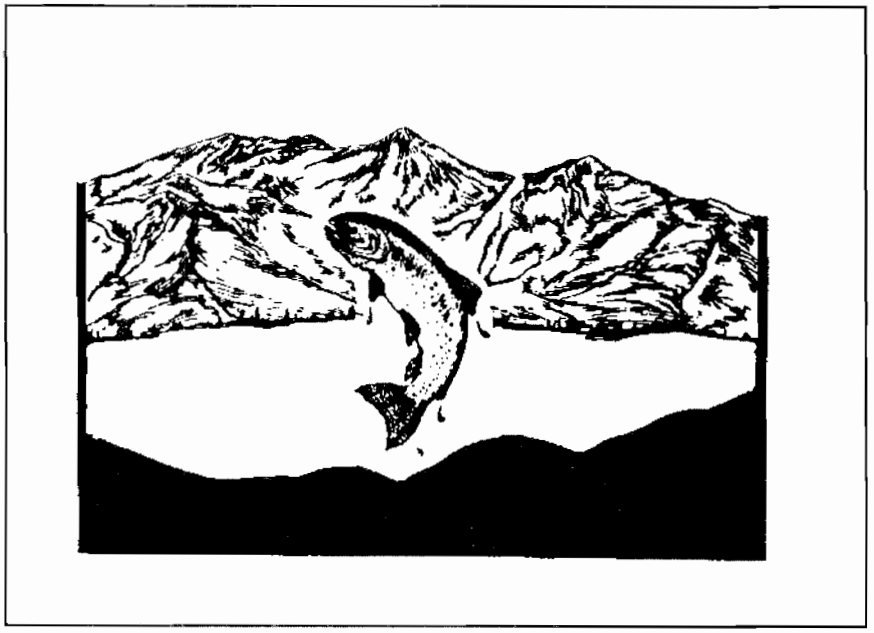
PROCEEDINGS OF THE

41ST ANNUAL

NORTHWEST FISH CULTURE CONFERENCE



IDAHO DEPARTMENT OF FISH AND GAME

BOISE, IDAHO

DECEMBER 4, 5, 6, 1990

TABLE OF CONTENTS

Hatcheries - Thomas L. Rogers, Session Leader

Fish Health - Kent Hauck, Session Leader

WELCOME

On behalf of The Idaho Department of Fish and Game, I would like to welcome you to Boise for the 41st Annual Northwest Fish Culture Conference. My name is Bill Hutchinson and I am the State Fish Hatcheries Manager and Chairman of this year's conference.

As you can see from the turnout, this conference is becoming ever increasingly popular as a forum for fish culture activities. It is estimated that over 400 participants are attending this year. Given the current economic situation, it is evident that the importance placed on this conference reflects the dedication and commitment that you all have made.

I recently read a fish culture article and I would like to quote some parts that seem appropriate for us here today. "During the past 10 or 12 years, applied science and mechanics have revolutionized fish hatchery operations. More advances have probably occurred during this period than since the very beginning of trout culture. The uses of new chemicals in treating diseases in hatcheries, eradicating undesirable fish populations, spawning and transporting fish, and the employment of labor-saving devices such as fish loaders, self graders, incubators, and dry feeds are only a few of the advances illustrating the progress made. They indicate that fish culture is at last beginning to receive the recognition and research that it deserves. With a greater demand for hatchery-reared fish each year, additional important advances are sure to take place." This excerpt was taken from The California Department of Fish and Game, Fish Bulletin 107, and written by Earl Leitritz in 1959. Even though this was written some 31 years ago, before almost everyone here began their career, I feel it is pertinent in reflecting attitudes in fish cultural advances during the 1980s.

The development and interest in aquaculture continues to grow, and as fishery scientists, we have advanced, at least I would like to think we have advanced, from ground horse meat diets to highly specialized feeds formulated for specific species or controlled growth. From spawning whatever you happened to get your hands on to high tech genetic engineering creating all female populations. From "boot-strap" fish culture using the S.W.A.G. method and hatchery records kept on scraps of paper filed in some obscure file cabinet to advanced computer programs with extensive data bases able to tell you everything you ever wanted to know about your fish and more. Fish now can carry their own social security numbers and extensive information by using the PIT tag. Pathologists are developing such rapid and sophisticated disease diagnosis that at times it seems every fish contains every pathogen. The ART of trout culture goes back to 1741, and despite all of today's high-tech advances, I hope we never lose the true ART in fish culture.

With that, I would like to begin this year's conference. I hope you all enjoy yourselves, and I think the presenters have something to share with us all.

INTRODUC

FISH CULTURE

PERFORMANCE OF RAINBOW TROUT REARED AT FOUR DENSITIES USING SUPPLEMENTAL OXYGEN

Greg A. Kindschi, Charlie E. Smith, and Robert F. Koby, Jr.
U.S. Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, Montana

ABSTRACT

Wild and domesticated strains of rainbow trout Oncorhynchus mykiss were reared in triplicate at four different fish loading densities. Fish performance and health, and water quality were monitored during the 4-month test. Instream dissolved oxygen was supplemented to maintain afternoon dissolved oxygen in the effluent at 6 to 7 ppm. The highest densities reached were 13.3 lb/ft3 by the wild strain and 18.4 lb/ft3 by the domesticated strain. Mortality increased with density in both strains. Decreased survival in the wild strain was due to cannibalism. The lower survival of fish of the domestic strain, was caused by mechanical failures resulting in decreased dissolved oxygen. Weight gain and total length improved with increasing densities in the wild strain, but the opposite held for the domesticated strain. Variability in length increased directly with fish density. Feed conversions of both strains were adversely affected by increased densities but neither dorsal fin erosion nor fish condition factor were affected. During both studies, neither un-ionized ammonia nor carbon dioxide reached critical levels. Although parasitic outbreaks of Ichtyobodo and Hexamita occured in the wild strain they did not appear to be related to fish density. Overall fish health did not appear to be affected by differences in density. There were two periods of daily maximum dissolved consumption--during the first daily feeding and after the last daily feeding. Rainbow trout of the wild Eagle Lake strain and domesticated Arlee strain consumed dissolved oxygen at an average rate of 221 and 233 mg/kg of fish/hour, respectively, for the duration of these two studies. No differences in average dissolved oxygen consumption of rainbow trout were caused by the different rearing densities. The daily dissolved oxygen consumption averaged 189.7 g/kg of feed for fish of the Eagle Lake strain and 240.5 g/kg of feed for those of the Arlee strain. Un-ionized ammonia or carbon dioxide never reached detrimental levels and did not appear to affect the fish. Recommendations are made to use back-up electrical systems, oxygen systems and constant water temperature controls when rearing fish at high densities with supplemental oxygen.

The above information has been taken from the following manuscripts already submitted for possible publication:

Dwyer, W.P., G.A. Kindschi, and C.E. Smith. In review. Evaluation of high and low pressure oxygen injection techniques. Proceedings of the American Fisheries Society Bio-Engineering Symposium, October 24-26, 1988, Portland, Oregon.

Kindschi, G.A., C.E. Smith, and R.F. Koby, Jr. In review. Effects of supplemental oxygen on the performance of two strains of rainbow trout reared at four densities. Progressive Fish Culturist.

Kindschi, G.A., C.E. Smith, and R.F. Koby, Jr. In review. Oxygen consumption of two strains of rainbow trout reared at four densities with supplemental oxygen. Progressive Fish Culturist.

STUDIES OF GROWTH IN RAINBOW TROUT

Kaiser H., J. de la Garza and G.W. Klontz
Department of Fish and Wildlife Resources,
University of Idaho
Moscow, Idaho

INTRODUCTION AND OBJECTIVES

In a hatchery it may become necessary to control fish growth which may lead to a more continuous production in a commercial food fish hatchery. One advantage is that the producer would become adaptable in responding to different market conditions and product price changes.

This study describes a method to control growth of rainbow trout. The trials were conducted in the laboratory at the University of Idaho. One objective was to study growth of rainbow trout after a period of reduced feeding. Also, the reliability of weight estimations based on length data shall be discussed under the situation of low and high feeding rates.

METHODS

The fish were held in 100 l (26.4 gal) tanks with a water inflow of 5 l/min (0.79 gal/min) at a constant water temperature of 15°C (59°F). Oxygen values did not reach critical levels for salmonids. Eyed eggs of Kamloops trout were obtained from Trout Lodge, Washington and all fish were hatched in the laboratory. All fish were hand-fed twice daily. The commercial diet was provided by Silver Cup, Murray, Utah. The fish density was not considered to be a growth limiting factor and never exceeded
1.8 $kg/m^3/cm$ of body length.

One feeding procedure was based on a method proposed by Haskell (1959). The feeding rate as % of body weight per day **(%BW)** depends on the daily length increase in mm/fish **(dL)**, the length of the fish in mm on day of feeding **(L)** and the feed conversion - expressed as the amount of feed as fed per kg gain - **(FCR)** (see equation 1).

$$\%BW = \frac{3 * dL * FCR * 100}{L} \qquad (1)$$

Another group of fish was fed to satiation twice daily. This method should assure the maximum growth potential of the fish under the given conditions.

The estimation of weight was done using a widely used method (Haskell 1959). Weight in g/fish **(W)** is a function of length in mm/fish **(L)** and the condition factor **(K)** - in this example 0.000011224 (equation 2).

$$W = K * L^3 \qquad (2)$$

Fish on a reduced feeding rate were fed intermittently: 7 days on feed and 7 days off. When on feeding they were fed for 50% of the allowable growth rate.

RESULTS

The fish fed at a reduced feeding regimen had slow growth. After 172 days there was a total length gain of only 30 mm per fish. The mean weight at the start of the period was 0.8 g/fish with an ending weight of less than 4 g/fish (Figure 1).

The average daily length increase during the 172-day period was 0.177 mm per fish. There was an average daily weight gain of less than 1% of body weight, which is a low value for fish of this size. The feed conversion (1.59 kg feed/kg gain) was lower than would be expected from this diet (Table 1). There was no mortality, disease outbreaks or evidence of fin damage during the period of growth reduction.

Table 1: Growth data for rainbow trout fed on a reduced feeding regimen

Average daily length increase	0.177 mm/fish
Average percent weight gain per day	0.94 %
Average feed conversion (kg feed per kg gain)	1.59
Duration of reduced feeding	172 days

After the period of reduced growth the fish were reassigned to high feeding regimens. They were divided into two treatment groups. One group was fed according to the feeding regimen by Haskell (1959) (equation 1) and the other fish were fed to satiation twice daily. Figure 2 shows the effect of full feeding. All groups had good weight and length gains during the next 6 months. There was an immediate reaction to the change in feeding regimen and no delay of growth. The fish reached a marketable size in the range of 250-300 g/fish within 169 days.

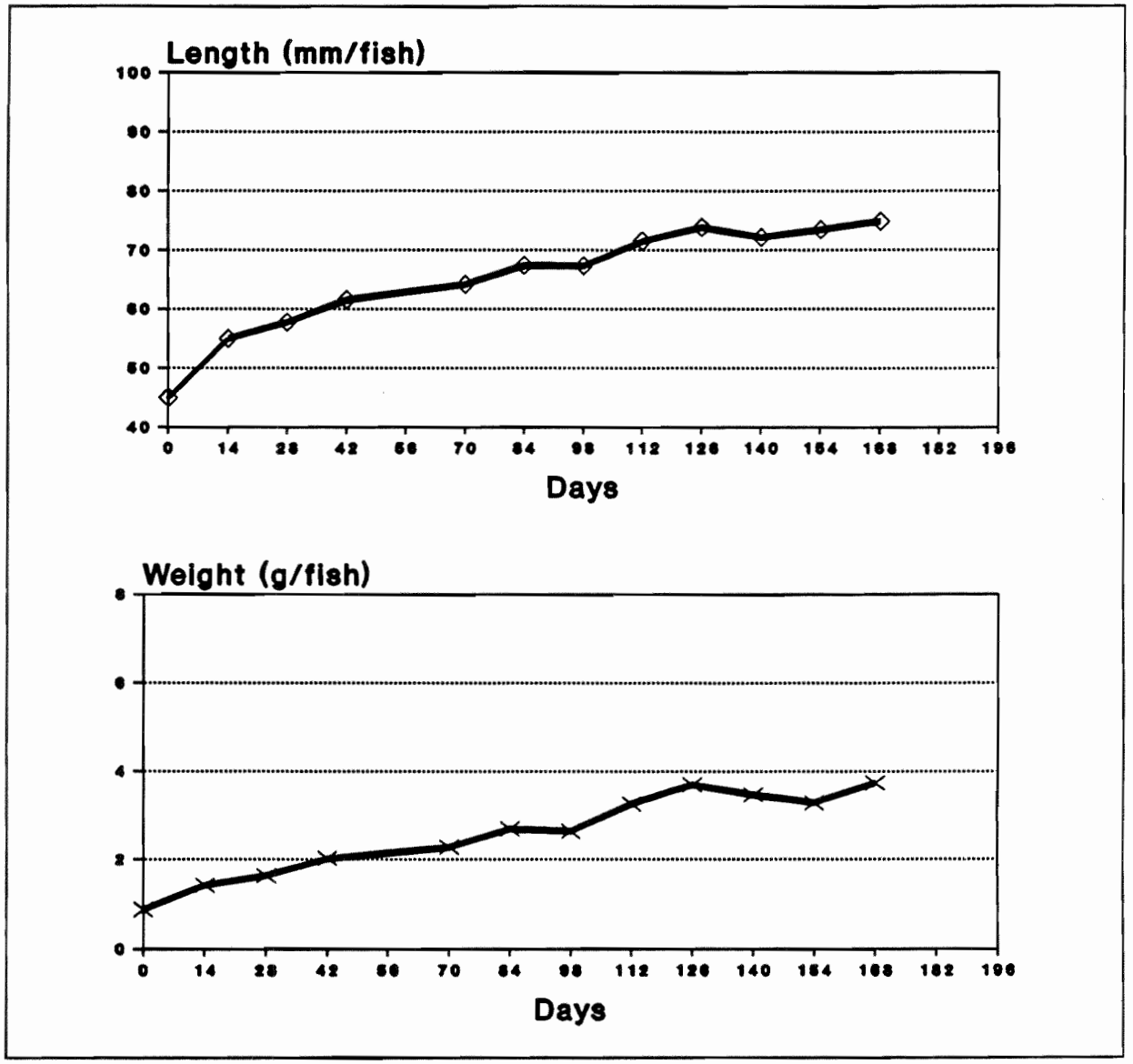


Figure 1: Length and weight development of rainbow trout fed at a reduced feeding regimen for 172 days

A comparison of growth data between these fish and other groups that had been fed at the same feeding regimen continuously is listed in Tables 2 and 3. The only difference was that the control group had not been on a reduced feeding regimen prior to the growth period.

The fish being fed to satiation had an average daily length gain of 1.03 mm/day (control group) and 1.08 mm/day (reassigned fish). The mean weight gain per day was 2.96 and 2.83% of body weight, respectively. The feed conversion data were comparable between the treatment groups (Table 2). Similar results were found for the fish that had been fed according a common feeding guide. All differences between treatments are not significant at a 5% probability of error. There was no significant increase in size variation despite different treatments of the fish prior to the period of full-feeding (Tables 2 and 3). This indicates that all fish that had been held on a reduced feeding regimen went back on feed without problems.

Table 2: Growth data for rainbow trout fed to satiation twice daily after a period of feed reduction compared to the growth of a control group that had been fed continuously.

Growth parameter	Continuous feeding	After reduced feeding
Average length gain (mm/day)	1.03	1.08
Average % weight gain per day	2.96	2.82
Average feed conversion (kg feed per kg gain)	1.06	0.93
Coefficient of variation (%)		
a) Length	8.30	8.76
b) Weight	29.80	26.20
Growth period (days)	196	162

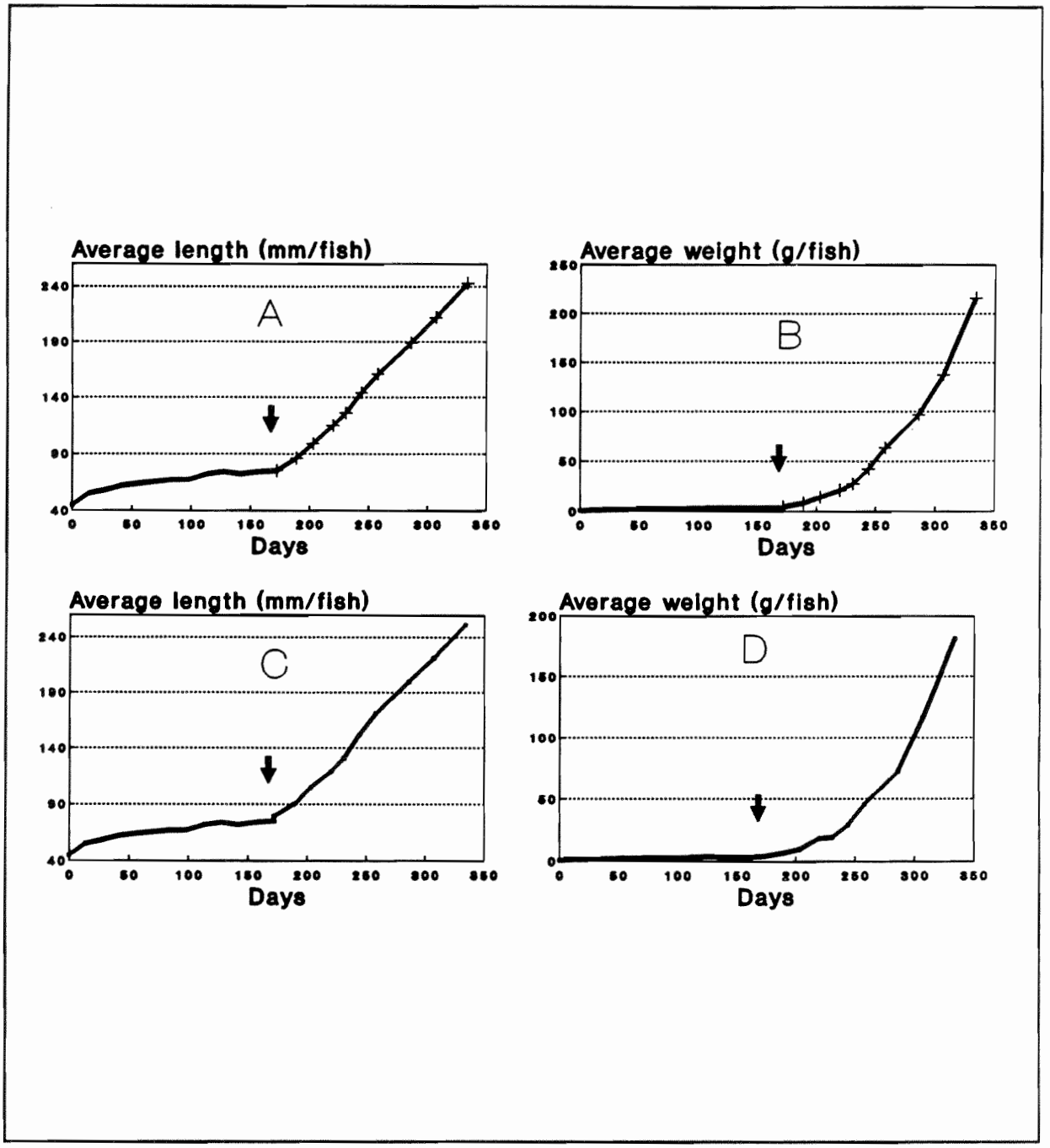


Figure 2: Length and weight changes of rainbow trout before and after full feeding.

A and B = Feeding according to the common feeding guide
C and D = Feeding to satiation twice daily

Arrows indicate the time of reassignment

Table 3: Growth data for rainbow trout fed according to the common feeding regimen after a period of feed reduction compared to the growth of a control group that had been fed continuously.

Growth parameter	Continuous feeding	After reduced feeding
Average length gain (mm/day)	0.97	1.04
Average % weight gain per day	3.15	2.85
Average feed conversion (kg feed per kg gain)	1.10	1.17
Coefficient of variation (%)		
a) Length	9.03	10.6
b) Weight	26.9	31.7
Growth period (days)	169	162

Figure 3 shows a graphical comparison of the growth curves before and after the resumption of full feeding. The growth curves start at approximately the same weight or length to make a valid comparison. It becomes clear that there was a comparable length and weight gain for all treatments. There appeared to be a tendency for improved weight gain in the group fed to satiation after a period of growth reduction, but the difference is statistically not significant (5% probability of error).

The second objective of the study was to test the reliability of weight estimation based on length data. The condition factor formula (equation 2) has been widely used. A comparison between the expected weight using the formula and the real measured weight as a function of length is shown in Figure 4. This comparison is done for fish fed at a reduced feeding rate and for fish during a period of full feeding. Also, the resulting error that would occur if the formula were used to estimate weight from length is provided.

The figure shows that there is increasing error at higher length data. The error increased up to 22% when fish were fed at the high feeding rates (e.g. satiation). The higher the length the more unreliable is the estimation. The same comparison was done for fish that have been held at low feeding rates. There is more constant error between 20 and 25%. Here, the weight of the fish would be underestimated (Figure 4, second graph).

DISCUSSION

The study has shown that it is possible to hold rainbow trout at a feeding rate that produces virtually no growth during a six-month period without negative effects on the fish. There were no mortalities or disease.

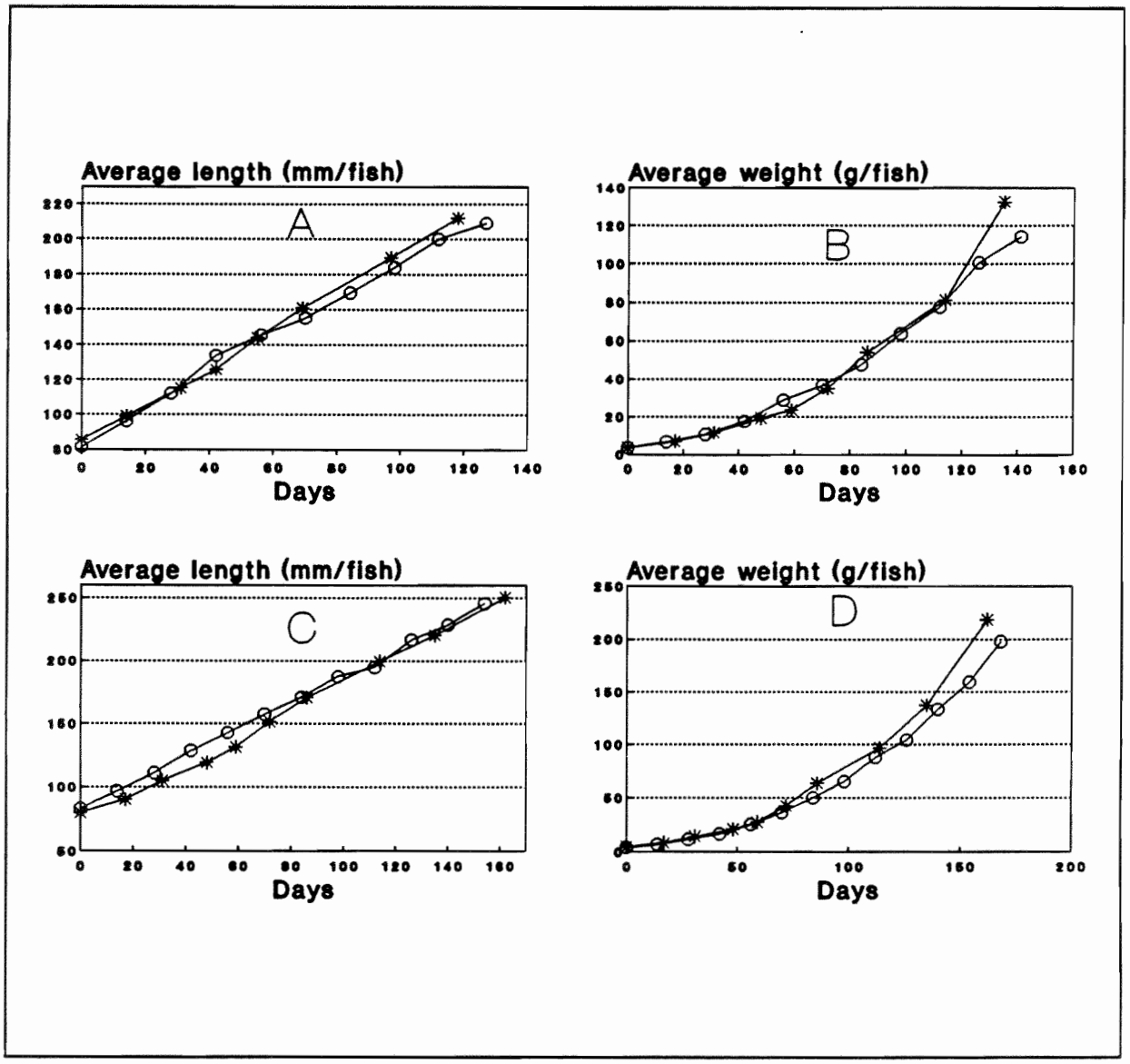


Figure 3: Comparison of length and weight changes of rainbow trout during a period of full feeding.

A and B = Feeding according to the common feeding guide
C and D = Feeding to satiation twice a daily
O = Continuous feeding
* = Full-feeding after a period of reduced growth

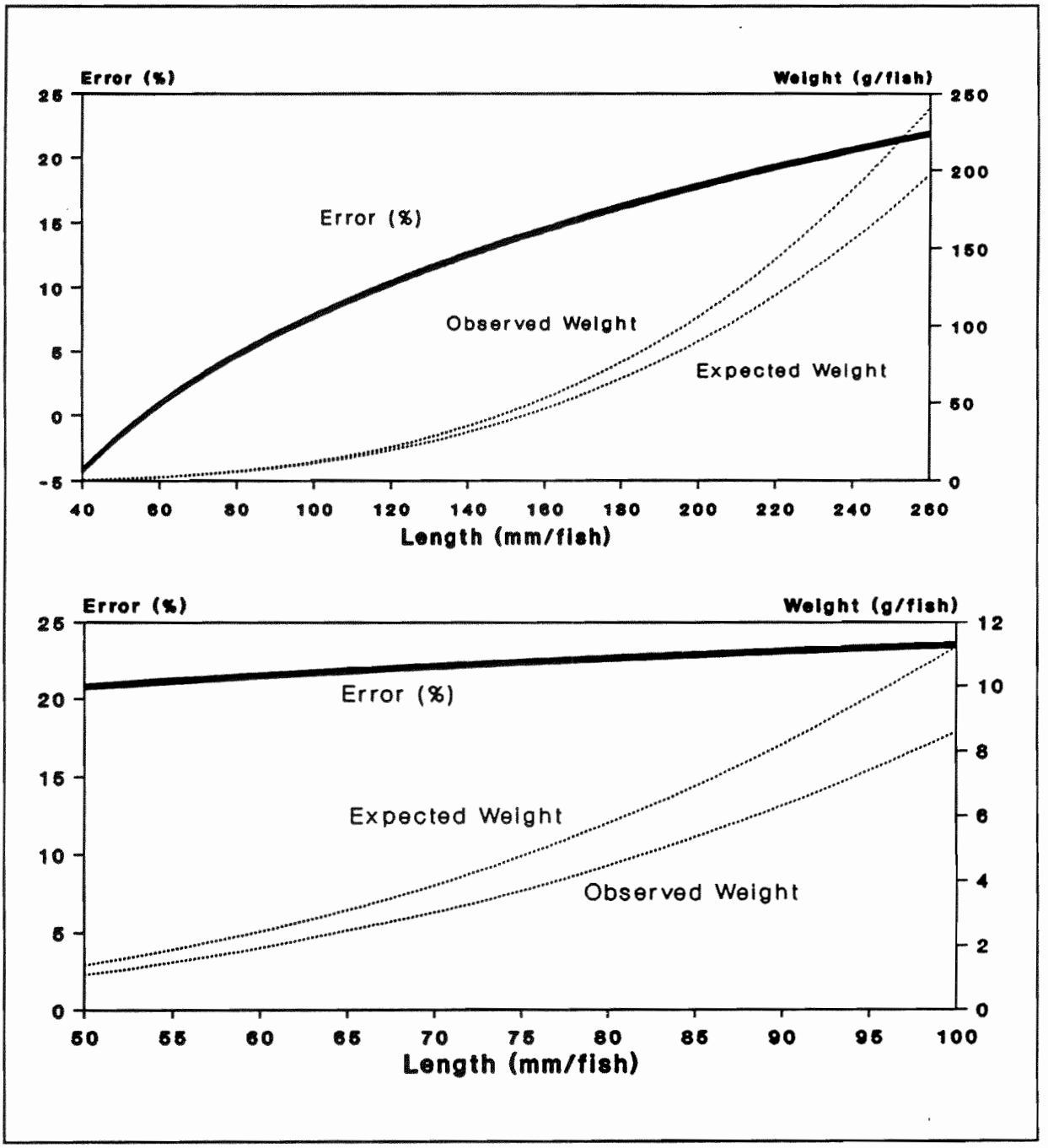


Figure 4: Comparison of the observed weight-length relationship and the theoretical estimation of weight and the resulting error in %.

First graph: Data for fish being fed at full feeding
Second graph: Data for fish being held on reduced feeding

After reassignment to a high feeding rate the fish started growing at a normal rate. This means that the growth rate remained predictable. Thus, there appeared to be no compensatory growth. All fish grew as well as those that had been fed continuously. The treatment did not result in a significant increase in size variation.

These findings are important for practical fish production and the results have several advantages:

1) A manager can buy eggs or fry and keep the fish "on hold" for a period of up to 6 months. The eggs can be purchased when the best quality and price is available and there would be only seasonal egg picking resulting in more efficient use of labor.

2) The introduction of diseases is limited.

3) During the production period equal portions of the fry can be transferred into the production units every month (see Figure 5). The growth rate remains predictable.

4) Fish are available most of the time which aids fast reaction to market price fluctuations.

A good product should be uniform, thus the quality of the product depends on the size variation or coefficient of variation. The proposed treatment did not increase the variability. This indicates that fish were feeding well after a period of slow growth, thus no fish were left behind.

The data have shown that it remains difficult to do an accurate weight estimation if one commonly used formula. Especially, if fish are held on feeding rates that are unusually low, it cannot be recommended to use length-weight tables. In this case the measurement of length should be accompanied by weight determination. This would reduce the risk of over- or underestimating the total weight of fish in the holding tanks.

For further studies it will be necessary to test our findings in a large-scale operation. Our results should be seen as a contribution to hatchery management and controlling growth of trout. We are continuing the study of the effects of different feeding rates on growth and length-weight correlation.

SUMMARY

Rainbow trout were held on a reduced feeding regimen for 6 months prior to reassignment to high feeding rates. During the following 6 months of full-feeding growth data were comparable to data from fish that had been fed continuously at the same rate. The paper deals with the problems of using common length-weight tables to estimate fish weight based on length data.

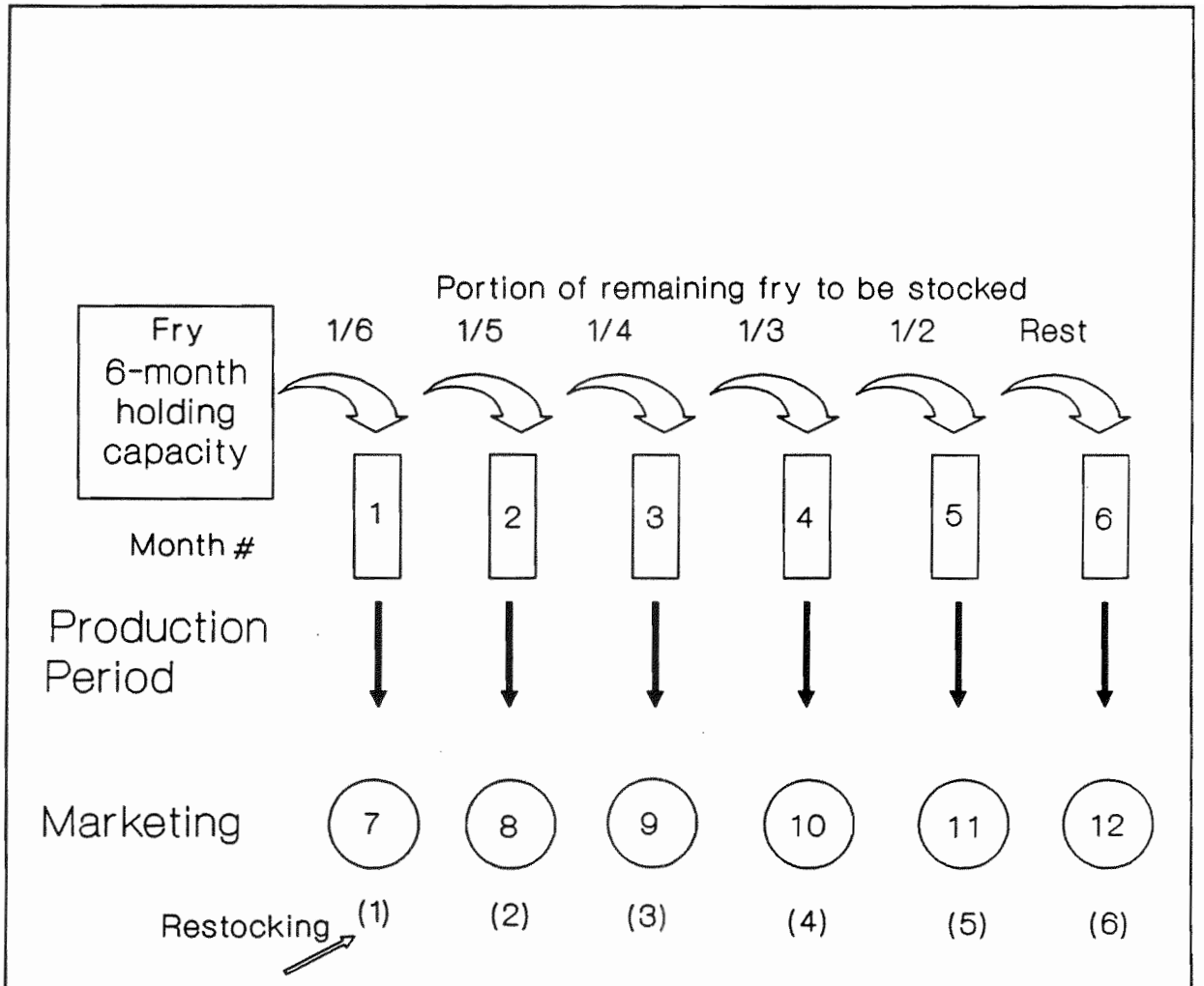


Figure 5: Management plan for a six-month production period

BIBLIOGRAPHY

Haskell, D.C. 1959. Trout growth in hatcheries. New York Fish and Game Journal, Vol. 6, No. 2, 204-237.

LIMITATIONS ON THE USE OF PURE OXYGEN IN FISH CULTURE

John Colt
James M. Montgomery, Inc.
Bellevue, Washington

ABSTRACT

In pure oxygen aeration systems, the build-up of carbon dioxide is important. The build-up of carbon dioxide has three primary effects: (1) increase in dissolved carbon dioxide concentrations, (2) decrease in pH, and (3) reduction in the mole fraction of un-ionized ammonia due to the decrease in pH. In a closed system with no carbon dioxide removal, the maximum carrying capacity is controlled by pH at low pHs, by carbon dioxide at intermediate pHs, and by un-ionized ammonia at high pH. Under pHs typical of salmonid systems, it is impossible to exceed an un-ionized ammonia criterion.

INTRODUCTION

Due to economic pressures and the lack of available water resources, the intensity of aquatic production facilities in many parts of the world has significantly increased over the last 10 years. Concurrently, use of aeration equipment has become common as dissolved oxygen is typically the first water quality parameter to limit production (Gowan, 1987; Severson et al., 1987). Intensification of production increases the concentrations of carbon dioxide, ammonia, fecal solids, and dissolved organic matter. The simultaneous impact of these parameters must also be considered when evaluating the carrying capacity and operation of high intensity production systems. Depending on environmental conditions (which are not well-understood by either biologists or engineers), the addition of oxygen may not increase the carrying capacity.The relationships developed in this paper can be used for the design and operation of more efficient rearing systems.

INTENSITY IN FISH CULTURE

Hatchery intensity in this paper will be discussed in terms of cumulative oxygen consumption (COC):

$$COC = \sum_{i=1}^{n} (DO_{in} - DO_{out})$$

$$COC = \frac{(6.944)(M)(FR)(OFR)}{Q}$$

$$L = \frac{COC}{(6.944)(OFR)(FR)}$$

Where:

COC = cumulative oxygen consumption (mg/liter)

DO_{out} = effluent dissolved oxygen from a rearing unit (mg/liter)

DO_{in} = influent dissolved oxygen to a rearing unit (mg/liter)

n = number of raceways in series

M = mass of fish in rearing unit (kg)

OFR = Oxygen-feed ratio (kg oxygen/kg feed)

FR = Feeding rate (%)

Q = Water flow (lpm)

L = Loading (kg fish/lpm)

WATER QUALITY CRITERIA

The modeling presented in this paper is based on the following water criteria (Colt and Orwicz, In Press):

Dissolved Oxygen Criterion	(low)	6.5 mg/liter
	(high)	300 mm Hg
Dissolved Carbon Dioxide Criterion		20 mg/liter
Un-ionized Ammonia Criterion		12.5 mg/liter NH_3-N
pH Criterion	(low)	6.0
	(high)	9.0
Suspended Solids Criterion		15 mg/liter

MATERIALS AND METHODS

The modeling approach used in this work is based on (1) water quality criteria for critical parameters, (2) metabolic characteristics of fish under production conditions, and (3) basic carbonate chemistry. The specific details of the modeling are presented by Colt and Orwicz (In Press).

pHs are discussed in terms of the equilibrium pH (pHe). This is the pH of a water in equilibrium with atmospheric carbon dioxide at a given temperature and barometric pressure. The final pH after addition of carbon dioxide was computed from the basic definition of alkalinity (Stumm and Morgan 1981).

Oxygen consumption is based on an oxygen-feed ratio (OFR) of 250 g oxygen/kg feed. The production of metabolic carbon dioxide was assumed to be 1.375 times the oxygen consumption (RQ=1) and total ammonia production was equal to 0.030 times the daily feed input.

OVERALL PRODUCTION CAPACTIY

Because of the dependence of cumulative oxygen consumption on the amount of carbon dioxide retained in the water, the overall production capacity will be presented in terms of the degree of gas transfer across the system boundaries.

Closed System (No Transfer of Gases)

A typical flow-through, single-pass culture system approximates a closed system with no transfer of gases. The maximum cumulative oxygen consumption for a closed system is presented in Figure 1. The available oxygen is assumed to be 90% of saturation. At a give equilibrium pH, the minimum of COC_{pH}, $COC_{ammonia}$, COC_{carbon}, and COC_{ss} is the limiting COC and therefore controls the amount of oxygen that can be used. This produces the sharp changes in the limiting COC curves at the intersections between different individual COC curves. The controlling factor depends on equilibrium pH and at 15 C are equal to:

Equilibrium pH (pH_e)	Controlling Factor
< 6.8	pH
6.8 - 8.7	available oxygen
> 8.8	un-ionized ammonia

Because available oxygen is low, carbon dioxide is not a limiting factor in a closed system. The COC in the three regions increases with decreasing water temperature.

Closed System with Pure Oxygen Addition

The maximum cumulative oxygen consumption for a closed system with pure oxygen addition is presented in Figure 2. Carbon dioxide removal in pure oxygen aeration systems is minimal due to the low gas-to-liquid ratios used in these systems. The controlling factor depends on equilibrium pH and at 15 C are equal to:

Equilibrium pH (pH_e)	Controlling Factor
< 7.4	pH
7.4 - 8.6	carbon dioxide
> 8.6	un-ionized ammonia

The COC in the three regions increases with decreasing water temperature.

Open System

A serial-reuse culture system with gravity aerators or a transport system with diffused aeration approximates an open system. These types of aeration can effectively remove carbon dioxide, but will have little impact on ammonia. Even though carbon dioxide is removed, the pH of the water will not be significantly changed due to metabolic activities. The maximum cumulative oxygen consumption for a open system is presented in Figure 3. The open system is controlled by the un-ionized ammonia criterion computed at the equilibrium pH. The maximum cumulative oxygen consumption is seriously limited at high pHs.

Influent Carbon Dioxide and Ammonia Concentrations

Influent carbon dioxide concentrations have been assumed to be 100% of saturation and total ammonia nitrogen concentrations have been assumed equal to zero. This approximates an unpolluted, shallow, fast-moving stream. The effect of increasing the influent levels of these two parameters is presented in Figure 4.

Increasing influent carbon dioxide concentrations reduces the maximum COC under most conditions, especially below an equilibrium pH of 7.2. The addition of ammonia to the influent water significantly changes the shape of the maximum $COC_{ammonia}$ curve. The maximum $COC_{ammonia}$ for the addition of 0.50 mg/liter of total ammonia nitrogen is 0.0 mg/liter over the pH range of 9.00 to 8.00. This is because this amount of total ammonia nitrogen results in an un-ionized ammonia concentration greater than the criterion over this whole pH range. But for

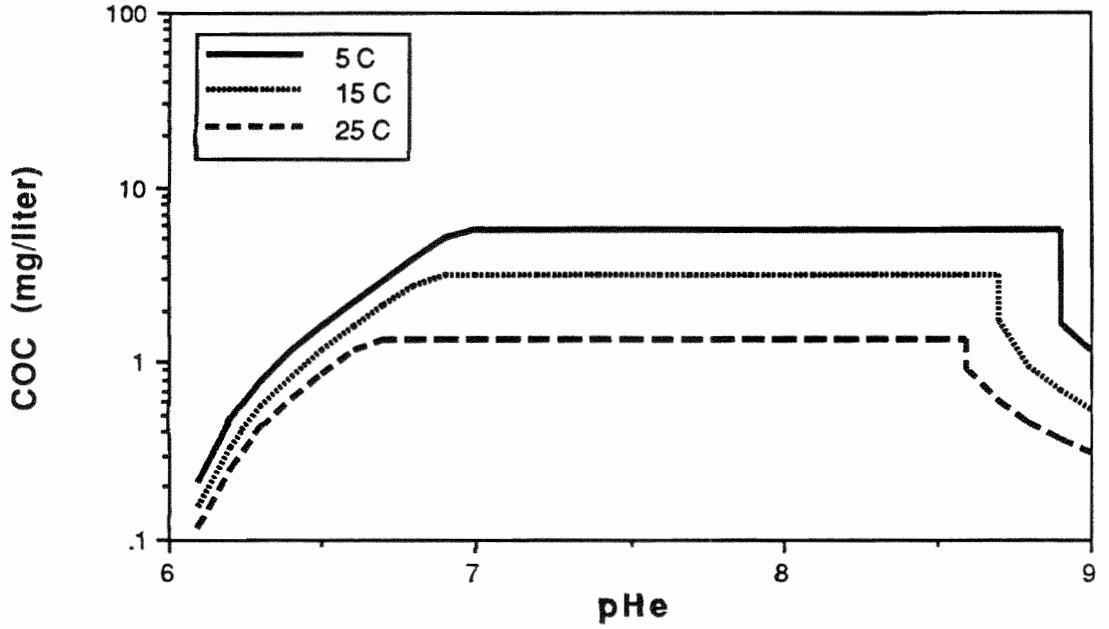


Figure 1 Overall cumulative oxygen consumption for a closed system as a function of equilibrium pH (assumes barometric pressure = 760 mm Hg, influent DO = 90% of saturation)

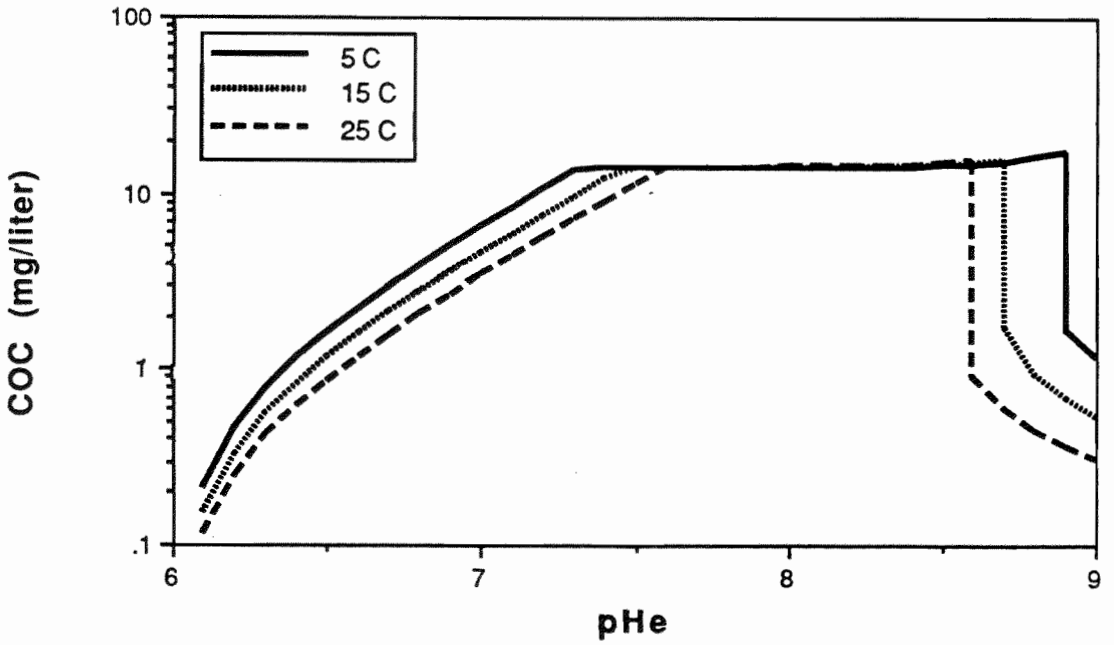


Figure 2 Overall cumulative oxygen consumption for a closed system with pure oxygen addition as a function of equilibrium pH

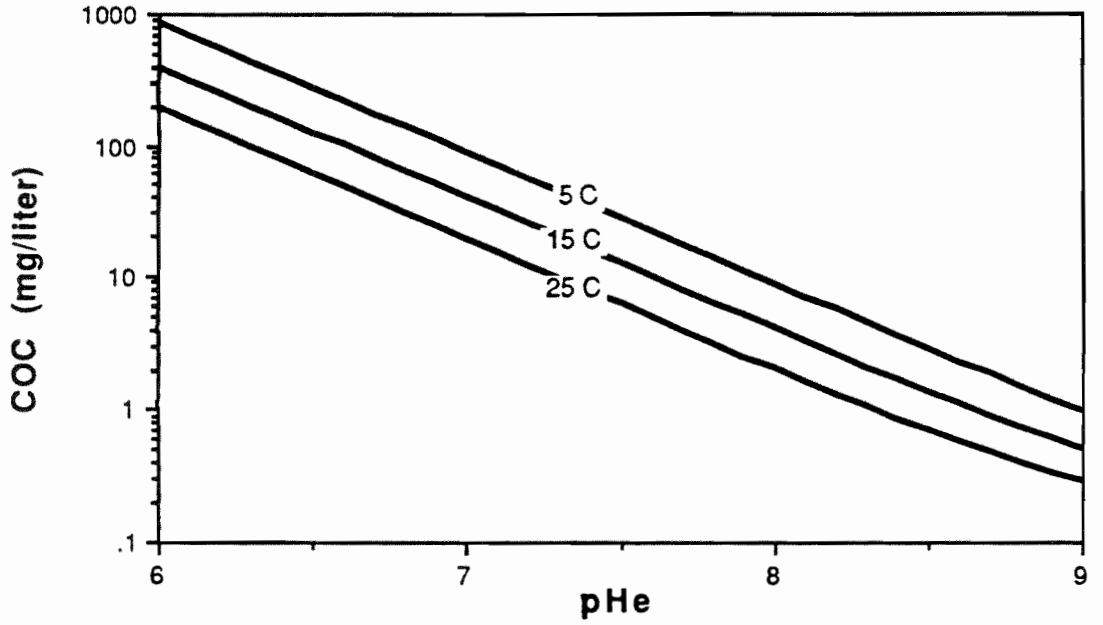


Figure 3 Maximum cumulative oxygen consumption for un-ionized ammonia ($COC_{ammonia}$) as a function of temperature and equilibrium pH (assumes complete removal of excreted carbon dioxide from the water and an un-ionized ammonia criterion equal to 12.5 µg/liter NH_3-N)

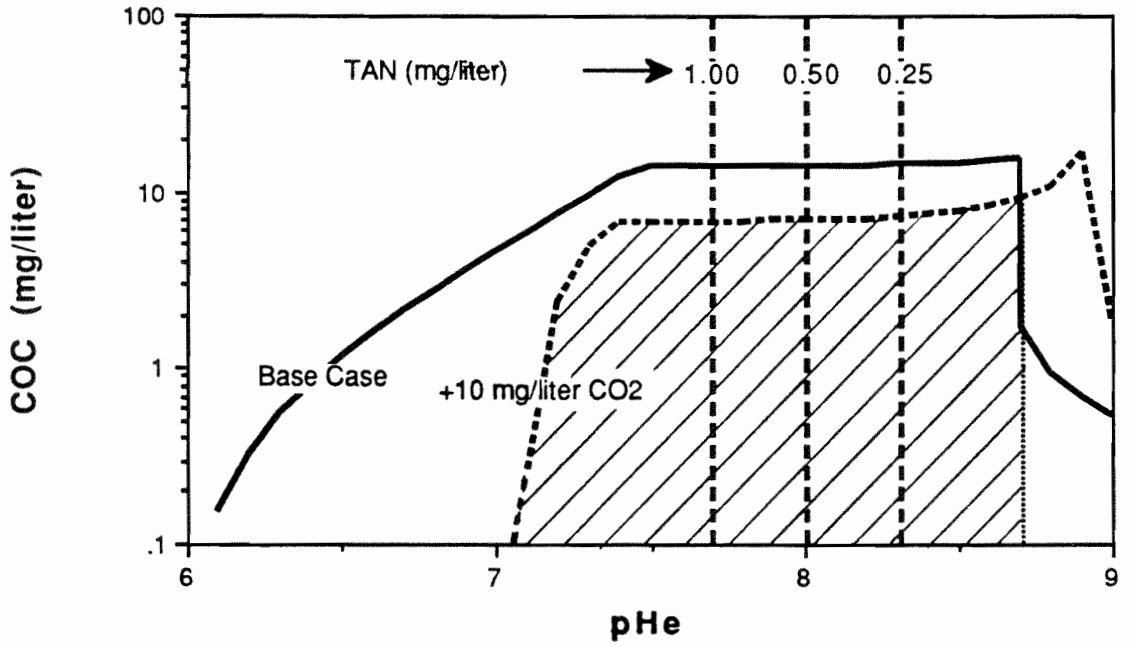


Figure 4 Effect of influent carbon dioxide and ammonia on overall cumulative oxygen consumption. The base case is a semi-closed system (Figure 2). The carbon dioxide case assumes the addition of 10 mg/liter of carbon dioxide to the influent water. The $COC_{ammonia}$ are presented for 0.25, 0.50, and 1.00 mg/liter of total ammonia nitrogen present in the influent water. The $COC_{ammonia}$ lines run vertically with very high values to the left of the respective line and zero values to the right of the line. The COC for 10 mg/liter of carbon dioxide and 1.00 mg/liter of ammonia-N is represented by the cross-hatched area (assumes water temperature = 15 C)

equilibrium pHs equal to or less than 8.0, the maximum $COC_{ammonia}$ approaches a large positive number due to the depression of the pH value resulting from metabolic carbon dioxide. Therefore, elevated influent ammonia concentrations significantly reduce the maximum COC at higher pHs.

Elevated influent ammonia concentrations have little effect on COC_{pH} or COC_{carbon}, but elevated influent carbon dioxide concentrations have a significant effect on $COC_{ammonia}$. Therefore, the maximum COC for elevated ammonia and carbon dioxide influent concentrations will be determined by the COCpH and COC_{carbon} curves for a given amount of influent carbon dioxide and the vertical $COC_{ammonia}$ curve translated to the right by the resulting depression in pH. Elevated influent carbon dioxide concentrations can significantly increase the acceptable pHs for culture when influent ammonia concentrations are elevated.

DISCUSSION

The most limiting water quality parameter in an aquatic production facility will depend on a variety of factors such as equilibrium pH of the water, the degree of carbon dioxide retention, influent DO concentration, and water temperature. Relatively small design and operational factors may have a major impact on the importance of these parameters. The carrying capacity of a flow-through system can be limited by oxygen, pH, carbon dioxide, and un-ionized ammonia. In systems that retain the majority of excreted carbon dioxide, un-ionized ammonia limitations will typically be unimportant. In fact, over normal pHs, it should be impossible to exceed the un-ionized ammonia criterion. pH limitations will be more important in water with low alkalinities and especially for egg incubation due to higher sensitivity to low pHs. At alkalinities < 10 mg/liter as $CaCO_3$ (0.20 meq/liter), pH limitations may occur even for single-pass non-aerated systems. The use of pure oxygen in waters with equilibrium pHs less than 6.5 should be carefully evaluated. Carbon dioxide will commonly be limiting in high-intensity systems because of the relatively small variation in COC_{carbon} over normal pHs.

In a single-pass system without aeration, dissolved oxygen will typically be the most limiting water quality parameter. In high intensity systems using pure oxygen or aeration, this may not be the case and other parameters such as pH, carbon dioxide, ammonia, or solids may be more limiting. The design of pure oxygen systems for waters with either low or high pHs must be done with extreme care.

REFERENCES

Colt, J. and K. Orwicz. In Press. Modeling production capacity of aquatic culture systems under freshwater conditions. Aquacultural Engineering.

Gowan, R. 1987. Use of supplemental oxygen to rear chinook in seawater. Pages 35-39 in Papers on the use of supplemental oxygen to increase hatchery rearing capacity in the Pacific Northwest, Bonneville Power Administration, Portland, Oregon.

Severson, R.F., J.L. Stark, and L.M. Poole. 1987. Use of oxygen to commercially rear coho salmon. Pages 25-34 in Papers on the use of supplemental oxygen to increase hatchery rearing capacity in the Pacific Northwest, Bonneville Power Administration, Portland, Oregon.

Stumm, W. and J.J. Morgan. 1981. Aquatic chemistry, 2nd Edition, ß, New York.

OXYGEN INDUCED GAS BUBBLE DISEASE IN RAINBOW TROUT *Oncorhynchus mykiss* (Richardson).

Donald A. Edsall and Charlie E. Smith
U.S. Fish and Wildlife Service
Fish Technology Center
Bozeman, Montana

ABSTRACT

An Aquatector oxygen injection unit was used to supersaturate a hatchery water supply to 200% oxygen saturation (18-20 mg/L) and increase the total gas pressure to 120% of saturation. Nitrogen saturation was reduced to near 100%. Rainbow trout, *Oncorhynchus mykiss* (Richardson), held in the treated water developed signs of gas bubble disease in 4 days, and 50% died within 20 days. We demonstrated that supersaturated total gas pressure due to excessive oxygen saturation causes gas bubble disease in the absence of supersaturated nitrogen gas. It is recommended that users of oxygen injection systems closely adjust the amount of oxygen added to the water to keep the total gas pressure near saturation.

This paper has been accepted for publication in Aquaculture and Fisheries Management.

FISH TRANSPORT IDAHO STYLE

Dave Bruhn
National Fish Hatchery
U.S. Fish and Wildlife Service
Hagerman, Idaho

INTRODUCTION

Many millions of fish are transported annually in the State of Idaho. Included are steelhead trout, chinook salmon rainbow trout, brown trout, catfish, and tilapia. Numberwise, the private sector undoubtedly transports the majority of fish, as their annual rainbow trout production alone probably exceeds 20 million pounds; or at least 40 million fish.

May of the Idaho fish transport operations involve cooperation between Idaho Department of Fish and Game (IDFG), Idaho Power Company (IPC), and the Fish and Wildlife Service (FWS). This paper will review the magnitude of these programs, the procedures and types of equipment utilized to accomplish these large scale operations.

TRANSPORT DATA

The combined 1990 transport totals for IDFG, IPC, and FWS amounted to 19,717,000, fish weighing 2,947,000 pounds and requiring 334,500 miles of vehicle operation.

PROCEDURES

Included in the transport operations is the standard practice of loading steelhead and rainbow trout directly from Hagerman Valley 59°F water into 40 to 42°F chilled water on the transport trucks (without tempering of the fish).

Additives to the transport water include anti-foam at a concentration of 12 ppm, and pro-poly aqua (polyvinyl pyrollidone) at a concentration of 100 ppm. The latter product serves to temporarily replace the protective fish slime that is adversely affected by handling. 1990 costs for these materials were anti-foam at $20.64/gal and pro-polyaqua at $13.98/gal.

Fish pumps are used almost exclusively for loading fish up to 12-14 inches in length. Some commercial operators incorporate a grading device into their fish pump loading procedure, thereby returning less than market-size fish to the rearing ponds.

TYPES OF EQUIPMENT

Conventional Straight Trucks

These units range from regular pickup trucks with slide-in tanks to 6- and 10-wheel trucks with frame- or bed-mounted tanks. The commercial trout industry utilizes the latter type extensively in transporting market-size fish (2/lb) from rearing facilities to processing plants.

Conventional Straight Trucks Pulling Trailers

IDFG utilizes these units in transporting salmon and steelhead smolts, as well as long-distance transport of rainbow trout from the southern Idaho hatcheries to central and north Idaho rearing/redistribution facilities.

Semi-Trailers

Idaho Power Company operates two trailer units in conjunction with anadromous mitigation obligations for the Hells Canyon hydropower complex. Salmon and steelhead smolts are transported from Rapid River and Niagara Springs hatcheries.

The U.S. Army Corps of Engineers, Walla Walla District, under the Lower Snake River Fish and Wildlife Compensation Plan provided five trailer units for operation in Idaho. These are utilized primarily at the IDFG McCall, Sawtooth, and Magic Valley hatcheries and the FWS Dworshak and Hagerman hatcheries for transporting salmon and steelhead smolts.

The trailers, costing $130,000 each in 1982, are 5,000-gallon capacity, double-walled (insulated), five compartment, stainless steel construction. Oxygen is supplied through carbon rods from liquid oxygen cylinders, with high-pressure oxygen cylinders as backup. The oxygen passes through 15 flow meters prior to reaching the carbon rods. Two diesel generators supply 110-volt electric power to ten Fresh-Flo aerators (two per compartment) to augment oxygen levels and to dissipate carbon dioxide from the water.

Steelhead smolt loadings for these units normally range from 6,500 to 7,000 pounds (1.54 to 1.68 pounds of fish per gallon of water) of 8-inch fish at water temperatures of 40 to 50°F. Time in transit may be up to 10-12 hours. Spring chinook salmon loadings normally are 3,300 pounds (o.78 lbs/gal) of 5- to 6-inch fish.

Aircraft

As with may agencies in the western United States, IDFG utilizes helicopters and fixed-wing aircraft for specialized fish plants into remote and inaccessible waters.

PANEL DISCUSSION: IMPLICATIONS OF CHANGES IN EFFLUENT MONITORING REQUIREMENTS

IMPLICATIONS OF CHANGES IN EFFLUENT MONITORING REQUIREMENTS

Don Gibbons
Environmental Protection Agency

No Abstract submitted.

EFFLUENT MANAGEMENT: AN AQUACULTURE INDUSTRY APPROACH

Dr. Randy MacMillan
Director of Research and Development
Clear Springs Trout Company
Buhl, Idaho

INTRODUCTION

Clear Springs Trout Company, located in south-central Idaho, is a vertically integrated company producing high quality rainbow trout Oncorhynchus mykiss for human consumption. It is the largest trout company in the United States producing over 18 million pounds of fish annually. As part of its commitment to environmental quality, Clear Springs Trout Company has made a substantial economic and technical commitment to minimizing solids and nutrient introductions in effluent discharged into the Snake River. Aquaculture is one of the few agricultural industries currently regulated by the U.S. E.P.A. and Idaho Dept. of Environmental Quality. We must obtain a National Pollutant Discharge Elimination System (NPDES) permit which specifically limits the amount of Total Suspended Solids (TSS) which can be discharged in our effluent. The newest permits further require that various dissolved nutrient discharges be monitored and therapeutant use documented although no discharge limits have been set. This information will undoubtedly be used in future regulatory agency efforts to limit effluent content. Should further limits be imposed, we request that regulatory decisions be based upon scientifically credible information (not scare tactics) and upon thorough understanding of Snake River hydrodynamics, other nutrient sources and best management practices. We suggest that there are multiple sources of nutrients into the Snake River including, but not limited to, irrigation runoff, sewage treatment, dairies and aquaculture. Some research suggests phosphorus concentrations in the Snake River above Shoshone Falls, an area preceding aquaculture effluent introductions, may be sufficient to cause significant phytoplankton blooms under low flow conditions. Recent concerns regarding Snake River water quality have been exacerbated by low water flows associated with drought. We support strong enforcement of existing discharge requirements to help improve the Snake River. The purpose of this presentation is to summarize current effluent management practice and describe some future directions we in the aquaculture industry and Clear Springs Trout Co. specifically, are pursuing.

CURRENT ACTIVITY

During the past 10 years, Clear Springs Trout Company has made continuous improvements in hatchery design, feed delivery systems, manure removal practices and production optimization. Current practices and design features allow ready compliance with NPDES permits. Our current NPDES permit allows for a TSS of no more than a monthly mean of 5.0 mg/L and no more than 15.0 mg/L instantaneous TSS maximum. Settleable solids, a component of TSS, must not exceed 0.1 ml/L for a monthly average. Since settleable solids are a component of TSS, we suggest reliance on this parameter is superfluous and may lead to confusion. Solids

discharge limitations were established in 1974. To capture solids, we utilize quiescent zones (settling basins) at the end of each cement raceway. Fish activity forces sediment into these zones where the solids settle to the bottom. No raceways are swept or flushed. Sediment is vacuum removed or siphoned from the settling basins weekly. Vacuumed material is collected into specially constructed settling ponds and when these ponds are full, manure is removed by pumping into a tank truck. Manure is hauled away and distributed as fertilizer onto agricultural land. In 1990, approximately 15% of our labor force was devoted to removal of 5.3 million gallons (8-10 % solids) of manure at a cost over $300,000.

Clear Springs Trout Company has recently instituted a new, automated feed system which has improved feed conversion. The new system allows small volumes of feed to be distributed at numerous time intervals and permits more fish access to feed. Ambient oxygen declines are limited and improved food conversion occurs. Improved food conversion can be expected to reduce solids generation and diminish leaching of nutrients into the water from sediment. Feed conversions have improved 80 % due to, amongst other things, feed management changes. These changes have occurred while maintaining optimal body composition.

Clear Springs Trout Company also has an aggressive Fish Health Management Program. Each farm has a Hatchery Technician responsible for the diagnosis and treatment of fish diseases. Clinical and subclinical conditions are monitored and treatments or prophylactics instituted to ensure as optimal fish health as economically feasible. Optimal fish health promotes better feed conversion.

Clear Springs Trout Company has also instituted a year-long study to examine the quantity of nutrients in our hatchery inffluent and effluent, at various locations along the Snake River and various tributaries. These data will supplement other public sponsored (DEQ) studies. Additional insight may also be gained to help us better define and manipulate management practices to reduce nutrient outflow.

FUTURE ACTIVITIES

A multidimensional research effort is underway to further reduce nutrient outflows from aquaculture facilities. These studies are being pursued by various university, federal, state and private research organizations. Researches are both short and long term. Significant effort is directed toward nutritional efficiencies or improved feed conversions. One approach to this is by increasing feed fat content. This may increase feed caloric value and allow protein concentration to be reduced. Effort must focus on fat, protein, and energy ratios to prevent problems of excess fat deposition in the fish. Research is also being directed at identification of optimal phosphorus concentrations in feed. This research is being conducted at the Tunison Laboratory in New York and should allow for reductions in feed phosphorus content. To reduce phosphorus content, alternate feed ingredients will need to be examined as well as different technologies for producing the feeds.

Various aspects of hatchery design, hydraulics, manure removal and disposal, effluent recycling and feeding technologies are also areas of current scientific endeavor. Various existing technologies for feeding are being

evaluated. These include feed regimen testing with new feeding systems, enhanced manure capture structures and manure removal systems. Automated manure removal technologies would provide more cost-effective practices.

CONCLUSIONS

Clear Springs Trout Company is an environmentally responsive aquaculture company doing its part to be good stewards of our resources. We encourage other aquaculturists to also be good stewards. Resolution of the environmental impact associated with nutrients are complicated by the multiplicity of nutrient sources, only some of which are regulated, and problems of water budget and low water flows. We encourage strong enforcement of existing water quality regulations and encourage the cooperation of policy makers, enforcement agencies, and all contributors of nutrients into the Snake River in developing an effective water quality management plan.

WATER QUALITY ISSUES AND ACTIVITIES MIDDLE SNAKE RIVER, IDAHO

Michael J. McMasters
Idaho Department of Health and Welfare
Division of Environmental Quality
Twin Falls, Idaho

ABSTRACT

Water quality problems have been identified in the Middle Snake River over the last three years. The problems have resulted from a combination of cumulative effects including irrigated agriculture, concentrated animal feeding operations, fish hatchery effluents, existing hydropower facilities, and point source discharges from municipal facilities. A number of activities are underway in this stretch of the river that will be utilized to address water quality conditions and water quality management activities in the future. The development of a comprehensive management plan is anticipated in the future and will require cooperation and coordination of local, state, and federal agencies to adequately address the problem.

Middle Snake River Issues

The area of concern is an 94 mile stretch of the Snake River from Milner Dam to King Hill. This river stretch is impacted by return flows from irrigated agriculture, runoff from confined animal feeding operations, hatchery effluent, hydroelectric development and point source discharge.

* **Irrigated Agriculture** - Approximately 560,000 acres are irrigated with water from the Snake River in the Magic Valley. Farmers irrigate roughly 370,000 acres from deep wells powered by pumps wired to hydroelectric dams on the Snake River. During the irrigation season, 13 perennial streams and over 50 agricultural drains contribute irrigation tailwater to the Snake River.

* **Confined Animal Feeding Operations** - A significant number of dairies and confined animal feeding operations are located in the four county area. The USDA 1989 estimate for all cattle and calves in the area was 270,000 head.

* **Fish Hatcheries** - Idaho Fish and Game has issued 120 permits for commercial fish culture facilities on the mainstem and its tributaries in this reach. There are also four state and federal hatcheries in the area. Of these facilities, 85 have NPDES permits.

* **Existing Hydroelectric Facilities** - Five hydroelectric dams are located in the study area, including Twin Falls Dam, Shoshone Falls Dam, Upper Salmon Dam, Lower Salmon Dam and Bliss. All of these projects are owned and operated by Idaho Power Company. Upper and Lower Salmon Falls Dam and Bliss Dam are scheduled for relicensing. Additional small facilities are located on the canals and tributaries in this area.

* **Proposed Hydroelectric Facilities** - Additional hydropower projects have been proposed for this reach. They include the A.J. Wiley, Empire, Dike, Auger Falls, Boulder Rapids, Kanaka Rapids, and Star Falls projects.

* **Point Source Discharge** - In addition to hatchery effluents, other point sources include the cities of Twin Falls and Hagerman which contribute direct discharges into the river.

* **Wetlands** - The study area contains seven areas that are on the list of Idaho Priorities of Wetland Acquisition prepared by the Idaho Department of Parks and Recreation. Several of these areas are threatened because of hydropower development, fish farm development, recreation and grazing.

Middle Snake River Activities

* **Middle Snake Planning Group** - This group consists of representatives of Twin Falls, Gooding, Lincoln and Jerome Counties. The objective of this group is to develop a management plan which will provide direction to the counties and resource agencies.

* **Division of Environmental Quality Monitoring Study** - DEQ is conducting a one-year water quality monitoring study. Information is being collected at 55 monitoring sites associated with agricultural drains, fish hatcheries, tributaries and the Snake River.

* **State Agricultural Water Quality Program** - The State Ag. Program makes grants to Soil Conservation Districts to assist in the development of water quality plans and for cost-sharing with farmers who apply Best Management Practices. In the Middle Snake River area, there are currently three implementation projects funded under this program, including Cedar Draw, Vinyard Creek and East Upper Deep Creek. The Scotts Pond planning project is also underway.

* **TMDL Activities** - A TMDL has been developed for Billingsley Creek, a tributary to the Snake River where a number of fish hatcheries are located. A portion of the Snake River will be listed as a water quality-limited segment in the next 305(b) report.

* **Hatchery NPDES Permit Monitoring** - Draft NPDES permits require the hatcheries to monitor their effluent and include a reopener clause which allows permit modification. This monitoring may be contracted out to the Aquaculture Institute at the University of Idaho.

* **Other Hatchery Studies** - The Clear Springs Trout Farm is monitoring its own effluent in more detail, the influent spring water, hatchery feed and feeding systems and instream water quality. The Idaho National Engineering Laboratory has initiated a study of the fish hatcheries with the focus on hatchery waste management and the sue of solids as soil amendments.

* **State Water Plan Activities** - The Idaho Department of Water Resources is involved in a planning effort which could result in state "protected" status for all or portions of this stretch of river. The 1991 Legislature will consider a bill to extend "interim" protected status for 48 months.

* **Potential Endangered Species Listing** - The U.S. Fish and Wildlife Service is evaluating several species of snail associated with Snake River springs for possible listed as endangered species.

* **U.S.G.S. Program** - The U.S. Geological Survey has initiated the national Water-Quality Assessment Program (NAWQA), designed to describe the status and trends in water resources. In Idaho, the Upper Snake River Basin which includes the Twin Falls stretch was chosen as a study unit.

* **Idaho Dept. of Fish and Game Activities** - The Department of Fish and Game is planning on enhancing white sturgeon populations in the Snake River between Shoshone Falls and Bliss Dam.

* **Bureau of Land Management** - The Bureau of Land Management has initiated the development of the Resource Management Plan for the Shoshone District, located in the downstream portion of the study area.

* **U.S. Park Service** - In November 1989, the Hagerman Fossil Beds were added to the National Park System. The U.S. Park Service is in the process of developing a management plan for the Fossil Beds.

* **Northwest Power Planning Council** - The Council has designated the area from Twin Falls to Bliss as a protected area for white sturgeon, bald eagles, and a number of candidate endangered species.

* **Idaho Power Relicensing Studies** - As part of the relicensing process for Bliss, Upper Salmon and Lower Salmon Dam, Idaho Power is conducting environmental studies in this reach of the Snake River.

NORTHWEST FISH CULTURIST CONFERENCE

John T. Heimer
Staff Biologist
Idaho Fish and Game Department
Boise, Idaho

ABSTRACT

Often we hear about the different things we hope to do as a result of monitoring programs currently in effect or ones we want to start. I submit that before we talk about the big "M" word (M for monitoring) we need to think about what we want to accomplish as a result of the sampling. In too many instances I have seen extensive sampling done then disagreements developed as to what the results means. If a little data is confusing or can be interrupted differently then more data is even a bigger problem. All to often more monitoring is recommended to assess what the results really mean as they can be and often are in question.

I feel we should look closely at monitoring programs. Often when the study results are finally obtained it is too late as decisions have already been made. Then one has to ask if the results were worth it from the standpoint of time and money. Often this is a good way to postpone politically sensitive decisions that should have already been made. Monitoring should be designed and implemented with a specific goal and plan of action incorporated into it. As an example water quality monitoring was conducted on Idaho's South Fork Salmon River with results such that logging would either be terminated or continue depending upon the results. Everyone knew far in advance what options were available. At American Falls Reservoir on the Snake River in eastern Idaho dissolved oxygen concentrations in the outlet water is checked every 15 minutes during the summer months. If standards are not met water is spilled to increase oxygen conditions in the river downstream.

In many cases we know what the problem is, but often have to wait for results to "prove" it. In the meantime the resource suffers and decisions are made by other people without the use of the information we have generated.

DIETARY CONTROL OF PHOSPHORUS IN HATCHERY EFFLUENTS

H. George Ketola
U. S. Fish and Wildlife Service
Tunison Laboratory of Fish Nutrition
Cortland, New York

The influence of fish hatcheries on phosphorus (P) discharges was identified in a 1979 report, "Water quality and phosphorus loading analysis of Platte Lake 1970-1978", by James Grant of the Water Quality Division of the State of Michigan. The report indicated that the Platte River Anadromous State Fish Hatchery, located on a tributary, contributed 41% of the phosphorus going into the Platte Lake. The average concentration of phosphorus in the river above the hatchery was 0.012 ppm and 0.033 ppm below it. Most, if not all, of this increase in phosphorus came directly or indirectly from the fish feed. The study indicated that this coho salmon and steelhead hatchery was accelerating the eutrophication process in this transitional mesotrophic lake by discharging 3,305 pounds of phosphorus (1,500 kg) into the Platte River, which flows into Platte Lake. Furthermore, the water quality report recommended that the National Pollutant Discharge Elimination System (within the Water Quality Division of Michigan) permit the hatchery to discharge no more than 1,400 pounds of phosphorus per year. Another study estimated the total annual discharge of P to be between 1,694 and 2,240 pounds with a recommended maximum permissible discharge of 1,980 pounds. (Kanaga and Evans, 1982). The value, 1400 pounds, was adopted as the maximum permissible level of P to be discharged from the hatchery.

Harry Westers, Chief of the Fisheries Division of the Michigan Department of Natural Resources, proposed that research be done on fish feeds to reduce phosphorus in fish hatchery effluents. Westers' evaluation indicated that water treatment or management of the effluent itself would not accomplish this reduction in phosphorus, hence the need to find ways to reduce the contribution of dietary phosphorus to the hatchery effluent. Wester's production records for the hatchery indicated that the annual feed use was 110 tons of feed. If we use the annual P discharge value from Grant (1979), 3305 pounds, that equates to a discharge of approximately 15 pounds of P per 1,000 pounds of feed fed (or about 15 kg P discharged/1,000 kg feed). If we use the 2,249 value from Kananga and Kane (1992), that equates to a discharge of approximately 10 kg P discharged/1,000 kg feed fed.

Estimates of phosphorus retention by Atlantic salmon were made based on a study by Ketola (1975) using feed/gain data and an average concentration of phosphorus of 0.45% in the whole fresh carcass of salmon. The calculated retention in body tissues of phosphorus from diets ranged from 16 to 18% when experimental diets contained moderate (1.3%) to high levels (1.6%) of phosphorus. For a commercial salmon diet containing 1.7% P, the retention of phosphorus was estimated to be 21% thereby suggesting that 13 kg P was wasted (solid and dissolved) per 1000 kg of feed. Many commercial feeds have excess phosphorus and a relatively high proportion of undigestible phosphorus, such as phytin. (Unavailable phosphorus includes those forms of phosphorus that are not readily digested and absorbed by the fish. Most of the animal and inorganic sources of phosphorus are readily available. In contrast, plants often contain as much as two-thirds of their phosphorus bound in phytin that trout and salmon cannot

digest. Phytin phosphorus is therefore not available.) Therefore it appeared likely that typical retention of dietary phosphorus may be very low, perhaps 20%, which means that up to 80% of dietary phosphorus may be discharged into hatchery effluent water. This observation indicated a great potential to markedly increase retention of phosphorus, and thereby greatly reduce phosphorus discharges in hatchery effluents.

METHODS

Experiments were conducted in the laboratory and in a salmon hatchery. The composition of the experimental diets is shown in Table 1. Each diet was assigned at random and fed at equal levels to replicate lots of fish. In all experiments, growth, survival, and feed conversions were determined. Experiments 1 and 4 were conducted in the laboratory, experiments 2 and 3 at the Platte River Hatchery. In experiment 2, increases in concentrations of P in water were determined by periodic measurements of differences between P in influent and effluent waters. In experiments 1, 3 and 4, phosphorus was analyzed in feed, fish carcasses and solid wastes. The amount of soluble and suspended phosphorus discharged in water was determined by balance as follows:

P discharged in water = P consumed - (P deposited in body tissue + P in settlable solid wastes).

RESULTS AND DISCUSSION

In experiment 1, the growth of trout fed commercial control OMP diet (Oregon Moist Pellet) was significantly greater than for trout fed diet A or C (Table 2). Supplementing basal diet C (Table 1) with feed grade dicalcium phosphate or defluorinated phosphate had no effect on growth of trout. Mortality was negligible throughout the study. Measurements of the distribution of phosphorus showed that the percentage feed phosphorus that appeared in effluent was significantly reduced for diets containing defluorinated rock phosphate (DRP) relative to the commercial control diet OMP. The phosphorus content (0.45%) of whole carcass was not significantly influenced by diet (data not shown).

The levels of phosphorus in the sludge from fish fed diets containing defluorinated rock phosphate were between 30 to 35%,
significantly higher than for fish fed feed grade dicalcium phosphate, 15%.

These results suggest that the reduced discharge of phosphorus was related to the relative insolubility of defluorinated rock phosphate and reduced water leaching of phosphorus from fecal and feed wastes. Comparative absorption rates were not determined.

Diets containing defluorinated rock phosphate supported growth 84 to 90% as good as that for the OMP feed, while phosphorus discharges were reduced by approximately 40 to 49% when expressed as amount of P discharged per unit of weight gain or feed fed.

Experiment 2 was a large-scale hatchery study conducted under carefully controlled conditions. In this study, basal diet C (Table 1) was modified to contain either 3.9 or 3.5% defluorinated rock phosphate or 5.8% bone meal. Defluorinated rock phosphate supplemented at 3.9% of diet and bone meal at 5.8% diet provided 0.7% supplemental phosphorus. The OMP diet was fed as the control. The results of this study (Table 3) show the effect of diet on growth of coho salmon and P discharges in effluent water in comparison with the standard hatchery diet (OMP) fed at the same level of dry matter. Growth of salmon fed OMP was significantly better than that for the T2 diets; however, P discharges appeared 2 to 3 times higher than those fed the T2 diets. There was no significant ($P > 0.05$) effect of the supplements to the T2 diets in terms of growth and P discharges in hatchery effluents.

A laboratory study (not shown) was conducted in an effort to determine whether the addition of low levels of fish meal to the T2 diet would improve growth. The level of P supplement was adjusted to compensate for the P supplied by fish meal. The results of the laboratory study indicated that the T2 diet containing 10% fish meal (diet T2M) significantly improved growth, comparable to that for OMP.

In experiment 3, salmon were fed either the T2M diet containing 10% fish meal or the OMP diet for 45 weeks. The mean final body weight of fish fed the T2M diet was 27 g, not significantly different from that of fish fed the OMP diet, 26 g (Table 4). Furthermore, diet had little influence on 45-week mortality (21 to 23%) or feed conversion (4.6 to 4.9) in this study (data not shown).

The amounts of phosphorus discharged (in dissolved and suspended forms) in the effluent, per 1000 kg of feed were about 4 kg for the T2M diet and 8 kg for the OMP diet. When expressed as amount per 1,000 kg of fish production, these values were 5 kg for the T2M diet and 12 kg for the OMP diet. Therefore, feeding the T2M diet to coho salmon in the hatchery significantly ($P < 0.01$) reduced the amounts of phosphorus discharged by approximately 49 to 61%. These discharge values were in close agreement with values obtained in a laboratory study with rainbow trout fed similar diets.

SUMMARY

In summary, two production-scale hatchery experiments with coho salmon fingerlings, showed that two economical diets (T2 and T2M), containing moderately reduced levels of available phosphorus (0.8 to 0.9% of diet) supplied in part as defluorinated rock phosphate, markedly reduced phosphorus discharges in the effluent water. The T2 diet contained no fish meal and supported growth equal to about 85% of that for a conventional hatchery feed (OMP). Supplementing the T2 diet with 10% fish meal (T2M diet) increased growth of coho salmon similar to that of fish fed the OMP diet while discharges of phosphorus decreased by about 49 to 61%.

By the time experiment 3 was completed, a new mandate by a court was issued to further reduce the maximum permissible level of P discharged from the Platte River Hatchery. The new limit was 923 pounds annually. Obviously, further improvements in diets relative to the effluent characteristics were needed.

Previous studies were based on an estimated available P requirement above 0.6 but not more than 0.9% of diet based on an earlier study with Atlantic salmon (Ketola, 1975). Therefore, it seemed that in order to further reduce the discharge of P in hatchery effluents, it would be necessary to determine more precisely the minimum dietary requirement for phosphorus. Another study (experiment 4) was conducted in the laboratory with 9 g rainbow trout which were fed a semi-purified diet (diet D, Table 1) containing 0.4% available phosphorus. In this study, highly available phosphorus ($NaH_2PO_4.H_2O$) was added to diet D to give incremental levels of P. The control diet was the T2M diet. The results (Table 5) show that growth and feed conversion were not significantly improved by supplements of P to basal diet D. Percentage bone ash content, however, was significantly increased by supplements of P indicating that 0.4% phosphorus contained in basal diet D was deficient even though it appeared to support normal growth. The growth of trout fed the T2M diet was significantly faster than that for trout fed the basal diet with or without supplemental P. The reason for this difference is not known but it appears to be unrelated to P content. The most striking results were in the levels of P discharged in the effluents. As shown in Table 5, the P discharges in effluents significantly increased as trout were fed increasing levels of P. The discharge for trout fed the diet containing 0.5% P (basal diet + 0.1% supplemental P) was only about 33 to 39% as great as that for the trout fed the T2M diet (P content 1.15%). For the unsupplemented basal diet (0.4% P), the values were even lower; however, that diet may be detrimental to the fish because it is probably deficient based on the bone ash data (Table 5). Although this experiment was conducted with semi-purified diet that supported growth at 75% of the T2M control, it suggests that the minimum requirement of trout for P is about 0.5% of diet. When such a diet was fed at a feed/gain ratio of 1.2, discharges of P were reduced by 60% or more compared with the T2M diet, and by almost 90% compared with a conventional salmon feed containing 1.3% P or more.

In conclusion, these studies show that:

1. Feeding coho salmon a conventional salmon feed (Oregon Moist Pellet) resulted in discharges of soluble and suspended phosphorus (P) into effluent water equal to 7 to 8 g of P/kg feed or 12 to 13 g of P/kg weight gain of salmon.

2. Salmon fed diet T2M, containing 10% fish meal, 2.5% defluorinated rock phosphate and about 0.8-0.9% available phosphorus, supported growth and survival in the hatchery comparable to that for the conventional commercial salmon diet. Phosphorus discharges in effluents decreased (by 50% or more), to 5 g P/kg of production or 4 g P/kg of feed.

3. Laboratory studies with fingerling rainbow trout fed semi-purified diets suggest that the requirement for available dietary phosphorus may be 0.5%, and when trout are fed such a diet, discharges of effluent phosphorus can be reduced much further, to 1.2 g P/kg of production or 1.1 g P/kg of feed.

Table 1: Composition of experiment diets (%).

Ingredients	A	C (or T2)[1]	T2M	D
Soybean meal	24	41	31	--
Corn gluten meal	--	30	30	--
Corn, yellow	6	--	--	--
Alfalfa meal	7	--	--	11
Blood flour	18	10	10	21
Casein	20	--	--	28
Egg white, dried	--	--	--	11
Whey, dried	--	--	--	5
Fish oil	17	11	11	7
Soybean oil	--	--	--	9
Lecithin, soybean	--	--	--	1
Arginine.HCl,L	0.2	--	--	1
Phenylalanine, L	0.4	0.3	--	--
Lysine.HCl, L	--	0.4	0.4	0.3
Methionine, L	--	--	--	0.3
Vitamin Mix[2]	1	1	1	1
Mineral Mix	+[3]	+[3]	+[4]	+[4]
P Source	+	+	+[5]	--
Herring Meal	--	--	10	--
Cellulose or Wheat middlings (to 100)	+	+	+	2

[1] Diet T2 had the same composition as diet C but lacked the phenylalanine supplement and contained supplemental P (0.7% of diet) as finely ground defluorinated rock phosphate.

[2] Ketola (1983).

[3] Mixture provided the following (in mg/kg of diet): $MnSO_4.H_2O$, 315; $ZnSO_4.7H_2O$, 680; $CuSO_4.5_2O$, 60; $FeSO_4.7H_2O$, 500; KIO_3, 8.5; Na_2SeO_3, 0.3; $NaMoO_4.2H_2O$, 8.3. It also provided (in g/kg of diet): $MgCO_3$, 3.4; NaCl, 4.9; KCl, 4.9.

[4] Mineral mixture provided the following (in mg/kg of diet): Mn, 100; Zn, 100; Cu, 10; Fe (ferrous), 100; I, 5; Se (selenite), 0.1; and Mg, 990.

[5] Diet T2M was supplemented with enough finely ground defluorinated rock phosphate (DRP) to provide a level of dietary P equal to the difference between 0.66% of diet and the amount supplied by fish meal (analyzed for P). (In other words, P from fish meal + P from DRP = 0.66% of diet.)

Table 2: Effect of diet on rainbow trout and effluent discharges of phosphorus in the laboratory (Experiment 1).

Diet[1]	Wt. Gain	Distribution of feed P: Sludge	Carc.	Effl.	g effluent P /kg: Gain	Feed
(12 wks)	(g)	(%)	(%)	(%)	(g/kg)	(g/kg)
OMP[2]	8.8a[6]	24a	20a	57a	13.3	7.4
A with DRP[3,4]	7.9b	30ab	30b	40b	6.3	4.2
C with DiCal[4,5]	7.5b	15c	25c	60a	11.3	7.4
C with DRP[4,5]	7.4b	35b	27c	38b	6.8	4.4

[1] Each diet was fed to triplicate lots of 50 fish each (initial weight, 2 g/fish).
[2] Oregon Moist Pellet; P content 1.3%.
[3] Diet A with DRP was analyzed to contain 1.05% total P.
[4] Diets A and C were fed with supplements of either DRP (defluorinated rock phosphate) or DiCal (feed grade $CaHPO_4.2H_2O$) to provide 0.7% supplemental P.
[5] Diet C contained by calculation approximately 0.5% P without P supplement; supplemented diets contained (by analysis) between 1.15 and 1.25% total P.
[6] Values not followed by the same letter are significantly different ($P < 0.05$).

Table 3: Hatchery study with coho salmon: Effect of diet on growth and P discharges in effluent water (Experiment 2).

Diet (8 wk)	Wt. Gain (g/fish)	Feed/Gain (g/g)	Discharge P (ug P increase/L)
T2[1]	6.2a	1.02a	10a
T2, 90%[2]	5.8a	1.11a	15a
T2, bone[3]	5.2a	1.21a	15a
OMP[4]	7.7b	1.14a	33b

[1] T2 diet contained 3.9% defluorinated rock phosphate (DRP) and no supplemental phenylalanine. DRP provided 0.7% supplemental P in diet. Total dietary P was 1.15%.
[2] T2 diet with 10% less DRP.
[3] Bone meal was supplemented at 5.8% of diet to provide 0.7% supplemental P.
[4] OMP, Oregon Moist Pellet (containing 1.3% P), commercial.
[5] Values not followed by the same letter are significantly different ($P < 0.05$). Each diet was fed to three replicates of 25,000 fish each (initial weight, 5 g/fish).

Table 4: Effect of diet fed to coho salmon on growth and on P discharges from hatchery (Experiment 3).

Diet [1]	Avg. Gain	Distribution[2] of feed P			g effluent P/kg of:	
		Sludge	Carc.[2]	Effl.	Gain	Feed
(45 wk)	(g)	(%)	(%)	(%)	(g/kg)	(g/kg)
T2M[3]	27	19	40**	38**	5**	4**[5]
OMP[4]	26	12	26	60	12	8

[1] Each diet was fed to 4 replicates of 199,000 fish each (initial weight, 4 g/fish).

[2] Distribution of feed phosphorus refers to the percentage partioning of P from feed into solid waste P (in sludge), carcass P and P discharged as suspended and dissolved in effluent water during the first 17 weeks of the study -- prior to winter when cold water resulted in low growth rates and greater wasting of feed. By 17 weeks, fish gained 50 to 60% of their total weight in the study. Values for carcass represent only those fish that survived.

[3] T2M diet; total P content 1.15% (see Table 1).

[4] OMP, Oregon Moist Pellet, P content 1.3%.

[5] Values followed by asterisks (**) are significantly ($P < 0.01$) different from those of the control diet (OMP).

Table 5: Effect of graded levels of P in diet of rainbow trout fingerlings (Experiment 4).

Diet[1]	Wt. Gain[2]	Feed/ Gain	Bone Ash	g effluent P/kg : Gain	Feed
(10 wks)	(g)	(g/g)	(%)	(g/kg)	(g/kg)
Basal D[3]	19a[6]	1.2a	45a	0.9a	0.8a
+0.1% P[4]	18a	1.2a	50b	1.3a	1.1a
+0.2% P	19a	1.2a	52bc	2.0b	1.8b
+0.4% P	19a	1.1a	56c	4.5c	4.0c
+0.8% P	19a	1.2a	54bc	9.4d	8.0d
T2M[5]	25b	1.0c	52bc	3.3e	3.3e

[1] Each diet was fed to 3 replicates of 30 fish each.
[2] Initial body weight was 9 grams.
[3] Basal diet D contained approximately 0.4% available P (non-phytin). For composition, see Table 1.
[4] P supplemented as $NaH_2PO_4.H_2O$ at the expense of $NaHCO_3$ and cellulose to maintain a constant level of Na.
[5] T2M diet; total P content 1.15% (see Table 1).
[6] Values not followed by a common letter are significantly different ($P < 0.05$).

REFERENCES

Grant, J. 1979. Water quality and phosphorus loading analysis of Platte Lake 1970-1978. Water Quality Division, Michigan Department of Natural Resources, Publication 4833-9792, Lansing.

Kananga, D., and E. D. Evans. 1982. The effect of the Platte River Anadromous Fish Hatchery on the fish, benthic macroinvertebrates and nutrients in the Platte Lake. Water Quality Division, Michigan Department of Natural Resources, Lansing. April.

Ketola, H. G. 1975. Requirement of Atlantic salmon for dietary phosphorus. Transactions of the American Fisheries Society. 104:548-551.

Ketola, H. G. 1985. Mineral nutrition: Effects of phosphorus in trout and salmon feeds on water pollution. Pages 465-473 in C. B. Cowey, A. M. Mackie and J. G. Bell, editors, Nutrition and Feeding in Fish, Academic Press, London.

Ketola, H. G. 1983. Requirement for dietary lysine and arginine by fry of rainbow trout. Journal of Animal Science. 56:101-107.

HATCHERIES

THREATEN WINTER CHINOOK PROGRAM AT COLEMAN NATIONAL FISH HATCHERY

J. Scott Foott
U. S. Fish and Wildlife Service
California-Nevada Fish Health Center
Anderson, California

Winter-run chinook salmon are one of four distinct runs (fall, late fall, winter, and spring) of chinook found in the upper Sacramento river (Hallock & Fisher 1985). On November 6, 1990, the National Marine Fisheries Service made a final ruling on this stock and listed it as a threaten species.

Historically, winter-run chinook spawned from April through July in the cold, spring-fed tributaries of the McCloud river (Fig. 1). In the 1942, Shasta and Keswick dams blocked this spawning migration. However, cold water releases from the reservoirs expanded spawning habitat for this stock. The increased population supported a major upper Sacramento river fishery in the 1950's and early 1960's. In 1967, Red Bluff Diversion Dam went into operation and impeded up-river migration of the winter chinook. Many adults were forced to spawn in the high water temperature region below the dam. The population declined to 2,000 in the early 1980's and in 1990 only 441 were counted past Red Bluff Diversion Dam (J. Smith, USFWS N. Central Valley FRO, pers. comm., 1990). Other factors which may have effected the winter chinook include loss of spawning gravel, pollution, predation, and out-migrate losses to irrigation diversions (Hallock & Fisher, 1985).

In 1988, a multi-agency recovery plan was developed which included a hatchery propagation program at Coleman National Fish Hatchery. The objective of this hatchery propagation program is to supplement the wild run while minimizing hatchery effects on the stock.

Limited attempts in the 1970's and 80's to spawn winter chinook at Coleman NFH were largely unsuccessful due to pre-spawn mortality and the effect of high water temperature on egg viability. In 1989, adult winter chinook captured at both Keswick and Red Bluff Diversion Dam were held in a 12,400 cf pond supplied with chilled water (45 - 58 F, January - June). This indoor pond was part of the recently completed spawning facility at the hatchery. Fish were identified by a passive integrated transponder (P.I.T.) tag and their condition recorded after each handling period. Malachite green was added to the MS-222 anesthetic (Finquel, Argent Laboratories, Redmond, WA) bath to help combat fungal infection. After furunculosis was detected, fish were given intraperitoneal oxytetracycline injections. Only one female of the 42 fish captured was successfully spawned that year. The average duration in captivity was 38 days with a range of 10 - 106 days. Mortality was attributed to bacterial and fungal infections.

In 1990, the California Department of Fish & Game imposed a 20 fish trapping limit on the propagation program. Four males and ten females were captured between January and May. A number of steps were taken to improve spawning success in addition to P.I.T. tagging and the use of Pro-Poly Aqua "artificial slime" (AquaVet, Hayward CA), malachite green dip treatments, and MS-

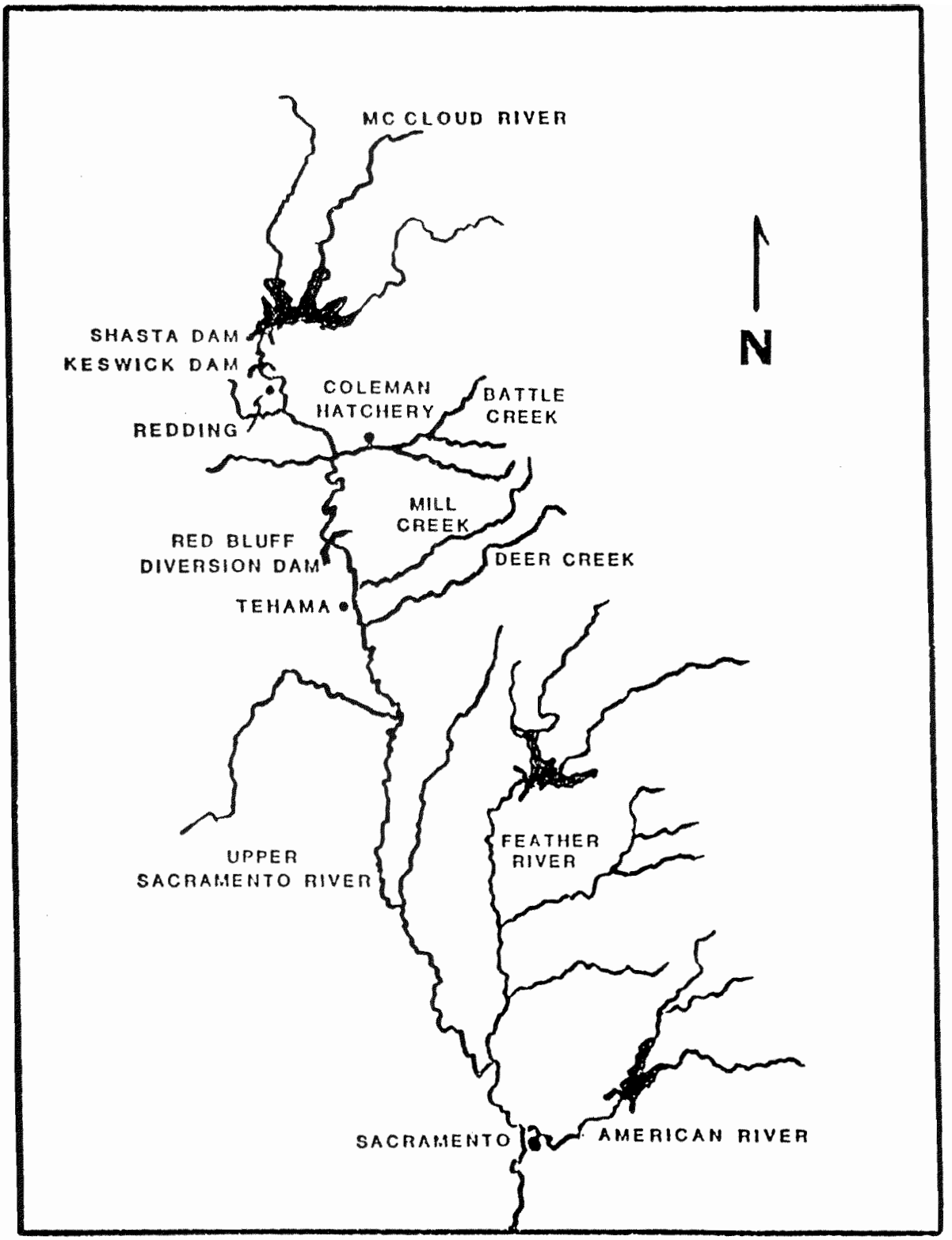


Figure 1. The Sacramento River and its tributaries.

222 anesthetic when handling adults. Disease control was attempted by vaccinating the adults soon after capture with a 0.5 mL/Kg fish intraperitoneal injection of bivalent (1:1 mix of formalin-killed Aeromonas salmonicida and Yersinia ruckeri) bacterin and oxolinic acid (2.4 mg/Kg fish). This technique has been effective with Atlantic salmon broodstock (Fletcher & Bullock 1987). Formalin treatment of the holding pond was done 3X/wk to control external fungus. The large volume of the holding pond and inability to completely restrict pond outflow made the formalin treatments ineffective and expensive.

Beginning in March, the photoperiod within the indoor holding area was advanced approximately 35 min/d to achieve a summer solace day length by late May. Two females were given two intraperitoneal injections 4 days apart of 5µg/Kg luteinizing hormone-releasing hormone analog (LH-RHa) des-Gly^{10} [D-Ala] LH-RH-ethylamide (Sigma Chemical Co.) in sterile saline. These last remaining females were in poor condition and showed no signs of imminent ovulation at the time of the first LH-RHa injection. Four days after the last LH-Rha injection the ripe females were spawned. As no male was alive
at this time, 1 mL semen pellets frozen on dry ice and held in liquid nitrogen twelve days earlier was used to fertilize the eggs. No fertilization occurred from this mating. Of the 14 fish captured, only one successful mating occurred with the average duration in captivity being 56 days (range 10 - 124 days). Survival of winter-run chinook eggs and juveniles in the hatchery has been high.

Besides disease, there are a number of factors which affect
spawning success of captive winter chinook. The natural spawning period of April - July (Slater 1963) decreases the likelihood that the few adults captured will mature at the same time and forces the hatchery to hold fish for up to four months. The sex ratio of this limited broodstock is often skewed. In the last two seasons, there were no live males available when the last females ovulated. Because the winter run can overlap the late fall and spring run chinook, stock identification at the time of capture can be difficult.

The high pre-spawn mortality in 1989 and 1990 due to fungal infections and the poor success of formalin treatment in the 12,400 cf holding pond, prompted several adult holding trials during the fall of 1990. The objective of these trials was to maintain adult chinook in containers which lend themselves to effective formalin treatment and fish handling. Adult fall chinook salmon were captured at Red Bluff Diversion Dam, transported to Coleman NFH, injected with oxolinic acid and bacterin, placed in 10 in. PVC pipe cages, and held in 96 cf tanks. There were three 4 fish groups. The first group was anesthetized in MS-222 and given a malachite green (5ppm) dip treatment 3X/week. The second group received a 15 min, 167 ppm formalin flush treatment 3X/week and the third group acted as a no treatment control. The average (Std. dev.) survival duration of the control, malachite green dip, and formalin treatment groups were 17 (5) days, 24 (11) days, and 32 (3) days respectively. The poor survival in all groups was due to abrasions which developed on the fish within 6 days of being placed in the PVC tubes. Another trial involved holding adults in 6 ft. circular tanks and 96 cf rectangular tanks in which black plastic was floated on the water surface and the tanks covered with lids. Jumping behavior, especially at dawn and dusk, resulted in severe abrasions on the fish.

Cryopreservation experiments with fall-run chinook utilizing a different methodology (Horton & Ott 1976, Scheerer & Thorgaard 1989) than that used in 1990 have shown a 40 % success rate. At the time of writing, an experiment to

monitor levels of plasma testosterone as an indicator of female maturation (Fitzpatrick et al. 1986) is in progress. Dr. Carl Schreck, of the Oregon State University Cooperative Fishery Research Unit, is performing the laboratory assays for this experiment. It is hoped that knowledge of the fish's testosterone level could be used to estimate when to check the female for ripeness or induce ovulation with LH-Rha injections.

In 1991, we hope to hold the adults in a 20 ft. circular tank surrounded by a tall, plastic curtain. The tank will be supplied with chilled, ozone-treated water and photoperiod will again be accelerated. In addition to the 1990 procedures, fish will be given oxolinc acid injections on a bi-weekly basis. Our basic approach will be to aggressively obtain gametes rather than wait for natural spawning. If necessary this will be accomplished by freezing semen or inducing ovulation with LH-RHa. The critical state of the winter-run chinook salmon in the Sacramento river require that natural resource agencies put forth our best efforts for preservation of this unique fish.

The following people are acknowledged for their efforts in the Winter-Run Chinook Propagation Program: Gene Forbes (Manager Coleman NFH), Roger Shudes (Asst. Mgr. Coleman NFH), Jim Smith and Keith Marine (N. Central Valley FRO), and the Coleman NFH staff.

REFERENCES

Fletcher, J. W. and G.L. Bullock. 1987. Utilization of oxolinic acid for control of Aeromonas salmonicida in Connecticut and Merrimack River Atlantic salmon broodstock. 1987 Joint Meeting, 11th Annual Fish Health Section/AFS, 12th Annual Eastern Fish Health Workshop, and 18th Annual Midwest Fish Health Workshop, Baton Rouge, Louisiana.

Fitzpatrick, M.S., G. Van Der Kraak, and C.B. Schreck. 1986. Profiles of plasma sex steriods and gonadotropin in coho salmon, Oncorhynchus kisutch, during final maturation. Gen. Comp. Endocrinol. 62:437-451.

Hallock, R.J. and F.W. Fisher. 1985. Status of the winter-run chinook salmon, Oncorhynchus tshawytscha, in the Sacramento river. Calif. Dept. of Fish and Game, Anad. Fish. Br. Office Report. 28 pp.

Horton, H.F. and A.G. Ott. 1976. Cryopreservation of fish spermatozoa and ova. J. Fish. Res. Board Can. 33:995-1000.

Scheerer, P.D. and G.H. Thorgaard. 1989. Improved fertilization by cryopreserved rainbow trout semen treated with theophylline. Prog. Fish-Cult. 51:179-182.

Slater, D. W. 1963. Winter-run chinook salmon in the Sacramento river, California with notes on water temperature requirements at spawning. Bureau of Sport Fisheries and Wildlife, Special Scientific Report - Fisheries No. 461.

TOUR OF CLACKAMAS FISH HATCHERY

George F. Nandor
Oregon Department of Fish and Wildlife

Presented a slide show tour of Clackamas Fish Hatchery.

HATCHERY ACCOUNTABILITY - USING VIDEO TO WELCOME HATCHERY VISITORS

Ralph S. Boomer
U.S. Fish and Wildlife Service

Presented a video of "Hatchery Accountability - Using Video to Welcome Hatchery Visitors."

"PROCESS CONTROL IN THE MODERN HATCHERY: SENSORS, TRANSMISSION AND DATA MANAGEMENT"

By:

Brian G. D'Aoust of Common Sensing, Inc.

and

Jim Conant of The Controls Group, Inc.

PART I: Sensor Considerations - Brian G. D'Aoust

INTRODUCTION

As aquaculture intensifies production, an increasing degree of automation is necessary. A number of factors can challenge the efficiency of aquaculture automation systems including design, installation, maintenance and reliability.

In this paper we wish to provide a logical framework - from the sensor end to the computer end.

We will consider:

1) the type of information needed for hatchery control,
2) the relevant sensors,
3) the transmission mode of the sensor signal to the control device,
4) sensor placement, and
5) methods of hatchery control and data management.

We hope to provide a better understanding of sensors, automation, and data management considerations for the modern hatchery.

SENSORS - THE PRIMARY SOURCE OF INFORMATION

These thoughts are in part an extension of some ideas presented by me in this workshop two years ago in which a limited number of sensors - those dealing with dissolved gases - were considered. It was soon clear that there would be benefit in presenting a more complete outline of the whole process, i.e.

1. How one chooses a sensor.
2. What mode of electrical transmission is required.
3. What one does with the signal when it arrives.

There are a number of different sensors to be found in a hatchery environment. Each may have different:

1. output signals,
2. connectors,
3. maximum lead distance,
4. preventive maintenance/service schedule.

A. Typical Water Quality/Physical Plant Sensors

Figure 1 lists common types of sensors in the hatchery environment. Note the column after the name which indicates analog voltage, analog current or digital (on/off) as a type of signal to be handled. Notice several types come with all types of output.

	TYPE of SIGNAL OUTPUT AVAILABLE			
SENSOR TYPE	ANALOG VOLTAGE	ANALOG CURRENT	DIGITAL (ON/OFF)	METER or EXTRA CIRCUIT
Temperature	X	X	Alarm Setpoint	N/A
Pressure	X	X	Alarm Setpoint	N/A
Oxygen	X	X	Alarm Setpoint	X
pH	X	X	X	N/A
TDGP (Dissolved Gas)	X	X	X	N/A
Flow	X	X	X	N/A
Level	X	X	X	N/A

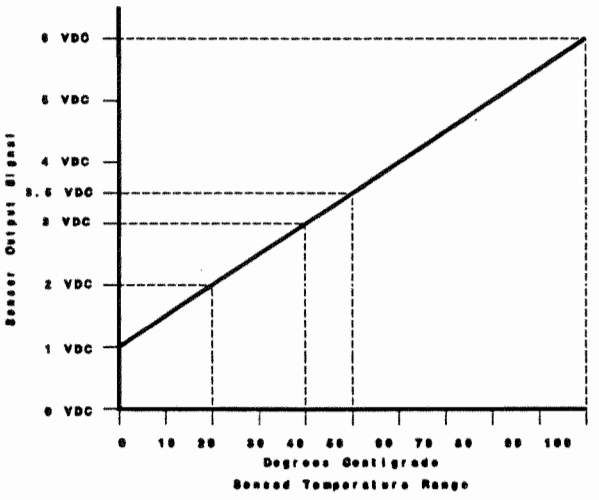
Figure 1. Analog is a range of sensed values. Digital is an On/Off signal only.

B. Signal Transmission Options:

The different methods of sending the sensor's signal shown in Figure 1 depend on whether an analog or digital value is being sent. Digital signals are either an "On" or "Off" electrical state. Analog signals express a spectrum of values (ie. 0-100%) and can be transmitted as either:

1. Current signal (ie. 4-20 milliamperes)
2. Voltage signal (1-6 volts dc).

In both cases the sensor signal "outputed" is proportional to the parameter's range of values being sensed. For example if 1.0 volt dc (VDC) sensor output indicates 0 Ĉ and 6.0 VDC indicates 100 Ĉ, the temperature range sensed is 0-100 Ĉ which is output over a corresponding voltage range of 1-6 VDC, or 5 VDC total signal range (ie. 6 VDC - 1 VDC = 5 VDC). Given this, each 1 VDC sensor output increment equals 20 Ĉ because a 100 Ĉ sensed temperature range is being "outputed" a 5 VDC output range, (or 100 Ĉ divided by 5 VDC = 20 Ĉ per 1 VDC). Therefore, 2.0 VDC would indicate 20 Ĉ, 3.0 VDC = 40 Ĉ, 3.5 VDC would indicate 50 Ĉ,, and so on...



Distance the Signal Must be Transmitted

An advantage in using analog *current* transmission is that it minimizes signal losses from longer distance signal transmission (greater than 50 feet). Analog *voltage* signals will be lost over longer distances due to electrical resistance of the wire used for signal transmission.

Another disadvantage of analog voltage signals is their susceptibility to electrical "noise" interference from electric motors and random electric signals. These interferences can give erratic and false sensor readings. This problem is compounded when the signal must be transmitted over longer distances. Analog current signals are relatively "immune" to these "noise" interferences.

D. Sensor Placement

Considerations here have to do mainly with relevance of the sensor position to reflect a true representation of the parameter sensed, and accessibility for maintenance and calibration.

Each sensor can have its own unique maintenance and/or calibration requirements. When siting two or more sensors in close proximity, a sensor's maintenance or calibration may affect the other nearby sensor(s).

E. Sensor Output Signal Determines . . .

A question that arises from Figure 1., is whether the sensor must be equipped with it's own special meter or display? A dedicated meter or display can be required of sensors that do not have an industrial standard output (ie. analog current or voltage) that can be read by the hatchery's monitoring/control system. For example many oxygen sensors can not be "plugged in to" standard monitoring/control devices because they have a non-standard signal output such as millivolts or ohms that require special translation circuitry.

On the other hand, many analog current transmitters available provide liquid crystal displays (LCD) for reading at the sensor location. This arrangement is often very convenient for reading values and calibration at the sensor's location. This same analog current transmitter can also be used to send a standard signal to a hatchery monitoring/control computer.

PART II: Hatchery Control and Data Management - Jim Conant

INTRODUCTION

I would like to discuss how data reported by various sensors control the conditions of a hatchery. I'll also illustrate how that same sensor data can be stored, reported, and made meaningful to the needs of a hatchery.

In the interest of de-mystifying this subject matter, certain terms and concepts require clarification, such as;

Control.....	the electronic manipulation of equipment to maintain desired site conditions.
Monitor.....	to report, but not control, a sensed condition
Control Setpoint.....	the desired operating level of those conditions (temp., DO, flow, etc.)
Control Bandwidth.....	the acceptable operating range above or below Control Setpoint
Alarm Setpoint.....	level at which a controlled or monitored condition is beyond its desired operating range (ie. control bandwidth)
Real-time.....	describes events as they occur in time with no delay (ie. current conditions)

In relating how these terms are applied to hatcheries, one must consider what conditions need to be maintained and what equipment maintains those conditions. For instance, we all know that dissolved oxygen, water temperature and water level are crucial operating parameters for a hatchery. These conditions are maintained by equipment such as aerators/oxygen generators, heat exchangers, and pumps and valves. The function of a control system is to automate appropriate equipment for the maintenance of optimal operating conditions.

To accomplish this task, the control system must "know" the controlled real-time conditions, the desired setpoint for each, and how to manipulate the respective equipment to achieve setpoint conditions.

We hope to illustrate the advantages microprocessors offer in:

1) achieving hatchery control objectives,
2) providing extensive data reporting and management capabilities,
3) improving hatchery organization, operation and output.

The last ten years have seen microprocessor based devices supplant electro-mechanical mechanisms as the predominant method of process control. There are many advantages microprocessor controls have that are the driving force behind this change.

These advantages include, but are not limited to:

* Greater control precision
* Ease of use through plain English menu-selected data entry screens
* Extensive reporting and management features
* Efficient hatchery performance that is a direct result of these advantages

HATCHERY SITE CONTROL

Recently, a simple, reliable, and cost-effective "tool set" that meets the broad range of process control needs, particularly for the hatchery, resulted from the combination of:

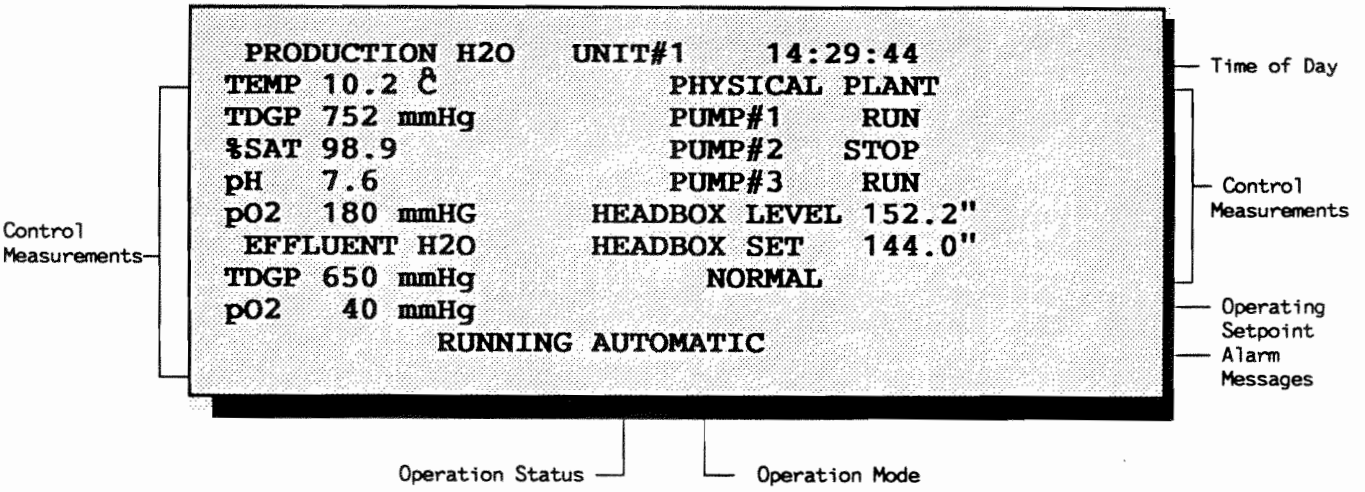
1. a specifically designed microprocessor hardware architecture for electronically sensing and manipulating the conditions of a controlled environment with,

2. a specifically designed software language for processing and manipulating the *real-time* conditions of a controlled environment,

This combination delivers extensive operating information and safety parameters for hatchery control that were never possible with electro-mechanical control devices.

A. A Microprocessor Must Control *Real-Time* Events.

Consider for a moment what *real-time* information is pertinent to the efficient operation and control of a hatchery site and should be readily displayed for viewing.



As displayed on the "Main Status Screen" of a microprocessor controller, (or microcontroller) at a minimum, you must know what are the physical plant conditions with respect to pump operation and headbox level. Additionally, you would want to monitor quality parameters for both production and effluent water.

Hatchery system electrical energy consumption data can be captured and displayed by a microprocessor.

```
ENERGY STATUS  17:01:55
MOTOR CURRENT  157.0 AMPS
PRESENT kW     1436 kW
DEMAND kW      1546 kW
TODAY'S kWH    7672 kWH
YESTERDAY'S    11700 kWH
RUNNING HOURS  12.5 HOURS
TOTAL HOURS    3190.6 HOURS
```

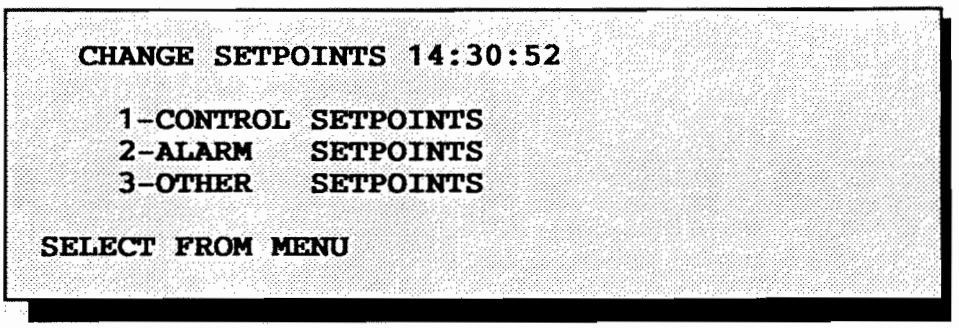
This energy usage data can aid equipment management and maintenance efforts. Information displayed includes the present site "Motor Current", the "Present" or immediate electrical consumption, a calculated average electrical "Demand "kWH" over the last fifteen minutes, plus the "Today's kWH" which is the total electrical energy consumed since 12 o'clock midnight and "Yesterdays" which is the electrical energy consumption for the previous day. Pump run-time information can also be tracked and reported for both the amount of pump operation time since it was last started ("RUNNING HOURS") and the pump run-time total.

B. Plain English Menu Selection

For ease of use, microcontrollers provide for the entry of operation and alarm setpoints via plain-English menus which guide the operator through the setpoint entry process. This circumvents any requirement for previous computer experience.

It is relatively simple for a user to program setpoints and other control parameters. This is done through a descriptive menu selection of the data you wish to enter.

Access to the control system can be regulated with a Password routine that assigns passwords for authorized users. Each operator's password defines the level, or amount, of access that a user may have, thereby restricting sensitive control areas. This prevents unwanted access and tampering.



C. Raceway Energetics

A hatchery production management feature delivered by the microcontroller is Raceway Energetics. By monitoring and controlling water temperature and oxygen content in conjunction with feed energy values, feeding rate, fish species and size, maximum fish production can be achieved.

Raceway Energetics is a biological energy consumption model that predicts fish growth based upon the parameters mentioned above. Each raceway or fish group monitored can have its own "feeding curve" based upon each group's conditions of water temperature and available oxygen. For example, if one fish group's water temperature changes, a corresponding change in the energy calculation is automatically made. So given a constant energy value for the feed, the feed rate would be altered accordingly.

The Raceway Energetics model constantly monitors water temperature, oxygen content, and flow to determine if there is enough available oxygen for the fish to consume the calculated amount of feed. If there is not enough available oxygen, the feeding rate is altered to the optimal rate consumable. Raceway Energetics thus automates maximum fish production.

Each fish group's predicted growth is tracked and reported during the entire production period. Feeding rates are adjusted according to the model's predicted fish size. Predicted growth can be compared to actual samplings, and adjustments to the Raceway Energetics model can be made at any time.

The energy value of the feed used is entered into the Raceway Energetic model by the hatchery manager. The manager can correct the energy value used in Raceway Energetics calculations as feeds and their energy value change. A manager may override the automated feeding program and manually enter the rate and schedule he desires.

Fish production automation using the Raceway Energetics model conserves resources and reduces excess feeding and the consequent wastes.

HATCHERY DATA MANAGEMENT - LOGGING AND REPORTING

The microcontroller can provide extensive hatchery reporting and management features. Large memory storage accommodates the recording and retrieval of historical operating information. This is valuable in fine-tuning production practices and costs.

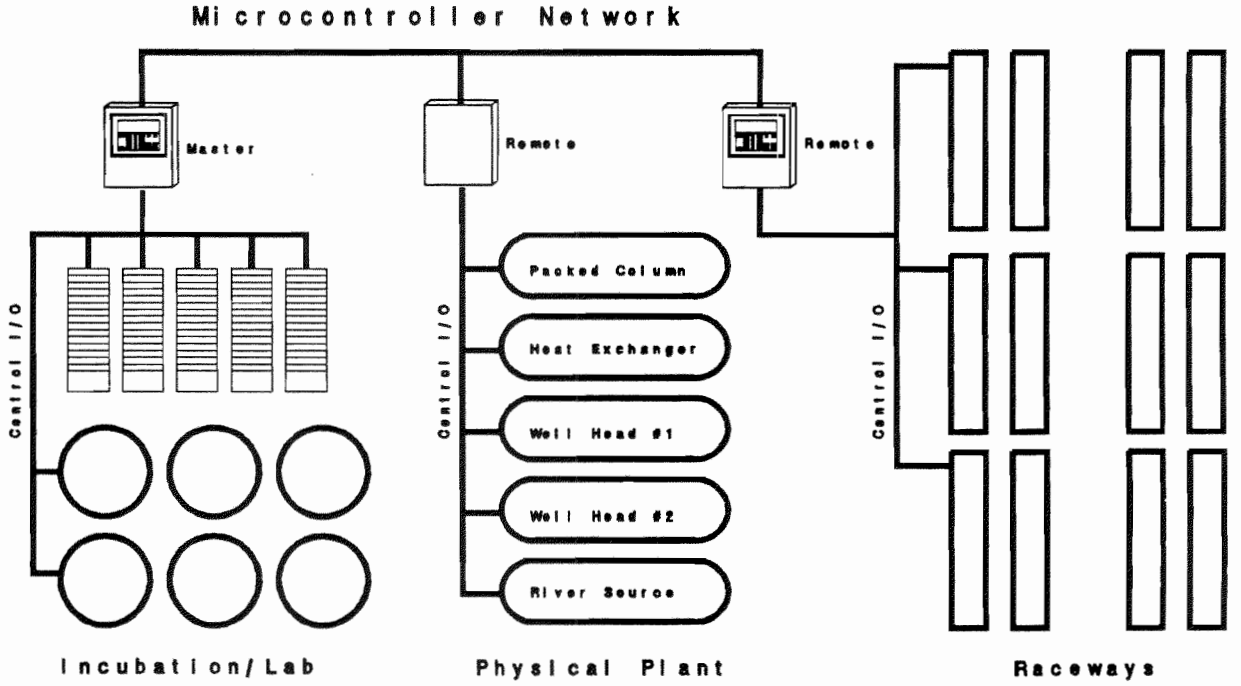
A microcontroller can provide a Trend Log showing all sensor measurements and equipment activity in user-defined time intervals. Each Trend Log displays any Alarm event and its source, plus the time and date of the log record. A Search feature allows a user to find data from a specific Trend Log record. This feature is valuable in reviewing and analyzing equipment and hatchery performance.

A separate log that records Alarm events aids in the detection and analysis of problem conditions in the hatchery. Information captured and recorded upon an Alarm includes the source with the time and date of the event.

D. Microcontroller Networks

The modular architecture of the advanced microcontroller permits customized "building block" construction for more extensive system control.

Many hatchery sites exist in unique production and equipment configurations. By "networking" several microcontrollers with various combinations of equipment control, and then linking them together through a data exchanging pathway, an intelligently integrated system control is achieved.



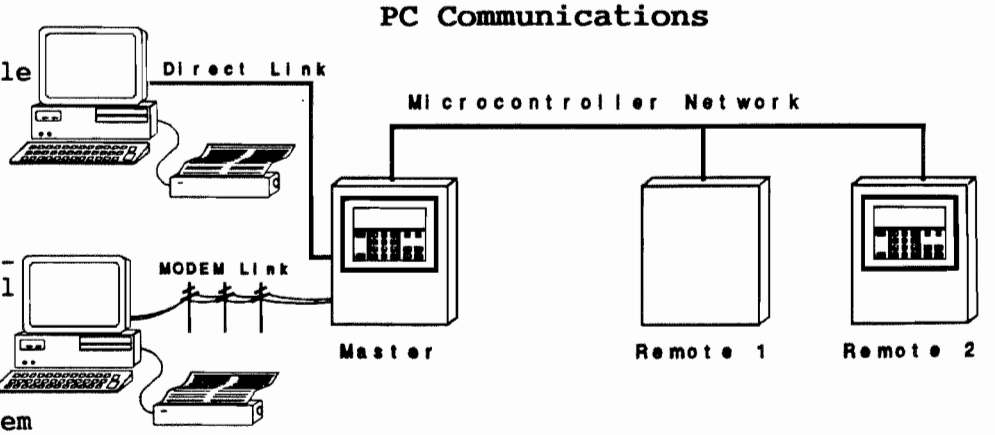
A typical network of integrated microcontrollers might consist of a "Master" microcontroller that has a keypad and a display, and one or more "Remote" microcontrollers which may or may not have their own keypad and display, each with its own equipment "Zone of Control".

Control of various combinations of equipment can be accomplished by a single microcontroller. Each "Zone of Control" is independently controlled by its respective microcontroller.

What we achieve with this networking of microprocessor controllers is the "distributed control" of an environment, vs. "centralized control". This "Distributed Control" means greater system control flexibility. Future facility expansion and equipment control needs can be accommodated with the addition of "Remote" units to an existing network. Conversely, centralized systems require a thorough re-engineering and the associated costs to accomplish the same task.

E. On-Site and Remote Hatchery Access with Real-Time Color Graphics

With the use of a Personal Computer a manager can observe and modify a hatchery's operating conditions and retrieve information. This can be done by an on-site Direct Link between the Master microcontroller and a PC that is within four-thousand feet of the hatchery Master. Remote PC access to the hatchery control system can also be established from anywhere in the world that has modern phone service via a MODEM Link. The Personal Computer serves as a user interface to the control equipment only. The PC allows changes in setpoints but the microcontrollers are what house the control logic and actually sense and control the hatchery conditions.



The MODEM Link is particularly valuable in monitoring remote aquaculture sites. Both Direct and MODEM Link communication can be done on the same site. In fact, someone can be communicating with the control system over a phone line while, simultaneously, someone else can be communicating with the system via an on-site Direct Link. This is valuable in helping a manager troubleshoot his system.

The MODEM may also be used to automatically call out an alarm or failure to a specific phone # or beeper. Pre-recorded messages detailing the nature of the alarm can be incorporated into this feature.

With either the MODEM or the Direct Link, comprehensive system information can be observed and retrieved. This information can be used to generate management reports and graphic analyses of hatchery operation.

A Color Graphic Screen on the following page representing the hatchery's equipment schematic and system flow shows the operator current *Real-Time* operating conditions.

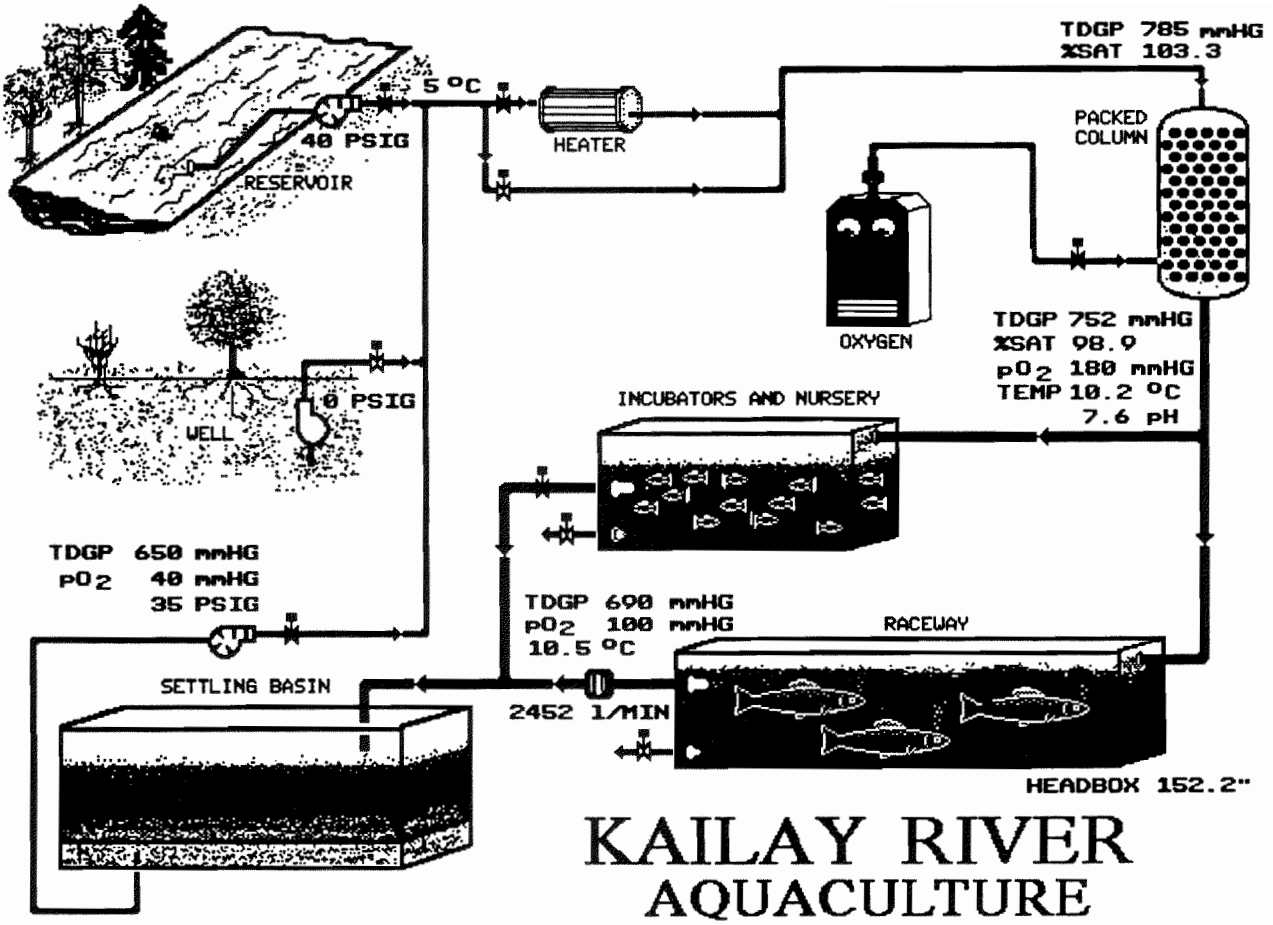
By color graphically displaying animated movement of machinery, and color coding the on/off status (ie. white/red) of system components, a manager gets immediate and intuitive information of the site's operating status. Numerical readouts of the system's sensor points on the color graphic screen augment this color graphic information to provide a complete "picture" of the hatchery's current conditions.

All of the system information that is monitored or controlled can also be recorded for up to a full calendar year. A manager can retrieve this recorded information into a Personal Computer for user-designed printing of system reports and graphical analysis of the data.

On-site locations can also opt to have automatic generation of operating data reports on a daily basis at user-specified times. This is useful for documenting hatchery operations.

On-site locations may also have the automatically generated printout report of each Alarm event.

All the information captured by the microprocessor control system can be sent, or "exported", in a form that can be used by other software programs that have extensive number handling and graphic capabilities. These programs include popular PC applications like Lotus 1-2-3, Microsoft Excel, dBase, and others.



CONCLUSION

The benefits that accrue from implementing advanced microprocessor hardware and software that target aquaculture's process control needs are apparent.

- Enhanced data management
- Easy reporting capabilities
- Complete safety and alarming features
- Convenient remote accessibility via PC
- Improved hatchery operation and profitability

In summary, the application of modern electronic sensing and automation technology to aquaculture facilities can play a vital role in satisfying the ever present demands on production efficiencies, output, and cost control.

FISH HEALTH

VACCINATION OF JUVENILE COHO SALMON AGAINST THE BACTERIAL COLD-WATER DISEASE AGENT, FLEXIBACTER PSYCHROPHILUS

R.A. Holt[1] and J.L. Fryer[2]
[1]Oregon Department of Fish and Wildlife
Department of Microbiology
[2]Oregon State University
Corvallis, Oregon

INTRODUCTION

Bacterial cold-water disease (BCWD) is a serious septicemic infection of hatchery-reared salmonids especially young coho salmon (Oncorhynchus kisutch) in the Pacific Northwest. The causative bacterium was first isolated by Borg (1960) from coho salmon with characteristic skin and muscle lesions. In recent taxonomic studies, Bernardet and Grimont (1989) further characterized the bacterium and proposed the name be changed from "Cytophaga psychrophila" to Flexibacter psychrophilus. This is the most frequently identified bacterial fish pathogen from salmonids in Oregon fish hatcheries. The reason for this is the pathogen is isolated from fish populations involving a number of different disease signs including: (1) the classic BCWD skin and muscle peduncle lesions or fin erosion which occurs in most species of salmonids, (2) spinal deformities usually found in populations which had previously suffered a BCWD epizootic, (3) mixed infections of F. psychrophilus and EIBS virus in yearling chinook (O. tshawytscha) and coho salmon; these fish are usually anemic, may display exophthalmia, dark pigmentation of the skin, pale livers and (4) more rarely, eroded gill tissue lesions in rainbow trout (O. mykiss). We also have isolated the bacterium from several stocks of spawning adult coho and chinook salmon.

Because BCWD is a widespread problem in hatchery alevin, juvenile and yearling coho and because satisfactory control of this disease is not always achieved with antibiotic medicated food, we decided to test vaccination for BCWD prevention. The methods to be tested including immunization by injection and immersion have proven successful for other fish pathogens such as Vibrio anguillarum and Yersina ruckeri. Also, the affect of fish size at vaccination was tested.

MATERIALS AND METHODS

Bacterin Preparation

The F. psychrophilus isolate SH3-81 chosen for the immunization studies was obtained from the kidney of a juvenile coho salmon with classic BCWD signs at Sandy Hatchery, Sandy, Oregon in 1981. A severe epizootic of BCWD was in progress at the time. The bacterium was grown in tryptone yeast extract broth (Becker and Fujihara, 1978) at 17°C for 72 hr., harvested by centrifugation, resuspended in 0.2% formalized saline and stored at 4°C.

Immunization and Challenge Procedures

Immunization experiments were conducted in pathogen-free 12°C water at the Clackamas and Corvallis Fish Disease laboratories. Juvenile coho salmon raised from the egg stage in spring water at Sandy Hatchery were used for each experiment. No previous BCWD had been noted in these fish. For immersion vaccination, fish were dipped 1 or 2 minutes in laboratory water containing 5 x 108 F. psychrophilus cells/ml. The injectable bacterin consisting of 1010 cells/ml diluted 50:50 with Freunds Complete Adjuvant (Difco) was injected at 0.05-0.1 ml intraperitoneally into each fish. These immunized and control fish were maintained in 460L circular tanks for about 28-50 days in 12°C water, then distributed into 68L tanks and challenged by subcutaneous injection with various concentrations of viable SH3-81 cells. Dead fish were removed daily and examined by inoculating Cytophaga agar plates with kidney tissue. The experiments were terminated after 14-36 days when no dying fish were evident in any group.

Vaccination of yearling coho salmon

In this test, our goal was to determine if a BCWD bacterin under optimal conditions would have any protective value. Groups of 100 coho (27g) each were given one of the following treatments: (1) intraperitoneal injection of the injectable bacterin, (2) 60 second exposure to the immersion bacterin, or (3) 60 second exposure to well water only. After 50 days each group was distributed into two tanks (50 fish each) and challenged by injection three days later with 8.6 x 105 cells per fish.

Vaccination of coho salmon at selected sizes

Experiments were conducted to determine if coho salmon at 4.1, 1.5, 0.9 and 0.5 g mean weight could be protectively immunized against F. psychrophilus. In general, fish at each size were vaccinated by immersion for 2 minutes, held for about 30 days, and subgroups challenged at one of four ten-fold dilutions of viable SH3-81 cells. Replicate groups of 25 fish each for each treatment and each challenge dose were included.

RESULTS

Vaccination of yearling coho salmon

As shown in Table 1, intraperitoneal injection of adjuvant and formalin-killed cells of F. psychrophilus provided complete protection in yearling coho salmon against a subcutaneous injection challenge of 8.6 x 105 viable cells compared to 43% loss in control groups. This experiment also demonstrated that vaccination by the immersion method resulted in lower losses (11%) compared to controls.

Table 1. Efficacy of formalin-killed F. psychrophilus bacterin when administered to yearling coho salmon by immersion or intraperitoneal (I.P.) injection with Freunds Complete Adjuvant.

	Treatment					
	Controls		I.P. injection of adjuvant and bacterin		Immersion in bacterin for 60 seconds	
Replicate	1	2	1	2	1	2
Fraction of Fish that died	17/50	26/50	0/50	0/50	5/50	6/50
Combined % loss	43		0		11	

Vaccination of coho salmon at selected sizes

Results of the immersion vaccination experiments for coho salmon at 4.1, 1.5, 0.9 and 0.5 g mean weight are shown in Figures 1-4. Each figure shows the percent survival for the combined replicates of controls and immersion vaccinated groups at a particular fish size, challenged at four different levels. Statistically significant greater survival was observed for the vaccinated groups for all fish sizes and at all challenge levels except the highest in the case of 1.5 and 4.1 g fish. Similar results to those obtained with other bacterins were observed in that best protection occurred in fish larger than 1 g. In 4.1 g coho (Figure 1), at the three lower challenge levels about 30% difference in mortality was observed between vaccinated and control groups. In 1.5 g coho (Figure 2), at the 1.6 x 105 cells challenge concentration vaccinates had a 24% loss compared to 75% for controls. The relative per cent survival (RPS) (Amend, 1981) for this test was 68 indicating acceptable potency. For fish vaccinated at 0.9 or 0.5 g (Figures 3 and 4) the difference in survival between vaccinated and control groups was smaller, usually 10-15%.

DISCUSSION

These studies demonstrate that protective immunity can be obtained with a bacterin consisting of formalin-killed cells of F. psychrophilus. An injectable bacterin of formalin killed cells and Freunds Complete Adjuvant when administered intraperitoneally provides the best protection. Immersion, a more practical method of immunizing large numbers of fish also provides protective immunity, however, size of fish at vaccination is important in determining the level of immunity achieved. We feel the immersion bacterin should be tested in a variety of field conditions with production groups of fish to determine the role this bacterin will play in the prevention and control of BCWD. A combination of techniques is probably need to minimize the impact of BCWD including: (1) use of

proper rates of water flow during incubation and substrates that minimize abrasion injuries, (2) administering registered therapeutants to the water or in the diet, and (3) vaccination when the fish reach a specific size. From other experiments not reported here (Holt, 1988) we have found that F. psychrophilus strains from geographically different areas differ in their antigens. Therefore, the strain or combination of strains of F. psychrophilus used in a bacterin will be important.

ACKNOWLEDGEMENTS

This research was funded by the Oregon Department of Fish and Wildlife and U.S. Fish and Wildlife Service under PL89-304, the Anadromous Fish Act, project AFS-78-1. We thank Mr. Dick Whitlatch and the Sandy Hatchery crew for experimental fish. Also, thanks to Ms. Glacier Tajwall for typing this manuscript.

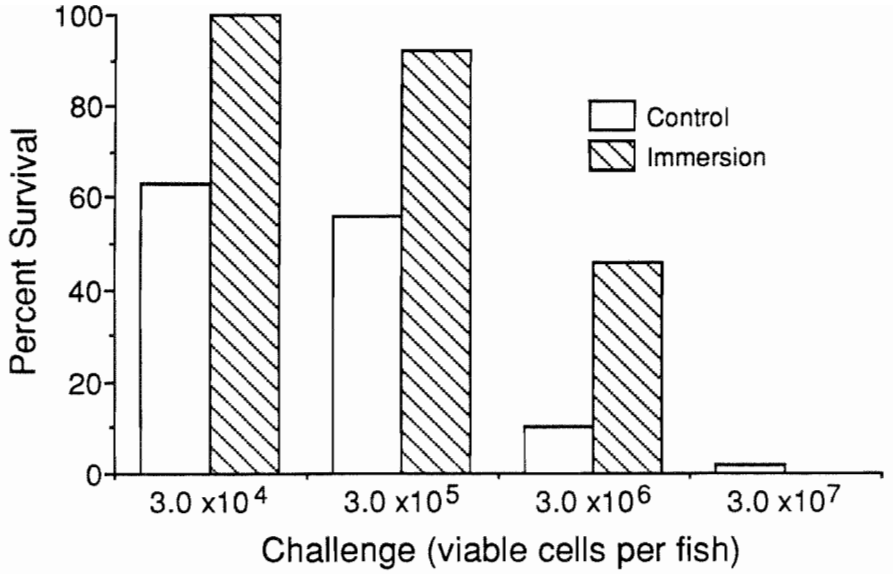


Figure 1. Survival of vaccinated and control 4.1G coho salmon challenged with *F. psychrophilus* 31 days after vaccination.

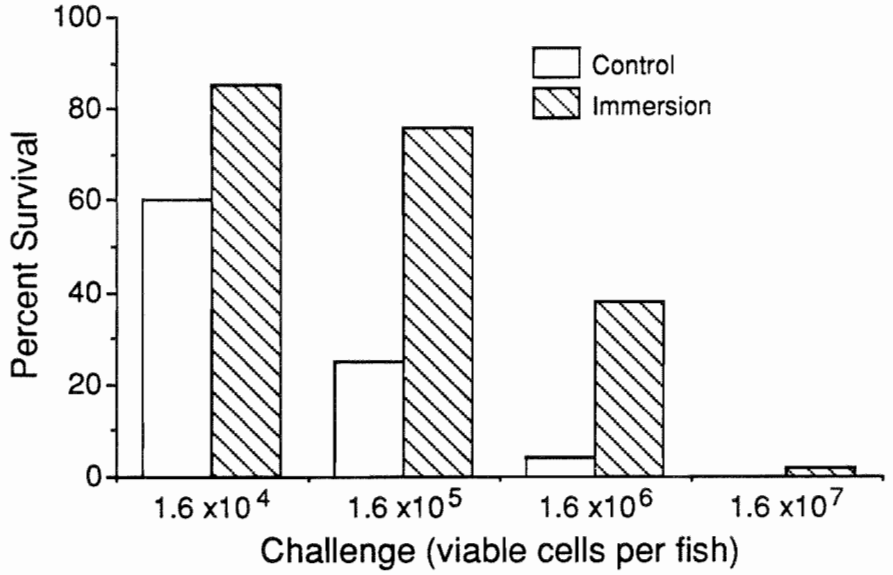


Figure 2. Survival of vaccinated and control 1.5G coho salmon challenged with *F. psychrophilus* 30 days after vaccination.

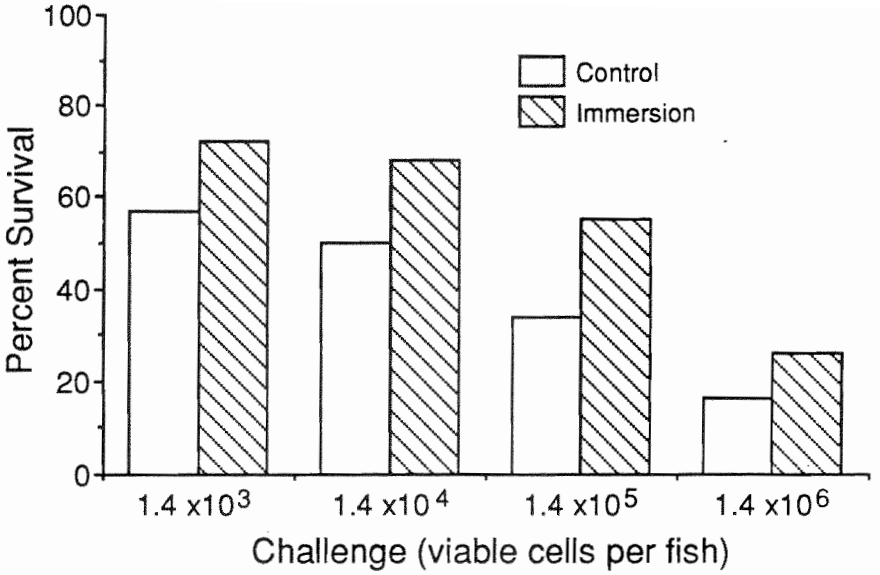


Figure 3. Survival of vaccinated and control 0.9G coho salmon challenged with *F. psychrophilus* 31 days after vaccination.

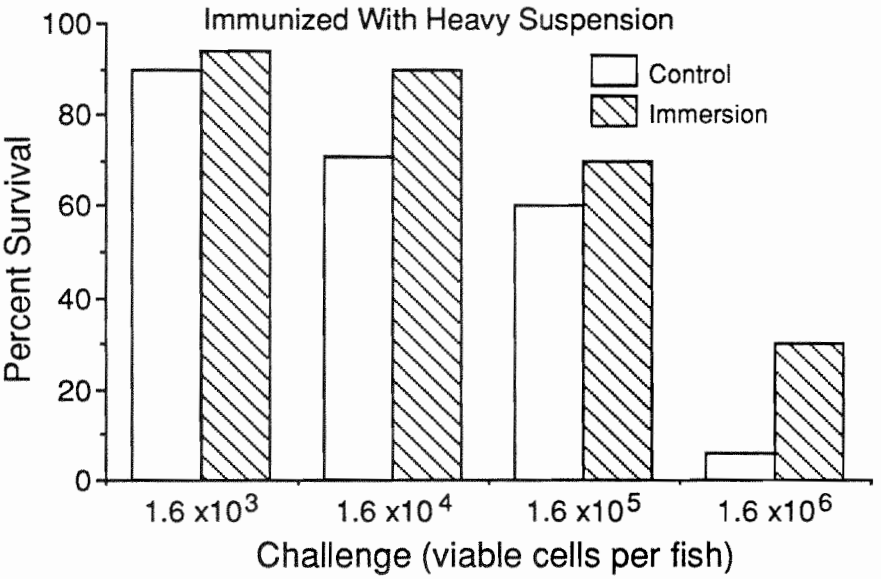


Figure 4. Survival of vaccinated and control 0.5G coho salmon challenged with *F. psychrophilus* 28 days after vaccination.

REFERENCES

Amend, D.F. 1981. Potency testing of fish vaccines. International Symposium in Fish Biologics: Serodiagnostics and Vaccines, Leetown, W. Va. Dev. Bio. Standard 49, pp. 447-454.

Becker, C.D. and M.F. Fujihara. 1978. The bacterial pathogen Flexibacter columnaris and its epizootiology among Columbia River fish. Monograph No. 2. Am. Fish. Soc. Washington, D.C.

Bernardet, J.F. and P.A.D. Grimont. 1989. Deoxyribonucleic acid relatedness and phenotypic characterization of Flexibacter columnaris sp. nov., nom. rev. Flexibacter psychrophilus sp. nov., nom., and Flexibacter maritimus. Wakabayashi, Hikida, and Masumura 1986. Int. J. Syst. Bacteriol. 39: 346-354.

Borg, A.F. 1960. Studies on myxobacteria associated with disease in salmonid fishes. Wildlife Disease 8:1-85, 2 microcards.

Holt, R.A. 1988. Cytophaga psychrophila, the causative agent of bacterial cold-water disease in salmonid fish. Ph.D. Thesis, Oregon State University, Corvallis, Oregon. 166 pp.

A UNIQUE STRAIN OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS FROM THE HAGERMAN VALLEY OF IDAHO

W.J. Groberg, Jr.
Oregon Department of Fish and Wildlife
Eastern Oregon State College
La Grande, Oregon

V.L. Ediger
School of Arts and Sciences
Eastern Oregon State College
La Grande, Oregon

S.E. LaPatra
Clear Springs Trout Company
Buhl, Idaho

ABSTRACT

Infectious hematopoietic necrosis virus (IHNV) is the most important viral pathogen affecting salmonid fish in North America. The catastrophic consequences of IHNV infections in hatchery populations of juvenile salmonids can be a limiting factor for the enhancement of anadromous salmonid populations. The virus also has a significant economic impact on the commercial trout industry in Idaho. Prior to 1990, four serological types of IHNV were documented in Oregon and the Columbia River basin. These serological types were based on the plaque reduction profiles attained with a monoclonal and a polyclonal antiserum. An isolate of IHNV acquired from a commercial hatchery in Idaho in February of 1990 displayed a unique plaque reduction profile. Thus this isolate represents a new serological strain of IHNV in the Columbia River basin. This unique strain shows no neutralization with either monoclonal or polyclonal antisera specific for a reference strain of IHNV. Vaccination of fish with a recently developed IHNV vaccine may not afford protection against this new IHNV strain.

INTRODUCTION

Infectious hematopoietic necrosis (IHN) is an extremely lethal viral disease affecting several species of salmonid fish in the Pacific Northwest including Oncorhynchus nerka (sockeye and kokanee salmon), O. tshawytsha (chinook salmon), and O. mykiss (rainbow trout and steelhead). The etiological agent, infectious hematopoietic necrosis virus (IHNV), is an RNA rhabdovirus which most often infects alevins, fry or fingerlings by attacking and destroying the hematopoietic tissue of the anterior kidney and spleen. Outbreaks of IHN in hatcheries can result in catastrophic losses with mortality rates greater than 90% in young fish. Since no therapeutic compounds exist for the treatment of IHN, infected populations are often destroyed followed by complete hatchery decontamination. Thus epizootics of IHN can have a significant economic impact on commercial trout operations

because of lost production, and the virus poses a threat to the enhancement of anadromous salmonid populations through losses of important public hatchery stocks.

As a member of the Rhabdoviridae family of viruses, IHNV is structurally similar to rabies and vesicular stomatitis viruses against which protective vaccines have been developed (Kelley et al., 1972; Wiktor et al., 1984). This similarity prompted efforts to develop a vaccine against IHNV and subsequent work resulted in a vaccine using IHNV's surface glycoprotein G as the antigenic determinant (Engelking and Leong, 1989a). This vaccine induces protective immunity in fish that have been immersed in a bath of the vaccine and is reportedly protective against five biochemical types of IHNV (Engelking and Leong, 1989b).

Infectious hematopoietic necrosis virus can be segregated into serotypes based upon plaque reduction profiles obtained by neutralization of virus isolates with a monoclonal antiserum designated Mab RB/B5 (Winton et al., 1988) and a polyclonal anti-IHNV glycoprotein rabbit antiserum (Anti-G). Both of these antisera were made against the Round Butte, Oregon Type I strain of IHNV. Plaque reduction data acquired over the past several years using over 70 IHNV isolates from Oregon and the Columbia River basin have revealed four distinct plaque reduction profile patterns (Groberg, 1990). Studies reported in this paper with a recent IHNV isolate, designated HV90, show that it has a unique plaque reduction profile and thus represents a fifth serotype of IHNV to be identified in this geographical area.

MATERIALS AND METHODS

Isolation and Confirmation of the IHNV Isolate

Rainbow trout thought to be dying from IHN were obtained from a commercial trout hatchery in the Hagerman Valley of Idaho in February of 1990. Kidneys and spleens from four fish were removed and prepared for inoculation onto cell cultures according to methods described in the Fish Health Section Bluebook (Amos, 1985). Samples were inoculated onto epithelioma papulosum cyprini (EPC) and chinook salmon embryonic-214 (CHSE-214) cell cultures. Known samples of a Round Butte reference strain (RB82) of IHNV were also used to infect EPC and CHSE-214 cell cultures. Cell cultures lacking virus were also prepared as negative controls. Cell cultures were incubated at 16° C and monitored for resulting cytopathic effects (CPE) which were used to establish a presumptive diagnosis.

The indirect fluorescent antibody test (IFAT) was then used to confirm this diagnosis (LaPatra et al., 1989). Fifty microliter aliquots from the supernatants of the primary kidney-spleen samples from the above cell cultures were subcultured onto EPC cell monolayers grown on microscope coverslips. Positive controls were similarly prepared by infecting EPC cells with the reference RB82 IHNV strain. Negative controls of uninfected EPC cells were also prepared by mock infection with cell culture medium (MEM-0). Following 24 hours of incubation at 16° C the cell preparations were prepared for IFAT examination following the procedure of LaPatra et al.(1989). An anti-IHNV

monoclonal antibody (193-110) was used as the primary antibody and a fluorescein labeled horse anti-mouse immunoglobulin antibody served as the secondary antibody. These preparations were observed under a fluorescent microscope and observations of the suspected IHNV isolate from the kidney-spleen samples were compared to preparations infected by the reference strain of IHNV and mock infected controls.

Plaque Reduction Assays and Serological Typing of the IHNV Isolate

Once confirmed to be IHNV by the IFAT, plaque reduction assays using the monoclonal Mab RB/B5 and the polyclonal Anti-G antibody were used to determine the serotype (Groberg, 1990) of the isolate designated HV90. Serial ten-fold dilutions of the HV90 IHNV isolate and the RB82 reference strain were prepared and 0.2 mL aliquots of each dilution were mixed with an identical volume of polyclonal Anti-G (diluted 1:50 with MEM-0), of monoclonal Mab RB/B5 (diluted 1:5 with MEM-0), or of cell culture medium (unneutralized virus). In separate assays these virus isolates were similarly mixed with polyclonal rabbit antisera (diluted 1:50 in MEM-0) made against the Coleman (California) strain of IHNV and against whole Round Butte IHNV. All virus-antiserum suspensions were incubated on a rocker platform for one hour at 16° C. Replicate 0.1 mL volumes of each dilution and each virus suspension were inoculated onto EPC monolayers in 24-well plates. These were incubated on the rocker platform for another hour. The inoculum was then decanted and 0.5 mL of overlay medium (cell culture medium/methyl-cellulose) was added to each well. The plates were incubated undisturbed for ten days at 16° C. They were then fixed and stained with formalin/crystal violet solution. Viral plaques were counted under a dissection microscope and the average of replicates used to determine the virus titer. Logarithmic differences in the IHNV titer between unneutralized and antiserum treated virus (neutralized) were then calculated.

Infection Trials of Juvenile Rainbow Trout with the IHNV Isolate

The HV90 IHNV isolate was used for water-borne infection experiments in two sizes of young rainbow trout by one of the co-authors (SEL) at Clear Springs Trout Company research facilities. It was included with seven IHNV isolates from other rainbow trout production hatcheries to make virulence comparisons among isolates from the same geographical area. The HV90 isolate, which was not from a Clear Springs facility, was propagated and prepared for experimental infections identically to procedures used for the seven other isolates. Triplicate groups of rainbow trout at 0.5 gm (840 fish/lb) and 1.7 gm (274 fish/lb) were exposed to separate isolates at a concentration of 1,000 plaque forming units per milliliter of aquarium water for one hour. One group of fish at each size were treated similarly except they were not exposed to IHNV and served as uninfected controls. Morbid fish were collected daily (twice daily during peak mortality) and a minimum of 20% per aquarium per day were assayed for IHNV and for culturable bacteria. Data for each IHNV isolate were used to calculate combined percent mortality and mean day from exposure to death.

RESULTS

Isolation and Confirmation of the IHNV Isolate

Cell cultures inoculated with the kidney-spleen homogenates from rainbow trout showed CPE typical of IHNV when compared to the IHNV reference strain. Negative controls showed no CPE.

Preparations of the HV90 isolate stained by the IFAT procedure were found to exhibit specific fluorescence. The intensity and distribution of this fluorescence in cells appeared identical to that observed in RB82 inoculated cell cultures confirming that the isolate was IHNV. No fluorescence was observed in mock infected cell cultures.

Plaque Reduction Assays and Serological Typing of the IHNV Isolate

Plaque reduction assays using the RB82 reference strain of IHNV showed a 2.3 log reduction of viral plaques with the monoclonal Mab antisera and a 2.0 log reduction of plaques with the polyclonal Anti-G antiserum. Plaque reduction assays of the HV90 isolate showed no reduction of plaques with either the Mab RB/B5 or Anti-G antisera (Figure 1). Identical assays using the polyclonal Anti-Coleman IHNV antiserum showed no neutralization of either virus isolate while the Anti-Round Butte IHNV antiserum showed a 1.5 log reduction of RB82 and a 1.3 log reduction of HV90.

Infection Trials of Juvenile Rainbow Trout with the IHNV Isolate

The combined percent mortality of the seven other IHNV isolates ranged from 30 to 68% in 0.5 gm fish and from 8 to 30% in 1.7 gm fish. The HV90 isolate produced a 71 and 40% mortality in fish at these same sizes, respectively. The higher rates with HV90 are not statistically significant compared to other isolates producing high mortality, however there were consistent trends between the three replicates in fish at both sizes towards a higher mortality with HV90. Control groups at each size had a 2% mortality.

There was also a tendency towards a shorter mean day to death with the HV90 isolate over that seen with the other seven, although it was again not supported by statistical analysis. In fish at 0.5 gm the mean day to death was 10.6 days with HV90 while the range was from 11.6 to 14.3 days with the other isolates. At 1.7 gm HV90 killed fish in an average of 11.8 days and the other isolates ranged from 13.9 to 14.5 days, with the exception of one isolate that showed a mean day to death of only 10.5 days. The combined mortality was only 8% with that isolate and it is believed the mean day to death is skewed to the short side because of the low mortality which occurred early in the experiments.

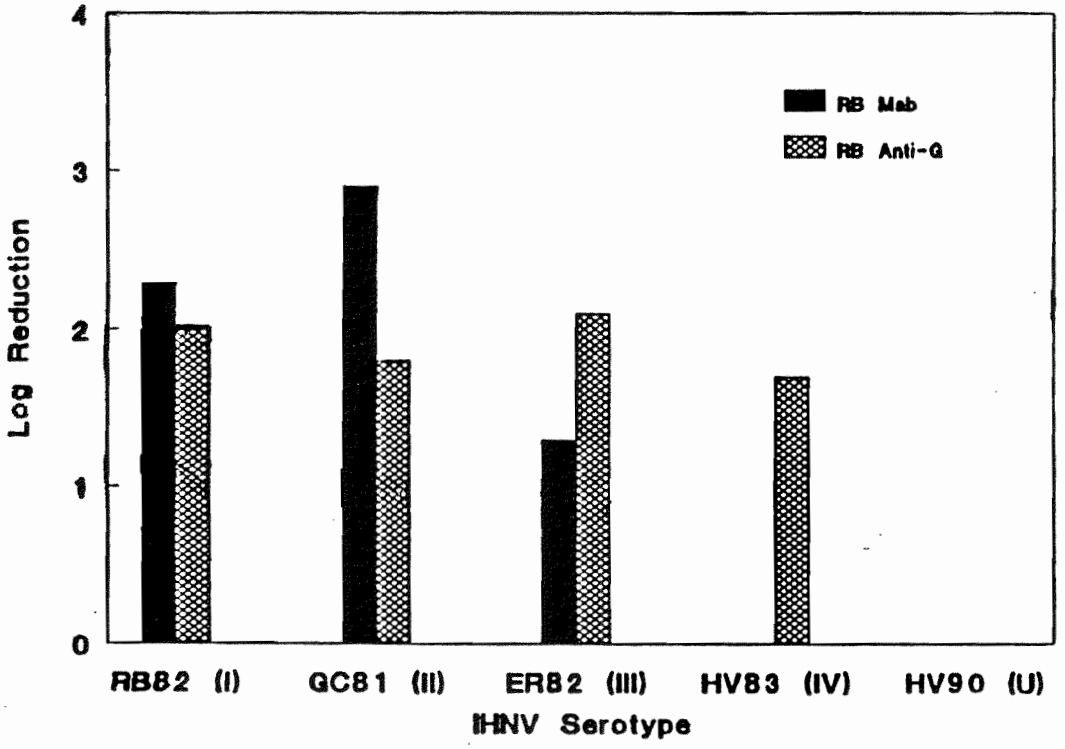


Figure 1. Plaque reduction profiles of four serotypes of IHNV designated I, II III and IV, and a unique serotype designated U. These profiles were obtained by neutralization assays using a Round Butte Anti-IHNV G protein polyclonal serum (RB Anti-G) and a Round Butte monoclonal IHNV antiserum RB/B5 (RB Mab). The Log_{10} reduction by each antisera with IHNV isolates is shown by the bar.

DISCUSSION

The rainbow trout in these studies were presumptively diagnosed to have IHN based upon the CPE observed in EPC and CHSE-214 cell cultures inoculated with tissue homogenates from the fish. The appearance of the CPE was similar to that of the reference IHNV strain. This diagnosis was confirmed since the HV90 isolate revealed specific fluorescence when stained with the IHNV antibody by the IFAT procedure.

Initial observations of the HV90 isolate on cell cultures were that the CPE was more advanced by 72 hours than CPE seen with any other IHNV isolate the authors had experienced. This suggested that this isolate may be a more virulent strain of IHN virus. Thus, it was evaluated with a limited number of contemporary isolates (1988-90) from the Hagerman Valley, for its ability to kill young rainbow trout in laboratory infection trials (results of these experiments will be presented by S.E. LaPatra at the Second International Symposium on Viruses of Lower Vertebrates in Corvallis, Oregon on July 29-31, 1991). Based on mortality and the average day at which death occurred, the HV90 IHNV was not significantly different than other highly virulent isolates tested when these data were compared statistically (J.E. Parsons, personal communication). Trends in the experiments, however, indicated the HV90 isolate was more lethal and further evaluation of its relative virulence should be made.

Serological characterization of the isolate was also of interest because it was perceived that the intensive fish culture environment of a large commercial facility could provide conditions favorable for the emergence of new strains of IHNV through mutational events and selective forces. Each month, rearing of several hundred-thousand newly hatched alevins is begun in a typical commercial operation. Most of these groups undergo epizootics of IHN with losses ranging from about 10 to 70% (J.L. Zinn, personal communication). Thus the frequent introduction of large numbers of susceptible hosts in an environment where the viral pathogen is continually present could, over time, allow for the appearance of new and variant strains of the virus. This would be especially evident if a mutant appeared that possessed enhanced virulence properties over that of the enzootic wildtype virus.

Further, because IHNV is an RNA virus and RNA viral genomes are known to exhibit high mutation rates on the order of 10^{-3} to 10^{-4} errors per genome doubling (Holland et al., 1982), the possibility that variant types might spontaneously occur is relatively high. It was of interest, then, to compare the plaque reduction profile of the HV90 isolate with that of an isolate obtained from the same facility in 1983 (HV83). Conceivably, mutational events over several years may have allowed for selection of a different strain of IHNV in this hatchery which could be discerned by serological assays. The intent of this study was to test that hypothesis and, indeed, the HV90 isolate proved to be significantly different from the 1983 isolate. The HV83 isolate showed a 2.0 log reduction with the polyclonal Anti-G reagent while HV90 showed no plaque reduction with this same antiserum (Figure 1).

Previous plaque reduction assays on IHNV isolates from Oregon and the Columbia River basin have resolved all isolates into four distinct serological groups designated I-IV (Groberg, 1990). The plaque reduction profile obtained

with HV90 revealed that this isolate could not be segregated into any of these four serological groups (Figure 1) including Type IV, shown to be typical of isolates from commercial hatcheries in southern Idaho. Thus HV90 appears to be unique among all previously examined IHNV isolates from this area.

Whether this strain of IHNV appeared through mutational and selective processes as discussed above, or simply as an introduction from an outside source cannot be determined. This virus was not neutralized by an IHNV antiserum specific for a Coleman (California) IHNV strain, providing some evidence (albeit not conclusive) it is not a California strain. Eggs are brought into the Hagerman Valley from numerous outside suppliers and there are daily movements of fish and equipment within the valley that could allow for the dissemination of a newly introduced pathogen. Facility effluents are also obviously a vehicle to support the spread of infectious organisms.

It is significant that HV90 is not neutralized by the polyclonal Anti-G antiserum which is specific for IHNV's surface glycoprotein G. A recently developed IHNV subunit vaccine (Engelking and Leong, 1989a) uses the surface glycoprotein G as the protective antigen. It is possible, therefore, that fish vaccinated with this vaccine may show little or no protection against the HV90 strain of IHNV. Experiments in fish vaccinated with the subunit vaccine and challenged by the HV90 isolate, however, have not yet been reported. A prelude to field trials with this product in the Hagerman Valley should include a thorough survey of the biochemical, antigenic, and virulence properties of enzootic varieties of IHN virus in the area.

ACKNOWLEDGMENTS

The La Grande Fish Pathology Laboratory is funded under an Augmented Fish Health Monitoring contract with the Bonneville Power Administration. Anti-sera were generously provided by Cathy Lannan, Jim Winton, Mark Engelking, and JoAnn Leong. Contributors of virus isolates and neutralization assays were James Esselstyn, Sam Onjukka, Nancy Wood, Harold Ramsey, Joe Lientz, Ray Brunson, and Jerry Zinn. Statistical analyses for the virulence trials were done by Jim Parsons.

REFERENCES

Amos, K.H. 1985. Procedures for the detection and identification of certain fish pathogens, 3rd edition. American Fisheries Society, Fish Health Section, Corvallis, Oregon. (Available from AFS, Bethesda, Maryland.)

Engelking, H.M., and J.C. Leong. 1989a. The glycoprotein of infectious hematopoietic necrosis virus elicits neutralizing antibody and protective responses. Virus Research 13:213-230.

Engelking, H.M., and J.C. Leong. 1989b. Glycoprotein from infectious hematopoietic necrosis virus (IHNV) induces protective immunity against five IHNV types. Journal of Aquatic Animal Health 1:291-300.

Groberg, W.J., Jr. 1990. A unique serotype of infectious hematopoietic necrosis virus from rainbow trout in the Snake River basin. American Fisheries Society, Fish Health Section Newsletter 18:1-2.

Holland, J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. VandePol. 1982. Rapid evolution of RNA genomes. Science 215:1577-1585.

Kelley, J.M., S.U. Emerson, and R.R. Wagner. 1972. The glycoprotein of vesicular stomatitis virus is the antigen that gives rise to and reacts with neutralizing antibody. Journal of Virology 10:1231-1235.

LaPatra, S.E., K.A. Roberti, J.S. Rohovec, and J.L. Fryer. 1989. Fluorescent antibody test for the rapid diagnosis of infectious hematopoietic necrosis. Journal of Aquatic Animal Health 1:29-36.

Wiktor, T.J., R.I. McFarlan, K.J. Regan, B. Dietzschold, P.J. Curtis, W.H. Wunner, M.P. Kieny, R. Lathe, J.P. Lecocq, M. Mackett, B. Moss, and H. Koprowski. 1984. Protection from rabies by a vaccinia virus recombinant containing the rabies virus glycoprotein gene. Proceedings of the National Academy of Sciences USA 81:7194-7198.

Winton, J.R., C.K, Arakawa, C.M. Lannan, and J.L. Fryer. 1988. Neutralizing monoclonal antibodies recognize antigenic variants among isolates of infectious hematopoietic necrosis virus. Diseases of Aquatic Organisms 4:199-204.

FIELD EVALUATIONS OF A SUBUNIT VACCINE FOR IHNV AT HAGERMAN HATCHERY, IDAHO DEPARTMENT OF FISH AND GAME

Keith A. Johnson, Fish Pathologist
Idaho Department of Fish and Game
Eagle Fish Health Laboratory
Eagle, Idaho

JoAnn Leong
Department of Microbiology
Oregon State University
Corvallis, Oregon

Two production field trials were conducted to evaluate an experimental subunit vaccine to control Infectious Hematopoietic Necrosis Virus (IHNV) of rainbow trout at Hagerman State Fish Hatchery. Fish were about 1 gram when vaccinated by immersion. Daily loss and the cause of death was monitored invaccinates and a control group for 180 days post vaccination in Trial I and 147 days for Trial II. A natural challenge of the virus occurred in vaccinates and controls of both trials.

The coldwater disease (CWD) agent, Flexibacter psychrophilus, occurred in both trials. Control using the antibiotic oxytetracycline was initiated in Trial I after disease signs were evident. The IHNV loss began about two weeks after loss to CWD. In trial I a 17% loss occurred in controls compared to 3.7% in the vaccinates during the period that IHNV occurred in the populations.

In Trial II, oxytetracycline was applied to the populations twice to control CWD and the loss was reduced. The IHNV loss period was delayed and occurred at a greatly reduced rate in both lots. Control loss was 1.8% due to IHNV compared to 0.8% in the vaccinates.

At Hagerman Hatchery, CWD infections preceded the IHNV outbreaks. Using antibiotic therapy early in the rearing period reduced the CWD loss, and consequently mortality to IHNV was brought down from about 25% to less than 5%. These trials demonstrated that populations with reduced levels of CWD infections were able to survive the natural virus challenge at a much higher rate. In both trials, the severity of IHNV mortality was reduced in vaccinates compared to controls and the loss occurred later in the vaccinates. The subunit vaccine appears to have promise for rainbow trout culture situations in which the fish can be raised free of the virus until protection can be developed.

A LIBRARY OF MONOCLONAL ANTIBODIES TO DETECT IHNV

Sandra S. Ristow and Jeanene Arnzen de Avila
Department of Animal Sciences
Washington State University
Pullman, Washington

Monoclonal antibodies are antibodies which have been developed by the immunization of inbred mice with a particular protein or organism. After immunization of the mouse, the spleen of the mouse is removed, and the lymphocytes isolated and fused with myeloma cells in the presence of a small amount of polyethylene glycol. This process results in the production of hybrid cell lines referred to as hybridomas, each of which produces a single antibody to a small sequence of amino acids on a protein (usually 6 to 12 amino acids in length). The sequence recognized by the monoclonal antibody is referred to as an epitope. Monoclonal antibodies are different from polyclonal antibodies because polyclonal antibody is a mixture of many different antibodies reacting with many different epitopes on a protein.

We have developed monoclonal antibodies to several electrophoretic types of IHNV as described by Hsu, Engelking and Leong (Applied and Environmental Microbiology, 52:1353, 1986). The collection of viral isolates used for monoclonal antibody production included representatives from different stocks of salmonid fish and different geographical areas. Isolates were expanded by growth on CHSE-214 cells, and the virus purified by ultracentrifugation. BALB/c mice were injected with IHNV glycoproteins or nucleoproteins which had been purified by gel electrophoresis. Spleen cells were isolated from immunized mice and fused to the P3X63Ag8.653 myeloma. Hybridomas were selected by their ability to immunoprecipitate proteins of the virus, react in tests of indirect fluorescence against the virus, or to react with the virus in ELISA and to neutralize viral infectivity.

To date, we have cloned 27 hybridomas which produce antibodies reacting with unique sequences (epitopes) on the glycoproteins (G) and nucleoproteins (N) of IHNV. One of the most useful antibodies which has been produced in our laboratory is 1NDW14D, which has identified over 150 isolates of IHNV. In order to use 1NDW14D in a direct fluorescence assay, sterile round coverslips are placed into 24 well tissue culture plates, a cell monolayer (CHSE-214) added, and 16 to 24 hours later the coverslips are infected with the virus. After the infection is established (16 to 24 hours), the coverslips are removed, rinsed in phosphate buffered saline, and fixed in cold acetone for 10 minutes. After fixation, the coverslips are stained. To use the antibody, 1NDW14D, in a direct fluorescence assay, it is conjugated to the dye, fluorescein isothiocyanate. A dilution of the conjugated antibody is added to the coverslip and incubated at 37°C for 30 minutes. After the incubation, the coverslip is washed 5 times with PBS to remove any unbound antibody and is counterstained with Evan's Blue. When viewed under an ultraviolet light from a fluorescence microscope, infected cells are stained a brilliant fluorescent apple green on a red background of uninfected cells.

To run the indirect form of the fluorescence assay with 1NDW14D or with other monoclonal antibodies in the library, a predetermined dilution of the

selected monoclonal antibody is incubated with the coverslip for 30 minutes, washed, and a dilution of fluorescein conjugated rabbit anti-mouse immunoglobulin added and incubated with the coverslip for an additional 30 minutes. The PBS washed coverslip is mounted on a slide and viewed under a fluorescence microscope.

A experiment was performed in which the ability of fluorescein conjugated 1NDW14D to detect IHNV from infected fish samples having either low or high infection was compared with the results from the traditional plaque assay. The sensitivity of the two assays was similar and good correlation of results on both assays was noted.

A time study was performed using the indirect fluorescence method to determine how quickly virus infection could be detected. It was apparent that by 6 to 8 hours "capping" could be seen on infected cells with either an antiglycoprotein or an antinucleoprotein antibody, and full cytoplasmic infection was always seen by 16 to 20 hours.

By fluorescence, several monoclonal antibodies made to the nucleoproteins universally identify the virus, while others are quite specific, identifying fewer isolates. For example, one of the antibodies (2NH105B) identifies electrophoretic type 2 virus (Hsu et al., 1986) while another antibody (2NC042C) identifies an epitope which originally appeared on electrophoretic type 4 virus. Other antibodies in the library, when used in indirect fluorescence on coverslips of infected cells, allow differentiation within electrophoretic subtypes because they react with one isolate within a subtype, but not another.

We have noted several changes in the nucleoprotein epitopes of isolates of IHNV from Dworshak National Fish Hatchery at Ahsahka, Idaho. In 1984, an isolate which was fluorescence positive with 2NH105B (indicative of type 2 virus) appeared at the facility. Since that date all isolates which we have tested possess the epitope identified by 2NH105B. More recently, an isolate appeared which has the epitope identified by 2NC042C, an epitope which was first noted by us on a 1979 isolate from Coleman Hatchery. The serum neutralization patterns with antiglycoprotein antibodies 3GH127B and 3GH92A of the G proteins of all of the isolates thus far tested from Dworshak Hatchery appear to be typical for Columbia River Strains, thus differentiating them from the "Coleman strain" of the virus.

Our anti-glycoprotein monoclonal antibodies are useful for differentiating among isolates in the indirect fluorescence assay and several of them are serum neutralizing. In a study of 17 isolates chosen to delineate the life cycle of IHNV, neutralization of isolates by monoclonal antibodies 3GH127B and 3GH92A revealed that the selected viruses could be divided into two regional groups, those isolates which were neutralized by both of the antibodies and which came from the Columbia Basin and those isolates which came from California/Southern Oregon. Neutralization by the two antibodies was not species related, but was more related to geography. [The single exception to this was a 1983 isolate from Rangen Research in southern Idaho.] Neutralization by the two antibodies did not strictly agree with their reactivity in indirect flourescence with the same 17 isolates. While 3GH127B reacted in indirect fluorescence with all 17 isolates, 3GH92A did not, revealing small differences in specificity between the two antibodies. Another antiglycoprotein monoclonal antibody, 1GH131A, reacts in indirect fluorescence with a number of isolates, but neutralizes only the

Hagerman isolate which was used for the immunization which produced the antibody. Another antibody, 1GCDR2P, predominately identifies Cedar River isolates in indirect fluoresecence. Utilizing both methods, indirect fluorescence and serum neutralization, we are able to further differentiate among isolates of IHNV.

We are also able to distinguish between viral hemorrhagic septicemia virus (VHSV), a European rhabodvirus which recently appeared in coho salmon in Washington, and IHNV, which is enzootic in the Pacific Northwest. We have developed, with Dr. P. E. Vestergard-Jorgensen of Denmark, an immunodot assay based on two monoclonal antibodies recognizing conserved epitopes on the nucleoproteins of VHSV and IHNV. Monoclonal antibody 1NDW14D, which recognizes a conserved epitope on the nucleoprotein of IHNV, has recognized 79 out of 80 isolates of IHNV spotted on nitrocellulose, but not isolates of VHSV. Monoclonal antibody 1P5B11, developed by Dr. Jorgensen's laboratory (Lorenzen et al. 1988) which recognizes a conserved epitope on the nucleoprotein of VHSV, reacted with all representative isolates of VHSV, but not with any of the isolates of IHNV. Neither monoclonal antibody bound to other rhabdoviruses: spring viremia of carp virus (SVCV), pike fry rhabdovirus (PFRV), a new Danish eicosid rhabdovirus unrelated to PFRV, or to rhabdovirus anguilla (EVX), spotted on nitrocellose.

Figures and photographs associated with this presentation, which was given by Jeanene Arnzen de Avila have appeared or will appear under copyright in the following journals under the titles cited below:

Ristow, S.S., and Arnzen J. M. Development of Monoclonal Antibodies that recognize a Type 2 specific and a common epitope on the nucleoprotein of infectious hematopoietic necrosis virus. Journal of Aquatic Animal Health 1:119-125, 1989.

Arnzen, J.M., Ristow, S. S., Hesson, C.P. and Lientz, J. Rapid Fluorescent Antibody Tests for Infectious Hematopoietic Necrosis Virus (IHNV) utilizing Monoclonal Antibodies to the Nucleoprotein and Glycoprotein. (In Press, Journal of Aquatic Animal Health)

Ristow, S.S., Arnzen de Avila, J. Monoclonal Antibodies to the Glycoprotein and Nucleoprotein of Infectious Hematopoietic Necrosis Virus (IHNV) reveal differences among isolates of the virus by fluorescnece, serum neutralization and electrophoresis. (submitted to Diseases of Aquatic Organisms)

Ristow, S.S., Lorenzen, N., Vestergaard Jorgensen, P.E.V. Monoclonal Antibody Based Immunodot Assay distinguishes between viral hemorrhagic septicemia (VHSV) and Infectious Hematopoietic Necrosis Virus (IHNV). (submitted to Journal of Aquatic Animal Health)

CHARACTERISTICS OF THE NORTH AMERICAN ISOLATES OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS

James R. Winton
U.S. Fish and Wildlife Service
National Fisheries Research Center
Seattle, Washington

In the fall of 1988, viruses were isolated from adult chinook salmon returning to a private hatchery on Orcas Island in the Puget Sound of Washington State and from adult coho salmon returning to the Makah National Fish Hatchery near Neah Bay, Washington. Electron microscopy of infected cell monolayers showed virions that were 53-65 nm in diameter and 156-300 nm in length with a bullet-like morphology typical of fish rhabdoviruses. Polyclonal rabbit antisera against the F1 European reference strain of viral hemorrhagic septicemia virus (VHSV) neutralized infectivity at serum dilutions of approximately 1:1000. Cells infected with the new isolates reacted with VHSV polyclonal and monoclonal antisera by the fluorescent antibody technique. The isolates and the F1 reference strain were purified in sucrose gradients and analyzed by polyacrylamide gel electrophoresis (PAGE) revealing that the isolates were composed of five structural proteins with molecular weights identical to each other and to VHSV. The molecular weights were: L 165,000; G 70,000; N 40,000; M1 26,000; and M2 23,000. Virion proteins separated by PAGE were transferred to nitrocellulose and analyzed by western blot assay using antisera against infectious hematopoietic necrosis virus, hirame rhabdovirus, and VHSV. The antiserum against VHSV reacted with all proteins of VHSV and all proteins of the new isolates. These data provided convincing evidence that the first two North American isolates were morphologically, serologically, and biochemically indistinguishable from European strains of VHSV.

In the fall of 1989, additional isolates of VHSV were recovered from adult coho salmon at the Lummi Tribal Hatchery in Puget Sound and the Soleduck Hatchery and Bogachiel River on the Washington coast. These appeared to be similar to the 1988 isolates and were neutralized by antiserum against VHSV strain F1. To determine the potential impact of these North American strains of VHSV upon stocks of Pacific salmonids, waterborne challenges of juvenile chinook, coho, pink, sockeye, and Atlantic salmon and steelhead and rainbow trout were conducted. We exposed replicate groups of 25 fish to each of the four North American isolates of VHSV at 10^5 PFU/mL for 1 hour in a waterborne challenge, observed mortality for 14 days, and titered fish that died. Temperatures during challenges ranged from 8-13 C. After 14 days, the combined total mortality for all strains was: chinook, 0%; coho, 5.5%; sockeye, 7%; pink, 0%; Atlantics, 0.5%; steelhead, 2.5%; and rainbow, 4.5%. These data showed that the North American isolates of VHSV were substantially less virulent than expected, based upon published data and challenges conducted in Europe. We injected replicate groups of 25 juvenile steelhead trout and juvenile coho salmon with 0.05 mL of the Makah isolate (approximately 10^7 PFU/fish) and held these fish in fresh water. In this experiment, it appeared that injection of a relatively massive dose of VHSV was able to induce mortality in steelhead trout (43%), but not in coho salmon (0%).

Because these new isolates were obtained from adult fish recently returning from the marine environment, we compared the stability of IHNV and the F1 and Makah isolates of VHSV in fresh and salt water. The viruses showed significant differences in stability at the end of 1 hour. For IHNV, the fresh water titer was reduced 20X and the salt water titer reduced 8X; for VHS F1, the reduction in fresh water was 50X and the salt water was 2X; while for Makah, the fresh water reduction was 200X compared to 10X in salt water. These data suggest the North American strains were much more stable in salt water.

The four North American strains of VHSV were not closely related to the F1 reference strain when analyzed by T1 ribonuclease fingerprinting. In this assay, genomic RNA is digested into fragments of various size that can be separated by two-dimensional electrophoresis to form specific spot patterns (fingerprints). The Makah, Orcas, and Lummi isolates were very similar (less than 10% spot differences) and the Bogachiel isolate was closely related (about 25% spot differences). All four isolates were significantly different from the F1 reference strain (over 50% spot differences). More European strains will be compared. The data suggest the isolates were not recent imports and that minor differences exist among the North American strains.

In the summer of 1990, an isolate of VHSV was obtained from Pacific cod caught by an angler in Prince William Sound, Alaska. This isolate will be compared with other North American strains. If this isolate is indistinguishable from the strains recovered from salmonid fish, our understanding of the biology of VHSV in North America will be significantly improved.

PROGRESS ON THE REGISTRATION OF ERYTHROMYCIN FOR USE IN FISH CULTURE

Christine Moffitt
University of Idaho

No Abstract submitted.

THE SUCCESSFUL USE OF ELISA-BASED BROODSTOCK SEGREGATION FOR THE CONTROL OF BACTERIAL KIDNEY DISEASE IN SPRING CHINOOK SALMON

Ronald J. Pascho and Diane G. Elliott
U.S. Fish and Wildlife Service
National Fisheries Research Center
Seattle, Washington

Transportation of juvenile salmonids downriver past Columbia River and Snake River hydroelectric dams by truck or barge has shown substantial benefits for fall chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss)*, as evidenced by greater adult returns of transported fish in comparison to nontransported fish. Adult returns of spring chinook salmon have remained low, however, even for transported smolts. There is increasing concern that bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, may be contributing to the poor survival of the spring chinook salmon smolts.

In 1988, the National Fisheries Research Center-Seattle began a study to evaluate the effects of BKD on the survival of the spring/summer chinook salmon stocks that were part of the U.S. Army Corps of Engineers juvenile fish transportation program. The study included monitoring the downstream migration of spring chinook salmon smolts originating from parents with known BKD infection levels.

At Dworshak National Fish Hatchery, Idaho, selected egg lots from the 1988 spring chinook salmon spawning were segregated into two groups, one containing eggs from females infected with very low levels of BKD and the other containing eggs from females infected with high levels of BKD. The level of a specific antigen fraction of *R. salmoninarum* in the kidney of each parent was quantified by the enzyme-linked immunosorbent assay (ELISA), and the concentration of *R. salmoninarum* in the ovarian fluid was quantified by the membrane filtration-fluorescent antibody technique. The creation of these progeny groups was based on experimental evidence that *R. salmoninarum* is transmitted vertically in association with the salmonid egg, and that transmission to the progeny is related to the infection level in the female parent. The progeny lots in the high and low BKD-level groups were maintained in separate water supplies, and were assigned to three raceways per BKD-level group (approximately 35,000 fish per group).

Total post-hatching mortality of the progeny of the high-BKD-level parents was 20% for the 17-month hatchery rearing period, significantly higher than the 10% mortality recorded during the same period for the progeny of the low-BKD-level parents. Mortality in the high-BKD group was most severe after the fish were moved from nursery tanks to raceways in April 1989, when clinical BKD became evident (Figure 1). During the 11 months of raceway rearing prior to release, 17% of the fish in the high-BKD group died, whereas only 5% of the fish in the low-BKD group died. The mortality curve for the high-BKD group in particular followed general trends in water temperature.

The juveniles were tested by the ELISA at regular intervals to follow the prevalence and levels of BKD in the two groups. The greatest separation in

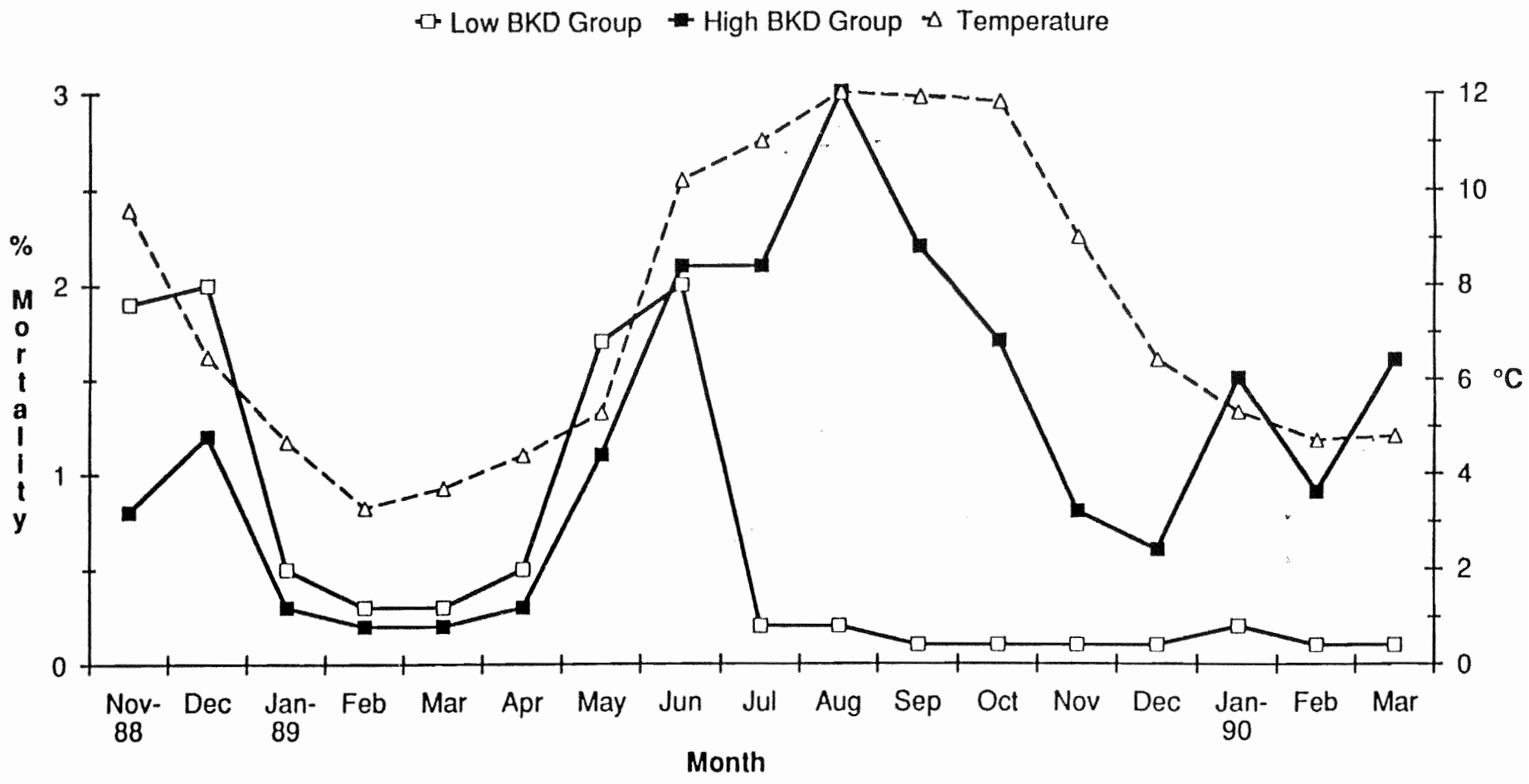


Figure 1. Monthly percent mortality and monthly mean water temperature during hatchery rearing of spring chinook salmon in the two BKD-level groups at Dworshak NFH. Clinical BKD became evident in fish from the high-BKD-level group during July 1989.

the prevalence and levels of the disease between the two groups occurred just before their release from the hatchery (Figure 2). Approximately 85% of the fish in the high-BKD raceways and 62% of the fish in the low-BKD raceways were infected with *R. salmoninarum*. A comparison of the prerelease ELISA profiles showed that 39% of the tested fish from the high-BKD raceways, but less than 1% of the fish from low-BKD raceways, had medium-to-high BKD infection levels.

Before release, approximately 80,000 fish in each group were marked with coded-wire tags to evaluate adult returns. In addition, about 4,500 fish in the high-BKD group and 2,300 fish in the low-BKD group were marked with Passive Integrated Transponder (PIT) tags so that their downriver migration could be monitored by tag detectors at several hydroelectric dams on the Snake and Columbia Rivers. Preliminary data from this monitoring suggested that inriver survival was higher in the low-BKD-level group than in the high-BKD-level group. Differences in recovery of tagged fish became more pronounced as the fish moved downstream, despite the fact that twice as many fish in the high-BKD-level group were PIT-tagged. Cumulative recoveries of PIT-tagged fish from the low-BKD-level group and the high-BKD-level group, respectively, were 31% and 28% at Lower Granite Dam (62 miles from Dworshak NFH), 44% and 36% at Little Goose Dam (110 miles), and 51% and 41% at McNary Dam (215 miles).

From this research, it appears that vertical transmission may be an important mechanism for establishing and maintaining *R. salmoninarum* infections in hatchery populations of spring chinook salmon. ELISA-based brood stock segregation to eliminate progeny from females highly infected with BKD may reduce the impact of this disease, and therefore increase the survival of juvenile spring chinook salmon during hatchery rearing and downriver migration.

ACKNOWLEDGEMENTS

This work was supported by research contract No. E8688047 from the U.S. Army Corps of Engineers, Walla Walla, Washington.

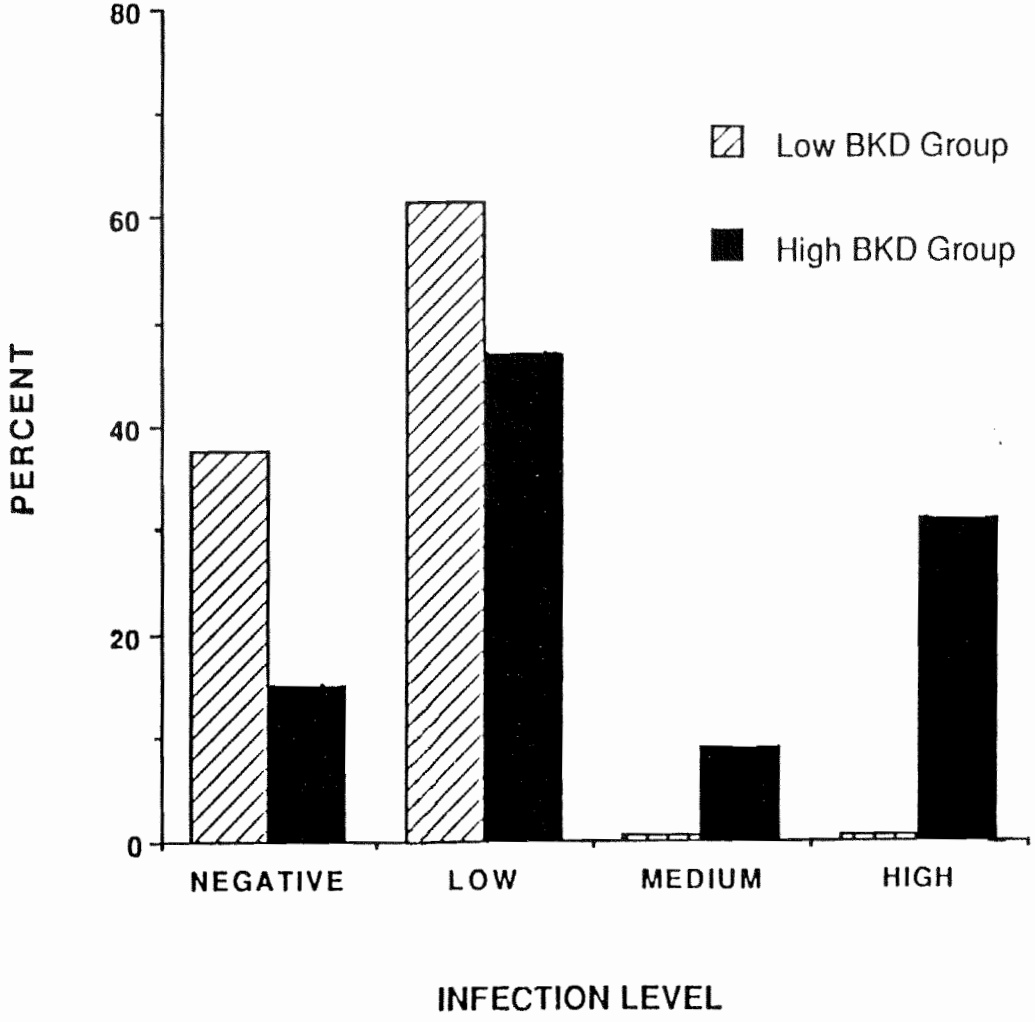


Figure 2. ELISA profiles of the two BKD-level groups after testing 210 fish from each group just before their release from Dworshak NFH in April 1990. Infection levels have been divided into negative fish (based on a comparison with equivalent samples from known negative fish), and positive fish with low, medium, and high infection levels

MANAGEMENT OF FURUNCULOSIS

Jim S. Nelson, Ph.D.
Research and Development
Biomed, Inc.

At BIOMED, we believe in an integrated fish health program. This is not unusual in medicine where a multiple discipline approach to health is practiced. Fish require the same type of care as any other animal. Vaccination can be an important part of a comprehensive fish health program. To determine how vaccination fits into a disease control program, we must understand the disease and as importantly the husbandry practices that impact the disease.

One of the difficult problems facing the aquaculture industry is the management and control of furunculosis caused by the bacterium, Aeromonas salmonicida. Rather than retracing the hundred or so years of history of this disease, let us consider what we can do about it and try to come-up with some solutions.

Furunculosis has both an acute and chronic character. The acute phase can claim as little as 5% of a population or can chronically kill, over time, all of your fish. The chronic problem is a result of the ability of the pathogen to sequester itself in the animal. This is a carrier. The carrier fish is partially immune. Aeromonas salmonicida produces an enzyme which kills immunity producing cells and therefore degrades the immunity in a carrier fish. When the immunity is broken, the harbored pathogen will infect and kill the fish. Then, because of the high load of bacteria present during an infection, the pathogen can infect other fish leading to further mortalities and more carrier fish. This yields cycles of infection. Because of these cycles, we feel no one control approach will be effective.

At all sites with furunculosis, a plan is needed to evaluate the situation. Each site is different and requires a specific plan of attack. For instance, a hatchery in Michigan had a spring source where fish in the spring were suspected carriers. To handle the problem, large rainbow trout were vaccinated and released into the spring. The large trout ate the carriers and the site has been relatively furunculosis free since. We would not necessarily recommend this. However, the same thought process is used when trying to manage the problem elsewhere. This is where a disease management plan and team approach is necessary.

A good disease management team includes all personnel on the farm. Look for possible sites of contamination and discuss them with each other. Make it understood this is in no way incriminatory. You are trying to find solutions to the problem. Have meetings when no disease is occurring to identify what you are doing right and reinforce those practices. Many of these practices are common on most facilities; however, we would like to go over them to show how furunculosis can be managed.

All infectious sources must be eliminated. This includes no common usage of buckets or nets between tanks of fish. Each pond has its own gear. Liberal use of disinfectants is recommended where no net or bucket is allowed to touch

fish unless it has been disinfected. Standing water such as mud puddles or other casual water in empty tanks should be disinfected, drained and dried. When a pond is emptied, it should be immediately cleaned, disinfected and dried. No fish should be transferred to a tank which has not been disinfected immediately before use. Because we often come in contact with fish, our hands and boots should be disinfected.

One of the largest sources of infection comes from the personnel on the site. It is difficult to train and watch yourself and is even more difficult to train and watch visitors on the site. Your friends may be fish farmers who have furunculosis problems. If they come over on Sunday morning to talk and have coffee and feed a few fish, you may be inheriting their problems. It is wise to quarantine your site and not have outside help.

Outbreaks of infection should be stictly monitored. Each pond experiencing furunculosis should immediately be put on approved antibiotics. This requires the isolation of Aeromonas salmonicida to determine if the antibiotic being used is potent against the strain causing the infection. Antibiotic resistance has been repeatedly proven with Aeromonas salmonicida. Monitoring the consumption of antibiotic is critical to determine if therapeutic doses are achieved. Each pond with furunculosis should be hand fed and the weight of medicated diet consumed should be recorded. Dead fish should be removed from the pond at least 4 times per day and recorded using log records at pond side. All dead fish should be disposed of in a manner which will not further contaminate the site.

What we have described so far is how to fight the fire when it happens. Long term solutions should be sought. These include looking at the overall husbandry practices. Look at loading densities and lower densities when possible to dilute the effects of carrier fish. Look at feed conversion rates. Slow growing fish may indicate sick or carrier fish. Once a complete analysis of the facility has been made, then a long term disease management plan can be formulated.

Included in this disease management plan is vaccination. Selection of the vaccine product is critical to achieve relief from furunculosis. Biomed has been developing Aeromonas salmonicida bacterin over the last 10 to 15 years. Biomed must meet USDA product standards which include producing a stable, sterile, safe and potent product. The US fish farmers are protected because Biomed must test its bacterins for sterility, safety and potency; AND the USDA also performs these same tests. This duplication of effort insures safe products for the US consumer. Biomed has choosen not to introduce a fully licensed immersible furunculosis bacterin because experimental products were shown to have short potency shelf-lives. We feel a better product should be licensed than what is presently available now. Autogenous immersible Aeromonas salmonicida bacterins when used fresh have shown efficacy in field use. Autogenous bacterins are made from isolates supplied by the fish farm on a per order basis. Biomed has an USDA license to produce autogenous bacterins. Careful product selection should be of prime consideration when vaccinating against furunculosis.

Vaccinations should be performed to break the cycle of infection. To start this process, brood stock should be vaccinated by injection. Furunculosis is frequently found on the skin. Handling brood fish can transfer the pathogen to the handler. The situation arises while taking eggs where somebody has to go feed fish. They cycle the disease back to young fish continuing the cycle of

infection. Juvenile fish are more susceptible than older fish. All fish on a site should be vaccinated before the spawn of that year are ponded. When furunculosis has been encountered over several years, it may be wise to use multiple vaccinations where swim-ups are vaccinated by immersion and then vaccinated again three of four more times up to 8 grams. These vaccinations should be performed whenever the fish are handled to minimize costs. At 10 to 15 grams, the fish can be injected with bacterin. This should afford your fish a degree of long term immunity.

And this is critical: Do not bring unvaccinated fish onto the site! Any fish carrying furnculosis will reinfect your population. You must break the cycle of infection and vaccination is one of the tools used to accomplish this. The proper application and use of vaccines in fish may not necessarily eliminate the problem under all circumstances but should significantly reduce the dependence on therapeutic drugs and the overall impact of the disease.

What if you have the disease? What is a furunculosis prognosis? Difficult disease problems like furunculosis will not be solved rapidly. Many months of constant surveillance and record keeping will be required to determine the success or failure of the disease management program. Monthly reviews of the problem will be required. Percentages of mortality should be calculated and plans for further vaccinations and antibiotic treatments should be considered. To break the furunculosis cycle of infection, constant vigilance over several years may be required.

Biomed cannot make management decisions for you. However, one of our four fish health experts would be happy to help find solutions to your disease management problems. We feel disease control through vaccination is economically sound when used with other disease control measures. No one method will be completely effective. A well balanced disease control program using all health technology will yield furunculosis as manageable on your farm.

NUTRITION

VITAMIN C REQUIRED FOR FINFISH GROWTH, DEVELOPMENT, AND ADAPTATION

Douglas Ramsey
Rangen Aquaculture Research Center
Hagerman, Idaho

Vitamin C or ascorbic acid is essential in numerous biochemical actions and physiological functions of both plants and animals. It is required for normal growth and development and it plays a role in fish's adaptation to various stressors and diseases. Most animals can synthesize ascorbic acid in sufficient amounts to prevent clinical symptoms of vitamin C deficiency, the condition know as scurvy. However, primates, guinea pigs, fish, shrimp, and some insects, bats, and birds require a dietary source of vitamin C to prevent or reverse scorbutic symptoms. Natural forage fulfills the ascorbic acid requirement of wild fish, but captive populations rely primarily on artificial diets for the necessary ascorbic acid levels. Dietary essentiality of ascorbic acid in these species likely results from an absence of L-gulonolactone ocidase, and enzyme required for biosynthesis of ascorbate from glucose or other simple precursors.

Fish are composed of significant amounts of collagen in the form of bone, cartilage, integument, and other connective tissues. Synthesis of this protein is dependent on ascorbic acid as a cofactor in hydroxylating the amino acids, lysine and proline. Reduced enzyme activity and subsequent impaired collagen formation result in the classic vitamin C deficiency symptoms of scoliosis and lordosis. Other symptoms include poor growth, hemorrhaging due to leaky blood vessels, and impaired wound healing. Prolonged periods of torpor develop within 12 to 14 weeks in rainbow trout on a C deficient diet. Scoliosis becomes evident in trout after 19 to 21 weeks without dietary vitamin C.

Ascorbic acid also serves as a cofactor in hydroxylation reactions involved in excretion of drugs and toxicants. Its role in detoxification of organochlorine pesticides was investigated by Wagstaff and Street in 1971 and other have shown that ascorbic acid activity is required by liver detoxification mechanisms involving cytochrome P450 and arylhydroxylase systems. Mayer et al. (1977) found that exposure to the pesticide toxaphene resulted in reduced levels of whole body vitamin C activity and decreased backbone collagen in fathead minnows and channel catfish. This led to the hypothesis that these hydroxylation reactions may compete for available vitamin C activity.

Ascorbic acid is a strong reducing agent that provides electrons to functional groups of other biochemicals and free radicals found in the aqueous phase of biological fluids. Studies have also shown that ascorbic acid plays a role in iron metabolism through reduction of the ferric ion to the ferrous ion. Hilton et al. (1978) reported increased spleen iron levels in scorbutic fish and liver iron levels that were positively correlated to dietary concentrations of ascorbic acid. These data led to the conclusion that ascorbic acid may control the release of iron within spleen tissues, thereby affecting the redistribution of iron stores. Free radical formation occurs in the epidermal layers of fish exposed to ultraviolet radiation from the sun. Free radicals cause the condition in fish known as sunburn. Reduction of these injurious chemical species becomes important for the maintenance of fish health.

These biochemical processes involving vitamin C have been shown to affect a number of physiological functions. Effects of dietary ascorbic acid on growth have been studied extensively in carp, catfish, salmonids, shrimp, tilapia, and other species. Figure 1 demonstrates the effect on growth in rainbow trout without dietary vitamin C. The data also show that relatively small amounts of vitamin C are needed for normal growth and development. However, under the stressful conditions of culture, fish require more ascorbic acid to successfully resist disease and maintain health.

Morphological consequences of a C deficient diet have been demonstrated. Incomplete hydroxylation of lysine and proline results in the condition commonly known as broken back syndrome. Scoliotic and lordotic fish in production are normally culled out. Otyher resulting deformities include crenulated and incomplete opercles, wavy cartilage in gill arches and filaments, and misshapen cartilage of the head.

Vitamin C action has been conclusively linked to reproduction in fish. Halver et al. (1975) showed that the eggshell membrane of rainbow trout is an active site of collagen synthesis. This was done through the observation that radioactive material was present in the eggshells after feeding radio labelled ascorbic acid to mature female trout. Vitamin C deficient females possessing leaky blood vessels may contaminate eggs during the stripping process, thereby reducing fertilization success. Sandnes et al. (1984) offered that conclusion when they observed a significantly lowered hatching success in eggs of broodstock fed a C deficient diet versus those fed adequate vitamin C.

A growing body of evidence shows that increasing concentrations of dietary vitamin C provides a higher level of physiological adaptation to deal with stressors such as infectious diseases. Li and Lovell (1985) reported correspondingly higher survival in channel catfish fed increasing concentrations of ascorbic acid in the diets, following a challenge of *Edwardsiella ictaluri*, the bacteria causing enteric septicemia in catfish. Fish fed the highest level (3000 ppm) had significantly enhanced antibody production and complement activity. Navarre and Halver (1989) obtained similar results with rainbow trout experimentally infected with *Vibrio anguillarum* bacteria. In their study, improved survival and increasing levels of antibody correlated directly with dietary levels of ascorbic acid from 0 to 2000 ppm.

The specific interactions between ascorbic acid and components of the immune system are still unclear. However, these studies show that vitamin C is responsible for enhancement of innate and adaptive immunity in fish. Innate immunity derives from cell-mediated functions such as phagocytosis, one of the first steps in eliminating pathogens and other foreign material from the body. End products of phagocytic processes are harmful to fish bet they are detoxified by ascorbic acid. Other ascorbic acid involvement includes steroid synthesis and active transport of ascorbic acid into white blood cells for various immune functions.

Satyabudhy et al. (1989) described an immunity response to increasing levels of ascorbic acid in rainbow trout experimentally injected with infectious hematopoietic necrosis (IHN) virus. They reported increasing resistance of 6-week-old trout to a lethal challenge of IHN virus in this study. Trout receiving the highest dietary level of vitamin C (a stable, phosphorylated form, ranging from 20 to 320 ppm ascorbate-equivalent) in both vaccinated and unvaccinated

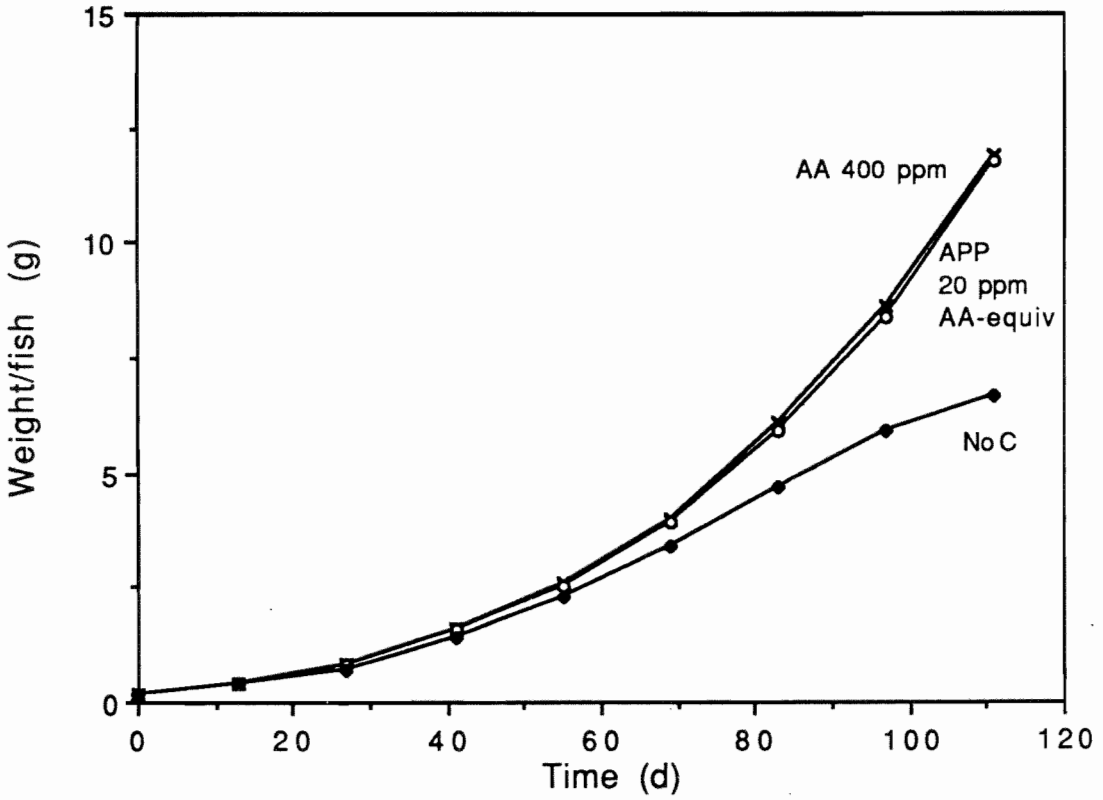


Figure 1. Weight of trout fed casein-base diets with 400 ppm AA, 20 ppm APP (AA-equivalent), or no vitamin C. Vitamin levels are those added to the mash. (From Grant et al., 1989)

populations experienced proportionately lower mortality during the 21-day post-challenge period (Figure 2).

Most vitamin requirements by fish are affected by size, physiological state, environmental factors, and nutrient interrelationships. When determining appropriate supplemental levels for cultured animals of any species, nutritionists should also take into account the desired response level and the whole body or indicator tissue concentration associated with that response. The concentration range of vitamin C required to sustain optimum growth and feed conversion in foodfish may be less than what would be required for maximal adaptive potential such as disease resistance and tolerance to environmental stressors, as shown in Figure 3. Maximum benefits may be obtained as we approach dietary levels depicted as "0" or overage (Figure 3), where excess vitamin is excreted or neutralized. However, we do not know the side effects of pharmacologic doses of vitamin C in fish nor fully understand their requirements. Our current knowledge states that vitamin C requirements in fish generally fall in the range of 50 to 200 ppm.

Vitamin C research has experienced two major breakthroughs in recent years. The most recent one is the development of stable forms of the vitamin. We are now able to provide prescribed levels of ascorbic acid without adding excessive amounts to compensate for the loss during feed milling and storage. Two products (phosphorylated analoges of vitamin C) have shown efficacy in bioavailability and good stability (Grant et al. 1989). The second breakthrough was the development of an accurate method for vitamin detection in feeds and fish tissues. These tools provide the capability to attain higher levels of understanding of vitamin C requirements in fish.

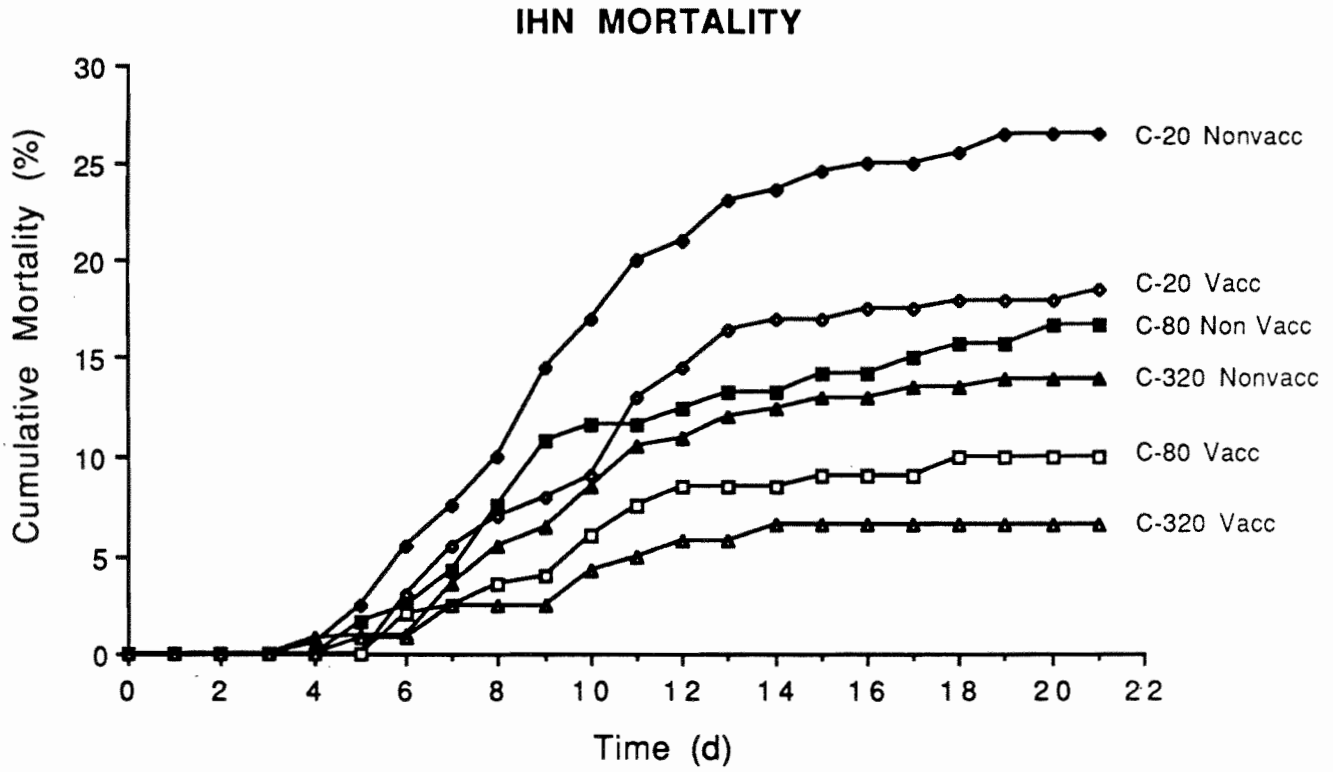


Figure 2. Cumulative mortality of rainbow trout challenged with IHN virus and fed diets with AsPP as vitamin C source. (From Satyabudhy et al., 1989)

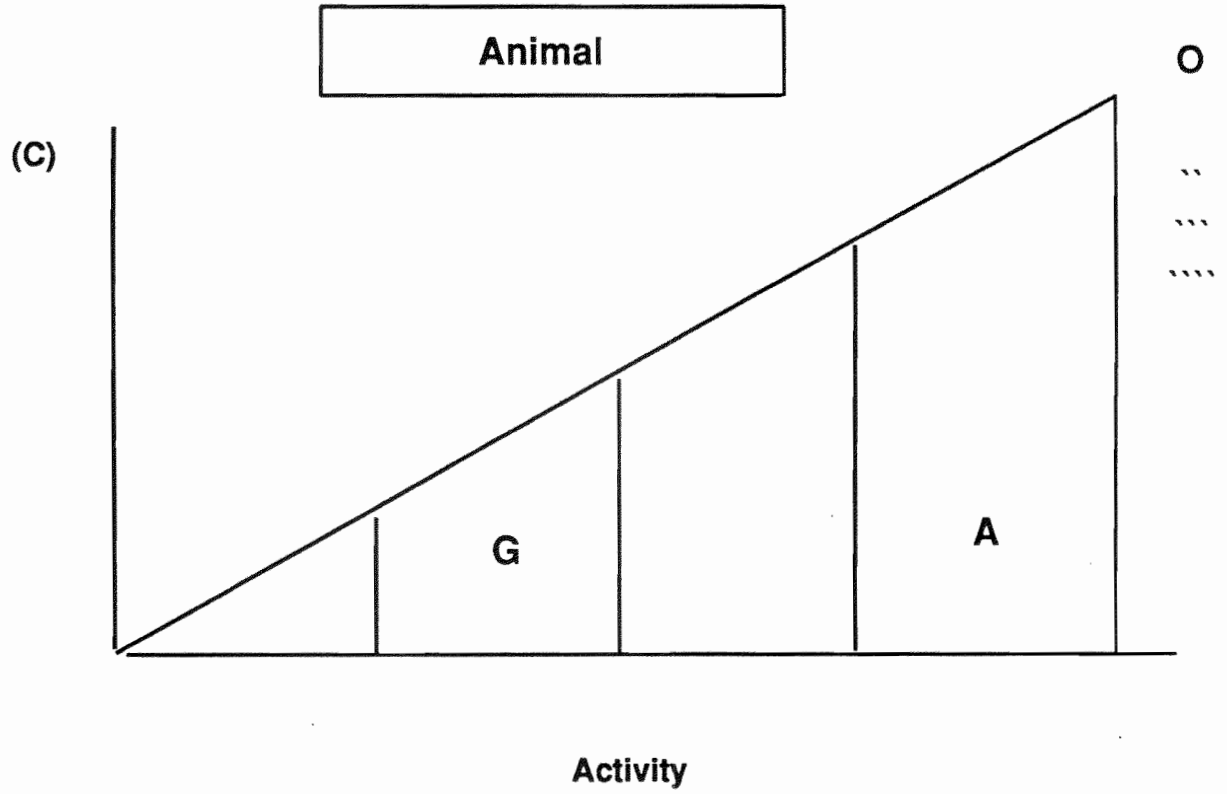


Figure 3. Ascorbic acid tissue concentrations (C) with respect to whole animal activities-growth (G), and adaptation (A). Excess vitamin (O) is excreted from body. (From Vitamin Technologies International, Buhl, Idaho, USA 1989)

LITERATURE CITED

Grant, B.F., Seib, P.A., Liao M.L. and K.E. Corpron. 1989. Polyphosphorylated L-ascorbic acid: a stable form of vitamin C for aquaculture feeds. J. World Aqua. Soc. 20(3):143_157.

Halver, J.E., Smith, R.R., Tolbert, B.M. and E.M. Baker. 1975 Utilization of ascorbic acid in fish. In: C.G. King and J.J. Burns (Editors), Second Conference on Vitamin C. Ann. NY Acad. Sci. 258:81-101.

Hilton, J.W., Cho C.Y., and S.J. Slinger. 1978. Effect of graded levels of supplemental ascorbic acid in practical diets fed to rainbow trout (*Salmon gairdneri*). J. Fish. Res. Board Can. 35:431-436.

Li, Y. and R.T. Lovell. 1985. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr. 115:123-131.

Mayer, F.L., Mehrle, P.M., and W.P. Dwyer. 1977. Toxaphene: Chronic toxicity to fathead minnows and channel catfish. Environ. Prot. Agency (US, Ecol. Res. Ser. EPA-600/3-77-069). 49pp.

Navarre, O. and J.E. Halver. 1989. Disease resistance and humoral intibody production in rainbow trout fed high levels of vitamin C. Aquaculture. 79:207-221.

Sandnes, K., Ulgenes, Y., Braikkan, O.R., and F. Utne. 1984. The effect of ascorbic acid supplementation in broodstock feed on reproduction of rainbow trout (Salmo gairdneri). Aquaculture. 43:167-177.

Satyabudhy, A.M.A., Grant, B.F., and J.E. Halver. 1989. Effects of L-ascorbyl phosphates (AsPP) on growth and immunoresistance of rainbow trout (Onchorhynchus mykiss) to infectious hematopoietic necrosis (IHN) virus. Pro. Third Int. Symp. on Feeding and Nutr. in Fish, Toba, Japan, Aug 28-Sept 1, 1989. pp 441-426.

Wagstaff, D.J. and J.C. Street. 1971. Ascorbic acid deficiency and inductions of hepatic microsomal hydroxylative engymes by organochlorine pesticides. Toxicol. Appl. Pharmacho. 19:10-19.

DIGESTION AND ABSORPTION OF L-ASCORBYL-2-SULFATE BY RAINBOW TROUT

J.E. Halver and S. Felton
School of Fisheries, University of Washington
Seattle, Washington

L-ascorbyl-2-sulfate dihydrate, provided by Pfizer Inc. Chemical Division, was incorporated into standard test diet H440 and fed to rainbow trout Oncorhynchus mykiss average weight 70 g confined in circular tanks supplied with 11-12° water at 5 L/minute. After 5 days, fish fed diets containing L-ascorbic acid (C_1) or L-ascorbyl-2-sulfate (C_2) were sacrificed and fecal wastes, blood, and liver were assayed for C_1 and C_2 content using an improved HPLC assay technique. Assuming standard digestibility coefficient of 0.85 for H440 diet consumed by rainbow trout of this size in this water temperature, the mean absorption coefficient for C_2 used in this procedure was about 70% of the C_2 fed. Discrete quantities of both C_1 and C_2 were detected in the blood of C_2 fed fish, indicating absorption and conversion of some C_2 material into C_1. Extension of trials to 10 days and to 21 days plus subsequent sampling of feces and tissues confirmed the absorption coefficient for C_2 from the test diet to be greater than 2/3 of the material consumed.

METHODS AND MATERIALS

Thirty-six rainbow trout Oncorhynchus mykiss average weight 70 grams (50-80 g) were acclimated to test diet H440 feed, containing 50 mg of L-ascorbic acid per kg dry weight (see Table 1). All fish were held in 3' x 3' circular tanks, with self-cleaning center drains, supplied with 12-12°C water flow at 3 liters per minute. After 2 weeks pretreatment, fish were feeding avidly on diet and were subdivided into 3 lots of 12 fish each. Lots were designated P89A (no C diet), P89B (C_1 at 1000 mg/kg diet), and P89C (C_2 at 500 mg/kg diet). Fish were fed daily to satiation at 0700-0800 hours and 1600-1700 hours for 6 days per week.

On day 5, three fish from each lot were removed, placed in tricaine methane sulphonate (MS222) until quiescent, then sacrificed for tissue and fecal sample analysis. Fish were stunned by a blow to the head, the caudal peduncle was amputated, blood was collected into a tared test tube and allowed to clot. Fish were opened at the midline and liver excised and placed in a tared container, and the fecal sample was collected in a tared watch glass by expressing the posterior one cm of large intestine contents.

All samples collected were held on crushed ice until homogenized (maximum 10-30 minutes). Samples were made to 10 g with distilled water, then homogenated with a Polytron homogenizer for one minute. Samples were deproteinized by treatment in a microwave oven for one minute.

Treated samples were centrifuged, supernatant collected, then precipitate made back to volume with water and residue rehomogenized. Rehomogenized samples were microwaved for one minute, then centrifuged and supernatant combined with

Table 1. Test Diet H440

Water-Soluble Vitamin Test Diet H-440[a]

Complete test diet (g)			Vitamin mix (mg)		Mineral mix (mg)	
Vitamin-free casein		38	Thiamin·HCl	5	USP XII No. 2	Plus
Gelatin		12	Riboflavin	20	$AlCl_3$	15
Corn oil		6	Pyridoxine·HCl	5	$ZnSO_4$	300
Cod liver oil		3	Choline chloride	500	CuCl	10
White dextrin		28	Nicotinic acid	75	$MnSO_4$	80
α-Cellulose mixture[b]		9	Calcium pantothenate	50	KI	15
α-Cellulose	8		Inositol	200	$CoCl_2$	100
Vitamins	1		Biotin	0.5	per 100 g of salt mixture	
	9		Folic acid	1.5		
Mineral mix		4	L-Ascorbic acid	100		
Water		200	Vitamin B_{12}[c]	0.01		
Total diet as fed		300	Menadione (K)	4		
			α-Tocopherol[d] acetate (E)	40		

[a] Diet preparation: Dissolve gelatin in cold water. Heat with stirring on water bath to 80°C. Remove from heat. Add, with stirring, dextrin, casein, minerals, oils, and vitamins as temperature decreases. Mix well to 40°C. Pour into containers; move to refrigerator to harden. Remove from trays and store in sealed containers in refrigerator until used. Consistency of diet adjusted by amount of water in final mix and length and strength of beating.

[b] Delete 2 parts α-cellulose and add 2 parts CMC for preliminary feeding.

[c] Add vitamin B_{12} in water during final mixing.

[d] Dissolve α-tocopherol in oil mix.

first extraction supernatant. An aliquot of the sample extracted was filtered through a 0.45 micron preparatory filter disk. An appropriate size aliquot (10-50 mL) was injected into the Spectrophysics model 8000 HPLC and passed through 2 tandem C_{18} columns.

Resolution of peak elution times and areas, after electrochemical detector for C_1 and uv detector set at 254 nm for C_1, C_2, and other uv sensitive compounds, was calculated by a coupled computer using reference standards of C_1 and C_2 for elution times and peak heights. Detection of C_1, C_2, and other compounds was recorded for quantitative content of each sample from each lot (see Table 2).

RESULTS

A modified extraction technique, using the microwave oven for 2 one-minute heating periods, then immediate cooling of the sample, indicated little loss of C_1 and no hydrolysis of C_2 during this rapid extraction procedure. Extraction with 5% trichloracetic acid or 5 perchloric acid indicated continuous hydrolysis of C_2 and other C derivatives during the extraction and subsequent preparative operations.

To validate the conversion of excess dietary C_1 into C_2 and subsequent excretion of C_2, leading to an overestimate of material absorbed, the P89B groups of fish were also assayed. Pooled blood samples from 3 fish, pooled fecal samples from 3 fish, and 3 individual fish livers were assayed for C_1 and C_2 content. Assay values of fecal waste and tissues obtained on day 5 were confirmed in a subsequent set of assays at day 15 and day 21 conducted of fish fed the various diet treatments for additional periods. Pool samples of fecal waste, fish liver, and fish blood were assayed for C_1, C_2, and other C intermediates.

Table 2 summarizes the assay results obtained. Assuming a standard digestibility coefficient of 0.85 for diet H440 fed to rainbow trout of this size in 10 to 12°C water systems, it was possible to estimate the absorption coefficient for C_2 used in these experiments. A conservative factor of 0.15 for undigested diet, was used in these calculations, and theoretically the unabsorbed C_2 from the diet would reside in this 15% of diet residue present in the feces. In the first 5 day experiment, it was necessary to pool the fecal samples to obtain a reasonable sample size for the assay. This pooled fecal sample contained approximately 1000 ug of C_2/g dry fecal waste, indicating .66 (66%) of the fed C_2 had been absorbed. Most of the digestible material capable of being absorbed, should have been absorbed, as fish had been fed to satiation 2 times daily.

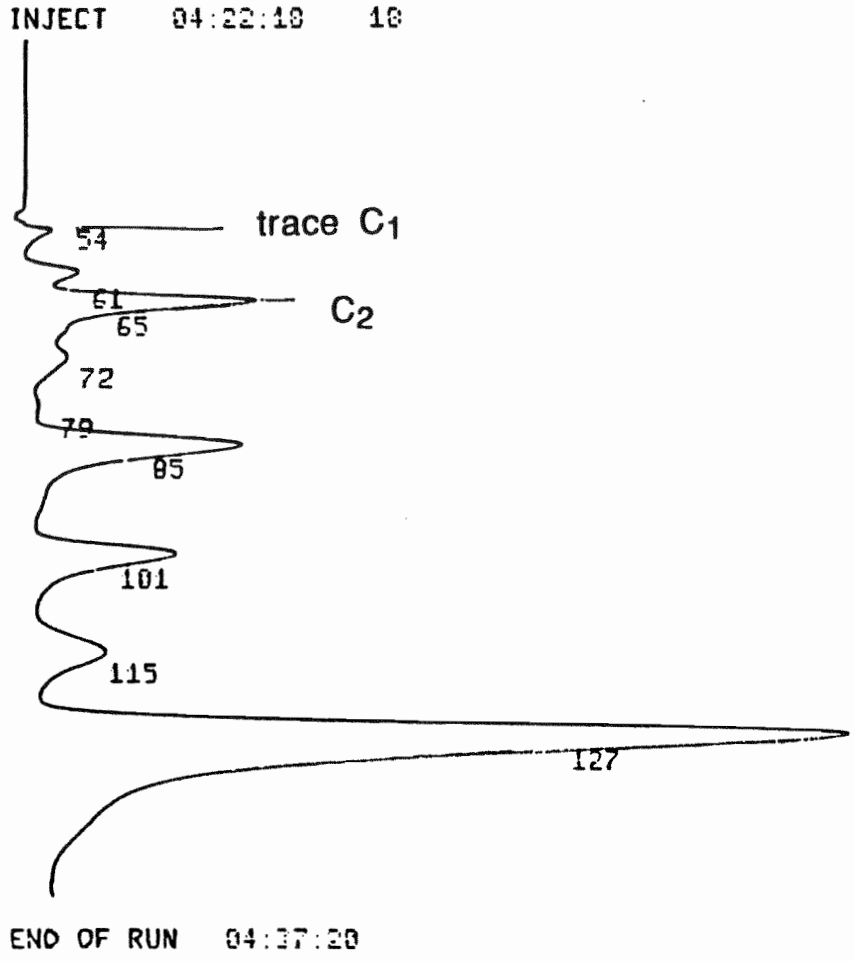
DISCUSSION

Approximately 4 hours after the morning feeding, the animals were removed, anesthetized, and sacrificed for sample collection. The fecal waste assays during the first 5 day experiment period were subsequently confirmed with fecal waste concentrations of C_2 varying between 800-1000 ug C_2/g dry fecal waste.

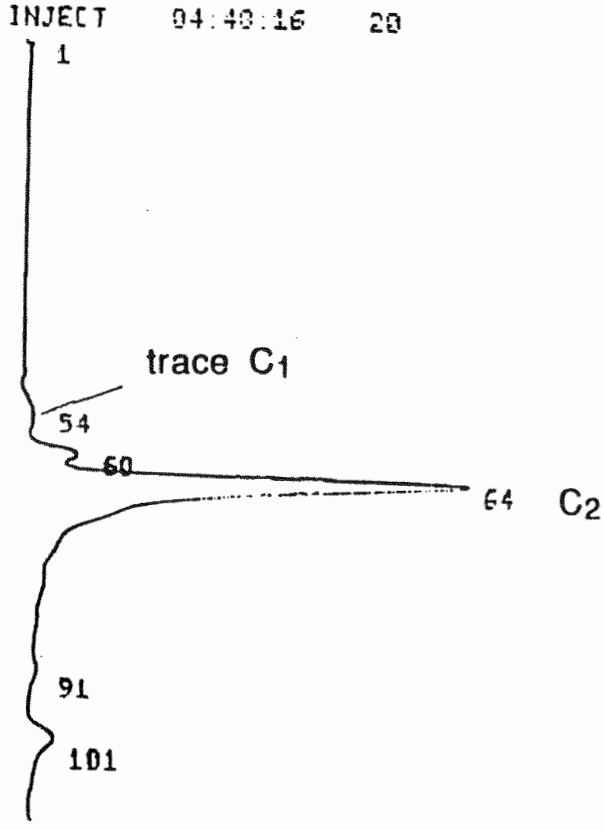
TABLE 2

ASSAYS FOR C_1 and C_2 (ug/g sample)

Code	Tissue	Day 5		Day 15		Day 21	
		C_1	C_2	C_1	C_2	C_1	C_2
P89A	Blood	trace	trace	trace	trace	trace	trace
	Liver	trace	16	trace	trace	trace	trace
	Dry feces	215	112				
P89B	Blood	trace	trace	4	trace	4	32
	Liver	106	60	33	trace	63	trace
	Dry feces	48	870	43	335	50	380
P89C	Blood	trace	482	4	13	5	48
	Liver 1	11	11	19.3	64.8	17.4	16.4
	Liver 2	(pooled)		20.3	37.2	(pooled)	
	Liver 3			15.5	28.5		
	Dry feces	44	997	n/d	1210	n/d	798
P89C Diet Dry 550 ug C_2 (calc)			536	not run		n/d	530



Example of P89C fed fish--blood sample

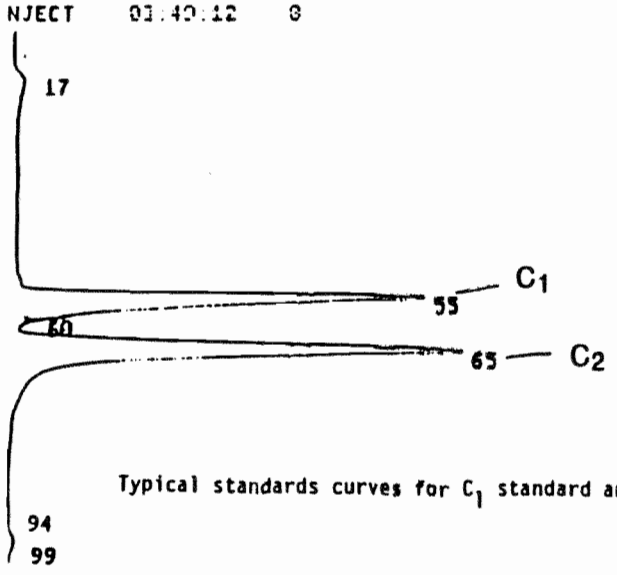


Example of P89C fed fish--feces sample.

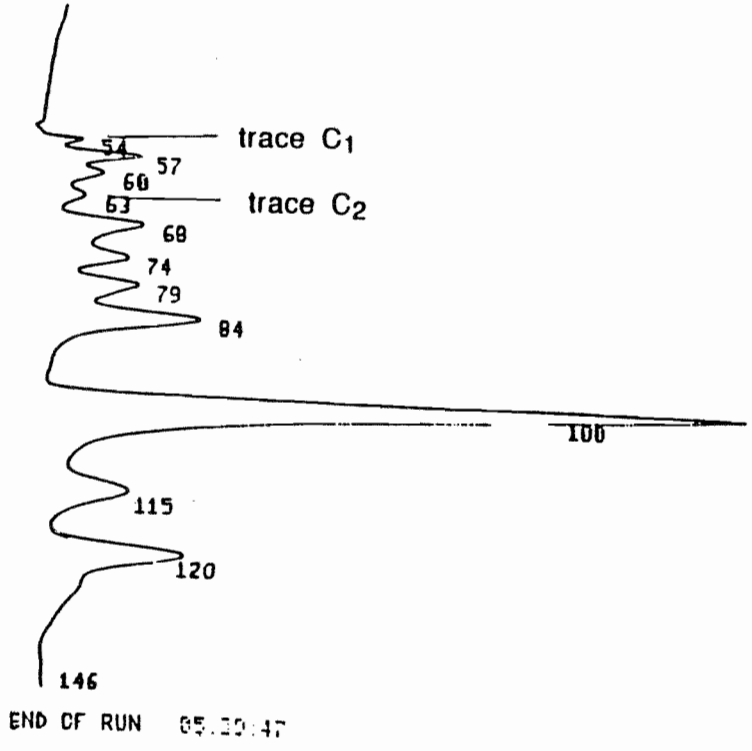
INDIVIDUAL FISH NUMBER 890011 HPLC Assay (Microwave extraction)

Fish had been fed diet P89C containing 500mg C_2/kg diet(536 ug/g dry wt)

This dietary intake level is approximately 2.5 vitamin C equivalent requirement



Typical standards curves for C_1 standard and C_2 standard combined sample.



Example of P89C fed fish--Liver sample

This type of variation is expected, since food passage through the gut, with concentration in the lower gut, may vary with activity, amount of diet eaten, and biological variation between fishes. Assay of the diet for C_2 content indicated 536 ug of material/g of dry diet, again within experimental error, and the figure of 536 was used as the calculation factor, to be coupled with the 85% digestibility factor for H440 diet. Therefore, the theoretical calculation would be digestibility coefficient = 1- ugC_2 in feces, divided by 536 x 6.6. Individual fish fecal waste varied from 400-1200 ug C_2/g dry feces, and from 800-1200 from pooled 3 fish fecal samples. Mean values were 1002 ug C_2/g dry feces, which resolves to 1- 1002/3538 = 0.72, or 72% of material absorbed.

To validate the conversion of excess C_1 into C_2, which has been postulated on the basis of radioactive ^{14}Carbon and ^{35}sulfur labeled C_1 and C_2 pulsed dose experiments, one would expect excretion of some C_2 in the feces of C_1 fed fish. Therefore, the P89B groups of fish were also assayed. Dietary levels of C_1 was approximately 10 times the requirement of rainbow trout of this size under these environmental conditions. The assays disclosed about 800 ug of C_2 was present per g dry feces of fish fed these levels of C_1 for two 6-day feeding periods. These results would indicate some of the C_2 found in the fecal waste may have originated from absorption of C_2 into the physiological system of the fish, subsequent hydrolysis into the C_1 found in the blood of the fish, and excess C_1 or C_2 then excreted by the fish. If these values were subtracted from the C_2 content found in the C_2 fed fish, the absorption coefficient would increase correspondingly, and probably would be in the area of about 80% or more of the C_2 fed. These experiments did not measure biological efficacy of C_2 as a C source, but did demonstrate that C_2 was absorbed, passed into and through the physiological system, was partially hydrolyzed at least to provide the elevated C_1 in the liver of the fish, and some of the C_2 fed was higher (a) not absorbed, or (b) absorbed and then excreted. Regardless, the absorption values conservative estimate at about 75% of the C_2 absorbed from these diets, would not be decreased. Under these experimental conditions, and using these diet treatments, L-ascorbyl-2-sulfate appears to be readily absorbed from the diet as this material passes through the gastrointestinal tract of rainbow trout. Typical elution graphs for C_2 in diet and in fecal wastes are enclosed.

Note: Estimated DC at 0.85 for H440 leaves 0.15 of diet as feces.
Assume all excreted and unabsorbed C_2 remains in feces.
Assume digestion rate is constant for each fish(may vary with size and individual
Assume last 1 cm of large intestine contents represents feces to be excreted.
With these assumptions DC for C_2 in diet for D5, D15 and D21 was:

1 - ug C_2 in dry feces/(6.6 x 536).

D5 DC = 1- 997/(6.6 x 536) = 0.72 or 72% absorbed.

D15 DC = 1- 1201/3538 = 0.66 or 66% absorbed.

D21 DC = 1- 798/3538 = 0.77 or 77% absorbed.

Mean value found in all fecal samples was 1002 ug C_2/ g dry feces.
Mean DC = 1 - 1002/3538 = 0.72 or 72% absorbed.
Analytical detection reliability about 2 ug/ g sample.

PANEL DISCUSSION: GENE CONSERVATION - WHAT DOES IT MEAN TO HATCHERY PRACTICES, A NORTHWEST UPDATE

GENE CONSERVATION-WHAT DOES IT MEAN TO HATCHERY PRACTICES-A NORTHWEST UPDATE

James R. Graybill
Mt. Hood Community College
Gresham, Oregon

Introduction to Panel Discussion

It seems as though at least once a week The Oregonian carries articles on the health and well being of our hatchery system. (See "Do hatcheries help or hurt salmon?", The Oregonian, Science Section, November 1, 1990) There are other articles about the "Salmon Summit" or speculation on what will happen if salmon in Idaho are listed as threatened or endangered. There are many reasons for the decline in the numbers of fish returning to the Snake River or other river systems, but it seems that many folks are pointing the accusing finger at the management policies and in particular genetic policies of our hatchery systems. For example, there are a few papers concluding that hatchery fish are not as capable of survival as wild fish. This has been partially responsible for "wild fish" policies in Oregon at least.

Numerous fish biologists have criticized hatchery practices that result in inbreeding, small population founder effects, and selection (intentional/unintentional) all of which result in a loss of genes at numerous loci. Also criticized are stock transfers which may result in hatchery fish spawning with wild fish. Conservation of genes or conservation of genetic variation is the aim for a wild or hatchery population that must cope with varying and rapidly changing environmental situations, particularly during the last fifty years. The media, public and fishery scientists are asking hatcheries to become aware of genetic principles which will lead to sound management of the hatchery product.

It is difficult for us to defend the current or future hatchery policies if we do not know what they are. Therefore, this panel will inform us of the hatchery practices that conserve genes that are already in place and some newer practices and policies that will be used in the future by their state or agency.

Other states or provinces have their own genetic policies, but in the interest of time we have had to limit the present discussion to the Columbia River and adjacent regions.

ARTIFICIAL PRODUCTION UNDER OREGON'S WILD FISH MANAGEMENT POLICY

Kathryn Kostow, Gene Resource Program Leader
Oregon Department of Fish and Wildlife
Presented by John Leppink

Oregon's Natural Production and Wild Fish Management Policy was adopted by the Oregon Fish and Wildlife Commission in January, 1990. The policy was adopted for the purpose of rebuilding natural fish production and conserving the genetic resources of wild fish. The policy was adopted as an Administrative Rule therefore implementation of it is mandated by law.

The Wild Fish Management Policy applies to Oregon's anadromous salmonids, trout, whitefish, sturgeon, and listed nongame species. "Wild fish" is defined as any naturally spawned fish belonging to an indigenous population of these species. The policy also directs the Department to work at the level of breeding populations. A breeding population is defined as a group of individuals with a 90 percent or higher probability of breeding with each other than with others outside of the group. Gene flow between breeding populations is recognized to occur in many cases. Therefore this approach does not require proof that genetic differences exist between populations in order for them to be identified as separate populations.

The Wild Fish Management Policy states that the protection of genetic resources shall be the priority in the management of wild fish. The policy addresses habitat, harvest, hatchery practices, and population introductions. The policy also mandates the development of a Gene Resource Conservation Policy and program. This presentation focused on those parts of both policies that relate to artificial production.

Hatchery Practices

The Wild Fish Management Policy recognizes that interbreeding of hatchery fish and wild fish poses risks to conserving and utilizing the genetic resources of wild populations. These risks will be limited by controlling gene flow from the hatchery populations to wild populations. Implementation options include:

* Eliminating the release of hatchery fish;

* Release fish that can be reproductively isolated from the wild population, limiting the portion mixing with the wild population to no more than 10% of the wild population size;

* Release fish that are genetically similar to the wild population and limit the number allowed to spawn naturally to no more than 50% of the wild population size.

A detailed definition of "genetically similar" is under development. The policy currently includes some guidance:

* The hatchery population lineage originates from the wild population;

* Wild fish are incorporated into the hatchery population each generation;

* Genetic changes in the hatchery population caused by genetic drift and artificial selection are avoided;

* Duration of the artificial program will also be considered as an indication of domestication. Additional criteria may also be added.

Fish Introduction

The policy also directs the Department to oppose fish introductions that will cause mortality to wild fish from competition, predation, or disease that will reduce the wild population to an unacceptable size as determined by natural production objectives. A minimum allowable population size of 300 fish is included in the policy.

Implementation Goals

ODFW has several implementation goals for the next few years:

1. Preliminary delineation of wild populations has been completed. Oregon has over 1,500 wild fish populations covered by the policy. These populations are currently under review to determine if they are in compliance with the policy. Breeding populations are being grouped for some management considerations, such as our sensitive listings.

2. Current hatchery populations are being reviewed to determine lineages, ages of captivity, current hatchery practices.

3. Hatchery practices are being evaluated and improved. Detailed guidelines for each hatchery program will be developed.

4. Hatchery/wild ratios on natural spawning grounds are being measured using fin marks, tags and scale analysis, and intensive spawning ground surveys.

5. An ODFW Gene Resource Conservation Policy is being developed.

The Gene Resource Conservation Policy

The Wild Fish Management Policy mandates the development of a Gene Resource Conservation Policy. This policy and program are currently under development. It is expected that the objective of the policy will say something like:

"Insure the stability of Oregon's natural aquatic ecosystems and the persistence of it's native fish species by conserving the current reproductive capacity of, and the potential for evolutionary adaption by, Oregon's wild fish. This will be accomplished by maintaining current population fitness and appropriate levels of genetic variation in wild populations."

The intent of this wording is to recognize that evolution is the process that is being managed, that genetic change is inherent in evolution and will not be avoided, but that it is the state's objective to avoid extinction of native species and destablization of ecosystems.

The Gene Resource Conservation Policy will also provide the conceptual guidelines for a Gene Resource Conservation Implementation Plan. Possible components of the Implementation Plan include:

1. A systematic risk analysis method for assessing the potential risks of management actions, including artificial production programs.

2. A genetics monitoring program that will provide data through time on the issues reviewed in the risk assessment.

3. A genetics research plan.

4. Hatchery operational guidelines specific for each hatchery program.

5. Genetic guidelines for the development of recovery plans that will be applied to populations that have declined to unacceptable levels. The guidelines will require that a limiting factor analysis be conducted before artificial production is used.

6. Guidelines for determining that wild populations are extinct and for designing reintroductions.

7. Genetic management guidelines for Oregon's species and subbasin plans.

Conclusion

The intent of Oregon's Wild Fish Management Policy is to insure that the state's native fish species will be managed for sustained, natural production in the state's lakes and rivers. Implementation of the policy will take time, funding, commitment, flexibility, public support, and, most importantly, a cooperative effort between fish managers and hatchery managers.

GENE CONSERVATION-WHAT DOES IT MEAN TO HATCHERY PRACTICES, A NORTHWEST UPDATE

Tom Sheldrake
United States Fish and Wildlife Service

I will start by reading the service fish gene resource protection policy.

> In implementing its mission to conserve, protect, and enhance fish and wildlife and their habitat for the continuing benefit of people, the Fish and Wildlife Service shall take appropriate measures to ensure the protection of gene resources of fish, the genetic variability within and between populations of fish species, and site specific adaptations within fish populations. As the basis for protecting gene resources of fish, each species and race shall be separated into populations based on hydrogeological units, gene frequency analyses, meristic characters, life histories, behavioral traits, or other suitable information as available.

And we do this! When I started in fish hatcheries thirty years ago I remember a policy that required random mating. I can remember long discussions over whether we could use the fungused males first and other genetic risk versus practical operation arguments. Over the years we have adopted other policies regarding minimum number of spawning adults, and a fish transport policy that instructs drivers to return fish to the hatchery or destroy them if they cannot be released at an approved site.

Our latest list includes:

A spawning ratio of one female with one male, and a spawning population of at least 200 males and 200 females.

Disease inspection of individual adults, and single female egg incubation.

All eggs water hardened in Iodophores.

Eggs from disease positive parents will not be culled and destroyed, but maintained in separate rearing units to decrease horizontal transmission.

Fish excess to the hatchery capacity will not be released over naturally reproducing populations, and only smolts released at the hatchery site.

Adults selected for production and future broodstock when excess adults are available are selected from all segments of the run.

Most Service hatcheries are mitigation facilities with one stock of fish, no habitat, and all progeny are released at the station so it's pretty easy for us to comply with these guidelines. In the interest of generating some panel discussion I will give you some examples of when we don't comply at some of our stations.

Spring Creek is a station where a lot of fish become ripe at the same time so can't always put only one female per bucket, and still spawn them all. They have 277 stacks of 16 tray Heath incubators and still have to put more than one female per tray. We make exceptions here because it is a recognized artificial hatchery stock, all their production is released into the tacks pool as smolts, have no serious infectious disease problems, and over the years have had almost no straying of returning adults outside of the pool.

Little White is another example. Here for some reason we have poor survival of males in the wild. To get the 1 on 1 ratio we would have to collect 2600 adults to acquire the number of males to mate with 450 females. We make an exception here in the interest of the fishery, and with the same reasoning as Spring Creek.

On the other end of the scale we spend one fourth of the Warm Springs budget to externally mark the hatchery fish so that the returning adults can be separated from the naturally spawning component of the run in the Warm Springs River, and sacrifice some hatchery capacity to accomplish the goal of maintaining the hatchery stock as similar genetically to the wild stock as possible.

GENE CONSERVATION

John Kerwin, Hatchery Resource Manager
Washington Department of Wildlife

There has been little, if any, accountability in fish genetics over the history of fish culture. Generally, it will take at least 4-5 generations for damage to exhibit itself. That will equal at a minimum 12-15 years by that time and the staff will have changed. A historical perspective of some of the activities that the Washington Department of Wildlife has been involved in and our current activities is summarized as follows.

HISTORY

SOUTH TACOMA WINTER STEELHEAD

In 1945, Clarence Pautzke, John Johansen, Charles Foster, and Tom Inions began a new steelhead program at the South Tacoma Hatchery. They used a 25 x 25 foot wooden box at the ladder at Chambers Creek. Arriving steelhead were taken from the trap from February through April and transferred to the nearby South Tacoma Hatchery where water temperature was a constant 56°F. The constant warmer water caused earlier maturation, thus producing eggs much earlier than would have been the case in their natural stream environment. During the 1940s and 1950s, fish were often held for 2 years prior to release.

By holding fish in warmer water at South Tacoma and selecting for the earliest maturing fish, it soon became possible to rear a steelhead to smolt size in one year. Through the continuous selection of early maturing fish, the majority of eggs are presently taken in December, January, and early February (runs typically enter in December and January). Wild stocks currently enter late February through June with peak spawning generally in April. Average fecundity is 4,443 (range 7,425 to 2,562).

SKAMANIA SUMMER STEELHEAD

The first adults were trapped from the Washougal River during 1957. Due to the small number of steelhead available, additional adults were trapped from the Klickitat River.

Adult steelhead were continuously subjected to a selection process for early gonadal maturation. Eggs were originally taken in March and April. Difficulties with achieving a suitable size caused the selection of those fish that matured earlier during each successive spawning season.

During 1970, earlier eggs were obtained through elongating the photoperiod with banks of spotlights. The lights were turned on in early December with males being subjected to a photoperiod two weeks earlier than the females.

In 1971, the hatchery manager began a specific selection process to produce "higrade" adults. Approximately 10% of the largest and esthetically pleasing females were selected and spawned with a similar single male. These fish were marked and reared separately. The goal of the program was to produce a larger percent return of three year ocean fish and thus shift the age structure from saltwater residency of 1, 2 and 3 years to predominantly 2 and 3 years.

LYONS FERRY HATCHERY

The ultimate goal for this program was to return like fish, in place and time to the Snake River. With that goal in mind, returning adult steelhead are trapped beginning in September with the last returns trapped in December (peak returns are generally late - September/early October). Adults are segregated into holding ponds by month of return.

There exist three adult holding ponds. Spawning is random with a percentage of fish that returned from each month equal to the percentage of the total return spawned. This is done to maintain run timing and avoid potential genetic impacts.

The Washington Department of Wildlife works with four different types of broodstocks. They can be broken down as follows:

* ANADROMOUS - Steelhead, Searun Cutthroat, etc.

* CAPTIVE RESIDENT - Rainbow, Brown, Brook, Cutthroat, etc.

* WILD RESIDENT - Twin Lakes Cutthroat, Lake Lenore Lahonton Cutthroat, Lake Whatcom Kokanee, etc.

* CAPTIVE ANADROMOUS - Coastal and Hood Canal Searun Cutthroat, etc.

The basis for the genetic management program is that:

GENETIC DIVERSITY IS THE PRIMARY RESOURCE

OF ANY SUCCESSFUL FISH CULTURAL PROGRAM

Genetic Diversity can be defined as the variation in the inherited basis of differences in appearance, size, behavior, life history, ecology and other traits characteristic of that species, population, and individuals. The primary issues of the Washington Department of Wildlife Genetic Management Program can be summarized as:

1. Maintain a large number of mated pairs in a spawning population.

2. Maintain a random spawning/mating technique.

3. Maintain a ratio of 1 male to 1 female (1:1).

4. Recruit future broodstock according to the proportion of the egg take per spawning period.

5. Equalize the contribution of each male used during the spawning process.

6. Should genetic supplementation be required, carefully consider all the available options.

THE ROLE OF HATCHERIES IN GENE CONSERVATION AS CONDUCTED BY THE WASHINGTON STATE DEPARTMENT OF FISHERIES

Andy Appleby
Washington State Department of Fisheries
Salmon Culture Division

In the 1970s, concern for genetic conservation began to develop both within and outside the Washington State Department of Fisheries. The Department looked seriously at the issue and determined that our hatchery system should be doing what ever was possible to maintain as much of the genetic diversity as existed. That is, we would make an effort to halt the collapsing of stocks into fewer and fewer groups of homogeneous mixes.

We decided to approach the problem from three avenues:

1) <u>Hatchery siting and operations.</u>
   a) Mother station concept.
      Now employed at Eastbank, Shale Creek, and Tucannon hatcheries.
      Up-coming Methow Hatchery.
      Gives us reproductive isolation.
      May not make most efficient use of incubators and ponds.

This idea consists of trapping adults on small tributaries, then hauling either adults or eggs to a central facility for holding, spawning, hatching, and early rearing, then transferring them back to those same tributaries for final rearing and release. May require obligating incubators and or ponds on the basis of maintaining separation and not on carrying capacity.

2) <u>Fish transfer policies.</u>
   a) Stock transfer policy.
      Develop list of acceptable donor stocks by species for each watershed/hatchery.
      List is prioritized, may be several acceptable stocks.
      Requires coordination with harvest to meet escapement goals for hatcheries that routinely provide eggs to other stations.
      May mean program short falls if no acceptable stocks are available for transfer.

3) <u>Fish cultural practices.</u>
   a) Develop spawning guidelines/genetics manual.
      Identify problems with current procedures.
      Specific set of techniques for use under given set of hatchery objectives.

While all of the above are currently used in Washington State, today I will confine my remarks to fish cultural practices and how the WDF spawning guidlines are helping to maintain genetic variation in our artificially cultured salmon stocks.

After review, the fish cultural practices used at that time were found to be lacking in at least some aspects given the above goal. With the help a Profs. Hershberger and Iwamoto of the Univ. of Wash., a genetics manual and a set of spawning guidelines for use at our facilities was created.

This manual, which was written in the early 80s, became hatchery policy in 1983. The manual runs just over 100 pages and covers topics from basic genetic concepts to use of genetics in fish culture. It was designed to explain enough of how genetics works so field personnel could understand their role in the goal of gene conservation, In addition, it gives specific rules so each manager has easy to follow guidelines to use on spawning day.

I will not try to cover all 100 pages of the manual today but I would like to cover the basic premiss on which it is founded and then show you the four cases which our hatchery programs use as well as the specific instructions for each case.

Slide PROCEDURES AND PRACTICES

1) Importance of population size
   at least 200 (100 males and 100 female)
   eggs taken throughout run

2) Determining ratio of males and females
   1 to 1 if < .5 million eggs taken on one day
   1 to 3 if > .5 million eggs taken on one day

3) Fertilization practices
   pool gametes
   do not delay fertilization

4) Selection of eggs to be retained on station
   1 to 1 male to female ratio
   keep as many egg takes as starting facilities will allow

5) Use of jacks in spawning
   spawn jacks at a level up to 2% of the number of
   males and females used to spawn that day.
   (this level should keep jack genes in population)

SPAWNING GUIDELINES

CASE 1

WHERE ADULT EGG TAKE POTENTIAL IS BELOW THE DESIRED ESCAPEMENT GOAL.

1) Male to female ratio used in spawning should be 1 to 1.
2) In order to ensure a 1 to 1 ratio, sex products should be pooled before fertilization. Do not delay fertilization.
3) Spawn jacks at a rate not to exceed 2% of total number of males and females spawned that day.

CASE 2

WHERE ADULT EGG TAKE POTENTIAL IS ABOVE THE DESIRED ESCAPEMENT GOAL, BUT EVERY FEMALE WILL BE SPAWNED.

1) Male to female ratio used in spawning should be 1 to 1 if less than 0.5 million eggs are expected to be taken. If more than 0.5 million eggs are taken then a 1 to 3 male to female ratio can be used.
2) Pool sex products before fertilization as in CASE 1.
3) Spawn jacks at a rate not to exceed 2% of total number of males and females spawned that day.
4) Egg take retained at station to perpetuate run should include as many spawning days as possible, commensurate with starting and incubation facilities.

CASE 3

WHERE ADULT EGG TAKE POTENTIAL IS WELL ABOVE THE DESIRED ESCAPEMENT GOAL AND THERE IS NO NEED TO SPAWN EVERY FEMALE.

This is the case where the impacts of random drift and in-breeding have the greatest opportunity to distort gene frequencies.

1) Male to female ratio for egg take to be retained for station release should be 1 to 1. Egg take to be used for co-ops, off-station plants, etc. should follow the criteria established in CASE 2.
2) Pool sex products before fertilization as in CASE 1.
3) Spawn jacks at a rate not to exceed 2% of total number of males and females spawned that day.
4) Egg take retained at station to perpetuate run should include as many spawning days as possible, commensurate with starting and incubation facilities.

CASE 4

WHERE THE STATION GOAL IS TO PRESERVE SPECIFIC RUN TIMING SEGMENTS OR WHERE CUT OFF DATES ARE USED TO SEPARATE RACES OR STOCK OF FISH.
FISH.

Each stock should be examined individually and relegated to the appropriate class- 1,2,3 and treated accordingly.

GENE CONSERVATION - WHAT DOES IT MEAN TO HATCHERY PRACTICES, A NORTHWEST UPDATE

Bill Hutchinson, Hatcheries Manager
Idaho Department of Fish and Game
Boise, Idaho

Anadromous fishery managers in the northwest are currently faced with a tremendous challenge that stems from a common issue: genetics. How do you properly manage hatchery and wild/natural stocks that often share the same watersheds? Genetic effects of hatchery fish on wild/natural fish, and vice versa, has long been a topic of heated debate.

As an agency, The Idaho Department of Fish and Game does not have a formalized genetic policy for anadromous fish. However, in the Department's Anadromous Fish Management Plan adopted by our Fish and Game Commission, several genetic goals are stated: certain waters within the state will be designated for wild fish, and no hatchery fish will be stocked in them; wild fish will have priority in all fish management decisions; hatchery programs will be managed to minimize any adverse impacts on wild fish; at least one-third of adults returning to hatchery weirs will be released upstream to provide natural production.

Genetic diversity is an important part of any successful fish management program. Maintaining sufficient numbers of spawning adults is critical to any program, and Idaho's first and foremost priority is to get fish back. Negotiating eight hydroelectric dams twice during their life is a major challenge for Idaho's fish, resulting in mortality rates of 99.99%. With this level of mortality and the number of returning adults at such a critical level, proper genetic management is ever so important.

Hatcheries play an important role in Idaho's fish management program. Idaho is currently using most of the commonly accepted broodstock selection and spawning methods used by Washington, Oregon, and the U.S. Fish and Wildlife Service. Methods used include: spawning ratios of one male to one female; random selection of mated pairs; use of jacks in spawning; eggs collected from the entire spectrum of the run; eggs are water-hardened in iodophore; whenever possible, integrate gametes of naturally-spawning fish into hatchery stocks; collect eggs from as large a number of mated pairs as possible. In addition, all wild/natural steelhead trapped at hatchery weirs are released upstream. Currently, the Department is working to identify chinook stocks (hatchery versus wild/natural) for the purpose of maintaining their genetic characteristics, and also to integrate them into hatchery stocks.

Idaho is committed to protecting it's wild/natural stocks and has given them priority over it's hatchery stocks. Maintaining genetic integrity and minimizing selective pressures are paramount to the survival of these fish.

Hatcheries have often been criticized for adversely effecting stocks of anadromous fish. In certain instances, this criticism was warranted. Management agencies are currently working hard to correct past mistakes and insure that decisions for any genetic manipulations are conscious ones. Genetic policies toward hatchery and wild/natural stocks are inevitable. However, one has to wonder just what kinds of selective pressures and genetic implications eight hydroelectric dams, reduced flows, large-continuous slack water areas and extensive harvest rates, all contributing to mortality rates in excess of 99.9%, are having on these same fish.

FISH CULTURE

VARIATION IN IODINE CONCENTRATION DURING WATER HARDENING OF SALMONID EGGS

Robert W. Rogers
Patrick F. Chapman
Washington Department of Fisheries
Salmon Culture Division
Olympia, Washington

Abstract.--Iodine concentrations in and about the egg mass during water hardening of salmonid eggs was measured. Varied volumes of egg:iodophor in static single use and reuse lots, and iodophor recirculation with unrinsed and rinsed eggs were examined. Data indicated that static iodophor water hardening in volumes of 1:1, 1:2, and 1:4 (egg:iodophor) did not expose all eggs to uniform concentrations of iodine and that iodophor reuse further reduced the exposure level. Iodophor recirculation with volumes of 1:1 and 1:4 (egg:iodophor) resulted in exposure of all eggs to higher concentrations of iodine for an extended period of time. Rinsing of eggs in iodophor immediately prior to water hardening in recirculated iodophor further increased the level of iodine exposure to the eggs (1:4, egg:iodophor). An increase in the volume of recirculated iodophor resulted in a higher concentration of iodine within the egg mass for a longer period of time.

Iodophor water hardening of salmonid eggs at concentrations of 50-100 ppm active ingredient iodine for up to one hour is a standard practice throughout the Pacific Northwest. Several different conatiners are presently used for the water hardening process, and because of this diversity, the egg:iodophor volume within the container is as diverse. The purpose of our study was to measure changes in iodine concentration above, within, and below the mass of eggs during water hardening in buckets while varying egg:iodophor volume; following iodophor rinsing of eggs; and during iodophor recirculation.

Methods

Coho and chinook salmon eggs for all test lots were taken at the hatchery by the usual spawning procedure, either by the hatchery crew or ourselves. Unfertilized, undrained eggs were pooled in 15 liter buckets, fertilized with pooled milt for 10 minutes, and drained of excess ovarian fluid and milt prior to exposure to iodophor. A newly prepared stock solution of iodophor was used for water hardening each day. Initial iodine concentration prior to exposure of eggs ranged from 105-110 ppm. We used 1% titratable PVP Iodine purchased from Western Chemical Company. Iodine concentrations were measured colorimetrically with a Bausch and Lomb Spectronic 20 spectrophotometer by the Iodine DPD method (Hach Company, 1987).

After fertilization and draining, each egg lot was measured volumetrically into a bucket. A measured volume of iodophor stock solution was then poured into the eggs to achieve mixing. Sampling began immediately after the initial mixing (designated as time 0) and continued at predetermined intervals throughout the 60 minute sample period. At the end of 60 minutes, the iodophor solution was

poured off, mixed well, and remeasured (this value designated as the end point). The sample sites for most tests were (1) in the iodophor column 100mm above the egg mass, (2) in the center of the egg mass and, (3) below the egg mass (at the bottom of the bucket). Initial tests used only the sample site above the egg mass.

Recirculation of the iodophor solution was achieved by using an undergravel aquarium filter and an air pump. For the recirculation lots, the filter assembly was first placed in the bucket, a measured volume of eggs was placed over the filter, the pump was started, and the iodophor solution was added. Complete recirculation of the solution occurred approximately every 2.5 minutes.

Iodophor rinsing of fertilized and drained eggs was achieved by briefly immersing the eggs, twice, into 10 liters of 100 ppm active ingredient iodine. After rinsing, the eggs were placed into a bucket, iodophor was added, and sampling began.

All results are averaged data from two replicates.

Results

Drained eggs in buckets: 4L eggs, 4L Iodophor

There was no appreciable change in the iodine concentration of the static control bucket throughout the sample period (Figure 1). By contrast, an immediate reduction in iodine concentration at time 0 from 108 ppm to 91.9 ppm was seen in the bucket containing eggs, followed by a gradual decrease in concentration throughout the sample period to 72.9 ppm (Figure 2). After the iodophor solution was poured off of the eggs and mixed, the end point iodine concentration was 24.5 ppm.

Drained Eggs in Buckets: Single Use and Reuse of Iodophor

Figure 3 includes the results of Figure 1 (the single use static 1:1 volume lot) as well as the results of the single use and reuse static 1:2 volume (2L eggs:4L iodophor) lot. Of note here is the slightly elevated levels of iodine throughout most of the sample period for the single use 1:2 lot compared with the single use 1:1 lot but particularly at time 60 and after mixing. The iodine concentration of the single use 1:2 (egg:iodophor) lot was 14.1 ppm higher at time 60 and 30.8 ppm higher after mixing than that of the single use 1:1 (egg:iodophor) lot. More notable, however, was the marked reduction in iodine concentration of the reuse 1:2 lot whereby a 25 ppm reduction in concentration was seen at time 0, and a total iodine concentration of 3.1 ppm at the end of the sample period. The reuse 1:2 lot involved exposure of an additional 2 liters of eggs to the "used" iodophor drained from the single use 1:2 lot.

Drained Eggs in Buckets: 2L eggs, 8L Iodophor

As seen in previous lots Figure 4 shows an initial drop in iodine concentration at all sample sites at time 0. Additionally, a measurable

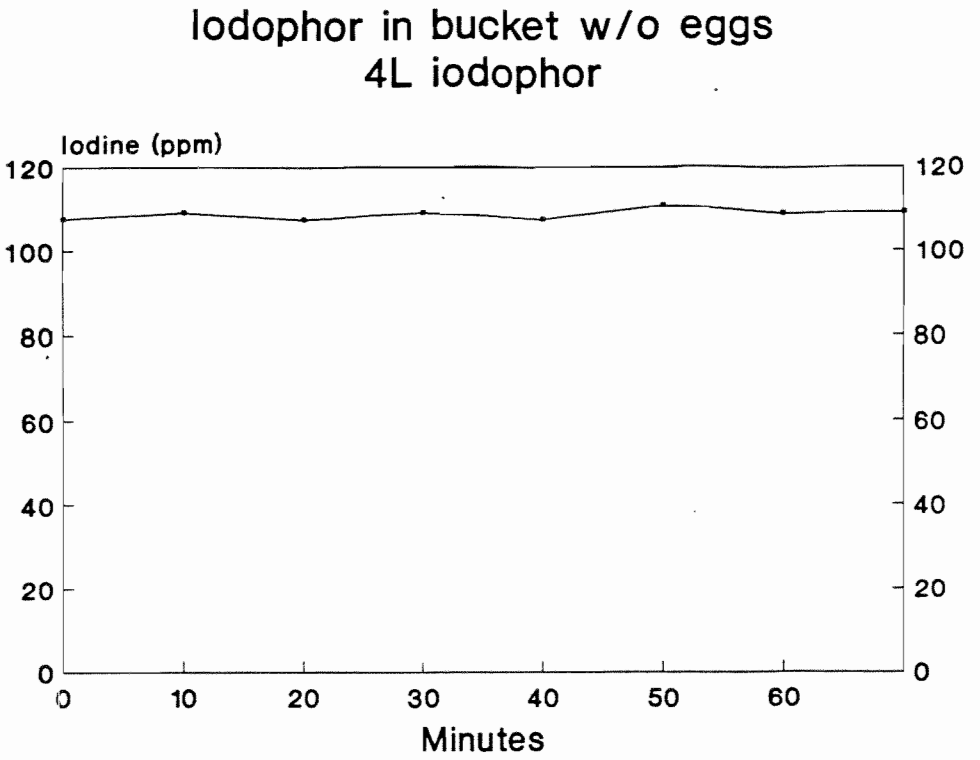


FIGURE 1

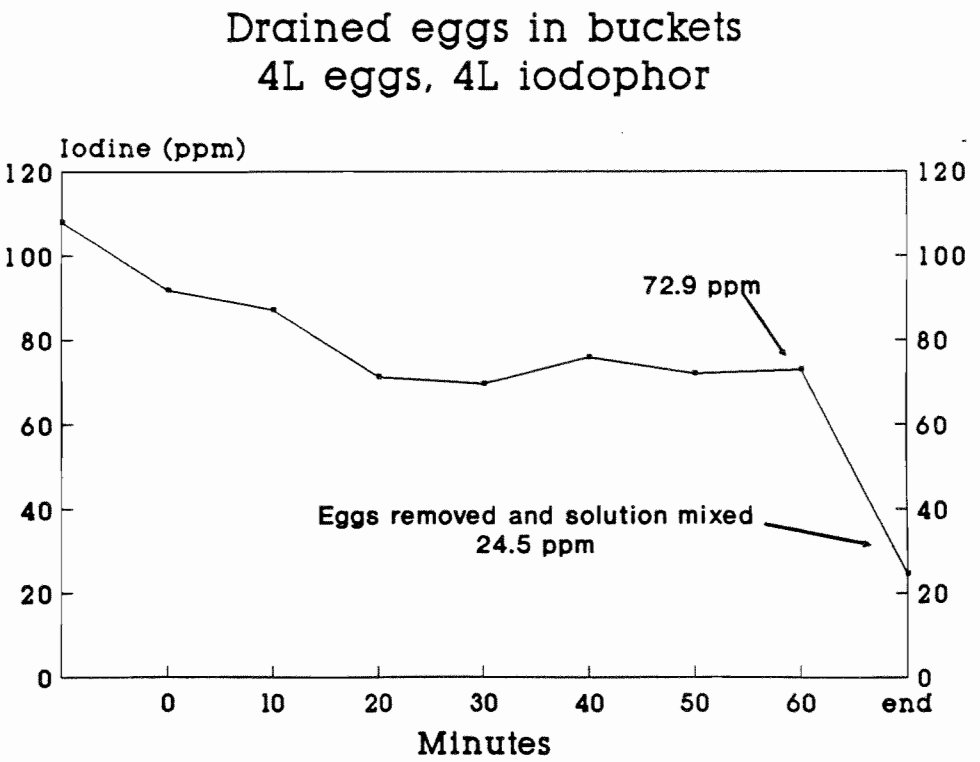


FIGURE 2

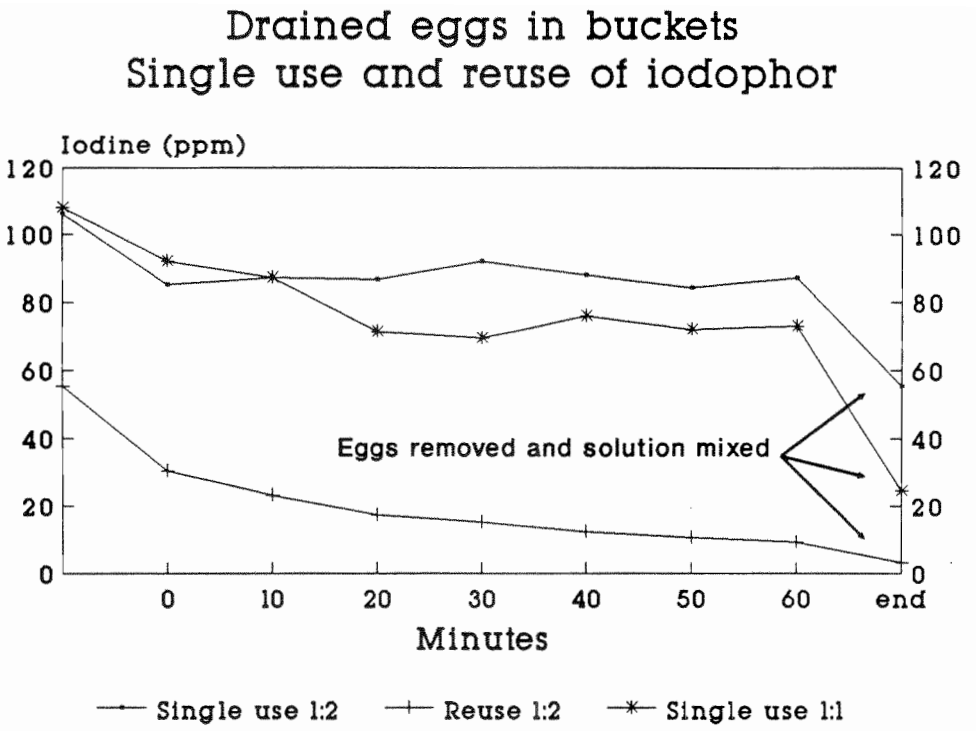


FIGURE 3

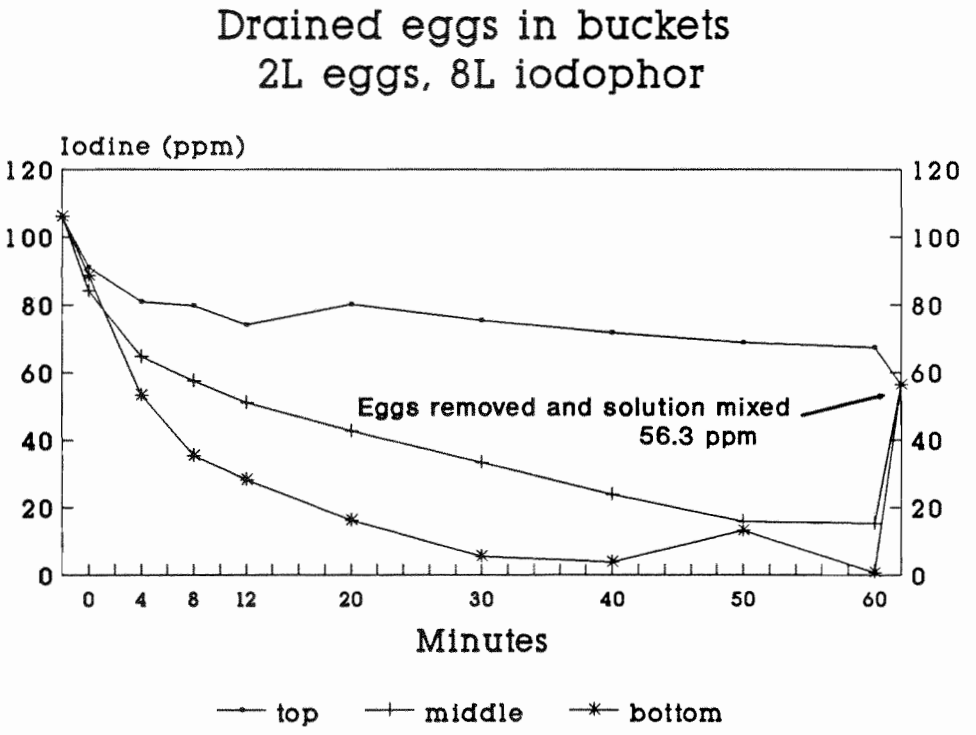


FIGURE 4

difference in iodine concentration was observed between the 3 sample sites. Throughout the sample period, the iodine concentration at each sample site decreased quicker the further the sample site was from the egg:iodophor interface, i.e., the eggs at the middle and bottom of the bucket were not exposed to the elevated levels of iodine in the iodophor column above the egg mass. Reduced exposure of portions of the egg mass to the solution is again suggested by an increase in the end point measurement of 56.3 ppm (41.1 ppm higher than time 60 of the middle sample site and 55.6 ppm higher than time 60 of the bottom sample site).

Drained Eggs in Buckets with Recirculation: 2L Eggs, 8L Iodophor

Recirculation of iodophor without exposure to eggs did not appreciably reduce iodine concentrations after one hour (Figure 5). When 8L of iodophor was recirculated through 2L of eggs, the initial reduction in iodine concentration at time 0, for all sample sites, was between 20-25 ppm (Figure 6), which was fairly typical of previous tests. However, throughout the 60 minute period the iodine concentration remained very similar at all sample sites. This was in sharp contrast to the results of Figure 4 and suggested that there had been improved exposure of all eggs to the iodine.

Rinsed Eggs in Buckets with Recirculated Iodophor:
2L Eggs, 8L Iodophor

By rinsing eggs in an iodophor solution prior to water hardening, an elevated iodine concentration was maintained at all sample sites throughout the sample period (Figure 7). In contrast to unrinsed lots, the rinsed lot did not show any appreciable reduction in iodine concentration at time 0 (Figure 8). Additionally, iodine concentrations in rinsed lots were approximately 30 ppm higher than unrinsed lots throughout the sample period.

Rinsed Eggs in Buckets with Recirculated Iodophor:
6L eggs, 6L Iodophor

Figure 9 shows the results of a 1:1 volume (egg:iodophor) rinsed and recirculated lot. This lot, as with previous rinsed and recirculated lots, exhibits fairly uniform iodine concentrations throughout the egg mass. There is, however, a drop of 5-10 ppm iodine at time 0. Most notable with this lot was the relatively quick reduction in iodine concentration at all sample sites when compared with rinsed and recirculated lots using 1:4 (eggs:iodophor) (Figure 10).

Discussion

Current water hardening practices, in general, involve the immersion of newly fertilized, drained eggs into an iodophor solution of 50-100 ppm active ingredient iodine for up to one hour. The more common incubation/water hardening units include either vertical incubators, shallow or deep troughs, or buckets.

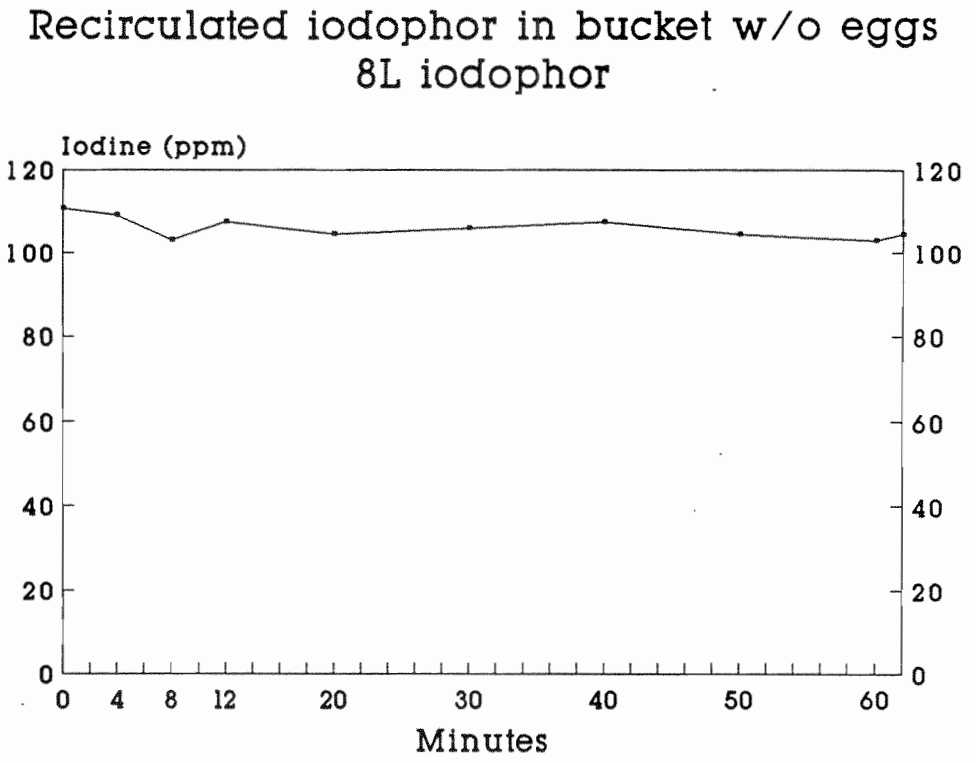


FIGURE 5

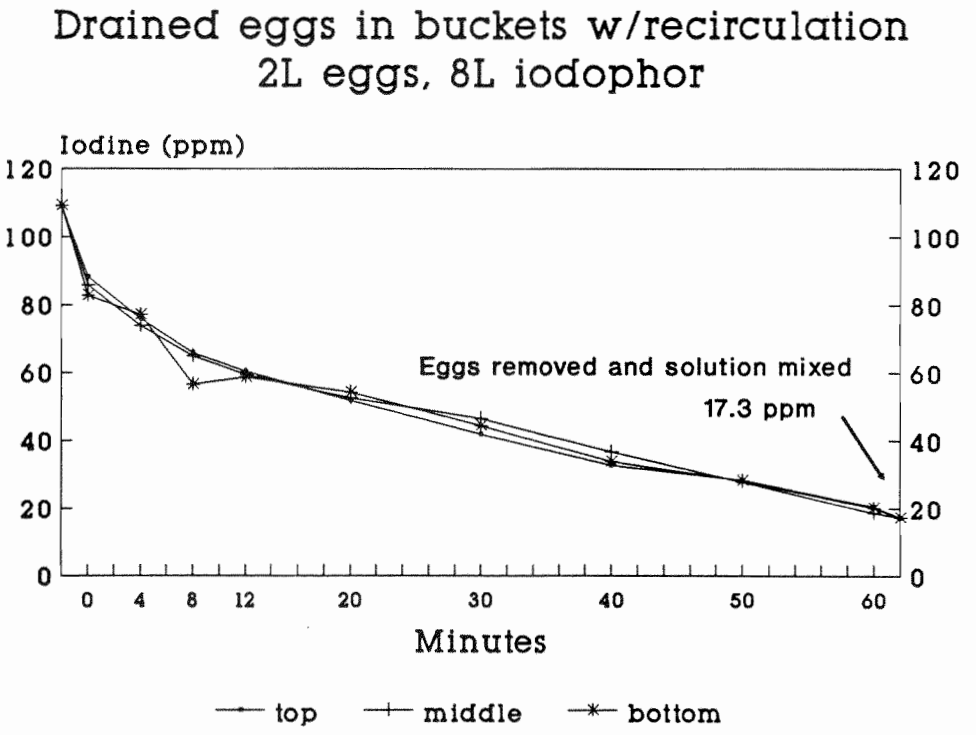


FIGURE 6

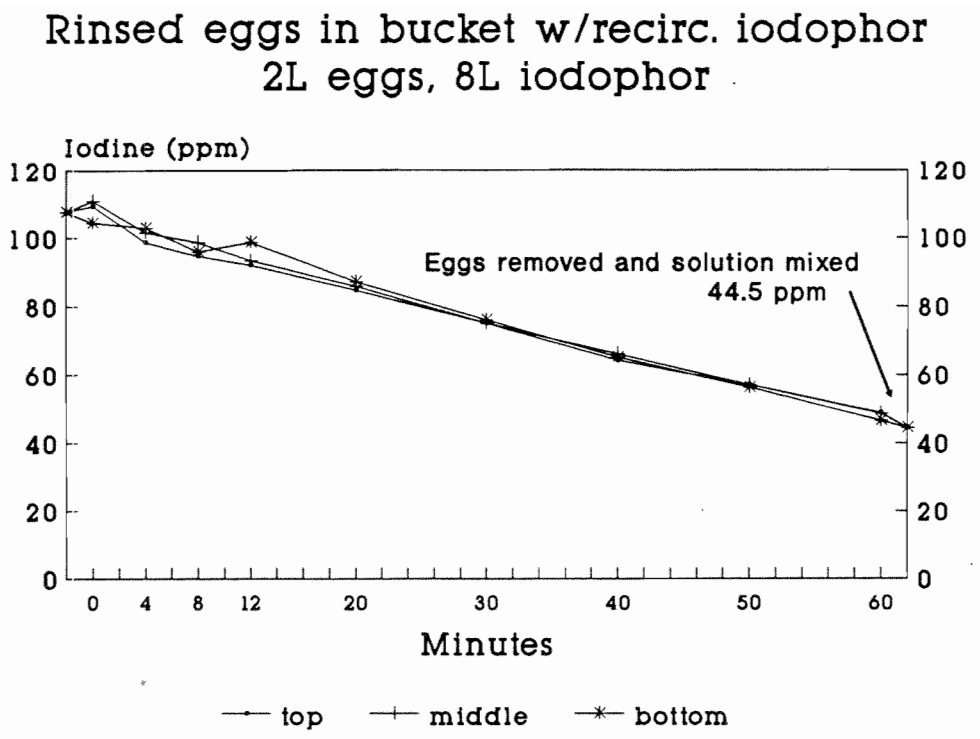


FIGURE 7

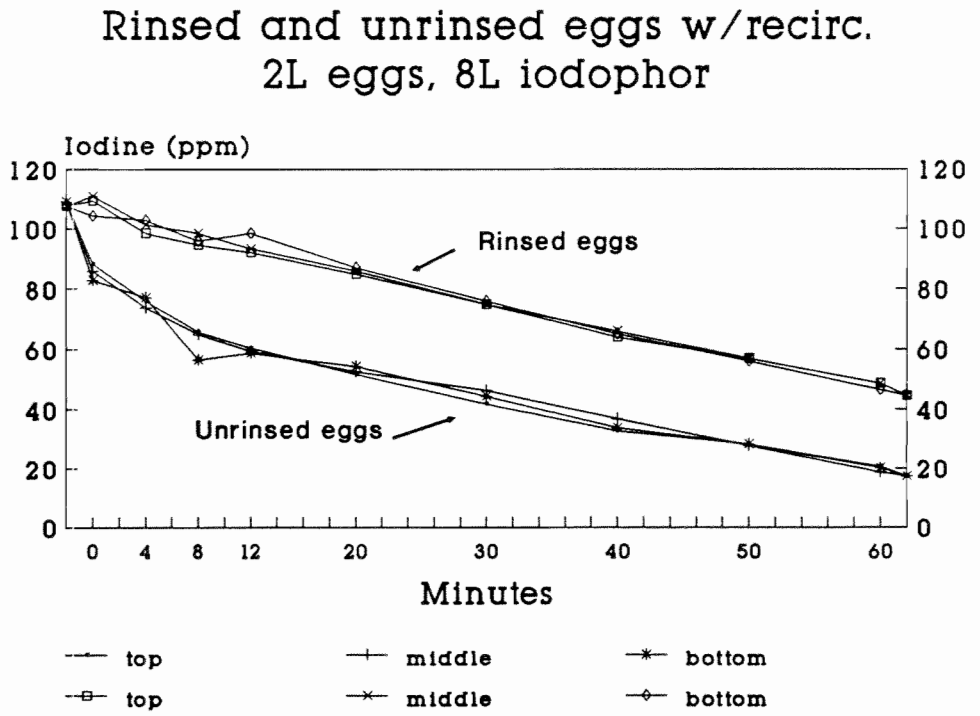


FIGURE 8

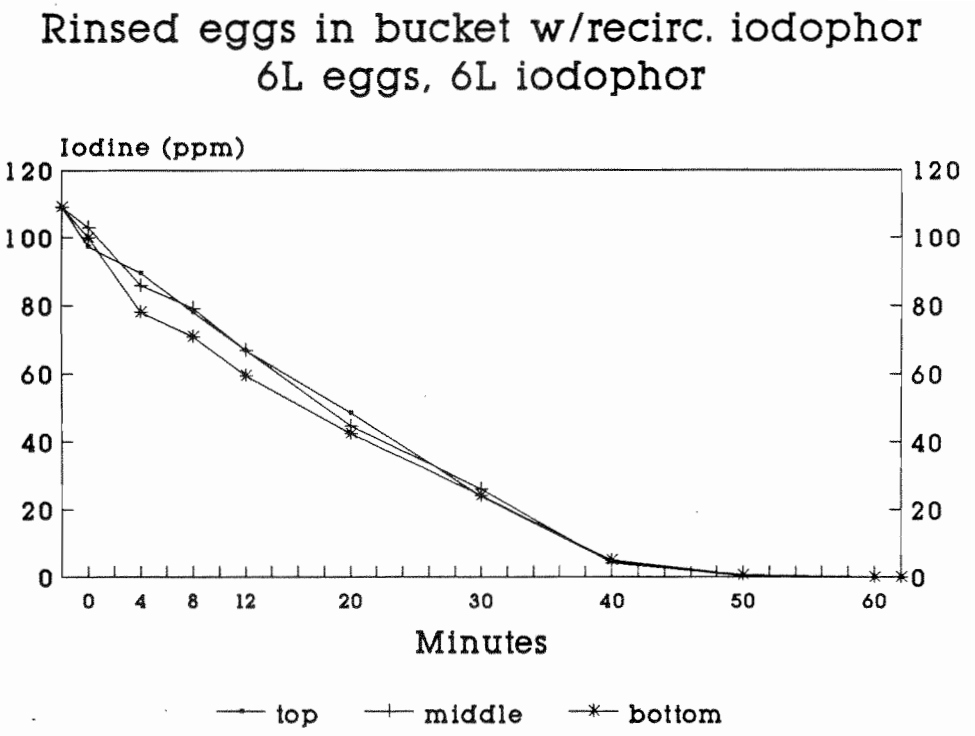


FIGURE 9

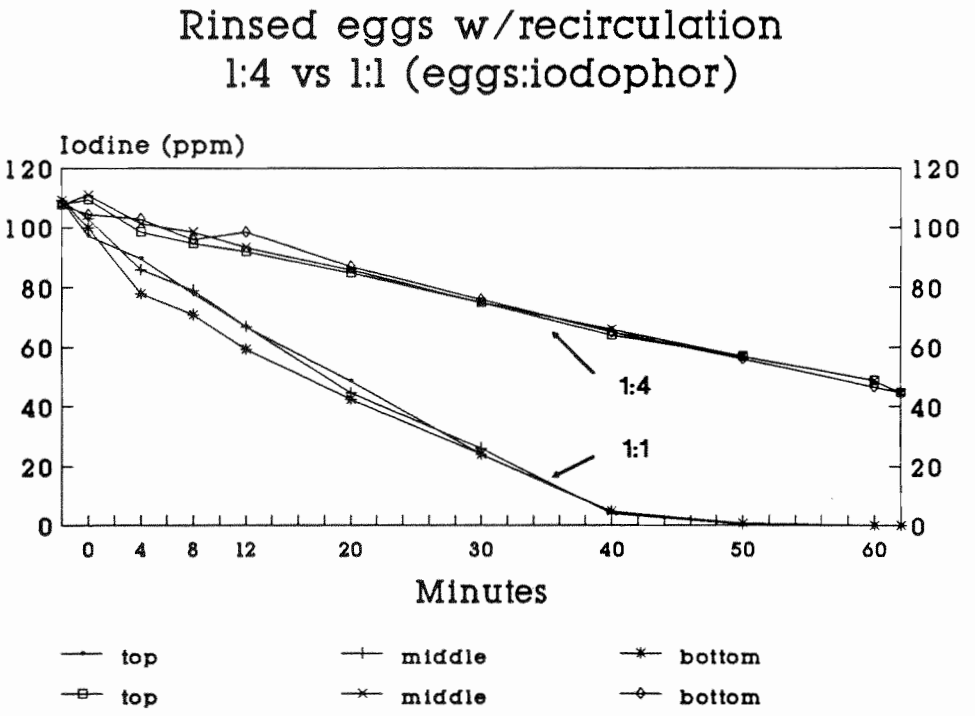


FIGURE 10

Based on observations made during iodophor water hardening tests this past season, this process does not result in exposure of all eggs to similar concentrations of iodine. Early in our testing process, this variability of exposure was evidenced by the results of the static 1:1, 1:2, and 1:4 (egg:iodophor) volume lots. Figures 2 and 3 suggested that the concentration of the iodine in the iodophor column above the egg mass was not reflective of the iodine concentration within or below the egg mass. This was apparent by a reduction in the iodine concentration of the mixed solution after completion of these tests.

The results of Figure 4 clearly demonstrate that an iodine concentration gradient does exist within the egg mass during static iodophor water hardening. This is seen by the progressively lower iodine concentration measurements between the three sample sites of the static 1:4 (egg:iodophor) lot. Throughout the full sample period, iodine concentrations decreased as the distance from the egg:iodophor interface increased. This difference between sample sites is due, in part, to displacement of most of the iodophor solution by the egg mass. As a result, only the eggs near the egg:iodophor interface were exposed to the reservoir of unused iodophor. Eggs below the interface were exposed to equivalent levels of iodine only at initial mixing (time 0). The iodine concentration reaching eggs below the interface had been reduced by exposure to other eggs. During static water hardening, only passive fluid movement occurs (as eggs water harden) and consequently, reduced iodine levels occur within and below the bulk of the egg mass.

Iodophor recirculation increased the concentration of iodine to eggs below the interface. Figure 6 demonstrates uniform exposure of all eggs to iodine for the sample period. Of particular note here, in addition to very similar iodine concentration measurements at all sites, is the relatively low end point concentration. Comparison of the end point values of the static 1:4 (Figure 4) and recirculated 1:4 (Figure 6) lots again suggest that increased exposure in the recirculated lot has been achieved.

Figure 7 represents further efforts to increase the level of iodine available to eggs during water hardening by prior rinsing of the eggs in an iodophor solution. Most notable with this lot was an increase in iodine concentration at all sites throughout the test period (approximately 30 ppm higher than the unrinsed recirculated lot, Figure 6). In addition, a drop in iodine concentration at time 0, seen in the unrinsed lots, was not seen in this lot. It is apparent that a quick rinse of drained eggs in an iodophor solution prior to water hardening will result in increased exposure of eggs to higher concentrations of iodine (Figure 8).

In an effort to determine what effect changes in egg:iodophor volume (of recirculated lots) has on iodine concentration we reduced the egg:iodophor volume from 1:4 to 1:1 (Figure 9). Figure 10 shows the comparative results of this change. Clearly, a reduction in the egg:iodophor volume reduces the length of exposure of eggs to higher concentrations of iodine.

A summary of iodophor water hardening techniques at Pacific Northwest hatcheries prepared by Groberg (1990) indicated that iodophor rinse and iodophor water hardening solutions of 50-100 ppm are used selectively depending on facility, species, and incubation unit types, and, that no one dosage or duration is recommended for all. Our results indicate that iodophor recirculation,

iodophor rinsing, and increased iodophor volumes all result in exposure of eggs to greater concentrations of iodine for longer periods of time. This presumably results in more effective disinfection of eggs than achieved by current methods, and should be considered in any program of iodophor water hardening. If a change of procedure is considered however, an effort must be made to determine what levels of increased iodine exposure are tolerable by first assessing egg/fry survival of experimental lots.

Amend (1974) demonstrated that rainbow trout eggs water hardened in 100 ppm iodine for 15 minutes resulted in a significant loss of eggs in the test lot. However, each test lot was small (approximately 400 eggs) and, as a result, may not be reflective of conditions that exist during water hardening of production-sized lots. Results of more recent work with larger lots of chinook salmon (standard loadings in vertical incubators) by Fowler (1988) and Fowler and Banks (1989) indicated that significant losses of eggs/fry did occur in fall chinook at Abernathy from eggs water hardened in 75 ppm iodine for either 30 or 60 minutes. Eggs water hardened in 50 ppm iodine for 30 minutes experienced no significant increase in loss in the test group (Fowler and Banks, 1990). These results are from lots water hardened in vertical heath incubators. Variability in both the egg:iodophor volume and the effective area of egg:iodophor interface between the vertical trays and other incubation units (including buckets), may well result in quite different exposure levels.

Preliminary results of water hardening tests of eggs in vertical incubators and deep troughs, though not included in this report, did indicate variabilty between unit types. It is conceivable that eggs water hardened in units that, by design, allow for a more uniform exposure of eggs to the iodophor reservoir (i.e., vertical trays, shallow trough screen baskets, or iodophor recirculation), will be exposed to increased iodine levels and subsequently suffer increased losses. It will, therefore, be important to determine what the maximum tolerable levels of iodine are for each type of incubation unit (or applied technique) to prevent increased egg/fry mortality from iodine toxicity. In addition, it may also be necessary to reduce initial iodine concentrations for egg lots water hardened in recirculated iodophor since exposure of eggs to higher concentrations of iodine has been demonstrated by this technique. While our results this year did not include egg/fry losses, continued studies are planned for next season.

References

Amend, D. F. 1974. Comparative Toxicity of Two Iodophors to Rainbow Trout Eggs. Trans. Am. Fish. Soc., 103:73-78.

Fowler, L. G. 1988. Spawning and Incubation Methods. Semiannual Progress Report. Abernathy Salmon Culture Technology Center.

Fowler, L. G., and J. L. Banks. 1989. Spawning and Incubation Methods. Semiannual Progress Report. Abernathy Salmon Culture Technology Center.

Fowler, L. G., and J. L. Banks. 1990. Spawning and Incubation Methods. Semiannual Progress Report. Abernathy Salmon Culture Technology Center.

Groberg, W. J., Jr. 1990. Water Hardening Salmonid Eggs in Iodophor. Oregon Department of Fish and Wildlife Informational Report 2 prepared for the Pacific Northwest Fish Health Protection Committee.

Hach Company. 1987. Procedures for Water and Wastewater Analysis. 2nd ed. Hach Company, Loveland, Colorado.

A DATABASE POND INVENTORY SYSTEM - LEAVING A PAPERLESS TRAIL

Stephen M. Pastor
U.S Fish & Wildlife Service
Vancouver, Washington

Abstract

Database methods are being applied to information collected and managed at U.S. Fish & Wildlife Service hatcheries and fisheries resource offices in the Columbia River Basin. Standard database files are used to record information during spawning and egg development, at time of release, and when fish return as adults. Newly developed data files and software form the basis of an active pond inventory system. It is possible to demonstrate the simplicity of following the paperless trial left by inventory record keeping. Retrieval of all information pertinent to any group of released fish, including the number and size of spawned fish, is possible with no "extra" record keeping .

Introduction

Database methods were developed to reduce redundancy and inconsistency found in traditional record keeping systems. Database Management Systems (DBMS) have been available for the current generation of microcomputers for several years. U.S Fish & Wildlife Service hatcheries and fisheries resource offices in the Columbia River Basin have begun using database methods to record information on returning adults, spawning, egg development and fish releases. Many events which occur at the hatchery are not recorded in a standard fashion, making it difficult to retrieve information on groups of fish.

Recording basic information in a standard format will not only create an active pond inventory system, but will create a "Paperless Trail" that can be followed from release back to spawned adults.

Data is entered on a daily basis using the browse, edit and append modes of dBASE III PLUS, the DBMS used by FWS fisheries personnel in the Columbia River Basin.

Data Files Used

Six different data files are used to maintain a pond inventory system. The Pond Inventory file, "PondInv" contains the date, pond, raceway or tank designation, number of fish, number per pound, and the two digit, sequential lot number to which the fish belong. A Mortality file "Morts", contains a date field, and a field for each tank, pond or raceway on the hatchery. A From To file, "FromTo" is used to document the movement of fish between rearing units. A Fry Out file, "FryOut", is used to document movement of fry from incubation trays, jars, etc, into rearing units such as tanks. It contains a Take field in addition

to the fields in the FromTo file. A "Growth" file contains the expected growth in inches per day, and the expected feed conversion rate for each lot of fish in the inventory. A "Sample" file makes it possible to record information when sample counts are taken.

The LN field occurs in each of the data files. This field contains a two digit sequential "Lot Number" assigned to each group of fish as it goes on feed. This field is used to link information in the different files.

Processing of Pond Inventory Information

After information is entered, it is processed by the dBASE program PondInv which is started by typing "do pondinv" at the dBASE dot prompt. PondInv calls other programs and performs the following operations:

PondCheck checks various dates to insure that dates in the PondInv file aren't advanced past the date of information in other files. PondGrow increments the size of fish in each pond or raceway, using information found in the Growth file and the LHstart or LHeom file. PondMove "moves fish" from one raceway to another in the PondInv file, if fish are moved on the current date. PondFry then performs the same operation for any fry that have been put out. PondMort then subtracts any mortality for the current date. PondSum will then list the current inventory numbers and the total for each lot of fish on hand.

The PondInv file will contain the number and size of fish on a daily basis.

Following the Paperless Trail

The Pond Inventory system can be used on a daily basis through the entire production cycle. Years later the results of coded wire tag studies will finalized. It will be possible to retrieve information on groups of fish by using the Pond Inventory data files in reverse. This is the Paperless Trail referred to in the title of this paper.

Let us assume that fish with a given coded wire tag were released from raceway 36 on July 17, 1990. When we look in the FromTo file, we see that fish were moved from R12 into R36 on 06/15/90. We also see these fish came from T01 on 02/01/90. If we check the FryOut file we see that the fish in tank 1 were a combination of fry from Take 01S and 02S, and were put out on 10/19/89 and 10/20/89.

Other files described in previous presentations are sources of more information. An Egg Activity file used to document the number of eggs at each stage for each take can be used to calculate percent eyed, etc.. The number of males and females spawned is available from the Fish Removal file used to document spawning activity and the number of adults returning to the hatchery.

Data files containing length and age information are created and maintained by fisheries resource personnel. Once the spawning date has been determined, these files can be consulted to retrieve further information.

The following information can be retrieved from data files originally used to maintain the Pond Inventory and other purposes:

which take of eggs the fish came from
how many males spawned
how many females spawned
average length of males and females spawned
age composition of males and females spawned
percent eyed
percent hatched
percent on feed
daily mortality for each tank and raceway
coded wire tagged fish - strays spawned

The Pond Inventory system, which is being used at two FWS hatcheries, is currently in the beta testing stage. Use of the programs and data files through one or several production cycles will undoubtedly reveal the need for modification.

Software to retrieve information as described in this paper has not yet been written. Although logically simple, it should provide a reasonably difficult programming challenge.

Data files Used by the Pond Inventory system

PondInv	DATE	LN	POND	NUMBER	PERLB	
Morts	DATE	R01	R02	R03	...	
FromTo	DATE	NUMBER	PERLB	FROM	TO	
FryOut	DATE	NUMBER	PERLB	FROM	TO	TAKE
Growth	LN	PERDAY	CONVER			
Sample	DATE	POND	PERLB			

PondInv calls the following programs:

PondCheck	check date in mortality file
PondGrow	increment sizes
PondMove	"move fish" in PondInv
PondFry	"move fry" into PondInv
PondMort	subtract mortality
PondSum	list current inventory and totals

FromTo data file

DATE	FROM	TO
02/01/90	T01	R12
06/15/90	R12	R36

FryOut data file

DATE	TAKE	FROM	TO
10/19/89	01S	inc	T01
10/20/89	02S	inc	T01

EggAct data file

TAKE	GOOD_EGGS	BAD_EGGS	LS_EE_2_HT	LS_HT_2_FD
01S	900000	8000	2000	100
02S	47000	2000	1000	200

Fish Removal data file

DATE	TAKE	NUM_TAKEN	SEX	USE
07/18/89	01S	116	1	1
07/18/89	01S	227	2	1
07/25/89	02S	59	1	1
07/25/89	02S	118	2	1

BioData file

DATE	LENGTH	SEX	ID	TAGCODE	T_AGE	FW_AGE
07/18/89	71	2			4	2
07/18/89	88	2			4	1
07/18/89	91	1			5	1

===

THE CULTURE OF TIGER MUSKIES IN WASHINGTON STATE

Jack Tipping
Washington Department of Wildlife

Abstract

Tiger musky (northern pike x muskellunge) hatchery rearing requirements include hatching in a bell jar, 68 F water temperature, feeding every five to ten minutes, a feeding rate of about 15 percent body weight per day, a feeding method to accommodate feeding style (only taking feed while falling in the water column), and the initial use of a Japanese freeze-dried feed. Due to the warm temperature and large amount of feed used, frequent trough cleaning and treatment for disease was required. Water temperature was accomplished using water heaters, feeding rate and style was obtain using Loudan feeders on a timer, and feed used included Biokyowa, Bioflake, and live brine shrimp initially, followed by Biodry diet after about three weeks. Troughs initially had to be cleaned twice a day which was accomplished by siphoning so that water quality was not degraded. Fry were treated with diquat daily for about the first two weeks to reduce bacterial gill problems. At about 3.0cm, fry were moved from troughs to an intermediate raceway, whereupon they were cleaned once per day, done by brushing. Rearing density was similar to trout culture. Growth of fish was rapid with fry increasing from about 35,000/lb in early May up to 14/lb by the end of August. Mortality has been between 1.6 and 3.8 percent in the last two years. Feed conversion has ranged between 1.19 and 1.44:1.

AN OVERVIEW OF PROBLEMS EXPERIENCED IN WALLEYE CULTURE

Greg A. Kindschi, Frederic T. Barrows, and Elizabeth MacConnell
U.S. Fish and Wildlife Service
Bozeman, Montana

Abstract

Walleye Stizostedion vitreum are becoming an important recreational and commercial fish throughout North America. Consequently the demands on fish production facilities to raise more and larger walleye are increasing. Several problems are presently experienced in walleye culture but research has provided several advancements and improvements as well. Captive broodstocks are very limited so most facilities rely on the unpredictable spring collection of gametes. However, females generally provide an abundant supply of eggs. Fry are stocked into fertilized production ponds or reared intensively on formulated feeds. Quantification of fry is difficult but Jensorter, Inc. has developed an accurate fry counter that does not appear to effect survival. In ponds, plankton productivity is weather dependent so cannibalism and survival may be variable. Fry will consume formulated feeds but swim bladder uninflation, cannibalism, and fish with bent caudal peduncles may prevent high percentages of survival. Three methods for the detection of swim bladder inflation have been developed for all sizes of walleye in various situations: a light table, MS-222, and radiographic methods. Swim bladder uninflation has also been identified in pond-reared walleye. A survey of incidence rates and causes of swim bladder uninflation in pond-reared walleye fingerlings is presently being conducted. Studies indicate that walleye with uninflated swim bladders have a suppressed immune response when stressed so may be more susceptible to disease. Walleye with inflated swim bladders seem to temporarily over-inflate their swim bladders when subjected to dewatering. Bacterial infections (e.g. Columnaris) continue to cause problems in walleye culture. Fin erosion of the dorsal spiny-rayed fin is also a problem when intensively rearing walleye fingerlings. Because most fish feed mills primarily produce trout feeds--walleye diets are special order feeds and are generally manufactured only one time each year. Vitamin C has a short storage life, consequently there is a good chance of feeding a Vitamin C deficient diet because the feed was stored too long. Recent studies indicate that walleye will use the stabilized form of Vitamin C which is ASPP. Use of this source of the vitamin could eliminate all future deficiency problems. Pathological changes in walleye associated with Vitamin C deficiency include: lordosis and scoliosis, dislocation of vertebrae, compressed spinal cord and displacement of adjacent kidney and skeletal muscle, twisted gill filaments, broken isthmus, and hemorrhagic fins and eyes. Recent research has shown that a diet containing about 100 mg of Vitamin C per kg of feed is sufficient for normal growth. Walleye remain an important sport fish and one which is very marketable. Continued walleye research will improve culture techniques and hopefully provide solutions to the problems presently being experienced.

The above information has been taken from the following manuscripts which have either been published or are presently in review:

Barrows, F.T. and G.A. Kindschi. In review. Methods for the detection of swim bladder inflation of fingerling walleye. Progressive Fish-Culturist.

Barrows, F.T., G.A. Kindschi, C.E. Smith, and R. Zitzow. 1990. Summary of walleye culture and diet development research conducted and coordinated by the U.S. Fish and Wildlife Service in 1989. Bozeman Information Leaflet #62.

Barrows, F.T., W.A. Lellis, and J.G. Nickum. 1988. Intensive culture of larval walleye with dry or formulated feed: note on swim bladder inflation. Progressive Fish-Culturist 50:160-166.

Coolwater Fish Culture Workshop Minutes. 1990. Bozeman, Montana, January 8-10, 1990.

Kindschi, G.A. and E. MacConnell. 1989. Factors influencing early mortality of walleye fry reared intensively. Progressive Fish-Culturist 51:220-226.

Kindschi, G.A. and F.T. Barrows. In review. Optimal screen mesh size for restraining walleye fry. Progressive Fish-Culturist.

Kindschi, G.A. and F.T. Barrows. In review. Evaluation of an electronic walleye fry counter. Progressive Fish-Culturist.

MacConnell, E. and F.T. Barrows. In review. Pathological changes in Vitamin C deficient walleye. Journal of Aquatic Animal Health.

SUSTAINABLE AQUACULTURE and the ENVIRONMENT: CLOSING THE LOOP

Alan Ismond, P.E.; A.T. Consultants,
Simplot Aquaculture
Caldwell, Idaho

The benefits of aquaculture for stock enhancement and food fish are becoming more and more evident as the degradation of the environment continues. Since aquaculture is also a resource consumptive industry, it is important to design facilities that do not further compound environmental resource depletion. The motivation to do so will be increasingly stronger in the ensuing decade. Government regulations concerning waste discharge will likely become more stringent. There is also a growing demand and awareness by consumers for environmentally sound industries and products. The declining availability of quality resources will necessitate more efficient use and reuse of aquaculture inputs such as water. Lastly, there is also the moral and ethical consideration for anyone involved in resource based industries to ensure that successive generations will be able to enjoy the same (or better) level of resource quality and abundance.

The primary resources required in aquaculture are water, energy, and feed which consists of aquatic and terrestrial resources. Land is also required but not consumed. The outputs from aquaculture, depending on whether the facilities is a hatchery, for enhancement only, or a food fish facilities, can include liquid effluent, and solid waste comprised of faecal material, mortalities, and fish offal. In general, mass balances have been done around the farm perimeter to gauge the efficiency with which resources were converted into fish: energy + water + feed = liquid effluent + solid waste + fish growth. If the perimeter of the farm is extended further to include the resources required to produce the feed, and the surrounding land/water mass that must absorb the wastes, a different picture emerges. On the input side: are the sources of feed sustainable, and on the output side: can the environment absorb the farm waste products without degrading the ecosystem?

With respect to feed, the aquatic and terrestrial components must be considered. How many pounds of fish must be harvested from the ocean to produce one pound of fish? More importantly, is the harvest sustainable and how can the depleted nutrients be restored? Terrestrial feed components are generally sourced from agricultural crops with the same question of sustainability. Crops remove nutrients from the soil, how are the nutrients replaced? If aquaculture is to become resource efficient, then consideration must be given to sourcing renewable and/or recycled feed components. Organic waste streams can be converted to single cell protein. Aquaculture effluent can be used as a substrate for producing algae. Crop and food processing by-products can be used as feed components. Ultimately, resource utilization is maximized when terrestrial feed components outweigh aquatic feed components. This may indicate that herbivorous fish, at least for food fish production, may be more desirable.

Water is a critical resource when it comes to fish rearing. The quantity available is diminishing and competing uses increasing, especially in some parts of the U.S.. The quality of the water resource is also declining due to

pollution. This will necessitate better control of aquaculture influent water. In order to prevent the effluent from compounding existing problems, better control will be required of the effluent water. In short, the trend will be towards a greater need for conservation through water reuse and recycling. Waste management will play a critical role in optimizing resource management. Whereas uncontrolled discharge of wastes has been the trend of the past, various scenarios must be assessed for the future. The dominant strategy has been to capture, contain, and destroy wastes. An example would be to use oxidation lagoons for treating effluent from the farm. No useful by-products are generated. A better strategy would be to contain, destroy, and recover a useful by-product. The ultimate goal should be to eliminate or minimize, recover, and reuse waste streams. The following examples for solid and liquid effluent waste treatment illustrate the various strategies.

Solid waste in the form of fish offal, faeces, and mortalities can no longer be discharged without accountability. The next step would be to contain these materials and allow them to degrade. A more resource efficient approach would be to combine the recovered faecal material with the mortalities to produce a soil amendment. The fish offal can also be recycled for fertilizer or animal feed. In the case of the water resource, the effluent treatment can range from untreated discharge (unacceptable), to land application for irrigation. In a once flow through system, these scenarios still require high water consumption. A more resource efficient approach would be to recycle a portion of the water and bioconvert dissolved nutrients into fish or animal feedstock. The reduced discharge water could then be used for irrigation and soil enhancement.

Obviously, the preceding scenarios are very much site specific. Other technologies such as anaerobic digestion to recover biogas or energy can also be considered. Ultimately, however, the ideal model would be to create an integrated farm design where aquaculture wastes are used to enhance surrounding soil conditions which in turn generate components for fish feed. The farm siting, size, and fish species selection would dictate the compatibility of such a model. However, if aquaculture is to be a viable endeavor for the future, it is time for resource management to become a driving force in planning and operating facilities rather than a consequence.

BROODSTOCKS/GENETICS

SEX REVERSAL AND PRODUCTION OF AN ALL-FEMALE POPULATION IN RAINBOW TROUT

Carmen Olito and Irvin Brock
Alaska Department of Fish and Game
Broodstock Development Center
Fort Richardson, Alaska

ABSTRACT

Three groups of testosterone-treated rainbow trout (Oncorhynchus mykiss) from brood-year 1988 were spawned to produce experimental groups of all-female fish. The three groups of treated fish comprised: (1) normal (XY) males, (2) sex-reversed (XX) males, and (3) gynogenetic (XX) males. Eggs and fry from the resulting 28 experimental lots were incubated and reared separately. Sex ratios in progeny from all groups were determined to identify the male parent genotype. To aid in future identification of XX and XY males, the genotype of each male parent was matched with the morphology of its testes. Generally, sex-reversed (XX male) testes are shortened and globular in texture and appearance as opposed to normal (XY male) testes which are long and flat with a smooth texture. Four experimental lots were selected for sex reversal, based on the morphology of the male parents' testes: 3 lots were the progeny of XX males and 1 lot was the progeny of an XY male. This was the expected result based on testes chosen. All lots selected for sex-reversal received testosterone-treated feed for 500 degree-days.

EFFECTS ON ADULT PRODUCTION FROM USING TWO YEAR OLD MALE COHO FOR SPAWNING

Andy Appleby and Paul Seidel
Washington State Department of Fisheries
Salmon Culture Division

ABSTRACT

An experiment was conducted over two years to determine the relationship of juvenile growth rate and parentage on the production of both adult and 2-year-old-male (jack) coho salmon (Oncorhynchus kisutch). Female coho were spawned normally and the eggs were randomly divided into two lots. One lot was fertilized with the sperm from jacks exclusively. The other lot was fertilized as per normal spawning practices at Cowlitz hatchery, which includes using up to 2% jacks in spawning operations. Additionally, 1/2 the progeny from each group were reared at an accelerated rate, thus producing smolts almost 50% larger than normal. The other half was reared to normal smolt size. Preliminary analysis of the returns of coded-wire tagged fish suggest that smolt size and parentage both play significant roles in producing 2-year-old-males and their effects may be cumulative. Other elements examined were: total percent survival, survival to adult, size of returning adults and sex ratio of returning adults.

INTRODUCTION:

The production of precocious 2-year-old male coho (jacks) is generally associated in varying degrees with all coho populations, both natural and cultured. Evidence indicates that the percentage of a cohort maturing as "jacks" is related to growth rates of fingerlings and parentage (Noble 1959; Salo 1958; Wallis 1968; Seidel 1977). This study was designed to better understand the roles that fingerling growth rates and parentage have on the production of adult and jack coho.

METHODS AND MATERIALS:

The study design required four identical ponds. Enough fish for two ponds (approximately 350,000) were sired by 2-year-old-male coho exclusively. Two additional ponds of fish (approximately 350,000) were sired by both 2 and 3-year-old-males following normal spawning practices at Cowlitz hatchery. Normal practices include using jacks for up to 2 percent of fish spawned each day (males and females). To insure that only 2-year-old-males were being used to sired the jack only populations, scale and length samples were taken from 50 suspected jacks. Based on this analysis, males less than 17 inches (43 cm) fork length were considered to be 2 years old. While this threshold excluded some 2-year-old-males which were larger than 43 cm, no 3-year-old-males fell below this limit.

After four rearing ponds had been populated with these fingerlings, one pond from each group,jack sired (JACK), normal sired (NOR.) was fed on an accelerated feeding schedule which was designed to produce smolts of 10 -12 fish per pound (38 g.) The other two ponds were fed as per normal hatchery procedure (NG.) and would produce smolts of 18-20 fish per pound (25 g.). The study design is presented in table 1. All groups were identified with a unique coded-wire tag and adipose fin clip prior to release. This study was repeated with the 1985 brood.

RESULTS:

JACK PRODUCTION:

The group producing the fewest jacks was the normal growth x normal cross (NG.x NOR.). This group produced 6.94% and 6.87% of total survival as jacks from the 1984 and 1985 broods respectively (Figure 1). The group with the highest production of jacks was the accelerated growth x jack cross (ACC.x JACK) which produced 29.83% and 28.91% jacks respectively. The two other cells in the study design, accelerated growth x normal cross (ACC. x NOR.) and normal growth x jack cross (NG. x JACK) produce jacks in approximately equal numbers for both years, (Figure 1).

PERCENT SURVIVAL:

The control group (NG. x NOR.) had the lowest total survival rates (adults plus jacks) from the 1984 brood and the second lowest total survival from the 1985 brood, 1.42% and 3.13% respectively, (Figure 1). Increasing the size of the smolts (ACC.x NOR.) did increase the survival in both years, 2.08% ,6.29% respectively. The normal growth x jack cross group (NG.x JACK) produced survivals similar to the control group in both years. Total percent survival was highest in the accelerated growth x jack cross (ACC.x JACK) group both years, 2.49%, 6.85% respectively.

Percent survival as adults was similar for all groups in 1984, ranging from 1.32% for the control group (NG.x NOR.) to 1.82% for the accelerated growth x normal cross (ACC.x NOR.). The survival to adult values fluctuated greatly with the 1985 brood ranging from 2.91% for the control (NG.x NOR.) to 5.32% for the accelerated growth x normal cross (ACC.x NOR.), (Figure 1).

ADULT PRODUCTION:

While total survival was highest for the accelerated growth groups, fewer fish could be reared in each pond due to loading criteria, consequently, the production strategy which would produce the most adults is not obvious. In 1984, the normal growth x jack cross (NG.x Jack) produced 3,611 adults followed in order by the normal growth x normal cross (NG.x NOR.) with 2,940, the accelerated

growth x normal cross (ACC.x NOR.)with 2,851 and the accelerated growth x jack cross (ACC.x JACK) with the fewest adults with 2,784, (Figure 2).

In 1985, distinctly different values were observed. The accelerated growth x normal cross (ACC.x NOR.) produced the highest number of adults with 8,208, this was followed by the accelerated growth x jack cross (ACC.x JACK) with 7,460 the normal growth x normal cross (NG.x NOR.) with 6,879 and finally the normal growth x jack cross (NG.x JACK) with 5,488 adults.

SIZE OF ADULTS:

Fork lengths of adult males returning to the hatchery were smaller than adult females in every group both years (overall average), 54.6cm vs 64.4cm. for 1984 and 53.5cm vs 63.7cm for the 1985 brood, (Figure 3). Returning adult males from the 1984 brood accelerated growth groups were the same size as returning adult males of the normal growth groups, (54.5cm vs 54.8cm). The 1985 brood males from the accelerated growth groups were slightly larger than the males from the normal growth groups, (54.4cm vs 52.7cm). The returning adult females from the 1984 and 1985 brood accelerated growth groups were slightly larger than the normal growth groups, 65.0cm vs 63.9cm in 1984 and 64.0cm vs 63.0cm for the 1985 brood. Number of adults measured was at least 100 from each group for each brood year.The statistical significance of the differences has yet to be determined.

SEX RATIO'S OF RETURNING ADULTS:

More males than females returned to the Cowlitz hatchery regardless of treatment or brood year. The 1984 average was 60.6% males, (range 58.6%-64.5%), (Figure 4). The 1985 average was 66.8% males (range 58%-75.6%). In both brood years the groups with the highest percentage of total survival as jacks (both the normal and accelerated growth jack crosses) had the lowest percentage of males in the adult return. Both the normal growth and accelerated growth x normal crosses had the highest percentage of males in the adult returns, (Figure 4).

LITERATURE CITED

1) Noble,R.E. 1959. Downstream migration of silver salmon, timing-age-size, jack silvers - growth or inheritance. Proc. 10th N. W. Fish Cult. Conf., pp 48-51.

2) Salo,E.O., and W.H.Bayliff. 1958. Artificial and natural reproduction of silver salmon, Oncorhynchus kisutch, at Minter Creek, Washington. Wash. Dept. Fish. Res. Bull. 4. 76p.

3) Seidel, P.R.,S.B.Mathews. 1977. Comparison of times of release of 1972-brood Cowlitz and Elokomin coho reared and released at the Klickitat hatchery. Unpublished report, Washington Department of Fisheries. 7p.

4) Wallis, J. 1968. Recommended time, size and age for release of hatchery reared salmon and steelhead trout. Fish. Comm. Ore., Clackamas, Proc. Rept. 61p.

POST SPAWNING MORTALITY AT ENNIS NFH, MONTANA

Wes Orr
U.S. Fish and Wildlife Service

Ennis National Fish Hatchery, with 15,000 gallons per minute of 54 degree F. Spring water, has suffered from heavy post-spawning losses over the years. The percent mortality has fluctuated dramatically from year to year, but has always been much higher in male fish. Some years the mortality of males has exceeded 80 percent. The losses are characterized by the appearance of dime sized round or half moon spots of fungus on the dorsal surface of the fish which spread very rapidly. The integrity of the skin is compromised, water floods into the fish, and in 3 to 5 days the fish is usually dead. This mortality reduced the number of fish available for the next years spawning program, and also reduced the number of retired broodstock which could be used for catch out programs.

Prior testing at the Bozeman Fish Technology Center suggested that increased doses of vitamin C may decrease stress caused mortality. We decided to test this theory, so in 1989 the 2 year old Erwin strain rainbows were split equally into 6 raceways(830 fish per raceway). Three raceways were fed a diet containing 100 ppm vitamin C, and the other 3 were fed the same diet containing 500 ppm vitamin C. The AaPP (ascorbic acid polyphosphate) was donated by Rangens, and the feed was manufactured by Murray Elevator. The feed was fed and mortality recorded for 156 days beginning about 2 months before spawning. Mortality in the group fed 100ppm vitamin C was 4.56 Percent. The group fed 500ppm vitamin C also exhibited a low mortality of 4.67 Percent. Growth and egg survival of both groups was almost identical. There was no detectable difference between the 100ppm and 500ppm group. There were 2 problems with this test however. First, there was no control group, and secondly, nitrogen degassers were installed on the outside raceways in 1989. Degassers, by relieving one stress on spawning broodstock (supersaturation) could be responsible for reducing post spawning mortality.

So, in 1990 another test was initiated. This time Erwin strain rainbows were split equally into 3 groups. The control group received the contract GR6 broodstock diet, the other groups received the same formulation with 20ppm vitamin C and 100 ppm vitamin C derived from AaPP. Feeding started on April 1, 3 months before spawning, and terminated on September 30 at the end of spawning.

See table I below:

	Controls	20 PPM	100 PPM
NO. START 4/1	1680 fish	830 fish	830 fish
WT. @ START	3.00	3.30	3.20
WT. EACH @ END	3.70	4.20	3.80
LBS. FEED FED	4482	2367	2326
MORTS(183 DAYS)	100	43	44
% MORTALITY	5.95	5.18	5.30
PERCENT EYEUP	78.43	81.27	76.49
EGGS/LB FEMALE	990	914	939

TABLE I.

Only 2 or 3 fish ever developed the classic fungusing pattern common to Ennis. Since we were not able to demonstrate the effect of vitamin C on post-spawning mortality, we assume that degassing the water may be responsible for the absence of high mortality; and in fact, overall mortality for all strains has improved measurably since this installation. This confirms our observations over the years about stress. We believe that when the sum total of all the stresses confronting a spawning population reach a certain level, it causes the fish to break. Therefore, reducing stress any way you can, for instance by reducing nitrogen saturation, will reduce the overall stress and reduce post-spawning mortality. I am also convinced that like Pacific Salmon, there are genetic and chemical components in at least some rainbow trout populations that cause them to"break" when they have spawned. Perhaps some day the endocrinologists can tell us how to overcome that phenomenon too!

HATCHERY BROODSTOCK DEVELOPMENT AND ITS IMPLICATIONS IN REESTABLISHING SUMMER STEELHEAD RECREATIONAL FISHERIES IN NORTHEAST OREGON

Rhine T. Messmer
Richard W. Carmichael
Michael W. Flesher
Oregon Department of Fish and Wildlife
Research and Development Section
LaGrande, Oregon

The summer steelhead hatchery program in Northeast Oregon was initiated in 1976 because summer steelhead escapement to the Grande Ronde Basin, as indexed by redd counts, had decreased from an average of 6 redds/mile in the early 1960's to less than 1 redd/mile in 1976. This decrease was attributed to dams constructed on the lower Snake and Columbia rivers during this time period. The Lower Snake River Compensation Plan (LSRCP) was initiated in the early 1980's to reestablish productive summer steelhead fisheries in the Grande Ronde basin similar to what existed prior to construction of the four lower Snake River Dams and to enhance natural production of steelhead in the Grande Ronde basin with hatchery supplementation. A description of LSRCP mitigation goals and facilities is found in the LSRCP Five-Year Study Plan (Carmichael 1989)

In the Grande Ronde basin, summer steelhead recreational fisheries traditionally occurred from August through December on the lower Grande Ronde River and during February and March on the upper Grande Ronde and Wallowa rivers. The majority of the harvest occurred in the fall on the lower Grande Ronde River where fish appeared to overwinter prior to making a spring migration to spawning areas (Figure 1). Peak catch occurred in the 1969-70 run year when an estimated 1,600 steelhead were harvested. Because of decreasing escapement, summer steelhead seasons in the Grande Ronde River basin were closed in 1974.

To accomplish LSRCP objectives, the Oregon Department of Fish and Wildlife (ODFW) began to develop a hatchery broodstock (Wallowa stock) for the Grande Ronde Basin in 1976. From 1976-1978, wild adults of unknown origin were collected early in the spring at Ice Harbor and Little Goose dams. Summer steelhead adults were collected at the dams because escapement to the Grande Ronde basin was at an all time low in 1976, and there were no populations of steelhead which could provide adequate numbers of broodstock. Broodstock could not be collected from the lower Grande Ronde River in the fall because there were no adult holding facilities in Northeast Oregon capable of holding adult steelhead. Fish could not be trapped from the lower Grande Ronde River in the spring because of high spring run off. Collecting broodstock form Ice Harbor and Little Goose dams may have inadvertently selected for adults that had migrational patterns different from wild Grande Ronde steelhead, since the majority of the wild steelhead destined for areas above Lower Granite Dam pass the dam from August through December. In 1979, eggs were received from Pahsimeroi Hatchery in Idaho. Adult returns from smolts released at Wallowa Hatchery provided broodstock for the 1980-1990 broods. Less than 70 adults per year were used for broodstock for the 1976-1981 broods.

Returns of hatchery summer steelhead to the Grande Ronde Basin gradually increased form the first returns in 1980 and in 1983 a catch and release fishery was opened on the lower Grande Ronde River. In 1986 consumptive fisheries for adipose clipped hatchery fish were reopened on the Grande Ronde and Wallowa rivers. Summer steelhead creel surveys were initiated in 1985. Results from creel surveys indicated that few hatchery fish were caught during the fall-winter period in 1985 and 1986 even though wild fish were caught and released in good numbers during this time period. Also, the contribution of hatchery steelhead increased from September to December and many fish were caught in the Wallowa River during the spring in 1986 and 1987. (Carmichael et al. 1986, 1987, 1988) Because of poor contribution of hatchery fish in the fall we hypothesized that Wallowa stock hatchery steelhead were either overwintering in areas outside of Oregon in the Snake or Grande Ronde rivers or were less catchable than wild fish during the fall-winter time period.

In the fall of 1987, ODFW and Washington Department of Wildlife (WDW) jointly conducted a radio telemetry study to determine the migratory patterns of Wallowa stock steelhead destined for the Grande Ronde Basin. Results of the radio telemetry study are found in Mendel and Schuck (1989) and Carmichael et al. (1990). The general migration pattern of Wallowa stock steelhead destined for Wallowa Hatchery is well represented by the complete tracking data of three radio tagged fish that are presented in Figure 2. After being tagged at Lower Granite Dam in the fall, the steelhead migrated at a direct and steady rate up the Snake River until they reached an area near the mouth of the Grande Ronde River where they ceased active migration and overwintered. The fish overwintered in this area until mid-February when they started a rapid migration up the Grande Ronde and Wallowa Rivers. The results of the radio telemetry study corroborated our early creel survey results. Because the Wallowa stock steelhead was developed from fish collected in the Snake River in the spring, the majority of the run does not enter the Oregon section of the Grande Ronde River until February, and therefore are not available for harvest in Oregon during the fall-winter period.

Creel survey results for the 1988-89 (Carmichael et al. 1989) and 1989-90 run years (Carmichael et al. in preparation) show that in years with average or above average flows in the Snake and Grande Ronde rivers in the fall, some hatchery fish enter and are caught in Grande Ronde River in the fall, but most of the hatchery fish are caught in the spring. Historically most of the catch occurred in the fall (Figure 1). The exploitation rate on fish that enter the Grande Ronde River in the fall is probably higher than for fish that enter in the spring because of the excellent fishing conditions and high angler effort during the fall. This high exploitation could result in fewer fall-run fish returning to Wallowa Hatchery and consequently low numbers of fall-run fish being incorporated into the Wallowa broodstock. Fisheries could be selectively reducing the fall-run component of the Wallowa stock.

CONCLUSIONS

1. The original broodstock source and selection procedure for Wallowa Hatchery, the fishery contribution pattern, and adult migration pattern may limit achievement of the two LSRCP management objectives for summer steelhead in the Grande Ronde Basin.

2. The majority of Wallowa stock summer steelhead are not available to Oregon anglers during the fall.

3. There appears to be substantial differences in the migration patterns between hatchery and wild stocks. There is the possibility that interbreeding of hatchery and wild fish may alter the life history characteristics and productivity of the wild populations.

4. The high exploitation rate of hatchery fish that enter the Grande Ronde River in the fall may result in depletion of this component of the run.

RECOMMENDATIONS

1. Continue to monitor contribution of Wallowa stock summer steelhead to Grande Ronde and Snake River fisheries. This will help determine to what extent environmental conditions affect run timing of Wallowa stock.

2. Incorporate wild Grande Ronde steelhead into the Wallowa Hatchery broodstock program.

3. Attempt to increase and maintain the segment of the hatchery run which returns to the lower Grande Ronde River in the fall. Some options are:
   a. Not marking (adipose clip) some of the hatchery smolts to protect them from sport harvest.
   b. Incorporate wild adults into Wallowa stock on a annual basis.
   c. Implementing protective regulations such as area and season closures.

4. Releases using Wallowa stock smolts should be limited to areas that will maximize sport harvest and minimize the potential of hatchery fish interbreeding with wild fish.

REFERENCES

Carmichael, R.W. 1989. Lower Snake River Compensation Plan--Oregon evaluation studies, Five-year study plan. Oregon Department of Fish and Wildlife, Fish Research Project, Portland.

Carmichael, R.W., M.W. Flesher, and R.T. Messmer. 1989. Summer Steelhead creel surveys in the Grande Ronde, Wallowa, and Imnaha rivers for the 1988-89 run year. Oregon Department of Fish and Wildlife, Fish Research Project AFFI-LSR-90-17, Annual Progress Report, Portland.

Carmichael, R.W., R.T. Messmer, and B.A. Miller. 1987. Lower Snake River Compensation Plan--Oregon evaluation studies. Oregon Department of Fish and Wildlife, Fish Research Project FRI/LSR-88-16, Annual Progress Report, Portland.

Carmichael, R.W., B.A. Miller, and R.T. Messmer. 1986. Lower Snake River Compensation Plan--Oregon evaluation studies. Oregon Department of Fish and Wildlife, Fish Research Project FRI/LSR-86-35, Annual Progress Report, Portland.

Carmichael, R.W., B.A. Miller, and R.T. Messmer. 1988. Summer Steelhead creel surveys in the Grande Ronde, Wallowa, and Imnaha rivers for the 1987-88 run year. Oregon Department of Fish and Wildlife, Fish Research Project AFFI-LSR-89-02, Annual Progress Report, Portland.

Carmichael, R.W., B.A. Miller, and R.T. Messmer. 1990. Migratory patterns of adult Wallowa stock summer steelhead in the Grande Ronde and Snake rivers during the 1987-88 run year. Oregon Department of Fish and Wildlife, Information Reports (Fish) 90-2, Portland.

Mendel, G., and M. Schuck, 1989. Migration patterns of Wallowa Stock hatchery steelhead in the Snake and Grande Ronde rivers of Washington. Washington Department of Wildlife, Fisheries Management Division Report 89-4, Dayton.

PRODUCTION OF TRIPLOID RAINBOW TROUT

Carmen Olito and Irvin Brock
Alaska Department of Fish and Game
Broodstock Development Center
Fort Richardson, Alaska

ABSTRACT

Inducing triploidy in fish through heat shocking techniques is a relatively simple and inexpensive way to sterilize fish. These experiments were conducted to identify the optimal combination of thermal shock temperature, time after fertilization the shock should be applied, and shock duration necessary to produce the highest yield of triploidy in rainbow trout (Oncorhynchus mykiss). The experimental design followed a matrix that varied four temperatures, four post fertilization times, and three shock durations, done over two years. Flow cytometry analysis was used to determine triploidy. A 20 min thermal shock of 26C applied to green eggs 20 min after fertilization produced 100% triploid rainbow trout with the highest survival. This technique was repeated on a production level with equal success.

PREPARED FOR NORTHWEST FISH CULTURE CONFERENCE
DECEMBER 1990
BOISE, IDAHO

INITIAL USE OF WASTEWATERS FROM ARCATA SEWAGE TREATMENT SYSTEM FOR IMPRINTING COHO SALMON SMOLTS

George H. Allen, Ph.D.
Professor Emeritus Fisheries, Humboldt State University
and Aquaculture-Fisheries Consultant, City of Arcata,
Department of Public Works

and

David Hull
Aquatic Resources Specialist, City of Arcata,
Department of Public Works

ABSTRACT

A mixture of Arcata marsh and oxidation pond effluents was used in April 1990 to imprint 7,700 coded-wire tagged coho salmon smolts. Smolts came from a 90 percent survival of parr reared in two brackish-water ponds from December through April. Parr were fed pellets at four percent body weight per day. Complete pond netting, good water quality, and the inclusion of geese within the pond perimeter all contributed to high pond survivals. A unique size distribution with modes at 12 and 17 cm developing in both pond populations. All indices of smolt quality suggested optimal condition in our 1990 population except condition factor (values about 1.25 rather than near unity). Smolts left the imprinting pond for Humboldt Bay within 48 hours after initiation of tidal flushing.

INTRODUCTION

Prior to 1990, wastewater from the City of Arcata (Humboldt Bay, northern California coast) sewage treatment plant (STP) was only used in the Arcata wastewater salmon culture for rearing smolts in brackish-water ponds (Allen 1983). The wastewater provided salinity control in the rearing ponds and provided nutrients for production of natural foods (mysids, amphipods, and other estuarine benthos).

In April 1990 we conducted the first experiment on imprinting of coho smolts with wastewater. Wastewater imprinting of smolts and operating a homestream for capture of returning adults had been envisioned for more than a decade (Allen 1984). Our initial studies in 1990 employed a temporary pump and piping system to deliver a mixture of disinfected Arcata oxidation pond and effluent from the Arcata Marsh and Wildlife Sanctuary (Allen and Couch 1988; Figure 1) to an imprinting pond (Figure 1, South Pond).

Reported here are the details of rearing and imprinting of coho smolts of the 1989 brood year using wastewaters, and monitoring of the coho smolt migration into Humboldt Bay in April 1990.

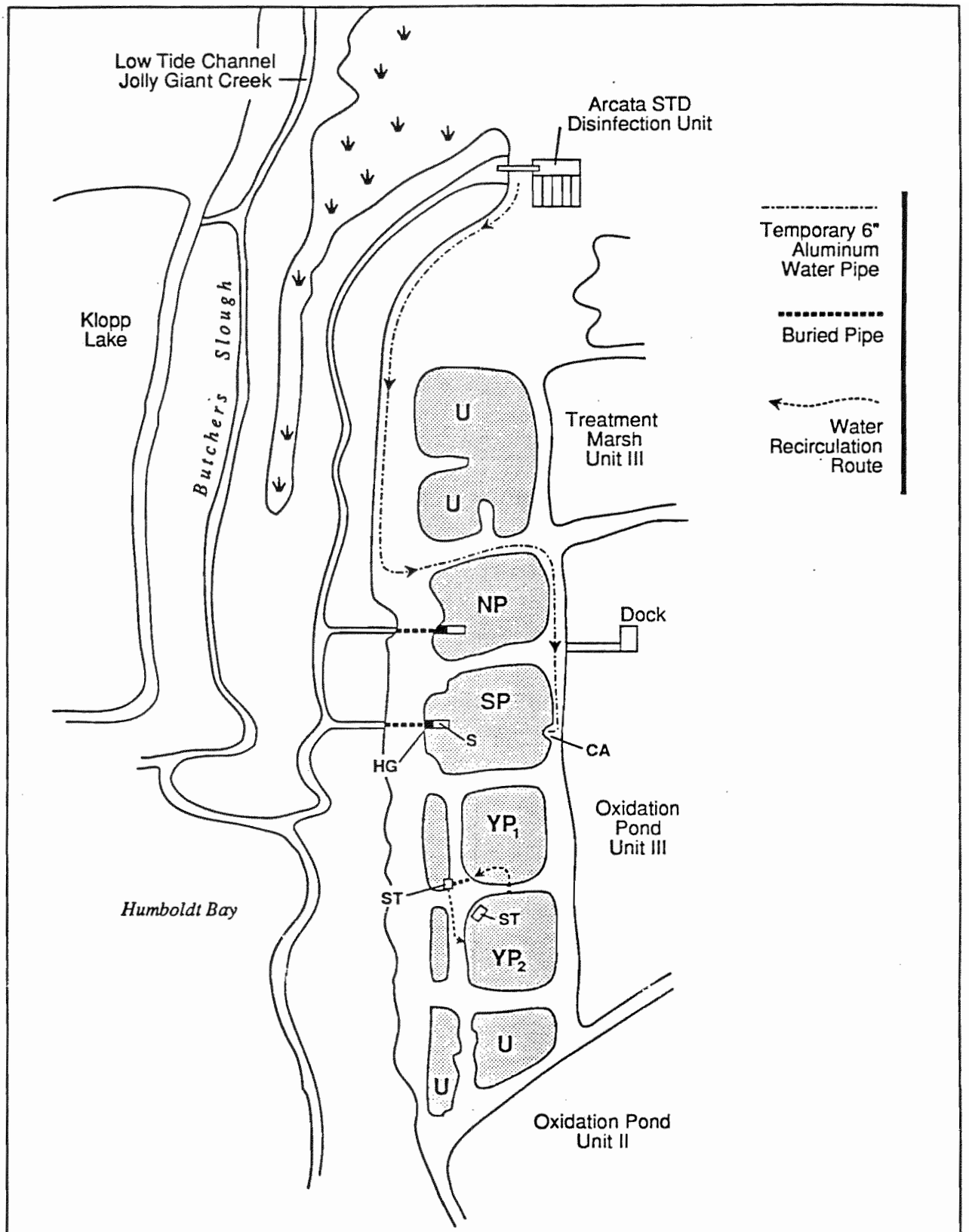


Figure 1. Physical facilities in operation, Arcata Wastewater salmon aquaculture facilities, May 1, 1990. [NP: North Pond; SP: South Pond; RF: Rock Filter; S: Concrete Sump; HG: Headgate; ST: Smolt Trap; YP1: Yearling (Winter Pond) No. 1; YP2: Yearling (Winter Pond) No. 2; U: Undeveloped future aquaculture units.]

MATERIALS AND OPERATIONS

Facilities

Ponds and associated facilities used in coho rearing and imprinting in 1989-90 are shown in Figure 1. Eggs and fry were incubated and reared in recirculating systems as previously described (Allen and Couch 1989). Fry were transferred to an outdoor pond in the spring of 1989 (Summer Pond No. 1 - SP_1) for rearing to late fall 1989. Fingerlings were marked by removal of an adipose fin and given a Coded Wire Tag (CWT) in November 1989 marked fish were divided into two lots and released into Yearling (Winter) Ponds (YP_1 and YP_2) for rearing to smolt stage. Summer Pond 1 was protected from otter and bird predation by a wire mesh fence around the periphery of the ponds, a one-strand electric wire at the base of the fence, and over-head netting. Winter Ponds were similarly protected. Grass along Winter Pond banks had been harvested during winter months by coots but were excluded in 1990 by pond fencing. In the 1989-90 winter rearing, we experimented with controlling pond-bank grass by the inclusion of three European domestic geese (Anser anser) in each pond.

Smolt traps were used to remove coho smolts in migrating condition during April prior to the pond draining scheduled for 27-28 April. Traps were the same as those previously described in Allen 1986. The trap in YP_1 was in operation during the entire rearing season because the trap's sump pump was being used to circulate water continuously between YP_1 and YP_2 during the 1989-90 winter rearing period (Figure 1). The smolt trap in YP_2 was placed into operation on 1 April 1990. YP_2 smolt trap did not operate at full efficiency in 1990 due to clogging of it's air-lift system water pumping system, and to deterioration in caulking in the seams of wood planks used in the trap construction.

Facilities added in April 1990 for imprinting smolts with Arcata STP effluent included submersible pump located in the Arcata STP effluent discharge chamber, 850 feet of 6"-diameter aluminum irrigation pipe to carry treated domestic wastewater to the imprinting pond (South Pond), and a "cascade aerator" to deliver STP effluent to the imprinting pond. The system delivered about 100 gpm to South Pond when operated.

The cascade aerator was our initial attempt at managing an expected high ammonia content in water associated with marsh and oxidation pond effluent. The cascade was constructed on a base built from the top of the dike to the pond bottom using surplus number 2 gravel and oyster shell mixture on site. This base surface was covered with hypolon (a rubberized fabric) forming a U-basin between retaining walls made of broken concrete blocks. Length of the cascade aerator was 21 feet, with the maximum width of 15 feet at the head and minimum width 1 1/2 feet where effluent entered the pond at mean pond level. The upper 8 feet of the cascade aerator was No. 2 gravel, and the bottom 13 feet of 2-5 inch broken concrete shards. Effluent entered the top of the cascade aerator via a 7-foot plastic pipe fitted with 1/4-inch drain holes drilled at 2-inch intervals.

Rearing ponds were fitted with air stones supplied with pumped air. Two sets of large air stones were placed in each pond, doubling the mixing capacity over previous years. An additional aeration unit consisting of an

8'-drilled pipe air diffuser and a 60'-floating plastic pipe fitted with 20 airstones of 1" x 1" x 2" dimension were placed across the east third of South Pond during smolt imprinting.

Operations

Coho salmon eggs for our 1989-90 operations originated from fish returning to the Humboldt County fish hatchery on Prairie Creek. Of the 50,000 eggs allocated to our project, only 13,000 fry (232 fish per pound) were produced from our indoor facilities. This relatively low survival rate was due to losses associated with an unknown disease or water quality problem whose symptoms mimic those of Cytophaga sp. (coldwater disease). Surviving fry placed into Summer Pond No. 1 resulted in 8,600 fingerlings (66 percent of fry introduced). Fingerlings (48 fish per pound) were given coded-wire tags in November 1989 and divided between Yearling Ponds Nos. 1 and 2 for rearing to smolt size.

Preparation of winter ponds for fingerling rearing was started in July 1989 when ponds were filled half full with saltwater pumped from Humboldt Bay. In October and November, freshwaters were slowly added to the ponds to establish a 1:1 salt to freshwater mixture. Both oxidation pond water and rainwater available in idle rearing ponds were used to establish initial pond salinities. The seawater-freshwater mixtures were aerated for six weeks prior to introduction of coho juveniles. Fingerling coho reared in winter ponds were fed at a four (4) percent body weight using both Silver Cup and Rangen dry pellets. During the last week of feeding in March, fingerling coho were fed Romet B, a commercial medicated-type feed carrying sulfadimethoxien and ormetoprin. The diet was fed at two (2) percent assumed body weight per day (6.7 pounds in YP_1 and 3.6 pounds in YP_2).

Wastewater Imprinting

Most smolts produced from our ponds have been captured on pond draining during late April or early May periods when pond-water temperatures reach 18-19 C and DSM-behavior has declined. With initiation of marsh effluent for imprinting smolts, times of pond draining were pre-set in 1990 to coincide with a new moon period just prior to the time of our historical peak out-migrations. This time also allowed for Humboldt State University students to assist pond personnel in seining, processing, and planting smolts. The period chosen for pond draining was in 1990 was April 26-27.

Initially smolts removed by trapping from YP_1 and YP_2 were to be placed in South Pond under tidal flushing with a constant addition of pumped STP effluent for imprinting. Delays in completing the STP effluent delivery system required the imprinting of trapped smolts by holding fish for 24-48 hours in a 500-gallon aerated aquaria filled with STP effluent and bay water at a 2:1 mixture. After tank imprinting, smolts were then released into South Pond operated only with tidally-flushed Humboldt Bay water. Smolts removed by pond draining were imprinted by holding in South Pond after the pond had been filled with a planned 1:1 mixture of STP effluent and bay water. We discovered that at low tide, the pond headgate installed in 1969, leaked so that the percent of wastewater in the pond at the time of introducing smolts

removed by pond draining was higher than planned. As a precautionary measure, additional saltwater was pumped into the pond during the time smolts were being held for imprinting.

Establishing the toxicity of Arcata STP effluent to smolts was undertaken by a standard static bioassay using a range of effluent-bay water mixtures (Young 1990). A field study of potential toxicity of wastewater-seawater mixtures actually used to imprint smolts in South Pond was conducted by holding smolts in cages placed in the pond. Static bioassay tested twelve effluent concentrations ranging from undiluted effluent to a 40 percent wastewater to 60 percent bay water mixture. Bioassay used four smolts per 5-gallon bucket of aerated test solution. Bioassay cages were located at the base of the cascade aerator in South Pond, and floated in surface water immediately adjacent to the South Pond headgate. Static bioassays were conducted in a shed housing a water storage and treatment unit of our egg incubation system.

Observations in previous years on the time of residence of smolts released into Jolly Giant Creek indicated that after 24 hours most smolts left the immediate area where released. We undertook to monitor the time of residence of smolts after release into South Pond for imprinting by making a standardized sweep at low tide with a 10-foot seine through a concrete sump located immediately inside the South Pond headgate. The sweep could not sample a small deep-well area of the sump located immediately adjacent to the headgate.

Smolts released into South Pond on 27 and 28 April 1990 were protected from bird predation by personnel staying overnight in a small trailer parked adjacent to the pond. In addition, a propane cannon, set at 5-minute intervals and directed across the pond, was used to minimize predation. No systematic bird deterrance was undertaken for a small lot of smolts held as a random sample and released May 8-10. On May 9, ospreys were allowed to fish (total dives 5, total smolts caught 5)! High tides occurred during the night following opening of the South Pond tide gate. We assumed migrants would reach deeper channels of mid-Humboldt Bay after two tidal flushings where they would not be subjected to heron predation but would subject to catch by cormorants and other diving species over which we had no control.

A random sample of juvenile coho removed from rearing ponds on 27-28 April were held in floating nets in a 500-gallon holding tank. On May 10, all coho were individually measured for length, and examined for obvious external pathology, and placed into South Pond as part of out-migration studies. Condition factor (L/W^3 x 1000) was determined on samples of smolts recovered from smolt traps in late April and for smolts removed from the ponds on draining on April 27 and 28.

Water Quality

Rearing Ponds

Physical water quality parameters in YP_1 and YP_2 varied little between ponds (Table 1). Salinities dropped steadily with dilution from seasonal rains. No periods of cold weather occurred in 1990, with pond-water

temperatures never falling below 7 C. Seasonal warming brought water temperatures up to 16 C by late-March. Mean pond temperatures fluctuated between 16 C and 18.5 C during April. Dissolved oxygen was always near 8.0 mg/l or higher. Visually observable higher phytoplankton densities in YP_2 as compared to YP_1, apparently reflected the slightly higher pH and oxygen values in YP_2 than YP_1, and the substantially lower Secchi disc readings. Despite the fact that water was constantly recirculated between the rearing ponds, a distinctly higher concentration of phytoplankton persisted in YP_2. During February and March, YP_1 became much clearer than YP_2 when YP_1 developed a considerable growth of Enteromorpha around the pond edges and on the upper portions of brush-bundgle reefs. With advent of clear, warm, and sunny periods in April, YP_1 became greener with phytoplankton, with a decreased clarity approaching that in YP_2.

Table 1. Water quality, brackish-water ponds, Arcata wastewater-seawater salmon aquaculture system, 6 November 1989-21 March 1990.

Factor	Yearling (Winter) Pond Number 1 Mean	1 Range	1 N	2 Mean	2 Range	2 N
Temperature (C)	10.5	7-16	19	10.5	8-16	19
Salinity (ppt)	12.7	7.1-14.9	9	13.6	8.4-14.9	9
pH units	7.7	7.0- 8.1	14	8.0	7.3- 8.7	14
Dissolved oxygen (mg/l)	8.8	6.2-10.6	12	10.7	7.8-15+	12
Clarity (secchi disc in cms)	90+	60-100+	13	70	40-100	13

Imprinting Pond

Water quality parameters were only sporadically collected during preparing South Pond for imprinting, and were collected by three different groups. Ammonia levels recorded from measurements using a Hach Aqaculture test kit Model FF-3 designed for saltwaters was useful only to suggest relative concentrations during the smolt imprinting operations. Studies by Peterson (1990) comparing Hack kits values against spectrophotometer values from standards showed Hach kit values higher than spectrophotometer values.

Arcata STP effluent was initially pumped into South Pond on 17 April and continued through 19 April. The pond had been filled partially with bay water prior to pumping. During this initial study of imprinting water mixtures, the ammonia index values were high (9 to over 15 mg/l readings), with dissolved oxygen levels near 6 mg/l (Table 2). On April 23, South Pond was drained and

Table 2. Physical and chemical characteristics of surface samples of smolt imprinting water o mixtures of Arcata STP effluent and Humboldt Bay water, 18 April-1 May 1990.

Date	Time	Temp. (C)	Salinity (ppt)	Dissolved oxygen (mg/l)	Total NH_3 (mg/l)	pH Units	Remarks
Apr 18	1030	-	15	5.6	-	-	Pumping STP effluent began April 17
19	-	15.0	6	6.2	>15	-	
20	-	17.0	-	6.0	9	7.5	Low tide. Cage bioassay with smolts near headgate started 20 April
25	0830	-	9	7.2	>10	-	Cage bioassay with smolts started at base of Rock Filter
26	0830	16.0	11	-	> 7	7.75	Values same for surface and bottom water
	(1030	-	22	-	0.4	-	Humboldt Bay water pumped into South Pond
27	-	-	6.3	11.9	> 7.5	7.75	YP_2 smolts added
	-	16.9	-	10.0	5	7.8	High tide
28	0700	16.5	9	6.6	-	-	YP_1 smolts added
	-	18.0	-	11.0	4	8.0	High tide
29	late pm	-	12	-	-	-	Headgate opened; bio-assays terminated
	-	18.0	-	11.0	3	8.0	High tide
30	ca 1600	21.5	-	-	-	-	Low tide
May 1	0830	14.5	-	-	-	-	Low tide

another imprinting mixture prepared in order to increase the ratio of saltwater to STP effluent. The pond was set at a four-foot depth using a guage located next to the headgate. STP effluent was added from April 23 through April 24. Readings during April 25-26 period showed total ammonia index values from 7 to 10 mg/l and oxygen near 7 mg/l. As noted previously, leaking of the pond headgate resulted in a greater ratio of effluent to bay water than could have been safe for the smolts in the pond. Thus we pumped bay water of 22 ppt and 0.4 mg/l NH_3 into South Pond during April 26. Smolts from YP_2 pond draining were placed into South Pond for imprinting on 27 April and YP_1 on April 28 as ammonia index values were declining (3 to greater than 7.5 mg/l) and oyxgen levels had elevated (mainly 10-11 mg/l). On 29 April, the headgate was opened allowing smolts to freely migrate to Humboldt Bay were they encountered ambient Humboldt Bay water conditions (Table 3).

Butcher Slough

Arcata aquaculture personnel did not monitor Humboldt Bay water quality but data were available from a study during April on the bacterial content of Butcher Slough waters (Darby 1990). Sampling sites were chosen in order to locate sources of bacterial contribution to Butcher Slough waters as follows: Site 1 - Jolly Giant Creek channel; Site 2 - Arcata STP effluent channel; Site 3 - imprinting pond (South Pond) discharge channel; and Site 4 - main channel of Butcher Slough below the confluence of the three channels noted above. Water samples were collected at weekly intervals between 26 March and 23 April inclusive. Samples were taken at both the highest and lowest tidal conditions occurring during daylight hours on sampling dates. Salinity and temperature encountered by smolts migrating from South Pond in April 1990 are shown in Table 3. High tide conditions probably were characteristic of open Humboldt Bay water (26-28 ppt salinity; 17-18.5 C temperature). Low tide conditions reflected the mixtures of freshwaters feeding each channel (Arcata STP freshwater effluent at Site 2; freshwater surface lens of Jolly Giant Creek water at Site 1; bay waters flowing from South Pond at Site 3; and predominantly freshwaters at Site 4 originating from mixtures of Arcata STP effluent and Jolly Giant Creek flows). Low tide temperatures were similar (17-18.5 C) to high tide water temperatures.

The only aberrant surface water temperatures recorded was a minimum value of 14.5 C recorded at low tide in South Pond on 1 May 1990 (Table 2). The value probably resulted from night-time cooling of surface water since Darby (1990) reported little temperature stratification in bay waters.

RESULTS

Production

Survival of fingerling coho placed in YP_1 and YP_2 during November 1989 survived at an overall 89 percent rate (7,700 total smolts recovered, Table 4). Survival was high in both ponds (87 and 92 percent respectively).

A total standing crop of 658 pounds of smolts was taken from the two ponds (Table 5). Dry pellets (464 pounds) produced a net gain of 478 pounds for a gross conversion ratio of about 1.0. The ratio indicates an efficient use of the pellets in the system probably from recycling of nutrients from

Table 3. Temperature (^{o}C) and Salinity (ppt) of Butcher Slough water sampled at four sites and high and low tide, March-April 1990 (from Darby 1990).

Tide	Site	Parameter	Sampling date, March-April 1990				
			3/26	4/2	4/9	4/16	4/23
	1	Salinity	21	20	21	20	16
		Temperature	17	18	18.5	18.5	18
	2	Salinity	1	1	1	1	1
		Temperature	17	17	17	17	17
	3	Salinity	26	28	27	28	21
		Temperature	18	18	18.5	18.5	18
	4	Salinity	4	4	6	4	3
		Temperature	17.5	18	18.5	18.5	18
Low		Tide Levels	0.6	-0.2	1.7	0.4	1.4
High		Tide Levels	6.7	5.1	5.9	4.7	5.5
	1	Salinity	27	27	28	28	17
		Temperature	18	18	18.5	18.5	17.5
	2	Salinity	27	27	26	26	17
		Temperature	18	18	18.5	18.5	17
	3	Salinity	27	27	26	26	23
		Temperature	18	18	18.5	18.5	17
	4	Salinity	27	27	26.5	26.5	17
		Temperature	18	18	18.5	18.5	17

Table 4. Number of coho smolts produced from two net-covered brackish-water ponds, Arcata wastewater salmon aquaculture system, November 1989-April 1990.

Factor	Yearling (Winter) Pond Number 1	2	Combined
Fingerlings released to ponds	5,031	3,580	8,611
Smolts recovered			
Trapping	603	252	855
Pond draining	3,793	3,054	6,847
Total	4,396	3,306	7,702
Percent recovered	87	92	89
Percent production trapped	14	8	11

Table 5. Weight of coho smolts produced from two net-covered brackish-water ponds, Arcata wastewater salmon aquaculture system, November 1989-April 1990.

Factor	Yearling (Winter) Pond Number 1	2	Combined
Pounds fingerlings released to pond	105	75	180
Pounds smolts recovered	413	245	658
Net pounds produced	308	170	478
Pounds pellets fed	287	177	464
Uncorrected dry-weight food conversion ratio	1.07	0.96	1.03

uneaten food, feces and urine through the food chain of the static pond system. The ratio was ubcorrected for natural pond food resulting from nutrients introduced with wastewater.

Smolt Condition

Smolts produced in 1990 were of uniformly high quality. Of 139 coho randomly sampled from fish recovered during pond drainings only one fish was found with any external signs of pathology. A 17 cm smolt with exopthalmia of one eye was considered as due to stress in the holding tank since samples were held in a suspended net for 10 days before examination. No missing or eroded fins were noted other than the adipose fin associated with CWT marking. Only one obviously half-clipped adipose was noted in the sample. The extremely healthy condition of the coho smolts removed on pond draining also characterized the smolt populations removed by trapping (Table 6). Excluding missing fins, only four (4) percent of smolts removed from YP_1 showed any external pathology, while less than one percent of smolts from YP_2 had any visually-appearing pathology. Neither smolts trapped or examined in random

Table 6. Rate of occurrence of "beak-marks", missing fins, and pathology in trapped coho salmon smolts, Arcata wastewater aquaculture system, March-April, 1990.

Factor	Yearling (Winter) Pond No. 1	2
Number examined	306	130
Beak-marked	0 (2)[1]	0
Missing fins (LP, RP)	3	0
Pathology		
Eroded operculum	4	1
Hemorrhaging (smolt)	1	0
Exopthalmia		
Parr	4	0
Smolt	2	0

[1]Black crown night heron gained access to smolt trap 20 April.

samples of smolts removed from the pond on draining produced fish with obvious signs of bird predation as measured by "beak marks". This is the first time when no such marks were recorded and contrasts sharply with previous years when a large number of such marks were recorded in both trapped smolts and smolts removed from the ponds (Allen et al. 1989; Table 14).

Under the 1989-1990 pond conditions and the heavy feeding regime, an unusual bimodal population developed in both ponds (modes at 12 and 17 cm) (Figure 2). Coho in YP_1 were on the average slightly larger than YP_2 because of more fish occurring in the intervals on the upper limbs of both modes. The largest coho we recovered measured 27.8 cm (from YP_1), showed no signs of gonad development and had an empty, atrophied stomach.

Smolt Migration

Rearing Ponds

Only 11 percent of our systems total smolt production was removed by trapping in 1990 (Table 4). In the continuously operated smolt trap in YP_1, initial catches were made in late March (Figure 3). A relatively large catch of smolts occurred in YP_2 smolt trap on 2 April after the trap was placed into operation on 1 April.

Smolt migratory behavior, as indicated by smolt trap catches, showed two peaks of activity in April (April 9-10; and April 24-27) (Figure 3). A slight rise in water temperature was associated with the April 9-10 peak, but not with the April 24-27 increase in catches. New moon fell on April 25 which correlated with the lunar hypothesis of a stimulus for the April 24-27 peak catches. Smolts may also have been stimulated by weak storm fronts and associated changes in barometric pressure during the April 9-10 and 24-27 peak trapping periods.

Although a new moon (dark phase) fell on March 26 in 1990 suggesting that the initial smolt movements in YP_1 were correlated with this physical factor, there was also a sharp rise in water temperature in late March. Most water temperatures were 16° C or above during peak smolt catches in April 1990 (Figure 3).

Only 22 parr occurred in smolt trap catches (12 - YP_1; 10 - YP_2, of which 6 were taken on 2 April, the first day of YP_2 trap operation). Only one parr was found in the sample of 139 fish sampled from the coho removed on draining ponds, although 10-20 coho in parr livery were known to have been placed in the holding tanks. The single parr recovered was 9.7 cm in size compared to the 8.8 cm size of the smallest smolt noted in the sample. This result is congruent with past experience that holding parr in cool water induces silvering, and probably also enhances DSM behavior. Minimum average smolt size of coho smolts trapped in both ponds was slightly over 10 cm (Figure 4).

Imprinting Pond

Monitoring time of out-migration of smolts imprinted in South Pond began with a trial seine sample on April 6. Many smolts were present but their time

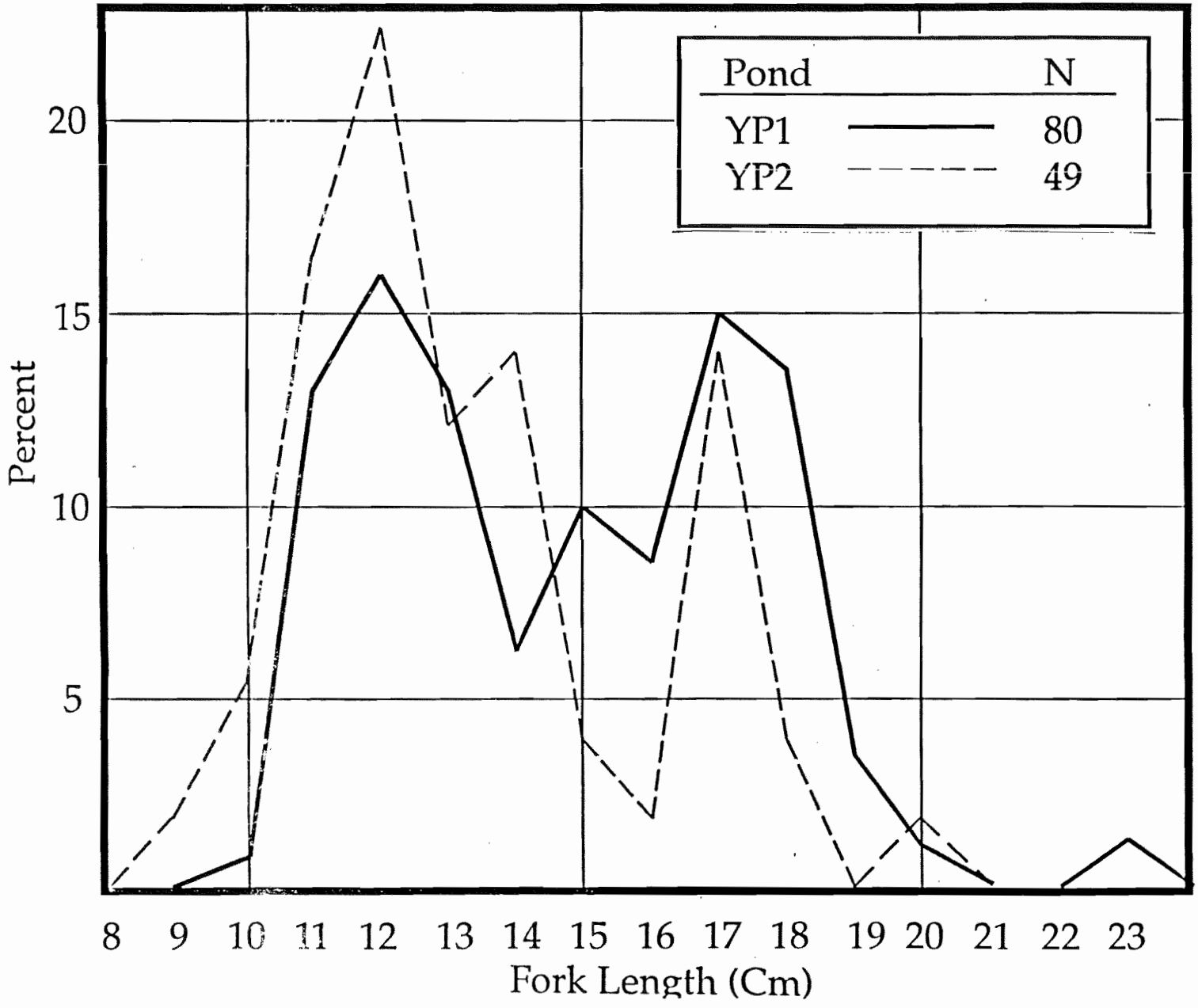


Figure 2. Length-frequency of random sample of coho salmon smolts removed from rearing ponds, 26 and 27 April 1990.

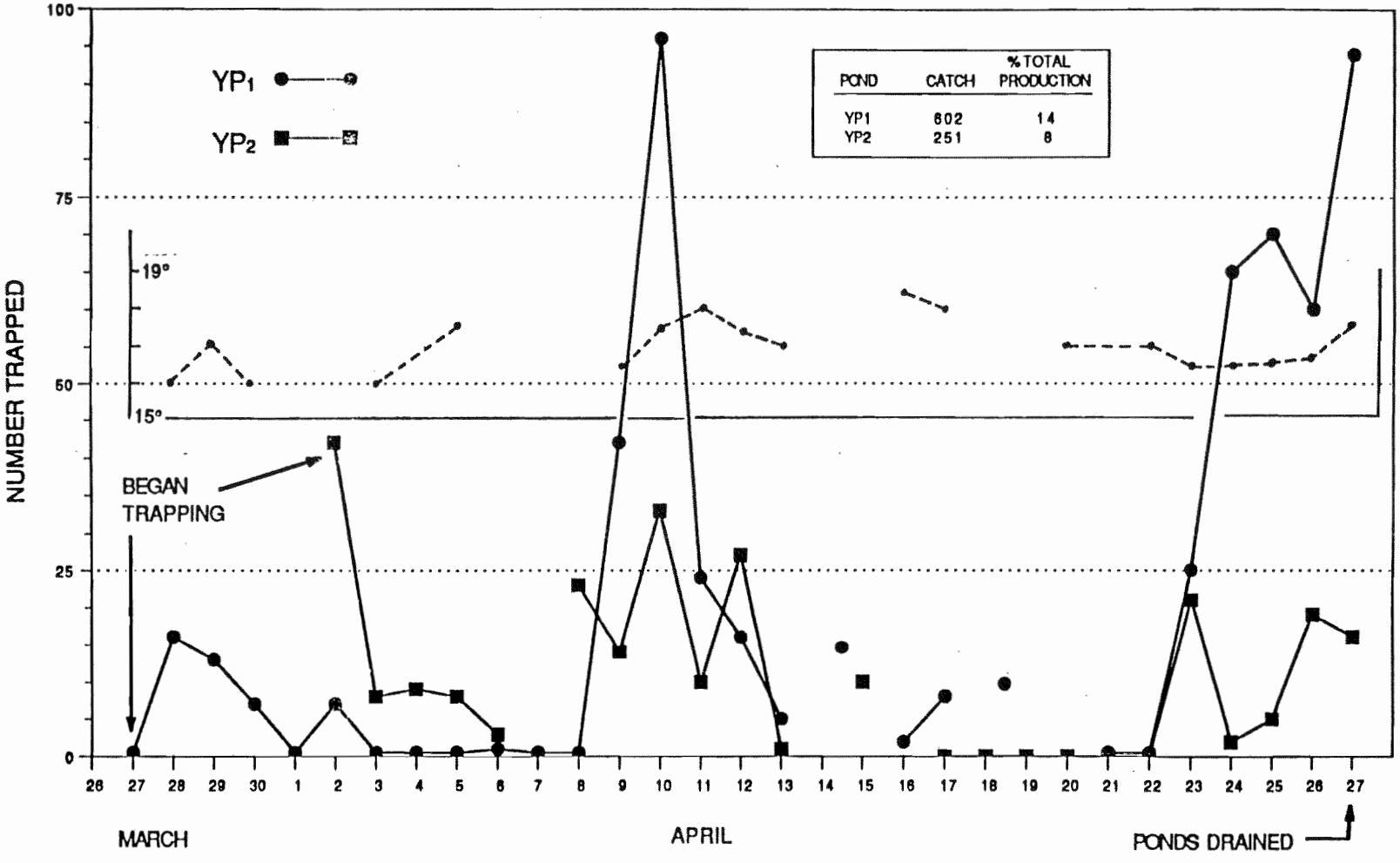


Figure 3. Number of coho smolts trapped from rearing ponds YP1 and YP2, 26 March - 27 April 1990.

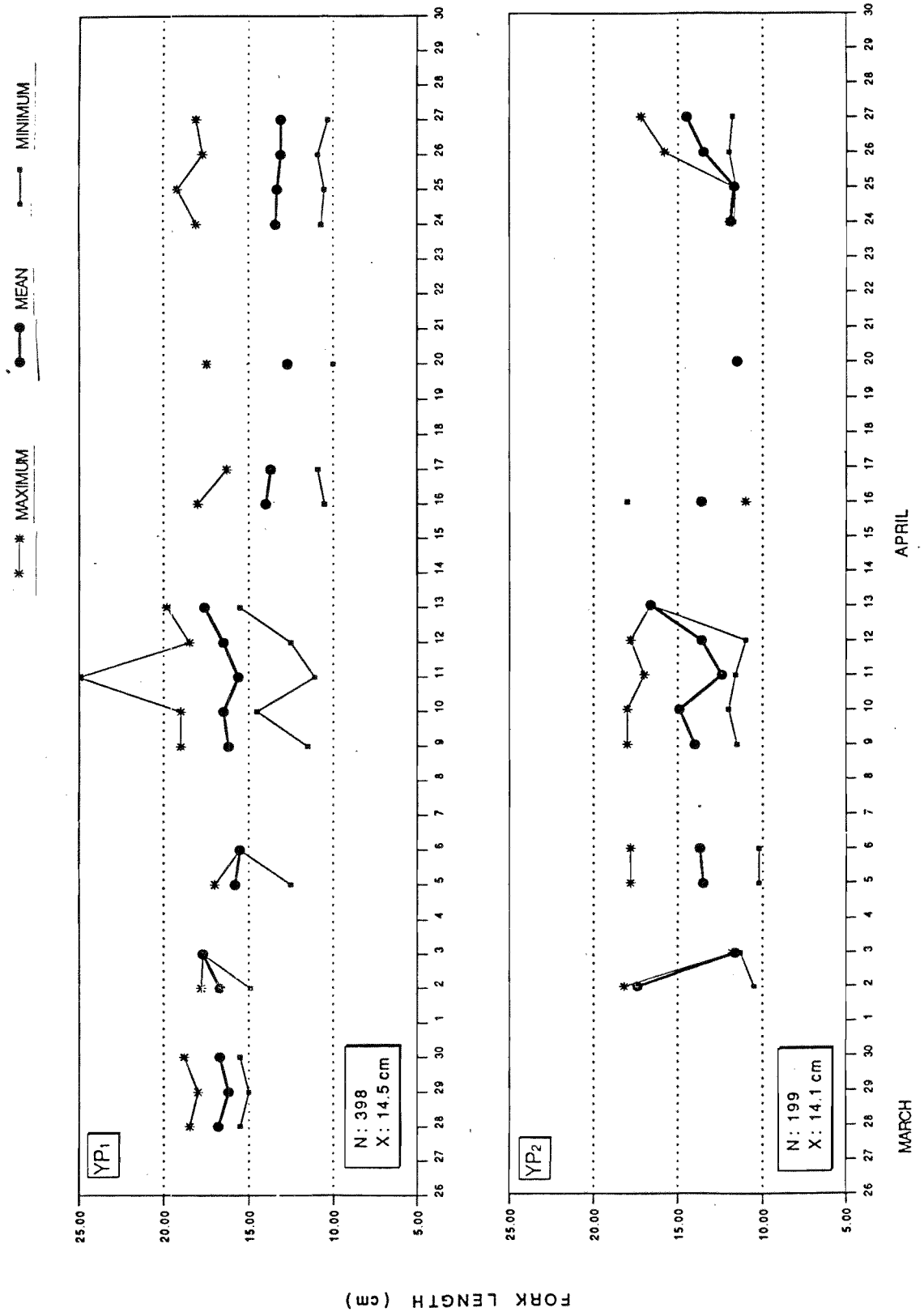


FIGURE 4 Maximum, mean, and minimum size of coho salmon smolts trapped from rearing ponds YP_1 and YP_2, 26 March - 27 April 1990

of migration was not followed by a second seine sample (Table 7). The major release of smolts into South Pond for imprinting on April 27 and 28 was followed by 2-3 days of imprinting in the wastewater-seawater mixture. On mid-morning of April 29 the headgate was opened on an out-going tide. By April 30, most coho had left the pond and on May 1, virtually no coho remained. The monitoring was repeated on May 9 and 10 when smolts from the random samples held in tanks were processed and released into South Pond on May 8. Again, most smolts had left the pond for Humboldt Bay within a 48-hour period (Table 7).

Table 7. Length of residence of coho smolts in South Pond, April 30 to May, as indicated by catch of one standardized sweep of 10-foot seine through pond sump at low tide.

Date	Time	Approximate Accumulated Coho Population	Temperature	Catch	Conclusion
April 6		100	-	20	None
April 30		7,000+	21.5	200+	Most left
May 1			14.5	0	All left
May 9		140	-	9	Most left
May 10			16.5	0[1]	All left

[1]Additional sweep over deep-well caught 3 smolts.

Smolt Size

Smolts produced ranged from a minimum of 8.8 cm to nearly 28 cm. Modal sizes were around 12.0-12.5 cm and 17.0-17.5 cm for both trapped smolts (Figure 5) and for smolts recovered during pond draining (Figure 2).

Larger smolts migrated first as clearly shown in 1990 by the catch from YP_1 smolt trap in March when no coho less than 15 cm were recorded (Figure 4). Smolts were much more variable in size in the initial YP_2 trap catches, with most smolts in the 10-12 size range. The overall larger mean size of coho in YP_1 was readily apparent by the smolt catches of April 9-11, when larger maximum, mean, and minimum sizes were recorded over YP_2 catches. During the last smolt trapping period of April 24-27, the mean size of coho trapped from both ponds decreased to about 13.5 cm.

Smolt populations migrating in the early, middle, and late portion of migratory period showed considerable relative sizes both within and between ponds (Figures 5 and 6). As noted previously, almost all smolts taken in the

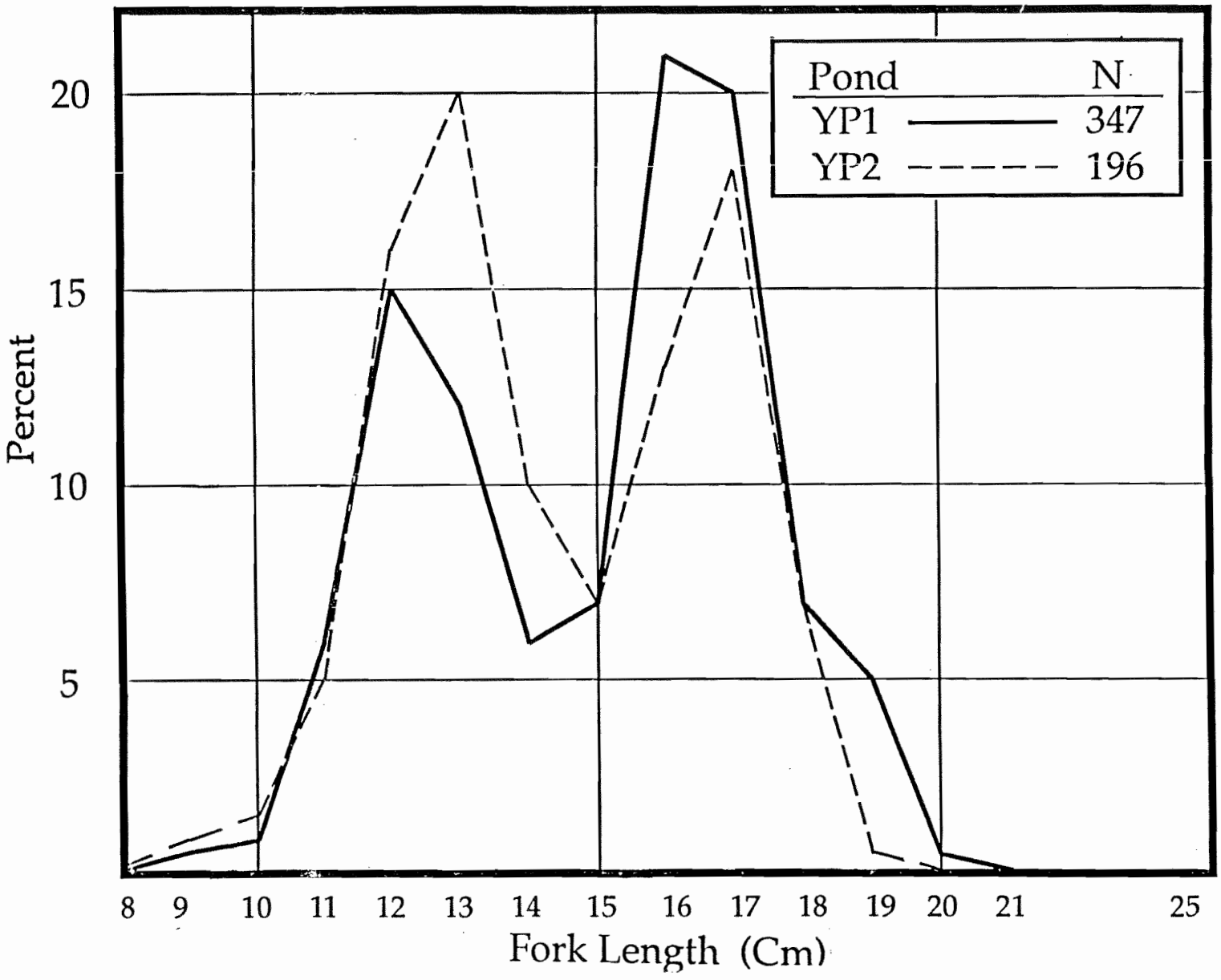


Figure 5. Length-frequency of coho salmon smolts removed from rearing ponds by trapping, 26 March - 27 April 1990.

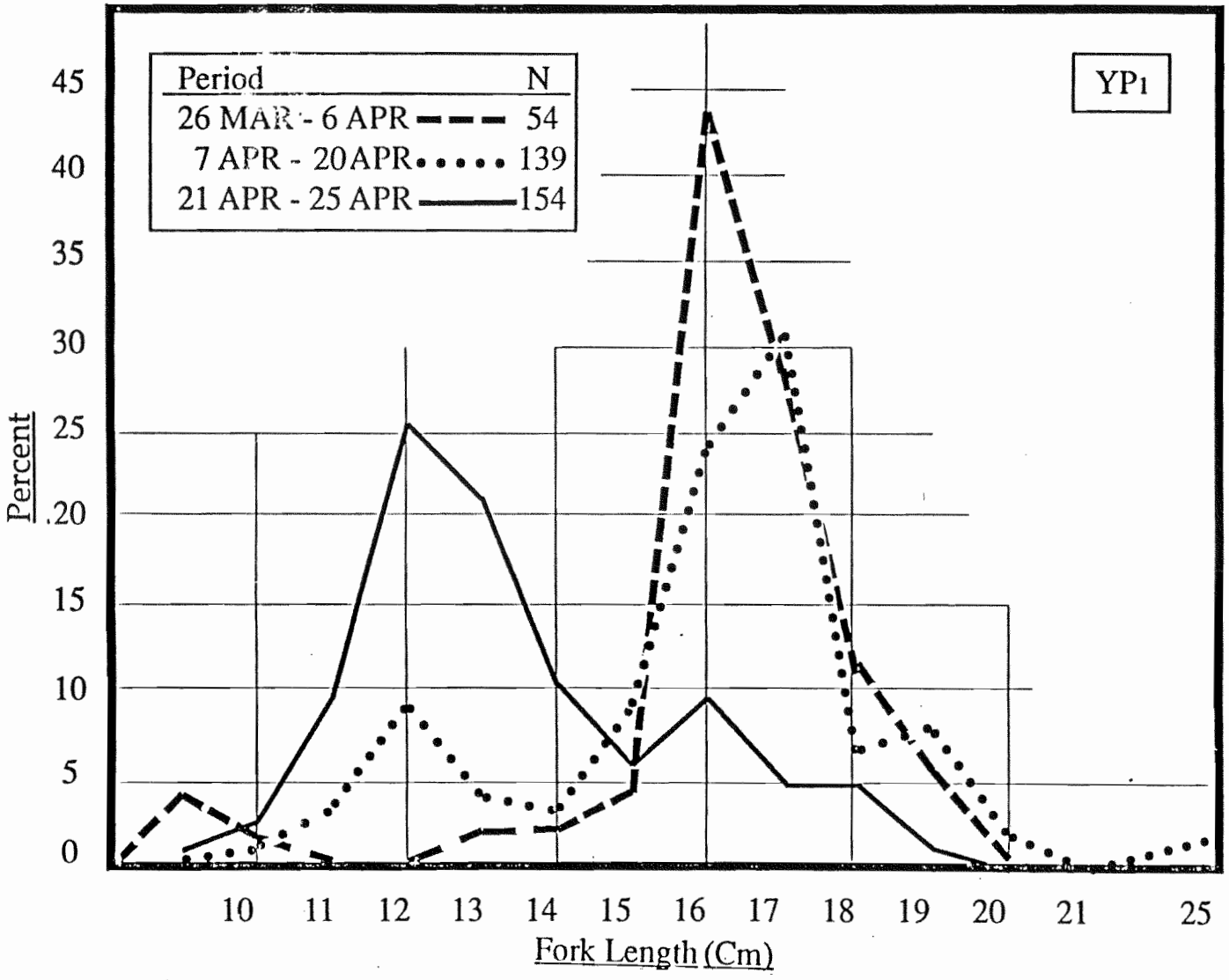


Figure 6. Comparison of size of coho salmon smolts trapped during early, middle, and late periods of smolt trapping operations, YP1 rearing ponds, 26 March - 27 April 1990.

early portion of trap catches from YP_1 were the larger-sized portion of the pond population. These larger-sized smolts continued to dominate trap catches during the middle portion of the run. Only in the last portion of smolt trapping did both the larger- and smaller-sized portion of the pond population appear equally in trap catches (Figure 6). In contrast, the early catch of smolts in YP_2, although mostly of the larger-sized fish, contained a noticeable number of the smaller-sized members of the pond population (Figure 7). Smolts in the middle portion of the run were primarily smaller fish, with a reduced percentage of the larger-sized smolts. The last smolts trapped were predominantly the smaller-sized fish, with a reduced percentage of larger-sized individuals left in the population.

The combined smolt trap catches displayed the bimodal size distribution found from smolts removed from both ponds found during pond draining. A relatively greater percentage of the smolts in YP_1 attained the larger-sized mode (Figures 5 and 6), accounting for the roughly 0.5 cm difference in average size between the two pond populations.

Marine Survival

Indications are that the 1990 smolts should have better than average marine survival based on all indices of smolt quality but one. Favorable indices included: high pond survival, no record of any period of impaired water quality to cause stress, virtually no recorded mortalities, no carcasses or skeletons recovered in the ponds after draining, only a small percentage of the smolt with any external pathology, a virtual 100 percent smolting in the two pond populations, and finally immediate migration of smolts from South Pond to Humboldt Bay. The single index not apparently within accepted norm was the condition factor. A C.F. near or below unity is associated with smolting since smolts are known to become slim during DSM and associated smolting stages. Some smolts produced in our ponds during 1990 obviously were slim but the population condition factor for samples measured in late April all exceeded a value of 1.25 (Table 8). Pond-reared coho in previous studies showed mean C.F. values of 0.99 during spring of 1976 and 1982 (Del Sarto 1980; Leonhardt 1984). As virtually no mortalities occurred during migration prior to saltwater entry, we had a highly effective release which should enhance the number of returning adults.

Quality of Smolt Imprinting Water

Undiluted Arcata STP effluent was toxic to coho smolts in static bioassays in April 1990 as reported in other years (Allen 1987; Tables 4 and 5). Smolts placed in undiluted effluent by Young (1990) all died within 24 hours. Total mortalities also occurred in mixtures equal to or less than 85 percent wastewater and 15 percent bay water. Dilutions equal to or greater than 80 percent wastewater and 20 percent baywater produced no smolt mortalities.

The ability of seawater to inhibit nitrite and ammonia toxicity to salmon smolts has been well established (Crawford and Allen 1977; Harader and Allen 1983). The ameliorating effect of seawater was demonstrated in 1990 by cage

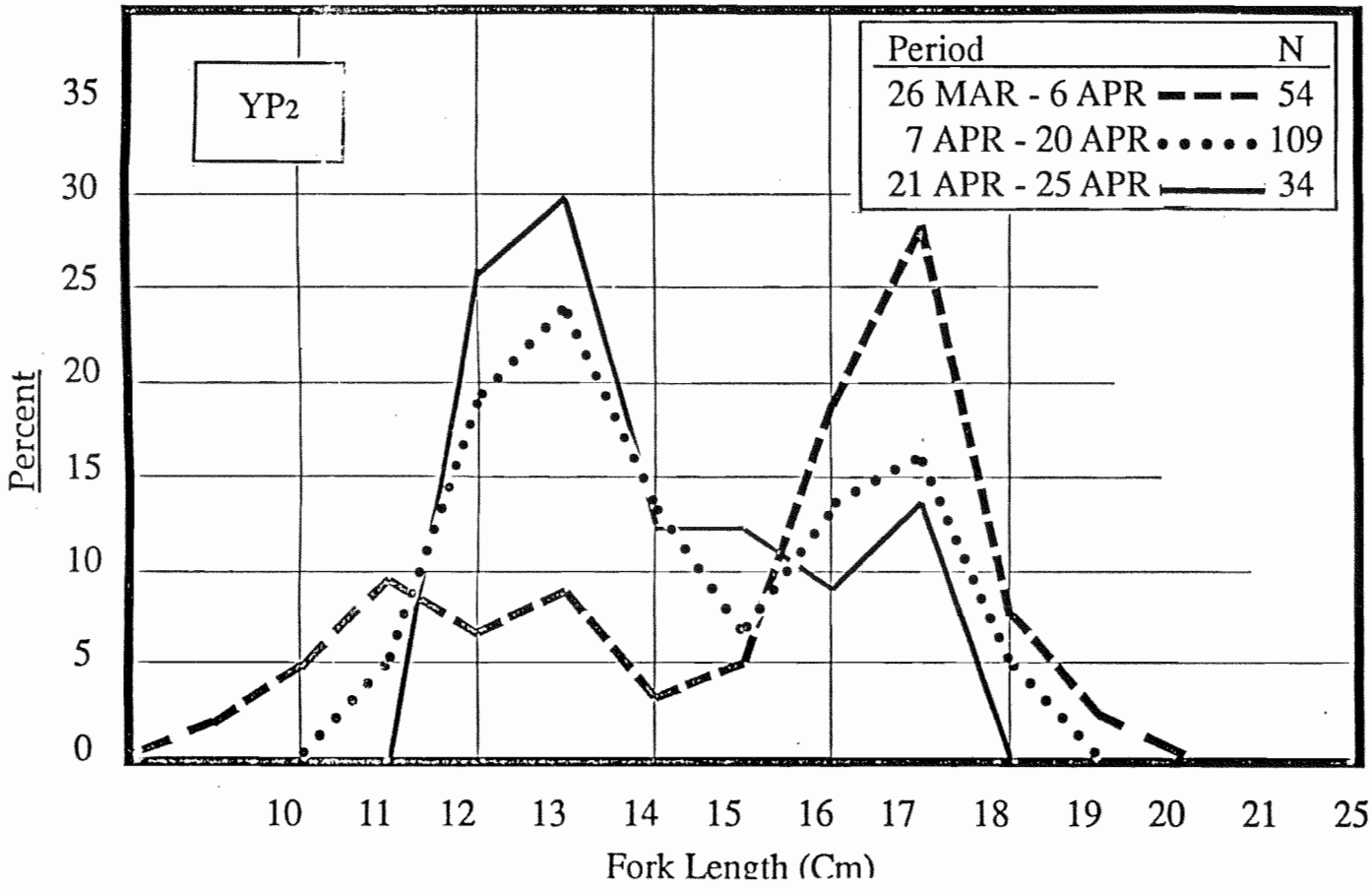


Figure 7. Comparison of size of coho salmon smolts trapped during early, middle, and late periods of smolt trapping operations, YP_2 rearing ponds, April 1 -27, 1990.

Table 8. Condition factor (L/W3 x 1,000) of coho smolts, YP1 and YP2 rearing ponds, 25-27 April 1990.

Group	Date	CF	Remarks
YP_1 Trap	25 Apr	1.25	Single sample of 40 smolts
YP_1 Trap	27 Apr	1.45	Single sample of 92 smolts
YP_1 Pond	26 Apr	1.29	Weight from 5 random sample of 50 smolts per sample; length from random sample of 80 smolts
YP_2 Pond	27 Apr	1.45	Weight from 3 random samples of 50 smolts per sample; length from random sample of 49 smolts

bioassay in South Pond and observation on smolts released into the pond for imprinting and migration. Three smolts placed in a cage adjacent to the headgate in South Pond on 20 April showed no signs of distress when examined on April 20 prior to pond draining in preparation of the static mixture of wastewater and bay water. On 25 April a second bioassay cage with seven smolts was placed at the foot of the cascade aerator directly in the path of incoming STP effluent. On 29 April, all ten smolts in the bioassay cages were alive and active when pond gate was opened and cage bioassays terminated. At no time were any coho smolts from the rearing ponds showing signs of stress during their residence in the ponds, or as noted in smolt samples seined from the pond sump at low tide. No concentrations of birds were reported April 26-29 during holding for imprinting again indicating no smolt stress since birds almost instantaneously are attracted to our ponds when stressed fish are present.

DISCUSSION

In 1990, survival of coho parr reared to fingerling size in the summer pond was average for our system. In contrast, survival recorded for rearing parr to smolt stage in Winter Ponds was the best ever recorded at the Arcata wastewater project. A major factor in this record survival had to be associated with the role of geese in reducing night-time penetrations into ponds by black-crowned night herons. This was evidenced by not a single beak-marked coho found in our 1990 smolt production. No evidence of any disease or parasites were also contributing factors to the high survival. Good fish health was correlated with good water quality in 1990.

The idiosyncratic nature of earthen fish ponds was illustrated by the relative difference between phytoplankton and macrophyte levels occurring

between the two rearing ponds despite the fact that water was constantly recirculated between the two ponds during the 1990 rearing period. Good water quality parameters were recorded from both ponds and high survival occurred in both ponds indicating that the kind of plant community may not be the critical factor in achieving the good quality smolts and high survival in our system.

Assignment of causes to the 10 percent mortality suffered in the yearling (winter) ponds was not possible, although canabalism brought on by the extreme range in size found in the smolts reared may be a possibility. Cannibalism in trout is regularly reported from observing larger fish in the process of swallowing smaller fish; however, we found no such evidence of cannabilism in the coho examined in 1990. Our large array of brush-bundle reefs may be providing diverse habitats for readily defended territories by coho parr during winter rearing. Fish eliminated may have been those most genetically inferior in the population.

Sporadic operational and mechanical problems continues to plague our egg and fry culture facilities using recirculated water. We will continue to implement improved emergency management and operations procedures to make the indoor rearing system consistently reliable.

Initial imprinting of smolts with a mixture of Arcata marsh and oxidation pond effluents was undertaken under conditions of uncertainty, particularly the potential of smolt mortalities due to ammonia toxicity. The possibility also existed of mortalities from free chlorine should there be malfunctions in the Arcata STP sulfonation process. Both physical (cascade aerator) and chemical (salinity) factors may have provided smolts with protection against imprinting water toxicity. The reduction in effluent ammonia by the cascade aerator probably was slight since the wastewater was spread only over about half the available surface area of the filter. Also the detention time through the filter was minimal. Despite the high total ammonia index values found in the imprinting water mixtures, there were no mortalities in coho smolts held in cages, nor were there any indications of stress occurring in either caged or pond fish. This result remains both a source of relief and puzzlement at this time. Marsh waters in natural environment in northern climates, especially during spring seasons, contain high ammonia levels. Fish populations in such areas have the option of temporarily leaving these high-ammonia aquatic habitats. Smolts released into a marsh-bay water imprinting pond do not have this option prior to opening pond gates. That the complex chemical constituents in bay saltwater protects smolts against ammonia levels found in laboratory studies seemed to be confirmed in studied on smolts in a field environment.

Future Activities

Previous pond management activities have been aimed at maximizing the juvenile fish survival in outdoor rearing ponds (winter and summer pond units). During the 1990-91 juvenile coho rearing season, we will begin experimenting on techniques to increase the percentage of smolt production removed by trapping. By increasing the percentage of smolt production that can be induced to migrate voluntarily, we will be reducing stress associated with seining and transport of smolts during pond draining. One approach will be to modify the entrances to our underwater smolt traps. An underwater fence will be placed perpendicular to the mouth of the smolt intake pipe in YP_1 in

order to direct smolt schools migrating along pond banks directly to the pipe entrance. In addition, a small cove in which the smolt inlet pipe is located will be covered with a black-plastic floating cover to darken the entrance. We also are planning to relocate our smolt-trapping facility in YP_1 so that trapped smolts can be diverted directly into imprinting waters without handling. The new system also will be designed so that smolt enumeration can be fitted with inexpensive smolt counters (Oulette 1987). By having smolts migrate directly into the imprinting pond, handling stress will be minimized, thus theoretically improving both imprinting and estuarine survival.

Poor adult coho returns during recent years was related to smolt losses in Jolly Giant Creek from non-point pollution (Allen 1988). We thus are predicting that the coho smolts released during the spring of 1990 should have marine survival at least equal to that of our previously better returns (0.3-0.5 percent adult return to Jolly Giant creek or closely adjacent stream). Thus we are anticipating sufficient returning adults to allow measurement of imprinting success from the pattern of recovery of returning adults in the 1990 and 1991 migratory seasons to streams entering Humboldt Bay. Whether adults will be attracted to the Arcata STP outfall rather than into the outfall pipe from yhe imprinting pond will be of major importance in planning future operations. Regardless of whether returning adults "home" to the Arcata STP outfall or return into imprinting pond, we plan on utilizing a much greater portion of the effluent in future operations of the adult capture facilities than the minimal flows to be used in the fall of 1990.

Our current egg and fry rearing facilities, even when operating at full capacity, will not be adequate to provide the fingerlings to test the capacities of existing and projected outdoor rearing ponds. Early thinking never envisioned that an Arcata system operating at full rearing capacity should duplicate existing egg and fry rearing capabilities already in excess capacity in Humboldt County. A basic concept was to cooperate with local agencies. Such cooperation would be to utilize the excess existing egg and fry rearing capacity in the county in return for surplus eggs to be produced by adults returning to Arcata resulting from an increased smolt production. The Arcata estuarine site has an advantage of continuous accessibility to adults regardless of local stream flow regimes. Delayed adult migrations have occurred in northern California coastal streams during recent low-flow years. Trapping facilities on local streams in turn suffer damage and become inoperable during flood flows during wet cycles.

Minimizing operational costs in the Arcata wastewater-seawater system has always been a major management objective. At the low rates of pond stocking during experimental work, feeding pellets at regular hatchery rates appears a waste of food. Our first major production of smolts, released in the spring of 1976, at an average size of only 11 cm, returned about 0.5 percent to Jolly Giant and Jacoby Creek watersheds. Maximizing the production of high-quality smolts of minimal size appears to be the strategy for attaining acceptable catch and escapement rates with least cost per smolt. We suggest that the overall carrying capacity of the Arcata yearling (winter) ponds with supplemental feeding and high aeration may easily approach or exceed 100,000 coho smolts per pond based on informal discussion with visiting fish culturists. We will be rearing a small complement of coho in Yearling Pond No. 1 in the 1990-91 rearing season without any supplemental feeding. The objective will be to produce smolts in a 10-12 cm size range from natural

feed. Adult returns will give additional data on how such minimal-sized smolts survive in the ocean as compared to 1990-smolt releases.

ACKNOWLEDGEMENTS

We are indebted to Steve Sanders, Superintendent, Prairie Creek Fish Hatchery, for supplying coho eggs for our 1989-90 studies. Hatchery manager Vincent Franklin and aquaculture technicians Vaden Jantz, Chuck Glasgow, Todd Flanigan, Tom Wallace, and Paul Kendall, were resposnsible for operations and assisted with draining ponds and processing fish. Students in Wastewater Aquaculture conducted term projects associated with coho rearing and assisted pond draining and smolt imprinting. We also acknowledge the assistance of David Couch, City of Arcata Sewage Treatment Technician; Larry Preston, CFG Eureka, California and Phil Baker and John Hays, CFG, Region I, Redding, California. We are indebted to Dr. Tom Hassler, California Cooperative Fishery Research Unit for use of their coded-wire tagging machine. Our thanks to Delores Neher,California Cooperative Fishery Research Unit, Humboldt State University, who assisted with manuscripts and prepared the final copy.

LITERATURE CITED

Allen, G.H. 1983. Arcata urban sports fisheries: A case history. pp. 208-243, In Allen, L.J. Ed., Urban Fishing Symposium Proceedings. American Fisheries Society,Bethesda, Maryland. 297 pp.

Allen, G.H. 1985. Artificial propagation in north coastal California citizen operated salmon and steelhead restoration and mitigation programs. pp. 5-66, In C. Toole, B. Wyatt, and S.Taylor, Eds., Report on the 3rd California Salmon and Steelhead Restoration Conference, February 23-24, 1985, Ukiah, California. California Sea Grant College Program, Marine Advisory Program, University of California, Davis. 155 pp.

Allen, G.H. 1986. Water temperature and downstream migration behavior of coho and steelhead smolts. Proceedings, 37th Annual Northwest Fish Culture Conference, 2-4 December 1986, Eugene, Oregon: Yearling Life Stages: 8-24.

Allen, G.H. 1987. Arcata wastewater treatment plant discharges for imprinting of salmonid smolts. Proceedings, 38th Annual Northwest Fish Culture Conference, 1-3 December, Tacoma, Washington: 174-210.

Allen, G.H. and D.R. Couch. 1988. Evolving management techniques at the Arcata wastewater salmonid aquaculture project. Proceedings, 39th Annual Northwest Fish Culture Conference, 6-8 December, Richmond, B.C.: 177-198.

Allen, G.H., D.R. Couch, and D.H. Hull. 1989. Controlling bird predation on one-third acre rearing ponds by total coverage with netting - 1989 results. Proceedings, 40th Annual Northwest Fish Culture Conference, 5-7 December, Shalishan, Oregon. In press.

Crawford, R.E. and G.H. Allen. 1977. Seawater inhibition of nitrite toxicity to chinook salmon. Transactions American Fisheries Society, Vol. 106, No. 1:105-109.

Darby, R.W. 1990. High and low tide bacterial analysis of Butcher Alough. Humboldt State University, Fisheries Department, Wastewater Aquaculture, Spring 1990. Term Projects Report. 37 pp. (G. Allen, Instructor).

Del Sarto, G.J. 1980. Time and development of smolting in salmon reared in brackish water. California, Humboldt State University, Fisheries Department. Master of Science Thesis. 143 pp.

Harader, R.R. and G.H. Allen. 1983. Ammonia toxicity to chinook salmon parr: reduction in saline water. Transactions American Fisheries Society, Vol. 112:834-837.

Leonardt, D.S. 1984. Evaluation of two-unit wastewater-seawater pond system for rearing juvenile coho salmon, Oncorhynchus kisutch. California, Humboldt State University, Fisheries Department, Master of Science Thesis. 76 pp.

Ouellette, D.A. 1987. Further studies on automatic counting of salmonid smolts. California, Humboldt State University, Fisheries Department, Master of Science Thesis. 79 pp.

Young, D. 1990. Toxicity of Arcata sewage plant effluent to coho smolts. Humboldt State University, Fisheries Department, Wastewater Aquaculture, Spring 1990. Draft Term Project Report. Unpublished. (G. Allen, Instructor).

LIST OF EXIBITORS

Company	Address	City/State	Zip Code
Argent Laboratories	8702 152 NE	Redmond, WA	98952
Bioproducts, Inc.	P.O. Box 429	Warrenton, OR	97146
Biosponge Aquaculture	P.O. Box 2989	Sheridan, WY	82801
Christensen Networks	230 Bayside Road	Bellingham, WA	98225
Common Sensing, Inc.	7595 Finch Road	Bainbridge Is., WA	98110
Electronic Data Solution	P.O. Box 31	Jerome, ID	83338
Industrial Plastics, Inc.	740 South 28th	Washougal, WA	98671
J.L. Darling Corp.	2212 Port of Tacoma Rd.	Tacoma, WA	98421
J.L. Eagar, Inc.	P.O. Box 476	Salt Lake, UT	84054
Jensorter, Inc.	20225 Harvest Lane	Bend, OR	97701
Magic Valley Heli-Arc, Inc.	P.O. Box 511	Twin Falls, ID	83301
Marisource	P.O. Box 9037	Tacoma, WA	98409
Moore-Clark	P.O. Box	La Conner, WA	98257
Ozone Research & Equip.	3840 N. 40th Ave.	Phoenix, AZ	85019
Pacific Rim Aqua Supplies	P.O. Box 774	Nanaimo, B.C.	V9R 5M2
Protect-A-Cover, Inc.	12310 Hwy 99 #124	Everett, WA	98204
Rangen, Inc.	P.O. Box 706	Buhl, ID	83316
Reiff Mfg., Inc.	Rt. 4, Box 183	Walla Walla, WA	99362
Silver Cup Fish Feed	P.O. Box 57426	Murray, UT	84157
Smith-Root, Inc.	14014 NE Salmon Ck. Ave.	Vancouver, WA	98686
Trondtech Marketing, Inc.	848 Superior Dr.	Campbell River, B.C.	V9W 7H3
VMG Industries, Inc.	858 Grand Ave.	Grand Junction, CO	81501
Waring Marine Ltd.	3113 2nd Ave.	Port Auberni, B.C.	V94 4C4

LIST OF REGISTRANTS

	T..B..A..	NORTHWEST INDIAN FISHERIES COMM.		OLYMPIA	WA 98506
	T.B.A.	CEDC FISHEREIS PROJECT	250 - 36TH STREET	ASTORIA	OR 97103
ABBOTT	PAUL	IDAHO POWER COMPANY	P.O. BOX 70	BOISE	ID 83707
ACOBA	BELINDA	BELLINGHAM VO-TECH	1717 TEXAS STREET #8	BELLINGHAM	WA 98226
ADAMS	ROBERT	WINTHROP NATIONAL FISH HATCHERY	P.O. BOX 429	WINTHROP	WA 98862
AITKEN	GARY	KOOTENIA RIVER W. STURGEON HATCHERY	P.O. BOX 1269	BONNERS FERRY	ID 83805
ALLEN	GEORGE	HUMBOLDT STATE UNIVERSITY	2711 SUNNY GROVE AVENUE	MCKINGLEYVILLE	CA 95521
ALSAGER	RICK	IDFG-SAWTOOTH HATCHERY	HC64, BOX 9905	STANLEY	ID 83278
AMOS	KEVIN	WASHINGTON DEPT. OF FISHERIES	115 G.A. BUILDING	OLYMPIA	WA 98501
ANDERSON	DICK	USFWS	1440 ABERNATHY RD	LONGVIEW	WA 98632
ANDERSON	RON	BIOPRODUCTS, INC.	P.O. BOX 429	WARRENTON	OR 97146
APPLEBY	ANDY	WASHINGTON DEPT. FISHERIES	ROOM 115 GENERAL ADMIN. BLDG.	OLYMPIA	WA 98504
ARNOLD	MIKE	QUILEUTE FISHERIES	P.O. BOX 187	LAPUSH	WA 98350
AURDAHL	GLEN	SVERDRUP CORPORATION	P.O. BOX 369	BELLEVUE	WA 98009
AUSTIN	ROBERT	BONNEVILLE POWER ADMIN.	796 NE BUNYARD PLACE	ESTACADA	OR 97023
BABIAR	MARC	WASHINGTON DEPT. OF FISHERIES	2640 KINNIKINNICK DRIVE	LEAVENWORTH	WA 98826
BAKER	ALEX	MUCKLESHOOT TRIBE	25305 SE MUD MOUNTAIN ROAD	ENUMCLAW	WA 98022
BANKS	JOE	USFWS	1440 ABERNATHY ROAD	LONGVIEW	WA 98632
BARBER	JAY	IDAHO DEPT. OF FISH AND GAME	2974 FISH HATCHERY ROAD	AMERICAN FALLS	ID 83211
BARE	BRYON	IDFG-RAPID RIVER FISH HATCHERY		RIGGINS	ID 83549
BARGER	LARRY	WASHINGTON DEPT. OF WILDLIFE	W 7570 EELLS HILL ROAD	SHELTON	WA 98584
BARRETT	DAN	OREGON DEPT. OF FISH AND WILDLIFE	STAR ROUTE B, BOX 1	CASCADE LOCKS	OR 97014
BASS	RAY	OREGON DEPT. OF FISH AND WILDLIFE	15055 S. CENTURY DRIVE	BEND	OR 97707
BAST	DALE	U.S. FISH AND WILDLIFE SERVICE	1325 FISH HATCHERY ROAD	JACKSON	WY 83001
BATES	DAVE	CAPILANO COLLEGE	2055 PURCELL WAY	N. VANCOUVER	BC V7J 3H5
BAXTER	MIKE	WASHINGTON DEPT. OF FISHERIES	2284 B SPENCER ROAD	SALKUM	WA 98582
BEERS	DAN	IDFG-CLARK FORK FISH HATCHERY	P O BOX 98	CLARK FORK	ID 83811
BERG	JERRY	US FISH & WILDLIFE SERVICE	P.O. BOX 1994	OROFINO	ID 83544
BERGSTROM	ROBERTA	IDAHO DEPT. OF FISH AND GAME	1800 TROUT ROAD	EAGLE	ID 83616
BILAS	JOHN	ALBERTA GOVT FISH AND WILDLIFE	1440 17A STREET S.E.	CALGARY ALBERTA	t26 4t9
BILLMAN	DAVE	IDFG-AMERICAN FALLS HATCHERY	2974 S. HATCHERY ROAD	AMERICAN FALLS	ID 83211
BILLUPS	BARRY	SIMPLOT AQUACULTURE	20875 WAGNER ROAD	CALDWELL	ID 83605
BLACK	TAMARA	WASHINGTON DEPT. OF FISHERIES	610 N. MISSION, STE. B8	WENATCHEE	WA 98801
BLODGETT	JOE	MT HOOD COMMUNITY COLLEGE	2901 SO. 118	PORTLAND	OR 97266
BODLE	JACK	LITTLE WHITE SALMON-WILLARD NFH	BOX 17	COOK	WA 98605
BOLDING	BRUCE	WASHINGTON DEPARTMENT OF WILDLIFE	600 CAPITOL WAY N	OLYMPIA	WA 98501
BOLDT	HAL	OREGON DEPT. OF FISH AND WILDLIFE	17330 SE EVELYN ST.	CLACKAMAS	OR 97015
BONNELL	GREG	DEPT. OF FISHERIES & OCEANS	555 W. HASTINGS STREET, ST.400	VANCOUVER	BC V3L 1N2
BOOMER	RALPH	U.S. FISH AND WILDLIFE SERVICE	2625 PARKMONT LANE, BLDG. A	OLYMPIA	WA 98502
BORNIA	COLIN	SIMPLOT AQUACULTURE	214 MARVIN STREET	CALDWELL	ID 83605
BOURBON	KENT	IDFG-CLEARWATER HATCHERY		AHSAHKA	ID 83520
BOYER	NORMAN	S.S.R.A.A.	1621 TONGASS	KETCHIKAN	AK 99901
BROCK	CAROLYN	RANGEN, INC.	115 13TH AVE S.	BUHL	ID 83316
BROCK	DAVID	RANGEN, INC.	115 13TH AVE. S.	BUHL	ID 83316
BROCK	IRVIN	ALASKA DEPT. OF FISH AND GAME	P.O. BOX 5267	FORT RICHARDSON	AK 99505
BROWN	ALLAN	U.S. FISH AND WILDLIFE SERVICE	1425 FISH HATCHERY ROAD	JACKSON	WY 83001
BROWN	ART	WASHINGTON WILDLIFE	1182 SPENCER ROAD	WINLOCK	WA 98596
BROWN	DOUGLAS	MUCKLESHOOT TRIBE	34900 212TH AVENUE SOUTH	AUBURN	WA 98002
BRUHN	DAVE	U.S. FISH AND WILDLIFE SERVICE	3059-D NATL FISH HATCHERY RD	HAGERMAN	ID 83332
BRUNSON	RAY	U.S. FISH AND WILDLIFE SERVICE	2625 PARKMONT LANE BLDG. A	OLYMPIA	WA 98502
BUCKMASTER	BRUCE	BIOPRODUCTS, INC.	P.O. BOX 429	WARRENTON	OR 97146
BURGE	HOWARD	IDAHO FISHERIES RESOURCE OFFICE	P.O. BOX 18	AHSAHKA	ID 83520
BURNS	ROD	ALBERTA FISH & WILDLIFE DIVISION	9945 108TH ST.	EDMONTON	AL T5K 2G6
BUSBY	CHRIS	OVA TECH	BOX 819	LUMBY	BC V0E 1G0

BYRNE	JIM	WASHINGTON DEPT. OF WILDLIFE	5112 NE 139 LOOP	VANCOUVER	WA 98682
CARLSON	CHRIS	GRANT COUNTY P.U.D.	P.O. BOX 878	EPHRATA	WA 98223
CARPENTER	SHIRLEY	CALIFORNIA DEPT. OF FISH AND GAME	1701 NIMBUS ROAD, SUITE A	RANCHO CORDOVA	CA 95670
CHAPMAN	JERRY	IDFG, ASHTON FISH HATCHERY		ASHTON	ID 83420
CHAPMAN	JOE	IDFG-CABINET GORGE HATCHERY	P O BOX 493	CLARK FORK	ID 83811
CHAPMAN	PATRICK	WASHINGTON DEPT. OF FISHERIES	115 GENERAL ADMIN BLDG	OLYMPIA	WA 98504
CHETTLEBURGH	PETER	CANADIAN AQUACULTURE MAGAZINE	4611 WILLIAM HEAD ROAD	VICTORIA BC	CN
CHILTON	GUY	OREGON DEPT. OF FISH & WILDLIFE	STAR RT, BOX 750	CASCADE LOCKS	OR 97014
CHRISTENSEN	SCOTT	CHRISTENSEN NETWORKS	230 BAYSIDE ROAD	BELLINGHAM	WA 98225
CHRISTIANSON	CHRIS	OREGEON DEPT. OF FISH AND WILDLIFE	8702 SW 49TH	PORTLAND	OR 97219
CHRISTOPHER	MARLA	BIOPRODUCTS INC	BOX 11 BUCCANEER	HALFMOON BAY	BC VON1YO
CISNEROS	GERALD	COWLITZ SALMON HATCHERY	2284 SPONSER ROAD	SALKUM	WA 98582
CLARINE	RONALD	U.S. FISH AND WILDLIFE	6970 HATCHERY ROAD	ENTIAT	WA 98822
CLEMENS	KATHLEEN	DWORSHAK FISH HEALTH CENTER	P.O. BOX 18	AHSAHKA	ID 83520
CLUNE	THOMAS	BONNEVILLE POWER ADMINISTRATION	103 S. 3RD STREET	YAKIMA	WA 98901
COCHRAN	JAMES O.	DEPT OF FISH AND GAME	P.O. BOX 3-2000	JUNEAU	AK 99802
COFFELT	ROBERT	SIMPLOT AQUACULTURE	20875 WAGNER ROAD	CALDWELL	ID 83605
COLEMAN	CAROL	WASHINGTON DEPT. OF FISHERIES	1435 PAVEL ROAD	BEAVER	WA 98305
COLT	JOHN	JAMES M. MONTGOMERY	2375 130TH AVE. N.E., STE 200	BELLEVUE	WA 98005
CONANT	JAMES	THE CONTROLS GROUP	10518 N.E. 68TH STREET	KIRKLAND	WA 98033
COONTS	PHIL	IDFG-SAWTOOTH HATCHERY	HC64, BOX 9905	STANLEY	ID 83278
CORN	ROBERT	CALIFORNIA DEPT. OF FISH & GAME	601 LOCUST ST.	REDDING	CA 96001
COSTELLO	RON	MOORE-CLARK	BOX M	LA CONNER	WA 98257
CRATEAU	ED	U.S. FISH AND WILDLIFE SERVICE	4696 OVERLAND ROAD, ROOM 560	BOISE	ID 83702
CRAWSON	MICHAEL	RANGEN AQUACULTURE RESEARCH CTR.	RT 1, BOX 264	HAGERMAN	ID 83332
CREER	RONALD	UTAH DIVISION OF WILDLIFE	1596 W. NORTH TEMPLE	SALT LAKE CITY	UT 84116
CRISLER	ROBIN	OREGON DEPT. OF FISH AND WILDLIFE	95183 ELK RIVER ROAD	PORT ORFORD	OR 97465
CUENCO	MICHAEL	CRITFC	975 SE SANDY BLVD., SUITE 202	PORTLAND	OR 97214
CUTTING	CHARLES	SVERDRUP CORP.	P.O. BOX 369	BELLEVUE	WA 98009
D'AGUST	BRIAN	COMMON SENSING, INC	7595 FINCH ROAD NE	BAINBRIDGE IS.	WA 98110
DAGGETT	WILLIAN	WASHINGTON DEPT OF FISHERIES	HCR 78, BOX 431	NASELLE	WA 98638
DAVIS	JOHN	U.S. FISH AND WILDLIFE SERVICE	CARSON NATIONAL FISH HATCHERY	CARSON	WA 98610
DE AVILA	JEANENE	ANIMAL SCIENCES, WSU	347 CLARK HALL, WA STATE UNIV	PULLMAN	WA 99164
DE LA GARZA	JUAN	UNIVERSITY OF IDAHO	DEPT. FISH AND WILDLIFE	MOSCOW	ID 83843
DEISHER	BOB	RANGEN, INC.	P.O. BOX 706	BUHL	ID 83316
DEISHER	CORAL	RANGENS		BUHL	ID 83316
DELARM	MIKE	NATIONAL MARINE FISHERIES SERVICE	13635 SE ELLIS STREET	PORTLAND	OR 97236
DELEBO	JOHN	OSMONICS	5951 CLEARWATER DRIVE	MINNETONKA	MN 55343
DEPAPE	DAVE	ALBERTA GOVERNMENT FISH & WILDLIFE	1440 17A STREET NE	CALGARY ALBERTA	T2G 4T9
DEWANDEL	RAY	SIMPLOT AQUACULTURE	20875 WAGNER ROAD	CALDWELL	ID 83605
DICKENSON	MIKE	OREGON DEPT. OF FISH AND WILDLIFE	HC 30, BOX 142	CHILOQUIN	OR 97624
DITTENTHOLER	THOMAS	MT HOOD COMMUNITY COLLEGE	24050 SE STARK STREET, #222	GRESHAM	OR 97030
DIXON	GLEN	INCH CR. HATCHERY - D.F.O.	BOX 61	DEWDNEY BC	VOM IHO
DORMAN	PAUL	IDFG, MCCALL FISH HATCHERY	P.O. BOX 1021	MCCALL	ID 83638
DREDGE	BRAD	IDFG HAYPUR FISH HATCHERY	071 US HIGHWAY 20	BELLEVUE	ID 83313
DUCEY	RONALD	CALIFORNIA DEPT. OF FISH AND GAME	2001 NIMBUS ROAD	RANCHO CORDOVA	CA 95670
DUNDAS	BAXTER	TAMGAS CREEK FISH HATCHERY	P.O. BOX 410	METLAKATLA	AK 99926
DUNNE	ANGELA	WASHINGTON DEPT. OF WILDLIFE	1700 SILVER BEACH ROAD	BELLINGHAM	WA 98226
DUOLOS	SPEROS	KOOSKIA NATIONAL FISH HATCHERY	P.O. BOX 18	AHSAHKA	ID 83520
EAGAR	ROY	J. L. EAGAR, INC.	P.O. BOX 476	NO. SALT LAKE	UT 84054
EDDY	BRUCE	PACIFIC POWER AND LIGHT	920 SW 6TH AVENUE	PORTLAND	OR 97204
EDSALL	DONALD	U.S. FISH AND WILDLIFE SERVICE	4050 BRIDGER CANYON ROAD	BOZEMAN	MT 59715
EDWARDS	VIRGIL	OREGON DEPT. OF FISH & WILDLIFE	39800 S.E. FISH HATCHERY ROAD	SANDY	OR 97055
ELDREDGE	JOAN	ELECTRONIC DATA SOLUTION	P.O. BOX 31	JEROME	ID 83338
ELLIOTT	TOM	TROUT LODGE	12000 MCCUTHEON ROAD EAST	SUMNER	WA 98390
ENGELHARDT	MEL	U.S. FISH AND WILDLIFE SERVICE	34286 SE RAINBOW ROAD	ESTACADA	OR 97023
ENGEMANN	DOUGLAS	IDFG, MACKAY FISH HATCHERY		MACKAY	ID 83251
ENNS	JOHN	ALBERTA GOVERNMENT FISH AND WILDLIF	1440 17A STREET S.E.	CALGARY ALBERTA	T2G 4T9
ERION	TERRY	WASHINGTON DEPT. OF WILDLIFE	2306 SOUTH 16TH AVENUE	YAKIMA	WA 98903

ESSELMAN	BOB	IDFG, HAYSPUR FISH HATCHERY	071 US HIGHWAY 20	BELLEVUE	ID 83313
ESTEY	DON	CALIFORNIA DEPT. OF FISH AND GAME	P.O. BOX 158	CLEMENTS	CA 95227
FARMER	RUSS	BIOPRODUCTS, INC.	P.O. BOX 429	WARRENTON	OR 97146
FARR	WINSTON	SUMMIT TECHNOLOGY INC.	615 2ND AVENUE	SEATTLE	WA 98104
FIES	TED	OREGON DEPT. OF FISH AND WILDLIFE	61374 PARRELL ROAD	BEND	OR 97702
FITZGERALD	JASON	TRONDTECH MARKETING, INC.	848 SUPERIOR DRIVE	CAMPBELL RIVER	BC V9W 7H3
FONT	MELODY	WASHINGTON DEPT. OF WILDLIFE	130 HATCHERY LANE	GOLDENDALE	WA 98620
FONT	RUDY	WASHINGTON DEPT. OF WILDLIFE	130 HATCHERY LANE	GOLDENDALE	WA 98620
FOOTT	J. SCOTT	U.S. FISH AND WILDLIFE SERVICE	COLEMAN FHC, RT. 1, BOX 2105	ANDERSON	CA 96007
FORNER	ED	U.S. FISH AND WILDLIFE SERVICE	911 N.E. 11TH	PORTLAND	OR 97232
FOULK	TIM	MT HOOD COMMUNITY COLLEGE	2016 SE 122ND	PORTLAND	OR 97266
FOWLER	L.G.	U.S. FISH & WILDLIFE SERVICE	127 SOUTH VISTA WAY	KELSO	WA 98626
FRANK	DENNIS	PROTECT-A-COVER, INC.	12310 HIGHWAY 99 #124	EVERETT	WA 98204
FREW	TOM	IDFG-HAGERMAN HATCHERY	ROUTE 1, BOX 247	HAGERMAN	ID 83332
GANZHORN	JACK	PENINSULA COLLEGE	1502 E. LAURIDSEN BLVD.	PORT ANGELES	WA 98362
GARCIA	AARON	LITTLE WHITE SALMON-WILLARD NFH	BOX 17	COOK	WA 98605
GARLIE	TODD	IDAHO DEPT FISH AND GAME	3806 S. POWERLINE ROAD	NAMPA	ID 83686
GARRETT	JAMES	WASHINGTON DEPT. OF FISHERIES	315 FISH HATCHERY ROAD	GLENWOOD	WA 98362
GARRISON	BOB	OREGON DEPT. OF FISH AND WILDLIFE	850 SW 15TH	CORVALLIS	OR 97333
GARTON	RONNIE	WASHINGTON STATE FISHERIES	13124 AUBURN BLACK DIAMOND RD.	AUBURN	WA 98002
GIBSON	JIM	SKAGIT SYSTEM COOP.	P.O. BOX 368	LACONNER	WA 98257
GILES	JERRY	CLEAR SPRINGS TROUT COMPANY	P.O. BOX 515	SODA SPRINGS	ID 83276
GILSTRAP	ALLEN	BIOSPONGE AQUACULTURE	23 E BRUNDAGE, P.O.BOX 2989	SHERIDAN	WY 82801
GOEDE	RON	UTAH DIVISION OF WILDLIFE RESOURCES	1465 WEST 200 NORTH	LOGAN	UT 84321
GORRIE	WAYNE	PACIFIC RIM AQUA SUPPLIES	P.O. BOX 774 STATION A	NANAIMO BC	CN
GRAHAM	MICHAEL	IDFG, MAGIC VALLEY FISH HATCHERY	RT 1	FILER	ID 83328
GRANDE	KERRY	U.S. FISH AND WILDLIFE SERVICE	P.O. BOX 665	SARATOGA	WY 82331
GRANT	BLAKE		RT. 1, BOX 264 A	HAGERMAN	ID 83332
GRAYBILL	JIM	MT. HOOD COMMUNITY COLLEGE	SCIENCE DIV., 26000 SE STARK	GRESHAM	OR 97030
GREGG	T.R.	ENGINEERED PRODUCTS	P.O. BOX 30	PHILOMATH	OR 97370
GRIBBLE	MIKE	OREGON DEPT. FISH AND WILDLIFE	STAR ROUTE B BOX 10	CASCADE LOCKS	OR 97014
GROBERG	WARREN	OREGON DEPT. OF FISH & WILDLIFE	BADGLEY HALL-EOSC	LA GRANDE	OR 97850
GUNTER JR.	E. ROYCE	CALIFORNIA DEPT. OF FISH & GAME	3246 SKAGGS SPRING ROAD	GEYSERVILLE	CA 95441
HAERLING	DON	WASHINGTON DEPT. OF FISHERIES	2284 SPENCER ROAD	SALKUM	WA 98582
HAGER	ROBERT	WASHINGTON DEPT. OF FISHERIES	424 EAST X STREET	TUMWATER	WA 98501
HALVER	JOHN	SCHOOL OF FISHERIES HF-15	UNIVERSITY OF WASHINGTON	SEATTLE	WA 98195
HAMILTON	MARK	MONTANA DEPT. FISH, WILDLIFE, PARKS	604 W. PENNSYLVANIA AVE.	ANACONDA	MT 59911
HAMOR	THOMAS	ALBERTA GOVT OF FISH & WILDLIFE	1440 17A STREET SE	CALGARY ALBERTA	T2P 4T9
HAMSTREET	CHARLES	U.S. FISH AND WILDLIFE SERVICE	P.O. BOX 549	LEAVENWORTH	WA 98826
HARRYMAN	BILL	IDFG-CLARK FORK HATCHERY	P O BOX 98	CLARK FORK	ID 83811
HASHAGEN	KEN	CALIFORNIA DEPT FISH AND GAME	1416 NINTH STREET	SACRAMENTO	CA 95814
HAUGEN	BILL	OREGON DEPT. OF FISH AND WILDLIFE	17330 SE EVELYN	CLACKAMAS	OR 97015
HAUKENES	ALF	UNIVERSITY OF IDAHO	113 STYNER #1	MOSCOW	ID 83843
HEIMER	JOHN	IDAHO DEPT FISH AND GAME	P.O.BOX 25	BOISE	ID 83707
HENDRIX	MICHAEL	NORTHEAST FISHERY CENTER	P.O. BOX 75	LAMAR	PA 16848
HERRIG	DAN	U.S. FISH AND WILDLIFE SERVICE	3116 WAGON WHEEL ROAD	BOISE	ID 83702
HERRON	TOM	IDFG-SAWTOOTH HATCHERY	HC64, BOX 9905	STANLEY	ID 83278
HEWITT	JOHN	RANGEN, INC.	P.O. BOX 706	BUHL	ID 83316
HICKEY	PAUL	TACOMA PUBLIC UTILITIES/LIGHT DIV	P.O. BOX 11007	TACOMA	WA 98411
HILL	JIM	CEDC FISHERIES PROJECT	250 - 36TH STREET	ASTORIA	OR 97103
HODGES	DARYL	MONTANA DEPT. FISH, WILDLIFE, PARKS	P.O. BOX 508	BIG TIMBER	MT 59011
HOLDER	TIM	WASHINGTON DEPT OF WILDLIFE	P O BOX 278	STARBUCK	WA 99359
HOLT	RICHARD	OREGON DEPT. OF FISH & WILDLIFE	DEPT. OF MICROBIOLOGY, OSU	CORVALLIS	OR 97331
HOOVER	ROBERT	IDFG, MACKAY FISH HATCHERY		MACKAY	ID 83251
HOPPER	KATHLEEN	WASHINGTON DEPT. OF FISHERIES	115 G.A. BLDG.	OLYMPIA	WA 98504
HOSKINS	JOHN	OREGON DEPT. OF FISH AND WILDLIFE	90700 FISH HATCHERY ROAD	LEABURG	OR 97489
HOUSEWORTH	DAVID	TAMGAS CREEK FISH HATCHERY	P.O. BOX 410	METLAKATLA	AK 99926
HUBLOU	WALLY	BIOPRODUCTS, INC.	P.O. BOX 429	WARRENTON	OR 97148
HUDSON	CRYSTAL	USFWS	P.O. BOX 917	FT. MORGAN	CO 80701

HURST	JACK	OREGON DEPT FISH AND WILDLIFE	RT 2, BOX 149	IRRIGON	OR 97844
HUTCHINS	JOHN	SUMMIT TECHNOLOGY	615 2ND AVENUE SUITE 518	SEATTLE	WA 98104
HUTCHINSON	BILL	IDAHO DEPT. OF FISH AND GAME	600 S. WALNUT	BOISE	ID 83707
ISMOND	ALAN	A.T. CONSULTANTS/SIMPLOT	20875 WAGNER ROAD	CALDWELL	ID 83605
JANSMA	KEN	WASHINGTON DEPT. OF FISHERIES	P.O. BOX 54	GRAYS RIVER	WA 98621
JENSEN	GREG	JENSORTER, INC.	20225 HARVEST LANE	BEND	OR 97701
JENSEN	LOREN	OREGON DEPT. OF FISH AND WILDLIFE	43863 GREER DRIVE	LEABURG	OR 97489
JERRY	CLIFFORD	MUCKELSHOOT TRIBE	34900 212TH AVENUE SOUTH	AUBURN	WA 98002
JOHNSON	DON	DIVERSIFIED OVA TECH	BOX 4022	LOWER NICOLA	BC V0K 1Y0
JOHNSON	KEITH	IDAHO DEPT. OF FISH AND GAME	1800 TROUT ROAD	EAGLE	ID 83616
JOHNSON	RANDY	OREGON DEPT. OF FISH AND WILDLIFE	3137 NE 60TH AVENUE	PORTLAND	OR 97213
JONES	BOB	OREGON DEPT. OF FISH AND WILDLIFE	RT. 1, BOX 279	ENTERPRISE	OR 97828
JONES	TERRY	OREGON DEPT. OF FISH AND WILDLIFE	STAR ROUTE BOX 71	IDANHA	OR 97350
KAISER	HORST	UNIVERSITY OF IDAHO	DEPT. FISH & WILDLIFE RESOURCE	MOSCOW	ID 83843
KANE	TOM	U.S. FISH AND WILDLIFE SERVICE	2625A PARKMOUNT	OLYMPIA	WA 98592
KEITHLEY	MARY ANNE	WASHINGTON DEPT. OF FISHERIES	BOX 3, AZWELL	PATEROS	WA 98846
KELSEY	SUZANNE	MARITIME HERITAGE CENTER	P.O. BOX 2034	CONCRETE	WA 98237
KENWORTHY	BRYAN	USFWS-LAHONTAN NAT. FISH HATCHERY	710 HIGHWAY 395	GARDNERVILLE	NV 89410
KEOWN	KEITH	WASHINGTON DEPT. OF FISHERIES	10119 STEILACOOM ROAD SE	OLYMPIA	WA 98501
KERWIN	JOHN	WASHINGTON DEPT. OF WILDLIFE	600 CAPITAL WAY N.	OLYMPIA	WA 98501
KETOLA	GEORGE	U.S. FISH AND WILDLIFE SERVICE	3075 GRACIE ROAD	CORTLAND	NY 13045
KIESER	DOROTHEE	DEPT. OF FISHERIES AND OCEANS	PACIFIC BIOLOGICAL STATION	NANAIMO	BC V9R 5K6
KINDSCHI	GREG	U.S. FISH AND WILDLIFE SERVICE	4050 BRIDGER CANYON ROAD	BOZEMAN	MT 59715
KOBBERSTAD	WAYNE	ALBERTA FISH & WILDLIFE DIVISION	9945 108TH ST.	EDMONTON	AL T5K 2G6
KOLLER	DIANA	OVA TECH	C9 GALENA BAY RR2	NAKUSP	BC V091R0
LABISKE	ED	OREGON DEPT. OF FISH AND WILDLIFE	15020 CHANCE ROAD	TILLAMOOK	OR 97141
LADOUCEUR	GRANT	D.F.O. CANADA	R. RT.3	QUALICUM BEACH	BC V0R 2T0
LAMOTTE	ED	U.S. FISH AND WILDLIFE SERVICE	SPRING CREEK NATL FISH HATCHER	UNDERWOOD	WA 98651
LANDON	SHARON	IDAHO DEPT. OF FISH AND GAME	1800 TROUT ROAD	EAGLE	ID 83704
LANGSTON	EDWARD	OREGON DEPT. OF FISH & WILDLIFE	RT. 4, BOX 594	ASTORIA	OR 97103
LANIER	ROGER	IDFG-CLEARWATER HATCHERY		AHSAHDA	ID 83520
LAPATRA	SCOTT	CLEAR SPRINGS TROUT COMPANY	P.O. BOX 712	BUHL	ID 83316
LARKIN	MIKE	IDAHO DEPT. OF FISH AND GAME	600 SOUTH WALNUT	BOISE	ID 83707
LAW	DUNCAN	CEDC FISHERIES PROJECT	250 - 36TH STREET	ASTORIA	OR 97103
LEEK	STEVE	U.S. FISH AND WILDLIFE SERVICE	MP 61.75R SR14	UNDERWOOD	WA 98651
LEITH	DAVE	ABERNATHY SALMON CULTURE TECH CTR.	1440 ABERNATHY ROAD	LONGVIEW	WA 98632
LEPPINK	JOHN	OREGON DEPT. FISH AND WILDLIFE	BOX 59, 2501 FIRST AVENUE	PORTLAND	OR 97207
LEWIS	MARK	OREGON DEPT. OF FISH AND WILDLIFE	850 SW 15TH STREET	CORVALLIS	OR 97333
LIEBERMAN	ELIOT	ARGENT LABORATORIES	8702 152NE	REDMOND	WA 98952
LIENTZ	JOSEPH	DWORSHAK FISH HEALTH CENTER	P.O. BOX 18	AHSAHKA	ID 83520
LINER	GLENN	WASHINGTON DEPT. OF FISHERIES	1404 KALAMA RIVER ROAD	KALAMA	WA 98632
LONG	KEN	OREGON DEPT. OF FISH AND WILDLIFE	ROUTE 1, BOX 764	ASTORIA	CA
LOOMIS	JEROME	OREGON DEPT. OF FISH AND WILDLIFE	726 SW LOWER BEND ROAD, BOX 15	MADRAS	OR 97741
LORD	JOHN	IDFG-GRACE HATCHERY	390 HATCHERY ROAD	GRACE	ID 83241
LOUDENSLAGER	ERIC	HUMBOLDT STATE UNIVERSITY	DEPT OF FISHERIES HSU	ARCATA	CA 95521
LOWELL	RICK	IDAHO DEPT FISH AND GAME	3806 S. POWERLINE ROAD	NAMPA	ID 83686
LUCAS	TOM	CLEAR SPRINGS TROUT COMPANY	RT 3, BOX 406 A	BUHL	ID 83716
MACMILLAN	RANDY	CLEAR SPRINGS TROUT COMPANY	PO BOX 712	BUHL	ID 83316
MALLAK	MARSHALL	OZONE RESEARCH AND EQUIPMENT, CORP.	3840 N 40TH AVENUE	PHOENIX	AZ 85019
MANUEL	JERRY		433 COUGAR ST S.E.	OLYMPIA	WA 98503
MARQUARDT	GARY	DOMSEA FARMS, INC	1656 KRAFT ROAD	POCATELLO	ID 83204
MARSHALL	BRUCE	VMG INDUSTRIES, INC.	858 GRAND AVENUE	GRAND JUNCTION	CO 81501
MARTIN	FRED	WASHINGTON DEPT OF FISHERIES	14949 148TH AVE S.E.	YELM	WA 98597
MASKILL	MARK	DWORSHAK NATIONAL FISH HATCHERY	P.O. BOX 18	AHSAHKA	ID 83520
MAY	LARRY		28415 SE W K ANDERSON ROAD	GRESHAM	OR 97080
MC ROBERTS	WALLY	CLEAR SPRINGS TROUT COMPANY	P.O. BOX 712	BUHL	ID 83316
MCCALL	JIM	REIFF MFG., INC.	RT. 4, BOX 183	WALLA WALLA	WA 99362
MCCARVER	DENISE	W.D.F. SALMON CULTURE	3405 SATURDAY AVE	MALAGA	WA 98828
MCCOLLUM	PAUL	VALDEZ FISHERIES DEV. ASSOC. INC.	BOX 125	VALDEZ	AK 99686

MCGEHEE	JERRY	IDFG-CLEARWATER HATCHERY		AHSAHKA	ID 83520
MCKNIGHT	SCOTT	MOORE-CLARK	BOX M	LA CONNER	WA 98257
MCLEOD	BRUCE	CARSON NATIONAL FISH HATCHERY	WIND RIVER HATCHERY	CARSON	WA 98610
MCMAHAN	STAN	WASHINGTON DEPT. OF FISHERIES	12710 124TH AVE CT KPN	GIG HARBOR	WA 98335
MCPHERSON	GENE	IDAHO DEPT. OF FISH & GAME	BOX 1021	MCCALL	ID 83638
MEADOWS	STEVE	QUILEUTE FISHERIES DEPARTMENT	P.O. BOX 187	LAPUSH	WA 98350
MENDOZA	ADAM	CRESTON N.F. HATCHERY		KALLISPELL	MT 59901
MESSMER	RHINE	OREGON DEPT. FISH & WILDLIFE	INLOW HALL, EOSC	LAGRANDE	OR 97850
MIETHE	HARLEY	OREGON DEPT. OF FISH & WILDLIFE	RT. 4, BOX 594	ASTORIA	OR 97103
MILLER	ARNIE	IDFG-GRACE HATCHERY	390 HATCHERY ROAD	GRACE	ID 83241
MOFFITT	CHRISTINE	UNIVERSITY OF IDAHO	DEPT. FISH & WILDLIFE RESOURCE	MOSCOW	ID 83843
MOORE	BOB	IDAHO DEPT. OF FISH AND GAME	BOX 85	ELLIS	ID 83235
MOORE	STEVE	WASHINGTON DEPT. OF WILDLIFE	RT. 3, BOX 3856	FORKS	WA 98331
MORINAKA	RON	BPA/FISH AND WILDLIFE	PO BOX 12094	PORTLAND	OR 97212
MORRIS	ROB	IDAHO DEPT. OF FISH AND GAME	2131 NIAGARA SPRINGS ROAD	WENDELL	ID
MORRISON	JOHN	US FISH AND WILDLIFE	2625 PARKMONT LANE	OLYMPIA	WA 98502
MORSE	JUNE		4757 FOOTS CREEK RD.	GOLD HILL	OR 97525
MOYER	MICHAEL	SSRAA	NEETS BAY	KETCHIKAN	AK 99950
MUNSON	DOUG	IDAHO DEPT. OF FISH AND GAME	1800 TROUT ROAD	EAGLE	ID 83616
NANDOR	GEORGE	CLACKAMAS FISH HATCHERY/ODFW	24500 S. ENTRANCE ROAD	ESTACADA	OR 97023
NEALEIGH	GEORGE	SILVER CUP FISH FEED	P.O. BOX 155	MANZANITA	OR 97130
NELSON	CHRIS	SILVER CUP FISH FEED	P.O. BOX 57426	MURRAY	UT 84157
NELSON	JIM	BIOMED, INC.	1720 130TH AVE. N.E.	BELLEVUE	WA 98005
NIELSEN	KEVIN	CH2M HILL	BOX 8748	BOISE	ID 83707
NOBLE	RICHARD	MINNESOTA AQUAFARM	P.O. BOX 592	CHISHOLM	MN 55719
NORMAN	FRED	DEPT. OF WILDLIFE	1416 14TH ST SW	PUYALLUP	WA 98371
NOVOTNY	A.J.	MARINKA INTERNATIONAL	1919 E. CALHOUN	SEATTLE	WA 98112
NYE	FRED	HOFFMANN LA ROCHE	5160 GREEN ACRES ST	BOISE	ID 83709
O'KEEFE	TIM	RANGEN, INC.	P.O. BOX 706	BUHL	ID 83316
OCHS	JIM	INDUSTRIAL PLASTICS, INC.	740 SOUTH 28TH	WASHOUGAL	WA 98671
OLSON	WAYNE	DWORSHAK NATIONAL FISH HATCHERY	P.O. BOX 18	AHSAHKA	ID 83520
OMAN	LENI	WASHINGTON DEPT. OF WILDLIFE	600 CAPITOL WAY N	OLYMPIA	WA 98501
OMAN	ROGER	MOUNT HOOD COMMUNITY COLLEGE	18700 MADRONA	OREGON CITY	OR 97045
OSBERG	KIMBERLEY	PENTEC ENVIRONMENTAL INC.	120 W. DAYTON, SUITE A7	EDMONDS	WA 98020
OSBORNE	GARY	WA DEPARTMENT OF FISHERIES	1435 TERRACE CT, NE	EAST WENATCHEE	WA 98802
OTTO	BILL	OREGON DEPT. OF FISH AND WILDLIFE	P O BOX 197	IDLEYLD PARK	OR 97447
OWENS	HAROLD	MAGIC VALLEY HELI-ARC MFG	P.O. BOX 511	TWIN FALLS	ID 83301
OWSLEY	DAVID	DWORSHAK NATIONAL FISH HATCHERY	P.O. BOX 18	AHSAHKA	ID 83520
PALMER	JACK	OREGON DEPT FISH AND WILDLIFE	RT 2, BOX 149-C	IRRIGON	OR 97844
PALMER	TED	COMMON SENSING, INC	7595 FINCH ROAD NE	BAINBRIDGE IS.	WA 98110
PARK	MARJORIE	U.S. FISH AND WILDLIFE	P O BOX 80	NEILTON	WA 98506
PARKE	CLYDE	ALBERTA FORESTRY LANDS & WILDLIFE	BOX 394, ALLISON CR. STATION	COLEMAN ALBERTA	TOK OMO
PARKER	DON	WASHINGTON DEPT OF WILDLIFE	12208 S.E. EVERGREEN HWY	VANCOUVER	WA
PARRISH	DAVE		1115 GRANT AVE.	BOISE	ID 83706
PATTERSON	TERRY	COLLEGE OF SOUTHERN IDAHO	P.O. BOX 1238	TWIN FALLS	ID 83301
PELLISSIER	RENE F.	PRINCE WILLIAM SOUND AQUACULTURE CO	P.O. BOX 649	WHITTIER	AK 99693
PENNY	JOHN	WASHINGTON DEPT. OF WILDLIFE	13246 LINCOLN ROCK ROAD	EAST WENATCHEE	WA 98802
PETERS	LINDA		RR1 FIELD ROAD C10	SECHELT BC	CN
PETERSEN	KRISTINE	WA DEPT. OF FISHERIES	P.O. BOX 313	DAYTON	WA 99321
PETERSON	JIM	MONTANA DEPT. FWP	P.O. BOX 2163	GREAT FALLS	MT 59403
PETERSON	PAUL	WASHINGTON DEPT OF FISHERIES	2284 SPENCER ROAD	SALKUM	WA 98582
PHILLIPS	RAY	BIOSPONGE AQUACULTURE	23 E BRUNDAGE, P.O.BOX 2989	SHERIDAN	WY 82801
POPOCHOCK	DENIS	WASHINGTON ST. DEPT. OF FISHERIES	12710 124TH AVE CT KPN	GIG HARBOR	WA 98335
POSEY	MIKE	OREGON DEPT. FISH AND WILDLIFE	RT 2, BOX 149	IRRIGON	OR 97844
POWELL	DAVID	RANGEN AQUACULTURE RESEARCH	RT 1, BOX 264	HAGERMAN	ID 83332
PRESTEGARD	ERIC P.	P.W.S.A.C.	P.O. BOX 1110	CORDOVA	AK 99574
PUBLACK	DOUG	ALBERTA FISH & WILDLIFE	1440-17A STREET S.E.	CALGARY ALBERTA	T26 4T9
QUEENER	MIKE	WASHINGTON DEPT. OF FISHERIES	5.68 N, WACHOUGAL RIVER ROAD	WACHOUGAL	WA 98671
QUINTON	BRYAN	DEPARTMENT OF WILDLIFE, WASHINGTON	600 CAPITOL WAY N	OLYMPIA	WA 98501

RAMSEY	DOUG		RT. 1, BOX 264 A	HAGERMAN	ID 83332
RAN	JASON	YIN FISHERIES	P.O. BOX 151	TOPPEMISH	WA 98948
RANKIN	JOHN	IDFG, MAGIC VALLEY HATCHERY	RT 1	FILER	ID 83328
RASMUSSEN	ULF	WDW - SOUTH TACOMA HATCHERY	7723 PHILLIPS ROAD SW	TACOMA	WA 98498
RAY	MICHAEL	SIMPLOT AQUACULTURE	1051 OWYHEE AVE	NYSSA	OR 97913
RENSEL	JULIA	IDAHO DEPT. OF FISH AND GAME	BOX 85	ELLIS	ID 83235
RHOADS	ROB	DOMSEA FARMS, INC	1656 KRAFT ROAD	POCATELLO	ID 83204
RICHE	MARTY	SIMPLOT AQUACULTURE	20875 WAGNER ROAD	CALDWELL	ID 83605
RIGG	JIM	DOMSEA FARMS	1656 KRAFT ROAD	POCATELLO	ID 83204
RIGGIN	JIM	SIMPLOT AQUACULTURE	20875 WAGNER	CALDWELL	ID 83605
ROBARDS	STEVE	WASHINGTON DEPT. OF WILDLIFE	HCR BOX 52	CHELAN	WA 98816
ROBART	RANDY	ODFW	RT 1, BOX 443	MAWPIN	OR 97037
ROBERTS	STEVE	WASHINGTON DEPT. OF WILDLIFE	3860 CHELAN HATCHERY	WENATCHEE	WA 98801
ROBINSON	JIM	OREGON DEPT. OF FISH & WILDLIFE	RT. 2, BOX 418	BANDON	OR 97411
ROCKOWSKI	JAMES	LITTLE WHITE SALMON-WILLARD NFH	BOX 17	COOK	WA 98605
ROGERS	JERRY	LITTLE WHITE SALMON-WILLARD NFH	BOX 17	COOK	WA 98605
ROGERS	ROBERT	WASHINGTON DEPT. OF FISHERIES	GENERAL ADMIN. BLDG. RM 115	OLYMPIA	WA 98504
ROGERS	TOM	IDAHO DEPT. OF FISH AND GAME	600 SOUTH WALNUT	BOISE	ID 83707
ROSS	CARL	WASHINGTON DEPT OF FISHERIES	P O BOX 175	STARBUCK	WA 99359
ROSS	LESLIE	WASHINGTON DEPT. WILDLIFE	WHATCOM FALLS PARK	BELLINGHAM	WA 98226
ROWAN	GERALD	CONFEDERATED TRIBES OF UMATILLA	P.O. BOX 638	PENDLETON	OR 97801
RUESCHMANN	PETER	WARING MARINE LTD.	3113 2ND AVENUE	PORT AUBERNI	BC V94 4C4
RUSH	MICHAEL	SIMPLOT AQUACULTURE	20875 WAGNER ROAD	CALDWELL	ID 83605
SCHAEFFER	DREW	OREGON DEPT. FISH & WILDLIFE	1723 N.E. 157	PORTLAND	OR 97230
SCHENK	WINFRED	ALBERTA GOVERNMENT FISH & WILDLIFE	1440 17A STREET S.E.	CALGARY ALBERTA	T26 4T9
SCHNEIDER	RICH	CLEAR SPRINGS TROUT COMPANY	RT. R, P.O. BOX 712	BUHL	ID 83316
SCHULTZ	MURRAY	OREGON DEPT OF FISH AND WILDLIFE	2501 S.W. 1ST	PORTLAND	OR 97201
SCHUYLER	SCOTT	SKAGIT SYSTEM COOP.	P.O. BOX 368	LACONNER	WA 98257
SCHWAB	DICK	RANGEN, INC.	P.O. BOX 706	BUHL	ID 83316
SELIN	JAMES		P.O. BOX 2034	CONCRETE	WA 98237
SELLARDS	GARY	US FISH & WILDLIFE SERVICE	34288 SE RAINBOW ROAD	ESTACADA	OR 97023
SEMPLE	ROBERT	USFWS, MAKAH NATIONAL FISH HATCHERY	P.O. BOX 730	NEAH BAY	WA 98357
SEVERINSEN	TROND	TRONDTECH MARKETING, INC.	848 SUPERIOR DRIVE	CAMPBELL RIVER	BC V9W 7H3
SEVERSON	RICHARD	ORE-AQUA, INC.	88700 MARCOLA ROAD	EUGENE	OR 97478
SHANAHAN	BEA	ARGENT LABORATORIES	8702 152NE	REDMOND	WA 98952
SHARPNACK	ROB	SIMPLOT AQUACULTURE	20875 WAGNER ROAD	CALDWELL	ID 83605
SHAVER	GARY	MONTANA DEPT. FWP	P.O. BOX 423	BRIDGER	MT 59014
SHAW	HARRY	HAGERMAN NAT. FISH HATCHERY	3059D NAT'L FISH HATCH. RD.	HAGERMAN	ID 83332
SHELDON	RAY	ODFW	17330 S.E. EVELYN ST	CLACKAMAS	OR 97015
SHELDRAKE	TOM	U.S. FISH AND WILDLIFE SERVICE	911 NE 11TH AVENUE	PORTLAND	OR 97232
SHIPPENTOWER	GENE	CTUIR FISHERIES	P.O. BOX 638	PENDLETON	OR 97801
SHUDES	ROGER	COLEMAN NFH	RT. 1, BOX 2105	ANDERSON	CA 96007
SILVER	TODD	J.L. DARLING CORP.	2212 PORT OF TACOMA ROAD	TACOMA	WA 98421
SIPLE	JOHN	IDAHO DEPT. OF FISH & GAME	P O BOX 543, RT 1	BONNERS FERRY	ID 83805
SMITH	BOB	PAYETTE RIVER FISHERIES	BOX 586	NEW PLYMOUTH	ID 83655
SMITH	DICK	LOST RIVER TROUT HATCHERY	STAR ROUTE	MACKAY	ID 83207
SMITH	KERRY	SMITH-ROOT, INC.	14014 NE SALMON CREEK AVE	VANCOUVER	WA 98686
SMITH	R.Z.		28395 WILLOW CREEK DRIVE	WILSONVILLE	OR 97070
SMITH	ROBERT	NATIONAL MARINE FISHERIES SERVICE	911 N.E. 11TH, SUITE 620	PORTLAND	OR 97232
SPARROW	HUGH		1655 WARREN GARDENS	VICTORIA	BC V85 159
STANLEY	CHARLIE	OREGON DEPT. OF FISH AND WILDLIFE	33465 HWY 22	HEBO	OR 97122
STEDRONSKY	WAYNE	OREGON DEPT. OF FISH & WILDLIFE	STAR ROUTE B, BOX 526	CASCADE LOCKS	OR 97014
STEELE	EARL	BELLINGHAM VO-TECH INST.	3028 LINGBURG AVENUE	BELLINGHAM	WA 98225
STEGE	EDWARD	US FISH & WILDLIFE SERVICE-HAGERMAN	3059D NATIONAL FISH HATCH.RD.	HAGERMAN	ID 83332
STEINER	RALPH	IDFG, HAGERMAN FISH HATCHERY	RT. 1, BOX 247	HAGERMAN	ID 83332
STERN	BARBARA	SMITH ROOT	14014 NE SALMON CREEK AVENUE	VANCOUVER	WA 98686
STEVENS	CARSON	MARITIME HERITAGE CENTER	2268 YEW ST. ROAD	BELLINGHAM	WA 98226
STICKELL	TRENT	OREGON DEPT. OF FISH & WILDLIFE	P.O. BOX 59	PORTLAND	OR 97207
STOUT	STEPHEN	WASHINGTON DEPT. OF FISHERIES	RT 1 BOX 140	ELMA	WA 98541

STRATTON	MIKE	OREGON DEPT. OF FISH & WILDLIFE	P.O. BOX 59	PORTLAND	OR 97207
STREUFERT	JONATHAN	DWORSHAK NATIONAL FISH HATCHERY	P.O. BOX 18	AHSAHKA	ID 83520
STROM	CHARLIE	MT HOOD COMMUNITY COLLEGE	2901 SE 118	PORTLAND	OR 97266
STRUCK	JAMES	OREGON DEPT. OF FISH & WILDLIFE	RT. 2, BOX 2198	CLATSKANIE	OR 97016
STUTZ	WILLIAM	IDFG-SAWTOOTH HATCHERY	HC64, BOX 9905	STANLEY	ID 83278
SULLIVAN	ROBERT	PARAMETRIX, INC	13020 NORTHUP WAY, N.E.	BELLEVUE	WA 98005
TALBOTT	IVAN	IDFG-RAPID RIVER FISH HATCHERY		RIGGINS	ID 83549
TANSLEY	BILL	OREGON DEPT. OF FISH AND WILDLIFE	P.O. BOX 130	CAMP SHERMAN	OR 97730
TAYLOR	RALPH	IDFG, FISH TRANSPORTATION	RT. 1	FILER	ID 83328
TBA	TBA	BIOPRODUCTS	P.O. BOX 429	WARRENTON	OR 97146
THOMING	JAY	OREGON DEPT OF FISH AND WILDLIFE	42921 N.E. 3RD	CORBETT	OR 97019
THORPE	MIKE	MARITIME HERITAGE CENTER	1106 W. HOLLY A-3	BELLINGHAM	WA 98225
TIPPING	JACK	WASHINGTON DEPT. OF WILDLIFE	2101 HWY 508	ONALOSKA	WA 98570
TODD	NEIL	DIVERSIFIED OVA-TECH LTD.	P O BOX 237	MERRITT	BC VOK 2B0
TOWNSEND	BILL	TROUT LODGE	12000 MCCUTHEON ROAD EAST	SUMNER	WA 98390
TRAYNOR	MARK	OREGON DEPT. OF FISH AND WILDLIFE	STAR RT B, BOX 9	CASCADE LOCKS	OR 97014
TURNER	NEIL	WASHINGTON DEPT. OF FISHERIES	1433 PAVEL ROAD	BEAVER	WA 98305
UNDERWOOD	JON	ALBERTA FISH AND WILDLIFE	COLD L. FISH HATCH., BOX 81595	COLD LAKE	AB
UPLINGER	RICHARD	CALIFORNIA DEPT. OF FISH AND GAME	P.O. BOX 910	BIG PINE	CA 93513
VALENTINE	JOE	UTAH DIVISION OF WILDLIFE RESOURCES	1596 WEST NORTH TEMPLE	SALT LAKE CITY	UT 84116
WADE	BRUCE	WASHINGTON DEPT OF FISHERIES	6263 MT. BAKER HWY	DEMING	WA 98244
WAGNER	HARRY	NW POWER PLANNING COUNCIL	851 SW 6TH AVE., STE. 1100	PORTLAND	OR 97204
WALKER	DERALD	OREGON DEPT. OF FISH AND WILDLIFE	61374 PARRELL ROAD	BEND	OR 97702
WALKER	SUSY	BIOPRODUCTS, INC.	P.O. BOX 429	WARRENTON	OR 97148
WALTERS	BRUCE	WASHINGTON STATE DEPT. OF WILDLIFE	1341 RINGOLD RIVER ROAD	MESA	WA
WALTERS	TIM	OREGON DEPT. OF FISH AND WILDLIFE	850 S.W. 15TH ST	CORVALLIS	OR 97333
WARREN	JIM	US FISH AND WILDLIFE SERVICE	911 N.E. 11TH AVE (AFF)	PORTLAND	OR 97232
WARREN	RON	WASHINGTON DEPT. OF FISHERIES	5935 FISH HATCHERY LN	MARBLEMOUNT	WA 98267
WAVRA	SHARON	IDAHO DEPT. OF FISH AND GAME	1800 TROUT ROAD	EAGLE	ID 83606
WELLS	JASON	VALDEZ FISHERIES DEV. ASSOC. INC.	BOX 125	VALDEZ	AK 99686
WENDT	JEFF	WASHINGTON DEPT OF WILDLIFE	P O BOX 270	STARBUCK	WA 99359
WESTERHOF	RICK	IDFG, HAYSPUR FISH HATCHERY	071 US HIGHWAY 20	BELLEVUE	ID 83313
WHITE	DAVE	CLEAR SPRINGS	159 10TH AVE N	TWIN FALLS	ID 83301
WILLINGHAM	EDDY	MARISOURCE	P.O. BOX 9037	TACOMA	WA 98409
WILLINGHAM	RAINER	MARISOURCE	P.O. BOX 9037	TACOMA	WA 98409
WINFREE	ROBERT	US FISH & WILDLIFE	3059 F NFH ROAD	HAGERMAN	ID 83332
WOODY	STAN	WASHINGTON DEPT. OF WILDLIFE	28 BEAVER CREEK ROAD	CATHLAMET	WA 98612
WRIGHT	TIM	OREGON DEPT. OF FISH AND WILDLIFE	90700 FISH HATCHERY ROAD	LEABURG	OR 97489
YASKOVIC	JOHN	OREGON DEPT. OF FISH & WILDLIFE	P.O. BOX 59	PORTLAND	OR 97207
YOUNG	DOUG	IDFG, OXBOW FISH HATCHERY	P. O. BOX 200	OXBOW	OR 97840
ZAJAC	DAVID	U.S. FISH AND WILDLIFE SERVICE	2625 PARKMONT LAND, BLDG A	OLYMPIA	WA 98502
ZIMMERMAN	BRIAN	PARADISE BAY SEAFARMS	2010 CLEVELAND	PORT TOWNSEND	WA 98368
ZINN	JERRY	AQUA HEALTH LTD.	RT 3, BOX 299	BUHL	ID 83316

ANNUAL NORTHWEST FISH CULTURE CONFERENCE

HISTORICAL RECORD

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