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Study of Disease and Physiology in the 1979 Homing Study Hatchery Stocks

A Supplement to: Imprinting Salmon and Steelhead Trout for Homing, 1979, by Slatick, Gilbreath and Walch

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INTRODUCTION

The National Marine Fisheries Service (NMFS), under contract to the Bonneville Power Administration, is conducting research on imprinting salmon and steelhead for homing (Slatick et al. 1979, 1980; Novotny and Zaugg 1979). The studies were begun with little background knowledge of the effects of disease or certain physiological functions on imprinting and homing in salmonids. Consequently, work aimed at filling this void was begun by the authors in 1978 (Novotny and Zaugg 1979) and continued in 1979.

In 1979, we examined random samples of normal populations of homing test fish at the hatcheries to determine the physiological readiness to migrate and adapt to seawater and general fish health. At the Manchester Marine Experimental Station, Manchester, Washington, we determined the survival of samples of the test fish maintained in marine net-pens after release from the hatcheries. Hatcheries and stocks sampled are listed in Table 1.

The data collected from random samples were as follows:

1. Physiology.

Gill Na^+-K^+ ATPase. Abnormally low values could be indications that the fish were either in pre- or post-smolt condition, or had been stressed in some way.

Plasma electrolytes. Lower than normal values of Na or Cl could indicate immediate problems of osmoregulation when the fish were introduced to seawater; high values may indicate some dehydration due to stress. Increases in levels of K can indicate kidney failure or nitrogen supersaturation stresses.

Table 1.--Hatcheries and stocks sampled in the 1979 homing studies.

Hatchery	Species	Pathology tag no.	Date[a/] arrived at Manchester	Date of[a/] viral assay	Date of[a/] blood sampling	Date transferred to seawater pens	ATPase profile	Hatchery release dates
Chelan-Leavenworth	Steelhead	6101-6160	4/25/79	4/26/79	4/26/79	4/27/79	Yes	4/26/79
Wells-Winthrop	Steelhead	3001-3060	5/10/79	5/11/79	5/11/79	5/12/79	No	5/9/79
Tucannon	Steelhead	6801-6860	5/14/79	5/16/79	5/16/79	5/16/79	Yes	5/17/79
Carson	Spring chinook	6401-6460	5/01/79	5/04/79	5/04/79	5/03/79	Yes	5/08/79
Big White Salmon	Fall chinook	6301-6360	5/18/79	5/19/79	5/19/79	5/22/79	Yes	5/21/79

a/ Held in fresh water at Manchester for sampling prior to transfer to seawater pens.

Hematocrits and hemoglobins. Values below or above normal ranges usually indicate anemia or dehydration, which can reflect nutritional disease or physiological changes.

2. Fish health

The incidence of diseases during freshwater rearing, as reported in hatchery records describing the treatment of fish, were examined.

The extent of latent bacterial kidney disease (BKD) as determined by indirect fluorescent antibody technique and the presence (or absence) of certain pathogenic viruses were of particular interest.

A histological determination of significant lesions or abnormalities in tissue from the gill, eye, liver, kidney, thyroid, brain, and olfactory sac was undertaken.

3. Survival in seawater net-pens

Periodic assessments of survival and growth were made, and the major causes of mortality were determined.

These surveys were conducted to provide a documentation of the health and physiological (smolt) condition of the populations of fish involved in the tests, especially at the time of imprinting and release. When the marked adult fish return, the data analyzed from the health and physiology surveys should provide us with information that would indicate any adverse influence on survival. Low survival in the marine net-pens caused by poor health or a low percentage of fish transformed through the smolting stages could bias any attempts to relate returns to imprinting.

METHODS AND MATERIALS

Hatchery Sampling

The sampling of fish from the hatchery stocks for health profiles was based on a combination of statistics and economics. Random sampling from

populations ranging as high as 100,000 or more showed that a population with a disease incidence of 5% or greater can be detected from a sample of 60 fish (Ossiander and Wedemeyer 1973). Health survey samples of 60 fish were taken at the hatcheries, and held in circular tanks in fresh water at Manchester. In most cases, the tissue and blood samples were collected within 24 hours after arrival at Manchester.

Sampling for Physiology

Plasma Electrolytes

Sodium, potassium, and chloride ion levels in plasma were determined for the Wells Dam Hatchery steelhead, Salmo gairdneri, and Big Creek Hatchery fall chinook salmon, Oncorhynchus tshawytscha, near the time of release. Profiles of plasma electrolytes of the Tucannon and Chelan Hatchery steelhead and Carson Hatchery spring chinook salmon were determined in fresh water and during seawater culture at Manchester. Plasma sodium and potassium values were determined by atomic absorption spectrometry and chlorides with a chloridometer.

Gill Na^+-K^+ ATPase

During 1979, selected stocks of fall and spring chinook salmon and steelhead trout being reared for release at state and federal hatcheries in the Columbia River drainage were monitored for changes in gill Na^+-K^+ ATPase to evaluate the state of smoltification at release.

From tagged releases, we determined the relationship between the state of smoltification at release and length of migration time from the hatchery to the estuary.

At approximately 2-week intervals during the spring and summer of 1979, 30 fish were removed by dip net from representative ponds or raceways at Tucannon, Carson, Leavenworth (Chelan), and Big White Salmon Hatcheries.

Steelhead from the Wells Dam rearing ponds were sampled only at release. Ten groups of three fish each were anesthestized or killed by a blow on the head at each sampling. After weights and/or fork lengths were determined, approximately equal quantities of gill filaments were removed from the gill arches of each of the three fish in the group (total weight of gill filaments-0.1 to 0.2 g) and processed as described in our previous report (Novotny and Zaugg 1979).

Disease Sampling

Life History of Hatchery Juveniles

Husbandry techniques, disease, and environmental history may have deleterious effects on fish health and smolt quality (Wedemeyer et al. 1979; Folmar and Dickhoff 1979). Many chemotherapeutic compounds used in the treatment of parasitic and bacterial diseases of fish may affect smoltification (Lorz and McPherson 1976), and subclinical infections may be exacerbated by the stress of seawater entry.

The information (Table 2) was obtained from hatchery management and is self-explanatory. Where information was not obtained, the entries have been left blank.

Blood Sample Collection

The fish were lightly anesthetized in an aerated 1:20,000 solution of MS-222. In the larger fish, blood was sampled from the caudal arch with a 1 cc heparinized syringe and a 25 gauge hypodermic needle. Small fish were bled by severing the caudal peduncle and collecting the blood in heparinized capillary tubes.

Blood samples taken for hematocrits (packed cell volume) were

Table 2.--Disease and life history data of juvenile salmonids during freshwater rearing.

Hatchery	Stock	Agency	Species	Date egg take	Date ponded	Feed	Water source	Water temp. °F	Percent mortality (all causes)	Size at release (no./lb.)	Date released (1979)
Chelan-Leavenworth	Chelan	WDG	Steelhead	----	----	Dry & OMP	River	34-56	15.4	4.0	4/26/79
Wells-Winthrop	Wells	WDG	Steelhead	----	----	Dry & OMP	Well & River	∿50	----	4.7	5/09/79
Tucannon	Skamania	WDG	Steelhead	----	----	Dry & OMP	River	40-62	22.0	11.4	5/17/79
Carson	Carson	USFWS	Spring Chinook	August 1977	January 1978	OMP & Dry	Spring & River	41-52	47.0	16.5	5/02/79
Big White Salmon	Spring Creek	USFWS	Fall Chinook	September 1978	----	OMP & Dry	Ground Water & River	42-52	7.6	69.0	5/21/79

a/ Bacterial gill disease.

centrifuged in microhematocrit tubes for 3 minutes in a Clay-Adams Autocrit II[1/] (Snieszko 1960).

Blood samples for hemoglobin determination were either read directly with an A-O hemoglobinometer or collected in 20ul capillary tubes to determine hemoglobin concentration by the colorimetric method described by Bauer (1970).

Viral Assays

In 1978, liver, spleen, and kidney tissues from 60 fish in each test group were sampled, pooled in 12 tubes of 5 fish each, and screened by a private laboratory (Rangen Research Laboratories) for viruses. In 1979, the tissue samples from each fish were aseptically divided into equal portions. One lot was submitted to Rangen Research Laboratories and the other to the National Fisheries Research Center. The results of these independent tests are reported in Appendix A.

Histopathology

Sixty individually numbered fish of each test group were preserved in fixatives and submitted to Bio-Med Research Laboratories. Gill, liver, eye, kidney, thyroid, brain, and olfactory tissues were sectioned, appropriately stained, and examined for any pathologic lesions or abnormalities. See Appendix B.

Bacteriological Assays

The sensitive and highly specific indirect fluorescent antibody technique (IFAT) was used to diagnose latent Bacterial Kidney Disease (BKD) in hatchery populations.

The individually identified fish were opened ventrally and the kidney

[1/] Reference to trade names does not imply endorsement by National Marine Fisheries Service, NOAA.

exposed. Thin smears of anterior and posterior kidney tissue were made on multi-spot slides after piercing the kidney with a sterile inoculation loop. The slides were air-dried and fixed in reagent grade acetone for 10 minutes. The acetone fixed slides were stored at -20°C until they were examined. Prior to the sampling season, 40 positive control slides were prepared in the same manner and stored at -20°C. The control slides were prepared from a clean kidney lesion from a spring chinook salmon from Carson National Salmon Hatchery that was tested and confirmed to have high numbers of BKD organisms.

The IFAT for BKD was originally described by Bullock and Stuckey (1975) and later modified by G. W. Camenisch (unpublished report) of the U.S. Fish and Wildlife Service (FWS), Eastern Fish Disease Laboratory. The complete procedure used in this study is described in our previous report (Novotny and Zaugg 1979).

All dead and dying fish in the seawater pens were collected daily. Each fish was opened from the vent, external and internal lesions noted, and the procedures for culturing vibriosis and other gram negative bacteria (Novotny, Harrell, and Nyegaard 1975) were followed.

The postmortems were classified as follows:

1. Negative (cause of death not determined).
2. BKD (from lesions).
3. Vibrio anguillarum--serotypes 775, 1669, or 7244.
4. Vibrio sp.
5. ERM (enteric redmouth).
6. Furunculosis.
7. Aeromonas hydrophilia (ex liquefaciens).

RESULTS AND DISCUSSION OF HATCHERY STEELHEAD SURVEYS

Chelan Hatchery (Transferred to Leavenworth Hatchery) Steelhead

Gill Na^+-K^+ ATPase

Since the phenomenon of elevation in gill sodium, potassium stimulated ATPase (Na^+-K^+ ATPase) activity was first reported to be associated with parr-smolt transformation in steelhead (Zaugg and Wagner 1973) and in in coho salmon, O. kisutch, (Zaugg and McLain 1970), numerous experiments have been conducted to verify these results and extend observations to other species. As a result, it has been conclusively shown that the rise in gill Na^+-K^+ ATPase activity is one of the many physiological changes which occur at the time of parr-smolt transformation.

The average gill Na^+-K^+ ATPase activity of Chelan Hatchery steelhead sampled in 1979 at Leavenworth Hatchery was not substantially different from 1978 (Table 3). Gill Na^+-K^+ ATPase showed only a small rise in late April (Figure 1) with a peak mean value of 9.4. The absence of a greater increase in activity may have resulted from water temperatures which remained at the upper limit (13°C) for good smoltification during late April and May (Zaugg et al. 1972). The average fork length (20.8 cm) was similar to 1978 (21.0 cm), but the average weight (98.1 g) was up from 1978 (79.4 g).

Plasma Electrolytes

A compilation of data on rainbow trout by Miles and Smith (1968) and Hickman et al. (1964) suggests expected normal or near normal plasma electrolyte values in fresh water of 130 to 172 meq (milliequivalents)/l for Na^+, 1.4 to 6.0 meq/l for K^+, and 111 to 155 meq/l of Cl^-.

Table 4 lists some known values for steelhead trout from available published literature, including data for the Dworshak Hatchery steelhead

Table 3.--Fish health and physiological data for the 1979 homing study fish, and a comparison with the 1978 homing study fish.[a/]

Stock and species	Year	% of latent BKD detectable in the kidney by IFAT				8-day post seawater entry gill ATPase activity-umoles pi/mg pr/h[b/]				Peak freshwater gill ATPase activity-umoles pi/mg pr/h		Hematological data (taken at Manchester upon arrival)		Plasma electrolytes 3/ (Meq/l)							
														Na			Cl			K	
		Anterior	Posterior	Both	Either/ both	Min.	Max.	$\bar{X}$	SD	Date	Activity $\bar{X}$	Mean hematocrit value (%)	Mean hemoglobin g/100 ml	n	$\bar{X}$	SD	n	$\bar{X}$	SD	n	$\bar{X}$	SD
Chelan-Leavenworth steelhead	(1979)	0	1.7	0	1.7	11.2	21.8	16.6	3.6	4-23-79	9.4±2.2	49.8	8.9	10	154.0	±2.2	10	124.8	±2.1	10	2.5	±1.8
	(1978)	13.3	16.7	56.7	86.7	14.2	28.6	19.5	4.3	5-03-78	7.5	43.3	8.9	60	165.0	±14.8	58	130.9	±17.2	60	1.1	±0.8
Wells steelhead	(1979)	1.7	1.7	0	3.4	Not sampled				5-19-79	16.5±9.2	50.8	9.6	39	138.2	±20.0	20	132.9	±11.2	59	1.61	±2.2
	(1978)	16.7	20.0	46.7	83.4	7.1	13.7	10.9	2.8	5-03-78	17.0	55.6	11.4	58	150.3	±11.1	58	107.9	±20.6	58	2.5	±2.6
Tucannon steelhead	(1979)	1.7	0	0	1.7	18.0	27.7	22.8	3.1	5-08-79	25.9±9	53.0	9.2	58	140.7	±11.3	59	127.0	±8.7	58	2.9	±1.7
	(1978)	8.3	3.3	10.0	21.6	10.9	23.4	17.6	4.6	5-08-78	18.2	48.5	9.7	60	159.5	±9.5	59	131.6	±6.5	60	2.4	±2.6
										5-22-78	11.7										
Carson spring chinook	(1979)	10.0	3.3	20.0	33.3	25.8	38.2	31.7	3.4	(up to release) 5-01-79	20.4±5.4	36.7	5.2	10	145.6	±4.2	10	134.1	±2.1	10	3.7	±0.3
Big White Salmon fall chinook	(1979)	0	8.3	0	8.3	Not sampled				5-09-79	13.5	43.6	7.1	53	170.3	±15.6	-	-	-	53	2.4	±1.4

a/ From Novotny & Zaugg 1979.
b/ 10 days for the 1978 homing study fish.
c/ Taken upon arrival at Manchester.

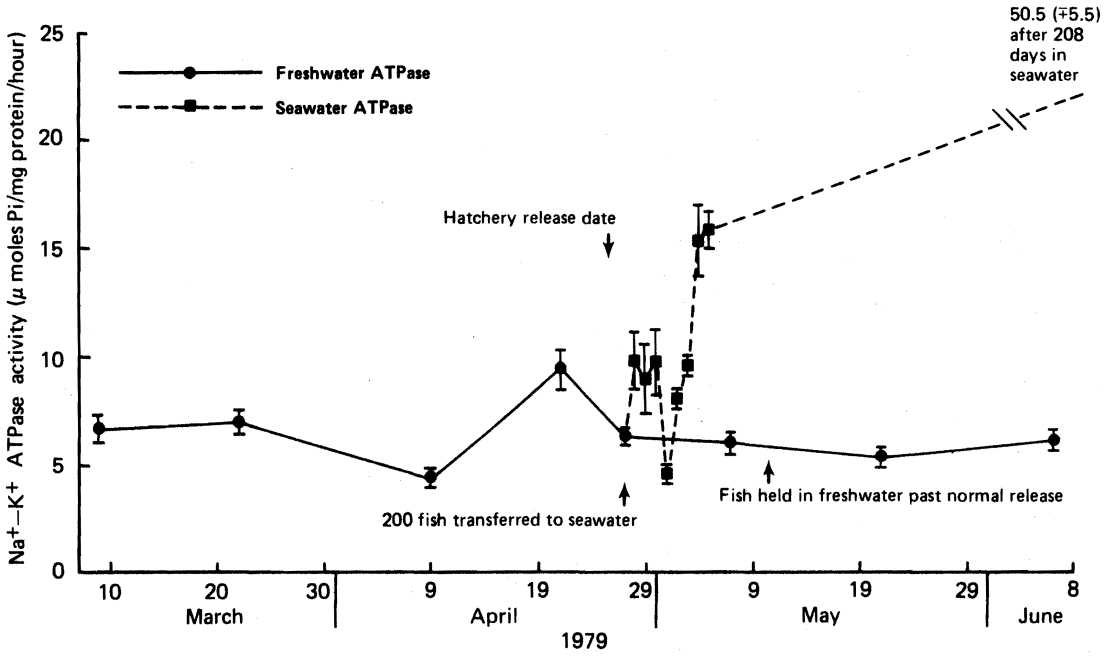

Figure 1.--Gill Na^+-K^+ ATPase activity (means and standard deviations) of the Chelan Hatchery steelhead in fresh water and seawater.

Table 4.--A summary of plasma Na^+, K^+, and Cl^- values in steelhead trout (from published sources).

Condition	Na^+	Cl^-	K^+	Reference
June-July (55 g fish)				
Laboratory test — Freshwater	$\bar{X}$ = 162	range: 140-160	$\bar{X}$ = 6.0	Houston (1959)
Laboratory test — Saltwater (after 36 hours)	$\bar{X}$ = 170	137-185		
March-May (13-15 cm fish)				
Laboratory tests Freshwater (range of mean values)	102-149	105-161		Conte and Wagner (1965)
Spring 1975				
Dworshak Hatchery (at release)		$\bar{X}$ = 134.2 range: 128-138		Newcomb (1978)
Captured at Little Goose Dam (downstream from Dworshak)		$\bar{X}$ = 134.2 range: 128-141		
Laboratory tests (control groups-Spring)	Mean values range from:			
	159 to 169	133 to 138	2.6 to 4.3	Newcomb (1978)
	Individual values range from:			
	155 to 182	128 to 144	2.3 to 5.2	

(Newcomb 1978). Newcomb's data are extensive, represent reasonably large sample sizes (15 to 25), and are probably good approximations of Columbia River steelhead.

The mean plasma Na^+ and K^+ values of the Chelan Hatchery steelhead were within normal limits (Table 3, Figure 2) at the time of release. The Na^+ levels increased in seawater, but returned to normal (in the survivors) after 1 week.

Hematology

There is considerable hematological data in the literature for rainbow trout, less for steelhead trout. From the data summarized in Table 5, it may be possible to estimate the range of hematocrit and hemoglobin values for healthy steelhead. The lower limit of mean hematocrit should not fall below 30%, and mean hemoglobin values below 6 would certainly be suspect. Upper levels are more difficult to define. Snieszko (1960) reports mean hematocrits of 53% and mean hemoglobin levels of 8.7 g/100 ml of blood in rainbow trout of a size comparable to large steelhead smolts. Although our values on steelhead trout (Table 3) were much closer to Snieszko's, Newcomb (1978) reported mean hematocrit levels in steelhead similar to that found by other researchers working on rainbow trout (Table 5). A number of authors (McCarthy et al. 1973; Wedemeyer and Nelson 1975; Wedemeyer and Yasutake 1977) repeatedly suggest that the hematocrit levels of clinically healthy rainbow trout should be between 24 and 43%, with hemoglobins ranging from 5.4 to 9.3 g/100 ml blood, and these values will be used as the expected range for individual fish for the purposes of this report.

The summarized data of the hematocrit and hemoglobin values for the Chelan Hatchery steelhead are presented in Figure 3. There was no difference in mean hemoglobin between 1978 and 1979 (8.9 g/100 ml) nor in

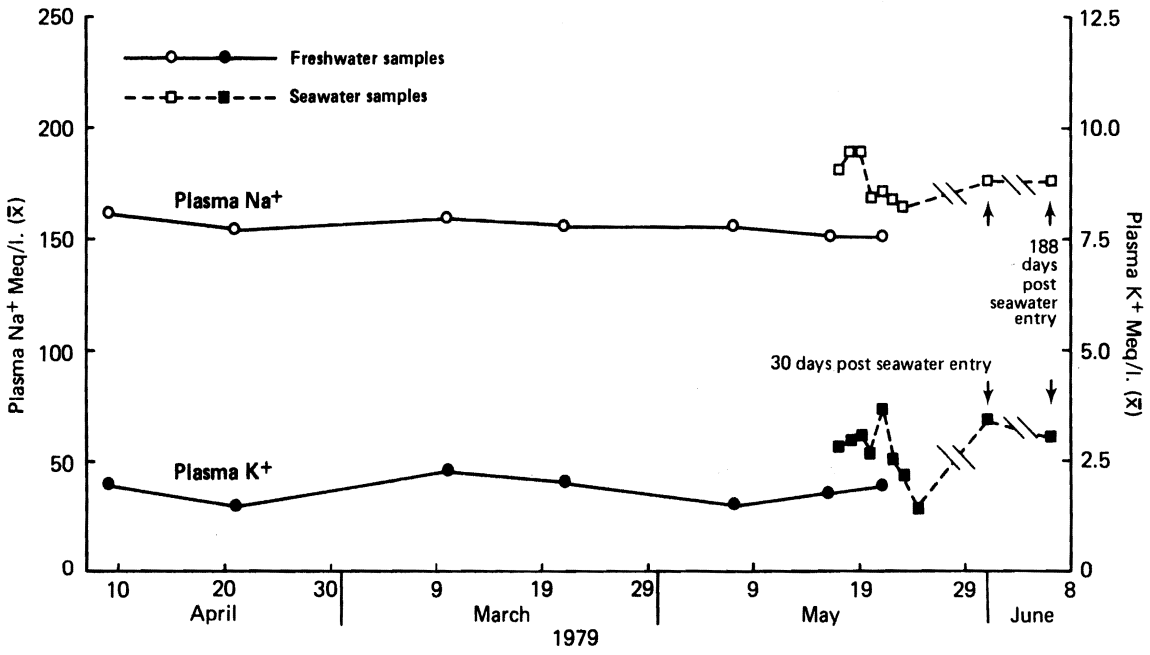

Figure 2.--Mean plasma Na^+ and K^+ levels in Chelan Hatchery steelhead sampled in fresh water at Leavenworth Hatchery and during seawater culture at Manchester.

Table 5.--A summary of hematocrit and hemoglobin values for rainbow and steelhead trout (from published sources).

Source of data	Hematocrit %	Hemoglobin (g/100 ml blood)	References
Rainbow trout	$\bar{X}$ = 31.6 S.D. = ± 0.3	$\bar{X}$ = 7.4 S.D. ± 0.15	Houston and DeWilde (1968)
Rainbow trout	$\bar{X}$ = 28.2 to 31.7 (Individuals:) (11 to 44%)	$\bar{X}$ = 6.5 to 7.7 (Individuals:) (2.2 to 13.0)	Barnhart (1960)
Rainbow trout (Kamloops strain)	$\bar{X}$ = 39.5 (30 to 49)	$\bar{X}$ = 7.5 (5.2 to 12.9)	McCarthy, et al. (1973)
Rainbow trout (Shasta strain)	$\bar{X}$ = 34.1 (24 to 43)	$\bar{X}$ = 7.6 (5.4 to 9.3)	Wedemyer and Yasutake (1977), and Wedemyer and Nelson (1975)
Rainbow trout (average 14.2 cm)	$\bar{X}$ = 45.3	--	Snieszko (1960)
(average 23.5 cm)	$\bar{X}$ = 53.0	$\bar{X}$ = 8.7	
Steelhead trout At Dworshak Hatchery (Spring)	$\bar{X}$ = 40.3 (36 to 47)		
At Little Goose Dam (Spring)	$\bar{X}$ = 35.6 (28 to 44)		Newcomb (1978)
Laboratory tests (Spring)	$\bar{X}$ = 31 to 37.8 Individual range:	28 to 45	

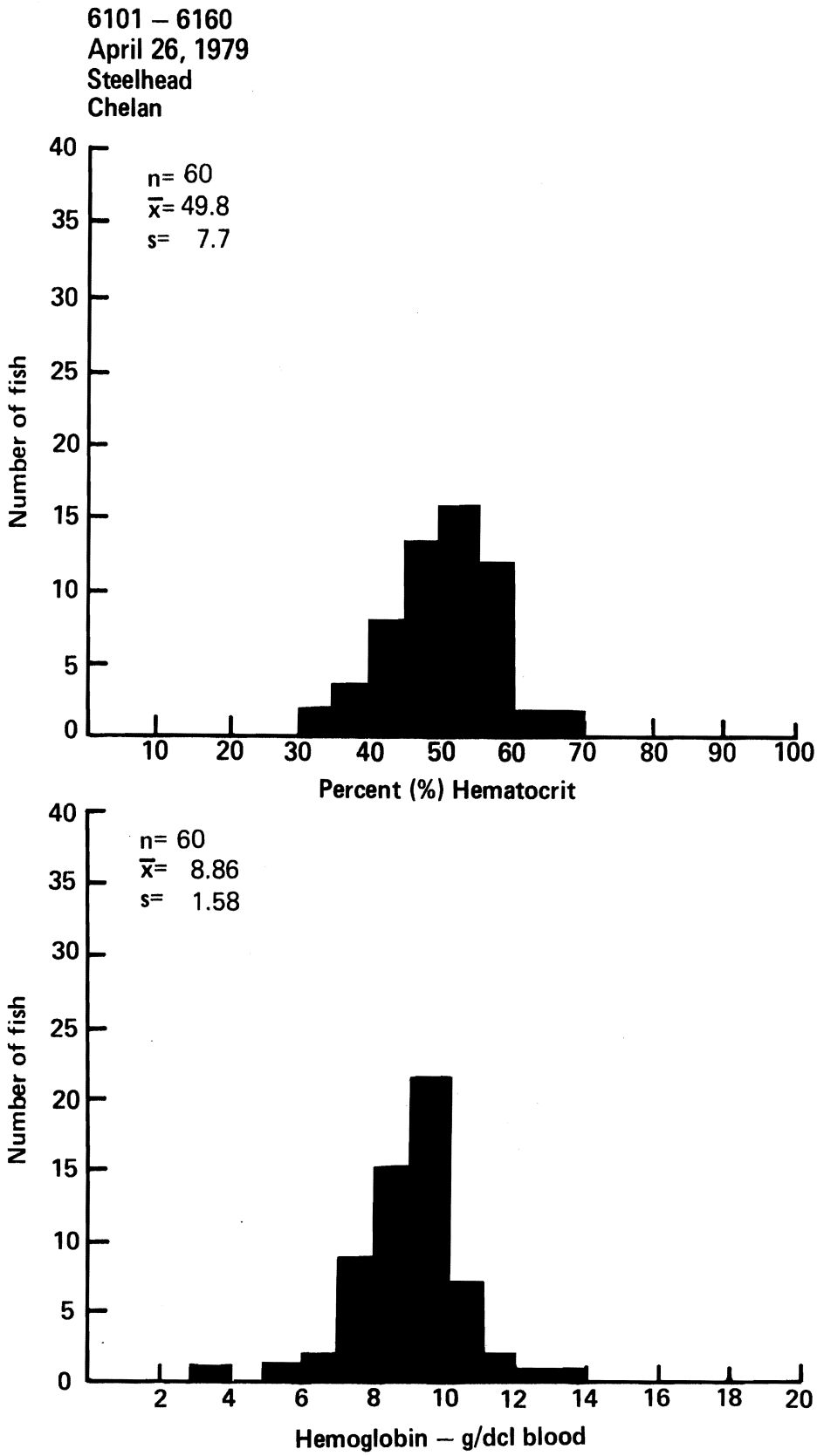

Figure 3.--Frequency histogram for hematocrit and hemoglobin values for the Chelan Hatchery steelhead in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\overline{X}$), and standard deviation (s) are also given.

the range of values. Mean hematocrit in 1979 (49.8%) was slightly higher than in 1978 (43.4%), and 85% of the fish had hematocrits above the expected maximum of 43% (for rainbow trout) in 1979 compared to 50% in 1978. None of the hematocrits or hemoglobins fell below the minimum expected values.

Viral Screening

Both Rangen Research Laboratories and the National Fisheries Research Center (USFWS) indicated that no IPN virus was present in the Chelan (Leavenworth) Hatchery steelhead.

Indirect Fluorescent Antibody Test for Bacterial Kidney Disease.

One posterior kidney smear (1.7%) was found to have a few BKD organisms in the Chelan (Leavenworth) Hatchery steelhead.

Histopathology

A detailed report on the examination and interpretation of selected tissue sections from the random samples is presented in Appendix B. A summary of the pathological conditions observed, their severity, and their frequency of occurrence is presented in Table 6. The severity is ranked as: I--recognizible (least severe), II--intermediate, and III--severe. Note that the incidence of rank II and III severity was low for all conditions encountered (Table 6).

The major pathological conditions encountered in the Chelan (Leavenworth) Hdatchery steelhead were of lymphocyte infiltration and epithelial hypertrophy in gill tissue and the presence of sporozoan parasites.

Records (Table 2) indicate the total mortality in the hatchery was 15%.

Table 6.--Pathological conditions observed in the homing stocks and their percentage of incidence.

Organ & pathology	Incidence (%)																			
	Tucannon steelhead severity a/				Chelan-Leavenworth steelhead severity a/				Wells steelhead severity a/				Big White fall chinook severity a/				Carson spring chinook severity a/			
	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total
Eye																				
Skeletal muscle lesions	0	0	0	0	10.0	1.7	0	11.7	0	0	0	0	0	0	0	0	13.3	1.7	0	15.0
Retrobulbar fat lesions	0	0	0	0	3.3	1.7	0	5.0	0	0	0	0	0	0	0	0	15.0	0	0	15.0
Acute focal hemorrhage	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Focal mononuclear cell infiltration	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0
Retrobulbar pyogranulomatous inflammation	0	0	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	20.0	0	0	20.0
Retrobulbar mononuclear infiltration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.7	0	0	6.7
Gill																				
Increased numbers of lymphocytes	81.7	18.3	0	100.0	71.7	23.3	0	95.0	80.0	0	0	80.0	95.0	1.7	0	96.7	98.3	0	0	98.3
Epithelial cell proliferation	78.3	20.0	1.7	100.0	68.3	26.7	0	95.0	98.3	1.7	0	100.0	95.0	5.0	0	100.0	93.3	6.7	0	100.0
Lymphatic telangiectasis of secondary lamellae	5.0	0	0	5.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solitary basophilic mass in secondary lamellae	6.7	0	0	6.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solitary eosinophilic mass in secondary lamellae	3.3	0	0	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nematode parasite in secondary lamellae	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Focal granuloma in secondary lamellae	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0
Sporozoan parasite in secondary lamellae	0	0	0	0	26.7	0	0	26.7	0	0	0	0	0	0	0	0	0	0	0	0
Focal inflammation at lamellae base	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	1.7	0	0	1.7
Vascular telangiectasis of the secondary lamellae	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0
Mucoputulent gill	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0
General inflammation	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0
Liver																				
Focal mononuclear cell infiltration	3.3	0	0	3.3	6.3	0	0	8.3	1.7	0	0	1.7	34.5	0	0	34.5	24.1	0	0	24.1
Sporozoan parasite	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0				
Nonsuppurative triaditis	0	0	0	0	3.3	0	0	3.3	0	0	0	0	0	0	0	0				
Increased parenchymal fat	0	0	0	0	3.3	0	0	3.3	0	0	0	0	3.4	0	0	3.4				
Lesions typically associated with bacterial kidney disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.2	0	0	5.2
Microgranulomas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.9	0	0	6.9
Capsular parasitic granuloma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Kidney																				
Lesions typically associated with bacterial kidney disease	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.8	0	0	8.8
Microgranulomas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0	0	1.8
Pyogranulomas nephritis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0	0	1.8
Pyogranulomatous periureteritis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0	0	1.8
Dilated tubule with giant bacteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0	0	1.8
Focal tubular degenerative giant cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0	0	1.8
Olfactory sac																				
Ciliated protozoan parasite	53.3	0	0	53.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nematode parasite	3.3	0	0	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyogranulomatous inflammation of the olfactory sac	1.7	0	0	1.7	5.1	0	0	5.1	0	0	0	0	0	0	0	0	25.4	0	0	25.4
Acute focal hemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Inflammation of the olfactory sac	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	1.7	0	0	1.7
Thyroid																				
Perifollicular thyroiditis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.2	0	0	6.2
Brain																				
Mononuclear menigeal infiltration	1.7	0	0	1.7	0	0	0	0	5.3	0	0	5.3	0	0	0	0	1.9	0	0	1.9
Encephalitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.8	0	0	3.8
Other																				
Pharyngeal sporozoan parasite	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pharyngeal nematode parasite	0	0	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a/ I - Recognizable (least severe)
II - Intermediate
III - Severe

Seawater Adaptation

At the time of introduction to seawater, the test group contained mostly transitional and smolted fish, with 7% classified as precocious males. These precocious fish had all died by 82 days of seawater residence. Cumulative losses due to osmoregulatory dysfunction during the first 30 days in seawater were about 28% in spite of 48% of the fish being smolted at seawater entry. Reversion from an apparent smolt or transitional stage to a transitional or parr stage, did take place after 82 days in seawater. This suggests that those fish which were judged to be smolts based upon external characteristics were not physiologically true smolts, or that high seawater temperatures may have forced reversion.

Vibrio Strain 775 was the bacterial pathogen most commonly isolated from moribund fish. The 30-day survival was 59%, and the survival after completion of the tests (207 days) was only 8% (Table 7).

Wells Hatchery (Transferred to Winthrop Hatchery) Steelhead

Gill Na^+-K^+ ATPase

In 1978, Na^+-K^+ ATPase activities were determined on representative samples of Wells Dam Hatchery (rearing pond) steelhead, sampled at Winthrop Hatchery on 3 May. These fish had average gill Na^+-K^+ ATPase activity of 17.0 ($\pm$ 5.1), with values ranging from 11.6 to 26.6. The sampled fish averaged 22.5 cm fork length and 102.2 g body weight and were judged to be in a good smolted condition.

In 1979, the average length and weight of samples transferred to Manchester were 21.7 cm and 97.0 g (respectively) on 11 May 1979. The average gill Na^+-K^+ ATPase activity from Wells Hatchery fish at Winthrop Hatchery on 19 May was 16.5 ($\pm$ 9.2), with values ranging from 8.2 to 37.1.

Table 7.--Survival during seawater culture periods and causes of mortality.

Stock and species	Seawater survival in net-pens						Causes of mortality during seawater culture					
	N (start) and date	Survival (%) 30 day post entry	N (end) and date	Days in seawater	% survival to end	% mortality per day	Negative for pathogenic bacteria (%)	BKD (%)	Vibrio (%)	ERM (%)	Furunculosis (%)	Other (%)
Chelan-Leavenorth steelhead	200 4-27-79	59	16 11-20-79	207	8.0	0.44	3.4	0	34.8	0	0	61.8
Wells steelhead	200 5-11-79	17.5	9 11-20-79	193	4.5	0.49	0	0.7	4.6	0	0	94.8
Tucannon steelhead	200 5-15-79	48.5	37 11-19-79	190	18.5	0.43	10.0	0	26.7	1.7	0	61.7
Carson spring chinook	200 5-02-79	56.5	41 11-20-79	202	20.5	0.39	Numbers examined were not sufficient to evaluate.					
Big White Salmon fall chinook	150 5-21-79	93.3	11 11-26-79	189	7.3	0.49	7.1	0	85.7	0	0	7.1

Plasma Electrolytes

As in 1978, there were noticeable differences in the plasma electrolytes of the Wells-Winthrop Hatchery steelhead when compared to the other steelhead stocks in these studies (Table 3). There was only one sample taken (at the time of release), but this stock again had the lowest mean Na^+ and K^+ values, even lower than in 1978 (Table 3).

There were no differences in transportation techniques or water quality in 1979 between stocks, thus no obvious stresses were involved. In 1979, plasma chloride was also measured. In 1978, the mean plasma chloride level (108 meq/1) was below the expected value of 111 meg/1 for healthy rainbow trout. In 1979, none of the chloride values ($\bar{x}$ = 133 meq/1) were below normal.

Hematology

The hematocrit and hemoglobin data for the Wells Hatchery steelhead are presented in Figure 4. In 1978, the Wells Hatchery steelhead had the highest mean hematocrit and hemoglobin values of any of the steelhead stocks that we studied (Table 3). In 1979, 84.9% were above the expected maximum (43%) hematocrit level and 51.7% above the maximum expected 9.3 g/100 ml blood hemoglobin value. None of the hematocrit or hemoglobin values were below the expected minimums.

Viral Screening

Rangen Research Laboratories reported IPN virus in 12 out of 12 pooled samples in the Wells Hatchery steelhead, and the National Fisheries Research Center (USFWS) reported all samples negative for IPN virus.

Indirect Fluorescent Antibody Test for Bacterial Kidney Disease

Two out of 60 fish sampled (3.3%) from the Wells (Winthrop) Hatchery steelhead were found to have light BKD infections in anterior or posterior kidney.

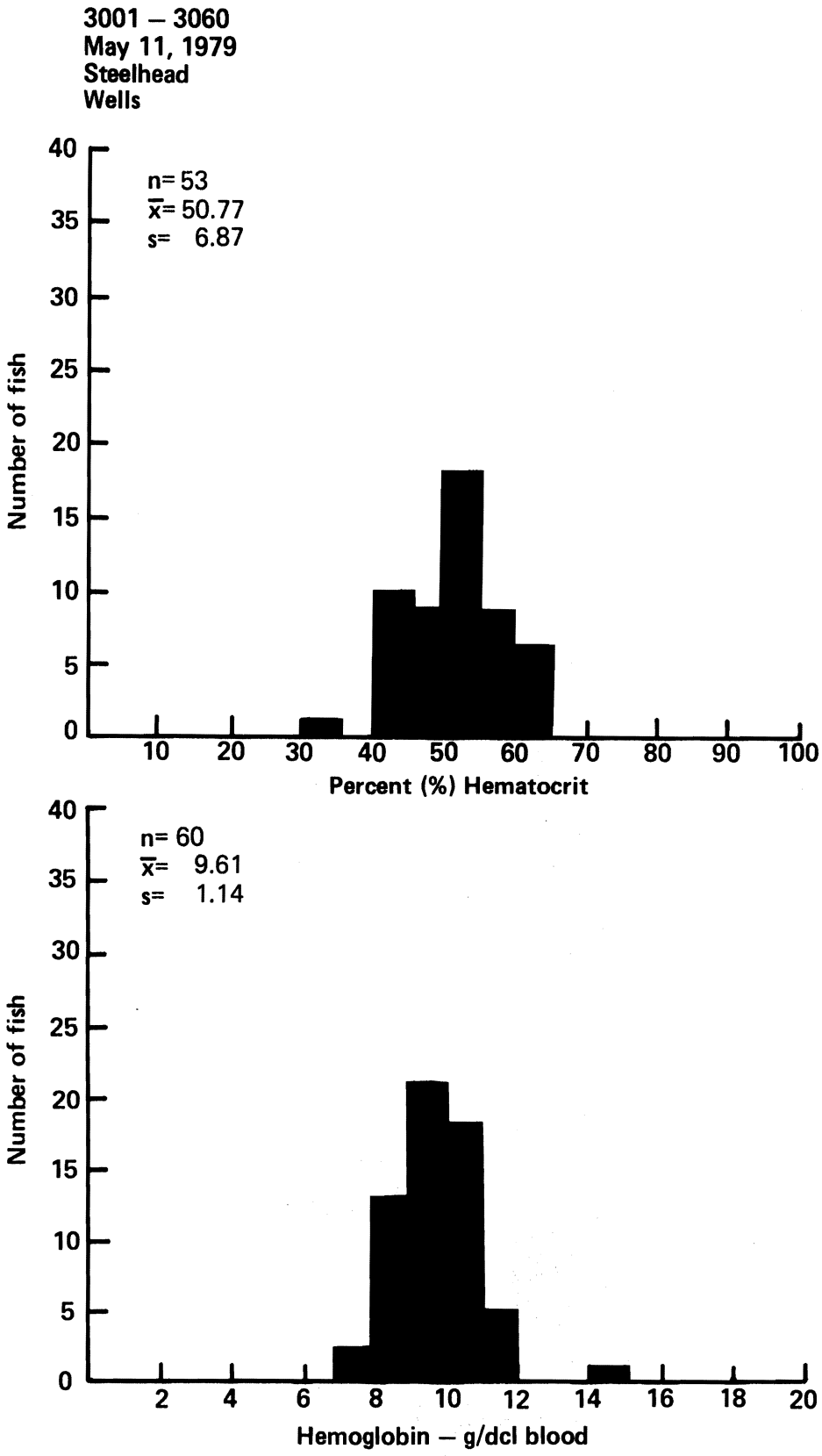

Figure 4.--Frequency histogram for hematocrit and hemoglobin values for the Wells (Winthrop) Hatchery steelhead in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviations (s) are also given.

Histopathology

The major pathological conditions encountered in the Wells (Winthrop) Hatchery steelhead were increased numbers of lymphocytes and epithelial hypertrophy in gill tissue (Table 6).

Seawater Adaptation

At entry to seawater, Wells-Winthrop Hatchery steelhead were primarily of transitional stage fish, based upon external characteristics. The cumulative mortality within 30 days of seawater residence attributable to osmoregulatory dysfunction was 73%. Vibriosis accounted for most of the other mortalities for the remainder of the study. The presence of precocious males was not a problem in this test group. The survival to test completion (193 days) was only 5% (Table 7).

Tucannon Hatchery Steelhead

Gill Na^+-K^+ ATPase

The gill Na^+-K^+ ATPase profile of summer-run steelhead from the Tucannon Hatchery in fresh water was qualitatively similar to that observed in 1978 with a distinct peak in enzyme activity in early May (Figure 5) followed by a sharp decline. A typical pulse of Na^+-K^+ ATPase activity was observed when these fish were transferred to seawater at Manchester. Little change occurred until the fourth day when activity began to rise (Figure 5). Fish held at the hatchery and sampled just 5 days after release of the main group averaged 17.2 cm fork length and weighed 43.1 g.

This was down from the average lengths and weights of 19.5 cm and 65.9 g, respectively, at the same time in 1978.

Plasma Electrolytes

The summary data for plasma electrolytes at the time of release are listed in Table 3. The mean values for Na, K, and Cl of the Tucannon

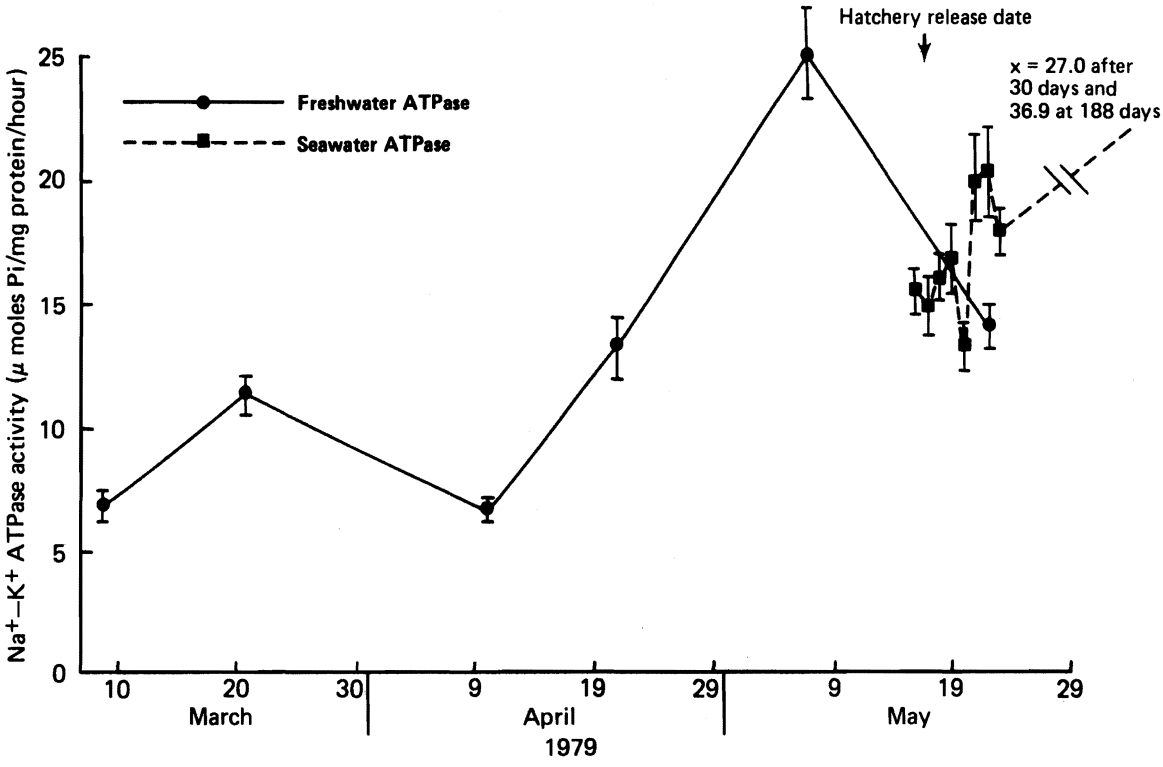

Figure 5.--Gill Na^+-K^+ ATPase activity (means and standard deviations) of the Tucannon Hatchery steelhead in fresh water and seawater.

Hatchery steelhead fall within the expected ranges for rainbow trout. There was little difference in the mean Cl and K values between 1978 and 1979 (Table 3). In 1978, 43.3% of the Tucannon Hatchery samples were below the minimum range reported for K in rainbow trout.

The plasma Na and K profiles of the Tucannon Hatchery steelhead in both fresh and seawater appeared to be within normal ranges (Figure 6). There was a typical rise in Na and K followed by a drop within the first week after transfer to the seawater pens, and a return to normal in the surviving fish after this initial stress period.

Hematology

The summarized data of the hematocrit and hemoglobin values for the Tucannon Hatchery steelhead are presented in Figure 7 and Table 3. The mean hematocrit was slightly higher in 1979 than in 1978 and the mean hemoglobin value was slightly lower than the 1978 mean.

Viral Screening

The National Fisheries Research Center (USFWS) reported all Tucannon Hatchery steelhead samples tested as negative for IPN virus. Rangen Research Laboratories reported IPN virus in 1 out of 12 pooled samples tested.

Indirect Fluorescent Antibody Test for Bacterial Kidney Disease

Only 1 out of 60 Tucannon Hatchery steelhead sampled (1.7%) was found to have BKD organisms in an anterior kidney smear.

Histopathology

The major pathological conditions encountered in the Tucannon Hatchery steelhead were lymphocytic infiltration and epithelial hypertrophy in gill tissue, and a 53% incidence of ciliated protozoan parasites in the olfactory sac (Table 6). Total mortality during rearing in the hatchery

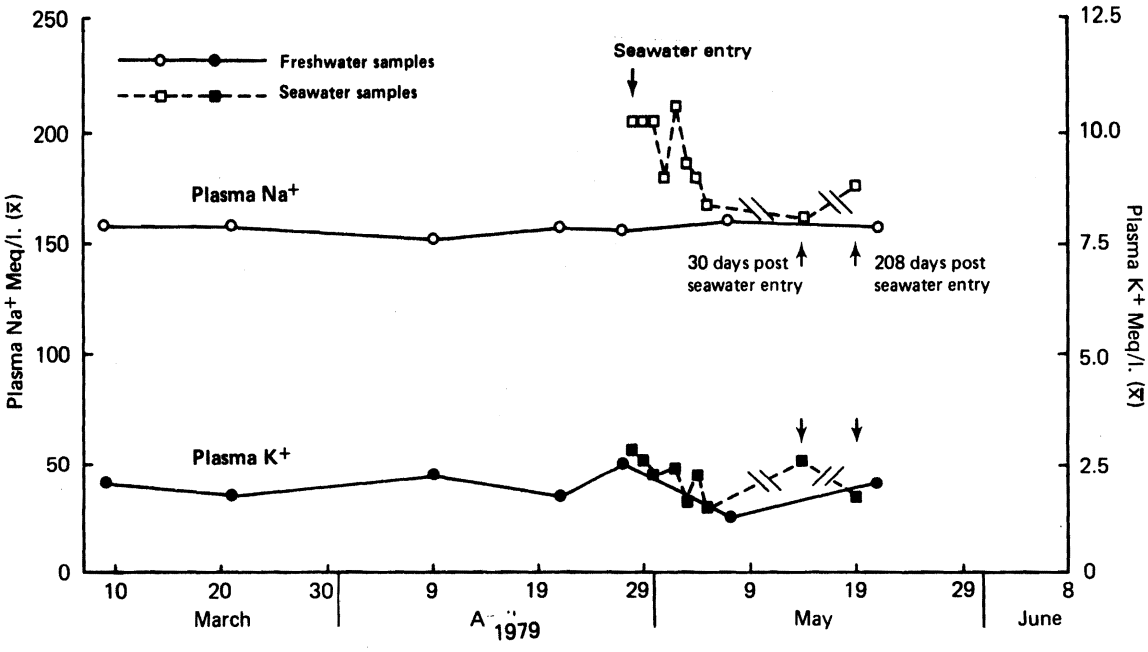

Figure 6.--Mean plasma Na^+-K^+ levels in Tucannon Hatchery steelhead sampled in fresh water at Tucannon Hatchery and during seawater culture at Manchester.

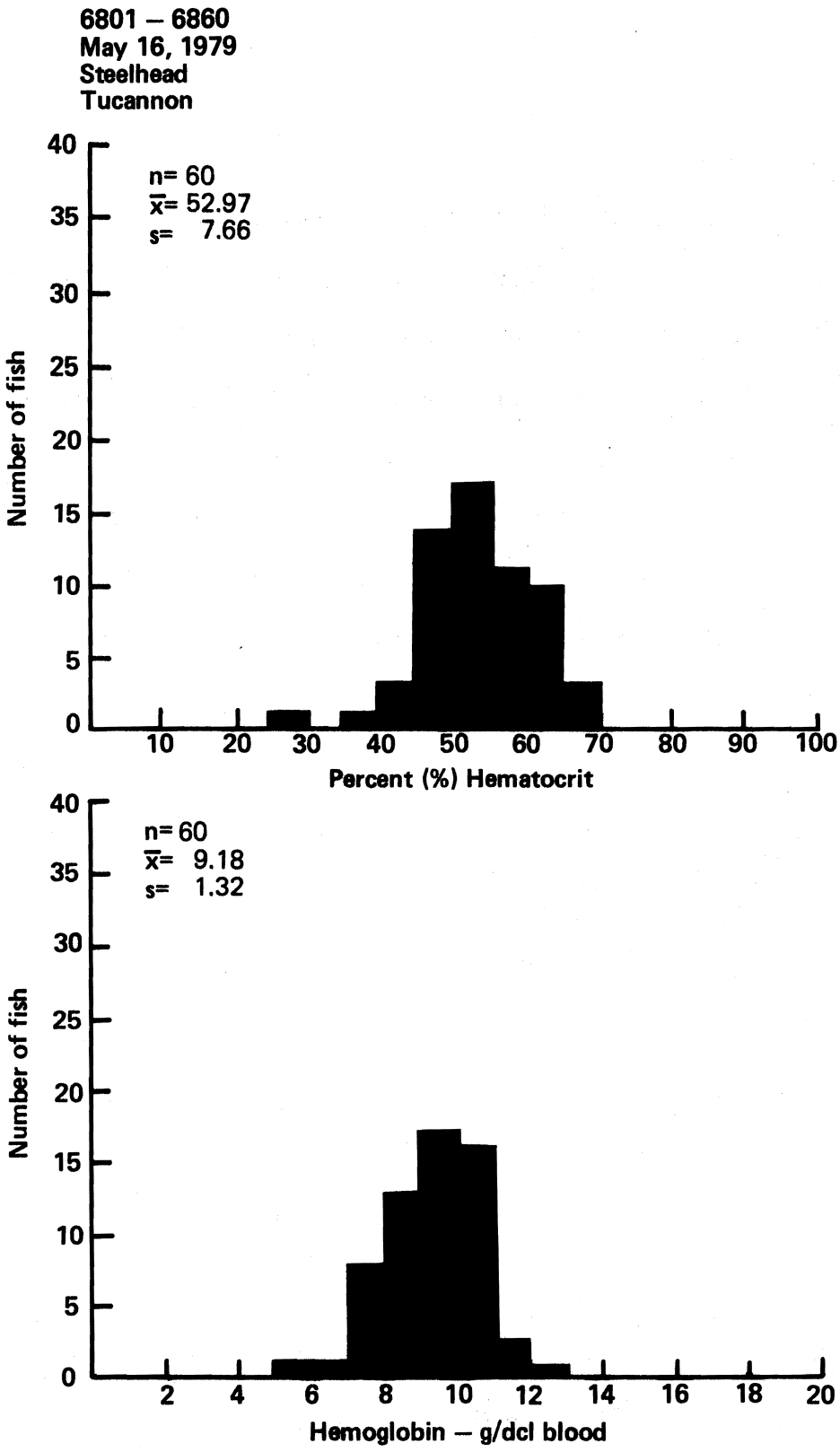

Figure 7.--Frequency histogram for hematocrit and hemoglobin values for the Tucannon Hatchery steelhead in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviations (s) are also given.

was 22%.

Seawater Adaptation

At seawater entry 66% of the Tucannon Hatchery steelhead were judged to be smolts. Within 30 days of seawater residence 51% of the population had died. Osmoregulatory dysfunction accounted for 19% of the initial mortality. Vibriosis was the pathogen most commonly isolated from moribund fish. A large number of fish (25%) were unaccounted for at the end of testing, and the loss may have been due to escape from the net-pen. At completion of the experiment (188 days), 18.5% of the fish had survived. The overall survival may have been closer to 40% if the losses from the net-pen are included.

Summary

The compilation of the 1978 and 1979 data and preliminary analysis of the 1980 data suggest that the generally high mean hematocrit and hemoglobin values in northwest steelhead stocks may reflect a normal hematological condition for these anadromous strains of the rainbow trout, or they may be associated with smoltification.

The pathologist did not find histological lesions typically associated with BKD in the kidney or liver tissue of the three steelhead stocks examined. Although the more sensitive IFAT tests of kidney tissue smears from the same specimens did reveal the presence of BKD organisims, the incidence of the disease was extremely low. There were no known mortalities due to BKD in any of the steelhead stocks sampled during seawater culture, except the Wells Winthrop Hatchery stock. Lesions symptomatic of BKD were found in 0.7% of the mortalities examined, but the disease was not confirmed by IFAT.

Analysis of the pathologist's data indicate that lesions of the eye were much reduced in comparison to 1978. Histopathological conditions observed in the three steelhead stocks were restricted to a few organ systems, and may not significantly effect homing response or survival. However, there were several conditions that appeared in all three stocks, and it may be of interest to summarize the probable causes.

The pathological conditions in gill tissue of the 3 steelhead stocks were predominately lymphocytic infiltration and epithelial hypertrophy. The incidence was higher in 1979, especially in the Tucannon Hatchery fish. These observations are probably indicative of exposure to antigens, including pathogenic and non-pathogenic microorganisms, irritants, or a

mild form of nutritional gill disease. Second in frequency of occurrence was the presence of sporozoan parasites in the gills of Chelan-Leavenworth Hatchery steelhead. Pathological conditions in other tissues were minor, with the exception of a high incidence of ciliated protozoan parasites in the olfactory rosettes or sacs of the Tucannon Hatchery steelhead.

Figure 8 compares the survival of all three steelhead stocks during seawater culture. The survival within the first 10 to 30 days after transfer to the seawater pens was highest in the Chelan-Leavenworth Hatchery stock and lowest in the Wells-Winthrop Hatchery stock. This trend is again apparent in Figure 9 which compares the known (removed) daily mortalities for the first 4 weeks after transfer to the seawater pens. The largest numbers of dead fish were removed from the pen of the Wells-Winthrop Hatchery steelhead. Over 72% of the mortalities that occurred within the first 4 weeks were removed within 1 week after transfer to seawater.

Figure 10 is a comparison (for all three stocks) of the average fork lengths (at release) of steelhead that could be separated into three states of development based on visual observation. These were: (1) heavy parr marks present; (2) fish in transition to smolting, with parr marks still discernible although faint; and (3) silvery smolts; parr marks absent. Although the average length of the Wells-Winthrop Hatchery steelhead was much higher than the Tucannon Hatchery stock, only 28% of the Wells-Winthrop Hatchery stock had the visual appearance of smolts versus 66% for the Tucannon Hatchery stock. The Chelan-Leavenworth Hatchery steelhead were in between, with 48% considered to be visibly smolted. However, the large average size of the Chelan-Leavenworth Hatchery smolts

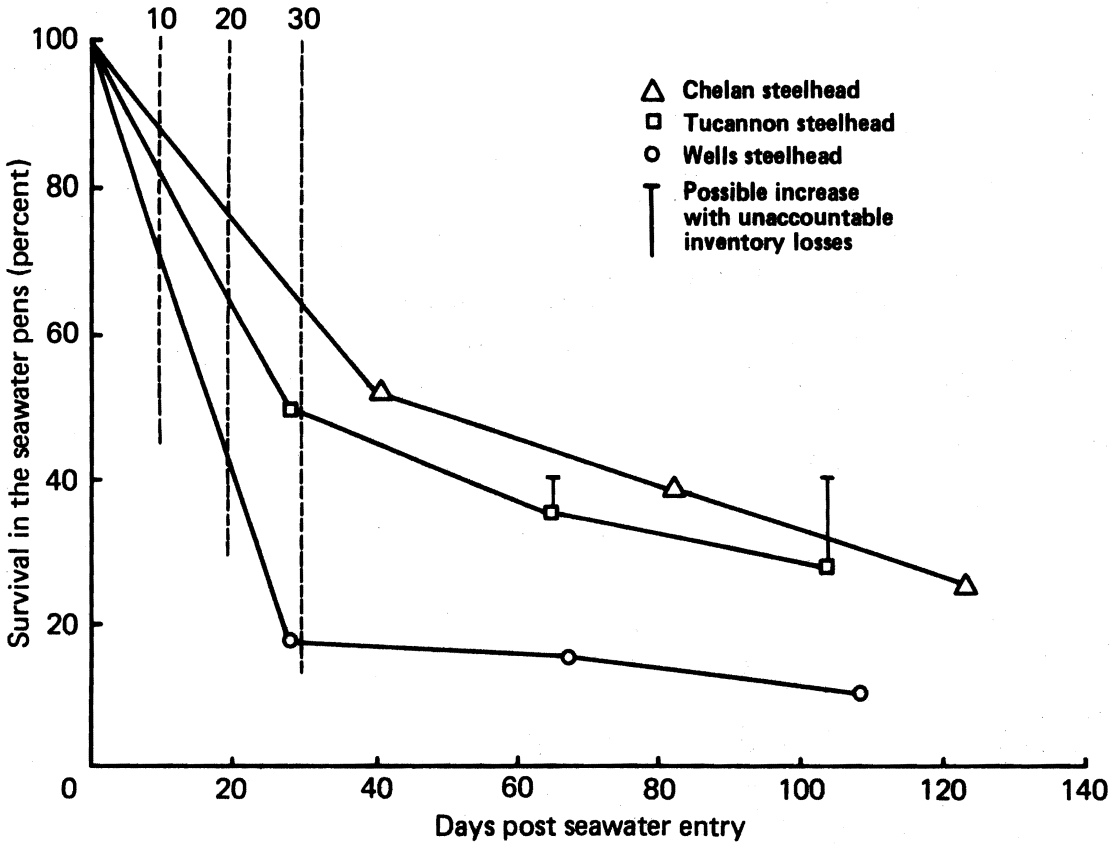

Figure 8.--A comparison of survival during seawater culture of the three steelhead stocks tested in 1979.

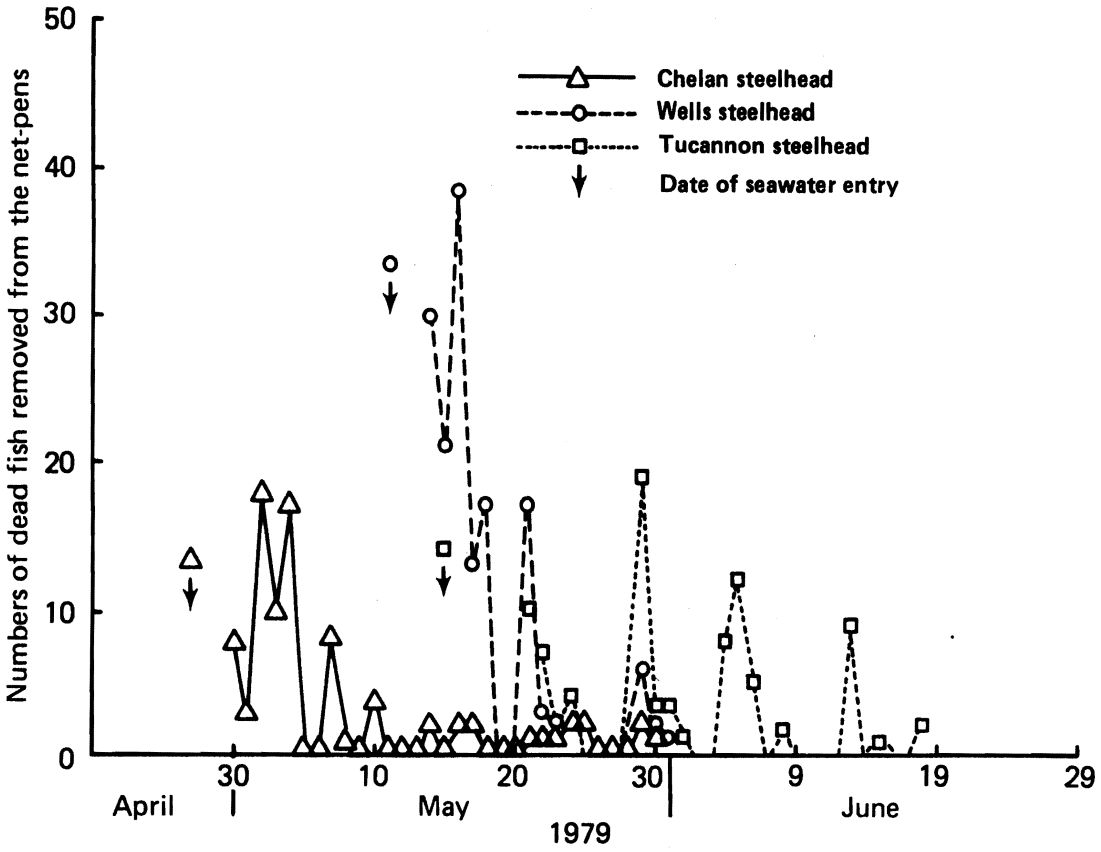

Figure 9.--A comparison of the known daily mortalities (removed) of the three stocks of steelhead after transfer to the seawater pens at Manchester. Each stock began with 200 fish. Mortalities are shown for (approximately) the first 30 days.

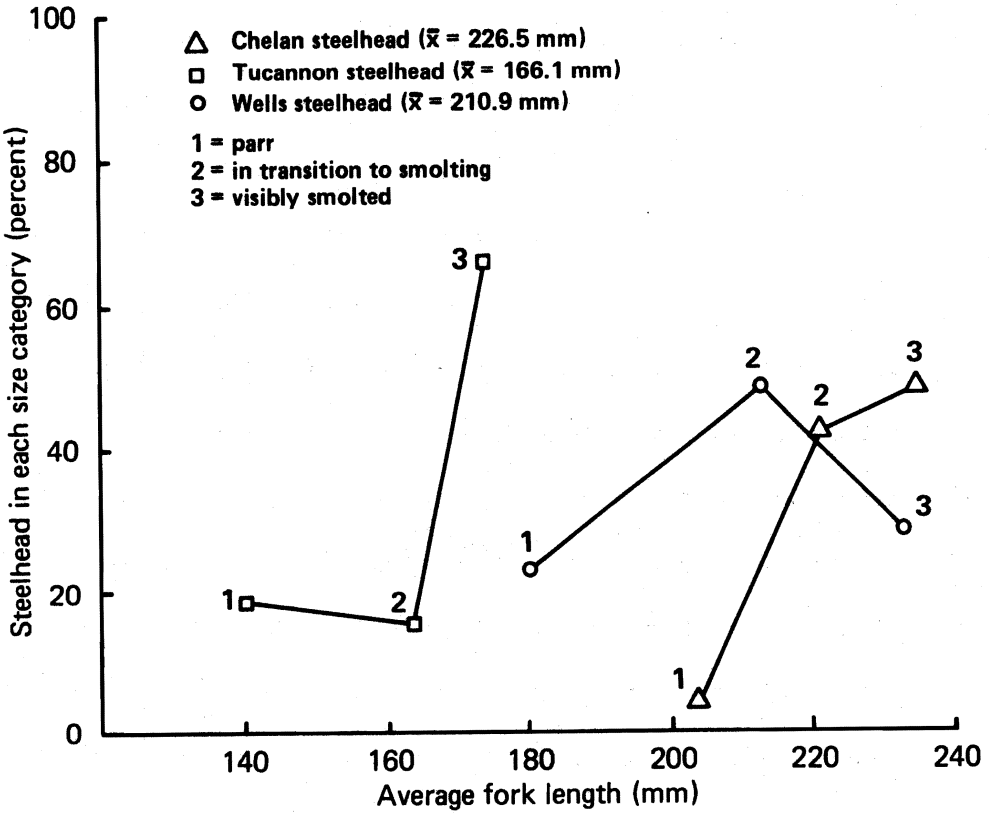

Figure 10.--Relationship of the percentage of abundance of the three visible physiological stages at the time of release to the average size of each state (for all three steelhead stocks studied in 1979).

probably contributed to the greater initial survival after transfer to seawater.

The clinical health status of the Wells-Winthrop Hatchery steelhead was satisfactory. The high initial mortality after transfer to seawater (in comparison to the other steelhead stocks) suggests, however, a severe osmoregulatory dysfunction. We cannot directly compare this type of immediate stress with a supposed transition through the Columbia River estuary because culturing samples of these hatchery test groups in net-pens in seawater is an artificial situation and is recognized as such. Lower survival may not be indicative of what is occurring in nature, as: (1) the fish are transferred directly from fresh to 28°/oo seawater without conditioning in estuarine water (as presumably might be the case in nature); (2) they are fed an artificial diet; and (3) they are contained in net-pens and stressed by frequent (monthly) measurement activities.

Nevertheless, one can assume that if the survival in the net-pens was high, the fish should be able to withstand the normal transition rigors in the wild, and that the tests may be a relative measure of seawater adaptability between treatments or stocks. In comparison to the other two stocks, the Wells-Winthrop Hatchery fish were much less likely to survive any early osmoregulatory stresses.

RESULTS AND DISCUSSION OF HATCHERY CHINOOK SALMON SURVEYS

Carson Hatchery Spring Chinook Salmon

Gill Na^+-K^+ ATPase

Figure 11 is a graph of the gill Na^+-K^+ ATPase activity in 1979. The enzyme values were somewhat higher in 1979 than in 1978. The time during which activity increased appeared to be the same in both years.

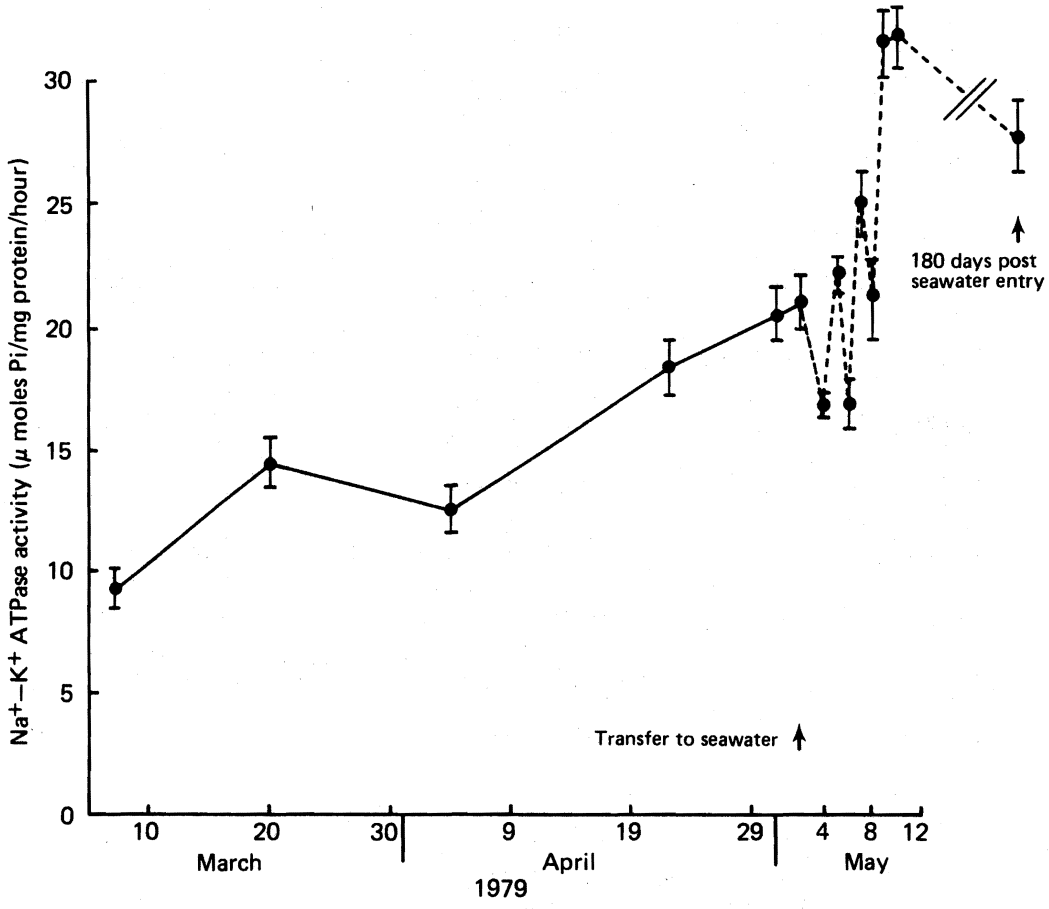

Figure 11.--Gill Na^+-K^+ ATPase activity (means and standard deviations) of Carson Hatchery spring chinook salmon in fresh water, and after transfer to seawater pens at Manchester.

Plasma Electrolytes

There is little published data on normal plasma electrolyte levels in hatchery chinook salmon. Table 8 is a summary of the mean plasma Na, Cl, and K values from chinook salmon that we previously examined. The exceptionally high K values in the Kalama Hatchery spring chinook salmon may be due to hemolysis that occurred after sampling.

There is a slight decrease of plasma Na in the Carson Hatchery spring chinook salmon from 158 meq/l in March until the fish were released in early May (Figure 12). The mean plasma Na value of 145.6 meq/l at this time (Table 3) is within the range of means encountered in other chinook salmon samplings (Table 8). As expected, plasma Na values rose abruptly after transferring the fish to seawater (Figure 12), but quickly returned to normal levels. The mean chloride level of 134.1 meq/l at release is higher than any previously observed mean levels in any of the species studied in 1978 or 1979. The mean plasma K value of 3.7 meq/l was also the highest for any species studied in the past two years, with the exception of the coho salmon. There were fluctuations in the mean K levels after transfer to seawater for the first week, and then a leveling off in the surviving fish to 3 to 3.5 meq/l.

Hematology

Unpublished data from salmon diet studies in Oregon indicate expected mean hematocrits for spring chinook salmon ranging from 24.2 to 38.0% and 35 to 39% for fall chinook salmon. Published data on small fall chinook salmon fingerlings (Banks, et al. 1971) indicate that hematocrit and hemoglobin values increase as the water temperature increases (Table 9).

Table 8.--Mean values of plasma Na, Cl, and K from other samplings of hatchery chinook salmon.

Sample	Millequivalents/l		
	Na^+	Cl^-	K^+
1978 Kalama Falls Hatchery spring chinook salmon (at release)	137	116	11.9*
1978 Kooskia Hatchery spring chinook salmon (at release)	114	104	-
1978 Leavenworth Hatchery spring chinook (at release)	150	108	1.7
1979 Leavenworth Hatchery spring chinook			
March	158	129	3.0
At release (late April)	149	125	0.8
June	148	130	2.3

* These were abnormally high potassium values, and may have been due to some hemolysis of the samples.

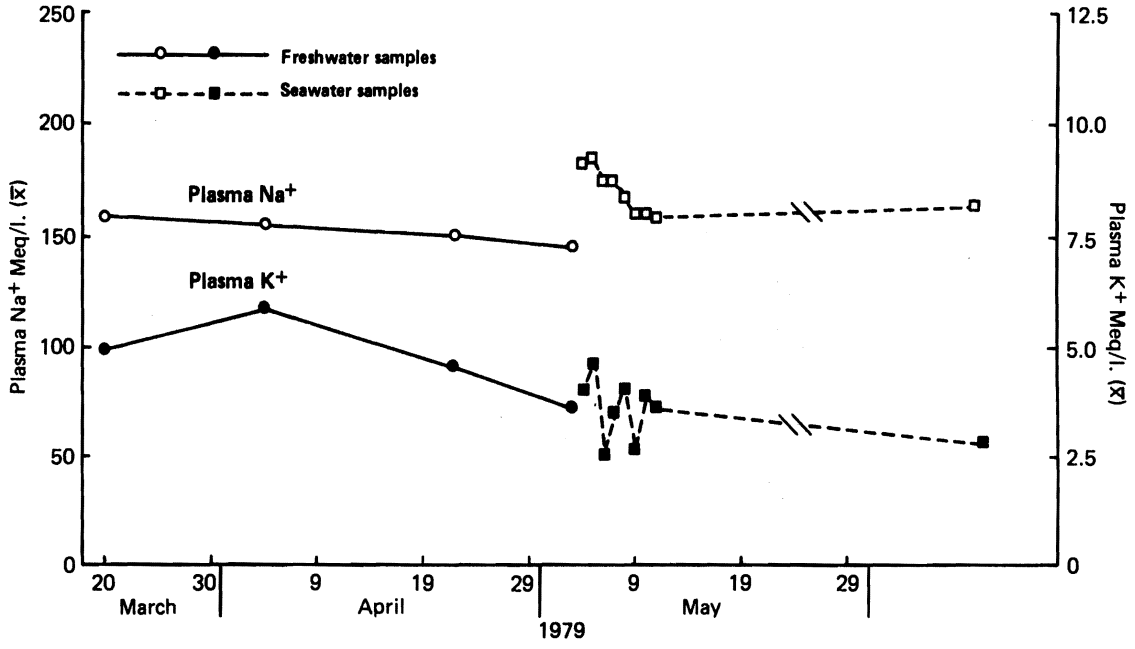

Figure 12.--Mean plasma Na^+ and K^+ levels in Carson Hatchery spring chinook salmon in fresh water at Carson Hatchery and during seawater culture at Manchester.

Table 9.--Hematocrit and hemoglobin values of fall chinook salmon cultured for four weeks at four water temperatures. The average weight of the fish was 3.2 to 4.0 g (from Banks et al. 1971).

Rearing temperatures (°C)	Number of fish	Hematocrit (%)		Hemoglobin (g/100 ml blood)	
		$\bar{X}$	Range	$\bar{X}$	Range
10.0	10	32.2	29-36	5.4	4.5-6.3
12.7	10	35.8	31-40	5.4	4.8-6.4
15.6	10	37.6	32-43	5.9	4.9-6.8
18.3	10	38.9	35-46	6.4	5.4-7.3

The mean values of fall and spring chinook salmon sampled in 1978 (for all studies) ranged from: (1) hematocrits - 36.7 to 59.4% and (2) hemoglobins - 5.2 to 8.9 g Hb/100ml blood. Hematocrit values below 28% in Pacific salmon may be the beginning stages of a number of problems and should signal a cautionary warning.

Although the mean hematocrits and hemoglobins of the Carson Hatchery stock were well within the expected limits, 18.3% of the fish had hematocrits below 28% (Figure 13). Most of the low hemoglobin values ($\leq$ 3.0 g Hb/100 ml blood) were associated with the low hematocrits. Bacterial kidney disease organisms were present in all samples with hematocrits below 25%.

Indirect Fluorescent Antibody Test for Bacterial Kidney Disease

The Carson Hatchery spring chinook salmon had the highest incidence of BKD (as determined by IFAT) for any of the homing stocks screened (Table 3). The total incidence was 33.3%, with 25% of these classified as level III (severe). All fish with hematocrits below 25% were positive for BKD. However, not all BKD infected fish had low hematocrits. Latent infections of BKD may require several years to develop into an active form capable of killing fish in the marine environment (Ellis, et al. 1978). On the basis of the number of fish with heavy intensities of BKD infection in our subsample, we would anticipate some ocean mortality from BKD.

Histopathology (See Appendix B)

The Carson spring chinook salmon had a high incidence of epithelial hyperplasia and lymphocytic infiltration in the gills (Table 6). This inflammatory response is probably the result of exposure to a number of pathogenic and/or non-pathogenic organisms. The relatively high incidence

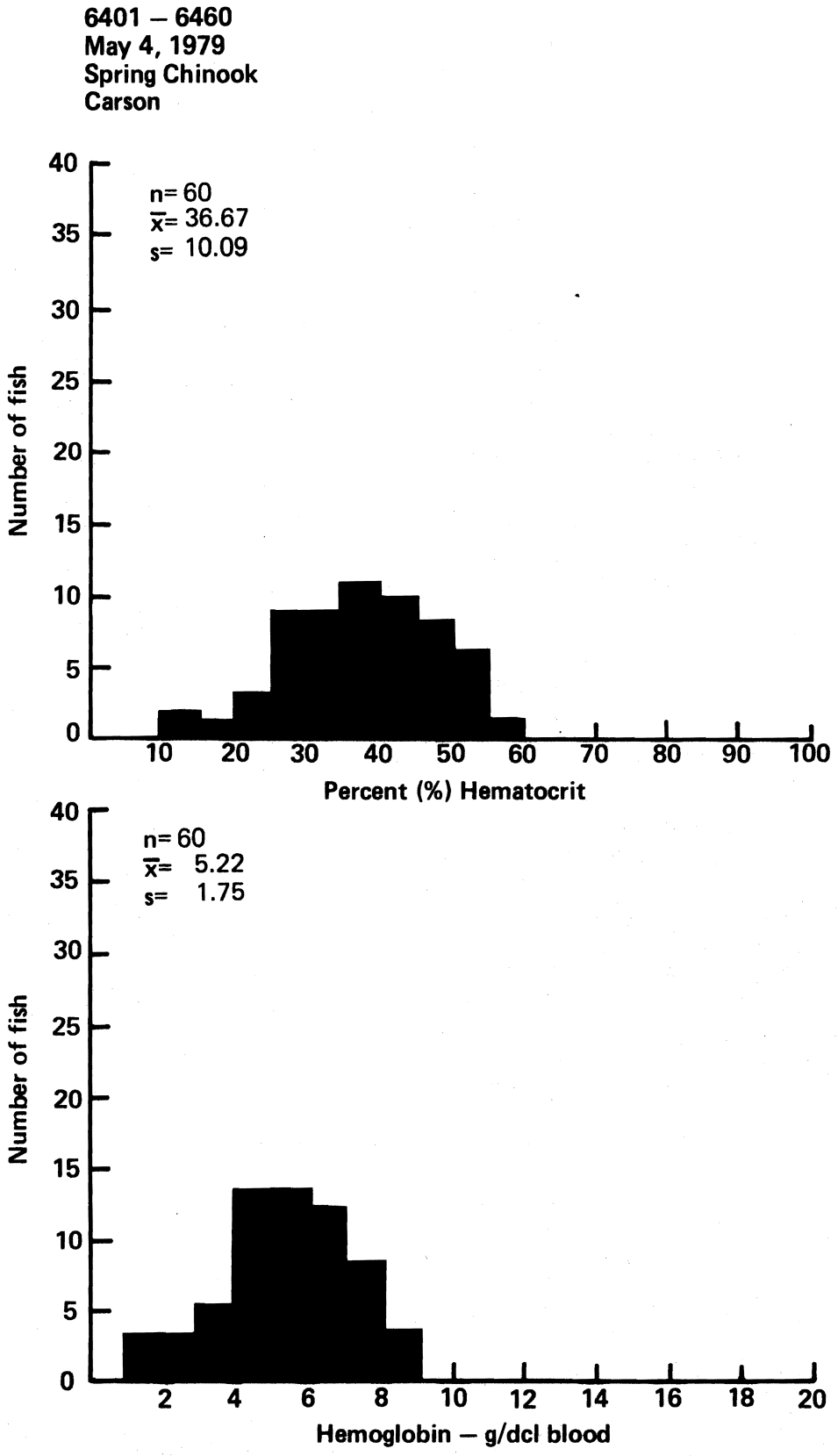

Figure 13.--Frequency histogram for hematocrit and hemoglobin values for the Carson Hatchery spring chinook salmon in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values (X), and standard deviations (s) are also given.

of focal mononuclear cells in the liver is also indicative of possible antigenic stimulation. This is supported by the confirmation of bacterial kidney disease in this stock. In addition, the Carson Hatchery spring chinook salmon had the highest incidence (25%) of granulomatous inflammation of the olfactory sac, which may be associated with gramulomatous lesions of the liver and kidney typical of BKD.

Seawater Adaptation

At the time of seawater entry, the Carson Hatchery spring chinook salmon were visually characterized as primarily transitional (55%) and smolted (39%) fish. The mean weight of the smolted fish was 25.8 g and the mean weight of the population sample was 23.0 g.

In early summer, 13 precocious males were observed in the surviving population. If this represented the maximum knumber in the original population, there would be a minimum loss of 6.5% from precocious maturation.

Initial losses due to osmoregulatory dysfunction were minimal (6%). Further losses occurring during the third and fourth week after seawater entry, were probably due to seawater diseases. The survival during seawater culture is shown in Figure 14, and indicates that approximately 60% of the fish were able to survive the first 30 days.

Big White Salmon Hatchery Fall Chinook Salmon

Gill Na^+-K^+ ATPase

Fall chinook salmon used in the homing experiments were tagged and moved from the Spring Creek Hatchery to holding ponds on the Big White Salmon River. Gill Na^+-K^+ ATPase activities are plotted in Figure 15. Gill Na^+-K^+ ATPase in the homing study fish showed an increase in

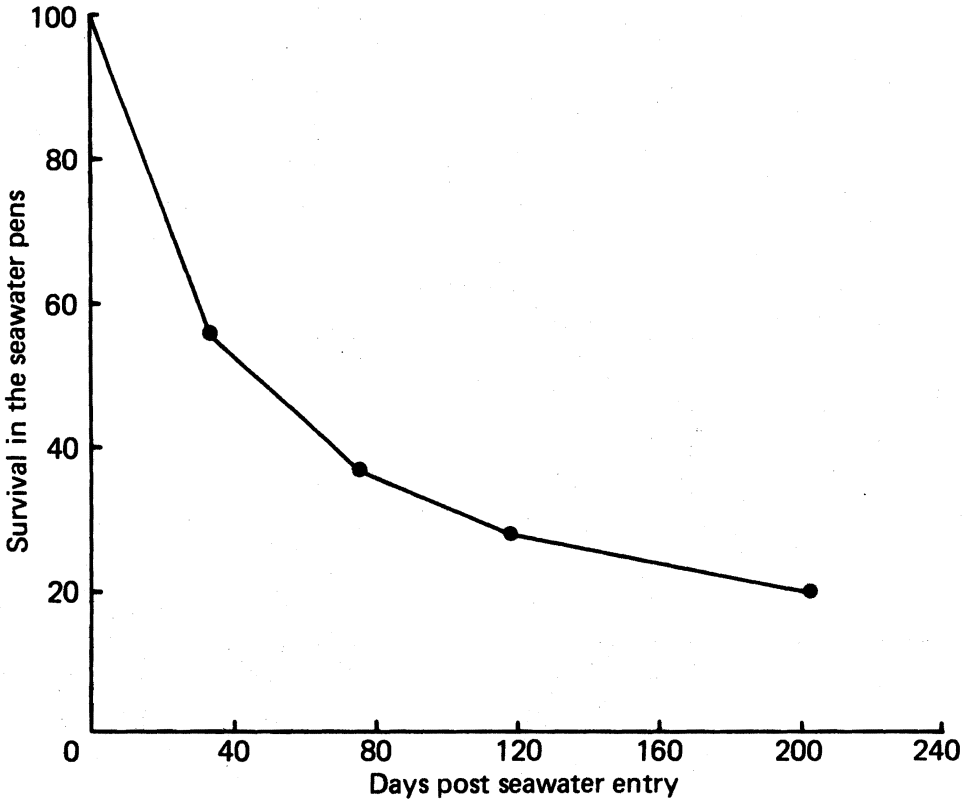

Figure 14.--Survival of the Carson Hatchery spring chinook salmon during seawater culture.

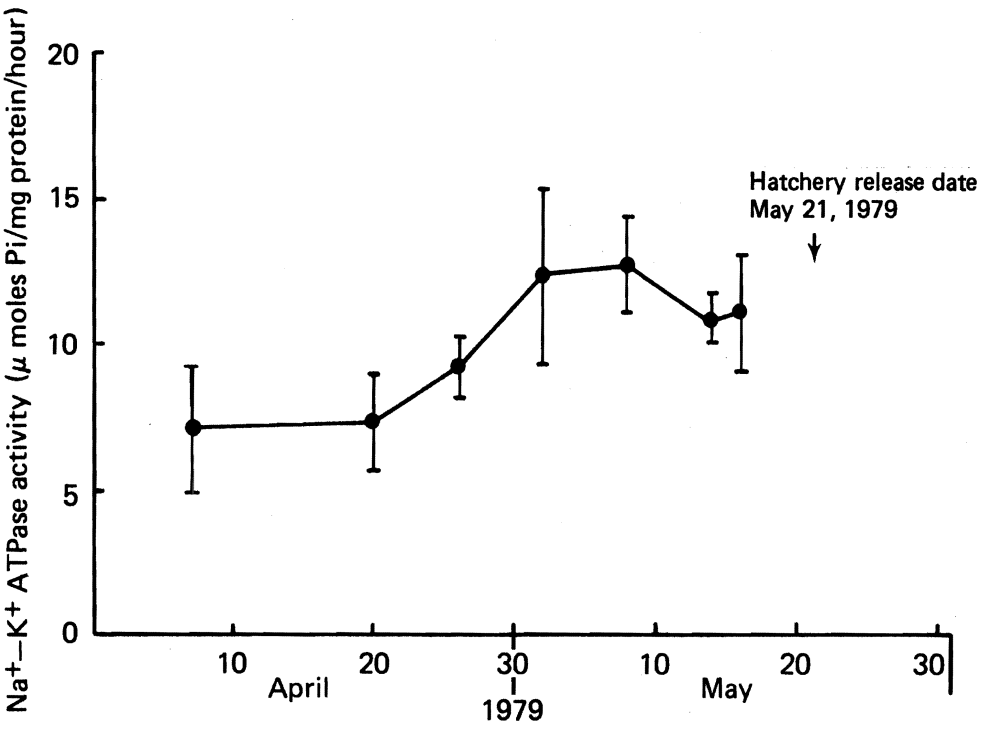

Figure 15.--Gill Na^+-K^+ ATPase activity (means and standard deviations) of Big White Salmon Hatchery fall chinook salmon reared at Spring Creek National Hatchery.

activity at approximately the same time as fish held at Spring Creek Hatchery, though the magnitude of that increase was less. Colder water at the Big White Salmon River and slower growth were factors that probably affected the gill Na^+-K^+ ATPase activity.

Plasma Electrolytes

The mean plasma Na levels of 170.3 meq/l from the Big White Salmon Hatchery fall chinook salmon at the time of release were the highest of any of the species of salmonids sampled in 1978 or 1979. The mean K level of 2.4 meq/l was within normal limits for chinook salmon. Only small amounts of plasma are available from fall chinook salmon, and the volumes were not sufficient to include chloride analyses. No samples were taken after transfer to seawater.

Hematology

The mean hematocrit and hemoglobin values (Figure 16) of the Big White Salmon Hatchery fall chinook salmon were in the upper level ranges for fall and spring chinook salmon sampled in 1978. None of the hematocrits were below 28%, and the hematological data suggested a healthy stock of fish.

Viral Screening

The National Fisheries Research Center (USFWS) reported all of the Big White Salmon Hatchery fall chinook samples negative for IPN virus. Rangen Research Laboratories reported 4 out of 12 pooled samples as positive for IPN virus.

Indirect Fluorescent Antibody Test for Bacterial Kidney Disease

There was an 8.3% incidence of BKD in the Big White Salmon Hatchery fall chinook salmon, all associated with posterior kidney. However, two (out of five) of the fish had moderate (level II) infections. This could represent an eventual loss from BKD after release.

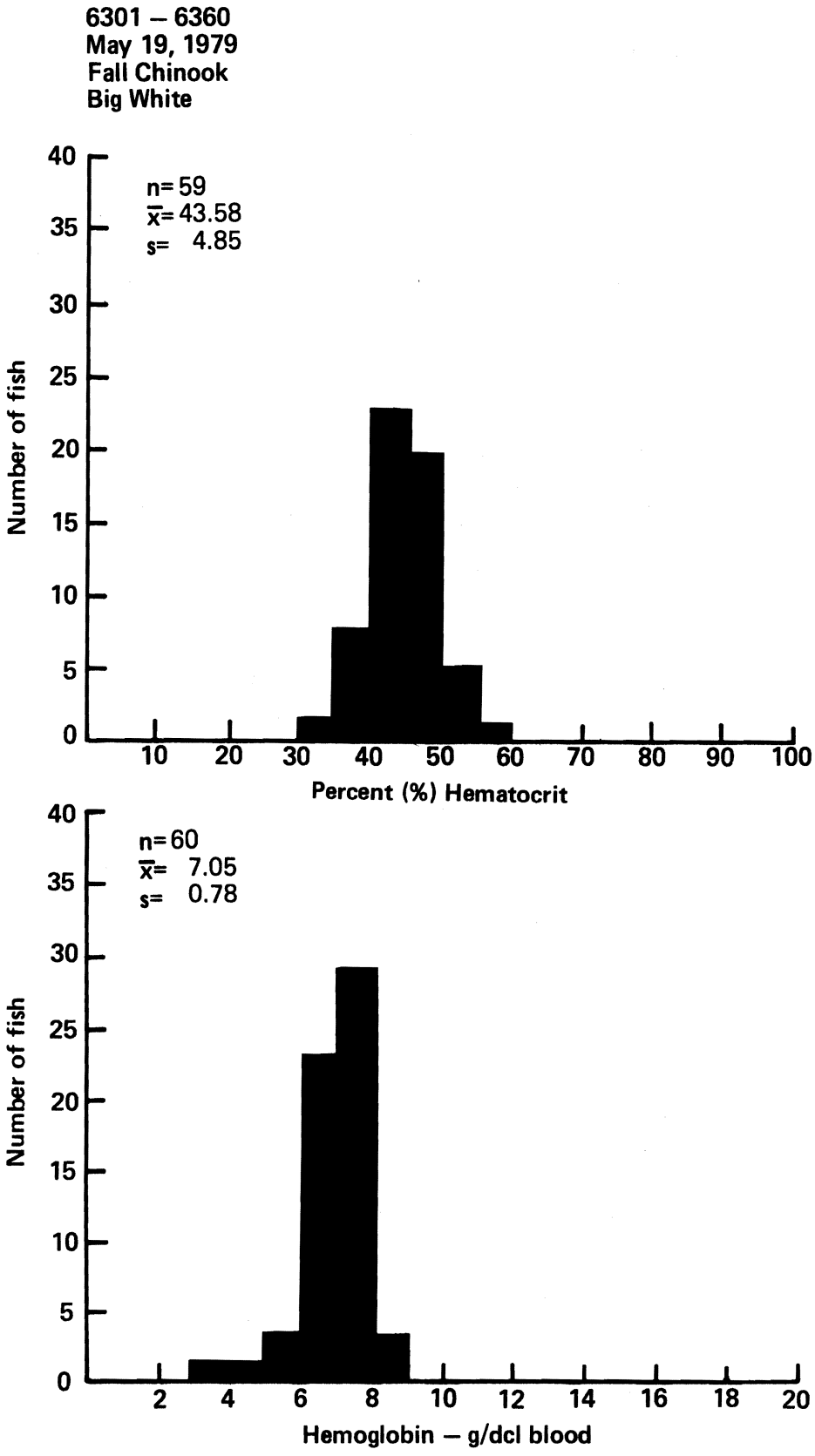

Figure 16.--Frequency histogram for hematocrit and hemoglobin values for the Big White Salmon Hatchery fall chinook salmon in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviations (s) are also given.

Histopathology

As reported for all homing test groups in 1979, the Big White Salmon fall chinook salmon had a high incidence of epithelial cell proliferation and lymphocytic infiltration in the gills (Table 6). This stock suffered mortalities from enteric red mouth disease (Yersinia ruckerii) after they were transferred to the Big White Salmon Hatchery rearing ponds, and this may have contributed to the development of these lesions. The high incidence of focal mononuclear cells in the liver is indicative of possible antigenic stimulation and is consistant with the confirmation of bacterial kidney disease by IFAT.

Seawater Adaptation

The mean weight of the Big White Salmon Hatchery fall chinook salmon sample was 7.6 g when introduced to seawater, and only 15% of the test group were visually characterized as smolts (mean weight of 9.7 g). The majority of the remaining fish appeared to be in the transitional stage. The immediate mortality from osmoregulatory stress was slight, and the survival during the first 30 days in seawater was over 90% (Figure 17). The mortality increased dramatically after 60 days in seawater, and was due primarily to seawater diseases. No precocious males were observed in this test group.

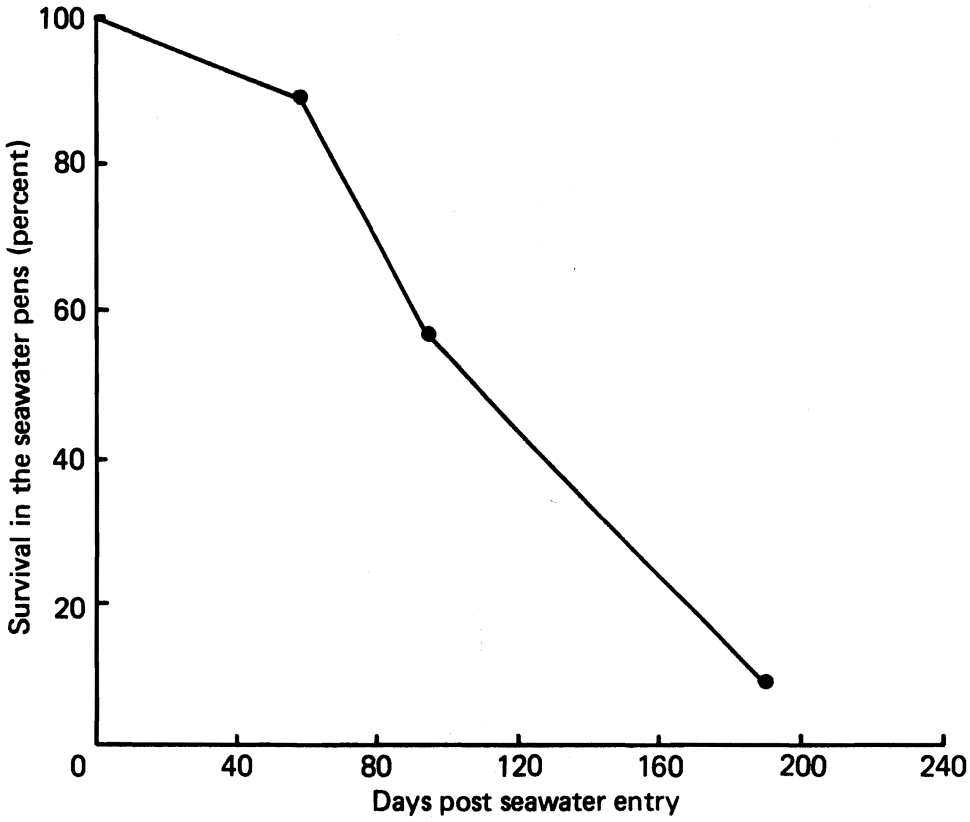

Figure 17.--Survival of the Big White Salmon Hatchery fall chinook salmon during seawater culture.

Summary

We began our first examination of the olfactory or sac in 1979, since this tissue may be the source of "cuing" for any olfactory imprinting. We were not able to measure responses to olfactory stimuli in fish with obvious damage to olfactory sac epithelial tissue, but we have been able to demonstrate that this sensory receptor can be the site of pathological conditions in normal populations.

For example, as mentioned in the section on steelhead, ciliated protozoans in the olfactory sac were found in 53% of the Tucannon steelhead. Histological evidence of any type of olfactory sac pathology was neglible in the two other steelhead stocks, and no pathology was found in the Big White Salmon fall chinook salmon. No parasites were found in the olfactory sacs of the Carson Spring chinook salmon, but evidence of some type of inflammatory lesion was found in over 25% of the Carson fish. The pathologist suggests that the lesions found in both the kidney and olfactory sac are highly indicative of bacterial kidney disease, and that the nares may be a portal of entry for the organism (Appendix B). As a result of this assessment, we examined tissue smears of the olfactory sac by IFAT of the Leavenworth and Carson Hatchery spring chinook salmon from the following year class. BKD organisms were found in many samples, including some in the Class II and III intensity. Until we have sufficient tag return data for any of the 1979 homing study releases of fish, it will be difficult to assess any problems that may have been due to pathology of the olfactory sac.

In 1979, the general health status of the Big White Salmon Hatchery fall chinook salmon stocks was good in comparison to the Carson Hatchery

spring chinook salmon. There were no problems with hematology in the fall chinook salmon, but over 18% of the spring chinook salmon had low hematocrits and hemoglobins. Bacterial kidney disease organisms were found in every kidney sample of fish with hematocrits below 25%. Histological examination of the gills and other organ tissues suggest a serious problem exists with BKD (Appendix B) in the Carson fish. Although the Big White Salmon Hatchery fall chinook salmon had been exposed to some microscopic organisms (Appendix B), this was probably Enteric Redmouth Disease, and at the time of release most of the fish were clinically healthy. There was no evidence of abnormal indicators of smoltification in either stock, but the short-term (30 day) survival of the Carson fish in the seawater net-pens was poor. In general, a combination of health status evaluation factors plus a high incidence of precocious males indicate that losses in the Carson Hatchery spring chinook salmon are going to occur prior to or during the first winter after release.

CONCLUSIONS

Steelhead

1. The general health of the three steelhead stocks in the 1979 studies was good, and there were no indications of any pathology that would impair survival or imprinting, with two possible exceptions: the presence of sporozoan parasites in the gills of over 25% of the Chelan Hatchery fish; and protozoan parasites in the olfactory sacs of over 50% of the Tucannon Hatchery fish. Unfortunately, we cannot evaluate the impact of such parasitic infestations at this time.

2. Observations of external appearances to determine the extent of smoltification indicated that less than 33% of the Wells-Winthrop Hatchery

steelhead were smolted at the time of transfer to seawater (no gill Na^+-K^+ ATPase profiles were available for this stock). Both the Chelan and Tucannon Hatchery steelhead were transferred shortly after peak gill Na^+-K^+ ATPase activities, and had a higher percentage of visible smolts than the Wells-Winthrop fish. This indicates that the Tucannon and Chelan fish were better prepared for the transition to seawater.

3. The survival of the Tucannon and Chelan Hatchery steelhead during the first 30 days after transfer to the seawater pens was two to three times greater than that of the Wells-Winthrop stock, even though the average size of the Wells-Winthrop fish was larger. This indicates that the expected survival of the Wells-Winthrop Hatchery steelhead will be less than survival from the Chelan and Tucannon Hatcheries.

Chinook Salmon

1. The incidence of latent BKD in the Big White Salmon Hatchery fall chinook salmon was low, basic hematology was normal, and the pathology of examined organ tissue reflects the probable exposure to ERM and BKD. The recovery from ERM was apparently successful, and the fish were healthy when transferred.

2. Approximately 20% of the Carson Hatchery spring chinook salmon were anemic, and BKD organisms were found in every fish with hematocrits below 25%. BKD was found in about 33% of the Carson Hatchery fish, and the intensity of infection was heavy in 25% of the infected fish. The presence of granulomatous lesions typical of BKD in 25% of the olfactory sacs also reflects the serious nature of this disease in the Carson fish, and may well affect imprinting as well as survival.

3. The gill Na^+-K^+ ATPase profiles were normal for both the Carson and Big White Salmon chinook salmon, and the major releases were made just after the peaks of Na^+-K^+ ATPase activity indicating good preparation for seawater adaptation.

4. About 90% of the Big White Salmon fish survived the first 30 days in the seawater pens. Slightly over 50% of the Carson fish survived the first 30 days in the seawater pens. This indicates that the chances for survival of the Big White Salmon Hatchery fall chinook salmon are excellent, and the chances for the Carson Hatchery spring chinook salmon are below normal.

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APPENDIX A

THE SURVEILLANCE OF VIRUS DISEASES IN SELECTED HATCHERY STOCKS OF SALMON AND STEELHEAD SMOLT IN THE COLUMBIA RIVER BASIN DURING 1979

CONTRACT NO. 9-79
(EFFECTIVE APRIL 1, 1979 TO MARCH 1, 1980)
USDC/NOAA PURCHASE ORDER NO. 79-ABB-00276

THE SURVEILLANCE OF VIRUS DISEASES IN SELECTED HATCHERY STOCKS OF SALMON AND STEELHEAD SMOLT IN THE COLUMBIA RIVER BASIN DURING 1979.

FINAL REPORT PREPARED JUNE 1, 1980

FOR

NATIONAL MARINE FISHERIES SERVICE
NORTHWEST AND ALASKA FISHERIES CENTER
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RANGEN RESEARCH, A DIVISION OF RANGEN, INC., CONSIDERS THIS REPORT AS SATISFACTION IN FULL OF ALL OBLIGATIONS UNDER CONTRACT NO. 9-79 (NOAA P.O. NO. 79-ABB-00276). WE HAVE APPRECIATED THE OPPORTUNITY OF BEING OF SERVICE TO YOUR ORGANIZATION AND HOPE THAT WE WILL HAVE THE OPPORTUNITY TO DO SO AGAIN IN THE FUTURE.

ANY QUESTIONS WITH REGARD TO THE CONTENTS OF THIS AND ANY RELATED REPORTS MADE UNDER CONTRACT NO. 9-79 SHOULD BE DIRECTED TO:

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I. INTRODUCTION

On the spring of 1978, the National Marine Fisheries Service (NMFS) Manchester, Washington laboratory began an annual study to evaluate various factors pertinent to the successful smoltification, ocean survival, and adult return of selected anadromous salmonid fish stocks of hatchery origin in the Columbia River basin. A portion of this study was devoted to ascertaining the general health profile of each stock at the time of smoltification and immediately prior to hatchery release and natural out-migration into saltwater. The purpose of this general health profile was to determine the occurrence and incidence of selected infectious diseases known to be potentially important to the growth and survival of salmonid fishes in general. The health profile data acquired is then used in the evaluation and interpretation of other data obtained with regard to the relative success of saltwater adaptation, ocean survival, and hatchery return potential.

Migrant hatchery fish stocks were examined for selected viral, bacterial, and parasitic disease agents using a wide variety of techniques as described in the parent NMFS project report. Summary results of the first year of this study pertaining to 1978-79 brood year stocks have been reported by Busch (1979), CZESD and ETSD (1979), Novotny and Zaugg (1979). This report concerns itself only with the work contracted by NMFS to Rangen Research under USDC/NOAA Purchase Order No. 79-ABB-00276 (Rangen Contract No. 9-79) and pertains to that portion of the general health profile dealing with the screening of selected populations for the presence of important viral pathogens known to infect salmonid fish in North America. Included among these pathogens are infectious pancreatic necrosis virus (IPNV), infectious hematopoietic necrosis virus (IHNV), *Herpesvirus salmonis*, and any additional replicating agents capable of inducing specific cytopathic effects (CPE) under the given conditions of surveillance.

The virus disease most commonly associated with Pacific salmon and steelhead trout stocks in the Pacific Northwest is infectious hematopoietic necrosis virus. IHNV was first recognized in 1951 in sockeye salmon (*Onchorhynchus nerka*) at Leavenworth National Fish Hatchery and kokanee salmon, a land-locked sockeye salmon, at Winthrop National Fish Hatchery. The virus was isolated for the first time from sockeye salmon in 1958 (Wood, 1974). The disease is known to cause significant mortality in hatchery populations of Pacific salmon and steelhead trout as well as other salmonid species all along the Pacific coast of North America from California to Alaska and is considered endemic to many watersheds including the Columbia River Basin.

IHNV infection characteristically results in a peracute to acute course of disease resulting in high levels of mortality in chinook salmon (*Onchorhynchus tshawytscha*) and an acute to subacute disease in sockeye salmon. The

disease is characterized, as its name implies, by destruction of the hematopoietic tissues resulting in an acute condition of hemorrhage, anemia, and often severe mortality among fry and fingerling fish under hatchery conditions. Infection seems to be primarily vertical in nature with the disease being transmitted with the eggs and reproductive fluids of asymptomatically infected returning broodstock. The coho salmon (*Onchorhynchus kisutch*) appears to be more resistant to IHNV infection than the other species of Pacific salmon but can function as an asymptomatic carrier in the maintenance and dissemination of the disease (Wolf, 1972).

Survivors of an epizootic infection of IHNV are known to carry the virus in an eclipse phase of intracellular infection typical of the rhabdovirus group of which it is a member. During this asymptomatic carrier state, the virus is not detectable by routine methods of surveillance and is presumed to be non-infectious. This period of subclinical infection includes the time of physiological transition to saltwater known as smoltification. The latent effects of its presence upon smoltification, ocean survival, and adult return are not known at this time. However, the detectable infectious virus has been shown to reappear immediately prior to spawning in infected populations of returning adults and is readily transmitted to the progeny at this time.

The early epizootiology of suspected viral diseases including IHNV in the Columbia River drainage is reviewed by Parisot et al. (1965). Several extensive surveys to determine the incidence and distribution of the virus have been conducted since that time in selected stocks of Columbia River trout and salmon. In 1972, Amend and Wood reported that no IHNV could be found in Columbia River stocks of Pacific salmon returning to 15 selected hatcheries in the state of Washington. These findings were based upon the extensive sampling of 130 to 150 ovarian fluid samples taken from each population at the time of spawning. Tebbit and McMichael (1973) found no evidence of IHNV in 10 Columbia River salmon stocks returning to hatcheries in the state of Oregon during 1971 and 1972. However, in 1973 they reported the confirmed isolation of IHNV from an adult spring chinook salmon stock returning to Oregon's Pelton Dam Holding Facility. Numerous additional studies have been undertaken by various state and federal agencies in subsequent years but have often failed to be comprehensive in design and execution and their results are often not readily available.

Even though IHNV has not been found to be a major problem for Columbia River stocks of chinook salmon, in general, it does continue to pose a threat to this specie as well as sockeye salmon under hatchery conditions. The real occurrence and incidence of IHNV is still not well defined due to problems in the detection of asymptomatic carrier states of infection during the eclipse stage of the virus. Knowledge is also lacking on the possible

residual effects of the carrier state infection on successful smoltification, saltwater survival, and adult return. The disease remains endemic to the watershed and has been increasing in its known host range, geographical distribution, and physical tolerances such as temperature. IHNV has recently been found to be pathogenic for rainbow trout (*Salmo gairdneri*) fry, fingerlings, and yearling fish at 14.5 C. (58 F.) in the Snake River drainage of the upper Columbia River basin. It has also been found in a subclinical infectious carrier stage in rainbow trout broodstock in this same area (Morton and Busch, 1980).

Another salmonid virus, infectious pancreatic necrosis virus (IPNV), has not often been associated with or considered a problem in Pacific salmon stocks. However, IPNV is known to be endemic in the Columbia river drainage where it is primarily recognized as a reovirus disease of trouts and chars. It is also known to be capable of infecting Atlantic, coho, and chinook salmon (Fish Health Section, 1974).

IPNV is considered to be the same disease originally described by M'Gonigle (1941) in brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) in hatcheries in the Canadian maritime provinces and later confirmed by MacKelvie and Artsob (1969). The pathogenesis of IPNV in Atlantic salmon has recently been described by Swanson and Gillespie (1979). The disease was first isolated in the western United States in 1963 by Parisot, et al.

IPNV disease is typically characterized by a peracute to acute course of infection and mortality of fry and fingerling fish or a subacute to chronic infection of larger fish up to and including yearling sizes. The infectious agent is readily transmitted horizontally through the water between fish by means of infected feces and urine from clinically diseased or asymptomatically infected carrier fish. It is also readily transmitted in a vertical manner from asymptomatically infected adult fish to their progeny via infected reproductive fluids and eggs. Survivors of an epizootic infection often remain asymptomatic carriers for life and continually shed the infectious virus into the water, a condition which has recently been modeled on a Chinook salmon cell line by Hedrick, et al. (1978), and established in rainbow trout under laboratory conditions by Reno, et al. (1978). The disease has been shown to be transmitted in the natural environment to susceptible stocks and maintained in wild and feral populations for extended periods of time with the incidence of infection gradually decreasing over time when no new introduction of the virus is made (Yamamoto, 1975). Sonstegard (1970) also showed long term survival of IPNV in the gastrointestinal tracts of selected non-salmonid fishes, picivorous birds, and aquatic invertebrates.

Until 1968, IPNV had not been known to occur in any of the Pacific salmon species. However, in July of that year, Wolf and Pettijohn (1970) isolated the virus from coho

salmon fingerlings at Lamar National Fish Hatchery in Pennsylvania. The eggs of the infected stock had been taken from a spawning population of landlocked adult salmon in Lake Michigan. The fish had been checked for virus as fry and were determined to be free of the infection. However, IPNV was enzootic among hatchery stocks of trout at the time and virus was soon isolated from the fingerling salmon during an epizootic of furunculosis disease (*Aeromonas salmonicida*). No mortality could be attributed to the presence of the virus and evidence indicated that infection was due to horizontal rather than vertical transmission.

Amend and Wood (1972) surveyed 15 Columbia River stocks of Pacific salmon returning to hatcheries in the state of Washington in the fall of 1970, selecting only for IHNV on primary screen and confirmatory cultures. Consequently, no IPNV was reported. In 1973, Tebbit and McMichael reported on the surveillance of Columbia River stocks of Pacific salmon returning to selected hatcheries in Oregon. Their comprehensive design included the examination of visceral tissues and ovarian fluids from 60 adult females from each of four discrete populations. Adult fish and progeny fry were also bled and the sera titered for specific neutralizing antibodies against the common viral diseases. IPNV virus was isolated from two of twelve five-fish tissue pools from adult coho salmon returning to the Bonneville Hatchery in 1971 and also from their progeny fry in thirty or thirty ten-fish pools indicating vertical transmission. It is interesting to note that McMichael (1974) was only able to isolate virus from the fry progeny at 30 and 60 days of age post hatch and that these same fish as fingerlings, when sampled at 90 and 210 days post hatch, no longer yielded detectable levels of virus but did demonstrate specific neutralizing antibodies against IPNV with titers in excess of 1:200 as confirming evidence of prior exposure. Attempts at horizontal transmission of the virus under hatchery conditions were unsuccessful at 15 C.

A report in the FAO Aquaculture Bulletin (1973) indicated that McMichael's coho salmon isolate of IPNV was unlike the standard ATCC UR-299 (American Type Culture Collection) IPNV trout isolate and more typical of French isolates of IPNV that are known to lose 99% of their infectivity in a single freeze/thaw cycle.

When Tebbit and McMichael (1973) continued their sampling program, they found returning adult populations and their progeny fry to be negative for IPNV at all of the selected hatcheries sampled in 1972 and 1973. However, they were able to demonstrate specific IPNV neutralizing antibodies in the sera of 270 adult fall chinook and coho salmon. The overall incidence was 74% in the returning coho and 92% in the returning fall chinook populations. In 1972. they were also able to isolate IPNV from adult coho salmon returning to Cascade Hatchery but the virus

could not be demonstrated in the fry progeny or in the adult returns the following year. Tebbit and McMichael (1973) also reported finding IPNV in adult chinook salmon returning to the Pelton Dam Holding Facility in 1973 after the adult returns and progeny fry were found to be free of the virus in the 1971 and 1972 brood years.

IPNV was isolated from steelhead trout being reared at Idaho Power Company's Niagria Springs Steelhead Hatchery in the Snake River canyon of Southern Idaho near Buhl in 1974. IPNV is known to be endemic to the commercial rainbow trout hatcheries in the local area and its appearance at the Niagria Springs station was not surprising. The virus has since reappeared at the station periodically and has been associated with significant mortality.

In 1974, Wood indicated that he had yet to make any positive IPNV isolations from Columbia River stocks in Washington state. However, in 1975, Tebbit reviewed McMichael's initial surveillance results and continued the program. Again looking at visceral tissue samples, ovarian fluids, and progeny fry, Tebbit was able to demonstrate IPNV in six of twelve five-fish tissue pools from adult coho salmon returning to the Cascade hatchery during the 1972 broodyear, but he could not demonstrate vertical transmission to the progeny fry. That same year Tebbit also isolated IPNV from a population of spring chinook salmon fry at the Pelton Dam Holding Facility that were the progeny of an adult stock that had been previously diagnosed with a confirmed asymptomatic infection of IHNV (Tebbit, 1975).

In 1967, Wolf and Quimby were first able to demonstrate the development of specific viral neutralizing antibodies in the serum of asymptomatically infected IPNV carrier fish. Presence of such specific antibodies are considered as presumptive serological evidence of prior or concomitant exposure to the virus. During the 1973 broodyear, Tebbit was able to detect IPNV specific neutralizing antibodies in the sera of adult coho salmon returning to both the Sandy Hatchery and the Bonneville Hatchery. None of these seropositive populations or their progeny fry yielded a confirmed viral isolation. Tebbit continued surveillance during the 1974 brood year but failed to isolate virus from any of the adult returns sampled but IPNV specific neutralizing antibodies were found in a stock of landlocked spring chinook salmon in the Detroit River impoundment on the North Santiam River in Oregon. An endemic infection of IPNV had previously been demonstrated in wild cutthroat trout populations in these same waters.

Based upon the findings of both IPNV and IHNV in stocks of anadromous salmonids in the Columbia River basin, the state of Oregon established a management policy prohibiting the transport of any Columbia River stocks of fish to a coastal river system for fear of disseminating an endemic viral disease problem.

In 1975, Mulcahy and Sanders reported isolating IPNV from

spring chinook salmon at the Oregon Fish and Wildlife Commission's Corvallis Research Laboratory. More recently, in the first year of this study, Busch (1979) was able to isolate IPNV from 16 of 28 stocks of Pacific salmon and steelhead trout smolts at 12 of 18 Columbia River hatcheries sampled. The incidence of infection among 11 coho salmon stocks sampled was 82%. Of five steelhead trout stocks examined, 75% were found to be infected with IPNV. Four spring chinook salmon stocks had an incidence of IPNV of 60% and eight fall chinook salmon stocks had an incidence of IPNV of 33%. All infections appeared to be asymptomatic carrier states and could not be associated with any significant mortality.

It has been shown time and again that IPNV can indeed infect Pacific salmon and steelhead trout. It is becoming recognized that IPNV may be all too common as an asymptomatic infection in a wide variety of discrete spawning populations in the Columbia River basin and that these infections may be easily missed or not clinically diagnosed in certain types of samples taken at certain stages in the life history of the Pacific salmon and steelhead trout. It still remains to be shown whether or not the presence of an endemic infection of IPNV has a detrimental effect upon the population. Mortality directly attributed to the presence of the virus under hatchery conditions has yet to be demonstrated for Pacific salmon stocks but is known to occur with steelhead trout populations.

The last of three recognized salmonid viruses to be potentially found in the Columbia River basin is *Herpesvirus salmonis*. This virus or other closely related viruses are known to infect rainbow trout and sockeye salmon at all stages in their life history and has been implicated in mortality of both fry and adult fish. The virus was first described in Japan as being endemic among certain stocks of sockeye salmon. It has only been diagnosed once in the United States as an asymptomatic infection of rainbow trout broodstock at the Winthrop National Fish Hatchery. The virus still remains a fairly unknown entity with regard to the general health of Pacific salmon and steelhead trout stocks as its true host range, geographical distribution, and general ecology and epidemiology has yet to be determined (Wolf, et al. 1975).

II. MATERIALS AND METHODS

Procedures of viral surveillance were designed to fit within the overall field sampling program and budget of the parent study and, at the same time, give optimum sensitivity and accuracy with regard to detecting the presence of any of the major salmonid viruses found within the Columbia River basin. Techniques for both surveillance of viral diseases including primary isolation and confirmed serological identification and surveillance of specific

viral neutralizing humoral antibodies were used.

A total of 5 stocks of chinook salmon, coho salmon, and steelhead trout from 5 different Columbia River basin hatcheries (Figure 1 and Table 1) were checked for the presence of virus at the time of smoltification and hatchery release during 1979. Field sampling was conducted by NMFS personnel according to procedures established by and with field sampling kits provided by Rangen Research (Appendix A). Hatchery stocks being sampled were first transported in a live haul container back to the NMFS Laboratory at Manchester, Washington. They were then maintained in freshwater flow-through systems (Beaver Creek water supply) for one to two days prior to lethal sampling. The field sampling diluent provided was formulated (see Appendix B) to provide optimum survival of any infectious virus under transport conditions while inhibiting the growth of any microbial contaminants. It was provided in sterile graduated polycarbonate screw-cap tissue culture grade centrifuge tubes (Corning #25310, Corning Glass Works, Corning, New York). The use of the graduated tube allowed for the calculation of accurate sample dilution factors based upon the displacement method as 0.2 g. of tissue was pooled from each of five fish into 9 ml of field sampling diluent and a total of twleve five-fish pools collected from the randomly selected 60 fish sample.

Serum samples were obtained by lightly anesthetizing 60 fish from each stock in an aerated solution of 1:20,000 tricaine methanesulfonate (Ayerst Laboratories, New York). In most cases, blood was sampled from the caudal arch by means of a 1.0 cc heparinized syringe fitted with a 25 G hypodermic needle. Small fish were bled by severing the caudal peduncle. All whole blood samples were immediately transfered to heparinized micro-capillary tubes, plugged at one end, and centrifuged for three minutes in a Clay-Adams Autocrit II centrifuge. The serum was then transfered to a second micro-capillary tube, plugged at both ends, and placed sequentially in a numbered tube rack.

Following completion of all sampling procedures and the Field Sampling Data Sheet (Appendix A), the complete refrigerated field kit and samples were returned to the Rangen Research Laboratory in Hagerman, Idaho via Greyhound NBO Package Express Service. The average time in transit was 20 hours and the temperature of the samples upon receipt was consistently between 6 and 8 C. A duplicate set of tissues samples were taken from the same fish as per the directions of Dr. Dan Mulcahy and sent to the National Fisheries Research Center in Seattle, Washington for confirmatory viral analysis by a comparable methodology.

Upon receipt into the Rangen Laboratory, the receiving temperature of the tissue samples was recorded. The raw serum samples were stored at 4 C. for a maximum time of five days prior to titration and serum neutralization. The twelve tissue sample tubes were assigned a group accession number and each tube in the group given a

FIGURE 1. GEOGRAPHIC LOCATION OF SELECTED COLUMBIA RIVER DRAINAGE SALMON AND STEELHEAD HATCHERIES SAMPLED FOR VIRUS DURING 1979.

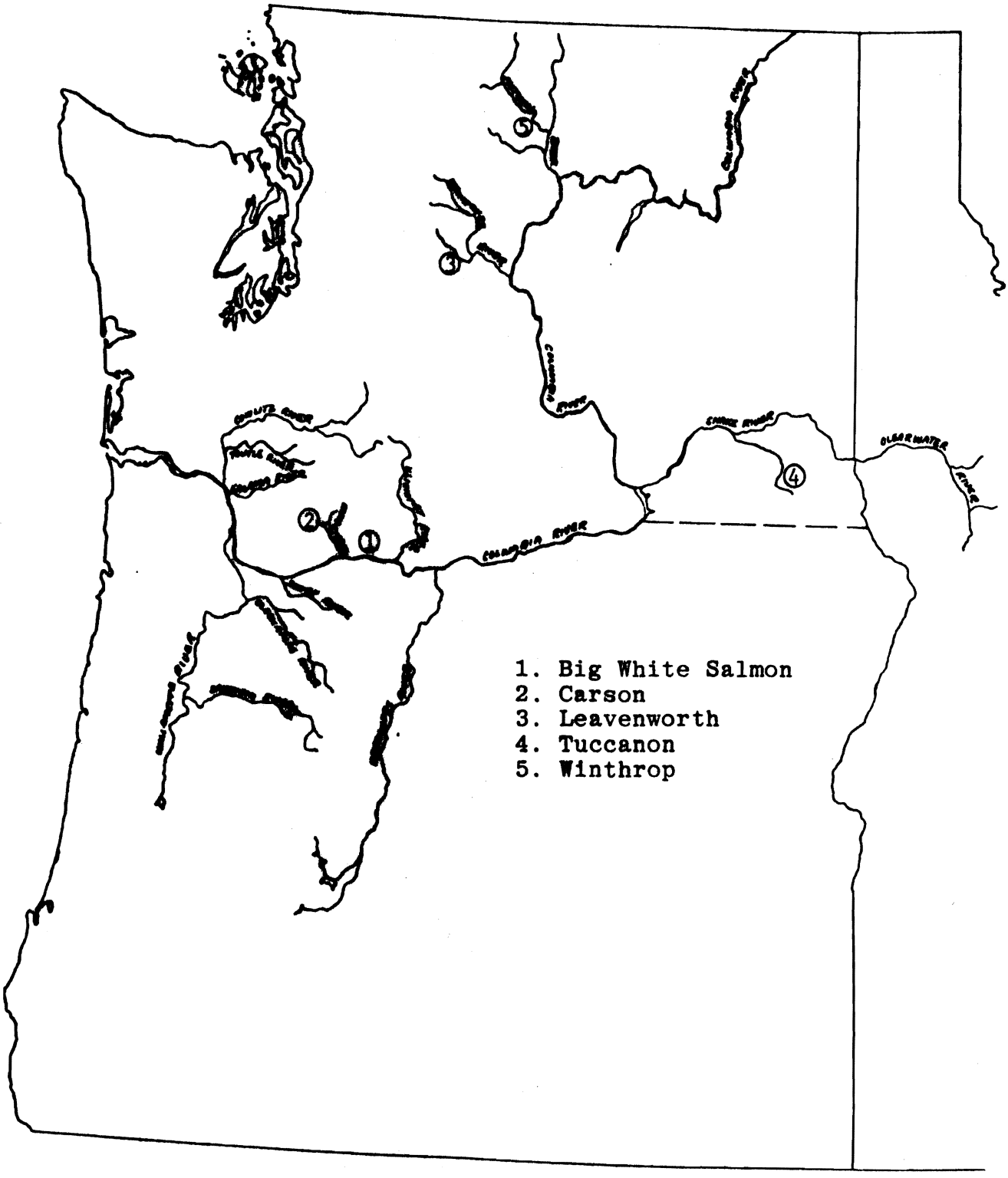

TABLE 1. STOCKS OF PACIFIC SALMON SMOLT EXAMINED FOR VIRUS AT THE TIME OF RELEASE FROM SELECTED COLUMBIA RIVER DRAINAGE HATCHERIES DURING 1979.

STATION	STOCK EXAMINED	DATE SAMPLED	RANGEN ACC. NO.
Big White Salmon	fall chinook salmon	5/24/79	208-79
Carson	spring chinook salmon	5/8/79	179-79
Leavenworth	steelhead trout (Chelan stock)	4/25/79	158-79
Tuccanon	steelhead trout	5/16/79	203-79
Winthrop	steelhead trout (Wells stock)	5/15/79	195-79

serial numeric identifying code. The refrigerated tissue pools were homogenized *in situ* with a Polytron PUC-2-110 homogenizer fitted with a steam sterilizable stainless steel PT-10 generator (Brinkman Instruments, Westbury, New York) for 30 seconds. The homogenized tissue samples were then centrifuged at 2,000 X g for ten minutes at 4 C. in a Sorval RC-5 refrigerated centrifuge with an HS-4 rotor and appropriate tube adapters (Dupont/Sorval, Newtown, Conn.) to remove large cellular debris. Two tenths of a milliliter of the supernatant was pipetted off with a sterile disposable polyethylene tip and diluted in 1.8 ml of a disinfecting diluent (Appendix B) and incubated at 4 C. overnight for decontamination of fungal and bacterial organisms. This procedure resulted in a final 1:100 working dilution of the original tissue sampled. All samples were maintained at 4 C. during all preparatory procedures.

Tissue samples received into the laboratory were screened for the presence of virus within seven days of sampling. Viral screen tests were conducted with a microculture procedure using the Chinook Salmon Embryo (CHSE-214) cell line in passages 267 through 277 and a MEM-10-Tris-PSF media (Appendix B) at 12.5 C. incubation for 21 days. Four replicate wells of each of two dilutions were run for each of the pooled tissue samples. Microculture screen tests were prepared by dropping 0.05 ml of the decontaminated and diluted 1:100 tissue pool into each of the first four wells of an eight well series on a sterile Microtest II (Falcon #3040, Becton, Dickinson, and Co.) tissue culture plate and 0.025 ml of the same sample into the last four wells of the eight well series using an Oxford 8000H sampler and a sterile Oxford 810S tip. During preparation, the microculture plates were temporarily covered with a Falcon #3041 sterile lid and maintained at 15 C. Appropriate IPNV and IHNV positive viral controls and negative media and cell controls were set up for each group tested. After the sample dilutions were delivered to the microculture plate, a stock flask of CHSE-214 cells that had been grown to 95% confluence at 20 C. in MEM-10-Tris-PSF media was examined under an inverted microscope for confluence and quality and dissociated in 4 ml of PAN/EDTA media (Appendix B). The PAN/EDTA cell suspension was centrifuged at 1500 x g for four minutes. The supernatant was decanted and the pelleted cell mass resuspended in a small volume of MEM-10-Tris-PSF media. The resuspended cells were further diluted in MEM-10-Tris-PSF media to a final 1:4 working concentration based upon the surface area split ratio and placed in a sterile 100 ml covered flask containing a sterile magnetic stir bar. The diluted cell suspension was placed on a refrigerated magnetic stir plate with slow stirring to maintain the cells in suspension. A sterile Minipet repipetting syringe (Manostat #71-500-010, Manostat, New York, N.Y.) was fitted with a sterile disposable 18g X 1½" hypodermic needle and primed with the chilled CHSE-214 cell suspension. Fifteen hundredths of a milliliter of the

diluted CHSE-214 cell suspension was pipetted into each well on the inoculated microculture test plate. The plate was immediately sealed with Falcon #3044 pressure sensitive film and incubated at 12.5 C. The suspended CHSE-214 cells were allowed to settle down through the sample material in the well, adsorb infectious virus present, and attach to the bottom of the well.

All viral screen tests were incubated at 12.5 C. for 21 days. All wells of both dilutions of both test and control series were periodically read under an inverted microscope for evidence of specific cytopathic effect (CPE), cytotoxicity, and microbial contamination. The daily observations were recorded on the Virological Examination Report Sheet (Appendix C). If more than four wells in an eight well series were found to be cytotoxic, the original decontaminated sample was diluted 1:2 with additional disinfecting diluent to a final working dilution of 1:200 and re-run on the microculture screen procedure as described above. If more than four wells in an eight well test series were found to be contaminated, the original sample was filter sterilized through a sterile 0.45u membrane filter and re-run on the microculture screen procedure.

At the end of the 21 day incubation period, the 96 wells on each plate (8 wells for each of 12 pooled tissue samples in the lot) were classified as being either positive (definite CPE), questionable (possible CPE or cytotoxicity), or negative (no evidence of CPE or cytotoxicity) and pooled into one of three tubes according to its classification. After the supernatant medias had been aspirated and pooled from the wells of the microculture screen plates for further testing, all wells of the plate were stained with a 1% alcoholic solution of crystal violet and dried as a permanent record of the screen results.

The presence or absence of infectious virus in each of the three classified screen pools was confirmed and identified by means of a microculture serum neutralization procedure. One quarter of a milliliter of sterile field sampling diluent was placed in each of the first three wells of an eight well series on a sterile microculture plate. Twenty five thousandths of a milliliter of EFDL #149 Polyvalent IPNV Antisera (Eastern Fish Disease Laboratory, Kearneysville, West Virginia) diluted 1:100 in sterile field sampling diluent was pipetted into the next two wells of each eight well series. Twenty five thousandths of a milliliter of EFDL #150 IHNV antisera diluted 1:100 in sterile field sampling diluent was pipetted into the next two wells of each eight well series and finally 0.025 ml of EFDL #100 normal rabbit sera diluted to a final 1:100 working concentration in sterile field sampling diluent was pipetted into the last well of each eight well series. Each of the three classified and pooled screen aspirates were then serially diluted out to 10^{-3} by pipetting 0.025 ml of the material into the first well of an eight well series, mixing with the diluent present, and transfering 0.025 ml

into the second well, mixing, and transfering 0.025 ml into the third well and mixing. Twenty five thousandths of a milliliter of the 10^{-3} dilution of the sample in the third well of each series was then transfered to each of the remaining five wells in the series containing the various antiseras. Appropriate positive IPNV and IHNV controls as well as negative media and cell controls were prepared in the same manner. The prepared microculture serum neutralization plates were then temporarily covered with a sterile Falcon #3041 lid and incubated at 15 C. for 60 minutes to affect appropriate neutralization of any virus present. CHSE-214 cells were then prepared to a 1:2 dilution based upon surface area as described above and 0.15 ml of the diluted cell suspension pipetted into the last five wells of each eight well test series on the plate. The plate was immediately sealed with film and incubated at 12.5 C. for five days.

The serum neutralization results were read under an inverted microscope at the end of the five day incubation period. The results were recorded on the Virological Examination Report Sheet (Appendix C). Destruction of the cell monolayer with characteristic CPE in well four, five and eight of a test series indicated the confirmed presence of IHNV in that sample pool. Destruction of the cell monolayer with characteristic CPE in wells six, seven, and eight indicated the confirmed presence of IPNV in that sample pool. Destruction of the cell monolayer with characteristic CPE in wells four, five, six, seven, and eight could indicate the presence of *Herpesvirus salmonis*, a mixture of viral agents, partial neutralization of a particular strain of a virus, or possibly a previously undescribed viral agent in which case additional procedures would have to be applied to confirm identity. All wells demonstrating CPE in an eight well sample series on the serum neutralization plate were pooled and lyophilized in a stabilizer as a reference stock culture. All wells of the serum neutralization plate were then stained with a 1% alcoholic solution of crystal violet and dried as a permanent record of the results.

Sixty individual raw refrigerated serum samples from each stock of fish were screened for the presence of virus specific neutralizing antibodies by means of a microculture modified procedure using the CHSE-214 cell line in passages 267 through 277 and a MEM-10-Tris-PSF media (Appendix B). A virulent IPNV stock culture originally isolated in 1978 from Leavenworth spring chinook salmon as Rangen Path. 138-78-3 (Busch, 1979), was used in all serum neutralization procedures.

The microculture neutralizing serum titration procedure was conducted by pipetting 0.025 ml of sterile field sampling diluent (Appendix B) into each well of an eight well series on a sterile Microtest II (Falcon #3040) tissue culture plate for each serum sample to be titered using an Oxford 8000H pipette and a sterile Oxford 810S tip. During

preparation, the microculture plates were temporarily covered with a Falcon #3041 sterile plastic lid and maintained at 15 C. Calibrated 0.025 microtiter loops (#1-220-33 microdiluters, Cooke Laboratory Products, Alexandria, Virginia) were flame sterilized, wetted in sterile 0.9% Hank's balanced salt solution, blotted on sterile towels, and loaded with individual raw serum samples from the plugged heparinized microcapillary tubes. The 0.025 ml of raw sera was then serially diluted through the eight well series of diluent on the microculture plate to give a two-fold series of dilutions from 1:2 to 1:256. Appropriate known positive and negative trout and rabbit serum controls were included with each test. Following dilution, all wells on the plate were then inoculated with 0.025 ml of a field sampling diluent suspension containing 10^6 $TCID_{50}$ units of Rangen Path. 138-78-3 Leavenworth spring chinook salmon IPNV virus previously grown out on CHSE-214 cells, covered, and incubated at 15 C. for 30 minutes and 4 C. for an additional 30 minutes to effect serum neutralization.

During the 60 minute serum neutralization period, a stock flask of CHSE-214 cells that had been previously grown out to 95% confluence at 20 C. in MEM-10-Tris-PSF was examined under the inverted microscope for confluence and quality and dissociated in 4 ml of PAN/EDTA media (Appendix B). The PAN/EDTA cell suspension was centrifuged at 1500 x g for four minutes. The supernatant was decanted and the pelleted cell mass resuspended in a small volume of MEM-10-Tris-PSF media. The resuspended cells were further diluted in MEM-10-Tris-PSF media to a final 1:2 working dilution based upon surface area split ratio and placed in a 100 ml covered sterile flask containing a sterile magnetic stir bar. The diluted cell suspension was placed on a magnetic stir plate with slow stirring to maintain the cells in suspension. A sterile Minipet repipetting syringe (Manostat #71-500-101, Manostat, New York, N.Y.) was fitted with a sterile disposble 18g X 1½" hypodermic needle and primed with the chilled CHSE-214 cell suspension. Fifteen hundredths of a milliliter of the diluted CHSE-214 cell suspension was pipetted into each well on the incubated serum neutralization plates. The plates were immediately sealed with Falcon #3044 pressure sensitive film and incubated at 15 C. for seven days. All wells of both the test and control dilution series were read under the inverted microscope periodically for evidence of specific cytopathic effect (CPE), cytotoxicity, and microbial contamination. Observations were recorded as the highest serum dilution demonstrating complete viral neutralization and a total lack of any specific CPE. All wells of the plate were stained with a 1% alcoholic solution of crystal violet and dried as a permanent record of the serum neutralization results.

III. RESULTS

A total of 300 Pacific salmon and steelhead trout smolts representing five discrete anadromous stocks at five Columbia River basin hatcheries were tested for the presence of infectious viruses during 1979. The results of these tests are summarized in Table 2. for each of the hatchery stocks examined.

Neither infectious hematopoietic necrosis virus (IHNV) nor *Herpesvirus salmonis* were found during the course of this study. Infectious pancreatic necrosis virus (IPNV) was confirmed at four of five hatcheries sampled and in four of five stocks of smolted fish examined. IPNV was isolated from two of three populations of steelhead trout examined with all twelve five-fish pools of tissue from the Winthrop steelhead trout stock being positive but only one five-fish pool of tissue from the Tuccanon steelhead trout stock being positive. All sixty fish sampled from the Chelan stock of steelhead trout from Leavenworth Hatchery were negative for virus. In addition, all twelve five-fish pools of tissue from the Big White Salmon stock of fall chinook salmon as well as the Carson stock of spring chinook salmon were found to be positive for IPNV.

Because of the statistically insignificant numbers of stocks included for surveillance in the 1979 study, further analysis of this data by itself is not justified. However, recognizing that the year class of stocks examined for virus in 1978 and 1979 are largely unique and discrete spawning populations, the data from both years has been combined and summarized in Table 3.

Since the year class stocks checked for virus during the 1978 and 1979 studies can be combined to increase sample size and statistical significance, Table 4. is used to summarize the combined incidence of IPNV infection among and within populations by specie. It is shown that among populations, by species, IPNV was most common in coho salmon smolt (82% incidence among populations sampled) and least common in fall chinook salmon smolt (33% incidence among populations sampled). The IPNV carrier incidence of infection within populations by species was determined on the basis of confirmed viral isolation from five-fish tissue pools within a sixty-fish lot for each population. On this basis, the IPNV carrier incidence within populations sampled was the highest for coho salmon smolt at 57% and lowest for fall chinook salmon smolt at 15%.

None of the confirmed IPNV isolations could be associated with any significant mortality or loss within the populations examined.

The results of serum neutralization testing are summarized is Table 5. IPNV specific neutralizing antibodies were detected in 2.44% of the sera from spring chinook salmon smolt at Carson Hatchery while IPNV was isolated from 33%

TABLE 2. SUMMARY OF PRELIMINARY SCREEN RESULTS AND CONFIRMED IDENTIFICATION OF VIRUS ISOLATED FROM SELECTED COLUMBIA RIVER DRAINAGE HATCHERY STOCKS OF ANADROMOUS SALMONID SMOLTS DURING 1979.

STATION	STOCK	NUMBER OF SCREEN POOLS			CONFIRMED VIRAL ISOLATION RESULT
		POSITIVE	SUSPECT	NEGATIVE	
Big White Salmon	fall chinook salmon	12	0	0	IPNV
Carson	spring chinook salmon	4	5	3	IPNV
Leavenworth	steelhead trout (Chelan stock)	0	0	12	negative
Tuccanon	steelhead trout	1	0	11	IPNV
Winthrop	steelhead trout (Wells stock)	12	0	0	IPNV

TABLE 3. SUMMARY OF CONFIRMED VIRUS ISOLATION AND IDENTIFICATION FROM SELECTED COLUMBIA RIVER DRAINAGE HATCHERY STOCKS OF ANADROMOUS SALMONID SMOLTS DURING 1978 AND 1979.

STATION	STOCK	1978 CONFIRMED RESULT*	1979 CONFIRMED RESULT
Big Creek	coho salmon	IPNV	
	coho salmon (Cowlitz stock)	IPNV	
Big White Salmon	fall chinook salmon		IPNV
Bonneville	fall chinook salmon (group 1)	negative	
Carson	coho salmon (yearling)	IPNV	
	spring chinook salmon	negative	
	spring chinook salmon		IPNV
Cowlitz	fall chinook salmon	negative	
Dworshak	steelhead trout	IPNV	
Kalama Falls	spring chinook salmon	negative	
	coho salmon	IPNV	
	fall chinook salmon	negative	
	fall chinook salmon	IPNV	
Klickatat	coho salmon	IPNV	
Kooskia	spring chinook salmon	IPNV	
Leavenworth	spring chinook salmon	IPNV	

(continued)

TABLE 3. (continued)

Leavenworth	steelhead trout	negative	
	steelhead trout (Chelan stock)		negative
Little White Salmon	fall chinook salmon	negative	
Rocky Reach	coho salmon	negative	
Sandy	coho salmon	IPNV	
Spring Creek	fall chinook salmon	negative	
Skamania	steelhead trout	IPNV	
Toutle	coho salmon	IPNV	
	coho salmon (Montlake stock)	IPNV	
	fall chinook salmon	IPNV	
Tuccanon	steelhead trout	negative	
	steelhead trout		IPNV
Willard	coho salmon (group II)	negative	
	coho salmon (group III)	IPNV	
	fall chinook salmon	negative	
Winthrop	steelhead trout	IPNV	
	steelhead trout (Wells stock)		IPNV

* from Busch, 1979.

TABLE 4. COMBINED INCIDENCE OF INFECTIOUS PANCREATIC NECROSIS VIRUS IN SELECTED STOCKS OF ANADROMOUS SALMONID SMOLT AT COLUMBIA RIVER DRAINAGE HATCHERIES DURING 1978 AND 1979.

	TOTAL STOCKS EXAMINED	STOCKS WITH CONFIRMED IPNV	INCIDENCE OF INFECTION AMONG POPULATIONS	INCIDENCE OF INFECTION WITHIN POPULATIONS
All Stations Sampled	19	14	74%	---
All Stocks Combined	33	20	61%	41%
coho salmon	11	9	82%	57%
fall chinook salmon	9	3	33%	15%
spring chinook salmon	5	3	60%	41%
steelhead trout	8	6	75%	52%

TABLE 5. SUMMARY OF CONFIRMED VIRAL AND SERUM NEUTRALIZATION RESULTS FROM FIVE SELECTED COLUMBIA RIVER DRAINAGE HATCHERY STOCKS OF ANADROMOUS SALMONID SMOLTS DURING 1979.

STATION	STOCK	CONFIRMED VIRAL ISOLATION RESULT	TOTAL SERA EXAMINED	TOTAL SERA IPNV POSITIVE	INCIDENCE OF SEROPOSITIVE SAMPLES	MEAN TITER
Big White Salmon	fall chinook salmon	IPNV	--------- no serum samples taken ----------			
Carson	spring chinook salmon	IPNV	41	1	2.44%	1:4
Leavenworth	steelhead trout (Chelan stock)	negative	--------- no serum samples taken ----------			
Tuccanon	steelhead trout	IPNV	56	17	30.36%	1:3.6
Winthrop	steelhead trout (Wells stock)	IPNV	38	0	0.00%	negative

of the homologous five-fish tissue pools tested. Steelhead trout smolts sampled at Tuccanon Hatchery had a 30.36% incidence of seropositive IPNV specific neutralizing antibodies among 56 individual sera while only 8.33% of the homologous five-fish tissue pools were positive for IPNV isolation.

Even though the Wells stocks of steelhead trout at Winthrop Hatchery had a 100% incidence of IPNV isolations among the twelve five-fish tissue pools sampled, no IPNV specific neutralizing antibodies could be found among the 38 homologous individual sera tested. As no sera were made available to the Big White Salmon or Leavenworth stocks, no serum neutralization tests on these two stocks were run.

IV. DISCUSSION

The failure to isolate either *Herpesvirus salmonis* or infectious hematopoietic necrosis virus (IHNV) during the course of this study, either during 1978 or 1979, is not unusual when considered within the given limitations of the experimental design applied. *Herpesvirus salmonis* or other closely related salmonid viruses have yet to be isolated from coho salmon, chinook salmon, or steelhead trout in the United States. In fact, the virus has only been isolated once in North America from a Columbia River basin salmonid stock and has not been seen since. Due to the fact that our knowledge of the ecology and epidemiology of the disease is still limited, it can only be said that the virus was not detected within the limitations of the experimental design applied. Possibly by broadening the scope of the study by including conditions known to be optimum for the recovery of the virus at all stages in the life history of Pacific salmon and steelhead trout as well as the application of more sensitive techniques of serological surveillance such as detection of specific neutralizing factors in the sera or other body fluids or the detection of specific antigens or antibodies in the various body tissues and fluids by means of enzyme linked immunoadsorbent assay (ELISA) or counterimmunoelectrophoresis (CIE) procedures could also give a better understanding of the ecology of the disease and its impact on anadromous stocks of salmonids.

Infectious hematopoietic necrosis virus (IHNV) is known to be endemic to the Columbia River basin but is usually considered a disease of fry and fingerling chinook salmon and steelhead trout. Coho salmon appear resistant but are known to function as asymptomatic carriers. IHNV is also known to enter into a non-infectious "eclipse" phase of infection in post-epizootic or convalescent populations and, as such, cannot be isolated and identified by routine methods of culture as applied in this study. In order to obtain a better understanding of the ecology, epidemiology, and overall impact of IHNV on the anadromous salmonid stocks

of the Columbia River basin, surveillance programs should include testing of ovarian fluids from adult spawming populations as well as progeny fry at the swim-up stage as these are the two stages in the life history of the fish when the virus is known to exist in the infectious form. Techniques of chemical induction or serological surveillance using serum neutralization, ELISA, and CIE procedures may be effective in detecting virus at these and other stages when the virus may be present in the non-infectious eclipse form.

Extensive recent isolations of IHNV from clinically diseased rainbow trout fingerlings and yearling fish at 14.5 C. (58 F.) (Morton and Busch, 1980) may indicate that strains of IHNV in the Columbia River basin are significantly extending their host, geographic, and temperature tolerance ranges and again points out the fact that the salmonids viruses are in a continuing dynamic state of adaptation. Descriptions and accepted facts of ecology and epidemiology based upon historical information may not necessarily continue to hold true in the future as the viral pathogens adapt to new hosts and environments.

Isolation of infectious pancreatic necrosis virus (IPNV) from the stocks examined and, in particular, the repeated finding of a relatively high incidence of the virus among the populations in comparison to some previous reports of surveillance again seems unusual upon initial examination as was pointed out in last year's study. However, when one takes into consideration that IPNV is: 1) known to be endemic to large areas of the Columbia River basin; 2) known to infect both Pacific salmon and steelhead trout under both experimental and natural conditions; and 3) has been detected in at least ten discrete populations of Pacific salmon and steelhead at seven different stations since 1971 and prior to this study; the results take on a more consistent image. It is also noted in this study and in several previous surveillance studies that IPNV has been isolated only from visceral tissues of infected returning adult stocks and their 30 to 60 day old progeny fry and not from reproductive fluid samples. However, our current understanding of the epidemiology of IPNV in anadromous stocks of Columbia River salmon and steelhead trout seems to be primarily based upon studies which relied primarily upon ovarian reproductive fluid samples and were specifically designed to monitor for IHNV. When McMichael (1974) and Tebbit (1975) sampled both reproductive fluids and pooled visceral tissues from returning adult salmon populations, they were able to isolate IPNV from four discrete populations. However, all four IPNV isolations were obtained exclusively from the pooled visceral tissue samples while all of the paired homologous reproductive fluids samples remained negative for the virus (Tebbit, 1979, personal communication). This observation would indicate that surveillance studies based solely upon

reproductive fluid samples, while being well suited to the detection of IHNV, may not accurately reflect the true incidence or occurrence of IPNV in a population of asymptomatic adult carriers.

It should also be noted that McMichael (1974) consistently failed to isolate IPNV from known infected fry populations of Pacific salmon after 60 days of age post feeding. Therefore, additional surveillance programs should be designed to sample fry and fingerling sized fish at 30 to 60 days of age post feeding and at smoltification prior to hatchery release in order to detect the presence of IPNV accurately.

IPNV as well as many other diseases of viral etiology are known to be stress mediated as well as being species and age specific. The period of smoltification in the life history of an anadromous salmonid fish is recognized as a time of major physiological change and stress to the animal, particularly under hatchery conditions of intensive culture and nutrition. It is also a time when hormonal balances are in a state of flux and these too have been correlated with the occurrence of certain diseases. Therefore, it is reasonable to assume that this period of smoltification and physiological stress, particularly when coupled with major changes in hormonal balances and behavior, could well exacerbate a preexisting subclinical infection of IPNV or even increase the relative susceptibility of an uninfected population to infection from an endemic source common to the hatchery or watershed. To the best of our knowledge, this is the first surveillance study to report on results obtained specifically from populations undergoing smoltification.

Another consideration for discussion is the demonstrated low incidence of IPNV to be found among the asymptomaticly infected populations. Tebbit and McMichael (1974) reported only two of twelve five-fish tissue pools taken from adult coho salmon returning to Bonneville Hatchery in 1971 were found to be positive for IPNV. Only six of twelve five-fish pools of tissue from adult coho salmon returning to Cascade Hatchery in 1972 were positive for IPNV (Tebbit, 1975). In last years study of 1978 broodyear fish seven of the 16 populations found to be infected with IPNV demonstrated virus in less than half of the five-fish tissue pools examined on initial screening. This year, in 1979 broodyear fish, two of the four populations found to be infected with IPNV demonstrated the virus in fewer than half of the five-fish tissue pools examined on initial screening. These findings would indicate that a full 60-fish sample, based upon hypergeometric sampling statistics, may be necessary in order to consistently detect the virus. Spot checks of fewer than 60 fish are apt to miss detecting an infection of low incidence as many seem to be.

A final consideration in the surveillance of IPNV in the Pacific salmon and steelhead trout stocks of the Columbia River basin is the demonstrated variability

in the occurrence of the virus between different year classes of a particular stock. During extensive sampling between 1971 and 1974, McMichael (1974) and Tebbit (1975) consistently failed to isolate IPNV from the same hatchery stocks during subsequent years even though neutralizing antibodies indicating prior exposure were found to be present at times. Therefore, it appears reasonable to assume that the demonstrated presence or absence of IPNV in a particular year class of a given hatchery stock of anadromous salmon or steelhead trout may not necessarily mean that all other year classes of that same stock will be similiarly infected or free of the disease. As was shown in Table 3. the 1978 year class of Carson spring chinook salmon were negative for IPNV, however, the 1979 year class was found to be positive. The same holds true for the Tuccanon steelhead trout stocks examined in 1978 and 1979.

It should also be noted that paired five-fish pooled tissue samples sent to Dr. Dan Mulcahy at the National Fisheries Research Center in Seattle were all reported as being negative for virus (see Appendix D). As Dr. Mulcahy indicated in his letter, this additional testing did nothing to clear up the question. It is suggested that additional intensive testing of 120 to 150 individual fish from one or two selected sites be undertaken by two or more laboratories using the identical cell lines, protocols, and procedures as used in this study by Rangen Research. A possible exchange of personnel may also be needed to insure complete and accurate duplication. Additional methods such as ELISA, CIA, FAT, etc. may also be considered.

The serum neutralization results obtained in this study indicate that this technique may be useful in additional surveillance studies. However, more sensitive serological techniques such as ELISA and CIA may be able to give higher titers and more definitive results. Samples also need to be handled and processed more rapidly to insure minimum loss of acitvity during storage.

Based upon these observations including the relatively low incidence of IPNV both among and within populations of anadromous Pacific salmon and steelhead trout in the Columbia River basin, the serological findings of McMichael (1974) and Tebbit (1975) utilizing neutralizing antibody techniques, and the variability of clinical infection between the different year classes and life history stages of a particular stock, it is suggested that future attempts at viral surveillance include both sampling of visceral tissues as well as reproductive fluids from spawning populations. Progeny fry and fingerlings should be sampled at 30 to 60 days of age post feeding and smolted fish should be sampled just prior to hatchery release. Attempts at isolation of virus during the various stages of the life history should be coupled with techniques of serological surveillance based upon serum neutralization, ELISA, and CIE procedures. All year classes of a particular stock should be examined over the course of the study.

Any additional studies of a viral surveillance nature undertaken in the future may also want to include procedures for the detection of piscine erythrocytic necrosis (PEN) virus disease, particularly if returning adult salmon and steelhead trout populations are included in the sampling.

The demonstrated presence of IPNV in smolted stocks of Pacific salmon and steelhead trout in the Columbia River basin should be of concern but not alarm to resource management agencies. IPNV has yet to be associated with any significant mortality in stocks of Pacific salmon under hatchery conditions and is not an altogether common cause of mortality in stocks of steelhead trout with the possible exception of one station in Southern Idaho. The disease does, however, hold the potential for significant losses of steelhead trout under hatchery conditions and significant harm to this species. The sublethal impact of the virus on Pacific salmon throughout their life history, particularly with regard to ocean survival and adult return, has yet to be determined.

V. CONCLUSIONS

1. Infectious pancreatic necrosis virus (IPNV) was isolated from four of five stocks of Pacific salmon and steelhead trout smolts at four of five Columbia River basin hatcheries during surveillance of 1979 brood year stocks.

2. The presence of infectious pancreatic necrosis virus (IPNV) in stocks of Pacific salmon and steelhead trout smolts at selected Columbia River basin hatcheries in 1979 was not associated with any significant mortality.

3. Infectious hematopoietic necrosis virus (IHNV) was not isolated from any of the five stocks of Pacific salmon and steelhead trout smolts at five Columbia River basin hatcheries in 1979.

4. *Herpesvirus salmonis* was not isolated from any of the five stocks of Pacific salmon and steelhead trout smolts at five Columbia River basin hatcheries in 1979.

5. Additional funded in-depth studies are needed to resolve questions concerning the ecology and epidemiology of infectious pancreatic necrosis virus (IPNV) in hatchery stocks of Pacific salmon and steelhead trout in the Columbia River basin.

VI. LITERATURE CITED

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RANGEN RESEARCH HATCHERY

TROUT AND SALMON DIETS • LIVE TROUT
FISH PATHOLOGY • DISEASE CERTIFICATION • CONTRACT RESEARCH

ROUTE ONE • TELEPHONE (208) 837-4464
HAGERMAN, IDAHO 83332

ERIES DIVISION

RANGEN, In

NMFS - MIGRANT SMOLT HEALTH INDEX STUDY

-Field Sampling Procedures-

A. Field Sampling Kit - each field sampling kit consists of 13 graduated plastic screw-cap tubes of sterile viral sampling and transport diluent (12 sample tubes and 1 replacement tube), 1 sample tube shipping rack, 1 preaddressed shipping label for return of field sampling kit and samples, 1 instruction and field sampling data sheet, 1 insulated shipping container. This kit is sufficient for sampling a single lot of 60 fish for virus screening. Field kits should be stored at 4 C. prior to use and at no time should they be frozen or held above 15 C. Gel packs of refrigerant should be held in the freezer prior tc packing for return shipment.

B. Sampling Procedure - 60 fish should be randomly selected from a defined lot and divided into 12 5-fish pools. The fish should be sacrificed by a blow to the head and aseptically opened to expose the kidney and viscera with sterile dissection instruments. Care must be taken during dissection not to cut into the gastro-intestinal tract or otherwise contaminate the internal tissues. Tissues for viral assay should be quantitatively sampled with the modified Russion Tissue Forceps provided. The forceps should be dipped in 70% isopropyl alcohol and wiped clean to disinfect between 5-fish pools. Tissues from each fish are sampled by taking one (1) full forcep of material and depositing it in the sterile viral sampling diluent. This tissue volume is critical and the tissue should only fill the cups of the forceps, no more, no less. Tissues to be sampled from each of the 5 fish in the pool in order of sampling are the liver (being careful to avoid the gall bladder and the introduction of bile into the sample), the spleen (being carefull to sample as little as possible of associated adipose or fat tissue), and the kidney. After all three tissues from each of the five fish in the pool are sampled and placed in the tube of sampling diluent, the tube is capped tightly and placed securely in the tube rack. Samples and diluent should be kept cool and out of the sun during the entire sampling procedure.

C. Shipping - When all of the samples are taken and the field sampling data sheet has been completed, all materials are placed back into the insulated shipping container together with sufficient frozen gel pack refrigerant to last for 48 hours. The preaddressed shipping label is placed on the outside of the container

and the container sent as soon as possible after sampling the single lot. Shipment should be by either Grayhound Package Express or United Parcel Service. Avoid shipping over weekends or holidays. If necessary, hold samples at 4 C. in a refrigerator and ship on the following Sunday or Monday.

D. If there are any questions or problems, contact:

Dr. Robert A. Busch
Rangen Research Station
Route 1, Box 264
Hagerman, Idaho 83332

Office: (208) 837-6192
Home: (208) 837-6370

NMFS - MIGRANT SMOLT HEALTH INDEX STUDY

-Field Sampling Data Sheet-

NMFS Sample Code:_____ Date:__/__/____

Sampling Location:_____ Time:_____ hours

_____ Technician:_____

Species Sampled:_____

Original Source and Identification _____

_____

_____

Sampling Notations, Observations, Gross Lesions, etc.:___

_____

_____

_____

_____

_____

_____

_____

Virus Disease History: Yes or No

1) Has a virus disease ever been diangosed in these fish stocks sampled? _____

2) Has a virus disease ever been diagnosed at the station of origin of these fish? _____

If the answer is "yes" to either or both of the above questions, please indicate which virus disease was diagnosed, on what date, and by whom:

_____

_____

_____

_____

_____

Sample Shipment Information:

Via:_____ Date: __/__/____

Point of Origin:_____ Time:_____ hours

_____

Sample Receipt Information:

Received Date:__/__/____ Time: _____ hours

Condition:_____

Formulation of Medias and Reagents

A. Field Sampling Diluent

Dulbecco's PBS (10X stock) Gibco #408	100 ml
T/C Grade Water	800 ml
Gentamicin (50 mg/ml stock) Shering Corp.	4 ml
Amphotericin B (250 ug/ml stock) Gibco #529L	4 ml
Phenol Red (0.5% stock) Gibco #510	4 ml

Adjust pH to 7.2 with sterile 1N NaOH

Adjust final volume to 1 liter with T/C grade water

B. Decontamination Diluent

Dulbecco's PBS (10X stock) Gibco #408	100 ml
T/C Grade Water	800 ml
Gentamicin (50 mg/ml stock) Schering Corp.	20 ml
Amphotericin B (250 ug/ml stock) Gibco #529L	2 ml
Phenol Red (0.5% stock) Gibco #510	4 ml

Adjust pH to 7.2 with sterile 1N NaOH

Adjust final volume to 1 liter with T/C grade water

C. MEM-10-Tris-PSF Tissue Culture Media

Eagle/Earle MEM (Auto-Pow) Gibco #410-1700	4.701 g
T/C Grade Water	421.3 ml

Autoclave at 121 C. for 15 minutes

Cool and aspetically add:

Fetal Calf Serum (mycoplasma and virus free) Gibco #614	50 ml
l-Glutamine (200 nM) Gibco #503	5 ml
Sodium Bicarbonate Solution (7.5% stock) Gibco #508	5 ml
Tricine Buffer (1M Tris) Gibco #573	5 ml
Antibiotic-Antimycotic (100X stock) Penicillin 10,000 U/ml	

Streptomycin 10,000 ug/ml Amphotericin B 250 ug/ml Gibco #600-5240	5 ml
Calcium Chloride (10% stock)	1 ml

Adjust pH to 7.2 with sterile 1N NaOH

Adjust final volume to 500 ml with T/C grade water

D. PAN/EDTA Dissociation Media

Versene (1:5000 solution) Gibco #670-5040	100 ml
Pancreatin (4X N.F. reconstituted to 2.5% in 20 ml sterile T/C water) Gibco #R13-5720-L	4 ml
Phenol Red (0.5% stock) Gibco #510	0.4 ml

( ) Diagnosis
( ) Certification
( ) Surveillance

VIROLOGICAL EXAMINATION
FISH PATHOLOGY LABORATORY
RANGEN RESEARCH HATCHERY

File No.:________

Acc. No.:________

Sampling Date:___/___/___ Time:________hours Technician:______________

Receiving Condition: ( ) live ( ) fresh
( ) frozen ( ) refrigerated
Holding condition: ______hours ______°C
Comment:

No. Sampled:				
Lot #:				
Morb.:				
Mort.:				
Asyp.:				

Tissue Sampled: ( ) whole fish ( ) mixed viscera ( ) liver ____%
( ) spleen ____% ( ) pyloric caeca ____% ( ) kidney ____%
( ) ovarian fluids ( ) seminal fluids ( ) other:

Pooling:

Lot #	Fish/Pool	No. Pools	Lot #	Fish/Pool	No. Pools

Inoculant Preparation:

Pool #	Tis. wt. g.	Buffer ml	Sup.vol. ml	pen/strep ml stock	mycostat ml stock	gentamicin ml prep	filter 0.45 u	final [illegible] fact[illegible]

Prep. Incubation: _____°C _____hours Inoculation Date:___/___/___ Time:____ h
Technician:______________ Inoc. Vol.: 0.025 0.05 0.1 0.2 ml ( ) wet ( ) dry
( ) single ( ) duplicate ( ) microtiter ( ) multiwell ( ) 60 mm dish
Absorbtion Time: _____min. _____°C.

Cell Line	Passage	Seed Date	Vessel	Growth Temp.	Conf.	Quality	Inc. Tem

Media:____________________ Control Virus:____________________

Lot #:____________________

RESULTS:

Date	Day																																				

VIROLOGICAL EXAMINATION (continued)

Serum Neutralization (microculture):

Set-up Date: __/__/____ Time: _____hours Technician: _____

Sample Handling: ( ) raw ( ) filtered ( ) fresh ( ) stored 4 C. ____day

Neutralizing Antisera: _____ Working Dil.: _____

_____ Working Dil.: _____

Control Sera: _____ Working Dil.: _____

Cell Line Passage Seed Date Vessel Growth Temp. Conf. Quality Inc.

_____

Neutralization: Viral Sup. Working Dilution: _____ Volume: _____ml

Antiserum Volume: _____ml Ratio: _____

Incubation Time: _____min. Temp.: _____C.

Inoculation: Inoc. Vol.: _____ml ( ) wet ( ) dry Absorb. Time: _____min.

Media: _____ Lot #: _____

Control Virus: _____

Plate Identification: _____

ROW	Sample No.	WELL TEST	A	B	C	D	E	F	G	H	RESULTS
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											

Remarks:

National Fisheries Research Center - Seattle
Bldg. 204, Naval Support Activity
Seattle, Washington 98115

June 28, 1979

Mr. John A. Miller
U.S. Fish and Wildlife Service
Olympia Area Office
2625 Parkmont Lane
Olympia, Washington 98502

Dear John:

As mentioned in our telephone conversation today, we have completed the virus testing of five Columbia River basin hatcheries. Mr. Tony Novotny, NMFS, collected the fish and live-hauled them to his facility. He supplied organ samples as 5 fish pools from each group. Each organ was split and half sent to us and half to Dr. Bob Busch, Ranger Research Hatchery, Idaho. One group was of such small size that splitting organs was not possible. Organs from separate test groups were supplied. Steve Leak, USFWS, obtained samples of one group at the time Novotny picked up the fish.

The sites and species tested and the results obtained are given here:

Species	Location	Results		
		Busch	Mulcahy	Leek
Steelhead	Wells Rearing Pond (held at Winthrop H)	+	-	NT*
Steelhead	Leavenworth H. (Chelan H. stock)	-	-	NT
Steelhead	Tucannon H.	-	-	NT
Sp. Chinook	Carson H.	+	-	-
Fall Chinook	Big White H.	+	-	NT

* Not tested

As you can see, this testing does not clear anything up. I would recommend selecting one site for a more intensive examination of about 150 individual fish. Both the usual detection methods and fluorescent antibody should be used. Additionally, a group should

SURNAME	DATE
	6/27

CONSERVE AMERICA'S ENERGY

Save Energy and You Serve America!

be returned to the lab for placement in salt water and any mortalities examined.

Please feel free to call me for any further assistance.

Sincerely,

Dan Mulcahy, Ph.D.
Research Virologist

cc: Steve Leek
Bob Busch
Tony Novotny

APPENDIX B

HISTOPATHOLOGY REPORT

APPENDIX B. Report of the Veterinary Pathologist

Correction:

References are made to the pathology of the olfactory "lobe" in the following report. The correct terminology should be the olfactory "pit," "organ," or "sac," as the tissue examined was from serial sections through the nares, which reveal the epithelium of the olfactory organ. The olfactory lobe is a projection of the anterior lower margin of the cerebral hemispheres.

BIOMED RESEARCH LABORATORIES

Biological Testing - Research & Development

1115 E. Pike Street **Seattle, Washington 98122** **(206) 324-0380**

January 11, 1980

National Marine Fisheries Service
Manchester Marine Research Station
Anthony Novotny,
Fisheries Research Biologist
P.O. Box 38
Manchester, Washington 98355

SUBJECT: Histopathological survey of 1979 Columbia River hatchery stocks involved in the National Marine Fisheries Service (NMFS) homing studies.

METHODS AND MATERIALS: The following five stocks of hatchery fish are evaluated in this histopathological study:

1) Tucannon Steelhead
2) Chelan Steelhead
3) Big White Salmon Fall Chinook
4) Carson Spring Chinook
5) Wells Steelhead

Sixty fish from each hatchery are tagged in the lower jaw with a numbered anchor tag, fixed in Bouin's solution for 24 hours, rinsed with tap water and stored in 20 liter buckets of 10% buffered formalin for a total of 300 fish.

The fixed tissue is trimmed into a set of four blocks which are parafin embedded, sectioned and stained with hematoxylin and eosin. Block #1 consists of a cross-section of the head containing the brain and both eyes and occasionally thyroid; block #2, the gill and thyroid (the second gill arch); block #3, the liver and kidney and block #4, a cross-section of the olfactory lobe.

LESIONS FROM BLOCK #1 (eye, periorbital muscles and fat, brain and optic nerve):

Lesions in the striated muscle and adipose tissue surrounding the eye are rare and occur in only the Chelan Steelhead and Carson Spring Chinook. The

evaluation of the muscle lesions is subjective and it is difficult to tell fixation artifacts from very mild degenerative changes. The typical (+) lesion is a mild one in which there is an irregular loss of myofibrills within the muscle fiber and a mild granularity of the sarcoplasm. The moderate (++) lesions are slightly more pronounced and in some instance have a few mononuclear cells between the muscle fibers.

The retrobulbar fat lesions vary from a mild infiltration of mononuclear cells to necrosis of the adipose tissue to actual inflammation which is usually a pyogranulomatous retrobulbar panniculitis (especially in the Carson Spring Chinook). This retrobulbar panniculitis is associated with a pyogranulomatous inflammation of the olfactory lobe in a high percent of the cases. No intraocular lesions are present in any of the fish.

One fish has an acute focal area of hemorrhage in the periocular muscles which appears to be the result of trauma immediately prior to death. One fish has a microfocal accumulation of mononuclear cells in the optic nerve, five have a mononuclear infiltration of the meninges and two have encephalitis. One fish has a sporazoan parasite in the pharyngeal epithelium and another has a nematode adhearing to the wall of the pharynx.

LESIONS FROM BLOCK #2 (gill and thyroid): In almost all of the hatchery fish, there is a slight (+) proliferation of the gill epithelium with subepithelial infiltration of a few lymphocytes. This is most pronounced where the gill filament joins the gill arch. In moderate cases (++) this lymphoid infiltrate increases to several cells in thickness. No severe (+++) cases of lymphoid infiltration are present in these five groups of fish. The gill epithelium proliferation tends to parallel the lymphoid infiltration in severity. The epithelial proliferation is most pronounced on the tips of the gill filament. In moderate (++) cases lamellar hypertrophy is present with some proliferation from the bases of the secondary lamellae. In marked (+++) cases this proliferation results in short club-like lamellae which are fused at the base. In the fish examined, none have lesions that have progressed to the point of complete fusion between adjacent gill lamellae.

OTHER SPORADIC LESIONS PRESENT ARE: Lymphatic telangiectasis (F.1) of the secondary lamellae in three of the Tucannon Steelhead. Vascular telangiectasis of the secondary lamellae in one of the Wells Steelhead. The lymphatic telangiectasis represents an acute terminal change related to impaired lymphatic return and probably results when the fish is not euthanized rapidly. The vascular telangiectasis is a chronic lesion associated with physical or chemical trauma to the gills. This is commonly found in farmed fish after grading, pond transfer or when they are exposed to metabolic waste, highlevels of feed particles in the water or chemical pollution. It results from the rupture of the pilaster cells which normally join the dorsal surface of the secondary lamellae to the ventral. This results in a dilation of the lamellar capillary pooling, of blood, thromboses and fibrosis.

Solitary basophilic masses in the secondary lamellae are present in 6.7% of the Tucannon Steelhead. These bodies appear to be an intracellular or subepithelial microsporidian protozoan parasite (F.2).

Solitary eosinophilic masses in the secondary lamellae are present in 3.3% of the Tucannon Steelhead. These bodies are individual gill epithelial cells which are undergoing necrosis (F.1 & 3). The significance of these lesions is unknown.

One of the Tucannon Steelhead has a nematode parasite in the gills, one of the Chelan Steelhead has a focal granuloma of undetermined etiology in the secondary lamellae, one Carson Spring Chinook has a mixed inflammatory lesion at the base of the second gill arch and one of the Wells Steelhead has a mucopurulent inflammation of the gill surface.

A sporazoan parasite is present in the gill epithelium of 26.6% of the Chelan Steelhead (F. 3). The majority of the thyroids examined have normal thyroid follicles with normal low cuboidal epithelium and eosinophilic colloid secretion. Because of the diffuse distribution of the thyroid follicles in telosts, thyroid tissue was not found in all of the tissue sections. There was no evidence of thyroid neoplasia or degeneration; however, two Carson Spring Chinook had a perifollicular thyroiditis which appeared to be a secondary infection and not a primary thyroid lesion.

LESIONS OF BLOCK #3 (liver and kidney): Microfocal accumulations of mononuclear cells in the hepatic parenchyma is present in the fish from all five hatcheries; however, the percentage of fish with the lesions varied considerably.

Tucannon Steelhead	3.3%
Chelan Steelhead	8.3%
Big White Salmon Fall Chinook	34.5%
Carson Spring Chinook	24.1%
Wells Steelhead	1.7%

Although no necrosis is associated with the focal accumulation of mononuclear cells, some of the larger foci show a tendency towards a granuloma formation. The Carson Spring Chinook have 6.9% of the fish with these microgranulomas and an additional 5.2% with well developed lesions comparable with those produced by Corynebacterium sp. (KD). One of the Chelan Steelhead has a nonsuppurative triaditis of unknown etiology, and one of the Tucannon Steelhead has a single sporazoan parasitic granuloma (F.5). Only the Carson Spring Chinook have any renal lesions. Although the abundant hematopoietic tissue make the granulomas more difficult to recognize, 8.8% of the Carson Spring Chinook have lesions characteristic of kidney disease (Corynebacterium sp.).

LESIONS OF BLOCK #4: The olfactory lobe appears to be an excellent organ to examine in a health screen because of the variety of etiological agents that appear to become established there. Ciliated protozoan parasites (F. 6 & 7) are present in 53.5% of the Tucannon Steelhead, and 3.3% of the fish have a nematode parasite present. The lot from the Chelan hatchery has nematode parasites in the olfactory lobe in 5.1% of the fish. Most of the hatcheries have a low incidence of inflammatory lesions in the olfactory lobe with no infections in the Chelan Steelhead and Big White Salmon Fall Chinook, only one of the Wells Steelhead have a mild inflammation and only one of the Tuccanon Steelhead have a pyogranulomatous inflammation of the olfactory lobe. The Carson Spring Chinook have 25.4% with a pyogranulomatous inflammation of the olfactory lobe, 1.7% with a mild inflammatory reaction, and 1.7% with acute focal hemorrhages which appeared to be traumatic in orgin. The high incidence of the pyogranulomatous olfactory inflammation correlates with the kidney disease lesions, and leads one to speculate that the Corynebacterium may enter the host through the olfactory epithelium in some cases.

Other spontaneous lesions found in the fish are a sporazoan parasite in the pharyngeal epithelium of a single Tucannon Steelhead and pyogranulomatous ureteritis, focal tubular degeneration and a focal dilated renal tubule containing large bacteria in three seperate Carson Spring Chinook.

DISCUSSION: The total incidence of the lesions in the fish examined is reported as the percent of lesion containing tissue in the total number of tissues examined. A detailed statistical analysis is not attempted. Some of the lesions are grouped in mild (+), moderate (++) and marked (+++) categories. Most of the lesions in the fish examined are mild to moderate with severe lesions being rare.

The skeletal muscle lesions of the eye are mild and not very common. The mild lesions could possibly be an artifact due to inadequate (slow) fixation. Some of the more severe lesions may be secondary degeneration due to periorbital inflammation; however, a few cases are suggestive of a primary muscle degeneration. This appears to be the case in the Chelan Steelhead which are from a selenium deficiency area of the state. A comparison of tissue selenium levels from the various hatcheries would help evaluate this problem and provide a guide for food supplementation.

The gills of the fish have only mild to moderate gill lamellar hypertrophy and lymphocytic infiltration which is expected in hatchery fish. Histological evaluation of the gills is subjective because the lymphoid cell population varies in density at different levels in the gill arch. Increased lymphoid cells in the gills are a non-specific indicator of exposure to antigens in the water. The lamellar epithelium hypertrophy is in response to physical or chemical irritation which is common in hatchery reared fish. Other sporatic gill lesions such as lymphatic telangiectasis and vascular telangiectasis of the secondary lamellae, solitary basophic and solitary eosinophilic masses of the secondary lamellae, and nematode parasites are of low incidence. Only the sporazoan parasites in the gill epithelium of 26.6% of the Chelan Steelhead have a high level of infestation.

The liver and kidney sections from the Carson Spring Chinook are the only ones that show any charasteric histological lesions of kidney disease. These fish also have the highest incidence of pyogranulomatous olfactory lobe inflammation, pyogranulomatous retrobulbar inflammation and brain inflammation. It may be that one portal of entry for the corynebacterium organism is through the olfactory lobe. While KD organisms cannot be detected in the H & E tissues, the correlation of these histological lesions with the incidence of positive FA procedures for KD will be important.

SUMMARY: This is a histopathological survey of sixty fish each from the following Columbia River Hatchery stocks involved in the National Marine Fisheries Service homing studies: Tucannon Steelhead, Chelan Steelhead, Big White Salmon Fall Chinook, Carson Spring Chinook and Wells Steelhead. In this study, particular emphasis, by request, is directed to the eye, gill, liver, kidney, olfactory lobe, thyroid and brain.

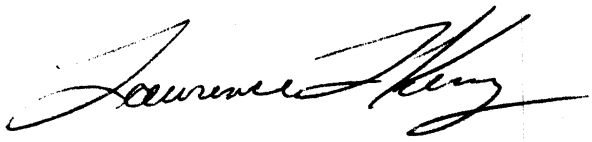
Periorbital striated muscle degeneration is present in the Chelan Steelhead and the Carson Spring Chinook. The Chelan Steelhead lesions may be a nutritional myopathy; however, the Carson Spring Chinook lesions appear to be secondary to a periorbital bacteria inflammation.

All of the hatchery fish have mild to moderate gill epithelial hypertrophy and lymphocytic infiltration. A few sporatic gill parasites are present; however, there is a high incidence (26.6%) of sporazoan parasites in the secondary lamellae of the Chelan Steelhead. A high number (53.3%) of the Tucannon Steelhead have ciliated protozoan parasites in the olfactory lobe and an occassional nematode parasite is seen in the Tucannon and Chelan Steelhead.

A few foci of mononuclear cells are present in the livers of the fish from all five hatcheries; however, only Carson Spring Chinook have liver and kidney lesions indicative of kidney disease. Marked pyogranulomatous inflammation of the olfactory lobe

or periorbital tissue is present in all fish with lesions suggestive of kidney disease. The overall health of these fish appears good except for the Carson Spring Chinook which have the highest incidence of disease.

Respectfully submitted,



Lawrence L. Kunz, D.V.M.
Diplomate, American College
of Veterinary Pathologists

INDEX OF DATA TABLES

LIST OF CODES USED IN DATA TABLES

AH	Acute focal hemorrhage
LTSL	Lymphatic telangiectasis of secondary lamellae
NPSL	Nematode parasite in secondary lamellae
SBM	Solitary basophilic mass in secondary lamellae
SEM	Solitary eosinophilic mass in secondary lamellae
FM	Focal mononuclear cell infiltration
SP	Sporozoan parasite
CP	Ciliated protozoan parasite
NP	Nematode parasite
PO	Pyogranulomatous inflammation of the olfactory lobe
MMI	Mononuclear menigeal infiltration
SPSL	Sporozoan parasite in secondary lamellae
FGSL	Focal granuloma in secondary lamellae
NT	Nonsuppurative triaditis
ROPG	Retrobulbar pyogranulomatous inflammation
ROMI	Retrobulbar mononuclear infiltration
O	Inflammation of the olfactory lobe (acute suppurative)
PT	Perifollicular thyroiditis
SLT	Secondary lamellae (vascular) telangiectasis

TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (%)

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN	
	Tucannon Steelhead Total Incidence																
-	98.3	100	100	0	0	85	100	100	100	95	100	100	100	43.3	100	98.3	SP = inpharngeal epith, of fish = 7.1%
+	0	0	0	81.7	78.3	LTSL 5	0	0	0	FM 3.3	0	0	0	CP 53.3		MMI 1.7	
++	0	0	0	18.3	20	NPSL1.7	0	0	0	SP 1.7	0	0	0	NP 3.3			
+++	0	0	0	0	1.7	SBM 6.7	0	0	0		0	0	0	PO 1.7			
	AH-1.7%					SEM 3.3											
	Chelan Steelhead Total Incidence																
-	88.3	95	98.3	5	5	71.7	100	100	100	88.3	100	100	100	94.9	100	100	Pharyngeal nematode = 1.7%
+	10	3.3	FM 1.7	71.7	68.3	SPL26.6	0	0	0	FM 8.3	0	0	0	NP 5.1			FM in optic nerve =1.7%
++	1.7	1.7		23.3	26.7	FGSL1.7	0	0	0	NT 3.4	0	0	0				
+++	0	0		0	0		0	0	0		0	0	0				
	Big White Salmon Fall Chinook Total Incidence																
-	100	100	100	3.3	0	100	96.6	100	100	65.5	100	100	100	100	100	100	
+	0	0	0	95	95		3.4	0	0	FM34.5	0	0	0	0	0	0	
++	0	0	0	1.7	5		0	0	0		0	0	0	0	0	0	
+++	0	0	0	0	0		0	0	0		0	0	0	0	0	0	
	Carson Spring Chinook Total Incidence																
-	85	85	73.3	1.7	0	98.3	100	94.8	93.1	75.9	91.2	100	100	71.2	94.1	94.2	Pyogranulomatous
+	13.3	15	ROPG 20	98.3	93.3		0	5.2	6.9		8.8	0	0	PO 25.4	PT 5.9	MMI 1.9	Uretheritis = 1.7%
++	1.7	0	ROMI6.7	0	6.7		0	0	0		0	0	0	0 = 1.7		E = 3.9	Focal tubular
+++	0	0		0	0		0	0	0		0	0	0	AH = 1.7			Degeneration = 1.7% with giant gacteria =1.7%
				Focal mixed inflam. base of lamellae = 1.7%						FM?4.1							
	Wells Steelhead Total Incidence																
-	100	100	100	20	0	96.6	100	100		98.3	100	100	100	98.3	100	94.7	
+	0	0	0	80	98.3	SLT 1.7	0	0		FM 1.7	0	0	0	0 = 1.7		MMI 5.3	
++	0	0	0	0	1.7		0	0			0	0	0				
+++	0	0	0	0	0		0	0			0	0	0				
				Mucoputulent gill inflammation = 1.7%													

Tucannon Steelhead

TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (%)

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD GRAN	LIVER MISC	KIDNEY KD GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN	
1	-	-	-	++	++	-	-	-	-	-	-	-	-	-	
2	-	-	-	+	+	SBM-SEM LTSL	-	-		-	-	CP	-	-	SP-pharyngeal
3	-	-	-	++	+	-	-	-	-	-	-	-	NA	-	
4	-	-	-	+	+	-	-	-	-	-	-	CP	-	-	
5	-	-	-	+	++	-	-	-	FM	-	-	CP	-	-	
6	-	-	-	++	+	-	-	-	-	-	-	CP	NA	MMI	
7	-	-	-	+	+	-	-	-	-	-	-	CP	NA	-	
8	-	-	-	+	+	SBM	-	-	-	-	-	CP	NA	-	
9	-	-	-	+	+	-	-	-	FM	-	-	CP	-	-	
10	-	-	-	+	+	-	-	-	-	-	-	CP	-	-	
11	-	-	-	+	++	-	-	-	-	-	-	CP	-	-	
12	-	-	-	+	+	-	-	-	-	-	-	CP	-	-	
13	-	-	-	+	+	-	-	-	-	-	-	-	-	-	
14	-	-	-	+	+	SBM	-	-	-	-	-	CP	-	-	
15	-	-	-	+	+	SBM	-	-	-	-	-	-	-	-	
16	-	-	-	+	+	-	-	-	-	-	-	-	-	-	
17	-	-	-	+	+	-	-	-	-	-	-	CP	-	-	
18	-	-	-	+	+	-	-	-	-	-	-	-	-	-	
19	-	-	-	+	+	-	-	-	-	-	-	-	-	NA	
20	-	-	-	+	+	-	-	-	SP	-	-	CP	NA	-	
21	-	-	-	+	+	-	-	-	-	-	-	CP	-	-	
22	-	-	-	+	+	LTSL	-	-	-	-	-	-	-	-	
23	-	-	-	+	+	-	-	-	-	-	-	CP	-	-	
24	-	-	-	+	+	-	-	-	-	-	-	-	NA	-	
25	-	-	-	+	+	-	-	-	-	-	-	CP	NA	-	
26	-	-	-	+	+	-	-	-	-	-	-	-	-	-	
27	-	-	-	+	+	-	-	-	-	-	-	CP	-	-	
28	-	-	-	++	++	-	-	-	-	-	-	CP	-	-	
29	-	-	-	+	++	-	-	-	-	-	-	CP	NA	-	
30	-	-	-	++	++	-	-	-	-	-	-	-	-	-	
SUBTOTALS															
-	30	30	30	0	0	25	30	30	30	SP =1	30	30	30	11	22	28	
+	0	0	0	25	24	SBM=4	0	0	0	FM=2	0	0	0	CP=19	NA=8	NA=1	
++	0	0	0	5	6	SEM=1	0	0	0		0	0	0				
+++	0	0	0	0	0	LTSL= 1	0	0	0		0	0	0			MMI=1	

Tucannon Steelhead cont'd TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (%)

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN
31	-	-	-	+	+	-	-	-	-	-	-	-	-	NP & CP	-	-
32	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	+	++	-	-	-	-	-	-	-	-	NP	-	-
34	-	-	-	++	++	-	-	-	-	-	-	-	-	-	NA	-
35	-	-	-	++	+++	-	-	-	-	-	-	-	-	-	-	-
36	-	-	-	++	++	-	-	-	-	-	-	-	-	CP	NA	-
37	-	-	-	++	++	-	-	-	-	-	-	-	-	CP	-	-
38	-	-	-	+	+	LTSL	-	-	-	-	-	-	-	CP	-	NA
39	-	-	-	+	+	SEM	-	-	-	-	-	-	-	-	-	-
40	-	-	-	++	++	-	-	-	-	-	-	-	-	CP	-	-
41	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
42	-	-	-	+	+	LTSL	-	-	-	-	-	-	-	CP	-	-
43	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
44	-	-	-	++	++	-	-	-	-	-	-	-	-	-	-	-
45	-	-	-	+	+	NPSL	-	-	-	-	-	-	-	-	NA	-
46	-	-	-	+	+	-	-	-	-	-	-	-	-	CP	NA	-
47	-	-	-	+	++	-	NA				-	-	-	-	NA	-
48	-	-	-	+	+	-	-	-	-	-	-	-	-	CP	-	-
49	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
51	-	-	-	+	+	-	-	-	-	-	-	-	-	PO	NA	-
52	AH	-	-	+	+	-	-	-	-	-	-	-	-	CP	-	-
53	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
54	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
55	-	-	-	+	+	-	-	-	-	-	-	-	-	CP	-	-
56	-	-	-	+	+	-	-	-	-	-	-	-	-	CP	-	-
57	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
58	-	-	-	+	+	-	-	-	-	-	-	-	-	CP	NA	-
59	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	+	+	-	-	-	-	-	-	-	-	CP	-	-
FINAL TOTALS																
-	59	60	60	0	0	51	60	59	60	57	60	60	60	26	44	57
+	0	0	0	49	47		0	0	0		0	0	0			
++	0	0	0	11	12		0	0	0		0	0	0			
+++	0	0	0	0	1		0	0	0		0	0	0			
NA	0	0	0	0	0		0	1	0		0	0	0		16	2

MISC AH = 1

LTSL = 3 SBM = 4 FM = 2
NPSL = 1 SEM = 2 SP = 1

CP = 32
NP = 2
PO = 1

MMI = 1 SP in pharyngeal epithelium.

Chelan Steelhead — TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (%)

#	EYE			GILL			LIVER				KIDNEY			OLFACTORY	THYROID	BRAIN	
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD GRAN	MISC		KD GRAN	Ca					
61	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	NA	
62	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
63	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
64	-	-	-	-	-	-	-	-	-	FM	-	-	-	-	NA	-	NP-pharyngeal
65	-	-	-	+	+	SPSL	-	-	-	-	-	-	-	-	NA	-	
66	-	-	-	+	+	-	-	-	-	-	-	-	-	NP	NA	-	
67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	
68	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
69	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	
71	-	-	-	+	+	SPSL	-	-	-	-	-	-	-	-	NA	-	
72	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
73	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
74	-	-	-	+	+	SPSL	-	-	-	-	-	-	-	-	NA	-	
75	-	-	-	+	+	-	-	-	-	NT	-	-	-	-	NA	-	
76	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
77	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
78	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
79	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
80	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
81	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
82	-	-	-	++	++	-	-	-	-	-	-	-	-	-	NA	-	
83	-	-	-	+	+	-	-	-	-	-	-	-	-	NP	NA	NA	
84	-	-	-	++	++	SPSL	-	-	-	-	-	-	-	-	-	-	
85	-	-	-	++	++	-	-	-	-	-	-	-	-	-	-	-	
86	+	-	-	++	++	-	-	-	-	-	-	-	-	-	NA	-	
87	-	++	-	++	++	SPSL	-	-	-	-	-	-	-	-	-	NA	
88	++	+	-	++	++	-	-	-	-	-	-	-	-	NA	NA	-	
89	+	-	-	++	++	SPSL	-	-	-	-	-	-	-	-	-	-	
90	-	-	-	+	+	SPSL	-	-	-	FM	-	-	-	-	NA	-	
SUBTOTALS																	
-	27	28	30	3	3	23	30	30	30	27	30	30	30	27	10	27	
+	2	1	0	20	20	SPSL=7	0	0	0	FM=2	0	0	0	NP = 2	NA =20	NA=3	Nematode = 1
++	1	1	0	7	7		0	0	0	NT=1	0	0	0	NA = 1			
+++	0	0	0	0	0		0	0	0		0	0	0				

Chelan Steelhead cont'd

TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (%)

	EYE			GILL			LIVER				KIDNEY						
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD GRAN	MISC		KD GRAN	Ca		OLFACTORY	THYROID	BRAIN	
91	+	-	-	++	++	SPSL	-	-	-	-	-	-	-	-	-	-	
92	+	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
93	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
94	+	-	-	++	++	-	-	-	-	-	-	-	-	-	NA	-	
95	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
96	-	-	*	+	+	-	-	-	-	-	-	-	-	-	NA	-	*FM-optic nerve
97	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
98	-	-	-	+	+	-	-	-	-	NT	-	-	-	-	NA	-	
99	-	-	-	++	++	SPSL	-	-	-	-	-	-	-	-	-	-	
100	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
101	-	-	-	++	++	SPSL	-	-	-	-	-	-	-	-	-	-	
102	-	-	-	++	++	SPSL	-	-	-	-	-	-	-	-	-	-	
103	-	-	-	++	++	SPSL	-	-	-	FM	-	-	-	-	NA	-	
104	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
105	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
106	-	-	-	++	++	FGSL	-	-	-	-	-	-	-	-	-	-	
107	-	-	-	+	+	-	-	-	-	-	-	-	-	NP	NA	-	
108	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
109	+	+	-	+	+	SPSL	-	-	-	-	-	-	-	-	-	-	
110	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
111	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
112	-	-	-	+	+	SPSL	-	-	-	-	-	-	-	-	NA	-	
113	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-	
114	-	-	-	+	++	SPSL	-	-	-	-	-	-	-	-	NA	-	
115	-	-	-	+	++	-	-	-	-	-	-	-	-	-	NA	-	
116	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-	
117	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
118	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
119	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
120	-	-	-	+	+	SPSL	-	-	-	-	-	-	-	-	NA	-	
FINAL TOTALS																	
-	53	57	59	3	3	43	60	60	60	53	60	60	60	56	23	57	Paryngeal nematode = 1
+	6	2		43	41		0	0	0		0	0	0				FM in optic nerve = 1
++	1	1		14	16		0	0	0		0	0	0				
+++	0	0		0	0		0	0	0		0	0	0				
NA	0	0		0	0		0	0	0					1	37	3	
MISC.			FM=1			SPSL=16 FGSL= 1				FM=5 NT=2				NP=3			

TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (1)

Big White Fall Chinook

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN
121	-	-	-	+	++	-	-	-	-	-	-	-	-	-	NA	-
122	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
123	-	-	-	+	++	-	-	-	-	-	-	-	-	-	-	-
124	-	-	-	+	+	-			NA		-	-	-	-	NA	-
125	-	-	-	++	++	-	-	-	-	-	-	-	-	-	NA	-
126	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
127	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
128	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
129	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
130	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
131	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
132	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
133	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
134	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
135	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
136	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
137	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
138	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
139	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
140	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
141	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
142	-	-	-	+	+	-	-	-	-	-		NA		-	-	-
143	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
144	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
145	-	-	-	+	+	-	-	-	-	-		NA		-	NA	-
146	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
147	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
148	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
149	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
150	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
SUBTOTALS																
-	30	30	30	0	0	30	29	29	29	23	28	28	28	30	16	30
+	0	0	0	29	27	0	NA=1	NA=1	NA=1	NA=1	NA=2	NA=2	NA=2	0	NA=14	0
++	0	0	0	1	3	0	0	0	0	FM=6				0		0
+++	0	0	0	0	0	0	0	0	0					0		0

Big White Fall Chinook cont'd

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN
151	-	-	-	+	+	-			NA		-	-	-	-	-	-
152	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
153	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
154	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
155	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
156	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
157	-	-	-	+	+	-	+	-	-	FM	-	-	-	-	NA	-
158	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
159	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
160	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
161	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
162	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
163	-	-	-	-	+	-	-	-	-	FM	-	-	-	-	NA	-
164	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
165	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
166	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
167	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
168	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
169	-	-	-	+	+	-	+	-	-	-	-	-	-	-	NA	-
170	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
171	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
173	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
174	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
175	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
176	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
177	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-
178	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	-
179	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-
180	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
180B	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
FINAL TOTALS																
-	60	60	60	2	0	60	56	58	58	38	58	58	58	60	30	60
+	0	0	0	57	57	0	2	0	0	0	0	0	0			
++	0	0	0	1	3	0	0	0	0	0	0	0	0			
+++	0	0	0	0	0	0	0	0	0	0	0	0	0			
NA	0	0	0	0	0	0	2	2	2	2	2	2	2		30	

MISC. FM=20

TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (%)

Carson Spring Chinook

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD GRAN	LIVER MISC	KIDNEY KD GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN
181	-	-	-	+	+	-	-	-	-	-	-	-	NA	-
182	-	-	-	+	+	-	-	-	FM	-	-	-	-	-
183	-	-	-	+	+	-	-	-	-	-	-	-	-	-
184	-	-	-	+	+	-	-	-	FM	-	-	-	-	-
185	-	-	-	+	+	-	-	-	-	-	-	-	-	-
186	-	-	-	+	+	-	-	-	-	-	-	-	NA	-
187	-	-	-	+	+	-	-	-	FM	-	-	-	NA	-
188	-	-	-	+	+	-	-	-	-	-	-	-	-	-
189	-	-	-	+	+	-	-	-	FM	-	-	-	NA	-
190	-	-	-	+	+	-	NA			-	-	-	NA	-
191	-	-	-	+	+	-	-	+	-	-	-	-	-	-
192	-	-	ROPG	+	+	-	-	-	FM	-	-	PO	-	-
193	-	-	ROPG	+	+	-	-	-	-	-	-	-	-	E
194	-	-	-	+	+	-	-	-	-	-	-	-	-	-
195	++	+	ROPG	+	+	Inflam.	-	-	FM	+	-	PO	-	MMI
196	+	+	-	+	+	-	-	-	-	-	-	-	NA	-
197	-	-	-	+	+	-	-	-	-	-	-	-	-	-
198	-	-	ROPG	+	+	-	-	-	FM	-	-	PO	PT	-
199	-	-	-	+	+	-	-	-	FM	NA		-	NA	-
200	-	-	ROMI	+	+	-	-	-	-	-	-	PO	-	-
201	-	-	-	+	+	-	-	-	-	-	-	-	NA	-
202	-	-	-	+	+	-	-	-	-	-	-	PO	-	-
203	-	-	ROMI	+	+	-	-	-	FM	-	-	PO	NA	E
204	-	-	-	+	+	-	-	-	-	-	-	-	NA	-
205	-	-	-	+	+	-	-	-	-	-	-	-	PT	-
206	-	-	-	+	+	-	-	-	-	-	-	-	NA	NA
207	-	-	-	+	+	-	-	-	-	-	-	-	-	-
208	-	-	-	+	+	-	-	-	FM	-	-	-	NA	-
209	-	-	ROMI	+	+	-	-	-	-	-	-	-	-	-
210	-	-	-	+	+	-	-	-	-	-	-	-	-	-
SUBTOTALS														
-	28	28	23	0	0	29	29	29	19	28	29	24	16	26
+	1	2	ROPG=4	30	30	Inflam			FM=10	1	NA=1 NA=1	PO=6	PT=2	MMI=1
++	1	0	ROMI=3	0	0		0	0		NA=1			NA=12	Encephalitis=2
+++	0	0		0	0		0	0						NA=1
							NA=1	NA=1	NA=1					

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN	
211	-	-	-	+	++	-	-	-	-	-	-	-	-	-	NA	NA	
212	-	-	-	+	+	-	-	-	+	-	-	+	-	PO	-	-	
213	-	-	-	+	+	-	-	-	-	-	-	-	-	AH*	-	-	*Focal hemorrhage (trauma)
214	-	-	-	+	++	-	-	-	-	-	-	-	-	-	NA	-	
215	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
216	-	-	ROMI	+	+	-	-	-	-	-	-	-	-	PO	NA	-	
217	+	+	ROPG	+	+	-	-	-	+	-		NA		PO	NA	NA	
218	-	-	-	+	+	-	-	-	-	-	-	-	-	0	-	NA	
219	-	-	-	+	++	-			NA		-	-	-	-	-	NA	
220	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
221	-	-	-	+	+	-	-	-	-	-	-	-	-	PO	-	-	
222	-	-	-	+	+	-	-	-	-	-	+*	-	-	-	NA	-	*Pyogranulomatous nephritis
223	+	+	ROPG	+	+	-	-	-	-	-	-	-	-	PO	-	-	
224	-	-	-	+	+	-	-	-	+*	-	-	-	-	-	NA	-	*Multifocal hepatic microgranulomas
225	+	+	ROPG	+	+	-	-	-	+*	-	-	**	-	-	NA	-	*Capsular parasitic granuloma. **Pyogranulomatous periureteritis.
226	-	-	ROPG	+	+	-	-	-	-	-	-	-	-	PO	NA	NA	
227	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
228	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
229	+	+	ROPG	+	+	-	-	+*	-	-	+	-	-	PO	-	-	*Pyogranulomatous triaditis
230	-	-	-	-	+	-	-	-	-	FM		NA		-	NA	-	
231	-	-	ROPG	+	+	-	-	-	-	FM	-	-	-	PO	-	-	
232	-	-	-	+	+	-	-	-	-	-	-	-	-*	-	NA	-	*dilated kidney with gram bacteria.
233	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-	
234	-	-	-	+	+	-	-	-	-	-	-	-	-	NA	-	-	
235	+	+	-	+	+	-	-	-	-	FM	-	-	-	-	-	-	

FINAL TOTALS

Continued on next sheet

Carson Spring Chinook cont'd

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN	
236	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
237	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	NA	
238	-	-	-	+	+	-	-	-	-	-	-	-	-*	-	NA	-	*Focal tubular degen. giant cells.
239	+	+	ROPG	+	+	-	-	+	-	-	+	-	-	PO	-	NA	
240	+	+	ROPG	+	++	-	-	+	-	-	+	-	-	-	NA	-	

FINAL TOTALS

	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN	
-	51	51	44	1	0	59	58	55	54	44	52	57	57	42	32	49	Pyogranulomatous
+	8	9		59	56		0	3	4		5		0	PO=15	PT=2	MMI=1	Ureteritis=1
++	1	0		0	4		0	0	0		0		0	O=1		E=2	Focal tubular degeneration=1
+++	0	0		0	0		0	0	0		0		0	AH=1			Dilated renal tubule with giant bacteria =1
NA	0	0		0	0		2	2	2	2	3	3	3	1	26	8	

MISC ROPG=12 ROMI= 4 *Focal mixed inflam. at base of lamellae=1 FM=14

TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (%)

Wells Steelhead

#	EYE			GILL			LIVER				KIDNEY			OLFACTORY	THYROID	BRAIN
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Ca			
241	-	-	-	+	++	-	-	-	-	-	-	-	-	-	NA	-
242	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
243	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
244	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-
245	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
246	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
247	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	MMI
248	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
249	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
250	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
251	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
252	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	NA
253	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
254	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
255	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
256	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
257	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
258	-	-	-	+	+	SLT	-	-	-	-	-	-	-	-	NA	NA
259	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
260	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-
261	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-
262	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-
263	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
264	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
265	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
266	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-
267	-	-	-	-	+	-	-	-	-	-	-	-	-	0	-	-
268	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-
269	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
270	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
SUBTOTALS																
-	30	30	30	8	0	29	30	30	30	30	30	30	30	29	9	27
+	0	0	0	22	29	SLT=1	0	0	0	0	0	0	0	O=1	NA=21	MMI=1
++	0	0	0	0	1		0	0	0	0	0	0	0			NA=2
+++	0	0	0	0	0		0	0	0	0	0	0	0			

Wells Steelhead

#	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	OLFACTORY	THYROID	BRAIN	
271	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-	
272	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
273	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-	
274	-	-	-	+	+	*	-	-	-	-	-	-	-	-	NA	MMI	Gill inflam.
275	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
276	-	-	-	-	+	-	-	-	-	-	-	-	-	-	NA	-	
277	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
278	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	MMI	
279	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
280	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
281	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
282	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
283	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
284	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	NA	-	
285	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
286	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
287	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
288	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
289	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	NA	
290	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
291	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
292	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
293	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
294	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
295	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
296	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
297	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
298	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
299	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
300	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
FINAL TOTALS																	
-	60	60	60	12	0	58	60	60	60	59	60	60	60	59	13	54	
+	0	0	0	48	59	SLT=1	0	0	0		0	0	0			MMI=3	
++	0	0	0	0	1	Mucopu-tulent gill	0	0	0		0	0	0				
+++	0	0	0	0	0	Inflam=1	0	0	0		0	0	0				
NA	0	0	0	0	0		0	0	0		0	0	0		47	3	
MISC.										FM=1				O=1			

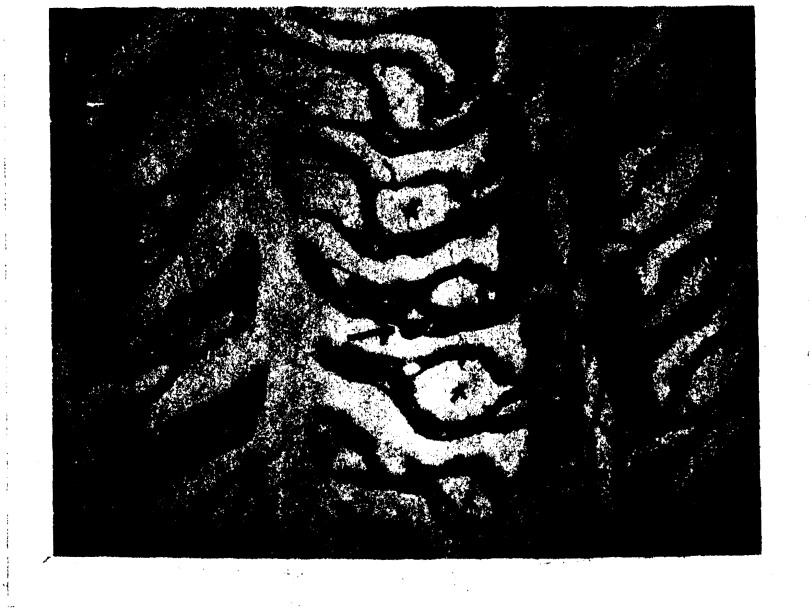


Fig. 1-Lymphatic telangiectasis (*) and a solitary eosinophilic mass of the secondary lamellae (arrow).

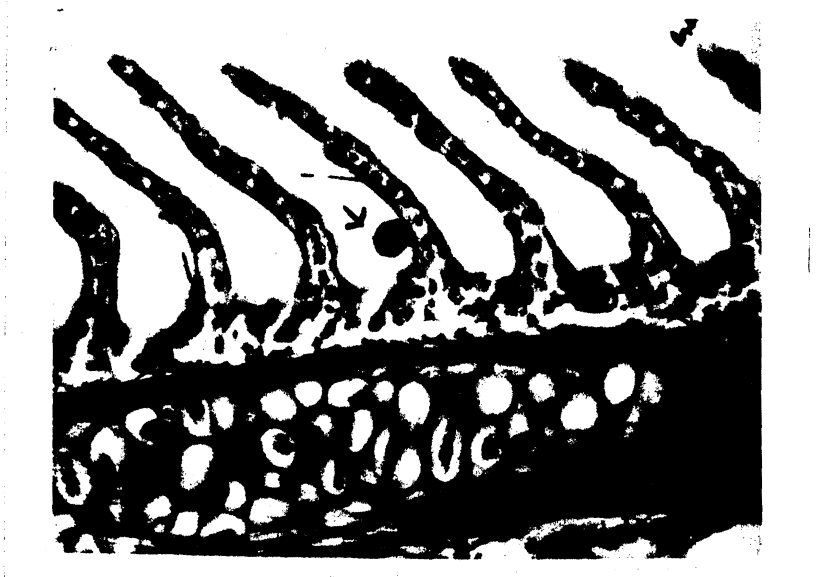


Fig. 2-Solitary basophilic mass in the secondary lamellae of the gill.

Fig. 3-Solitary eosinophilic mass in the gill secondary lamellae (arrow).

Fig. 4-A sporazoan parasite in the gill secondary lamellae.

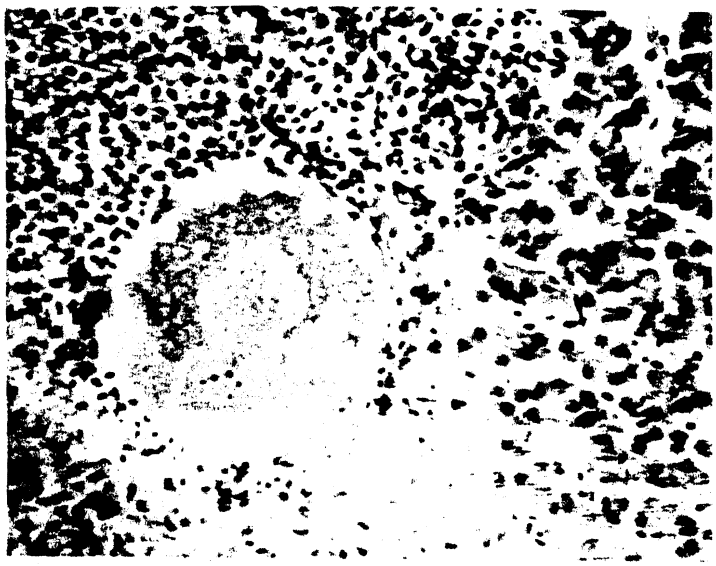


Fig. 5-Hepatic sporazoan parasitic granuloma.

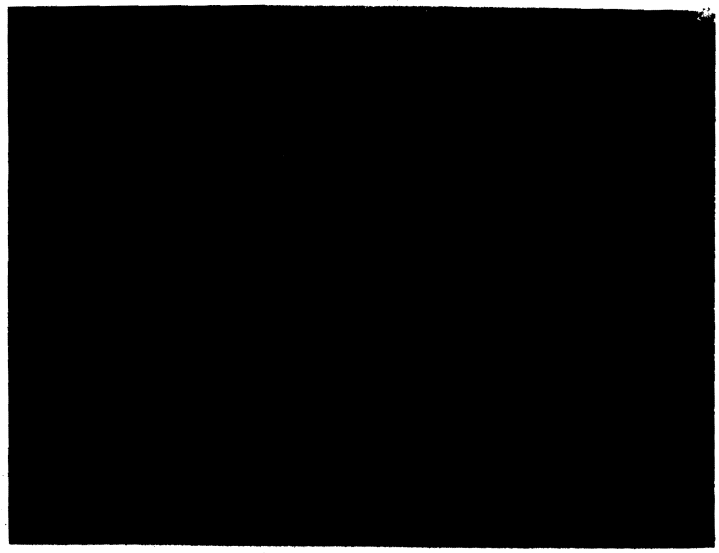


Fig. 6-Ciliated protozoan parasite in the olfactory lobe.

Fig. 7-Ciliated protozoan parasite in the olfactory lobe.