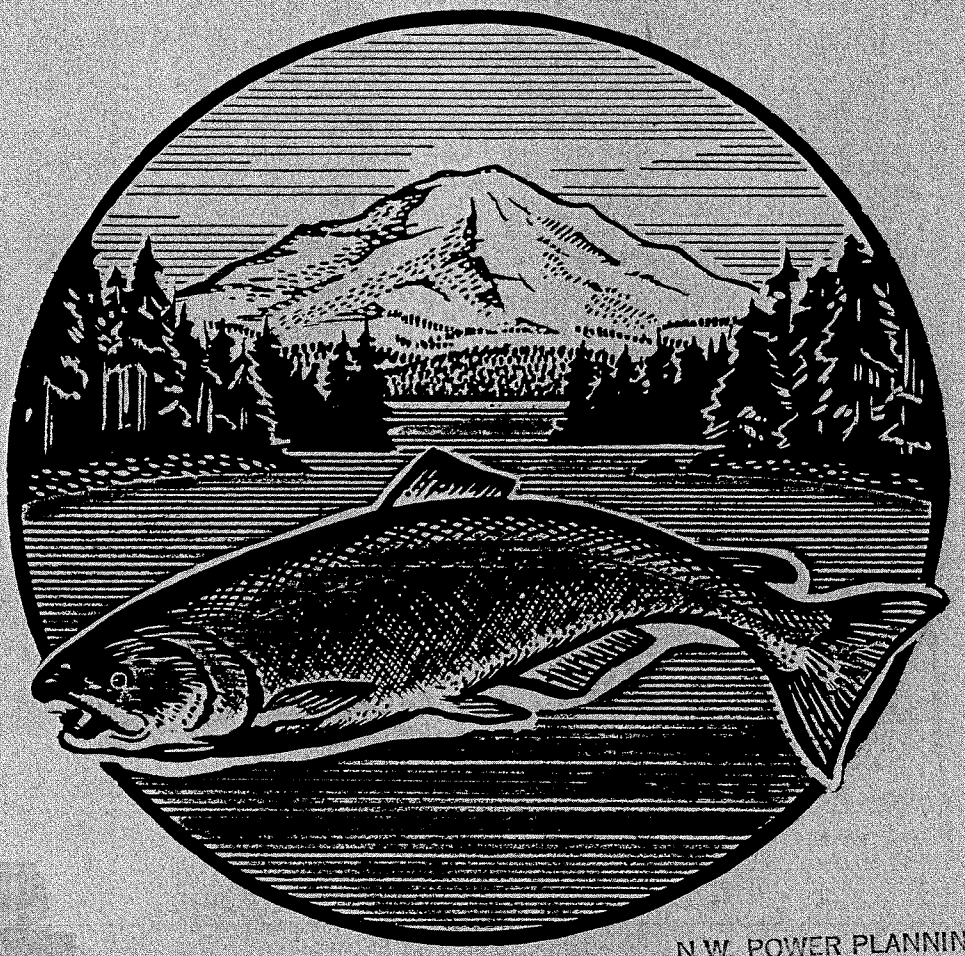
PROCEEDINGS OF THE

38TH ANNUAL NORTHWEST FISH CULTURE CONFERENCE



TACOMA, WASHINGTON

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PROCEEDINGS

of the

Thirty-Eighth Annual

NORTHWEST FISH CULTURE CONFERENCE

December 1-3, 1987

Fife, Washington

Chairman

Wilbur C. Ashcraft

Washington Department of Fisheries

THE NORTHWEST FISH CULTURE CONFERENCE

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

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

PREFACE

The 38th Annual Northwest Fish Culture Conference was highlighted by the registration of slightly more than 500 people representing fishery agencies, tribal entities, and the private aquaculture sector. Over thirty industry representatives supported a three-day exhibition of state-of-the-art fish culture equipment and technology.

Preplanning for the 1987 conference was the responsibility of hatchery personnel, salmon culture researchers and Quality Control staff. Hatchery field personnel also actively participated in the planning of the conference, and I am especially appreciative of their contribution.

Looking ahead, the traditional hosts of the annual fish culture conferences need to respond to a request of the California Department of Fish and Game to serve as host of a future meeting. Representatives attending the 1987 session made this request and we have since received a more formal request from California's Director, Mr. Pete Benedetti. This is "your" meeting and as such, your input in this matter should be provided to the Chairman of the 1988 conference.

We have received a lot of very positive feedback on the meeting and want to pass on a special thank you to panel participants for a job well done. Finally, I would also like to extend a very special thanks to all of the gracious suppliers of door prizes and to those hosting after-hour events which assure interest and great fellowship.

We had a great meeting and we are eagerly looking forward to the 1988 meeting in British Columbia.

Wilbur C. Ashcraft

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Fish Marking--Bob Vreeland, Panel Leader

A welcome by Washington Department of Fisheries Director Joseph R. Blum December 1, 1987.

Fish culture is one of the cornerstones of fish management, and fish culture in the Pacific Northwest is a gem. Without it, we would not be where we are today in this arena. With it, we have progressed much further than many people anticipated we ever could. Your job and my job are not done--in one sense they are just beginning. The information you exchange at this conference will move us into the future. We need the best talent, the best thinking, the best collective decision-making we can get, to accomplish our goals in the 1990s and the twenty-first century.

What has changed in fish culture, and what has changed in fisheries management in the North Pacific in the past decade, is described in a term that was coined by my predecessor, Bill Wilkerson, and by those with whom he worked, as "cooperative management." Cooperative management extends into fish culture, and is something you and I must work with. It is good for the resource. It is good for the citizens, the taxpayers who pay the bill for salmon culture and other kinds of fish culture, and it is the way to the future.

The 1960s, '70s, and the early '80s were times of little cooperation. Too much of our business was conducted before single individuals in long black robes, or nine individuals in long black robes, namely judges, who were making key decisions for us because we let them. It is up to us to continue in the way shown by Bill Wilkerson and his co-workers. I have continued in the past year, working cooperatively, to remove from those judges the prerogative of making the decisions that need to be made in our business.

No single entity, no single agency, no individual has a corner on the brain power, on the creativity that is needed to lead us into the future. Collectively, we have it. Collectively, we can develop, and we can grow--where we cannot grow alone. Who cares? A broad array of people who depend on us. I hear from many of them, and the managers and the agencies that you represent also hear a lot from them. They are the individual fishermen, they are the crew members on the fishing boats, they are the Sierra Club. They are my wife, your spouses, your children. They are future generations. How we do our job will determine how people rate the legacy we have left them.

I am challenging you with a very big task. The task goes beyond science. It goes beyond pond cleaning. It requires that we all continue to learn about being part of a cooperative society that is working for the good of all the people that I have identified. You have met the task in the scientific area. The challenge has been identified to you in the recent past, and I am re-identifying it for you for the future. Share your information. Seek out creativity, and get the job done for the resource and for the people who depend upon that resource.

Over the next two or three days you are going to exchange a great deal of information about fish culture. It is a key part of our business. You are extremely important people doing critical work. There are many fisheries that would not exist today, were it not for fish culture, but culture is not the only part of the equation. We need to keep that in mind as well. There is room for the natural component just as there is room for the culture component.

Fish in Washington, Lower British Columbia, and parts of Oregon have been going through a rather difficult time in the past six or seven months, with low water. Fish culture is helping the resource come through this difficult time. We don't know whether today is going to be the beginning of a December, January, and February that will get us back on a normal moisture path. We do know that through your efforts, through the jobs you have done over the past few years, we have in our facilities a resource that will help to provide for the 1989-'90-'91 time frame, when the natural runs may be reduced because of changes in the natural environment.

Again, I want to welcome you to this 38th Annual Conference. I was a mere nine years old when you held your first one, and some of you may have attended that first annual conference. You have a challenge before you that you have met during those 38 years. I believe that you are prepared to meet the challenge in the future. You also need to challenge me, and to challenge the other managers in the agencies--the decision-makers--to make sure we are doing our jobs. We are in tight financial times, and we may have to start cutting budgets. It is up to you to tell us where we can cut, if we must cut, and still accomplish our mission.

The last thing I am going to say on cooperative management is that with budget cuts it simply makes good sense that we cooperate. The federal government is facing budget cuts, the state is, and the tribes have for some time. Individually, we are lost. Collectively, we can still get the job done.

Thank you very much, and have a good conference.

CURRENT AND FUTURE PERSPECTIVES ON CHEMOTHERAPEUTANTS IN AQUACULTURE

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Registration of Chemicals and Drugs

The major laws involved in the clearance of chemicals and drugs for fisheries use are the Federal Food Drug and Cosmetic Act of 1906 (and its amendments) for drugs regulated by the U.S. Food and Drug Administration (FDA) and the Federal Insecticide, Fungicide and Rodenticide Act of 1910 (and its amendments) for pesticides and disinfectants regulated by the U.S. Environmental Protection Agency (EPA). The jurisdiction over the chemical depends upon the method of application, not the purpose of application. For example, Bisazir, a chemosterilant for sea lampreys was classified as a pesticide in 1980 by EPA. However, in 1986, EPA reversed that decision and claimed that FDA should regulate the compound because its use was as a drug.

Drugs registered for fisheries use must meet regulations developed by FDA. During research phases, a drug must be studied under an Investigational New Animal Drug (INAD) permit and a New Animal Drug Application (NADA) is filed when the registration data package is complete. Requirements for registration include data on human safety, efficacy against target organisms, toxicity to nontarget organisms, residues in food-producing animals, and effects on the environment. For drugs to be used on food-producing animals, data need to be generated that will establish tolerances and withdrawal times. All studies to generate these data must be accomplished according to Good Laboratory Practices (GLP) and the drug must be produced according to Good Manufacturing Practices (GMP) regulations. GLP and GMP requirements significantly raise the cost of developing a new drug. Cost has been the primary factor that has kept pharmaceutical and chemical companies out of the minor-use arena.

Since 1983, FDA has been operating under a policy of registering drugs for fishery use as minor uses. This policy permits extrapolation of data from a major animal species such as beef, pork or chickens to minor species, allows the use of inherent withdrawal times on a case-by-case basis, and provides different guidelines from major species for the preparation of data. Also, in 1983, the Interregional Research Project Number 4 (IR-4) of the U.S. Department of Agriculture (USDA) included drug registration for minor species as part of their mission. USDA has joined forces with FDA in a program to enhance the development of needed minor-use drugs. IR-4 and FDA have jointly sponsored four workshops since 1983. IR-4 has provided funding for studies on some aquaculture drugs (chloramine-T, Romet-30, erythromycin, and oxytetracycline for lobsters). FDA has also funded studies to generate basic

metabolism and pharmacokinetic data that may be useful in extrapolating information between animal species. Aquatic species studied include rainbow trout, channel catfish, and lobsters.

Recently, the Joint Subcommittee on Aquaculture of USDA established a special Task Force on Therapeutic Compounds to prepare an action plan to accelerate the registration of compounds needed in aquaculture. The plan will include an assessment of current efforts, the institutional and human resources available, a priority list of needed therapeutic compounds, and the steps that are required to obtain clearance for each compound, with budgets needed to get the job done. The plan will be presented to the U.S. Congress in spring of 1988.

Development of Priority Drugs

The Aquaculture Work Group within IR-4 established its priorities for the registration of needed aquaculture drugs at the third IR-4/FDA Workshop for Minor Uses of New Animal Drugs, September 25-26, 1985, Rockville, Maryland. Those priorities were based on the needs of all aquaculture groups represented at the Workshop and were reviewed at the 1987 meeting. Only streptomycin was added after the 1985 Workshop to reflect needs in bivalve culture. The top eight chemicals, in priority order, were: 1) a replacement for malachite green, 2) oxolinic acid, 3) chloramine-T, 4) erythromycin, 5) oxytetracycline for lobsters, 6) formalin for penaeid shrimp, 7) benzocaine and 8) streptomycin. Although only oxytetracycline achieved registration for use on lobsters since 1985, some progress has been made on nearly all of the compounds. The atmosphere at FDA and IR-4 is one of sincere cooperation. The 1987 Aquaculture Work Group saw no need to change its list of priorities or to identify additional needed compounds. The work needed on the seven priority compounds already far exceeds the available funding. A presentation on the status of each priority drug follows.

1. A replacement for malachite green

The number one priority in all aquaculture groups at IR-4 is a replacement for malachite green, primarily for its use as a fungicide. Because abnormalities have developed in a wide variety of animal species after exposure to high levels of malachite green and because malachite green could represent a potential hazard to humans, a replacement is needed (Meyer and Jorgenson 1983). FDA has indicated that it would be extremely difficult, if not impossible, to obtain a full registration for malachite green. A limited or restricted registration may be possible sometime in the future.

The National Fisheries Research Center-La Crosse (NFRC-L) has tested over 200 candidate compounds for fungicidal properties but only 20 were found effective *in vitro*. The most promising compounds have been tested *in vivo* on infected fish eggs, but only 8-quinolinol sulfate and Formalin-F were

effective against fungal infections. At 70 ppm for 15 minutes, 8-quinolinol sulfate controlled infections, but the fry died shortly after the eggs hatched. Formalin-F was effective at 250 ppm for 60 minutes. Only a 4% post-treatment infection rate was observed and 82% of the eggs hatched. Further delineation of promising compounds will continue. Candidate chemicals include dichlorophene, alpha-terthienyl, Alcide, Herbisan, and potassium permanganate. All will be tested alone and in combination with Formalin-F.

The Fish Farming Experimental Station (FFES) at Stuttgart, Arkansas, has identified a candidate that they feel shows promise as a protozoicide and possibly, as a fungicide. Over 250 chemicals were tested in a system that involved the use of cultured protozoans exposed to different concentrations of the test chemicals. One compound, dichlorophene, killed all protozoans in preliminary studies at minimum concentrations of 0.5 to 0.7 ppm. The minimum lethal concentration of malachite green, by comparison, was 1 to 3 ppm. Toxicity of the minimum effective concentration was assessed in static bioassays with golden shiners and fathead minnows. The minimum effective concentration determined by the protozoan assay was not toxic to either fathead minnows or to golden shiners in 24 hours. Malachite green caused 100% mortality of both fathead minnows and golden shiners at concentrations of 1 ppm or more.

The NFRC-L tested dichlorophene in in vitro tests using fungal challenges. The compound effectively inhibited fungal growth in the range of 10 to 100 ppm. The effectiveness of dichlorophene in controlling fungus on channel catfish eggs was tested by the FFES. Concentrations of 50 and 100 ppm were toxic and killed the eggs within 48 hours after four 15-minute exposures. Concentrations of 5 and 10 ppm of the compound caused no visible signs of toxicity either during or after hatching. These concentrations effectively arrested the spread of fungus that had appeared on the eggs before the first treatment and eliminated the fungus by the final treatment. Combined egg survival was 34% higher in the treated eggs than in the untreated controls.

2. Oxolinic acid

Oxolinic acid is a broad spectrum antibacterial registered for fishery uses in Europe and Japan. Various attempts were made without success to interest the sponsors (Parke-Davis and Pfizer) to pursue a registration in the United States. However, if the toxicology data on oxolinic acid on file with FDA for a human drug registration will be made available from Warner-Lambert, another firm has expressed interest in pursuing the registration. The National Fish Health Research Laboratory (NFHRL) in Kearneysville, West Virginia, has conducted some in vitro tests with oxolinic acid with very favorable results against many gram-negative bacterial pathogens.

3. Chloramine-T

Chloramine-T is a candidate compound for the control of bacterial gill disease in fish, especially salmonids. IR-4 has funded studies on efficacy,

toxicity, effects of environmental factors, and residue dynamics. Laboratory and field tests conducted to determine efficacy at the NFHRL yielded good results. In the field trials, less than 5% mortalities were observed among 46,000 treated salmonids; whereas, 30 to 40% mortalities occurred in the untreated controls (6,000 fish).

No mortalities or abnormal responses were observed in rainbow trout tested by NFRC-L at 1, 3, and 5 times the use pattern (12 ppm for 1 hour). In 96-hour LC50 tests, chloramine-T was more toxic at high water temperatures and slightly less toxic in hard than in soft water.

The NFRC-L also conducted studies to determine what effects environmental factors may have on the toxicity of chloramine-T. Toxicity increased as the loading rates increased from 0.52 grams to 2.07 grams of fish per liter of water in 24-hour exposures. Lower toxicity (loss of effectiveness) was observed in the presence of high levels of feed and fecal material. The greatest loss of activity occurred when fish and feed or fish and fecal material were present.

Protocols from NFRC-L were reviewed and accepted by FDA for studies to develop residue methodology and to establish residue dynamics for chloramine-T. An HPLC method developed by NFRC-L detects chloramine-T in water, but further effort will be needed before a method for tissue residues is available.

4. Erythromycin

Erythromycin is known to control bacterial kidney disease (BKD) in salmonids. Several INAD permits are in effect on the West Coast. Under INAD 4333 held by the Oregon Department of Agriculture, approximately 17,000 pounds of Gallimycin 50 were fed in 1986 to 30 million chinook salmon and 22 million coho salmon. The University of Idaho (INAD 2957) has found that erythromycin is highly effective in reducing BKD mortalities at 0.1 gram per kilogram of body weight per day for 21 days. Preliminary results of tagged returning salmon indicate a 3 to 1 survival advantage for the treated versus the controls. The University of Idaho has a contract with IR-4 to delineate the proper dose and to develop tissue depletion information.

5. Oxytetracycline for lobsters

FDA amended its animal drug regulations on June 30, 1987 to approve a supplemental NADA filed by Pfizer, Inc. to permit the use of oxytetracycline in lobster feed for the control of gaffkemia.

6. Formalin for penaeid shrimp

A revised amendment to the NADA on formalin to expand the use to penaeid shrimp for control of external parasites was reviewed by FDA in 1986, but no

response to FDA's comments has been issued to date. The University of Arizona is coordinating the response for Natchez Animal Supply Company, the sponsor of Formalin-F.

7. Benzocaine

On November 18, 1986, FDA assigned INAD number 4890 to the U.S. Fish and Wildlife Service (Service) for studies on the use of benzocaine as an anesthetic in fish. Benzocaine is a possible replacement for MS-222 for use in situations that cannot accommodate the required 21-day withdrawal time of MS-222. Laboratory tests at NFRC-L have shown that benzocaine is as effective as MS-222 at lower concentrations. FDA has expressed optimism that benzocaine can be registered. Protocols for residue studies have been reviewed and accepted by FDA and a residue method is under development. Field test sites are under consideration for the needed efficacy tests.

8. Streptomycin

No known work is underway on this compound.

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The 1985 Aquaculture Work Group also raised questions about iodophors and hormones and requested that FDA work out a system whereby it would be possible to register or use these compounds. FDA has ruled that iodophors are neither an animal drug nor a food additive and thus are not regulated under FDA jurisdiction (January 13, 1987). EPA has registered the iodophors for general sanitizing purposes and may want a specific registration if the compounds are sold with claims for fishery uses. Iodophors are sometimes used to prevent the horizontal transmission of fish pathogens on gear and equipment and in water used to handle eggs during spawn-taking operations. The iodophors used in these operations are polyvinylpyrolidone iodine compounds. Betadine and Wescodyne are two iodophors that have been used by fish hatcheries and tested for efficacy and toxicity.

FDA has not made a final decision on how they plan to regulate hormones in aquaculture; however, any potential users of hormones should contact FDA's Division of Therapeutic Drugs for Food Animals for instructions.

Status of Malachite Green

Malachite green has never been registered as a fishery drug; however, since 1981, the Service has held an INAD that permits data collection related to the use of malachite green to control fungal diseases on certain species of fish. The INAD specifically limits the use of malachite green to certain hatcheries and to species of fish or populations that are threatened or endangered.

On March 16, 1987, FDA ruled that the states of Washington, Oregon, and Idaho would be allowed to use malachite green to control fungal infections under the Service's INAD 2573 at selected state fish hatcheries where Pacific and Atlantic salmon are raised. The Service is required to monitor the use of malachite green in accordance with all provisions of the original INAD as defined in Memoranda of Agreements with the states. Requirements include proper labeling, safety precautions, no release of malachite green in hatchery effluents, filing reports on use, and documenting efforts to install charcoal absorption systems by 1990. Complete records are mandated.

Under the INAD on malachite green, NFRC-L is conducting studies to determine the tissue distribution and depuration of malachite green residues from eggs and tissues of fish.

Safety of Formalin

Recently, questions have been raised concerning worker safety and the environmental acceptability of effluents resulting from the use of formalin in fish culture. Some misinformation has also been released regarding the potential for carcinogenicity related to formalin uses in fisheries. Because of the problems associated with malachite green, more hatcheries are using formalin for control of fungal infections on eggs and some of them have had little experience in handling the compound.

A New Animal Drug regulation issued by FDA on April 3, 1986, approved the use of formalin solution (approximately 37% by weight of formaldehyde gas in water) for parasite control on fish and fungal control on fish eggs. Issues concerning effluents, carcinogenicity, and worker safety were addressed by FDA at that time.

The discharge of formalin-treated effluents into the environment poses no problem when the compound is used as directed on the label. FDA addressed the discharge issue when they approved formalin for use in fish culture and found that no significant impact would occur. The Service provided the environmental impact analysis used by FDA to arrive at its decision to allow formalin use in fisheries. The label and package insert state that formalin-treated water should be diluted 10 times after fish treatments and 75 times after egg treatments before it leaves a hatchery. In a report by EPA on the effects of formaldehyde in the environment, formalin decomposes in water in approximately 30 hours under aerobic conditions (Kitchens et al. 1976). Anaerobic decomposition takes approximately 48 hours. All evidence points to rapid biodegradation if biological systems in waterways are not overloaded. Formalin does not bioaccumulate or biomagnify. It is a naturally occurring, metabolic product.

The question of carcinogenicity also was addressed by FDA. The Service provided background information on human safety in the Freedom of Information Summary on formalin. That information was accepted by FDA as adequate to meet

concerns about the use of formalin in fish culture. In 1980, FDA ruled that it would take no regulatory action against formalin because the major exposure to FDA-regulated products is from ingestion at low levels, not from inhalation, the route that induced tumors in the nasal passages of rats. In 1981, EPA stated that their concerns on formaldehyde related to carcinogenicity are limited to exposures other than by water routes. In essence, they said fishery uses did not constitute an exposure concern. Formalin (formaldehyde solution) was listed in Category III, a chemical group that did not warrant investigation unless new, adverse information came to EPA's attention. In a recent article by Takahashi et al. (1986), rats exposed daily to 0.5% formalin (5,000 ppm) in their diet for 8 weeks developed gastric tumors. At the normal treatment levels in fish culture (250 ppm formalin in the water for 1 hour), people would be exposed to less than 0.002 ppm in the air for only 1 hour and none orally from eating the fish. In a study done by the Service, no residues of formaldehyde above background levels were detected in fish exposed to formalin at treatment levels. In fact, formaldehyde occurs naturally in fish at levels of 3 to 12.8 ppm.

The Formaldehyde Institute, Inc. (1987) also provided justification why formaldehyde does not warrant a cancer warning on use labels. Their conclusions are based on tests sponsored by the Chemical Industry Institute of Toxicology (CIIT). In the CIIT studies, rats continuously exposed to formaldehyde gas for 2 years showed a 44% incidence of nasal cancer at 14.3 ppm, 0.9% at 5.6 ppm, and zero incidence at 2.0 ppm. In mice, the CIIT found a 0.9% incidence at 14.3 ppm and zero incidence at 5.6 and 2.1 ppm. Other studies in hamsters, mice, and monkeys showed no adverse effects from low level formaldehyde exposure.

Another aspect of the CIIT study was that the rats were exposed to highly reactive formaldehyde gas, not to formalin (formaldehyde in a water solution) for 6 hours a day, 5 days a week for up to 2 years. Because of the extremely irritating properties of formaldehyde gas, humans are unlikely to be exposed to high levels of formaldehyde for long periods. The threshold level for irritation in humans is 0.1 ppm formaldehyde and above 3 ppm it becomes highly irritating to humans. No one would be able to work in an atmosphere of 3 ppm.

The U.S. Occupational Safety and Health Administration (OSHA) issued a revised occupational safety and health standard for occupational exposures to formaldehyde on December 4, 1987 (U.S. Occupational Safety and Health Administration 1987). This standard reduces the permissible exposure level (PEL) in the workplace from 3 parts formaldehyde per million parts in the air to 1 ppm for an 8-h time-weighted average (TWA). The short-term exposure limit (STEL) for 15 min has been reduced from 5 to 2 ppm. An action level of 0.5 ppm, measured as an 8-h TWA, has been added to minimize compliance requirements for employers whose workers are subjected to very low exposures of formaldehyde gas.

The Service has calculated the maximum amount of formaldehyde (worst case) to which a fishery worker could be exposed in normal hatchery treatments using formalin. The calculation is based on the laws of partial pressures, the assumption that the partial pressures are linear, and the assumption of complete equilibrium with no ventilation. To determine the maximum amount of formaldehyde gas that could possibly be available to fishery workers, an equation and a table with data on partial pressures and air saturation from aqueous formaldehyde was used (Walker 1975). Based on these calculations, the maximum possible amount of formaldehyde that could theoretically be released into the air from a hatchery treatment would be a TWA of 0.117 ppm (μL/L), well below the PEL or action levels set by OSHA. The Service has implemented a monitoring program to verify the exposure levels for hatchery workers. The STEL for 15 min is 2 ppm formaldehyde. The only value that could be of concern is a 2,000 ppm formalin treatment at 20°C for 15 min (3.75 μL/L formaldehyde). The calculated figure is based on total saturation of the air (complete equilibrium) and no ventilation, neither of which will occur in a hatchery situation. To achieve complete equilibrium, the air would have to be saturated with formaldehyde, a situation that is very unlikely. Thus, hatchery workers would not be exposed to hazardous levels from most hatchery treatments.

Hatchery workers are required, however, to wear face shields, impervious gloves and aprons when opening drums and setting up transferring and dispensing systems. A half-mask respirator with a vapor cartridge for formaldehyde should be at hand for use in the event of an accidental spill. If small amounts of concentrated formaldehyde solution (formalin) are needed, the compound should be drawn from a supply drum in a well-ventilated area and containers should be filled and capped by workers wearing protective clothing (U.S. Fish and Wildlife Service 1987). The exposure from handling the drums or small containers would be minimal because the period of handling would last for only a few minutes and the protective clothing would protect skin and face from accidental exposure. If formalin is accidentally spilled, it can be cleaned up and deactivated with household ammonia or sodium bisulfite.

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"An Update on the Malachite Green Removal Study"

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"Malachite green is an effective fungicide used for the treatment of fish and fish eggs. It has been used for over 40 years by fish culturists at federal, state, and private hatcheries. Recently, this compound was identified as a potential teratogen and the U.S. Fish and Wildlife Service (Service) has restricted its use on national fish hatcheries. However, because there is no effective fungicide to replace malachite green, the Service was granted an Investigational New Animal Drug permit (INAD) #2573 to allow the use of malachite green only at specified fish hatcheries that produce fish for restoration of depleted stocks of Atlantic salmon. The restricted use permit requires close monitoring of the malachite green used at fish culture facilities. Recently, state hatcheries in Idaho, Oregon, and Washington were allowed to use malachite green under the conditions of the Service's INAD.

Specifically, the permit requires keeping of accurate records of the success or failure of treatments, species and numbers treated, inventories and quantities used, assurance that workers will be protected, and assurance that measures will be taken to prevent releases of malachite green in the hatchery effluents. An annual report is required.

The continued use of malachite green for treating adults and eggs of important or endangered fish species is contingent on the development of systems to remove the therapeutant from hatchery effluents. Oxidizing and reducing agents are no longer acceptable for the treatment of malachite green-treated water because the basic structure of the molecule remains intact. The permit from the U.S. Food and Drug Administration requires that all hatcheries using malachite green under the INAD for the treatment of eggs or adults must be in full compliance with conditions for malachite green removal by March 1, 1989.

The development of removal systems will be accomplished in two phases. The first involves (1) hatchery site visits to identify and review facility sizes, layouts, and water flow; (2) identification of

specific needs for malachite green and potential approaches to minimize usage; (3) a search of the literature to identify practical procedures for removing malachite green from treated water; (4) identification of existing sources of filtration systems, feasibility of removal, and methods for disposal of spent carbon; (5) evaluation of needs for continued use of malachite green; and (6) recommendations for the development of a commercial or experimental prototype unit.

The second phase of the project will focus on prototype testing and development (Leifing 1987)."

The U.S. Fish and Wildlife Service has entered into a contract with the Department of Energy, Bonneville Power Administration to test activated carbon filtration for the removal of malachite green. The scope of work has four main objectives: (1) develop specifications for granulated activated carbon and operational criteria for filters by conducting a microcolumn simulation study. This study will be conducted at the National Fishery Research Center in La Crosse, Wisconsin; (2) based upon the results of the microcolumn simulation study, construct and test an activated carbon filter for removing all malachite green from fish incubator effluent. The flow range will be from 50 to 150 gpm. This phase of the study will be conducted at the Abernathy Salmon Culture Technology Center in Longview, Washington; (3) assemble and test an activated carbon filter for removing malachite green from water containing adult spring chinook salmon. The flow range will be from 500 to 1000 gpm. This phase of the study will be conducted at the Carson National Fish Hatchery in Washington; (4) furnish the Bonneville Power Administration with a final report. The report will include operational manuals for each activated carbon filtration system, engineering specifications and drawings, power requirements, costs and sources of equipment.

The schedule for completing the contract is:

Objective		Approximate Dates Start	End
1	Microcolumn simulation study	Aug. 1, 1987	Oct. 1, 1987*
2	Incubation study	Oct. 1, 1987	Mar. 1, 1987
3	Brood stock study	Jun. 1, 1987	Oct. 30, 1987
4	Final report	Nov. 1, 1987	Dec. 30, 1988

*Telecon with Leif Marking at La Crosse on November 3, 1987 indicates that object 1 is approximately 3 months behind schedule.

For more information on the study, principal persons on the study are:

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Designing Effective Immunization Programs for Salmonids

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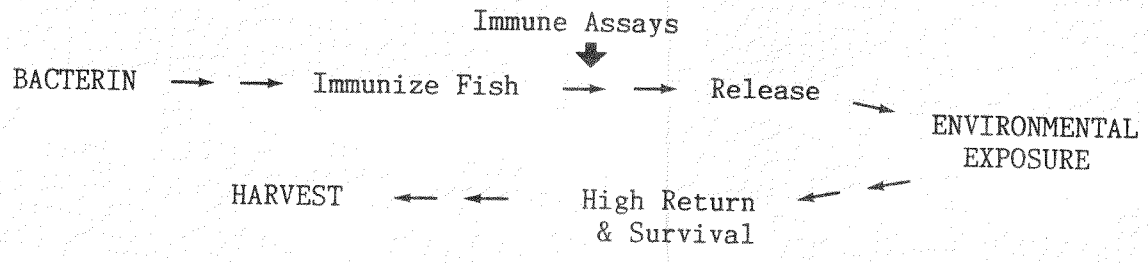
Salmonids have active immune systems that monitor the environment and are armed for encountering and destroying pathogens. By present immunization programs, fish can be protected against a specific disease when we are in control of the potency of the immunogen and the timing of exposure to the targeted pathogen. Upon release from the hatchery, however, we loose control of knowing what disease-causing agents the fish are exposed to and have little information concerning the long term progress of the fishes' immune response. The following diagram shows the steps of an immunization program and lists questions that should be approached:



IMMUNOGEN:

How was it prepared? If a bacterin, was it formalin-killed, for instance, or was the preparation further refined to enhance antigens which might be most effective in making a potent bacterin?

Should the immunogen be commercially purchased or produced inhouse?

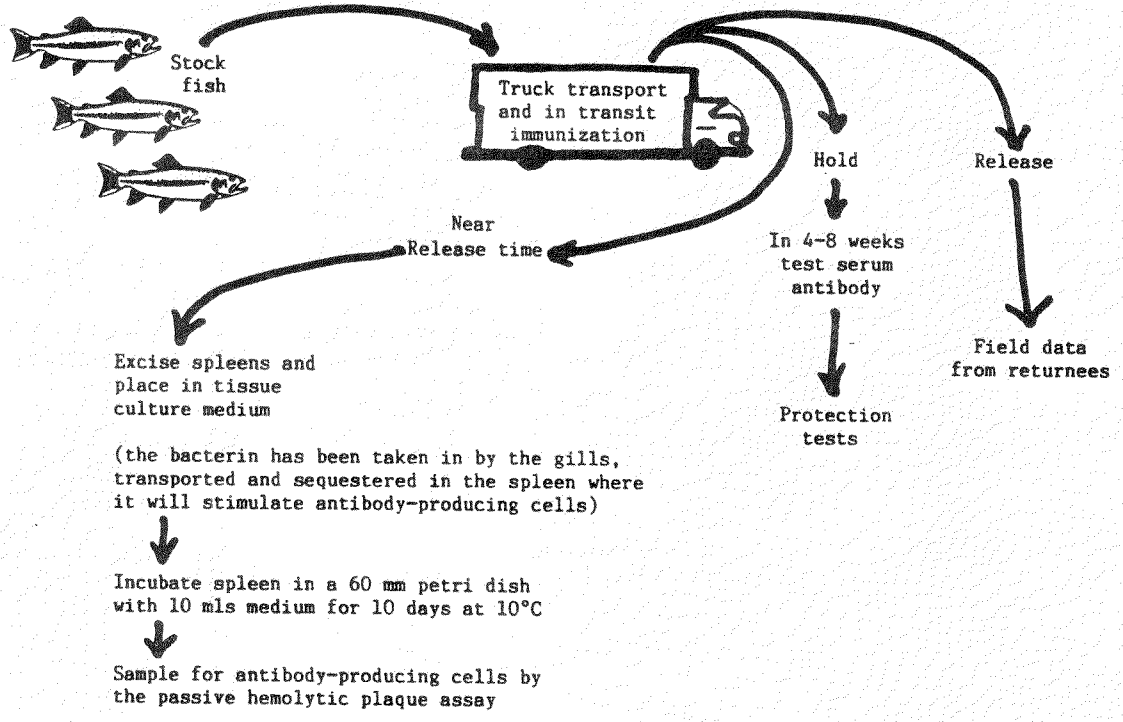
FISH EXPOSURE:

Should the immunogen be given by bath, spray, flush exposure, orally, or injected? What are the water temperatures and other environmental factors that might hinder the immune response?

IMMUNE ASSAYS:

Can the fish be sampled immediately after immunization to assess if the immungoen is effective? What serological tests might be used? Should (and can) protection tests be done, or is it necessary to statistically analyze return of immunized fish differentiated by markers?

To fill in these information gaps and ensure effective immunization regimens, we are designing immunological assays that tell us if the animals' immune systems are activated. Spleen organ culture makes possible sampling for bacterin efficacy near the time of immunization and release. Also, in vitro assays with multiple spleen sections from the same fish give better statistical data about potency comparisons of different bacterin batches. In other experiments, we are attempting to further serologically and genetically define pathogens the fish will encounter in the rivers and bays in order to design our immunogens accordingly. The goal is to promote the survival of our fish by activating their own defensive mechanisms.



IN VITRO ASSAYS

Immunological/Serological Assays Used with Fish Disease Antigens

Immune System Physiological Step	Immune Event	Immune Assay and Variations
Afferent immune system		
Macrophage functions		
Phagocytosis	Engulfed particles	Counting particles in cells, capillary tube migration
Pinocytosis	Engulfed solutes	Counting vacuoles
Killing	Death of microorganisms	Chemiluminescence, plate counts
Monocyte transport	Movement of antigens	Microscopic examination
Melano-macrophage centers	Aggregations of melano-macrophages in kidney and spleen	Histological examination
Efferent immune system		
T-lymphocytes		
Blastogenesis	Cell growth and proliferation	Direct counts, radioimmunoassay
Cell compatibility	Transplant rejection	Scale transplants
Cytotoxicity	Destruction of target cell	Counting target cells
B-lymphocytes		
Blastogenesis	Cell growth and proliferation	Direct counts, radioimmunoassay
Antibody-producing cells	Production of antibody	Passive hemolytic plaque assay (Jerne assay), Cunningham technique, rosette test
Humoral antibody	Release of antibody into serum	Agglutination, passive hemagglutination, passive agglutination, co-agglutination
		Precipitation, double immunodiffusion, radial, counterimmunoelectrophoresis rocket electrophoresis
		Fluorescent antibody technique, direct/indirect, different fluorescent dyes
		Enzyme-linked immunosorbent assay (ELISA), immunoblot, Western blot, different enzyme labels, fogging (plastic adherence)
		Radioimmunoassay
		Complement fixation
		Neutralization of microorganisms
Survival	Protection against disease	Disease challenge, exposure to specific microorganism, or to nonspecific, uncontrolled, environmental exposure

12/1/87

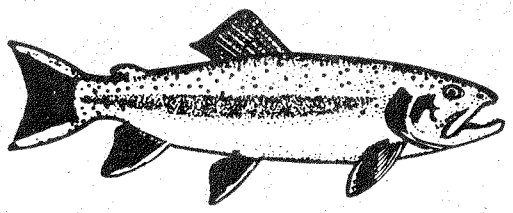
DESIGNING EFFECTIVE IMMUNIZATION PROGRAMS FOR SALMONIDS

Doug Anderson, U.S. Fish & Wildlife Service
National Fish Health Research Laboratory
Kearneysville, WV 25430 USA

QUESTIONS CONCERNING ENVIRONMENTALLY-INDUCED DISEASE OUTBREAKS; AN IMMUNOLOGIST'S PERSPECTIVE

1) Are these fish infected?

2) Can pathogen be detected?



STRESSOR

DEATH

3) How can they avoid stress?

4) If they can't avoid it, how can we help them survive?

SURVIVAL

Four important questions that concern fish immunologists when analyzing problems of adult fish returning to spawning areas and encountering potentially harmful stressors. The answer to question #1 is almost always "yes" as salmon are carriers of such pathogens as Renibacterium salmoninarum, the causative agent of bacterial kidney disease, Aeromonas salmonicida (furunculosis), and IPNV (Infectious Pancreatic Necrosis Virus). Improved biochemical and serological assays help confirm infections (question #2), but it often difficult to detect few numbers of pathogens, especially in large fish populations. For question #3, we realize that natural-occurring stressors such an unseasonable flooding or high water temperatures are difficult to avoid; however, man-made stressors such as pollutant dumping or fortuitous fish-ladders may be altered. Survival of salmon in artificial holding ponds until eggs are ripe can be improved by injecting antibiotics and is an example of how man can improve spawning yields - for question #4. In the future, protection of fish against diseases may be helped by effective immunization programs or the fish may be given drugs that will stimulate the immune response and/or nonspecific protective factors to assist them through stressful periods.

Elimination of Chronic Furunculosis by Disinfection of Eggs with an Iodophor: 2 Case Histories

Christopher Horsch, U.S. Fish & Wildlife Service

McFadden (1969) and Ross and Smith (1972) found that various iodophor products were effective disinfectants against several superficial egg-born fish pathogens. Among these pathogens were Aeromonas salmonicida, causative agent of Furunculosis, and Yersinia ruckeri, causative agent of Enteric Red Mouth. As a result of these works, the U.S. Fish & Wildlife Service, in 1984, incorporated into it's Salmonid Fish Health Protection Program, the use of iodophor egg disinfection to remove superficial egg-born bacteria such as A. salmonicida, to allow transfer of eggs from enzootic areas for these pathogens to other areas without fear of pathogen transfer.

The following are two case histories of the successful elimination of chronic Furunculosis by disinfecting newly fertilized eggs with an iodophor.

Steelhead Trout (Salmo gairdneri Richardson)

Prior to 1985, Furunculosis had been a chronic problem in the early rearing of steelhead trout fingerlings at the Coleman National Fish Hatchery, Anderson, California. Epizootics would begin in early May about one month after first feeding while the fish were still in hatchery nursery tanks. Mortalities would last for approximately two weeks until antibiotics, administered through the feed, would suppress the infection. Average mortality for this period was close to 5%.

Once the fish were ponded in outside raceways in June, outbreaks of Furunculosis would occur in late late July or early August when water temperatures reached 60 F. Mortalities of close to 5% were again encountered until suppressed by antibiotics.

During the 1985 spawning season (December 1984 to February 1895) scrapings from sediments in incubator trays in which the steelhead eggs were held, yielded abundant, viable cells of A. salmonicida. Close examination of steelhead adult mortalities and spawned out fish revealed a high prevalence of Furunculosis-induced enteritis. Many without the enteritis were found to be carrying low numbers of bacteria in kidney and/or lower intestine. As a result of these disciveries, all incubator trays were disinfected with 250 ppm sodium hypochlorite and all newly fertilized eggs were disinfected by

water-hardening in 75ppm iodine (from Argentyne@) for 30 minutes.

This process has been repeated for the past 3 years and there has been no evidence of Furunculosis nor low level carriers in the juvenlie steelhead trout being reared at the hatchery. The adult steelhead returning this season (December 1987- present) are the 1985 brood which were disinfected with iodophor as eggs. To date, there have been no isolations of Furunculosis from these adults.

Cutthroat Trout (Salmo clarki henshawi)

In 1982, the Pyramid Lake Indian Paiute Tribe desired to shift the program at the Lahontan National Fish Hatchery from domesticated strains of Lahontan Cutthroat trout to the "wild", newly restored Pyramid Lake strain. There was much concern over the possible transfer to the Lahontan hatchery of Furunculosis on the eggs of fish spawned at Pyramid Lake (enzootic for Furunculosis).

From 1982 to 1985, small egg lots were spawned & fertilized and then water hardened for 60 minutes in 100ppm iodine (from Wescodyne or Argentyne@). After disinfection, the iodophor solution was drained and the eggs were packed on ice in styrofoam crates and transfered to the Lahontan hatchery. Upon receipt at the hatchery, the eggs were rinsed briefly in pathogen free well water and then placed into incubator trays in an isolation building. Eggs and resultant fish were maintained in the isolation building until stocking once the fish reached 6 inches in length.

These fish were exposed to harsh handling procedure and poor water quality in an attempt to surface a latent infection into an epizootic. After one year of biweekly examinations of kidney/intestine by culture and FA (fluorescent antibody), no infections, clinical or carrier state, could be detected.

Therefore, in 1986, the program at the hatchery was changed from the domesticated strains to the Pryamid Lake strain. All eggs transfered to the Lahontan hatchery from Pyramid Lake are water hardened for 60 minutes just after fertilization in 100ppm iodine (from Argentyne@). No rinsing occurs until eggs are received at the hatchery.

Again, kidney/intestine samples were screened from mid-1986 until md-1987 and no evidence of infection could be detected. To date, no incidence of Furunculosis has been observed as a result of egg transfers to the hatchery from known positive adult populations at Pyramid Lake.

As an epilogue to these two success case histories, it should be duely noted that there is no evidence of Furunculosis-laden carrier fish in the water supplies of either of these two hatcheries. The success of any production program using egg disinfection for removal of egg-born pathogens requires that those pathogens be absent from the water supply.

References

McFadden, T.W. 1969. Effective disinfection of trout eggs to prevent egg transmission of Aeromonas liquifaciens. J. Fish. Res. Bd. Canada 26: 2311-2318.

Ross, A.J. and C.A. Smith 1972. Effect of two iodophors on bacterial and fungal fish pathogens. J. Fish. Res. Bd. Canada 29:1359-1361.

Observations on Water-Hardening Salmonid Eggs in Iodophor

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The practice of water-hardening salmonid fish eggs in iodophor disinfectants began in the early 1980's primarily as a response to the spread of Infectious Hematopoietic Necrosis Virus (IHNV) in the Pacific Northwest. The procedure was seen as a means to reduce the levels of virus in the egg environment as early as possible after fertilization thus preventing outbreaks of IHN in the emerging juveniles. It is impossible to assess the efficacy of the procedure for preventing IHN because it has not been possible to experimentally induce outbreaks of the disease in progeny derived from infected parents thereby allowing comparison of treated versus nontreated eggs. Early removal of fish pathogens and proliferating microorganisms in the egg environment is a sound disease control measure, however, and water-hardening eggs in iodophor appears to be beneficial. It has become a standard fish culture practice amongst several fisheries management agencies and hatcheries where Pacific Salmon are propagated.

Several variations for water-hardening eggs in iodophor have evolved depending upon the facilities and incubation system used. Ideally, ovarian fluid is removed by spawning the eggs into a collander over a bucket. The eggs from several females may then be pooled into a disinfected container and milt and pathogen-free water added to achieve fertilization (fertilization should not be attempted using water containing iodophor as high egg loss has occurred in several cases where this was done). Rinsing of eggs with a stock solution of iodophor (75-100 ppm) may then be done and the eggs transferred to the incubation container (tray, basket, etc.). The type of incubation system avaiable dictates how the water-hardening in 75-100 ppm iodophor is attained. Where tray incubation in stacks is used it is most convenient to pour the eggs into the tray containing the iodophor solution beginning at the top tray. The tray is pushed into its shelf just short of the incoming water for 15 minutes after which time the tray is pushed completely into the shelf. Normal incubator water flow will then gradually dilute out the iodophore. Where other types of incubation systems are used it has most often been necessary to hold the eggs in the iodophor for one hour to prevent trauma to the eggs during water-hardening. No adverse effects have been apparent from holding the eggs in iodophor for as long as one hour.

Variations of this procedure have successfully been conducted on eggs from cutthroat and rainbow (including steelhead) trout, fall, summer and spring chinook salmon, and sockeye (including kokanee) salmon without excessive egg loss or deformities. Results with coho salmon eggs are equivocal and further tests need to be made. Fish culturists are encouraged to test the technique on small lots of eggs the first year it is used to insure it does not result in significant egg loss. The benefits from water-hardening eggs in iodophor have been reported to be a more robust fish and in some cases reduced furunculosis.

GARRISON SPRINGS DOWN-WELL ISOLATION BUCKET INCUBATORS

Darrell Mills

INTRODUCTION

Down-well isolation bucket incubators were adapted for use at Garrison Springs Hatchery in response to an Infectious Hematopoetic Necrosis virus (IHN) outbreak in 1985 brood steelhead at Washington Department of Wildlife's South Tacoma and Chamber's Creek hatcheries. Garrison Springs is located on a tributary of Chamber's Creek and our adult trapping facility is located on lower Chamber's Creek.

We began isolation bucket (I.B.) incubation with the 1987 brood fall chinook in an effort to eliminate or greatly reduce the horizontal transmission of IHN from adult salmon held and spawned in Chamber's Creek. Our I.B. incubation system is very closely fashioned after the Washington Department of Wildlife system now in use at many of its hatcheries. The bucket design is very similar to those discussed at previous Northwest Fish Culture Conferences (A. J. Novotny etal, NMFS, 35th Annual Fish Culture Conference; M. Baxter, Cowlitz Salmon Hatchery, 37th Annual Fish culture Conference.) The purpose of this report is to demonstrate how easily this system of incubation can be adapted to existing hatchery systems using materials which are readily available.

METHODS

A four gallon (15 liter) square plastic bucket, with the bottom cut out and replaced with a 1" X 1/8" slotted aluminum plate was placed inside another four gallon square plastic bucket. We chose to use slotted aluminum plates for the bottom of our I.B. units instead of Vexar as used by the Cowlitz and Washington Department of Wildlife hatcheries. We found that aluminum plates took less time to install, required fewer rivets to secure and were considerably more durable. The outside bucket was drilled with twelve $\frac{1}{2}$" holes near the rim for water drainage. The bucket incubators were set side-by-side in a standard shallow trough. About one foot of trough length was allowed for each I.B. unit. Two pairs of shallow troughs were set up in a concrete rearing pond (10' X 64' X 5' deep).

Water was supplied to each I.B. unit by a 3/8" polyethylene tube that was plumbed into a 2" PVC pipe. Each pair of troughs was serviced by one 2" PVC line and contains 30 incubation buckets. Each PVC pipe was connected to a fiberglass head box which receives its water from the existing pond water manifold. The water supply to the I.B. units was entirely gravity fed. Each unit received 0.8 gallons per minute.

Water flowed down onto a floating 1½" foam pad to dissipate energy, trap debris and distribute the water which down-welled through the eggs and exited at the bottom. The water then rose between the inner and outer buckets and exited through the holes at the top of the bucket.

In an effort to detect and eliminate IHN positive fish from our system, our 1987 brood fall chinook females were spawned in five-fish pools and ovarian fluid samples were taken from all of them. Each five-fish pool sample was numbered. This number remained with that individual group of eggs until they were determined to be virus free. On occasion the eggs from a five-fish pool will extend above the water level when water hardened. This did not seem to effect overall egg survival. A 15 minute 1:600 formalin drip was used to control fungus.

Newly spawned eggs from our Chamber's Creek trap were transported to Garrison Springs as mixed eggs and sperm. Each receiving I.B. unit was filled with a 1% Argentyne solution after the 3/8" water line had been corked. The eggs were then poured into the I.B. units and allowed to water harden for 30 minutes before the water flow was restarted.

Incubation loss for the 1987 season averaged about 12% and which is normal for Garrison. Each pair of troughs is covered with black visqueen to keep developing embryos out of the sunlight.

COMMENTS

We conducted a prototype test with our 1986 brood Chamber's Creek chum. We tested white buckets against blue buckets. Eggs used were pooled into a common spawning container, fertilized and then split into the I.B. units. Eggs in the white units sustained a 10% loss and eggs in the blue units sustained 5% loss. The eggs were incubated in a building supplied with Sylvania "Cool White" fluorescent bulbs. This possible light related effect has not been demonstrated at Wildlife hatcheries. We chose blue units to minimize the effects of light because our buckets are located outside.

Where waterborn debris is a problem, the foam pads may be changed periodically by corking the water supply, carefully removing the dirty pad and replacing it with a clean one. The flow may then be resumed.

When an egg/sperm mixture contains a great deal of ovarian fluid or sperm it is advantageous to drain this excess fluid to prevent dilution of the iodophor solution. The slotted inner bucket may be removed from the solid bucket. When the egg/sperm mix is poured in, the excess fluid can then drain before treatment begins. Iodophor treatment and the subsequent 30-minute water hardening will be done when the drained egg bucket is replaced in the outer bucket.

ACKNOWLEDGEMENTS

I wish to thank the following for their assistance in preparing this report. They will be able to provide further assistance for those interested in further information.

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Phone: (206) 964-7268

Mr. Mike Baxter
Washington Department of Fisheries
Cowlitz Salmon Hatchery
Phone: (206) 985-2655

ISOLATION BUCKET INCUBATION DETAILS

A. Inner bucket

B. Outer bucket

C. Foam pad

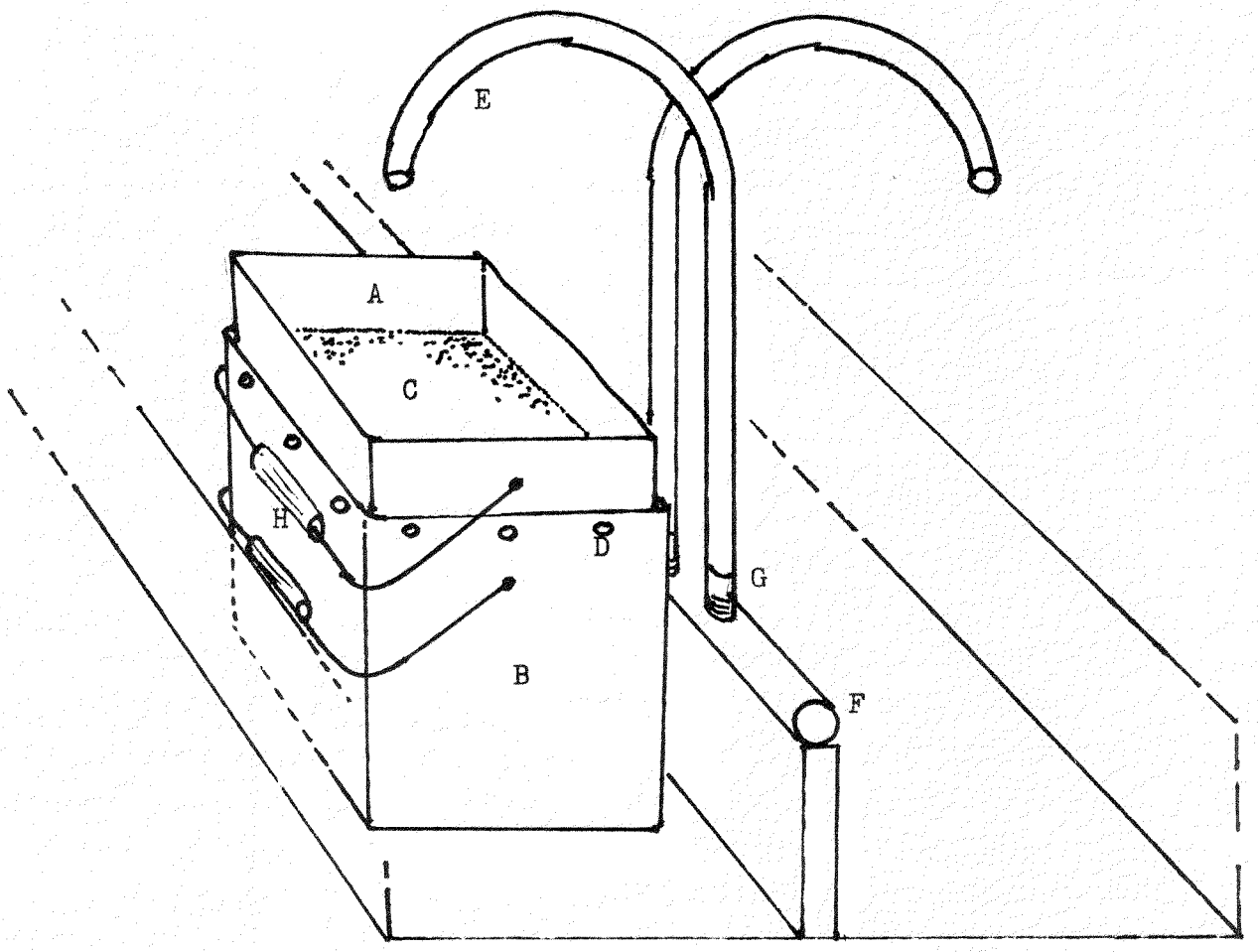
D. Drain hole

E. Polyethylene water supply

F. P.V.C. supply line

G. Brass adapter nipple

H. Bucket handle



A Synopsis of Formaldehyde Injection Systems Currently Used by the WDF for Control of Fungus on Salmon Eggs.

By Richard G. Kolb
Department of Fisheries
Washington

In 1986, the Washington Department of Fisheries changed from the traditional use of Malachite Green for control of fungus (Saprolegnia parasitica) to formaldehyde for health and environmental reasons. Initially drip and flush methods were tried with varying degrees of success.

Based on the results of the work done in 1986, the Department has decided to use formaldehyde injection into the water as the method of treatment. The treatment consists of a 1:600 concentration for 15 minutes, seven days a week. It is hoped this will successfully control the fungus and also reduce the amount of fumes associated with previous drip or flush treatments.

Two basic systems are being used: gravity or pressurized. Both systems use PVC piping and fittings with various control injectors. The gravity system starts with a barrel placed above the incubation water supply and has valves for each incubator. This system requires a stand pipe at one end to help prevent air locks (Fig. 1).

The pressurized system is used at locations with longer pipe line runs. It requires a pump, regulation device and/or return line (Fig. 2). One advantage of this system is the formaldehyde storage tank can be easily located outside the building to reduce fumes. Two types of pumps are used: diaphram and tubing (Fig. 3).

The injectors come in various designs depending on site demands and individual preferences. They are as standard as tubing with a clamp, or as modern as irrigation drip emitters (Fig. 4) which deliver a relatively constant flow under various pressure ranges. Many facilities are using hypodermic needles (Fig. 5) in sizes 18 to 25 as their regulators.

At WDF, the injection of formaldehyde into the head end of the incubators is still in its formative stage. The initial results are encouraging, but the various methods will all be examined and final recommendations will then be available.

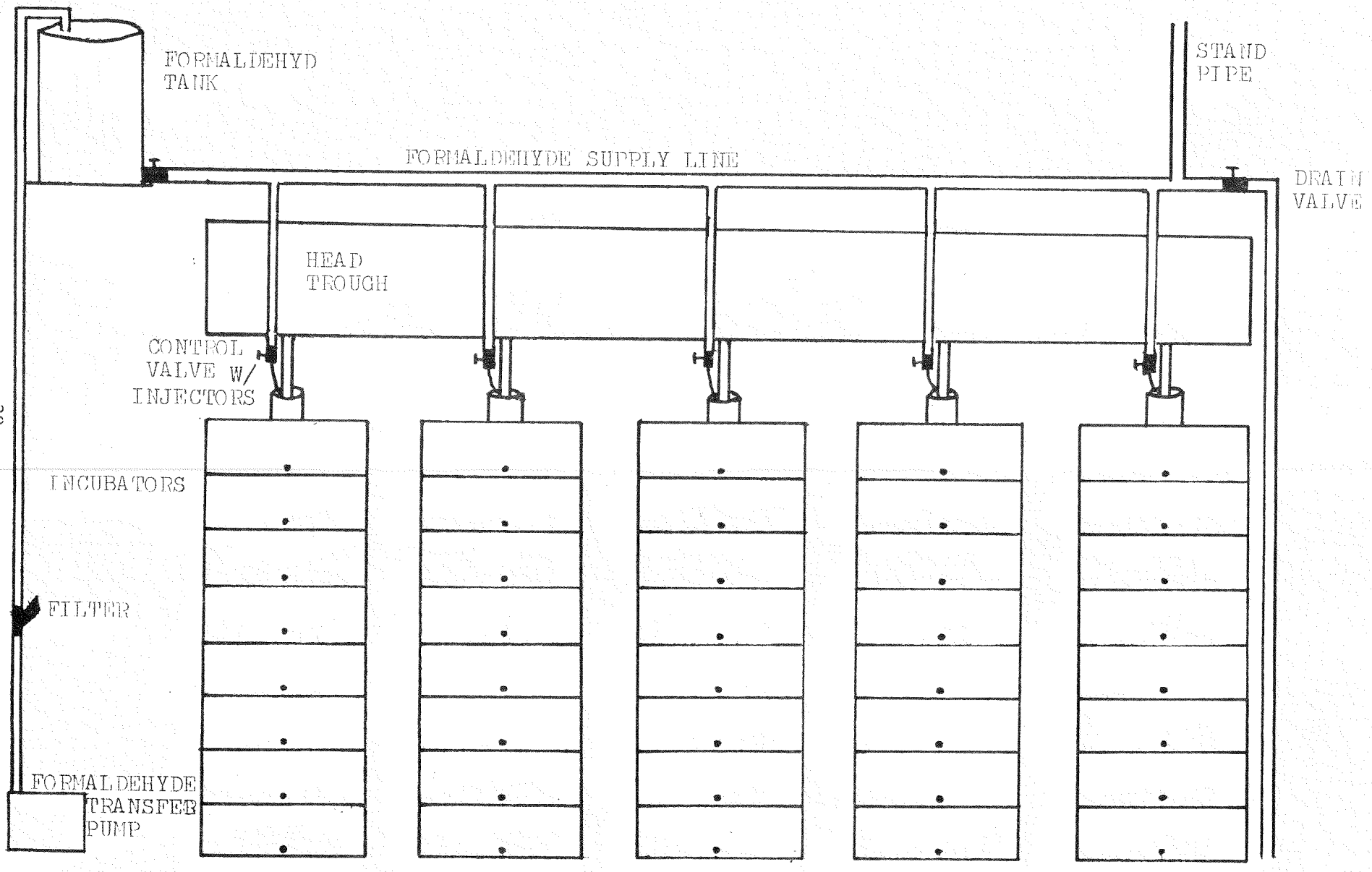


Figure 1: Schematic diagram of gravity injection system.

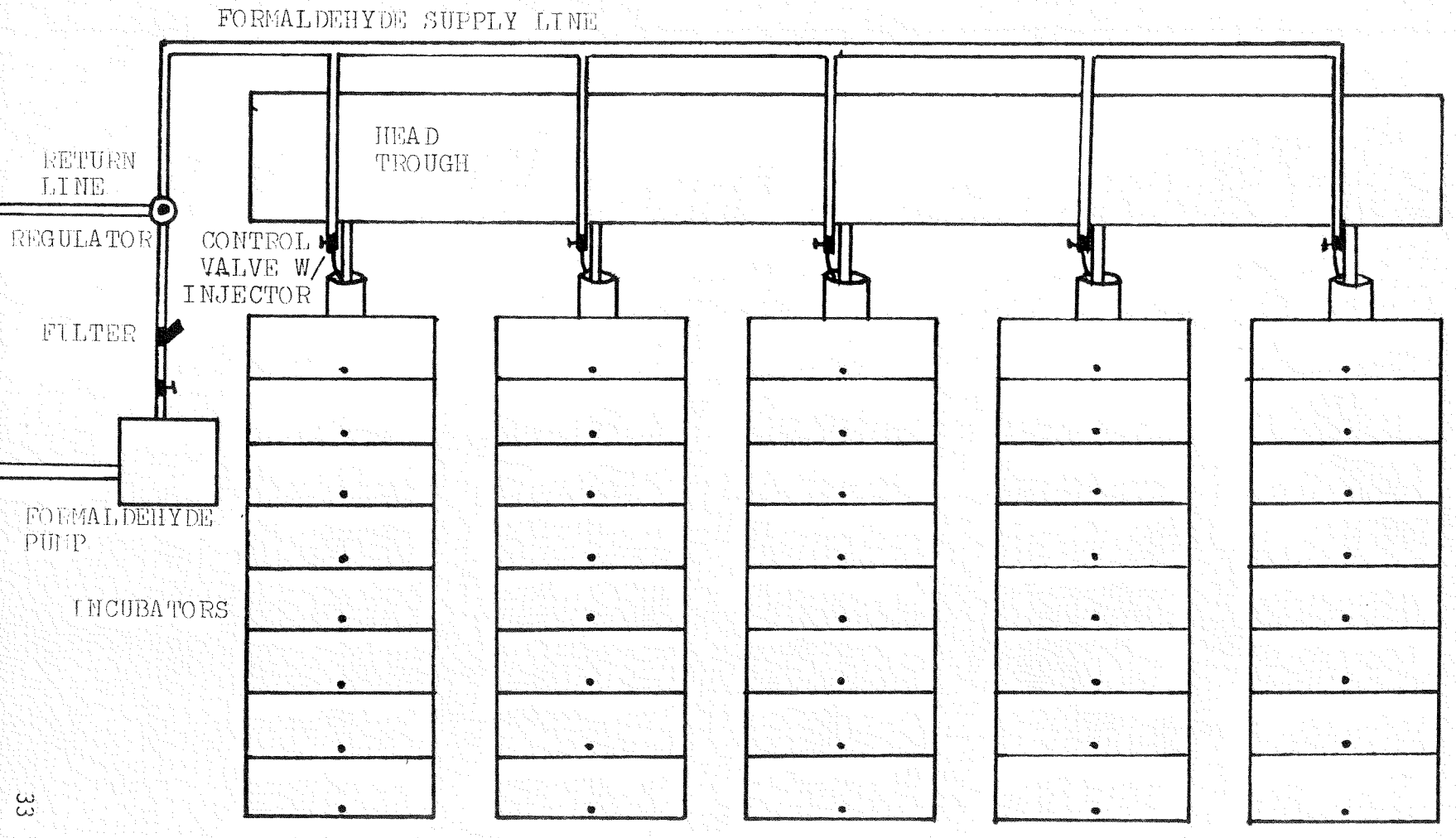


Figure 2: Schematic diagram of pressurized injection system.

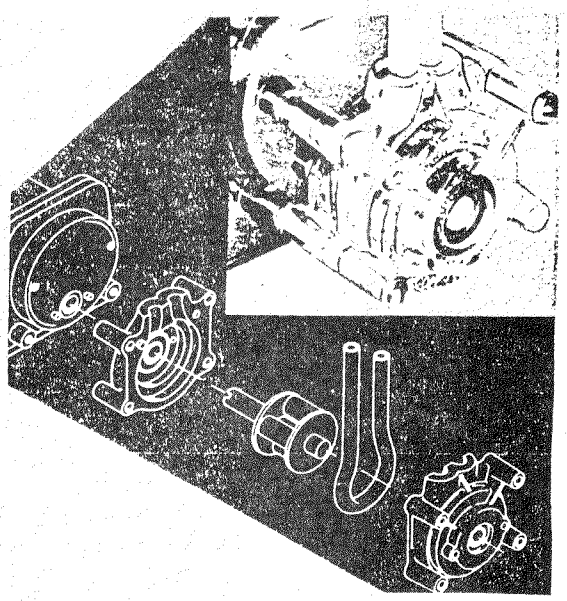


Figure 3: Schematic diagram of a tubing pump. The tubing fits into the pump housing between the outer wall and the rollers. As the shaft rotates, the rollers squeeze the tubing and force the formaldehyde into the system without coming into direct contact with it (the pump is self priming).

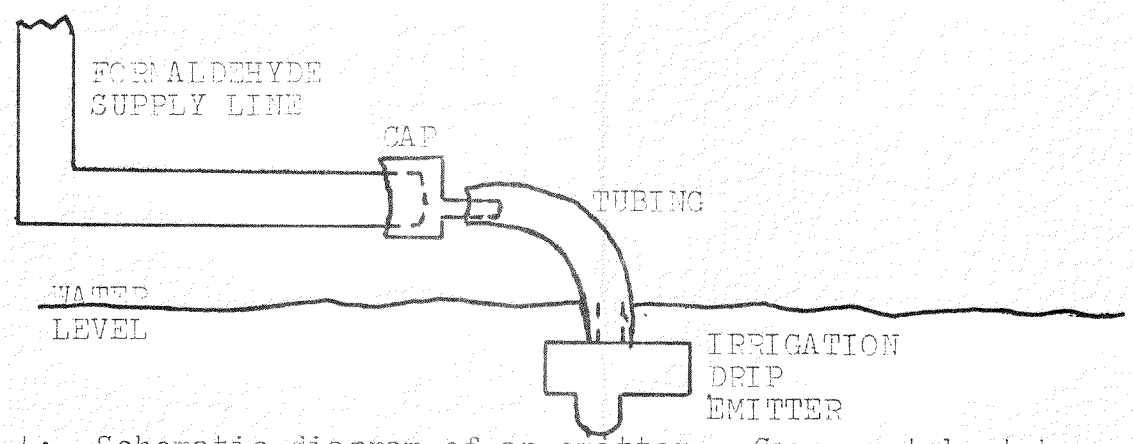


Figure 4: Schematic diagram of an emitter. Care must be taken to ensure that the control diaphram material in the emitter is compatible with formaldehyde.

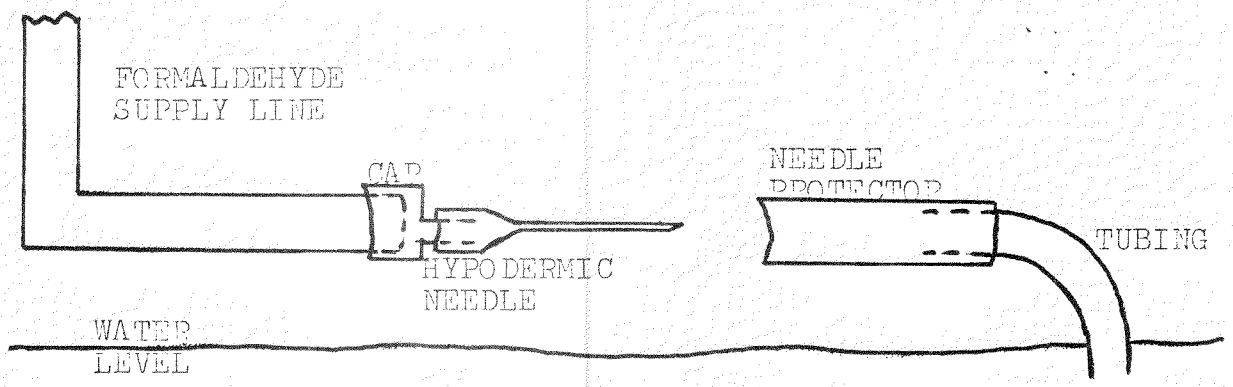


Figure 5: Schematic diagram of a hypodermic needle used as a regulator.

Do it Yourself Transport Tank Renovation

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The plan from the begining of the project was to completely renovate our hatchery transport tank to provide trouble free fish transport, fire suppression, low maintenance in the long term, and to reduce stress on the drivers. To accomplish these goals, every aspect of the project was carefully thought out ahead of time and the best parts and materials available were used.

The crew at the Yakima Hatchery set out to accomplish what was normally done by the engineering division or private metal fabricators. The un-insulated 1,200 gallon steel tank was mounted on a '69 Chevy 2 1/2 ton chassis that was in very bad condition. Of the 2 gasoline motors for recirculating water one was not operable and the other motor and impeller was virtually worn out. The carbon stone system lacked good air tight fittings and sealed ends. To top off all the problems the inside and outside layers of paint were peeling down to the metal.

The first priority came in replacing the truck with an International diesel powered truck chassis rated at 25,000 pounds. Safety cannot be stressed enough when choosing the correct truck to haul heavy loads in on and off highway service.

The next task was the removal of the old recirculating systems 2 gasoline motors, gas tanks, inlet piping, welded inlet screens, the outlet piping in the top of the tank, and 18 brass valves that controlled the system. By doing this approximatly 500 pounds of materials were removed. The recirculating system was replaced with three 12 volt Fresh-Flo 75 g.p.m. aerators, one in each of the three baffled areas of the tank, which were supplied with power from the truck's 2 batteries and 105 amp alternator. Each motor was wired with a individual switch and supplied water by lifting it from the bottom of the tank and "fanning" it across the water surface from slots in the top of the unit.

The many benefits in changing from internal combustion recirculating systems to electric systems are; low maintenance, no gasoline, no oil, no pump impellers to wear out, no noise, no heat transfer to water from the road, the air, and the motor, no danger of plumbing freezing, no contaminants are trapped inside pumps or pipe systems, and finally there is no problem in starting the motors. Since there are fewer moving parts on a electric system the costs of repair will be significantly lower than a re-build of a gasoline motor costing about $400.00. The only problems I have encountered with the new motors are that moss or other debris can clog the outlet slots in the top of the P.V.C. tube which requires periodic cleaning. Also water can weep out of the top bearing in the support tube and can be corrected by forming a bead of silicone around the propeller shaft just below its lower plastic bearing to deflect water from being forced up the support tube.

When all modifications were completed the tank was sandblasted and painted by a commercial painting contractor that guaranteed his work. For the inside tank surface a potable water epoxy paint was used providing a smooth hard surface, that has excellent resistance to salt water immersion, which can be expected to hold up to wear for 10 years. The outside was painted with 2 prime coats and then 2 coats of acrylic urethane epoxy which gave a extremely high gloss surface that simplifies cleaning and reflects sunlight and heat when a light color or white paint is used. If inside storage is available for the tank and the surfaces are cleaned regularly a 15 year paint life can be expected for the outside of the tank. The higher costs for the best paints available can be justified by the durability of the industrial coatings compared to the costs of poor surface preparation and re-painting using cheaper inferior paints.

The old carbon stone oxygen supply system was replaced using new longer lengths of carbon which reduced the number of fittings and increased the capacity of the system. All thread to stone fittings and the end surfaces were coated with a layer of J B Weld epoxy to make air-tight seals. The previous oxygen tank was clamped to the deck on one side of the tank and was usually under foot when loading or planting fish. After the gas motors were removed this provided an area to mount two oxygen cylinders vertically which increased work space and increaced the length of time for hauling fish when away from a oxygen distributors.

Other items added to the truck were; new sections of 4" smooth I.D. pipe and cam lock fittings for positive sealing, 6" P.V.C. storage tubes for the planting hose to increase their life by excluding sunlight, C. B. radio, back-up alarm for safety, and a state common frequency radio for fire and emergency use. All external wiring is protected by plastic convoluted tubing to protect from short circuits and to extend their life.

The approximate cost of tank renovation excluding new truck was $3,800.

Major materials used:

Valspar HI-BUILD epoxy 78 series tank lining immersion service, 2 coats 4.0-8.0 mils per coat. cost - approx. $25.00/gallon

Valspar Phenolic primer 13 W 22 series, exterior surfaces, 2 coats 1.0-2.0 mils per coat, cost - approx. $20.00/gallon

Valspar Acrylic Urethane Epoxy 43 series, exterior top coat, 2 coats 1.5-2.0 mils per coat, cost - approx. $47.00/gallon

Fresh-Flo aereators model TT, 12 volt, 10 amp current draw, custom tube and shaft lengths made to order.
Contact - Gene Guenther, Fresh-Flo Corp., RT 1, Hwy 28 SW, Cascade, WI 53011 Ph. (414) 528-8236

Use switches rated to 30 amps for motors, stranded automotive wiring of at least 8 gauge to switch pannel - at least 12 gauge wire to motors from switch pannel.

Evaluation of Saltwater Acclimation Strategies for Young-of-the-Year Chinook

Bruce Bachen
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With over 200 inches of rain in parts of southeast Alaska, this is not an area where one would expect hatchery water supplies to be limiting production capacity. Yet, there are certain times of the year, notably the late winter and summer periods, when annual low flows can limit hatchery capacity. At our Medvejie Hatchery near Sitka, we have developed a culture strategy that minimizes freshwater demand during the late winter period when freshwater supplies are lowest. For the chinook program, this has meant moving juveniles to saltwater net pens prior to late winter low flows and holding the fish until the spring release. We have done this for five years with mixed results so we designed experiments last year to better define the parameters needed for successful introduction in the late summer and early fall.

The experiments focussed on two controlled variables, time and introduction stategy. A series of experiments was done between late June and mid-October. There were three types of introduction strategies tested. The first was direct entry where fish were exposed to full-strength saltwater by placing them in cages and lowering fish to a depth where maximum salinity was found, generally about 10 ft. Daily mortality records were kept for 1 month after introduction. These fish wre moved directly from freshwater to full-strength saltwater to test the inherent ability of the fish to adapt. The second introduction stategy was stepwise acclimation. Chinook were exposed to controlled increases in salinity starting with freshwater and ending at full-strength saltwater. Salinity was increased in equal increments over 30 or 60 days and adjusted weekly (Fig. 1). Fish were then challenged for 3 weeks in full-strength saltwater. The purpose of this introduction strategy was to test the response of chinook exposed to low salinity water in a controlled environment.

The third introduction strategy used net pens which contained a gradient of salinity, being low near the surface and full-strength at the bottom of the pen. This strategy allowed the fish to select exposure salinity depending on their depth. After three weeks in net pens the fish were transferred to full-strength saltwater for two weeks.

The uncgntrolled variable in these experiments was size. In late June these chinook averaged 3 g (150/lb) and grew to an average size of 11 g (41/lb) by mid-October when the the experiments were completed.

The results of the experiments are summarized in Table 1. Survival in the direct entry experiments was similar during the first two periods (43 and 36%) but declined markedly in the last period (16%). The stepwise acclimation experiments followed the same trend. The thirty day acclimation allowed 88.5% to survive a direct saltwater challenge during the July-August period. By the September-October period only 15% of the 30-day and 6% of the 60-day acclimated fish could survive full-strength saltwater. No fish died in net pens in the first two periods during the three weeks the fish were held. During the July-August period fish were then subjected to full-strength saltwater and 17% died over two weeks. We were unable to challenge the fish in the August-September period due to heavy sea lice infestation. No net pen trials were started for the September-October period because of the presence of sea lice.

From our data it was evident that the tolerance of sub-yearling chinook salmon to exposure to full-strength seawater diminished from June through October. With all introduction stategies the best results occurred in July. One interesting observation made during these experiments was the significance of salinities above 25 PPT to survival. Of 1200 test fish subjected to the range of introduction strategies and times of entry, less than 1% died at salinities less than or equal to 25 PPT. The response to salinities at 29 and above was much different. If fish were not fully acclimated, these higher salinities caused substantial losses where concentrations of 25 PPT did not. This finding could be important to those who use bioassays to assess the readiness of smolts prior to liberation.

There is an important relationship between size and ability to tolerate saltwater. The mean size of the mortalities in these experiments

was always less than the overall mean of the population at the beginning of the experiment. This indicated that the smaller fish were subject to greater loss than the larger fish. Further support for the importance of size comes from the observation that the only two successful fall introductions of this stock to saltwater were done with fish with a mean size of 20 g or about twice as large as the fish in these experiments. It is appropriate to think of a moving critical size that is neccessary for successful acclimation to saltwater. This size is fairly small through the summer, but increases fairly dramatically towards fall. It is also probable that critical size varies with stock.

Acclimation affected both the timing and the extent of mortality. Direct entry chinook generally showed all of the loss in the first week of exposure. Mortality in acclimated fish typically did not peak until the second week. With the exception of the September-October period acclimation was highly effective at reducing losses. The reason that acclimation was not effective in the September-October period probably has to do with the size of the fish being too small for that time period.

As an acclimation strategy, net pens having a low salinity surface lens appeared to be as effective as controlled step-wise salinity adjustment. This was the desired result since costs are far less for net pen acclimation than for providing intermediate salinity water in raceways.

Based on these results, we plan to alter the timing of our saltwater introductions next year from fall to mid-summer. We will provide an artificial low salinity lens to facilitate acclimation. With this strategy we would see five fold increase in the capacity of our freshwater system for we will be releasing fish to saltwater at 3 g instead of 15 g. The costs of providing additional net pens is relatively small compared to freshwater raceways.

We do foresee some problems with long term saltwater holding. There appears to be two intrinsic growth rates in the population. The faster growing fish can grow very large by release time adding substantial feed costs. Controlling growth in that segment of the population is a problem to overcome. Sea lice, also, will be a problem from time to time as it

has been to fish farmers around the world. Despite these concerns, overwintering chinook in net pens is an outstanding opportunity for coastal hatcheries to increase their smolt capacity relatively inexpensively.

Finally I would like to acknowledge Dail Hurdlow, Allen Edsall, Jim Seeland and Dan Goodness for their conscientious efforts to carry out this study.

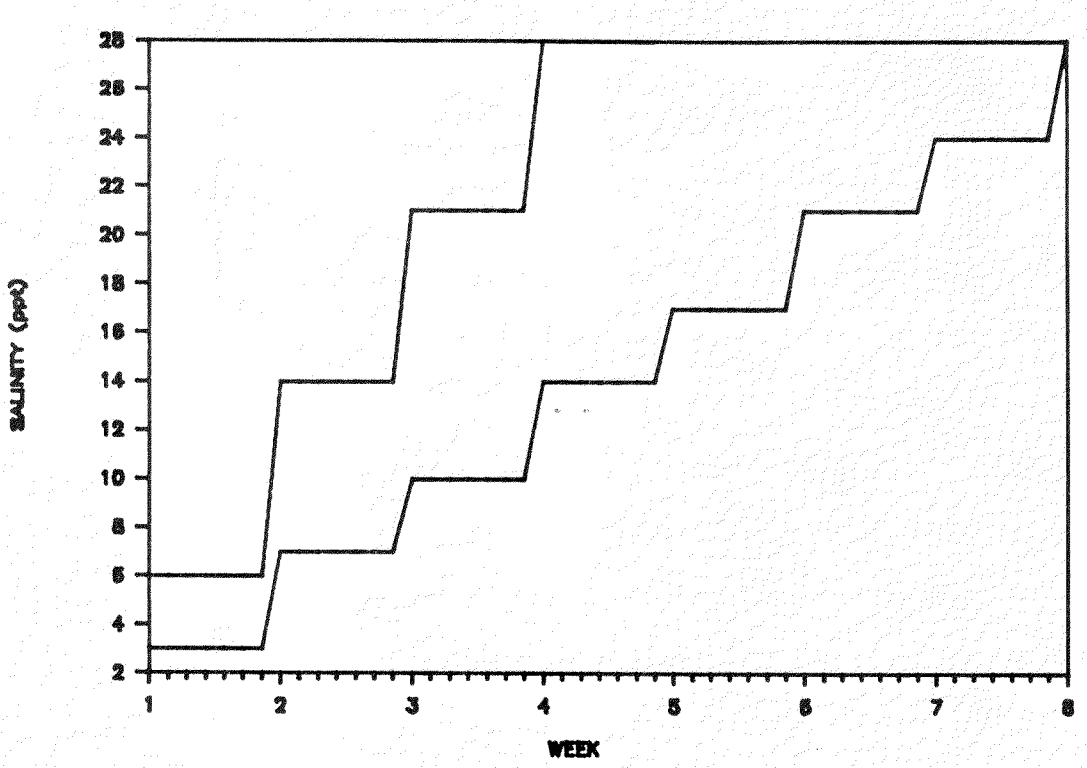

Fig. 1. Target saltwater concentrations during 30 and 60 day stepwise acclimation experiments.

Table 1. Percent survival of chinook exposed to full-strength saltwater.

		Acclimation Strategy		
		Stepwise Increase		Net Pen
Time period	Direct Entry	30 day	60 day	Exposure
mid-July to mid-August	43	88.5	ND	83
mid-August to mid-September	36	84	65	100[1/]
mid-September to mid-October	16	15	6	ND

1/ Experiment terminated prematurely due to sea lice infestation. These fish were placed in net pens for three weeks but were not challenged with full-strength saltwater for two weeks as were those in the first period.

WATER QUALITY IN AQUACULTURE

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Abstract

There are three elements to be considered when discussing water quality for aquaculture. These are: 1) the source water, 2) the rearing water, and 3) the effluent water.

Source Water: Many water quality parameters have been considered and delineated. For instance, thirty-nine are listed in the EPA Water Quality Standards Criteria Digest (U.S. EPA 1979-80), for aquaculture. Although it is very important to do extensive water quality checks before initiating an aquaculture project, it may need to be followed by testing with the appropriate species, even when all parameters appear to check out positive.

Rearing Water: Fish metabolism causes water quality changes. The changes must stay within the acceptable tolerance range of the species cultured. This requires knowledge of the metabolic characteristics and tolerances of the species involved. From this, we can determine the optimum production capacities. However, hatchery design and operational modes also play important roles in this criterion.

Effluent Water: The environment, which receives the discharge, must be protected from excessive metabolic by-products. Each situation is unique but limitations must be recognized beforehand and adhered to. This too affects design, modes of operation, production levels, metabolic characteristics and diets.

This paper identifies these three elements and addresses them as fundamental factors for site selection, facility design, operational modes, and production potential.

Water Quality in Aquaculture

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Introduction

Water quality for aquaculture has three elements of concern:

1. Source Water: this is the single, most important factor in selecting a hatchery site. Unfortunately, because of its complexity, no simple recipe is available for each species cultured.
2. Rearing Water: water quality changes occur as a result of fish rearing. Such changes are self-limiting. Economics (i.e. survival) forces fish production facilities to discharge relatively high quality water.
3. Effluent Water: although environmental considerations dictate the discharge be of good quality, hatcheries often are highly consumptive of water. In terms of volume, fish hatchery effluents can impact receiving waters. Sensible effluent control measures can alleviate the impact.

Source Water

Unfortunately, there is no simple recipe which lists all water quality parameters for optimum rearing conditions by species. The effects of water quality on fish are too complex for that. Synergistic and antagonistic considerations alone presently defy comprehension. Each water supply is unique in its physical and chemical makeup. Criteria, such as identified in Table 1, are general guidelines that help identify potential problems. As new information becomes available, these criteria may require modification and expansion. When in doubt about the suitability for a specific species, bio-assays should be conducted.

Table 1. Water quality standards for salmonid aquaculture

Parameter	A	B	C	D
Alkalinity (as $CaCO_3$)	20 mg/l	undetermined	20-200 mg/l	120 - 400 mg/l
Aluminum (Al)	.01 mg/l	.01 mg/l	--	
Ammonia (NH_3)	.02 mg/l	.0125 mg/l	.012 mg/l	.0125 mg/l
Arsenic (As)	.05 mg/l	.05 mg/l	--	
Barium (Ba)	5.0 mg/l	5.0 mg/l	--	
Cadmium (alk 100)	.0005 mg/l	.0005 mg/l	--	.0004 mg/l
Cadmium (alk 100)	.005 mg/l	.005 mg/l	--	.003 mg/l
Calcium (Ca)	52 mg/l	--	52 mg/l	4 - 160 mg/l
Chloride ( )	--	4.0 mg/l	--	
Chlorine (Cl)	.003 mg/l	.003 mg/l	--	.03 mg/l
Chromium (Cr)	.03 mg/l	.03 mg/l	--	
Carbon dioxide (CO_2)	1.5-15.0 mg/l	1.0 mg/l	2.0 mg/l	0 - 10.0 mg/l
Copper (alk 100)	.006 mg/l	.006 mg/l	.006 mg/l	
(Cu) (alk 100)	.03 mg/l	.03 mg/l	.03 mg/l	
Dissolved Oxygen (DO)	75%, never below 5.0 mg/l	7.0 mg/l --	5.0 mg/l --	5.0 - sat. mg/l
Fluoride (F)	.5 mg/l	.5 mg/l	--	
Hydrogen cyanide (HCN)	.005 mg/l	--	--	
Hydrogen sulfide (H_2S)	.003 mg/l	.003 mg/l	.002 mg/l	0 mg/l
Iron (Fe)	.1 mg/l	.1 mg/l	1.0 mg/l	.5 mg/l
Lead (Pb)	.02 mg/l	.02 mg/l	--	
Magnesium (Mg)	15 mg/l	15 mg/l	--	needed
Manganese (Mn)	.01 mg/l	.01 mg/l	--	0 - .01 mg/l
Mercury (Hg)	.2 mg/l	.0002 mg/l	--	.002 mg/l
Nitrogen (N)	110% TDG 103% N_2	110% TDG 103% N_2	110% TDG --	110% TDG
Nitrate (NO_3)	1.0 mg/l	1.0 mg/l	--	0 - 3.0 mg/l
Nitrite (NO_2)	1.0 mg/l	.1 mg/l	.55 mg/l	.1 - .2 mg/l
Nickel (Ni)	.01 mg/l	.01 mg/l	--	
PCB	.002 mg/l	--	--	
pH	6.7-8.6	6.5-8.0	6.7-9.0	6.5 - 8.0
Potassium (K)	5.0 mg/l	5.0 mg/l	--	
Salinity	5.0 ppt	5.0 ppt	--	
Selenium (Se)	.01 mg/l	.01 mg/l	--	
Silver (Ag)	.003 mg/l	.003 mg/l	--	
Sodium (Na)	75 mg/l	75 mg/l	--	
Sulfur (S)	1.0 mg/l		--	
Sulphate (SO_4)	50 mg/l	50 mg/l	--	
Total dissolv. solids (TDS)	400 mg/l	400 mg/l	400 mg/l	
Total susp. solids (TSS)	80 mg/l	80 mg/l	80 mg/l	80 mg/l
Uranium (U)	.1 mg/l		--	
Vanadium (V)	.1 mg/l		--	
Zinc (Zn)	.005 mg/l	.005 mg/l	.04 m/gl pH7.6	.03 mg/l
Zirconium (Z)	.1 mg/l		--	
Temperature	--	0°-15°C	--	

A: Daily, J.P. and P. Economon, 1983.
B: Fish Culture Manual, Alaska Dept. Fish and Game, FRED Div., June, 1983.
C: Wedemeyer and Wood, 1974.
D: Piper, G. P., et. al. 1982.

Fish rearing considerations go beyond such a list of physical and chemical parameters. To include all factors which minimize stress and promote fish health under all conditions is difficult, if not impossible. There are too many physiological, chemical, behavioral and physical factors which affect a fish's response to stress.

As far as water chemistry is concerned, more detailed information is available on what substances are toxic to the fish than on those that promote fish health.

The following guideline for water quality evaluations has been taken from the manual: "Summary of water quality criteria for salmonid hatcheries" (1983).

1. Check the drainage basin of surface waters for potential sources of pollution Occasionally, groundwaters could be contaminated by a distant pollution source.
2. Conduct a sampling program that detects seasonal and "event related" variations in water quality (eg. the effects of high rainfall, snowmelt, groundwater table variations, etc.).
3. Anticipate possible changes in groundwater quality that could occur with extensive pumping (eg. the risk of increases in iron concentrations).
4. Perform a few very comprehensive and accurate water analyses to check for possible toxicity problems. It would be desirable to cross-check results using at least two laboratories.
5. If the suitability of a water source is uncertain, give a high priority to long-term on-site biological testing to determine both acute and chronic effects on growth, condition and fish health.

The State of Michigan selected to go to groundwater sources (springs and wells) to the largest extent possible. This permits specific pathogen-free rearing, stable water temperatures; and consistent water chemistry. Groundwater sources are often high in nitrogen gas and low in dissolved oxygen levels, such conditions are most successfully controlled with pure oxygen.

The Rearing Water

A number of water quality parameters are adversely affected by the fish culture processes. Table 2 shows those parameters we are most concerned with.

Table 2. Water quality parameters undergoing changes as a result of fish rearing.

Parameter	Ideal Inflow Quality	Increase	Decrease	Fish Health Concern	Environmental Concern	Cause of Change
Dissolved Oxy.	100%		X	Yes	Yes	Respiration and Organic decomposition
Ammonia	non-detectable	X		Yes	Seldom	excretory product
pH	7.0-8.5		X	Seldom	Seldom	Due to CO_2 increase
Carbon dioxide	20 mg/l	X		Seldom	No	Respiration
Phosphorus	--	X		No	Yes	excretory product
Solids	--	X		Yes	Yes	food & feces
Nitrite	non-detectable	X		Yes	No	incomplete nitrification of NH_3

Dissolved oxygen and ammonia are the most significant parameters in intensive fish culture operations, while nitrite toxicity can occur in recirculating systems and high production pond culture. Levels should not reach the range where they are harmful to the health of fish. In that sense, a fish production system is self-regulating since environmental quality must remain high enough to protect the fish.

In most cases, dissolved oxygen is the first limiting factor. In salmonid culture, rearing unit effluent recommendations vary from 5.0 to 7.0 mg/l D.O. These variations reflect species and water temperatures. Oxygen transfer efficiency across gill membranes depend on the partial pressure of the oxygen dissolved in the water. At 100 percent, the partial (pO_2) pressure is 159.2 mm Hg at an atmospheric pressure of 760 mm Hg (.2095 x 760).

Downey and Klontz (1981) selected a minimum of 90 mm Hg pO_2 for rainbow trout. What this means in terms of available oxygen to the fish for a low versus a high temperature is shown on the following page.

1. At 6°C, oxygen saturation at sea level is 12.62 mg/l. At a barometric pressure of 760 mm Hg, 90 mm Hg pO_2 equals 7.14 mg/l.
2. At 16°C, dissolved oxygen saturation is 10.13 mg/l and 90 mm Hg. pO_2 equals 5.73 mg/l.

In case 1, the available oxygen to the fish is 12.62-7.14 or 5.48 mg/l; in case 2 it is 10.13-5.73 or 4.40 mg/l. Despite the fact that the minimum effluent oxygen level for 6°C must be as high as 7.14 mg/l, versus 5.48 mg/l for 16°C, more oxygen is available to the fish at the lower temperature. Hence more fish can be produced. Furthermore, since lower temperature means lower metabolic rate, the fish do not consume as much oxygen at 6°C as they do at 16°C. Metabolic rates and food requirements appear to be directly related in fish as is probably true of all coldblooded animals. There is considerable data available that demonstrate that salmonids consume from 200 to 250 g of oxygen per kg of food, esocids (northern pike and muskie) about 110 g (Pecor, 1979) and the common carp, a warmwater species, consumes about 230 g. (Huisman, 1974). Such values are independent of fish size and water temperature. But, these two factors, size (weight) and temperature are used to establish feeding levels, as reflected in the equation:

$$\% \text{ Body weight (BW) to feed} = \frac{C^\circ \times 2.0}{100k \times L_{cm}},$$

where K is the metric condition factor ($K = \frac{W}{L^3}$), the value 2.0 is a constant derived from the Hatchery Constant formula of Haskell (1959) and modified by Westers (1984).

Therefore, a 10 cm trout with a K-factor of .01 reared at 6°C requires a feeding level of:

$$\% \text{ BW} = \frac{6 \times 2.0}{1.0 \times 10} = 1.2\%;$$

at 16°C the level is:

$$\% \text{ BW} = \frac{16 \times 2.0}{1.0 \times 10} = 3.2\%$$

Since the oxygen consumption per unit (kg) of food is constant, a loading formula which expresses the maximum kg of fish per unit of flow in liters per minute (lpm) can be expressed as:

$$\text{kg/lpm} = \frac{\text{Avail. D.O. in mg/l}}{2.0 \times \% \text{ BW}} \quad \text{or:}$$

$$\text{kg/lpm} = \frac{\text{Avail. D.O.} \times 100\text{k} \times \text{L}_{\text{cm}}}{4.0 \times \text{C}^{\circ}}$$

This is based on 200 g O_2 per kg food. The maximum loading for 10 cm trout at 6°C is:

$$\text{kg/lpm} = \frac{5.48 \times 1.0 \times 10}{4 \times 6} = \underline{2.28} \text{ kg};$$

for 16°C it is:

$$\text{kg/lpm} = \frac{4.4 \times 1.0 \times 10}{4 \times 16} = \underline{.69} \text{ kg};$$

a reduction of 330% in carrying capacity!

All of the above is based on a DO minimum of 90 mm Hg pO_2. If a straight minimum of 6.0 mg/l is used, the contrast is even more significant, nl. 2.7 kg/lpm for 6°C versus .65 kg/lpm for 16°C, a 415 percent difference! Adding oxygen to the water, especially at the optimum temperature range for growth can be extremely significant in terms of fish culture productivity. It is here that the application of pure oxygen has its greatest merit. This was demonstrated in a case with channel catfish production, where Collins, et. al. (1983) applied liquid oxygen and realized a 300 percent increase in production. Production increase is not the only benefit derived from added oxygen. Significantly better growth in channel catfish is realized when D.O. levels are maintained at 100 percent saturation

(Andrews, et. al. 1973). When pure oxygen is used, one must strive to retain a total dissolved gas pressure of 100 percent or less to prevent gas bubble disease. Oxygen application eventually will shift the limiting factor to ammonia. Ammonia production too is directly related to the food intake. Data indicates that salmonids generate from 25g to 35g of ammonia per kg of food consumed. It is the undissociated ammonia that is toxic to the fish. Fortunately, most of the ammonia ionizes, but the degree of ionization is strongly influenced by pH, and less by temperature. At a pH of 8.0 there is more than 30 times as much un-ionized ammonia than at a pH of 6.5. However, the concentration of un-ionized ammonia at the gill surface at eleveated pH may be considerably less than the concentration in the surrounding water. The micro environment at the gill surface has a depressed pH due to carbon dioxide respiration. Whenever the free CO_2 is relatively low in the source water, this could be a significant factor.

Considerable debate has been generated as to what maximum level un-ionized ammonia should be allowed. In an extensive review of this topic Mead (1985) concluded that the "traditional" level of .0125 mg/l is too conservative and he proposes instead .020 mg/l. This position appears sound and is the value used in this presentation. Whenever oxygen levels are maintained at or above the recommended minimum, .020 mg/l un-ionized ammonia for salmonids should not cause any stress on these fish. For ammonia the following loading formula is proposed:

$$kg/lpm = \frac{10}{\%\ UA \times \%\ BW}, \text{ or}$$

$$kg/lpm = \frac{10 \times 100k \times L}{\%\ UA \times C^{\circ} \times 2^{cm}}$$

This equation is based on the following assumptions:

1. Oxygen consumption per kg food is 200 g.
2. Ammonia generated per kg food is 28.8 g.
3. Maximum allowable un-ionized ammonia is .020 mg/l.

Should oxygen consumption be as high as 250 g, and ammonia production 35 g per kg of food, the value 10 in the numerator would change to 6.6. Thus one can assume that for salmonids the value will range somewhere between 7.0 and 10.0.

Table 3 shows the percentage of un-ionized ammonia at different pH's and temperatures.

Table 3. Percentage of un-ionized ammonia values for different pH's and temperatures.

Temperature °C	pH Value						
	6.0	6.5	7.0	7.5	8.0	8.5	9.0
2	.0098	.0309	.0977	.308	.968	3.00	8.90
4	.0115	.0364	.115	.363	1.14	3.52	10.3
6	.0136	.0429	.135	.427	1.34	4.11	11.9
8	.0159	.0503	.159	.501	1.57	4.79	13.7
10	.0186	.0589	.186	.586	1.83	5.56	15.7
12	.0218	.0688	.217	.684	2.13	6.44	17.9
14	.0254	.0802	.253	.796	2.48	7.43	20.2
16	.0295	.0933	.294	.925	2.87	8.54	22.8
18	.0343	.108	.342	1.07	3.31	9.78	25.5
20	.0397	.125	.396	1.24	3.82	11.2	28.4

For 6°C and a pH of 7.5 the percent un-ionized ammonia is .427, for 16°C it is .925. The maximum loading, based on ammonia (for 10 cm trout) for 6°C is:

$$\text{kg/lpm} = \frac{10 \times 1.0 \times 10}{.427 \times 6 \times 2} = 19.5 \text{ kg/lpm}$$

for 16°C:

$$\text{kg/lpm} = \frac{10 \times 1.0 \times 10}{.925 \times 16 \times 2} = 3.4 \text{ kg/lpm}$$

The loading based on DO limitation is 2.28 kg/lpm for 6° and .69 kg/lpm for 16°.

At the higher temperature oxygen can be added to nearly 4 times the original level of 100 percent saturation before un-ionized ammonia is limiting. In this case, the use of oxygen would allow for a 400 percent increase in production!

A pH around neutral results in very low un-ionized ammonia levels. Ideally the pH should remain below 8 and above 7. In poorly buffered waters the pH may be subject to significant fluctuations. A low pH, although advantageous with respect to ammonia toxicity, increases the fish's susceptability to infectious as well as non-infectious diseases.

Carbon dioxide produced in respiration, in water yields carbonic acid causing the pH to decline in poorly buffered waters. Carbon dioxide also affects the pH of the blood interfering with oxygen uptake. However, the fish will compensate and can acclimatize and grow normal in high carbon dioxide environments, providing the water is well oxygenated and has sufficient buffering capacity.

In some studies (Randall, et. al. 1982) rainbow trout did not survive when exposed to CO_2 tension of 7.5 mm Hg (equivalent to 22 mg/l CO_2 at 10°C) at an oxygen tension of 100 mm Hg or less. This is above the 90 mm Hg recommended by Downey and Klontz.

Lethal levels of CO_2 for various species of fish range from 20 to 200 mg/l according to McKee and Wolfe (1963) with the lower levels applying to situations with low dissolved oxygen conditions. In today's fish culture situations with feeding of pelleted diets, CO_2 seldom creates problems as a result of fish respiration. This was not the case when fish were fed wet diets based on a study by Haskell and Longacre (1984).

The aforementioned components, DO, NH_3, pH and CO_2 are the most significant ones with respect to their potential negative influence on the quality of the fish rearing environment caused by fish productivity. One other parameter, nitrite, should be mentioned. In recirculating systems and high density pond culture, as in commercial catfish production, nitrite has the potential to increase to damaging levels. Levels as low as .1 to .2 mg/l can produce signs of methemoglobinemia, known as brown blood disease. Nitrite is produced as a result of incomplete nitrification. In intensive, flow-through fish culture operations nitrite toxicity does not occur.

Effluent Water

The third area of interest is the effects fish culture effluent water has on the quality of the receiving water or the environment.

Table 2 lists those parameters which have the potential to adversely affect the environment, i.e. settleable solids, phosphorus, dissolved oxygen and ammonia nitrogen. Table 4 compares the quality of hatchery effluents with river water (typically unpolluted river in Denmark) and untreated domestic waste in Denmark as reported by I. Warrer-Hansen (1982). All values are in mg/l.

Table 4. A comparison of hatchery effluents with river water and untreated domestic waste in Denmark

Parameter	River Water	Hatchery Effluent	Domestic Waste Water
BOD	1.0-5.0	3.0-20	300
Total-N	1.0-2.0	.5-4.0	75
NH_3-N	--	.2- .5	60
Total P	.02-.10	.05-.15	20
Susp. Solids (SS)	--	5.0-50.0	500

From this it can be concluded that hatchery effluents are rather clean, with concentrations much lower than those of domestic waste water. This is of great significance when considering treatment methods.

However, fish production systems are very consumptive of water. For instance, a hatchery which produces from 50 to 75 metric ton of fish a year has a daily water consumption equal to a population of 170,000 people. How serious then is the potential of negative environmental impact caused by hatchery effluents?

A survey on European fish-farm effluents conducted by Alabaster (1982) concluded that only a minority of fish farms clearly impacted the environment and then only for a limited distance.

The potential to degrade the environment depends greatly on the character of the receiving water. Discharging organic solids, phosphorus and nitrogen into very pure oligotrophic waters are of particular concern. In Finland fish production systems according to Sumari (1982), contributed about 60 tons of phosphorus annually. This is equal to 9 percent of P loading from all industry.

The fish farms contributed more P than fertilizers and chemical industries combined. It was concluded that the P contribution from fish production is out of proportion to the national economic significance of the fish farming industry.

In Denmark, the organic discharges from 530 trout farms equates to the untreated organic loading from a population of over half a million people. For these reasons, farm effluents are the main factor limiting the extension of this industry in Finland and Denmark.

In France, the imposition of stringent standards for ammonia nitrogen in farm effluents could result in a substantial cut in production. It is rather surprising to see constraints placed on ammonia. Although algal requirements for N are about 16 times greater than for P on a molecular basis (6 to 8:1 on a weight basis), phosphorus is most often the principal factor limiting production in temperate as well as tropical fresh waters. Fish derive their phosphorus requirements principally from food. Requirements of available phosphorus range from .29 to .90 percent of the diet according to Beveridge (1984). From 60 to 80 percent of the total P in plant material exists as phytin, a phosphorus salt bound to Ca or Mg; this form is usually unavailable to the fish since they lack the enzyme phytase. Surplus dietary phosphorus is largely excreted through the kidneys while unavailable phosphorus is passed out in the feces. The mean P content of trout diets is 1.45 percent, while some diets are over 2 percent.

Data indicates that only 32 percent of P ingested is assimilated and utilized, the rest is passed out via feces and urine per Penczek, et. al. (1982).

Because P dilution in effluent water is so great, (.05-.15 mg/l) there is presently no feasible technology available to remove it. The Tunison laboratory of fish nutrition of the U.S. Fish and Wildlife Service in collaboration with the State of Michigan is involved in studies to reduce the P output of hatcheries by

means of P adjustments in the fish diets. Studies thus far by Ketola et. al. (1985) indicate that reductions of 40 to 50 percent can be realized economically.

Fish farms produce significant volumes of solids. The output of suspended solids per metric ton of fish produced equates with the treated waste of 859 persons (Solbe, 1982). The most effective solution to effluent quality is solids control by means of settling and physical removal. The settling velocity of trout excreta, when intact, is generally high. The larger the particle, the quicker the settling. It is, therefore, imperative to prevent fragmentation. If solids are allowed to settle and remain within the fish rearing unit, resuspension and fragmentation often occurs due to fish activity. Solids should leave the rearing unit immediately after being "produced", this will make later settling easier.

Rectangular rearing ponds can be made self-cleaning by means of baffles which create a high velocity along the bottom (Boersen, et. al. 1985). Solids will quickly leave such rearing units and resettle in a fish-free section of the raceway behind the fish retaining screen. From here they can be removed directly or via a drain to a common settling basin. In Michigan, it was found that pumping solids directly from the raceway settling section is an acceptable procedure. Baffled rearing units are totally self-cleaning and save many manhours in cleaning. This also promotes fish health since frequent practice of cleaning and flushing of rearing tanks can cause stress on the fish.

Although effluents from fish farms seldom are harmful to the environment, it is important that solids generated be separated from the discharge. It is also prudent to consider the use of low-P ("environmentally friendly") diets. However, solid removal, which simultaneously intercepts a significant portion of the phosphorus as well, in and of by itself almost always will result in quality effluent control for fish production systems.

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Improvements in Artificial Fertilization Technologies in Rainbow Trout

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Abstract

An artificial replacement for coelomic/ovarian fluid was used as a fertilization medium and shown to significantly increase fertilization success in eggs from commercial rainbow trout broodstock. In addition, short term storage of rainbow trout sperm (up to 35 days post-spawning) was achieved and successfully expanded to a commercial broodstock operation. The implications of these technologies on the efficiency and success of a commercial broodstock operation are discussed.

Introduction

Despite the fact that the practice of salmonid husbandry is over a century old, artificial fertilization practices continue in much the same manner today as in the past decades. Detailed information on improved fertilization practices and gamete handling, as reviewed by Billard (1985) Scott (1980), and Scott and Baynes (1981), have largely been ignored in terms of application to production-scale facilities.

The objective of this study was to apply some of the published techniques to a commercial rainbow trout genetic selection program in order to (a) allow matings between individuals temporally separated in maturation by up to five weeks, and (b) to increase overall fertilization success. This paper also describes the expansion of these techniques to our production-scale broodstock facility and the resultant increase in operating efficiency.

Methods and Materials

The rainbow trout broodstock used in these studies were part of a commercial genetic selection program ongoing at Clear Springs Trout Company, Buhl, Idaho. At the time of gamete collection, fish were anaesthetized with MS-222. Semen and egg takes were collected in individual 700ml Whirl Pak bags (VWR, Salt Lake City, Utah) and stored in a cooler with ice until further treatment. All studies were initiated within 1 h of gamete collection.

The short term sperm storage studies involved dilution of the collected semen 1:1 with complete Modified Cortland Solution (Table 1, modification of Parsons and Thorgaard, 1985). The diluted sperm was flushed with gaseous oxygen, sealed in the collection bag and placed in a 1° C. incubator. Samples were analyzed for motility weekly for five weeks. At the end of five weeks of storage a small quantity of sperm was removed and used to fertilize a sample of eggs.

Table 1. Composition of Modified Cortland Extender

	Grams/liter
SOLUTION A	
KCl	9.00
NaCl	2.35
NaH_2PO_4	0.51
$MgSO_4 * 7H_2O$	0.29
$CaCl_2 * 2H_2O$	0.29
SOLUTION B	
$NaHCO_3$	5.00
Glucose	5.00
Streptomycin sulfate	6.00
Benzylpenicillin (Na salt)	5 million I.U.

To make complete extender add 1 part of Solution B to 4 parts Solution A and mix thoroughly

The initial fertilization diluent studies involved the replacement of the ovarian/coelomic fluid with a fertilization diluent (Table 2, modification of Billard, 1977). Eggs from two females were pooled and divided approximately in half. In one lot (treatment) the natural ovarian fluid was drained and replaced with 200ml of artificial fertilization diluent. Freshly collected sperm from three males was pooled and added to the eggs at a 10^{-3} dilution rate (0.2ml). The other lot (control) was fertilized using the normal dry spawn technique (Lietritz and Lewis, 1976). Incubation proceeded until eye-up was completed.

Table 2. Composition of Artificial Fertilization Diluent

	Grams/liter
NaCl	5.00

Made up with a solution of
50 mM Glycine
20 mM Tris
adjusted to pH 9.0

The large-scale expansion of the fertilization diluent study involved four trials with pooled egg takes from 36, 35, 72, and 71 females, respectively. Each pooled lot was divided approximately in half and treated as described above except that fertilization diluent was added to the treatment groups at a rate of 1 litre/10 females. Survival was monitored to the eyed stage in each trial.

Results

Motility and fertilizing ability of sperm stored for five weeks as described above indicates that success with this technique may vary greatly between individuals and could be related to initial sperm quality (Table 3). However, it appears that sperm stored in such a manner can be equally as viable as fresh sperm.

Table 3. Motility and Fertilizing Ability of Sperm from Three Rainbow Trout Males Stored for 35 Days

Male No.	Motility*	Eyed	Dead	Total	p(live)
1	(+/-)	93	113	206	0.451
2	(+)	137	5	142	0.965
3	(--)	0	216	216	0.000
Control**	(++)	208	13	221	0.941

* Subjective estimate of % sperm showing strong, forward motility. (++) = > 80%; (+) = 60-80%; (+/-) = 40-60%; (-) = 20-40%; (--) = < 20%

** Pooled sperm freshly collected from five males

The initial fertilization diluent trial demonstrated a highly significant ($P < 0.01$) improvement in fertilization success (as measured by eye-up) when replacing ovarian fluid with artificial fertilization diluent (Table 4). The expansion of this experiment to the four production trials showed equally promising results (Table 5). In each trial, the increased success in eye-up when replacing ovarian fluid with artificial fertilization diluent was highly significant ($P < 0.01$).

Table 4. Initial Fertilization Diluent Trial Results

	Eyed	Dead	Total	p(eyed)
Treatment	450	92	542	0.830
Control	595	262	857	0.694

$X^2 = 31.9$ ($P < 0.01$)

Table 5. Results of Production Scale Fertilization Diluent Trials

Trial Number	Treatment	n	p(eyed)	X^2
1	Diluent	62767	0.6532	5967
	Control	52136	0.4254	$p < 0.01$
2	Diluent	54868	0.6013	2288
	Control	48505	0.4515	$p < 0.01$
3	Diluent	108688	0.6331	22760
	Control	91864	0.2541	$p < 0.01$
4	Diluent	131484	0.7383	37400
	Control	124975	0.3341	$p < 0.01$

Discussion

The successful short-term storage of sperm for up to five weeks, allowing matings between individuals with different spawning times, has improved the efficiency of our genetic selection program.

It has previously been reported that ovarian/coelomic fluid is of variable composition and quality between individual females,(Satia, et al., 1974) and does not appear to be better than a properly buffered mineral diluent when used in artificial fertilization (Billard, 1983). The fertilization diluent used in this study appears to have provided a more uniform and stable environment for artificial fertilization than pooled ovarian fluids of rainbow trout reared at 58°F. The relatively poor eye-up results reported for the control lots in the fertilization diluent experiments are consistent with normal results reported for rainbow trout broodstock held at 58° F (J. Parsons, unpublished data).

The techniques described have been expanded to our production brood facility. We collect the anticipated amount of sperm needed at the beginning of the week from at least 50 males, dilute and store it in appropriate aliquots in a refrigerator. Eggs are stripped directly into a colander (draining the eggs of ovarian fluid) and transferred to a container having fertilization diluent. Eggs are added to the fertilization diluent at the rate of 10 females/liter of diluent. Sperm is added to the egg/diluent mixture at a $5x10^{-2}$ dilution (5 ml sperm/liter diluent) and thoroughly mixed by pouring back and forth between a second container. This mixture is allowed to stand for 15 minutes before quick rinsing and transfer to incubators.

The advantages of using sperm storage and a fertilization diluent in a production brood station appear to be:

1) Better utilization of gametes, particularly sperm, resulting in the possible reduction in the number of males carried at the brood site (equally considering genetic consequences).

2) Improvement and uniformity of fertilization rates due to an optimum fertilization environment.

3) Simplified spawning procedure using sperm of a broad and consistent genetic background and separate handling of male and female broodstock.

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OPERATIONS OF A COMMERCIAL PRODUCER OF RAINBOW TROUT

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Abstract-15 minute film showing the farm, feed, processing, distribution, and research divisions of Clear Springs Trout Company. Followed by 15 minutes of slides showing facility design and equipment applications in the farm operations of the company.

FRESHWATER REARING OF ATLANTIC SALMON

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Here in the Puget Sound we are on the verge of becoming successful competitors in the world aquaculture marketplace. This season about 1,000 tons of pen-reared Atlantic salmon will be harvested from Puget Sound, with only a few producers in business. By 1989 about ten times that amount will be harvested, a small increase compared to the projected growth in Norwegian exports to the U.S. from 5,800 tons to 40,000 tons for the same period. In addition to imports, Puget Sound growers will see pen-reared product from east coast U.S. and freshwater raised salmon from Minnesota and Idaho competing in the marketplace.

Growth and diversification in the industry has meant innovation and change in the way fish culture is practiced in the hatchery and on the farm. In fact, that is my theme, people have changed the way fish are managed but in comparison little has changed in the technology we use. I hope to make that point evident while doing some justice to the rather broad topic of freshwater rearing of Atlantic salmon. I will note a few highlights of Atlantic salmon culture history and will describe how Atlantic salmon are being reared by a local company, Sea Farm Washington, whom I wish to thank for the invitation to speak today on their behalf.

The subject of freshwater rearing of Atlantic salmon covers more ground geographically and historically than any other cultured anadromous salmonid. In Europe the natural range of this specie extends from Portugal to the White Sea north of the arctic circle, in North America from New York state north to Hudson Bay. In terms of fish culture history the record precedes that for Pacific salmon: In 1834, two Scottsmen (Shaw and Young) bred Atlantic salmon in wooden boxes to prove that parr fish were really young salmon. For those of you interested, I have an original publication of similar early biological work conducted in England by Malloch in 1912.

Here in America, (according to my trusty Manual Of Fish Culture published by the U.S. Fish and Fisheries Commission in 1897) the Atlantic salmon was first cultured on a large scale on the Penobscott River at Craige Brook Hatchery, founded in 1871. I recommend this manual, in fact I came across it at Dick Noble's office - he was using it to write a hatchery feasibility report! Indeed, that is my theme today, we have the technology and the resource, it has been around a long

time and it does not matter if it is a million dollar facility or the backyard mom and pop operation, what is needed is cooperation of the people involved. In March we will be talking in Ocean Shores about that topic, today however, lets look at a few examples of Atlantic salmon technology from the 1897 Manual Of Fish Culture.

> Regarding water for a salmon hatchery; "The very best is the water from a stream fed by a clean lake of considerable depth, taken a short distance below the outlet of the lake with an intervening rapid... After this, the water of a brook fed largely by springs... But it is better to have the water flow a long distance in an open channel that it may be well aerated, and in cold weather cooled down from the temperature with which it springs from the ground. The next best is pure spring water; but in all cases a cooling and aeration pond is necessary. Lastly, choose ordinary river or brook water, as clean as possible."

Water temperature and cleanliness are keys to Atlantic salmon culture; temperature cycles are needed to entrain the smoltification process, and clean water to rear the bottom dwelling fry which are very susceptible to Saprolegnia. In these terms, commercial hatcheries are using "next best... pure spring water"; the reason being to avoid infectious disease, which we have been successful in doing, however, the cost is in smolt management made more complex by constant temperature water. Hence, the old way - surface spring water, is the best biologically but the more difficult politically. We are working hard on both fronts, to manage Atlantic salmon smolts in constant temperature water and to assess the opportunities for surface water commercial sites.

Another example from the 1897 Manual Of Fish Culture regarding conditions affecting fecundation of Atlantic salmon eggs: "In 1872 experiments were made bearing on the duration of the capacity for fecundation of the eggs... The rates of impregnation ranged from 92.5 % down to zero." How true it is, the commercial fish farmer raising a captive broodstock today is faced with performing the same standard tests to assess the fecundity of gametes produced under a variety of fish culture conditions. Fifty one percent of captive brood Atlantic males tested this year in a delayed fertilization trial produced motile sperm at the seawater farm site and only fifty eight percent of those were motile when tested four hours later at the hatchery. Less variation occurred when brood fish were transferred to cool freshwater just prior to ovulation, in comparison to seawater spawned or constant temperature freshwater spawned. Again, the old method is the best biologically but the more difficult politically. As the industry diversifies, we will see improvements in the options for management of captive broodstock during the critical maturation phase of production.

Another example from the 1897 Manual Of Fish Culture - regarding Saprolegnia: "The best precaution against the growth is the careful picking out of dead eggs... Great loss from it can only occur in consequence of neglect of the duty of picking out the dead eggs... In case of serious attack on fry or older fish to treat them with an exterior application of salt, which, while not a cure-all, is very efficacious and a safe remedy for fish that have reached the feeding stage." As Jim Dills (Manager at Sea Farm Washington's Rochester Hatchery) can testify, Atlantic fry will sit on the bottom and swallow fungus until their mouths plug up or gills fungus over, it is up to the culturist to keep all surfaces clean. Combitanks with biomat substrate are loaded with 50 - 60K eggs and 25K fry, requiring about 10 man hours per day per half million fry for sanitation purposes. Like the manual says, salt baths are effective and safe.

One last example from the disease section: "One of the most uncontrollable diseases attacks salmon fry midway in the sac stage... The most evident symptom is the appearance of scattered white spots in the sac... In 1890 about a third of them (died)... In 1891 there was not a trace of it... In 1892 it returned again... In 1890 this epidemic appeared to run in families... All the eggs from a particular mother would have a common degree of liability to the disease." Today we know that incubator substrate could have prevented much of the Craige Brook Hatchery loss to white spot disease. Nonetheless, the practical fish culture analysis of the problem was applied: "It did not appear to be infectious, as several lots of fry, separated by screens, would occupy a single trough, and in some cases those at the head... Would be totally destroyed, or nearly so, and those below them escape from the attack." In actuality, flow rates were regulated at Craige Brook Hatchery by the number of eggs in a trough such that some troughs probably suffered white spot disease due to high flow rates.

So what's new, and that is just the point, every new technology is simply an improvement on a very old theme, one that we are working daily to adapt to Atlantic salmon in the Pacific Northwest commercial fish farms. Actually, this process began 38 years ago with the transfer of Canadian Gaspe Bay stock to Wizard Falls Hatchery for stocking Hosmer Lake, Oregon. In 1972 the National Marine Fisheries Service, (Manchester, WA) began pen-rearing the Gaspe Bay stock from Wizard Falls as part of a conservation program to restore the east coast Atlantic salmon stocks. Since then, Manchester has produced over a dozen Atlantic salmon broodstocks, some in their fourth generation now. Surplus eggs have provided the original broodstock for commercial growers: The revenue has been used to offset production costs at the Big Beef Hatchery (U.W.). In recent years, commercial growers have purchased fewer eggs and produced more from their own broodstock. Sea Farm Washington has developed Norwegian, Penobscott, and Gaspe Bay Atlantic salmon stocks. This year the spawning was conducted at the Port Angeles farm and water hardened eggs were transferred to the Rochester

hatchery for disinfection and incubation. Eggs are eyed up in Cowlitz style buckets then pooled for hatching into one meter slotted screen combitanks at 50K eggs per tank with 10 liter/minute flow and biomat substrate. Prior to swim up, fry are ponded into indoor 1 meter tanks at 25K/tank with 10-12 liter/minute flow. Water depth is gradually raised from 3 to 20 inches as feeding from automatic feeders progresses. Fish are transferred to 3 meter circular tanks then graded into 5 meter tanks for rearing to smolt sizes of 9/lb. for S-1 and 3/lb. for S-2's. Smolts are transported for direct release to sea pens in 500 gallon tanks equipped with liquid oxygen and air blowers.

Current hatchery development includes practical application of Atlantic salmon biology to identify and separate by grading S-1, S-2, and precocious male populations. Also, we have implemented a seawater hyperchallenge procedure to evaluate the onset of smoltification in constant temperature conditions. Hatchery monitor groups have been established for monitoring freshwater physiological status and survival in seawater pens.

MARINE NET PEN REARING OF ATLANTIC SALMON

By: Jan I. Wiese-Hansen, Scan Am Fish Farms

The farming of Atlantic Salmon on a commercial scale has been developed over the last twenty-five years, first in Norway and later in other countries. Numerous pen designs and rearing tecniques have been developed, but I am sure that we will see a continued evolution within the industry.

I will show pictures of a few different pen designs used in Norway and Washington and discuss some of the difficulties and advantages that I experience as particular to the Pacific Northwest.

The basic idea of pen-rearing Atlantics is to put smolts which range in size from one to four ounces into the sea, feed them well and harvest them at six to thirty pounds one and a half to two years later, doesn't that sound easy?

Before you can put your fish into the water, however, you need pens and for that, a D.N.R. lease is required. The difficulties associated with obtaining that lease are currently the major obstacle for the expansion of the industry in Washington. Even so, the existing farms' productions could reach 10,000 tons, with a projected market value of 80 to 100 million dollars by 1991. That is more than the total imported quantity of salmon from Norway in 1986. Most of the production will be sold December through May, when there are almost no wild runs available to the market.

There are special tecniques required for farming Atlantics in the Pacific Northwest and the necessary ground work has been done, first by National Marine Fisheries Service through Connie Mahnkens program, and later by Sea Farms Washington and ourselves. A broodstock has been established and the necessary number of eggs for commercial ventures has become available.

I am now going to talk about the most important factors for salt-water performance, survival and growth and will be expres--sing my personal opinions. These are not necessarily shared by

everyone else, or backed up by hard scientific facts.

The determining factors can be divided into three main areas:

A. Smolt Quality
B. Husbandry Tecniques
C. Site Performance.

Smolt quality is determined by the following:

1. Stock of Origin: Effects: acceptance of full salinity, disease resistance, grilsing (jacking) rate, suitability for pen-rearing due to low stress level and a rapid growth rate.

The East Coast Gaspe stock seems to be an almost perfect fish for farming, and may well be the fish of choice in the future.

2. Saltwater Readiness: We challenge fish by actually moving a group to the saltwater site. For fish being accustomed to a fluctuating temperature rate, a vigorous feeding response should be observed within a day or two. Smolts coming from a water source with a constant temperature should begin feeding within four or five days. The traditional 48 hour challenge, considered a success if the fish do not die does not tell us much at all. When you become familiar with a particular stock, you can normally assess the readiness of a group by looking at their color, shape and behavior.

3. Disease Immunity: Most fish culturists dream of having a disease-free hatchery and disease-free fish. The reality is, however, that when fish enter salt water, they are going to be exposed to existing pathogens. Those fish without a developed immune response will suffer severe losses. Our experience clearly indicates that immunities built up by a combination of vaccinations and exposure will provide for a survival rate of 75 to 90%.

The second determining factor for salt-water performance is your husbandry tecniques. A primary rule for farming Atlantics is to avoid unnecessary stress. The most stressful experience in a farmed salmons life is during the transfer from the hatchery into the saltwater. The handling, transport and the direct entry into a totally different environment will normally cause some mortalities. During the first six months following saltwater entry, recorded losses in Puget Sound have been as high as 80%, perhaps higher for some groups, and as low as 8% for others. We therefore, almost religiously, pay close attention to all details when we move our smolts.

Specifically, we try to reduce stress and physical damage to the fish by:

A. Making sure that they are ready for saltwater.

B. Avoiding grading and handling for a period prior to anticipated transport.

C. Discontinuation of feeding 2 or 3 days before transport.

D. Avoiding excessive crowding and the use of dip nets when loading the transport tanks.

E. Carefully observing loading densities, temperatures and dissolved oxygen levels.

F. Avoiding sudden changes of the salinity, temps and D.O. levels by gradually pumping saltwater into the transport tanks prior to the direct discharge of the smolts into the sea pens.

G. Avoiding the introduction of smolts into the sea when strong currents could occur before the smolts have adjusted to their new environment.

When these details have been properly followed, smolts will normally show a feed response in a very short time and can then be medicated, if necessary, for any outbreaks of furunculosis. They should have absolutely no further handling for the next six months or more. After 7 or 8 months in saltwater, Atlantics can again tolerate some handling.

That is the time to split up groups, size grade and inventory. After this point, there are really no problems with these fish. They don't develop B.K.D. problems, they are very resistant to Vibrio and we have never seen severe outbreaks of "furunc" among the larger fish, although there are visible signs of a mild infection in a percentage of the population.

Site Performance: The third important factor for the success of a fish farming operation is the quality of the site. Ideally, it should be reasonably sheltered, even though off-shore pens have been developed. The depth should not be less than 45 feet, but 100 to 140 feet is preferred. Currents should vary between one-half to three knots most of the time. Frequent and sudden changes of salinity and temperature will stress the fish. It is most important to avoid areas where algae blooms are likely to occur. With the most optimistic site, an algae bloom can still, on occasion, occur with distressing results. A bloom can cause a sudden drop in the oxygen level or, as with chaetoceros, direct physical damage to the gills of the fish. In either case, severe losses may occur. Algae blooms represent, by far, the most serious threat faced by this industry in Washington. After experiencing several blooms, one quite severe, we are working to find methods of minimizing the problem.

Some methods we are currently using, or implementing are:

1. The introduction of oxygen into the water column.
2. Airlift systems to move water from depths with less algae present.
3. Hanging nets with a growth of mussels, caprela, etc. to reduce the concentration of algaes.

By the combination of these tecniques with a monitoring and prediction program, I am confident that we can minimize this problem.

In conclusion, it is my opinion that the pen rearing of Atlantic Salmon in Puget Sound is here to stay. We are ready and willing to develop a 500 million dollar industry when it becomes politically feasible.

The Pansize Salmon Business

by

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My topic today is "The Pansize Salmon Business". I will start by giving a little history on the industry. In the late 1960's Domsea Farms, along with National Marine Fisheries Service, did a feasibility study on the production and marketing of pansize salmon raised in saltwater net pens. By the mid 1970's there were a number of operations involved in this activity. They included Domsea, Pacific Ocean Farms, Mariculture N. W. and Aquasea. Weyerhaeuser Company did a feasibility study in Henderson Inlet near Olympia and later purchased Oregon Aqua Foods in Newport, Oregon, which had been involved in pansize salmon, but was just then switching to ocean ranching. Because of the development of the saltwater industry, a need for smolts became apparent and a number of small freshwater growers went into production.

Swecker Salmon Farm was one of these. We started our operation in 1974 with 250,000 eyed coho eggs from the lower Kalama Hatchery. We had five acres, two dirt ponds and one well which produced 500 gallons per minutes. In contrast, today, we have 70 acres, 49 ponds and 2500 gpm of fresh water. In 1974 we had a contract to produce 200,000 fifteen gram smolts. Today our annual production is:

200,000 pounds pansize salmon

400,000 Atlantic salmon smolts

400,000 coho & chinook fingerlings & smolts

Next year we plan to double the number of Atlantic Smolts we produce. As you can see our operation has diversified a great deal from our original plan of raising a few coho smolt.

This diversification is an example of what is happening generally in aquaculture as well as the pansize industry. When we originally started raising pansize salmon, everyone thought that saltwater net pens were the only way to go. In 1973 people in the industry dissuaded Debby and I from attempting to raise pansize coho in fresh water ponds. Today these fish are raised in saltwater ponds and pens and freshwater ponds and tanks fed by pumped wells, springs and by diverting water from streams. All of these production options have advantages and disadvantages. However, by working together this diversity helps to provide year round production of high quality product. I believe that aquaculture will continue to find ways to raise more products in more and varied environments in a cost effective manner.

For Swecker Salmon Farm new products and services has allowed us to continue with our pansize program on a more cost effective basis. We are able to pump our water for the production of fingerlings and smolts and then allow it to gravity feed through large dirt growout ponds. Coho seem to be very hardy and resistant to disease. Even with this water reuse plan we have not needed to do any diagnostic work on coho for more than five years. They also seem particularly adaptable to many culture strategies. When we first started raising pansize, the industry standard was to harvest a 12 ounce fish in two to three years. Now we produce this same fish in one to two years. Natural size variations in each

population gives us year round production from the end of the first year to the end of the second year.

Some examples of different companies using different rearing strategies are as follows. In saltwater, Blue Water Farms at Port Townsend has a small floating cage complex in saltwater and is using pansize as a means of generating cash flow while they develop a production strategy for a larger fish. Domsea Farms has two net pen sights near Bremerton. One of these is the largest pen complex in the world at approximately five acres. Domsea has been the leader in the culture of pansize salmon and was one of the few companies which was able to withstand the early shakeout in the 1970's and stay with the program. They recently negotiated the sale of their fresh and saltwater sites to a Norwegian company, Global Aqua, which plans to convert them to Atlantic Salmon. Domsea is experimenting with the production of pansize salmon in fresh water in Idaho. Near Domsea is an operation called Viking Salmon Farm also raising pansize coho and trying out larger fish of other species. In Ssouth Puget Sound the Squaxin Indian Tribe is producing this same product along with delayed release coho and steelhead in their saltwater net pens near Squaxin Island.

In freshwater, Seasprings Company near North Bend is a small fresh water grower of pansize using a cool-cold water spring as its water source. They are experimenting with the development of a fresh-water brood stock. The Rochester area, because of its high quality aquifer has proven to be a good place to rear all kinds of salmon. There are three farms which produce fingerlings for their own salt water operations. They include two Domsea facilities and

the Seafarms of Norway operations. Three other sites are raising pansize, including Swecker, Steelhammer and Carlson Salmon Farms. All of the Rochester operations use circular ponds or tanks which can be cement, fiberglass or doughboys. We pump ground water directly into these from wells about 100 feet deep. Some also gravity flow their water through additional dirt raceways to growout larger fish. Two other operations in the Morton area, Cascade Aqua Farm and Glenoma Trout Farm, are using stream water diverted through dirt ponds to raise pansize. Cascade is a highly successful company while Glenoma has faltered through several owners. Other small pond culture operations have started up, but have not produced any fish yet.

Oregon is also producing pansize salmon. This is being done in fresh and saltwater ponds by Oregon Aqua Foods and in saltwater raceways and seacages by Anadromous, Inc. This diversified production of pansize salmon has allowed for the development of many supporting industries to supply, process and market these products. One example would be the processing company owned by Swecker Salmon Farm, Inc. in Tumwater called Aqua Pak. We will process about one million pounds of pansize salmon in 1988 from most of the growers mentioned above.

Pansize salmon is not viewed as the best option for farming by the aquaculture industry for many reasons. These include requirements for intense grading, special processing and unique marketing strategies. The main problem however, has been price and the cost per unit of production. In spite of this, however, there continues to be a vital economic niche occupied by this product. In

many ways, I think pansize salmon can be viewed as a stepping stone to economic development. Here is a list of some of the parallel industries which have developed with the significant contribution of "baby coho salmon" as they are sometimes called.

- Primary fish processing, valued added processing and repackaging of fish products.
- Packaging material manufacturers.
- Marketing operations of both live and finished product.
- Fish smoking and canning.
- Management services, production services, consultive services.
- Feed manufacturing and waste recycling.
- Cold storage facilities for fresh products.
- Freight consolidation and forwarding to truck lines and airlines.

In summary, the diversity and change which has occurred in the pansize industry over the past nearly twenty years is really a model for the future development of the salmon aquaculture industry. New methods of production and product integration will result in higher profits and wider participation in fish farming. As an industry, we must plan to grow, change and innovate to remain competitive.

Aquaculture - A Worldwide View

Natalie Fobes, Seattle Times

Thank you for having me here today. I am a photojournalist at the Seattle Times. I saw my first salmon in 1981 at Ore-Aqua while doing a story on ranching for the paper. I was enthralled. Since that time I've tried to get every assignment that dealt with salmon and its habitat, or its people: the fishermen, Indians and biologists.

In 1986 I applied for and received a $25,000 grant from the Alicia Patterson Foundation which enabled me to take a leave of absence from the paper and spend 14 months photographing and writing about the Pacific salmon. My goal was simply to see what the status of Pacific salmon was around the Pacific Rim. I soon found out it was not that simple.

The project was divided into five general areas. The biology of the Pacific salmon, the Pacific Rim cultures that grew around the salmon, habitat degradation, commercial and sport fishing and salmon ranching and farming. To further focus the stories, I assigned a geographical area to each topic, with exception of the biology which was shot anywhere I could find it. I concentrated on the Yakimas and Tulalips for the cultural aspect, focussed on Washington for habitat degradation. Commercial and sport fishing I photographed in Alaska and for salmon ranching and farming I went to New Zealand and Japan. The completed project was published in the Seattle Times November 22. A few copies are on the back table. More can be obtained by writing the Times.

Salmon culture is here to stay. All countries now supplement their wild salmon runs with hatchery produced fish. Japan and the Soviet Union introduce approximately 2 billion smolts into the system each year, the United States releases about 800 million from government hatcheries and Canada produces about 500 million.

Salmon farming and ranching will increase in the future. Six countries are engaged in large-scale private Pacific salmon culture. Over 12,700 metric tons of farmed Pacific salmon were harvested in 1986 throughout the world. In 1980 only 2,400 metric tons were harvested.

New Zealand both ranches and farms chinook, what they call quinnats. The quinnat was introduced to the country in 1876 from Sacramento River stock. The South Island citizen group responsible for the introduction waited patiently for three years for the fish to return. Just as they were about to give up hope, a hunter brought good news. A 15 pound quinnat had been caught on the Rakaia River. Shot, actually, by a confused member of a hunting party.

In recent years, seven companies have invested in ranching and farming. New Zealand has good conditions for salmon culture: the stock is virtually disease free, the water supply is plentiful and unpolluted, there are no

commercial fishermen targeting salmon and there are very few neighbors to complain of views cluttered up by netpens and hatcheries. In fact, there are only 300 residents on the southernmost island in the country, Stewart Island, where most of the salmon farming takes place.

New Zealand Salmon Company is the largest in the country. It, like 6 out of seven of the other companies involved in ranching, operates a farm at Stewart Island, the southernmost island in the country. The company owns four seperate farms with 28 or 30 pens each. Each pen is 8 meters in diameter and 7 meters deep and holds a biomass of 2 ton of salmon.

The beauty to farming is the efficiency of harvest. New Zealand Salmon Company stuns the fish with CO2, bleeds them and within hours tons of fish are on a plane bound for Seattle or Japan. The harvest was 200 metric tons in the 86-87 season. It can be even more efficient than this. One farmer in Canada transports live fish from his pens to the processor where the salmon are subsequently killed and dressed.

A number of problems, both long and short term, can befall the fledgling industries of ranching and farming. New Zealand's biggest problem is the large incidental by-catch of salmon by commercial fishermen targeting for blue cod and tuna. Fully one-twelfth of the ranched salmon are caught and sold by the fishermen. A long range worry is that unregulated growth in the industry will put too many salmon in the feeding grounds of the continental shelf. And another fact the Kiwis can't do anything about is that New Zealand is 17 hours flying time from the marketplaces of North America's west coast. New Zealand, unlike Japan, exports most of its salmon.

Japan celebrates one hundred years of salmon culture this year. It operates the largest hatchery system in the world. Sixteen prefectures and the island of Hokkaido release over 2 billion smolts per year. The return in 1985 totaled 49.3 million chum. The recovery rate was 2.71%. Essentially one hundred percent of the chum, and almost all of the pink and cherry, are hatchery produced in Japan. As the government stepped up its production of fish, beginning with the first of the five year plans in 1954, the fishermen became dependent on the increased number of returning salmon. The short coastal rivers, which suffered from overfishing and pollution throughout most of the century, will never be able to produce the fish now necessary to sustain the runs.

The Japanese government owns the rights to the salmon. Rights of salmon harvest in the coastal areas and within Japans boundary waters are passed to the prefectural governments which distribute them to fisherman's cooperatives. The Japanese Fishery Agency gives the far seas harvest rights of the Bering Sea directly to the companies involved. These

contracts are renewed every five years. These laws are almost a century old.

Ocean coops harvest salmon in traps, known as setnets, over 300 meter long. This fish is sent to the markets. Eggs are taken from fish caught in river traps, v shaped nets which stretch the width of the river, or those seined. Over the last few years the peak of the runs have been getting later. Biologists attribute this to the efficiency of the traps. Most salmon are unable to make it past the traps while the devices are operating. Biologists have suggested that the traps shut down two days a week. This would allow a more even distribution of early and late fish from the run for use as brookstock.

Iwate prefecture has one of the highest recovery rates in Japan. Chum fry are raised in freshwater pens until approximately 1.5 grams when they are tranported to the ocean pens. After being reared in temporary ocean pens for almost two months, they are released in the ocean at 4 to 5 grams. The return rate for Iwate Prefecture ranges as high as 7 percent. The average for the prefecture is 4 percent. The feed is a dry pellet made of fishmeal, corn, minerals and vitamins.

The Japanese have been farming salmon since 1975. Currently, 8000 metric tons are produced. The 15 gram yearling coho fry are placed in saltwater pens 10 meters by 10 meters by 7 meters deep for 8-9 months. At that time, the water becomes too warm and the salmon are harvested. The average weight is 2.5 to 3 kilograms and the fish are 55 cm. long.

Pollution is of concern. At the Shizugawa area coop, eleven years of fish farming has resulted in a build up of 15 to 30 cm. of debris on the bottom of the 14-22 meter deep bay. The fisheries coop is concerned what this pollution might mean for other kinds of aquaculture in which the coop engages. Different solutions have been discussed include something as simple as moving the pens around the bay or experimental as installing a charcoal filter on the bottom to neutralize the salmon feces and food debris.

The Japanese are content with the number of salmon the system is producing. What they are trying to do now is raise the quality of the fish. This is being attempted in three ways. The government is propagating different species such as coho and cherry. Researchers are trying to increase the oil content by transferring eggs from late-returning salmon to rivers where the peak of the run is traditionally earlier. And, in an attempt to keep the integrity of the gene pool, the government is making plans to set aside stock on four Hokkaido rivers for use as "gene bank." Commercial fisherman would be forced to work under new regulations and are fighting the proposal.

The Japanese system is simpler to manage than any in North America for many reasons. 1. Almost all fish are hatchery produced so there is not the problems associated with managing mixed stocks. 2. Salmon is produced for only one user group: the commercial fishermen. The aboriginal people of Japan, the Ainu, have no special rights to the salmon even though their ancestors depended on the fish as much as the native peoples of North America did. The salmon legends and ceremonies of the Ainu and Indians are astoundingly similar. Also, there is virtually no recreational fishing for salmon. I only heard of one case where salmon were caught legally by citizens. Once a year in Iwate prefecture, people pay about 6 dollars to wade into a stream and grab a very dark chum. Only one fish per person please. 3. The Japanese government in most cases doesn't have to worry about habitat degradation as all salmon are caught in the setnets or in traps at the mouths of the rivers. As a result, gravel from the river's bottoms has been dredged for building material. Dams block many streams and urban pollution is put in the rivers. Well water is used for hatchery operations.

Although most of the public is not allowed to see the salmon, the mystery of the creature continues to hold an allure for the citizens of Japan. School kids learn about salmon in their science classes. Many schools in Honshu and Hokkaido raise salmon from eggs. One Sapporo junior high has a salmon hatchery on the premises and the Come Back Salmon Society, formed to pressure the government into cleaning up the Toyohira River, has built a museum totally devoted to Pacific salmon.

But the resource has been taken from the people. More than one person asked me if I had ever seen a salmon spawn in the wild. More than one person was astounded when I described the frenzy of the spawning salmon. Anthropologist and Come Back Salmon President Masakazu Yoshizaki is distraught that the salmon is now only an efficient protein machine. He complains the Japanese people used to live with nature and states that they have forgotten how. "The lack of pure nature destroys the human mind," said Yoshizaki. And pure nature is the essence of Pacific salmon.

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Fish and Game, Washington Department of Fisheries; Chikaro Iioka, Iwate Prefecture; William C. Atkinson; Connie Mahnken, NMFS; Jon Lindbergh; Masakazu Yoshizaki, Come Back Salmon; Fishermen from Kensencho and Shizugawa fisheries cooperatives; Kazuyuki Shoji, Sapporo teacher; Pat Bell, North Vancouver School District; Tom Murdoch, Adopt-a-stream; Tom May, Royal Pacific Salmon.

BACTERIAL INFECTIONS AND LESIONS ASSOCIATED WITH AIR SPAWNING OF TROUT

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A mortality of about 20% occurred in female rainbow trout at the Ennis and Creston, Montana National Fish Hatcheries. Clinically affected fish were lethargic and occasionally, but not always, showed fungal infections. External hemorrhagic lesions were also seen on some fish. Gross pathology consisted of a whitish-colored pseudomembrane formation on spleens and often livers and hearts. The pericardial cavity of affected fish was often filled with a granular whitish-colored fluid. Pyloric caeca were inflammed and adherent to each other. Many of the affected fish had large numbers of eggs that were undergoing resorption and often adherent to each other. These lesions were present only in females; they were not observed in males.

Histologically, the most common lesion found in tissues was the extreme cellular inflammatory response resulting in pseudomembrane formation on spleens, livers and hearts. Lymphocytes and polymorphonuclear leukocytes were present, but the most common cells were macrophages, many of which contained ingested gram positive bacteria and cellular debris. A similar cellular response was present around and between pyloric caeca causing them to adhere to each other.

Innoculation of tryptic soy agar and blood agar with material from the pseudomembranous growths covering the organs

and fluid from the visceral cavity yielded both gram negative and gram positive organisms. The gram positive organisms were identified as Aerococcus viridans and Lactobacillus sp., and appeared to be causing the extreme cellular inflammatory response noted. The gram negative organisms were of the genus Aeromonas and Pseudomonas.

Ross and Toth (1974), isolated a Lactobacillus sp. from moribund three-year-old female rainbow trout at the Hot Creek, California State hatchery; they called the disease Pseudo-kidney disease. A similar observation was reported by Holt (1970). Both authors suggested that the organism appears to be low in virulence requiring that fish be sufficiently stressed before an infection will proceed.

More recently the name Lactobacillus piscicola has been proposed for a group of seventeen bacterial strains that were isolated from diseased rainbow and cutthroat trout, as well as chinook salmon (Hui, et al. 1984).

Evidence suggests that the disease seen in the fish from Ennis and Creston hatcheries may have been transmitted during air spawning of female brood fish. The stress of spawning, along with the bacterial infection, appears to be sufficient to cause mortality.

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Introduction to IHNV Broodstock Culling Panel

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IHN is a serious disease of salmonids, causing catastrophic losses in some situations. IHN stands for Infectious Hematopoeitic Necrosis. Infectious means transmissable, hematopoeitic means blood cell forming tissue, necrosis means destruction of tissue. The disease is caused by a virus given the simple name of IHN virus or IHNV.

Viruses are pathogens which are not technically living, like bacteria or parasites, and we cannot eliminate them with the chemicals used to treat fish. Viruses also cannot be seen by light microscopes, which are those used to see most bacteria and parasites on our fish. In addition, we cannot simply inoculate nutrient media with viruses and see them grow. They need the host cell to replicate.

The method used to detect IHNV is called tissue culture assay. We take healthy fish cell cultures, which have been developed for consistancy and longevity, and inoculate them with sample tissue, such as ovarian fluid. If the cells die in a manner typical of IHNV, we do another confirmatory test, using the cell cultures and antiserum for IHNV to confirm the isolation of the IHN virus.

I'm telling you the details of the detection of IHNV because it explains part of the difficulty in giving cut and dried answeres on whether or not you have IHNV in your fish.

Another part of the detection difficulty is that if a population of fish has IHNV, you cannot always find it. The two best times to test for the virus are when fish are spawning or when they are actually dying from the disease. For this reason, broodstocks of both anadromous and non-anadromous salmonids are routinely sreened for IHN virus at spawning.

We know that prevention of IHN is only accomplished by eliminating infected fish and contaminated water supplies. For this reason, a procedure called broodstock culling was developed. Broodstock culling basically means separating eggs during incubation while tests on the parents are completed. Then if a test, say for IHN, is positive, you can either destroy those infected eggs or rear the infected eggs separately from the non-infected.

The procedure is not complicated, but requires much attention to detail. Here's how it's done: each person handling fish must disinfect his or her hands, spawning knife, bucket and anything else that could contaminate samples from one fish or pool of fish to another. Gametes must go into a numbered container, which corresponds to a numbered sample. Water from one fish or pool of fish must not flow over another group during incubation.(This is why the bucket incubation system was developed.) Then, in the lab, the same care must be taken not to cross-contaminate samples. This means numbering test tubes and plates, using different pipets or pipet tips and paying as much attention to detail as was done on the hatchery. Because the procedure is so de-

tailed, it can limit the number of fish spawned per day and cause many extra hours in the lab.

In the early 1980's, broodstock culling for meant all eggs from infected adults were destroyed. We have developed better isolation incubation and egg disinfection techniques and are now rearing progeny from infected aduts. We at the Dept. of Fisheries are struggling with IHN related decisions right now, and many of you on the hatchery as well as the administrators probably wonder why we can't seem to make up our minds on reccomendations. That is why we invited these three fish virologists to speak about this subject.

Production Trials of Rearing Progeny from Adult Salmonids Infected with Infectious Hematopoietic Necrosis Virus

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Extensive laboratory experiments and field trials have failed to demonstrate that progeny from parents infected with infectious hematopoietic necrosis virus (IHNV) develop clincial IHN disease or carry the virus in a form normally detected by cell culture assays. The risk of using gametes from carrier adults in a hatchery production regime was the next step in assessing this possibility. Trials were conducted with fall chinook salmon at one experimental facility and one production hatchery and with steelhead trout at a production hatchery. In each case the eggs were disinfected with 100 ppm iodophor after water-hardening and egg incubation, hatching and early rearing of juveniles was in water known to free of IHNV.

In two consecutive years Elk River stock fall chinook salmon from individual mating pairs of adults in which one or both parents were IHNV carriers were reared to release without any evidence of IHNV. The total number of smolts from IHNV positive mating pairs released over the two brood years was approximately 135,000.

In 1986 family groups of Wallowa stock summer steelhead trout containing gametes from IHNV positive adults were reared in tandem raceways at Irrigon Hatchery also without any evidence of IHNV up to release. Approximately 500,000 smolts were produced from the family groups in which 22% of the females and 48% of the males were IHNV positive.

Also in 1986 fall chinook salmon (up-river bright stock) with about a 50% IHNV positive carrier rate in parental groups were reared under normal production procedures at Bonneville Hatchery without any evidence of IHNV present in the offspring. Progeny from IHNV positive parental groups in this case was approximately 3.5 million which were released.

Progeny from IHNV negative parents serve as control groups in each of the trials and differential fin clipping and coded-wire tagging of positive and negative groups were done to identify them as mature adults. This will afford the opportunity to evaluate the prevalence of IHNV in the adult fish to determine whether or not covert transmission of the virus is occurring.

PANEL DISCUSSION-IHN CULLING

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The concept of broodstock selection has been used in fisheries for many years and the elimination or "culling" of adults with undesired traits provides a tool to improve fish stocks. This process assumes that the undesired traits have a genetic basis. In recent years, the practice of broodstock selection for control of fish diseases, especially IHN virus, has been adopted at several hatcheries. This technique involves testing each adult for IHNV, discarding gametes from IHNV-positive fish and separate rearing of individual families, significantly increasing both cost and effort required to operate hatcheries.

Fish health workers differentiate between horizontal transmission of infectious agents (transmission through the water from fish to fish) and vertical transmission (transmission from adult to progeny via the gametes). Most fish diseases are relatively easily transmitted horizontally and a few are known to be efficiently transmitted vertically (e.g. infectious pancreatic necrosis or bacterial kidney disease). Just as broodstock selection will not be effective for traits that are acquired from the environment, hatchery workers will not be able to prevent diseases that are horizontally transmitted unless the fish can be protected from exposure to water containing the infectious agent. Conversely, the use of pathogen-free water supplies will not

prevent diseases that are vertically transmitted. Effective control of fish diseases requires an understanding of the biology of the infectious agent including the role of both vertical and horizontal transmission. Diseases that are transmitted by both methods require a combination of pathogen-free water supplies, adult inspection, and use of disease-free gametes.

Hatcheries designed and operated to include: individual adult screening, the spawning of one male with one female, surface disinfection of fertilized eggs, and individual rearing of single family groups in pathogen-free water will provide hatchery workers with the maximum flexibility for broodstock selection whether for fish disease or for important genetic traits. The hatcheries of the future will regard adult selection as one of the most important parts of the hatchery operation. Hatcheries will rapidly assay adults for a variety of genetic traits and infectious agents. Matings will be made on the basis of the information obtained.

Broodstock selection for disease control can be expected to be successful for diseases that are vertically transmitted at high efficiency provided that the hatchery is supplied with pathogen-free water. Examples where this technique has shown benefit include infectious pancreatic necrosis virus and bacterial kidney disease. The failure of broodstock selection alone to control IHNV is due to the lack of virus-free water supplies at several of the locations where the method has been tried. Control of IHNV

has been achieved at some facilities by surface disinfection of fertilized eggs and rearing fry in virus-free water, suggesting vertical transmission of IHNV is relatively inefficient.

BUSCH, R.A. 1987. PANEL DISCUSSION - THE RELATIVE VALUE OF CULLING IHN VIRUS INFECTED FISH STOCKS AS A MEANS OF DISEASE CONTROL. 38TH ANNUAL NORTHWEST FISH CULTURE CONFERENCE, DECEMBER 1-3, TACOMA, WASHINGTON.

ABSTRACT

The culling and destruction of disease infected fish has been extensively practiced for many years as a means to limit the incidence and occurrence of disease in both hatchery and free living populations. The practice of culling adult spawning broodstock and production stocks of eggs, fry, and fingerling fish infected with infectious hematopoietic necrosis (IHN) virus has become an accepted hatchery management procedure, particularly with anadromous salmonid stocks at state and federal hatcheries in the Pacific northwest. However, the value and effectiveness of this practice has not yet been clearly demonstrated.

One practice is to cull IHN virus infected adult fish at the time of spawning in order to prevent infection in the progeny. This procedure assumes that we achieve 100% accuracy in initial detection, that the IHN virus is vertically transmitted, and that this is the best time to prevent infection of the progeny stock. In fact, our current tissue culture based virus assay methods for reproductive fluids and tissues may not efficiently detect a virus titer of less than 1.0 X 104 PFU/cc and, as such, many low level infections will probably remain undetected. Secondly, IHN virus has not been conclusively shown to be vertically transmitted. In fact, if vertical transmission occurs at all, it is certainly only a rare occurrence. Lastly, it has been repeatedly demonstrated that eggs taken from IHN infected broodstock can be routinely incubated, hatched, and reared under virus free conditions and produce a certifiable virus-free stock of fish. Therefore, the culling of IHN virus infected broodstock at the time of spawning does not appear to be a potentially effective or necessary method of controlling the disease, particularly when broodfish are not in excess.

A second practice is to cull IHN virus infected production lots as eggs, fry, or fingerlings to control the disease in the hatchery, limit the effects of the effluent on the receiving waters and fish stocks, and prevent the progeny returning to spawn as infected adults. This procedure assumes that the infected stock cannot be effectively isolated in the hatchery, that reducing the level of IHN virus in the effluent waters will limit its impact on the resident fish stocks, and that IHN virus infected smolts return as IHN virus infected adult spawners. In fact, if the hatchery is not specifically designed and operated to isolate diseased stocks of fish, other stocks will most likely have already been infected by the time the disease is first detected due to the high titer of virus released into the water from an infected population, the high survival rate of the virus in the water, and the very effective and efficient horizontal transmission of the virus through the water and across the gill epithelia to susceptible stocks. IHN virus is generally

considered to be a hatchery disease that is exacerbated by the environment, loading density, and stress associated with a production hatchery operation. Even though the disease can be routinely found in feral and wild stocks of salmonid fish, with some exception given to certain strains of the virus in sockeye salmon, it does not appear to have an adverse effect on resident populations. Lastly, IHN virus has not been shown to be a recurrent disease. There is no evidence that fish which originally become infected with the virus as eggs, fry, or fingerlings, will return as infected adults at the time of spawning. In fact, it appears that those returning adult fish that are shown to be infected with the virus at the time of spawning are actually virus-free fish that have only recently acquired the infection during their up-stream migration or, more commonly, while being held in virus infected waters prior to spawning. This fact has been amply demonstrated on captive rainbow trout broodstock. When a certified disease-free spawning population is infected with the virus for the first time as adult fish, they demonstrate a high incidence of the virus at the time of their next spawning but are negative for the virus thereafter including all remaining spawnings. Conversely, when the stock is initially infected with the virus as fry or fingerlings, the incidence of infection at the time of first spawning two to three years later is 0% to 5%.

Based on the above arguments, I would conclude that the current practice of culling IHN virus infected hatchery stocks of fish most likely does not significantly reduce the incidence or severity of the disease in either hatchery or downstream resident populations. I would recommend that IHN virus infected stocks only be culled from production when there is an obvious excess of fish available and the culling is only for the purpose of limiting labor costs associated with epizootic disease occurrences.

A CASE STUDY: SALMONID HATCHERY EFFLUENT TREATMENT IN SECHELT, B.C.

Abstract

A self cleaning particulate filter (Triangle Filter Model TF48RB) was installed at United Hatcheries Salmonid Facility to treat the effluent prior to discharge to the marine environment.

The B.C. Ministry of Environment and Parks, Waste Management Branch set maximum allowable effluent discharge concentrations of BOD5 10 mg/L, TSP 10 mg/L, NH3 1 mg/L, NO3 3 mg/L, and PO4 0.5 mg/L. In all cases the post treated effluent concentrations were acceptable. For example, total suspended particle (TSP) concentrations ranged from 23 to 91 mg/L prior to the filter treatment and were reduced to less than 1 mg/L after the filter.

In addition to the United Hatchery study, a summary of case studies from Scandinavia is included.

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Introduction

The environment is affected by the effluent discharge from intensive aquaculture systems. The unchecked effluent may lead to the eutrophication, deoxygenation and sediment growth in the receiving aquatic environment (Liao, 1970; Enell, 1987; Enqvist and Larsson, 1987).

The B.C. Ministry of Environment and Parks, Waste Management Branch has addressed this concern and has established objectives for the discharge of effluent to marine and fresh waters from fish hatcheries (Table 1).

Liao and Mayo (1972) point out that typicallly effluent treatment processes involve either one or a combination of the following:

- Aeration
- Sedimentation
- Filtration
- Disinfection
- Activated Sludge
- Lagooning (settling ponds)
- Digestion or Equivalent (such as dewatering and incineration)
- Coagulation
- Absorption (taste or odor removal by activated carbon)

The focus of this case study was the success of a self-cleaning particulate filter (Triangle Filter, Model TF48RB) in treating the effluent from a salmon hatchery prior to discharge to the marine environment. The criteria for success were simply the ability of this particular filtration process to meet the objectives as laid down by the B.C. Ministry of Environment and Parks, Waste Management Branch.

Materials and Method

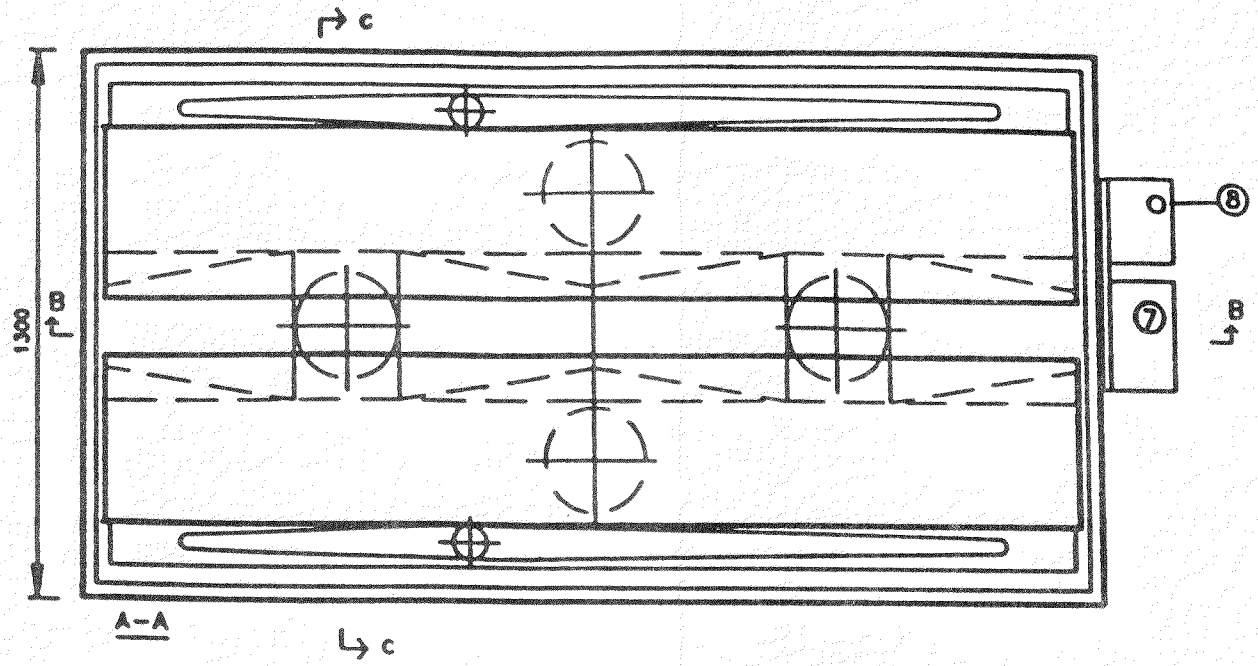
Site: United Hatcheries

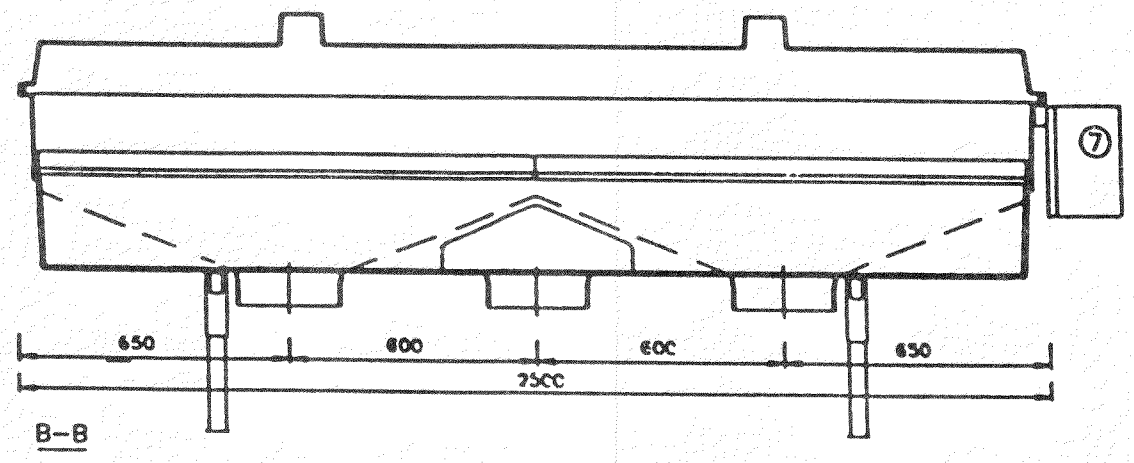
United Hatcheries is located in Sechelt, B.C. on the western bank of Sechelt Inlet.

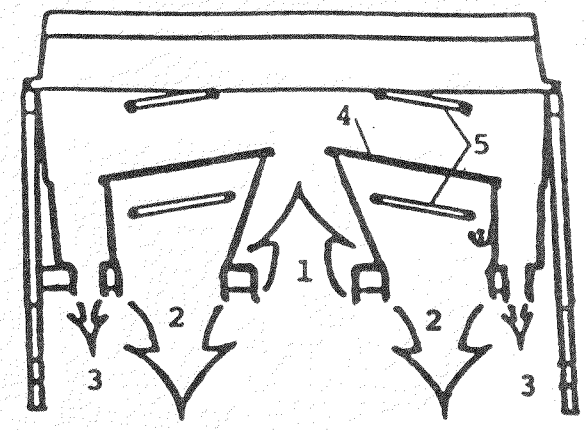
The hatchery, in 1987, had in production approximately 525,000 chinook salmon (Oncorhynchus tshawytscha) and 30,000 coho salmon (Oncorhynchus kitsutch) smolts. At the time of this study, early spring of 1987, the chinook and coho averaged 1.6 and 0.8 grams, respectively. The fish were fed daily at a rate of 2.3% body weight.

Diagram 1

The Triangle Filter TF48RB







EXPLANATIONS

1 INLET WATER OD 250 MM (2 PCS)
2 OUTLET PURIFIED WATER OD 250 MM (2 PCS)
3 OUTLET SLUDGE WATER OD 75 MM (2 PCS)
4 FILTER PLATE 1200x400 MM (4 PCS)
5 WASHING NOZZLES
6 ADJUSTABLE STAND
7 CONTROL BOX 220 V AC
8 RINSE WATER 3/4 "

Daily tank cleaning consisted of brushing the walls and bottom of the tanks, removing the standpipe and flushing the detritus to discharge. The total flow rate of discharged effluent varied from 600 liter/min. to 1200 litre/min depending on the degree the tanks required flushing.

Filter Installation

The self-cleaning particulate filter (Triangle Filter, Model TF48RB, Diagram 1) is a simple flow through system and was installed downstream of the tanks.

The 25 cm (10 inch) pipe which channelled the discharge from each of the tanks was connected to a manifold beneath the filter. Water for the wash cycle was provided 5 litre/ min. and 4-5 bar (50-60 psi) and finally power (220 volt/single phase) was supplied.

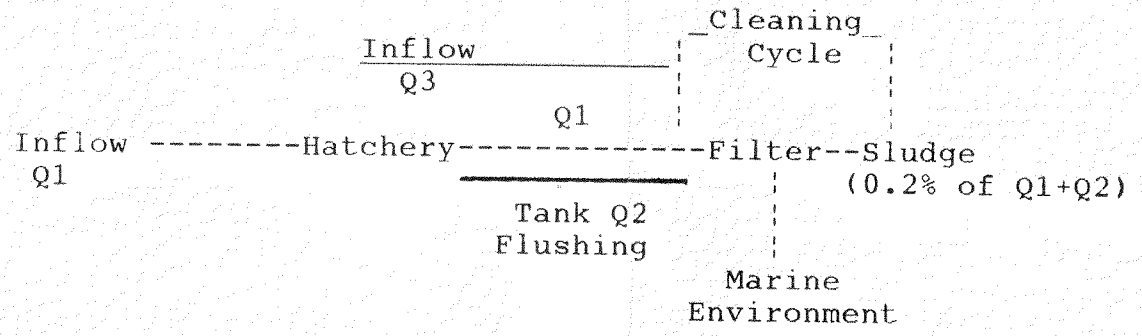
Filter Operation

Basically, the effluent from the hatchery travels up from the manifold through the filter reservoir and then down through two sloped pairs of 60 micron filter plates. The filtered effluent then flows into the marine environment.

When the filter plates become clogged with particulate matter, the effluent runs off the plates into a channel where two electrical probes lay. When the channel fills, the circuit is completed across the probes and the cleaning cycle is automatically activated.

When the cleaning cycle is activated, an electrical motor drives a ram the length of the unit. Attached to the ram and located above and beneath the filter plates are spray nozzels which move back and forth across the length and width of the filter plates. The plates are sprayed clean concentrating the particulate matter with the wash water. The resulting wash effluent is channelled to a tank (approximately 6 m x 0.8 m x 0.8 m deep) where the sludge is collected and concentrated.

Filter Process



Note: The rinse cycle can either be automatically initiated each time the filter plates blind or the period of the cycle can be pre-set.

Sampling

Water samples were taken at a maximum of four points:

1) Inflow to hatchery
2) Before the filter
3) After the filter (discharge to environment)
4) Wash water flow to sludge

Samples were analyzed for:

pH
Total Suspended Solids
Total Phosphate as P
Nitrate and Nitrite as N
Biological Oxygen Demand (BOD5)
Ammonia Nitrogen N
Total Kjeldahl Nitrogen N

The samples were analyzed by Can Test Ltd. in accordance with the procedures described in "Laboratory Manual for the Chemical Analysis of Water, Waste Water, Sediments and Biological Materials" (2nd edition), published by the Government of B.C., Ministry of Environment, Water Resources Services, 1976 and "Standard Methods for the Examination of Water and Waste Water", 15th edition, 1980 and 14th edition, 1975, published by the American Public Health Association.

Results and Discussion

Table II provides the site specific maximum effluent discharge concentrations for United Hatchery as determined by the B.C. Ministry of Environment and Parks.

Table III provides the results of the sample analysis from United Hatchery. In all cases the effluent discharge concentrations after the filtration process were below the critical, maximum allowable concentrations (Table II). In addition, Table III reveals the highly concentrated nature of the sludge. For example, total suspended solids were as great as 348 mg/L.

This filtration process is extremely efficient as a particulate filter, appearing to remove up to 100% of the total suspended solids. In addition, the ammonia-nitrogen concentration was reduced from 3.56 mg/L to 0.34 mg/L. Total phosphate concentrations were reduced from 0.71 mg/L to 0.21 mg/L and from 3.48 mg/L to 0.17 mg/L. In all cases BOD5 concentrations were less than 10 mg/L.

Table IV summarizes a series of case studies performed in Scandinavia. Again, the efficiency of this filtration process is illustrated from these studies.

In summary, a space efficient, low maintenance, self-cleaning particulate filtration process is a viable option for effluent treatment. Not only is such a process suitable for effluent treatment, but it is also applicable to influent treatment and as part of a treatment process in recirculating systems.

Table I

Objectives for the Discharge of Effluent to Marine and Fresh Waters From Fish Hatcheries

Level	A
Parameter	
BOD5 lb/100 lb fish/day	0.40
Suspended solids, lb/100 lb fish/day	0.40
Ammonia nitrogen, lb of N/100 lb fish/day	0.04
Nitrate nitrogen, lb of N/100 lb fish/day	0.12
Total phosphate phosphorus, lb. of P/100 lb fish/day	0.020
pH range	6.5-8.5

(Department of Lands, Forest and Water Resource Services, Victoria, B.C., 1980.)

Table II

Maximum Allowable Effluent Discharge Concentrations for the Sechelt Salmonid Hatchery as Determined by the B.C. Ministry of Environment and Parks

Parameter	Concentration
BOD5	10
Total Suspended Solids	10
NH3	1
NO3	3
PO4	0.5
pH	6.5-8.5

- Other than pH, all Concentrations are in Mg/L

Table III

Results of Sample Analysis at United Hatchery,
Sechelt, B.C.

SAMPLE SOURCE	INFLOW	OUTFLOW	HATCHERY/INFLOW	BEFORE FILTER	AFTER FILTER	SLUDGE	BEFORE FILTER	AFTER FILTER
DATE SUBMITTED	MAR 31/87	MAR 31/87	APR 3/87	APR 3/87	APR 3/87	APR 3/87	APR 6/87	APR 6/87
DATE SAMPLED	MAR 30/87	MAR 30/87	-	-	-	-	APR 5/87	APR 5/87
TEST								
pH	-	-	7.03	6.64	6.82	6.62	6.17	6.56
Total Suspended Solids	L 1.0	L1.0	L 1.0	23.0	L 1.0	348.	91.0	L 1.0
Total Phosphate as P	-	-	0.051	0.71	0.21	8.49	3.48	0.17
Nitrate & Nitrite as N	-	-	0.092	0.041	0.084	0.16	0.032	0.059
Biochemical Oxygen Demand (BOD5)	-	-	L10.	L10.	L10.	183.	78.	L10.
Ammonia Nitrogen N	-	-	-	-	-	-	3.56	0.34
Total Kjeldahl Nitrogen N	-	-	0.094	2.83	0.75	30.6	-	-

L = Less than = not detected
mg/L = milligrams per litre

All results expressed as milligrams per liter except for pH, expressed in pH units.

Table IV

Summary of Case Studies Conducted in Scandinavia Concerning the Triangle Filter in Effluent Treatment
(TSP - Total Suspended Particles, TP - Total Phosphate, TN - Total Nitrogen)

Facility	Species	No. & Filter Type	Litre/Sec.	Influent Mg/L			Before Filter Mg/L			After Filter Mg/L		
				TSP	TP	TN	TSP	TP	TN	TSP	TP	TN
Akvaforsk/ Norway	Trout	1-TF24RS/80 micron	17	0.0			47.9±38.8			6.0±4.8		
	Atlantic Salmon	TF24RB/63 micron	17	0.0 0.0			202.5±110.7 3.5±2.8			12.25±10.59 0.0		
Saltviken/ Sweden	Trout	TF24WS/80 micron	13		0.07	0.36±0.34		2.33±4.41	1.36±1/08		0.32±0.32	0.86±0.46
Laholm/ Sweden	Altantic Salmon	TF24RS	10	5.0 3.33±1.37 0.13±0.09	0.01		14.93±13.35 7.67±0.75	0.07±0.04		5.93±1.07 4.58±1.34	0.04±0.02 0.19±0.04	
Laurra/Findland		TF24WS			0.02			0.59+0.26			0.12±0.02	
Scandinavia/ Sweden	Eel	6-TF24RS/80&100 micron	230		0.07±0.02		10.80±0.98	0.51±0.11	2.52±1.37	1.50±0.71	0.30±0.12	1.09±0.79
Orkelljunga/ Sweden	Trout and Atlantic Salmon	TF24RS/100 micron	20				9.50±1.50			2.0±1.0		

(Personal Communication, N. Perssons, 1987)

Note: TF - Triangle Filter
RS - Electrically Driven Ram/Top Spray Only
WS - Hydraulically Driven Ram/Top Spray Only
RB - Electrically Driven Ram/Top and Bottom Spray

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AN APPLICATION OF LARGE SUBMERSIBLE PUMP TECHNOLOGY IN HATCHERY DESIGN

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Rising lake levels in Pyramid Lake, a natural lake system, placed a shore based fish rearing facility in jeopardy of flooding. Redesign of the facility was based on the concept of creating a rearing facility that would handle significant changes in lake level fluctuations while minimizing the distance water had to be pumped to fish rearing containers. The redesigned facility incorporated the use of a submersible pump to replace a land based centrifugal pump station and a series of vinyl lined raceways to replace concrete raceways which had been flooded. The submersible pump was chosen because:

(1) it could operate over a wide range of lake water levels without encountering cavitation problems which arise in centrifugal pumps when suction head pressure changes;

(2) it eliminated the danger of a shore based pump station being flooded;

(3) it eliminated the problem of priming the pump system.

Earthen channels, lined with vinyl, were chosen as rearing containers because the vinyl liners could be moved and reused in response to changing lake levels. Incorporating the concept of a movable and reusable rearing container allowed the rearing system to be placed close the the lake which minimized the energy required to deliver water to fish rearing containers.

Evaluation of the Aquatector: A High Pressure Oxygen Injection System for Use in Fish Culture

By

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The Aquatector oxygen injection unit was tested at the Bozeman, MT Fish Technology Center and Saratoga, WY National Fish Hatchery. Tests were conducted to evaluate: ease of operation, efficiencies in a reuse situation, and effectiveness in maintaining dissolved oxygen levels above saturation.

The Aquatector was invented in West Germany by Franz-Josef Damann for use in the champagne fermentation process. They presently come in several different sizes for the treating of various water supplies. These are some of the Aquatector applications:

1. In aquaculture it can be used to increase production, improve fish quality, and enhance hatchery effluent.
2. In sewage treatment facilities it can increase capacity, improve purification, and remove odors.
3. The Aquatector can be used to oxidize and precipitate iron and manganese.

4. With ozone it can be used for disinfection and sterilization of water supplies.

Stainless steel Aquatectors are available for ozone use but are not necessary as this increases the expense.

The Aquatector Model 50/225 is the largest unit available at this time. Two-2 hp pumps supply water to the top of the Aquatector column where the water is supersaturated with pure oxygen. The supersaturated water leaves the bottom of the column through a needle valve which regulates pressure and flow. A by-pass valve on the pumps can also regulate pressure and flow. Aquatector internal pressure is indicated by a pressure gauge on the column itself. For safety reasons its important not to exceed 87-100 psi internal pressure because fittings may break. A closed system with no leaks is important to prevent big bubble formation.

The Aquatector Model 40/160 is the second largest unit which sells for $3755 (1987). Model 50/225 costs $4904 (1987) and this price does not include shipping, pumps, setup or any plumbing hardware.

An oxygen controller unit is included with the Aquatector which regulates oxygen flow through a solenoid valve. If water flows become low the oxygen shuts off so it is not wasted. In the winter the sight tube in which the oxygen controller is housed is the first thing to freeze so heat tape is needed to assure oxygen flow. Ideally the unit should be enclosed to protect it from cold and sunlight.

For getting the supersaturated Aquatector water into the fish rearing areas we tried perforated and open ended PVC at the

head end of the raceways. A flexible hose was used to this point because elbows in this line will break up small bubbles creating big bubbles which reduces efficiency.

The end of the pump intake line had a valve on it for making pump priming easier. Mats of algae will plug the Aquatector internal screen so depending on water supply the intake may need to have a fine mesh screen over it to prevent debris from entering the Aquatector. The internal screen needs to be cleaned frequently if pressure drops are noted. Fecal material and fish feed go right through the Aquatector with no problem so it does work in reuse situations.

The Aquatectors were tested in a production situation at Saratoga NFH, in south central Wyoming. The elevation there is 6700 ft. and oxygen saturation is 8.6 mg/l at 52 ^{o}F in late summer. Saratoga's spring pond water supply can fluctuate from 5.8 to 11.5 mg/l dissolved oxygen over a 24 hour period due to the plant influence in the pond. Water is used several times in serial or terrace type raceways and dissolved oxygen levels have dropped to 4.5 mg/l in a lower brood pond in the morning hours.

The Aquatector was placed at the head end of the production raceways (Location #2) and also at the head end of the brood ponds (Location #5). Dissolved oxygen readings were then recorded at each station both during the morning and night (See Figure 1). For example, during the morning hours the Aquatector increased dissolved oxygen levels from 6.5 to 11.7 mg/l for an efficiency reading of 90.7 % when at Location #5. Generally efficiencies varied from 55-75 %. The following equation was used for determining oxygen efficiencies:

$$\% \text{ O2 Efficiency} = \frac{\text{Raceway Water Flow (L/M)} \times \text{Raceway Dissolved O2 Increase} \times 100}{1428 \text{ mg O2/L} \times \text{O2 L/M Added}}$$

Retention time of the supersaturated water was monitored in a 6 X 60 ft. raceway with 100 gpm flow. Dissolved oxygen levels decreased by only 10 % after going 60 feet. However, as much as 1 mg/l was lost in water falling 8 inches over a dam board.

A liquid oxygen bottle holds 4500 cubic ft. of oxygen and one lasted about 4 days at Saratoga and cost $44.00/day. Using the same amount of oxygen cost $26.00/day at Bozeman so prices vary considerably according to geographic location.

A modified Winkler titration method (A.P.H.A. 1985) was used to test supersaturated water greater than 20 mg/l dissolved oxygen. Aquatector effluent has been as high as 55 mg/l dissolved oxygen in our tests.

A stress test was conducted on a raceway with 10 to 14 in. Snake River cutthroat trout. Water dissolved oxygen saturation was increased to 438 % with the Aquatector. Figure 2 indicates some of the variables measured during this test. We were adding 42 mg/l dissolved oxygen to 70 gpm flow which increased total gas to 155 % and decreased nitrogen gas to 81 %. The increase in saturation stessed fish in only 30 minutes and started killing a few in an hour. Histological analyses of the gills showed distinct emboli or air bubbles in the lammellae and gill capillaries. Aneurysms and stagnation of blood was caused by the emboli and was the reason for eventual suffocation. Air bubbles and blood could be seen along the perimeter of the caudal fins of most fish.

Aquatector efficiencies were monitored at various settings with different incoming dissolved oxygen levels both in the morning and at night (Figure 3). The lower the dissolved oxygen in the incoming water the greater the oxygen efficiencies. A drop in incoming dissolved oxygen of 1 mg/l increased efficiency about 25 %. The Aquatector output and oxygen efficiencies appear to depend on elevation. The results presented here were for elevations of 4900 and 6700 ft. Efficiency readings at other elevations will probably be different.

An oxygen flowmeter tester was devised for monitoring flowmeter accuracies (Figure 4) because these readings are important when determining oxygen efficiencies. Initially it was thought that internal Aquatector pressures would affect the oxygen flowmeter output. This was not the case but the flowmeters tested were always adding more oxygen than what was indicated on the gauge. In using this flowmeter testing method we recorded the amount of time for a known volume of water to be displaced at various flow meter settings. Weighing the oxygen tanks periodically is another method to determine the amount of oxygen actually used.

There is much potential for these types of oxygen injection systems. Whether its on distribution trucks, as a means for incorporating ozone into the water or in raising dissolved oxygen levels in fish production waters. Aquatector testing will continue both at the Saratoga NFH and Bozeman FTC utilizing Nitrox Oxygen Generators. We also plan on testing another unit called the Bicone which is distributed by Liquid Air Corporation.

Acknowledgments

We would like to thank the staffs at the Saratoga NFH and Bozeman FTC for their technical assistance during this study and also Mr. Bill Mebane of Zeigler Brothers, Inc. for donating one of the Aquatectors.

References

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Figure 1. Dissolved oxygen readings (mg/l) taken at different sample locations in the morning with and without the Aquatector oxygen injection unit.

SARATOGA NFH OXYGEN INJECTION (MG/L) INTO 1250 G.P.M.

SAMPLE LOCATION	A.M. No O_2 INJECTION	A.M. O_2 INJECTION LOCATION #5		A.M. O_2 INJECTION LOCATION #2
1	5.9	6.8		7.1
2	6.5			11.3*
3	6.0			10.8
4	5.8	6.5		10.2
5	6.3	11.7*	EFFICIENCY 90.7%	9.3
6	6.0	11.0		8.9
7		10.5		8.8
8		9.6		
SATURATION	8.6	9.5		9.1
TEMPERATURE (^{o}C)	11°	6 °		9°

*AQUATECTOR LOCATION

Figure 2.

FISH STRESS TEST

AQUATECTOR SETTINGS: 50 GPM
50 PSI INTERNAL PRESSURE
15 L/MIN O_2

AQUATECTOR EFFLUENT D.O. WAS 42 PPM ADDED TO 70 GPM FLOW IN RACEWAY WITH SNAKE RIVER CUTTHROAT TROUT.

	WITH AQUATECTOR	NORMAL
SATURATION	438%	100%
TOTAL GAS	155%	107%
NITROGEN GAS	81%	104%

STRESS SYMPTOMS BEGAN IN 30 MINUTES -- MORTALITY IN 60 MINUTES

Figure 3. Aquatector Model 50/225 oxygen efficiencies taken with different incoming dissolved oxygen levels and internal column pressures both in the morning and the evening. The amount of pure oxygen added was 19.5 L/M in each situation.

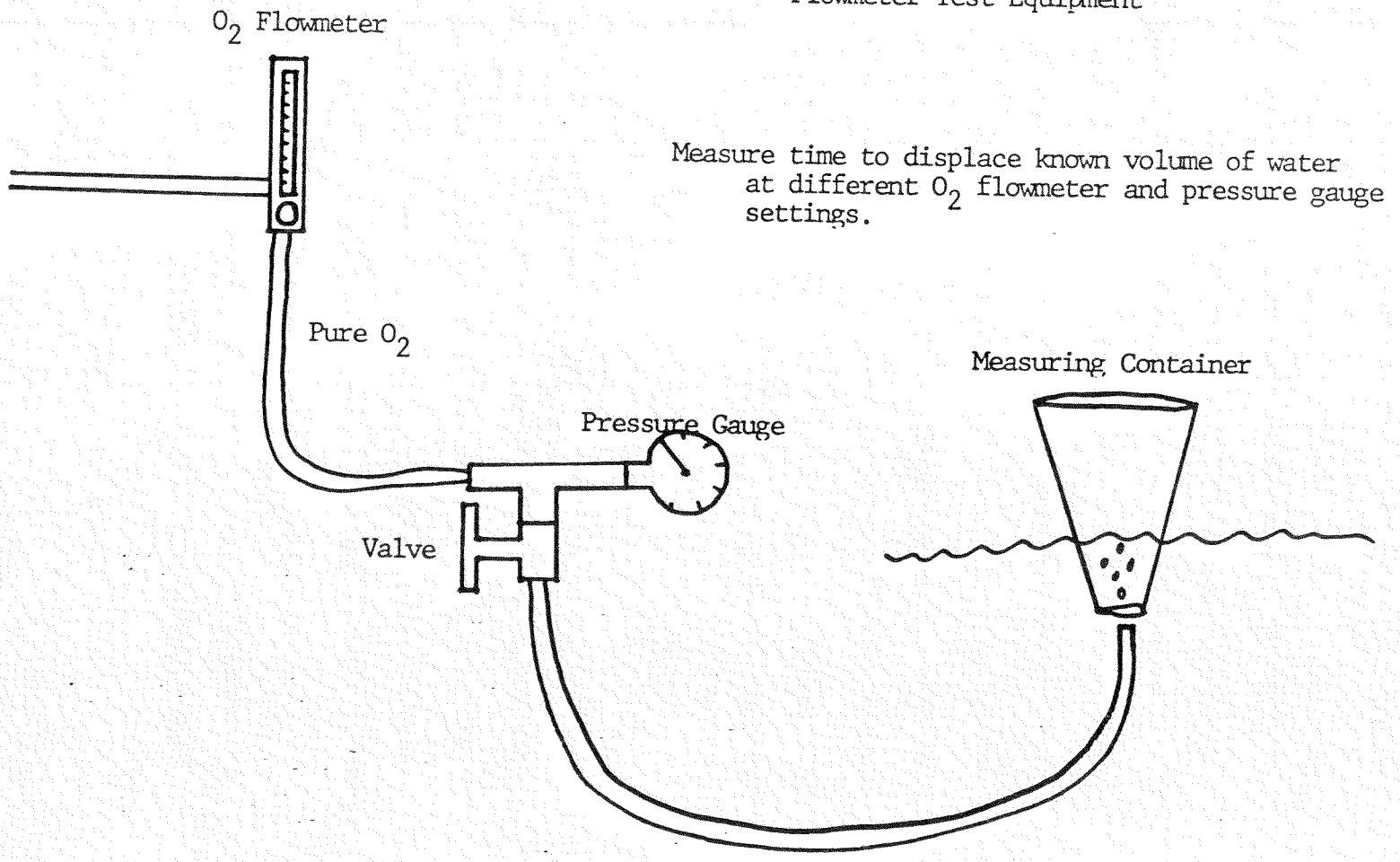
AQUATECTOR MODEL 50/225

EFFICIENCIES

COLUMN PRESSURE	O_2 INPUT	D.O. INCOMING		% EFFICIENCY
3.0 BAR	19.5 L/M	7.3 MG/L	A.M.	62.9
3.0	"	7.5	P.M.	52.7
3.2	"	7.0	A.M.	76.5
3.2	"	7.3	P.M.	59.5
3.4	"	6.5	A.M.	90.7
3.4	"	7.4	P.M.	71.4
3.6	"	6.9	A.M.	71.4
3.6	"	7.7	P.M.	47.6
3.8	"	6.7	A.M.	68.0
3.8	"	7.7	P.M.	42.5

Figure 4.

Flowmeter Test Equipment



THE LIGHT PIPE: A NOVEL APPROACH TO LIGHTING FOR STUDIES OF SALMON BEHAVIOR WITH APPLICATION TO FISH CULTURE

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Information on the behavior and behavioral interactions of juvenile salmonids lags far behind our knowledge on the production of these fish from hatcheries and rearing facilities. Only recently have we become concerned about the relevance to survival of topics such as voluntary migration or migration tendency of fish that have been physiologically defined as being "smolts". Even less is known of the behavior of hatchery - released smolts during early estuarine residence; a period considered to be critical to survival. It is here that physiological adaptation to sea water occurs, feeding on live prey is learned and predators must be avoided.

As a complement to our research at West Vancouver on the microhabitat requirements of chinook salmon (Macdonald et al, 1987) and on sublethal effects of wastes discharged into estuarine waters, we are using a large (1200 gal.) experimental aquarium to study the behavior of salmon in a sharply stratified vertical salinity gradient (Birtwell and Kruzynski, 1987). Among questions we seek to answer are: when do these fish choose to move into sea water, will a reduction in dissolved oxygen in the surface freshwater layer force them to move into sea water prematurely and if so what are the consequences of this

behavior? Will pollutants and hypoxia render them more susceptible to predation?

In order to study these responses in our water column simulator, we can vary and control temperature, salinity, water velocity, dissolved oxygen and total gas pressure. We have also tried to reproduce indoors, the photoperiod which our outdoor fish stocks encounter. Moreover, we wish to record fish behavior at very low light intensities since feeding and predation in many species peaks during dawn and dusk. Lighting control for this purpose was achieved by adapting and modifying a recently-invented lighting system, the TIR Light Pipe (Hughes, 1985) to meet our special research requirements.

The TIR Light Pipe consists of a high intensity discharge lamp housed in a reflector which directs the light output into a long acrylic box lined with a patented reflective film which effectively "pipes" the light along the length by total internal reflection (T.I.R.). The light emitted along the length of the bottom diffuser provides overhead illumination for our aquarium (LWH 6'x2'x6'). A shutter mechanism, installed between the light source and the prism light guide, provides photoperiod control including dawn and dusk periods. By passing the incident light beam through a deep red filter and using a high sensitivity video camera we have been able to monitor the behavior of juvenile salmonids at low light intensities and at night.

Thus by combining some of the unique characteristics of the TIR Light Pipe with certain physiological properties of fish vision, we have managed to fulfill the following requirements:

1) simulate daylight spectrum
2) provide uniform lighting
3) control day length including dawn-dusk simulation
4) facilitate observation at night
5) allow safe operation in a corrosive environment

Some additional advantages of our Light Pipe system over conventional lighting may render it particularly useful to fish culturists and behavioral researchers. These range from such basic considerations as providing light without heat and ease of bulb replacement in difficult locations, to the design of precise yet variable horizontal light intensity gradients, to tracking the sun and piping its light into indoor spaces (Panet-Raymond and Peddle, 1986).

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Tracking Gases During Supplemental Oxygen Delivery - Practical Approaches

by

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Abstract:

Current interest in supplemental oxygen addition for purposes of restoring water quality and/or increased production per volume of water necessitates reliable and consistent techniques for monitoring all relevant parameters. The chief hazard in systems for enhancing oxygen concentration is that the total gas pressure exceeds atmospheric pressure due to the inability to remove sufficient Nitrogen to make up for the extra oxygen. In such situations it is necessary to monitor both oxygen and total gas,(Pt)unless it can be demonstrated that nitrogen is undersaturated. Alternative strategies for multiparametric monitoring must often be considered in relation to the specific physical plant design. Some common denominators such as sensor placement,sensor reliablility, theory of operation, maintenance schedule and compatibility with other devices must all be considered, again in relation to system function and design. A typical flow situation is considered and suggestions made for such a system in relation to cost,available techniques, their reliability and required maintenance schedules. It is suggested that in many systems the simplest most reliable and most cost effective means which will at least give the required information in all cases is a measurement of total gas combined with wet Winkler titration. This can be easily accomplished on a daily basis with minimal effort.

Introduction:

Hyperoxygenation of water in hatchery operations usually necessitates monitoring for total dissolved gas as well as oxygen at several points in the system. This in turn depends on the fortunes of the water supply, the degree to which denitrogenation is necessary, and the analytical options, manpower and budget available. I want to limit my remarks today to only a general consideration of these constraints in order to provide a general strategy which will in all cases provide the minimal information necessary to keep "on top" of the system.

Supersaturation hazard of oxygen addition:

Although it is known that supersaturation with oxygen is less stressful (as indicated by LD/50's) to aquatic organisms than supersaturation with air (Rucker 1972), the general consensus these days seems to be that the target should be normal saturation to as great an extent possible -the more subtle stresses present the worse off one is.

To do this it is desireable to have inflowing water as close to atmospheric

saturation as possible particularly when considering hyperoxygenation for increased production. Depending on the water source, for example well or spring water, reservoir water, flowing river water, pretreatment by collumn, vacuum degassers or some of the simple countercurrent systems available may be necessary. In order to avoid considering each of these options in detail, a very general schematic is provided in Figure 1 which outlines the minimal schedule and location of monitoring necessary.

Figure 1, shows a schematic representation of a flowing water system in which fish are grown, together with a hypothetical oxygen - add system and the desireable points of monitoring (M) to provide a continuous picture of what is happening.I will go once over the picture quickly, then consider some of the points in more detail.

Water is added on the left and exits on the right. The pure oxygen add system is shown at the top left entering near the main stream. The volume of flow is deliberately pictured as lower which would allow using a high pressure add system to supersaturate the water with oxygen and thereby provide more oxygen with lower water flow. In cases where some supersaturation exists it may bemore desireable to subject a larger fraction of the water flow to the hyperoxygenation thereby decreasing the nitrogen further in the process. As we progress left to right fish respiration is seen depleting the water of oxygen and bringing the total gas level down to ambient or near saturation by the amount indicated as "DELTA-PO2". This is shown by the three successive bar-graphs at the beginning, just after the O-2 add, and at the exit of the hypothetical raceway. In each of them N2 saturation is assumed to be at ambient saturation, and oxygen is shown at normal saturation at the entrance. The four arrows (M) show the minimal points where some checks need to be made, of 1) total gas pressure,2)oxygen presure and 3) nitrogen pressure or saturation. The question I want to address is what is the minimal equipment needed to do this reliably in all cases?

While it is true that the intensity of monitoring necessary will dictate to a great extent the type and sophistication of the equipment needed, a consideration of both the analytical task at hand, and the budget should lead us to the common denominators which will show the minimal analyses required.

Figure 2 shows schematically the mole fraction and therefore the pressure fraction of gases in water which can and must be measured to determine the total dissolved gas pressure.

Figure 3 is a schematic representation of the direct method for measuring gases common to saturometer, gasometer, Tensionometer or whatever other wonderful names have been thought up to describe this device! The method is basically the same regardless of which equipment is used, although some instruments give only delta P and others give absolute pressure, and so on. All depend on creating a gas phase in the water and measuring its pressure mechanically or electrically. The figure shows the sum of all gases present adding up to what I call Pt, the total dissolved gas pressure, from which, if oxygen and temperature are known all required parameters can be calculated. Figure 4 shows the successive calculation of remaining gas pressure as each known quantity is subtracted from the total ABSOLUTE pressure. This indicates the utility of using low internal volume Absolute pressure sensors, because the TOTAL PRESSURE rather that the DIFFERENTIAL is provided and is identical to the expression of Dalton's law in

Figure 4., which accounts for 99.94% of all atmospheric gases.

Considering Figure 2, which is a scale bar graph of the saturated gas pressures in water at normal pressure, it is clear that when water vapor and oxygen are subtracted from the equation in figure 4, one is left with a quite accurate estimate of nitrogen pressure. This allows a clear picture of how much the total gas will rise with each additional pressure increment of oxygen. Table 1 indicates the minimal contribution ofpCO2 at pH and Carbonate alkalinities normally associated with salmonid culture. It is clear that at pH values and Carbonate Alkalinity expected in salmonid culture, pCO2 amounts to a very low pressure ,usually less than 5.mm Hg.

This means that if we have a reliable O2 measurment we can accurately estimate nitrogen with a precision equivalent and sometimes better than accurate quantitative analysis (such as water sample extraction and gas chromatographic analysis). This brings me to the point of considering the options (briefly) for O2 analysis. The simplest option seems to be a portable kit-type Winkler titration which is inexpensive, has a reasonable shelf life and is accurate to about 0.2 ppm. Using the equation of Weiss (1970) one can convert the oxygen to its partial pressure, subtract from the total gas pressure and calculate both nitrogen pressure and saturation. If this is done at each of the points in figure 1, a clear picture of the gas equilibration will result. This approach is inexpensive, accurate and will be acomplished with minimal time and labor. In fact we may well ask why not leave well enough alone at this point?

Remembering however that Figure 1 is indeed an ideal situation, and that atmospheric equilibration is assumed pushes us toward the real world where labor costs also present themselves as an important factor. We then quite naturally escalate the complexity of monitoring needs and push to the next step which is more automated monitoring. Can this be dome reliably?

The answer of course is yes, but we now get into a consideration of the reliability of the monitoring system and the need for a commitment to maitaining the sensors. I cannot stress the importance of this factor enough, that automation -any form of automation - demands a schedule of preventive maintenance and service be built into the schedule of operations. The critical sensor in this situation, that is the sensor with what is probably the shortest required maintenance or calibration interval is the oxygen sensor, although total gas probes also need a periodic dry-out to eliminate internal condensation build up. Modern pressure sensors however drift very little over a year in comparison to dissolved oxygen sensors. I will limit this consideration to a quick enumeration of continuous methods and/or sensors for oxygen, shown in Table 2. The numbers preceding the different types indicate my subjective estimate of reliability based on my personal experience - no objective study has been made.

		Available techniques-Oxygen	
Clark Electrode	(4)	continuous, diffusion	scheduled calibration cleaning
Leeds & Northrup	(3)	continuous, balanced balanced non-consumptive	scheduled calibration minimal cleaning

Fuel Cell (validyne)(2)	consumptive(gas phase only)		scheduled calibration easily replaced
Paramagnetic (gas phase)(1)	reliable limited to gas phase	"	"
Gas Chromatograph	accurate,versatile	cost effective	needs technician
Mass Spectrometer	"	Xpensive	NEEDS TECHNICIAN

There are essentialy three options for the measurement of Total Dissolved Gas by the direct method:

"Saturometer"	Provides Delta P, requires concurrent measurement of Barometric Pressure for calc'n of % Saturation	Periodic Membrane washing or replacement
"Gasometer"	In - line device Provides Delta-P requires calculations of % Sat'n	same
"Tensionometer" or Total Dissolved Gas Meter	Probe Device in-line or free probe Absolute Pressure sensor gives Total Gas Pressure Directly	same

The merits of these methods all depend on one's resources of patience, manpower and money but there are very definite differences in reliability and cost. In any case those with an asterix are those which can be made to work with automated and semi-automated systems. Using any of these methods on a continuous basis scheduled frequent maintenance to a reliable standard is essential! They are not magic wands and will only give as good service as they get!

Another source of uncertainty in choosing a total monitoring system involves the number of monitoring sites anticipated to be needed, and this depends on the extent to which the water in question has to be handled. The following outline summarizes the different types of water sources which may impact on the degree of monitoring from the purest pristine river to the most intensely recycled system.

Typical sources of hatchery water where O2 may be added

1) well or spring water - oversaturated with N2 - undersaturated with O2

- oversaturated with both gases

- undersaturated with both gases

2) geothermal
- (hot)
- heated then cooled before use

3) reservoir with Hydro
a) thermostratifying

b) stable saturation state

c) varyations in water level through season often leads to problems late in season

4) reservoir without Hydro
a), b) c, as above

5) river with approximately constant saturation

a) large seasonal temperature/flow variation

b) little seasonal temperature/flow variation

6) Recycled Water
a) dilution source - all above variations

b) dilution fraction 0-100% - all above variations

In light of these considerations the scheme shown on Figure 1 will appear very simple and absolutely minimal in scope. The system chosen will also depend on the number of separate sites to be monitored, how critical they are in relation to the operation of the facility (Do they warrant alarm functions etc) and the alternative cost of the manpower to do them manually vs the manpower to monitor the monitors. There is as yet no formula to be applied, just good sense applied to one's own experience and that of others. A good rule of thumb to apply when considering automated systems and particularly automatic sensors is to determine the reliabilitu of the sensor and wether it has already been used in such capacity in other locations. While the reliability guide presented in Table 1 is an indication of my own and others experience it is by no means inclusive and I would urge anyone interested in this question to search both technical and scientific literature for the past six months as there are often new developments in other fields which can provide assistance to this one.

Reliability of the direct sensing methods is well established and there is now three years of experience on the Columbia with Automated Sattelite telemetry which is available through the corps of Engineers , North Pacific Division, Portland.

In summary my message is this: adding oxygen requires at least a minimal monitoring schedule be set up. This minimal analysis requires 1)total dissolved gas pressure, Temperature, and oxygen be measured at EACH location indicated in Figure 1, and where any new treatment of the water is put into operation. The minimal equipment for this need only be a direct measurement technique and a wet chemistry (Winkler) Kit, however if more advanced systems are set up, it is

essential to also set up a schedule of maintenance and calibration which will 1) establish the reliability and integrity of the sensors and 2) allow one to fine tune this schedule according to the drift or maintenance requirements of each sensor.

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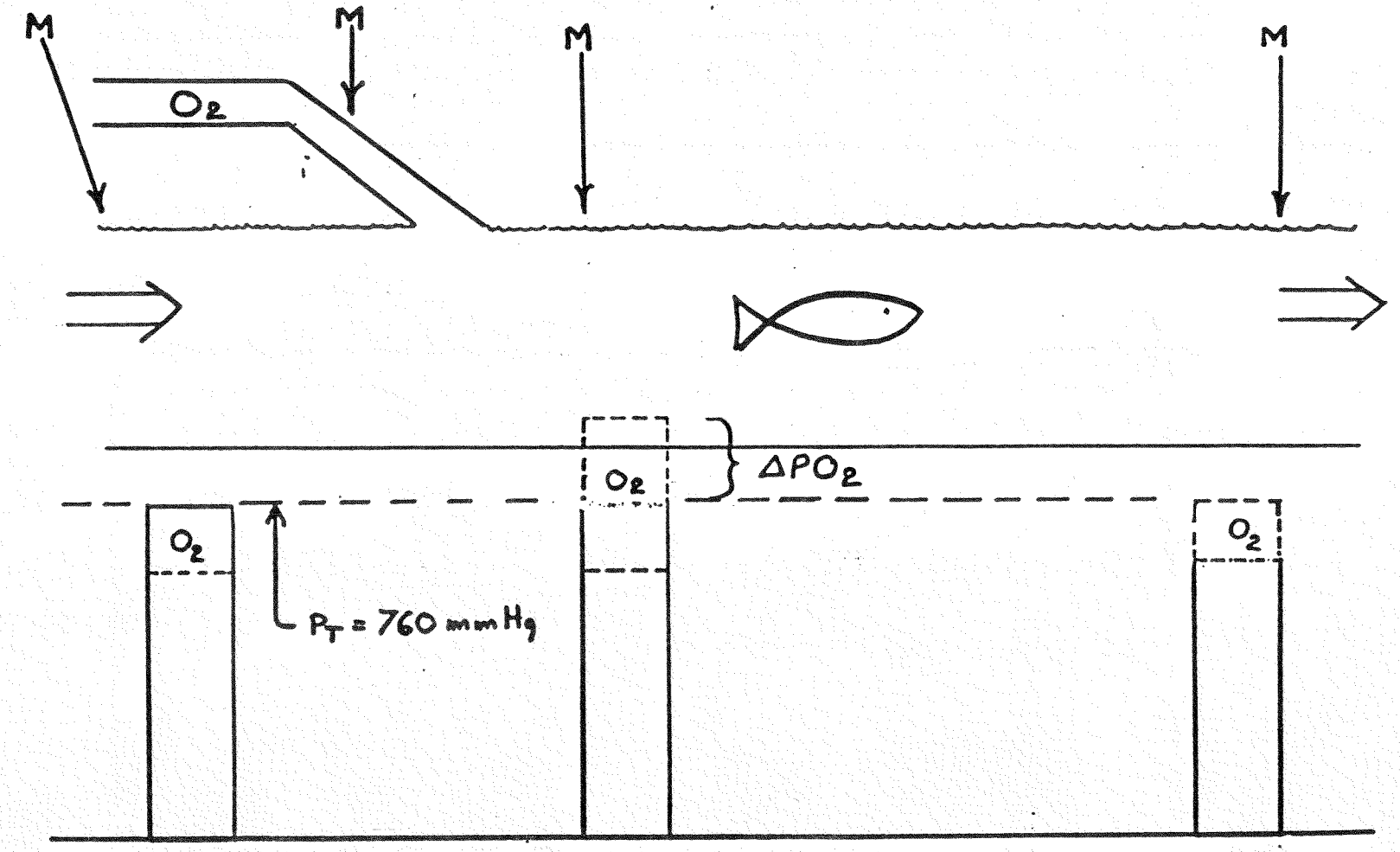

Fig 1

FIGURE 2

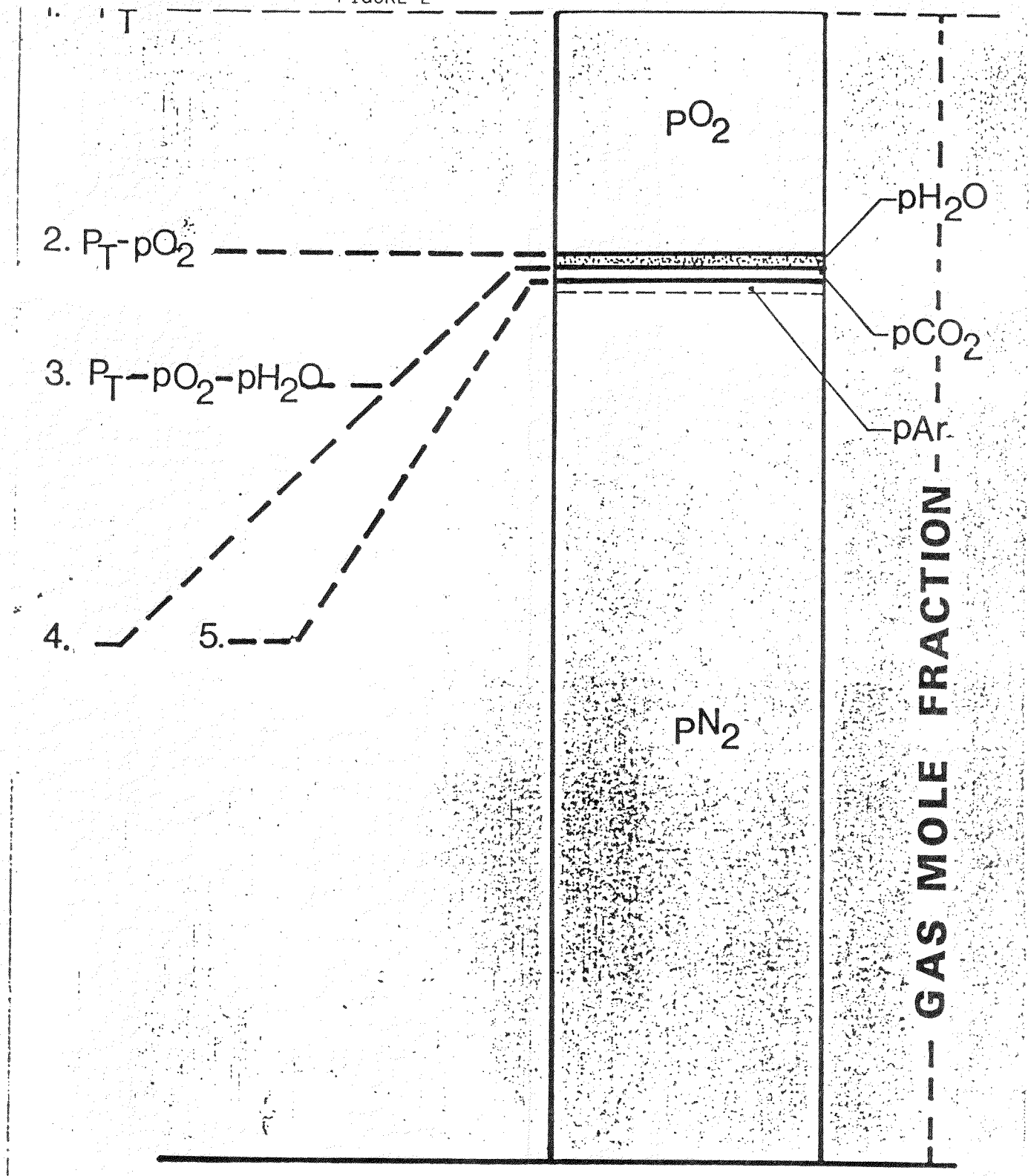
1. T
2. $P_T - pO_2$
3. $P_T - pO_2 - pH_2O$
4.
5.
pO_2
pH_2O
pCO_2
pAr
pN_2
GAS MOLE FRACTION

FIGURE 3

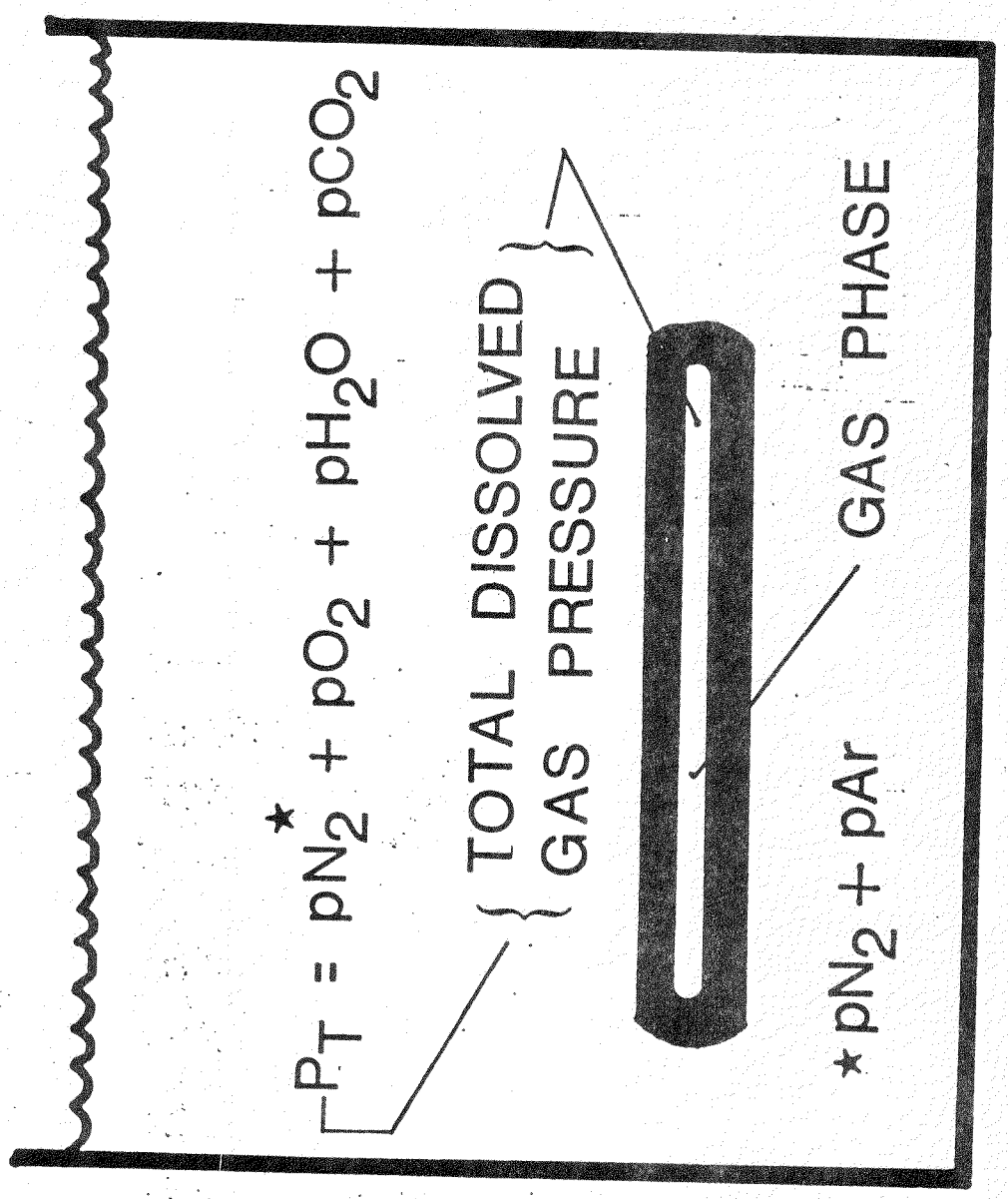
$P_T = pN_2^{\star} + pO_2 + pH_2O + pCO_2$
TOTAL DISSOLVED GAS PRESSURE
GAS PHASE
$\star pN_2 + pAr$

FIGURE 4

(1) Total Dissolved Gas Measurement:

The total dissolved gas measurement provides the total pressure measureable due to dissolved gases including water vapor (Fig 1).

$$PT = \boxed{pN2 + pAr + pO2 + pH2O + pCO2} = 99.94\% \text{ of all atmospheric gases}$$

(2) Total Dissolved Gas Pressure - pO2:

$$PT - pO2 = \boxed{pN2 + pAr + pH2O + pCO2}$$

Since Ar is inert, bears a constant ratio to N2 and only amounts to 0.93% of atmospheric pressure, it is convenient to include it with the pN2 measurement:

$$PT - pO2 = \boxed{pN2 + pH2O + pCO2}$$

(3) Reference to Table I gives the water vapor pressure for temperature (t) which can then be subtracted from the right hand side of the equation to give:

$$PT - pO2 - pH2O = \boxed{pN2 + pCO2}$$

(4) Reference to TABLE II indicates the very low pCO2 at most pH and alkalinity levels found in fresh water, and allows the use of equation (4) as an approximation of pN2.

(5) HOWEVER, when there is reason to believe that the levels of CO2 are significant, a simple check can determine the pCO2; by adding alkali to a bucketful (approximately two gallons) of water upon which the above measurements have been made (use approximately 10 cc. of 20% (w/v NaOH), the alkali will not substantially alter the tension of the other gases present but will absorb all the CO2 thus causing a decrease in PT numerically equal to the pCO2.

NEED OF OXYGEN IN AQUAFARMING

by
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Good morning ladies and gentlemen. It is a pleasure for me to share with you my thoughts on the "NEED OF OXYGEN IN AQUAFARMING". I have structured this presentation to address the aquafarmers in the audience. The underlying assumption I'm using in this talk is, "aquafarmers are in business to make money, not because they like raising a particular cash crop for the fun of it!" My presentation will cover the following topics:

1. The future of water supplies is important to you.
   * Quality and quantities are declining.

2. Oxygen is the primary factor in limiting your productivity.
   * Water is only a vehicle for transporting oxygen.

3. Oxygen in the air is not free.
   * Bubble and splash techniques do not minimize costs.

4. Maximize oxygen efficiency to maximize profits.
   * The ATEC ZX OXYGENATION SYSTEM is a proven and reliable delivery system of energy efficient oxygen which will increase production capacity.

5. How can ATEC help you.

Water is the LIFE BLOOD of the aquaculture industry, in fact, without it you are out of business! Water is the vehicle by which you transport oxygen and food to your cash crops whether they be catfish, trout, crawfish, shrimp or other species.

The quality and quantity of water available to you are under attack. You now not only compete for your water supplies, but you must also be aware of how many times and in what way it has been used before it reaches you. Therefore, there are two questions which you must answer if you are to be successful in aquafarming: first, how do you maintain your production levels with decreasing water quality, and second, how do you expand your production levels to meet the growing markets with declining quality and quantities of water.

If lower quantities of water were the only problem, then the reuse of the water would be an acceptable solution. Unfortunately, this in not the case. Water is only a vehicle for transporting oxygen and oxygen is the primary factor in limiting production in a defined quantity of water. Therefore, you will need some means of introducing oxygen into the water before each use. In fact, if your prime water source lacks sufficient oxygen at any time during the growth cycle of your crops, it will also have to be oxygenated or you may face a possible loss of your total crop.

Recognizing this problem, aquafarmers have made every effort to take advantage of natural and artificial water falls, splash boards and pumping in order to

increase oxygen levels. Since air APPEARS to be a free source of oxygen, a variety of bubble and splash techniques including aerators, blowers, bubblers and diffusers have been used to marginally increase the oxygen levels. In many cases these techniques are used to just maintain survival levels of oxygen in the water.

Just what is the level of oxygen necessary in aquafarming? It most likely varies from specie to specie, however, the majority of researchers agree that saturation levels are preferred. Since saturation itself varies with temperature, altitude, salinity and other factors, its practical ranges are from 7 to 11 ppm (parts per million) oxygen. Researchers also agree that if this level drops to less than 80% of saturation, health, growth and feed conversion will suffer.

The use of air to reoxygenation water demonstrates an indisputable scientific law of nature. If you start with 0 ppm of oxygen in water, it is relatively easy to increase the level to 1 ppm using air. To add the next ppm is harder. As you get closer and closer to saturation, it gets increasingly more difficult to add the next increment of oxygen and ENERGY COST TO DO SO INCREASE EXPONENTIALLY.

As an example of this law, we will examine the case for a 3 pound per horsepower, per hour aerator. An aerator's efficiency is measured/rated by the number of pounds of oxygen the aerator can transfer per hour into 0% oxygen saturated water using one horse power of energy. Aerators are rated in this manner because the percentage advantage (efficiency) between aerators capacity to transfer oxygen using one horse power of energy can be calculated. Further, this percentage advantage remains constant at various levels of oxygen saturation up to 100% saturation, i.e., an aerator that is 50% more efficient than its competitor using the same amount of energy will transfer 50% more oxygen at any given level of oxygen saturation up to 100% saturation.

Based on the preceding (oxygen transferred at 0% saturation), one usually evaluates the capital verses operating costs and oxygen delivered to determine which aerator to buy and use. This is a mistake! Let's look at the 3 pound per horsepower, per hour aerator to see why.

We now know the aerator will deliver/transfer 3 pounds of oxygen to the 0% oxygen saturated water using 1 horse power of energy where the CASH CROP IS IN IMMEDIATE PERIL OF DYING. As the aerator continues to work and increase the oxygen saturation to 50%, where the CASH CROP IS BARELY SURVIVING, it now only transfers 1½ pounds of oxygen per horse power per hour. Remember the indisputable scientific law - "it takes more energy to transfer the next constant increment of oxygen (1ppm) into water". Another way of stating this law is: "as the oxygen content in the water approaches saturation, one horse power of energy will only transfer a smaller and smaller per unit amount of oxygen." If the aeration system is operated to 90% of saturation, then the 3 pound per horse power per hour aeration will only deliver 3/10 of a pound of oxygen per horse power per hour. NOTE: IT TAKE 1000% MORE ENERGY TO TRANSFER 1 PPM OF OXYGEN AT 90% SATURATION VERSES 1 PPM OF OXYGEN AT 0% SATURATION. In fact, the most efficient aeration system will not reach saturation.

If you will recall, an aeration system needs to provide not only a certain concentration of oxygen in the water, but it also needs to provide the pounds

of oxygen required to support the quantity of fish being reared. In a cold water operation where 15 pounds of oxygen is required to support an additional 100,000 pounds of fish, the requirement to support the 100,000 pounds of fish from the most efficient aeration equipment is 50 horse power or 50 one horse power machines. At this level, the combination of purchase price, maintenance cost, and power cost are prohibitive.

CONCLUSION -- THE OXYGEN CONTAINED IN AIR IS NOT FREE. Once it is understood oxygen in air is not free, a cost comparison between aerators (using oxygen in air) and pure oxygen delivery systems can be made to determine the most cost effective method of transferring oxygen into water, i.e. the lowest cost oxygen delivered to your cash crop. Therefore, the efficient delivery of oxygen into the water now becomes the problem.

The use of pure oxygen in aquafarming waters has several advantages. Most notable is that pure oxygen adds a new dimension. The aquafarmer can now increase his production given the same quantity of water by increasing the concentration of oxygen up to saturation. Other advantages are: the oxygen carrying capacity of water is increased by a factor of five while it's in contact with the pure oxygen, and pure oxygen is readily available.

Oxygen can be supplied as a liquid from industrial gas companies and stored in an insulated tank which they will lease you. The liquid oxygen is released on demand as a gas. Oxygen can also be produced as a gas on site in oxygen generators called PSA units. Now you only have to determine which is the lowest cost source of pure oxygen.

ATEC has developed its ZX OXYGENATION SYSTEM to take advantage of the lowest cost source of pure oxygen by providing the most economical method of delivery of dissolved oxygen for aquafarming operations. In sketch 1, the oxygen and the water are contacted in the oxygenation column. This creates a quantity of highly oxygenated water flowing from the column which is then distributed to the water flowing in the feed channels or the start of the individual raceways to bring it to the desired oxygen concentration. This oxygen rich water can also be fed to the start of second, third and fourth usage of water to take best advantage of the limited water supplies you have while providing the fish with a healthy growth environment. The important concept is to let the highly oxygenated rich water into the main body of water without a loss of oxygen, i.e. bubbles breaking out. THIS SYSTEM ACHIEVES THIS AND THE RESULT IS 100% OXYGEN UTILIZATION.

In tests at the Sate of Utah's experimental hatchery in Logan, Utah, this oxygen delivery system demonstrated that it could maintain whatever oxygen concentration level we selected. More importantly, the system was capable of maintaining levels of saturation up to 185%. There were no bubbles and no measured oxygen loss. As we attempted to go above this level, the raceway began to turn milky and was obviously losing oxygen, but, up to this point, 100% oxygen utilization was achieved. A full scale unit was then designed and installed at Utah's Midway Hatchery. During 1987 their fingerlings grew 30-50% faster in an oxygen environment near saturation as compared to previous years at a lower level of oxygen.

Full comparative tests are now being run at various oxygen concentrations in six test raceways. A preliminary report on those results should be available after the first of the year and will be reported at the World Aquaculture

meeting in Hawaii (See 1) in January of 1988. The tests should be completed by early spring and a full report issued after that.

Although the above examples all relate to raceway type operations, the same problem exists with regard to catfish and other aquatic species in pond culture systems where the bulk of the oxygen is supplied by algae. As seen in sketch 2, the comfort zone concept provides the necessary ppm level and quality of oxygen in a small portion of the pond to maintain healthy fish during the periods when oxygen production by the algae is insufficient. Much higher densities of fish can be supported by adding additional oxygen, thereby increasing productivity and profits. The bottom line to you is the efficient delivery of oxygen can increase your productivity through better health, better conversion and higher rearing densities in the same facilities and using the same water.

In summary, we have talked about why the future of water supplies are important to you; why oxygen is the primary factor in limiting your productivity; that oxygen in air is NOT FREE; why you must maximize oxygen efficiency to maximize profits, and how to go about it.

(1) Oxygen Injection: It's use in water quality improvement and growth enhancement for raceway culture of Rainbow Trout (Salmo Gardneri), John D. Leppink and Joe Valentine, Utah Division of Wildlife Resources, 1596 West North Temple, Salt Lake City, Utah 84116

A STATUS REPORT OF CONSTRUCTION AND OPERATIONS OF THE LOWER SNAKE RIVER COMPENSATION PLAN

Dan Herrig, U. S. Fish and Wildlife Service

The Lower Snake River Project, consisting of Ice Harbor, Lower Monumental, Little Goose, and Lower Granite Locks and Dams, was authorized by Congress in March 1945. Although authorized in 1945, project construction was delayed for many years while design studies occurred and construction monies were sought. The first dam, Ice Harbor, was not completed until 1962; and the last dam, Lower Granite, was closed in 1975. In spite of the Corps' efforts to maintain the anadromous fish resources through adult fish passage facilities and other measures, it became obvious with the operation of Ice Harbor Dam that major losses to fish and wildlife resources were occurring, and that the losses were increasing with the completion of each succeeding dam.

In accordance with the Fish and Wildlife Coordination Act, the Fish and Wildlife Service and National Marine Fisheries Service prepared a special report for the Corps on the impacts of the Lower Snake River dams. The report was completed in 1972 with input from the Columbia River Basin Fishery Technical Committee. Among other findings, it indicated that anadromous fish populations in the Snake River System had decreased by one half in the first ten years of operation of the Lower Snake River Project. Consequently, to compensate for the project's large fish and wildlife losses, Congress authorized the Lower Snake River Fish and Wildlife Compensation Plan (LSRCP) as part of the Water Resources Development Act of 1976. The following summarizes the major features of the fish propagation segment of the compensation plan and gives a brief update of the status of hatchery construction and operations.

The 1976 legislation authorized the Corps to construct sufficient anadromous fish hatcheries and associated trapping and holding facilities to return 18,300 fall chinook adults, 58,700 spring and summer chinook adults, and 55,100 steelhead adults back to the project area. In addition, residence fish hatcheries and other projects were authorized to produce 93,000 pounds of trout annually to replace lost resident sport fisheries in Washington and Idaho. During the design phase of the program, the federal agencies involved agreed that the FWS would budget for and administer the fisheries portion of the LSRCP.

As shown on the map below, the program required expansion or construction of twelve hatcheries and numerous satellite facilities in Idaho, Oregon, and Washington (Figure 1). When completed Idaho Department of Fish and Game will operate four hatcheries, Oregon Department of Fish and Wildlife--three hatcheries, Washington Department of Game--two hatcheries, Washington Department of Fish--one hatchery and U.S. Fish and Wildlife Service--two hatcheries. Construction is complete and fish production is underway at eleven of the twelve hatcheries; Clearwater Anadromous Fish Hatchery (FH) to be completed by 1991 (Table 1). The four satellite facilities

yet to be constructed will be completed between 1988 and 1990 (Table 1).

When all are completed, the facilities will produce about 550,000 pounds of chinook smolts and 1,380,000 pounds of steelhead smolts. (Tables 2 through 4). The numbers of smolts will be about 9 million fall chinook, 1 million summer chinook, 6.2 million spring chinook, and 9.5 million steelhead (Tables 2 through 4).

The estimated total cost for development of the LSRCP will be $177 million for construction features and about $8.5 million annually for facilities operations and maintenance (Figure 2). All anadromous fisheries compensation and most resident fisheries compensation was allocated to project power costs. Consequently, the Congressionally-authorized FWS budget expenditures are reimbursed to the Federal treasury from Bonneville Power Administration power revenues. To date, funding for the program has been growing with project needs (Figure 2). Funding for FY 1988 is expected to be adequate.

Included in the current and future O&M costs is a hatchery evaluation study program being conducted by the operating agencies and tribes. The program began in FY 1982 and is expected to continue for some time after the entire LSRCP has been developed and is fully operating. The studies are being conducted not only to determine if established hatchery production goals are appropriate and if the authorized compensation level is being met, but also to assist in developing new procedures to overcome fish production and fishery management problems that are often inherent in developing successful hatchery programs with native wild stocks of anadromous fish. It is the latter purpose, problem-solving, which has received most of the evaluation study emphasis to date.

Although the majority of the program has been underway only a couple years, a status report of releases and returns is appropriate. Figures 3 and 4 illustrate the releases and estimated returns of summer, spring, and fall chinook and steelhead produced at LSRCP hatcheries through 1987. Please note that release figures consist of pre-smolts and smolts only--they do not include fry or fingerling releases for any race or species. Regarding adults, fall chinook adult returns are estimates of returns to Ice Harbor. These counts include some wild and naturally produced fish along with hatchery returns. Summer chinook adult returns are to the South Salmon River trap and also include some wild and naturally produced fish. Similarly, some spring chinook return estimates may include wild and naturally produced fish (e.g. Sawtooth FH rack returns). The steelhead adult returns are our best estimates of total returns to the Snake River Basin, i.e. above Ice Harbor.

NWFCC87.FWS

Table 1. Remaining LSRCP Construction Activities

Facility	Agency	Spp	Construction Schedule
Clearwater FH	IDFG	Chk Sth	Complete Dec 1991
Crooked R. Trap/Release	IDFG	Chk Sth	Complete May 1990
Powell Trap/Release	IDFG IDFG	Chk Sth	Complete May 1989
Imnaha Trap/Release	ODFW	Chk	Complete Jul 1988
Little Sheep Trap/Release	ODFW	Sth	Complete Mar 1988

Table 2. LSRCP FACILITIES IN THE STATE OF IDAHO

Hatcheries	Spp	Design Capacity (1000 lbs)	Production Releases (millions)	Adult Return
Sawtooth FH	Chk	149.0	2.24	19,232
Dworshak Expansion	Chk	70.0	1.05	9,000
McCall FH	Chk	61.3	1.00	8,000
Clearwater FH	Chk	91.0	1.37	12,200
	Sth	350.0	2.50	14,004
Magic Valley FH	Sth	291.5	2.00	11,660
Hagerman NFH	Sth	340.0	2.40	13,600
TOTALS	Chk	371.3	5.7	48,432
	Sth	981.5	6.9	39,264

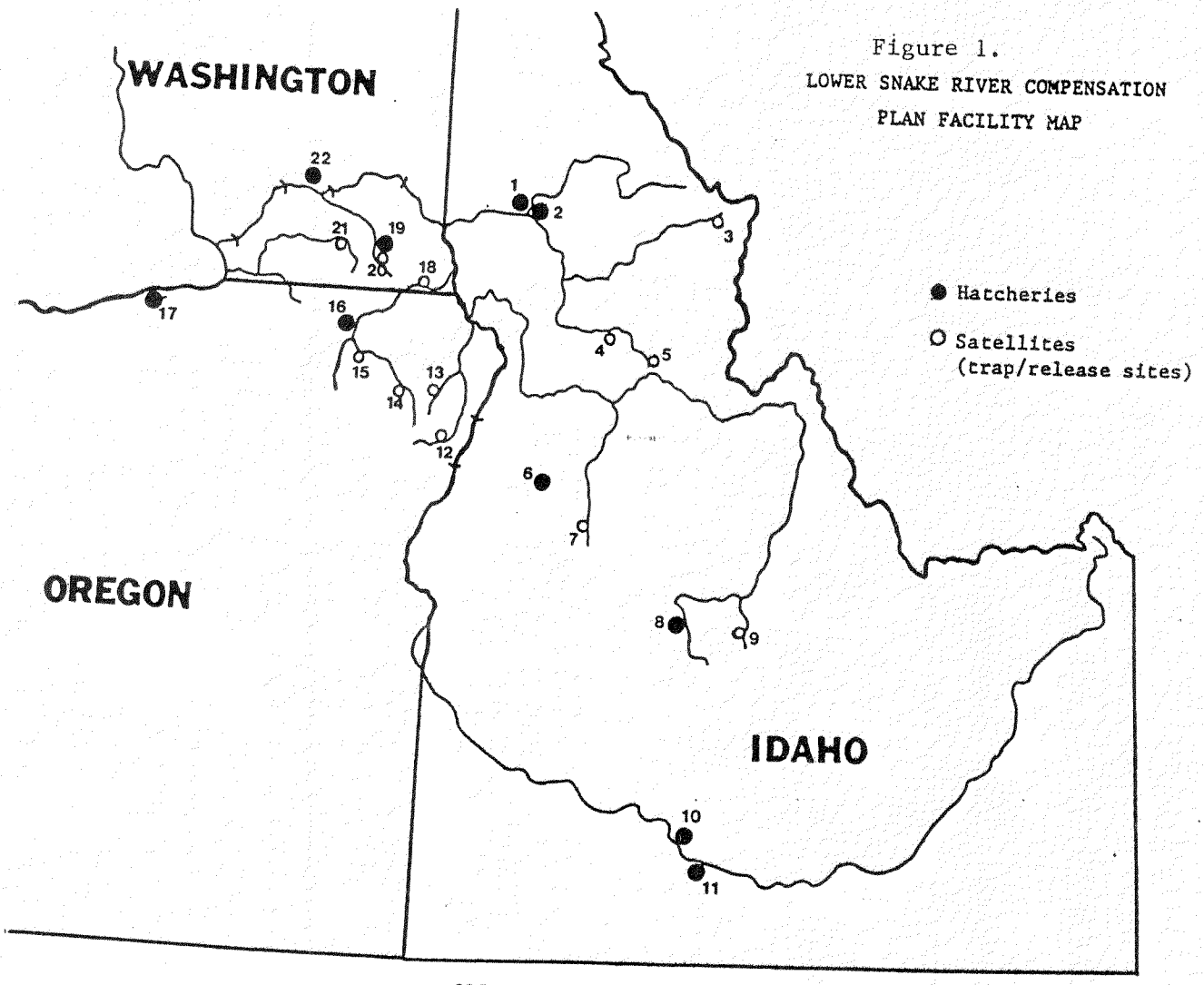
Table 3. LSRCP FACILITIES IN THE STATE OF OREGON

Hatcheries	Spp	Design Capacity (1000 lbs)	Production Releases (millions)	Adult Return
Lookingglass	Chk	69.6	1.39	9,072
Irrigon/Wallowa	Sth	279.6	1.68	11,184

Table 4. LSRCP FACILITIES IN THE STATE OF WASHINGTON

Hatcheries	Spp	Design Capacity (1000 lbs)	Production Releases (millions)	Adult Return
Lyons Ferry FH	Chk			
	Fall	101.8	9.162	18,300
	Spg	8.8	0.132	1,152
Lyons Ferry FH	Sth	116.4	0.931	4,656
Lyons Ferry/ Tucannon	Resident trout	86.0	0.258	
Instream Improvements		7.0	0.021	

Figure 1.
LOWER SNAKE RIVER COMPENSATION
PLAN FACILITY MAP



OPERATING AGENCIES

Idaho Department of Fish & Game

1. Clearwater FH
3. Powell
4. Crooked River
5. Red River
6. McCall FH
7. South Fork Salmon River
8. Sawtooth FH
9. East Fork Salmon River
11. Magic Valley FH

U.S. Fish and Wildlife Service

2. Dworshak NFH Expansion
10. Hagerman NFH

Oregon Department of Fish & Game

12. Imnaha
13. Sheep Creek
14. Wallowa FH
15. Big Canyon
16. Lookingglass FH
17. Irrigon FH

Washington Department of Fish

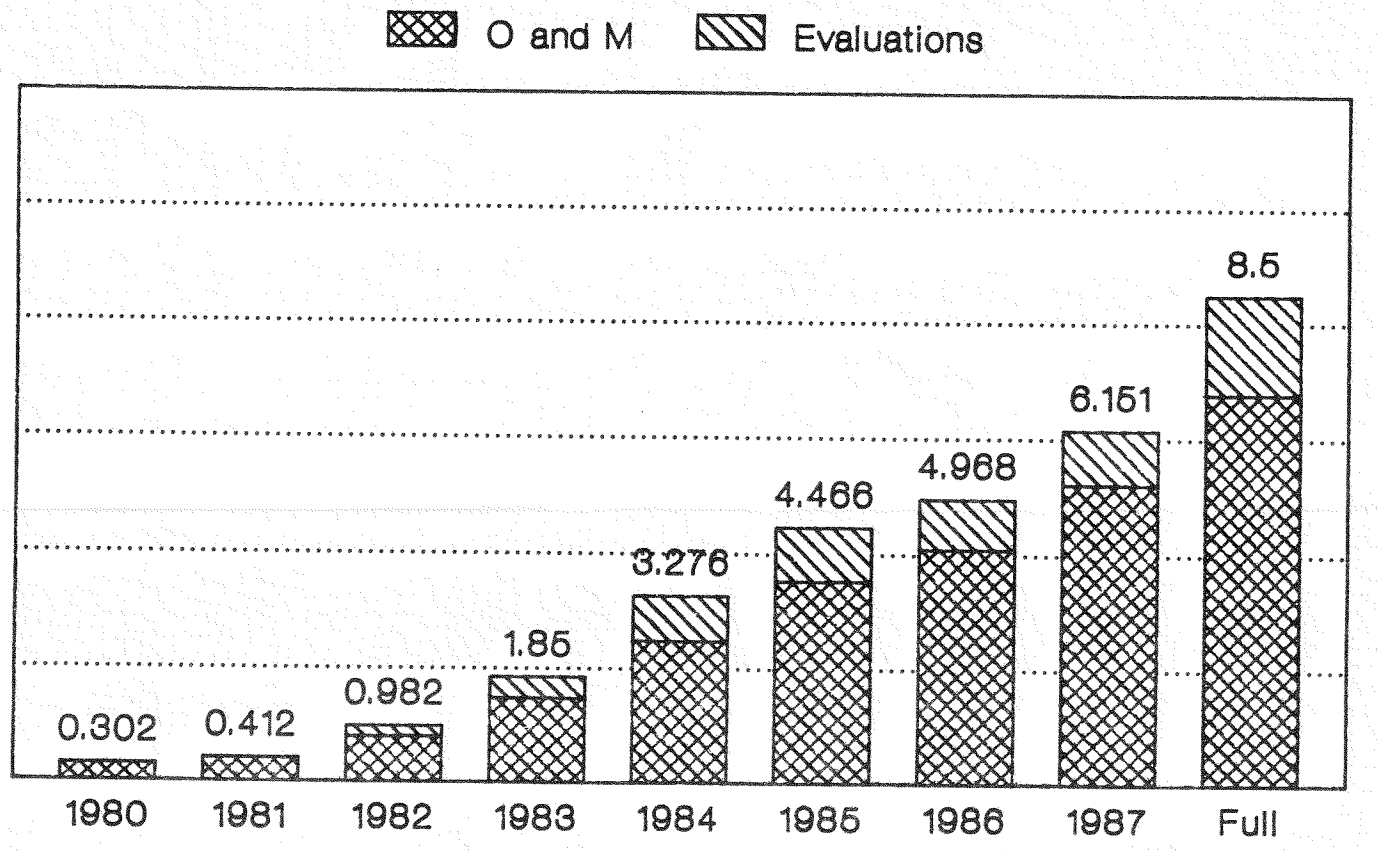
22. Lyons Ferry FH - Salmon

Washington Department of Game

18. Cottonwood Creek
19. Tucannon FH
20. Curl Lake
21. Dayton Pond
22. Lyons Ferry FH - Steelhead

Figure 2.

LSRCP Funding Summary
(In Millions of Dollars)



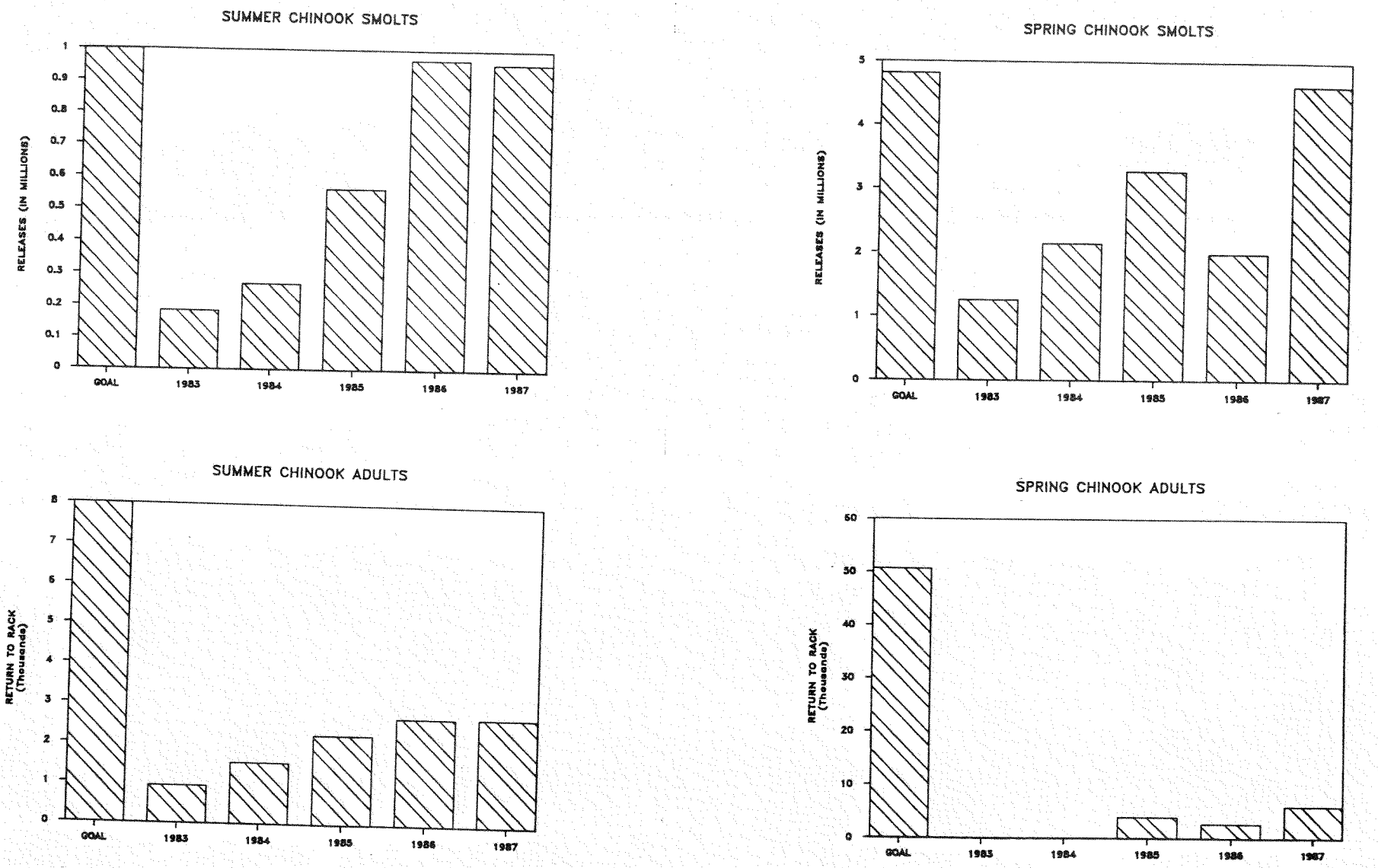


Figure 3. Estimates of LSRCP hatchery releases and adult returns.

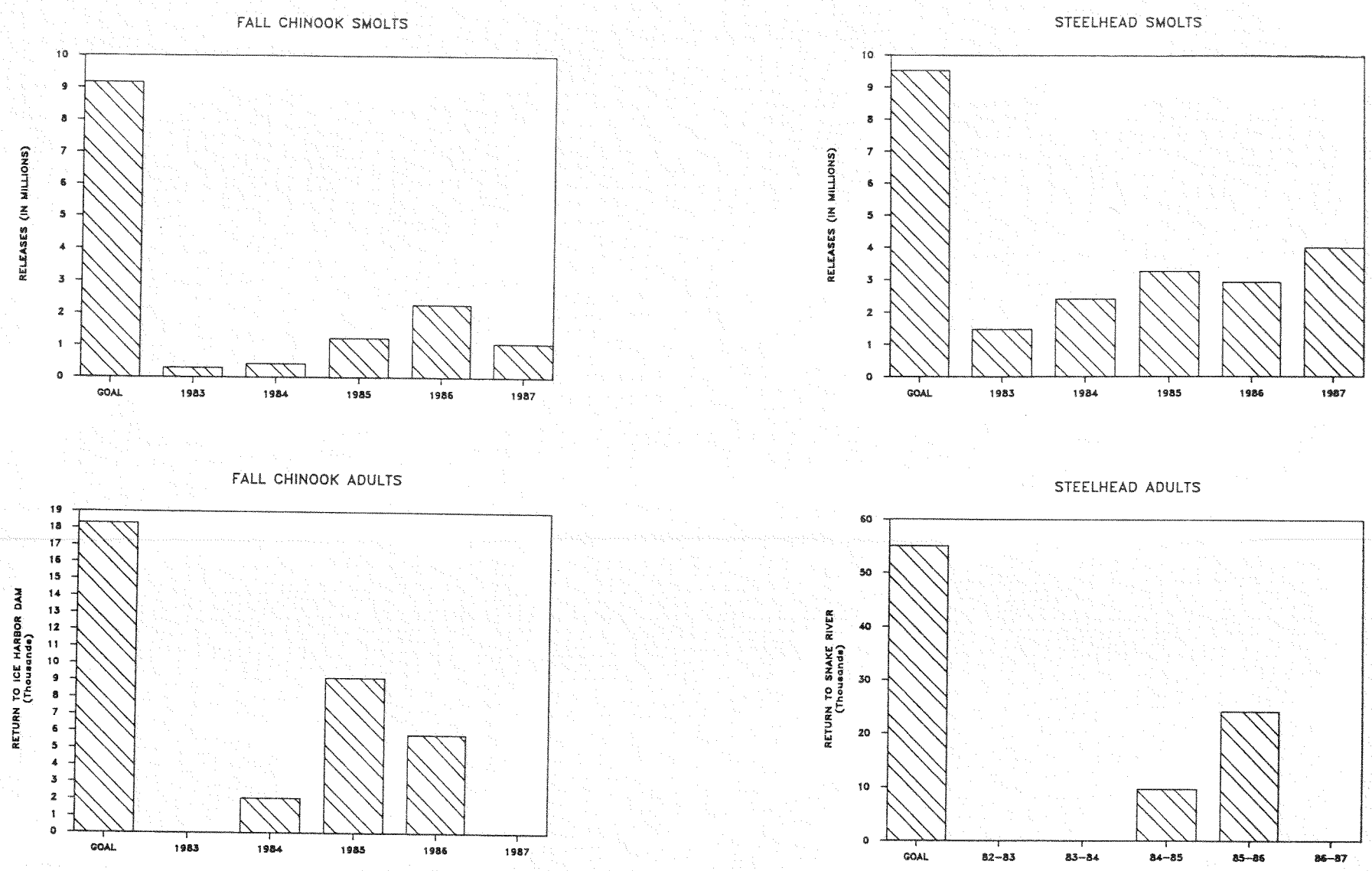


Figure 4. Estimates of LSRCP hatchery releases and adult returns.

KEEPER CHANNELS: An Evaluation of two densities and three loadings.

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INTRODUCTION

In 1986 and 1987 a new plastic substrate designed specifically for use in Keeper Channels was evaluated. The substrate, called "The bringing up apparatus for yolk sac fry" or BUA was developed by the Japanese as a replacement for gravel. The substrate is made of a durable PVC plastic, which is placed on the bottom of the keeper channel. Fry reside between the lateral vanes of the substrate (Fig 1).

Use of BUA was advertised as a way of increasing loading capacity in keeper channels. Also, because BUA is lightweight, it is more easily handled and cleaned than gravel. To assess the use of BUA and to determine maximum loading levels in keeper channels supplied with one of two gravel sizes or the BUA the following study was initiated.

METHODS

Three substrate and two density treatments were evaluated. The three substrates were :BUA , Large gravel (LG; 6-14 cm), and Small gravel (SG; 3-6cm). The two loadings were : Normal density (ND; 880,000 alevins/ channel) and High density (HD; 1,360,000 alevins / channel).Well water (8-10 C) was supplied to each of the six channels at a flow rate of 80-100 gpm.

Three identical sampling sites were established in each of the six channels. Alevins were sampled from each station on three dates during development. Additionally, dissolved oxygen determinations were made at each site on two dates. Mortalities were counted at each site and expanded for the entire channel.

Both live and preserved alevins and fry were used to determine wet length and weight. The preserved alevins and fry were dissected to seperate yolk and tissue portions, dried in an oven at 100 C and weighed. The same procedure was done on eyed eggs collected from each channel.

One way analysis of variance was used to test the hypothesis of equality of several variables within treatments. A two- way (randomized) Model 1 ANOVA tested the hypotheses of equality of several variables: fork length , wet weight, dry yolk,tissue and total weight.

Yolk absorption rate (YAR, dry egg yolk weight-dry yolk weight of alevins / number of elapsed days)and yolk conversion efficiency (YCE, Tissue weight increase/ Yolk weight decrease) were calculated using pooled data from each treatment. Analysis of covariance (Zar 1974) was used to test for differences in yolk absorption rate between treatments.

RESULTS

There were significant differences in stage of development (as measured by the Kd index) due to substrate and density and the interaction of both variables.Within the high density groups Kd values were identical (2.110). Two of the normal density groups were at a lesser stage of development than the high density groups (Table 1).

At swim-up there were significant differences in mean length due substrate, density and the interaction of both variables (Table 1). Fry incubated on the BUA were largest regardless of density. Within the gravel groups, fry incubated at normal densities were slightly larger than fry incubated at high densities. The smallest fry were found in the SGND treatment.

Fry weight was significantly affected by both substrate and density (Table 1). However, there was no significant interaction between the two variables. Fry incubated at heavier densities weighed less than fry incubated at normal densities. Within densities, fry incubated on BUA were heavier than fry incubated on gravel. Fry incubated on large gravel were heavier than fry incubated on small gravel.

There were no significant differences in yolk absorption rates between treatments due to either substrate or density (Table 2). Alevins in both BUA treatments had the highest YAR's. Alevins incubated at normal densities had higher YAR than alevins incubated at high densities.

There were small differences in yolk conversion efficiencies between treatments (Table 2). The maximum difference between

treatments was only 3%. Within substrates, the small gravel groups had slightly higher YCE's than the other substrate groups. There was no difference in YCE due to density.

Mortality was highest in the high density groups. The LGHD and SGHD had the highest overall mortality , 3.02% and 2.72%, respectively.Mortality in the BUAHD group was 0.61 %. Mortality in the normal density groups did not exceed 0.51 %. Most of the mortality was confined to the upper portion of the channels and appeared to be caused by smothering.

Fry emigration was estimated visually. Fry in both BUA supplied channels vacated the channels within two weeks of the onset of swim-up. In contrast, fry in the gravel treatments took 3-4 weeks to vacate the channels, even though they were at the same stage of development as the BUA fry.

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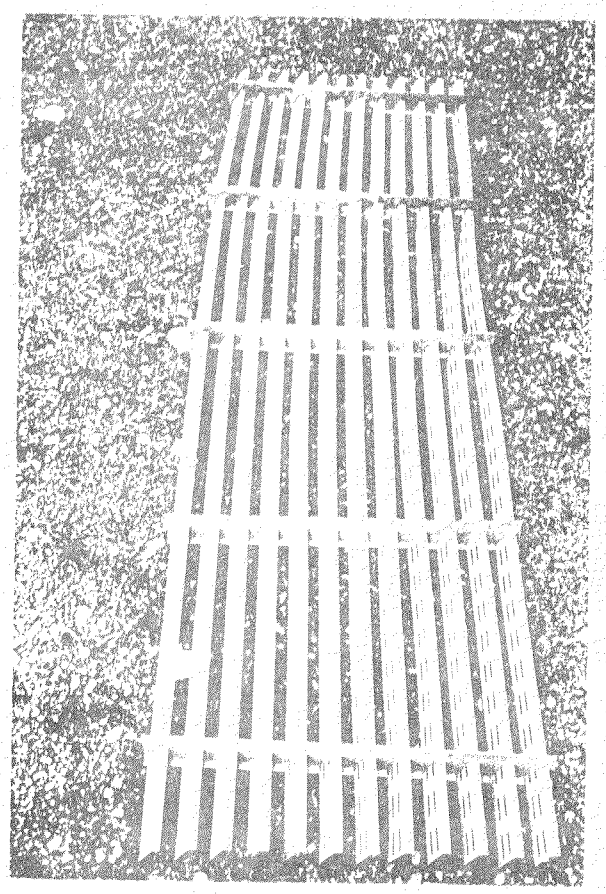
Zar,J.H. 1974. Biostatistical analysis.Prentice-Hall, Inc. Englewood Cliffs, N.J. 620p.

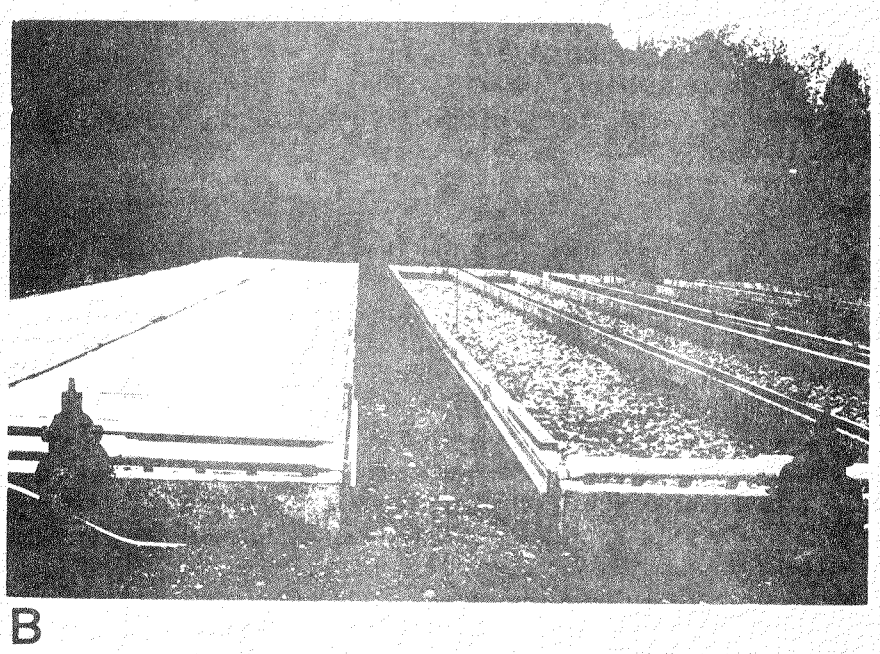
Table 1. Mean length weight and Kd index for swim-up fry sampled at 1700 temperature units.

	Length		Weight		Kd	
Group	mm	sd	mg	sd	mean	sd
BUAND	35.0	1.0	419.9	39	2.13	0.05
LGND	34.7	1.1	411.2	46	2.14	0.05
SGND	34.8	1.3	399.5	45	2.11	0.05
BUAHD	34.9	1.2	399.6	42	2.11	0.05
LGHD	34.5	1.2	388.4	45	2.11	0.05
SGHD	33.8	1.2	368.7	41	2.11	0.05

Table 2. Yolk absorption rates and yolk conversion efficiencies for the treatments.

Group	YAR(mg/day)	YCE (%)
BUAND	1.370	71.4
LGND	1.241	69.3
SGND	1.251	71.6
BUAHD	1.347	69.5
LGHD	1.282	70.8
SGHD	1.079	71.5
Group Means:		
BUA	1.359	70.5
LG	1.306	70.1
SG	1.247	71.6
Normal density	1.287	70.8
High density	1.236	70.6





B

Fig 1. A. View of single section of BUA.
B. View of Keeper channels containing gravel substrate.

CAN PACIFIC HALIBUT BE REARED IN CAPTIVITY ?

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Early in 1985, the International Pacific Halibut Commission (IPHC) contacted the National Marine Fisheries Service (NMFS) Manchester Field Station and the U.S. Fish and Wildlife Service (USFWS) Marrowstone Field Station and asked if we would be intrested in the conducting a study to determine if spawning and rearing of Pacific halibut (Hippoglossus stenolepis) would be feasible. We agreed to conduct the work at the Marrowstone Field Station because of the availability of good quality sea water from Admiralty Inlet. The University of Washington (UW) was later invited in as an additional cooperator because of their expertise in flat fish spawning and culture, and because there was a very good chance that a graduate study project would evolve.

In August of 1985, we received our first group of halibut from an IPHC charter. Five small fish were transported in the lined, seawater-filled bed of a pick-up from the offloading pier to the USFWS Marrowstone Field Station on Marrowstone Island near Port Townsend. Large dip nets and a homemade nylon net sling/cradle were used to move the fish from the catcher boat to the truck and ultimately to the holding tank. The transport took approximately 10 minutes.

The fish were placed in a 16'x 4' deep circular plastic lined swimming pool for the duration of the study. One fish, a 20lb female, died within the first week, presumably from handling stress. Caudal fin tips and the white underside were severely hemorrhaged.

Subsequent groups of fish were captured and delivered to Marrowstone in November 1985, October 1986, September and November 1987. No attempt was

made to spawn the fish during the winter of 1985-86 (existing literature indicates they are winter spawners) because we did not know what sex the fish were or if they were mature or not. We were content the first year to observe them. The fish were fed live and frozen Pacific herring (Clupea harrengus) during the first year. They had no problem catching the live herring, but readily took the frozen herring if live fish were not available. We eventually dropped the live herring from the diet for fear of producing a serious BOD if the seawater system failed and flows were reduced.

The halibut were initially tagged with colored spaghetti tags through the operculum. We have since retagged the older groups and the last two groups with the pit tag which was recently developed at NMFS Manchester. A total of 14 fish are currently being held in the pool at Marrowstone.

The fish are periodically treated with a 1:4000 solution of formalin for the monogenetic trematode Entobdella hippoglossi which is very specific to the Pacific halibut. Most treatments appear to be approximately 98% effective; however, we have decided not to use a stronger solution because we don't yet know how formalin will effect the overall survival of the fish.

In July of 1987, we attempted to convert the fish to artificial feed. We made a series of 4" long x 1/2" dia sausages using Moore-Clark OMPII and a TRESPADE sausage stuffer. The fish initially took the sausages but we soon discovered that they were spitting them back out. After two weeks, the fish visually rejected the sausages. We have since learned that Norwegian experiments with artificial feed have faired similarly. We will continue to pursue an artificial food with various components, possibly fish oils and/or krill.

In December of 1986, we started monthly blood sampling to determine sex and hormonal levels. Since the literature indicates that Atlantic halibut (Hippoglossus hippoglossus) are winter spawners, we assumed that the Pacific halibut are too. We now have a full year of data on monthly blood samples for male testosterone and female estradiol (Figure 1). Our results correlate with Norwegian studies and indicate elevated hormone levels between December and March. As indicated in Figure 1, we currently have only one mature male and possibly three mature females. In fact, the mature females were quite easily spotted once their gonads reached full development because of the apparent distension of the visceral cavity. Vitellogenin, an egg-yolk protein precursor, was also observed to increase in the female blood samples. IPHC data indicates that the females become mature near 12 years of age and the males at 8 years of age, or approximately 20 and 4kg respectively. Their data also indicates that a 25kg female will produce roughly 500,000 eggs while a 125kg female may produce four million eggs.

In March of 1987, toward the end of the spawning season, one small male and two females were injected with 15,000 IU human chorionic gonadotropin (HCG). Eight days later the same fish were injected with synthetic luteinizing hormone releasing hormone (LhRh-a) at 1.5 mg/fish. Milt was

subsequently released from the male. No eggs were released from the females, though the viscereal cavities were extremely distended. Both females later died, the injection dosages being to large. Fish will again be injected during the winter of 1987-88, but at lower levels. Mature controls will be held aside.

A graduate student from the UW is on board and will devote the entire winter of 1987-88 to spawning, rearing, care, and feeding of the Pacific halibut.

FIGURE 1

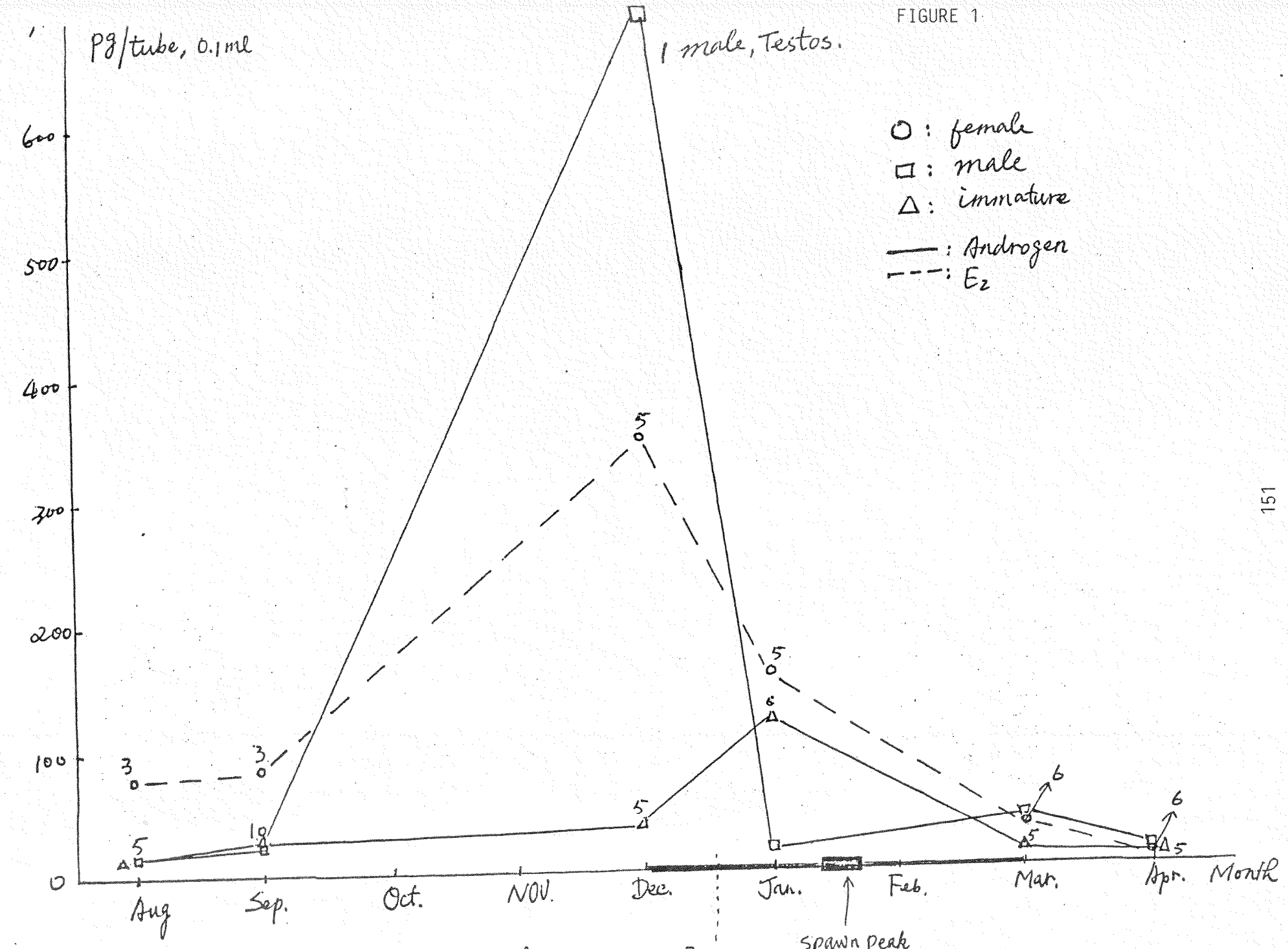

Fig. Summary of Halibut RIA data. 11-8-87.

Pen Rearing and Imprinting of Fall Chinook Salmon

by Thomas L. Macy

Introduction

Pen rearing of upriver bright fall chinook in a backwater and acclimation pond of the John Day Pool of the Columbia River has been funded by the Bonneville Power Administration. The purpose of this study is to evaluate the potential use of backwaters and acclimation ponds for rearing upriver bright fall chinook above the John Day Dam. Applications of results would include increased hatchery production, utilization of excess eggs and fish, and in-place-in kind fish mitigation. At the time this study was started upriver bright fall chinook were a depressed stock and, therefore, the use of these production fish was approached cautiously. We decided to use two methods of rearing: (1) pen rearing and (2) natural rearing behind a barrier net.

Methods

The first year, 1983, we looked for two types of sites (1) an existing acclimation pond and (2) a backwater. The areas we examined included (1) the Snake River below Ice Harbor Dam, (2) lower Yakima River, (3) lower Umatilla River and (4) the Columbia River between John Day and Priest Rapids Dams. We examined aeriel photos and various maps before actually travelling the shoreline. Study site selection criterion included size, depth, water quality and uniqueness of water source. We selected two sites, both within John Day Pool: (1) Rock Creek, a backwater; and (2) Social Security Lake, an existing pond in a Corps of Engineers Wildlife Park.

During the second year, 1984, we started rearing fish while testing densities and feeding rates. In fed pens at Rock Creek and Social Security Lake we tested two densities and two feeding rates. Densities were based on estimated water exchange and feeding rates were (1) full hatchery ration as determined at our control hatchery, and (2) 3/4 hatchery ration. In addition, Rock Creek fish were reared behind a barrier net, and in pens without feed (pens without feed excluded all preditors). Densities within both the Barrier net and unfed pens were based on available zooplankton.

During the third year, 1985, we reared fish in fed pens at both Rock Creek and Social Security Lake. Densities and feeding rates were based on results of 1984 rearing trails. At Rock Creek we also reared fish behind a barrier net with a surface area of approximately one acre and within net pens without feed. Densities (numbers of fish) within the barrier net and unfed pens were twice those of 1984 trials because growth during the 1984 trials was very good.

During the fourth year, 1986, we reared fish in fed pens as we had in 1985. Other rearing senerios were changed, based upon data collected

in previous years. Within the barrier net we removed preditors before stocking. We did this because of high mortality experienced the first two years. Unfed pens were reared at higher densities because growth in 1984 and 1985 was indicating no growth limitations. Two additional densities of pen reared fish, with feed, were also added. Two pens with 38,000 fish per pen (target release density .15 pounds per cubic foot) and two pens with 55,000 fish per pen (target release density .2 pounds per cubic foot). We tested these two densities because (1) it appeared density was not limiting based on our trials, (2) pen rearing operations in other locations were rearing chinook at these higher densities, and (3) pen rearing appears to be more cost effective when utilizing higher densities.

In 1987 our fifth year, IHN was diagnosed in our parent stock at Little White Salmon National Fish Hatchery (LWSNFH) and no progeny were to be shipped upriver. We shifted our entire compliment of pen rearing equipment from Rock Creek to Drano Lake, a backwater of the Bonneville Pool which is located at the effluent of LWSNFH. Here we pen reared fish (1) at densities tested in 1984 trials, (2) at triple densities of 1984 trials 55,000 fish per pen (target release density .2 pounds per cubic foot) (3) at 75,000 fish per pen (target release density .3 pounds per cubic foot), and (4) without feed at densities the same as were reared at Rock Creek in 1986. Because our barrier nets were custom made to the Rock Creek location we did not rear behind a barrier at Drano Lake.

During all four years of rearing all pen reared fish (except in the unfed low density pens) were coded wire tagged as were each group reared behind a barrier net. In addition each year a control group from LWSNFH was coded wire tagged. In 1987 the hatchery control group was destroyed when IHN was detected as the cause of elevated mortality. Non of the 1987 pen reared fish tested were found to have IHN and releases were made on schedule.

Results

Barrier net reared fish and fish reared in pens without feeding grew poorly when stocked at approximately .001 pounds per cubic foot in 1985 and 1986. However, in 1984 fish reared at slightly less than .001 pounds per cubic foot grew very well (these fish were stocked at a later date when zooplankton concentrations were higher).

Without removing preditors, mortality within the barrier net ranged from 30% (1984) to 49% (1985). Mortality was negligible when preditors were removed in 1986.

Growth rates of fish in pens without food were similar to that in the barrier net. Growth was best for all unfed fish when zooplankton numbers increased later in rearing.

Our testing of densities and feeding rates has shown that we can pen rear fall chinook in backwaters and ponds to at least .29 pounds per cubic foot at a full hatchery ration. There was very little difference in growth or mortality at any density that we tested.

At temperatures above 59° F. we experienced epizootics of Enteric Redmouth and culminaris which were controlled with a feed treatment of 2.75% TM50. Fish were reared to a target release size above 100 per pound during the four rearing seasons before sustained water temperatures exceeded 59° F.

It appears that pen rearing upriver bright fall chinook is cost effective when compared to hatchery rearing and that cost effectiveness is directly related to rearing densities.

The barrier net was both costly and labor intensive, however, it served the purpose for evaluating natural rearing without the need for large numbers of fish.

Other pen rearing operations throughout the world have proved successful; now it is up to the agencies to apply these techniques to rearing upriver brights in the Columbia Basin.

CO-MANAGEMENT OF SALMON IN ALASKA

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Decisions in a democracy can involve active public participation of the people being governed (participitory democracy), or it involves delegating the decisions to elected officials who in turn delegate the implementation of policy to appointed staff. The larger the population base the more likely the decisions are made by elected officials whose policies are carried out by staff. Frequently, staff is given broad latitude in interperting policy. This latter approach frequently removes people from the decision making process who are most affected the by the decisions and frequently results in conflict between the government bureaucrat and the public. This is the practice commonly followed in most states. However, in Alaska a decision making process is still being used which allows active, broad public participation and people have an effect on the decisions which directly affect them. This has allowed the formation of a number of agreements between the governing state and the people being governed. For example, the Alaska Department of Fish and Game enhancement effort which is copperatively conducted with the commercial fishermen through the Regional Aquaculture Associations. This I refer to as co-management in my presentation.

The Regional Aquaculture Associations are private non-profit corporations (PNP's). They were formed during a time when salmon stocks in Alaska were severely depressed and the State of Alaska was planning the enhancement activities to bring the harvest rates back to its historical level. Fishermen wanted to have direct imput into this planning and wanted to become directly involved themselves. Legislation was created due to fishermen lobbying efforts that created the formation of PNP's. This legislation allowed the fishermen to build hatcheries, restore wild stocks, harvest fish to pay for operations, tax themselves to support the activities, and gave them a voice directly to the commissioner through the Regional Planning Teams.

This cooperative effort has, in my opinion, greatly improved the management structure in Alaska. The PNP's have hired professional staffs who can competently carry out the biological functions and act as technical advisors to the fishermen in dealing with political or governmental issues. Through this process, fishermen have gained a better knowledge of management concerns and must deal with similar issues in conducting the business of the Regional Association. In addition, the Department of Fish and Game has had to improve their management practices because they have to deal with a more informed public. The Department has also gained much information on mixed stock management because of the intensive tagging program conducted by the associations. Separation of stocks and management decisions have greatly improved. This process of co-management has benefitted and continues to benefit the State of Alaska.

WHIRLING IN THE NORTHWEST

By
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An old fish disease, long feared by fish growers, pathologists and administrators has appeared in the Northwest. Whirling disease, causative agent Myxobolus cerebralis , has been found in Oregon and Idaho. The disease has not only caused salmonids to chase their tails but also has put concerned agencies and private sector fish growers in a whirl.

Confirmed cases have been identified in the Grande Ronde drainage including the Wallowa and Lostine Rivers and the recently remodeled Wallowa Hatchery. It has been found and confirmed in the Imnaha River including Sheep Creek and Little Sheep Creek. Private ponds have shown positive in several watersheds including the John Day, Powder River, Willow/Rhea Creek, and North Santiam. Although fish from these private ponds have been demonstrated to be positive, the river systems have not turned up any positive samples to date.

In Idaho, confirmed cases have been identified in two hatcheries, Sawtooth on the upper Salmon River and Pahsimerol on the Pahsimerol River, a tributary of the Salmon. Samples to date taken from adjacent streams and elsewhere in Idaho have not turned up positive results.

Although extensive searching has taken place in Washington hatcheries and southeastern Washington streams, no positive samples have been located as yet.

Two other species of Myxobolis having slightly different spore size and causing different pathologies have caused local fish doctors fits in the search for whirling disease. Myxobolus cerebralis spores have a specific spore size range and for confirmation must be found in cartilage in the head through histological examination of sectioned material.

No one knows for sure how Myxobolus cerebralis arrived in the Northwest. Imports from positive regions, straying anadromous fish from positive systems or early stocking of positive fish from Nevada could have been the source of the Northwest problem.

The Pacific Northwest Fish Health Protection Committee (PNFHPC) was introduced to the problem at their February 1987 meeting. A special meeting was held in June to update information and to discuss alternative actions.

Because whirling disease had been exotic to the Northwest and there has been considerable fear for the fisheries resource and private sector salmonid production, the disease has had emergency status. Emergency action, however, has been limited. The disease has been found in areas where eradication is not possible or practical. Although one lot of catchable rainbow at the Wallowa hatchery has been destroyed to prevent spread of the disease, no such action has been deemed practical elsewhere. Important stocks of upriver chinook are

now involved and destruction of these stocks would be difficult to justify considering the extent of the problem.

At the most recent meeting of the PNFHPC the latest findings were presented. Locations where positive and suspect samples had been taken were identified. The question of how serious whirling disease is as a threat to fish in hatcheries and wild stocks was discussed. Those who have witnessed the problem in hatcheries have told us that it does not cause high mortalities. Representatives of the agencies and tribes present at the meeting developed alternative language for the Model Fish Health program which takes whirling disease out of an emergency status (Class A) and puts the disease into certifiable status (Class B) along with such diseases as IHN and IPN. Although fish destruction might still be the best option in some cases it is not required in every case under the proposed reclassifications.

New guidelines for egg movements have been proposed by the PNFHPC participants. (See attachment)

At present the membership is in the process of ratification of the proposed amendments to the model program. This ratification process should soon be complete.

Although additional searching may turn up more cases of whirling disease and natural processes may eventually spread the disease to new locations, fish culturists, if they adhere to the proposed guidelines, should not have to worry about being accused of the spread of this difficult to manage problem.

Attachment

GUIDELINES FOR THE TRANSFER OF FERTILIZED EGGS FROM PARENTS, FISH STOCKS OR FACILITIES WHICH MAY BE POSITIVE FOR *Myxobolus cerebralis* INTO AREAS OUTSIDE ITS KNOWN GEOGRAPHIC RANGE

September 1987

1. Transfer and release of salmonid fish infected with or exposed to *Myxobolus cerebralis* into waters not known to be positive for the presence of *M. cerebralis* should be strictly prohibited.

2. Transfer of gametes from positive adults or from adults from positive stocks or facilities should be prohibited because of the inability to surface-disinfect gametes and associated fluids.

3. Only disinfected fertilized eggs, incubated in pathogen-free water and facilities, should be transferred from within geographic areas positive for the presence of *M. cerebralis* to areas outside the known range of the pathogen.

4. Although there is, at this time, no evidence that *M. cerebralis* can be transmitted vertically (from parent to progeny) through properly disinfected eggs incubated in pathogen-free water, it is recommended that only fertilized eggs, from pathogen-free parents, be incubated and shipped from positive stocks, facilities or geographic areas to areas outside the known geographic range of *M. cerebralis*.

5. In all situations where fertilized eggs from *M. cerebralis*-positive parents, stocks, facilities or geographic areas must be transferred to facilities outside the known geographic range of the disease, the following procedures must be followed:

   a. Water harden all fertilized eggs in 100 parts per million active iodine in a water solution of polyvinyl pyrolidone iodine (iodophor) for at least 15 minutes followed by rinsing in pathogen-free water and incubation in pathogen-free facilities prior to transfer.

   b. If disposable egg-shipping containers are used, they must be destroyed by incineration following use. If impervious reusable containers are used they must be disinfected as indicated below.

c. Disinfect all fertilized eggs upon receipt at the receiving facility in a 100 ppm active iodine solution (as above) for at least 15 minutes.

d. Disinfect all fluids remaining in the shipping container with chlorine and disinfect any reusable shipping containers by washing and thorough drenching in at least a 200 part per million solution of active chlorine. Unpacking of eggs and all sanitary measures should be carried out in an area isolated from routine fish cultural activities.

e. At M. cerebralis-positive facilities the "strict sanitary measures" described on page VI-3 of the Model Program section on Emergency (Class A) Disease Control are recommended.

Washington Department of Fisheries

Augmented Fish Health Monitoring

Patty Michak
Washington Department of Fisheries

INTRODUCTION

In 1986 Washington Department of Fisheries began its' Augmented Fish Health Monitoring project. This project, funded by Bonneville Power Administration (BPA), was developed to fill a void of information in the area of artificial production.

An interagency technical committee was formed to determine the minimum level of fish health monitoring needed in the Columbia Basin. Members include both administrative and technical personnel from four state agencies and the U.S. Fish and Wildlife Service. The committee developed a monitoring program which includes agreed upon levels of testing in these areas: specific fish health parameters, water quality and prevalence of certain pathogens.

BENEFITS OF PROJECT

This project will augment previous studies of Columbia Basin anadromous hatcheries funded by BPA. Data provided will allow proposals to be made to improve artificial production in the Columbia Basin. The overall goal is to increase smolt-to-adult survival by 20 percent. This will be evaluated by the coded wire tagging project.

Additionally, the project will increase communications among personnel in hatcheries, research and management. Obstacles to fish health will be identified and plans to resolve them will be developed. The project will develop a standardized interagency system for collection, storing and analyzing fish health data.

SUMMARY OF ANALYSIS

Washington Department of Fisheries has divided the sampling and data collection into three major groups: pre-release analysis, adult analysis and juvenile rearing analysis. The pre-release analysis involves pathogen incidence sampling on 60 fish per stock and completion of the Organosomatic analysis (Geode) on four index stocks (Figure 1). The adult analysis done at spawning includes screening for viral pathogens and Bacterial Kidney disease (BKD). Pre-spawning mortalities are sampled for the presence of bacterial pathogens and parasites (Figure 1). The juvenile rearing analysis involves collecting water quality and rearing parameters, monthly monitoring visits, parasite analysis, viral analysis when clinical signs exist, and a mid-term rearing check for BKD (Figure 1).

NEW SAMPLING TECHNIQUES AND PROBLEMS

To complete the above analyses we had to develop new techniques in sampling for and identifying certain pathogens. Viral sampling of adults included the addition of blood films for Erythrocytic Inclusion

Bodies Syndrome (EIBS). Adults were bled either from the gills or by severing the caudal peduncle. Sampling of juveniles for viral pathogens presented some unique problems. The whole kidney and spleen were removed for IHNV and IPNV analysis. Blood films for EIBS were made by severing the caudal peduncle. It was necessary that the fish be anesthetized in small groups, approximately 10, to obtain free flowing blood. We had some problems obtaining blood from very small fish if they died in the anesthetic.

The Bacterial Kidney Disease analysis presented us with the most problems initially. Kidney smears from juveniles were first attempted by blending the whole kidney then smearing the tissue across a slide. These slides were generally uneven and too thick. Next we removed the whole kidney squashed it between two slides and then smeared the tissue across one slide with the second slide making an even film of kidney cells. This method worked fine when the kidney tissue was only used for BKD sampling, but we also needed the kidney for viral testing and it was time consuming and messy to scrape the remaining kidney into a vial. So our current method of obtaining kidney smears is to run a cotton swab the length of the kidney and then smear an even film across a slide. The kidney can then be removed for viral assay if desired.

In screening adults for BKD we developed a method, with the help of Diane Elliott of the USFWS, to sample ovarian fluid. We chose ovarian fluid because of the ease of sampling and the close association of the fluid to the eggs. Approximately a one ml sample from each female was taken from a cup of eggs using a disposable bulb pipettor. The BKD sample was taken before the viral sample to prevent cross contamination from the viral sampling syringe. The one ml sample was then placed in a microcentrifuge tube. These tubes were frozen on return to the lab to await later processing. Processing involves spinning the samples in a high speed centrifuge (13,000 x g) to pelletize the cellular material. After centrifugation nearly all fluid is decanted leaving only a small amount of fluid to resuspend the pellet. A drop of the resuspended material is then placed on a spot slide to dry. After drying the slide is heat and acetone fixed and either stored refrigerated for later reading or read immediately using a fluorescent antibody technique (FAT).

The volume of sampling made it necessary to refine our fluorescent antibody technique. Pre-release and mid-term analysis alone totaled approximately 3,300 slides. It was imperative that our technique be as exacting and streamlined as possible. The main changes that we implemented were: filtering the conjugate weekly through a .2 micron filter to remove fluorescing dye debris, a conjugate staining time of 10 minutes, and changing our counterstain from Rodamine to Evans Blue for better contrast of fluorescing bacteria to the background.

In addition to the required sampling we ran hematocrits on all pre-release groups. We felt that valuable base line information could be obtained with a minimum of effort. Since we were already taking blood samples in microhematocrit tubes for the blood films we simply took enough blood to run hematocrits. We encountered a problem with obtaining enough blood from small fish. It was necessary to use very little anesthetic and kill small fish with a blow to the head, then bleed immediately to obtain a sufficient volume of blood.

DATABASE DEVELOPMENT

An inherent part of this project is collection and evaluation of fish health data. We developed three forms to fulfill our data collection needs. Form FH01 Fish Health Monitoring Report (Part 1) allows the pathologist to describe the current status of the stock and list sampling done in the field or samples sent to the lab during a monthly monitoring visit. The form is uniquely numbered with a case history number to track sampling data and results (Figure 2). Part 2 of this form FH02 is used on station to record hatchery rearing parameters and to account for cause of loss and any medication used (Figure 3). Form FH03 the Medication and Mortality Report is a daily worksheet for recording loss by cause, medication usage and magnitude and duration of epizootics. Both form FH02 and FH03 are completed by hatchery personnel and sent to our main office monthly for each station stock.

To compile hatchery rearing data, monthly sampling data, pre-release, mid-term and adult sampling data and lab results we needed a database management system. Using R-Base System 5 and consulting with a computer programming specialist we have developed a data compilation and report generation system. At present we are routinely producing the following reports: Disease Prevalence Summary, Hatchery Rearing Parameters and Mortality Summary (by hatchery or species), Yearly Medication Report, and Viral Certifications.

SAMPLING RESULTS AND DISCUSSION 1986-1987

Results for the 1986 pre-release exams are completed with the exception of the whirling disease samples. A total of 34 pre-release exams were conducted. These were evenly divided between 1985 and 1986 brood fish. Results are presented in Figure 5 by brood year for all species combined. The 1985 brood releases include early and late coho, spring and fall chinook. The 1986 brood releases are predominantly fall chinook with one group each of spring and summer chinook.

The only variation in results by species was for the 1985 brood spring chinook. These groups had an average incidence of EIBS of 92.3%, considerably higher than the overall average. We were surprised to find a fairly high incidence of inclusions bodies in all stocks and broods, with the yearling (1985 brood) stocks having a very high incidence of 72.3 %.

The BKD results for the 1985 brood are suspect do to a processing artifact. The first ten groups processed and read were completed before we refined our technique. These groups show considerable higher number of positives than any groups read following refinement of our technique. We feel that some of what was called positive was actually fluorescing debris. The BKD results for 1986 brood were generally negative. The few positives were not evenly distributed throughout the samples. The 5 % positives were found mainly in three groups.

Midterm BKD exam results are also completed. Twenty stocks were sampled and the results are .2 % positive.

The whirling disease samples are not yet completed because of equipment failures. We have new equipment on order and have not received it to date.

COMMENTS

The Augmented Fish Health Monitoring Project conducted by Washington Department of Fisheries is now in its' second year. We feel that we have worked out most of the problems we encountered in the first year. With the collection of a second year of data we will have a better understanding of the 1986 results.

The overall project goals are very large and will not be fully realized until all participating agencies are on line and the collective information is evaluated. We are very proud of the work we have accomplished, but it could not have been done without the help of the following hatchery crews:

Cowlitz Hatchery - Paul Peterson, manager
Elokomin Hatchery - Ed Jouper, manager
Grays River - Dick Aksamit, manager
Kalama Falls - Bob Ready, manager
Klickitat - Doug Loucks, manager
Lewis River - Rob Nicolay, manager
Lower Kalama - John Norton, manager
Lyon's Ferry - Carl Ross, manager
Priest Rapids - Ernie Davis, manager
Ringold - Frank Anderson, manager
Rocky Reach - Don Rapelje, manager
Speelyai - Steve Decker, manager
Washougal - Dick Johnson, manager
Wells - Jerry Moore, Manager

Figure 1. Summary of Analysis

I. PRE-LIB ANALYSIS: all lots
- A. Organosomatic Analysis at index stations.
- B. Viral Assay:
  1. IHNV & IPNV: 60+ fish. Tissue culture, AFS (1985). Sample 60 healthy fish plus a separate group of moribund fish.
  2. Erythrocytic Necrosis (EIB): 60 fish. Blood smear, AFS (1985).
- C. BKD Analysis: 60 fish. Kidney smear, FAT, AFS (1985). One fish pool.
- D. M. cerebralis: examine most susceptible species for spores. In the case of more than one hatchery in a watershed, the most susceptible species will be examined to certify the watershed. Refer to AFS (1985).

II. ADULT ANALYSIS: all lots
- A. Viral Assay:
  1. IHNV & IPNV: 60 fish. Tissue culture, AFS (1985).
  2. Erythrocytic Necrosis (EIB): 60 fish. Blood smear, AFS (1985).
- B. Bacterial Analysis:
  1. BKD: 60 fish. Kidney smear, FAT, AFS (1985). One fish pool.
  2. Furunculosis: take cultures when pre-spawning mortalities indicate, AFS (1985).
  3. Enteric red mouth: take cultures when pre-spawning mortalities indicate, AFS (1985).
- C. Ceratomyxosis (C. shasta): sample pre-spawning mortalities, AFS (1985).

III. JUVENILE REARING ANALYSIS: all stations, all lots
- A. Water Parameters
  1. Flow Index: monthly, all lots, Piper et al (1982).
  2. Loading Density: monthly, all lots, Piper et al (1982).
  3. Sample Water supply: twice yearly, refer to water sampling schedule.
- B. Monthly Visits: all lots will be examined. Attempt to examine 10 moribund fish. If < 10 moribund fish are available make up the difference with healthy fish. Appropriate diagnostic techniques will be used at the discretion of the examiner (tissue culture, gram stain, bacterial culture, blood smear, FAT, etc.).
- C. Parasitic Analysis:
  1. Ceratomyxosis: 10 fish, June through October in surface water supplies only.
  2. PKX: 10 fish, morts in surface water supplies, if kidneys appear swollen.
- D. Viral Analysis:
  1. IHNV & IPNV: 10 fish, if clinical signs exist. Tissue culture, AFS (1985).

2. Erythrocytic Necrosis (EIB): sample moribund if anemic, CWD, BKD, or fungus are present. Blood smear AFS (1985).

E. BKD: mid-term, 60 fish. Kidney smear, FAT, AFS (1985). One fish pool.

Form FH01 FISH HEALTH MONITORING REPORT (Part 1)

Location: _____ Date: _____ / _____ / _____
month day year

Lot Description: _____ _____ _____ _____
species stock brood sub-group

Exam Type: _____ _____ Size: _____ Mortality: _____
[Monit-M,Diag-D,Pre-P,Cert-C] [Egg-E,Juv-#/lb,Adult-A] [Nor-N,Inc-I,Epi-E]

Total loss on day of exam: _____

*** SAMPLE SIZE ***					*** SAMPLE SIZE ***				
Type Pathogen	Hlthy	Morib	Total	#Pos	Type Pathogen	Hlthy	Morib	Total	# Pos
*	*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*	*

[Viral: IHN,IPN,EIB Bact: BKD,CWD,FUR,ERM Paras: M.c,C.s,PKX]

POND	TEMP	LOSS	POPUL	%LOSS	F/LB	FLOW	LBS/GPM	LBSFED/DAY

Diagnosis:

Recommendations:

Remarks:

Examiner: _____ Case Number: _____

Form: FH02

FISH HEALTH MONITORING REPORT (Part 2)

Location: _____

Information for: _____ month _____ year

Lot Description: _____ Species _____ Stock _____ Brood _____ Sub-group

Pond #(s)	Number of fish	Fish/ Pound	Total Pounds of Food for month	Water Inflow gpm	Pond Volume cu ft	% Reuse	Total Number of Morts for Month	Remarks
TOTAL:								LAST DAY OF MONTH

FISH INFO: Ave Lth _____in.

DIET INFO:
Type _____

Pellet Size _____

Lot Number _____

WATER INFO:
Max Temp Range _____ lowest - _____ highest

Agent	* MORTALITY * Number OR Pct	**** MEDICATION RECORD **** Type	Dosage	Amount	Remarks

Compiled by: _____ Case No. _____ Pathologist: _____

FORM: FH03

MEDICATION AND MORTALITY REPORT

Location: _____

Information for: _____ month _____ year

Lot Description: _____ Species _____ Stock _____ Brood _____ Sub-group

Day	****** Medication ****** Type	Dosage	Amount	** Total ** ** Mortality ** Number	Cum	** Daily Mortality Due to Specific Agent ** IHN	IPN	EIB	BKD	CWD	FUR	ERM	C.s	other (specify)	
1															
2															
3															
4															
5															
6															
7															
8															
9															
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26															
27															
28															
29															
30															
31															
TOTAL															

Figure 5. Pre-release Analysis Results 1986-1987

1985 brood - 17 stocks			1986 brood - 17 stocks		
IHNV	-	negative	IHNV	-	negative
IPNV	-	negative	IPNV	-	negative
EIBS	-	72.3 % positive	EIBS	-	48.4 % positive
BKD	-	31 % positive	BKD	-	5 % positive

IS ADULT RECOVERY OF COHO AND CHINOOK SALMON AFFECTED BY STATUS OF SMOLTIFICATION AT RELEASE?

by

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ABSTRACT

The degree of smolt development at release greatly influences rates of seaward movement for juvenile salmon in the Columbia River. Minimal smoltification results in slow migration along the shoreline whereas more completely transformed fish tend to move to mid-river and migrate faster. As migrants move downstream the smoltification process is accelerated and rates of migration increase. Coho salmon released in June of 1979 from the Washougal (WDF), Toutle (WDF), and Cascade (ODFW) hatcheries migrated to Jones Beach (Oregon; RKm 75) faster than fish released in May. Migration rates of fish released in July were similar to fish released in June. All fish released from these hatcheries experienced rapid increases in gill $Na^+ - K^+$ ATPase activity while migrating downriver, an indication of further smolt development which, up to the time of release, had been inhibited or prevented by the hatchery environment. Migration rates and Jones Beach recapture information indicated that fish released in June and July were probably more completely developed smolts than those released in May. Adult recoveries of fish released from these three hatcheries and from the Big Creek hatchery (ODFW) were greater for fish released in June than May, and similar for May and July. Failure of the July-released fish to show recovery rates comparable to June fish may have resulted from ocean conditions or from high river temperatures (18-20° C) which have been

shown in laboratory studies to be detrimental to retention of smolt condition in coho salmon.

From 1978 to 1982 subyearling fall chinook salmon released from the Spring Creek National Fish Hatchery (USFWS) into the Columbia River during March, April, and May were monitored for development of gill $Na^+ - K^+$ ATPase activity before release. Fish released in 1978, 1979, and 1980 generally showed development of elevated ATPase activity during the spring, prior to the May release. In 1981 there was no development of elevated gill ATPase activity prior to and including the May release. In 1982 there was no elevation in activity until at the time of the May release. Adult recoveries for 1981 (all releases) and 1982 (March and April releases) were low (0.14- 0.37 %) whereas recoveries from fish released in 1978, 1979, 1980, and May 1982 were higher (0.54 - 2.12 %). These data suggest that pre-release smolt development, as indicated by elevated gill $Na^+ - K^+$ ATPase activity, is important to post-release survival of subyearling fall chinook salmon.

EXPERIMENTAL ACCELERATION OF OVULATION IN CHINOOK SALMON AND STEELHEAD TROUT AT PRODUCTION HATCHERIES IN BRITISH COLUMBIA

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The use of high potency gonadotropin releasing hormone analogues (LHRHa) has been identified as a means of inducing ovulation and synchronizing spawning in salmonids, thus reducing broodstock holding time and pre-spawning mortality.

We have tested a synthetic LHRH analogue (D-Ala^{6}-desGly^{10}-LHRH ethylamide) to accelerate ovulation and spawning in chinook salmon (Oncorhynchus tschawytscha) and steelhead trout (Salmo gairdneri) at two production hatcheries in B.C.

At Chilliwack Hatchery (April 1986), 16 adult female steelhead trout were divided into two groups (day 0). Eight fish were injected with saline as controls and eight were injected with LHRHa (0.02 mg/kg) in 0.4 ml/kg saline solution. The treatment was repeated three days later. By day 13, 100% of the hormone treated fish had spawned compared with 12.5% of the controls. Mean percent survival of the induced eggs (84.9%) was lower than the mean of the controls (95.8%), but not significantly different.

At Kitimat Hatchery (August 1986) 80 adult female chinook salmon were divided into four groups of 20 fish (day 0). In this experiment the effect of an antibiotic (Liquimycin LA) was also tested. Group 1 received intraperitoneal

injection of the LHRHa (0.02 mg/kg) plus 0.1 ml of antibiotic injected into the dorsal sinus. Group 2 received the hormone alone, group 3 received the antibotic alone and group 4, a saline injection. The hormone treatments were repeated three days later. By day 7, 75 and 80% of the LHRHa treated fish had ovulated compared with 30 and 35%, respectively, in the antibiotic alone and saline injected fish. Pre-spawning mortality was negligible in all four groups. Mean survival to eyed egg stage was higher in the hormone and antibotic treated groups (90.4 - 94.1%), but not significantly different than the mean survival of the eggs from the control group (86.7%).

The mechanism of action of gonadotropin releasing hormone in the process of final maturation in salmonids, alternative GnRH and LHRH analogues currently available and the advantages of these in terms of cost efficiency and the optimization of broodstock management in production hatcheries will be discussed.

ACKNOWLEDGEMENTS

We acknowledge the contribution of Don Buxton and Leslie Schubert in the test conducted at Chilliwack Hatchery, Edward Britton and Mark Westcott for assistance in the test at Kitimat Hatchery. Thanks are due to Ms. H.M. Dye for the preparation of computer generated diagrams, and to M. Booth for typing this abstract and preparation of the tables.

ARCATA WASTEWATER TREATMENT PLANT DISCHARGES FOR IMPRINTING OF SALMONID SMOLTS

George H. Allen

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Introduction

In September 1987, the City of Arcata, a small community of about 15,000 population located on the north arm of Humboldt Bay (Arcata Bay), northern California coast, received a Ford Foundation Innovative Grants award and also an Environmental Merit Award from the International City Managers Association. Both awards were in recognition of development of a system that successfully integrated city sewage treatment plant (STP) and effluent disposal with wildlife enhancement and salmon culture based on wastewater reuse. The system began operating July 1986 when disinfected secondary-level treated oxidation pond effluent began entering three freshwater wetlands of the Arcata Marsh and Wildlife Sanctuary (AMWS) (Figure 1). Freshwater now leaving the marsh system is pumped to a disinfection unit for chlorination and discharge to Humboldt Bay via Butcher's Slough (Figure 1). Freshwater from the AMWS is planned for operation of a fishway and holding ponds for returning adults, and for smolt imprinting to complete an anadromous salmonid wastewater aquaculture facility begun in 1969. Land allocated for development of these facilities lies within 100 feet of the pipe carrying marsh water to the chlorination chamber (Figure 2). The completed facilities would allow for investigation of whether AMWS freshwater contains chemical cues that could improve salmonid smolt imprinting and thus minimize straying of returning adults. The proposed fishway would allow study of reactions of migrating salmonids to residual free chlorine and chlorinated hydrocarbons produced in disinfected wastewaters from the closely adjacent STP outfall (Figure 2).

This paper documents the history of planning the location of STP discharge points where fresh water would be potentially available for fishway operation. Preliminary studies on the effects of chlorination on AMWS water quality required for smolt imprinting and fishway operation are presented. Beneficial uses of Humboldt Bay waters to be enhanced by proposed use of freshwater from the AMWS in fishway operation are described.

In November 1987, the North Coastal Regional Water Quality Control Board adopted a permit for the upgraded Arcata STP and disposal system effluent standards and operating parameters as required under the federal National Pollution Discharge Elimination System (NPDES) program. This paper outlines how the proposed use of marsh effluent in aquaculture is consistent with extant policies on wastewater discharges to California bays and estuaries as outlined in the NPDES permit.

Finally, I describe the two kinds of adult holding ponds proposed for use with the wastewater-operated fishway.

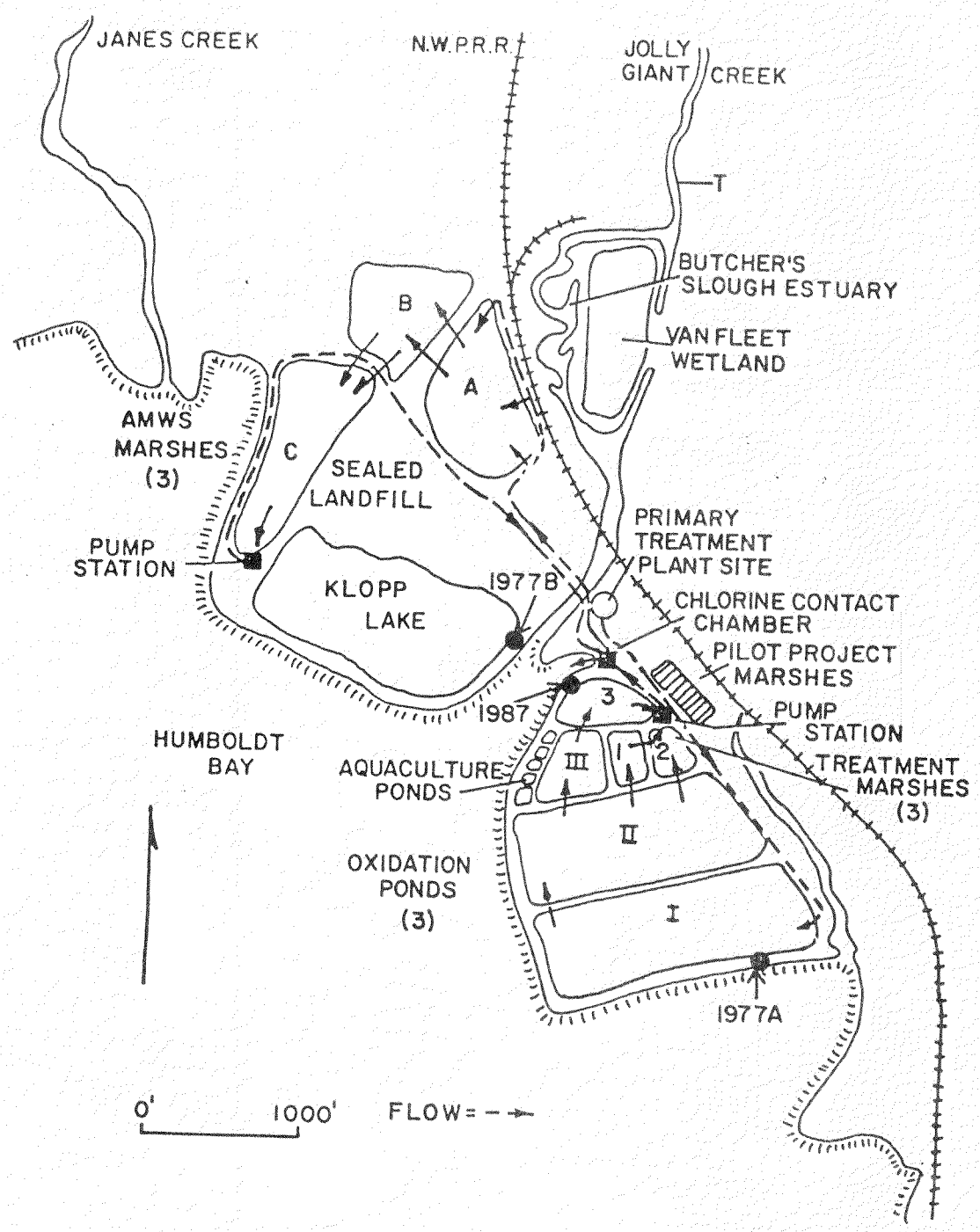

Figure 1. Plan view of Arcata sewage treatment system and wetlands disposal facility (Arcata Marsh and Wildlife Sanctuary) as of July 1986, showing locations of outfalls identified potential sites for fishway and pond operation using wastewaters (Salmon ranching feasibility study-1977A; Marsh Task Force proposal-1977B; Present proposal based on final STP and disposal system-1987) (T-temporary smolt release site and adult collecting weir upper Butcher Slough estuary 1977).

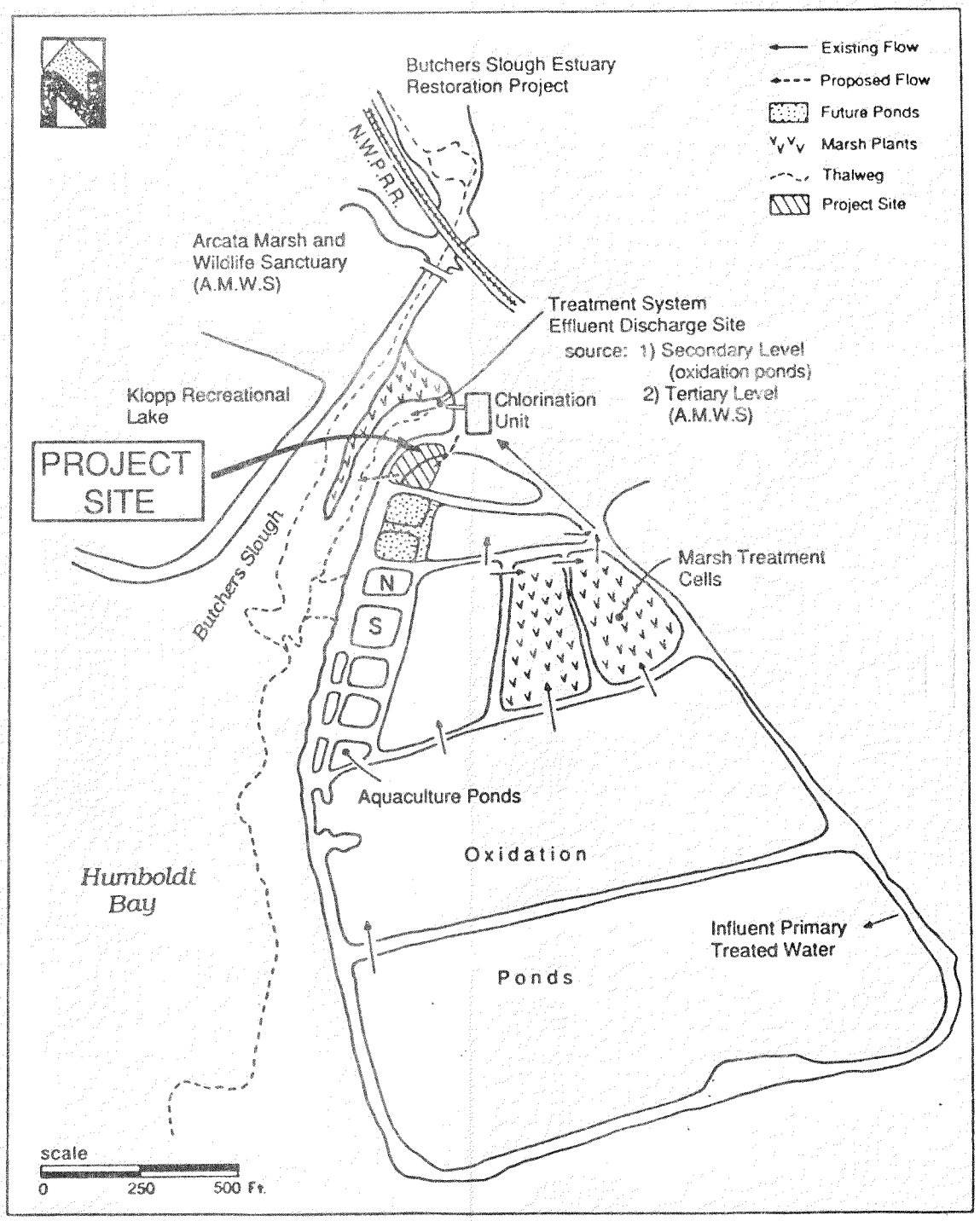

Figure 2. Details of Arcata secondary sewage treatment system, aquaculture ponds, and location of proposed adult fishway and holding facilities to be operated with AMWS effluents (Project Site) August, 1987. (Pilot Project Ponds - N,S).

Fish Culture History

In 1963, I proposed to the City of Arcata a plan to locate ponds within the periphery of an existing 55-acre oxidation pond sewage treatment system for development of an experimental juvenile salmonid rearing system using oxidation pond water mixed with seawater from Humboldt Bay (Allen 1984). Choice of the oxidation pond location for an anadromous salmon culture system arose from the fact that no feasible site on a stream adjacent to the Humboldt State College campus could be located for possible development of an instructionally-related anadromous culture system. On acceptance by the city, the 1963 proposal was submitted to the U.S. Public Health Service where it became an approved project for funding as a demonstration project. The project had to be withdrawn, however, when floods ravaged northern California in 1964 and matching funds provided by Humboldt County had to be withdrawn. My interest in the general topic of wastewater aquaculture, however, was continued while on a sabbatical leave to Europe in 1966-67 through library research (Allen 1969). On returning to Humboldt County, capital funding for pilot-project ponds was obtained from the California Department of Fish and Game, Wildlife Conservation Board (Figure 2, N.S. ponds). Experimental operation of pilot-project ponds using coho, chinook, and steelhead trout began in 1971 and continued through 1976 under Sea Grant funding. In 1977, the city of Arcata assumed funding of wastewater salmon culture within the Department of Public Works. Two major factors allowed the council to financially support such a municipal wastewater salmon culture operation. The first factor was a sizeable return in 1977 of coho salmon to a temporarily selected "home-stream" (Jolly Giant Creek) where pond-reared smolts had been released in 1976. This return demonstrated the viability in the ocean of wastewater-reared juvenile salmon (Miyamoto 1979). The second factor was non-technical. Salmonid aquaculture with wastewater could be viewed as "enhancement of receiving waters". An "enhancement" of Humboldt Bay water was going to be required for Arcata's continued wastewater discharge into Humboldt Bay under a policy promulgated in 1974 by the California State Water Quality Control Board (Gearheart et al. 1983). The policy prohibited wastewater discharges to certain California bays and estuaries except under certain conditions to be detailed later in this report.

Under city funding, experimental rearing of juvenile salmonids continued in pilot project ponds while two demonstration-stage rearing units were developed at minimal capital cost through use of waste materials (Leonhardt 1984). Pilot-project ponds (N,S, Figure 2) have been converted to tidal flushing ponds for studies on marine rearing of juveniles and adults.

During pilot and demonstration stages of wastewater-seawater rearing of juvenile salmonids, major aquatic rehabilitation and restoration projects adjacent to the aquaculture ponds were completed. Under California Coastal Conservancy funding, the AMWS doubled the acreage of freshwater wetlands around Humboldt Bay (Gearheart et al. 1983). In 1986, the estuary to Jolly Giant Creek (Butcher Slough) was restored (Allen and Hull, 1987). As noted previously, wastewater began entering the AMWS, as well as STP effluent into Butcher Slough when the upgrade STP plant and disposal system began operation in 1986. When the proposed facilities for utilization of AMWS freshwater for operating imprinting ponds, fishway, and adult holding ponds are constructed,

all of the physical elements for a complete anadromous salmonid wastewater aquaculture project initiall proposed in 1963 will be in place.

"Home Stream" for Wastewater-Produced Smolts

During pilot project studies smolts were released into a number of creeks and sloughs adjacent to the oxidation pond where returning adults could not be recovered in any systematic manner. With release of smolts in 1976 into Jolly Giant Creek and construction of trapping facilities located in the upper estuary of the creek, consistent recovery of marked adults became possible (Miyamoto 1979). Located on private property, this estuarine trapping site was abandoned and moved to city property located about two miles inland (Allen 1984 Plates E and F). Points-of-release and points-of-return on Jolly Giant Creek were always considered temporary since the creek is too small to accommodate adults that will arise from full utilization of pond rearing capacity now on hand. Thus, freshwater to be produced in an upgraded Arcata sewage treatment system had always been regarded as the final source of water for operating a fishway.

Considerable uncertainty has existed over the nature of the future STP and the location of the final discharge point. A first tentative suggestion was advanced in 1977 when I was asked to provide the City of Arcata with a feasibility study on establishing a salmon aquaculture system based on the use of Arcata STP wastewaters (Allen 1977). The city was exploring alternatives to a regional collection and treatment system for Humboldt Bay being developed in response to two water pollution control policies established in the early 1970's (PL 92-500, Federal Clean Water Act of 1972, and as noted earlier the 1974 California State Water Quality Control Board policy on discharges to Bays and Estuaries). The Bays and Estuaries policy as initially worded prohibited discharges unless "enhancement" of receiving waters above conditions which would have existed in the absence of a discharge could be demonstrated. What consisted an "enhancing" act was not defined (Allen and Gearheart 1978; Environmental Research Consultants 1974; Pequenat and Butler 1979) but eventually became those beneficial uses listed in pollution control plans for bays and estuaries as established by regional water quality control boards. Arcata was always a reluctant participant to the regional collection and disposal system under consideration because Arcata was meeting federal discharge requirements with a treatment system consisting of primary treatment, secondary treatment in lagoons, and direct discharge to Humboldt Bay after chlorination. Algal cells, and Daphnia (crustacean-water fleas) however, were responsible for technical violations of effluent standards (Biological Oxygen Demand (BOD), Nonfiltrable Residues (NFR) also termed Suspended Solids, and Bacteriological Indicators). A continued discharge to the bay was being viewed as violation of the Bays and Estuaries policy. Salmon aquaculture certainly appeared a technique to enhance beneficial use of bay waters accompanying an upgrade of the sewage treatment system under the bays and estuaries policy. The proposed plan had a discharge direct to Humboldt Bay (Figure 1, Point 1977A) and originated in ideas developed prior to the 1974 bays and estuaries policy (Allen 1973) (see also Environmental Research Consultants 1974, Appendix A). The plan assumed that all effluent had to pass through the salmon aquaculture facility, and that final effluent disinfection might not be necessary because of the extra treatment provided by

the aquaculture ponds. Neither assumption was valid. Also techniques for ammonia control were not clearly formulated. The direct use of oxidation pond water through the salmon rearing ponds was summarily eliminated from any further consideration in subsequent planning of alternatives for meeting Humboldt Bay water quality protection needs. Aquacultural Recycling, however, was mentioned in discussing alternatives for a regional system (Environmental Research Consultants 1974, pages IV-13 to IV-14). Although the review gave aquaculture techniques support, the plan as available at that date for the EIR review was insufficiently developed to merit serious consideration by reviewing authorities. A concluding remark "This technique may provide an excellent ultimate solution to the local domestic waste disposal system" certainly may have provided some psychological energy for subsequent planning efforts.

A second plan in 1977 was proposed with a discharge point on the west side of Butcher Slough (Figure 1, Point 1977B) (Klopp No date). This second plan (Figure 3) proposed an integrated sewage treatment, wastewater disposal and salmon ranching project utilizing an existing abandoned county sanitary land fill located immediately west of the Arcata oxidation ponds. The scheme involved only land owned by the city, contained only two marshes, and had marsh effluent flowing through a 17-acre lake before being discharged into Humboldt Bay via Butcher's Slough. Water from the 17-acre lake (Klopp Lake) was to pass through a fishway and ponds before entering Butcher Slough. The "enhancement" values associated with wetland development offered by this proposal were highly significant because of the relative lack of freshwater wetlands around Humboldt Bay (Allen and Hull 1987). The 1977 proposal (Figure 3) was designed to employ gravity flow wherever possible in order to minimize energy and electrical costs associated with pumping. Pumping in the plan would be required to lift influent sewage water to primary clarifiers from which supernatant passed through the lagoons by gravity flow. Pumping was also needed to furnish water to the first cell of the two wetlands. Pumping would also have been needed to operate the proposed fishway facilities at the outlet of the recreational lake (Figure 3, Fishway) since during high tides water levels in Butcher Slough were above those in Klopp Lake. This 1977 plan specifically mentioned scientifically studying imprinting of smolts with wastewater (Allen 1984).

The complicated history of administrative policy and political events leading to the final adoption of the integrated sewage treatment and disposal system now in place (Figure 1) has been sufficiently documented elsewhere and will not be repeated here (Bretnall 1984; Gearheart et al. 1983, In press). The system as completed in 1986 has three-marshes in series instead of two as originally proposed in 1977. This was due to the enlarged wetland (AMWS) project constructed independent of wastewater issues, by the California Coastal Conservancy because of the value of increasing freshwater wetland habitat around Humboldt Bay. There was a significant design change, however, in the established Arcata system over that proposed in 1977 which was highly beneficial to planning the fishway elements of Arcata's salmon culture system. This was returning of marsh effluent by pumping back to a dual-chambered disinfection unit located immediately adjacent to the aquaculture ponds (Figure 2). This change made by State Water Resources Control Board staff during design review now has pressurized water from the AMWS being available immediately adjacent to the northern end of the aquaculture facilities (Figure

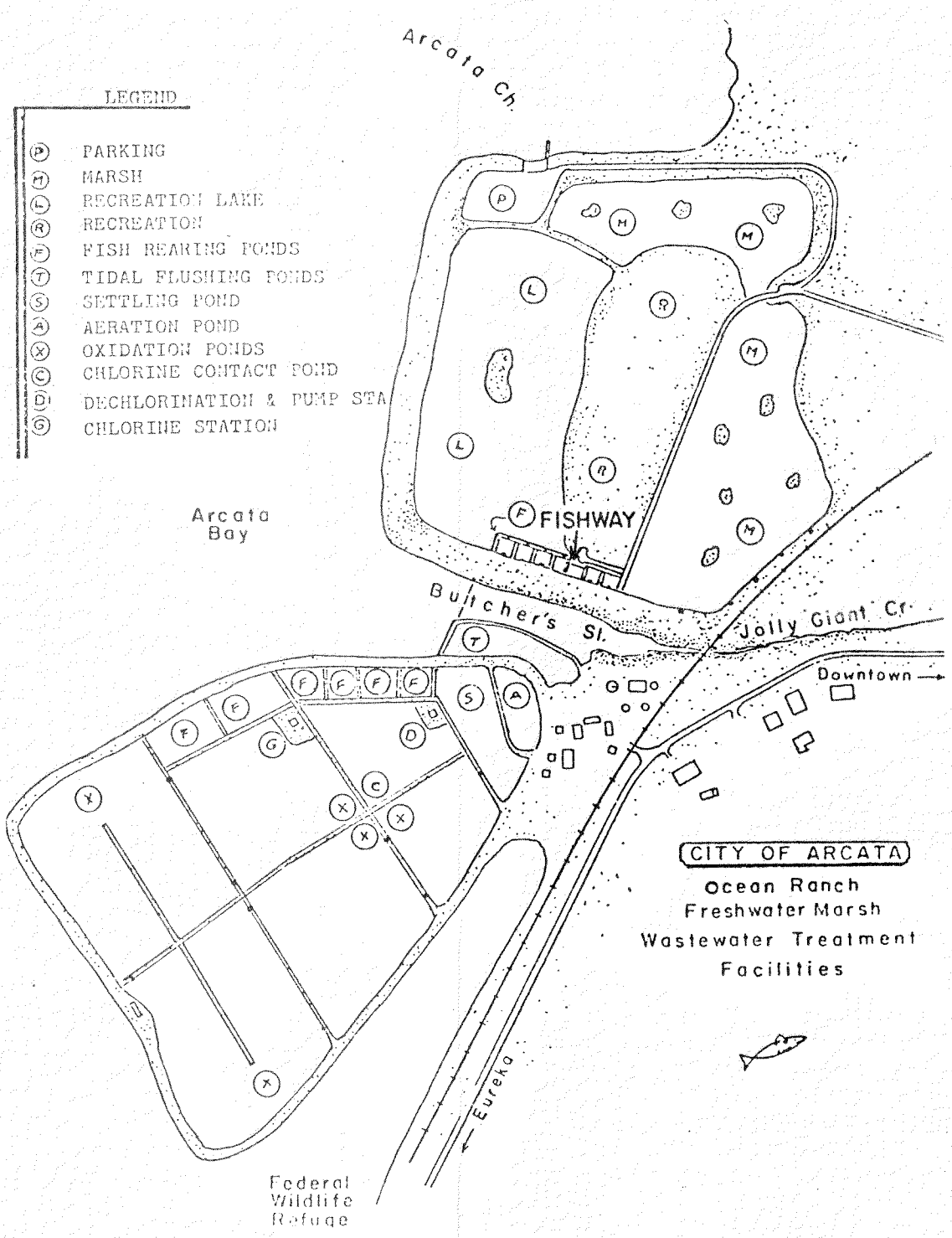

Figure 3. Location of fishway in an upgraded Arcata oxidation pond system of sewage treatment, freshwater marsh and lake system for wildlife enhancement, and anadromous salmonid aquaculture system based on wastewater utilization as developed by Arcata Marsh and Wildlife Task Force for City of Arcata, 1977 (Klopp, No date).

2). As previously noted, this will allow easy integration of AMWS freshwater into the Arcata wastewater aquaculture project.

Marsh Effluent in Culture Operations

Three major uses of freshwater from the AMWS can enhance the operation of the Arcata wastewater salmonid aquaculture system beyond that of fishway operation. These uses are: (1) provide freshwater for salinity control in rearing ponds, and (2) provide an experimental location for systematic study of the efficacy of an artificial home-stream for reducing rate of straying of adults through improved smolt imprinting, and (3) provide a location to study behavior of returning adult salmonids in a chlorinated wastewater discharge field.

Homing and Straying

The location of the Arcata aquaculture system on the lower reaches of the Butcher Slough estuary is an optimal site for avoiding conflicts in managing natural and artificial anadromous salmonid stocks (Allen 1985; Lister et al. 1980). Straying of adults returning to Jolly Giant Creek to adjacent creeks was predicted in initial proposals to the California Department of Fish and Game for the pilot-project wastewater aquaculture ponds. Straying was considered beneficial since stocks of salmon in streams entering Arcata Bay were at extremely low levels (Allen 1984). Recent increase in salmon restoration programs on Humboldt Bay has reduced the need for seed stock to Arcata Bay tributary streams (Hull 1987). If marsh water can improve imprinting, and therefore improve homing, the technique would not only benefit Arcata, but would be useful for other agencies in considering similar schemes at other estuarine-sited STPs.

There have been two opportunities to measure the rate of straying of coho salmon by adults returning into Arcata Bay from smolts released into Jolly Giant Creek. In the fall 1977 - winter 1978 migratory season, 0.3 percent of smolts released were recovered in Jolly Giant Creek (Miyamoto 1978), and 0.2 percent in Jacoby Creek in temporary trapping facilities (Harper 1980). In the fall 1984 - winter 1985 migratory season, 0.5 percent of smolts released were recovered in Jolly Giant Creek and 0.3 percent in three closely adjacent streams (Allen 1985). Thus locating a permanent point-of-return on a site along Jolly Giant Creek would only be expected to collect 60 percent of the returning adults. This would be an unacceptabaly high loss (40 percent) of returning adults anticipated in expanded operations.

There has been only one opportunity to measure chinook straying. Fall chinook of Iron Gate, Klamath River stock, were reared in wastewater ponds and at the Humboldt State University fish hatchery. Unequal sized lots of fin-marked chinook were released in the experiment which originally was designed to have equal numbers in four categories (Table 1). The uneven-sized lots came from mortalities suffered from a bacterial outbreak in the HSU hatchery stock, while an error in pond management released most of the Arcata wastewater-readied chinook as unmarked fish in June. No jacks were taken in

Table 1. Number of 1982-brood year fall chinook Iron Gate stock released into Jolly Giant Creek, summer 1983, in experiment planned for 10,000 fish to be released in each category.

	Rearing Location					
	Humboldt State University hatchery			Arcata Wastewater-Seawater Ponds		
Month of Release	Number Released	Mean Wt(gm)	Mark	Number Released	Mean Wt(gm)	Mark
June	2,035	8.3	LV	16,700	5.0	UM
November	2,500	15.2	RV	0	-	LP or RP
Total	5,535			16,700		

Jolly Giant creek from these plants. Below normal rainfall during the 1985-86 and especially the 1986-87 migratory seasons seriously effected the ability of salmon to migrate from Humboldt Bay into Jolly Giant creek (Table 2). Only two adult chinook were recovered from Jolly Giant creek. On 23 January 1986, a 14.4 pound 87 cm over-mature unmarked female chinook was sighted in one of the creek's sediment basins and recovered upstream from the basin by electro-fishing. On 29 October 1986, a 12.0 pound 81 cm male was dip-netted from the upper section of the Butcher Slough estuary during an out-going tide following a heavy rain of short duration. In contrast, in 118 days from October 22, 1985 through February 17, 1986, four chinook were taken during 111 days of trap operationon Freshwater Creek, and from 62 days of trapping between 21 November 1986 and February 1, 1987, a total of 21 chinook were taken (Table 2). The last chinook in the 1986-87 migrating season trapped at Freshwater was January 19, 1987. The degree of straying to Freshwater Creek by Jolly Giant Creek chinook could not been determined since all chinook captured were unmarked. No fin-marked HSU hatchery-reared chinook from the experiment were ever recorded. Since a chinook release program had not been instigated at Freshwater Creek at a time to account for the number captured, some of Freshwater Creek recaptures could have been of Jolly Giant origin. Iron Gate stock used in the experiment migrate to freshwater in August-September and spawn in October-November. Such fish in Humboldt Bay could have been seeking an alternative stream when low flows impeded access to the creek at their usual time of migration and maturation.

Straying induced by lack of water in small coastal streams on Humboldt Bay probably is a direct function of the degree of freshwater runoff from major storm events occurring during a receding tide. Miyamoto (1979) found in a very wet migratory season (1977) there was a normally distributed return around a late November peak to the upper Butcher Slough estuary, with fish trapped about two hours following high tide. Only three such shared high tide-high stream discharge events occurred in the 1986-87 migratory seasons and each event produced either a chinook or a steelhead adult in Jolly Giant creek (29 Oct. 86, 2 Jan. 87, and 11 Feb. 87). Thus use of AMWS water to create an entirely artificial home-stream with a constant flow of water at a lower estuarine location will create a point-of-return for adult anadromous

salmonids whose accessability will be independent of streamflow produced by seasonal rains. The proposed facility will increase the overall reliability of the salmon culture system by reduction of straying due to inadequate attractant flows. Chinook salmon should be particularly aided by a constant flow of attractant water since they will move into hatchery facilities without the necessity of freshet or rainfall conditions typically associated with coho (Allen 1959).

Table 2. Comparison of fall chinook salmon recovered at trapping facilities on Freshwater Creek and from Jolly Giant Creek by fishing during two low-flow years (fall 1985 to winter 1987).

Species	Jolly Giant Creek		Freshwater Creek	
	85/86	86/87	85/86	86/87
Chinook	1	1	4	21
Coho	0	0	74	109
Steelhead	1	3	21	29

Water Quality Reliability

During recent years the water quality in Jolly Giant Creek in spring months has either deteriorated or poor conditions went undetected during earlier smolt releases. Adult fish returning in 1977 came from smolts released to the upper Butcher Slough estuary (Figure 1, Point T) while returning fish in 1984 came primarily from smolts released inland that migrated through the culvert section of the creek (Allen 1985). Smolts released inland in the spring of 1985, 1986, and 1987, however, encountered toxic conditions in the creek as measured by the capture of either dead or moribund smolts in downstream migrant traps located within the culvert section of the creek (Craig 1985; Waller, Personal Communication 1986 and Table 4 in Allen 1987). The source of contaminants and their identification are under investigation but the problem will be difficult to solve. Craig (1985) reported three 1-3 day periods between April 11 and May 16, 1985, when most fish trapped in the upper estuary of Jolly Giant Creek were dead or moribund. We believe that there is not only a point source of pollution from some ancient or unauthorized connection to the creek but that illegal dumping of toxic cleaning or cutting fluids by commercial operations or private individuals has been occurring. Thus the use of marsh effluent for future smolt migration to Humboldt Bay will assure that smolts released will not be subject to mortalities from unpredictable non-point sources of pollutants.

Constraints

Effluent Standards

Two legally designated outfalls to "waters of the state" have been established for the Arcata integrated system in a federal NPDES standards issued by the North Coastal Regional Water Quality Control Board November 1987. Water to Humboldt Bay (Outfall No. 001) has a standard of median fecal coliforms not to exceed 14 MPN/100 ml (shellfish growing water standard). Water entering the AMWS (Outfall No. 002) has a effluent standard of median total coliforms not to exceed 23 MPN/100 ml (public recreation standard). These two outfall standards are consistent with an informal state policy that discharge requirements need only to be met once in a system prior to discharges to a receiving water. The upgraded Arcata STP and wetland disposal system has been designed and constructed so that water returning from the AMWS will always be rechlorinated before entering Butcher Slough, either as undiluted marsh water or as water blended with oxidation pond water. Oxidation pond water, however, can be discharged directly to Butcher Slough after disinfection. This requirement for double chlorination of the oxidation pond effluent used in marsh enhancement probably arose because the concept of wetland treatment technology was new and that some redundancy was necessary in case high levels of treatment in pilot-project marshes (Gearheart et al. 1982, 1986) might not prove attainable in a full scale project. Oxidation pond water discharged to the AMWS has almost always been less than 2 MPN/100 ml fecal coliforms. Marsh effluent prior to second chlorination has attained the same level of quality as pilot project cells (Allen et al. In press). Sporatic technical violations of bacteriological standards for discharge to Butcher Slough have occurred in the non-chlorinated AMWS effluents. Undoubtedly elevated fecal coliform levels are related to conditions expected to occur in a productive wetland heavily utilized by a wide variety of wildlife, especially mammals and birds (Table 3, e.g., May, September).

Chlorine Toxicity

Free chlorine, chloramines, and by-products produced by chlorination of STP effluents, are known or suspected to be toxic to salmonids at low concentrations. Free chlorine at 0.02 mg/l is toxic and behavior is affected at even lesser concentrations (Tsai 1973).

Toxicity of Arcata oxidation pond water prior to disinfection currently is determined on monthly grab samples by a certified public laboratory using trout bioassay. Water leaving the AMWS has been monitored by Arcata personnel by continuous cage culture using three species of fish (trout, stickleback, mosquitoefish). Virtually no mortalities in 96-hour bioassay of oxidation pond effluent have been reported by the certified public laboratory, and no mortalities in AMWS water until the approach of summer when elevated temperature and lowered dissolved oxidation levels became unsuitable to trout. Thus, water leaving the AMWS and entering the disinfection unit has been shown reliable for salmonid culture.

Table 3. Fecal coliform (MPN/100 ml) concentrations in water discharged from the Arcata Marsh and Wildlife Sanctuary supplied with disinfected STP effluents having total coliforms 2 MPN/100 ml or less, May-September 1986.

	MPN Level[1]					
Month	<23	>23-<100	>100-<700	>700-<2,400	≥2,400	N
May	4			1	2	7
June	5	2	1	1		9
July	5	1				6
August	3	2				5
September	1		4			5
Total	18	5	5	2	2	32
Percent less than category	56	72	88	90	100	

[1]Standards:

Outfall No. 001: (to Butcher Slough): fecal coliforms - 14 MPN/100 ml, median value (shellfish growing waters).

Outfall No. 002: (to AMWS): total coliforms - 23 MPN/100 ml, median value (recreational use).

Wastewater entering Humboldt Bay after disinfection has an effluent standard for free chlorine residual of 0.01 mg/l after treatment with SO_2. Automatic sampling and chlorine analyzing equipment was provided in the treatment plant upgrade to continuously monitor the free chlorine residual existing in water taken from the downstream side of the sulfonation process. To date, this equipment has been inoperative due either to improper installation or from incompatible instruments and recorders installed. During initial period of startup, STP plant operators routinely conducted standard idometric analysis probably only accurate to $\pm$0.5 mg/l to determine chlorine residuals. The method would be useless for monitoring water to be used in aquaculture with salmonids sensitive to values 0.02 mg/l or less. Consequently, in the spring of 1987, we undertook a bioassay to confirm our hypothesis that desulfonated Arcata effluent would be sufficiently toxic to coho parr and smolts to negate its use in fish culture.

Bioassays were conducted in 30-gallon containers (bathtubs) testing effluent toxicity under three methods of dechlorination (Table 4). Control fish (coho parr and smolts from the Arcata rearing ponds) were held in fish pond water pumped into a container located adjacent to the pond. Test fish were bioassayed in a container mounted in the chlorine analyzer room where

Table 4. Bioassay of Arcata sewage treatment plant effluent under three different dechlorination treatments March-April 1987 (after Ferguson 1987).

Test No. 1. Norman SO_2 application rate in use; continuous flow; no aeration; 10 fish, March 20-21.

Factors	Effluent		Fish pond water	
	Start	End	Start	End
Temp.	12.1	13.0	12.4	13.2
DO	13.0	13.8	8.9	9.4
pH	9.1	9.2	6.8	6.8
24-hour survival	0		10(3)*	
Percent mortality	100		0	

*Three fish jumped from tank.

Test No. 2. Disinfected effluent dechlorinated at a rate of 1 ml thiosulfate per 5 gallons of effluent; static test; aeration; 5 fish; 26-30 March.

Factors	Effluent		Fish pond water	
	Start	End	Start	End
Temp.	15.5	15.5	14.1	17.0
DO	10.8	9.0	12.9	12.4
pH	7.3	8.1	9.1	9.4
5 Day survival	5		5	
Percent mortality	0		0	

Test No. 3. Doubling normal SO_2 application rate; continuous flow; aeration; 5 fish in effluents, 3 in control; 9-11 April.

Factors	Effluent		Fish pond water	
	Start	End	Start	End
Temp.	16.0	16.0	16.2	16.8
DO	10.1	8.4	10.0	9.4
pH	7.2	7.0	9.6	9.5
48-hour survival	00**		3	
Percent mortality	100		0	

**Power surge during experiment shut off wastewater flow but not chlorinator or sulfonator. Mortalities probably from some combination excessive SO_2 or free chlorine.

effluent could either be obtained by grab sampling or from the intake line sampling water downstream from the sulfonation process. Tests run during March and April utilized smolts that were being removed from fish pond by trapping. A second series of tests conducted in May studied the toxicity of effluent being discharged into Butcher's Slough at tide levels sufficiently high to produce mixing at the outfall pipe (Table 5). Water was grab sampled while standing on the outfall pipe. Undiluted effluent was compared to effluent mixed with bay water, water from a fish pond, and water from Humboldt Bay taken away from the outfall. Smolts and parr used in the May tests were from a sample of fish being held after pond draining specifically for use in the bioassay tests. Bioassays were conducted in 5-gallon buckets with water oxygenated with air pumped through air-stones .

Effluent being discharged into Butcher's Slough in late March under normal dechlorination operations quickly produced mortalities (Table 4, Run 1). Repeating the experiment under a controlled dechlorination process using thiosulfate produced no mortalities or stressed fish after five days (Run 2, Table 4). A third run to study efficacy of doubling the normal amount of SO_2 being used by treatment plant operators for dechlorination showed no mortalities in an initial 24-hour period, but a power surge interfered with rate of effluent flowing through the disinfection unit and all fish were killed in test tank (Run 3, Table 4). In early May, undiluted effluent leaving the treatment plant was lethal to coho juveniles during short periods of exposure (Table 5). Effluent diluted roughly 1:1 with bay water showed toxicity on one day (Run II), but no toxicity on a second day (Run III). Mortalities also occurred in comparison water from fish ponds but not in bay water during Run II. Pond water in Run II slightly more saline and slightly warmer than bay water might have caused the mortality due to increased sensitivity in smolts.

These preliminary tests were sufficient to document that it would be hazardous to use chlorinated-dechlorinated effluent for imprinting smolts under normal treatment plant operation and monitoring. Free chlorine also causes avoidance behavior in adult anadromous salmonids and therefore chlorinated wastewaters would be unacceptable for "home-stream" attractant flows for capturing returning adults. Chlorinated wastewaters adversely affect blood chemistry and morphology of coho salmon (Buckley et al. 1976; Buckley 1977). Use of non-chlorinated AMWS freshwaters would insure reliable high-quality water supply needed for smolt imprinting and adult attractant water.

Chlorinated Hydrocarbons

A preliminary study (Gilbert 1987) on the occurrence of potentially toxic compounds formed during chlorination of Arcata STP effluents was conducted during July 1986, during the first month of start-up operations (Table 6). The values reported in our preliminary study (644 ug/l) are substantially higher than those found in effluents using a wide variety of non-wetland sewage treatment system (<2-19 ug/l) (Table 7).

Since wastewaters from the AMWS do not enter groundwaters being used for domestic water supplies, there are no potentially adverse effects to public health via drinking water. Assessment of public health risk of trihalomethanes in public water drinking supplies is under study but

Table 5. Preliminary bioassay results of Arcata chlorinated-dechlorinated STP effluent sampled at outfall to Butcher's Slough, May 7-13, 1987.

Test Number — Description

1 Water sampled from end of outfall pipe with 5-gallon bucket. Three juvenile coho salmon (97-108 mm FL) placed in unaerated water at 1000 on May 7, 1987. By 1300, all three fish stressed, and on 1000 on May 8, all three fish were dead. Temp. 19°C.

2 Water sampled from four sites, and bioassayed with three coho salmon smolts per 5-gallon bucket. Water aerated.

Factors	Undiluted effluent	Diluted effluent	South Pond (control)	Humboldt Bay (Control)
Salinity (°/oo)	0	15.6	29.8	28.5
Temperature	20	21	22	20
Mortalities				
8 May 1030 Start				
1215	3	0	0	0
1400	-	0(2)[1]	0(1)	0
1645	-	1(2)	0(1)	0
1745	-	1(2)	0(1)	0
1900	-	3	0	0
11 May 0700	-	-	2	0
1300 End	3	3	2	0
Fish size (mm FL)	12.0-12.8	12.0-12.4	10.9-13.4	11.5-12.0

3 Water sampled from two sites and bioassayed with three coho salmon parr per 5-gallon bucket. Water aerated.

Factors	Undiluted effluent	Diluted effluent adjacent to outfall
Salinity (°/oo)	0	17.1
Temperatures (°C)	19.0	19.2
Mortalities		
11 May 1345 Start		
11 May 1630	3	0
12 May 1330	-	0
13 May 1300 End	3	0
Fish size (mm FL)	11.0-12.0	10.8-11.22[2]

[1] ( ) Number of fish showing signs of stress (abnormal swimming behavior, at surface, lethargic).

[2] All three parr turned silvery during course of bioassay.

Table 6. Average concentration of Trihalomethanes (ug/L) in Eureka and Arcata STP effluents after dechlorination, June-July 1986 (from Gilbert 1987).

Compound	Eureka (N = 12)	Arcata (N = 4)
$CHCl_3$	6.3	46
$CHBrCl_2$	2.3	106
$CHBR_2Cl$	0.6	258
$CHBr_3$	0.0	234
Total THM	9.2	644

Table 7. Median or average concentration of Trihalomethanes (ug/L) in municipal wastewaters based on values from no more than three samples (from Robeck 1987).

		Municipality				
			Orange County Water District			
Source	Compound	Washington D.C.	2nd Period	3rd Period	Phoenix	Palo Alto
Table V-1	$CHCl_3$	1.5	1.6	3.5	3.5	13
	$CHBrCl_2$	<0.3	0.1	0.5	0.3	0.2
	$CHBr_2Cl$	<0.2	0.2	0.7	0.2	0.1
	$CHBr_3$	0.0	0.1	0.5	0.1	0.0
	Total	<2.0	2.0	5.2	4.1	13.3
		San Jose Creek	Whittier Narrows		Pomona	LA Glendale
Table V-2	$CHCl_3$	14	5.1		7	5.5
	$CHBrCl_2$	0.4	1.4		2.5	5.8
	$CHBr_2Cl$	0.2	0.6		1.8	5.6
	$CHBr_3$	0.2	0.4		0.9	2.0
	Total	15	8		12	19

assessment of risk is difficult (Robeck 1987). A recent federal study of wastewater treatment policy suggested uncertainties exist on receiving water effects from chlorination by-products (Levenson 1987, p. 138). The major public health concern for Arcata-produced THM compounds would be from these compounds entering human foods through consumption of contaminated bivalves. Virtually no recreational bivalve fisheries now exists in Butcher Slough and adjacent channels although one is planned for development in Butcher Slough (Allen and Hull 1987).

Assessment of potential risk to commercial oyster beds by THM compounds might be made in reference to bacterial indicators but such an assessment is confounded by non-point sources of indicator organisms and other deleterious substances entering Humboldt Bay via Jolly Giant Creek. Relatively little is known on the inhibitory effects of chlorinated hydrocarbons on salmon migratory behavior in contrast to that of free chlorine. Thus availability of a non-disinfected water in a fishway operation in relatively close proximity to discharges of disinfected water (Outfall No. 001) would provide a site for scientific studies of salmonid behavior as affected by THM, free chlorine and other by products found in a chlorinated wastewater discharge field.

Bays and Estuary Policy

Enhancement of Beneficial Uses

The Arcata NPDES permit adopted November 1987 by the California North Coast Water Quality Control Board (Order No. 87-125) states:

> E. Arcata Marsh Wildlife Sanctuary
>
> 1. The Arcata Marsh Wildlife Sanctuary shall be operated, maintained, and managed to provide maximum positive benefits for freshwater habitat, recreation, education, scientific study and water quality while at the same time having no adverse impacts on any other beneficial uses of Humboldt Bay.
>
> 2. A comprehensive Arcata Marsh Wildlife Sanctuary management plan shall be submitted to the Regional Board on or before February 28, 1988. The Plan shall clearly outline a program and its implementation that will provide compliance with E. 1. above.

The use of the AMWS effluent proposed for the furthering anadromous salmonid culture clearly complies with the board order to maximize beneficial uses and maintain existing beneficial uses. Out of eleven legally established beneficial uses of Humboldt Bay waters, marsh water for aquaculture use clearly enhances three beneficial uses (Table 8). Two additional uses probably also qualify as being enhanced by the proposed use of marsh effluent (No. 2 and 7). The Regional Board specifically found that use of treated wastewater in the AMWS project provided positive benefits for scientific study which would also be manifest, along with education, in any scientific study of imprinting of anadromous juvenile salmonids, and their subsequent rates of homing and straying. The AMWS waters would be expected to possess distinctive chemical cues necessary for imprinting smolts and attracting returning adults

Table 8. Beneficial uses of Humboldt Bay enumerated in Water Quality Control Plan for the North Coastal Basin (IB) on March 20, 1975, to be enhanced from operation of adult fishway and smolt imprinting pond using non-disinfected freshwater from Arcata Marsh and Wildlife Sanctuary discharge.

Beneficial use	Enhanced: Yes	Enhanced: No	Comments
1. Navigation		x	
2. Water contact recreation	x(?)		Release of surplus juveniles to Klopp Lake for recreational fishing.
3. Non-water contact recreation		x	
4. Ocean commercial and sport fishing	x		Catch of adult fish in Humboldt Bay and Ocean.
5. Cold freshwater habitat (tributaries)	x		Development of a new salmonid ecosystem based on a wastewater stream.
6. Wildlife habitat		x	
7. Preservation of rare and endangered species	x(?)		Brackish wastewater ponds to be used in coastal cutthroat brood stock development program.
8. Marine habitat		x	
9. Fish migration	x		Allows migration during periods of low stream flows due to yearly or seasonal droughts.
10. Fish spawning		x	
11. Shellfish harvesting		x	

[1]State Water Resources Control Board Water Quality Control Policy for Enclosed Bays and Estuaries of California 1974: the policy prohibits the discharge of municipal and industrial discharges of waste to enclosed bays such as Humboldt Bay, with exceptions granted by Regional Boards if it is found that the treated wastewater discharged will enhance the quality or beneficial uses of the receiving water above that which would occur in the absence of the discharge.

(Scholz et al. 1976; Hasler and Scholtz 1983). Studies on homing and straying would be able to use existing fish trapping facilities on Arcata Bay for recovering marked adults (Figure 4).

Protecting Beneficial Uses

Arcata's discharge to Butcher Slough was allowed under the Bay and Estuaries Policy with the expectation that no degrading of any existing beneficial uses of bay waters would occur. A major beneficial use to be protected is that of commercial oyster culture, as well as recreational harvesting of several kinds of bivalves. Protection of public health from human consumption of contaminated bivalves in Humboldt Bay is achieved by not harvesting when the concentration of bacterial indicator species is low in waters overlying beds or in bivalve meats. Certificiation of oyster beds for harvesting by the California Department of Public Health requires overlying water to show 14 MPN/l fecal coliforms or less, and oysters to have less than 230 MPN/fecal coliforms/gram of meat.

Potential human pathogens that could contaminate Humboldt Bay bivalves originate from two major sources: (1) freshwaters entering the bay from creeks and sloughs (non-point sources) and (2) wastewaters discharged from sewage treatment and disposal systems (point sources). Contribution of non-point sources of bacteriological indicators from two areas (Mad River Slough and Jacoby Creek) in a 1975-76 period were presented in Gearheart (1981, Table 6). All three bacterialogical indicators studied were in much higher concentrations in Jacoby Creek non-point water than found in Mad River Slough water (Table 9, A,B,C). A commonly used ratio (FC/FS) in water quality studies is used to locate suspected sources of human fecal contamination. Ratio values suggest human versus non-human animal sources as follows: >4.0, human $\geq$0.6, non-human; >0.6-<4.0, mixed; references in Musselman et al. 1978). Twice as many ratios greater than 4.0 were found in the agricultural drainage water of Mad River Slough than water originating from a drainage characterized by rural residences, some agriculture use, but largely commercial timberland (Jacoby Creek). This was unexpected since human contamination would be expected more in Janes Creek water than Mad River Slough. It points up difficulties in assessing the significance of water quality control monitoring data from indicators. A major study aimed at characterizing bay water quality in relation to shellfish harvesting was carried out on bay waters by federal shellfish authorities (Musselman et al. 1978). Although serious deficiencies were documented in STPs existing at that time in their ability to insure constant disinfection of effluents (reliability) - "It was found that most of these deficiencies did not have definitive bearing on the opening or closing of the conditionally approved portion of Arcata Bay" (underlining in original). Harvesting closures in Humboldt Bay occurs for five days following 0.5-inches of rainfall in any 24-hour period. Non-point contributions of bacteriological indicators in freshwater inflows in Jolly Giant Creek have been shown to increase dramatically with the ascending discharge curve associated with storm events (Roberts 1985). Jolly Giant creek non-point contamination (Table 10) includes runoff from urban surfaces, soil bacteria, raw sewage surcharging in older portions of the collection system, or surcharging when the collection system is blocked. Since the city is only now programming for a staged identification and repair of degraded collection

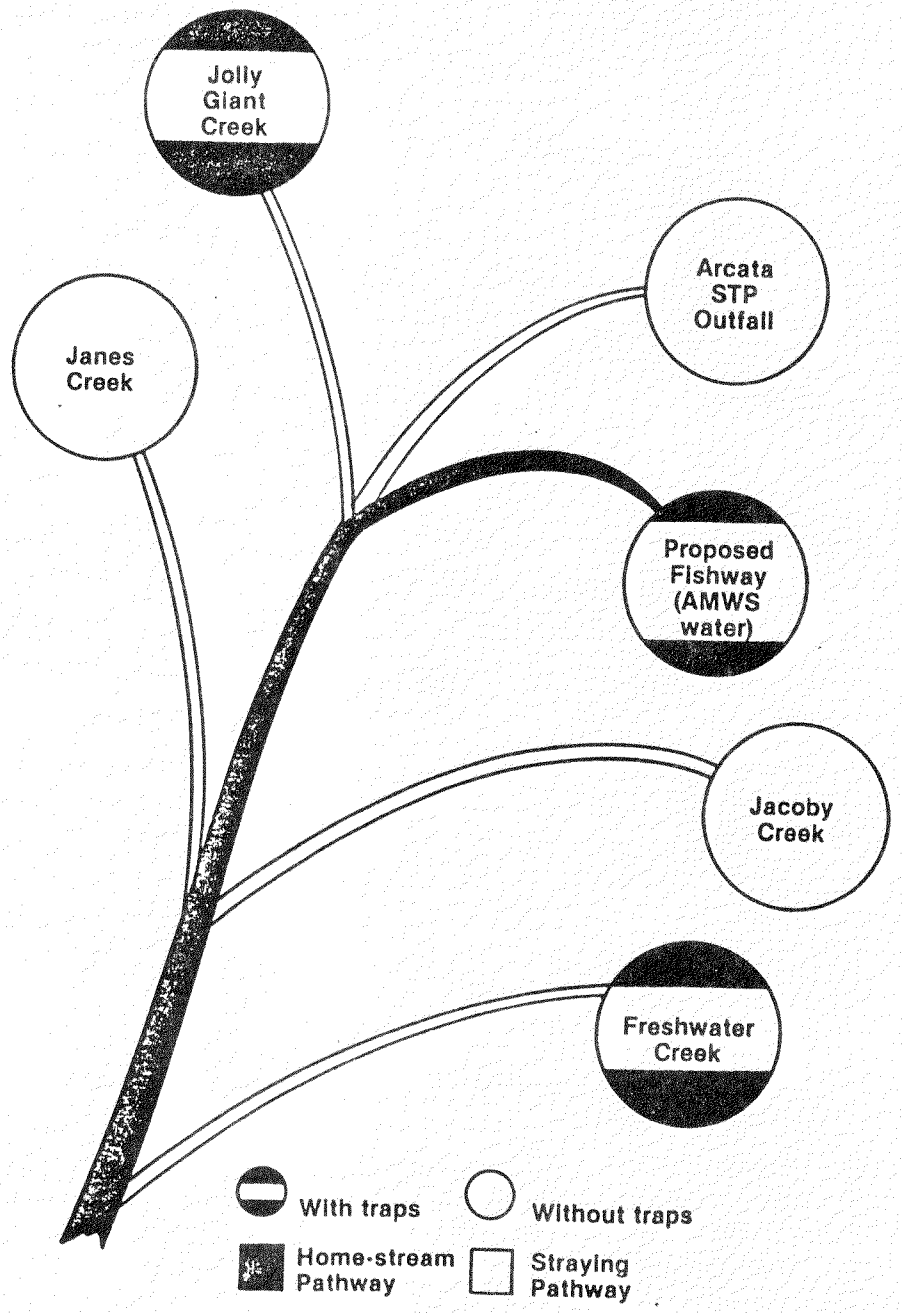

Figure 4. Alternative migration routes avaiable to adult salmon returning to proposed Arcata smolt imprinting ponds using AMWS water, and permanent adult salmonid trapping facilities on streams entering Arcata Bay available for marked fish recoveries for homing and straying studies.

Table 9. Frequency of concentrations of bacteriological indicator organisms in water sampled from Mad River Slough and upper Jacoby Creek Channel, and fecal coliform/fecal streptoccus ratios, November 19, 1975 to September 22, 1976 (from Gearheart 1981, Table 6).

A. Total Coliforms

Category	Mad River Slough	Jacoby Creek
<23	26	2
≥32 >250	10	27
>250-≥2400	4	16
>2400	1	6
	41	41

B. Fecal Coliforms

Category	Mad River Slough	Jacoby Creek
<14	32	5
≥14 <50	4	5
>50-<230	5	11
>230-<500	0	3
>500	0	17
	41	41

C. Fecal Streptococci

Category	Mad River Slough	Jacoby Creek
<14	11	0
≥14 <50	14	1
>50-<230	10	19
>230-<500	4	6
>500	2	15
	41	41

D. FC/FS

Category	Mad River Slough	Jacoby Creek
<2	14	19
≥2 <4	11	15
>4 <6	8	5
>6 <8	1	0
>8 <10	2	1
>10-<12	2	0
>12	3	1
	41	41

lines, there is no reason to believe water quality passing through Butcher Slough during major storm events has changed over that reported by Roberts (1985). Thus heavy concentrations of bacteriological indicators in Jolly Giant Creek water routinely pass through Butcher Slough and the Arcata STP outfall during wet weather periods.

Use of a new technology of sewage treatment and disposal based on wetlands proposed by Arcata required major consideration of risks involved to bivalve contamination (Gearheart 1981) (Appendix I). With an assumed disinfected discharge having a 20 MPN/100 ml concentration of total coliforms, Gearheart showed that the Arcata STP would only have been contributing 0.005 percent of the total coliform load to the bay where 99.9 percent of the total coliforms loading was coming from non-point sources (Table 11). A similar

Table 10. Bacterial indicator organisms in water sampled from Jolly Giant Creek from upper Butcher's Slough estuary, during storm and non-storm flows, October 10, 1980 - March 9, 1981 (from Roberts 1985).

Indicator organisms					
Total coliforms		Fecal coliforms		Fecal streptococci	
Interval	Number	Interval	Number	Interval	Number
<230	1	<250	15	<250	17
230 - 1,000	1	250 - 1,000	46	250 - 1,000	36
1,000 - 10,000	43	1,000 - 5,000	35	1,000 - 5,000	39
10,000 - 50,000	54	5,000 - 10,000	12	5,000 - 10,000	11
50,000 -100,000	5	10,000 - 20,000	7	10,000 - 25,000	5
100,000 -200,000	3	20,000 - 50,000	3	>25,000	2
>200,000	1	>50,000	1		
Total samples	108		109		100

analysis if now performed on the upgraded STP with wetland disposal through the AMWS would produce even a smaller percentage of Arcata Bay coliform load originating from the Arcata STP.

On leaving the human body, the rates of deactivation, destruction, attentuation, sequestering, or disappearance of human pathogens is heavily dependent on the time of exposure to environmental stresses and processes. Data collected by Musselman et al. (1978) provided an opportunity to estimate a gross disappearance rate for total coliforms in channels adjacent to the Arcata STP. This was done by Gearheart (1981) by aligning concentration values in the direction which tides would have moved the water column over sampling points (Table 12). Knowing the concentration of wet-period non-point total coliform concentrations in Jolly Giant Creek (12,000 MPN/100) plots were then made of the incoming concentrations and concentrations at the two uppermost stations by day of sampling (Figure 5). The linearity of these log plots allowed computation of K_{10} values (rate of disappearance) using an equation of the form $N_t = N_0 10^{-K_{10}t}$ (Allen and Gearheart 1982). The daily K_{10} values found, ranged from 0.60 to 0.75. These disappearance rates are needed for my complete risk analysis of human pathogens passing through the AMWS to Butcher Slough and becoming available for ingestion by commercially-cultured oysters.

Increasing attention is focusing on actual pathogens rather than indicator species in assessing public health risks. From December 1984 to August 1985, identification of the actual kinds of bacteria associated with isolates of bacteriological indicators used in effluent standards and monitoring (total coliform, fecal coliform) was made on weekly samples of influent and effluent water in ten cells of the pilot project marsh system fed with unchlorinated oxidation pond effluent (Gearheart et al. 1986) (Figure 1).

Table 11. Comparison of total coliform contributions to Humboldt Bay from non-point sources with levels from non-point sources with levels from sewage treatment plant discharges (point sources) under two different operating conditions (from Gearheart 1981, Table 3).

Drainage	Area	C Value ▲	Flow (ft^3 per day 10^6)	Concentration (MPN/100 ml 10^6) Condition 1*	Concentration (MPN/100 ml 10^6) Condition 2+	Contribution (MPN/day 10^6) Condition 1*	Contribution (MPN/day 10^6) Condition 2+	Percent Contribution to bay 1*	Percent Contribution to bay 2+
Eureka	43,100	.75	115	.010	.010	345	345	58	52
Arcata	29,960	..75	80	.010	.010	240	240	41	36
Eureka SSTP (Murray)	-	-	13	.00002	.02	.078	.78	.01	11
Eureka SSTP (Hill)	-	-	4.7	.00002	.0003	.028	.423	.004	.06
Eureka SSTP (Mc Cullen)	-	-	2.4	.00002	.0014	.014	1.00	.002	.15
Arcata SSTP	-	-	6.2	.00002	.00002	.037	.037	.006	.005
Rainfall on Bay	6,100	1.0	11	0	0	0	0	0	0
Totals			167			585	664	-100	-100

▲ Rainfall - Runoff coefficient assuming a saturated ground condition with 0.5 in/day of rainfall.

* Condition 1 - Sewage treatment plants producing 20 MPN/100 ml Total coliform effluent.

+ Condition 2 - Sewage treatment plants producing median total coliform level found during the 1978 FDA sanitary survey of shellfish waters - Arcata Bay.

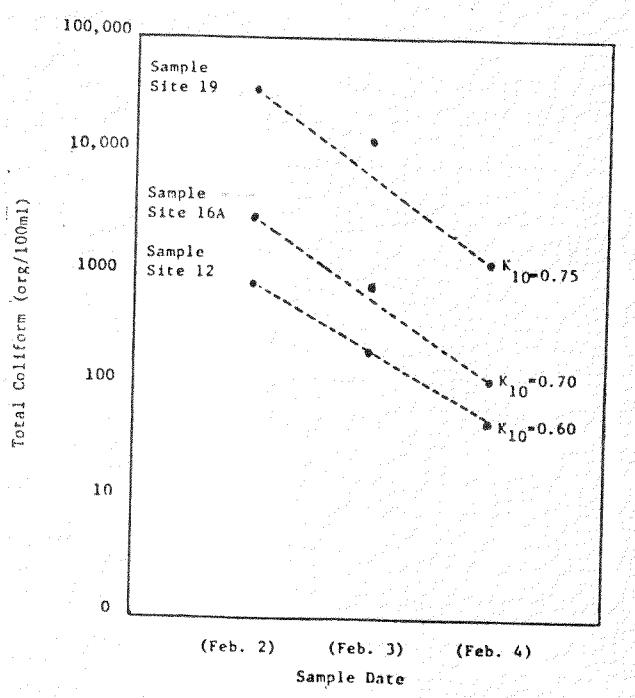

Figure 5. Total coliform disappearance rates calculated from concentrations found in February during wet-weather period in Arcata Channel - Janes Creek - Arcata Boat Basin drainage (Gearheart 1981, Figure 4; based on data collected by Musselman et al. 1978).

Table 12. Trends in bacteriological indicator organisms in Arcata Bay and FC/FS ratios from concentration values at stations aligned with predominant tidal movement (data from Musselman et al. 1978, as analyzed in Gearheart 1981.

Channel and Stations	Indicator Organisms (MPN/100 ml) Total Coliform	Fecal Coliform	Fecal Streptococcus	FC/FS Ratio
Arcata Channel - Janes Creek - Arcata Boat Basin				
Up Bay Station				
19	5,400	490	330	1.5
16A	490	49	49	1.0
12	750	49	17	2.9
10	330	46	23	2.0
34	340	25	21	1.2
Down Bay Station				
Arcata Channel - South Arcata Bay (Bracut Area)				
Up Bay Station				
24	330	130	9.3	14.0
22	330	23	14.0	1.6
21	795	87	22.0	4.0
31	1,950	250	125.0	2.0
27	385	20	13.0	1.5
Down Bay Station				
Mad River Slough				
Up Bay Station				
6	790	78	45	1.7
3	725	87	33	2.6
34	340	25	21	1.2
27	385	20	13	1.5
Down Bay Station				

Speciation of bacteria on total coliform isolates was performed using the API 20E technique. In the 31-week study, there were no isolates of pathogenic bacteria known to be strictly residents in humans within the 39 categories (genera, bacteria) investigated. The only identification of a strictly human pathogen was that of Shigella boydii in a special study on the regrowth of bacteria following disinfection. In a 60-liter sample of chlorinated/dechlorinated Arcata STP effluent, the pathogen represented 6 percent of the species identified from total coliform isolates obtained after a week of regrowth. The study suggested Shigella spp. may be more resistant

to chlorination than bacteriological indicators. Attentuation of this form in marsh cells was attributed to increased detention of water in the cells. "Marsh effluent could possess a minimum health risk when compared with the oxidation pond effluent chlorinated or unchlorinated in Arcata".

Bacterial speciation was also carried out on non-point sources of water by Gearheart et al. (1986) (Mad River, Jolly Giant Creek). No Shigella spp. or Salmonella spp. were isolated in these studies. More total isolates, a larger number of kinds of bacteria, and higher spikes in counts of coliforms were found in Jolly Giant Creek as compared to Mad River water. This was attributed to the greater soil surface/water volume ratio of the smaller Jolly Giant Creek as compared to Mad River.

A second study on bacterial speciation has been completed in 1985 on water in Jolly Giant Creek and bivalves from Butcher Slough (LeGouvello 1986). From 2 October-5 December 1985, 16 samples of pooled meats from 5-10 clams showed no Salmonella. Seven samples showed no potential pathogens but in nine samples at least one identification was made of either Pseudomonas putrefaciens, Proteus mirabilis, Klebsiellae, Citrobacter sp., Citrobacter freudii, or Enterobacter cloacae. Pathogens were not isolated from 13 sediment samples taken at sites where clams were dug, but were isolated in three sediment samples. Speciation was similar to that found in clam meats (Pseudomonas putrefaciens, Citrobacter freundii, Enterobacter cloacae, Vibrio parahaemolyticus). During the study, Jolly Giant creek water was sampled 12 times every two hours during two seasonal storm events. Salmonella was isolated in two samples from a storm event sampled October 20-21. These storm waters contained total coliforms ranging from 7 to 28×10^4 MPN/100 ml. A single water sample from a storm event studied on December 1-2 containing 0-$1{,}100 \times 10^4$ MPN/l total coliforms also showed Salmonella present. Although Jolly Giant creek water contained Salmonella, they were absent in estuarine bivalves. This strongly suggested that the chances of a Jolly Giant creek pathogen reaching commercial oysters beds was highly unlikely. Likewise these results support the contention it would be very unlikely that any pathogenic organisms found in oysters would have originated in STP effluents that had passed through disinfection on entering the AMWS and discharged to Butcher Slough through an aquaculture operation.

A risk analyses of contamination of oysters from viral particles passing through the treatment and disposal system has been completed using disappearance rates as published in the literature of viruses through wetland and oxidation ponds (Allen and Gearheart 1977, 1982; Allen et al. In press). The analysis indicated that $(1.1)(10^{11})$ viral particles in disinfected wastewater would be required in a flow of 1.5 MGD to the STP to have one viral particle leave the AMWS. A second way of summarizing results of the analysis was that the average expected viral load entering the Arcata STP in raw sewage would not be sufficient to overwhelm all of the units in the treatment and disposal system.

The most recent study of viral attentuation rates in a wetland environment under Humboldt Bay weather conditions took place in a marsh pilot project cell (61 m long, 6.1 m wide, 0.6 m deep) from April to September 1986 (Ives 1987). The cell was seeded with coliphage MS-2 and sampled monthly at eight points located along the cell which contained a typical emergent macrophyte community. Percent virus removal ranged from 79 percent in May to

96 percent in August and September, with a mean virus removal of 91.5 percent. Removal rates were not constant either across or along the length of the cell. Vegetation at the influent region of the marsh was more effective in removing viruses than at the effluent region, with a non-vegetated area located within the center of the cell consistently showing an increase in plague forming units. Date of viral disappearance in the pilot project marsh cell was overall about the same as found for coliform in channel waters (Figure 5). Viral removal, however, occurred mainly in the first day with initial contact with marsh plants. Emergent vegetation removed virus-laden suspended particles from the water column, transferring them to sediments where presumably they are inactivated. The periphyton on the plants undoubtedly is a key to such processes (Tojimbara 1986). These studies supported conclusions drawn from the literature on the efficacy of viral inactivation in marshes, and the utility of placing marshes as pretreatment to disinfection with chlorine to facilitate maximum deactivation of viral particles (Gearheart 1981). Wetland treatment units are under development in the Arcata oxidation ponds to provide pre-treatment of oxidation pond water prior to disinfection and discharge to the AMWS (Figure 2). A hard-stem bulrush bed has been hand-planted for similar pre-treatment of water entering the pumping station at the terminal end of the AMWS. The Arcata technology of long detention time with wetland treatment units is one of the emerging techniques to overcome incomplete kills associated with disinfection by chlorination, to deactivate viral particles, and prevent addition of carcinogens to receiving waters from chlorination by-products (Levenson p. 139).

Most of the risk analysis to human health from potential pathogens originating non-disinfected AMWS water to be used in aquaculture has addressed the reliability of the STP and AMWS units to prevent pathogens from appearing in Butcher Slough. The analysis is incomplete without considering the die-off (disappearance) that occurs between the discharge point and the oyster beds. Some preliminary evidence has been presented in this paper for performing such an analysis. There still remains, however, one other element for a complete risk analysis -- the probability that any ingested human pathogenic particles reaching a susceptible human would cause illness. The sequence of events that needs to be considered has been outlined by public health authorities (Appendix II) but I am in no position to make an estimation of the probability that an Arcata-STP derived particle would ever cause an infection.

In summary, the evidence presented in this paper that human pathogens originating in Arcata sewage, passing through the STP units, the chlorine disinfection process on entering the AMWS, passing through the three marshes, and entering Butcher Slough via undisinfected marsh water used for fishway and pond operation, would enter a bivalve, and reach a susceptible human being, is highly unlikely, and probably zero.

Facilities Design

Selection of a run size as a planning target in the 1977 feasibility report (Allen 1977) used a return of 10,000 adult salmon as suggested for a viable family-operated commercial enterprise during a world aquaculture conference held by the Food and Agriculture organization of the United Nations in Kyoto, Japan, 1976. Pond space for rearing the necessary smolts for such a

return was based on an assumed rate of adult return per smolt planted of 1.0. Pilot project results, however, have only produced a rate of less than 0.5 adults per smolt planted (Allen 1984, 1985). With currently available pond space, a run size of 2,000-5,000 still appears a reasonably attainable goal with our established coho return rates. No location on Jolly Giant Creek would be available to handle such a potential run size but the abandoned aeration pond immediately adjacent Butcher Slough has sufficient space for the necessary holding ponds.

Two pond designs are envisioned. First is a large circular unit similar to that existing at the fish hatchery at the University of Washington School of Fisheries. Water jets will be placed along the sides of the pond to provide a slow circular flow. The second pond will be long and deep, patterned after the "fall-back" system pioneered at northwest salmon culture stations for holding and sorting chinook salmon as described by Sheldon (1970). Aeration will be provided in headboxes prior to delivering water to the ponds, primarily to insure adequate oxygen during periods of increqasing water temperature and lowered oxygen in marsh waters known to occur in spring months during time of pond use for smolt migration.

The volume of freshwater from the AMWS required for operating the facilities will vary with function. The least volume is anticipated when the system is employed for smolt imprinting (probably not more than 100 gallons per minute necessary). A larger volume probably will be needed for attracting returning adults, but even this can be minimal (200-300 gpm). Plumbing, however, would be sized so that maximum volumes could be available for experimental work, and for future use in salinity control in adjacent rearing ponds. A denil fishway is envisioned, with the fishway entrance at the end of channel recessed into the west end of the holding-pond complex.

Costs will be minimized by completing the pond dikes in phases using waste material as they become available. The first diking needed will isolate the site for drying and removal of sludges. The second phase will be to complete pond dikes, and to stabilize pond banks with concrete rubble and slabs routinely disposed at the ponds. The system is designed to operate by gravity flow. Pumping will be provided through the normal operation of AMWS return-flow system. The final phase will be constructing connecting runways, sorting pen, spawning shed, and upwelling manifold. along with associated pipes and valves. These elements will represent the major out-of-pocket expenses for the system.

Since returning adults will not be fed, metabolic wastes would be no more than in a natural stream, while feed wastes would be non-existent. Smolts placed in the ponds will be in their down-stream migration life history stage and are expected to migrate quickly to the bay (Allen 1987). Therefore, metabolic, fecal, and feed wastes would also be minimal from smolts placed into the system for imprinting and volitional migration to the sea. Water quality discharge problems from fish culture operations associated with fishway and associated ponds will be virtually non-existent.

The forthcoming request to the California Regional Water Quality Control Board for use of disinfected AMWS water for fish culture operations most likely will be viewed as risk assessment in the context outlined by Comar (1979) (Appendix III). The data now available shows that risk to public

health from human pathogens originating from proposed fish culture operation would be negligible. On the other hand, chlorinated wastewater is now being considered toxic as an "Industrial Organochlorine" (Meyers and Hendricks 1982), and THM compounds can now be found listed on the national interim primary drinking water standards as a potential carcinogen (D'Itri F.M. and L.G. Lolfson 1987). By-products of wastewater chlorination in marine environments have been little studied but are becoming increasingly of concern to public health authorities (Levenson 1987, Robeck 1987). Thus reduction of chlorinated wastewaters entering receiving waters actually would be a protection of beneficial uses. The proposed increasing use of unchlorinated AMWs water to enhance fish culture through use in smolt imprinting and attractant water for returning adults should be an action readily supportable by the public and concerned regulatory agencies since it both enhances and protects beneficial uses of Humboldt Bay waters.

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I wish to specifically acknowledge the long and dedicated involvement in the Arcata programs of Robert A. Gearheart, Professor of Environmental Engineering, Humboldt State University. A background in ecology, limnology, sanitary engineering, and especially lagoon and wetland treatment technologies, have been invaluable in understanding the sewage treatment and water quality protection issues germane to the Arcata program. Mr.David Hull, Aquatic Resources Specialist, City of Arcata Department of Public Works, who coordinates the multiple objectives of the Arcata integrated sewage treatment and wastewater reuse schemes provided operational details and data. Mr. Jud Ellingwood, Humboldt Fish Action Council, gave technical support, supplied eggs when needed, and has been very helpful in recovering and reporting marked salmon straying to Freshwater Creek. The North Coast (California) Water Quality Control Board staff supported our early work in wastewater aquaculture with informal water quality standards for unchlorinated pond effluents released to the bay. Critical reviews of Arcata proposals by Board staff required unequivocal documentation in meeting technical and policy constraints. This process was instrumental in obtaining regulatory acceptance of the Arcata innovations. I wish to extend heartfelt recognition to the many volunteers (city employees, elected officials, citizens, and HSU faculty), and especially those assisting the Arcata Marsh and Wildlife Task Force. Undoubtedly their true reward lies in the satisfaction of the public values and services being provided by the Arcata programs. Especially noteworthy is the fact that I have had the privilege of working with two Directors of Public Works who were supportive of embryonic ideas. Special thanks to Delores Neher, California Cooperative Fishery Research Unit, Humboldt State University, for assistance in preparation of this paper.

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APPENDIX I. Fundamental wastewater treatment and reuse concepts underlying Arcata STP and effluent disposal efforts (reproduced from Gearheart 1981).

USE OF VASCULAR PLANTS FOR TREATMENT AND RECLAMATION OF OXIDATION POND EFFLUENT AND NON-POINT SOURCE POLLUTION LOADS

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Low Cost Wastewater Treatment Systems

In recent years, there has been an increasing need for the development of improved methods for wastewater treatment, specifically for those communities which could be categorized as small to medium in size. While the "progress" of our industrial society continues at a rapid rate, technological advances in treatment methods for the new variety of toxic chemicals, exotic organics, and general domestic sewage seems stymied. Initial construction cost and continuing operational costs of wastewater treatment plants are the most significant factors affecting technology selection process. The cost to small communities for reaching the same level of wastewater treatment as large communities using standard technology is disproportionately high (Tchobanoglous, 1974). Although large sums of money are presently being made available by the Federal and State governments for pollution control systems, relatively few funds are being applied to advance research and development of improved treatment technolgoy (Ruckelshaus, 1976). Since present wastewater treatment systems are primarily designed after "natural" mechanisms for pollution abatement (trickling filters, activated sludge, oxidation ponds, etc.), it is ironic that practical, cost effective, and efficient treatment of wastewater utilizing controlled nutrient uptake by macrophytes in a marsh, is not in wider use and encouraged by regulatory and funding agencies.

Westman (1977) recently reviewed the progress of the United States government in improving the quality of the nation's surface and ground waters under the Clean Water Act of 1972 (PL-92-500). Excessive capital cost in treatment systems designed to meet nationwide effluent standards has been a major factor in preventing the program from achieving 1977 goals. Rising energy costs now are contributing to heavy operating expenses required by these treatment systems. Westman also noted a bias in federal and state agencies against innovative wastewater treatment schemes capable of reducing these costs. Moreover there is a reluctance to implement Section 201 of the law mandating priority to systems that can generate revenue to offset treatment costs by reuse of treated wastewaters in silvicuture, agriculture, aquaculture, and habitat restoration (McGaughey, 1967; Amramy, 1968; Stone, et al., 1952).

The resource values of waste must be utilized in a safe and beneficial manner to assist in the support of various life systems. There exists opportunities to use wastewater to assist various ecological systems, forestry projects, wildelife habitat, botanical gardens wetland process, etc. The use of biological systems should be encouraged if they substantially reduce the capital cost, energy cost, chemical cost, and/or labor cost of the treatment requirement (International Conference of the Renovation and Recycling of Wastewater, 1975).

The Clean Water Act of 1977 contains a major redirection of the sewage treatment construction grant program. The new emphasis supports innovative and alternative treatment technologies such as land treatment, wetlands, marsh systems, and aquaculture. The intent of the lawmakers is to force and encourage alternative and natural processes which are ecologically sound, i.e. the maximum utility of nutrients and water are obtained prior to ultimate disposal. An ecologically sound alternative should also be energy efficient in the construction, operation, and maintenance costs. In the proposed study, freshwater marsh treatment systems are considered alternative and innovative technology. These systems are energy efficient and provide for reclamation and recycling of wastes and elimination of pollutants.

APPENDIX II. Circumstances that must occur for persons who ingest wastewater-contaminated food to become ill (from Bryan 1974).

The following circumstances must occur for persons who ingest wastewater-contaminated foods to become ill:

(1) The infectious agent must be present in citizens of a community or in animals on farms, or toxic agents must be used for industrial or agricultural purposes; and wastes from these sources must reach sewerage or drainage systems.

(2) The agents must survive and pass through all wastewater treatment processes to which they are exposed.

(3) The waste-treatment effluent or watercourse receiving the effluent must be used as irrigation water for crops or as a growing environment for fish or watercrops or for washing or freshening harvested foods. Thus, the agents must survive in the receiving watercourse.

(4) The agents must survive in the soil in which irrigated foods are grown.

(5) The agents must contaminate a food product.

(6) Then one of the following events must occur:

  (a) The agents must be present on the contaminated food in sufficient numbers to survive storage and preparation and still cause illness.

  (b) Bacteria on foods in insufficient numbers to cause illness must multiply and reach levels that are necessary to cause illness.

  (c) Bacteria, and perhaps other organisms, enter food preparation areas on raw foods, where they may be transferred to workers' hands or to equipment surfaces which if inadequately washed will then contaminate other foods that they subsequently touch.

(7) Sufficient quantities of the contaminated food that contain enough of the agent to exceed a person's susceptibility threshold must be ingested. Ingestion of foods contaminated to this level may result in sporadic cases of illness as well as epidemics. When insufficient numbers of pathogens to cause illness are ingested, the infected individuals may become carriers and subsequently contaminate other foods that they touch.

APPENDIX III. Editorial on risk analysis and regulatory decision making germane to proposed Arcata use of non-disinfected wastewaters from the Arcata Marsh and Wildlife sanctuary in aquaculture (Colmar 1979).

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Risk: A Pragmatic De Minimis Approach

Society is becoming increasingly well informed and anxiety-prone about technology-associated risks, which leads to desire for their elimination. The logical and traditional approach is first to estimate the risk, a scientific task. Then comes the issue of risk acceptance, a most difficult step—moving from the world of facts to the world of values. Ideally, judgments involving risk acceptance should be made on society's behalf by a constitutionally appropriate body. But no such public decision-making process exists. We make do with disparate efforts of individuals, special-interest groups, self-appointed public interest groups, and legislative, judicial, and regulatory systems. However, if at least very large and very small risks were dealt with on the factual basis of effects, the individual and social value systems could be accommodated to some degree and much confusion avoided.

It is human nature to be concerned primarily with effects on our own person and family and secondarily with effects on the population at large. Unfortunately, although we can predict statistical effects on populations, there is no way to predict effects on individuals. This is why fortune-tellers never become as rich as insurance companies. We need then to define actuarially the existing state of well-being and calculate effects on it.

Each person has a probability of dying in any particular year, the value depending mainly on age. The existing probabilities are well known for the United States. For example, in 1975, 1.89 million died out of a population of 213 million, giving an overall probability of 1 in 113. For some specific age groups the values were: 1 to 4 years, 1 in 1425; 5 to 14 years, 1 in 2849; 25 to 34 years, 1 in 692; 55 to 64 years, 1 in 67. We can now answer the question, What does changing a risk do to a person's existing probability of dying? For instance, if a young child were exposed to an additional risk of 1 in 100,000 (0.014 in 1425) in 1975, his overall risk for that year would be 1 in 1425 plus 0.014 in 1425, or 1.014 in 1425.

For the purpose of discussion some guidelines, which may depend somewhat on age, can now be stated in terms of numerical risk:

1) Eliminate any risk that carries no benefit or is easily avoided.

2) Eliminate any large risk (about 1 in 10,000 per year or greater) that does not carry clearly overriding benefits.

3) Ignore for the time being any small risk (about 1 in 100,000 per year or less) that does not fall into category 1.

4) Actively study risks falling between these limits, with the view that the risk of taking any proposed action should be weighed against the risk of not taking that action.

Clearly, these suggested guidelines are a gross oversimplification. The unfortunate, overtaken by a one-in-a-million catastrophe, have a 100 percent chance of harm. The hard fact is that attempts to eliminate risks for the unfortunate few tend to markedly increase them for the rest of a large population. This idea is most difficult to defend politically, especially when the unfortunate few are known and the unfortunate many are nameless. In addition, it is necessary to take into account such matters as validity and uncertainty in risk estimates, nonlethal and esthetic effects, voluntary versus involuntary risks, societal abhorrences, and the strange versus the familiar.

Nevertheless, other than depriving the news media of a ready source of attention-grabbing items, the pragmatic de minimis approach should serve to promote understanding about how to deal with risk in the real world: encourage identifiers of risk to provide risk estimates: focus attention on actions that can effectively improve health and welfare and at the same time avoid squandering resources in attempts to reduce small risks while leaving larger ones unattended: and prevent anxiety, apathy, or derision as a response to the increasing recognition that we apparently live in a sea of carcinogens (the "today" risk).—CYRIL L. COMAR, *Professor Emeritus, Cornell University, and Director, Environmental Assessment Department, Electric Power Research Institute, Palo Alto, California 94303*

Integrating the Information Needs of Fish Hatcheries and Fisheries Managers - an Application of Microcomputer Technology

Stephen M. Pastor
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The basic premise of this work is that fish hatcheries and fisheries managers have commom data needs and are working toward the same goals. By working together the amount of effort expended for data collection can be kept to a minimum, and the quality of the data collected can be enhanced.

The Vancouver Fisheries Assistance Office serves the federal fish hatcheries located in the lower Columbia River Basin. Fortunately, four of the seven hatcheries are equiped with on site computing equipment. The concepts described in this paper are currently being implemented at these hatcheries.

The history of computer use at fish hatcheries parallels that of other professions, although a strong argument for being somewhat behind can easily be made. Initial use of computers focused on individual applications and used files unique to those applications. The concept of using a common database to supply the needs of several or many applications evolved some time ago in the field of data processing, and is now becoming increasingly common in applications related to fish hatcheries.

Federal hatcheries document fish distribution with several reports: Lot History Production, Hatchery Production Summary, Monthly Distribution Summary and the Quarterly Fish & Egg Distribution Report. The introduction of coded wire tagging has necessitated further reporting of tagged fish with a Marking Form, one of which is filled out for each group of tagged fish released. This is quite a list of reports, all of which document the same basic information in various formats.

Upon close examination it becomes obvious that one database file can be constructed to fulfill the reporting requirements for all of these reports. One entry per coded wire tag would fullfill the reporting requirement for tagged fish. Information for fish releases unrelated to coded wire tagging should also be placed in this file, as should any fish transfered rather than released. The file now contains all information on fish distribution.

This one file can now be used by several different programs which extract or total data to conform to the formats of various reports. Total fish shipped can be determined for Lot Histories by electronically adding all of the entires in the database for a given month and lot. Rather than printing and sending reports, copies of the file itself can be supplied to offices interested in the information. An example would be Fisheries Assistance offices interested in tagged releases. In this application information on tagged releases can be extracted electronically. Information on untagged releases will simply be ignored.

Another opportunity to integrate the information needs of hatcheries and fisheries assistance offices is in accounting for fish

returning to the hatchery. The primary source of information is again at the hatchery, which creates and maintains a database file containing information on date, sex, and disposition of fish removed from the run. This one file can contain nearly all of the information the hatchery needs to document both run size and spawning activity (figure 1). With the proper applications software this file can provide a logical, convenient view of the data for the hatchery staff (figure 2). When passed on to a fisheries assistance office, the file can also be used in conjunction with age and length samples to determine the age composition of the entire run.

It is important to note that in both examples the hatchery staff creates, maintains and uses the database files as an important internal source of information which is used to meet multiple reporting requirements. Since the hatchery actually uses the data in the database, they have a vested interest in maintaining its accuracy. A key concept is that the hatchery operates as a "quality control circle" insuring the quality of a "product" which consists of information in the database files. Another important benifit is that data is only entered once, reducing both input effort and the opportunity to make mistakes, and is then used for several reports . Each report produced is an opportunity to spot inaccuracies in the data, and any corrections made to the database will be used in all subsequent reports.

Record#	DATE	NUM_TAKEN	SEX	SPECIES	DISPO
1	05/03/87	1	F	SCS	9
2	05/04/87	1	M	SCS	5
3	05/04/87	1	M	SCS	9
4	05/10/87	1	F	SCS	9
5	05/13/87	1	M	SCS	5
6	05/26/87	3	F	SCS	5
7	05/26/87	1	J	SCS	5
8	05/27/87	105	M	SCS	2
9	05/27/87	282	F	SCS	2
10	05/27/87	1	J	SCS	2
11	05/28/87	355	F	SCS	2
12	05/28/87	145	M	SCS	2
13	05/31/87	1	F	SCS	5
14	06/01/87	1	F	SCS	5
15	06/02/87	163	M	SCS	2
16	06/02/87	378	F	SCS	2
17	06/04/87	122	M	SCS	2
18	06/04/87	346	F	SCS	2
19	06/04/87	2	F	SCS	5
20	06/11/87	4	F	SCS	5
21	06/11/87	1	M	SCS	5
22	06/13/87	1	F	SCS	5
23	06/14/87	2	F	SCS	5
24	06/14/87	1	M	SCS	5
25	06/17/87	5	F	SCS	5
26	06/17/87	1	M	SCS	5
27	06/21/87	5	F	SCS	5
28	06/21/87	5	M	SCS	5
29	06/22/87	3	F	SCS	5
30	06/23/87	4	F	SCS	5
31	06/24/87	1	F	SCS	5
32	06/25/87	2	F	SCS	5
33	06/26/87	2	F	SCS	5
34	06/27/87	6	F	SCS	5
35	06/27/87	4	M	SCS	5
36	06/28/87	5	F	SCS	5
37	06/29/87	4	F	SCS	5
38	06/29/87	1	M	SCS	5
39	06/30/87	4	F	SCS	5

Figure 1

Figure 2

11/30/87 Spawning and Run Summary for SCS from LWFR87

Date	Males Spawned	Females Spawned	Green Females	Bad Females	Estimated Eggs Taken	Actual Eggs Taken
07/14/87	24	97	1	0	368,600	416,615
07/21/87	27	131	4	0	497,800	461,644
07/28/87	32	157	1	4	596,600	654,376
08/04/87	10	90	0	0	342,000	338,186
08/13/87	112	248	0	10	942,400	960,075
08/14/87	52	96	1	3	364,800	340,896
08/20/87	109	213	6	8	809,400	842,311
08/28/87	118	228	0	0	866,400	896,528
Totals	484	1,260	13	25	4,788,000	4,910,631
	1.0	: 2.6				

	Jump Outs	DIPS	Killed as Surplus
Males	1	170	658
Females	2	481	1,461
Jacks	0	5	6
Unknown	0	0	0
Percent of Total Removed	3	656	2,125
	0.0	14.3	46.5

		Percent of Run
Total Males	1,313	28.7
Total Females	3,242	71.0
Total Jacks	11	0.2
Total of Unknown Sex	0	0.0
Total Number Removed to Date	4,566	

Date of last entry 11/01/87

Inducement of Unique Otolith Banding Patterns as a means to Mass-Mark Juvenile Pacific Salmon.

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Abstract

We investigated the potential of deliberately inducing specific otolith banding patterns as a means to identify hatchery populations of pacific salmon. Initial experiments with embryos and alevins of chum salmon clearly showed that simple manipulations of incubation water temperature produced distinctive and unique patterns in the otolith microstructure of these fish. Similarly, intervals of starvation and feeding imposed upon fingerling-sized fall chinook also produced recognizeable banding patterns in their otoliths. These environmental manipulations appear to affect all individuals in a treatment group, impose no obvious stress upon the juvenile fish, and produce easily distinguishable patterns between the different experimental groups. Experiments with altered photoperiod schedules were less effective at producing obvious patterns in otolith microstructure.

We have begun implementing this marking technique on a hatchery production scale using thermal manipulations of incubation water temperature to induce identifiable otolith banding patterns on up to 800,000 juvenile pacific salmon. This technique represents a minimal intrusion upon normal hatchery operations and based upon the success of these trials, the mass-marking of several million juveniles appears to be quite feasible. This method is viewed as an efficient means whereby large numbers of juvenile salmonids can be mass-marked simultaneously, consequently reducing the cost of fish marking while increasing the effectiveness and scope of the marking program.

Radio Tracking Technology in Fisheries Research

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Radio telemetry as a research tool has it's greatest application in the area of in situ behavior studies. Use of the tool depends upon physical and data application requirements. UThe primary concerns are physical, 1) the size of the fish studied, 2) the radio tag battery life and 3) the transmitter range of the tag. The next consideration should be the expected behavior of the test species. From a fisheries management standpoint, the most easily obtained radio telemetry data is limited to qualitative aspects of fish behavior and there is a reluctance to apply information of this nature. Technical developments that enable us to release larger groups of radio tagged fish are aiding in the development of models and statistical procedures that will help promote the use of radio tags for quantitative in situ behavior studies.

STATUS OF THE PIT TAG SYSTEM

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INTRODUCTION

Since 1983, the National Marine Fisheries Service (NMFS) has been involved in a cooperative research program with the Bonneville Power Administration (BPA) to evaluate a new uniquely coded miniature identification system that can be used in tagging fish. The system is referred to as a passive integrated transponder (PIT) tag. The PIT tag has undergone extensive biological testing and technical (systems) development between 1983 and the present. This paper presents an overview of the current status of the PIT-tagging system--detailed information and test results can be found in Prentice et al. (1984, 1985, 1986, and 1987) and Prentice (1987a and 1987b).

The primary use of the PIT tag to date has been in pilot studies by NMFS and other researchers to address fish migration issues within the Columbia River System. However, the PIT tag has a strong potential wherever the repetitive recognition of individuals is required. The PIT tag is a passive system which should stay with the fish throughout its life-cycle and can be detected and decoded in living fish, potentially eliminating the need to anesthetize, handle, restrain, or sacrifice fish during data retrieval.

PIT TAG OPERATION AND INTERROGATION

The PIT tag consists of an integrated micro-chip that is bonded to an antenna coil. The electronic components of the tag are encapsulated in a glass tube about 12mm long and 2mm in diameter. Each tag is preprogrammed at the factory with one of a potential 34 billion unique (10 digit alpha-numeric) code combinations. The tag is passive, having no power of its own and, therefore, must rely upon an external source of energy to operate. A 400 kHz detector signal energizes the tag, and a unique 40 to 50 kHz signal is transmitted (from the tag) to the interrogation equipment where the tag code is immediately processed, displayed, and/or stored to a computer.

Several types of PIT tag interrogation systems are available. Portable battery powered (hand-held) scanners are used in a variety of tagging and detection applications, and larger portable AC units are being developed for release monitoring from raceways and fish pumps. A system of fixed loop

PIT tag monitors have been installed at the juvenile fish bypass facilities at McNary, Little Goose, and Lower Granite dams [these dams are among a series in the Columbia River System that have been modified to collect and/or divert juvenile outmigrants as a method of increasing overall survival in the system]. In addition, a monitoring system has been installed at the adult collection facility at Lower Granite Dam. The monitoring systems at these dams are capable of detecting 95%, or more, of all PIT-tagged fish, with a tag code reading accuracy of 99%.

The interrogation range of the PIT tag varies with the monitoring equipment used. With the hand-held scanner the tag detection range is up to 7.6cm whereas with a fixed loop interrogator the range is about 19cm. The tag can be read through soft and hard tissue, liquid (seawater and fresh water), glass, and plastic, but not through metal. Extremes in temperature (-273° to 6Ø° C) does not affect the detection or reading of the tag. In addition, the tag is not affected by instantaneous pressure changes of Ø to 5 atmospheres. Successful tag monitoring can take place at velocities up to 3.6m per second. The tag's operational life is unknown at this time, however, it is thought to be at least 1Ø years.

To our knowledge, the interrogation system is completely safe to both the operator and tagged animal. Portable scanners and release monitors require no special permits. However, large fixed loop detection systems, such as those installed at hydroelectric dams, require permits from the Federal Communications Commission for the operation of low-powered transmitting devices. No special training or licensing of the operator is required to operate the tag monitoring equipment.

PIT TAGGING SYSTEMS

PIT tags are injected into fish using a 12 gauge hypodermic needle. A modified hypodermic syringe was developed for portable applications. This hand-held unit requires each tag to be manually inserted into the needle and is satisfactory for small numbers of fish. An automated (bench mounted) tagging machine has also been developed and has advantages in rapidly tagging large numbers of fish. With this system, tags are housed in a removable clip that gravity feeds to an air-ram activated plunger which pushes the tag through the needle. The tagging rate using the automated system is more than double that of the hand held injector (up to 4ØØ fish/h).

The body cavity was selected as the best area to implant the PIT tag. At tagging, the needle is positioned just posterior of the pectoral fins and slightly off-set from the mid-ventral line. The needle is then inserted to place the tag posterior to the pyloric caeca in the proximity of the pelvic girdle. Properly implanted PIT tags have up to a 99% retention rate and little affect on the fish's survival.

After tagging, tag presence and code identity are obtained

using a detector/decoding system. The system can be a portable (battery powered) scanner unit or a computer-interfaced detection system. Computer interfaced detection stations are normally used and allow automated entry of tag code, length, weight, and other comments. This computer-based system was developed by NMFS and makes it possible to electronically maintain records on large numbers of individual fish. Of particular interest is the integration of a sonic digitizer to document length and a computer compatible electronic balance to automatically record weight. This automated weighing and measuring station can be run independent of PIT tag recording and should have broad application in fisheries for the automated processing of fish size information.

BIOLOGICAL EVALUATION OF THE PIT TAG

Laboratory and field tests were conducted using juvenile and adult chinook salmon (Oncorhynchus tshawytscha) and steelhead (Salmo gairdneri), juvenile sockeye salmon (O. nerka), juvenile coho salmon (O. kisutch), and adult Atlantic salmon (S. salar). Fish ranging in size from 2 to 10,000g were tagged, and all tests indicated that the PIT tag does not adversely affect growth or survival in otherwise healthy fish. Serial tagging studies showed that fish as small as 1.3g can be PIT-tagged and high (95-99%) survival and tag retention normally achieved. For instance, groups of chinook salmon PIT-tagged (at 3g) in fresh water, transferred to seawater net-pens, and monitored for over 1 year in captivity had 100% tag retention with all tags reading properly. In addition, swim-chamber stress tests showed no significant effect of the PIT tag on swimming ability or survival of juvenile salmonids.

No appreciable host tissue response or evidence of infection due to tagging procedures has been observed in salmonids. Tagging wound condition, tag placement within the body cavity, and histological effects of the tag were documented by examining groups of PIT-tagged juvenile fall chinook and sockeye salmon over time. The tagging wound appeared to close almost immediately and as early as 2 weeks after tagging there was little evidence of external trauma at the injection site. Histological sampling indicated that within 3 weeks after tagging, the injection site usually consisted of normal scar tissue replacing the dermis and underlying muscle tissue damaged during injection of the tag. By 4-6 weeks after tagging the injection site was normally difficult to locate histologically, and complete healing usually occurred by this time. Tag placement studies showed the location of the PIT tag to be relatively stable over time, with the majority of the tags occurring near the posterior end of the pyloric caeca.

Mature PIT-tagged Atlantic salmon, which were hand-stripped of sperm and eggs, showed tag retention (after spawning) of 100% for males and 83% for females. Four tags were passed with the

eggs during the first stripping of the females and four tags during the second to fourth stripping. When a PIT tag was passed at spawning, it was easily recognized or electronically detected among the eggs and, therefore, could be re-inserted into the fish. Evidence suggests the PIT tag is a viable procedure for inventory control of brood stocks.

COMPARISON OF PIT TAG TO TRADITIONAL TAGGING AND MARKING METHODS

A series of tests comparing the PIT tag to traditional methods of marking and tagging salmonids were conducted under field conditions using active outmigrating juvenile spring and fall chinook salmon and steelhead. Freeze branded and PIT-tagged fish were released into the Snake River and McNary Dam reservoir and subsequently monitored at juvenile collection facilities at Lower Granite and/or McNary dams. PIT-tagged fish were detected in situ, and the tag information and time, date, and location of the fish were automatically stored on a computer and printer. Recovery of information from branded fish required large random subsamples of migrating (marked and unmarked) juveniles to be individually handled and inspected.

It was concluded that neither migration or survival of PIT-tagged fish was compromised. Evaluation of the data showed the PIT tag provided more uniform (between replicate) information than traditional freeze brand marking methods. In addition, the application of data expansion factors to brand subsample information are required to estimate relationships in the total population. The retrieval of PIT tag information, however, is based on (passively) monitoring 100% of the fish passing the collection facility and no expansion factors are required. Our tests suggest that, for migration studies, the PIT tag can provide more reliable data than traditional methods while allowing a 90 to 95% reduction in required sample size.

FUTURE APPLICATIONS

In 1987, NMFS and other agencies began utilizing the PIT tag to estimate in-river survival and travel timing for salmonids within the Columbia River System. These pilot studies were successful, and use of the PIT tag is projected to expand. Increased use of the PIT tag will have the added benefit of reducing by over 90% the numbers of fish that are currently freeze branded at hatcheries for many in-river studies. In addition, since PIT tag monitoring is passive, considerable handling and stressing of migrating fish (necessary for brand monitoring) can be avoided.

Based on biological and technical information gathered to date, the use of the PIT tag will not be limited to salmonids. Prawns and crabs have also been successfully tagged, and the PIT tag has application to any animal that can accept and retain the tag. Examples of advantages and applications of the PIT tag

include: individual identification of brood stock; growth and genetic studies; reduction in and combination of replicated treatments; and many other studies where repetitive identification of individuals is important.

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NATURAL AND ARTIFICIAL GENETIC MARKS IN FISHERIES MANAGEMENT

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Most fishes exhibit considerable genetic variability, both among individuals and among populations. One can consider fish stocks (populations) to be self-sustaining breeding units within a species which are at least partially reproductively isolated from one another. Given that such stocks are genetic units, one of the most direct tests of stock structure is to examine their genetic profiles. At present, the approach of choice for this purpose is the analysis of protein variation by starch-gel electrophoresis.

Electrophoresis is a process whereby charged molecules (such as enzymes and other proteins) are separated in an electric field. Using electrophoresis, it is possible to document the genetic characteristics of individuals (and populations) because of the relationship between the genetic code (DNA) and the biochemical phenotype, as expressed after electrophoresis and enzyme staining, in the form of the banding patterns on gels. Each enzyme (protein) subunit is encoded by a specific segment of DNA -- a gene locus -- which specifies its structure. Thus, electrophoretic characteristics have a direct genetic basis and are inherited from generation to generation. Electrophoresis can be applied to the culture, management, and conservation of fish stocks in several different ways, including: a) stock identification using naturally occurring genetic marks, b) stock identification using artificial genetic marks, and c) monitoring of hatchery broodstock practices.

Genetic stock identification (GSI) relies on the combined use of these biochemical and genetic procedures to distinguish among stocks based on the amount and pattern of genetic variation they possess. For the past several years, GSI has been successfully used by several agencies to estimate proportional stock contributions to numerous salmonid mixed-stock fisheries from California to Alaska. Each such analysis consists of three different parts: 1) the collection of baseline data (genetic profiles for each potentially contributing stock), 2) the collection of fishery data (genetic profiles for the fish in the fishery sample), and 3) the statistical analysis of both data sets using maximum likelihood estimation to generate estimated stock contributions (Milner et al., 1985. Mar. Fish. Rev. 47(1):1-8).

Through selective breeding, it is possible to alter the amount and/or pattern of naturally occurring genetic variation intentionally and thereby create a distinctive genetic mark for a

previously indistinguishable stock. The Washington Department of Fisheries has successfully used this approach with the Kennedy Creek stock of chum salmon to estimate the contribution of artificial enhancement activities to total production by this stock and to estimate total juvenile production by all chum stocks in the Totten Inlet area of south Puget Sound (Seeb et al., 1986. Trans. Amer. Fish. Soc. 115:448-454).

Just as intentional genetic marking of a stock is possible via selective breeding, unintentional genetic degradation of a stock can result from inappropriate or careless hatchery operations. Such changes can take many forms but one of the most common is the detrimental loss of overall genetic variation (reduced heterozygosity and/or polymorphism) due to the use of a small number of parents and inbreeding. In most cases this is a direct result of spawning practices which do not maximize the number of parents (males and/or females) used to produce each subsequent generation. A second problem, that of directional change in genetic characteristics, can result from hatchery practices which result in one or more portions of the run being underrepresented in the hatchery spawning population. Electrophoretic characterization of each hatchery stock coupled with regular screening of the genetic profile of the stock can be used to monitor the genetic characteristics of each hatchery stock and thereby identify potential problems before they have an adverse effect on the future of the stock.

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Brock	Irving R.	ADFG	PO Box 5-337	Ft. Richardson	AK	99505
Brown	Peter	BC Fisheries	Kootenay Trout Hatchery	Wardner	BC	V0B 2J0
Brown	Douglas	Muckleshoot Tribe	34900 212th AVe. SE	Auburn	WA	98002
Bruce	Ian	General Sea Harvest Corp.	1575-200 Granville	Vancouver	BC	V6C 1S4
Bruhn	Dave	USFWS	Rt 1 Box 256	Hagerman	ID	83332
Brunson	Wayne D.	WDW	4912 192nd St SW	Lynnwood	WA	98036
Brunson	Ray	USFWS	2625 Parkmont Lane Bldg A	Olympia	WA	98502
Bugert	Bob	WDF	Box 474	Waitsburg	WA	99361
Burr	Bob	Cypress Salmon	3013 Mountain View N	Renton	WA	98056
Busch	Bob	Clear Springs Trout Company	PO Box 712	Buhl	ID	83316
Capri	Jack	Newport Pacific Corp	PO Box 1028	Newport	OR	97365
Carlile	Kermit H.	Walton's Mtn Farm	Rt 3 Box 278-M	Sherwood	OR	97140
Carlson	Chris	Grant Co. PUD	PO Box 878	Ephrata	WA	98823
Carlson	Leonard	Carlson Salmon Farm	10630 176th Ave SW	Rochester	WA	98579
Chaney	Bruce W.	Montana FW & P	Box 2163	Great Falls	MT	59403
Chapman	Patrick	WDF	2415 Bush Ave NW	Olympia	WA	98502
Chapman	Joe	IDFG	Rapid River Hatchery	Riggins	ID	83549
Charley	Larry	Tulalip Tribe	10610 Waterworks Rd	Marysville	WA	98270
Chase	Don	WDW	600 N Capitol Way	Olympia	WA	98504
Christianson	Chris	ODFW	4325 NW Terra Lynda	Albany	OR	97321
Cisneros	Gerald Alan	WDF	1319 101st East	Tacoma	WA	98445
Clay	Herbaldon		1822 South Hosmer	Tacoma	WA	98405
Clemens	Kathy	USFWS	Rt 1 Box 2105	Anderson	CA	96007
COMMON SENSING		D'Aoust, Brian	7595 Finch Road N.E.	Bainbridge Is.	WA	98110
Cormier	Charlotte	WDF	17329 SE 265th ST	Kent	WA	98042
Costello	Ron	Moore Clark	P.O. Box M	LaConner	WA	98257
Couture	Richard	Envirocon Pacific Ltd	PO Box 819	Lumby	BC	V0E 2G0
Crateau	Ed	FWS	PO Box 1731	Weaverville	CA	96093
Crawley	Doug	BC Fisheries Branch	34345 Vye Road	Abbotsford	BC	V2S 4N2
Curtis	Lyle E.	ODFW	Rt. 2 Box 41	Otis	OR	97368
Dahrens	Dennis	ODFW	17330 S.E. Evelyn St.	Clackamas	OR	97015
Dallas	Bob	WDF	5925 Glenmore Drive	Olympia	WA	98501
DeCew	Mark	WDF	PO Box 149	Salkum	WA	98582
Delarm	Mike	NMFS	13635 SE Ellis St	Portland	OR	97236
Demaris	A.J.	ODFW	214 Cole River Drive	Trail	OR	97541
denBreejen	Harry	Shuswap Fish Culture Ltd	RR 1 Deep Creek Rd	Enderby	BC	-0-
denBreejen	Jack	Shuswap Fish Culture Ltd	RR 1 Deep Creek Rd	Enderby	BC	-0-
Dills	Jim	Sea Farm Washington	17846 Danby Dr SW	Rochester	WA	98579
Dimesky	Robert S.	NITROX	159 John Downey Drive	New Britian	CT	06051
Dimmitt	Kent	WDF	3800 14th SE B-80	Lacey	WA	98503
Dingwall	Loren	WDW	45300 Reiter Rd	Sultan	WA	98294
Dixon	Glen	DFO	Box 61	Dewdney	BC	V0M 1H0
Doerr	Bill	IDFG	State Fish Hatchery	Mackay	ID	83251
Dompier	Douglas W.	Col. Inter Tribal Fish Comm	975 SE Sandy	Portland	OR	97214
Dordic	Alex	Union Carbide Canada Ltd.	Suite 280 10991 Shelbridge Way	Richmond	BC	V6X 3C6
Dorn	Paul	Suquamish Tribe	P.O. Box 498	Suquamish	WA	98392
Ducey	Ron	CDF&G	124 Elderbrook Lane	Sacramento	CA	95670
Dunne	Delbert R.	Mt Tamgas Creek Hatchery		Metlakatla	WA	99926
Duplaga	W.K.	WDF	189 Reno Cutoff Road	Washougal	WA	98674
J.L. EAGAR INC.		Eagar, Roy	PO Box 476	N Salt Lake	UT	84054
Edenstrom	Charles A.	WDF	4418 Montclair	Lacey	WA	98503
Eggers	Ron	NW Planning Council	850 SW Broadway Suite 1100	Portland	OR	97205
Eicher	George	EICHER Associates	8787 SW Becker Dr.	Portland	OR	97223

Elam Jr	Tom E.	Maritime Heritage Center	2013 Marine Place	Sedro Woolley	WA	98284
Ellis	C.H. (Bud)	WDF-Retired	2621 37th Ave W	Seattle	WA	98199
Eltrich	Rich	WDF	Room 115 GA Bldg	Olympia	WA	98504
ENERTECH		Mattison, John	Box 230089	Anchorage	AK	99507
ENGINEERED PRODUCTS		Gregg, Mike	5065 SW Nash	Corvallis	Or	97333
ENVIROCON PACIFIC			#205 2250 Boundary Road	Burnaby	BC	V5M 3Z3
Estey	Don F.	CDFG	P.O. Box 158	Clements	CA	95227
Evans	Steve	WDF	10119 Steilacoom Rd SE	Olympia	WA	98503
Everson	Michael D.	ODFW	236 Cole Rivers Drive	Trail	OR	97541
Farr	Winston	Summit Technology	1075 Dexter Horton Building	Seattle	WA	98104
Ferg	Russell G.	USFWS	PO Box 58	Quilcene	WA	98376
Ferster	Donald		1580 Rowan Street	Victoria	BC	V8P 1X3
Finkbonner	Bill	Lummi Tribe	6498 Saxon Rd	Acme	WA	98220
Fisher	Jerry	ODFW	29014 Fish Hatchery Rd.	Alsea	OR	97324
Flagg	Thomas	NMFS	PO Box 38	Manchester	WA	98353
FLEX-A-LITE, CONSOLIDATED			4540 S. Adams St. P.O. Box 9037	Tacoma	WA	98409
Font	Rudy	WDW	32915 SE 309th St	Palmer	WA	98051
Foster	Bob	WDF	Rm 115 GA Bldg	Olympia	WA	98504
Fowler	L.G.	USFWS	1440 Abernathy Road	Longview	WA	98632
Fuss	Howard	WDF	1428 Mercantile NE	Olympia	WA	98506
Gale	Grant	BC Recreational Fisheries	34345 Vye Rd.	Abbotsford	BC	V2S 4N2
Garner	Mike	Domsea Farms Inc	4398 W. Old Belfair Hwy	Bremerton	WA	98312
Garrett	James E.	WDF	1435 Pavel Road	Beaver	WA	98305
Garrison	Robert L.	ODFW	OSU 303 Extension Hall	Corvallis	OR	97331
Garton	Ronnie	WDF	13124 Auburn Black Diamond Rd.	Auburn	WA	98002
Gatz	Henning	Aquatech Systems	PO Box 4315	Bellingham	WA	98227
Gearheard	Jim	WDW	Campus Mail GJ-11	Olympia	WA	98504
George	Raymond	Suquamish Tribe	P.O. Box 498	Suquamish	WA	98392
Gibson	Jim	Skagit System Coop.	P.O. Box 368	LaConner	WA	98257
Godby	Bill	USFWS	PO Box 25486	Lakewood	CO	80225
Goldberg	Harry	Aquatess Canada Ltd	1350 East Kent	Vancouver	BC	V5X 2Y2
Goodrich	Tom	BIOMED Research	1720 130th Ave NE	Bellevue	WA	98005
Gowan	Ron	Anadromous	500 SW Madison	Corvallis	OR	97333
Graf	Dennis V.	BC Government	Loon Creek Hatchery RR 1	Cache Creek	BC	V0H 1K0
Grande	Kerry	USFWS	3059 C National Fish Hatchery	Hagerman	ID	83332
Graybill	James R.	Mt. Hood Comm. College	26000 SE Stark St	Gresham	OR	97030
Gregg	T. R.	Engineered Products	5065 SW Nash	Corvallis	OR	97333
Greiner	Darren	BC Recreational Fisheries	34345 Vye Rd	Abbotsford	BC	V2S 4N2
Griffith	Glen	WDF	Samish Hatchery	Burlington	WA	98233
Groberg	Warren	ODFW	Badgley Hall-EOSC	La Grande	OR	97850
Gross	Steve	WDF	3900 Kalama River Rd	Kalama	WA	98625
Grosse	Daniel	UW	UW School of Fisheries WH-10	Seattle	WA	98105
Grover	Jerry C.	USFWS	500 NE Multnomah St.	Portland	OR	97232
Hager	Bob	WDF	Rm 115 GA Bldg	Olympia	WA	98504
Halloran	Bill	SSRAA	1621 Tongass Ave. Rm 103	Ketchikan	AK	99901
Hamilton	Glen		587 Tait St	Victoria	BC	-0-
Hammer	Stan	WDF	335 Island Blvd	Fox Island	WA	98333
Hansen	Sheryl	Cran-Mar Trout Farm	28633 216th Ave SE	Kent	WA	98042
Hansen	Corky	Sound Realty	7605 Spurgeon Crk. Rd. SE	Olympia	WA	98503
Hansen	Todd	Mt Hood C.C.	1710 SE 212th	Gresham	OR	97030
Hanson	John S.	USFWS	Box 1050	Red Bluff	CA	96080
Hanson	John	Moore Clark	P.O. Box M	LaConner	WA	98257
Harmer	Paul A.	Utah Wildlife	PO Box 536	Glenwood	UT	84730
Harrell	Lee	NMFS	Box 38	Manchester	WA	98353
Harris	Steve O.	WDF	HCR 78 Box 431	Naselle	WA	98638

Harvard	Ivan L.	WDW	Whatcom Falls Park	Bellingham	WA	98226
Hatch	Randy	Suquamish Tribe	P.O. Box 498	Suquamish	WA	98392
Hatfield	Doug	WDF	Rm 115 GA Bldg	Olympia	WA	98504
Haugen	Bill	ODFW	17330 SE Evelyn	Clackamas	OR	97027
Heggelund	Per		1515 Dexter Ave N #406	Seattle	WA	98109
Henderson	Dutch	WDF	10500 Skookumchuck Rd	Tenino	WA	98589
Henderson	Rod	WDF	105 W. Sunset Way	Issaquah	WA	98024
Henry	Mark	WDF	429 West Main	Concrete	WA	98267
Herrig	Daniel		4696 Overland Road RM 560	Boise	ID	83705
Hill	Jim	CEDC Fisheries	250 36th St	Astoria	OR	97103
Hill	Raymond L.	ODFW	Rt. 2 Box 149	Irrigon	OR	97844
Hillaire	Ed A.	Lummi	1642 Kwina Rd	Bellingham	WA	98226
Hinton	Joseph	Makah Fisheries	PO Box 115	Neah Bay	WA	98357
Hocking	Richard	Seattle Aquarium	Pier 59 Waterfront Park	Seattle	WA	98101
Holt	Richard	ODFW	OSU Dept of Microbiology	Corvallis	OR	97331
Holway	Jim		32500 SE Rainbow Rd.	Estacada	or	97023
Hopley	Bill	WDF	Rm 115 GA Bldg	Olympia	WA	98504
Hopper	Kathy	WDF	Room 115 GA Bldg	Olympia	Wa	98504
Horsch	Christopher	USFWS	Rt 1 Box 2105	Anderson	CA	96007
Hoskins	John G.	ODFW	90700 Fish Hatchery Road	Leaburg	OR	97489
Houseworth	Dave	Tamgas Creek Fish Hatchery	PO Box 416	Metlakatla	AK	99926
Hublou	Wally	Bioproducts	PO Box 429	Warrenton	OR	97146
Huddleston	Terry	Clear Springs Trout Company	PO Box 712	Buhl	ID	83316
Hudson	Greg	Domsea Farms	4398 W. Old Belfair Hwy	Bremerton	WA	98312
Hughes	Dan	ODFW	P.O.Box 130	Camp Sherman	OR	97730
Hurdlow	Dail	NSRAA	823 Charles	Sitka	AK	99835
Hurley	Bob	WDF	Box 24	Tenino	WA	98589
Hutchinson	Bill	IDFG	600 S. Walnut	Boise	ID	83707
Hutchins	John	Summit Technology	1075 Dexter Horton Building	Seattle	WA	98104
INDUSTRIAL PLASTICS			740 S. 28th	Washougal	WA	98671
INNOVAC TECHNOLOGY		Ward, Vern	1124 NW 53	Seattle	WA	98107
Isaac	Dennis L.	ODFW	17330 SE Evelyn St	Clackamas	OR	97015
Isaksson	Ken	WDW	1182-C Spencer RD	Winlock	WA	98596
Ives	Gary	Suquamish Tribe	P.O. Box 498	Suquamish	WA	98392
Ives	Charlene	Suquamish Tribe	P.O. Box 498	Suquamish	WA	98392
Jakola	Karl-Johan	Vinn	1415 38th Ave	Seattle	WA	98122
James	Bill	WDF	Rm 115 GA Bldg	Olympia	WA	98504
Jansma	Ken	WDF	PO Box 129	Humptulips	WA	98552
Janson	Vince	WDW	1182 Spencer Rd	Winlock	WA	98596
Jaquez	Joel K.	WDF	PO Box	Kalama	WA	98625
Jateff	Bob	WDF	Rt 1 Box 192	Raymond	WA	98577
Jensen	Loren	ODFW	Rt 1 Box 195	Bandon	OR	97411
JENNSORTER INC			20225 Harvest Lane	Bend	OR	97702
Johnson	Chuck	WDF	8537 Rhondo SW	Olympia	WA	98502
Johnson	Patrick	Bluewater Farms	104 14th Ave East	Seattle	WA	98122
Johnson	Pete	Pfizer Inc	3418 S. Chapman	Greenacres	WA	99016
Johnson	Randall L.	ODFW	17330 SE Evelyn St	Clackamas	OR	-0-
Johnson	Carl Eugene	WDF	7212 S 128th	Seattle	WA	98178
Johnson	Dick	WDF	5.63 Washougal River Rd	Washougal	WA	98671
Jones	Terry F.	ODFW	Star Route Box 71	Idanha	OR	97350
Jouper	Ed	WDF	1318 HWY 407	Cathlamet	WA	98612
Kaczynski	Vic	CH2M Hill	2020 SW 4th Avenue	Portland	OR	97201
Kelly	Melvyn D.	ODFW	Rt. 2 Box 2198	Clatskanie	OR	97016
Keown	Keith	WDF	10119 Steilaccom Road	Olympia	WA	98506
Kerwin	John	WDW	600 N. Capitol Way	Olympia	WA	98504

Kidder	Jay S.	R.W. Beck and Associates	2121 4th Ave	Seattle	WA	98121
Kienow	Stewart E.	Montana FW & P	100 Spring Cr. Drive	Somers	MT	59932
Killebrew	Kip	Stillaquamish Tribe	3439 Stoluckquamish Ln	Arlington	WA	98223
Kimbel	Mark A.	WDF	Rm 115 GA Bldg	Olympia	WA	98504
Kindschi	Greg	USFWS	4050 Bridger Canyon Rd	Bozeman	MT	59715
Kinzie	Katie	WDF	HCR 78 Box 413	Naselle	WA	98638
Kinzie	Katie	WDF	HCR 70 Box 413	Naselle	WA	98638
Kolb	Rich	WDF	13030 Auburn Black Diamond Roa	Auburn	WA	98002
Kolschefsky	Frank	Montana FW & P	Rt #1 96-4	Eureka	MT	59917
Kriss	Aron	Bioproducts	PO Box 429	Warrenton	OR	97146
Kruzynski	George	DFO	4160 Marine Drive	West Vancouver	BC	V7V 1N6
Labiske	Ed	ODFW	15020 Chance Road	Tillamook	OR	97141
Lamotte	Ed	USFWS	Spring Creek NFH	Underwood	WA	98651
Landon	George A.	ODFW	24500 S. Entrance Road	Estacada	OR	97023
LaRiviere	Paul	WDF	416 E. Dayton	Dayton	WA	99328
LaRiviere	Mark G.	Makah Fisheries Management	P.O. Box 115	Neah Bay	WA	98357
Larrick	Walter	Roza Irrigation Dist	PO Box 810	Sunnyside	WA	98944
Larson	Dale	BC Rec. Fisheries Branch	34345 Vye Rd	Abbotsford	BC	V2S 4N2
Latta	Steve	Bioproducts	PO Box 429	Warrenton	OR	97146
Law	Duncan	CEDC Fisheries	250 36th St	Astoria	OR	97103
Lee	Jim	WDW	1182 Spencer Road	Winlock	WA	98596
Leek	Steve L.	USFWS	Box 17	Cook	WA	98605
Leeland	Greg	Sound Environmental Inc	PO Box 355	Tacoma	WA	98401
Leith	David	USFWS	1440 Abernathy Road	Longview	WA	98632
Leitz	Michael	SSRAA	Neets Bay	Ketchikan	AK	99950
Lennox	J.D. (Doug)	Union Carbide Canada Ltd	Suite 280- 10991 Shellbridge W	Richmond	BC	V6X 3C6
LeVander	Lori J.	WA Dept of Ecology	4350 150th Ave. NE	Redmond	WA	98052
Lientz	Joe	USFWS	PO Box 18	Ahsahka	ID	83520
Linderoth	Amanda	Scan Am Fish Farms	P.O. Box 961	Anacortes	WA	98221
Long	Dan	F-A-L	PO Box 9037	Tacoma	WA	98409
Longley	Thomas	WDF	12711 124th Ave CT KPN	Gig Harbor	WA	98335
Lorz	Harold	ODFW	4192 N. Umpqua Hwy	Roseberg	OR	97470
Loudenslager	Eric	Fisheries Dept. HSU	HSU Fisheries Dept.	Arcata	CA	95521
Ludtke	Martin	WDF	MP 5.68 R Washougal River Rd	Washougal	WA	98671
LUMMI FISHERIES	SUPPLY, INC.	Adams, Dave	851 Coho Way	Bellingham	WA	98225
MacQuarrie	Don	Seastar Res. Corp	2620-1066 W. Hasting	Vancouver	BC	V6E 3X2
Macy	Thomas L.	USFWS	9317 Hwy 99 Suite I	Vancouver	WA	98665
Majnarich	John	BIOMED Research	1720 130th NE	Bellevue	WA	98005
Malee	Sibley A.	Montana FW & P	Route 1 Box 4630	Arlee	MT	59821
Marquardt	Gary	Domsea Farms	13700 Fagerholm Lane SE	Olalla	WA	98359
Marshall	Jack C.	Rainbow Ranch	194 Middle Fork Rd	Chehalis	WA	98532
Martin	Brian	Min. of Environment & Parks	RR #6 Boys Road	Duncan	BC	V9L 4T8
Martin	Fred A.	WDF	Room 115 GA Bldg	Olympia	WA	98504
Martin	Phillip	USFWS	P.O. Box 80	Nellton	WA	98566
Mason	B. John	Mount Hood C.C.	1310 NE Lynden Apt 56	Gresham	OR	97030
Massee	Ken	Domsea Farms	4398 W Old Belfair Hwy	Bremerton	WA	98312
Mattice	Scott A.		#124 401 Westview	Coquitlam	BC	V5K 3W3
Maxey	Douglas W.	WDW	1182-B Spencer Road	Winlock	WA	98596
Maxwell	Ed	WDF	HCR 78 Box 432	Naselle	WA	98638
Mazur	Carl	Brumar Sea Farms	2925 W 11th Ave.	Vancouver	BC	V6K 2M4
McAuley	Carlin	Domsea Farms	4398 W. Old Belfair Hwy	Bremerton	WA	98312
McBride	Marge	USFWS	PO Box 80	Neilton	WA	98566
McKay	Michael	MHCC	2236 SW Brink	Troutdale	OR	97060
McKee	Phil	ODFW	HC 60 Box 12	Idleyld Park	OR	97447
McKnight	Scott	Moore Clark	P.O. Box M	LaConner	WA	98257

McLeod	Bruce M.	USFWS	Rt 1 Box 98A	Kooskia	ID	83539
McLeod	Verna	Quadra Sea Farms Ltd	Box 649	Quathiaski	BC	V0P 1N0
McRoberts	Wally	Clear Springs Trout Company	PO Box 712	Buhl	ID	83316
McSweyn	Keith	WDF	13030 Auburn Black Diamond Roa	Auburn	WA	98002
Meadows	S.	Quileute Tribe	P.O. Box 279	La Push	WA	98350
Mencke	Brian	Cascade Aqua Farms	814 King Rd	Winlock	WA	98546
Merz	Nancy Dee	WDF	520 Englert Rd	Woodland	WA	98674
Meyer	Alan	ODFW	HC 30 Box 142	Chiloquin	OR	97624
Michak	Patty	WDF	Rm 115 GA Bldg	Olympia	WA	98504
Middaugh	Gene	ODFW	43182 N. River Dr.	Sweet Home	OR	97386
Middagh	Kevin N.	WDF	22115 NE 28th ST	Camas	WA	98607
MIDWEST SPECIALTY PRODUCTS		Balcain, Scott A.	100 State St.	Peoria	IL	61602
Miller	John A.	USFWS	6304 NW Bernie Drive	Vancouver	WA	98663
Miller	Bill	USFWS	PO Box 163	Ahsahka	ID	83520
Mills	Darell	WDF	PO Box 94141	Fort Steilacoom	WA	98445
Mitchell	Todd	Mt Hood C.C.	3326 Orendale NE	Salem	OR	97303
MITSUBISHI INTERNATIONAL		Fukano, Bob	1001 Fourth Ave Suite 2828	Seatle	WA	98154
Miyamoto	Joe	Puyallup Tribe	6824 Pioneer Way W.	Puyallup	WA	98371
Mofitt	Christine M.	Idaho Cooperative Fish	University of Idaho	Moscow	ID	83843
Molony	Bob	WDF	6263 Mt Baker Hwy	Deming	WA	98244
Montgomery	Carole	WDF	2284-C Spencer Rd.	Salkum	WA	98582
Moore	Dennis	Muckleshoot Tribe	34900 212th Ave. SE	Auburn	WA	98002
Moore	Jerry E.	WDF	Azwell Route Box 3	Pateros	WA	98846
MOORE-CLARK			P.O. Box M	LaConner	WA	98257
Morinaka	Ron	BPA	P.O. Box 12094	Portland	OR	97212
Muir	Pauline	Sound Environmental Inc	PO Box 355	Tacoma	WA	98401
Muller	Mike	WDF	Room 115 GA Bldg	Olympia	WA	98504
Murray	Ross	Envirocon Pacific Ltd	#205 2250 Boundary Road	Burnaby	BC	V5M 3Z3
MURRAY ELEVATORS		Nelson, Chris	PO Box 7428	Murray	UT	84107
Nandor	George F.	ODFW	2418 E. Fall Creek Rd	Alsea	OR	97324
Nealeigh	George W.	ODFW-Retired	P.O. Box 155	Manzanita	OR	97130
NEILSON METAL INDUSTRIES		Neilson, Jack	3501 Portland Rd NE	Salem	OR	97303
Nelson	Carol	UBC Animal Science	#248-2357 Main Mall	Vancouver	BC	V6T 2A2
NITROX			159 Downey Crive	New Britian	CT	06051
Noble	Dick	Salmon/Trout Advisory	P.O.Box 6232	Olympia	WA	98502
Norgard	Michael C.	Mt Lassen Trout Farm	Rt 5 Box 36	Red Bluff	CA	96080
Norman	Fred	WDW	1416 14th St. SW	Puyallup	WA	98371
North	John	CEDC Fisheries	250 36th St	Astoria	OR	97103
NORTHWEST SCALE SYSTEMS		White, Dan	2100 W. Broadway #5	Eugene	OR	97402
Novotny	Anthony(Tony)	Marinka International	1919 E. Calhoun	Seattle	WA	98112
O'Sullivan	Danny	B.C. Fish & Wildlife	Summerland Trout Hatchery	Summerland	BC	V0H 1Z0
Ochs	Gordon	Industrial Plastics	740 S 28th	Washougal	WA	98671
Ochs	Jim	Industrial Plastics	740 S. 28th	Washougal	WA	98671
Olson	Ron	NWIFC	6730 Martin Way	Olympia	WA	98506
Olson	Todd	CEDC Fisheries	250 36th St	Astoria	OR	97103
Olson	Wayne H.	USFWS	140 Boulder Drive	Orofino	ID	83544
Oman	Leni	University of Idaho	2415 Bush Ave. NW	Olympia	WA	98502
Osborne	Gary L.	WDF	Star Rt 16	Beverly	WA	99321
Owsley	David E.	USFWS	PO Box 18	Ahsahka	ID	83520
Page	David	WDF	15072 52nd St E	Sumner	WA	98390
Parke	Clyde	AFLW	Box 394	Coleman	ALTA	T0K 0M0
Parsons	Jim	Clear Springs Trout Company	PO Box 712	Buhl	ID	83316
Pastor	Steve	USFWS	9317 NE 99th Suite I	Vancouver	WA	98682
Patterson	Roberta	Malaspina College	#607- 1680 Dufferin Creek	Nanaimo	BC	V9S 5N3
Pearce	Brian	DFO	555 W. Hastings	Vancouver	BC	-0-

Peck	Larry	WDF	Room 115 GA Bldg	Olympia	WA	98504
Pedersen	Paul	WDF	Minter Creek Hatchery	Gig Harbor	WA	98335
Pennell	William	BC Salmon Farmers Assoc.	2459A Bellevue Ave.	West Vancouver	BC	V7V 1E1
Peterson	Don	BC Rec. Fisheries Branch	780 Blawshard St	Victoria	BC	V8V 1X5
Peterschmidt	Caroline	Domsea Farms	4398 W Old Belfair Hwy	Bremerton	WA	98312
Peterson	Paul	WDF	2284 Spencer Rd.	Salkum	WA	98582
Peterson	Donald L.	WDF	Klickitat Salmon Hatchery	Glenwood	WA	98619
Petersen	Karl	Envirocon Pacific Ltd	#205 2250 Boundary Road	Burnaby	BC	V5M 3Z3
Peterson	Larry	WDW	249 Fish Hatchery Road	Mossyrock	WA	98564
Pflug	Dave	R.W. Beck and Associates	2121 4th Ave	Seattle	WA	98212
Phillipson	Ken	NWIFC	6730 Martin Way E.	Olympia	WA	98506
Pigott	Roy K.	WDF	19110 Pioneer Way E	Orting	WA	98360
Piper	Robert G.	Prog. Fish Culturist/AFS	P.O. Box 3706	Bozeman	MT	59772
Poppleton	John	Envirocon Pacific Ltd	#205 2250 Boundary Road	Burnaby	BC	V5M 3Z3
Postlewait	Dana	Sverdup Corp	P.O. Box 369	Bellevue	WA	98009
Povey	John C.	Mt Lassen Trout Farm	Rt 5 Box 36	Red Bluff	CA	96080
Powe	Don	Peaceful Valley Recreation	1418 16th Ave.	Lewiston	ID	83501
Pratschner	Greg	USFWS	Box 549	Leavenworth	WA	98826
Price	Clifford W.	Muckleshoot Tribe	34900 212th Ave. SE	Auburn	WA	98002
Probst	Duane	Neilsen Metal Industries	3501 Portland Rd NE	Salem	OR	97303
PROTECT-A-COVER		Frank, Dennis	12711 Hwy 99	Everett	WA	98204
Rains	Dave	Rains Quality Trout Farm	Star Rt Box 31	Pateros	WA	98846
Rains	Barb	Rains Quality Trout Farm	Star Rt Box 31	Pateros	WA	98846
Raistakka	Wesley L.	USFWS	Rt 1 Box 2105	Anderson	CA	96007
Rapelje	Don	WDF	Rt 3 Box 3136	East Wenatchee	WA	98801
Rasch	Tony	WDF	15200 NE 209th Place	Brush Prairie	WA	98606
Rasmussen	Ulf	WDW	P.O.Box 44109	Tacoma	WA	98444
Ready	Bob	WDF	3900 Kalama River Road	Kalama	WA	98625
Reece	Mark L.	WDF	11001 Lewis River Road	Ariel	WA	98603
Rensel	Jack	Rensel Associates	2412 N. 77th Street	Seattle	WA	98103
Richer	William	WDF	5941 Fish Hatchery Rd	Marblemount	WA	98267
Rideout	Jay	FAL/HEATH	PO Box 9037	Tacoma	WA	98409
Rieben	David	ODFW	Rt 4 Box 594	Astoria	OR	97103
Robards	Steve C.	WDW	HCR Box 52	Chelan	WA	98816
Roberts	Steve	WDW	580 Nelson Place	E. Wenatchee	WA	98801
Robert	Randy	ODFW	Rt.1 Box 443	Maupin	OR	97037
Rogers	Robert	WDF	2020 Conger Ave NW	Olympia	WA	98502
Rogers	David	ODFW	43863 Greer Drive	Leaburg	OR	97489
Roley	Dennis	Bioproducts	PO Box 429	Warrenton	OR	97146
Ropp	Gene	WDF	Azwell Route Box 3	Pateros	WA	98846
Ross	Leslie	WDW	Whatcom Falls Park	Bellingham	WA	98226
Ross	Carl	WDF	PO Box 175	Starbuck	WA	99359
Rotter	Dan	Global Aqua	11405 Gate Rd S.	Olympia	WA	98502
Rowan	Gerry	Anadromous Inc	500 SW Madison	Corvallis	OR	97333
Rowland	Richard A.	ODFW	2917 SE 142nd Place	Portland	OR	97236
Roy	Hugh G.	ROY-ALL HATCHERIES	1287 212 St RR 14	Langley	BC	V3A 7R2
Rudholm	Paul E.	BVTI	3148 Bay Rd.	Ferndale	WA	98248
Rudnick	Don	WDF	14418 383rd Ave SE	Sultan	WA	98294
Russum	Jerry D.	ODFW	95163 Elk River Road	Port Orford	OR	97465
Rutledge	Ray	EnerTech	Box 230089	Anchorage	AK	99507
Sawtell	Tom A.	Salmon/Trout Advisory	1515 Dexter Ave. N	Seattle	WA	98109
Schaeffer	Drew	ODFW	1843 SE 37th Avenue	Portland	OR	97214
Schaefer	Mark	WDF	14418 383rd Ave SE	Sultan	WA	98294
Schamber	Tim	ODFW	660 South F St	Independence	OR	97351
Scheer	Ken	BC Rec. Fisheries Branch	34345 Vye Rd	Abbotsford	BC	V2S 4N2

Schenk	Winfried	Alberta Fish and Wildlife	1440 17a St. SE	Calgary	ALTA	T2G-4T9
Schirato	Margie	WDW	HCR 63 Box 714	Naselle	WA	98638
Schmidt	Carl	Bluewater Farms	508 Lincoln St	Port Townsend	WA	98368
Schneider	Rich	Clear Springs Trout Co	PO Box 712	Buhl	ID	83316
Schnick	Rosalie	USFWS	PO Box 818	LaCrosse	WI	54602
Schroder	Larry	WDF	5939 Fish Hatchery Lane	Marblemount	WA	98267
Scott	Tom	Clear Springs Trout Company	PO Box 712	Buhl	ID	83316
Scott	Marilyn	WDF	Rt 2 Box 10-A	Glenwood	WA	98619
Scott	Brian	Swecker Salmon Farm	6601 198th St SW #16	Rochester	WA	98579
Scribner	Tom	Yakima Indian Tribe	P.O.Box 151	Toppenish	WA	98948
Seabolt	Kristina M.	Trout Lodge Inc	PO Box 11	McMillan	WA	98352
Seidel	Paul	WDF	Room 115 GA Bldg	Olympia	WA	98504
Sepeda	Robert	Anandromous	203 Costa Court	Coos Bay	OR	97420
Shake	Bill	USFWS	Lloyd 700 Bldg	Portland	OR	97232
Shearer	Joel T.	Mt. Hood Community College	2950 NE 23rd Apt. #18	Gresham	OR	97030
Sheldrake	Tom	US Fish and Wildlife	700 NE Multnomah Street	Portland	OR	97232
Sheldon	Ray	ODFW	17330 SE Evelyn St.	Clackamas	OR	97015
Sherry	Kathleen	Malaspina College	6254 Forest Creek	Nanaimo	BC	V9S 5N7
Shores	David J.	WDF	12615 124th Ave. Ct	Gig Harbor	WA	98335
Siemens	Mark	BC Rec. Fisheries Branch	34345 Vye Rd	Abbotsford	BC	V2S 4N2
Sinclair	D. C.	J. S. McMillan Fisheries	2199 Commissioner St.	Vancouver	BC	V5L 1A4
Slatick	Emil	NMFS	Big Pasco Industrial Park #900	Pasco	WA	99301
Smith	Charlie E.	USFWS	4050 Bridger Canyon Road	Bozeman	MT	59715
Smith	Stan	USFWS	Bldg. 204 Naval Station	Seattle	WA	98115-5007
SMITH-ROOT INC		Smith, Kerry	14014 NE Salmon Creek Ave.	Vancouver	WA	98686
Smith	Quentin E.	ODFW	Rt. 1 Box 764	Astoria	OR	97103
Smith	Robert Z.	NMFS	847 NE 19th Suite 380	Portland	OR	97232
Snyder	Bruce	UW	9744 Manley Rd.	Bremerton	WA	98310
Solar	Igor I.	DFO-West Vancouver Lab	4160 Marine Drive	West Vancouver	BC	V7V 1N6
Sorenson	Dan	Makah NFH	PO Box 730	Neah Bay	WA	98357
SOUND ENVIRONMENTAL INC		Stroud, Wendell	PO Box 355	Tacoma	WA	98401
Sparrow	Hugh	BC Rec. Fisheries Branch	780 Blanshard St.	Victoria	BC	V8S 1S9
Spielman	John	Mt Hood C.C.	PO Box 292	Fairview	OR	97024
Stanley	Charlie	ODFW	33465 Hwy 22	Hebo	OR	97122
Stedronsky	Wayne A.	ODFW	Star Route B Box 526	Cascade Locks	OR	97014
Steele	Earl	BVTI	3028 Lindbergh Ave	Bellingham	WA	98225
Stevie	Michelle	WDF	7440 Fairview SW	Olympia	WA	98502
Stickell	Trent	ODFW	Star Route B Box 1	Cascade Locks	OR	97014
Stilwater	Rick	WDW	2306 S. 16th Ave.	Yakima	WA	98903
Stippl	Stefan	Falkemeckl	6749	Wieslauten	FRG	-0-
Stratton	Mike	ODFW	1916 SE 20th	Portland	OR	97214
Sundet	Steinar	Ma-Bo Aqua Inc	7125 Vanvikan	-0-	NOR	-0-
Swecker	Dan	Swecker Salmon Farm Inc.	10420 173rd SW	Rochester	WA	98579
Sweeney	Mark	Montana FW & P	606 West Pennsylvania	Anaconda	MT	59711
Tansley	Bill	ODFW	Rte. 1 Box 449	Maupin	OR	97037
Taylor	Gib	USFWS	1221 Ebb Tide Terrace	Olympia	WA	98502
Terry	Justin	BVTI	2115 Yelm Road	Olympia	WA	98501
TEXAS REFINERY CORP		Nash, Doug	PO Box 545	Redmond	OR	97756
Thorsen	William	USFWS	6970 Hatchery Drive	Entiat	WA	98822
Thygesen	Ted	WDF	E. 41 Coulter Creek Rd.	Belfair	WA	98528
Tipping	Jack	WDW	2101 Hwy 508	Onalaska	WA	98570
Todd	Neil L.	Diversified Ova-Tech Ltd	Box 237	Merritt	BC	V0K 2B0
Toedtemeier	Guy	WDF	Rt 2 Box 90	Glenwood	WA	98619
Townsend	Bill	Trout Lodge	Box 11	McMillan	WA	98352
Trimble	Robert	WDF	HCR Box 385	Southbend	WA	98586

Turk	Earle	WDF	PO Box 6645	Kennewick	WA	99336
Turner	Neil	WDF	4404 Lewis River Rd	Woodland	WA	98674
Turner	Lynda L.	WDF	1435 Pavel Rd.	Beaver	WA	98305
Turner	Rich	ODFW	17330 SE Evelyn St	Clackamas	OR	-0-
Turvey	Doug	DFO	Box 225	Sandspit	BC	V0T 1T0
Van Meter	Jerry	USFWS	700 NE Multnomah Street	Portland	OR	97232
Van Ree	Gary A.	Global Aqua	11405 Gate Rd S.	Olympia	WA	98502
Van Slyke	Dan	Anandromous	934 Garfield	Coos Bay	OR	97420
Van Tussenbrook	Lee	WDW	12208 SE Evergreen Hwy	Vancouver	WA	98684
Vincent	D.	Malaspina College	900 5th Ave	Nanaimo	BC	V9R 5K2
Visscher	Larry	USFWS	13905 W. Atlantic	Lakewood	CO	80228
Vogel	Dave	USFWS	PO Box 667	Red Bluff	CA	96080
Volkhardt	Gregory C.	NWIFC	6730 Martin Way	Olympia	WA	98506
Voskuilen	Reece	Sverdup Corp	P.O. Box 369	Bellevue	WA	98009
Wade	Bruce	WDF	6263 Mt Baker Hwy	Deming	WA	98244
Wagner	Paul	Pyramid Lake Fisheries	Star Route	Sutcliffe	NV	89510
Wainwright	Duane L.	USFWS	710 Hwy 395	Gardnerville	NV	89410
Walker	Derald	ODFW	65885 W. Highway 20	Bend	OR	97701
Walliem	Bill	USFWS	Box 429	Winthrop	WA	98862
Walters	Bruce D.	WDW	1341 Ringold River Rd.	Mesa	WA	99343
Ward	Tim	WDF	10119 Steilacoom RD SE	Olympia	WA	98503
Ward	Tim	WDF	10119 Steilaccom Rd SE	Olympia	WA	98503
Warner	Steve		4200 Old Gate Rd.	Lake Oswego	OR	97034
Warren	Jim	USFWS	500 NE Multnomah ST Suite 1692	Portland	OR	97232
Warren	Keith	CEDC Fisheries	250 36th St	Astoria	OR	97103
Wastel	Mike	Domsea Farms	4398 W. Old Belfair Hwy	Bremerton	WA	98312
Watson	Lynn	IDFG	HC 66 Box 150	Island Park	ID	83429
Watson	Rich B.	WDF	PO Box 177	Humptulips	WA	98552
Weaver	Dewey	Domsea Farms	PO Box 4789	Rolling Bay	WA	98061
Weist	Guy E.	WDF	Rt 1 Box 263	Raymond	WA	98577
Wells	Steve	ODFW	580 Fish Lake Road	Butte Falls	OR	97522
Westers	Harry	Michigan DNR	PO Box 30028	Lansing	MI	48909
Westgard	Dick	WDF	10408 Marine View Drive	Everett	WA	98204
White	Gary R.	USFWS	PO Box 790	Warm Springs	OR	97761
Whitlatch	Richard A.	ODFW	39800 SE Fish Hatchery Road	Sandy	OR	97055
Wiese Hansen	Jan	Scan Am Fish Farms	P.O. Box 961	Anacortes	WA	98221
Wilcoxen	Don	ODFW	26915 Trask River Rd	Tillamook	OR	97141
Williams	Norman F.	Muckleshoot Tribe	34900 212th Ave. SE	Auburn	WA	98002
Willow	John	Pacific Aqua Foods Ltd	350-601 Cordova St	Vancouver	BC	V6B 1G1
Witczak	Daniel B.	WDF	PO Box 129	Humptulips	WA	98552
Wold	Einar	NMFS	847 NE 19th Ave Suite 350	Portland	OR	97232-22
Wolff	Klaus	BC Rec. Fisheries Branch	Site 11 RR#1	Summerland	BC	V0H 1Z0
Wolski	Stefan					
Wolski	Szczepan	Envirocon Pacific Ltd	Rt 2 Box 2142	Clearwater	BC	V0E 1N0
Wood	James W.	WDF-Emeritus	8124 NE 157th St	Bothell	WA	98011
Wood	James L.	Mt Hood Comm College	1320 NE 110	Portland	OR	97220
Woody	Stanley C.	WDW	28 Beaver Creek RD	Cathlamet	WA	98612
Wright	Terry	NWIFC	6730 Martin Way E	Olympia	WA	98506
Yasutake	Tosh	National Fish Research Cen.	Building 204 Naval Station	Seattle	WA	98115
Yeager	Gary	ODFW	PO Box 142	Nehalem	OR	97632
Youngren	Jim	Glenwood Springs	Rt 1 Box 126	Eastsound	WA	98245
Young	Steven J.	Tulalip Tribe	10610 Watersworks Rd	Marysville	WA	98270
Zajac	David	USFWS	2625 Parkmont Lane Building A	Olympia	WA	98502
Zimmerman	Brian	Anadromous Inc.	500 SE Madison	Corvallis	OR	97333
Zirjacks	Don L.	USFWS	Carson NFH	Carson	WA	98610