was performed. Equipment operated fine throughout the study. This was the year that IHN did not cause severe mortality in the nursery tanks. All of the ozone fish survived along with the control group. The only results were that the ozone system operated perfectly.

A decision was made to try ozone with reuse in 1988 after experiencing good success in 1987 testing. The combination had possibilities of maintaining all early rearing at Dworshak. The reuse system at Kooskia NFH, with well water, had produced excellent results since 1982. The ozone/reuse system started out without problem. However, the reuse water was later found to create too much of a demand and it was difficult to keep a desired residual



of ozone in the system. A month into the study, the main electrical supply line to the hatchery faulted and all power was lost. The reuse system was exposed to raw untreated water for the second time; a motor failure on a supply pump created the first raw water exposure. The reuse ozone study was terminated after two months with similar IHN losses as the control group due to raw water exposure and an apparent over-estimated ozone demand. It is believed that if enough ozone could be generated to offset the reuse ozone demand and leave a low residual of ozone in the system, the system would offer protection from IHN virus.

#### Iodophore Treatment

Proven an effective disinfectant, use of iodophore treatment has been common in fish culture activities for years. Extensive use of iodophore began in 1984 to facilitate culling operation and to limit accidental cross contamination of gametes with IHN. The aseptic procedures employed are now standard protocol during spawning, incubation, and culling of IHN positive egg lots.

Procedures include disinfection of fish handlers' hands in iodophore (250 mg/l) solution and disinfection of all equipment used in a 500 mg/l solution between uses. Both males and females are swabbed with iodophore (250/mg/l) before collection of gametes. Water hardening of individual egg lots in 100 mg/l iodophore for one hour is also performed. Use of iodophore in disinfecting equipment used for nursery and outdoor pond rearing is also employed when rearing units lack individually assigned nets, brushes, etc.

#### **FUTURE**

In 1989, the hatchery will initiate early rearing of all steelhead at other locations having an IHN-free water supply. Return of these fish will be at a size and time when IHN losses are expected to be lower. Dworshak will not hatch eggs and early rear steelhead when high IHN virus losses normally would occur in the hatchery. Breaking the early virus cycle may prove highly beneficial towards fewer losses in fish returned to Dworshak at a later date; the host species having been removed.

Iodine will be tested on a flow-through basis for the first time at Dworshak. A pilot system consisting of a chemical metering pump, flow meter, pump control and iodine batch tank will be tested on a 150 gallon per minute single-pass flow. A dosage rate will be maintained for a residual iodine level of 0.3 to 0.5 ppm in the fish tanks. The system will be tested without fish for operational problems for 2 weeks. After operational testing, rainbow trout will be introduced for one month. Fish will be observed and examined histologically for adverse effects of iodine. Upon successfully rearing rainbow trout, steelhead will be tested from eggs taken in the latter part of the run. These fish will be reared to ponding size of 250 fish per pound following the same rearing regime as with ozone testing. There will be a control group for both the rainbow and steelhead trout. Water temperature will be 54°F. Iodine for controlling IHN is currently being studied and tested at the SNFRC. Various iodine levels in

different types of water have been used. Results are very positive and support the efforts for testing on a production basis at Dworshak. Iodine has been used successfully by a commercial trout grower in the Hagerman valley. It was used to treat bacterial gill and found to be very effective. It was later used to treat IHN and again results were favorable.

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The 1985 ozone study will be repeated. Single-pass ozonated water (600 gallons per minute) will be used in the nursery rearing. Further testing of ozone on IHN virus will continue for possible later incorporation on a production scale, if necessary.

A 6400 gpm water supply furnished from Dworshak Reservoir, as part of the new Clearwater hatchery Lower Snake River Compensation Plan design, is planned for the Dworshak hatchery. This "clean" supply, in absence of suspected IHNV carriers (spring chinook and steelhead trout anadromous fish), should be highly beneficial to egg incubation and early nursery rearing; a segment of production that experiences high epizootic losses to IHN virus when untreated river water is pumped from below Dworshak Dam.

Table 1. Summary of IHN incidence in sampled adult steelhead, 1983-1988.

1983*				1984				1985				1986				1987				1988				TOTALS			
No.		%		No.		%		No.		%		No.		%		No.		%		No.		%		No.		%	
Date	No.	IHN+	IHN+	No.	IHN+	IHN+	IHN+	No.	IHN+	IHN+	IHN+	No.	IHN+	IHN+	IHN+	No.	IHN+	IHN+	IHN+	No.	IHN+	IHN+	IHN+	No.	IHN+	IHN+	IHN+
1/26	30	25	83.3	—	—	—	33.3	21	7	33.3	—	—	—	—	—	—	—	—	—	51	32	62.7	—	—	—	—	—
2/02	7	7	100.0	4	0	0	0	27	0	0	2	0	0	0	0	45	0	0	—	85	7	8.2	—	—	—	—	—
2/08	7	3	42.8	—	—	—	—	33	1	3.0	5	0	0	0	0	51	0	0	—	96	4	4.2	—	—	—	—	—
2/15	31	2	6.4	—	—	—	—	38	6	15.8	12	0	0	0	0	84	0	0	—	165	8	4.8	—	—	—	—	—
2/22	43	5	11.6	18	3	16.7	3	26	1	3.8	25	0	0	0	0	124	0	0	—	236	9	3.8	—	—	—	—	—
3/01	122	3	2.5	10	2	20.0	2	39	2	5.1	38	0	0	0	0	131	0	0	—	367	7	2.0	—	—	—	—	—
3/08	160	5	3.1	16	2	12.5	4	93	4	4.3	236	0	0	0	0	102	0	0	—	642	13	2.0	—	—	—	—	—
3/15	169	15	8.8	83	1	1.2	280	18	6.4	6.4	236	0	0	0	0	100	0	0	—	926	34	3.7	—	—	—	—	—
3/22	200	54	27.0	160	7	4.4	310	3	1.0	236	0	0	0	0	203	12	5.9	141	0	1250	76	6.1	—	—	—	—	—
3/29	186	35	18.8	253	13	5.1	245	8	3.3	263	1	0.4	215	29	13.5	276	1	0.4	1438	87	6.0	—	—	—	—	—	—
4/05	221	81	36.6	423	16	3.8	207	12	5.8	368	10	2.7	282	7	2.5	338	4	1.2	1839	130	7.1	—	—	—	—	—	—
4/12	145	32	22.1	397	23	5.8	472	37	7.8	271	20	7.4	251	10	4.0	371	3	0.8	1907	125	6.5	—	—	—	—	—	—
4/19	120	44	36.6	353	43	12.2	240	40	16.7	116	7	6.0	492	52	10.6	296	0	0	1616	187	11.6	—	—	—	—	—	—
4/26	190	117	61.5	434	83	19.1	304	124	40.8	130	16	12.3	240	32	13.3	260	2	0.8	1558	374	24.0	—	—	—	—	—	—
5/03	88	84	95.4	398	116	29.1	157	76	48.4	59	7	11.9	80	10	12.5	341	54	15.8	1123	347	30.9	—	—	—	—	—	—
5/10	17	17	100.0	444	181	40.8	—	—	—	—	—	—	—	—	—	40	11	27.5	649	249	38.4	—	—	—	—	—	—
5/17	—	—	—	36	9	25.0	—	—	—	—	—	—	—	—	—	—	—	—	—	36	9	25.0	—	—	—	—	—
FMS	1736	428	24.6	1537	311	20.2	1258	139	11.0	1073	28	2.6	1360	86	6.3	1499	56	3.7	8463	1048	12.4	—	—	—	—	—	—
MALES	—	—	—	1492	188	15.3	1234	200	16.2	924	33	3.6	1000	77	7.1	793	50	6.3	5523	548	9.9	—	—	—	—	—	—
	1736	428	24.6	3029	499	18.0	2492	339	13.6	1997	61	3.0	2440	163	6.7	2291	106	4.6	13985	1596	11.4	—	—	—	—	—	—
TOTAL NUMBER IHN+ EGGS DESTROYED																											
	367,638		3,614,964			1,788,000		1,097,133		754,000		1,014,548		8,636,283													

\*Females sampled only



Federal/Provincial (British Columbia) Policy for Transplant of Finfish  
within British Columbia in Relation to Fish Disease Concerns

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Unregulated transplants of fish between natural water bodies are of concern to fisheries management agencies because of the potentially harmful effects on existing fish populations in the receiving waters. Specifically these concerns include, but are not limited to, potential disease introduction and spread, competition between fish species, predation and genetic effects. Because disease information (absence or presence of organisms pathogenic to fish) is readily obtained through disease surveys of donor and recipient waters, it is the most frequently evaluated factor when adjudicating transplant requests. In B.C., transport and introduction of fish are controlled by both Provincial and Federal legislation. With the rapid growth of salmon farming on the B.C. coast the need to develop new guidelines governing the movement of fish among facilities in a way that would reduce the risk of disease transfer was soon evident.

To meet this need, the Federal/Provincial Transplant Committee, which adjudicates on transplants, drafted guidelines to serve the following objectives:

1. to reduce the risk of fish disease transfer from cultured fish to wild stocks;
2. to reduce the risk of fish disease transfer between culture facilities and;
3. to facilitate the movement of healthy stocks between culture facilities and for stocking natural waters.

To facilitate the application of these guidelines the province has been subdivided into nine zones (Appendix 1) mostly based on watersheds (or adjacent watersheds in the coastal zone). Within each zone a set of conditions have to be met for immediate transplant approval whereas more stringent conditions are required for transplants between zones (Appendix 2).

The basic considerations leading to the proposed conditions are:

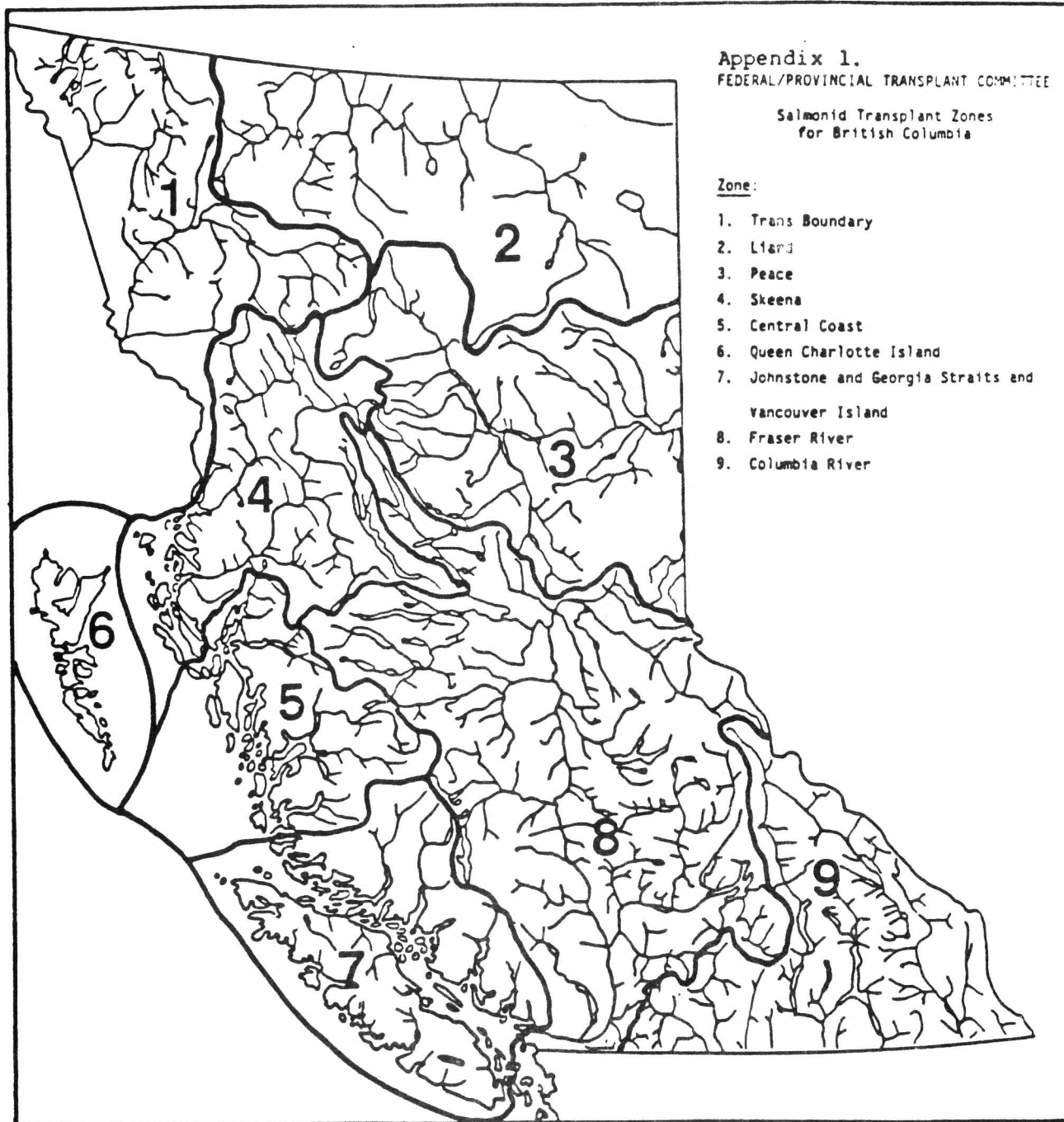
1. That screening of broodstock for diseases of which the causative agent is vertically transmitted will lower the risk of pathogen transmission via the egg. While screening is not 100% effective, due to lack of suitable diagnostic methods, disease transmission is reduced.
2. Knowing that causative agents for bacterial kidney disease, furunculosis and enteric redmouth disease are present at low prevalence in most of the wild stocks examined in B.C., transplants carrying the pathogen are not likely to introduce the agent to new areas. For endemic disease agents it is the pathogen load that is considered to be critical in impacting on wild stocks in the area or to be of concern to nearby farms.
3. The emergency diseases (Appendix 3) include diseases and disease agents not known to occur in this area or non-treatable diseases (e.g. IHN).

Especially, the introduction of an exotic pathogen can be very deleterious to stocks. Transfer of stocks is therefore not permitted from facilities where such a pathogen has been found.

4. Losses of 1% per day for 4 consecutive days are considered very high. Although such losses may be due to environmental problems rather than fish disease, the Committee requests that the facility ensure that the losses are not due to a very virulent disease agent.

If all conditions are met, fish are free to be transported. Otherwise, special conditions (such as a health check of stocks prior to transplanting) are required and must be met or the application may be rejected entirely.

Based on: Proposed Transplant Policy and Guidelines for Finfish. A discussion paper prepared by the Federal/Provincial Transplant Committee, October 1988.



SCALE

20 0 20 40 60 80 100 200 miles

## Appendix 2. General Conditions for the Transport of Live Fish or Eggs.

1. The person transporting them must be the holder of a permit issued by the Transplant Committee that authorizes the transport of that species, alive.
2. The originating facility had received only screened eggs which were surface disinfected upon water hardening.
3. Mortalities in any stocks currently reared at the originating facility did not exceed 1% per day for any four-consecutive-day period.
4. The stock to be transported is not known to have clinical signs of bacterial kidney disease (BKD), furunculosis, or enteric redmouth disease (ERM).
5. The originating facility is not known to have any emergency diseases.

### Application

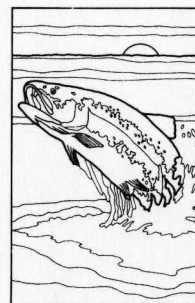
1. Within a zone, live fish or eggs may only be transferred if all general conditions have been met.
2. Between zones, live fish or eggs may only be transported if:
  - a) all general conditions have been met; and
  - b) fish have been reared exclusively on ground water, surface water free of fish or water treated in a manner to kill fish pathogens.

Appendix 3. List of Emergency Diseases and Disease Agents.

1. Infectious hematopoietic necrosis (IHN);
2. Infectious pancreatic necrosis (IPN);
3. Viral hemorrhagic septicemia (VHS);
4. Herpes virus salmonis;
5. Spring viremia of carp (SVC);
6. Oncorhynchus masou virus (OMV);
7. Any filtrable agent causing cytopathic effects in tissue culture other than above;
8. Whirling disease;
9. Hitra disease;
10. Other diseases of concern not listed above.

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# Chemicals, Drugs and Hormones



A NEW PROCESS FOR EGG HARDENING OF RAINBOW TROUT  
(SALMO GAIRDNERI) EGGS AND COMPARISONS OF RINSING  
AND EXTENDER SOLUTIONS.

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ABSTRACT

The effect of several compounds used to improve the fertilization rate and later survival of rainbow trout (*Salmo gairdneri*, Richardson) was investigated. Rinsing the eggs before fertilization with a 3% NaCl- 0.05% EDTA solution made it possible to achieve a good fertility rate when used with an extender solution composed of three ingredients: NaCl 6 g/L, 0.2 g/L CaCl<sub>2</sub> and 0.4 g/L B-glucose. Use of this extender solution resulted in a 10-15% increase in the number of eyed eggs.

Washing the eggs after fertilization firstly in Zn 0.75 mg/L, 0.025 g/L vitamin C and 0.2 g/L NaCl solution and following it in a similar solution except that instead of NaCl its contains CaCl<sub>2</sub> improved eyed egg survival. Utilization of the extender and washing the eggs resulted in a 5%-10 % increase of the number of eyed eggs.

Application of Zn 0.15 mg/L, 0.04 g/L CaCl<sub>2</sub> and 5 mg/L vitamin C solution for egg hardening resulted in a further increase of 5-10% in the number of eyed eggs.

Iodophor compounds must be tested in each hatchery for egg hardening before their routine application is accepted.

The possibility of increasing the number of eyed eggs by using selected minerals and vitamins as a drip treatment during incubation is discussed.

KEY: rainbow trout, egg fertilization enhancement, incubation.

INTRODUCTION.

Maintaining trout broodstock is labour-intensive, expensive and requires space and special feeding. The number of fry per kilogram of broodstock is an indicator of the economical success of the program. The number of egg per female can be increased by



feeding, but there is an upper limit physiologically as well as an economic limiting factor introduced by the cost of the feed and the consequences resulting from the intensive feeding. More eggs are produced by fish of smaller egg size, but mortality of fry from small eggs has been demonstrated to be significantly greater than of fry from large trout eggs (Kincaid 1970, Small 1978,).

The success of an aquaculture or a fish hatchery can be improved, however, with an increase in the percentage of successfully developing eggs. In many hatcheries and aquaculture operations worldwide the percentage of eyed eggs is between 35-55 %. The percentage of eyed eggs is a reliable measurement of operational success because, generally, the survival is about 90 % from that stage up to feeding.

The eyed egg percentage increases when the success of fertilization rate improves. The success of any economically productive, artificial insemination program depends upon maximum use of available gametes. The success of fertilization is influenced by several known and unknown environmental factors: feeding condition of the parents; water temperature; ion content (Hamor 1966, Hwang and Idler 1969) illumination; individual differences; agglutination of spermatozoa by autoantibodies; and for males, the number of spermiation (Buyukhatipoglu and Holtz 1984).

Because of a high concentration of sperm cells and the small semen volumes, fertilizing large numbers of eggs is often difficult with untreated semen. Diluting semen has long been investigated as a means of increasing the number of eggs that can be fertilized from a given volume of semen. Ellis and Jones (1939) reported that a diluted seawater activator suppressed fish sperm motility; they attributed this effect partly to osmotic pressure of the salt solution. When distilled water was used as an activator, maximum motility was reached for a short time, but morphological distortion (swelling) was very evident 20 second after activation (Guest 1973).

Pond water containing  $Ca^{++}$  ions is known to inhibit the permeability of the sperm cell membrane (Hoar 1969). Saline solutions have been used with success to enhance sperm motility and egg fertilization (Guest et al. 1976, Hamor 1969, Kafuku, Rieniets and Millard 1987, Woynarovich 1955, 1962). A field trial with rainbow trout semen showed that semen diluted in a seminal plasma-mimicking medium maintained control values of fertility after 30 minutes of storage. Interference from non-motile cells was insignificant (Erdahl and Graham 1987).

Variation in the  $K^{+}$  concentration in an artificial medium indicated that a high concentration ( $>6.8$  mM) decreased egg fertility, while a low concentration ( $<3.4$  mM) had no effect on fertility (Erdahl et. al 1987).

Billard (1978) reported that the duration of sperm motility is related directly to fertilization success for several fish species. Leitritz and Lewis (1976) suggested that salt in fertilization diluents increases the fertilization rate by keeping the proteins from broken eggs in solution and therefore keeping the micropyle from becoming clogged.

The developing embryo is not completely sealed from its

environment, but there is little evidence that material is absorbed from the water McCay (1936) and Tunison et. al. (1936) found that before the yolk sac of *Salvelinus fontinalis* is absorbed (that is, well before feeding starts), there is marked increase in calcium within the embryo. This must originate in the environment as Rudy and Potts (1969) showed that the eggs of *Salmo salar* absorbed calcium from water at a rate that increased as the egg matured.

The first successful results, where the rinsing of the eggs was done with a combination of a salt-sugar extender, was reported at the Canadian Aquaculture Conference in Quebec (Hamor 1987).

Information regarding the composition of water on egg survival is scanty, but detrimental effects caused by ammonia were noted by Charlie Smith of Fish Cultural Development Center, Bozeman, Montana a long time ago (unpublished report). Groberg (1987) reported that a one hour exposure of 125 ppm iodophor compound (Betadine) resulted in an effective level of treatment. It was also tested by Evelyn et al. (1986), and no detrimental effects was found on egg survival when it was used as a hardening solution.

#### MATERIALS AND METHODS.

Eggs and semen were collected originally from our second and third generation rainbow trout of Mount Lassen or Trout Lodge strains from California and Washington respectively. Eggs were collected by strainers, separating ovarian fluid. After the eggs were stripped from each female, they were either rinsed in salt solution or as controls, transferred into plastic or stainless steel pans. For every four or five female we use two or three male. The eggs and semen were stirred, then 0.5L of an extender solution was added and stirring continued for about one minute. Pond water, ovarian fluid or extender solution was added to control groups. The eggs were left undisturbed for five minutes in order to complete fertilization. They were then washed and collected into a large container and left one to two hours for hardening. Test lots were placed on trays that were divided into nine compartments, with about 9000 eggs on each tray.

Fertilization success was determined by measuring the eyed egg percentage.

Sperm movement was observed under a compound microscope and a stopwatch was used for timing the movement.

For addition of Zinc the 10 mg "Wampole Zinc Tablets" and for vitamin C the 500mg "Stanley Vitamine C Tablets" were used.

#### RESULTS.

The effect of some salts and sugars on sperm movement time were investigated in order to develop an efficient extender solution. Movement of sperm increased five-fold compared to

Figure 1.

### Effect of salt and sugar

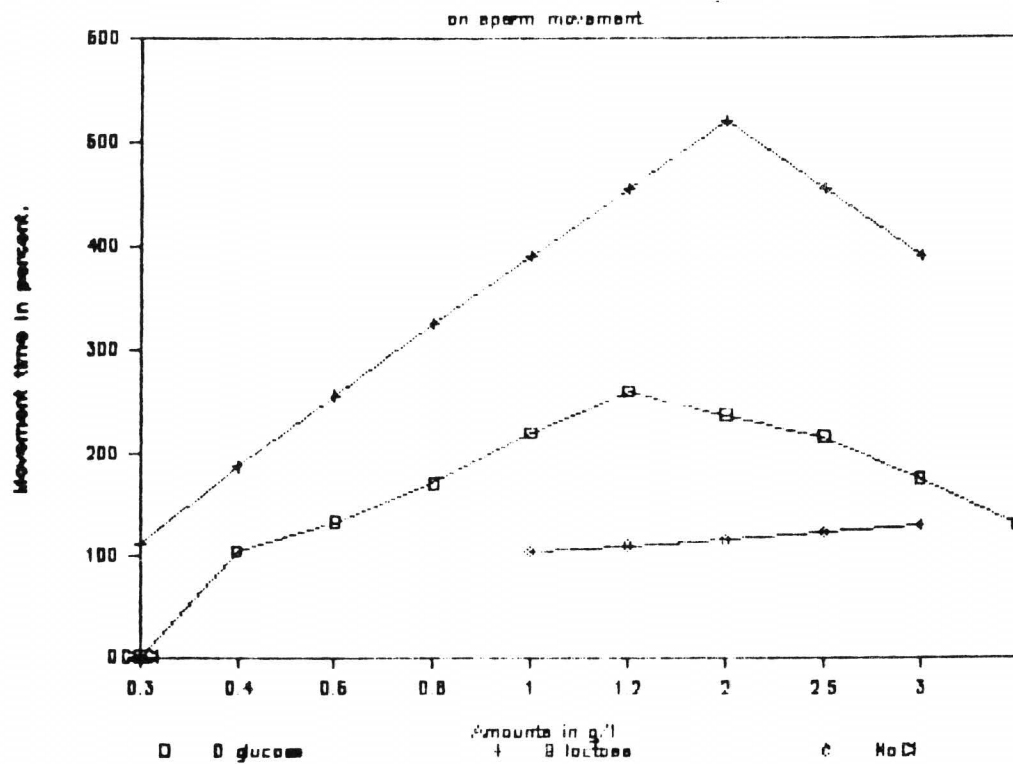
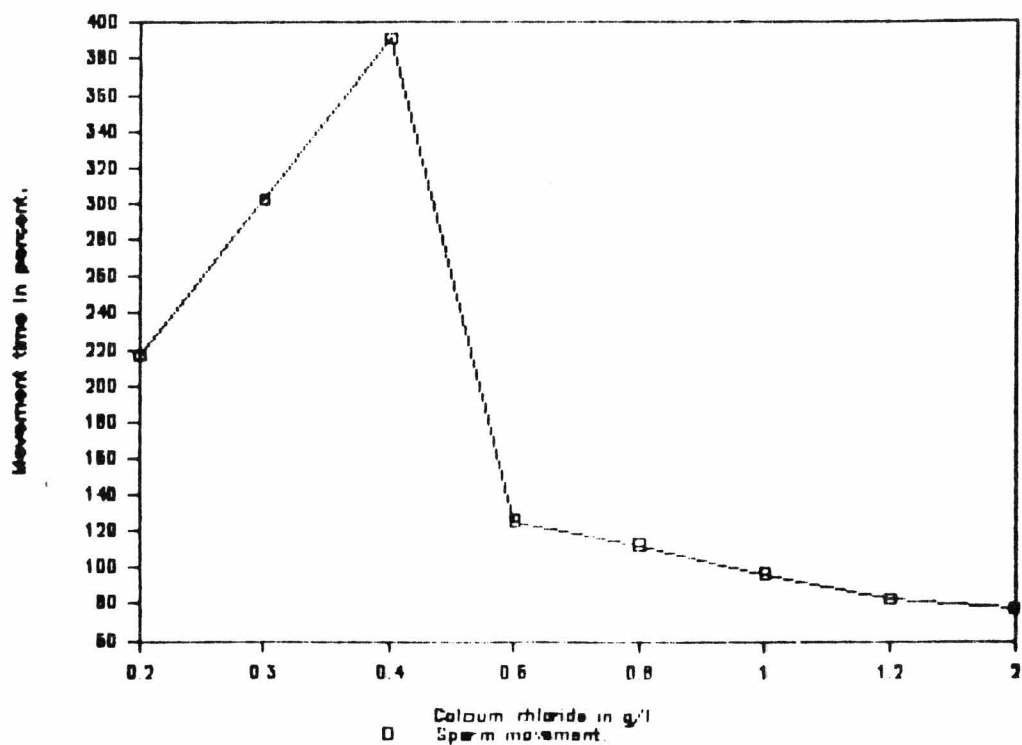


Figure 2.

### Effect of Calcium on sperm movement.



movement of control sperm in hatchery water with 2 g/L B-lactose added. Addition of greater or lesser amount of B-lactose was also found to be effective. The same was found when D-glucose, salt or calcium chloride was added (Figures 1 and 2). After testing several combinations, the following solution was found to increase sperm movement about ten folds:

6.0 g/L	NaCl
4.5 g/L	CO(NH <sub>2</sub> ) <sub>2</sub>
0.2 g/L	CaCl <sub>2</sub>

While comparing the effectiveness of salt solutions by counting the number of eyed eggs, it was found that the solution giving the best sperm movement did not necessarily give the highest percentage of eyed eggs.

The following combination was the most successful in our trials, and yielded the highest number of eyed eggs;

6.0 g/L	NaCl
0.2 g/l	CaCl <sub>2</sub>
0.4 g/l	B-lactose

This extender solution has been used in our hatcheries, but the improvement was much less than we expected. We did have a high success with several lots (75-95% eyed eggs), but a much lower success with others. More study is required.

In trials where semen was introduced into the pan first there was approximately a 3% increase in eyed eggs; another 3% increase was noted when the eggs and semen were mixed immediately (Dave DePape pers. comm.).

Surprisingly, a delicate mixing of sperm and eggs for one minute in the extender solution did not cause any damage to the eggs, but yielded a 5-10% increase in the number of eyed eggs. The use of an extender solution saturated with oxygen resulted in an additional increase of 5-10% of eyed eggs.

Examining temperature differences of the water as they affected the eggs, semen and extender solution did not yield any positive results. It was found that temperature differences of up to 6C between the water and extender solution unless the temperature exceeded 14-16C.

Water quality is an important factor. Water from different sources yielded markedly different results (Table 1).

Table 1.

Percent of eyed eggs using three different  
hatchery water for sperm activation.

Origin of male and female spawned.	Origin of hatchery water.		
	Raven Brood Station.	Sam Livingston Fish Hatchery.	Allison Station.
Brood	Percent of eyed eggs.		
Mount Lassen	41	48	60
Mount Lassen	54	68	69
Mount Lassen	75	77	76
Trout Lodge	35	41	45
Trout Lodge	40	43	52
Trout Lodge	23	55	60
Mean	44	55	60
S.D.	17.12	14.43	11.18

The differences within the percentage of eyed eggs are significant when the Wilcoxon's signed rank test is used. Parametric tests are unsuitable because the various egg qualities mask the effects of water quality. The difference in water is more pronounced between Raven and Allison, or Raven and Sam Livingston, than it is between that from Sam Livingston and Allison. This shows that water quality can be a very significant factor in the success or failure of fertilization. Distilled or de-ionized water proved to be better than hatchery water for the extender solution.

It was found that the success percentage is influenced by the solutions used for rinsing the eggs. Some of the results are presented in Table 2.

Table 2.

Percent of eyed eggs using several rinsing agents.

Rinsing solution.			Combination of solutions.							
NaCl	3.0 %	+	-	-	+	-	-	-	+	-
Urea	0.5 %	+	+	-	-	-	-	-	-	-
Protease	0.01%	+	-	+	-	-	-	-	-	-
Alcohol	10.0 %	-	-	-	-	+	-	-	-	-
MgSO4	3.0 %	-	-	-	-	-	+	-	+	-
Acetic acid		-	-	-	-	-	-	+	-	-
Eyed eggs %		71	54	9	72	9	19	22	63	66

Several more solutions were tested and compared. A 3% NaCl solution with 0.5% EDTA (Ethylene diamine tetra acetic acid) was used for rinsing the eggs. This gave a significantly higher percentage of success than did other solutions. The most important trials are summarized in Table 3;

Table 3.

Success of fertilization expressed as percent of eyed eggs using different combination of extender solution and rinsing processes.

Group.	Mean	S.D.	P	N
1/ Rinse, extender (dist. water)	78.1	8.7	0.001	40
2/ Rinse. extender(hatchery water)	72.4	7.4	0.05	40
3/ Eggs rinsed with NaCl	51.1	12.00	0.05	40
4/ Eggs rinsed with NaCl + EDTA	58.9	11.1	0.05	40
5/ Control;no rinse, no extender	52.7	11.9	0.01	40

T-tests showed significant differences within all but the third and fifth group. There is a distinct advantage if distilled

Figure 3.  
Success of fertilization.

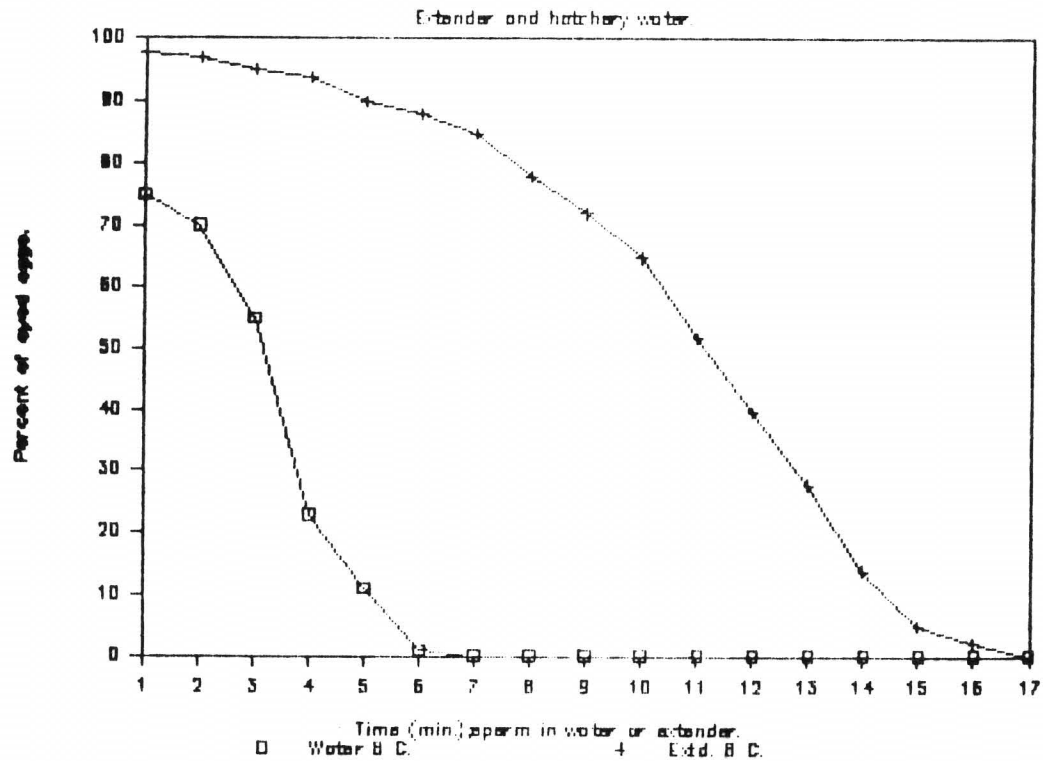
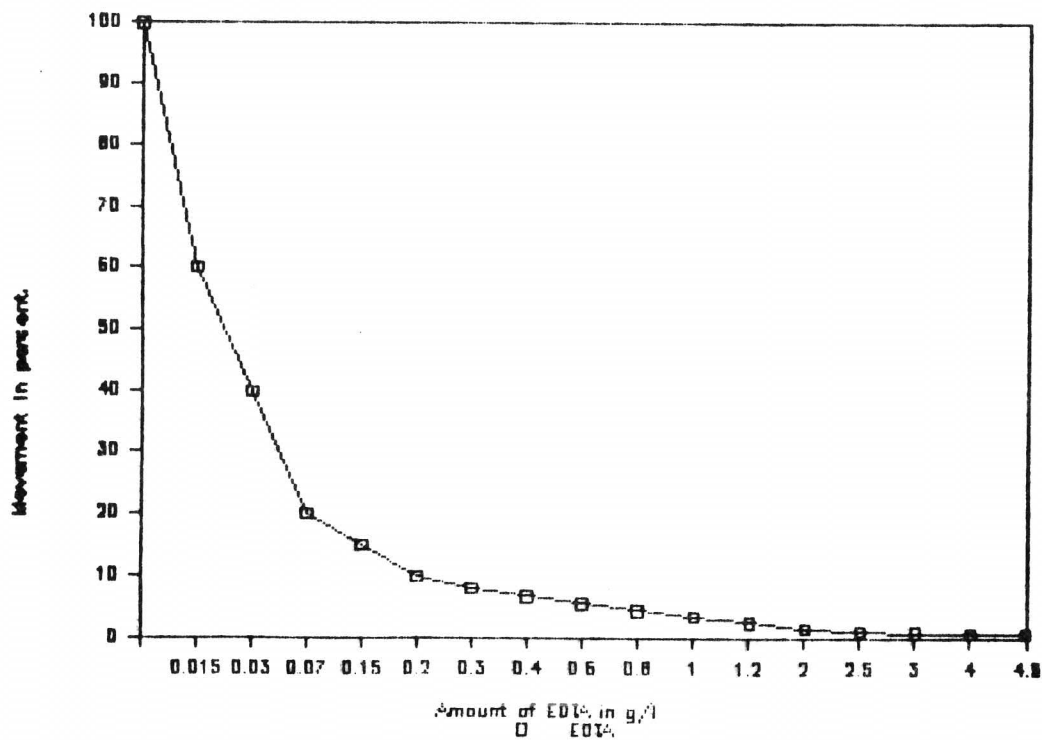


Figure 4.

Effect of EDTA on sperm movement.





or de-ionized water is used to make the extender solution. Extender solution made by bubbling oxygen into the extender solution for about 15 minutes just before it is used also increased the percent of eyed eggs by about 3-5%.

Use of the extender solution delayed the micropyle closure by up to 15-20 minutes longer than was observed when eggs were placed in fish hatchery water. Tests were run at three different temperatures. In these experiments, eggs from the same female were kept in the water from 1-26 minutes, and semen from the same male was added to the eggs at a specified intervals (Figure 3). The explanation for the fertilization peak after the eggs are in the extender for five minutes is that it takes about four minutes to free the micropyle surroundings from traces of rinsing solution in which the EDTA proved to have a detrimental effect on sperm movement (Figure 4).

An experiment on the ability of the sperm to fertilize eggs it was found that there was a greater number of fertilized eggs in the extender solution than there was in plain hatchery water. In these experiments, eggs from the same fish were added to a water-sperm or extender-sperm mixture for times from 1 to 17 minutes. Sperm in hatchery water failed to fertilize eggs seven minutes after activation, but they were still active after nine minutes in extender solution, only failing after 17 minutes (Figure 5). We confirmed Billard's (1978) observation that sperm moving for a longer time in water gave better fertilization results than those that moved for a shorter time. The percent of eyed eggs was significantly higher in trials where the sperm movement exceeded two minutes at 10C (Figure 6).

Further improvement was found with a modification of the egg hardening process. Where eggs of the same origin were washed and hardened in ammonia-free water versus water containing 0.2 mg/L of ammonia there was always a higher percentage of eyed eggs (Table 4).

Table 4.

Percent of eyed eggs obtained using water with and without ammonia content for washing and hardening of eggs.

Process:		Percent of eyed eggs.		
Washing.	Hardening.	Mean	S.D.	N
Am. free	Am. free	68	8.35	12
Am. present	Am. free	64	6.2	12
Am. free	Am. present	54	7.9	12
Am. present	Am. present	46	11.3	12



Success of fertilization.

Time (min.)	0 (□)	10 (+)	20 (Δ)	5 (●)	7 (×)
1	80	90	80	80	70
2	85	85	60	12	18
4	100	90	82	6	8
6	90	88	85	2	2
8	88	85	78	0	0
10	85	82	70	0	0
12	65	60	50	0	0
14	48	45	38	0	0
16	32	28	22	0	0
18	20	18	12	0	0
20	10	5	4	0	0
22	4	2	1	0	0
24	0	0	0	0	0
26	0	0	0	0	0

### Effect of sperm movement time

on fertilization success.

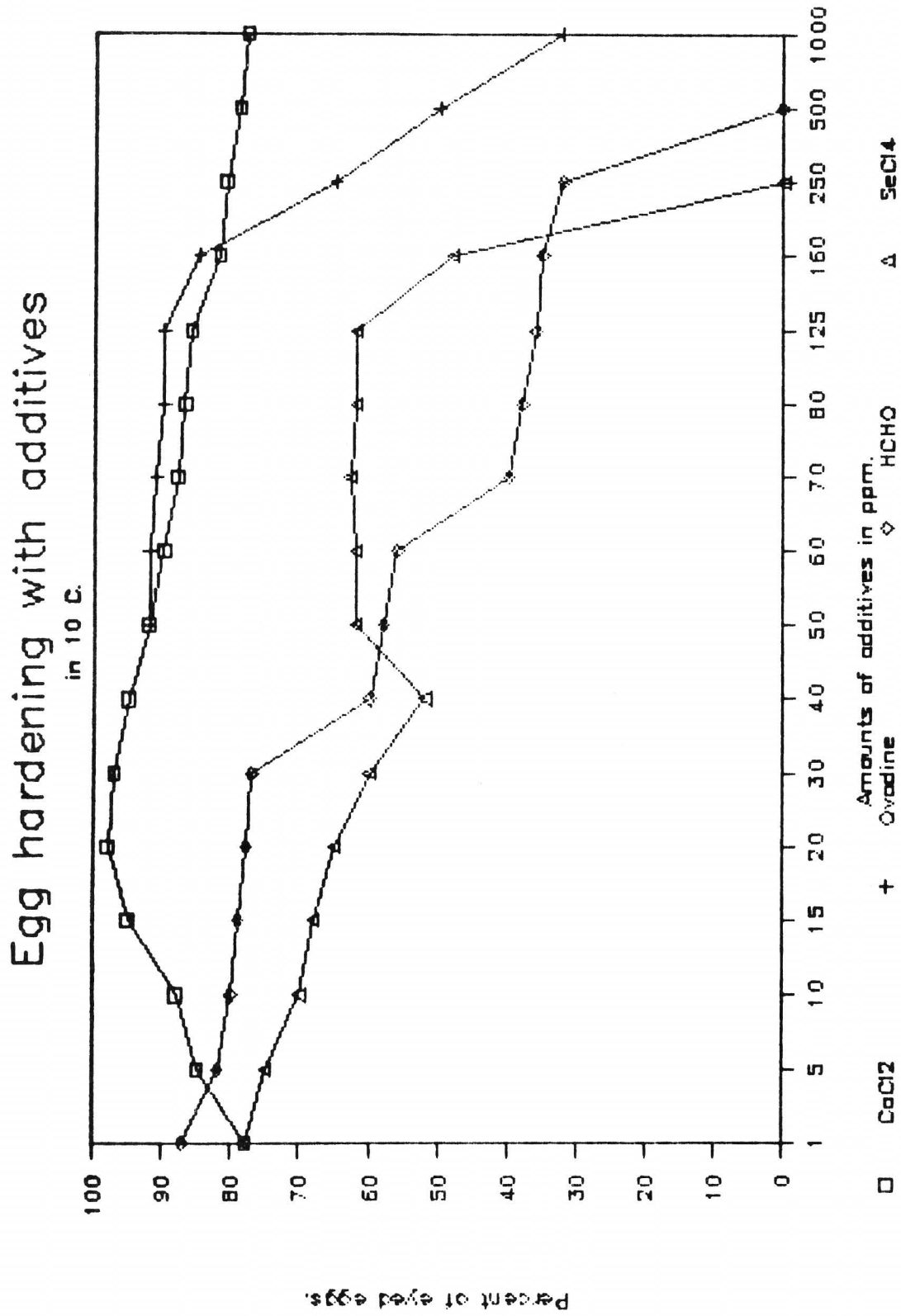
Movement time in seconds	Percent eggs fertilized
30	45
45	50
62	52
78	56
98	58
112	72
120	77
160	75
205	78
215	79
240	80

Percent eggs fertilized

Movement time in seconds

Pool of 10

Figure 7.



The water in which ammonia was present contained 2 mg/L total suspended solids, 0.05 mg/L P as total phosphate, 0.2 mg/L N as ammonia and 0.17 N as nitrate. The temperature of the water was 10 C with a pH of 7.5, and the BOD (biochemical oxygen demand) was less than 1 mg/L. (Chemical & Geological Laboratories LTD 1987).

The difference between the use of wash water with or without ammonia is slight. This is because the eggs are exposed to ammonia for only a short time. In the hardening process, however, the eggs are given a 20 to 30-fold exposure. Further improvement was found when the eggs were washed after fertilization with two different solutions, the first containing 0.75 mg/L Zn, 0.2 g/L NaCl, 0.025 g/l vit C, and the second, a similar one, except of the CaCl<sub>2</sub>.

Calcium chloride gave an unexpectedly high increase in the percentage of eyed eggs, even though the Sam Livingston hatchery water is hard (<30 mg/L CaCO<sub>3</sub>; conductivity readings 280 - 350 micro-ohms). Vitamin C further improved the embryonic survival. The best results were noted with an egg hardening solution containing 0.75 mg/L Zn, 0.004 g/L CaCl<sub>2</sub> and 5 mg/L vitamin C.

Alternatively formalin, cobalt or selenium chloride have a detrimental effect, but Ovodine appears to be harmless unless it is more than 160 ppm in the solution (Fig 7).

The estimated LD<sub>50</sub> levels (Keleti and Lederer 1974) are 13375 ppm for Argemone and 1460 ppm for Ovodine in waters originating from the Sam Livingston Fish Hatchery and Raven Brood Station, respectively.

## DISCUSSION.

The history of artificial spawning of salmonids goes back to the eighteenth century. From the beginning, the stickiness of the eggs presented difficulties in the man-made environment. Treatment of the eggs with tannic or humic acid (extracted from plant material) was soon developed to clean the egg surface thus preventing them from either sticking together or to the surface of the incubating jars. This treatment was applied after fertilization because the acids are detrimental to the sperm. But, it was logical to expand the treatment in order to improve the success of fertilization. Application of a saline solution to extend sperm motility, with a combination of carbamide (urea) to make the eggs free-floating, improved the success of fertilization for carp (Woynarovich 1955, 1962). Several salt solutions have been developed worldwide. These solutions significantly increase semen movement time, thus extending fertilization time.

Studies of different combinations of salts and sugar have shown KCl inhibitory effects on sperm movement. The mechanism by which high K<sup>+</sup> concentration results in a decrease in egg fertility is unknown. Erdahl et al. (1987) listed four possible

explanations: (1) disruption of the sperm-egg interaction; (2) interference with possible potassium channels that may affect the eggs increased membrane permeability to  $K^+$ ; (3) sealing of the internal micropyle orifice; and (4) disruption of the plasma membrane  $Na^+ - K^+$  mechanism.

The use carbamide for salmonid eggs wasn't necessary. This led to the modification of the extender solution introduced by Woynarovich (1955, 1962; Hamor 1969, 1987). The solution of B-lactose is more efficient than that of D-glucose (Figure 1), and calcium chloride also has a positive effect up to 0.4 g/L (Figure 2). However extended sperm movement does not have an absolute correlation with the success of fertilization. Therefore, it was necessary to find a process which not only improved the environment for the semen, but also aided fertilization. By using extender solution instead of hatchery water, the sperm were able to fertilize the eggs for a longer time (Figure 3), and the micropyle remained open longer as well (Figure 5).

At the beginning of the experiments, the success of fertilization was determined by the percentage of eggs starting cell division. However, it was found that the number of eyed eggs can be significantly less than the number starting to develop because many of them arrested in early embryonic stages. Fish survival from the eyed stage to the early feeding stage averages about 95% in our hatcheries; therefore, it is practical to use the percentage of eyed eggs as a measurement for fertilization success.

There are several excellent investigations which have shown that the introduction time of semen to the eggs results in differences in the success of fertilization (Alderdice et al. 1986). Our experiments support these observations. Therefore, we always introduce a squirt of semen to a dry pan before introducing the eggs. We then mix the eggs and sperm together, adding semen from two or three males, then adding the extender solution and mixing them constantly for one minute. Surprisingly, neither the gentle mixing nor a 5C warmer extender solution seemed to negatively affect the fertilization success.

The differences in eyed egg percentages caused by the different origins of water (Table 1) confirmed what Dave Ackerman of the Auburn Fish Hatchery said at the 38th Northwest fish culture meeting. He reported that "water appears to be the worst medium for egg fertilization especially when conditions are severe" (Ackerman 1987).

It is also known that the quality of eggs can vary (Smith et al. 1983, Morrison 1983). They can become overripe three days from the time of maturation (Craik nad Harvey 1984). In addition proteins in the ovarian fluid can plug the micropyle of the ovum. Trace elements in the water can disable the semen. Rienietz and Millard (1987) noted the importance of water quality on fertilization, they could not, however readily attribute the large differences in egg fertility to variations in water quality, but instead to differences in egg or semen quality. These findings gave us the idea, that the eggs should be cleaned before being fertilized.

Although we did not examine the success of rinsing to clean the eggs of surface contamination (such as BKD bacteria), the

method described here might be more efficient than the rinsing described by Evelyn et al.(1986).

As expected the use of water resulted in early closure of micropyle. Salt solutions improved fertilization results, but still some uncertainty remained when the number of eyed eggs were compared (Table 2). Further experiments indicated that the combination of NaCl with EDTA is the most effective solution for rinsing the eggs (Table 3). However the presence of EDTA reduces the sperm movement (Figure 4), which explains the delayed peak for success of fertilization. Following the rinsing process, the eggs were kept in the extender solution and the semen was added at two minute intervals to 57 separate batches, because approximately four minutes are required for the egg surface to be totally free of remaining EDTA (Figure 5). This peak did not appear when the eggs were rinsed in a 3% salt solution following the salt-EDTA solution (Figure 3); however these step used in everyday practice would overcomplicate the procedure and not offer any real benefit.

Although the sperm movement time is influenced by water quality and temperature (Hamor 1966), Billard's observation (1978) that a longer movement time results in a higher percentage of fertilized eggs (Figure 7) should be confirmed. High oxygen content enhanced sperm survival (Billard et al. 1981).

All the results showed that the percentage of fertilized eggs can be influenced by rinsing them and by using an extender solution. The observation that the number of eyed eggs declined if water containing ammonia was used for hardening suggested that survival can be influenced after fertilization occurred. To reduce transmission of disease, the use of chemical disinfectant (Betadine) - 125 ppm for one hour - attained an iodine concentration which was reported high enough to destroy all known salmonid viruses without causing any ill effects to egg survival (Groberg 1987). Our results and verbal information from staff at other hatcheries indicate an adverse effect at that level. However, when we consider treatment with iodophor compounds, we should not forget that chemical toxicity is influenced by water quality and also differences between egg lots and fish strains. Therefore the same compound can be more toxic in one hatchery than in another. These effects are also demonstrated in work by Groberg (1987), where there was 21% error for the controls and only 4.5 for the iodophor compound-treated group. The different toxicity (LD-50) noted between Ovodine and Argentine does not necessarily mean that one is less toxic than the other, but might reflect differences between genetic stocks and water quality. This inconsistency indicates that application of any iodophor compound at the time of egg hardening must be properly tested in each hatchery before routine use is accepted.

The addition of zinc, vit C and CaCl<sub>2</sub> resulted in further improvement in eyed eggs percentage. This is not surprising if we consider that ascorbic acid significantly increases the number of hatched eggs compared to eggs from fish without dietary supplements of ascorbic acid.

Reports regarding the activity in the zona radiata (Hamor and Garside 1973), the ability of eggs to absorb calcium during development (Rudy and Potts 1969) and egg sensitivity towards

heavy metals (Michibata et al. 1987, Rombough and Garside 1982, Shazili and Pascoe 1986) all indicate a possible existence of constant material exchange and transport during incubation. Zinc, calcium and vitamin C (Sandnes et al. 1984, Smith et. al. 1979, Wekell et. al. 1986) are known to be important in the growth and development of fishes, therefore adding them in the time of washing and hardening the eggs can improve embryonic survival. A word about caution should be said about this procedure in waters with high metal contents.

The treatment of eggs at the time of egg hardening by using a drip treatment to add minerals and vitamins promises new possibilities for improvements in eyed egg survival. Drip treatment for incubating eggs is in progress; however, we should realize that because of variation in water quality the composition of additives for such treatments might require modification. Our procedure for fertilization and egg treatment can be summarized as follows:

- 1/. Check for ripe fish at least once, but no more than twice, a week.

- 2/. Do not keep ripe fish more than three days in cages. If possible spawn them the day after sorting.

- 3/. While spawning, collect the eggs in a strainer, separating them from the ovarian fluid.

- 4/. Rinse the eggs in a solution of 3% NaCl - 0.05% EDTA moving the strainer up and down a few times in the solution. After the eggs are rinsed they should be drain for about one minute. The rinsing solution can be made using pond water from where the spawning fish has been held. About 10 L solution are necessary to rinse every 300 000 eggs.

- 5/. Use a dry pan for spawning. Squirt semen from one male into the pan, then introduce the eggs. The eggs from 3 to 5 females can be fertilized using 2 to 5 males. Mix the eggs and semen immediately after the semen from each male is introduced. Then add the oxygen bubbled extender solution : 6 g/L NaCl, 0.2 g/L CaCl<sub>2</sub>, and 0.4 g/L B-glucose. Mix it for one minute. Let these stand for about five minutes, but not more than 15 minutes. Distilled or de-ionized water should be used to make the extender solution which can be stored in a cool place for 2-3 weeks. Before use, the extender solution should be oxygenated by bubbling air (preferably oxygen). Its temperature should be adjusted to approximate that of the water in which the spawning fish are kept. Use about 50 ml of solution for 3000 rainbow eggs.

- 6/. Thoroughly but gently wash the eggs clean from the extender solution and semen using by 30 cm diameter sieves in pond water. We filled two containers with pond water and gave the eggs a first and a second washing. We are adding 0.75 mg/L Zn, 0.2 g/L NaCl, 0.025 g/L vitamin C to the first, and 0.75 mg/L Zn, 0.2 g/L CaCl<sub>2</sub> and 0.025 g/L vitamin C, to the second washing. The eggs are washed in a sieve for about four minutes in each solution. A rope handle can facilitate working with the sieves. Change the water after washing 100 000 eggs.

- 7/. Harden the eggs in a solution of 0.15 mg/L Zn, 0.04 g/L CaCl<sub>2</sub> and 0.005 g/L vit C for one to two hours. The egg hardening water should be saturated with oxygen and free from ammonia.



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# THE ORAL ADMINISTRATION OF PRODUCTION-ORIENTATED PROTEINS & PEPTIDES TO SALMONIDS

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## INTRODUCTION

The teleost gut is known to be permeable to ingested proteins and peptides. The first recorded evidence of intact proteins entering absorptive cells of the gastrointestinal (GI) tract of salmonids, following oral administration, was supplied by Nagai & Fugino in 1983. The passage of intact proteins into the bloodstream of salmonids, following oral delivery, was first described by McLean & Ash (1987). Although the precise mechanism by which proteins and peptides are absorbed by the fish gut remains a contentious issue, the possible benefits of applying this phenomenon to the aquaculture industry are obvious. If this mechanism could be used as a method of orally delivering production-orientated proteins and peptides to fish, it may provide a convenient means of not only vaccinating fish, but also influencing their growth and sexual development. A brief consideration of these possible applications to the aquaculture of salmonids is provided below.

## A) Oral Vaccination

One of the major problems which awaits a solution in fish culture is the development of cheap, easy to handle, safe, efficacious vaccine preparations. While some commercially available products have proved highly effective (notably those against vibriosis and enteric redmouth), many have proved a) too expensive, b) difficult to administer, c) a health risk or d) of inadequate potency in the field. In general, vaccination as currently practiced, requires that the antigenic component be delivered by immersion, spray or injection. However, the provision of an effective oral vaccination procedure would decrease stock loss due to stress caused by the above procedures and also provide considerable financial savings by reducing labor costs. Although, to date, a certain degree of success has been achieved experimentally, in using live vaccines delivered via the oral route (e.g. Braaten & Hodgins, 1976), it is apparent that the majority of the vaccine's antigenic component is degraded by the action of the gastric and pancreatic secretions of the GI tract. That portion of the vaccine that is absorbed in an immunogenically reactive form, when presented orally, often provides weak protection to secondary challenge. Furthermore, it appears that the immune response elicited following oral vaccination of fish results in local immunity rather than more desirable anamnestic responses (Ellis, 1988). Nevertheless, the potential that oral vaccination offers as an effective and convenient method of protecting fish has been indicated by Johnson & Amend (1983). These workers vaccinated sockeye salmon with a Vibrio anguillarum preparation using rectal intubation techniques. Following secondary water-borne challenge, fifty-nine days

post-vaccination, 55% of control, untreated animals died, whereas in the vaccinated fish, no mortalities were recorded. Supportive quantitative evidence for the findings of Johnson & Amend has been provided by studies which compared the absorption of the soluble protein antigen horseradish peroxidase (HRP) following either its oral or rectal administration to rainbow trout (Figure 1.).

The indications of the latter studies are that by-pass of digestive secretions results in a two-to-four-fold increase in the amount of antigen absorbed by the gut (and presumably presented to the immune system). It is contended therefore, that it may be possible to increase the net amounts of antigen delivered to the immune system by protecting the antigenic component of the vaccine. That GI absorption is not limited to specific antigens of small size has recently been indicated by McLean et al. (1988a). In these studies (Figure 2.) the high molecular weight lipoglycophosphoprotein, vitellogenin, was detected in the bloodstream of chinook salmon following oral intubation. The importance of this study relates to the molecular size of the vitellogenin molecule, which is greater than, for example, certain antigenic proteins of the IPN virus, and approaches those of some bacterial antigens. Thus, when combined with other studies that have mapped the permeability of the salmonid gut, our findings indicate that it is a feasible proposition to orally deliver antigenic components of vaccines. Such investigations provide reasons for optimism with respect to the future development of oral preparations. Whether oral vaccines become a viable proposition in the near future however, will ultimately depend upon the discovery or development of effective methods of

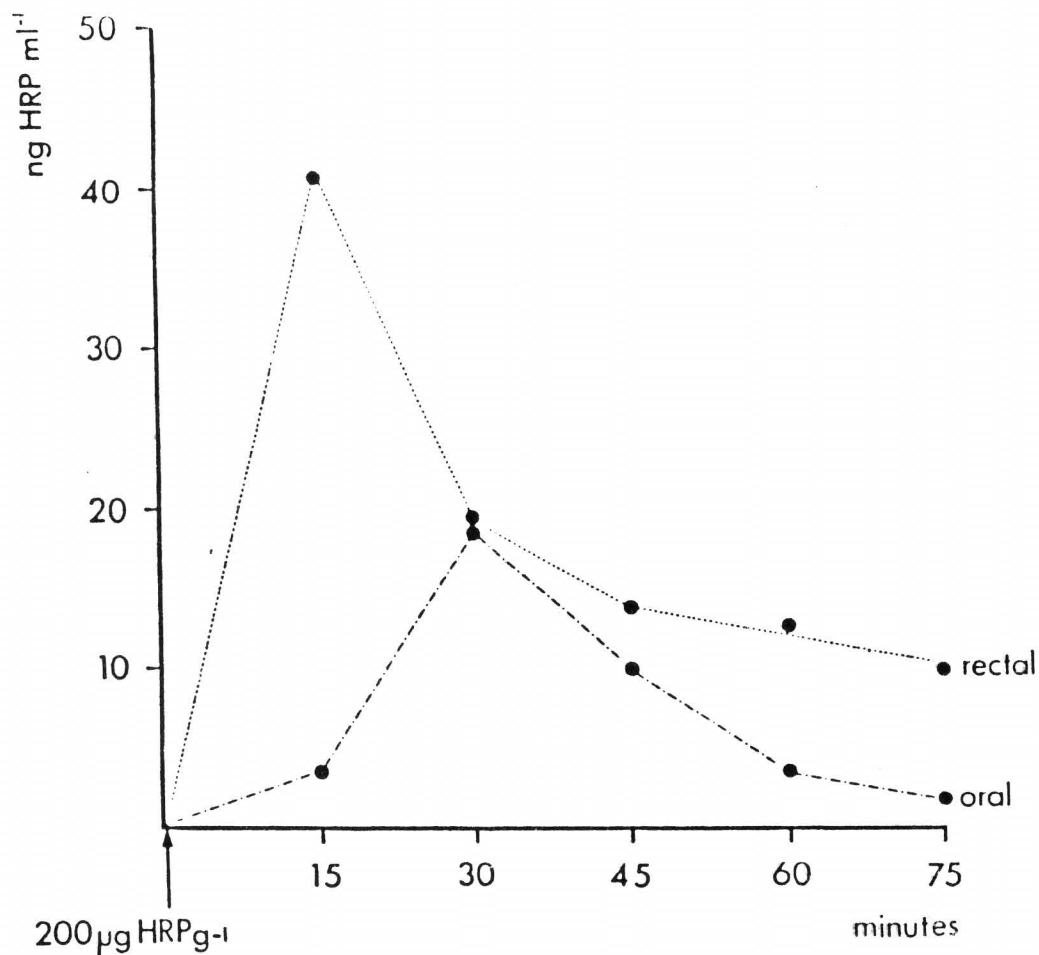


Figure 1. The time-course of appearance and net plasma presence of HRP following either oral or rectal administration of 20 mg HRP ml<sup>-1</sup> in 1 ml 0.9% saline (McLean & Ash, 1987; unpublished).

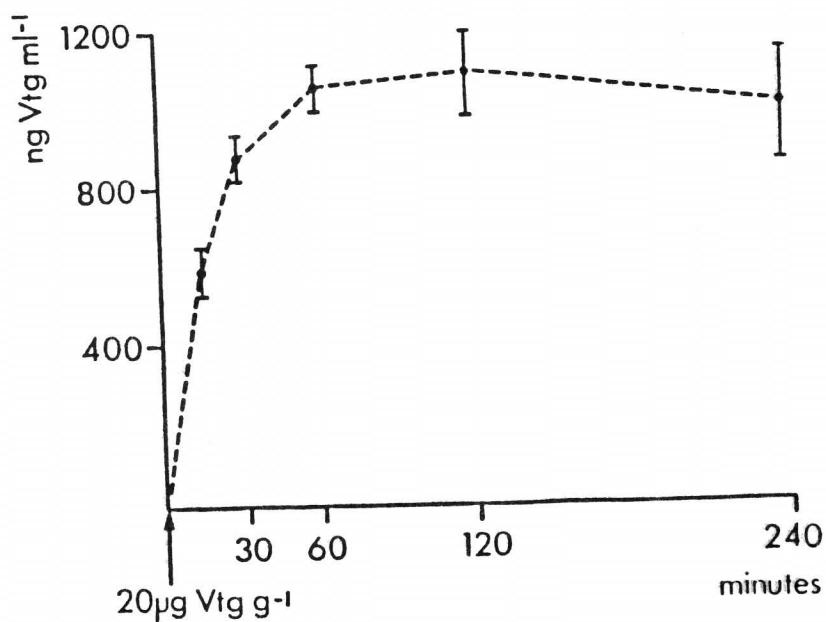


Figure 2. The time-course of appearance of vitellogenin (Vtg) in the plasma of chinook salmon following a single oral intubation of 20  $\mu$ g Vtg g<sup>-1</sup> body weight in 200  $\mu$ l of 0.9% saline (McLean *et al.*, 1988a).

either by-passing or manipulating the action of the digestive processes of the salmonid gut. It should be emphasised, however, that under oral loading, it is difficult to control the dose of vaccine delivered to individual fish. Thus, greater amounts of the antigenic component of the vaccine may be required for effective vaccination. Furthermore, there may exist species differences with respect to vaccine efficacy when delivered by the oral route.

## **B) Growth Enhancement**

A variety of techniques have been examined in attempts to manipulate the growth performance of teleosts. Of those examined, the most promising appears to be the delivery of various forms of somatotropin (for review see Donaldson et al., 1979). The potential benefits of accelerating growth are many, but in particular, they provide a means of compressing the time span required for hatchery fish to obtain appropriate body mass prior to release or seawater transfer. Alternatively, somatotropin treatment may be used to increase the size of fish released at the normal time. Both scenarios offer management advantages; the former allowing sustained release of the year class and the latter providing individual fish better equipped for survival. Indeed, resource enhancement studies have shown that release at the appropriate size and time has a major influence upon ocean survival (Bilton et al., 1982). Notable success has been achieved in accelerating growth of both coho salmon and rainbow trout with the topical and parenteral applications of both native and recombinant, bioengineered somatotropins (e.g. Gill et al., 1985; Down et al., 1988). But, such



procedures are not without their disadvantages. Somatotropin administration by injection is often time-consuming, logistically demanding, requires a certain amount of expertise to perform, and may be stressful to recipient animals due to repetitive anaesthesia, handling and confinement. An ability to orally administer somatotropins to fish would certainly overcome many of the foregoing constraints. A prerequisite to the development of an oral system of delivery, however, is the availability of "base-line" data with respect to the normal capacity of the fish gut to absorb somatotropins.

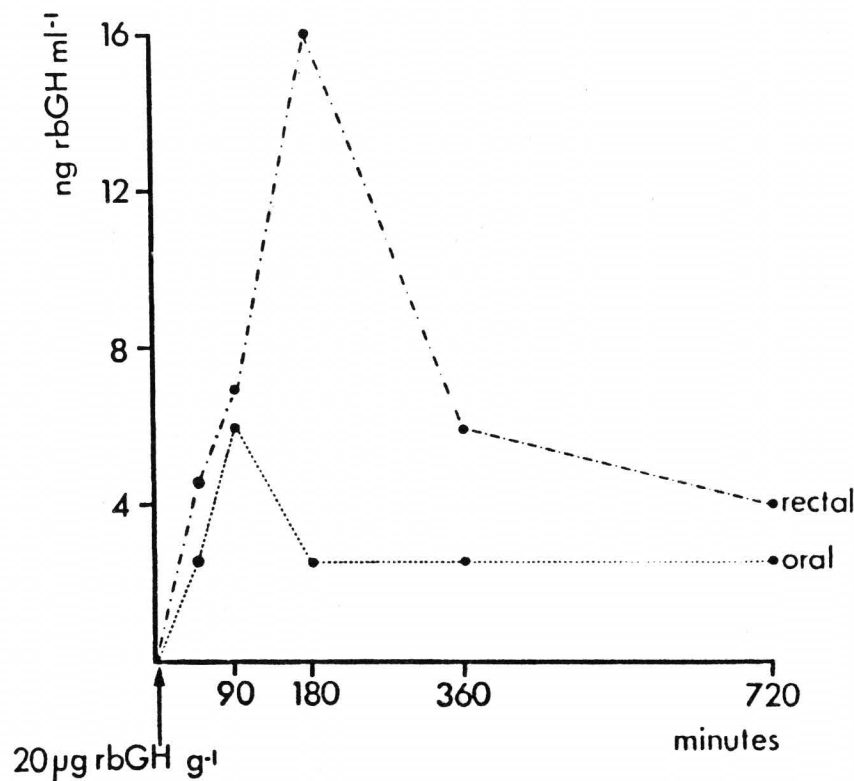


Figure 3. The time-course of uptake and net plasma presence of orally and rectally administered recombinant bovine growth hormone (rbGH) in coho salmon parr. rbGH was delivered at a dose of  $20 \mu\text{g g}^{-1}$  body weight in  $200 \mu\text{l}$  0.9% saline (McLean *et al.*, 1988b).

Figure 3 summarises a study undertaken to quantitatively assess the oral bioavailability of rbGH to coho salmon parr. Comparison of oral and rectal delivery techniques suggests that the maximum absorptive capacity of the salmon gut for the delivered dose is, as is the case with other proteins, two-to-four-fold greater than actual, under oral administration. The data presented thus confirms previous suspicions: the digestive secretions of the salmonid GI tract decrease the amount of protein available for absorption in an intact form. For the dose delivered in the above experiment, plasma levels of rbGH appear to be below that required for influencing growth performance in salmonids. However, it is evident that oral delivery of such production-orientated proteins is not without promise. It should also be noted that recent studies have determined that plasma levels of biologically active peptides and proteins are not, necessarily, a reliable criterion with respect to quantifying GI uptake (Gardner & Wood, 1987). As is the case with oral vaccines, it is apparent that to achieve the objective of delivering a specific dose which provides the desired effect, it will be necessary to develop effective techniques of enhancing somatotropin uptake by the salmonid gut. Such techniques may take the form of encapsulation, combined with the judicious manipulation of the digestive processes. It is possible that specific doses of recombinant and, or native forms of somatotropin may be more effective in terms of gaining access to the bloodstream than others; while different "species" of somatotropin, when delivered orally, may be more potent than others.

### C) Controlled Reproduction

Effective methods of controlling the sexual maturation of salmonids (see Donaldson 1988) command the interest of not only the reproductive physiologist, but also the commercial producer. The productivity of an individual salmon growing unit is dictated, among other things, by the stage at which fish reach sexual maturity. During the latter stages of the maturational process, not only is there a loss in food conversion efficiency, body coloration and flesh quality (colour and texture) but, for salmonids in particular, the onset of secondary sexual characteristics which ultimately reduce or limit the marketability of the product. Many techniques (e.g. surgical manipulations, chemosterilization, hormone administration, hybridization, chromosome set manipulation, irradiation) have been investigated in attempts to produce sterile fish, but often these techniques demand considerable expertise to perform, or are not applicable to the production situation. One method of producing sterile fish, which could feasibly utilise oral delivery techniques, concerns the administration of monoclonal antibodies raised against fish germ cells (see Secombes et al., 1987). Theoretically, target cells -in this case gonad germ cells- are bound by a specific antibody and subsequently destroyed by the body's (complement- mediated) natural defences. The induction of this autoimmune response, which may result in the complete destruction of germ cells, could thus provide a means of controlling precocious maturation (jacking). Concomitantly, high flesh quality may be retained while the development of secondary sexual characteristics is prevented, resulting in a more marketable, and desirable product. An ability to orally deliver such

antibodies will, needless to say, depend upon the provision of an effective means of delivery as outlined above. It should be noted that more effort has to be invested in this experimental technique to assess and justify its application to the industry, in any form. In comparatively recent times, a variety of techniques have also been employed to manipulate the latter stages of the reproductive cycle of teleosts. Success has been achieved with single or multiple injections of pituitary extracts or gonadotropin preparations, and also with LH-RH or GnRH analogues with or without dopamine antagonist in inducing ovulation of a variety of species. The benefits of such manipulations are many fold; in salmonids, they provide a means of synchronizing maturation of broodstock and in other species ovulation is induced under conditions where it would not normally occur.

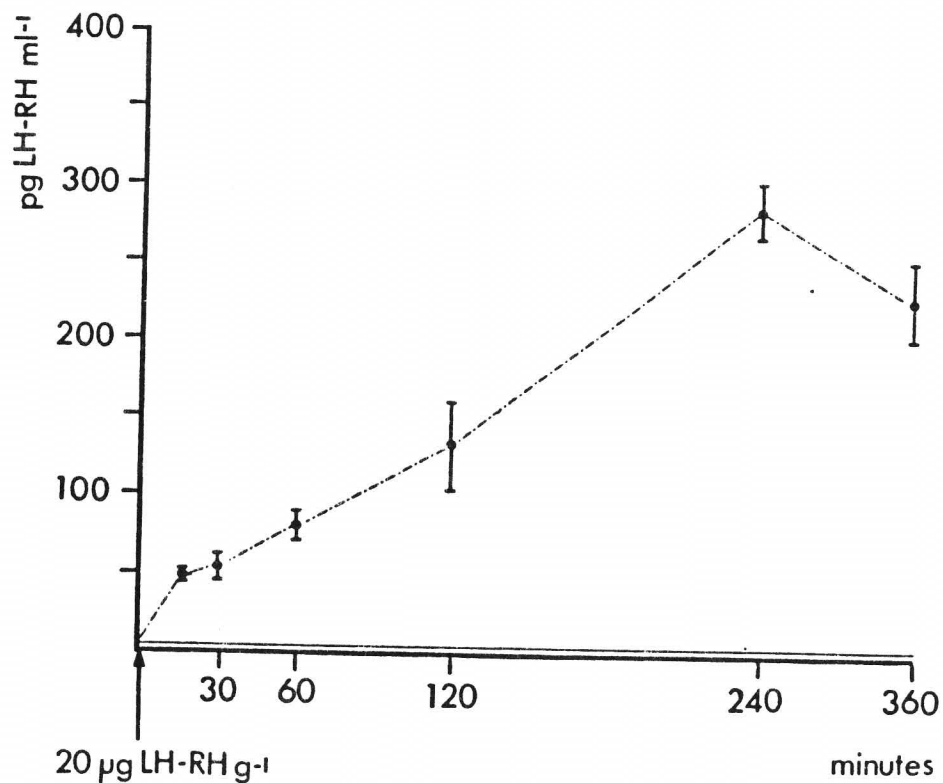


Figure 4. The time-course of absorption and net plasma presence of LH-RH delivered via the oral route to coho salmon.

Induced ovulation and spermiation thereby provides a method of not only reducing labour costs, but also the number of fish lost due to pre-spawning mortalities. Concomitantly, methods of controlling reproductive cycles also represents a powerful management tool in providing techniques of synchronizing and separating the maturational windows of two or more species. Although less applicable to salmonids, the oral delivery of maturational hormones represents a goal in many aquaculture systems which offers considerable potential. Recent studies utilizing LH-RH and LH-RHa, undertaken at the West Vancouver Laboratory and University of Victoria demonstrate that these highly potent peptides enter the salmonid circulation following oral delivery (figure 4). Further studies are, however, needed to determine whether the biological activity of the absorbed peptide is retained.

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EFFECT OF 17 $\alpha$ -METHYLTESTOSTERONE ON GONADAL DIFFERENTIATION OF THE  
CHINOOK SALMON (Oncorhynchus tshawytscha)

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**ABSTRACT**

Chinook salmon alevins were immersed one or five times in a solution of 400 ug/l 17  $\alpha$ -methyltestosterone (MT), followed by dietary treatment for periods of 28, 56 or 84 days at doses ranging from 0 to 80 mg MT/kg diet. Masculinization in the range of 90 to 100% was achieved by immersion treatment alone. Predominantly males and males showing reduced gonadal tissue development (semi-sterile) resulted by combined immersion and dietary treatment at lower doses (10-20 mg MT/kg diet) during the whole range of durations, and at higher doses (40-80 mg MT/kg diet) for the shorter durations (28 and 56 days). Sterility was attained in 91-97% of fish treated at doses of 40 and 80 mg/kg when the oral treatment lasted for 84 days.

Survival in all the experimental groups was comparable to controls. Mean weight of the experimental groups during treatment, measured every 4 weeks, was not significantly different from controls.

**INTRODUCTION**

Most of the research on sex control on commercially important Oncorhynchus species at the West Vancouver Laboratory has been on the sterilization of coho salmon (O. kisutch) and on the production of monosex female stocks of chinook salmon (O. tshawytscha) by the use of "female



sperm". To complement this research and in response to present needs of the industry we have oriented our current research efforts towards the hormonal sterilization of chinook salmon and the development of a source of coho female sperm. This paper is a preliminary report on the effect of immersion and dietary treatment with 17 -methyltestosterone on the gonadal development, masculinization and sterilization of chinook salmon.

## **MATERIALS AND METHODS**

Chinook salmon eggs from Big Qualicum B.C. stock were transported and incubated at the West Vancouver Laboratory in 10°C well water. Replicated groups of approximately 150 eggs were immersed one or five times in a solution of 400 ug/l methyltestosterone for 2 hours (531 ATU) starting at 25-35% hatch, then at weekly intervals (to 821 ATU) during yolk-sac absorption. Dietary steroid administration was started at first feeding at dosages ranging from 0 to 80 mg/MT/kg diet and lasted for 28, 56 or 84 days. Diets were prepared by spraying the steroid dissolved in 95% ethanol solution onto a dry experimental diet (P10) (Higgs et al., 1985).

The fry were sampled for weight at the end of each treatment period and also at 119 days from first feeding when samples were taken for histological analysis.

Histological analysis of cross sections cut between the operculum and a point anterior to the dorsal fin were fixed in buffered formalin and stained with hematoxylin-eosin. Percentages of gonadal morphology at the time of sampling are shown in Fig. 1 and 2.

The different gonadal conditions observed in the treated groups are described as follows:

Male:        Normal testicular development. Gonad consisting of densely

packed cysts of spermatogonia. Size and tissue structure of the testis is the same or very similar to control males. In some cases further development to spermatids and spermatocytes can be observed in extensive areas of the gonad.

Female: Similar or equal to control ovary. Gonad contains oogonia and perinucleolar oocytes.

Intersex: Gonad consisting mostly of clusters of testicular germinal tissue interspersed with one to several oocytes.

Partial

Sterile: Gonad showing medium to extensive reduction of germinal tissue. Clusters of remaining spermatogonia among areas of connective tissue occupy between 10 to 70% of the gonad. The gonad is often reduced in size and shows thickening of the hilum and tunica.

Sterile: Gonad completely or nearly completely devoid of germinal tissue. Size of the organ is either small or alternatively large but with large hollow areas. Hilum and tunica are thickened.

## RESULTS

Close to 100% masculinization was achieved with one immersion treatment alone at the time of hatching. One or five immersions only or 5 immersions followed by dietary treatments of short duration (28 days) produced a range of conditions including variable numbers of intersex fish and partially sterile fish. Low proportions of sterilized fish were also produced (8.1 to 27.3%).

Sterility was generally low (0-56%) at all dietary dosages extending

for shorter durations (28-56 days) and at low doses (10-20 mg/Kg) even for the full duration of the experiment. Sterility was highest (91-97%) at the highest dosages when the treatment was extended for 84 days.

Mean weight of the fish at the time of sampling for histology (17 weeks from first feeding), compared with controls, was higher in the groups that received the low dietary MT concentration (10 mgMT/Kg diet) and lower in the groups that were fed the highest dose (80 mgMT/Kg diet). However, differences at this time were not statistically significant.

Mortalities during treatment were generally low (97.5% overall survival) and showed no relation to dose or duration of the treatment.

## CONCLUSIONS

With regards to masculinization these results confirm previous successes in the masculinization of chinook salmon by single 2hr. androgen immersion (Baker et al., 1988; Pifferer and Donaldson, 1988).

For sterilization we conclude that the effective dose required to achieve a high percentage of sterilization in chinook salmon is about 8 times higher than the effective dose to sterilize coho salmon (Donaldson and Hunter, 1982). We also conclude that, compared to one immersion alone, several immersions contribute to further inhibit gonadal development at lower doses and short durations of dietary treatment. This effect, however is no longer evident at the higher doses (40-80 mg/Kg) for the long duration (84 days). Finally, treatments did not seem to significantly affect the overall performance of the fish, during early development.

Selected groups (based on degree of sterilization achieved) were PIT (passive integrated transponder) tagged and are currently being reared in a net pen at the Pacific Biological Station (PBS), Nanaimo, B.C. for a long

term study of growth, survival and gonadal development.

#### ACKNOWLEDGMENTS

We wish to thank R. Humphries and the staff of the Rosewall Creek Experimental Hatchery, A. Solmie and staff of the Experimental Fish Farm at PBS, for care of the fish used in this study. We acknowledge the assistance of Dr. D.A. Higgs in providing the dry diet used in the oral administration of the androgen. The contribution of Dixon Mclean and Karima Mulji for the histological processing and preparation of computer diagrams for this report are also greatly acknowledged.

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Fig. 1 EFFECT OF IMMERSION<sup>1</sup> AND DIETARY TREATMENT WITH 17 $\alpha$ -METHYLTESTOSTERONE ON SEX DIFFERENTIATION AND GONADAL MORPHOLOGY OF CHINOOK SALMON

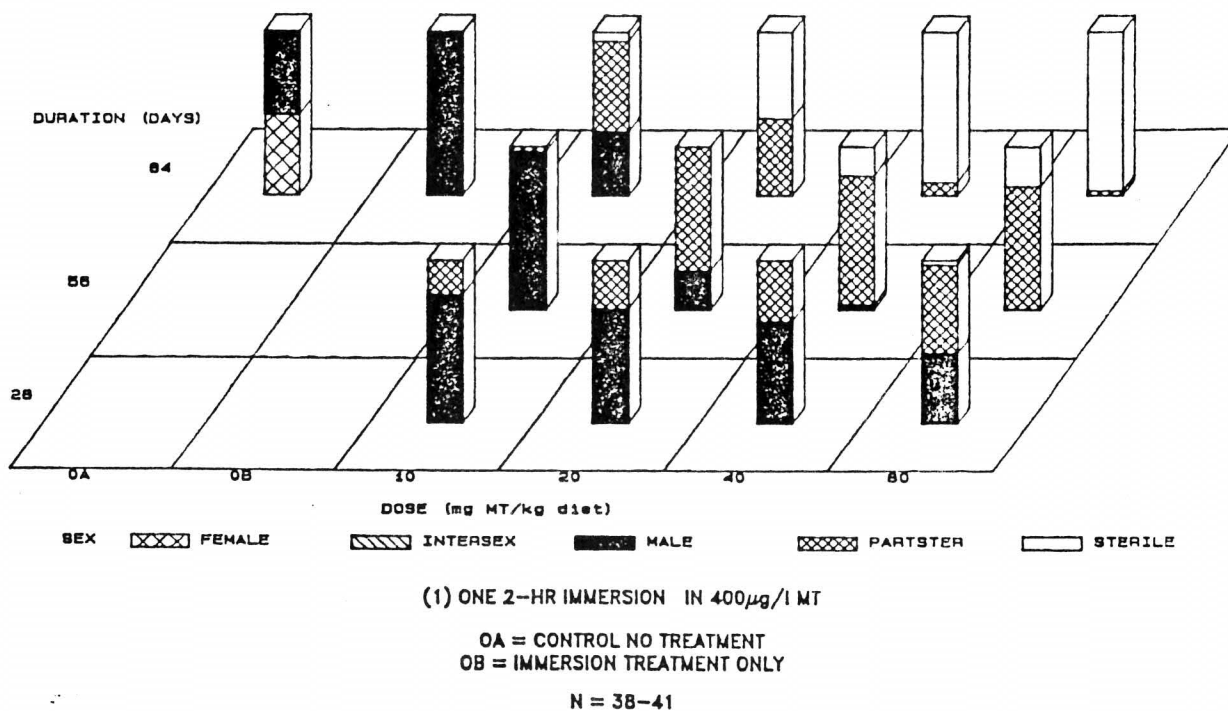
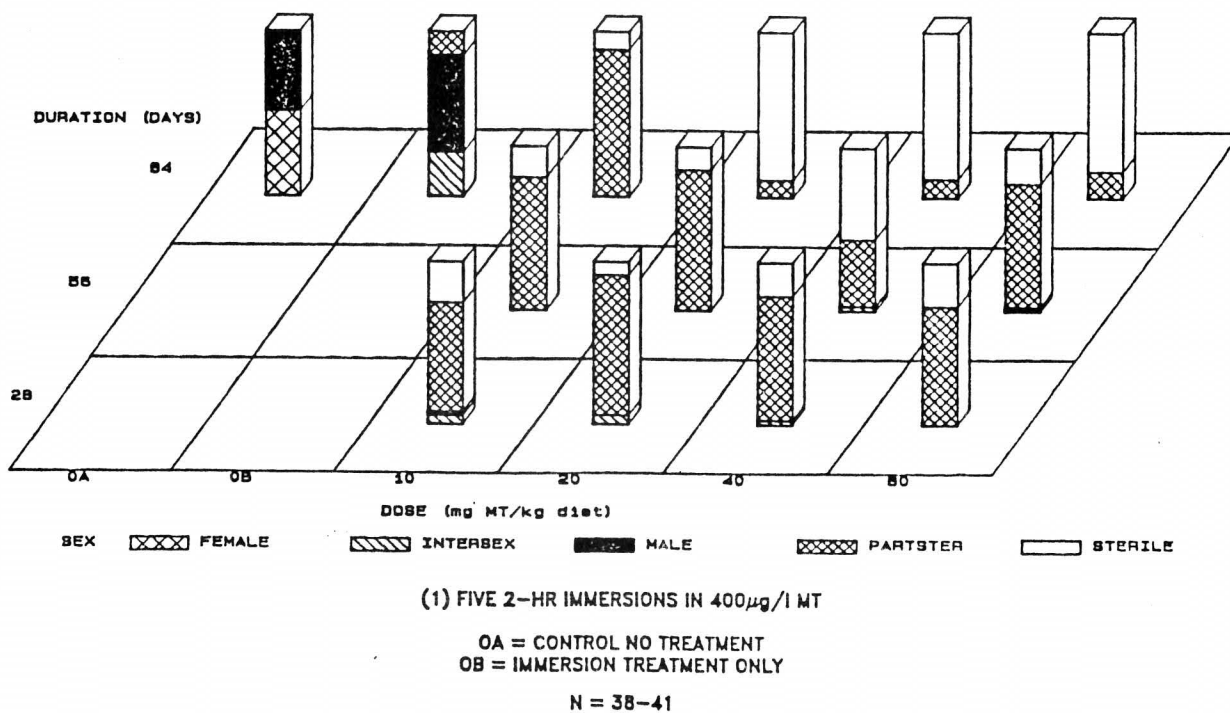


Fig. 2 EFFECT OF IMMERSION<sup>1</sup> AND DIETARY TREATMENT WITH 17 $\alpha$ -METHYLTESTOSTERONE ON SEX DIFFERENTIATION AND GONADAL MORPHOLOGY OF CHINOOK SALMON



# S A L T     P R E S E N T A T I O N

by  
Phil Edgell

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Box 1100  
Port Alberni, B.C.  
V9Y 7L9

- Malachite Problems - Worker Health - Implicated as a carcinogen mutagen and teratogen.
- Environmental damage - Effluent into rivers and streams.
  - Residuals in fish - Sale fish.

- Alternatives
- Formalin - presents the same problems as malachite green. ie: Alaskan workers sensitizing to formalin.
  - Malachite - Filter effluent. Costly and not a complete solution.
  - Salt

## Method

Pooled production loadings of 8,000 Chinook eggs or 12,000 coho eggs per tray, in eight-tray Heath stacks.

- Mix up salt solution - Grams per liter. Measure with conductivity meter and specific gravity hydrometer.
- Shut off flow to Heath stack.
- Pump salt solution through the stack until desired concentration is reached in bottom tray.
- Static bath for 15 minutes - 2 hours.

- Overhead #1 - We know from treatment of adults at West Van Lab that concentrations of less than 28 ppt. are effective. The lower the concentration, the lower the cost.
- Malachite 1 ppm. for one hour 3 x a week.

- Overhead #2 - Results measured in percent mortality.
- 5 ppt. and 10 ppt. were ineffective both visually and statistically, so not included.
  - Best seasalt performed as well as the malachite.

- Overhead #3 - % mortality.
- Control visually having lots of fungus.

- Overhead #4 - Our best treatment on the Chinook was our strongest concentration for one hour. Maybe not strong enough?
- 28 ppt. - 24 ppt. over 6 trays.
  - Unpooled due to time.
  - Again salt comparable.

Overhead #5 - Note downward trend in mortality as salinity decreases.

Break: We have had successful fungus control comparable to malachite with concentrations between 20 - 25 ppt.

Objective 1988 - Reduce cost - Increase duration with a weaker concentration.  
- Reduce frequency.  
- Introduce cheaper salt.

- Increase the size of our experiment
  - same procedure.
  - pooled eggs.
  - 3 replicates.

4 NaCl & CaCl<sub>2</sub> treatments  
10 seasalt treatments  
1 Malachite  
1 Control

Overhead #6 - % mortality used to measure results.  
- 2 hour treatments not doing well.  
- 20 ppt. out-performing the 25 ppt.

Overhead #7 - 2 hour treatments not doing well.  
- 1 hour treatments no significant difference.

Overhead #8 - Same trend with 2 hour treatments.

Overhead #9 - 10 ppt. ineffective. Visually lots of fungus.  
- Increased duration did not work.  
- Malachite effective.  
- Control lots of fungus - high mortality.

Overhead #10 - 3 best treatments.  
- Malachite lowest in 88 but salt in 87. No statistical difference.  
- All three very acceptable for % mortality. Robertson Creek mean % mortality for production treated with malachite is over 10%.

Overhead #11 - Lower durations of NaCl and CaCl<sub>2</sub> are as good or better than seasalt, with 2 hour treatments being hard on eggs, especially NaCl and CaCl<sub>2</sub>.

Results: Our salt treatments have been successful enough to do a production run in 1989.

Concentration - Optimum somewhere between 17 & 23 ppt. at 3 times a week.

Duration - Optimum somewhere between 30 minutes and 1 hour.  
No cost benefits to reducing time.

Frequency - Optimum seems to be 3 times a week.

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White spot (Coagulated yolk) - Two 100 alevin samples were checked.

- Control - 7% occurrence.

- Salt - 9% occurrence.

- Malachite - 15% occurrence.

Not enough samples for statistical significance, but worth a good look.

Overhead #12 - Cost - We still feel we can bring the cost down.

- Bulk NaCl bought cheaper.

- Recirculation.

\* \* \* \* \*



## Chinook Seasalt Treatments 1987 Brood

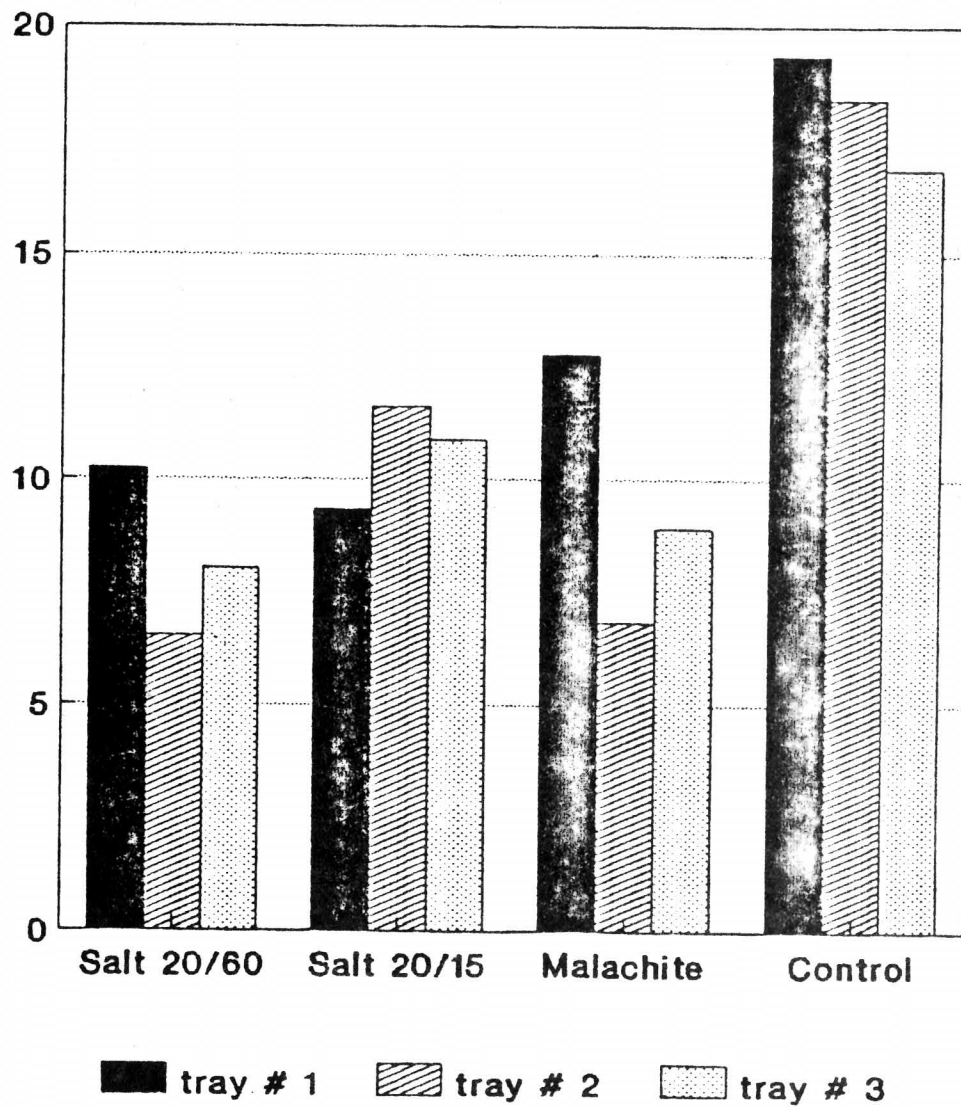
A	Malachite
B	Control (No Treatment)
C	20 ppt. for 60 minutes
D	20 ppt. for 15 minutes
E	10 ppt. for 60 minutes
F	10 ppt. for 15 minutes
G	5 ppt. for 60 minutes
H	5 ppt. for 15 minutes

# Seasalt Experiment

## 1987 Brood Chinook

Chinook Treatments	Tray #	% Mortality	Mean
Seasalt at 20 ppt. for 60 minutes.	1	10.22	8.26
	2	6.53	
	3	8.03	
Seasalt at 20 ppt. for 15 minutes	1	9.36	10.62
	2	11.63	
	3	10.88	
Malachite at 1 ppm.	1	12.80	9.52
	2	6.83	
	3	8.92	
Control (No treatment)	1	19.36	18.21
	2	18.39	
	3	16.87	

## RCH CHINOOK EGG TREATMENTS 1987 BROOD

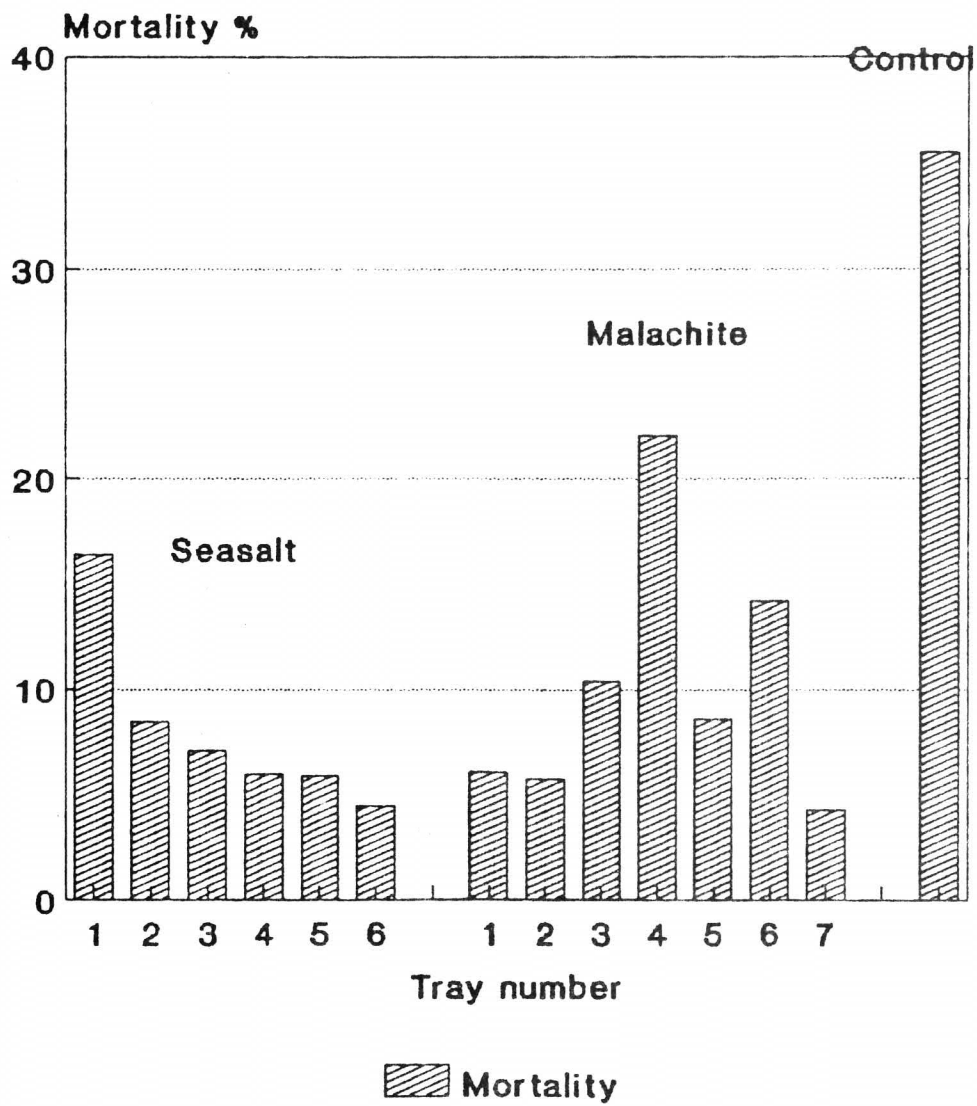


# Seasalt Experiment

## 1987 Brood Coho

Coho Treatments	Tray #	% Mortality	Mean
Seasalt at 28 ppt. for 60 minutes	1	16.4	8.07
	2	8.5	
	3	7.1	
	4	6.0	
	5	5.9	
	6	4.5	
Malachite 1 ppm.	1	6.1	10.1
	2	5.8	
	3	10.4	
	4	22.1	
	5	8.6	
	6	14.2	
	7	4.3	
Control (No Treatment)	7 Trays		35.5

## Mortality 1987 Brood Coho SeaSalt vs Malachite



# NaCl & CaCl<sub>2</sub> Experiment

## Brood 1988 Chinook

NaCl & Hours CaCl <sub>2</sub>	Freq.	Tray#	%Mort	Mean
25 ppt. 2	3	1	63.7	61.6
25 ppt. 2	3	2	65.1	
25 ppt. 2	3	3	55.8	
25 ppt. 1	3	1	10.4	9.7
25 ppt. 1	3	2	9.5	
25 ppt. 1	3	3	9.2	
20 ppt. 2	3	1	25.0	22.4
20 ppt. 2	3	2	22.5	
20 ppt. 2	3	3	19.6	
20 ppt. 1	3	1	6.5	6.2
20 ppt. 1	3	2	5.3	
20 ppt. 1	3	3	6.9	

# Seasalt Experiment

## Brood 1988 Chinook

Seasalt	Hours	Freq.	Tray#	%Mort	Mean
25 ppt.	2	2	1	26.8	32.7
25 ppt.	2	2	2	36.1	
25 ppt.	2	2	3	35.1	
25 ppt.	2	3	1	16.0	14.5
25 ppt.	2	3	2	14.4	
25 ppt.	2	3	3	13.1	
25 ppt.	1	2	1	11.7	9.3
25 ppt.	1	2	2	9.0	
25 ppt.	1	2	3	7.1	
25 ppt.	1	3	1	11.2	9.6
25 ppt.	1	3	2	7.3	
25 ppt.	1	3	3	10.3	

## Seasalt Experiment Brood 1988 Chinook

Seasalt	Hours	Freq.	Tray #	% Mort	Mean
20 ppt.	2	2	1	18.0	19.5
20 ppt.	2	2	2	19.6	
20 ppt.	2	2	3	20.9	
20 ppt.	2	3	1	14.1	13.5
20 ppt.	2	3	2	13.6	
20 ppt.	2	3	3	12.9	
20 ppt.	1	2	1	14.2	14.0
20 ppt.	1	2	2	13.0	
20 ppt.	1	2	3	14.8	
20 ppt.	1	3	1	8.4	8.1
20 ppt.	1	3	2	8.2	
20 ppt.	1	3	3	7.6	

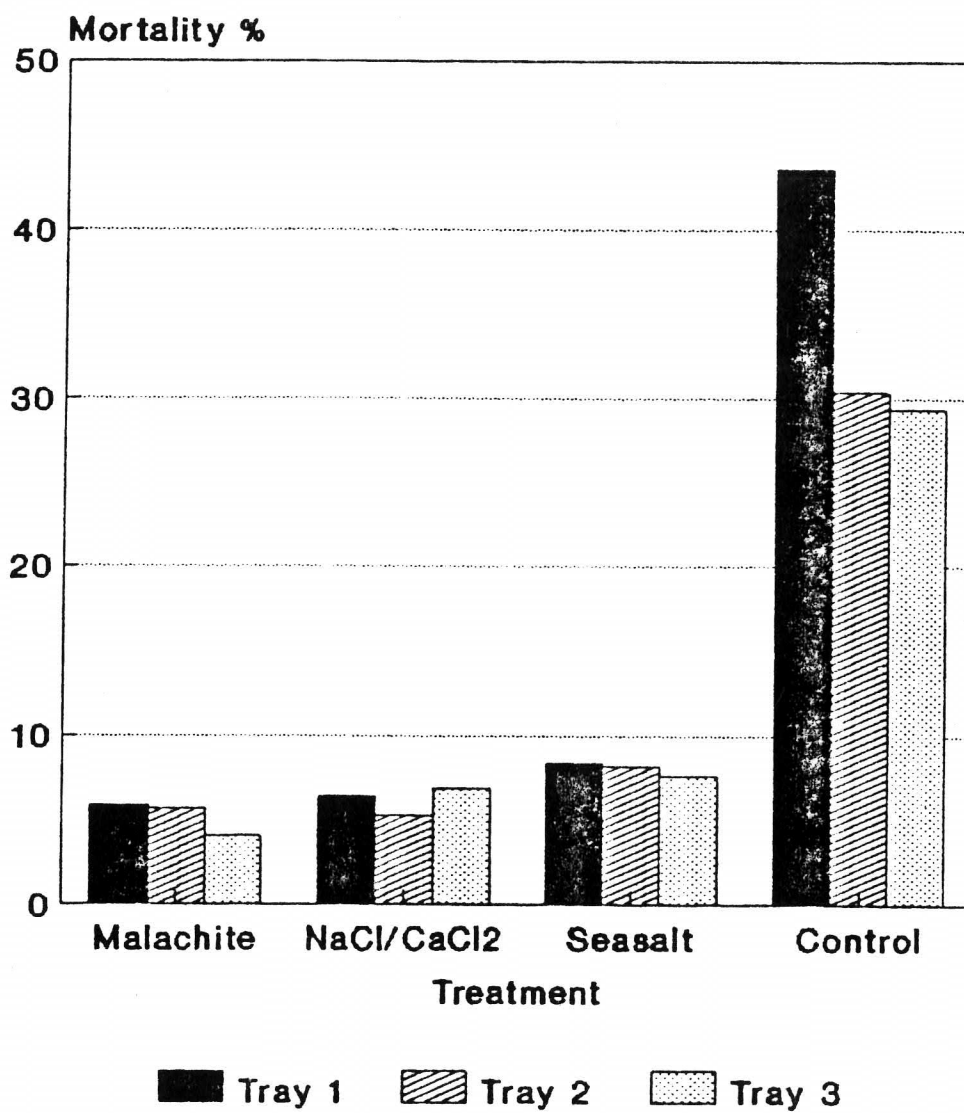


# Seasalt Experiment

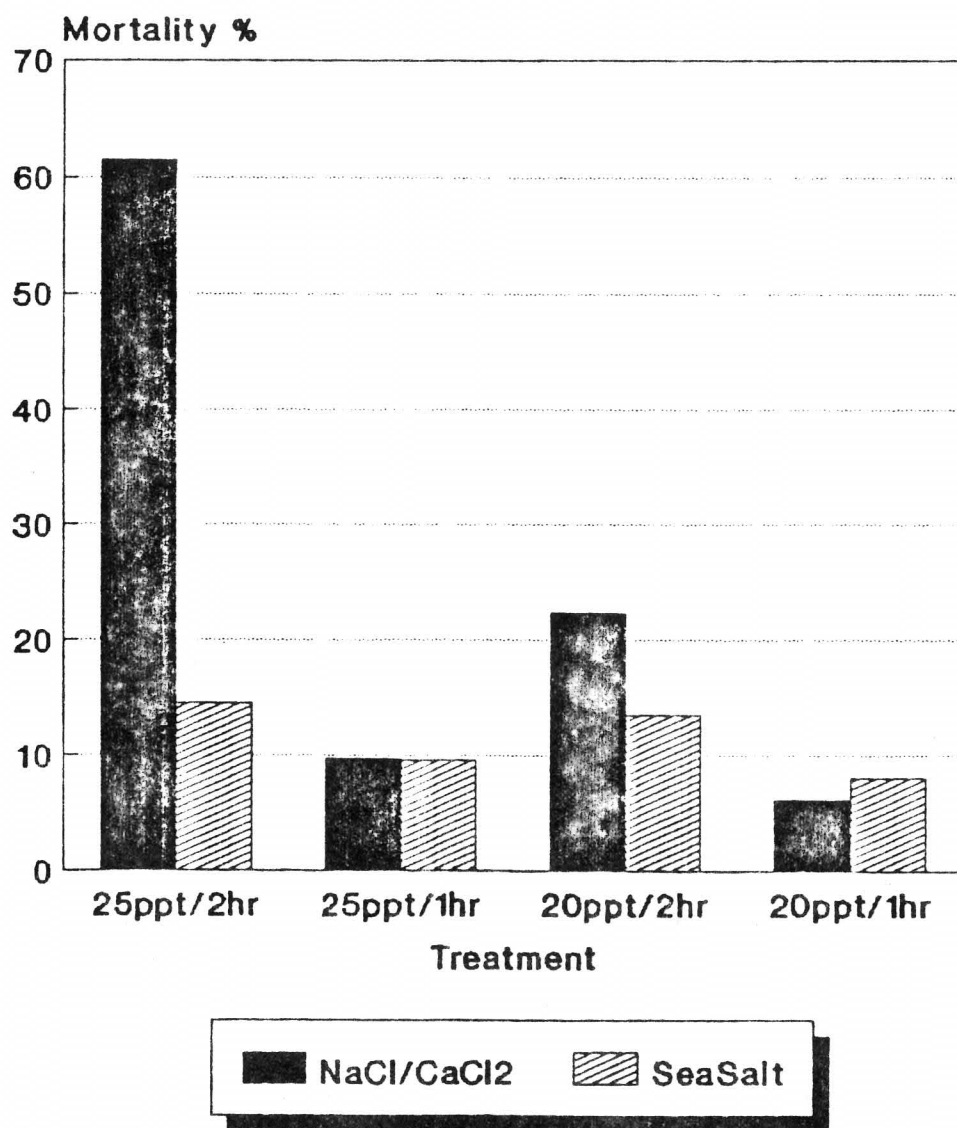
Brood 1988 Chinook

Seasalt	Hours	Freq.	Tray#	%Mort	Mean
10 ppt.	2	2	1	45.6	46.1
10 ppt.	2	2	2	43.4	
10 ppt.	2	2	3	49.3	
10 ppt.	2	3	1	46.1	41.7
10 ppt.	2	3	2	32.0	
10 ppt.	2	3	3	47.0	
Malachite					
1 ppm.	1	3	1	5.9	5.2
1 ppm.	1	3	2	5.7	
1 ppm.	1	3	3	4.1	
Control			1	43.7	34.5
Control			2	30.4	
Control			3	29.4	

## Rob Cr Salt vs Fungus Exp 1988 Brood Chinook



## NaCl/CaCl<sub>2</sub> vs SeaSalt 3 treatments per week



## Cost ; One eight tray Heath stack

NaCl & CaCl<sub>2</sub> at 20 ppt.

NaCl = \$0.16 per kilo.

CaCl<sub>2</sub> = \$1.40 per kilo.

Total cost per stack = \$7.58.

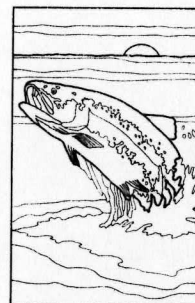
64000 Chinook eggs.

96000 Coho eggs.

Bulk NaCl which makes up 75% of the cost can be purchased at a lower cost.

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## Oxygenation/Water Quality



## Oxygen Delivery Systems in B.C. Transport Trucks

Grant Gale, Fish Culturist

BC Min of Environment Fisheries Branch

Fraser Valley Trout Hatchery

Abbotsford, BC

### Introduction

The Fish Culture Section of the B.C. Ministry of Environment Fisheries Branch is responsible for stocking the lakes and rivers with trout for sportsfishermen. Species reared by provincial hatcheries include Rainbow, Cutthroat (Anadromous, Coastal and Yellowstone), Steelhead, Brown, Brook, Dolly Varden, and Lake Trout. All of the eggs for these fish come from wild sources, with the exception of the Coastal Cutthroat and two stocks of Rainbows, which are domesticated from originally wild stocks. In 1987, the F.C.S. released approximately 11.3 million fish, weighing about 83,000 kilograms, into 1070 lakes and streams throughout the entire province. Since only six hatcheries, located in Duncan, Abbotsford, Cache Creek, Summerland, Cranbrook, and Hudson Hope (mostly in the southern part of the province), produce these fish, the stocking program requires an effective live fish transport system.

### Transport Equipment

The F.C.S. currently has several different styles of fish transport equipment, ranging from pick-up trucks with

removable 100, 150, and 200 gallon (450, 700, and 900 liter) tanks to a 3200 gallon (15,000 liter) converted milk tanker. The list includes 2-ton trucks with 500 gallon (2200 liter) tanks, 5-ton trucks with 1000 gallon (4500 liter) units, and a flat deck semi-trailer with 5 tanks capable of carrying 1800 gallons (8200 liters). The smaller tanks can be carried by a forklift for moving fish in the hatchery, while the semi-trailers are generally used to move fish between facilities. Aircraft, both fixed-wing and helicopter, are required to fly fish into areas which are inaccessible by truck or short hike. Sometimes, a fair bit of ingenuity may be required to put fish into some lakes. Fish are carried by bucket, in back-packs lined with plastic bags, and on various types of all-terrain vehicles.

### Loading

The loading rate is generally 1 kilogram of fish per 10 liters of water, which can be adjusted as to our experience with a specific stock of fish, the condition of the fish, and the duration of the trip. We have gone as high as 2 kilos per 10 liters (200 grams per liter) for 2 - 3 gram fry loaded into 50 liter fry cans for air liberations (short flights). Certain stocks and species handle transport better than others, and robust fish tend to travel better than less vigorous fish.

Transport times are generally less than 10 hours, but are frequently over 30 hours. In emergency situations,

we've held fish as long as 50 to 60 hours. Holding fish in transport overnight is a common practice. Air flights usually last about 2 hours, but can be up to 5 or 6 hours.

The fish are starved for a minimum of 36 hours to reduce ammonia output and reduce particle contamination of the water. We've found that ammonia output is reduced by half after about 48 hours, and doesn't decrease appreciably after that. Fish tanks are usually flushed with fresh water after loading, and if necessary, may be flushed during transit if a secure water source can be located.

#### Oxygen Delivery System - Past

The former oxygen delivery system used, for diffusion, 1/4" Tygon tubing perforated with several rows of needle holes (3/8" spacing). This Tygon was strung through either aluminum racks, or loops welded to the tank bottom. The holes are the restricting, therefore the regulating, points in the system. The volume of oxygen delivered to the fish is controlled by adjusting the pressure in the system, forcing more or less oxygen through the holes as desired. This system releases a bubble with a large surface to volume ratio, hence, there is considerable wastage.

In the bottle compartment, there are two bottles with adjustable regulators supplying lines which feed separately and directly into a manifold in the truck cab. This manifold is divided into two separate units, each with a single supply line, a pressure gauge, and valves for each



tank. Tygon lines run from the valves to the fish compartments where they connect to the perforated Tygon. The oxygen is turned on for both units of the system, and one unit is allowed to run, while the other is held in reserve. The pressure gauge is monitored for a decrease which indicates the supply is depleted in that bottle, and the other unit must be manually turned on. The oxygen delivery to each unit is controlled by the regulator on the bottle, which with only one control, is adjusted to compensate for the weakest tank.

#### Oxygen Delivery System - Present

The redesigned system has the two oxygen bottles, with adjustable regulators, teeing together to feed into a pre-set (50 psi) regulator. A single line runs from this regulator, mounted in the bottle compartment, to the truck cab and attaches to a manifold on the dash. The manifold, which is a single unit, has a pressure gauge and enough flow meters for each fish compartment. From each flow meter, two lines run from a 'Y' connector through shut-off valves to the fish compartments where they attach to Wilfley Weber ceramic micro-pore diffusers. Both oxygen bottles are turned on with the regulators set at slightly different pressures (80 psi and 90 psi) to allow for sequential operation. The flows to the tanks are adjusted with the flow gauges in the cab.

The advantages of the flow gauge system include:

- 1) independant tank adjustment cuts down on waste, by allowing reduction of the oxygen flow to tanks with fewer fish. This also permits these fish to travel easier as the oxygen levels can be maintained at closer to normal levels.
- 2) more precise adjustment of oxygen flow allows easier maintenance of dissolved oxygen levels in the tanks.
- 3) easier consumption calculations for planning.
- 4) automatic switching of bottles by differential gas pressures, reducing the risk of bottles running empty unnoticed. This also enables more complete use of the contents of the bottle as any bottle with less than 500 psig was considered empty and not used.

The ceramic micro-pore diffusers are about twice as efficient as the perforated Tygon for allowing oxygen to dissolve into water. In an experiment conducted at the Fraser Valley Trout Hatchery, 72% (by weight) of the oxygen supplied through the diffuser dissolved in the water as opposed to 38% of that supplied through the Tygon. Two 5-ton tankers were equipped with similar flow meter delivery systems to test the diffusers and Tygon in general use over a 3 month period. The truck fitted with diffusers required, in general, about 1/2 the amount of oxygen required by the Tygon to maintain an average load of fish. That is, 5.1 liters per minute flow through the Tygon versus 2.6 liters

per minute flow through the diffusers for a 500 kilogram load of 60 gram smolts loaded at 100 grams per liter.

One question mark concerns the ability of the diffusers to stand up to netting abuse and road vibration (particularly when the truck is empty). Another problem concerns the flow meters in which the flow indicators, particularly the T-type, stick when the flow drops. The ball-type riders stick also, but not quite as bad. Different makes of meters don't stick as bad as others. There is an operational error of 10% of scale for these meters, that is, a 5 liter per minute flow meter has a potential error of 1/2 liter per minute flow. When working at 1 liter per minute, this can be of serious concern.

And of course, there is always the concern of cut and pinched lines. This can be countered by inserting pressure gauges on the outlets of the flow meters. In the event that one of the lines or one of the diffusers is damaged, the valve can be closed and there will still be a supply of oxygen to the tank. Another complete supply line runs from the bottle compartment to the manifold, as a back-up in case of a line or regulator failure.

### **Future Additions**

The next step for us is to add a continuous oxygen monitoring system, with an oxygen probe mounted inside the fish compartment, and a digital readout panel mounted inside the cab. DO's can be continuously monitored and the oxygen

delivery adjusted to maintain, as close as possible, a stable oxygen environment during the various conditions encountered on a trip. Upper and lower DO parameters can be set, and attached to alarms for extra safety, aiding in the detection of changes before they become problems. This will also allow us to sleep better when overnighting fish, as a portable alarm can be carried into the sleeping quarters. There is not only the concern of system failures, but vandalism is a distinct possibility when the trucks are left unattended in motel parking lots at night.

#### Summary

The flow meter system is more complex than the old pressure system, however, it is a better system for fish transport. It is easily mounted in transport trucks, and in addition to the ceramic micro-pore diffusers, creates a reasonably efficient oxygen delivery system. The additional DO monitoring equipment delivers a continuous supply of data which allows fish culturists to manipulate the fishes' environment in transit. The alarm systems attached to the monitoring equipment creates a desirable safety indicator in the event of system changes. The results are long term cost savings, and most importantly, healthier fish.

## MANAGING A HATCHERY WITH OXYGEN INJECTION

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### ABSTRACT

An ATEC ZX oxygenation unit was installed at the Midway Hatchery in January, 1987. It has been in continuous use since then. Fish quality and growth have been enhanced. The spring source for the hatchery has a variable flow of 5 to 15 cfs and normally contains less than 5 ppm oxygen with nitrogen levels of up to 120%. With the addition of oxygen, the management of fish and rearing facilities has changed. Pounds of fish per gallon per minute and per cubic foot have increased from 4.37 to 9.5 and from .77 to 3.0 respectively. Conversions have fallen from 1.35 to 1.1. Growth rates have increased from .020 to .023 inches per day. Autopsies of rainbow trout showed no gas bubble disease problems in spite of above normal nitrogen levels, up to 110%, with oxygen levels at 8.6 ppm at an elevation of 5,400 ft. Total gas pressure was kept below 108% to avoid the stress of gas supersaturation.

### INTRODUCTION

The goal of a hatchery manager is to produce given numbers of fish of various sizes and species at a given time in the most efficient manner possible. The fish at the time of harvesting or stocking are to be of top quality to guarantee a suitable product for the market or to guarantee survivability in the wild to achieve the best possible return to the creel.

Stocking from state and federal hatcheries is usually seasonal and production has not been as intensive as in large commercial hatcheries that are processing and shipping on a regular basis.

In our state hatcheries we maintain annual production quotas that are considered to be an optimal loading level for each station. In most cases the carrying capacities of these stations have been derived by trial and error methods using experience and limited technology.

Our stations had been designed and constructed to operate at a given weight per cubic foot of water, weight per gallon per minute flow, and three to four exchanges per hour. Little consideration was given to water quality, temperature, or oxygen concentration.

Increased demands are being placed on our hatcheries by a growing population, increased popularity of fishing, and new management philosophies. Program changes such as year-round fishing, urban fisheries, new species, increased stocking size, extended stocking period, and new reservoir development are all leading towards more intensive culture at our stations.

Space is not the factor which is limiting our ability to adjust to these new demands. Rather, limited water resources are preventing these new fish needs, therefore, we must better manage our water systems and strive to improve water quality by "stripping" harmful

gasses and introducing oxygen to the concentrations needed for increased production.

In 1955, Haskell stated that carrying capacity is based on the following two assumptions:

1. Carrying capacity is limited by (a) oxygen consumption and (b) accumulation of metabolic products.
2. Amount of oxygen consumed and quantity of metabolic products are proportional to the amount of food fed.

Speece, 1981, states that dissolved oxygen (D.O.) is just as basic a necessity in fish production as feed, and excess dissolved nitrogen (D.N.) can be as lethal as "ick". However, the management of these two gasses does not generally receive the same priority in hatchery design and operation as do feed and "ick".

## HISTORY

Midway Hatchery is located at an elevation of 5,430 ft. above mean sea level and the water temperature averages 56.5° F. Oxygen levels of incoming spring water averages 5.8 ppm: saturation at this elevation should be 8.6 ppm. Flow varies from 5-15 cfs with the

high flows in late summer. Percent nitrogen saturation averages 117% and CO runs from 14-48 ppm with an average of 28.6 ppm.

Three series of cement raceways are available. At a depth of 1.5 ft. we would have approximately 83,500 cubic ft. of pond space. Ponds were constructed near the spring source and there is very little fall from the spring to the head of the raceways and between series of ponds. The system allows for very little aeration or nitrogen removal from cascading.

It is evident that Midway Hatchery has several adverse conditions involving water quality and hatchery design. These raceways have not been utilized to full capacity because of low oxygen and high nitrogen in the incoming water.

A hatchery with a flow of 12 cfs of good quality water could produce approximately 30 to 40 pounds of fish per gpm flow, or 162-216,000 pounds per year. Previous records indicate that only 12 pounds per gpm flow were produced in cement raceway systems, due to poor water quality and physical structure of cement raceway systems. The total production at the station was approximately 192,000 pounds per year. Only 1/3, or 64,000 pounds were being produced in cement raceway systems. Yet, 129,000 pounds of fish were being produced in earthen systems below cement raceways.

New and improved technology and equipment has been developed in



the last decade which have given the culturalist more opportunity to effectively and economically manage water quality. As a result, our people began looking for an aeration system to improve water quality to allow full utilization of water and pond space available in cement systems.

### OXYGENATION

The ATEC ZX Oxygenation System was selected for use at the Midway Hatchery and was put into full operation in January 1987. This unit met the constraints listed by Speece (1981) and several other criteria set up by administrators.

A very small portion of the water supply is pumped through the ZX Unit where it is supersaturated with oxygen, returned and mixed with the main flow to achieve an oxygen concentration equal to 100% saturation. The unit is fully automatic including the control of low water/high water level, low oxygen/high oxygen, and power out alarms. An auxiliary power system was supplied by the state which comes on line automatically when there is a power failure.

### OBSERVATIONS

As we inject high concentrations of oxygen into the water we find that we are also stripping dissolved nitrogen. As shown in Figure 1 as the oxygen increases % total gas saturation increases while the % saturation of  $N_2$  decreases.

Growth of rainbow trout was compared in three levels of oxygen - 5.15 ppm, normal spring water low, 7.5 ppm medium, and 8.4 high. Each level was duplicated, using 2,000 fish in each unit. Flow and density indices were held constant, and the same hatchery constant was used was used to regulate amounts of feed to be fed. Fish health was monitored by using AUSUM, a Necropsy Based Fish Health Assessment System by Goede (1987).

Preliminary analysis of data at 253 days was as follows:

Production Parameters	Raw Water	Medium $O_2$	High $O_2$
Avg. daily length increase	.0150	.0230	.0225
Avg. Weight/fish	22.63	52.90	51.70
Feed Conversion	1.89	1.21	1.20
Fat Index (Goede 1987)	2.90	2.95	3.00
Condition factor (Piper et al)	4010	4047	4173
$K_{tl}$	1.11	1.12	1.16

One can observe the increased performance of rainbow trout using oxygen injection at Midway Hatchery under experimentally controlled conditions. Our main concern at this time is to monitor benefits of oxygen injection under normal production circumstances.

We have selected one raceway to compare old carrying capacity with

new carrying capacity after oxygen injection. We reached 5.0 ppm D.O. at the tail end of this system with 5024 lbs of 6 in. fish with 530 gpm inflow, resulting in a flow index (Piper, et al) of 1.58.

Comparison of this information to previous production reads as follows:

Production Parameters	Old	New
D.O.	6.2-5.0	8.6-5.0
Gal/min.	530	530
Lbs. fish	2,316	5,024
Length fish	6"	6"
Flow index	.73	1.58
Lbs/gpm	4.37	9.5
Volume cu.ft.	3,000	3,000
Lbs/cu.ft.	.77	1.68
Density	.13	.23
Number fish	27,792	60,288
Daily Length Increase	.020	.023
Conversion	1.35	1.10

## DISCUSSION

Using a density index of .5 and a volume of 3600 cu.ft we should be able to raise 3 lbs/cu.ft of 6" fish in this raceway with an inflow of 1139 gpm. The weight of fish would be 10,800 lbs @12\lb. for a total of 129,600 fish.

This data was from a single pass situation in one series of ponds. We could use the 12 cfs in one series to an oxygen level of 5.0 ppm introduce oxygen to saturation of 8.6 ppm and deliver it to the

next series. We would then have the possibility of a triple pass situation using oxygen saturated water to operate each series.

Using a flow index of 1.58 and a flow of 12 cfs we find that we could possibly raise 153,490 lbs. of 6 in. fish compared to 23,535 lbs at flow index of .77.

Using a density index of .5 we find that we will need 51,163 cu. ft. of pond space. We have 83,500 cu.ft. of pond space available, therefore, we would be using 61% of our pond space rather than 28%.

#### CONCLUSION

Managing a fish hatchery with oxygen injection is very challenging and the increased production potential astounding.

There is no question that oxygen injection improves water quality and increases production capabilities. We are continuing to evaluate oxygen injection and planning expansion of the system to further increase production. Some reconstruction will be needed to change delivery channels between systems and additional injection sites will be selected. We feel that oxygen injection will help meet increased demands on our limited water resources.

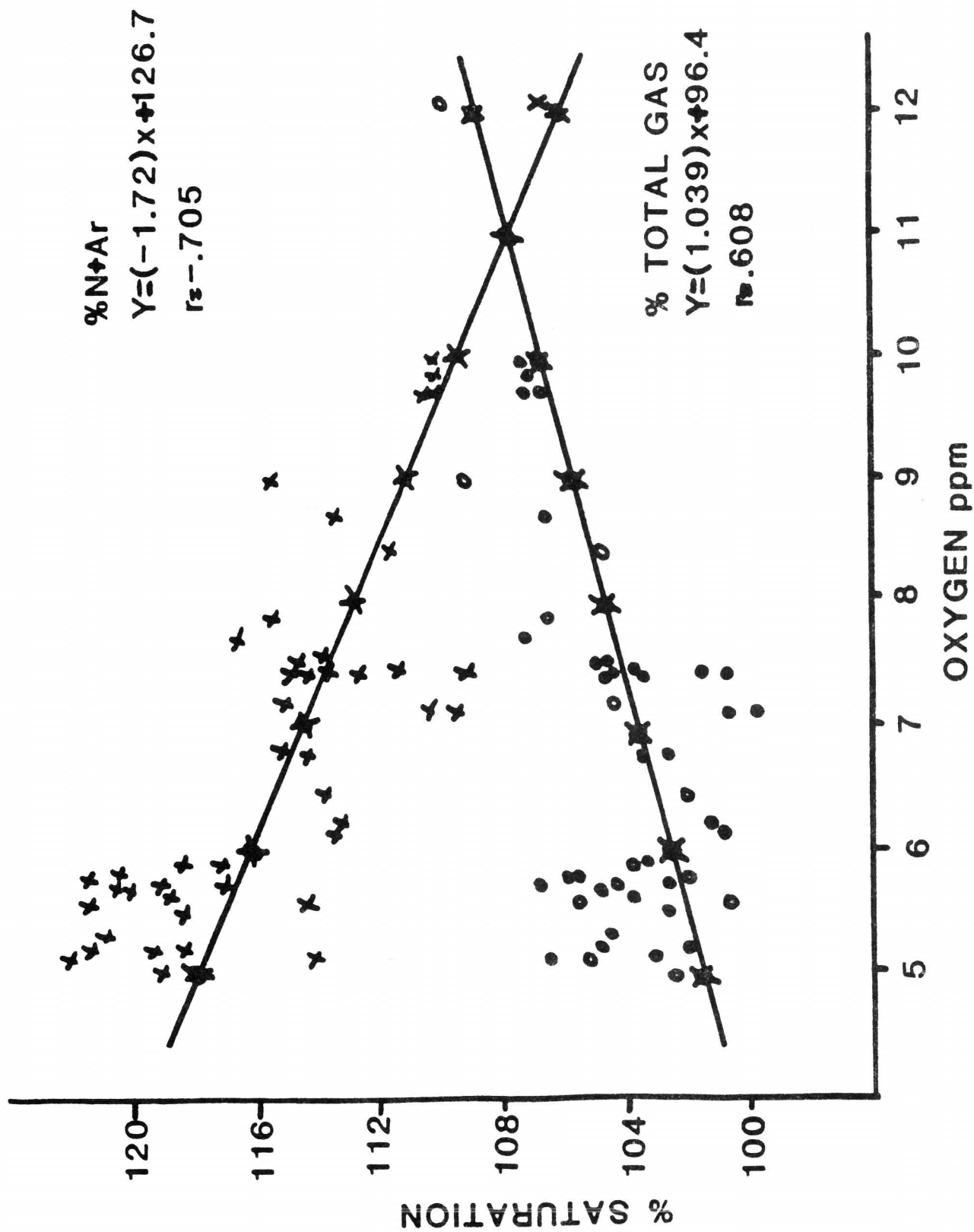


Figure 1. % Saturation total gas and nitrogen at various levels of injected oxygen.

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Performance of rainbow trout and Snake River cutthroat trout  
reared in oxygen supersaturated water

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### Abstract

Rainbow trout Salmo gairdneri reared in water containing oxygen at 180% or 94% of saturation at the Bozeman Fish Technology Center for 125 days showed no difference ( $P < 0.05$ ) in growth or feed conversion. Hematocrits were reduced in fish held in the supersaturated oxygen environment, but returned to normal levels within 35 days after fish were returned to water below oxygen saturation; mortality was not affected. Hemoglobin levels were not statistically different between treatments.

Cutthroat trout Salmo clarki reared in 183%, 127%, or 97% oxygen supersaturated water at the Jackson National Fish Hatchery for 91 days also showed no differences in growth or feed conversion. Hematocrits were lower as dissolved oxygen was increased. There were no differences in mortality during handling or distribution stress tests.

Gas bubble disease was not detected in fish from either experiment. Results of these trials indicate that long term culture in water saturated up to 183% oxygen has no adverse impact on growth or survival of these two species.

## Introduction

Oxygen supplementation of hatchery water supplies is receiving increased attention now that the technology for efficiently injecting oxygen into water is readily available. Benefits include increased carrying capacity, better water quality and improved fish health. Although information is available describing various oxygen injection system designs and efficiencies (USFWS 1987), little has been published on how fish performance and health are affected by high oxygen levels.

When water supersaturated with oxygen is used in water reuse situations, fish held in first-pass water are subjected to supersaturated oxygen. Oxygen is usually at or below saturation after the water has been used once or twice. Fish hatcheries of the Michigan Department of Natural Resources have been rearing trout in water supersaturated with up to 180% oxygen, with no apparent ill effects. The result has been an overall savings in feed costs due to improved feed conversion (H. Westers, personal communication). However, data on survival of fish after stocking into natural waters have not been collected.

We conducted two studies; one a laboratory trial at the U.S. Fish & Wildlife Services' Fish Technology Center, Bozeman, MT, and the other a production scale test at the Jackson National Fish Hatchery, Jackson, WY. The main objective was to determine the effect of supersaturated oxygen levels on growth and feed efficiency of trout. Effects on hematocrit, hemoglobin, and post-rearing survival were also investigated.



## Methods

### Bozeman tests

Kamloop strain rainbow trout Salmo gairdneri averaging 6.3 g were divided into six lots of 300 each and placed in 20 gal rectangular aluminum tanks. The initial density index (weight of fish/(volume of rearing space x length of fish); Piper et al. 1982) was 0.38. Three tanks received 51 F spring water that had been supersaturated by injecting oxygen supplied by a model T-2 Nitrox oxygen generator into the water supply line. Water and oxygen were mixed in a closed column of polyvinylchloride pipe (length, 5.5 ft; diameter, 4 in) containing 5 ft of Koch rings, 1 in in diameter and collected in a 20 gal head box. The other three tanks (controls) received unaltered 51 F spring water.

To obtain the desired effluent dissolved oxygen levels of 6 mg/L in the control tanks and 12 mg/L in oxygen supplemented tanks, the initial water flow was set at 1 gpm, resulting in a flow index (weight of fish/(length of fish x water inflow); Piper et al. 1982) of 1.2. Oxygen flow was set at 0.15 L/min into the oxygen supplemented water. The water flow was later adjusted to 1.5 gpm to maintain flow indices below 1.5.

The average inflowing oxygen saturation was 180% (17.0 mg/L), in the supplemented water and 94% (8.6 mg/L) in the control water. Nitrogen saturation was 82% in the treated water and 108% in the control water. Total dissolved gas saturation was 103% in the treated water and 105% in the control water.

Fish were hand fed 6 times per day at a hatchery constant of 9 according to the method of Buterbaugh and Willoughby (1967). Silver Cup Trout Feed (Murray Elevators, Inc., Murray, UT) was the diet used throughout the trial. Tanks were cleaned and mortalities recorded daily. Fish were weighed every 2 weeks and the amount of feed fed adjusted weekly.

When density indices reached 1.0 after 63 days, the number of fish in each tank was reduced to 200. Hematocrits were determined on 10 fish per tank (30 per treatment). Another 10 fish from each tank were fin clipped and stocked into a 0.1-acre pond (temperature, 38 F) on the Center grounds, to test for differences in survival. The number of surviving fish was determined when the pond was drained after 37 days. The rest of the excess experimental fish were placed in tanks receiving regular spring water. After 11 days, hematocrits were determined on 40 of these fish (20 per treatment).

After 103 days, when density index again reached 1.0, fish numbers were reduced to 100 per tank. Hematocrit and blood hemoglobin levels (cyanmethemoglobin method; Miale 1982) were again determined on 10 fish per tank (30 per treatment). The pond experiment was repeated as before. Fish were removed from the pond after 35 days. Hematocrit and hemoglobin levels were determined from 10 fish per treatment, and condition factors from 15 fish per treatment.

The trial was terminated after 125 days. Hematocrit and hemoglobin levels were determined from 10 fish per tank and condition factors from 12 fish per tank. Weight gain and feed

conversion was calculated for each treatment (average of three tanks). The data was analyzed by analysis of variance (Hintze, 1987).

#### Jackson tests

Snake River strain cutthroat trout Salmo clarki averaging 1.4 g were divided into six lots of approximately 47,000 each and placed in concrete hatchery tanks holding 1,635 gallons of water. The initial density index was .32. Approximately 87 gpm of pumped well water (temperature, 48 F; initial dissolved oxygen, 8.87 mg/L) was introduced to each tank via polyvinylchloride columns (length, 5.5 ft; diameter, 10 in) that were packed with 2 in diameter Koch rings.

The columns to four of the tanks were closed (sealed) to the atmosphere. Supplemental oxygen supplied by a Nitrox model T-8 oxygen generator was injected at the top of the columns and mixed with water inside. Two tanks received the highest amount of oxygen (175% of saturation) and the other two tanks an intermediate amount (125%). Two control tanks that received no supplemental oxygen were outfitted with "degassing" columns open to the atmosphere.

For the trial, the average gas saturation level was 183% oxygen, 79% nitrogen, and 101% of total gas pressure in the high oxygen water. In the intermediate oxygen water, saturation was 127% oxygen, 93% nitrogen, and 100% total gas. Saturation of the control water was 97% oxygen, 101.5% nitrogen, and 100.5% of total gas pressure.

Fish were fed 8 times per day with automatic feeders.

Silver Cup Trout Feed was fed throughout the trial. A hatchery constant of 6 was used to determine the initial feed rate. After the first two weeks the feeding rate was lowered (hatchery constant of 5) to avoid feed wastage. Each lot of fish was sample counted every two weeks, and the amount to feed was adjusted weekly.

After 42 days, when the density index reached 0.5, the weight of fish in each tank was reduced to 175 lb. Hematocrits were obtained from 30 fish per tank (60 per treatment). Mortalities due to handling stress were recorded for each treatment. An effort was also made to evaluate differences in distribution stress and stocking survival. Excess fish from each of the experimental tanks were placed in separate compartments of a distribution tank and held there for three hours before release into outdoor raceways. Mortalities from each group were recorded for 4 days. Another 200 fish per tank (400 per treatment) were marked with fin clips and stocked into a five acre reclaimed gravel pit pond (temperature, 12 C; dissolved oxygen, 10.3 mg/L) located 20 minutes from the hatchery. Wyoming Game and Fish Department employees later attempted recapture with electro-shocking equipment and gill net sets.

The trial was terminated after 91 days. Average weight gain and feed conversion were determined for each treatment. Hematocrits were determined from 30 fish per tank. Condition factors and dorsal fin index, ((height of dorsal fin in cm x 100) /total length in cm; Kindschi 1987) a measurement of fin

erosion, were determined from another 30 fish per tank. Ten fish per tank were preserved in Bouins' solution for later histological examination. The data was analyzed by analysis of variance and t-tests (Hintze, 1987).

### Results

Bozeman tests There were no differences ( $P < 0.05$ ) in average weight gain, feed conversion, survival, or condition factor of rainbow trout raised for 125 days in oxygen supplemented water or in water below oxygen saturation (Table 1). Survival of fish after removal from the trial was unaffected by oxygen level. There were no mortalities in fish reared in oxygen supersaturated water and then placed in tanks with unsupplemented water. In two tests in which fish were removed from trial tanks and stocked into a hatchery pond there were no differences in survival rates between treatments after the 37- and 35-day periods (Table 2). Condition factors of fish recovered from the second pond stocking were similar between the two treatments.

Hematocrit levels remained lower ( $P < 0.05$ ) in fish held in oxygen supplemented water throughout the trial (Table 3). Hematocrits taken 11 days after the fish were removed from oxygen supersaturated water increased marginally from previous levels (39.0 to 40.0), as did those of control fish removed from the trial at the same time (42.1 to 42.7). Fish surviving 35 days in a hatchery pond showed no difference between treatments in hematocrits, but levels were considerably higher

than those of cohorts measured before the stocking (Table 2).

Hemoglobins of fish held in oxygen treated water for 103 days was depressed compared to that of control fish (Table 3), but there was no difference after 125 days. Hemoglobin increased from 8.21 to 9.77 g/100 mL in the oxygen supersaturated group and from 8.79 to 10.05 g/100 mL in the control group after fish spent 35 days in a hatchery pond. There was no significant difference in hemoglobin among treatments after this test.

#### Jackson tests

There were no differences ( $P < 0.05$ ) in average weight gain, feed conversion, or condition factor of Snake River cutthroat trout raised for 91 days in 183%, 127%, or 97% oxygen saturated water (Table 4). Mortality was low; any differences between treatments could not be determined because the sampling error in inventorying large numbers of fish was higher than the number of mortalities. Measurements of dorsal fin lengths showed slight erosion had occurred since initial measurements were made before the trial (Table 4) but there were no differences between treatments.

The only fish losses during the inventory on the 42nd day were due to physical injuries; handling stress was not a factor. Mortality was very low following the simulated fish distribution trip; fish showed no effect of oxygen supplementation on tolerance to distribution stress.

Electroshock sampling of the gravel pit pond 60 days after stocking failed to turn up any fish. The fish were thought to

be too deep to be reached with the electrodes because of warm mid-summer surface temperatures. Gill nets set 120 days after the stocking turned up one 8.5" fish that was from the control group. Due to the length of time elapsed before recapture efforts began, no conclusions could be drawn from this test.

Hematocrits were lowest ( $P < 0.05$ ) in fish raised in high oxygen saturated water (Table 4), and were highest in those raised in lower oxygenated water.

Examination of stained, 5-micron sections of preserved tissues of fish from each treatment showed no histological changes due to prolonged exposure to the oxygen levels used in this study.

#### Discussion

Fish growth was not affected by the high oxygen in either test. Since the water used for the control groups was well oxygenated, fish in all treatments were probably receiving all the oxygen necessary for metabolism. If control fish would have been held in poor quality water (<70% oxygen saturation) their level of feeding activity may have been reduced. Since the same hatchery constant was used throughout the trials to determine feed rate for fish in each treatment, any improvement in fish performance would be reflected in improved growth and feed conversion efficiency. In the present studies, neither feed conversions nor growth were improved ( $P < 0.05$ ).

Exposure of fish to lower dissolved oxygen increases oxygen affinity of the blood (Riggs 1970). In this study decreased hematocrits and hemoglobins in fish held in super-

saturated water probably resulted from abnormally high oxygen. This was expected, since fish have less need for oxygen transport capability when environmental oxygen is increased. Both hematocrits and hemoglobins returned to that of controls when fish were moved to unsupplemented water. Results of these studies suggest that fish reared in water oxygenated up to 180% of saturation may be stocked into natural waters with no increase in mortality if dissolved oxygen concentration remains satisfactory.

No visible adverse affects from exposure to high oxygen were noted. Generally, total gas pressure must be 110% or more of saturation to cause gas bubble disease (Weitkamp and Katz 1980). Adding oxygen to water through a sealed column has the additional advantage of displacing nitrogen below saturation and maintaining total gas pressure close to 100% of saturation (Dwyer, et al. 1988). This was evidenced in both the Bozeman and Jackson tests.

#### Acknowledgments

We thank the staffs of the Bozeman Fish Technology Center and Jackson National Fish Hatchery for their assistance in collecting data and caring for the fish.



Table 1. Performance of rainbow trout raised in oxygen supplemented water supply for 125 days.

Oxygen saturation (%)	Weight gain (g/fish)	Feed conversion factor	Survival (%)	Condition factor (Kx10-4)
180	31.5	1.24	91.7	0.1015
94 (control)	29.5	1.29	94.3	0.0998

All values are means of three replicates;

initial average weight, 6.3 g;

feed conversion = feed offered/weight gained;

condition factor (K) = weight (g)/length (mm)<sup>3</sup>

Table 2. Survival, hematocrit, hemoglobin, and condition factors of rainbow trout stocked in hatchery pond after removal from oxygen supplemented water (2 test replicates).

Oxygen saturation (%) and test	Survival (%)	Hematocrit	Hemoglobin (g/100 mL)	Condition factor (Kx10-4)
180				
1st	40.0			
2nd	90.0	50.0	9.77	0.0889
94				
1st	36.7			
2nd	93.3	53.0	10.05	0.0908

There are no significant differences in any column;  
 1st test, 63 d in trial and 37 d in pond;  
 2nd test, 103 d in trial and 35 d in pond.

Table 3. Hematocrit (Ht; %) and blood hemoglobin (Hb; g/100 mL) of rainbow trout reared at two oxygen levels.

Oxygen saturation (%)	Variable	Days after start of trial		
		63	103	125
180	Ht	39.0a	43.5a	38.3a
	Hb		8.21x	8.58
94 (control)	Ht	42.1b	49.4b	41.2b
	Hb		8.79y	8.64

In each column, values followed by a different letter are significantly different from each other ( $P < 0.05$ ).

Table 4. Performance of Snake River cutthroat trout raised in oxygen supplemented water for 91 days.

Oxygen saturation (%)	Wt. gain (g/fish)	Feed conversion factor	Hematocrit		Condition factor (Kx10 <sup>-4</sup> )	Dorsal fin index
			6 wk	13 wk		
183	7.6	0.811	37.0c	35.2b	0.1007	9.83b
127	7.9	0.844	42.6b	35.4b	0.0988	9.90b
97	8.0	0.845	45.0a	37.6a	0.0990	10.16a

Initial average weight, 1.4 g; initial condition factor (K), 0.0935 x 10<sup>-4</sup>; initial dorsal fin index, 10.9

dorsal fin index = (dorsal fin length (cm) / total length (cm))100

In each column, values followed by a different letter are significantly different (P<0.05).

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## EVOLVING MANAGEMENT TECHNIQUES AT THE ARCATA

### WASTEWATER SALMONID AQUACULTURE PROJECT

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#### ABSTRACT

Three coordinated studies of urban pollution in Jolly Giant Creek in the spring of 1988 documented for the fourth year a serious loss of Arcata wastewater-seawater reared coho and steelhead smolts. Over one-third of marked out-migrant juvenile coho and steelhead sampled were moribund or dead. Results of an analysis of past data on smolts released and numbers of returning adults suggested that impaired imprinting and homing may have resulted from smolts being subjected to poor water quality in Jolly Giant Creek. Land use changes in 1988 at the Arcata STP utilized a site for locating an artificial home-stream to be operated with marsh effluent to avoid use of Jolly Giant Creek as a home-stream. Pilot project ponds are now designated for the site for imprinting smolts with treated wastewaters and for operating the fishway and holding ponds for returning adults. An initial study on assessing the effectiveness of complete netting of one of our 0-33 acre rearing ponds resulted in only a 5-8 percent greater survival in juveniles as compared with an adjacent unnetted pond, but we were unable to accurately partition mortality between possible causes. A newly redesigned 2,000-gallon recirculating aquarium system produced nearly 53,000 fry and fingerlings with a less than 5 percent mortality. Application of a commercial airstones (FAT CAT) in new configuration improved our pond aeration capabilities.

#### INTRODUCTION

The Arcata wastewater salmonid aquaculture facilities is located in the center of about 170 acres of former tidelands of the north arm of Humboldt Bay (Arcata Bay) northern California coast (See Allen, 1984, Figure 1). These tidelands provided space for a sewage treatment system (mainly lagoons and wetlands), and for lumber-related industries now re-developed into a mixture of freshwater, brackish and estuarine aquatic systems (Allen 1988; Allen et al. 1988). The area also contains vestigial estuarine and bay marshes (Gearheart et al. 1983). The entire complex is now called the Arcata Marsh and Wildlife Sanctuary (AMWS) (Figure 1). These habitats have been highly

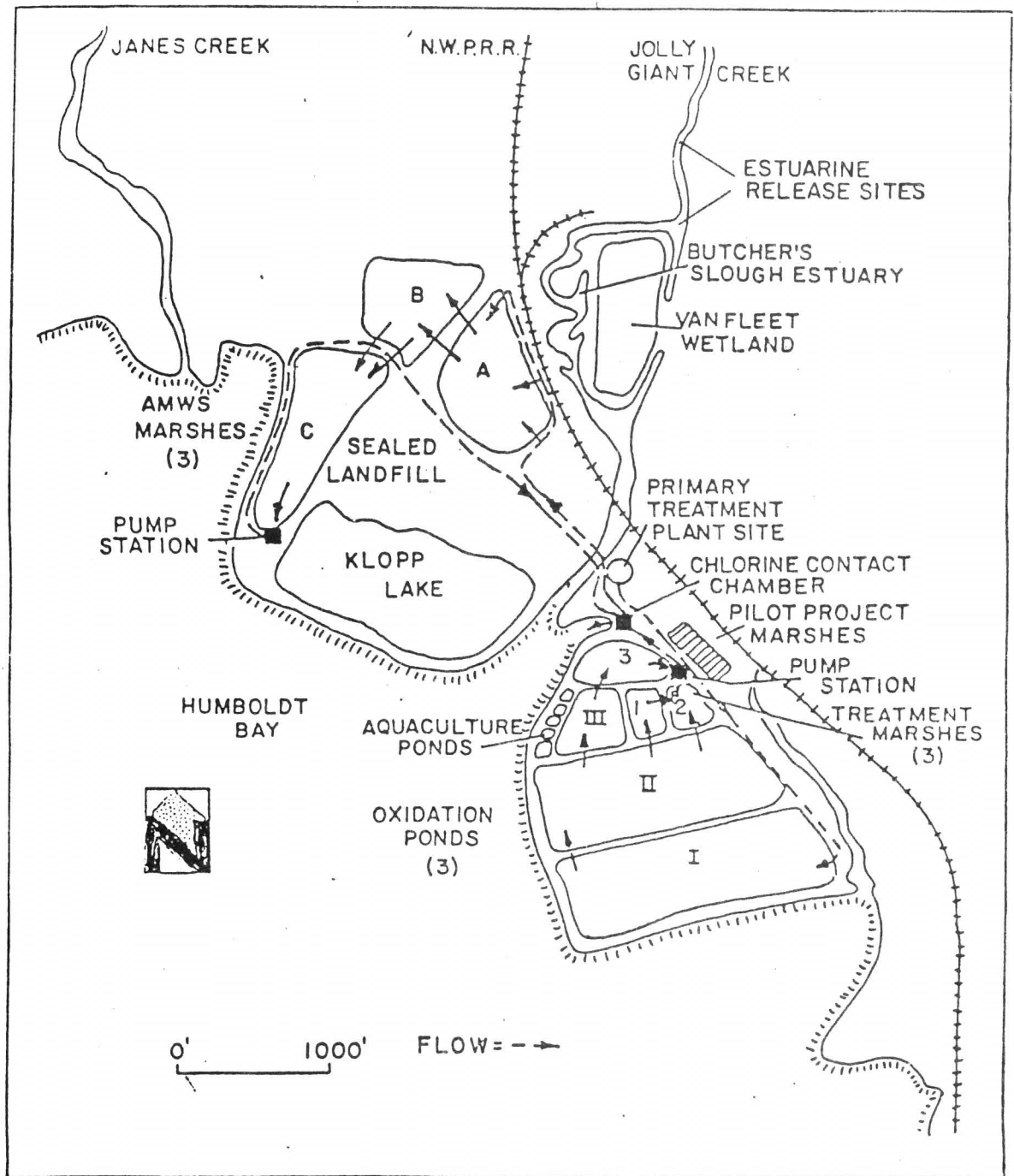


Figure 1. Butcher Slough estuarine release site for smolts and out-migrant juveniles, spring 1988, and location of Arcata Wastewater aquaculture ponds within the Arcata Marsh and Wildlife Sanctuary and Arcata sewage treatment system.

successful in attracting aquatic birds, many of which are fish-eating species (herons, egrets, cormorants, mergansers, ospreys, gulls, terns, grebes, kingfishers). The Arcata wastewater-aquaculture fish ponds, located within the periphery of the city's oxidation pond treatment complex, have always attracted these fish predators (Blankinship 1973). Initial defense against bird predation was primarily creating vertical pond banks, eliminating wading areas at the base of pond banks, and placing nets along the banks (Lutz 1985). Smolts and downstream-migrant juveniles produced in the ponds have been released into a small urban stream (Jolly Giant Creek) chosen as a temporary home-stream for returning adults (Allen 1984). Recent monitoring of the out-migration of juvenile salmonids released in the creek using downstream-migrant traps has shown non-point pollution causing serious mortalities during spring months (Allen 1987).

This paper reports on the results of studies on smolt outmigration and water quality in Jolly Giant Creek during spring of 1988, and an analysis of possible impairment of imprinting and homing of coho smolts from petroleum pollution. Plans on how our project ponds can be converted into a site for adult fishway and holding pens, and as a smolt imprinting migration route are presented. We also report on our first effort to evaluate the complete netting of a rearing pond to reduce bird predation, on the construction and initial testing of a redesigned recirculating system for increasing our project's fry production capacity, and finally on some improvements in pond aeration techniques.

#### WATER QUALITY AND SMOLT MIGRATION 1988

During the spring of 1988, we completed three studies to further document the impact of non-point pollution on juvenile salmonid downstream migrants in Jolly Giant Creek. Sax (1988) released groups of marked coho and steelhead trout at one estuarine and several inland sites (Table 1). Estuarine releases were into a sediment basin or in the main channel of a recently reconstructed estuary (Butcher Slough; Allen and Hull 1987) immediately upstream from the sediment basin (Figure 1). All inland release sites were located above the culverted downtown section of Jolly Giant Creek (see McFadden 1987), with the farthest upstream location at an adult fish trap located on the creek (Allen 1984, Plate F). Time of out-migration of released coho and steelhead juveniles was studied by sampling a total of eleven times between February 17 and May 1, 1988 at the release sites or closely adjoining habitats using seines. Yeoh (1988) compared mortality rates between these same groups of released juveniles by capturing out-migrants using two McBane DSM traps operated in parallel at a downtown site as reported in Allen (1986, p. 11) (Table 2). A third study (Denton 1988) bioassayed water of the creek using steelhead and coho juveniles held in small cages located in a sediment basin, in the main channel of the creek, and in a small wetland adjacent to the sediment basin (Figure 2).

Juveniles released into the estuarine site migrated soon after release (Left Dorsal Tag and RP coho, and LM steelhead, Table 1). Following release on 16 February, two LDT coho were recovered within two days and no more were recovered during the remainder of the sampling. Pond-reared coho (RP mark) released into the estuary between March 18 and April 27 were also virtually



Table 1. Species, number, and place of release of coho and steelhead smolts, and percent recovered by seining, Jolly Giant Creek, February-May 1988.

	Pond reared			Steelhead		Hatchery-reared (Growth accelerated)	
	Inland		Estuary	Estuary		Inland (Fish trap)	Coho Estuary
	Fish trap	Other sites					
Number released	46	167		610	365	144	83
Mark	LP	LP		RP	LM	Right dorsal tag	Left dorsal tag
Date released	8 Apr	18 Mar- 29 Apr		18 Mar- 1 May	13-16 Apr	16 Feb	16 Feb
Percent recovered by seining	6.5	2.9 <sup>1</sup>		2.2 <sup>2</sup>	5.6 <sup>3</sup>	4.9 <sup>4</sup>	3.6 <sup>5</sup>

<sup>1</sup>For all inland sites.

<sup>2</sup>Excluding large catch on May 1 when fish from pond draining released.

<sup>3</sup>No fish recovered after April 18.

<sup>4</sup>Recoveries only made from inland sites.

<sup>5</sup>No recoveries made upstream from estuary.

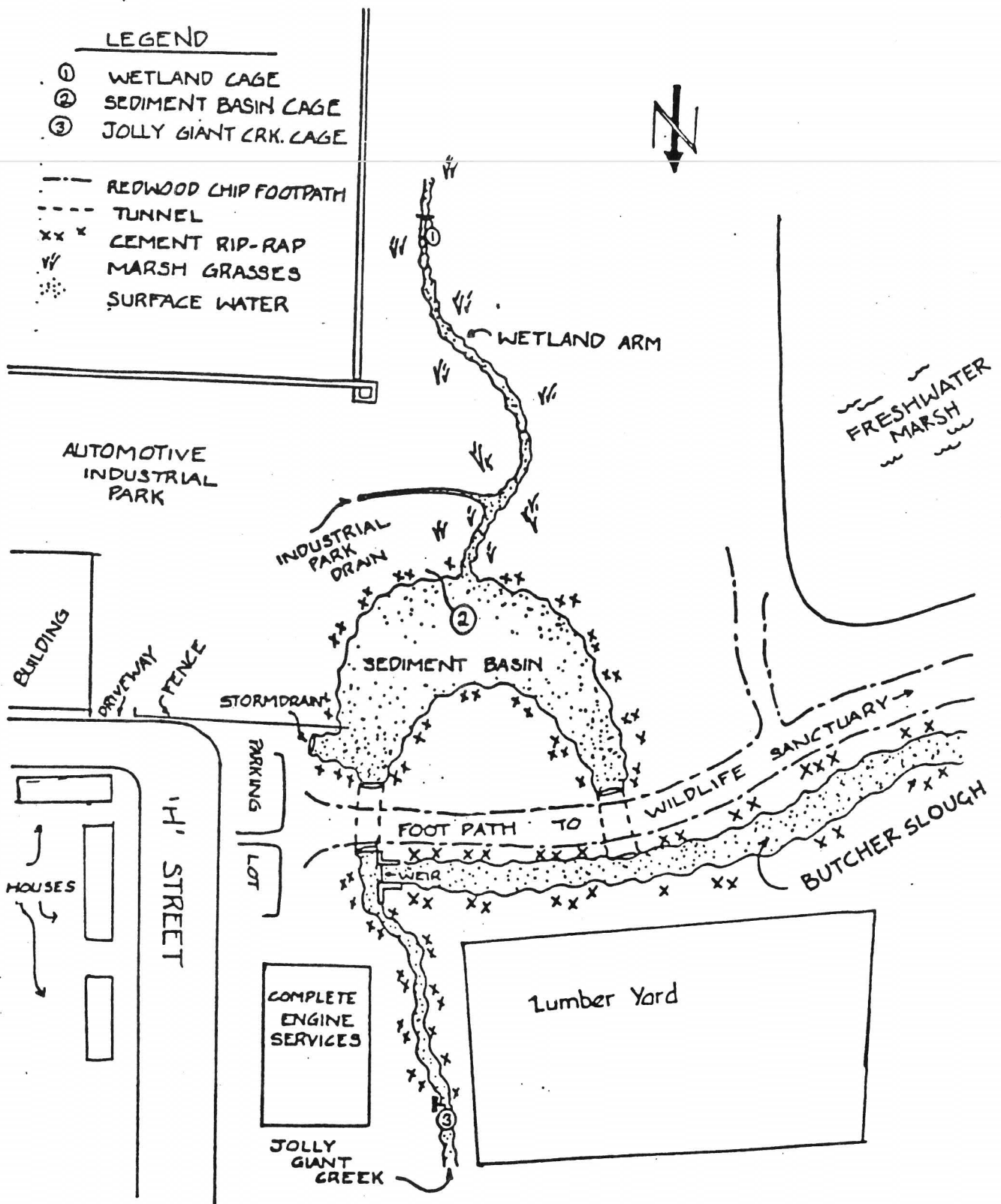


Figure 2. Bioassay stations at planting location of smolt and out-migrant juveniles released into upper Butcher Slough, spring 1988. (from Denton 1988, Figure 2).

absent from estuary seine sampling (three marks recovered through April 30). On May 1, a final plant of 291 RP-marked coho produced 53 marked fish on the final day of seine sampling (May 1). Of the 365 LM-marked steelhead juveniles released between April 13-16, 21 were recaptured on 18 April, with none captured on our last sampling day (May 1).

Table 2. Number by mark and percent moribund juvenile coho salmon recovered in McBane DSM-traps located in downtown Arcata, Jolly Giant Creek, 5 March-7 May, 1988 (adapted from Yeoh 1988).

Trapping period	Fin mark or tag						Moribund	
	LP	Right dorsal tag	RP	Ad	Um	Total	Number	Percent
5-21 Mar	0	0	0	0	0	0	-	-
23 Mar-3 Apr	Trap Inoperative							
7 Apr	2	1	0	0	0	3	1	33
8-30 Apr	29	2	1	0	3	35	15	43
1 May	6	-	-	1	0	7	0	0
2-9 May	0	0	0	0	0	0	0	-
Total	37	3 <sup>1</sup>	1	1	3	45	16	36

<sup>1</sup>All recoveries prior to April 10.

Groups of coho released at inland sites showed differences in time of out-migration. Growth-accelerated coho (Right Dorsal Tag) released February 16 (Table 1), were recovered at the release site for 52 days following planting. The last recoveries (3) were made on April 8. An additional three recoveries were made in the downstream-migrants traps between April 7-12, 1988. The pattern of out-migration correlated with a major increase in out-migration rate of coho smolts taken in pond smolt traps on April 8. The large coho (RDT mark) were subject to illegal fishing by juveniles (two fish observed caught, and fishing gear found on two occasions at the adult fish trap where fish were released). The large inland-planted coho delayed their out-migration until the normal period of downstream migration (April). In contrast the same fish when released into the estuary migrated quickly. The smaller-sized pond-reared smolts migrated rapidly from the creek on release as would be expected. Of the total of 213 LP-marked coho planted above the DSM traps, 37 were recovered (Table 1). The largest daily releases of these fish occurred between April 8-11 (79 fish), which correlated with the peak daily catch in the DSM trap of 9 fish on April 14. From a release of 46 LP-marked

coho made into the adult fish trap on April 8, 1988, seining captured three fish on April 13, and no LP-marked coho thereafter, indicating little delay in out-migration after release.

Heavy losses in 1986 releases of smolts was caused in part by a single illegal dumping of an industrial cleaning fluid mixture (xylene, benzene, toluene) into Butcher Slough sediment basin during the peak period of out-migration (Allen 1988). Recovery of moribund smolts in McBane traps located in the middle (downtown) section of the creek was associated with an unidentified petroleum compound(s). Such non-point urban pollution now of widespread concern (Bruhn 1988) continued to cause stress and mortalities in out-migrating smolts in the spring of 1988. Yeoh (1988) held coho smolts in a small cage downstream from her trapping site over a three-day period during a rainy period and found a 50 percent mortality. This indicated trapping stress was not the only possible source of mortalities in the trap-caught migrants. Between March 5 and May 9, 1988, a total of 45 out-migrant coho were caught of which 16 were either dead or dying (Table 2, 36 percent morbidity rate). Juvenile fish mortalities were also recorded in and around the estuary release site (Denton 1988). Caged steelhead and coho placed on the bottom of the sediment basin or in the main channel of Butcher Slough (Figure 3). showed a 67 percent mortality during studies conducted from March 3 to May 13, 1988. In contrast, caged fish placed on the bottom of a small wetland channel directly off the sediment basin had a 100 percent survival, with fish feeding and growing during the bioassay period.

Exact cause in mortality in Jolly Giant out-migrants was not determined, although circumstantial evidence continued to indicate a petroleum substance. Both peak smolt catches and high incidence of moribund fish were correlated with periods of light rainfall at the mid-town trapping site, although periods of no petroleum film alternated with periods of clear water during times of no rainfall. Denton, however, was not able to correlate stream freshets with mortalities in caged fish, although oxygen as low as 3 mg/l was recorded in bottom water of the sediment basin. Salinity meter assigned to the study malfunctioned as the wide range of salinities known to occur in the Butcher Slough channel was not documented for this study. Periods of floating grease and petroleum fluids were found intermittently in the sediment basin and main channel of Butcher Slough but these substances did not penetrate to the wetland bioassay site.

#### IMPRINTING AND HOMING

Repeated non-point pollution in Jolly Giant Creek during spring (April) outmigration periods now seriously compromises use of the creek for establishing any substantial or sustained run from our wastewater pond-reared smolts. This is seen by comparing the numbers of coho juveniles released into the creek (Table 3) to the number of adults recovered from Butcher Slough estuary or from an adult fish trap located inland on Jolly Giant Creek (Table 4). The lack of any recoveries either of jacks or as three-year-olds, of coho juveniles released in 1986, was reported previously (Allen 1987). The recovery, however, of unmarked coho released into the creek in 1986 was not reported. Large juvenile coho (3.2 fish/pound) from the Mad River hatchery were planted into the creek during November 1986. Behavior of the fish in the

Table 3. Number and mark of juvenile coho salmon reared in Arcata wastewater-seawater pond and released into Jolly Giant Creek, spring 1986-88.

Year of Release	Marks				
	Ad-CWT	RV	RP	LP	Unmarked
1986	3,900	400	-	-	(350) <sup>1</sup>
1987	607	-	-	-	-
1988	-	-	622	244	-

<sup>1</sup>Accelerated-growth smolts (3 fish/lb) from CFG hatchery on Mad River released December 18, 1986.

Table 4. Number of mark and age of adult coho salmon recovered in Jolly Giant Creek trap, creek, or closely adjoining regions, 1986-1988 migratory seasons.

Migratory season	Mark and Age							
	Ad-CWT		RV		RP		LP	
	1/1	1/2	1/2	1/2	1/1	1/1	1/1	1/2
1986/87	0	-	0	-	-	-	0	-
1987/88	(1) <sup>1</sup>	0	0	0	-	-	(1)	(6) <sup>2,3</sup>
1988 <sup>4</sup>	0	0	-	-	1	0	-	-

<sup>1</sup>Stray from smolt released by Fish Action Council into Freshwater Creek.

<sup>2</sup>Stray, unknown origin.

<sup>3</sup>Four females, two males.

<sup>4</sup>Recoveries through December 31, 1988.

creek was monitored by seining for their out-migration pattern. This was a cooperative study with California Department of Fish and Game, Mad River Hatchery. Within a few weeks these smolts were found to have left the creek during a period of heavy rain and associated storm runoffs. Since these fish were sub-yearlings they would have been expected to return as jacks in 1987 and three-year-olds in 1988. One such fish was recovered (Table 4) but might have been a stray since we recovered a Ad-CWT fish from Freshwater Creek also. Heavy rains beginning in late November and continuing through December 1988 produced runoffs sufficient to allow unimpeded adult salmon migration into the creek on three occasions. No unmarked salmon were recovered, but one marked RP-marked coho jack was recovered from the adult trap on November 28, 1988. This would represent a 0.6 percent return of jacks from the pond-reared coho released into Butcher Slough estuary. The return in 1987, however, of six unmarked three-year-old coho was both puzzling and intriguing. One carcass was recovered from Jolly Giant Creek in the center of the city, another carcass was found on the west bank of the oxidation pond just a few hundred feet from the Arcata STP discharge point, while four fish were taken inland from the adult fish trap. In July 1986, the Arcata STP discharge point was changed from direct discharge into Humboldt Bay at the south end of oxidation pond No.1 to a small inlet just north of the wastewater aquaculture ponds (Figure 1). Because there was a large return of coho to Humboldt Bay and to the Freshwater Creek drainage in 1987 (Table 5), it is possible that the wastewater discharge into Butcher Slough could have been an attractant, with adult coho then migrating up the creek under high flows.

Table 5. Total estimated return of adult coho salmon, and number of Ad-CWT tagged coho recovered, Freshwater Creek (east shore of north Humboldt Bay (compared with return and tags to Jolly Giant Creek, fall 1987-winter 1988<sup>1</sup> (from Brumback 1988).

Return site	Total run	CWT tagged fish recovered	Remarks
Freshwater Creek	834 est.	48 (1/2 age) (HFAC only) <sup>2</sup>	HFAC contribution estimate: 430 fish (0.9 percent return on smolts released)
Jolly Giant Creek	8	0	Two age 1/1 (jacks) recovered, including one CWT-HFAC coho released spring 1987.

<sup>1</sup>Includes data from Humboldt Fish Action Council trapping station and carcass surveys of entire watershed during 87-88 migratory season.

<sup>2</sup>One CWT from Mad River and Klamath River strays recovered.

In contrast to the potential of wastewater to attract migrating salmon, the complete analysis of the 1985-brood Ad-CWT returns suggested polluted water may impair imprinting and homing. Although heavy losses of 1985-brood

coho smolts were recorded in Jolly Giant Creek in spring of 1986, some Ad-CWT fish did reach the ocean as shown by the recovery of two tags in the Oregon ocean commercial troll and recreational fisheries (Table 6). No tags were reported from the California ocean commercial and recreational fisheries, nor were any found in a sample of 48 Ad-CWT tagged coho salmon recovered from a

Table 6. Summary of recoveries of Ad-CWT marked coho salmon smolts released spring 1986 in Jolly Giant Creek in Oregon ocean troll and recreational fisheries in 1987.

	Fishery		Totals
	Recreational	Commercial Troll	
Recoveries	1	1	2
Date caught	2 July	13 August	
Location	Brookings	Coos Bay	
Fork length	62.0	63.5	
Estimated contribution to fishery	1.65	4.12	6

trap located on Freshwater Creek (east side of Arcata Bay) (Hull 1987) and from carcasses sampled through the Freshwater Creek drainage (Table 5). Lack of tags from Freshwater Creek is significant since straying to nearby streams occurs from the release of smolts into the Butcher Slough estuary. In 1977 Jolly Giant Creek coho salmon strayed to Jacoby Creek at a 45 percent rate (Miyamoto 1979). In 1984, four Ad-CWT tagged Jolly Giant Creek three-year-old smolts were recovered as adults from nearby streams (Freshwater and Janes Creek) compared with a known total of 17 fish recovered from Jolly Giant Creek (Allen 1985). Lack of recoveries in Humboldt Bay streams of Ad-CWT smolts released in the spring of 1986 which were subjected to poor water quality in the upper estuary of Jolly Giant Creek appeared to avoid California waters and especially Humboldt Bay in 1987. Possible explanations for such adult coho behavior in 1987 would include the hypothesis that:

1. smolt imprinting was seriously impaired by the deleterious water quality conditions in Jolly Giant Creek during out-migration,
2. adversely conditioned smolts simply refused to re-enter Humboldt Bay waters, or
3. a combination of both factors plus random error from small numbers involved in the experiment.



We encourage other workers to examine records for aberrant results that might also be explained by an assumption of imprinting and homing impairment from adverse water quality.

#### WASTEWATER OPERATED HOME-STREAM

The as yet unresolved non-point pollution source in Jolly Giant creek has made the creation of a permanent home-stream for the Arcata wastewater salmonid culture system a major priority for our project. Use of effluent from the last of three AMWS enhancement marshes as operated in series with STP effluent has always been considered as a potential water source for operating both a smolt imprinting facility and an adult fishway and trapping system (Allen 1984). Availability of space, location of the final Arcata STP effluent pipe, quality of marsh water, conservative administrative policies in setting effluent standards, and availability of funding, have all made completion of the adult fishway and imprinting ponds a slow process (Allen 1988). The most recent site for locating the wastewater fishway (Allen 1988, Figure 2), is not now available. The space had to be used in 1988 to hold sludge removed from a former facultative pond during conversion to a wetland treatment pond. The sludge will be dried out, capped with impervious material, and the area utilized for vehicle and equipment storage. This change in land use forced another site for the fishway. Our latest plans now utilizes our North and South Pilot Project ponds as a site for using effluent for smolt imprinting as shown in Figure 3. AMWS water piped under pressure along the east side of our aquaculture ponds will have outlets to any of the ponds. The initial fishway will be designed into an existing two-foot diameter culverts connecting South pond with the bay. This new plan will allow for installation of smolt collecting and enumeration facilities between ponds. Juveniles will only have to be handled briefly for enumeration and marking on migrating into Arcata Bay via Butcher Slough, thus minimizing handling stress. The system will be especially appropriate for fall chinook salmon which has been the target species for the Arcata wastewater culture system since its inception. Chum salmon is another species well-adapted for use of our system as shown by the success of estuarine-located chum salmon aquaculture facilities around the north Pacific Ocean.

Unchlorinated marsh effluent is obviously the most desirable water for future smolt imprinting and adult fishway operation. The city will be approaching regional water quality control authorities for such beneficial reuse of marsh effluent soon (Allen 1988).

#### BIRD PREDATION

From mid-November 14 to mid-December 1986, a total of 4,500 coho juveniles were removed from one of our summer-rearing habitats (Summer Pond No. 1) marked with Ad-CWT and released into Winter Pond No. 1. Winter Pond No. 1 was dominated with a small green algae, *Nanochloris*. We have commonly called the form L.G.C. (little green cells) (Ramsey 1987). When L.G.C.'s are dominant, ponds are in a "green phase", as compared to early pilot-project



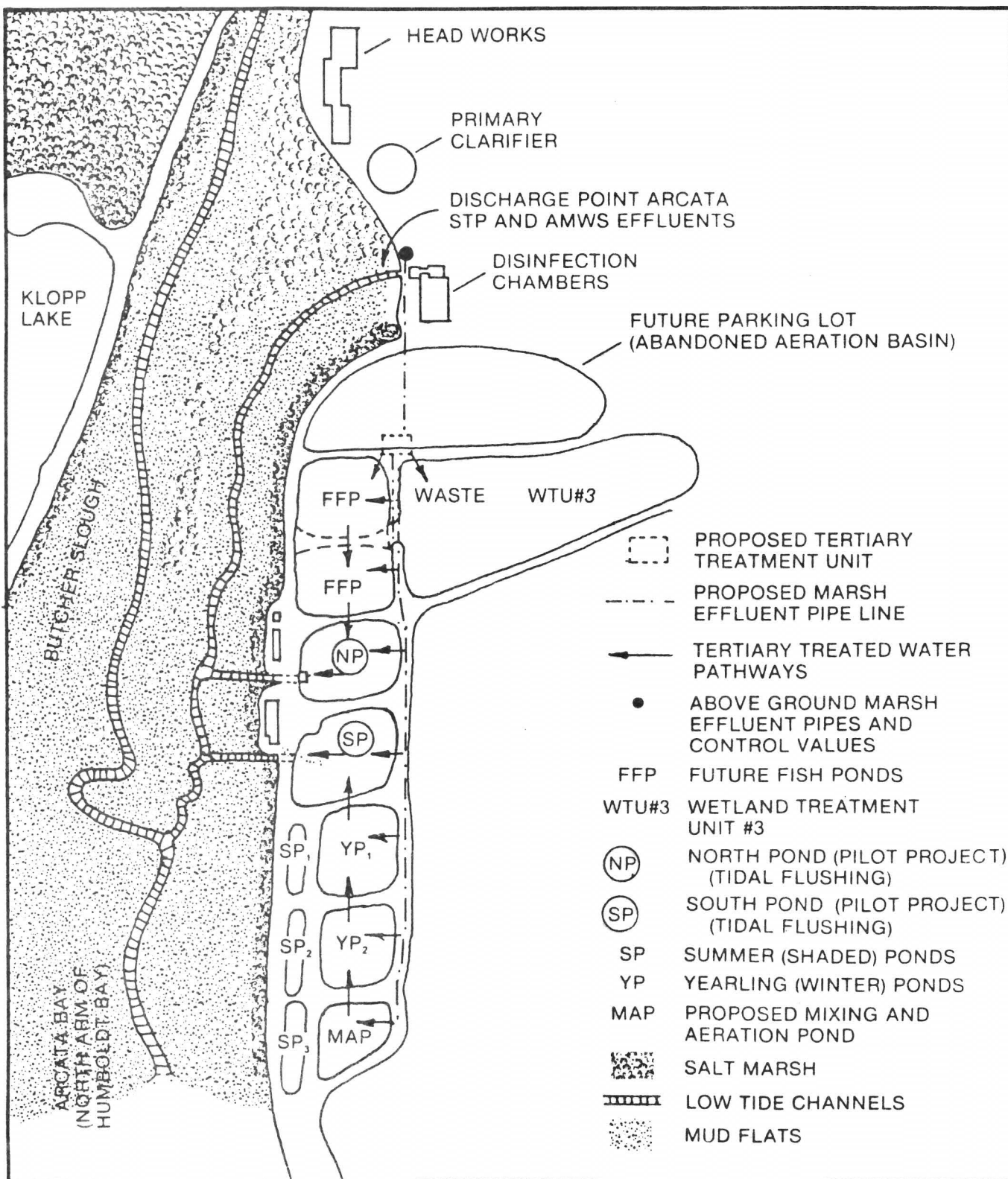


Figure 3. Proposed pathways of marsh effluent for smolt imprinting, and for operating adult fishways and holding ponds.

studies when ponds were usually dominated by the macrophyte Enteromorpha which produces a "clear phase" (Hedgepeth 1983). Enteromorpha provides a food base for gammarid amphipods and has appeared as a large percentage of stomach contents of steelhead-rainbow trout juveniles reared in Enteromorpha-dominated ponds (Allen et al. 1981). The overall contribution of L.G.C.'s to the food chain of juvenile salmonids has not been investigated. Dense algal cells has always been of concern as a possible gill filament irritant. Species succession, especially dinoflagellates, often causes sudden low dissolved oxygen resulting during population crashes. Pond mortalities also occur from toxic decomposition components when dinoflagellate blooms crash. With these factors in mind, we decided on using Winter Pond No. 1 for a rearing experiment using "green phase" water but with no supplementary feeding to test growth and survival. Previous work indicated at least 10,000 coho smolts could be produced in pilot project ponds without supplemental feeding. We considered our pond loading (4,500) well within the ponds food-producing capacity. We also reared 650 large coho juveniles (3.2 fish per pound) from the Mad River hatchery Winter Pond No. 2 to rear coho without supplemental feeding. This was primarily an outmigration study, using a smolt trap in which water was drawn into the catch box by use of water flow created with an air-lift water-pumping system (See Figure 2 in Allen 1987). Survival of coho in both winter ponds was very low (Table 7). In late March and early April unusually clear, sunny, and windless days produced intense phytoplankton blooms in all our ponds. Water in Winter Pond No. 2 in a one day period went

Table 7. Numbers of coho salmon planted, recovered by trapping, and from pond draining, Winter Ponds No. 1 and 2, winter-spring, 1987, City of Arcata wastewater aquaculture system.

	Winter Pond	
	1	2
Number planted	4,500	650
Number Recovered:		
Smolt Trap	302	41
Pond Draining	233	16
Total	535	57
Percent Recovered	12	9

from green to brown. An excessive oxygen demand accompanying photosynthetic reduction lowered pond dissolved oxygen levels to less than 2 ppm. Available aeration was not able to alleviate the problem in time to prevent serious losses signalled by over 40 floating dead coho recovered from along the periphery of the pond from April 7-9, 1987. Pond draining on May 27 only recovered 16 fish (3.0 fish/pound). We also suffered water quality problems

in North Pond being operated on ambient Humboldt Bay water. The pond was temporarily shut off from tidal exchange for a brief period. During a warm weather period a dinoflagellate bloom developed which subsequently crashed causing a mass mortality. An experimental mixture of adult cutthroat trout, and yearling steelhead, chinook, and coho salmon were all lost. Bird predators frequented all the project ponds during periods when stressed fish were present. Ospreys that regularly feed on fish fauna of Klopp Lake (17-acres brackish-lake water of the AMWS complex), were particularly active at this time over North Pond and Winter Pond No. 2.

Bird predation on Winter Pond No. 1 in spring 1987 appeared most egregious from day-time feeding cormorants. We have felt that elimination of initial cormorant predators can result in cessation of predation by this species. We have a hypothesis that individual cormorants probably communicate to other birds on the location of productive feeding areas. In the fall of 1986 our usual unofficial control measures were prevented by an unusual occurrence of a variety of Asian species of birds never or rarely seen in North America. Sighting of an Asian green finch among a flock of local finches residing in bushes along the banks of the Arcata oxidation pond directly adjacent to our fish ponds was of international significance. Knowledge of this first American record of the Asian finch was transmitted widely over the Audubon hot-line. Bird watchers from across the United States swarmed the Arcata marshes and the oxidation ponds, morning and night, weekdays and weekends, for about three months. Consequently control measures against cormorants were confined to sling shot, rock throwing, and verbal insults. Cormorants were utterly unimpressed with these efforts and fished eagerly while dodging missiles. Although we felt that cormorant predation was probably the major source of mortality of coho reared in Summer Pond No. 1, we were not able to partition mortality between cormorants, other bird species, or water quality. Our pond records show no oxygen levels less than 8 ppm in Winter Pond No. 1, although levels of 2 ppm were found during known periods of fish losses in both North Pond and Winter Pond No. 2. Transient low periods of oxygen may have been missed by our weekly grab-sample monitoring procedure but we did not record any periods of fish mortalities as were recorded for other ponds. Since heavy public use of the AMWS areas is expected to occur indefinitely, we have initiated a program of complete netting of all ponds to minimize fish loss to birds.

Winter Pond No. 1 was netted in the summer of 1987. Cresoted lodge-pole pine posts 6" diameter, were spaced on 16' centers on the north and south banks of Winter Pond No. 1. High-tensile stainless steel wire stretched between posts and was tightened to 300 pounds of tension by permanently installed ratchets. Black-plastic bird netting was purchased in 6'-wide rolls was strung across the steel wires, woven together with 3/8" polypropylene rope at one side of the pond, then worked to the opposite of the pond. After final stretching of the panels, ends were tied to a 2"x2" chain link fence installed entirely around the pond. Chain link fence was used to allow for trimming weeds and grass, and to partially impede egress of birds walking into the pond from the banks. The fence may possibly deter otters. Cost was \$800 for materials to bird-proof the 0.33 acre pond.

Fish in both ponds were fed Silver Cup pellets at 50% of the company's recommended feeding rates. No adjustments were made for mortalities during the rearing period. Overall survival rates of juveniles reared in both winter

ponds in the 1987/77 season (Hopson 1988) (Table 8) was greatly improved over the 1986-87 season (Table 7). The 87/88 rates were at the lower range of survivals recorded for the coho reared in ponds in previous years. Although there was a slightly greater overall survival from the protected (netted) pond of 5-8 percent, we still were unable to partition mortalities between bird predation or to pond water quality because of unreliable data obtained from our control pen. A slightly too-large mesh size for complete segregation of smaller-sized fish used in the control experiment was a source of error for which we could not adjust (Table 9). Winter Pond No. 2 will be covered with netting for the 1989 rearing program and the pond-netting studies continued.

Table 8. Summary of results of rearing juvenile salmonids in a protected (netted) wastewater-seawater pond and in pond unprotected (not netted), December 1987-May 1988.

Comparison	Winter Pond Number		
	1		2
Pond covered with bird netting	Yes		No
Species reared	Coho		Steelhead
Origin	Jolly Giant Creek	Mad River Hatchery	Jolly Giant Creek
Releases Date	13 Jan 88	21 Dec 87	13 Jan 88
Number	437	1,505	1,480
Mark	LP	UM	UM
Recoveries Smolt trap	208	340	116
Pond draining	<u>34</u>	<u>292</u>	<u>429</u>
Total recovered	242	632	545
Percent Recovered	55	42	37

Table 9. Summary of control group of coho salmon reared in floating cage (4' x 4' x 6') in Winter Pond No. 1, December 1987-April 1988.

Comparison	Mark		Total
	LP	UM	
Released			
Date	8 Jan 88	20 Dec 87	
Number	18	45	63
Counted 5 Marh 88	not recorded	not recorded	33
Recovered 29 Apr 88	7	25	32
Mean size			
On release	11.5	12.8	
On recovery	15.4	16.0	
Apparent survival (%) <sup>1</sup>	40	56	51

<sup>1</sup>Fish at least 11 cm or less were able to pass through cage mesh. Error potentially greatest for small LP-marked coho entering and leaving cage.

#### RECIRCULATING FRY-REARING SYSTEM

During pilot-project operations we constructed a smolt-holding system of four 500-gallon tanks using recirculated water. Water conditioning was through a 1-foot deep pea-gravel filter bed in each tank. Water down-welled through the bed by water being pulled to the surface with air-lifts fitted into two corners of each tank with two tanks also attached to a macrophyte tank (Blood 1984). We also used the tanks to rear fry and hold adults during our pilot-project work. These 500-gallon tanks had to be operated continuously otherwise filter-bed bacteria would die and the beds would become clogged, with subsequent cleaning and biological recharging of the pea-gravel tedious and time-consuming. In the summer of 1988, pilot-project facilities were redesigned and tested with rainbow trout fry. We replaced three of the original 500-gallon tanks with three-100 gallon standard fiber-glass circular tanks, and four 70-gallon rectangular fiber-glass troughs. Water quality control units were based on principles of sewage treatment technology. Unit processes of sedimentation, ammonia removal, dissolved organic fraction removal, fine sediment removal, sterilization, and a nutrient removal were designed into the fry-rearing facility. The new system can also be used to hold smolts for marking.

## Sedimentation Basin

Water from each rearing tank and trough is routed to a common drain which enters a shallow rectangular sediment basin 35" wide, 72" long, with a water depth of 8". The unit was constructed of plywood lined with hypolon, a rubberized water-proof canvass product. Internally the unit has two sets of baffles formed by concrete blocks. Fecal material and uneaten food settle to the bottom of the basin and are removed by daily cleaning with a siphon.

## Trickling Filter

Ammonia removal is provided primarily by three trickle filters. Filters were constructed of 18-diameter plastic pipes 24" in height filled with whole oyster shell as a substrate. Water from the sediment basin is delivered through rotating sprinkler arms of PVC plastic with small drain holes drilled into the underside of the arms. The units are operated in parallel. Ammonia is reduced to nitrate in the unit.

## Protein Skimmer

An 18"-diameter piece of concrete well-casing 6 feet in length was fitted with a fine-bubble air diffuser at the base of the unit. Water flowing into the unit near the top of the pipe, descends as a counter-current against air bubbles. Foam produced by the rising bubbles overflows from the top of the unit and leaves the culture system. Foam contains dissolved organic material (DOM, or "crud") (Wheaton 1977).

## Submerged Filter Bed

The smaller of two pre-existing sump chambers (43" x 36" x 40") was converted to a second filter bed by filling the chamber with 1/2"-diameter porous lava rock. Water from either the Trickling Filters or the Protein Skimmer flows into the top of the filter bed. Water downwells through the filter and enters an adjacent water storage unit (second and larger of the pre-existing sump) underneath a submerged baffle.

## UV-Sterilization

A "purchase-of-opportunity" allowed us to design into the system an inexpensive "out-of-pocket" UV-sterilization unit to control pathogens. Water is delivered to the sterilizer from the storage chamber by pumping. Sterilized water under pressure is then piped back to culture tanks.

## Macrophyte Nutrient Removal Unit

Part of the water in the recirculating system is delivered by the sump pump to a 26" x 16" x 144" tank for removal of nutrient from the water by plants. This unit is located in a newly constructed greenhouse positioned along the outside of the south wall of the fish barn. This is the same site



that Blood (1984) located tanks to study macrophytes for nitrogen removal efficiencies. Our new tank unit will also use a plant species but with the function of removing CO<sub>2</sub> to help control pH as our recirculating system tends to become acidic. We have had the opportunity to watch at least two species of plant colonize the tank by "self-design" (a filamentous algae growing on plastic clothers baskets, and a lawn grass (perennial rye) growing hydroponically). No studies have been conducted yet on nitrogen or CO<sub>2</sub> removal capacities of these plants. Plant species to be employed in this unit has not yet been selected.

### System Testing

The new recirculating system contains roughly 2,000 gallons of water. Our water pump was selected to produce four turn-overs per hour of operation. The system was tested using surplus rainbow trout fry (Eagle Lake stock) available through cooperation of the College-of-the-Redwoods fish hatchery. Testing began by distributing 42.4 pounds of fry (1,200 fish per pound) into the troughs. As fish grew the population was spread out to the circular tanks. As further growth occurred, fingerlings were removed from the system and planted into Summer Pond No. 1. Mortalities during the rearing study was less than 5%.

In addition to rainbow fry, we also reared a small lot of coho in the one remaining 500-gallon tank. This tank was looped into the system with the water entering and returning to the storage sump via a single oyster-shell trickle-filter unit. From the few returning unmarked coho adults recovered in 1987 in Jolly Giant Creek, only a single female produced a normal compliment of eggs. From 3,200 eggs, fry hatched were initially reared in one trough then reared in the 500-gallon tank. A total of 890 juveniles were produced (average fork length 13.1 cm) marked by freeze-branding technique and released into Jolly Giant Creek. Of these 122 were of larger average size (15.8 cm) and were smolt-like in external appearance. This coho production was part of the overall loading of the system during our test run.

The system was successful in rearing a heavy load of rainbow fry (about 53,000). We estimated that the number of fish removed was larger than that placed in the system (Table 10). This was due to an error in the data given to us on the amount of fish delivered. The redesigned system significantly increased our fry rearing capacity of our operations to nearly 55,000 fish (rainbow and coho loads combined). Since our existing egg incubator capacity is roughly 300,000 eggs, we will be planning additional fry rearing facilities when increased production is undertaken.

Table 10. Summary of initial testing of redesigned recirculating fry-rearing system, May 2-December 21, 1988.

Experiment Stage	Date	Number of Fish	Number per pound	Comments
Loading System	2 May	50,000	1,180	Troughs only
Removals	24 May	24,200	507	To Summer Pond
	11 Jul	24,400	413	To Summer Pond
	21 Dec	4,150	49	Vaccinated for Vibrio and to Summer Pond
Number Reared		52,750		

#### POND AERATION

Prior to 1988, aeration to Winter Ponds No. 1 and 2 was provided by two aerators in each pond. These aerators consisted of 8-foot lengths of 1" PVC pipe with 3/16" holes drilled every 4 inches. Aerators were secured to the pond bottoms and supplied with air through 1" Poly pipe. This system was primarily designed to prevent stratification of the ponds. With this system working, our ponds have never stratified, but low dissolved oxygen values have been recorded. Air bubbles produced by these aerators were large in diameter. Small air bubbles are more efficient at oxygen transfer (Wheaton 1977). In late 1988 the aerators were replaced with a new design which produces small bubbles. New aerators are made of four glass air stones each 6" by 1" by 1" spaced 8" apart. Airstones have 1/2" inlets and are screwed into a manifold of 3/4" PVC pipe. We will be studying oxygen levels during future phytoplankton crashes associated with species succession to determine if the new system is adequate or whether an emergency oxygen supply will have to be made available during critical periods when our ponds are going through species succession.

A "FAT CAT" (Patent held by Aquatic Ecosystems of Apopka Florida) aeration system has also been installed on Summer Pond No. 1. Two floating 1/2"-poly pipe airlines run the length of the pond. Every 6 feet the airline is tapped and a 24" long, 1/4" dropper line supplies air to a 1.5" by 1" by 1" glass airstone. There are 28 airstones in this pond. The system rises and falls with pond water levels and is designed to oxygenate the top 24" of the pond to saturation.



Feeding of large salmon and coastal cutthroat broodstock held in 8' x 4' x 4' floating cages in summer pond No. 2 has been aided by a "FAT CAT" system modification. The floating airline was bent into a circle and four airstones were placed around the circle. Feed thrown into the circle is caught in the circulation pattern and retained rather than being carried out of the cage. We have found this a simple but effective way of both aerating cages and improving food delivery to the fish.

#### ACKNOWLEDGEMENTS

With our temporary home-stream subject to increasing urban pollution and at least partly responsible for poor adult returns, our project relies on locally available surplus eggs, fry, or adults for many of our programs. We wish to thank those who have enabled us to continue our studies through assistance with fish stocks: California Department of Fish and Game Mad River Hatchery, Humboldt County fish hatchery at Prairie Creek, Humboldt Fish Action Council operations located on Freshwater Creek, and the College of the Redwoods Aquaculture Program. We wish especially to note the contribution of Dr. Terry Roelofs and students, Humboldt State University Fisheries Department, who conducted a major survey of the Freshwater Creek drainage during the fall 1987 and spring 1988 migratory season. Delores Neher, California Cooperative Fishery Research Unit, typed manuscript drafts and prepared our final copy.

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GERRARD RAINBOW TROUT BROOD PROGRAM  
KOOTENAY TROUT HATCHERY

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To understand the impetus behind the Gerrard rainbow trout brood program, one needs to know a little background of the strain. Kootenay Lake has historically supported a prime fishery for rainbow trout, Dolly Varden char, and kokanee salmon. Several distinct strains of rainbows and kokanee inhabit the lake. Kokanee to 8-10 lbs have been taken as have rainbows to 35 lbs. Environmental factors over time have allowed this strain of rainbows to develop and they have become specialized in their behavior, especially in regard to spawning. This strain does not mature until at least their fifth year and they utilize only a few hundred metres of spawning gravel located 40 kilometers upstream from Kootenay Lake, at the townsite of Gerrard after which the strain was named. These large fish have of course created special demands on the fishery, and the continuance of this strain has been a provincial priority.

In the late 1970's biologists were looking for a late maturing stock of rainbow trout which would provide trophy-type fisheries in certain lakes. In 1979, it was decided to collect eggs from the remaining wild stock at Gerrard. However, due to the relatively small number of returning adults to Gerrard, between 250-600 per year, it was not feasible to collect enough eggs from wild adults to satisfy provincial requirements without damaging the run. Therefore it has evolved that approximately 5,000 eggs are taken annually from wild Gerrard stock at the spawning grounds, which provides us with sufficient fish for our program. An attempt is made to get eggs and sperm from several fish, but because there is no breeding back into the brood stock, large numbers of adults and a wide genetic background are not critical to our criteria. Our major criteria for our brood stock is the age of maturation, which is set at 5 years of age minimum first time spawning.

The fish are sorted from 2 years of age onward to normal spawning time and any fish that show signs of maturation prior to 5 years of age are culled from the program. Females that mature at 4 years of age are

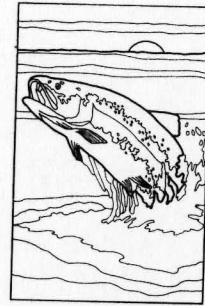
spawned, with the resulting eggs and fry being utilized in lower priority situations. Of the fish that do make it to our 5 year initial maturity target, all males are spawned and kept for subsequent spawning seasons, but females are only kept until they have spawned once and then are released. All fish are released once they reach 7 years of age.

For our actual spawning of this stock we air spawn each female separately and collect milt from each male separately with all males being checked for sperm motility before being added to the eggs for fertilizing. Also by crossing 5, 6 and 7 year old fish we do reduce the chance of crossing siblings.

Besides provincial interest in this strain, there appears to be some demand from other agencies, and this brood stock may be a viable way of producing those eggs for distribution. To date we have been able to maintain good health in this stock.

The Gerrard strain is unique. We are fortunate to have the opportunity to work with them and we must remember in our management of them that we are caretakers of a very special resource.

## Unique Topics



Stress of Carbon Dioxide Anaesthesia  
on Coho Salmon Smolts

by

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CANADA

ABSTRACT

Coho salmon smolts (mean wt = 18 g) anaesthetized in dissolved carbon dioxide gas ( $\text{CO}_2$ ) for coded-wire tagging exhibit an extreme hyperactive response when first introduced into the anaesthetic bath. The physiological stress of this response was measured using plasma cortisol as an index and compared to two other common anaesthetics, tricaine methanesulphonate (TMS) and 2-phenoxyethanol (2PE), as well as different methods of  $\text{CO}_2$  application.

It was concluded that the least stressful anaesthetic (in terms of peak level of plasma cortisol and rapidity of recovery) was the highest level of  $\text{CO}_2$ , mainly because the fish were anaesthetized most rapidly and therefore the biochemical chain of events constituting the stress response was shut down most quickly. Buffering the anaesthetic bath to reduce the drop in pH caused by  $\text{CO}_2$  addition, or pre-dosing the fish in a low level of  $\text{CO}_2$  (which drastically reduces the hyperactive response), both increased the stress response. However, high  $\text{CO}_2$  (above 400 mg/L) has a very small window of safety and can cause significant mortalities within 10 min.

Carbon dioxide gas at 300-400 mg/L is recommended as an anaesthetic for salmonid juveniles. Both stress and hyperactivity can be reduced by minimizing handling of the fish prior to anaesthesia.



-----WHITE RIVER SPRING CHINOOK RESTORATION PROJECT-----

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Historically, spring chinook have been economically important to the Puget Sound Region. Sport, commercial and Indian treaty fisheries once flourished on healthy runs of spring chinook to Puget Sound.

In recent decades factors such as overfishing, habitat degradation, and dams and diversions, have reduced spring chinook stocks in the Puget Sound to a level where their very existence is threatened. ( Unpublished 1980 )

At the present time tribal, state, and federal agencies are working together on efforts to restore depleted stocks on selected river systems throughout the Puget Sound. Three river systems have been targeted for restoration. They are the Nooksack and Skagit rivers in North Puget Sound and the White River drainage in South Puget Sound. This paper will focus on the White River enhancement project being conducted by the Washington Department of Fisheries (WDF) in cooperation with Federal and Tribal agencies.

-----A Declining Run-----

The White River drainage originates in the Emmons glacier on the Northeastern face of Mount Rainier. From the glacier the river flows west and then south until it meets the Puyallup River just below Sumner. Several man-made



perturbences have occurred on this river since the turn of the century. Flood control projects, clear-cut logging practices, and diversions of large amounts of water for power generation are only a few of a long list of environmental impacts which have contributed to a declining spring chinook population. Trap counts recorded at the Buckley diversion dam since 1940 illustrate the steady decline of the run over the last 4 decades (see figure 1). In the mid 1960s, escapement to the White River was reduced to fewer than 600 fish. By 1977, escapement had declined to around 50 adults and has remained below 100 ever since. ( Unpublished, 1987)

#### -----Early Project History-----

The W.D.F. began restoration efforts on the White River as early as 1974. From 1974 to 1976 adults were captured at the Buckley trap, transferred downstream to the Puyallup hatchery, and later spawned. Progeny were reared at the Minter Creek hatchery located on Henderson Bay in South Puget Sound. As yearlings the fish were released back into the White River. From 1974 to 1976 a total of 96,475 yearlings were released into the White River.

#### -----Current Project Status-----

In 1977 the restoration project changed direction. Environmental concerns which had contributed to the declining run were not being addressed on the White River. Because of the unsatisfactory performance of the smolt plants this program was ended and a broodstock maintenance and restoration project was created at Minter Creek Hatchery.

Since 1977 White River Spring Chinook have been planted into Minter Creek in hopes of rebuilding the population which can eventually be returned to the White River.

At present three facilities are involved in maintaining the run. Minter creek hatchery traps returning adults and also incubates the eggs. The adults are moved to Hupp Springs Hatchery two miles upstream from Minter Creek where they are held and eventually spawned. Hupp Springs Hatchery provides cool spring water to hold adults and rear yearlings. Progeny are reared and released into Minter Creek from Hupp Springs as zero's and yearlings. The National Marine Fisheries Service operates a saltwater rearing program at Manchester, Washington. White River Spring Chinook are raised in net pens from yearlings to 4 and 5 year old adults. This leg of the project provides an egg bank to supplement the Minter Creek program.

#### -----Adult Returns-----

The present broodstock program began by capturing adults at the Buckley fish trap and Transporting them to Hupp Springs for holding. Since 1986 the run has been maintained from adults returning to Minter Creek and adults held at the saltwater pen site. Figure 2 depicts the returns to the trap at Buckley and the increasing returns to Minter supplemented by fish from Manchester.

#### -----Trapping Adults At Minter and Manchester-----

Beginning in late May returning adults are captured at Minter Creek and transported to Hupp Springs. Prior to

transfer each fish is given a one time injection of erythromycin and oxytetracycline to combat bacterial infections during the long holding period. At Hupp Springs fish are kept in 10 by 100 concrete raceways. Water depth is kept at 2 1/2 ft. and sprinklers are used for shade. Females are separated from males in the ponds.

Adults from Manchester are inspected for maturity beginning in late July. Mature fish are transported to Hupp Springs Hatchery through August. Manchester fish are also given an antibiotic injection before transport. Manchester fish are kept separate from Minter fish at Hupp Springs.

-----Prespawning Mortality-----

Maintaining healthy adults for up to 4 months in concrete raceways has been a real challenge for this program. Figure 3 depicts the percent mortality of the adults from 1982 to 1988. These numbers are somewhat overestimated since spawned out males were included. The primary cause of mortality for adults returning to Minter Creek has been skin abrasions and ulcers primarily on the peduncle and ventral areas. Females seem to be the most susceptible to this problem. Prior to 1985, BKD and Furunculosis caused some of the losses. After 1985 drug treatments were given. Since then these pathogens have not been observed in adult mortality.

In an effort to reduce the skin abrasion problem a nylon tarp was used as a pond liner in 1987. Even with the tarp skin abrasions were still a major cause of loss. In 1988

losses were dramatically reduced even though returning adult numbers had increased. Again, most loss in 1988 was due to skin abrasion and ulceration.

#### -----Spawning Operations-----

All returning adults are 100% coded wire tagged. On a typical spawning day males are killed and bled first. Their heads are removed and each tag is read to insure the fish is of White River stock. Then females are killed and bled. Females are spawned into individual buckets. Milt is pooled and the eggs fertilized. The eggs are transported to Minter Creek Hatchery where they are disinfected prior to incubation. Tags from the females are then read to determine if they are White River stock.

#### -----Egg Losses-----

Egg losses in fish from Minter Creek have averaged between 5-10% since 1982. In contrast, egg losses from fish reared in saltwater pens have averaged between 35 and 67% since 1983. The latter eggs are often very soft, mushy, and transparent in color. Ovarian fluid is quite watery and the eggs from some females have severe soft shell problems. Both Diet and disease are suspected in causing the poor egg quality of the pen reared broodstock. Daily 15 minute formalin drips (1:600) are used to control fungus and reduce soft shell.

#### -----Rearing And Release-----

Fry are ponded in late December through January. Bioproducts' Biodiet starter feed and grower feeds are used

until the fish are 100/lb.. The fish are then fed Bioproducts Biomoist feed until release.

During the rearing period fish released as yearlings are given three prophylactic erythromycin treatments. Fish released as 0's are given one treatment. The treatment rate is 4.5% drug strength fed at 2% of body weight/day for 14 days. Treatments are given in March, June, and September. Fish are approximately 180/lb., 55/lb., and 20/lb. at the time of treatment.

All fish are coded wire tagged. Zero's are tagged with a different code than yearlings and each group (Manchester or Minter) is given a unique code. Fish are tagged in April when they are about 120/lb.. All fish are started in 10ft.x100ft. concrete raceways, But the yearling fish are transported to a 0.2 acre dirt pond for completion of rearing. Zero age fish are raised to the largest size possible ( 55/lb. - 70/lb. ) and released in late May or early June. Yearlings are liberated in March-April at sizes of 8/lb. - 5/lb.. Yearlings are allowed to emigrate volitionally. One month prior to any release Minter Creek water is pumped into the ponds to acclimate the smolts. Production figures since 1977 are given in Figure 4..

#### -----Rearing Loss-----

The percent loss since 1979 is shown in figure 5. Up until the 1986 brood BKD had caused a chronic low level loss throughout the rearing period. Prophylactic treatments were started beginning with the 1985 brood. Even though we treated

the 1985 brood several times during the rearing period BKD still contributed to most of the loss. Since all fish were tagged we were able to determine if fish of Manchester origin or Minter origin were contributing to the loss at differential rates. Yearling fish in the dirt pond consisted of 46% Manchester fish and 54% Minter fish. Just prior to release (March 1987) the loss due to BKD began to increase in this population. After analyzing over 300 mortalities we found that Manchester progeny accounted for 85% of the loss. In 1988 this group of fish returned as three year old adults. Interestingly the Minter progeny returned at a much higher rate (78%) than the Manchester group (22%). This data suggests that BKD may have contributed to the decreased survival of the Manchester progeny fish. It is not uncommon to find gross evidence of BKD in spawned adults reared at Manchester.

Beginning with the 1986 brood fish rearing practices were changed to try and halt BKD related losses. Several steps were taken: 1) The .20 acre pond was limed at the end of the rearing period 2) The diet was changed to Bioproducts' Biodiet and Biomoist feeds 3) Most Manchester fish were released as 0's instead of yearlings. Also treatments with erythromycin were continued. The 1985 brood losses were 10.0%, however the 1986 brood losses were reduced to 0.5%. Currently, losses for the 1987 brood group are below 1% and BKD has not been observed in the mortality of these groups.

-----Saltwater Broodstock Rearing Program-----

At the present time Hupp Springs transfers 3500

yearlings to the Manchester net pens. Fish are moved to the sea pens during the winter months. Upon arrival the fish are placed in a pen with a vinyl skirting around its perimeter. Fresh water is pumped into the pen creating a lens on the saltwater. Fish seek the level of saltwater they want until they are completely acclimated. After approximately two weeks the vinyl skirt is removed and the fish are given their first innoculations against Vibrio and BKD. Inoculations and medicated feeds are given routinely throughout the rearing period. During the summer, treatments are given at 60 to 90 day intervals. During the winter the periods are from 90 to 120 days.

The smolt to adult mortality rate has averaged above 90%. The high mortality occurs at three periods of rearing: (1) after one year in marine net pens, up to 25% loss is attributable to BKD. (2) after 18 months in seawater, up to 25% percent loss is attributable to precocious males (jacks); and (3) up to 90% of the remaining population is lost to marine fungal pathogens ( Harrel et.al., 1985 ).

The low survival and egg viability have steadily improved since the beginning of the saltwater program. Improved diets and feeding practices, larger rearing pens and healthy smolts transferred from Hupp Springs Hatchery have helped to increase production quality. This is evident in the

1986 brood fish where survival and fish quality have been excellent when compared with past broods. Since 1981 over 100,000 spring chinook smolts have been planted in Minter Creek from adults raised in saltwater pens.

-----Summary-----

The White River Spring Chinook is the last remaining stock of spring chinook in the southern basin of Puget Sound. The White River stock is genetically distinct from all other Puget Sound stocks as shown by starch gel electrophoresis and is therefore believed to be uniquely adapted to southern Puget Sound river systems. (Hopley, 1986)

The main goal of the White River Spring Chinook restoration project is to restore the native population within the White River watershed. To achieve this objective two complimentary broodstock programs are operating to maintain and build upon the White River Spring Chinook run.

The Minter Creek-Hupp Springs project raises and releases smolts and depends upon returning adults to enhance the run. The Manchester project rears broodstock in saltwater pens and is used as an egg bank. Since 1986 these facilities have supplied a combined egg take in excess of 160,000 per year. All indications are that this number will more than double in 1989. White River spring chinook are scheduled for reintroduction to the White River system in the early 1990's. It appears at this time that restoration efforts will succeed in restoring a sustainable population of spring chinook to the White River in the next decade.



Figure 1. Number of Adult White River Chinook Returning to White River Trap

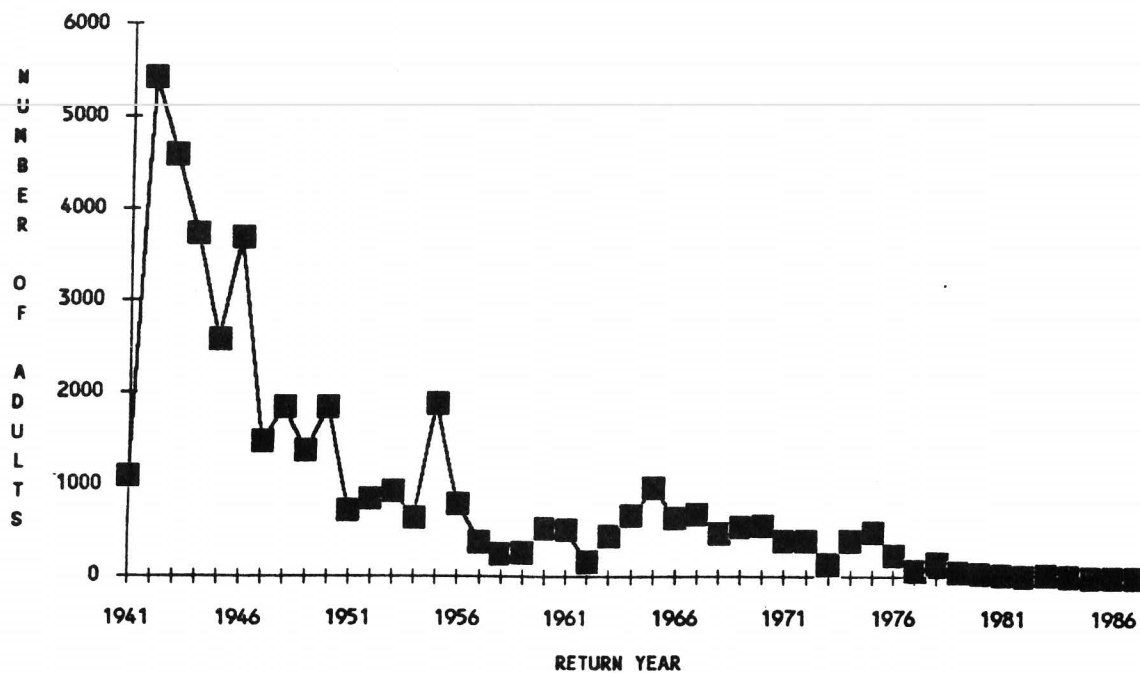


Figure 2. Number of Adult White River Spring Chinook Broodstock trapped at White River, returned to Minter and hauled from Manchester.

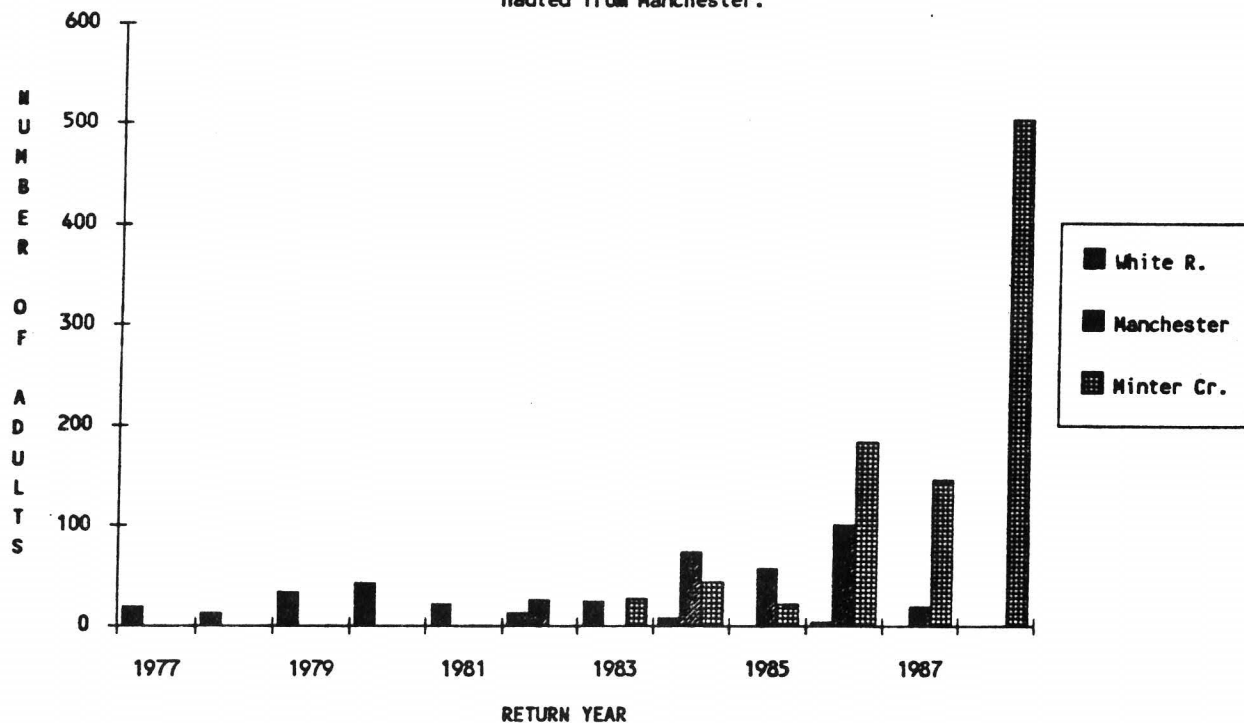


Figure 3. Percent Adult Loss

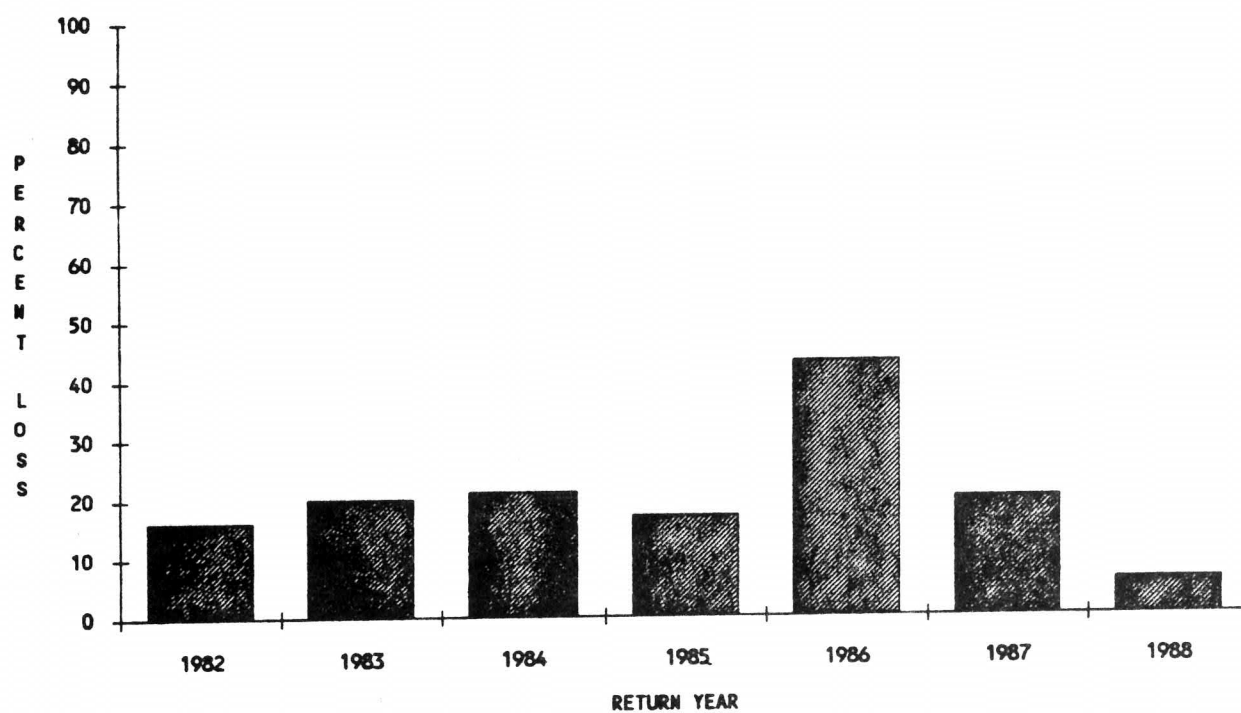


Figure 4. Number of White River Spring Chinook Smolts planted into Minter Creek.

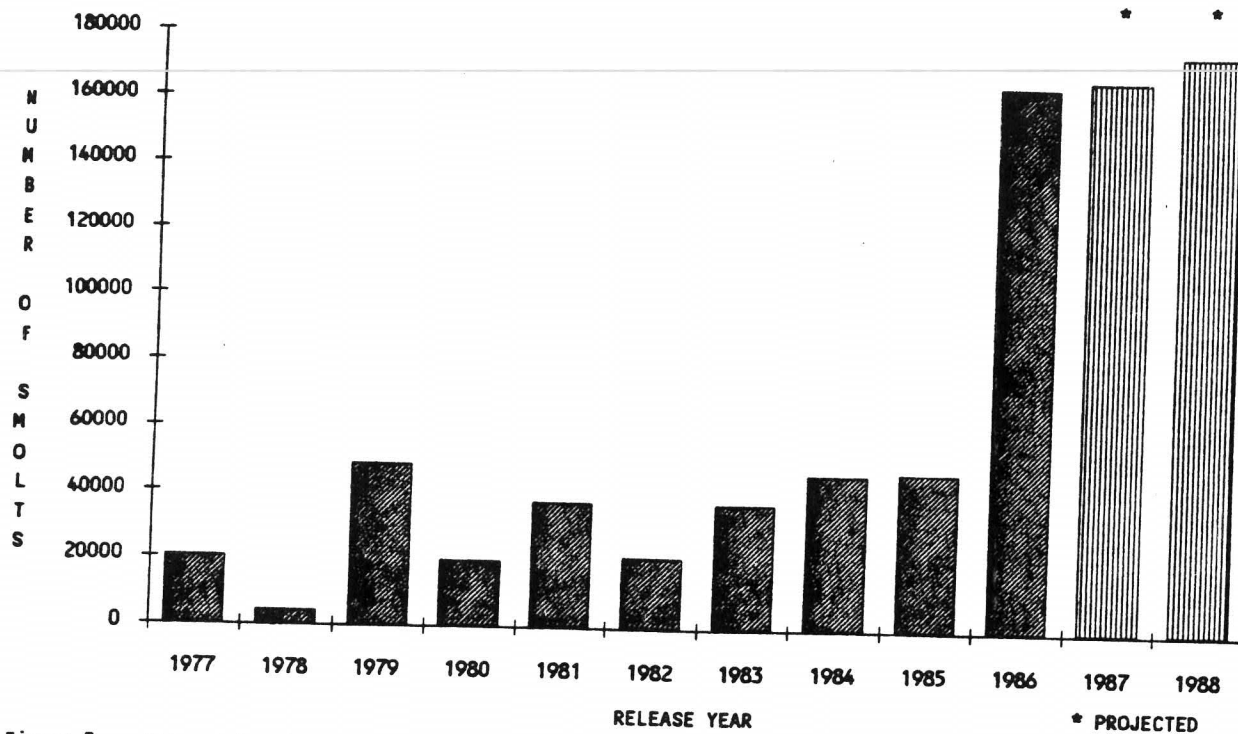
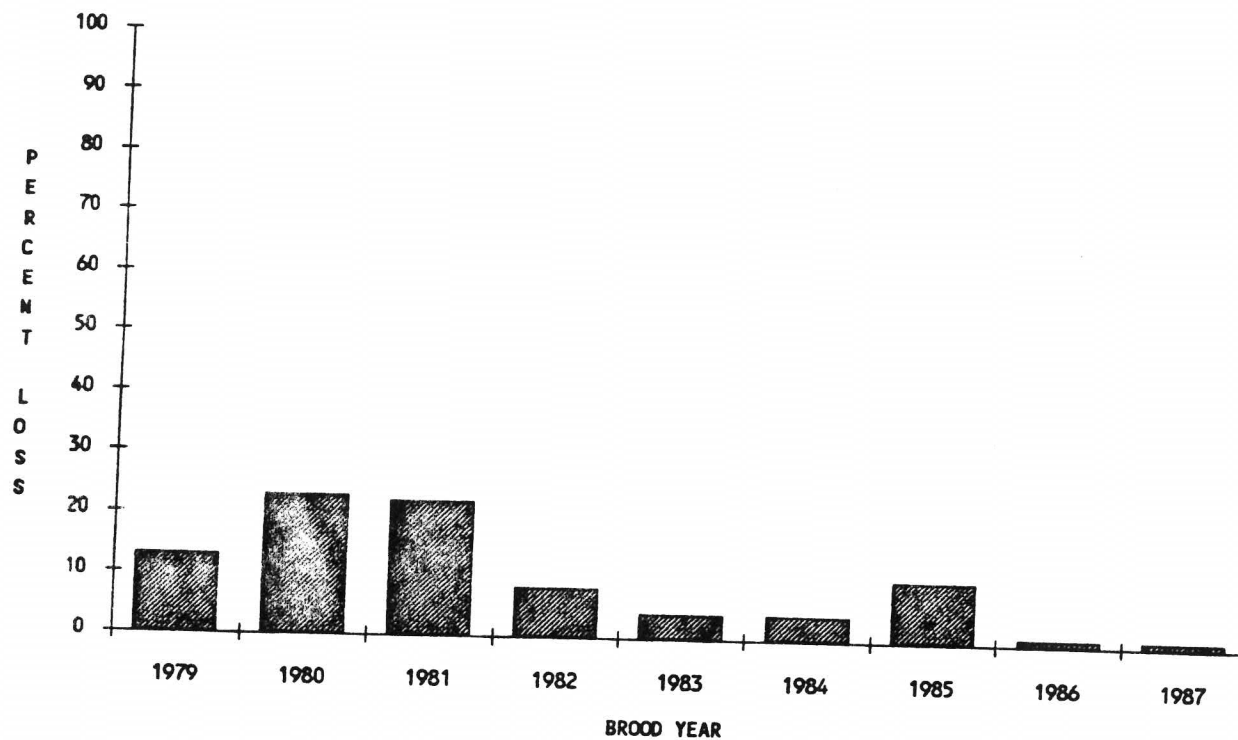


Figure 5. Percent Rearing Loss



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# A Relational Database Approach to Documenting Fish Runs, Spawning Activity and Egg Development through Initial Feeding

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## ABSTRACT

Database management techniques can now be applied to the needs of fish hatcheries that must document the numbers of fish returning to the hatchery, the numbers of fish spawned, and the disposition of eggs taken. A system is described in which raw data is organized into three files. These files correspond to distinct events during the taking and incubation of eggs. Interactive techniques are used to input and update information. Software relates the files and provides reports in a traditional format. Most of the information in the reports, including the total number of eggs taken, "true" percent of eyed eggs and "true" percent of eggs that produce fry, is computer calculated. The structure of the data files facilitates transfer of information to other offices.

## INTRODUCTION

Recent developments in microcomputer hardware and software make it possible to implement advanced information management techniques at fish hatcheries. These techniques will not only increase productivity at the hatchery, they will facilitate the exchange of information among various offices. This paper describes the use of relational database management software

describes the use of relational database management software to document spawning activity and egg development.

A database is a set of data organized to serve many applications efficiently by centralizing data and minimizing redundancy.

There are three types of databases: hierarchical, network, and relational. Relational databases and the associated Data Base Management Systems (DBMS) are the most recent development, and are the simplest to use. In a relational database data is stored in two-dimensional tables. Data in one table can be related to data in other tables as long as the tables share a common data element.

Two years of experience has shown that hatchery staff can create and maintain database files with relative ease, if files are properly structured and organized.

Three database files and two programs (Figure 1) are used in the system described in this paper.

#### FISH REMOVAL / SPAWNING

Record keeping begins as fish which have returned to the hatchery are "permanently removed from the population". DIP's, or fish which are found "Dead In Pond" are entered in the Fish Removal file by date and sex along with the usage code "5". When fish are removed for spawning, the date, take designation, sex, number of fish, and usage code "1" are entered. The estimated number of eggs per female can be entered once each spawning session, or for each take. Usage codes have been established for bad females, green females,

passed upstream, killed as surplus, etc.. The structure of the Fish Removal file facilitates daily data entry.

Runsum is a program which processes the information in the file, and produces a highly readable, traditional view of that information. By entering and processing data on a daily basis a hatchery manager will have an updated, detailed accounting of spawning activity, and the composition of the fish run being "removed", and eventually an accounting of the entire run (Figure 2).

#### EGG DEVELOPMENT

Once eggs are being incubated and "worked" a file named the Egg Activity file is used. One record is entered in this file for each take of eggs. Use of the take designation simplifies data entry and editing.

The number of eggs discarded because of disease considerations if any, is entered in the DISCARDED field. After the initial pickoff the number of good eggs and bad eggs is entered in the respective fields. Fields are also provided for loss from initial pick off to hatching, and from hatching to initial feeding.

Since eggs may or may not be transferred to other facilities, and since there may be multiple transfers from one take, a separate Egg Transfer file is used. Egg take, date of transfer, etc., are entered into this file.

The Eggsum program was developed to process information contained in the Fish Removal, Egg Activity, and Egg Transfer

files. This program produces the Egg Summary report in Figure 2. The take field located in each file is used to locate information for each take of eggs. The number of females spawned is found in the Fish Removal file. The total number of eggs taken, and the number of eggs taken per female is determined for each take by adding and subtracting the appropriate fields from the Egg Activity and Egg Transfer files.

The Egg Summary contains three sections: one documents females used, the number of eggs per female (both estimated and actual), the number and percentage at various stages, and the number of eggs and fry on hand for each take; the second section documents "egg loss" per take; and the third section lists all egg transfers.

Calculation of the actual, or "true" percent of eggs which reach each stage is complicated by the discarding or transfer of eggs or unfed fry from any number of stages of development.

#### POSSIBLE ENHANCEMENTS

Future development of this system would involve removing the DISCARDED field from the Egg Activity file, and placing this information in a Discard file. Files which contain information on water temperatures, and the dates on which various takes are shocked should also prove useful.



## Files

## Programs

## Fish Removal / Spawning

TAKE  
DATE  
NUM\_TAKEN

## RUNSUM

SEX  
SPECIES  
USAGE  
EST\_EGG\_FM  
OTHER\_ID

produces a detailed accounting  
of spawning activity and  
run composition

## Egg Activity

BY  
TAKE  
DISCARDED  
BAD\_EGGS  
GOOD\_EGGS  
LS\_EE\_2\_HT  
LS\_HT\_2\_FD

## Egg Transfer

TAKE  
DATE  
NUMBER  
STAGE  
SPECIES  
BY  
HC  
LN  
OTHER\_ID  
TO

## EGGSUM

produces a detailed accounting  
of the development of all  
eggs taken

# Spawning and Run Summary

=====

Broodyear 88  
Species Fall Chinook Salmon  
Location Test Data

file XXFR88  
Date 11/21/88  
Time 15:26:29

1988	Males	-----Females-----		Spawned		Estimated	Male	Female
Date Take	Spawned	Spawned	Green Bad	Out		Eggs Taken	ID	ID
09/23 05B	64	492	6	1	0	0	BT	BH
09/24 06A	16	211	0	0	0	1,055,000		Ab e
09/25 07B	63	391	6	1	0	1,955,000	BT	BH
09/25 07S	14	54	1	2	0	270,000		
09/28 09A	16	92	0	0	0	460,000		Ab e
09/28 09B	65	341	0	4	0	1,705,000	BT	BH
09/28 09S	21	55	0	0	0	275,000		
09/30 11B	19	75	2	0	0	375,000	BT	BH
09/30 11S	7	31	2	1	0	155,000		
10/02 13B	12	39	23	0	0	195,000	BT	BH

Totals 297 1,781 40 9 0 6,445,000

Ratio 1.0 : 6.0

	Jump		Killed as
	Outs	DIPS	Surplus
Males	0	118	195
Females	0	104	0
Jacks	0	0	4
Unknown	0	0	0
	=====	=====	=====
Percent	0	222	199
of Total			
Removed	0.0	8.7	7.8

Percent  
of Run

Total Males	610	23.9
Total Females	1,934	75.9
Total Jacks	4	0.1
Total of		
Unknown Sex	0	0.0

=====

Date of  
last entry 10/02/87

Total Number  
Removed to Date 2,548

# Egg Summary

Broodyear 88  
Species Fall Chinook Salmon  
Location Test Data

file XXFR88  
Date 11/21/88  
Time 15:52:12

T a k e	Current Females Spawned	Eggs Taken	Eggs per Female	Number Eyed	% Eyed	Number Hatched	% htcd	Number on Feed	% on Feed	Number on Hand
05B	492	2,134,788	4,339	1,807,061	84.6	1,806,061	84.6	1,805,161	84.5	1,805,161
06A	211	1,050,000	4,976	1,000,000	95.2	998,500	95.1	0 **.*		998,500
07S	54	539,000	9,981	250,000	92.9	0 **.*		0 **.*		250,000
07B	391	1,860,769	4,759	1,592,733	85.6	0 **.*		0 **.*		1,592,733
09S	55	275,000e	0e	0	**.*	0 **.*		0 **.*		0
09B	341	1,601,336	4,696	1,310,703	81.8	0 **.*		0 **.*		1,310,703
09A	92	911,000	9,902	450,000	99.7	0 **.*		0 **.*		450,000
11S	31	156,000	5,032	155,000	99.3	0 **.*		0 **.*		155,000
11B	75	366,825	4,891	332,037	90.5	0 **.*		0 **.*		332,037
13B	39	195,000e	0e	0	**.*	0 **.*		0 **.*		0

T a k e	Unfertilized Eggs	Green Eggs Shipped	Green Eggs Discarded	Loss as Green Eggs	Eyed Eggs Shipped	Loss as Eyed Eggs	unfed fry shipped	unfed Fry Loss
05B	0	0	0	327,727	0	1,000	0	900
06A	0	0	0	50,000	0	1,500	0	0
07S	0	0	270,000	19,000	0	0	0	0
07B	0	0	0	268,036	0	0	0	0
09S	0	275,000	275,000	0	0	0	0	0
09B	0	0	0	290,633	0	0	0	0
09A	460,000	0	0	1,000	0	0	0	0
11S	0	0	0	1,000	0	0	0	0
11B	0	0	0	34,788	0	0	0	0
13B	0	0	0	0	0	0	0	0

Take Stage	Number	Date	Transferred to	State
09A UNFER	460,000	09/29/88	L White Salmon NPH	WA
07S GREEN	270,000	09/30/88	Eagle Creek NPH	OR
09S GREEN	275,000	09/30/88	CL ODFW	OR

## THE USE OF CHILLED CO2 ANAESTHETIC IN A MARKING TRAILER

Shary Stevens

Robertson Creek Hatchery  
Department of Fisheries & Oceans

At Robertson Creek Hatchery, we have put together an indoor mobile tagging unit to try and eliminate the inconvenience and discomfort of our normal tagging operations, which seemed to be taking toll on our equipment, crew and the general well-being of the fish. Our 10 x 20 foot Britco trailer has 3 tagging units in operation.

An 890 liter live box runs the length of one side, with an independant water supply off the main line. The tank is also aeriated through perforated tubing supplied by a one-horse-power motorized air pump, mounted on the outside of the trailer. Fish enter the live box with the aid of a front-end loader through a trap pipe on the outside of the trailer.

Our anaesthetic tank is mounted in one of the corners and has a volume of 78 liters. Colour coded lines supply surface water to the tank. Another line, connected to a "Little Giant" submersible pump, takes the anaesthetic from the tank, and pumps it to the table circulating back to the tank. An exhaust fan is mounted just above the anaesthetic tank to remove vapours, created by any anaesthetic, out of the trailer.

With combined flow from the tank to the sorting troughs or sinks, we have a total volume of 150 liters.

During indoor tagging operations, the temperature of the anaesthetic bath will increase due to heat exchange from room temperature, if not properly controlled.

We found that, if you start with 8° Celcius water in the circulatory system, it will increase to 15° Celcius in approximately 1-2 hours. This prompted us to install a cooler unit.

After the sinks and the anaesthetic tank have been filled, the cooler unit is turned on to bring the temperature of the water down two degrees lower than the creek temperature and to maintain that temperature for as long as the unit is running.

CO2 charge rates and times will vary greatly with hatchery water qualities, volume of anaesthetic tanks, temperature of water and type of airstones used. We are generally aiming for a concentration of 350 parts per million at 150 liters; - a charge of 10 cubic feet per hour for 20 minutes gives us a charge of 350ppm.

We measure the levels of CO<sub>2</sub> with titration or a P.H. meter. As CO<sub>2</sub> is bubbled in, our pH drops, so we add a buffer of sodium bicarbonate to bring the pH back to 6.0. Oxygen is then bubbled in throughout the system to maintain a good supply, since the quality will drop with additions of fish to the anaesthetic.

With a circulating anaesthetic system, the CO<sub>2</sub> concentrations can dissipate in as little as 20 minutes. So, after the initial charge, we run a continuous trickle charge of 5 cubic feet per hour, to keep the anaesthetic bath with a constant level of CO<sub>2</sub>. The Condenser of our water-cooled reffridgerator unit is mounted on the outside wall of the trailer. Inside the anaesthetic tank is the chiller unit, which is approximately 33 feet of 1/2" copper tubing, which chills the tank down, or lets it warm up to the desired temperature, monitored by manually controlled thermostats.

When setting up for a reffridgeration unit, care and consideration must be taken to install a unit that is large enough to compensate for heat accumulation through air exchange.

Halfway through the day's tagging operation, or after about every 12,000 fish, we change the anaesthetic, because it will become cloudy and slightly foamy.

Chilled CO<sub>2</sub> in an enclosed trailer has begun to make a positive difference in our tagging operations.

General costs are on the decline, due to improved efficiency.

Tagging and post-tagging mortalities for coho have dropped from .30% to .06%, and, for chinook, from .93% to .43%.

Down time because of equipment malfunction has been minimized because the machines are kept in one spot, and not jostled around or exposed to harsh climatic changes.

Gumming up of the moveable fixtures, quite often caused by chemical anaesthetics, has been eliminated through the use of CO<sub>2</sub>.

With employee health and safety a major concern of the late 80's, the use of chilled CO<sub>2</sub> gas as an anaesthetic comes as a welcome alternative.

- oOo -

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HAMMER, STAN	Fox Island Net Pens/WDF	335 Island Blvd.	Fox Island, WA	9833
HAMOR, THOMAS	Livingstone Fish Hatchery	1440 17A St. S.E.	Calgary, Alta.	T2G 4T9
HANNIGAN, GEORGE	Mt. Hood Comm. College	1801 N.E. 162nd	Portland, OR	97230
HANSON, BRIAN	Mt. Hood Comm College	2950 N.E. 23rd, Apt. #82	Gresham, OR.	97030
HANSON, RED	Moore-Clarke Company	P.O. Box M	La Conner, WA	98273
HANUSE, KENNY	Village of Four Seasons Landscaping	309 1333 E 7th Ave.	Vancouver, B.C.	V5N 1R6
HARDING, DAVID	Fisheries & Oceans	555 W. Hastings St.	Vancouver, B.C.	V6B 5G3
HARGROVE, JANET	Bon Accord Enterprises	R.R. 2 Site 230, C29	Qualicum, B.C.	VOR 2T0
HARGRAVE, JOHN	Little Qualicum Spawning Channel	R.R. 2, Site 230, C29	Qualicum, B.C.	VOR 2T0
HARKNESS, DAVID E	Bellingham Voc. Tech. Inst.	2115 Hoogdal Rd.	Sedro Woolley, WA	98284
HARRELL, LEE W.	NMFS	Box 38	Manchester, WA	98353
HARRISON, COLIN	Dept. of Fisheries & Oceans	Box 1, R.R. #1	Malakwa, B.C.	VOR 2J0
HASHAGEN, K.A.	CA. Dept of Fish & Game	1416 Ninth St. Room 1251	Sacramento, CA.	95814
HAYWARD, DAN	Min. of Env. - Vancouver Is. Hatch.	R.R. #6, Boys Road	Duncan, B.C.	V9L 4T8
HEFNER, TOM	Bellingham Voc. Tech. Inst.	2516 North Shore Rd. #8	Bellingham, WA	98226
HENDERSON, DUTCH	WA. Dept of Fisheries	13030 Auburn Black Dia Rd.	Auburn, WA	98002
HEWITT, JOHN	Ranger Inc.	350 Redwood CT #212	Boise ID	83712
HILL, RAYMOND	OR. Dept of Fish & Wildlife	RT 2 Box 149	Irrigon, OR.	977844
HILLANO, RUSS T.	Snootli Hatchery	P.O. Box 95	Bella Coola, B.C.	VOT 1C0
HIRSCH, BRIAN	Canadian Liquid Air Ltd.	8390 Manitoba St.	Vancouver, B.C.	V5X 3A7
HOGG, WILLIAM	Bellingham Vo. Tech. Inst.	3035 Cherrywood Ave.	Bellingham, WA	98225
HOLDER, TIM W.	WA Dept of Wildlife	Rt. 1 Box 32	Pomeroy, WA	99347
HOLLICK NENYAN SANDRA	DFO Capilano Hatchery	4500 Capilano Park Road	N.Vancouver, BC	V7R 4L8
HOLWAY, JIM	Salmonid Technology	32500 S.E. Rainbow Rd.	Estacada, OR.	97023
HOPKINSON, SARAH	ESL Environmental Sciences Limited	407-1155 Robson Street.	Vancouver, B.C.	V6E 1B9
HOWARD, COLLEEN	WA Div. Fish.-Nooksack Salmon Hatch.	6263 Mt. Baker Hwy.	Danang, WA.	98244
HUBLOW, WALLACE F.	Bioproducts, Inc.	8686 S.E. Owen Drive	Portland OR.	97266
HUFFAKER, STEVEN	Idaho Fish & Game	P.O. Box 25	Boise, ID	83707
HUDLOW DAIL	NSRAA	1308 Sawmill Creek	Sitka, Alaska	99835
HUTCHINS, JOHN	Summit Tech. Consulting Eng.	615 2nd Ave. Suite 580	Seattle, WA	98104
HUTCHISON, BILL	Idaho Fish & Game	600 S. Walnut Box 25	Boise, Id	83707
ISAAC, DENNIS	OR. Dept of Fish & Wildlife	17330 S.E. Evelyn Street	Clackamas, OR	97015
ISMOND, ALAN	Aquatechnology Consultants	9-1355 W 4th Ave.	Vancouver, B.C.	V6H 3Y8
JACKSON, RUSSELL G.	Bellingham, Voc. Tech. Inst.	1011 High St. #11	Bellingham, WA.	98225
JANSON, VINCE	WA. Dept of Wildlife	1182 Spencer Rd.	Winlock WA	98596
JARVIS, JANICE	Seymour Salmonid Society	23924 Fern Crescent	Maple Ridge, B.C.	V2X 7E7
JENKINS, MICHAEL	Bellingham Voc. Tech.	1733 Electric Ave.	Bellingham, WA.	98226
JOHNSON, DON	Diversified Ova-Tech	Box 72	Lower Nicola, BC	VOK 1Y0
JOHNSON, MARK J.	WA Dept of Fisheries	301 Fish Hatchery Rd.	Glenwood, WA	98619
JOHNSON, MILDRED	Seymour Salmonid Society	4287 Greta St.	Burnaby, B.C.	V5J 1N7
JOHNSON, PETE	Pfizer, Inc.	3418 S. Chapman	Greenacres, WA	99016
JOHNSON, RANDY		5932 W. Bell Rd. Ste D-106	Glendale, AZ	85308
JOHNSON, RICHARD E.	WA State Fisheries	5.63 R-Washougal RV-Rd.	Washougal, WA.	98671
JOHNSTON, BRUCE	Seymour River Hatchery	308-700-4th Ave.	N. Westminster, BC	V3M 1S6
JONES, TERRY	OR. Dept. of Fish & Wildlife	Star Rt. Box 71	Idanha, OR.	97350
JOUPER, ED	WA Dept. of Fish.-Salmon Culture Div.	W 40 Skokomish Valley Rd.	Shelton, WA.	98584
KACZYNSKI, VIC	CH2M-Hill	2020 SW 4th Ave.	Portland, OR	97201
KAHL, LARRY	D.F.O., Chehalis R. Hatchery	R.R. 1, 16250 Morris Va. Rd.	Agassiz, B.C.	VOM 1A0
KIESER, DOROTHEE	Dept of Fisheries & Oceans	Hammond Bay Road	Nanaimo, B.C.	V9R 5K6
KIMBEL, MARK A.	WA. Dept of Fisheries	115 General Admin. Bldg.	Olympia, WA	98504
KINDSCHI, GREG	U.S. Fish & Wildlife Service	4050 Bridger Canyon Rd.	Boseman, MT	59715
KIRKS, ROBERT	Fish. Tech.-Bellingham Voc. Tech.	1333 Lincoln St., #104	Bellingham, WA	98226
KIRSCH, GEORGE	Montana Dept. Fish, Wildlife & Parks	Route 1, Box 96-7	Eureka, Mt	59917
KLONTZ, G.W.	Dept of Fish & Wildlife Resources	University of Idaho	Moscow, ID	83843
KLUBE, LARRY	WA. State Dept of Wildlife	37501 SE Fall Cty-Snoqualmie	Fall City, WA	98024
KLUMPH, RICK	OR. Dept of Fish & Wildlife	4909 3rd St.	Tillamook, OR	97141

KNORR, ELEANOR	Summerland Trout Hatchery	R.R. #1, Site 11, Lakeshore Summerland, B.C.	VOH 120
KOLLER, DIANA	Diversified Ova-Tech.	C-9 Galena Bay, R.R. #2	VOG 1R0
KOLODYCHUK, BARRY	Min.of Env. - Fraser Va. Trout Hatch.	34345 Vye Rd.	V2S 4N2
LABISKE, ED	OR. Dept of Fish & Wildlife	15020 Chance Rd.	Tillamook, OR 97141
LADOUCEUR, GRANT	D.F.O., Big Qualicum Hatchery	R.R. #3	Qualicum, BC VOR 2T0
LAMONT, CAROL	Univ. of B.C.	Suite 248-2357 Main Mall	Vancouver, B.C. V6T 2A2
LAND, BOB	Min.of Env.-Fisheries Research	34345 Vye Rd.	Abbotsford, B.C. V2S 4N2
LARIVIERE, PAUL	WA State Dept of Fisheries	416 E. Dayton Ave.	Dayton, WA 99328
LARRICK, WALTER	ROZA Irrigation District	P.O. Box 810	Sunnyside, WA 98944
LARSON, DALE	Fraser Valley Trout Hatchery	34345 Vye Rd.	Abbotsford, B.C. V2S 4N2
LAW, DUNCAN	CEDC Fisheries	250-36th Street	Astoria, OR 97103
LAWSETH, DON	Dept of Fisheries & Oceans	Box 1100	Port Alberni, BC V9Y 7L9
LAWSON, CHARLOTTE	Fraser Valley Trout Hatchery	826 Dever Drive	Kamloops, B.C. V2B 6R1
LEE, JIM	WA. Dept of Wildlife	2306 S. 16th Ave.	Yakima, WA 98903
LEMKE, LARRY	Summerland Trout Hatchery	R.R. #1	Summerland, B.C. VOH 120
LEWIS, ADAM	Envirocon Pacific Limited	205-2250 Boundary Road	Burnaby, B.C. V5M 3Z3
LINDEROTH, AMANDA	Scan Am Fish Farms	P.O. B 961	Anacortes, WA 98221
LINDSAY, BOB	Ministry of Env. Fisheries Branch	310 Ward St.	Nelson, B.C. V1L 5S4
LOBELLO, JON	Bellingham Voc-Tech.	1411 Grant St. #2	Bellingham, WA 98225
LOUCKS, DOUG	Dept of Fisheries	1435 Pavel Road	Beaver, WA 98305
LUTZ, SHARON	Northwest Indian Fish.Commission	6730 Martin Way E.	Olympia, WA 98506
LYNN, OTTO	Rangen Inc.	P.O. Box 1204	Lamoille, NV 89828
MACEY, SCOTT	Wood Bay Salmon Farms	Box 18, Buccaneer, R.R.1	Halfmoon Bay BC V0W 1Y0
McKINLAY, DON	D.F.O.	555 W. Hastings St.	Vancouver, B.C. V6B 5G3
MACLEAN, IAIN	D.F.O.	Box 247	Tahsis, B.C. V0P 1X0
MacQUARRIE, DON	Darcy Springis Hatchery	Melmore Rd.	Bowen Island, BC V0N 1G0
MAHONE, BILL	Makah Fisherie Mangement	P.O. B 115 Neah Bay,	Neah Bay, WA 98357
MAJNARICH, JOHN J.	Biomed Inc.	1720 130th Ave. N.E.	Bellevue, WA 98005
MANUEL, JERRY	Tri L Ltd.	433 Cougar St. S.E.	Olympia, WA 98503
MARKET, JACK	Moore Clark Co.(Canada)	1350 E. Kent St.	Vancouver, B.C. V5X 2Y2
MARQUARDT, GARY	Global Aqua U.S.	421 - 355 Erickson Ave.	Bainbridge Is. WA 98110
MARTIN, ALAN	Aquatess ( Canada) Ltd.	302 -4464 W. 10th Ave.	Vancouver, B.C. V6R 2H9
MARTIN, BRIAN	Min.of Env.-Vancouver Is.Hatch.	R.R. #6, Boys Road	Duncan, B.C. V9L 4T8
MATTHEWS, JIM	Quileute Fisheries Dept.	P.O. Box 187	La Push, WA 98350
MATTICE, SCOTT	Univ. of B.C.	R.R. 1, Site Q, Box 25	Bowen Island, B.C. V0N 1G0
MAXWELL, ED	WA Dep.of Fish-Naselle Salmon Hatch.	HCR 78 Box 430	Naselle, WA 98638
MAZUR, CARL	Univ. of B.C.	248-2357 Main Mall	Vancouver, B.C. V6T2A2
MCDONALD, MICKEY	Ministry of Environment	Kootenay Trout Hatchery	Wardner, B.C. V0B 2J0
McGEER, JIM	UBC	2490 Sherbrooke St.	Vancouver, B.C. V5X 4E4
McKIM, DEREK	BUTI-Student	5575 34 B Ave.	Delta, B.C. V4K 3N2
McKNIGHT, SCOTT	Moore-Clark Company	P.O. Box M	La Conner, WA 98273
McLEAN, EWEN	W. Vancouver Laboratories, D.F.O.	4160 Marine Drive	W.Vancouver, BC V7V 1N6
McMAHAN, STAN	BVTI	4226 Grassmere Rd	Concrete, WA 98237
McNAIR, JOHN	Alaska, Dept of Fish & Game	P.O. Box 20	Douglas, AK 99824
McNEIL, DAVID	D.F.O.	Box 197	Kitimat, B.C. V8C 2G7
MEADOWS, STEVE	Quileute Fisheries Dept.	P.O. Box 187	La Push, WA 98350
MEEHAN, MIKE	Icicle Seafoods Inc.	P.O. Box 8	Seward, AK 99664
MEYER, ALAN	OR Dept of Fish & Wildlife	HC 30 Box 142D	Chiloquin, OR 97624
MICHUK, PATTY	WA Dept. of Fisheries	115 GA Building AX-11	Olympia, WA 98502
MIDDAUGH, GENE	OR Dept of Fish & Wildlife	43182 N. River Drive	Sweet Home, OR 97386
MOLONY, BOB	WA Dept of Fisheries	6263 Mt. Baker Hwy	Deming, WA 98244
MOON, MEL	Quileute Fisheries Management	P.O. Box 87	LaPush, WA 98350
MOORE, EDWARD J.	WA Dep.of Fish.-Dungeness Hatch.	301 FishHatchery Rd.	Sequim, WA 98382
MOORE, JERRY	WA Dept of Fisheries	Box 3, Azwell, Rt.	Pateros, WA 98846
MORGAN, JOHN	UBC- Dept of Animal Science	209-2065 West 5th Ave.	Vancouver, B.C. V6J 1P8
MORINAKA, RON	Bonneville Power Adm.	P.O. Box 12094	Portland, OR 97212
MOSER, JEFFREY	BVTI-Student	601 N. Jennifer Lane	E.Wenatchee, WA 98802
NANDOR, GEORGE F.	OR Dept of Fish & Wildlife	24525 S. Entrance Rd.	Estacada, OR 97203
NEALEIGH, BILL	Murray Elevators	P.O. Box 155	Manzanita, OR 97130
NELSON, CHRIS	Murray Elevators	P.O. Box 7428	Murray, UT 84107

NEWSHOLME, KELLY	Diversified Ova-Tech	Box 750	Hudson Hope, B.C. VOC 1VO
NORBURY, DEAN	Hardy Sea Farms/Ocean Farms	720-1140 W. Pender Street	Vancouver, B.C. V6E 4G1
NORMAN, FRED	WA State Dept of Wildlife	1416 14th St. S.W.	Pulallup, WA 98371
NORTH, JOHN	CEDC Fisheries	250-36th Street	Astoria, OR 97103
NOVOTNY, TONY	Biomed, Inc.	1720 130th Ave. NE	Bellevue, WA 98005
NYARA, BILL	OR Fish & Wildlife	P.O. Box 15	Madras, OR 97741
OCHS, JIM	Industrial Plastics	740 S. 28th St.	Washougal, WA 98671
OLSON, WAYNE H.	Dworshak National Fish Hatchery	P.O. Box 18	Ahsanka, ID 83520
O'NEIL, JOSEPH	Mount Hood Fisheries	1950 NE 23rd #97	Gresham, OR 97030
O'NEIL, MIKE	Toboggan Creek Hatchery	R.R. #1	Smithers, B.C. VOJ 2NO
ONGLIN, KEVIN	Saga Seafarms Ltd.	P.O. Box 94	Garden Bay, B.C. VON1SO
ORDENDORFF, JOHN	Bioproducts, Inc..	P.O. Box 429	Warrenton, OR 97146
ORENIUS, HARRI		102, 1400 Wingrove St.	Nanaimo, B.C. V9J 3L7
ORR, WES	USFWS-Ennis Nat.Fish Hatch.	180 Fish Hatchery Road	Ennis, MT 59729
OWSLEY, DAVID	Dworshak National Fish Hatchery	P.O. Box 18	Ahsahka, ID 83520
PALMER, TED	Innovac Technology Inc.	1124 N.W. 53rd	Seattle, WA 98107
PARRISH, EVAN M.	Crystal Springs Trout Ranch	P.O. Box 99	Springfield, ID 83277
PARRISH, MICHAEL	Mt. Hood Comm College	3260 N.E. 8th	Gresham, OR 97230
PASTOR, STEPHEN M.	US Fish & Wildlife Service	9317 Highway 99, Suite I	Vancouver, WA 98665
PAVLSEN, ROBERT R.	WA State Dept Wildlife	4203 Central Pk Dr.	Aberdeen, WA 98520
PEARCE, BRIAN	DFO	555 W. Hastings St.	Vancouver, B.C. V6B 5G3
PECK, DANNY	Mt. Hood Comm. College	2950 N.E. 23rd	Gresham, OR 97030
PETERSON, DON	Rec. Fisheries Branch	780 Blanshard St.	Victoria, B.C. V8V 1X5
PHILLIPS, RITCH	Armstrong-Keta, Inc.	P.O. Box 21990	Juneau, AK 99802
PHILLIPSON, KEN	Northwest Indian Fish.Commission	6730 Martin Way E.	Olympia, WA 98502
PIEL, VINCENT	Yakima Indian Agency	P.O. Box 151	Toppenish, WA 98948
PITTERS, GARRY	Aloutte River Correctional Centre	P.O. Box 1000	Maple Ridge, B.C. V2X 7G4
POWE, DONALD	Peaceful Valley Recreation	1418 16th Ave.	Lewiston ID 83501
POWE, ELEANOR	Peaceful Valley Recreation	1418 16th Ave.	Lewiston, ID 83501
PRATSCNER, GREG	US Fish & Wildlife Service	P.O. Box 549	Leavenworth, WA 98826
PRESSEAU, ROLAND E.	Ibec Aquaculture Corporation	P.O. Box 789	PortMcNeill, B.C. VON 2RO
PRUITT, THOMAS A.	USFWS-Garrison Dam Nat.Fish.Hatch.	P.O. Box 918	Riverdale, ND 58565-0
RAISTAKKA, WESLEY L.	USFWS, Coleman NFH	RT. 1, Box 2105	Anderson, CA 96007
RALFS, GREG	Min.of Env.-Vancouver Is.Hatch.	R.R. 6, Boys Road	Duncan, B.C. V9L 4T8
RASMUSSEN, ULF	Dept of Wildlife/Skamania Hatchery	M.P. 0.39-L Steelhead Road	Washougal, WA 98671
RAIN, JASON	Y.I.N. Fish. Management Resource	Rt 1, Box 1207	Toppenish, WA 98948
REY, RALPH	Moore-Clark Co(Canada) Ltd.	5314 Westhome Rd	Victoria, B.C. V8X 4M6
RHEM PHILLIP	Micrologix International	Box 2460	Sidney, B.C. V8L 3Y3
RIDEOUT JAY	Rideout Pacific	P.O. Box 88574	Steilacoom, WA 98388
ROBARDS, STEVE	WA State Dept of Wildlife	HCR Box 52	Chelan, WA 98816
ROBART, RANDY	OR Dept of Fish & Wildlife	Rt.1, box 443	Mavpin, OR 97037
ROBINETTE, KAREN	Pr.WilliamSound Aqua. Corp.	P.O. Box 670	Whittier, AK 99693
ROBINSON, JIM	OR. Dept of Fish & Wildlife	Rt. 1, Box 195	Bandon, OR 97411
ROCKOWSKI, JAMES J.	Dworshak National Fish Hatchery	P.O. Box 18	Ahsahka, ID 83520
ROGERS, JERRY C.	USFWS-Spring Cr. Nat.Fish Hatch.	622 NW LincolnStreet	White Salmon, WA 98672
ROGERS, ROBERT	WA Dept of Fisheries	2020 Conger Ave. NW	Olympia, WA 98502
ROLEY, DENNIS	Bioproducts, Inc.	P.O. Box 429	Warrenton, OR 97146
ROMEY, DAN	Annette Island Reserve	P.O. Box 348	Metlakatla, AK 99926
ROSS, LANCE A.	WA Dept of Fisheries	Rt. 3 Box 204	Dayton, WA 99328
ROSS, LESLIE E.	WA Dept of Fisheries	Whatcom Falls Park	BellinghamWA 98226
ROWAN, GERRY	Anadromous, Inc.	P.O. Box 437	Fort Klamath, OR 97626
ROWLAND, RICK	OR Dept Fish & Wildlife	17330 S.E. Evelyn Street	Clackamas, OR 97015
SADECKI, MELVIN	ID Fish & Game	State Fish Hatchery	Ashton, ID 83420
SAMS, LINDA	Royal Pacific Seafarms Ltd.	Box 19	Egmont, B.C. VON 1NO
SANDERCOCK, F.K.	DFO	555 West Hastings	Vancouver, B.C. V6B 5G3
SANDS, SHELAGH	Gunnuk Creek Hatchery	Box 505	Kake, AK 99830
SCHAEFFER, DREW	OR Dept of Fish & Wildlife	1843 SE 37th Ave.	Portland, OR 97214
SCHAMBER, TIMOTHY W.	OR Dept of Fish & Wildlife	2418 E Fall Cr Rd	Alsea, OR 97324
SCHEER, KEN	B.C. Fish Culture Section	34345 Vye Rd.	Abbotsford, B.C. V2S 4N2
SCHENK, WINFRIED	Alta F&W - Livingston Hatchery	1440 17A St. S.E.	Calgary, AB T2G 4Y9

SCHNEIDER, RICH	Clear Springs Trout Co.	Rt 4 Box 712	Buhl, ID	83316
SCHRODER, LARRY	WDF	5939 Fish Hatchery Rd	Marblemount, WA	98267
SCRIBNER, TOM	Yakima Indian Nation	FRM P.O. Box 151	Toppenish, WA	98948
SEABOLT, KRISTINA	Troutlodge, Inc.	P.O. Box 11	McMillin, WA	98352
SEEL, SYBIL	Royal Pacific Sea Farms Ltd.	Box 2510	Sechelt, B.C.	VON 3A0
SEGAL, JOHN	Malaspina College	900 Fifth St.	Nanaimo, B.C.	V9R 5S5
SERRANO-PRINE JOSE'	R.Montana Dept.of Fish.Wild.&Parks	P.O.Box 2163	Great Falls, MT	59403
SHAW, HARRY TOM	US Fish & Wildlife -Hagerman NFH	3059-D Nat.Fish Hatchery Rd.	Hagerman, ID	83332
SHAW, VERN	N.Van.Island Salmonid Enhance.Soc.	Box 1409	Port Hardy, B.C.	VON 2P0
SHELDON, RAY	OR. Dept of Fish & Wildlife	17330 S.E. Evelyn St.	Clackamas, OR	97015
SHELDRAKE, TOM	US Fish & Wildlife Service	1250 - 700 Multnomah St.	Portland, OR	97232
LARSON, DALE	Fraser Valley Trout Hatchery	34345 Vye Road	Abbotsford, B.C.	V2S 4N2
SINCLAIR, DON	J.S. McMillian Fisheries Ltd.	2199 Commissioner St.	Vancouver, B.C.	V51 1A4
SMITH, DAVID	Smith-Root, Inc.	14014 N.E. Salmon Creek Ave.	Vancouver, WA	98686
SMITH, EARL J.	Bellingham VO Tech	P.O. Box 1792	Marysville, WA	98270
SMITH, QUENTIN E.	OR Dept of Fish & Wildlife	Rt. 1, Box 764	Astoria, OR	97103
SMITH, ROBERT Z	National Marine Fisheries Service	847 NE 19th , Suite 350	Portland, OR	97232
SMITH, RORY A.	Prov. of B.C.	Loon Creek Hatchery R.R. 1	Cache Creek, B.C.	VOH 1Z0
SOLAR, IGOR I.	DFO W. Vancouver laboratory	4160 Marine Drive	W.Vancouver, BC	V7V 1N6
SOERENSON, DAN	USFWS-Makah Nat.Fish Hatchery	P.O. Box 730	Neah Bay , WA	98357
SPARROW, R.A.H.	B.C.R.F.	1655 Warren GDS	Victoria, B.C.	V8S 1S9
STANLEY, CHARLIE A.	OR Dept of Fish & Wildlife	33465 Hwy 22	Hebo, OR	97122
STANTON, BOB	DFO Capilano Hatchery	4500 Capilano Pt Rd.	N.Vancouver, BC	V7R 4L3
STANTON, DAVID	Min. of Env.-Fish Culture	65 Government St.	Victoria, B.C.	V8V 2X5
STEDRONSKY, WAYNE A.	OR Dept of Fish & Wildlife	Star Route, B Box 527	Cascade Locks, OR	97014
STEELE, EARL	Bellingham Voc. Tech. Inst.	3028 Lindbergh Ave.	Bellingham, WA	98233
STICKELL, TRENT	OR Dep.of F&W-Bonneville Fish Hatch.	Star Rt. B, Box 12	Cascade Locks, OR	97014
STILWATER, RICK	WA Dept of Wildlife	12209 S.E. Evergreen Hwy.	Vancouver, WA	98684
SWAFFORD, JERRY	OR Dept of Fish & Wildlife	HC GO Box 13	Idleyld Park, OR	97447
SWEENEY, MARK	Montana Dept.of Fish. Wild & Parks	606 W. Pennsylvania	Anaconda, MT	59711
TAYLOR, GIB	USF&WS - Ret	1221 Ebb Tide Terrace	Olympia, WA	98502
THORSON, WILLIAM M.	U.S. Fish & Wildlife Service	6920 Fish HatcheryDr.	Entiaia, WA	98320
THUNSTROM, KATHY	Bellingham Vo Tech Inst.	5211 Strand Rd.	Deming,WA	98244
TIPPING, JACK	WA Dept of Wildlife	2101 Hwy 508	Onalauha, WA	98570
TODD, NEIL L.	Diversified Ova-Tech Ltd.	Box 237	Merritt, B.C.	VOK 2B0
TOWNSEND, BILL	Trout Lodge Inc	Box 11	McMillan, WA	98352
TURNER, LYNDA L.	WA Dep.of Fish McKernan Hatch.	411 Deyette Road	Shelton, WA	98584
UNDERWOOD, JON	Alta F&W-Cold Lake Fish Hatch.	Box 1259	Cold Lake ,AB	TOA OVO
VANSLYKE, DAN	Anadromous, Inc.	777 NE 2nd Street	Corvallis, OR	97330
VAN TINE, JIM	Quinsam Hatchery	Box 467	Campbell R., BC	V9W 5C1
VINGE, KURT	Divers.Ova-Tech-Shuswap Hatchery	Box 819	Lumby, B.C.	VOE 2G0
VOLKHARDT, GREG	Northwest Indian Fish.Commission	6730 Martin Way E.	Olympia, WA	98506
WADE, BRUCE	WA Dept of Fisheries	3324 N.W. Ave. #12	Bellingham, WA	98225
WAGNER, JIM	Env.Pacific-Big Tree Cr.Hatch.	Box 227	Campbell R. BC	V9W 5B1
WALLIEN, BILL	U.S. Fish & Wildlife	Box 429	Winthrop, WA	98862
WARREN, KEITH	CEDC Fisheries	250-36th Street	Astoria, OR	97103
WARREN, RON	WA Dept of Fisheries	5935 Fish Hatchery LM	Marbelemont,WA	98267
WATSON, BARB	Armstrong-Keta,Inc.	P.O. Box 21990	Juneau, AK	99802
WESTGARD, RICHARD L.	WA State Dept of Fisheries	10408-Marine View Drive	Everett, WA	98204
WHITLATCH, RICHARD	OR Dept of Fish & Wildlife	39800 S.E. Fish Hatchery Rd.	Sandy, OR	97055
WHITTAKER, JERRY	Colorado Division of Wildlife	6060 Broadway	Denver, CO	80216
WILCOX, KELLY	N.Van.Island Salmonid Enhancement	Box 1409	Port Hardy, B.C.	VON 2P0
WILLIAMS, BRIAN	Kispiox Band Council Hatchery	Box 25 Kispiox Rd.	Hazelton, B.C.	VOJ 1Y0
WILSON, DUWAYNE	Clear Springs Trout Co.	Rt 4 Box 712	Buhl, ID	83316
WILSON, THOM	University of WA	School of Fisheries WH-10	Seattle , WA	98195
WITHERS, GRANT T.	Seymour Salmon Hatchery	1711 Delta Ave.	Burnaby, B.C.	V5B 3G5
WOLF, KLAUS	Min. of Env.	R.R. 1 Site 11	Summerland, B.C.	VOH 1Z0
WOLSKI, LUCYNA	Env.Pacific-Clearwater R. Hatch.	R.R. 2, Box 2142	Clearwater ,B.C.	VOE 1N0
WOODY, STANLEY, C.	WA Dept of Wildlife	28 Beaver Creek Road	Cathlamet, WA	98612
WRIGHT, HAL	Industrial Plastics	740 S. 28th St.	Washougal, WA	98671

WRIGHT, TERRY	Northwest Indian Fish.Commission	6730 Martin Way E.	Olympia, WA	98506
ZAMLUK, RITA	Syndel Laboratories	690 Homewood Road	Campbell R., BC	V9W 3N5
ZIMMERMAN, BRIAN	Anadromous, Inc.	P.O. Box 1007	North Bend, OR	97459
ZIRJACKS, DON	US Fish & Wildlife Service	Carson Nat.Fish Hatchery	Carson, WA	98610

# PRIZE DONATIONS AND WINNERS

<u>Prize</u>	<u>Donor</u>	<u>Winner(s)</u>	<u>Agency</u>
Beverage	Pacific Western Breweries Prince George, B.C.	Gene Middaugh Stan McMahan Steve Arnold Ken Phillipson Ray Bass Lynda Turner	Oregon (D.F.W.) Bellingham (V.T.I.) Min. of Env. (B.C.) N.W. Indian Commission (WA) Oregon Dept. F&W Wash. Dept. Fisheries
Beverage	Columbia Brewing Creston, B.C.	Phillip Rhem Phill Edgell Mark Kimbell	Micro-Logix, Sidney, B.C. DFO, Robertson Creek Wash. Dept. Fisheries
Beverage	Brites Wines Oliver, B.C.	William Thorson Bob Molony Dr. Klontz	U.S. Fish & Wildlife Wash. Dept. Fisheries University of Idaho
Beverage	Casabello Wines Penticton, B.C.	Ed Moore	Wash. Dept. Fisheries
Beverage	Calona Wines Vancouver, B.C.	Paul LaRiviere Wilbur Ashcroft	Wash. Dept. Fisheries Wash. Dept. Fisheries
Berkley Graphite STHD Rod	Hub Sports Abbotsford, B.C.	Jack Tipping	Wash. Dept. Wildlife
Gerber Folding Knives	Bio Products, Oregon	Sally Goldes Don Johnson Mildred Johnson Rob Foster Henn Gruenthal	Min. of Env. (B.C.) Spius Ck. Hatchery Seymour Hatchery (B.C.) Wash. Dept. Fisheries Jackson Fish Hatchery (WY)
Trip for Two Victoria/Seattle	B.C. Stena Line, Victoria, B.C.	Kurt Vinge	Shuswap Hatchery (B.C.)
Back-Pack	Mountain Man Sports, Cranbrook, B.C.	Robert Paulsen	Wash. Dept. Wildlife
Fly Box & 3 dozen flies	Min. of Env. (B.C.) B. Chan, B. Hatch, N. Basok	Steve Meadows	Quizeote Tribe Fisheries (WA)
Floating Flashlight	Acklands, Victoria, B.C.	Grant Ladouceur	DFO, Canada
Avi-Air Hats	Avi Air, Kamloops, B.C.	Phil Edgell William Thorson Bob Molony Dr. Klontz	DFO, Canada U.S. Fish & Wildlife Wash. Dept. Fisheries University of Idaho



"Salmon for All" Sweatshirts	Salmon For all Group Oregon	Ray Hill Dale Hurdlow	Oregon Dept. of F&W Alaska - NSRAA
Peetz Rod & Reel Trolling Combo	Peetz Manufacturing Victoria, B.C.	Don Powe	Idaho Hatchery
\$25 in "Loons"	NWFCC	Jerry Moore	Wash. Dept. Fisheries
Neoprene Waders Certificate	Perfection Tackle Abbotsford, B.C.	Bryan Ludwig	Min. of Env. (B.C.)
Jacket	Pacific Western Brewing, Prince George, B.C.	Ken Phillipson	NWest Indian Comm. (WA)
Fly Fishing Vest	Ruddicks Fly Shop Burnaby, B.C.	Don Lawseth	DFO, Port Alberni, B.C.
Single Action Reel	Gord's Tackle, Sardis, B.C.	John Cox	Mt.Hood Comm.College(WA)
Fraser Valley Trout Hatchery Fishing Trip	Fraser Valley Trout Hatchery Abbotsford, B.C.	Don Buxton	DFO Chilliwack River Hatchery
Scotty Downrigger and Rod Holder	Scotty Marine Victoria, B.C.	Stan Woody	Wash. Dept. Wildlife
Cedar Loon Carving	Kadian Kraft, Burnaby, B.C.	Duncan Law	CEDC Fisheries, Oregon
Wooden Plate Carvings	Waring Marine, Port Alberni, B.C.	James Rockowski Ron Ek Laird Siemens Chris Carlson	U.S.F&W Service Min. of Env. (B.C.) Min. of Env. (B.C.) Grant County, Public Utilities, Dept. of Wash
Guided Sturgeon Fishing Trip and 1 Night Accom.	Fred's Tackle Vedder Crossing, B.C. Bakerview Motor Inn Abbotsford, B.C.	Terry Wright	Northwest Indian Fish. Comm. Washington
Duffle Bags Books-The Gilly Avi Air Ball Hats Fish Knives	Okanagan Helicopter Min. of Env. - B.C. Avi Air, Kamloops, B.C. Int. Knives & Darts, Victoria, B.C.	Ron Creer and Doug Loucks	Utah Div. Wildlife Wash. Dept. Fisheries
Avi Air Airtime Gift Certificate	Avi-Air, Kamloops, B.C.	L. Wolski	Envirocon (B.C.)

## 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	Oregon Game Commission	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, W.
1967	Seattle, Washington	University of Washington	Donaldson, L.
1968	Boise, Idaho	Idaho Fish and Game Department	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 Fish and Game Department and University of Idaho	Parrish, E. & Klontz, G.
1984	Kennewick, Washington	Washington 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	Hager, B.