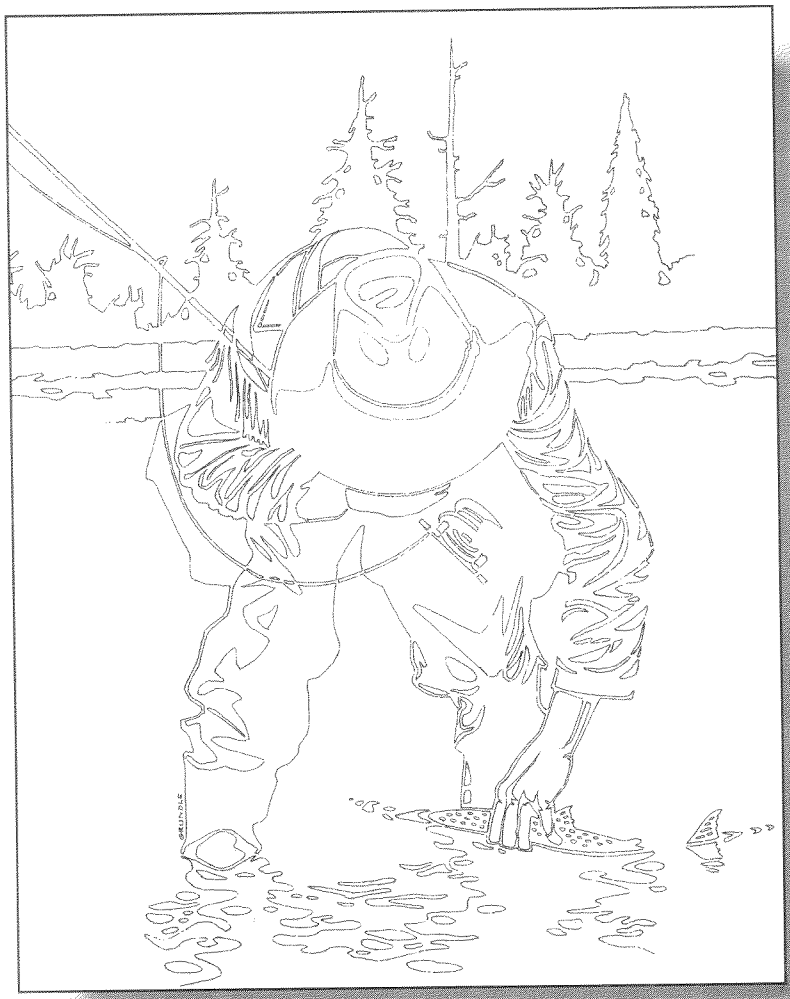
PROCEEDINGS

of the

47th Annual
NORTHWEST FISH CULTURE CONFERENCE



BC Environment

Fisheries and Oceans

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Victoria, B.C.

Proceedings of the 47th Annual NORTHWEST FISH CULTURE CONFERENCE

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The Northwest Fish Culture Conferences are informal meetings for exchange of information and ideas concerning all areas of fish culture. The Proceedings contain unedited reports to accompany oral and poster presentations given at the Conference. The papers have not been peer reviewed and should not be cited as primary publications.

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Preface

The 47th Annual Northwest Fish Culture Conference is hosted by the Canadian Department of Fisheries and Oceans, Habitat and Enhancement Branch (DFO) and the British Columbia Ministry of Environment, Lands and Parks, Fisheries Branch (MELP).

The Honourable Paul Ramsey, Minister of Environment, Lands and Parks, has agreed to provide the welcoming address to the delegates. Brian Riddell, Research Scientist, DFO, will give the keynote address on the history of fish culture and its potential for the future.

A special thanks to all the speakers, poster authors and session leaders. The quality and variety of the presentations will make for a great conference program. Thanks also to the proceedings authors for their timely responses and cooperation in meeting our formatting and printing needs - your efforts have resulted in a quality publication.

We also wish to thank all the trade show vendors and the generous prize donors for supporting the conference. The major feed companies (Moore-Clark, Nelson and Sons Inc., Bioproducts Inc. and Rangen Inc.) deserve special mention for also agreeing to support an evening social activity.

As Conference Directors, we were very fortunate indeed to have an excellent organizing committee. Bryan Ludwig (MELP) and Don MacKinlay (DFO) have done a great job in putting together an excellent program and producing the Proceedings. Sue Billings (MELP) has handled registration; Cathy Hohnsbehn (MELP) has arranged the facilities and socials; Ray Billings (MELP) has organized the trade show; Greg Ralfs (MELP) is responsible for audio visual arrangements; Nick Basok (MELP), Tony Massey (MELP) and Glen Dixon (DFO) have secured door prizes and will conduct the draws; Tim Yesaki (MELP) has organized all souvenirs for the conference; and Dave Stanton (MELP) has been responsible for the poster session.

We sincerely hope you find the 47th Annual Northwest Fish Culture Conference, and your stay in Victoria, to be a rewarding and enjoyable experience!

Your 1996 NWFCC Conference Directors,

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Greg Bonnell
Salmonid Enhancement Program
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THE FIRST HUNDRED YEARS, FROM GREAT EXPECTATIONS TO MUCH MALIGNED,

... AND NOW FOR SOMETHING COMPLETELY DIFFERENT!

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The arts and sciences of managing Pacific salmon are in a period of critical evaluation and, less critical, prognostication. But there has probably not been any aspect of salmon management that has undergone the change in perspective that the use of hatchery production has. The critics of this production cite concerns about the mixed-stock over-harvest of natural populations, ecological interactions between hatchery and wild salmonids, and even resort to the mysteries of genes and biodiversity. Given the history of production and user support for hatcheries, these criticisms initially generated debate, frequently defensive and polarized. Recently though, a more objective assessment of the use of hatcheries and how they are integrated into salmon management has developed. Hatchery production can not be viewed as operating independent of the natural environmental limits, the local natural populations, or fisheries management systems. The ocean ranching of hatchery production obviously exposes these fish to the uncertainties of Nature and management (for lack of a better word) of our fisheries. It is equally as obvious that hatcheries have the potential to produce large numbers of fish, but if this production poses a threat to the conservation of natural populations, shouldn't that threat be assessed like any other threat (for example, the famous 4-H club of the Columbia River ... hydro, habitat, harvest, hatcheries)? Hopefully, we agree that the answer to this rhetorical question is YES ... but we don't abandon hatcheries just because we evaluate them and identify problems. Evaluation involves analysis, learning, and application/adaptation where necessary.

I suggest even that the re-assessment of hatchery production is only one issue in a larger, and growing, malaise about the past management of Pacific salmonids and the emergence of new management philosophies for conservation (for example, see Olver et al. 1995). Certainly there has been an emergence of new buzz-words (sustainability, ecosystem management, watershed management, biodiversity, genetic effective population size, population viability analysis, and risk assessment... to cite a few). In practical terms, these changes indicate a shift from a single stock, utilitarian perspective to a more balanced and ecosystem-based management addressing conservation of population diversity and production for use (including aesthetics) by society. In fact, many recent definitions of conservation explicitly recognize the need for population diversity and habitats in order to sustain production and natal ecosystems. I mention this here, because to consider a role for fish culture in the future, we should both learn from past experience and anticipate future management goals.

Salmon management objectives in the future will almost assuredly involve: maintaining and expanding population diversity, maintenance or restoration of ecosystems, and sustaining production for fisheries (the scale of which maybe highly variable). The objectives likely varying by species, site, and situation. However, in planning a role for fish culture there seem to be three basic questions to consider:

1) What is problem that fish culture is to address? (specific the program objectives and identify appropriate management strategies);
2) What fish culture "tools" are appropriate? (integrate the tool with the size of the natural populations and the capabilities of fishery management); and
3) What are the likely outcomes of the culture activity? (ensure expectations are realistic, monitor results, and be flexible to change)

Experience and ingenuity have already developed numerous fish culture tools that will likely be remain useful in the future, possibly much more useful when integrated more fully with habitat, harvest, and public expectations. But

there are also new and emerging technologies or opportunities that may change how fish culture is applied. I will consider some of these in my talk, and only list them here:

- establishment of hatchery production regions versus natural conservation areas,
- development of conservation hatcheries (for small population recovery) or more natural-like hatchery environments,
- application of mass marking for exploitation directed on hatchery production,
- use of non-reproductive fish or development of all-female groups for increased egg production,
- use of mariculture for the development of captive brood stock (ex-situ gene banks?)

How much the role of fish culture changes in the future will probably depend more on social and resource policy decisions (balancing fish production and biodiversity with other resource uses), than on technological capabilities. In general, my expectation is for the scale of fish production from hatcheries to decrease in many areas, for a diversification of enhancement activities to assist conservation, and for substantially greater integration of all components of salmon management. During these changes, however, I hope we can maintain a commitment to scientific and assessment programs (e.g., marking or genetic studies) and in the education programs frequently associated with fish culture. The former has been essential in the assessment and management of many salmonid populations, and the latter will continue to be important in fostering a conservation ethic. This ethic and the maintenance of our social values for Pacific salmonids may be essential to sustaining salmonid ecosystems and production in the face of expanding human populations and their desire for economic growth and recreation.

Reference:

Olver, C.H., B.J. Shuter, and C.K. Minns. 1995. Toward a definition of conservation principles for fisheries management. Can. J. Fish. Aquat. Sci. 52: 1584-1594.

SPAWNING PROTOCOLS FOR THE 21ST CENTURY

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Introduction

Historically, the anadromous salmon runs of the Pacific Northwest have been one of its greatest resources. This is evidenced by the huge commercial fisheries (peak annual catch of 43 million pounds) and cannery operations which existed at the beginning of the 20th century (National Research Council, 1996). The Columbia River commercial salmon fishery began with two gill-net boats in 1866 and reached a peak of 2,800 gill-net boats in 1910 (National Research Council, 1996). During this period, the number of canneries processing the commercial salmon harvest increased to 40. Fish hatcheries were introduced to the Columbia and Snake river basins to further increase the size of existing fish runs. Bonneville Fish Hatchery, Oregon's first production hatchery, began operation in 1909. Between 1910 and 1930, this facility released over 100 million spring chinook fingerlings into Tanner Creek alone (Wallis, 1964). The underlying hatchery theory was that by eliminating mortality at early life stages, increased numbers of salmon would be available for harvest.

Even with production hatcheries in place, it soon became evident that salmon populations were declining. As early as 1919, Columbia River fishing regulations in both Oregon and Washington included gear restrictions and closed seasons (National Research Council, 1996). This decline was exacerbated by the building of dams on the mainstem Columbia and Snake rivers. In 1938 Bonneville Dam was completed without the benefit of fish passage facilities, although a fish ladder was constructed later. Grand Coulee Dam was completed in 1942 and effectively blocked passage to the entire north third of the Columbia River basin. The completion of Brownlee Dam in 1958 permanently blocked anadromous fish migration in the Snake River to portions of Oregon and Idaho (Sayre, 1970). A total of 14 mainstem Columbia River and 13 mainstem Snake River dams had been constructed by the mid 1970s .

Salmon losses at the dams, combined with increased levels of anthropogenic activities such as fishing, overgrazing, road building, logging, and mining, resulted in declining salmon runs and fisheries (Nehlsen et al., 1991). Population declines, particularly those attributable to dams, led to the development of large-scale hatchery systems. These mitigation hatcheries were to replace the anadromous fish lost by the construction of dams (National Research Council, 1996). Many mitigation facilities initially were designed to compensate for huge losses of upriver fish runs by producing large numbers of juvenile salmonids for release in the lower Columbia River.

Continued human alterations of the environment, as well as industrial and agricultural pollution, have further degraded fish habitat and many runs have continued to decline. Hatcheries have been able to produce large numbers of fish which reproduce successfully at hatchery facilities but not necessarily in the wild. Thus, many runs influenced by hatchery programs are not self-sustaining, and if hatchery programs are terminated production may be lost (Chilcote et al., 1986).

The philosophies of hatchery production and mitigation, over time, are being renovated with the concept of supplementation, the use of artificial propagation to rebuild naturally reproducing fish populations, and manage for

wild fish. Matrix spawning protocols can be an important component of supplementation operations. The Imnaha chinook salmon program in Northeast Oregon embodies the concept of hatchery-supported supplementation. Currently, most hatchery operations in the Pacific Northwest fall under one of three classifications. Production hatcheries are designed to produce hatchery-sustained runs of fish for increasing harvest. Mitigation hatcheries are designed to produce fish for consumption, replacing those lost to human activities. Supplementation hatcheries are designed to produce naturally reproducing fish and rebuild depressed fish populations.

Production and mitigation hatcheries enhance salmon numbers for the purpose of sustaining or enhancing commercial and recreational fisheries (Carmichael and Messmer, 1995). Traditional spawning practices are commonly used, including pooling eggs and milt from varying numbers of males and females, selecting for early sexual maturation by spawning the fish that return first, then terminating spawning once production goals are met and excluding or culling small, non-robust appearing fish from the spawning pool. These traditional spawning methods are likely to result in artificial selection. Artificial selection is the practice of preferentially segregating fish with certain traits for a specific use, and results in the loss of genetic variability of the population. Since the specific gene combinations important for natural population health are not known, it is imperative that hatchery operations that are integrated with natural production do not intentionally select for any trait and, in essence, preserve the entire range of genetic material. In addition, unknown genetic correlations between the selected trait and other traits could lead to surprising and possibly harmful changes in non-selected traits (Kapuscinski and Miller, 1992). Artificial selection, intentional or not, combined with the above-normal survival of fish in hatchery environments, favors hatchery traits and increases the occurrence of gene combinations that may be selected against normally in the wild (Steward and Bjornn, 1990). Fish produced by production and/or mitigation facilities return to their natal hatcheries to be used as broodstock, and artificial selection may not be detrimental for their short-term existence.

Supplementation hatcheries are oriented toward maintaining naturally reproducing fish populations. Supplementation has been defined as the use of artificial propagation to maintain or increase natural production while maintaining the long term fitness of the target population (RASP, 1992). Spawning practices are governed by principles of gene conservation. Practices include: collecting broodstock from across the entire population, using unpooled milt from individual males, and following non-selective breeding guidelines by not selecting spawners on the basis of physical characteristics. These spawning practices minimize artificial selection and maximize the genetic variability of the population. Some of the fish produced at supplementation hatcheries return to reproduce in the natural environment. Artificial selection for genes not suited to the wild can compromise the survival of these fish. Maintaining genetic variability in a population preserves an array of material on which natural selection can work.

Genetic material is an integral part of the physical and behavioral characteristics of a natural population. For example, there is a genetic component to homing ability, ocean distribution of stocks, and directional preferences exhibited by some species of salmon fry upon emergence from gravel (Goodman, 1993). Interactions between the genetic makeup of a fish and its environment determine which traits will be preserved and passed on to the next generation of fish. These interactions are complex and poorly understood. As our knowledge of natural populations is insufficient to predict which physical traits and gene combinations are optimum for survival, it is critical that fish culturists and managers attempt to preserve the entire genetic composition of a given fish population. This process allows fish with many genotypes the opportunity to survive and flourish in the hatchery environment. In theory, this may provide genetic diversity necessary for adaptation in the future.

Genetic material is selected and passed on during spawning. Thus, spawning protocols are critically important activities influencing the genetic resources of a population. When artificial propagation is used to rehabilitate a natural population, the selection of spawners and spawning protocols can determine success or failure. The Imnaha River chinook salmon supplementation program provides an example of how the Oregon Department of Fish and Wildlife attempts to maintain the genetic diversity of and successfully enhance a natural population This paper will focus on the spawning protocols used in this program.

The Imnaha River Chinook Salmon Program

The Imnaha River basin drains approximately 950 square miles of the eastern Wallowa Mountains and the plateau between the Wallowa River drainage and Hells Canyon of the Snake River. Most major tributaries in the basin are contained within the Hells Canyon National Recreation Area (De Shazo et al., 1986). The Imnaha Basin has enjoyed atypical protection from degradation due to its remoteness and diminutive human population. Relatively minor damage has occurred from road building, livestock grazing, feedlots, logging, and water diversion, but the river habitat is generally considered pristine (Nez Perce Tribe et al., 1990). Fish that return to the Imnaha River to spawn must first make their way through eight dams on the mainstem Columbia and Snake rivers.

Four mainstem Snake River dams were built between 1961 and 1975: Ice Harbor, Lower Monumental, Little Goose and Lower Granite. It is estimated that these dams resulted in a 48% reduction in annual production of all chinook populations upriver of Lower Granite Dam, including Imnaha River spring chinook (Carmichael and Messmer, 1995). The Lower Snake River Compensation Program (LSRCP) was established in 1975 to compensate for fishery losses stemming specifically from the construction of these four dams. The hatchery supplementation program was initiated in 1982 under the LSRCP. The LSRCP program originally was designed with to provide hatchery fish for harvest (Carmichael, 1989). However, the focus of the Imnaha River component of this program has shifted to supplementing and recovering the natural population (Carmichael and Messmer, 1995). Management objectives are: to restore natural populations of chinook salmon in the Imnaha River basin to historic abundance levels; to reestablish traditional tribal and recreational fisheries for chinook salmon; to maintain genetic and life history characteristics of the endemic wild population; and to ensure that the genetic and life history characteristics of hatchery produced fish mimic wild fish (Carmichael et al, 1995). These are to be accomplished with the support of artificial propagation

The Imnaha Fish Facility, located at river kilometer 72 on the Imnaha River, is an adult collection and acclimation satellite associated with Lookingglass Fish Hatchery. The Imnaha Fish Facility was completed in 1989 and includes a picket weir that directs fish up a stepped ladder and into an adult trap. The adult handling facility consists of a fish elevator, an anesthetic tank and transfer tubes for release of adults back into the river above the weir. The weir is installed annually, as early in June or July as water conditions permit. Adults are collected weekly, counted, measured, and either held for broodstock or released upstream. The Imnaha chinook salmon run contains both naturally-produced (unmarked) fish and hatchery-produced (fin-clipped) fish.

The Imnaha chinook salmon population is one of the Lower Snake River populations designated as threatened and given protection under the Endangered Species Act (ESA). Broodstock collection is performed annually according to guidelines that integrate ESA and Oregon Department of Fish and Wildlife (ODFW) policies. Two hundred sixty-four Imnaha chinook salmon were projected to return in 1996, and ODFW was authorized to retain 50% of both naturally- and hatchery-produced returning adults for broodstock. These criteria were applied to each age and sex of adult Imnaha chinook salmon. Adult salmon not retained for broodstock were passed above the weir as long as the number of hatchery-produced adults did not exceed the number of naturally-produced adults.

During broodstock collection, all returning fish were anesthetized and examined for marks. In addition, their age was estimated using fork length and their sex was determined visually. Fish of the same mark, age, and sex (i.e., all 4-year-old, adipose-clipped, males) were alternately passed upstream or collected for use as broodstock, based solely on the order in which they were removed from the anesthetic tank. This was done to avoid selection for size, apparent health, or other physical traits associated with each fish. This strategy allowed ODFW to collect a representative sample of the natural run. Fish for broodstock were collected from all components of the population without bias toward run timing, age, or size to retain the greatest genetic diversity (Reisenbichler and McIntyre, 1986). It was also deemed important not to weed out jacks and precocious parr intentionally. The mechanism for early sexual maturation evolved naturally and these fish may contain genetic material important for the long-term fitness of the population (Kapuscinski et al., 1991). However, the number of jacks and precocial males that were included in the broodstock was balanced against their presumed contribution rate to natural populations (see Gross, 1991).

Guiding Principles for Imnaha Chinook Salmon Spawning Protocols

The following principles guide our conduct in regard to spawning the Imnaha chinook salmon broodstock. Fish for broodstock are collected and spawned in an attempt to augment the abundance of the naturally-reproducing population. We believe use of these principles will produce progeny with the greatest fitness and highest potential for long-term health of the population.

Principle 1: Spawn all available, fully mature broodstock. Since Imnaha chinook salmon are collected from a population governed by the ESA, we need to use all of the individuals we remove from nature. Therefore, our goal is to spawn all broodstock over the course of the mating season. We attempt to use all fully mature fish each spawning day and not exclude individuals for any reason. Fully mature Imnaha chinook salmon adults are spawned without regard to age, size or other physical characteristics.

Principle 2: When possible, spawn at least 100 of each sex. If the broodstock contain less than 100 fish of either sex, a matrix spawning protocol is implemented. In theory, an effective breeding population is the minimum number of successfully reproducing adults at which most rare gene combinations are maintained Steward and Bjornn, 1990). Kapuscinski and Jacobson (1987) suggest 100 fish as a minimum spawning pool, whereas Allendorf et al., (1987) recommend 200 individuals, split evenly by sex. Thus, approximately 100 fish of each sex appears to be an effective breeding population for Imnaha chinook salmon. Spawning less than this number of individuals can result in the loss of genetic material from the population. If there are more than 100 individuals of each sex the population appears to be large enough to maintain genetic diversity using one-by-one matings.

Principle 3: Attempt to avoid artificial or intentional selection. The goal of the Imnaha chinook salmon supplementation program is to produce fish that can successfully reproduce in the wild. Natural mating patterns are so complex and so little understood that it is extremely unlikely they could be mimicked correctly in the hatchery environment (Kapuscinski and Miller, 1993). All types of selection should be avoided in order to minimize adaptation to hatchery conditions. Imnaha chinook salmon taken for broodstock are collected from all components of the population, and are representative of the natural population in terms of run timing, age, and sex.. Small, unattractive, or sexually precocious fish are not intentionally culled from the spawning population in this program.

Principle 4: Each individual should have the opportunity to make an equal contribution to the next generation. In other words, to maintain the full diversity of genetic material collected from all components of the population, no individual spawner should be given an artificial advantage over any other. For example, if 98 male spawners are available in a broodstock, each male should be given the chance to fertilize approximately the same number of eggs. If a matrix spawning protocol is used, matrix structure should be kept as similar as possible throughout spawning, so that the family size of each male-female cross is approximately the same. We select adult spawners randomly, without bias toward observable traits, in order to decrease the potential for a given fish receiving a human-induced advantage. If matrix spawning is deemed to be the most effective protocol, a matrix structure is selected at the beginning of the spawning season and every attempt is made to remain with the same matrix throughout the season.

Principle 5: Avoid pooling semen from multiple males. There are large differences among the capabilities of males to fertilize eggs (Gharett and Shirley, 1985). If equal volumes of milt from several males are pooled and mixed with a single portion of eggs, different males may fertilize different numbers of the available eggs. In cases where milt is pooled sequentially, the order in which milt is added has a mild effect on fertilization success but does not determine what portion of progeny will be fertilized by each male (Withler and Beacham, 1994). In order to ensure each male an opportunity to make an equal genetic contribution, milt from individual males should be combined with equal aliquots of eggs. In the Imnaha chinook salmon program, males are spawned individually, milt is not pooled, and an attempt is made to combine each male's milt with an approximately equal number of eggs.

Given these principles, we recognized that using traditional spawning protocols may make it difficult to preserve the genetic diversity of the broodstock while using all available spawners. Thus, the concept of matrix spawning

was considered for use in some situations. Matrix spawning involves crossing the gametes from multiple individuals of one sex with the gametes from multiple individuals of the opposite sex. Using a given number of parents, matrix spawning allows for a greater number of genetic combinations than one-by-one spawning. In cases where the number of available Imnaha chinook salmon broodstock falls short of 100 fish per sex, it is critical to attempt to preserve the genetic combinations which provide the greatest potential for survival and reproduction in the wild. Since it is not known what specific genetic combinations best contribute to the long-term survival of the population, the best defense is to make all crosses possible. Another consideration is the ESA mandate to use all Imnaha chinook salmon broodstock collected. In cases where the female:male ratio is not equal, one-by-one spawning methods are not adequate. A matrix spawning protocol can maximize genetic diversity while providing a mechanism to spawn unequal numbers of males and females in a way that equalizes each individual's contribution to the progeny. Thus, matrix spawning is a protocol suited for use in the Imnaha chinook salmon program.

The number of females and males, as well as the female:male ratio, are used to determine the proper spawning protocol for each spawning season. If the sex ratio equals 1:1 (one female to one male) and there are at least 100 of each sex within a broodstock, one-by-one spawning may be used. If the sex ratio does not equal 1:1, even if the numbers of each sex equal or exceed 100, a spawning matrix should be used. Regardless of the sex ratio, if the numbers of either sex do not equal or exceed 100, a spawning matrix should be used, with at least two of the least numerous sex in each matrix.

Matrix development takes place at the beginning of the spawning season. The number of male and female spawners is determined and the sex ratio calculated. Since each individual needs to contribute equally to the next generation in order to avoid artificial selection, the preferred strategy is to use as many identical matrices as possible. Given the total number of broodstock and the sex ratio, select a matrix structure which will be workable throughout the entire spawning season. For example, for a broodstock of 300 which is composed of 100 females and 200 males, a 2 x 4 matrix would be selected. This matrix accounts for the 1:2 sex ratio and follows the recommendation that there should be two of the least numerous sex (in this case, females) per matrix. The majority of spawning matrices for the season should be made up of 2 x 4 matrices. However, there may be an odd number of females or males left at the end of each spawning day, due to uneven sexual maturity and/or mortalities, and the matrix may be adjusted to compensate.

Goals for matrix development are to make each parent's contribution to the next generation as equal as possible, increase the numbers of fish in each matrix, ensure that females are fertilized by more than one male and use the highest feasible numbers in each cell for a given number of spawners, i.e., a 2 x 2 matrix is preferred over a 1 x 3 matrix. Increasing the size of the matrix provides theoretical improvements if good fish culture practices are followed and extra time and handling does not substantially reduce egg survival. However, there should be a balance between genetic diversity and logistic constraints. For example, a matrix of 20 females x 20 males may provide the maximum genetic advantage, but it is probably too large to be feasible in a hatchery situation. The primary objective is to use all spawners collected and spawn them in a way that maximizes genetic combinations while limiting mortality caused by handling, time delay, or disease (McPherson, 1992).

The 1996 Imnaha spring chinook sex ratio was approximately 1:2, one female to two males. Twenty-seven females and 61 adult males were retained for broodstock. Since the number of females was well below 100, and the sex ratio did not equal 1:1, matrix spawning with at least two females used in each matrix was the protocol of choice. The choice of a 2 x 4 matrix reflected the 1 x 2 sex ratio of the total broodstock. Spawning by a 2 x 4 matrix requires splitting the eggs of each female into four approximately equal amounts, one bucket or subset for each male used. Milt from each of the two males is used to fertilize a subset of each female's eggs. The genetic result of a 2 x 4 matrix might look like the one shown in Table 1.

Table 1. Conceptual schematic of a 2 x 4 spawning matrix.

	Female X	Female Y
Male A	AX	AY
Male B	BX	BY
Male C	CX	CY
Male D	DX	DY

A 2 x 4 spawning matrix creates the maximum number of genetic combinations possible from crossing two females and four males. In contrast, the use of pseudo-matrix (Table 2) or non-matrix (Table 3) spawning would result in a maximum of four or two genetic combinations, respectively. Any of the eight combinations in Table 1 could be derived from the same six parents; however, the use of pseudo-matrix or non-matrix spawning will limit the outcome. Other problems result from attempting a 2 x 4 spawning without the structure of a full matrix. Pseudo-matrix (Table 2) generally uses pooled sperm, and may result in a single female being fertilized by multiple males. All males may not contribute equally to the progeny, however, which will skew the genetic contribution of the parents. Non-matrix spawning (Table 3) of these same parents would result in a surplus of two males, causing further loss of genetic material. Since it is not known what genetic combinations are critical for survival, future reproduction, or fitness in the Imnaha River system, the greatest number of genetic combinations that can be created should have the best effect on the long-term health of the population.

Table 2. Conceptual schematic of 2 x 4 pseudo-matrix spawning.

	Female X	Female Y
Male A	AX	--
Male B	BX	--
Male C	--	CY
Male D	--	DY

-- equals no progeny produced.

Table 3. Conceptual schematic of 2 x 4 non-matrix spawning.

	Female X	Female Y
Male A	AX	--
Male B	--	BY
Male C	--	--
Male D	--	--

-- equals no progeny produced. In this case, Males C and D are surplus.

Matrix spawning may also protect the population from loss of specific genes caused by low fecundity or poor fertilization. If Male A's sperm is nonviable, the greatest loss is suffered by the non-matrix spawning scenario, as shown in Table 3. In this case, all of Female X's eggs would be lost. In the pseudo-matrix spawning scenario, Male A's nonviable sperm would cause the loss of a full 50% of Female X's eggs. In the 2 x 4 spawning matrix schematic (Table 1), losing Male A's sperm eliminates only about 25% of Female X's eggs and 25% of Female Y's eggs. The total number of eggs lost is the same, but the risk of losing entire gene combinations is reduced. Non-matrix matings are especially affected by fertility problems, since the loss of a single male or female completely wipes out the genetic material of another contributing adult.

Given the previous principles, the following text illustrates the appropriate use of different spawning protocols and gives examples of different scenarios that might occur in the Innaha program. Before selecting a protocol to use, one should assess the total number of males and females in the broodstock, as well as he sex ratio. Table 4 summarizes the possible outcomes of this assessment. The number of males and females is related to retaining

maximum genetic diversity within the broodstock. The sex ratio of the broodstock is related to our ability to spawn each individual.

Table 4. Spawning protocol scenarios and selection.

No. females No. males	≥ 100 ≥ 100	< 100 < 100	≥ 100 ≥ 100	< 100 < 100
Sex ratio	1:1	1:1	Not 1:1	Not 1:1
Outcome	No matrix used. Spawn 1 x 1.	Use matrix. Spawn 2 x 2.	Use matrix. Spawn 1 x ?, depending on sex ratio.	Use matrix. Structure depends on sex ratio (usually not more than 2 x 4)
Category	A	B	C	D

Scenario 1: The broodstock population is composed of 40 females and 60 males. Number of each sex < 100, and the sex ratio not 1:1. These numbers are not large enough to preserve genetic diversity unless a spawning matrix is employed. Also, the uneven distribution of males and females requires a matrix to ensure that all broodstock are used. Since the number of the least available sex (females) is well under 100, a minimum of 2 females must be included in each matrix. Given these conditions, this scenario falls under category D. The selection of a 2 x 3 matrix would be appropriate for this broodstock.

Scenario 2: The broodstock population totals 100 females and 300 males. Number of each sex ≥ 100, and the sex ratio is not 1:1. The large size of the broodstock is sufficient to protect genetic diversity. However, the uneven sex ratio requires matrix spawning to enable the use of all spawners. Since the least available sex equals 100, it is not necessary to include at least two females in each matrix. Given these conditions, this scenario falls under category C. The selection of a 1 x 3 matrix would be appropriate for this broodstock.

Scenario 3: The broodstock population is evenly split with 150 spawners of each sex. Number of each sex spawned ≥ 100, and the sex ratio is 1:1. Numbers are large enough that genetic diversity is not an issue. The even sex ratio makes spawning all individuals straightforward. Given these conditions, this scenario falls under category A. A One-by-one spawning scenario would be appropriate for this broodstock.

Scenario 4: The broodstock population is composed of 80 males and 80 females. Number of each sex spawned < 100, and the sex ratio is 1:1. This population size is not large enough to provide the desired genetic diversity unless spawned in a matrix. Since numbers are small, a minimum of two of the least available sex should be included in each matrix. The even sex ratio lends the population to an even-sided matrix. Given these conditions, this scenario falls under category A. The selection of a 2 x 2 matrix would be appropriate for this broodstock.

Summary

The Imnaha chinook salmon population is a depressed Lower Snake River population designated as threatened under the Endangered Species Act. The goal of ODFW's Imnaha River chinook salmon supplementation program is to use artificial propagation to augment the numbers of the naturally reproducing population. Our primary goal is to maintain the genetic diversity of this unique population. Given this goal, a number of principles guide our conduct. We attempt to spawn all fully mature broodstock, maintain an effective breeding population by spawning at least 100 individuals of each sex, avoid artificial or intentional selection, allow each individual the opportunity to make an equal contribution to the next generation, and avoid pooling milt. The use of a matrix spawning protocol allow us to maximizs the preservation of genetic diversity, and spawn all of the broodstock. Thus, matrix spawning is our protocol of choice for most scenarios involving Imnaha chinook salmon.

Conclusions

Spawning practices and protocols are valuable tools to aid in the preservation of genetic diversity of depressed salmon stocks. As the focus slowly changes from traditional management objectives of production and mitigation, the objective of supplementation is gaining importance in fish culture. The use of spawning matrices may be critical in supplementation programs. However, a continuous evaluation of what works and what doesn't each breeding season, and adjustment of activities if needed (adaptive management), are necessary for a commitment to healthy fish populations. For the present, there are enough established guidelines available that any hatchery with an interest, an eligible population, and clear goals can use types of protocols described above. These protocols are flexible enough for a hatchery manager to mix and match at his/her discretion, according to the station's unique needs. They can be adapted to virtually any hatchery program a critical eye. In closing, Meffe (1987) reminds us that the science of conservation genetics is in its infancy and we will make mistakes. The pain of these mistakes can be minimized if we maintain maximum genetic diversity for maximum flexibility in future research and management.

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OPTIMIZING INCUBATION

AT SALMON HATCHERIES

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Introduction

Incubation is the process of turning a bag of chemicals into a living organism. This "miracle of life" transformation, the most profound event in the history of the universe, occurs every time an egg is successfully fertilized. While over 3 billion years of evolution has done a pretty good job of making the process fairly robust, fish culturists can still play an important role in giving each individual miracle its best shot at success.

Some points should be kept in mind when thinking of how to optimize conditions for incubation:

First, the process of incubation evolved with a number of assumptions about the environment in which it would occur, and is adapted to work well in that environment. For salmonids, this usually means the environment of fairly pristine mountain streams, and for Pacific salmon, it means cold, clear, high-oxygen, high-flow gravel beds. This is usually the kind of environment we try to mimic in fish culture facilities, using ultra-clean water and avoiding temperature extremes.

Second, this 'typical' environment is not necessarily 'ideal' for the incubation process. The reason that salmonids (a marine fish) sought out freshwater streams in which to lay their eggs probably had more to do with avoiding the abundance of predators in the ocean than that streams are a perfect incubation environment. (t is in fact the inhospitable-to-life nature of mountain streams, with their lack of nutrients, shifting substrate and cold temperatures, that is appealing to salmonids. Neither predators nor pathogens can survive in these locations during the long periods when salmon eggs are not available.

Third, optimization is an arrow that needs a target before deciding which way to aim the bow. Usual targets are perfect survival or maximum fry weight, but targets such as highest disease resistance or shortest swim-up timing might require aiming in a different direction. The fish culturist does not have to blindly follow nature's lead but has many options to manipulate incubation outcomes to serve whichever 'optimal' target is selected.

Fourth, while the water supply at every hatchery is undoubtably not 'optimal' for incubation, embryonic development is so efficient and low-key that it has little impact on the water used for it. Therefore, water supplies can be fairly inexpensively re-engineered to be more 'optimal' than what they were originally, using recirculation technology.

The incubation process starts off with the production of the eggs and sperm in the bodies of the broodstock, includes the stripping and fertilization of the eggs and their placement and care in incubation containers, and ends when the fry have swum up and started to feed. Each of these steps can be improved by improving the environment (physical and chemical) in which they occur, by removing deleterious effects (like pathogens or toxins) and by providing stimuli to accelerate (or decelerate) development.

Adult Holding

The role of the adult in incubation is to furnish the process with good-quality eggs and sperm, or more correctly, to take the best care of itself so that good quality eggs and sperm can be produced.. The role of the fish culturist is to take the best care of the adults so that they are in fit condition to produce good quality eggs and sperm.

The processes of egg and sperm production within maturing adults is a long and complex sequence of precisely timed and executed events. These events are signalled and triggered by a cascade of hormone releases that start before the germinal cells begin to undergo meiosis to produce gametes and are still going on while eggs and sperm are being shed from the adults' bodies.

We have to understand that the adult salmon has other things on its mind, in addition to procreation, while it is creating eggs or sperm, some of which, like survival, take precedence. The amount of energy that an adult puts into gamete production is dependent on the amount of energy that it has to spare. The fish culturist should seek ways to minimize the requirement for energy use for other things by the adults, to maximize the effort available for gamete production.

Some guidelines are:

Provide an optimal environment:

Temperature - cool enough to keep oxygen levels high, metabolic rates low and reduce invasion by pathogens, but warm enough to allow thorough gamete development.

Light - low enough to reduce excitement.

Space - room enough to move and avoid rubbing against each other and the container walls but not enough to establish territories.

Water Flow - enough for sufficient oxygen supply and waste removal.

Chemical properties: ionic content of the water should be sufficient to minimize leaching of essential ions needed for egg development.

Protect from pathogens:

Minimize handling to avoid the abrasions, stress and trauma that can encourage infection.

Maintain physical separation between broodstock and sources of pathogens or toxins (birds, rodents, wild fish, people).

Inject or bath with antibiotics to control pathogen growth on adults being held.

Stimulate development

Manipulate degree of isolation from each other to stimulate or delay maturation, depending on the preferred timing of spawning.

Inject hormones (e.g. LHRH) to stimulate final maturation and synchronize timing of egg-takes.

Provide nutrients in food or water that will aid in gamete development.

Fertilization

The first requirement of successful fertilization is good quality eggs and sperm, which is mainly determined by how the adults were treated. Once removed from the adults, the gametes may or may not have all that is required to produce a living embryo. The fish culturist has many options available to mae the fertilization process as near perfect as the quality of the eggs and sperm will allow.

Some guidelines:

Provide an optimal environment

Ensure that water temperature, oxygen content, other dissolved gas pressures and light are suitable at every stage of the process. Test the egg's environment with an oxygen probe and thermometer every minute during a typical (or extreme) egg-take procedure to convince yourself that the eggs (and sperm) are well taken care of at all times.

Manipulate the ionic content of the water used for fertilization, washing and hardening, to ensure that there is enough sodiu, calcium and other ions for proper fertilization (Brown and Lyman, 1981) and hardening (Li et al., 1989). Such a small amount of water is needed for these activities that almost every hatchery could benefit from using a prepared mix rather than just using the available water (Rieneits and Millard, 1987).

Use procedures that eliminate occurence of blood or broken eggs in the fertilization buckets. Use a bicarbonate soda wash (Wilcox et al, 1984) to dilute any cytoplasm (potassium) in the ovarian fluid, since it severely inhibits sperm activation (Morisawa et al., 1983).

Protect from pathogens

Use test kits to screen for known pathogens and eliminate carriers from the egg pool.

Select only those eggs that are at their peak of ripeness. Do not use eggs from over-ripe females with watery ovarian fluid, nor eggs that have had to be torn out of the skein. Test the fertilizability of eggs from different parts of the abdomen and taken from fish with different degrees of softness to determine the best time and procedure for extracting eggs.

Handle eggs and sperm carefully during extraction, storage and mixing to minimize physical damage.

Make sure that your wash water, fertilization water and hardening water are all sterile.

Stimulate development

Consider using an activator solution to stimulate or prolong sperm activity (Moccia and Munkittrick, 1987).

Manipulate the content of the harding water content to see if you can provide essential nutrients Ronnestad and Fyhn, 1993) or ions to the egg at the only time when it is taking in large amounts of external water. After hardening, the egg becomes quite impervious to movement of all but the smallest chemicals through the shell.

Egg and Alevin Incubation

Much of the fish culture procedures and criteria for incubation is determined by the containers in which the fertilized eggs are kept over the incubation period. Biologically, the choice of container should not make much difference to the fish, since every kind should provide the same kind of even, gentle flow that brings oxygen and removes wastes from the area around every egg. Care needs to be taken that a container or the way it is loaded does not crush or deform eggs.

The stages of development that an egg goes through after fertilization, from the combining of the sperm and egg haploid nuclei, through the first cell division and formation of the blastula, the gastrula, the neural fold, the eyes, etc. are incredibly complex (Velson, 1980) and are made up of, and controlled by biochemical reactions that were pre-set, all ready to go, in the bag of chemicals (Hamor and Garside, 1977) that was produced by the female salmon (Brachet and Alexandre, 1986).

Provide an optimal environment

Ensure that water quality is kept at the highest standards, including oxygen, nitrogen and total gas pressures, ammonia, nitrite and carbon dioxide. Micro-environments within an incubation container can be very different from one another due to the pattern of water flow or stagnation within the incubation container.

Add ions to your process water if it is very soft or acid and soften the water if it is extremely hard (Gunn and Keller, 1980).

Keep the temperature within the metabolic limits of the fish (Weatherly and Gill, 1995), with the understanding that this is one area where the environment can be manipulated to suit your needs, since those of the fish are very plastic.

Minimize disturbance by keeping light and sound (vibration) levels very low.

Provide media for alevins to lean up against, reducing energy wasted in thrashing around.

Protect from pathogens

Thoroughly disinfect both the containers and the fertilized eggs at the beginning of incubation. Start off with a clean water supply and make it even cleaner with disinfection.

Take every step possible to ensure a pathogen-free environment, including limiting work around and access to the incubation area.

Pick dead eggs out of the system as soon as possible. Pre-eyed picking has proven to be very useful in stocks with poor fertilization, if conducted extremely carefully.

Minimize handling and disturbance to only those events that are essential (Jensen and Alderdice, 1983). There are always a great deal of extremely complex biochemical events going on in eggs and, even though the egg is very robust much of the time, a perfectly healthy egg can be killed if the timing of a hard bump occurs at a sensitive period for only one of its millions of cells.

Stimulate development

While the egg is encased in its shell, it might be possible to provide it with some ions or nutrients that will aid in its development. Once the shell is gone, alevins are much more intimately connected with, and sensitive to, the water around it. Treatment with hormones at this stage can alter the sexual development of salmon and it is likely that other chemicals can be utilized by the alevin in its development.

Experiment with adding nutrients, hormones and ions to the incubation water to see if they direct development closer to the incubation target.

Recirculation

Many of the suggested improvements to the environment for adult holding and incubation listed above involve altering the basic characteristics of the water used in the hatchery. While this might be considered to be a shopping list for the type of water that would make up the perfect water supply (but impractical to implement in facilities that do not have the suggested type of water), most of the changes described above can be accomplished fairly easily at any hatchery using water recirculation technology. In fact, complete control of the incubation environment probably depends on the application of water reuse, since to alter the characteristics of any flow-through supply to such an extent would be prohibitively expensive.

The appeal of recirculation for incubation water is that it is much simpler to treat incubation waste water than it is to treat rearing waste water. During rearing, massive amounts of extraneous material is added to the system in the form of feed, and over half of the feed is not incorporated into fish flesh and becomes waste, mainly solids (uneaten food and feces) and ammonia (Timmons and Losordo, 1994). During incubation, no extra material is added to the system whatsoever, and only a very small fraction of the existing egg is excreted as waste, mainly ammonia. For a short period of time, the egg shells are shed and can be removed either as solids, or after they have disintegrated, as a foam fraction. This means that a recirculation plant for an incubation system needs to be much simpler, smaller and cheaper than one for a rearing system.

Conclusion

Fish culturists have the opportunity to make major improvements in the quality of incubation in salmon hatcheries. Any hatchery with a history of poor incubation success should take a more active role in the control of the physical, chemical and biological components that make up its system. Recirculating the incubating water can make such control relatively inexpensive.

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CHILLED, RECIRCULATED INCUBATION FOR COHO SALMON

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Purpose

The well water supply at Inch Creek Hatchery, located in the lower Fraser River valley 14 km east of Mission, BC, has a reverse temperature profile, making it warm (up to 13.5°C) in the winter and cool (down to 5°C) in the summer. The two major problems with culturing coho salmon at this facility are that:

1. The warm water causes an early swim-up and very long rearing period for the fish, requiring that they be fed at a very low ration level to keep their release weight to the target 20 g. We do not consider such near-starvation rearing to be good for the fish;
2. Periodic myxobacterial infections occur, usually during early rearing in February and March and again during June and July of the first year of rearing. The virulence of these infections has been variable from year to year, from stock to stock and from container to container within the same year and stock, but the infections are a nuisance, and require that we take an extra 20-30% more eggs than our target to compensate for rearing mortalities.

Delaying the onset of rearing would allow for a more normal feeding regime and might avoid the time period during which myxobacterial infections usually occur. In 1994, we conducted a small-scale test to delay the swim-up of fry from the usual January-February period until May, by chilling the incubation water to 4°C. The eggs/alevins in the test group survived as well as the normal production groups that were incubated in normal well water (averaging 10°C), but the fry that swam up in May contracted the myxobacterial infection in June along with the normal production group that were several grams larger.

Since we could only borrow a small chiller, we recirculated the incubation water at about the 95% reuse level, based on flow. In 1995, we scaled up our recirculating, chilled system to a capacity of half of the coho production, and to be able to chill to less than 2°C, so that rearing would not commence until July. This paper reports on the results of that test.

Methods

Two batteries of incubator trays were used, one for the chilled water (which was recirculated) and one for the ambient well water (which was flow-through). The flow-through system was a standard set of stacks of Heath-style incubators fed by a head tank that received aerated well water and drained through a floor drain.

The recirculation system consisted of these major components:

1. Sump: a 100 L aluminum box was placed on the floor to receive the outflow from the upper battery of Heath-style incubator trays. The sump also received a small amount (less than 1.0 L/min) of make-up flow and was designed to overflow to the regular floor drain.
2. Pump: a 1.5 hp, in-line pump was plumbed to receive water from the bottom of the sump and deliver it through the chiller and sterilizer to the aeration box.
3. Ultra-violet sterilization: all process water passed through a UV sterilization unit consisting of six 3 ft long bulbs.
4. Chiller: two 3 hp chiller units in series had the capacity to keep the water temperature at about 1.5°C in a room

that had an air temperature anywhere between 10° - 20°C.

5. Supply piping: including flow meter, valves, bypass loop around the sterilizer and make-up water supply.
6. Aeration box: the aeration box consists of a calibrated distribution plate and a media bed (1.5" diameter flexi-rings) with louvred walls to allow ventilation (MacKinlay, 1991). This type of media-filled box can also serve as a biological filter. The aerator fed water to a header tank that distributed it to the incubators.
7. Incubators: the Heath-style incubator trays were arranged in stacks of 8 trays each, receiving approximately 15 L/min of flow per stack, with a capacity of 8-10,000 coho eggs per tray.
8. Ammonia filter: some empty incubator trays were filled with clinoptilolite, a mineral that is purported to absorb very large amounts of ammonia from water. When total ammonia concentration approached 1.0 mg/L (the safe level was estimated to be anything less than 10 mg/L, Sigma, 1983), the clinoptilolite was changed.

Standard methods for egg-take and incubation operation were used, including disinfection before placement in the incubator containers and picking of dead eggs after the eyed stage. Water temperature was controlled mainly by adjusting the amount of make-up water entering the system, although the chiller unit was thermostatically controlled. An in-line flow meter indicated that the system was pumping about 250 L/min.

Results and Discussion

Our major concerns about the recirculated, chilled water were that placing the eggs in cold water might kill them, that a long period of incubation in cold water might kill or deform the fish, or that long-term exposure to elevated levels of nitrogenous wastes might have detrimental effects on the fish. We also understood that the prediction of development timing would not follow our usual model that is based on events occurring at certain accumulated thermal units (°C times days) for each development stage and species.

Cold water effect on survival

Several authors (Weatherly and Gill 1995, Hubert and Stonecypher 1994, Hubert and Gern 1995, Beacham and Murray 1990) warn that plunging eggs into very cold water (<4°C) would cause high mortality. Although salmonids are known to withstand extremely cold temperatures in the wild, the consensus seems to be that at least the very early stages of development require warmer (>6°C) water. Weatherly and Gill (1995) suggest that it takes 4-6 days at 6°C to reach the blastula stage of development, which is the first stage that can tolerate colder temperatures. For this reason, we started off the incubation of all groups at 6°C and four days after the last group was placed in the system, we decreased the temperature to 2°C.

We conducted an experiment to test the effect of plunging eggs into 2°C water after various lengths of time in 10°C water; 0 days, 5 days, 10 days and 23 days. There was no clear trend to survival versus length of time at the warmer temperature, but since only one of the 15 lots from the pooled group of eggs had survival over 80%, we considered that the group of eggs that was used were substandard and will try the same experiment again this year.

Table 1 indicates equivalent survivals for fish that were incubated in the chilled water and those that were incubated in the ambient water. The survival during rearing was fairly poor for the chilled fish immediately following ponding, and then mortalities levelled off to the same level as those of the ambient fish. This just made up for the longer rearing period during which the ambient fish had been accumulating mortalities.

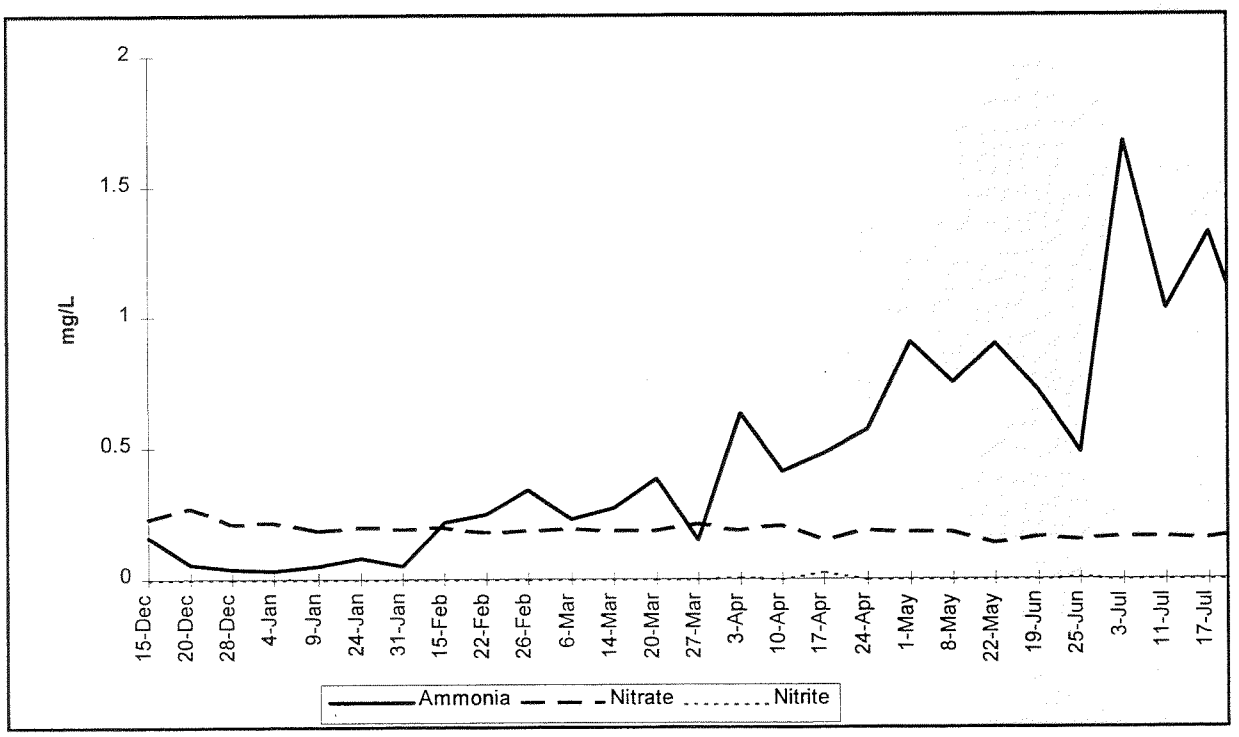
Table 1. Survival of Chiller and Ambient groups of Inch Creek Coho for 1995 Brood.

Group	Eggs Taken	Fry Ponded	Survival to Ponding	Fry Rearing in November	Still Rearing to date
Chiller	382224	322198	84.3%	305054	94.7%
Ambient	348057	258395	81.4%	243000	94.0%

Metabolite build-up

Oxygen and temperature monitoring were carried out at least twice daily during incubation. Water samples were taken weekly and sent to the lab for analysis for ammonia, nitrite, nitrate, pH, and hardness. At no time did the ammonia or nitrite levels reach toxic levels (Figure 1). Even though our water reuse rate was extremely high, the nitrite never increased much above the barely detectable level and nitrate did not continue to build up over time, as would normally be expected of a recirculation system (Muir, 1982). Changing the clinoptilolite did not seem to make a noticeable difference to the ammonia levels, but we suspect that the system developed a population of nitrifying bacteria on the containers and the aerator media, since the nitrite and nitrate levels remained fairly stable. Since it only takes a few minutes to flush the entire system clear of metabolic wastes using fresh water, we will probably not use the clinoptilolite in the future.

Figure 1. Concentration of Nitrogen metabolites during incubation recirculation at Inch Creek in 1996.



The main justification to using a very simple recirculation system during incubation is that metabolic waste does not build as quickly nor as high as it does in a system involving rearing. There is no need for solids removal and the expelled egg shells that are not picked out by the fish culturists rapidly disintegrate into dissolved substances that essentially remove themselves by foaming. While this can prove to be a nuisance in a large-scale operation, the foam removal can be accelerated by incorporating a foam fractionator somewhere in the system loop.

Development Timing

Even though the usual practice in our hatcheries is to predict that hatch of coho will occur at about 450 ATU (accumulated thermal units, in degrees centigrade times days) and ponding will occur at about 850 ATU (Shepherd, 1984), we expected that a lower incubation temperature would require fewer ATU's and therefore expected the fish to be ready for ponding well before they reached 850 ATU. The developmental timing models reviewed by Beacham and Murray (1990) indicate that, at 2°C, hatch should occur at 370 ATU and ponding at 530 ATU. These models overestimated incubation timing of this experiment (Table 2), but a computer model by McLean et al (1991) predicted ponding at 480.4 ATU for 1.9°C water, which was right on the mark for the last group.

Table 2. Development Timing of Inch Creek Chiller Coho for 1995 Brood. All eggs were started at 6°C and then dropped to 1.8°-2.0°C for the duration of their incubation.

Group	Egg Take Date	Days at 6°C	50% Hatch Date	Ponding Date	Hatch ATU (Days)	Ponding ATU (Days)
1	Nov 17/95	28	Mar 29/96	Jun 13/96	375 (133)	510 (209)
2	Nov 27/95	18	Apr 23/96	Jul 6/96	350 (148)	495 (222)
3	Dec 7/95	8	May 16/96	Jul 18/96	330 (161)	459 (224)
4	Dec 11/95	4	May 25/96	Jul 29/96	320 (166)	482 (231)

Conclusions

Both the chilling and the recirculation components of this system worked very well. The build-up of metabolic waste can be easily handled with periodic flushing of the system with fresh water. This year we intend to monitor ammonia build-up with a simple chemical test kit rather than expensive lab analyses, and flush when the ammonia gets up to 0.5 mg/L. We have also re-plumbed the chiller system to initially chill the water in a group of Atkins-style bulk-box incubators to increase the capacity of the system for early incubation. After the eyed stage, the eggs will be moved to the Heath stacks and the chiller will be switched over to that water system.

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FORT BABINE HATCHERY - INSTREAM REARING AND INCUBATION

Prepared by : Brenda Donas, Community Advisor, Habitat and Enhancement Branch, D.F.O.

The Fort Babine Hatchery is located approximately 140 kms. north-west of Smithers, B.C., near the Fort Babine Band Reserve. The hatchery is one of the Community Economic Development Program facilities operated under contract to D.F.O..

The Fort Babine Hatchery produces 85,000 chinook yearlings, 80,000 coho yearlings, 100,000 fed coho fry and 25,000 fall release chinook fry. All of the tributaries to the Babine River in the area of the hatchery are basically zero gradient ie. meandering. Due to this low head, it is not possible to pipe surface water to the hatchery for conventional (on land) incubation and rearing containers. At the time of hatchery construction, there was no electrical service to Fort Babine therefore pumping water was also not possible. (Electrical service reached Fort Babine about seven years ago, however, the cost of pumping water is impractical). The alternative was to use the resource that was there ie. the Babine River.

Incubation at the Fort Babine Hatchery

Chinook salmon spawn in the Babine River during September. Adults are captured at the Babine River counting fence and egg takes are done at the fence site. Initial incubation occurs in a moist incubation system. The moist incubation system consists of a large aluminum holding reservoir which is filled with water pumped from the Babine River and a moist incubation system which contains trays and a timer system. The eggs are misted once every six hours from fertilization to the eyed stage. The reservoir water is kept cool by a Taylor Refrigeration aquarium style cooling unit.

Coho salmon spawn in the Babine River throughout November and December. Initial incubation occurs in the moist incubation system.

At time of eyeing, eggs are shocked, picked and weight enumerated. The eyed eggs are then transferred to the instream cassette incubation system. The cassette incubation system consists of screened trays that slide into a floating aluminum frame. The cassette frame sits in the upstream section of the floating raceway and is covered with a plywood lid. The top and bottom cassettes are left empty as these trays are most likely to suffer predation from otters. Cassettes are cleaned every few days with a paint roller. Fine organic debris tends to cling to the screening of the cassettes and so in order for adequate water exchange to occur the screens must be kept clean.

The eggs remain in the cassettes right through to the button-up stage. The winters are very harsh in the North and water temperatures can dip as low as 0.5 degrees Celsius. Thus far, there have not been icing problems with the cassette system.

Rearing

At time of ponding, the cassettes are treated with a 1 hour, static, 8.5 PPM choramine-T bath. The fry usually develop a myxobacterial infection after ponding and we are finding that by doing a Chloramine-T treatment at time of ponding, very few fish become infected.

After the Chloramine-T bath, the fry are ponded into the floating raceways. These raceways are of aluminum construction with perforated ends and bottoms and solid aluminum sides. The raceways are suspended within a large float by chains such that raceway depth can be altered by changing the level setting of the chains.

The raceways vary in size from 12.7 to 15.4 cubic metres. Float construction varies with some of the floats being of styrofoam block and wood construction while others were constructed using "Dock Blocks" and 2"*6" wood. The raceway floats look like large rectangles with a pointed front i.e. are pentagonal in

shape with the point of the pentagon pointing into the river flow. Each raceway is anchored using large concrete blocks which sit on the river bottom. The raceways float in relatively shallow water ie. about 6 to 10 feet deep and the raceways themselves are only four feet deep when fully lowered. Several of the raceways are also anchored to land. A system of wooden docks and walkways provide access to land and between raceways.

The raceways are covered with sloping wooden lids that have 4 sections which can be opened to accomodate feeding and cleaning activities. The sloping lids allow for easier snow removal as well. The lid openings can be locked to deter vandalism.

The Pros and Cons of Instream Incubation and Rearing on the Babine River

There are some inadequacies with instream incubation and rearing as follows :

- no fine control over flows going through incubation and rearing
- cassette incubators are more difficult to clean as compared to Heath stack type incubation
- eggs are subject to predation
- snow removal is necessary
- increased bacterial load due to spawning adults in the vicinity of the raceways
- no temperature control
- difficult to treat for disease ie. static bath treatments are inconvenient

There are also positive points to the instream incubation and rearing :

- no piping necessary to supply containers with water for incubation or rearing
- no electrical service required, therefore can be used in remote locations
- fish have access to natural feed organisms (plankton)
- no head required to operate the system
- no water alarms required, therefore no after hours emergency stand-by time
- low cost to operate due to low maintenance and no energy costs
- rearing containers can be moved around by towing them behind a boat ie. site location is flexible

Survivals from Fort Babine hatchery have been comparable to on-land facilities in Northern British Columbia, (as per results from coded wire tagging programs which have been conducted on coho and chinook at the hatchery).

Design of the system at the Fort Babine Hatchery is attributed to Matt Foy.

MANIPULATION OF GROWTH AND ADIPOSITY OF JUVENILE CHINOOK SALMON

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Introduction

In the Pacific Northwestern United States and Western Canada, hatchery reared chinook salmon (*Oncorhynchus tshawytscha*) return from the ocean to spawn at a lower rate than wild fish. Possible reasons include; increased incidence of disease, behavioral differences and differences in physiology. Hatchery reared salmonids are normally larger than wild fish at the time of release and contain higher levels of lipid. Size and adiposity (stored lipid) have been shown to affect physiological factors such as smoltification and early maturation in salmonids. The objective of the current study was to determine if growth and adiposity could be manipulated independently in juvenile chinook salmon by simultaneously varying amount of ration and dietary lipid level in high protein diets. Specifically, we wished to produce four groups of fish; two groups similar in size to those released from hatcheries, one with lipid levels similar to those of hatchery fish (high lipid) and the second, with lipid levels similar to wild fish (low lipid); and two groups of smaller fish, similar in size to wild fish at the time hatchery fish are released, which would contain subgroups with high and low lipid.

Materials and Methods

Duplicate groups of chinook salmon (*Oncorhynchus tshawytscha*) fry (0.7 g) were fed high protein diets (Table 1) containing high lipid (HL) (65% protein, 23% lipid) or low lipid (LL) (85% protein, 3% lipid) at high ration (near satiation= HR) or low ration (one half of high ration= LR) for 247 d to determine if growth and adiposity could be independently controlled.

Table 1. Experimental Diets.

	g/kg/dry wt[1]
Fish meal[2]	563
Wheat gluten	50
Vitamin C	11
$CaHPO_4$	25
Vitamin mix[3]	15
Arg	5
Gelatin	100
Fish oil	30 or 230
Choline Cl	10
Trace mineral mix[4]	1
Carboxymethyl cellulose	20
Algibind[5]	20

[1] Water added (500 ml/kg dry diet), [2]Supplied by NMFS, Kodiak, AK; 94% protein, 2% lipid, 4% ash.

[3] USFWS, Abernathy, [4] USFWS, No. 3, [5]Algea Produkter A/S, Lier, Norway.

This combination of diet and ration produced four treatment groups: HR/HL, HR/LL, LR/HL, LR/LL. Each diet was fed to duplicate groups of fish. In contrast to a traditional nutrition experiment where the effect of various diets on growth and feed efficiency are examined, our objective was to manipulate size and adiposity by feeding diets differing in nutrient composition and possibly feed efficiency. This required employment of a iterative feeding regime. On day 1 of the experiment, the amount of feed eaten by the fish fed the high ration diets was noted. For the remainder of the first month, the high ration groups were fed 10% less than the group that consumed the least feed on day 1, and the low ration groups were fed half this amount. The day after the first monthly sampling, and each subsequent sampling, the amount of feed to be fed to each tank, for the remainder of the month, was calculated as follows;

in the high ration tank with the lowest feed consumption/fish,

$$(C \times 0.9)N$$

where:

C = the lowest average amount of feed consumed/fish in the high ration tanks the first day after sampling

0.9 = a factor to help insure that all feed fed is consumed

N = the number of fish in the tank

in the other high ration tanks,

$$(C \times 0.9)N + \frac{\left(\frac{(W_{HR} - W) \times N}{F}\right)}{d}$$

where:

W_L = the largest mean fish weight in the tanks fed the high ration

W = the mean weight of the fish in the tank

F = the feed efficiency in the tank the previous month

in the low ration tank with the largest mean fish weight,

$$\frac{(C \times 0.9)N}{2}$$

in the other low ration tanks,

$$\frac{(C \times 0.9)N}{2} + \frac{\left(\frac{(W_{LR} - W) \times N}{F}\right)}{d}$$

where: W_{LR} = the largest mean weight of fish in the tanks fed the low ration

Fish were generally fed 5 days per week. Feed was supplied twice each day until fish ceased to feed or had consumed their allotted ration. If a group of fish failed to consume their feed allotment in five days they were fed on day 6 (Saturday). The low ration groups received one half of the amount of feed fed to the high ration groups fed the same dietary lipid level. The effects of ration and dietary lipid level on; weight gain, adiposity, feed efficiency and protein retention efficiency were assessed using regression and two-way ANOVA ($P<0.05$).

Results

Our results show that, when high protein diets were fed, ration level controlled growth and dietary lipid level controlled adiposity. Amounts of dry feed fed were; 17.5, 15.4, 6.7 and 6.1 g/fish; final mean fish weights were; 20.2, 19.5, 9.2 and 8.7 g (Fig 1) and final whole body lipids were; 11.3, 5.4, 7.1, 3.9 % for the HR/HL, HR/LL, LR/HL, LR/LL treatments (Table 2). Fish fed the LL diets were fed slightly less than fish fed the HL diets due to under estimation of feed efficiency in the LL treatments during the final month of the experiment (Table 2). Overall feed efficiency (109-127%) was lowest in the HR/HL groups but differences among treatments were not significant (Table 2).

Table 1. Final weight[1,2], mortality, feed fed, Feed efficiency (weight gain/feed fed), final whole body protein (wet basis) productive protein value (PPV) and final whole body lipid of juvenile chinook salmon fed a high or low ration containing high or low amounts of lipid.

Treatment	Final wt. (g)	Feed Fed (g)	Feed efficiency %	Final lipid %
High ration/High lipid (HR/HL)	20.2±0.1[a]	17.5±0.5	109±1.5	11.3±0.2[a]
High ration/Low lipid (HR/LL)	19.5±0.3[b]	15.4±0.2	123±0.5	5.4±0.1[c]
Low ration/High lipid (LR/HL)	9.2±0.3[c]	6.7±0.1	118±8.5	7.1±0.1[b]
Low ration/Low lipid (LR/LL)	8.5±0.2[c]	6.1±0.1	127±1.5	3.9±0.1[d]
P (probability)				
Ration	<0.0001	<0.0001	0.21	<0.0001
Lipid	0.02	0.02	0.06	<0.0001
Ration x Lipid	1.0	0.07	0.60	0.0001

[1] Initial whole body protein 12.5%, final protein (%): HR/HL, 16.6±0.7; HR/LL, 17.2±0.6; LR/HL, 15.8±0.6; LR/LL, 15.5±0.7.
[2] n=2 for all analysis.

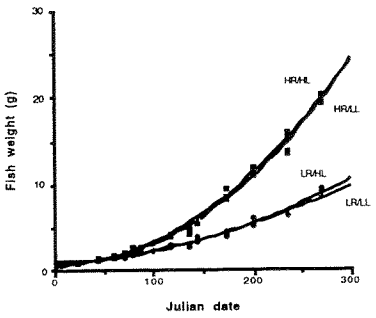

Figure 1. Growth of juvenile chinook salmon fed a high (HR) or low (LR) ration containing high (HL) or low (LL) lipid.

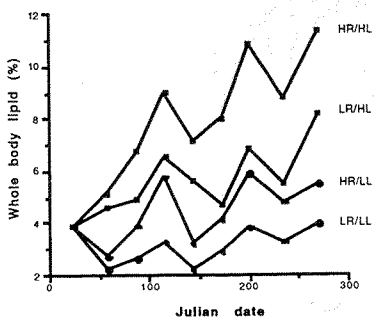

Figure 2. Whole body lipid of fish fed high ration (HR) or low ration (LR) diets containing high lipid (23%=HL) or low lipid (3%=LL).

Discussion

Our study indicates that growth and adiposity can be varied independently in juvenile chinook salmon by varying ration and diet composition. This will allow examination of the independent and combined effects of these factors on metabolism, smoltification, early male maturation and immunocompetence in juvenile salmonids. If further research indicates that growth rate at a specific time of the year, a specific size at release or a specific adiposity improve smolt quality, then similar diets and a similar feeding regime can be used to improve hatchery effectiveness.

Acknowledgements

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THE OPTIMAL FEEDING LEVEL FOR JUVENILE WHITE STURGEON *Acipenser transmontanus* AT 12°C USING A MODIFIED ABERNATHY SALMON DIET

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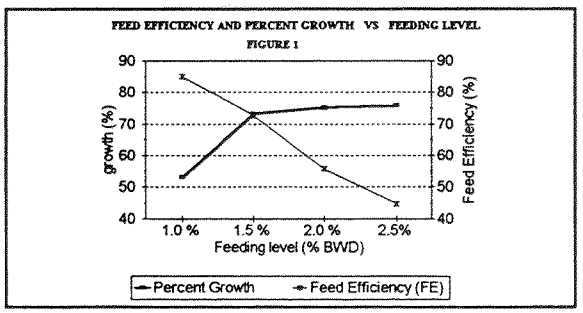
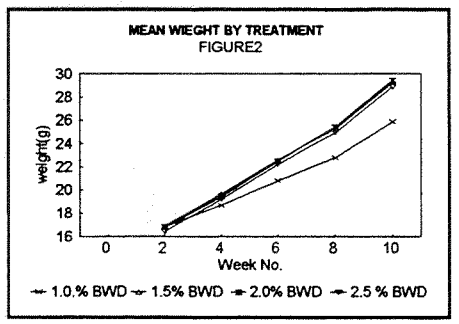
With the decline of several native populations of white sturgeon in the Pacific Northwest, fishery managers are considering hatchery supplementation as an option for restoration. Being a relatively new cultured species, only limited data has been published on their optimal feeding rate at various life stages. No published data is available on feeding rates for this species at temperatures typically found in Columbia River basin supplementation hatcheries (9-15°C); the most likely location for future enhancement efforts. The purpose of this study was to identify the optimal feeding rate for post larval (~15g) white sturgeon at 12°C.

Fingerling white sturgeon graded as "mediums" (~60% of the population met the length criteria) were transferred from a soft-moist diet to the standard Abernathy Salmon diet for 30 days. Fingerlings that had adapted to the diet were randomly stocked into 12 tanks of eighty fingerlings each. Each tank was randomly assigned to one of four feeding levels (1, 1.5, 2, or 2.5 percent body weight per day (BWD)). Rearing units were 2.5 ft fiberglass circular tanks with a 210 liter capacity. Well water was supplied at 3 liters per minute via a surface spray bar. After a two week acclimation period the 8-week feeding trial began. Fish were fed a version of the Abernathy diet modified to meet known white sturgeon nutrient requirements (52.1% protein, 14.5% fat, 6.6% ash, 7.4% moisture). Feed was distributed by automatic feeders 24 hr per day at 2 hr intervals. Rations were adjusted daily for growth and mortality. Stocking densities were kept below 15g/liter.

The mean water temperature and dissolved oxygen levels were 11.9°C and 9.4 ± .2 mg/liter, respectively. There were no significant differences (P= .05) in mean weight, length or percent growth for the 1.5%, 2.0%, and 2.5% feeding levels. The 1% feeding level had significantly slower growth rates and lower mean weights than the other treatment groups. There were no significant differences in the final whole body protein and lipid levels.

The best feed efficiency was realized at the 1% feeding level. At the 1.5% feeding level growth was significantly higher and feed efficiency significantly lower than the 1% feeding level. A feeding level of 1% BWD for white sturgeon juveniles in the size range from 15-30g at 12°C appears to be optimal (See figures 1 and 2).





COMPARISON OF GLUCANS FROM THREE SOURCES IN DIETS FOR FALL CHINOOK SALMON *ONCORHYNCUS TSHAWYTSCHA*

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Glucans administered by intraperitoneal injection or as a bath have been used to enhance the nonspecific immune response in Atlantic salmon, *Salmo salar*, coho, *Oncorhynchus kisutch*, channel catfish, *Ictalurus punctatus* and brook trout, *Salvelinus fontinalis*. Little work examining the effects of orally administered glucans on the fish's immune response has been done. The objective of the present trial was to examine the effect of orally administered glucans on the survival and nonspecific immune response of fall chinook salmon, *Oncorhynchus tshawytscha*, juveniles. Mean time to 50% mortality and lysozyme increases were the responses monitored.

Initially, Abernathy stock fall chinook salmon, *Oncorhynchus tshawytscha*, average weight 5.1 g, were tested for serum lysozyme, mucus lysozyme from the skin, nare and intestinal wall, and for gill ATPase. Groups of thirty fish were weighed and transferred to 228 liter circular tanks having 12-13°C well water with flows of 6 liter/min. Fish were randomly assigned to the eight experimental tanks, with duplicates of the treatment groups. The glucans were administered as percent of the diet: VitaStim-Taito (VST), 0.1%; *Schizochytrium*, 1.0%; Levucell, 0.5%. The fish were fed these diets at 2% body weight and the dosage period for each compound was 14 days.

Fish were bath challenged with *Vibrio anguillarum* after the dosage period. In exactly 1 hour, fish were returned to their experimental tanks. Feeding was resumed and all groups were fed Abernathy control diet at 2% body weight/day. When mortality reached 50% the remaining fish in each tank were anesthetized and sampled for the following: serum lysozyme, mucus lysozyme from the skin, nare and intestine.

Skin lysozymes increased in all groups after the 14 day dose period. However, at the time of 50% mortality the skin mucus lysozymes were low in all groups. Intestinal mucus lysozymes were lowest in the survivors of the group fed Levucell but this group had a mean survival time to 50% mortality significantly ($P<0.05$) longer (163 days) than the groups fed VST (120 days) or *Schizochytrium* (123 days). Growth of the fish was also significantly different. The VST and the *Schizochytrium* fed fish were significantly ($P<0.05$) larger than the control and the VST fed fish were significantly larger than the Levucell fed fish. These results may reflect the digestibility of the glucan preparations, the fish's stage of smolt or the dosage level/length of feeding period.

1992 BROOD COHO SALMON FISH FOOD STUDIES AT CAPILANO SALMON HATCHERY, NORTH VANCOUVER, BRITISH COLUMBIA: PRELIMINARY RESULTS

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Abstract

Coho salmon were reared for a 325 - 329 day period from the 2.5 gram weight to release smolt weight of 15.8 - 18.1 grams with five commercial feed types; BioDry 1000, BioMoist, Ewos Vextra Smolt, Oregon Moist Pellet (Canadian formula, CMP), White Crest. BioDry and CMP replicates were reared in outdoor 104 cubic meter Burrows Ponds. BioMoist, Ewos, CMP and White Crest replicates were reared indoors in 10.1 cubic meter raceways with artificial light that matched natural photo period. Raceways were loaded with 11,000 fry and Burrows Ponds were loaded with 62,000 fry for the feed trials. Daily feed calculations were adjusted to account for individual feed type caloric value. Growth modeling was used to determine days to feed to reach the target size for smolt release. All groups were coded wire tagged except for the indoor CMP replicates.

Rearing mortalities ranged from .67% to 4.72 % for all feed types except for one group of BioMoist that registered a 13.47 % mortality.

Dry weight feed conversions ranged from 1.00 to 1.73 for all feed types except for BioMoist. The BioMoist replicates were noticeably different from all the other feed types with poor conversions of 1.69 and 1.99 (Figure 1).

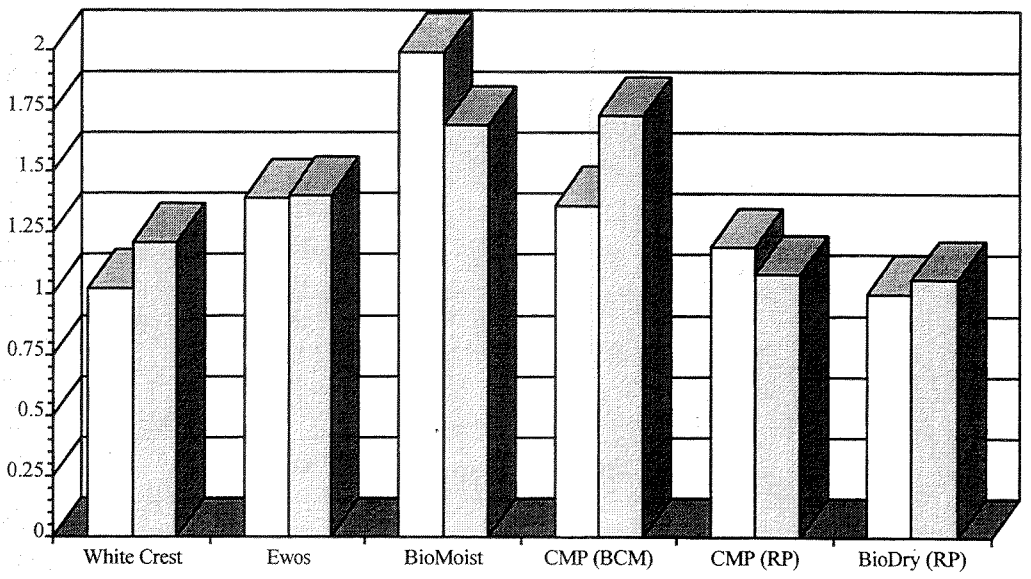

Figure 1. Dry Weight Feed Conversions 1992 Brood Capilano Coho Feed Study

Cost to feed individual groups were tracked by monitoring time to hand feed and cost of food fed. Cost to grow the fish to release ranged from $.96 to $5.36 CDN / kg in fish growth (Figure 2). BioDry was on the low end and the BioMoist was the most expensive. Comparing pond types showed it was more efficient to grow fish in the outdoor Burrows Ponds versus the indoor raceways. Coho grown with BioDry was noticeably more efficient than CMP in the Burrows Ponds. There seemed to be no difference between the CMP and White Crest groups in the indoor raceways and they were noticeably more efficient than the other feed types fed.

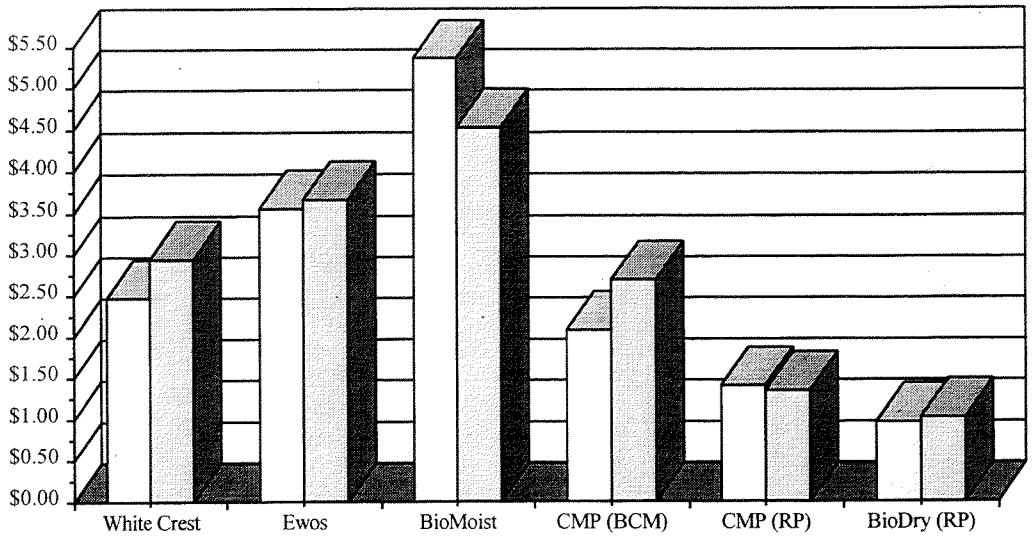

Figure 2. Cost per kg of Fish 1992 Brood Capilano Coho Feed Study

Survival rate from smolt release to adult recruitment varies between 2.76% to 5.73% (Figure 3). This data includes hatchery rack, commercial and tidal sports catch and do not include aboriginal or fresh water sport harvest. However there is no significant difference in the survival rates.

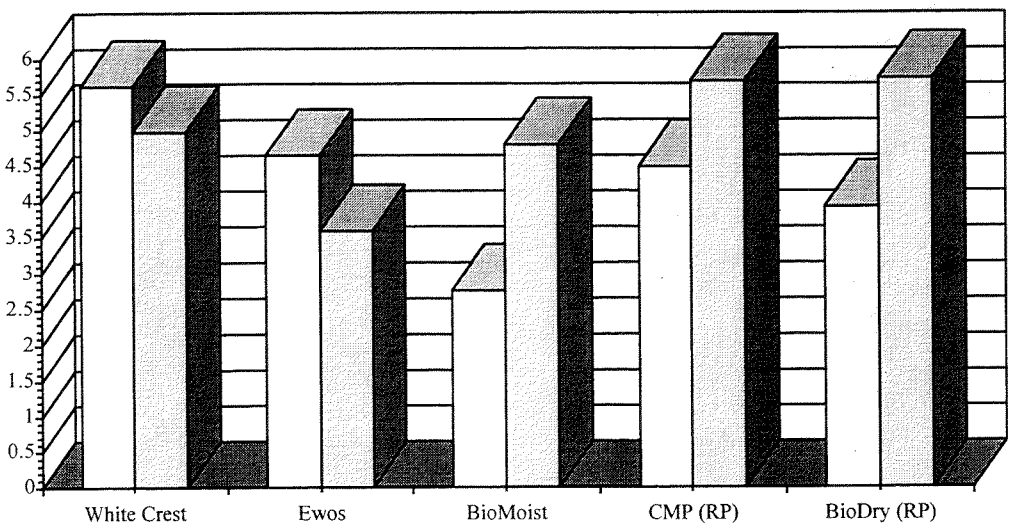

Figure 3. Percent Survival to Adult by Diet 1992 Brood Capilano Coho Feed Study. Data does not include aboriginal fishery and freshwater sport catches.

Coho feed trials are continuing at Capilano with BioDry, Ewos and Moore Clark Fry feed versus the traditional CMP diet. The poor food conversions resulted in dropping BioMoist from further studies. White Crest's manufacturer ceased operation that eliminated further use of this feed.

SURVIVAL OF HATCHERY-REARED SEA-RUN CUTTHROAT TROUT FROM AN EARTHEN POND VERSUS A BAFFLED AND STANDARD RACEWAY

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Abstract- Sea-run cutthroat trout *Oncorhynchus clarki* at the Cowlitz Trout Hatchery were reared in and released from a standard raceway, a raceway with baffles and an earthen pond. Adult returns from two releases showed that rearing pond fish survived at 161% of standard raceway reared fish and baffled raceway fish survived at 87% of the standard raceway reared fish. Fish growth was measured at the hatchery in the winter following spring release; rearing pond fish grew about 0.5 cm longer than the standard raceway group while no difference was apparent between the standard and baffled raceway reared fish.

Introduction

Many salmonid hatcheries use several types of fish rearing vessels, including concrete raceways and earthen rearing ponds. Typically, fish production per cubic meter of water is greater in raceways than in ponds and conversely, based on water inflow, production is greater in ponds than raceways. In Washington State, Millenbach (1966) investigated and concluded that ponds were a low-cost means to produce juvenile steelhead *Oncorhynchus mykiss* smolts. Ponds are commonly thought to be a more natural rearing environment and produce a better quality fish (Piper et al. 1982; Maynard et al. 1995). However, there is little literature on the relative post-release performance of fish reared in the two environments. Millenbach (1966) stated that some experimental evidence shows that survival to adult of pond reared smolts may be greater than for hatchery reared smolts. Griffith (1991) reviewed the survival of juvenile steelhead released from hatcheries in British Columbia and indicated that best performance was from channels, followed in declining order by channel/pond combinations, netpens, raceways and then ponds, although side by side comparisons were not conducted. Hesthagen and Johnsen (1989) found that post-release survival of brown trout *Salmo trutta* released in two lakes was greater for fish reared in an earthen pond on natural feed than for those reared in circular tanks on artificial feed.

At the Cowlitz Trout Hatchery, sea-run cutthroat trout *O. clarki* are reared in concrete raceways until late fall when they are usually transferred to an earthen rearing pond until release in spring. However, the fish are sometimes reared in concrete raceways until release. This study compared the relative post-release survival of cutthroat trout reared in a pond versus a raceway.

Baffles in raceways have been used to reduce cleaning time by using water flow to move solids to the lower end of the raceway (Boersen and Westers 1986; Kindschi et al. 1991; Wagner 1993; Tipping 1994). The baffled raceway rearing environment may be more natural than standard raceways; water swirls between baffled sections, and feed pellets move and have to be chased to be consumed. This study also compared the relative postrelease survival of cutthroat trout reared in a baffled versus a standard raceway.

Methods

In each of 1994 and 1995 at the Cowlitz Trout Hatchery, Washington, a group of about 22,000 sea-run cutthroat trout was reared in a 6.1 x 30.3 x 0.9 m concrete raceway with a water flow of about 74 L/s of re-used well water mixed with river water. A second group of about 23,000 fish was reared in a similar raceway except that it contained five 6.1 x 1.2 m steel baffles. The first baffle was 0.6 m from the upper raceway end, with remaining baffles spaced 3.0 m apart, which ended halfway down the raceway. Spacing under the first baffle was 4.1 cm, and 2.2 cm thereafter. Both groups of fish were fed to satiation with dry feed using five Babington demand feeders per raceway. In the baffled raceway, one feeder was placed behind each baffle except the first. The fish were held in raceways until mid-April when they were released; fish were pumped into a tanker truck, transported about 0.6 km in 5 minutes, and released.

A third group of cutthroat trout, 43,971 fish in 1994 and 58,366 fish in 1995, was placed in an earthen rearing pond in November, along with about 220,000 steelhead juveniles (standard practice at the hatchery). The rearing pond was 440 x 50 x 2 m with an inflow of about 227 L/s. Fish were fed with eight Babington demand feeders stationed around the pond. Rearing density (kg/m^3) was much higher in the raceways than in the pond while flow index (kg/L/s) was much lower in the raceways than in the pond (Table 1). In mid-April, the fish were counted with an electronic fish counter as they were released from the pond. Smolts from all three groups were measured for fork length and weight at release, and condition factor ($K=[10^2 weight,g]/[length,cm]^3$) was determined.

The three groups of fish were tagged with a magnetic wire tag in the left cheek, right cheek, or snout. A wand detector (Northwest Marine Technology, Olympia, Washington) was used to determine presence and location of tags. Tagged adult cutthroat trout were obtained from recreational anglers from August through October. Cutthroat trout entering the Cowlitz Trout Hatchery were examined for tags in November and December. The ventral fin of fish returning to the hatchery was excised so they would not be counted in a subsequent year.

Growth of cutthroat trout was determined by subtracting the mean fork length at release from the mean length at first hatchery return for both releases.

Chi-square ($P=0.05$) was used to compare the return rates of raceway reared cutthroat trout with the pond reared fish and to compare the raceway reared fish to those reared in the baffled raceway. Student's t-test ($P=0.05$) was used to compare growth rates from fish in the three rearing environments.

Results

For both years combined, an average of 3.14% of pond reared sea-run cutthroat trout were recovered compared to 1.95% of fish reared in the standard raceway and 1.69% of fish reared in the baffled raceway (Table 2). Pond reared cutthroat trout survival was an average of 161% of standard raceway fish while baffled raceway fish returned at 87% of standard raceway fish. Survival of pond reared fish was significantly greater than the standard raceway fish for the 1994 release ($X^2=160.5$ $p<0.001$) and 1995 release ($X^2= 37.2$ $p<0.001$). Survival of raceway cutthroat trout was higher than baffled raceway fish in both years ($X^2=5.2$ $p<0.025$ for 1994 release; $X^2=4.2$ $p<0.05$ for 1995 release).

Growth of cutthroat trout, measured from release to first return at the hatchery, averaged 12.6 cm for pond reared males while standard raceway and baffled raceway males averaged 12.1 cm and 11.9 cm, respectively (Table 3). Females from the rearing pond averaged 10.9 cm growth while standard raceway and baffled raceway fish averaged 10.2 cm and 10.5 cm, respectively. In 1994, growth was significantly greater for rearing pond males ($p<0.001$) and females ($p<0.05$) compared to males and females from the standard raceway. In 1995, growth was significantly greater for rearing pond females ($p<0.001$) compared to standard raceway females; growth of standard raceway males was greater than baffled raceway males ($p<0.05$) while growth of baffled raceway females was greater than standard raceway females ($p<0.01$).

Table 1. Release parameters of rearing pond and raceway reared Cowlitz Hatchery sea-run cutthroat trout.

	Rearing Pond		Standard Raceway		Baffled Raceway	
	1994	1995	1994	1995	1994	1995
Mean Length(cm)	23.5	23.2	22.9	23.3	22.6	22.9
Mean Weight (g)	124.7	121.1	118.3	124.0	117.1	120.9
Mean Condition Factor	0.96	0.97	0.98	0.98	1.01	1.01
N=	453	963	300	352	300	352
No. Fish Released	43,971	58,366	22,170	22,739	23,061	24,302
Rearing Parameters						
Density (kg/m^3)	0.5	0.5	17.5	18.6	17.0	18.0
Flow (kg/L/s)	105.7	101.9	39.7	42.2	38.6	40.8

Table 2. Adult recoveries of tagged Cowlitz Hatchery sea-run cutthroat trout reared in a pond and raceways.

	Release group and year of release					
Year	Rearing Pond		Control Raceway		Baffled Raceway	
Recovered	1994	1995	1994	1995	1994	1995
1994	1,191	--	263	--	222	--
1995	33	2,044	10	604	10	574
1996	0	NA	0	NA	0	NA
TOTAL	1,224	2,044	273	604	232	574
Percent	2.78	3.50	1.23	2.66	1.01	2.36
Average	3.14%		1.95%		1.69%	

Table 3. Growth(cm) of Cowlitz Hatchery sea-run cutthroat trout from release to first hatchery return. N=100 unless otherwise noted.

	Rearing Pond		Control Raceway		Baffled Raceway	
	M	F	M	F	M	F
1994 release	12.5	10.6	11.6	10.1[1]	11.8	9.9[2]
1995 release	12.7	11.1	12.5	10.3	11.9	11.0
Average	12.6	10.9	12.1	10.2	11.9	10.5

1/ N=79 2/ N=61

Discussion

The improved smolt-to-adult survival of earthen pond reared sea-run cutthroat trout compared to raceway reared fish supports Piper et al. (1982) and Maynard et al. (1995) in that the pond environment produced a better quality fish. However, the parameters influencing that survival are uncertain and need to be determined. Some possibilities include: 1) lower rearing density in the pond; lower density has been shown to enhance survival of juvenile chinook salmon *O. tshawytscha* (Banks 1994; Ewing and Ewing 1995) but not usually coho *O. kisutch* (Hopley et al. 1993; Ewing and Ewing 1995); 2) pond reared fish had slightly lower condition factors, associated with migrating versus nonmigrating steelhead smolts (Ewing et al. 1984; Tipping et al. 1995); 3) cryptic coloration for pond fish may be different, helping them avoid predation (Donnelly and Whoriskey 1991; Maynard et al. 1995); and 4) increased exposure to natural feed organisms may help postrelease foraging ability (Maynard et al. 1996); rearing pond cutthroat trout grew about 0.5 cm more than raceway fish as measured at initial hatchery return. However, the transition of hatchery fish to natural feed may not be problematic: Paszkowski and Olla (1985) found that a majority of hatchery-produced coho salmon smolts readily recognized, captured, and ingested natural prey and seemed capable of foraging successfully upon release into the marine environment.

Although the baffled raceway was thought to be a more natural environment than the standard raceway, fish survival was contrary to this idea. The cause of higher condition factors in fish from the baffled raceway is unknown but may have impaired survival.

Acknowledgments

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EFFECTS OF REARING DENSITY ON POST-RELEASE SURVIVAL AND ADULT CONTRIBUTION OF FALL CHINOOK SALMON REARED AT SPRING CREEK NATIONAL FISH HATCHERY: A PRELIMINARY REPORT

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Four broods of fall chinook salmon were reared at Spring Creek National Fish Hatchery from the first-feeding stage at densities of 91,000, 182,000, 273,000, and 364,000 fish per pond to evaluate crowding effects on survival and adult contribution after release. Spring Creek Hatchery is located in the mid-Columbia River Gorge area in Washington State and employs a water recycle and biological reconditioning system to rear about 16 million smolts annually. At each test density, fingerlings were reared in paired rectangular recirculating ponds and received pond-discrete coded wire-tags and adipose fin clips prior to release. To duplicate the customary multiple release strategy used at Spring Creek, about one-third of the population in each test pond was released in mid-March, a second one-third was released in mid-April, and the final one-third was released in mid-May.

Mean biomass at release ranged from 0.25 lb/ft^3 and 1.1 lb/gal-min of inflow for fish reared at the lowest density to 0.95 lb/ft^3 and 4.4 lb/gal-min of inflow for fish held at the highest density (Table 1). Fingerlings reared at the lowest density were significantly larger at release than fish reared at any of the other densities (Table 2). No difference was found in size at release among test groups stocked at densities of 182,000, 273,000, and 364,000 fish per pond. Dissolved oxygen in pond effluents at release (Table 3) reflected the different densities, and were well within recognized acceptable limits for salmon culture. To analyze percent mortality during rearing, losses were partitioned into three stages:

(1) from stocking in mid-December to first release in mid-March,
(2) from the first release in mid-March to the second release in mid-April, and
(3) from the second release in mid-April to the final release in mid-May.

Using this format, density effects on mortality during rearing were not significant (Table 4). Losses were significantly higher, however, from stocking to first release than at any other stage. Mortality was also slightly, but significantly higher from mid-March to mid-April than during the period from mid-April to mid-May.

Although tagged fish recoveries are incomplete, some data are available from each of the four broods of test fish. To date, survival rates have been highest in ponds where 91,000 fish were reared (Table 5). No statistically significant differences have been found for post-release survival in groups of fish stocked at densities of 182,000, 273,000, or 364,000 fish per pond have not been found. The lack of substantial differences in survival after release between fish reared at different densities has resulted in a direct relationship between increased rearing density and increased adult contribution (Table 6). A four-fold increase in rearing density has produced a three-fold increase in adult contribution. Based on the number of tagged fish returns to date, future changes in this relationship seem unlikely.

Table 1. Biomass per unit of rearing space and per unit of water flow at release of four broods of fall chinook reared at four densities . Values are means of combined 1989 through 1992 broods reared at each density in paired ponds.

Release date	Rearing density (Fish stocked/pond X 1000) 91	182	273	364	Mean
	Pounds of biomass/ft^3				
Mid-March	0.22	0.45	0.63	0.86	0.54
Mid-April	0.29	0.55	0.83	1.09	0.69
Mid-May	0.25	0.46	0.68	0.91	0.58
Mean	0.25	0.49	0.71	0.95	
	Pounds of biomass/gpm.				
Mid-March	1.0	2.1	2.9	4.0	2.5
Mid-April	1.3	2.4	3.8	5.0	3.1
Mid-May	1.1	2.1	3.1	4.2	2.6
Mean	1.1	2.2	3.3	4.4	

[1] Total rearing space/pond was 3224 ft^3 Water inflow/pond was 700 gpm.

Table 2. Mean weight (g/fish) at three releases of four broods of fall chinook salmon reared at four densities[1]. Values are means of combined 1989 through 1992 broods reared at each density in paired ponds

Release date	Rearing density (Fish stocked/pond X 1000) 91	182	273	364	Mean
Mid-March	3.7	3.6	3.6	3.6	3.6
Mid-April	7.1	6.7	6.7	6.7	6.7
Mid-May	12.1	11.4	11.1	11.1	11.4
Mean[1]	7.6a	7.2b	7.1b	7.1b	

[1] Means in this row followed by different letters are significantly different (P <0.05)

Table 3. Dissolved oxygen (ppm) in pond effluents at three relearns of four broods of fall chinook salmon reared at four densities. Values are means of combined 1989 through 1992 broods reared at each density in paired ponds.

Release date	Rearing density (Fish stocked/pond X 1000) 91	182	273	364	Mean
Mid-March	11.2	10.4	9.8	9.0	10.1
Mid-April	10.5	9.7	8.9	8.1	9.3
Mid-May	11.2	10.5	9.7	9.0	10.1
Mean	11.0	10.2	9.5	8.7	

Table 4. Percent mortality during three stages of rearing four broods of fall chinook salmon at four densities. Values are means of combined 1989 through 1992 broods reared at each density in paired ponds.

Release date	Rearing density (Fish stocked/pond X 1000) 91	182	273	364	Mean[1]
Stocking to mid-March	2.8	2.7	3.0	2.9	2.9a
Mid-March to mid-April	0.4	0.5	0.5	0.7	0.5b
Mid-April to mid-May	0.2	0.3	0.4	0.4	0.3c
Mean[2]	1.1	1.2	1.3	1.3	

[1] Means for rearing stage across densities with different letters are significantly different (P<0.05).
[2] Means for rearing density across rearing stage are not significantly different (P<0.05).

Table 5. Estimated post-release percent recovery of four broods of fall chinook salmon reared at four densities. Cell values are means of paired ponds.

Brood year	Rearing denslity (Fish stocked/pond x 1000) 91	182	273	364	Mean
1989	0.43	0.26	0.28	0.31	0.32
1990	0.22	0.22	0.17	0.09	0.18
1991	0.15	0.15	0.15	0.13	0.15
1992	0.21	0.08	0.13	0.22	0.16
Mean[1]	0.25a	0.18b	0.18b	0.19b	

[1] Rearing density means followed by different letters are significantly different (P<0.05).

Table 6. Estimated adult contribution per rearing pond from four broods of fall chinook salmon reared at four densities. Cell values are means of paired raceways.

Brood year	Rearing density (Fish stocked/pond X 1000) 91	182	273	364	Mean
1989	381	456	731	1078	662
199~	192	376	458	305	333
1991	130	250	397	443	305
1992	182	132	333	740	347
Mean[1]	221a	304b	480c	642d	

[1] Rearing density means followed by different letters are significantly different (P< 0.05).

ACCLIMATING SALMONIDS IN THE WILDS NEAR HOOD RIVER, OREGON

Mick Jennings and Michael Lambert

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Introduction

The Hood River Production Program (HRPP) is a fish supplementation project in the lower Columbia Basin funded by Bonneville Power Administration (BPA) and jointly implemented by the Confederated Tribes of the Warm Springs Reservation of Oregon (CTWS) and the Oregon Department of Fish and Wildlife (ODFW). The primary goals of the HRPP are to (1) re-establish naturally sustaining spring chinook salmon using Deschutes stock in the Hood River subbasin, (2) rebuild naturally sustaining runs of summer and winter steelhead in the Hood River, (3) maintain the genetic characteristics of the population, and (4) contribute to tribal and non-tribal fisheries, ocean fisheries, and the Northwest Power Planning Council's (NPPC) goal of doubling salmon runs in the Columbia Basin.

In accepting the Hood River Production Master Plan, the NPPC recommended adopting a three-phased approach which included collecting three years of baseline information, project implementation and facilities construction, and follow-up monitoring and evaluation studies. Comprehensive collection of data began in the Hood River subbasin in late, 1991, including information on the life history and production of anadromous salmonid stocks returning to the Hood River subbasin (Olsen et al. 1994). Information collected by the HRPP was used to prepare an environmental impact statement (EIS) evaluating the program's impact on the human environment.

In 1995, following three years of collecting baseline information, the HRPP moved into project implementation and facilities construction. Among other things, this phase of the project includes utilizing Hood River native steelhead for hatchery broodstock and converting the spring chinook hatchery program from Carson to Deschutes stock. Winter steelhead broodstock development actually started in 1992 because of concerns for the availability of wild winter steelhead in the Hood River based on low returns back to the Powerdale Dam adult trap. Also, techniques such as acclimation and volitional release of hatchery fish were to be used to improve survival and homing ability and to reduce residualism with wild juvenile salmonids.

As mentioned earlier, the project is a cooperative one with ODFW. They have taken the lead on developing the hatchery broodstock and rearing programs. CTWS has focused its attention on acclimation and volitional release of hatchery winter steelhead and spring chinook. Summer steelhead broodstock development has been delayed until facilities are built to handle the production.

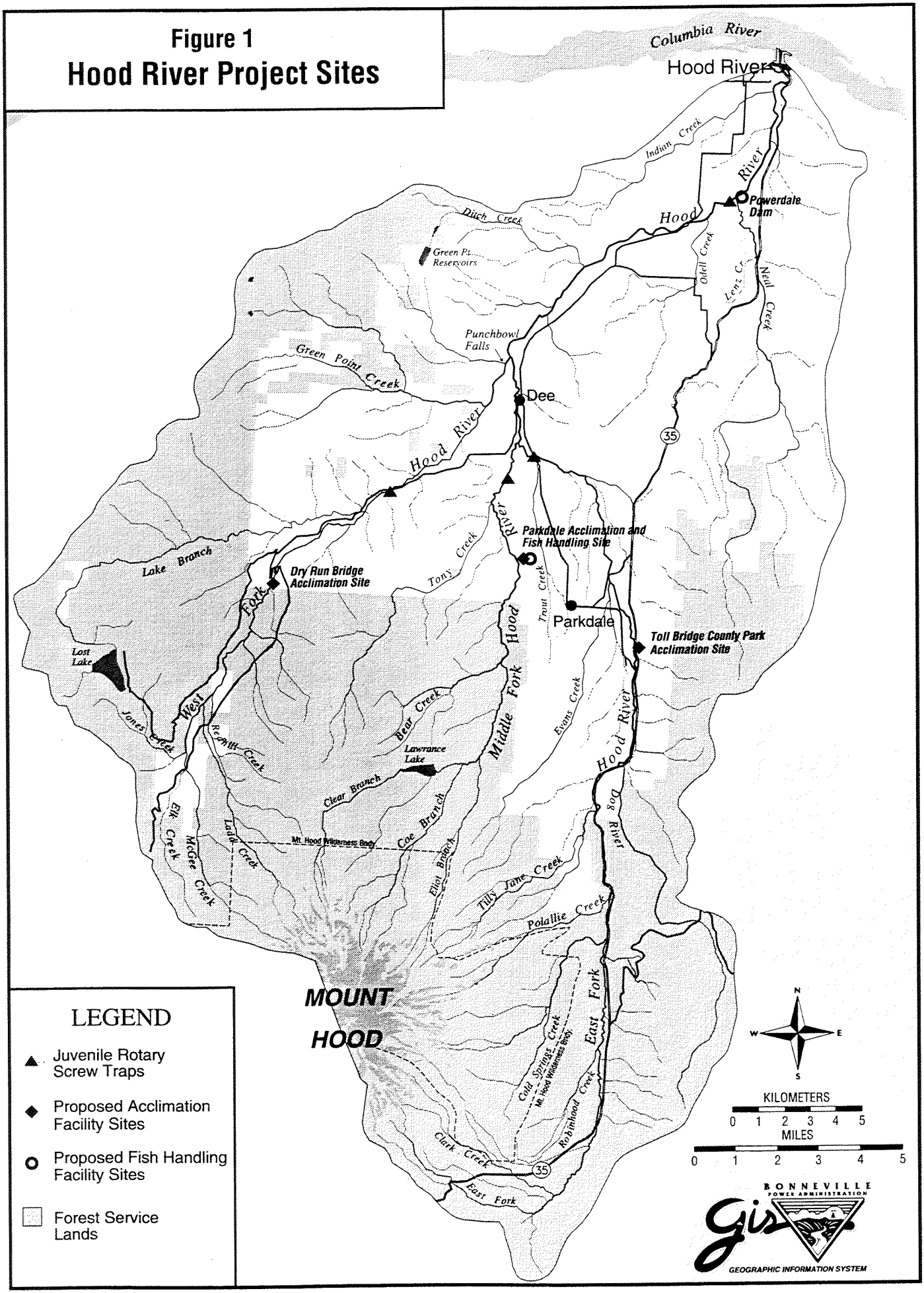
Materials and Methods

Two acclimation and release sites were chosen in the Hood River subbasin as shown in Figure 1. One portable pond for winter steelhead smolts was located at Toll Bridge County Park on the East Fork Hood River and two portable ponds for spring chinook salmon smolts were located near Dry Run Bridge on the West Fork Hood River. These sites were chosen because of their close proximity to prime spawning and rearing habitat for each species.

The three portable ponds were purchased from ModuTank, Inc. located in Long Island, New York. Each pond has the dimension 11'9" x 49'3" x 4'9" and has a capacity of 19,500 gallons of water. The ponds are constructed of four foot galvanized steel panels bolted together, "L" braces and stainless steel cables for support, a 36 mil reinforced polypropylene liner and a six inch PVC flange for draining the raceway. This type of portable pond was used successfully by ODFW on the Siuslaw River in Oregon (Lindsay et al., 1991-94).

East Fork Hood River. During mid-March one pond was assembled at Toll Bridge County Park near the East Fork Hood River and about 20 miles from the Columbia River. It was an excellent location not only because it was close to preferred winter steelhead habitat, but it required minimal site preparation before setting up the pond. Figure 2 displays the East Fork acclimation site. The set-up included running an underground power line to the site to provide electricity for two 400 gal/min sump pumps and a camp trailer. Once the pond was erected, a four foot high, six inch diameter

Figure 1
Hood River Project Sites



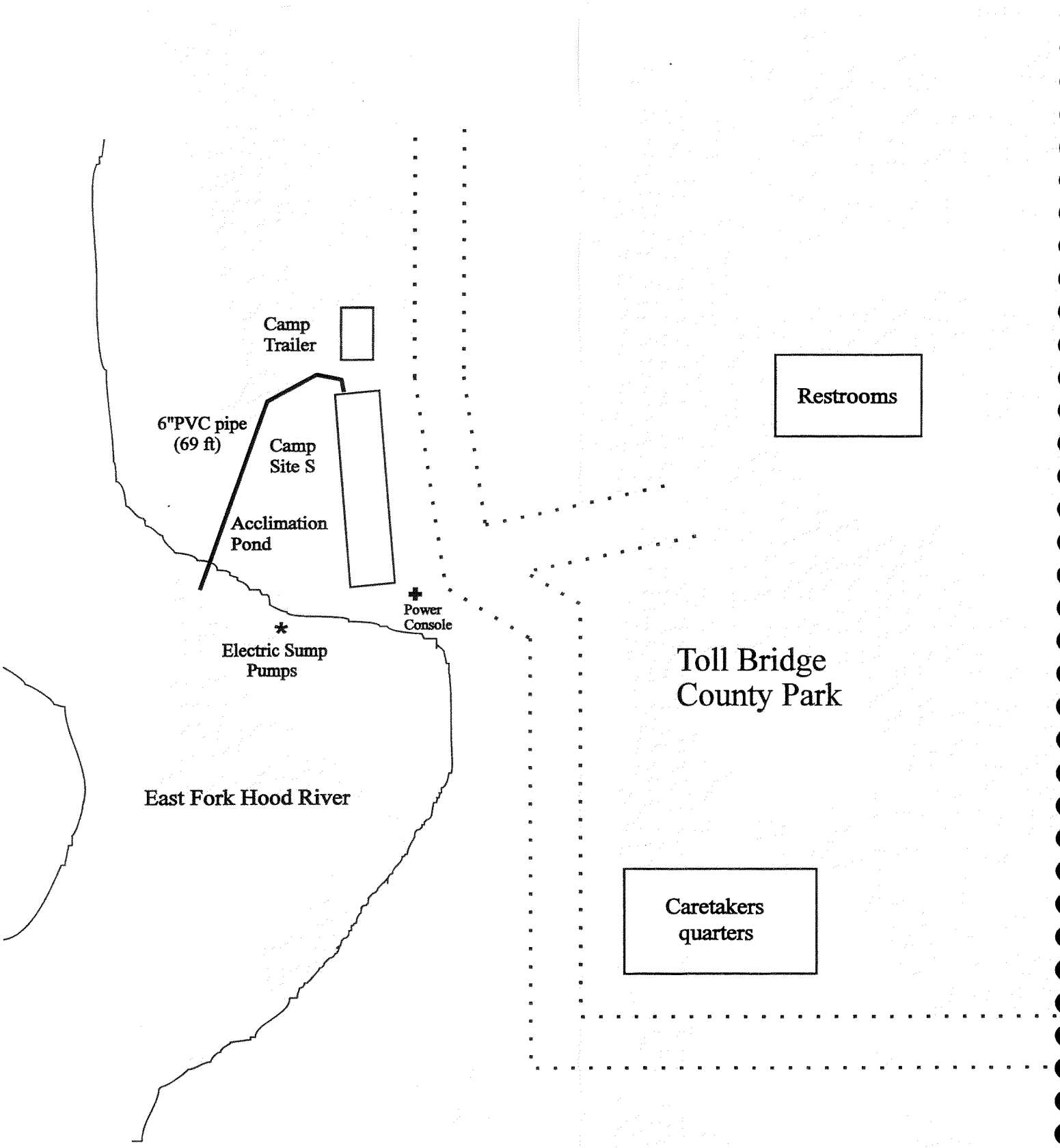


Figure 2. A diagram of the East Fork Hood River acclimation site (20 rivermiles from the Columbia River), located at Toll Bridge County Park.

PVC standpipe was connected to the outlet flange to control the water level inside the raceway and to use it to release fish or drain it when needed. About 70 feet of pipe was used for the outlet back to the East Fork. Also, an electric float alarm was attached to the pond wall to warn the caretaker of changes in water level. Finally, the pond was covered with a fine mesh net to prevent fish from jumping out and also protecting them from predators. A camp trailer was moved to the site to provide housing for the caretaker who was on duty 24 hours per day during acclimation. Including help from volunteers, the set-up took about 100 hours.

Winter steelhead smolts were transported from Oak Springs Hatchery to theToll Bridge acclimation site beginning April 1, 1996. With approximately 51,000 smolts at 5.3 fish per pound scheduled for release in Hood River, the group was split and acclimated one-half at a time. The first group of 24,000 fish were held in the pond until they were released volitionally between April 12-22. Approximately 2,000 fish failed to migrate from the pond. These fish were left to acclimate with the second group. The second group of 27,000 winter steelhead smolts arrived between April 22-24 and were held until their volitional release between May 1-8. On May 8 the remaining 6,000 juveniles were trucked to RM 0.5 and released.

Two electric trash pumps pumped 700 gpm of water from the East Fork into the pond. Loading density in the pond at time of the first transfer was 6.5 lbs/gpm (1.9 lbs/cu ft). Pond loading from the second group was slightly higher at 7.8 lbs/gpm (2.4 lbs/cu ft). Water temperatures ranged between 38°F - 51°F (3.5° C - 10.7° C).

Smolts were volitionally released from the portable pond and standpipe utilizing an untried technique. An aluminum hopper (or funnel) was constructed with a rectangular "V" shaped bottom, three vertical sides, one open side and the "V" bottom connected to a six inch diameter pipe. The hopper dimensions were about two feet square by one foot high. During volitional release one section of standpipe was removed to lower the water level in the pond to approximately a three foot depth. The hopper was placed on top of the remaining standpipe so that the opening to the standpipe was enlarged and provided easier fish emigration.

Duties of the acclimation caretaker included monitoring water temperatures and dissolved oxygen, checking water supply and pond level, picking mortalities, and feeding fish. Fish were fed as much as they would consume of #4 Bio Moist pellets but were taken off feed three days prior to release. Length/weights were taken from juveniles just prior to transfer to the acclimation raceway, from non-migrant juveniles (6,000 transferred to RM 0.5) and from migrants caught in downstream smolt traps.

West Fork Hood River. An old rock quarry site near Dry Run Bridge on the West Fork about 21 miles from the Columbia River was chosen for acclimating spring chinook. This location is near the preferred spawning and rearing habitat . Water quality and quantity in this stream is considerably better than in the East Fork because it is not influenced by glacial runoff or irrigation withdrawal. However, this stream is in a remote canyon with no electricity which made acclimation set-up extremely difficult. Land ownership included both a private landowner and US Forest Service and required special use permits from both groups. A permit was also required from Hood River County.

Assembly of two ModuTank portable ponds identical to the one on the East Fork began on March 18 and took over 700 hours to erect. Figure 3 shows a detailed diagram of the West Fork acclimation site. Unlike the East Fork pond, water to the West Fork tanks was supplied through a screened head box and a 930 foot gravity flow pipeline diverted in Blackberry Creek, tributary of the West Fork. Between the intake and ponds there was about 38 feet of head differential which provided 400 gal/min into the west pond and 350 gal/min into the east pond.

In addition, about 360 feet of pipe was used for the return flow back to the West Fork. Control valves regulated water at the head box, the junction of the two ponds and at each pond outlet. An elaborate bracing and support system for the pipeline took much of the assembly time. Also, the base for the ponds required considerable filling with sand, leveling and compacting. Both acclimation ponds were set up as described for the East Fork acclimation site with bird netting, alarm systems, standpipes with screens, and a hopper attached to the standpipe when spring chinook salmon smolts were volitionally released. A caretaker was on-site 24 hours per day.

Approximately 129,000 Deschutes stock spring chinook averaging 9.9 fish/lb were acclimated in two separate groups. The first group of 85,080 smolts were transported from Pelton Ladder near Round Butte Hatchery to the West Fork acclimation ponds between April 8-10, 1996. Fish were volitionally released between April 15 and 22 using the hopper

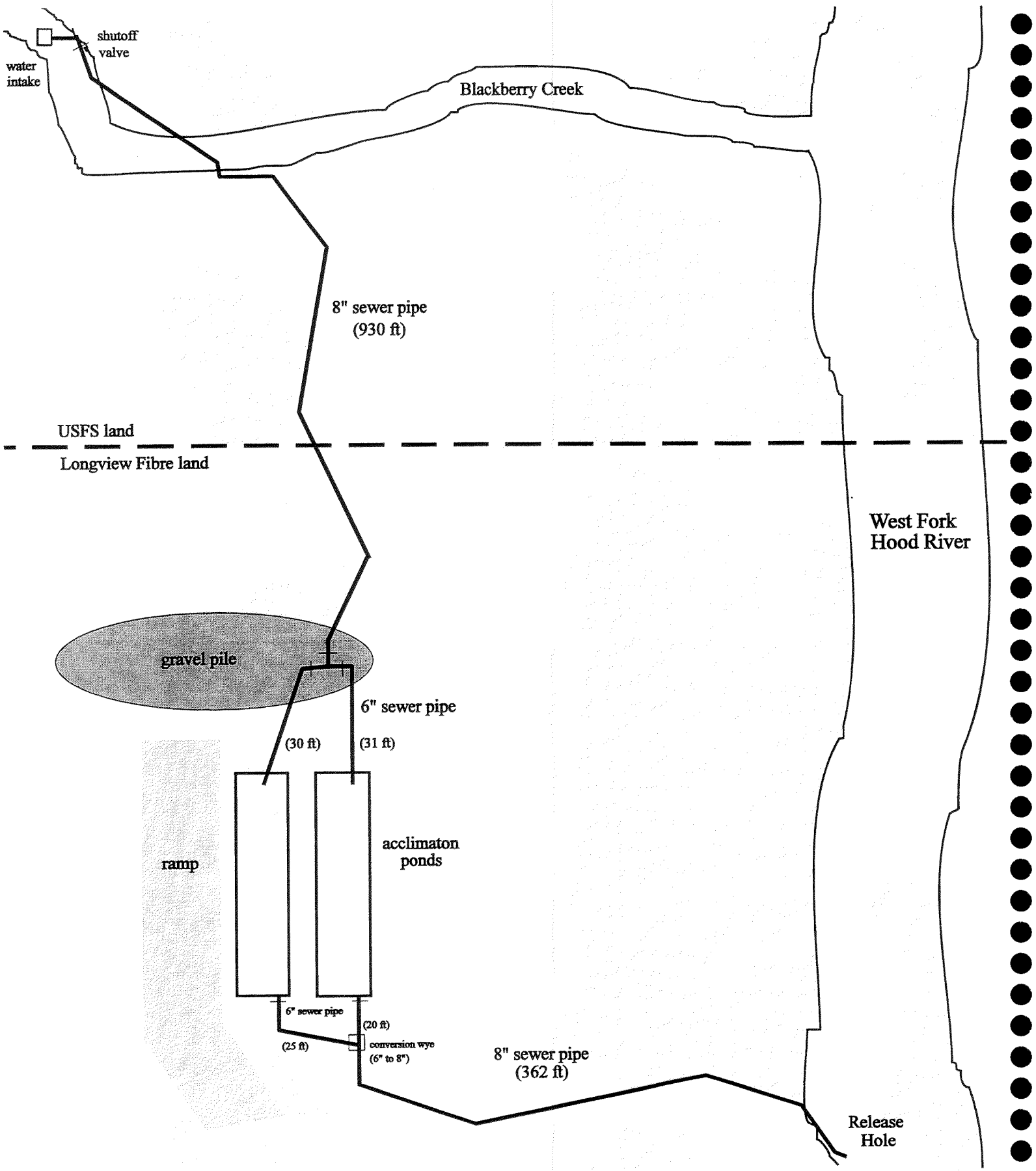


Figure 3. A diagram of the West Fork Hood River acclimation site (21 rivermiles from the Columbia River) located near Dry Run Bridge.

technique described previously. An estimated 2,000 fish failed to migrate. The second group of 44,838 spring chinook smolts arrived at the West Fork on April 22 and 23 and were ponded with the non-migrants. These fish were acclimated until April 29 and volitionally released for a 10 day period. The remaining 2,000-3,000 spring chinook smolts were forced into the West Fork Hood River. Pond densities varied from a maximum of 11.9 lbs/gpm (2.1 lbs/cu ft) for the first group (west pond) to a minimum of 3.5 lbs/gmp (0.6 lbs/cu ft) in the second group (east pond). Water temperatures ranged from 39°F to 43°F (4.0° C to 6.0° C). Fish were fed a #4 Bio Moist diet but were taken off feed three days prior to release.

Results and Discussion

East Fork Hood River. Of the 51,022 winter steelhead smolts transported to the East Fork acclimation pond, 44,916 smolts emigrated volitionally, 5,988 fish remained in the pond and were trucked to RM 0.5 and released, and 118 fish died in the pond. A downstream migrant screw trap (RM 4.5) operated by ODFW estimated 33,612 or 73 percent of the volitionally released hatchery winter steelhead passed the trap. Estimates of 1994 and 1995 trap catches of unacclimated hatchery winter steelhead were 32 and 38 percent, respectively. Differences in size at release may account for some of the variability in trap catches. Winter steelhead hatchery smolts averaged 5.9 fish/lb in 1994, 5.4 fish/lb in 1995 and 5.3 fish/lb in 1996. Table 1 displays the trap catch information.

Table 1. Estimated numbers of hatchery winter steelhead smolts migrating past a juvenile migrant trap located at RM 4.5 in the mainstem Hood River (Olsen et al., 1995).

Race year	Hatchery production release	Fish/lb	Estimated number of smolts past mainstem migrant trap			
			Estimate	95% C.I.	% of production release Estimate	Range
Winter,						
1994	38,034	5.9	12,201	5,739 - 18,664	32.1	15 - 49
1995	42,860	5.4	16,344	1,173 - 31,515	38.1	3 - 74
1996[a]	44,916	5.3	33,612	23,359 - 43,864	74.8	52 - 98

[a] 1996 data unpublished.

Casual observation of juveniles leaving the pond through the hopper and standpipe rated this volitional release technique very successful. Fish aligned to the shaded hopper area and actively migrated during the late afternoon and evening hours. Fish were timed leaving the pond at eight fish/min.

Condition factors as shown in Table 2 for the volitional migrants were 0.97 while the non-migrants trucked to mouth of the river were 1.00.

Table 2. Condition factors for hatchery winter steelhead volitional migrants collected in the mainstem Hood River juvenile screw trap and non-migrants trucked to the mouth of the Hood River.

Group	N	Mean	Range	95% C.I.
Volitional migrants	327	0.97	0.80 - 1.28	± 0.01
Non-migrants	207	1.00	0.84 - 1.15	± 0.01

West Fork Hood River. A total of 129,211 Deschutes stock hatchery spring chinook smolts were transported to the West Fork acclimation ponds. Of that group, 123,211 smolts emigrated volitionally, 6,000 smolts failed to migrate and were forced out of the ponds at the end of acclimation, and 707 smolts died in the pond.

When the ponds were lowered a foot to begin volitional release, smolts began moving out immediately. Shading the back portion of the ponds, especially when it was sunny, helped position fish near the outlet standpipe and hopper. An estimated 50,000 fish moved out of the ponds during the first afternoon and evening. Within 24 hours hatchery smolts

began showing up at the Powerdale screw trap, a distance of 17 miles. However, because of this mass migration, the trap was not fished and consequently no estimate was collected for the number of chinook smolts that left the basin.

Subsequent snorkel and electrofishing surveys in the West Fork and nearby tributaries have failed to find any hatchery spring chinook smolts.

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IMPROVING SUMMER STEELHEAD EMIGRATION FROM THE CLEARWATER RIVER, IDAHO
- a continuing education in length frequency

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Introduction

B-run steelhead (*Oncorhynchus mykiss*) in the Clearwater River provide a nationally renown fishery. Anglers from Montana, Idaho, Oregon, and Washington regularly travel to this area for the opportunity to catch 20 pound plus steelhead. This unique run of steelhead is completely dependent on Dworshak National Fish Hatchery (NFH) for its continued existence and genetic integrity. The run was permanently denied access to its historical spawning grounds in 1969 with the completion of Dworshak Dam on the North Fork Clearwater River. Dworshak NFH, constructed as mitigation for that lost habitat, annually releases 2.3 million steelhead smolts into the Clearwater River Basin to maintain this economically and genetically important resource (USFWS 1996).

During many years a large percentage of these steelhead never arrive at emigration detection facilities on the Snake River, 116 km downstream from the hatchery. Recent research indicates that the majority of smolts not successful in emigrating even to Lower Granite Dam are the smallest production fish (less than 170 mm total length at time of release; Bigelow 1995 and unpublished data). Sampling conducted by Idaho Department of Fish and Game (IDFG) in the lower Clearwater mid April through mid August, 1995, found 79% of *Oncorynchus mykiss* collected were hatchery steelhead smolts (Cochnauer 1996).

On the other hand, large steelhead also may not be successful emigrants. Partridge (1985 and 1986) found a high percentage of creeled fish in the upper Salmon River were residualized steelhead smolts. The majority of these residuals were large steelhead (>250mm).

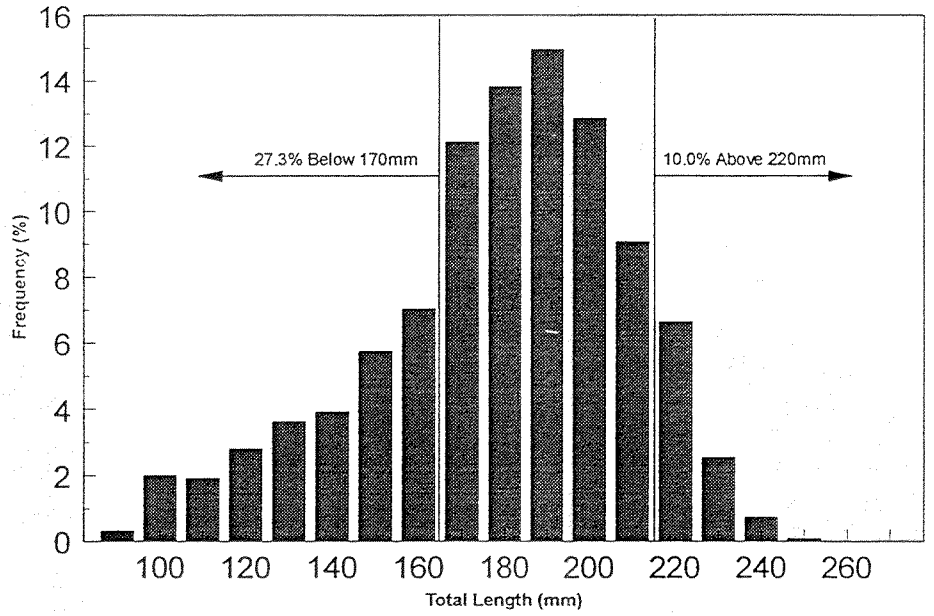
Steelhead smolts which do not successfully emigrate from the system are contributing nothing to the perpetuation of steelhead in Idaho. In addition, they may negatively impact sensitive species such as the endangered Snake River fall chinook, spring/summer chinook, or the proposed threatened wild A-run steelhead. Potential exists for these large steelhead smolts to prey on wild fry (Cannamela 1992). Also, any size residual smolt may be negatively impacting wild chinook or steelhead through displacement, competition for food, and behavioral influences (Partridge 1986, Cannamela 1993).

For these reasons and because NMFS' size recommendations contained in the *Proposed Plan for Snake River Salmon*

(NMFS, 1995), length frequency distribution of steelhead released from Dworshak NFH has recently become a concern. The recovery plan states that all fishery agencies within the Snake River basin "should only release steelhead smolts that are 170 to 220 mm in total length" (chapter V, section4.5b) in order to avoid residualization of smaller fish and predation by larger fish. Cannamela (1992) also recommends tightening length frequencies of released steelhead smolts in order to reduce residualism at both ends of the length spectrum.

Historically, Dworshak NFH has had wide length frequencies in released steelhead smolts. Data as far back as 1984 show approximately 25% of the smolts at time of release would not be within the recovery plan goals. Dworshak NFH releases last year show that 27% of the fish released fall below 170 mm (figure 1). Dworshak's Hatchery Evaluation Team is in the second year of investigating methods of tightening steelhead length frequencies by using different fish culture practices. Various practices used last year had little effect on length distributions of released fish (Bouchard et al. 1995).

Figure 1. Length Frequency of steelhead at release, Dworshak NFH, Brood Year 1995



In 1995, the Dworshak Hatchery Evaluation Team conducted a series of pilot studies on the development of wide length frequency distributions. One of the team's observations was steelhead length frequency distributions were relatively narrow during nursery rearing and began to increase noticeably after fish were transferred to outside rearing containers. A review of literature revealed that rainbow trout are territorial and set up social hierarchies. This behavior results in high size variability with dominant and subordinate individuals (Abbot et al. 1984;1989). However, at higher densities the establishment of these hierarchies is depressed and individual growth rates are much more uniform. This may be one of the main reasons why length frequencies in the nursery are relatively narrow. When the fish are transferred outside, at much lower densities, the opportunity to establish territories becomes available. It is at this point where size variability becomes quite noticeable.

Currently, steelhead at Dworshak NFH are transferred from indoor nursery tanks to outside rearing facilities at an average size of 100 fish per pound. Density indices (DI) in the nursery can reach as high as 0.6 or even 0.7 by the time of transfer due to limited space. Steelhead are usually ponded into Burrows ponds at double the final rearing number and are split at the time of adipose fin clipping. At ponding, the DI is about 0.1, gradually increasing until it reaches about 0.35 by the time of release in April. The dramatic decrease in DI from the nursery to outside rearing may be partially responsible for the development of wide steelhead length distributions by the time of release.

We hypothesize, that by maintaining high rearing densities, we can reduce variability in steelhead growth. By

transferring steelhead from the nursery to outside rearing facilities at a much higher initial DI resembling that in the nursery, then slowly allowing density to decrease until time of release, the establishment of territories may be delayed or eliminated. Hopefully much narrower length frequency distributions will result. This study will compare length frequency distributions of steelhead transferred to outside raceways according to established production protocols (controls with DI = 0.1) and length frequency distributions of steelhead transferred to outside raceways at higher densities (treatments with DI = 0.4).

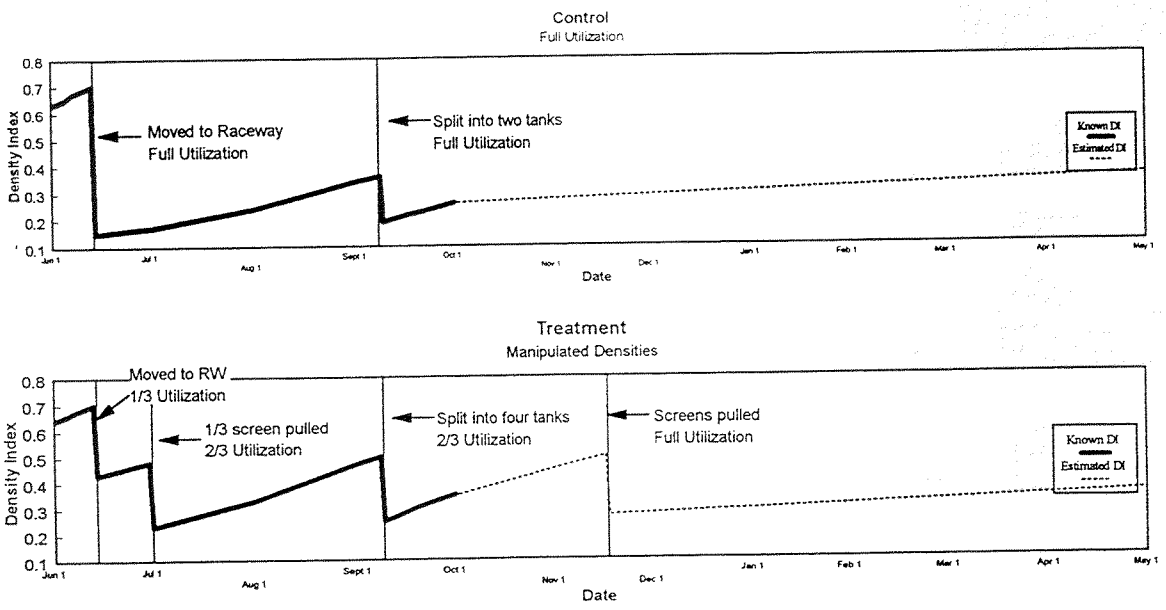
Methods

During June, 1996, Brood Year 96 steelhead were moved from nursery tanks to an outdoor raceway. To ensure an even mix of genetics, all fish were placed in one raceway for a day. Total length was sampled (n=225) to determine baseline data. These fish were then equally split into three raceways.

Control fish were allowed to utilize the entire raceway, giving an initial DI of 0.15. DI gradually increased to 0.36 in September. These fish were then adipose fin clipped and split into two raceways, decreasing the DI to 0.19 (Figure 2). They will be reared in these raceways until release in April (DI approximately 0.35).

Two treatment raceways were fitted with screens that allow the fish to utilize only the top third of the raceway. The initial DI in these ponds were 0.43. Once these fish reached a DI of 0.5 the top screen was removed allowing the fish to utilize two thirds of the raceway (DI of 0.23). At fin clipping, the DI was 0.50 and fish were split into four raceways with screens which allowed two third utilization, dropping the DI back to 0.25. The bottom screens in the treatment ponds will be removed when DI = 0.50 (mid November). The steelhead will then be allowed to use the entire length of the raceways with a DI 0f 0.26 which will gradually rise until release (DI = 0.36).

Figure 2. Density Indices of Control and Treatment ponds by month



Total length will be sampled (n=100) in all study raceways at every major change in DI and at release in April. Comparisons will be made using analysis of variance and Chi-square analysis to determine if manipulating rearing densities will tighten length frequencies of released steelhead.

Results

Results through November will be presented at the Northwest Fish Culture Conference in December. Final results will be available after release in May 1997.

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DIFFERENTIAL PERFORMANCE OF VENTRAL FIN CLIPPED AND ADIPOSE FIN CLIPPED/ CODED-WIRE TAGGED SPRING CHINOOK SALMON

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Abstract. -Warm Springs National Fish Hatchery is operated by the U.S. Fish and Wildlife Service, and is located on the Warm Springs River within the Confederated Tribes of the Warm Springs Reservation of Oregon. For three broodyears (1987-89), we applied ventral fin clips and adipose fin clips/coded wire tags in order to evaluate the two marks on hatchery performance of spring chinook salmon (*Oncorhynchus tshawytscha*). Each broodyear, approximately 100,000 fish received a ventral fin clip and 100,000 were marked with an adipose fin clip/coded wire tag. For the 100,000 ventral fin clipped fish, 50,000 received a left ventral fin clip and 50,000 received a right ventral fin clip. The mark study was also nested within a diet study, where fish in ponds containing left ventral fin clips were fed one diet and fish in ponds containing right ventral fin clips were fed another diet. From our sampling at the hatchery, we found that: 1) each mark group within a diet had variable length frequency distributions at release; 2) age, length, and sex composition at return were similar for each marked group within a diet; and 3) survival to adult was affected by diet but not by type of mark. In closing, spring chinook salmon marked with a ventral fin clip performed as well as fish marked with an adipose fin clip/coded wire tag; but sample sizes were small and survival to adult was low for all groups.

CARBON DIOXIDE ANAESTHESIA DURING CODED-WIRE TAGGING

DOES NOT REDUCE SURVIVAL OF COHO SALMON

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Introduction

Dissolved carbon dioxide gas (CO_2) has been used as an anaesthetic for fish for over 50 years . Even though CO_2 anaesthesia is used extensively in the Salmonid Enhancement Program, it has not achieved wide-spread acceptance mainly because fish that have been pre-stressed (handled) exhibit an extreme hyperactive response when exposed to levels of CO_2 high enough to anaesthetize them. Evaluations of the stress of CO_2 anaesthesia on rainbow trout (Iwama et al.) and chinook salmon (MacKinlay, et al. 1994), as measured by cortisol response, have shown that the physiological response is not much different than that of other anaesthetics such as tricaine methane sulphonate (TMS, or MS222), benzocaine, metomidate and 2-phenoxyethanol.

Since the high levels of carbon dioxide (over 150 mg/L) used to induce anaesthesia in fish also cause a rapid drop in blood pH (acidosis), some authorities (Wedemeyer, 1996) have warned against its use, speculating that a delayed negative effect might occur that is not detected by stress response testing. The ultimate test of the suitability of an anaesthetic (or any fish culture procedure) is whether fish exposed to it have the same survival rate to the adult stage as fish exposed to traditional methods. This experiment was designed to test that effect.

Methods

For three years (1991, 1992 and 1993) approximately 10,000 12 g pre-smolt coho salmon were anaesthetized either with about 200 mg/L of CO_2 or 90 mg/L of TMS. Standard procedures for handling, tagging and adipose-fin-clipping the fish were used. The fish were starved for two days prior to tagging, anaesthetized in one or the other chemical, injected with coded-wire tags and returned to rearing containers. The tagging temperature was approximately 8°C. Following tagging, the fish were held for two days (30 days in 1993) and checked for tag loss. Tag retention in all cases was over 98%.

Recovery of tagged fish occurred through the Mark Recovery Program (MRP) (Kuhn, 1988), an international, coast-wide effort to sample the commercial, Indian and sport fisheries in the eastern Pacific Ocean. The survival rates reported here came from the MRP database.

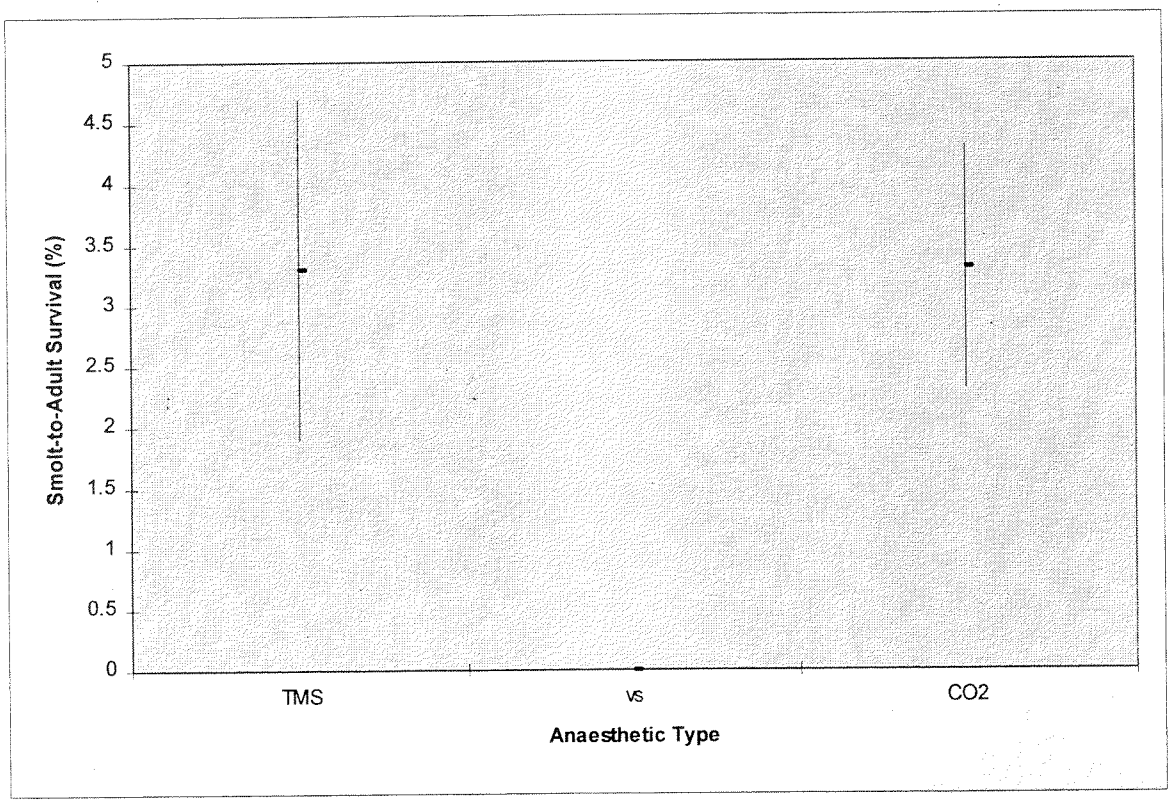
Results

The mean survival for the two anaesthetics was not significantly different, if the three years are considered as replicates (Means and 95% confidence limits are: for TMS 1.3% ± 1.4%, for CO_2 1.3% ± 0.4%). It may appear (Table 1) that the first two years had slightly higher survivals for the TMS-tagged fish and the last year had higher survival for the CO_2-tagged fish, but the survivals reported in the MRP are not very precise. There are so many sources of error in the complex process of recovering tags from the fisheries and in estimating sampling rates of the many contributing sectors to the final tally of tag recoveries that potential error could easily exceed 40% of the recorded numbers. The survivals reported here range from an 8% to 41% difference from each other. Since the 95% confidence limits overlap (Figure 1), there is no significant difference between CO_2 versus TMS survivals.

Table 1. Survival of Coho salmon anaesthetized during tagging using different anaesthetics. Results are in percent survival from smolts released to adults returned.

Year	TMS	CO_2
1991	5.1%	4.7%
1992	3.2%	2.6%
1993	1.6%	2.7%

Figure 1. Mean Survivals of fish anaesthetized with two different anaesthetics. Error bars indicate 95% confidence.



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USE OF JACK COHO AS BROODSTOCK INCREASES

JACK CONTRIBUTION TO ADULT RETURNS

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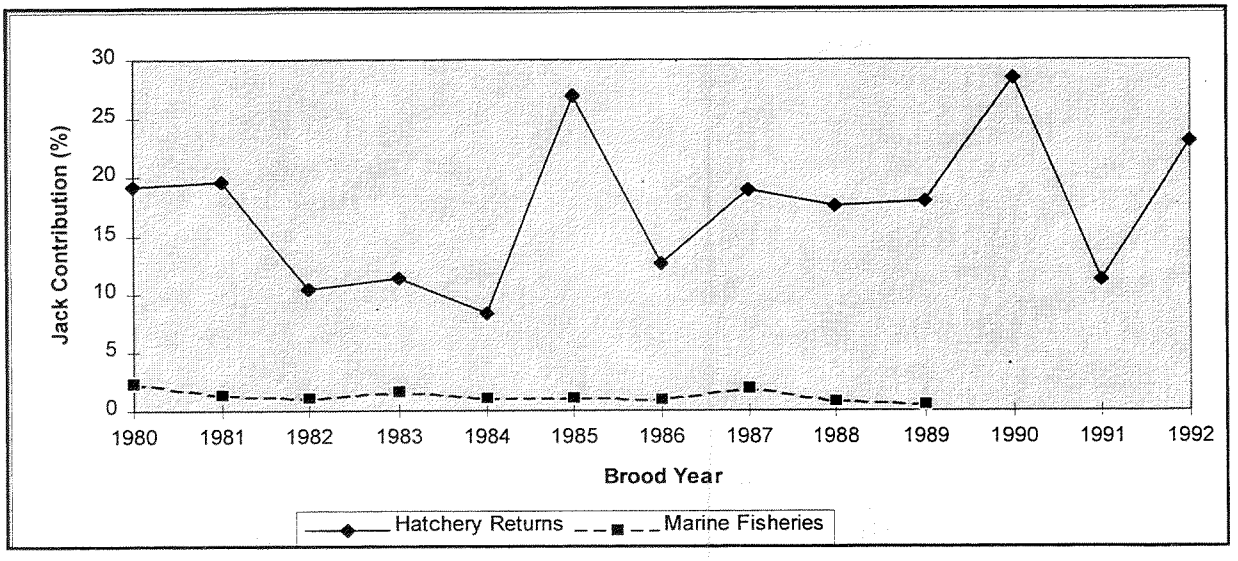
Background

Precocious 2-year-old males (jacks) make up a substantial proportion of the returns to the Chilliwack River Hatchery. Almost all of the rest of the adult returns are 3-years old. It has been recommended that, in order to maintain genetic diversity, hatcheries use jacks as part of coho broodstocks. This approach, to get as many fish as possible to contribute to the genetic pool of a stock, is probably more applicable for a marginal or small stock of fish that is totally dependent on hatchery production than it is for a large, mixed hatchery/wild stock like that of the Chilliwack River. Since jacks can make up more than half of the male returns to the hatchery, and since that high jacking rate is probably related to the hatchery techniques used rather than to a natural characteristic, we questioned the wisdom in encouraging the occurrence of more jack returns by using them as broodstock.

There is a long-standing controversy as to whether the age-at-return of salmon is determined by the animal's genes or by its environment (Gardner, 1976). Even though jacking is reported to be higher in hatchery-origin stocks than in wild stocks, supposedly as a consequence of rapid-growth during early rearing, the reporting of jacks in wild populations is probably underestimated because jacks are not caught by conventional fishing techniques. Certainly, the jacks from our hatchery are not detected in the marine fisheries (Figure 1).

Figure 1. Contribution of jacks as percent of the total numbers of coho salmon that swim in to the Chilliwack Hatchery, and to marine fisheries as reported by the Mark Recovery Program.



Jacks have been seen to contribute sperm to the spawning act of full-sized adults in the wild but the importance of this contribution is probably not possible to evaluate. It has been postulated that jacks may represent the fastest growing fish in a population and by not using them in the spawning process, a decrease in large fish in the population would result. The converse argument is that if those rapid-growing fish keep returning as jacks, their fast-growing genes are wasted (in terms of fishery contribution) anyway.

While most studies show that age-at-return is controlled by a combination of inherited and environmental factors (Hager and Noble 1976, Iwamoto et. 1984, Bailey et al. 1980), no studies specifically showed the difference in jack contribution to groups of fish who had been fertilized with jack compared to adult sperm and had been released into the wild. This study tested whether using jack sperm to fertilize eggs would result in a greater proportion of jacks returning than a group fertilized with adult sperm.

Methods

All of the eggs were taken in one egg take, pooled and then divided into equal lots, one was fertilized with jack sperm (the jack/adult group) and the other with adult sperm (the adult/adult group). Standard disinfection procedures were used and the eggs were placed in separate, side-by-side Heath stacks. Similar ponding and rearing regimes were used for both groups.

A proportion of the fish was tagged by injecting a binary-coded wire segment into the nose cartilage. Each tagged fish also had the adipose fin removed to flag it as a tagged fish when it was caught in a fishery or when it returned to the hatchery. Normally, in evaluating the returns of coded-wire tagged fish, we use the information from the coast-wide Mark Recovery Program, that includes commercial and sport catches in addition to the hatchery returns (Kuhn et al. 1988). In this case, since jacks are not caught in the marine fisheries, we used only the returns to the hatchery to evaluate the proportion that were jacks. This gives a more accurate estimation of the population proportions.

Results and Discussion

Survival to the eyed stage was very poor in both groups: 75.5% for the jack/adult group, and 78.1% for the adult/adult group. The cause of the low survival was poor fertilization — 96% of the dead eggs checked were unfertilized. Survival from ponding to release was slightly poorer in the jack/adult group when compared to the control, the survivals were 93.8% and 97.6% respectively. The main losses occurred shortly after ponding. The sizes at release were not significantly different between the jack/adult group and the control, at 18.8 g and 19.9 g, respectively.

The number of marked coho of each experimental group to return to the hatchery is shown in Table 1, along with the return from the rest of the production-scale fish from the 1986 brood year releases from the hatchery.

Table 1. CWT release and recovery of Jack/Adult and Adult/Adult crosses of coho at Chilliwack River Hatchery.

Group	Number Tagged (#)	Marks recovered as jacks (#)	Marks recovered as adults (#)	Proportion of jacks in total marked return
Jack/Adult	9582	98	132	42.6%
Adult/Adult	10044	45	193	18.9%
Hatchery Production	49032	92	510	15.3%

The hatchery production group is included to show the proportion of jacks in the "normal" hatchery population, but this may not represent a true comparison because the experimental groups were taken from a one-day "late-timing"

egg take (Dec 5, 1986), while the production lot represents eggs taken from both early and middle timing groups (Sept 1 to Nov 30, 1986). The proportion of jacks in the returning population is quite variable (Figure 1).

Since the jack/adult group produced twice as many jacks as the adult/adult group, it could be assumed that the jack/adult group would produce fewer adult males and this appears to be the case with the experimental groups. The jack/adult group returned to the hatchery at a male to female ratio of 0.81:1 while the adult/adult group returned at a ratio of 0.97:1. This is 20% fewer harvestable males, or 10% fewer total pieces. Since we release about 2 million coho smolts that have a survival of at least 10%, this would represent a loss of 20,000 harvestable pieces to the fisheries if we used jack sperm during spawning.

The survival rates varied considerably between the groups (Table 2) with the adult/adult cross surviving at 17% while the jack/adult group was only 12.6%. There may not be any significance to this difference between the experimental groups because the production group survived at about the same as the jack/adult group at 12.1%.

Table 2. Total production, including catch and rack recoveries of Jack/Adult and Adult/Adult crosses of Chilliwack River Hatchery coho.

Group	Marks Released	Jacks Recovered	Adults Recovered	Total Survival
Jack/Adult	9582	108 (1.1%)	1096	12.6%
Adult/Adult	10044	55 (0.5%)	1649	17.0%

* This table does not include escapement that spawns in the wild.

Conclusions

Coho eggs fertilized with jack sperm produced over twice as many jacks (2.3:1) as normal adult/adult fertilization. The occurrence of precocious coho males (jacks) appears to be influenced by heredity.

Production of adult males appears to have been reduced by the higher production of jacks in the jack/adult cross when compared to the adult/adult control.

We will continue to use only 3+-year old broodstock since avoiding jacks during spawning does not seem to have had a noticeable effect on the proportion of jacks in our returning population.

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LAKE REARING SALMONIDS IN BRITISH COLUMBIA

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Introduction

Salmon farming has become a multi million dollar industry in BC. By implementing aggressive fishculture techniques, salmon production is now the number one export item, by dollar value, of agriculture products in BC, exporting more meat tonnage than either the cattle or poultry industries. Despite government restrictions on farm sites and hatcheries, annual production has increased and is presently holding at 20-30,000 tonnes of salmon. One half of the total dollarvalue of salmon exports for BC comes from salmon farms where biological and market problems have not been the main stumbling block. The salmon farming industry has developed its own fishculture technology, has an abundance of broodstock and salmon while lacking full government support. In contrast the salmon enhancement programs which are blessed with full government co-operation, experience declining commercial and sport harvests.

BC.'s salmon enhancement efforts seemingly are choked by new directives for conservation rather than aggressive enhancement, minimal numbers for release, budget cuts, regulations with regards to saltwater survival, genetic diversity, disease containment, etc. The salmon farming industry, on the other hand, by using different fishculture strategies like lake rearing of smolts, different year classes of salt water introductions, captive broodstock and extensive disease prevention through vaccination, may have something to offer.

Our intensive netpen fishculture operation in Lois Lake, BC, has been in production for eight years. Freshwater netpens in other lakes are operated for the culture of salmon and trout. I believe that we are the largest of these and supply approximately two million large smolts annually for marine fish farms in BC.

Lois Lake

Lois Lake, located 18 km east of Powell River, at an elevation of 131 m, is a large deep coastal lake, 29 km long by 3.6 km wide, regulated by a dam. The lake was dammed in 1930 and its nutrient level is low and considered unproductive due to a lack of biological productivity following impoundment (Truscott, 1988). Good water, clarity and high oxygen levels prevail.

Flushing is moderate to high with lake water being drawn through a pipe 30 m below the surface for hydro generating purposes. This deep outlet aids in lowering the thermocline during the summer to about 10 m which benefits fish growth due to higher surface temperatures. Active logging is ongoing around the lake causing some temporary phosphorus loading. Fish samples taken in 1976 by Fish & Wildlife indicated the presence of kokanee, cutthroat, and rainbow trout, three spine sticklebacks and prickly sculpins.

Permits for the location of lease sites were obtained from the Ministry of Environment, Lands and Parks and approval to operate was required from the BC Ministry of Agriculture, Fisheries & Food with numerous other ministries having regulatory input through this agency.

Net Pens.

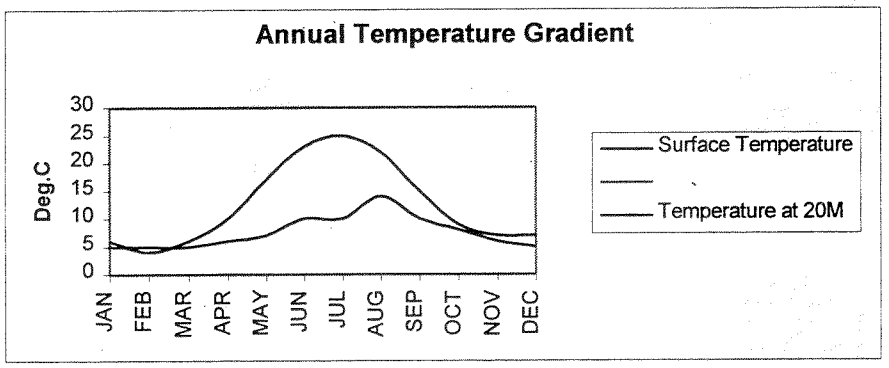
The site has 60 octagonal net pens, approximately 12 m across, constructed of wood. The nets are sewn from 1 to 2 cm stretched nylon mesh. They are 15 to 20 m deep and hang just below the thermocline to allow the fish to find their own level of comfort. This generally results in the smolts utilizing about 1500 m^3 of the available space. The

individual, hinged pens, are moored in clusters of 10 along a 3 m wide workdeck which is anchored in the allocated locations at one end only . This type of anchor system accommodates swinging of the pen system around the anchor in the center of the lake where exposure to different wind directions creates water movement and high oxygen levels during the warm summer months.

Stocking Densities, Feeding & Grading

Fry and smolts are stocked at densities of up to 2.0 kg /m^3. This prevents disease, increases survival and ensures faster growth rates. Coho, chinook and Atlantic salmon and rainbow trout are the species grown. We start at 150,000 fry per pen in April/May and after extensive grading end up with 35,000 smolts of about 80 g each, depending on the species, in October. Grading is ongoing to avoid cannibalization and fin damage. Slow growers are ruthlessly destroyed and can amount to 15% of the total. All pens are fitted with automatic feeders which handle 75% of the feed, the balance being done manually. To obtain our objective of producing large smolts, conversion levels are high—up to 1.4 including mortalities and culls. In the summer, during high growth periods, feeding levels go up to 5% of biomass per day. Surface temperatures rise to 24° C during the summer months. See Figure 1. Feeding continues throughout daylight hours for maximum growth. Daily feed levels are based on individual sample weights and divers continually check for overfeeding while checking the stock and removing mortalities.

Figure 1. Temperatures of Surface and 20 m deep water at Lois Lake.



Predation.

Predator nets are fitted over the pens to deter birds, such as great blue herons.

Disease avoidance and treatment.

Health management includes careful selection of disease-free broodstocks from clean hatcheries. Samples of mortalities are checked by culture method and PCR for furunculosis or IHN on a regular basis. Most stocks are moved into the lake, from different hatchery locations at 1.0-2.0 g after the fry have learned to feed on artificial fish food, thus avoiding active pursuit of copepods, which are proven carriers of nematode worms. Fry are dip-vaccinated against redmouth, hydrophyla and furunculosis, both prior to transport into the lake and again at 5.0 g. At 20.0 g, all smolts are intraperitonealy injected against furunculosis, IHN and vibriosis.

All smolts remain in freshwater at least six weeks after injection, prior to transportation into saltwater, to aid in the build-up of antibodies. This intense initial immunization program has effectively reduced the extended use of antibiotics throughout the salmon farming industry.

The production cycle at Lois Lake includes a 4-6 week fallowing period in the spring , without any smolts in the water. We also monitor the levels of *Saprolegnia* fungus, by incubating water samples and counting spores and by

avoiding handling the fish when spore counts are high. All smolts are sampled and tested for osmoregulation by counting retained chloride ions(Clarke and Blackburn). Reverting of smolts after saltwater introduction has decreased extensively since we adopted this method of testing for blood sodium levels.

Transports.

All smolts are pumped through counters and transported out of the lake by tank trailers to saltwater farms around BC, Long distance hauls, which include ferry trips and barging, could take up to 20 hours.
Loading densities are kept at 50-80 kg/m^3.

Escapes of salmon smolts.

Despite all attempts to avoid escapes, spillage does occur sometimes during grading, transporting, storms and fish transfers. Dropnets are extensively used to avoid this but occasional accidents happen and smolts have an excellent chance of surviving in the lake due to the absence of predators. Coho smolts, who escaped broken netpens during a major storm in 1989, have now established themselves in the lake and tributaries. A sportfishery has developed for these escapees with a lot of positive local reaction.

An extensive literature review called the Environmental Effects of Salmon Netcage Escapements (Hatfield and EVS Consultants,1996) concluded that it takes a very large number of atlantic salmon escapees or repeat escape events to establish a selfsustaining Atlantic salmon population. Due to the general distribution of other species such as rainbow and cutthroat trout, etc., it is highly unlikely that Atlantic salmon would ever get established in Lois Lake (Needham, 1995) to the same extent..

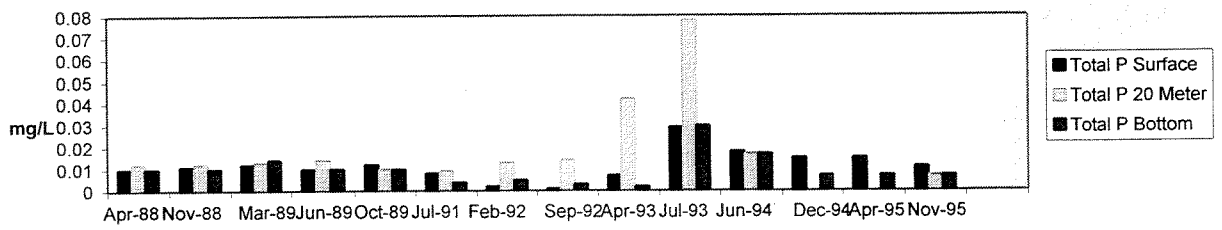
Environmental monitoring

A monitoring program for Lois Lake water quality has been ongoing since the farm's inception. Water samples are sent on a regular basis to an independent laboratory for the following analysis;

Nitrogen: ammonia, nitrate and nitrite
pH
Phosphorous: soluble reactive and total (see Figure 2)

All sampling data have been sent to the Ministry of Agriculture Fisheries and Food for analysis and the effect of the farm on the surrounding water quality has been undetectable. On the other hand helicopters dumping logs into the lake, heavy precipitation, runoff, and fertilizing of the nearby forest tree farm, coincide with increased phosphorus concentrations in the lake. Before and after this phosphorus spike, concentrations are characteristic of a system with low productivity. Lake rearing has proved to be environmentally sustainable. Over the eight years that our farm has been in production, output has increased ten fold while the phosphorus levels have remained constant. In general, Logging operations have had no effect on the health of our smolts.

Figure 2. Phosphorus levels in Lois Lake 1988-1996 (highest points were after helicopter dumping of logs).



Conclusions

Our total production of 160 tonnes is about twice the tonnage produced in a government-sponsored major salmon enhancement facility. Lake rearing could effectively compliment the Salmonid Enhancement Program hatcheries. These could be expanded at a low capital investment cost. Supplemental Lake rearing could open up additional release windows by rearing S0, S½, or S2 smolts for optimum survival and or predator avoidance.

Another physical problem in salmon enhancement is the lack of broodstock returns. Netpens have proven an excellent environment to contain selected fry and smolts for grow-out to broodstock. For example, enough broodstock were held in two netpens in Lois Lake to supply a 5-million coho egg take in 1991. This type of broodstock containment in fresh or saltwater should prove an excellent supplement to the lack of wild salmon returns.

We are presently doing 8-10 different stocks for six separate customers and all these batches are kept apart. If production were dedicated to enhancement rather than salmon markets, lake rearing could centrally grow many different depleted stocks in separate cages, for biodiversity and for transportating to and imprinting at their rivers of origin. Of course, lake rearing is already used for trout and salmon on a smaller scale in various locations, such as Georgie Lake. Comparing output levels, we could produce 10 million 20 g coho, 30-40 million 7 g chum, or 20 million 10 g chinook. Production in lakes is only limited by the environmental factors and the number of approved cages.

Future options are for enclosed bag systems with low head pumping for better containment of diseases like fungus, control of effluent discharge and increased production. Lake rearing of salmonids as practiced in Lois Lake should be a valuable asset to salmon enhancement programs. Drawbacks include the lack of control for isolating groups from diseases like *Saprolegnia* and others, however, the technology is available and the cost per smolt for enhancement purposes could be effectively reduced.

Acknowledgments

I would like to thank my partners at Lois Lake for all their help and input in the preparation of this publication.

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CHINOOK SMOLT SEAPEN TRANSLOCATION AN ALTERNATE HATCHERY RELEASE STRATEGY TO REDUCE MACKEREL PREDATION

by

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Introduction

The increased presence of chub mackerel in the waters off the West Coast of Vancouver Island (WCVI) and their subsequent predation, has seriously affected the survival of hatchery released chinook smolts. Conuma Hatchery typically releases approximately 3 million chinook smolts from the Conuma River estuary, located 25 km inland from the entrance to Nootka Sound in Moutcha Bay at the head end of the Tlupana Inlet. When Mackerel are present at release, the predation pressure on these migrating chinook smolts through this long, narrow inlet, can be devastating. In an attempt to reduce this veritable "bottleneck" of predation at release, we have translocated a seapen of estuary, salt water reared chinook smolts by towing the entire pen 17 km along the Tlupana Inlet to the opening of Eliza Passage, 8 km from the entrance to Nootka Sound and the open Pacific Ocean (figure 2). These fish were representatively marked with a coded wire tag and will be compared to a similar group of fish that were not translocated.

Background

In 1986, Science Branch of the DFO initiated the MASS research project to obtain a better understanding of the processes that control survival and recruitment of salmon in the ocean (Healey, 1988). The consensus of the participants at the onset of the MASS program was that survival of salmon during the first year in the ocean was critical to the ultimate recruitment of salmon to the fisheries (Hargreaves et al, 1995). Extensive sampling was conducted in the Alberni Inlet and Barkley Sound each year during 1987-1993. Purse seining was used to determine the abundance, distribution and migration timing of all species of juvenile salmon. Gillnetting, balloon trawling and purse seining were used to determine the abundance, distribution, and rates of predation of fishes feeding on juvenile salmon. Stomach contents of predators were analyzed for presence and abundance of juvenile salmon (Hargreaves et al, 1995).

Mackerel - *The Predator* - and El Nino

The Pacific (chub) mackerel spawn each year off southern California and Baja, Mexico, they are highly migratory, very fast and can live as long as ten years. Heargraves and Hungar (1995) noted that "the intense El Nino oceanographic conditions in the North Pacific were probably the main "trigger" for the influx of mackerel into B.C. waters, temperatures were 1-2 degrees higher during January - August 1992 than the long term average temperatures from 1934 - 1993". During the El Nino conditions, they also noted that chub mackerel were abundant in large numbers as far north as Yakatut Alaska. They also discovered that "mackerel predation was particularly intense on juvenile chinook salmon".
"Many mackerel we examined had eaten several chinook, and some mackerel had eaten as many as five juvenile chinook in locations where chinook were abundant". They estimated that the total number of chinook eaten by mackerel in Barkley Sound in 1992 was somewhere between 3.4 and 8.1 million, or between 40% and 95% of the 8.5 million chinook released that year by Robertson Creek Hatchery. A similar pattern of events was also found in 1993. This heavy predation by mackerel is believed to have

devastated survivals of chinook not only of Robertson Creek stock, but other WCVI stocks as well, notably the Conuma and Nitinat stocks (figure 1).

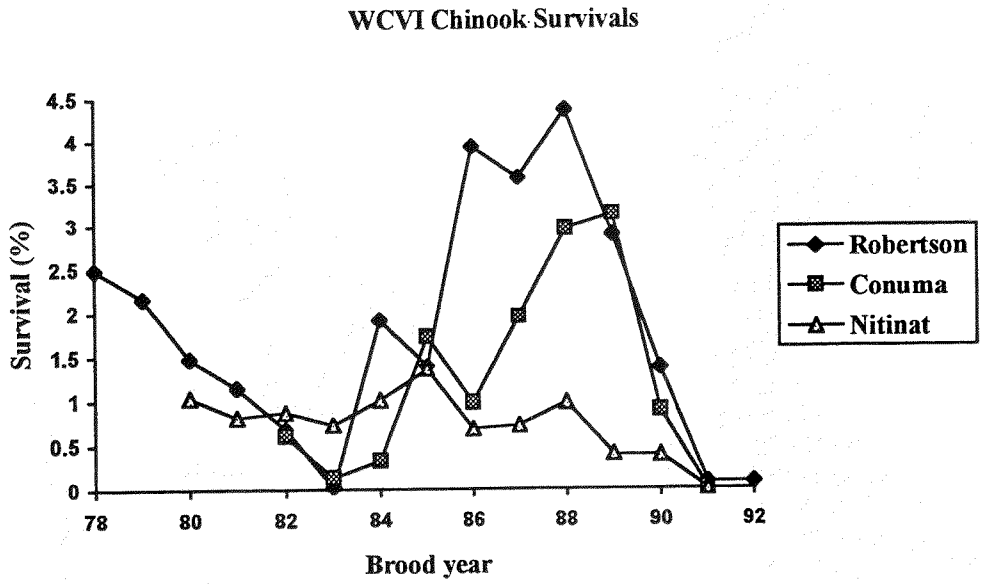

Figure 1. WCVI hatchery survival rates by brood year.
((Exp, CWT + Catch + Escapement) / Total Released)

In the Tlupana Inlet, mackerel have been observed, captured and have had hatchery chinook smolts identified in their stomach contents by Conuma Hatchery staff from 1992 to 1996. We have literally had to release millions of chinook smolts into the waiting mouths of chub mackerel. Hargreaves and Hungar (1995), in their paper entitled "Robertson Creek Assessment and Forecast for 1994", made some recommendations based on the forecasted drastic declines of adult returns in 1994 through 1996. Of particular interest to us was the recommendation that "the Salmonid Enhancement Branch should seriously consider alternative hatchery production and release options" and "are there any alternative rearing or release strategies that may reduce predation mortality of chinook if mackerel return in 1994, or in the future". To follow up with this recommendation, we initiated an alternate release strategy whereby a netpen of ocean reared chinook smolts were towed (translocated) to a release site closer to open ocean, with the hope of bypassing some of the predation pressure due to mackerel in the Tlupana Inlet.

The Translocation

The netpen used for the translocation was a 100 ft x 100 ft hexagonal pen, 15 ft deep with half inch mesh. The Conuma stock chinook were initially reared, marked and vaccinated for Vibrio at Conuma Hatchery then transferred to the hexpen on May 15 and 16, 1996 after 90 days of fresh water rearing. Fish health and pen conditions were closely monitored during the salt water rearing stage. Mackerel were observed in the Tlupana Inlet and around the seapens both before and during this stage. Several of these mackerel were caught, their stomach contents were examined, revealing that many hatchery chinook smolts from a previous seapen release had been eaten by them.

The translocation hexpen was towed June 03, 1996 from Moutcha Bay to the entrance of Eliza Passage, a total distance of 17 km (figure 2). During the trip, the net and fish were monitored and the tow speed adjusted so as not to allow the net to bag excessively and to make sure the fish were not stressed and were able to keep up. The entire operation took two days. At the end of the first day, the pen was moored at Critter Cove overnight and the next morning, the remainder of the translocation journey was completed. Total towing time was 18 hours giving an average tow speed of 0.94 km/hr. An interesting

observation made by the participants during the trip was that mackerel were seen in the wake of the hexpen and seemed to be following it. The number of mackerel could not be accurately estimated, however, the crew felt there were not large numbers present. Of the 652,500 fish released on June 04,1996 at 14:00 hrs, 23,500 were CWT marked, the average release weight was 6.9 gm and length was 84 mm. This group of fish, as well as all Conuma stock chinook, were also post-hatch thermal/otolith marked with a 5 band 24hr ambient mark. A similar group of chinook smolts were marked with a different CWT code, were salt water reared during the same time period but were not translocated. The returns from these two groups and subsequent translocation groups will be monitored over the next several years and will be examined for differences in survival.

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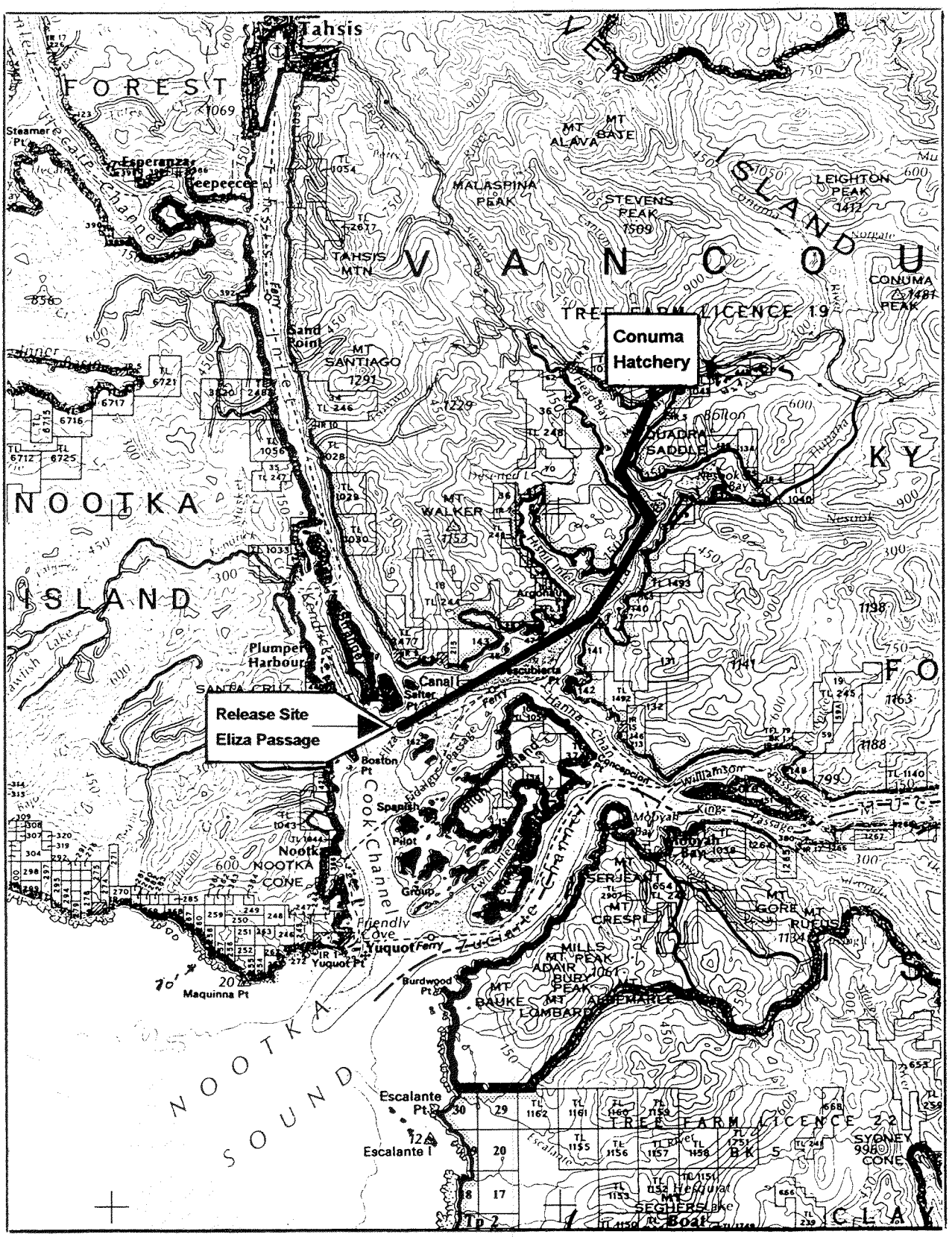

Figure 2. Translocation route of Conuma stock chinook June 03 and 04, 1996

CHEHALIS RIVER HATCHERY

SITE DESCRIPTION AND PRODUCTION

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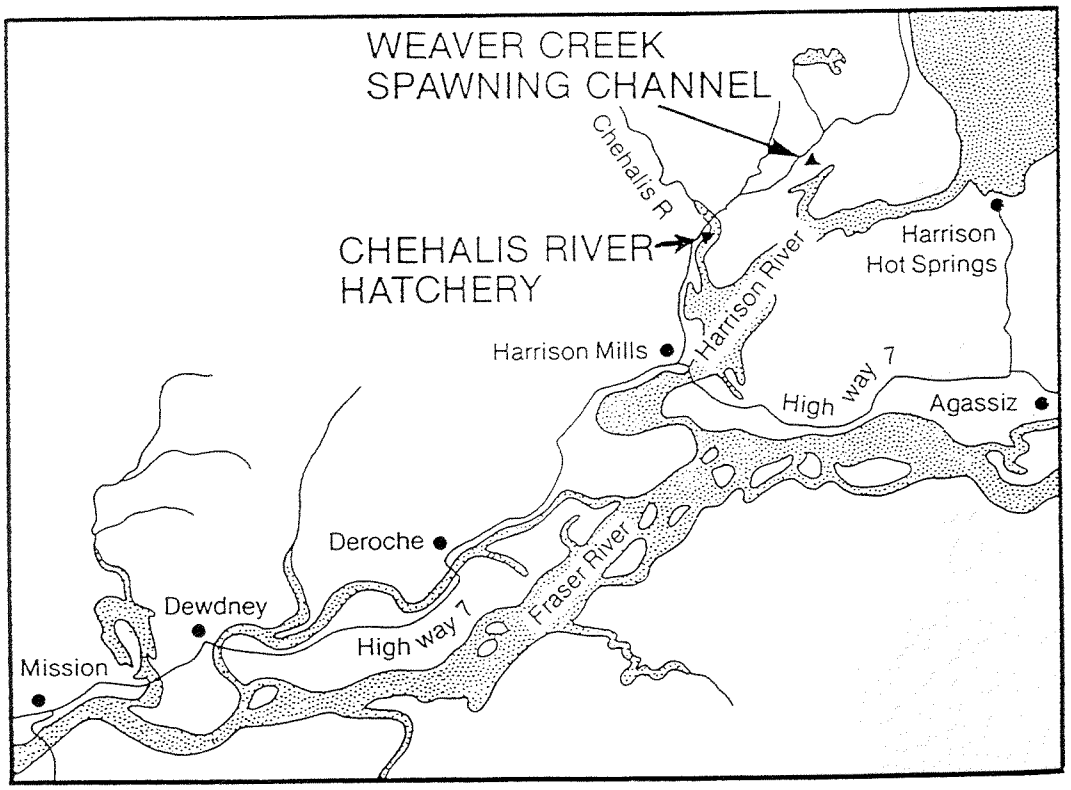
Chehalis River Hatchery Location

The hatchery is located in the Fraser Valley between Vancouver and Hope, British Columbia. It is on the north side of the Fraser River just 6 km north of Harrison Mills.

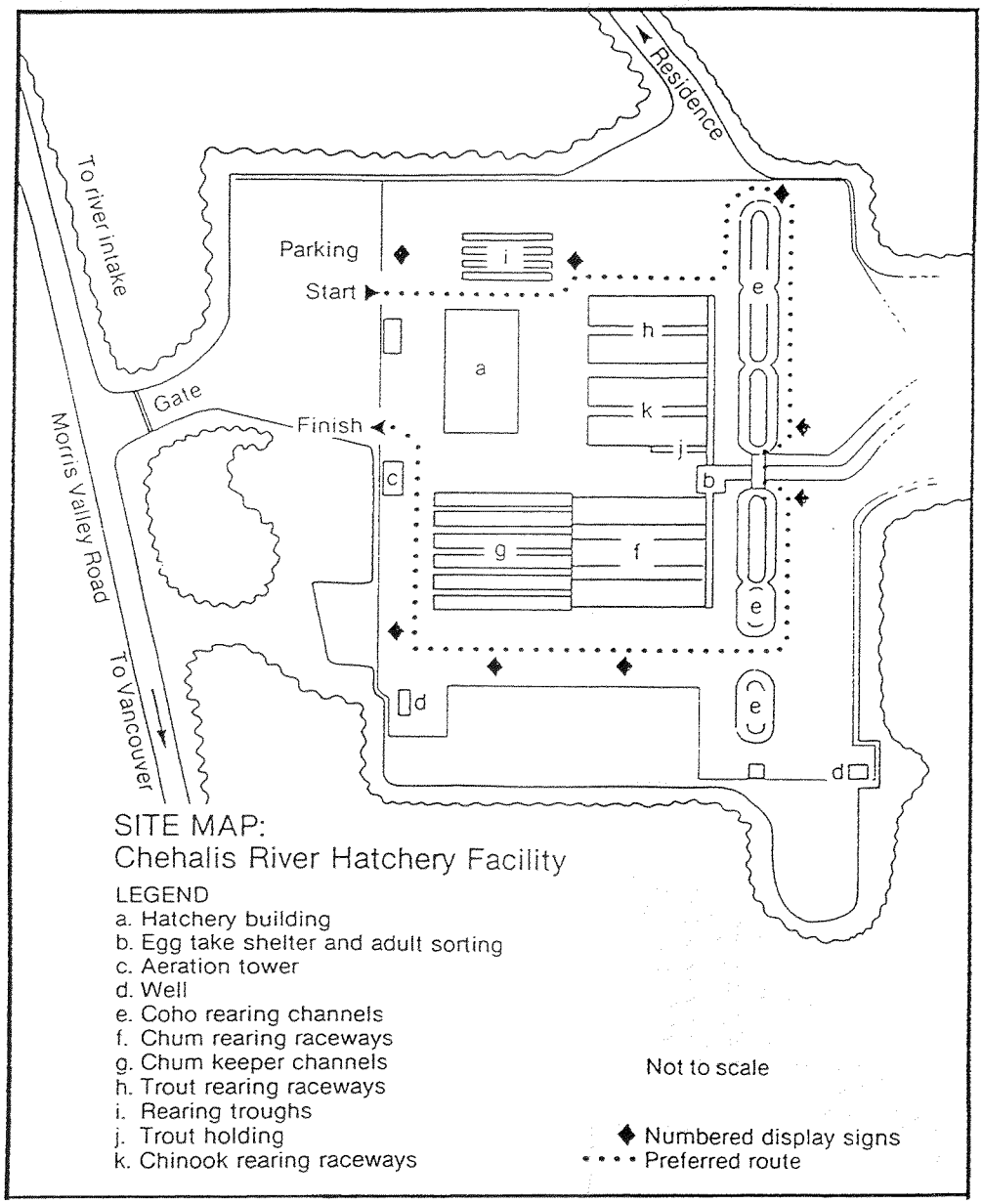


Chehalis River Hatchery Site

The hatchery was approved for construction in 1980 and commencement of operations began in 1982. It is a salmonid enhancement facility of the Government of Canada. The site is constructed on 26.5 ha (66 acres).

The capital cost of the project was $6,500,000, with an annual operating cost of $650,000 per year ($330,000 for materials and services, $15,000 capital for new or replacement equipment, and $305,000 for wages (seven person years)).

The hatchery site has;
-a gravity feed, riverbank fixed-screen intake, on the Chehalis River which supplies 25,000 to 50,000 lpm (15-30 cfs) of river water depending on water demand.



-3 pump stations (20, 60, 100 hp) which supply up to 17,000 lpm (10 cfs) of well water.
-an aeration tower for aeration of well water and distribution of well and river water.
-a hatchery building containing offices, washrooms, lunchroom, laboratories, workshop, dry storage areas, mechanical/electrical room, incubation room with "Heath" trays and "Atkin" cells , and a freezer (20,000 kg capacity) for fish food.
-12 keeper channels for chum and chinook salmon egg hatching and alevin incubation. (Approximately 4 to 5 inch cobble substrate in a single loose layer is used in all channels.)
-32 aluminum rearing troughs for initial fry rearing for mainly coho and chinook fry.
-14 concrete raceways for rearing (all species) and adult holding (chum, chinook, and coho).
-3 concrete holding channels for steelhead and cutthroat
-3 asphalt lined channels for coho rearing.
-a fish brailer and lifter, anesthetic tank, sorting table and transfer pipes for adult salmon handling, and an egg-take shelter used for adult sorting and spawning,
-a sludge lagoon into which waste from cleaning the raceways is pumped.
-a fuel shed, a residence (for crew standby), and display panels for public information and self-guided tours.

Chehalis River Hatchery Production

The hatchery produces approximately 9,000,000 chum fed fry, 2,500,000 chinook fed fry, 1,100,000 coho smolts, 89,000 steelhead smolts, and 24,000 cutthroat smolts.

Chum eggs are taken from spawners returning to the hatchery. They are incubated to the eyed stage in "Atkin" Cells and then moved to the keeper channels to hatch and incubate until buttoned at which time they are flushed to the rearing channels. Fry are reared to 1.2 g in size and then released directly from the site.

Chinook eggs are taken from spawners returning to the hatchery and Harrison River. They are incubated in "Heath" trays and "Atkin" cells to the eyed stage and then are moved to the keeper channels to hatch and incubate to the buttoned stage when they are either moved or flushed to various rearing areas. The Chehalis "red" chinook are reared to approximately 7 g in size and the Harrison "white" chinook are reared until 2 g. Both are released directly from the site.

Coho eggs are taken from spawners returning to the hatchery. They are incubated in "Heath" trays, ponded into troughs until 1.2 g in size, and then moved to various rearing raceways and ponds until release at 20 g.

Steelhead and cutthroat broodstock are collected from adults returning to the Chehalis River. Only wild adults are used for steelhead broodstock. The adults are taken to the Fraser Valley Trout Hatchery where they are spawned. The juveniles are incubated and reared to approximately 3 g in size before being brought back to this site to rear. The steelhead are between 60 and 80 g at release and the cutthroat are between 75 and 100 g at release.

Hatchery Benefits

The site provides a significant year round freshwater sport fishery near a large population centre by producing coho, summer and fall chinook, summer-run and winter-run steelhead, cutthroat, and chum.

It provides an educational experience and recreational outing to hundreds of school children, visiting tourists from many countries, and the general public daily year round.

Stimulates the local economy through the sport fishery, tourism, the purchasing of supplies for operations, and by providing employment.

Provides information for fisheries management and research. Harrison chinook tagged at the facility provide management with an important source of information relevant to the large wild Harrison chinook population that drives the Georgia Strait chinook fishery. Chehalis tagged coho and chum provide data on exploitation rates and survival rates.

Produces chinook, coho, and chum for the saltwater sport and commercial fisheries. Saltwater sport head recoveries for Chehalis coho and chinook indicate most fish are taken in the Deep Bay, Campbell River, and Nanaimo areas. Some Chehalis fish are caught in Washington, Oregon, and Alaska, although Canadian catch is 80-90% of the total catch. coded-wire tag recoveries in 1992 in sport fisheies and commercial fisheries showed a catch of 87,100 coho, 12,600 chinook, and 46,800 chum from Chehalis Hatchery.

Produces chinook, coho, and chum for the Fraser River Indian food fishery.

Starting in 1992, in co-operation with the Fraser Valley Trout Hatchery, rears 30,000 summer-run steelhead fry to smolt size for release in the Coquihalla River near Hope.

Restored the red summer-run chinook population in the Chehalis river by the use of transplants. In co-operation with Fraser Valley Trout Hatchery, produced a fishable summer-run hatchery steelhead population.

Provides information on water conditions, fish numbers, angling success, and river access to anglers on a daily basis.

Provides a centre for the reporting of fisheries violations on the river.

Provides eggs and fry to Community Advisor and small projects. Currently providing 200,000 chum fry for release into the Serpentine River and 50,000 chum fry for release into the Nicomekl River.

Provides advances in fish culture through isolation incubation techniques, fish rearing and release strategies, disease treatments, pond cleaning, and adult salmon sorting and enumeration procedures.

THE METHOW SALMON HATCHERY

"SUPPLEMENTATION FACILITY"

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ABSTRACT

As part of the Methow River Spring Chinook Enhancement Project, the Methow Salmon Hatchery was built for the sole purpose of enhancing the natural production of spring chinook salmon in each of three sub-basins in the Methow River, without affecting the genetic characteristics of each stock. The project also included the construction of remote satellite acclimation ponds and adult trapping facilities. The hatchery makes use of innovative design and operations features, which closely resemble conditions found in nature. The hatchery and associated facilities were designed by Fish Pro Inc., built with funding from Douglas Co. PUD, and operated by the Washington Department of Fish and Wildlife.

INTRODUCT ION

Location/Description

The main production hatchery is located in Northcentral Washington near the town of Winthrop along the Methow River. Facilities consist of a hatchery building with 300 iso-buckets, 45 vertical incubators and 24 - 3' X 15' X 2.5' fiberglass starter tanks. Outside, there are 12 - 8' X 80' X 4' covered concrete raceways, three - 8' X 80' X 4' covered concrete adult ponds, one - 110' X 40' X 4.5' on-site hypalon-lined rearing pond, two - 110' X 40' X 4.5'. off-site gravel-lined acclimation ponds, three off-site adult collection facilities, a surface water intake, and ground water supply. The hatchery program calls for a production goal of 750,000 spring chinook smolts @ 15 fish/lb divided equally between the Twisp, Chewuch and Methow Rivers.

Water Supply/Intake

Surface water is supplied to the hatchery by an intake that virtually takes care of itself. The five main intake screens are set at a 45° angle to the flow of water allowing for a sweeping action across the screens. Leaves and other floating debris are easily flushed past the screens to a partially open gate. An overflow weir upstream of the intake, within the structure, sets the minimum head needed to operate all production facilities by gravity flow. In addition, there is an ice prevention system which utilizes a small air compressor to charge diffusion piping located on a support rack under the intake screens. At low temperatures the system produces fine bubbles of air that rise past the intake screens helping in the prevention of icing. Similar intakes are also in operation supplying the Chewuch and Twisp Acclimation Ponds. Surface water temperatures range from 32-65°F.

The ground water supply system for the Methow Salmon Hatchery consists of four production wells. All water is stabilized via a packed column system located in the main headtank prior to gravity distribution to the raceways and

all other hatchery rearing or incubation units. Ground water temperature is a constant 46° F.

ADULT COLLECTION AND HOLDING

Capture and Transport

There are adult traps located upstream at each of the three rivers (Twisp, Chewuch, and Methow). Barriers to fish passage at each site help to divert returning adults into collection areas for subsequent transport back to the main hatchery. A floating barrier is utilized on the Twisp, a modified/remodeled denile-type fishway on the Chewuch, and a vertical slot fishway, with trapping bypass, on the Methow. Once in the collection area, the adults are marked and placed into an innertube to be carried up to a 300 gal tank used in transport. Adults which are not needed for the hatchery program can also be easily passed upstream through a small opening in the trap area.

Adult Ponds

After a short hauling time, the adults are placed into one of 3 adult ponds by allowing them to travel down a PVC tube from the tank into the pond. Having three ponds allows us to keep each of the three stocks separate throughout the holding process. Adult ponds are 8' X 80' X 4' concrete with both a surface water and well water supply. A spray system along the sides helps to keep the adults calm during the holding period. Barriers along the sides and at both ends of the pond keep adults from jumping out. A perimeter fence with ground pressure sensors and motion lighting help to add security to the system. An epoxy paint seals the walls of the ponds and eliminates any abrasions on the adults.

SPAWNING AND INCUBATION

Spawning Area

There is an individual spawning area at the head end of each adult pond. This allows greater ease in disinfection between ponds on spawning days. Two separate drains from the spawning area allow spawning wastes to be diverted into the septic system if needed. Spawning protocol calls for 1:1, male to female spawning ratio of all fish, with disinfection between individual females. Iso-buckets are used for mixing eggs with milt prior to water hardening.

Disinfection Room

Iso-buckets are then brought into the disinfection room one stock at a time for water-hardening in iodofor. After 1 hr, the buckets are placed in an individual incubation room depending on the particular stock.

Incubation Rooms

There are three separate incubation rooms with the ability to isolate-incubate up to 100 females per room. 150-buckets are used until viral and ELIZA test results are known. Eyed eggs are then transferred to 15 vertical incubators and arranged based on those test results. An automated formalin delivery system can treat the iso-buckets as well as the vertical incubators through a system of stainless steel piping and valves. The formalin mixing and delivery point is located in a separate, specially-designed room in the hatchery. Each incubation room is positioned so that fry transfer to the start tank room is easy.

REARING

Early Rearing/Start tanks

From the vertical incubators fry are transferred to starter tanks. The tanks are molded fiberglass, dark blue in color, and sized 3' X 15' X 2.5'. With 24 of these tanks, the ability to separate different egg lots based on fry size and/or propensity for disease problems is greatly enhanced. Loadings are kept at or below 0.125 lbs/cu ft/in at all times.

Overhead lighting is adjustable to simulate natural conditions during first feeding. Twin drain sumps can divert waste water to the clarifier during cleaning as can an in-line vacuum system. CWT marking takes place in these tanks just prior to transfer to outside raceways.

Covered Raceways

Twelve covered raceways are located away from active areas and available for rearing until yearling transfer to acclimation ponds. Each pond is 8' X 80'x 4' with a well water and surface water supply. Brown-pigmented concrete creates a more subdued environment which the fish respond well to. PVC-vinyl covers also add to the natural shading of the ponds from bright sunlight. Baffles in the ponds create currents which help to clean the bottom as well as giving the fish different velocities to choose from. Water temperatures can be tempered to closely match the river cycle. After a year the fish are then transferred to their respective acclimation pond for release.

ACCLIMATION

Release Ponds

There are three acclimation ponds; one on each of the Twisp, Chewuch and Methow Rivers. The ponds are all 40' X 110' X 4' with predator fencing around all sides and low-level overhead bird protection. Yearling spring chinook are transferred in March from the main hatchery and allowed to leave the pond volitionally after 6 weeks of acclimation time. Size and time of release are 15 fish/lb and April 15th respectively. The acclimation ponds incorporate a variety of features which help to mimic conditions found in the natural environment.

Natural Rearing Additions

All acclimation ponds have a camouflage-type netting over certain areas. The shading that is produced allows the fish to avoid the bright sunlight and to experience different environments. The netting is easy to feed through and is tough enough to withstand severe temperature changes. The vegetation around and inside of the ponds has been allowed to grow up contributing to an increase in insect production. The weed growth also creates areas for fish to rest and/or hide. Gravel substrate along the sides and bottom of each pond also help to acclimate the fish to conditions which they will find upon final release into the river. River water is used to further acclimate the fish to temperature changes and to increase homing success of adults.

RETURNS

Since the hatchery's first brood year was 1992, adult returns are only just beginning to show up in the Methow system. We did see some 4-year-old fish this year from both the Chewuch and Twisp releases in 1993. Next year's 5-year-old returns should give us some additional data with which to make some observations on the overall program.

SUMMARY

The goal of the Methow Salmon Hatchery is to increase the number of returning adults to the Methow System without sacificing a loss in the genetic integrity of the three target stocks. Isolation techniques, small manageable starter tanks, acclimation facilities, stringent spawning guidelines, and natural rearing will only add to our ability to acheive that goal.

Leavenworth National Fish Hatchery
Leavenworth, Washington

Avian Predation Pond Covers

Terri Judd
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The Leavenworth National Fish Hatchery, like any other hatchery, has its problems with predators. During the winter of 1995, high numbers of fry Chinook Salmon were lost to bird predation. There was an immediate need to develop an affordable and effective anti-bird covering system for the raceways.

The fish production crew combined ideas from other hatcheries and ideas of their own. The main concerns were design, manufacture, and cost. Another challenge was to make the protective covers strong enough to withstand the weight of the heavy snowload that is common to the Leavenworth area.

The cost aspect fortunately was not a problem. Many volunteer hours were spent on building the new predation covers. The Upper Valley Chapter of Trout Unlimited, several high school students, community service providers, and others helped with the project.

How predation covers were built:

* Size of panel ----10' x 7'4"

* Panel corners welded, not clamped

* Holes drilled in pipe (3" apart on 10' side and 6" apart on 7'4" side)

Procedures:

1) Cut pipe to designated lengths.

2) Drill holes as specified above.

3) Weld 4 pipes together to form rectangular frame .

4) Thread wire through all holes, and clamp end.

5) Tie rope to center of 10' pipe, and attach "S" hook.

6) Place 2 galvanized hooks on pond walls (2 on each side of panel).

7) Cover raceway with several predation screens (we have 7 covers to a raceway).

Materials used to build one predation cover:

* 34'8" of 3/4" galvanized round stock

* 7' of 1/2" galvanized round stock for center support

* 1/16" galvanized 7-strand braided wire (as needed)

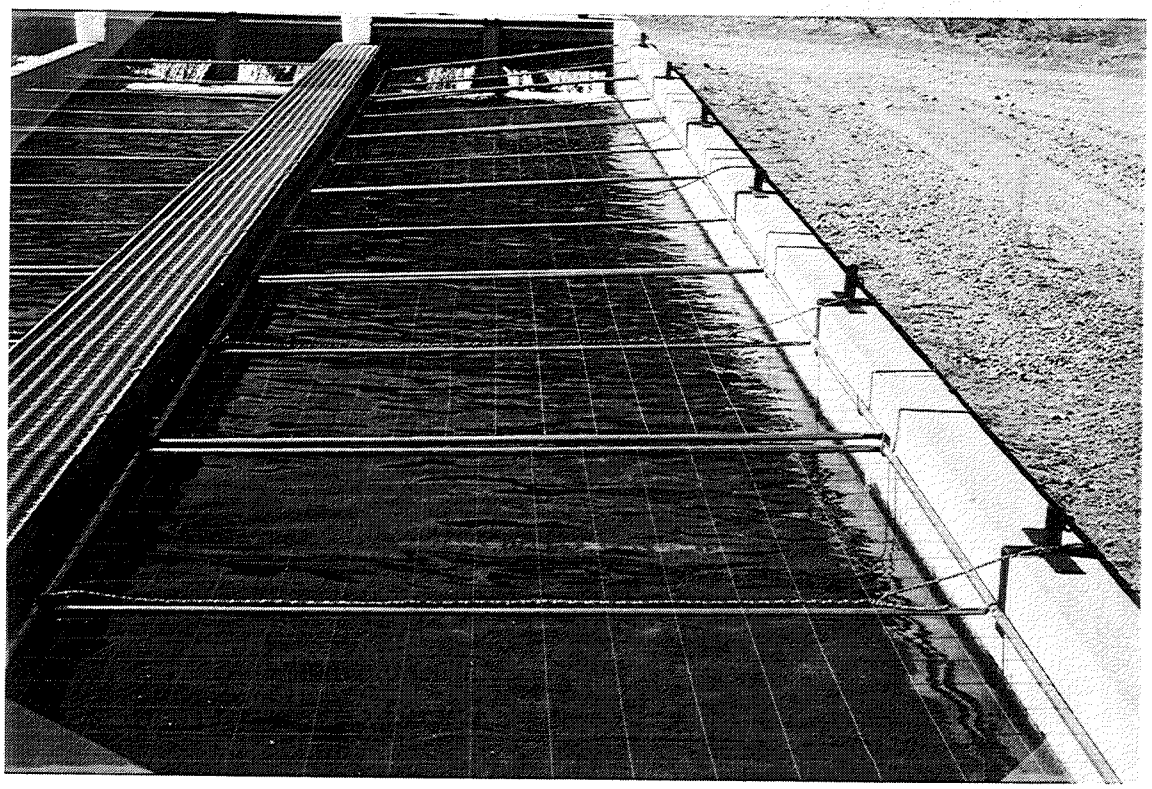
* Wire clamps

* 25' poly rope

* 2" S hooks

* Galvanized hooks

Some fine tuning may still be required on parts of the covers, but over all they have met our needs in a satisfactory manner. The first heavy snow will put our design to the test.



VANCOUVER ISLAND TROUT HATCHERY

EFFLUENT TREATMENT

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ABSTRACT

A sequential batch, lamella clarifier was installed in a provincial trout hatchery in order to remove settleable solids from its effluent. The system was developed to optimize gravity sedimentation and removal of biosolids while at the same time minimize head loss, land area and mechanical complexity. The design rationale, design criteria, system operation and performance evaluation are presented. Potentially, the system can be used in water supply and waste water treatment applications in many minicipal, industrial, and aquacultural settings.

Introduction

The Vancouver Island Trout Hatchery is a groundwater fed, single pass, facility. Built in 1992, it was designed for the incubation and rearing of Steelhead, Rainbow, Cutthroat, and Brown Trout for release into Vancouver Island lakes and rivers. Of critical importance in the design of this facility, as it is in any modern hatchery, is the quality of the effluent being discharged. Suspended solids concentration was identified as the key determinant of this effluent water quality. Therefore, suspended solids separation became the focus of the waste treatment technology incorporated in the facility design.

The sources, quantities and physical characteristics of solids generated in fish culture operations have been researched in depth and the performance of existing solids control mechanisms is well documented. It is also recognized by most fish culturists that the design of rearing structures has a major influence on the physical characteristics of solids, in particular particle size. Particle size is, in turn, of primary importance in the choice of a solids removal technology.

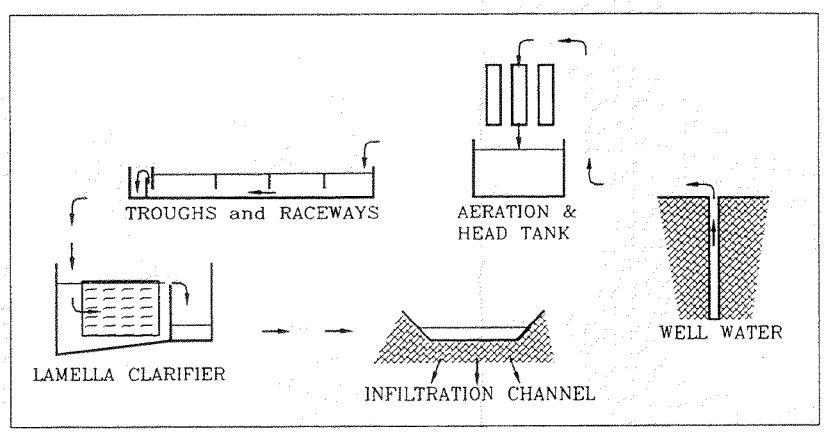

Figure 1. VANCOUVER ISLAND TROUT HATCHERY - FLOW SCHEMATIC

The objective of the solids removal processes at this facility was to produce an effluent that met waste discharge permit criteria. The entire facility (Figure 1), not just the waste treatment component, was designed from the ground up to control the physical characteristics of the solids (feces and uneaten food) within the system. Raceways and troughs incorporating baffles were chosen rather than circular tanks in order to minimize the abrasion and breakup of solids and to facilitate their rapid and continuous movement out of the rearing areas. There was a conscious effort made to avoid turbulence and pumping in the effluent stream in order to minimize particle breakup. To minimize operations and maintenance costs and head loss, gravity sedimentation as opposed the mechanical separation was chosen for solids removal. Clarified effluent discharged from the waste treatment process is delivered to infiltration channels located on a river terrace adjacent to the site, while solids recovered in the process are removed from the site and used as fertilizer on nearby farm land.

Clarifier Design

Theory aside, for a given discharge (Q), a gross estimate of the required surface area of a gravity settler (A) can be calculated using the formula $A = VoQ$, where Vo, the overflow rate, which is related to the settling velocity, is expressed as a discharge per day, per unit surface area (Figure 2). For this facility,

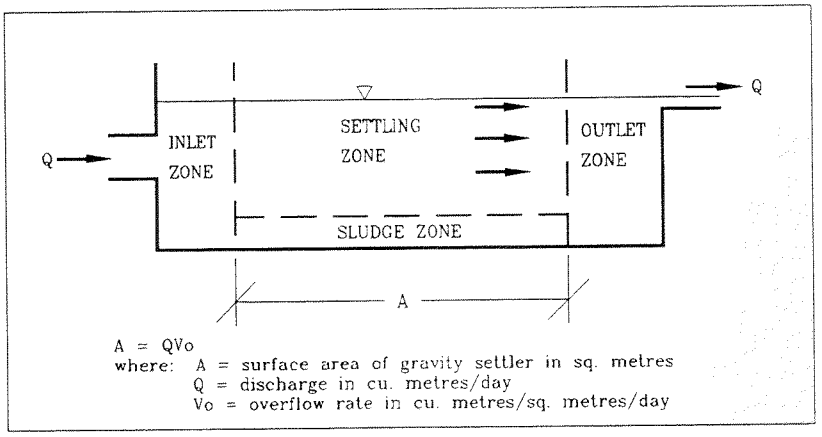

FIGURE 2. CALCULATION OF REQUIRED SURFACE AREA OF A GRAVITY SETTLER

the overflow rate was conservatively chosen to be 35 cu.m./sq.m./day - values typically range from 30 to 60 for municipal sewage treatment facilities. On the bases of this overflow rate, and an eventual (expanded facility) discharge capacity of 30,000 l/min. (43,200 cu.m./day), the horizontal surface area required for settling was determined to be 43,200/35 = 1234 sq.m. To provide this area using a conventional settling pond would require a rectangular pond roughly 20 m x 60 m (65 ft. x 200 ft.) or a circular one 40 m (130 ft.) in diameter. It was also determined in pilot studies, that settled solids would have to be removed within three days of deposition in order to avoid biological decay and release of nutrients to the water column. Structures of this size compounded by the operational problem of frequent removal of settled solids made conventional settling ponds out of the question.

In order to overcome the size and operating constraints inherent in a conventional clarifier, a modified lamella type clarifier was developed. Lamella clarifiers, like tube settlers, are designed to maximize settling area in a minimum of space, and space was a consideration at this site. They achieve this by layering horizontal surface area rather than running it out linearly - ie stacking it one on top of the other rather than end to end. Conventional industrial settlers such as this are often used to settle inorganic solids. They are normally installed on an incline in order to allow for the continuous gravity removal of settled solids (Figure 3a). However, the tendency of such systems to plug due to biofouling has restricted their use in the settling of organic material such as municipal and hatchery wastes which are subject to rapid decay.

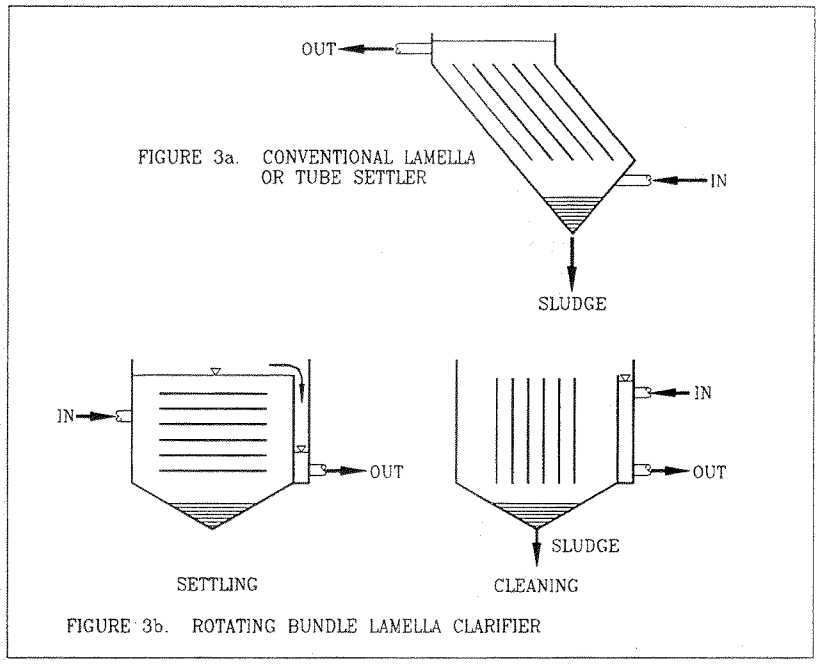

FIGURE 3a. CONVENTIONAL LAMELLA OR TUBE SETTLER

FIGURE 3b. ROTATING BUNDLE LAMELLA CLARIFIER

FIGURE 3a & 3b. CONVENTIONAL AND ROTATING BUNDLE LAMELLA SETTLERS

To overcome this biofouling problem, the lamella concept was modified so that it would operate on, what could best be termed, a batch bases (Figure 3b). The lamella plates are oriented horizontally during normal continuous flow operation. This allows solids to settle out on the plates like dust under and on furniture. However, periodically clarifier inflows must be interrupted so that the plates can be cleaned. The desired settling area is developed by the construction of lamella plate bundles. Each bundle consists of 32 - 1.2 m x 2.4 m aluminum plates stacked one on top of the other 39 mm apart to create a matrix of plates roughly 1.2 m wide x 1.2 m deep x 2.4 m long. There are 12 such bundles in the clarifier tank providing a total settling area of 1100 sq.m. (Figure 4).

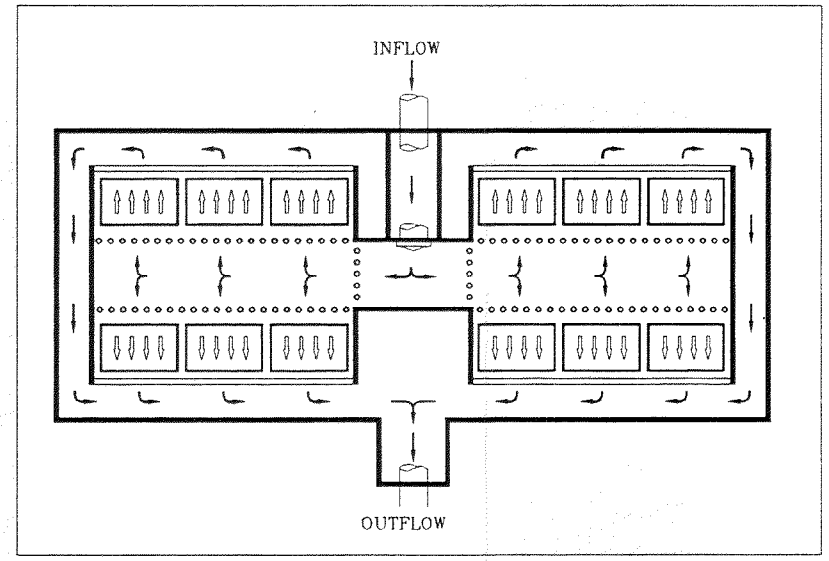

FIGURE 4. CLARIFIER FLOW SCHEMATIC DURING NORMAL OPERATION

In order to clean the lamella bundles and remove settled solids, it is necessary to divert untreated flows around the clarifier for a short period (Figure 5). The cleaning sequence, which takes place in the morning before feeding has begun, is as follows:

1) flows are diverted around the clarifier tank containing the lamella bundles;
2) standing water in the clarifier tank is decanted into the effluent trench;
3) the lamella plate bundles are rotated into the vertical position;
4) accumulated solids are washed off the lamella plates and tank bottom and the resulting sludge is pumped to a secondary thickener;
5) the lamella bundles are rotated back into the horizontal position; and,
6) the flows are diverted back into the clarifier tank.

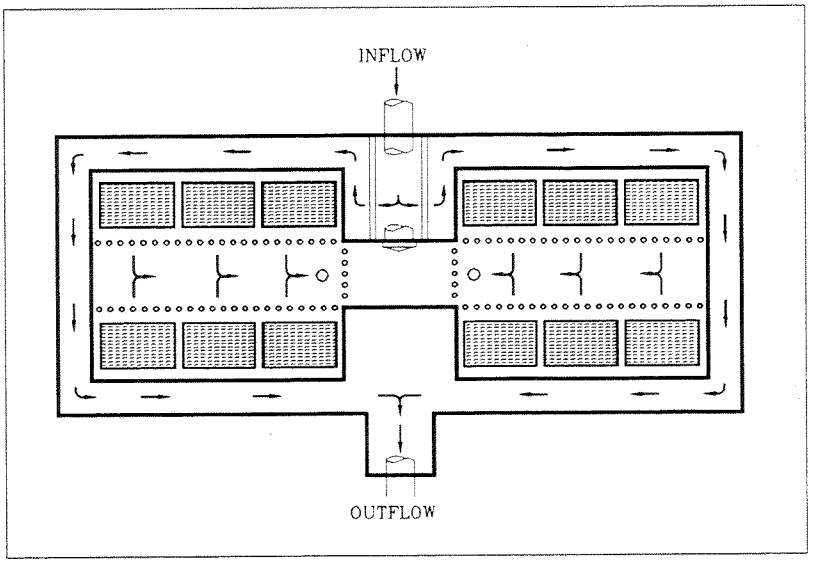

FIGURE 5. CLARIFIER FLOW SCHEMATIC DURING CLEANING OPERATION

Cleaning takes less than one hour and is required two or three times per week depending on the fish loading.

Sludge removed from the clarifier during cleaning undergoes further processing (Figure 6). It is stored

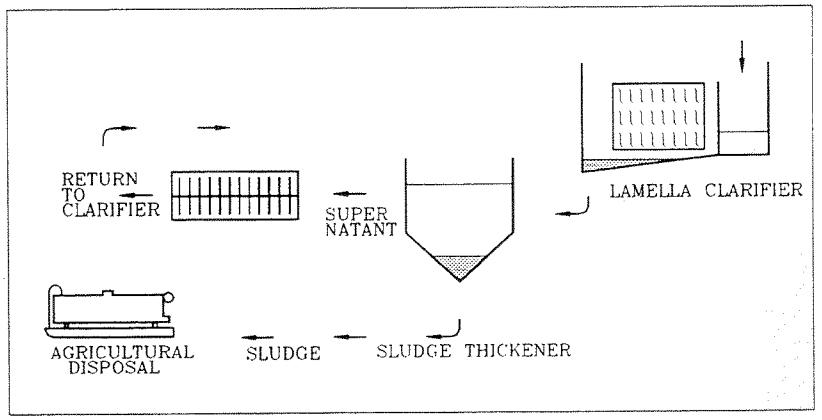

FIGURE 6. CLARIFIER CLEANING AND SLUDGE TREATMENT

in a conical thickener where it is allowed to form a concentrated sludge and a clarified supernatant. The thickened sludge is removed from the thickener and stored in a container which, when full, is taken to a

local farm for use as a fertilizer. Over the period between cleanings, the supernatant is metered out of the thickener and into a rotating biological contactor for biological treatment. This secondarily treated waste stream is then redirected into the lamella clarifier to settle biosolids and mix with the larger effluent flows.

Discussion

Weekly effluent sampling for a period of 10 months gave the following test results:

	TEST	PERMIT OBJECTIVE
BOD	< 10 mg/l	<10 mg/l
SS	< 1	<1
TN	< 0.32	<0.40
TP	< 0.018	<0.07

Fish loading and flows varied widely over the sampling period. Loading was always less than the 1 kg/l/min. design and flows varied from a minimum of 7000 l/min. to a maximum of 20,500 l/min.

A detailed evaluation of the efficiency of separation (% solids removed) has not been carried out. However, effluent water quality and operations and maintenance objectives have been met. The installation has performed as projected. After three years of operation this unique lamella settler has been shown to be a practical alternative for suspended solids control. With minor modifications similar systems could become an option in other waste water treatment process streams requiring high performance sedimentation in space restricted installations.

TREATMENT OF EFFLUENT GENERATED DURING GRAVEL CLEANING OPERATIONS AT A SPAWNING CHANNEL

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Abstract

Spawning channels accumulate sediment and must be routinely cleaned to maintain high egg to fry survival rates. At Little Qualicum, spawning gravel is cleaned yearly by scarification with a bulldozer. During this process water flows are maintained so that sediment is dislodged from the gravel and swept downstream. As the bulldozer works its way down the channel, the concentration of suspended solids in the outflow increase to very high levels (peaks of 12,000 mg/L). To minimize effects on the downstream receiving environment (steelhead rearing area, municipal water supply and recreational use), this effluent must be treated. This has been achieved by pumping the heaviest portion of the effluent stream to a large (8 acre) field. The field is heavily vegetated with grasses, bushes and small trees. Earthen baffles have been constructed to disperse the flow and it is surrounded by dikes. With an inflow of 15 cfs the suspended solids concentration is typically reduced from 1350 to 8 mg/L at the point of discharge to the Little Qualicum River. Last year 85% of the effluent was intercepted and pumped to the field (90 million liters). Downstream suspended solids concentrations were monitored throughout the cleaning and impact on fish was predicted using the methods described in the "Provisional Field Guide for Assessment of Risk and Impact" (Newcombe, 1996). With the present level of effluent treatment, this model predicts that the fish in the river experience a sublethal effect characterized as moderate physiological stress.

Introduction

Spawning channels are routinely cleaned to maintain high egg to fry survival rates. The porous gravel that lines these long shallow meandering channels acts as a large horizontal filter so that fine sediment is removed and gradually accumulates in the interstices. Settling basins at the inflow slow done the siltation process but are usually not large enough to completely protect the channel. At Little Qualicum the settling basin removes all the coarse and medium sand and a large proportion of the very fine sand but allows much of the silt and clay to enter the channel. A very high proportion (> 80%) of this material is removed as the water flows through the 14,000 ft long channel.

Sediment begins to accumulate after the adults (chum, chinook, coho) spawn in the fall. The amount of sediment entering over the winter depends on land use activity in the upper watershed and on rain fall patterns. Since the early 1980's the degree of winter siltation has been affected by logging, road construction, the natural gas pipeline crossing, agriculture, hydro-line construction and by natural bank erosion. Over the past 10 years the amount of fine sediment entering the channel (after the settling basin) has varied between 30 and 150 Tonnes per year. Reducing siltation events in the upper watershed is of prime importance -- increased siltation during the winter decreases egg to fry survival and greatly complicates the subsequent gravel cleaning operation. There is an ongoing effort by operations staff to identify sediment sources and initiate remedial action.

If sediment is allowed to accumulate year after year, the gravel permeability is reduced and the channel quickly becomes non-productive. To maintain productivity the Little Qualcium channel is cleaned every year in late June. Cleaning is

performed at this time because the fry/smolt migration out of the channel is complete and because there is still enough water flow to carry out the cleaning operation.

A number of gravel cleaning methods have been tested over the years. Most of these involve mechanically dislodging the sediment and flushing it to the river. This approach is unacceptable at Little Qualicum because of impacts on downstream water users. There are a large number of domestic water users (including the Town of Qualicum Beach) downstream of the channel. The lower river is also used intensively for recreation at that time of year. Furthermore newly emerged fry from late-run steelhead that spawn below the channel are vulnerable to cleaning effluent.

Because of these concerns an intensive effort has been made at Little Qualicum to minimize the amount of effluent entering the river. An alternative approach is to shut the flow off, drain the channel and truck the gravel to a screening plant on land. This is also unacceptable at Little Qualicum because large numbers of coho fingerlings utilize the channel year around.

At Little Qualicum spawning gravel is cleaned by scarification with a bulldozer. When the bulldozer starts at the head of the channel the water flow rate is increased to 45 cfs so that dislodged sediment is swept downstream. Most the this material resettles further down the channel. However some remains in suspension and eventually enters the river. As the bulldozer works its way downstream, the concentration of sediment increases to very high levels (approximately 12,000 mg/L). Before this occurs the channel inflow is reduced to 15 cfs and pumps are used to intercept this concentrated effluent. In 1996 two large submersible pumps were operated. The entire operation takes about 90 hours (10 work days) and is usually spread over the last two weeks of June.

Effluent is pumped to a large field (8 acre) that is densely vegetated. Treated effluent is discharged to the river from this field. Most of the heavy effluent that bypasses the pumps is directed to a large rearing pond for treatment. Although not as effective as the field, the pond does remove a high proportion of the sediment if the flow is low. Failure of a pump at this time results in surge of effluent through the rearing pond and an increase of sediment in the river.

Effluent from the cleaning operation enters the river at two points. Treated effluent from the field enters via culverts just upstream of the main fishway and a combination of treated (from the rearing pond) and untreated effluent enters the river at the main fishway. Downstream conditions are monitored throughout the cleaning operation. Samples are collected and measured immediately for turbidity -- later the samples are measured for the suspended solids concentration (mg/L) (note: suspended solids is also referred to as NFR or non-filterable residue). Using a relationship based on previous years data, the approximate suspended solids concentration (mg/L) is calculated from turbidity. This technique allows us to monitor the suspended solids concentration in the river with a fair degree of accuracy during cleaning.

Tentative guidelines developed several years ago require that the cleaning is stopped temporarily if the suspended solids concentration in the river reaches 200 mg/L. They also require that the cleaning operation be terminated for the year if the cumulative stress index in the river reaches 8.0. The cumulative stress index (CSI) is just a shorthand way of expressing the effect of concentration (mg/L) and duration of exposure (hr.) (Newcombe 1994). It is the natural log of C*T -- so if fish are exposed to a concentration of 30 mg/L for 99 hours the CSI is ln(30*99) = 8.0. These guidelines have been superseded by methods described in the "Provisional Field Guide for Assessment of Risk and Impact" (Newcombe, 1996). This method predicts the impact of siltation on a particular salmonid life-stage using empirical models relating severity of effect to the concentration of suspended solids and the duration of exposure. Model predictions have led to renewed efforts to further reduce the discharge of suspended solids to the Little Qualcium River.

Effluent Characteristics

Fig. 1 shows the NFR concentration of effluent pumped to the field in June 1995. The average NFR was 1,350 mg/L and the peak NFR reached 10,816 mg/L on June 30/95. The NFR of treated effluent leaving the field and entering the LQ river is also shown in Fig. 1. The average NFR was only 8 mg/L and the peak was 27 mg/L..

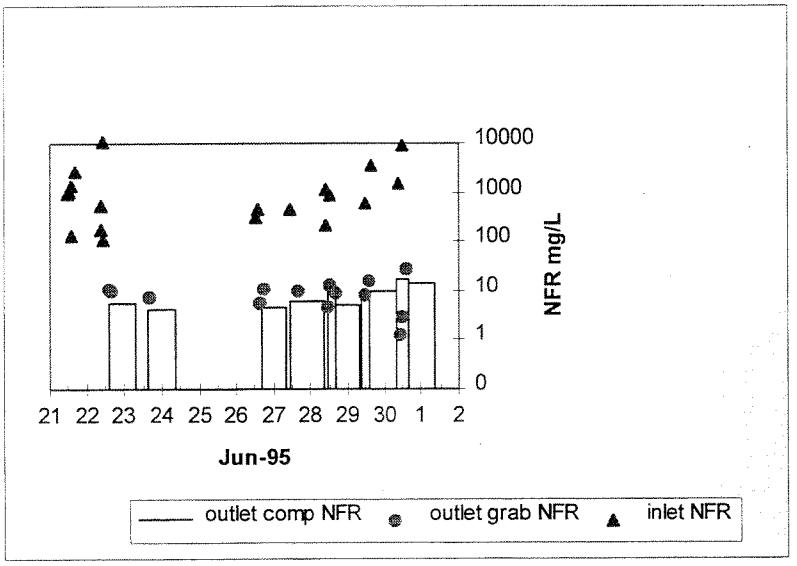

Fig. 1. NFR at the inlet and outlet of the settling field.

The bulk of the sediment entering the river comes from discharge at the main fishway. This is a combination of untreated and treated effluent. Fig. 2 shows composite and grab NFR's for this site.

NFR averaged 36 mg/L while the peak was 1157 mg/L. Grab samples show the variation in NFR while composite samples show the average NFR over a period. Composite samples are formed by pooling small grab samples taken every 10 minutes.

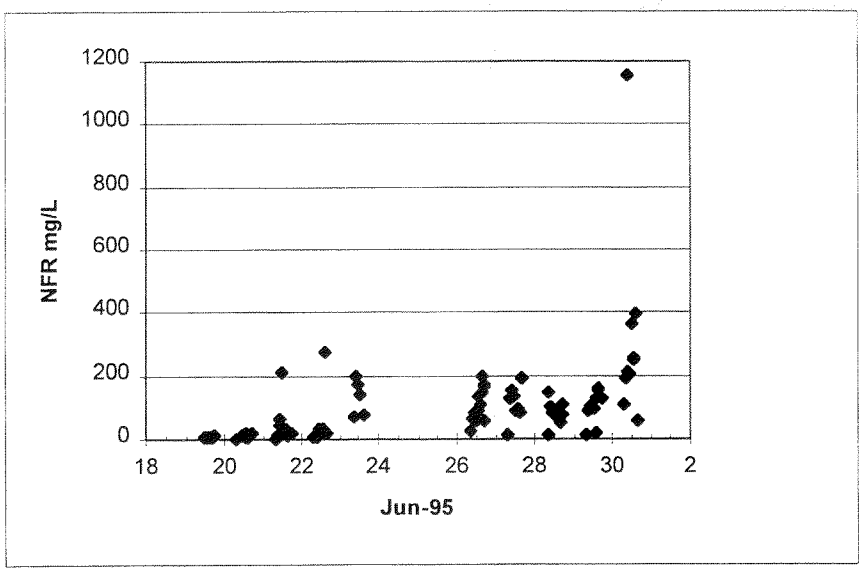

Fig. 2. Grab NFR (mg/L) of cleaning effluent at the main fishway

Effluent Treatment

The 8 acre settling field is densely covered by tall grass, bush and small trees. It is surrounded by an earthen dike and has two internal earth baffles to disperse the flow. The outflow structure consists of three culverts (one 16 inch diameter and two 24 inch diameter). The field is operated at a water depth (at the outflow) of 40 cm.

In 1996 two submersible Flygt pumps (model C-3201) were used to pump approximately 120 million liters of effluent to the field. In 1995, 90 million liters of effluent was pumped. 99% of the sediment entering the field (over 100 Tonnes) was removed. Most of this sediment was very fine material (silt/clay). This material is normally very difficult to settle however the tall grass acted like a filter and achieved very high removal efficiencies. Although the 1996 data is not yet analyzed, it was clear that the field was approaching an upper limit. As more effluent is pumped removal efficiency drops.

Overall, in 1995, 85% of the sediment dislodged from the spawning gravel was intercepted by the field (or rearing pond) while 15% entered the river. Much of the discharge to the river occurred prior to the start of pumping when the bulldozer was at the upstream end of the channel and the inflow rate was 45 cfs. To reduce the percentage going to the river more effluent will have to be pumped to the field.

At present much of the 8 acres is poorly utilized and the internal baffle structures could be extended so that more of the available settling area is used. Also the field will have to be given some attention if its capacity is to be maintained. Accumulated sediment will have to be redistributed and a method of draining standing water during winter will have to be developed. Any modifications to the field will have to made the previous summer so that lush growth is reestablished before pumping begins in June. Improvements to the field will become more critical as the volume pumped increases.

Impact on the River

Grab samples were taken routinely from the Little Qualicum River during the 1995 gravel cleaning program. Sites were established 100 m downstream of the spawning channel outlet (just upstream of the confluence with Whiskey Creek) and upstream of the channel. NFR of grab samples taken between June 19 and June 30 are shown as scattered points in Fig. 3. A peak of 91 mg/L was measured at 10:00 on June 30. The solid line in Fig. 3 reflects the daily NFR. It was assumed that two hours after the bulldozer stopped scarification, the NFR fell to a background concentration of 0.8 mg/L. This concentration was also assumed over the weekend.

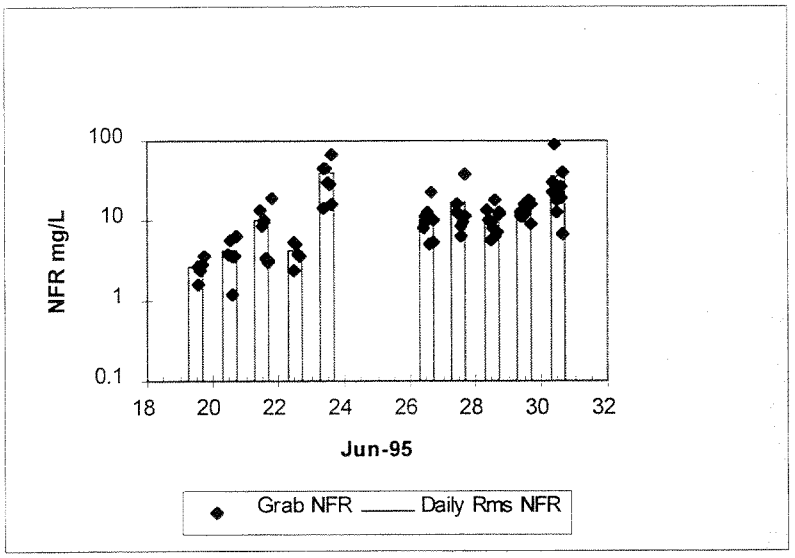

Fig. 3. Grab and daily RMS suspended solids concentration mg/L for Little Qaulicum River.

Because the NFR data is so variable, it is recommended that the daily NFR be calculated by taking the root mean square (RMS) rather than the simple average of the daily grab samples (Newcombe per. comm.). The RMS value is just the square root of the average of the squares. If the NFR is constant over the day the simple average and the RMS value are similar. However if the data is variable the RMS gives more weight to high values and is therefore greater than the simple average. The solid line in Fig. 3 shows the daily RMS values.

In 1995 NFR concentrations were elevated over the background value for a period of 114 hours (Fig. 3) and the average concentration (simple average of the daily RMS values) over this period was 13.7 mg/L. The cumulative stress index was $\ln(114*13.7) = 7.4$. Therefore the tentative guidelines (peak NFR < 200 mg/L; CSI < 8.0) for discharge to the Little Qualicum River were met.

It should be noted that to reduce impact of the cleaning effluent, river flows were increased by releasing clean water from Cameron Lake. In 1995 the river flow was increased from 145 cfs to 224 cfs. Although this flow increase only lasted for a few days it lowered NFR concentrations in the river at a critical time.

Using the methods of Newcombe (1996), the impact of this discharge on the river can be predicted. Firstly, the most vulnerable life stage present in the river at the time of the sediment event must be identified. Steelhead fry are present at Little Qualicum in late June. To predict the severity of effect index (z) on juvenile salmonids the following relationship was used: $z = 0.7262 + 0.7034*x + 0.7144*y$, where: $x = \ln(\text{concentration mg/L})$ and $y = \ln(\text{duration in hours})$. With a concentration of 13.7 mg/L and a duration of 114 hours the severity of effect index is 6. Using tables developed by Newcombe (1996), this corresponds to a sublethal effect characterized as a moderate physiological stress. An important assumption of Newcombe's model is that the stress from the daily sediment events is cumulative. The fact that NFR falls to background levels at night and on the weekend (Fig. 3) does not alleviate the impact of the daily sediment events.

For life stages that are committed to the gravel interstices (eggs or alevins) the model predicts more severe effects. Therefore to minimize impact on steelhead, it may be necessary in some years to delay cleaning so that alevins have time to move out of the gravel.

The turbidity in the river increases as the NFR increases (Fig. 4). This relationship allows NFR to be calculated from turbidity. It is very useful because NFR can not be determined in the field while turbidity can be easily and quickly measured.

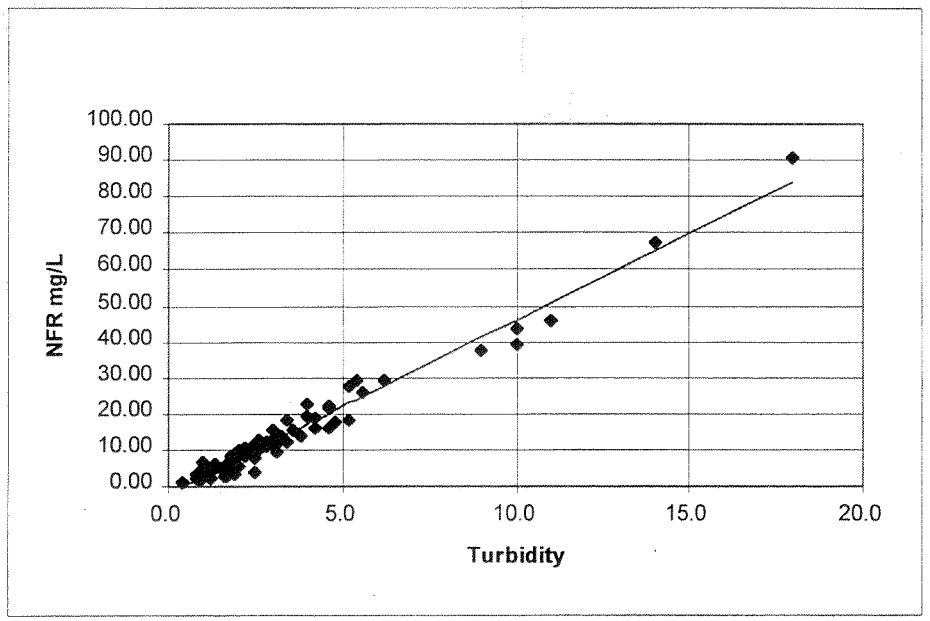

Fig. 4 NFR (mg/L) vs turbidity (NTU) for Little Qualicum River.

Recommendations

The present effluent treatment system is very effective. Last year over 90 million liters of concentrated effluent were treated at a 99% removal efficiency. This degree of effluent treatment substantially reduces the impact of gravel cleaning on the river. To achieve further reductions, the field must be modified so that a greater area is used for settling. This would allow more effluent to be pumped to the field while maintaining high removal efficiency. Improving the reliability of the pumping system would also reduce the effluent load to the river. Intakes must be protected so that they do not clog. Loss of a pump at a critical time results in an instantaneous surge of concentrated effluent to the river. Improved flow monitoring throughout the facility is also required so that staff can easily balance the operating flow with the pump rate thereby minimizing the discharge of effluent to the river.

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B.C. ENVIRONMENT

FISH STOCKING PROGRAM

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The B.C. Environment, Fish Culture Section stocking program serves B.C. by enhancing twelve hundred lakes and streams with twelve million freshwater fish every year. Fish stocking supports twenty-five percent of the four hundred and fifty million dollar freshwater fishing industry in British Columbia.

Seven species and over thirty stocks of trout, char, and Kokanee are reared at five major provincial hatcheries located in Duncan, Abbotsford, Summerland, Cache Creek, and Bull River (Cranbrook).

The stocking program is unique in North America as it utilizes wild and semi-wild brood stocks, rather than domestic strains, for ninety-five percent of its egg collections.

Fish Culturists employ several methods of brood capture, but the most common are the in-stream trap and net trap. Adult capture is also conducted by electro-shocking, angling, seining, and tooth entanglement nets.

Chemical free incubation, which is new to the provincial fish culture section, has led the way to upwelling incubators. This incubation method has proven successful in controlling fungus in most stocks.

Liberations from provincial trout hatcheries take place year round, but are most heavily concentrated in the spring and fall of each year. During these times, fish are transported province wide by means of trucks, helicopters, fixed-wing aircraft, and on occasion, boats. Constant oxygen monitoring and periodic visual observation ensures that live, healthy fish are released into B.C. waters.

A specially designed semi trailer, capable of hauling 1,300 kilograms (2,866 pounds) of fish in five tanks, is used for transporting live fish between major hatcheries, and to a staging area in Prince George, for redistribution. Transport times can exceed eighteen hours not including loading or unloading time.

In 1996, three Fish Culturists left the Fraser Valley Trout Hatchery driving two, five ton transport trucks. **Where to?!** To stock six thousand rainbow trout into six lakes in the Fort Nelson area. This is a 1,500 kilometer (930 mile) haul - one way!! It took four days and one hundred and forty seven working hours to complete this trip.

The fisheries program will continue to deliver stocking programs that provide diverse angling opportunities but that are consistent with wild fish conservation objectives. Future activities will include developing non-reproductive stocks to minimize potential impacts of stocking programs on wild fish and developing special stocks suited to specific habitats such as alkaline lakes, or lakes with populations of non-game fish.

SALMONID ENHANCEMENT PROGRAM

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Overview of the Program

SEP was launched as a federal-provincial initiative during 1977 in response to chronic declines in salmon stocks. It's major business is production of Pacific salmon, including steelhead and cutthroat, in cooperation with the Province. Production is intended to help rebuild specific stocks or to maintain or increase fisheries.

SEP operates 22 government hatcheries, 60 spawning channels and 46 fishways. In addition, SEP contracts 21 community groups to operate hatcheries, 13 of which are native communities (Community Economic Development Program), and provides technical guidance and some financial support for some 160 volunteer projects.

SEP staff design, implement and, where necessary, maintain numerous habitat improvement projects ranging from water storage and side channel development to bank stabilization and estuary improvement. This year SEP has over 50 new projects scheduled most of which are joint with the Province, B.C. Hydro, the Fraser and Skeena Green Plans or the Aboriginal Fisheries Strategy.

SEP also funds the Lake Enrichment Program to increase the survival and production of selected sockeye salmon stocks.

About 600 million juvenile salmon are released from SEP projects annually.

Catch of enhanced salmon is usually 4-5 million fish in B.C. recreational and commercial fisheries annually, accounting for 10-20% of the Canadian catch. Additional fish are harvested in U.S. waters and in Aboriginal fisheries, or escape to their spawning streams.

The Program developed and continues support of the Salmonids in the Classroom educational package which has been endorsed by the B.C. Ministry of Education. It is estimated that some 1 million children throughout B.C. and the Yukon Territory have studied this material and a large percentage have had hands-on experience through a related classroom incubator program.

A new Streamkeepers program, similar to the "Adopt-a-Stream" program in Washington State, has been initiated to facilitate public involvement in fish habitat protection and improvement.

ALASKA SALMON ENHANCEMENT - AN UPDATE FROM ANOTHER PLANET

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Introduction

Salmon enhancement in Alaska differs from other West Coast programs in some dramatic ways. During the last few years, wild and hatchery salmon runs have reached all-time records in Alaska at the same time some of the runs in the Pacific Northwest were at record lows. While hatchery programs in Alaska seek to benefit all user groups, commercial fisheries are targeted as the primary beneficiaries. Nearly all of Alaska's hatcheries are operated by private non-profit organizations rather than by government. Hatchery funding and species emphasis are very different in Alaska compared to other areas on the West Coast. Alaska's effort to create self-funded enhancement has created greater vulnerability to the economic impacts of changing salmon markets. In this paper we will briefly review the history and development of the Alaska enhancement program by examining three key elements: contribution to fisheries, financing and impacts to wild stocks. Some successes and problems will be highlighted and the current State review of the hatchery program will be explained.

The Alaska salmon enhancement program was developed in the 1970s when returns were at historic lows and fishermen were clamoring for something to be done to save the salmon industry. Alaska established a two prong approach to increase salmon production. In 1971, the Fisheries Rehabilitation and Enhancement Division was formed within the Department of Fish and Game to significantly expand the State hatchery system which, up until then, amounted to five hatcheries. In 1974, the Alaska legislature passed enabling legislation for development of private non-profit (PNP) hatcheries and funded development of the program through Alaska's fisheries enhancement revolving loan fund ("the loan fund"). Abundant oil revenues provided the resources to develop facilities at a rapid rate and by 1987 a total of 20 state hatcheries and 20 PNP hatcheries had been built. Beginning in 1988, with oil revenues falling, the State began to shift the operating expenses and responsibility for its production facilities to PNP hatchery programs. Transfers were accomplished via long term operational contracts where the PNP organizations agreed to take over operations and the State retained ownership of the facilities. In some cases, where no operators stepped forward to take on the financial burden of operations, hatcheries were simply closed. Since 1988, twelve State hatcheries were transferred and three were closed. The State continues to operate 4 hatcheries, while the PNP sector now operates 31 enhancement sites (CFMD 1996). The Alaska salmon enhancement program is approximately 25 years old and has already undergone fundamental change.

In several respects, Alaska's salmon industry couldn't be facing more different circumstances that it did when the enhancement program began. Alaska's salmon harvests have improved dramatically since the 1970s, due in part, but not primarily to hatchery production. In 1979, the statewide harvest of wild salmon amounted to 87.5 million and an additional 980,000 salmon from hatcheries were caught (CFMD 1996). By 1995, the statewide wild salmon harvest had grown to 183.4 million and the harvest of enhanced salmon was 25.0 million (Fig. 1). While Alaska's runs improved, world wide salmon production also increased. Japanese chum production increased from about 10 million fish in the 1970s to 56 million in 1994 (Fisheries Agency of Japan 1994; NPAFC 1996). Farmed salmon, in 1995, represented 41% of the world salmon market (Smoker et al, in review) even at these high levels of wild and ranched production. This huge increase in supply has placed downward pressure on prices in Alaska, not only affecting fishermen, but hatcheries as well.

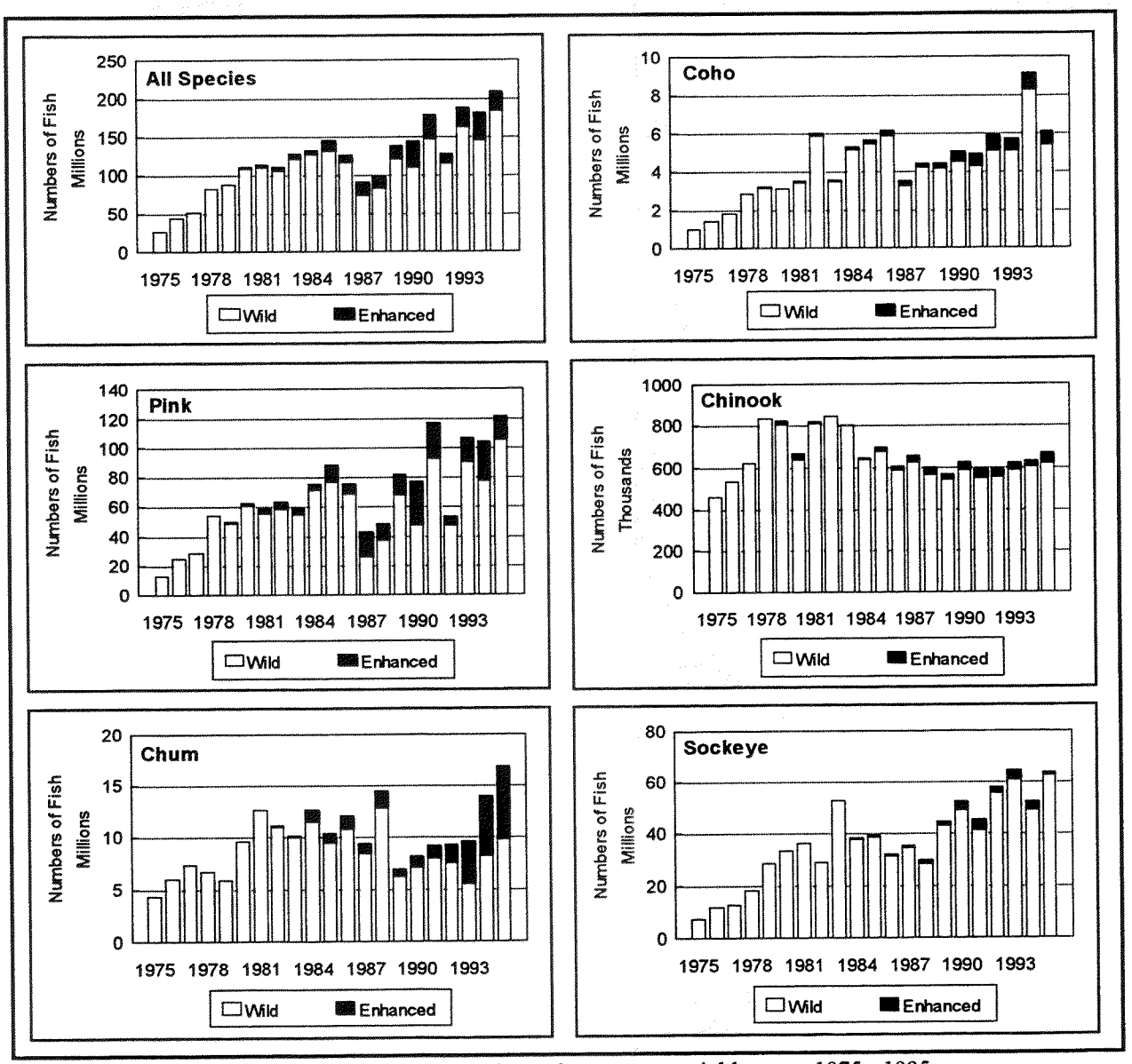

Figure 1. Wild and enhanced components of Alaskan salmon commercial harvest, 1975 - 1995.

Contribution to fisheries

One of the primary goals of the salmon enhancement program was to provide significant contributions to the common property fisheries: commercial, sport, and subsistence users. Policy makers clearly did not want hatcheries to operate merely to sustain themselves. While all five species of Pacific salmon are included in the Alaskan program, pink and chum salmon are by far most numerous. The coastal locations of most hatcheries allows short term rearing to be done in saltwater net pens, so large numbers of pinks and chums can be cultured at low cost using relatively little freshwater and limited shore-based facilities. Overall egg takes have steadily climbed since inception of the enhancement program while adult production has trended higher with greater variation (Fig. 2). Pink fry production has been relatively stable since 1990, ranging from 787 to 920 million per year and annual returns have fluctuated between 14.8 and 41.2 million adults since then (CFMD 1996). Most pink production is located in the Prince William Sound region. Chum production has steadily increased to 473 million fry in 1995 and 9.8 million adults returned in that year. Most chum production is in the Southeast Alaska region. Coho smolt production was 17.4 million in 1995 and 1.3 million adults returned to enhancement projects in that same year.

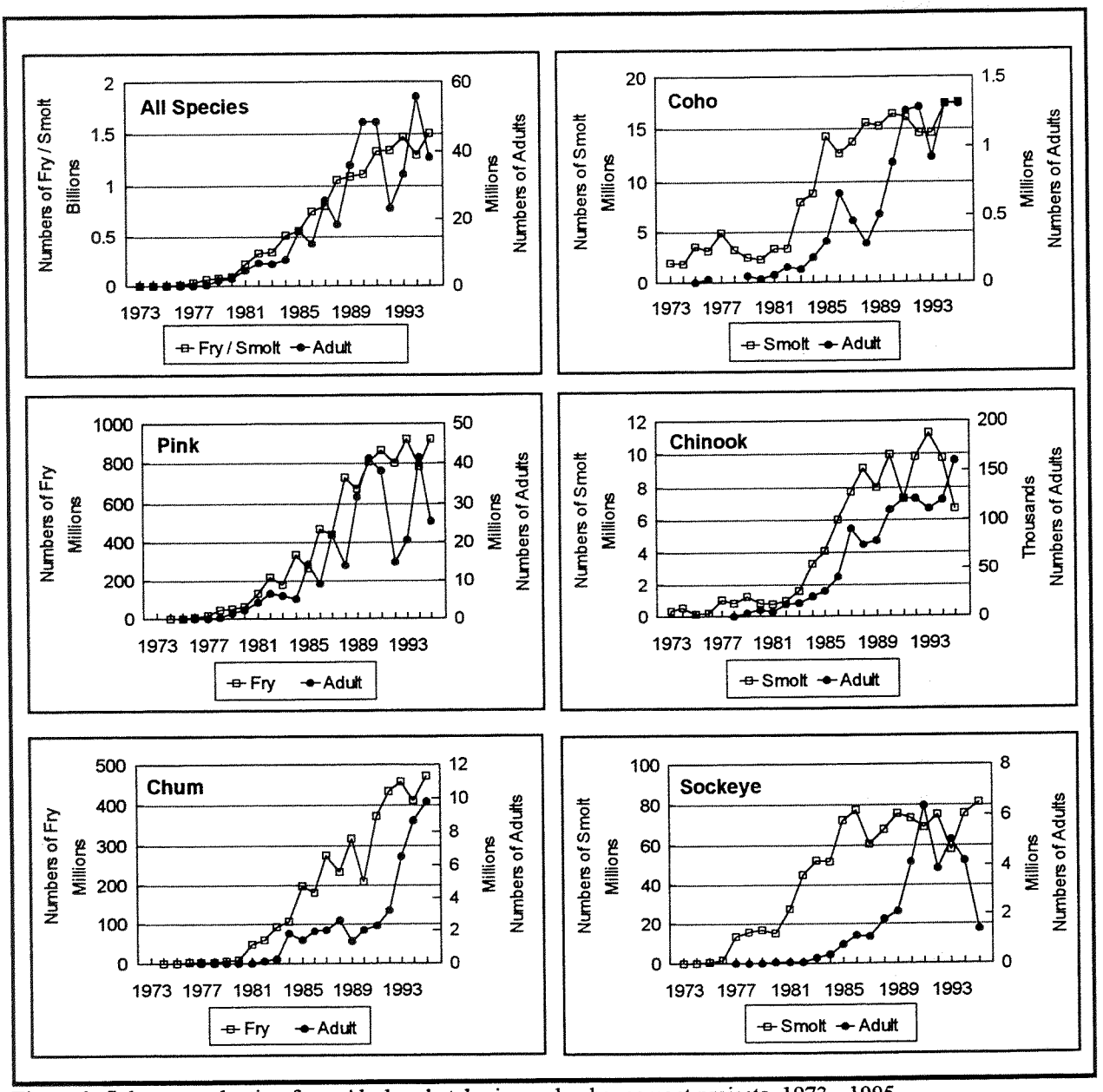

Figure 2. Salmon production from Alaskan hatcheries and enhancement projects, 1973 - 1995.

Overall chinook production has declined somewhat from highs that occurred in the late 1980s. In 1995, 6.7 million chinook smolts were released and 160,000 adults returned. Sockeye fry production has trended higher, reaching 81.3 million in 1995, but adult returns have declined. In 1995, 1.4 million adults returned, well down from the peak of 6.4 million in 1991. Clearly, the Alaska salmon enhancement program has been successful in producing fish that have contributed substantially to the state's fisheries (Fig. 1).

By producing large numbers of salmon for harvest, the program has generally yielded outstanding returns on the enhancement tax dollars that have been invested in enhancement projects. Through 1995, fishermen provided $15 million in enhancement taxes to Northern Southeast Regional Aquaculture Association (NSRAA) whose enhancement programs generated $47 million of salmon caught in commercial fisheries (Fig. 3). Fishermen in this region have benefited by catching salmon from this program that were worth an average of $11.9 million for the past 3 years, despite low prices. Enhancement has been successful in extending fishing periods, decreasing competition in fishing areas and providing economic benefit for the State (Bachen and Linley 1995).

Financing

The unique structure of Alaska's enhancement program is founded on the expectation that hatcheries would become self-funded. The State hatchery loan program was established to finance construction and initial startup costs, but hatchery programs were expected to generate their own revenues in a reasonable length of time to pay for ongoing operations and repay state loans. There are two types of PNP organizations, regional associations and independents, distinguishable in terms of funding by the provision that only regional associations receive an enhancement tax collected by the State from fishermen of a participating region. Nearly all PNP organizations raise additional funds through the sale of a portion of the returning fish they produce. Most regional associations limit their take to 30-40% of the return to ensure that common property fisheries will receive substantial benefit from the program. Both the enhancement tax collections and direct sales of returning fish are influenced negatively as general market prices fall.

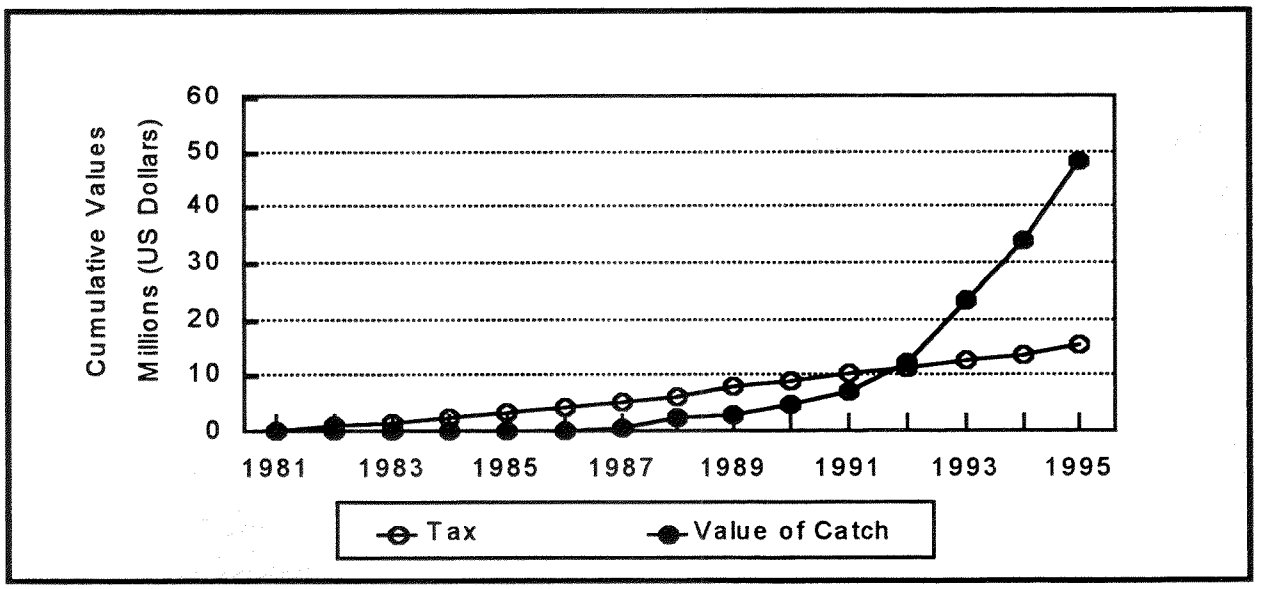

Figure 3. Cumulative values of NSRAA production and enhancement taxes, 1981 - 1995.

The loan fund has provided substantial support for Alaska's hatchery program. Between 1976 and 1995, the State loaned $101 million to PNP operators for capital and operational expenses (DCED 1995). Borrowing by hatchery operators peaked in FY 1985 and after falling for a time has been increasing for the past five years. In 1995, $8.8 million was loaned to PNP operators (Fig. 4). Of the total borrowed, the balance still owed to the State in 1995 collectively by all PNP organizations is $91.8 million.

While some hatcheries have achieved financial independence, others have not. Some organizations are able to generate enough revenue to fund ongoing operations and capital improvements as well as pay back state loans. Those that have had problems have been challenged by poor siting, terminal harvest limitations due to wild stock concerns, poor fish culture practices and low returns. However, the biggest challenge is the fact that the market price assumptions that went into development of business plans for Alaska's PNP hatcheries have fallen dramatically over time. Thus, actual income from fish sales is far less than projected in many cases and has been inadequate to meet financial obligations.

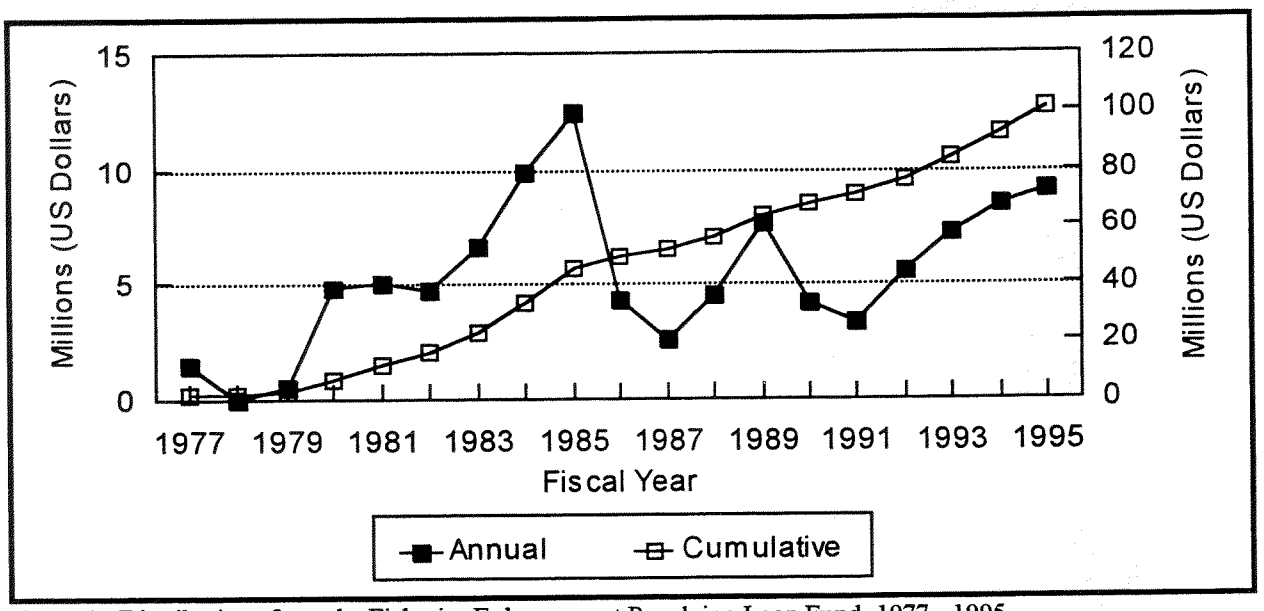

Figure 4. Distributions from the Fisheries Enhancement Revolving Loan Fund, 1977 - 1995.

In response to the financial challenges brought on by low prices, some hatcheries have tried alternative processing and marketing strategies to try to generate more revenue from their returns than could otherwise be generated from directly selling fish to processors. Some hatcheries have tried to derive greater value from the fish by controlling the processing and marketing of their fish themselves or by selling roe instead of whole fish. For a time, hatcheries were increasing production to try to generate the necessary revenue by selling more fish. These efforts have met with limited success so far and some have created negative reactions from the processing industry and media.

As some PNP organizations have struggled to become self-sufficient they have had to borrow more money and refinance their loans. Few PNP hatcheries have actually closed for lack of funds, but some are facing such high levels of debt that loans will not be fully repaid unless market conditions change dramatically.

The transfer of State hatcheries to PNP organizations has helped to provide financial stability in some cases and had the opposite impact in others. Over a brief period, the State transferred the responsibility for funding $5.46 million of annual operational expense from its general fund to PNP operators (personal communication, Betty Abbel, Administrative Officer, Commercial Fisheries Management and Development Division, Alaska Department of Fish and Game, Juneau, Alaska). In many cases, fishermen-led PNP organizations felt that they had little choice but to take on the additional financial obligation because of the adverse impacts to fisheries if the State hatcheries were closed. In the case of Hidden Falls Hatchery, an established chum return provided an immediate source of revenue that was adequate to fund operations. In contrast, the transfer of the Gulkana sockeye egg incubation box project brought no funding mechanism and these costs had to be born by other projects under the management of Prince William Sound Aquaculture Association. In some cases overall revenue has not been adequate to absorb the increased liability of funding state hatcheries and has resulted in additional borrowing by PNP operators.

Even the hatcheries that are struggling to find ways to be financially self-sufficient are providing significant public benefit through the salmon they produce for common property fisheries. Had salmon prices held at levels within historic ranges, fewer hatcheries would be having difficulty in becoming self-supporting.

Impacts to Wild Stocks

While there is concern over hatchery/ wild stock interactions in Alaska, the level of concern is generally lower than what it is in the Pacific Northwest. There may be several reasons for this difference. Perhaps most important is the opportunity in parts of Alaska to site hatchery development in coastal areas where terminal harvest can be directed

on hatchery returns that are spatially or temporally separated from wild stocks. Other factors include conservative genetic and pathology policies to guide enhancement activities.

By state policy, maintaining the health of Alaska's wild stocks is the foremost concern of fisheries managers. Since not all regions of the State have the same opportunities for isolating hatchery from wild stocks, there are varying levels of concern over hatchery impacts. The greatest level of concern over hatchery/wild stock interactions has developed in areas where intensive net fisheries operate on a mixture of wild and hatchery stocks as they migrate home. In some areas of Prince William Sound, mixtures of hatchery and wild stocks pass through sequential gauntlet fisheries before reaching their spawning streams (Heard, 1996). This has led to concern that wild stocks are being overharvested. In response, greater emphasis on stock identification through micro-wire tagging and otolith marking has provided managers with better tools to identify hatchery returns.

Chums returning to an enhancement project in Sitka Sound, southeast Alaska are aggressively harvested because they are returning to a bay that has no sustainable salmon production. The steep topography of the area provides essentially no freshwater habitat for salmon. Locating enhancement projects in areas like this has proved to be a good way of creating isolated harvest areas where hatchery returns can be taken without adverse consequences to wild stocks.

State Review of Hatchery Policy

In October, 1996, the Hatchery Policy Group met to hear testimony on two aspects of hatchery operations: production levels and financing. This group is charged with making recommendations regarding State Policy to the Salmon Industry Task Force appointed by the Governor. The Governor seemed to be responding to concerns from certain groups that hatcheries are ruining markets by producing too many fish and that some hatcheries are unfairly competing with processors.

The contribution of enhanced fish to fisheries has not been uniform throughout the State and disparities have recently led to conflict between fishermen in regions that benefit from hatcheries and other areas where they do not. Hatchery-produced chum salmon are heavy contributors to the southeast Alaska fisheries, comprising 67.2 % (6.2 million fish) of the commercial catch of chum in 1995 (CFMD 1996). In contrast, the enhanced percentage of the commercial chum catch in the Arctic-Yukon-Kuskokwim area was only 3.1% (57,000 fish) in the same year. Different regions of the State have vastly different enhancement programs ranging from virtually non-existent to providing most of the catch in some fisheries. Planning and implementing enhancement activity has been done on a regional basis and different areas have had different opportunities and interests in enhancement. Trying to assess and address the impacts of enhancement in one area on another will be very difficult.

The charges of unfair competition are coming from seafood processors that dislike seeing hatcheries sell fish or roe in direct competition with themselves. These hatcheries that are doing this believe that they face a tough choice: accept the prices that they would receive from the processors and not generate enough revenue to meet financial obligations or take on the additional risks of marketing and hope for a better price. Direct marketing and processing by hatcheries has evoked a strong response by processors who feel that non-profit hatcheries that receive state subsidized loans should not be on the same playing field with them trying to sell salmon or roe.

Summary

Hatcheries in Alaska face some very different challenges and circumstances than those to the south. Favorable ocean conditions have driven marine survival rates to historic highs, helping to create a glut of fish on the market. In some situations in 1996, a portion of hatchery and wild returns went unharvested due lack of market demand. Two species, pink and chum, were selling ex-vessel for less than $.10/lb this past year. Alaska no longer dominates the salmon markets and salmon prices are determined by world supply and demand. High abundance and low prices is better than low abundance and low price. In periods of abundance, it is easy to see why the need for hatcheries would be questioned. However, participants from the processing and harvesting sectors of the industry in Alaska voiced strong support for continuing hatchery production at the recent Hatchery Forum. They recognized that marine survivals of salmon in Alaska will probably decline at some point and that hatchery production will be

needed to meet market demand. This raises the difficult problem of financing hatchery production and Alaska policy makers face a choice: fund current hatchery production by providing subsidies to assist hatcheries that haven't become financially self-sufficient or allow market forces to determine which hatchery programs will survive.

Acknowledgments

We wish to thank Steve Reifenstuhl and Janette Byrd for their help in preparing this paper.

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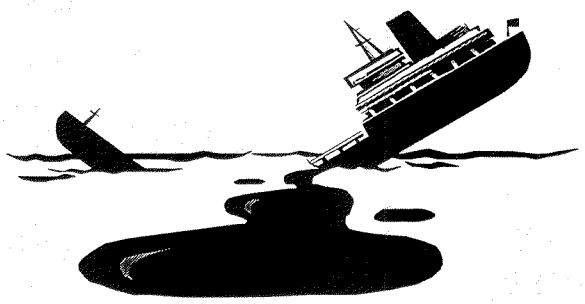
Fish Culture/Salmon Enhancement And The *Exxon Valdez* Oil Spill



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On March 24, 1989 the *T/V Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, Alaska, spilling eleven million gallons of North Slope Crude oil, making it the largest tanker spill in United States history. Currents moved the oil along the coastline of Alaska, contaminating portions of the shoreline of Prince William Sound, the Kenai Peninsula, lower Cook Inlet, the Kodiak Archipelago, and the Alaska Peninsula. Oil eventually reached shorelines nearly 600 miles southwest from Bligh Reef.

During the spring and summer of 1989, efforts focused on containing and cleaning up the spill and rescuing oiled wildlife. Booms were placed around salmon hatcheries in Prince William Sound, Cook Inlet and Kodiak to keep oil from reaching the net pens. The "Mosquito Fleet," a fleet of private fishing vessels, played an important role in protecting these hatcheries, assisting the oil skimmers, and capturing oiled wildlife and transporting them to rehabilitation centers.

In 1991, the U.S. District Court approved the settlements of joint state and federal lawsuits against Exxon concerning the 1989 *Exxon Valdez* oil spill. The terms of the civil settlement required Exxon Corporation to pay the United States and the State of Alaska $900 million over a ten year period to restore the resources injured by the spill. The criminal settlement added another $100 million, in a one time deposit, to the state and federal coffers ($50 million to each).

Under the court approved terms of the civil settlement, a Trustee Council of three state and three federal members was appointed to administer the monies received from the settlement and to restore the resources injured by the spill. State agencies represented on the council are the Alaska Department of Fish and Game, the Department of Law and the Department of Environmental Conservation. The three federal agency members are from the National Oceanic and Atmospheric Administration, the United States Forest Service and the Department

of the Interior. The two governments will undertake restoration efforts together and they "shall jointly use such monies for purposes of restoring, replacing, enhancing, rehabilitating or acquiring the equivalent of natural resources injured as a result of the oil spill and the reduced or lost services provided by such resources."

The Trustee Council's mission has three main elements. The first includes restoration, replacement and enhancement of affected resources, acquisition of equivalent resources and services and long term environmental monitoring and research programs.

The second element is that a unanimous decision from the six trustees is required before a project or other expenditure of money is approved.

The final element is all funds must be spent on projects within Alaska, unless the trustees unanimously agree that a specific task cannot be performed within the state.

The $900 million civil settlement has been allocated to several different areas. $214 million was spent on damage assessment during the first three years following the spill. An additional $180 million is in the process of being spent on research, monitoring and restoration of the injured resources and services. Using $375 million to purchase and protect forests and land for habitat protection will consume more than a third of the civil settlement. The administration, science management and public information process will cost $35 million and a total of $108 million is being set aside in a special restoration reserve for work beyond the 10 year payment cycle from the Exxon Corporation. The $180 million projected to be spent on research, monitoring and restoration includes approximately $11 million or 6% for salmon enhancement and fish culture projects.

Chenega Chinook Salmon Release

The Chenega Chinook Salmon *Oncorhynchus tshawytscha* release was proposed and implemented as a subsistence project. The recovery objective for subsistence is defined as *a return to healthy and productive resources at pre-spill levels, and when people are confident that those resources are safe to eat.* Animals such as birds, pink salmon *O. gorbuscha,* clams and seals are the traditional subsistence resources, but some have not fully recovered from the oil spill so the chinook salmon will provide a replacement until the more traditional resources recover.

To implement this project, 50,000 20-gram chinook smolts are produced at Prince William Sound Aquaculture Association's (PWSAC) Wally Noerenberg Hatchery and transported to Chenega net pens (Ferren et al, 1996). The smolts are reared for about two weeks to insure imprinting and then released. This smolt release is projected to produce nearly 1,000 adult fish to be utilized as a replacement resource for the subsistence users.

Experimental Fry Release

Pink salmon hatcheries, operated by the Prince William Sound Aquaculture Corporation, annually release hundreds of millions of fry into Prince William Sound. The fact that release timing, location, size and number released per day can be controlled makes the hatchery pink salmon attractive as an experimental tool.

In 1994, a total of 15 million experimental fry were reared for an extended period (80 days), at the Wally Noerenberg and Armin F. Koernig hatcheries, to 1.5 grams for release in mid-June (Ferren et al, 1996). Two hundred and forty million fry were short term reared (45 days) and released by mid-May at 0.3 grams. The

normal hatchery practice is to release the fry during the peak of the plankton bloom in mid May so they can take advantage of this food source. Fry released a month later do not have this abundant food source available to them.

The marine survivals of the experimental fry and production fry were determined with the use of coded wire-tags. The adults were recovered in the brood stocks and common property fisheries the following year and the marine survivals of the two treatment groups were compared.

The production releases had a marine survival of 0.37% while the experimental release had a survival of 15% (Willette et al, 1996). Preliminary data in 1996 indicates similar marine survivals for the two different releases. Although the larger fry are released after the peak of the plankton bloom they still have a higher marine survival. One theory to explain the increased survival is the ability of the larger fry to avoid predators. Another is the abundance of predators has decreased by mid June. Expanding this scenario may allow the hatcheries to rear and release fewer fish while maintaining the same number of returning adults.

Tatitlek Coho Salmon Release

Subsistence resources available to residents of Tatitlek village were severely disrupted by the oil spill. This project was initiated to enhance subsistence resources near Tatitlek by creating a coho salmon *O. kisutch* return to the village. The 2,000 returning salmon will lessen the impacts of continued harvests on other subsistence resources injured by the spill, such as harbor seals.

The coho salmon return was created through an annual release of 20,000 coho smolts near the village. The smolts are produced at the Solomon Gulch Salmon Hatchery under an agreement between its operator, the Valdez Fisheries Development Corporation, and the Tatitlek Council. The sea ready smolts are transported from the hatchery to Tatitlek by boat, placed in net pens for about two weeks, and then released into the wild. Coho are currently returning to Tatitlek and are being utilized by the subsistence fishermen.

Port Graham Pink Salmon

The oil spill clean-up effort had a negative impact on the Port Graham pink salmon as it did on the local coho and sockeye *O. nerka* runs. Boom deployment during the early phases of the clean-up trapped a large number of outmigrating pink salmon fry behind the boom curtain causing high levels of mortality on the ebbing tides.

The Port Graham Pink Salmon project will supply pink salmon for subsistence use during the pink broodstock development phase of the hatchery. The more traditional subsistence resource, coho and sockeye salmon, are at low levels so pink salmon are heavily relied upon by the subsistence users. This project will help ensure that pink salmon remain available for subsistence use until the more traditional species are restored.

The principal mission for the Port Graham hatchery is to enhance the fry to adult survival of the pink salmon to accelerate the broodstock development phase. This increased survival will allow a portion of the return to be used for subsistence and also build the pink run back up to levels that will allow commercial exploitation. To accomplish this goal in the shortest time available, the hatchery is releasing long term reared fry (longer than 45 days). Studies undertaken at the hatchery in 1993 and 1994 indicate that pink salmon released in October at 8 grams had a marine survival approaching 7.0%. The fry reared to 0.4 grams and released at the peak of the zooplankton bloom survived at less than 0.5%. Long term rearing of the fry does increase the risk of diseases such as vibrio so the size goal may be adjusted downward to minimize potential losses. Based on studies done in

Prince William Sound, this should still allow significant increases in survival to permit the project to meet its goal.

English Bay Sockeye

Sockeye have historically been an important subsistence and commercial species to the local people of Nanwalek and Port Graham. Boom deployment during the oil spill also trapped and killed large numbers of sockeye smolts in the ebbing tides exacerbating declining returns and limiting fishing opportunities.

The English Bay Sockeye project was designed to increase the number of returning adults to the local area. The program was started in 1989 as a spring fry release project but changed in 1991 to a fall presmolt project. Fry are reared in the lake in net pens and released in late fall as presmolt, migrating to the sea in the spring. This is a very successful program with marine survivals estimated to be as high as 30-40%. Returns of 25-30 thousand adults in the past two years has allowed the commercial fishery to reopen and the subsistence fishery to flourish.

Port Dick Spawning Channel

Lower Cook Inlet has a significant number of estuarine and intertidal nursery areas important to pink and chum *O. keta* salmon production. Port Dick was chosen for rehabilitation because it is considered one of the most important pink and chum salmon production streams in the area and it was moderately to heavily oiled by the oil spill.

Port Dick Creek was spawning limited due to several factors including wide fluctuations in water levels, extreme flooding effects, inadequate water flow and freeze out conditions. Adults would spawn but their eggs and progeny would not survive. This project has improved the spawning habitat by rehabilitating formerly used tributaries which will provide a much more consistent and stable habitat than what was previously there.

The channel was completed in the summer of 1996, and in the fall, populations of pink and chum salmon were utilizing the new spawning habitat. The new channel should supply an additional 22,000 pink and 8,000 chum salmon to the fishery.

Little Waterfall Creek Fish Pass

Several beaches on Afognak Island, including Little Waterfall Bay, were heavily oiled in 1989. This project increased the spawning and rearing habitat for coho and pink salmon by modifying an existing fish pass (Honnold, 1996). Three barriers in Little Waterfall Creek were bypassed in 1981 with fish passes to allow increased pink and coho salmon passage to previously unused spawning and rearing habitat. One of the sections, however, has not operated efficiently and salmon have not been able to fully utilize the largest portion of the habitat. This project modified this section of fish pass by decreasing its grade from 27% to 17%. Modification has opened up habitat that will produce an additional 24,000 and 2,700 pink and coho salmon, respectively. Although the pink return in 1996 was weak (preliminary estimate - 9,000) approximately 37% used the modified ladder compared to previous years usage of 19%.

Fort Richardson Hatchery Water Supply Improvements

The Fort Richardson hatchery was extensively renovated in 1982 but adequate clean water has always been in short supply. The facility has raceway capacity to produce 250 thousand pounds of chinook, coho and rainbows,

but poor water supply limits the production to approximately 125 thousand pounds annually. This project will fund a water treatment system which will bring the hatchery up to the original design capacity. Three to six thousand gallons of water a minute will be drawn from Ship Creek, filtered and ozonated. Additional water, up to two thousand gallons a minute, will also be available from the Army power plant and this water will require dechlorination. The increased fish production made possible by this project will primarily be utilized by the sport fisherman in the south central and interior regions of the state.

Coghill Lake Fertilization

The Coghill Lake fertilization project (Kyle, et al 1996) was initiated to restore the sockeye run to historical levels by using established and proven lake fertilization technology. This would provide an important replacement resource for sport and commercial fisheries in Prince William Sound. Coghill Lake has historically been the major producer of sockeye salmon in the sound and there is no evidence that the productivity of the lake was impacted by the oil spill. Historical returns average 177,000 adults but in recent years the returns have dipped to an average of 33,000 adults. Reasons for this decline are not known but some theories include climatic effects on freshwater and marine survival (e.g. temperature, lake turbidity), smolt migration through oil contaminated water of the sound and overharvest of returning adults. The most plausible explanation for this decline, however, is that high escapements into the lake in the early 1980's essentially cropped off the zooplankton levels leading to poor freshwater survival and subsequent decline in the sockeye stock.

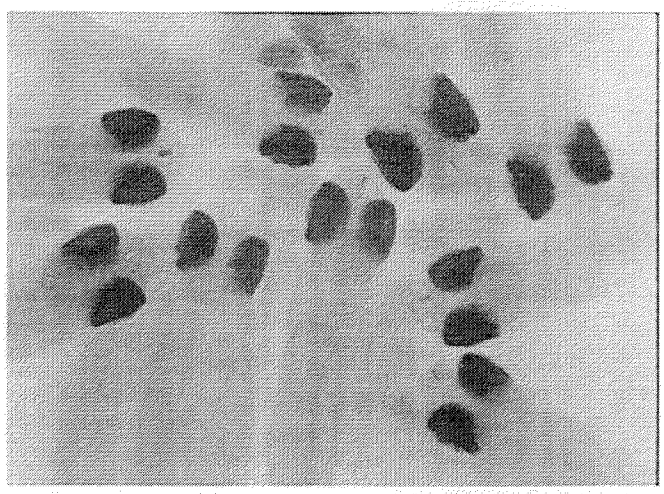
Liquid fertilizer was added to the lake via a fixed wing aircraft over six week summer periods of 1993 through 1995. Comprehensive limnological surveys were conducted following the application through late fall. Phytoplankton and zooplankton were sampled for species composition and biomass and sockeye fry were sampled for abundance, distribution and stomach contents. Sockeye smolt were sampled, using an inclined plane trap, for size and age information and returning adults were counted through a weir.

The effects of the fertilization were positive with a mean increase of phosphorus of 22% and mean algal biomass (chlorophyll a) increase of 250%. This increase in zooplanktors was utilized by rearing sockeye fry and contributed to increased smolt production. The average of the pre-fertilization smolt production was 275,000 and the average smolt production after fertilization was 1,400,000.

Restoration of Coghill Lake will be achieved when the densities of the in-lake juveniles are balanced with the available forage base. Revised management strategies were recently implemented to allow adequate escapement into the lake and this will accomplish several things. Sufficient numbers of spawning adults will produce the optimum number of fry which the lake can support and the decomposition of the carcasses will enrich the lake and keep the cycle going.

Coded Wire Tagging

The late 1980's heralded in the cultural success of the pink salmon hatcheries in Prince William Sound. Fry releases of up to half a billion produced huge harvests (17 million) dwarfing the 3.4 million average harvest when hatcheries were absent from the sound (Riffe et al, 1996). The hatchery returns were welcomed by the fisherman of the sound and the aquaculture associations, but unfortunately they complicated management of the smaller and much more numerous wild stocks. The oil spill further confounded the issue as many of the wild stocks were damaged by contamination of the intertidal portions of the spawning streams. To protect the wild stocks, fisheries managers needed a way to differentiate them from the hatchery stocks and micro coded wire tagging was the most practical and reliable way to mark large numbers of small pink salmon fry.

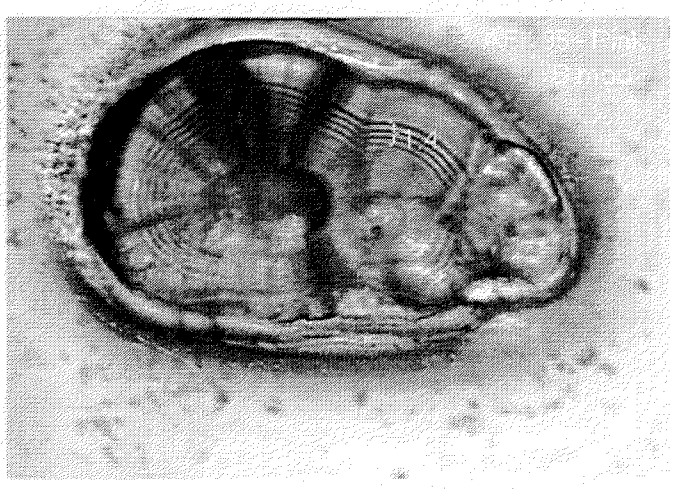


A coded wire tag program was initiated in 1986 and was expanded after the 1989 oil spill. As coded wire tagging is expensive and labor intensive an average of only 1 in 500 fry were tagged. In 1995, approximately 23% of the return was examined and the data indicated that of the 17 million pink salmon caught, all but four million were from the hatcheries. This data provided the fishery managers with accurate real time and post season estimates of hatchery and wild contributions to commercial harvests. Combined with Fish and Game aerial surveys the managers were able to reduce exploitation on wild stocks and target the fishing effort on the hatchery stocks.

Otolith Mass Marking

Although coded wire tagging provides the fisheries managers with a useful tool to differentiate between hatchery and wild stocks, there is a degree of concern that the precision is not great enough. There is also evidence that coded wire tagging may cause some straying (Habicht et al, 1996). To increase the management precision and alleviate the straying concerns the Trustee Council funded the otolith mass marking project to effectively mark all hatchery produced pink salmon in Prince William Sound.

Otoliths are small bones in the inner ear of fish which can be marked through systematic changes in water temperature during egg incubation. The temperature change causes bands of light and dark material, similar to the bands in a tree, to be laid down in the otolith. Controlling the duration of the heating period as well as the number of cycles permits distinguishing marks to be applied to the fry from each facility as well as different lots within the same hatchery.



Boilers were installed at the four Prince William Sound pink salmon hatcheries to heat the water and in the fall of 1995, approximately 685 million pink salmon eyed embryos had marks applied with a sustained 4°C change in water temperature for a 24 hour period (Joyce et al, 1996).

The development of otolith mass marking will be used as an in-season stock separation tool for pink salmon in the sound. In addition, there will be a two year over lap between the otolith marking and coded wire tagging. This will allow the managers to compare the two methods and draw conclusions as to the pros and cons of each method. In 1997 the fisheries managers will use these marks to achieve exploitation rates appropriate for less productive wild populations while still harvesting the hatchery stocks.

Acknowledgments

Additional information on all of the projects mentioned in this paper is available from the Oil Spill Public Information Center at 645 G St., Suite 401, Anchorage AK 99501, 1-800-283-7745. Current information is also available on the Oil Spill Public Information Center's home page on the Internet. Their address is www.alaska.net./~ospic/index.html

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SPRING CHINOOK SALMON CAPTIVE BROOD REARING

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The continued decline of wild spring chinook salmon in Idaho spawning grounds prompted the initiation of a captive brood program as a short term protection action. This program was designed so that only first generation progeny would spend a portion of their lifetime in a hatchery. Fall migrants of wild chinook salmon were captured as parr from the Selway river, in an area called the McGruder corridor. Three consecutive brood years were captured, Brood Year 1991, 1992 and 1993.

The first obstacles to overcome in this program were in design and construction of a captive brood facility within the parameters of our existing production operation. The initial concept was to place the captive brood rearing program in a specially modified chinook raceway. Plumbing would supply water from two sources and carry it to four - six foot diameter circular tanks and two - 6' x 33' rectangular tanks.

One of the anticipated problems with this new project was the uncertainty whether the wild fish would convert to a pellet diet. For the first brood year we went to extreme measures in making up a diet of live blood worms, premium shrimp cat food, anchovy paste and freeze dried krill. Then over the course of two weeks a pellet diet was added to the mixture. After the third week we had successfully transferred these fish from the initial mixture to 100% pellet diet. The subsequent Brood Years 1992 and 1993 were transferred over to a pellet diet in about a week, using only a mixture of freeze dried krill and a pellet diet. The pellet diet we used was the Biodiet closed formula feed.

Brood Year 1991 were reared part of their life in circular tanks. Then due to budget and constructions constraints, they were reared for a portion of their lifetime in a steelhead raceway. While in the raceway they experienced water temperatures in the mid to high sixties. They were also displayed very skittish behavior and shied away from any type of movement or activity that occurred during normal rearing practices of the steelhead in the adjacent raceways. The 6' x 33' rectangular trough was eventually purchased and installed in the chinook raceway. The Brood Year 1991 fish were then moved into this rearing tank.

The Brood Year 1991 fish, being reared in the rectangular tanks, seemed to be continually uneasy and easily frightened. These tanks were fit with additional shading about eight inches above the water in an effort to provide more cover and security for these fish.

Rearing and egg taking results of Brood Year 1991 fish were a bit discouraging. The fish were much smaller than anticipated and the egg quality was very poor. Eye-up ranged from 0% to 86.5%. Seven females were spawned. These females yielded 6,563 green eggs, and after shocking and egg picking 2,600 eyed-eggs remained for an overall eye-up percentage of 39.6. Two of these females had zero percent eye-up (no fertilization). Approximately 1,400 fish are currently on hand.

After the first spawning season we reviewed the condition of the fish to decide if we were going to continue this program. It was decided something needed to be done to give these fish more of a 'natural' rearing condition. We decided to deepen the water, add more shading and perhaps a circular tank with continual directional flowing water. At this time we purchased a five foot deep, ten foot wide portable swimming pool and installed it in the existing adult holding pond.

The Brood Year 1992 fish were the first to be placed into the swimming pool. Within a few weeks a noticeable calmness of behavior could be observed. A small number of the Brood Year 1992 fish and the remaining 1991 fish were left in one of the 33 foot rectangular tanks as a comparison to the swimming pool.

Another item of discussion for improved fish quality and egg quality was their diet. We continued to feed the special brood diet manufactured by Bioproducts, which had the fish meal replaced with Krill meal. This diet also had an added level of selenium. This is the same diet which was formulated for Idaho's captive brood sockeye project. To simulate ocean conditions we debated the option of feeding them live disease free rainbow trout. Some concern was that the chinook would only want to feed on the live rainbow trout and would not continue feeding on the specially modified brood diet. The overall consideration for the need to improve the fish quality and egg quality seemed to warrant the risk to try this option. The initial feeding frequency was once per week of 150 rainbow trout fingerlings fed to 69 captive brood chinook. A training period was needed to have these fish successfully feed on the rainbow trout. At first we needed to stun them because the chinook would chase the rainbow but not eat them. Eventually they became very proficient at feeding on fresh fish. Now when the rainbow trout are poured into the swimming pool it gives the appearance feeding an alligator pit. The rainbow trout were also fed the special brood diet. We felt that as the feed was assimilated through their digestive system it may add enhanced nutrients to the chinook's diet.

The Brood Year 1992 chinook were to have mature four-year-old spawning females in the summer of 1996. During March, 1996 the 150 fish per week ration was increased to twice per week in an effort to help add to the egg quality. During late June, 1996 a noticeable reduction of the number of fish being eaten could be observed. We felt this coincided with the maturing of a portion of these fish.

A comparison of Brood Year 1991 to Brood Year 1992 spawned females shows a very improved overall condition of the 1992 fish and egg quality. The eggs from the twelve Brood Year 1992 females were much larger and had a healthier appearance. At this time final egg numbers are not available. Egg survival to eye-up stage is expected to be similar to our other brood stock at about 80% survival.

At first there was a concern if chinook reared to adults in these conditions would spawn at the correct time. We found these fish did spawn at the correct time, in fact spawning coincided with chinook spawning at our three satellite facilities.

Acknowledgments

This project has been one of the greatest team efforts that I have been a part of. The success of this project came from the sheer determination of the entire hatchery staff that this project would not fail. I would like to thank the Fish Culture staff for their progressive fish culture ideas and implementation of these. I would like to thank the maintenance staff for their design and engineering to make this project fit within the parameters of a production hatchery. I would like to thank the secretary for assisting in data accumulation, purchasing of equipment and supplies and clerical support in the writing reports and letters related to this project. Then I would like to thank the Regional Fish and Game Staff and Nez Perce Indian Tribe for their work and effort to collect the out-migrating par in the fall and holding them safely for transport back to Clearwater Hatchery.

UPPER GREEN RIVER STEELHEAD RESTORATION
WILD ABOUT HATCHERIES

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Background

The Green River flows west out of the Cascade Mountains entering Puget Sound at Seattle.This river provides an important regional fish resource supporting commercial and recreational fisheries. Anadromous fish were free to navigate the 90 miles of mainstem river until 1911 when the City of Tacoma constructed a diversion dam at river mile 61. In 1962 the US Army Corps of Engineers completed Howard Hanson Dam at river mile 64.5. In 1983 native wild winter steelhead trout (*Oncorhynchus mykiss*) were re-united with their native habitat above both dams.This stock of steelhead is considered healthy in the lower river below the dams. (see Figure 1. and Table 1.).

Green River Wild Winter Steelhead (Oncorhynchus mykiss) Runsize Summary - By Year

Return Year	Green R. Return	Total Harvest	Total Escape
1984	2,589	426	2,163
1985	3,082	796	2,286
1986	3,500	722	2,778
1987	2,871	1,186	1,685
1988	3,321	943	2,378
1989	3,006	1,090	1,916
1990	2,822	1,338	1,484
1991	1,369	425	944
1992	2,282	340	1,942
1993	2,015	293	1,722
1994	2.094	273	2,367
1995	2,525	225	2,300

Table 1.

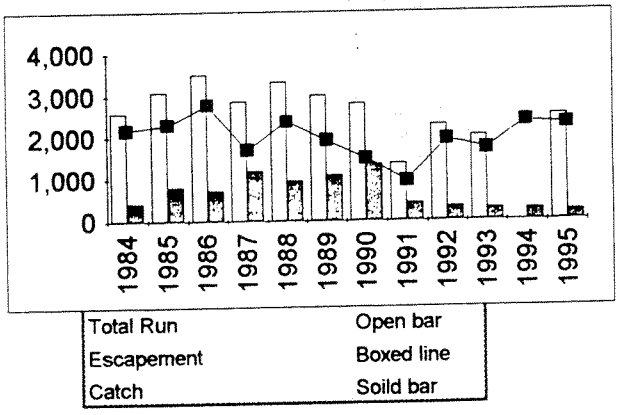

Figure 1.

Although re-introduction of fish above the dams started thirteen years ago, much of the available habitat in that area remains underutilized.. Juvenile passage at Howard Hanson is considered marginal. The dam is used for flood control and storage of water in the spring to meter out to the lower river in late summer. The filling of the reservoir in spring coincides with the timing of fish trying to outmigrate creating delays and possibly residualization. Can the upper river produce enough fish to compensate for juvenile survival problems and still support a self-sustaining population? Natural production of steelhead in the upper Green River is estimated using habitat based methodology. An escapement goal of 582 adults is based on a production potential of 10,499 smolts. Tom Cropp of Washington Department of Fish and Wildlife calculated these numbers based on his estimate of 100 miles of main-

stem and large tributaries and 0.0168 smolts per square meter. These figure are used as an estimate as others have reported values up to 0.045 smolts per square meter.

Project Design

The Muckleshoot Tribe and the Washington Department of Fish and Wildlife joined together to try to make the system productive once again. Project cooperators include Trout Unlimited and the City of Tacoma. Brood fish collection occurs in March when up to 60 wild steelhead adults are captured by hook and line in the middle valley area of the Green River. These fish are transported to the restoration facility (Muckleshoot's Keta Creek Hatchery) for ripening and spawning. The offspring are hauled as fed-fry to the upper reaches of the watershed for release(Figure 2. and Table 2.). Successful outmigrants that return as adults are collected in a temporary trap at Tacoma's Dam (Figure3. and Table 3.).

Green River Wild Steelhead Juvenile Plants

Brood Year	Numbers Planted
1994	12,100
1985	21,783
1986	49,500
1987	41,360
1988	45,965
1989	47,190
1990	32,652
1991	40,906
1992	32,652
1993	57,196
1994	55,105
1995	83,774
1996	76,518

Table 2.

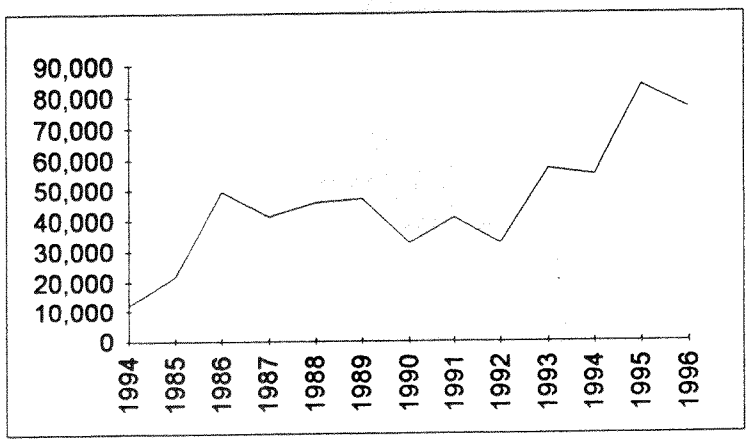

Figure 2.

Wild Steelhead Hauled to the Upper Green River - 1992- 1996

Return Year	Number Hauled
1992	74
1993	20
1994	39
1995	102
1996	133

Table 3.

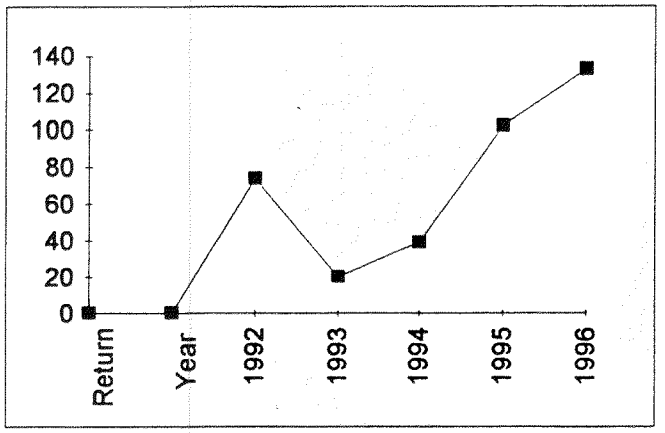

Figure 3.

Discussion

The focus of this paper is describing techniques used to lessen pre-spawning mortality. Since the first year that fish were brought to Muckleshoot's Keta Creek hatchery, we have been the student, learning to handle these wild creatures that are not adapted to the hatchery environment. They will not last long without proper handling. Every phase of the operation is critical, from catching the fish to stripping the eggs. Mortalities may occur from hooking wounds, stress to fish that were difficult to play or land, scrapes and bruises from netting and handling, holding tank and divider roughness creating wounds, and fish leaping from tanks.

The protocols developed and put into practice are as follows:

* Not using fish over 25 pounds
* Bringing fish to the boat or bank as soon as possible to place into perforated PVC tubes
* Using cotton mesh nets on the river and hatchery
* Using barbless hooks in the capture
* Using smoothing agents on all surfaces in the holding / ripening tanks
* Securing holding tanks with heavy lids
* Maintaining at least 8 cubic feet per adult in holding
* Maintaining at least one turn-over per hour in flows in holding tanks
* Checking fish for ripeness once per week except near the end of spawning
* Using consistant chemical treatments right up to final spawning
* Having plenty of males on hand that are ripe when females are ready

During spawning a general fish health screen is conducted. Ovarian fluid and kidney / spleen samples are sent to the Northwest Indian Fisheries Commission Fish Health Center for analysis. To date all samples have been negative. General fish condition has been poor to excellent over the years. Prior to cotton mesh net use, fish would come in bleeding from the eyes and more often die prior to spawning. Barbless hook use on the river has prevented wound damage and severe bleeding. Preparing the holding tanks to be fish friendly has gone a long way. Painting all concrete surfaces with a smooth epoxy cuts abrasion problems to near zero. Another help has been using a fine mesh vexar sandwiching a stronger stainless steel mesh screen in all dividers. An early management decision was not to use hormone injections to induce female ripening. This option was rejected to make conditions as natural as possible, to let fish ripen over a longer time frame to mimic the native stock as close as possible. The issue of whether to hold sexes together or separate was settled keeping them apart. When separate, the males do not have to be handled every time females are checked.

Prophylactic treatments start the day following adults enter the holding tanks and continue until spawning completion. The malicite green used in early years was replaced in 1989 with formalin and salt. This shift has greatly enhanced fish survival to spawning and helped to calm fish for handling. Treatment methodology follows the pattern of 1:10,000 formalin on day one, a 25 pound salt block added on day 2, 1:8,000 formalin on day 3, salt on day 4, 1:6,000 formalin on day 5, salt on day 6, and then continue the 1:6,000 formalin and salt rotation to the end. Table 4. shows the actual percentage change in female survival from early years to present .

Green River Wild Winter Steelhead Broodstocking History By Year

Brood Year	Total Adults	Female In	Females Spawned	Female Morts	Percentage Female surv.	Female Spawn O	Females Release	Males In	Male Morts	Males Release
1984	20	8	6	1	85.7	0	1	12	2	10
1985	42	25	12	11	52.2	2	0	17	7	10
1986	36	25	20	4	83.3	0	1	11	6	5
1987	34	20	17	3	85	0	0	14	7	7
1988	47	27	16	8	66.7	3	0	20	13	7
1889	20	13	13	0	100	0	0	7	0	7
1990	37	26	19	0	100	7	0	11	7	4
1991	19	9	9	0	100	0	0	10	2	8
1992	41	23	10	8	55.6	4	1	18	4	14
1993	35	17	17	0	100	0	0	18	4	14
1994	51	28	14	5	73.7	8	1	23	17	6
1995	46	27	18	2	90	7	0	19	4	15
1996	58	32	22	5	81.5	5	0	26	7	19
Ave.	37	18	15	4	82.6	3	0.3	16	6	10

Table 4.

Through this project, we hope to restore native steelhead to the upper Green river where they flourished some 70 years ago. Much has been learned and we have a long way to go to say it worked . Items on our agenda include a new trap and haul facility at Tacoma's Diversion Dam. A new Fish Restoration Facility on the Green will apply natural rearing and training techniques to produce a more wild like fish to increase post release survival. On a separate track the Army Corps is studying the juvenile passage problems at Howard Hanson Dam .

Acknowledgements

Many thanks to Richard Johnson, Assistant Fish Enhancement Chief at Muckleshoot Tribe for reviewing the draft ,and Tom Cropp and Steve Foley of the Washington State Department of Fish and Wildlife as chief cooperators. Thanks also to Paul Hickey of Tacoma Public Utilities, Frank Urabeck and company of Trout Unlimited, and Chief Pathologist Bruce Stewart and staff at the Northwest Fish Health Center. The many years we have all worked together are paying off to make restoration a possibility.

EVALUATION OF THE SUCCESS OF RESTORING SPRING CHINOOK SALMON NATURAL PRODUCTION IN LOOKINGGLASS CREEK, OREGON

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Abstract.-- Hatchery-produced adult Rapid River stock spring chinook salmon that returned to Lookingglass Hatchery was used to evaluate the restoration of natural production in Lookingglass Creek. To quantify the success of the restoration effort, we compared life history characteristics of the hatchery adults and their naturally-produced progeny in Lookingglass Creek with those of the extinct Lookingglass Creek natural population and other extant natural populations in Columbia River basin tributaries. We released 133 adult Rapid River stock spring chinook salmon above the Lookingglass Hatchery weir in 1993, 99 in 1993, and 112 in 1994. We estimated the total adult spring chinook salmon population above the hatchery weir to have been 220 in 1992, 297 in 1993, and 121 in 1994. There was no significant difference in mean adult-per-redd estimates among the Rapid River hatchery stock, the extinct Lookingglass Creek natural population, or other natural populations in the Columbia and Snake River basins. The estimated juveniles-per-adult for the 1993 brood of naturally-produced Rapid River stock was higher than the mean for the 1965-1969 broods of the extinct Lookingglass Creek natural population while the 1994 brood of naturally-produced Rapid River stock was lower. Monthly median fork lengths of naturally-produced Rapid River stock juveniles from the 1993 and 1994 broods in Lookingglass Creek were consistently similar to or significantly greater than the maximum of median fork lengths observed from the 1964 to 1969 broods of the extinct Lookingglass Creek natural population. Outmigration timing from Lookingglass Creek of the 1993 and 1994 broods of naturally-produced Rapid River stock juvenile spring chinook salmon peaked 1 to 2 months later in the fall than what was observed for the median of the 1965 to 1969 broods of the extinct Lookingglass Creek natural population. Both the naturally-produced Rapid River stock and the extinct Lookingglass Creek natural population, however, migrated past the trap site predominantly as subyearlings. Juvenile spring chinook salmon from the 1992 to 1994 broods of naturally-produced Rapid River stock that were PIT-tagged in Lookingglass Creek exhibited arrival timing at, and survival indices to, Lower Granite Dam within the range observed for most naturally-produced juveniles PIT-tagged in some Grande Ronde River tributaries.

Introduction

The Grande Ronde River Basin historically supported large populations of fall and spring chinook (*Oncorhynchus tshawytscha*), sockeye (*O. nerka*) and coho (*O. kisutch*) salmon and steelhead (*O. mykiss*) (Nehlsen et al. 1991). Escapements of anadromous salmonids to the Grande Ronde River Basin (Oregon Department of Fish and Wildlife (ODFW), unpublished data), as well as escapements to the entire Snake River Basin (Nehlsen et al. 1991), have declined, several to extinction. Hatcheries were built in Oregon, Washington, and Idaho under the Lower Snake River Compensation Plan (LSRCP) to compensate for losses of anadromous salmonids due to the construction and operation of the lower four Snake River dams. Lookingglass Hatchery (Figure 1) was completed under the LSRCP in 1982 and is the only hatchery in northeast Oregon that releases spring chinook salmon in the Grande Ronde River basin. The upstream migration of adult spring chinook salmon entering Lookingglass Creek has been almost completely blocked by a picket weir located at the hatchery intake (Figure 1) and, until this study was initiated in 1992, almost no adult spring chinook salmon captured at Lookingglass Hatchery were released above that weir to spawn. This study was developed by the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and the ODFW in consultation with the Nez Perce Tribe (Lofy et al. 1994). The goal of this study is to determine the success of using a non-endemic hatchery stock for reestablishing natural production, by comparing life history characteristics of the hatchery adults and the naturally-produced progeny of

these fish to other naturally-reproducing populations in the Columbia River basin. We used data collected from 1992 to 1996 on outplanted Rapid River stock adults in Lookingglass Creek and their naturally-produced progeny (Lofy and McLean 1995a, 1995b, and McLean and Lofy 1995), detailed life history data on the extinct Lookingglass Creek natural population that was collected from 1964 to 1974 (Burck 1993; Burck 1964-1974), and data collected from 1984 to 1996 on spring chinook salmon from natural populations in the Columbia River basin.

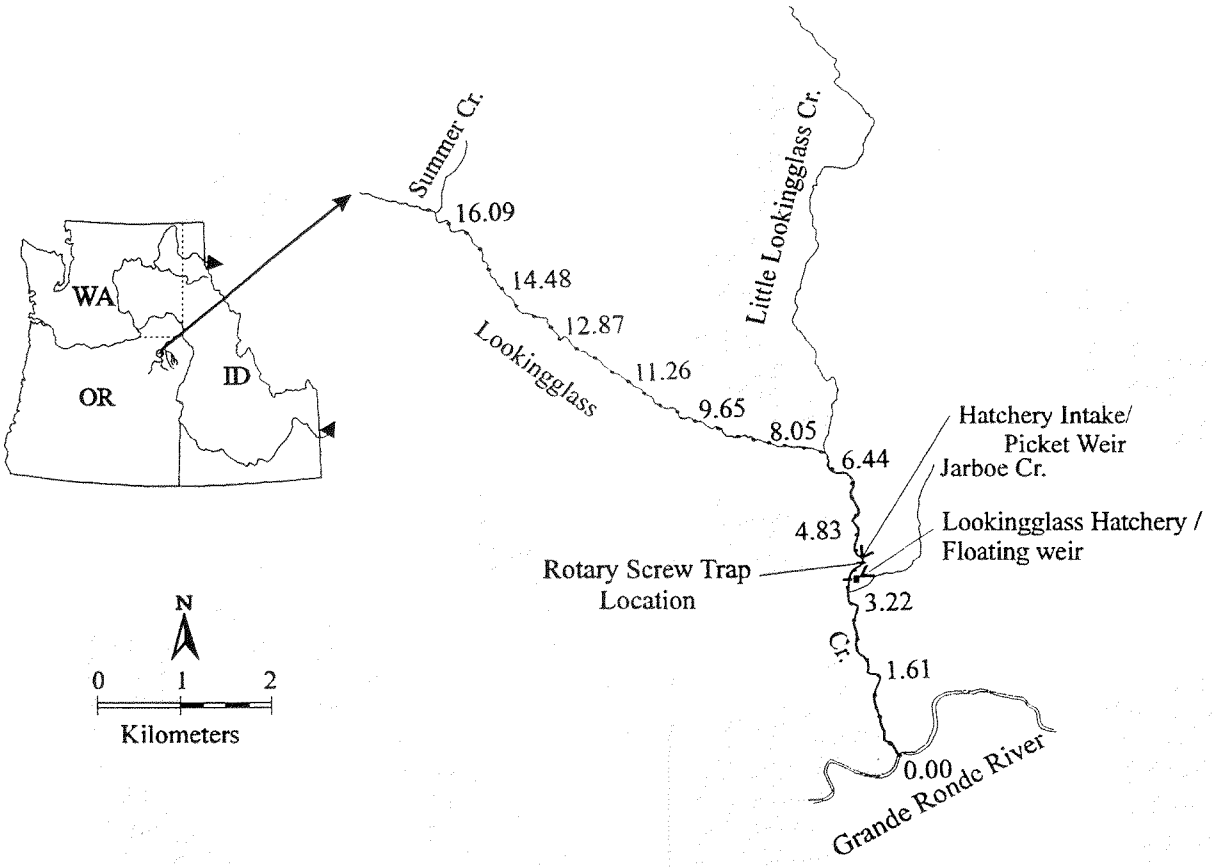

Figure 1. Map of the Lookingglass Creek basin showing the location of the Lookingglass Creek Hatchery and the rotary screw trap. The (•) show 0.4-river km sections of Lookingglass Creek.

Methods

Release of adults above the weir

During 1992, 1993, and 1994 a portion of the adult Rapid River stock spring chinook salmon returns to Lookingglass Hatchery were released above the hatchery weir (Figure 1). The fish were marked before release to distinguish them from fish that escaped above the weir without being handled so a total population above the weir could be estimated. A mark-recapture technique (Brower and Zar 1977), using marked and unmarked carcasses recovered on spawning ground surveys, was used to estimate the total number of males and females above the weir each year.

Spawning ground surveys

Spawning ground surveys were conducted to document the number of redds and to recover carcasses. Surveys were conducted every two weeks before the first digging activity was observed and weekly after that date. Digs were categorized as incomplete or complete based solely on physical characteristics. The number of redds and an estimate of the total population above the weir were used to calculate an adult-per-redd estimate. A mean adult-per-redd and 95% confidence interval was then calculated for the 1992 to 1994 releases.

Juvenile fork lengths

Monthly median fork lengths of juveniles from the 1993 and 1994 broods of naturally produced Rapid River stock were compared to those observed for the 1964 to 1969 broods of the extinct Lookingglass Creek natural population (Burck 1964-1974). Monthly sampling was completed around the 20th of each month and usually took 2 to 3 days to complete. Sampling occurred from the first April after emergence to October each year (7 months). The juvenile sampling was conducted in the same areas used by Burck (1993), from about rkm 7.25 to 7.65 in Lookingglass Creek (Figure 1). A Kruskal-Wallis test (Wilkinson 1992) and post hoc multiple comparisons (Dunn 1964, cited by Daniel 1990) were used to determine if the 1993 and 1994 broods had significantly different fork lengths than the 1964 to 1969 broods.

Juvenile outmigration

To compare juvenile-per-adult and juvenile-per-redd estimates to those observed for the 1965 to 1969 broods of the extinct Lookingglass Creek natural population the total number of juvenile spring chinook salmon from the 1992 to 1994 broods of naturally produced Rapid River stock that passed the rotary screw trap site was estimated. Monthly estimates of the total number of juvenile chinook salmon from the 1993 and 1994 broods that passed the trap site. This allowed comparison of the median outmigration timing from Lookingglass Creek to what was observed for the 1965 to 1969 broods. The juvenile migration past the trap site was divided 13 periods: January through May of the juveniles first year of migration combined (1 period); June of the first year of migration through April of the second year of migration (11 periods); and May through June of the second year of migration combined (1 period). Fish captured in the trap were marked throughout most of the periods to calculate the total number of outmigrants for each period using a Peterson-Lincoln estimator (White et al. 1982). Using the variance of the outmigrant estimate for each period, which was calculated using a bootstrap method (Efron and Tibshirani 1986), we calculated 95% confidence intervals for each period. When no fish were recaptured during a period a recapture of one was used to estimate trap efficiency, and if no releases were made, the release and recapture numbers of the previous period were substituted.

Arrival timing and survival to Snake and Columbia rivers dams

Juvenile spring chinook salmon captured in the Lookingglass Creek basin from the 1992 to 1994 broods of naturally-produced Rapid River stock were tagged with Passive Integrated Transponder (PIT) tags to determine arrival timing at, and minimum survival indices (minimum survival estimate) to, Lower Granite Dam. From September 18 to September 29 each year, fish were captured from above, or just below the weir, PIT-tagged, and released in approximately the same area where they were captured. Arrival timing was graphed as the weekly percentage of the total number of Lookingglass Creek detections at Lower Granite Dam. Survival indices (detection rate) to Lower Granite Dam were calculated for the 1992 to 1994 broods of naturally produced Rapid River stock using cumulative unique detections at all Snake and Columbia river dams. Survival indices were also calculated by dividing the cumulative number of unique detections by the total number of the juveniles tagged. Ninety-five percent confidence intervals for survival indices were calculated using methods described in Ott and Mendenhall (1985).

Unmarked adult returns to Lookingglass Hatchery

Adult returns to Lookingglass Hatchery in 1995 and 1996 were monitored to identify possible returns of Rapid River stock adult spring chinook salmon from releases above the weir in 1992 and 1993. Based on pre-release sampling conducted by ODFW (ODFW unpublished data) the percentage of the total Rapid River stock released from the hatchery that had no recognizable fin clip was calculated in order to determine how many of the unmarked fish returning may be from the hatchery release and not from natural production in Lookingglass Creek. Age at return to the hatchery was calculated using fork length data because the scale analysis is not yet complete.

Results and Discussion

Release of adults above the weir

One hundred thirty three adult Rapid River stock spring chinook salmon were marked and released above the hatchery weir in 1992, 99 in 1993, and 112 in 1994 (Table 1). The estimated total spring chinook salmon population above the weir in 1992, 1993, and 1994 was estimated to be 220, 297, and 121 respectively (Table 1). The total spring chinook salmon

population above the weir in 1967, 1968, and 1969 was 252, 400, and 812 respectively (Burck 1993). Our population estimates for the 1992 to 1994 broods of the Rapid River stock fell within the lower end of the range observed for the 1967 to 1969 broods of the extinct Lookingglass Creek natural population.

Spawning ground surveys

Completed redds observed above the weir in Lookingglass Creek in 1992, 1993, and 1994 were 49, 132, and 40 respectively (Table 1). The adult-per-redd estimate above the weir was 4.5 in 1992, 2.3 in 1993, and 3.0 in 1994. The mean adult-per-redd estimate for Rapid River stock adults released from 1992 to 1994 was compared to the mean observed for the extinct natural population from 1967 to 1971 and the mean for some other natural populations in the Columbia River basin using overlap of the 95% confidence intervals. Mean adult-per-redd estimates for other natural populations in the Columbia River basin were not significantly different from the Rapid River stock released in Lookingglass Creek above the hatchery weir (Figure 2). The lack of any significant difference in the mean adult-per-redd estimate from other natural populations indicates that the Rapid River stock adults released above the Lookingglass Hatchery weir survived and could build redds with similar success to other natural populations in the Snake and Columbia river basins, however, our estimate was more variable than the adult-per-redd estimates from the other Columbia River basin natural populations (Figure 2).

Table 1. Release and recovery information for the population estimate above the weir.

Year	Sex	Total Released	Population estimate	Population SEM	Total redds
1992	male	49	141[a]	54	
	female	44	79[b]	9	49
1993	male	49	138	22	
	female	50	159	18	132
1994	male	56	59	5	
	female	56	62	4	40

[a] Total population includes an additional 18 male spring chinook salmon that stayed above the weir from a release of 20 after the population estimate was made.

[b] The female total population includes an additional 19 fish that stayed above the weir from a release of 20 after the population

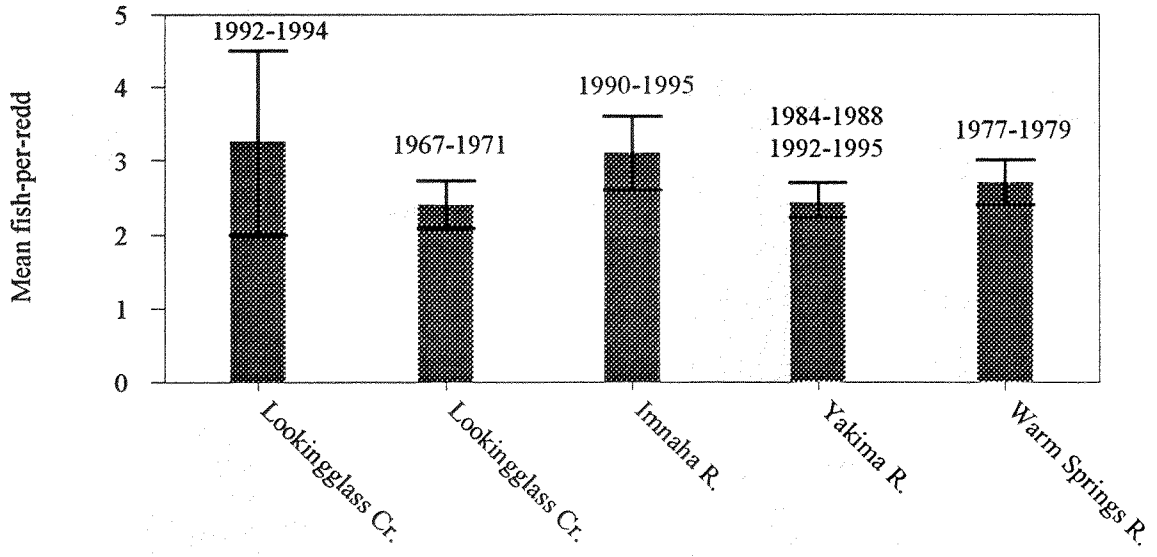

Figure 2. Mean adult-per-redd estimates for some natural populations (spawning in the wild) of spring chinook salmon found in the Columbia River basin, years used in the calculation of the mean are above the bars. Error bars indicate a 95% confidence interval.

Juvenile fork lengths

Monthly median fork lengths from the 1993 and 1994 broods of naturally produced Rapid River stock were consistently near the maximum median fork lengths observed for the 1964 to 1969 broods of the extinct Lookingglass Creek natural population (Figure 3). From April through July for the 1993 brood and June through October for the 1994 brood, median fork lengths were significantly greater than the maximum median fork lengths for the 1964 to 1969 broods (Figure 3). The significantly larger median fork lengths for the 1993 and 1994 broods may be attributed to low densities of juveniles in the stream compared to the 1964 to 1969 broods. These fork length comparisons show that the naturally-produced Rapid River stock has slightly different timing and amount of growth through the year than the extinct Lookingglass Creek natural population (Figure 3), although we aren't able to discern whether the differences are genetic, environmental, or both.

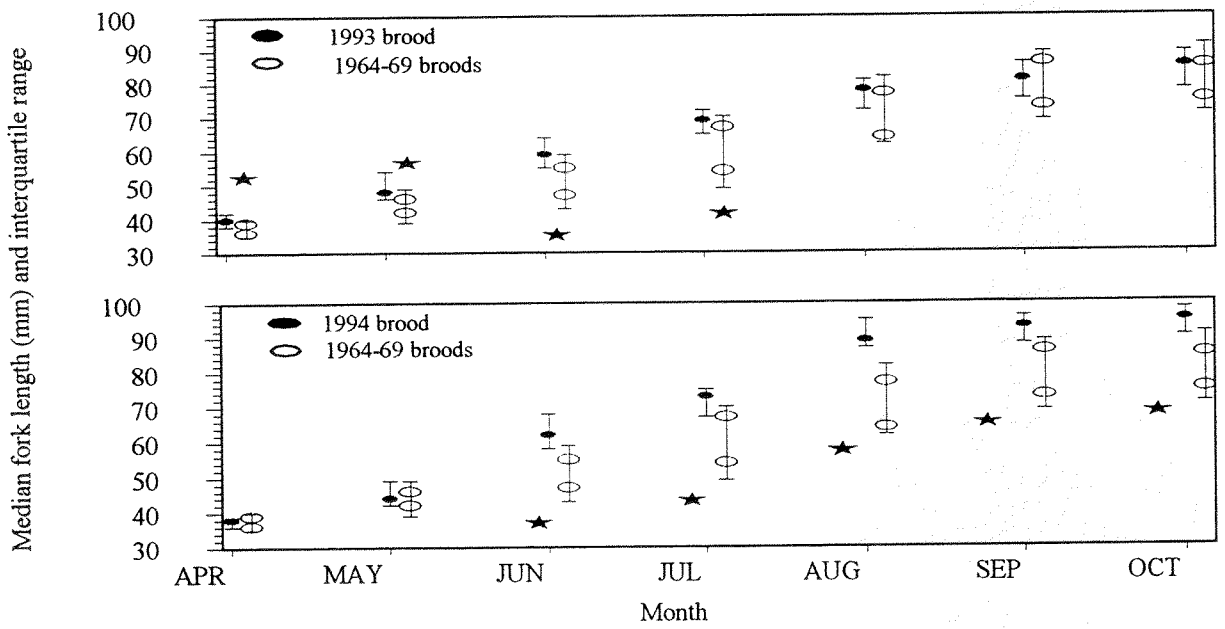

Figure 3. Monthly median fork lengths of juvenile spring chinook salmon in Lookingglass Creek for the 1993 and 1994 broods of naturally produced Rapid River stock and the range from the 1964 to 1969 broods of the extinct Lookingglass Creek natural population. Stars indicate significant differences between the 1993 and 1994 broods and the 1964 to 1969 broods.

Juvenile outmigration

The total number of juvenile spring chinook salmon from the extinct Lookingglass Creek natural population estimated to have passed the trap site in Lookingglass Creek for the 1965 to 1969 broods ranged from 44,789 ± 3,774 to 145,586 ± 55,589 (Burck 1964-1974). Because we were not able to operate the trap before 29 October, 1993, our estimates for the number of 1992 brood naturally produced Rapid River stock include only fish that passed the trap site after that date. For the 1992, 1993 and 1994 broods of naturally produced Rapid River stock we estimated that 8,506 ± 1,853, 117,525 ± 27,782 and 7,418 ± 1,827 fish left Lookingglass Creek. The mean juvenile-per-adult estimate for the 1967 to 1969 broods was 134 ranging from 94 to 178, and for the 1993 to 1994 broods it was 229 ranging from 61 to 396 (Figure 4). The mean juvenile-per-redd estimate for the 1965 to 1969 broods was 569 ranging from 277 to 1,286, and for the 1993 to 1994 broods it was 538 ranging from 185 to 890 (Figure 4). Estimates of juvenile-per-adult and juvenile-per-redd indicated that the Rapid River stock adults produced juveniles, that were able to survive to outmigration from Lookingglass Creek, at similar levels as the extinct Lookingglass Creek natural population. The outmigration from Lookingglass Creek for the 1993 and 1994 broods of naturally produced Rapid River stock occurred primarily during 2 peak time periods, January through May (J-M) and October (O) (Figure 5). For the 1965 to 1969 broods of the extinct Lookingglass Creek natural population passage by the trap, the peak occurred in August (A)(Figure 5). For both the 1993 to 1994 and 1965 to 1969 broods, most of the fish left Lookingglass Creek before December in their first year of life (Figure 5). The large percentage of outmigrants

during the January through June periods for both the Rapid River stock and the extinct Lookingglass Creek natural population may have been a result of high spring flows causing small fry to be swept from the stream. The first peak in migration timing for the 1993 and 1994 broods may be due to error, as indicated by the high coefficient of variation (Figure 5). The similarity in juvenile migration patterns of the two naturally-produced populations indicate that the Rapid River stock has the ability to respond similarly to environmental factors that influence migration timing as the extinct Lookingglass Creek natural population.

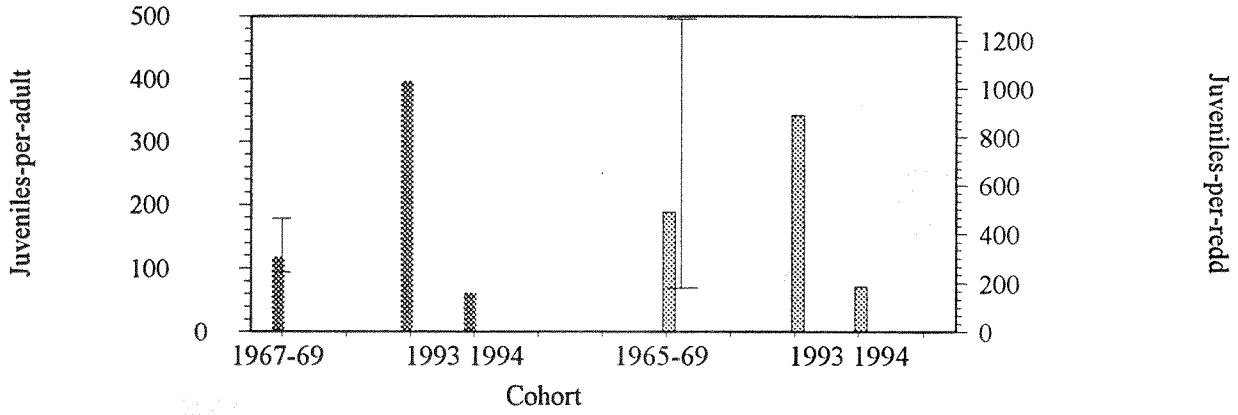

Figure 4. Juvenile production indices from Lookingglass Creek for the 1993 and 1994 broods of Rapid River stock and the mean and range (narrow vertical lines) for the 1965 to 1969 broods of endemic stock. Darker bars are the juveniles-per-adult index while the grey bars are the juveniles-per-redd index.

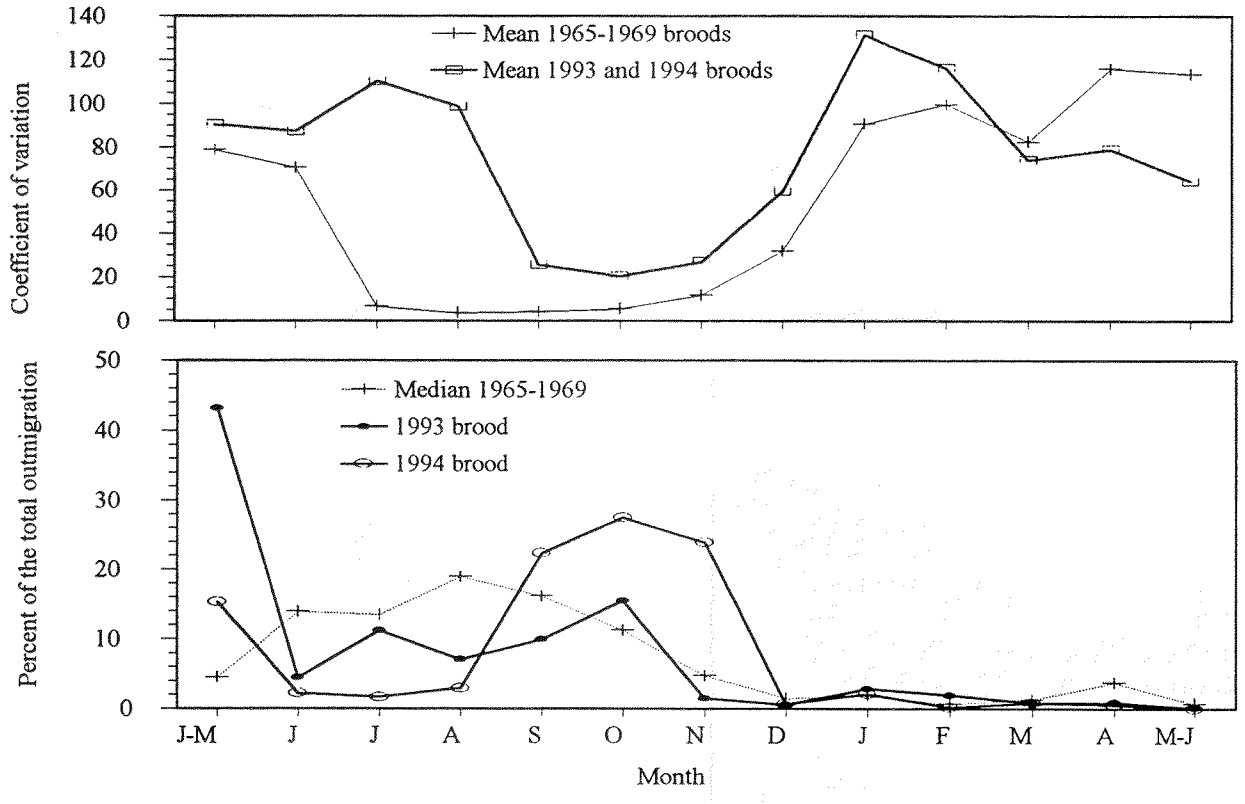

Figure 5. Coefficient of variation and percent of the total outmigration from Lookingglass Creek estimated by period.

Arrival timing and survival to Snake and Columbia rivers dams

There were 1,022 juvenile spring chinook salmon from the 1992 brood, 997 from the 1993 brood, and 1098 from the 1994 brood of naturally produced Rapid River stock PIT-tagged in Lookingglass Creek (M°Lean and Lofy 1995). The first of the 1992 brood arrived at Lower Granite Dam the week of 15 April, and the last fish arrived the week of 13 May (Figure 6). The arrival timing of the 1993 and 1994 broods of naturally produced Rapid River stock was similar to the 1992 brood, with the first fish arriving the first of April and the last fish arriving at the end of May. The numbers of fish detected at Lower Granite Dam for the 1992, 1993, and 1994 broods was 102, 59, and 33 fish respectively. The arrival timing of juveniles from Lookingglass Creek was most similar to the natural populations from the Lostine, Minam, and Wenaha rivers for the 1992 brood (Walters et al. 1994) (Figure 6) with similar results for the 1993 (Sankovich et al. 1995) and 1994 broods (Sankovich et al. in press). The similarity of arrival timing between the naturally produced Rapid River stock and the 3 natural populations of Grande Ronde River tributaries may be the result of geographic location and travel distance to Lower Granite Dam.

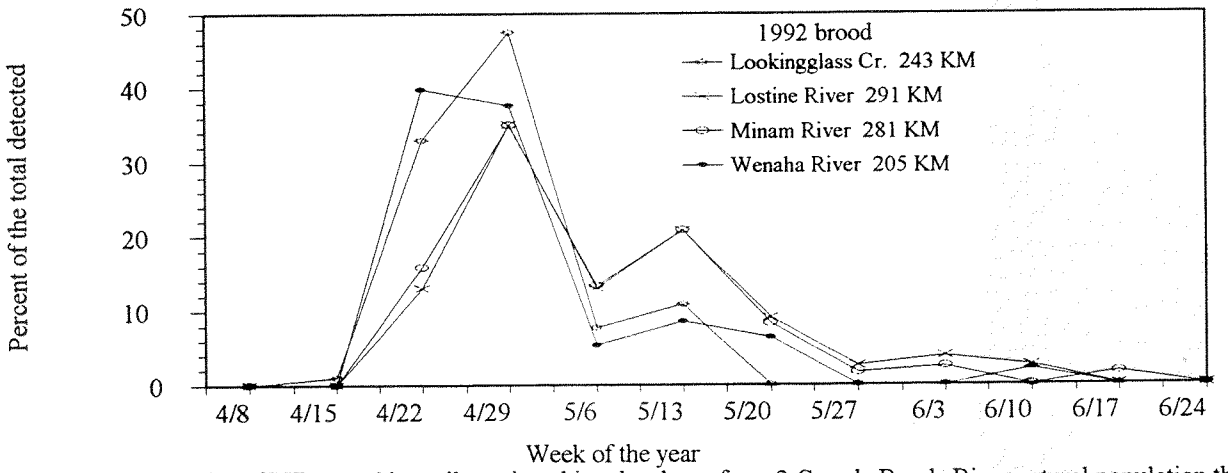

Figure 6. Arrival timing of PIT-tagged juvenile spring chinook salmon from 3 Grande Ronde River natural population that were most similar to the arrival timing of naturally produced Rapid River stock juveniles from Lookingglass Creek. Due to the similarity between the 1992 to 1994 broods only the 1992 brood is shown.

Survival indices of PIT-tagged juvenile chinook salmon from the 1992 to 1994 broods of naturally produced Rapid River stock were 17.4, 12.5, and 16.9 % respectively (Figure 7). The survival indices for fish from Lookingglass Creek were not significantly different from most of the other natural populations of Grande Ronde River tributaries for all broods (Figure 7). The reduction in survival indices for the 1993 brood compared to the 1992 and 1994 broods of naturally produced Rapid River stock from Lookingglass Creek was similar to the reduction in survival indices of the same broods from other natural populations of tributaries of the Grande Ronde River except for the Grande Ronde and Lostine rivers. The similarities seen in the survival to Lower Granite dam between the naturally produced Rapid River stock from Lookingglass Creek and other natural populations of Grande Ronde River tributaries indicates that the fish produced from hatchery adults released into the natural environment can survive as well other natural populations in the Grande Ronde River basin to Lower Granite Dam.

Unmarked adult returns to Lookingglass Hatchery

Unmarked adult spring chinook salmon return data at Lookingglass Hatchery that could have returned from the 1992 and 1993 broods of naturally produced Rapid River stock has been monitored. All hatchery reared juveniles released from Lookingglass Hatchery have been fin-marked prior to release since the 1992 brood. Based on prerelease subsampling conducted by ODFW the percent of the total fish released that did not have any recognizable fin clip was 0.001% for the 1992 brood and 0.000% for the 1993 and 1994 broods (ODFW unpublished data). Therefore the estimated unmarked adults that were of hatchery origin will probably be 0 for all years. Eight unmarked jacks and 96 unmarked 4-year-olds from the 1992 brood returned to Lookingglass Hatchery in 1995 and 1996. Only 1 unmarked jack returned to the hatchery in 1996 from the 1993 brood. The unmarked adults could be from three sources: naturally produced from Lookingglass Creek; naturally produced strays from other populations; or unmarked hatchery fish. We are unsure of the proportions of each type, however, it is likely that a high proportion are naturally produced fish from Lookingglass Creek.

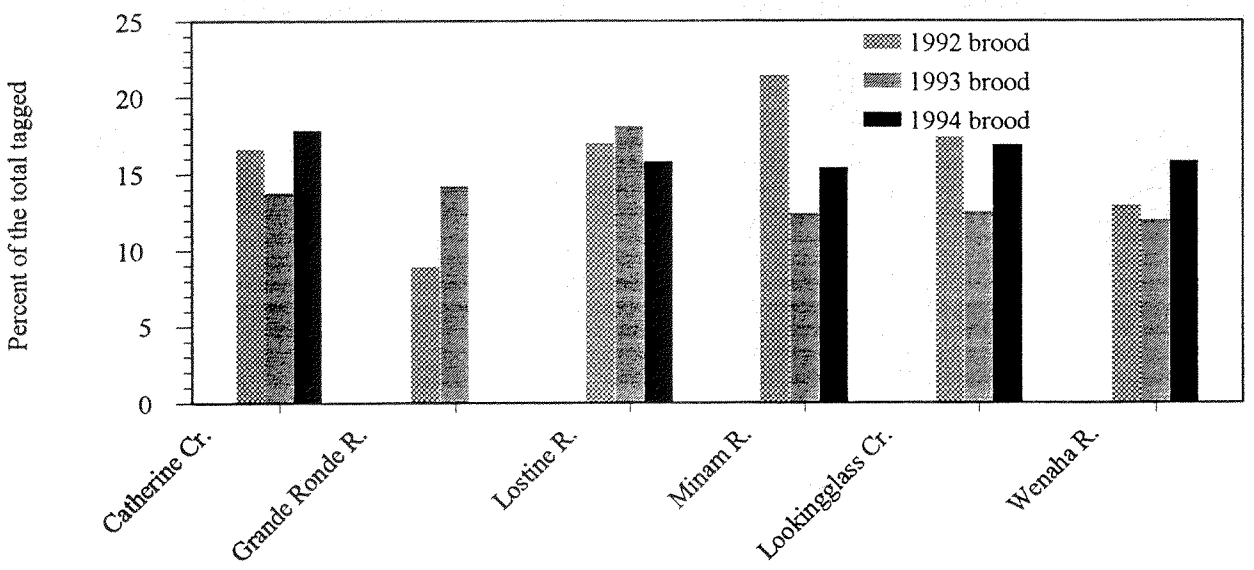

Figure 7. Survival indices for natural populations of Grande Ronde River tributary juvenile spring chinook salmon and naturally produced Rapid River stock from Lookingglass Creek. Survival indices are first time PIT tag detections at Snake and Columbia River dams divided by the total number tagged.

Summary

The releases of adult Rapid River stock spring chinook salmon above the Lookingglass Hatchery weir from 1992 to 1994 have shown promising results. These hatchery adults released into the wild have shown that they can survive to spawning and produce juveniles that grow at similar rates and can survive to outmigration from Lookingglass Creek. The naturally produced juveniles have shown similar outmigration timing from Lookingglass Creek as the extinct Lookingglass Creek natural population. The arrival timing at, and survival to, Lower Granite Dam was similar to other natural populations of Grande Ronde River tributaries. There have also been adult returns from the releases made from 1992 to 1994 above the Lookingglass Hatchery weir. The adult returns however, are incomplete and need to monitored until outmigrant to adult survival rates can be estimated.

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THE ROLE OF OREGON DEPARTMENT OF FISH & WILDLIFE COASTAL HATCHERIES IN REBUILDING NATURALLY SPAWNING COHO POPULATIONS

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Introduction

The depressed status of Oregon's wild coastal coho salmon stocks has led to the closure of many ocean and freshwater fisheries. Various groups are working on methods of restoring the health of wild coastal coho salmon populations, and providing an opportunity to harvest coho salmon. As part of the efforts to achieve these goals, management objectives for Oregon Department of Fish and Wildlife (ODFW) coastal hatcheries are being reviewed. Historically these hatcheries have been operated to augment ocean and freshwater fisheries, or as mitigation for habitat loss. Recently there has been much discussion of the impact of these hatchery operations on wild salmon populations. This discussion has led to efforts to reduce negative impacts on wild populations while maintaining the objective of fishery augmentation. This paper will discuss some of the changes in hatchery objectives being considered by ODFW, and how stock differences influence the implementation of these objectives.

There are two main objectives being considered for ODFW coastal coho hatcheries. The first is a modification of the historic objective, fishery augmentation, to minimize negative impacts on wild salmon populations. The second is to use hatchery technology to assist in restoring depressed wild salmon populations. An evaluation of the status of the freshwater habitat, the wild population, and the history and characteristics of the hatchery population would determine which of the objectives would be appropriate for each hatchery. Some hatcheries may rear different coho salmon stocks for the different objectives. Following are some of the techniques being considered for implementing these objectives.

Objective 1) Augment salmon fisheries while minimizing negative impacts on wild populations.

Develop and Maintain Hatchery Brood stock From Local Wild Population: Since local wild fish have evolved to best utilize the local environmental, hatcheries should develop brood stocks from the wild population where the hatchery fish are released. Even a hatchery stock developed from the local wild population will experience some level of domestication over time. These changes may or may not effect the survival of the hatchery fish but do pose a risk to the wild population through interbreeding of the hatchery and wild fish. Once the hatchery brood stock is developed the hatchery should maintain it's genetic diversity using the following guidelines:

1) Incorporate 5% to 30% wild fish in the hatchery population every year. Except that no more than 25% of the wild population may be taken for hatchery use.
2) Collect and spawn fish from the entire hatchery run.
3) Maintain a minimum return to the hatchery of 500 fish for spawning.
4) Spawn one male to one female (or use a 2x2 matrix if desired).
5) Include jacks in spawning (based on % in the wild spawning population).
6) Retain the same proportion of each female's eggs based on the ratio of (eggs needed / total egg take).

These recommendations incorporate most of the suggestions made by Simon (1991) and Kostow (1996). Simon (1991) made three other suggestions not incorporated above; (1) spawn all females, (2) use sperm from adjacent wild or hatchery populations for crosses with the hatchery brood stock, and (3) independently coded-wire tag the juveniles from 20 separate families to collect data on the variation in reproductive success of the hatchery stock.

Mark All Hatchery Fish: Although this increases hatchery costs and imposes some extra mortality on hatchery fish the benefits gained are substantial. This will help create an opportunity for selective harvest of hatchery fish, assist

in hatchery brood stock selection and maintenance, and assist in monitoring the interactions between hatchery and wild fish (both as adults and juveniles).

Appropriate Location for Hatchery or Release Site: Hatcheries and/or release sites should be located low in the river system, and in areas that allow for segregation of returning hatchery fish from the wild population. This will help to reduce straying to wild spawning areas, create an opportunity for selective harvest of hatchery fish, and reduce competition and disease interactions of hatchery and wild fish.

Link Hatchery Production Level to Status of Wild Population: When the wild population is depressed enough to preclude harvest of excess hatchery fish there is no reason to produce more hatchery fish than are needed to maintain brood stock. When the wild population is healthy enough to allow harvest of excess hatchery fish, production levels at the hatchery can be increased to provide harvest opportunities.

Objective 2) Use hatchery technology to assist in restoring depressed wild salmon populations.

Explore Alternative Rearing Strategies to Maintain A Wild Phenotype: Traditional hatchery rearing techniques are efficient at producing large numbers of smolts with limited resources. However, they can result in behaviors significantly different from wild fish. This can have consequences for both the hatchery and wild fish. Alternative rearing strategies include; low density rearing, pond cover, in pond structures, predator training, more natural feeding/growth schedules, and supplemental feeding with natural prey items. Besides maintaining wild-type behaviors these strategies may result in improved survival, reduced pond cleaning needs, reduced disease, and better feed conversions.

Use Hatchery Fish to Supplement Wild Production: The use of hatchery fish to rebuild wild salmon populations has not been adequately evaluated, but does offer a tool in certain situations. Because some evaluations have shown hatchery supplementation to actually have negative effects on wild populations (Nickelson et al., 1986), this technique should generally not be used in areas with existing wild populations. Appropriate areas include localized extirpations (poor habitat, barrier, etc.) where small scale projects could help to bridge gaps between the existing wild spawning areas, and major basin/subbasin extirpations where large scale restoration could be done with little threat to existing wild populations. Any re-introduction project will need to be evaluated for success. Kostow (1996) listed some general comments on use of hatchery fish for re-introduction's.

1) Fish should not be introduced outside their historic, native range.
2) Causes of the extirpation or fragmentation must be corrected before the re-introduction.
3) Adequate sized founding populations must be used.
4) These are limited duration programs and must be evaluated each generation.
5) Expect a slow process. Hatchery fish will not be well adapted to natural production and initial survivals and breeding success will be low.
6) Such programs are dependent on adequate hatchery brood stocks. May need to "reserve" brood stocks for future use.

Establish Captive Wild Brood stocks: This is a new and potentially expensive technique that may be appropriate in some situations. It is currently being used for Snake River chinook salmon at the Lookingglass hatchery.

Nehalem Hatchery

Located on the Northern Oregon coast this hatchery produces coho salmon, steelhead and rainbow trout. The hatchery began operation at its current site in 1966. It is located at river mile 10.3 of the North Fork Nehalem River, a tributary of Nehalem Bay. There are three recognized coho populations in the Nehalem River Basin; North Fork Nehalem, Lower Nehalem (mainstem and tributaries below Hwy. 26), and Upper Nehalem (mainstem and tributaries above Hwy. 26). The North Fork Nehalem and Lower Nehalem populations are considered very depressed to extirpated, with substantial natural spawning of hatchery coho. The Upper Nehalem population is healthier with a November through January run timing and an estimated population of 1,850 fish (1990-1995 average). The hatchery rears two coho stocks. North Nehalem stock is a long-time domesticated hatchery stock

healthier with a November through January run timing and an estimated population of 1,850 fish (1990-1995 average). The hatchery rears two coho stocks. North Nehalem stock is a long-time domesticated hatchery stock based on North Fork and Lower Nehalem wild coho with introductions of foreign stocks through the 1952 brood year. The Fishhawk stock is a recently domesticated stock (begun in 1978) based on wild Upper Nehalem coho. The North Nehalem stock is reared exclusively in two brood cycles (1994 and 1995). Fishhawk stock is reared exclusively in the other brood cycle (1993) and has a later run timing than North Fork Nehalem stock (Figure 1).

Implementation of Objective 1

Both hatchery stocks were developed from local wild fish, but show different levels of domestication. Hatchery records from the 1950's document a late October through January spawning timing for the North Nehalem stock. That timing has been shifted to late September through November. The Fishhawk stock and wild coho still display a broader and later spawning timing. Although, the Fishhawk stock appears to have lost the latest spawning component.

Starting with the 1995 brood year all coho salmon released from Nehalem hatchery will be marked. This will help document hatchery/wild ratios on the spawning grounds and at the hatchery, and help develop fisheries targeting returning hatchery coho.

Figure 1. Spawning timing of Fishhawk and North Nehalem stock coho (1993 and 1994 run years respectively) and wild coho (1994 run year).

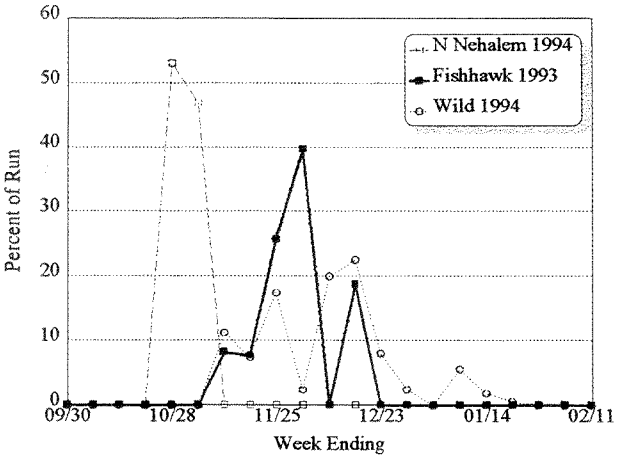


Table 1. Punchcard catch estimates and hatchery return data for Nehalem coho salmon. Harvest rate is [catch/(hatchery returns + catch)]*100.

Run Year Stock	Hatchery Return	N Fk Nehalem Punchcard Catch	N Fk Nehalem Harvest Rate
1995 N Fk Nehalem	5,022	N.A.	--
1994 N Fk Nehalem	5,464	569	9.4%
1993 Fishhawk Lake	5,191	578	10.0%
1992 N Fk Nehalem	3,353	373	10.0%

The location of the hatchery allows for segregation of returning hatchery fish from the main wild coho spawning areas in the Upper Nehalem basin. This location will also help to develop fisheries targeting returning hatchery coho. Current freshwater fisheries harvest about 10% of the fish entering the North Nehalem River (Table 1).

Coho release numbers, based on compliance with wild fish management plans and good fish culture practices, call for releasing a total of 605,000 smolts split between a March and April release. Because of the inability to harvest these fish in any but North Nehalem fisheries, Nehalem hatchery coho production has been reduced to brood stock maintenance levels. The brood stock maintenance release level is 200,000 smolts split between a March and April release. This should maintain a return of at least 500 adult coho, which would provide enough eggs to return to full production.

Implementation of Objective 2

The substantial shift in spawning timing for North Nehalem stock indicates a level of inadvertent selection and domestication of this stock. The initial response to this would be to discontinue rearing this stock in favor of the Fishhawk stock. The Fishhawk stock could then be used for both fishery augmentation (objective 1) and restoration of wild populations (objective 2). Further data analysis suggest a second approach. Although there has clearly been selection and domestication of the North Nehalem stock, comparison of the two stocks shows egg size ($p=0.78$), egg loss ($p=.93$), and post release survival to ocean fisheries ($p=0.94$) were not significantly different. This suggests no loss in performance of North Nehalem stock. North Nehalem stock did have

significantly higher fecundity (2,941 versus 2,523 p=0.00). Although survival to ocean fisheries were very similar, survival to hatchery return was consistently higher, but not statistically significant (p=0.10), for North Nehalem stock (Figure 2). This suggests a higher rate of straying for the Fishhawk stock. Since the 1987 brood year only one stock has been released each year. Thus, the percent of hatchery fish on the spawning grounds since the 1990 return year can be used to compare straying rates for the two stocks (Figure 3). Both comparisons (Figures 2 and 3) indicate higher straying rates for the Fishhawk stock.

Figure 2. Survival (coded-wire tag data) for Fishhawk versus North Nehalem stock coho. Survival is (estimated adult recoveries/tagged fish released)*100.

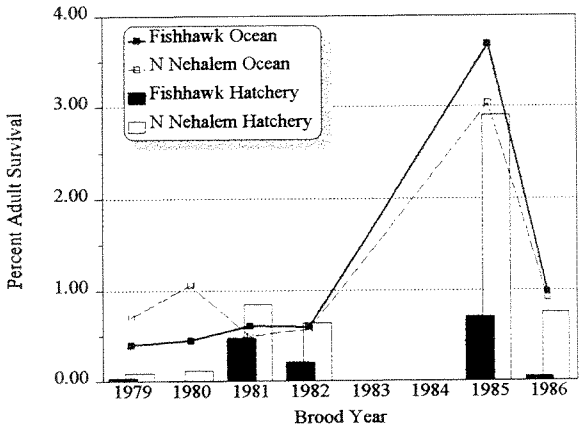
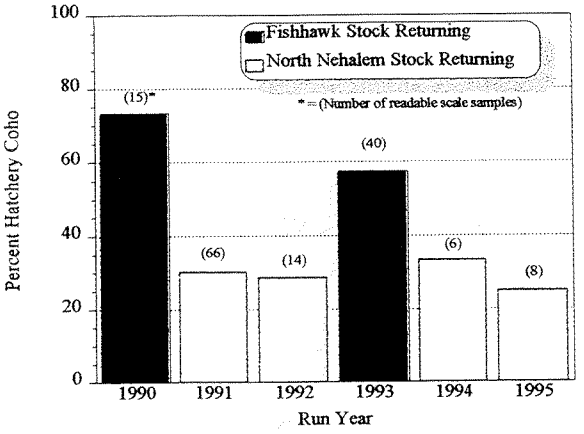


Figure 3. Percent hatchery fish on spawning ground surveys in years when either Fishhawk or North Nehalem stock was released. Date is from scale samples recovered on spawning ground surveys.



The earlier run timing and lower straying rate for the North Nehalem stock make it a good stock for fishery augmentation. The later run timing and higher straying rate of the Fishhawk stock mean it would not be a good stock for fishery augmentation. The Fishhawk stock could be developed for restoration of wild populations.

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PENNASK LAKE - "A POT OF GOLD"

AND NO END TO THE RAINBOW?

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This poster attempts to depict the importance of the Pennask Lake rainbow trout run to the British Columbia fish stocking program with it's emphasis on utilization of wild egg sources. The historic, recreational, and physical aspects of Pennask Lake and it's wild rainbow trout are also presented.

With an annual spawning run of between 20,000 and 25,000 wild adult rainbow trout, Pennask Lake may be arguably the largest run of wild rainbow trout in North America. The Government has operated an egg collection program at this lake since 1929. With an average annual egg collection of 2 million, this station accounts for 20% of total rainbow trout eggs collected by the province, and is the only truly wild egg source available to the Province (Andrusak, Blann,1992). Stocking of Pennask rainbow trout includes over 400 of the approximately 1000 lakes stocked in British Columbia each year. Reknowned for their fighting ability, the Pennask Lake rainbow trout are the world famous "*Kamloops Trout*" sought after by many thousands of recreational anglers. Eggs taken by the Fish Culture Section from Pennask Lake have an annual value in excess of $12,000,000 (Pennask L.R.U.P 1993)

The egg collection at Pennask Lake has become the single most important rainbow trout egg station to the Province, and together with it's lengthy and interesting history this site could be likened to the "Yankee Stadium" of egg collection sites. Beyond Fish Culture, the lake has a colourful history that is inextricably tied to James Drummond Dole (Dole Pineapple Ltd.), and the Pennask Lake Fishing and Game Club (later named the Pennask Lake Company). A Royal visit by Queen Elizabeth II and Prince Philip to the Great Lodge in 1959, and the near disaster of a proposed mine and water storage for mining at Pennask Lake are a couple of noteworthy events that raised the public awareness of the area. A Class "A" Provincial Park was established on the southeast corner of the lake in 1974, to secure the only public access to the lake, and thereby monitor and regulate angling effort and development.

The lake provides an excellent fly-fish only fishery. Creel survey indicates approximately 15,000 angler days of use per year, between the public and the Pennask Club members. Catch per angler day by public anglers averages over 3.0 fish per day, while catch by the Pennask Club members averages over 4.0 fish per day. An estimated 50,000 fish are harvested from Pennask Lake annually (B. Chan pers. com.).

The Pennask area has always attracted the attention of various user and consumer groups. Just as the Provincial Fisheries Program leadership embraced the concept of wild stock conservation long before it became popular with many provincial and state agencies (Peterson. 1996), the Fish Culture section recognized the importance of the Pennask Lake rainbow. As a result efforts have been made to help protect this unique system for future generations. These include:

- a comprehensive Land Resource Use Plan developed and endorsed by all key user groups of the area, in 1993.

- a Pennask Lake Brood Management Plan developed in 1995 encompassing egg collection procedures and data collection to maintain the temporal, demographic, and genetic integrity of the spawning run.

- a genetic refugia for Pennask rainbow trout established in a remote, previously barren lake in the British Columbia Interior.

- a "genetic fingerprint" for Pennask rainbow established by protein electophorisis to monitor population parameters, heterozygosity levels, and allelic diversity (Volpe, 1995).

- stringent egg disinfection procedures and regular pathological examinations of spawning adults conducted to ensure fish health concerns are satisfied.

- no fish stocking of Pennask Lake or it's nearby tributary systems to keep the run entirely wild.

- ongoing interaction, communication, and consultation between the Province and the various user groups of the Pennask area.

- attempts to raise the level of public awareness of this unique fish population and to have the area designated as "sensitive and worthy of protection".

In 1927 James Dole explored British Columbia in search of "one spot of perfect beauty, - one lake of perfect (protected) fishing - one region teeming with attractions - that he and his friends could call their own...". He settled on Pennask Lake and found it to be "nearest to being the pot of gold at the end of the rainbow of any lake seen or heard of" (Read, 1977). With proper care and consideration there need be no end to the rainbow at Pennask Lake.

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THE AGATE PASS SEAPENS COHO PROGRAM: REARING HISTORY, CONTRIBUTION RATES, AND WASHINGTON STATE REVENUES AND BENEFITS

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Introduction

Marine net pens have been used since 1972 in Washington State to increase survival rates of coho yearlings, promote residency, and to imprint populations to specific geographic areas (Appleby et al, 1989), (Buckley and Haw, 1978). The Agate Pass Seapens are one of 19 marine net pen facilities producing approximately 4,000,000 coho annually (1995 data). These facilities range in capacity from 50,000 to 2,200,000 salmon and are operated either by Washington Department of Fish and Wildlife (WDFW), a Tribe, or jointly as a WDFW cooperative with a Tribe or regional group.

The Suquamish Indian Tribe (SIT) has operated the Agate Pass Seapens continuously since 1981. This program has been made possible by a cooperative agreement with WDFW. WDFW provides the smolts and fish food and the SIT provides the facility and staffing. The Agate Pass Seapens are located directly west of Seattle in Puget Sound adjacent to the Kitsap Peninsula. These waters comprise an important usual and accustomed fishing area for the Suquamish people and for local sport fishers.

This report presents an overview of the Agate Pass Seapens rearing program, fish health and marine mortality, and multiple interpretations of coded-wire tag data. Contribution rates to all fisheries are presented using recovery data of Agate Pass Seapens CWT groups for 11 of the 14 brood years between 1979 and 1992. Total catches of Agate Pass Seapens coho by gear and areas are detailed, with economic values calculated for Washington fisheries. Recoveries of Agate Pass Seapens coho strays are reported. Estimated survival and fishery contribution rates are compared to similar facilities and parent hatchery broodstock. Finally, a planned facility design change to a spar buoy system is discussed.

Program Overview

Two Puget Sound coho stocks (Wallace River and/or Minter Creek) are incubated at Minter Creek Hatchery, reared at Coulter Creek Hatchery, and transferred to Agate Pass Seapens as yearlings in January of each year. Weight at transfer is 15 g/fish (30 fish/lb). Freshwater is replaced by ambient saltwater (28 g/L or 28 ppt salinity) during the 45 minute tow to the Agate Pass aboard the transport barge.

The Agate Pass Seapens consist of four 8.5 m (28 ft) square pens that are 5 m (16 ft) deep and are suspended from wooden surface floats. The full rearing volume of each pen is 361 m^3 (12,500 ft^3) at slack or low current, but is reduced by 50% during full ebb or current flow. Maximum current velocity is 2 knots. The coho are usually feed a frozen diet at 1.2% body weight daily. Hand feeding spans a 3-hour period in the morning and again in the afternoon. Average food conversion is 1.4:1. Loading densities are kept below 1.5 kg/m^3 (1 lb/ft^3) at full volume, with rearing density adjusted by early releases.

Mortalities are removed two to three times weekly by scuba diver which permits enumeration of adipose fin clipped fish. The diver inspects the fish, repairs nets, and examines the seabed below the pens for food wastage. If necessary, adjustments are made in feeding rates to avoid wastage. The fish are inspected monthly by Northwest Indian Fisheries Commission (NWIFC) pathologists, or more often during epizootics or other events. Target release weight is 45-57 g/fish (8-10 fish/lb) by mid June or earlier if daily surface water temperature exceeds 13^0 C. Feeding information, growth rates, mortality, water quality, fish health data, and related operational data are entered into a hatchery management database.

Fish Health and Marine Rearing Mortality

Coho held at Agate Pass Seapens have experienced cumulative mortality levels ranging from a low of 0.3% in the 1981 brood to a high of 27.4% in the 1991 brood (Figure 1). Bacterial kidney disease (BKD), abdominal distention syndrome (Bloat), and the inability to adapt to the saltwater environment at the time of transfer have been the three major causes of mortality. Additional complications have been associated with anemia due to unexplained causes (BY 91).

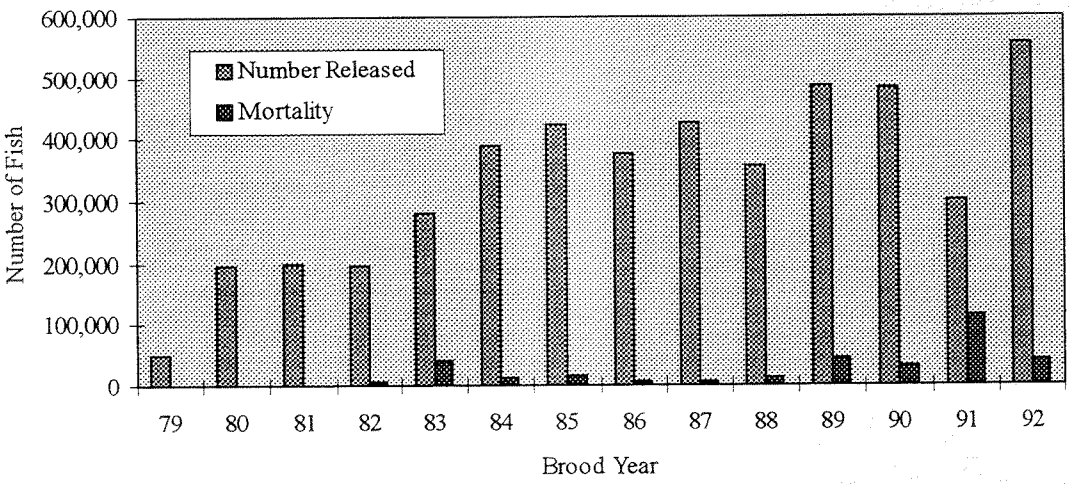

Figure 1. Agate Pass Seapens total coho release and marine mortality by brood year

The level of severity of these conditions has varied over the years. Mortality due to osmoregulatory problems at the time of saltwater transfer has been as high as 16% (BY 91). The condition known as bloat has been a chronic problem and a significant contributor to mortality levels. This condition results in fish with fluid filled stomachs and distended abdomens. Fish with bloat can be found throughout the rearing cycle and do not seem to recover. Bacterial kidney disease progresses rapidly once the fish enter the saltwater netpen environment and ultimately becomes the primary cause of mortality. Mortality due to BKD can be quite devastating. In an effort to reduce the severity of BKD infections, the 1989 and 1990 brood were experimentally treated with the antibiotic oxytetracycline. Fish were fed medicated feed (4g oxytetracycline/45.4 kg fish/day for 21 days) shortly after saltwater transfer. BKD levels were analyzed using the Quantitative Fluorescent Antibody Technique (Cvitanich, Fish Health Lab) which indicated some degree of benefit. Projected mortality due to BKD after release was estimated to be from 1 to 3%.

Other pathogens isolated from fish held in the pens have been *Aeromonas salmonicida* (causative agent of furunculosis) and *Vibrio anguillarum*. In both cases, no signs of disease occurred. Starting with brood year 1987 the coho have received a one hour immersion vaccination against *Vibrio anguillarum* during truck transport to the dock (maximum of 136 kg fish/L of vaccine at a dilution of 1:1000).

Predation accounts for less than 0.5% total mortality. Coho mortality from river otters predation is controlled by electric fences around the perimeter of the floats. Avian predation is restricted by the use of bird nets. Prompt removal of mortalities from the pens alleviates scavenger fish damage to the nets.

The mortality at Agate Pass Seapens follows an annual pattern as represented by brood year 1992 (Figure 2). Early season mortality is characterized by high initial losses due to inability to adapt to saltwater or injury during transfer. Mortality rates decrease to 0.1%/week until water temperatures rise in late April and through May. The increased temperature stress accelerates mortality in diseased or non-smolted fish. The weekly mortality rates continue to increase until release. The late season mortality rates in Figure 2 decline due to a partial release in week 20. The mortality rate for week 22 represents one day.

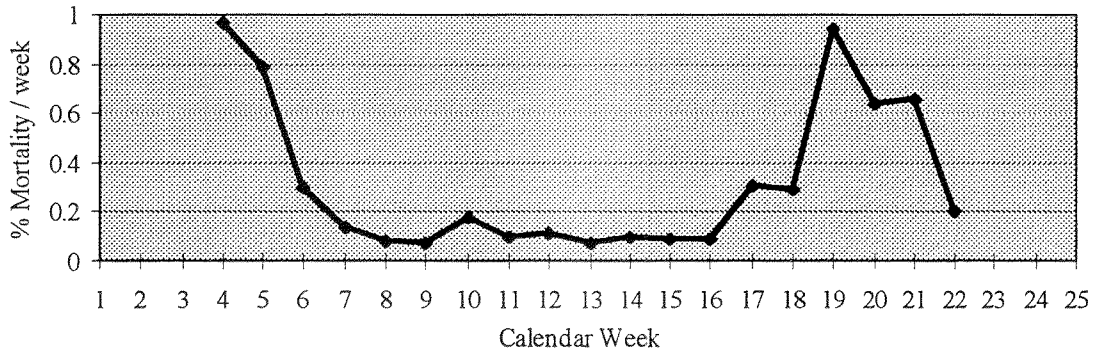

Figure 2. Brood year 1992 Agate Pass Seapens coho mortality by calendar week

Contribution to All Fisheries

Coho releases from Agate Pass Seapens have been represented by CWT groups of 29,000 to 50,000 fish per year, except for three brood years (1979, 1984, and 1991) (Figure 3). The CWT groups ranged from 8.0% (BY 90) to 24.6% (BY 80) of the total release.

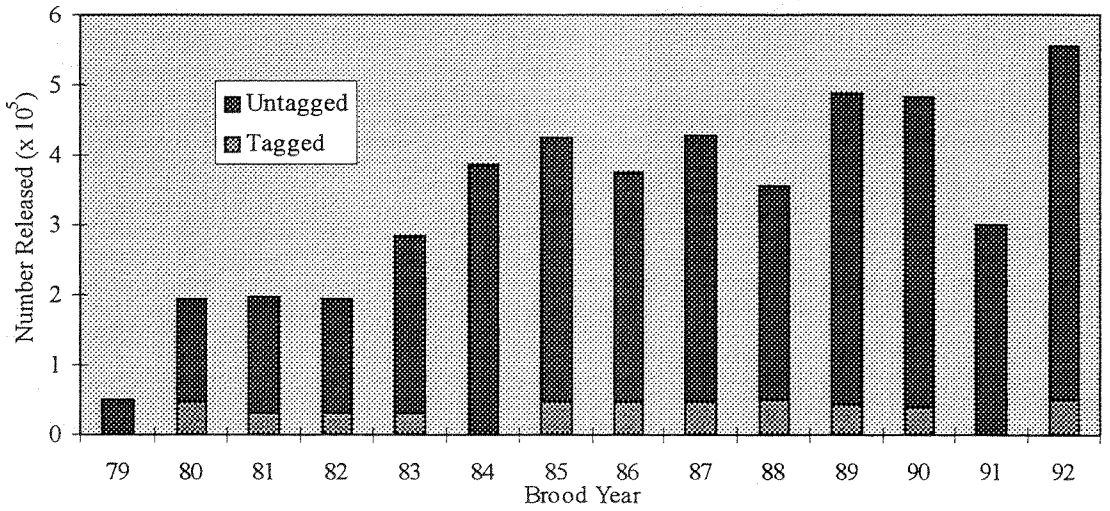

Figure 3. Coho Released by Agate Pass Seapens, showing tagged and untagged fish.

Estimates of total catch and fishery contribution rates (Table 1) and individual fishery catches (Table 2) were made from CWT recovery data obtained from the Pacific States Marine Fisheries Commission.

Brood Year	Number Tagged	Number Released	Estimated Contribution Rate by Area												
			Alaska	Canada	WA Coast and South	Strait Net and Troll	Strait Sport	Hood Canal	North Puget Sound Net	North Puget Sound Sport	Mid Puget Sound Net	Mid Puget Sound Sport	South Puget Sound Net	South Puget Sound Sport	All Freshwater
79	0	49,855	*	*	*	*	*	*	*	*	*	*	*	*	*
80	48,130	195,720	0.0	32.4	2.2	0.9	3.6	0.9	2.0	0.3	45.0	9.8	1.0	0.3	1.5
81	30,029	197,984	0.0	48.8	3.0	2.4	1.5	0.5	4.2	0.0	33.0	3.4	0.2	0.0	3.1
82	29,843	194,560	0.0	40.8	5.4	2.1	1.3	0.3	7.0	0.0	39.1	2.4	0.6	0.0	1.1
83	30,089	282,202	0.0	40.2	3.2	2.0	2.5	3.1	3.8	0.0	40.7	1.3	1.7	0.0	1.6
84	0	387,042	*	*	*	*	*	*	*	*	*	*	*	*	*
85	48,015	424,191	0.0	41.4	4.5	0.3	2.3	0.0	2.2	0.2	45.4	1.1	1.6	0.0	1.0
86	48,494	375,059	0.0	46.9	2.1	1.4	3.7	0.4	1.9	0.3	40.5	1.1	0.5	0.1	1.1
87	47,260	426,806	0.0	40.0	3.3	0.5	6.3	0.0	0.8	0.0	46.2	2.2	0.4	0.0	0.3
88	49,668	355,679	0.0	49.1	3.7	1.8	5.3	0.0	5.8	0.2	31.0	2.0	0.5	0.0	0.5
89	44,809	487,662	0.0	53.1	8.6	0.1	3.6	0.0	0.6	0.5	28.2	4.3	0.5	0.0	0.6
90	38,483	482,959	0.2	60.6	13.8	0.0	4.1	0.0	0.6	2.0	8.7	5.6	1.3	0.0	3.2
91	0	299,487	*	*	*	*	*	*	*	*	*	*	*	*	*
92	49,051	554,987	0.0	67.2	5.1	1.6	0.0	0.0	0.4	0.0	22.0	0.0	3.4	0.0	0.4
		Average	0.0	47.3	5.0	1.2	3.1	0.5	2.7	0.3	34.5	3.0	1.1	0.0	1.3

Table 1. Agate Pass Seapens coho contribution rates by brood year and area (* = No CWT releases)

Brood Year	Total Catch	Estimated Total Catch by Area												
		Alaska	Canada	WA Coast and South	Strait Net and Troll	Strait Sport	Hood Canal	North Puget Sound Net	North Puget Sound Sport	Mid Puget Sound Net	Mid Puget Sound Sport	South Puget Sound Net	South Puget Sound Sport	All Freshwater
79	*	*	*	*	*	*	*	*	*	*	*	*	*	*
80	36,672	0	11,894	799	330	1,320	330	733	110	16,502	3,594	367	110	550
81	19,476	0	9,496	585	467	292	97	818	0	6,427	662	39	0	604
82	21,703	0	8,853	1,165	456	282	65	1,519	0	8,486	521	130	0	239
83	45,685	0	18,345	1,478	891	1,142	1,416	1,713	0	18,571	594	777	0	708
84	*	*	*	*	*	*	*	*	*	*	*	*	*	*
85	76,039	0	31,512	3,414	228	1,749	0	1,673	152	34,522	836	1,217	0	760
86	61,540	0	28,846	1,288	862	2,277	246	1,169	185	24,924	677	308	62	677
87	96,271	0	38,478	3,186	481	6,065	0	770	0	44,477	2,118	385	0	289
88	44,915	0	22,055	1,654	808	2,380	0	2,605	90	13,924	898	225	0	225
89	43,957	0	23,320	3,783	44	1,582	0	264	220	12,396	1,890	220	0	264
90	23,682	41	14,350	3,278	0	971	0	142	474	2,060	1,326	308	0	758
91	*	*	*	*	*	*	*	*	*	*	*	*	*	*
92	13,509	0	9,078	684	216	0	0	54	0	2,972	0	459	0	54
	Average	4	19,657	1,937	435	1,642	196	1,042	112	16,842	1,192	403	16	466

Table 2. Agate Pass Seapens estimated total coho catch by brood year and area (* = No CWT releases)

Washington State Revenues and Benefits

Agate Pass Seapens coho contribute substantially to fisheries outside of Washington State as illustrated in Tables 1 and 2. However, Agate Pass Seapens operation is contingent upon benefits to Washington fishers exceeding the costs of providing these benefits. Revenues and benefits to Washington fisheries were calculated using values from Tables 10-12 in the 1988 Washington State Department of Community Development (DCD) Report "Economic impacts and net economic values associated with non-Indian salmon and sturgeon fisheries" . Total revenue and benefit per coho to each fishery is shown in Table 3. These values are estimates and limited to non-Indian salmon harvested within Washington waters.

Fishery	Revenue and Benefit
NPS Sport	$178.41
NPS Net	$5.60
SPS Net	$5.81
SPS Sport	$245.53
Strait Net & Troll	$6.42
Straight Sport	$147.63
WCS Charter	$115.53
WCS Net	$13.09
WCS Private	$70.06
WCS Troll	$13.51

Table 3. Total Washington State revenue and benefits per fish by selected fishery (NPS=North Puget Sound, SPS=South Puget Sound, WCS=Wa Coast and South)

Commercial revenue was calculated as total revenue generated per area divided by catch. Recreational sport benefits were calculated as total recreational benefits per area divided by catch. The values were calculated for the period 1982-1985.

These revenue and benefit values are used for discussion purposes only. It is assumed that if these values were adjusted for the Agate Pass Seapen coho brood years 1980-1992 they would be different, but within the same order of magnitude. No argument is being made that one fishery should be favored over another fishery. In addition to not valuing Canadian harvests, no value is calculated for escapement.

Tribal net catches were added to the DCD report to calculate estimated revenues and benefits to selected Washington State fisheries (WDFW memo, 1996) (Table 4). These values do not include spiritual, religious, and cultural attributes which increase the real value of salmon to Native American fishers.

Brood Year	NPS Sport	NPS Net	SPS Net	SPS Sport	Strait Net and Trol	Strait Sport	WCS Charter	WCS Net	WCS Private	WCS Troll	Total Value
80	$306,227	$12,090	$91,253	$325,329	$3,655	$187,816	$7,914	$0	$3,154	$661	$938,099
81	46,978	5,355	36,760	81,179	2,923	41,798	2,287	181	4,577	5,429	227,467
82	40,611	9,022	46,610	57,801	2,934	36,479	19,555	0	10,086	9,776	232,876
83	25,211	11,927	59,970	41,355	3,555	76,075	9,730	0	2,600	10,778	241,199
83	40,058	8,880	48,208	20,887	2,166	85,857	4,350	730	1,954	4,935	218,026
85	54,331	10,957	191,402	106,524	1,553	252,512	17,832	819	9,645	31,555	677,130
86	129,811	9,347	140,910	24,864	5,609	333,335	9,099	589	3,153	9,625	666,341
87	219,558	23,385	245,992	183,381	2,696	905,305	20,591	0	12,487	26,196	1,639,592
88	105,970	14,919	80,644	79,469	5,205	369,146	15,615	0	15,947	12,683	699,599
89	93,092	2,813	70,097	347,229	344	218,865	38,874	0	32,114	27,797	831,226
90	109,427	2,326	14,221	252,574	0	136,898	75,325	0	31,805	15,917	638,492
92	1,980	2,611	19,371	21,802	1,247	23,761	6,412	0	3,110	4,723	85,017

Table 4. Estimated revenues and benefits to selected Washington State fisheries

Actual Agate Pass Seapens coho harvest and value to the Suquamish Tribal fishers is calculated from Salmon Management Area 10E Tribal fish ticket data (Zischke, 1996) (Table 5). Table 5 does not

include the value of Tribally caught Agate Pass Seapens coho harvested in mid Puget Sound.

Brood Year	# Coho Caught	Coho Value
80	1,314	$3,626
81	2,084	$11,085
82	1,927	$13,084
83	8,411	$13,251
84	18,032	$141,842
85	14,368	$299,741
86	7,957	$93,131
87	8,685	$139,174
88	2,720	$64,164
89	1,634	$44,812
90	2,298	$12,743
91	8,676	$54,216

Table 5. Suquamish Tribal Area 10E commercial coho harvest and value

Observed Straying

The Agate Pass Seapen coho CWT data provide an opportunity to observe straying patterns. Straying is defined for this paper as freshwater recoveries outside of Washington Salmon Management Area 10E. These coho were Wallace River, Minter Creek, or George Adams Hatchery stock, transferred to Coulter Creek Hatchery for freshwater rearing. Coulter Creek Hatchery is now part of the Minter Creek Hatchery Complex. Minter Creek Hatchery stock is now the dedicated stock for the Agate Pass Seapens. Recovery locations of Agate Pass Seapens coho strays are enumerated in Table 6.

Mid and South Puget Sound	Distance from Agate Pass (km)	Wallace River Stock	Minter Creek Stock	Minter + George Adams Stock
Grovers Creek Hatchery	6	62	1	93
Cowling Creek Hatchery	6	13		
Blackjack Creek	20	1		
Seattle Aquarium	25	1		
Univ of WA Hatchery	25	3		
Garrison Springs Hatchery	55	5		3
Soos Creek Hatchery	70	1		1
Minter Creek Hatchery	75	38	1	25
North Puget Sound				
Tulalip Hatchery	50	4		
Wallace River Hatchery	130	237		
Hood Canal				
Big Beef Creek Research Hatchery	80	56		1
Quilcene National Fish Hatchery	95	1	1	
Hoodsport Hatchery	120	4		1
George Adams Hatchery	125			9
Total Number of Tagged Recoveries		50,576	1,194	9,018

Table 6. Freshwater recovery locations of Agate Pass Seapen coho by broodstock, brood years 80-92 combined, including distance from the Agate Pass Seapens

The recovery patterns of Agate Pass Seapen coho observed in Table 6 cannot be used to determine straying rates because (1) each tagged fish does not have an equal probability of being recovered during spawning ground surveys and (2) each fish in a run can not be classified as a home or stray recovery. The data support observations of Vander Haegen and Doty (1995) that hatchery salmon do not stray randomly, but return to their natal hatchery or another hatchery. The two nearest hatcheries, Grovers Creek Hatchery and Cowling Creek Hatchery, together received proportionally more strays than any other recovery locations, except for Wallace River Hatchery. All Agate Pass Seapens coho straying to Wallace River Hatchery were of Wallace River origin, suggesting the genetic component of hatchery straying. Similar results are observed for George Adams Hatchery strays from Agate Pass Seapens-- the only recoveries at that hatchery were of the single year that George Adams Hatchery broodstock was used.

Survival and contribution rate analyses

The Agate Pass Seapens estimated survival rate and estimated total fishery contribution rate was compared to three similar net pen facilities and the parent broodstock hatcheries (Figure 4). The results are based on a computer model and show that extended marine rearing may have a positive effect on survival and fishery contribution over freshwater releases. The high estimated survival of Agate Pass Seapens coho, relative to the other net pens, may be in part due to better quantification of CWT mortalities in the pens.

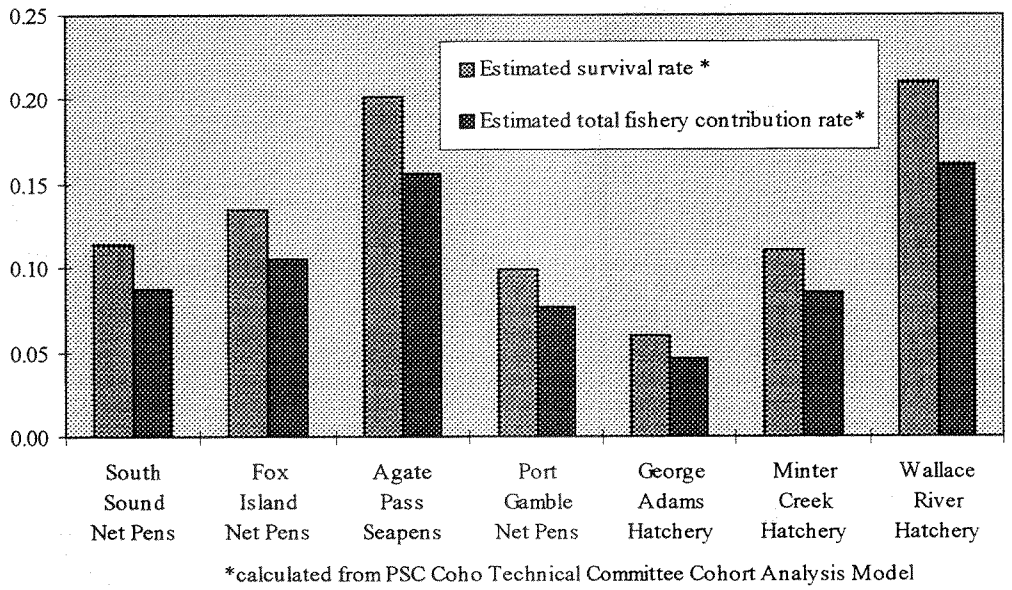

Figure 4. Survival and contribution rates of 7 selected Puget Sound and Hood Canal coho facilities.

Summary

WDFW has determined the average direct cost of salmon smolts produced in Washington State to be $3.00/lb. The cost to produce 500,000 smolts is therefore $50,000. SIT has determined the Agate Pass Seapens program direct cost to be $35,000. WDFW provides $30,000 for fish food during the extended marine rearing period. These figures total $115,000. The revenue and benefit to selected Washington fisheries ranged from $85,017 to $1,639,592 and averaged $591,255 for brood years 1980 to 1992. Given the assumptions of this simple analysis, the Agate Pass Seapens have a benefit costs ratio of 5:1 for the Washington fisheries alone.

Planned Facility Modifications

The SIT plans to replace the wood surface floats of the current Agate Pass Seapen facility with a Ocean Spar three-pen complex in the near future (Figure 5). The SIT and the Muckleshoot Indian Tribe own and operate an Ocean Spar complex in Elliott Bay, adjacent to downtown Seattle. These systems provide a constant rearing volume and more protection from predators and storms, thereby reducing stress and promoting fish health. The new Agate Pass Seapens will operate at half the rearing density of the existing system.

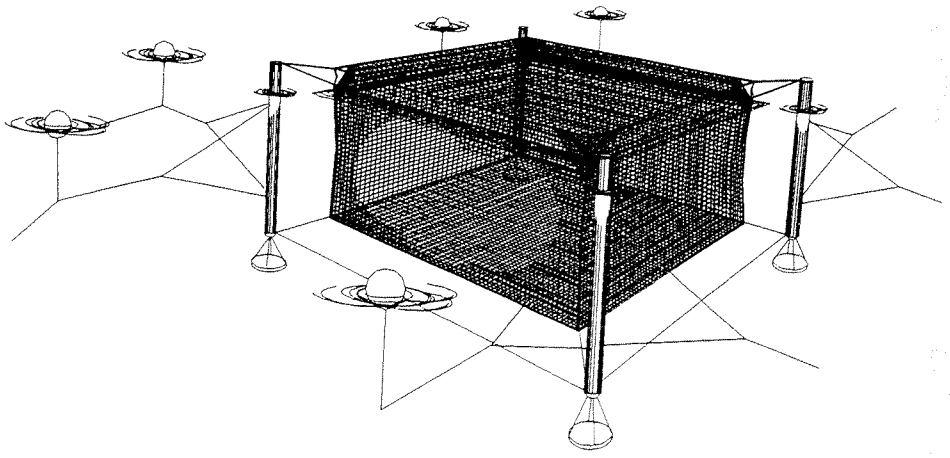

Figure 5. Schematic of an Ocean Spar net pen. The new Agate Pass Seapens will consist of three in series.

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ATLANTIC SALMON RECOVERY OPERATIONS IN MAINE RIVERS

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Abstract

Atlantic salmon (*Salmo salar*) recovery operations in Maine include restoration programs on large industrial rivers where original native stock has been extirpated, and rehabilitation programs on several small rivers where remnant populations have been reduced to critically low levels.

A "river specific" approach has been implemented to match management and stock enhancement techniques to meet the needs of each individual river. The Green Lake and Craig Brook National Fish Hatcheries utilize captured sea run broodstock, and captive wild juveniles held to maturity, to produce eggs for use in smolt and fry stocking programs.

Introduction

During the early 1800's, at least 34 of the numerous coastal rivers in what is now the State of Maine contained self sustaining Atlantic salmon (*Salmo salar*) populations. As colonization and development accelerated, habitat was degraded, destroyed or made inaccessible. Early stocking programs failed to halt the decline. By 1947, less that 10% of the original habitat remained accessible to Atlantic salmon adults (Beland, 1984).

In the 1960's, the marine feeding grounds for North American and European stocks of salmon were discovered off Greenland by the commercial fleets, and exploitation grew rapidly. The few remaining Maine stocks continued to dwindle and through the 1950's the Penobscot River stock virtually disappeared. The Penobscot had historically been the most productive of all Maine salmon rivers.

The U. S. Fish and Wildlife Service (USFWS) and Maine Atlantic Sea Run Salmon Commission (ASRSC) developed the "Model River" concept in 1967. The Penobscot was selected as the model for salmon restoration on large industrialized rivers. Improvements in water quality, installation of fish passage facilities, and increased hatchery production all contributed to an atmosphere of optimism. During the 1970's and early 1980's, returns and recreational fishing success in Maine increased, however, Atlantic salmon were being heavily exploited at sea by interception fisheries. Canadian and West Greenland fisheries had considerably reduced spawning escapement. In addition, the recreational fishery harvested 15-25% of the returning spawners. Those factors, coupled with declining ocean survival have drastically reduced Atlantic salmon abundance in all Maine rivers during the past decade.

Recently, the high seas interception fishery has been reduced and "no kill" regulations enacted on all Maine salmon rivers, but salmon survival in the marine environment remains poor. The sustained decline resulted in the potential Endangered Species listing of Atlantic salmon populations in seven small Maine rivers (Dennys, East Machias, Machias, Narraguagus, Pleasant, Ducktrap and Sheepscot). It is hoped that the poor marine environment is cyclical in nature and will improve. In the interim, the two USFWS hatcheries in Maine have reconfigured their programs to address the rehabilitation of remnant wild populations in addition to continuing to work toward restoration goals on the Penobscot.

Current Restoration Activities

The Penobscot River restoration continues to be the major restoration effort in Maine. Since the original Penobscot strain was extirpated, a synthetic strain was developed using donor stocks from local rivers in Maine and Canada. The Green Lake National Fish Hatchery annually stocks 600,000 yearling smolts and 300,000 fall parr in the Penobscot drainage. The yearling smolts are produced using heated water to accelerate growth. The hatchery features water filtration and ultraviolet treatment of rearing water.

Of approximately 64,000 salmon known to have returned to 18 U.S. rivers since 1970, approximately 47,000 (73%) have returned to the Penobscot (Kimball, 1996). Three out of every four adult salmon presently returning to U.S. waters, return to the Penobscot. Since 1980, with few exceptions, between 1,000 and 4,500 sea run fish have annually entered the Penobscot River. Of the returning fish, 500-600 are recaptured for hatchery broodstock and the remainder are passed up river to spawn.

In spite of significant spawning escapement over two decades, the adult run in the Penobscot is still hatchery dependent, with adults of hatchery smolt origin comprising 90% of the run (Figure 1).

In an attempt to accelerate natural reproduction, annual headwater stockings of 2,000,000 swim-up fry have been added to the smolt stocking program. Most of this stocking is done by canoe due to the inaccessibility of much of the habitat. It is hopeful that this technique will imprint returning adults to high quality, remote spawning and nursery habitat.

The priority information need for the Penobscot program is a thorough evaluation of natural reproduction by returning adults and subsequent juvenile survival for each of the major tributaries.

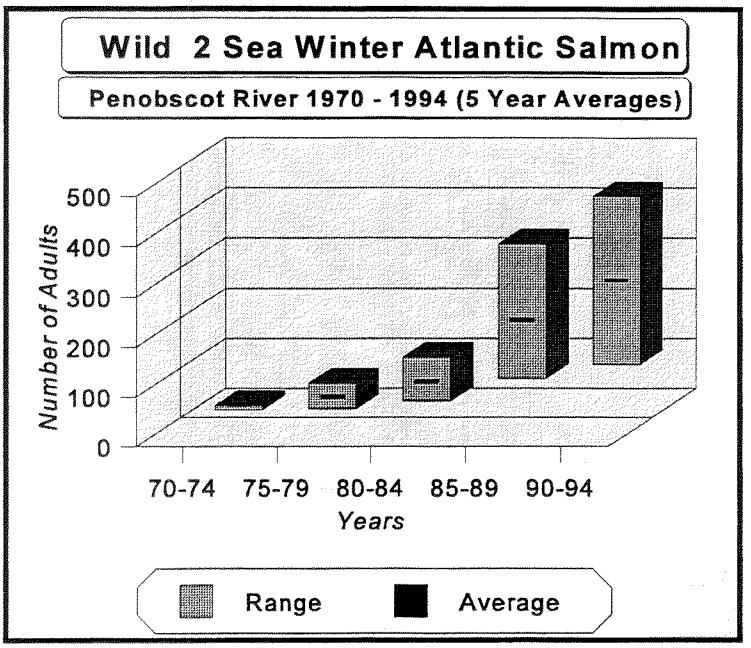

Figure 1 Wild 2 sea winter adult returns, Penobscot River, 1970-1994 (5 year averages) .

Current Rehabilitation Activities

There are seven small coastal rivers in Maine which contain the last remaining wild stocks of Atlantic salmon in the U.S. Declining returns through the 1980's and 1990's prompted the U.S. Fish & Wildlife Service to designate these river stocks as Category 2 candidate species under the Endangered Species Act in 1991.

As part of the pre-listing recovery plan, the Craig Brook National Fish Hatchery was reprogrammed from Penobscot River broodstock holding and smolt production to multi-river broodstock holding, egg and fry production. Facilities were expanded and retrofitted to hold broodstock for five of the candidate rivers in addition to the existing Penobscot sea-run broodstock program.Because of the very low number of returning adults as evidenced by sharp decline in redd counts an alternative to sea-run broodstock was needed (Figure 2).

Wild parr were collected throughout each of the drainages and brought back to the hatchery. The wild parr were held in a low stress environment and induced to feed on freeze dried krill (both *Euphasa pacifica* and *E. superba* are used). The fish are gradually weaned to a dry pelletized Atlantic salmon diet and eventually adapt to routine hatchery environment.

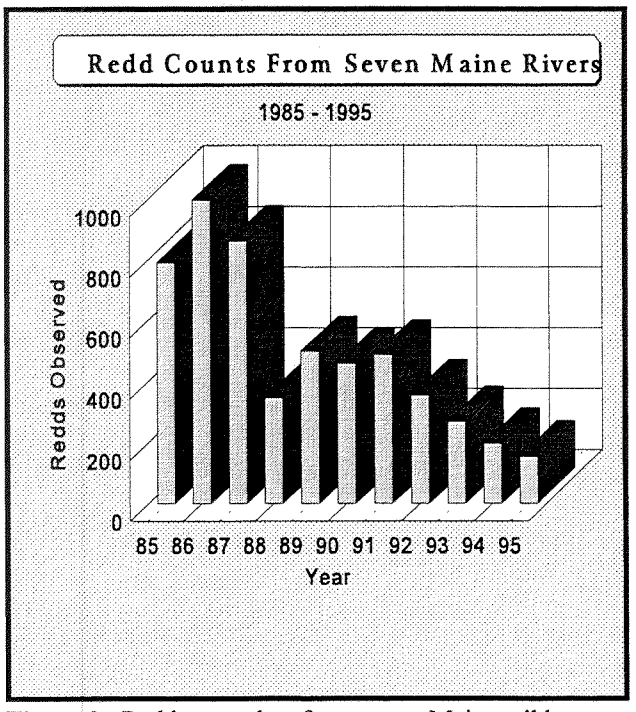

Figure 2. Redd count data from seven Maine wild salmon rivers, 1985-1995

Since 1992, more than 4,100 wild-origin Atlantic salmon parr have been collected from six Maine rivers and reared to maturity in freshwater. Survival, growth and maturity of parr was variable, possibly reflecting local adaptations in individual stocks. Overall, about 65% of the parr matured after two years in captivity at age 3. Age 3 females produced 2,140 eggs per spawner, while females maturing at age 4 and repeat spawners, combined, produced 3,680 eggs per spawner. A total of 1.2 million feeding fry have been stocked into their rivers of origin during the last two years of this program. Each parr which survived to maturity (both sexes combined) has resulted in the production of about 1,000 feeding fry for restocking purposes. Assuming 5-10% survival between fry stocking and the parr stage, a minimum of 50-100 parr will replace each of the original parr collected for the captive broodstock rearing program. Growth and survival of stocked fry originating from captive broodstock was comparable to that observed for fry originating from traditional USA stocking programs, which utilize adult, sea run broodstock as the primary egg source for restocking programs (Baum et al., 1996).

Fry stocking sites were selected to avoid competition for wild juveniles. Areas where good numbers of redds were observed the previous fall are not stocked. In many cases barriers such as beaver dams and log jams have prevented spawning access. These barriers are removed where possible and the habitat seeded with fry.

The Future

There will be more Atlantic salmon in Maine waters during the next decade than any time in history. Commercial aquaculture presently generates more gross revenues in Maine than lobster fishing. Although aquaculture has had the positive effect of decreasing the profitability of commercial salmon fishing, there is concern that sea cage escapees may genetically swamp the remaining wild stocks.

A State Atlantic Salmon Conservation Plan is being prepared as a possible alternative to Endangered Species listing. Some of the proposals include construction of weirs to stop the influx of sea cage escapees, habitat improvement and protection, and increasing public involvement in watershed stewardship activities.

Return rates for hatchery smolts showed a significant increase in 1996. Hopefully, this is a sign of improving conditions in the North Atlantic. If marine survival does not improve and we cannot achieve meaningful regulation of the high seas fishery, then the wild Atlantic salmon in the United States may well disappear in the next decade.

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CONSERVATION AQUACULTURE OF ENDANGERED WHITE STURGEON (*Acipenser transmontanus*) FROM THE KOOTENAI RIVER, IDAHO.

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Abstract.- The white sturgeon (*Acipenser transmontanus* Richardson) population in the Kootenai River was listed as endangered by the U.S. Fish and Wildlife Service on September 6, 1994 (59 FR 45989) under the authority of the Endangered Species Act of 1973. This population was listed due to the virtual absence of recruitment during the past two decades, declining population size, and post-development habitat loss and degradation. The last substantial year-class was produced naturally in 1974, the year prior to operation of Libby Dam. In 1990, the population size was estimated to be 880 individuals (88-274 cm TL; 95% CI 638-1,211), of which > 80% were older than age 21. During the late 1980's, assessment of conservation aquaculture with Kootenai River white sturgeon was proposed. During the early 1990's, conservation aquaculture began in order to maintain the population's genetic variability, reduce the threat of extinction, and counteract the lack of recruitment and decline in the size of the Kootenai River white sturgeon population. In 1991, 1992, 1993, and 1995 progeny from wild Kootenai River white sturgeon broodstock were successfully produced and reared in the Kootenai Hatchery. During these years, 7 females were mated with 14 males, producing 9 families of progeny. To date, 208 hatchery-reared progeny of wild Kootenai River white sturgeon have been stocked in the Kootenai River. Thirteen of 104 age 1 hatchery reared Kootenai River white sturgeon released in 1992 were recaptured during 1995. Following three years in the Kootenai River, these fish grew from 19 to 38 cm (TL). Fifteen of 90 age 2 hatchery reared fish were released in 1994 were also recaptured during 1995. Annual growth of these fish ranged from 0 to 10 cm (TL). Given the current status of the Kootenai River white sturgeon population (lack of recruitment, aging population, unknown and declining effective population size) and the uncertainties of recovery efforts to re-establish natural recruitment (augmented discharge), conservation aquaculture is a prudent and necessary recovery tool to ensure the existence of this endangered white sturgeon population for future generations.

The white sturgeon (*Acipenser transmontanus* Richardson) population in the Kootenai River was listed as endangered on September 6, 1994 (59 FR 45989) under the authority of the Endangered Species Act of 1973. This population was listed due to the virtual absence of recruitment during the past two decades, declining population size, and post-development habitat loss and degradation. The last substantial year-class was produced naturally in 1974, the year prior to operation of Libby Dam. The dam impounds the Kootenai River near Libby, Montana, forming Lake Koocanusa (Figure 1). Construction and operation of Libby Dam has drastically altered the hydrograph, thermograph, and downstream nutrient loading rates in the Kootenai River. Research since 1991 has confirmed natural spawning in four of the past five years; however, natural recruitment has been virtually absent since 1974 (n=16).

The Kootenai River white sturgeon population was estimated to be 4,000-6,000 individuals in 1981 (Graham, 1981). Using tag recovery data from 1979-1981, Partridge (1983) estimated the population to be 1,148 fish (50-224 cm TL; 95% CI 907-1503). In 1990, the population size was estimated to be 880 individuals (88-274 cm TL; 95% CI 638-1,211) (Apperson and Anders, 1991). The 1990 estimate was not statistically different from the previous estimate of 1,148, however, these estimates were not directly comparable since they covered different geographic areas (Giorgi, 1993). Apperson and Anders (1991) reported that natural mortality was 3.74%, sex ratio was approximately 1:1, and that 7% of female and 30% of male white sturgeon in the Kootenai River reproduce annually. Assuming a 3.74% annual mortality rate and the 1990 population estimate of 880 fish, 153 wild white sturgeon died between 1990 and 1995. The number of female spawners was approximately 25 in 1995, assuming 7% of the female population was reproductive. Accordingly, another 101 fish will be lost from the population during the next five years (1996-2000) resulting in only 21 reproductive females by the year 2000. No recent population estimates for this population exist. Updated estimates should be completed by the fall of 1996.

Since the formation of Bonnington Falls on the Kootenay River (west of Kootenay Lake B.C.) approximately 10,000 years ago, white sturgeon in the Kootenai River system are believed to have been landlocked between Kootenai Falls, Montana, and Bonnington Falls B.C. (Figure 1). Genetic analysis (electrophoresis) of white sturgeon populations has revealed that the Kootenai River population has the lowest average heterozygosity (0.54) compared to that of white sturgeon populations in the Columbia River (0.74; Setter and Brannon, 1990; Setter and Brannon, 1992). Subsequently, the Kootenai River white sturgeon population has been reported by the U.S. Fish and Wildlife Service as a genetically separate population (59 FR 45989).

During the late 1980's, assessment of conservation aquaculture was proposed. During the early 1990's, conservation aquaculture began to maintain the population's genetic variability, reduce the threat of extinction, and counteract the lack of recruitment and decline in the size of the Kootenai River white sturgeon population. In 1991, an experimental aquaculture program began to assess Kootenai River water quality, white sturgeon gamete viability and the feasibility of aquaculture as a component of population recovery. In 1991, 1992, 1993, and 1995 progeny from wild Kootenai River white sturgeon broodstock were successfully produced and reared in the Kootenai Hatchery. In 1993, a breeding plan to preserve the genetic variability of the Kootenai River white sturgeon population was prepared (Kincaid, 1993) to guide operations at the Kootenai Hatchery.

In light of the need to prevent extinction and preserve the remaining genetic variation of the Kootenai River white sturgeon population, the objectives of this paper are to describe benefits and techniques of conservation aquaculture as a component of population recovery.

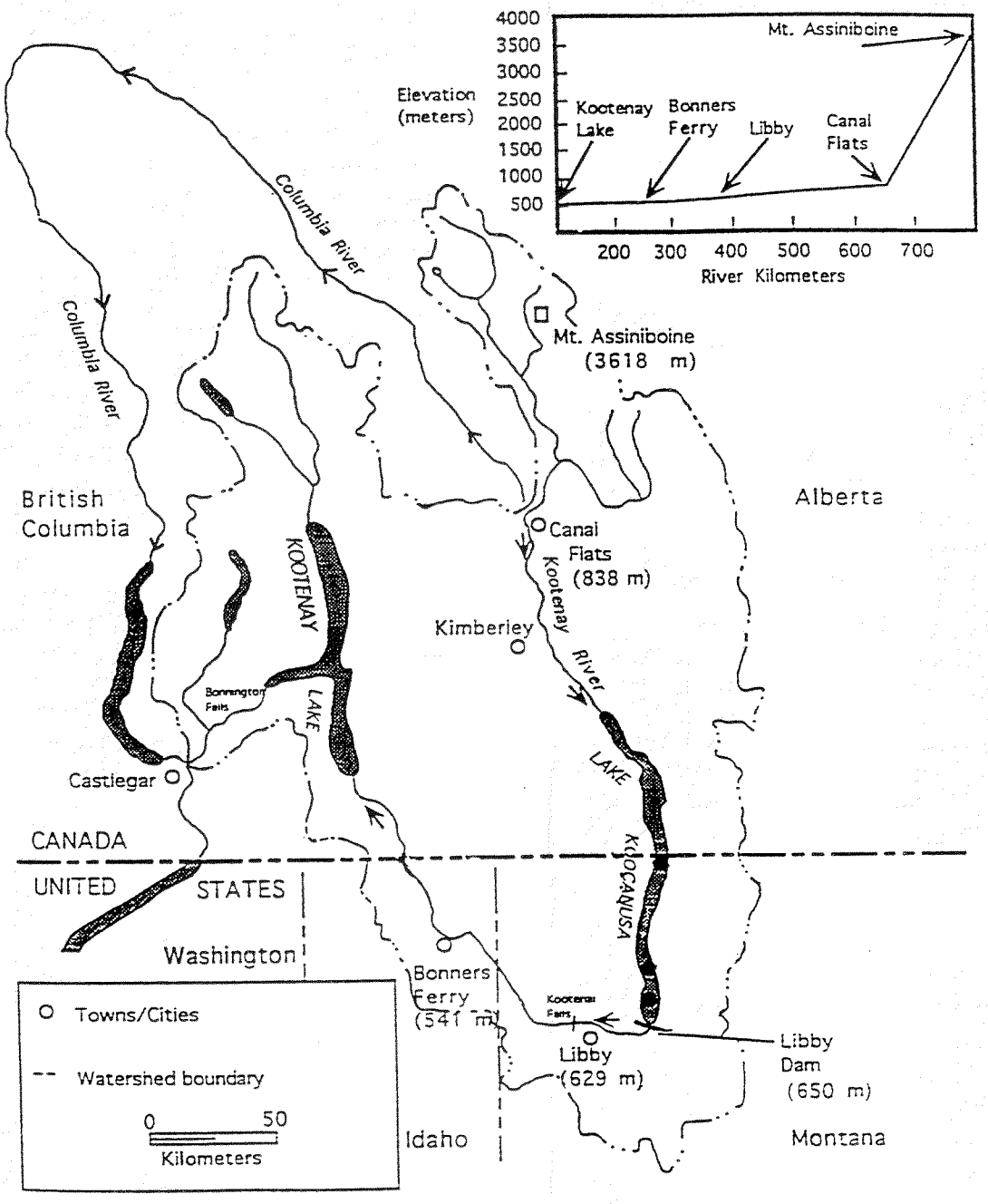

Figure 1. Kootenai River drainage.

Study Area

From headwaters in southeastern British Columbia, the Kootenai River flows south into northwest Montana where it is impounded by Libby Dam near the town of Libby, forming Lake Koocanusa (Figure 1). Downstream from Libby Dam, the Kootenai River flows westerly into the northeast corner of Idaho, turns north, enters British Columbia and Kootenay Lake. The Kootenay River then flows out the West Arm of Kootenay Lake, joining the Columbia River at Castlegar, B.C. Kootenai Falls, Montana forms an upstream migration barrier and Bonnington Falls, B.C. blocks downstream migration isolating white sturgeon to within a 270 km reach of the Kootenai River system in Montana, Idaho, and B.C.

The Kootenai River consists of three habitat types or reaches: canyon, braided channel, and meandering. Immediately downstream from Libby Dam, the river flows through a narrow steep-sided canyon into Idaho. This canyon reach is characterized by swift water and substrates of gravel and larger particle size. The braided channel reach is located from Bonners Ferry, Idaho upstream 10 km and is characterized by reduced gradient, and shallow braided gravel channels. The meandering reach of the Kootenai River exists from Bonners Ferry downstream to Kootenay Lake (Figure 1).

Pre-impoundment

Prior to impoundment (before Libby Dam completion in 1974) the Kootenai River hydrograph was characterized by average spring discharge peaks of approximately 1,600 cubic meters per second (m3/s; 58,000 cfs) (Figure 2). The pre-impoundment Kootenai River was also characterized by a 4 to 6 km wide floodplain in the furthest downstream 128 km of the river. Diking of the furthest downstream 128 km of the Kootenai River (from 1920's to 1950's) in Idaho eliminated approximately 50,000 acres of natural floodplain from Bonners Ferry, Idaho to the International Border. Estimates of floodplain area lost to diking in B.C. may be equal or greater. Naturally produced year classes of white sturgeon were frequently documented in the Kootenai River before 1974. The self-sustaining provided a viable commercial and recreational fisheries.

Post-impoundment

The Kootenai River can be characterized as a collapsed aquatic ecosystem following diking (channelizing), impoundment, and resulting denutrification. The effects of ecosystem collapse on early life stages and lack of juvenile recruitment remain unknown. After Libby Dam was completed in 1974 peak spring discharges were reduced up to 67% of pre-impoundment values (Figure 2). Libby Dam (and the impounded Lake Koocanusa) have acted as a nutrient sink effectively reducing downstream transport of phosphorous and nitrogen by 63 and 25 % (Woods, 1982), with sediment trapping efficiencies exceeding 95 % (Snyder and Minshall, 1995). A drastic shift in fish species composition downstream from Libby Dam (favoring omnivorous species) has also accompanied impoundment of the Kootenai River (Paragamian, 1994). The last substantial natural year class of white sturgeon in the Kootenai River was produced in 1974 (Apperson and Anders, 1991).

Methods

Broodstock Collection

From April through June 1991, 1992, 1993, and 1995, wild Kootenai River white sturgeon broodstock were collected by angling. Captured fish were placed ventral side up in a hooded vinyl stretcher suspended across the boat gunwales. Fish in the stretcher were periodically provided with fresh river water. Sex and reproductive development of captured fish were determined by visual observation through a 2-3 cm midline incision on the ventral surface of the fish between the 3rd and 4th ventral scutes anterior to the vent. In preparation for examination, this area was treated with a 4% nitrofurazone antibacterial solution. Alison forceps were used to collect a gonadal tissue biopsy to confirm gonad development. All captured males were checked for maturation on the river by inserting a tygon tube (5 mm diameter) into the genital opening and siphoning sperm out with an attached 20 ml plastic syringe. If sperm was collected, or if a gonad inspection determined that male and female

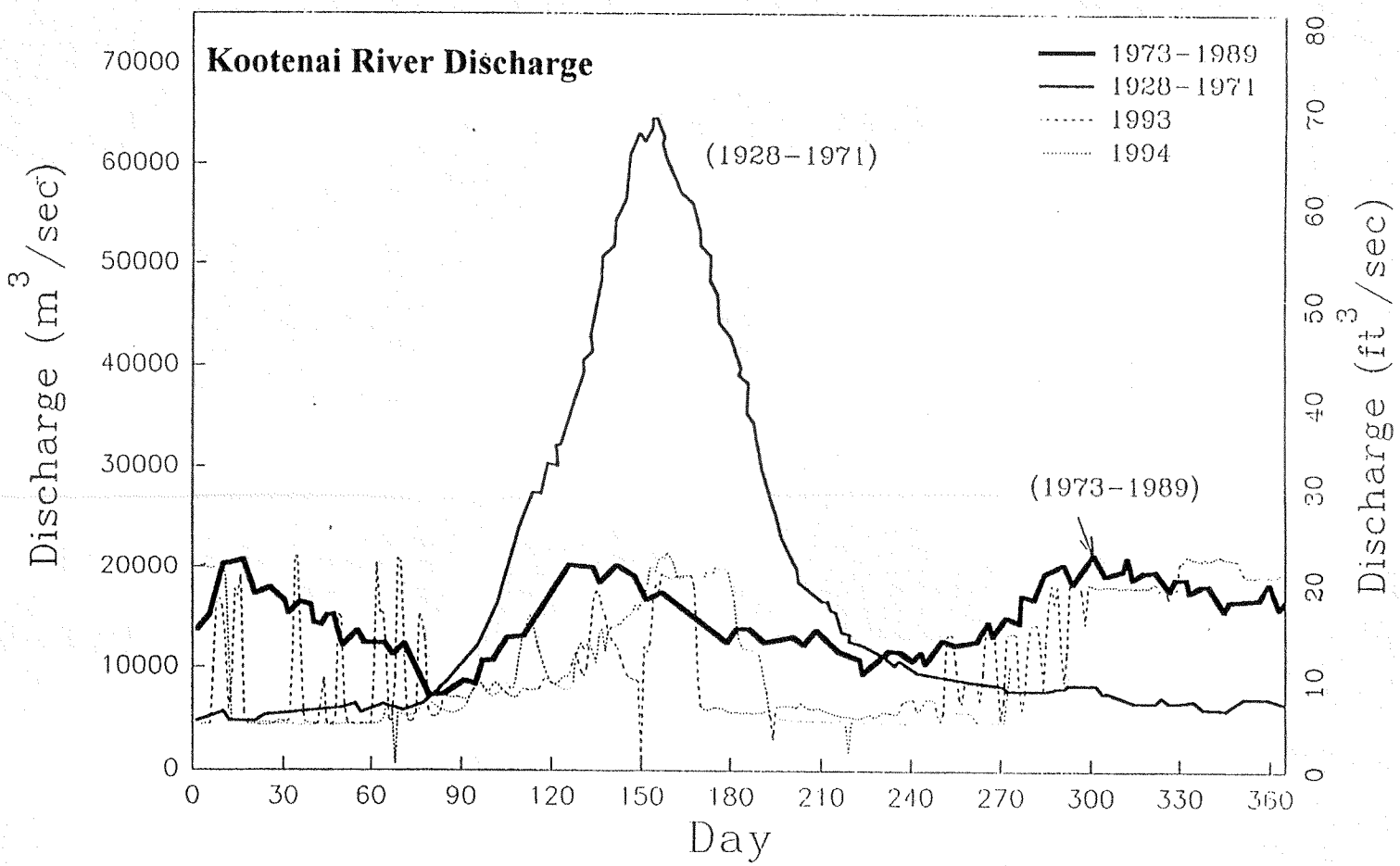

Figure 2. Discharge records from pre- (1928-1971) and post- (1973-1989; 1993-'94) Libby Dam.

white sturgeon were sexually mature, they were transported to the hatchery in an oxygenated tank truck. Non-reproductive males and females were immediately released back into the river.

Reproductive development of males and females was categorized according to criteria reported by Conte et al. (1988). All fish examined were checked for recapture, the removal of a scute, and marked with a PIT tag (Passive Integrated Transponder, Destron Inc. Boise, ID. USA) in the dorsal musculature ventral to the dorsal fin. All fish were measured (FL, TL cm) and weighed, and a pectoral fin ray section was removed for age determination. Each fish was also marked with an individually numbered external dart tag (Floy Tags Inc. Seattle, WA. USA) and by removal of the second anterior left lateral scute. Once all data were collected and sex and reproductive status were determined, fish were either brought to the hatchery for spawning in an oxygenated tank truck or released immediately back into the river.

Adult female white sturgeon brought into the hatchery were periodically examined for oocyte development and degree of maturation. Examined fish were guided into a hooded vinyl stretcher with the anterior end of the fish covered by the hood. Once the fish was secured in the stretcher it was rolled ventral side up, declined slightly at the anterior end to keep the head submerged. A piece of tygon tubing (3 cm dia.) was then fitted in the mouth of the fish to provide a constant source of oxygenated water over the gills. Using the surgical procedure described above to determine reproductive status of wild broodfish in the field, 10 to 20 eggs were removed and microscopically observed to determine maturation and timing for hormonal induction of ovulation.

Spawning Broodstock

All adult white sturgeon spawned in the Kootenai Hatchery were wild, collected from the Kootenai River by angling one to two months prior to spawning. Adult white sturgeon were spawned in the Kootenai Hatchery during June and July and released back into the Kootenai River after several weeks of post-spawning observation.

The following criteria were used to determine timing of hormone injections to most effectively induce ovulation: 1) appearance, shape, and attresia of eggs; 2) egg diameter through long axis; 3) position of the germinal vesicle (GV); 4) progesterone egg maturation assay producing germinal vesicle breakdown (GVBD), and 5) Oocyte Polarization Index (PI) values (Siple and Aitken, 1992; Conte et al. ,1988; Van Eenaanam et al., 1996).

Females

Once eggs from female broodstock exhibited GVBD (Conte et al., 1988) and a PI value of ≤ 0.12 (Van Eenaanam, 1996), hormone injections to induce ovulation were initiated. Two injections, primary and resolving doses of lutenizing hormone releasing hormone analogue (LHRHa) were administered. The primary dose consisted of 10% of a 0.1 mg/kg body weight injection, followed in 12 hours by a resolving dose of 90% of 0.1 mg/kg body weight LHRHa. Following the resolving dose injection, the female was returned to a 1.0 X 0.67 X 3.0 m holding tank, which was viewed hourly to observe the onset of ovulation. Ovulation was defined by the presence of at least 100 eggs shed into the spawning tank. Depending on water temperature in the spawning tank, egg removal (hand stripping) began 4 to 7 hours after the first signs of ovulation. This 4 to 7 hour delay provided time for eggs to be shed inside the body cavity, allowing subsequent hand-stripping to massage them through the oviduct.

Males

To date, male white sturgeon in the Kootenai Hatchery have not received hormone injections. A 30 ml sperm sample from each male was collected several hours after administering the females LHRHa resolving dose. A portion of sperm from each 30 ml sample was checked microscopically to determine motility duration when water activated. Sperm motility greater than 2 minutes was required for fertilizing eggs. The remainder of unused, motile sperm samples were stored in sealed plastic bags with pure oxygen and refrigerated at 4-5° C.

Egg Removal and Fertilization

During 1991 and 1992 Cesarean surgery was used to remove eggs from the body cavity of ovulating white sturgeon broodstock. In 1993, eggs were collected by Cesarean surgery and hand-stripping. In 1995, eggs were removed only by hand-striping. The Hand-stripping greatly minimizes stress associated with Cesarean surgery and reduces the recovery period of post-spawning adult white sturgeon prior to release back into the wild.

Results

In 1991, 1992, 1993, and 1995 progeny from wild Kootenai River white sturgeon broodstock were successfully produced and reared in the Kootenai Hatchery. During these years 7 females were mated with 14 males producing 9 families of progeny (Table 1). In all years except 1995, sperm from more than one male was combined to fertilize eggs. In 1995, sperm from each male was used to fertilize a separate batch of eggs producing four families, (or two pairs of half-sibling families, each with a shared female parent).

To date, 208 hatchery-reared progeny of wild white sturgeon broodstock have been released into the Kootenai River (Table 2). To date, 208 hatchery-reared progeny of wild Kootenai River white sturgeon have been stocked in the Kootenai River. Thirteen of 104 age 1 hatchery reared Kootenai River white sturgeon released in 1992 were recaptured during 1995. Following three years in the Kootenai River, these fish grew from 19 to 38 cm (TL). Fifteen of 90 age 2 hatchery reared fish were released in 1994 were also recaptured during 1995. Annual growth of these fish ranged from 0 to 10 cm (TL).

Table 1. Crosses of wild Kootenai River white sturgeon in the Kootenai Hatchery, 1991-1995 as part of the conservation breeding program.

Year	Females	Males	# Families Produced
1991	1	2[a]	1
	1	2[a]	1
1992	1	2[a]	1
	1	1	1
1993	1	3[a]	1
1994[b]	0	0	0
1995	1[c]	2	2
	1[c]	2	2
Total	7	14	9

a = Sperm from > 1 male was pooled to fertilize eggs from each female.
b = No fish spawned during 1994.
c = Eggs from each female were fertilized separately with sperm from two different males.

Table 2. Data from twenty-eight hatchery-reared white sturgeon recaptured from the Kootenai River during 1995.

Year class	Number	Release year	Capture year	Percent (#) Recaptured
1990	14	1992	none	0
1991	104	1992	1995	12.5 (13)
1992	90	1994	1995	13.3 (15)
Total	208			13.5% (28)

Discussion

The risks and benefits of conservation aquaculture must be evaluated on a case by case basis. The Kootenai River white sturgeon population is aging (>80% is older than 20 years) and has very limited natural recruitment (n=16) represented since 1974. This population condition will result in a missing generation of spawners in another 15-20 years because the youngest reproductive male and female in this population were estimated to be 16 and 22 years of age. Augmented discharge regimes in the Kootenai River during 1991, 1993, 1994, and 1995 designed to stimulate natural spawning and recruitment have not restored natural recruitment. Juvenile recruitment (natural or artificial) is needed to rebuild the age-class-structure of the Kootenai River white sturgeon population to reduce the threat of extinction and preserve the remaining genetic variation.

To address this problem, a breeding plan to preserve the genetic variability of the Kootenai River white sturgeon population and to begin rebuilding the natural age class structure was developed (Kincaid, 1993). This plan is designed to maximize the number of different adults contributing progeny to the population over time while minimizing contribution of any one sibling group. Under this plan 3 to 6 different females will be spawned annually, and a sufficient number of fish will be released to produce an estimated 10 spawners per family at age 20.

In addition to preserving genetic variability, rebuilding a healthy natural-age-class-structure and preventing extinction, conservation aquaculture of Kootenai River white sturgeon provides other important benefits. Artificial production of early life stages and juvenile white sturgeon using a genetically sound conservation aquaculture program (Kincaid, 1993) will allow identification of limiting factors for wild egg incubation, hatching, and rearing and survival of early life stages in the Kootenai River. Release of hatchery-reared juvenile white sturgeon into the Kootenai River also provides valuable information on growth, habitat use, and feeding ecology.

Given the current status of the Kootenai River white sturgeon population (lack of recruitment, aging population, unknown and declining effective population size), and the uncertainties of recovery efforts to re-establish natural recruitment (augmented discharge), conservation aquaculture is a prudent and necessary recovery tool to ensure the existence of this endangered white sturgeon population for future generations. The Kootenai River white sturgeon conservation aquaculture program, in combination with augmented discharge, (Marotz et al. 1996) is the most reasonable approach to population recovery. Kincaid stated "the notion that these two approaches are incompatible is a misconception. There is no biological reason to prevent simultaneous implementation of both approaches. Indeed, when the advantages and disadvantages of both approaches are considered in light of the current "endangered" status of Kootenai River white sturgeon, simultaneous implementation of both approaches (augmented discharge and conservation stocking) seems to offer the highest probability to protect and preserve the genetic variability of the Kootenai River white sturgeon population".

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EFFECT OF HABITAT DEGRADATION ON SALMON ENHANCEMENT EFFORTS IN THE TSOLUM WATERSHED

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Abstract

The Tsolum River has abundant spawning and rearing habitat. Historically this river supported large runs of pink and coho salmon and steelhead and cutthroat trout. Declines of pink salmon in the late 1960's led to enhancement efforts in the 1970's. This program was initially successful and runs of pink salmon were rebuilt. Despite continued enhancement, populations declined rapidly through the 1980's. This declining trend led to a review of the program that revealed two outstanding environmental problems. Firstly leachate from an abandoned copper mine in the upper watershed degrades water quality in the Tsolum River. Secondly extreme low flows in drought years block adult migration and reduces useable rearing habitat. The situation is made worse by water extraction for domestic and agricultural use. These environmental problems combine to greatly reduce the effectiveness of enhancement efforts on the Tsolum River.

Introduction

The Tsolum River is a low gradient stream that flows parallel to the coastal plain of Vancouver Island for approximately 31 km. It originates in a series of marshes and small lakes (Blue Grouse, Lost and Helldiver) and enters the Puntledge river near Courtenay (Fig. 1). The major tributaries are: Murex, Constitution, Dove, Headquarters and Portuguese Creeks. The stream has good spawning gravel and a total spawning area of 200,000 sq. m. (Brown et al. 1977). There is extensive coho rearing habitat in the upper reaches and in the tributaries.

There is intensive agricultural and urban development along the lower reaches and water is withdrawn both for domestic use and for irrigation. The river is naturally subject to low summer flows and so a concrete dam was constructed in 1964 to store water in Wolf Lake. This water is released during summer and fall to augment flow in the lower Tsolum. Adequate flows are required for upstream migration and spawning of pink salmon.

Murex Creek is an important tributary that flows from the Mt. Washington area to the upper Tsolum River. When the snow melts in the spring the flow in Murex Creek greatly increases and forms the bulk of the flow in the lower Tsolum. This tributary is also important because it drains an abandoned copper mine on Mt. Washington (Fig 1). This mine was operated from 1964 to 1967 by Mt Washington Milling Co. Ltd. and copper leaching experiments on the waste rock were conducted

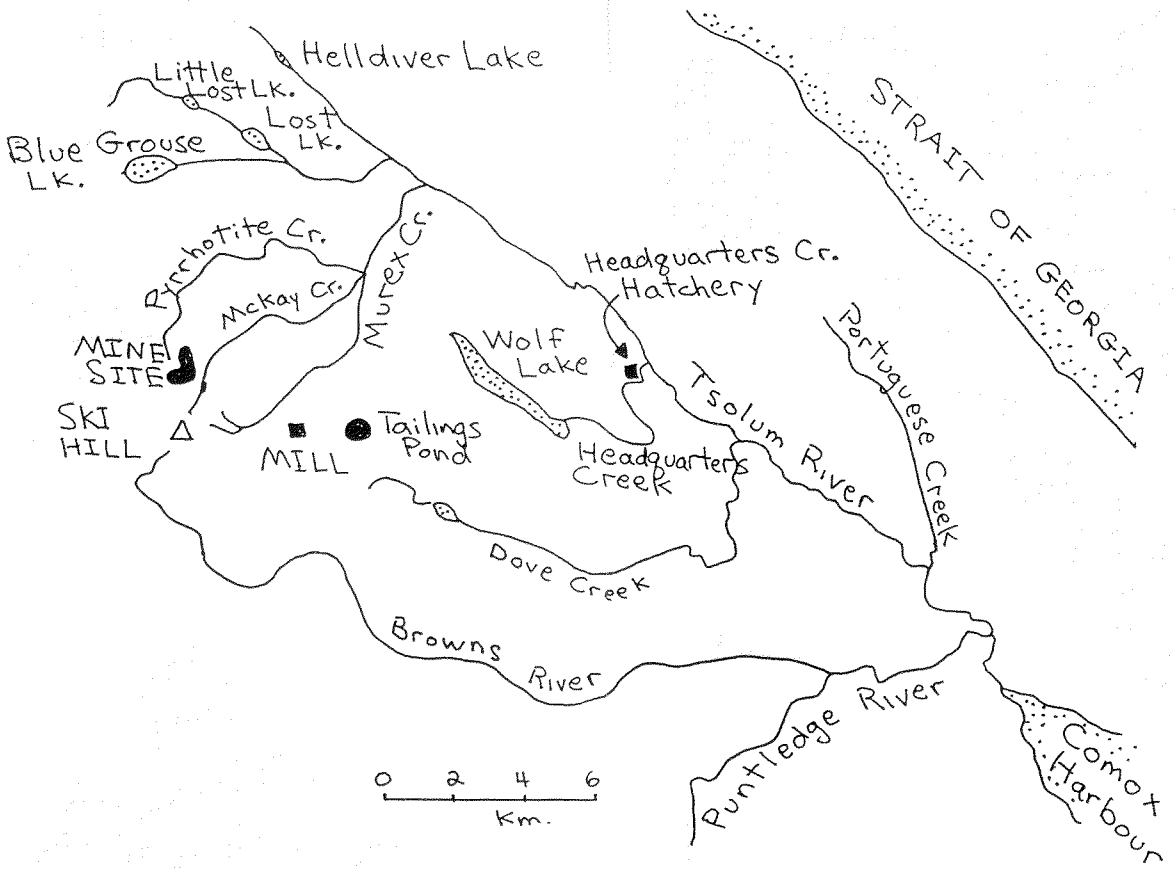

Fig.1. Tsolum watershed

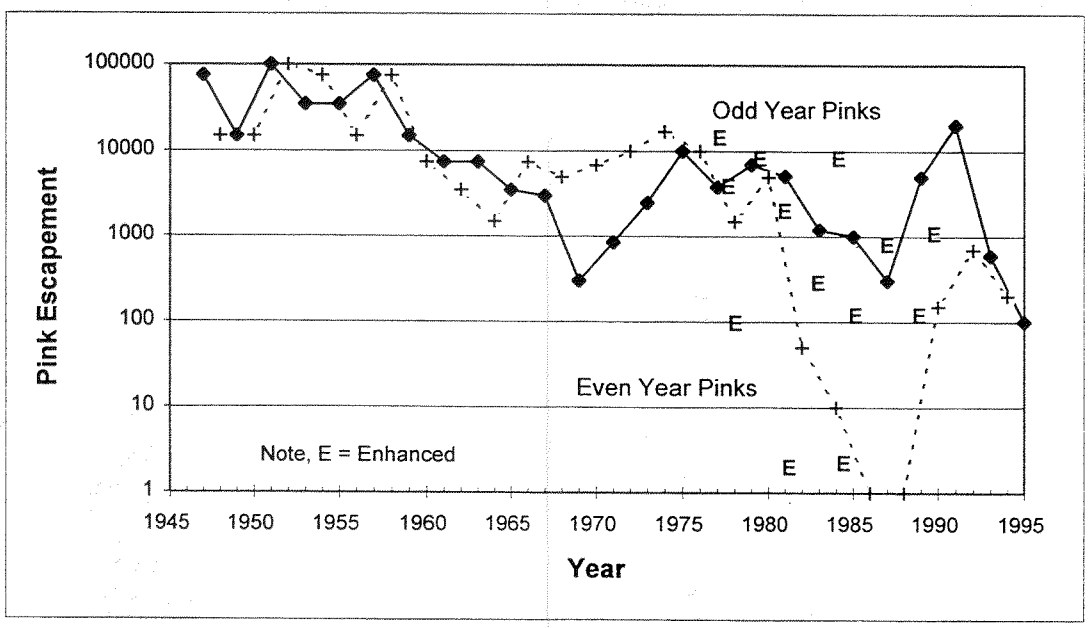

Fig. 2. Odd and even year pink escapements for Tsolum River

at the site by Esso Resources Ltd. in 1978/79. Sulfuric acid was injected and waste-rock was inoculated with bacteria to enhance the leaching of copper. When these experiments were completed in 1979 the site was neutralized with lime and acid generation was presumably terminated. However by the mid 1980's exposed pyrite at the mine site was again generating acid and high concentrations of copper were detected in Murex Creek and in the downstream 18 km of the Tsolum River.

Salmon enhancement on the Tsolum started in 1964 with the construction of the Wolf Lake flow control structure. Since that time a variety of techniques have been used for enhancement of pink, coho and steelhead populations. Because of limited success, efforts have been redirected to solving the environmental problems in the watershed.

Enhancement Efforts

The Wolf Lake reservoir theoretically can supply 25 cfs to Headquarters Creek for 6 weeks during low flow periods (3200 acre ft). This reservoir was designed to augment flows in the lower Tsolum and assist pink salmon during their upstream migration. In 1968 gravel boxes were built on Headquarters Creek and an egg incubation program was started for pinks (Bams and Crabtree 1976, Bams 1972). This program was continued in 1970 and 1972 (Bams 1979). Approximately 200,000 to 330,000 unfed fry were released to Headquarters Creek per year from gravel boxes. In 1969 the odd year cycle collapsed and in 1971 500,000 pink eggs were moved from Kakweiken River (Bams 1976). Most of these eggs were fertilized with sperm from Tsolum males and incubated in gravel boxes. By the middle seventies both odd and even year runs were re-established to approximately 10,000 fish (Fig. 2).

In the fall of 1979 enhancement of pink salmon was started again and eggs were collected from Tsolum Pinks. This resulted in 52,000 fry being released to the Tsolum in the spring of 1980. In the fall of 1980 a full scale enhancement effort began with the collection of over 1.2 million eggs. Eggs were incubated in gravel boxes or in keeper channels. Unfed fry were released to the Tsolum (668,000) and to Puntledge (126,000) in the spring of 1981. Some fry (93,000) were also reared in seapens and released at a larger size to increase ocean survival. Also 129,000 fry from natural spawning in Headquarters Creek migrated downstream to the Tsolum river. Table 1 summarizes enhancement effort on the Tsolum. Results of wild fry enumeration at Headquarters Creek fence are also shown. It should be noted that since 1985, large numbers of eggs have been imported yearly from Quinsam Hatchery.

Pink escapements are shown for odd and even years from 1964 to 1995. Years where there was significant enhancement are labeled with an E. Returns in a particular year are the result of natural spawning and enhancement effort two years before.

Coho fingerlings were transplanted to the Little Lost Lake, Helldiver Lake and Blue Grouse in the summer of 1984 (Aug 31/84, 7.4 g), 1985 (Aug 31-Sept 18/85, 2.8 - 4.2 g), 1986 (July 14-15/86, 3.7 - 3.9 g) and 1990 (Dec 12/90, 11 g) from Puntledge Hatchery. Smolts (> 20 g) were also released from Headquarters Creek Hatchery on May 21/86 (54,000) and on May 6/90 (101,000). Coho smolts from these releases would have migrated down the Tsolum river to the sea in the spring of 1985, 86, 87, 90 and 1991.

Effects of Copper Contamination

Acid generation at the Mt Washington Mine site mobilizes copper which flows into Pyrrhotite Creek. This stream contaminates Murex Creek which flows to the Tsolum River (Fig. 1). With snow melt in the spring, Murex Creek is the bulk of the flow in the Tsolum River and so downstream copper concentrations remain high. Copper concentrations in Murex Creek, mid Tsolum, lower Tsolum and upper Tsolum over a typical spring are shown in Fig. 3. Copper is elevated from April to June and peaks in May or June often reach 100 ug/L. During this period the copper concentration in the Tsolum River upstream of the Mine site is below 2 ug/L (labelled Upper Tsolum in Fig. 3).

Copper has a variety of sublethal effects and is lethal at high concentrations. The LC50 depends on species and life stage and on water hardness, temperature and humic acid concentration. Bioassays performed at Puntledge hatchery and throughout the Tsolum watershed by

Table 1. Pink enhancement on Tsolum River. Wild fry migrating from Headquarters Creek are also shown. These fry are result from previous years escapement and also from adults trucked from Puntledge Hatchery.

Brood Year	Number of fry released the following year: Unfed	Fed	Wild (fence count)	Adults moved from Puntledge
1979	51,547			
1980	668,317	93,299	128,898	
1981	571,459	142,494		
1982	No enhancement			
1983	41,156			
1984	No enhancement			
1985	No enhancement			
1986	No enhancement			
1987			580,000	9,764
1988			386,000	2,741
1989	No enhancement			
1990				3,705
1991	No enhancement			
1992	816,000			
1993	100,000			
1994	100,000			
1995	No enhancement			
1996				5,400

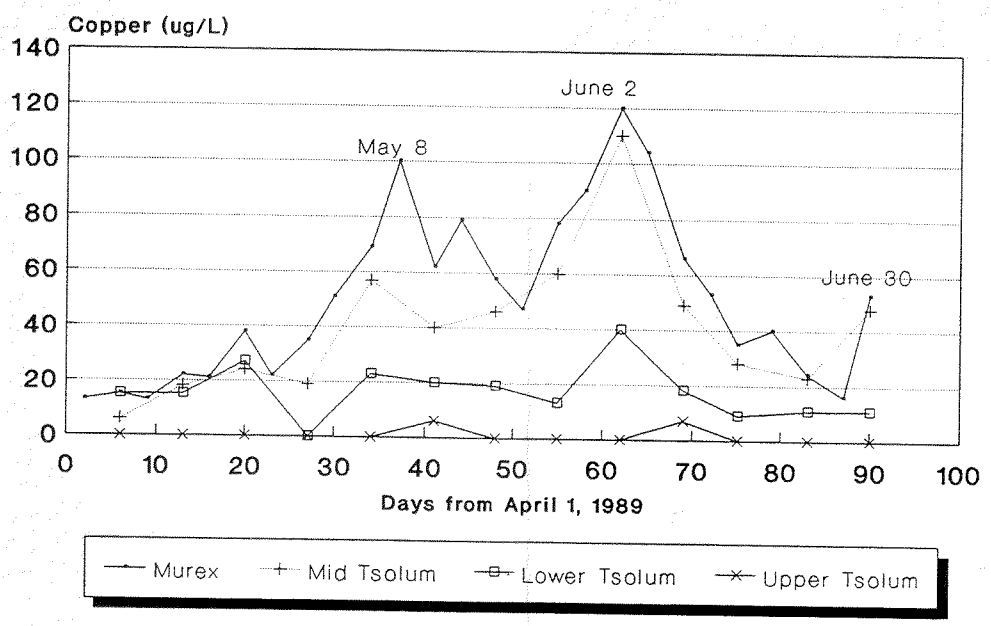

Fig. 3. Copper concentrations during the spring of 1989.

Ministry of Environment staff have been performed (Deniseger et al. 1995). Fish preparing to make their migration to seawater (smolts) were the most sensitive life stage. The copper concentration to kill 50 % of a sample of coho smolts in 96 hours (96hr LC50) was 23 ug/L (95% confidence interval 19-28 ug/L). These results were for soft water with little complexing capacity (humic acids). It should be noted that the ability to make a successful migration to seawater would be impaired at even lower copper concentrations.

These bioassays as well as a review of the scientific literature were used to develop a set of copper objectives for the Tsolum watershed. These objectives provide target concentrations for the mine clean-up program. Target values take background water quality, species and life stage into account. To protect aquatic resources in the watershed the maximum copper concentration must be less than 7 ug/L and the 30 day average must be less than 11 ug/L (Deniseger et al. 1995). To achieve these objectives there must be a 95% reduction in the discharge of copper at the mine site.

From these results it is speculated that coho and steelhead are more affected by copper contamination than pink salmon. Coho and steelhead fingerlings reside in the river year around and make the transition to seawater in the mid to late spring. This would coincide with peak copper levels. Pink salmon fry however move to sea between late March and early April -- prior to high copper. However this is not always the case, on some years copper concentrations of 50 ug/L have occurred in early April. These levels are high enough to detrimentally affect the downstream pink migration.

Copper concentrations have not been intensively monitored at other times of the year. It is known that high copper levels can occur in Murex Creek in the fall and during winter thaws. Downstream effects of these events on the Tsolum may not be as dramatic as during the spring because there is probably more dilution. However, if copper is elevated in the Tsolum during fall when adults are migrating upstream, it is likely that the fish would avoid the Tsolum and spawn in the Puntledge.

Steelhead and coho returns to the Tsolum have declined to very low levels in recent years. Steelhead escapements have declined from a high of 3,500 in the 1950's to 0 in 1991 (Deniseger et al. 1995) while coho populations have declined from 7,500 to a few hundred spawners.

Effect of Low Flow During August and September

Extreme low flows over the last two weeks of August and during September make it difficult for returning pink salmon to migrate up-river. Riffles are exposed and water temperatures are high. Migration is blocked and pre-spawn mortality is very high. Low flow also reduces rearing habitat for coho and steelhead fingerlings.

Flows of 15 cfs on the lower Tsolum are sufficient for upstream migration and spawning. Flows below 10 cfs are critical especially if they occur for an extended period during the upstream migration. Average monthly flows for August and September between 1964 and 1994 are shown in Fig. 4. If the average monthly flow is below 10 cfs for both August and September it is likely that upstream migration will be severely inhibited. This occurred in 1986, 1987 and 1990 (Fig. 4).

In 1990 there was an extreme drought and flows in the lower Tsolum were less than 10 cfs (and often less than 5 cfs) for August and September. There was unusually low rainfall for August (11.9 mm) and a record low of 0.6 mm for September. Only 150 fish returned from 2741 spawners in 1988. Most of the returning adults probably strayed to the Puntledge and contributed to the record escapement (63,000 fish). It should be noted that ocean survival was good for the 1988 brood year and adults returned to the nearby Puntledge and Quinsam Hatcheries in record numbers in 1990.

Low flows in 1990 were worsened by water extracted for agriculture and domestic use. There are 34 water licences on the lower Tsolum. Water released from Wolf lake in 1990 failed to increase flows on the lower Tsolum. Fig. 5 contrasts water flow rates at Headquarters Creek (released from Wolf Lake) with flow measured in the lower river (at Water Survey of Canada gauge). In mid August a flow of over 20 cfs resulted in a flow of 2 cfs in the lower river (Fig. 5). Although not proven, this discrepancy was probably due to water extraction.

Extreme low summer flows in the lower Tsolum would have less effect on coho populations. Adult migration occurs much later in the fall when flows are high. The rearing capacity of the Tsolum

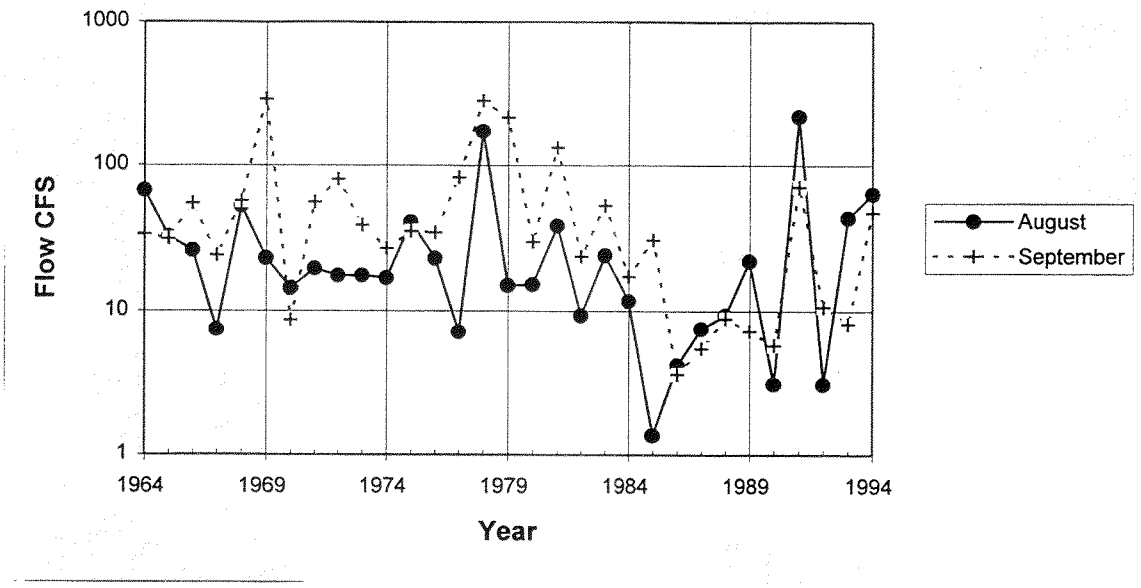

Fig. 4. Mean monthly flow for August and September -- 1964 to 1994.

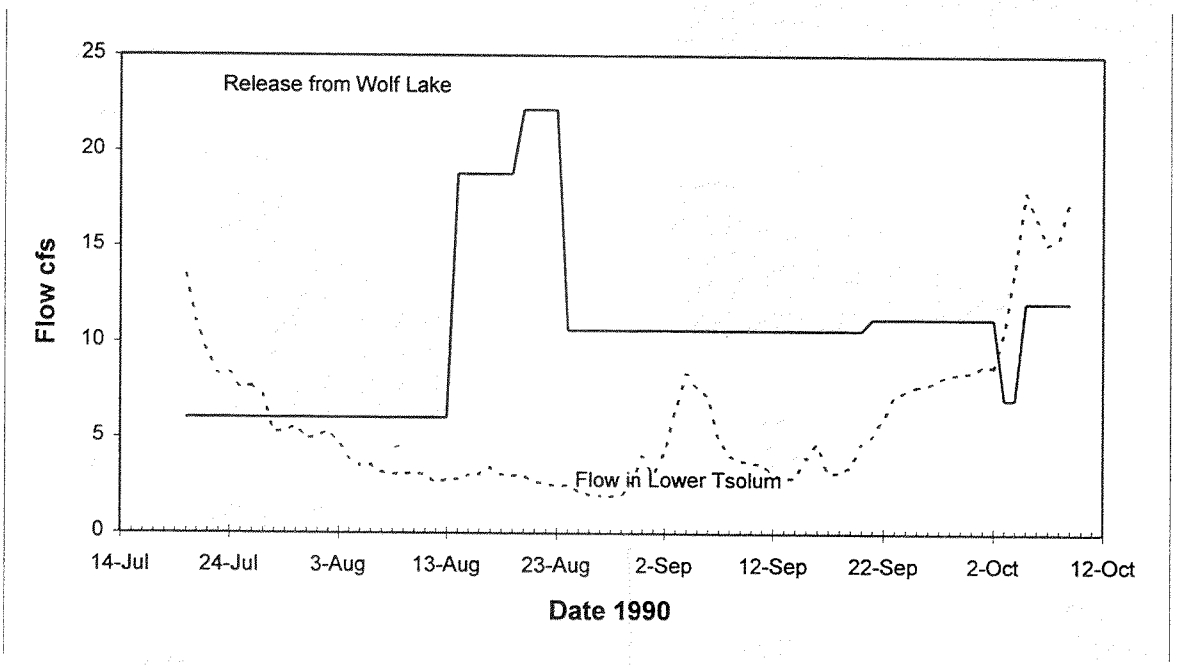

Fig. 5. Flow in the lower Tsolum vs flow released from Wolf Lake to upper Tsolum -- August and September 1990.

would be reduced by low flows however many juveniles rear in the upper river and in tributaries and are not affected by conditions in the lower river.

Low flow in August and September can explain some but not all of the poor returns to Tsolum. In 1982, 1983, 1993 and 1994 flows were excellent but returns were very low. In 1994 for example, summer flows were ideal averaging 64 cfs in August and 48 cfs in September (Fig. 4). However only 200 pinks returned from a significant enhancement in 1992 (Table 1). The fry released in 1992 were equivalent to approximately 9,100 spawners (assuming fecundity of 1,400 eggs/female and an egg to fry survival rate of 13%). The run also collapsed in 1982 after the equivalent of over 11,000 fish spawned in 1980 (Table 1). This occurred despite near ideal flows (over 20 cfs) during late August and September of 1982. Flows were also adequate in 1993 when only 600 fish returned from a spawning run of 20,000 in 1991. In 1983 1,200 fish returned after the equivalent of 13,700 adults spawned in 1981. Once again water flows in the Tsolum were ideal during August (mean = 24 cfs) and September (mean = 53 cfs). This data shows that even when summer flows are adequate, a whole range of enhancement techniques (fed and unfed fry release, egg transfers, adult transfers and natural spawning) have failed on the Tsolum River. It should also be noted that during this period ocean survival rates have generally been good as indicated by returns to other facilities in the area.

The only successful return to date occurred in 1991. 20,000 pinks returned from 5,000 spawners in 1989. In 1991 flows were exceptionally high through the entire spawning migration. A severe summer storm in early August resulted in flood conditions. High flows prevailed until another storm struck on August 28. The mean monthly flows for August and September 1991 were 218 cfs and 71 cfs respectively.

Conclusions and Recommendations

There is strong circumstantial evidence that copper contamination is having detrimental effects on the steelhead and coho populations of the Tsolum Watershed. Bioassays in the field and laboratory show a range of effects from direct lethality at higher concentrations to impaired smoltification at lower levels. Intensive monitoring over a 10 year period shows that steelhead and coho smolts are exposed to detrimental copper concentrations every spring.

Pink salmon populations on the Tsolum may be intermittently affected. Although low summer flows are the most important environmental factor, pink runs have failed in years when flow was ideal (1982, 83, 93, 94). It is conceivable that in some years, copper levels are high enough in the early spring to affect the pink fry migration.

Copper concentrations in Murex Creek and the lower Tsolum must be reduced. This can be accomplished by controlling acid generation at the abandoned mite site on Mt. Washington or by treating the effluent further down the mountain. This effluent is highly toxic and is flowing directly into salmon bearing waters. Furthermore it has been monitored for many years.

Critical low flows in the Tsolum can be alleviated by releasing more water from Wolf Lake (starting in mid August) and by stricter enforcement of water withdrawal sustems on the lower Tsolum. This may require modifications to the Wolf Lake flow control system. The outlet structure may have to altered to allow more draw-down. Also instrumentation will have to be installed for measurement of flow and lake level so that operators can have better control over the release of water. Also investigations will have to undertaken to see how much the lake can be lowered without affecting the trout populations.

A strategy for releasing water from Wolf Lake must be developed so that the reservoir is fully utilized for the benefit of the fish. Release of water in August is critical because upstream migration is starting and the effects of irrigation withdrawals in the lower river must be overcome. However if all the storage is exhausted before the first fall rains there is the danger of attracting fish into the river and then running out of flow. In a dry year, releases from Wolf Lake are the major source of water from mid-August to early October.

If these improvements do not alleviate flow problems in the Tsolum then either the storage capacity will have to be increased or an alternate supply for irrigation will have to found. The nearby Puntledge river is controlled for Hydro generation and has a minimum summer flow of 500 cfs.

Irrigation withdrawals from this source would have an insignificant effect on the Puntledge River and would spare the Tsolum.

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MASS MARKING AND SELECTIVE FISHERIES:

RECENT HISTORY, CURRENT STATUS, AND FUTURE

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In recent years, concern for declining wild salmonid stocks and loss of fishing opportunity has led to the development of a management tool we refer to as mass marking and selective fisheries. The basic concept is simple and appealing. The idea is to mass mark harvestable hatchery fish with a mark that is easily identified by fishers and implement selective fishery regulations that allow retention of marked hatchery fish and release of unmarked wild fish.

Unfortunately, the implementation of mass marking and selective fisheries is not simple. Numerous complex technical issues and obstacles exist which have prevented widespread acceptance and usage. Important issues and concerns include types of fishing gear and their associated release mortality, types of suitable marks, cost, changes in allocation between user groups, Tribal Treaty rights, and maintaining the viability of the existing coded-wire tag (CWT) program. Much analysis and debate over these issues has occurred and will continue to occur in the future. Because of time allotments, I will limit my presentation to discussing the marking and recovery aspects, to key components of mass marking and selective fisheries.

The complexity of the issues surrounding mass marking and selective fisheries is driven by the species. Steelhead salmon, *Oncorhynchus mykiss*, present the least technical difficulties because they are not targeted in mixed-stock ocean fisheries. Additionally, the CWT does not play as critical of a role in steelhead salmon management as it does with chinook, *O. Tshawystscha*, and coho, *O. Kisutch*, salmon. Mass marking and selective fisheries for steelhead salmon were started about 15 years ago. Their successful implementation has led to common acceptance as a management tool from California to Alaska.

In October 1993, the Pacific Salmon Commission (PSC) established an ad hoc committee to do an assessment of mass marking and selective fisheries because of "the importance of conservation and potential implications of selective fisheries for the coastwide CWT system" (PSC 1995). The PSC had concerns about maintaining the viability of the CWT program because it is central to management of chinook and coho salmon.

The PSC Selective Fishery report concluded that "Selective fisheries should not be considered for chinook at this time". This was based upon the fact that the logistics of mass marking hatchery chinook salmon are not feasible at this time simply because there are too many fish to mark in too short of a time frame. As an example, in Washington Department of Fish and Wildlife (WDFW) hatcheries we have 35 million hatchery coho salmon that would need to be marked over a ten-month time frame. With chinook salmon we have about 150,000 million that would need to be marked in about 10 weeks. It would not be practical to hire that many people to mark that many fish in that amount of time.

Work was started three years ago however to address this problem. The goal of this work was to produce a machine that would process fish without human handling, without anesthesia, at a rate of a fish every two seconds, and at a cost significantly less than what it would cost to do manually. A bright spot on the horizon is that such a device has been successfully tested in the laboratory and is ready for production testing.

The PSC committee recommended that as with steelhead salmon, the adipose fin should be used as the mass mark for hatchery coho salmon. The PSC recommendation was based upon ease of application, cost of application, ease of recognition, induced mortality, and mark stability.

The most critical factor when comparing the ventral fin to the adipose fin is the induced mortality. Based on an extensive literature review, Jacobs (1990) estimated that ventral fin removal reduced survival by 20 percent to 50 percent. Recent WDFW studies confirm this rate. Relative to adipose clipped CWT'd coho salmon, ventral clipped CWT'd fish survived significantly less. The study included two competitive broods at three Puget Sound hatcheries. Ventral clipped fish had 6 percent to 19 percent lower survivals for the first brood year and 15 percent to 32 percent the second year. One brood year of chinook salmon data has been gathered which shows a 60 percent differential mortality. Besides the high mortality, a very disturbing factor is that it is significantly variable between hatcheries and between years. These variable results suggest that every hatchery group would have to be CWTd to account for the viability. Presently, only one hatchery in a region is CWTd and used as an index for other hatcheries with similar migration and fishery contribution. In addition to the mortality factor, ventral fins regenerated to the point that about 15 percent would be unrecognizable as a marked fish.

Another important PSC tagging recommendation was the use of double index CWT groups. Currently most wild stock groups are represented by tagged hatchery groups which have similar migration patterns and fishery distribution. These index groups are adipose clipped and CWT'd. In a selective fishery, the adipose clipped fish would be retained by fishers so a different tag group is also needed to represent wild fish. Hence in addition to the adipose clip plus CWT index group to represent the hatchery, a second index group is needed with a CWT but no adipose clipto represent wild fish. The double index groups also serve as an important means of evaluation for selective fisheries. Upon return, if selective fisheries were successful at returning more fish to the spawning grounds, the group of CWT fish without an adipose clip should return in greater numbers than the CWT group with an adipose clip.

The PSC committee recommended the use of electronic detection to sample CWTs. Electronic detection will utilize wand and tube detectors. Wands will be used in low volume situations like dock sampling of recreational fisheries. Tubes are designed for high volume situations like hatchery rack returns or sampling commercial fisheries. The PSC committee recommended 1.5 length CWTs be used to increase reliability of electronic detection.

In 1995 legislation was passed in Washington and Oregon mandating mass marking 1995 brood hatchery coho for selective fisheries. Puget Sound Tribal governments represented by the Northwest Indian Fisheries Commission and the Canadian Department of Fisheries and Oceans (DFO) requested WDFW to limit the marking of north coastal and Puget Sound hatchery coho salmon until September, 1996. Canada wanted time to test and determine the feasibility of electronic sampling in the West Coast Vancouver Island troll fishery. Washington agreed to limit the marking to 25 percent of the total production. Due to reduced effort and catches, DFO determined that they would not be able to determine feasibility of the electronic sampling equipment until at least the end of the 1997 season. WDFW resumed marking in October. Several Puget Sound Tribes asked for, and received, a Temporary Restraining Order (TRO) in Federal Court. The TRO was determined justified and a permanent halt to marking was ordered by the court at least in part because Canada had not yet agreed to substitute electronic sampling for visual methods. The court determined that loss of CWT data under such conditions would pose irreparable harm to the viability of the CWT program.

The future of mass marking and selective fisheries is unclear. WDFW strongly feels that if we are to have viable mixed stock recreational coho salmon fisheries in the future we will have to have mass marking and selective fisheries. Very limited or complete closures for the last four years have already started a collapse of the recreational infrastructure. Without meaningful opportunity, state taxpayers and legislators will question the expense of hatcheries. The probability of chinook and or coho listings in Washington waters under the Endangered Species Act will, at a minimum require selective fisheries for any measurable opportunity, in mixed-stock fisheries.

WDFW feels other agencies will also come to this same understanding. Recent Endangered Species Act listings of coho salmon in California and the recent Slaney, et.al. article showing the long-term decline of wild stocks and precipitous decline in catch of coho salmon for British Columbia underscore this idea.

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A REVIEW OF WASHINGTON DEPARTMENT OF FISH AND WILDLIFE'S EFFORTS TO MASS-MARK 1995 BROOD HATCHERY COHO WITH AN ADIPOSE FIN CLIP

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Introduction

On May 16, 1995 Washington State Governor Mike Lowery signed into law Senate Bill 5157 directing the Washington Department of Fish and Wildlife to accomplish the following: (1) protect wild stocks of chinook and coho salmon by (2) identifying all hatchery chinook and coho with an external mark that would (3) allow a selective harvest on hatchery chinook and coho (Chapter 372, Laws of 1995). The adipose fin clip was selected to mark these fish because it had the least affect on the health and survival of the fish, and because sportsmen were familiar with identifying the missing adipose fin (Blankenship, 1993). We also expect to receive information on stray rates of hatchery fish and stock contribution to fisheries in regions within the state (Fuss, pers. comm., 1996). Approximately 30 million coho were identified as being available for mass-marking. New mass-marking trailers were built to accommodate the increased clipping requirements, crews were trained, and workers were hired. A variety of factors influenced the decision as to when, where, and how many fish were to be marked, such as the difficulty of marking fish in release ponds, the size of the fish at marking, budget constraints, and agreements with tribal entities. This report summarizes the mass-marking effort and results to date.

Methods and Materials

Criteria for Marking

Based on the annual hatchery rearing programs within the purview of the Washington Department of Fish and Wildlife, about 30 million 1995 brood year coho were available to be clipped with about 6.5 million of the total in the coastal area, 11.4 million in Puget Sound, and 12.1 million in the Columbia River (Figure 1). These fish represent the bulk of yearling coho releases for the Washington Department of Fish and Wildlife. The criteria used to determine when to clip fish were: (1) Clip fish when they reach 2.3 grams/fish; (2) Clip fish before they are transferred to large release ponds; (3) Clip fish in spring or fall according to the work schedule of the respective facility manager; (4) Clip no more than 25% of the Puget Sound coho in the spring because of agreements with tribal entities, and (5) Do not clip fish when increased water temperature is likely to cause stress or mortality.

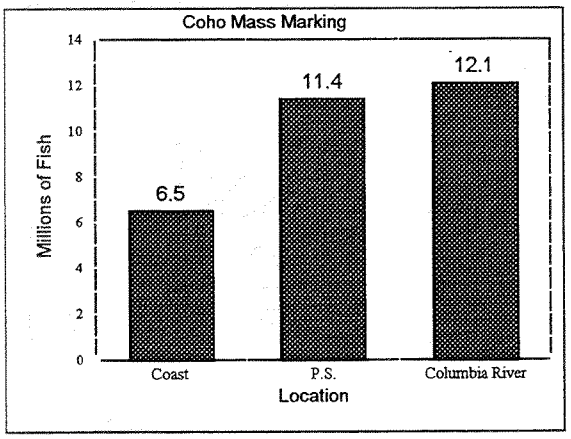

Figure 1. Numbers of coho to be marked by region.

Fin-clipping

Trailers designed for mass-marking were used. The trailers are 26 feet long and 8 ½ feet wide. Each trailer is equipped with a 2 3/4 hp pump, a 84 gallon anesthetic tank, and two " pre-knock-out" basins. They were made by Wells Cargo Company of Ogden, Utah. Each trailer accommodates 14 workers and costs approximately $ 50,000 (U. S.) (G. Schurman, pers. comm., 1996). Fish to be clipped were crowded to the end of the pond, and hauled in screen buckets to the clipping trailer. From 10,00 to 15,000 fish were held in holding tanks within the trailer up to three to four times per day depending on the speed of the workers. "Knee-bend" or "iris" scissors were used to clip the fish. Numbers of fish were estimated by the total pounds of fish weighed into the trailer. Quality control (QC) checks were made during the fin-clipping, and a final QC check will be made on groups of 2,000 fish before release at each facility.

Fish Health

We attempted to clip fish by considering the fish health requirements associated with each facility.

Results

Cost

The total cost of this project, excluding the capital cost for the tagging trailers, was $ 125,471.42 or $ 10.88./ 1,000 clips. Temporary workers were hired from Kelly Services, a contract labor firm, at a cost of $ 8.70/hour. Goods and services accounted for 10.3 % of the total or $ 12 ,943.36. Hatchery personnel supervised the employees in the marking trailers, thus no additional charges for supervision were accrued. There were indications of a direct impact on normal hatchery operations due to the involvement of hatchery personnel (E. Maxwell, pers. comm.,1996.).

Table 1. Total number of clipped fish by region.

Hatchery	Number of clipped fish
Bingham Creek	856,800
Satsop Springs	470,000
Humptulips	1,928,000
Willapa	2,254,710
COAST TOTAL	5,509,510
Hood Canal	450,000
Marblemount	660,000
Minter Creek	1,195,187
Soos Creek	600,000
PUGET SOUND TOTAL	2,905,187
Elochoman	519,200
Lewis River	2,600,000
COLUMBIA RIVER TOTAL	3,119,200
STATE TOTAL	11,533,897

Number of clipped fish

A total of 11,533, 897 fish were clipped in the spring clipping season which began April 1, 1996 at Humptulips Hatchery near Aberdeen, Washington and ended August 6, 1996 at Lewis River Hatchery, near Woodland, Washington. The coastal region facilities clipped 5, 509, 510 fish , 2,905,187 fish were clipped in Puget Sound, and 3,119, 200 fish were clipped at facilities in the Columbia River region (Table 1).

Daily Clips/ Worker Rate

The average daily clips/worker was 6,181 with a range from 4,702 to 7,140 (Table 2). Most facilities reported increases in the daily clip rate as the workers became more experienced . The daily average number of clips/trailer/facility was 71,487 with a range from 44,400 to 90,000 (Table 2). The daily totals also increased as the workers improved their efficiency.

Fish Size

Fish size at the time of marking ranged from 2.3 gms/ fish to 18.1 gms/fish with workers preferring a range from 2.8 gms/fish to 5.7 gms/fish because of the increased ease of handling fish.

Fish health

To date, fish health problems associated with mass-marking have been minimal. In fact, they have been less than what we had initially anticipated. Problems identified to date include some fungal infestations as a result of abrasion due to handling and some instances where the fin clipping was too deep and resulted in damage to the epidermis, dermis and underlying musculature. Fish health has been managed by avoiding marking fish at inappropriate times such as high-water temperatures in the summer (K. Amos, pers. comm., 1996).

Table 2. Daily clip rates by hatchery.

Hatchery	Average number of clipped fish /day.	Average number of clipped fish/worker/day.
Bingham Creek	77,800	7,140
Hood Canal	90,000	6,429
Humptulips	54,103	6,800
Satsop Springs	67,143	5,053
Willapa	73,900	6,302
Marblemount	44,400	6,000
Minter Creek	70,305	6,226
Soos Creek	80,000	6,154
Elochoman	85,000	7,000
Lewis River	72,222	4,702
STATE AVE.	71,487	6,181

Conclusion

The initial efforts to mass-mark the 1995 brood hatchery coho required a tremendous effort by many people. We believe we have the equipment and trained personnel to efficiently mark large numbers of salmonids. The cost of this initial effort, excluding the capital cost for the trailers, was $ 125,471.42 or $ 10.88/1,000 clips to mark 11,533,897 fish. The specially designed trailers efficiently facilitated the clipping of large numbers of fish. In addition to allowing a selective harvest on hatchery coho, we should be able to gain data on stray rates and on contributions to regional fisheries by hatchery fish.

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* Howard Fuss, Andy Appleby, and Dave Knutzen critiqued this report and contributed many helpful suggestions.

A REVIEW OF THE PERFORMANCE OF GENETICALLY ALTERED RAINBOW TROUT IN THE HATCHERY AND STOCKED IN ALASKAN LAKES

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Introduction

Salmonid populations have disappeared or their numbers have declined drastically in many of their historic spawning streams in the West. For some it has been necessary to invoke the Endangered Species in an attempt to protect them. There are myriad reasons for this decline, but one major concern is the loss of genetic resources and the negative impact that hatcheries may have through genetic introgression.

Absolute reproductive isolation does not exist with salmonids because natural straying occurs. The recent huge hatchery releases of salmon from Japan, United States, Russia, and Canada add to this straying. The infiltration of genes from hatchery populations into naturally spawning (or wild) populations can adversely affect their genetic diversity (Windsor and Hutchinson 1990; Allendorf 1991; Hindar et al. 1991). This problem is less obvious in freshwater lakes stocked with species like rainbow trout and grayling. However, hatchery-stocked fish will impact indigenous populations whether in lakes or streams through interbreeding and directly affecting the genetic diversity of the same species and through competition with all species for scarce resources.

Most species (rainbow trout, lake trout, Arctic grayling, and non-anadromous coho and king salmon) stocked into Alaskan lakes are destined for local sport fisheries. Fish are stocked as catchables, ready to be caught during the current season, or as fingerling or fry to be recruited into the fishery in later years. A number of these fish will survive to mature and breed. To mitigate the impact of these hatchery populations on wild stocks, the Alaska Department of Fish and Game's Genetics Section recommends using proactive measures such as stocking sterile or non-reproductive fish in sensitive areas.

Rainbow trout, originally taken in 1982-1985 from the Swanson River on the Kenai Peninsula, Alaska, are the strain held as broodstock at the Fort Richardson Hatchery Broodstock Development Center (BDC). This single stock of fish is used for lake stocking programs throughout the state of Alaska. To protect indigenous stocks from genetic introgression by these hatchery fish, alternative non-reproductive fish needed to be developed. In the late 1980's, the BDC was assigned the task of developing the protocol for producing sterile fish, primarily rainbow trout, for use in lake stocking programs. The most promising and economical method to produce sterile fish was to create triploid fish using heat shock shortly after fertilization (Chourrout 1980; Chourout 1984; and Thorgaard and Jazwin 1981). Heat at the appropriate time induces the egg to retain the second polar body during its second phase of meiotic division resulting in a fertilized egg with three sets of chromosomes. The assumption is that these triploid fish develop and grow normally. Growth should continue after they reached the age and size when most of their diploid counterparts mature sexually and breed. At this time, the triploid fish should continue to put energy into growth rather than sexual maturation. These fish are sterile because the third set of chromosomes inhibits the gonads from undergoing meiosis to produce viable gametes, ultimately preventing sexual maturation. This paper is a summary of our experience with triploid fish: the production and performance in terms of growth and survival both in the hatchery and the lakes after stocking.

Production of genetically altered populations at Fort Richardson Hatchery

Triploid Production

In 1988 we began to experiment with the feasibility of producing sterile rainbow trout through heat shock shortly after fertilization. This method disrupts the spindle apparatus during the second phase of meiosis in the egg, causing a set of chromosomes to be retained. The result of a successful procedure is a fish with 3 sets of chromosomes: 2 from the female parent and 1 from the male parent. The only special apparatus necessary for this technique is a large hot water bath and an accurate timer.

Our initial experiments in 1988 and 1989 were matrices using different shock duration times and post-fertilization timing at 4 different temperatures. The heat shock durations were 10 and 20 minutes; post fertilization timing was 10, and 20 minutes; and the shock temperatures were 26°C, 27°C, 28°C, and 29°C. We took blood samples when the fry from these eggs reached 2g and submitted them for flow cytometry analysis to verify ploidy (Thorgaard et al. 1982). We found that triploid fish could be made at any of the test temperatures as long as we waited at least 10 minutes after fertilization and allowed the eggs to remain in hot water at least 10 minutes. The incidence of triploidy increased at each temperature with increasing shock duration and post fertilization time (Figure 1). Temperature seemed to have more of an effect on egg survival than it did on triploidization. Survival decreased as the temperature increased with increasing shock duration. The most effective combination of parameters to create triploid rainbow trout at the Fort Richardson Hatchery is a 20 minute shock, 20 minutes after fertilization, at 26°C. We have used this protocol for rainbow trout since the initial experiments and have had 97-100% triploidization rates. At least 450 fish are screened annually from various groups to confirm this rate.

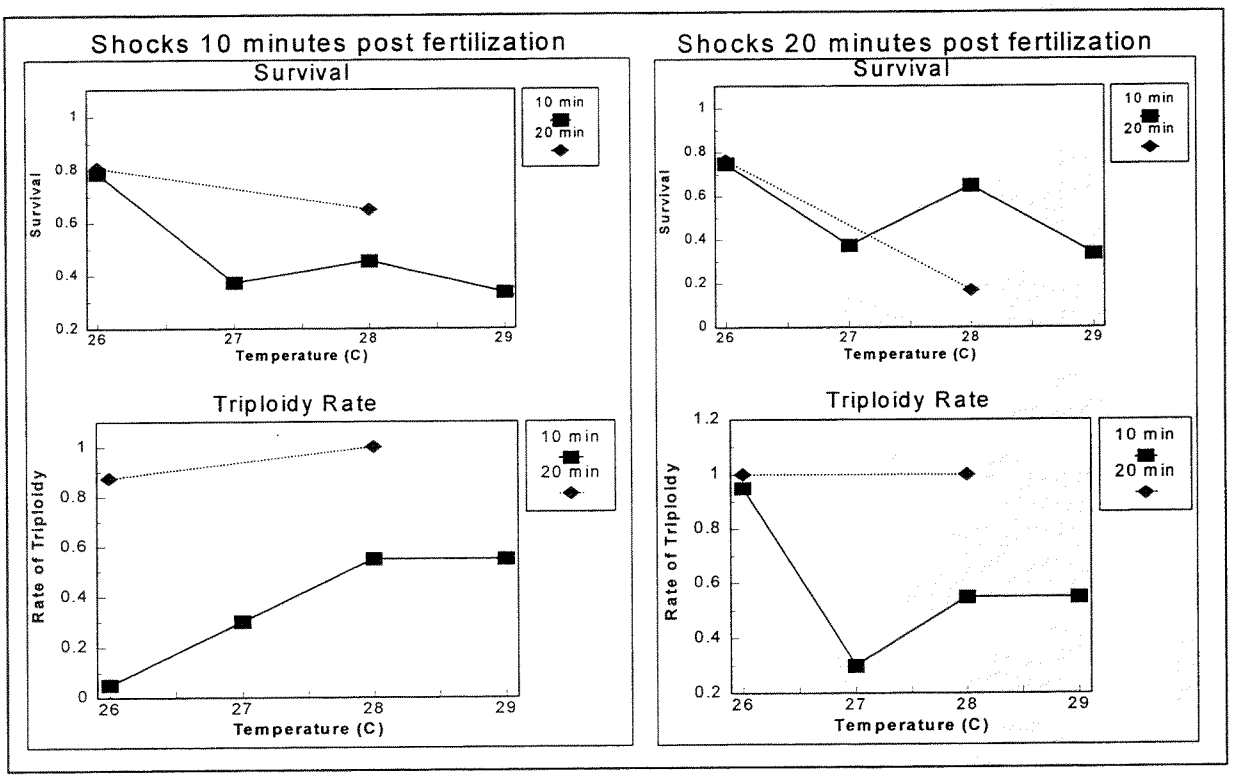

Figure 1. Average rates of survival and triploidy in rainbow trout at various temperatures with 10 and 20 minute shock durations, at 10 and 20 minutes post fertilization. Unpublished data from BDC, 1988-1989.

All-Female Production

Precocious male mortality is a problem with the Swanson River rainbow trout broodstock held at the Fort Richardson Hatchery. Since 1985 two-year old male mortality has ranged from 20% to 95%. In the spring of 1988, we began producing an all-female population of rainbow trout through a process of gynogenesis in an attempt to alleviate this problem. In this process, milt was irradiated with UV light before fertilization, and the fertilized eggs were heat-shocked using the triploidization protocol. The resulting population was all-female. All of these fish were fed the male hormone testosterone at emergence to reverse their sex, creating functional males. These males, designated XX males, are used to fertilize the eggs of normal females. All offspring of these matings are normal, untreated females. Stocking female fish is advantageous because they do not mature early and are available to the fishery for a longer period of time. Our success rate in creating all-female populations is virtually 100%.

Triploid All-Female Production

There was indication that some triploid fish, particularly males, could mature sexually and produce gametes (Lincoln and Scott, 1984; Simon et al., 1993). In 1991 we began producing all-female triploid rainbow trout to circumvent this potential problem. Triploid all-female rainbow trout are the result of normal eggs fertilized with XX males to create all-females, then heat shocked to produce triploid fish. Using this population was additional security if triploidization was not 100%; the chance of triploid all-female rainbow trout reproducing is minimal. For these reasons, and to minimize the number of different groups of fish held, Fort Richardson Hatchery has produced only all-female triploid rainbow trout since 1992.

Performance of triploid fish populations

Hatchery:

After the triploidization protocol was well established in 1991, a number of triploid fish were produced as family groups. Controlled experiments were conducted with these groups in the hatchery to determine growth and survival differences relative to normal diploid fish. These experiments were designed to minimize genetic variation among groups and set up as follows: eggs from one female were divided into two groups, one fertilized with milt from a normal (XY) male and the other fertilized with milt from a sex-reversed (XX) male. Each of these groups was subdivided and one subgroup heat shocked to produce triploid fish, the other left to produce diploid fish. This procedure was repeated until at least 20 females were spawned. The progeny of these matings were used for in-hatchery evaluations in 1991 through 1993.

Initial observations of triploid rainbow trout growth and survival in the hatchery during early rearing in 1988-90 indicated triploid fish tended to be slightly smaller and less hardy than diploid fish (Table 1). The controlled experiments in 1992-93 showed a significant difference ($P<0.06$) in growth only between the diploid and triploid rainbow trout, confirming the initial observations. Differences between diploid all-female and diploid mixed-sex groups were not significant (Figure 2). Although growth differences between diploid and triploid are significant, muscle growth dynamics studies looking at the ultimate capacity for growth conducted at Southern Illinois University show no differences between diploid and triploid rainbow trout (Sheehan, pers com). This indicates that there are other factors, perhaps behavior, inhibiting triploid growth.

Mortality in the production facility was thought to be greater in triploid fish populations. However, in all controlled experiments there were no significant differences in survival between diploid and triploid rainbow trout throughout their hatchery rearing phases (Brock et al, 1994). Stress tests done at the hatchery to test tolerance for crowding, ammonia, and agitation also showed no difference between diploid and triploid rainbow trout.

	Size (g)	Fingerling Survival
Triploid	1.67	93.1%
Diploid	2.04	96.3%

Table 1. Diploid and triploid fingerling size and survival after 2 months hatchery rearing (1990).

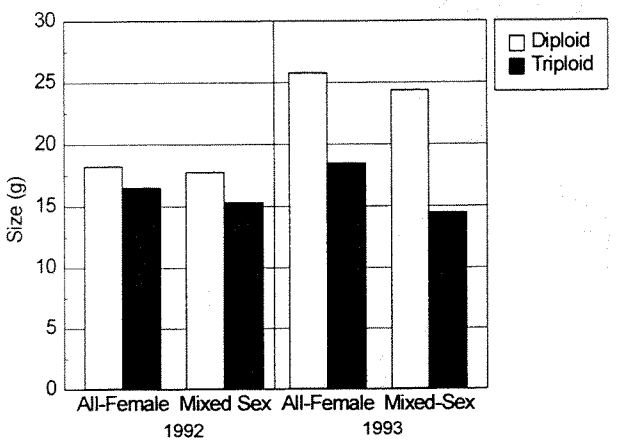

Figure 2. Average size of diploid and triploid families in 1992 and 1993. Differences between diploids and triploids are significant; those between all-female and mixed-sex are not.

One group of 500 triploid all-female rainbow trout (brood year 1991) was held at the BDC for 3.5 years to verify sterility as well as monitor growth and survival to the age of sexual maturity. In June, 1994, all fish were sacrificed to size, examine gonads, and take blood for ploidy analysis. Sampling 60 fish from each group, we found no significant difference between diploid and triploid all-female populations of fish in growth or survival before spawning in March 1994. Triploid fish did not spawn and had no mortality during the post-spawning period, while the diploid all-female population spawned and subsequently suffered almost 30% mortality. Flow cytometry analysis of blood taken from putative triploid fish verified that 97% were triploid. Gonads of triploid fish examined were very immature, string-like, and many had to be examined under a microscope to verify sex. A measure of gonad development is the gonadosomatic index (GSI=(gonad weight/body weight)*100; see Lincoln and Scott, 1984). Normal diploid female rainbow trout at this age at the hatchery have gonads packed with eggs, and a gonadosomatic index ranging from 9 to 24. The GSI of these triploid females averaged 0.08.

Field: Johnson Lake

Johnson Lake: In cooperation with area biologists in the Matanuska-Susitna Valley, diploid and triploid, as well as all-female rainbow trout were differentially fin clipped and stocked into various lakes in 1988 through 1993. In 1988-1990, our primary experimental lake was Johnson Lake (Figure 3). This lake is closed to sport fishing. In 1988 we stocked approximately 1,000 each of diploid mixed-sex and triploid mixed-sex rainbow trout fingerling; and in 1989 we stocked 2,000 of each group into Johnson Lake.

Brood year 1988 data was not considered here because the triploidy rate averaged 37% in the stocked group. Although brood year 1989 triploid fish were smaller at stocking, the size difference between diploid and triploid fish after one year of growth in Johnson Lake was not significant (Havens, 1991). There was a significant difference in survival between the two groups in brood year 1989. Triploid survival to 1 year was 46% while diploid survival was 53% for brood year 1989 fish ($P<.50$). The number of triploid fish in this population continued to decline until they comprised only 25% of the population as 2-year olds in 1991 (Figure 4). This trend reversed completely after the age when these rainbow trout spawn. As 4-year olds, triploid fish comprised 70% of the population; although they continued to be smaller than the diploid fish. Curiously, 63% of the triploid 4-year old population was male; the reverse of what we saw in the diploid population where only 40% were male. In this particular strain, both in the hatchery and on the Swanson River, precocious males mature at two years of age and many die. The last year we stocked mixed-sex triploid rainbow trout was in 1990 into Johnson Lake. This brood year's population followed the same pattern as that of brood year 1989. After one year in the lake, triploid survival was 58% and diploid survival was 69%, a significant difference ($P=.05$). I have no data for these fish as 2-year olds. However, they comprised 89% of the population as 3-year olds and 72% of these fish were male.

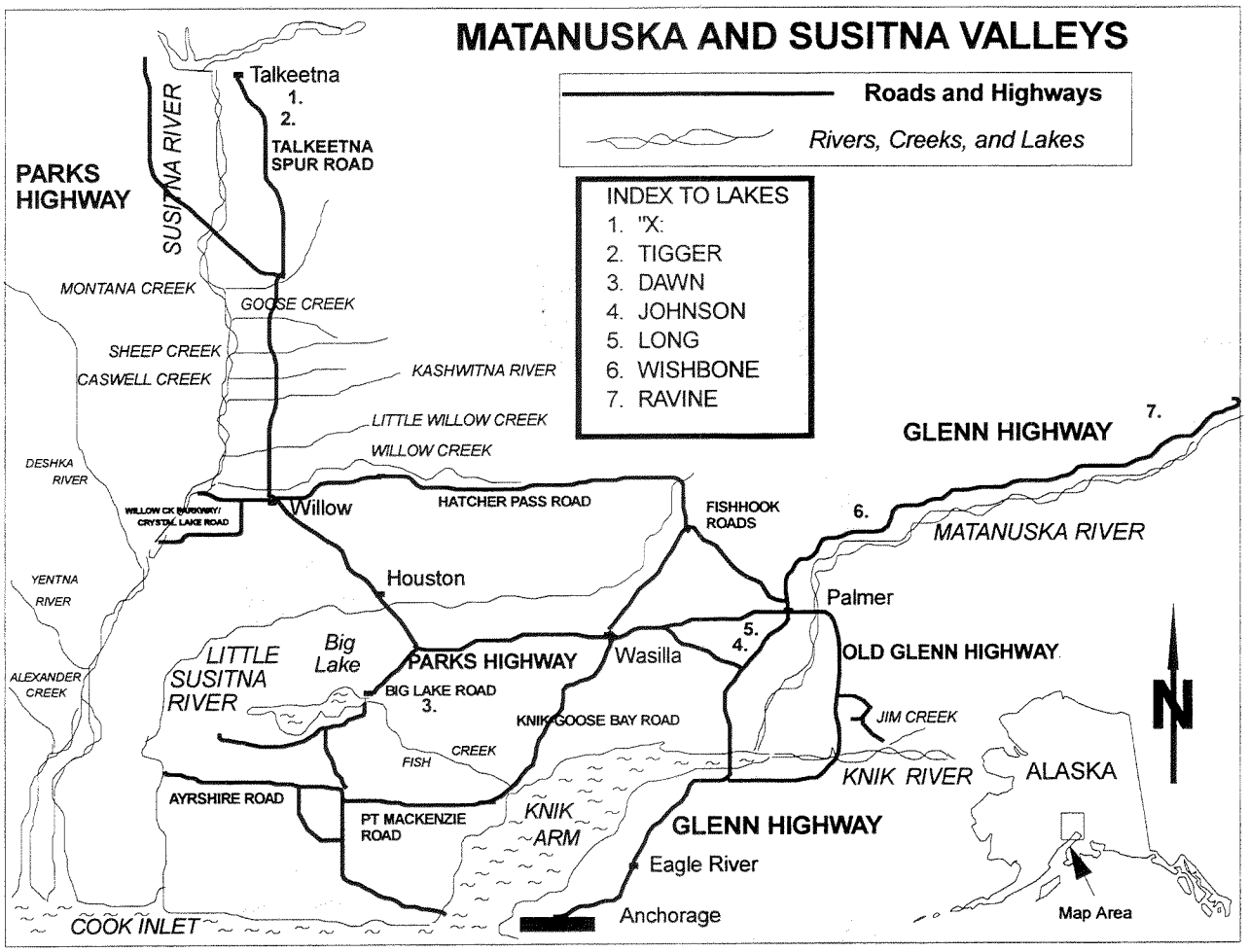

Figure 3. Location of lakes in Matanuska-Susitna Valley used for field studies.

In 1991 we sacrificed 113 of the 2-year old fish caught to examine gonadal development. This is the age at which many Swanson River males mature sexually, spawn and die in the hatchery. We found no significant difference in size between diploid and triploid males, but a significant difference between females (P=.04). The diploid females averaged 321g, over 100g larger than the triploid females who averaged 218g. The GSI of triploid females (0.04) was also significantly less than that of diploid fish (3.8), with no indication of gonadal development in the triploid fish. The GSI in triploid males (0.54) was also significantly less than that of diploid males (1.33). Ploidy in all triploid fish was verified through flow cytometry.

Field: Other Lakes

In July 1991, we stocked Long, Wishbone, and "X" Lakes (Figure 3) with diploid mixed-sex and triploid all-female rainbow trout at ratios of approximately 50:50. Fish were differentially clipped at the hatchery before release. Although these lakes vary in size, they were all stocked with about 200 fish per surface acre (Havens and Sonnichsen, 1992). These lakes are restricted to catch and release fishing only. We repeated this experiment in 1992 stocking Dawn, Ravine, and Tigger Lakes. These lakes are open to sport harvest and received a combined estimated total of 523 angler-days of fishing effort in 1992 (Mills, 1993).

Diploid fish in 5 of the 6 lakes stocked were significantly larger than triploid fish at stocking, as well as at 1 and 2 years of age (Table 2). The average length of triploid fish was 4% less than diploid fish at stocking and 11% less when the fish were 1 and 2 years old (Brock et al. 1994). As 1-year olds, significantly more diploid rainbow trout were caught in 5 of the 6 lakes, indicating a higher survival. Ravine Lake was the exception in this experiment. As 2-year olds there was no significant difference between the number of diploid and triploid fish

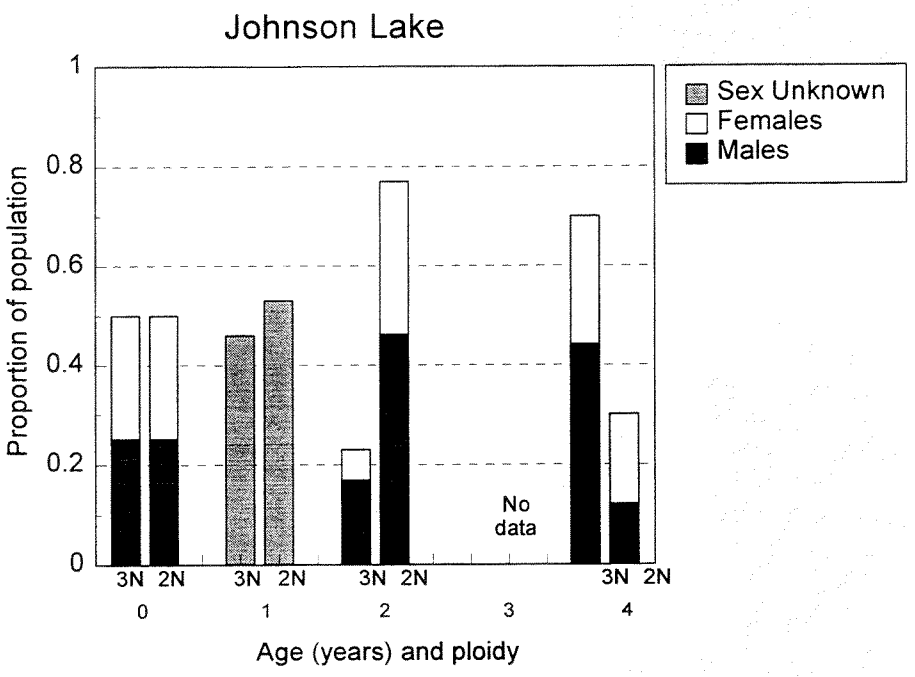

Figure 4. The proportion of males and females and the proportion of triploid (3N) and diploid (2N) fish in the population at each age. These fish belong to broodyear 1989.

	Lake					
	Long	Wishbone	"X"	Dawn	Ravine	Tigger
Surface Area (acres)	74.4	52.7	101.4	11.8	12.3	18.9
Number Stocked						
Diploid	7,277	5,304	10,152	1,146	1,202	1,881
Triploid	7,451	5,265	10,074	1,147	1,189	1,868
Mean Length at Stocking						
Diploid	55(1)	56(1)	54(1)	49(1)	49(1)	50(1)
Triploid	54(1)	53(1)	54(1)	47(1)	47(1)	48(1)
Catch Age 0						
Diploid				189	110	111
Triploid				134	103	33
Mean Length Age 0						
Diploid				97(1)	100(1)	86(1)
Triploid				88(1)	91(1)	78(1)
Catch Age 1						
Diploid	560	599	689	176	274	47
Triploid	265	284	376	103	389	9
Mean Length Age 1						
Diploid	195(1)	179(1)	188(1)	254(3)	213(2)	223(5)
Triploid	167(2)	155(1)	170(1)	219(3)	181(1)	189(9)
Catch Age 2						
Diploid	107	234	126			
Triploid	92	127	102			
Mean Length Age 2						
Diploid	305(5)	268(3)	280(3)			
Triploid	275(4)	229(2)	252(2)			

Table 2. Summary of stocking and sampling of mixed-sex diploid and all-female triploid rainbow trout stocked in six lakes in southcentral Alaska. Standard errors of the means are in parenthesis. (From Brock et al., 1994.)

caught in 2 of the 3 lakes sampled, indicating higher survival of triploid fish between age 1 and age 2 (Table 2). The reduced survival of diploid 2-year olds was probably due to early sexual maturation and spawning activity in the diploid population The west side of this lake is strewn with boulders which could have offered more protective cover against predators for the smaller triploid all-female fish, an advantage the other lakes with gentle vegetated slopes do not have (Brock et al. 1994). Studies done by Simon et al. (1993) in South Dakota are generally consistent with our results.

Performance of all-female rainbow trout

In the hatchery there has been no significant difference in the growth and survival between diploid all-female and diploid mixed-sex populations of rainbow trout since we began rearing production lots of all-females in 1991 (Figure 2). In 1993 we stocked Long, Johnson, and "X" Lakes with all-female and mixed-sex rainbow trout at 50:50 ratios. Stocking density was approximately 200 fish per surface acre. These fish were differentially clipped for identification before stocking.

In Johnson and "X" lakes mixed-sex rainbow trout grew significantly more than all-female rainbow trout during the first year. As 2-year olds sampled in the fall of 1995, mixed-sex fish were still significantly larger in 2 of the 3 lakes, while the all-female fish were larger in "X" Lake (Figure 4). The proportion of all-female fish was not significantly greater than the proportion of mixed-sex fish in Long and Johnson lakes in 1994; and not significantly greater in all 3 lakes in 1995 (Rutz and Baer, 1996). The assumption that precocious males would fall out of the mixed-sex population as 2-year olds was not realized. We also noticed several behavioral differences of all-female fish in "X" Lake. After stocking, many all-female fish swam toward the beach rather than deep water to escape warm surface waters. These fish also did not appear to mix well throughout the lake; the majority were captured in a few fyke nets in one vicinity, while the mixed-sex fish were found throughout the lake.

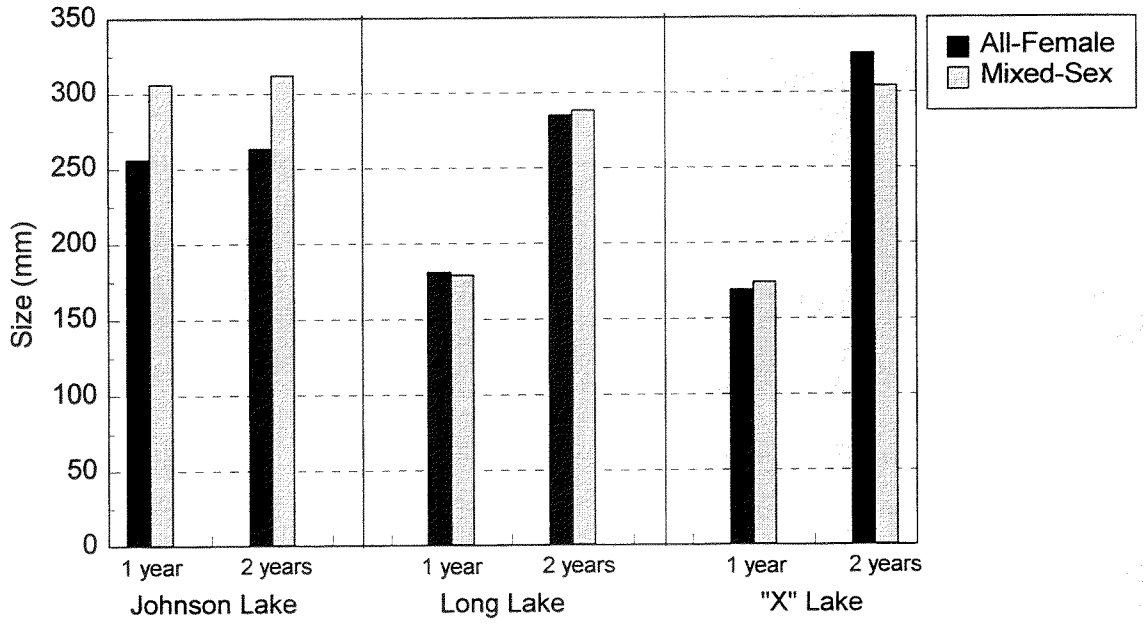

Figure 4. Length of all-female and mixed-sex rainbow trout stocked into 3 lakes at 1 and 2 years of age. (Data from Rutz and Baer, 1996)

Conclusions

Results are mixed for all groups of fish we tested in the hatchery. In controlled experiments triploid fish had significantly lower survival or no difference in survival relative to their diploid counterparts. In most cases, triploid

fish also tended to be smaller than diploid fish. Overall, all-female populations were not significantly different in growth or survival from mixed-sex populations. Both of these performance measures can be insignificant in the hatchery. In the wild they can be exaggerated or reversed, depending on the lake into which they are stocked. Triploid fish held through the age of normal maturity show no indication of spawning. This pattern continues to be exhibited in lakes after fish are stocked.

The Alaska Department of Fish and Game has developed guidelines for lake stocking. Open systems with indigenous rainbow trout or competitor sport fish species should not be stocked. Landlocked lakes with ephemeral outlets or lakes that are judged likely to flood more often than once every ten years should only be stocked with all-female triploid fish. Completely landlocked lakes can be stocked with fish capable or reproducing. We began experimenting with sterile fish to satisfy both the demand for fish by the public and the guidelines set down by the department's geneticists. The often slower growth and diminished survival of sterile fish make them poor candidates for widespread stocking programs, but in areas where they can potentially affect other populations, they are the only option available today for stocking.

Acknowledgments

I would like to thank the crew at the Fort Richardson Hatchery for all of their care with special project fish, the field crew from the Palmer, Alaska office for their diligence in sampling, Chris Habicht from Genetics for his help in designing the experiments, and Pat Hansen for biometrics support. I would also like to thank Larry Peltz and Sandy Sonnichsen for all of their editorial comments. These studies were partially funded through the Federal Aid in Sport Fish Restoration Act.

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TRIPLOID FISH FOR FISHERY ENHANCEMENT IN WASHINGTON

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Introduction

Researchers and fish culturists have been producing sterile fish by inducing triploidy shortly after fertilization. There are numerous situations where sterile fish are useful. WDFW is particularly interested in using sterile fish to enhance fisheries in situations where it is unacceptable for hatchery fish to interbreed with wild fish or where population sizes need to be limited. Our goals are to: (1) reliably produce 100% triploid fish; (2) compare growth, survival, and fishery contributions of triploid and diploid salmonids; and (3) determine which hatchery practices need to be changed to successfully rear triploid fish. WDFW's program is still in the experimental stage, and we are developing or testing the methods to produce triploid fish and evaluate their performance for possible use in situations where we want to reduce the gene flow between hatchery and wild fish.

Methods

Our program has included coho, fall chinook, cutthroat trout, and kokanee, and this year we are also going to add brook, rainbow and brown trout. We chose to induce triploidy using a thermal shock shortly after fertilization because it is inexpensive, portable and simple. Our methods are similar to those described by Crozier and Moffett (1989), Thorgaard and Jazwin (1981), and Utter et al. (1983). In general, the eggs are fertilized, held for a certain amount of time in cool water, shocked in a warm water bath, then placed in a stack or trough for incubation. Whenever possible, we monitored mortality and compared the growth of control and triploid fish.

Results

Coho

We began our triploid program with coho because WDFW has an extended rearing program for coho marine net pens in Puget Sound. Those fish are released from the net pens at about 10 fpp in June and contribute mainly to commercial and sport fisheries in Puget Sound. Using this program allows us to observe the triploid fish for an additional year because they are released after two years rather than the usual one year. This program is also appropriate for studying triploids because they are released from marine waters without having imprinted to a unique river and may stray to surrounding streams when they return. Using sterile fish in the net pens may provide a solution to potential problems from straying without eliminating the program.

We are now beginning the third of three consecutive brood years of producing triploid coho for release on-station at Minter Creek Hatchery and for extended rearing at Fox Island Net Pens. Each year, we have produced about 10,000 control and triploid fish for release on-station, and 5,000 of each for transfer and extended rearing at the net pens. In the hatchery, we have been able to rear the control and triploid fish in separate portions of the raceway for the first year, until the fish are tagged, and space is limited. At that point, the tagged fish to be released on-station are combined with the hatchery's regular production, and those to be transferred to the net pens are combined into part of a single raceway.

Each year, we tested the ploidy of 60 treated fish by flow cytometry. All were triploids. We compared lengths of the triploid and diploid control fish until they were combined, and found that the triploids grew as well as the diploid fish, but there was more variation in the triploid fish (Figure 1). The 1994 brood of coho were transferred to one pen at Fox Island in May, 1996. It is no longer practical to sample growth regularly, but when possible, we are collecting dead fish and reading the tags. In 1997, we will begin to recover tags from escapement and fisheries.

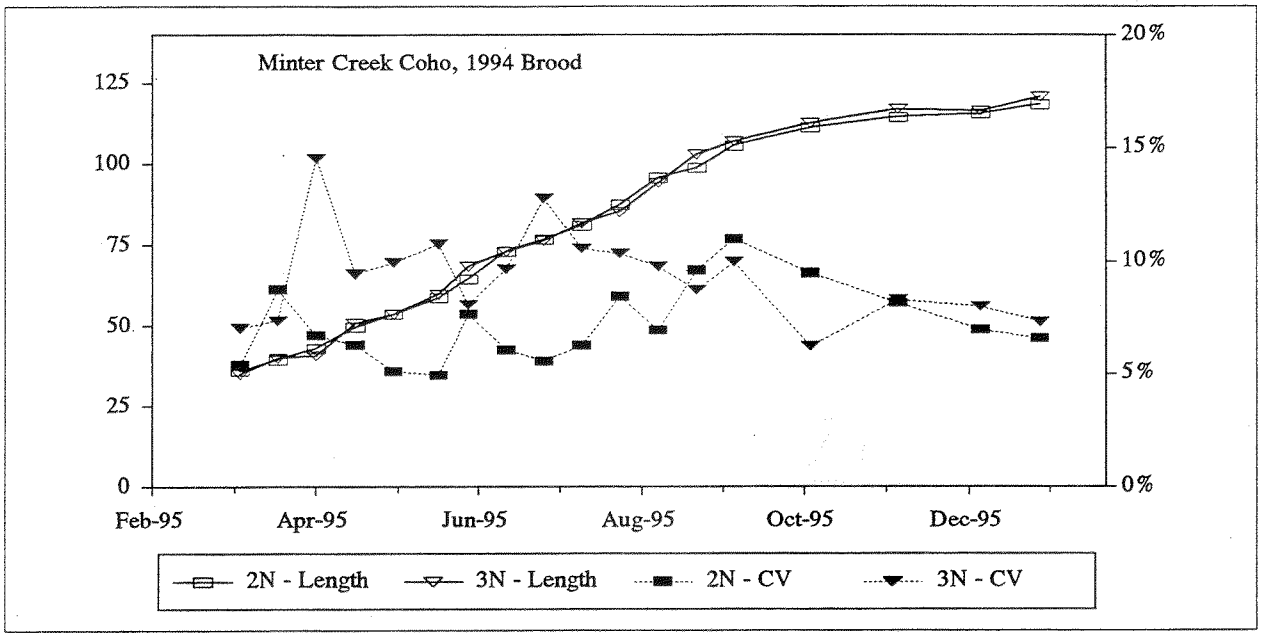

Figure 1: Comparison of growth of triploid and control coho reared in separate portions of a raceway at Minter Creek Hatchery.

Fall Chinook

In 1995, we added fall chinook to our program because they are also reared and released from marine net pens. That year, we had 75% survival of the treated eggs, but only 50% were triploids. We did not evaluate those fish any further. This year, we tried different treatments, and those eggs are now incubating. If we successfully produce a high proportion (90%+) of triploid fish, we will also be rearing them at Fox Island, mixed in a pen with control fish.

Kokanee

In 1995, we treated kokanee eggs from Lake Whatcom, north of Seattle. This lake contains a rather unique broodstock of kokanee that are planted in numerous lakes in western Washington. We achieved about 87% triploids, but the mortality was very high - 50% of the controls and 85% of the treated fish. At planting, the treated fish were slightly smaller than the controls, and seemed to be thinner. We did not see any deformed fish. This year, we are continuing our work with Lewis River kokanee.

Cutthroat

Among the resident trout used for stocking lakes in Washington, there is most demand for triploid Twin Lakes cutthroat. In 1995, 85,000 were planted into 64 different lakes. The broodstock for these fish are reared naturally in Twin Lakes, in eastern Washington, near Chelan. On the first day of spawning in 1995, we collect eggs for heat shocking at a remote site where the fish are spawned. The normal procedure is to fertilize the eggs at Twin Lakes, then to allow them to water harden before they are taken to Chelan Hatchery. However, there is no electrical source there so we had to transport the green eggs to Chelan Hatchery, about 4 to 5 hours away where we heat shocked and reared the eggs. Unfortunately, the delay was too long, and about 85% of both the control and the treated eggs died; most of these were blank eggs. We obtained 70% 3N in one treated group and 90% 3N in another treated group. Before continuing with Twin Lakes cutthroat, we are going to try to perfect the technique and evaluate triploid Tokul Creek cutthroat, another strain which is planted in Western Washington lakes.

At the time of writing, we have plans to work with Eastern brook trout, rainbow trout and brown trout. All of these fish will be planted into lakes in western Washington, and their performance will be evaluated.

Conclusions

WDFW's program to produce triploid salmonids is very much in the experimental phase, although there is already a demand for using triploid trout for planting into lakes. We still have many problems to solve before we are ready to begin general production of triploid fish. These include improving survival and obtaining consistently high triploid conversion, reorganizing rearing space to accommodate these smaller groups of fish, determining appropriate rearing practices for triploid fish, ensuring that the expected benefits of sterile fish are realized, and that they contribute to the target fisheries, and finally, that using triploid fish, a genetically modified organism, is acceptable to Washington's citizens.

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STERILE TROUT PROGRAMS IN IDAHO:

MINIMIZING GENETIC RISKS TO NATIVE STOCKS

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Overview and Rationale

Over the last 20 years, the production and use of sterile fish as a fishery management tool has received increasing attention. Much of the early interest in sterile fish stemmed from speculation that they would divert energy resources from reproductive products to somatic growth, and would therefore have potential to grow faster, live longer, and attain larger sizes than normal fertile fish. Although these fishery benefits by and large have not been realized, the advantage of sterility alone can make such fish an invaluable tool for fishery management. All sterile hatchery trout could provide directed harvest opportunity while protecting native trout from overharvest or genetic impacts.

Despite recent improvements in production techniques, and the availability of sterile triploid rainbow trout *Oncorhynchus mykiss* trout from commercial egg suppliers, the performance of sterile fish in recreational fisheries remains largely undocumented in the peer reviewed literature. Sterile fish must perform at or near levels of normal hatchery fish to be acceptable to fishery managers and anglers. In 1996, the Idaho Department of Fish and Game (IDFG) initiated a project to evaluate the performance of sterile triploid trout in recreational fisheries and to develop methods to produce triploid rainbow trout with our own in-state broodstocks.

Sterile hatchery trout have potential applications in several IDFG fishery management programs. In the last decade IDFG stream stocking has been reduced from over 2,000 to about 850 stream miles, mostly in response to increased emphasis on wild trout fisheries where habitat can support a fishery. Despite this reduction, about 40% of our current stream catchable plants occur in streams with viable wild trout populations. Although IDFG recognizes possible genetic concerns, stocking often continues in these streams in response to public demand or political pressure. Using only sterile catchable trout in such cases would eliminate the concern for genetic impacts, but still meet the demand for consumptive fishing.

Henrys Lake in eastern Idaho supports a fishery for native Yellowstone cutthroat trout *O. Clarki*. The lake has an egg taking station and wild cutthroat production is supplemented with 2,000,000 hatchery cutthroat annually. In the 1950's IDFG began stocking rainbow x cutthroat hybrids to diversify the fishery and provide a higher trophy component. These hybrids are fertile, and at least some introgression with cutthroat has occured, although the wild cutthroat population and fishery remain strong. In the early to mid 1980's, IDFG made several attempts to produce and evaluate sterile triploid hybrids. Results were inconclusive, but minimizing further introgression remains an important management goal.

IDFG manages ten lakes and reservoirs with trophy trout regulations (two fish over 20 inches, barbless lures and flies only). If sterile trout do grow faster and reach larger sizes, they represent a way to improve size structure and trophy potential in these and other waters. From a research perspective, an attractive aspect of our trophy waters is that restrictive harvest regulations permit long-term monitoring of stocked fish which is difficult in standard yield fisheries with no length limits. One of our objectives is to evaluate relative survival and growth of triploid and normal rainbow trout in nine lakes and reservoirs statewide, including six waters with restrictive harvest regulations.

IDFG maintains two rainbow trout broodstocks, the Hayspur strain and a domestic Kamloops strain. Although commercial egg suppliers can provide sterile fish for evaluation purposes, developing sterile fish from our own broodstocks would be more cost effective in the long term. We are attempting to create a tetraploid Hayspur strain broodstock to produce sterile triploid progeny, both rainbow trout and rainbow x cutthroat hybrids. This would

decrease our dependence on expensive commercial triploids while providing a strain of fish which is known to perform well in Idaho fisheries.

1996 Activities

Sterile Stream Catchables

In spring 1996, IDFG purchased 20,000 triploid and 20,000 control diploid rainbow trout eggs from Mount Lassen Trout Farms, Inc. These fish are being reared at Nampa Fish Hatchery to catchable size (250 mm) and will be available for stream stocking in spring 1997. We will verify sterility of the triploid group in fall 1996 and are monitoring hatchery performance (hatch rate, growth, conversion) for triploids and controls. Fish remaining in spring 1997 will be allocated to paired stocking evaluations in ten or more streams statewide. Approximately 250 triploid and 250 control fish will be jaw-tagged and stocked in each stream. We will use a paired-t test on tag return data to detect overall differences in triploid versus control returns.

Henrys Lake Sterile Hybrids

In March, 1996 approximately 68,000 cutthroat trout eggs were fertilized with rainbow trout sperm and subjected to heat shocks to induce triploidy. Treatments were at 28.5 and 29.5°C, 10 min after fertilization, and 10 min duration. Another 30,000 control eggs were handled identically at ambient temperatures (7.7°C). Eye-up and hatch rates for treated groups were 20-25% of control eggs. In September, flow cytometry of blood samples indicated 35% triploids in the 28.5°C group and 60% triploids in the 29.5°C group. Treatment and control fish were differentially fin-clipped and stocked into a 10 ha pond in mid-September. Despite the low percentage of triploids in the treated groups, we feel we can monitor performance of steriles versus controls if blood samples from treated fish are collected during future sampling. We will also to attempt refine our heat shock treatments in 1997 to improve the rate of triploidy induction.

Lakes and Reservoirs

In April, 1996 IDFG received 60,000 triploid and 60,000 control rainbow trout eggs from Trout Lodge. These fish were hatched and reared at Nampa Hatchery. In August, triploids and controls were differentially grit marked with fluorescent dye. In October, equal numbers of triploid and control fish were stocked into nine lakes and reservoirs statewide, six of which have restrictive regulations including length limits. Periodic sampling and creel surveys over the next several years will allow us to assess relative growth, survival, and contribution to the creel.

Tetraploid Broodstock

In February, 1996 we used hydrostatic pressure shock to induce tetraploidy in Hayspur strain rainbow trout eggs. Pressure shocks were 8000, 8500, 9000, and 9500 psi starting 4 hr 46 min after fertilization and lasting 5 min. Treatment groups were reared separately until blood analysis. Flow cytometry of blood samples taken in July indicated very low induction of tetraploidy (one of a total of 136 sampled fish). We will modify our treatment regime this fall to include both thermal and pressure shocks, and will also attempt to refine the timing of treatments.

Discussion

This is the first year of a multi-year project on sterile fish production and evaluation in Idaho. The most critical aspect of this work will be documenting the performance of sterile triploid trout in recreational fisheries, and communicating that information to fishery managers. Even a somewhat lower level of fishery performance may be acceptable if genetic risks from hatchery fish are reduced or eliminated. Fishery managers will always face political or public pressure to stock hatchery fish to meet consumptive demands. Short of ignoring those demands, using sterile hatchery products may represent the best way to minimize genetic impacts on wild and native stocks.

PRODUCTION-SCALE PRESSURE SHOCKING OF RAINBOW TROUT (*Oncorhynchus mykiss*) IN BRITISH COLUMBIA

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Introduction

British Columbia's Fisheries Program Strategic Plan (1996-2000) consists of four strategic objectives:

1. Conserve wild fish populations and their habitat
2. Manage for the sustainable use of fish
3. Build support for resource stewardship
4. Support cooperative arrangements with First Nations

Strategic objectives 1 and 2, plus Fisheries Program policies such as the Conservation Policy (1993) and the Wild Indigenous Fish Policy (1993) have directed the Fish Culture Section (FCS) to "deliver stocking programs that are consistent with wild fish conservation objectives" (Fisheries Program Strategic Plan, 1996). As a result, the FCS is in the process of developing non-reproductive stocks to minimize potential impacts of stocking programs on wild fish and also to diversify angling opportunities.

Research into non-reproductive stocks in B.C. Fisheries began in 1989 when Research Biologist Kanji Tsumura conducted heat shock trials in order to establish a protocol for creating triploid rainbow trout (*Oncorhynchus mykiss*). Triploidy rates of up to 80% were obtained through these heat shocking trials. Recently, at the Fraser Valley Trout Hatchery (FVTH), production sized groups (~15,000 eggs per treatment) of rainbow trout eggs were heat shocked producing triploid rates ranging from ~90.0 to 98.2%. To date, heat shocking has not produced 100% triploidy rates in rainbow trout, however, 100% triploidy rates have been produced by heat shock with cutthroat trout (*Oncorhynchus clarki*) and brook trout (*Salvelinus fontinalis*). To address conservation concerns of the B.C. Fisheries Program, a triploidy rate of 100% for production fish is or will be required. As a result, it became necessary to look at alternative triploidization methods. An effective production scale triploidization method would need to:

1. repeatedly produce 100% triploid rates
2. shock large numbers of eggs in a short period of time
3. transport and set up with ease (in B.C. there are five trout hatcheries and many egg take stations)

Currently, hydrostatic pressure shock (HPS) is the only alternative method that meets the above criteria. Benfey et al., (1988) indicate that HPS is the most effective method (in terms of survival and triploid rate) for

producing triploid salmonids. The only limitation to the use of HPS is the limited volume of the pressure chamber (Thorgaard, G.H., 1986). To avoid this limitation, the FCS purchased a portable, hydrostatic pressure shocker with a 2.7 liter pressure vessel capable of shocking approximately 20-25,000 eggs per treatment (TRC Hydraulics Inc., Dieppe, N.B.). The maximum length of pressure shock treatments is usually five minutes, therefore, it is possible to pressure shock five groups of eggs per hour for a total of 125,000 eggs per hour. To date, we have completed three trials with rainbow trout.

Preliminary Trial

Objective: To familiarize staff with the machine and to find a suitable treatment protocol.

Chilliwack River steelhead (*Oncorhynchus mykiss*) were the only spawning salmonids available in early April, 1996. Gametes from 8 males and 8 females were taken, placed in a cooler and transported (two hours) by vehicle to the FVTH. A total of 976.6 grams of eggs were pooled and then divided into six groups. The pooled groups of eggs were fertilized with pooled sperm samples to standardize viability of the treatment groups. Test parameters were set through discussions with Dr. Ed Donaldson and Mr. Igor Solar from West Vancouver Lab, Department of Fisheries and Oceans. Ambient water temperature throughout this experiment was 10° Celsius. After treatment, each group was incubated separately in a vertical tray incubator. In terms of yield (% survival * % 3N), treatment number 6 was the most effective (Table 1). Ploidy analysis was determined via flow cytometry (Allen, 1983). After ploidy analysis the remaining fish were terminated.

#	Treatment	# of eggs	% Surv. to eye	% Surv. to hatch	% 3N	Yield (%)
1	Control	1266	97.7	96.1	--	--
2	Heat: 26.5°C/ 20 min Post Fert / 25 min	1260	94.8	79.5	80.0	63.6
3	Pressure: 8500 psi/ 25 min Post Fert/ 5 min	1228	98.2	86.6	5.0	4.3
4	Pressure: 8500 psi/ 40 min Post Fert/ 5 min	1195	96.0	59.5	68.4	40.7
5	Pressure: 9500 psi/ 25 min Post Fert/ 5 min	1262	95.0	82.7	80.0	66.2
6	Pressure: 9500 psi/ 40 min Post Fert/ 5 min	1238	96.0	84.7	100.0	84.7

Table 1: Treatments and resulting % survivals and 3N% for the preliminary trial conducted with Chilliwack River steelhead.

Production Trial #1

Objective: To determine the impact of normal production procedures (extended holding and transportation of fish, fertilization with masculinized female milt) on the yield of HPS.

Based on the results from the preliminary study, a production size HPS trial was conducted using a treatment of 9500 psi, 40 minutes post fertilization and five minute duration (10°C ambient water). Blackwater River strain rainbow trout were trapped and held at Dragon Lake (near Quesnel, B.C.) for two days until they were transported (7 hours) by tanker truck to the FVTH. At the hatchery, the fish were held for a period of five days. Eggs from four females were pooled to form each treatment group. Each pooled group of eggs was fertilized with pooled samples of excised testes from masculinized female Blackwater River strain rainbow trout broodstock. After treatment, each group was incubated separately in a vertical tray incubator. Total percent survival to eyed egg and troughing for the HPS treatments was lower by approximately 10% and 20%, respectively, than control values (Table 2). Previous studies have also determined that survival of triploid embryos from the eyed stage to hatching was variable but generally lower than survivals in the controls (Lou and Purdom, 1984). Variability in percent survivals was observed in pressure shock treatment #3 (Table 2). Lou and Purdom (1984) suggest this variability provides further evidence that egg quality is vital to successful

ploidy manipulation. The results of the flow cytometric ploidy analysis for this trial are currently not available but, will be discussed at the conference.

#	Treatment	# of eggs	% Surv. to eye	% Surv. to trough
1	Pressure: 9500 psi/ 40 min Post Fert/ 5 min	10 336	87.0	all HPS
2	Pressure: 9500 psi/ 40 min Post Fert/ 5 min	10 944	89.9	treatments
3	Pressure: 9500 psi/ 40 min Post Fert/ 5 min	9 200	55.6	combined
4	Pressure: 9500 psi/ 40 min Post Fert/ 5 min	8 903	80.4	(see below)
	Total egg # & mean % Survivals	39 383	78.2	61.0
5	Control	1 097	88.6	81.3

Table 2: Treatments and resulting % survivals for production trial #1.

Production Trial #2

Objective:
A) To compare the yield of production size HPS and heat shock treatments on Pennask all-female rainbow trout embryos.
B) To compare the yield of two HPS treatments, high pressure (9500 psi) and lower pressure (8500 psi).

On June 27, 1996, the HPS unit was transported to the Summerland Trout Hatchery (SLH) to treat eggs taken from rainbow trout at the Pennask Lake egg station. The females were stripped and the green eggs were transported to SLH via truck. Approximately 3 hours later, the initial groups of eggs were fertilized with milt from masculinized female Pennask Lake strain rainbow trout. Eggs and milt were pooled in order to standardize viability of treatment groups. Ambient water temperature was 10° Celsius. After treatment, each group was incubated separately in trays suspended in flow through troughs. The high pressure and heat shock groups exhibited similar survival rates to eye and ponding (Table 3). The low pressure shock and control groups exhibited higher survival rates. Using a similar protocol, Teskeredzic et al. (1993) also observed decreased survival rates as the pressure treatment increased. Flow cytometric ploidy analysis results and conclusions regarding yield from both pressure treatments will be discussed at the conference.

#	Treatment	# of eggs	% Surv. to eye	% Surv. to trough
1	Control	855	88.0	84.9
2	Heat: 26.5°C/ 20 min post Fert / 25 min	151 501	86.5	79.8
3	Low Pressure: 8500psi/ 40 min Post Fert/ 5 min	9 653	95.2	93.0
4	Low Pressure: 8500psi/ 40 min Post Fert/ 5 min	9 997	82.5	79.8
5	Low Pressure: 8500psi/ 40 min Post Fert/ 5 min	1 003	91.8	89.0
	Total egg # & mean % Survivals	29 653	89.8	87.3
6	High Pressure: 9500/ 40 min Post Fert/ 5 min	9 719	97.0	90.5
7	High Pressure: 9500/ 40 min Post Fert/ 5 min	9 788	74.6	64.0
8	High Pressure: 9500/ 40 min Post Fert/ 5 min	9 865	83.6	73.4
	Total egg # & mean % Survivals	29 372	85.1	76.0

Table 3: Treatments and resulting % survivals for production trial #2.

Discussion

In the future, 100% triploidy rates for production rainbow trout will be necessary in order to minimize the impacts of these fish on wild fish stocks. Within the FCS, heat shocking has not produced 100% triploidy rates, on a consistent basis, in rainbow trout. Teskeredzic et al. (1993) speculated that percent triploidization

and survival of the heat shocked eggs is directly related to egg size. As a result, smaller volume eggs receive a greater thermal shock than larger volume eggs within the same treatment, leading to inconsistent triploidy rates. In contrast, HPS subjects all eggs within a treatment, regardless of size, to the same pressure shock. To date, the FCS has induced 100% triploidy rates in steelhead with HPS and is in the process of confirming the effectiveness of this protocol for rainbow trout. With the large egg capacity and ease of transportation and set up of this particular unit, HPS meets the criteria of an effective production scale triploidization method. Finally, regardless of the triploidization method utilized, consistent survival results are intimately related with egg quality or fitness (Lou and Purdom 1984, Teskeredzic, 1993). Therefore, in order to obtain maximum yield from any triploidization method, it is paramount that steps must be taken to ensure optimum gamete quality.

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Alteration of ploidy in rainbow trout with heat and hydrostatic pressure.

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Abstract.

Ploidy of rainbow trout was modified with heat and pressure treatment. Heat treatment at plus 26 Celsius for twenty minutes duration from six to forty minutes after fertilization produced different percentages of triploids and tetraploids. The eggs were heat treated in polyethylene sandwich bags (ziplock type). After the heath treatment was applied, the eggs were washed, cleaned, and held at normal egg development temperatures (rainbow trout 6 - 12 C). The hydrostatic pressure treatment of 715 (KPA) or 10,000 (PSI) for 30 minutes post-fertilization produced the highest number of triploids. Ploidy measurements were determined by flow-cytometry and Coulter Counter techniques. The authors discuss the interpretation of the results.

Introduction

Fisheries management in Alberta has a problem in several lakes, prairie ponds and other water bodies where rainbow trout is planted. High losses in the water bodies occur. One of the observations indicated that rainbows are dying because they consuming their energy in the spawning and therefore they have not enough energy to survive in the winter or if they spawning in the spring then they losing the remaining energy reserves. The other reason for the mortality was speculating, that rainbows do not have suitable spawning grounds, therefore their eggbound condition is the cause of the mortality. It was also speculated, that triploid fish will give a faster growth rate than triploid. To explore these theories we started a triploid production program. We have now only preliminary results and insufficient date about our field experiments.
We hope that sterilization will eliminate the fishery management problems associated with sexual maturation of fish. Prevention of fish maturation may maximize growth by diverting resources which would be otherwise used for gonadal development, eliminate uncontrolled reproduction, and present new management options by extending the life cycle beyond the normal time of spawning and death in salmonid species (Benfey and Donaldson 1988, Donaldson and Hunter, 1982, Donaldson and Benfey, 1987, Ihssen et al., 1991).

Triploids are expected, to grow faster and live longer than diploids as they reach the age of sexual maturity, because they spend no energy to produce gonads. The faster growth of females, if simply measured as total body weight at age, may be partly due to the greater accumulation of fat in the body cavity rather than the desired increase in muscle tissue (Ihssen et al, 1991). Some studies have found inferior early growth in triploid salmonids (Solar et al., 1984) while others have found no difference in normal condition, but triploid rainbow trout did not survive or grow as well as diploids in chronic high water temperature conditions (Ojolick et al., 1995).

Whether adult triploids could provide trophy fish or more economical production of fish is still not certain, because the results to date are inconsistent. It appears that the growth advantage of triploids is species dependent, even for closely related species. Also, the growth advantage may be held only by females, not by triploid males that develop pronounced secondary sexual characters and for some species, even sperm. Triploid tiger trout (Brown males x Brook female trout) appeared to survive better than diploid hybrids at the alevin stage (Scheerer and Thorgaard, 1987, McKay et al, 1992.).

In the triploid induction we had several contradictory results that forced us to understand better the process. Another surprise is the constant and relatively high level of triploids in the untreated control. We also looked into how the possibilities to manipulate chromosomes to induce polyploidy involves techniques to suppress normal meiosis or mitosis to retain additional haploid sets of chromosomes (polar bodies). Induced polyploidy refers to the production of individuals

with extra sets of chromosomes. This can be done by treating fertilized fish eggs with either temperature shock (Varadaj and Pandian, 1988), hydrostatic pressure, chemical or electrical shock treatments (Chourrout, 1986, Teskeredzic et al., 1991). Physical treatments, such as temperature shock, electric shock and hydrostatic pressure are easily applied and do not require the use of harmful chemicals or hormones. The only other method used to produce triploid fish without harmful side effects is the crossing of tetraploid with diploid fish.
Temperature-shock treatments are particularly advantageous for situations in which large volumes of eggs need to be treated. Heat shock and hydrostatic pressure are the most practical because of less expensive treatment to produce sterile fish. Applications of these treatments, produces polyploids without the use of harmful chemicals and hormones. However, high mortality in eggs by heat shocking is reported, requiring extra labour in egg picking. Hydrostatic pressure has been used for induction of both triploidy and tetraploidy. A limitation of using hydrostatic pressure is that appropriate pressure vessels may not accommodate large volumes of eggs.

The timing of the treatments is critical. If the treatments are applied shortly after fertilization, triploids can be produced due to retention of the first or second polar body of the egg. If they are applied at or later than anaphase II, the second meiotic division cannot be stopped and no gynogenetic diploids or triploids will result. If the treatments are applied before the first cleavage division, tetraploids can be produced. Typically, treatments have been applied at variable times after fertilization. The duration of the heat treatment is usually shorter than the cold treatment. This is primarily because of the excessive egg mortality associated with prolonged heat treatments. Heats shocking techniques are more effective than cold treatment for salmonids. The upper lethal temperatures of sperm and eggs of rainbow trout are two minute's exposures between 40-42°C (Hamor 1964, 1988).

Based on results and other information from the literature, acceptable rainbow trout egg survival occurs within the heat induced temperature range of 26-28°C. The most successful induction method for rainbow trout has been application of an 8-min. pressure treatment (Table 1).

TABLE 1 - Methods for Manipulation of Rainbow Trout Ploidy

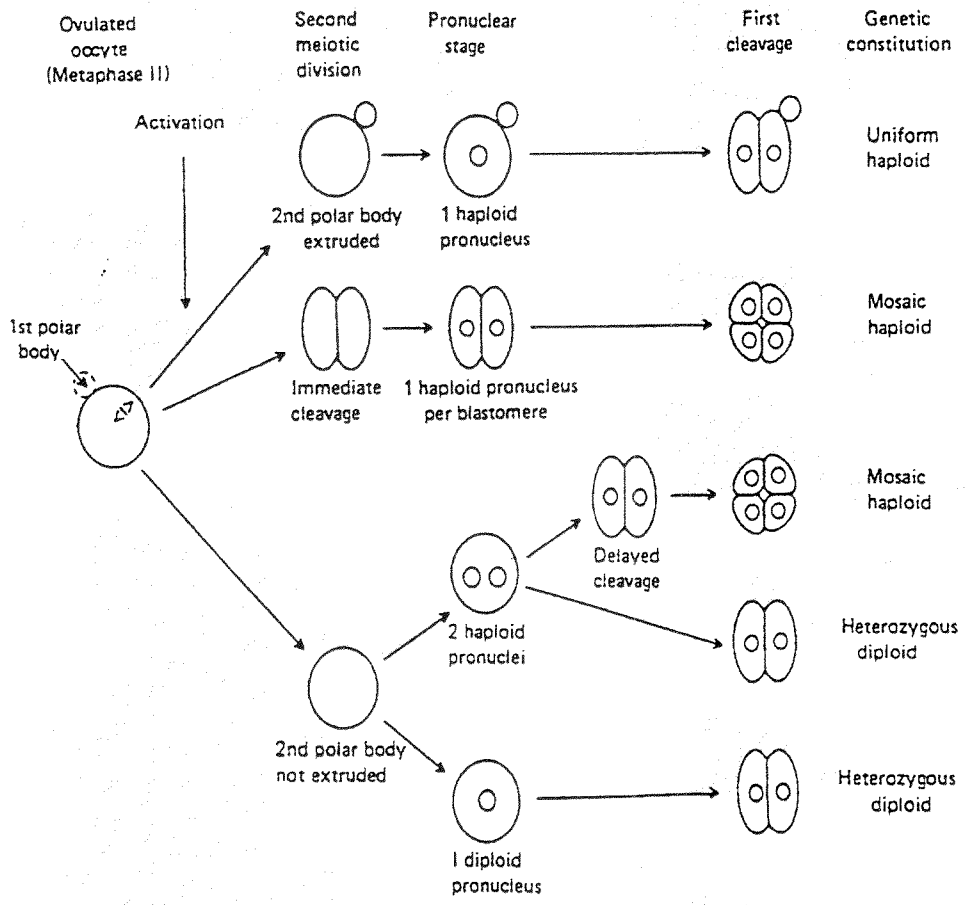
(Ihssen et al 1990)

Early heat 27-30°C, 1=0-70 min, D=10 min.	50% triploids
Early heat 36°C, 1=20 min, D=1 min.	43% triploids; 33% survival of embryos
Early heat 26°C, 1=25 min, D=20 min.	98% triploids; 87% survival
Early heat 26-38°C, 1=1 or 40 min., D=10 min.	83-100% triploids; 50-70% survival
Early pressure, 420 kg/cm^2, 1=10 min, D=8 min.	100% triploids; 53% survival
Late heat 36°C, 1=5 h, D=1 min.	16% tetraploids; 28% survival of embryos
Late heat 28°C, 1=8.5 h, D=14 min.	8% tetraploids, 25% survival
Late pressure 490 kg/cm^2 1=5.8 h, D=4 min.	100% tetraploids; 40% survival

Eggs impregnated by completely inactivated sperm are developing into gynogenetic haploids (Figure 1). Ploidy manipulation can result in a mixture diploid, triploid, tetraploid, and sometimes even higher ploidy levels and mosaics (Figure 2). Therefore, reliable methods for ploidy identification are required.

Findings show that heat and pressure shocks exert a negative influence on growth that is independent of changes in ploidy,

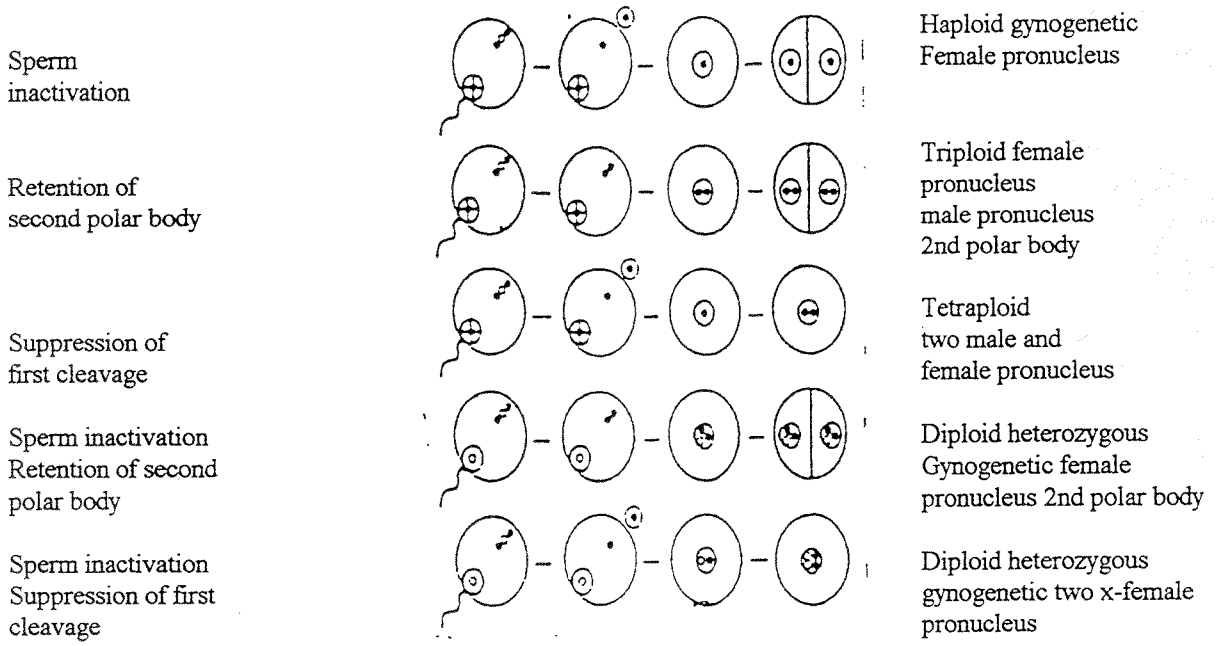
Figure 1. Parthenogenetic fertilization.



Classification of parthenogenomes.

Possible routes of development, and their genetic constitution after activation of unfertilized mouse eggs. Normal meiotic division leads to polar body extrusion, leaving one haploid pronucleus. When second polar body extrusion is suppressed, eggs either undergo immediate cleavage or develop two haploid pronuclei or one diploid pronucleus. (After Browder, 1985).

Figure 2. Production of gynogens, triploids and tetraploids.



Three operations are required for producing gynogenetic individuals, triploids and tetraploids in lower vertebrates, sperm inactivation, retention of second polar body and suppression of first cleavage. From Chourrout (1984). In salmonid populations the male- female ratio is determined by the male gametes. That ratio is different in strains, populations and can change from one year to the other in the same population. In cases where investigators are testing the survival of triploids and not concerned about the male-female ratio, the results are skewed. Female triploids are not developing gametes but males do. That can make a difference between the male and female triploid fish survival. Consequently, investigators working with populations with differing male-female ratios are getting different results. That is the reason why all female triploid populations or populations having more females than males are showing better survivals.

Figure 3. **Timing of triploid production**.

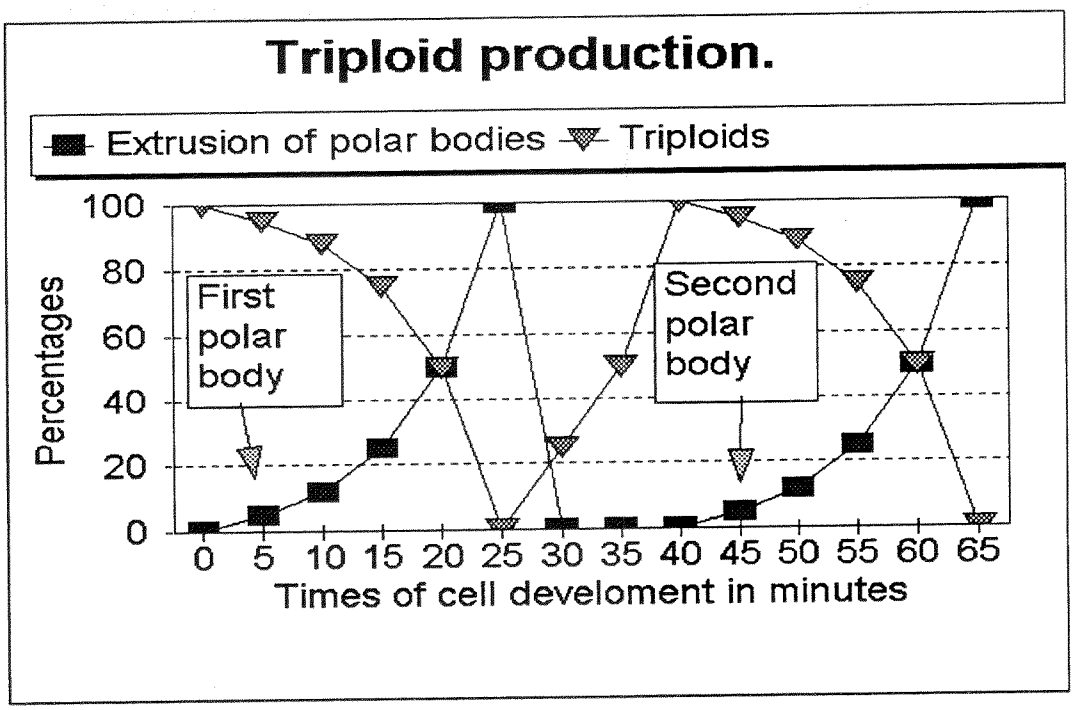
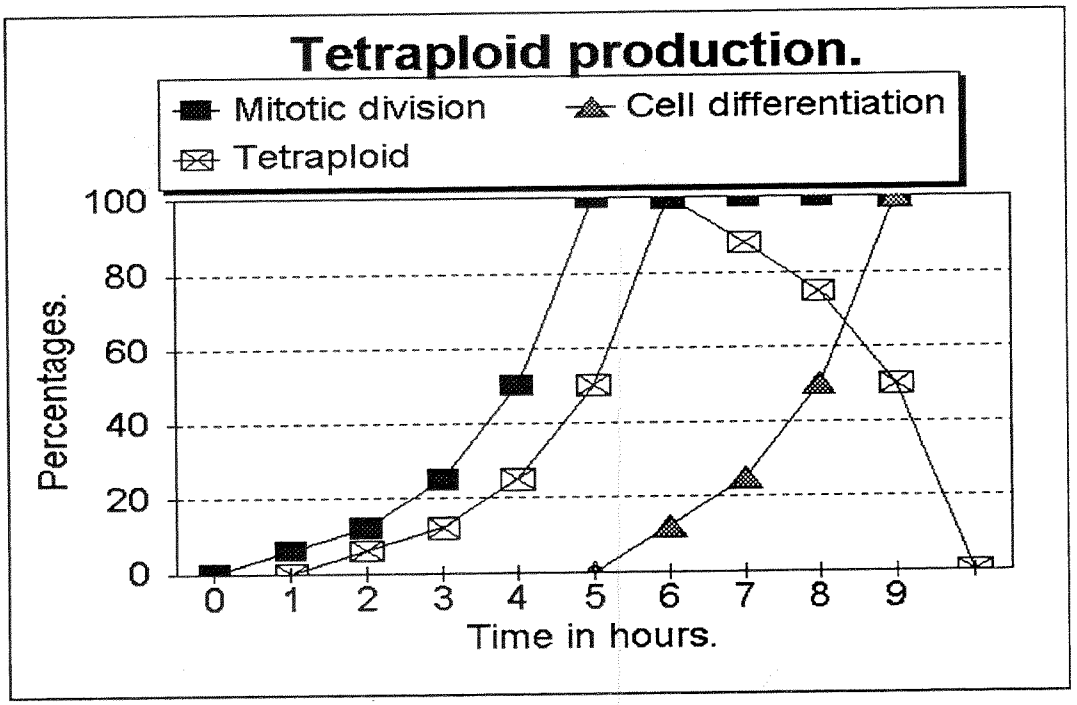


Figure 4.. **Timing of tetraploid production**.



and that triploid perch may have the potential to outgrow diploids if the negative effects of such shocks can be avoided (Malison et al., 1993). Significantly greater mean weight was observed at 17 months for diploid coho salmon (16.6 g) when compared to their triploid siblings (14.5 g), (Utter at al. 1983).

No differences were detected in the ability of triploids to smoltify and to successfully adapt to seawater under normal conditions in coho salmon (Oncorhynchus kisutch) by Johnson et al., 1986. Diploid and heat-induced triploid treatment groups of rainbow trout (Oncorhynchus mykiss), coho salmon (Oncorhynchus kisutch), and reciprocal hybrid crosses were produced and monitored for survival and growth through early life stages. The triploid rainbow trout female X coho salmon male hybrids showed a significant increase in IHN resistance when compared to pure-species rainbow trout groups. Early growth results suggest a poorer growth rate for the triploid hybrid (Parsons et al., 1986).

Egg and fry mortality of triploid rainbow trout was higher than diploid mortality but after sexual maturation triploid females grew better than diploids of either sex or triploid males (Ihssen et al., 1991). Sterility is also advantageous in situations where the control of reproduction is desirable.

Several methods are available for the feminization and sterilization of fish. Non-hormonal feminization can be achieved by gynogenesis (Hunter et al., 1983, 1986, Johnson and Write, 1986, Johnstone et al., 1979, Refstie et al., 1982). Some other sterilization methods are: irradiation, chemosterilization, hormone antagonists or specific anti-androgens or anti-estrogens, anesthetics (Ihssen et al., 1990), gonadal autoimmunity, surgical methods and chromosome set manipulation. The production of entirely female stocks requires phenotypically male brood stock of female genotypes to fertilize eggs from untreated females. The sex-inversed males are produced by feeding fry for 700°C days with food containing 3 mg kg^{-1} 17 methyltestosterone. The majority of the males lack sperm ducts, requiring the semen to be removed surgically, but reduced duration androgenization increases the proportion of males with intact ducts. No fish destined for human consumption are treated with hormones. Sterile trout is produced by the induction of triploidy in female eggs. Eggs are heat shocked at 28°C for 10 min., commencing 40 min. after fertilization. Commercially acceptable levels of survival and sterility are achieved (Bye and Lincoln 1986).

Eggs fertilized with sperm partially inactivated sperm develop into aneuploids (Benfey et al., 1986, Echelle et al., (1988), having chromosome numbers other than exact multiples of the haploid set (monosomic, double trisomic, tetrasomic, pentatomic, etc.). Such progenies probably have lower viability than the triploids. Hertwig (in Ihsseen et al., 1990) has shown that if the eggs of the frog (Rana fusca) are fertilized with sperm treated with high dosages of gamma-radiation, they develop more normally than those fertilized with sperm treated with lower dosages. This paradoxical phenomenon, called the "Hertwig effect," is due to the egg being able to tolerate sperm that is completely genetically inactivated better than partially inactivated sperm.

Tetraploidy results from the suppression of the second mitotic division and the retention of the first or second polar body, similar to the mechanism proposed for the establishment of triploidy in cold-shocked fish. Crossing tetraploids with diploids is a method that holds promise as a simple and economical solution to produce triploids. This procedure requires that viable, fertile tetraploids can be produced for the species of interest.

Methods

We produced triploid rainbow trout using heat and hydrostatic pressure. In the laboratory trials, heat treatments were applied after the fertilization occurred in the second, fourth and sixth minutes and so on in every two minutes intervals up to twenty-four minutes. Following that, in the first production serial in 1993, we heat-treated the eggs twelve minutes following fertilization. In both treatments the eggs were enclosed in polyethylene bags. In the second production series the treatments of the eggs were applied in the fourteenth, twenty fourth and thirty-eight minutes following fertilization. All treated egg lots were placed in a floating screen inside a large (40L) water bath. The durations of all treatments were twenty minutes for each application and the heat applied was 28 C°. Rainbow trout eggs were treated with 429 KPA (6000 psi) hydrostatic pressure at ten, twenty and thirty minute post-fertilization intervals for the duration of four minutes. The temperatures in these treatments were between 9.4 - 11.3 C°, about the same as the pond temperatures were the eggs producing fish were located. Similar procedures are applied to grass carp eggs where 8000 psi. for the duration of two minutes is applied within four minutes after fertilization (Table 2).

In the 1992 laboratory trials, ploidy was determined from homogenized embryos, followed by chromosome staining procedures (Thorgaard and Disney, 1990). Ploidy was determined in 1993 - 1996 either with flow-cytometry, Coulter counter methods or both. In both methods we compared our measurements with commercially available standards.[1] In every analyzed group we measured the percent of ploidy in the treated and the control groups. Statistical analysis applied to the results is the "Statistix for Windows" by Analytical Software.[2]

Results and Discussion

Ploidy manipulation started about in the nineteen sixties. Chourrout (1984 and 1986) gave very good comprehensive assessments of triploid and tetraploid creation. The male and female gametes (sperm and egg) have a meiotic division before they are ready for fertilization forming a union, the zygote. The zygote then produces its own copies duplicating into two cells, four cells and so on. Early in embryonic development, the cells are undifferentiated, meaning that each of them is alike. During this phase, separating the dividing cells can produce individual fish from each cell. This is well known in hatcheries, as fishes may be attached to each other like Siamese twins or with two heads, depending what point and how the separation occurs. The science to dealing with those aberrations is the teratology.

However there are still many unanswered questions remained. We know that in gamete division the oocyte (egg) produces the haploid condition (1N instead of 2N) plus a polar body. A polar body contains one nucleus derived from the first and/or second division of meiosis, but has no cytoplasm. Therefore, by retaining a polar body, the cell will have one extra chromosome set. A haploid cell contains one set of chromosomes (1N), diploid=two (2N), triploid= three (3N), tetraploid= four (4N), pentaploid= five (5N) hexaploid (6N). Pentaploids, hexaploids, and tetraploid gynogenetics result if the antimeiotic and antimitotic treatments are applied (Chourrout 1986).

Ploidy manipulation causes changes in the cell composition. There are even differences in mitochondrial DNA and allozyme variation in rainbow trout spawning in different season's Ferguson et al., (1993). Consequently, the requirements for nutrients and trace elements are changed and that can explain the different results reported in the growth and survival of polyploids. Triploids created by the retention of first or second polar bodies have a male or female pronucleus respectively (Chourrout 1984, 1986). Our approach taking heterozygous as only measurement for vitality might be misleading. Alterations are observed from the traditional genetical rules. Ferguson et al., (1993) did not fund reduced enzyme heterozygosity relative to the naturalized population in Ontario cultured rainbow trout. Moran et al., (1996) found a relationship between population size and mitochondrial DNA variation in wild and hatchery brown trout (Salmo trutta L.). Between-population haplotype differences found in this work support the hypothesis of a recent divergence of all the Spanish brown trout from a common ancestral genotype. Saavedra and Guerra (1996) found no significant differences between multi locus heterozygosity, viability and growth in a cultivated population of the European oyster. In the hatchery produced giant clams there was no consistent correlation of individual heterozygosity and growth rates within batches (Benzie and Williams 1996).

In our opinion, the Hertwig effect is not a paradox, but contrary is well expected, because a partially inactivated sperm carries unevenly matching chromosomes. Likewise, these ploidy manipulations also cause chromosome aberrations that can result in malformation and stunted growth. Better understanding of the mechanisms and the role of altered chromosomes will lead us to overcome the undesired effects (Malison et al., 1993).

The results of our heat and hydrostatic treatments are summarized in Table 3 and Figures 5 and 6. Our results are in agreement with those of Poisil and Chourrout (1992), that higher pressure results higher percentage of ploidy change, results less survival and that the timing of the treatment is the most important variable. It came first as a surprise that the triploid fish supposed to be our controls in the readings showed an average 3% triploidity. First we suspected some error in our readings, but both methods (Coulter Counter and flow-cytometry) were giving the same results from two different hatcheries. In populations from 1992 to 1995 we found about 3% triploid in most of the untreated hatchery population and in 1996 about 15%. Spontaneous triploidity is reported earlier by other authors. Thorgaard and Gall (1979), Thorgaard et al., (1982 a and b) found more than 6% spontaneous triploids among 30 adult trouts that failed to mature

[1] CytoBioTechnics inc., 2176 Hixon Street, Oakville, Ontario L6L 1T4. (Blood samples Coullter Corp. standard beads)

[2] Analytical Software P.O. Box 12185 Talahassee Florida, 32317 USA T:904-8939371

in a hatchery. We believe that spontaneous triploids occurring in hatcheries are the result of the manipulation/handling of eggs. To prevent unwanted triploidy, we suggest giving attention to the egg handling. The explanation for the few tetraploids found in egg lots that were chemically treated prior to the exclusion of the first polar body or before the mitotic division is that the eggs retained both of the polar bodies. This is a very rare phenomenon, with occurrences of those individuals less than one percent. In our opinion, producing those intentionally is difficult or impossible because of the problems of egg survival.

Following the laboratory trials the first batch of heat treated eggs in 1993 (twenty minutes treatment, twelve minutes after fertilization at 28°C) resulted in 17% percent triploids. The three egg lots treated in 1994 (14, 24 and 38 minutes post fertilization) resulted in 46.0 %, 33.5% and 71.9 % respectively. Effectiveness of heat shock would appear to be time dependent due to the timing of polar body exclusion (Fig. 3 and 4).

Table 2. **Hydro pressure experiment Allison Brood Station 20th of January 1995.**

Time after fertilization (minutes)	Pressure (KPA) (psi)	Volume eggs(ml)		Water temp(C)	Lot
10	429	6000	60	10.2	AD1
23.5	429	6000	40	10.4	BD1
33.5	429	6000	30	10.8	CD1
CONTROL: - CTRL1					
10*	429	6000	30	11.0	AD2
10*	429	6000	30	11.0	AD3
20*	429	6000	30	11.2	BD2
20*	429	6000	30	11.2	BD3
30*	429	6000	30	11.3	CD2
30*	429	6000	30	11.3	CD3
CONTROL: - CTRL2					
10*	572	8000	30	11.4	AE2
10*	572	8000	30	11.4	AE3
20*	572	8000	30	9.4	BE2
20*	572	8000	30	9.4	BE3
30*	572	8000	30	9.7	CE2
30*	572	8000	30	9.7	CE3
CONTROL: - CTRL3					
10*	715	10000	30	9.9	AF2
10*	715	10000	30	9.9	AF3
20*	715	10000	30	10.1	BF2
20*	715	10000	30	10.1	BF3
30*	715	10000	30	10.2	CF2
30*	715	10000	30	10.2	CF3
CONTROL: - CTRL4, CTRL5					

* - Samples split into two 30ml groups. 3ml sperm used for following sample groups. Strain: RBTR/BEBL Duration of pressure; 4 minutes per sample.

The triploid condition occurs when the first or second polar body fails to leave the zygote. We estimated that eggs started to release the first polar body between the first and twenty-fifth minutes in our experiments. After that time in about the fortieth minute the releasing of second polar body starts. As more percentage of egg looses the polar bodies so the percentage

of possible poliploid creation goes down (Figure 3). Tetraploidy is produced either, when both polar bodies are retained in the zygote or when the first mitotic division occurs, prior to cell differentiation. As cell differentiation increases in more and more percent of eggs so the percentage of teraploidity decreases (Figure 4). Cell division can be meiotic, when the number of chromosomes is halved or mitotic when the number of chromosomes remains the same.

Table 3. **Results of hydrostatic pressure experiments.**

Percent of polyploid.

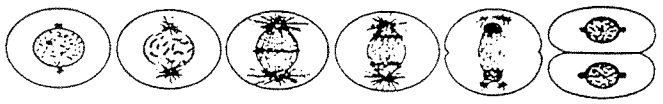
Measured(M) and predicted(P) values.

Pressures	6000		8000		10000	
Minutes	M	P	M	P	M	P
10	17%	16%	37%	36%	60%	56%
20	43%	38%	50%	58%	80%	77%
30	60%	60%	77%	79%	100%	99%

$$P = -145 + 0.0116Hs + 3.59Te + 0.81V + 2.36Ti \qquad R^2 = 0.93$$

Where; P= ploidy in percent (≈ % of triploids); Hs= hydrostatic pressure in psi;
Te= temperature in C°; V= volume of eggs in ml;
Ti= time in minutes when the pressure treatment is applied following the fertilization.

Figure 5. **Mitotic cell division.**



Mitotic cell division.

1-2 Prophase, 3 Metaphase 4-5 Anaphase

6 Telophase.

Eventually the interference to manipulate ploidy should be introduced in or before the stage of anaphase when the number of chromosomes is doubled but they still are in the same cellular envelope. Heat or pressure treatments applied during this stage would result in either karyokinesis (chromatid separation) or cytokinesis (cleavage) inhibition. Karyokinesis is the separation of two daughter strands of duplicate chromosomes before cell wall formation, whereas cytokinesis is the separation of individual cell membranes. The retainment of even (2n or 4n) chromosome number result in mostly tetraploids as the literature and our own experience shows. The difference between the first and second type tetraploid (karyokinesis or cytokinesis)is due to the presence of one or two centromere RNA's and DNA's (female or male pronucleus) in the nucleolus. We think that the karyokinetic tetraploids have fewer methallothioneins (Olsson et al., 1990) and therefore are less viable than the cytokinetic tetraploids. Consequently, their survivability is poorer. Hypotethicaly, productions of uneven number of chromosome sets are also possible through the phenomenon called an anaphase lag. The anaphase log is the delay of in the movement of one or more chromosomes from the metaphase plate during anaphase, often resulting in chromosome loss. We think the anaphase log is responsible for the production of mosaics; individuals that contain diploid, triploid and/or tetraploid cells. The amount and distribution of those cells in different ploidy stages depend on the time and stage when the alteration in cell division happens (Figures 3 and 4).

In later development, the cells differentiate and instead of producing a whole individual they will produce only specific organs or tissues (ex. multiple eyes, extra fins etc.). The germ cells giving rise to the sex organs appear in very early embryonic stages. However, the development of sexuality depends on several complicated genetic mechanisms. The rate of division of cells is time specific in different fish species (Ignateva 1969, 1973).

It is altered by environmental temperature, developmental stage, cell type, presence or lack of several ions, hormones, barometric and gas pressures. The different factors (heat, pressure, chemical) that trigger polyploidy is explained by these variables. Inconsistency in polyploid percentages also occurs because of the magnitude of the heat shock. Important variables are the ripeness, temperature of the eggs to be treated, temperature of the holding pond and the temperature applied in the treatment (Johnstone 1987, Diaz et al., 1993). Also, the volume of the eggs treated may alter the results. In heat treatment the centre of an egg mass may have a time lag in temperature change. It was for this reason that in our second heat treatment (1993), we placed the eggs in screens in the warm water, instead of suspending them in plastic bags. Also, if the treating container is larger, its temperature is less affected by the temperature of a treated product. Mixing of the treating water is also important to ensure even treating temperatures. We think these factors may cause the different success in triploid production. Proper understanding, interpretation and application of these variables are required to achieve high percentages of polyploids.

We think that production of triploids is an answer to some problems in fishery management and the negative results connected with the procedures can be diminished or eliminated by further studies and improvements in the techniques. We are far from the final evaluation of our triploid induction experiments on rainbow trout eggs. In our studies, there were no obvious high losses in egg survival but we still have to work on our final analyses. Slower development was

minimal (98%) and deformations in our rainbow trout progeny were not evident. In other studies completed on a grass carp, we have observed deformities and slower developments in the early life history stages.

Conclusions

The existence of spontaneous polyploids in the population interferes with the success of the brood stock programs. Culling out the not productive polyploids from the population would increase the average fecundity. Taking granted that all the untreated and/or unmarked fish is diploid without determining the presence or lack of spontaneous triploidity gives misleading results. The available data suggest that the spontaneous poliploids are created in the hatcheries by the egg handling. Because we know, that the first two polar bodies are extruded in the first hour after fertilization, than it is logical to watch egg handling practices in that time slot. The second time slot to watch is between four and six hours after fertilization, the time for mitotic division. Further evaluation of spontaneous triploidity is necessary.

The vitality of poliploids is influenced by the genetical makeup of the population used to create them, conditions of the parents, nutrients in the egg, technics of treatment the eggs, nutrition available for the poliploids including trace elements, general water quality and water temperatures.

The time of treatment determines the type of the polyploid produced. In our opinion induction of triploids.in the times of the second polar body extrusion is more efficient than in the first one. Natural and artificial influences are creating all kinds of chromosome combinations that are not always viable and have a normal or deformed appearance. Triploids can be produced by the retainment of the first or second polar body, teraploids by preventing the mitosis. However there are several other combinations are possible, such as those tetraploids that retained both of the polar bodies.

Hydrostatic pressure was more efficient to us to modify ploidy and should be considered to use with adequate vessel size and procedures when creation of a large number of triploid required.

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The Leavenworth National Fish Hatchery Complex (LNFHC) manages three federal salmon hatcheries located in north central Washington state. They include the Leavenworth, Entiat, and Winthrop National Fish Hatcheries. All are located on tributaries of the mid-Columbia River. The Complex shares a commitment to building a very extensive public outreach program. Partnerships have proven themselves to be at the very foundation of any successful information and education program. Partners joining the LNFHC come from many government agencies, non-profit organizations, service clubs, sportfishing groups, private industry, large-scale corporations, schools, American Indian tribes, Chambers of Commerce, and local communities. Projects range from outdoor education to predator control, facility improvements to school mentorships, the annual Wenatchee River Salmon Festival to Kids in the Creek events, correctional community service work to stream restoration activities and opportunities for the disabled.

More specifically, the LNFHC receives recognition from such organizations as the national Sportfishing and Boating Partnership Council, American Fisheries Society, Wenatchee National Forest, International Festivals and Events Association, Environmental Education Association of Washington, Audubon, City of Leavenworth, North Central Washington Educational Service District schools, and Trout Unlimited. It is with tremendous appreciation to these partners that the Leavenworth Complex is successful in blending community service with fish production and resource protection.

Partnerships are invaluable. They offer abundant resources. Each provides a specialty in an area others may lack. It is rewarding to share our hatchery mission with so many... that is to increase the awareness and appreciation for natural resources throughout the entire northwest. By understanding the fragility (in our case) of aquatic resources and their importance to human life, our communities learn to protect, care for, and personally take a part in the conservation of the natural world around them.

Leavenworth Complex partnerships show up in many ways. Monetary donations, in-kind contributions, volunteer labor, and construction services all provide avenues in which our agency and our partners can work side by side in challenges facing our resources today.

TROUT UNLIMITED....A PARTNERSHIP CELEBRATING 32 YEARS

The focus of this presentation is to offer an in-depth view of a partnership that began more than thirty years ago at the Leavenworth National Fish Hatchery. Red Pittack, an avid fisherman and hatchery supporter, became one of the first volunteers to give personal time assisting in hatchery operations. Founder of the Upper Columbia Chapter of Trout Unlimited (TU), Red was the driving force behind the establishment of a permanent relationship with Trout Unlimited. Members of the group helped control Leavenworth gill disease in sockeye in 1964.

Workload periods are very cyclic at the hatchery and at key times of the year the work requires a great amount of labor- intensive activity. Volunteer assistance during these heavy periods have saved several thousands of dollars each year. Examples of this include adipose fin clipping of steelhead, spawning of salmon and steelhead, egg sorting, construction projects such as avian-predation control panels, hatchery clean up work and more.

Both the Upper Columbia chapter of Trout Unlimited and the Leavenworth based Icicle Valley Chapter have worked on the following projects highlighted below:

ADIPOSE FIN CLIPPING STEELHEAD

*Leavenworth NFH raised 100,000 steelhead which required fin clipping each year. TU volunteers and school students assisted in accomplishing this work at no cost to the hatchery.

STEELHEAD AND SPRING CHINOOK SALMON SPAWNING

*Spawning adult fish is a very labor intensive task because of the detailed fish sampling procedures the hatchery deals with. Each spawned female is given a number which corresponds to a fish health sample and individual incubator. To keep the technical tracking of these fish during spawning requires up to 16 people. Trout Unlimited has donated labor each spawning period and makes the arrangements to transport spawned steelhead to the local food bank.

SORTING EGGS

*The hatchery does not have available staff to process eyed eggs fast enough before they hatch. Volunteers are utilized in the sorting and placing of eyed eggs in incubators during this time.

CONSTRUCTION OF AVIAN PREDATION PANELS FOR THE PONDS

*Due to the location of the Leavenworth NFH, winter migrating ducks find the hatchery the perfect feeding ground. In the spring of 1995, hatchery personnel waged a friendly war against the bird predation. A unique aluminum framed wire mesh screen was designed to cover the raceways suffering the most impact. Each panel measures 8 feet by 10 feet and wire was woven in 3 inch by 6 inch rectangles. Weaving the wire mesh is extremely time consuming and labor intensive. Volunteers provided the labor to complete this task. Trout Unlimited purchased over $3,000 worth of materials to cover yet another bank of raceways.
(See poster session exhibiting these panels by Terri Judd, LNFH)

THE 1995 FLOOD AFTERMATH

*Another "one-hundred year" flood event hit the Wenatchee River Valley hard in November of 1995. Logs and debris were scattered over the entire river area to and around the fish hatchery ladder and the surrounding perimeter cyclone fence. Trout Unlimited and student volunteers removed all debris and fencing and replaced 3 gates and 250 feet of security fencing along the ladder.

INTERPRETATION AND EDUCATION ACHIEVEMENTS

*Trout Unlimited volunteers provide hatchery tours during annual open house events. TU members purchased materials and constructed a handicapped- accessible viewing platform overlooking the adult holding ponds. This platform sits beside the hatchery

spawning shed and offers an excellent observation location for hatchery tour and visitor use.

HANDICAPPED FISHING PLATFORM ON THE ICICLE RIVER

*One of the largest volunteer projects at the Leavenworth NFH has been the handicapped fishing platform constructed on the Icicle River just downriver from the hatchery ladder. Trout Unlimited volunteers spent one full year planning and six months building the ramp and fishing platform. The estimated value of this project came to $60,000. The actual cost was just under $10,000. The construction work included an asphalt pad, a 300 foot sloped asphalt wheelchair ramp and a platform extending over the Icicle River. Over 2,000 volunteer hours were expended to complete this project in 1991. Unfortunately, during the flood of 1995, four feet of the bank was lost and the platform damaged beyond repair. Trout Unlimited is currently in the process of aquiring new permits to begin this project all over again.

LEAVENWORTH PARTNERSHIP & VOLUNTEER HOURS

IN LABOR HOURS:

YEAR	MAIN-TENANCE	RESOURCE SUPPORT	PUBLIC USE	ADMINI-STRATIVE	TOTAL HOURS	DOLLAR VALUE
1990	3764	2533	216	80	6,593	39,500
1991	2698	4572	224	50	8,044	48,264
1992	1220	4580	4080	310	10,190	96,805
1993	2840	1600	6650	1200	12,290	116,755
1994	2500	1700	6800	1300	12,300	117,355
1995	2660	1155	7001	1660	12,476	158,268
1996	NOT COMPLETE AT THIS TIME					

During a period of time approaching the 1970's, federal regulations changed regarding volunteers at federal fish hatcheries.Volunteers were not allowed to work at federal facilities due to injury and liability issues. Leavenworth's own local Trout Unlimited member fought hard against the limiting of volunteer opportunities at the hatchery and wrote and called Senators and Congressman to reinstate the volunteer program. It was not until 1970-71 that volunteers were, once again, allowed to officially help at the hatchery.

IN CLOSING

Even the change in volunteer programs did not stop the determination of our Leavenworth NFH Trout Unlimited partners. It only goes to prove that when motivated people begin acting like neighbors, communities are reinvigorated and work hard towards reaching common goals. The Leavenworth National Fish Hatchery Complex is dedicated to offering opportunities to become ***partners for our fish!***

THE ROLE OF FISH CULTURE/ENHANCEMENT CENTRES IN PUBLIC INVOLVEMENT AND STEWARDSHIP IN EASTERN CANADA

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Introduction

The enhancement and restoration of wild (particularly salmonid) fisheries was the driving factor behind the development of fish culture in Atlantic Canada over 130 years ago (Prince, 1906) and remains the focus of fish culture activities at the Department of Fisheries and Oceans' (DFO) Maritime hatcheries. In recent years, the realization that active public involvement and co-operation is integral to the success fisheries enhancement and conservation activities has resulted in a change of the traditional roles of hatcheries and their programs.

In the early 1980's, budgetary reductions and the adoption of a client participation philosophy prompted DFO's Gulf Region to develop and strengthen ties with public groups and/or private groups (non-government organizations - NGOs). A result of this program redirection was the development of the Salmonid Enhancement Centre (SEC) concept. Under this concept the hatcheries would not only provide fish for stocking; they would serve as a focal points for regional enhancement and conservation projects. SEC personnel expanded their roles to include the provision of technical advice and support for off-site fish culture and enhancement projects. NGO involvement was promoted in hatchery activities as well as off-site enhancement programs. NGO participation was integrated into broodstock collection and stocking activities, stream and river habitat improvement projects, the marking (fin clipping) of fish for project assessment, and the operation of fish fences and fish traps in their 'adopted' rivers. Advisory committees were established to allow NGO input into the direction and operation of the SECs and their programs.

The amalgamation of DFO's Gulf and Scotia-Fundy Regions to form the Maritime Region in 1994 has seen an expansion of the SEC concept and NGO participation in other areas of the Maritime provinces.

To facilitate public involvement and education, DFO's Maritime Region hatchery and enhancement personnel have developed and/or refined a number of enhancement techniques which can be operated by NGOs. The purpose of this paper is to describe some of these techniques and their use, and outline the future of fish culture in support of public fisheries enhancement in Canada's Maritime provinces.

Streamside incubation

Streamside incubation devices have been employed throughout Atlantic Canada in support of stock enhancement endeavors for over 20 years and have ranged in complexity from Vibert or Whitlock-Vibert boxes (Whitlock, 1974) to deep substrate incubators (Gray & Cameron, 1987; O'Connel & Bourgeois, 1987).

In New Brunswick, DFO's Charlo and Miramichi Salmonid Enhancement Centres provide eyed Atlantic salmon eggs to streamside incubation projects utilizing upwelling incubation boxes (Porter and Meerburg, 1977). These projects, operated in collaboration with the Nepisiguit Salmon Association, the Northumberland Salmon Protection Association, Noranda Inc., the Northwest Salmon Association, and Management of Salmon on the Restigouche and its Tributaries, result in the stocking of over 700,000 swim-up fry annually to the Miramichi, Nepisiguit, and Restigouche River systems. Egg-to-fry survival rates commonly exceed 90% (Baker, 1991; 1992).

Satellite rearing

Satellite rearing involves the establishment of small rearing facilities operated by NGOs at remote "satellite" sites. The sites vary in complexity from simple single tank operations with gravity feed water sources to multi-tank facilities with gravity feed surface water and pumped ground water. The tanks are stocked with feeding fry from a local DFO or Provincial hatchery. The fry are fed through until the autumn, at which time they are usually marked (adipose fin-clipped) and stocked into appropriate habitat. Broodstock for these projects are collected (often angled) from the systems into which the resultant fry will be stocked. This follows a 'discrete stock' policy followed by DFO hatcheries.

Satellite rearing of Atlantic salmon was initiated in 1984 in conjunction with the Miramichi Lumber Co. at their lodge on Rocky Brook, a tributary of the Miramichi River. The program was expanded in 1985 to include an additional facility on another Miramichi tributary, Black Brook, sponsored by the Black Brook Salmon Club. Initial results indicated a significant rate of adult returns from the fry stocked (Sherer, 1990). Based on these results, and the obvious merits of satellite rearing as both a conservation and educational tool, a cooperative agreement between the Miramichi Salmon Association and DFO resulted in the expansion of Miramichi satellite rearing to include 15 sites in 1991. The satellite rearing concept has been well accepted, and has expanded to include the rearing of other species. In New Brunswick and Prince Edward Island the St. John Fish Culture Station, and the Miramichi, Charlo, and Cardigan Salmonid Enhancement Centres provide fry to over 25 satellite sites rearing Atlantic salmon and an additional 14 sites rearing speckled trout which are operated in collaboration with over twenty NGOs. Trout from 3 of the satellite sites in Prince Edward Island are stocked into semi-natural rearing ponds for further rearing prior to stocking.

The potential benefits of satellite rearing in terms of enhancing fish stocks are variable and highly dependent on the quality of the fish reared and the habitat into which they are stocked. However, the benefits in terms of instilling conservation ethics and involving the public in conservation activities are widely accepted. Recognizing that "A society that is cognizant of conservation issues will insist upon more effective management of our fisheries", the Atlantic Salmon Federation and Orvis Services Inc. announced a program which would provide up to $100,000 for the expansion of satellite rearing in 1996/97.

Semi-natural rearing

The semi-natural rearing of salmonids through the stocking of lakes or ponds with juveniles is not a new approach. The rearing of coho (*Oncorhynchus kisutch* Wabaum) and chinook (*O. tshawytscha* Walbaum) smolts in large, natural , and man made ponds was adopted in the early 1960's in Washington and Oregon (Lemier, 1967; Korn & Smith, 1970).

In Atlantic Canada, experiments were initiated on Prince Edward Island (P.E.I.) in 1983 to determine the feasibility of utilizing abandoned mill ponds for Atlantic salmon culture. The first of these "semi-natural rearing ponds" was constructed in western P.E.I. by modifying an existing pond, Profitt's Pond through the installation of a water control dam and screening at its outflow and screening at its inflow. This experimental pond, operated in

collaboration with the O'Leary Wildlife Federation, is approximately 1.8ha in size and only 2m deep. The pond was stocked in the early summer with up to 50,000 yearling parr. The parr were reared through to 2 year old smolts which are stocked out the following spring. Although the fish were fed several times a day with a commercial pellet, natural food was found to constitute a large part of the diet (Cameron, 1987). Parr to smolt survival was variable over the next several years (38-63%) but the smolts produced were more similar to wild smolts in terms of their fin condition and condition factor than those reared in a hatchery (Davidson *et al*, 1995). The difference in quality between the semi-naturally produced and hatchery produced smolts has translated into differences in mean adult returns (8.8% and 3.1% respectively, Davidson and Bielak, 1992).

The success of Profitt's Pond, combined with an increasing demand for smolt stocking, prompted the construction of a second semi-natural pond in 1990 at Mooney's Pond in eastern P.E.I. Mooney's Pond's construction and subsequent operation has been a collaborative undertaking led by the Morell River Coop. The success of Profitt's and Mooney's Pond has led to the construction of one additional semi-natural salmon rearing pond, and three semi-natural speckled trout ponds. Funding and operational support for these ponds is generated within the community by NGOs. DFO's Cardigan SEC has partnered with these projects by supplying the fish. The total output of smolts on P.E.I. from semi-natural rearing now exceeds 80,000 and the combined trout production attributed to semi-natural ponds exceeds 30,000.

In a novel extension of this technique, a project was initiated in 1991 at the Atlantic Institution in Renous, a maximum security prison operated by Correctional Services Canada situated on a tributary of the Miramichi River. Two ponds were created along the banks of the river and water diverted to flow through them. Local NGOs provide the operational support for the ponds while an indoor facility at the prison grows out fry to parr to stock the ponds. This indoor facility, operated in a secure portion of the prison, provides employment for up to eight long term offenders. The Miramichi SEC provides the fry and technical advice to the project.

Lake cage rearing

Cage rearing has been accepted as the preferred method of commercial culture of adult Atlantic salmon in Atlantic Canada but very little has been published on the use of cages for freshwater rearing of juveniles.

Cage rearing was initiated at the DFO Mersey Hatchery in Nova Scotia as part of the hatchery's efforts to produce a better quality smolt. The project was successful and was extended to other DFO hatcheries throughout the Maritimes. Smolts are stocked in the summer with 1+ parr or large grade 0+ parr and are reared through to smolts the following spring. Fin condition, condition factors, and return rates were found to be similar to those found in semi-natural rearing. In 1991, the Northumberland Salmon Protection Association, in conjunction with DFO's Miramichi SEC and Heath Steele Inc. established a lake cage rearing facility for of Atlantic salmon at McCormack Lake on New Brunswick's Miramichi River system. Lake cages have been adapted for rearing of speckled trout supplied by the Charlo SEC for a joint DFO/Nepisiguit River Management Committee project aimed at enhancing trout populations in New Brunswick's Nepisiguit River.

Lake cage rearing was initiated in P.E.I. in a joint DFO/Montague Watershed Enhancement Co-op program. In this program the group has installed three cages in a large pond in Eastern P.E.I. The three cages are stocked separately with salmon, speckled trout, and rainbow trout. The rainbow trout are sold in the local aquaculture markets, thereby providing the operating funds to grow and distribute the other two species for enhancement projects. The Cardigan SEC currently provides the salmon parr and yearling trout for this project free of charge, but a mechanism is being developed for the group to purchase the rainbow trout from DFO.

Fish Friends

In the winter of 1991, the staff of the Cardigan SEC in P.E.I. initiated a salmon incubation and rearing program in eight elementary schools across the province. In 1992 the Atlantic Salmon Federation (ASF) adopted the incubators designed for this program and integrated its curriculum(adopted from the B.C." Salmonids in the

Classroom" program) into their Fish Friends schoolroom incubation and education program. This was funded by their Atlantic-wide Educational and Public Awareness Program (EPAP). Fish Friends has resulted in the establishment of schoolroom incubation units in 254 Maritime schools. All nine of DFO's Maritime hatcheries supply eggs and assist in the stocking of the fry produced from the Fish Friends program.

The Future

As active as public and private groups have become in fisheries conservation and enhancement in the Maritime provinces in the past, their participation is about to take a quantum leap in both scope of activities and management responsibility.

In response to Canada's budgetary crisis, and to be consistent with its fundamental shift towards focusing on the marine environment and reducing its freshwater activities, DFO has announced its intent to withdraw from operating fish hatcheries in the Atlantic Canada by 1998. DFO notes that it has been the client groups, not the resource, which have been the major beneficiaries of salmonid stocking, and hence feels that it should be the client groups who should assume responsibility for this activity in the future. DFO's primary objective is to build upon partnerships such as those noted above, and divest its nine Maritime hatcheries to interests that will ensure that they continue to operate for the benefit of the public fisheries.

The divestiture process is currently underway. Client consortia have formed to investigate taking over the operation of four of the hatcheries and these consortia are currently developing business/management plans outlining the proposed operational goals and funding for the facilities in question. It is expected that interested client groups/consortia will be established to investigate the operation of the remaining five hatcheries by early 1997.

After the completion of the divestiture process DFO will maintain a core group of personnel who will provide improved technical expertise, assistance, and technology transfer to the NGOs involved in hatchery operations, This will include a stock assessment program enhanced through NGO involvement. DFO will also continue to regulate fish introductions and transfers to protect the fisheries resources against fish disease, genetic, and ecological impacts; will ensure the rights and interests of First Peoples are not impeded by enhancement activities and related management practices; and will maintain its habitat management role, including that pertaining to fish passage and habitat enforcement.

In summation, the role of Federal fish culture facilities in public involvement and stewardship of our fisheries resources has evolved over the past twenty years. This evolution has seen a transition from government led and operated programs with little public input to the adoption of new techniques and the development of collaborative programs. The completion of the process, the future, will see the major fish culture role shift from government to NGOs and more NGO involvement in, and responsibilities for, all aspects of fisheries management including, and most importantly... the decision making process. Public "involvement" will switch to public "lead" - but maybe that is where the lead should have been all along!

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THE BELLA COOLA COHO WORKING GROUP

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The Bella Coola Coho Working Group is responsible for devising a local, multi-faceted, multi-year and successful coho initiative. The group was formed in 1994 and is made up of salmon enhancement volunteers, rod & gun club members, CWF representatives, fishers (both sport and commercial) and provincial and federal government professionals and technicians. The following sections will describe the impetus for local action, give suggestions about key components of the process which got things going and a sample of the results to date.

Impetus for Local Action

1. Concern from local volunteers over declining escapements in the lower valley tributaries.
- for the second year in a row river walks in lower valley creeks showed few adults where 100's should have been.
2. Concern by Snootli Hatchery staff (from review of mark data) about exploitation rate of local coho stocks.
- review of MRP in-season data and rough calculations indicated it could be as high as 80%
3. Decision by SEP Operations to drop coho production at Snootli Hatchery (first round of SEP cuts).
4. Pressure from SFAB and volunteer groups relating to hatchery program.
- sport opportunity/pressure had increased due to production from hatchery program and there was concern over the economic and biological implications of discontinuing enhancement
5. Concern from fisheries branch about coho enhancement eg. impact on wild stocks, masking of basic problem and potential pressure for net fishery of any surplus adults.
6. Frustration of DFO Community Advisor and volunteers in getting consensus and clear direction for developing a long term plan when dealing with everyone individually.

Step 1: Call Letter for Participants

1. Do some homework before sending the letter so you can give information which validates the need for local action (estimated exploitation rates, graph of declines in escapement, no plan by DFO to fund a program etc.).
2. Ask for help in developing something of long tern significance and indicate what commitments have already been made in time and effort eg. local volunteers will raise money for marking and do creek walks for six years.
3. Inform participants you are organising a "workshop". The focus is on information and action.
4. Remind participants that the process will be constructive, focused, objective and consensus building.
5. Ask for input into the agenda but give participants a basic outline to build on.
6. Give the date you will check back with invited participants and ask about preferred meeting dates/times.
7. Send the letter months ahead of when you think the workshop could take place as many people people are booked well in advance and must consider travel etc.

STEP 2: Setting up the Workshop

1. Be flexible about scheduling the date but set some limits or you will go crazy going back and forth between people and dates. Remember that fisheries personnel are busiest during herring and salmon season and that volunteers often cannot make meetings during week days. Consider doing workshop over two evenings or on a weekend.
2. Be prepared to carry on without someone. You can always include comments by sending an agenda and getting input prior to the meeting and/or can organise a speakerphone and have them check in at a certain time.
3. Allow at least a day for the workshop with some preparation and follow-up time for people presenting information and putting out the minutes.
4. Ask everyone to bring something and be sure they get a chance to contribute (data, pictures, anecdotal and historical information). This increases commitment and sense of ownership of process.
5. Insure location has adequate room and AV facilities (projector & screen, flip charts and chalk board).

6. Arrange for a facilitator(s). In this case the DFO Community Advisor shared role with DFO Species Co-ordinator. Volunteers were favourable to Fisheries Management sharing role as this indicated a willingness to be involved and contribute. It was also very helpful to have two people so one could be at the chalk board and flip charts to record important points and the other could distribute information, operate projector etc.

Step 3: At the Workshop

1. Welcome the group, introduce participants and re-iterate ground rules, then update Agenda (certain people may be constrained by time so may have to flip some things around).
2. Set agenda items on the chalk board or flip sheets and keep track on input to each item. Write down all suggestions, contributions and let the group key in on "keepers" later. Also may help to have "ground rules" posted and some questions which need answering through the group.
3. Summarise key points of each item before moving on and list where consensus was reached. Any action items should be listed with responsibility centre and timeline identified. If you get stuck on a certain point, leave it and come back to it later, don't get bogged down. Some of our consensus points were:

- there seemed to be a trend of declining #'s of coho spawners in the lower Bella Coola River
- this decline appeared to be caused by high harvest rate in various commercial fisheries
- CWT marking and release of enhanced coho was a way to document which fisheries responsible for catch of local stocks and have something to take to fisheries management and international discussions if necessary
- enhancement (production) should be confined to smolt releases to minimise impact on wild fry

4. Develop short and long term action list. Some of our action items were:

- all the fishers, volunteer groups and DFO staff would assist with doing spawner counts in local creeks at least twice each winter during peak spawning periods for a period of six years starting that fall
- one of the local volunteer groups would collect broodstock, raise funds for fish food and CWT marking for selected coho stocks for a period of three years starting that fall
- Snootli Hatchery to supply facilities and technical/fish culture assistance to volunteers for coho enhancement for at least three brood years.
- volunteers would assist DFO assessment biologists in collecting better escapement data on systems where adult fish were returning for six years
- DFO (STAD), assessment biologist to lobby for more funds for central coast and for an adult fence in Bella Coola
- DFO (PCAD, assessment biologist to lobby for CWT tags for group
- DFO (HEB) to assist volunteers with funds for some of the fish food, plumbing supplies & equipment needed to increase capacity for coho culture at Snootli Hatchery

5. Commit to putting out minutes in a timely manner (ideally within a week).
6. Set a tentative schedule for follow-ups on information (get a mailing list) and the next workshop (can be after significant work or program is finished or annually etc.).

Step 4: The Follow-up

1. Do an article for the local paper and other publications.
2. Have community event to highlight volunteer efforts and show appreciation for support and invite media.
3. Include opportunities for ongoing involvement and education of school children in initiative (increase value to community).
4. Do mail-outs when individual programs are completed and goal posts reached.

A Sample of the Results So Far

Marked smolts released from new rearing ponds at Snootli Hatchery in May 95, marked 95 brood coho fry on site and 96 broodstock program underway, three years of juvenile assessment data and fall spawner surveys, funding support from HCF and PSF for marking , fish food and habitat enhancement projects, a visible increase in focus on Central Coast stocks by Fisheries Management and Assessment at higher levels and a continued high level of commitment and participation by the group members in the third year of the initiative. Consensus is, there's more to come!

VANCOUVER ISLAND CLASSROOM INCUBATION

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Overview

The Classroom Incubation Program (CIP) brings 120 liters of a local stream into the classroom so that students can observe salmonid development from eyed egg to fed fry stage. Grade level varies from kindergarten to university, and you'll find most projects in elementary classrooms.

This year, program coordinators will deliver eggs to about 250 Vancouver Island classroom incubation projects.

Purpose

The CIP is one of three core education programs designed by Fisheries and Oceans Canada (DFO) to foster a sense of stewardship within participating students. We are in the business of attitude enhancement.

DFO also sells a number of supporting texts and teaching aids through the BC Teacher's Federation.

Calendar of Events

By this time (early December), and with the help of school district coordinators, participating teachers have examined their incubation kits for signs of wear, and the required materials and components have been purchased.

At present, we are in the middle of distributing materials and providing inservice training for new participants.

Normally, teachers set up tanks before the Christmas break to ensure that the system functions well and that a healthy biofilter has been established before egg delivery. Students observe conditions daily in preparation for this event.

In most cases, coordinators pick up eyed eggs for delivery some time in January from a local enhancement facility. Sometimes, teachers observe an egg take and take green eggs back to the classroom, or coordinators incubate green eggs in a classroom "mother tank" and deliver from there at the appropriate time.

Hatch normally occurs in February, and swim-up anywhere between late March and late April.

During the entire incubation period, students continue to monitor conditions, make regular water changes, and record ATU's. Because of the range in age among projects, the concept of "monitor" varies greatly, and the program lends itself to every imaginable scientific sophistication. And though we may chuckle to think that a gang of 5 year olds takes a good hour to exchange 2 or 3 gallons of water, we need to remember that the process is still critical to the development of an "attitude".

Once most of the fry have emerged and are buttoned-up, they are fed for a week to ten days before they are released to their native stream.

The teacher uses a dip net to gather the fry into a bucket of water taken from the tank. Once the class arrives at the stream, each student partly fills a ziplock bag with stream water and waits in line to receive a small number of fry

which he/she will carefully release back to Nature.

The fry release likely has more impact on participants than any other chapter in the program. The stewards bear a personal investment in their salmonids, and releasing them is an emotional and important experience, especially for adults.

The stewards prepared a suitable incubation environment pending the arrival of the eggs, then nurtured them to be healthy and strong for their return to Nature. The fry release reminds them of the purpose of their efforts, and teaches them an important distinction between ownership and stewardship.

Support

When I began work with this program in 1989, I had 27 projects and was able to provide technical support for all of them and costs were covered through the Salmonid Enhancement Program's community advisor. To encourage program growth and maintain program quality, I have established funding partnerships for material costs and technical support.

School districts and DFO now share the cost of substitute teachers so that district level coordinators can leave the classroom to check equipment, assist me with inservices and material distribution, deliver eggs and provide support during incubation.

In addition to the support that my company and its staff provides, the funding partnership provides .5 days per project year of substitute time so that coordinators spend an equivalent time servicing projects. Therefore, 250 Vancouver Island projects require 125 substitute days - about $25,000 total or $100 per project year.

The Vancouver Island Watership Foundation secures funds from DFO and NGO's to purchase materials and equipment. Watership uses DFO funds to buy and/or manufacture components for the basic incubation kit. Each kit costs about $150, and annual replacement costs average $40 per project. Projects receive new gravel and dechlorinator annually.

The chiller cost of $855 is shared between the Pacific Salmon Foundation and the school via the Watership Foundation's chiller purchase plan.

Egg Requirements

Program coordinators from eleven of thirteen Vancouver Island school districts forward information to me on egg deliveries and survival to the fed fry stage. Last year, eighteen enhancement facilities provided 31,500 chum and coho eggs for about 220 incubators, and 23,780 fed fry were released. Overall survival rate was 75%. Individual project survival tended to be either greater than 90% or less than 40%.

Acknowledgements

I must acknowledge Don Lawseth, Tom Rutherford and Trevor Morris for their professional support over the past eight years, and the paid and volunteer staff in Vancouver Island's enhancement facilities for their commitment to this program and their contribution to the evolution of an environmental ethic.

FISH CULTURE FACILITIES AND STAFF:

OPPORTUNITIES FOR THE EFFECTIVE DELIVERY OF WILD FISH STOCK MESSAGES TO THE PUBLIC

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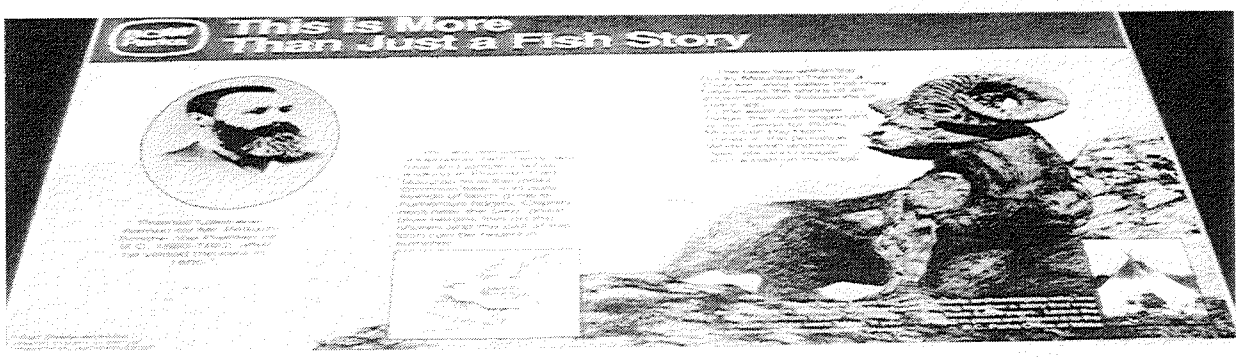
Background

A market survey conducted in 1991 in British Columbia for the Department of Fisheries and Oceans found that two-thirds of the population had visited a hatchery. Traditionally visitor areas and programs at fish culture facilities have focused on fish culture, missing an opportunity to communicate a broader fisheries message. The British Columbia government capitalized on the great potential to reach people through hatchery facilities by conducting a $2.5 million dollar upgrade of visitor areas at their hatcheries in the early 1990s. This included exhibit installations at the Fraser Valley, Kootenay, Loon Creek and Summerland fish culture facilities and the building of the Freshwater Eco-Centre as part of the construction of their new Vancouver Island facility. The Alberta government has embarked on a similar project: examining the potential of its fish culture facilities to communicate a fish and fish habitat conservation message.

Fish Culture Only a Small Part of the Story Told

British Columbia fish culture staff have been involved in planning, building and operating exhibits and programs on fish and fish habitat conservation for over five years now. Early on they recognized a need to involve staff from other sections within Fisheries, other departments within their Environment Ministry and even other Ministries. Their approach was to portray fish culture as only one important tool in a whole host of fish management tools. But to understand the need for fisheries management they realized that most people require some basic information on fish biology and fish habitat. This must be followed with a description of how human activities impact fish and fish habitat. Only then can the role of government in fisheries management, including fish culture be appreciated.

Telling the fish story from an ecosystem perspective is not easy. There are bureaucratic and political boundaries which must be crossed. In the B.C. situation there was mistrust of fish culture staff who were perceived by some to have worked in isolation in the past serving there own needs and not those of the wider department. There was also professional jealousy since fish culture staff were given control over budgets and thus perceived to have complete control over how 'others' stories were told. Even when it was evident that other departments were being invited to join the project as equal partners there was reluctance on the part of some staff.



Fish Culture Facilities and Their Programs Are Parts of Communities

In addition to inviting other government representatives into the fold, there were efforts in even the smallest projects to invite representatives of the people who would be using these facilities. Representatives of seniors and youth groups, conservation agencies, tourism, related nearby attractions, and schools were invited to review the designs and concepts. Major changes were made as a result of feedback obtained during interviews, open houses and meetings.

Facilities like the Freshwater Eco-Centre, located in a suburb of Duncan, B.C., will never be 'major' tourist attractions. The majority of people who visit the Centre or participate in its programs are residents. Even the tourists that do visit might be accompanied by a resident who is 'showing them around'. As the facility grows in age, more and more of the visits are repeats. To continue to attract repeat visitors, staff must be well aware of other facilities and their programs. Duplication must be avoided at all cost. Working with other facilities, even creating themes to which all facilities can contribute, is the order of the day. The Centre has become an integral part of the community, aware of its interests and its needs.

Even if tourists constitute a major component of a facilities visitation, staff must work cooperatively with other area attractions and the community to ensure tourists reach their destination. If the attendant at the local gas station doesn't know anything about the facility then they can't give directions to a tourist. The federal Capilano Fish Hatchery in North Vancouver is an example. From May to October thousands of tourists that might not have visited the hatchery alone, visit the facility as part of a tour that includes Grouse Mountain, the Cleveland Dam and the Capilano Suspension Bridge. Even tourists in automobiles find out about the hatchery through brochures that advertise all of the area attractions. Being located near a large urban centre and using a joint marketing approach this facility receives a visitation of almost a half million people annually.

Raising Awareness Only a First Step

Visitor areas and programs at fish culture facilities are usually built with the goal of raising awareness. However awareness is the only the first step in a traditional approach to environmental education. Awareness is followed by knowledge gain and action. (Hungerford and Volk)

The Sam Livingston Fish Hatchery Volunteer Society, which is administering a grant from the Fisheries Management Enhancement Program in Alberta to capitalize on the potential of fish culture facilities there, is starting out with this larger objective of 'action'. Not only is the Society going to try and make Alberta residents more aware of the habitats in their own backyards, but they are going to provide them with resources for conserving fish habitats.

Plans are in their initial stages, but the project designs may not only include exhibits but also resource libraries, kits for data collection and computer support. Hands-on participation is an opportnity to integrate awareness and knowledge.

Programs Heart of a Facility

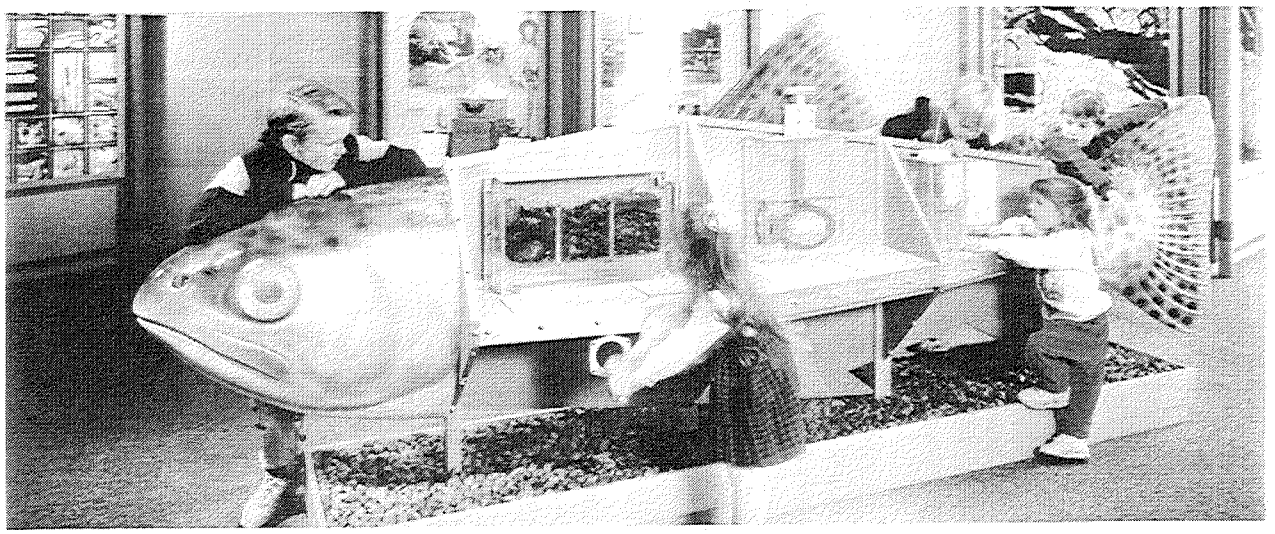
Many of the B.C. fish culture facilities are finding that their programs, not their exhibits, are what attract visitors. Research conducted at the Royal B.C. Museum found that visitors were attracted to 'concrete' experiences. (Peart and Kool) Interacting with a 'real live' person is more 'concrete' than reading text on a panel. Staff, docents and guest speakers can also act as powerful mentors. Special events organized by Freshwater Eco-Centre staff last fall which combined live presentations and hands-on activities like constructing bird boxes received record attendance.

Conclusions

To create vibrant, well-attended visitor facilities and programs fish culture staff must work hard, crossing bureaucratic boundaries, integrating themselves into communities, and creating authentic action projects for their visitors. This often comes at a cost of volunteering time on evenings and weekends. Many fish culture staff should be commended for their willingness to make these contributions. The results are evident in the meaningful experiences of their visitors. The 'payback' is a wealth of new knowledge and experiences for these dedicated staff.

The challenge in the years to come will be to move away from the traditional approach of self-guided exhibits and to incorporate more public programming, including the sponsorship of stewardship projects. Self-guided exhibits may result in large numbers of visitors, but so what? Is the goal of raising awareness enough?

Fish and fish habitats are being altered or are disappearing at an alarming rate. Programs at fish culture facilities can direct people into habitat conservation efforts that will help arrest this trend. Fish culture programs are part of larger departments whose goals usually include habitat conservation. Staff can play an active role in helping to achieve this goal through their visitor facilities and programs.



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Acknowledgments

Many of the conclusions made in this paper are the result of first-hand experiences obtained while coordinating the upgrade of B.C. fish culture facilities. This upgrade would not have been possible without the unending support of BC Environment staff especially Harvey Andrusak, Don Peterson, Dale Larson and Ray Billings. Many consultants also contributed ideas, but Anne Curran must be given special mention. Professionally Interpretation Canada and the International Visitor Studies Association have provided conferences, journals and the opportunity to network with colleagues all over North America. In relation to the ideas developed in this paper, Bob Peart, Rick Kool and Kerrie Post have been instrumental. Finally involvement in the Alberta project is offering new opportunities to develop these ideas further. I'd especially like to mention Dave DePape, Bev Yee and Cal Kullman and the rest of the Core members of this project.

REGISTRATION OF AQUACULTURE CHEMICALS:

WILL WE EVER GET TO THE FINISH LINE?

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The community of aquaculturists, researchers, and fish health practitioners has experienced difficulty securing adequate funding for aquaculture chemical development and meeting requirements of registration. Increased enforcement by the U.S. Food and Drug Administration (FDA) of existing laws regarding use of unregistered chemicals in aquaculture in the early 1990's created several responses. One response by "unregistered chemical users" was to request Investigational New Animal Drug Applications to allow for continued use of these substances. Another response by the aquaculture community was to join in cooperative registration/research ventures funded annually by regional or federal initiatives, such as the International Association of Fish and Wildlife Agencies' effort with NBS-La Crosse.

However, the overuse of INADs as a vehicle to provide compounds for regular use rather than research leading to registration caused concern at FDA and resulted in discontinuation of permits that were not linked to a manufacturing sponsor or were not making due progress toward registration.

These approaches all suffered from the fact that the diverse aquaculture community was largely naive to the drug registration arena, and participants competed for limited public funding to accomplish many objectives. Although FDA was slow to respond to these problems, the agency tried to become pro-active, but found a moving target. The wealth of environmental conditions, species and life stage differences, and political attitudes toward aquaculture chemical use and development have all complicated the attainment of significant progress towards completion of registrations. Recent responses in Canada toward net pen aquaculture may make the situation even more precarious.

Researchers at the University of Idaho in their publicly funded research efforts to register erythromycin have spent more than 10 years, and nearly 3 million dollars working on aspects of erythromycin registration. Although scientific studies are completed and nearly all submitted, erythromycin is still not approved in the FDA as a therapeutic compound to treat *Renibacterium salmoninarum* causative agent of bacterial kidney disease in salmonids.

The most significant problem that the aquaculture community faces is the drug and chemical company's quest for short-term profits. Business decisions that are linked to rapid return on investments and high profit margins are driving corporate takeovers. Reassessment of chemical development by previously supportive companies has resulted in the discontinuation of aquaculture projects.

How do we develop a future market when short term corporate profits drive many business decisions? We need some revolutionary decisions from governments and private aquaculture to resolve this dilemma.

RESULTS OF INAD INVESTIGATIONS EVALUATING THE EFFICACY OF CHLORAMINE-T TREATMENT

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Abstract

A total of 123 chloramine-T treatment trials were conducted by U.S. Fish & Wildlife Service (Service) facilities to control mortality in a variety of fish species caused by a variety of infectious pathogens during a one-year period. Approximately 58% of the trials appeared efficacious, 31% appeared ineffective and 11% were inconclusive. Overall, when chloramine-T was used at treatment concentrations of 6, 10 or 15 mg/L, efficacy was observed in 56%, 65% and 65% of the trials, respectively. When treatment frequency of chloramine-T was one, two or three times, efficacy was observed in 44%, 61% and 88% of the trials, respectively.

Introduction

Chloramine-T has historically been the drug of choice when diagnostic evidence shows salmonids to have bacterial gill disease (BGD) and flexibacteriosis. Diseases such as these can pose a serious threat to fish survival in intensive culture programs. Although death is caused by damage from a massive infection of the gills, stressors associated with intense fish culture predispose fish to infection. No single pathogen appears to be responsible for BGD or flexibacteriosis, but all known agents are gram-negative bacteria, including flexibacteria and flavobacteria. Moreover, the inflamed gills associated with the diseases are susceptible to secondary infections by opportunistic fungi. Integrated fish health management practices usually prevent the occurrence of these diseases. However, numerous factors can lead to severe disease outbreaks requiring prompt treatment to prevent losses of fish valuable to natural resource stewardship.

Chloramine-T is effective in controlling BGD in cultured fishes (From 1980, Bullock et al. 1991). Salmonids are relatively tolerant of the chemical. Bills et al. (1988) established that the 1-hour LC50 value of chloramine-T to rainbow trout as greater than 60 mg/L except in soft acidic (pH 6.5) waters where the 1-hour LC50 was 55.8 mg/L. Bullock et al. (1991) recommend a bath treatment of 8.5 mg/L of chloramine-T for 1 hour as an effective treatment for BGD in cultured salmonids. Results are best when treatment is begun in the early stages of an outbreak. Bullock et al. (1991) stated that a second or third treatment may be required if an outbreak is in an advanced stage or the fish are under stress.

Chloramine-T is also considered to be effective in controlling flexibacteriosis in cultured fishes. Observations by staff at the National Fish Health Laboratory, Leetown, WV and anecdotal observations by hatchery managers throughout the United States indicate that chloramine-T treatment is an effective method of controlling flexibacteriosis. They consider the use of 8.5 mg/L for 1-hour in a flow-through or flush system adequate to control the external phase of the diseases.

Chloramine-T is used throughout the U.S. Fish and Wildlife Service (Service) to control mortality in a variety of intensively cultured fish species caused by BGD/flexibacteriosis. The objective of treatment with chloramine-T was to minimize the impacts of disease on fish health, fish quality, and survival in order to fully meet fishery management objectives. Results presented in this report will be used to support a new animal drug application (NADA) for chloramine-T.

Background

Ten National Fish Hatcheries participated in developing clinical field trial data using chloramine-T flow-through or static bath treatments under INAD exemption #4000 during the past year (06-Jan-95 to 03-Jun-96). These facilities conducted 18 studies consisting of data from a total of 152 trials. Treatment trials were conducted on eight different cultured species which involved a total of 7.4 million fish (Table 1). Species evaluated included rainbow trout (*Onchorhynchus mykiss*), cutthroat trout (*Onchorhynchus clarki*), apache trout (*Onchorhynchus apache*), bull trout (*Salvelinus confluentes*), kokanee salmon (*Onchorhynchus nerka*), chum salmon (*Onchorhynchus keta*), coho salmon (*Onchorhynchus kisutch*), and striped bass (*Morone saxatilis*). Treated fish ranged in age from fry to adult. Fish size categories were defined as < 2", between 2 - 6" and > 6". Only rainbow trout and kokanee salmon were represented in all three size groups, indicating that infectious pathogens afflict a wide range of fish sizes. All treated chum and coho salmon were less than 2.2 inches, possibly indicating these fish are more susceptible to disease while still small and when they do not possess a fully developed immune system. Trials were characterized as treatment trials, such as therapeutic or prophylactic or controls. There were 123 trials that entailed treatment with chloramine-T, and an additional 25 trials that were classified as non-infected, untreated "controls".

Table 1. Fish species treated with chloramine-T during the reporting period, total number of fish treated, and number of fish from each size group (< 2", between 2 - 6" and > 6").

Species Treated*	Total Number of Fish	Number of fish (< 2 in.)		Number of fish (2 - 6 in.)		Number of fish (> 6 in.)	
RBT	2,657,800	984,485	**37%**	1,504,865	**57%**	157,287	**6%**
APT	186,400	54,000	**57%**	132,400	**71%**		
BLT	65,000					65,000	**100%**
CTT	37,000			37,000	**100%**		
CHS	219,400	207,300	**94%**	12,100	**6%**		
COS	197,000	196,970	**100%**				
KOE	4,055,600	1,367,000	**34%**	1,464,100	**36%**	1,224,500	**30%**
STB	4,864						

* Fish species abbreviations: RBT - rainbow trout; APT - apache trout; BLT - bull trout; CTT - cutthroat trout; CHS - chums salmon; COS - coho salmon; KOE - kokanee salmon; STB - striped bass.

Although theses controls were not "true" controls because they were not diseased, they were beneficial from a basal mortality comparison standpoint. Treatment methods used were either flow through or static bath. Fifteen trials were flow through while two were static bath treatments. Treatments were conducted in circular tanks or cement raceways, depending on the particular hatchery. The purpose of the trials was to collect clinical field trial data that will be used to determine the most appropriate treatment regime for controlling BGD and flexibacteriosis in a variety of cultured fish species. Chloramine-T concentrations and dosing intervals tested during these trials were either 6, 8, 10 or 15 mg/L applied 1 - 3 times.

Materials and Methods

Akzo Chemical, Inc. is the manufacturer who supplied chloramine-T for use in treatment therapy. Chloramine-T for use in treating BGD and flexibacteriosis in fish is a pure, water soluble compound and is not formulated in any way. During use, chloramine-T is dissolved in water and applied as a bath solution at a specific concentration for 1 hour and then flushed from the fish-holding container, or metered for 1 hour at a flow adequate to achieve the desired treatment concentration in a flowing system. Storage conditions, handling procedures and investigational labeling are outlined in the Study Protocol for a Compassionate Aquaculture Investigational New Animal Drug (INAD) Exemption for Chloramine-T - INAD #4000 (Study Protocol). According to the Study Protocol, chloramine-T treatments were typically initiated when disease signs were evident, mortality increased and the causative agent was identified during a pre-treatment pathological examination. Although a definitive diagnosis of BGD/flexibacteriosis is always recommended, occasionally treatment was initiated following a presumptive diagnosis only. In most cases, mortality data was the only component of the listed primary response parameters to have been collected to determine efficacy of treatment. Although pre-treatment data was to be collected for at least 10 days and post-treatment data was to be collected for at least 14 days, this was not always the case. However, in each trial, diligent effort was directed towards compilation of this source data.

Treatment trials were characterized as effective if the post-treatment mortality was lower than pre-treatment and during treatment mortality. A decrease in mortality had to be at least 30% to be considered effective. In the trials in which only post-treatment mortality was submitted, the average daily mortality was determined for both the first half and the second half of the post-treatment period. The same principles were applied to determine effectiveness when comparing average daily mortality during the second half of the post-treatment period with the first half. Mortality decreases that were not at least ~ 30% were characterized as ineffective. Trials in which there was no substantial mortality before or after treatment, or when fish were moved to other rearing units were considered inconclusive.

Results

Overall, there were 127 treatment trials and 25 trials that used non-infected, untreated "controls" resulting in data from 152 trials. Of the 127 treatment trials, 123 were for therapeutic treatment and four were for prophylactic treatment. Of the therapeutic treatments, 71 of the 123 treatment trials (58%) appeared effective, 31% appeared ineffective and 11% appeared inconclusive (Table 2). Approximately 16% of the trials were conducted using 6 mg/L, 51% conducted using 10 mg/L and 33% conducted using 15 mg/L of chloramine-T. Chloramine-T was administered one, two or three times, regardless of concentration, in 26%, 49% and 25% of the trials, respectively.

There was a total of 66 treatment trials that involved trout, and of these 65% were effective, 29% were ineffective and 6% were inconclusive. Trials which involved trout and were characterized as inconclusive were done so because there was virtually no mortality before, during or after treatment. A total of 55 treatment trials were administered to diseased rainbow trout, and 65% (36 of 55 trials) of the treatments were effective while 27% (15 of 55 trials) were ineffective. Results from the remaining four trials (7%) which involved rainbow trout were inconclusive. Sixty percent of the trials which involved apache trout (6 of 10 trials) were effective. The only trial which involved cutthroat trout was effective while the only trial which involved bull trout was ineffective.

There was a total of 54 treatment trials which involved salmon, and of these 46% were effective, 35% were ineffective and 19% were inconclusive. Trials which involved salmon and were characterized as inconclusive were done so for several reasons. There was either virtually no mortality before, during or after treatment, fish were moved to different rearing units (affecting culture conditions resulting in biases), or fish were diagnosed with another disease. In the later case, kokanee salmon held at Creston NFH were treated for furunculosis during the early stages of the chloramine-T post-treatment period, rendering this data inconclusive. A total of 26 treatment trials were administered to diseased kokanee salmon, and 42% of the treatments were effective, 30% were ineffective while 28% were inconclusive. A total of 25 treatment trials were also

administered to diseased chum salmon, and 56% of the treatments were effective while 44% were ineffective. All three trials that involved coho salmon were inconclusive.

Table 2. Overall chloramine-T therapeutic treatment efficacy.

Species	No. of Effective Trials	No. of Ineffective Trials	No. of Inconclusive Trials	Total
RBT	36	15	4	55
APT	6	4	0	10
CTT	1	0	0	1
Subtotal (trout)	43 (65%)	19 (29%)	4 (6%)	66
KOE	11	8	7	26
COS	0	0	3	3
CHS	14	11	0	25
Subtotal (salmon)	25 (46%)	19 (35%)	10 (19%)	54
STB	3	0	0	3
Total (all fish)	71 (58%)	38 (31%)	14 (11%)	123

All three trials that involved treatment of striped bass with chloramine-T appeared efficacious. A single lot of fish was treated for several recurring outbreaks of BGD. Results from the first trial showed that mortality dropped immediately after treatment, and returned to zero within 5 days of treatment. In addition, fish no longer swam near the surface and the investigator commented that the fish "looked better" following treatment. Treatment following the second and third outbreak also appeared efficacious, although mortality was not high prior to treatment. However, these fish also ceased "riding high" in the water following treatment.

Efficacy was demonstrated using 6, 10 and 15 mg/L when administered one, two and three times (Table 3). Overall, in terms of treatment concentration, treatment appeared effective in 56% (9 of 16) of the treatment trials when 6 mg/L chloramine-T was used, in 65% (34 of 52) of the treatment trials when 10 mg/L was used, and in 65% (22 of 34) of the treatment trials when 15 mg/L was used. In terms of treatment frequency, treatment appeared effective in 44% (12 of 27) of the treatment trials in which chloramine-T was administered a single time, in 61% (31 of 50) of the treatment trials in which it was administered two times, and in 88% (22 of 25) of the treatment trials in which it was administered three times. This data suggests that the number of times chloramine-T is administered may be as much, or more, of a factor as treatment concentration in controlling mortality caused by BGD/flexibacteriosis. There was no substantial difference in degree of efficacy when comparing different treatment concentrations, and virtually no difference between 10 and 15 mg/L. However, there appeared to be a profound difference with regard to the number of times chloramine-T was administered. There appeared to be a strong positive correlation between the number of times chloramine-T was administered and treatment efficacy. This observation seems to hold somewhat true when efficacy-treatment frequency is evaluated for each treatment concentration. Trials conducted at 6 mg/L chloramine-T:one time resulted in 46% efficacy (6 of 13 trials). However, 100% of the trials in which chloramine-T was administered two times (1 of 1 trials) and three times (2 of 2 trials) appeared effective. Trials conducted using 10 mg/L chloramine-T showed similar results. When chloramine-T was administered a single time, 0% (0 of 4 trials) of the trials appeared effective. But, 63% of the trials appeared effective when chloramine-T was administered two times (21 of 33

trials) and 87% appeared effective when it was administered three times (13 of 15 trials). This relationship was not as strong when results from trials in which 15 mg/L was evaluated. When chloramine-T was administered a single time, 60 % (6 of 10 trials) of the trials appeared effective. However, only 56% of the trials in which chloramine-T was administered two times (9 of 16 trials) appeared effective while 88% (7 of 8 trials) of the trials conducted ion which chloramine-T was administered three times appeared effective.

Table 3. Overall effectiveness of chloramine-T use by the Service - 1995 .

Treatment Conc.	6 mg/L			10 mg/L			15 mg/L			Overall % Efficacy
No. of Treatments	Effect. Trtmnt	Not Effect. Trtment	% Effect.	Effect. Trtmnt	Not Effect. Trtment	% Effect.	Effect. Trtmnt	Not Effect. Trtment	% Effect.	
1	6	7	46%	0	4	0%	6	4	60%	44%
2	1	0	100%	21	12	63%	9	7	56%	61%
3	2	0	100%	13	2	87%	7	1	88%	88%
Total Efficacy	9	7	56%	34	18	65%	22	12	65%	

There did not appear to be a correlation between fish size and efficacy of treatment. Results for both trout and salmon had shown that treatment of fish between 2 - 6 inches resulted in the highest incidence of efficacy. Eighty-seven percent of trials that involved trout between 2 - 6 inches were efficacious, as compared with 63% and 31% when treatment was administered to fish less than 2 inches and greater than 6 inches, respectively. Similarly, 83% of the trials that involved salmon between 2 - 6 inches were efficacious, as compared with 55% and 50% when treatment was administered to fish less than 2 inches and greater than 6 inches, respectively.

Four prophylactic trials were also conducted. Three trials involved treatment of kokanee salmon, and appeared effective. A single trial involved treatment of bull trout, and did not appear effective. However, efficacy was difficult to determine because no controls were run.

Conclusions

Results from Service INAD studies conducted in 1995 again showed efficacy with regard to treatment therapy. There were 127 treatment trials, 123 of which were therapeutic treatment trials, conducted in 1995 as compared with 37 similar type trials conducted in 1994. Results from 1995 treatment trials were similar to results from 1994. In 1995, 58% of the trials conducted appeared efficacious while 31% appeared ineffective. In 1994, 49% of the trials conducted appeared effective while 32% appeared ineffective. In 1995, 11% of the trials were inconclusive while 19% of the 1994 trials were inconclusive. There was a greater degree of conscientiousness by investigators in carrying out treatment trials and recording/submitting data. This may have been partially responsible for the higher degree of efficacy in 1995. It probably was a major reason why only 14 of the 127 trials conducted in 1995 were inconclusive. Typically, results are inconclusive because of misdiagnosis of disease, treatment using more than one chemical or moving fish before ample post-treatment data is generated. Although these situations arose in 1995, there were not as many as expected based on the 1994 data.

Treatment of diseased trout apparently resulted in a higher incidence of effective trials than treatment of

diseased salmon. Sixty-five percent of the trials that involved treatment of trout appeared effective while only 46% of the trials that involved treatment of salmon appeared effective. This is probably a result of differences in culture conditions, such as water source, density and flow index, and not because chloramine-T was more efficacious in controlling mortality caused by BGD/flexibacteriosis in trout than in salmon.

Some of the most profound results focus on treatment concentration and treatment frequency with regards to efficacy. Use of chloramine-T at 6 mg/L resulted in the lowest level of efficacy because only 56% of the treatment trials appeared effective. The level of efficacy was higher when chloramine-T was used at 10 or 15 mg/L. Trials conducted using the higher treatment concentrations resulted in approximately 65% efficacy. Although dose-titration studies have not been conducted to determine optimal treatment concentrations, this data suggests that treatment concentrations in this range may be within the optimal treatment range. Data results that were most surprising was the relationship between apparent efficacy and treatment frequency. When treatment trials were conducted with chloramine-T, and the chemical was administered at one, two or three times, a total of 44%, 61% and 88% efficacy was achieved, respectively. These results were achieved regardless of treatment concentration. There appears to be a very strong positive linear correlation (R = .992) between treatment frequency and level of efficacy. Based on these observations, it appears that the highest degree of efficacy can be achieved using 10 or 15 mg/L chloramine-T:three times. However, there are many factors that potentially play a role in treatment efficacy that may be adequately addressed only by conducting pivotal field trials. Results from ancillary trials, such as every trial conducted in 1995, are supportive at best. Some of these factors include determining the level of disease infection. Many times treatment follows a presumptive diagnosis, while a definitive diagnosis has never been made. Even during a definitive diagnosis, no assurances are made regarding uniformity of disease level in fish among all test units. This leads to wide variability in treatment efficacy results, and can greatly influence mortality among non-treated fish. Without some measure of disease uniformity among test units, decreases in post-treatment mortality can only be presumed to be a result of chloramine-T treatment. Timeliness of treatment also plays a role in treatment efficacy. Several times, following treatment, the investigator noted that results would have appeared more efficacious had treatment been initiated before the disease got too "bad". Service facilities in close proximity to Fish Health experts, such as Quilcene NFH, Quinault NFH and the Bozeman FTC, or in a position to conduct frequent fish health exams utilizing their own staff usually treat diseases before they get out of hand. This often results in a greater likelihood of effective treatment. There are many other factors that may affect treatment efficacy that can only be factored out by conducting pivotal trials. Results from Service efforts during 1995 reveal some interesting considerations when treatment with chloramine-T has been deemed necessary to control mortality caused by a variety of infectious pathogens. In particular, additional insight may have been gained on the relationship between treatment concentration/treatment frequency and trial efficacy. However, this data and similar data are supportive, and pivotal data is necessary before any definitive conclusions can be drawn based on treatment efficacy.

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IS *FLEXIBACTER PSYCHROPHILUS*, CAUSAL AGENT OF SYSTEMIC BACTERIAL COLD-WATER DISEASE, VERTICALLY TRANSMITTED IN SALMONIDS?

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Abstract

Eggs and embryos from steelhead broodstock from a hatchery in California were examined internally and externally for evidence of contamination due to the causal agent of Bacterial Cold Water Disease, *Flexibacter psychrophilus*. There was surface-contamination on some eggs (28%), despite iodine disinfection. Although killed at 100 ppm iodine, *F. psychrophilus* is resistant to lower iodine concentrations, and complete egg surface disinfection may not always be achieved. *F. psychrophilus* was isolated from within some eggs and embryos (4-13%). *F. psychrophilus* is resistant to lysozyme (found within a salmonid egg), and could survive and be transmitted within eggs. The progeny of fish injected with erythromycin prior to spawning had lower mortalities than fry from non-injected fish. The results of this study indicate that *F. psychrophilus* may be transmitted both horizontally and vertically within salmonid hatcheries.

Introduction

High mortalities due to systemic bacterial cold-water disease (BCWD) were documented in steelhead trout (*Oncorhynchus mykiss*) at Big Creek Hatchery (Davenport, CA). Losses up to 85% during the first two months of rearing were observed in some lots of fish. All steelhead lots developed systemic BCWD within 1 to 4 weeks of button-up. The eggs and young fish had been incubated in sand filtered spring water, which is fish-free. Yellow colonies typical of *Flexibacter psychrophilus*, causal agent of BCWD, were isolated on medium inoculated with surface-disinfected steelhead egg homogenates. The purpose of the study presented here was to determine if *F. psychrophilus* could be transmitted within steelhead eggs, and if this was the route of the observed infections due to *F. psychrophilus*. Another objective of this study was to determine if injecting antibiotics into broodstock female steelhead would reduce the prevalence of intra-ovum infections (if any) due to *F. psychrophilus*.

Materials and Methods

Two stocks of steelhead trout were examined. Five female fish from the Scott Creek (SC) stock were injected with erythromcyin (20 mg kg^{-1} fish weight) upon receipt and at 30 d intervals thereafter. Seven females from the San Lorenzo (SL) stock were injected with oxytetracycline (20 mg kg^{-1} fish weight), following the same regime. Five more SL females were left uninjected.

At spawning samples of eggs (unfertilized) were taken from each fish. Eggs were also sampled from each fish after they were fertilized, surface-disinfected and water-hardened with 100 ppm iodophore for 1h, and then rinsed in the hatchery water. Additional samples were taken from the progeny of each fish at the eyed and hatch stage.

We examined the eggs/embryos for surface or internal *F. psychrophilus* contamination, following a modified protocol of Evelyn *et al.* (1984). Eggs or embryos (unfertilized, newly fertilized and surface-disinfected, eyed and newly hatched) were placed directly into selective media (TYE - tryptone yeast extract broth with fetal calf serum). Five additional newly-fertilized, surface-disinfected eggs, eyed eggs, and newly hatched sac fry from each fish were surface-disinfected a second time with iodophore, after which they were rinsed with sterile distilled water. The twice-disinfected eggs/embryos were placed in TYE broth as above. All TYE tubes were incubated at 17 °C for 72h and then examined for turbidity (indicating surface contamination). The TYE broth from all tubes was examined for bacterial growth by streaking onto TYE plates, which were incubated a further 72h. If the TYE broth in a given tube was clear and free of turbidity and there was no evidence of bacterial growth, the egg/embryo was homogenized, and then incubated at 17 °C for an additional 72h. After that the homogenate was examined for bacterial growth.

All growth on all plates was examined to determine if it was due to *F. psychrophilus*. Growth was deemed to be *F. psychrophilus* if it met the following criteria: 1) Gram negative short rods, 2) yellow pigment produced on TYE medium, 3) the yellow pigment turned red when streaked onto filter paper soaked in 1N NaOH (indicative of flexirubin, the pigment produced by *Flexibacter spp.*), 4) growth at 17 °C, but not at 30 °C, and 5) a positive slide agglutination result. The slide agglutination was done with a saline suspension of the growth to be examined, and antisera raised against *F. psychrophilus* in rabbits.

To examine the possibility that *F. psychrophilus* could survive inside salmonid eggs, the contents of 50 eggs from the uninjected SL group were taken aseptically. The egg contents were spiked with *F. psychrophilus* cells after which the tubes were then incubated at 17 °C for 7d. After that, the spiked egg contents were streaked onto TYE plates which were incubated at 17 °C for 72h. Any growth was confirmed as being due to *F. psychrophilus* according to the criteria described in the preceding paragraph.

To determine whether *F. psychrophilus* is resistant or susceptible to lysozyme, which is found in salmonid eggs and is probably responsible for passive defense of the developing embryos against bacterial pathogens, we followed a modification of the procedure described by Yousif *et al.* (1994). A suspension of *F. psychrophilus* cells was made in phosphate-buffered saline (PBS). We also suspended *Aeromonas salmonicida* (a bacterium known to be susceptible to lysozyme) in the same way, as a verification of activity in the lysozyme used. The bacteria (both *F. psychrophilus* and *A. salmonicida*) were then diluted 1/10 in selected solutions of hen egg white lysozyme in PBS (concentrations of 0, 0.1, 1.0, and 2.0 mg ml^{-1}). Samples of the bacterial suspensions in each lysozyme concentration were taken at 0, 30, 60, and 90 minutes. The cells were washed in PBS, serially diluted 100-fold to 10^{-6}, and then 25 µl of each diluted sample were dropped, in triplicate, onto TYE plates. The plates were incubated at 17 °C for 48h, after which colonies were counted. Results are expressed as the percentage reduction in cell number, using the colony counts from the control tubes (0 mg ml^{-1} lysozyme) as the standard (0%) reduction.

At the outset of this study it became apparent that *F. psychrophilus* was contaminating the surface of some of the eggs/embryos (see **Results and Discussion**), despite iodophore disinfection procedures at the hatchery. In order to determine the susceptibility of *F. psychrophilus* to iodophore the above experiment was repeated, except that the bacterial suspensions (both *F. psychrophilus* and *A. salmonicida*) were exposed to iodophore concentrations of 0, 10, 100, and 500 ppm in sterile, distilled water. The procedure was as above, except that samples were taken at 0, 30 and 60 minutes only.

The remaining progeny of the 17 experimental fish were reared in the hatchery according to standard hatchery practice and mortalities were monitored. Any mortalities were determined to be due to BCWD by culture and immunoassays, in addition to noting characteristic pathological signs and direct observation of typical *Flexibacter spp.* cells in fish tissues, examined at 600 - 1000x by phase microscopy.

Results and Discussion

Twenty-eight percent of all of the newly spawned and fertilized eggs, eyed eggs, and yolk sac fry were surface contaminated with *F. psychrophilus* (Table 1). This was despite the fact that the eggs had been surface-disinfected with iodophore at the hatchery. This suggests that a source of *F. psychrophilus* contamination was the hatchery water. We also isolated *F. psychrophilus* from the surface of 10% of eggs that had only been in contact with ovarian fluid from the spawning female, indicating that the females themselves were the source of infection in these cases (Table 1).

Table 1 Percentage (%) of eggs or embryos positive for *Flexibacter psychrophilus*. Scott Creek (SC) or San Lorenzo (SL) female steelhead trout were injected before spawning with erythromycin (E) (n=5 fish), oxytetracycline (O) (n=7 fish), or were not injected (N) (n=5 fish). Eggs and embryos examined for surface or internal bacterial contamination (3-5 per stage per fish). Results for surface contamination are expressed as the average proportion of samples at each stage that yielded positive results for surface contamination due to *F. psychrophilus*. Results of ovarian fluid contamination refer to percentage of eggs positive for *F. psychrophilus* contamination from ovarian fluid. Results for internal contamination are expressed as results from individual stages. n.d. = not determined, SE = standard error

Stock	Injected with	+ve for surface contamination	+ve for ovarian contamination	+ve for internal contamination		
				newly fertilized egg	eyed egg	50% hatch
SC	E	23	9	15	12	8
SL	O	33	12	12	8	2
SL	N	28	10	11	0	2
Average of all 3 stocks (± SE)		**28 ± 4**	**10 ± 1**	**13 ± 2**	**7 ± 5**	**4 ± 3**

Table 2. Susceptibility of *Flexibacter psychrophilus* and *Aeromonas salmonicida* to iodophore

Bacterial species	Iodophore concentration (ppm)	% Reduction of cfu after exposure to iodophore		
		Time of exposure:		
		0 min	30 min	60 min
Aeromonas salmonicida				
	0	0	0	0
	10	0	100	100
	100	0	100	100
	500	0	100	100
Flexibacter psychrophilus				
	0	0	0	0
	10	0	50	100
	100	0	98	100
	500	0	100	100

Evidence of surface contamination despite normal disinfection procedures prompted the iodophore experiment. The data indicate that *F. psychrophilus* is not resistant to iodophore at 100 ppm (Table 2); there was a 98% reduction in the number of *F. psychrophilus* cells (Table 2). However, there was only a 50% reduction in cell numbers at 10 ppm. In routine hatchery practices iodophore disinfectants are used at concentrations varying from 50 - 100 ppm (Groberg 1988), but the egg:iodophor volume may vary considerably, and therefore not all eggs within a container are exposed to equal iodophor concentrations (Rogers & Chapman 1991). It may well be that in certain hatcheries, a percentage of eggs are not exposed to sufficiently high iodine concentrations and are therefore not effectively surface-disinfected. Such eggs could act as reservoirs for horizontal transmission of *F. psychrophilus*.

F. psychrophilus was isolated from the contents of 13% of newly spawned eggs, as well as from 7% of eyed eggs and from 4% of newly hatched alevins (Table 1). Previously it was thought that *Renibacterium salmoninarum*, causal agent of bacterial kidney disease, may be the only bacterial pathogen of salmonids that could survive within salmonid eggs (Evelyn *et al.* 1984, Barker *et al.* 1991, Yousif *et al.* 1994) Most other bacterial salmonid pathogens are Gram negative, including *F. psychrophilus*. Susceptibility to lysozyme is a characteristic of many fish pathogens (Grinde 1989), and Yousif *et al.* (1994) have shown that a number of Gram negative fish pathogens are susceptible to lysozyme purified from coho salmon (*O. kisutch*) eggs. However, those authors did not test *F. psychrophilus* for lysozyme susceptibility. Our *in vitro* experiments for lysozyme susceptibility indicate that *F. psychrophilus* is resistant to lysozyme. Exposure of *F. psychrophilus* to 2 mg ml^{-1} for 90 minutes resulted in only a 44% reduction in the number of viable cells, as compared to a 99% reduction in *Aeromonas salmonicida* viability when *A. salmonicida* was exposed under the same conditions (Table 3). These data were supported by the fact that *F. psychrophilus* was isolated from 100% of the samples of egg contents that had been spiked with *F. psychrophilus* cells. It is evident that *F. psychrophilus* is resistant to the defense systems that are present within salmonid eggs.

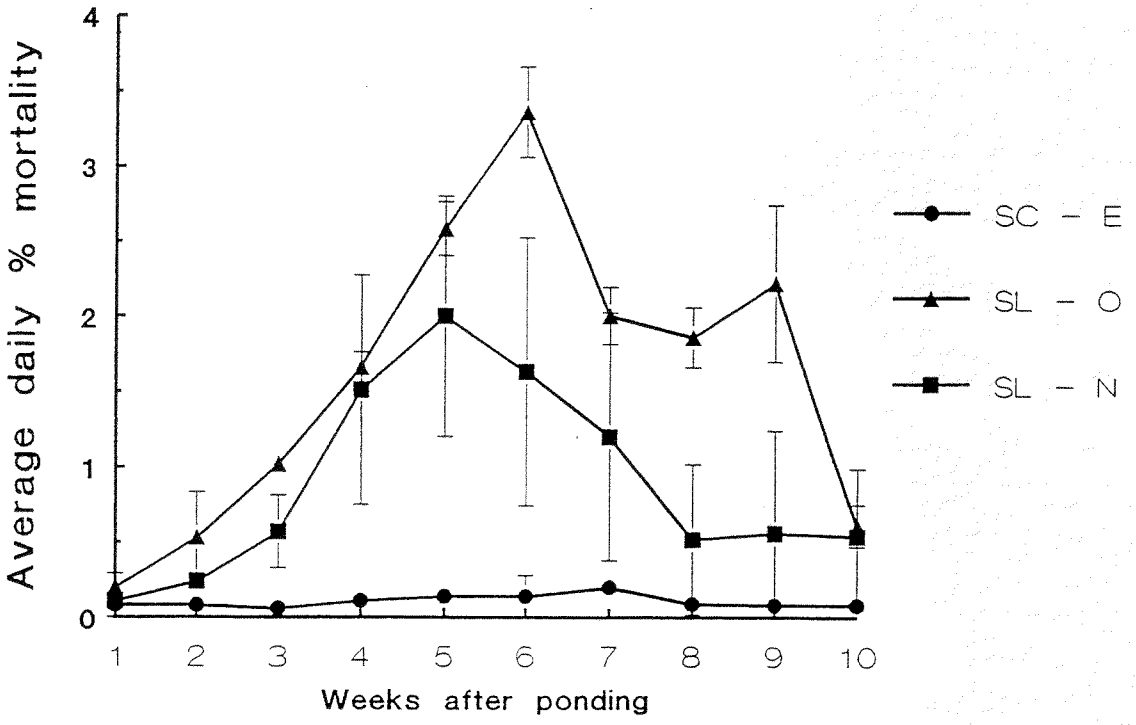
Table 3. Susceptibility of *Flexibacter psychrophilus* and *Aeromonas salmonicida* to hen egg white lysozyme

		% Reduction of cfu after exposure to lysozyme			
		Time of exposure:			
Bacterial species	Lysozyme concentration (mg ml^{-1})	0 min	30 min	60 min	90 min
Aeromonas salmonicida					
	0.0	0	0	0	0
	0.1	1	56	74	53
	1.0	2	56	78	67
	2.0	1	99	98	99
Flexibacter psychrophilus					
	0.0	0	0	0	0
	0.1	0	20	0	31
	1.0	0	10	60	46
	2.0	0	30	55	44

There was no significant difference in the prevalence of intra-ovum infection due to *F. psychrophilus* within eggs and embryos from antibiotic injected, versus non-injected broodstock (Table 1). This was the case with both the Scott Creek (injected with erythromycin) and San Lorenzo (approximately half injected with tetracycline) stocks. Nor was there any significant difference in the prevalence of surface contamination due to *F. psychrophilus* within the ovarian fluid of antibiotic injected versus non-injected female broodstock (Table 1). However, there were significantly fewer mortalities due to BCWD within the progeny of the Scott Creek (injected with erythromycin)

stock than that of the San Lorenzo stock (injected with oxytetracycline or not injected - Figure 1). The data from the hatchery indicates that injecting broodstock with erythromycin before spawning may be effective in reducing mortalities due to BCWD.

Figure 1 Average daily % mortalities due to bacterial cold-water disease within progeny of Scott Creek (SC) or San Lorenzo Creek (SL) steelhead broodstock that were injected with either erythromycin (E), oxytetracycline (O), or were not injected (N), before spawning. n = 16,000 - 25,000 per stock. Error bars = standard error.



Surface contamination due to *F. psychrophilus* within the water is a serious concern for the hatchery in question, and this may well be the case for other hatcheries. This hatchery has a fish-free water source. However, amphibians, insects, snails, and possibly other animals may be reservoirs of infection, releasing *F. psychrophilus* into the water. *F. psychrophilus* was isolated from the brain tissue of Pacific salamanders and newts taken from the site of the hatchery water source (data not reported here). From the data presented here it seems likely that there are multiple routes of infection due to *F. psychrophilus* in juvenile salmonids and vertical transmission may be included in these routes. Broodstock erythromycin injected may be effective as a preventive measure.

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THE USE OF HYDROGEN PEROXIDE TO CONTROL EXTERNAL FUNGUS AND COPEPODS IN SUMMER STEELHEAD AT THE MERWIN HATCHERY ARIEL, WASHINGTON

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Introduction

Hydrogen peroxide has been listed by the U. S. Food and Drug Administration (FDA) as a low regulatory compound (LRP) for the control of fungus on fish and eggs. The Washington Department of Fish and Wildlife (WDFW) has had limited experience using this compound on steelhead. An opportunity occurred to test the efficacy of hydrogen peroxide for controlling fungus in adult steelhead at the Merwin hatchery in Ariel, Washington.

The Merwin hatchery, located in southwestern Washington near the Merwin Dam on the Lewis River, was built and is funded by Pacificorp. It is operated by WDFW. The facility contains four rearing ponds, ten production raceways, and four adult holding raceways, and uses reservoir water disinfected with ozone gas for its water supply.

Adult steelhead are trapped at the base of the dam and are transported via truck to the nearby hatchery in June and July for gamete maturation until spawning in December. Originally, it was believed that the water disinfected with ozone would eliminate the need for chemical treatments in the adult fish, however the trucking and handling resulted in fungal infestations which occurred within a few days of fish being brought to the hatchery.

In 1993, formalin was used at 167 mg/l for one hour drips to control fungal infestations in these fish. Hydrogen peroxide was first listed as an LRP compound in the fall of 1993. In 1994 a small group of adult steelhead were placed into a separate brood raceway at the hatchery and hydrogen peroxide was administered. It became apparent that bath treatments of 50 mg/l, 75 mg/l, and 100 mg/l were not effective in controlling saprolegniasis in these fish. However, fish exposed to one hour bath treatments of 125 mg/l survived in good condition to spawning, and the gametes were viable. These preliminary results generated strong interest in conducting a side by side comparison between formalin and hydrogen peroxide using normal operational population densities during the 1995 adult holding period.

Methods

The side by side comparison between formalin and hydrogen peroxide was made using identical rearing units and nearly equal numbers of fish per treatment type. Approximately one half of the fish received formalin at 167 mg/l for one hour drip treatments, while the other half received hydrogen peroxide at 125 mg/l administered as a bath treatment. Formalin was used as a drip treatment due to concerns of oxygen depletion during the treatment. Hydrogen peroxide was used as a bath treatment to conserve on chemical use and to make the cost per treatment more similar to the cost of formalin.

Initially, the first fish receiving hydrogen peroxide were treated with 75 mg/l on day one, then 100 mg/l on day 2, followed by daily treatments of 125 mg/l. This treatment strategy was used because it was not known if fish naive to hydrogen peroxide could tolerate a 125 mg/l bath treatment upon first exposure.

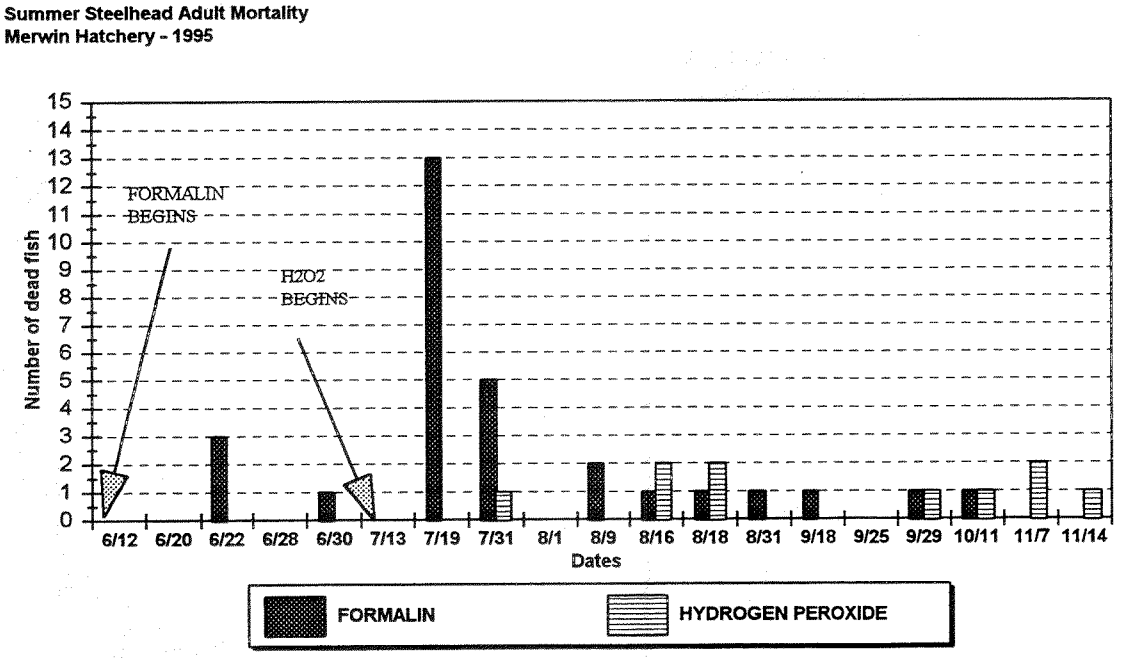
Treatments were made daily to the formalin group of fish from mid June through August 31, 1995. The adults receiving hydrogen peroxide were not collected until July 13th, so daily treatments began at this date and lasted through August 31. The treatments were then administered on alternate days from the first of September through spawning in December.

The level of hydrogen peroxide in the treated water was measured by using test strips (Quantofix made by Macherey-Nagel) which could only measure up to 100 mg/l. Measurements were made every two weeks to verify that the level of peroxide remained at least 100 mg/l during the treatments.

Results

The level of hydrogen peroxide remained at least 100 mg/l, as determined by the test strips, during all of the bath treatments. This is a strong indication that the hydrogen peroxide did not dissipate significantly during the bath treatment.

Total cumulative mortality during the period of daily treatments indicated that adults being treated with hydrogen peroxide had much better survival. There were 5 mortalities out of 125 adults (4 %) treated with hydrogen peroxide during this period, compared with 27 mortalities out of 119 adults (22.7 %) being treated with formalin. Saprolegniasis was the cause of death in nearly all of the fish. It should be noted, however, that most of the adults receiving formalin were trapped 3 and 4 weeks earlier than the fish that were given hydrogen peroxide. This early group had the highest mortality of the entire population. Most of the adults receiving hydrogen peroxide were trapped on July 13. After this date, the level of mortality was similar (See figure below). High mortality occurred in the formalin group on July 19, six days after the side by side



comparison began. The loss was primarily due to external fungus, but the fish were already showing signs of the infection prior to the initiation of the use of hydrogen peroxide. After August 31, the mortality rate was very low in both groups of fish. There were 3 mortalities in the formalin group, and 5 mortalities in the hydrogen peroxide group after August 31.

Physical appearance of adults at spawning was notably different between the two groups of fish. Adults receiving hydrogen peroxide were much brighter in color than those receiving formalin treatments. The gills of fish receiving hydrogen peroxide were completely free of copepods (*Salminicola* sp.), while those in the formalin group were heavily infested. The hatchery crew observed that fish receiving formalin could not withstand 40 minutes of no water flow without showing signs of low blood oxygen. This was most likely due to the level of copepods on the gill tissue.

There were no differences in fecundity, gamete survival, and hatching rate between the two groups of fish. The hatchery

crew noted however, that there were 6 to 10 females in the formalin group that were sterile. The cause of this phenomenon was not determined. Fecundity ranged from 3,000 to 4,000 eggs per female in both groups. Hatching rates were at 90 percent in eggs from both groups of adults. Survival beyond hatching was not monitored.

The cost of each treatment was comparable on a per treatment basis. Formalin treatments required 2.2 gallons per treatment for a cost of $ 7.17 each. The hydrogen peroxide treatments used 1.9 gallons per treatment, for a cost of $ 9.98 each. Costs are based on the 1995 per barrel price of $ 179.49 for formalin, and $ 289.00 for hydrogen peroxide. There were 134 total treatments made to the fish on formalin, for a total cost of $ 960.78. The adults receiving hydrogen peroxide were administered 110 treatments, for a total cost of $ 1097.80.

Water temperature was recorded daily and averaged high and low for the months of June through December, 1995. (Table 1). The Merwin hatchery generally reaches its highest temperature in October, due to the nature of the reservoir which supplies the hatchery. There is very little daily fluctuation at this facility.

Table 1. Average daily water temperature at the Merwin hatchery from June through December, 1995.

Month	avg. high	avg. low
June	54.7	53.9
July	53.1	53.0
August	54.7	54.6
September	57.6	57.4
October	58.6	58.3
November	53.1	53.0
December	46.0	45.9

Discussion. Overall, the use of hydrogen peroxide was just as effective as formalin for controlling *Saprolegnia* when both groups of fish were receiving treatments. Mortality was similar in the two groups after the side by side comparison began in mid July. The advantages of using hydrogen peroxide include the control of copepods in the gills, and the assumed belief that hydrogen peroxide is not as detrimental to the environment as formalin. Hydrogen peroxide also shows more usefulness in low oxygen conditions than does formalin. The cost of hydrogen peroxide was higher, but the benefits may justify the expense.

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ELEMENTAL IODINE AS A FUNGICIDE FOR CHINOOK AND COHO SALMON EGGS.

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Abstract

Ongoing tests with elemental iodine for fungicidal efficacy on chinook and coho eggs are presented. A combination of lab and on-site tests were conducted, with constant exposure to iodine (range 0.020 - 0.310 mg/l) from fertilization to the eyed stage (i.e. 27 to 39 days, depending on species and water temperature). In addition, the influence of iodine-water contact time prior to egg exposure (i.e. 0 to 30 min) and pH (i.e. pH = 6,7, and 8) also were tested. Fungicidal efficacy was evaluated by careful documentation of fungus growth, egg mortality, observations of hatching success, egg capsule degradation rates, egg volume displacement, and alevin mortality. Findings from this work are evaluated and recommendations for further tests are made.

Introduction

For the past several years we have been testing the efficacy of elemental iodine as a fungicide on salmon adults and eggs (Jensen et al., 1996). Iodine has several advantages over other chemical disinfectants, including its effectiveness at very low concentrations(i.e., ~0.2 mg/L), its long persistence (e.g. compared to ozone), and its low reactivity with organic compounds. At treatment levels, residual iodine in hatchery effluent would not be detectable and would not be hazardous to downstream aquatic organisms or to human water consumption. Tests at Salmon Enhancement hatcheries (Big Qualicum (BQ), Puntledge River (P), and Robertson Creek (RC)) and at the Pacific Biological Station (PBS) on salmon adults and eggs have yielded promising results, with iodine concentrations in the 0.1-0.2 mg/L range (Jensen et al., 1996). However, the techniques for ensuring uniform mixing and stable concentrations for treating adult (or juvenile) salmon pose some problems. First, fish are very sensitive to low iodine concentrations. Second, these low iodine concentrations are difficult to measure. In addition, iodine treatment during incubation cause egg capsules to harden, which interferes with hatching.

This paper discusses tests conducted at PBS and BQ during the fall and winter (1995, 1996) with chinook and coho eggs. The objective was to determine effective levels of iodine for fungus control and to prevent egg capsule alteration (i.e., hardened). At both PBS and BQ, the influence of contact time (i.e. the time from when the iodine stock solution is diluted to treatment level to when the eggs are exposed) was tested (i.e. ~1 - 30 min.). By varying contact time, we hoped to determine the range of effectiveness as the iodinated water passes through several incubator trays or through large incubation boxes or channels. In addition, at PBS, the influence of pH was included (i.e. pH = 6,7, and 8). This latter factor influences the chemistry (Black et al., 1965) of iodine (e.g. @ 25 °C and pH=6 with 0.5 mg/L iodine in solution, $\%I_2$=90 and %HIO=10; while @ 25 °C and pH=8 with 0.5 mg/L iodine in solution, $\%I_2$=12 and %HIO=88). Hence, by varying pH we hoped to determine whether or not the fungicidal efficacy and capsule hardness were effected.

Methods

Iodine Dosing and Measurement

Iodine introduction into the test water (at both BQ and PBS)was achieved by passing water through 2 cartridges filled with elemental iodine (Iomech Ltd., PO Box 14054, 2398 Lakeshore Blvd. West, Etobicoke, ON M8V 4A2) connected in series and diluted to target concentrations (see Figures 1 and 2). Iodine concentration was measured spectrophotometrically using the DPD method (Palin, 1967).

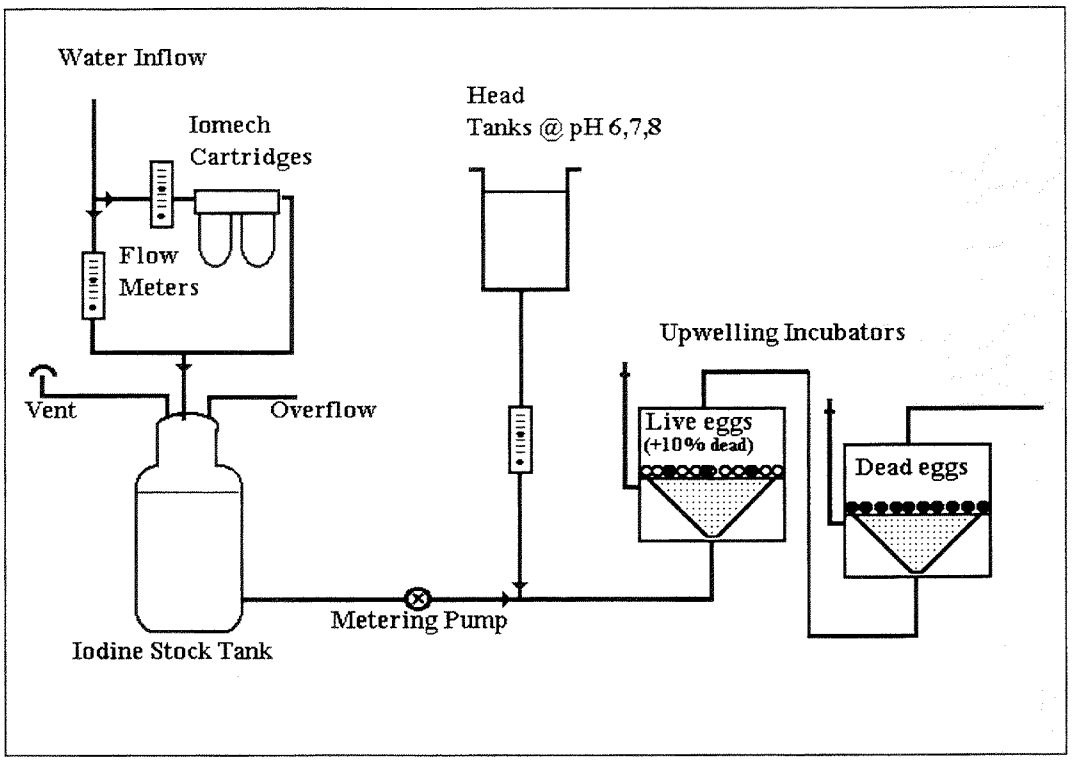

Figure 1. Schematic of PBS Iodine Experimental Setup

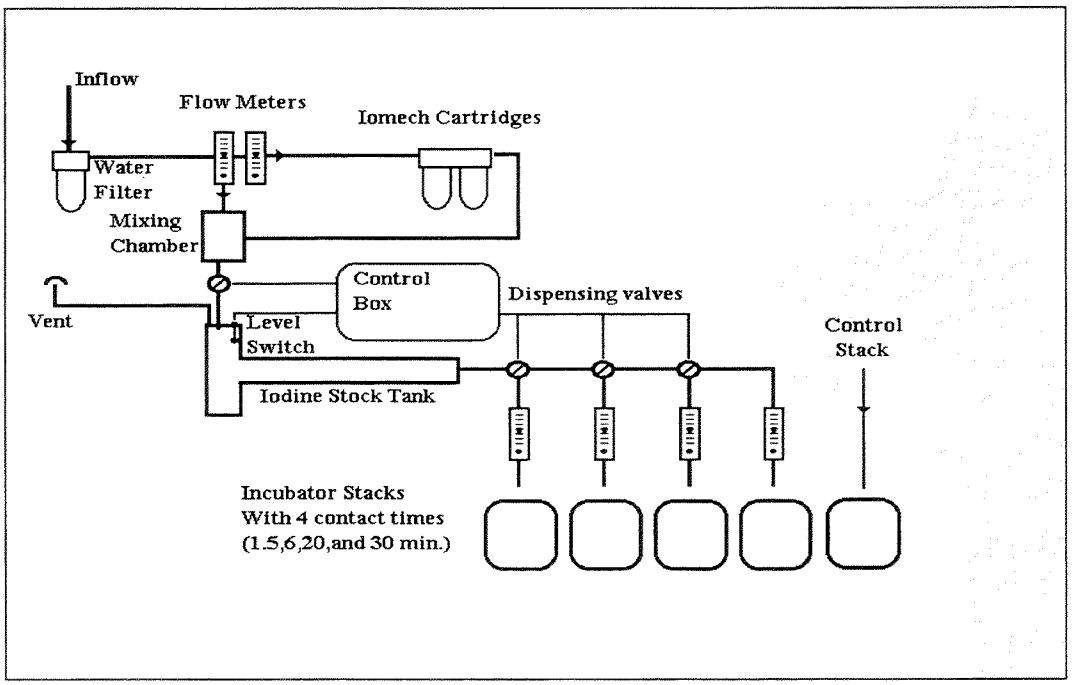

Figure 2. Schematic of BQ Iodine Experimental Setup

Egg Source, Fertilization Procedures, and number of Eggs and Replicates

Two species of Pacific salmon were tested, namely chinook (*Oncorhynchus tshawytscha*) and coho (*O. Kisutch*) from the Big Qualicum river. Eggs were fertilized and placed in incubators (i.e. standard "Heath" trays at BQ and small experimental upwelling incubators (Jensen and Groot, 1991) at PBS). At BQ, each treatment was replicated 3 times, with about 500 chinook eggs and about 700 coho eggs placed in 15-cm diameter PVC rings in each incubation tray to simulate normal egg-loading density. At PBS, each experimental incubator was partitioned into 4 replicate treatments, with about 120 chinook eggs and about 180 coho eggs per treatment.

To encourage fungus growth, unfertilized eggs (i.e.,50 unfertilized eggs per 700 test eggs, or about 7 % were added) were water-hardened and placed in with the fertilized test eggs for the coho experiment at BQ (no unfertilized eggs were used in the chinook exp. at BQ). At PBS, about 10 to 12 % unfertilized and water-hardened eggs were added to chinook and coho test eggs, respectively. In addition, at PBS, to further assess fungus growth, a duplicate incubator (each with 4 replicate partitions) was completely filled with unfertilized water-hardened eggs as a substrate for fungus growth. To assess fungus growth a quantitative fungus code was developed as follows:

Fungus assessment scale:	Description
0	no fungus
1	surface layer difficult to see on some eggs
2	clumps of fungus covering less than 50% of eggs
3	partial matting covering more than 50 % of eggs
4	fungus surrounding all eggs
5	thick matting of fungus surrounding all eggs

BQ 95/96 Test

Four Heath stacks (plus a "control" untreated stack) were employed for iodine disinfection tests at BQ. The treatments consisted of continuous exposure to iodine from fertilization to the eyed stage (i.e. about 4 weeks for chinook eggs at about 10 °C, and about 5 weeks for coho eggs at about 7 °C). Iodine (i.e. 0.4 mg/L) was applied to each of the 4 disinfection stacks. Incubation trays (with eggs) were positioned in the stack to achieve 4 levels of iodine-water contact time (i.e., about 1, 6, and 20 min, with an additional contact-tank used for the 30-min treatment) before eggs were exposed. Measurements of iodine concentration, flows, and pH were made routinely, once or twice per week, while temperature was recorded daily.

Assessment of response to iodine treatment consisted of recording egg mortality, alevin mortality, qualitative assessment of fungus growth, and assessment of egg capsule persistence or resistance (by volume) to degradation after hatching.

PBS 95/96 Test

At PBS, a more complex experimental design was employed, to include the influence of pH. The full factorial experimental design was as follows:

Treatment Factor	Treatment Levels
Iodine concentration	0.2 mg/L
Contact time	1, 10, and 30 min.
pH	6, 7, and 8.

Therefore the experiment consisted of 9 treatment levels (achieved by careful adjustment of metering pumps and flow meters) and a control (i.e. untreated water). The lower levels of pH were controlled by bubbling CO_2 into the supply water, while the higher pH was achieved by addition of sodium bicarbonate. Temperature (about 8 °C) and pH was computer-monitored. Iodine concentrations were measured daily.

Assessment of response to treatments consisted of recording egg mortality, alevin mortality, fungus growth, egg capsule persistence (by dry weight), and egg hardness by measurement of displaced egg volume using the technique described by Alderdice et al. (1984).

Results

BQ 95/96 Test

The experimental conditions, mortality responses, and fungus growth (for coho eggs) are summarized in Table 1. For chinook eggs during the iodine treatments (i.e. 27 days of exposure), the average temperature was 10.51 °C and the average pH was 7.57. For the coho eggs (i.e. 39 days of exposure), the average temperature was 6.85 °C and the average pH was 7.54. Fungus growth assessment on the coho eggs ranged from 2 to 3.5, with the lowest incidence of fungus occurring at the highest iodine concentration.

Table 1. Summary of experimental conditions, mortality responses, and fungus growth (for coho eggs) at BQ.

Chinook BQ Site Fall 1995

Treatment	pH	temp	Iodine mg/L	exposure days	exposure hr/day	C x T mg.hr.L	Mortality Egg	Alevin	Sum
T11	7.57	10.51	0.178	27	24	111.07	6.20	4.06	10.26
T12	7.57	10.51		27	24	111.07	3.53	2.60	6.13
T13	7.57	10.51	0.092	27	24	111.07	4.02	1.61	5.63
T21	7.57	10.51	0.109	27	24	68.02	1.14	2.09	3.23
T22	7.57	10.51		27	24	68.02	2.21	1.10	3.31
T23	7.57	10.51	0.080	27	24	68.02	3.90	0.93	4.83
T31	7.57	10.51	0.099	27	24	61.78	2.82	0.40	3.22
T32	7.57	10.51		27	24	61.78	2.76	1.06	3.82
T33	7.57	10.51	0.088	27	24	61.78	2.25	0.82	3.07
T41	7.57	10.51	0.085	27	24	53.04	2.99	0.00	2.99
T42	7.57	10.51		27	24	53.04	2.28	1.04	3.32
T43	7.57	10.51	0.063	27	24	53.04	3.06	0.82	3.88
C1	7.57	10.51	0.000	27	24	0.00	6.50	1.52	8.02
C2	7.57	10.51	0.000	27	24	0.00	7.31	1.04	8.35
C3	7.57	10.51	0.000	27	24	0.00	13.32	1.61	14.93

Coho BQ Site Fall 1995

Treatment	pH	temp	Iodine mg/L	exposure days	exposure hr/day	C x T mg.hr.L	Mortality Egg	Alevin	Sum	fungus
T11	7.54	6.85	0.082	39	24	71.14	9.37	0.60	9.97	2.0
T12	7.54	6.85		39	24	71.14	7.25	1.67	8.92	2.0
T13	7.54	6.85		39	24	71.14	11.98	3.31	15.29	2.0
T21	7.54	6.85	0.046	39	24	36.50	29.90	0.15	30.05	3.0
T22	7.54	6.85		39	24	36.50	26.72	0.42	27.14	2.5
T23	7.54	6.85		39	24	36.50	13.28	0.99	14.27	2.0
T31	7.54	6.85	0.047	39	24	40.25	32.13	0.00	32.13	3.0
T32	7.54	6.85		39	24	40.25	17.15	0.29	17.44	2.0
T33	7.54	6.85		39	24	40.25	11.71	0.69	12.40	2.0
T41	7.54	6.85	0.025	39	24	18.72	29.52	0.14	29.66	3.0
T42	7.54	6.85		39	24	18.72	21.76	0.28	22.04	3.0
T43	7.54	6.85		39	24	18.72	21.48	0.52	22.00	3.0
C1	7.54	6.85	0.000	39	24	0.00	46.92	0.42	47.34	3.5
C2	7.54	6.85	0.000	39	24	0.00	53.09	0.14	53.23	3.5
C3	7.54	6.85	0.000	39	24	0.00	36.06	0.00	36.06	3.5

Trends for eggs, alevins, and total (i.e. combined egg and alevin) mortality generally followed a parabolic relationship, with increases in mortality occurring at low and high levels of iodine (Figure 3). The parabola models ($y = a + bx + cx^2$, where y = mortality and x = iodine) with predicted minima (mg/L Iodine) and corresponding mortality (%) are listed in Table 2.

Table 2.

Model	Species	a	b	c	r^2_{DF} Adj[a]	Minimum[b] Iodine (mg/L)	Predicted[b] Mortality(%)
stdmrt(tot.)[c]	BQcn[d]+co[d]	77.078842	-1140.9933	6177.5317	0.7047	0.092	24.39
egg mrt[e]	BQcn	9.0324605	-127.24136	625.1436	0.6650	0.102	2.56
alevin mrt	BQcn	1.3718233	-24.715585	229.81039	0.7417	0.054	0.71
total mrt	BQcn	10.404296	-151.95694	854.954	0.7038	0.089	3.65
egg mrt	BQco	43.809824	-667.38476	3171.2494	0.6570	0.105	8.70
alevin mrt	BQco	0.2367214	-12.896337	394.23737	0.4187	0.016	0.13
total mrt	BQco	44.029341	-676.51039	3523.6743	0.6467	0.096	11.56

[a] r^2 is adjusted for the degrees of freedom, based on the number of model coefficients.

[b] Calculation of the minimum value for the 7 parabola models ($y=a+bx+cx^2$) was achieved by solving for the first derivative ($y=b+2*c*x= 0$). Therefore the minimum $x=(-1*b)/(2*c)$ and the predicted mortality was calculated for the minimum.

[c] Stdmrt = Standardized mortality is expressed as a percentage of the maximum total mortality (i.e., 14.93% for chinook and 53.23% for coho).

[d] cn = chinook and co = coho.

[e] mrt = mortality.

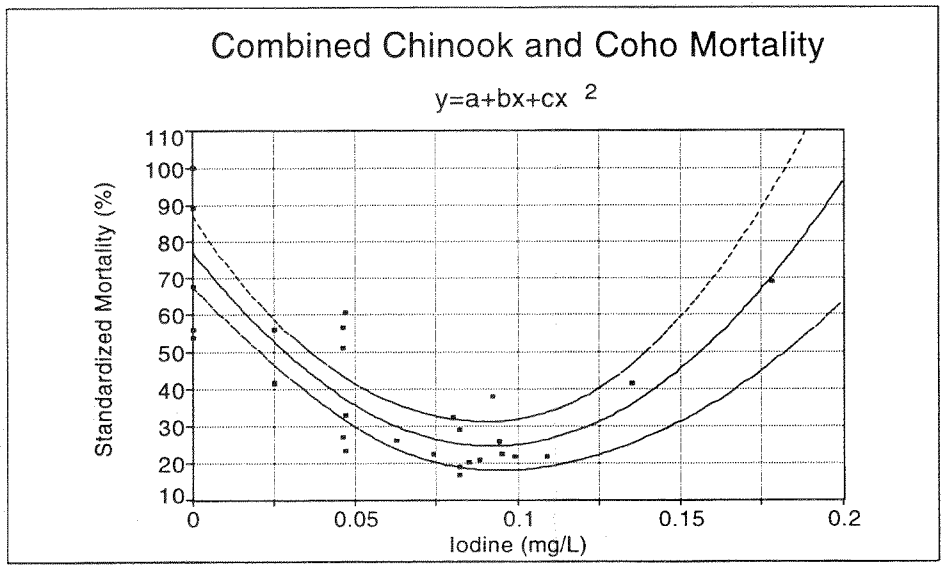

Figure 3. Standardized "total" mortality and 95% confidence limits of BQ eggs. Model coefficients are listed in Table 2, above.

Egg Capsule Persistence

At 99 % hatch (Feb. 28, 1996), coho egg capsule material (about 60 ml of settled volume) was collected from each treatment replicate (except for control trays, where capsules had already decomposed and only about 5 ml of egg capsule material remained for the 3 replicates). After 47 days, the volume of egg capsule material remaining for each treatment was as follows:

Iodine (mg/L)	Mean Volume Remaining (%)
0.082	90.24
0.046	86.75
0.047	69.47
0.025	61.87
control	0.00

PBS 95/96 Test

The experimental conditions, mortality responses, and fungus growth are summarized in Table 3. The exposure time for chinook was 10 days longer and for coho was 8 days shorter compared to BQ due to differences in water temperatures (mean temperatures ranged from 7.54 to 8.45 °C). The iodine demand for PBS water was very low and resulted in higher iodine levels (mean iodine ranged from 0.14 to 0.31 mg/L) than at BQ.

Table 3. Summary of experimental conditions, mortality responses, and fungus growth at PBS.

Chinook Fall 1995

Treatment	pH	temp	Iodine mg/L	exposure days	exposure hr/day	C x T mg.hr.L	Mortality Egg	Alevin	Sum	fungus code avg	fungus code data[a]	dead egg assessment[b] avg	fungus code data[a]
pH 6													
0 min	6.1	7.82	0.31	38	24	285.27	8.4	0.6	9.0	0.00	0,0,0,0	0.00	0,0,0,0
10 min	6.1	7.98	0.26	38	24	235.57	9.7	0.6	10.3	0.50	2,0,0,0	0.75	2,0,0,1
30 min	6.1	8.16	0.23	38	24	213.13	6.5	1.5	8.0	0.00	0,0,0,0	0.00	0,0,0,0
ctrl	6.1	7.91	0.00	38	0	0.00	11.6	1.4	13.1	1.75	0,2,2,3	4.00	4,4,4,4
pH 7													
0 min	7.0	7.91	0.30	38	24	272.96	14.5	2.5	17.0	0.75	0,0,3,0	1.00	0,0,2,2
10 min	7.0	8.14	0.27	38	24	250.53	4.4	0.0	4.4	0.00	0,0,0,0	1.25	2,0,1,2
30 min	7.0	8.39	0.24	38	24	216.69	6.5	0.0	6.5	0.00	0,0,0,0	1.50	1,1,2,2,
ctrl	7.0	8.10	0.00	38	0	0.00	4.6	0.0	4.6	0.25	1,0,0,0	4.75	5,5,4,5
pH 8													
0 min	8.0	8.16	0.29	38	24	268.22	13.5	3.6	17.1	0.75	3,0,0,0	0.75	1,1,1,0
10 min	8.0	8.37	0.26	38	24	232.92	2.2	0.5	2.7	0.00	0,0,0,0	1.00	0,1,1,2
30 min	8.0	8.45	0.16	38	24	145.83	5.9	0.6	6.6	0.25	0,0,0,1	2.00	2,2,2,2
ctrl	8.0	8.05	0.00	38	0	0.00	21.8	0.0	21.8	2.75	3,4,4,0	5.00	5,5,5,5

Coho Fall 1995

Treatment	pH	temp	Iodine mg/L	exposure days	exposure hr/day	C x T mg.hr.L	Mortality Egg	Alevin	Sum	fungus code avg	fungus code data[a]	dead egg assessment[b] avg	fungus code data[a]
pH 6													
0 min	6.1	8.01	0.28	31	24	211.59	13.6	3.6	17.2	0.00	0,0,0,0	0.25	0,0,0,0
10 min	6.1	8.13	0.21	31	24	155.72	16.8	2.5	19.3	0.00	0,0,0,0	2.50	2,2,3,3
30 min	6.1	8.40	0.23	31	24	170.60	17.8	2.1	19.9	0.00	0,0,0,0	0.00	0,0,0,0
ctrl	6.1	8.03	0.00	31	0	0.00	10.9	5.1	15.9	0.00	0,0,0,0	3.00	3,3,3,3
pH 7													
0 min	7.1	7.62	0.29	31	24	212.56	14.4	7.4	21.9	0.00	0,0,0,0	1.50	2,2,1,1
10 min	7.1	7.95	0.29	31	24	212.11	9.4	6.7	16.1	0.00	0,0,0,0	0.50	0,0,1,1
30 min	7.1	8.11	0.21	31	24	156.46	10.0	2.1	12.0	0.00	0,0,0,0	1.75	1,2,2,2
ctrl	7.1	7.80	0.00	31	0	0.00	10.8	1.6	12.3	2.00	1,2,2,3	3.50	3,3,4,4
pH 8													
0 min	8.1	7.61	0.24	31	24	175.06	8.4	19.6	28.1	0.00	0,0,0,0	1.25	1,0,2,2,
10 min	8.1	7.94	0.21	31	24	153.19	9.8	17.8	27.6	0.00	0,0,0,0	3.00	3,3,3,3
30 min	8.1	8.11	0.14	31	24	105.87	10.3	2.7	13.0	0.75	0,1,1,1	2.25	3,3,1,2
ctrl	8.1	7.54	0.00	31	0	0.00	31.4	0.5	31.9	3.00	4,2,2,4	4.00	4,4,4,4

[a] Fungus code data for each replicate.

[b] Fungus code data for unfertilized egg groups.

High pH caused an increase in fungus growth, as indicated by the increased egg mortality and fungus growth on both live and dead eggs in the control treatments. The fungicidal efficacy of iodine was most dramatically evident in the incubators with all dead eggs. In these incubators (for both chinook and coho), fungus growth codes ranged from 0 (at high iodine levels) to 4 or 5 (in the pH 8 control). Fungus growth was modeled using simple multiple linear regression ($z = a + bx + cy$, where z = fungus code , x = pH, and y = iodine (mg/L)) to yield the following 2 models:

Chinook egg model: $z = 2.0101 + 0.3451(pH) - 13.4587(\text{iodine, mg/L})$ $r^2_{DF\ Adj} = 0.8332$

Coho egg model: $z = 0.2018 + 0.4719(pH) - 9.1086(\text{iodine, mg/L})$ $r^2_{DF\ Adj} = 0.6387.$

The effect of pH and iodine concentration on fungus growth on unfertilized chinook eggs yielded a highly significant 2-factor model, indicated by the high r^2 value above, and illustrated in Figure 4 below.

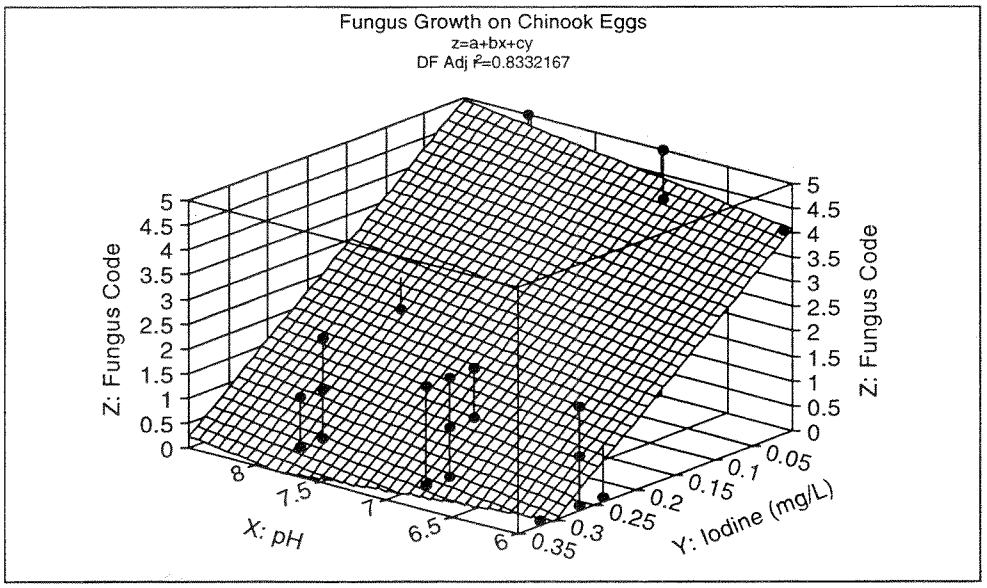

Figure 4. A 3-dimensional plane, illustrating the influence of pH and iodine on fungus growth on "dead"chinook eggs.

Egg Capsule Persistence and Egg Volume Displacement

In a similar fashion as described for BQ, egg capsule persistance was measured for coho eggs at PBS. However, instead of quantifying egg capsules volumetrically, the material was collected (30 days after hatch) and dried (at 60 °C for 24 hr) to yield dry weight of remaining capsule material per egg. These measurements were made for all but 2 of the iodine treatments (plus no material was recovered from the control treatment) to yield the following:

Iodine(mg/L)	pH	Contact Time (min)	Egg capsule remaining (dry weight per egg, µg)
0.28	6.05	0	134
0.21	6.05	10	8
0.23	6.05	30	124
0.29	7.05	0	346
0.29	7.05	10	597
0.21	7.05	30	406
0.24	8.07	0	227

In attempt to further assess the influence of iodine on egg capsules, displaced egg volume with a 10-gm load was measured for chinook eggs from each treatment (Table 4):

Table 4. Mean displaced chinook egg volume.

pH	Iodine (mg/L)	Mean disp. vol(%)	STD
6.1	0.000	1.12	0.23
6.1	0.310	1.36	0.25
6.1	0.260	1.21	0.19
6.1	0.230	1.31	0.40
7.0	0.000	1.07	0.16
7.0	0.300	1.30	0.38
7.0	0.270	1.14	0.19
7.0	0.240	1.06	0.12
8.0	0.000	1.13	0.13
8.0	0.290	1.27	0.37
8.0	0.260	0.85	0.22
8.0	0.160	1.15	0.24

Discussion

The results from the tests at both BQ and PBS indicate that elemental iodine is effective in controlling fungus growth. Similar to results reported earlier (Jensen et al., 1996), untreated eggs experienced greater losses due to fungus than eggs treated with iodine from fertilization to the eyed stage. However, hardened egg capsules still caused hatching problems with resultant alevin mortality as well as creating a potential flow blockage problem in fully loaded trays.

At BQ, chinook egg mortality ranged from 1.1 to 6.2 % when treated with iodine ranging in concentration from 0.063 to 0.178 mg/L for 27 days, compared to untreated egg mortality ranging from 6.5 to 13.3 % (Table 1). Hardened egg capsules caused increased alevin mortality, particularly at higher iodine concentrations (i.e., 4.1 % alevin mortality occurred at 0.178 mg/L). The coho test at BQ (with 3 % unfertilized eggs added to encourage fungus growth) resulted in significantly increased egg mortality (i.e., untreated egg mortality ranged from 36 to 53 %) with iodine-treated egg mortality reduced, but not effectively (i.e. egg mortality ranged from 7.3 to 32.1 %). This lack of fungicidal efficacy was likely due to the generally lower iodine concentrations (i.e. 0.025 to .082 mg/L) that occurred due to increased iodine-demand in the water. Increased alevin mortality, caused by hardened egg capsules, occurred only at the highest iodine levels. However, egg capsule persistance (as observed for coho) showed a consistent trend with iodine concentration, with 62 to 90 % of egg capsule material remaining 47 days after hatch when eggs had been treated with 0.025 to 0.082 mg/L.

The analysis of egg and alevin mortality from the tests at BQ (Table 2) indicated that for eggs, the optimum iodine concentration for fungus control was 0.102 and 0.105 mg/L for chinook and coho, respectively. The optimum iodine concentration (during egg exposure) to minimize alevin mortality was 0.054 and 0.016 mg/L for chinook and coho, respectively. This much lower optimum for alevins is associated with the hardened egg capsule discussed above. By standardizing the total mortality values for both chinook and coho, the combined positive and negative influences of iodine treatments are clearly illustrated (Figure 3) with the optimum iodine concentration being 0.092 mg/L.

At PBS, the influence of pH on fungus growth was dramatically apparent for both chinook and coho tests. Untreated "control " egg mortality doubled (for chinook) and tripled (for coho) at the higher pH 8 treatment. Similarly, fungus growth increased in directly proportion to pH. This may be an explanation for differences in severity of fungus problems at different sites. The overall assessment of egg and alevin mortality appears to indicate that iodine treatment concentrations were too high (i.e., ranging from 0.16 to 0.31 mg/L). This was due to the low iodine demand of the PBS water supply. This conclusion is consistent with the BQ mortality analysis discussed above. The egg and alevin mortality data at pH 6 and 7 were not consistently reduced with iodine treatment (Table 3). However, at pH 8, iodine treatment was effective in reducing fungus growth and egg mortality. The influence of both iodine concentration and pH significantly influenced fungus growth (Table 3), with increasing iodine levels and decreasing pH levels dramatically reducing fungus growth. Egg capsule persistance, for iodine-treated eggs, was greater at higher pH. However, further work with various iodine concentrations and pH levels are necessary to fully

understand this phenomenon. Displaced egg volume (Table 4), when modeled against the % I_2 (Chang, 1958) yielded an r^2=0.9263 over the pH range 6 to 8 and iodine concentration of 0 to 0.3 mg/L. This indirect measurement of egg capsule elasticity or hardness suggests that the % I_2 and % HIO influence the egg capsule. This should be determined histologically.

In summary, elemental iodine is effective in controlling fungus growth on chinook and coho eggs. However, the problems associated with egg capsule alteration have not been solved by increasing contact times or by altering pH levels. Perhaps iodine can be employed to disinfect water, followed by addition of thiosulphate to chemically remove the iodine, prior to coming in contact with the eggs. This approach would act on pathogenic organisms and would not affect the eggs.

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Fish Pills -- Medication Possibilities for the Future

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Abstract

Medicated pellets containing concentrated levels of antibiotic (erythromycin) were fed to spring chinook salmon (*Oncorhyncus tshawytscha*) to evaluate the tissue levels attained and the uniformity that could be achieved in the population after being fed for 14 days. When compared to the tissue levels attained and the population uniformity achieved after feeding a commercially manufactured medicated diet (4.5% Aquamycin, BioProducts Inc., Warrenton, Oregon), no significant difference was observed in the levels reached or in the population uniformity. In addition, preliminary data indicates that the formulation and feeding of the medicated "fish pills" can provide significant economic benefits and more effective medicated treatments.

Introduction

Medicated "fish pills" containing two levels of Gallimycin-100p were formulated and pelletized to be fed **with** regular unmedicated salmon feed pellets to investigate an alternative method of administering medication orally to juvenile salmonids. Evaluation of the tissue (kidney) levels attained and the uniformity that could be achieved in the population was compared to the tissue levels attained and the population uniformity achieved after feeding a commercially manufactured medicated diet. Other potential benefits are discussed.

Methods and Materials

Two levels of medicated pellets were formulated and pelletized, a 30% Gallimycin-100p pellet and a 60% Gallimycin-100p pellet. The semi-moist " fish pills" were extruded to be 2.0 mm in size. Four small groups of juvenile spring chinook salmon were used in the feed trial. Groups contained 250 fish at 42.5 fish/pound (93.8 fish/kg) and were fed for 14 consecutive days (see Table 1.) The fish used in the trial represented every female adult spawned in 1995 (51 females) that had low too very low enzyme-linked immunos sorbent assay (ELISA) results. The fish in each group were fed a 1.3% body weight (BW)/ day ration. The target treatment level for each group was 100 mg/kg. Each group was held in 5 foot diameter (49 ft^3 , 1.39 m^3) fiberglass tanks until the end of the investigation. All feed was kept frozen prior to feeding and was weighed out daily based on a computer generated feed charts. At the end of the 14-day trial, 20 fish from each group were examined and kidney samples were taken and analyzed for antibiotics by microbiologic assay.

Table 1: Medicated "fish pills" vs. 4.5% Aquamycin: four treatment groups, 14-day feeding trial

Treatment Group	Control BioMoist Feed	30% Gallimycin-100p	4.5 % Aquamycin Manufactured feed	60% Gallimycin-100p*
# of fish	250	250	250	250
size	42.5 f/lb 93.8 f/kg	42.5 f/lb 93.8 f/kg	42.5 f/lb 93.8 f/kg	42.5 f/lb 93.8 f/kg
Total weight	2670.6 g	2670.6 g	2670.6 g	2670.6 g
Feeding rates Body weight/day	1.3%	1.3%	1.3%	1.3%
Medicated feed	0	2 grams/lb. of fish	1% Body weight/day	1 gram/lb. of fish
Total medicated feed	0	174 g	399 g	37 g

**Note: The group fed 60% Gallimycin were fed three days with medicated feed, four days without twice. Previous test feedings indicated possible toxicity problems at the 60% level.*

Results

Tissue assays from the fish sampled from the 4.5% Aquamycin group and the group fed the 30% Gallimycin-100p pellet indicated no significant difference between the levels attained and the uniformity reached in two treatment groups (See Figure 1). The levels in the 60% Gallimycin group were detectable but low. The average micrograms/gram of erythromycin in kidney tissue in the two groups was 101.97 ug/g (See Table 2).

Table 2: Average micrograms of erythromycin per gram of kidney tissue sampled at the end of the 14-day feeding trial.

4.5% Aquamycin = 100.95 ug/g kidney
30% Gallimycin-100p = 103 ug/g kidney

The fish from all groups fed very well throughout the feed trial. No losses of any kind occurred in any of the groups either from natural mortality or from any toxic side effects.

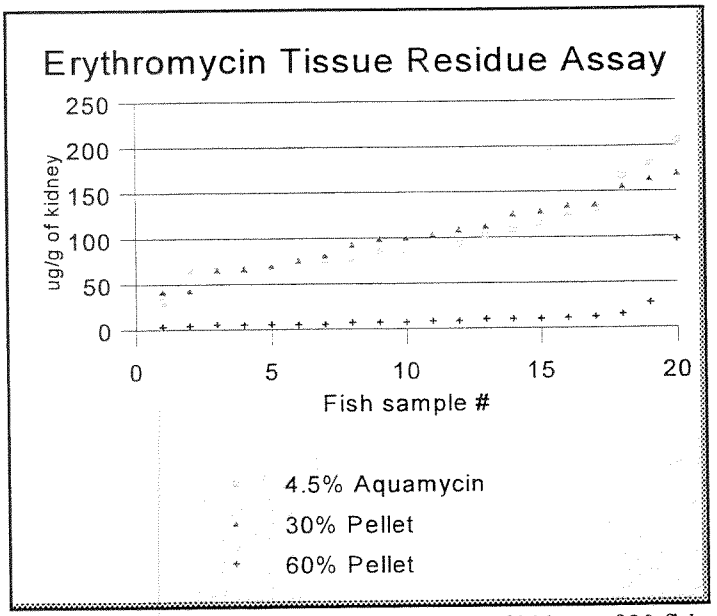

Figure 1: Erythromycin tissue assay in ug/g of kidney of 20 fish sampled per group at the end of the 14-day feeding trial.

Discussion

In an attempt to improve the administration of antibiotics orally to juvenile spring chinook salmon, medicated pellets were formulated to be fed **with** regular salmon feed. A 30% Gallimycin-100p and a 60% Gallimycin-100p "fish pills" were formulated and extruded. A 14-day feed trial was designed to compare the levels of erythromycin that could be attained the kidney tissue of juvenile SCS from feeding the medicated pellets compared

to the levels that were attained feeding manufactured medicated feed. The distribution of levels reached both within and between treatment groups was also examined. Results indicated no significant difference in the levels and/or the distribution in the population between the group of fish fed the 30% Gallimycin-100p pellet and the 4.5% Aquamycin manufactured feed. An apparent advantage is, regardless of the amount of unmedicated feed fed to the group fed the 30% pellet, levels of erythromycin in kidney tissued sampled could be the same as in the group fed the 4.5% Aquamycin pellets which require feeding rates of at least 1% body weight per day. The problem of less than 1% BW feeding rates can be negotiated with the use of the medicated pellets (e.g., cold water temperatures, reduced feeding rates).

Initially, the scope of this investigation was designed only to examine the levels of erythromycin in the tissues and the distribution in the population that could be attained. A comparison between each effective method was performed with interesting results (see Table 3). The example in Table 3 represents a typical treatment scenario for the Entiat NFH. As stated earlier in this discussion, medicated feed treatments are often recommended by the Fish Health Biologist during the time when the fish are approximately 20 f/lb and when feeding rates are less than 1% body weight per day. The feeding of the 4.5% Aquamycin at 1% body weight per day would cost more than $5,000.00 and if feeding rates are less than 1% BW/day there is a potential for waste. Also, the recommended duration for treatment is 21 days which would result in even greater costs and potential economic waste when using the 4.5% Aquamycin. Uneaten and/or unfed commercially produced, medicated feed becomes a controlled waste therefore difficult and expensive to be disposed.

Table 3: Cost comparison between the commercially manufactured 4.5% Aquamycin and the 30% Gallimycin-100p extruded "fish pills" for a typical production scenario at the Entiat NFH.

Cost Comparison		
Production Example: 400,000 SCS @ 20 f/lb - 14 -day treatment		
	4.5% Aquamycin	30% Gallimycin Pellet
Cost/ lb.	$1.88	$1.51
Medicated Feed	2800 lbs. (1% BW/day x 14 days)	1233 lbs. (2 grams / lb. x 14 days)
Treatment Cost	$5264.00	$1862
Regular Feed Cost	$0.00	$635.00 1567 lbs. @ $0.405/lb
Total Cost	**$5264.00**	**$2497.00**

The feeding of the medicated "fish pills", in this case the 30% Gallimycin-100p allows greater flexibility when administering medication. When feeding conditions are not optimal, the pellets can be kept for greater periods of time without a loss in the potency of the drug. Small groups and/or lots of fish (i.e., progeny of females with High ELISA readings) can easily be treated. Feeding **with** regular feed, ensures that the nutritional quality of the daily diet. The use of veterinary drug prescriptions can also be investigated. There appears to be a considerable cost benefit when using the Gallimycin-100p "fish pills" (See Table 3); however, the difference in cost is due to the basic costs of Aquamycin and Gallimycin. Presently, Aquamycin costs are nearly double the cost of Gallimycin which leaves us with the question: If Gallimycin-100p is effective, why pay double for Aquamycin?

Medicated "fish pills" may offer considerable cost benefits, flexible feeding regimes, and the potential for future treatment possibilities pending access to other antibiotic combinations that could be used to treat BKD.

Acknowledgments

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DORSAL FIN EROSION IN NORMALLY PIGMENTED AND ALBINO STEELHEAD TROUT

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ABSTRACT

Differences in levels of dorsal fin erosion have been observed between albino and normally pigmented steelhead at WDFW hatcheries. In the summer of 1996, an experiment was conducted to evaluate dorsal fin erosion. Equal numbers (N=250) of Skamania strain summer steelhead were put in three aquaria at Elochoman Hatchery. One aquaria consisted of normally pigmented fish, one albinos, and a third aquaria half albino and half normally pigmented fish. Fish ($n \geq 20$) were sampled biweekly for length, weight, K-factor, dorsal fin length, dorsal fin erosion and ratio of dorsal fin length to overall fork length. Means were subject to Analysis of Variance and if significant differences were indicated, a Newman-Keuls multiple comparison test was used to determine which treatments were significantly different. Dorsal fin erosion was exacerbated with increasing density and light levels. By the experiment's end, albino groups had significantly ($p \geq 0.001$) longer dorsal fin lengths, greater ratios of dorsal fin length vs fork length and significantly less fin erosion than normally pigmented groups. Focus should not be placed on the albinos themselves, but as indicators of what high density and high light levels engender in normally pigmented fish. Fin nipping is sight dependent and increases with density and enhanced light levels. Because albinos have minimum pigment in their dorsal fin membranes, these fins are virtually invisible to other fish and albinos suffer minimal fin nipping.

INTRODUCTION

This experiment is an outgrowth of a series of observations made during the summer of 1995. Steelhead in one raceway at Beaver Creek Hatchery had a number of albinos present. When dorsal fin condition of albinos in the raceway was compared to normal pigmented fish in the same raceway, the condition of albino dorsal fins was superior. Albinos showed little or no evidence of fin nipping while normally pigmented fish showed severe erosion. Both groups were of similar size.

Albinism is a simple autosomal recessive character (Bridges and von Limbach, 1972). It becomes manifest when inherited from both parents. Fish inheriting the gene from only one parent become carriers of the gene, but do not display the characteristic albino pink eyes or lack of pigmentation. Higher than normal levels of albinism were introduced into Skamania summer steelhead stock through intensive selection for increased growth during the 1960-70's.

Density effects on steelhead and rainbow trout dorsal fins were reported by Bosakowski and Wagner (1944) and Kindschi et al. (1991). Soderberg et al. (1993) indicated density effects on Atlantic salmon dorsal fins. Stringer and Hoar (1955) reported fin nipping in Kamloops rainbow trout to be related to light level and victim coloration.

METHODS

On March 19,1996, Skamania strain summer steelhead *(Oncorhynchus mykiss)* were placed into three aquaria at the Elochoman Salmon Hatchery. Two hundred fifty albinos, and 250 normal fish were placed into separate aquaria. A third aquarium held a mixed population of 125 albinos and 125 normally pigmented individuals. Aquaria measured 61 cm long by 76 cm wide. Two were 64 cm deep but the third, containing all normal fish was 59.5 cm deep. Normal pigmented fish were 570 fish per pound and albinos were slightly smaller at 590 fish per pound. Four fish groupings were evaluated: normally pigmented fish, albinos, and mixed normals and mixed albinos in the combined aquarium.

At two week intervals, 20-25 fish random samples were subject to one way analysis of variance (ANOVA) for length, weight, and condition factor (K-factor). When dorsal fin erosion became apparent; measures of dorsal fin length

(mm), the ratio of dorsal fin length to fork length (mm), and a dorsal fin erosion scale were also subject to ANOVA. If the ANOVA indicated a significant difference existed between groups, the Newman-Keuls Multiple Comparison test was used to identify which group or groups were statistically different.

The dorsal fin erosion scale is similar to that used by the State of Maine (1992). A perfect dorsal fin is given a '0' on the scale. A completely eroded dorsal fin is given a value of '4'. Intermediate erosion is given a '2' or '3' value. Rips, tears or a ragged fin margin places the fin in a higher category. The higher the value; the greater the incidence of fin erosion.

Routine feeding levels were determined and culture duties were performed by hatchery staff.

RESULTS

Regularly scheduled sampling began on April 8, 1996. On April 10, a single fluorescent shop light was installed over the middle aquaria to provide additional light to all aquaria. Sample means and groups which Newman-Keuls determined to be statistically different are presented in Table 1. At the initial sampling both normally pigmented groups were significantly longer and heavier than albinos (Figures 1&2). By the second sample date, April 22, only the mixed normal group had a significantly greater ($p<0.001$) length, which continued throughout the experiment. By the experiment's end, the mixed normal group was significantly longer than the albino which was significantly longer than the normals and mixed albinos. The mixed normals were significantly heavier and mixed albinos significantly lighter than normal or albino groups.

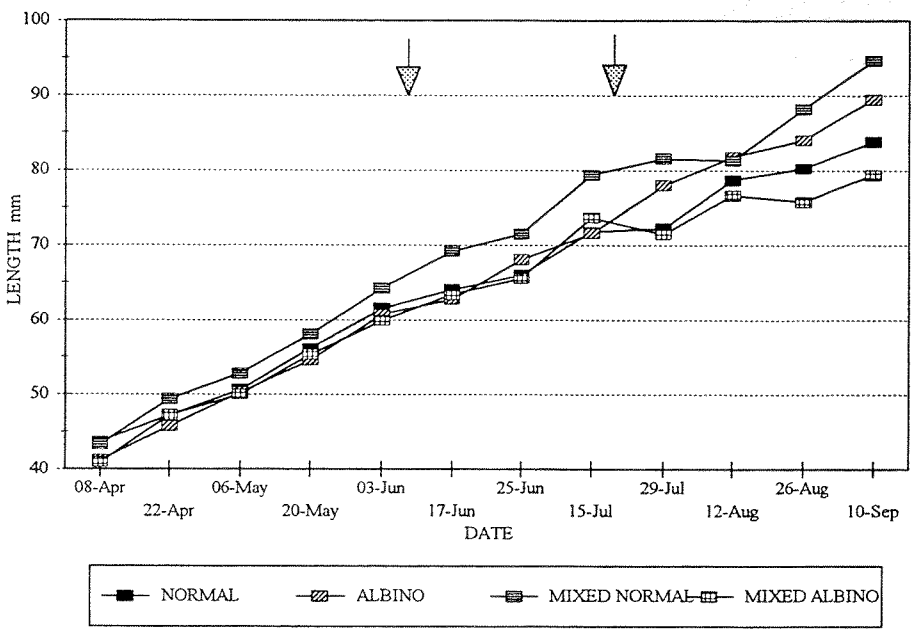

Figure 1. Mean sample lengths (mm) of Elochoman albino and normal summer steelhead April-Sept. 1996. First arrow indicates placement of crowder into aquaria. Second arrow indicates placement of additional shop light.

Table 1. Mean sample values for length (mm), weight (g), K-factor, dorsal fin erosion, dorsal fin length (mm) and dorsal fin to fork length ratio (%). Statistically significant values are indicated.

	Length				Weight			
Date	Normal	Mixed Normal	Albino	Mixed Albino	Normal	Mixed Normal	Albino	Mixed Albino
08-Apr	* 44	* 43	41	41	* 0.8	* 0.8	0.7	0.7
22-Apr	47	* 49	46	47	1.2	1.4	1.1	1.3
06-May	51	* 53	50	50	1.6	* 1.8	1.5	1.5
20-May	56	* 58	55	55	1.8	* 2.2	1.8	1.8
03-Jun	62	* 64	61	60	2.6	* 3.1	2.5	2.4
17-Jun	64	* 69	63	63	3.0	* 3.8	2.9	2.8
25-Jun	66	* 71	68	66	3.4	* 4.4	3.6	3.4
15-Jul	72	* 79	72	74	4.0	* 5.5	4.1	4.3
29-Jul	72	* 82	* 78	71	4.3	* 6.3	* 5.6	4.2
12-Aug	79	81	82	77	6.1	7.0	6.4	5.4
26-Aug	80	88	84	76	6.2	8.3	7.0	4.9
10-Sep	84	** 95	* 89	79	6.7	** 9.6	7.8	* 5.4
	K-factor				Dorsal Fin Erosion			
08-Apr	0.95	0.96	0.98	0.96	0.0	0.0	0.0	0.0
22-Apr	1.10	1.17	1.16	1.23	0.0	0.0	0.0	0.0
06-May	1.21	1.22	1.20	1.18	0.0	0.0	0.0	0.0
20-May	1.02	1.09	1.08	1.05	0.0	0.0	0.0	0.0
03-Jun	1.10	1.14	1.09	1.10	0.0	0.0	0.0	0.0
17-Jun	1.12	1.14	1.17	1.11	** 0.9	* 0.6	0.0	0.0
25-Jun	1.17	1.19	1.12	1.18	0.6	* 1.5	0.1	0.2
15-Jul	1.05	1.07	1.10	1.05	* 1.1	** 3.0	0.5	0.5
29-Jul	1.10	1.14	1.16	1.11	0.7	* 2.6	0.2	0.4
12-Aug	1.23	1.24	* 1.16	* 1.17	1.4	** 3.1	0.3	* 1.0
26-Aug	1.14	1.16	1.15	1.11	2.8	2.9	* 0.0	* 1.0
10-Sep	1.10	1.10	1.07	1.05	3.1	3.2	** 0.1	* 2.1
	Dorsal Fin Length				Dorsal fin to fork length ratio X 10			
17-Jun	* 4.4	5.5	5.9	5.6	** 69%	* 80%	93%	88%
25-Jun	6.5	6.4	** 8.1	* 7.9	* 99%	** 90%	118%	120%
15-Jul	7.7	* 4.4	8.4	8.1	107%	* 56%	118%	110%
29-Jul	7.6	** 5.2	* 9.0	7.9	105%	* 65%	116%	111%
12-Aug	7.7	** 4.8	* 10.2	8.1	99%	** 61%	* 124%	107%
26-Aug	4.5	4.4	** 9.2	* 7.9	56%	51%	* 110%	* 104%
10-Sep	4.0	4.5	** 9.9	* 6.8	48%	49%	** 111%	* 87%

* indicates the Newman-Keuls multiple comparison test has identified this value as being significantly different.

** indicates a second statistically significant group distinct from other groups.

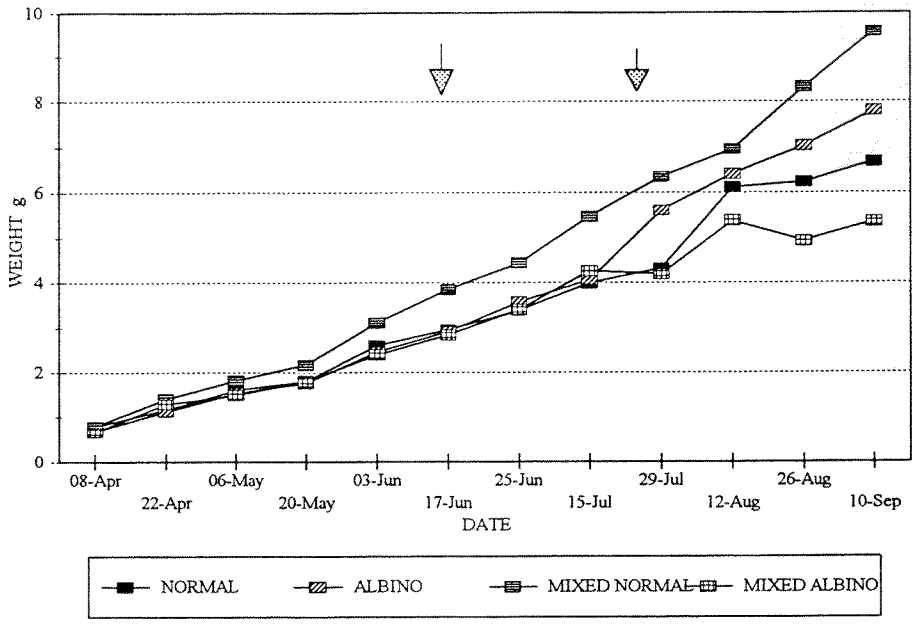

Figure 2. Mean sample weights (g)of Elochoman albino and normal summer steelhead April-Sept. 1996. First arrow indicates placement of crowder into aquaria. Second arrow indicates placement of additional shop light.

Condition factors (K-factor) are presented in Figure 3. ANOVA indicated significant differences did exist on the April 22, June 3, July 15, and Sept. 10, but the multiple comparison test was unable to identify which group or groups were significantly different. The Aug. 12 sampling showed both groups of albinos had significantly lower K-factors than normally pigmented groups. At the experiment's end ANOVA indicated a significant difference existed between groups (P=0.025) but Newman-Keuls was unable to distinguish which groups differed. Table 1 and Figure 2

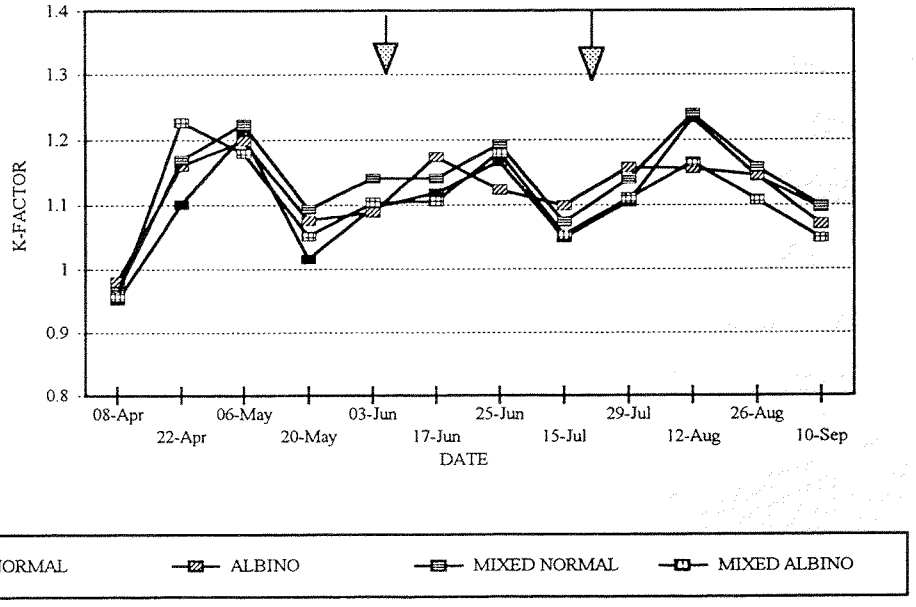

Figure 3. Mean condition factors (K-factor) of Elochoman albino and normal summer steelhead April-Sept. 1996. First arrow indicates placement of crowder into aquaria. Second arrow indicates placement of additional shop light.

indicate reduced K-factors for albino groups.

As of June 3, no differences in fin erosion were apparent in experiment fish (Figure 4), although substantial fin erosion was observed in steelhead transported to the Elochoman Hatchery from Vancouver Hatchery and also in steelhead held at Beaver Creek Hatchery. Dorsal fin erosion has been reported to be density related. On June 5, a crowder was placed in each aquaria to concentrate fish and increase density in an attempt to initiate fin nipping.

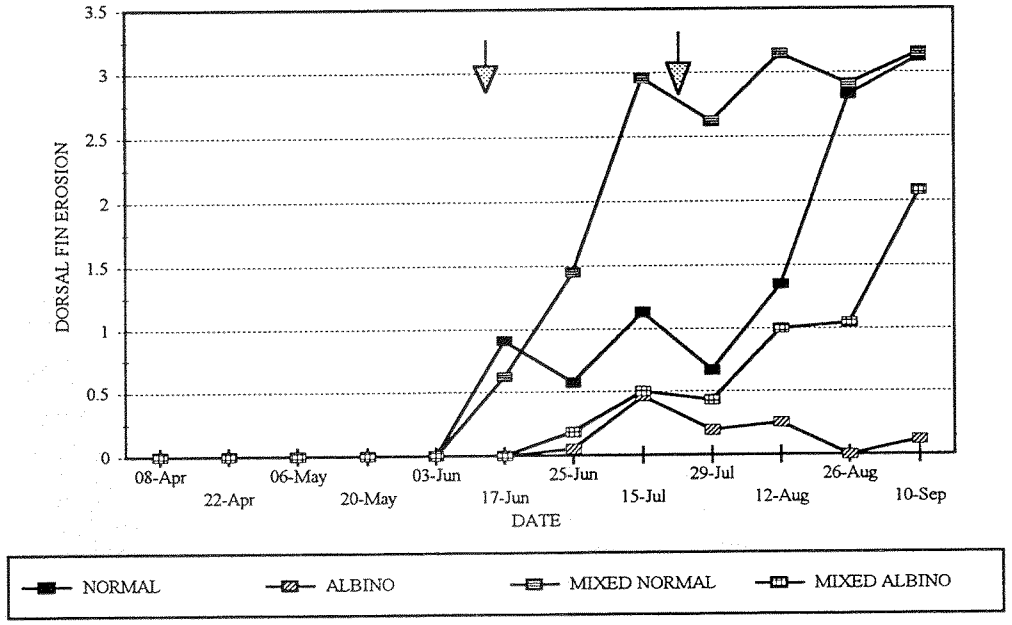

Figure 4. Mean dorsal fin erosion of Elochoman albino and normal summer steelhead April-Sept. 1996. First arrow indicates placement of crowder into aquaria. Second arrow indicates placement of additional shop light.

Prior to the installation of the crowder, mixed and albino fish had 10.48 cu^3 ft. of rearing space and were at 24 fish per cu^3 ft. Normal fish had 9.74 cu^3 ft. of space and were 26 fish per cu^3 ft. After the insertion of the crowder, mixed and albino fish had 5.15 cu^3 ft. of rearing space and were 48 fish per cu^3 ft. The normal tank was configured slightly different and space dropped to 5.27 cu^3 ft. and density became 47 fish per cu^3 ft. With the installation of the crowder, rearing area was approximately halved and density doubled.

At the next sampling, June 17, two weeks later, evidence of increased dorsal fin erosion was apparent. The normal and mixed normal groups showed immediate significant ($p<.001$) increases in erosion (Figure 4), which persisted throughout the experiment. By July 15, the albino group in the combined aquarium showed slight fin erosion but by this time dorsal fins of normally pigmented fish in that aquarium no longer existed. The all normal and mixed normal groups showed significantly higher ($p<.001$) levels of dorsal fin erosion than albino groups. Dorsal fin erosion of normally pigmented fish in the combined aquarium was far more severe than that of the normal group. The mixed albinos showed only slight dorsal fin erosion compared to the albino only group, which had virtually no dorsal fin erosion. Mixed normals, while having the greatest fin erosion were substantially larger (7 mm longer, 1.8 g heavier) than the next biggest group, the mixed albinos.

Prior to June 17, dorsal fins were quite similar among all groups based on visual observation. After this sample date, measurements of dorsal fin lengths (mm) were recorded. Since larger fish have a tendency to have longer dorsal fins, I chose to also monitor the ratio of dorsal fin length to overall fork length (as a percentage) to standardize erosion level and remove size bias. Dorsals of mixed normals were significantly shorter than normals and either albino group (Figure 5) and dorsal fin to fork length ratio was significantly less from June 25 through August 12 (Figure 6).

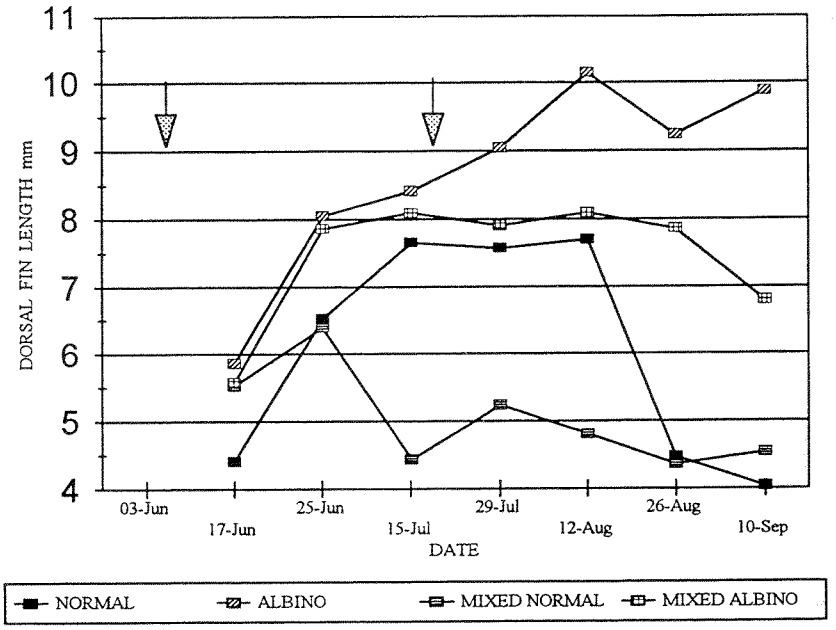

Figure 5. Mean dorsal fin lengths (mm) of Elochoman albino and normal summer steelhead April-Sept. 1966. First arrow indicates placement of crowder into aquaria. Second arrow indicates placement of additional shop light.

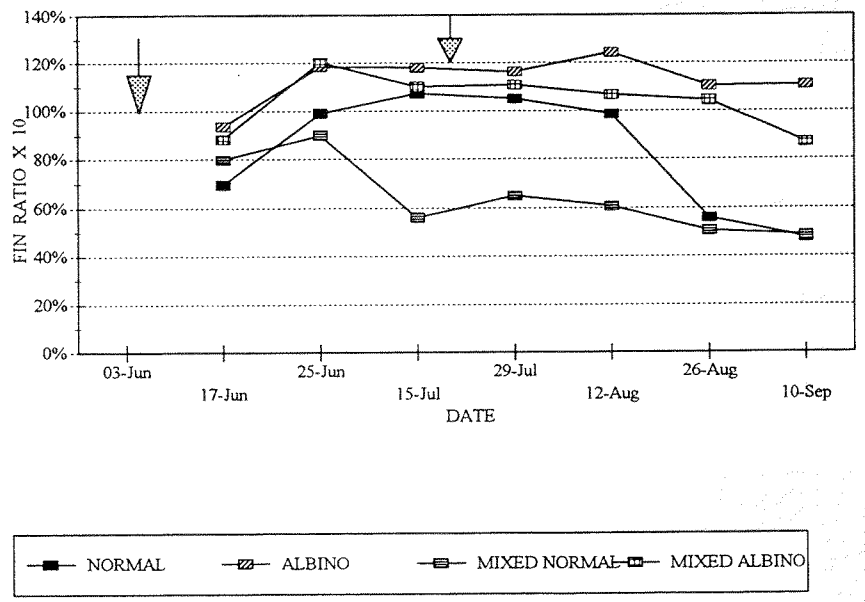

Figure 6. Mean ratio of dorsal fin length /fork length (mm)X 10 as a percentage in Elochoman albino and normal summer steelhead April-Sept. 1996. First arrow indicates placement of crowder into aquaria. Second arrow indicates placement of additional shop light.

By late-July, it seemed odd that the erosion levels of both normally pigmented groups were not more similar. The mixed normals had significantly worse dorsal fins than the normal group (Table 1, Figure 4). Light level was then considered. The all normal aquarium was in an area that was shaded some of the day. An additional shop light was

installed overhead on July 19 to balance light distribution. The additional light promoted major fin erosion in the normal only group. By Aug. 26, both normal groups showed equal, and significantly greater dorsal fin erosion, significantly decreased dorsal fin lengths and significantly lower dorsal fin ratios than albinos. This persisted until the experiment's termination. The additional shop light further increased illumination in the mixed aquarium, which may have exacerbated fin erosion in the mixed albino group as seen in Figure 4. By the experiment's end normally pigmented fish had significantly smaller dorsal fins and significantly lower dorsal fin to fork length ratios than albinos.

DISCUSSION

The purpose of this experiment was not to highlight albinos as subjects for fish culture. Rather, the albinos should be seen as controls, or indicators, illustrating the effects of density and light levels upon the rearing of normal steelhead.

Albino and normally pigmented summer steelhead were monitored for eight weeks without any evidence of dorsal fin erosion. It was not until a crowder was inserted into each aquarium on June 5, effectively doubling fish densities, that dorsal erosion began. The response was immediate. By the next sample date June 17, dorsal erosion was statistically significantly greater ($p<0.001$) in both groups with normally pigmented fish (Figure 4). Halving aquaria volume and doubling density, caused normally pigmented steelhead to fin nip each others dorsal fins. Kindschi et al. (1991) reported steelhead reared in isolation show no evidence of dorsal erosion, and the use of baffles provided some reduction in dorsal fin erosion. Bosakowski and Wagner (1944) reported crowding to have a detrimental effect on fin health, especially the dorsal fin in rainbow trout. Soderberg et al. (1993) indicated Atlantic salmon raised at higher densities had greater levels of fin erosion.

Once the crowder was in place and density doubled from 24 to 48 fish per cubic meter, dorsal fin erosion became evident in normally pigmented groups. However, the extent of erosion in the all normal aquaria was less than in the mixed normal group. Two explanations might explain this disparity. Although all aquaria held ≈250 fish (there were discrepancies due to mortality), in the mixed aquarium only 125 dorsal fins were visible. Albino dorsals lack pigment, making them imperceptible. If one were to assume that both albinos and normally pigmented steelhead fin nip, then the 125 normally pigmented fish would be subject to double fin nipping while albinos would not be nipped. In the all normal aquarium, 250 visible dorsal fins were available for nipping, spreading the targets and mollifying the damage on individual fins.

A second explaination for the difference in dorsal erosion between the all normals and mixed normals lies in background light levels. The all normal aquarium was located where it received the least reflected light. This was not intentional, but a condition of the layout of the aquarium room. Stringer and Hoar (1955) reported increases in fin nipping in Kamloops rainbow trout with increased lighting intensity. Kamloops trout and steelhead are the same species *(Oncorhynchus mykiss)*. In addition, they reported coloration to be an important component of fin nipping, with darker fish more prone to attack than lighter colored fish. Steelhead albinos are light colored. To balance light intensity a second shop light was installed on July 19. With the additional light, the erosion level in the all normal group climbed so that by Aug. 26 it was nearly identical to the mixed normal group (Figure 4). Both mean dorsal fin length and the dorsal fin to fork length ratio for this group plummeted (Figure 5 and 6). The gain in light intensity triggered the increase in fin nipping and may also have improved the all normal groups ability to forage. As feed pellets became more visible, a rapid weight gain became evident (Figure 2).

The additional light also enhanced visibility in the mixed aquarium, potentially making albino dorsals more visible and subject to nipping. At the latter stages of this experiment, when normally pigmented fish in this aquarium had little dorsal fin left to nip, some erosion was seen in the dorsals of albino trout. The additional light may have finally illuminated albino dorsals; or normally pigmented fish which were experienced in fin nipping, learned to fin nip albinos. Throughout the experiment, the all albino aquarium was subject to the greatest overall light level and dorsal fins of this group were virtually perfect. Erosion of the mixed albino dorsals could have been due to learned nipping behavior of the normally pigmented fish in enhanced light levels in this aquarium.

Dietary effects as a cause of dorsal fin erosion can be eliminated, since all experiment fish received the same ration, yet albinos retained their dorsal fins. Albinos do not have some superior ability to utilize nutritive components in

feed compared to their normal counterparts to benefit fin health. If this were so, both groups of albinos should have had similar levels of fin erosion at the same time. This was not the case. The mixed albinos suffered more fin erosion than the all albino group; indicating fin erosion was due to fin nipping, since feed and density were similar. Dorsal fin erosion is due to nipping, a behavioral rather than nutritional component.

Dorsal fins of albinos are difficult for rival fish to see in water. They provide a poor target for fin nipping. Unfortunately, the albino's characteristic pink eyes seem to provide a good target. Six eyes were plucked out of fish in the albino group and two from the mixed normal group.

In summary : 1.) Dorsal fin erosion is caused by fin nipping. 2.) It is sight dependent and did not begin until fish densities were increased, and was exacerbated with increased high light levels, particularly in the all normal group. Light is an important factor in fin nipping. 3.) If naturally appearing dorsal fins are desired in hatchery reared rainbow and steelhead trout, then reduced density and a subdued light regime is recommended.

ACKNOWLEDGMENTS

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SPRING CHINOOK SALMON - BKD - ERYTHROMYCIN

LONG TERM BENEFIT ???

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Abstract

Three broodyears ('92, '93, '94) of spring chinook salmon were reared under regular (variable year to year) production schemes at the U.S. Fish and Wildlife Service, Winthrop National Fish Hatchery located at Winthrop, Washington. For broodyears '92 and '93, different antibiotic (erythromycin) therapy protocols were administered to specific groups of fish. For broodyear '94 no antibiotics were administered and fish were reared under what was considered "low stress", "quality" environments. Prior to programmed release (April '94, '95, '96), fish from each specific test group were transferred to the National Biological Service, Marrowstone Field Station, (Puget Sound, Washington) for sea water survival evaluation over the next 14-16 weeks. When administered relatively early in the rearing period, erythromycin therapy controlled mortality and pathogen amplification due to *Renibacterium salmoninarum,* in freshwater. For broodyear '92 and '93 groups, which were progeny of parents with a low risk of vertical transmission of *Renibacterium salmoninarum* and had been reared for a significant period of time on untreated river water and at moderately high densities (DI 0.15 - 0.20), mortality increased and bacterial kidney disease (BKD) amplified during the sea water evaluation period, regardless of previous erythromycin therapy. However, when broodyear '94 fish were reared without erythromycin and under specific "low stress" , "quality" rearing criteria: 1. reared on well (infiltration gallery) water until a few months just prior to release, 2. reared at relatively low densities (DI 0.1), and 3. were progeny of parents with a low risk of vertical transmission of *Renibacterium salmoninarum*, mortality did not significantly increase, BKD did not amplify, and significant extended rearing benefit was observed.

Methods

Freshwater Rearing

Brood Year 1992 - 3 groups - 3 raceways per group - 60,000 fish / group (20,000 fish / raceway)

Group #1 - Medicated in October 1993 with erythromycin (14 day therapy).

Group #2 - Medicated in February 1994 with erythromycin (20 day therapy).

Group #3 - Controls - never fed medicated feed.

Prior to transfer to Marrowstone Field Station on April 6th, kidney samples from 60 salmon from each group were collected for ELISA-BKD (enzyme-linked immunosorbent assay for bacterial kidney disease) analysis.

Brood Year 1993 - 3 groups - 3 raceways per group - 75,000 fish / group (25,000 fish / raceway).

Group #1 - Medicated once (August 1994, 20 day therapy) with erythromycin.

Group #2 - Medicated twice (August 1994, 20 day therapy and February 1994, 19 day therapy) with erythromycin.

Group #3 - Controls, never fed medicated feed.

Prior to transfer on April 4th, kidney samples from 100 salmon from each group were collected for ELISA-BKD analysis.

Brood Year 1994 - single group - 2 raceways - 22,600 fish (11,300 fish / raceway)

No medication.

Prior to transfer on April 3rd, kidney samples from 60 salmon were collected for ELISA-BKD analysis.

Saltwater Rearing

Brood Year 1992

On April 6, 1994, 300 fish (100 / raceway) from each treatment group (900 total) were transported to the National Biological Service (NBS), Marrowstone Field Station. At Marrowstone, each group was divided equally into 4, 10 ft^3 (0.28 m^3) circular tanks (75 fish / tank). Acclimation to sea water began on April 11, 1994. Study was terminated after fish had been in sea water for 14 weeks.

Brood Year 1993

On April 4, 1995, 300 fish (100 / raceway) from each treatment group (900 total) were transported to the National Biological Service (NBS), Marrowstone Field Station. At Marrowstone, each group was divided equally into 4, 10 ft^3 (0.28 m^3) circular tanks (75 fish / tank). Acclimation to sea water began on April 10, 1995. Study was terminated after fish had been in sea water for 16 weeks.

Brood Year 1994

On April 3, 1995, 240 fish (120 / raceway) from regular production were transported to the National Biological Service (NBS), Marrowstone Field Station. At Marrowstone, fish were divided equally into 4, 10 ft^3 (0.28 m^3) circular tanks (60 fish / tank). Acclimation to sea water began on April 9, 1996. Study was terminated after fish had been in sea water for 16 weeks.

At termination, in all years, all surviving salmon were killed, weighed, measured, and a portion of the kidney was collected for ELISA-BKD analysis.

Therapy effect was evaluated by using mortality data and ELISA-BKD analysis results.

ELISA-BKD Analysis Interpretation - For the purposes of this evaluation, survival potential as it relates to the degree of infection with *Renibacterium salmoninarum,* was based on a risk analysis scale or "Rank" that forecasts the probability of disease occurring in a fish (spring chinook) infected with *Renibacterium salmoninarum.* The "Rank" value is derived from two values: 1. The optical density (O.D.) reading of the sample and 2. The "Multiple". The O.D. is a direct reading from the analysis and its value reflects the amount of *Renibacterium salmoninarum* antigen present. The higher the O.D. value, the larger the amount of antigen and , generally, the more severe the infection. The " Multiple" is the number of statistical units (based on a "t-test") a samples O.D. value is from a known negative value. Finally, the "Rank" is equal to the Log_2 of the "Multiple". Rank values fall between 0 and 12 where 0 is negative and 12 will be "Pushing up Daisy's" in the very near future. Table 1 presents a general relationship between these values.

Table 1.

Risk	O.D. (Approximate)	Multiple	Rank
Very Low	< 0.100	< 16	< 4
Low	0.100 - 0.250	< 64	< 6
Moderate	0.250 - 0.500	< 128	< 7
High	0.500 - 1.000	< 256	< 8
Very High	1.000 +	256 +	8 +

Results

All Brood Years - Survival Potential

Tables 2 shows survival data for the 3 broodyears. The first columns show the cumulative percent survival in freshwater, while fish were being reared at the Winthrop NFH. The second column shows the cumulative survival of freshwater rearing minus the cumulative mortality that occurred in salt water while the fish were at the Marrowstone Field Station. The third column represents the fresh and salt water cumulative survival minus a projection of long term mortality based on individual ELISA-BKD values of the survivors at study termination. At termination, a surviving fish with a "Rank" value of 6 or greater was considered to have a high probability of dying of BKD prior to adulthood and therefore was actually a swimming "MORT".

Table 2.

BY '92 Cumulative Survival Data

	Freshwater	Fresh + Salt Water	Fresh + Salt Water + ELISA
Control	69.6 %	19.3 %	3.7 %
October 1993	88.7 %	26.0 %	8.3 %
February 1994	68.6 %	38.5 %	13.7 %

BY '93 Cumulative Survival Data

	Freshwater	Fresh + Salt Water	Fresh + Salt Water + ELISA
Control	64.6 %	25.5 %	12.2 %
Erythromyicn 1x	98.2 %	21.7 %	10.2 %
Erythromycin 2x	96.9 %	46.5 %	25.3 %

BY '94 Cumulative Survival Data

	Freshwater	Fresh + Salt Water	Fresh + Salt Water + ELISA
Control	99.8 %	87.7 %	87.7 %

Discussion

As directed in several Laws, Treaties, and Agreements the production goal for the Winthrop NFH was set, many years ago, at 1,000,000 yearling spring chinook smolts.

Given the physical resources at this hatchery and the information gathered in the above evaluations, the way to best accomplish this would appear to be by following the two erythromycin treatment scenario of BY '93. In this case, starting with 1,032,000 fry, the goal could be met and the directives would be satisfied, "Mission Accomplished". The problem is that while 1,000,000 smolts would be released from the hatchery, only 262,000 would have the potential to survive to adulthood "solely because of BKD".

In an alternative case, starting with 340,000 fry, using the rearing scenario of BY '94 and utilizing physical resources at the hatchery in a different way, 298,000 smolts with the potential to survive to adulthood "solely because of the lack of BKD", would be produced . Unfortunately, the production goals set by the several Laws, Treaties, and Agreements would not be met "Mission Failure" even though an increase of 13.7 % in survival potential would be realized.

Conclusion

So much emphasis has been devoted to erythromycin as the answer for the bacterial kidney disease ills over the past 20 plus years that the development of specific and significant long term fish culture management strategies have been stifled. There are situations in which medicating with erythromycin, or other antibiotics, in conjunction with significant improvements in fish culture management, like applying reduced carrying capacity parameters (specific for each rearing station or situation) and reducing physical parameters which favor disease amplification (overcrowding, poor water quality, etc.), may effectively and significantly increase long term survival, but significant long term benefit that can be attributed exclusively to feeding erythromycin has yet to be clearly and consistently demonstrated.

Feeding erythromycin has some significant benefits; (1) it can reduce the amplification of *Renibacterium salmoninarum* in hatcheries and receiving waters, (2) it is important ethically and compassionately in that it reduces visible pain and suffering (for those who believe fish experience pain and suffering), (3) it is important in the politics of fish culture because dead and dying fish don't make for good press nor do they generate positive public support. I list this last benefit cautiously because it can promote symbolism over substance.

Feeding erythromycin to spring chinook salmon at hatcheries with historic problems with bacterial kidney disease and involved in natural resource supplementation programs reduces the visible mortality while the animals are under captive control - but achieving consistent and significant long term survival benefit remains questionable. Only after site or hatchery specific environmental obstacles are identified and corrected (rather than ignored) and stresses are reduced to the greatest degree possible, can antibiotic therapy potentially have a significant impact.

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WHIRLING DISEASE PREVENTION AND CONTROL:

A REVIEW

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Whirling disease is caused by the myxosporean protozoan *Myxobolus cerebralis*. The disease has been associated with significant declines in rainbow trout *Oncorhynchus mykiss* populations that have been closely monitored on the Madison River in Montana and in Colorado. In the Middle Park, Colorado reach of the Colorado River, Walker and Nehring (1995) observed high mortality of young-of-the-year rainbow trout, reducing age 1+, 2+, and 3+ cohorts to 0.7, 0.5, and 9.7% of the 1994 population, respectively. Subsequent live-cage studies showed that mortality varied among the species tested: brown trout *Salmo trutta*, 2%; Colorado River cutthroat trout *Oncorhynchus clarki pleuriticus* , 10%; Colorado River rainbow trout, 23%; Tasmanian strain rainbow trout 50%. The caged fish exhibited typical signs of the disease, including whirling behavior, skeletal deformities, and black tails. According to an account by H. Novick in the 18 August 1994 "The River Reporter", 95 to 97% of all wild rainbow trout from 1991 to 1993 had been lost in the upper Colorado River. In Montana, rainbow trout densities have dropped 90% in infected reaches of the Madison River (6 April 1995, Missoulian).

Clearly, whirling disease is a significant problem. Its control is difficult given the tenacity of the spore and its longevity in wet muds. The spores can tolerate freezing at -20°C for at least 3 months and the spores are still viable after passage through the guts of predators such as northern pike *Esox lucius*, black-crested night herons *Nycticorax nycticorax*, or mallard ducks *Anas platyrhynchos* (Taylor and Lott 1978; El-Matbouli and Hoffman 1991). There have been reports from Europe of spores remaining viable in dry pond beds for as many as twelve years (Bauer 1962). This article summarizes control methods attempted to date.

For the fish culturist, there are a variety of ways of dealing with the parasite. Since the alternate host for *M. cerebralis* is the oligochaete worm *Tubifex tubifex*, avoiding earthen ponds for culturing fish and keeping concrete systems free of organic waste and sediment are good common sense solutions (Markiw 1992a). Fish are less susceptible to the disease as they grow older, since the cartilage attacked by the trophozoite is largely converted to bone in older fish. Therefore, stocking dirt ponds with larger fish is an option for fish not destined to be stocked in the wild; Hoffman (1990) recommends using fish that are at least 6 cm long. Rasmussen (1965) reported success in Danish trout farms by rearing rainbow trout to 5 cm in concrete tanks before stocking into infected dirt ponds.

Disinfection of in-coming water is possible with ultraviolet radiation. Hoffman (1974) found that 2537 Angstrom units of UV light at dosages of 35,000, 43,000, and 112,000 microwatt sec/cm^2 were effective in controlling infection of rainbow trout fry. Filtration of water through a 25 µm commercial filter cartridge did not reduce or eliminate the disease (Hoffman 1974). However, Hoffman et al. (1962) noted that sand-charcoal filters had been used successfully in France.

Disinfection of hatcheries and ponds is feasible with chemicals (Table 1). Calcium cyanide was effective in disinfecting ponds, whereas quicklime was less effective (Bauer 1962). Calcium cyanamide (488 g/m^2) used for disinfection of dirt ponds and chlorine gas (300 ppm) for disinfection of incoming spring water were effective in preventing the recurrence of whirling disease the following year in a Pennsylvanian trout hatchery (Hoffman and Dunbar 1961). Tests with quicklime in simulated tests by Hoffman and Hoffman (1972) were effective in preventing infection of rainbow trout. Treatment of simulated earthen ponds with either 4550 g/m^2 hydrated lime (CaOH) or 1200 ppm chlorine on the wet mud did not destroy all the spores (Hoffman and O'Grodnick 1977).

However, when the spores were not protected by the mud, 10 ppm chlorine was sufficient to kill the spores (Hoffman and O'Grodnick 1977). Chlorine at 200 ppm gave variable results (Hoffman and Putz 1969). Chlorine at 400 ppm killed 36-90% of spores (Hoffman and Hoffman 1972).

Table 1. A list of chemicals causing distortion and probable death of *Myxobolus cerebralis* spores.

Chemical	Concentration	Citation
Calcium hydroxide	0.5 and 2.0%	Hoffman and Putz (1969)
Calcium oxide (quicklime)	0.25, 0.5, and 1.0% 380 g/m2 (3360 lb/acre)	Hoffman and Hoffman (1972)
Potassium hydroxide	0.25, 0.5, and 1.0%	Hoffman and Hoffman (1972) Uspenskaya (1957)
Sodium hydroxide	1.0%	Uspenskaya (1957)
Available chlorine as sodium hypochlorite	1,600 ppm	Hoffman and Putz (1969)
Alkyl dimethylbenzylammonium chloride (Roccal)	200 and 800 ppm	Hoffman and Putz (1969)
Calcium cyanide	4,000 kg/ha	Bauer (1962)

Prophylactic treatment with chlorine can be effective. Chlorine was effective in reducing infection by 73% in one group and 63% in another group of trout treated weekly with 0.5 ppm for 2 hr over a 4 mo period. Presumably, the triactinomyxon stage of whirling disease and tubeficids were killed by the chlorine, whereas the treatment was not toxic to the fish (Markiw 1992a). The use of chlorine may be hampered by the U.S. Food and Drug Administration (FDA) and state water quality regulations, as it is not an approved compound for discharging from hatcheries.

Table 2. A list of drugs tested for use against *Myxobolus cerebralis*.

Drug	Concentration	Results (% reduction of incidence)		Citation
		Lot 1	Lot 2	
Acetarsone (Stovarsol)	10-1000 mg/kg fish/d (3 d/wk for 6 mo)	suppression		Hoffman et al. 1962
Amprolium	13-18 mg/kg B.Wt	17	0	Taylor et al. 1973
	24-44 mg/kg B.Wt.	50	0	Taylor et al. 1973
Fumagillin DCH	1 g/kg feed fed at 1%B.W.	73		El-Matbouli and Hoffman 1991
Furazolidone	152-194 mg/kg B.Wt.	100	39	Taylor et al. 1973
Merck 930	8-15 mg/kg B.Wt.	0	---	Taylor et al. 1973
	33-64 mg/kg B.Wt.	0	0	Taylor et al. 1973
Nicarbazin	6-14 mg/kg B.Wt.	17	0	Taylor et al. 1973
	30-60 mg/kg B.Wt.	22	0	Taylor et al. 1973
Oxytetracycline	68-152 mg/kg B.Wt.	39	---	Taylor et al. 1973
Sulfamerazine	15-36 mg/kg B.Wt.	0	0	Taylor et al. 1973

Various drugs have also been tested, with limited success (Table 2). The antibiotic Fumagillin (dicyclohexylamine) fed to rainbow trout (medicated pellets contained 0.1% Fumagillin) reduced clinical infections of whirling disease; 73-100% of non-medicated fish had severe infections whereas only 10-20% of medicated fish harbored spores (El-Matbouli and Hoffman 1991). In drug efficacy tests with rainbow X cutthroat trout hybrids fed medicated feed, Taylor et al. (1973) found that furazolidone inhibited spore formation. However, the drug affected palatability and growth in this group was half that of controls. Also, some fish on furazolidone still had trophozooites and granulomas. Russian literature (Bauer 1962) suggested that osarsol added to feed was effective in controlling the disease. Acetarsone (Stovarsol) suppressed the disease, but did not eliminate it (Hoffman et al. 1962). Similarly, Markiw (1992a) noted that furoxone, benomyl, proguanil and clamoxyquin reduced losses and infection of young

salmonids, but none prevented or totally eliminated the disease. Even if these drugs were effective, registration of the drug through the FDA generally requires many years of testing and millions of dollars.

For those trying to manage whirling disease in natural waters, the options are fewer. For programs relying upon stocked fish, stocking larger fish (> 6 cm) should be evaluated. Fish should not be transferred from positive sites. The disease is not considered egg-transmissible, so expansion programs for sensitive species such as cutthroat trout may benefit from egg transfer if no other disease-free sources are available. Stocking of infected fish into infected areas is not recommended. This practice may exacerbate problems by increasing the dose of triactinomyxons. This hypothesis needs to be tested in the wild, but Markiw (1992b) demonstrated that rainbow trout exposed to low numbers (1 or 10) of triactinomyxon did not develop spores. Higher doses of triactinomyxon resulted in proportionately more spores being recovered from infected fish, presumably overwhelming the immune system. In Utah, removal of trout from the upper Fremont River drainage to break the life cycle of the parasite is being tried, but the experiment is still in progress.

For management of naturally reproducing populations in positive waters, selection of resistant species or strains is one of the few options currently available. Salmonids vary in susceptibility, with the following list ranking the most common in order of decreasing susceptibility: rainbow trout, sockeye salmon *Oncorhynchus nerka*, golden trout *O. aguabonita*, cutthroat trout *O. clarki*, brook trout *Salvelinus fontinalis*, steelhead *O. mykiss*, chinook salmon *O. tshawytscha*, Atlantic salmon *Salmo salar*, brown trout, coho salmon *O. kisutch*, lake trout *Salvelinus namaycush*, and splake (lake X brook trout hybrid) (O'Grodnick 1979; Markiw 1992a). Walker and Nehring (1995) reported that the kokanee in Colorado are more resistant to whirling disease than previous literature indicates.

Cutthroat trout are more resistant to the disease than rainbow trout. Walker and Nehring (1995) noted that Snake River finespotted cutthroat trout in a state hatchery that shared a similar lot history with similar-sized rainbow trout were negative ($\underline{n} = 20$) whereas rainbow trout had infection rates of 65-70%. In a single trial with greenback cutthroat trout *O. clarki stomias*, M. Markiw noted that rainbow trout yielded 15.6 times more spores than the cutthroat trout (Walker and Nehring 1995). With these susceptibility differences in mind, cutthroat trout may be better candidates for stocking or wild-fish management in infected waters, especially in the West.

The best management is to avoid infecting negative waters, containing the infection through enforcement of disease regulations, public education, and disinfection. Thorough drying of contaminated mud can kill spores (Hoffman and O'Grodnick 1977). Heat has been effective in causing the distortion and probable death of spores. Hoffman and Putz (1969) examined spores after heating in 0.85% saline to 60°, 80°, and 100°C. These temperatures were effective in killing spores whereas temperatures of 40°C or room temperature were not. Later tests by Hoffman and Markiw (1977) indicated that heating spores for 10 min at 90°C was effective in killing the spores as determined by methylene blue staining (killed spores take the stain, live spores do not). Heating at lower temperatures progressively reduced the percentage killed (80°C, 98%; 70°C, 60 %; 60°C, 34%; 50°C, 24%) in five trials. Heating for longer periods (up to 100 min) at 70°C increased the percentage of spores that were stainable, but still did not reach 100% (Hoffman and Markiw 1977). Smoking fish at 66°C for 40 min was effective in killing spores (Wolf and Markiw 1982)

Future research into control of the disease is needed. Immunological studies have indicated that rainbow trout produce antibodies against *M. cerebralis*, but protection against infection has not been demonstrated (Griffin and Davis 1978; Markiw 1992a). Enhancement of the immune response may be one avenue of research. Hybrids of resistant salmonid hybrids are being evaluated in Utah for use in infected reservoirs. Resistance of various strains of rainbow trout need to be determined. A greater understanding of the environmental determinants influencing the severity of the disease should lend greater insight in control measures that minimize mortality. Until future research provides additional approaches to controlling whirling disease, the data summarized above should be helpful in the control and eradication efforts.

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