

Annual Research Progress Report for 2002

Macaulay Salmon Broodstock Laboratory
Juneau Center, School of Fisheries and Ocean Sciences
University of Alaska Fairbanks

by

Dr. William W. Smoker
Professor of Fisheries

Ivan A. Wang
Research Associate

Juneau Center, School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
11120 Glacier Highway
Juneau, AK 99801

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I. Introduction

University research and teaching at the Macaulay Broodstock Laboratory are concerned with the biology of Pacific salmon. In particular, the Lab applies research to artificial culture of salmon and the wise use of salmon resources. In Alaska, artificial culture is synonymous with its ocean ranching program; Douglas Island Pink and Chum, Inc., (DIPAC) is one of Alaska's private non-profit organizations in that program. DIPAC provides the Lab with its basic support, facilities.

This progress report is prepared at the end of the Lab's twelfth year of operation, 2002. It is written for several purposes: for the information of the several extramural funding agencies that support research at the Lab, to satisfy the reporting requirements of our operating permits, and to serve researchers as a record of progress.

A. Support

Primary support for research at the Lab comes from DIPAC, which provides space and utilities to the Lab. The National Marine Fisheries Service, The Alaska Science and Technology Foundation, Teck Cominco Alaska Inc. and the University of Alaska Fairbanks/School of Fisheries and Ocean Sciences provided financial support to research in the Lab during 2002.

Sources of support for research at Macaulay Salmon Broodstock Laboratory in 2002.			
Agency	Grant No.	Amount	Title
Douglas Island Pink and Chum, Inc. (DIPAC)	Memorandum of Understanding, May 1991.	\$24,000 *	*In kind award of space and utilities
Alaska Science and Technology Foundation	99-3-097	\$96,000	Effects of Total Dissolved Solids on Salmonid Fish
NOAA-NMFS	50ABNF600123	\$224,000	Outbreeding Effects in Pacific Salmon
Teck Cominco Alaska Inc.	360816	\$60,000	Effects of Total Dissolved Solids on Fertilization Rates of Salmonids in the Red Dog Mine Area

B. Permits

Permits valid in 2002 at Macaulay Salmon Broodstock Laboratory and references to reports on activities under the permit.		
Permit Number	Purpose	Numbers, Disposition, Released
ADF&G P-02-003	Expose Arctic grayling gametes to Total Dissolved Solids	Arctic grayling, 6,000 eggs and milt from 10 males. No releases All embryos humanely euthanized.
ADF&G FTP 02J-1002	Transport Arctic grayling gametes from Chena River, Fairbanks to Juneau	See P-02-003 above.
ADF&G P-02-003 – Amendment #1	Expose Arctic grayling, Dolly Varden, and chum salmon gametes to Total Dissolved Solids	Arctic grayling, 8,000 eggs and milt from 10 males Dolly Varden, 6,000 eggs and milt from 3 males; chum salmon, 10,000 eggs and milt from 9 males. No releases. All fish humanely euthanized.
ADF&G FTP 02J-1006	Transport Arctic grayling gametes from Red Dog Creek (Red Dog Mine) to Juneau	See P-02-003 Amendment #1 above.
ADF&G FTP 02J-1005	Transport Dolly Varden gametes from Ikalukrok Creek (Red Dog Mine) to Juneau	See P-02-003 Amendment #1 above.
ADF&G FTP 02J-1007	Transport chum salmon gametes from Ikalukrok Creek (Red Dog Mine) to Juneau	See P-02-003 Amendment #1 above.
ADF&G P-02-008	Compare survival and fitness of intercrossed juvenile Chinook salmon	Released 10,000 tagged parr (5,000 wild and 5,000 hatchery) into Twin Lakes in June. Captured parr in September. 3,000 parr cultured at Macaulay Salmon Broodstock Laboratory from July to September. All fish humanely euthanized.
ADF&G P-02-010	Recover experimental coho salmon from Sheep Creek and nearby streams.	Recovered 58 juveniles and 4 jacks. All fish humanely euthanized.
IACUC #02-26	Assures humane treatment of Outbreeding Depression experimental animals.	500 jacks/adults and 150,000 fry. Over 90,000 fry were reared and released and 62 juveniles and jacks were humanely euthanized.
IACUC #02-51	Assures humane treatment of Total Dissolved Solids experimental animals.	See P-02-003 and Amendment #1.

C. Personnel

The following people took part in activities at the Lab under its Scientific/Educational and Fish Resource and Transport Permits:

Barbi Failor, BS Graduate Research Asst., UAF	Will Hayes, BS Lab/field technician, UAF
Cara Rodgveller, BS Graduate Research Asst., UAF	William W. Smoker, PhD Professor, UAF
Michael S. Stekoll, PhD Professor, UAF/UAS	Ivan Wang, BS Research Associate, UAF

II. Research Projects

A. Genetic Mechanisms of Outbreeding Depression In Pacific Salmon

W. W. Smoker, A. J. Gharrett, I. A. Wang, S. L. Walden, supported by NMFS

1. Background

We made experimental intercrosses between populations of Pacific salmon, releasing them to complete their life cycle in the ocean, and have been observing their performance (growth, survival, etc.) for two generations. Three populations of coho salmon from Southeast Alaska were used to make the nine possible first-generation crosses (Table 1) and fifteen second-generation crosses (Table 2). Tests of hypotheses about fitness effects of outbreeding depression will be possible from observations of these salmon.

Table 1. Table of first generation crosses. Parent's origins abbreviated Gastineau (G), Hidden Falls (H), Neets Bay (N). Generation 1: 9 groups (female x male) parental crosses boldface

		Male's Population		
		H	N	G
Female's Population	H	HH	NH	GH
	N	HN	NN	GN
	G	HG	NG	GG

Table 2. Table of 15 second-generation lines, made in 2000 from returning BY 97 adults. Parents' origins abbreviated Gastineau (G), Hidden Falls (H), Neets Bay (N), female parent indicated first. Three parental lines in boldface on diagonal, six F₂ intercross lines off diagonal in four letters indicating maternal and paternal source lines, six repeat F₁'s in italics off diagonal.

Female's Pop	Male's Population								
	HH	HN	HG	NH	NN	NG	GH	GN	GG
HH	HH				<i>HN</i>				<i>HG</i>
HN				HNNH					
HG							HGGH		
NH		NHHN							
NN	<i>NH</i>				NN				<i>NG</i>
NG								NGGN	
GH			GHHG						
GN						GNNG			
GG	<i>GH</i>				<i>GN</i>				GG

The first generation hybrids were released in the spring of 1999 (Brood Line 97). When the fish were one year old, each of the nine groups of crosses, reared separately as embryos, fry, and parr, were tagged with coded micro wire (CWT). Each group received a different tag code. After the end of the summer the parr were transferred from Macaulay (formerly Gastineau) Hatchery to the Sheep Creek Hatchery, 15 km distant but also a tidewater facility on Gastineau Channel. (Gastineau Channel is a fiord in southeast Alaska devoid of significant natural production of coho salmon). At Sheep Cr. the aggregate group of tagged salmon was evenly distributed into two identical raceways. The returning adults will give us information on differences in ocean survival, a direct measure of fitness, and were used to produce the second generation of hybrid intercrosses, control lines, and some back crosses. Brood Line 97 adults returned from August to November 2000 and recovery of Brood Line 2000 fish will be complete in 2003.

2. Broodyear 2000

a) Rearing and release

Broodyear 2000 fry were reared in three raceways at Sheep Creek Hatchery from October 30, 2001 to May 21, 2002. Broodyear 2000 fry had low mortalities and moderately high weight weight gain during rearing (Table 3). The data shown are pooled for all crosses. Since fish from all crosses were reared together, it was not possible to track growth by cross without a destructive sample. Fish were moved to a covered outdoor raceway at Sheep Creek Hatchery on May 20, 2002. Heavy rains reduced the water quality in the outdoor raceway on May 21, 2002. Therefore, we decided to release the fish into Sheep Creek that day. We recovered experimental juveniles and jacks from streams nearby and including Sheep Creek, and Macaulay Hatchery's coho harvesting starting from August 2002 to November 2002.

Table 3. Rearing weights, numbers, and mortalities for Broodyear 2000 fry.

Date	Weight (g)	# of Fish	# of Mortalities
9/27/01	6.21	96,230	
10/30/01	6.67	95,954	276
11/29/01	8.78	95,805	149
12/17/01	8.31	95,792	13
1/8/02	9.14	95,777	15
2/28/02	10.09	95,757	20
4/2/02	10.68	95,757	0
4/30/02	12.10	95,754	3
5/21/02	12.34	95,749	5

b) Stream recoveries

Beginning on August 19, 2002, we set baited traps in Sheep Creek, the release site, and four other streams in the Gastineau Channel area. We set four to six traps at each site once a week for approximately 24 hours (Table 4). We used two types of traps in each stream. The first type is a galvanized minnow trap with the openings enlarged to 75mm. The second is a mesh/wire fish trap (Aquatic Eco Systems, www.aquaticeco.com, part number "FTA"). We weighted the traps with rocks and anchored them with rope, carabiners, and/or PVC stakes and placed them in locations likely to hold coho jacks. We baited the traps with cured or fresh chum or coho salmon roe. We concluded trapping on November 6, 2002 after we were no longer catching target fish and adults in the streams were absent or spawned out.

We recovered 58 coho juveniles and four coho jacks with adipose fin clips (Table 4). Of those fish, 33 juveniles and four jacks carried coded wire tags (CWTs) from this experiment (Table 5). Two fish either had no tag recovered, one fish had a CWT from a Macaulay Hatchery production release, and twenty had CWTs from an experimental release of 10,000 coho presmolts from an Alaska Department of Fish and Game (ADF&G)/Auke Bay Laboratory-NMFS environmental impact study for an airport expansion plan. When the identity of the fish in Jordan Creek was determined, we discontinued sacrificing adipose fin-clipped coho juveniles and jacks caught at Jordan Creek. Two adipose fin-clipped coho juveniles were caught and released at Jordan Creek.

c) DIPAC commercial harvest sampling

Beginning on August 23rd and ending September 20th, we sampled 85 totes from Douglas Island Pink and Chum's (DIPAC) commercial harvest at Macaulay Hatchery as well as the raceway used for collecting DIPAC's coho broodstock. From this sampling, we found 102 coho jacks and juveniles in the totes and 117 coho jacks and juveniles in the raceway. Out of the 219 jacks and juveniles, 34 were adipose fin-clipped and turned in to ADF&G's Tag Lab for processing. Three of the fish we turned in had CWTs related to this project (Table 5).

Table 4. 2002 Trap Record

Date set	Date retrieved	Location	clipped jacks	unclipped jacks	clipped juveniles	unclipped juveniles	Dollies	Sculpin	Other	Traps deployed	
										mesh	wire
19-Aug	20-Aug	Lawson Creek	0	0	0	0	2	0		3	2
20-Aug	21-Aug	Salmon Creek	0	0	0	0	0	0		3	2
20-Aug	22-Aug	Salmon Creek	0	0	0	0	0	2		-	-
21-Aug	22-Aug	Switzer Creek	0	0	0	0	0	2		3	2
22-Aug	23-Aug	Jordan Creek	0	0	0	12	2	8		3	1
26-Aug	27-Aug	Lawson Creek	0	0	0	0	0	11		2	2
27-Aug	28-Aug	Sheep Creek	0	0	4	0	0	14		3	2
28-Aug	29-Aug	Salmon Creek	0	0	0	1	0	25		3	2
29-Aug	30-Aug	Switzer Creek	0	0	0	0	4	0	13 fry	3	2
29-Aug	30-Aug	Jordan Creek	0	0	13	5	5	14		3	1
3-Sep	4-Sep	Lawson Creek	0	0	0	0	8	27		3	2
3-Sep	4-Sep	Sheep Creek	0	0	2	0	0	0		3	1
4-Sep	5-Sep	Salmon Creek	0	0	0	0	1	9		3	2
5-Sep	6-Sep	Switzer Creek	0	0	0	1	21	0	11 fry	3	2
5-Sep	6-Sep	Jordan Creek	0	0	0	3	1	3		3	1
9-Sep	10-Sep	Sheep Creek	0	0	0	0	0	1		4	1
10-Sep	11-Sep	Lawson Creek	0	0	0	0	6	3		4	2
11-Sep	12-Sep	Salmon Creek	0	0	0	0	1	2		3	2
11-Sep	12-Sep	Switzer Creek	0	0	0	1	8	0		4	1
12-Sep	13-Sep	Jordan Creek	0	0	0	2	2	4		3	2
16-Sep	17-Sep	Lawson Creek	0	0	0	0	8	4		3	2
16-Sep	17-Sep	Sheep Creek	0	0	0	0	0	8		4	1
17-Sep	18-Sep	Salmon Creek	0	0	0	0	1	6		2	2
17-Sep	18-Sep	Switzer Creek	0	0	0	3	7	1	7 fry	4	1
18-Sep	19-Sep	Jordan Creek	0	0	5	6	38	0	1cutthroat	3	1
17-Sep	19-Sep	Sheep Creek	0	0	19	0	0	0		1	0
23-Sep	24-Sep	Lawson Creek	0	0	1	0	4	5		4	2
23-Sep	24-Sep	Sheep Creek	0	0	3	0	0	1		2	1
24-Sep	25-Sep	Salmon Creek	0	0	0	0	3	10		2	2
24-Sep	25-Sep	Switzer Creek	0	0	2	3	11	0	2 fry	2	2
25-Sep	26-Sep	Jordan Creek	0	0	0	0	0	7	1 stickleback	3	1
24-Sep	26-Sep	Sheep Creek	0	0	0	0	0	0		1	0
30-Sep	1-Oct	Lawson Creek	0	0	0	0	1	4		4	2
30-Sep	1-Oct	Sheep Creek	1	0	1	0	0	0		2	1
1-Oct	2-Oct	Salmon Creek	0	0	0	0	1	23		2	2
1-Oct	2-Oct	Switzer Creek	0	0	0	2	4	0	5 fry	4	1
2-Oct	3-Oct	Jordan Creek	0	0	1	1	0	2		3	1
1-Oct	3-Oct	Sheep Creek	0	0	0	0	0	0		1	0
8-Oct	9-Oct	Lawson Creek	0	0	0	0	2	4		3	2
3-Oct	8-Oct	Sheep Creek	0	0	1	0	0	0		1	0
8-Oct	9-Oct	Sheep Creek	2	0	1	0	0	0		3	1
9-Oct	10-Oct	Switzer Creek	0	1	3	2	1	0		4	1
10-Oct	11-Oct	Jordan Creek	0	1	1	0	1	7		3	1
8-Oct	11-Oct	Sheep Creek	0	0	0	0	0	0		1	0
11-Oct	14-Oct	Sheep Creek	0	0	0	0	0	0		1	0
14-Oct	15-Oct	Lawson Creek	0	0	0	1	2	15		3	2
14-Oct	15-Oct	Sheep Creek	1	0	1	0	0	1		3	1
15-Oct	16-Oct	Salmon Creek	0	0	0	0	2	3		2	2
15-Oct	16-Oct	Switzer Creek	0	0	0	1	3	0		4	1
16-Oct	17-Oct	Jordan Creek	0	0	0	0	2	0		3	1
15-Oct	17-Oct	Sheep Creek	0	0	0	0	0	0		1	0
17-Oct	22-Oct	Sheep Creek	0	0	0	0	0	0		1	0
22-Oct	23-Oct	Lawson Creek	0	0	0	0	4	1		3	0
22-Oct	23-Oct	Sheep Creek	0	0	0	0	0	1		4	0
23-Oct	25-Oct	Sheep Creek	0	0	0	0	0	0		1	0
23-Oct	24-Oct	Salmon Creek	0	0	0	0	0	4		2	2
23-Oct	24-Oct	Switzer Creek	0	0	0	1	4	0	1 fry	4	1
24-Oct	25-Oct	Jordan Creek	0	0	0	2	1	3		3	1
25-Oct	28-Oct	Sheep Creek	0	0	0	0	0	0		1	0
28-Oct	29-Oct	Lawson Creek	0	0	0	0	0	7		3	2
28-Oct	29-Oct	Sheep Creek	0	0	0	0	0	10		4	1
29-Oct	30-Oct	Salmon Creek	0	0	0	0	0	5		2	2
29-Oct	30-Oct	Switzer Creek	0	0	0	2	4	1		4	1
30-Oct	31-Oct	Jordan Creek	0	0	0	0	0	5		3	1
29-Oct	31-Oct	Sheep Creek	0	0	0	0	0	0		1	0
31-Oct	4-Nov	Sheep Creek	0	0	0	0	0	0		1	0
4-Nov	5-Nov	Lawson Creek	0	0	0	0	1	0		3	2
4-Nov	5-Nov	Sheep Creek	0	0	0	0	0	7		2	2
5-Nov	6-Nov	Salmon Creek	0	0	0	0	0	7		2	2
5-Nov	6-Nov	Switzer Creek	0	0	0	2	5	1		3	1
6-Nov	7-Nov	Jordan Creek	0	0	0	0	0	0		3	1
Total			4	2	58	51	171	278			

Table 5. 2002 CWT recoveries. Jacks in gray background. Lengths are mid-eye to fork in mm.

Cross	Recovery Location	Date Caught	Length
GGxNN & NNxGG	SHEEP CR	9/23/2002	103
HNxNH & NHxHN	SHEEP CR	10/1/2002	103
HNxNH & NHxHN	SHEEP CR	9/19/2002	106
HNxNH & NHxHN	SHEEP CR	8/28/2002	110
HNxNH & NHxHN	MACAULAY	10/7/1998	133
HNxNH & NHxHN	MACAULAY	10/7/1998	136
GGxHH & HHxGG	SHEEP CR	9/23/2002	101
GGxHH & HHxGG	SHEEP CR	9/19/2002	104
GGxHH & HHxGG	SHEEP CR	9/19/2002	115
GHxHG & HGxGH	LAWSON CR	9/24/2002	101
GHxHG & HGxGH	SHEEP CR	9/19/2002	118
NNxNN	SHEEP CR	9/19/2002	98
NNxNN	SHEEP CR	9/19/2002	99
HHxNN & NNxHH	SHEEP CR	9/19/2002	94
HHxNN & NNxHH	SHEEP CR	9/19/2002	104
HHxNN & NNxHH	SHEEP CR	9/19/2002	114
GHxHG & HGxGH	SHEEP CR	8/28/2002	102
GHxHG & HGxGH	SHEEP CR	9/19/2002	105
GHxHG & HGxGH	SHEEP CR	9/19/2002	121
GHxHG & HGxGH	SHEEP CR	9/19/2002	121
GHxHG & HGxGH	SHEEP CR	9/19/2002	129
GGxNN & NNxGG	SHEEP CR	9/19/2002	103
GGxHH & HHxGG	SHEEP CR	9/23/2002	86
GGxHH & HHxGG	SHEEP CR	9/4/2002	103
GGxHH & HHxGG	SHEEP CR	9/19/2002	104
GGxHH & HHxGG	SHEEP CR	8/28/2002	113
GGxHH & HHxGG	SHEEP CR	10/9/2002	118
HNxNH & NHxHN	SHEEP CR	9/19/2002	101
HNxNH & NHxHN	SHEEP CR	9/19/2002	103
HNxNH & NHxHN	SHEEP CR	10/9/2002	110
HNxNH & NHxHN	SHEEP CR	9/19/2002	126
GGxGG	SWITZER CR	9/25/2002	115
HHxHH	SHEEP CR	9/4/2002	104
HHxHH	SHEEP CR	10/15/2002	112
HHxHH	SHEEP CR	9/19/2002	135
GGxHH & HHxGG	SHEEP CR	10/1/2002	238
GHxHG & HGxGH	SHEEP CR	10/9/2002	283
GNxNG & NGxGN	SHEEP CR	10/15/2002	235
GNxNG & NGxGN	SHEEP CR	10/9/2002	247
GNxNG & NGxGN	MACAULAY	10/28/1998	261

3. Morphometrics

We completed recording digital landmarks from photos of all of the Broodyear 1997 and 2000 parents using TPSDIG32 version 1.31 and analysis using TPSRELW version 1.29 is in progress. (Rohlf 2002, Department of Ecology & Evolution, State University of New York, Stony Brook 11794-5245 (<http://life.bio.sunysb.edu/morph/index.html>)).

4. DNA Analysis

To date, we have run four loci for all 270 of the 1997 broodyear parents and a fifth locus is being run for some individuals not identifiable from the first four loci (Table 6). From coded-wire tags, we identified 219 individuals from the 1997 crosses that returned in 2000. We have run four loci from these individuals (Table 7). From these data, we determined the parentage for 217 of the returns. The last two individuals will have DNA reisolated and rerun.

Table 6. Preliminary DNA Analysis of Broodyear 1997 parents (270 individuals)

Locus	Number of alleles
OTS 101	30
OKI 1	11
OKI 10	26
OKI 16	21
OKI 13	6

Table 7. Preliminary DNA Analysis of Broodyear 2000 parents (219 individuals)

Locus	Number of alleles
OTS 101	29
OKI 1	10
OKI 10	23
OKI 16	12

B. Effects of Inbreeding on Chinook Salmon Fry

Cara J. Rodgveller and W.W. Smoker, Supported by NOAA-NMFS Auke Bay Laboratory

1. Background

Researchers from the National Oceanic and Atmospheric Administration (NOAA) - National Marine Fisheries Service (NMFS), at their research station at Little Port Walter (LPW), Baranof Island, developed a geographically isolated Chinook salmon (*Oncorhynchus tshawytscha*) broodstock for the Alaska salmon enhancement program. The broodstock was originally taken from a wild population of spring Chinook salmon in the Chickamin River in 1976 and has been in artificial culture for five generations. Gametes have been taken at LPW, fertilized and incubated there. The resulting fry, parr, and smolts were cultured there for more than a year for each generation before being released into the ocean.

In 1996, NMFS scientists collected gametes from wild Chinook salmon in the Chickamin River and produced F₁ hybrids between the hatchery-raised and wild fish (wild x hatchery and hatchery x wild) and F₁ control crosses (wild x wild and hatchery x hatchery). In 2001, new artificial matings were created; the wild- and hatchery-controls were maintained, reciprocal F₁ hybrids were reproduced and reciprocal F₂ hybrids were produced, and are now in the hatchery (Table 1). F₁ hybrids were reproduced because the adults used to create the F₁ hybrids in 1996 were raised in different environments; the wild fish reared in the Chickamin River and the hatchery fish were cultured at LPW. The 2001 F₁ reproductions were from wild and hatchery parents that were raised in the same environment.

Table 1. Number of each experimental cross of hatchery-bred and wild Chinook salmon from the Chickamin River, Alaska, transported to Macaulay Hatchery Juneau, Alaska, 2002. H designates hatchery-bred fish; W designates fish of wild ancestry.

Cross Type		# of fish
F1 hybrid (reproduction)	HH X WW	500
F1 hybrid (reproduction)	WW X HH	500
Control	HH X HH	5500
Control	WW X WW	5500
F2 hybrid	HW X HW	500
F2 hybrid	WH X WH	500

2. Twin Lakes Experiment

On June 3, 2002 we transported 13,000 Chickamin River Chinook fry (Table 1) from LPW to DIPAC's Macaulay Hatchery by floatplane. We held all 13,000 fry at the University of Alaska Fairbanks' lab at Macaulay Hatchery. On June 21-22, 2002, I marked 5,000 wild-origin fish and 5,000 hatchery-origin fish with a photonic tagging gun. I used the gun to mark the fish according to their cross and release location (north or south lake in Twin Lakes, Juneau, AK). I also clipped a pelvic fin to differentiate our experimental Chinook from DIPAC'S Chinook broodstock (planted in Twin Lakes for sport fishing), and as a verification mark for the photonic tags.

On June 28, 2002, I released 2,500 of each cross type (wild and hatchery) into each of the Twin Lakes, 5,000 fry in each lake. To keep fish from migrating between lakes we placed a 28' by 8' seine with 1/4" mesh between the lakes for the duration of the experiment.

On September 28-29, 2002 I seined both of the Twin Lakes with baited hauls of a beach seine along the shore. I recaptured 106 (54% HHxHH, 45% WWxWW, 1% unknown) experimental fish from the southern lake with 28' by 8' beach seines with 1/4" mesh. No fish were recovered from the northern Twin Lake. The fish were euthanized with MS-222. Length, weight, and photonic tag data were recorded. Length and weight analyses have not been completed.

3. Common Garden Experiment

From July-September 2002, I conducted a common garden growth experiment in the 114-liter tanks at Macaulay Hatchery. On July 1-3, I weighed 2,550 fish (to the nearest hundredths of a gram) and measured lengths (to the nearest millimeter). I then used these fish to populate seven low-density tanks (150 fish per tank) and five high-density tanks (300 fish per tank). Each tank included equal numbers of the six experimental crosses (Table 1). I fed the fish to satiation using automatic feeders. Artificial lighting was set for ambient day length.

On September 22-24, 2002, I euthenized all fish with MS-222, weighed, and measured them, and examined them for photonic tags. I also kept a caudal fin clip for genetic analysis. Length and weight analyses have not been completed.

C. Salmon as a Bioassay Model of Effects of Total Dissolved Solids

M.S. Stekoll, W.W. Smoker, I.A. Wang, B.J. Failor, Supported by ASTF

Summary of Project Findings

We completed the study of the lethal and sublethal effects of exposing salmonid juveniles to a specific mixture of total dissolved solids (TDS) modeled after discharge from Red Dog Mine (northwest of Kotzebue). The need for this test came from two State of Alaska regulatory agencies (Department of Fish and Game and Department of Environmental Conservation) working in concert with industry's Alaska Council of Producers to revise discharge limits for total dissolved solids in the Alaska mining industry. Little work had been done previously on the effects of TDS on salmonids, particularly in Alaska. Their health, growth, and development were regarded as an appropriate biological indicator of water quality related to TDS levels.

We found that for short (24- to 96-hour) exposures, fertilization was the most sensitive juvenile stage to this TDS mixture. We observed reduced fertilization rates in concentrations of this TDS mixture as low as 250 ppm. Natural background levels of TDS in our control water (Macaulay Hatchery) typically ranged from 20 to 60 ppm. For fertilization, we observed differences in sensitivity to this TDS mixture between species of salmonids. King, pink, and coho salmon were most sensitive, and Arctic char were least sensitive. Likewise, coho salmon chronically exposed to this TDS mixture at 2500 ppm had lower fertilization rates as well as higher posthatch mortality rates.

These results apply only to the populations tested with the specific TDS mixture we used. Extensions to other populations may not be true. Applying this work to situations with different TDS mixtures, especially differing ratios of ions, additions or deletions of ions, and additional toxicants like heavy metals, is even more problematic. It is likely that other species will be adversely affected by TDS, especially during fertilization, but it is not possible to extrapolate our results to predict the effects of a given population at a given concentration.

III. Protocols and Research Animal Care

Fish in the lab are maintained according to best fish cultural practices recommended by the American Fisheries Society and other professional agencies. The laboratory is periodically inspected by the University of Alaska Fairbanks Institutional Animal Use and Care Committee. Macaulay Salmon Broodstock Lab includes rooms at Macaulay Hatchery-Channel Drive and at Sheep Creek hatchery-Thane. These are two separate buildings owned by Douglas Island Pink and Chum Inc. Each is operated by DIPAC under permit from the state as a salmon hatchery; the University uses research space in each building under conditions of agreements with DIPAC.

The rationale for our use of live animals in this research is that empirical studies require observation of live fish; alternative model systems are not available and are not feasible; most studies are of variation of fitness traits in natural and cultured populations of salmonid fish. Salmonid fish are the basic resource for many Alaskan cultures and societies as well as for one of Alaska's most important industries; Conservation of salmonid biodiversity is self-evidently important; efficient operations of fishery enhancement projects are important; research on salmonid species is necessary.

Experiments are designed according to accepted standards in animal science, numbers of individuals, treatment groups, and replicates are those necessary to achieve adequate statistical power to minimize error. Proposed designs are peer-reviewed under the direction of funding agencies. Designs are also reviewed by State of Alaska Chief Fish Pathologist, by the State of Alaska Chief Fish Geneticist and by appropriate resource managers for compliance with state regulations governing conservation of salmon resources.

A. Gamete Collection

Fish used in research are generally obtained on site from the Macaulay Salmon Hatchery, a facility operated by Douglas Island Pink and Chum Inc. by permission and under supervision of the State of Alaska; any transport, usually of unfertilized eggs, from another location is governed by state permits.

Mature females are taken from the broodstock facility. They are killed by a blow to the head. Egg takers dried the bellies with paper towels and took the eggs by excision into clean, dry, labeled containers, retaining only eggs from females free of "over mature" (a visual determination) eggs. These containers

were carried into the Broodstock Laboratory. In the Lab eggs were kept on ice or under refrigeration (4°C) in a covered container until use.

From each donor adult we generally collect tissues (eye, heart, white muscle, liver) for biochemical analysis, and we collect size information (mid-eye to fork of tail length, weight).

Males are also collected from the Hatchery broodstock raceways and killed by a blow to the head. Semen (milt) is collected dry into separate containers and taken into the Lab. Semen was kept on ice or refrigerated in a covered container and samples are routinely examined for evidence of motility before use. Small-volume samples may be extended 100 fold in Cortland Extender (KCl 7.2 gram, NaCl 1.880 g, NaH_2PO_4 0.408 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.232g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.232g, NaHCO_3 1.0g, Glucose 1.0g, In distilled water to 1.00 L, pH 8.2.)

B. Fertilization and Activation

Eggs from individual females are poured into plastic containers and mixed with semen from individual males (one to one matings unless otherwise specified). The mixed gametes might be flooded with Activator Solution (NaCl 9.0 gram, TRIS 1.21 g, Glycine 1.5g, in distilled water to 1.00 L, pH 9.0).

C. Care of Embryos

Blank (white, dead) eggs are removed from incubators (FAL™ Heath Incubators) 24 hours after fertilization. Eggs are treated with formalin fungicide as required during early development. After the eyed stage dead eggs are removed from each incubator. Daily checks for condition of embryos are made.

D. Care of Fry, Parr, Smolts, Juveniles, Adults

Fish culture practice at Macaulay Lab, in all research projects, follows the best known professional standards, minimizing stress by providing optimum water quality, nutrition, and freedom from pathogens. Fish are fed commercial salmon diet (various makers) of the appropriate size and ration. Densities are maintained at or below appropriate recommended limits in an excess of flowing water. Aquaria and rearing tanks can be supplied with supplemental air as a guard against low water flow. A continuous log of temperature is maintained for all culture systems.

E. Pathology/Health

Disease diagnostic services are provided by the State of Alaska Department of Fish and Game Fish Pathology Laboratory under the supervision of the Chief Fish Pathologist, located in Juneau. The Pathologist reviews research proposals as part of the permitting process and the Pathologist inspects the laboratory periodically. Diseased fish are necropsied and are regularly taken to the Pathology Laboratory for diagnosis and advice.

Samples of ovarian and seminal fluid are taken from gamete donors for detection of the causative agents of disease, particularly bacterial kidney disease, by the Alaska Department of Fish & Game Fish Pathology Laboratory. Families made from gametes of heavily infected fish are destroyed under the direction of the ADF&G Chief Pathologist.

Antiseptic foot baths are maintained at the boundary between the Lab and the fish culture spaces of Macaulay Hatchery. Antiseptic baths are maintained for tools--nets, brushes, etc.

F. Disposition of research animals.

Euthanasia. Methane Tricaine Sulfonate, MS 222, 1g/kg. Adult fish on occasion are dispatched by a blow to the head, by immersion in anaesthetic or by electroanaesthesia following the Alaska industry standards and published international standards (Animal protection aspects on killing of fish [Tierschutzaspekte bei der Toetung von Fischen] Von Bernoth, E-M; Wormuth, H-J DTSCH. TIERAERZTL. WOCHENSCHR., vol. 97, no. 4, pp. 154-157,1990. Carcasses not required as specimens are disposed of through the Macaulay Hatchery disposal system.

IV. Facilities - Macaulay Hatchery Wet Laboratory

- A: 3 Dimensional Video Recording Tank
 B: FAL Heath™ Vertical Incubation Systems (2 x 16 trays)
 C: 100 Liter Culture Systems: Individual Light, Photoperiod, and Food Control
 D: Living Stream™ 200 L fiberglass tanks
 E: Sink
 F: Work Tables
 G: Shelves
 H: Flammable Liquid Storage Locker
 I: Ultra Cold Freezer. -80C SoLow™ 11 Cu. Ft.
 J: Chest Freezer, 20 Cu. Ft.
 K: 1000 Liter fiberglass tanks

