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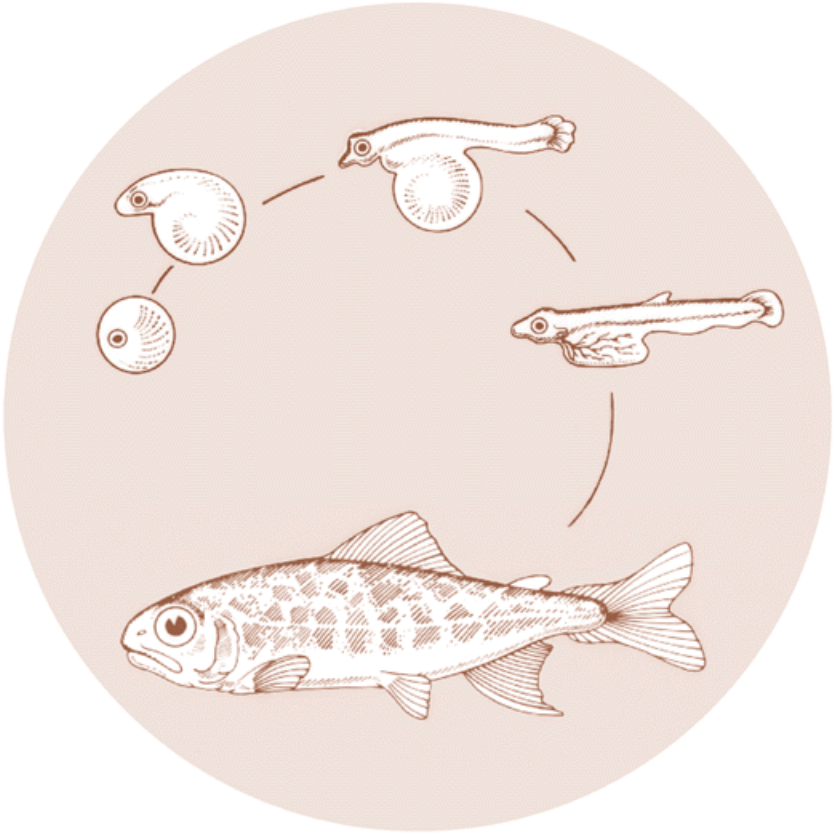
ELISA - BASED SEGREGATION OF ADULT SPRING CHINOOK SALMON FOR CONTROL OF BACTERIAL KIDNEY DISEASE

Annual Report 1991



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ELISA-BASED SEGREGATION OF ADULT SPRING CHINOOK SALMON FOR CONTROL OF BACTERIAL KIDNEY DISEASE

ANNUAL REPORT 1991

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ABSTRACT

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum (RS),* is a serious disease of salmonid fish worldwide. The disease has a major impact on spring chinook salmon populations in the Columbia River system. There is strong evidence that RS can be transmitted from parent to progeny, and segregation of progeny based on levels of antigen detected in adult fish may obviate this mode of transmission.

Results are presented from the third year of a four year study to investigate segregation of broodstock as a tool for controlling BKD. Segregation of adult fish infected with RS has been achieved using enzyme-linked immunosorbent assays (ELISAs) optimized in the first and second year of this project. Gametes from both 1990 and 1991 broodstock, either injected with erythromycin or receiving no antibiotic injection were successfully segregated into groups having either high or low levels of the RS soluble antigen. Offspring have been monitored every three months from the 1990 broodstock and are being monitored from the 1991 broodstock. Antigen levels in the offspring from the 1990 segregation experiment at Marion Forks Hatchery were low and clinical BKD was not observed in any of the juvenile fish. At Carson National Fish Hatchery, antigen levels were also low in fish which were sampled December 1990 through July 1991. Total mortality was low throughout these sampling periods. An increase in mortality was observed in November-December 1991, and preliminary evidence suggests that motality may have been due BKD. The epizootic appears to have equally effected both offspring from high and low RS antigen level parents. Antigen levels in moribund fish are being examined to confirm the prevalence of RS infection.

INTRODUCTION

Bacterial kidney disease (BKD) is responsible for major losses of intensively cultured salmon and trout *(Oncorhynchus* spp.) in the Pacific Northwest and worldwide (Bullock and Herman, 1988; Fryer and Sanders, 1981). The disease is caused by a fastidious, gram-positive bacterium, *Renibacterium salmoninarum* (RS), that produces a chronic, systemic infection. Salmonids are susceptible at all life stages in both freshwater and marine environments (Banner et al., 1986). Vaccination has not been effective in controlling the disease and current treatment methods rely on antibiotic therapy, principally erythromycin, and other management strategies (Elliott et al., 1989). Control is complicated by the survival of RS within host cells and within unfertilized and fertilized eggs (Young and Chapman, 1978; Evelyn et al., 1984). Intracellular survival and the presence of bacteria within the egg are viewed as contributing to bacterial persistence and vertical transmission, respectively.

Recently, segregation of infected gametes has been shown to be an effective means of reducing mortality due to BKD during hatchery rearing (Pascho et al., 1991). This report presents the results of the work completed during the third year of a four year study evaluating the use of enzyme-linked immunosorbent assay (ELISA) as a procedure for the segregation of spring chinook (*Oncorhynchus tshawytscha*) broodstock. Segregation experiments were carried out at two separate hatcheries: Marion Forks Hatchery in Oregon, and at Carson National Fish Hatchery in Washington. A schematic of the experimental design is presented in Figure 1. Briefly, the project was designed to identify and segregate gametes from *R. salmoninarum* infected and uninfected fish using enzyme-linked immunosorbent assays (ELISAs). Additionally, adults injected with erythromycin were examined to investigate the effect of antibiotic therapy on ELISA-based

segregation of progeny. A first year of segregation was initiated with 1990 broodstock. Each group resulting from the segregations have been reared separately and the presence of antigen and clinical disease have been monitored throughout the fish's development. The major portion of this year's work was devoted to determining the levels of *R. salmoninarum* soluble antigen present in offspring from the 1990 broodstock. Smolts from Carson Hatchery were tagged prior to release for identification of the effects of segregation on ocean survival and return rate. A second year of segregation has been started at Marion Forks and Carson Hatchery with the 1991 broodstock.

The ELISA systems used for segregating the adults incorporate specific antibodies to detect *R. salmoninarum* antigens in tissues of infected fish. Soluble proteins produced by *R. salmoninarum* during infection have been used for many years as dependable diagnostic markers for the presence of the bacteria in diseased fish (Chen et al., 1974; Bullock et al., 1980). The principal soluble antigens produced by the bacterium are a 57 kilodalton protein (p57) and its breakdown products (Wiens and Kaattari 1989; Rockey et al., 1991). The segregation experiment at Marion Forks Hatchery was based on the use of a monoclonal antibody based ELISA (monoclonal ELISA; Rockey et al., 1991) designed specifically to detect p57. The segregation at Carson hatchery utilized a polyclonal antibody based ELISA (polyclonal ELISA), modified from the protocol described by Pascho and Mulcahy (1987). In addition to the segregation experiments, this report describes further use of a confirmatory Western blot technique and a ELISA system which can be used in the field.

This research has been a joint project between the laboratories of Dr. S. L. Kaattari of the Department of Microbiology at Oregon State University and the laboratory of Dr. J. R. Winton at the National Fisheries Research Center in Seattle, WA.

FLOW CHART FOR THE SEGREGATION EXPERIMENTS

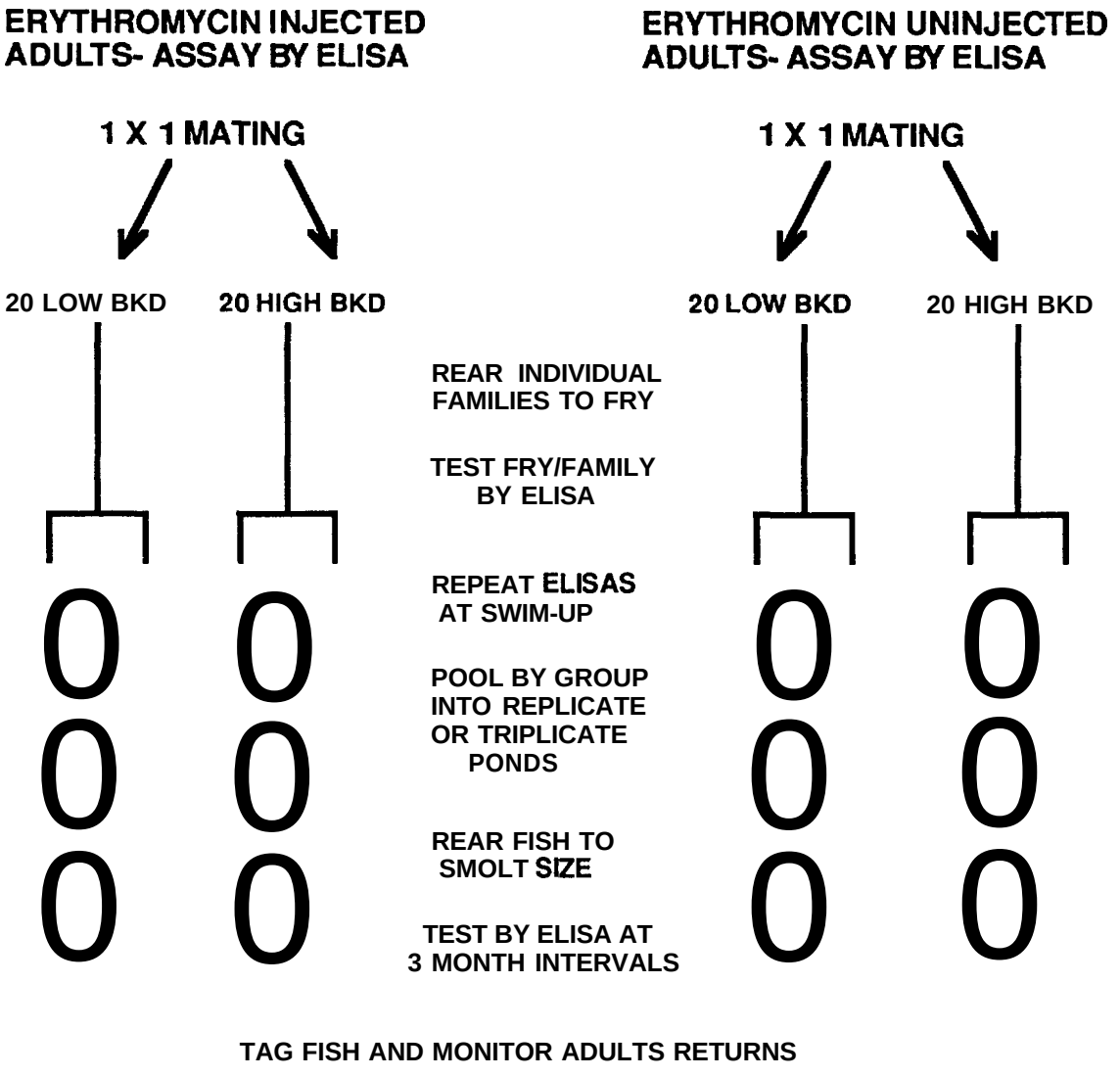

Figure 1. Schematic outline of the segregation experiments being conducted in this study. (Matings varied from 20-26 to accommodate hatchery production requirements.)

MATERIALS AND METHODS

I. Development and Standardization of ELISAs and Western Blot Procedures

Comparison of the Monoclonal and Polyclonal ELISA. In order to independently cross-check the polyclonal ELISA and monoclonal ELISAs, 55 adult chinook kidneys were tested at NFRC and OSU according to standard protocols described in Winton et al., (1990)

Use of a Sensitive Western Blot Procedure for the Confirmation of ELISA Positive Tissue Samples. Kidney tissues were collected from spawning chinook salmon during the course of the segregation experiments at Marion Forks Hatchery. Concentrations of p57 were determined using the monoclonal ELISA. Polyacrylamide gel electrophoresis and Western blot analysis of the kidney samples were conducted using tissues stored at -200C. Briefly, samples were diluted 1 :1 (v:v) with sample buffer, boiled for 3 min, and insoluble precipitates were removed by centrifugation at 15,000 x g for 0.5 min. A total volume of 7 µl of sample was electrophoresed at 1 OOV on 10% sodium dodecyl sulfate-polyacrylamide gels. Proteins were electroblotted onto PVDF membranes (Millipore Corp, Bedford, MA) for 1 h with 100V, and nonspecific sites were blocked with 5% nonfat dry milk-0.1% Tween 20-Tris buffered saline (T-TBS) for 1 h at 37°C. Blots were washed for 15 min and probed with 1 mg ml^{-1} of 3 monoclonal antibodies 4D3, 3H1, and IA1 (Wiens & Kaattari, 1991) diluted in 1% bovine serum albumin (BSA)-T-TBS. Excess primary antibody was removed by rapidly washing twice with T-TBS, followed by one wash for 15 min and one for 5 min. A secondary sheep anti-mouse horshradish-peroxidase conjugate (Amersham Corp., Arlington Heights, IL) was diluted 1 :1500 in 1% BSA-T-TBS and used to probe the blots. The secondary antisera was preabsorbed with 0.08% chinook salmon kidney tissue

supernatant (previously tested low by monoclonal ELISA) to reduce background staining. After 45 min, blots were washed followed by a final rinse in Tris buffered saline. Enhanced chemiluminescent substrate was applied and Hyperfilm was exposed for I-IO min, according to the manufacture's directions (Amersham Corp.). Blots which were to be exposed for longer than 10 min were washed for an additional 1 h to reduce background luminescence.

Development of a Rapid, Field ELISA. The protocol for this assay is outlined in Appendix 1. Briefly, a cotton swab was inserted into the kidney of the fish in the manner used for fluorescent antibody technique (FAT). Swabs were placed in 5 ml polystyrene tubes which had been precoated with 500 µl of 3.2 µg ml^{-1} MAb 4D3 in PBS. After the 30 min incubation, swabs were removed, tubes were washed, and 500 µl of a 1 µg ml^{-1} biotinylated 3H1 second antibody applied for 30 min. Excess second antibody was subsequently removed and 500 µl of a 1:200 dilution of streptavidin-conjugated horseradish peroxidase (SA-HRPO) was added for 30 min. Substrate was added and incubated for 10 min. Positive samples were identified by visual comparison to a reference standard. Chinook salmon kidney and ovarian fluid samples from females used in the 1991 segregation experiment at Marion Forks Hatchery were used to further test the sensitivity and specificity of the Field ELISA.

II. Selection and Modification of Hatcheries for Segregation Experiments

Installation and Maintenance of Individual Rearing Units; Pooling of Experimental Groups.

In order to examine the transmission of BKD from adults to progeny, a subsample of the progeny from each individual mating pair at Carson Hatchery was maintained as an individual group. From the 1990 brood, a sub-sample of approximately 250 eggs were removed from each lot and

held from December, 1990 through mid-June, 1991, in an individual rearing tank. The remaining eggs were pooled into four experimental groups, along with four replicate groups. All fish were held indoors on spring water at 7°-8° C. Pooled groups were tagged in late April, 1991 and transferred to outdoor ponds having the same water supply as their indoor rearing tanks. The setup and specifications for the individual rearing units were outlined in Winton et al. (1990).

Individual tanks were cleaned daily to remove unused food and collect dead fish. Numbers of moribund fish from each of the tanks, excluding developmentally defective individuals, were low and no outbreaks of disease or parasitism were observed. Samples collected periodically for testing by polyclonal ELISA were taken to obviate cross-contamination among the tanks, as were the daily maintenance activities.

At Marion Forks Hatchery, 80 individual rearing units were installed. All progeny from each adult cross were placed in separate 5 gallon buckets where the yolk-sac fry remained until first feeding. Subsequently, families from each treatment group were randomly distributed into one of three triplicate ponds.

III. Determination of the Extent of Vertical Transmission of RS from Adult to Progeny

Protocol for Processing Samples to Determine Antigen Levels In 1990 Offspring from Marion Forks Hatchery. The segregation of the 1990 broodstock at both Marion Forks and Carson Hatcheries, and the procedures for processing and ELISA analysis of antigen levels of eggs from the 1990 broodstock has been reported in Winton et al. (1990). The sampling schedule and numbers of samples processed at each time point for Marion Forks Hatchery are listed in Table 1. After the swim up, fry were sampled from each bucket and each treatment group was randomly assigned to one of three triplicate outdoor raceways, except for the

injected high group, for which there was only enough fish for two replicate raceways. Fish were sampled every three months after ponding.

Table 1. Sampling schedule at Marion Forks Hatchery.

1990 Broodstock

Date	Event	# Sampled
Sept. 90	Spawn #1	440 Adults
Oct. 90	Shocked Eggs	NS[1]
Nov. 90	Sampled Eggs	60/bucket
Jan. 91	Picked Dead Eggs	NS
March 91	Sampled Swim-up Fry	60/bucket
	Ponded fish	
May 91	Fed Erythromycin	
June 91	Sampled Pond Fry	60/pond
July 91	Fed Erythromycin	
Sept. 91	Sampled Parr	60/pond
Dec. 91	Sampled Smolts	60/pond
Feb. 92	Sampled Smolts	60/pond
March 92	Release S1 Smolts	
	60/pond	

1991 Broodstock

Date	Event	# Sampled
Sept. 91	Spawn #2	416 Adults
Oct. 91	Shocked Eggs	NS
Nov. 91	Sampled Eggs	30/bucket
Jan. 92	Picked Dead Eggs	NS
Feb. 92	Samoled Swim-uo Fry	60/bucket

'N.S. = not sampled

The methods for monoclonal ELISA sample preparation of swim-up fry, parr, pre-smolts and smolts are listed in Appendix 2. Sample analysis has been completed for progeny from the 1990 brood year, while analysis of the 1991 brood year is underway. Juveniles at Marion Forks hatchery were treated as production stock and fed etythromycin twice: the first time for a three week regimen in May and a second time for a three week regimen in July, as per standard hatchery practice.

Collection of Samples of Fry and Fingerlings for Testing at Carson Hatchery. Samples of progeny from the individual tanks of the 1990

brood were collected periodically to test a spectrum of developmental and rearing stages. Samples of 30 fish from each tank were taken 1) in October-November, 1990 as eyed eggs: 2) in December-January, 1990-I 991 as sac fry; 3) in March, 1991; 4) in May, 1991; and 5) in June, 1991. The protocol and results from the testing of eggs by polyclonal ELISA were presented in Winton et al. (1990). Due to the small size of the fish, whole fish were homogenized and tested as sac fry. Fish collected subsequently were tested individually, with the sample consisting of the kidney combined with portions of the back and tail.

For the 1990 brood at Carson Hatchery, a sample of 30 eggs was collected from each of 99 individual tanks approximately 69 days following fertilization. Average daily thermal unit (DTU) value for these eggs was 900 and water temperature averaged approximately 7°-8°C. Handling and processing were described in last year's report (Winton et al. 1990). No eggs were sampled from the 1991 brood.

Fish in the pooled groups were sampled periodically to determine the levels of BKD. Samples of 60 fish were collected 1) in December-January, 1990-1991 as sac fry; 2) in April, 1991; 3) in July, 1991; and 4) October, 1991. Two additional samples will be taken before the fish are released in April, 1992. Samples taken through July, 1991 have been thus far been tested.

To obtain a production density of 40,000 fish per raceway at Carson Hatchery, the progeny of the experimental fish for the 1990 brood were pooled. To achieve this density, the eggs from 12-13 females were needed in each of the experimental groups. Eggs were held separately from fertilization through eye-up, and were combined at the time of shocking (in November). In order to obtain adequate numbers of progeny to stock experimental raceways at production levels, disparate fecundity made it necessary to select varying numbers of females to construct each group. The protocol for the collection and handling of the progeny

is as described in the above sections.

Determination of the Extent to Which BKD-ELISA Segregation is Affected by the Injection of the Adult Fish with Erythromycin. The protocol outlined in the section above applies to the collection and processing of tissues from fry and fingerlings to examine the effect of administering erythromycin to females prior to spawning.

IV. Determination of Pond Mortality and the Cumulative BKD-Caused Mortality

Total monthly mortality at both hatcheries was recorded from each pond and a subsample of mortalities were saved for ELISA analysis. Water temperature was monitored by hatchery personnel during the course of rearing.

V. Determination of BKD Infection of Tissue from Adult Spring Chinook Salmon for the 1991 Brood Stock Segregation Experiment

Distribution of Polyclonal ELISA Values Among Adult Spring Chinook Salmon at Carson Hatchery Based on Samples of Kidney Tissue. Sampling of the returning adults in 1991 followed the procedure for handling and spawning, implemented in 1990, as described in Winton et al. (1990), with the following changes noted. Adult chinook salmon were spawned at weekly intervals beginning August 5, 1991, at Carson Hatchery. The density in the holding pond was higher in 1991, and the number of times fish were handled was increased over that in 1990. Production methods currently practiced at the hatchery were adjusted to integrate this study and minimize its impact on customary practices. Fish used in this segregation experiment represented a portion of the hatchery production and were handled accordingly. Adult fish began

returning to Carson Hatchery on May 29, 1991, three weeks later than in 1990. Fish were confined in holding ponds from their arrival until spawned or discarded. Returns continued at the hatchery through spawning, which was completed on August 26.

On June 13, 1991, 910 returning adult fish were examined, uniquely tagged with colored nylon cable ties (Panduit Corp., Tinley Park, Illinois) placed on the caudal peduncle, injected with erythromycin (approximately 11 mg/kg body weight), and returned to the holding pond. A corresponding sample of 254 adult fish was tagged and did not receive the antibiotic injection. On July 18, 1991, approximately 1300 fish entering the hatchery since June 13 were tagged and injected. A second injection was administered to the surviving, previously injected adults. At this time, 250 additional fish were marked as receiving no injection. Fish returning after July 18 possessed no mark and received no injection. This procedure was done to provide a spectrum of the returning adults to be used as experimental fish.

During the month of August, fish were checked at weekly intervals for ripeness and returned to the holding pond if green, or spawned if ripe. A total of 1080 females and 773 males were spawned. Fewer males were used due to the standard practice of fertilizing multiple females with the milt of one male.

Adult fish were anesthetized, selected for spawning, and labeled with an individual number. Tag information, representing antibiotic treatment and timing of return to the hatchery, was recorded at this time.

Both males and females were killed by cerebral concussion. Females were bled by severing the caudal artery. Eggs from a single female were collected, held separately, fertilized using the milt from one male, and transported to the brood facility at the hatchery. Contamination between females was avoided by having the person spawning the female wear a new disposable glove for each fish and by

thoroughly disinfecting all common equipment with iodophor between fishes.. For each family of fertilized eggs, a sample of male kidney and female kidney was collected from the parental fish for testing by polyclonal ELISA.

In the hatchery building, the eggs were rinsed in water to remove any extraneous tissue, hardened by immersion in 75 ppm iodophor for 20 min, and placed into racks with a separate container and water supply for each individual family of eggs. Eggs from each family group were held in isolation from the time of spawning until tissues had been tested by polyclonal ELISA for the presence of antigen to RS, using the methods described in Winton et al. (1990). At this time, experimental families were selected. Tagging information for each fish provided data on antibiotic treatment and timing of return to the hatchery. Optical density data from polyclonal ELISA and information on antibiotic treatment of adults were used to apportion eggs from individual matings into four experimental groups: 1) antibiotic injection/low ELISA (OD c 0.080) (INLO; N = 24); and 2) antibiotic injection/high ELISA (OD >0.300) (INHI; N = 24); 3) no antibiotic injection/low ELISA (OD ~0.080) (UNLO); 4) no antibiotic injection/high ELISA (OD >0.400) (UNHI; N = 23). Fish were selected for the above groups based on timing of return to the hatchery: 1) May 29-June 13; 2) June 14-July 18; 3) July 19-spawning date. The polyclonal ELISA value for the female parent was dominant for assignment to low or high groups. Male polyclonal ELISA values were used only to exclude eggs from matings of low female with a high male.

Distribution of Monoclonal ELISA Values Among Adult Spring Chinook Salmon at Marion Forks Hatchery Based on Samples of Kidney Tissue. Adult chinook salmon returning to the Minto collection facility of Marion Forks Hatchery typically remain in the North Santiam River until spawning and are not injected with antibiotics. To accommodate the design of this project, approximately 450 adults were encouraged to enter the facility early and were injected once with erythromycin and oxytetracycline (100 mg if fish weighed less than 8.2 kg and 200 mg if fish weighted more than 8.2 kg). Fish were injected 9-21 days prior to spawning. Fish were spawned using delayed fertilization techniques. Eggs and milt were stored in zip-lock plastic bags supplemented with oxygen and placed in ice coolers for transport to Marion Forks Hatchery. On the same day that fish were spawned, kidney samples were removed, transported to Oregon State University, and assayed by the monoclonal ELISA, as described in Winton et al. (1990). Based on results from the monoclonal ELISA, crosses between fish were completed on the following day. Eighty crosses were made to provide families that could be distributed among four treatment groups as follows: 1) no antibiotic injection/low ELISA (N=20); 2) no antibiotic injection/high ELISA (N=20); 3) antibiotic injection/low ELISA (N=20); and 4) antibiotic injection/high ELISA (N=20). A total of 416 fish were screened to obtain the fish necessary for the 80 crosses.

VI. Tagging and Evaluation of Returns from Experimental Groups

Acquisition of Funding, Tagging, and Monitoring of Returning Fish from each of the Experimental Groups. Originally included as an optional objective of the project, discussions with various agencies and individuals indicated strong support for the tagging and analysis of returning adults as part of this study. Funds for the tagging of all experimental fish at Carson Hatchery were

provided through the Bonneville Power Administration. Each of the pooled treatment groups and each of their replicates received a uniquely coded tag lot, facilitating identification of returns from individual raceways. Progeny from the pooled groups were tagged in late April and early May, 1991, as they were being transferred from rearing tanks inside the hatchery building to their outside rearing ponds. The tagging process took approximately 1-2 days per pooled group. Stocking densities for each of the pooled groups ranged from 36,557 to 41,847 fish per pond. Funds for tagging of the experimental groups were not allocated for the 1990 brood at the Marion Forks facility.

RESULTS AND DISCUSSION

I. Development and Standardization of ELISAs and Western Blot Procedures

Comparison of the Monoclonal and Polyclonal ELISA. A total of 30 female and 25 male kidney samples were cross checked by the polyclonal and monoclonal ELISAs. Generally, there was a high correlation ($r^2 = 0.78$) between the estimated antigen levels between the two ELISAs (Figure 2). While the two assays specifically recognized Rs antigens, differences exist between the two assays. The monoclonal ELISA detects two epitopes on a specific soluble antigen 57 kDa protein (p57) while the polyclonal ELISA may recognize a number of soluble antigens produced by Rs in addition to p57. This possible differential recognition may be responsible for the variations between the two assays.

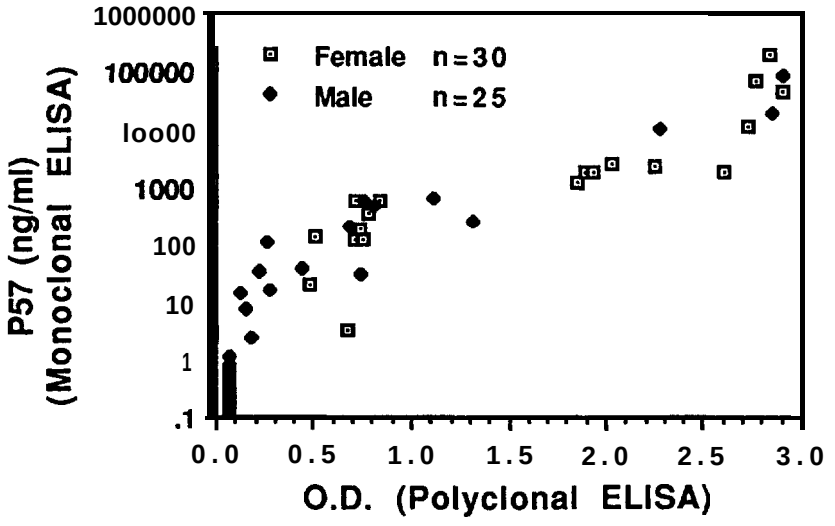

Figure 2. Comparison of 55 adult kidney samples by the polyclonal and monoclonal ELISA. Samples with antigen concentrations above 3 ng ml^{-1} are considered to be positive in the monoclonal ELISA while samples with an OD. of greater than 0.080 are considered to be positive by the polyclonal ELISA.

Use of a Sensitive Western Blot Procedure for the Confirmation of ELISA Positive Tissue Samples. Detection methods for RS have grown increasingly sensitive as new technical developments have occurred. Currently, the technique with the highest sensitivity is considered to be the ELISA (Pascho et al., 1987). In many laboratory situations, this increased sensitivity has led to the identification of lightly infected fish as positive for RS, but this cannot be confirmed by other detection techniques. Western blotting is an ideal technique for the confirmation of p57 in ELISA positive samples as both the appropriate molecular weight and antigenic identity must be coincident for a positive test (Sakai et al., 1990; Wiens et al., 1990). A limitation of the Western blot has been the lack of sensitivity (Rockey et al., 1991). As described previously (Kaattari et al. 1989), it is difficult to surpass limits of 250 ng ml^{-1} of the 57 kDa protein using standard blotting procedures. However, recent advances in detection technology have

increased the sensitivity of the Western blot. A fluorogenic peroxidase substrate was used in place of the conventional chromogenic enzyme substrate in the final step of the blotting protocol. Using a substrate which can be cleaved to produce a chemiluminescent product, the sensitivity has been increased 50-l 00x over the conventional peroxidase substrate (Winton et al., 1990). In the third year of this project we have used this technique for a more accurate and sensitive confirmation of ELISA-positive fish. The presence of p57 in adult samples was confirmed at concentrations as low as 13 ng ml^{-1} in kidney tissue and 10 ng ml^{-1} in ovarian fluid of adult chinook (Table 2). Difficulty still exists with low level detection due to the lengthy exposure times required and the subsequent increase in background luminescence.

Development of a Rapid, Field ELISA. In order to further simplify testing for RS in hatchery situations, a rapid Field ELISA was developed which requires little training and no electronic equipment. One of the major rate limiting steps in the quantitative ELISA is the physical removal of the kidney sample for processing. In the field ELISA this step was simplified by the use of a cotton swab which was inserted into the kidney in a similar manner as in preparing swabs for fluorescent antibody analysis. The swab is placed in detecting reagents and color development is compared visually to a standard positive and negative tube. This assay takes approximately two hours to complete and can be performed at the spawning facility.

Further work was done this year to test the efficacy of the Field ELISA using ovarian fluid samples obtained at Marion Forks Hatchery. Ovarian fluid from ELISA kidney-positive and kidney-negative females from the 1991 segregation experiment at Marion Forks Hatchery were tested and compared to both the quantitative ELISA and Western blot. Low levels of p57 were observed in ovarian fluid samples as compared to kidney tissues from the same fish (Table 2).

Inconsistencies in antigen levels of ovarian fluid have also been reported by Pascho et al. (1991) who were unable to detect antigen in ovarian fluid samples with bacterial counts below 1 x 10^5 ml^{-1}. The authors speculate that undefined ovarian fluid factors may influence the detection of antigen. Armstrong et al. (1989) have reported the presence of agglutinin titers to heat killed Rs as high as 1:36 in chinook salmon ovarian fluid. In a preliminary report, Griffiths & Lynch (1990) identified Rs reactive antibodies in the ovarian fluid. Assuming that the low level of antigen in our study was not due to an artifact in the collection process, one possible explanation might be that the antigen was being masked by ovarian fluid components. Western blotting was used in an attempt to identify antigen as it was assumed that the denaturing and heating treatment of the samples prior to electrophoresis would liberate any antigen non-covalently complexed to antibody or any other protein. Western blot analysis confirmed that QELISA-positive ovarian fluid samples contained p57, however, p57 was not detected in QELISA-negative ovarian fluid samples even though fish had kidney antigen concentrations greater than 10,000 ng ml^{-1}. A second possible explanation for the low quantities of p57 observed in the ovarian fluid may be the proteolysis of antigen, however, this does not appear likely as little degradation of p57 was observed in ovarian fluid or kidney samples confirming the earlier findings of Rockey et al., (1991). A third possibility may be that p57 was bound by components present on the surface of the egg. Little is known about the function of p57 in vivo or the tissue binding capacity of p57. P57 has been shown to bind fish leukocytes (Wiens & Kaattari, 1991) and spermatocytes in vitro (Daly & Stevenson, 1989). A final explanation may be that the synthesis of p57 may be down-regulated by the bacteria after contact with egg components. Regardless, these experiments suggest that the detection of antigen in ovarian fluid may be of a limited utility for the determination of systemic levels of RS p57 in the adult fish.

While this assay is less sensitive than the quantitative ELISA, the advantages of an easier sample preparation and short assay time may lend this assay as a rapid field tool for segregating salmonids. In an effort to transfer this technology to fish health workers, Oregon State University has licensed both the monoclonal antibodies and the ELISA technology to DiagXotics, Inc. Wilton, Ct. 06897. The technology is expected to be commercially available in the near future.

Table 2. Comparison of p57 concentrations in kidney tissue and ovarian fluids from spawning chinook salmon returning to Marion Forks Hatchery. Kidney tissue was collected and antigen levels determined by the quantitative ELISA (QELISA). Based on the QELISA results, ovarian fluid samples were taken the following day and assayed by the field and QELISA. The Western blot was used for the confirmation of antigen levels in samples.

Fish #	QELISA (ng ml-l&EM)	Field ELISA	Western Blot	QELISA (ng ml^{-1} ± SEM)	Field ELISA	Western Blot
44	120,350 ± 28,043	+	+	88 ± 22	+	+
27	13,938 ± 1,750	+	+	10 ± 2	-	+
47	13,215 ± 1,730	+	+	593 ± 177	+	+
12	10,123 ± 859	+	+	< 3[2]		
170	4,484 ± 737	+	+	150 ± 28	+	+
79	4,102 ± 908	+	+	< 3		
99	1,287 ± 128	n.t[1]	+	32 + 13	+	+
106	635 ± 88	n.t	+	< 3		
80	227~64	+	+	< 3		
117	187 ± 16	n.t	+	< 3		
114	63 ± 9	n.t	+	< 3		
15	60 ± 6	+	+	< 3		
91	53 ± 8	+	+	< 3		
144	51 ± 10	+	+	< 3		
97	46 ± 7	n.t	+	< 3		
142	31 ± 8	+	+	< 3		
21	23 ± 6		+	< 3		
4	19 ± 2		+	< 3		
141	13 ± 1	n.t	+	< 3		
70	4 ± 1	-	-	< 3		

[1] n.t.= not tested

[2] <3 = antigen level was below the sensitivity of the QELISA.

II. Determination of the Extent of Vertical Transmission of RS from Adult to Progeny

Analysis of Antigen Levels in Samples of 1990 Offspring from Marion Forks Hatchery. Fish were collected from each of the four treatment groups from the eyed egg stage in November, 1990, through pre-release in April, 1991. Samples of the eggs, swim-up fry , and parr were processed whole and tested individually. No significant levels of antigen were detected in the eggs, swim-up or parr stages. The kidneys of the pre-smolts, smolts and pre-release were individually tested. A low percentage were identified which had antigen levels above 3 ng ml^{-1} (Table 3). The highest percentage of positive fish (7.5%) were from the injected high group. However, positive fish were also identified in the two groups from low positive

Table 3. Percent of samples with antigen levels above 3 ng ml^{-1} homogenate in each treatment group. N is the number of individual samples tested in each treatment group.

Developmental Stage	Sample Date	Uninjected Low	Uninjected High	Injected Low	Injected High
Eggs	Nov. 90	0 (N = 1200)	0 (N = 1140)	0 (N = 1020)	0 (N = 660)
Swim-up	March 91	0 (N = 1200)	0 (N = 1140)	0 (N = 1020)	0 (N = 660)
Parr	June 91	0 (N = 180)	0 (N = 180)	0 (N = 180)	0 (N = 120)
Pre-smolts	Sept. 91	1.11 (N = 180)	0 (N = 180)	0 (N = 180)	0 (N = 180)
Smolts	Dec. 91	0.56 (N = 180)	1.11 (N = 180)	0 (N = 180)	7.5 (N = 120)
Pre-release	Feb. 92	1.11 (N = 180)	0.56 (N = 180)	.56 (N = 180)	0.85 (N = 117)
Total number >3 ng ml-1		5	3	1	10
Total number Sampled		3120	3000	2760	1797

parents. Antigen levels in all of the positive fish were between 3 and 10 ng ml^{-1}. Further confirmation of the low antigen levels is being performed by Western blot analysis. In summary, the antigen levels found were low and are not indicative of clinical disease. Clinical disease was not observed in any of the juvenile fish sampled at Marion Forks hatchery from the 1990 brood year.

Collection of Samples of Fry and Fingerlings for Testing by ELISA at Carson Hatchery. The results from the polyclonal ELISA testing of eggs from individual parents, collected in October-November, 1990, were discussed in last year's report (Winton et al. 1990). Samples of the 1990 brood were collected at various developmental and life history stages (Table 4).

Additionally, samples from the individual tanks were taken from December, 1990 through June, 1991. All fish were processed individually. ELISA data for samples collected were approximately equivalent for all groups and times of sample collection. Data from samples taken in May, 1991 and June, 1991, and thus far tested, are shown in Figures 3-4. These data are representative and were chosen as they were the latest samples tested. The data show that the levels of BKD present in the fish examined remained very low with narrow ranges.

Data from the pooled groups also showed low levels of BKD in the sac-fry (Figure 5, December, 1990). A sample taken in July, 1991 had slightly elevated means for the OD readings of all the groups (Figure 5). The range of OD values also increased from December to July, indicating spread of the disease. Table 5 presents the data for the July, 1991 sample from the pooled groups.

Table 4. Percentage of samples from individual tanks and raceway groups at Carson National Fish Hatchery with polyclonal ELISA OD level above 0.100 in each treatment group. N is the number of individual samples tested in each treatment group.

Individual Tanks

Developmental Stage	Sample Date	Uninjected Low	Uninjected High	Injected Low	Injected High
Eggs	Oct.-Nov. 90	0[1] (N = 720)	0[1] (N = 750)	0[1] N = 750)	0[1] (N = 750)
Swim-up Fry	Dec.-Jan. 90-91	2	<1	<1	2
Parr	March 91	0 (N = 720)	0 (N = 750)	0 (N = 750)	0 (N = 750)
Parr	May 91	0 (N = 720)	0 (N = 750)	0 (N = 750)	0 (N = 750)
Parr	June 91	0 (N = 720)	(N = 750)	(N = 750)	0 (N = 750)
Total Number OD >0.100		0	0	0	0
Total Number Sampled		3600	3750	3750	3750

Raceway Groups

Developmental Stage	Sample Date	Uninjected Low	Uninjected High	Injected Low	Injected High
Parr	April 91	0 (N = 120)	0 (N = 120)	0 (N = 120)	0 (N = 120)
Parr	July 91	0 (N = 120)	0 (N = 120)	0 (N = 120)	0 (N = 120)
Pre-smolt	October 91	0 (N = 120)	0 (N = 120)	0 (N = 120)	0 (N = 120
Total Number OD >0.100		0	0	0	0
Total Number Sampled		360	360	360	360

Table 5. Comparison of adult female fish (spawned August, 1990) and progeny for pooled groups (sampled July, 1991) at Carson National Fish Hatchery.

Treatment Group	Mean for Adult Fish	Mean for Pooled Progeny	Sample Number Pooled Progeny	Percentage with OD >0.100
Injection				
Low Adult OD				
I	0.069	0.099	60	17
II	0.079	0.087	58	16
High Adult OD				
I	0.485	0.085	60	15
II	0.606	0.110	60	32
No Injection				
Low Adult OD				
I	0.072	0.108	60	28
II	0.077	0.079	60	12
High Adult OD				
I	1.407	0.091	60	22
II	1.395	0.092	60	22

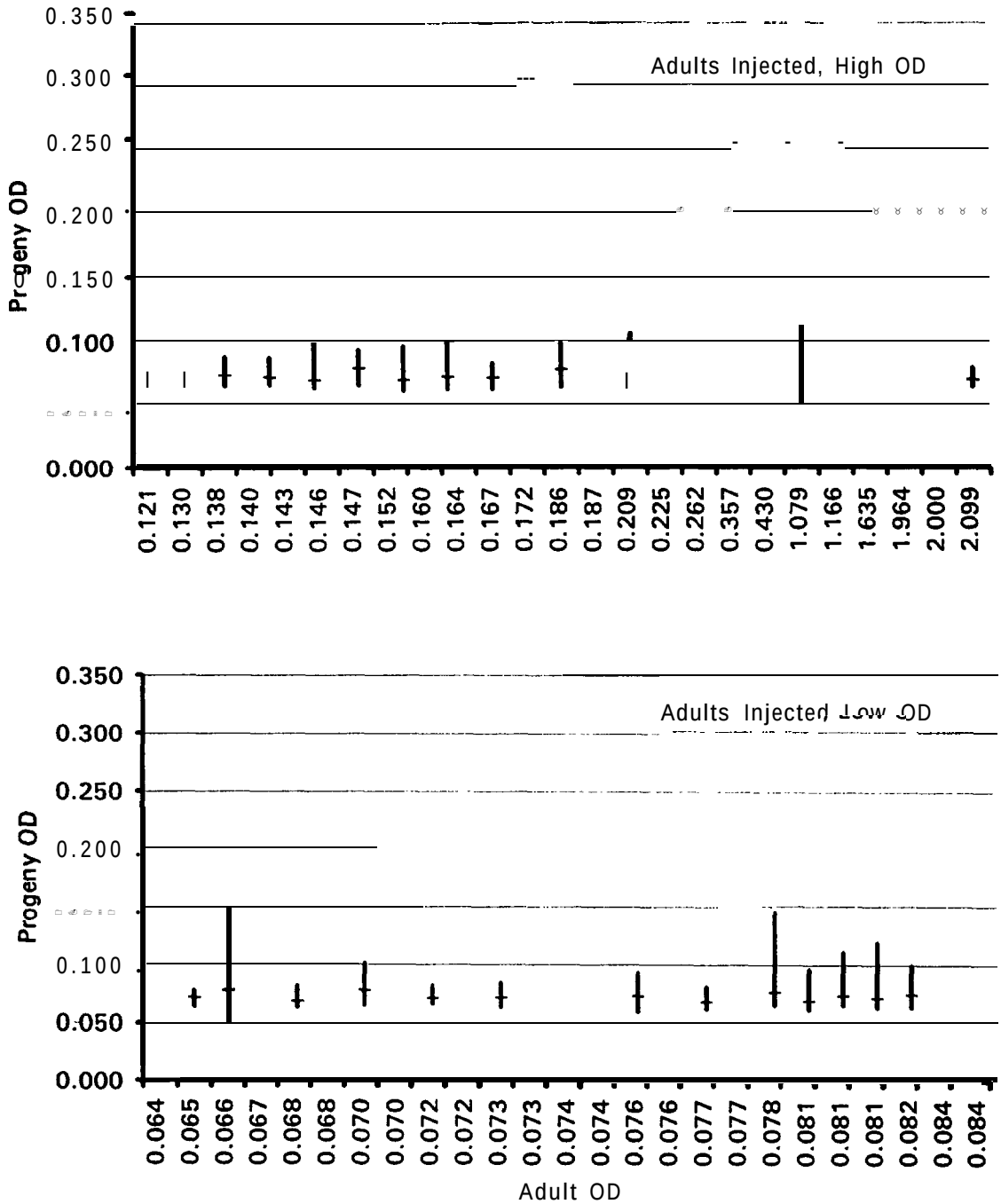

Figure 3. ELISA optical density values of samples of progeny (N = 30), collected May, 1991, from replicate treatment groups of female chinook salmon receiving pre-spawning injection(s) of erythromycin. (Vertical bars indicate range of ELISA values and dash indicates mean; missing values indicate incomplete testing or missing sample).

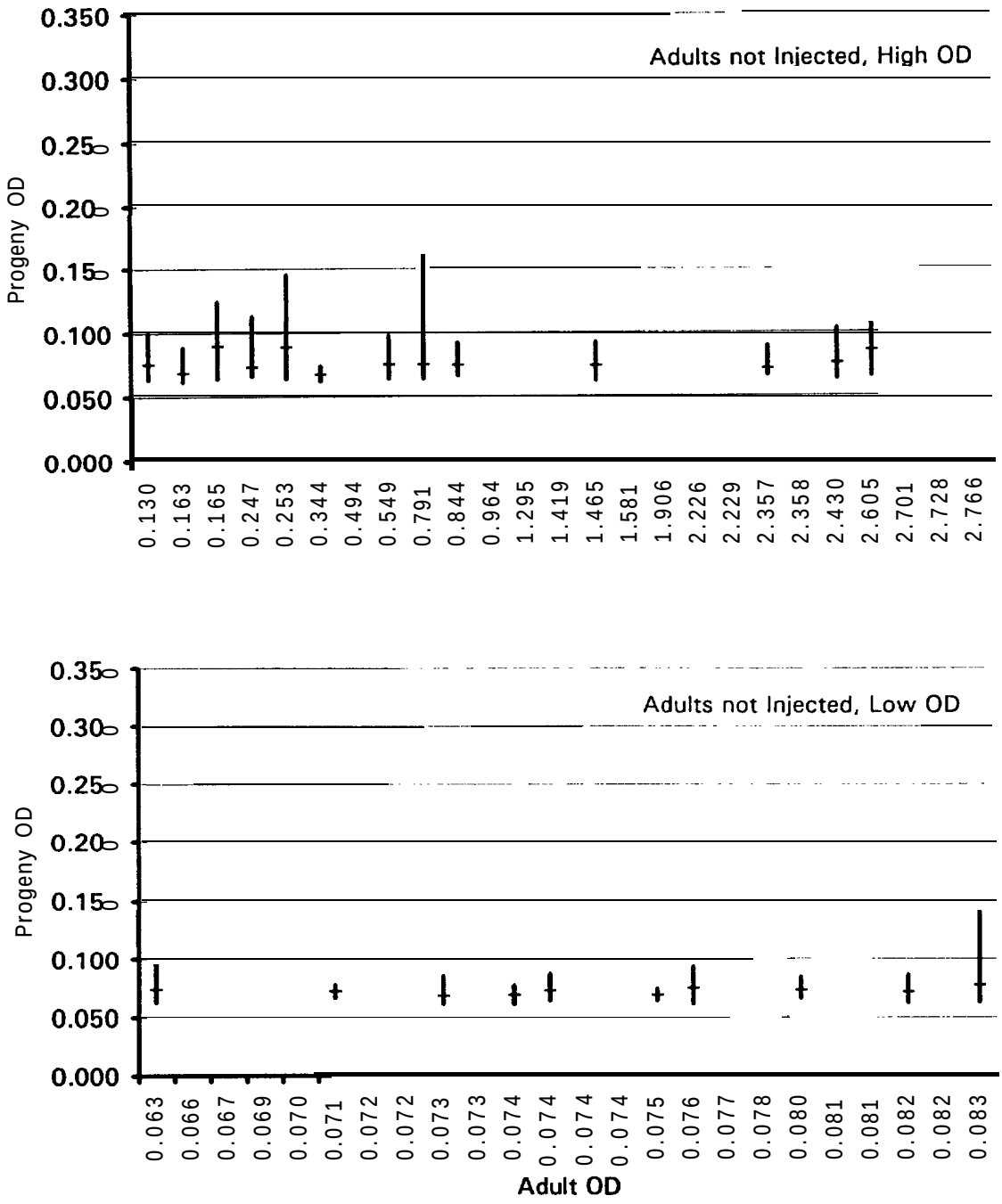

Figure 4. ELISA optical density values of samples of progeny (N = 30), collected May, 1991, from replicate treatment groups of female chinook salmon receiving no pre-spawning injection of erythromycin. (Vertical bars indicate range of ELISA values and dash indicates mean; missing values indicate incomplete testing or missing sample).

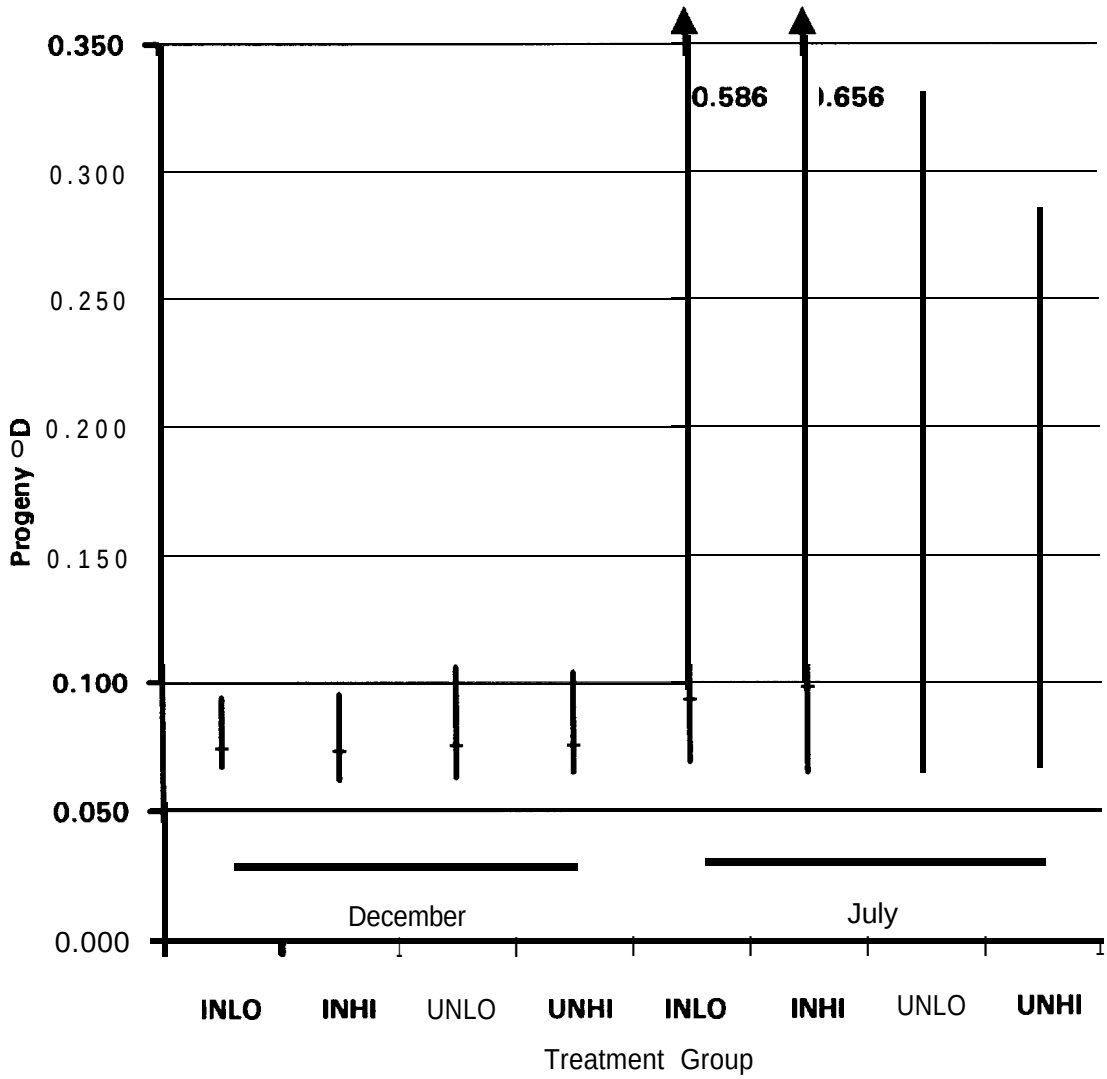

Figure 5. ELISA optical density values of samples of progeny (N = 60) tested as sac fry in December, 1990 and parr in July, 1991 from replicates of pooled groups of female chinook salmon at Carson National Fish Hatchery. (Abbreviations: INLO-adult injected with antibiotic/low ELISA (OD < 0.080); INHI-adult injected with antibiotic/high ELISA (OD > 0.300); UNLO-adult not injected with antibiotic/low ELISA (OD < 0.080); UNHI-adult not injected with antibiotic/High ELISA (OD > 0.400)).

Water temperature is an important environmental factor which influences the progression of BKD. Sanders et al. (1978) found that water temperature is inversely correlated with mean time to death of coho salmon (0. *kisutch*), sockeye salmon (0. *nerka*), and steelhead (*O. mykiss*). The low water temperature at Carson Hatchery (7-8°C) may affect the progress of the disease, keep infection levels low, and make detection more difficult.

Determination if Differences in ELISA Titers Between Adult Spring Chinook Salmon are Retained by the Progeny During Extended Rearing. Preliminary results from the pooled fish at Carson Hatchery show an increase in the OD range for all experimental groups approximately one year following fertilization (through July, 1991). The slow progress of the disease at Carson Hatchery may be related to water temperature during rearing. Losses from each of the raceways declined from May-October and began to increase in November and December (Figure 6). A subsample of daily mortality from these raceways was collected and processed for ELISA. Results from these fish will indicate if mortality during November and December is BKD related.

In the research done by Pascho et al. (1991), mortality for progeny of both low and high BKD groups remained similar until water temperature increased during the summer. Following exposure to these higher temperatures, losses of fish from both the high and low BKD group increased and peaked, returning to lower levels as the water temperature dropped in the fall. The loss in the high group remained significantly higher than in the low group. Sanders et al. (1978) mentioned that outbreaks of the disease occurred in the fall in water temperatures of 1 0°C, and reported 93-l 00% mortality for artificially infected fish held at 6.7°C, 60-71 days following infection.

The water temperature at Carson Hatchery remains at 7°-8°C during the entire rearing period and may obscure or repress any clear-cut differences in the

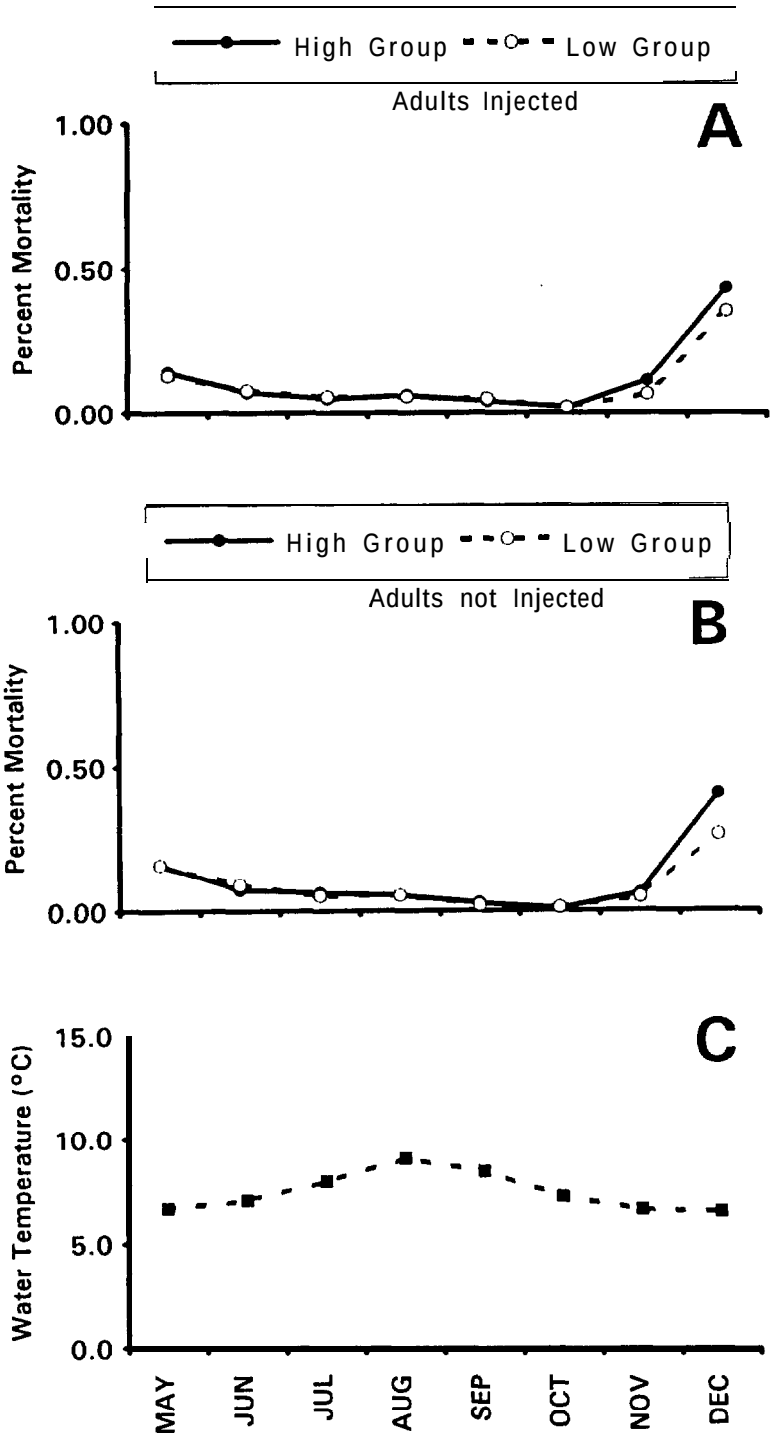

Figure 6. Percentage monthly mortality of juvenile spring chinook salmon for pooled groups, Carson National Fish Hatchery, May-December, 1991 and average monthly water temperature. A. Progeny of uninjected adults with high or low antigen levels. B. Progeny of injected adults with high or low antigen levels. C. Average monthly water temperature.

incidence of BKD infection between the experimental groups. Testing of the samples processed from later in 1991 and those collected and tested in 1992 will show if the pattern seen by Pascho et al. (1991) becomes evident with the fish from Carson Hatchery.

Determination of the Extent to Which BKD-ELISA Segregation is Affected by the Injection of the Adult Fish with Erythromycin.

Etythromycin is commonly administered in hatcheries for the control of losses due to bacterial kidney disease, as injection of pre-spawning adults and/or as medicated food administered during the rearing of the progeny (Elliott et al. 1989). Investigation on the techniques for the control of vertical transmission of bacterial kidney disease from adult to progeny have indicated that the injection of broodstock females with antibiotics prior to spawning is beneficial (Bullock and Leek 1986; Evelyn et al. 1986; Elliott et al. 1989; and Brown et al. 1990), with timing and dosage of critical importance. Concerns have been raised that the widespread use of erythromycin may result in the development of resistant strains of RS, negating the efficacy of the drug as a control measure for the disease (Evelyn et al. 1986).

Results from the experimental fish held in the individual tanks, as mentioned previously, showed little difference between groups (Figures 3-4). The pooled fish sampled and tested through July, 1991, indicate that the groups from adults that were not injected prior to spawning may be experiencing increased levels of BKD, but results are quite preliminary (see Figure 5).

III. Determination of Pond Mortality and the Cumulative BKD-Caused Mortality

Estimate of BKD mortality during rearing. At Marion Forks Hatchery, mortality was monitored in each group from June, 1991, to February, 1992 (Figure 7). Total mortality was low for all four groups at Marion Forks Hatchery. In addition, little difference in total mortality was observed between treatment groups from either the uninjected adults (Figure 7.A) or the injected adults (Figure 7.B). A subsample of all mortalities were frozen and later analyzed by the monoclonal ELISA to determine the incidence of Rs antigen positive fish (Table 6). There was no clear trend in the numbers of antigen positive mortalities per treatment groups. Two groups, uninjected high and injected low, had the highest total numbers of Rs positive fish. However, BKD was not a major source of mortality among the spring chinook salmon at MFH in the 1990 brood year.

Table 6. Percentage and number of offspring mortalities at Marion Forks Hatchery which were positive for RS antigen.

	Treatment Group			
Date	No Injection Low	No Injection High	Injected Low	Injected High
June 91	0 (N = 3)	0 (N = 4)	0 (N = 0)	0 (N = 0)
July 91	0 (N = 210	21 .o (N = 19)	0 (N = 9)	10.0 (N = 10)
Aug. 91	0 (N = 1)	0 (N = 3)	0 (N = 2)	0 (N = 5)
Sept. 91	0 (N = 2)	33.3 (N = 3)	0 (N = 4)	0 (N = 5)
Oct. 91	0 (N = 71	27.3 (N = 11)	5 (N = 20)	0 (N = 5)
Nov. 91	0 = 10) (N	0 (N = 2)	35.3 (N = 17)	0 (N = 2)
Total Number >3 ng ml-t	0	8	7	1
Total Sampled	44	52	42	27

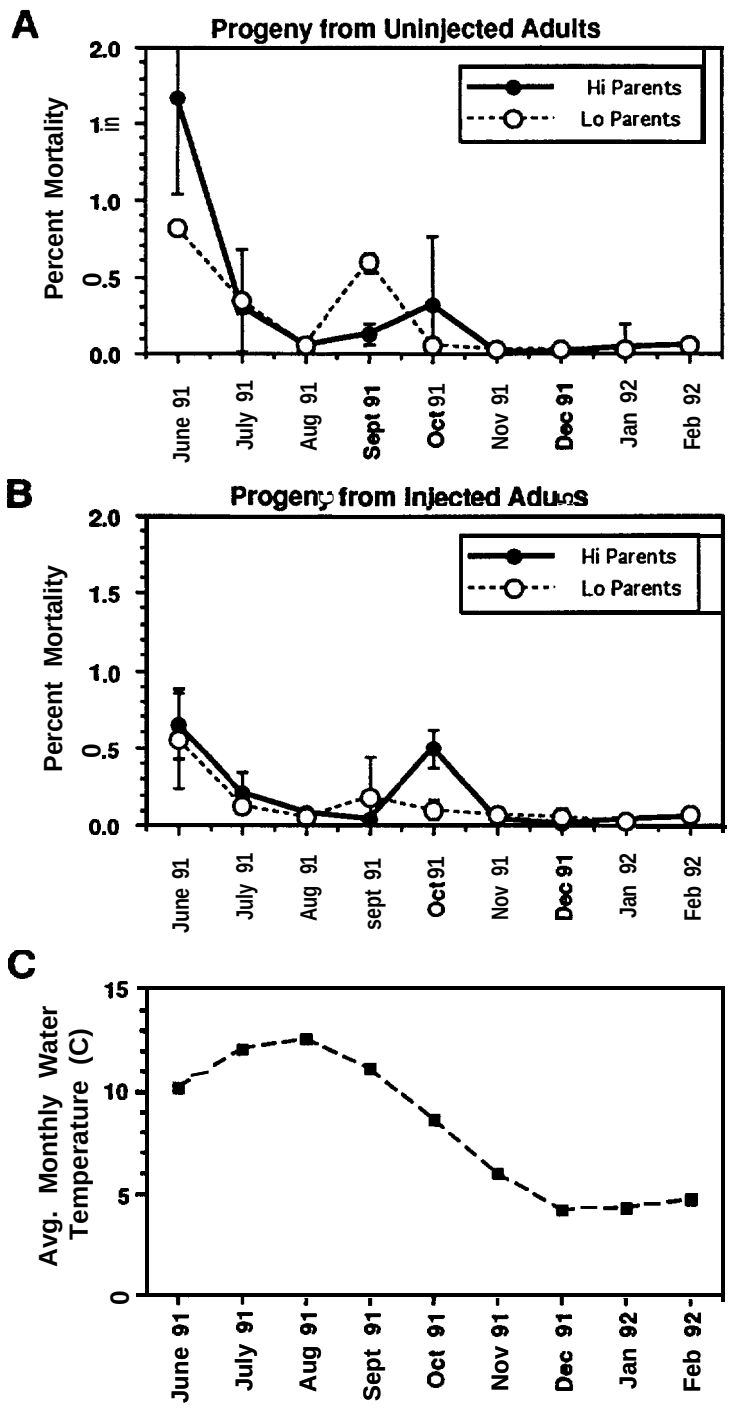

Figure 7. Percent monthly mortality of juvenile spring chinook salmon at Marion Forks Hatchery from June 1991 to Feb 1992. Mortality data were averaged between ponds for a particular treatment. A. Progeny of uninjected adults with hi or low antigen levels. B. Progeny of injected adults with hi or low antigen levels. C. Average monthly water temperature of Marion Creek.

IV. Determination of Antigen levels in Kidney Tissue Adult 1991 Spring Chinook Salmon Brood Stock

Segregation of the 1991 Spring Chinook Salmon Broodstock. A second year of segregation experiments were successfully performed at Carson National Fish Hatchery and Marion Forks Hatchery. A total of 416 adults returning to Marion Forks Hatchery were screened by ELISA (Appendix 3). The distribution of antigen levels is shown in Figure 8.

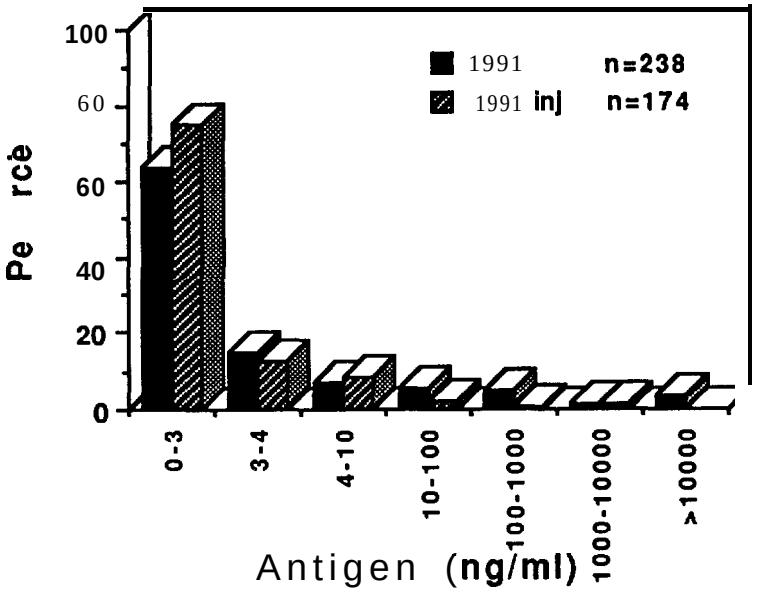

Figure 8. Comparison of the percent prevalence of antigen levels in injected and uninjected adults returning to Marion Forks Hatchery in 1991.

Adult chinook with the highest levels were crossed as described previously (Winton et al., 1990). The antigen levels of the adult crosses are indicated in Figure 9. The progeny from these crosses are currently being reared and samples have been taken as described in Table 1.

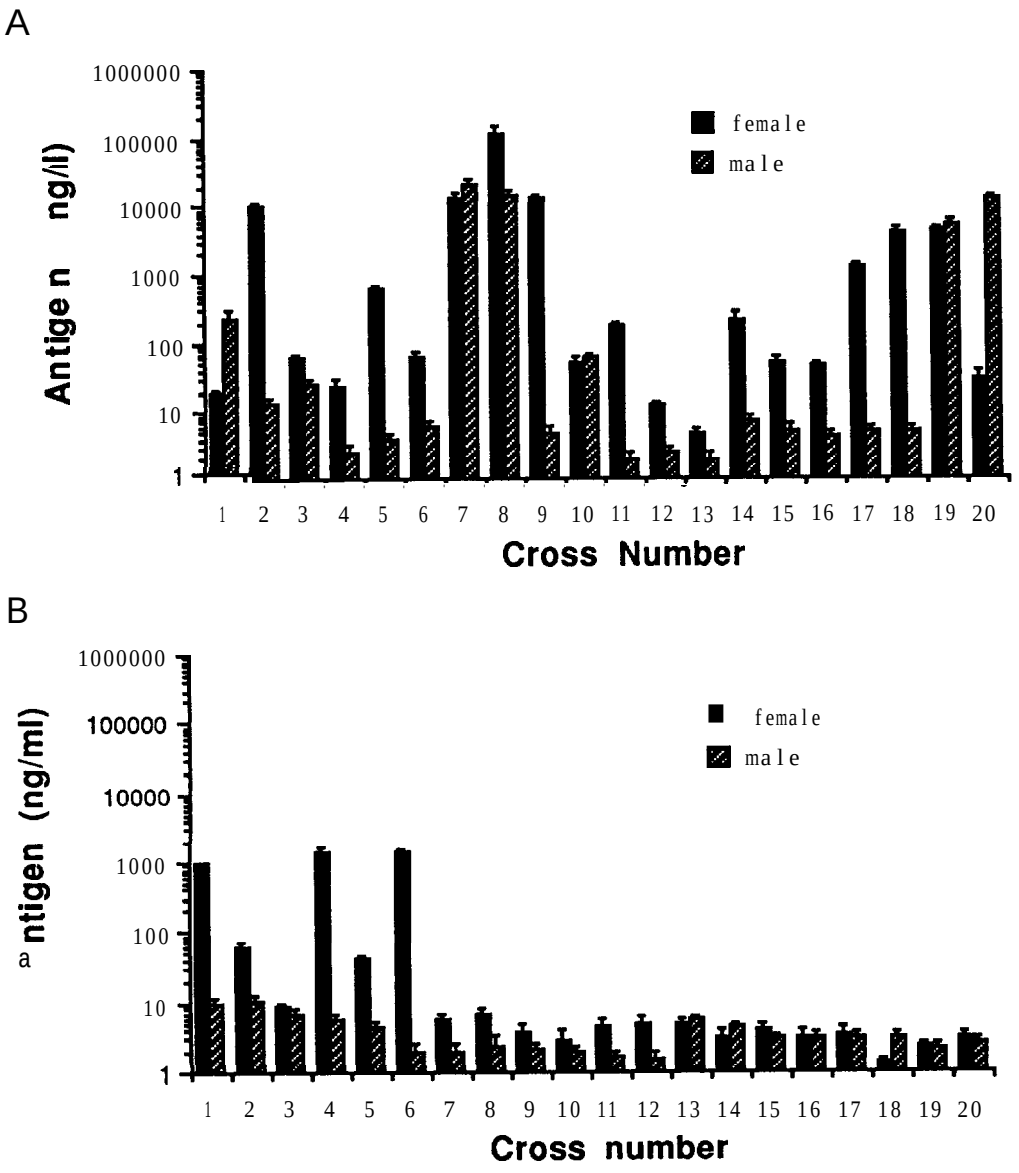

Figure 9. Levels of Rs antigen in adult chinook salmon selected for segregation at Marion Forks Hatchery determined by the monoclonal antibody-based ELISA. Gametes from each cross in the 1991 segregation experiment were incubated in individual buckets and assigned a number. A) Uninjected adults with high levels of antigen. B) Injected adults with high levels of antigen.

Distribution of ELISA Values Among Adult Spring Chinook Salmon Based on Samples of Kidney Tissue. The results for 1853 returning adult fish (773 male, 1080 female) at Carson Hatchery in 1991 are presented in Figure IO. Fish having polyclonal ELISA values below 0.100 OD predominated for both sexes, males 92% and females 83% (nearly identical percentages compared to the 1990 adults). Numbers and mean OD for adult male fish are presented in Figure 11. The overall mean OD for fish injected with erythromycin (once[INJ1] or twice [INJ2]) was lower than for the fish receiving no injection (UN, returning early (E), mid(M), or late(L) in the run).

A higher incidence of infection was detected among the returning adult female fish considered positive (OD >0.100), and all of the females sampled after the second week's spawning had positive OD values (Figure 12). All the fish holding in the river and returning to the hatchery after mid-July showed a consistent increase in OD values.

Selection of spawning pairs used for the segregation experiment was based primarily on polyclonal ELISA results for the female fish as for 1990. For the low groups, no crosses were included which mated males with high values with females having low values. Distributions of ELISA values for each of the second year's treatment groups are presented in Figure 13. The ELISA values for individual fish, timing of return, mating, and spawning date are presented in Appendix 4. Selection of fish for the experimental groups in both 1990 and 1991 was based the polyclonal ELISA OD of the female parent and selection was based on individuals with the lowest (LO groups) or highest (HI groups) OD readings (Appendix 5).

Figure 10.

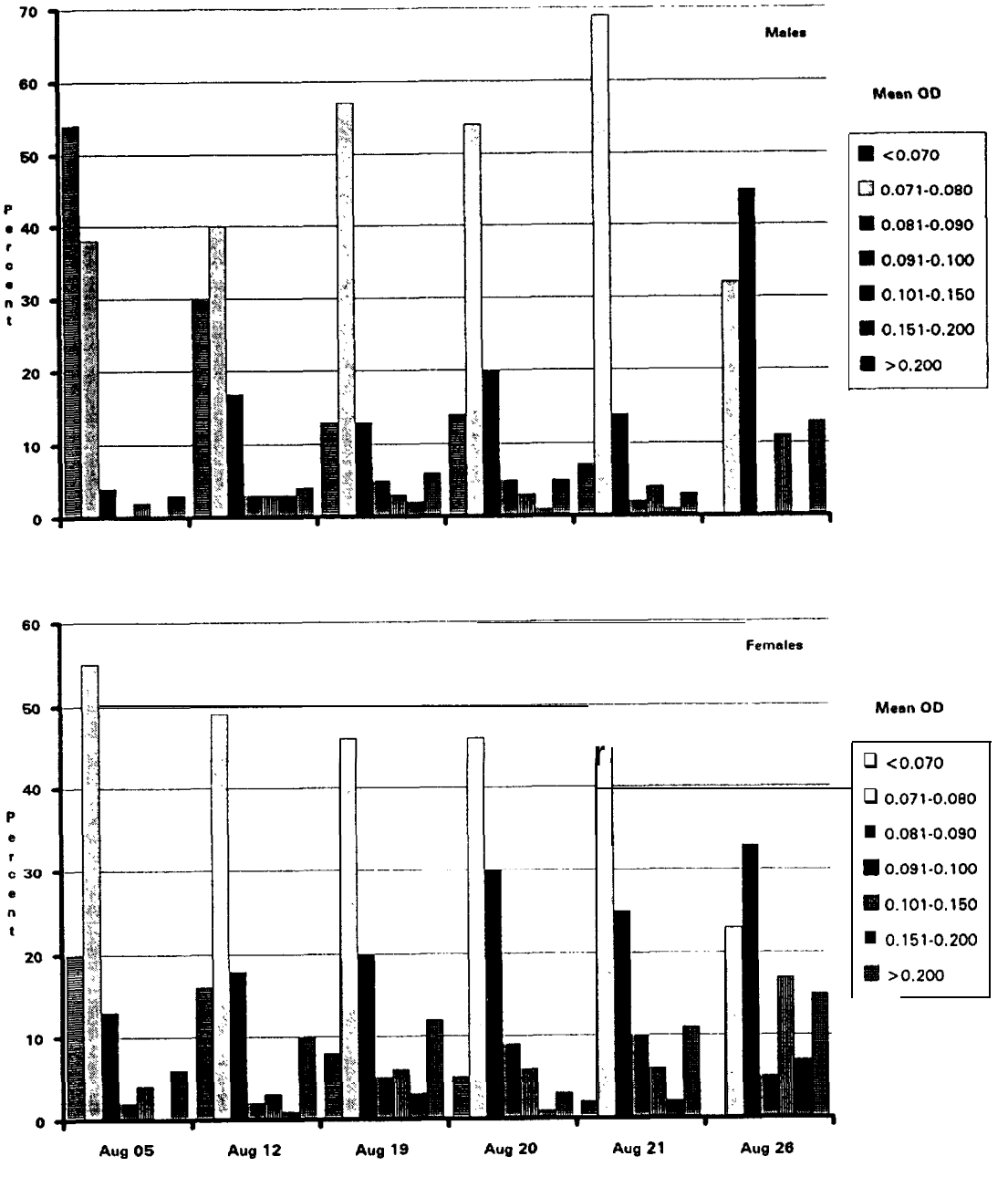

Figure 10. Percent occurrence of optical density values determined by polyclonal antibody-based ELISA for adult chinook salmon at Carson National Fish Hatchery, spawned August 5-26, 1991.

Figure 11

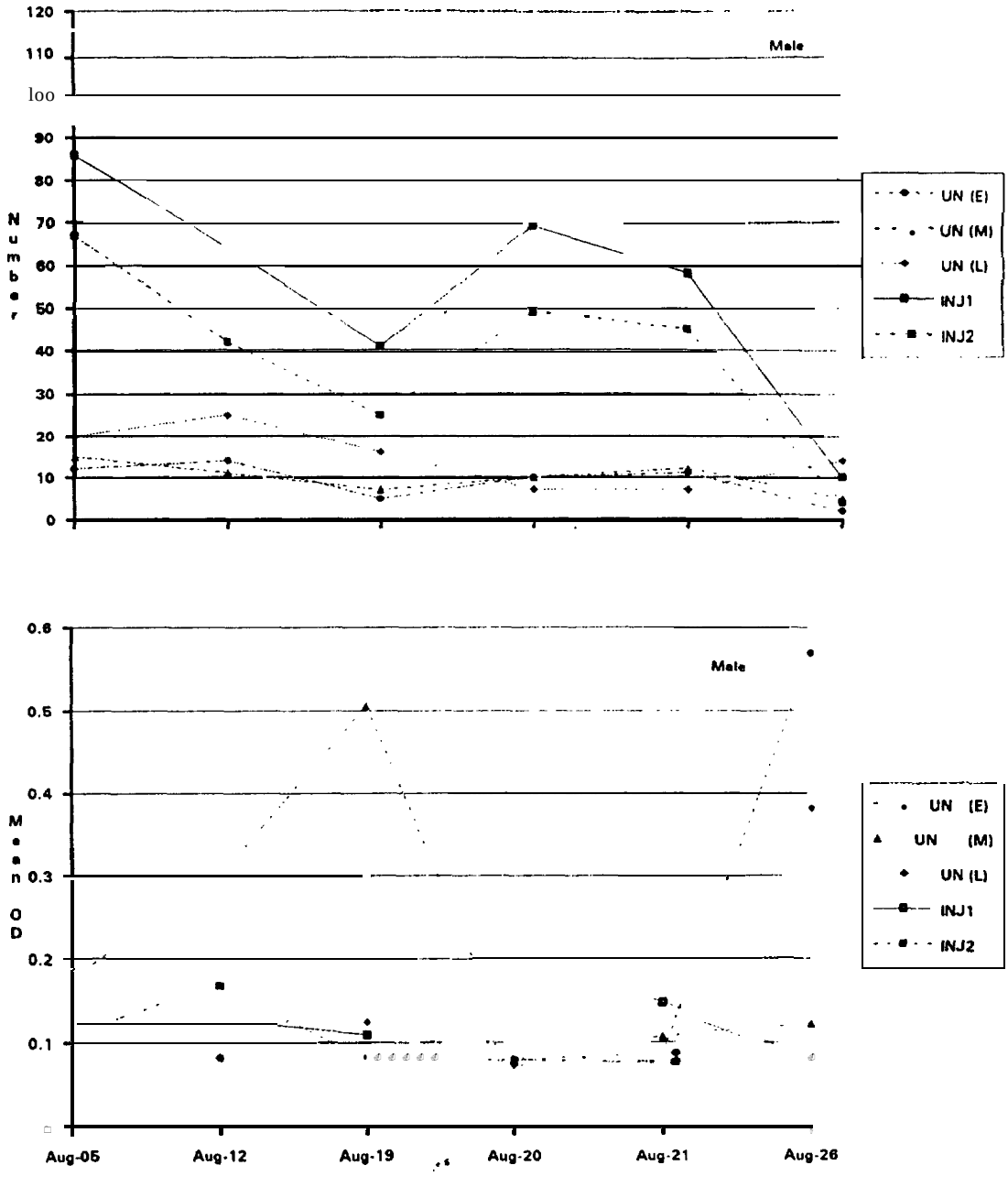

Figure 11. Number and mean optical density values determined by polyclonal antibody-based ELISA for adult male chinook salmon at Carson National Fish Hatchery, grouped by antibiotic treatment and timing of return, spawned August 5-26, 1991. (Abbreviations: UN-no injection of erythromycin; INJ1/2-injected once or twice with erythromycin; (E)-returning to hatchery May 29-June 12, 1991; (M)-returning to hatchery June 13-July 17, 1991; (L)-returning to hatchery after July 17, 1991).

Figure 12

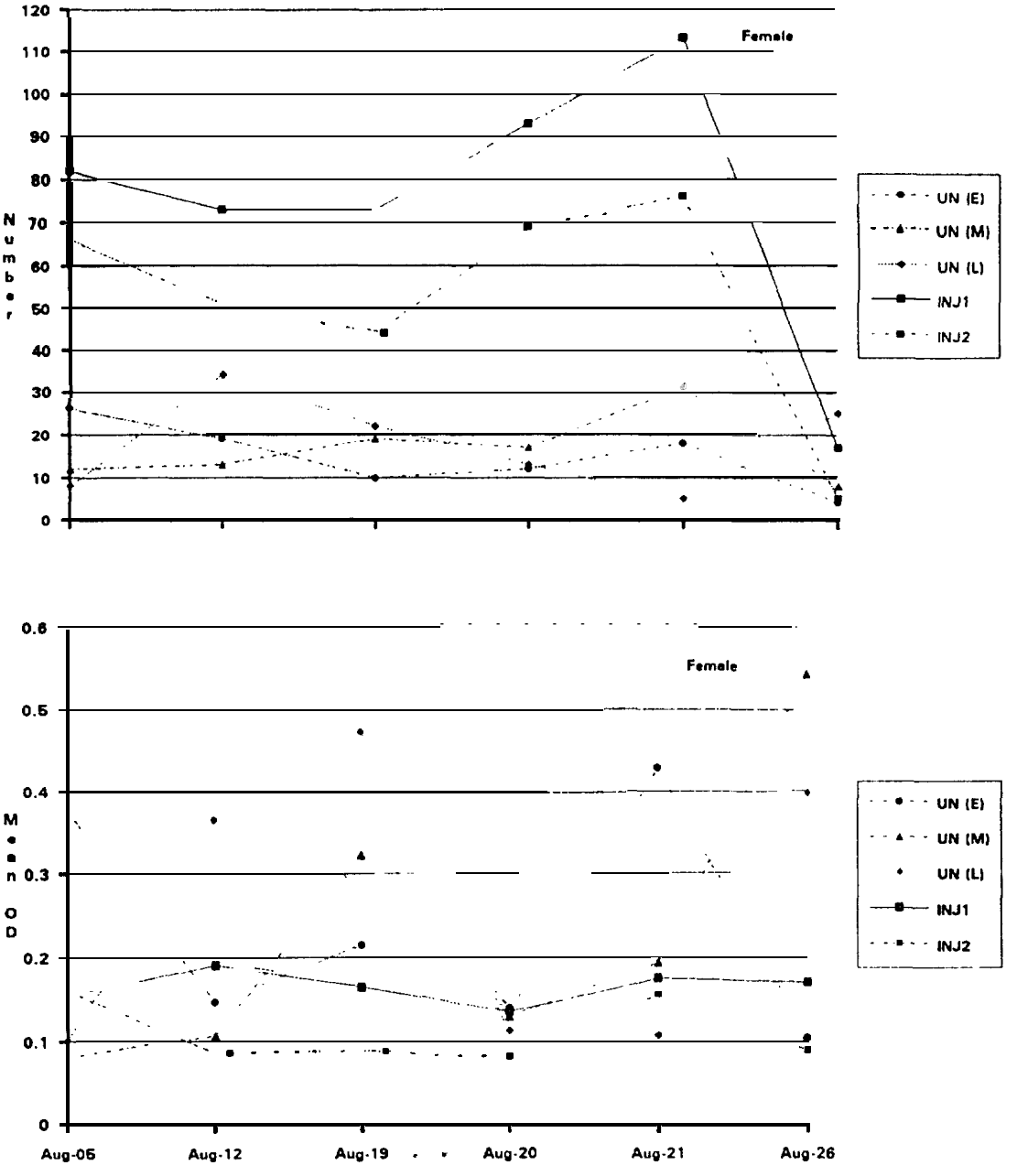

Figure 12 Number and mean optical density values determined by polyclonal antibody-based ELISA for female chinook salmon at Carson National Fish Hatchery, grouped by antibiotic treatment and timing of return, spawned August 5-26, 1991. (Abbreviations: UN-no injection of erythromycin; INJ1 /2-injected once or twice with erythromycin; (E)-returning to hatchery May 29-June 12, 1991; (M)-returning to hatchery June 13-July 17, 1991; (L)-returning to hatchery after July 17, 1991).

Figure 13

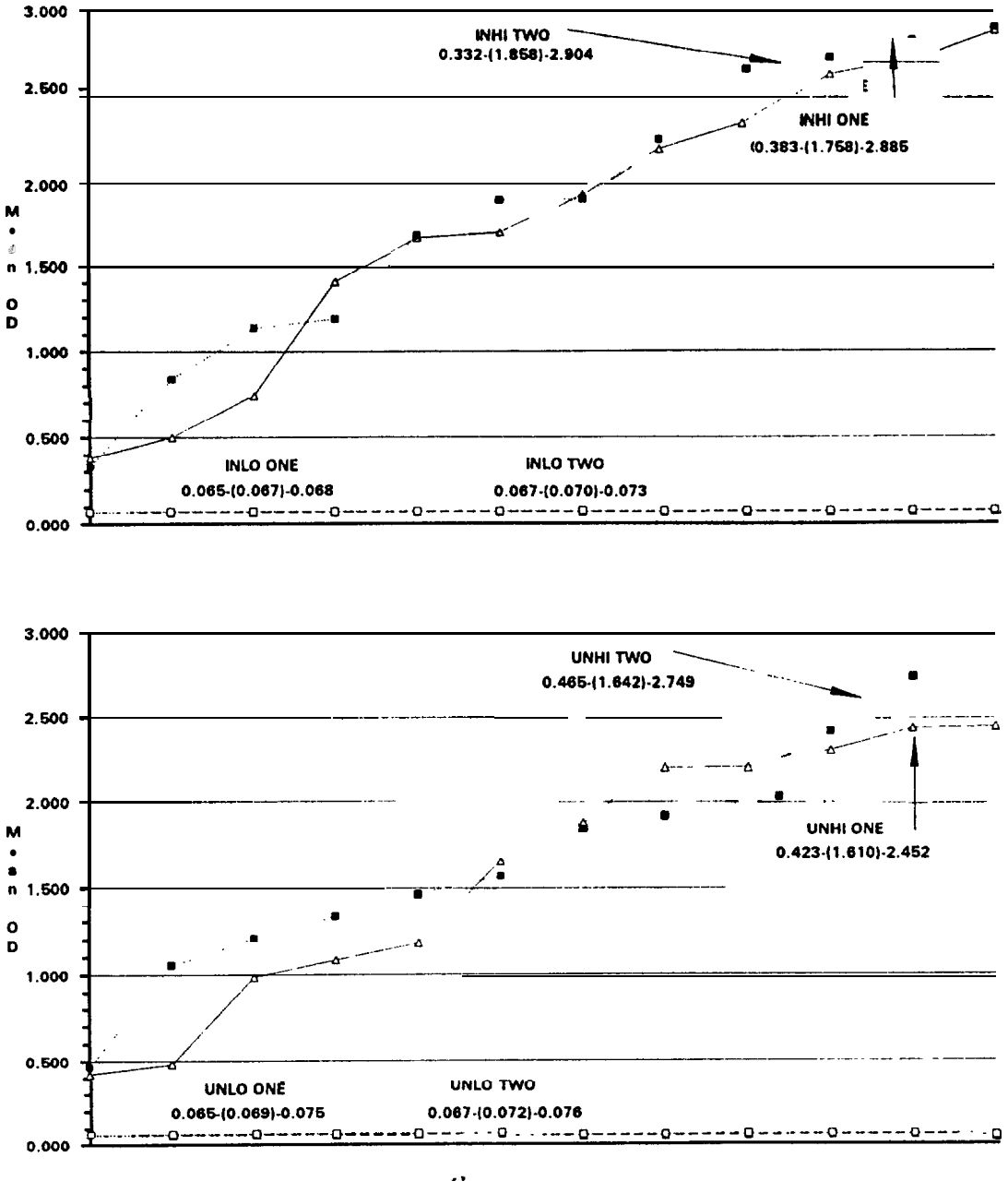

Figure 13. Distribution of mean optical density values determined by polyclonal antibody-based ELISA for female chinook salmon selected for segregation at Carson National Fish Hatchery, grouped by antibiotic treatment,spawned August 7-27, 1990. (Abbreviations: IN-injected with erythromycin; UN-no injection; LO-ELISA OD <0. 100; HI-ELISA OD > 0.100; ONE/TWO-replicate groups).

VI. Tagging and Evaluation of Returns from Experimental Groups

Acquisition of Funding, Tagging, and Monitoring of Returning Fish from each of the Experimental Groups. Losses ranged from 500-2000 fish per group during tagging. Subsequent monthly losses were elevated initially, but returned to low levels until November-December, 1991, when mortality increased for all groups. Fish will be released in April, 1992. Funds have been procured for tagging both the 1991 segregation experiments at both Carson and Marion Forks Hatcheries.

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Appendix 1. Protocol for the monoclonal antibody-based Field ELISA.

Reagent	Volume (Conc)	Incubation Conditions	Incubation Time	TBBS Wash
MAb 4D3 in PBS	500 µl (3.2 µg/ml)	17°C	overnight	No
BSA in TBBS	4.0 ml (0.01 g/ml)	ambient temp.	30 minutes	No
Sample	Swab in 500 µl BSA-TBBS	ambient temp.	30 minutes	5x
MAb in 3H1 in PBS	500 µl (1 µg/ml)	ambient temp.	30 minutes	5x
SA-HRPO in PBS	500 µl (0.25 µg/ml)	ambient temp.	30 minutes	5X 1X TBS
Substrate	500 µl ABTS	ambient temp. dark	10 minutes	No

Appendix 2. Methods for the preparation of fish samples for analysis by the monoclonal-based ELISA.

Swim-up fry. Sixty fry were collected from each bucket and transported to OSU. Fish were anethized with 3 ml l^{-1} of a 10% Ethyl p-aminobenzoate (Sigma, St. Louis, MO) 95% ethanol solution. Fry were rinsed with double distilled water and individually weighed, followed by an equal wt:vol addition of 1% BSA-TTBS. Supernatant was tested by ELISA for each fish.

Parr. Sixty parr were collected from each rearing pond on June , 1991. Fish were lethally anethized with 10% Ethyl p-aminobenzoate and transported to OSU on ice. Parr were individually weighed and placed in wirl-pak sample bags and homogenized using a rolling pin.

Pre-smolts and Smolts. Sixty fish were collected from each rearing pond on Sept. x,1 991, and as smolts in Dec 1991 and Feb x, 1992. Fish were sprayed with 70% Etoh and the entire kidney removed and mixed 1 :1 with 1% BSA-TTBS. Kidney samples were homogenized with applicator sticks and centrifuged five minutes at 500 x g and frozed at -200C until ELISA analysis.

Appendix 3. Kidney tissue antigen levels (ng ml^{-1}) of adult chinook salmon returning to Minto ponds of Marion Forks Hatchery. Fish were either injected with both erythromycin and oxytetracycline (Y) or uninjected (N).

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
1	MINTO	1991	N	F	1.93	0.45
2	MINTO	1991	N	F	3.02	0.60
3	MINTO	1991	N	F	2.50	0.54
4	MINTO	1991	N	F	19.21	1.63
5	MINTO	1991	N	F	1.58	0.40
6	MINTO	1991	N	F	1.61	0.40
7	MINTO	1991	N	F	2.90	0.59
8	MINTO	1991	N	F	2.45	0.53
9	MINTO	1991	N	F	1.88	0.45
10	MINTO	1991	N	F	2.80	0.57
11	MINTO	1991	N	F	1.18	0.32
12	MINTO	1991	N	F	10123.90	859.83
13	MINTO	1991	N	F	3.12	0.61
14	MINTO	1991	N	F	1.69	0.42
15	MINTO	1991	N	F	59.92	6.43
16	MINTO	1991	N	F	1.91	0.45
17	MINTO	1991	N	F	2.32	0.51
18	MINTO	1991	N	F	1.54	0.39
19	MINTO	1991	N	F	3.06	0.61
20	MINTO	1991	N	F	3.19	0.62
21	MINTO	1991	N	F	22.88	6.41
22	MINTO	1991	N	F	1.99	0.46
23	MINTO	1991	Y	F	2.32	0.51
24	MINTO	1991	N	F	1.28	0.34
25	MINTO	1991	Y	F	1.68	0.58
26	MINTO	1991	N	F	1.84	0.62
27	MINTO	1991	N	F	13938.30	1750.35
28	MINTO	1991	N	F	1.11	0.42
29	MINTO	1991	Y	F	958.49	83.48
30	MINTO	1991	N	F	1.30	0.48
31	MINTO	1991	N	F	1.16	0.44
32	MINTO	1991	N	F	2.24	0.70
33	MINTO	1991	N	F	2.46	0.75
34	MINTO	1991	N	F	2.22	0.70
35	MINTO	1991	Y	F	1.28	0.48
36	MINTO	1991	Y	F	1.60	0.56
37	MINTO	1991	Y	F	0.88	0.34
38	MINTO	1991	Y	F	60.34	11.24
39	MINTO	1991	N	F	1.01	0.39

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
40	MINTO	1991	N	F	1.05	0.40
41	MINTO	1991	Y	F	2.14	0.68
42	MINTO	1991	Y	F	6.64	1.31
43	MINTO	1991	N	F	2.02	0.66
44	MINTO	1991	N	F	120351.0	28043.4
45	MINTO	1991	N	F	2.10	0.68
46	MINTO	1991	N	F	2.25	0.71
47	MINTO	1991	N	F	13215.74	1702.63
48	MINTO	1991	N	F	3.46	0.91
49	MINTO	1991	N	F	1.38	0.42
50	MINTO	1991	Y	F	8.78	1.23
51	MINTO	1991	N	F	0.89	0.29
52	MINTO	1991	Y	F	2.46	0.61
53	MINTO	1991	Y	F	3.05	0.69
54	MINTO	1991	Y	F	2.93	0.67
55	MINTO	1991	N	F	1.73	0.49
56	MINTO	1991	N	F	2.75	0.65
57	MINTO	1991	Y	F	1.63	0.47
58	MINTO	1991	Y	F	4.28	0.83
59	MINTO	1991	N	F	3.84	0.78
60	MINTO	1991	N	F	2.32	0.59
61	MINTO	1991	Y	F	1.12	0.36
62	MINTO	1991	N	F	2.11	0.55
63	MINTO	1991	Y	F	2.02	0.54
64	MINTO	1991	Y	F	3.74	0.77
65	MINTO	1991	Y	F	1.89	0.52
66	MINTO	1991	Y	F	3.08	0.69
67	MINTO	1991	N	F	1.71	0.48
68	MINTO	1991	N	F	3.03	0.68
69	MINTO	1991	N	F	2.27	0.58
70	MINTO	1991	N	F	4.54	0.86
71	MINTO	1991	Y	F	2.80	0.66
72	MINTO	1991	Y	F	1457.24	179.10
73	MINTO	1991	Y	F	1.86	0.74
74	MINTO	1991	Y	F	4.54	1.30
75	MINTO	1991	Y	F	2.18	0.82
76	MINTO	1991	Y	F	2.28	0.85
77	MINTO	1991	Y	F	2.40	0.88
78	MINTO	1991	N	F	3.65	1.14
79	MINTO	1991	N	F	4101.93	907.77
80	MINTO	1991	N	F	227.33	63.85
81	MINTO	1991	N	F	3.13	1.04
82	MINTO	1991	N	F	2.18	0.82
83	MINTO	1991	Y	F	2.77	0.96

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
84	MINTO	1991	N	F	2.23	0.84
85	MINTO	1991	Y	F	1.93	0.76
86	MINTO	1991	Y	F	2.45	0.89
87	MINTO	1991	?	F	3.51	1.12
88	MINTO	1991	Y	F	2.33	0.86
89	MINTO	1991	Y	F	2.18	0.82
90	MINTO	1991	N	F	2.19	0.83
91	MINTO	1991	N	F	53.32	8.03
92	MINTO	1991	N	F	3.05	1.02
93	MINTO	1991	N	F	2.57	0.92
94	MINTO	1991	Y	F	4.75	1.33
95	MINTO	1991	Y	F	41.12	6.94
96	MINTO	1991	N	F	5.74	1.48
97	MINTO	1991	N	F	46.27	7.42
98	MINTO	1991	Y	F	1.70	0.68
99	MINTO	1991	N	F	1286.93	128.39
100	MINTO	1991	Y	F	2.31	0.80
101	MINTO	1991	N	F	2.02	0.74
102	MINTO	1991	Y	F	5.84	1.29
103	MINTO	1991	Y	F	1.12	0.54
104	MINTO	1991	N	F	3.58	1.00
105	MINTO	1991	N	F	1.82	0.70
106	MINTO	1991	N	F	634.88	88.3
107	MINTO	1991	Y	F	3.87	1.04
108	MINTO	1991	Y	F	1.62	0.66
109	MINTO	1991	N	F	1.34	0.60
110	MINTO	1991	Y	F	1.26	0.58
111	MINTO	1991	Y	F	2.37	0.81
112	MINTO	1991	?	F	1.09	0.53
113	MINTO	1991	Y	F	2.23	0.78
114	MINTO	1991	N	F	62.81	8.78
115	MINTO	1991	N	F	3.11	0.93
116	MINTO	1991	Y	F	1358.78	132.06
117	MINTO	1991	N	F	187.12	15.58
118	MINTO	1991	N	F	2.08	0.76
119	MINTO	1991	?	F	2.98	0.91
120	MINTO	1991	Y	F	1.50	0.63
121	MINTO	1991	N	F	2.22	0.63
122	MINTO	1991	Y	F	2.53	0.69
123	MINTO	1991	N	F	1.73	0.54
124	MINTO	1991	N	F	1.36	0.46
125	MINTO	1991	Y	F	2.354	0.244
126	MINTO	1991	N	F	1.40	0.47
127	MINTO	1991	Y	F	2.73	0.72

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
128	MINTO	1991	N	F	2.26	0.64
129	MINTO	1991	Y	F	1.46	0.48
130	MINTO	1991	Y	F	0.76	0.27
131	MINTO	1991	Y	F	3.47	0.83
132	MINTO	1991	N	F	1.38	0.46
133	MINTO	1991	Y	F	3.60	0.84
134	MINTO	1991	Y	F	1.17	0.41
135	MINTO	1991	N	F	1.82	0.56
136	MINTO	1991	N	F	1.47	0.48
137	MINTO	1991	N	F	6.14	1.13
138	MINTO	1991	Y	F	1.46	0.48
139	MINTO	1991	Y	F	1.41	0.47
140	MINTO	1991	?	F	1.75	0.54
141	MINTO	1991	N	F	12.73	1.20
142	MINTO	1991	N	F	31.36	7.75
143	MINTO	1991	N	F	5.41	1.14
144	MINTO	1991	N	F	50.81	10.01
145	MINTO	1991	N	F	2.96	0.83
146	MINTO	1991	Y	F	2.62	0.78
147	MINTO	1991	N	F	3.85	0.96
148	MINTO	1991	Y	F	2.13	0.70
149	MINTO	1991	N	F	3.75	0.94
150	MINTO	1991	Y	F	3.11	0.85
151	MINTO	1991	N	F	2.89	0.82
152	MINTO	1991	N	F	2.98	0.83
153	MINTO	1991	Y	F	3.07	0.85
154	MINTO	1991	N	F	1.78	0.63
155	MINTO	1991	Y	F	3.35	0.89
156	MINTO	1991	Y	F	2.74	0.80
157	MINTO	1991	N	F	2.06	0.69
158	MINTO	1991	Y	F	4.83	1.07
159	MINTO	1991	N	F	389.82	87.33
160	MINTO	1991	N	F	2.89	0.82
161	MINTO	1991	N	F	0.86	0.42
162	MINTO	1991	Y	F	2.68	0.79
163	MINTO	1991	N	F	3.26	0.87
164	MINTO	1991	Y	F	3.97	0.97
165	MINTO	1991	N	F	3.14	0.86
166	MINTO	1991	N	F	3.26	0.66
167	MINTO	1991	N	F	2.80	0.34
168	MINTO	1991	N	F	1.97	0.25
169	MINTO	1991	N	F	2.02	0.26
170	MINTO	1991	N	F	4484.28	737.33
171	MINTO	1991	N	F	2.18	0.28

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
172	MINTO	1991	Y	F	2.37	0.30
173	MINTO	1991	N	F	2.49	0.31
174	MINTO	1991	N	F	2.52	0.31
175	MINTO	1991	N	F	1.66	0.22
176	MINTO	1991	Y	F	2.04	0.26
177	MINTO	1991	N	F	2.22	0.28
178	MINTO	1991	N	F	2.81	0.34
179	MINTO	1991	N	F	4.08	0.44
180	MINTO	1991	N	F	2.25	0.29
181	MINTO	1991	N	F	3.51	0.40
182	MINTO	1991	Y	F	2.83	0.34
183	MINTO	1991	Y	F	2.59	0.32
184	MINTO	1991	N	F	2.62	0.32
185	MINTO	1991	Y	F	1.90	0.25
186	MINTO	1991	Y	F	2.81	0.34
187	MINTO	1991	N	F	2.24	0.28
188	MINTO	1991	Y	F	2.58	0.32
189	MINTO	1991	N	F	2.81	0.34
190	MINTO	1991	N	F	2.08	0.21
191	MINTO	1991	N	F	4.64	0.39
192	MINTO	1991	N	F	1.84	0.19
193	MINTO	1991	N	F	23370.71	1750.76
194	MINTO	1991	N	F	1.83	0.19
195	MINTO	1991	N	F	1.75	0.18
196	MINTO	1991	N	F	2.24	0.23
197	MINTO	1991	N	F	2.07	0.21
198	MINTO	1991	N	F	193.74	15.91
199	MINTO	1991	N	F	2.64	0.26
201	MINTO	1991	Y	F	1.41	0.24
202	MINTO	1991	Y	F	1.37	0.24
203	MINTO	1991	Y	F	1.37	0.24
204	MINTO	1991	Y	F	2.31	0.34
205	MINTO	1991	Y	F	2.33	0.34
206	MINTO	1991	Y	F	2.94	0.40
207	MINTO	1991	Y	F	1.04	0.18
208	MINTO	1991	Y	F	1.21	0.21
209	MINTO	1991	Y	F	2.35	0.35
210	MINTO	1991	Y	F	0.90	0.16
211	MINTO	1991	Y	F	1.21	0.21
212	MINTO	1991	Y	F	1.42	0.17
213	MINTO	1991	Y	F	1.42	0.17
214	MINTO	1991	Y	F	1.91	0.30
215	MINTO	1991	Y	F	1.63	0.27
216	MINTO	1991	Y	F	1.44	0.24
217	MINTO	1991	Y	F	1.09	0.19

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
218	MINTO	1991	Y	F	3.22	0.42
219	MINTO	1991	Y	F	1.55	0.26
220	MINTO	1991	Y	F	0.92	0.16
401	MINTO	1991	N	M	241.94	68.04
402	MINTO	1991	N	M	3.69	0.85
403	MINTO	1991	N	M	2.59	0.69
404	MINTO	1991	N	M	1.83	0.56
405	MINTO	1991	N	M	6.15	1.13
406	MINTO	1991	N	M	2.37	0.56
407	MINTO	1991	N	M	1.48	0.39
408	MINTO	1991	N	M	1.95	0.49
409	MINTO	1991	N	M	2.90	0.64
410	MINTO	1991	N	M	2.62	0.60
411	MINTO	1991	N	M	1.29	0.34
412	MINTO	1991	N	M	2.56	0.59
413	MINTO	1991	N	M	3.51	0.72
414	MINTO	1991	N	M	5.10	0.90
415	MINTO	1991	N	M	1.76	0.45
416	MINTO	1991	N	M	1.42	0.38
417	MINTO	1991	N	M	4.46	0.83
418	MINTO	1991	N	M	1.24	0.33
419	MINTO	1991	Y	M	4.84	0.87
420	MINTO	1991	Y	M	1.81	0.46
421	MINTO	1991	N	M	2.37	0.56
422	MINTO	1991	N	M	1.70	0.44
423	MINTO	1991	N	M	1.46	0.39
424	MINTO	1991	N	M	2.40	0.56
425	MINTO	1991	N	M	2.60	0.59
426	MINTO	1991	Y	M	3.29	0.69
427	MINTO	1991	N	M	3.26	0.69
428	MINTO	1991	N	M	3.98	0.78
429	MINTO	1991	N	M	2.66	0.60
430	MINTO	1991	Y	M	2.64	0.82
431	MINTO	1991	Y	M	1.29	0.47
432	MINTO	1991	N	M	3.14	0.92
433	MINTO	1991	Y	M	3.68	1.01
434	MINTO	1991	N	M	2.72	0.84
435	MINTO	1991	N	M	4.08	1.08
436	MINTO	1991	N	M	2.92	0.88
437	MINTO	1991	N	M	5.10	1.23
438	MINTO	1991	N	M	7.29	1.50
439	MINTO	1991	N	M	2.94	0.88
440	MINTO	1991	Y	M	2.94	0.88
441	MINTO	1991	N	M	4.91	1.20
442	MINTO	1991	N	M	1.77	0.62

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
443	MINTO	1991	N	M	14.69	2.17
444	MINTO	1991	N	M	4.74	1.18
445	MINTO	1991	N	M	2.44	0.78
446	MINTO	1991	Y	M	2.48	0.79
447	MINTO	1991	N	M	24.49	6.84
448	MINTO	1991	Y	M	1.98	0.67
449	MINTO	1991	N	M	1.40	0.51
450	MINTO	1991	Y	M	3.25	0.94
451	MINTO	1991	Y	M	2.49	0.79
452	MINTO	1991	N	M	2.71	0.83
453	MINTO	1991	N	M	2.64	0.82
454	MINTO	1991	N	M	3.01	0.73
455	MINTO	1991	Y	M	6.65	1.16
456	MINTO	1991	N	M	1.15	0.36
457	MINTO	1991	N	M	1.90	0.54
458	MINTO	1991	Y	M	5.53	1.05
459	MINTO	1991	Y	M	1.58	0.47
460	MINTO	1991	N	M	1.68	0.50
461	MINTO	1991	N	M	1.63	0.49
462	MINTO	1991	Y	M	1.70	0.50
463	MINTO	1991	Y	M	1.98	0.56
464	MINTO	1991	Y	M	2.35	0.63
465	MINTO	1991	N	M	1.08	0.34
466	MINTO	1991	N	M	134.80	16.44
467	MINTO	1991	N	M	2.88	0.71
468	MINTO	1991	Y	M	1.61	0.48
469	MINTO	1991	Y	M	1.31	0.41
470	MINTO	1991	N	M	2.40	0.64
471	MINTO	1991	N	M	1.83	0.53
472	MINTO	1991	N	M	2.03	0.57
473	MINTO	1991	Y	M	4.30	0.91
474	MINTO	1991	N	M	3.95	0.86
475	MINTO	1991	Y	M	10.67	1.49
476	MINTO	1991	N	M	1.98	0.56
477	MINTO	1991	Y	M	2.10	0.58
478	MINTO	1991	Y	M	2.08	0.43
479	MINTO	1991	N	M	1.41	0.32
480	MINTO	1991	N	M	1.49	0.34
481	MINTO	1991	Y	M	2.48	0.83
482	MINTO	1991	N	M	2.68	0.50
483	MINTO	1991	N	M	1.95	0.41
484	MINTO	1991	N	M	3.22	0.56
485	MINTO	1991	N	M	2.47	0.48
486	MINTO	1991	Y	M	2.11	0.43

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
487	MINTO	1991	N	M	1.21	0.29
488	MINTO	1991	N	M	1.28	0.30
489	MINTO	1991	Y	M	1.97	0.41
490	MINTO	1991	N	M	3.08	0.55
491	MINTO	1991	N	M	1.52	0.34
492	MINTO	1991	N	M	20618.80	3645.80
493	MINTO	1991	N	M	14843.80	3104.87
494	MINTO	1991	Y	M	1.48	0.34
495	MINTO	1991	Y	M	1.65	0.37
496	MINTO	1991	Y	M	10.06	1.05
497	MINTO	1991	Y	M	1.59	0.36
498	MINTO	1991	N	M	1.78	0.38
499	MINTO	1991	N	M	4.67	0.39
500	MINTO	1991	Y	M	5.89	0.46
501	MINTO	1991	Y	M	3.21	0.31
502	MINTO	1991	N	M	267.99	55.02
503	MINTO	1991	N	M	3.44	0.32
504	MINTO	1991	Y	M	1.73	0.18
505	MINTO	1991	N	M	2.66	0.27
506	MINTO	1991	N	M	2.71	0.27
507	MINTO	1991	N	M	2.34	0.24
508	MINTO	1991	N	M	151.91	14.04
509	MINTO	1991	N	M	60.62	8.69
510	MINTO	1991	Y	M	2.46	0.25
511	MINTO	1991	N	M	2.54	0.27
512	MINTO	1991	N	M	2.05	0.23
513	MINTO	1991	N	M	3.21	0.32
514	MINTO	1991	N	M	2.93	0.30
515	MINTO	1991	N	M	3.12	0.32
516	MINTO	1991	Y	M	2.20	0.24
517	MINTO	1991	N	M	5486.74	823.10
518	MINTO	1991	N	M	2.79	0.29
519	MINTO	1991	N	M	1.49	0.16
520	MINTO	1991	N	M	1.81	0.20
521	MINTO	1991	N	M	2.44	0.26
522	MINTO	1991	N	M	11.78	0.71
523	MINTO	1991	Y	M	2.04	0.23
524	MINTO	1991	N	M	2.75	0.29
525	MINTO	1991	Y	M	2.14	0.24
526	MINTO	1991	N	M	4.54	0.41
527	MINTO	1991	N	M	3.06	0.32
528	MINTO	1991	N	M	2.56	0.27
529	MINTO	1991	Y	M	2.91	0.30

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
530	MINTO	1991	N	M	2.13	0.23
531	MINTO	1991	N	M	3.67	0.35
532	MINTO	1991	N	M	2.42	0.26
533	MINTO	1991	N	M	1.87	0.21
534	MINTO	1991	Y	M	4.36	0.40
535	MINTO	1991	N	M	3.17	0.53
536	MINTO	1991	N	M	13078.70	1300.42
537	MINTO	1991	N	M	1.74	0.32
538	MINTO	1991	N	M	256.49	59.56
539	MINTO	1991	N	M	2.04	0.37
540	MINTO	1991	N	M	3.55	0.58
541	MINTO	1991	Y	M	1.41	0.24
542	MINTO	1991	N	M	2.10	0.38
543	MINTO	1991	N	M	1.74	0.32
544	MINTO	1991	N	M	2.40	0.43
545	MINTO	1991	Y	M	2.20	0.40
546	MINTO	1991	Y	M	2.52	0.55
547	MINTO	1991	N	M	2.99	0.51
548	MINTO	1991	N	M	1.62	0.29
549	MINTO	1991	N	M	1.88	0.34
550	MINTO	1991	N	M	1.62	0.29
551	MINTO	1991	N	M	2.62	0.46
552	MINTO	1991	N	M	2.38	0.43
553	MINTO	1991	Y	M	3.15	0.53
554	MINTO	1991	N	M	544.55	94.08
555	MINTO	1991	N	M	3.18	0.54
556	MINTO	1991	Y	M	2.32	0.42
557	MINTO	1991	N	M	1.94	0.36
558	MINTO	1991	Y	M	3.14	0.65
559	MINTO	1991	Y	M	1.92	0.20
560	MINTO	1991	Y	M	2.74	0.59
561	MINTO	1991	Y	M	1.86	0.30
562	MINTO	1991	Y	M	2.09	0.32
563	MINTO	1991	Y	M	1.77	0.29
564	MINTO	1991	Y	M	6.82	0.79
565	MINTO	1991	Y	M	1.46	0.26
566	MINTO	1991	Y	M	2.04	0.36
567	MINTO	1991	Y	M	4.56	0.62
568	MINTO	1991	Y	M	1.89	0.34
569	MINTO	1991	Y	M	1.56	0.28
570	MINTO	1991	Y	M	2.27	0.39
571	MINTO	1991	Y	M	2.04	0.36

I.D. NUMBER	LOCATION	YEAR	INJECTION	SEX	QELISA	MSE
572	MINTO	1991	Y	M	3.11	0.49
573	MINT0	1991	Y	tvl	3.09	0.49
574	MINT0	1991	Y	M	1.56	0.28
575	MINT0	1991	Y	M	2.94	0.47
576	MINT0	1991	Y	M	1.80	0.32
577	MINT0	1991	Y	M	2.44	0.41
578	MINT0	1991	Y	M	1.13	0.19
579	MINT0	1991	Y	M	1.76	0.32
580	MINT0	1991	Y	M	2.12	0.37
581	MINT0	1991	Y	M	3.22	0.50
582	MINTO	1991	Y	M	1.64	0.30
583	MINTO	1991	Y	M	3.08	0.49
584	MINTO	1991	Y	M	1.80	0.32
585	MINTO	1991	Y	M	3.48	0.53
586	MINTO	1991	Y	M	2.38	0.41
587	MINTO	1991	Y	M	1.22	0.21
588	MINTO	1991	Y	M	2.53	0.37
589	MINTO	1991	Y	M	1.65	0.26
590	MINTO	1991	Y	M	2.23	0.34
591	MINTO	1991	Y	M	0.93	0.11
592	MINTO	1991	Y	M	1.55	0.25
593	MINTO	1991	Y	M	2.71	0.39
594	MINTO	1991	Y	M	1.69	0.27
595	MINTO	1991	Y	M	1.69	0.27
596	MINTO	1991	Y	M	1.51	0.24
597	MINTO	1991	Y	M	2.19	0.33

Appendix 4. ELISA Values for Adult Spring Chinook Salmon Returning to Carson National Fish Hatchery, May-August, 1990.

Return to Hatchery :
1 A) May 1 O-July 11, not injected with erythromycin;
1 B) May 1 O-June 13, injected twice with erythromycin on June 13 and July 11;
2) June 14-July 11, injected once with erythromycin on July 18;
3) July 12-August 27, not injected with erythromycin;
4) unknown date of return, unknown injection.

Week 1, August 7 Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
1	1A	0.073	1	1A	0.071
2	3	0.075	2	3	0.073
3	1A	0.072	4	4	0.071
4	1B	0.070	6	4	0.078
5	3	0.071	5	3	0.079
6	1B	0.121	7	4	0.074
7	1B	0.084	11	4	0.079
8	3	0.074	8	1A	0.070
9	1A	0.077	9	1A	0.071
10	1A	0.070	10	3	2.862
11	2	0.076	13	2	0.070
12	3	0.070	12	3	0.071
13	4	0.074	3	4	0.978
14	3	0.072	14	3	0.068
15	1B	0.069	18	1B	0.067
16	3	0.074	15	3	0.070
17	1A	0.090	16	3	0.069
18	3	0.072	17	3	0.069
19	3	0.074	19	1A	0.073
20	2	0.182	20	2	0.077
21	1B	0.082	21	1B	0.070
22	1A	0.077	22	1A	0.069
23	2	0.072	23	2	0.071
24	3	0.073	24	3	0.074
25	3	0.074	25	3	0.070
26	3	0.076	26	3	0.067
27	3	0.073	29	3	0.068
28	1B	0.068	27	1B	0.084
29	3	0.074	31	1A	0.069
30	2	0.067	30	2	0.074
31	2	0.082	32	2	0.082
32	4	0.070	28	1A	0.067
33	3	0.079	33	3	0.070
34	1B	0.079	28	1B	0.067
35	1B	0.074	34	1B	0.068
36	1B	0.078	35	1B	0.066
37	1B	0.067	36	1B	0.070
38	1A	0.069	37	3	0.076
39	1A	0.071	38	1A	0.071
40	1B	0.068	39	1B	0.071
41	2	0.071	40	2	0.079

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
42	2	0.069	41	2	0.073
43	2	0.071	42	2	0.069
44	4	0.069	43	4	0.069
45	1A	0.066	44	3	0.069
46	1B	0.070	45	1B	0.087
47	2	0.073	46+47	2+2	0.074+0.065
48	2	0.073	47	2	0.065
49	3	0.068	48	1A	1.216
50	3	0.069	49	1A	0.075
51	1A	0.085	50	3	0.065
52	2	0.091	51	2	0.066
53	1B	0.074	52	1B	0.069
54	2	0.074	53	2	0.061
55	1B	0.075	54	1B	0.072
56	NS	NS	55	4	0.063
57	2	0.071	56	2	0.065
58	2	0.075	57	2	0.116
59	1B	0.098	58	1B	0.060
60	4	0.073	59	4	0.069
61	1B	0.072	60	1B	0.062
62	2	0.077	61	2	0.059
63	1A	0.077	62	1A	0.062
64	2	0.076	63	2	0.071
65	1B	0.071	64	1B	0.066
66	2	0.073	65	2	0.062
67	2	0.074	66	2	0.064
68	4	0.067	67	1B	0.062
69	1B	0.073	67	1B	0.062
70	3	0.071	68	1A	0.065
71	2	0.075	69	2	2.295
72	1B	0.094	70	1B	0.065
73	1A	0.067	71	1A	0.062
74	3	0.070	71	1A	0.062
75	1B	0.085	72	1B	0.082
76	3	0.082	73	3	0.229
77	1A	0.080	74	1A	0.072
78	2	0.079	75	2	0.068
79	2	0.069	76	2	0.074
80	1A	1.814	77	3	0.082
81	2	0.084	78	2	0.076
82	2	0.070	79	2	0.070
83	2	0.081	80	2	0.240
84	1B	0.186	81	1B	0.073

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
85	1B	0.079	82	1B	0.070
86	2	0.077	83	2	0.067
87	3	0.081	84	1A	0.069
88	1B	0.081	85	1B	0.083
89	1B	0.089	86	1B	0.071
90	2	0.078	87	2	0.070
91	2	0.086	88	2	0.069
92	2	0.078	89	2	0.069
93	3	0.084	90	1B	0.067
Week 2, August 13					
94	3	0.073	91	3	0.061
95	1B	0.070	92	1B	0.064
96	3	0.067	93	3	0.965
97	3	0.069	94	3	0.065
98	3	2.358	95	3	0.064
99	3	0.066	96	3	0.067
100	3	0.549	97	3	0.068
101	1B	1.917	98	1B	0.073
102	3	0.068	99	3	0.069
103	3	0.791	100	3	0.075
104	1B	0.066	101	1B	0.075
105	3	0.067	102	3	0.078
106	3	0.064	103	3	0.068
107	1A	0.072	104	3	0.077
108	1A	0.071	105	3	0.132
109	3	0.074	106	3	0.072
110	3	0.065	107	3	0.069
111	3	0.067	108	3	2.662
112	3	0.074	109	3	0.074
113	3	0.073	110	3	0.084
114	2	0.083	111	2	0.065
115	2	0.070	111	2	0.065
116	3	0.076	112	3	0.094
117	3	0.069	113	3	0.070
118	3	0.065	114	3	0.064
119	3	0.080	115	3	0.069
120	3	0.073	116	3	0.073
121	3	0.064	117	3	0.069
122	1B	0.065	118	1B	0.066
123	3	0.069	119	3	0.068
124	3	0.247	120	3	0.084
125	3	0.068	121	3	0.646

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
126	3	0.074	122	3	0.077
127	1B	0.088	123	1B	0.086
128	1B	0.066	124+127	1B+1B	0.080+0.095
129	3	0.065	125	3	0.071
130	3	0.066	126	3	0.068
131	2	0.066	127	1B	0.095
132	3	0.065	128	3	0.081
133	3	0.066	129	3	0.066
134	2	0.064	130	2	0.073
135	1A	0.063	131	1A	0.068
136	2	0.076	132	2	0.066
137	3	0.067	133	3	0.069
138	1B	0.070	134	1B	0.063
139	2	0.071	135	2	0.061
140	1A	0.067	136	3	0.062
141	3	0.066	137	3	0.062
142	3	0.071	138	3	0.067
143	2	0.072	139	2	0.087
144	1B	0.083	140	1B	0.078
145	1B	0.071	141	1B	0.066
146	1B	0.086	142	1B	0.061
147	3	0.083	143	3	0.061
148	2	0.076	144	2	0.081
149	1B	0.148	145	1B	0.086
150	3	2.099	143	3	0.069
151	2	0.072	146	2	2.569
152	1B	0.078	147	1B	0.064
153	2	0.070	148+150	2+2	0.064+0.067
154	3	0.074	149	1A	0.062
155	2	0.073	150	2	0.067
156	3	0.076	151	3	0.065
157	3	0.068	152	3	0.061
158	2	0.079	153	2	0.064
159	3	0.073	154	3	0.067
160	3	0.071	155	3	0.062
161	2	0.065	156	2	0.063
162	3	0.070	157	3	0.063
163	3	0.073	158	3	0.083
164	3	0.068	159	1A	0.067
165	1A	0.066	160	1A	0.066
166	1A	0.069	161	3	0.064
167	2	0.072	162	2	0.070
168	3	0.086	163	3	0.063

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
169	3	0.083	164	3	0.066
170	2	0.071	165	2	0.066
171	2	0.077	166	2	0.072
172	1B	0.068	167	1B	0.071
173	1B	0.073	168	1B	0.066
174	1B	0.076	169	1B	0.068
175	2	0.067	170	2	0.062
176	2	0.067	171	2	0.067
177	3	0.075	172	3	0.071
178	1B	0.064	173	1B	0.062
179	3	0.068	174	3	0.065
180	1A	0.065	175	1A	0.066
181	3	0.068	176	3	0.066
182	1A	0.068	177	1A	0.063
183	1B	0.070	178	1B	0.068
184	2	0.067	179	2	0.075
185	1A	0.065	180	3	0.068
186	4	0.083	181	1A	0.071
187	1A	0.062	182	1B	0.069
188	1A	0.062	183	1A	0.065
189	2	0.063	184	1B	0.067
190	1B	0.062	185	1B	0.067
191	1B	0.072	186	1B	0.068
192	2	0.071	187	2	0.067
193	1A	0.064	188	1A	0.066
194	3	0.081	189	3	0.072
195	3	0.074	190	3	0.082
196	3	0.067	191	3	0.086
197	1B	0.077	192	1B	0.073
198	1A	0.068	193	1A	0.066
199	1B	0.072	195	1B	0.066
200	1B	0.068	196	1B	0.071
201	1A	0.068	197	3	0.062
202	1A	0.068	198	1A	0.065
203	2	0.069	199	2	0.070
204	1B	0.066	200	1B	0.066
205	2	0.076	201	2	0.066
206	1A	0.066	202	1A	0.073
207	4	0.074	203	4	0.072
208	1A	0.069	204	1A	0.066
209	1B	0.075	205	1B	0.067
210	1B	0.070	206	1B	0.069
211	3	0.063	207	3	0.068

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
212	1A	0.062	208	1A	0.064
213	1B	0.067	210	2	0.068
214	2	0.071	210	2	0.068
215	1A	0.092	211	1A	0.064
216	1B	0.065	212	1B	0.068
217	1B	0.072	213	1B	0.069
218	1B	0.077	214	1B	0.091
219	2	0.064	215	2	0.064
220	4	0.068	216	4	0.069
221	2	0.065	217	2	0.067
222	1B	0.063	218+219	1B+1B	0.075+0.075
223	1B	0.068	219	1B	0.075
224	3	0.067	220	1A	0.063
225	2	0.066	221	2	0.064
226	3	0.072	222	3	0.095
227	1B	0.065	223	1B	0.069
228	1B	0.067	224	1B	0.063
229	3	0.070	225	3	0.068
230	1A	0.070	226	1A	0.068
231	1A	0.069	227	1A	0.068
232	1B	0.074	228	1B	0.065
233	3	0.064	229	3	0.068
234	3	0.069	230	1A	0.063
235	1A	0.074	231	1A	0.065
236	1A	0.064	232	3	0.068
237	2	0.070	233	2	0.077
238	1B	0.068	234	1B	0.069
239	2	0.074	235	2	0.067
240	1B	0.073	236	1B	0.064
241	1B	0.071	237	1B	0.065
242	1B	0.065	238	1B	0.064
243	1B	1.166	239	1B	0.071
244	1A	0.064	240	1A	0.193
245	2	0.068	241	2	0.061
246	1A	0.067	242	1A	0.066
247	3	0.064	243	3	0.098
248	2	0.061	244	2	0.067
249	1B	0.066	245	1B	0.066
250	2	0.070	246	2	0.069
251	2	0.066	247	2	0.067
252	2	0.099	248	2	0.064
253	1B	0.064	249	1B	0.072
254	3	0.065	250	3	0.065

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
255	1A	0.065	251	1A	0.064
256	3	0.066	252	3	0.076
257	1B	0.073	253	1B	0.057
Week 3, August 20					
258	1A	0.070	254	3	0.065
259	2	0.078	255	2	0.060
260	1A	0.068	256	1A	0.066
261	1B	0.082	257	1B	0.073
262	1A	0.075	258	1A	0.064
263	1B	0.089	259	1B	0.061
264	3	0.069	260	3	0.076
265	1B	2.000	261	1B	0.064
266	1B	0.065	262	1B	0.067
267	2	0.069	263	2	0.064
268	3	0.074	264	3	0.081
269	1A	2.701	265	3	0.099
270	4	0.078	266	4	0.081
271	3	0.082	267	3	0.081
272	3	0.078	268	3	0.080
273	3	0.089	269	3	0.075
274	3	0.076	270	3	0.085
275	3	0.074	271	3	0.074
276	3	0.078	272	3	0.080
277	1A	0.085	273	1A	0.076
278	3	0.074	274	3	0.076
279	3	0.193	275	3	0.081
280	3	0.071	276	3	0.077
281	3	0.086	277	3	0.075
282	3	0.073	278	3	0.074
283	3	0.073	279	3	0.080
284	3	0.144	280	3	0.077
285	3	0.077	281	3	0.081
286	3	0.080	282	3	0.078
287	3	0.075	283	3	0.079
288	3	0.075	284	3	0.085
289	3	0.078	285	3	0.292
290	3	0.072	286	3	0.075
291	3	0.071	287	3	0.083
292	3	0.072	288	3	0.081
293	3	0.076	289	3	0.078
294	4	0.082	290	3	0.074
295	1B	1.964	291	1B	0.080

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
296	3	0.074	292	3	0.078
297	3	0.078	293	3	0.078
298	3	0.075	294	3	0.076
299	1A	0.075	295	3	0.081
300	1A	0.075	296	3	0.080
301	1B	0.084	297	1B	0.080
302	3	0.075	298	3	0.075
303	3	0.075	299	3	0.075
304	3	0.074	300	3	0.073
305	3	0.071	301	3	0.077
306	3	0.071	302	3	0.082
307	1B	0.077	303	3	0.077
308	3	0.071	303	3	0.077
309	3	0.072	304	3	0.078
310	1B	0.085	304	3	0.078
311	3	0.076	305	3	0.079
312	3	0.082	305	3	0.079
313	3	0.078	306+307	4+3	0.071
314	3	2.229	307	3	0.102
315	3	0.095	308	3	0.079
316	3	1.581	308	3	0.079
317	3	0.102	309	3	0.081
318	3	1.419	309	3	0.081
319	3	0.080	310	3	0.079
320	3	0.344	310	3	0.079
321	3	0.075	312	3	0.098
322	1B	0.085	311	1B	0.078
323	3	0.071	313	3	0.078
324	3	0.077	314	3	0.079
325	3	0.089	315	3	0.089
326	3	0.071	316	3	0.114
327	3	0.074	317	3	0.083
328	1B	0.073	318	1B	0.083
329	3	0.074	319	3	0.098
330	3	0.237	320	3	0.078
331	3	0.085	321	3	0.085
332	2	0.084	322	2	0.092
333	2	0.187	322	2	0.092
334	3	0.076	323	3	0.076
335	4	0.068	324	4	0.078
336	3	0.073	325	3	0.083
337	4	0.089	326	3	0.082
338	1A	0.080	326	3	0.082

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
339	4	0.072	327	3	0.082
340	3	0.080	327	3	0.082
341	3	0.080	328	3	0.086
342	4	0.081	328	3	0.086
343	3	0.094	329	3	0.079
344	3	0.078	329	3	0.079
345	3	0.087	330	1A	0.078
346	1A	0.083	330	1A	0.078
347	1B	0.085	331	1B	0.082
348	1B	0.082	331	1B	0.082
349	1B	0.080	332	1B	0.081
350	1B	0.080	332	1B	0.081
351	3	0.076	333	3	0.092
352	3	0.076	333	3	0.092
353	4	0.080	334	3	0.079
354	3	0.282	334	3	0.079
355	1A	0.090	335	3	0.079
356	3	0.078	335	3	0.079
357	1B	0.076	336	1B	0.079
358	1B	0.085	336	1B	0.079
359	3	0.079	337	3	0.078
360	3	0.075	337	3	0.078
361	3	0.078	338	3	0.081
362	3	1.906	338	3	0.081
363	3	0.076	339	3	0.081
364	3	0.080	339	3	0.081
365	1B	0.080	340	1B	0.085
366	1B	0.081	340	1B	0.085
367	1B	0.086	341	1B	0.080
368	1B	0.076	341	1B	0.080
369	1A	0.071	342	1A	0.088
370	3	0.075	342	1A	0.088
371	1B	0.077	343	1B	0.080
372	1B	0.092	343	1B	0.080
373	2	0.075	344	2	0.082
374	2	0.075	344	2	0.082
375	3	0.086	345	3	0.086
376	1A	0.080	346	3	0.076
377	1B	0.084	347	1B	0.084
378	1B	0.080	347	1B	0.084
379	3	0.913	348	3	0.077
381	3 2	0.078 0.077	348 349	3 2	0.077 0.084

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
382	2	0.087	349	2	0.084
383	2	0.077	350	2	0.085
384	2	0.087	350	2	0.085
385	2	0.078	351	2	0.076
386	3	0.074	351	2	0.076
387	1B	0.076	352+354	1B+1B	0.074
388	1B	0.084	354	1B	0.075
389	1A	0.074	353	3	0.079
390	1A	0.077	353	3	0.079
391	1A	0.081	355	1A	0.080
392	3	0.084	356	3	0.083
393	1B	0.078	357	1B	0.087
394	1B	0.085	358	1B	0.072
395	4	0.073	359	4	0.077
396	3	2.605	360	3	0.073
397	1A	2.412	361	3	0.074
398	4	0.077	362	4	0.071
399	4	0.229	363	3	0.086
400	1A	0.074	364	1A	0.082
401	3	0.077	365	3	0.082
402	3	0.073	366	3	0.538
403	1B	0.077	367	1B	0.085
404	4	0.076	368	3	0.073
405	4	0.097	369	3	0.072
406	2	0.073	370	2	0.075
407	1B	0.077	371	1B	0.081
408	3	0.075	372	3	0.074
409	3	0.083	373	1A	0.074
410	2	0.073	374	2	0.073
411	3	0.071	375	3	0.073
412	3	0.072	376	3	0.072
413	2	0.073	377	2	0.071
414	3	0.071	378	3	0.083
415	3	0.095	379	3	0.077
416	1B	0.078	380	1B	0.072
417	1B	0.078	381	1B	0.073
418	1B	0.073	382	1B	0.073
419	3	0.075	383	3	0.074
420	2	0.069	384	2	0.069
421	3	0.844	385	3	0.071
422	3	0.072	386	3	0.078
423	1B	0.076	387	1B	0.074
424	1A	0.090	388	1A	0.080

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
425	2	0.087	389	2	0.073
426	2	0.080	390	2	0.072
427	1A	0.075	391	3	0.076
428	1A	0.076	392	3	0.074
429	4	0.076	393	4	0.071
430	1B	0.072	394	1B	0.078
431	3	0.074	395	3	0.077
432	3	0.077	396	3	0.074
433	1B	0.073	397	1B	0.099
434	3	2.357	398	1A	0.077
435	4	0.078	399	3	0.079
436	3	0.072	400	3	0.115
437	3	0.077	401	3	0.076
438	3	0.075	402	3	0.074
439	1B	0.073	403	1B	0.079
440	4	0.075	404	3	0.077
441	3	0.075	405	3	0.094
442	2	0.076	406	2	0.072
443	1B	0.078	407	1B	0.085
444	2	0.075	408	2	0.073
445	3	0.071	409	4	0.074
446	1A	2.430	410	3	0.075
447	2	0.080	411	2	0.075
448	3	0.071	412	4	0.074
449	2	0.072	413	2	0.080
450	2	0.073	414	2	0.072
451	1B	0.074	415	1B	0.075
452	1B	0.101	415	1B	0.075
453	4	0.069	416	4	0.078
454	3	0.074	416	4	0.078
455	1B	0.082	417	1B	0.072
456	1B	0.070	417	1B	0.072
457	2	0.076	418	2	0.081
458	2	0.080	418	2	0.081
459	4	0.078	419	3	0.072
460	3	0.082	419	3	0.072
461	1A	0.078	420	3	0.071
462	3	0.078	420	3	0.071
463	3	0.093	421	3	0.071
464	3	0.078	421	3	0.071
465	1B	0.071	422	1B	0.074
466	1B	0.078	422	1B	0.074
467	1B	0.073	423	1B	0.081

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
468	1B	0.078	423	1B	0.081
469	4	0.071	424	1A	0.074
470	3	0.070	424	1A	0.074
471	4	0.074	425	4	0.075
472	4	0.080	425	4	0.075
473	2	0.085	426	2	0.071
474	2	0.076	426	2	0.071
475	3	0.075	427	3	0.095
476	1A	0.081	427	3	0.095
477	3	0.084	428	3	0.084
478	3	0.090	428	3	0.084
479	1B	0.081	429	1B	0.075
480	1B	0.077	429+430	1B+1B	0.075
481	1B	0.081	430	1B	0.075
482	1B	0.075	430	1B	0.075
483	3	0.076	431	1A	0.073
484	3	0.124	431	1A	0.073
485	1B	0.096	432	1B	0.076
486	1B	0.084	432	1B	0.076
487	1B	0.079	433	1B	0.075
488	1B	0.066	433	1B	0.075
489	3	0.085	434	1A	0.077
490	3	0.076	434	1A	0.077
491	3	0.076	435	3	0.078
492	3	0.075	435	3	0.078
493	1B	0.079	436	1B	0.078
494	1B	0.075	436	1B	0.078
495	1B	0.078	437	3	0.102
Week 3, August 21					
496	1A	0.073	438	1A	0.074
497	1B	0.071	439	1B	0.083
498	1A	0.075	440	4	0.074
499	4	0.081	441	4	0.071
500	1B	0.075	442	1B	0.075
501	1B	0.059	443	1B	0.075
502	1B	0.077	444	1B	0.073
503	3	2.226	445	3	0.076
504	2	0.083	446	2	0.080
505	1A	0.074	447	3	0.080
506	1A	0.073	448	3	0.080
507	1B	0.072	449	1B	0.093
508	1A	0.115	450	1A	0.078

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
509	1B	0.082	451	1B	0.077
510	4	0.076	452	4	0.080
511	2	0.070	453	2	0.077
512	2	0.072	453	2	0.077
513	1A	0.072	454	1A	0.077
514	1B	0.076	455	1B	0.079
515	1A	0.074	456	3	0.080
516	3	0.072	457	4	0.085
517	1B	0.071	458	1B	0.076
518	1A	0.074	459	1A	0.081
519	4	0.075	460	4	0.085
520	4	0.070	461	3	0.078
521	2	0.073	462	2	0.086
522	1A	1.295	463	3	0.078
523	1B	0.076	464	1B	0.078
524	1B	0.072	465	1B	0.087
525	2	0.083	466	2	0.080
526	1A	0.076	467	3	0.085
527	3	0.071	468	3	0.088
528	2	0.076	469	2	0.074
529	1A	0.069	470	1A	0.077
530	1B	0.077	471	1B	0.074
531	4	0.085	472	4	0.078
532	1B	0.079	473	1B	0.076
533	1B	0.082	474	1B	0.076
534	1B	0.072	475	1B	0.078
535	3	0.080	476	3	0.090
536	1A	0.079	477	3	0.080
537	2	0.087	478	2	0.073
538	2	0.081	479	2	0.074
539	1B	0.091	480	1B	0.089
540	1B	0.089	481	1B	0.071
541	1B	0.075	482	1B	0.069
542	1B	0.086	483	1B	0.068
543	2	0.078	484	2	0.071
544	4	0.081	485	4	0.069
545	4	0.057	486	4	0.074
546	1A	0.077	487	1A	0.072
547	3	0.058	488	4	0.072
548	2	0.091	489	2	0.073
549	1A	0.056	490	1A	1.110
550	2	0.087	491	2	0.069
551	3	0.075	492	1A	0.071

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
552	4	0.082	493	4	0.072
553	2	0.075	494	2	0.089
554	3	0.091	495	3	0.070
555	1B	0.089	496	1B	0.071
556	2	0.076	497	2	0.067
557	1B	0.084	498	1B	0.073
558	4	0.080	499	4	0.067
559	4	0.076	500	4	0.069
560	3	0.088	501	3	0.069
561	2	0.078	502	2	0.068
562	1A	0.073	503	1A	0.071
563	1A	0.087	504	1A	0.072
564	3	0.079	505	3	0.073
565	1B	0.072	506	1B	0.070
566	1B	0.078	507	1B	0.071
567	1B	0.076	508	1B	0.071
568	1B	0.077	509	1B	0.071
569	1B	0.087	510	1B	0.083
570	2	0.072	511	2	0.079
571	4	0.057	512	4	0.082
572	1A	0.075	513	1A	0.074
573	3	0.075	514	3	0.075
574	1B	0.086	515	1B	0.076
575	3	0.078	516	3	0.078
576	1B	0.074	517	1B	0.072
577	1B	0.074	517	1B	0.072
578	2	0.074	518	2	0.077
579	2	0.073	518	2	0.077
580	1A	0.071	519	1A	0.070
581	1A	0.089	519	1A	0.070
582	1B	0.090	520	1B	0.073
583	1B	0.086	520	1B	0.073
584	1A	0.096	521	3	0.075
585	1A	0.083	521	3	0.075
586	1B	0.086	522	1B	0.072
587	1B	0.085	522	1B	0.072
588	3	0.083	523	3	0.079
589	4	0.086	523	3	0.079
590	1B	0.085	524	1B	0.074
591	1B	0.085	524	1B	0.074
592	4	0.084	525	3	0.074
593	1A	0.081	525	3	0.074
594	1B	0.086	526	1B	0.085

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
595	1B	0.094	526	1B	0.085
596	1B	0.097	527	1B	0.073
597	1B	0.088	527	1B	0.073
598	4	0.086	528	3	0.079
599	4	0.094	528	3	0.079
600	4	0.100	529	3	0.077
601	4	0.095	529	3	0.077
602	1A	0.092	530	1A	0.090
603	3	1.465	530	1A	0.090
604	1A	0.089	531	1A	0.080
605	1A	0.964	531	1A	0.080
606	1B	0.140	532	1B	0.076
607	1B	0.093	532	1B	0.076
608	1B	0.094	533+538	1B+1B	0.074
609	1B	0.091	538	1B	0.084
610	2	0.105	534	2	0.081
611	2	0.101	534	2	0.081
612	3	0.085	535	4	0.072
613	3	0.088	535	4	0.072
614	1B	0.090	536	1B	0.074
615	1B	0.098	536	1B	0.074
616	2	0.092	537	2	0.073
617	2	0.083	537	2	0.073
618	1B	0.090	539	1B	0.083
619	1B	0.086	539	1B	0.083
620	1A	0.084	540	3	2.013
621	1A	0.093	540	3	2.013
622	3	0.095	541	3	0.074
623	4	0.088	541	3	0.074
624	2	0.091	542	2	0.083
625	2	0.083	542	2	0.083
626	1A	0.084	543	3	0.077
627	3	0.095	543	3	0.077
628	1B	0.095	544	1B	0.078
629	1B	0.088	544	1B	0.078
630	1B	0.091	545	1B	0.074
631	1B	0.099	545	1B	0.074
632	2	0.093	546	2	0.077
633	2	0.096	546	2	0.077
634	1B	0.091	547	1B	0.278
635	1B	0.122	547	1B	0.278
636	2	2.099	548	2	0.072
637	2	0.100	548	2	0.072

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
638	3	0.095	549	3	0.072
639	4	0.091	549	3	0.072
640	2	0.091	550	2	0.076
641	2	0.088	550	2	0.076
642	1B	0.089	551	1B	0.082
643	1B	0.102	551	1B	0.082
644	4	0.114	552	4	0.075
645	4	0.095	552	4	0.075
646	4	0.091	553	3	0.080
647	4	0.098	553	3	0.080
648	2	0.088	554	2	0.073
649	2	0.090	554	2	0.073
650	1B	0.089	555	1B	0.070
651	1B	0.094	555	1B	0.070
652	1B	0.094	556	1B	0.073
653	1B	0.082	556	1B	0.073
654	3	0.089	557	4	0.078
655	4	0.087	557	4	0.078
656	2	0.085	558	2	0.073
657	2	0.090	558	2	0.073
658	2	0.098	559	2	0.075
659	2	0.095	559	2	0.075
660	1B	0.090	560	1B	0.073
661	1B	0.088	560	1B	0.073
662	1B	0.084	561	1B	0.085
663	1B	0.092	561	1B	0.085
664	1B	0.101	562	1B	0.089
665	1B	0.094	562	1B	0.089
666	2	0.136	563	2	0.083
667	2	0.087	563	2	0.083
668	2	0.087	564	2	0.078
669	2	0.084	564	2	0.078
670	1A	0.103	565	3	0.072
671	1A	0.086	565	3	0.072
672	1B	0.089	566	1B	0.080
673	1B	0.097	566	1B	0.080
674	3	0.086	567	3	0.075
675	4	0.089	567	3	0.075
676	1B	0.105	568	1B	0.077
677	1B	0.088	568	1B	0.077
678	1A	0.091	569	4	0.081
679	1A	0.087	569	4	0.081
680	1A	0.088	570	4	0.100

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
681	1A	0.101	570	4	0.100
682	2	0.095	571	2	0.078
683	2	0.186	571	2	0.078
684	1B	0.108	572	1B	0.076
685	1B	0.104	572	1B	0.076
686	1B	0.093	573	1B	0.076
687	1B	0.104	573	1B	0.076
688	1B	0.096	574	1B	0.078
689	1B	0.209	574	1B	0.078
690	3	0.093	575	4	0.075
691	1A	0.092	575	4	0.075
692	2	0.097	576	2	0.075
693	2	0.092	576	2	0.075
694	4	0.134	577	4	0.068
695	4	0.096	577	4	0.068
696	3	0.097	578	3	0.078
697	4	0.103	578	3	0.078
698	1B	0.088	579	1B	0.082
699	1B	0.096	579	1B	0.082
700	1B	0.098	580	1B	0.075
701	1B	0.100	580	1B	0.075
702	1B	0.092	581	1B	0.077
703	1B	0.092	581	1B	0.077
704	1A	0.086	582	1A	0.073
705	1A	0.090	582	1A	0.073
706	4	0.090	583	4	0.079
707	3	0.086	583	4	0.079
708	1B	0.091	584	1B	0.074
709	1B	0.094	584	1B	0.074
710	1B	0.091	585	1B	0.076
711	1B	0.086	585	1B	0.076
712	2	0.357	586	2	0.077
713	2	0.087	586	2	0.077
714	1A	0.101	587	1A	0.072
715	1A	0.095	587	1A	0.072
716	2	0.105	588	2	0.073
717	2	0.103	588	2	0.073
718	1B	0.107	589	1B	0.071
719	1B	0.101	589	1B	0.071
720	2	0.144	590	4	0.084
Week 3, August 22					
721	2	0.110	591	2	0.081

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
722	4	0.093	592	4	0.079
723	1B	0.103	593	1B	0.077
724	1A	0.097	594	3	0.099
725	1B	0.100	595	1B	0.097
726	1A	0.089	596	3	0.074
727	1B	0.090	597	1B	0.075
728	2	0.091	598	2	0.071
729	1B	0.093	599	1B	0.074
730	1B	0.093	600	1B	0.076
731	4	0.093	601	3	0.073
732	2	0.102	602	2	0.082
733	2	0.096	603	2	0.073
734	4	0.856	604	1A	0.074
735	1B	0.090	608	1B	0.075
736	2	0.093	606	2	0.085
737	1B	0.092	607	1B	0.090
738	1B	0.111	608	1B	0.075
739	4	0.091	609	4	0.076
740	3	0.092	610	4	0.084
741	1B	0.093	611	1B	0.093
742	2	0.116	612	2	0.076
743	4	0.088	613	4	0.076
744	4	2.342	613	4	0.076
745	1B	0.098	614	1B	0.078
746	1B	0.105	614	1B	0.078
747	1B	0.094	615	1B	0.074
748	1B	0.095	615	1B	0.074
749	1B	0.097	616	1B	0.074
750	1B	0.097	616	1B	0.074
751	1B	0.092	617	1B	0.075
752	1B	0.094	617	1B	0.075
753	2	0.092	618	2	0.079
754	2	0.089	618	2	0.079
755	1A	0.095	619	1A	0.071
756	1A	0.094	619	1A	0.071
757	1B	0.090	620	1B	0.075
758	1B	0.121	620	1B	0.075
759	4	0.100	621	3	0.071
760	4	0.092	621	3	0.071
761	3	0.096	622	4	0.072
762	1A	0.092	622	4	0.072
763	2	0.121	623	2	0.076
764	2	0.099	623	2	0.076

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
765	1B	0.105	624	1B	0.075
766	1B	0.152	624	1B	0.075
767	4	0.114	625	1A	0.069
768	1A	0.112	625	1A	0.069
769	1B	0.113	626	1B	0.072
770	1B	0.099	626	1B	0.072
771	2	0.103	627	2	0.075
772	2	0.106	627	2	0.075
773	1B	0.103	628	1B	0.069
774	1B	0.108	628	1B	0.069
775	1B	0.104	629	1B	0.079
776	1B	0.101	629	1B	0.079
777	2	0.101	630	2	0.073
778	2	0.098	630	2	0.073
779	3	0.277	631	4	0.082
780	4	0.096	631	4	0.082
781	1B	0.102	632	1B	0.083
782	1B	0.105	632	1B	0.083
783	4	0.109	633	3	0.073
784	4	0.103	633	3	0.073
785	1B	0.097	634	1B	0.073
786	1B	0.143	634	1B	0.073
787	3	0.105	635	4	0.076
788	3	0.096	635	4	0.076
789	1B	0.107	636	1B	0.068
790	1B	0.106	636	1B	0.068
791	4	0.098	637	3	0.084
792	3	0.107	637	3	0.084
793	1B	0.164	638	1B	0.076
794	1B	0.097	638	1B	0.076
795	1B	0.152	639	1B	0.078
796	1B	0.106	639	1B	0.078
797	1B	0.098	640	1B	0.071
798	1B	0.120	640	1B	0.071
799	1A	0.098	641	3	0.075
800	4	0.092	641	3	0.075
801	3	0.105	642	3	0.073
802	3	0.101	642	3	0.073
803	2	0.096	643	2	0.073
804	2	0.098	643	2	0.073
805	1B	0.104	644	1B	0.077
806	1B	0.105	644	1B	0.077
807	4	0.095	645	3	0.073

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
808	4	0.095	645	3	0.073
809	1B	0.105	646	1B	0.072
810	1B	0.111	646	1B	0.072
811	1A	0.116	647	1A	0.073
812	3	0.092	647	1A	0.073
813	1B	0.095	648	1B	0.083
814	1B	0.104	648+649	1B+1B	0.083+0.072
815	1B	0.098	649	1B	0.072
816	1B	0.093	650	1B	0.072
817	4	0.097	650	1B	0.072
818	4	0.106	651	4	0.074
819	2	0.096	651	4	0.074
820	2	0.095	652	2	0.075
821	1B	0.093	652	2	0.075
822	1B	0.091	653	1B	0.073
823	3	0.094	653	1B	0.073
824	4	0.097	654	4	0.075
825	2	0.094	654	4	0.075
826	2	0.091	655	1B	0.073
827	1B	0.104	655	1B	0.073
828	4	0.106	656	4	0.075
829	1B	0.113	657	1B	0.074
830	1B	NS	NS	NS	NS
831	1A	0.102	658	4	0.085
832	4	0.103	658	4	0.085
833	4	0.114	659	4	0.079
834	4	0.097	659	4	0.079
835	1B	0.138	660	1B	0.079
836	1B	0.107	660	1B	0.079
837	2	0.097	661	2	0.101
838	2	0.094	661	2	0.101
839	4	0.191	662	4	0.080
840					
	4	0.094	663	1B	0.080
841 842	1B	0.108 0.102	663	1B	0.079
843	1B	0.103	664	1B	0.077
844	1B	0.101	664+666	1B+1B	0.077+0.079
845	4	0.102	665	4	0.210
846	1A	0.104	665	4	0.210
847	1B	0.106	666	1B	0.079
848	1B	0.124	666	1B	0.079
849	2	0.120	667	2	0.075
850	2	0.097	667	2	0.075

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
851	1B	0.111	668	1B	0.075
852	4	0.104	668	1B	0.075
853	4	0.126	669	4	0.083
854	4	0.107	669	4	0.083
855	2	0.102	670	2	0.081
856	2	0.123	670	2	0.081
857	1B	0.113	671	1B	0.083
858	1B	0.102	671	1B	0.083
859	1B	0.103	672	1B	0.118
860	1B	0.117	672	1B	0.118
861	1A	0.098	673	4	0.077
862	1A	0.102	673	4	0.077
863	2	0.100	674	2	0.075
864	2	0.108	674	2	0.075
865	1B	0.104	675	1B	0.083
866	1B	0.100	675	1B	0.083
867	3	2.766	676	1A	0.075
868	3	0.100	676	1A	0.075
869	4	0.103	677	4	0.081
870	4	0.110	677	4	0.081
871	1A	0.099	678	3	0.076
872	1A	0.099	678	3	0.076
873	1B	0.106	679	1B	0.075
874	1B	0.106	679	1B	0.075
875	1B	0.104	680	1B	0.083
876	1B	0.102	680	1B	0.083
877	1B	1.635	681	1B	0.077
878	1B	0.114	681	1B	0.077
879	4	0.107	682	4	0.081
880	3	0.115	682	4	0.081
881	3	0.100	683	4	0.075
882	4	0.095	683	4	0.075
883	1B	0.104	684	1B	0.078
884	1B	0.130	684	1B	0.078
885	2	0.097	685	2	0.080
886	2	0.094	685	2	0.080
887	1A	0.097	686	4	0.082
888	1A	0.097	686	4	0.082
889	4	0.120	687	4	0.076
890	3	0.099	687	4	0.076
891	1B	0.103	688	1B	0.076
892	1B	0.104	688	1B	0.076
893	4	0.119	689	4	0.077

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
894	3	0.098	689	4	0.077
895	2	0.097	690	2	0.079
896	3	0.097	691	4	0.087
897	2	0.100	692	2	0.077
898	1B	0.098	693	1B	0.085
899	1B	0.127	694	1B	0.080
900	4	0.130	695	4	0.076
Week 4. August 27					
901	1B	0.105	696	1B	0.102
902	1B	0.091	697	1B	0.089
903	1B	0.089	698	1B	0.083
904	1B	0.097	699	1B	0.084
905	2	0.430	700	2	0.089
906	1B	0.088	701	1B	0.085
907	1B	0.085	702	1B	0.150
908	2	0.092	703	2	0.091
909	1B	0.082	704	1B	0.184
910	1B	0.088	705	1B	0.090
911	1A	0.090	706	3	0.153
912	1A	0.097	706	3	0.153
913	1B	0.083	707	1B	0.176
914	1B	0.084	707	1B	0.176
915	1B	0.170	708	1B	0.090
916	1B	0.136	708+709	1B+3	0.090+0.081
917	1A	0.106	709	3	0.081
918	1A	0.082	710	3	0.080
919	1A	0.494	710	3	0.080
920	1A	0.082	710	3	0.080
921	1A	0.084	711	3	0.081
922	1A	0.078	711	3	0.081
923	1B	0.090	712	1B	0.086
924	1B	0.160	712	1B	0.086
925	1A	0.253	713	3	0.112
926	1A	2.728	713	3	0.112
927	1B	0.079	714	1B	0.155
928	1B	0.085	714	1B	0.155
929	1B	0.106	715	1B	0.083
930	1B	0.088	715	1B	0.083
931	1A	0.082	716	3	0.084
932	1A	0.095	716	3	0.084
933	2	0.123	717	2	0.163
934	2	0.086	717+719	2+2	0.163+0.081

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
935	1A	0.165	718	3	0.096
936	1A	0.084	718	3	0.096
937	2	0.080	719	2	0.081
938	2	0.088	719	2	0.081
939	1B	0.081	720	1B	0.091
940	1B	0.081	720	1B	0.091
941	1B	0.084	721	1B	0.085
942	1B	0.086	721	1B	0.085
943	1B	0.083	722	1B	0.159
944	1B	0.095	722	1B	0.159
945	1B	0.082	723	1B	0.309
946	1B	0.146	723	1B	0.309
947	1A	0.130	724	3	0.081
948	1A	0.163	724	3	0.081
949	1A	0.083	725	3	0.113
950	1A	0.086	725	3	0.113
951	1A	0.089	726	3	0.082
952	1A	0.089	726	3	0.082
953	2	0.082	727	2	0.081
954	2	0.084	727	2	0.081
955	1B	0.101	728	1B	0.086
956	1B	0.088	728	1B	0.086
957	1A	0.090	729	3	0.084
958	1A	0.086	729	3	0.084
959	2	0.086	730	2	0.087
960	2	0.086	730	2	0.087
961	1B	0.081	731	1B	0.086
962	1B	0.092	731	1B	0.086
963	2	0.172	732	2	0.085
964	2	0.089	732	2	0.085
965	2	0.125	733	2	0.086
966	2	0.086	733	2	0.086
967	2	0.091	734	2	2.266
968	2	0.097	734	2	2.266
969	1B	0.225	735	1B	0.085
970	1B	0.092	735	1B	0.085
971	1B	0.088	736	1B	0.086
972	1B	0.100	736	1B	0.086
973	1A	0.090	737	3	0.080
974	1A	0.087	737	3	0.080
975	1A	0.080	738	1A	0.094
976	1A	0.081	738	1A	0.094
977	1B	0.083	739	1B	0.082

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
978	1B	0.090	739	1B	0.082
979	1B	0.084	740	1B	0.092
980	1B	0.084	740	1B	0.092
981	1B	0.085	741	1B	0.085
982	1B	0.090	741	1B	0.085
983	1B	0.097	742	1B	0.084
984	1B	0.097	742	1B	0.084
985	1B	0.086	743	1B	0.086
986	1B	0.088	743	1B	0.086
987	1A	0.084	744	1A	0.082
988	1A	0.081	744	1A	0.082
989	1B	0.087	745	1B	0.084
990	1B	0.085	745	1B	0.084
991	1B	0.108	746	1B	0.084
992	1B	0.087	746	1B	0.084
993	1B	0.116	747	1B	0.085
994	1B	0.087	747	1B	0.085
995	1B	0.089	748	1B	0.135
996	1B	0.085	748	1B	0.135
997	1B	0.085	749	1B	0.093
998	1B	0.100	749	1B	0.093
999	1B	0.094	750	1B	0.093
1000	1B	0.090	750	1B	0.093
1001	1A	0.092	751	3	0.083
1002	1A	0.093	751	3	0.083
1003	1A	0.084	752	3	0.086
1004	1A	0.088	752	3	0.086
1005	1B	1.079	753	1B	0.120
1006	1B	0.096	753	1B	0.120
1007	1B	0.108	754	1B	0.082
1008	1B	0.094	754+758	1B+1B	0.082+0.090
1009	1B	0.124	755	1B	0.083
1010	1B	0.089	755	1B	0.083
1011	1A	0.084	756	3	0.083
1012	1A	0.084	756	3	0.083
1013	1A	0.089	757	3	0.710
1014	1A	0.088	757	3	0.710
1015	1B	0.167	758	1B	0.090
1016	1B	0.088	758	1B	0.090
1017	1B	0.087	759	1B	0.085
1018	1B	0.086	759	1B	0.085
1019	1A	0.085	760	1A	0.087
1020	1A	0.087	760	1A	0.087

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
1021	1A	0.085	761	1A	0.088
1022	1A	0.083	761	1A	0.088
1023	1B	0.100	762	1B	0.086
1024	1B	0.262	762	1B	0.086
1025	2	0.108	763	2	0.084
1026	2	0.088	763	2	0.084
1027	1B	0.096	764	1B	0.086
1028	1B	0.087	764+765	1B+1B	0.086+0.083
1029	1B	0.088	765	1B	0.083
1030	1B	0.083	765	1B	0.083
1031	1A	0.089	766	3	0.084
1032	1A	0.238	766	3	0.084
1033	1A	0.094	767	3	0.081
1034	1A	0.090	767	3	0.081
1035	1B	0.085	768	1B	0.082
1036	1B	0.092	768	1B	0.082
1037	1A	0.084	769	3	0.086
1038	1A	0.084	769	3	0.086
1039	1A	0.088	770	3	0.081
1040	1A	0.083	770	3	0.081
1041	2	0.086	771	2	0.082
1042	2	0.084	771	2	0.082
1043	2	0.102	772	2	0.082
1044	2	0.085	772	2	0.082
1045	1B	0.084	773	1B	0.080
1046	1B	0.124	773	1B	0.080
1047	1A	0.083	774	3	0.088
1048	1A	0.097	774	3	0.088
1049	2	0.094	775	2	0.082
1050	2	0.084	775	2	0.082
1051	2	0.089	776	2	0.087
1052	2	0.090	776	2	0.087
1053	1B	0.084	777	1B	0.085
1054	1B	0.089	777	1B	0.085
1055	1A	0.092	778	1A	0.089
1056	1A	0.087	778	1A	0.089
1057	1B	0.087	779	1B	1.726
1058	1B	0.088	779	1B	1.726
1059	1B	0.087	780	1B	0.093
1060	1B	0.094	780	1B	0.093
1061	1B	0.099	781	1B	0.090
1062	1B	0.086	781	1B	0.090
1063	1B	0.082	782	1B	0.089

Female Number	Return to Hatchery	ELISA OD	Male Number	Return to Hatchery	ELISA OD
1064	1B	0.084	782	1B	0.089
1065	1A	0.084	783	3	0.086
1066	1A	0.135	783	3	0.086
1067	1B	0.095	784	1B	0.092
1068	1B	0.090	784	1B	0.092
1069	1B	0.083	785	1B	0.088
1070	1B	0.090	785	1B	0.088
1071	1A	0.082	786	1A	1.763
1072	1A	0.096	786	1A	1.763
1073	1B	0.093	787	1B	0.087
1074	1A	0.086	788	1A	0.085
1075	1B	0.090	789	1B	0.087
1076	1B	0.082	790	1B	0.084
1077	1B	0.081	791	1B	0.089
1078	1B	0.083	792	1B	0.088
1079	1B	0.091	793	1B	0.088
1080	1B	0.084	794	1B	0.090

Appendix 5. ELISA Values for Adult Female Chinook Salmon Returning to Carson National Fish Hatchery in 1991 of which gametes were taken and used in a second year of adult segregation. Salmon returning to the hatchery were coded as follows: 1) May 29-June 13; 2) June 14-July 18; 3) July 1 9-August 26.

NO INJECTION, LOW OD

Female Number	ELISA OD	Return to Hatchery	Egg Take
22	0.073	3	Aug-05
24	0.066	1	Aug-05
33	0.068	2	Aug-05
82	0.067	1	Aug-05
134	0.072	3	Aug-05
178	0.068	2	Aug-06
212	0.067	3	Aug- 12
221	0.065	3	Aug- 12
256	0.068	2	Aug- 12
257	0.066	1	Aug-12
259	0.075	1	Aug-12
308	0.071	1	Aug-12
Mean	0.069		
Std Dev	0.003		

NO INJECTION, LOW OD

Female Number	ELISA OD	Return to Hatchery	Egg Take
395	0.070	3	Aug-19
410	0.068	3	Aug-19
416	0.067	3	Aug-19
428	0.076	2	Aug- 19
459	0.073	1	Aug-19
584	0.072	1	Aug-20
586	0.076	2	Aug-20
628	0.069	3	Aug-20
678	0.068	1	Aug-20
686	0.068	3	Aug-20
734	0.076	2	Aug-20
957	0.075	2	Aug-21
Mean	0.072		
Std Dev	0.004		

NO INJECTION, HIGH OD

Female Number	ELISA OD	Return to Hatchery	Egg Take
15	2.452	1	Aug-05
48	1.083	1	Aug-05
72	2.316	1	Aug-05
73	2.210	1	Aug-05
207	1.178	3	Aug- 12
213	2.211	3	Aug-12
214	1.880	3	Aug-12
219	2.445	3	Aug-12
239	1.659	3	Aug-12
270	0.423	3	Aug-12
305	0.480	1	Aug-12
350	0.986	1	Aug-13
Mean	1.610		
Std Dev	0.753		

NO INJECTION, HIGH OD

Female Number	ELISA OD	Return to Hatchery	Egg Take
434	1.206	1	Aug-19
458	1.844	2	Aug-19
483	1.925	3	Aug-19
554	1.326	3	Aug-19
555	1.053	3	Aug-19
817	1.468	1	Aug-21
896	2.032	1	Aug-21
1010	1.570	2	Aug-21
1032	2.426	3	Aug-26
1038	0.465	3	Aug-26
1055	2.749	3	Aug-26
Mean	1.642		
Std Dev	0.646		

Appendix 5. cont.

INJECTION, LOW OD

Female Number	ELISA OD	Return to Hatchery	Egg Take	Number Injections
21	0.065	2	Aug-05	1
25	0.068	1	Aug-05	2
52	0.068	1	Aug-05	2
57	0.067	2	Aug-05	1
62	0.065	2	Aug-05	1
63	0.068	1	Aug-05	2
228	0.067	2	Aug-12	1
229	0.067	1	Aug- 12	2
233	0.068	2	Aug- 12	1
260	0.067	1	Aug- 12	2
309	0.066	1	Aug-12	2
311	0.067	2	Aug-13	1
Mean	0.067			
Std Dev	0.001			

INJECTION, LOW OD

Female Number	ELISA OD	Return to Hatchery	Egg Take	Number Injections
389	0.072	1	Aug-19	2
392	0.069	2	Aug-19	1
402	0.071	1	Aug-19	2
408	0.067	1	Aug-19	2
409	0.068	1	Aug-19	2
421	0.069	2	Aug-19	1
430	0.068	2	Aug-19	1
461	0.072	1	Aug-19	2
463	0.073	2	Aug-19	1
482	0.072	2	Aug-19	1
488	0.068	1	Aug-19	2
505	0.071	2	Aug-19	1
Mean	0.070			
Std Dev	0.002			

Appendix 5. cont.

INJECTION, HIGH OD

Female Number	ELISA OD	Return to Hatchery	Egg Take	Number Injections
8	2.629	1	Aug-05	2
26	2.718	2	Aug-05	1
75	2.344	2	Aug-05	1
123	1.410	1	Aug-05	2
141	1.694	2	Aug-05	1
173	1.664	1	Aug-06	2
217	2.198	2	Aug-12	1
237	1.929	2	Aug-12	1
263	0.500	2	Aug-12	1
273	2.885	2	Aug-12	1
280	0.383	2	Aug-12	1
312	0.744	2	Aug-13	1
Mean	1.758			
Std Dev	0.861			

INJECTION, HIGH OD

Female Number	ELISA OD	Return to Hatchery	Egg Take	Number Injections
401	0.332	1	Aug- 19	2
		2		1
413	0.606	2	Aug-19	1
619	2.726	2	Aug-20	1
684	1.894	2	Aug-20	1
768	1.903	2	Aug-21	1
779	1.138	1	Aug-21	2
833	2.904	2	Aug-2 1	1
877	2.829	2	Aug-21	1
907	2.252	1	Aug-21	2
959	1.678	2	Aug-21	2
986	1.191		Aug-21	1
Mean	1.858			
Std Dev	0.846			

Appendix 6. Publications and Presentations.

PUBLICATIONS

Rockey, D.D., L.L. Gilkey, G.D. Wiens, and S.L. Kaattari. 1991. Monoclonal antibody-based analysis of the *Renibacterium salmoninarum* p57 protein in spawning chinook and coho salmon. J. Aquat. Anim. Health. 3:23-30.

Rockey, D.D., P.S.D. Turaga, G.D. Wiens, B.A. Cook, and S.L. Kaattari. 1991. Serine proteinase of *Renibacterium salmoninarum* digests a major autologous extracellular and cell-surface protein. Can. J. Microbiol. 37:758-763.

Wiens, G.D. and S.L. Kaattari. 1991. Monoclonal antibody characterization of a leukoagglutinin produced by *Renibacterium salmoninarum.* Infect. and Immun. 59:631-637.

Wiens, G.D., L.L. Gilkey, D.D. Rockey, J.K. Bishop, J.R. Heidel, and S.L. Kaattari. Qualitative detection of p57 in kidney tissue and ovarian fluid of spawning chinook and coho salmon using a novel field ELISA. Submitted to Diseases of Aquatic Organisms.

Wiens, G.D. and S.L. Kaattari. Bacterial Kidney Disease *(Renibacterium salmoninarum). In:* Fish Diseases II: Viral, Bacterial and Fungal Infections. Eds, R.M.W. Stevenson and P.T.K. Woo. Commonwealth Agricultural Bureau International, Wallingford, Oxon, United Kingdom.

PRESENTATIONS

Gilkey, L.L., G.D. Wiens, D.D. Rockey, J.K. Bishop, and S.L. Kaattari. Development of field ELISA system for the detection of *Renibacterium salmoninarum* in adult chinook and coho salmon. Ann. Meet., Oregon Chapt., Amer. Fish. Soc., Bend, OR, February, 1992.

Kaattari, S.L. The role of a secreted protein (p57) in pathology and diagnosis of bacterial kidney disease. Washington State University, Veterinary School, Pullman, WA. Oct. 7, 1991.

Kaattari, S.L. Development of a rapid immunodiagnostic for monitering of bacterial kidney disease. Northwest Fish Health Protection Agency, Winatchee, WA. Sept. 24-36, 1991.

Kaattati, S.L., J.D. Piganelli, L.L. Gilkey, D.D. Rockey, J.K. Bishop, and G.D. Wiens. The role of a major secreted and cell-associated protein in bacterial kidney disease pathology and diagnosis. USDA, US-Irish Bilateral Agreement: Biotechnological approaches to the culture and the diseases of fish and shellfish. Sept. 14-l 7, 1992.