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# RESEARCH AND RECOVERY OF SNAKE RIVER SOCKEYE SALMON

ANNUAL REPORT FOR APRIL 1993 - APRIL 1994

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# ABSTRACT

Research and recovery activities for Snake River sockeye salmon Oncorhynchus nerka conducted by the Idaho Department of Fish and Game (IDFG) during the period of April 1992 to April 1993 are covered by this report. These activities include continued upgrading of the fish holding facilities at Eagle, Idaho, culture of fish held in the captive broodstock program, trapping of feral juveniles and an adult sockeye in the Stanley Basin, and trapping residual sockeye from Redfish Lake.

Smolts were trapped exiting Redfish Lake during April through June 1992. The traps captured 161 smolts with a trap efficiency of 13%. Half of the smolts were incorporated into the broodstock program, while half were released with PIT tags for continued migration. Smolts presumed to originate from Alturas Lake were trapped on the Salmon River and were similarly handled.

A single male adult sockeye was trapped from Redfish Lake Creek. This fish was held to maturity, and harvested milt was cryopreserved for future use in the brookstock program.

Residual sockeye were captured in spawning condition from Redfish Lake and a few fertilized eggs were obtained. These were also included in the captive broodstock being held to maturity. Genetic analysis demonstrated that the residuals are indistinguishable from the anadromous form.

The performance of the broodstock groups brought into culture during the first year of this program has been satisfactory. Survival has been 79% for outmigrant groups, with the primary causes of mortality being Bacterial Kidney Disease (BKD) in a single lot of outmigrants, other bacterial infections, and tumors classified as lymphosarcomae. Survival for the progeny of the sockeye adults spawned in 1992 has been 97%.

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## INTRODUCTION

The drastic decline in number of returning adult sockeye salmon Oncorhynchus nerka to Redfish Lake in the Stanley Basin of central Idaho over the last decade prompted the Idaho Department of Fish and Game (IDFG) and the Shoshone-Bannock Tribe to embark on a captive broodstock recovery effort in the spring of 1991. This effort was subsequently funded through the Bonneville Power Administration. The first annual report which covered the first year's activities was recently published (Johnson 1993).

The activities during the second year are covered by this annual report for the period of April 1992 through April 1993. The groups of O. nerka taken into captivity during the first year of the captive broodstock program include outmigrants trapped from Redfish Lake in 1991, outmigrants trapped from the weir on the Salmon River upstream from the confluence of Redfish Lake and presumably originated from Alturas Lake, and the progeny from the spawning of the four anadromous adult sockeye which returned in 1991. These were cultured during the subsequent year. Three other groups were added in 1992. These include Redfish Lake outmigrants, smolts trapped from the Salmon River, and the progeny of residual sockeye, captured in Redfish Lake in November 1992 and spawned.

The other aspects covered in this report include modifications to the Eagle Fish Hatchery facility and those relating to the liaison between the other research efforts in the recovery of Snake River sockeye salmon.

## FACILITY MODIFICATION

One aspect of the water system for the hatchery building at Eagle, which made the system vulnerable to failure, was the lack of a second pump and degassing tower. This weakness was remedied by the installation of both. There remain some additional plumbing modifications to allow for the selection of any combination of the four wells as water supply. This is needed since the output and gas saturation level should be varied by well selection. A centrifugal sand separator was installed as part of the second tower since sand has always been a problem with these wells.

A delivery of holding tanks was received from Aquafarms Ltd. in anticipation of spawning in 1992. This shipment included forty-eight 0.6-m, twenty 1-m, twelve 2-m, and four 4-m tanks. While no spawning occurred in 1992, most of the 2-m tanks have been used for the Redfish Lake 1991 brood year sockeye.

The expansion of rearing water and spacing using wells 1 and 2 at Eagle is in progress. The concept is to use four existing old concrete double raceways to hold 3.4-m tanks. This required that the common wall be removed and a drain installed in its place. Each raceway should hold 14 tanks.

Modification of two raceway sets is being done in the first phase, and the structural work was completed by IDFG Engineering Bureau. The pump and degassing tower was also completed. Two wells exist on the grounds of the Eagle Hatchery which could provide water for expansion of the broodstock and progeny culture program. These wells were scanned by video camera to inspect the casing and accumulation of sand. The casing turned out to be serviceable, and there was no sand accumulation detected. The decision was made to add a pump to each well to pressurize the water line using a common degassing tower. The engineering for this is being done by the firm of KCM of Seattle, Washington.

Bids were put out for twenty-eight 3.4-m tanks, and they are currently under construction. The goal is to have one set of 12 running this summer.

The water level alarm system has worked very well during the year. Users elected to exchange the pagers that each employee uses for a style which is capable of giving a message rather than simply a tone. This allows the user to differentiate between a real water system emergency from a wrong number. Users were getting quite a few wrong number calls, and the exchange has corrected the problem.

## FISH CULTURE ACTIVITIES

### Juvenile Trapping for 1992

The trap at Redfish Lake Creek was considerably modified for the trapping of juvenile outmigrants. A Krey-Meekin trap was used in 1991, and the resulting high velocities killed about 8% of the total outmigrants caught. The trap site used by IDFG in the 1960s was renovated to accommodate five incline plane traps placed in five of the nine trap bays. The goal was to increase trapping efficiency, accommodate high flows, and trap without mortality. Aluminum conduit pieces were spaced 1/4 inch apart in the trap inserts with a fish collector at the back of the trap. This was covered with a section of conduit pieces spaced 1 inch apart to allow the outmigrants to fall through, but to exclude bull trout which might prey on the outmigrants. The only mortalities (e) occurred in the first two days of trapping before the spacing was permanently set at 1/4 inch by welding a reinforcing bar beneath the upper set of conduits. After this was completed, no further mortalities occurred (Figure 1).

Trapping commenced on April 15 and continued until June 10, 1992. A total of 161 outmigrants were trapped with 3 mortalities. The remaining 158 were PIT-tagged and equally divided between those retained for broodstock and those released for trap efficiency estimation and to continue migration. Trap efficiency was estimated at 13% from the recovery of 3 fish out of 23 released above the trap site. Using the expansion of 100/13, the point peak period of outmigration was April 30 through May 6. Redfish Lake became ice-free on April 16, 1992. The mean fork length of the outmigrants was 98 mm (Figure 2). Outmigrants retained for broodstock were transported to Eagle Hatchery weekly and placed into culture with the designation Redfish Lake 1992 outmigrants.

Outmigrants which continued the journey toward the ocean were intercepted by the PIT tag detectors at three Snake River dams (Figure 1). Eighteen of the 79 released (22%) were interrogated in 1992, which compares to 57% interrogated in 1992. Mean travel time to Lower Granite Dam was 16.9 days and was longer for the other collection sites (Figure 1).

### Smolt Trapping At Sawtooth Weir

*O. nerka* smolts were trapped at the weir at the Sawtooth Fish Hatchery on the Salmon River above the confluence of Redfish Lake Creek. These presumably originated from Alturas Lake. The operational period for this trap spanned the entire run since it was operated for wild chinook. Smolts were trapped from April 20 through May 18, 1992 (Figure 3). A total of 147 were trapped, of which 96 were retained for broodstock, 37 fish were released with PIT tags for migration timing, and 14 died. Most of the mortality occurred on April 30 when a Krey-Meekin trap was used to attempt to increase the fish count for a trap efficiency estimate. Nine fish died due to high water velocities in the trap and it was removed after one night of service. The trap efficiency estimate was 5.4% based on the recapture of 2 of the 37 fish released and was approximately the efficiency obtained at the same trap for chinook (Kiefer 1993). This was used to yield a total run estimate of 2,720 outmigrants for 1992. The interception

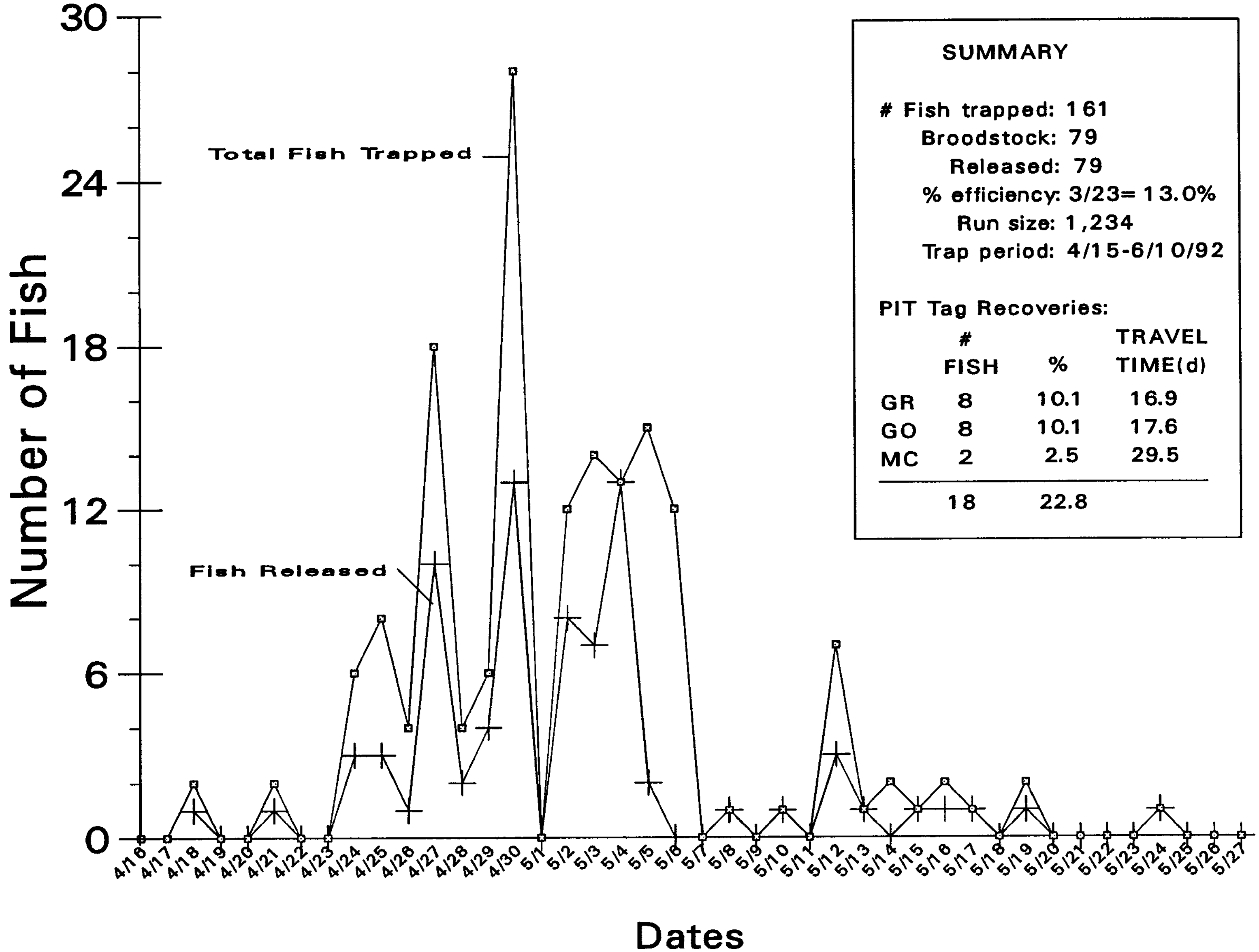


Figure 1. O. nerka trapped at Redfish Lake Creek (1992 daily counts).

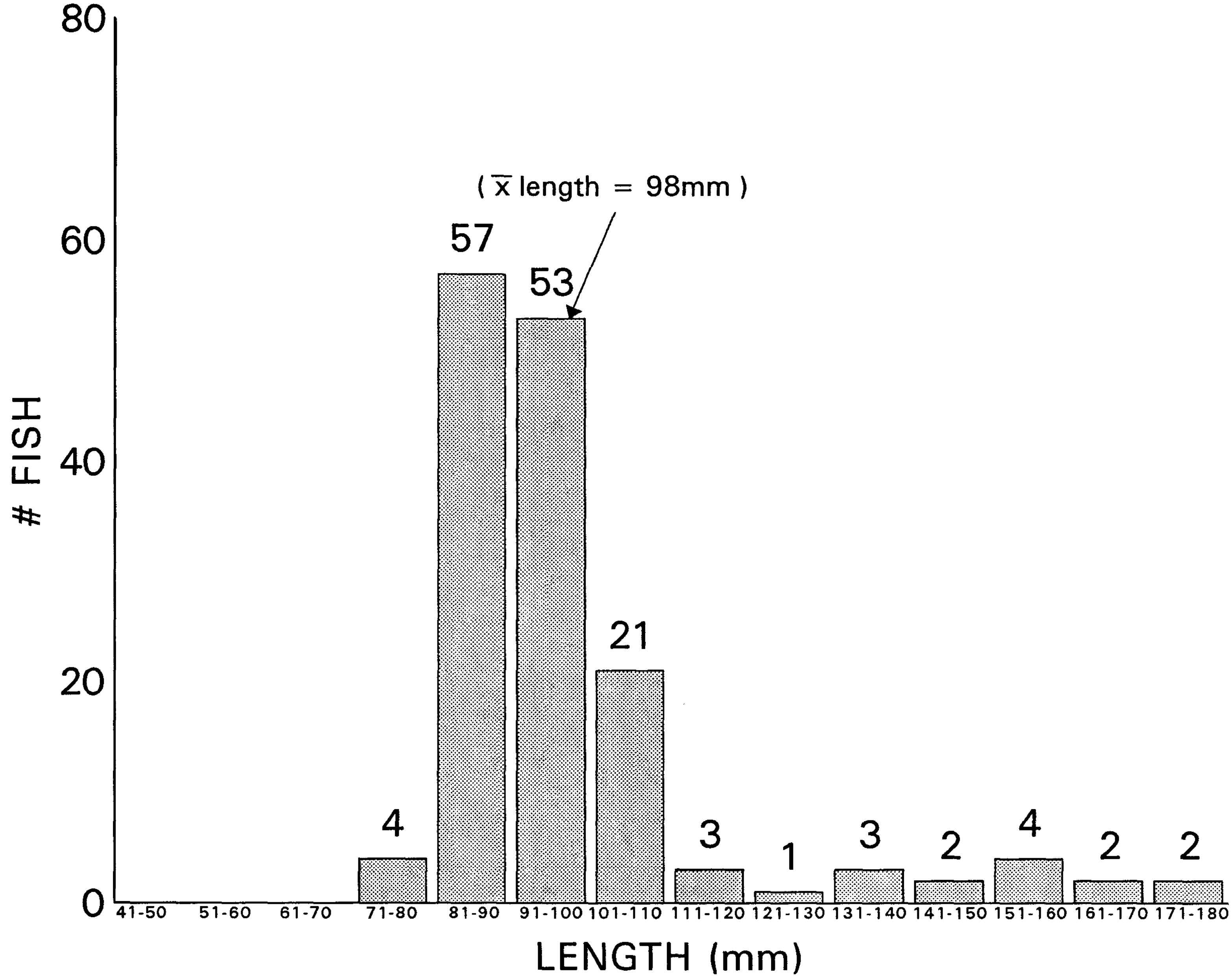


Figure 2. Redfish Lake outmigrant 1992 length distribution.

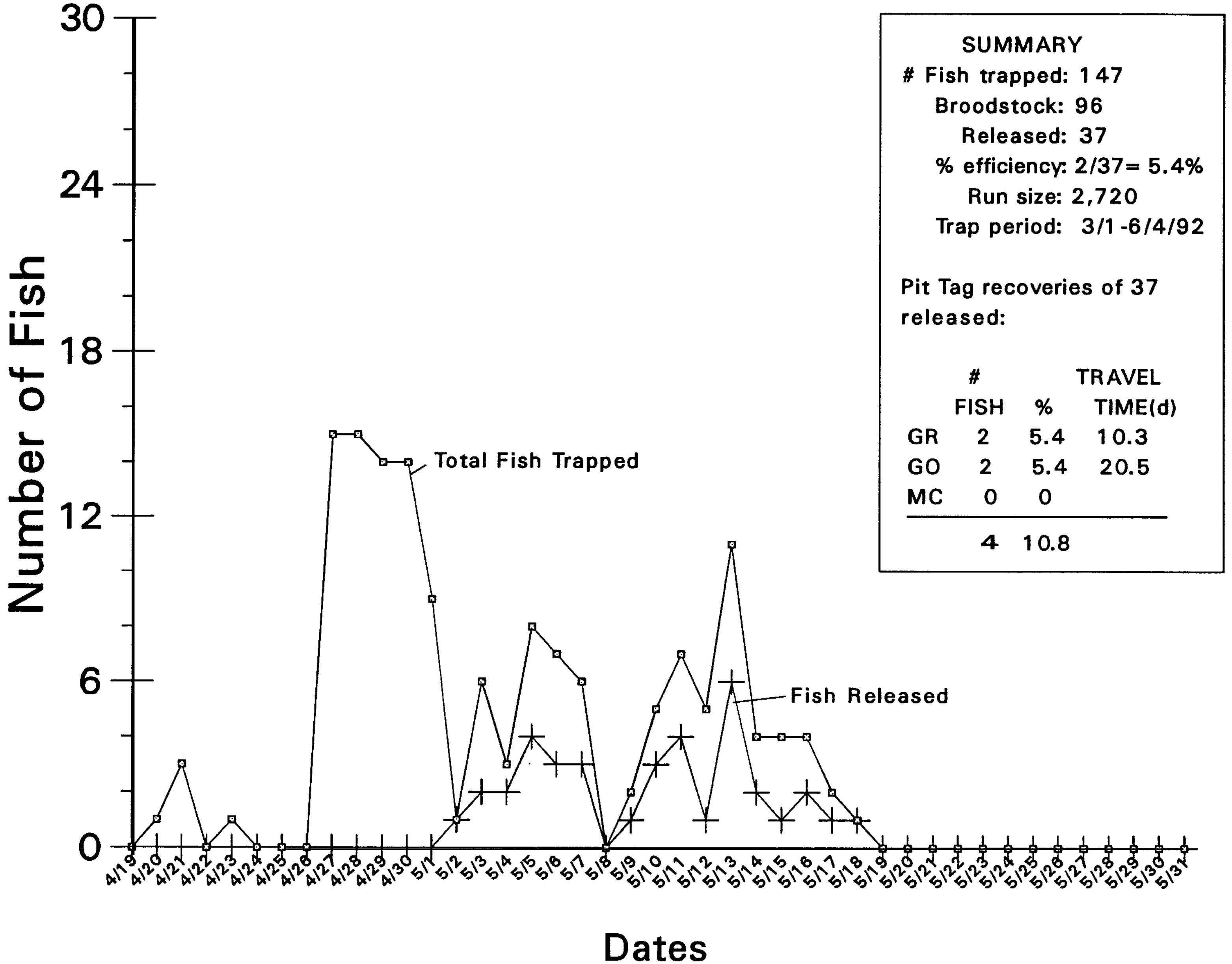


Figure 3. O. nerka trapped at Sawtooth Fish Hatchery (1992 daily counts). Krey-Meekin installed at diversion April 30 and removed May 1.

rate to the Snake River dams was 10.8%, with a travel time of 10.3 days to the fish collection facility at Lower Granite Dam. The distribution of fork lengths for these outmigrants is presented in Figure 4. The smolts which were retained for broodstock were transported to Eagle Hatchery and designated as Alturas Lake 1992 outmigrants.

## Adult Trapping at Redfish Lake Creek

The identical trap was used in 1992 as had been used in previous years. This was installed on June 25 and operated through October 30, 1992. A single male sockeye, given the name Lonesome Larry, was trapped on August 3 and was transported to Sawtooth Fish Hatchery. This fish was held in well water with gravel from Sockeye Beach of Redfish Lake as substrate. This technique was used successfully in 1991. The observations and milt harvests are listed in Appendix 1. An implant containing gonadotropin releasing hormone (GnRH) was provided by Dr. Penny Swanson, National Marine Fisheries Service (NMFS) Montlanke Laboratory, and installed by dorsal incision midway through the milt harvest to increase the volume of milt. Since there was no maturing female sockeye available of the appropriate stock, all milt was frozen and held for cryopreserved storage. The techniques and extender solution for this procedure have been investigated for several species of salmonids. These protocols were performed in duplicate by the laboratories of Dr. Joe Cloud, University of Idaho (UI), and Dr. Gary Thorgaard, Washington State University (WSU). Sperm motility was examined before freezing and, as a quality control measure, the ability of the frozen milt to fertilize eggs was also evaluated.

O Eggs and sperm were obtained from Lake Pend Oreille kokanee . *nerka kennerlyi* and brought fresh to both laboratories. Eggs were divided into lots of 50 to 200 eggs and exposed to thawed milt of the anadromous male from selected dates of cryopreservation. A positive control of fresh kokanee milt was used with one lot of eggs at each site. The lots of eggs were separately incubated to the eyed stage and the percent fertilization results determined. The dates of milt harvest are given in Appendix 1 and the fertilization results are given in Appendices 2 and 5. Milt from three of the six cryopreservation dates tested by Dr. Cloud yielded fertilizations rates of about 35% of the control level. This is the rate expected with other salmonids.

There was no fertilization obtained from straws frozen on the remaining three dates. Straws from three cryopreservation dates frozen by Dr. Thorgaard's group failed to fertilize kokanee eggs. Cryopreserved milt of the residual males (termed "beach spawners" in Appendix 5) fertilized kokanee eggs at about half the rate as the fresh sperm control. These results will be used to select frozen milt for use in the broodstock program in future years and suggests that further development is warranted for the cryopreservation of sockeye sperm.

Appropriate duplicate tissue samples for genetic identification and pathology were removed when the male was sacrificed after no further milt was obtained. These were distributed to Dr. Waples (NMFS), Dr. Brannon (UI), and the Eagle Fish Health Laboratory (IDFG) for analysis.

There was an increase in the volume of milt following the GnRH implant, although the milt appeared to be more watery. The fertilization results with the cryopreserved sperm before and after the implant was applied were too low to give an accurate idea of whether there was a reduction in the quality of the sperm following the GnRH application.

The carcass was delivered to a taxidermist for mounting. The mount is used in the "Nose To Nose" educational program for school-aged children conducted by IDFG and resides on permanent display in the Morrison-Knudsen Nature Center. This location in Boise, Idaho receives 200,000 visitors annually.

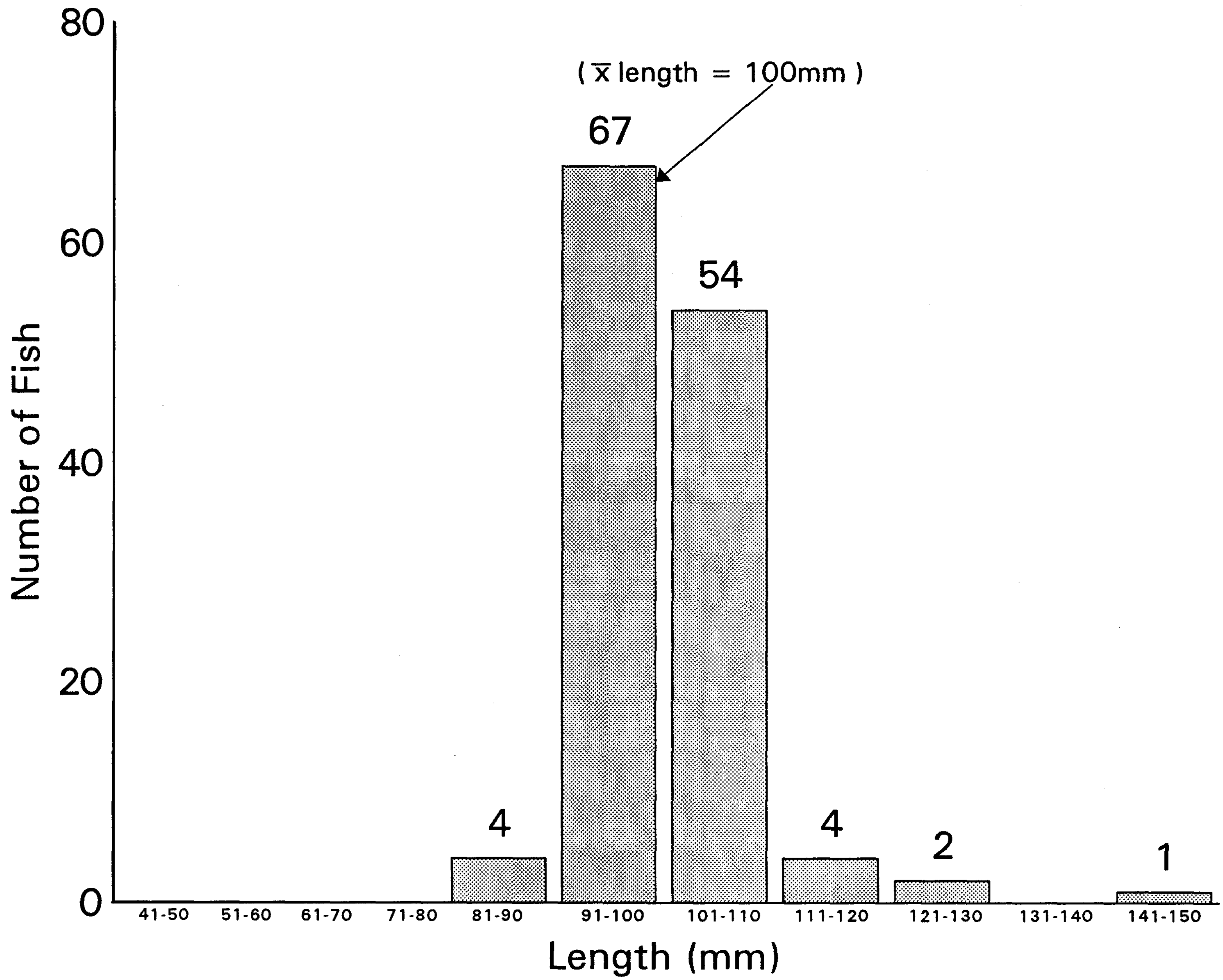


Figure 4. Alturas Lake outmigrant 1992 length distribution.

## Residual Sockeye In Redfish Lake

There was considerable circumstantial evidence that a third deme of O. nerka existed in Redfish Lake. The primary observation leading to this conclusion was that the smolts continued to migrate from the lake in the spring months even though no anadromous adults returned to spawn. Thus Dr. Rieman (IDFG) and Dr. Brannon (UI) (Appendix 3) sought to demonstrate the presence of this form of sockeye. They observed small, non-anadromous O. nerka in spawning condition in the vicinity of Sockeye Beach of Redfish Lake during the normal October-November spawning time for sockeye. Five of these fish were captured and blood samples taken for genetic analysis. Four of these were transported to Eagle Fish Hatchery. One was a spawned-out female and the others were ripe males. Thirty-six eggs were removed by incision and fertilized with milt from two males. Tissues of these fish were taken for genetic and pathologic analysis. Genetic determinations were made by DNA (Brannon 1992) and protein electrophoresis (Waples 1992) and demonstrated that this population is very closely related, if not identical, to the anadromous adult sockeye which have returned to Redfish Lake in 1991 and 1992. The eggs were incubated at Eagle Hatchery and given the group designation of Redfish Lake residual sockeye, 1992.

The population of residual sockeye are treated as part of the "Evolutionarily Significant Unit" under the Endangered Species Act following recommendations from NMFS in December 1992.

# PERFORMANCE OF CULTURED GROUPS

## Redfish Lake Outmigrants 1991

The Redfish Lake outmigrants retained for broodstock in 1991 started this report period with an inventory of 703 fish and ended with 597 for 85% survival during the year and 79% since the start. Fish weight increased from 335 g to 1,035 g (Figure 5). Bacterial Kidney Disease (BKD) had been detected in group 4 in 1991 and caused considerable loss in this group during 1992-1993. Erythromycin treatments were applied to group 4 and showed efficacy (Figure 6). The interval between treatments was shortened to a month, and there appeared to be better reduction in mortality. This will continue to be the practice until spawning. The other groups have remained free of detectable BKD using ELISA and FAT diagnostic techniques. Strict quarantine practices are applied to these groups and probably are the reason that BKD has not been detected in the other groups.

Several significant events occurred during the reporting period in the culture of the Redfish Lake outmigration 1991 groups. The first was that a high prevalence of tumors has been detected. Tumors are generally rare in salmonids, but at least six individuals of groups 1, 2, 3, and 5 developed tumors. The most prevalent was a thyoma which appears as an enlargement of the thymus on one side. The growth attained the size of a golf ball in fish of about 900 g body weight. This has previously been reported by Warr et al. 1984 in rainbow trout and was classified as a lymphosarcoma. These occurred during the winter months in 5 of the 608 fish held at Eagle. Histological sections demonstrated that the cell type was a lymphocyte and the tumor was sent for confirmation to the Institute For Tumors Of Lower Vertebrates. Attempts to culture the cells of this tumor are in process at several fish health research laboratories.

A second type of tumor was collected from the isthmus of one fish during the winter also. This was identified as a fibroma which has also been reported previously in salmonids. These tissues were also submitted for verification. There has been some speculation that the susceptibility to tumors has a genetic

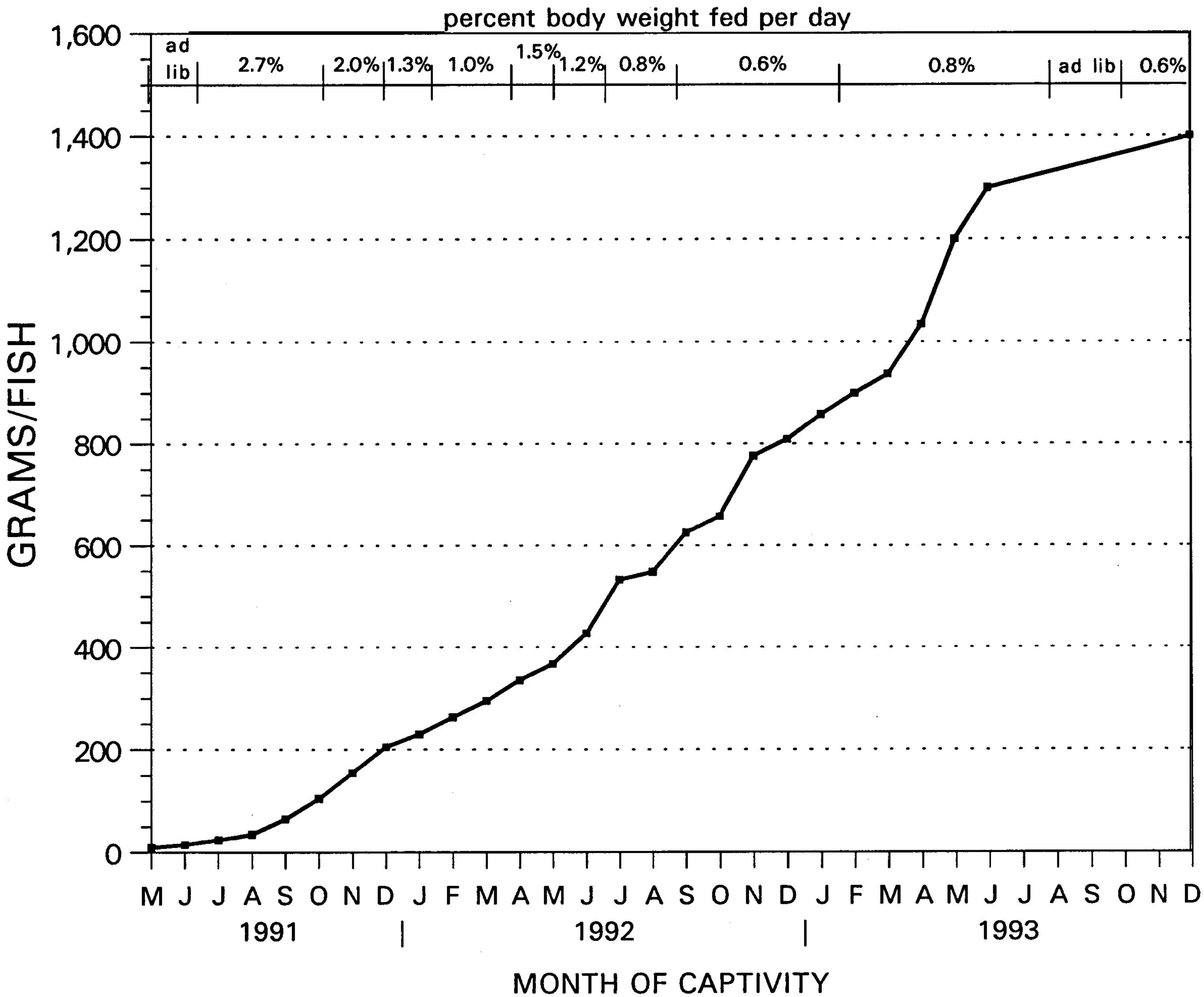


Figure 5. Redfish Lake outmigrant 1991 monthly growth (May 1991-December 1993).

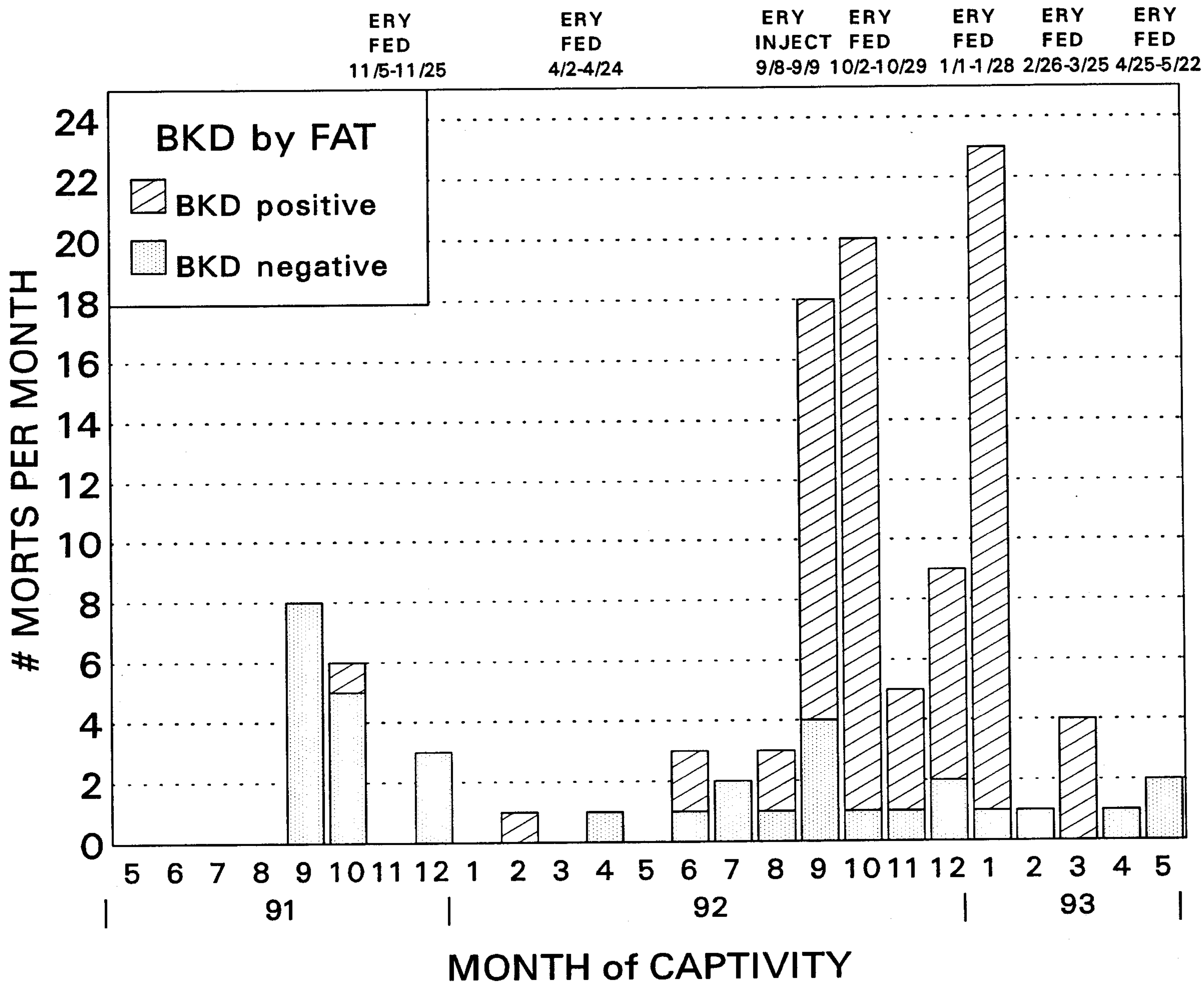


Figure 6. Redfish Lake outmigrant 1991 group 4 mortality by month (cumulative mortality May 1991-May 24, 1993 = 56.1%).

base in salmonids, and this may be evidence that this population has undergone a single or a series of genetic bottlenecks previously. If that were true, there also should be a high incidence of tumors in the progeny of these broodstocks. It may be difficult to examine this occurrence since the progeny will mostly be at sea if these tumors develop at the fourth year of life as they did in captivity. Some progeny are planned to remain in captivity, and these will be available to answer this question.

Space and water constraints due to delays in the completion of the expansion of the facilities at Eagle force the move of Redfish Lake outmigrants 1991 groups one and two to Sawtooth Fish Hatchery in April 1993. This was not an appropriate time of the year for such a move since the water temperatures at the two stations are so different (Eagle Fish Hatchery=12.8°C; Sawtooth Fish Hatchery=4.5°C). This change in temperature caused the fish to go off feed for several weeks, and a Pseudomonas sp. infection was exacerbated by the stress. The loss over the next two months amounted to 37%. Once feeding was initiated by the use of a highly palatable feed formulated by Bioproducts , Oxytetracycline was used to counter the infection. Mortality decreased as the fish adapted to the rising well water temperatures as the spring progressed.

Each mortality in the Redfish Lake outmigrant 1991 group was sampled for genetics and pathology. No BKD has been demonstrated in groups other than group 4, but bacteria causing Bacterial Coldwater Disease (BC), motile aeromonad septicemia, and bacterial gill disease have been the primary cause of death. Mortalities were also processed for gill raker counts, flesh tone due to the carotinoid added to the diet, number and distribution of dorsal spots, and condition of the gonads. Most of these data were collected to add power to the differentiation between the anadromous and resident forms of O. nerka. These may offer a more rapid test than protein electrophoresis or DNA based tests. Most data have not yet been analyzed, but there had been enlarging of the ovaries in about 60% of the females, which died during the late winter and spring. Ova were increased in diameter in the ovaries from large females. This is of interest as a predictor of impending sexual maturation in the fall of 1993.

## Alturas Lake 1991 Outmigrants

The outmigrants which were captured in the spring of 1991 were cultured at Eagle Fish Hatchery in 1992, then moved to Sawtooth Fish Hatchery due to space and water constraints at Eagle Fish Hatchery. The starting inventory was 136 and the ending inventory was 125. The overall survival has been 90% for the two years of culture and a weight gain of 12 to 900 g for the period (Figures 7 and 8). The causes of mortality included jumping out of rearing tanks and motile aeromonad septicemia. No BKD was detected in the mortalities, but two prophylactic treatments with Erythromycin were applied during the year. The mortalities were examined and sampled as described above for Redfish Lake outmigrants 1991, and the increase in ovary size was noted for the females of this group as well. Some of this group are expected to attain sexual maturity in the fall of 1993. These fish should be predominantly 5-year-olds at this time since otolith aging demonstrated that they migrated as 2-year-olds in 1991.

## Redfish Lake Brood Year 1991

This group is the progeny of the single female and three males which returned to Redfish Lake as adult sockeye in 1991. These were cultured as separate subfamilies until they were PIT-tagged in June 1992. The subfamilies were from male A, B, and C; a group which was fertilized with pooled milt from all three males (ABC); and those eggs recovered from the gravel as volitional spawned eggs (natural). These fish averaged 1.0 g with an inventory of 937 at

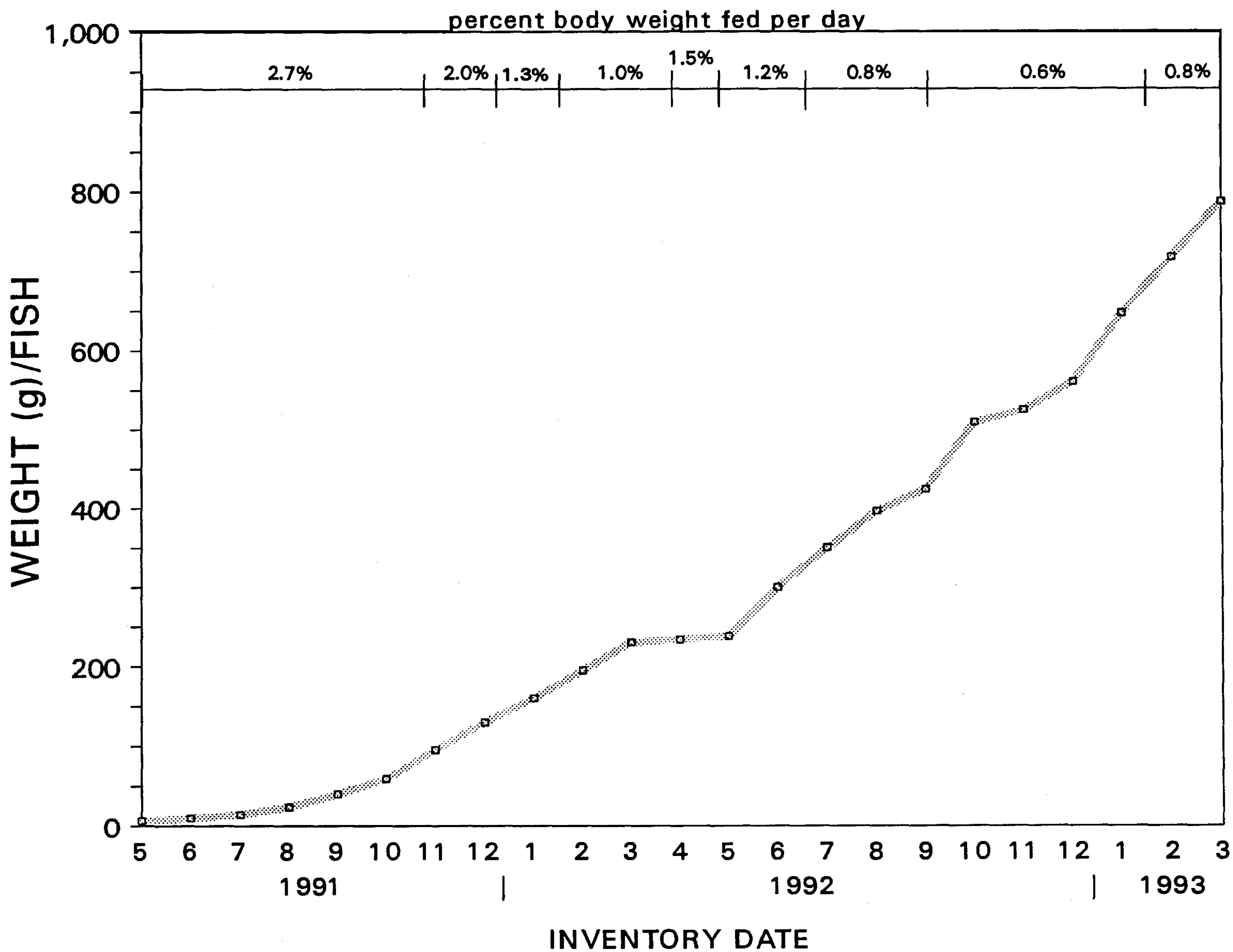


Figure 7. Alturas Lake outmigrant 1991 growth (May 1991-March 1, 1993).

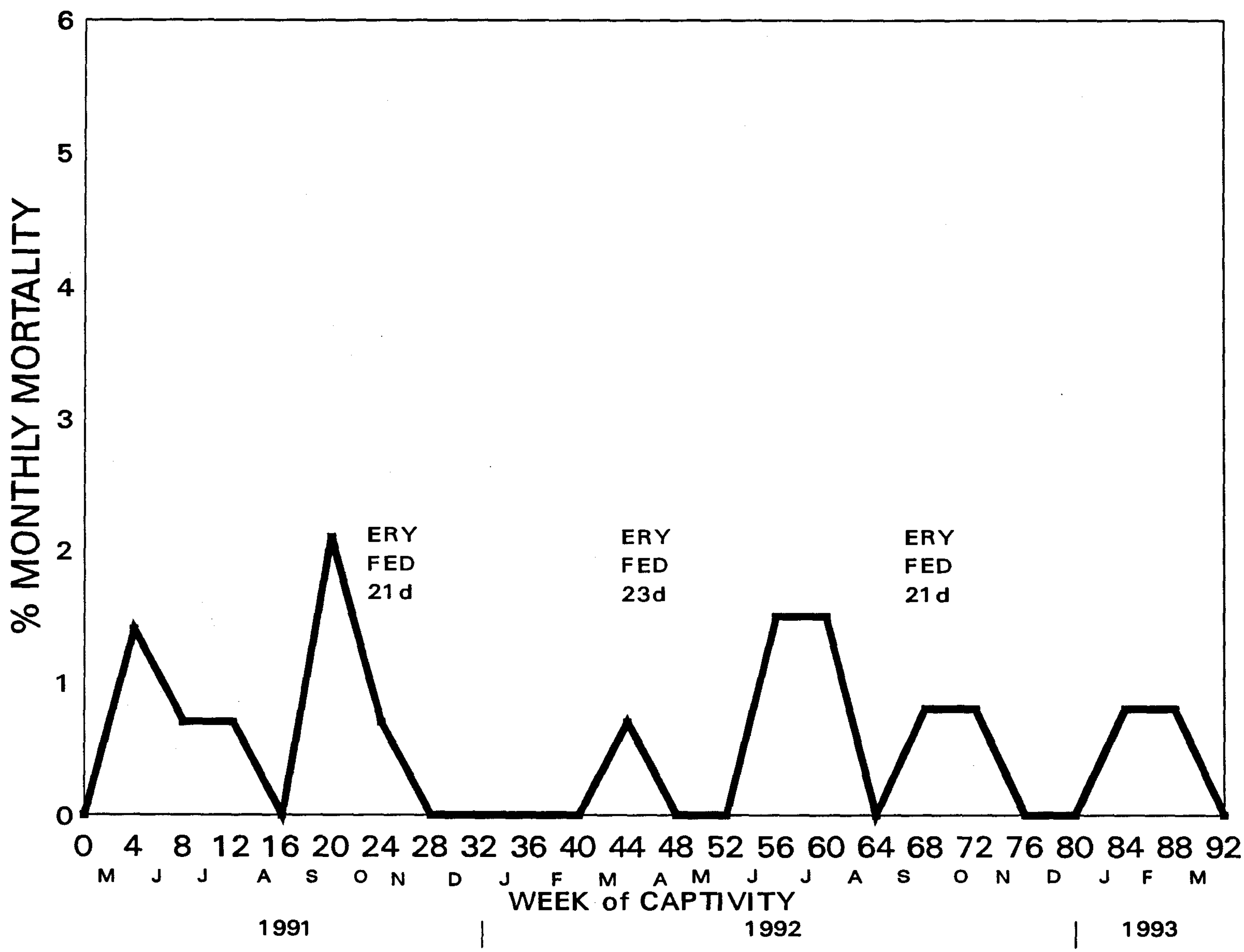


Figure 8. Alturas Lake outmigrant 1991 mortality by month (cumulative mortality May 1991-March 9, 1993 = 11.8%).

Eagle Fish Hatchery in April 1991 and grew to 182 g by April 1993 with a loss of 12 fish. They were PIT-tagged at 7 g and randomized into groups to prevent loss of an entire genetic subfamily in the event of a single tank disaster. The loss and growth of this group are shown in Figures 9 and 10. Survival for this group since being on feed has been 97%. This group is also represented by a similar number of fish being reared by NMFS at the Montlake Laboratory in Seattle (Flagg 1993).

The cause of death in the 12 mortalities was varied and included a few due to BC, motile aeromonad septicemia, bacterial gill disease, pin-heads, and jump outs. All mortalities were examined for BKD and viruses with all negative results. No tumors have been seen in these fish as well. The PIT-tagging process and the resulting motile aeromonad septicemia was probably responsible for one-third of the total loss.

## Redfish Lake Outmiqrants 1992

Outmigrants captured from Redfish Lake Creek in 1992 averaged 8.7 g for the 79 fish, and by the end of April 1993, there were 69 remaining averaging 252 g (Figure 11). The causes of this loss included pin-heading, bacterial gill disease, and a jump out. No BKD has been detected in this group. Growth has been uniform, except there was a slowing of growth in the first winter. This may have been due to a physiological effect since the well water temperature at Eagle Fish Hatchery only decreases slightly in response to lower air temperature.

## Alturas Lake Outmiqrants 1992

Ninety-five outmigrants were taken into culture at Eagle Fish Hatchery averaging 10 g in May 1992. The final inventory in April 1993 was 88 fish which averaged 211 g. The cause of death included two pin-heads, two with surface Pseudomonas lesions, two with gill cysts resembling Loma, and one which died from handling during a monthly inventory. The growth of these fish demonstrated a biphasic growth pattern with a decrease in winter months as was seen with Redfish Lake outmigrants 1992 (Figure 12).

## Redfish Lake Residual Sockeye 1992

Thirty-six fertilized eggs were obtained from the mating of the residual sockeye captured from Sockeye Beach of Redfish Lake by Dr. Brannon (UI). The number of cumulative temperature units (TU) to eyeing was 318, to hatch was 614, and 932 to swim-up and first feeding. Yolk absorption was fatal to one fish, but all others have done well (Figure 13). The fecundity for the female could not be determined since she was mostly spawned-out when collected. These will be cultured at Eagle Fish Hatchery for use in the captive broodstock program.

# PROJECT LIAISON ACTIVITIES

## Technical Oversiqht Committee

The IDFG has had at least one representative at all monthly meetings of the Stanley Basin Technical Oversight Committee. The notes of these meetings are available through the Bonneville Power Administration (BPA). The project has been greatly enhanced through the interactions at the meetings, and much of the progress to date has been the result of these interactions.

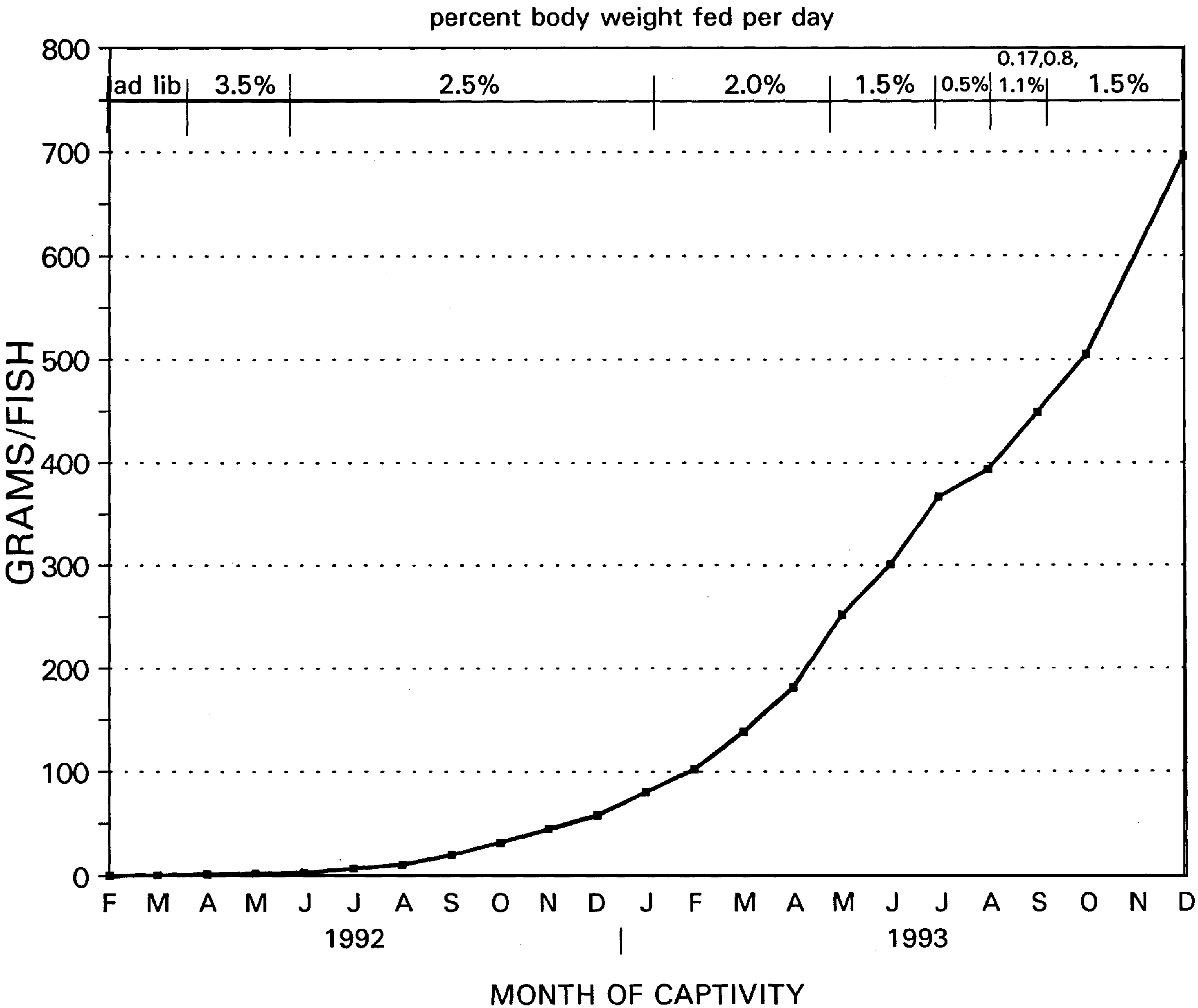


Figure 9. Redfish Lake brood year 1991 growth (January 28, 1992-December 1, 1993).

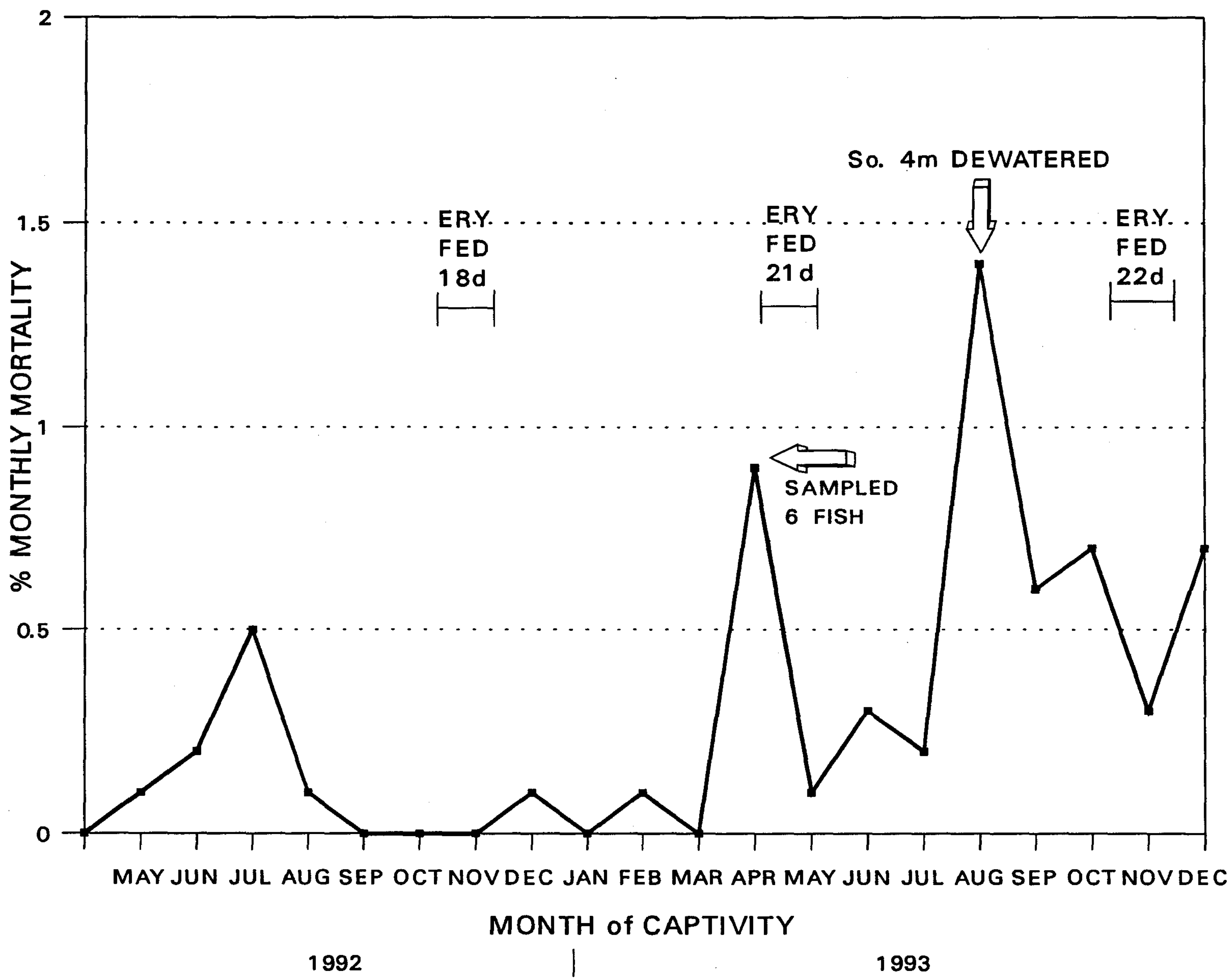


Figure 10. Redfish Lake brood year 1991 mortality by month (cumulative mortality May 1992-December 9, 1993 = 6.3%).

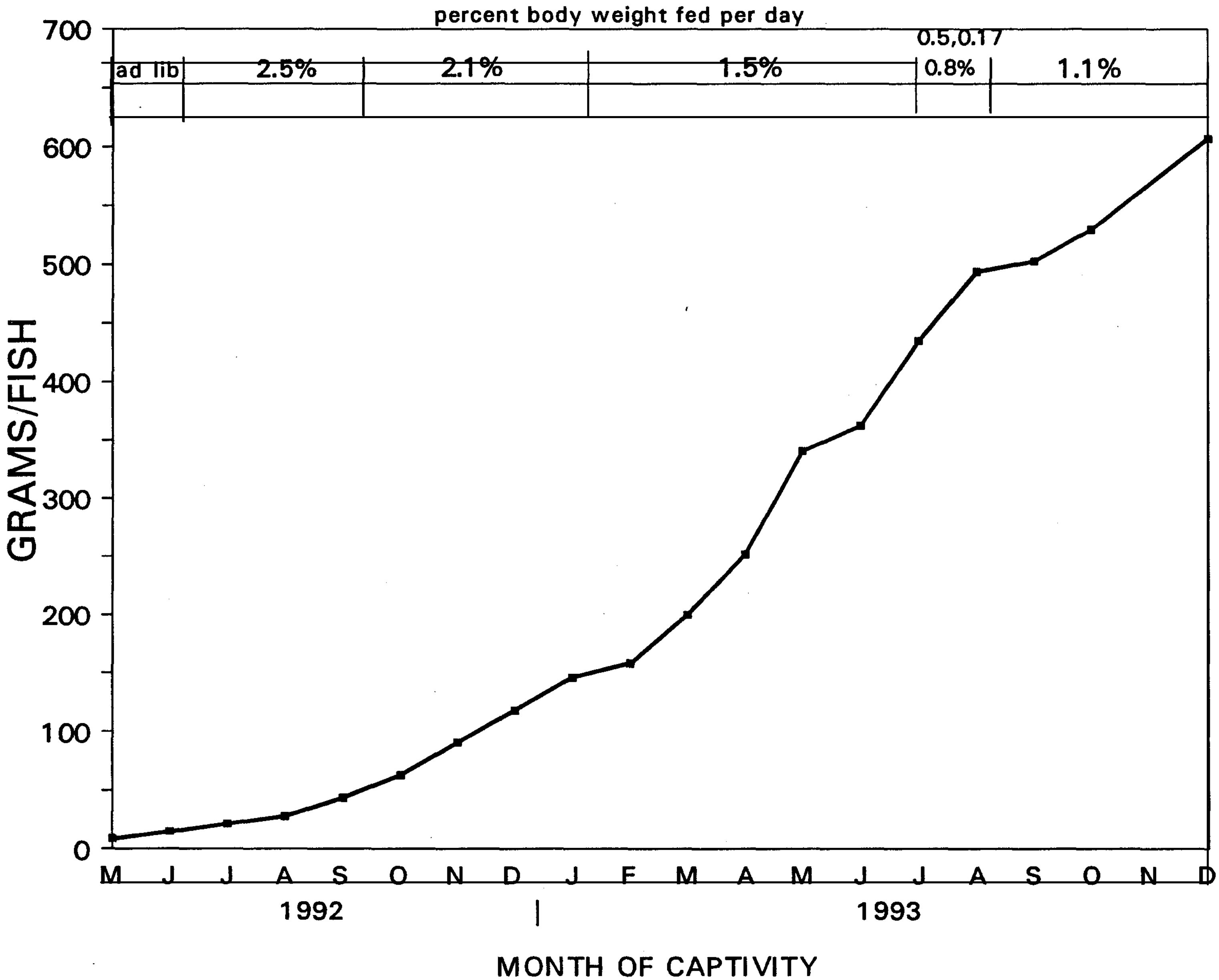


Figure 11. Redfish Lake outmigrant 1992 growth (May 1, 1992-December 1, 1993).

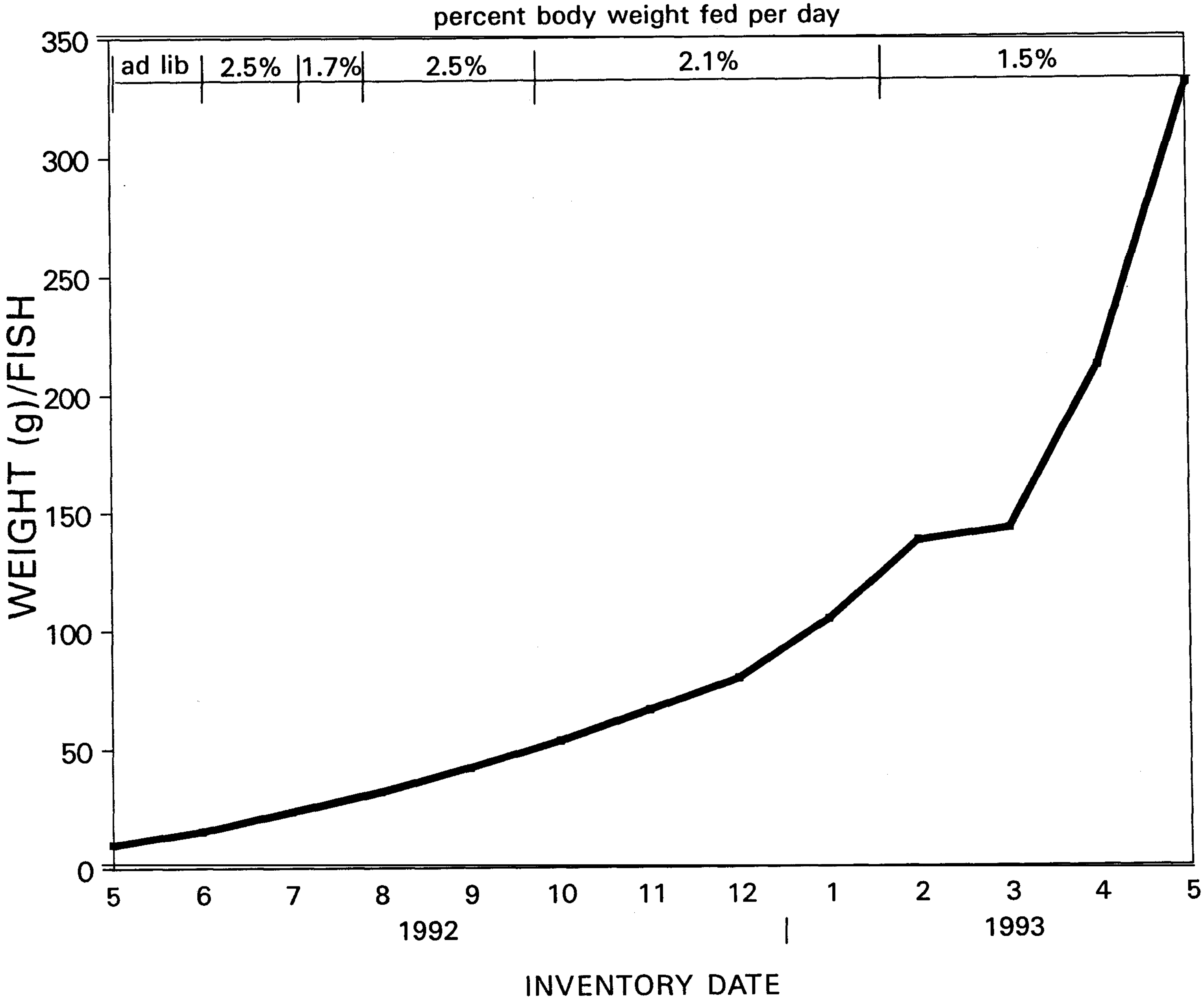


Figure 12. Alturas Lake outmigrant 1992 growth (May 1, 1992-May 11, 1993).

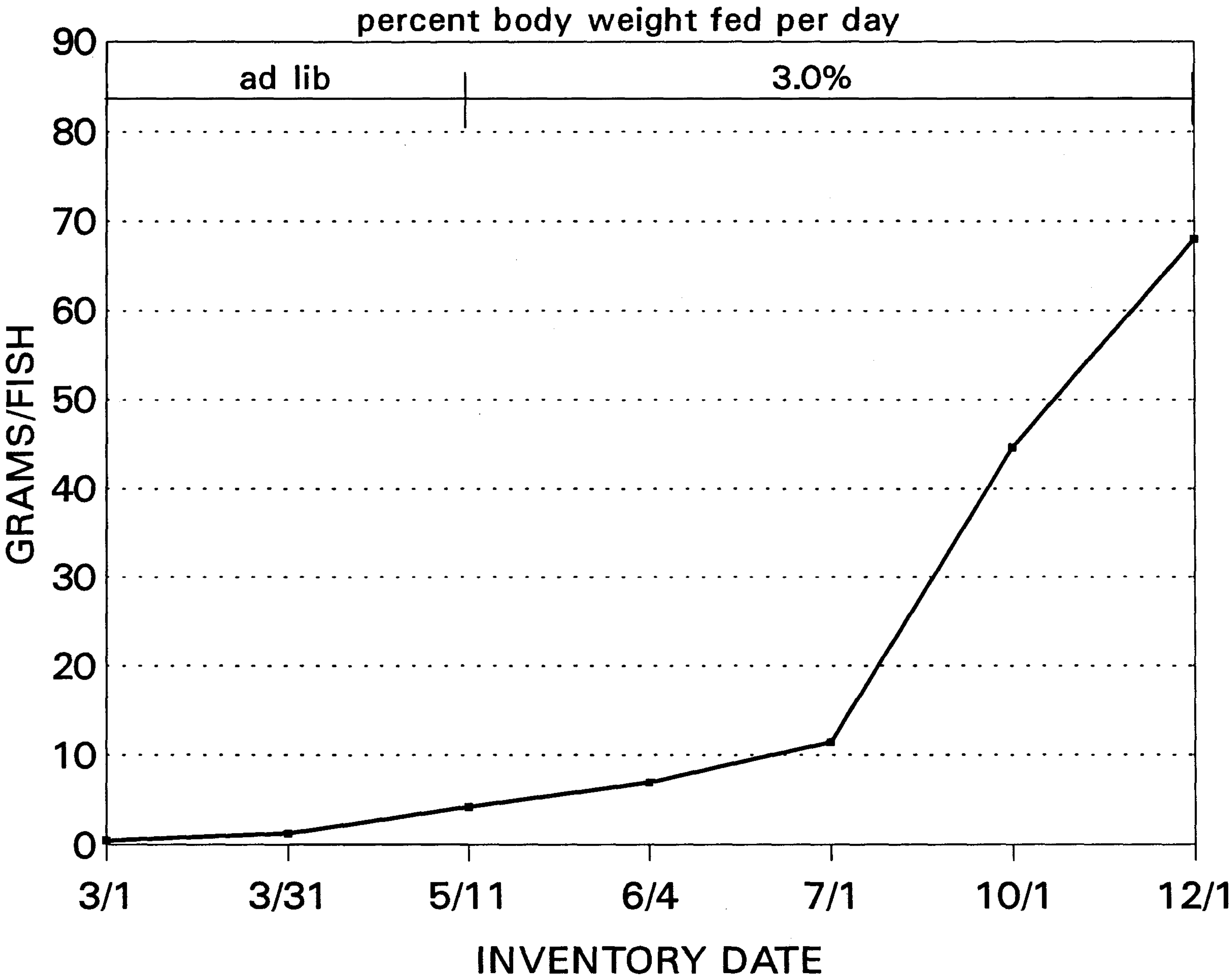


Figure 13. Redfish Lake brood year 1992 residual sockeye salmon growth (March 1-December 1, 1993)(n=35 fish).

## Broodstock Nutrition Consultation Team

A meeting was held in February 1993 on broodstock nutrition to provide suggestions on modifications to the captive broodstock program. The minutes are included as Appendix 4 to this report. Generally, the group favored the approach of including a brood diet from a second manufacturer but that the progress to date in the program has been satisfactory. This suggestion was implemented by including a semi-moist brood diet made by Rangen Inc. of Buhl, Idaho in the feeding protocol. Other directions given dealt with handling frequency, densities, and the experience of the fish culturists on broodstock management. Most of these have also been included in the rearing protocols.

## Presentations

The subject of captive broodstocks for recovery of stocks with low numbers has been the subject of considerable interest in the Northwest. Presentations were given to the Idaho and North Pacific International Chapters of the American Fish Society, Canadian Aquaculture Association, Project Status Review for BPA, Idaho Aquaculture Association, and several school groups.

## National Marine Fisheries Service Propaqation Permit

The final propagation permit for the captive broodstock program was received in July 1992. The activities which relate to release of progeny from the spawning of Redfish Lake outmigration 1991 were not covered in the first permit. These activities were applied for as a modification submitted to NMFS in April and the review is in process. The other modifications included not having to adhere to a set number of fish used for trapping efficiency evaluations, use of residual sockeye in the captive broodstock, and evaluation of the volitional spawning of outmigrants reared to maturity and released back into the lake of origin. A report on the trapping activities for 1993 is due to NMFS by the July following the trapping season.

## Broodstock Database

A computer programmer was hired to construct a database file which interacts with the PIT tag reader to call up data on each fish held in the captive broodstock. This will in turn allow for the generation of a bar code label for each sample which will come from the fish. This was considered essential because 18 samples will originate from each fish and be needed at different times. The probability and consequences of an error required that a mechanical labeling system be used. This program is nearing completion and will be field tested before spawning season this fall.

# STAFFING

The sockeye program is being run with three permanent employees, Dr. Keith Johnson, Ryan Johnson, and Dr. Rob Dillinger. Three temporary fisheries technicians have been used at Sawtooth Fish Hatchery and Eagle Fish Hatchery for trap monitoring and fish culture duties.

# ACKNOWLEDGEMENTS

This is the second year of this project and, as usual, many people have given their expertise to this program which has been greatly appreciated and has contributed to the successes to date. Financial support has come from the Bonneville Power Administration through Dr. Jeffrey Gislason who has been very cooperative in arranging budgeting for the activities. Cooperation of the PIT-tagging crew on the Smolt Monitoring Project has been arranged through Russ Kiefer, and the expertise of his taggers, Steve Warren and Vincent Pero, has resulted in very low mortality and excellent results in culturing feral sockeye as captive broodstock. The crew at Sawtooth Fish Hatchery operated both the smolt and adult weirs on Redfish Lake Creek in a manner that is consistent with the sensitive nature of the fish and without incident. The staff of the Eagle Fish Health Laboratory has processed all the pathology samples generated in this program. Their help has allowed for timely response to impending health and quality problems for the fish reared in this project. Without the assistance of these and many others, the fish would not be doing as well as they are.

This upcoming year should see spawning of two groups of outmigrants and the return of progeny to lakes in the Stanley Basin. The efforts of everyone associated with this program has brought these groups of fish to this point. It is now their responsibility to do their part.

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# A P P E N D I C E S

Appendix 1. Adult Redfish Lake male sockey maturation events.

| Date | Maturation events |
|---|---|
| 8/3/92 | Captured male, 605 mm fork length. Held in Sawtooth Fish Hatchery (SFH) well water with Redfish Lake gravel. |
| 9/27/92 | Checked for maturity. Coloring when lights on for 20 minutes. No milt. |
| 10/8/92 | Small drop of milt; not enough even for motility check. |
| 10/13/92 | First milt harvest; small volume (1-2 ml). First bag mostly urine; second bag was milky. Motility estimated at 20% using activator. Sent milt to Washington State University (WSU) (motility estimated at WSU = 30%). |
| 10/16/92 | 3-4 ml of milt harvested; motility estimated at 5% using activator and well water. Sent to WSU (motility estimated at WSU = 85%). |
| 10/19/92 | 3-4 ml milt harvested; motility estimated at 10% using activator. Sent to WSU (motility estimated at WSU (motility estimated at WSU = 30%). |
| 10/22/92 | 4-5 ml of good milt harvested. Motility estimated at 75%. |
| 10/24/92 | Hormone induction. Placed implant (GnRHO, from Penny Swanson, at 2 p.m. (Made 1/4 in x 1/4 in incision and inserted pellet). Fish recovered and was swimming after 20 minutes. |
| 10/26/92 | Milt harvest - 5 ml urine and sperm, 10 ml sperm. Motility was at least 85% and volume increased about 3-fold. |
| 10/27/92 | SFH crew did milt harvest. Volume approximately 15 ml with motility of 85%. |
| 10/28/92 | Milt harvest - volume 12 ml with motility of 50%. |
| 10/29/92 | Fish losing equilibrium sometimes. Milt harvest volume approximately 8 ml with motility of 60%. Killed fish, took bllld and sent to Brannon (UI), and removed tested and sent to Paul Wheeler (WSU). Genetics and pathology samples taken; scales and otoliths retained. Total of about 63 ml. milt harvested for cryopreservation. |

Appendix 2. Fertility of 1992 cryopreserved sperm, Joe Cloud, University of Idaho, by telephone.

| Expressed as % of eggs hatched (number of eggs) by cryo date. | | |
|---|---|---|
| control kok/kok | 51.9 | (77) |
| | 45.5 | (44) |
| cryo date 10/16 | 3.9 | (78) |
| | 0.0 | (87) |
| 10/22 | 17.5 | (63) |
| 10/24 | 28.2 | (54) |
| 10/25 | 0.0 | (59) |
| 10/27 | 16.3 | (49)[a] |
| 10/28 | 0.0 | (101)[a] |

[a] milt harvested after gonadotropin release hormone induction.

Appendix 3. Observations concerning the existence of a beach spawning, non-anadromous deme of O. nerka in Redfish Lake.

**State of Idaho**
**Department of Fish and Game**
**Eagle Fish Hatchery**
**Eagle, Idaho**

**M E M O R A N D U M** **November 17, 1992**

**TO:** Staff file

**FROM:** Keith A. Johnson, IDFG Eagle Hatchery, 208-939-4114

**CC:** Steve Huffaker, Bill Hutchinson, Virgil Moore, Dexter Pitman, Jim Lukens, IDFG; Jeff Gislason, BPA; Ernie Brannon, UI; Bruce Rieman, USFS:

**SUBJECT:** Redfish Lake Resident Sockeye, 1992

**Introduction**

The existence of a beach spawning, non-anadromous deme of O.nerka in Redfish Lake (RFL) was postulated from the number of outmigrant smolts trapped in RFL creek during 1991 and 1992 because the smolt production potential of anadromous adult sockeye should not have produced the number and that the outmigrants differed electrophoretically from the Fishhook Creek kokanee population. This prompted investigation on the existence of this group.

**Observations**

Dr. Bruce Rieman, USFS, snorkeled sockeye beach on the nights of October 16 and 20 and observed five O. nerka on October 16 and two on October 20 which resembled kokanee in size (8-10") but were dusky green in color. Some of these fish were paired for spawning. No collections were made.

Dr. Ernie Brannon, UI, toured sockeye beach on October 22 with a boat with lights. I was present on this trip. Two O. nerka of 8-10" were observed but not captured. He again observed the sockeye beach area on October 27 and saw four and captured one female which had partially spawned. A blood and adipose fin sample was obtained and the fish released the following morning. I consulted Steve Huffaker and NMFS on how to proceed. We decided if further fish could be collected and eggs obtained, they would be fertilized with sperm from the single anadromous RFL male Sockeye and/or other resident sockeye from sockeye beach, RFL.

Dr. Brannon, Keya Collins, and I went back on the night of October 28. Two "resident sockeye" were observed from the boat but were not caught in two blind sets with the beach seine.

The anadromous RFL male sockeye was near death after eight milt harvests and sacrificed on October 29.

Dr. Brannon seined sockeye beach on the night of November 4. Four resident sockeye were captured and one of these was a spawned-out female. Four others were observed but were not captured. Blood and adipose fin samples were obtained from the four for DNA tests. They were transported to Eagle Hatchery on November 5 in RFL water by the crew at Sawtooth Fish Hatchery.

Milt was obtained from the three males and the fish cold branded as follows: one dot=D, two dots=E, three dots=F. This is used to identify the origin of the milt and subsequent samples.. Motility tests yielded: D=0%, E=80%, F=50%. Thirty six eggs were obtained by expression and incision of the female. There were fertilized with pooled milt from E and F diluted with WSU's activator solution for two minutes, decanted, and water hardened with 100 ppm Iodine (1:100 Argentyne, diluted with Eagle Hatchery water).

**The female carcass was then processed for samples as follows:**

**1. Meristics: body proportions: Fork length 214mm**
**Total length = 233mm**
**Head length = 48mm**
**Eye width: Horiz. 12.2mm; vert. = 10.8mm**
**gill raker counts: L = 32 R = 29**
**Skin color: dusky green**
**Flesh color: 0, very pale pink**
**Spot pattern: documented by photograph**

**2. Pathology samples: Viro - KSPC + Br and OF with milt E,F and activator BKD - FAT and ELISA WD - brain case**

**3. Genetic samples**
**Blood: 3 vials in SSC, sent to WSU/UI**
**Henri:/liver/muscle/eye: duplicated and held -80c for NMFS and UI.**

**4. Aging and origin**
**Otoliths and 10 scales removed for microchemistry and aging.**

**The milt which remained after fertilization portion removed was oxygenated and sent to WSU/UI for cryopreservation.**
**Male D: 2 bags: urine and milt, milt**
**Male E: 2 bags: urine and milt, milt**
**Male F: one bag: milt with some blood contaminating**

**MILT SCHEDULE**
**Nov. 5: Listed above.**

**Nov. 9: male D; small volume, poor quality.**
**male E; poor volume and quality, tail erosion***
**male F; fair volume and quality***
*** cold brand misread; lables on milt were reversed, modify lables on milt in storage.**

**Nov. 12: male D; nearly all urine**
**male E; volume and quality fair**
**male F; nearly all urine**

**All three males terminated on 11/12/92 and tissues sampled with the same protocol as the female previously. Samples sent 11/12/92 to NMFS and UI.**

**MERISTICS ON MALES**

| Body Proportions: | MALE D | MALE E | MALE F |
|---|---|---|---|
| Fork length(mm) | 209 | 219 | 220 |
| Total length(mm) | 227 | 238 | 219 |
| Head length(mm) | 49 | 52 | 51 |
| Eye deminsions(mm) | 13x11 | 14x11 | 13x11 |
| **Coloration:** | | | |
| Skin | dusky green | dusky gold/green | dusky gold/green |
| Flesh | pearl white | pearl white | pearl white |
| Spot pattern: Dorsal- | entire dorsal | upper 1/3 | less numerous as D |
| Tail- | entire tail | not prominant | all of tail |
| Gill Raker: left,right | 32,30 | 32,33 | 33,32 |

**ADDITIONAL SOCKEYE BEACH OBSERVATIONS:**

Nov 11: Steve Wingert (SFH) did not observe any fish on sockeye beach, but did see a flock of mergansers in the area.

Nov. 14: K. Johnson at dawn also failed to observe schools or carcasses at sockeye beach, but a single bald eagle was feeding on a merganser carcass on the beach.

KAJ:rl

Appendix 4. Idaho Fish and Game sockeye broodstock nutrition workshop meeting notes.

Idaho Fish and Game

Sockeye Broodstock Nutrition Workshop

February 5, 1993

Meeting Notes

Idaho Fish & Game Headquarters
Boise, Idaho

# SOCKEYE BROODSTOCK NUTRITION WORKSHOP

## LIST OF WORKSHOP PARTICIPANTS

| Name | Affiliation | Phone |
|---|---|---|
| Ron Anderson | Bioproducts Inc. | 503/861-2256 |
| G.R. Bouck | Bonneville Power Administration | 503/230-5213 |
| Richard C. Bull | University of Idaho | 208/885-7653 |
| Joe Chapman | Idaho Dept. of Fish & Game | 208/774-3684 |
| Jeff Davis | IDFG | 208/334-3700 |
| Dave Erdahl | U.S. Fish & Wildlife Service | 406/587-4265 |
| Bob Esselman | IDFG | 208/788-2847 |
| Laurie Fowler | Rangen Inc. | 206/577-6324 |
| Brad George | IDFG | 208/476-3331 |
| Jeff Gislason | BPA | 503/230-7463 |
| David Groves | Sea Spring Salmon Farm Ltd. | 604/246-9191 |
| Ron Hardy | NMFS -- NWFSC | 206/553-7626 |
| Bill Hutchinson | IDFG | 208/334-3700 |
| Keith Johnson | IDFG | 208/334-3700 |
| Ryan Johnson | IDFG | 208/334-3700 |
| Keith Keown | Washington Dept. of Fisheries | 206/58G-2801 |
| Jeff Kuechle | BPA Writer-Editor | 503/232-6008 |
| Mike Larkin | IDFG | 208/334-3791 |
| Carlin McAuley | National Marine Fisheries | 206/842-7181 |
| Scott Patterson | IDFG | 208/265-7228 |
| Tom Rogers | IDFG | 208/334-3791 |
| Paul Siri | University of California | 707/875-2211 |
| Ralph Steiner | IDFG | 208/266-1431 |
| Bill Townsend | Trout Lodge | 206/863-0446 |
| Rick Westerhof | BPA | 503/230-5061 |
| Bob Winfree | U. S. F. W. S. | 208/837-4457 |

Sockeye Broodstock Nutrition Workshop
Boise, Idaho February 5, 1993 Meeting
Notes

**Keith Johnson** thanked everyone for coming. After a round of introductions he began with some background on the Red Fish Lake broodstock. Basically, he said, we're at a phase in this program where we want to be sure we're going in the right direction as far as the feed we are using, although everything seems to be working fine with our Biodiet feed. We know we only have one shot at this and there are a lot of people watching how this is going, as well as a lot of critics of the program.

When I get feeling uneasy about things, **Johnson** said, I usually try to rely on other people's experience and expertise. That's why you're here today. I'd like us to have an open discussion about your experiences, as well as whatever research data you can provide to support or refute what we're doing. I want you to give us some direction as far as where we should go from a nutritional and feeding rate standpoint on these captive broodstock sockeye.

Mow, there aren't that many people that have tried captive brood sockeye, **Johnson** said, but there are several examples in the literature that have been successful. I've read over most of those papers, and it doesn't seem to be a major problem. Maybe I'm overly worried about things that might go wrong with our program, because there are a lot of people looking over our shoulders. So if you see something that you think we're making a mistake on, please let us know. That's why we're here today. If you have some suggestions as far as how we may be able to improve the product -- the eggs and sperm that come out of these fish, and will go into recovery program -- please let us know.

To begin, I'd like to give you a quick rundown on where we are in the program, he continued. **Johnson** discussed the topography of the Stanley Basin, where Redfish Lake is located. From a habitat standpoint, he said, there has been a small amount of development, but essentially the habitat has remained in pretty pristine condition. Historically, there were sockeye runs in the 20,000 range. There are also kokanee that spawn in Fishhook Creek at the north end of the lake.

The lake is a very unproductive system, **Johnson** explained. It is *very* ligotrophic for a sockeye system -- actually, I would say ultra-ligotrophic. There is some work being done now, sponsored by BPA, examining the feasibility of fertilizing of the lake in anticipation of this sockeye recovery.

During the 50's, he said, we had counts that were in the 3,000 to 4,000 range. Over the course of the 1960s through the 1980s, there has been a steady decline, to the point now where in the last half a decade we could easily consume all the sockeye that had returned to that lake in a lunch. In 1991 we had four fish back, one female and three males; last year we had a single male come back. We called him "Lonely Larry."

The historic range of sockeye in the Stanley Basin area included at least five lakes, said **Johnson:** Stanley, Redfish, Yellowbelly, Petit and Alturas Lakes. Sunbeam Dam was in operation from about 1910 to 1924; there is some disagreement about whether or not that was a complete barrier. Probably not, because there were observations of sockeye in Redfish Lake during those years. The dam was dynamited in the early 1930s. Genetically, it's a classic bottleneck population, and it's likely the existing population reflects the effects of that bottleneck.

Our goal in the program, of course, is to bring naturally-spawned adults back to Redfish Lake, and any other lakes in the Stanley Basin that can still accommodate sockeye, said **Johnson.** Our department's goal is to have 6,000 naturally-produced adults coming back to the Stanley Basin. So essentially, our hatchery program is a kick-start attempt. It's also an attempt to maintain that sockeye genotype while improvements can be made in downstream passage.

**Johnson** described IDFG's trapping operation over the last few years. We use an incline screen trap or incline bar trap, he said; our trapping efficiency last *year* was about 16 percent. In 1991, we trapped 861 fish here, likely the offspring of demonstrated sockeye in Redfish Lake in 1989. They came out as 1 plus, averaging about 9-10 grams, and there isn't very many of them.

Half the fish trapped in a day are PIT-tagged and released downstream, he said, in an attempt to monitor successful migration into and through the hydro system. The other half of the capture smolts are transported and included in our captive broodstock program, and are PIT-tagged at that time. That way, we can identify day of capture and size of migration in our captive broodstock. All our fish have been PIT-tagged to date.

The captured fish are brought to Eagle Hatchery, ***Johnson*** continued; most of you saw it today. It's an old facility; as in most hatchery operations, I wish we had more space and more water. The thing I like about the layout, though, is its flexibility -- we can mix and match, because nothing is attached to the ground.

Since there wasn't a lot of preparation time for this project, I went through a major panic on how to convert wild fish onto a commercial diet, he said. I'd like to thank the people in this room who helped me with this. After taking their suggestions, I was able to formulate my own conversion diet. The main components are krill, bloodworms and Biodiet; it also included -- although I'm not really sure why -- anchovy paste and premium cat food. It smells terrible to me, but I have to admit that, of the 1,000 wild fish that we brought into this program, only three failed to convert on that diet. We feed that diet for about 10 days to two weeks, and I have to admit that, from a pathologist's standpoint, I see the amount of solubles in there and it makes me want to get them off that as fast as possible.

After the fish are actively feeding, said **Johnson,** we eliminate the cat food and anchovy paste and go to just krill, bloodworms and Biodiet for a relatively short period of time -- about another five to seven days. After that, those fish will take Biodiet pellets as a first offering, and by that time we're home free.

I don't like using krill, he continued. In '91 we started seeing a lot of intestinal parasites -- tapeworms. It didn't seem to kill anything, but there was some hemorrhaging in the gut. We're not positive it came from the frozen krill. But I don't like feeding non-pasteurized things. Still, it worked. That's what we start with and usually within three weeks we're on the Biodiet.

We start off in May of the trapping year at around six to ten grams, **Johnson** said. Then they take off in pretty much a normal growth rate. I thought getting these wild fish to eat was going to be the major hurtle in this program, but that seemed to be a real piece of cake.

Now, we do feed carotenoid, he continued. Last April we started using Biodiet's brood diet with canthaxanthin at 30 milligrams per kilogram and then natural carotenoid -- the astaxanthin complex -- at five milligrams per kilogram. The fish are starting to get reddish now.

So that tells you a little about the outmigrant component of the program, **Johnson** said. The second genetic component of the program is the wild fish that return. These fish were held at the Sawtooth Hatchery, where we have good holding conditions and water temperatures (50 degrees or 10 c.). We've had excellent holding of those fish up there. A lot of people have had high levels of pre-spawning loss in sockeye; we haven't seen that. The fish are trapped around the 10th to the 20th of August with a spawning date at two months later at least -- about the 20th of October. So we have roughly a two to two and one-half month holding period. They're held inside, with ambient light -- it's dark, but it's still an ambient photoperiod.

They seem to spawn right on with the normal wild sockeye spawn timing in Redfish Lake, he continued. In 1991, we had one female and she had 2,100 eggs. They were incubated as sub-families. Each male was used, sperm was checked for motility prior to fertilization, and then we did individual sub-family incubation. The incubation to eye was done at Sawtooth and then those eggs were split up -- half went to Eagle, and half went to National Marine Fishery Service at Montlake.

So fecundity for a 1.1-kilo female was 2,177 eggs, **Johnson** said. The eggs were incubated separately and were reared at Eagle until they were 7 grams as separate sub-groups and then they were PIT-tagged, pooled together and then redivided into the 6 lots. The fertilization rate was around 90 percent; right now we're running at 91 to 92 percent survival to the current size at 104 grams. In short, we've had very good success with the program to date.

The third genetic type we can deal with in the program are what I'll call residuals, Johnson said. There are the fish that were captured this year on Sockeye Beach in late October, early November. I call these residuals for several reasons. First, genetically they are almost identical to the outmigrants, and to the five adults for which we had genetic typing done. That genetic typing is a DNA fingerprinting being done at the University of Idaho and Washington State.

**Johnson** showed a series of slides, comparing the presumed residuals to other stocks of kokanee indigenous to the area. Genetically they're different, phenotypically they're different, he said. We're not sure at this point if it can be used in a recovery program.

So that's a brief review of where we are in the program, he said. I guess I'm looking at this and asking myself, genetically is it too late to try to recover a stock that has gone this far down in numbers. I don't know the answer to that and I know a lot of geneticists that will battle that issue back and forth. From my standpoint, it would be nice to have more fish to work with. Does anyone have any questions or comments?

I had to research this a bit, said **Jerry Bouck,** and if you go back through the histories that we can dig up, there are a couple of important things to consider. One is the observation of Everman in the late 1880's, which I think has a very important bearing on this situation. Now, at that time, there were no dams on the river, and there was a good run of sockeye coming in there. And there are records of people really hitting on those fish and hauling loads of fish down to the miners to sell them. So there was heavy fishing pressure on those fish in the late 1880's. By the 1890's, when Everman visited Redfish Lake and some of the others, he observed only 14 adults that returning. Another year they didn't see any. And that collapse long before Sunbeam Dam was built in 1911. After that, there is a big debate about whether any fish passage really occurred until 1924. So there was a period again where there should have been total reproductive failure.

So how could there still be sockeye in Redfish Lake? asked **Bouck.** The National Marine Fisheries Services status report came up with six hypothesis. I rejected all six hypotheses for a variety of reasons, and suggest a seventh: the residual sockeye. Residual sockeye have been reported in the literature, primarily by Ricker and his associates as well as Ted Bjornn.

The other thing is, how do you account for the number of juvenile fish coming out of there? **Bouck** asked. Now we have enough genetic data to indicate that some may be kokanee, but most of them seem to be electrophoretically similar to the residual sockeye. When you look at the number of fish leaving the lake, compared to the number of anadromous fish that have returned to the lake in the last few years, you're getting too many smolts coming out of there.

So something is contributing to this, and it looks to me like it must be residual sockeye, **Bouck** said. What that means, though, I don't know. Since they don't produce that many eggs, I would suggest, **Keith**, that if you're getting normal rates of recruitment from their eggs, then you've got to have a much larger population in that lake. I would suggest that you very likely have a higher population of residual sockeye there then you might think at first.

So I really commend you for looking for them, **Bouck** said. I predicted that if you looked for them you would find them. Another piece of evidence is the fact that spawn timing is inheritable with the kokanee spawning at a different time, different place, and different water temperature than the sockeye which spawns much later and at a different time, place and temperature.

So does that argue that they may be in Alturas, Stanley, and Yellowbelly Lake? asked **Ron Hardy**. Well, Alturas is still putting out outmigrants -- we know that, said **Johnson.** Quite a few outmigrants, in some years. Alturas is isolated, because downstream from Alturas Lake is 100 percent de-watered during the adult return period. It has good water flow going out during the smolt outmigration, but adults would have to walk on rocks for half a mile. That situation is in the process of being corrected. The water did flow during adult season this year. We trap down below, so if any adults got up we would know.

So we do know that Alturas is still putting out outmigrants, **Johnson** continued. We don't know about the other lakes. We haven't looked at Petit Lake, and Yellowbelly was recently poisoned with a re-introduction of cutthroat. We didn't see any kokanee during that poisoning. Stanley Lake has had a barrier on it since the 1960's, so that would have isolated that population. It does have a kokanee population; it's been planted over the years with a number of stocks from outside the basin.

I guess what I'm getting at, said **Bouck,** is that we've got something different than we've ever worked with before. We really don't know much about residual sockeye and where they come from. At the end of the glacial period that lake was probably covered by several hundred feet of ice, so the population that's up there is probably fairly recently derived. I don't think the original stock is there; I think they must be residual sockeye, and that for this reason they should not be put protected under the Endangered Species Act.

(The residuals) need protection, don't misunderstand me, **Bouck** continued. But as long as they are safely ensconced in that lake, I don't think they necessarily qualify for protection under the ESA. If you don't have protection under the Act, you have a great deal more flexibility in terms of what you can do with those fish. I have been told repeatedly that animals that spend their time in fresh water are not covered by the National Marine Fisheries Service. They are not covered by the Section 10 Permit. So you could have both biologically and administratively an entirely different situation.

Now, that said, I'd like to add that I think that the run can be brought back, **Bouck** said. I don't mean to imply anything dire. But we're dealing here with something mostly unknown.

Next, **Johnson** spent a few minutes on growth rates and mortality. 1991 was the year they were captured. What I'm trying to do with the growth rate, he said, is to mimic nature, as far as how those fish would grow in the wild, by adjusting percent bodyweight fed per day. What I was looking for was a growth rate that would be relatively rapid in the summer and early fall, plateau off until March or so, start up again and grow well until through October, and then plateau again. I want to repeat this until the fish spawn.

Outmigrants from Red[f]ish Lake are 1+, he said. So at outmigration, they are 16 months from fertilization. In the wild, the stock typically *spawns* as fours or fives. However, my experience with things in captivity *is they* spawn a year earlier than you would normally get in the wild, because of good nutrition. What we try to do is adjust percent body weight to try to give us a somewhat stepped growth rate over a three-year period.

So my goal is to produce a fish that's about 1,200 to 1,400 grams as a 4 year old, **Johnson** continued. Now, that's not a monster sockeye, but Redfish Lake stock isn't a particularly large stock. The adults that are coming back currently are between 1.1 and 1.6 kilos, and I wanted to aim for that.

If we continue this growth rate, say through June 15th, I would expect that we will hit our target of about 1.2 kilos, he said. That's a mean weight; we probably have some fish in there that are that size already. They seem to be growing fairly uniformly, compared to some of the observations done on sockeye growth in New Zealand. So I don't feel we're doing really badly.
There's another side to what we're trying to do growth rate-wise, **Johnson** continued. Our goal is to get high survival, as opposed to large fish. We are feeding, in round numbers, about 70 percent of the recommended growth chart. The reason for that is to try to keep down the amount of uneaten feed and fecal material. To me, the culture environment is paramount. I think that our mortalities have reflected that.

So from May of '91 through January '93 we're looking at 90 percent survival in all but one of our groups, he said. Groups 1 through 5 were formed simply by the date or the week they were transported down to our hatchery. That is all the difference there is. We did have a bacterial gill disease outbreak, caused by screens that were too small. There has also been some bleeding loss; we bled 20 fish to get blood samples for the National Marine Fisheries lab and University of Idaho for the DNA work. And I killed 11 out of 20. I am not real proud of that; I've bleed a lot of fish in the past without killing them, but these things bled a lot better than I've seen before. So with groups 1,2,3 and 5 we've done pretty well. We have practiced erythromycin feedings spaced roughly six months apart in all populations.

Group 4 is a different story, **Johnson** continued. We have serious bacterial kidney disease problems in group 4. Our overall loss as of February 1 is 50 percent. We saw the first BKD positive in October, 1991. The first BKD-caused mortality occurred 40 weeks after they were taken into captivity. What I learned was, don't raise sockeye in the presence of BKD. Once it's established in the population, you can try as hard as you want by feeding or injecting antibiotics but you can't stop it.

I kept these fish because I wanted to find out if you could control BKD in a captive broodstock, **Johnson** said. And the answer would seem to be no. One problem with wild fish is that they are not as bacteriologically clean as many anti-hatchery people would like to think. This population, for example, carries a low level of BKD. It looks like one fish out of 759 that we brought into Eagle had the disease.

I also wanted to look at how frequently you need to feed erythromycin to control the disease, he continued. At this point, it looks like every other month for 28 days. That's a lot of erythromycin. My final conclusion from this is, if the fish aren't critical, the best thing you can do is get them off your station as soon as possible.

So we're going to have to deal with segregation throughout this whole program if we spawn these fish, **Johnson** said. We started out with 196 fish in Group 4, and right now we're at just below 100.

Could you inject those fish just prior to spawning, and save the eggs? asked **Bill Townsend.** That's our intention, **Johnson** replied. I would still treat those progeny as positive for the first year of culture, before I would feel good about pooling them with anything else.

**Johnson** talked for a few minutes about his Alturas Lake fish. We trapped them originally because we felt we needed to have a control group, fish that we could dissect or sacrifice, or use to test a treatment we weren't comfortable using on the sockeye. Now, the National Marine Fisheries Service recovery plan isn't out yet, but the preliminary draft places a fair amount of emphasis on trying to utilize the Alturas Lake stock. If that does become fact, then we do have 128 fish. They are at Sawtooth Fish Hatchery and are about 636 grams. They're two-year-old outmigrants, so they're actually a year older than the Redfish outmigrants. Yet last year, at about 500 grams, we did not see any maturity in those fish. I thought I had seen some information from **Dave Groves** that he got spawning at two years old. Actually, said **Groves,** these were sockeye that were 10 grams as zeros. At two years old, they were males. They all were spawned at the end of the third year -- both male and female. We didn't zee any precocity as three-year-olds, **Johnson** said. Well, looking at those fish today, they look to me like fish that in our experience would spawn next fall, **Groves** said.

Well, said **Johnson,** this is what we're anticipating. What I don't know is what percent will spawn. I'm hoping they don't all spawn; from a functional standpoint we need to have incubators and space to rear the progeny of all of those fish.

But that's another question I have for the group, he continued. If we drop back so that we're on a very low growth rate, could we take half that population and put it at a different growth rate and expect to have spawning a year later? The National Marine Fisheries Service is looking at things like that in its Wenatchee sockeye captive broodstock program, but those fish are behind us by a year in size.

We have the two groups of outmigrants that were captured this year, **Johnson** said. They're at Eagle; we refer to them as the outmigrant '92s from Redfish Lake and Alturas Lake. These were running 143 grams as of January 1. It's a pretty uniform growth rate; we've tapered them now back to less than 2 percent body weight to try to moderate that growth. At the moment, the growth rate is pretty predictable.

Is that consistent with what we saw in the '91 outmigrants? asked **Bill Hutchinson.** It's a little slower, said **Johnson.** The '91 outmigrants January 1st were about 240 grams; the '92 are running about 140. That's intentional -- we're trying not to feed them as rapidly, to stretch that out for an extra year. I would add that the mortality for that group has been minimal.

There were a relatively small number of outmigrants captured from Alturas in '92, **Johnson** continued -- about 193. Growth rate has been very similar; they're a little smaller. I don't know why; they seem to start out a little bit less but they grow in parallel.

Then we have our brood year '91 fish, he said. These are the progeny of that single female and the three males. Their growth rate is quite similar to what we were seeing with the other groups; again, we've started to taper these off in the winter time. They were 104 grams as of February 1. Mortality has been quite low. Cumulative mortality is less than two percent during the first eight to nine months of culture. There is no real pattern to it, except that these fish were PIT-tagged, which is the intrusion of something about the size of a baseball bat into the body cavity if we were the fish. There was about 0.6 percent mortality as a result of that. We did have one other fish die, and we did have a jump out recently. Altogether, at our facility, we have 924 of these fish.

I wouldn't say we have done an exhaustive literature search in relation to sockeye culture, **Johnson** said. Most sockeye broodatocks are raised to smolt size, released to the ocean, and brought back. I think our challenge here, at least from a nutritional standpoint, is that we're providing all the nutritional opportunities the fish are going to have.

I guess that's my concern, and where I want to throw it open to comment from the group. Are we doing the right thing? Should we be adding natural foods to our program? he continued. From my standpoint, I see a risk in using unpasteurized product. But would the benefits outweigh the risk? I'd like to hear from the people here about what we can do from the growth rate standpoint. When we have some fish that we can separate out, should we put them on a lower nutritional profile to spread out spawning by a year? If so, what would you suggest we do as far as a feeding regimen? He asked **Groves** to begin.

What do the temperature profiles of Redfish Lake look like? asked **Groves.** Is that considerably colder than the holding tanks at the hatchery? Redfish Lake itself will warm up in the summer to a surface temperature of about 17 degrees C., **Johnson** replied. The thermocline typically is about 20 meters, dropping to around 4 degrees C. in the deepest layers.

The distribution pattern of sockeye in that lake are similar to what you would see elsewhere, he continued. In the winter, you would expect the lake to ice over in early December; average ice-out is the 10th or so of May. Outmigration typically occurs in late April toward the end of May. From that standpoint, it is not an unusual lake, except that it is very unproductive. The adults come into that lake at about its maximum temperature, when the surface temperature is about 17 degrees C.

Well, they experience considerably higher temperatures on their way there than they do once they get there, said **Houck.** When the adults go into the lake they probably adjust to the lower temperature, said **Groves.** Well, I can't say for Redfish, **Johnson** said, but I know for every other sockeye population I've ever seen that they hang right below the thermocline, stay in that strata for weeks, and then start to ball up in front of the creeks. I know in southeast Alaska, we used to see lake surface water temperatures of 15 to 17 degrees C. and then when the creek temperature dropped, they'd go blowing up the creek, and they'd spawn in the next couple of days. It seemed like that drop in creek temperature -- down to **10** or 11 degrees C. -- was a major trigger for sockeye secondary migration.

**Groves** then gave a short overview of his own Chinook broodstock program, which led gradually into a discussion of the relationship between nutrition and reproductive success. In 1936, **Groves** said, we reformulated our broodstock ration. The principal thing we did was to add half a part per million of selenium to a ration that tested out to approximately one and a half to two parts per million of selenium already -- probably that was in bound form. We increased the ascorbic acid level, as well as the vitamin E level. We cut the iron level from nearly 700 mg per kilo down to 250 mg. And that year, our egg survival and fertility rates jumped up to about 80 percent from green eggs to hatch.

From that point on, said **Groves**, we continued to formulate our own ration for about another year. Then we began to use commercial rations which were more increasingly available in Canada and the J.S. We began to notice that there wasn't a lot of difference from that point on in the kind of broodstock production we had on the commercial rations, compared to what we got on our own formulated rations. In latter years we've used entirely commercial rations, and not usually commercial broodstock rations. There's been a progression in the quality of the commercial grower rations available in the salmon culture industry.

One thing we have noticed, however, he continued, is that the first chinook we spawned looked entirely different phenotypically compare to wild fish. They were short, fat, chubby. As our rations improved, three-year-old fish would be typically about 15 pounds, a nice long, lean shape. One of the other opportunities we had, as a result of selling smolts to the salmon farming industry at large, is that we sold fish to people who fed them under a number of different degrees of intensity and care, **Groves** said. In 1987, we brought back some brood fish from that first 1984 egg lot, of which we had kept some ourselves. Instead of being 15 pounds as three-year-olds, they were 30 pounds. We were very excited about these fish -- they were the most magnificent-looking chinook I'd ever seen coming out of culture. But they weren't very good for broodstock. The eggs looked beautiful, but they had soft shells, and they had horrible results through the incubation phase. The ones we were growing as market fish, about half that size, had much better survival.

So broodstock nutrition for salmon is very similar to broodstock nutrition in cattle, or sheep, or other animals, **Groves** continued. The conditions under which you raise them for maximum growth rate, where you're really pushing them... those aren't the fish you want to produce your eggs. You need to keep another sub-group that you don't push, that you grow to a good size but not an excessive one.

In terms of nutrition, our observations have been fairly general, **Groves** said. We started thinking about selenium in 1985, partly because we had a herd of beef cattle that were also in a selenium-deficient area. They began to have typical selenium-deficiency problems -- early embryonic mortalities and early calving. Those stopped when we gave them selenium injections.

With the chinook, fertilization looked to be OK, but again, we were .experiencing a lot of early embryonic mortality, he continued. There were some differences in fatty acids, but the biggest difference between the eggs was in their selenium levels. The only other difference was that the domestic fish had higher levels of fat in their eggs, and higher levels of saturated fat within the egg fat. We never had any definite proof that it was a lipid problem, but by feeding the domestic fish the way we were feeding them at that time, we were probably loading the system with higher saturated fats.

Those early fish must have been deficient in something -- they would feed voraciously right up to spawning, so they must have been *trying* to compensate for something, **Groves** said. We haven't had that problem with subsequent generations -- the appetite of the hatchery fish now seems to taper off naturally, about the same time you'd expect to see it taper off in wild fish.

Just a few words about our sockeye, **Groves** continued. At a very early stage in B.C. salmon culture, we thought about sockeye, because they seemed to do so well in fresh water. Early attempts to rear sockeye in saltwater net-pens were a dismal failure, but that was before we had any Vibrio vaccines -- they would die of everything that came by. It was for that reason that the industry began to work with coho, and then chinook. Around 1989, the Federal Department of Fisheries tried to rekindle interest in the sockeye in the salmon-farming industry, starting with eggs from wild fish. And once again, those fish behaved extremely well in fresh water -- they looked like Keith's fish. They did very well at very high densities. Once again, though, we put them to sea as age-zero smolts, about 10 grams. They grew fine for a couple of months, then began to taper off. BKD was a real problem -- they had two or three times the mortality of chinook reared under the same conditions. By the end of the second year, almost all of them had died.

The other thing that was disappointing was the pigmentation, **Groves** continued. One of the points of reintroducing sockeye to the salmon-culture industry was that, even though they wouldn't reach the size of the chinook, they would be a very saleable commodity at two or three pounds because they were so bright red. But none of our efforts to pigment them had much success -- the best we seemed to be able to do was white or pink, never really red. We fed them dose of astaxanthin and canthaxanthin that would have turned chinook or coho red several times over. For whatever reason, they just *didn't* absorb the pigment -- perhaps their normal diets are so high in carotenoids that they're extremely inefficient at absorbing them.

In terms of spawning, he continued, I'd be wary of trying to hold the sockeye back an extra year by feeding them a little less. In our experience with broodstock, we don't like to overfeed them, but if they're hungry, we give them maybe 80 percent of satiation. We don't push the feed on them, but underfeeding them will give you lower fecundities and higher disease problems. 75-80 percent of maximum would be a much safer bet.

One other thing, said **Groves:** if you happen to have some mortalities, we've found a very simple way to tell which fish are going to spawn next fall, as early as mid-February or March: total ovary weight in the females, and egg diameter. If you multiply those two together, you get an index, which kind of amplifies. As early as right now, you could probably see a quite significant difference between the fish that were going to spawn in the next cycle, and the ones that weren't. In chinook, if the index was less than 10, they weren't going to spawn the next year. If it was greater than 10, they were going to spawn that next cycle. the break is very clear, and it gets bigger as the season goes on.

**Johnson** said he had begun in September to plot ovary area (length times width) verses fish length and weight. It looks like we're starting to see a change, although it isn't real dramatic yet. He described last year's efforts by Dr. Beall of Virginia Polytechnic to determine maturity via ultrasound. It's a fascinating technique, **Johnson** said, but we didn't see any gonads. **Jerry Houck** said that, in his experience working with wild Columbia River sockeye in the late '60s, there was no difficulty in telling when those wild fish were going to be ripe. You'd work with them just like your hatchery people now, handling them, and you could tell very easily which fish weren't quite ready, and which you needed to spawn tomorrow.

**Ron Hardy** said that, in the U.S. at least, the FDA limits the amount of selenium in animal feed to 0.3 parts per million. That doesn't limit the total amount from natural sources, of course, just what you can add to feed. In Canada they allow 0.5 parts per million, said **Groves. Hardy** added that feed manufacturers now have at their disposal stable forms of ascorbic acid, which have been denied them in the past. This has eliminated a lot of the problems we *used* to see, he said, which probably were associated with the loss of ascorbic acid during storage.

**Bouck** talked for a few minutes about his sockeye rearing experience, saying, among other things, that they had excellent survival, and had no trouble at all maturing the fish. They were held at a constant 10 degrees C on a standard Oregon Moist Pellet diet. It got a little colder in the winter, but 10 C. was the maximum. He said they had cut off the feed around July 4 of the third year, because that's what they thought wild fish would do. He didn't remember producing any jacks; basically, all the fish matured at three years. The first brood we brought through that way, he said, I don't suppose we had reproductive success with more than five percent of the eggs. The problem was that the males came ripe early, and the females weren't ready yet. We had problems with coagulated semen, or the males *would* just die. Then when the females did come ripe, we had problems with fragile eggs. We didn't know what went wrong.

We did several broods over about seven years, **Bouck** said, and that was pretty much the pattern, time after time. There were probably four broods that we had reproductive failure on, even though these fish were just beautiful in the tanks. Then one day, **Bouck** *said,* they made a connection between high levels of testosterone in the diet, and early-ripening males. That was about February, he said, and as quickly as I could arrange it, I bought raw frozen Pacific shrimp. We divided our brood -- we had about 75-80 fish. The control group stayed on OMP; the experimental group was fed raw frozen shrimp from that time forward until July. The difference was overtly observable. The control group had the same early ripening and egg-quality problems we saw before; with the shrimp-fed fish, both sexes came ripe at the same time, the semen looked good, they fertilized well, and everything was fine and dandy until my technician had a horrible car accident. Mo one knew the experiment was back there, and we were never able to salvage them.

**Bouck** also described an incident that points out the crucial importance of water quality to these fish in a broodstock situation. A cadmium-plated bolt holding together a stack of Heath trays fell into one of his 4'-diameter tanks. After that, he said, we had a chronic mortality in that tank. We checked those fish for everything we could think of, and found nothing. Finally we decided to dump them, and when we did, we found this little bolt. We couldn't believe that was really the problem, so I tossed the bolt in with another group of fish we weren't concerned about. Sure enough, after a month or so, we started seeing a chronic low-level mortality.

Last but not least, said **Bouck,** this is not a research project. It's a very serious high-bucks endeavor. Several lawsuits are pending. The bottom line is, when everything's said and done, do we still have an anadromous fish? Will they still tolerate seawater? _ mean, we can raise them in the hatcheries. But when we get through, what do we have" I think we have to evaluate them physiologically, and know whether or not these fish can tolerate seawater. If they can't survive, and do well, in the ocean, they're not sockeye.

After a brief break for lunch, **Keith Keown** spoke for a few minutes about the Washington Department of Fisheries' White River spring Chinook_ salmon broodstock program. We're in our second year of full production, he said, and basically they give me a small allotment -- 3,500 -- of one-plus age smolts, that we bring out to saltwater pens. Our goal is to generate the maximum escapement we can get from those 2,500 smolts. The intent is to bring those fish to adult size; then, the month prior to spawning, we move them back to freshwater sites for spawning. Their eggs are supposed to be put back in the indigenous systems from which they originated, with the hope eventually that the anadromous run will right itself.

So it's a volume operation, **Keown** said. We produced 1.4 million eggs in the fall of '91, 1.2 million in 1992. In terms of feeding rates, we drive the fish pretty hard in the beginning -- around two to three percent a day for the first year and a half. Temperature isn't a big factor in this operation; we do see some warmer temperatures in August, when we'll back the feed off a little. Year four is the crucial one for us, so at around three and a half years we're really driving the feeding rate down, and take them off feed right before we take them to fresh water.

Out of the 1.4 million eggs in '91, we saw 66 percent viability, **Keown** said. That was on a grower diet. There was some thought that we could fine-tune that a little, and so last year we started to explore dietary concerns. Last year, we switched the fish that were going to be maturing as four-year-olds to a formulated broodstock diet in the spring. We fed them that broodstock diet for three months, and the viability on those 1.2 million eggs increased to 72.5 percent. We're not saying that's solely due to diet, said **Keown,** but it may have had an effect.

He spent a few minutes discussing sex ratios, saying that it had been necessary to cross four females with three males to get enough milt for the program. Our guidelines call for a one-to-one ratio, **Keown** said, but right now we're having to mix sperm to make it work.

That brings up the second dietary factor, he continued. There's quite a bit of literature about using diet -- lower winter feed rates -- to encourage males and females to mature simultaneously. By feeding one group only .15 percent of body weight per day during January and February (one feeding per week). The hope was to take the decision-making process away from the males, he said supposedly, the studies are showing that the fitness factor of the fish in winter does sometimes play a role. The results? The percentage of maturing males stayed exactly the same -- within one percent. The percentage of maturing female three-year-olds, however, dropped by almost half -- from 11 percent to six percent. So it had little or no effect on the males, but quite a noticeable effect on the females. And that, of course, made my problem worse, because it gave me even more four-year-old females.

How was your egg quality? asked **Carlin McAuley.** Did you have any problems with soft shell, or... Yes, absolutely, **Keown** replied. We only had two-third viability, so we obviously had soft shell and blank egg problems. Did the soft shell occur around the eye -up stage? **McAuley** asked. All the way through, **Keown** replied.

Any other questions? asked **Johnson.** Not a question, but a comment about the difference between wild and domestic fish, said **Groves.** One of the characteristics we've always noticed with domestic chinook eggs is that even though they look very close to the wild ones that we've also handled, the shells of the wild eggs disintegrate within a couple of days of hatching -- they're never a problem in terms of having to clean the trays. The domestic shells, on the other hand, stay around like crushed ping-pong balls. Even though we have good survival from the domestics eggs, we always see this difference, **Groves** said. It must be some kind of nutritional or physiological difference. And there does seem to be a small amount of additional hatching mortality with the domestic eggs -- the wild alevin get out very quickly, whereas some of the domestic ones get stuck halfway out. It's not a big loss, but it's characteristic. In response to a question, **Groves** said his water hardness is about **30 ppm. Johnson** said he had encountered similar problems with wild chum salmon in Alaska, and by putting calcium hydroxide into the water, they were able to significantly increase their hatching success.

Since our product is so heavily involved, I'll make a few comments on nutritional strategy, said **Ron Anderson.** He discussed the shortcomings of Oregon pellets, and said that the nutritional strategies his company uses today in the manufacture of brood diets have no relationship to that used for Oregon pellets.

I also wanted to give you an idea of how our brood diet thinking differs from a formulation point of view from a grower diet, **Anderson** said. In general, the protein levels in brood and grower diets are similar. The quality -- the source of that protein – is clearly better in the brood diet. For one thing, it's almost all from marine fish. So it's not really different in quantity, but it's clearly better in that, a fish protein is better than a plant protein. So grower diets contain some of both in most cases and brood diets generally don't.

The fat difference in the two feeds is in both the area of quality and quantity, **Anderson** continued. It is our belief that brood diets should be as low in fat as we can technically manufacture. We are limited by the extrusion process -- we have to have a certain amount of fat in the product. In our brood diet, we aim at a fat level of about 13 percent. A comparable good-quality salmon grower diet might be up at 16 to 18 percent. That three or four percent matters -- the idea here is to hold down residual fat accumulation. Obviously, that's related to the feeding rate of that diet, but if we can reduce up front, it's an advantage. We'd bring it down to 10 to 11 percent if we could.

The source of fat in our brood diet is marine-based, **Anderson** explained -- best-quality fresh, unprocessed marine fat. The vitamins we add are C and E, because of their value as anti-oxidants. These are the two that matter and they're supplemented at a very high rate. Pigments are a highly controversial area; obviously growers diets don't include it. Pigments aren't cheap - they add 3-5 cents a pound to a fish food, no matter how you cut it. But we sort of think that if mother nature feels salmon eggs ought to be pigmented, then that's enough for us.

We use canthaxanthin from the synthetic source and anthoxanthin from krill, he continued. The total pigment load is about 35 parts per million. That's less than a typical commercial diet, which would typically be about 50 ppm. So you wouldn't expect these brood fish to appear marketable in coloration, per se. They are being fed on lower rates to begin with, and they're consuming less pigment up front.

The last thing we consider is palatability, **Anderson** said. Our feed is used under many different conditions, and where there may be very cold temperatures or harsh rearing conditions, flavor and palatability seem to matter in the broadest sense but not in every case. I don't think it matters out at Eagle. I don't think the flavor is an issue except perhaps in early rearing. Those brood fish are well conditioned. Flavor wouldn't matter a bit out there. We add krill to flavor our food.

Because of the migration the fish undertake, and the fact that something is happening to the eggs during the migration, I don't think it would be a good idea to starve the fish from July 1st on, **Anderson** continued. There is no way we should assume that those fish at July 1st at Eagle have had the same quality nutrition as that fish that migrated from the North Pacific and arrived at the Columbia on July 1st. I think they should be fed at some declining rate as we go through the summer period, trying to help those- fish begin to utilize their fat as the egg development process occurs. I don't know what the magic number is, or when exactly you should stop feeding them. Hopefully, the animals will give us some cues.

I think the holding strategy is just as important, **Anderson** said. From my experience in working with customers, the most successful brood programs involve keeping fish very quiet, undisturbed, providing clear visual barriers from people and other activities or animals or light. You might be consider providing some floating barriers in the tank -- just strips of polyethylene. Give them areas where they can break up the visual patterns, and their social interactions with one another. I think they need to be fed in a way that minimizes all human contact with those fish from mid-summer on -- once a day, at a time when it seems most prudent and reasonable. And the rest of the time they really ought to be left alone.

How about the issue of testosterone? asked **Johnson.** Testosterone is very much an issue at Oregon Pellet. Oregon Pellet requires certain ingredients, some of which contain be very high levels of testosterone. The ingredient processing strategy used today in diets doesn't include the restrictions that Oregon Pellet included. Mostly because you just can't afford to include that much herring, said **Anderson.**

**A** discussion followed concerning the problems a company faces in funding brood stock diet research. I would like to put forward another consideration, said **Bouck.** The cost of the feed is peanuts compared to the cost of the rest of the program. Considering the number of stocks of fish that are being considered for some special assistance, there is probably a need for research. I would say that cost is not the issue.

I think from the private sector's point of view, when you talk about juvenile spring chinook, there is active research going on, said **Ron Anderson.** That's the main stream of the fish feed opportunity in the Northwest. Maybe, said **Bill Hutchinson,** but I don't think Bob's program was limited only to juveniles, was it? Well, if you're talking about the Hagerman program, **Winfree** replied, Tunison (?) had 6 Ph.Ds. They worked with Atlantic salmon, spring chinook, rainbow trout, striped bass -- basically, you've got less than one person per species, and that doesn't go a very long way when you consider that just a few years ago, the Western Fish Nutrition Lab had about 25 people working on Pacific Salmon alone.

So what you're saying is, the baby got thrown out with the bath water, said **Bouck.** You certainly have to pay attention to what the wild fish do, and try to match that, said **David Groves.** But -

with our little salmon enhancement project, we're an embarrassment to the Department of Fisheries and Oceans -- we get about 6.5 percent of those fish returning to the fishery, while the percentages at many of the big hatcheries are down on the order of half a percent. Mow, we do have a little higher water temperatures, so we produce a bigger smolt. But the other factor is that we're selling smolts to the industry, and when someone buys an Atlantic salmon smolt for $2.50, or an age zero Chinook for 50 or 60 cents, they really do expect that it's going to live, **Groves** said. If it doesn't you're going to hear about it, and quickly. We can't afford to have two kinds of fish in the hatchery, so we treat our wild broodstock exactly the same way we treat our domestic broodstock. The stocking densities and sanitation levels are exactly the same. I suspect that a lot of the Federal SEP fish are counted, but are not necessarily of anything like the same quality, especially in terms of disease control.

Well, for a lot of years, the emphasis in public hatcheries has been pounding the numbers, said **Winfree.** I think we all recognize that that's no longer what needs to be done. We're at a stage now where product quality needs to be defined, measured and enhanced. That can be achieved, through nutrition, through temperature, fish culture... I don't think there are many limits on that at all. It's just a matter of defining what we want our product to look like physiologically, and then producing it.

Right -- the real issue is harvest management, not hatcheries, said **Bouck.** If you listen to people, you get the impression that, on moonlit nights, hatcheries somehow rise up and do evil things. The real problem is mixed-stock fishery management. And one other thing, talking about shielding them -- we used a four-foot-high plastic barrier, so that they couldn't see us, and also used a spray to dimple the surface of the water. They were under a roof, so that they only got ambient light -- they really weren't disturbed much at all.

One other comment, about something you might want to concentrate on, said **Dick Bull --** the effect of nutrition on the immune system. With other species, it's really become quite important to look at the condition of that animal as far as the condition of that animal, and its ability to produce the antibodies it needs to resist disease. Some that have come to light include trace minerals, particularly the form in which those trace minerals are presented to the animal. **A** good example is cupric oxide -- it's very poorly absorbed compared to the sulfate form -- only about 4 percent absorbable. There are some organic ones that are even better than the sulfate form. Selenium is also very important to the immune system, **Bull** said. Copper, zinc, manganese are all involved in the immune process; there are also some vitamins --

vitamin E, for example. I would suggest that you make sure that whatever formula you use meets those nutritional needs, and you'll go a long way toward heading off some of your potential disease problems.

**Johnson** asked **Scott Patterson** to say a few words about IDFG's kokanee captive broodstock programs at Cabinet Gorge and Sandpoint. There were two main issues I was curious about, **Johnson** said: spawn timing, *and* how the three-year-old spawners you've seen might differ from what you've seen in the wild.

Well, as Keith said, we did hold about 4,000 kokanee back, said **Patterson.** At my station I had about 550. I kept them on the Biodiet 4000 grower, an Atlantic salmon grower diet with no pigment; I fed them quite heavily, almost doubling their body weight these last months. From a fish 32 centimeters long, I was able to produce 1,000 eggs. 60 percent of those 550 -fish matured as three-year-olds, compared to the wild stocks, which mature as 4s and 5s. So we have advanced them a little bit in that time.

The majority of the fish that matured were males, **Patterson** continued. If I have a 50-50 sex ratio, all the remaining immature fish will have to be female to match that 50-50.
As I said, I raised them on the Biodiet, feeding at around 0.8 percent body weight, he said. That produced a substantially larger female kokanee than we would see in the wild -- 32 cm, compared to 25-27. At 1,000 eggs per fish, I also doubled my fecundity. As far as egg quality is concerned, the eggs were smaller in the wild fish that mature as 4s and 5s. They were running around 18 eggs per gram; the four-year-olds we spawned in Coeur d'Alene were around 16 eggs per gram, and the Pend Oreille fish were around 12 eggs per gram. So even though they're larger, they're producing a smaller egg compared to my results. **Patterson** said the color of his eggs was down around the one to two yellow range; he added that the first few tanks have started to eye up, and they're around 80 percent at the eye-up stage. Water temperature averaged around 7 degrees, he added.

The fish we held back came from the January 3 spawning take -- one of the very last spawning takes we took in the wild, he continued. I still have green fish on hand.

I don't know if you guys know this, said **Anderson,** but canthaxanthin is the only one you can legally add to salmon diets in the United States at the moment. It only pigments the flesh – it won't pigment the skin, whereas astaxanthin pigments both. In other words, both will pigment the flesh and eggs, but only astaxanthin will transfer to the skin of maturing fish. Right, and these fish never turned red, **Patterson** said.

Bouck said he had read somewhere that residual sockeye don't necessarily die after spawning -- particularly the males. You may be able to feed them, and they'll keep growing. They may not produce any milt, but you might keep them alive. **Johnson** said he would be interested in seeing the reference if **Bouck** could dig it out.

**Johnson** said he had read of a similar study in New Zealand, where researchers had kept 50 fish alive after spawning. They did OK for the first three months, he said, but in the fourth month they went from 50 fish to one fish. It's a pretty important issue, though, because if you can tap sperm off and keep the fish alive, you can keep tapping sperm -- at least for awhile.

It's important for another reason as well, said **Bouck --** you see an immediate difference in coloration between residual and kokanee and sockeye. It would imply some basic metabolic differences, some fundamental differences. There are a lot of people that just want to lump all those fish together as O. nerka, and I suspect that if we could study them for awhile, we might find out something.

**Johnson** discussed Chris Wood's work in Nanaimo, which involved crossing sockeye with kokanee in an effort to evaluate the .neristic and physiological differences between the groups. One thing they noticed, he said, was that the deposition of carotenoid pigments is significantly lower in sockeye than it was in kokanee.

**Ralph Steiner** spoke for a few minutes about the results from the Cabinet Gorge kokanee project, basically confirming **Patterson's** comments. He said about 2.5 percent of his males matured precocially, but other than that, his results were much the same as **Patterson's.**

**Joe Chapman** added a historical synopsis of Idaho's involvement in kokanee broodstock, saying that the kokanee population in Lake Pend Oreille wasn't returning to the rack in expected numbers. We had some fish we were going to hold over and stock as one-year-olds, he said, clipped differentially so we could see whether or not they were coming back. Then we started thinking, the way these things aren't returning, we'd better think about holding on to some of them. That's how we got into this broodstock program. And it's on the increase, Steiner added. We have 55,000 one-year fish and 36,000 two-year fish, plus the 4,000 we spawned this year. There's a real concern that we need to protect these fish.

Next **Johnson** called on **Paul Siri,** who provided an overview of the northern California winter chinook project. You'll have to bear with me, he said, because this particular species has the most variable life-history characteristics of any chinook run in the eastern Pacific. This project is very visible in California, he said, because *as* you know we've had seven years of drought. The ESA authorization is limiting water deliveries to southern California and dredging activities in San Francisco Bay, so it's a pretty hot item.

In terms of background, 15 years ago, we were seeing adult returns in the 170,000 range, Siri said. 10 years ago, it was 100,000 animals. In 1990, only 189 fish came back. From that year-class, we were permitted to take 20 adults. From that, we were able to get about 1,200 fry, with one male parenting 58 percent of them.

Because of permitting delays, we weren't able to get the 1991 fish to Bodega Bay Laboratory until last September, Siri said. As a result, our first successful broodstock year is one year delayed from smoltification -- we won't be smolting those animals until probably some time in March. That's going to push some animals to mature early, and of course to increase the number of jacks.

The reason our lab was called in was that there was a strong desire on the part of the agencies to *rear* those animals side-by-side in an identical treatment, half in fresh water and half in salt water, Siri continued. Bodega's is a closed system; we felt that would be a good idea, to try to reduce the number of ambient pathogens. That means we're using biofilters, which of course is something of a headache, particularly when BKD is present. Unfortunately, all the animals in our '91 year class are BKD-positive. We've had cumulative mortality of about 6.8 percent since they've been transferred to us.

The fish range in size from about 80 grams to 280 grams, **Siri** said -- they're all over the map. About 10 percent look to be phenotypic jacks. The highest mortality we experience with this fish is from Saproligna, the freshwater fungus. 95 percent of those mortalities were precocious males.

Siri described current lab conditions for the fish: tank dimensions, water temperature, densities etc. He described some of the behavioral/environmental innovations he and his colleagues had introduced to the tanks to more closely mimic these wild fish's natural environment. For one thing, he said, we wanted to provide some behavioral options that would allow the fish to remove themselves from the stream and park themselves in a backwater. They have installed some small substrate areas with plates to block the flow of water, so that the fish can rest just as they would do in the wild. We think this will help in a couple of ways, Siri said. It will allow the smaller fish to escape the real aggressive bruisers, and it should also allow the animals to convert their feed more efficiently.

In terms of a temperature regime, said **Siri**, the fish are being held at a range of temperatures, from 9 to 13 degrees. We have some ideas about providing some slight environmental stresses to the animals, to allow them to develop the same type of natural resiliency they would have to find in the wild. He said University of California smolt quality researchers **Howard Bern** and **Richard Ishioka** have done some very interesting work on the physiological response of smolts to static water like that found in hatcheries and at the bases of dams. In natural situations, **Siri** said, their counterparts are better-prepared to deal with smoltification. What we want to do is to provide an upper and lower window of temperature variation.

But they're extremely delicate animals, and we're still not quite sure how we'll deal with mature animals, **Siri** said. It took the Fish & Wildlife Service two years before they had any successful spawning at all, because of the difficulty in keeping the adults alive. Smoltification will be done in three parts per thousand seawater per day for roughly 10 to 14 days. Then we'll be moving seawater-adapted animals down to Steinhart Aquarium in about three weeks.

The question of diet is interesting, he said. We would like to feed krill; however, our Fish & Wildlife Service colleagues feel that if we take at least the '91 animals off their diet (Biodiet Brood), that we would have a difficult time getting them to take their medicated feed for the bacterial kidney disease. We're medicating them four times a year, with 28-day applications of Gallimycin. It seems to be working quite well, he added; our mortalities dropped to three fish last month.

They're in a completely-enclosed environment, with the exception of a meter-wide strip along the east side of the building, **Siri** continued. That's for incidental light and photoperiod. We wanted to wait until the increasing photoperiod before we smolted the '91 animals, because we think they need those cues.

Why did you want to switch the fish over to krill, and eliminate the commercial diet? asked **Ron Hardy.** We didn't want to eliminate it -- we just wanted to supplement it, **Siri** replied. Just one comment, said **Johnson,** and I think we all understand this: the biological requirements of the animal have nothing to do with the permitting process (general laughter).

So far most of our discussion has centered on the needs of saltwater fish going into fresh water to spawn, said **Groves.** I'd like to hear from Bill Townsend, who has been growing Atlantic salmon in fresh water, whether he has any suggestions about the management problems you may run into with your sockeye broodstock, Keith. Are there any particular management or nutrition conditions that you can anticipate for them, **Bill**?

Well, we've set up a system that seems to work pretty well, **Townsend** said. We're feeding a prepared salmon broodstock diet. We raise them to around five pounds in 55 to 58-degree water. I thought we should wait until the fish were just about ready to spawn before moving them from the hot water, he said, but it doesn't seem to make that much difference. This year we moved some three months prior to spawning, and some we moved after they were actually ripe. We still got around 80 percent eye-up from those eggs. We're getting 4-5,000 eggs from those five-pound fish. Virtually all of them spawn at three years at that temperature; if we raise them in 48-degree water, we don't get any of them to spawn at three. That might be one way for Keith to move his back. And again, this is strictly freshwater rearing.

What do you think we should be doing? asked **Johnson.** Maybe you can give us a critique. You mean about preparing your broodstock to spawn? **Townsend** asked. Well, the first thing that came to mind was, you'll never get away with that warm water, but I didn't know you could move them. That's probably your number one thing – getting those fish out of there. Other than getting the temperature down, just give them lots of room and a good feed.
You might consider hormones, as well.

**Johnson** described the hormone implant treatments used on "Lonely Larry" this year, saying that they had been very successful in terms of increasing milt volume for cryopreservation. To me it looked a lot more dilute, so I'm not sure we wound up with a net increase in spermatozoa, he said.

Keith, when you called me, I did a fairly extensive search of the literature on brood fish nutrition, said **Winfree.** As I've been sitting here listening, I've been deliberately holding back, because I wanted to hear what the people in commercial production had to say. And I have to say there are a lot of similarities as far as brood fish nutrition. Obviously, nutrition is vital to successful reproduction. Feed composition is as important as water quality or temperature, in my experience. As we've heard today, rapid juvenile growth does accelerate maturation, and increases the percentage of maturing fish for both males and females. Maturation appears to be triggered by body nutrient stores such as lipid content or growth rate, six to 12 months before spawning. We have a tendency to think that nutrition is more important the closer the fish is to breeding; that may be true, but there is dispute as to how important those nutrients are that close to breeding.

There seems to be little good data about whether or not to discontinue feeding just before spawning for captive salmonids, **Winfree** continued. Sockeye will continue feeding voraciously right up to spawning, as long as feed is offered. There's a possibility that continued feeding may help reduce pre-spawning mortality. Feeding to excess, of course, is not recommended; by the same token, deliberately restricting feeding rate has been shown to have some pretty severe effects. Formula feeds do differ in their ability to prepare fish for breeding. In the past, OMP feed and its variations have been considered better than some other commercial diets. However, as a nutritionist, I find it very difficult to make meaningful interpretations of differences between closed-formula diets. Some companies have consistently good reputations; others don't. The closed-formula diets can change from year-to-year. Frozen supplements like krill or squid would bring you closer to the sockeye's natural diet, if that's what you're trying to do. 75 percent of the adult sockeye diet is crustaceans; large amounts of fish aren't typical of the sockeye diet.

Results from feeding krill vary a lot, **Winfree** said. Some people have had no results, but I've never read a study that said feeding krill was disadvantageous for broodstock. The studies I've read have either said it had no effect, or that it was valuable. If you're concerned about disease organisms in krill, even frozen feeds can be disinfected by using radiation. High levels of dietary ash, any amount vegetable oil, and high levels of animal fats do cause problems in breeder diets. Most problems with larval mortality seem to be caused by lipid composition. Lipid is a very broad group of nutrients, but basically, vegetable oils are bad; fish oils are good. The Japanese have found that krill oil and squid oil are both superior to fish oils for breeder diets.

The papers I've looked at have said that the vitamin and mineral premix specifications in U.S. fish and wildlife production diets seem to be adequate for breeders, he continued. He reiterated the point that stabilized vitamin C and vitamin E are both essential to a healthy diet. High levels of vitamins A and D, on the other hand, have been correlated with problems with salmon fry mortality.

I also think, personally, that it's a good idea to assay your feed periodically to see what *you're* actually getting, **Winfree** said. Frozen storage never hurts, even for shelf-stable feeds. Protein doesn't seem to be as critical for breeders as it would be in a starter diet. And there's a great deal of disagreement about what level of protein is needed **in** the breeder diet -- probably somewhere between 35 and 45 percent protein and 12 to 18 percent fat. The androgenic hormones we were talking about --

testosterone etc.     if they're present in fish meal, they may skew the se:: ratio toward more males. It may also stimulate precocious development in males.

I just have one more primary comment, **Winfree** said. No matter what you think is absolutely the best thing to do, don't stick just to that, because you'll be wrong. Always have more than one treatment, and divide your fish.

Next to speak was **Carlin McAuley,** who described his experiences with coho broodstock while working at Domsea. He said that attempts at seawater rearing had proved disastrous, and that eventually Domsea switched entirely to constant-temperature freshwater rearing. The fish had continued to feed right up to spawning. A group of fish were separated out and not fed for a period prior to spawning; **McAuley** said there was no discernable difference between the two groups.

**Ron Hardy** was up next, expressing a pair of dietary concerns. First, he said, I think I would zap up your natural astaxanthin, if that's possible, or see if you can get a variance and give them some artificial. I don't know for sure how essential it is, but it may have some effect on male spawning, over and above its effects on the eggs. We know that your current levels of canthaxanthin are sufficient to get good quality eggs. The only other thing is, since these are wild fish, there is a connection between stress and the levels of certain water-soluble vitamins. We've checked this in the Domsea coho by feeding higher levels of vitamins to certain groups. Now, we could never really find significant differences in gamete quality or survival, but we did see slightly higher numbers of eggs and slightly higher survival tin the fish getting extra vitamins]. From what we could tell, **Hardy** said, it seems to be especially critical about six months prior to spawning, during the period when proteins are being mobilized for egg production.

**Hardy** said he had some experiments planned with NMFS's coho, which, a year older than **Johnson's** Redfish Lake sockeye, should spawn this fall. One of the experiments he wants to try, he said, will involve feeding Antarctic krill to at least one group. We've got a whole bunch of krill, plus some powdered.

Here's a question for you, **Keith** -- how are you going to get those smolts to the ocean? asked **Townsend.** That's why we've got these BPA guys here, **Johnson** replied. Well, are you considering trucking them, or are you really going to put them into the river? **Townsend** asked. Well, that brings up something that makes me awfully nervous, said **Bouck.** When we drafted that biological plan last year, it was best guess. And we said the juveniles that come from these fish will go back into the lake to outmigrate.

Now, if I was king of the world, I would have at least four treatments, **Bouck** said. I realize that these fish have been taken to two separate locations, to assure against mechanical failure, but biologically they're on the same program, aren't they? So if the food is a problem, it will be a problem for both groups.

The other thing is, said **Houck,** the a ility to recognize when they're ready to spawn has been a problem. It's not nice to fool mother nature. Again, if I were king of the world, I think I might divide **Keith's** fish up into four groups, in a sort of two-by-two arrangement. One would be on whatever it's on, nutritionally, while the other would be on something else. Personally, I think I ' d go for some form of sterilized natural food.

The other thing is, said **Bouck,** I'd have those as replicates, and I'd take half those fish as adults and put them back into Redfish Lake and let them spawn on their own. I would mark them -- try to build in some technology so you could track them. I think we need to know more about exactly where the spawning grounds are, and where the residuals are spawning, and protect them. But it seems to me that the two critical areas that need to be diversified are nutrition and spawning.

Other meeting participants reiterated points made earlier: the importance of vitamins C and E, of micronutrient, of astaxanthin for color, of not putting all the eggs in one basket. **Dave Erdahl** cautioned against the earlier argument that the sockeye should be screened off from human contact as much as possible. You're going to have to spawn those fish eventually, he said, and you don't want them to totally freak out when you go to handle them And while we're on the subject, he added, have you given any thought to exactly how you'll spawn them?

Well, each fish is PIT-tagged, **Johnson** said. Through otolith microchemistry and other data, we know that 60 percent of the outmigrants have a female parent of resident origin. Somewhere around 30 percent had an anadromous origin. My hope is that we'll get a good natural spawning window, and be able to use that six or seven-week difference in spawn timing to be able to discriminate between sockeye and kokanee. We don't want to mix stocks, because their progeny would no longer be a useful stock for ESA purposes.

As far as the mechanics of the process, **Johnson** continued, we're planning to subdivide based on a spawning matrix recommended by Ni'FS. We'll subdivide the eggs from each female by two or three, and to use available males of the same biotype to spawn with them. Then everything will be held as separate sub-families, until we get the data back from the geneticists, the pathologists and the microchemical analysts, so that when we pool the groups, we pool them like to like. He said his team was currently developing a bar-code based computer program to track each individually PIT-tagged fish, so that he can pinpoint the origins of individual fish.

The discussion turned to the potential for problems stemming from the sockeye's naturally aggressive spawning-time behavior. **Groves** suggested "salmon condos," individual PVC pipe sections to hold male salmon. Otherwise, he said, they tend to get awfully banged up.

**Bob Esselman** suggested that the people who are actually doing the feeding may notice a decrease in appetite somewhere around three or four weeks prior to spawning -- that might give you a clue that it's time to do some sorting, he said. Others, however, said they had seen no observable loss of appetite, and that their fish had continued to feed voraciously right up to the time they spawned.

With no further comments forthcoming, **Johnson** thanked all for coming, and for contributing to a very productive session. With that, the workshop was adjourned.

Appendix 5. An evaluation of the ability of selected cryopreserved milt samples was performed by both laboratories. Kokanee gametes were obtained from the Idaho Department of Fish and Game spawning at Granite Creek, Lake Pend Orielle and transported on ice to Dr. Joe Cloud's laboratory at the University of Idaho and Dr. Gary Thorgaard's laboratory at Washington State University.

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TO: Keith Johnson, Idaho Department of Fish and Game
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FROM: Gary Thorgaard Gary

DATE: July 6, 1993

SUBJECT: Fertilization Trials with Cryopreserved 1992 O. nerka sperm

The current inventory and fertilization trial results with O. nerka sperm cryopreserved at WSU in 1992 are provided below. This is in addition to the 1991 results provided to you earlier. We did not conduct any additional trials with the 1991 sperm. Note that we did not have any success in our trials with Sockeye D, "Lonely Larry". Not all samples were tested (indicated below by NT) and it is possible that some will still be successful. In particular, the 10/13 and 10/27 samples had excellent motility and were not tested (althought the 10/13 sample has only two straws). We did, however, have relatively good success with cryopreserved sperm from the three beach spawners.

| Sample/ Date | # Straws Cryopreserved | % Motility Pre-freeze | % Fert. with Cryo. Sperm [1] |
|---|---|---|---|
| Sockeye D | 2 | 90 | NT |
| Sockeye 10/1 | 17 | 80 | 0 (0/12 |
| Sockeye 10/1 | 12 | 15 | NT |
| Sockeye 10/1 | 7 | 30 | NT |
| Sockeye D 10/2 | 7 | 0 | NT |
| Sockeye D 10/2 | 12 | 50 | NT |
| Sockeye 10/2 | 8 | 0 | NT |
| Sockeye D 10/2 | 20 | 85 | 0 (0/193 |
| Sockeye D 10/2 | 24 | 85 | NT |
| Sockeye D 10/2 | 21 | 30 | NT |
| Sockeye D 10/2 | 35 | 50 | 0 |
| Sockeye D 10/2 | 4 | (from testis) | NT |
| Three. Beach Spawners | | | |
| D 11/6 | 6 | 95 | 46 |
| D 11/9 | 7 | 70 | NT |
| E 11/6 | 4 | 95 | 44 |
| E 11/9 | 7 | 70 | NT |
| F 11/6 | 4 | 95 | 34 |
| F 11/9 | 1 | 95 | NT |
| F 11/13 | 1 | 95 | NT |

[1]Note: Control fertilization with non-cryopreserved kokanee sperm gave 83% fertilization (110/132)