

# Proceedings

# of the 45th Annual Northwest Fish Culture Conference



December 6-8, 1994 Sunriver, Oregon

SH 151 .N67 1994



Hosted by: Fish Propagation Section Oregon Dept. of Fish & Wildlife P.O. Box 59 Portland, Oregon 97207

# PROCEEDINGS

## OF THE

# FORTY-FIFTH ANNUAL

# NORTHWEST FISH CULTURE CONFERENCE

# DECEMBER 6-8, 1994

# SUNRIVER, OREGON

Drew Schaeffer Fish Propagation Section Oregon Department of Fish and Wildlife

# THE NORTHWEST FISH CULTURE CONFERENCE

- The Northwest Fish Culture Conference is an annual informal meeting by and between fish culturists for the exchange of information and ideas about all aspects of fish culture. These conferences are hosted on a rotating basis by the various fisheries agencies and entities of the Northwest. At the conferences, progress reports of management practices and problems, new developments, and research studies are presented. Both within the meeting and outside the formal meeting setting, active discussion, constructive criticism, and personal contacts are not only encouraged but actively cultivated. All persons interested in or associated with fish husbandry are invited to attend and to actively participate. The subject matter is limited to topics that have a direct application to fish culture.
- This proceedings contain abstracts and or talks presented at the conference. They are unedited, contain progress reports of uncompleted programs, and as such, should not be considered a formal, peer-reviewed publication.
- Mention in these proceedings does not indicate approval, recommendation, or endorsement of any proprietary product or material.

# **PROCEEDINGS CONTENTS**

- Section I Fish Management
- Section II Hatchery Operations and Technology
- Section III Fish Health Management/Nutrition
- Section IV The Changing Role of Artificial Propagation in Fisheries Management
- Section V Fish Culture Practices
- Section VI Educational Activities/Opportunities At Hatcheries
- Section VII Author/Subject Index 1982 1992
- Section VIII Raffle Donation Vendor List

## SESSION I

## FISH MANAGEMENT

- Feasibility of Implanting Blank Wire Tags in the Body of Juvenile Fall Chinook Salmon - Shannon Focher, Oregon Department of Fish and Wildlife
- Mass Mark Evaluation Simpson Hatchery Coho Stan Hammer, Washington Department of Fish and Wildlife
- South Fork Coquille Steelhead Management A Case Study Jim Muck, Oregon Department of Fish and Wildlife
- To Restore a Legacy; the Struggle for the Snake River's Salmon and Steelhead - Ed Crateau, U.S. Fish and Wildlife Service
- Trout Producer Quality Assurance Program Gary Fornshell, University of Idaho Extension Service

#### "Feasibility of Implanting Blank-wire Tags in the Body of Juvenile Fall Chinook Salmon."

Shannon M. Focher Richard W. Carmichael Michael C. Hayes MaryLouise Keefe

#### Oregon Department of Fish and Wildlife Route 2 Box 2541 Hermiston, OR 97838

#### ABSTRACT

Fall chinook salmon are released annually into the Umatilla River to partially mitigate for fish losses attributable to mainstem Columbia River Dams. Upper River Bright stock fall chinook salmon from early releases (1983-1990) were reared at Bonneville Hatchery. Fall chinook salmon from recent years were reared at Irrigon Hatchery (1991) and Umatilla Hatchery (1992-present). Each year varying numbers of these Umatilla fall chinook salmon have been found to stray into the Snake River. Concomitant with the endangered species listing of Snake River stock fall chinook salmon there was a need to separate lower river strays from Snake River stock at mainstem dams. Thus, we initiated a study to examine the utility of potential mass marking techniques. This presentation overviews the evaluation of body tagging as a mass marking tool and compares body tagging to other mass marking options.

Body tags are blank-wire tags injected into the body of a fish. For this evaluation, we implanted body tags into the right shoulder of juvenile fall chinook salmon. To separate the effects of individual marks, we compared body tagging with numerous marking combinations utilizing fin clips, body tags, and coded-wire tags (Table 1). The body tagging study was initiated in 1991 on fall chinook salmon reared at Irrigon Hatchery and continued in 1992 and 1993 at Umatilla Hatchery.

After the first three years of body tagging juvenile fall chinook salmon it is evident that implanting body tags is too costly and time consuming to make them effective as a mass marking tool. Estimated costs per 1,000 fish for body tags + left ventral fin clip, body tags only, and left ventral fin clip only were \$87, \$70, and \$17 respectively. Time required for marking 1,000 fish is estimated at 1.2 h

tagging.			
Mark	Irrigon Hatchery 1991	Umatilla Hatchery 1992	Umatilla Hatchery 1993
Left ventral	-	69,816 74,408	61,801 <sup>a</sup> 66,204 <sup>a</sup>
Body tag & left ventral	-	65,749 67,144	68,644 70,442
Body tag	147,586	70,435 65,184	69,225 69,518
Adipose & coded-wire tag	104,258	-	-
Adipose & coded-wire tag & right ventral	103,980	31,982 32,287	29,594 29,360
Adipose & coded-wire tag & body tag	145,048	-	· _

Table 1. Numbers of fall chinook salmon fin clipped and recognizably adipose and coded-wire tagged at Irrigon and Umatilla Hatcheries to study the effects of tagging.

<sup>a</sup> Adjusted for fin clip quality.

for fish marked body tag + left ventral fin clip, 0.85 h for fish marked body tag only, and 0.13 h for fish marked left ventral only. For Umatilla Hatchery production, mass marking 2.3 million fall chinook salmon would require 115 days for fish marked body tag + left ventral clip, 81.4 days for fish marked body tag only and 12.4 days for fish marked left ventral clip only. Tag retention for body tagged fish was similar to that found in coded-wire tagged fish.

In 1993 we began examining adults returning to the Umatilla River for wire tags and fin clips. Because we are in the early stages of this study, few adults have been recovered and the effects of marking on smolt to adult survival is inconclusive. We have encountered some problems detecting body tags in live adults because of variability in the accuracy of the hand-held tag detector. In 1994 National Marine Fisheries Service requested that all fall chinook salmon from Umatilla Hatchery be given a blank-wire nose tag and a right ventral fin clip. Consequently, the tagging study has been discontinued. We will continue to monitor adult returns in future years to study the effects of fin clips, body tags, and coded-wire tags on smolt to adult survival.

# MASS MARK EVALUATION-SIMPSON HATCHERY COHO

Stan Hammer

Washington Department of Fish And Wildlife

#### ABSTRACT

A left ventral (LV) fin clip was applied to one hatchery's annual production of coho salmon ( $\Omega$  kisutch) to determine the feasibility and effects of such an effort. The project was completed in 37 days. The fish were clipped at Simpson Hatchery, a Washington Department of Fish and Wildlife (WDF&W) facility located near Matlock ,Washington. The cost of this experiment was \$ 52, 031.05 or \$ 22.64/1,000 clips. A total of 2, 297, 700 coho were fin clipped with a daily average clip rate of 62, 100 fish. The daily average number of clips/worker was 4, 607 clips/day. Fish size ranged from 1.8 gms to 2.6 gms/fish. Quality Control (QC) was calculated as the daily percentage of substandard fin clips ( bad clips ). The initial rate was 1.3 % and the final bad clip percentage ( at release ) was 3.3 %. Fin clipping exacerbated the effects of furunculosis (Areomonas salmonicida).

#### INTRODUCTION

For almost as long as salmonids have been artificially cultured, there has been efforts to identify specific groups of fish by removing one or more fins or parts of maxillary bones. Literature is replete with studies chronicling problems associated with early fin clipping work, such as decreased survival of adult fish and inaccurate counts because of fin regeneration (Blankenship, 1993). With growing public interest in the mass-marking of hatchery salmon, and because the adipose fin (Ad) clip presently serves as a flag for

identifying coded-wire-tags ( CWT ), other fin-clipping options were reviewed. An internal document from the Washington Department of Fish and Wildlife (formerly WDF) sumarizing fin-clipping literature (Blankenship, 1993) rates the mortality associated with each mark from highest to lowest mortality as follows: anal fin, maxillary bones (left and right), dorsal fin, pectoral fins (left and right), ventral fins (left and right), and adipose fin.

The left ventral (LV) clip is one mark which has been used in the past to identify salmon with conflicting results (Blankenship, 1993). Because the LV clip had the least affect on survival after the adipose fin, the Washington Department of Fish and Wildlife (WDF&W) decided to test the effects of the LV clip on one hatchery's annual production of coho to answer the following questions: (1) Could we mark the entire annual production ?; (2) What effect would it have on the fish ?; and (3) How much would it cost?

Simpson Hatchery, located on the East Fork of the Satsop River, was chosen as the site for this test, because marking that facility's coho would directly benefit stock assessment requirements for the Chehalis River Basin. (Both the normal returning (fall) and the late returning (winter) Satsop River coho were marked.) The goal of the Simpson mass marking effort was to place a definitive mark on all hatchery coho in the Chehalis River side of the Grays Harbor Basin. The mark would help Freshwater Production Group biologists improve the accuracy of their estimates of wild coho harvest and escapement by excluding hatchery-origin fish from estimates of total production. Improving our ability to estimate the wild coho run is a critical management need as it drives run forcasts which affect fishing opportunities for all user groups. Identifying hatchery stocks reduces the error associated with this calculation, and provides a more accurate tool for managing mixed stocks of fish.

After clipping at Simpson Hatchery, about 67 % of the fish were transferred to their respective rearing and release ponds throughout the Chehalis River Basin; the remaining

33 % were released at the hatchery site. This report summarizes the fin-clipping effort and results.

### METHODS AND MATERIALS

#### **Fin-clipping**

Fish to be clipped were crowded to one end of the pond, and hauled in screen buckets to a tagging trailer. The fish were held in holding tanks within the trailer at the rate of about 10,000 to 15,000 fish per loading and the trailer was reloaded three to four times per day depending on the number and speed of the workers. A standard WDF&W trailer was used (Thompson, 1989). After fish were anaesthetized (using MS-222), workers began removing the left ventral fin when fish could be handled without exerting too much pressure. "Knee-bend" or "iris" scissors were used to clip the fish. Each clipper had an individual counter to record their efforts.

#### Quality Control Sampling on Fin Clips

#### During clipping

Four, two hundred fish groups were checked daily for percentage of bad clips. These clips were defined as having any part of the ventral fin remaining after clipping. The clipper's daily goal was to have 1.0 % or less bad clips. If the bad clip rate exceeded 1.0 %, clipping was stopped and a survey of the entire crew was taken. The goal of this check was to identify workers who did not realize they were making bad clips, then persuade them to do a better job.

# After clipping

To check for fin regeneration and the accuracy of earlier checks, we performed additional Quality Control (QC) checks after the clipping work was completed. A QC check is defined as the work and calculations needed to determine the daily percentage of bad clips. For these tests performed after completion of the clipping project, the following definitions were used: good clip--no part of the ventral fin remains; partial clip--part of the ventral clip remains, but it can be identified by a professional checker; bad clip--the ventral fin appears to be unclipped. Each check required examining a minimum of 2,000 fish .

#### Fish Health

Simpson coho have a history of outbreaks of cold-water-disease (<u>Flexibacter</u> <u>psychrophilus</u>) in early spring and furunculosis (<u>Areomonas salmonicida</u>) in late summer, so we attempted to clip the fish in the time window between the expected onslaught of the two pathogens. We began clipping May 20, 1993 and we completed the project July 13, 1993.

#### RESULTS

Cost

#### Contract Labor

The total cost of contract labor was \$ 36,495.84 or \$ 15.88/1,000 clips (Table 1, Figure 1).

(This amount represents the wages of the clipping crew.)

#### Supervisor Labor

The total cost of supervisor labor was \$ 10,699.20 or \$ 4.66/1,000 clips (Table 1, Figure 1).

#### Temporary Labor

The total cost of temporary labor was \$ 4,757.80 or \$ 2.07/1,000 clips (Table 1, Figure 1).

(This amount represents the cost of a temporary worker needed to supplement the regular hatchery crew during the clipping project.)

#### Goods and Services

The total cost of goods and services was \$78.21 or 0.03/1,000 clips (Table 1, Figure 1).

#### Total Cost

The total cost of this project was \$ 52,031.05 or \$ 22.64/1,000 clips (Table 1). Costs may be reduced further by analyzing the cost of using the trailer vs. use of the hatchery building, and comparing the level of supervision requirements.

# Quality Control Sampling on Fin Clips

#### During clipping

The average initial bad clip percentage for the project was 1.3 % with a range of 0.1 % to 4.5 % (Table 2, Figure 2). The initial bad clip percentage was high, a 2.70 % average for the first five days (Table 2, Figure 2), but declined to a 1.0 % average for the next eight days (Table 2, Figure 2) as the crew gained experience. Several days into the experiment, I became concerned that the daily bad clip percentage might be increasing between the regularly scheduled QC checks. I initiated an additional round of four QC checks. The daily bad clip percentage declined to a 0.8 % average for seven days (Table 2, Figure 2), with the addition of this series of checks.

#### After clipping

Two weeks after completion of the clipping experiment, a QC check revealed a bad clip percentage of 0.6 % and 0.3 % for normal (fall) and late (winter) returning coho, respectively (Table 3).

A mixed group of normal/late returning fish, formed by placing both the normal and late returning fish in a release pond, were checked twice, (Table 3). The total percentage breakdown for both QCs is 71.80 %--good clips, 24.90 %--partial clips, and 3.3 %--bad clips (Table 3, Figure 3).

#### Daily Clips/Worker Rate

The daily clips/worker average was 4,607 with a range of 2,014 to 6,428 (Table 2, Figure 4). We believe the initial low daily clip rates were due to inexperience, and there was quite a range in the ability of the clippers,--some struggled to make 3,000 clips/day and other workers averaged more than 9,000 clips/day. The daily average total number of clips was 62,100 with a range of 22, 151 to 96, 915 (Table 2, Figure 5). The clipping rate increased when we switched scissors from "knee-bends" to the "iris" type scissor. The clips/worker data was lost for the dates 6-18 -93 to 6-21-93 (Table 2), so I averaged the numbers to produce an estimate for those days.

#### Fish Size

#### Normal Returning Coho

We began clipping the normal returning coho on May 20, 1993 when they were 1.80 gms/fish or 250 fish/lb (Table 4, Figure 6). We completed clipping this group on June 30, 1993 when they were 3.50 gms/fish or 130 fish/lb (Table 4, Figure 6). The smaller fish (1.80gms/fish or 250 fish/lb. Table 4) were quite hard to clip, and even harder to QC. These smaller fish may have accounted for some of the early, high, bad clip percentages. The percentage of bad clips decreased as fish size increased, and as the skill of the clippers increased. Clipping quality improved when fish size approached 2.3gms/fish or 197 fish/lb (field observation).

#### Late Returning Coho

We began clipping the late returning fish on June 30, 1993 when the fish were 2.20gms/fish or 204 fish/lb (Table 4, Figure 6). We completed clipping this group on July 13, 1993 when the fish were 2.60 gms/fish or 175 fish/lb (Table 4, Figure 6). We had fewer bad clips on these fish (Table 2). This may be due to larger fish, improved quality control technique, improved clipping ability, or all three.

Fish Health

In late July there was an outbreak of furunculosis. An agency Fish Health Specialist reported no signs of cold-water disease but felt the fin-clipping exacerbated the affects of the furunculosis outbreak. Care should be taken to manage clipping projects to avoid historical disease outbreak patterns at respective hatcheries. Personnel should observe fish frequently for signs of stress during the clipping project.

#### CONCLUSION

We believe we will be able to identify 96 % of the fish with clipped ventral fins ( the identification rate for adipose fin clips is 99.6 %, D. Thompson, WDF&W, unpub. data, 1993 ) upon return in 1994 and 1995. The overall cost of the project was \$ 52,031.05 or about \$ 22.64/1,000 clips ( The current cost of applying coded-wire-tags and adipose fin clipping is \$ 113.00/1,000 clips, G. Schurman WDF&W , pers. comm., 1994 ). Most of this cost was due to contract labor, ( \$ 15.88/1,000 clips), but supervisory labor contributed approximately 20 % of the total. I recommend a minimum size for ventral fin clipping of 2.3 gms/fish or 200 fish/lb.( field observation ). It should be determined if the overall cost could be reduced if hatchery staff was responsible for supervising the clipping crew rather than additional biological staff. The speed of fin clipping directly relates to the number and experience of the clippers and the size of the fish. Well designed clipping trailers or hatchery clipping areas will greatly increase the speed of the operation.



Figure 1.





FIGURE 2.

# FINAL QUALITY CONTROL CHECK



FIGURE 3.

# DAILY NUMBER OF CLIPS/WORKER



CLIPS/WORKER





FIGURE 5.

# FISH SIZE NORMAL/LATE RETURNING FISH



FIGURE 6.

Table 1. Cost of Simpson Mass Mark Project.

Category	Cost
Contract Labor	\$ 36,495.84 ( \$ 15.88/1,000 clips)
Supervisor Labor	\$ 10,699.20 ( \$ 4.66/1,000 clips)
Temporary Labor	\$ 4,757.80 ( \$ 2.07/1,000 clips)
Goods and Services	\$ 78.21 (\$ .03/1,000 clips)
GRAND TOTAL	\$ <u>52,031.05 ( \$ 22.64/1,000 clips)</u>

Table 2. All data collected during Simpson Project.

<u>Day#</u>	Date	#Clips	<u>#Workers</u>	<u>#Clips/Wrker</u>	Daily OC
Day# 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18	Date 5-20-93 5-21-93 5-24-93 5-25-93 5-26-93 5-27-93 5-28-93 6-01-93 6-02-93 6-02-93 6-03-93 6-04-93 6-07-93 6-09-93 6-10-93 6-10-93 6-11-93 6-14-93 6-15-93	<u>#Clips</u> 30,185 42,207 51,946 62,257 59,957 61,469 53,059 70,408 85,326 69,899 80,573 72,837 74,343 65,723 71,843 64,264 96,915 22,151	9.2 12.33 16.0 15.6 14.67 14.53 11.67 14.20 15.53 13.0 14.4 11.53 13.26 13.23 13.73 14.0 17.0 11.0	3,281 3,423 3,247 3,991 4,087 4,230 4,547 4,958 5,494 5,377 5,595 6,317 5,607 4,968 5,233 4,590 5,701 2,014	2.5 3.43 4.54 1.22 2.03 1.08 0.97 0.82 1.22 1.34 0.94 0.58 0.84 1.88 0.47 0.95 1.17 0.0
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37	6 - 16 - 93 6 - 17 - 93 6 - 18 - 93 6 - 21 - 93 6 - 22 - 93 6 - 23 - 93 6 - 24 - 93 6 - 25 - 93 6 - 29 - 93 6 - 30 - 93 7 - 01 - 93 7 - 02 - 93 7 - 07 - 93 7 - 08 - 93 7 - 09 - 93 7 - 12 - 93 7 - 13 - 93	22,151 22,151 22,151 72,099 58,014 77,654 59,821 86,615 73,998 75,402 81,117 59,046 55,103 66,841 78,498 84,264 72,705 63,919 39,899	11.0 $11.0$ $14.57$ $10.4$ $16.73$ $11.97$ $13.87$ $13.0$ $11.73$ $14.73$ $13.47$ $14.0$ $11.27$ $16.07$ $13.90$ $14.40$ $15.73$ $15.2$	2,014 2,014 2,014 4,958 5,578 4,642 5,011 6,245 5,692 6,428 5,507 4,384 3,935 5,931 4,885 6,062 5,049 4,064 2,625	0.0 0.0 0.83 1.17 1.53 0.12 0.45 0.60 0.58 3.11 0.92 0.74 1.11 0.34 1.04 0.98 0.34 0.30
Average		62,100	13.50	4,607	1.30
Total		2,297,70	00		

<sup>1</sup> QCs lost for dates 6-18-93 to 6-21-93.

DATE	STOCK	GOOD CLIP	PARTIAL CLIP	BAD CLIP
7-13-93	Simpson			
(Cum.Total)	Normal	98.70	N/A <sup>1</sup>	1.30
	Late	99.10	N/A	0.90
7-27-93	Normal	99.40	N/A	0.60
	Late	99.70	N/A	0.30
			249.5. • Selater	
	Simpson			
	Mixed Normal/			
3-1-94	Late	74.60	22.10	3.30
4-14-94		69.00	27.70	3.30
TOTAL	Mixed Normal/			
	Late	71.80	24.90	3.30
<b></b>		5		

Table 3. Quality Control checks made on Simpson Coho.

No partial QCs for dates 7-13-93 and 7-27-93.

1

<u>Date</u>	<u>Stock</u>		<u>Fish Size(gms)</u>	<u>Fish Size(F/LB)</u>
5-20-93		Returning	1.80	250
6-30-93		Returning	3.50	130
6-30-93	Late	Returning	2.20	204
7-13-93	Late	Returning	2.60	175

Table 4. Fish Size Range-Normal/Late Coho.

#### ACKNOWLEDGEMENTS

Many people contributed to the completion of this report. The following people provided invaluable assistance:

- \* Ross Fuller and Chuck Johnson managed this project and coordinated maintenance and technical support.
- Howard Fuss supervised me during this effort, contributed many encouraging comments and helpful ideas, and edited this report.
- \* Gary Schurman and his tagging supervisor crew, Paul Graham. Julie Hoof, Jennifer Hulet, and Jeff McGowan, trained me to supervise the trailer. They also provided equipment and ideas to improve the operation of the trailer.
- \* The Simpson Hatchery crew, Bob Ready, Joel Jaquez, Joe Rothrock, and Gary Barker, provided outstanding assistance for the duration of this fin-clipping experiment. They provided numerous valuable suggestions, and supervised the trailer in my absence. They also assisted in the cleaning of the trailer at the end of each day.
- \* The fin-clipping crew, Lillian Amell, Elizabeth Brinegar, Kim Brinegar, Judy Dale, Alicia Denton, Elsie Gamber, Tami-Lea Pattison-Gerou, Nora Gouley, Glenda-May Hall, Kate Hawthorn, Amber Hawk, Robin Jaquez, Clydette Markie, Rhea Lewis, Clint Pettis, Peggy Renish, Dena Rothrock, Teddy Rothrock, Connie Shelton, Robbie Tompkins, Teresa Tupper, Leslie Van Brunt, Reba Van Brunt, and Honore Wright, performed their work in an outstanding manner, and they have earned special rewards for their consistently fine efforts.
- Dick Westgard and Andy Appleby critiqued this report and contributed several helpful suggestions.

#### REFERENCES

- Blankenship, L. 1993. Assessment of mass-marking 1992 brood Puget Sound coho. Washington Department of Fish and Wildlife Internal Document.
- Schurman, G. 1994. The cost of applying coded-wire-tags and adipose fin clips to salmonids in Washington State. Personal communication.
- Thompson, D. 1993. Unpublished data. Comparison of mortalities of adipose fin clipped and ventral fin clipped coho at three Washington Department of Fish and Wildlife hatcheries.
  1989. Mobile Tagging Trailers Design and Construction.
  Washington Department of Fisheries Technical Report #107.
  39pp.

#### SOUTH FORK COQUILLE STEELHEAD MANAGEMENT A CASE STUDY

#### Jim Muck Oregon Department of Fish and Wildlife PO Box 5430 Charleston, OR 97420

The Coos and Coquille River systems in southwestern Oregon support popular winter steelhead fisheries. Catch in the Coquille and Coos Rivers for the run years 1981-1992 averaged about 4,500 and 1,600, respectively. However, fish managers recently became concerned that the level of hatchery fish present in the naturally spawning population of winter steelhead exceeded levels set in guidelines of the Wild Fish Management Policy (OAR 635-07-525 through 635-07-529). Traps set in tributaries to the Coquille River showed that hatchery fish represented 95% of the run in 1990-91 and 76% in 1991-92. A creel survey conducted on the Coquille in 1991-92 determined that hatchery winter steelhead composed 83% of the run. The volunteer scale program showed an average of 81% hatchery and 19% wild for the run years 1982-1989. To reduce the effects of hatchery winter steelhead interactions on wild steelhead populations in these basins the following four management strategies were implemented:

- 1. Localized broodstock conversion
- 2. Acclimation of steelhead smolts prior to release
- 3. Catch and release regulation for wild steelhead
- 4. Reduction of release numbers of hatchery oriented steelhead smolts

Coos River stock steelhead were released into the Coquille basin from 1948 until 1968. Alsea stock was used from 1968 until 1982. The hatchery release program converted to Coquille stock steelhead in 1982 and was segregated in 1990 into two stocks, South Fork stock and a North and East Fork Coquille stock. The hatchery stock of Coquille winter steelhead was completely converted to localized stock in 1994. By using a localized in-system stock of winter steelhead, compliance with the Wild Fish Management Policy can be met by not allowing the hatchery component in the naturally spawning portion of the run to exceed 50%. This compares to 10% if an out-system stock was used.

Wild adult winter steelhead were captured for broodstock conversion by a variety of means. A weir trap was constructed in the South Fork Coquille. Angling was tried but proved unproductive. Beach seines were used in the larger pools and were effective in the East and North Fork Coquille. Walking gill nets downstream through holding pools or long reaches of stream was very successful. Adults trapped in the gill nets were immediately retrieved and either moved directly to a holding tank or placed in large diameter PVC tubes to rest instream before transfer to the Bandon Hatchery. All methods were labor intensive and couldn't have been conducted without the assistance of STEP volunteers and Forest Service personnel.

Acclimation and release sites were established on several tributaries to both the Coquille and Coos River basins. This management strategy ("blackhole acclimation") is aimed at attracting hatchery fish back to specific areas as adults. Fishery managers can then remove the fish from the river for release into nearby lakes to diversify angling opportunities. Sites in the Coquille were developed at Laverne Park - a mainstem in-channel site on the South Fork, Hantz Creek, Beaver Creek, and Ferry Creek. Acclimation sites in the Coos basin were established on the West Fork Millicoma, Big Creek, and Noble Creek. All sites are located downstream of important spawning grounds to minimize hatchery fish interactions with wild spawners.

Acclimation sites located in the stream channel can block passage of downstream juvenile migrants and upstream bound adults. To accommodate passage, a surface bypass channel was installed in the Beaver Creek site on the South Fork Coquille and in Big Creek on the South Fork Coos River. It consisted of a floating trough which extended from the downstream to the upstream end of the acclimation pond. The top was covered with netting to prevent the acclimating steelhead in the holding pond from escaping, and migrating fish from entering the holding pond.

Catch and release for wild steelhead was implemented on the South Fork Coquille in January of 1993. This regulation was adopted for the entire Coquille basin in January of 1994. A complete closure to steelhead fishing was implemented on the South Fork upstream of the Siskiyou National Forest boundary near the town of Powers. This section of the river is an important spawning area for wild fish. Collectively these management strategies are used to enhance and sustain wild steelhead production, and to comply with the WFMP.

Winter steelhead smolt release numbers in both the Coquille and the Coos basins have recently been reduced to decrease the number of adult hatchery steelhead returning to these rivers. Coquille river releases averaged about 149,000 from 1982-1992 and are presently113,000. Results of this strategy will be available in 1995 and 1996.

A two-year creel survey conducted on the South Fork Coquille River and funded by the Forest Service determined that the hatchery component in the catch of winter steelhead was 64% in 1993 and dropped to 55% in 1994. For purposes of the creel study the river was broken into five sections with section one located in the lower portion of the basin and section five located in the upper river. The hatchery component of the catch was shown to decrease overall with distance upstream. This suggests that the hatchery component of the run decreases further upstream in the watershed where important wild steelhead spawning is known to take place. Adult winter steelhead recovered in trap sites established in the Coquille basin followed a similar trend. The proportion of hatchery steelhead collected in traps was 95%, 76%, 65% and about 30% for run years 1991-94 respectively.

Adult steelhead were captured in tributary spawning grounds to the Coquille River in 1994 between January and May to determine the hatchery component in the spawning population. Results from 21 steelhead collected in Sucker, Rock and Coal creeks showed a composition of 67% wild and 33% hatchery.

Results from the first few years of the evaluation suggest that strategies implemented to manage winter steelhead in the Coquille and Coos Rivers in compliance with the Wild Fish Management Policy have been successful in reducing the hatchery component in the steelhead run. The success of this program will be more conclusive in later years when more data becomes available. The study will continue to determine trends in wild and hatchery steelhead abundance. Much of the effort directed at the project has gratefully come from volunteers and through cooperative agreements with the Forest Service. Continued assistance from these groups is necessary.

## LOWER SNAKE RIVER COMPENSATION PLAN OVERVIEW

Ed Crateau U.S. Fish and Wildlife Service Lower Snake River Comp. Office 4696 Overland Rd., Rm. 560 Boise, Idaho 83705

The Lower Snake River Compensation Plan (LSRCP) is the largest fish restoration program administered by the U.S. Fish and Wildlife Service (FWS). Authorized by the water Resource Development Act of 1976, the LSRCP was designed to replace salmon, steelhead, and resident trout losses caused by the construction and operation of Ice Harbor, Lower Monumental, Little Goose, and Lower Granite Lock and Dam projects on the lower 150 miles of the Snake River. The LSRCP program prescribed the construction of new facilities or the modification of existing hatcheries to produce enough smolts and resident trout to return 18,300 fall chinook, 58,700 spring and summer chinook adults and 55,100 steelhead adults back to the project area above Lower Granite Dam and 93,000 pounds of resident trout that were lost annually by the construction of the four lower Snake River dams. The program required expansion or construction of 12 hatcheries and 11 satellite facilities in Idaho, Washington, and Oregon. Under contract with FWS, the Idaho Department of Fish And Game operates four hatcheries, Oregon Department of Fish and Wildlife operates three hatcheries, Washington Department of Fish and Wildlife operates three hatcheries and the FWS itself operates two hatcheries. Construction costs alone for the 23 facilities totaled nearly \$180 million.

This single largest cooperative restoration venture of the FWS includes the three N.W. state agencies, the U.S. Army Corps of Engineers who constructed the facilities, Bonneville Power Administration who collect revenue from rate payers who buy power that ultimately funds the program and three Indian tribes the Nez Perce Tribe, Confederated Tribes of the Umatilla Indian Reservation and the Shoshone Bannock Tribes. The state and tribal agencies, co-mangers of the fishery resources, also operate a comprehensive hatchery evaluation program to collect information to evaluate the progress of the hatchery production programs.

The LSRCP program has produced as much as 20,000,000 salmon, steelhead and resident trout weighing approximately 2,000,000 pounds within its relatively short 14 year history. The video "To Restore a Legacy; the Struggle for the Snake River Salmon and Steelhead" narrated by Robert Wagner explains the LSRCP, its progress to date, the problems being encountered, and the massive federal, state and tribal involvement that make up the program

#### AQUACULTURE QUALITY ASSURANCE

#### Gary Fornshell Extension Aquaculture Agent University of Idaho 246 3<sup>rd</sup> Ave East Twin Falls, ID 83301

Aquaculture quality assurance (QA) programs are educational programs designed to ensure continued production of high-quality, wholesome farm-raised aquaculture products. It is important to understand the principles involved in quality assurance and how you, the producer can participate in your particular species' QA program.

#### What is a Quality Assurance Program?

For aquaculture producers, it is an educational program emphasizing good management practices in the handling of animal health care products and encourages a careful evaluation of current production practices. QA is a systematic analysis of those production practices that might compromise the wholesomeness of aquaculture products. The major emphasis of all food product QA programs is the avoidance of drug, pesticide and microbial residues.

Quality assurance is important for all industries. QA programs are developed to help ensure the cars we drive, the food we eat, and the medicines we take are safe, will last and will do the things they are designed to do. Within the last decade, several of agriculture's livestock producer groups have initiated QA programs. These include the beef, veal, dairy, pork, lamb and poultry industries. Most of these programs were developed in response to food safety issues. Fortunately, the aquaculture industry is positioned proactively, rather than having to react to safety issues. This is an excellent opportunity for aquaculture producers to demonstrate that their practices are safe, well monitored and the safety and quality of farm-raised aquaculture products can be assured.

#### Quality Assurance and Food Safety

Consumers of all foods have become concerned about the safety of their food supply. They are concerned about microbial contamination, drugs, pesticides and any residues in their food. This is especially true for consumers choosing to eat healthier and more wholesome food. Fortunately, aquaculture products are recognized for their high nutritional value and quality.

Quality and safety can only be achieved through a team effort. Producers, feed manufacturers, harvesters, transporters, processors, distributors and retailers must all do their part. Farm production of aquaculture species is only the first step - but an essential first step. If the product becomes contaminated during production, it may be difficult to "fix it" before harvest and impossible during processing or at the retail seafood counter.

The aquaculture industry may use various animal health care products and water treatment chemicals during production. Most aquaculture products are produced under environmentally controlled conditions, which allows considerable control over product quality. The safety of aquaculture products has been well established. The U.S. Food and Drug Administration (FDA) and the U.S. Center for Disease Control consider seafood the safest choice of muscle foods. However, if drugs and chemicals are not used properly, according to label directions, it is inevitable that unacceptable residues will occur. Good management practices will minimize the use of drugs and water treatment chemicals. Violative residues (i.e., those exceeding acceptable tolerances) may also occur in aquaculture products produced in contaminated water or fed contaminated feed.

But, as a producer, it is possible for you to prevent contamination and to assure the public and yourself, of your product's safety and quality. That is the purpose of an aquaculture QA program. What could be more important? That is why your participation is essential.

#### **Quality Assurance Starts With Commitment**

The high quality of the finished aquaculture product requires a team effort. That effort starts with you - the producer. Everyone must commit to produce the best quality aquaculture product possible; one that satisfies the consumers' high quality standards.

Commitment to quality assurance is fundamental to ensure continued marketing success. Consumer concerns for food safety have increased federal and state efforts to ensure consumer protection. Regulatory agencies have developed and continue to develop drug residue testing procedures and on-site inspection programs that specifically target all farm-raised aquaculture products. These same testing and inspection procedures apply to imported products. A producer-based QA program is intended to help maintain the aquaculture industry-wide violation-free status and ensure superior products. This is a timely opportunity for the aquaculture industry to participate in QA programs that will prevent consumer concerns and assure that the industry continues to flourish.

#### **Current Regulatory System**

There are four federal agencies that help ensure the safety and wholesomeness of aquaculture products for consumers. You, the producer, are ultimately responsible for providing a safe, wholesome product.

The four agencies are the U.S. Food and Drug Administration (FDA); U.S. Environmental Protection Agency (EPA); U.S. Department of Commerce (USDC); and the U.S. Department of Agriculture's (USDA), Animal and Plant Health Inspection Service (APHIS). The FDA is responsible for regulating medicated feeds, drugs, color additives and other fish health products. FDA's Center for Food Safety and Applied Nutrition houses the Office of Seafood which may soon administer a mandatory HACCP (Hazard Analysis of Critical Control Points) seafood inspection program for all seafood processing plants.

The EPA has primary jurisdiction for disinfectants, sanitizers and pesticides such as algicides. The EPA is also responsible for regulating water quality and may issue National Pollutant Discharge Elimination System (NPDES) permits. The EPA also sets limits on the discharge of some commonly used water treatments.

The USDC currently offers a voluntary, fee-based inspection program to the aquaculture processing industry. This inspection ensures proper sanitation and product quality for the consumer.

USDA-APHIS, under the Virus-Serum-Toxin Act, regulates all veterinary biological products shipped into, within, or from the United States. Veterinary biological products include vaccines and disease diagnosis test kits.

The current regulatory system provides the American consumer with the safest food supply in the world. Today, however, the consumer wants assurance that the food supply is free from drugs and chemicals. There is pressure for increased regulation of products and even for removal of some products that are currently in use. For this reason, aquaculture producers must understand the importance of proper drug and chemical usage and prevention of contamination from any outside sources.

#### The Critical Considerations in a QA Program

There are several critical considerations that must be addressed by any QA program. These considerations are similar in function to critical control points used by HACCPbased seafood processors. Proper decision making at each critical consideration decreases the possibility of environmental contamination and drug or chemical residues in aquaculture products and is vital to good management and responsible resource stewardship. These considerations include the following:

- Production site selection
- ♦ Water supply selection
- ◆ Water quality management
- ♦ Waste management
- ♦ Maintenance of the various life stages

- Feed quality and feeding practices
- ◆ Integrated animal health management
- Proper drug and chemical use
- ♦ Harvesting, holding and transporting
- ♦ Accurate and detailed recordkeeping

Each producer's QA program must be developed through careful evaluation of their respective operation. Discussion with extension and fish health professionals can be important as you tailor the QA program to your own operation.

Why Should You - The Producer, Participate in an Aquaculture QA Program?

Specific benefits to your participation in an aquaculture QA program are:

- Being part of a nationwide program to ensure continued availability of high quality wholesome aquaculture products for consumers and other customers.
- ♦ Avoid harmful drug or chemical residues.
- Improved production and waste management practices.
- ◆ Decreased production costs.

#### Other benefits include:

- ♦ An aquaculture QA program will allow the industry to showcase those practices it is already doing to ensure quality and educate consumers about the controlled conditions under which aquaculture products are produced.
- The FDA supports voluntary aquaculture QA programs and QA programs may provide for proactive involvement in the regulatory process.
- Employee confidence and pride increase when a QA program is implemented.
- Exported products will meet the high safety and quality requirements of the receiving country.
- By participating in your specie's QA program and maintaining QA records, you will meet the needs of your processor's effort to comply with the HACCP seafood safety program.

Remember that you as a food producer have a responsibility to consumers of the products you grow. The number of animals that you grow does not change this responsibility. The high quality of aquaculture products requires a team effort and you are the first step toward that effort.

#### How Can You Participate in a QA Program?

Several aquaculture QA programs are either already available or in development. The Catfish Farmers of America and the U.S. Trout Farmers Association each have species-specific programs. The American Tilapia Association and the Striped Bass Growers Association are developing producer QA programs. A group of cooperating universities and fishery product organizations in the Northeast is establishing a HACCP-based QA program for growers of pen-raised salmon, farmed and relayed molluscan shellfish and hybrid striped bass. The National Aquaculture Association is developing generalized QA materials for finfish and shellfish growers.

These QA programs are *completely voluntary*, but all aquaculture producers should participate. The goal of the aquaculture industry is to enroll every aquaculture producer in QA. The benefits to you and your farm are obvious, and the benefit to your industry will also be obvious.

So join today and work to make QA a vital part of this important industry. Contact your local or state aquaculture association about existing or proposed quality assurance programs. Your State Cooperative Extension Service or Sea Grant Marine Advisory Service can also provide information.

#### **Resource Publications and Videos:**

Aquaculture Quality Assurance: Become Involved. 1994. A 14-minute video available through your State Cooperative Extension Service or Sea Grant Marine Advisory Service.

Aquaculture Quality Assurance: A National Teleconference. 1994. A video available from Department of Fisheries & Allied Aquaculture, Swingle Hall, Auburn University, Auburn AL 36849-5628.

Federal Regulation of Drugs, Biologicals, and Chemicals Used in Aquaculture Production. 1993. Joint Subcommittee on Aquaculture, Working Group on Quality Assurance in Aquaculture Production. Available through the National Agricultural Library.

*Guide to Use of Drugs, Vaccines and Pesticides in Aquaculture.* 1994. Produced by the Joint Subcommittee on Aquaculture. Available through National Agricultural Library or your State Cooperative Extension Service or Sea Grant Marine Advisory Service.

#### Additional Information:

FARAD - Food Animal Residue Avoidance Databank. North Carolina State University, College of Veterinary Medicine, Raleigh NC 27606; (919) 829-4431, FAX (919) 829-4358.

Department of Environmental Toxicology, College of Agriculture and Environmental Sciences, University of California, Davis CA 95616; (916) 752-7507.

University of Illinois, 1220 Veterinary Medicine Basic Sciences Building, 2001 S. Lincoln Ave., Urbana IL 61801; (217) 333-6731.

#### **Aquaculture Information Center:**

National Agricultural Library USDA. Room 304, Beltsville MD 20705 (301) 504-5558.

#### **Producer Organizations:**

Catfish Farmers of America 1100 Highway 82 E. Suite 202 Indianola MS 38751

National Aquaculture Association P.O. Drawer 1569 Sheperdstown WV 25443

U.S. Trout Farmers Association P.O. Box 220 Harper's Ferry WV 25425

American Tilapia Association 4943 Cosgrove Rd. SW Kalona IA 52247

Striped Bass Growers Association P.O. Box 5452 Raleigh NC 27650-5452

#### SESSION II

#### HATCHERY OPERATIONS AND TECHNOLOGY

- Food for Thought Bill Klontz, University of Idaho, Professor Emeritus of Aquaculture
- The Effects of Three Rearing Densities on the Growth, Health and Mortality of Spring Chinook Salmon at Dworshak National Fish Hatchery - Peter Long, U.S. Fish and Wildlife Service
- The Effects of Three Rearing Densities on the Migration Time and Survival of Spring Chinook Salmon Released at Dworshak National Fish Hatchery - Ray Jones, U.S. Fish and Wildlife Service
- Integrated Hatchery Operations Team Policies, Procedures and Implementation Plan - Chris Christianson, Columbia Basin Fish and Wildlife Authority
- Umatilla Hatchery Operations Overview Jack Hurst, Oregon Department of Fish and Wildlife

#### FOOD FOR THOUGHT

#### G. W. (Bill) Klontz Moscow, Idaho

According to available data, the post-release survival of hatchery-raised spring chinook salmon in the Salmon River and Snake River systems is alarmingly low, especially in the reaches between the Sawtooth and Rapid River Hatcheries to the entry into the Lower Granite reservoir at Lewiston, Idaho (Buettner and Nelson, 1989 and 1991). Given the environmental conditions in these systems, the losses are surprisingly high; e.g., there are no static pools loaded with silt and predators, no major man-made obstacles such as dams, and no nitrogen supersaturation. There are, however, marked differences between the riverine environments and the hatchery environments. The major differences are water velocity and turbulence and food availability. Thus, it would be logical to conclude that the hatchery fish were ill-prepared for the riverine environments and succumbed because of their inability to cope with it. It is this premise that I base my forthcoming proposition to enhance the downstream survival of these fish.

Many researchers have considered swimming stamina of hatchery-raised and wild fish a very important criterion for performance in the natural environments (Horak, 1972; Greenland and Thomas, 1972; Green, 1964; Flagg et al, 1983; Besner and Smith, 1983; Black, 1965; Poston, 1975; Houlihan and Laurent, Evaluation of swimming stamina, in most studies, was 1987). accomplished by forcing the fish to swim against high water velocities to the point of exhaustion. The end-point was the maximum velocity that induced exhaustion during swimming periods of 2 to 80 minutes. Although most researchers deduced from their studies that better post-release survival might be correlated with swimming stamina, few studies were conducted to confirm this conclusively. Horak (1972) examined swimming stamina versus post-release survival but was unable under the test conditions used to demonstrate the relationship between stamina and postrelease survival. Creswell and Williams (1983) examined the effects of pre-test exercise on swimming endurance. Thev concluded that hatchery fish should be conditioned to stream velocities at least two days prior to release if survival were to be increased. However, better post-release survivals were recorded following 14 days of pre-release exercise. This e confirmed the observations of Burrows (1964), who reported This effect increased adult return rates in populations raised for 7 months in circular tanks with high water velocities. Burrows also reported that the fish raised in circular tanks exhibited improved growth rates and increased swimming stamina.

I question the validity of assuming that swimming stamina per se is a realistic criterion for post-release riverine survival, an environment with which free-living populations of fish cope continuously. The majority of testing conditions used to evaluate swimming stamina primarily measured the ability of the fish to tolerate elevated levels of white muscle lactic acid with its attendant decrease in blood pH (Johnston, 1982). The light muscle, used primarily for short-lived burst swimming, functions anaerobically and is quite susceptible to fatigue. The dark muscle, used primarily for sustained swimming activity, functions aerobically and is very resistant to fatigue.

The observations of Burrows support the contention that long-term physical conditioning has merit. There are conclusive data to demonstrate that fish can be conditioned for increased growth performance and sustained swimming capacity. For example, fish required to swim in sustained water velocities of 1.0 - 1.5 body lengths per second exhibited higher growth rates than did their counterparts in still waters (Leon, 1986; Jobling, 1991; East and Magnan, 1987; Davison and Goldspink, 1977; Houlihan and Laurent, 1987; Greer-Walker and Emerson, 1978; Christiansen et al., 1989). There was agreement by all authors that prolonged exercising improved feed conversions and increased the amount of feed required for satiation (Jobling, 1991). More significantly, there were exercise-associated changes in body composition; i.e., significant increases in muscle mass (Davison and Goldspink, 1977), increases in body protein and decreases in lipid (Jobling, 1990; Christianson et al., 1989).

In addition to quantitative changes in proportions of muscle tissue, there were exercise-associated histological changes in the light and dark muscles (Johnston, 1982; Davison and Goldspink, 1977). Others reported associated changes were a decrease in oxygen demand in stressor conditions (Woodward and Smith, 1985) and an increased stamina to high water velocities and turbulences (Burrows, 1964).

Since the foregoing citations conclude that several weeks of exercise are required to create a significant proportional increase in dark muscle, the plan presented here proposes to condition the fish throughout the majority of their pre-smolt period under controlled hatchery conditions.

The second major investigative conditions should be irregular feedings and sprinklers providing a visual barrier between the fish and the feeder. Fish in the wild feed irregularly with periods of high expenditure of swimming energy to cope with the water velocity and to avoid predators. Studies conducted under laboratory conditions have demonstrated the efficiency of irregular over regular feedings (Klontz et al., 1991). Fish fed irregularly displayed less interfish aggression (as evidenced by fin nipping). Also, there were less size variations within populations and no negative effects on feed conversions. Intermittent feeding also generated positive effects on growth performance and body condition.

There is little information on the effects of exercise combined with irregular feedings or short periods of starvation. Black (1965), in studies of the effects of short-term exercise and starvation on glycogen metabolism, observed significant decreases in blood glucose levels following two minutes of strenuous swimming by starved fish.

There are no known studies addressing the combined effects of long-term exercise and feeding regimen. Also, there are no known reports of post-release survival following long-term exercise and irregular feeding regimens.

Thus, the proposed approach to evaluate the effects of longterm exercise on post-release survival and adult returns consists of pre- and post-release evaluations. At the test hatcheries, identifiable groups of fish on the exercise and not exercised regimens should be established.

In the pre-release portion, ponds - whether they be raceways or circulating ponds - should be configured to provide specified water velocities by altering either the cross-sectional area of the pond (raceways and Burrows ponds) or the angle of water inflow (circular ponds). Raceways can also be configured to provide specified water velocities by inserting two sets of longitudinal panels in the ponds to provide an S-shaped flow. In addition, ponds, as mentioned before, should be provided with sprinklers to reduce the visibility of the staff at feeding times. Ponds used for the non-exercised (Control) fish can be used unmodified. Prior to release from the hatchery environments, sufficient numbers of fish in both groups - Test and Control - should be tagged for downstream monitoring after release and return as adults some years later.

During the pre-smolt period, data to evaluate the following performance parameters should be collected on a regular basis; i.e., biweekly or monthly. I am suggesting the consideration of 15 performance indicators, which are:

1) <u>Growth</u>, which can be measured as a function of changes in length, weight and condition factor. The daily length and weight increases may be affected by the combination of exercise and feeding regimens. The condition factor is considered to be directly related to fitness and should be within a definite range for post-release survival. The exercise and feeding regimens should affect this parameter. It should be considered as an important response variable for future hatchery practices.
2) <u>Size variation</u>, which is primarily the result of food availability and the individual feeding aggressiveness of the fish. It can be measured as the coefficient of variation for population lengths and weights.

3) Feed conversion ratio, which is not one of the prime considerations of these studies although reports in the literature imply that there will be improved feed conversion ratios with exercise.

4) <u>Specific growth rate</u>, which can be calculated the method developed by Focht (1983). The formula accounts for decreasing age-dependent growth rates and has been shown to be quite useful under hatchery conditions. The formula is:

SGR =  $((1 + (W_e - W_i) / W_i)^{(1/n)} - 1) * 100$ 

Where:	SGR	=	Specific growth rate (%/day)
	We	=	Weight (g/fish) at the end of the
	-		growth period.
	Wi	=	Weight (g/fish) at the beginning
	-		of the growth period
	n	=	Number of days in the growth period
	100	=	Decimal-removing factor

5) Organosomatic indices, which can be determined using the Goede method described and implemented by Warren (1990). These morphometric, physiological and chemical values can serve to document the physical condition of the fish. These data should contribute to the interagency data bank.

6) <u>Muscle mass and histology</u>, which can be determined on a bi-monthly basis. These evaluations should also be done on recovered post-release hatchery fish and wild fish.

Fish experiencing long-term exercise have been shown to have an increase in muscle mass and alterations in proportions of dark (sustained swimming) and light (burst swimming) muscle (Davison and Goldspink, 1977). There was also an attendant increase in dark muscle fiber diameter with sustained exercise (Johnston, 1982; Davison and Goldspink, 1977).

7) <u>Hematological parameters</u> consisting of hematocrit (% packed cells), hemoglobin (g / 100 ml blood), erythrocyte count (n / mm<sup>3</sup>), mean corpuscular volume (nm<sup>3</sup>), mean corpuscular hemoglobin (uug), mean corpuscular hemoglobin concentration (%), and serum proteins (g / 100 ml) can be determined using standard methods. These parameters are considered to be invaluable physiological criteria when employed properly for assessing the physiological status of fish.

8) <u>Proximate analysis</u> of whole fish, muscle, and viscera can be determined. This is to evaluate relative changes in lipid and protein levels during the course of the study. It is assumed that lipid contents will be negatively correlated to sustained swimming ability and post-release survival. Protein content is directly correlated with muscle mass, thus exercise should generate increases in protein levels apart from those occurring as growth.

9) Oxygen demand (Standard Metabolic Rate) (SMR), which can be determined using the method described by Klontz et al (1983a). The SMR is an indicator of the ability of fish to respond to stressful situations. Woodward and Smith (1985) noted that exercised fish exhibited a decreased SMR during short-term periods of stress than did their unexercised counterparts.

10) <u>Prevalence of environmental gill disease</u>, which is a frequent clinical manifestation of a chronic stressful situation, also affects the SMR by requiring an increased  $pO_2$  to satisfy the blood oxygen demand (Klontz et al 1985). Thus, the SMR data could be influenced by this condition. Examination can be made by wet mounts of the gill tissues. Quantification can be based on severity using a scale of 1 - 5 (1 + no change; 5 = occlusive interlamellar hyperplasia).

11) <u>Prevalence of latent bacterial infections</u>, particularly *Renibacterium salmoninarum*, which can affect the physiological responses to stressful situations. Examinations for *R*. *salmoninarum* and other organisms endemic to the study hatcheries can be conducted by the ELISA method.

12) <u>Mortality</u>, which is a response to the inability of the fish to cope with its environment or to the occurrence of a microbial pathogen. Thus, the exercised fish should have a lower cumulative mortality than their unexercised counterparts.

13) <u>Social behavior patterns</u>, which can be measured by the prevalence of fin nipping at times of inventory. Post-release behavior in the wild should be recorded for comparative purposes. This will require the cooperation of agency field personnel.

14) <u>Feeding behavior</u> of post-release fish, which can be evaluated by examining the stomach contents of recovered postrelease hatchery fish and wild fish. The questions to be answered are:

- a. Did the hatchery fish ingest the same foodstuffs as the wild fish?
- b. How rapidly following release did the fish begin to eat?

c. Is there a relationship between in-hatchery feeding regimen and post-release feeding behavior and food preferences?

15) <u>Recovery</u> of tagged fish released from the study hatcheries, which is the one of the main purposes of these studies. The collected fish should be sampled for the same physical and physiological examinations conducted on the fish in hatchery studies.

Thus, there is my approach to reducing the downstream migrating smolt loss and increasing the adult returns no matter what man-made modifications are implanted along the way. To some, it may seem a bit facetious to say that one of the best ways to reduce fish mortality is to teach the little beggers to swim. Also, to some the list of performance indicators may seem a little excessive and more appropriate to the university laboratory, although, if one thinks about it, they are not excessive and they belong in the hatchery study.

In conclusion, what I have offered is food for thought. If but one of the readers thinks the approach has merit and implements it or another like it, I think I have been successful in my efforts.

#### Literature Cited

- Besner, M. and L.S. Smith, 1983. Modification of swimming mode and stamina in two stocks of coho salmon (Oncorhynchus kisutch) by differing levels of long-term continuous exercise. Can. J. Fish. Aquat. Sci. 40:933-939.
- Black, E.C. 1965. Combined effect of starvation and severe exercise of glycogen metabolism of rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada 23(9):1461-1463
- Beuttner, E.W. and V.L. Nelson. 1989. Smolt condition and timing of arrival at Lower Granite Reservoir. Annual Report, 1988. Proj. No. 83-323 B., BPA
- Beuttner, E.W. and V.L. Nelson. 1991. Smolt monitoring at the head of Lower Granite Reservoir and Lower Granite Dam. Annual Report, 1991. Proj. No. 83-323 B., BPA
- Burrows, R.E. 1964. Effect of fingerling stamina on adult survival. Proc. NW Fish Cult Conf., pp. 54-55, Corvallis, OR.
- Christiansen, J.S., E. Ringo and M. Jobling. 1989. Effects of sustained exercise on growth and body composition of firstfeeding fry of Arctic charr, Salvelinus alpinus L., Aquaculture 79:329-335.

- Cresswell, R.C. and R. Williams. 1983. Post-stocking movements and recapture of hatchery-reared rainbow trout released into flowing waters: Effect of prior acclimation to flow. J. Fish Biol. 23:265-276.
- Davison, W. and G. Goldspink. 1977. The effect of prolonged exercise on the lateral musculature of brown trout (Salmo trutta). J. Exp. Biol. 70:1-12.
- East, P. and P. Magnan. 1987. The effect of locomotor activity on the growth of brook charr (*Salvelinus fontinalis*, Mitchell). Can. J. Zool. 65:843-846.
- Flagg, T.A., E.F. Prentice, and L.S. Smith. 1983. Swimming stamina and survival following direct seawater entry during parr-smolt transformation of coho salmon. Aquaculture, 32:383-396.
- Focht, R. 1983. Trout growth in aquaculture systems: Methods of estimating daily percent weight gain. M.S. Thesis, Fishery Resources, Univ. Idaho. 98 pp.
- Green, D.M. 1964. A comparison of stamina of brook trout from wild and domestic parents. Trans. Amer. Fish. Soc. 93:96-100.
- Greenland, D.C. and A.E. Thomas. 1972. Swimming speed of fall chinook salmon (Oncorhynchus tshawytscha) fry. Trans. Amer. Fish. Soc. 1972(4):696-700.
- Greer-Walker, M. and L. Emerson. 1978. Sustained swimming speed and myotomal muscle function in the trout (Salmo gairdneri). J. Fish Biol. 13:475-481.
- Horak, D.L. 1972. Survival of hatchery-reared rainbow trout (Salmo gairdneri) in relation to stamina tunnel ratings. Prog. Fish-Cult. 4:217-220.
- Houlihan, D.F. and P. Laurent. 1987. Effects of exercise training on the performance, growth, and protein turnover of rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci. 44:1614-1621.
- Jobling, M. 1991. Fulfilling the impossible dream. Fish Farmer. 13(4):52-53.
- Johnston, I.A. 1982. Physiology of muscle in hatchery-raised fish. Comp. Biochem. Physiol. 73B(1):105-124.

- Klontz, G.W., C.M. Herr and T.J. McArthur. 1983. Aquaculture techniques: Preliminary studies on carrying capacities and census-taking in serial reuse ponds. Tech. Comp. Rept. A-075IDA, IWRRI, 44pp.
- Klontz, G.W., M.H. Maskill and H. Kaiser. 1991. The effects of reduced continuous versus intermittent feeding of rainbow trout. Prog. Fish-Cult. 53(4):229-235.
- Klontz, G.W., B.C. Stewart and D.W. Eib. 1985. Chap. 20: On the etiology and pathophysiology of environmental gill disease in juvenile salmonids. In Fish and Shellfish Pathology (A.E. Ellis, Ed.), Academic Press, pp. 199-210.
- Leon, K.A. 1986. Effect of exercise on feed consumption, growth, food conversion and stamina of brook trout. Prog. Fish-Cult. 48:43-46.
- Poston, H.A. 1975. Influence of dietary protein and energy on swimming stamina, growth and body composition of brown trout. Prog. Fish-Cult. 37:257-261.
- Warren, J.W. 1990. Augmented Fish Health Monitoring Annual Report. BPA. 132 pp.
- Woodward, J.J. and L.S. Smith. Exercise training and the stress response in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 26:435-447.

## THE EFFECTS OF THREE REARING DENSITIES ON THE GROWTH, HEALTH AND MORTALITY OF SPRING CHINOOK SALMON AT DWORSHAK NFH, IDAHO - A PROGRESS REPORT 1993

#### Peter Long Dworshak National Fish Hatchery Ray N. Jones Idaho Fishery Resource Office

## U.S. Fish and Wildlife Service Ahsahka, Idaho

#### INTRODUCTION

In an effort to produce the healthiest, most viable smolts possible, the Idaho Fishery Resource Office (FRO) and Dworshak National Fish Hatchery (NFH) developed a project to evaluate the effect of rearing density on hatchery performance, post-release performance, and adult returns of spring chinook salmon (SCS) reared and released at Dworshak NFH in Idaho.

The objectives were to compare the growth, health, mortality, migration rate, smolt survival, and smolt to adult survival rates between three different rearing densities.

In this summary, we present some of the results of the hatchery rearing phase of the project, which includes growth, health, and mortality. Data on smolt migration, smolt survival, and adult returns is presented in the accompanying companion summary.

# SITE DESCRIPTION

Dworshak NFH is located at the confluence of the North Fork and the main-stem of the Clearwater River near Ahsahka, Idaho. Construction of the hatchery was included in the authorization for Dworshak Dam and Reservoir (Public Law 87-847, October 23, 1962) to mitigate for losses of anadromous steelhead (*Oncorhynchus mykiss*) caused by the dam and reservoir.

The hatchery was designed and constructed by the U.S. Army Corps of Engineers (COE) and has been administered and operated by the U.S. Fish and Wildlife Service (USFWS) since the first phase of construction was completed in 1969.

Additional construction was completed in 1982 under the Lower Snake River Compensation Plan (LSRCP) to provide rearing facilities for spring chinook salmon (*O. tshawytscha*). A total of 30 8-foot by 80-foot raceways were constructed. Dworshak NFH was originally designed to produce 1.4 million spring chinook salmon smolts (70,000 lbs @ 20 fish/lb) annually.

#### METHODS AND MATERIALS

Three rearing densities were tested: (1) low density or 1/3 normal loading at 15,000 fish per raceway (2) medium density or 2/3 normal loading at 30,000 fish per raceway (3) and high density or normal loading at 45,000 fish per raceway. A total of nine raceways were set up at low density, six raceways at medium density, and three raceways at high density.

Growth was monitored by conducting sample counts and measuring length frequency distributions in at least one raceway of each density group each month during rearing. Random samples were collected by crowding raceways and taking a sub-sample. Total lengths were measured to the nearest 5mm. The prevalence of *Renibacterium salmoninerum*, the causative agent of bacterial kidney disease (BKD) in juvenile populations during raceway rearing was determined for both brood years using the enzyme-linked immunosorbant assay (ELISA) technique. ELISAs were performed on a monthly basis from December 1990 to April 1991 and November 1991 to April 1992 for Brood Years (BY) 1989 and 1990, respectively.

ELISA data were converted to logarithms for statistical analysis to reduce variability. ANOVA was used to determine if mean monthly ELISA values were significantly (P<.05) different. Posthoc pair-wise differences in means were compared between densities using the Bonferroni procedure.

Total monthly mortality for each density group was calculated by summing the daily mortalities for each raceway in each respective density group for each month. Monthly mortality rates were calculated by dividing the total monthly mortality in each density group by the total number of fish in each density group at the beginning of each month.

#### RESULTS

For BY89, low, medium, and high density groups had mean lengths at time of release of 140, 137, and 134mm, respectively. Although the low density group was larger, differences were not significant.

For BY90, low, medium, and high density groups had mean lengths at time of release of 155,154, and 149mm, respectively. Again, the low density group was larger, but differences were not significant.

Mean sizes at release were greater for BY90 than for BY89 for all three density groups. Mean lengths for each density group for both brood years are listed by month in Table 1.

	Low De	nsity	Medium	Density	High Density				
Date	BY89 1		BY89	BY90	BY89	BY90			
June 1	74	69	75	67	72	67			
July 1	85	87	84	84	82	83			
August 1	94	98	89	97	89	97			
September 1	105	110	100	108	101	107			
October 1		114		113		114			
November 1	121	127	119	126	116	127			
December 1	130	134	124	134	123	134			
January 1	132	141	128	. 140	127	139			
February 1	134	145	129	144	128	139			
March 1	136	150	129	149	128	142			
April 1	140	153	137	152	134	147			
April 16		155		154		149			

Table 1.Mean monthly total length (TL[mm]) of Brood Year 1989 and 1990 spring<br/>chinook salmon reared at three different densities at Dworshak National Fish<br/>Hatchery, Ahsahka, Idaho.

ELISA did not exhibit any consistent trends for BY89. No clear pattern of *R. salmoninarum* prevalence was observed for any one density group. However, the high density group for BY90 showed a consistent trend of higher *R. salmoninarum* levels throughout the rearing period. The high density group always had significantly higher ELISA values than low or medium density groups. Overall ELISA values were higher for BY90 compared to BY89. The monthly mean, variance, and standard deviation of ELISA for BY89 and BY90 are listed in Table 2 for each density group.

For BY89, mortality rates did not appear to differ markedly between rearing densities with rates ranging from .006 to >.0001 per month during the raceway rearing period. The trend in mortality over the rearing period was similar among all three densities.

For BY90, mortality rates did not appear to vary much between low and medium density groups and ranged from .012 to .001 per month during the rearing period. However, fish in the high density raceways experienced higher mortalities than fish in the low and medium density raceways during part of the rearing period. From January to August 1991, fish in all three densities had similar mortalities. From September 1991 to April 1992, the fish in the high density raceways had higher mortalities than fish in low or medium density raceways.

#### DISCUSSION

Based on the ELISA data, the high mortality rate during raceway rearing of the high density group for BY90 was probably due to BKD. The higher prevalence of BKD may have been caused by increased stress due to a higher density. However, an examination of the individual raceway mortality records for the BY90 high density group suggests another possible source of mortality unrelated to density. The high density experiment was conducted in three separate raceways. Of the total mortalities from June 1, 1991 to March 1, 1992, about 60 percent occurred in one raceway, 30 percent in the second, and 10 percent in the third. The disproportionate distribution of mortality is strongly suggestive of an effect other than density. When the density experiments were initiated, individual nursery tank populations were not taken randomly from the total population of button-up fry. Although no eggs from females with ELISA greater than 0.5 were used in the study, one or more females could have made a disproportionate contribution to particular nursery tanks. If some females had higher levels of BKD (0.4 vs 0.1, for example), progeny from those females with different BKD levels could possibly exhibit different propensities for mortality. This could explain the disproportionate mortality rates between the high density raceways for BY90 and the consistently lower performance of the high density group overall.

#### CONCLUSIONS

The data do not consistently demonstrate any significant difference in hatchery performance of spring chinook salmon reared at the densities tested. Differences that were observed could not be conclusively related to a density effect. Results of this study are still preliminary.

				ELI	<u>SA</u>	
Brood Year	Date	Density	N	x'	S <sup>2</sup> ~	S
1989	12/90	Low	16	0.071	0.000	0.002
1909	12/20	Med	10	0.072	0.000	0.003
		High	5	0.072*	0.000	0.002
	1/91	Low	22	0.073	0.000	0.004
		Med	6	0.071	0.000	0.002
		High	5	0.076	0.000	0.004
	2/91	Low	18	0.076 <sup>•,b</sup>	0.000	0.004
		Med	30	0.080 <sup>b</sup>	0.000	0.007
		High	30	0.075	0.000	0.006
	3/91	Low	30	0.129	0.078	0.280
		Med	30	0.061	0.000	0.003
		High	30	0.079 <sup>b</sup>	0.008	0.089
	4/91	Low	30	0.061	0.000	0.004
		Med	30	0.136 <sup>b</sup>	0.100	0.316
		High	30	0.054*	0.000	0.007
1990	11/91	Low	15	0.092*	0.000	0.011
1770		Med	13	0.103	0.002	0.048
		High	5	0.550 <sup>b</sup>	0.673	0.820
	12/91	Low	15	0.095	0.000	0.016
		Med	15	0.085	0.000	0.004
		High	17	0.659	0.602	0.776
	1/92	Low	28	0.085	0.000	0.004
		Med	30	0.086*	0.000	0.006
		High	27	0.233 <sup>b</sup>	0.198	0.445
	2/92	Low	18	0.090	0.000	0.005
		Med	30	0.091	0.000	0.013
		High	27	0.560 <sup>b</sup>	0.524	0.724
	3/92	Low	30	0.092 <sup>b</sup>	0.000	0.010
	2.2.2	Med	29	0.171*	0.019	0.138
		High	30	0.245*	0.229	0.479
	4/92	Low	30	0.102*	0.002	0.050
		Med	29	0.087*	0.000	0.012
		High	27	0.306	0.251	0.501

Table 2. Mean, variance, and standard deviation of ELISA for spring chinook salmon at Dworshak NFH reared at three different densities.

<sup>1</sup>Mean ELISA values with different letters are significantly different at the .05 level.

# THE EFFECTS OF THREE REARING DENSITIES ON THE MIGRATION TIME AND SURVIVAL OF SPRING CHINOOK SALMON RELEASED AT DWORSHAK NFH, IDAHO - A PROGRESS REPORT 1993

#### Ray N. Jones Idaho Fishery Resource Office U.S. Fish and Wildlife Service Ahsahka, Idaho

#### INTRODUCTION

In an effort to produce the healthiest, most viable smolts possible, the Idaho Fishery Resource Office (FRO) and Dworshak National Fish Hatchery (NFH) developed a project to evaluate the effect of rearing density on hatchery performance, post-release performance, and adult returns of spring chinook salmon reared and releases at Dworshak NFH in Idaho.

The objectives were to compare the growth, health, mortality, migration rate, smolt survival, and smolt to adult survival rates between three different rearing densities.

In this summary, we present some of the results of smolt migration and survival after release. Data on growth, health, and mortatlity during hatchery rearing is presented in the accompanying companion summary. These data are preliminary and are subject to change.

#### SITE DESCRIPTION

Dworshak NFH is located at the confluence of the North Fork and the main-stem of the Clearwater River near Ahsahka, Idaho. Construction of the hatchery was included in the authorization for Dworshak Dam and Reservoir (Public Law 87-847, October 23, 1962) to mitigate for losses of anadromous steelhead (*Oncorhynchus mykiss*) caused by the dam and reservoir.

The hatchery was designed and constructed by the U.S. Army Corps of Engineers (COE) and has been administered and operated by the U.S. Fish and Wildlife Service (USFWS) since the first phase of construction was completed in 1969.

Additional construction was completed in 1982 under the Lower Snake River Compensation Plan to provide rearing facilities for spring chinook salmon (*O. tshawytscha*). A total of 30 8-foot by 80-foot raceways were constructed. Dworshak NFH was originally designed to produce 1.4 million spring chinook salmon smolts (70,000 lbs @ 20 fish/lb) annually.

# METHODS AND MATERIALS

Three rearing densities were tested: 1) low density or 1/3 normal loading at 15,000 fish per raceway (2) medium density or 2/3 normal loading at 30,000 fish per raceway (3) and high density or normal loading at 45,000 fish per raceway. A total of nine raceways were set up at low density, six raceways at medium density, and three raceways at high density.

About 1500 BY89 fish and about 600 BY90 fish in each of the three density groups were marked with PIT tags to determine migration time and survival to Lower Granite Dam after release.

Mean migration times to Lower Granite, Little Goose, and McNary dams were compared between density groups for both years using ANOVA. For those cases where significant differences were observed, post-hoc pair-wise differences in means were compared using the Bonferroni procedure. A two-sample T-Test was used to compare migration times between years for each density group. A significance level of .05 was used to reject the null hypotheses.

Survival of smolts to Lower Granite Dam was estimated by using PIT-tag interrogation rates. Interrogation rates at Lower Granite, Little Goose, and McNary dams were accumulated and used as a minimum estimate of survival to Lower Granite Dam. A chi-square test for goodness-of-fit was used to compare interrogation rates between density groups for both years. Comparisons were made for each interrogation facility separately and for the cumulative rate at all three facilities. A significance level of 0.05 was used to reject the null hypotheses.

#### RESULTS

Migration time for all three density groups from Dworshak NFH to the IDFG smolt trap at the mouth of the Clearwater River was about one day (55 km/day) for both BY89 and BY90. Migration times increased significantly between the Clearwater River smolt trap and Lower Granite Dam, the first mainstem PIT-tag interrogation facility on the Lower Snake River. For both BY89 and BY90, migration times were longer for the low density groups. The high density groups had the shorter migration times to each interrogation facility both years (Figure 1).

The number and percent of PIT-tagged fish in each density group that were interrogated at each dam in 1991 and 1992 are listed in Tables 1 and 2, respectively. For BY89, interrogation rates at individual dams and cumulative interrogation rates did not vary significantly between density groups. Overall, the medium density group had the fewest interrogations. The total number of PIT-tags interrogated ranged from 52-49 percent. The null hypothesis could not be rejected for the BY89 smolt release.

In contrast, interrogation rates for BY90 releases differed noticeably from BY89 releases. The chi-square tests for goodness-of-fit resulted in the null hypotheses being rejected in all cases but one. Interrogation rates were significantly (P<.05) different between density groups at each interrogation facility individually (except at Little Goose Dam) and cumulatively. For all tests where the null hypothesis was rejected, the low and medium density groups had observed values consistently higher than expected while the high density group had observed values consistently lower than expected. The low density group had the highest rate of interrogation, 57%, followed by the medium density group, 53%, and the high density group, 39%. Compared to BY89, the low density group had a higher interrogation rate for BY90 and the high density group had a lower interrogation rate. If comparisons are made between years for only low and medium density groups, cumulative interrogations for BY89 and BY90 were 50.3 and 55.0, respectively.

#### DISCUSSION

In general, the estimated post-release survival rate was higher for BY90 smolts compared to BY89 smolts. However, comparing interrogation rates of the various density groups between years is complicated by several factors. First, flows into Lower Granite pool were higher in 1992 than in 1991 during a significant portion of the smolt migration period (Figure 2). Second, BY90

smolts were larger than BY89 smolts, 16 fish per pound vs. 21 fish per pound, respectively. With higher flows and larger fish, it would be reasonable to expect a higher survival rate. For BY90 low and medium density groups, this was the case. Survival rates to Lower Granite Dam were higher for BY90 compared to BY89. The only exception was the high density group for BY90.

Based on smolt interrogation rates, it does not appear that the rearing densities we tested had an effect on survival. Even though we observed significantly different interrogation rates between density groups for the BY90 releases, it appears that the difference was due primarily to the low interrogation rate for the high density group. The pattern of smolt survival for BY90 after release was very similar to the pattern of fish performance we observed during hatchery rearing (see companion summary on hatchery growth, health, and mortality). The same factors that effected fish performance during hatchery rearing seemed to carry over and affect fish performance after release.

#### CONCLUSIONS

The data do not consistently demonstrate any significant difference in post-release performance of spring chinook salmon reared at the densities tested. Differences that were observed could not be conclusively related to a density effect. Results of this study are still preliminary and subject to change.

		١		1		,							1				1			
d from	(m)															×.				
release	n released Goods (C		JAL	%	49	57	50	49	48	49	51	50	55		52	49	52			
k salmon	), Little (		TOTAL	No.	243	287	181	243	240	247	253	249	277		782	730	611			
g chinoo	g cninook te (GRJ),		Cl	%	9	9	9	<b>5</b>	9	5	9	5	7		9	5	9			
89 spring ver Grani		MCJ	No.	29	29	31	26	31	23	28	25	37		89	80	60				
gged BY	Number (No.) and Percentage (%) of PIT-tagged BY89 spring chinook salmon released nom Dworshak NFH in 1991 that were interrogated at Lower Granite (GRJ), Little Goods (GOJ), and McNary (MCJ) dams.		ſ	ſ	ſ	%	14	17	16	11	12	14	14	16	14		16	12	15	
of PIT-ta			GOJ	No.	70	85	78	56	61	68	71	81	71		233	185	223			
ge (%) (			J	%	29	35	29	32	30	31	31	29	34		31	31	31			
ercenta	91 that	lams.	GRU	No.	144	173	143	161	148	156	154	143	169		460	465	466			
No.) and P	No.) and Pe NFH in 199 rv (MCJ) da	IcNary (MCJ) dams.	Number	Released	500	501	502	501	501	500	500	500	502		1503	1502	1502			
Number (	Dworshak	and McNa		Density	Low	WO.1	WO. ]	Med	Med	Med	Hioh	Hioh	High	b	Inw	Med	High			
Table 1.				Raceway	L L	- 00	ο σ	17	13	14	PC	74	D7		TOTAL	TOTAL	TOTAL			

•

d from 11/0

Number (No.) and Percentage (%) of PIT-tagged spring chinook salmon released from Dworshak NFH in 1992 that were interrogated at Lower Granite (GRJ), Little Goose (GOJ), and McNary (MCJ) dams. Table 2.

	AL	%	57	59	57	59	50	50	40	36	42	57	53	39	
	TOTAL	No.	113	118	113	117	66	100	80	71	83	344	316	234	
	1	%	10	10	7	10	9	80	7	4	7	6	∞	4	
	MCJ	No.	20	19	14	19	12	16	ę	80	13	53	47	24	
	ſ	%	13	11	13	13	11	15	7	12	10	12	13	6	
	GOJ	No.	26	21	26	26	21	29	13	23	19	73	76	55	
	5	%	34	39	37	36	33	28	32	20	26	36	32	26	
dams.	GRJ	No.	67	78	73	72	66	55	64	40	51	218	193	155	
and McINary (MUJ) dams.	Number	Released No.	200	200	200	200	200	200	200	200	200	600	600	600	
and McN	8	Density <sup>1</sup>	Low	Low	Low	Med	Med	Med	High	High	High	Low	Med	High	
		Raceway	7	80	6	10	11	12	26	29	30	TOTAL	TOTAL	TOTAL	

.



Migration Time (Days)

Figure 1. Mean migration times (days) from Dworshak NFH to Lower Granite, Little Goose, and McNary Dams for BY89 and BY90 spring chinook salmon.



Figure 2. Mean daily inflow (KCFS) into Lower Granite Reservoir during April, May, and June, 1991 and 1992.

WEAN DAILY INFLOW (KCFS)

# Presentation to the Northwest Fish Culture Conference Sunriver, Oregon December 7, 1994

by

Integrated Hatchery Operation Team W. H. "Chris" Christianson, CBFWA Fisheries Resource Coordinator

# Policies and Procedures for Columbia Basin Anadromous Salmonid Hatcheries

# Introduction

There are over 90 hatchery facilities in the Columbia River Basin currently used to produce salmon and steelhead. These facilities are funded, co-managed, and operated by several different entities for many different purposes. Most of these hatcheries were originally authorized and built to mitigate for fish habitat losses caused by construction and operation of dams and other water projects. Today, these facilities produce fish for many different management objectives, including supplementation, restoration, harvest, egg banking, and research.

Because hatcheries are operated by several entities and for different management purposes, these facilities have often used different operating guidelines. The need to improve the coordination and operation of these facilities was formally recognized in the Northwest Power Planning Council's *Strategy for Salmon*. The salmon strategy is a regional effort to double Columbia River Basin adult salmon populations without losing biological diversity.

As part of the salmon strategy, the Council called for the creation of an Integrated Hatchery Operations Team (IHOT). This multi-agency group was given several duties related to hatchery operations. In particular, IHOT was asked to develop regionally integrated hatchery policies for operating all Columbia Basin anadromous salmonid hatcheries.

One of the key products from this IHOT effort is a new manual entitled *Policies and Procedures for Columbia Basin Anadromous Salmonid Hatcheries.* This manual outlines a series of policies designed to both improve hatchery practices and make them consistent throughout the Columbia Basin. Its purpose is to help ensure that hatchery operations

will be consistent with the regional goal of rebuilding wild and naturally spawning fish runs.

The policy and procedures manual is organized into chapters that address the following policy categories identified by the Northwest Power Planning Council:

- Hatchery coordination
- Hatchery performance standards
- Fish health
- Ecological interactions
- Genetics

Within each policy chapter, IHOT identifies a policy statement and goals, performance standards and performance measures. The *policy statements* and *goals* reflects an overall policy direction that IHOT has agreed to pursue in operating the region's fish hatcheries. The actual procedures and guidelines that will be used to operate the hatchery facilities are identified as *performance standards*. The *performance measures* describe the types of information that will be used to evaluate the hatchery's compliance with the standards. Most chapters conclude with an *implementation plan* detailing actions that will be used to implement the individual policies and procedures.

The hatchery policies only provide guidance for the technical operation of hatcheries. They are not intended to include setting specific production priorities. These priorities are developed by the fisheries co-managers to meet specific fish management objectives, such as mixed-stock or terminal-area fisheries, supplementation of weak stocks, or in-river recreational or net fisheries. Production priorities may also be established to meet rebuilding schedules as called for in the Council's *Strategy for Salmon* or Endangered Species Act recovery plans. The production decisions must be provided by fishery comanagers through a comprehensive plan that addresses both natural and hatchery production. These plans are developed through negotiations, primarily under the Columbia River Fish Management Plan's Production Advisory Committee (PAC).

What follows is a summary of key elements from the policies and procedures manual. This summary includes the policy statements and goals, as well as an overview of the performance standards and measures.

# **Regional Hatchery Coordination Policy**

Basinwide resource needs can be most effectively addressed when hatchery operations are coordinated throughout the region. This coordination can be within an individual agency or between several agencies and co-managers. Coordination can also be used at different levels to meet various organizational needs. For example, staffing or equipment needs can be coordinated to meet a common goal. Coordination can also occur at the programmatic or administrative levels to achieve broader regional goals.

# **Policy Statement**

It shall be the policy of the management entities of the anadromous salmonid resources in the Columbia Basin to coordinate the operation of fish hatchery programs to meet basinwide resource management needs.

## Goals

- 1. Coordinate the operation of salmonid hatchery programs to meet basinwide resource management goals and objectives.
- 2. Develop administrative agreements for improved sharing of facilities, manpower, equipment and/or supplies to meet basinwide management program goals and objectives.
- 3. Foster open and frequent communication between managing entities to coordinate and jointly resolve technical issues relating to artificial production.
- 4. Operate hatchery programs in compliance with regionally adopted hatchery performance standards, fish health, ecological interactions, and genetics policies.

## **Performance Standards**

The performance standards in this section reflect the need for both operational and programmatic coordination. On an operational level, coordination involves establishing a common forum to share information, facilities, manpower and equipment. The programmatic coordination includes a review of hatchery operations to meet goals expressed in legal agreements, hatchery operational plans, regional policies, and agency/tribal programs. The performance standards also call for regular IHOT meetings to discuss programmatic and administrative matters, including the hatchery audit reviews.

#### Performance Measures

Several reports and operational procedures will be needed to measure the effectiveness of the regional hatchery coordination standards.

- 1. Within one year of ratification, IHOT will create a memorandum of understanding between members for the sharing of facilities, manpower, and equipment.
- 2. IHOT will prepare an annual report that details shared resources.
- 3. The IHOT facilitator will serve as a central distribution point for reports pertaining to fish hatchery operations.
- 4. Meetings of the full IHOT membership will occur regularly to discuss programmatic and administrative matters. The meeting schedule will be established by IHOT chairperson.
- 5. The IHOT facilitator will maintain an electronic bulletin board for sharing the future and current brood documents, and for the free distribution of information among members.
- 6. The IHOT facilitator will provide the means for timely reporting of fish escapement, transfer, and release goals and the progress being made to meet the objectives. The coordination of personnel and equipment sharing will be recorded and records maintained through the IHOT facilitator.
- 7. Agencies will update hatchery operation plans yearly.
- 8. Co-managers that operate fish rearing facilities will adopt common formats for reports developed within the basin (e.g., Annual Brood Planning Report, U.S. v. Oregon, etc.).
- 9. IHOT members will present a yearly report of all fish culture research proposed, in progress, or completed.
- 10. IHOT members will present annual updates of current fish survival information.

# Hatchery Performance Standards Policy

Hatchery operations must be consistent with fishery management goals as well as the regional polices established for hatchery coordination, fish health, ecological interactions, and genetics. This requires a review of each hatchery's purpose, goals, and objectives in light of these regional policies. It also requires a process for monitoring and evaluating hatchery compliance with the regional standards.

# **Policy Statement**

It shall be the policy of the management entities of the anadromous salmonid resources in the Columbia Basin to ensure that all hatchery practices are based on regional standards.

# Goals

- 1. All fish produced and released are consistent with management goals.
- 2. Physical facilities and equipment are operated consistent with standards to maximize fish quality.
- 3. Ensure compliance with hatchery coordination, fish health, ecological interactions, and genetics policies.
- 4. Ensure the use of an audit framework to evaluate the compliance of hatchery operations with regional standards.

# **Performance Standards**

This section addresses performance standards in three categories: program objectives, facility requirements, and hatchery operations. Performance standards for program objectives reflect the need to ensure that hatchery production programs meet the specific and collective requirements of existing programs and statutes.

Under the category of facility requirements, the performance standards address the physical components that play an important role in hatchery operations. These include water quality; adult collection, holding and spawning facilities; incubation, rearing and release facilities; predator control measures; and food storage and quality control. Performance standards for hatchery operations address major hatchery activities, such fish rearing, maintenance of fish health, fish transportation, and staff training.

#### **Performance Measures**

Compliance with hatchery performance standards will be measured through an independent hatchery audit. This audit will compare the hatchery's performance against standards specified in the policies and procedures manual or those identified in the hatchery's operational plan.

# Fish Health Policy

Fishery resources must be protected from the adverse effects of disease. Fish populations, whether cultured or free-swimming, are exposed to bacteria and viruses. Under certain conditions, these pathogens can cause disease outbreaks that lead to fish mortality. This can ultimately result in a significant impact on the fishery resource. Consequently, it is important that managers of a watershed, river, or hatchery facility be constantly aware of potential disease problems.

#### **Policy Statement**

It shall be the policy of the management entities of the anadromous salmonid resources in the Columbia Basin to protect those resources by restricting the importation, dissemination, and amplification of pathogens and diseases known to adversely affect fish.

# Goals

- 1. Strive to produce healthy fish for release or transfer.
- 2. Ensure that all fish produced are under a specific fish health management program.
- 3. Monitor and evaluate the health of wild, natural, and cultured fish populations.
- 4. Foster open and frequent communications among managing entities to jointly resolve fish health related issues.

# **Performance** Standards

Health care standards must be followed in order to prevent the introduction or spread of fish diseases. These standards include hatchery monitoring visits by fish health specialists; a fish health inspection program; hatchery sanitation procedures; water quality parameters; general cultural practices; and egg/fish transfer and release requirements.

# **Performance Measures**

Compliance can be monitored by answering the following questions:

- 1. Are monthly hatchery monitoring visits conducted by a qualified fish health specialist?
- 2. Are annual broodstock inspections conducted for *Renibacterium* salmoninarum and reportable viral pathogens?
- 3. Is the hatchery following accepted sanitation procedures?
- 4. Are water quality parameters outlined in the Hatchery Performance Standards Policy being followed?
- 5. Are rearing standards outlined in the Hatchery Performance Standards Policy being followed?
- 6. Are egg and fish transfer/release requirements met?
- 7. Is there a fish health monitoring and evaluation program in place?

# **Ecological Interactions Policy**

Hatchery facilities and programs should avoid adverse interactions between wild, natural, and hatchery fish populations. These interactions could involve predation, or competition for food and habitat. Hatcheries should also maximize the post-release survival of hatchery fish by using appropriate rearing and release strategies.

#### **Policy Statement**

It shall be the policy of the management entities of the anadromous salmonid resources in the Columbia Basin that artificial propagation programs will be designed and implemented to minimize ecological interactions that adversely affect the productivity of aquatic ecosystems.

#### Goals

- 1. Ensure that all fishes produced and released are under a specific management program.
- 2. Consider the ecological effects attributable to the specific hatchery products following release.
- 3. Consider how specific release strategies affect aquatic ecosystems.
- 4. Monitor and evaluate implementation of ecological interactions guidelines and ecological effects of artificially propagated fish on wild, natural, and cultured fish populations.
- 5. Foster open and frequent communications among managing entities to jointly resolve related issues.

# **Performance Standards**

Performance standards will vary between hatcheries, depending upon each hatchery's unique program objectives. Consequently, all existing hatchery programs will need to be reviewed in light of the policy statement and goals previously identified. Hatchery operations that can influence ecological interactions include (1) the location of fish releases, (2) fish size and age at release, (3) release density, (4) imprinting strategies, and (5) hatchery rearing conditions. Achieving the policy goals may require operational changes to hatchery programs, as well as structural changes to hatchery facilities.

# **Performance Measures**

The performance measures outlined in this section address operational procedures that the hatchery directly controls, while also reflecting the importance of meeting management plan goals. The performance standards are measured by answering the following questions:

- 1. Is the hatchery's program outlined in a subbasin management plan (e.g., Umatilla Basin Artificial Production Plan or Lower Snake River Compensation Plan)?
- 2. Is the hatchery operating under a current hatchery operational plan?
- 3. Is a hatchery monitoring and evaluation plan in place?
- 4. Does the hatchery program meet requirements established in the regional hatchery policies and subbasin planning documents in the following areas: species, stock, broodstock collection location, broodstock numbers, broodstock collection strategy, and spawning and egg-take protocols?
- 5. Does the hatchery's performance meet requirements outlined in the regional hatchery policies and in subbasin and hatchery plans for the following areas: percent smoltification, rearing density, disease condition, and the number, size, date(s), and location at release?
- 6. Are fish reared in the subbasin or acclimated in the subbasin?
- 7. Is the release strategy appropriate for the program?

# **Genetics Policy**

Maintaining genetic diversity in fishery populations is important for the conservation of existing genetic traits needed for long-term sustainability. Therefore, hatchery facilities should use operational procedures that avoid adverse genetic effects on wild, natural and hatchery fish populations.

# **Policy Statement**

It shall be the policy of the management entities of the anadromous salmonid resources in the Columbia Basin to operate artificial propagation programs that maintain adequate genetic variation and fitness in populations and protect the biological diversity of wild, natural, and cultured anadromous salmonid populations.

## Goals

- 1. All fish produced and released meet identified management objectives for specific artificial production programs and follow genetic guidelines.
- 2. Monitor and evaluate implementation of genetic guidelines and genetic effects of artificially propagated fish on wild, natural, and cultured populations.
- 3. Foster open and frequent communications among managing entities to jointly resolve related issues.

## **Performance Standards**

Genetic performance standards are designed to protect the capacity of a fish population to evolve, and thus persist in the face of environmental variability. Hatchery operations that can affect genetic diversity include (1) donor stock selection, (2) adult collection procedures, and (3) spawning strategies. The guidelines in this section address broodstock collection and spawning practices.

# **Performance Measures**

Compliance with genetics performance standards can be monitored by answering the following questions:

- 1. For new programs, has a broodstock collection plan been developed?
- 2. For new programs, was the donor selection outline followed in selecting the hatchery broodstock?
- 3. For existing programs, were broodstock collection procedures followed?
- 4. Were the appropriate number of spawners, male/female ratios, and fertilization protocols used?
- 5. Is there a genetic monitoring and evaluation program in place?

# **IHOT Members**

IHOT is comprised of representatives from the fisheries co-managers and cooperating entities listed below.

# Fisheries Co-Managers

Confederated Tribes of the Colville Reservation Confederated Tribes of the Umatilla Indian Reservation Confederated Tribes of the Warm Springs Reservation of Oregon Confederated Tribes and Bands of the Yakama Indian Nation Idaho Department of Fish and Game National Marine Fisheries Service Nez Perce Tribe of Idaho Oregon Department of Fish and Wildlife Shoshone-Bannock Tribes of Fort Hall U.S. Fish and Wildlife Service Washington Department of Fish and Wildlife

<u>Cooperating Entities</u> Bonneville Power Administration Mid-Columbia Public Utility Districts U.S. Army Corps of Engineers Northwest Power Planning Council Pacific Northwest Utilities Conference Committee Columbia River Inter-Tribal Fish Commission Columbia Basin Fish and Wildlife Authority

#### UMATILLA HATCHERY OPERATIONS OVERVIEW

#### Jack Hurst Umatilla Hatchery Manager Oregon Department of Fish and Wildlife

The Umatilla River is one of the most severely impacted tributaries of the Columbia River concerning fish populations. Since the early 1900's, irrigation development has turned local areas into highly productive agricultural properties, while eliminating stream flows needed for survival. Spring and fall chinook, coho and summer steelhead populations declined to the elimination of all except a remnant summer steelhead run. Continued operation of poorly constructed unscreened water diversions, habitat loss and hydroelectric operation in the mainstream Columbia, further prevented rebuilding of these stocks.

A plan was developed in 1986 to comprehensively cover the issues needed to address the reestablishment and rebuilding of salmon runs in the Umatilla Basin. This plan was prepared by the Oregon Department of Fish and Wildlife, in cooperation with the Confederated Tribes of the Umatilla Indian Reservation, National Marine Fishery Service, U.S. Fish and Wildlife Service, Bureau of Reclamation, and Forest Service. The plan developed became part of the Columbia River strategies, as set by the N.W. Power Planning Council, to double the fish runs within the basin.

#### INTRODUCTION:

A Umatilla Hatchery master plan was developed by Oregon Fish and Wildlife in cooperation with BPA and the Confederated Tribes of the Umatilla Indian Reservation. This plan addresses the production profile of the facility as well as design criteria. Along with this, are guidelines of operation based on fish health monitoring, research and evaluation of production and genetics and stock composition.

Umatilla Hatchery was originally designed to produce 290,000 pounds of fish. Design and engineering work for the facility and water supply was completed by the U.S. Army Corps of Engineers. The site chosen for the facility is adjacent to the Irrigon Fish hatchery, near Irrigon, Oregon. This site was chosen for the potential of adequate pathogen free well water, with temperatures ranging from 51 - 61° F. and for the close proximity in which to share manpower with Irrigon Hatchery.

Umatilla Hatchery is unique, in that twenty-four of the thirtyfour ponds are designed after Michigan style oxygen supplementation rearing units. The remaining ten ponds are designed as standard 20'x100' ponds. Michigan ponds are designed at roughly half the area of a standard or "Oregon" pond. They also have nine baffles evenly spaced for each pond, to facilitate self cleaning. Each Michigan pond also has two oxygen contactor columns, supplied with water via one 950 g.p.m. submersible pump. Each pond also has its own waste removal sump, complete with a gravity fed vacuum line.

Both pond types are of reuse configuration. Michigan ponds are in a three pond array, and Oregon ponds are at two ponds per pass. Michigan ponds are designed to rear chinook at three times the density of standard ponds, and summer steelhead at five times standard density. This is accomplished by oxygen supplementation and pond turn-over rates of four times per hour.

Due to the design of the Hatchery, questions need to be answered as to the effectiveness of Michigan rearing v.s. standard pond rearing. Currently, monitoring and evaluation of hatchery practices is ongoing, concerning differences in rearing performance of Michigan and Oregon ponds, including a vigorous tagging and marking program of all groups reared (100%). Additionally, a comprehensive and systematic fish health monitoring plan has been developed to monitor brood fish and monthly and preliberation exams. Every effort has been made to protect the genetic diversity of Umatilla wild steelhead, and consistent stocks of spring and fall chinook are being reintroduced. All chinook egg sources in the future will be from the Umatilla River, as runs are established. Currently, all summer steelhead are produced from Umatilla stock.

Initial production strategies for Umatilla Hatchery called for both subyearling and yearling spring chinook, fall chinook subyearlings, and yearling summer steelhead. One rearing uncertainty existed concerning rearing of spring chinook yearlings and, due to the warm (51-61 degree F.) well water available, it was felt that incubation temperatures would need to be reduced to delay ponding of this group. The shortened rearing time would enable us to meet release size resembling a standard sixteen month smolt.

Umatilla design called for and provided four incubation water chillers, three of which provide one hundred g.p.m. each of forty-five degree water, and one that provides sixty g.p.m. of thirty-eight degree water. These chillers enable us to not only prolong incubation, but to bring up to eight weeks of egg takes together, during their development.

Engineering design and review was completed in 1989, and construction was started in April 1990. The U.S. Army Corps of Engineers were contracted through Bonneville Power Administration for all design and construction phases. The BPA was the funding source for design and construction of Umatilla Hatchery, and is contracted with the Oregon Department of Fish and Wildlife for all funding of operation and maintenance of the facility. Fish production began in October 1991, with the arrival of spring chinook eggs for incubation. During this time, testing of well water supplies showed a total of 7,700 g.p.m. that was available. As 15,000 g.p.m. was needed to produce 290,000 pounds of fish annually, our program was reduced and diversified to produce 176,000 pounds.

Fish production is reviewed yearly, and an annual operation plan is developed through the cooperation of Oregon Department of Fish and Wildlife, Confederated Tribes of the Umatilla Indian Reservation, and the Bonneville Power Administration. Umatilla Hatchery is funded for seven full time employees and for two seasonal employees. One Fish and Wildlife Manager III oversees operations, and one Trades Maintenance Coordinator oversees maintenance for Umatilla and Irrigon Hatcheries.

# UMATILLA HATCHERY REDUCED PRODUCTION (7,700 g.p.m.) 1993-1994

Summer Steelhead	150,000
Fall Chinook Subyearlings 2	,682,000
Spring Chinook Fall Release	492,000
Spring Chinook Subyearlings	720,000
Spring Chinook Spring Release Yearlings	210,000
UMATILLA HATCHERY FULL PRODUCTION (15,000	g.p.m.)
Summer Steelhead	010 000

Cummon Charles

Summer Steelhead	210,000
Fall Chinook Subyearlings	5,940,000
Spring Chinook Subyearlings	1,000,000
Spring Chinook Yearlings	210,000

#### UMATILLA HATCHERY PERSONNEL

Ray Hill	Fish and Wildlife Manager 3 (Irrigon)
Jack Hurst	Fish and Wildlife Manager 1
Randy Winters	Fish and Wildlife Tech-2
	Fish and Wildlife Tech-1
Steve Banghart	Fish and Wildlife Tech-1
Cliff Miller	Fish and Wildlife Tech-1
Jennifer Mesteth	Fish and Wildlife Tech-1
Gary Huser	Fish and Wildlife Tech-1 Seasonal
Bob Gallup	Trades/Maintenance Coordinator (Irrigon)
Wesley Cone	Maintenance Worker 2
Michael Pearsall	Maintenance Worker 1 Seasonal

## SESSION III

## FISH HEALTH MANAGEMENT/NUTRITION

- Progress Report on Investigation of New Animal Drugs in the Western States - Jim Warren, Columbia Basin Fish and Wildlife Authority
- Lack of Evidence for the Horizontal Transmission of *Renibacterium* salmoninarum, the Causative Agent of Bacterial Kidney Disease, Among Raceways of Juvenile Spring Chinook Salmon Reared at Umatilla Hatchery - Warren Groberg, Oregon Department of Fish and Wildlife
- Treatment Of Bacterial Gill Disease With Hydrogen Peroxide Steve Roberts, Washington Department of Fish and Wildlife
- Evaluation of Formalin and Hydrogen Peroxide in a Production Setting for the Management of Fungal Infections in Eggs of Chinook Salmon -Paul Waterstrat, U.S. Fish and Wildlife Service
- Coho Anemia Disease (CAD) Rich Holt, Oregon Department of Fish and Wildlife

# **PROGRESS REPORT ON WESTERN PROJECT INADS**

The Western Regional INAD Project (Western Project) was created in November 1993 to sponsor and manage clinical field trials on three therapeutic compounds under U. S. Food and Drug Administration (FDA) Investigational New Animal Drug (INAD) exemption permits. These studies may aid in the registration of Chloramine-T and in the approval of new uses of Formalin and Oxytetracycline. Initial participants include 234 facilities operated by six state conservation agencies, three tribal groups, and ten private fish producers in Alaska, California, Idaho, Montana, Oregon and Washington.

The Western Project established a Board of Directors consisting of representatives from each of the 18 participating entities. On September 23, 1994 the Board met in Juneau, Alaska and approved Bylaws for the Western Project and revised the Cooperative Agreement on the responsibilities of the parties involved. The Board also approved an "open season" from October 1 to December 31, 1994 for applications to continue current enrollments or to enroll new facilities. Facilities currently enrolled will be assessed a 1995 calendar year fee of \$375 for each INAD compound needed at each facility. A one-time new facility fee of \$200 (same as 1994) will again be charged for each facility enrolling in the Western Project for the first time in 1995.

FDA approval was received on 10/18 for an INAD exemption for Chloramine-T (INAD 9321 - for external bacterial infections at 119 facilities). Applications are still pending for Formalin (INAD 9346 - for label expansion to cover its use as a fungicide for fish/eggs at 91 sites) and oxytetracycline feed additive (INAD 9332 - for label expansion to cover additional bacterial diseases, not now covered by the current label, at 191 facilities). Clinical trials on these compounds will help to control disease losses among more than 1.3 *billion* salmon and trout at participating Western Project facilities. Salmon and steelhead trout hatcheries rear and release young fish (smolts), usually too small and/or illegal for harvest, that must be healthy enough to survive migration to the ocean. After one to five years of ocean life survivors are harvested or return to the facility to spawn. Private fish producers in California, Idaho, and Washington use INAD compounds to protect millions of pounds of trout and some Atlantic salmon produced for processing and shipment for human consumption around the world. Other participants in the Western Project include trout egg producers and producers of other fish species such as sturgeon, bass, walleye, and perch.

Individual clinical field trials are guided by broad INAD protocols with several specific study objectives. Drug and chemical accountability and data collection will be facilitated by the use of standard forms and specific reporting procedures. These studies should provide many opportunities for the conduct of productive trials, especially where recurring disease problems enable convenient research involvement in study design and in the coordination of sampling schedules and data collection. All data will be shared publicly with researchers nation-wide. In addition, the Western Project is aggressively seeking the ways and means to accelerate and broaden critically needed New Animal Drug Application (NADA) research that meets stringent FDA drug registration and labeling requirements. To do this, a strategy is being developed to link together clinical field work with the research community and arrange the funding necessary to complete NADA research data submissions at the earliest time possible.

For further information contact Jim Warren, Western Regional INAD Coordinator, at 503/326-7031.

Lack of Evidence for Horizontal Transmission of *Renibacterium salmoninarum,* the Causative Agent of Bacterial Kidney Disease, Among Raceways of Juvenile Spring Chinook Salmon Reared at Umatilla Hatchery

W. J. Groberg, Jr., N. L. Hurtado, S. T. Onjukka and K. Waln

Oregon Department of Fish and Wildlife ODFW Fish Pathology Laboratory Badgley Hall Eastern Oregon State College La Grande, OR 97850

#### Summary

Constructed and operated under funding by the Bonneville Power Administration, Umatilla Hatchery uses two very different raceway designs to rear juvenile salmonids. One design uses two raceways in tandem with the second raceway supplied by reuse water from the first raceway. These are called Oregon raceways and fish are reared at conventional densities (i.e. 1 lb./cu ft). The second design uses three raceways in tandem with reuse water in the second and third raceways. All three raceways are supplemented with oxygen at the influent of each raceway. These are called Michigan raceways and the rearing density is three times that in Oregon raceways. Replicates of each raceway design are being used to evaluate the performance of fish reared in Michigan raceways compared to those reared in Oregon raceways. A comprehensive and systematic fish health monitoring program is part of the hatchery evaluation for Umatilla. This is important because infectious and parasitic diseases might be expected to be more severe in lower raceways on reuse water.

The fish health project for Umatilla includes examinations of a subsample of the brood fish for culturable viruses, particularly infectious hematopoietic necrosis virus, which is thought be vertically transmitted under certain conditions; for *Renibacterium salmoninarum* (Rs), the causative agent of bacterial kidney disease (BKD), which is known to be vertically transmitted; and for erythrocytic inclusion body syndrome, a viral infection, for which it is unknown if vertical transmission occurs. Juveniles are monitored monthly throughout rearing for the agents above as well as for systemic and gill bacteria, and for fungal and parasite infestations. A final preliberation examination is done on 30 grab-sampled fish per raceway for the same agents as adults are examined for.

Examinations of the 92 brood year adult spring chinook at Carson National Fish Hatchery, the source of spring chinook eggs for Umatilla Hatchery, revealed high levels and prevalences of Rs antigen as measured by the enzyme-linked immunosorbent assay (ELISA). This translated into significant losses to BKD throughout the rearing cycle at Umatilla. Losses were particularly severe in populations reared as subyearlings for release in November. These were ponded in one Oregon raceways in April, then split to two Oregon raceways in June, then to four Oregon raceways in July, and finally split among four Oregon and six Michigan raceways in August. The cumulative mortality from ponding in April until their release in November was 14.2%. Water temperatures during this period ranged from 10 to 15<sup>o</sup>C. The average

cumulative mortality in four Oregon raceways from August through their release in November was 1.87%. During the same period fish in six Michigan raceways averaged a 3.22% cumulative mortality. These differences in cumulative mortality between Oregon and Michigan raceways are significant (p = .001). There were no significant differences in mortality rates among the four Oregon raceways nor among the six Michigan raceways (p > .05).

The ELISA results from 30 grab-sampled fish from each of the four Oregon and six Michigan raceways one week prior to their release into the Umatilla River revealed no significant differences in the prevalences and levels of Rs antigen among all raceways, with one exception (comparisons of the mean ELISA values were made using an independent t test). Statistically significant differences were found between one upper Oregon raceway and one lower Michigan raceway. Significantly, fish in the two lower Michigan raceways receiving second pass water from the middle and upper raceway of the series showed no higher levels or prevalences of Rs antigen when compared to the middle and upper Michigan raceways. The ELISA and the cumulative mortality data thus do not indicate that fish in the lower Michigan raceways had increased infection with Rs as a result of horizontal transmission from the middle and upper raceways.

Several possibilities have been considered to explain this. 1) The degree of vertical transmission of Rs from maternal parents to their progeny overwhelmed any effects that horizontal transmission may have had. This is plausible considering the high infection levels and prevalences in the brood fish. If so, this would still suggest that horizontal transmission was minimal. 2) Horizontal transmission resulted in infection of the more Rs susceptible fish while the were together in Oregon raceways and minimal horizontal transmission occurred later among Michigan raceways. During April and May the entire population was in a single Oregon raceway, during June they were in two Oregon raceways, and during July they were in four Oregon raceways. There was considerable opportunity, therefore, when the fish were young for Rs susceptible fish to become infected. Potentially, most of the fish that were susceptible had already been infected prior to their final distribution among four Oregon and six Michigan raceways. This alternative has considerable merit. 3) Horizontal transmission within a raceway occurred at such a rate that it overwhelmed any effects that horizontal transmission "downstream" to lower raceways may have had on increased infection rates in these lower raceways. The crowded conditions in Michigan raceways could seemingly magnify this effect. At a high rate of horizontal transmission within a raceway downstream effects may not be discernible. 4) Erythromycin feeding of these fish limited horizontal transmission. These fish received two erythromycin feedings: An initial treatment was administered in June when they were in one Oregon raceway and a late treatment was given in October prior to their release. The 30 grabsampled fish per raceway were sampled for ELISA only a few days following this last treatment. Erythromycin is most likely effective against extracellular Rs that are probably the predominant form of the bacteria horizontally transmitted. It is most likely ineffective against intracellular Rs which would less likely be horizontally transmitted. Thus, horizontal transmission during and soon after erythromycin therapy might not be expected. It would seem, however, that ample opportunity for horizontal transmission would have occurred during August, September and that part of October before their second treatment. The sensitivity of the ELISA should detect increased infection over such a period and it didn't. This suggests that measurable horizontal transmission downstream was not occurring. 5) Environmental conditions at Umatilla are not favorable for horizontal transmission of Rs. It is known that organic material and

salinity profoundly affect the survivability of Rs in aquatic environments. In fresh water with no organic material its survival is very limited. Addition of 0.1% peptone to such water can actually result in growth of the bacterium (Trevor Evelyn, personal communication). Umatilla has relatively soft water which may limit survival of the organism and it receives many days of intense sunshine from which ultraviolet radiation could contribute to Rs inactivation. 6) The ELISA is not measuring parameters of infection which would reveal horizontal transmission. Sampling fish for ELISA soon after a erythromycin treatment may produce infection prevalences and levels that are lower than would be obtained at other times. Erythromycin inhibits protein synthesis, thus protein antigens normally produced by the bacterium, and detected by ELISA, may be significantly reduced following a treatment. The ELISA did reveal, however, levels of Rs antigen ranging from negative to very high in every raceway. It should, therefore, be indicative of relative infection levels and prevalences between groups of fish.

Most likely, several factors led to a failure to detect significant horizontal transmission of Rs among raceways at Umatilla following this BKD episode. In this particular case, it appears that vertical transmission and possible within raceway horizontal transmission contributed most significantly to the losses to BKD. It raises important questions, however, concerning the relative significance of vertical versus horizontal transmission in the epidemiology of BKD among populations of salmonid fish. Host-pathogen-environment interactions are complex. In fish these interactions are often perceived in the context that an infected host in close proximity to an uninfected host results in infection of the uninfected host via the water. This simplistic perception is probably inappropriate with the Rs bacterium and BKD. This has become an especially important concept for fish health professionals to convey to the fisheries community as there seems to be a prevailing notion within this community that the arrow of disease transmission points one-way from hatchery to natural fish.

#### Acknowledgments

The fish health monitoring project for Umatilla Hatchery Evaluation is funded under a Bonneville Power Administration contract number DE-BI79-91BP23720. Assistance by the Umatilla Hatchery crew is greatly appreciated. Dr. Richard Ettinger of Eastern Oregon State College generously provided the statistical analyses for comparisons of the ELISA data.

# Treatment of Bacterial Gill Disease with Hydrogen Peroxide

Steve Roberts, Washington Department of Fish and Wildlife, 122 S. Mitchell Ct. Liberty Lake, WA 99019 Phone\Fax 509-255-5907

#### Introduction

Hydrogen peroxide recently was found to be effective in controlling fungus on eggs and fish ( Schreck, C.B. et al., 1993 ). This information led to U.S. Food and Drug Administration to rule that hydrogen peroxide is a low regulatory priority drug when used at levels to 500 mg\L to control fungi on all species and life stages of fish, including eggs.

Bacterial gill disease causes significant mortality in fish reared in Washington state fish hatcheries. No registered chemicals are available to treat bacterial gill disease of salmonids. The objective of this study was to test hydrogen peroxide as a treatment for bacterial gill disease.

#### Materials and Methods

## **Toxicity Test**

The toxicity of hydrogen peroxide was tested in a simulated hatchery treatment of brook, brown, and rainbow trout. Replicated groups of fifty fish were treated for three consecutive days with a one hour drip treatment of either 100 or 250 mg\L hydrogen peroxide. Replicate groups of untreated controls were also maintained. Fish sizes averaged 1,621 fish\lb (0.28 g) for brook trout, 811 fish\lb (0.56 g) for brown trout, and 478 fish\lb (0.95 g) for rainbow trout. Water quality parameters were: water temperature 51 F (10.6 C), pH 7.5, and alkalinity 139 mg\L. Mortality was monitored for 14 days following the administration of the third treatment.

## **Small Scale Test**

Bacterial gill disease was diagnosed in cutthroat trout (*Oncorhychus clarki*) from a production circular pond. Diseased fish from the production pond were moved to hatchery troughs. Replicate groups of 50 fish were treated with either a 1 hr drip treatment with 100 or 250 mg\L hydrogen peroxide. Treatments were administered on three consecutive days. Replicate groups of untreated control fish were also maintained. Test and control fish averaged 35 fish\lb (13 g). Fish were not feed on the days that treatments were administered. Water temperature during the test were 47 to 54 F (8.3 to 12.2 C). Water flow was 5 gpm per trough. Mortality was monitored for seven days following the treatment.
# **Production Test - Ford Hatchery**

Rainbow trout (*Onchorhychus mykiss*) reared in four large circular ponds at Ford Hatchery were diagnosed with bacterial gill disease. Three ponds of rainbow trout were treated with hydrogen peroxide in a 20 minute bath for three consecutive days. Fish were treated with either 50, 75, or 100 mg\L hydrogen peroxide. One pond was maintained as an untreated control. The fish size varied from 73 to 105 fish\lb (4.2 to 6.2 g). The number of fish per pond also varied from 25,900 to 50,300. Fish were not feed on the days that treatments were administered. Water temperature during the test ranged from 48 to 60 F (8.9 to 15.6 C). Water flow was 145 gpm per pond. Mortality was monitored for 10 days following the last treatment.

# **Production Test - Columbia Basin Hatchery**

Rainbow trout reared in three raceways at Columbia Basin Hatchery were diagnosed with bacterial gill disease. Two raceways was treated with hydrogen peroxide at either 50 or 100 mg\L in a 1 hr drip for three consecutive days. One raceway was maintained as an untreated control. The fish averaged size was 1,500 fish\lb (0.3 g). The number of fish per pond also varied from 93,800 to 101,200. Fish were not feed on the days that treatments were administered. Water temperature during the test ranged from 59 F (15 C). Water flow was 450 gpm per raceway. Mortality was monitored for 10 days following the last treatment.

# **Production Test - Spokane Hatchery**

Rainbow trout fingerling reared in four large circular ponds at Spokane Hatchery were diagnosed with bacterial gill disease. The ponds were treated with a single 50 mg\L bath of hydrogen peroxide. One pond required a additional single 50 mg\L bath of hydrogen peroxide. The fish size and number ranged from 14 to 19 fish\lb (24 to 32 g) and 20,200 to 29,900 fish per pond. The fish were not fed on the day of treatment. Water temperature was 51 F(10.6 C). Water flow was 130 gpm per pond. Mortality was monitored for 14 days following the last treatment.

# **Results and Discussion**

# **Toxicity Test**

No apparent toxicity was noted in all species tested with both 100 and 250 mg\L hydrogen peroxide with the exception of the 250 mg\L hydrogen peroxide treated rainbow trout (Table 1). In the rainbow trout treated with 250 mg\L hydrogen peroxide, four percent mortality occurred in one replicate and no mortality in the other replicate.

Species	Treatment	Dose (mg\L)	Mortality Number	Mortality Percent	Mean Mortality
Brook	Control-1 Control-1	0 0	0\50 1\50	0% 2%	1%
Brook	H2O2-1 H2O2-2	100 100	0\50 0\50	0% 0%	0%
Brook	H2O2-1 H2O2-2	250 250	0\50 0\50	0% 0%	0%
Brown	Control-1 Control-1	0 0	0\50 0\50	0% 0%	0%
Brown	H2O2-1 H2O2-2	100 100	0\50 0\50	0% 0%	0%
Brown	H2O2-1 H2O2-2	250 250	0\50 0\50	0% 0%	0%
Rainbow	Control-1 Control-1	0 0	0\50 0\50	0% 0%	0%
Rainbow	H2O2-1 H2O2-2	100 100	0\50 0\50	0% 0%	0%
Rainbow	H2O2-1 H2O2-2	250 250	2\50 0\50	4% 0%	2%

Table 1. Toxicity of hydrogen peroxide treatments to brook, brown, and rainbow trout.

# **Small Scale Test**

The total mortality was lower in the fish treated with 100 mg\L hydrogen peroxide groups then the control or 250 mg\L hydrogen peroxide treated groups (Table 2.) Unfortunately, transfer of the fish to the hatchery troughs probably reduced the stress and the overall mortality of the control fish.

Treatment Group	Dose (mg\L)	Mortality Number	Mortality Percent	Mean Mortality
Control-1	0	2\50	4.0%	2.0%
Control-2	0	0\50	0%	
H2O2-1	100	0\50	0%	1.0%
H2O2-2	100	1\50	2.0%	
H2O2-1	250	8\50	16.0%	9.0%
H2O2-2	250	1\50	2.0%	

Table 2. Treatment of bacterial gill disease infected cutthroat trout with hydrogen peroxide.

# **Production Test - Ford Hatchery**

The total mortality for the 14 day post treatment was lower in the fish treated with 50 mg\L hydrogen peroxide compared to higher doses and the untreated control. (Table 3). However, the production test should be view with caution since the fish size and number varied between the ponds.

Table 3. Treatment of bacterial gill disease infected rainbow trout with hydrogen peroxide.

Treatment Group	Dose (mg\L)	Mortality Number	Mortality Percent
Control	0	782\49,300	1.6%
H2O2	50	90\39,100	0.2%
H2O2	75	152\25,400	0.6%
H2O2	100	942\48,100	1.9%

# **Production Test - Columbia Basin Hatchery**

Mortality declined in the raceways of fish treated with 50 and 100 mg\L hydrogen peroxide. At two days post-treatment the mortality increased in the 50 mg\L hydrogen peroxide treated fish; the fish were treated with 100 mg\L hydrogen peroxide for three additional days. The mortality in the control raceway increased to 12.5% on day three and the hatchery manager elected to treat the fish with 150 mg\L hydrogen peroxide for three consecutive days. The mortality in the fish treated with 100 mg\L hydrogen peroxide for three weeks.

# **Production Test - Spokane Hatchery**

Mortality in three of our ponds declined and remained at a low level following a single hydrogen peroxide treatment (Figure 1). In one pond, the mortality initially declined but subsequently increased and an additional single hydrogen peroxide treatment was administered (Figure 2).





Figure 2. Treatment of bacterial gill disease in rainbow fingerlings with two 50 mg\L hydrogen peroxide baths.



## Conclusions

In summary, hydrogen peroxide was effective in treating bacterial gill disease in cutthroat and rainbow trout. A treatment regiments of three consecutive days of 50 mg\L hydrogen peroxide administered in a 30 minute bath or 100 mg\L hydrogen peroxide in a 1 hr drip were recommended for fish smaller than 50 fish\lb. A treatment regiment of a single 50 mg\L hydrogen peroxide administered in a 30 minute bath was recommended for fish larger than 50 fish\lb.

# Acknowledgements

Valuable assistance was provided by Art Brown, Columbia Basin hatchery manager and the Columbia Basin hatchery staff, Bob Johns, Ford hatchery manager and the Ford hatchery staff, and Mike Albert, Spokane hatchery manager and the Spokane hatchery staff.

## Reference

 Schreck, C., M.S. Fitzpatrick, R.L. Chitwood, L.L. Marking, J.J. Rach, and T.M.
Schreier. 1993. Research to identify effective antifungal agents. Annual report 1993. Bonneville Power Administration, Portland, Oregon. 30 pp.

## EVALUATION OF FORMALIN AND HYDROGEN PEROXIDE IN A PRODUCTION SETTING FOR THE MANAGEMENT OF FUNGAL INFECTIONS IN EGGS OF CHINOOK SALMON

P.R. Waterstrat, Emily Coble and David Leith U.S. Fish and Wildlife Service Abernathy Salmon Culture Technology Center 1440 Abernathy Road Longview, WA 98632 206-425-6072

#### Abstract

The effectiveness of formalin and hydrogen peroxide in controlling fungal infection in chinook salmon eggs was evaluated under standard hatchery rearing conditions. The trial involved the daily 15 min treatment of eggs with either formalin at concentrations of 1,667 ppm or hydrogen peroxide at concentrations of 500 ppm or 1,000 ppm. Formalin at 1,667 and hydrogen peroxide at 1,000 ppm appeared effective in controlling fungal infections in eggs during both natural and artificial exposures to <u>Saprolegnia</u>. Hydrogen peroxide at 500 ppm while effective against natural exposure to <u>Saprolegnia</u>, failed to provide control upon the artificial challenge of eggs with fungus. Concentrations of both hydrogen peroxide and formalin declined as treated water passed down through the incubator stack. Respective concentrations of 1,667 formalin and 1000 ppm hydrogen peroxide at the bottom tray of an incubator stack were 50% and 33% of that observed in the top tray. While hydrogen peroxide at a dosage of 1000 ppm daily for 15 minutes appears be an effective alternative to the standard hatchery practice of treating eggs with 1,667 ppm formalin, both the concentration of hydrogen peroxide and formalin should be monitored to insure that effective concentrations are achieved throughout the incubation system.

Reference to a trade name or commercial product does not constitute Government endorsement

### Introduction

Problems associated with fungal infections in eggs are prevalent in the hatchery rearing of a wide variety of fish species. With the removal of malachite green as an approved drug by the U.S. Food and Drug Administration (FDA), the chemical treatment of fungal infections is restricted to the use of formalin on eggs of salmonids or esocids (Schreck et al. 1993). The health and environmental hazards associated with formalin coupled with an increasing scrutiny of formalin by regulatory agencies has prompted the screening of a wide variety of less hazardous compounds for use as antifungal agents in hatchery operations. Evidence from both laboratory studies (Marking et al. in press) and clinical trials (Waterstrat and Marking in press) indicate that hydrogen peroxide may be an effective alternative to formalin in the management of fungal infections in eggs. The recent declaration of hydrogen peroxide as a "low regulatory priority drug " for the control of fungus in all fish species and life stages by the FDA (Schnick 1994) means that hydrogen peroxide may be used without an investigation new animal drug permit (INAD). To further establish the efficacy of hydrogen peroxide and to develop effective protocols for its use, clinical trials evaluating its use in controlling fungal infections were conducted at the U.S. Fish and Wildlife Service, Abernathy Salmon Culture Technology Center (ASCTC) using eggs of fall chinook salmon (Oncorhynchus tshawytsha).

## Methods

### Test Chemicals:

The specifications and concentrations of the chemicals evaluated in the study were formalin, 1,667ppm (Paracide-F: 37% Formaldehyde, 6-13% Methanol, Argent Chemical Laboratories, Redmond WA 98052) and hydrogen peroxide, 500 ppm and 1000 ppm (35% Technical Grade Hydrogen Peroxide Solution, Eka Nobel Inc. Marietta GA 30062).

## Efficacy Tests:

Eggs from fall chinook salmon (<u>O. tshawytsha</u>) obtained during normal spawning operations at ASCTC were utilized. Following spawning and fertilization, eggs from

individual females were placed in separate Heath vertical incubator trays (F.A.L./ Heath, MariSource, Tacoma WA, 98409). The individual egg incubator trays were randomly assigned to an untreated control group or to treatments of either 1,667 ppm formalin, 1000 ppm hydrogen peroxide, or 500 ppm hydrogen peroxide. Treatments consisted of individual incubator stacks arranged with an empty mixing tray on top of each stack followed by 15 incubator trays containing eggs. Upon assignment to the incubator stacks, the eggs were water-hardened for 30 min in 50 ppm iodophor solution (Argentyne, Argent Chemical Co. Redmond WA 98052). Following water hardening, water flow was turned on and calibrated to a flow of 11.4 liters/min. Well water supplying the incubator stacks had the following characteristics: mean temperature, 12° C; total hardness, 90 ppm; alkalinity, 76 ppm; and pH, 7.7. The trial comprised 16 incubator stacks; 8 of the incubator stacks were artificially exposed to <u>Saprolegnia</u> by seeding the top mixing tray of each stack with 6 hemp seed cultures of Saprolegnia parasitica (ATCC 22284) contained in a tissue processing cassette (Uni-Cassette, Miles Diagnostic Div. Elkhart IN 46515) (Challenge Stacks), while the remaining 8 incubator stacks received no artificial challenge (Unchallenged Stacks). The respective treatments and untreated controls were replicated twice in both the Challenge Stacks and Unchallenged Stacks.

Dosages of hydrogen peroxide and formalin were calculated from the calibrated water flows measured at the inlet of the respective incubator stacks using both the percentage of active ingredient and the specific gravity of the test compounds. The dosages of formalin and hydrogen peroxide were delivered to the upper mixing tray of the respective incubator stacks using calibrated metering pumps (Model A141-152S, Milton Roy, Acton MA 01720 ). Water flow to the incubator stacks was calibrated and readjusted periodically throughout the experiment. Daily 15 min treatments with the test compounds were initiated on the day following fertilization and continued until day 35 of incubation, just prior to the initiation of hatching. Treatments were delivered to the upper mixing tray of the respective incubator stacks. Treatment concentrations were monitored using commercial test kits (Formaldehyde:Model FM-1, Hydrogen peroxide: Model HYP-1; Hach Co. Loveland CO 80539) adjusted by dilution for the concentrations used in the trial. To compensate for the

movement of treated water through the incubator stack, water was sampled from the top, middle and bottom trays of the incubator stacks at 5, 10, and 17 min, respectively, after the initiation of treatment.

Effectiveness of the test compounds was evaluated at eye-up on day 21 of incubation by visually examining the incubator trays and counting the number of eggs infected with fungus. Following examination for fungus infected eggs, eggs were shocked by mechanical agitation and evaluated for viability on day 22 of incubation by hand picking dead eggs and using a mechanical egg counter to count viable eggs. Criteria used to evaluate the treatments included the number of fungus-infected eggs and the total eyed-egg mortality. Data were analyzed using analysis of variance with the SigmaStat statistical software package (SigmaStat for Windows, Jandel Scientific Software, San Rafael CA 94912). Differences among treatments were evaluated at a significance level of  $p \le 0.05$  using Scheffe's procedure (Milliken and Johnson 1984).

### Results

## Treatment Concentrations

The means and standard errors (S.E.) of formalin and hydrogen peroxide concentrations observed in the top (Tray 15), middle (Tray 8) and bottom (Tray 1) of incubator stacks are presented in Table 1. A statistically significant decline in concentration was observed among the top, middle and the bottom of the incubator stack in all the treatment groups. Among incubator stacks treated with formalin and 500 ppm hydrogen peroxide, the middle and bottom trays were significantly lower than the top trays. Among stacks treated with 1000 ppm hydrogen peroxide, statistically significant differences in concentration were observed among the top, middle and bottom trays. Evaluation of concentrations among empty stacks treated with formalin and 1000 ppm also exhibited a decrease in concentration among the top, middle and bottom trays.

### Treatment Efficacy

The efficacy of formalin and hydrogen peroxide in the management of fungal infections in

eggs is presented in Table 3. The number of fungus infected eggs was significantly greater in the stacks artificially challenged with <u>Saprolegnia parasitica</u> (Challenged Stacks). Formalin appeared to be effective in controlling fungus in both challenged and unchallenged incubator stacks. Among the challenged stacks, 500 ppm hydrogen peroxide did not appear effective in controlling fungal infection. No statistically significant differences were detected between stacks treated with 500 ppm hydrogen peroxide and the untreated control stacks. Although not as effective as formalin, 1000 ppm hydrogen peroxide appeared to significantly reduce fungus in the challenged stacks. No significant difference between 1000 ppm hydrogen peroxide and 1,667 ppm formalin was detected. In unchallenged stacks all treatments appeared effective in reducing fungal infection relative to the untreated controls; no statistical differences were detected among 1,667 formalin or hydrogen peroxide at concentrations of 500 ppm or 1000 ppm.

## Discussion

Daily 15 min treatments of either 1,667 ppm formalin or 1000 ppm hydrogen peroxide appeared effective in controlling fungal infections of fall chinook salmon eggs challenged with artificial or natural exposure to Saprolegnia. Although effective under conditions of natural exposure, 500 ppm hydrogen peroxide did not appear effective in controlling fungus infection when eggs were exposed to artificial challenge. The lack of effectiveness of 500 ppm hydrogen peroxide may be due to either the level of fungal exposure experienced during the artificial exposure or to the effective concentration delivered to trays within the incubator stacks (Table 1). Levels of both hydrogen peroxide and formalin dropped considerably as the treatment flowed down the incubator stack. Levels of 1,667 ppm formalin, 1000 ppm hydrogen peroxide and 500 ppm hydrogen peroxide in the middle tray of the incubator stack were 57%, 46% and 59% of the respective concentrations observed in the top tray. The mean value observed in the bottom tray of incubator stacks treated with 500 ppm hydrogen peroxide was 195 ppm, considerably below the target concentration of 500 ppm. The decline in concentration in both formalin and hydrogen peroxide may be attributed to both the demand created by the presence of eggs and by the volatilization of the chemicals as the water tumbled from tray to tray. The loss in concentration may be partitioned by

comparing Tables 1 and 2. The drop in formalin concentration from the top to the middle tray of empty stacks was 30.8% while the loss between similar stacks loaded with eggs was 42.6%, producing an estimate that eggs accounted for 27.7% of the drop in concentration. Among stacks treated with 1000 ppm hydrogen peroxide the decline in concentration from the top to middle tray of empty stacks was 28.9%, while the decline in stacks loaded eggs was 51.6%, yielding an estimate that eggs accounted for 43.9% of the loss in concentration. To compensate for the decline in concentration due to either eggs loading or the volatilization of the chemical as it passes down the incubator stack, the treatment dosages may have be adjusted to achieve a target or a minimal effective concentration over all eggs in an incubation system. While not statistically significant, all fungal treatments experienced a trend toward a dose dependent increase in the cumulative number of dead eggs observed over the 22 day incubation period.

Hydrogen peroxide was effective in controlling fungal infections in fall chinook eggs at concentrations of 1000 ppm upon both natural and artificial challenge. The use of hydrogen peroxide presented few problems under the standard hatchery practices used in the study. Equipment currently used for the formalin treatment of incubator tray stacks was easily and readily converted for use with hydrogen peroxide. While hydrogen peroxide is a strong oxidizer and must be handled with reasonable care, it is "user-friendly" when compared to the risks associated with formalin. Decomposition products of hydrogen peroxide (water and oxygen) are relatively innocuous and are considered to be environmentally compatible (Eka Nobel 1993). The recent classification of hydrogen peroxide as a " low regulatory priority" compound by the U.S. Food and Drug administration (Schnick 1994) makes use of hydrogen peroxide especially advantageous for use in the management of egg fungal infections. Inexpensive commercial assay kits for formalin and hydrogen peroxide are readily adaptable for hatchery use. To ensure that both formalin and hydrogen peroxide are at effective concentrations and to prevent the loss of eggs due to overdosage, treatment concentrations should be monitored throughout the incubation system.

## Acknowledgments

The authors thank Jeff Poole and John Holmes for their help and suggestions in conducting the study.

### References

Eka Nobel. 1993. Hydrogen peroxide product information manual. Eka Nobel, 2622 Nashville Ferry Road, Columbus, MS 39701, 20 pp.

Marking, L.L., J.J. Rach and T.M. Scheier. in press. Evaluation of antifungal agents for fish culture. Progressive Fish Culturist.

Milliken, G.A. and D.E. Johnson. 1984. Analysis of Messy Data. Van Nostrand Reinhold Co. New York, NY 10003.

Schnick, R.A. 1994. Low regulatory priority declared for hydrogen peroxide as a fungicide. Notice from U.S. Department of Interior, National Biological Survey, National Fisheries Research Center, 2630 Fanta Reed Road, P.O. Box 818, La Crosse WI 54602-0818.

Schreck, C., M.S. Fitzpatrick, L.L. Marking, J.J. Rach and T.M. Schreier 1993. Research to identify effective antifungal agents. Annual Report 1991 Bonneville Power Administration, Project Number 89-054. 30 pp.

Waterstrat, P.R. and L.L. Marking (in press). Clinical evaluation of formalin, hydrogen peroxide and sodium chloride for the treatment of Saprolegnia in fall chinook salmon eggs. Progressive Fish Culturist

Table 1. Treatment concentrations measured at the top, middle and bottom trays of egg incubator stacks containing fall chinook eggs during daily 15 min treatments with the respective chemicals. Means not sharing a common letter are statistically different at a significance level of p = 0.05.

# Formalin

Dose	1667nnm	Maan (nnm)	C F	<b>D</b> ( )
Dose.	<u>1667ppm</u>	<u>Mean (ppm)</u>	<u>S. E</u> .	<u>Range</u> (ppm)
	Тор	1215.0 a	125.2	250 - 2750
	Middle	696.6 b	82.5	200 - 2000
	Bottom	610.0 b	65.3	150 - 1750
<u>Hydrogen p</u>	eroxide			
Dose: ]	1000 ppm	<u>Mean (ppm)</u>	<u>S. E</u> .	Range
	Тор	828.6 a	26.9	600 - 1150
	Middle	400.0 b	45.1	50 - 1000
	Bottom	272.0 c	29.9	100 - 650
<u>Hydrogen p</u>	eroxide			
Dose:	500 ppm	<u>Mean (ppm)</u>	<u>S. E.</u>	Range

			-	
Тор	364.3 a	29.9	150 -	600
Middle	217.9 b	18.6	150 -	400
Bottom	195.0 b	18.9	100 -	300

Table 2. Treatment concentrations measured at the top, middle and bottom of empty egg incubator stacks during 15 min treatments with the respective chemicals. Means not sharing a common letter are statistically different at a significance level of p = 0.05.

# Formalin

<u>Dose: 1667ppm</u> (p = 0.072)

	<u>Mean (ppm)</u>	<u>S. E</u> .
Тор	1083.3 a	83.3
Middle	750.0 b	144.3
Bottom	666.7 b	83.3

# <u>Hydrogen peroxide</u> (p = 0.033)

<u>Dose: 1000 ppm</u>	<u>Mean (ppm)</u>	<u>S. E</u> .
Тор	633.3 a	44.1
Middle	450.0 a	50.0
Bottom	416.3 b	44.1

Table 3. The efficacy of formalin and hydrogen peroxide as measured by the number of fungus infected eggs and the number of dead eggs at eye-up, day 22 of incubation. Challenged Stacks were artifically exposed to <u>Saprolegnia</u> <u>parasitica</u>. Unchallenged Stacks recieved no artifical exposure.

A. Challenged Stacks	<u>Fungus Inf</u>	ected Eggs	Dead E	ggs
•	Mean	<u>S.E</u>	Mean	<u>S.E</u>
Formalin:1667 ppm	0.7 a	0.1	259.2	63.0
<u>Hydrogen Peroxide</u>				
1000 ppm	51.0 ab	21.1	380.6	97.9
500 ppm	157.2 bc	84.4	221.3	35.6
Untreated Control	180.1 c	37.7	149.2	30.0
Significance	(p =	0.024)	(p = 0.0)	0731)

B. Unchallenged Stacks:	Fungus	Infected Eggs		De	ad Egg	<u>s</u>
	Mean	<u>S.E</u>		Mean		<u>S.E</u>
Formalin:1667 ppm:	0.2 a	0.1	3	211.3		95.5
Hydrogen Peroxide						
1000 ppm	1.4 a	0.5		270.4		98.0
500 ppm	11.7 a	4.4		117.1		21.8
Untreated Control	153 <sub>.</sub> 0 b	32.5		171.8		30.0

Significance	(p = 0.0001)	(p = 0.4877)
Diginneance	(p = 0.0001)	(p - 0.48)

# COHO ANEMIA DISEASE (CAD)

Richard A. Holt<sup>\*</sup> and Lyle D. Curtis<sup>\*\*</sup> Oregon Department of Fish and Wildlife

<sup>\*</sup>Corvallis Fish Pathology Laboratory, Nash Hall 220 Oregon State Univerity Corvallis, Oregon 97331-3804 and <sup>\*\*</sup>Salmon River Hatchery 575N, North Bank Road Otis, Oregon 97368

The following information on coho anemia disease was obtained and summarized from several sources including: our observations of CAD since 1980 in Oregon, a summary of information on this disease prepared by Bob Rogers, WDF&W, and discussions with Mel Eklund, NMFS, Ted Meyers, AF&G and Mel Willis, CF&G.

Synonyms- Fall Creek Syndrome, Shock Syndrome, Salmon Anemia Disease

**Disease Signs**-In affected groups, prior to adding feed, fish in the ponds appear normal, but during or soon after feeding some of the fish begin to show signs of stress. "Nice-looking" robust appearing fish begin to skim the surface, swim erratically or collect along the pond edge. Some display pigmentation changes but most look normal. The affected fish have very pale gills with about 20% or less showing a hemorrhaged spot in the ventral base of the filaments. When a dying fish is placed in a bucket of water, the gill color seems to progress from pink to almost white in a few minutes. When the peduncle is severed to collect blood, little or no bleeding of fluid occurs. This lack of fluid is quite different than what is seen with EIBS infected fish where plenty of fluid is present but with varying amounts of red blood cells.

Internally, the gut usually contains much fresh food and the organs are somewhat pale. Hematocrits of dying fish are less than 20%, often less than 10%. Active chummed fish from the same population have hematocrits usually in the normal range of 30-40%; however there may be some slightly low findings in the upper 20s. During a severe outbreak of CAD at Salmon River Hatchery in 1992, 29% of a sample of active fish taken from the upper end of the pond had hematocrits less than 30% and 12% had hematocrits less than 20%. It appears those fish that are severely anemic are still interested in feeding and when they feed, the lack of red blood cells results in anoxia and death. However, handling should induce a similar anoxia problem in these anemic fish when they are handled but losses do not increase significantly.

**Histology**- Some immature red blood cells are evident in blood smears but very few cells of any kind are found. Some hemosiderin is present in the spleen and no severe liver pathology is observed except some oxygen depletion lesions. The anterior kidney in one case showed no mitotic figures and in another was hyperplastic with evidence of increased red blood cell production similar to that seen after EIBS infections.

**Historical Occurrence and Distribution-** Fish with CAD signs have been observed in northern California, western Oregon, western Washington, and southeast Alaska. A majority of hatcheries where CAD has been observed have surface water supplies; however, in Washington there are two spring water fed hatcheries where signs of CAD occur in fish. All of the locations in Oregon have surface water supplies.

This disease historically has occurred in coho at ODFW central coast hatcheries usually beginning in late July or August and continuing until October or November. It was first observed at Fall Creek Hatchery, located on a Oregon central coastal stream, in 1980. Next it was observed at Nehalem Hatchery in 1981. Since then we have not seen it at Nehalem until fall 1993. Most of the occurrences have been at Fall Creek or Salmon River hatcheries in the mid-tolate 1980s. It has consistently occurred at Salmon River Hatchery in the last five years. In Washington, the disease had previously been observed at Lewis River Hatchery in 1988 and 1989 during the months of October through December. In fall 1993, the distribution of this disease changed dramatically in the Northwest. For the first time, we began to observe disease signs in coho at every location where coho were reared from Butte Falls and Cole Rivers hatcheries in the Roque River drainage, Rock Creek Hatchery in the Umpgua drainage to Klaskanine, Big Creek, Sandy, Oxbow, Cascade and Bonneville hatcheries in the lower Columbia River. Similar to Oregon's experience, in October- December 1993, CAD appeared at several hatcheries in western Washingon including Lewis River, Klickitat, Cowlitz, Hupp Springs, and George Adams (information obtained from a summary prepared by Bob Rogers, WDF&W). Ted Meyers reported CAD signs in three coho hatcheries in Alaska during the fall of 1993. He believes fish with these signs may have occurred two years ago at one of these locations. Mel Willis reported CAD signs in a very low level loss first observed in October 1993 in coho at Trinity Hatchery.

Pathologists from British Columbia, Canada indicate they have not seen this disease in their coho.

**Species Affected**- At nearly all locations where coho are affected other species such as steelhead and chinook show no signs or loss. Exceptions to this are occurrences in chinook at two hatcheries: White River spring chinook at Hupp Springs Hatchery in Washington and up-river fall chinook and Carson spring chinook at Bonneville Hatchery.

Loss Rate- Most outbreaks of this disease in coho in Oregon result in about 1-6% loss overall, however the most severe loss occurred at Salmon River Hatchery beginning in June 1992 and continuing until February 1993, accumulating a 30% overall loss. In 1993, loss from CAD in Salmon River Hatchery was very low (less than 2%).

**Effect of Water Temperature** - In Oregon most CAD outbreaks have occurred beginning in late July and August and continuing into October or November. Usually, the warmer the water temperature the higher the loss from this disease. In 1992 at Salmon River Hatchery unusually hot days in May and June seemed

to trigger a severe outbreak simultaneously in two different stocks of coho held in three asphalt ponds.

Effect of Diet- In Oregon, coho affected with CAD in 1993 and early 1994 were on one of the following diets: BMF2, BMF, BD500, SC Salmon or MCNAS. Diet may affect the severity of CAD but to date we have too few comparisons of different diets at the same hatchery to draw any meaningful conclusions. Some of these same diets are being fed in western Canada and in various coho hatcheries where CAD has not been observed in Washington and California.

Changing the schedule of feeding will affect losses. If the fish are not fed during a day, the losses will be minimal but as soon as they are fed again losses increase. A schedule of three days off feed followed by one day fed to satiation may have reduced the overall loss slightly at one or two locations in Oregon.

**Effect of Handling-** The increased loss observed after feeding has not occurred when the fish are handled such as during marking operations, lowering the pond for cleaning, or transporting the fish in trucks to saltwater netpens. There have been a few observations where populations of fish with chronic CAD appeared to stop dying from CAD when transferred to different ponds. At George Adams Hatchery in Washington, coho suffering from CAD in a dirt-bottom pond were split into three groups. Those transferred to saltwater netpens and those placed in a dirt-bottom pond located in the effluent of the original pond stopped dying of CAD. However, the third group placed in a concrete pond and held at a higher density continued to display CAD signs.

## Speculation Regarding the Cause-

1. Infectious agent? Since 1980, we have been unable to isolate or identify any infectious agent from CAD affected fish. On many occasions, samples have been placed on a variety of cell cultures with no development of suspicious viral cytopathic effect. No common bacterial fish pathogens are consistently isolated from these anemic fish which is rather strange in itself and in contrast to EIBS-caused anemia where secondary infections of *Flexibacter psychrophilus* and external fungi are common.

In some of the outbreaks of CAD, infections of EIBS virus have occurred several weeks prior to the onset of CAD. We have speculated that these fish have suffered the lysis of blood cells due to EIBS virus and their blood forming tissues have not recovered. Because they are so anemic, the stress of feeding causes them to die. No inclusions of EIBS can be found in the CAD affected fish. Thin sections of blood cells collected from CAD affected fish and observed with the electron microscope revealed no virus particles. However, we do not in all situations find EIBS preceding or during CAD outbreaks. Likewise, we have observed EIBS epizootics with no subsequent CAD. In preliminary transmission experiments, blood, kidney and spleen tissue have been injected into apparently healthy coho with no development of CAD or EIBS signs.

The simultaneous appearance of this disease last fall in many hatcheries from California to Alaska in some ways points to a non-infectious etiology. It does not make sense that an infectious agent could spread that rapidly without transfers of fish among all the locations.

2. Toxin? Dr. Mel Eklund of the NMFS, Seattle MontLake Lab, has found a bacterium by enrichment culture from the intestine of coho with CAD and from the bottom sediment of ponds containing these fish. Samples from Fall Creek Hatchery coho in 1980 were found to contain this bacterium. Recently, he has found this organism in affected fish from several hatcheries in both Washington and Oregon. This unidentified bacterium is a Clostridium, but different than the botulism-causing bacterium. It produces a toxin from this enriched culture which when injected into fish or mice causes death within 6-30 minutes. Dr. Eklund referred to this disease in salmon as "shock syndrome." He has observed this toxin producing bacterium in the last 30 years in locations throughout the Northwest during his surveys for the botulism bacterium. This included samples collected from ocean sediments off the Oregon and Washington coast. Dr. Eklund has cautioned we must consider the evidence of a toxin as the cause of this anemia still circumstantial. Experiments need to be completed on: (1) the administration of the toxin by injection and feeding routes and observation for typical CAD signs (2) inoculation of spores of this bacterium into the lower intestine of fish, sacrificing the fish and allowing time for the toxin to develop after the fish are left on the tank bottom. Healthy fish would then be allowed to cannibalize the carcasses to demonstrate if fish can develop this disease by feeding on mortality on the pond bottom (3) diet components that might enhance the growth of this bacterium or contain the toxin, such as the meal obtained from adult salmon carcasses (4) environmental sources. Have the recent warmer ocean temperatures resulted in increased prevalence of the toxin producing bacterium? Hopefully, the cause of this disease will be elucidated soon!

# SESSION IV

# SYMPOSIA: THE CHANGING ROLE OF ARTIFICIAL PROPAGATION IN FISHERIES MANAGEMENT

- The Changing Role of Artificial Propagation in Managing Pacific Salmon in the Pacific Northwest: The Shifting Paradigm - Dan Herrig, U.S. Fish and Wildlife Service; Rich Carmichael, Oregon Department of Fish and Wildlife
- Artificial Propagation of Pacific Salmon Under the Endangered Species Act: Constraints and Opportunities - Jeff Hard & Robin Waples, National Marine Fisheries Service
- Captive Broodstocks for Recovery of Snake River Sockeye Salmon -Keith Johnson, National Marine Fisheries Service; Tom Flagg, Idaho Fish and Game
- Operation of Pacific Salmon Compensation Hatcheries Within a Conservation Framework - Ed Bowles, Idaho Fish and Game
- Managing Hatcheries Within Oregon's Wild Fish Management Policy Guidelines: From Theory to Practice - Rhine Messmer, Oregon Department of Fish and Wildlife
- Integrating Artificial Propagation Programs into Ecosystem and Natural Production Management: Can it be Done? - Larry Lestelle, Jim Lichatowich, Lars Mobrand, Mobrand Biometrics Inc.

# The Role of Artificial Propagation in Managing Pacific Salmon in the Pacific Northwest A Shifting Paradigm

Daniel M. Herrig U.S. Fish and Wildlife Service Lower Snake River Compensation Plan Office Boise, Idaho

Richard W. Carmichael Oregon Department of Fish and Wildlife Research and Development La Grande, Oregon

## ABSTRACT

The role of artificial propagation of Pacific salmon has changed greatly over the last 100 years, and the role continues to change was we proceed toward the 21<sup>st</sup> Century. Social, cultural, and economic values of the people of the northwest and the nation have guided these changes by influencing and directing fisheries management objectives and fisheries managers. One major influence has been continual change of the roles and demands of our hatchery programs. In our presentation, we will briefly review the changing roles of hatcheries from the 1880's to the present, noting the major factors which effected these changes. The remaining presentations by panel members will describe potential future roles of hatcheries and the constraints and guidelines under which they might operate.

Before Europeans arrived, use of salmon by Native Americans was primarily for local subsistence and trading; there was no artificial production and no wholesale changes in aquatic habitat. This type of use persisted for some years after European settlement until the mid-1860's when the first cannery opened on the Columbia River. By 1883 there were 39 canneries and the salmon resource was becoming a significant local food source and an important national and international trade commodity.

The first artificial propagation facilities were built in the late 1880's to bolster returns for commercial fisheries. The industry believed that human intervention was necessary to sustain runs, and this attitude was supported by the public, fishermen, canneries, politicians, and conservation officials. Confidence of success was very high among all parties. As we proceeded into the 20<sup>th</sup> Century, we began to take ever increasing portions of natural runs; habitat destruction through small dams, logging, etc. became more widespread and severe; and catches began to decline. In response to the waning confidence of the public and others in artificial production programs, fish managers began investigating new rearing practices beginning about 1910 which resulted in releasing fish at larger sizes. Improvements in fisheries followed and a renewed confidence was instilled in hatchery programs.

During the next 10 to 30 years there were major losses of habitat due dam construction and

land uses and many runs again began to decline. During this period a large number of hatcheries were built or upgraded to compensate for habitat blocked, destroyed, or altered by dams. Sport fishing and Tribal hunting (for fish) interests began to have more influence on fish managers. In 1968, for example, the Columbia River tribes began a successful effort to demand their fishing rights through the courts. Hatchery technology improved vastly in the 1950's and 1960's: adult collecting and holding became more sophisticated, disease controls and improved feeds increased survival on the facilities, marking programs allowed some off-station evaluation, and rearing time was extended. Increases in run sizes and catches of some species in the 1960's and 1970's were attributed to hatcheries and there was renewed enthusiasm for their construction and use.

From the mid-1970's to the present, while more hatcheries were built in the mid-Columbia and Snake rivers, we have experienced the collapse of many hatchery and natural runs. Several fisheries have been curtailed and legislation has been passed (or, in the case of the Endangered Species Act (ESA), applied) in an effort to correct the problems, adding many new "players" in the process. Doubts about hatcheries have raised calls by some for a moratorium on constructing more. Others wonder about the ecological and genetic impacts of hatchery programs and call for major changes or even halting all operations. Still others have proposed that hatcheries may be the <u>only way</u> we can conserve and protect the genetics and numbers of the remaining wild populations. They promote hatcheries as conservation tools and recommend captive brood programs as an extreme measure.

The diversity of opinions on the future of hatcheries is immense. Social, political and biological uncertainties surround the future of hatcheries in the Pacific Northwest. What will be the future role of hatcheries? Can they assist in the conservation of threatened and endangered species? Can they realistically and efficiently operate with major ESA and wild fish policy constraints. Can they play an active role in ecosystem management efforts? And finally, will fish and hatchery managers have any say in guiding their future?

## Artificial Propagation of Pacific Salmon Under the Endangered Species Act: Constraints and Opportunities

## Jeffrey J. Hard and Robin S. Waples National Marine Fisheries Service Northwest Fisheries Science Center 2725 Montlake Boulevard East Seattle, Washington 98112

Artificial propagation of Pacific salmon (*Oncorhynchus* spp.) has a long history in the Pacific Northwest. Traditional objectives have focused on fisheries enhancement and mitigation for losses in abundance due to habitat removal or degradation, and two prominent characteristics are the liberation of large numbers of hatchery fish to the wild and mixed-stock harvest of migrating fish and returning adults. However, despite the reliance on artificial propagation of Pacific salmon to enhance fisheries and mitigate for habitat loss, there is little empirical evidence to indicate whether this technology can be used to generate sustainable increases in abundance of naturally spawning populations. Recognition of this fact is important, because demand is increasing for the use of artificially propagated fish to supplement natural salmon populations.

According to recent reports, many salmon populations have been extirpated in western North America and many others are at risk of extinction. One population of sockeye salmon (O. *nerka*) and three populations of chinook salmon (O. *tshawytscha*) are currently listed as threatened or endangered under the Endangered Species Act (ESA). To address the conservation of salmon populations in a more comprehensive way, the National Marine Fisheries Service recently announced it is initiating ESA status reviews of all U. S. (excluding Alaska) populations of all seven species of Pacific salmon.

Artificial propagation may assist the conservation and recovery of salmon populations at risk of extinction in several ways, including: (1) reducing the short-term risk of extinction of distinct populations; (2) supplementing existing natural populations to speed recovery; (3) providing a means of protecting populations and maintaining their genetic diversity until habitat is restored or other factors causing species decline are corrected; and (4) reestablishing self-sustaining populations in suitable but currently vacant habitat. Indeed, the ESA [Sec. 3(3)] explicitly recognizes the value of artificial propagation for the recovery of threatened or endangered populations. However, at the same time that possible applications of salmon artificial propagation on natural salmon populations. The ESA requires that conservation efforts focus on natural populations and "the ecosystem upon which [they] depend" [Sec. 2(a)(5)(b)]. Consequently, greater scrutiny of artificial propagation is required than has been customary to assess and limit adverse consequences to natural populations. Furthermore, under the ESA, artificial propagation can be a means to conservation, but it is not an end in itself.

The problems faced by natural salmon populations and the potential application of artificial propagation to these problems provide novel research and management opportunities. However, these problems also present some unique challenges regarding which components of biological diversity should be conserved and which approaches are likely to result in sustainable recovery. The clear mandate of the ESA to conserve natural populations of Pacific salmon and preserve their genetic and ecological distinctiveness must guide the responses to these challenges. Whether for evaluating existing hatchery programs for unlisted populations or determining whether to use artificial propagation as a recovery tool for listed populations,

this overriding goal requires an objective assessment of the risks of using, and failing to use, artificial propagation. The genetic risks associated with artificial propagation include extinction, loss of diversity within and among populations, and domestication; ecological risks include disease transfer, competition (for food, habitat, or mates), predation, altered migration, and displacement of natural fish. An objective risk assessment should weight these risks against the risk of forgoing the possibility of rapid recovery and, possibly, extinction of the population.

A full assessment of risks to natural salmon populations must consider not only specific aspects of the ways in which broodstock are selected and collected, juveniles are raised and released, and fish are harvested and intercepted during their migrations; such an assessment must also seek to determine the root causes of the populations decline. Although it has been common to respond to a salmon population's low or declining abundance with artificial propagation without addressing the factors contributing to decline, under the ESA, artificial propagation cannot be a substitute for remedying these factors. It is therefore essential that these factors be identified and, if possible, addressed <u>before</u> a decision is made about the role artificial propagation will play in efforts to conserve the population. Once these factors have been identified and a decision has been made on artificial propagation's role, adequate monitoring and evaluation of the population's status should be initiated. Monitoring and evaluation are necessary to determine the nature and extent of threats to sustained recovery; these activities should also provide some unique opportunities to increase our understanding of salmon biology in nature.

Unfortunately, the risks faced by salmon populations, both in the presence and absence of artificial propagation, are difficult to evaluate adequately because of the lack of definitive information on their relative threats to natural fish. Given this uncertainty, a conservation strategy should strive to minimize both immediate and long-term threats to these fish. A risk-averse conservation strategy requires evaluating evidence for these threats. A serious error may result if lack of evidence for a threat to a population is equated with the absence of this threat. The consequences of this error may be far more serious for a threatened or endangered population than the consequences of concluding that a threat may exist when it does not, because the status of threatened or endangered populations leaves little chance to recover from incorrect management decisions. The main point to remember is that weighing the anticipated costs associated with making these different errors in conserving threatened or endangered populations should be used as a guide to determining the appropriate management strategy. The costs considered should include the direct costs of making the error in evaluating the available information plus the costs of responding to the error once it is recognized.

Artificial propagation of Pacific salmon is at a crossroads. While traditional forms of artificial propagation may still have a place in enhancing salmon fisheries or mitigating for habitat loss, future operations will require greater consideration of their impacts on salmon populations in their natural ecosystem. The changes necessary to accomplish this goal will not be made easily, but they are necessary both to better integrate natural and artificial production of salmon and to sustain the viability of natural salmon populations.

## Captive Broodstocks For Recovery of Snake River Sockeye Salmon

## Keith A. Johnson and Thomas A. Flagg

## Idaho Department of Fish and Game, Eagle Hatchery 1800 Trout Road Eagle, ID 83616

## National Marine Fisheries Service Northwest Fisheries Science Center 2725 Montlake Blvd. East Seattle, WA 98112

The Idaho Department of Fish and Game and the National Marine Fisheries Service have established several captive broodstocks to aid recovery of the last known remnants of Snake River sockeye salmon (*Oncorhynchus nerka*). Broodstocks have been established from the few adults returning to Redfish Lake in central Idaho in 1991-1994, from smolts captured in 1991-1993, and from a few residual sockeye captured in 1992 and 1993. Residual sockeye are those which demonstrate genetic and behavioral patterns of sockeye yet are non-migratory. The first spawning of individuals of two broodstocks occurred in 1993 and about 12,000 progeny were returned to the nursery lake in 1994. A higher percent of adults have matured in 1994 and spawning has yet to be completed. Several general statements can be made from the progress to date for illustration of the relative risks in applying this experimental technology to other endangered stocks of Pacific salmon.

The concept of captive broodstocks for recovery of stocks of salmon cannot be considered without the commitment to correct the causes which underlay the endangered status. It is unrealistic to expect the progeny of a captive program to fare any better than their feral counterparts unless the problems are corrected with a few generations. In the case of Snake River salmon stocks this requires a dramatic increase in smolt-to-adult survival through dramatically improved survival in the downstream Snake River migration corridor.

Cornerstones of fish culture practices for captive broodstocks of sockeye are embodied in the *Sockeye Salmon Culture Manual* developed by the Alaska Department of Fish and Game. These include using a disease-free water source, rigorous disinfection at every opportunity, and rearing small groups in many containers in order to reduce losses when culling for disease is required. Application of these practices allowed containment of bacterial kidney disease to a single group over three years in one broodstock reared by IDFG. The application of these principles requires a facility which is not production oriented but rather oriented to producing a small number of quality progeny. Another important consideration is security. The Eagle facility is not open to access by the public and water alarms are backed with a paging system for security. A third criterion is flexibility in facility design. This has been accomplished for these broodstocks by having rearing tanks which can be moved and substituted for ones of another size. It is very difficult to envision what lay-out will be needed due to the variable number and sources of broodstock available.

Broodstocks were sourced from feral smolts and from eggs spawned from a few anadromous adults. Maturation and survival rates have been very different depending on the origin. Females of the outmigrant broodstocks have had a high proportion of ovaries with atresic, reabsorbing, and variable-sized eggs. Age at maturity has also been more variable for broodstocks of outmigrant origin. Maturation occurred during January-July 1994 although the

fish were held on natural photoperiod. No reasonable explanation of this is apparent. Males of one broodstock of residuals matured precociously as two year olds; this has not occurred in outmigrant or anadromous groups.

Genetic considerations for these broodstocks are paramount. The timing of the initiation of a captive broodstock must be done before the number of fish is so low that every adult is critically important. This, of course, is counter to the endangered status concept. In the case of Redfish Lake sockeye, every opportunity to broaden the genetic diversity of the broodstock has been explored but not at the expense of excessive risk from disease.

Technologies for these captive broodstock programs include identification of each brood fish with PIT tags for genetic purposes, identification of individuals by DNA, allozyme, and otolith methods, and evaluation of the performance of a special diet to improve gamete quality of captive broodstocks. While these have been helpful in improving performance of the broodstocks, the usual ingredients for good fish culture are also needed and must be in place before such a program is entertained. Many of the mortalities in the broodstocks at Eagle have occurred due to the lack of facilities being in place before they were needed.

Further work is needed in several areas of captive broodstock technology. Some are technical questions which have arisen as the result of this work, while others reflect improvement in administrative procedures:

- 1. Prediction of maturation rates based on growth rates at critical seasons.
- 2. Explanation for off-season maturation (January-July) on natural photoperiod.
- 3. Definition of temperature, nutritional, and rearing conditions for normal gonad development, especially for females.
- 4. Development of sperm cryopreservation methods for males reared in captivity.
- 5. Explanation of the occurrence of four types of tumors which have developed at Eagle but not at Big Beef Creek for groups of the same origin.
- 6. Imprinting of juveniles to direct resulting adults to spawn in areas of lakes with demonstrated success.
- 7. Commercially available sub-family incubators with a capacity for 600 eggs.
- 8. Causes of soft eggs incapable of resisting shock or mechanical injury from captive broodstocks.

Captive broodstock applications require some changes in attitude for fish culturists. There has to be a shift toward a nurturing, not production, mentality. There has to be adequate time and careful attention given to details which will yield quality animals. Inattention to details can cause compounding problems over a long time. There are also administrative attitude changes needed. The most important is not to get greedy in sizing these programs. Match the numbers of fish to be held in a broodstock to what the receiving environment can support. Ethics require that any fish above this number should be returned to the habitat. Finally, this has to be perceived as experimental and cannot be relied on to perpetuate a stock beyond a few generations since no fish culture program can duplicate natural selection. If these programs are undertaken, they should not be construed as substitutes for improving the conditions which led to the near-extinction of the stocks. These must be solved as soon as possible.

## OPERATION OF PACIFIC SALMON COMPENSATION HATCHERIES WITHIN A CONSERVATION FRAMEWORK

#### Ed Bowles

## Idaho Department of Fish and Game 600 South Walnut Street Boise, ID 83707

Abstract: Chinook salmon hatcheries in Idaho were constructed to provide harvest opportunities lost from hydropower development. Realization that mitigation is highly unlikely without survival improvements to the mainstem Columbia and Snake rivers' migration corridor, and concern about the continued erosion of natural production has prompted fish managers to develop interim conservation objectives. These include: maximize the number of fish retaining local genetic characteristics, optimize utilization of natural spawning and rearing habitat, and minimize adverse impacts to existing natural populations.

Successful use of compensation hatcheries to help meet conservation objectives requires that: all three groups of returning fish (natural, supplementation, reserve) are recognized as important to recovery; hatchery practices provide a survival advantage; broodstock strategies do not "mine" natural populations below critical levels; releases do not "swamp" target or non target natural populations; hatchery practices do not change natural genetic, behavior or health characteristics; monitoring and evaluation can resolve uncertainties, contain risks and allow managers to adapt accordingly. This approach represents a substantial shift from the original mandate for mitigation hatcheries.

#### ISSUE

What is the best use of compensation hatcheries located in natural production areas for threatened salmon, and the fish returning to these facilities?

#### BACKGROUND

Five of Idaho's chinook hatchery programs are located in important natural production areas for spring and summer chinook salmon listed for recovery under the auspices of the Endangered Species Act (ESA). Sawtooth Hatchery includes the upper Salmon River and East Fork Salmon River programs, and McCall Hatchery the upper South Fork Salmon River program. Both hatcheries are part of the Lower Snake River Compensation Plan (LSRCP). Pahsimeroi and Rapid River hatcheries are part of The Idaho Power Company (IPC) mitigation. These hatcheries were constructed to compensate for fish losses from hydropower development on the lower Snake River (LSRCP) and Hells Canyon reach of the Snake River (IPC). Broodstock for Rapid River Hatchery was derived from chinook that are not native to the Rapid River drainage. This distinction makes Rapid River Hatchery inappropriate for much of the approach outlined in this issue paper. Broodstocks for the other four hatchery programs were derived primarily from local stock endemic to the natural production areas where the hatchery programs operate. These stocks may also include genetic material from nonendemic chinook resulting from stock transfers (Bowles and Leitzinger 1991).

The approach for mitigation was to spawn and rear a portion of the historically productive local broodstock to produce a large number of smolts to compensate for lost smolt production (IPC) or reduced smolt-to-adult survival (LSRCP) from hydropower development. Annual broodstock management included retaining 67% of unmarked adults for hatchery production, up to escapement needs, and passing 33% of unmarked adults to spawn naturally. This strategy was implemented in an attempt to reduce domestication effects and maintain long term fitness of the locally evolved stock. All marked adults (0% to 25% of total) were retained and incorporated into the hatchery broodstock.

By the late 1980s it became evident that Snake River basin mitigation/compensation hatcheries were falling dismally short of mitigation objectives for chinook salmon (Herrig 1991). Even if significant improvements were made in fish health and husbandry, meeting mitigation expectations was highly unlikely until mainstem survival conditions were improved (Cannamela 1992). In addition, managers were concerned with continued erosion of natural production and the potential effects of these compensation programs on stock identity and natural productivity. Preliminary genetic monitoring studies have not detected obvious differences between hatchery and naturally reared fish (Waples et al. 1993, Marshall 1993), although pre-hatchery genetic information is not available. Analysis of spawning escapement data from before and after hatchery production began shows that more adults are returning to these production areas than would be expected without hatchery contributions. This hatchery benefit has only slowed the decline of total chinook production in the upper Salmon River, whereas in the upper South Fork Salmon River the hatchery benefit has actually increased total production. Although the hatchery programs are providing an adult-to-adult survival advantage, it was recognized that a greater emphasis needed to be put on the purely natural component of the local stock to insure its long term viability.

In response to this concern, additional conservation measures were implemented and natural production objectives became the driving force of compensation programs (Bowles and Leitzinger 1991, IDFG 1992). These programs utilize modified broodstock strategies, supplementation, naturally oriented rearing and release strategies, and intensive monitoring and evaluation to help meet conservation objectives. Beginning with brood year (BY) 1991, all hatchery chinook were externally marked prior to release. By 1995, all but 3-ocean returning adults will be distinguishable into three groups: naturally reared fish (unmarked), hatchery reared fish for supplementation (pelvic fin clip) and hatchery reared fish for reserve production (adipose fin clip).

### OBJECTIVES OF COMPENSATION HATCHERIES

Chinook hatcheries in Idaho were constructed with the goal of using artificial production to provide harvest opportunities lost through hydropower development. Restoring fisheries remains an important product of recovery (Idaho Code, Title 36) Managers now recognize that the assumed mitigation benefits expected from these upper basin hatcheries cannot be realized until juvenile chinook survival through the mainstem hydrosystem is improved (Petrosky and Shaller 1993).

Interim conservation objectives for these areas with mitigation hatcheries are to utilize natural and hatchery production to maintain as much locally evolved genetic material as possible until system improvements allow for legitimate recovery (IDFG 1992). The overriding priority is recovery of sustainable naturally reproducing chinook populations. This is not only vital to the preservation of the chinook species, but also the foundation for sustainable harvest opportunities in the future.

#### OPTIONS

There are several basic options regarding use of facilities and the three groups of returning adults in an attempt to meet conservation objectives.

- 1. Shut down hatchery facilities and allow all returning adults to spawn naturally.
- 2. Allow all unmarked fish to spawn naturally and retain all marked fish for hatchery production.
- 3. Utilize the hatchery to supplement naturally spawning fish, preserve genetic material and maintain viable populations.
- 4. Retain all fish for hatchery production.

Each of these options must meet the interim objective to maximize effective size of the locally evolved population. This objective has several facets: maximize the number of fish retaining local genetic characteristics, optimize utilization of natural spawning and rearing habitat, and minimize adverse impacts to adjacent populations. Several basic assumptions must be met to minimize risk and successfully integrate hatchery and natural production systems.

- Hatchery reared fish have an adult-to-adult survival advantage over naturally reared fish.
- Mining of natural fish for hatchery broodstock does not reduce the effective population size below critical level or result in loss of within population diversity.
- Release of hatchery reared fish does not swamp the natural target population and reduce within population diversity or alter population identity, and does not swamp non target natural populations through straying and loss of among population diversity.
- Hatchery practices do not promote genetic drift and artificial selection, which may cause loss of within population diversity and population identity.
- Hatchery practices and products do not impair the natural behavior or health of the locally evolved stock.
- Monitoring and evaluation has adequate power to resolve critical uncertainties, contain risks and allow managers to adapt the program accordingly.

#### APPROACH

Sustainable recovery of the number and inherent diversity of naturally reproducing chinook is only possible by improving mainstem juvenile migration conditions (Petrosky and Shaller 1993). Other options, such as artificial propagation and habitat improvement, may be important but are limited in potential benefits and focus on enhancing only a small portion of the natural diversity structure (Kapuscinski et al. 1991, Bowles 1992, RASP 1992).

Within this constraint, managers have recognized the potential benefits supplementation may provide as an interim measure to stem the decline of naturally reproducing populations (CBFWA 1990, CRITFC 1990, IDFG 1992, NPPC 1993). Existing mitigation/compensation facilities provide a unique opportunity to assess the utility of supplementation. Risks are more manageable and acceptable in these areas because natural populations have already been influenced by hatchery production.

We believe using these compensation/mitigation hatcheries in a manner that successfully integrates natural and artificial production is the best option for meeting conservation objectives. This decision is based on the assumptions that the criteria listed above for successful integration can be met, and that all three groups of chinook salmon returning to these production areas are important to recovery (i.e., locally evolved genetic material is relatively intact and represented in both hatchery and naturally reared fish).

The unmarked naturally reared group is our top priority, and afford the highest protection and care. Our second priority is the supplementation fish reared in a hatchery to enhance natural production. Natural fish make up at least half of this group's parents, and they are genetically similar to the unmarked group. The last group of fish are the "reserve" fish, which are third in priority but still important. This group is the closest genetic material available, should the natural and supplementation fish collapse. The reserve bank may play a vital role in avoiding severe bottlenecks from stochastic events and natural variability.

How do we best utilize these three groups to attain optimal conservation benefits without causing unacceptable risks? As stated, the primary emphasis should be placed on the unmarked naturally reared adult returns. This is the target group and true measure of recovery success. Genetically conservative criteria to protect these fish and established quide should be These criteria and the response of this supplementation efforts. group of fish should drive the entire program, -- not hatchery capacity, egg availability or harvest considerations.

Management of supplementation fish should not compromise these natural production criteria. The number of natural fish determine how many supplementation fish are integrated into the naturally rearing and reproducing group. The number of natural adults also determines the size of the supplementation broodstock, which should be comprised of at least 50% natural fish. This supplementation broodstock is the "bank" used to rebuild the naturally spawning group, but at a rate that avoids swamping (e.g., "50:50 rule", hatchery reared fish spawning or rearing in the natural habitat should not exceed the number of natural fish).

The third group of fish serves as a "reserve bank" to preserve locally evolved genetic material and augment natural production during severe bottlenecks when the natural and supplementation groups drop below established thresholds for population viability. This group, which has primarily hatchery-by-hatchery (HxH) parental crosses, is only useful in this role if it maintains genetic and ecological similarity to the local naturally spawning group, and does not compromise adjacent naturally spawning populations from straying. Fish culture and monitoring activities should all focus toward meeting these demands.

### MANAGEMENT OF FISH GROUPS

We have developed criteria to guide the management of these three important groups of fish (Table 1). These criteria reflect current conservation concepts and standards within the Columbia River Basin (CBFWA 1990, Kapuscinski et al. 1991, RASP 1992, Hard et al. 1992,NPPC 1993), but are understandably conceptual. Refinement of these standards through quantitative modeling and experimentation is necessary but, until this is done, we have chosen to err on the side of conservancy.

## Naturally Spawning Adults

The role of natural fish is to preserve the natural productivity and diversity that make these locally evolved fish vital to the perpetuation of the chinook species and future harvest opportunities.

- At least 67% of naturally produced adults (unmarked) from throughout the run should be allowed to spawn naturally. This minimizes the risk of "mining" and subsequent loss of effective natural spawners below acceptable levels.
- At least 50% of adults spawning naturally should be of natural origin (unmarked). Adult returns from the supplementation group can be used to make up the remaining 50% of fish spawning naturally. This criteria minimizes the risk of "swamping" and subsequent loss of within population diversity and population identity. This criteria should only be compromised when numbers of natural and supplementation fish fall below thresholds where risk of extinction from stochastic events or genetic bottlenecking overrides risk of swamping. In this situation, the 50:50 rule would be violated to maintain a minimum population level, utilizing fish from the genetically similar "reserve" bank.
- Harvest should never target naturally produced fish until recovery is secure.

#### Supplementation Fish

The role of these fish are to utilize the survival advantage gained in the hatchery to increase the number of fish available to spawn naturally. These fish will be integrated directly with the natural fish, so success is dependent on these fish remaining genetically and ecologically similar to the natural fish.

- The number of fish reared for supplementation should be determined by natural fish escapement and the 50:50 rule to minimize risk of "swamping". If supplementation fish are released as parr, their numbers should not exceed the number of natural parr or rearing capacity of the habitat. If `able 1. Conservation framework for management of three groups of chinook salmon returning to compensation hatcheries in Idaho.

	FISH GROUP	
	Hatcher	y-Reared
Natural-Reared	Supplementation	Reserve
\$		
	Purpose	
Preserve natural productivity and diversity as unique components of chinook species, and foundation for future harvest opportunities	Increase natural production of local populations without impairing natural productivity	Maintain/enhance effective size of local genetic material and augment natural production when at critical levels
	Priority	
First	Second	Third
	ESA Designation	
Listed	Listed	Listed
	Adult Allocation	
$\geq$ 67% allowed to spawn naturally	Fish allowed to spawn naturally cannot exceed number of naturally-reared spawners	None allowed to spawn naturally, unless natural and supplementation spawners are below critical threshold
$\leq$ 33% retained for supplementation proodstock	Remainder of fish retained in hatchery for supplementation broodstock	Remainder retained for "reserve bank" broodstock
	Broodstock	
≥50% natural origin	≥50% natural origin	Nearly 100% hatchery origin
≤50% hatchery origin (supplementation fish)	$\leq$ 50% hatchery origin (supplementation fish)	Small portion (3%?) natural origin, to avoid genetic drift Note: male natural gametes may be adequate
	Spawning	
Natural	Non-selective for size, age, origin	Non-selective size, age
	1:1 sex ratio	1:1 sex ratio
	Factorial crosses, if necessary	Factorial crosses, if necessary
	Rearing	
Natural	Separate from reserve fish	Separate from supplementation fish
	Natural-oriented techniques	Natural-oriented techniques
	Natural growth schedule	Natural growth schedule
	Innoculation and treatment	Innoculation and treatment
	Marking	
Up to 2,000 PIT tags in parr, presmolts,	100% pelvic fin clipped	100% adipose fin clipped
or smolts	$\geq$ 500 PIT tags	Portion CWT for U.S. v. Canada
		$\geq$ 500 PIT tags

Table 1. Continued.

	FISH GROUP			
	Hatchery-Reared			
Natural-Reared	Supplementation	Reserve		
	Releases			
N/A	Volitional or timed to coincide with natural emigration	Volitional or timed to coincide with natural emigration		
	Off-station releases scattered throughout target natural production areas.	Acclimation where feasible		
	Parr releases cannot exceed natural parr numbers and carrying capacity	Smolt or fall presmolt stage		
	Fall presmolt and smolt releases should represent adult equivalents expected from natural production			
	Harvest			
None targeted	None targeted	Utilized as a tool to maintain spawning escapement below thresholds established for straying and rearing criteria		
	Monitoring and Evaluation	#		
Genetics	Genetics	Same as supplementation fish, except:		
Health	Health	Adult escapement to Lower Granite Dan to predict rack returns		
Juvenile abundance/density	In-hatchery performance and survival	-		
Juvenile distribution and habitat utilization	Release characteristics: Size Location/date Method			
Emigration characteristics	Post-release behavior and distribution			
Adult run/spawner characteristics: Number Age structure	Adult run/spawner characteristics			
Sex ratio Run timing Spawning distribution and timing	Straying into non-target areas			
Survival characteristics: Prespawn Egg to parr Parr to emigrant Emigrant to smolt Smolt to adult	Post-release survival characteristics: Release to smolt Smolt to adult Prespawn			

.

.

supplementation fish are released as smolts, their numbers should be designed to bring back only as many adults as anticipated from naturally reared fish.

- Spawning and rearing strategies should focus on minimizing genetic and behavioral divergence from the target population being supplemented.
  - At least 50% of the supplementation broodstock should be comprised of unmarked adults that were reared in the natural environment. The remaining 50% of the supplementation broodstock can be comprised of hatchery reared fish (marked). This criteria will help avoid domestication, genetic drift, and loss of effective population size.
  - Rearing strategies should be designed to circumvent random natural mortality events, but mimic selective natural mortality events.
- Release strategies should be designed to minimize first generation interaction and "swamping" effects (e.g., release at smolt stage, distribute releases throughout target natural production area, parr or presmolt releases should match natural fish size, good health).
- Harvest should not target these fish until recovery is secure.

### Reserve Fish

The role of reserve fish is to maximize locally evolved genetic material available to recovery, and provide a reserve "bank" to augment natural production if levels drop below critical thresholds. This latter need may result from stalled recovery actions, stochastic environmental events, or natural variability. To serve this function, reserve fish must remain genetically and ecologically similar to the target population, and must be maintained at levels that will not adversely impact target and non target populations through straying and interactions. The challenge is to maintain as much reserve as possible without genetic drift, inadvertent hatchery selection and domestication, and without harm to adjacent natural populations. This challenge is accentuated because the reserve group will be predominantly HxH crosses.

### Minimize Genetic Drift

The reserve group currently has similar genetic and ecological characteristics as the target natural population. This similarity results from an inability to differentiate hatchery and natural returns and broodstock strategies promoting constant and thorough mixing. As marked fish return and differentiation becomes possible, risk of genetic drift also increases.

To avoid random genetic divergence, gametes from natural origin adults should be infused into the reserve group at a rate designed to maintain drift within acceptable detection limits. This rate of infusion can be quantified based on genetic monitoring of each group of fish. Initially, the rate should be quite low (<3%) because the groups are genetically similar. As multiple generations of reserve fish occur, the infusion rate will likely increase to maintain this similarity.

The rate of infusion to avoid genetic drift may determine the maximum number of reserve fish to propagate. Our top priority is natural fish and only a limited number of natural fish can be "mined" to maintain the integrity of the reserve fish. Innovative techniques, such as partial male spawning prior to release above the weir, may help reduce this constraint.

#### Avoid Domestication and Inadvertent Hatchery Selection

Natural oriented rearing techniques should be utilized to minimize inadvertent hatchery selection and avoid behavior modifications that may result in adverse interactions with natural fish (Cannamela 1993, RASP 1992, Bowles 1993). Our ability to culture fish to meet these specifications may also determine the maximum number of reserve fish to propagate (e.g., low density).

### Minimize Adverse Interactions

Although maximizing effective population size is important, it is also vital to maintain numbers and quality of this reserve group at levels that will avoid harmful straying, disease transmission, and genetic and behavioral divergence.

To contain risk of straying, managers should: .

- establish acceptable straying rates based on genetic similarity, potential outbreeding depression, loss of among population diversity and population identity;
- 2) develop and incorporate release strategies that maximize return integrity (e.g., imprinting cues, acclimation, smoltification, etc.);
- 3) Monitor straying and limit production of reserve fish to maintain straying within specified limits.

To contain risks of disease transmission, health management should incorporate state-of-the-art prophylactic and therapeutic techniques. Strategies to contain risk of genetic and behavioral
divergence were discussed above and include conservative broodstock management and natural oriented rearing techniques.

## Management of Reserve Fish Numbers

Managing levels of reserve fish to avoid unacceptable risk to natural production is vital to the success of the program. Obviously, the upper limit of fish maintained in the reserve bank will not necessarily be determined by hatchery capacity. Quantifiable limits are currently lacking, but may be below facility capacities. Until uncertainties are better resolved, initial criteria should err on the side of the natural component and maintain conservative numbers of reserve fish.

Production can be managed to minimize surpluses in most years. Managing at levels that will never produce surpluses is risky and undesirable because of high annual variability in system productivity. Management contingencies must be in place to handle potential escapement surpluses of reserve fish that variable system productivity may cause.

One option for surpluses is to spawn all reserve fish and dispose of eggs in excess of established criteria. This approach is undesirable because it fails to remove surplus fish before straying effects occur.

Fisheries may be the most effective tool for managing spawning escapement of reserve fish. Fisheries can remove surplus fish prior to straying and thus keep straying rates into adjacent drainages at acceptable levels. This approach would also allow exercise of lawful harvest privileges and responsibilities during the recovery process. Harvest would be appropriate during years when returns of reserve fish are high enough to cause unacceptable risk from straying, hatchery rearing capacities, and criteria relating to fish quality and behavior.

This approach represents a substantial conceptual shift from the original mandate for construction of mitigation/compensation hatcheries. The shift is from managing production as a tool for harvest, to managing harvest as a tool for production. Although this represents a conceptual shift, in practice the shift is much less substantial. Severe system constraints (i.e, mainstem survival bottlenecks) have precluded legitimate harvest opportunities since 1978. Compensation facilities have typically operated in a conservation mode well below capacities, attempting to maintain the maximum number of adult chinook possible until system survival improvements are made.

#### UNCERTAINTIES

Successful utilization of harvest as a tool to manage production is dependent on several assumptions.

- 1. Run size of reserve fish can be predicted accurately and with enough sensitivity to implement harvest strategies.
- 2. Harvest can be selective for reserve bank fish.
- 3. Catch and release of natural and supplementation fish can be managed at low enough levels to avoid unacceptable prespawn mortality and behavioral changes (e.g., temporal or spatial shift in spawning).
- 4. ESA guidelines are flexible enough to accommodate harvest of listed fish, recognizing that lack of harvest during high return years may result in unacceptable risks to recovery. Note: Although predominantly HxH crosses, the reserve fish would be "listed" because of periodic incorporation of natural fish to avoid genetic drift.

Federal regulations for conservation of threatened species may provide for direct take (16 USC, 1533 d). The Ninth Circuit Court upheld regulations which authorized "a carefully controlled and limited sport hunt of grizzly bears in designated geographical regions..." (Christy v. Hodel 857F.2d 1324, 1988). Permitting limited sport hunting in particular areas was consistent with the goal of conserving the species.

Numerous other uncertainties exist which preclude completely confident and risk free integration of compensation/mitigation programs into the recovery process. Some of these include:

- Can routing fish through a hatchery environment provide an adult-to-adult survival benefit over allowing fish to spawn and rear in the natural environment?
- Can spawning, rearing and release strategies be implemented to insure long term genetic and ecological similarity between hatchery and natural fish?
- How much and how frequent must natural genetic material be introgressed with reserve fish to avoid genetic drift?
- What are acceptable straying rates (as a percentage of local escapement) into non-target production areas?
- Can straying rates be maintained within acceptable levels by limiting production of the reserve fish and utilizing appropriate release strategies?

# MONITORING AND EVALUATION

Monitoring and research should be designed and implemented to resolve these uncertainties and identify unacceptable risks. We believe the uncertainties and risks associated with this approach are manageable within a monitoring and evaluation program so that implementation can proceed. This feedback loop allows the program to adjust accordingly if risks become too high or success too unrealistic. Much of the structure for monitoring and evaluating is already in place in Idaho through the Idaho Supplementation Studies (Bowles and Leitzinger 1991) and LSRCP Hatchery Evaluation Study (Cannamela 1993).

# IMPLEMENTATION

The underlying premise supporting this approach is that all three groups of fish (natural, supplementation, reserve) returning to upper basin production areas are important to maintain recovery options. If this premise is accepted, we believe this approach is the most logical and effective way to preserve the locally evolved population until mainstem hydrosystem constraints to productivity are remedied and recovery occurs.

For this approach to be feasible and successful, several conditions must be met.

- Ongoing mitigation/compensation programs provide at least a slight adult-to-adult survival benefit over naturally reared fish.
- The natural production area is not currently limited by spawning or rearing habitat.
- External differentiation is possible among natural, supplementation and reserve fish.
- Hatchery and natural fish currently share similar genetic and ecological characteristics.
- Spawning, rearing and release strategies are implemented to minimize risk of inadvertent hatchery selection and domestication, and promote natural behavioral characteristics.
- Intensive monitoring and research is in place to allow individual programs to adjust as uncertainties are resolved and risks identified. This should include:
  - genetic characteristics/profiles of both hatchery groups, natural target fish, and adjacent natural populations;
  - straying rates;
  - temporal and spatial spawning distributions;
  - health status and disease transmission;

- hatchery/natural interactions;
- hatchery and natural life history characteristics; and
- hatchery and natural survival coefficients.

Fish managers in Idaho began recognizing the need to shift to this approach in the late 1980s and are thus in a good position to meet these considerations for all upper basin chinook hatcheries located in critical natural production areas. Ongoing monitoring and research programs are already in place to meet the majority of evaluation requirements. These programs have been designed to provide adequate analytical power to detect adverse effects and allow managers to adjust accordingly.

- Bowles, E. 1992. Upriver supplementation and recovery options. The Osprey. Newsletter of the Federation of Flyfishers, Issue 16, September 1992, Seattle, Washington.
- Bowles, E.C. 1993. The hatchery challenge. Proceedings of Salmon Management in the 21st Century: Recovering Stocks in Decline. 1992 Northwest Pacific Chinook and Coho Workshop, Idaho Chapter of the American Fisheries Society, Boise, Idaho.
- Bowles, E.C. and E. Leitzinger. 1991. Salmon supplementation studies in Idaho rivers (Idaho supplementation studies). Idaho Department of Fish and Game, Experimental Design, Bonneville Power Administration Project 89-089, Boise, Idaho.
- Cannamela, D.A. 1992. Fish Hatchery evaluations Idaho. Annual Report for 10/89 - 9/90. Prepared for USFWS Lower Snake River Compensation Plan, Idaho Department of Fish and Game, Boise, Idaho.
- Cannamela, D.A. 1993. Natural rearing experimental design. Appendix A in FY1993 - FY1995 Three-year Plan, Lower Snake River Compensation Plan Hatchery Evaluation Studies, Idaho Department of Fish and Game, Boise, Idaho.
- Columbia Basin Fish and Wildlife Authority. 1990. Integrated system plan for salmon and steelhead production in the Columbia River basin. Public Review Draft. Prepared for Northwest Power Planning Council, Portland, Oregon.
- Columbia River Inter-Tribal Fish Commission. 1990. Integrated tribal production plan, Volume 1: production proposal for recovery of Snake River stocks. Columbia River Inter-Tribal Fish Commission, Portland, Oregon.
- Hard, J.J., R.P. Jones, Jr., M.R. Delarm, and R.S. Waples. 1992. Pacific salmon and artificial propagation under the Endangered Species Act. U.S. Department of Commerce, NOAA Tech. Memo. NMFS-NWFSC-2, 56 p.
- Herrig, D.(editor). 1991. Snake river hatchery review 1990 workshop summary. USFWS Lower Snake River Compensation Plan Office, Boise, Idaho.
- Idaho Department of Fish and Game. 1992. Anadromous fisheries management plan 1992-1996. Idaho Department of Fish and Game, Boise, Idaho.
- Kapuscinski, A.R., C.R. Steward, M.L. Goodman, C.C. Krueger, J.H. Williamson, E. Bowles, R. Carmichael. 1993. Genetic conservation guidelines for salmon and steelhead

supplementation. Fishery Bulletin: in review. Synthesis paper from Northwest Power Planning Council Sustainability Workshop, 1991, Portland, Oregon.

- Marshall A.R. 1993. Genetic analysis of 1992-93 Idaho chinook salmon baseline collections, and a comparative analysis with 1991 collections. Annual Report to Idaho Department of Fish and Game. Washington Department of Fisheries, Seattle, Washington.
- Northwest Power Planning Council. 1993. Columbia River Basin fish and wildlife program - strategy for salmon - volumes I and II. Document 92-21 and 92-21A, Northwest Power Planning Council, Portland, Oregon.
- Petrosky, C.E. and H.A. Shaller. 1993. A comparison of the productivities for Snake River and lower Columbia River spring and summer chinook stocks. Proceedings of Salmon Management in the 21st Century: Recovering Stocks in Decline. 1992 Northwest Pacific Chinook and Coho Workshop. Idaho Chapter of the American Fisheries Society, Boise, Idaho.
- Regional Assessment of Supplementation Project. 1992. Summary report series for the regional assessment of supplementation project. Prepared for the Bonneville Power Administration, Portland, Oregon.
- Waples, R.S., O.W. Johnson, P.B. Aebersold, C.K. Shiflett, D.M. VanDoornik, D.J. Teel, A.E. Cook. 1993. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River basin. Annual Report to the Bonneville Power Administration, Project 89-096, National Marine Fisheries Service - CZESD, Seattle, Washington.

Extended Abstract for 1994 Fish Culture Conference, Sun River Oregon.

Managing hatcheries within Oregon's Wild Fish Management Policy Guidelines. From theory to practice. Rhine T. Messmer. Oregon Department of Fish and Wildlife, 211 Inlow Hall, EOSC, 1410 L Avenue, LaGrande OR, 97840.

In N.E. Oregon, the Lower Snake River Compensation Plan annually produces thousands of summer steelhead and spring chinook salmon juveniles for release in the Grande Ronde and Imnaha River basins. These juveniles are produced under conventional and supplementation types of hatchery programs. The primary objective of a conventional type of hatchery is to maximize production of adult fish which contribute to commercial, sport, and tribal fisheries. The primary objective of supplementation hatcheries is to enhance natural production through artificial propagation and secondarily to produce adult fish for commercial, sport, and tribal fisheries. In recent years, some hatchery operations have come under criticism because of potential affects of hatchery produced fish on native fish populations. The main area of concern involves the interbreeding of hatchery with wild fish which may pose a risk to conserving and protecting the genetic resources of wild populations. Hatchery fish that are poorly suited to perform in the wild and that spawn with wild fish may reduce wild population performance. In order for fish populations to maximize production potential, they must retain characteristics which promote survival in the natural environment. It is also important that the natural populations retain genetic variation in order to persist under changing environmental conditions.

Oregon's Wild Fish Management Policy was passed by the Oregon Fish and Wildlife Commission for the main purpose of protecting the genetic resources of Oregon's native fish species. The Wild Fish Management Policy establishes guidelines for the percentage of naturally-spawning hatchery fish based on the genetic characteristics of the hatchery population. Management options under the Wild Fish Management Policy include 1) release no hatchery fish, 2) release hatchery fish that meet the following standards and limit their abundance to 50% or less of the breeding population. Standards for hatchery fish are as follows: originates from the endemic stock, a minimum of 30% wild parentage on average across brood years, no more than 25% of the wild donor population taken for hatchery broodstock in any year, no intentional artificial selection and unintentional artificial selection is avoided, and wild-type phenotypes are maintained in hatchery fish. Option 3 is to release hatchery fish which do not meet standards in option 2, but restrict hatchery spawners to 10% or less of the naturally-spawning population. A conventional hatchery program would generally operate under option 3, whereas a supplementation hatchery program would operate under option 2.

The LSRCP program has changed some of the management practices and hatchery operations in order to comply with the Wild Fish Management Policy. For conventional hatcheries, changes have occurred which are targeted at reducing the number of hatchery origin spawners to 10% or less of the natural spawning populations. For the Grande Ronde Basin summer steelhead program, we have reduced the number of direct stream summer steelhead smolt releases and reallocated these fish to existing acclimation ponds. We have also recommended exploring the possibility of locating acclimation ponds and adult traps in areas currently receiving direct stream releases (Catherine Creek and upper Grande Ronde River). For Grande Ronde spring chinook salmon program, straying of hatchery origin adults into natural production areas exceeded acceptable levels for wild fish management and the Endangered Species Act. This problem has been magnified since wild returns of spring chinook to the Grande Ronde Basin are severely depressed. To prevent straying of Rapid River stock spring chinook released from Lookingglass Hatchery, we have marked all smolt releases so returning adults can be trapped at Lower Granite Dam and hauled to Lookingglass Hatchery. We have also reduced the number of smolts released from Lookingglass Hatchery. This management strategy should result in very few hatchery fish straying into natural production areas. We also plan to develop endemic stocks for hatchery production, but sources of broodstock are severely limited at this time.

The summer steelhead and spring chinook salmon supplementation programs in the Imnaha River Basin have more constraints placed on them by the Wild Fish Management Policy because they intentionally release adults into the naturally-spawning population and therefore have greater inherent risks. The summer steelhead and spring chinook programs in the Imnaha River Basin utilize hatchery broodstock which originated from endemic stocks and therefore 50% of the natural spawning population can be of hatchery origin if the hatchery stock meets the standards of the Wild Fish Management Policy. Supplementation programs are most often started with populations of wild fish which have depressed production potential. Thus, wild numbers of fish are often not available to allow for full achievement of production goals because only 25% of the wild run can be retained for hatchery broodstock under Wild Fish Management Policy guidelines. The challenge in implementing supplementation programs is to develop adult broodstock collection and release strategies which will meet management and research objectives and still comply with the Wild Fish Management Policy. These strategies require extensive planning and coordination. For both the Little Sheep Creek summer steelhead and Imnaha River spring chinook salmon programs, preseason estimates of hatchery and wild returns, adult run timing, sex and age ratios, and spawning timing are developed. We then determine trapping and release strategies which will meet Wild Fish Management Policy guidelines. The number of hatchery and wild fish retained for broodstock is then determined and production goals are developed. Spawning strategies are utilized which will meet the broodstock requirements of the Wild Fish Management Policy. Since estimates of returning adults are often inaccurate, contingency plans have to be developed which allow for in-season adjustments in broodstock collection and release guidelines. Returns of adults are closely monitored in order to determine if contingency plans have to be implemented. It is essential that close communications are maintained between hatchery staff and research/management biologists in order to effectively and efficiently implement hatchery operations and achieve management and Wild Fish Management Policy goals.

Oregon's Wild Fish Management Policy is designed to protect the genetic integrity of our wild populations. Our hatchery programs often rely on wild populations in order maintain broodstock which are best adapted for supplementing depressed wild populations. Also, additional wild fish may become sources of hatchery broodstock to help rebuild and preserve severely depressed stocks of anadromous fish. Under Oregon's Wild Fish Management Policy, hatchery programs can no longer operate independently of wild fish populations, but have to be managed as part of the entire system in which they operate. It is possible that hatchery programs can comply with the Wild Fish Management Policy and in some cases still achieve adult return goals to compensation areas if the productivity and habitat of wild fish populations is improved and maintained.

# Integrating Artificial Propagation Programs into Ecosystem and Natural Production Management: Can It Be Done?

Mobrand Biometrics Inc.

Larry Lestelle, Jim Lichatowich and Lars Mobrand

# ABSTRACT

We propose an approach to the development of restoration programs for Pacific anadromous salmon that recognizes the importance of an ecosystem perspective. Important concepts such as habitat complexity and self organizing capacity of the stock are reviewed. A planning process comprised of six steps is described. The approach includes a comparison of historic and current habitat complexity and connectivity and intrapopulation life history diversity. Uncertainties are incorporated into the planning process through assumptions which are clearly identified. Risk of project failure is determined through a qualitative or quantitative weighing of the critical uncertainties. We emphasize the concept that restoration planning is an iterative process that must be continued after implementation. We examine the role of artificial propagation in the context of this planning approach. Uncertainties and risks associated with the use of artificial propagation are discussed within the premise of our conceptual framework.

# SESSION V

# FISH CULTURE PRACTICES

- The Use of Various Fertilization Media and Their Effects on Viability of Erwin Strain Rainbow Trout Eggs - Wes Orr, U.S. Fish and Wildlife Service
- Eye-up Success of Arlee Rainbow Trout Eggs as Affected by the Stage of Expulsion - Dan Brown, U.S. Fish and Wildlife Service
- Effect of Varied Milt Volume on Eye-up Success of Kamloop Rainbow Trout Eggs - Dan Brown, U.S. Fish and Wildlife Service
- Diel and Circannual Changes in Ammonium Excretion by Spring Chinook at Willamette Hatchery - Dick Ewing, Biotech Research and Consulting, Inc.
- Purina Floating Feed Diet Test at San Joaquin Hatchery Anna Kastner, California Department of Fish and Game
- Hatching Jars, Another Alternative Anna Kastner, California Department of Fish and Game
- Dworshak Hatchery 25 Years Later Dave Owsley, U.S. Fish and Wildlife Service
- The Effects of Light Intensity and Photoperiod on the Growth Response of Fingerling White Sturgeon - Brian Hickson, U.S. Fish and Wildlife Service

# THE USE OF VARIOUS FERTILIZATION MEDIA AND THEIR EFFECTS ON VIABILITY OF ERWIN STRAIN RAINBOW TROUT EGGS

Daniel R. Brown, John B. Shrable, and Wes H. Orr

U.S. Fish and Wildlife Service Ennis National Fish Hatchery 180 Fish Hatchery Road Ennis, MT 59729

# ABSTRACT

Brown, D. R., Shrable J. B., and Orr, W. H. 1994. The use of various fertilization media and their effects on the eggs and sperm of erwin strain rainbow trout eggs.

Artificial insemination of salmonids is a common, though unperfected, fish cultural practice well over 100 years old. Over the years much of the effort to improve insemination methodology has focused on the fertilization process.

This study evaluates egg survival to the eyed stage using 5 different fertilization diluents at dilution rates of 10<sup>-2</sup> and 10<sup>-3</sup>. It also examines the duration of forward and total sperm motility achieved with each diluent.

There was no significant difference in percent eye-up when saline or modified saline diluents were administered, but when water was used percent eye-up was significantly and adversely affected at both dilution rates (P<0.01). There was a significant effect of diluent on both forward and total sperm motion (P<.0001).

Water chemistry and pH at different hatcheries.or laboratories can influence the efficacy of any fertilization medium. With this in mind we recommend testing simple saline solutions or other diluents besides water for their possible value as fertilization mediums.

# INTRODUCTION:

Artificial insemination of salmonids is a common, though unperfected, fish cultural practice. Much of the research effort improve embryo survival has focused on the fertilization to process. Sperm dilution in saline solutions similar in composition to physiological saline levels (0.89%) has been reported to improve fertilization rate (Nomura 1964). Billard et al. (1974) studied the effects of various fertilization diluents on eggs and sperm and reported that a modified saline diluent (organically buffered to pH  $9\pm0.5$ ) allowed for use of a minimum amount of sperm to obtain a maximum rate of fertilization in rainbow trout eggs. Billard (1980) reported that sperm motility was intensified and prolonged by adding theophylline to the saline diluent. Scheer and Thorgaard (1989) reported the addition of theophylline to the fertilization media improved fertilization rate with cryopreserved RBT sperm. Billard and Cosson (1989) reported that sperm motility was prolonged beyond 30 s when 1 mM Ca++ was added to a modified saline diluent. Petit et al. (1973) reported that pH and osmotic pressure determining of factors in the success important а were fertilization diluent. At Ennis NFH, using a 0.75% saline fertilization diluent in place of water resulted in a 15% increase in egg survival to the eyed stage (Orr & Shrable unpublished 1988). The literature reports wide variation in sperm:egg ratios for optimum fertilization. Wharton (1957) and Plosila & Keller (1974) recommend about 10,000,000 sperm/egg for maximum fertilization. Billard et al. (1974), reports the minimum number of spermatozoa per egg for fertilization to be 200,000, and later (1977) recommends providing 500,000 to 1,000,000 sperm per egg for optimum fertilization success. Moccia et al.(1987), reported that when sperm:egg ratios were > 200,000:1 neither sperm motility nor sperm density were major factors in determining the percent of eggs reaching the eyed stage. They also reported that late in the reproductive season there was a significant correlation between sperm density and fertilization rate, but no significant relationship between sperm motility and fertilization rate.

Spawning seasons at Ennis are characterized by low fertilization rates and few spermiating males at first, high fertilization rates with many spermiating males during the peak, and low fertilization rates with many spermiating males at the end of the spawning season. What causes lower egg survival at the beginning and end of the spawning cycle?

Objectives were 1) evaluate several fertilization mediums for their effect on survival to the eyed stage of development, 2) examine the effect of sperm dilution on eyeup, and 3) determine if there is a correlation between sperm motion and egg survival using the different diluents described.

# MATERIALS AND METHODS:

PART I. Diluents and Dilution Rate Procedures:

Five different fertilization media: 1) spring water, 2) 0.75% saline, 3) D532 (a commercial diluent composed of 0.75% saline, 20 mM Tris, and 50 mM Glycine), 4) D532 + 5 mM Theophylline and

5) D532 + 10 mM CaCl<sub>2</sub> were prepared in one liter quantities and refrigerated at  $48^{\circ}$  F.

Twelve spermiating erwin strain rainbow trout were stripped, the milt was pooled into a plastic container and mixed gently by swirling, then placed in an insulated box cooled with ice. The eggs from 24 ripe Erwin females were air stripped, gently but thoroughly mixed together in a plastic container and placed in an insulated container cooled with ice. All gametes were transferred indoors. Thirty, 1 gallon buckets were labeled for 3 replicates each of the 5 diluents at both dilution rates of 10<sup>-2</sup> and 10<sup>-3</sup>. One hundred ml of eggs (approximately 2700) were randomly added to each of these buckets from the pooled egg lot. Six buckets of eggs received 50 ml of the first diluent, 6 buckets received 50 ml of the second diluent, etc.

Using a serological pipette, 0.05 ml of milt (10<sup>-3</sup> dilution) was added to each of 15 egg/diluent mixtures (3 replicates of 5 diluents), and 0.50 ml of milt (10<sup>-2</sup> dilution) was added to the remaining 15 replicates (Table 1). All replicates were gently swirled after milt addition to mix the contents. Each replicate was allowed to stand undisturbed for 1 min, decanted, and rinsed with fresh water. Eggs in each replicate were then water hardened in 500 ml of a 50 mg/L iodophor solution. After 30 minutes the eggs from each replicate were carefully poured into separate 1 L capacity upwelling incubators (54° F). Eggs were treated for 15 minutes daily with 1200 mg/L formalin to prevent fungus, and were exposed to weekly iodophor treatments of 40 mg/L active iodine for 10 minutes to prevent soft shell disease. They were mechanically shocked on the 15th day of incubation (330 TU), and sorted on day 16 with an electronic egg picker (Jensorter Model JX-4). Numbers of eyed and non-viable eggs were enumerated with an electronic egg counter (Jensorter Model BC). Data was statistically analyzed with the Statistical Analysis System (SAS 1988).

PART II. Sperm Motility Procedures:

Using a small eye dropper, 1 drop of diluent was expressed onto a clean glass slide and aligned under the microscope. The point of a regular teasing needle was inserted vertically into a container of milt 1/8" thick. The tip of the needle was then inserted into the drop of diluent on the slide and gently stirred in a circular motion 10 times. This method was calculated to be a sperm dilution of 1:500, intermediate between 10<sup>-2</sup> and 10<sup>-3</sup>. The needle was cleaned and dried after each application. Using low power, a phase contrast setting of 10 and a fiber optic light intensity setting of 2, motility was easily observed. A stop watch was used to record the time from activation to when forward motion (a period of intense flagellar activity which propels the sperm forward) ceased in 95% of the sperm cells in the field of view. A second timed reading recorded the total time from activation to when no further sperm motion (static sperm movement caused by slight flagellar activity) could be seen in the field of view. This was repeated 5 times for each diluent.

#### RESULTS AND DISCUSSION:

Of the five diluents tested, water was the only diluent that caused a significant difference in egg survival (P<0.05). There was also an effect of dilution rate when water was used. When the milt dilution rate was  $10^{-2}$  percent eyeup was 51.4. Using a dilution rate of  $10^{-3}$  egg survival dropped to 22.4%.

Using an average sperm concentration of  $10^{10}$ /ml and an egg size of 20,000/L the dilution rate of  $10^{-3}$  provided about 185,000 sperm per egg while the dilution of  $10^{-2}$  provided an estimated 1,850,000 per egg. It appears that the number of sperm cells provided for fertilization were adequate at both dilution rates in every medium except water. The sperm dilution rate routinely used at Ennis is  $2(10^{-2})$  (20ml sperm: 1L diluent: 2L eggs) or an estimated 5,000,000 sperm cells/egg. Does Ennis use more milt than necessary? If so, then the operation could be more efficient if less milt was used and the number of males on hand were reduced. However, experience has shown that gametes may be inferior in quality at the beginning and end of the reproductive season, so a dilution rate providing a higher sperm to egg ratio may be beneficial to eyeup success.

The motility test showed a significant correlation between the diluent used and both forward and total motion. (P<.0001). It was interesting that the increased forward motion gained with D532 + Theophylline, and the extra static motion gained with 0.75% saline made no difference in percent eyeup at either dilution rate.

This investigation seems to have generated more questions than answers but based on our results in naturally buffered spring water at Ennis, we recommend testing a saline fertilization medium as a means of increasing egg survival. Water chemistry and pH may influence the result so we emphasize "testing" before implementation!

#### ACKNOWLEDGEMENTS:

Special thanks to Dr. Rick Barrows of the Bozeman Fish Technology Center, Bozeman, Montana for his effort and assistance in experimental design, review and statistical analysis of this study.

#### REFERENCES

- Billard, R., 1992. Reproduction in rainbow trout: sex differentiation, dynamics of gametogenesis, biology and preservation of gametes. Aquaculture, 100: 263-298.
- Billard, R., 1980b. Reproduction and artificial insemination in teleost fish. 9th Int. Cong. Animal Reproduction and Artificial Insemination. Madrid. Vol. II: 327-337.
- Billard, R., 1977. A new technique of artificial insemination for salmonids using a sperm diluent. Fisheries, Vol II, No. 1: 24-25.
- Billard, R., Petit, J., Jalabert, B. & Szoiloci, D., 1974. Artificial insemination in trout using a sperm diluent. In: Early Life History of Fish (J.H.S. Blaxter, ed.), pp. 715-723. Berlin: Springer Verlag.
- Billard, R. and Cosson, M.P., 1989. Measurement of sperm motility in trout and carp. In: N. de Pauw, E. Jaspers, H. Ackefors and N. Wilkins (Editors). Aquaculture, a Biotechnology in progress. European Aquaculture Society. Bredene, Belgium. pp 499-503.
- Moccia, R. D. and Munkittrick, K. R. 1987. Relationship Between the Fertilization of Rainbow Trout Eggs and the Motility of Spermatozoa. Theriogenology April 1987 Vol 27 No. 4
- Nomura, M., 1964. Studies on reproduction of rainbow trout, Salmo gairdneri, with special reference to egg taking. VI: The activities of spermatozoa in different diluents and preservation of semen. Bull. Jpn. Soc. Sci. Fish., 30: 723-733.
- Petit, J. B. Jalabert, B. Chevassus, R. Billard, 1973. Ann. Hydrobiol. 4:201-210.
- Scheerer, P.D. and Thorgaard, G.H. 1989. Improved fertilization by cryopreserved rainbow trout semen treated with theophylline. Prog. Fish Cult., 51:179-182.

EFFECT OF VARIOUS ACTIVATION MEDIA SPERM MOTILITY DURATION

ACTIVATION MEDIUM	z	FORWARD MOVEMENT*	SD	ALL MOVEMENT**	SD
WATER	S	23.0 A	2.55	<b>55.4</b> D	12.36
SALINE	S	28.4 A	3.28	>600.0 E	00.0
D532	S	26.8 A	2.17	127.O F	25.80
D532+THEO.	4	52.5 B	9.04	<b>252.8</b> G	32.76
D532+CACL	4	<b>34.5</b> C	3.70	148.5 F	16.30

DIFFERING SUPERSCRIPT DENOTES SIGNIFICANT DIFFERENCE (P<0.05) \* PERIOD OF FORWARD MOVEMENT (SEC) \* \* PERIOD OF FORWARD MOVEMENT PLUS STATIC MOTION (SEC)

# EFFECT OF VARIOUS DILUENTS ON % EYE-UP ERWIN STRAIN RAINBOW TROUT EGGS

D532+CAL. 10 <sup>-2</sup>	D532+THEO. 1	D532	0.75% SAL.	WATER	D532+CAL. 1	D532+THEO. 1	D532 1	0.75% SAL.	WATER 1	DILUENT TYPE/ DILUTION RATE
0 2	10 <sup>-2</sup>	10 -2	10 <sup>-2</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	10 -3	10 <sup>-3</sup>	III.C
8.4	8.4	Со 57	7.8	7.8	8.4	8.4	හ .රා	7.8	7.8	DILUENT pH
ť	=	-	=	=	=	=	=		ω	NUMBER OF REPLICATES
62.7 <sup>D</sup>	62.0 <sup>D</sup>	64.4 <sup>D</sup>	61.9 <sup>D</sup>	51.4 <sup>C</sup>	64.0 <sup>в</sup>	66.5 <sup>B</sup>	66.4 <sup>B</sup>	62.5 <sup>B</sup>	22.4 <sup>A</sup>	MEAN EYE-UP %*
0.64	4.20	2.67	0.74	6.63	3.30	1.84	1.44	3.23	14.24	SD

\*Differing superscript denotes significant difference (P<0.05)

# EYE-UP SUCCESS OF ARLEE RAINBOW TROUT EGGS AS AFFECTED BY STAGE OF EXPULSION

# Daniel R. Brown, Wesley H. Orr and John B. Shrable U.S. Fish and Wildlife Service Ennis National Fish Hatchery Ennis, Montana 59729

#### ABSTRACT

Brown, D. R., Orr, W. H. and J. B. Shrable. 1993. Eye-up success of arlee rainbow trout eggs as affected by stage of expulsion.

At present, factors affecting egg quality in salmonids as well as other fishes are not well understood. Of concern to some fish culturists is whether or not egg quality (viability) remains uniform throughout the "stripping episode." In-other-words, is the viability of the first eggs collected from a female the same as eggs from the middle and last stages of the spawn. This study provides evidence that significant differences (P<0.01) in egg viability, measured as eye-up success, can be expected from a particular female at different stages of egg expulsion. Although these findings may not significantly effect overall hatchery reproductive performance, they may impact studies conducted investigating reproductive performance. The methodology of studies evaluating egg viability should include procedures to account for variation in egg quality with respect to stage of expulsion.

# Introduction

To date, there is limited data to explain variation in egg quality in rainbow trout. The majority of data available pertains to the effect of over-ripening on egg viability (Bromage et al. 1992). Kato and Kamler (1983) reported that water content of freshly fertilized rainbow trout eggs (non-waterhardened) was negatively correlated with embryo survival to hatch. Springate et al. (1984) found maximum egg fertilization success could be attained if eggs were stripped 4-6 days post ovulation. According to Bromage et al. (1992), a slight reduction in fertilization rate results in eggs stripped immediately after ovulation because of underripness.

Eggs that exhibit low mortality at fertilization and subsequent early life stages are generally considered to be high quality eggs. However, there is not a general consensus as to what mortality levels are permissible to qualify as high quality eggs. In addition, there is no clear understanding of the factors within the parent fish or the egg itself which may affect egg quality (Bromage et al., 1992).

The objective of this study was to determine if eggs obtained from individual females are uniform in viability depending on the order of expulsion. That is, are the first eggs collected of equal viability as the last, as well as those collected in the middle of the spawn? Uniform egg quality from the first to the last few eggs expelled would allow for eggs to be stripped from females directly into treatment replicates in studies where reproductive performance is measured. Variation in egg viability introduced by order of expulsion would not allow for this simple procedure.

# Materials and Methods

Six 2-yr-old female Arlee rainbow trout were individually stripped by injecting compressed oxygen (air spawning) into the body cavity. The eggs were expelled in lines approximately 50 cm in length. Follow-up "hand passes" were not performed to remove the last few eggs remaining in the body cavity. The spawn from each female was divided into three approximately equal portions by order of expulsion (A; first 1/3; B second 1/3; C last 1/3). Each individual portion from each female was gently stirred then divided into duplicates of equal volume. This process resulted in a total of six lots of eggs from each female, two replicates from each of three stages of the spawn. Saline (100 ml of 0.75%) was added to each lot of eggs (36 lots), followed by two ml of pre-collected milt (pooled from 12 males). Each gamete mixture was gently, but thoroughly stirred and allowed to stand undisturbed for 1 min prior to rinsing in fresh water (54°F). A 50 mg/L iodophor solution (500 ml) was then added to each lot of eggs. Each lot remained in the iodophor solution for 30 min, and was then decanted and rehydrated with fresh water. Each of the 36 egg lots of was randomly assigned to individual upwell incubators (1 L capacity). All incubators received 1/2 gpm waterflow. A formalin bath was administered daily (1200 mg/L for 15 min) to prevent fungal infection. Egg lots were mechanically shocked at the early eyed stage of embryonic

development (330 T.U.). On the following day eggs were sorted for viability with the aid of an electronic picker (Jensorter Model JX-4, Jensorter Inc., 20225 Harvest Ln., Bend, OR 97701). Blank eggs (translucent without visual eye development) were manually removed from each lot and counted. Viable (eyed) eggs were counted with the aid of an electronic counter (Jensorter Model BC). Resulting data were analyzed using the Statistical Analysis System (SAS, 1986). All percentage data were arc-sine transformed prior to analysis.

# <u>Results</u>

Egg viability measured as survival rate at the eyed stage of embryonic development was significantly affected (P<0.0001) by the stage of expulsion. Mean percent eye-up decreased with each successive stage of egg expulsion (Table 1). Frequency of blanks was not significantly affected by stage of expulsion. An interaction (P<0.0001) between stages of expulsion and individual females spawned was also observed (Table 2).

# Discussion

In some cases the action of "hand" stripping can have a negative impact on egg viability. This is most likely to occur in eggs that are the last to be expelled, when several "passes" have been administered and broken or ruptured eggs are the result. Not only are these eggs lost, but egg yolk material (vitellus) from the broken eggs can hinder the fertilization of other eggs (Wilcox et al., 1983). In this study, the last few eggs remaining in the body cavity were not removed by "hand stripping" and could not have impacted the data observed.

Conversely, reduced eye-up success in the last stages of expulsion in this investigation might have been due to "underripness" (Bromage et al., 1992; Springate et al., 1984).

Eye-up success was unaffected by stage of expulsion in eggs collected from three of the females spawned in this study (Table 2). It is conceivable that these three females ovulated a few days before the others allowing for more complete ripening. Another possibility might be that as a result of a greater time interval from ovulation, these three females produced more ovarian fluid than the others which facilitated more "egg mixing" action within the body cavity.

This study offers preliminary evidence that significant variation in egg quality can result in different segments of the spawn from a particular female. When measuring reproductive performance in salmonids, stripping eggs directly into randomly assigned replicates from a female could possibly bias test results. The authors suggest that when eggs are to be used for testing purposes, an individual female be fully stripped, the eggs gently stirred to homogenize the lot, and then randomly allocated to individual treatment replicates.

#### REFERENCES

- Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (<u>Oncorhynchus mykiss</u>). Aquaculture, 100: 141-166.
- Kato, K., and Kamler, E., 1983. Criteria for evaluation of fish
  egg quality as exemplified for <u>Salmo gairdneri</u> (Rich). Bull.
  Natl. Res. Inst. Aquaculture, No 4: 61-78.
- Springate, J.R.C., Bromage, N.R., Elliott, J.A.K. and Hudson, D.H., 1984. The timing of ovulation and stripping and their effects on the rates of fertilization and survival to eyeing, hatch and swim up in the rainbow trout (<u>Salmo gairdneri</u>). R. Aquaculture, 43: 313-322.
- Wilcox, K.W., Stoss, J., and Donaldson, E.M., 1983. Broken eggs as a cause of infertility of coho salmon. Aquaculture, 40: 77-87.

Table 1. Mean percent eye-up of arlee strain rainbow trout eggs as affected by stage of expulsion.\*

Stage of expulsion	N	Mean Eye-up %** SD
A (First 1/3 of spawn)	12	71.48 <sup>a</sup> 31.05
B (Second " " )	12	67.80 <sup>b</sup> 32.03
B (Third " " " )	12	64.24° 29.76

\*Effect of Stage: P<0.0001; CV=6.42; R-Square=0.99.

\*\*Differing superscript denotes significant difference.

Table 2. Comparison of percent eye-up in eggs collected from arlee strain rainbow trout: Interaction between individual females and different stages of expulsion.

<u>Female</u>	Stage of expulsion	N	Eye-up%	SD
l	A	2	98.4	0.0
1	В	2	92.9	2.2
1	С	2	75.6	4.2
2	А	2	85.0	2.6
2	В	2	76.9	0.6
2	С	2	73.9	0.4
3	А	2	88.9	3.3
3	В	2	91.8	2.5
3	С	2	88.0	0.2
4	А	2	36.5	10.2
4	В	2	28.5	7.3
4	C	2	29.9	9.5
5	А	2	95.7	0.4
5	В	2	94.6	1.1
5	C	2	96.5	1.4
6	A	2	24.4	0.9
6	В	2	22.4	0.4
6	С	2	21.7	0.6

Effect of treatment: P<0.0001; CV=6.42; R-square=0.99

# EFFECT OF VARIED MILT VOLUME ON THE EYE-UP SUCCESS OF KAMLOOP RAINBOW TROUT EGGS

Daniel R. Brown, John B. Shrable and Wesley H. Orr

U.S. Fish and Wildlife Service Ennis National Fish Hatchery 180 Fish Hatchery Rd. Ennis, MT 59729

## ABSTRACT

Brown, D. R., Shrable, J.B. and W.H. Orr. 1994. The effect of varied milt volume on eye-up success of Kamloop rainbow trout eggs.

Opinion varies among fish culturists and researchers alike regarding ratios of milt, diluent and eggs necessary to obtain optimal fertilization success in rainbow trout (Oncorhynchus mykiss) and other salmonids. Concern over what is "minimal" and "excessive" in terms of milt volume has prompted this study. Eyeup success in Kamloop strain rainbow trout eggs at milt application rates of 1, 5, and 10 times that normally employed at the hatchery were compared. There were no significant effects (P>0.05) of different milt concentrations tested on survival rate of eggs measured at the eyed stage of embryonic development. These findings indicate that in normal spawning situations, concern about "excess" milt application is probably unwarranted.

# Introduction

wild salmonids is characterized by high in Spawning concentrations of spermatozoa relative to the number of eggs in a redd. A trout spermatozoon has the ability to travel a distance of about 3 mm by flagellar motion. This is a shorter distance than the diameter of a single egg (4-6mm). Thus, eggs must be engulfed by spermatozoa in order to ensure the greatest chances for fertilization success (Billard 1992). Scott and Baynes (1980) in review of artificial insemination methods, reported that early studies indicated that 1 ml of semen per 1500-2000 eggs which equates to approximately 5,000,000 sperm per egg (assuming a sperm concentration of 1010 sperm per ml of semen) would yield maximum Billard et al. (1974) reported fertilization in salmonids. significantly lower fertilization success when sperm "in excess" was mixed with eggs when compared to small amounts of sperm  $(10^{-2}$ and 10<sup>-3</sup>); and that 70-80% fertilization could be achieved by adding 0.01 ml of semen to 10 ml of 20 mM carbonate-bicarbonate buffered (pH 9) saline extender and then added to batches of 800 eggs (approx 200,000 sperm/egg).

The sperm concentration normally used at the Ennis NFH is  $2(10^{-2})$  or 20 ml milt: 1 L diluent: 2 L eggs which provides 5 to 10 million sperm per egg with egg sizes of 20,000 to 10,000 per liter respectively assuming a sperm density of  $10^{10}$  spermatozoa/ml semen (Billard et al. 1974). Erdahl and Graham (1987) reported that rainbow trout semen diluted at 1:8 with a seminal fluid mimicking extender gave results equal to control values of fertilization

obtained without semen dilution. When semen was diluted (extended) prior to fertilization 1:1 (extender:milt), fertilization rate was higher than control values. Billard (1974, 1985) defined the minimum amount of sperm necessary to obtain optimum fertilization success with rainbow trout as  $10^{-3}$  (1 ml semen:1 L diluent:3 L of eggs). In a more recent investigation, Ciereszko and Dabrowski (1994) studied relationships between many aspects of rainbow trout semen and fertilization rate. In one case, they varied milt volume using two concentrations; 0.1ml and 0.02ml on separate batches of (531  $\pm$  23) eggs from the same female per 3ml of 0.7% NaCl. No significant differences in fertilization success between the two milt volumes tested were reported.

The objective of this study was to evaluate eye-up success of Kamloop strain rainbow trout eggs when applying milt at 5 and 10 times the concentration normally utilized in spawning activities at the Ennis NFH.

# Materials and Methods

In February 1994, during the peak of the spawning period milt was stripped from eighteen 3-yr old Kamloop rainbow trout males, pooled and transferred to 500 ml plastic containers and stored in an ice chest. Milt depth in each container did not exceed 1/8 inch. Approximately 80 ml of eggs (10,000 eggs per L egg size) were stripped from each of ten 3-yr old Kamloop females and pooled. The pooled lot was divided randomly into six replicate groups of approximately 1000 eggs each. Fifty ml of a 0.75 % saline fertilization medium and 1, 5 or 10 ml of semen were combined with eggs in each of two replicates. Dilution rates were 2(10<sup>-2</sup>), 10<sup>-1</sup>, and  $2(10^{-1})$  respectively, as defined by Billard et al (1974). All gamete mixtures were gently stirred after combination, and allowed to stand 1-2 min, then rinsed in fresh water. All replicates were then hardened in a 50 mg/L iodophor solution for 30 min then transferred to individual upwelling incubators. The fifteenth day following fertilization (330 TU) all eggs were exposed to mechanical shock and returned to individual incubation. On the following day all eggs in each replicate were picked (sorted) with the aid of an electronic picker (Jensorter Model JX-24, Jensorter Inc., 20225 Harvest Ln., Bend, OR, 97701) and counted with the aid of an electronic counter (Jensorter Model BC). Blank eggs (translucent without eye development) were separated, counted and removed manually. Resulting data was recorded and later analyzed using SAS (1993) system of statistical analysis. All percentage data were arc-sine transformed prior to analysis.

# Results and Discussion

Milt volume did not significantly affect (P>0.05) fertilization rate at dilution rates tested (Table 1). Treatments with 1 ml milt : 50 ml diluent : 100 ml eggs (1S) were considered to be equivalent to the normal dilution utilized in spawning practices at the Ennis NFH. The 1S dilution provided about 10 million sperm per egg. Increased milt application at 5 (5S) and 10 (10S) times the normal (1S) quantity did not significantly alter fertilization rate (Table 1). The frequency of blanks (eggs which are translucent without visual eye development after mechanical shocking) also was not significantly affected (P>0.05) by varied milt volume during artificial insemination (Table 1). These findings resolved our concerns that increased milt volume might be deleterious to fertilization success.

#### <u>Acknowledgements</u>

The authors wish to express their gratitude to Dr. Rick Barrows at the Bozeman Fish Technology Center, Bozeman, Montana; who always finds the time to assist in experimental design, review and statistical analysis of our investigations.

#### Literature Cited

- Billard, R., Petit, J., Jalabert, B. & Szoiloci, D., 1974. Artificial insemination in trout using a sperm diluent. In: Early Life History of Fish (J.H.S. Blaxter, ed.), pp. 715-723. Berlin: Springer Verlag.
- Billard, R., 1985. Artificial insemination in salmonids. In: R.N. Iwamoto and S. Sower (Editors), Salmonid Reproduction, Washington Sea Grant Program, Seattle, WA, pp. 116-128.
- Billard, R., Cosson, M.P. and Christen, R., 1987. Some recent data of the biology of trout spermatozoa. In: D.R. Idler, L.W. Crim and J.M. Walsh (Editor), Reproductive Physiology of Fish. Mem. Univ. ST. John's Nfld., Canada, pp. 187-190.
- Billard, R., 1992. Reproduction in rainbow trout: sex differentiation, dynamics of gametogenesis, biology and preservation of gametes. Aquaculture, 100: 263-298.
- Ciereszko, A. and Dabrowkski, K., 1994. Relationship between biochemical constituents of fish semen and fertility: the effect of short-term storage. Fish Physiology and Biochemistry, Vol. 12 No. 5, pp. 357-367.

- Erdahl, A.W. and Graham, E.F., 1987. Fertility of teleost semen as affected by dilution and storage in seminal plasma-mimicking medium. Aquaculture, 60: 311-321.
- Scott, A.P. and Baynes, S.M., 1980. A review of the biology, handling and storage of salmonid spermatozoa. J. Fish Biology., 17: 707-739.

Table 1. Effect of Varied Milt Volume on the Eye-up Success of Kamloop strain Rainbow trout eggs.

Treatment	(milt:diluent:eggs)	Eye-up%	* SD	Blank%*	SD
1S	(1ml:50ml:100ml)	81.3ª	0.6	2.91 <sup>b</sup>	0.3
5S	(5ml:50ml:100ml)	79.6ª	3.0	2.56 <sup>b</sup>	0.4
105	(10ml:50ml:100ml)	80.5ª	0.9	2.65 <sup>b</sup>	0.1
	• Production and the second of				

\*Like superscript denote no significant difference (P>0.05).
Diel and circannual changes in ammonium excretion by spring chinook salmon at Willamette Hatchery

> R. D. Ewing Biotech Research and Consulting, Inc. 2340 SE Ryan St. Corvallis, OR 97333

> > and

#### J. E. Sheahan Oregon Department of Fish and Wildlife Hamlet Route, Box 360 Seaside, OR 97138

#### ABSTRACT

Pollution of water by hatchery effluents has become more widely recognized in recent years because of the greater numbers of aquaculture facilities and the greater concern for the quality and use of water. In Europe and Asia, these concerns are becoming critical. Here in the Northwest, we still have an adequate supply of water, but these supplies are daily encroached upon by user groups who compete more and more for available water. One can foresee that in the not-too-distant future pollutants from hatchery effluents that were previously overlooked will be the subject of wide environmental concern. Hatchery operations will include the monitoring and/or removal of waste products from the effluent water.

One of the pollutants of concern in the effluent water of hatcheries is nitrogen generated from fish feed and excreted as a by-product of the metabolism of fish. Nitrogen build-up leads to the eutrophication of the water in which it is released and the gradual deterioration of the water quality if left unchecked.

We began a study of nitrogen excretion by spring chinook salmon reared at Willamette Hatchery as part of a study on the effects of oxygen supplementation in hatchery systems, funded by Bonneville Power Administration. Our measurements looked at both diel and circannual excretion of ammonium and urea from ponds of chinook salmon.

The experimental design at Willamette Hatchery consisted of a series of seven replicated ponds containing variable numbers of chinook salmon. Experimental conditions are presented in Table 1. Each week during the rearing season from July until March, samples at the inflow and outflow of each raceway were taken and analyzed for ammonium. This sampling continued from 1990 to 1994. In addition, on selected days in 1993 and 1994, samples were taken from the seven experimental ponds at hourly intervals for a thirty hour period. These samples were analyzed for ammonium and urea. Temperatures and water quality conditions were also taken simultaneously.

Results indicated that ammonium is excreted in a diel cycle (Fig. 1) Maximum ammonium excretion occurs about 4-5 in the

afternoon, when water temperature reaches a maximum. Ammonium excretion increased with pond density (Fig. 2) and as the water was recycled through Michigan-style raceways (Fig. 3).

While the ammonium levels increase dramatically in the higher densities and in the recycled water, the respiration of the fish causes the pH to decrease at the same time. This shifts the equilibrium from ammonia production to ammonium production. Thus, the deleterious ammonia concentrations are always kept lower than that which begins to produce developmental and health abnormalities in the fish populations.

Ammonium excretion also changes with season (Fig. 4). Most of this change can be accounted for by changes in temperature. There are excellent correlations between the excretion of ammonium and the water temperature.

Presently, we are calculating nitrogen flow through the system. Almost all of the nitrogen in the system is derived from fish feeds. In these feeds, about 36% of the weight is protein and 28% is water. In fish, about 76% of the weight is water and 18% is protein. Protein is comprised of about 5.7% nitrogen. By using the amount of fish feed fed to the fish, the amount of growth, and the amount of ammonium and urea flowing from the raceway, we should be able to derive a stoichiometry for nitrogen utilization and nitrogen wastage. In addition, we should be able to determine whether changes in the metabolism occur at various times of the year and whether fish feeds should be altered to meet these demands.



Figure 1. Ammonium excretion over a 27-hour period on September 16, 1993. Solid squares are concentrations of ammonium in effluent water from a raceway containing spring chinook salmon at a density of about 1 1b/cu ft. Asterisks show water temperature. Arrows indicate feeding times.







Figure 3. Ammonium excretion by spring chinook salmon reared in a series of three Michigan style ponds. Ammonium concentration was measured at the effluent from each pond in series. Temperature of the incoming water is presented as asterisks.



Figure 4. Ammonium excretion per kilogram of chinook salmon reared at three different densities. Solid squares represent fish reared at about 1 lb/cu ft, open squares represent fish reared at about 0.5 lb/cu ft, asterisks represent fish reared at about 3.0 lb/cu ft. Seasonal changes depend largely on decreasing water temperatures (not shown) during the winter months.

## PURINA FLOATING FEED DIET TEST AT SAN JOAQUIN HATCHERY

Anna Kastner California Department of Fish and Game PO Box 10 Friant, CA

#### Introduction

A feed comparison test was done at the San Joaquin Hatchery, from May 17, 1994, through July 5, 1994, to determine the effects on water quality of the two Purina feeds, with their new floating feed and their standard 5/32nd pellet. Also, a cost analysis, of price per pound of gain, was formulated.

#### METHODS AND MATERIALS

A feed test was set up to copy standard fish production at San Joaquin Hatchery comparing standard 5/32 purina pellets and their new floating feed (5105). No adjustments of numbers or sizes of fish were made. E series contained 102,100 RTE-93 at 4.5 to the pound and was fed Purina's new floating feed. F series was the control series and was fed the standard purina 5/32 pellet. E and F series were fed utilizing demand feeders. Both feed diets had the same ingredients and the feed chart was calculated using Purina's standard chart.

Each series (E & F) were cleaned each Monday morning and the depth of waste material measured below the screen in pond 5. PH, D.O., temperature, and ammonia were tested Tuesday and Thursday of each week using a YSI model 51B, Hach ammonia test kit, and a pocket PH meter.

The feed test lasted approximately 48 days and was stopped due to heavy pond loading in both test series. E and F series were thinned with 30,000 fish being removed from each series. These fish will remain on their respective diets until they are planted.

#### RESULTS

At the end of the feed experiment (48 days), The Purina floating feed (E series), had higher D.O's, lower waste material depths, lower ammonia levels and a slightly higher PH. These values remained consistant through out the entire experiment. Just before terminating the feed experiment, F series which had 20 percent fewer numbers and pounds showed signs of stress after feeding each day. E series showed no signs of sress after feeding, due to cleaner ponds, higher D.O's and lower ammonia levels.

#### DISCUSSION

The floating feed test showed remarkable results in the area of water quality with crowded and over weight conditions existing at the end of the feed experiment. Most all fish health problems in State Fish Hatcheries stem from poor water water quality conditions causing manifestations of all stress mediated diseases. Today, with the restrictions on the use of chemicals, stress mediated diseases may have to be controlled by lower pond loading, and better quality feeds, resulting in higher water quality conditions.

Purina floating feed had a conversion ratio of of 1.09 and a cost of \$0.34 per pound of gain compared to 1.33 conversion ratio and \$0.25 per pound of gain for their standard pellet. The State sees only the cost per pound where we as aquaculturists, look at water quality and fish health. Also, the cost of chemical treatment, the labor of chemical treatment, and the paper work that will follow chemical usage is not factored in as a hatchery cost.

The Purina floating feed we purchased came sacked, not bulk, which resulted in a higher cost, \$0.31 per pound. Bulk prices should be lower.

Fall spawning rainbow trout have a tendency to bleed when crowded or loaded. When our test series (E & F series) were thinned, only the fish on the Purina standard diet bled during the moving and loading process.

#### NON IONIZED AMMONIA CALCULATIONS

NH3 + HOH ----- NH4 + OH TOXIC WATER NON TOXIC

#### NH3= TOTAL AMMONIA x % UNIONIZED AMMONIA DEVIDED BY 100 = UNIONIZED FROM TABLE B TEMP & PH DEPENDANT

EXAMPLE: PH=6.9 TEMP= 11 C TABLE B=0.160 TOTAL AMMONIA=0.8

0.8 x 0.160 DEVIDED BY 100=0.00128

DANGER ZONE=0.0125 NON IONIZED AMMONIA

DATE	SERIES	D.O.	PH	TEKP	TOT/NE3	NH3	WASTB	FEED	CLEAN
5/17	E	7.4	7.0	11	.4	.000804	8.5	FLOAT	5/16
5/17	F	5.9	6.7	11	.5	.000505	11.5	5/32	5/16
5/19	E	6.6	6.8	11	.5	.000635 .	5	FLOAT	5/16
5/19	F	5.5	6.7	11	.6	.000606	7	5/32	5/16
5/24	E	6.5	6.9	11	.3	.000480	7.5	FLOAT	5/23
5/24	F	5.4	6.7	11	. 5	.000505	10	5/32	5/23
5/26	E	6.7	7.1	11.5	.5	.001315	4.5	FLOAT	5/23
5/26	F	5.0	6.7	11.5	?	UNKNOWN	6	5/32	5/23
5/31	E	6.0	7.4	12	.3	.001632	10	FLOAT	5/30
5/31	F	5.2	7.0	12	. 5	.001085	13	5/32	5/30
6/02	E	6.6	6.8	12.25	.3	.00041925	5	FLOAT	5/30
6/02	F	6.3	6.8	12.25	.5	.00069875	7	5/32	5/30
6/07	E	6.4	6.8	11.75	.15	.00020175	9.5	FLOAT	6/06
6/07	F.	5.6	6.7	11.75	. 4	.000428	10	5/32	6/06
6/09	E	5.8	6.6	12.25	.35	.00030914	5.5	FLOAT	6/06
6/09	F	4.7	6.6	12.75	.55	.0005047	7.5	5/32	6/06
6/14	E	5.0	6.6	12	.3	.0002598	9.5	FLOAT	6/13
6/14	F	3.9	6.6	• 12	5	.000433	11	5/32	6/13
6/16	E	6.4	6.6	12	. 4	.0003464	3.5	FLOAT	6/13
6/16	F	4.9	6.5	12	.6	.0004128	4.5	5/32	6/13
6/21	E	5.9	6.7	13.25	. 4	.000481	1.5	FLOAT	6/20
6/21	F	5.0	6.6	13.25	.6	.00057225	3.5	5/32	6/20
6/23	E	5.3	6.6	13	.45	.00042075	6	FLOAT	6/20
6/23	F	4.4	6.6	13	.65	.00060775	2.5	5/32	6/20
6/28	E	4.9	6.7	14	.45	.0005715	<u> </u>	FLOAT	6/27
6/28	F	4.1	6.7	14	.65	.0008255	_	5/32	6/27
6/30	E	5.1	6.6	13.75	. 4	.0003815	6.5	FLOAT	6/27
6/30	F	4.4	6.6	14	.6	.000606	5.5	5/32	6/27
7/05	E	5.1	6.8	14	.4	.00064		FLOAT	-
7/05		3.8	6.7	14.25	.65	.00084175	_	5/32	-

•

E SERIES (PURINA FLOATING FEED)

TEST DATE MAY 18-JULY 4 NUMBER OF FISH ON MAY 18, 1994 102,100 WEIGHT ON MAY 18, 1994 22,689 SIZE ON MAY 18, 1994 4.5 NUMBER OF FISH ON JULY 5, 1994 101,718 FISH LOSS FOR TEST PERIOD 382 WEIGHT ON JULY 5, 1994 35,207 SIZE ON JULY 5, 1994 2.9 POUNDS GAINED 12,518 PRICE OF PURINA FLOATING FEED 31.00/100# POUNDS FED DURING TEST PERIOD 13,665 FEED COSTS FOR FEED TEST \$4236.15 CONVERSION RATE 1.09 PRICE PER POUND OF GAIN \$0.34

#### F SERIES (PURINA STANDARD 5/32 PELLET)

TEST DATE MAY 18-JULY 4, 1994	
NUMBER OF FISH ON MAY 18, 1994	81,900
WEIGHT ON MAY 18, 1994	22,135
SIZE ON MAY 18, 1994	3.7
NUMBER OF FISH ON JULY 5, 1994	81,570
FISH LOSS FOR TEST PERIOD	330
WEIGHT ON JULY 5, 1994	31,500
SIZE ON JULY 5, 1994	2.6
POUNDS GAINED	9,365
PRICE OF PURINA STANDARD FEED	\$18.83/100#
POUNDS FED DURING TEST PERIOD	12,454
FEED COSTS FOR FEED TEST	\$2345.08
CONVERSION RATE	1.33
PRICE PER POUND OF GAIN	\$0.25

San Joaquin Hatchery Floating Feed Analysis, 1994



Total Ammonia ppm

San Joaquin Hatchery Floating Feed Analysis, 1994



ph Comparison

San Joaquin Hatchery Floating Feed Analysis, 1994



Waste Material Depth

San Joaquin Hatchery Floating Feed Analysis, 1994



**Dissolved Oxygen** 

#### HATCHING JARS, ANOTHER ALTERNATIVE

Anna Kastner California Department of Fish and Game PO Box 10 Friant, CA 93626

Our involvement with hatching jars arose from the need of finding an alternative method of hatching eggs without any chemicals. This was brought forth by the Food and Drug Administration's requirement to use approve drugs for aquaculture; also Water Quality discharge regulations are becoming rigorous when it comes to chemical monitoring of our effluent water discharge.

The first thing I would like to mention is that all the jars mentioned in this presentation are very successful with eyed eggs; Our problem arose when we tried to incubate green eggs.

We started with Eager 12" jars. The first problem we encountered was the filter that came with the jar; debris and silt accumulated in the filter blocking the water flow, thus producing what we call "hot spots". The "hot spots" are uneven distribution of water causing violent and jolting movement in the eggs. After eliminating the filter another problem was encountered, the concave screens that came with the jars caused "hot spots". Different types of media ( marbles, gravel and sand) was tried to even the flow, but non of these materials were successful. We realized that the plate was the key to the water flow problem. Discussing this problem with a major salmonid egg producer/shipper in Northern California we became familiar with a plate design used specially in the incubation of green eggs. Thanks to their innovation we constructed several different plates (circular and square patterns)

to see which would serve our needs. The best plate was square pattern on 1" centers with 1/8" holes and some with 3/16" holes.

The next step was to find a way to secure the plate to the jar. Silicon was use with some success until the jars needed to be cleaned. After several trials a stainless steel bolt was used to secure the plate in place. "o" ring material was used between the bottom of the jar and the plate to prevent the water from going up the sides of the jar.

At the same time we were working on the Eager jars we acquired some 6" Midland jars. These jars were use to cultivate Striped Bass. Some modifications had to be make on the jars. A plate was made similar to that of the Eager jar, but with 1" hole in the center to accommodate a 1/2" pvc inflow pipe. The water for the jar is piped in from the top of the jar to the bottom. The plate is used to diffuse the flow of water evenly and to hold the eggs away from the bottom. A 1/2" female adapter was place at the bottom of the pvc pipe to force the water in a even, upward manner.

At this time, the Mcdonald and eager jars seemed to be working well and the need for more jars was evident thus the monetary aspect needed to be considered. John Modin brought the hatchery the first "home made" jar. This jar was made of clear 6" acrylic tubing material. This jar went through its own evolution, many different types of jars were made and a conclusion was made: the less complicated the better. Another conclusion was made that making the jars was far less expensive that purchasing commercial jars. The jars are made out of 8" x 24" acrylic tube material. A

2" acrylic sleeve is inserted at the bottom of the jar and a 3/4"hole is drilled and tapped. A 3/4" poly nipple is connected to the tapped hole at the bottom of the jar. Two acrylic plates-ABS, polycarbonate, pvc or other flat stock can be substituted in plate construction- are needed, one with 5/32" holes with 1" center and one solid; both need a 1/4" center hole. If using acrylic for plate, drilling holes under water is suggested. It is important to note that the hole pattern and the size of the hole are critical when incubating green eggs, the present pattern appears to minimize turbulence and still yield adequate flow. This plates are placed between the acrylic sleeve producing a water chamber. At the top of the sleeve material, 3/16" O-ring material is used to prevent leaks in the upper plate. A 1/4" x 3" stainless steel bolt with 1/4" wing nut is used to hold the plates together (see acrylic diag). Stainless steel bolts are highly recommended due to their resistance to electrolysis. When using acrylic, fine tooth saw blades are recommended. A piece of plastic wrapped around the jar is use to protect eggs from sunlight.

Cost beingg one of the problems, a 8" pvc pipe was used to make a jar. This model encourage the thought of building jars out of pvc instead of acrylic, for one, the jars would be less expensive to build, ( see attach. A ), and the eggs would be protected from the sun. Thanks to the Kokanee foundation, the funds were available to build several jars. The jars were cut from a class 125 pvc pipe, thinner pipe like class 100 can be used. The dimension of the jar is 6" x 18". It requires two ABS plates, both with a 1/4" center hole, the other with 1/8" holes on 3/4" centers distributed evenly across the plate; The two plates are separated by a 6" x 3" sch.40 piece of pvc pipe. This piece is glued inside the bottom part of the jar. To make the 6" x 3" piece fit, a 1/2" slot must be cut; The purpose for this piece is to separate the two plates and to use it as a "O" ring seat. A 3/4" hole is drilled and tapped at the bottom of the 6" x 18" pipe, between the two ABS plates; Attached to the hole is a 3/4" hose barb, A 3/4" pvc 90 elbow is attached to the inside of the hose barb by a 3/4" nipple. This elbow forces the water evenly across the plate, the two plates are held together by a 1/4" x 5" round head stainless bolt and wingnut (see pvc diag.).

One word of caution, clear acrylic, though expensive (8" tube costs \$16.00/ft), would be the material of choice in the introduction and training period due to the need to see and get familiar with turbulence and flow patterns of the eggs. Eggs in the tender stage need to be rolled gently with a minimum water flow, about 6 gpm in a 12" jar. Watching this process is highly recommended before building pvc jars.

The advantages of this jars are that they have a low capital cost, a low operating cost and most important no chemical treatments required for fungus control. The low capital cost could be seen in the cost of the jars, an 8" x 24" jar cost about \$11.50 (see attach. B) and it will hold about 300-350 oz. of eggs. Three of these jars could hold a 14 tray incubator stack if each tray held 70 oz. of eggs. The low operating cost can be seen the elimination of time required to clean the jars. Many dead/unfertilized eggs come to the surface and they are easily removed and because they are constantly moving the need to pick eggs is decreased. Siltation in much less problematic and the air blockage problem is eliminated. The jars can be place in individual troughs thus allowing the eggs to hatch in the jars; when hatched the egg shells flow from the jar and do not impede the flow allowing a constant exchange of oxygen.

The disadvantages of the hatching jars are minor but must be recognized and observed. A screen or filter is essential and needed to remove large material (ie. leaves , insects, snails, etc.) from water. Partial blockage of the plate holes will cause irregular flows and can/will kill green tender eggs. The incoming pressure must be constant. Any fluctuation of head pressure will affect the water flow to the jars and it could be fatal to the tender green eggs. This can be eliminated by supplying jars with water from a head box.

This jars have been a plus in our operation, from allowing less people to take care of more eggs to the elimination of chemical in egg incubation. Each facility is different and results might need to be altered to meet the facility's needs, but I strongly encourage to try the jars. As of this time, we are still trying different plate patterns to find the optimum flow for the green eggs. At this time I would like to publicly thank the crew at the San Joaquin Hatchery and the pathology department for all their help, because without them, this paper wouldn't be possible.

#### ATTACHMENT A

# EIGHT INCH PIPE \$1.21/FT TWELVE INCH PIPE \$2.72/FT ACRYLIC 1/4" FLAT STOCK \$2.78/SQFT "O" RING 3/16" STOCK \$0.21/FT 3/3" POLY NIPPLE \$0.20/EA. 3/4" HOSE TO PIPE FITTING \$1.80/EA. 5" X 1/4" BOLT ASSEMBLY \$1.00/EA.

.

# COST ANALYSIS PVC LOW HEAD (THIN WALL)

#### ATTACHMENT B

COST OF 8" AND 12" PVC LOW HEAD JARS

	EIGHT	'I	NC	H J	AR	
TUBE	\$1.21	X	2	FT	=	\$2.42
FLAT STOCK	\$2.78	X	2	EA	=	\$5.56
"O" RING STOCK	\$0.21	X	2	FT	=	\$0.42
3/4" NIPPLE	\$0.20	х	1	EA	=	\$0.20
3/4" H/P	\$1.80	Х	1	EA	=	\$1.80
<u>5" X 1/4" BOLT</u>	\$1.00	X	1	EA	=	\$1.00
TOTAL					=	\$11.40

TWELVE INCH JAR

TUBE	\$2.78	X	2	FT	=	\$5.44
FLAT STOCK	\$2.78	x	2	EA	=	\$5.56
"O" RING STOCK	\$0.21	х	3	FT	=	\$0.70
3/4" NIPPLE	\$0.20	х	1	EA	=	\$0.20*
3/4" H/P	\$1.80	x	1	EA	=	\$1.80*
<u>5" X 1/4" BOLT</u>	\$1.00	X	1	EA	=	\$1,00
TOTAL					=	\$14.70

\* MAY REQUIRE LARGER INLET FOR LOW PRESSURE SYSTEMS PRICE LIST AS OF OCT. 1994

#### ATTACHMENT C

# HATCHING JARS FLOW RATE AND EGG CAPACITY

SIZE OF JAR	TYPE OF JAR	FLOW RATE	CAPACITY
		GREEN/EYED	IN OUNCE
6" X 18"	SJH JARS	1.5GPM/3GPM	130 OZ
6" X 18"	MIDLAND	2.5GPM/5GPM	100 OZ
8" X 24"	SJH JARS	4GPM/6GPM	250-300 OZ
12" X 18"	EAGER	8GPM/14GPM	600 OZ

.

#### ATTACHMENT D

## SJH PVC HATCHING JARS

SIX INCH JAR

TUBE 6" X 18"	\$2.45 X 1.5 FT	= \$3.68
SLEEVE 3"	\$3.32 X .33 FT	= \$1.18
PLATE	\$2.15 X 2 EA	= \$4.30
3/4" HOSE BARB	\$1.24 X 1 EA	= \$1.24
3/4" 90 ELBOW	\$0.34 X 1 EA	= \$0.34
3/4" NIPPLE	\$0.47 X 1 EA	= \$0.47
"0" RING STOCK	\$0.41 X 6" EA	= \$0.20
<u>5" x 1/4" BOLT ASS.</u>	\$0.66 X 1 EA	= \$0,66
TOTAL		= \$12.05

. •





#### David E. Owsley, P.E. Environmental Engineer

Dworshak Nation Fish Hatchery is located in north central Idaho downriver from Dworshak Dam, at the confluence of the North Fork and the main stem Clearwater River. Dworshak Dam was constructed by the Corps of Engineers in 1966-70. Operation of the hatchery by the Fish and Wildlife Service was authorized by the Corps of Engineers in a Memorandum of Understanding of 1969. The hatchery has since served primarily as mitigation hatchery for steelhead trout (*Oncorhynchus mykiss*); a unique run of the North Fork "B" strain threatened by the construction of Dworshak Dam. The Fish and Wildlife Service has endeavored over the past 25 years to meat the "mitigation goal" of providing 20,000 adult steelhead to the Clearwater River and maintain the unique genetics of the stock.

Dworshak National Fish Hatchery consists of a complete up-to-date mechanical, electrical, water reuse and reconditioning system employing filtration, sterilization, biological nitrification, pollution control and monitoring facilities, alarm system, water chillers, heaters, and numerous pumps. Initial construction at Dworshak National Fish Hatchery included 84 Burrows ponds, 64 nursery tanks, and 9 adult holding ponds. Twenty-five (25) Burrows ponds (System I) were operated on a heated recycled water flow, for rearing steelhead smolts to the required size of 180 mm in only one year. In 1973, System II (25 ponds) and System III (34 ponds) were converted from a single-pass of water, two-year rearing cycle, to water reuse and heating for accelerated production growth. This second phase construction, with added mechanical systems (biological filters, electric grid, sand filters, U.V. lamps, chillers, and boilers), increased production capacity and allowed all three water systems to be environmentally controlled. The three biological systems operating at the facility included a 3963 L/MIN fluidized bed system, a 5389 L/MIN submerged upflow system with 8.89cm Koch rings, and a 3963 L/MIN bead media upflow submerged bed filter system. Make-up for each system is 10 percent and the reason for reuse is temperature control. By controlling temperature with reuse, the facility can produce a steelhead trout smolt in one year. Under normal water temperatures in the river, it would take two years to produce this same smolt.

During the mid-1970's, with Dworshak National Fish Hatchery not meeting production goals nor meeting mitigation goals, major operational changes were made. Review and studies of the reuse systems, water temperature regime, water quality, and fish culture techniques were done by hatchery staff and university scientists. Corrective measures followed which removed the computerized pneumatic feed system, eliminated the ultra violet treatment of water reuse, redesigned the water flows to maximize single-pass use and a return to a more hands on basic fish culture. Selecting cooler water temperatures from Dworshak Reservoir during the summer, adding minerals (sodium chloride and potassium chloride) to a soft water supply, removing supersaturated nitrogen gas along with other designed mechanical changes, and more involvement of hatchery staff in monitoring fish culture, all contributed positively towards improving the hatchery's program.

Column degassers, using "Koch" rings as a diffuser, were designed and tested and placed in operation for aeration and for protection from high nitrogen gas. The Dworshak hatching jar was developed for the nursery tanks for incubating eggs and allowing fry to swim directly to the tanks. Excellent results on trials of demand feeders warranted placing all steelhead production ponds on these types of feeders. Dworshak National Fish Hatchery personnel developed the concept of

using overhead wire to support a net enclosure of nearly 130,000 square feet of rearing area from bird predation.

Further construction in the 1980's added 18,000 square feet of nursery building, doubling the number of inside rearing tanks to 128. A new concept of biological filtration, known as a fluidized san filter, replaced the oyster shell media in System I. An additional thirty 8' x 80' raceways were constructed under the Lower Snake River Compensation Plan to provide production facilities for spring chinook salmon (*Oncorhynchus tshawytscha*).

The uniqueness of Dworshak National Fish Hatchery's water systems provides several options for egg incubation and rearing. Three (3) temperature schematics are available for egg development through the incubators. Three (3) different temperature regimes are also available to the nursery tanks with ozone treated water to 32 of the 128 inside tanks. The domestic drinking water for the hatchery and four residences is also treated with ozone. The availability of the ozone makes this drinking water system the best in Idaho for disinfection requirements. The ozone system has been successfully operated since 1984. The outside steelhead ponds are furnished single-pass river water from May through October when desired temperatures can be obtained through selector gates at Dworshak Dam. A pump station on the North Fork, one mile downriver from the Dam, is capable of providing 90,000 gpm of water. Water reuse, used extensively during the colder months of November until fish are released in the spring, is made up from 10 percent new water to allow heating for desired fish growth. Each of the three outside ponding systems is independent of each other for temperatures when on reuse and heated water. Beginning in 1992, the hatchery was supplied with an additional 6400 gpm of gravity flow Dworshak Reservoir water directly by pipeline. This "clean" water furnishing egg incubators and nursery rearing has afforded disease protection from Infectious Hematopoietic Necrosis (IHN) virus in the early production stages.

The Kooskia hatchery, 35 miles upriver from Dworshak on the Clearwater River, has operated as a Complex with Dworshak since 1978 to restore the spring chinook fishery in the Clearwater. The biological filtration system consists of a 951 L/MIN submerged upflow filter system with expanded shale for the media. Kooskia National Fish Hatchery raises spring chinook salmon in the reuse system with temperature control and water shortage as the reason for reuse. Kooskia also uses 10 percent make-up water. In addition to spring chinook, the facility also serves as a temporary site for the rearing of rainbow trout for stocking Dworshak Reservoir while Dworshak's IHN virus problem continues to exist. The administrative headquarters for the Dworshak-Kooskia Complex is located at the Dworshak hatchery. The Dworshak Fish Health Center and the Idaho Fishery Resource Office are co-located at the Dworshak station.

The Dworshak hatchery has capacity for producing 2.3 million steelhead smolts; 1.4 million yearling chinook salmon smolts; and 200,000 sub-catchable rainbow trout for stocking Dworshak Reservoir. The hatchery's annual production capacity exceeds 550,000 pounds. Mitigation adult fish goals to the Clearwater River are 20,000 returning steelhead and 9,000 spring chinook. Steelhead goals are being satisfied, however, spring chinook returns continue to remain well below mitigation levels.

#### The effects of light intensity and photoperiod on the growth response of fingerling White Sturgeon

Brian Hickson Abernathy Salmon Culture Technology Center 1440 Abernathy Rd. Longview, Wa. 98632

#### BACKGROUND

In 1993 the Abernathy SCTC initiated a research program to evaluate the culture requirements for white sturgeon under rearing conditions found in Columbia Basin Hatcheries. State and Federal researchers evaluating the population dynamics of white sturgeon in the impounded waters of the Columbia and Snake river have concluded that some populations are in decline or are experiencing poor recruitment. Recommendations evolving out of these studies call for development of mitigation strategies to improve recruitment in "under seeded "reservoirs. One option being explored is hatchery supplementation.

A review of the scientific literature and discussions with established sturgeon culturists indicated that there was limited information available on the influence of photoperiod and light intensity on the growth and development of fingerling sturgeon. Some groups insisted that maintaining subdued lighting improved performance while others felt it was unimportant. Recent data from life-history studies on white sturgeon suggest that young of the year are most abundant in deep, holes (11-15 m) in the lower Columbia during daylight hours (McCabe 1993). Incident light intensity levels measured at 10 meters ranged from 1-3 lux (Brian Hickson, personal observation). It has been demonstrated that the primary sensory systems used in the detection and capture of prey by juvenile white sturgeon are olfactory, gustatory and tactile suggesting that vision and hence light is relatively unimportant for feeding in this benthic dweller (Brannon, 1985: Buddington 1985). Behavior studies reported by Brannon (1986) suggest that residence in dark environments improves the predatory sturgeon's ability to approach and capture fish whose avoidance responses are visually mediated. In related experiments larval sturgeon were more successful at avoiding predation by visual feeders under low light conditions. Semenov (1957) reported that sturgeon larvae developed as expected with colored light, ambient light levels, subdued light and total darkness. Sturgeon larvae reared in direct sunlight experienced abnormal development and repressed growth. When sturgeon are raised for enhancement it might be beneficial to maintain dark conditions to 1) minimize stress 2) train juveniles to seek out low light conditions to avoid post release predation, and (3) hone olfactory and tactile senses used in foraging.

This approach should be adopted only after careful study. Studies on salmonids found that exposure to constant darkness retarded growth and increased mortality. (Kwain 1975, Bilton, 1972) There is an increasing body of evidence for teleosts suggesting that light detection

through the pineal gland and it's related secretory function of releasing melatonin is important in mediating hormonal releases from other endocrine organs that control metabolism and reproduction. Physiological functions that have been linked to secretions from the pineal gland are circadian and seasonal control of growth and reproduction, carbohydrate metabolism, gonadotropin release, and growth hormone release. There is some evidence that the light sensing and secretory functions of the pineal gland have diverged in some species with expected hormonal releases continuing at the expected time in the absence of light. In a primitive fish such as the sturgeon these functions may not have diverged and a light stimulus may be required for normal metabolism and reproduction to proceed.

In light of the recent findings regarding the preference of juvenile white sturgeon for deep water, benthic habitats and the lack of consensus on the importance of controlling light intensity and duration to optimize growth and survival in the hatchery environment we felt it would be beneficial to conduct some preliminary, controlled tank studies.

#### STUDY OBJECTIVES

1. Evaluate the growth characteristics and survival of white sturgeon fingerlings reared long term (16 weeks) in the absence of light.

2. Evaluate the growth characteristics and survival of white sturgeon fingerlings reared long term (16 weeks) with 24 hours light.

3. Compare the growth and survival characteristics of the white sturgeon fingerlings reared with a natural photoperiod under a subdued light pattern (2 lux) to those reared with normal room light (50 lux)

4. Compare the proximate body compositions of white sturgeon fingerlings reared under the above light regimens.

5. Evaluate the effects of tank lighting on within tank size variation.

#### MATERIALS AND METHODS

Tank Illumination:

Light proof hoods with two internally mounted 2 ft., 20 watt fluorescent lights( Sylvania Model No F20T12 CW), were constructed to fit over all culture tanks except those used in the 24 hr darkness treatment. Fluorescent lights were mounted 15 inches from the waters surface. Light intensity levels were adjusted in individual tanks by covering the bulbs and/ or a clear fiberglass lid with layers of brown or black plastic as light filters. All replicates within a treatment had similar light /filter configurations. Light hoods were calibrated to target light intensity levels using an under water light meter reading in FT/candles. Readings were converted to the lux scale. All measurements were taken 12" off the tank bottom. Hoods for treatments requiring a natural photoperiod were wired into an existing circuit controlled by a photocell mounted outside the hatchery.

All light exposed surfaces of tanks in the 24 hour dark treatment were covered with two layers of black plastic sheeting to prevent penetration of overhead hatchery lighting. Tank cleaning and biweekly weight samples were conducted with the overhead lights off. Dark tanks were observed when necessary with a flashlight fitted with a red light filter. A special cover was constructed for the lid to allow for recharging the feeder without light penetration. The sides of tanks in the 24 light treatment were covered with black plastic to prevent the escape of light during the dark cycle.

#### General Culture Practices:

Seven month old sturgeon fingerlings hand grading into large, medium and small fingerlings based on length frequency distributions. Stocks were pooled by size into four- foot diameter circular fiberglass tanks until they are needed for stocking experimental tanks. Lots of 5 fingerlings each were netted from stock tanks graded as" mediums" for random distribution to the twelve, 2.5 ft circular tanks with a capacity of 217 liters. This process was continued until each tank contained twenty fingerlings. Each group was weighed after stocking to obtain an initial starting weight. If necessary larger or smaller individuals were substituted into the tanks until variations in initial starting weight between had a coefficient of variation of less than 5%. Three replicates were randomly assigned to each experimental treatment. Water was distributed via a surface spray bar at 3 liters per minute. Densities were maintained below 25 Kg/m3(Conte 1988).Tanks were cleaned weekly

Diet and Feeding Practices:

Fingerlings were fed Rangen semimoist diet (Rangen Inc. Buhl, ID) for the duration of the study. Automatic cartridge type feeders (Double A- Brand, Model 100, Dallas, TX.) were used to present feed continuously over a 24 hour time frame supplemented by three hand feedings per day. All tanks were fed the same % BWD. Daily rations for fingerlings were estimated using a computer generated growth program with feeding levels being adjusted for mortality on a daily basis. Biweekly adjustments to the feeding program were made based on actual growth rates determined from weight samples. A new specific growth rate(SGR)(Ricker 1975) was assigned to each treatment biweekly based on the mean of individual SGR's for replicates within a treatment.

#### Data Collection:

All fish were weighed in total on a biweekly basis. Weights were measured on a Saltorius 3000 electronic balance recording to the nearest .1 gram. After 16 weeks the experiment was closed. All fish from each treatment were anesthetized with 75mg/liter Tricaine

Methanesulfonate (MS-222) and measured individually for lengths(mm) and weights (g) to evaluate differences in size distributions within and between treatments. Dissolved oxygen and temperature were recorded biweekly. Tank light intensity levels were checked periodically and recalibrated to the target level.

#### Proximate Composition:

Five fingerlings from each treatment were anesthetized with a lethal dose of MS-222. Lengths and weights were recorded and blood samples taken from all individuals. Fingerlings were individually wrapped in plastic bags and frozen. When frozen, individual fish were ground up completely in a meat grinder. The product was blended to a smooth homogenate. Equal weight sub samples were taken from each homogenate within a treatment and pooled into one treatment sample. Duplicate samples were assayed for moisture, protein, ash and total lipid using standard AOAC (AOAC ,1984) procedures.

#### Histological examination:

Five fish from each treatment were anesthetized in a lethal concentration of MS-222. Lengths and weights were recorded and blood samples taken from all individuals. Hematocrit values were determined for individuals and blood smears prepared. Blood plasma samples were segregated by centrifuge and stored in an ultra cold freezer. Fingerlings were necropsied and examined for morphological differences. The viscera was dissected out and weighed. Liver tissue was segregated from the viscera and weighed. Samples of tissue from the major organ systems were collected in histology cassettes and preserved in buffeted formalin for later processing.

#### **RESULTS:**

#### General culture parameters

Water temperature during the study remained fairly constant with a mean temperature of  $12.5^{\circ} C \pm .37$ . Dissolved Oxygen levels remained close to saturation for most of the study. At the end of the study some of the 24 hr dark tanks had lower D.0. levels than other treatments. Mean D.O. levels for all groups was 9.3 mg/liter  $\pm$  .3. Light levels in the treatments calibrated with 50 lux light intensity tended to drop throughout the study requiring recalibration of the filters to attain target levels. This was primarily due to mineral deposits darkening the sides of the tanks and reductions in reflected light caused by growing sturgeon. There was no mortality in any of the treatments. We did observe wide within tank size variation and dropout of smaller individual fish in all treatments. This was characterized by fish going off feed and losing condition factor. The length -weight frequency data has not been analyzed completely to determine if there are significant treatment influences.

#### Growth

Sturgeon fingerlings in all groups grew well on the diets provided with a mean conversion ratio of 1.06. The 24 hr light treatment had the best overall conversion ratio and weight gain. Individuals in the 24 hr dark treatment had the worst conversion ratio and weight increase. The differences were not significant at the (P=.05) level. Conversion ratios and weight increase were intermediate for the two treatments with a natural photoperiod. A summary of growth data is provided in Table 1. Incremental specific growth rate and mean individual weight are charted in graphs 1 and 2 respectively.

# Table Number 1:Summary ofproduction parameters by treatment.

Treatment	50 lux /24 hour light	0 lux/24 Hour Dark	2 lux/natural photoperiod	50 Lux/natural photoperiod
Initial Biomass(g)	1245.6± 1.22*1	1247.7 ± 5.1	1248.36 ± 2.02	1251.7± 4.2
Final Biomass (g)	4464.7 ±132.0	4101.3 ± 155.6	4366.00 ± 110.7	4450.3± 311.2
weight gain(g)	3218.8 ±131.4	2853.6 ± 151.8	3117.6 ± 109	3198.5± 313.6
Individual weight(g)	223.2± 6.6	205.1 ± 7.8	218.3 ± 5.5	218.7± 9.1
total feed(g)	3344 ± 48.6	3165.9 ± 72.9	3277.7± 33.3	3291.1 ± 242.3
Mean SGR	0.011	0.011	0.011	0.011
Conversion Ratio	1.04 ± .05	1.09 ± .06	1.05 ± .03	1.05±.05
% Mortality	0	0	0	0
Max Density (KG/m3)	21.3± .63	20.3± .98	20.8± .56	20.8 ± .86

\*1 standard deviation

#### Proximate Analysis

Major differences in composition of individuals under different light conditions were not observed. The data will need to be analysed for statistical differences. There were significant differences between values we obtained for total lipid and those obtained by Hung(1986). Our protein, ash and moisture levels were similar but less than those obtained from cultured sturgeon in California. Results are summarized in Table 2.

#### Table: Number 2.

Treatment	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Total (%)
50 lux/ natural photoperiod	14.5	10.9	2.5	75	102.9
24 hr/dark	14.6	10.8	2.6	74.9	102.8
24 hr light	14.5	10	2.2	73.5	100.2
2 lux/ natural photoperiod	15.5	11	2.3	74	102.8
Mean	14.8	10.7	2.4	74.4	102.2
STD	0.4	0.4	0.2	0.6	1.1

# Whole Body Proximate Analysis

#### Histological Examination:

External examination of the fingerlings revealed that 22.9 % of the experimental population had varying degrees of inflammation and hemorrhaging along the ventral sautes. This condition was often accompanied by deformed paired and caudal fins with "corns". It is speculated that condition was caused by rubbing against the tank or each other in an attempt to maintain contact with the substrate. The wounds healed when they were moved to larger tanks at a lower density. The livers of all fish in all groups were creamy white and had large deposits of fat or glycogen. We did not assay for these compounds from the liver. Samples have been taken for histological examination. Larval white sturgeon livers with a similar appearance had large numbers of intercellular vacuoles. Hematocrit values were significantly (P=.05) higher in the 50 lux / Natural Photoperiod treatment than the other three treatments. The mean hepatosomatic index we obtained from sampled fish was twice that reported by Hung(1986) for 11 month old sturgeon fingerlings with a mean weight of 431 g. A summary of the data is provided in Table 3.

Treatment	Mean Wt.(g)	Mean Length(mm)	viscera wt.(g)	Liver wt.(g)	HSII	K	hematocrit
24Hr / dark	210.2	384	20.4	8.9	4.4	3.7x10 <sup>-6</sup>	23.7
24Hr. / Light	238	399	20.6	8.7	3.9	3.7x10 <sup>-6</sup>	23.8
50 lux / Nat.	232.7	396	25.1	10.9	4.2	3.6x10-6	27.6
5 lux / Nat.	235.5	397	22.4	8.8	3.3	3.7 x 10 <sup>-6</sup>	25.5
mean	229.1	394	22.1	9.3	4.0	3.7 x 10 <sup>-6:</sup>	25.2
Standard deviation	9.90	5.25	1.69	0.82	0.37	0.00	1.42
C.V.(%)	4.32	1.33	7.62	8.75	9.40	1.19	5.64

 Table Number 3: Summary of Morphological and Physiological

 Measurements

#### CONCLUSIONS:

An initial review of the data indicates that white sturgeon fingerlings can successfully feed and grow under a diverse range of light intensities and photoperiod and that survival in the short term is not compromised by being raised in complete darkness or 24 hr. light. The fact that they appear to grow better with 24 hr light is consistent with the findings for many teleosts. Pyle (1969) found no differences between growth in trout raised under 24 hour light and 24 hour dark for the first 30 weeks. After 30 weeks those in constant light outperformed those in 24 hr dark. The relationship developed much earlier in sturgeon indicating that light may have a greater influence on growth.

It is unclear why sturgeon reared under 24 hr darkness had inferior performance to those reared under the other light regimens as light is not required for feed capture and they typically occupy dark /murky environments. One possible explanation is that they were more active than their counterparts consequently dedicating more energy to movement. Young sturgeon were observed to be more active and moved from the bottom into the water column when the lights were shut down in the hatchery. No attempt was made to quantify differences in movement between treatments. There was a greater prevalence of ventral scute hemorrhaging in fingerlings in the 24 hr light treatment to those in the 24 hr dark treatment . If sturgeon in the 24 hour dark treatment were spending more time in the water column they would be less susceptible abrasion type injuries resulting from contact with the tank. The lower D.O. levels in dark tanks at the end of the study would also indicate higher levels of activity.

The data on length -weight frequencies and histological examinations has not been completely analyzed therefore it would be premature to comment on the significance of light intensity or photoperiod on these parameters.

The unusual structure of the liver in our fish appears to be influenced by factors other than light as all treatments had a similar structure and the white livers also develop during the fry stage. We hope to obtain livers from wild sturgeon larvae to determine if this is a natural condition in more temperate ranges. Data obtained by Hung (1986) indicated that wild sturgeon had higher hepatosomatic indexes than their hatchery reared counterparts. These indexes were still less than those found at the Abernathy SCTC.

For production white sturgeon rearing facilities increasing photoperiod may offer a benefit by allowing for faster growth of stocks. For facilities focused on sturgeon enhancement the data indicates that white sturgeon fingerling in this size bracket will perform adequately under subdued light conditions. More rigorous trials will be required to identify the optimal lighting environment for hatchery reared sturgeon and its impact on post release behavior and survival.

#### **REFERENCES**:

1. AOAC, 1984. Official Methods of Analysis of the Association of Official Analytical Chemist, 14th Edition. S.Williams (Editor). Association of Official analytical Chemist, Inc. Arlington, VA.

Bilton, H.T. 1972. A Comparison of body and Scale growth of young Sockey Salmon (*Oncorhynchus nerka*) reared under two light periods and in total darkness. Fish. Res. Board Can. 26: 330 :12p

Brannon, E. et al. 1985 Columbia River White Sturgeon- Early Life History and Genetics Study. Project report: Bonneville Power Administration. Department of Energy. Portland, Or.

Brannon, E. et al. 1986. Columbia River White Sturgeon (*Acipenser transmontanus*) Population Genetics and Early Life History. Project report: Bonneville Power Administration. Department of Energy. Portland, Or. Buddington, R.K. and J.P Christofferson, 1985. Digestive and feeding characteristic of the chondrosteans. In F.P Binkowski &S.I. Doroshov: North American Sturgeons. Junk Publishers Netherlands.

Conte, F.S., S.I. Doroshov, P.B. Lutes and E.M. Strange. 1988. Hatchery Manual For White Sturgeon. University of California Press. Oakland

Hung, S.O., P.B. Lutes and F. Conte. 1986. Carcass Proximate Composition of Juvenile White Sturgeon (Acipenser transmontanus). Comp Biochem. Physiol. Vol 88B, No1, pp269-272

Parsley, M.J. L. Beckman and G.T. Mc Cabe. 1992. Habitat use by spawning and Rearing White Sturgeon in the Columbia River Downstream From Mc Nary Dam. In: Status & Habitat Requirements of the White Sturgeon Populations in the Columbia River Downstream from Mc Nary Dam. Final Report U.S. Department of Energy, Bonneville Power Administration. Portland, Oregon.

Pyle, E. 1969. The effects of Constant Light or Constant Darkness on the Growth and Sexual Maturity of Brook Trout. Fish. Res. Bull. No31, pp 13-19

Mc Cabe G.T. and S.A. Hinton. 1990, Report D. In: A.A.Nigro. Status and Habitat Requiremnts of white sturgeonpopulations in the Columbia River Downstream from McNary Dam. Annual Report to the Bonneville Power Administration. (Project 86-50) Portland, Oregon.

Ricker, W.E. 1979. Computation and interpretation of Biological statistics of fish populations. Fisheries Research Board of Canada, Bulletin 191

Kwain, W. 1975. Embryonic development, early growth and meristic variation in rainbow trout exposed to combinations of light intensity and temperature. J. Fish Res. Board Can 32:397-402

Semenov, K.I. 1957. The effects of different light conditions on the development of Sturgeon Larvae in the period from hatching to transition to active feeding. Doklady Akademia Nauk SSSR. Biological Sciences Section Volume No. 4 pp. 248-251



