IMPRINTING HATCHERY REARED SALMON AND STEELHEAD TROUT FOR HOMING, 1978 - 1983

VOLUME III of III: DISEASE AND PHYSIOLOGY SUPPLEMENT.3

Final Report of Research

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Funded by

Tom Vogel, Project Manager
U.S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife
P.O. Box 3621
Portland, Oregon 97232
Project No. 78-l
Contract No. DE-AI79-84BP39646

February 1988

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PREFACE

Because of the scope of this report, it was prepared in three separate volumes. The Narrative is contained in Volume I, Volume II summarizes the data in tabular form, and Volume III contains the supplemental information on disease and physiology relating to the juvenile salmonids used in the study.

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Study of disease and physiology in the 1978 homing study hatchery stocks.

Study of disease and physiology in the 1979 homing study hatchery stocks.

Study of disease and physiology in the 1980 homing study hatchery stocks.

INTRODUCTION

The reports presented in this volume are designed to be used in conjunction with Volume I which contains the narrative report.

CZES Coastal Zone and Estuarine Studies

STUDY OF DISEASE AND PHYSIOLOGY IN THE 1978 HOMING STUDY HATCHERY STOCKS--A SUPPLEMENT TO: "IMPRINTING SALMON AND STEELHEAD TROUT FOR HOMING" BY SLATICK, NOVOTNY, AND GILBREATH, JANUARY 1979

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NOVEMBER 1979

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Annual Report of Research
Financed by
Bonneville Power Administration

and

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November 1979

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INTRODUCTION

The main functions of the National Marine Fisheries Service (NMFS) Aquaculture Task biologists and contractual scientists involved in the 1978 homing studies were primarily a surveillance of fish physiology, disease, and relative survival during culture in marine net-pens, to determine if there were any unusual factors that might effect imprinting and homing behavior. The studies were conducted with little background knowledge of the implications of disease and physiology on imprinting and homing in salmonids. Hatcheries and stocks sampled are listed in Table 1.

The data collected from random samples were as follows:

I. PHYSIOLOGY.

A. Gill Na+-K+ ATPase activities were measured at the time of release, with the exception of the Willard Hatchery coho salmon and Tucannon Hatchery steelhead, where freshwater Na+-K+ ATPase profiles were conducted throughout the spring. Abnormally low values could be indications that the fish were either in pre- or post-smolt condition, or had been stressed in some way.

B. Plasma electrolytes. Low 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 K levels can sometimes indicate nitrogen supersaturation stresses.

C. Hematocrits and hemoglobins. Values below or above normal can indicate possible conditions of anemia or dehydration, or reflect nutritional status, disease conditions, or both.

TABLE 1.--Hatcheries and stocks sampled in the 1978 homing studies,

Hatchery	Species	Pathology tag nos.	Date arrived at Manchester	Date of viral assay	Date of blood sampling	Date transferred to seawater pens	ATPase profile
Carson	Coho	4401-4460	4/25/78	4/25/78	4/26/78	4/27/78	No
Willard	Coho	4661-4700 4351-4370	6/ 8/78	6/12/78	6/12/78	6/10/78	Yes
Kooskia	Spring chinook	4001-4060	4/25/78	4/25/78	4/25/78	4/27/78	No
Dworshak	Steelhead	4061-4120	4/25/78	4/26/78	4/26/78	4/27/78	No
Leavenworth (Chelan)	Steelhead	3901-3960	5/ 2/78	5/ 3/78	5/ 4/78	5/ 4/78	No
Winthrop (Wells)	Steelhead	4501-4560	5/ 2/78	51 5/78	5/ 9/78	5/ 4/78	No
Tucannon	Steelhead	4601-4660	5/15/78	5/16/78	5/17/78	5/17/78	Yes

II. DISEASES.

A. Incidences of diseases based on hatchery records describing the culture and treatment of fish.

B. Random sampling of hatchery populations to determine:

1. The extent of latent bacterial kidney disease (BKD) as determined by indirect flourescent antibody techniques,

2. The presence (or absence) of certain pathogenic viruses.

3. A determination of significant lesions, abnormalities, or pathology in gill, eye, liver, and kidney tissue.

The presence and extent of the above disease organisms or lesions may have a detrimental effect upon the physiological mechanisms involved in imprinting and homing behavior.

III. SURVIVAL DURING CULTURE IN SEA-PENS.

A. Periodic assessment of survival and growth.

B. Necropsy of mortalities.

C. Assessment of the major causes of mortality.

Culturing subsamples of these hatchery test groups in net-pens in seawater is an artifical 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 30°/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.

METHODS AND MATERIALS

VIRAL ASSAYS

Liver, spleen, and kidney were sampled from 60 fish in each test group, pooled in 12 tubes of 5 fish each, and screened by a private laboratory (Rangen Research Laboratories) for viruses, See Appendix A.

HISTOPATHOLOGY

Sixty individually numbered fish of each test group in the homing study were preserved in successive fixatives and shipped to a private laboratory. Gill, liver, eye, and kidney tissues were sectioned and examined for any lesions, pathology, or abnormalities by a veterinary pathologist. See Appendix B.

EARLY LIFE HISTORY

Wherever possible, pertinent data concerning the culture and treatment of the hatchery groups were collected from the hatchery managers. These data are presented in Table 2.

SAMPLING

The sampling of fish from the hatchery stocks for health profiles was based on a combination of statistics and economics. The random sample of 60 fish from populations ranging as high as 100,000 or more was based on the work of Ossiander and Wedemeyer (1973). A single disease incidence of 5% or

TABLE 2.--Available desease and life history data of the homing study hatchery Juveniles.

Hatchery	Stock	Agency[1]	Species	eRR	Date take	Date ponded	Feed[2]	Water source	Water temp °F	Percent mortality (all causes)	Size at release (no./lb.)	Date released (1978)	Date transferred to Manchester (1978)	Diseases[3]	Disease treatment[4]
Tucannon	Skamania	WDG	Summer Steelhead		1976	March 1976	OMP Clark's Silver Cup	----	33-70	43.0	1.2	May 15	May 15	Costiasis Fungus BCD Columnaris Ichthyophthirius (June, 1979)	Malachite green Formalin Sulfas TM-50 (daily; last 2 weeks prior to release)
Dworshak	Dworshak	USFWS	Steelhead	----		----	CUP	River	40-60	----	8.0	April 25	April 25	External parasites	Formalin
Chelan (Leavenworth)	Chelan	WDG	Summer Steelhead		1976	----	Clark's Salmon Clark's Trout Silver Cup Salmon Silver cup Trout OMP	----	50-58	31.5	7.0	May 3	May 2	Vitamin C deficiency External parasites Unidentified systemic infection BCD	TM-50 Sulmet
Wells (Winthrop)	Wells	WDG	Steelhead		1977	1911	OMP Clark's Silver cup	Well and River	50	28.0	5.5	May 6	May 5	----	----
Kooskia	Kooskia	USFWS	Spring Chinook		----	----	OMP Abernathy dry	Yell and River	32-60	----	17.0	April 25	April 25	BKD Ichthyophthirius	Formalin
Carson	Carson	USFWS	Coho		----	1977	OMP Abernathy dry	Well	44	----	----	April	April 25	Furunculosis	TM-50 Roccol
Willard	Willard	USFWS	Coho		1976	1977	OMP	Well and River	44	16.0	21.6	June a	June a	CWD BKD Furunculosis	TM-50

1/ WDG - Washington Department of Game, USFWS - United States Fish and Wildlife Service. 2/ CUP - Oregon Moist Pellet.

3/ BCD - bacterial gill disease, BKD - bacterial kidney disease, CWD - cold water disease. 4/ TM-50 - oxytetracycline (dietary).

greater can be detected, provided the detection method is accurate. Subsamples of at least 60 fish were taken at the hatchery, or were drawn from the transported population for the health survey and held in circular tanks with running Beaver Creek water at ambient creek temperatures. When the average fish size was small, two subsamples of 60 fish each were required to provide sufficient material (blood and tissue) for the analyses. In most cases, the tissue and blood samples were collected within 24 hours after arrival at Manchester. However, some stocks were held as long as 6 days prior to processing. The fish from some of the hatcheries (primarily fall chinook salmon) were small. To obtain the required volumes of blood and tissue samples, we discarded any undersized fish, but still maintained a 60 fish sample size.

The fish subsampled for viral assays were individually weighed and measured. Fish that were subsampled for hematology and BKD were measured, but not weighed. Homing study fish preserved for histological sectioning were identified by numbered tags. Collected data on all parameters are available on an individual fish basis.

BLOOD SAMPLE COLLECTION

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

Whole blood smears were collected on microscope slides, labelled, air dried, fixed and stained in Diff-Quick," oven dried overnight at 45°C, and permanently mounted for future reference. The final data for hematocrits and hemoglobins were frequently based on sample sizes of less than 63 due to capillary tube breakage.

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

HEMATOCRITS

Blood samples taken for hematocrits (packed cell volume) were centrifuged in microhematocrit tubes for 3 minutes in a Clay-Adams Autocrit II (Snieszko 1960).

HEMOGLCBINS

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).

SAMPLING FOR LATENT BACTERIAL KIDNEY DISEASE (BKD)

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

The individually indentified fish were opened ventrally and the kidney 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 Hatchery that was tested and confirmed to have high numbers of pure BKD organisms.

The IFAT for BKD was originally described by Bullock and Stuckey (1975) and later modified by G. W. Camenisch of the U.S. Fish and Wildlife Service (FWS), Eastern Fish Disease Laboratory (See Appendix C for the complete procedure).

Basically this diagnostic procedure employs the following steps:

1. Application of specific BKD rabbit antisera to the unknown smear of kidney material.

2. Application of goat anti-rabbit IgG flourescein conjugate to the unknown smear. The specific antibody in the first antisera application will attach to any BKD organisms present in the unknown kidney smear. The second antisera application will then attach to the rabbit antibody (IgG), and the fluorescein serves as a label for the BKD organisms when exposed to flourescent microscopy.

3. The control slide was scanned first to insure that all procedures went normally, and the intensity of flourescence in the BKD control was subjectively noted. Only those organisms that were recognized as having the typical FA-BKD appearance were counted as positive. Any suspect organisms or probable debris were passed over as artifacts. Counts of the number of BKD organisms encountered in the first 150 microscope fields (mfs) were recorded. Samples were counted as positive if only one BKD organism was found. Both anterior and posterior kidney were examined and only those fish that had both kidney samples free of BKD organisms were classed as "negative".

NECROPSIES

All of the mortalities in the saltwater pens were collected daily. Each fish was opened asceptically from the vent; external and internal lesions were 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 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*)

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 coho salmon, O. kisutch, (Xaugg 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 salmonids demonstrate migratory behavior and an increased ability to tolerate seawater.

COLLECTION AND STORAGE OF GILL FILAMENTS

During 1978, selected stocks of coho and chinook salmon, O. tshawytscha, and steelhead, Salmo gairdneri, being reared for release at state and federal hatcheries in the Columbia River drainage were monitored for changes in gill Na^+-K^+ ATPase activities in an attempt to evaluate the state of smoltification at release.

In addition, where possible through tag identification, the relationship was determined between state of smoltificatin at release and length of migration time from the hatchery to the estuary.

At approximately 2-week intervals during the spring and summer of 1978, 30 fish were removed by dip net from representative ponds or raceways at Willard and Tucannon Hatcheries. Carson, Winthrop (Wells), Leavenworth (Chelan), Kooskia, and Dworshak Hatcheries were sampled only at release. The fish were grouped into ten groups of three fish each (approximately according to size) and they were killed by a blow on the head. After fork lengths and weights were determined, tails were servered to allow bleeding and 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 placed into a vial to which was then added 1 ml of a solution (labeled SEI) containing 0.3M sucrose, 0.2M Na2 EDTA, and 0.1M imidazole, pH 7.0. The samples of filaments in DEI were kept cold (on ice) until all 30 fish had been processed, at which time they were frozen on dry ice. The samples were later transferred to a freezer at −23°C where they were stored until processed for ATPase activities (stable up to at least 6 weeks). The analytical procedure for measuring ATPase activity is presented in Appendix D.

THE 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 smoltif ication (Lorz and McPherson 1976). Subclinical infections may be exacerbated by the stress of saltwater 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.

I. STEELHEAD

A. GILL ENZYME ANALYSES

1. TUCANNON HATCHERY (PATHOLOGY SAMPLE NUMBERS 4601-4660).

Summer run steelhead from the Tucannon Hatchery (Pond 1) showed increases in Na^+-K^+ ATPase activities through 8 May (Figure 1). Although the average activities dropped somewhat on 22 May and 5 June,

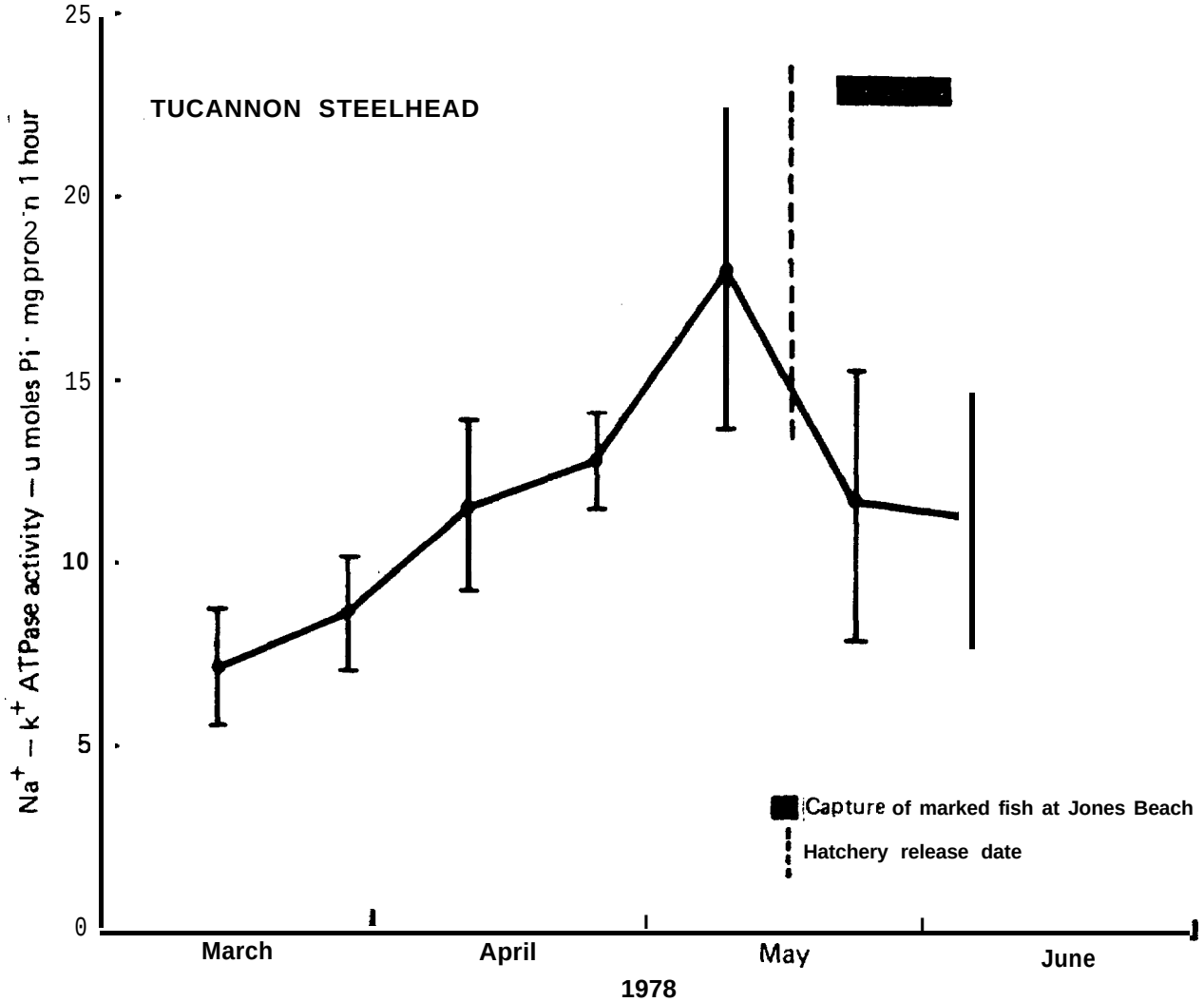


FIGURE 1. Gill Na^+ -K^+ ATPase enzyme activity in Tucannon steelhead sampled at the hatchery. Enzyme activity was measured at approximately 2-week intervals from meid-March through early June. The data shown for each sampling period are the mean and variance of 10 analyses from 30 fish (pooled- 3 fish /analysis).

the majority of these fish had elevated values. A few had either not smolted or had reverted early, having activities in the 6 to 7 range, resulting in a depression of the average activity. This group of fish (coded tag, W-O-Y-R) was released below Bonneville Dam on 17 May, slightly after the peak of the gill Na^+-K^+ ATPase activity and moved rapidly through a recapture area in the Columbia River estuary at Jones Beach where tagged fish were caught only from 20 May to 2 June (Figure 1). Although the activity was declining at release, the activity of fish transferred to seawater increased (Table 3). Fish held at the hatchery and sampled on 22 May just after release of the main group averaged 19.5 cm fork length and weighed 65.9 g.

These fish were all sampled by anesthetizing with MS-222, clipping the necessary amount of filament from one or two gill arches, then returning the fish to the pond. Only about 10% of the 210 animals tested failed to survive.

2. DWORSHAK HATCHERY (PATHOLOGY SAMPLE NUMBERS 4061- 4120)

Steelhead were sampled once at Dworshak National Fish Hatchery on 24 April from pond 42, System II, and generally appeared to be parr. The average gill Na^+-K^+ ATPase activity was 5.0 ± 1.1, ranging from 3.5 to 7.2. Only one group of 3 fish out of the 10 groups on which the enzyme activity was determined had a high enough value (7.2) to suggest even initial stages of smolt transformation. Lack of physical and biological signs of smoltification were undoubtedly due to water temperature which had been ranging from 13° to 15°C, and the upper limit which will permit smolting in steelhead is about 12°C (Zaugg, et al. 1972). The post-seawater Na^+-K^+ ATPase activity increased dramatically (Table 3).

TABLE 3 .--Summary data of viral assays, BKD a nalyses, ATPase ● aly8ca. hemotological analyses , Plasma ● lectrolytma, and survival In the . eawater pens of the 1978 homing study of fish.

Stock and species	Specimen nos.	Viral assays (results)	% of latent BKD detectible in the kidney by PAT				10-day post . eawater entry gill ATPase activity-umoles pi/mg pr/h				Freshwater gill ATPase activity-umoles pi/mg pr/h		Hemotological data (taken at Manchester upon ● [illegible])		Plasma electrolytes Meq/l								
															Na			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
Dworshak steelhead	4061-4120	IPN-confirmed	10.0	5.0	1.7	16.7	14.8	23.0	18.4	2.6	4-24-70	5.0	52.4	11.3	59	166.4	7.8	57	133.3	9.1	59	1.0	0.8
Leavenworth-Chelan[illegible] ruclhtad	3901-3960	Negative	13.3	16.7	56.7	86. 7	14.2	28.6	19.5	4.3	5- 3-78	7.5	43.3	8.9	60	165.9	14.8	58	130.9	17.2	60	1.1	0.8
Winthrop-Wells steelhead	4501-4560	IPN-confirmed	16.7	20.0	46.7	83.4	7.1	13.7	10.9	2.8	5- 3-78	17.0	55.6	11.4	58	150.3	11.1	58	107.9	20.6	58	2.5	2.6
Tucannon steelhead	4601-4660	Negative	8.3	3.3	10.0	21. 6	10.9	23.4	17.6	4.6	5- 8-78 5-22-78	18.2 11.7	48.5	9.7	60	159.5	9.5	59	131.6	6.5	60	2.4	2.6
Kooskia spring chinook	4001-4060	IPN-confirmed	11.7	8.3	70.0	90.0	17.0	35.0	22.6	5.0	4-24-78	18.1	39.1	6.6	37	114.0	19.2	31	104.1	7.9	not conducted		
Willard coho I	K01-K60	Not ● mmyed	6.7	3.3	5.0	15.0	-	-	*16.4	1.5	5- 7-78	12.8	41.5	6.4	-	143.8	-	-	120.4	-	-	7.5	-
Willard coho II	N01-N60	Negative	6.7	3.3	6.7	16.7		-	● 14.6	1.5	5-22-78	15.0	45.7	7.2	-	154.3	-	-	123.7	-	-	6.9	-
Willard coho III	4661-4700 4351-4370	IPN-confirmed	11.9	20.3	8.5	40.7	17.4	25.3	22.5	2.9	6- 8-78	8.9	42.3	8.4	52	144.3	10.9	43	115.4	7.6	52	5.8	2.7
Carson coho	4401-4460	IPN-confirmed	0	1.1	3.3	5.0	20.7	33.6	20.2	4.0	4-27-78	11.1	32.9	4.7	39	139.4	11.3	33	124.4	11.3	39	4.1	1.4
Expected ranges for clinically healthy rainbow trout													24-43	5.4-9.3	130-170			111-155			1.4-6.0		

• 8 days

TABLE 3 .--Continued.

Stock and species	Seawater survival (in net-pens)					Causes of mortality during seawater culture					
	N (start)	Survival (%) 30 day post entry	Survival (%) as of 9-24-78	N (end)	% Survival to termination (October-1978)	Negative for pathogenic bacteria (%)	% BKD	Vibrio (%)	ERM (%)	Furunculosis (%)	Aeromonas hydrophila (%)
Dworshak steelhead	178	46.4	10.8	7	3.9	73.4	0	15.6	7.8	3.1	0
Leavenworth-Chelan steelhead	180	80.0	20.0	28	15.6	44.9	0	55.1	0	0	0
Winthrop-Wells steelhead	179	10.5	0	4	2.2	2.0**	0	1.3	0	0	0.7
Tucannon steelhead	330	85.8	44.5	120	36.4	30.5	0	69.5	0	0	0
Kooskia spring chinook	180	98.8	50.0	66	36.7	36.8	10.5	42.1	5.3	5.3	0
Willard coho I	300	95.0	36.7	103	34.3	19.4	5.6	75.0	0	0	0
Willard coho II	300	96.0	46.3	124	41.3	51.4	2.9	45.7,	0	0	0
Willard coho III	300	97.0	43.3	118	39.3	34.2	7.9	57.9	0	0	0
Carson coho	180	98.2	88.0	83	46.1	52.4	4.8	42.9	0	0	0

**96X were classed as osmoregulatory stress.

3. CHELAN HATCHERY (TRANSFERRED TO LEAVENWORTH HATCHERY) (PATHOLOGY NUMBERS 3901-3960).

Chelan Hatchery steelhead sampled at Leavenworth Hatchery on 3 May gave and average gill Na^+-K^+ ATPase activity of 7.5 + 1.8, with values ranging from 4.2 to 11.1. Without having previous samples, it is difficult to determine the degree of smoltification from ATPase activities. However, values of 4 to 6 (3 groups) probably reflected little or no smolting. Values of 7 and 8 (6 groups) may have indicated some degree of transformation, while one group with 11 may have been completely transformed. General appearance suggested that most fish were in a transitional state. Average fork length was 21.0 cm and weight 79.4 g.

4. WELLS HATCHERY (TRANSFERRED TO WINTHROP HATCHERY) STEELHEAD (PATHOLOGY NUMBERS 4501-4560).

Na^+-K^+ ATPase activities were determined on representative samples of Wells Hatchery steelhead, sampled at Winthrop Hatchery on 3 May. These fish had an 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 weighed 102.2 g and were judged to be in a good smolted condition. However, this was the only group of fish sampled in which the gill Na^+-K^+ ATPase value declined significantly after transfer to seawater.

B. PLASMA ELECTROLYTES.

Sodium, potassium, and chloride ion levels in plasma were determined for fish near the time of release. This may be the first collected data on plasma electrolyte levels for steelhead; the published literature contains data for rainbow trout only.

A compilation of data on rainbow trout by Miles and Smith (1968) suggests expected values (in fresh water) of 130-170 meq (milliequivalents) /l for Na; 1.4-6.0 meq/l for K; and 115-155 meq/l for Cl. Hickman, et al. (1964) reported plasma Na ranges of 152-172 meq/l; Cl ranges of 111 to 145 meq/l; and K ranges of 3.5 to 4.2 meq/l at temperatures of 6 and 16°C.

Combining these reported ranges, we could expect the following ranges to be normal or near normal: Na^+ 130 to 172 meq/l; Cl- 111 to 155 meq/l; and K+ 1.4 to 6.0 meq/l.

1. TUCANNON HATCHERY.

The summary data for plasma electrolytes for the homing stocks are listed in Table 3. The mean values for Na, K, and Cl of the Tucannon steelhead fall within the expected ranges for rainbow trout. However, 43.3% of the Tucannon Hatchery samples were below the minimum range reported for K in rainbow trout.

2. DWORSHAK HATCHERY.

The mean plasma Na values for Dworshak Hatchery steelhead (Table 3). were considerably higher than Tucannon Hatchery steelhead (166.4 meq/l); mean Cl values were similar to the Tucannon fish (133.3 meq/l), but both are within the expected range for rainbow trout. The mean plasma K values were considerably lower (1.0 meq/l) than the Tucannon steelhead, and definitely lower than the lowest expected values for rainbow trout. There were no samples with K levels above the maximum expected for rainbow trout and 76.2% were below the minimum expected value for rainbow trout. The latter could be stress related or since the fish were sampled at the hatchery, a general hypokalemic (low blood K^+) condition.

3. CHELAN HATCHERY.

The plasma electrolytes of the Chelan Hatchery steelhead were quite similar to the Dworshak Hatchery fish (Table 3). The mean sodium and chloride values were near the upper limits reported for clinically healthy trout. The potassium values were low, as 63.3% of the samples were below the minimum for clinically healthy rainbow trout, again indicating possible stress factors. None of the potassium values were above the maximum reported for rainbow trout.

4. WELLS-WINTHROP HATCHERY.

There were noticeable differences in the plasma electrolytes of the Wells-Winthrop steelhead when compared to the other steelhead stocks in these studies (Table 3). First, the mean plasma K levels were almost identical to the Tucannon Hatchery stock (which were within the lower ranges expected in healthy rainbow trout), but again with a large variance; 41.3% were below the minimum expected value, and 8.6% were above the maximum expected value. The mean Na value of 150.3 meq/l is well within the expected range for healthy rainbow trout, but the mean Cl value fell below the minimum expected value for healthy rainbow trout. The mean Na and Cl values were the lowest of any of the steelhead stocks surveyed and statistically they were significantly different from the mean values of the other steelhead stocks. There was a high mortality in transit to Manchester and even though the survivors were rested for 7 days prior to sampling, these lower comparative values may be a reflection of that or some other stress factor.

C. HEMATOLOGY

There is no published information available on hematological values for normal wild or hatchery reared steelhead. Therefore, we can only make comparisons to the wide range of hematological values that appear in the literature for rainbow trout.

Houston and DeWilde (1968) reported the means and variances of hematocrits and hemoglobins for rainbow trout as 31.6 ± 0.3% and 7.4 ± 0.15 g hemoglobin/100 ml blood, respectively. Wedemeyer and Yasutake (1977) point out the clinically healthy rainbow trout can be expected to have hematocrits ranging from 24 to 43% and hemoglobin values ranging from 5.4 to 9.3 g/100 ml blood. Barnhart (1969) sampled several strains of rainbow trout fed two different diets and found mean hematocrit values ranging from 28.2 to 31.7%, with individual hematocrits ranging from 11 to 44%, and mean hemoglobin values ranging from 6.5 to 7.7 g/100 ml blood, with individual hemoglobins ranging from 2.2 to 13.0 g/100 ml blood.

McCarthy et al. (1973) studied the Kamloops variety of rainbow trout and found mean hematocrits of 39.5% (range: 30-49%) and mean hemoglobin values of 7.5 g/100 ml blood (range: 5.2-12.9 g/100 ml). The 96% percentile ranges were: Hct - 24.0-43.0; Hb - 5.4-9.3.

Wedemeyer and Nelson (1975) reported similiar values for the Shasta strain of rainbow trout. The means and percentile range estimates were: Hct - 34.1% (24.0-43.0); Hb - 7.6 g/100 blood (5.4-9.3).

Snieszko (1960) studied different size groups of rainbow trout, and found that fish averaging 142 mm long had mean henatocrits of 45.3%; and, fish averaging 235 mm in length had mean hematocrits of 53%. Approximately

10% of the smaller trout and 73% of the larger trout had hematocrits of 50% or more. The mean hemoglobin value of the larger fish was 8.7 g/l00 ml. Most steelhead smolts would be between these two size groups,

1. TUCANNON HATCHERY.

The summarized data of the hematocrit and hemoglobin values for the Tucannon steelhead are presented in Figure 2. The mean hematocrit (48.6%) and hemoglobin (9.9 g/100 ml blood) values were higher than the maximum expected values for rainbow trout reported by Wedemeyer and Yasutake (1977); 72.2% of the hematocrit values were above 43%, and 55.0% of the hemoglobin values were above 9.3 g/100 ml blood.

The blood samples were taken 48 hours after they arrived at Manchester (see Table 1) and there was no great deviation in water temperatures. Therefore, these high mean values should not be due to transport stress. Other evidence suggests that the fish were reasonably healthy, and these data may reflect normal values for steelhead, or they may be related to smoltification.

2. DWORSHAK HATCHERY,

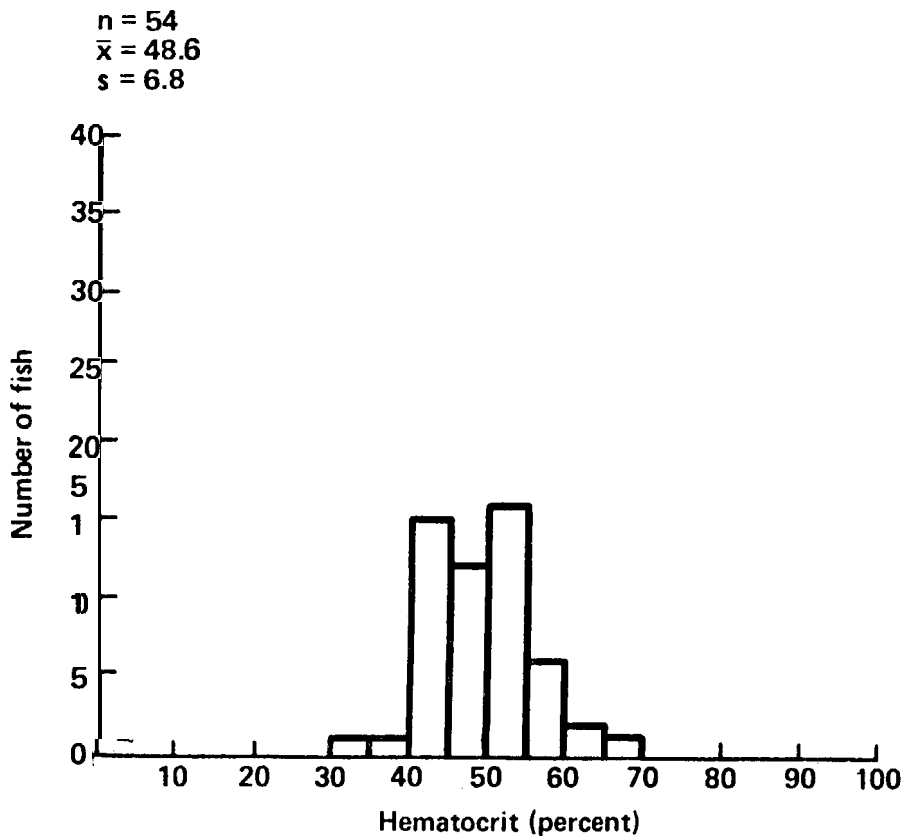
Wedemeyer (unpublished data) found mean hematocrit values of 40% (±3) and hemoglobins of 9 g/100 ml (+3) when he sampled Dworshak steelhead smolts in 1977. This is considerably lower than the values we obtained in 1978. The summarized data for the hematocrit and hemoglobin values for the Dworshak Hatchery steelhead are presented in Figure 3. The mean hematocrits (52.4%) and hemoglobins (11.5 g/100 ml blood) are higher than the Tucannon Hatchery steelhead and 78.0% of the hematocrit and 88.3% of

DISEASE LAB CODE 4601–4660

DATE SAMPLED May 17,1978

SPECIES STEELHEAD

HATCHERY STOCK TUCANNON



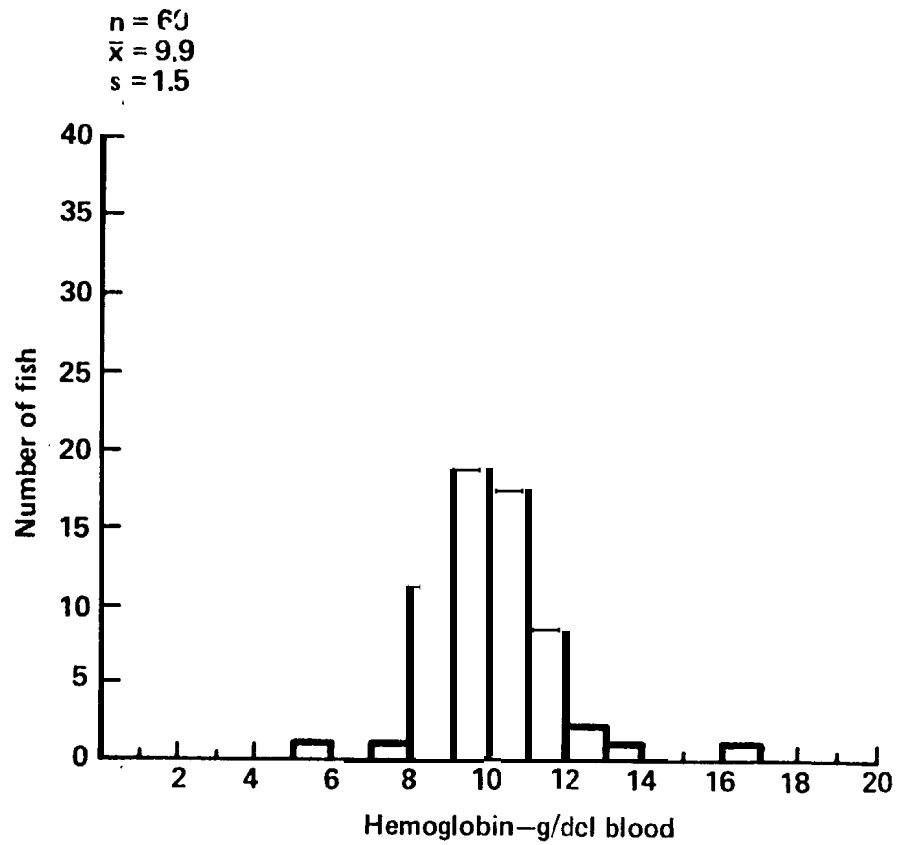


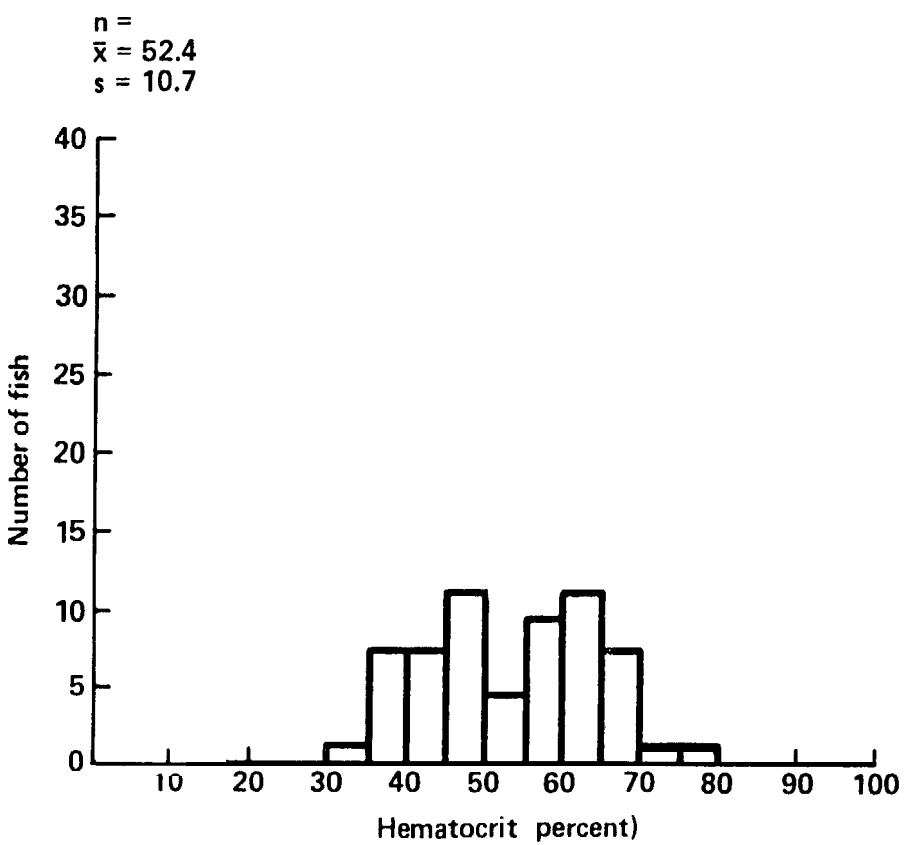
FIGURE 2.--A histogram and other data for hematocrit and hemoglobin values of the Tucannon steelhead.

DISEASE LAB CODE 4061–4120

DATE SAMPLED April 28,1978

SPECIES STEELHEAD

HATCHERY STOCK DWORSHAK



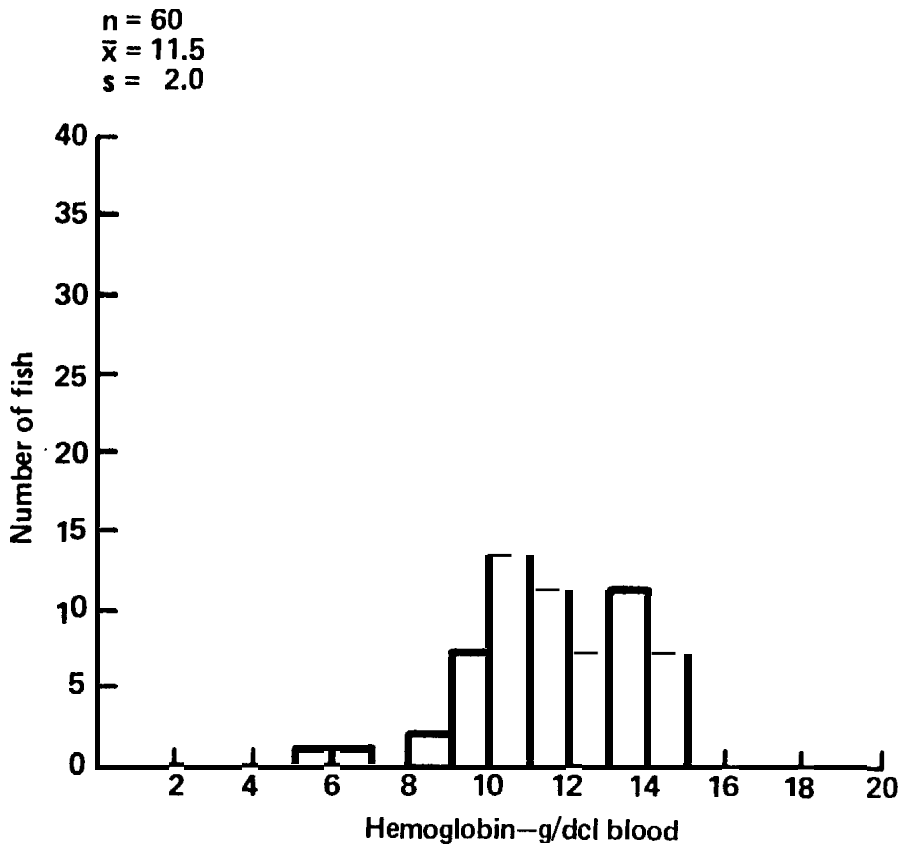


FIGURE 3 - A histogram and other data for hematocrit and hemoglobin values of the Dworshak steelhead

the hemoglobin values were higher than the maximum expected values reported by Wedemeyer and Yasutake (1977) for clinically healthy rainbow trout. None were lower than the minimum. All blood samples for hematology and plasma electrolytes were collected at the Dworshak Hatchery. Therefore, these elevated values cannot be attributed to transportation stress.

3. CHELAN HATCHERY.

The summarized data for the hematocrit and hemoglobin values for the Chelan-Leavenworth Hatchery steelhead are presented in Figure 4. The mean values are within the ranges that Wedemeyer and Yasutake (1977) reported could be expected for rainbow trout, but 50.0% of the hematocrit values are above the expected maximum of 43%, and 31.7% of the hemoglobin values were above 9.3 g/100 ml blood. None of the hematocrit values were below the minimum expected (24%), and only 1.7% were below the minimum hemoglobin value.

4. WELLS-WINTHROP HATCHERY.

The summarized data for the hematocrit and hemoglobin values for the Wells-Winthrop Hatcheries steelhead are presented in Figure 5. The mean values were the highest of any of the steelhead groups studied, as 94.7% of the hematocrits were above the expected maximum (43%) for rainbow trout, and 85.0% of the hemoglobins were above the maximum value expected (9.3 g/100 ml).

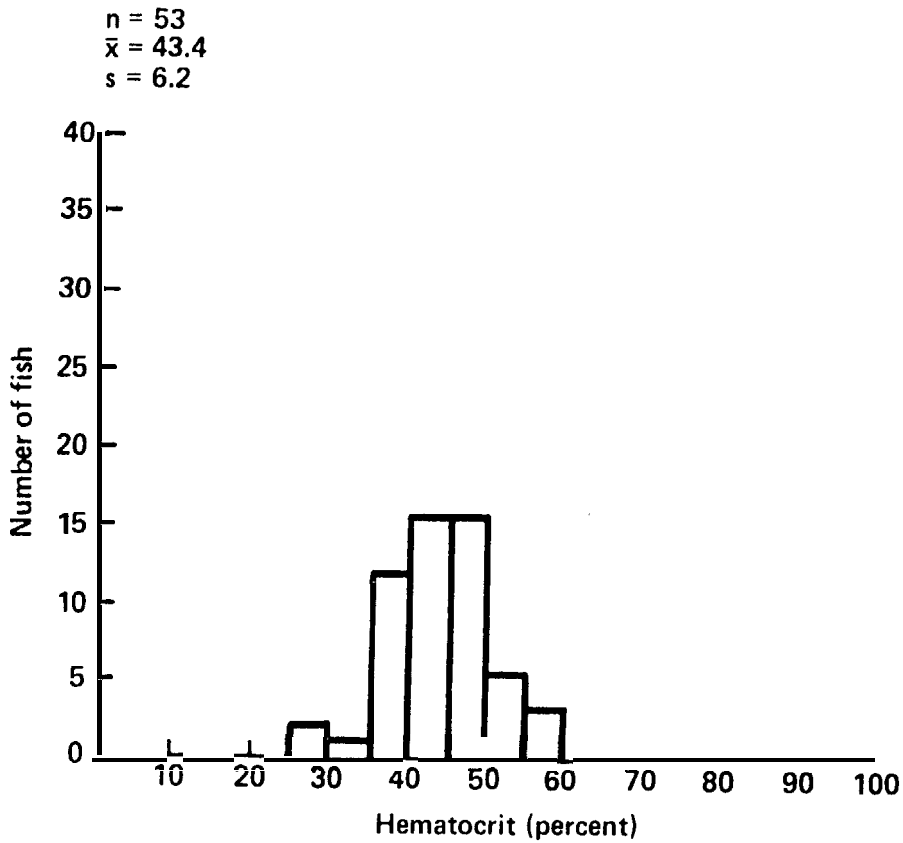
The generally high mean hematocrit and hemoglobin values for all of the steelhead stocks may reflect a normal hematological condition for these anadromous strains of the rainbow trout; or, they may be associated with mild dehydration as one physiological aspect of smoltification.

DISEASE LAB CODE 3901–3960

DATE SAMPLED May 4,1978

SPECIES STEELHEAD

HATCHERY STOCK LEAVENWORTH (Chelan)



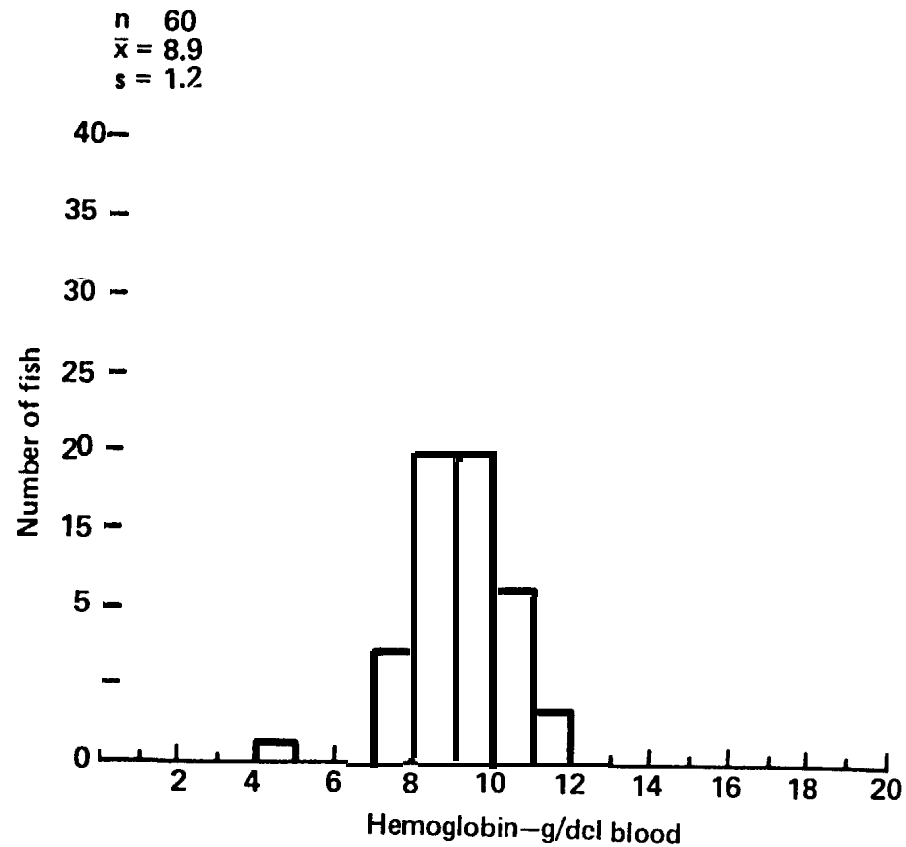


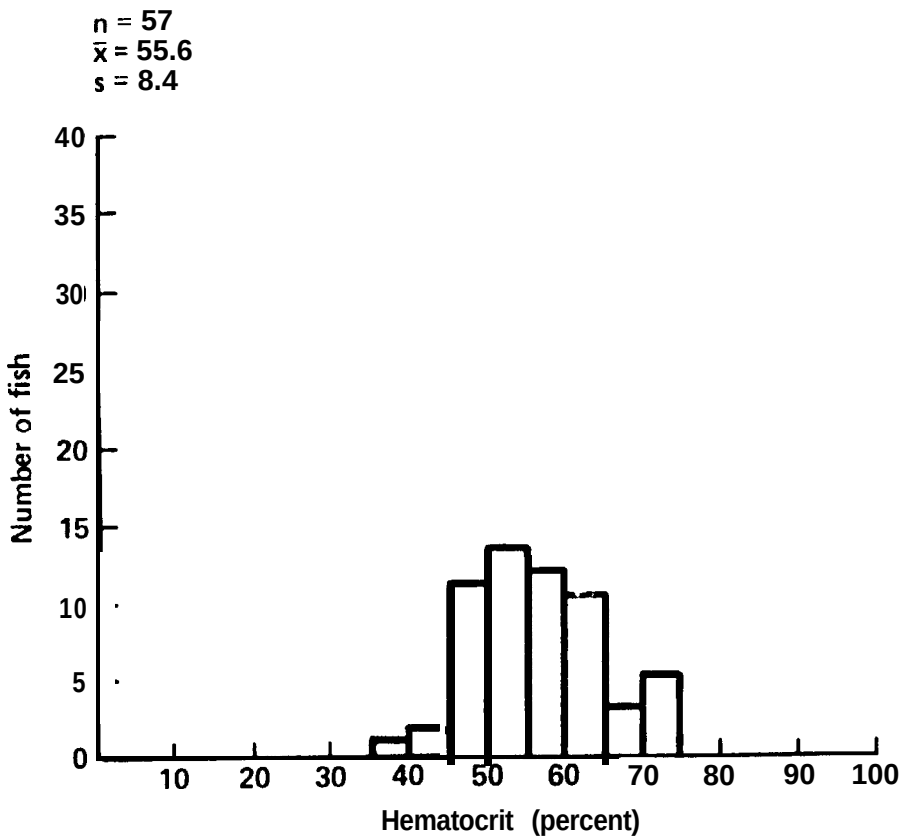
FIGURE 4.--A histogram and other data for the hematocrit and hemoglobin values of the Chelan-Leavenworth steelhead

DISEASE LAB CODE 4501–4560

DATE SAMPLED May 9, 1978

SPECIES STEELHEAD

HATCHERY STOCK WINTROP (Wells)



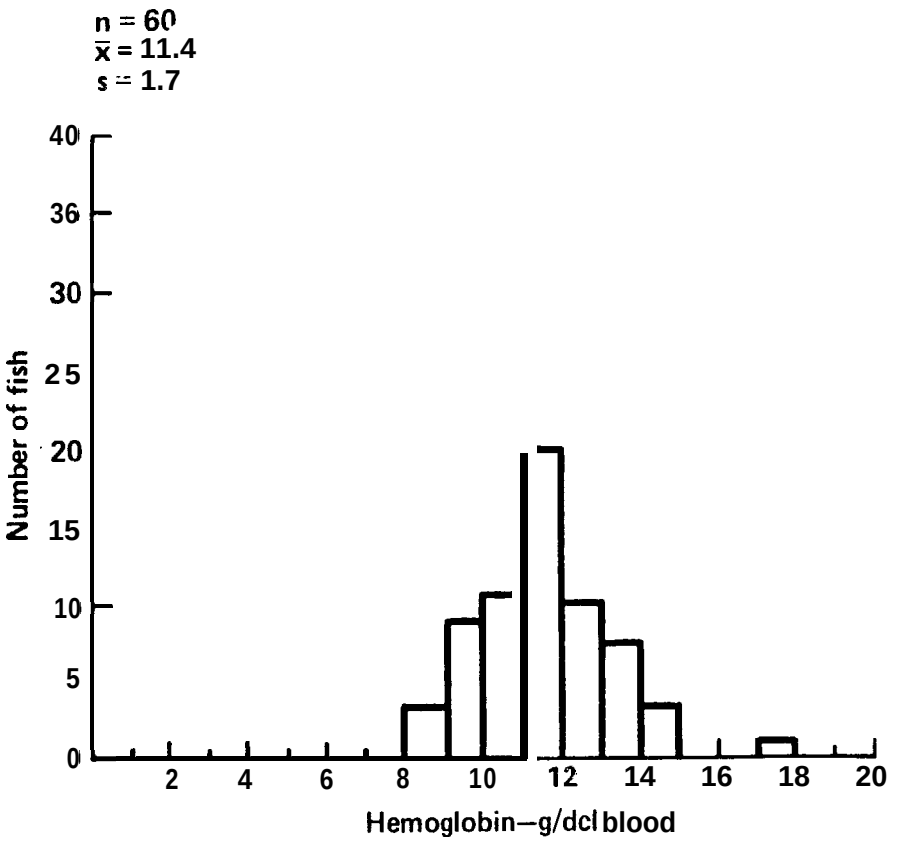


FIGURE 5.--A histogram and other data for the hematocrit and hemoglobin values of the Wells-Winthrop steelhead.

D. VIRAL SCREENING.

No confirmed evidence of common viral pathogens could be found by the private contract laboratory in the Tucannon or Chelan-Leavenworth Hatchery steelhead, but infectious pancreatic necrosis (IPN) virus was confirmed in the Dworshak and Wells-Winthrop populations. The complete results of the viral screening are presented in Appendix A.

E. INDIRECT FLOLJRESCENT ANTIBODY TEST FOR BACTERIAL KIDNEY DISEASE (IFAT-BKD), AND RELATIVE SURVIVAL OF STEELHEAD IN THE SEAWATER PENS.

1. TUCANNON HATCHERY.

The incidence of BKD as determined by IFAT was 13 out of 60 (21.6%), with 6 fish (10.0%) positive for both anterior and posterior kidney involvement; 5 (8.3%) with anterior kidney involvement only; and 2 (3.3%) with posterior kidney involvement only (Table 3).

The 30-day survival of the fish cultured in the seawater net-pens was 86%; the full term survival was 36% (which is relatively high for steelhead cultured in net-pens); and, no gross BKD lesions were found in necropsied fish (see Table 3).

2. DWORSHAK HATCHERY.

The incidence of BKD in this group as detected by IFAT was 16.7%. The level of infection was light, as only 1.7% of the fish had involvement in both posterior and anterior kidney; 10.0% in anterior kidney only; and 5.0% in the posterior kidney only (Table 3).

The 30-day survival of the Dworshak steelhead in the seawater net-pens was 46.4%, and the survival through termination was approximately one-tenth that of the Tucannon Hatchery steelhead. None of the mortalities

posted could be attributed to BKD, and only 26.6% of the mortalities posted could be attributed to bacterial pathogens (Table 3). A small percentage of the mortalities posted were due to ERM disease or furunculosis. Whether these were latent or transmitted in seawater is not known, as the hatchery records (Table 2) made available only indicate a treatment for external parasites with Formalin.

3. CHELAN-LEAVENWORTH HATCHERY.

The incidence of BKD in this group as detected by IFAT was 86.7% with 56.7% involvement in both anterior and posterior kidney (Table 3). The 30-day survival of the fish cultured in the seawater net-pens was 80.0%; the full term survival was 15.6% and no BKD lesions were seen in the necropsied fish (see Table 3).

The hatchery records (Table 2) indicate that this stock suffered a 37.5% mortality during freshwater rearing and were fed medicated diets to treat a number of diseases.

4. WELLS-WINTHROP HATCHERY.

The incidence of BKD in this group as detected by IFAT was 85.0% with 46.7% involvement in both anterior and posterior kidney (Table 3). The 30-day survival of the fish cultured in the seawater net-pens was 10.5%, the full term survival was 2.2%, and no BKD lesions were seen in posted mortalities. The large mortality during the first period was attributed to osnoregulatory stress (Table 3).

No statistically significant correlations were found between the incidence of BKD by IFAT and hematocrits or hemoglobins in the steelhead stocks studied.

F. HISTOPATHOLOGY.

A detailed report on the examination and interpretation of selected tissue sections from the random samples is presented by the contract veterinary pathologist in Appendix B. A summary of the pathological conditions observed, their severity, and their frequency of occurrence is presented in Table 4. The severity is ranked as: I. Recognizable (least severe); II. Intermediate; and III. Severe. Note that the incidence of rank II and III severity was low for all conditions encountered (Table 4).

Although the pathologist did not find gram-positive bacteria in either the kidney or liver tissue of the three steelhead stocks examined, the more sensitive IFAT tests of kidney tissue smears from the same specimens did reveal the presence of BKD organisims.

In general, the pathological conditions observed in the three steelhead stocks were not wide-ranging and may not significantly effect homing response or survival. However, there were several dominating conditions that appeared in all three stocks, and it may be of interest to summarize the probable causes.

Analysis of the pathologist's data indicate that all of the steelhead stocks had lesions (both degenerative and regenerative) in the skeletal muscle of the eye; both recognizable and of intermediate severity.

Recognizable retrobular fat necrosis (death of fatty tissue behind the eyeball) was evident in the Dworshak steelhead. These ocular lesions may be the result of an unfulfilled nutritional requirement and/or possible nutritional disorder.

TABLE 4.--Pathological conditions observed in the homing stocks and their percentage of incidence.

Organ & pathology	Incidence (%)																							
	Tucannon steelhead				Dworshak steelhead				Wells-Winthrop steelhead				Kooskia spring chinook				Carson coho				Willard coho			
	Severity 1/				Severity 1/				Severity 1/				Severity 1/				Severity 1/				Severity 1/			
	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total
Eye																								
Skeletal muscle lesions	48.3	8.3	0	56.7	53.3	10.0	0	63.3	50.0	0	1.7	51.7	10.0	0	0	10.0	27.1	0	0	27.1	36.8	3.5	0	40.3
Retrobulbar fat necrosis	0	0	0	0	27.1	3.3	1.7	32.1	6.7	0	0	6.7	6.7	1.7	0	8.4	0	0	0	0	6.9	0	0	6.9
Minimal subacute iridocyclitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Choroid gland inflamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Focal mononuclear cells in optic nerve	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chronic active ophthalmitis		0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corneal scleritis	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Optic nerve neuritis	0	0	0	0	3.3	0	0	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sugmental vasculitis	1.7	0	0	1.7	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chronic conjunctivitus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Gills																								
Increased number of lymphocytes	23.3	0	0	23.3	63.3	10.0	3.3	76.6	65.0	1.7	0	66.7	73.3	20.0	1.7	95.0	74.6	0	0	74.6	22.4	0	0	22.4
Epithelial cell proliferation	31.7	0	0	31.7	66.7	16.7	1.7	85.1	76.7	11.7	0	88.4	10.0	6.7	0	16.7	61.1	0	0	61.1	41.4	3.5	0	44.9
Basophillic granular organism	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60.7	16.1	0	76.8	5.2	3.5	1.3	10.4
Filamentous bacteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Ciliated protozoan parasites	0	0	0	0	23.3	0	0	23.3	0	0	0	0					0	0	0	0	0	0	0	0
Possible protozoan parasites	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0.3	0	0	3.3	0	0	0	0	0	0	0	0
Sporozoan parasites	0	0	0	0	3.3	0	0	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Local capillary dilation of the secondary lamellae	1.7	0	0	1.7	0	0	0	0	8.3	0	0	8.3	0	0	0	0	0	0	0	0	0	0	0	0
Microsporidian-sporozoan parasites	0	0	0	0	0	0	0	0		0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0
Neutrophils in secondary lamellae	1.7	0	0	1.7	0	0	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Congestion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
LIVER																								
Increased parenchymal fat	8.3	0	0	8.3	11.7	0	0	11.7	3.3	0	0	3.3	3.3	0	0	3.3	1.7	0	0	1.7	0	0	0	0
Bacterial kidney disease with gram pos. bacteria	0	0	0	0	0	0	0	0	0	0	0	0	3.3	1.7	0	5.0	0	1.7	0	1.7	0	0	0	0
Granulomatous lesions w/o gram pos. bacteria	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	1.7	1.7	3.4	0	0	3.4	1.8	0	0	1.8
Focal mononuclear cell infiltrate	20.0	0	0	20.0	0		0	0	0	0	0	0	48.3	15.0	5.0	68.3	5.1	0	0	5.1	7.0	0	0	7.0
Focal necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Gram stain	0	0	0	0	0	0	0	0	0	0	0	0	3.3	0	0	3.3	1.7	0	0	1.7	0	0	0	0
KIDNEY																								
Bacterial kidney disease with gram pos. bacteria	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	3.3	5.0	0	1.7	0	1.7	0	0	0	0
Granulomatous lesions w/o gram pos. bacteria	6.7	0	0	6.7	1.7	0	0	1.7	1.7	0	0	1.7	23.3	3.3	0	26.6	1.7	1.7	0	3.4	1.7	1.7	0	3.4
Nephrocalcinosis	0	0	0	0	0		0	0	0	0	0	0	23.3	8.0	3.3	34.6	0			0	0	0	0	0
Gram pos. debris in tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.8	0	0	6.8	0	0	0	0
Debris in excretory duct	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0
Gram stain	0	0	0	0	0	0	0	0	0	0	0	0	3.3	0	0	3.3	3.4	0	0	3.4	0	0	0	0

1/ I = Recognizable (least severe)
II = Intermediate
III = Severe

The pathological conditions in gill tissue were dominated by increased numbers of lymphocytes in gill tissue and epithelial cell proliferation. These observations may be indicative of very mild exposure to antigens, including pathogenic micro-organisms, and the mildest possible form of nutritional gill disease. Second in frequency of occurrence was the presence of ciliated protozoan parasites in the Dworshak steelhead. The incidences of gill pathology were the least frequent in the Tucannon steelhead.

The available hatchery records (Table 2) indicate that the steelhead mortality in the Tucannon Hatchery was 43% and in the Wells-Winthrop pond and hatchery, 28%.

II. SPRING CHINOOK SALMON: KOOSKIA HATCHERY (PATHOLOGY SAMPLE NUMBERS 4001-4060).

A. GILL ENZYME ANALYSES.

Spring chinook salmon from Kooskia Hatchery had an average gill Na+-K+ ATPase activity of 18.1 + 2.8 on 24 April. Values ranged from 14.0 to 22.7 (Table 3), and the activity continued to increase after transfer to seawater. A small group of O-age spring chinook salmon sampled at the same time had an average activity of 6.2.

B. PLASMA ELECTROLYTES.

Only plasma Na and Cl were determined for the Kooskia Hatchery spring chinook salmon (Table 3). The mean values (Na = 114 meq/l; Cl = 104 meq/l) were apparently lower than a Leavenworth Hatchery spring chinook salmon stock (Na = 150 meq/l; Cl = 108 meq/l), and the Kalama Falls Hatchery spring chinook salmon stock (Na = 137 meq/l Cl = 116 meq/l) sampled during the same period. There are apparently very little published data on plasma electolytes of spring chinook salmon.

C. HEMATOLOGY.

The summarized data for the hematocrit and hemoglobin values are presented in Figure 6. The mean values (39.1%; 6.6 g/dcl blood) are probably normal. 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. Our own experience has indicated that hematocrit values below 28% in Pacific salmon may be the beginning stages of a number of problems, and only 8.3% of the Kooskia Hatchery fish fell below this level.

D. VIRAL SCREENING (SEE APPENDIX A).

Rangen Laboratories found only 5 out of 12 pools negative for virus on the first screening, and IPN virus was confirmed in this stock.

E. IFAT-BKD.

The Kooskia Hatchery spring chinook salmon had the highest incidence of BKD (as determined by IFAT) for any of the homing stocks screened,with a total incidence of 90% and a 70% incidence of infection in both kidneys (Table 2). It was not possible to determine if there was any correlation of BKD to hematology because of the low number of noninfected fish in the sample for comparative analysis.

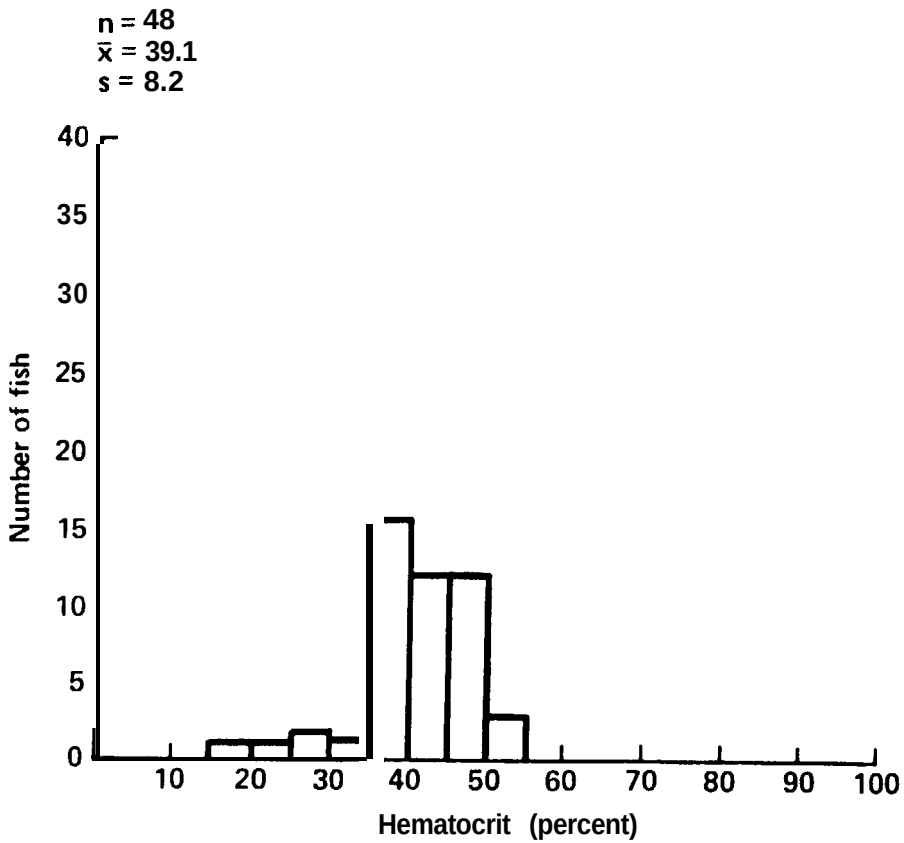
The 30-day survival of this group (Table 3) in the seawater net-pens was the highest of any homing stock tested in 1978 (98.8%). The seawater survival through termination of the tests (October 1978) was 36.7%, and 10.5% of the mortalities posted had grossly visible BKD lesions in the kidney, spleen, or liver. This follows the typical pattern that we

DISEASE LAB CODE 4001–4060

DATE SAMPLED April 25, 1978

SPECIES SPRING CHINOOK

HATCHERY STOCK KOOSKIA



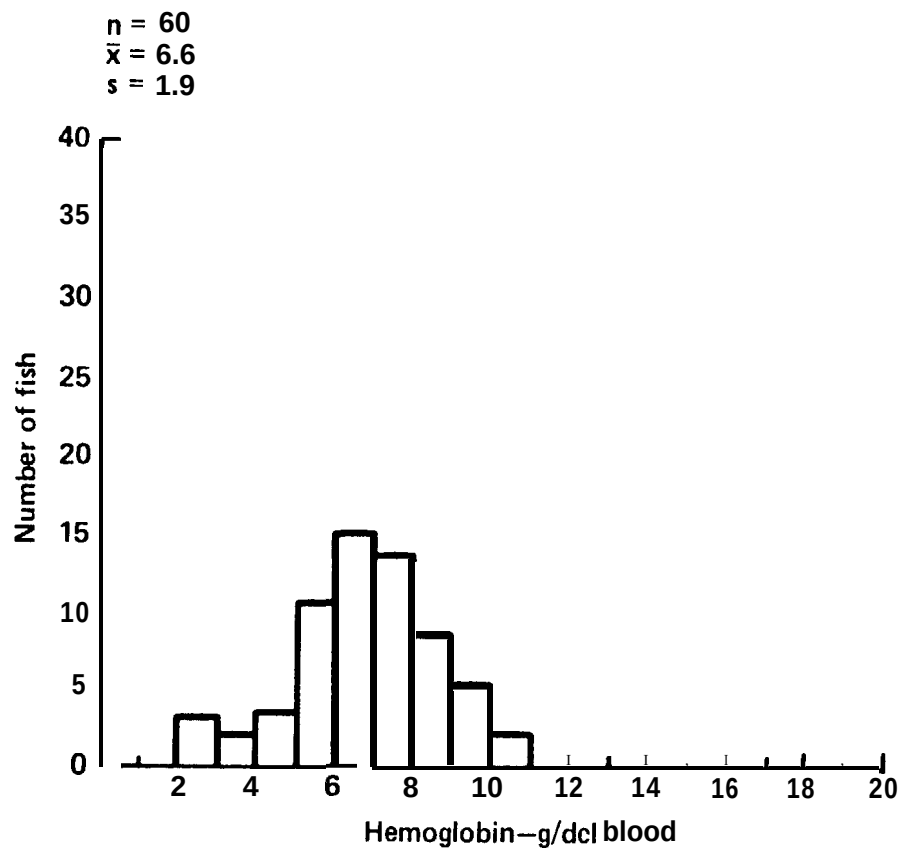


FIGURE 6.--A histogram and other data for hematocrit and hemoglobin values for the Kooskia spring chinook salmon.

have seen in the past: mortality in seawater due to latent BKD infections generally begins in the fall and may take more than 2 years (Ellis et al. 1978). A small percentage of the mortalities posted from this group (Table 3) were from ERM disease or furunculosis. Whether this was latent or transmitted in seawater is not known.

F. HISTOPATHOLOGY (SEE APPENDIX B).

The major difference between the other homing stocks and the Kooskia Hatchery spring chinook salmon (Table 4) are: 1) the incidence of muscle lesions of the eye is l/5-1/6 that of the steelhead and l/3-1/4 that of the coho salmon; 2) there is a very high incidence (95%) of increased lymphocytic activity in the gills; 3) the pathologist was able to find BKD (by tissue gram stain) in both liver and kidney (5%), and even though the sensitivity was approximately l/20 that of the IFAT, the level of infection must have been comparatively greater than that in the steelhead; 4) granulomatous lesions (26.6%) and nephrocalcinosis (34.6%), a condition of calcium phosphate precipitation in the renal tubules, dominated any pathology of the kidney. If the latter conditions are secondary results of low-level BKD infections, they could impair the process of excretion.

The hatchery records (Table 2) indicate that this group was treated for external parasites with Formalin which may account for some of the increased lymphocytic activity in the gill tissue.

III. COHO SALMON.

A. GILL ENZYME ANALYSES.

1. CARSON HATCHERY (PATHOLOGY SAMPLE NUMBERS 4401-4460).

The Carson Hatchery coho salmon were sampled on 27 April. Gill Na^+-K^+ ATPase activities averaged 11.1 ± 1.3, ranging from 8.8 to 12.4.

Some parr marks were still visible, but ventral fins were becoming clear. These indications suggested that smolt transformation had begun.

2. WILLARD HATCHERY (PATHOLOGY NUMBERS 4661-4700 AND 4351-4370).

A complete freshwater gill Na+-K+ ATPase profile was conducted on the Willard Hatchery coho salmon.

The first external signs of smolting were noticed on 26 April, at a time when gill Na^+-K^+ ATPase activity was 10.2. Three releases were made to coincide with groups used in the homing study. A high percentage of the fish were at or near smolt stage on the first two releases (Figure 7), one cooing before peak activity and the other (24 May) coming just as the Na^+-K^+ ATPase decline started. At the time of the third release (8 June) parr reversion was well in progress as indicated by the average Na^+-K^+ ATPase activity (about 9). Very few fish from these three releases were obtained from the Jones Beach recapture operation. Only one, from the third release, was obtained for Na^+-K^+ ATPase determination.

Average sizes of fish sampled near the three release times were: 1) 12.5 cm, 21.8 g; 2) 12.5 cm, 22.4 g; 3) 13.6 cm, 27.9 g.

B. PLASMA ELECTROLYTES.

Miles and Smith (1968) reported on plasma electrolytes in hatchery coho salmon. Spring samples of fish in fresh water averaging 12.5 g were as follows:

	X (meq/l)	Range (meq/l)
Na+	146.7	130.0-168.0
Cl-	117.3	90.9-132.6
K+	8.6	1.8- 19.0

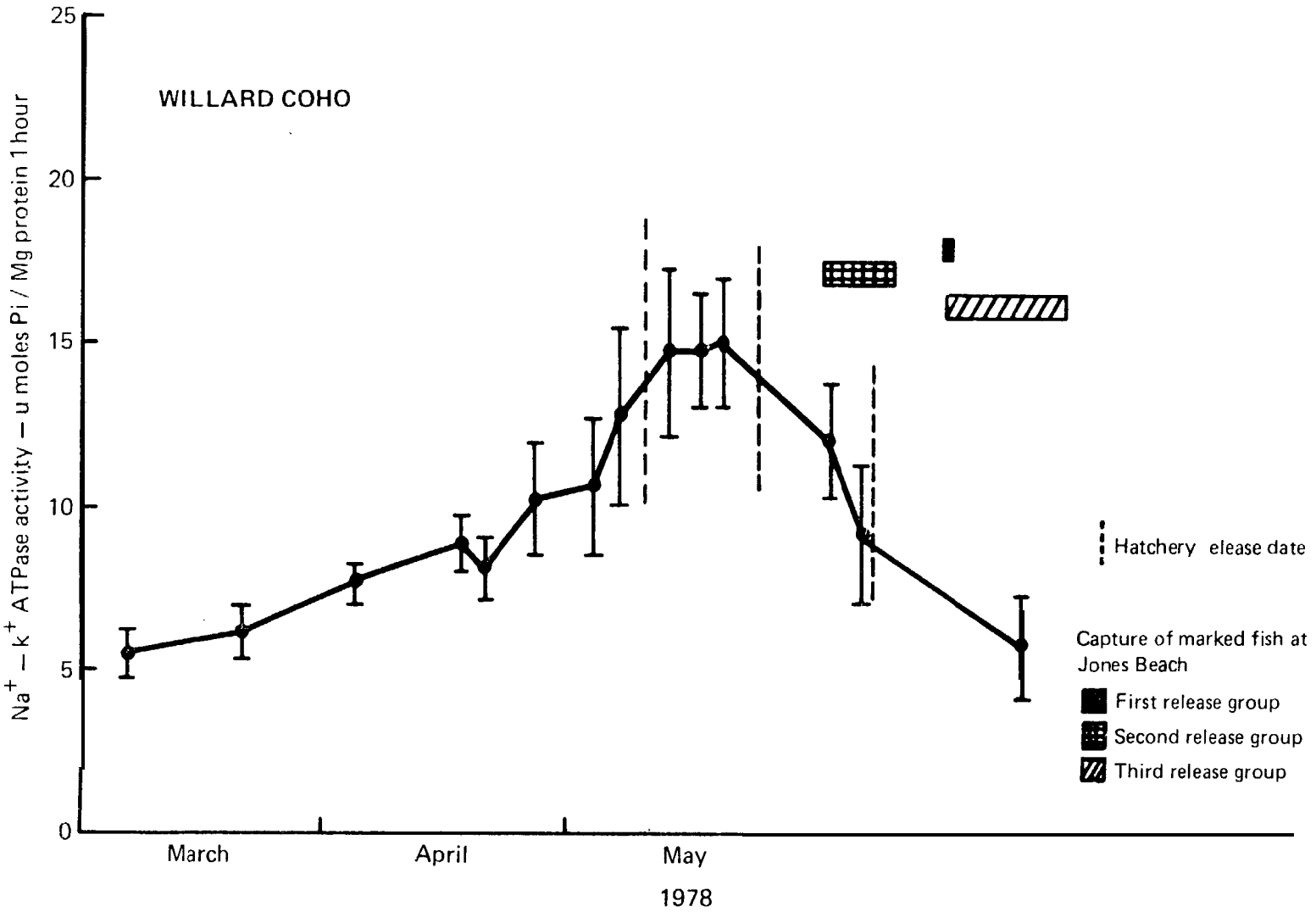


FIGURE 7.--Gill Na^+ -K^+ ATPase enzyme activity in Willard coho salmon sampled at the hatchery. Enzyme activity was measured at approximately 2-week intervals from early March through late June. The data shown for each sampling period are the mean and variance of 10 analyses from 30 fish (pooled--3 fish/analysis).

Our own studies of Columbia River Hatchery coho salmon in 1978 resulted in the following ranges of mean values for plasma electrolytes:

Na+	140.8 - 170.0
C1-	113.4 - 140.8
K+	5.7 - 8.4

1. CARSON HATCHERY.

The mean plasma electrolytes for the Carson Hatchery coho salmon (Table 3) were well within these ranges. The Na+ values ranged from 126 to 178 meq/l ($\bar{X}$ = 159.4). Cl' values ranged from 102 to 137 meq/l ($\bar{X}$ = 124.4), and K^+ values ranged from 0.8 to 7.8 meq/l (x = 4.1).

2.' WILLARD HATCHERY.

The mean Na+ (144.3 meq/l), Cl- (115.4 meq/l), and K+ (5.8 meq/l) values were well within the expected levels for clinically healthy coho salmon as prescribed by Miles and Smith. (1968).

C. HEMATOLOGY.

Wedemeyer and Chatterton (1971) list normal expected values (for coho salmon) in fresh water of 32.5 to 52.5% for hematocrits and 6.5 to 9.9 g/100 ml blood for hemoglobins.

1. CARSON HATCHERY.

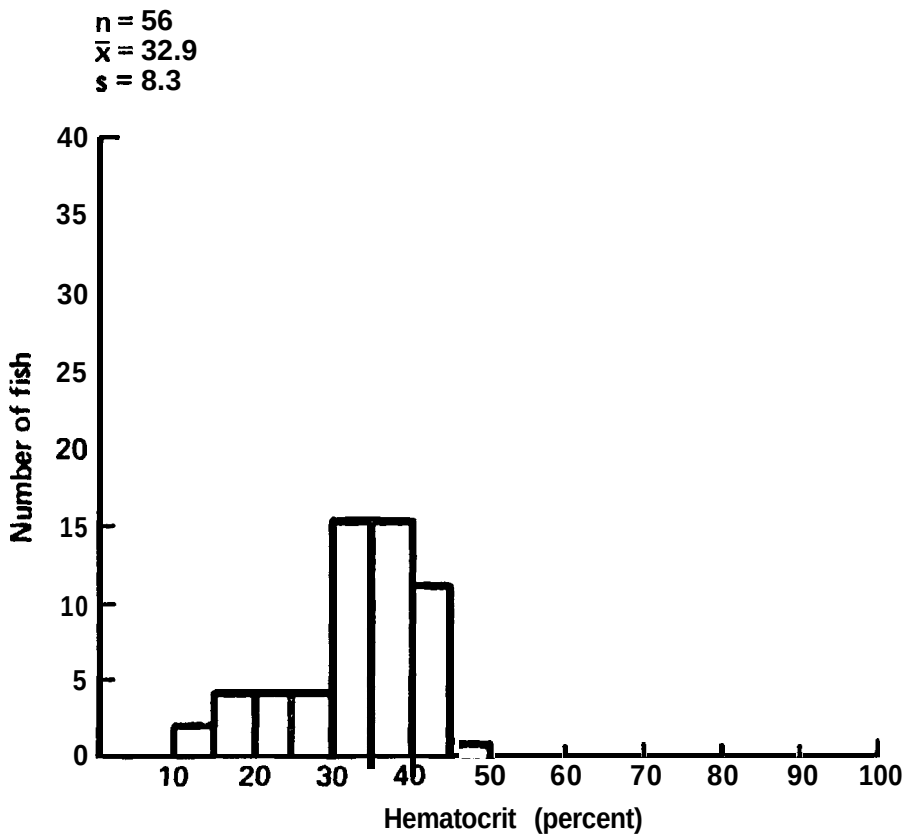
The mean hematocrits (32.9%) of the Carson coho salmon were close to the expected low, and the mean hemoglobin values (4.7 g/100 mg blood) were below the lowest expected value for clinically healthy fish (Figure 8). There were 41.3% of the hematocrit values below the expected low value (32.5%), and none of the fish were above the high (52.5%). About 91.4% of the hemoglobin values were below the expected minimum for clinically healthy coho salmon (6.5 g/100 mg), and none were above the high (9.9 g/100 ml).

DISEASE LAB CODE 4401-4460 4401–4460

DATE SAMPLED April 26, 1978

SPECIES COHO

HATCHERY STOCK CARSON



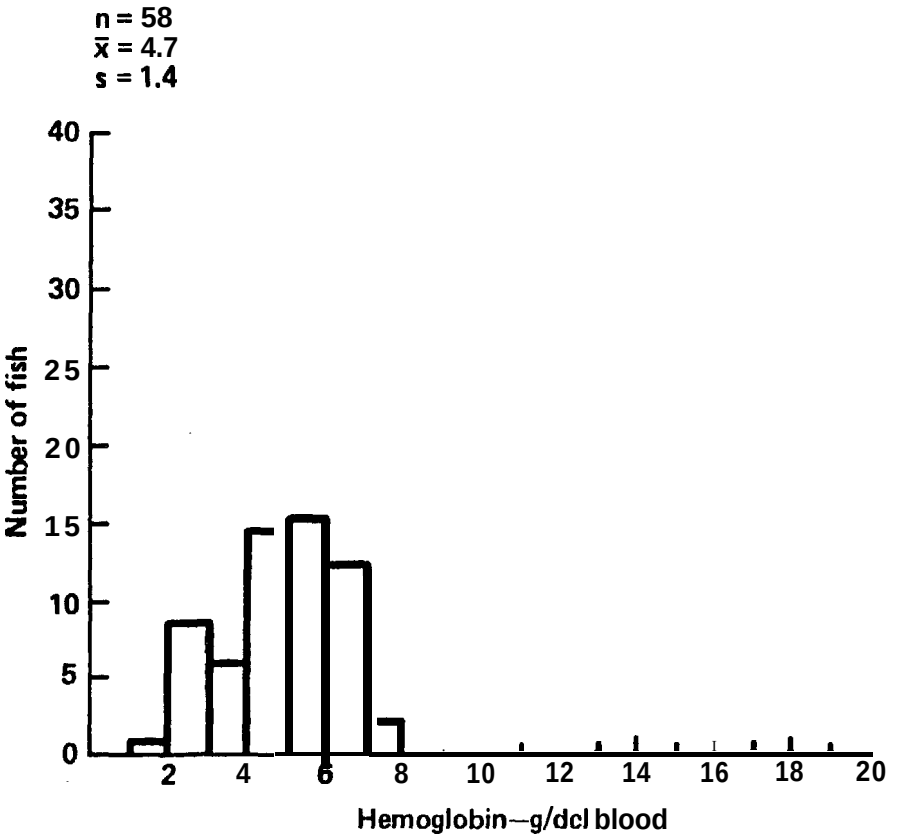


FIGURE 8.--A histogram and other data for hematocrit and hemoglobin values for the Carson coho salmon.

2. WILLARD HATCHERY.

The summarized data for the hematocrit and hemoglobin values are presented in Figures 9a, b, and c. Mean hematocrits from all three release groups (Table 3) were in the middle of the range expected for clinically healthy coho salmon (32.5 to 52.5%) as proposed by Wedemeyer and Chatterton (1971). The mean hemoglobin values (6.4 g/100 ml blood) of the first release group (I) were slightly below the expected (6.5 to 9.9 g/100 ml). The hematocrit values (Figure 9a) of the Willard I coho salmon were widely spread.

The change in the percentage of fish above or below the expected values of hematocrit and hemoglobin are listed below. Miles and Smith (1968) reported a decrease in hematocrits in coho salmon yearlings in early April followed by an increase in early May, but they did not report on hemoglobin values. In the case of Willard Hatchery coho salmon, there is a continuing shift of both mean and expected values to a higher level with the progression of spring. This could be a physiological adjustment to minor decreases in available oxygen.

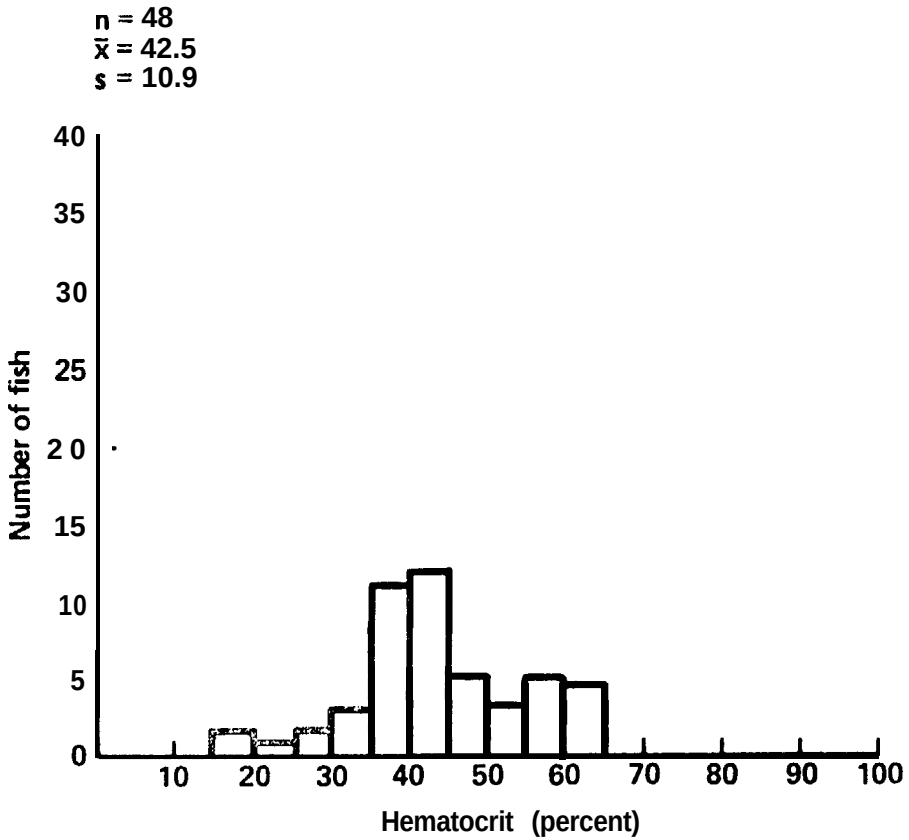
	Hematocrits		Hemoglobins	
	% of normal expected values		% of normal expected values	
Release group	Below (%)	Above (%)	Below (%)	Above (%)
I	10.2	18.4	56.3	0
II	5.7	9.4	29.3	3.4
III	6.9	3.4	5.1	13.5

DISEASE LAB CODE K01–K60

DATE SAMPLED May 15, 1978

SPECIES COHO

HATCHERY STOCK WILLARD I



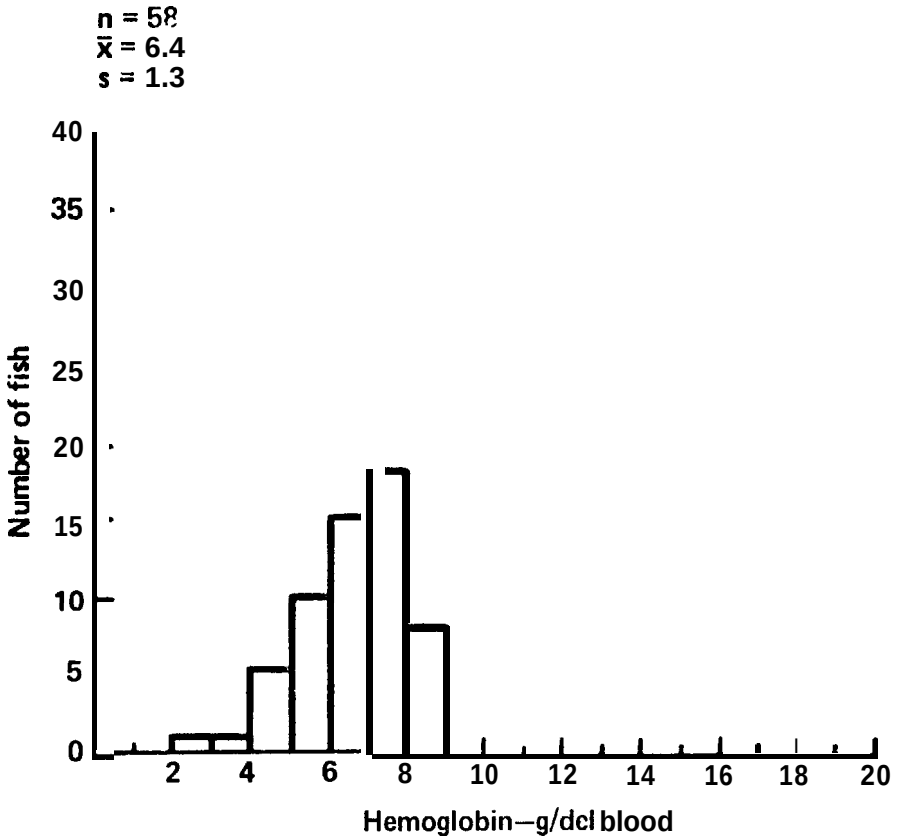


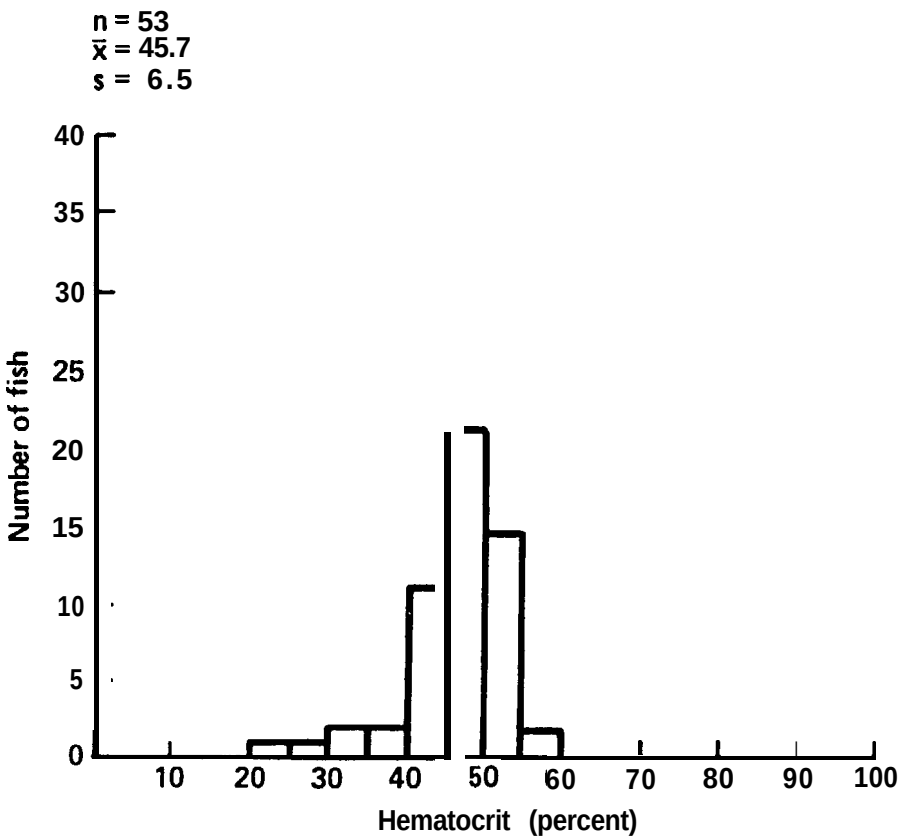
FIGURE 9a.--A histogram and other data for hematocrit and hemoglobin values for the Willard (I) coho salmon-first release group.

DISEASE LAB CODE N01–N60

DATE SAMPLED May 25,1978

SPECIES COHO

HATCHERY STOCK WILLARD II



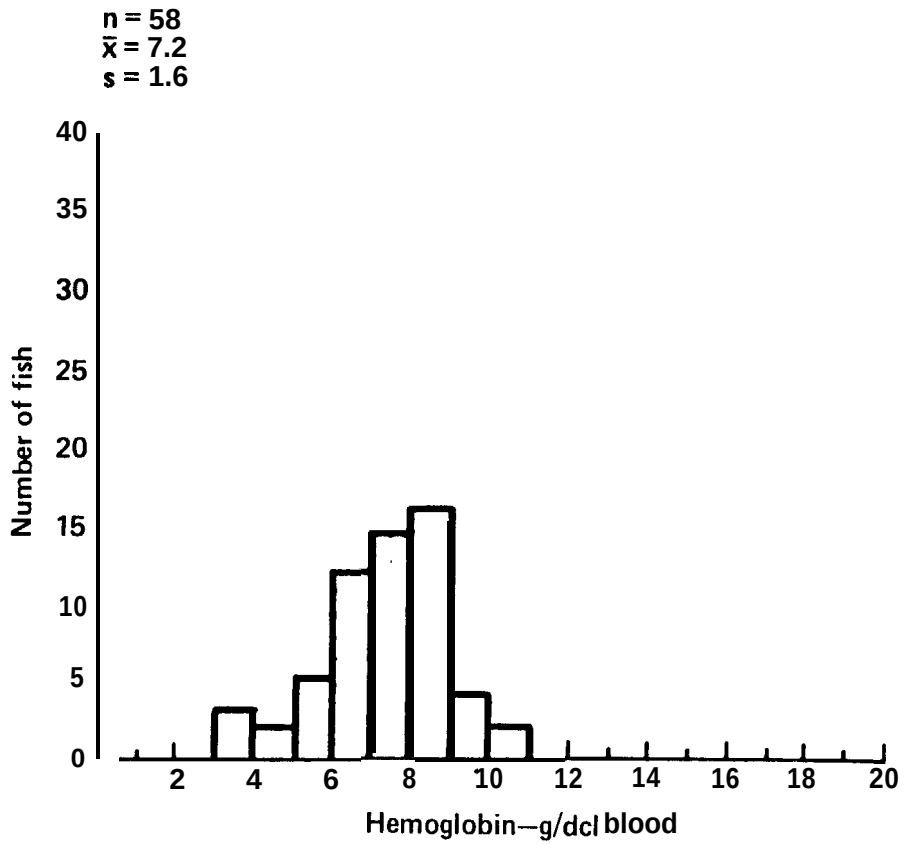


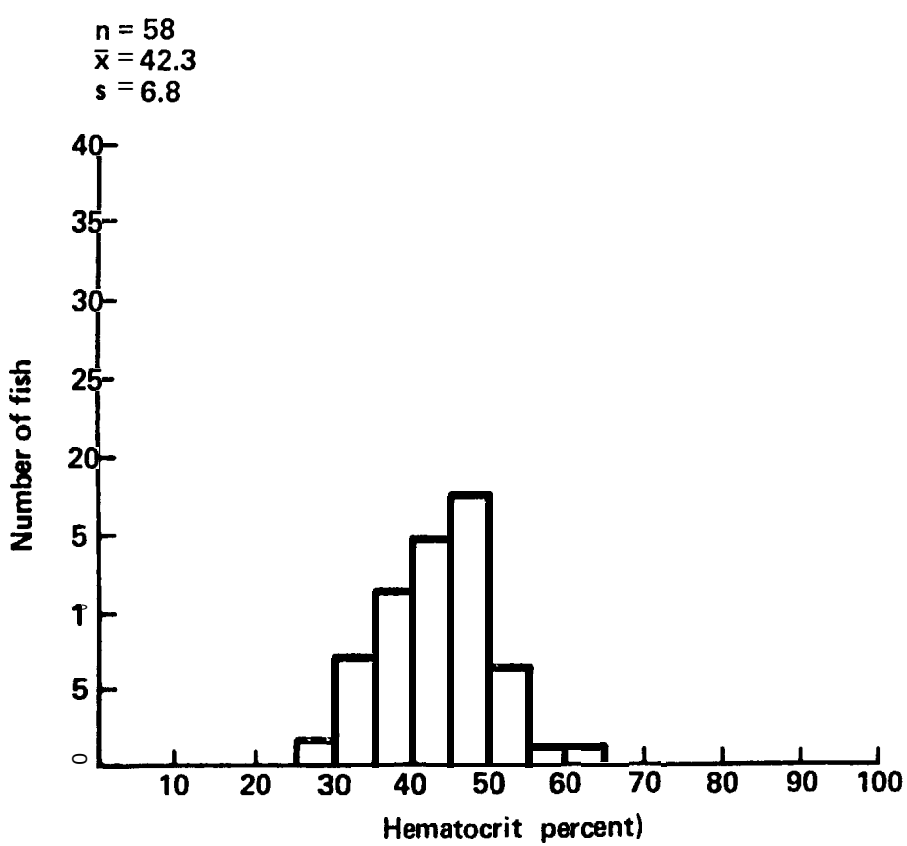
FIGURE 9b.--A histogram and other data for hematocrit and hemoglobin values for the Willard (II) coho salmon-second release group.

DISEASE LAB CODE 4351–4370 4661–4700

DATE SAMPLED June 12,1978

SPECIES COHO

HATCHERY STOCK WILLARD III



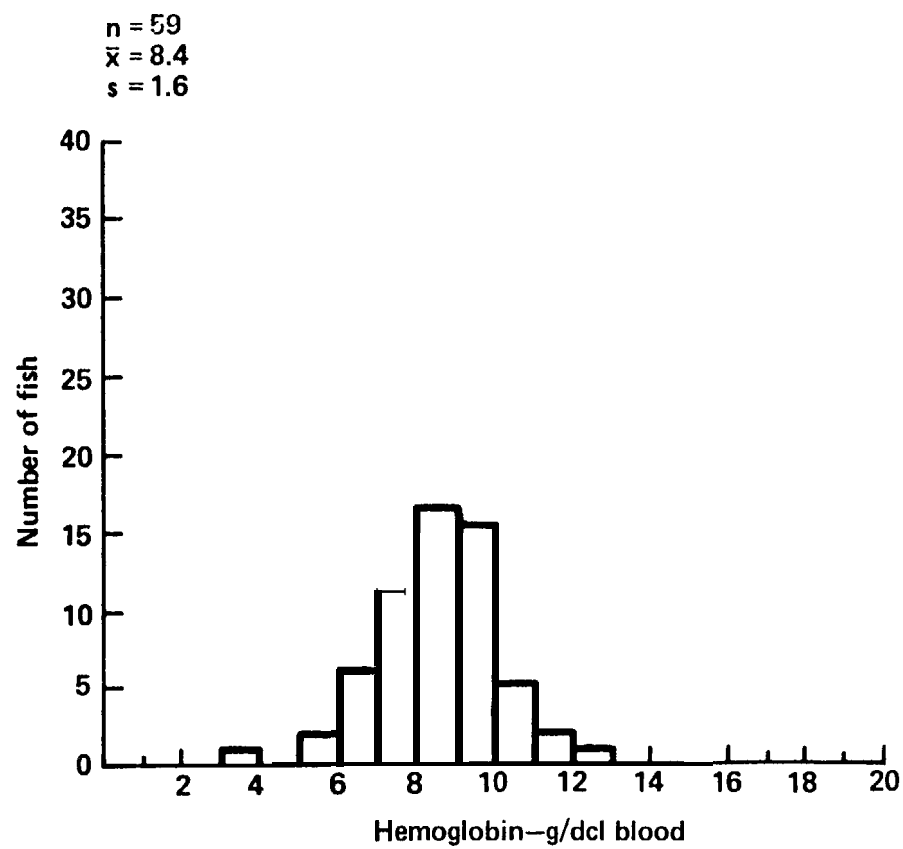


FIGURE 9c.--A histogram and other data for hematocrit and hemoglobin values for the Willard (III) coho salmon-third release group.

D. VIRAL SCREENING (SEE APPENDIX A).

1. CARSON HATCHERY.

The Rangen Laboratories only found 6 out of 12 pools to be negative for virus in this stock, and IPN virus was confirmed.

2. WILLARD HATCHERY.

Rangen Laboratories conducted tests on the Willard Hatchery II and III release groups only. In the II group, there were no negative pooled samples, and IPN viruses could not be confirmed. In the III group, there were five negative pooled samples, and IPN virus was confirmed in some of the remaining positive groups.

E. IFAT-BKD.

1. CARSON HATCHERY.

The incidence of BKD as determined by IFAT was only 5%, and there were no demonstrably abnormal hematocrit or hemoglobin values in the infected fish.

The 30-day survival for the Carson coho salmon was 98.2%, and the full-term survival (46.1%) was the highest of any of the 1978 homing stocks examined (Table 3). In spite of the low incidence of BKD, 4.8% of the mortalities examined were determined to have gross BKD lesions in the kidneys.

2. WILLARD HATCHERY.

Kidneys were sampled from all three release groups for BKD screening. The incidence of infection between group I and II was almost identical (Table 2), but 2 weeks later (group III), the percentage of BKD infected fish rose sharply. Most of this increase was in what might be

considered new hosts (anterior or posterior only). Because of the small sample size, it may not be possible to relate the incidence of BKD to hematocrit or hemoglobin levels. However, the data (below) do show a trend of decreasing hematocrit values with increasing latent BKD infection in the kidney.

	% Hematrocrit			g hemoglobin/100 ml blood		
	n	Mean	Variance	n	Mean	Variance
No detectable BKD	34	43.8	7.1	35	8.9	1.4
Anterior or posterior BKD only	18	41.3	5.2	18	7.8	1.2
Both posterior and anterior kidney infected	5	37.6	6.8	5	8.2	1.2

There were mortalities from gross BKD in all three groups cultured in the seawater net-pens (Table 3), and the percentage of mortalities with grossly detectable BKD was highest for the group III fish. The initial (30 day) survival in all 3 groups was quite high (and similar), but the survival to termination of the tests was only 34 to 41%. This high early survival in all 3 groups at least indicates that osmoregulatory stress was minimal. The 7.9% mortality in the group III fish due to gross BKD is disturbing in that it indicates a possible delayed mortality due to latent BKD in this stock.

F. HISTOPATHOLOGY (SEE APPENDIX B).

1. CARSON HATCHERY.

There was a recognizable incidence of skeletal muscle lesions of the eye (27.1%), recognizable increased numbers of lymphocytes (74.6%) and epithelial cell proliferation (61.0%) in the gills, and a 76.8% incidence of basophillic granular organisms in the gills (Table 4). These latter structures stained gram negative and may represent microsporidian protozoan parasites.

The incidence of BKD as detected by tissue gram stain was 1.7% in the liver and 1.7% in the kidney. Even though the incidence of BKD as detected by IFAT and tissue gram strain was low, tissue involvement in individually infected fish in the total population must have been significant because we would not expect a 4.8% mortality in the sea-pens from BKD with such a low incidence level (5% by IFAT). There was gram-positive debris in the kidney tubules (6.8%), and other gram staining material in the tissue (3.4%).

Table 2 is the hatchery record for the Carson Hatchery coho salmon, and indicates the fish were treated for furunculosis and probably some external parasites, but not BKD.

In general, these fish were in excellent condition, but the total evidence suggests that there could be a long term mortality due to latent BKD of at least 5%.

2. WILLARD HATCHERY (GROUP III FISH ONLY).

Recognizable pathological conditions of the eye were at similar levels to those of the Carson Hatchery coho salmon, but there was a considerable reduction in the percentage of fish with any gill pathology,

particularly those fish with evidence of basophillic granular organisms (Table 4). There were no detectable incidences of BKD organisms in liver or kidney (as determined by tissue gram stain), no evidence of gram positive staining debris, and no evidence of other gram stained material in tissue. This would seem to indicate that the severity of individual BKD infections was less than that of the Carson Hatchery coho salmon. However, the fact that the known BKD mortalities in seawater culture ranged from 2.9 to 7.9%, and that the IFAT-BKD levels ranged from 15.0 to 40.7% should be sufficient evidence to indicate that latent BKD could be a serious problem and might be the direct cause of a 5 to 10% mortality in the ocean.

Table 2 lists the available hatchery records of the Willard Hatchery coho salmon. Although BKD and furunculosis were two of the diseases detected in these fish, there was no evidence of furunculosis in seawater mortalities posted. This may have been the result of effective medication (for gram negative organisms) with TM-50.

COMPARATIVE VIRAL ASSAYS FROM SURVIVING FISH IN SEAWATER NET-PENS

Three hundred fish from each of the stocks of fish sampled during the course of this study had been maintained in salt water at the NMFS Manchester Laboratory since the time of sampling as part of the test for saltwater adaptation and survivability. In an attempt to expand upon the results of the viral isolation tests during the course of the freshwater smolt phase of the study, those stocks surviving saltwater introduction in sufficient numbers were sampled by USFWS personnel from the National Fisheries Research Center in Seattle, Washington, according to the methods described previously in this report. Paired tissue pool samples were tested for the presence of virus by both the National Fisheries Research Center and the Rangen Research Laboratory.

A total of 11 saltwater stocks and one additional stock that had been held in fresh water at the NMFS Montlake Laboratory in Seattle were examined. All twelve stocks had been found to be infected with IPN virus at the time of hatchery release as reported in the foregoing report but were found (by both USFWS and Rangen) to be negative for virus after a saltwater maintenance period of 155 to 200 days. Based upon the evidence for variation in the clinical presence of IPN virus and infectious hematopoetic necrosis (IHN) with regard to age, species, and stress conditions, it is not unreasonable to assume that the virus could well have returned to subclinical undetectable levels following a reduction in smoltification stress by acclimation to salt water. Serological surveillance of these same stocks may have provided a tool with which prior exposure could have been demonstrated in the absence of any clinical isolations of the virus.

SUMMARY AND CONCLUSIONS

The health status of the stocks was quite variable as could be expected. The Dworshak and Wells Hatcheries steelhead suffered from some early stresses in seawater, probably osmoregulatory. The incidences of latent BKD in the Wells and Chelan Hatcheries steelhead and Kooskia Hatchery spring chinook salmon were extremely high, and how these will effect survival in the ocean is not known. Gill enzyme activity in the Dworshak and Chelan Hatcheries steelhead at release was low. Of the steelhead, survival in the Tucannon Hatchery stock will probably be the highest, with Dworshak Hatchery stock the lowest.

The analyses conducted by the veterinary pathologist indicate that overall there was no evidence of serious pathological conditions that might be disastrous to any given stock, but at this time it is also difficult to interpret the results of certain types of clinical pathology that have either not been previously reported or extensively studied. For example, if the 77% incidence of basophillic granular organisms in the gills of the Carson coho salmon does represent an infestation of microsporidian protozoan parasites, is the intensity of infestation severe enough to cause irrepairable damage that might affect survival?

The results of the viral assays are questionable because the Rangen Laboratory is the only one that found evidence of viruses in these stocks (however, the veterinary pathologist did find evidence of a pox-type virus in one kidney from the Kooskia Hatchery spring chinook salmon). Secondly, even if the virus identification were substantiated, we cannot be sure of the significance of positive test results.

However, this variation is food for thought in how survival of individual stocks might be improved in the future through a closer examination of dietary and environmental requirements, and monitoring the incidence of sub-clinical diseases by random sub-sampling of populations.

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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 1978

CONTRACT NO. 2-78
(EFFECTIVE FEBRUARY 1, 1978 TO SEPTEMBER 30, 1978)
USDC/NOAA PRUCHASE ORDER NO. 02-78-M02-00189

THE SURVEILLANCE OF VIRUS DISEASES IN SELECTED HATCHERY STOCKS OF SALMON AND STEELHEAD SMOLT IN THE COLUMBIA RIVER BASIN DURING 1978

FINAL REPORT PREPARED FEBRUARY 1, 1979

FOR

NATIONAL MARINE FISHERIES SERVICE
P.O. Box 38
MANCHESTER, WASHINGTON 98353

BY

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RANGEN, INC. CONSIDERS THIS REPORT AS SATISFACTION IN FULL OF ALL OBLIGATIONS UNDER CONTRACT NO. 2-78. WE HAVE APPRECIATED THE OPPORTUNITY OF BEING OF SERVICE TO YOUR ORGANIZATION AND HOPE THAT WE MIGHT HAVE THE OPPORTUNITY OF DOING SO AGAIN IN THE FUTURE.

ANY QUESTIONS WITH REGARD TO THE CONTENTS OF THIS AND RELATED REPORTS MADE UNDER CONTRACT NO. 2-78 SHOULD BE DIRECTED TO:

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I. INTRODUCTION

In the spring of 1978, the National Marine Fisheries Service (NMFS) Manchester, Washington laboratory began a 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 can then be used in the evaluation and interpretation of other data obtained having to do with the relative success of saltwater adaptation, 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. This report concerns itself only with the work contracted by NMFS to the Rangen Research Laboratory under Ranges Contract #2-78 (USDC/NOAA Purchase order No. 02-78-MOZ-00189) and titled THE SURVEILLANCE OF VIRUS DISEASES IN SELECTED HATCHERY STOCKS OF SALMON AND STEELHEAD SMOLT IN THE COLUMBIA RIVER BASIN DURING 1978. It 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 (IPVN), infectious hematopoietic necrosis virus (IHVN), and Herpesvirus salmonis as well as any additional agents capable of inducing specific cytopathic effect (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 (IHVN). IHVN was first recognized in 1951 in sockeye salmon (*Onchorhynchus nerka*) at Leavenworth Hatchery and kokanee salmon (land-locked sockeye salmon) at Winthrop 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 system.

IHVN infection results in a peracute to acute course of infection resulting in high levels of mortality in chinook salmon (*Onchorhynchus tshawytscha*) and an acute

to subacute infection of sockeye salmon, The disease is characterized, as its name implies, by destruction of hematopoietic tissues resulting in an acute anemia, hemorrhage, 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 vertically with the eggs and reproductive fluids of asymptomatically infected returning broodstock. The coho salmon (*Onchorhynchus kisutch*) appears to be more resistant to IHVN 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 typical of the rhsbdovirus group of which it is a member. During this asymptomatic carrier state, the virus is not detectable by present 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 than 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 species as well as sockeye salmon under hatchery condi-

tions. 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. Knowledge is also lacking on the possible residual effects of the carrier state infection on successful **smolt-**ification, 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.

Another salmonid virus, Infectious Pancreatic Necrosis Virus (IPNV), has not often been associated with or considered a problem in Pacific salmon stocks, IPNV is known to be endemic in the Columbia River drainage but 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 disease was first isolated in the Western United States in 1963 by Parisot, et al.

IPNV disease is typically characterized by a paracute 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. 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 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 has 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 the virus was soon isolated from the fingerling salmon during an epizootic of furunculosis (*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 Mchlichael reported on the surveillance of selected 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 examined for specific neutralizing antibodies against the common virus 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 of 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 as titers in excess of 1:200 evidencing prior exposure. Attempts as horizontal transmission of the virus under laboratory conditions were unsuccessful at 15 C.

A report in the FAO Aquaculture Bulletin (1973) indicated that McMichael's coho isolate was unlike the ATCC UR-299 (American Type Culture Collection) IPNV trout isolate and more typical of French isolates of IPNV that loose 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 fry progeny at all of the selected hatcheries sampled were negative for IPNV in 1972 and 1973. However, they were able to demonstrate specific 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 nor 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 were found to be free of the virus in the 1971 and 1972 broodyears.

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 come up with 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 verticle 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 diagnosed with a confirmed asymptomatic infection of IHNV (Tebbit, 1975). During the 1973 broodyear, Tebbit was able to detect IPNV specific neutralizing antibodies in the sera of adult coho salmon returning to the Sandy Hatchery and in the sera of adult coho salmon returning to the Bonneville Hatchery. None of these seropositive populations *nor* their fry progeny yielded a confirmed viral isolation. Tebbit's continued surveillance during the 1974 broodyear failed to yield 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 finding of 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 once again docementing the fact 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 diversity of discrete spawning populations in the Columbia River basin and that it may not be easily recognized or clinically diagnosed in certain types of samples 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 know to occur with steelhead trout populations.

The last of three salmonid viruses to be found in the Columbia River basin is *Herprresvirus salmonis*. This virus or other closely related viruses are known to infect rainbow trout (*Salmo gairdneri*) 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 brood-stock at the Winthrop National Fish Hatchery. The virus still remains a fairly unknown entity with regard to the general health of Pacific salmon and steelehad 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 give optimum sensitivity and accuracy with regard to detecting the presence of any of the three major salmonid viruses found within the Columbia River basin.

A total of 28 stocks of chinook salmon, coho salmon, and steelhead trout from 18 different Columbia River basin hatcheries (Figure 1 and Table 1) were examined for the presence of virus at the time of smoltification and hatchery release during 1978, Field sampling was conducted by NMFS Personnel according to procedures established by and with field sampling kits provided by the Rangen Research Laboratory (Appendix A). Most of the hatchery stocks being sampled were first transported in a live haul container back to the NMFS Laboratory in Manchester, Washington. They were then maintained in freshwater flow-through systems (Beaver Creek water supply) for one to two days prior to sampling and saltwater introduction. A few stocks were sampled at the site of the production hatchery prior to transport back to the Manchester Laboratory. 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

FIGURE 1. GEOGRAPHIC LOCATION OF SELECTED COLUMBIA RIVER DRAINAGE SALMON AND STEELHEAD HATCHERIES SAMPLED FOR VIRUS DURING 1978,

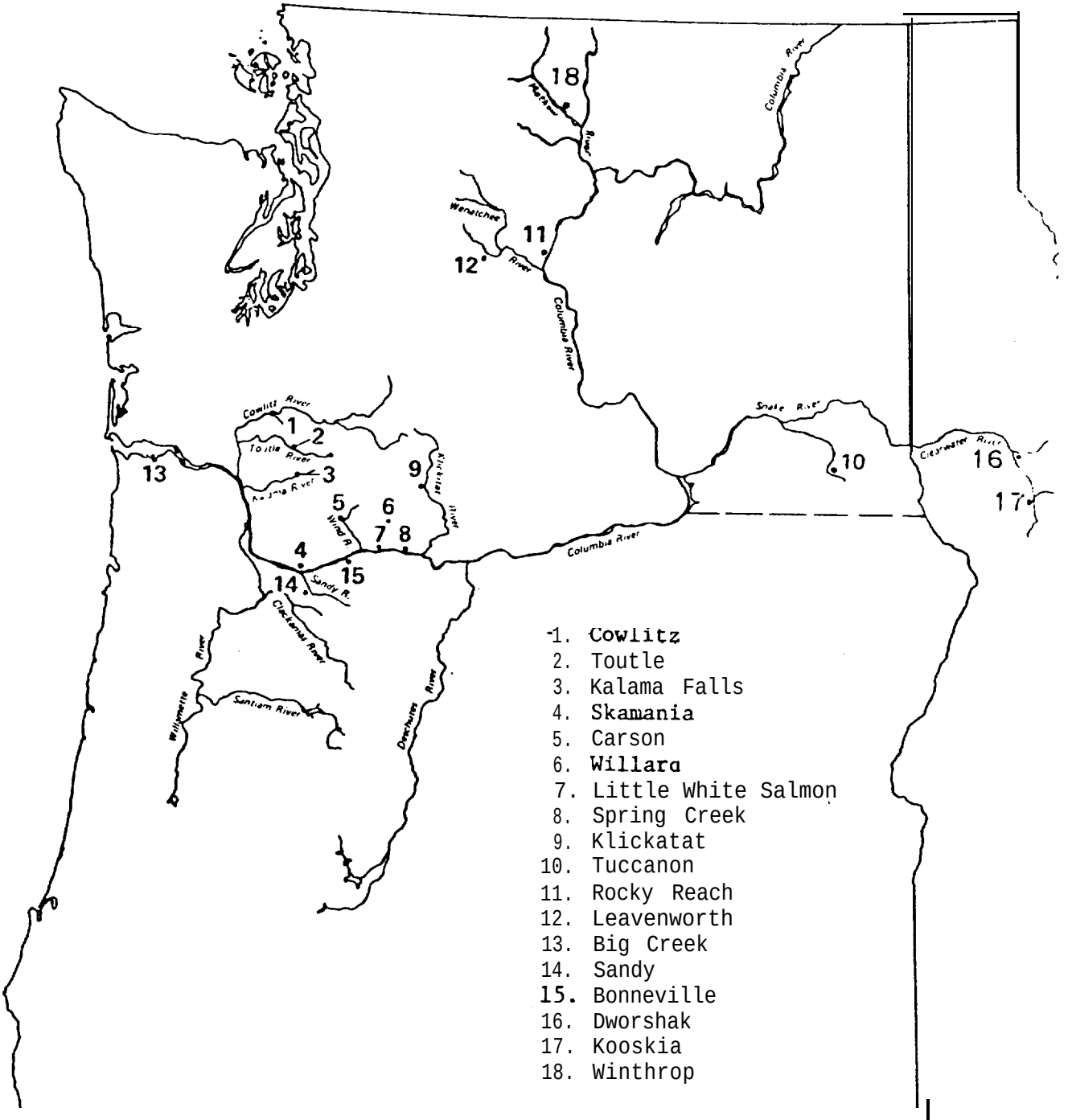

TABLE 1. STOCKS OF PACIFIC SALMON SMOLT EXAMINED FOR VIRUS AT THE TIME OF RELEASE FROM SELECTED COLUMBIA RIVER DRAINAGE HATCHERIES DURING 1978.

STATION	STOCK EXAMINED	DATE SAMPLED	RANGEN ACC. NO.
Big Creek	coho salmon	5/9/78	177-78
	coho salmon (Cowlitz stock)	5/9/78	178-78
Bonneville	fall chinook salmon (group I)	5/26/78	216-78
Carson	coho salmon (yearling)	4/26/78	146-78
	spring chinook salmon	5/2/78	158-78
Cowlitz Salmon	fall chinook salmon	6/19/78	269-78
Dworshak	steelhead trout	4/26/78	147-78
Kalama Falls	spring chinook salmon	3/3/78	070-78
	coho salmon	5/6/78	168-78
	fall chinook salmon	7/11/78	197-78
	fall chinook salmon	9/15/78	356-78
Klickatat	coho salmon	5/1/78	153-78
Kooskia	spring chinook salmon	4/25/78	148-78
Leavenworth	spring chinook salmon	4/22/78	138-78
	steelhead trout	5/3/78	162-78
Little White Salmon	fall chinook salmon	5/25/78	115-78
Rocky Reach	coho salmon	5/4/78	161-78
Sandy	coho salmon	5/7/78	165-78
Spring Creek	fall chinook salmon	3/21/78	099-78
Skamania	steelhead trout	5/7/78	166-78
Toutle	coho salmon	5/3/78	159-78
	coho salmon (Montlake stock)	5/18/78	199-78
	fall chinook salmon	6/9/78	250-78
Tuccanon	steelhead trout	5/16/78	189-78

TABLE 1. (CONTINUED)

Willard	coho salmon (group II)	5/25/78	214-78
	coho salmon (group III)	6/13/78	249-78
	fall chinook salmon	7/12/78	298-78
Winthrop	steelhead trout	5/5/78	167-78

for an accurate sample dilution factor calculation 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 twelve five-fish pools collected from the randomly selected 60 fish sample.

Following completion of all sampling procedures and the Field Sampling Data Sheet as outlined in Appendix A, the complete refrigerated field kits were returned to the Rangen Research Laboratdry in Hagerman, Idaho via Grayhound NBO Package Express service. The average time in transit was 20 hours and the temperature of the samples upon receipt was consistantly between 6 C. and 8 C.

Upon receipt into the Rangen Research Laboratory, the receiving temperature of the samples was recorded. The twelve sample tubes were assigned a group accession number and each tube in the group given a 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. One half milliter of the supernatant was pipetted off with a sterile disposable polyethylene tip and diluted in 2 ml of a disinfecting diluent (Appendix B) and incubated at 4 C. overnight for decontamination from fungal and bacterial agents. This procedure resulted in a final 1:50 working dilution of the original tissue sampled. All samples were maintained at 4 C. during all preparatory procedures.

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 system using the Chinook Salmon Embryo (CHSE-214) cell line in passages 268 through 279 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 1:50 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 controls and negative media and cell controls were set up for each group tested. After the sample dilutions had been 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 was

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 4 minutes. The supernatxnt 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 sensative film and incubated at 12.5 C. The suspended CHSE-214 cells were allowed to settle down through the sample material and attach to the bottom of the well.

All tests were incubated at 12.5 C. for 21 days. All wells 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 disinfecting diluent to a final working dilution of 1:100 and re-run on the microculture screen procedure as described above. If more than four wells in an eight well test series was found to be contaminated, the original sample was filter sterilized through a 0.45u membrane filter and re-run on the microculture screen procedure. If any of the wells in the control series demonstrated any unusual or inconsistant results at any time during the 21 day incubation period, the microculture screen procedure was repeated with the original samples.

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 of cytotoxicity) and pooled into one of three tubes according to its classification. After the supernatant media had been pooled from 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 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 a 1:100 working dilution of EFDL #149 Polyvalent IPNV Antisera (Eastern Fish Disease Laboratory, Kearneysville, West Virginia) was pipetted into the next two wells of each eight well series. Twenty five thousandths of a milliliter of a 1:100 working dilution of EFDL #150 IHN Antisera was pipetted into the next two wells of each eight well series and finally 0.025 ml of a 1:100 working dilution of EFDL #100 normal rabbit sera was pipetted into the last well of each eight well series. Each of the three classified and pooled screenmaterialswere then diluted to 10^{-3} by pipetting 0.025 ml of the material into the first well of an eight well series, mixing, and transfering 0.025 ml into the second well, mixing, and transferring 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. 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 covered temporarily with a sterile 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. Distruction of the cell monolayer with characteristic CPE in wells six, seven, and eight indicated the confirmed presence of IPNV in that sample pool. Distruction 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, or partial neutralization of a particular strain of a virus in which case additional procedures would have been applied to confirm identification. All wells demonstrating CPE in an eight well series on the serum neutralization plate were pooled and lyophilized in a stabolizer 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.

III. RESULTS

A total of 336 Pacific salmon and steelhead trout smolts representing 28 discrete anadromous stocks at 18 Columbia River basin hatcheries were tested for the presence of infectious viruses during 1978. 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 identified during the course of the study.

Infectious pancreatic necrosis virus (IPNV) was confirmed at 12 of 18 hatcheries sampled and in 16 of 28 stocks of smolted fish examined as summarized in Table 3. IPNV was most commonly found in populations of coho salmon (82% incidence among populations sampled) and least likely to occur in populations of fall chinook salmon smolt (25% of the populations examined). The incidence of infection among five-fish sample pools within each 60 fish lot was the lowest for fall chinook salmon smolt with an average of 12.5% in the two infected populations examined. The same measure of carrier incidence for infected spring chinook salmon smolt populations was 75%, 64% for infected coho salmon smolt populations and 61% for infected steelhead trout populations. None of the confirmed IPNV infections could be associated with any significant mortality or loss in any of the populations.

IV. DISCUSSION

The failure to isolate either *Herpesvirus salmonis* or infectious hematopoietic necrosis virus (IHNV) during the course of this study 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 from a Columbia River basin salmonid stock and has not been seen since. Due to the fact that our knowledge of the disease is still limited in terms of its occurrence, incidence, and pathogenesis, it can only be said that the virus was not detected within the limits of the experimental design. Possibly by broadening the scope of the study to include conditions known to be optimum for the recovery of the virus at all stages in the life history of Pacific salmon and steelhead and the application of more sensative 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

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 1978.

STATION	STOCK	NUMBER OF SCREEN POOLS			CONFIRMED RESULT
		POSITIVE	SUSPECT	NEGATIVE	
Big Creek	coho salmon	6	0	6	IPNV
	coho salmon (Cowlitz stock)	4	5	3	IPNV
Bonneville	fall chinook salmon (group I)	12	0	0	negative
Carson	coho salmon (yearling)	3	3	6	IPNV
	spring chinook salmon	0	3	9	negative
Cowlitz Salmon	fall chinook salmon	1	2	9	negative
Dwarshak	steelhead trout	0	8	4	IPNV
Kalama Falls	spring chinook salmon	0	0	12	negative
	coho salmon	12	0	0	IPNV
	fall chinook salmon	12	0	0	negative
	fall chinook salmon	3	3	6	IPNV
Klickatat	coho salmon	4	4	4	IPNV
Kooskia	spring chinook salmon	6	1	5	IPNV
Leavenworth	spring chinook salmon	12	0	0	IPNV
	steelhead trout	9	1	2	negative
Little White Salmon	fall chinook salmon	12	0	0	negative
Rocky Reach	coho salmon	0	0	12	negative

TABLE 2. (CONTINUED)

Sandy	coho salmon	12	0	0	IPNV
Spring Creek	fall chinook salmon	4	5	3	negative
Skamania	steelhead trout	12	0	0	IPNV
Toutle	coho salmon	9	1	2	IPNV
	coho salmon (Montlake stock)	12	0	0	IPNV
	fall chinook salmon	0	1	11	IPNV
Tuccanon	steelhead trout	0	10	2	negative
Willard	coho salmon (group II)	12	0	0	negative
	coho salmon (group III)	7	0	5	IPNV
	fall chinook salmon	12	0	0	negative
Winthrop	steelhead trout	10	1	1	IPNV

TABLE 3. INCIDENCE OF INFECTIOUS PANCREATIC NECROSIS VIRUS IN SELECTED STOCKS OF ANADROMOUS SALMONID SMOLTS AT COLUMBIA RIVER DRAINAGE HATCHERIES DURING 1978,

	TOTAL STOCKS EXAMINED	STOCKS WITH CONFIRMED IPN	INCIDENCE OF INFECTION
All Stations Sampled	18	12	67%
All Stocks Combined	28	16	57%
Coho Salmon	11	9	82%
Fall Chinook Salmon	8	2	25%
Spring Chinook Salmon	4	2	50%
Steelhead Trout	5	3	60%

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 primarily considered a disease of fry and fingerling chinook salmon and steelhead trout. Coho salmon appear resistant but can function as asymptomatic carriers. IHNV is 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 get a better understanding of the ecology 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 spawning 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 serological surveillance using serum neutralization, ELISA, and CIE procedures should be effective in detecting the virus at these and other stages when the virus may be present in the non-infectious eclipse form.

The isolation of infectious pancreatic necrosis virus (IPNV) from the stocks examined and, in particular, the finding of a relatively high incidence of the virus among the populations in comparison to previous surveillance studies seems unusual on initial examination. However, when one considers 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; and 3) has been previously detected in at least ten discrete populations of Columbia River salmon and steelhead at seven different stations since 1971, the results take on a more consistant 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 understanding of the epidemiology of IPNV in anadromous stocks of Columbia River salmon and steelhead trout seems to be primarily based upon studies which utilized ovarian reproductive fluid samples exclusively 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 but all four IPNV isolates obtained came exclusively from pooled visceral tissue samples while all of the paired reproductive fluid 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 of IPNV in a population of asymptomatic adult carriers.

It should also be noted that McMichael (1974) consistantly failed to isolate IPNV from known infected fry populations of Pacific salmon after 60 days of age post feeding. Consequently, surveillance programs based upon the sampling of fry and fingerling sized fish between 60 days of age and prior to smoltification may easily fail to detect the presence of IPNV.

IPNV as well as many other diseases of viral etiology are known to be stress mediated as well as species and age specific. The period of smoltification in the life history of an anadromous salmonid fish is recognized as a major time of physiological change and stress to the animal, particularly under hatchery conditions of intensive culture and nutrition. It is therefore 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 susceptibility of an uninfected population to infection from an endemic source. 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 asymptomatically 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 pools of tissue from adult coho salmon returning to Cascade Hatchery in 1972 were positive for IPNV (Tebbit, 1975). In the present study, seven of the sixteen populations found to be infected with IPNV demonstrated fewer than half of the tissue pools to be positive for virus on initial screening. This finding would indicate that a full 60 fish sample, based upon hypergeometric sampling statistics, may be necessary in order to consistantly detect the virus. Spot checks of fewer than 66 fish are apt to miss an infection of low incidence.

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. In extensive sampling between 1971 and 1974, McMichael (1974) and Tebbit (1975) consistantly failed to isolate IPNV from the same hatchery stocks during subsequent years even though neutralizing antibodies indicating prior exposure were at times found to be present. The presence or absence of IPNV in a particular year class of a given stock of fish may not necessarily mean that all other year classes of that same stock will be similiarly infected or free of the disease.

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 life history stages and year classes of a particular stock, it is suggested that future attempts at viral surveillance include sampling of both visceral tissues and reproductive' fluids from spawning populations. Progeny fry should be examined at 30 days of age post feeding and fingerling fish at a time just prior to hatchery release during smoltification. Attempts at isolation from the various stages in 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.

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 16 of 28 stocks of Pacific salmon and steelhead troutsmoltsat 12 of 18 Columbia River hatcheries in 1978.

2. The presence of infectious pancreatic necrosis virus (IPNV) in stocks of Pacific salmon and steelhead trout smolts at Columbia River basin hatcheries in 1978 was not associated with any significant mortality.

3. Infectious hematopoietic necrosis virus (IHNV) was not isolated from any of 28 stocks of Pacific salmon and steelhead trout smolts at 18 Columbia River basin hatcheries in 1978.

4. *Herpesvirus salmonis* was not isolated from any of 28 stocks of Pacific salmon and steelhead trout smolts at 18

Columbia River basin hatcheries in 1978.

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

IERIES DIVISION

RANGEN

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 to 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 careful1 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

VII. APPENDIX A

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: _____

VII. APPENDIX B

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.: ________

mpling Date: / ____ / ____ Time: ________ hours Technician: ________________

Receiving Condition: ( ) live ( ) fresh No. Sampled:
( ) frozen ( ) refrigerated
Holding condition: ___ hours _____ °C
Comment:

Lot #:				
Morb.:				
Mort.:				
Asyp.:				

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

Pooling:

Lot #	Fish/Pool	. 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	fina...

Prep. Incubation: ___ °C ____ hours Inoculation Date: / / Time: ____

...hnician: ________ Inoc. Vol.: 0.025 0.05 0.1 0.2 ml ( ) wet ( ) ...

( ) single ( ) duplicate ( ) microtiter ( ) multiwell ( ) GO mm dis...

Absorbtion Time: _____ min. _____ °C.

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

Media: ________________ Control Virus: ________________

Lot #: ________________

RESULTS:

Date	Day

VII. APPENDIX C

VIROLOGICAL EXAMINATION
(continued)

Serum Neutralization (microculture):

Set-up Date: / / m e :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. (TEST	WELL A	B	C	D	E	F	G	H	RESULTS
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										

Remarks:

Rangen Inc.
P.O. BOX 706 • 115 13 AVE. SO. • BUHL, IDAHO 83316 • (208) 543-6421

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(208) 837-6 19 1 HATCHERY

February 13, 1979

Mr. Tony Novotny
National Marine Fisheries Service
P.O. Box 38
Manchester, Washington 98353

Dear Tony;

Enclosed are two (2) copies of the final report on our contract #2-78 titled: The Surveillance of Viral Diseases in Selected Hatchery Stocks of Salmon and Steelhead Trout Smolt in the Columbia River Basin During 1978. I hope that it meets with your approval and is of pertinence to the parent study with regard to interpretation of the rest of the data. If you have any questions with regard to the study in general or this report in particular, please feel free to give me a call.

With regrad to the possibility of a major proposal to undertake a more complete study on the ecology and epidemiology of viral diseases, particularly IPNV, among Columbia River basin stocks of Pacific salmon and steelhead trout, I have mentioned basic considerations for its design in the discussion portion of this report and would be most happy to prepare a detailed proposal should a source of funding be available.

We have appreciated the opportunity of being of service to the National Marine Fisheries Service and your Laboratory during the course of this project. I hope that we have the opportunity of working together again in the future.

Sincerely,

Bob

Robert A. Busch, Ph.D.
Director of Research

I. COMMODITIES DIVISION: GRAINS • SEEDS • BEANS II. FERTILIZER DIVISION: CHEMICALS • CUSTOM APPLICATION • SPRAYING • SOIL TESTING • MICRONUTRIENTS • FEED FEED • IQUIDS DRY FERTILIZERS • AERIAL APPLICATION III. FISHERIES DIVISION: TROUT FEED • LIVE TROUT • FISH DISEASE RESEARCH AND PATHOLOGY LABORATORY IV. GENERAL FEEDS DIVISION: CUSTOM STEAM ROLLING • GRINDING • MOLASSIFYING • FEEDING • LOCKING • BULK FEED DELIVERY V TRUCK BROKERAGE & COMMODITY TRADING DIVISION VI TRUCK & EQUIPMENT DIVISION RANGEN MOTRUCKS INC • PARTS & SERVICE • I.C)Mh'Lt TEDIESEL & AUTOMOTIVE REPAIR • I:,b.4hliN:, • CATS • TROUT • SEL • ALL ON • TRUCK BROKER & EQUIPMENT VII. TRUCKING DIVISION: RANGEN TRANSPORTATION INC

APPENDIX B

HISTOPATHOLOGY REPORT

BIOMED RESEARCH LABORATORIES

Biological Testing - Research & Development

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

October 24, 1978

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

SUBJECT: Six groups of salmon with 60 fish in each group formalin fixed.

METHODS AND MATERIALS:

Six groups of salmon with 60 fish in each group were received in formalin fixative following a 24 hour fixation in Bouin's fixative. Three blocks of tissues were parafin-embeded, sectioned and stained by standard methods. Block #1 contained the eye; block #2 the gill (generally the second gill arch) and block #3 the kidney and liver. Sections from each block were stained with hematoxylin and eosin, and a gram stain was made on the sections of liver and kidney. All blocks of tissue were prepared identically except for those of the eye. Initially, it was thought that only lesions within the eye itself would be found, and each eye was removed from the fish head before processing. It became apparent that there were important lesions adjacent to the eye, and the sections made late in the study also contained a full cross-section of the head to better study these lesions.

After scanning several groups of fish, a table of the lesions being found was established, and the lesions categorized by the severity. A minus (-) indicated no lesion, a plus (+) indicated the mildest recognizable lesion, two pluses (++) indicated intermediate severity and three pluses (+++) indicated the most severe lesion found. Gram stains were either positive or negative.

RESULTS: The lesions found frequently enough to warrent tabulation are identified on the table for each group of fish as follows: (A detailed description for each lesion is included later.)

1) Eye:

a. Myo - degenerative and regenerative lesions in the skeletal muscle adjacent to the eye.

b. Fat - fat necrosis and inflammatory inflitrates in the retrobulbar fat.

c. Misc-miscellaneous sporadic lesions.

2) Gill:

a. Lymph - increased numbers of lymphocytes.

b. Epith - Epithelial cell proliferation.

c. Misc - miscellaneous sporadic lesions.

3) Liver:

a. Fat - increased amount of fat in the liver

parenchyma.

b. KD - definite kidney disease w/gram plus bacteria.

c. Gran - granulomatous lesions w/out gram plus bacteria.

d. Misc - miscellaneous sporadic lesions.

4) Kidney :

a. KD - as in 3b.

b. Gran - as in 3c.

c. Ca - nephrocalcinosis.

5) Gram:

a. L - gram positive bacteria in the liver.

b. K - gram positive bacteria in the kidney.

A two page table for each 60 fish group identifies each fish and the severity of any lesions noted. At the bottom of each table is a total of the lesions found by severity.

A summary table presents the incidence of lesions within each group. It is expressed as a percent of the total fish in that group, and again broken down by severity of the lesions.

In general, it should be noted that these fish were well-fixed and provided excellent material for histological

study except when cross-sections of heads were made late in the study where intracranial hemorrhage was noted, perhaps as an artifact secondary to being “thumped” on the head during sampling.

Lesions from Block #1: The lesions in the skeletal muscle adjacent to the eye consisted primarily of irregular loss of myofibrills within muscle fibers. In cross-section, the sarcoplasm of affected fibers would be either granular or lost completely. In longitudinal sections of muscle, the muscle fibers would vary greatly in thickness with sudden abrupt loss of well-defined myofibrils. In one case (#4543, Winthrop Steelhead) marked basophillia and increased numbers of muscle cell nuclei indicated an attempted regeneration. The amount of muscle available for evaluation varied from slide to slide; therefore, the estimation of severity is subjective, but the typical (+) lesion is a very mild one.

The lesions of the retrobulbar fat can be broken down into fat necrosis and actual inflammation (panniculitis). In fat necrosis, variable numbers of the fat cells would contain faintly eosinophillic, often granular deposits interpreted as the formation of soaps as the result of fat breakdown. The panniculitis consisted of cellular inflammatory infiltrates between fat cells. Monocytes and lymphocytes were the most typical inflammatory cells noted. These lesions were mild and over-lapping, and it did not appear important to tabulate them separately.

Miscellaneous lesions found in block #1 from small numbers of fish included several inflammatory lesions of the eye itself. Chronic inflammation with mononuclear cell infiltrates were seen in the corneoscleral junction region of several fish, and a more widespread chronic inflammatory infiltrate was seen in the iris and ureal tract (iridocyclitis) of one fish. Another fish had more widespread chronic ophthalmitis involving much of the globe. The optic nerves of several fish contained large numbers of mononuclear cells, another fish had focal mononuclear cell infiltrates in the choroid gland, and another fish had a chronic inflammatory infiltrate within the wall of a retrobulbar blood vessel. Intracranial hemorrhage or meningeal hemorrhage was noted in several fish.

Lesions from Block #2: In many section of gills, increased numbers of lymphocytes were noted. Small numbers of lymphocytes can be found beneath the gill epithelium of almost any salmon, typically in the areas where the gill filament joins the gill arch. In mild (+) cases, this lymphoid infiltrate increases to several cells in thickness, and in more severe (++ & +++) cases, the mass of lymphocytes may obliterate a portion of the space at the base of the gill filament and form recognizable collections of lymphocytes higher on the gill filament.

The gill epithelial proliferative lesion tended to parallel the lymphoid infiltrative lesions of the gill in severity.

The epithelial proliferation tended to develope on the tips of the gill filaments rather than adjacent to the gill arch. It also tended to develope on the surface of the filament between the secondary lamellae. In no case did this lesion progress to the point of causing fusion between adjacent gill filaments. Miotic figures could be found in the proliferating epithelium and often the areas of lymphoid infiltration and epithelial cell proliferation overlapped.

The most common sporadic lesion of the gills was found only in coho salmon. This lesion is identified as BGO in the tables because it presents as a basophillic granular mass of organisms up to 40 u in diameter located intracellularly or subepithelially on the gill filament between the bases of secondary lamellae without any obvious cellular reaction. This structure is gram negative, and may represent a microsporidian protozoan parasite.

In the spring chinook and Dvorshak steelhead, a large ciliated protozoan parasite was noted. This parasite varied in size up to approximately 150 microns, was oval to round in shape and was covered with cilia. It was found both free in the interfilamentous space and also embedded beneath a layer of proliferating epithelium. The Dvorshak and Winthrop steelhead also had microsporidian protozoan parasites, differing from the BGO in that they appeared to be surrounded by a more definite cyst wall, and the spores within the cyst

measured two to three microns in diameter. The numbers of these structures found was soo small to allow further study. Fish parasitologists are being consulted to help identify these parasites.

Miscellaneous non-protozoan gill lesions included occasional fish in which one to several secondary lamellae would have greatly dialated secondary lamellar capillaries. One fish had generalized capillary congestion of the gills. Another had sludging of granulocytic leukocytes in the secondary lamellar capillaries.

Lesions of the Liver: Fat deposition in the livers of these fish was uncommon, but when present was identified either as individual fat droplets in random hepatocytes, or as a descrete compact group of hepatocytes filled with fat vacuoles.

Grossly or histologically apparent lesions of corynebacterial kidney disease were uncommon in the liver, but when recognized consisted of areas of necrosis containing large numbers of small gram positive bacteria. Also, noted were several granulomatous lesions consisting of compact aggregates of macrophages organized into a granuloma, but without any detectable bacteria present. Much more common than either of the prece'eding lesions was a focal accumulation of mononuclear cells (FM) in sinusoids or around portal tracts.

Lesions of the Kidney: The lesions of corynebacterial kidney disease and granulomas in the kidney were essentially as described for the liver. The abundant hematopoietic tissue made the granulomas more difficult to recognize. Also, occasionally areas of disrupted architecture and hemorrhage were seen. These lesions appeared to be secondary to the sampling of the kidney for the fluorescent antibody test for kidney disease bacteria and were not tabulated.

In one fish (#4025, spring chinook), the kidney contained a focal area of spindle-cell proliferation. These spindle cells appeared to be fibroblasts, and occasional cells within this area contained oval eosinophillic intracytoplasmic inclusion bodies approximately 50 microns in diameter.

Gram Stains: Interpretation of the gram-stained sections of kidney and liver is exceedingly difficult. Where the typical KD lesion is seen in the H & E section, gram positive bacteria are abundant and readily apparent. In sections without these lesions few definite bacteria could be recognized. Nuclear debri, cellular debri within kidney tubular lumens, and other small fragments of material tended to stain gram positive. The melamin granules of the macrophages also caused some difficulty in differentiating bacteria from other material.

In the discussion, suggestions for improving the accuracy of this proceedure will be discussed. Without this additional work, the percent of livers and kidneys harboring individual bacteria cannot be determined. An attempt was made to do so on the first three groups (Carson coho, spring chinook and

Dvorshak) but this data should not be entered as it is not significant (see discussion).

DISCUSSION:

A detailed statistical analysis was not attempted, being better left to the computerized data analysis, It should be noted that while the lesions were grouped into mild to severe categories, the (+++) category implies the most severe lesion found, but not necessarily that a severe lesion was found. In general, even the most severe lesions seen could not be interpreted as severe or life endangering. The significance of the data presented here will come when it is compared with data from other studies. Therefore, this discussion will deal primarily in generalities.

In the sections from block #1, the skeletal muscle lesions of the eye are interesting. The mildest (+) lesions possibly could be artifact of inadequate fixation. However, the more severe lesions do not appear to be artifact, and one case demonstrated regeneration, irrefutable evidence of muscle injury. Also, a more severe muscle lesion with frank necrosis and mineralization were recently found in salmon which had been in sea water approximately two months. This lesion was a more severe form of the same disease process seen in the fish of this study, again supporting the opinion that it is a genuine lesion. This lesion in a mammal would be strongly suggestive of a vitamin E/

selenium deficiency state, and has been reported to occur in fish although the known reports are not strongly documented. With regards to further studies, some consideration should be given to evaluating the vitamin E/selenium status of these fish. With respect to the differences between the groups, the steelhead fish have a higher incidence of these myodegenerative lesions.

The lesions in the fat also could have a pathogenesis involving vitamin E/selenium, similar to the steatitis or "yellow-fat" disease of mammals. For adequate study of both the skeletal muscle and fat lesions in the future, cross-sections of the head will be made to include the eyes, brain and structures adjacent to the eyes. No parasitic lesions were found in the eyes, and only sporadic mild inflammation was found within the eyes. The hemorrhage found in the calvarium, when cross-sections of the head were made, are probably the result of trauma at the time of collection. However, because brain hemorrhage is one lesion seen with thiamine deficiency, in the future it would be best to avoid trauma to the head to allow better evaluation of the brain.

In the sections of gills (block #2), the lymphoid cell populations were quite variable. Some of this variation is an unavoidable result of sectioning a gill arch at different levels because the lymphoid cell population varies in density at different levels in the gill arch;

therefore, this data is subjective and to be interpreted with some caution. While difficult to acess, the lymphoid cell numbers do vary markedly and this data should be carefully compared via computer analysis with eventual survival data and other parameters which may correlate to the overall fish health. This lymphoid tissue would by its location be comparable to the bronchial-associated lymphoid tissue (BALT) of the mammalian lung. The BALT is the collection of unstimulated lymphocytes in the peribronchial tissue which respond to inhaled antigens by multiplying the producing colones of memory T cells, and B cells which produce specific antibodies against that antigen. Lymphoid cell increases noted in many of these fish gills are a non-specific indicator of exposure to antigens in the water.

The proliferative lesion of the gill epithelium suffers the same problems of interpretation that the gill lymphoid lesion did, e.g. that of the plane of section. Even the most severe (+++) lesion noted is relatively mild in degree. This lesion was not associated with histological evidence of bacterial disease and is a response to undefined damage to the gill epithelium with resultant reparative proliferation. This lesion could be compared to the proliferation of type II alveolar lining cells of the mammalian lung in response to damage, and is compatible with a very mild form of

the nutritional gill disease described in fish. The various parasites of the gills did not appear to be causing significant injury to the gill itself. Their importance may be as a subclinical disease state which will cause heavily infested populations to be unable to handle other stresses as well as less affected populations. For example, the Carson coho may not do as well as Willard coho when exposed to some additional insult because of their higher incidence of the basophillic granular organisms. These parasites have not been specifically identified, but work is continuing in this area.

A rare fish had greatly distended blood filled capillaries in occasional secondary lamellae of the gills. This lesion has been termed "hemorrhagic gill disease", and later "toxic gill disease" by investigators who have produced the lesion with DDT and aflatoxins.

In the sections of liver and kidney (block #3), the incidence of histologically detectable kidney disease lesions was extremely low, as was the incidence of discrete granulomas. Several groups of fish had signficant although mild infiltrates of mononuclear cells. The spring chinook salmon had the highest incidence of gill lymphoid cell lesions, mononuclear cell infiltrates in the liver and granulomas in the kidney. These inflammatory lesions may be inter-related and may

affect the overall survival of this population of fish. The focal mononuclear cell infiltrates may represent a proliferation of macrophages attempting to phagocytize material reaching the liver. This material could also be stiumlating the granuloma production in the kidney; and therefore, may represent an undetected blood-born infectious agent. While KD organisms could not be detected in these lesions, the correlation of these histological lesions with the incidence of FA positive KD infected fish.

The interpretation of gram stains is a definite problem area. The data generated is not reliable in my opinion because of technical difficulties in differentiating gram plus bacteria from other debri in the sections. I think the accuracy of this portion of the study could be improved by direct comparison of serial frozen sections of kidney tissue, staining one section with the gram stain and the adjacent section with the fluorescent antibody technique. This would need only be done on a few fish next year with some known positives to allow the pathologist to conclusively determine how much of the gram plus material is indeed coryneybacteria. This could be done at no additional charge, and the gram stains from this years fish re-evaluated. The frozen section work would also allow more precise localization of the bacteria within the tissues.

The proliferative lesion in the kidney with intracellular inclusion bodies may represent a viral infection. The morphology of the inclusions is similar to mammalial pox viruses, A portion of the lesion is being processed for electron microscopy to better define it.

SUMMARY: In this study, six groups of sixty fish each were examined histologically with particular emphasis on the eye, gill, liver and kidney. Frequent, but mild skeletal muscle myodegeneration was seen adjacent to the eye. Minimal gill epithelial lesions of the "nutritional gill disease" type were found in the gills, along with variable lymphoid hyperplasia and occasional protozoan parasites. A few fish had lesions typical of "toxic gill disease." Rare lesions of "bacterial kidney disease" were seen in the livers and kidneys plus some nonspecific granulomas. The overall health of these fish appeared good, but the spring chinook had the highest incidence of lesions in several disease categories. The gram stain did not prove useful in detecting low numbers of gram positive bacteria in tissue sections.

Respectfully submitted,

John T. Boyce, D. V. M.
Diplomate, American College
of Veterinary Pathologists

INDEX OF DATA TABLES

		DATA TABLE NUMBER	DATA TABLE PAGE
Totals in % (A summary of the incidence of lesions in all Six populations of fish)		A	107
Carson Coho Salmon	(4401-4430)	B1	108
	(4431-4460)	B2	109
Spring Chinook	(4001-4030)	C1	110
	(4031-4060)	C2	111
Dworshak Steelhead	(4061-4090)	D1	112
	(4091-4120)	D2	113
Winthrop Steelhead	(4501-4530)	E1	114
	(4531-4560)	E2	115
Tucannon Steelhead	(4601-4630)	F1	116
	(4631-4660)	F2	117
'Willard Coho	(4351-4670)	G1	118
	(4671-4700)	G2	119

TOTALS IN % A

	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 Call	KIDNEY K	COMMENTS:	
	CARSON COHO TOTAL INCIDENCE														BGO (GILL)	FM (LIVER)
-	73%	100%	99%	25%	39%	27%	98%	98%	97%	92%	98%	98%	100%	98%	95% 27%	92%
+	27%	0%	--	73%	61%	--	2%	0%	3%	--	0%	2%	0%	2%	5% 59%	8%
++	0%	0%	--	2%	0%	--	0%	2%	0%	--	2%	0%	0%	-	- 14%	0%
+++	0%	0%	--	0%	0%	--	0%	0%	0%	--	0%	0%	0%	-	- 0%	0%
	SPRING CHINOOK TOTAL INCIDENCE														CP (Gill)	FM (Liver)
-	88%	92%	100%	5%	83%	97%	96%	97%	98%	30%	97%	74%	100%	97%	97% 97%	30%
+	12%	7%	0%	73%	10%	--	2%	0%	0%	--	0%	23%	0%	3%	3% 3%	50%
++	0%	19%	0%	20%	7%	--	2%	3%	0%	--	0%	3%	0%	-	- 0%	15%
+++	0%	0%	0%	2%	0%	--	0%	0%	2%	--	3%	0%	0%	-	- 0%	5%
	DVORSHAK STEELHEAD TOTAL INCIDENCE														GILL	FM (LIVER)
-	37%	68%	87%	22%	47%	77%	100%	100%	100%	75%	100%	98%	100%	100%	100%	75%
+	53%	27%		65%	68%	--	10%	0%	0%	--	0%	2%	0%	0%	0% CP 18%	22%
++	10%	3%		8%	15%	--	0%	0%	0%	--	0%	0%	0%	-	- SP 3%	3%
+++	0%	2%		5%	4%	--	0%	0%	0%	--	0%	0%	0%	-	-	0%
	WINTHROP STEELHEAD TOTAL INCIDENCE														GILL (FE) GILL (MT)	FM (LIVER)
-	43%	93%	100%	33%	8%	90%	97%	100%	100%	100%	100%	98%	98%		90% 90%	100%
+	48%	7%	0%	65%	78%	--	3%	0%	0%	0%	0%	2%	2%		8% 2%	0%
++	2%	0%	0%	2%	13%	--	0%	0%	0%	0%	0%	0%	0%			0%
+++	2%	0%	0%	0%	0%	--	0%	0%	0%	0%	0%	0%	0%			0%
	TUCANNON STEELHEAD TOTAL INCIDENCE															FM (LIVER)
-	43%	100%	88%	78%	67%	97%	92%	100%	98%	82%	100%	93%	100%		12% intracranial hemorrhage*	82%
+	48%	0%	12%	22%	33%	3%	8%	0%	2%	-	0%	7%	0%		2% CD in gill	16%
++	9%	0%	0%	0%	0%	0%	0%	0%	0%	-	0%	0%	0%		2% neuts in gill	2%
+++	0%	0%	0%	0%	0%	0%	0%	0%	0%	-	0%	0%	0%			0%
	WILLARD COHO SALMON (58 total fish)														GILL	FM (LIVER)
-	60%	93%	97%	78%	55%	88%	100%	100%	97%	97%	100%	96%	100%		BGO + 5%	97%
+	36%	7%	--	22%	41%	--	0%	0%	3%	3%	0%	2%	0%		BGO ++ 5%	3%
++	4%	0%	--	0%	4%	--	0%	0%	0%	0%	0%	2%	0%			0%
+++	0%	0%	--	0%	0%		0%	0%	0%	0%	0%	0%	0%			0%

*ICH

Carson coho salmon

4401-4430	EYE MYO	EYE FAT	EYE MTSC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	GRAM L	GRAM K	COMMENTS:
4401	-	-	-		+	-	-	-	-	-	-	-	-	-		
02	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	BGO	= Basophillic granular organism
03	-	-	-			BGo++	-	-	-					-	-	
04	-	-	-			BGO++	-	-	-					-	-	
05	+	-	-		-	BGO++	-	-	-	-	-	-	-	-	-	
06	+	-	-	+	+									-	-	
07	+	-	-		+	FB	-	-	-	-	-	**		-	FB	=Filamentous bacteria. **See below
08	+	-	-		+					FM			-	-	-	FM = Focal mononuclear cell infiltrato
09	-	-	-	+	+	BGO	-	++	-	-	++	-	-	+	+	
4410	-	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
11	-	-	-	+	+								-	-	+	Rare G+ bacteria in kidney
12	+	-	-	+	+	BGO++	-	-	-				-	-	-	
13	+	-	-			BGO	-						-	-	-	
14	+	-	-	-	+								*	-	-	*G+ debri in tubles
15	+	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
16	-	-	-			BGO	+	-	-	-	-	-	-	-	-	
17	+	-	-	+	+	BGO	-	-	-	FM	-	-	-	-	-	
18	-	-	-			BGO	-	-	+	-	-	-	-	-	-	
19	+	-	-	+	+	BGO	-	-	-	-	-	-	-	-	+	
4420	-	-	-			BGO	-	-	-	-	-	-	*	-	-	*G+ debri in tubles
21	+	-	-			BGO	-		+				-		-	
22	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
23	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
24	+	-	-	+	+	BGO	-						-	-	-	
25	-	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
26	-	-	-	+	+	BGO	-	-	-	-	-	+	-	-	-	
27	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
28	-	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
29	-	-	SI/0	+	+	BGO	-	-	-	-	-	-	-	-	-	SI = minimal subacute iridocyclitis
4430	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
SUBTOTALS:																
	18	30	29	12	8	5	29	29	28	28	29	29	27	28	26	
+	12	0	-	18	22	16	1	0	2	2	0	1	-	1	3	
++	0	0	-	0	0	8	0	1	0	0	1	0	-	-	-	
+++																

Carson Coho Salmon

4 1-4430 4 6	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	GRAM L	GRAM K	COMMENTS:
4431	-	-	-	-	+	BGO	-	-	-	-	-	-	-	-	-	
32	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
33	+	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
34	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
35	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
36	-	-	-	-	-	BGO	-	-	-	-	-	-	-	-	-	
37	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
38	-	-	-	+	+	BGO	-	-	-	-	-	++	-	-	-	
39	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
4440	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
42	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	*	*Possible G+ bacteria in tubular lumen
43	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
44	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
45	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
46	+	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
47	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
48	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
49	-	-	CI	+	-	BGO	-	-	-	-	-	-	-	-	-	CI = choroid gland inflammation
4450	-	-	-	+	-	BGO	-	-	-	FM	-	-	-	-	-	
51	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
52	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
53	-	-	-	+	-	BGO	-	-	-	FM	-	-	-	-	-	
54	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
55	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
56	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
57	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
58	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
59	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
4460	No fish found															
FINAL TOTALS:																
	43	59	58	15	23	15	58	58	59	54	58	57	55	58	56	
+	16	0	-	43	36	BGO35	1	0	2	5	0	1	-	1	3	
++	0	0	-	1	0	BGO 8	0	1	0	0	1	1	-	-	-	
+++	0	0	-	0	0		0	0	0	0	0	0	-	-	-	

 C1
Spring chniook Kooskia

4001-4030	EYE NVO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	GRAM L	GRAM K	COMMENTS:
4001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
02	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	FM = Focal mononuclears
03	-	-	-	+	-	-	-	-	+++	-	-	+	-	-	-	Granulomatous hepatitis
04	+	++	-	++	-	-	-	-	-	FM	-	-	+	-	-	
05	0	0	0	+	-	-	-	-	-	-	-	-	+	-	-	
06	-	+	-	++	+	-	-	-	-	FM+++	-	-	-	-	-	Hepatocytic necrosis
07	-	+	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
08	-	-	-	++	++	-	-	-	-	-	-	-	-	-	-	
09	-	+	-	++	-	-	-	-	-	FM	-	-	-	-	-	
4010	-	+	-	++	++	-	-	-	-	-	-	-	-	-	-	
11	-	-	-	+	+	-	-	-	-	FM	-	-	+	-	-	
12	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
13	-	-	-	++	+	-	-	-	-	-	-	++	+	-	-	
14	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
15	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
16	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
17	-	-	-	++	-	-	-	-	-	FM	-	-	++	-	-	
18	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
19	-	-	-	+	-	-	-	-	-	FM++	-	-	-	-	-	
4020	-	-	-	-	-	-	-	-	-	FM	-	-	-	-	-	
21	+	-	-	+	-	-	-	-	-	FM	-	-	+	-	-	
22	-	-	-	++	+	-	-	-	-	FM	-	-	+	-	-	
23	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	
24	-	-	-	++	-	-	+	-	-	FM++	-	-	+	-	-	
25	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	
26	+	-	-	+++	++	-	-	-	-	FM++	-	++	-	-	-	
27	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
28				+			-	-	-	FM	-	+	-	-	-	
29	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
4030	-	-	-	+	-	-	-	-	-	-	-	+	+++	-	-	
SUBTOTALS:																
-	27	25	30	3	22	30	29	30	29	10	30	21	16	30	30	
+	3	4	0	17	5	0	1	0	0	13	0	7	11	-	-	
++	0	1	0	9	3	0	0	0	0	6	0	2	2	-	-	
+++	0	0	0	1	0	0	0	0	0	2	0	0	1			

Spring Chinook Roosevelt

4031-4060	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	GRAM L	GRAM K	COMMENTS.
4031	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
32	+	-	-	+	-	-	-	-	-	FM	-	+	++	-	-	
33	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
34	-	-	-	++	-	-	-	-	-	FM	-	+	-	-	-	
35	-	-	-	+	-	-	-	++	-	FM	+++	-	-	+	+	
36	-	-	-	+	-	-	-	-	-	FM+++	-	-	-	-	-	
37	-	-	-	+	-	-	-	-	-	FM	-	+	+	-	-	
38	-	-	-	+	-	-	-	-	-	-	-	-	++	-	-	
39	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
4040	-	-	-	++	++	CP	++	++	-	FM	+++	-	-	+	+	CP = Ciliated protozoan
41	+	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
42	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
43	+	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
44	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
45	-	-	-	+	-	-	-	-	-	FM	-	-	+	-	-	
46	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	
47	-	-	-	+	-	CP	-	-	-	FM	-	-	-	-	-	
48	-	-	-	++	-	-	-	-	-	FM+++	-	-	++	-	-	
49	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
4050	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
51	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
52	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	
53	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
54	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
55	-	-	-	+	+	-	-	-	-	FM	-	+	+	-	-	
56	-	-	-	+	-	-	-	-	-	-	-	-	+++	-	-	
57	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
58	-	-	-	+	-	-	-	-	-	FM++	-	-	-	-	-	
59	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
4060	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
FINAL TOTALS:																
-	53	55	60	3	50	58	58	59	59	18	58	39	25	58	58	
+	7	4	0	44	6	CP2	1	°	°	3°	°	18	28	2	2	
++	0	1	0	13	4	0	1	2	0	9	0	2	5	°	°	
+++	0	0	0	1	0	0	0	0	1	3	2	1	2	0	0	

Dvorshak steelhead

4061-4090

	EYE			GILL			LIVER				KIDNEY			GRAM		
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Cap	L	K	COMMENTS.
4061	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
62	-	+	-	+	+	-	+	-	-	FM	-	-	-	-	-	
63	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
64	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
65	+	-	-	+	++	-	-	-	-	-	-	-	-	-	-	
66	+	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
67	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
68	++	+++	**	-	-	-	-	-	-	-	-	-	-	-	-	**Chronic active opthalmitis
69	-	-	-	+	+	CP	-	-	-	-	-	-	-	-	-	CP = Ciliated protozoan
4070	-	-	-	+	++	-	-	-	-	FM	-	-	-	-	-	
71	+	++	-	+	+	-	-	-	-	-	-	-	-	-	-	
72	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	
73	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
74	+	-	-	-	+	**	-	-	-	FM	-	-	-	-	-	**Questionable protozoan parasite
75	+	+	-		+	CP	-	-	-	-	-	-	-	-	-	
76	+	+	-	-	++	-	-	-	-	FM	-	-	-	-	-	
77	+	-	-	-	+	CP	-	-	-	-	-	-	-	-	-	
78	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	
79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4080	-	-	-	+	++	-	-	-	-	-	-	-	-	-	-	
81	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
82	++	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
83	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
84	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
85	-	-	-	+	+	**	+	-	-	-	-	-	-	-	-	**Solitary basophilic mass in SL
86	-	-	-	++	++	CP	-	-	-	-	-	-	-	-	-	
87	-	+	FM	+	+	-	-	-	-	FM	-	-	-	-	-	Focal mononuclears in optic nerve
88	+	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
89	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	
4090	-	-	-	+	+++	-	-	-	-	FM	-	-	-	-	+	Gram bacteria (?) in kidney
SUBTOTALS:																
-	15	21	28	10	3	24	24	30	30	22	30	30	30	30	30	
+	13	7	-	19	21	CP4	6	0	0	FM8	0	0	0	0	0	
++	2	1	-	1	5	0	0	0	0	0	0	0	0	-	-	
+++	0	1	-	0	1	0	0	0	0	0	0	0	0	-	-	

Dvorshak steelhead

4091-4020	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	GRAM L	GRAM K	COMMENTS:
4091	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
92	+	+	-	+	+	CP	-	-	-	-	-	-	-	-	-	CP = Ciliated protozoan
93	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	
94	++	+	-	+	+	-	-	-	-	-	-	-	-	-	-	
95	+	-	-	++	++	-	-	-	-	-	-	-	-	-	-	
96	+	-	-	+	++	-	-	-	-	-	-	-	-	-	-	
97	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
98	++	-	-	+	+	CP	-	-	-	-	-	-	-	-	-	
99	-	+	**	+	+	CP	-	-	-	-	-	-	-	-	-	**Cornea-scleral inflammation
4100	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
01	+	+	-	+	-	-	-	-	-	FM	-	-	-	-	-	
02	++	++	-	++	++	CP/SP	-	-	-	-	-	-	-	-	-	SP = sporozoan protozoan parasite
03	+	+	**	+++	++	-	-	-	-	-	-	-	-	-	-	**Neuritis (optis nerve)
04	-	-	-	++	-	-	-	-	-	FM	-	-	-	-	-	
05	+	+	**	+	-	-	-	-	-	-	-	-	-	-	-	**neuritis (optic nerve)
06	-	-	-	++	+	-	-	-	-	FM++	-	-	-	-	-	
07	-	-	-	+++	++	-	-	-	-	-	-	+	-	-	-	
08	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
09	-	-	-	+++	+	SP	-	-	-	-	-	-	-	-	-	Sporozoan protozoan parasite
[illegible]	+	-	-	-	-	-	-	-	-	FM	-	-	-	-	-	
11	++	-	IHC	+	+	-	-	-	-	-	-	-	-	-	-	IHC = Brain hemorrhage
12	+	-	-	+	+	CP	-	-	-	FM++	-	-	-	-	-	
13	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
14	+	-	-	+	+	CP	-	-	-	-	-	-	-	-	-	
15	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
16	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
17	+	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
18	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
19	+	+	FM	+	+	CP	-	-	-	-	-	-	-	-	-	FM-Focal mono's in brain
4120	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	
FINAL TOTALS:																
-	22	41	54	13	8	46	54	6°	6°	45	60	59	60	60	60	
+	32	16	-	20	2°	CP7	°	°	°	FM13	0	1	0	0	0	
++	6	2	-	4	4	°	°	0	0	FM 2	0	0	0	-	-	
	0	1	-	3	1	0	0	°	°	0	0	0	0	-	-	

Winthrop Steelhead

4501-4530	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	GRAM L	GRAM K	COMMENTS
4501	+	-	-				-	-	-							
02	-	-	-	-	-	FD	-	-	-	-	-	-	+			Fd = Focal capillary dilation in S
03	-	-	-	+	+	-	-	-	-	-	-	-				
04	-	-	-	++	++	FD	-	-	-	-	-	-	-			
05	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
06	-	-	-	±	+	-	-	-	-	-	-	-	-			
07	++	*	-		+	-	-	-	-	-	-	-	-			*Focal hemorrhage in fat
08	-	-	-	-	+	-	-	-	-	-	-	-	-			
09	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
4510	-	-	-	-	+	FD	-	-	-	-	-	-	-			
11	-	-	-	-	+	-	-	-	-	-	-	-	-			
12	+	-	-	+	+	-	-	-	-	-	-	-	-			
13	-	-	-	-	++	-	-	-	-	-	-	-	-			
14	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
15	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
16	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
17	+	-	-	+	++	-	-	-	-	-	-	-	-			
18	+	-	-	-	+	-	-	-	-	-	-	-	-			
19	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
4520	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	
21	-	-	-	-	+	-	-	-	-	-	-	-	-			
22	+	-	-	-	+	-	-	-	-	-	-	-	-			
23	-	-	-	+	+	-	-	-	-	-	-	-	-			
24	+	+	-	-	+	-	-	-	-	-	-	-	-			
25	-	-	-	-	++	-	-	-	-	-	-	-	-			
26	-	-	-	+	+	-	-	-	-	-	-	-	-			
27	+	-	-	+	++	-	-	-	-	-	-	-	-			
28	+	-	-	+	+	-	-	-	-	-	-	-	-			
29	-	-	-	+	+	-	-	-	-	-	-	-	*			*De-ri in excretory duct
4530	-	-	-	+	+	FD	+	-	-	-	-	-	-			
SUBTOTALS:																
	19	2	3	14	2	2	29	30	30	30	30	29	28	-	-	
+	10	2	0	15	22	FD4	1	0	0	0	0	1	1	-	-	
++	1	0	0	1	6	0	0	0	0	0	0	0	0			
+++	0	0	0	0	0	0	0	0	0	0	0	0	0			

Winthrop Steelhead

4531-4560	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	GRAM L	GRAM K	COMMENTS:
4531	+	-	-	+	+	-	-	-	-	-	-	-	-			
32	+	-	-	-	+	-	-	-	-	-	-	-	-			
33	-	-	-	+	++	-	-	-	-	-	-	-	-			
34	+	-	-	+	+	-	-	-	-	-	-	-	-			
35	+	-	-	+	+	-	-	-	-	-	-	-	-			
36	+	-	-	+	+	-	-	-	-	-	-	-	-			
37	+	-	-	+	+	-	+	-	-	-	-	-	-			
38	-	-	-	-	-	-	-	-	-	-	-	-	-			
39	+	-	-	+	-	-	-	-	-	-	-	-	-			
4540	+	-	-	+	+	-	-	-	-	-	-	-	-			
41	-	+	-	+	+	-	-	-	-	-	-	-	-			
42	-	-	-	+	+	-	-	-	-	-	-	-	-			
43	+++*	-	-	+	+	-	-	-	-	-	-	-	-			*Marked muscle regeneration
44	+	-	-	+	+	-	-	-	-	-	-	-	-			
45	+	-	-	+	+	-	-									
46	+	-	-	-	+	-	-									
47	-	-	-	+	+	-	-	-	-	-	-	-	-			
48	-	-	-	-	-	-	-	-	-	-	-	-	-			
49	+	-	-	+	+	MP*	-	-	-	-	-	-	-			*Microsporidian protozoan parasite
4550	+	-	-	+	+	FD	-	-	-	-	-	-	-			FD = Focal capillary dilatation
51	+	-	-	+	++	-	-	-	-	-	-	-	-			
52	+	-	-	-	+	-	-	-	-	-	-	-	-			
53	-	-	-	-	+	-	-	-	-	-	-	-	-			
54	+	-	-	+	+	-	-	-	-	-	-	-	-			
55	+	-	-	+	+	-	-	-	-	-	-	-	-			
56		-	-	+	+		-	-	-	-	-	-	-			
57	-	-	-	+	+		-	-	-	-	-	-	-			
58	+	-	-	+	+	-	-	-	-	-	-	-	-			
59	+	-	-	+	+	-	-	-	-	-	-	-	-			
4560	-	-	-	+	+	-	-	-	-	-	-	-	-			
FINAL TOTALS:																
-	29	56	60	20	5	54	58	60	60	60	60	59	60			
+	29	3	0	39	47	FD5	2	0	0	0	0	1	0			
++	1	0	0	1	8	0	0	0	0	0	0	0	0			
+++	1	0	0	0	0	0	0	0	0	0	0	0	0			

 F1
Tucannon steelhead

4601-4630	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	GRAM L	GRAM K	COMMENTS.
4601	-	-	MH	-	+	-	-	-	-	FM	-	-	-			MH = Meningeal hemorrhage (ICH)
02	-	-	MH	-	-	-	-	-	-	-	-	-	-			
03	+	-	-	-	-	CD	-	-	-	-	-	-	-			CD = Capillary dilitation in S. L.
04	+	-	-	-	-	-	-	-	-	-	-	-	-			
05	-	-	ICH	-	-	-	-	-	-	-	-	+	-			ICH-Cerebral hemorrhage
06	+	-	-	-	+	-	-	-	-	-	-	-	-			
07	+	-	-	-	-	-	-	-	-	-	-	-	-			
08	+	-	-	+	+	-	-	-	-	-	-	-	-			
09	+	-	-	-	+	-	+	-	-	-	-	-	-			
4610	+	-	-	-	-	-	-	-	-	-	-	-	-			
11	+	-	-	-	-	-	-	-	-	-	-	-	-			
12	++	-	-	-	-	-	+	-	-	-	-	-	-			
13	+	-	-	-	-	-	+	-	-	-	-	-	-			
14	-	-	-	+	+	-	-	-	-	-	-	-	-			
15	-	-	-	+	+	-	+	-	-	-	-	-	-			
16	+	-	-	-	-	-	+	-	-	-	-	-	-			
17	-	-	-	+	+	-	-	-	-	FM	-	-	-			
18	+	-	-	-	+	-	-	-	-	-	-	-	-			
19	+	-	-	-	-	-	-	-	-	FM	-	-	-			
4620	+	-	-	+	+	-	-	-	-	FM	-	-	-			
21	+	-	-	-	-	-	-	-	-	FM	-	+	-			
22	+	-	-	-	-	-	-	-	-	-	-	-	-			
23	-	-	-	+	+	**	-	-	-	-	-	-	-			**Neutrophiles in secondary lamellae
24	-	-	-	-	-	-						-	-	-	-	
25	+	-	-	-	-	-						-	-			
26	-	-	ICH	-	-	-	-	-	-	FM	-	-	-			
27	++	-	ICH	-												
28	++	-	-	-	-	-	-	-	-	-	-	-	-			
29	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4630	-		ICH	-	-	-	-	-	-	-	-	-	-	-	-	
SUBTOTALS:																
-	10	30	24	24	20	28	25	30	30	25	30	28	30			
+	10	0	ICH6	6	10	0	5	0	0	FM5	0	2	0			
	4	0	0	0	0	0	0	0	0	0	0	0	0			
	0	0	0	0	0	0	0	0	0	0	0	0	0			

Tucannon steelhead

4631-4660	EYE MYO	EYE FAT	EYE MISC	GILL LYMP	GILL EPITL	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	GRAM L	GRAM K	COMMENTS:
4631	+	-	-		+	-	-	-	-	FM						FM = Focal mononuclears
32	-	-	-		+	-	-	-	-	-	-	**	-			**Foci of neurophiles
33	-	-	-	+	+	-	-	-	-	-	-	-	-			
34	-	-	-	-	-	-	-	-	-	-	-	-	-			
35	-	-	-	+	-	-	-	-	-	-	-	-	-			
36	-	-	-	-		-	-	-	-	-	-	-	-			
37	+	-	-	+		-	-	-	-	-	-	-	-			
38	+		-	-	-	-	-	-	-	-	-	-	-			
39	-	-	-	-	-	-	-	-	-	-	-	-	-			
4640	-	-	-	-	-	-	-	-	-	-	-	-	-			
41	+	-	-	+	-	-	-	-	-	-	-	+	-			
42	+	-	-	-	-	-	-	-	-	-	-	-	-			
43	-	-	CH	+	+	-	-	-	-	-	-	-	-			CH = cerebral hemorrhage
44	-	-	-	+	-	-	-	-	-	-	-	-	-			
45	-	-	-	-		-	-	-	-	-	-	+++	-	-	-	
46	-	-	**	-	+	-	-	-	+	-	-	-	-	-	-	**Segmental vasculitis
47	+	-	-		+	-	-	-	-	-	-	-	-	-	-	
48	-	-	-	+	+	-	-	-	-	-	-	-	-			
49	+	-	-	-	-	-	-	-	-	-	-	-	-			
4650	+	-	-	-	-	-	-	-	-	FM	-	-	-			
51	+	-	-	-	-	-	-	-	-	-	-	-	-			
52	-	-	-	-	-	-	-	-	-	-	-	-	-			
53	+	-	-	-	+	-	-	-	-	FM	-	-	-			
54	+	-	-	-	+	-	-	-	-	FM++	-	-	-			
55	++	-	-	-	-	-	-	-	-	FM	-	-	-			
56	+	-	-	-	-	-	-	-	-	-	-	-	-			
57				-	-	-	-	-	-	-	-	-	-			
58	+	-	-	-	-	-	-	-	-	FM	-	-	-			
59	-	-	-	-	-	-	-	-	-	-	-	-	-			
4660	-	-	-	-	+	-	-	-	-	-	-	-	-			
FINAL TOTALS:																
	26	6°	52	47	40	58	55	60	59	29	60	55	60	3	3	
	29	0	ICH7	13	20	-	5	0	1	FM10	0	3	0	0	0	
	1	0	0	0	0	-	0	0	0	FM 1	0	0	0	0	0	
	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	

Willard Coho salmon

4351-4670	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER [illegible]	LIVER [illegible]	LIVER [illegible]	KIDNEY [illegible]	KIDNEY [illegible]	KIDNEY [illegible]	GRAM [illegible]	COMMENTS
4351	-	-	-	-	-	BGO	-	-	-	FM	-	-	-		BGO = Basophillic granular organism
52	-	-	-	-	-	-	-	-	-	-	-	-	-		
53	+	-	-	-	+	-	-	-	-	-	-	-	-		
54	-	-	-	-	-	-	-	-	-	-	-	-	-		
55	+	-	-	-	+	-	-	-	-	-	-	-	-		
56	-	-	-	-	-	-	-	-	-	-	-	-	-		
57	+	-	-	-	-	-	-	-	-	-	-	-	-		
58	-	-	-	-	-	-	-	-	-	FM	-	-	-		
59	+	-	-	-	-	-	-	-	-	-	-	-	-		
4360	-	-	-	+	+	-	-	-	-	-		-	-		
61	-	-	-	-	+	-	-	-	-	-		-	-		
62	-	-	ICH	-	-	-	-	-	-	-	-	-	-		ICH = Intracranical hemorrhage
63	-	-	-	-	+	-	-	-	-	-		-	-		
64	-	-	-	-	-	-	-	-	-	-		-	-		
65	+	-	-	-	-	-	-	-	-	FM	-	-	-		
66	+	-	-	-	-	-	-	-	-	-	-	-	-		
67	-	-	-	+	-	-	-	-	-	-	-	-	-		
68	-	-	-	-	-	-	-	-	-	-	-	-	-		
69	-	-	-	-	-	BGO++	-	-	+	-	-	-	-		
4370	+	-	-	-	-	-	-	-	-	-	-	-	-		
4661	-	-	-	-	+	-	-	-	-	-	-	-	-		
62	+	+	-	-	-	BGO	-	-	-	-	-	-	-		BGO = Basophillic granular organism
63	-	-	-	-	-	-	-	-				-	-		
64	+	-	-	-	+	-	-	-	-	-	-	-	-		
65	-	-	-	-	-	-	-	-	-	-	-	-	-		
66	++	+	-	-	-	-	-	-	-	-	-	-	-		
67	-	-	-	+	+	-	-	-	-	-	-	-	-		(Slide mislabeled as 4067)
68	+	-	-	-	-	-	-	-	-	-	-	-	-		
69	-	-	-	-	+	BGO+++	-	-	-	-	-	-	-		
4670	-	+	-	-	+	-	-	-	-	-	-	-	-		
SUBTOTALS:															
-	19	27	29	27	20	26	30	30	29	27	30	30	7		
+	1	3	ICH1	3	10	3	0	0	1	FM3	0	0	7		
++		0	0	0	0	1	0	0	0	0	0	0	0		
+++	0	0	0	0	0	1	0	0	0	0	0	0	0		

Willard Coho salmon

4671-4700	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	GRAM L K	COMMENTS:
4671	-	-	-	-	++	-	-	-				-	-		
72	-	-	-	+	+		-	-				--	-		
73	-	-	-	+	++	-	-	-	-	-	-	-	-		
74	-	-	-	-	-	-	-	-	-	-	-	++	-		
75	+	-	-	-	-	-	-	-	-	-	-	-	-		
76	+			+	+		-	-		FN	-	-	-		FN = Focal necrosis
77	+	-	-	+	+		-	-				-	-		
78	+	-	-	+	+		-	-				-	-		
79	++	-	-	-	+		-	-				-	-		
468	+	-	-	+	+		000			o-		--			(No liver imbedded)
81	--	+	-	-	+	-	-	-	-	-	-	+	-		
82	-	-	-	+	+	-	-	-	-	-	-	—			
83	-	--		-	-	-	--		-	-	-	—			
84	No fish found														
85	+	-	-	-	+	-	-	-	-	-	-	-	-		
86	+	-	-	-	-	CG	-	-	-	-	-	-			CG = Congested gills
87	-	-	-	+	+	BGO++	-	-	-	-	-	-	-		
88	-	-	-	-	+	-	-	-				-	-		
89	+	-	-	-	+	BGO	-	-	+	-	-	-	-		
4690	-	-	-	-	-	-	-			FM	-	-	-		FM = Focal mononuclear cell infiltrat
91	-	-	-	+	+	-	-	-	-	-	-	-	-		
92	-	-	CC	-	-	-	-	-	-	-	-	-	-		CC = Chronic conjunctivitis
93	-	-	-	-	-	-	-	-	-	-	-	-	-		
94	+	-	-	-	-	-	-	-	-	-	-	-	-		
95	-	-	-	-	-	-	-	-	-	-	-	-	-		
96	-	-	-	+	+	-	-	-	-	-	-	-	-		
97	+	-	-	-	-	-	-	-	-	-	-	-	-		
98	No fish found														
99	-	-	-	-	-	-	-	-	-	-	-	-	-		
4700	+	-	-	-	-	-	-	-	-	-	-	-	-		
FINAL TOTALS:															
	35	54	46	45	32	51	57	57	55	52	58	26	58		
+	21	4	0	13	24	3	0	0	2	2	0	1	0		
++	2	0	0	0	2	3	0	0	0	0	0	1	0		
+++	0	0	0	0	0	1	0	0	0	0	0	0	0		

APPENDIX C

INDIRECT FLUORESCENT ANTIBODY TECHNIQUE (IFAT) FOR BACTERIAL KIDNEY DISEASE (BKD)

APPENDIX C. Indirect fluorescent antibody technique (IFAT) for bacterial kidney disease (BKD).

A. REAGENTS.

1. Rabbit anti-BKD serum. Biomed Research Laboratories (Seattle). Titer of 1:512. Hold at -20°C. Store in 0.1 or 0.2 ml aliquots. Dilute aliquots 1:50 in phosphate buffered saline as needed. Holds for 2 to 4 weeks at 2-4°C.

2. Goat anti-rabbit fluorescein isothiocyanate (FITC). Difco Laboratories (Detroit). (goat anti-rabbit serum; fluorescein conjugated IgG fraction). Rehydrate as per instructions. Freeze in 0.1 ml aliquots until needed.

3. Rhodamine counterstain. Difco Laboratories. Rehydrate as per instructions. Freeze in 0.1 ml aliquots until needed.

4. Carbonate-bicarbonate buffer (C-BB).

26.5 g Na_2CO_3 with distilled water to 500 ml final volume. Refrigerate until needed.

21.0 g NaHC03 with distilled water to 500 ml final volume. Refrigerate until needed.

For fresh C-B buffer, mix 1 part Na_2CO_3 solution with 4 parts NaHC03.

5. Phosphate buffered saline (Kawamura's) (PBS).

8 g NaCL; 0.2 g KCl; 1.15 g anhydrous Na_2HPO_4; and 0.2 g KH_2PO_4 dissolved in 1,000 ml distilled water (pH is 7.2-7.4). Prepare fresh, or as a 10-fold concentrate without NaCl. Store concentrate at 4°C until needed; dilute and add NaCl.

6. Fluorescent antibody (FA) mounting fluid. pH 9. Difco Laboratories or: 0.5 M carbonate buffer (C-BB) (pH 9.5).....1 volume; plus 9 volumes reagent grade glycerine. Mix thoroughly.

7. Low fluorescence immersion oil.

8. Rabbit antisera for furunculosis or enteric redmouth (ERM) disease as desired for control tests. Dilute approximately 1:50 with PBS.

9. Normal rabbit antiserum (RAS). Dilute 1:50 with PBS for control tests.

B. PROCEDURE FOR PREPARING IFAT-BKD SLIDE MATERIAL.

1. Mix 0.1 ml of goat FITC with 0.1 ml of rhodamine counterstain and dilute with 4.8 ml PBS. This is a 1:20 dilution.

2. The bottom row of the 12 well multi-spot control slide is used to replicate the following controls on the top row:

(a) goat FITC conjugate only; (b) normal RAS; (c) Furunculosis RAS; (d) ERM-RAS; (e) BKD-RAS; (f) BKD-RAS.

3. Layer a plastic box with wet sponges to provide a moist chamber, place a wire rack over the sponges, and place the slides on the rack.

4. Place one drop of dilute BKD-RAS on each well of the test groups and the appropriate control wells.

5. Place drops of the appropriate antisera on the remaining control wells (except the FITC conjugate only).

6. Seal the moist chamber and incubate the slides for 15 to 30 minutes at room temperature.

7. Flush the slides with PBS and transfer to slide staining dishes filled with PBS for 10 minutes.

8. Remove the slides and dry with a portable (cosmetic) hair dryer (with no heat).

9. One drop of the goat FITC counterstain conjugate is placed on each well of both test and control slides, and the slides are returned to the moist chamber for 15 to 30 minutes.

10. Rinse the slides with freshly prepared C-BB and transfer to slide staining dishes filled with C-BB for 10 minutes.

11. The slides are blower dried again, and one drop of FA mountant is placed on each well. Cover slips of 22 x 22 mm and 22 x 50 mm are used to completely cover the 25 x 75 mm slides. The slips are pressed down, butted, and small drops of cosmetic nail polish are used on corners to prevent the cover slips from moving or used to seal the entire edge. The lacquer is allowed to dry, and the slides are stored at 4°C until they are read.

c. EXAMINATION OF IFAT-BKD SLIDE MATERIAL.

Generally, slides are read within 24 to 48 hours of mounting, although no problems have been noted with BKD fluorescence for test storage periods of mounted material in excess of 30 days at 4°C, provided that the entire slide is sealed with lacquer.

The microscope used for reading the IFAT-BKD slides is a Zeiss with an pie-illuminator (UV), 12.5x oculars, and a 100x planachromatic lens with a numerical aperature of 1.25. Zeiss low fluorescence immersion oil is used.

The control slide is scanned first to ensure that all of the procedures went normally, and the intensity of fluorescence in the BKD control is subjectively noted. Generally, there has never been a problem with the control slides using this procedure.

We examined approximately 250 microscope fields (mfs) of anterior and posterior kidney in the slides from the first groups of fish. However, we noted that if any BKD organisms were going to be encountered, it would probably be within the first 100 mfs. Therefore, we felt that we could safely reduce the field scanning to 150 mfs, and all slides are now examined in this manner. Currently, we are also counting the number of BKD organisms/l50 mfs's in an attempt to determine the relative intensity of infection.

The only serious BKD-RAS cross-reactivity that was occasionally encountered was a bacillus that was easily recognized by its large size (2 to 3 times that of BKD) and perfect shape.

APPENDIX D

ANALYTICAL PROCEDURE FOR MEASURING GILL NA^+-K^+ ATPASE ACTIVITY

APPENDIX D. Analytical Procedure for Measuring Gill Na^+-K^+ ATPase activity.

ENZYME PREPARATION

For ATPase determinations, samples of gill filaments were thawed and briefly homogenized in a conical glass homogenizer (7 to 12 strokes). The homogenizer was reused with 1 ml SEI which was then added to the original homogenate. The combined value (2 ml total) was placed in ice water in a centrifuge tube. To each 2 ml volume of homogenate was added 2 ml distilled water and the contents were mixed. The homogenates were centrifuged (room temperature) at approximately 3,900 rpm (2,000 RCF) for 5 minutes (some homogenates from chinook salmon required 7 minutes), after which the supernatant liquid was decanted and discarded. The pellet was suspended in 0.5-0.8 ml (depending on size) of a solution containing 0.3M sucrose, 0.02M EDTA, 0.1M imidazole, and 0.1% deoxycholate, pH 7.0 (room temperature) by thorough homogenization in a conical glass homogenizer (30 strokes). The suspension was centrifuged (room temperature) at 3,900 rpm (2,000 RCF) for 5 minutes. An appropriate volume of the resulting supernatant liquid was withdrawn, placed in a small test tube on ice, and then used as the enzyme preparation.

ATPASE DETERMINATION

Na^+-K^+ ATPase activity was calculated as the difference between activities observed in the presence and in the absence of ouabain. One set of reaction mixtures (0.65 ml in each test tube) consisted of (mM) $MgCl_2$, 23; NaCl, 115; CKl, 75; imidazole, 115; and ouabain, 0.58; adjusted to pH 7.0. A second set contained the same volume and reagents without ouabain.

Appropriate reagent blanks were included. All tubes containing the above solutions were placed in an ice water bath. Enzyme preparation (10 ml) was added to all reagent tubes followed by 0.1 ml of 0.03M Na_2ATP (adjusted to pH 7.0). The rack of tubes was then withdrawn from the ice bath, shaken, and placed in a constant temperature, circulating water bath at 37°C for 10 minutes. After the incubation period, the rack was again immersed in the ice bath. After reaction mixtures had thoroughly chilled, 0.1 ml 17.5% HC104 was quickly added to each tube, followed by 1.75 ml deionized water (room temperature) and 3.0 ml 2-octanol. All tubes were then placed in a specially constructed wooden rack to facilitate extraction. Without delay, 0.25 ml ammonium molybdate reagent was dispensed into each tube. The tubes were then covered with a sheet of plastic film. A wooden lid containing plastic foam was placed atop the plastic film (foam in contact with plastic film) such that pressure applied to the lid formed a seal over each tube. The rack of tubes was then shaken vigorously for 30 seconds to extract the phosphomolybdate complex into the octanol phase. The lid and plastic film were then removed and 0.5 ml citrate'reagent added to each tube. A new sheet of plastic film was placed over the tubes and the extraction process repeated for 30 seconds. Molybdate reagent was prepared by adding 200 ml concentrated HCl to 58.4 g $(NH_4)_6 MO7 O_{24} 4.H_2O$ (ammonium molybdate), dissolving completely, then adding deionized water to 1 liter. The citrate reagent contained 143 g citric acid $(H_2C_6H_5O_7.H_2O)$ per liter, adjusted to pH 2.9 with NaOH (approximately 14 g).

Following the extraction procedure, all tubes were centrifuged briefly to facilitate complete separation of the aqueous and organic phases. The rack of tubes was then placed in a water bath (27°–$28^{o}C$) to maintain constant temperature while determining absorbance. Aliquots of the octanol layer were withdrawn and read in a Beckman DB spectrophotometer at 312 mu (UV) against a reagent blank. Appropriate standards containing known quantities of phosphate were used to determine amounts of phosphate liberated from ATP during the reactions. Molar absorptivity of the phosphomolybdate complex under these conditions is 20,400. Protein concentrations in the enzyme preparations were determioned by the method of Lowry et al. (1951) as modified by Miller (1959), using bovine serum albumin as a standard. Each assay contained 25 ul of the enzyme preparation.

ATPase activities were calculated and expressed as micro-moles (um)ATP hydrolyzed per mg protein per hour. Na^{+}-K^{+} ATPase activity is the value determined in the reaction containing no ouabain minus the value obtained in the reaction with ouabain present.

CZES Coastal Zone and Estuarine Studies

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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Annual Report of Research
Financed by
Bonneville Power Administration
(Contract DE-A179-79BP10682)

and

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National Marine Fisheries Service
Northwest and Alaska Fisheries Center
Coastal Zone and Estuarine Studies Division
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September 1981

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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 nrior 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 Z.--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 6 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 III[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 calorimetric 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, 0. 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/1)								
														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	53	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.

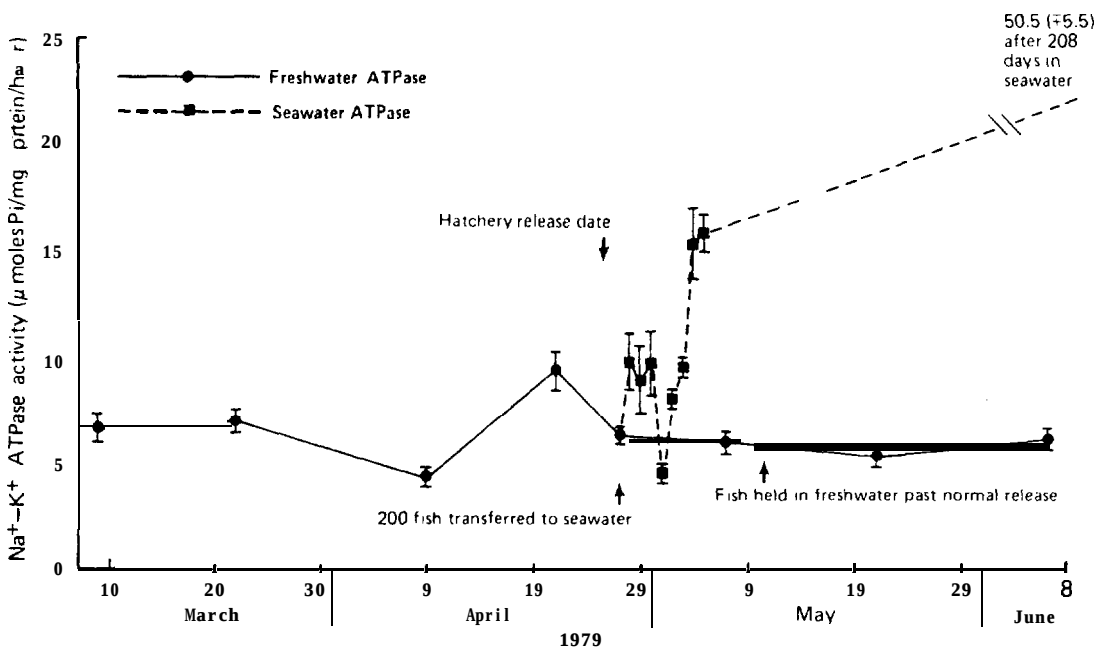

Figure 1.--Gill Na^+-K^+ ATPase activity (means and standard deviations) of the Chelan Hatchery steelhead in fresh water and seawater.

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)
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

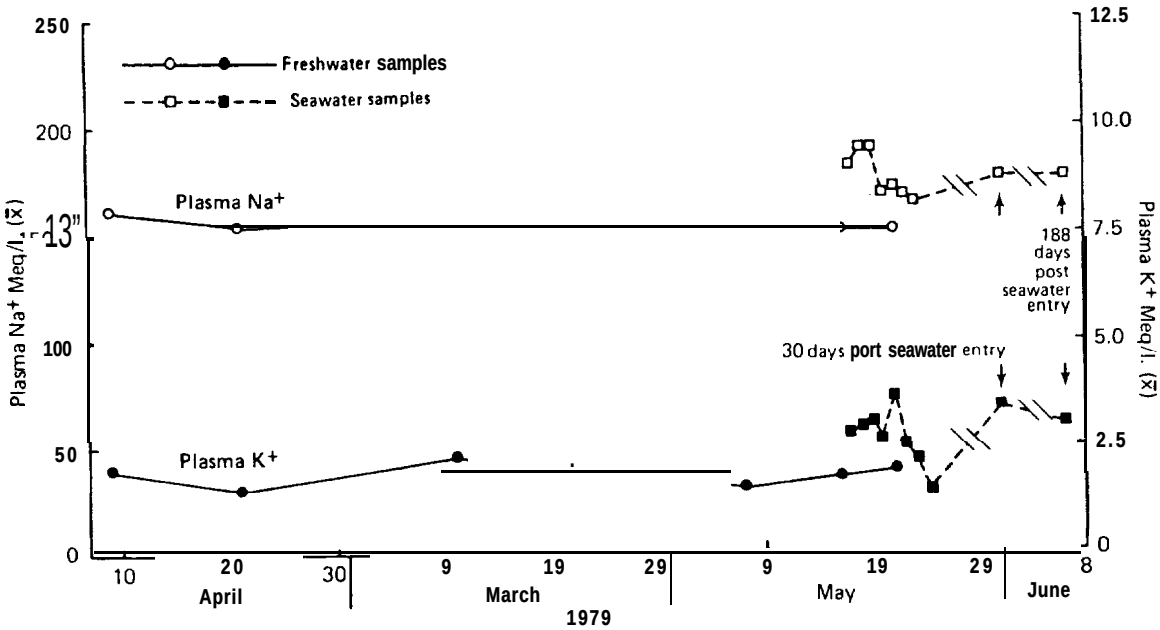

Figure 2.--Mean plasma Na^+ and K^+ levels in Chelan Hatchery steelhead sampled in fresh water at Leavenworth Hatchery and during seawater culture at Manchester.

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	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)	X = 35.6 (28 to 44)		Newcomb (1978)
Laboratory tests (Spring)	$\bar{X}$ = 31 to 37.8 Individual range:	28 to 45	

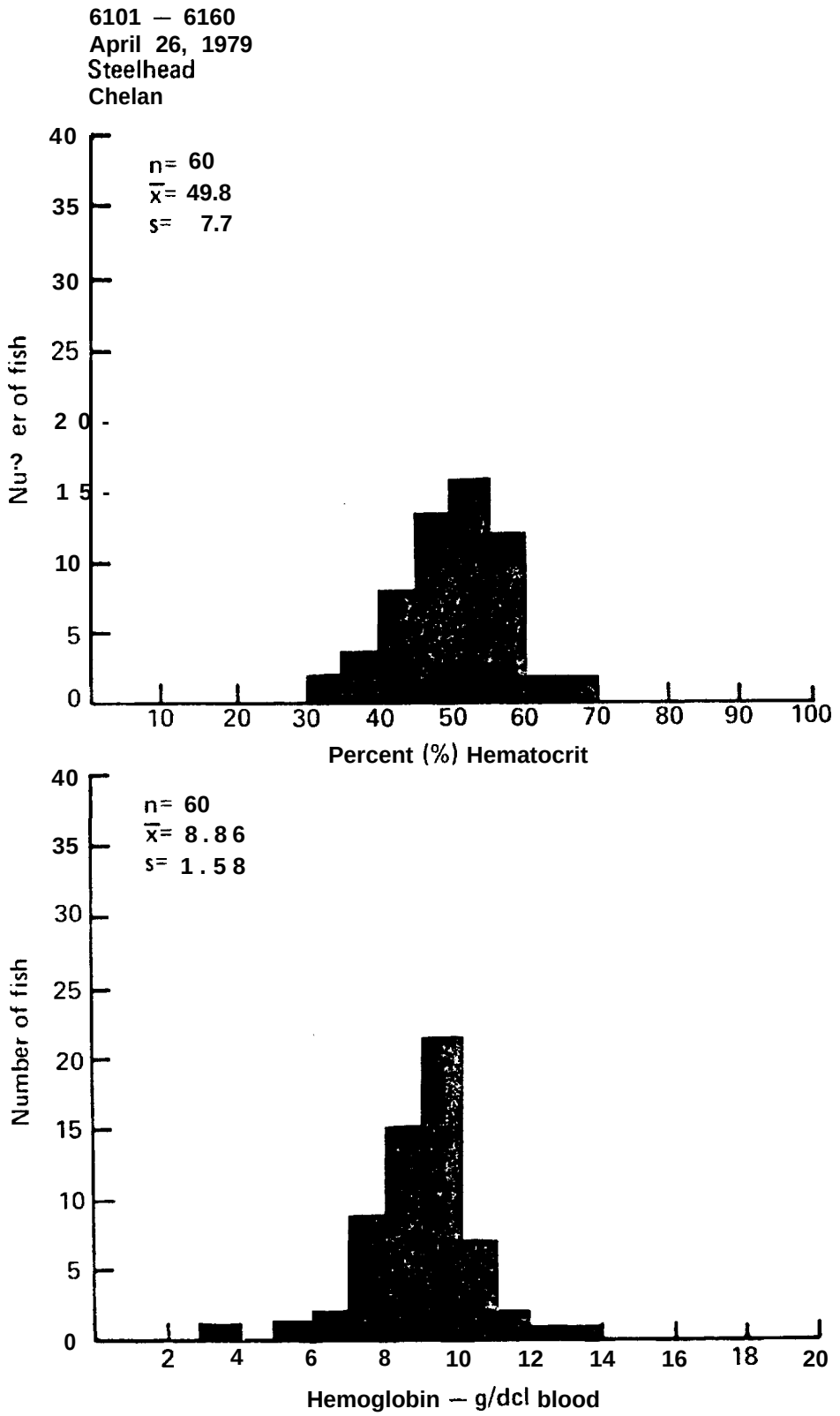

Figure 3.--Frequency histogram for hematocrit and hemoglobin values for the Chelan Hatchery steelhead in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviation (s) are also given.

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	.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	8.3	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 cellular infiltration	0	0	0	0	0	0	0	0	5.0	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	.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: N (start) and date	Survival (%) 30 day post entry	N (end) and date	Days in seawater	% survival to end	% mortality per day	Causes of mortality during seawater culture: 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	94.0
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	61 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/l) was below the expected value of 111 meg/l for healthy rainbow trout. In 1979, none of the chloride values ($\bar{x}$ = 133 meq/l) 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.

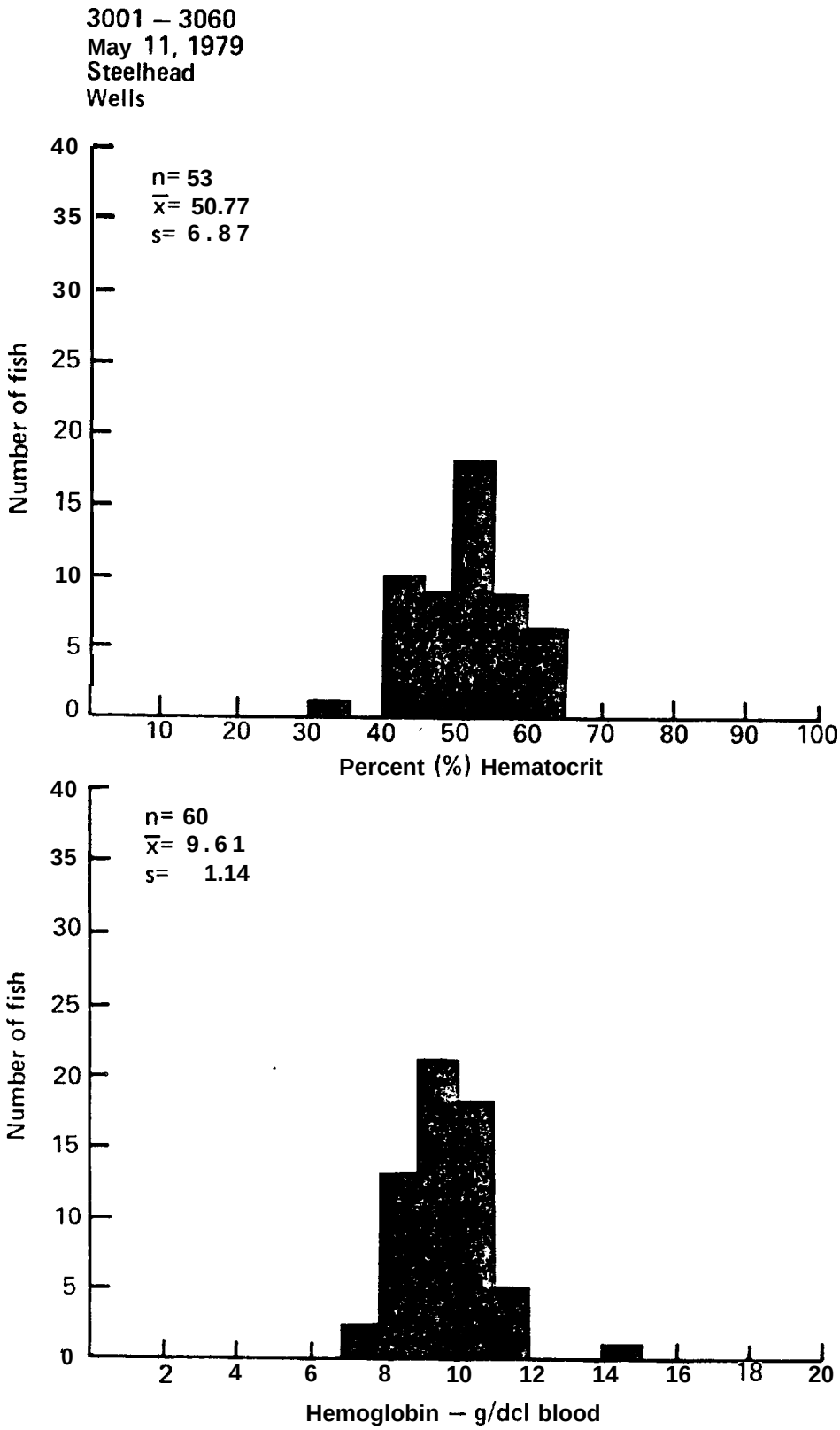

Figure 4.--Frequency histogram for hematocrit and hemoglobin values for the Wells (Winthrop) Hatchery steelhead in 1979. Number of fish samnled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviations (s) are also given.

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

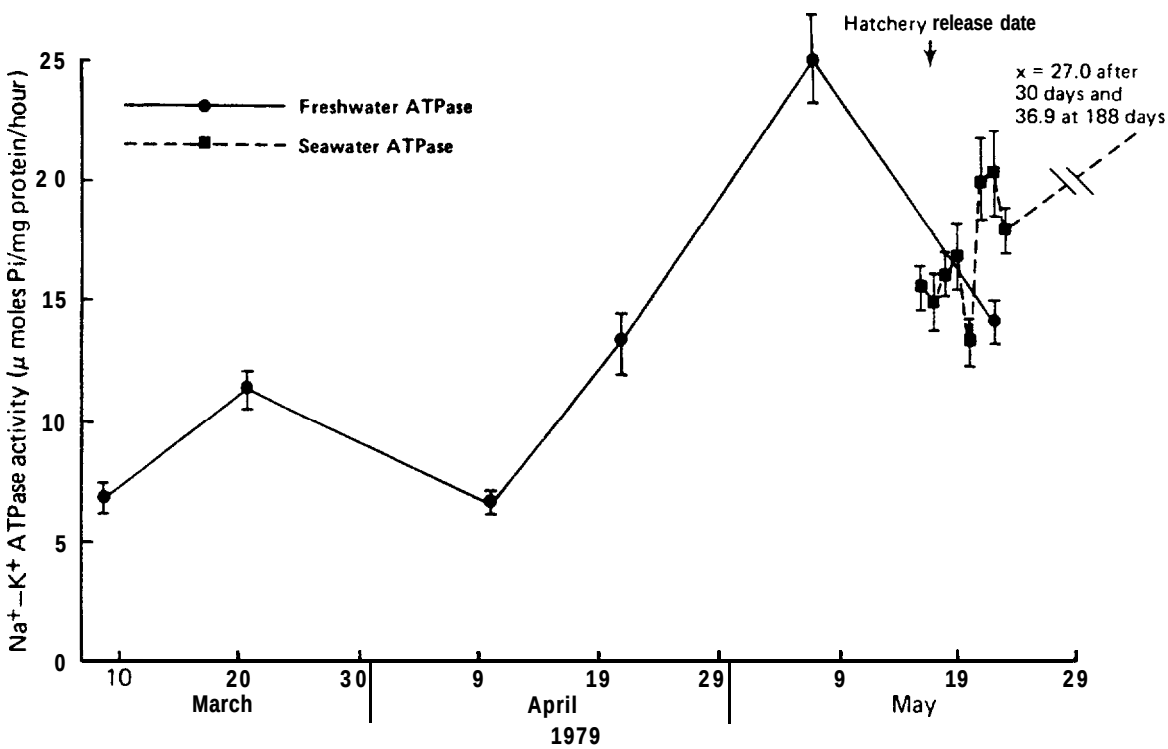

Figure 5.--Gill Na^+-K^+ ATPase activity (means and standard deviations) of the Tucannon Hatchery steelhead in fresh water and seawater.

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

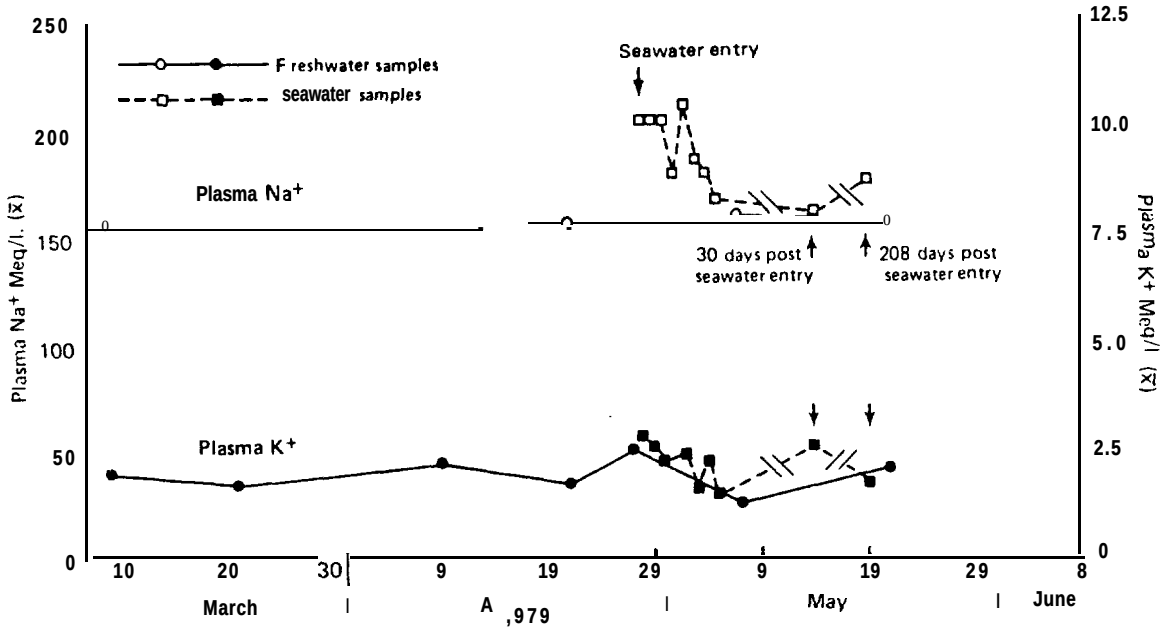

Figure 6. --Mean plasma Na^+-K^+ levels in Tucannon Hatchery steelhead sampled in fresh water at Tucannon Hatchery and during seawater culture at Manchester.

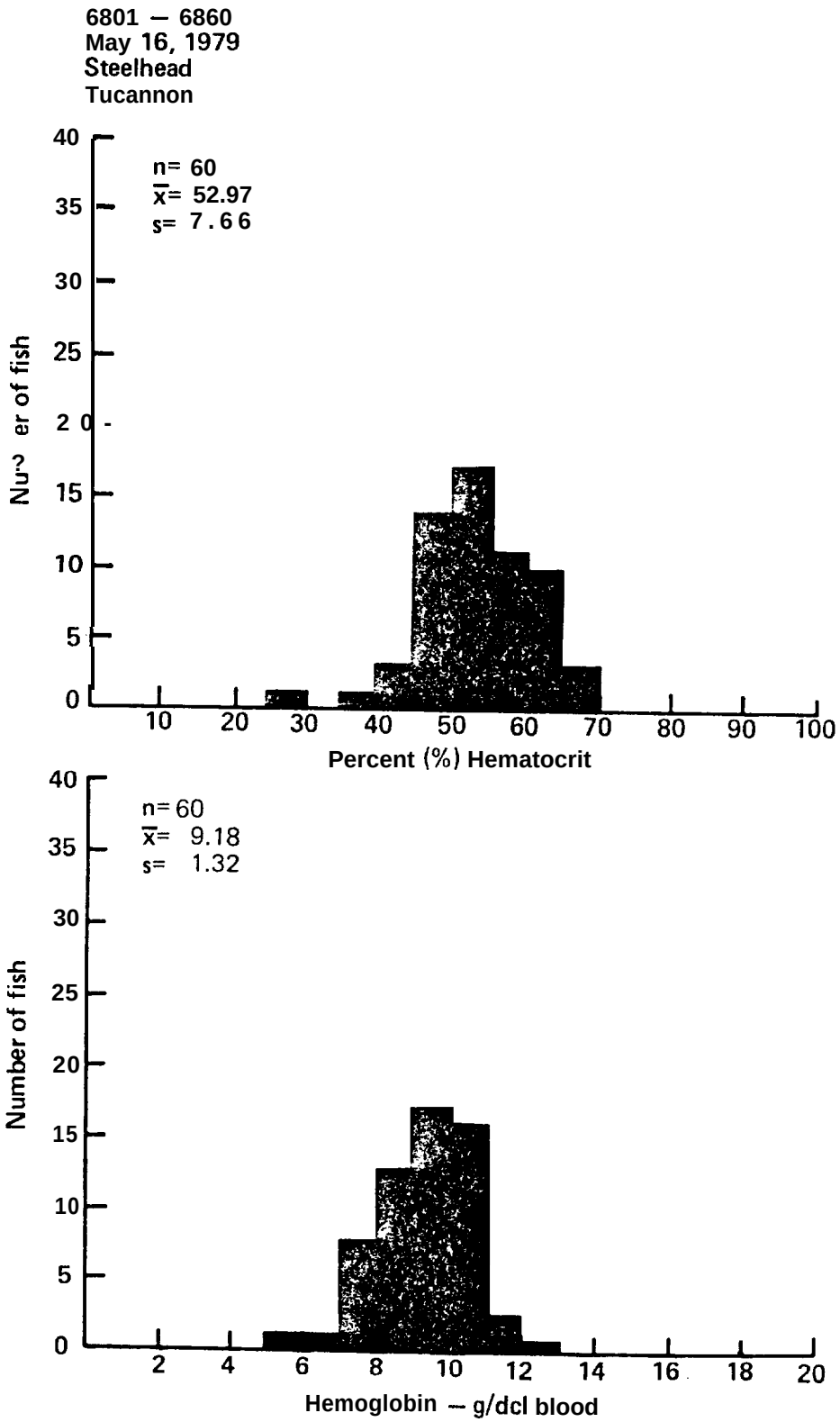

Figure 7.--Frequency histogram for hematocrit and hemoglobin values for the Tucannon Hatchery steelhead in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviations (s) are also given.

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

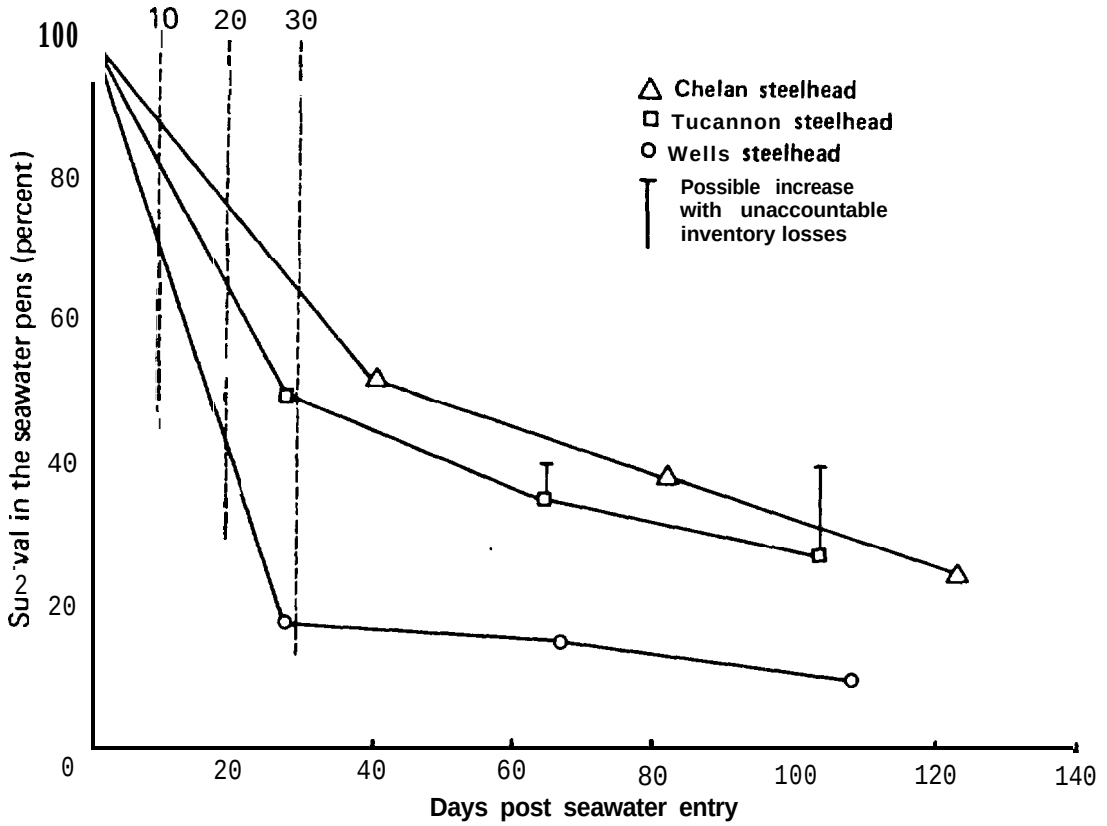

Figure 8.--A comparison of survival during seawater culture of the three steelhead stocks tested in 1979.

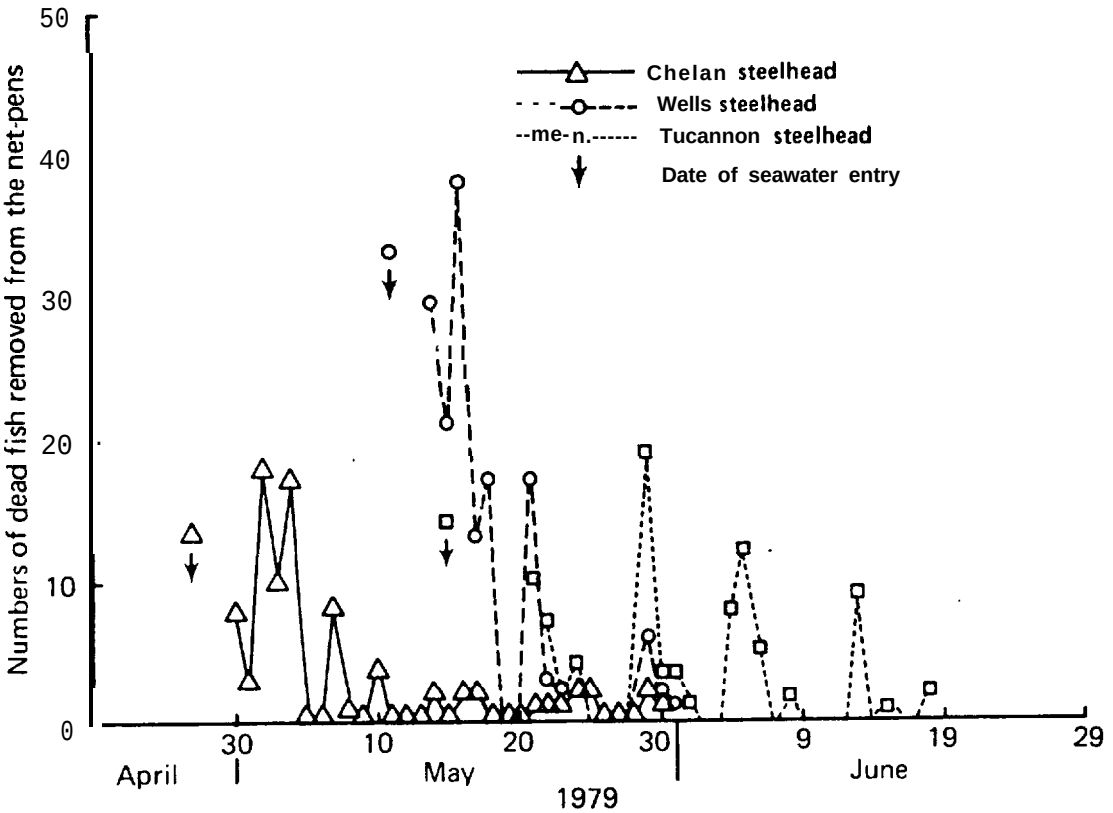

Figure 9.--A comparison of the known daily mortalities (removed) of the three stocks of steelhead after transfer to the seawater pens at Manchester. Each stock began with 200 fish. Mortalities are shown for (approximately) the first 30 days.

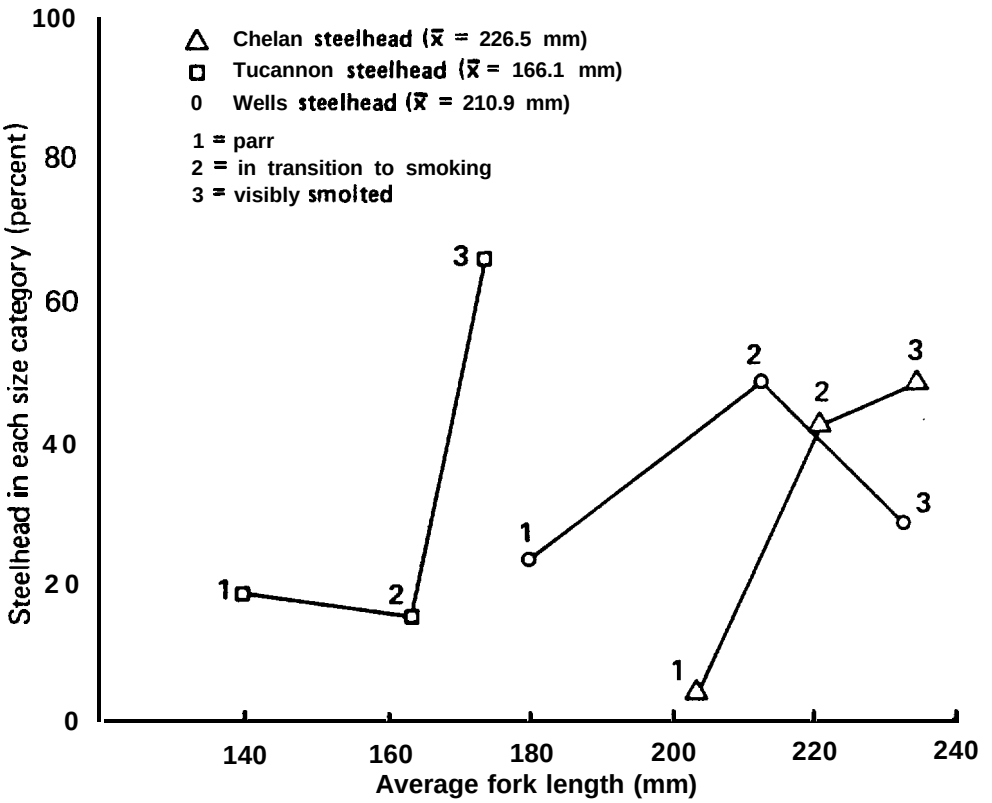

Figure 10.--Relationship of the percentage of abundance of the three visible physiological stages at the time of release to the average size of each state (for all three steelhead stocks studied in 1979).

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 $28^{o}/oo$ 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.

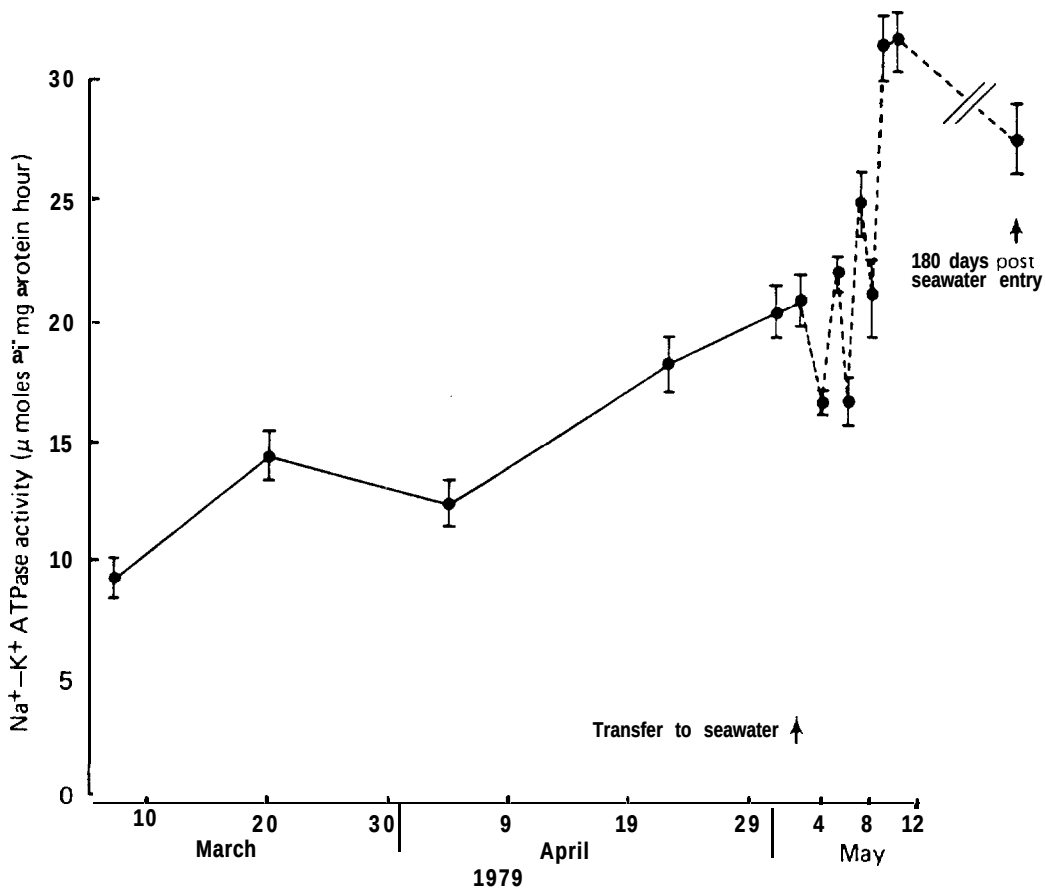

Figure 11. --Gill Na^+-K^+ ATPase activity (means and standard deviations) of Carson Hatchery spring chinook salmon in fresh water, and after transfer to seawater pens at Manchester.

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.

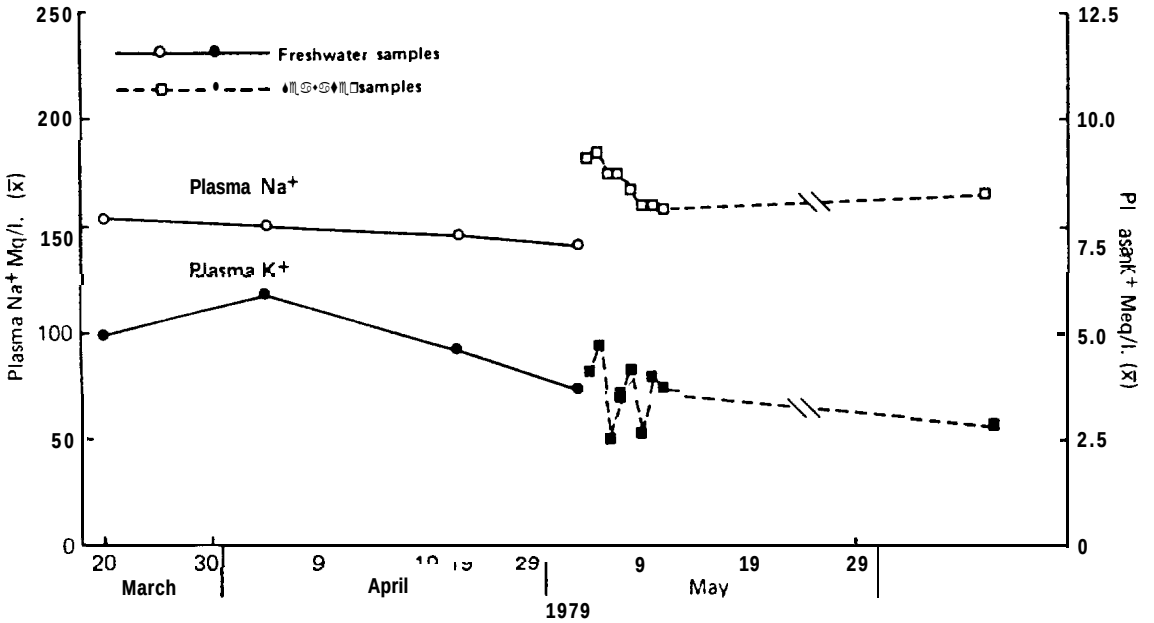

Figure 12.--Mean plasma Na^+ and K^+ levels in Carson Hatchery spring chinook salmon in fresh water at Carson Hatchery and during seawater culture at Manchester.

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

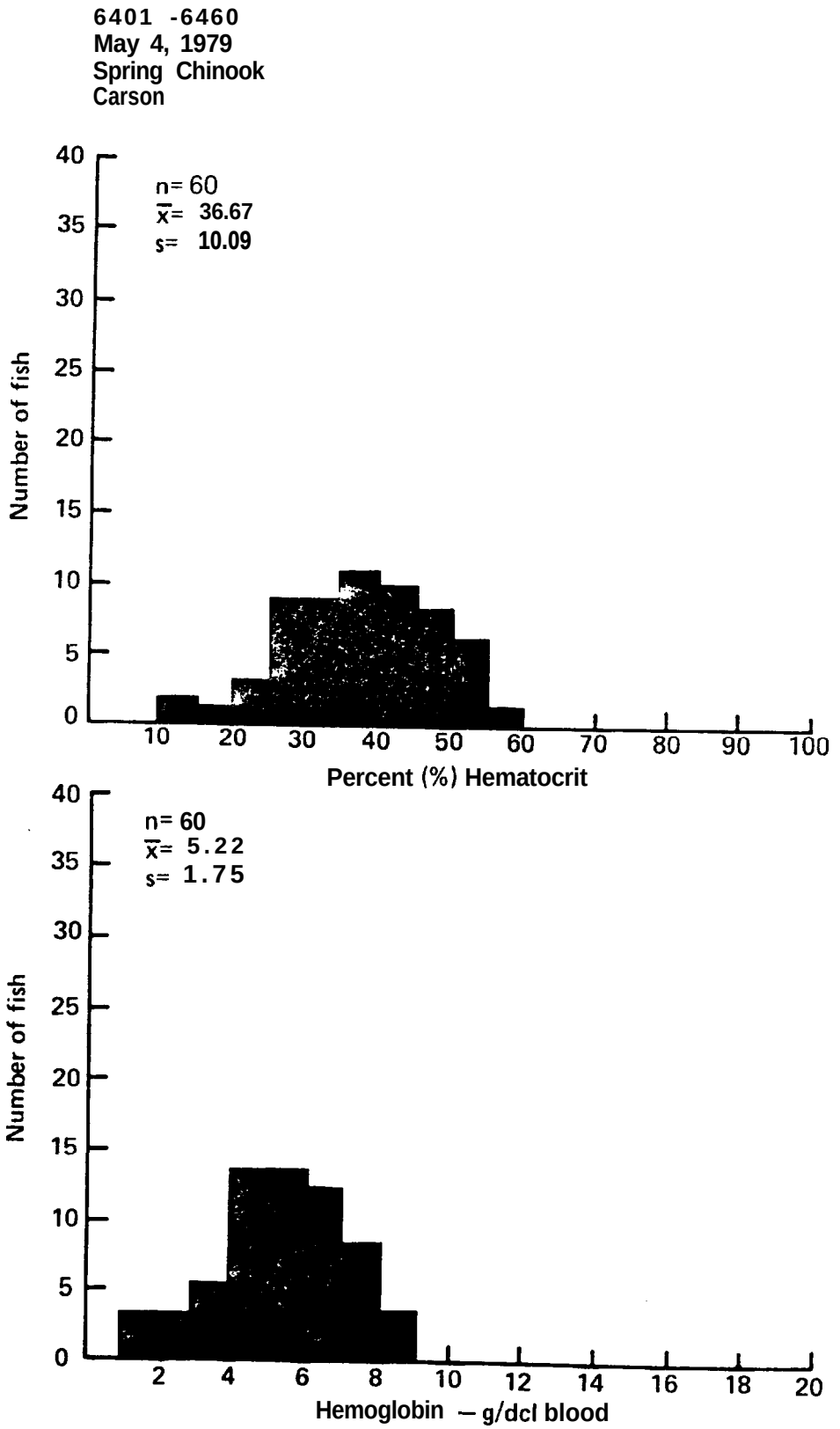

Figure 13.--Frequency histogram for hematocrit and hemoglobin values for the Carson Hatchery spring chinook salmon in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviations (s) are also given.

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 eummer, 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

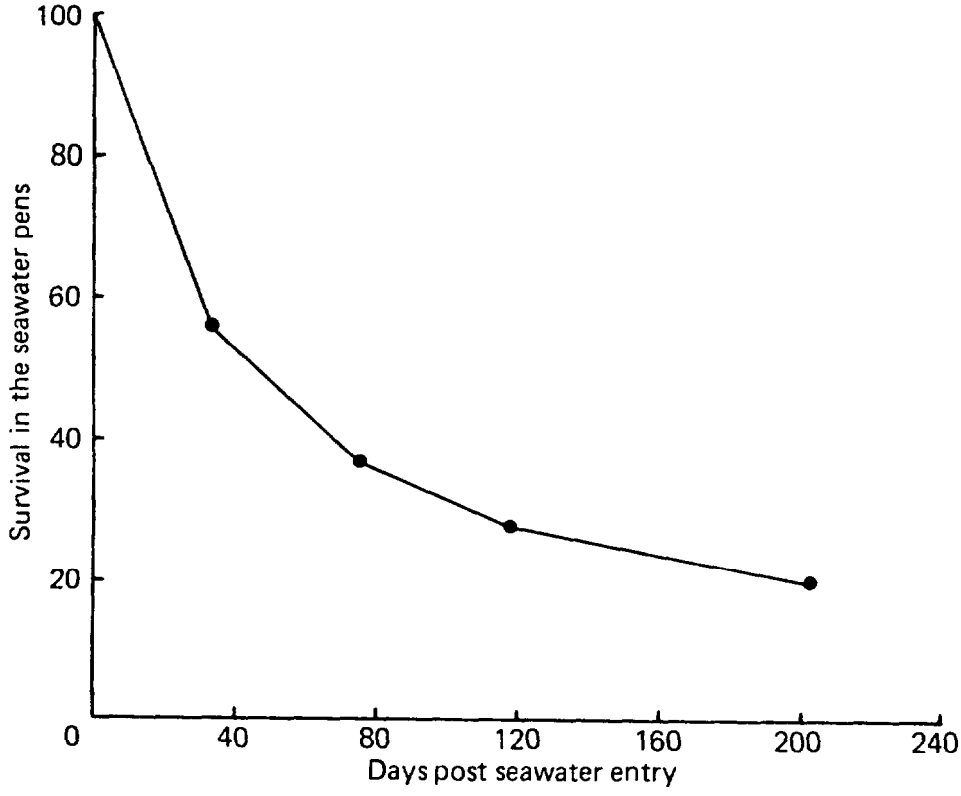

Figure 14.--Survival of the Carson Hatchery spring chinook salmon during seawater culture.

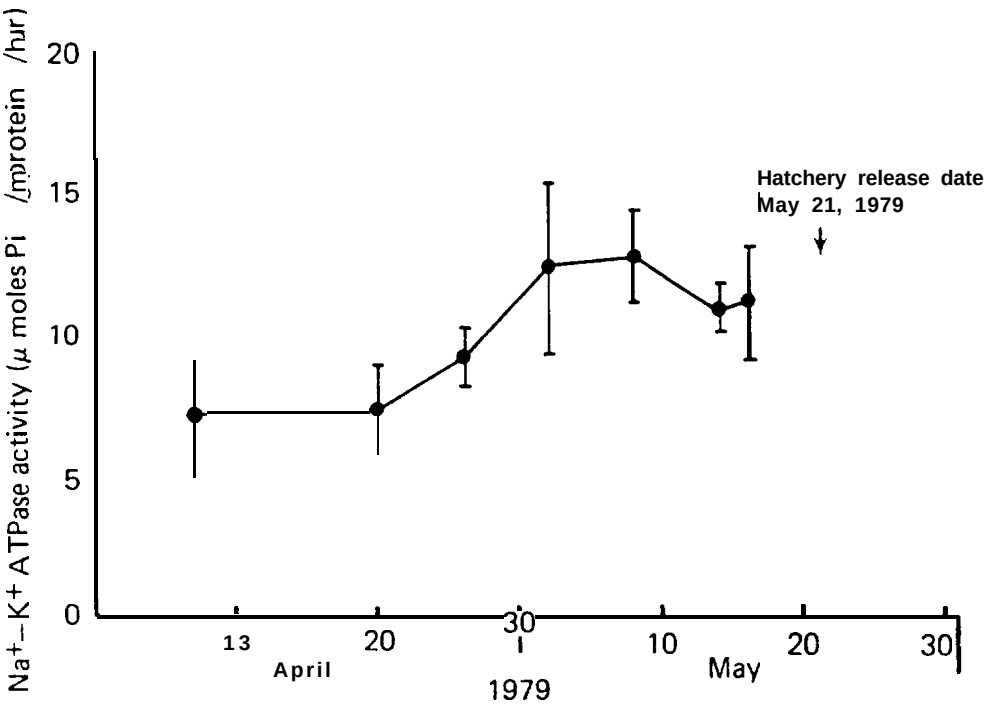

Figure 15.--Gill Na^+-K^+ ATPase activity (means and standard deviations) of Big White Salmon Hatchery fall chinook salmon reared at Spring Creek National Hatchery.

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%m 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.

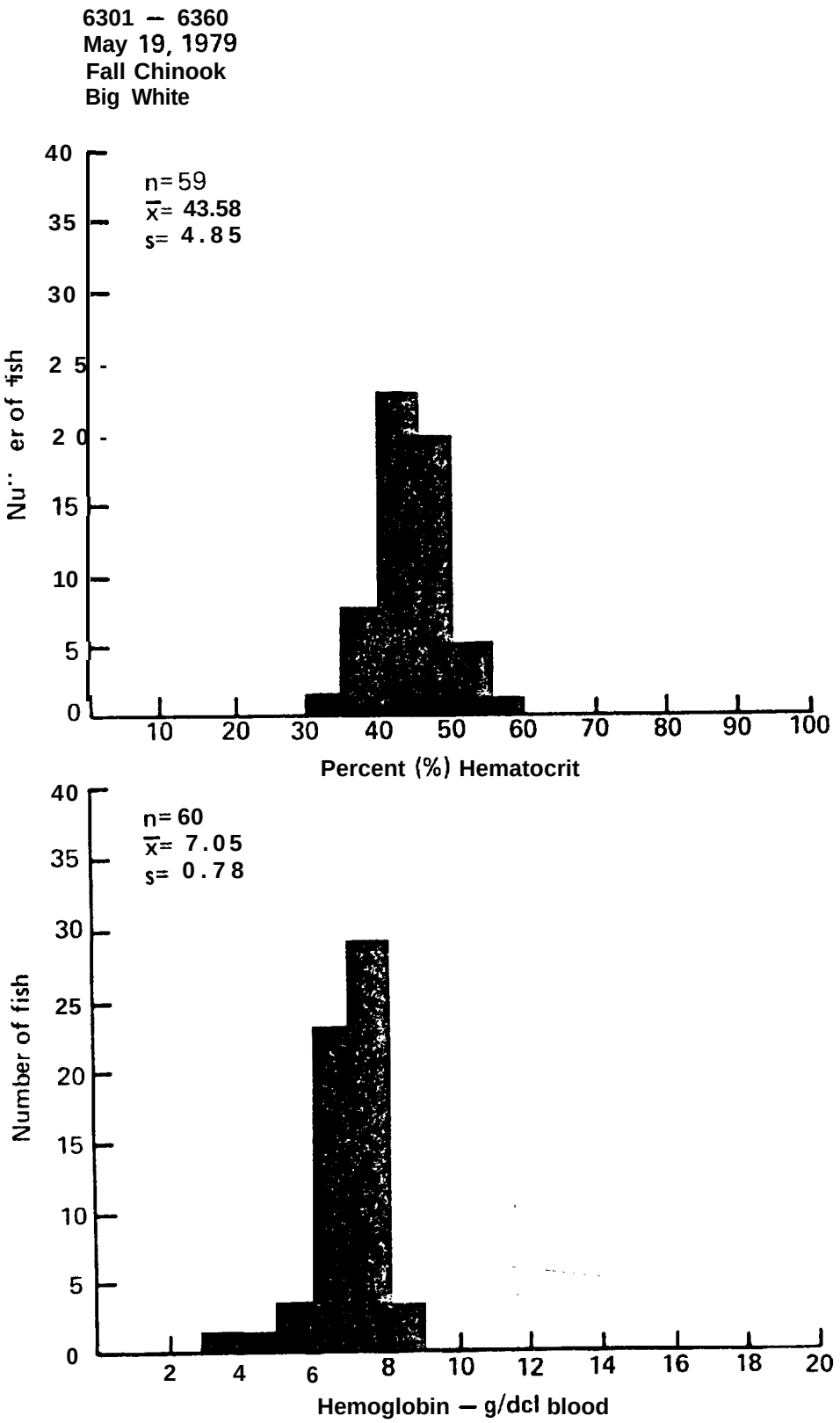

Figure 16.--Frequency histogram for hematocrit and hemoglobin values for the Big White Salmon Hatchery fall chinook salmon in 1979. Number of fish sampled (n), mean hemoglobin and hematocrit values ($\bar{X}$), and standard deviations (s) are also given.

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.

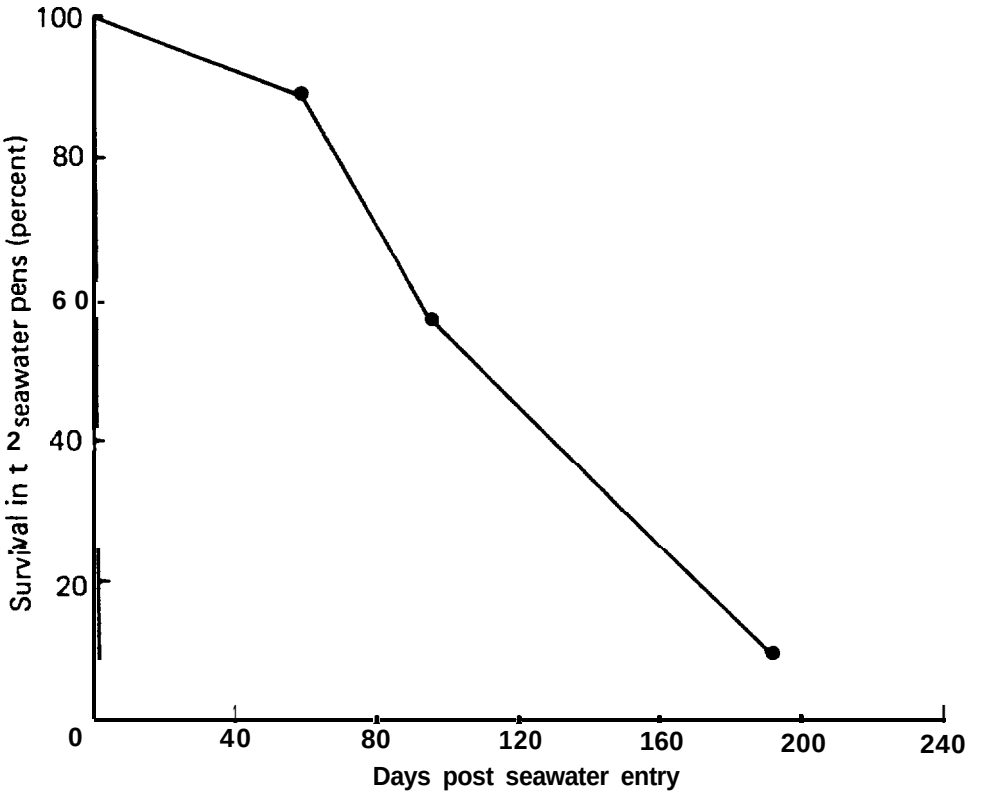

Figure 17.--Survival of the Big White Salmon Hatchery fall chinook salmon during seawater culture.

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 IT 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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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 wasableto 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 personnelaccordingto 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.

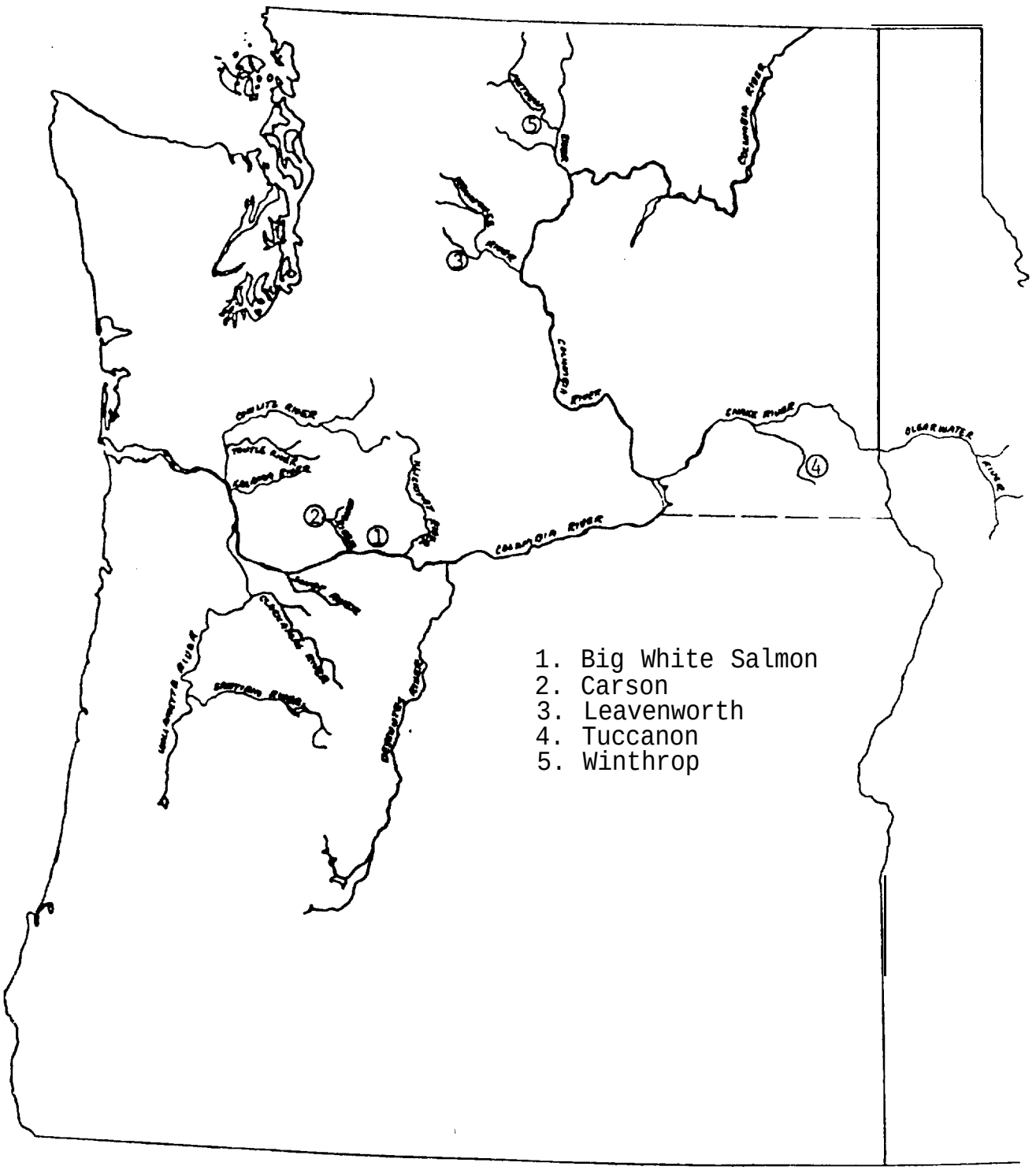

TABLE 1. STOCKS OF PACIFIC SALMON SMOLT EXAMINED FOR VIRUS AT THE TIME OF RELEASE FROM SELECTED COLUMBIA RIVER DRAINAGE HATCHERIES DURING 1979.

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. 'Iwo 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 8iOS 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 *Herpesvirussalmonis* were found during the course of 'his 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 yearclassof 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 yearclassstocks 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 oroccurrenceof 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 theoccurrenceof 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.

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VII. APPENDIX A

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

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 careful1 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

VII. APPENDIX A

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: _____

VII. APPENDIX B

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

VII. APPENDIX C

( ) 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 Lacto

Prep. Incubation: ____ °C _____ hours Inoculation Date: ___/___/___ Time: ____

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. Temp

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.l) 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.l & 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 healthscreen 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.

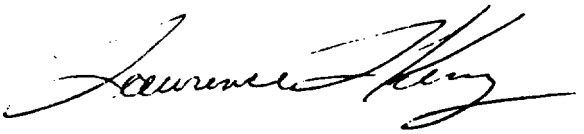
Periorbital striated muscle degeneration is present in the Chelan Steelhead and the Carson Spring Chinook. The Chelan Steelhead lesions may be a nutritional myopathy; however, the Carson Spring Chinook lesions appear to be secondary to a periorbital bacteria inflammation.

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,



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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
0	Inflammation of the olfactory lobe (acute suppurative)
PT	Perifollicular thyroiditis
SLT	Secondary lamellae (vascular) telangiectasis

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	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY 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	

TOTAL INCIDENCE OF LESIONS IN FISH 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 Uretheritis = 1.7%
+	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	
++	1.7	0	ROMI6.7	0	6.7		0	0	0		0	0	0	0 = 1.7		E = 3.9	Focal tubular Degeneration = 1.7% with giant gacteria =1.7%
+++	0	0		0	0		0	0	0		0	0	0	AH = 1.7			
				Focal mixed inflam. base of lamellae = 1.7%						FM24.1							
	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 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 CP = 32 NMI = 1 SP in pharyngeal epithelium.
NPSL = 1 SEM = 2 SP = 1 NP = 2
PO = 1

TOTAL INCIDENCE OF LESIONS IN TISSUE EXAMINED (C)

Chelan Steelhead

	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			OLFACTORY	THYROID	BRAIN	
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Ca				
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 (%)

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

Carson Spring Chinook

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	OLFAC	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	29	19	28	29	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							

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	
211	-	-	-	+	++		-	-	-		-	-	-		NA	NA	
212	-	-	-	+	+		-	-	+		-	+	-	PO	-	-	
213	-	-	-	+	+		-	-	-		-	-	-	AH*			*Focal hemorrhage (trauma)
214	-	-	-	+	++		-	-	-		-	-	-		NA		
215	-	-	-	+	+		-	-	-		-	-	-				
216	-	-	ROMI	+	+		-	-	-		-	-	-	PO	NA		
217	+	+	ROPG	+	+		-	-	+			NA		PO	NA	NA	
218	-	-	-	+	+		-	-	-		-	-	-	O	-	NA	
219	-	-	-	+	++				NA		-	-	-	-	-	NA	
220	-	-	-	+	+		-	-	-		-	-	-				
221	-	-	-	+	•				-		-	-	-	PO	-	-	
222	-	-	-	+	+		-	-	-		+*	-	-		NA	-	*Pyogranulomatous nephritis
223	+	+	ROPG	+	+		-	-	-		-	-	-	PO			
224	-	-	-	+	+	-	-	-	+*	-	-	-	-		NA		*Multifocal hepatic micro granulomas
225	+	+	ROPG	+	+	-	-	-	+*	-	-	**	-	-	NA	-	*Capsular paraganglioma. **Pyogranulomatous periuteritis.
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 giant 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																	
	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

Wells Steelhead

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
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	-	-	-	-	+	-	-	-	-	-	-	-	-	O	-	-
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	NA	
289	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
290	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
291	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
292	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
293	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
294	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
295	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
296	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
297	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
298	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	
299	-	-	-	+	+	-	-	-	-	-	-	-	-	-	NA	-	
300	-	-	-	+	+	-	-	-	-	-	-	-	-	-			
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	Mucopurulent 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			

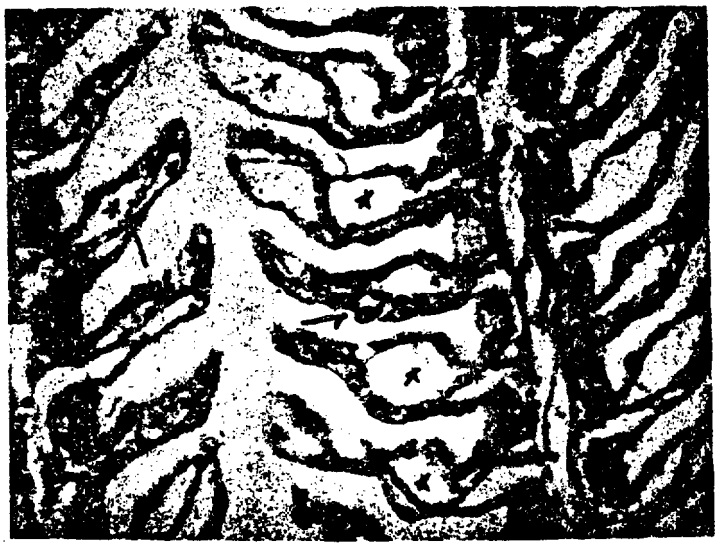

Fig. l-Lymphatic telangiectasis (*) and a solitary eosinophilic mass of the secondary lamellae (arrow).

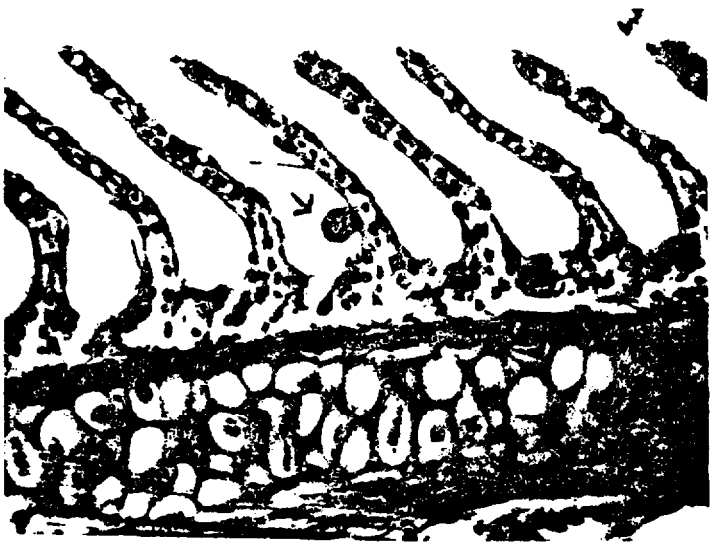

Fig. Z-Solitary basophilic mass in the secondary lamellae of the gill.

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

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

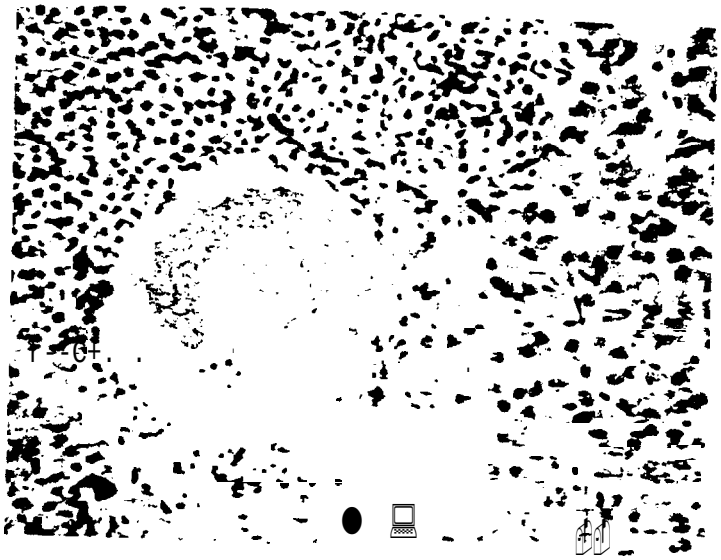

Fig. 5-Hepatic sporazoan parasitic granuloma.

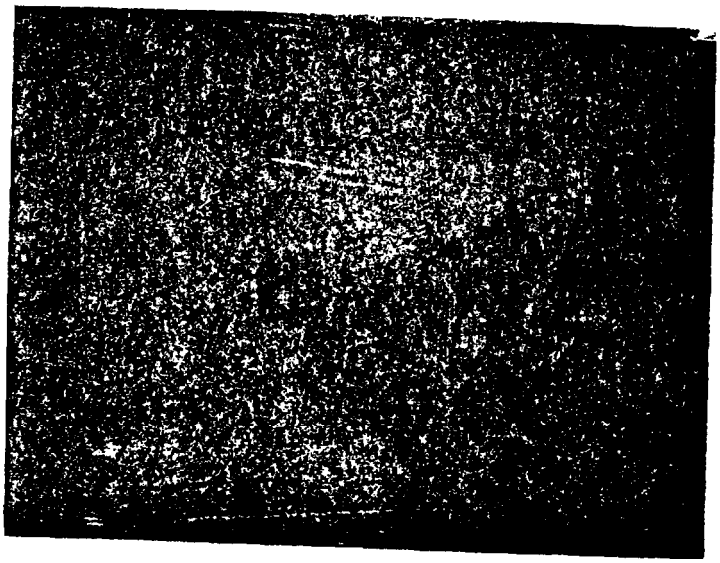

Fig. 6-Ciliated protozoan parasite in the olfactory lobe.

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

Study of Disease and Physiology in the 1980 Homing Study Hatchery Stocks– A Supplement to: "Imprinting Salmon and Steelhead Trout for Homing, 1980 by Slatick, Gilbreath, and Walch"

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Annual Report of Research
Financed by
Bonneville Power Administration
(Contract DE-A179-80BP18236)

and

Coastal Zone and Estuarine Studies Division
Northwest and Alaska Fisheries Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, Washington 98112

September 1984

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INTRODUCTION

The National Marine Fisheries Service (NMFS), under contract to the Bonneville Power Administration, has conducted research on imprinting salmon and steelhead for homing (Slatick et al. 1979, 1980; Novotny and Zaugg 1979, 1981). This supplement completes a 3-year study to determine the status of fish health and certain physiological functions that might affect imprinting and homing in salmonids. The field work was begun by the authors in 1978 and concluded in 1980.

In 1980, we examined random samples of normal populations of homing test fish at the hatcheries at frequent intervals to determine their general health and physiological readiness to migrate, but did not conduct seawater challenge tests as in previous years. In 1978 and 1979, we were unable to sample at frequent intervals, but we did determine the survival of samples of test fish maintained in marine net-pens after release from the hatcheries. The number of hatcheries and stocks involved in the 1980 study was about double that of 1978 or 1979 (Table 1).

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-smolting 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; higher 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 stock sampled in the 1980 homing studies.

Hatchery	Species	Date sampled	Pond no.	Date released	Histopathology tag no.
Dworshak	Steelhead	03/04/80	59	04/17/80	7061 to 7120
Dworshak	Steelhead	03/18/80	61		-- --
Dworshak	Steelhead	04/01/80	61		73 to 7420
Dworshak	Steelhead	04/15/80	61	04/17/80	-- --
Dworshak	Steelhead	04/29/80	--	(Held over in the	7661 to 7720
Dworshak	Steelhead	05/13/80	--	hatchery for post	-- --
Dworshak	Steelhead	06/04/80	--	release sampling)	-- --
Tucannon	Steelhead	03/07/80	01		-
Tucannon	Steelhead	03/07/80	04		-- --
Tucannon	Steelhead	03/07/80	02		-- --
Tucannon	Steelhead	03/21/80	01		-- --
Tucannon	Steelhead	03/21/80	04		-- --
Tucannon	Steelhead	03/21/80	02		-- --
Tucannon	Steelhead	04/04/80	01		7541 to 7560
Tucannon	Steelhead	04/04/80	04		7561 to 7580
Tucannon	Steelhead	04/04/80	02		7581 to 7600
Tucannon	Steelhead	04/10/80	01	04/08/80	-- --
Tucannon	Steelhead	04/10/80	02		-- --
Tucannon	Steelhead	04/10/80	04		-- --
Tucannon	Steelhead	04/18/80	04		-- --
Tucannon	Steelhead	04/18/80	02		--
Tucannon	Steelhead	05/02/80	04		-- --
Tucannon	Steelhead	05/02/80	02		-- --

Table 1.--Continued

Hatchery	Species	Date sampled	Pond no.	Date released	Histopathology tag no.
Tucannon	Steelhead	05/07/80	04	05/08/80	7781 to 7810
Tucannon	Steelhead	05/07/80	02		7811 to 7840
Tucannon	Steelhead	05/16/80	02		-- --
Tucannon	Steelhead	05/30/80	02		-- --
Tucannon	Steelhead	06/06/80	02		-- --
Tucannon	Steelhead	06/12/80	02	06/12/80	7841 to 7900
Leavenworth	Spring chinook	11/01/79	46	Fall marked fish' (sampled in fall)	-- --
Leavenworth	Spring chinook	11/14/79	06	Spring marked fish (sampled in fall)	-- --
Leavenworth	Spring chinook	03/03/80	17		7001 to 7060
Leavenworth	Spring chinook	03/17/80	17		-- --
Leavenworth	Spring chinook	03/31/80	03	04/24-05/01/80	7301 to 7360
Leavenworth	Spring chinook	04/14/80	17		--
Leavenworth	Spring chinook	04/23/80	CH	04/24-05/01/80 (Released in channel)	-- --
Leavenworth	Spring chinook	04/23/80	46	04/24-05/01/80	-- --
Leavenworth	Spring chinook	04/23/80	17	04/24-05/01/80	-- --
Leavenworth	Spring chinook	04/23/80	CL	04/24-05/01/80 (Control held in channel 48 h)	-- --
Leavenworth	Spring chinook	04/28/80	15	(Held over for	7601 to 7660
Leavenworth	Spring chinook	05/12/80	15	post-release	--
Leavenworth	Spring chinook	05/29/80	15	sampling)	-- --
Leavenworth	Spring chinook	06/17/80	15		-- --
Kooskia	Spring chinook	03/05/80	10		7121 to 7180
Kooskia	Spring chinook	03/19/80	10		-- --
Kooskia	Spring chinook	04/02/80	10	04/16/80	7421 to 7480
Kooskia	Spring chinook	04/16/80	07	04/23-05/05/80	-- --

Table I.--Continued

Hatchery	Species	Date sampled	Pond no.	Date released	Histopathology tag no.
Kooskia	Spring chinook	04/30/80	07	(Held over for	7721 to 7780
Tucannon	Steelhead	05/07/80	07	post-release	-- --
Kooskia	Spring chinook	06/05/80	07	sampling)	-- --
Rapid River	Spring chinook	03/06/80	01		7181 to 7240
Rapid River	Spring chinook	03/20/80	01		-- --
Rapid River	Spring chinook	04/03/80	01		7481 to 7540
Rapid River	Spring chinook	04/17/80	01	04/15/80	-- --
Rapid River	Spring chinook	05/01/80	01	(Held over in	-- --
Rapid River	Spring chinook	05/15/80	01	outside tanks for post-release sampling)	-- --
Carson	Spring chinook	12/13/79	--		--
Carson	Spring chinook	03/04/80	31		8051 to 8108
Carson	Spring chinook	04/04/80	31		8289 to 8349
Carson	Spring chinook	04/17/80	31		8501 to 8560
Carson	Spring chinook	05/12/80	31	05/12-05/15/80	8861 to 8920
Willard	Coho	12/12/79	--	Fall marked group	-- --
Willard	Coho	03/05/80	25		8109 to 8168
Willard	Coho	04/02/80	25		8229 to 8288
Willard	Coho	05/20/80	50	05/14-05/25/80	8661 to 8782
Spring Creek	Fall chinook	03/10/80	20		8169 to 8228
Spring Creek	Fall chinook	04/10/80	35		8351 to 8400
Spring Creek	Fall chinook	04/10/80	35		-- to --
Spring Creek	Fall chinook	05/08/80	22	05/09-05/19/80	8801 to 8860
Big Creek	Fall chinook	04/08/80	22		8401 to 8500
Big Creek	Fall chinook	04/23/80	22		8601 to 8660
Big Creek	Fall chinook	05/06/80	22		8701 to 8760

Table 1.--Continued

Hatchery	Species	Date sampled	Pond no.	Date released	Histopathology tag no.
Big Creek	Fall chinook	05/13/80	22	05/12-05/23/80	8921 to 8980
Hagerman	Fall chinook	06/02/80	11	06/03-06/05/80	-- --

Hematocrits and hemoglobins. Values below or above normal ranges usually indicate anemia or dehydration, respectively, which can reflect nutritional status, 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.

Fish were examined for subclinical bacterial kidney disease (BKD) using an indirect fluorescent antibody technique (IFAT). Viral assays were not conducted in 1980.

Histological determinations of significant lesions or abnormalities in tissue from gills, eyes, and olfactory sacs were undertaken.

These surveys were conducted to provide a documentation of the health and physiological (smolt) condition of populations of fish involved in the tests, especially at the time of imprinting and release. When marked adult fish return, data analyzed from the health and physiology surveys should provide us with information that would indicate any adverse influence on survival.

METHODS AND MATERIALS

Sampling Fish in the Hatcheries

The method of sampling fish from 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 has shown that a disease incidence of 5% or greater can be detected with a sample of 60 fish (Ossiander and Wedemeyer 1973). Health survey samples of 60 fish were taken at the hatcheries.

Sampling of fish for health profiles began the first week in March 1980 and ended the second week of June 1980. A mobile laboratory was used to sample upper river hatcheries every 2 weeks. A similar procedure was used by our Willard Laboratory staff at the lower river hatcheries. All of the required samples were collected and completely or partially processed on site.

Each fish and each of the assorted samples taken were assigned a unique number for identification and data processing.

Blood Sample Collection

Fish were Lightly anesthetized in an aerated 1:20,000 solution of MS-222 in groups of 10 to 15 fish. In the Larger fish, blood was sampled from the caudal arch with a l-cc ammonium heparinized syringe and a 25-gauge hypodermic needle. In small fish, blood was collected in ammonium heparinized capillary tubes after severing the caudal peduncle. Subsamples of blood taken for hematocrits (packed cell volume) were collected in microhematocrit tubes and centrifuged for 3 minutes in a Clay-Adams Autocrit II[1/] (Snieszko 1960). Subsamples of blood for hemoglobin determination were either read directly with an A-O hemoglobinometer, or collected in 20 ul capillary tubes and determined colorimetrically (Bauer 1970).

Plasma Electrolytes

Subsamples of blood were centrifuged in plastic serum vials, and the supernatent plasma was drawn off by disposable pipette, dispensed into plastic vials, sealed, and held frozen until they were shipped to a private

---

1/ Reference to a trade name does not imply endorsement by the National Marine Fisheries Service.

laboratory for analysis. Plasma sodium and potassium values were determined by atomic absorption spectrometry and chlorides with a chloridometer. Before analysis, each. plasma sample was ranked into four increasing levels of hemolysis.

Gill Na^+-K^+ ATPase

During 1980, selected stocks of coho, fall, and spring chinook salmon and steelhead trout at state and federal hatcheries in the Columbia River drainage were monitored for changes in gill Na^+-K^+ ATPase to estimate the state of smoltification at release.

At approximately 2-week intervals during the spring and summer of 1980, 30 fish were removed by dip net for Na^+-K^+ ATPase analysis from representative ponds or raceways at hatcheries that participated in the homing studies (see Table 1). Ten groups of three fish each were anesthesized at each sampling. After fork lengths were determined and blood samples taken, approximately equal quantities of gill filaments were removed from gill arches of each of the three fish in the group (total weight of gill filaments, 0.1 to 0.2 g), snap-frozen in Liquid nitrogen, and processed as described by Novotny and Zaugg (1979). Fall chinook salmon from the Hagerman National Fish Hatchery (NFH) were not sampled as extensively as the others, but a gill Na^+-K^+ ATPase profile was obtained.

Life History of Hatchery Juveniles

Husbandry techniques, disease, and environmental history may have deleterious effects on fish health and smolt quality (Wedemeyer et al. 1980; Folmar and Dickhoff 1979, 1981). 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.

Information in Table 2 was obtained from hatchery management and is self-explanatory. Where information was not obtained, the entries have been Left blank.

Histopathology

Sixty individually numbered fish of each test group were fixed in 10% buffered Formalin and submitted to Bio-Med Research Laboratories. Gill, eye, and olfactory tissues were sectioned, appropriately stained, and examined for any pathologic lesions or abnormalities. All Lesions were evaluated as described in Novotny and Zaugg (1979, 1981)[2/].

Bacteriological Assays

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

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

[2/] A copy of the complete veterinary pathologist's report for the 1980 studies can be obtained by written request from the Director, Coastal Zone and Estuarine Studies Division, NWAFC, NMFS, 2725 Montlake Boulevard East, Seattle, Washington 98112.

Table 2.--Available disease and life history data of homing study hatchery juveniles

Hatchery	Stock	Agency[a]	Species	Date of egg take	Date ponded	Feed[b]	Water source
DWORSHAK NATIONAL	Dworshak	USFWS	Steelhead	4/10/79	7/1/79	OMP	System III re-use (conditioned N. Fork Clearwater River)
TUCANNON	Skamania	WDG	Steelhead	March, 1978	---	DRY & OMP	River
LEAVENWORTH NATIONAL	Leavenworth	USFWS	Spring chinook	8/10-8/30, 1978	3/27/79	OMP	Well & river
KOOSKIA NATIONAL	Carson & Kooskia	USFWS	Spring chinook	August, 1978	--	OMP & DRY	Well & river
RAPID RIVER	Rapid River	IDFG	Spring chinook	8/10-9/11, 1978	Raceways 11/27/78, Earthen ponds 5/15/79	OMP	River
CARSON NATIONAL	Carson	USFWS	Spring chinook	8/21-8/28, 1978	1/18/79	OMP & DRY	Springs, creek, river
WILLARD NATIONAL	Little White salmon	USFWS	Coho	1978	--	OMP	Well & river
SPRING CREEK NATIONAL	Spring Creek	USFWS	Fall chinook	9/17-9/29, 1979	12/10- to 12/18/79	OMP & DRY	Springs
BIG CREEK	Big Creek	ODFW	Fall chinook	9/20-10/4, 1979	1/3- to 1/18/80	OMP	Big Creek
HAGEMAN NATIONAL	Snake River	USFWS	Fall chinook	Sept. 1979 (Tucannon Hatchery)	Hagerman	OMP	Spring

[a] WDG--- Washington Department of Game
USFWS- United States Fish & Wildlife Service
ODFW-- Oregon Department of Fish and Wildlife
IDFG-- Idaho Department of Fish & Game

[b] OMP--- Oregon Moist Pellets

[c] BGD-Bacterial Gill Disease
BKD--Bacterial Kidney Disease
ERM--Enteric Red Mouth
ICH--Ichthyophthirius

[d] TM50-Dietary Oxytetracycline

Table 2.--continued

Water temp-°F	Percent mortality (all causes)	Size at release (no/lb)	Date released (1980)	Diseases[c/]	Treatments
50-60 for rearing; 43-48 for conditioning for release	35% before ponding; 5% after ponding.	8.6	4/22-4/30	N2 oupersaturation (early) Whitespot (early)"ich" Epistylis	--
--	--	--	May	Epistylis	Formalin & malachite green
32-63	5.2%	17.6	4/24-4/30	BGD, BKD	--
--	--	--	April	BKD	--
34-60	13% to ponding	15.0	207,000 Sept 1979; 2.6 million March-April 1980.	BGD	3% salt/bath & 1/2 ppm/ malachite/ green dips.
44-51	32%	23.3	4/28-5/16	BKD & Costiasis	TM50; sulfamerazine; formalin.
--	--	--	--	--	--
46-52	24%	from 1140 to 19	10 releases 12/19/79 to 8/7/80	ERM; Finrot; gill amoeba	Formalin; Roccol; TM50.
39-50	2%	77.5	5/13/80	None	None
58	--	--	Early June	--	--

The IFAT procedure used in this study for BKD, 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, is described in Novotny and Zaugg (1979).

RESULTS AND DISCUSSION OF STEELHEAD SUKVEYS

Dworshak National Fish Hatchery

General

In the early part of the season, water re-use at the hatchery was about 90%, but the temperature was held down. Sampling from the ponds began in early March and continued until release in mid-April. At that time, approximately 600 fish were transferred to indoor circular tanks and held for additional sampling until June. Hatchery personnel fed and maintained these fish and tried to provide at Least 12 hours/day of light.

Table 3 summarizes major data (means and standard deviations) collected during each sampling period in 1980 for Dworshak NFH steelhead (histopathology and BKD summaries are presented elsewhere).

We noted a visible erosion of gill filaments in many fish on the second trip (18 March), but almost none on the third trip (1 April). These changes may be related to increases and decreases in water temperature which are shown in Figure 1[3/].

Average fork lengths of both control (163 mm) and test release (167 mm) groups (Table 3) were much smaller than release groups sampled in 1978 (193 mm). This probably can be attributed to the Lower water temperature in 1980.

[3/] Summary profiles are presented as "Number of Days Post 1 January 1980" for convenience of computing and plotting. Whenever possible, the sampling periods are presented next to the data points (see Table 1 for the dates of the sampling periods). Appendix A contains a cross reference chart for converting number of days post 1 January 1980 to calender date.

Table 3.--Summary data for the spring (1980) sampling of the Dworshak Hatchery steelhead (n=60), with means, standard deviations ( ), and ranges.

Item	Period 1	2	3	4*	5**	6	7
Date	4 March	18 March	1 April	15 April	29 April	13 May	4 June
Days>Jal[a]	63	77	91	105	119	133	154
Temp.-°C[b]	8.3	9.7	6.0	7.5	7.5	10.0	10.0
Avg. Fk Ln[c]	158.5	167.1	157.1	162.6	166.9	172.3	178.6
	(21.3)	(18.1)	(24.6)	(21.2)	(23.0)	(22.5)	(21.1)
(Range)	115-207	117-212	115-213	130-210	114-206	122-214	125-228
Avg. ATP Fk Ln[d]	165.9	164.3	157.7	168.1	163.3	173.1	178.6
	(18.5)	(20.1)	(25.0)	(20.0)	(25.4)	(25.3)	(22.7)
(Range)	147-182	128-181	129-190	141-198	119-198	137-202	138-207
Avg. ATP[e]	6.28	5.07	6.27	7.48	8.58	7.16	6.92
	(0.81)	(0.53)	(1.23)	(7.48)	(8.58)	(1.42)	(1.26)
(Range)	4.9-7.5	4.1-6.0	4.6-8.5	6.1-8.6	6.4-10.6	5.2-9.2	4.3-8.4
Avg. Hct[f]	48.7	48.1	58.8	48.6	52.7	43.0	47.8
	(6.15)	(7.61)	(5.21)	(5.85)	(7.40)	(6.42)	(5.37)
(Range)	35-63	29-66	41-72	34-62	35-67	26-56	36-58
Avg. Hb[g]	8.6	8.2	10.9	8.5	9.1	7.7	8.1
	(1.34)	(1.26)	(1.05)	(1.50)	(1.32)	(1.26)	(1.02)
(Range)	6.0-12.0	6.0-11.0	8.4-12.9	5.0-12.3	6.8-12.6	4.3-10.0	6.0-10.3
Avg. MCHC[h]	17.6	17.3	18.6	17.6	17.3	18.0	17.1
	(1.70)	(1.55)	(1.55)	(2.60)	(1.75)	(2.14)	(1.46)
(Range)	11.1-20.6	13.8-21.4	13.5-22.2	12.3-27.3	13.7-23.1	9.6-23.7	12.5-21.8
Avg. Na^+[i]	154.2	146.4	146.5	157.2	144.3	149.9	155.8
	(10.9)	(9.0)	(6.8)	(9.5)	(11.5)	(16.1)	(5.6)
(Range)	120-176	125-165	134-162	125-173	123-162	73-200	133-169
Avg. K^+[j]	1.70	0.80	1.50	0.60	0.60	1.10	0.60
	(0.96)	(0.32)	(0.63)	(8.96)	(0.18)	(1.07)	(0.31)
(Range)	0.36-4.50	0.45-1.72	0.51-2.50	0.25-1.98	0.37-1.13	0.34-4.80	0.20-1.75
Avg. Cl^-[k]	129.1	123.9	128.6	130.0	114.2	132.3	130.6
	(11.1)	(14.7)	(7.2)	(9.0)	(9.1)	(10.0)	(6.5)
(Range)	92-150	87-154	116-141	96-146	99-135	97-162	117-144
Na^+/Cl^-[l]	1.20	1.20	1.14	1.21	1.27	1.14	1.20
	(0.12)	(0.13)	(0.06)	(0.06)	(0.08)	(0.07)	(0.07)
(Range)	1.00-1.68	0.97-1.51	1.06-1.29	1.01-1.40	1.17-1.54	1.02-1.31	1.06-1.38

* Normal (control) release group
** Test release group
a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp. -°C: Water temperature (in °C) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. ATP: The average gill ATPase levels for that period (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour).
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl^-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratios of the plasma sodiums to chlorides for that period, averaged.

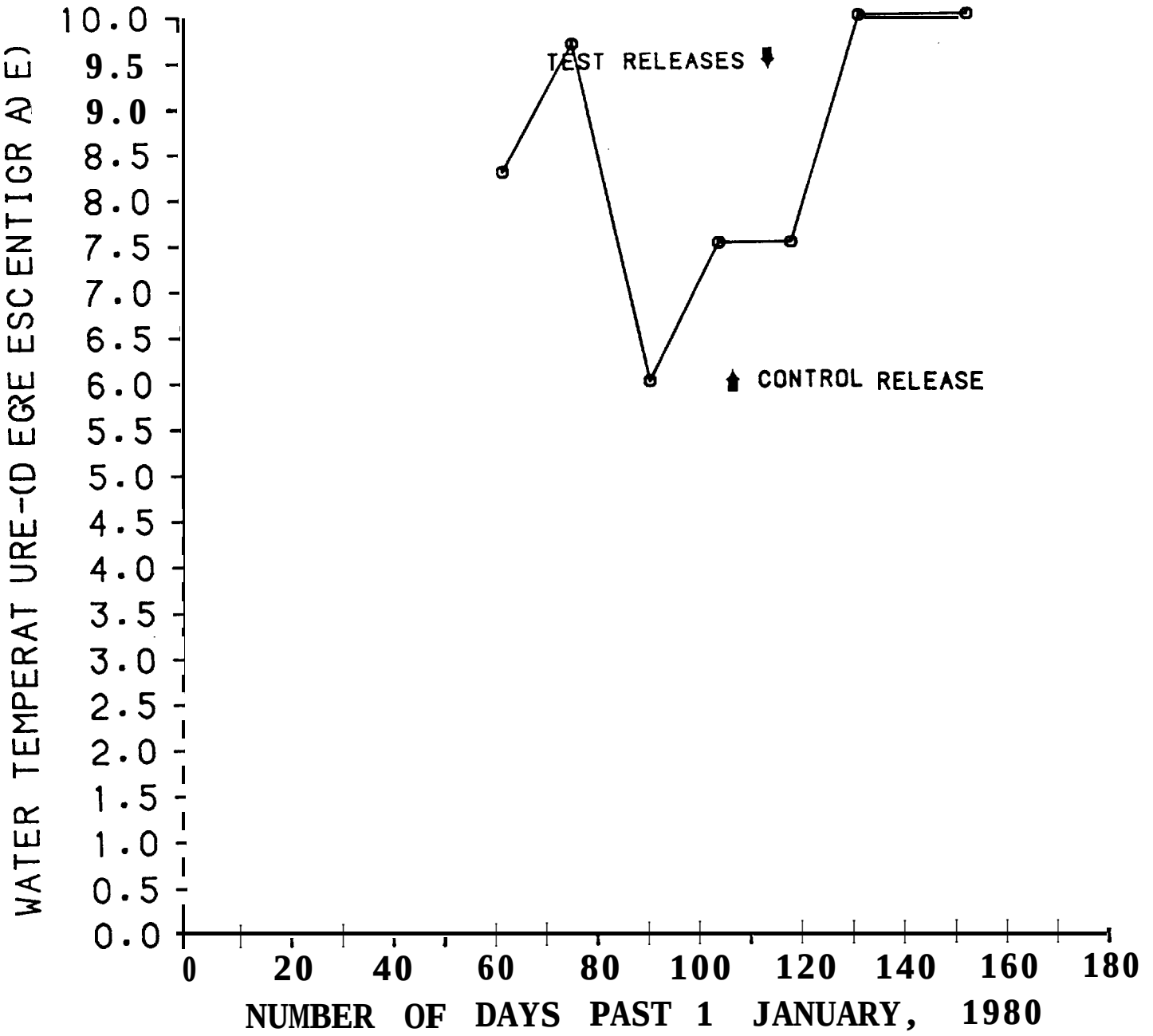


Figure 1.--Water temperatures at Dworshak NFH during spring 1980.

Gill Na^+-K^+ ATPase

Since the phenomenon of elevation in gill Na^+-K^+ ATPase activity was first reported to be associated with parr-smolt transformation in steelhead (Zaugg and Wagner 1973) and in coho salmon (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 physiological changes which occur at the time of parr-smolt transformation.

In 1978, the mean gill Na^+-K^+ ATPase activity was 5.0 ($\pm$1.1) u moles Pi/mg protein/hour on 24 April. The activity measured in 1980 (Table 3) indicates a peak on 29 April (5th period). However, this peak activity (average 8.6 $\pm$ 1.5) is far below expected values for highly developed smolts. Transfer of fish at this release time from the raceway to an inside holding tank for further sampling resulted in significant environmental changes, including an increase in water temperature from 7.5° to 10°C. That this change in holding conditions caused a reversal in Na^+-K^+ ATPase development is quite likely. Those fish transported and released undoubtedly experienced continuing increases in gill Na^+-K^+ ATPase activity as they migrated seaward. Nevertheless, fish held under the conditions used for extended sampling showed decreased activity after 29 April, thus producing a small peak on that date. We can state that the migration-transport group was released during a period of rising gill Na^+-K^+ ATPase activity, but that maximal levels had not yet been achieved. The range of activity in 1978 was from 3.5 to 7.2, but in 1980

during the same period the average was from 6.4 to 10.6. Higher Na^+-K^+ ATPase values in 1980 during the late April period may be due to a cooler temperature regime. In 1978, water temperatures ranged from 13° to 15°C, and the upper limit which will permit rising gill Na^+-K^+ ATPase activities in steelhead is about 12°C (Zaugg et al. 1972). The migration-transport group was released as gill Na^+-K^+ ATPase activity was rising, and the control group was released 17 April, just after the 4th sampling period.

We could find no significant correlations between the average gill Na^+-K^+ ATPase data and the averages of other data collected throughout the spring. When individual gill Na^+-K^+ ATPase activities from pooled 3-fish samples were compared with the average fork lengths of the pooled 3-fish samples, significantly higher correlations ($P = 0.001$; $P < 0.02$) were found only during the fourth and last sampling periods (Table 4). If smolting was initiated between the 4th and 5th periods as indicated by the Na^+-K^+ ATPase activity in Table 3, an approximate Na^+-K^+ ATPase index of smolting between those periods would be a value of 7.5. This would suggest that those steelhead larger than 168 mm were beginning to smolt (Figure 2).

On the basis of this information, approximately 38% of the Dworshak NFH steelhead would have started smolting in the 4th period (Figure 3).

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.--Correlation coefficients between gill Na^+-K^+ ATPase values for each sampling period, and the average fork lengths of the Dworshak Hatchery steelhead used to provide the gill samples in 1980 (3 fish/Na^+-K^+ ATPase analysis: 30 fish/period).

Item	Period 1	2	3	4	5*	6*	7*
Correlation coeff.	-0.33	0.03	0.22	0.87	0.36	0.34	0.75
P (DF = 8)				<0.001		<0.02	

* Groups held inside the hatchery (in small tanks) for post-normal release sampling. Stresses of handling, confinement, and artificial light conditions may have affected these fish.

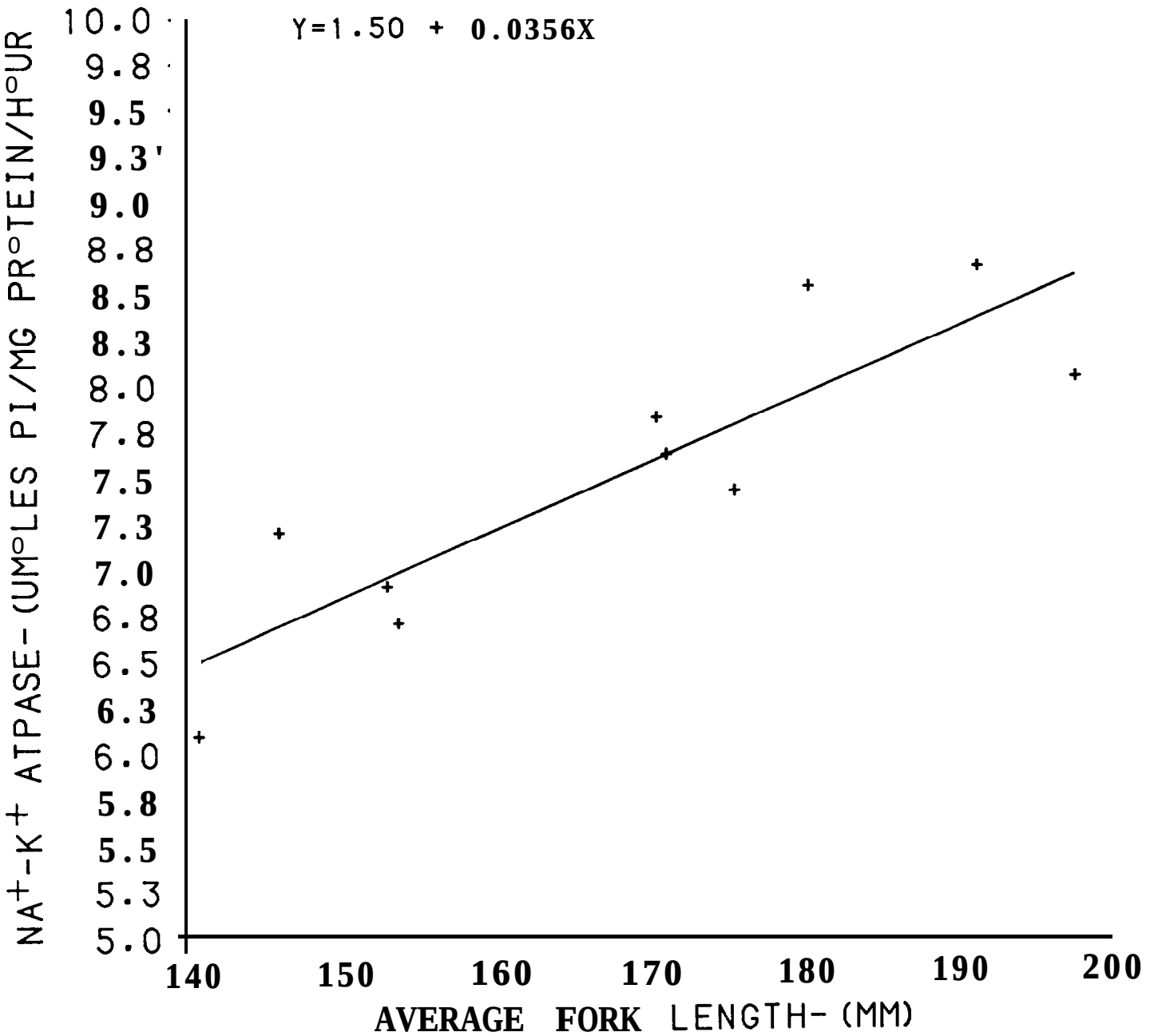


Figure 2.--Regression of gill Na^+-K^+ ATPase activity on average fork length in the 4th sampling period (3 fish pools for each Na^+-K^+ ATPase analysis) - 15 April 1980, Dworshak NFH steelhead. r = 0.87; P = 0.001.

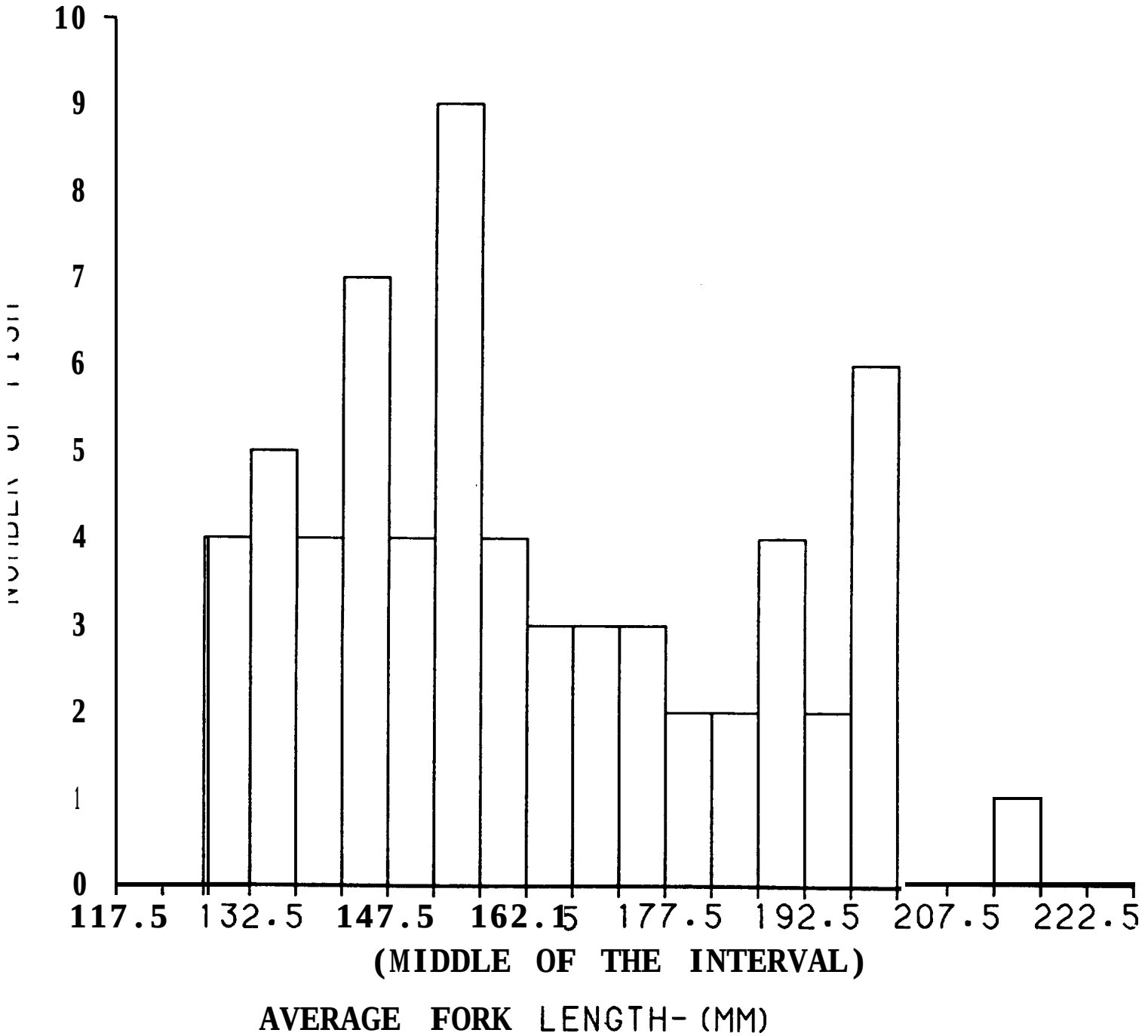


Figure 3.--Length-frequency histogram of steelhead sampled from Dworshak NFH in the 4th period.

Table 5 lists some known values for steelhead from available published literature, including data for the Dworshak NFH steelhead (Newcomb 1978). Newcomb's data are extensive, represent reasonably large sample sizes (15 to 25), and are probably good approximations of Columbia River steelhead.

Average plasma Na^+ values for Dworshak NFH steelhead throughout the season during 1980 were well within the previously published ranges (Table 3), but are below the mean value (166.4 mEq/l) of samples collected at release in 1978. Mean plasma Cl' values (Table 3) are also well within the suggested range and the mean value at release in 1978 (133.3 mEq/l). The early fluctuations in plasma Na^+ (Table 3) may have been due to changes in water quality, but there is definitely a coincidental sharp decline in both plasma Na^+ and Cl^- between the 4th and 5th periods which is also coincidental, but inverse to a significant rise in gill Na^+-K^+ ATPase activity.

There were no significant correlations of plasma Na^+ or Cl' or plasma Na^+/Cl^- ratios to fork lengths in any period. Correlations of plasma Na^+ to Cl^- gradually increased with each period, were highly significant ($P < 0.001$) from the 3rd to the 6th period, and reached a peak of $r = 0.69-0.70$ during the 4th and 5th periods (Table 6). Wedemeyer (1980) reports that a marked decline in plasma Cl' can be expected in anadromous salmonids during smoltification and Houston (1959) reports the same for steelhead.

Mean plasma K^+ values were highly erratic throughout the sampling period, but the general trend was downward (Table 3). The fact that average plasma K^+ values during early 1980 were 1.7 mEq/l or less (lower range was 0.5 mEq/l), and in 1978 (Novotny and Zaugg 1979) were similarly

Table 5.--A summary of plasma Na^+, K^+, and Cl^- values in steelhead (from published sources).

Condition	Na^+	Cl^-	K^+	References
June-July (55 g fish)				
Laboratory tests				
Freshwater	$\bar{X}$ = 162	Range: 140-160	$\bar{X}$ = 6.0	Houston (1959)
Saltwater	$\bar{X}$ = 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 Harchery (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	

Table 6.--Correlation coefficients between plasma Na^+ and Cl^- for each sampling period in 1980 for Dworshak NFH steelhead.

Item	Period 1	2	3	4	5	6	7
Correlation coeff.	0.377	0.454	0.587	0.687	0.696	0.607	0.158
P	<0.005	<0.001	<0.002	<0.001	<0.001	<0.001	n.s.

low ($\bar{x}$ = 1.0; sd = 0.8), suggests that plasma K^+ values reported by other researchers (Table 5) are high, or that a unique situation exists at Dworshak NFH.

There were no significant correlations between any plasma electrolyte measurements and water temperature.

Hematology

There are considerable hematological data in the literature for rainbow trout, Less for steelhead trout. From data summarized in Table 7, it is possible to estimate lower limits of mean hematocrits of 30% and lower mean hemoglobin values of 6 for healthy steelhead. Upper levels are more difficult to define. Snieszko (1960) reports mean hematocrits of 53% and mean hemoglobin Levels of 8.7 g/dl of blood in rainbow trout of a size comparable to large steelhead smolts. Although our values for Dworshak NFH steelhead (Table 3) were much closer to Snieszko's, Newcomb (1978) reported mean hematocrit levels in steelhead similar to that found by other researchers for rainbow trout (Table 7). A number of authors (McCarthy et al. 1973; Wedemeyer and Nelson 1975; Wedemeyer and Yasutake 1977) repeatedly suggest that hematocrit levels of clinically healthy rainbow trout should be between 24 and43%, 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 steelhead in this report.

Summarized hematological data for Dworshak NFH steelhead are presented in Table 3. Since we have spatial data for the 1980 hatchery samplings, we are including an analysis of the Mean Corpuscular (or cell) Hemoglobin Concentrations (MCHC) in this report. MCHC is the amount of hemoglobin (in g/dl) in the Packed Cell Volume (PCV, which is synonymous with the

Table 7.--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 (1969)
Kainbow 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)	Wedemeyer & Yasutake (1977), Wedemeyer & Nelson (1975)
Rainbow trout (average 14.2 cm)	$\bar{X}$ = 45.3	--	Snieszko (1960)
(average 23.5 cm)	$\bar{X}$ = 53.0	$\bar{X}$ = a.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		

hematocrit, Hct), and is calculated from the total hemoglobin (Hb) and hemotocrit (Hct) as:

$$\frac{\text{Hb (in g/dl)}}{\text{PCV (in ml/dl blood)}} \times 100 = \text{MCHC (g/dl)}$$

(Schalm 1975).

Steele (1978) reports that MCHC is a sensitive indicator of stress in fish, and that a sharp increase typically occurs under stressful conditions.

Average hematocrits and hemoglobins at the time of release in 1978 (20-25 April) were 52.4% and 11.3 g/dl blood, respectively. Similarly high values dominated the Dworshak NFH steelhead throughout the 1980 sampling periods (Table 3), and the largest percentage of samples to fall below any expected range was 3.3% (for Hb $\leq$ 5.4 g/dl blood) during the 6th period. The general trend for both Hct and Hb was a sharp early rise, followed by a downward trend after the 3rd period. All of the mean values were within suggested limits, but indicate that normal data for steelhead Hct and Hb may be significantly higher than for non-anadromous Salmo gairdneri. There were two sharp increases in MCHC (Table 3), both shortly after changes in environmental conditions (changing water supplies and habitat). MCHC values at release (Periods 4 and 5) appear to be normal.

Average Hct and average Hb were positively correlated ($r = 0.97$; $P < 0.001$, Figure 4). Hematocrits and hemoglobins were positively correlated throughout the sampling season ($P < 0.001$), with r values ranging from 0.580 to 0.850.

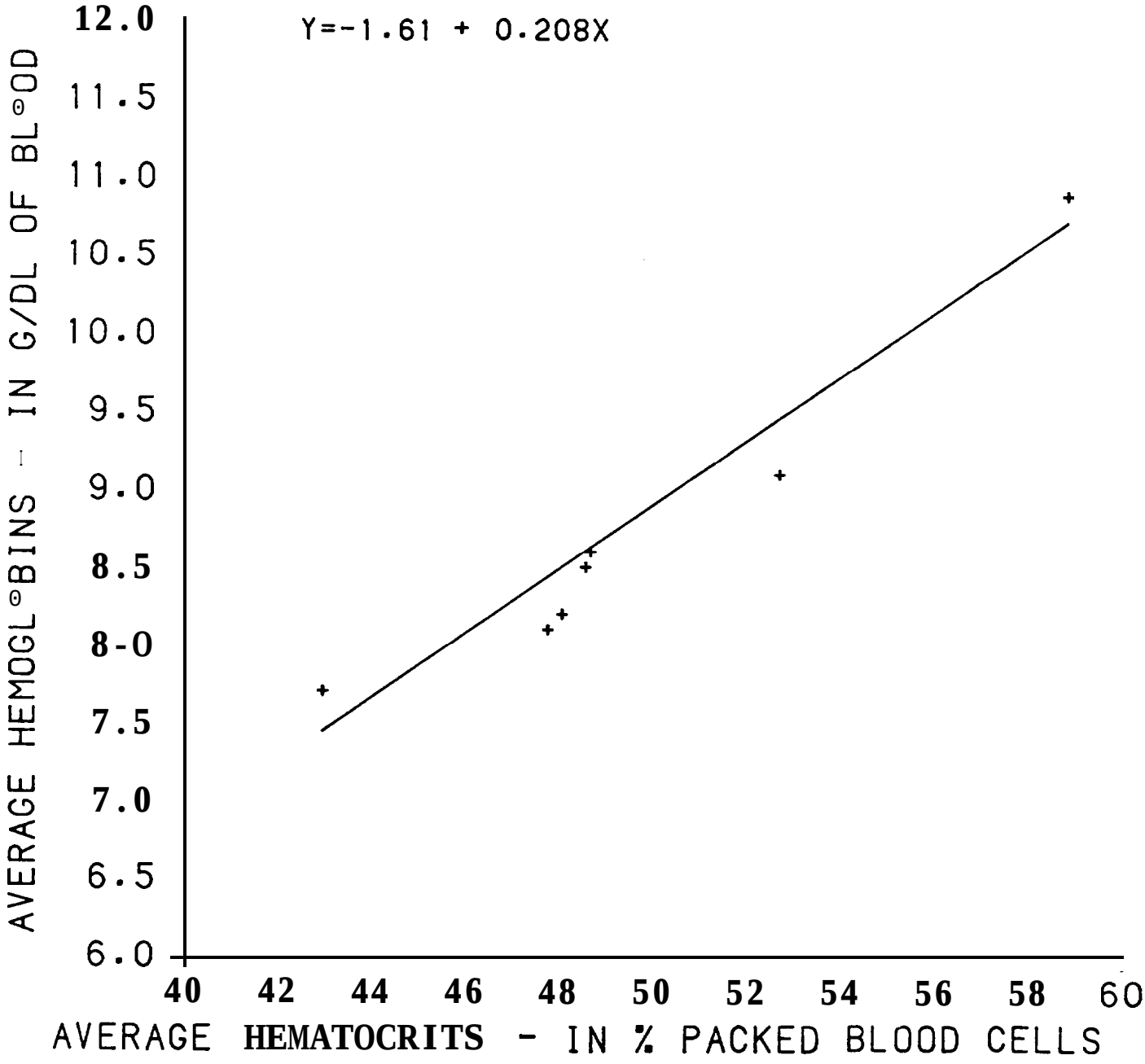


Figure 4.--Regression of the average hemoglobins on average hematocrits for the Dworshak NFH steelhead during spring 1980. $r = 0.972$; $P < 0.001$.

There were highly significant negative correlations between water temperature and average hematocrits ($r = 0.87$; $P < 0.020$) and average hemoglobin values ($r = 0.89$; $P < 0.010$) throughout the sampling periods (Figure 5). There were no significant correlations between average fish size and average Hct, Hb, or MCHC, nor between MCHC and water temperature or gill Na^+-K^+ ATPase activity.

Clinical data on hematology throughout the sampling period indicated that the stock was in good health at the time of release.

IFAT-BKD

Specimens of Dworshak NFH steelhead from the first, third, and fifth sampling periods were examined for the presence of BKD organisms by the IFAT. The incidence and intensity of infection was light in most cases.

During the first period (3 March 1980), 7 out of 60 fish examined (11.7%) had BKD organisms in either the anterior or posterior kidney, and the Level of intensity ranged from 1 to 5 organisms/l50 microscopic fields. No BKD organisms were found in the third period samples, and only 2.7% of the samples were infected in the fifth period. Intensities of infection in samples from the latter period were much higher, ranging from 53 to 148 organisms/l50 microscopic fields. Total infection for the 180 fish examined during the three periods was 5%, which is low.

Histopathology

A summary of the pathological conditions observed, their severity, and their frequency of occurrence is presented in Table 8. The severity is ranked as: I--recognizable (least severe), II--intermediate, and

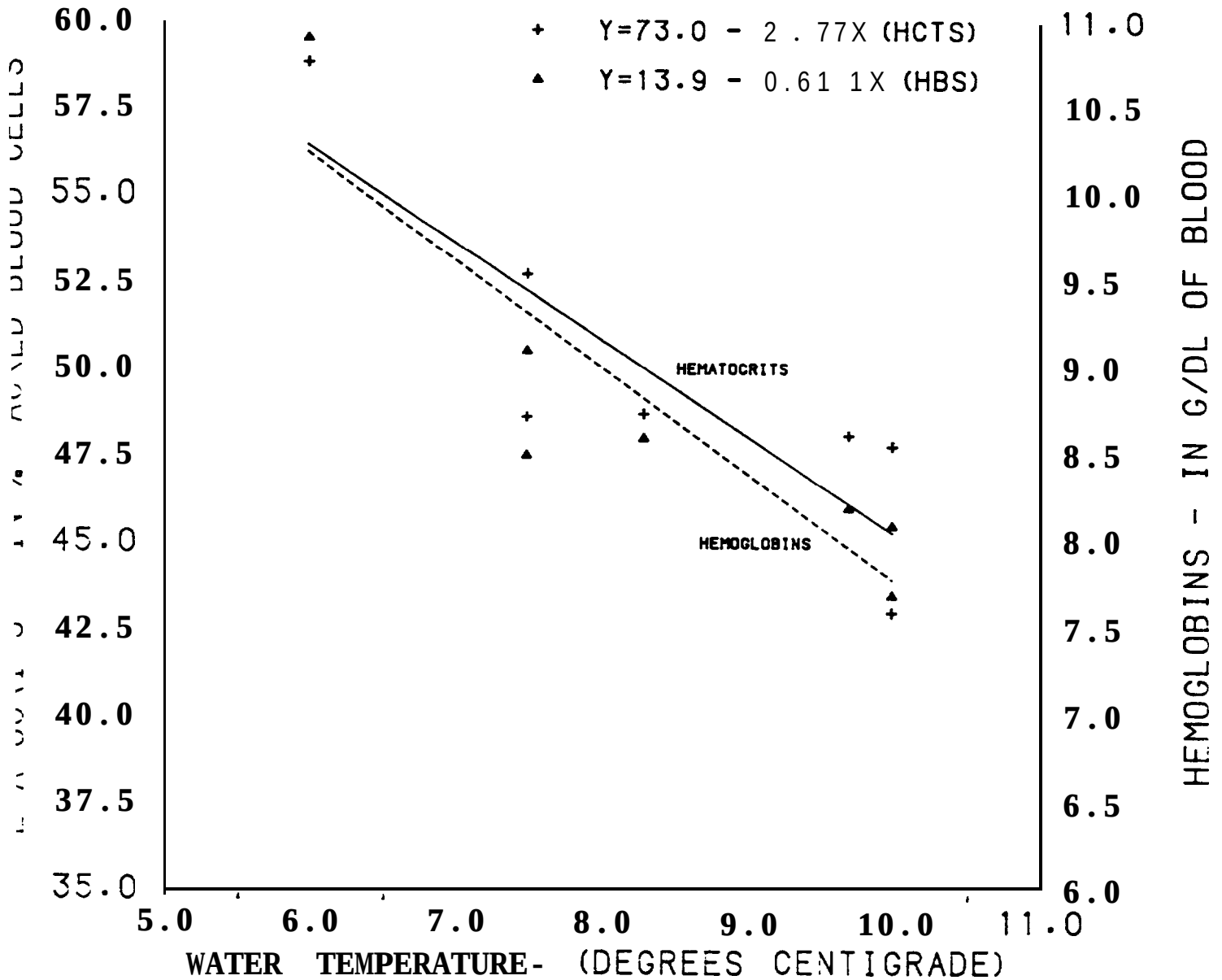


Figure 5.--Regressions of average hematocrits (x) and hemoglobins (^) of juvenile steelhead on water temperatures at Dworshak NFH during spring 1980. D.F. = 5. r = -0.866; P < 0.20 (hematocrits); r = -0.892; P < 0.10 (hemoglobins).

Table 8.--Pathological conditions observed in Dworshak Hatchery steelhead in 1980, and their percentage of incidence.[a]

Organ and pathology	Incidence (%) Period 1 (severity)[b] I	II	III	Total	Period 2 (severity) I	II	III	Total	Period 5 (severity) I	II	III	Total
Eye												
Skeletal muscle lesions	68.3	0	0	68.3	65.0	13.3	0	78.3	47.1	0	0	47.1
Gills												
Increased number of lymphocytes	60.0	25.0	0	85.0	25.0	66.7	6.7	98.3	51.7	36.7	0	88.4
Epithelial cell formation	45.0	33.3	6.7	85.0	0	33.3	66.7	100	36.7	41.7	16.7	95.1
Solitary basophilic organism	3.3	0	0	3.3	0	0	0	0	1.7	0	0	1.7
Vascular telangiectasis secondary lamellae	50.0	13.3	0	63.3	18.3	1.7	0	20.0	6.7	1.7	0	8.4
Acute focal hemmorhage	0	0	0	0	3.3	0	0	3.3	0	0	0	0
Bacteria present	0	0	0	0	3.3	0	0	3.3	1.7	0	0	1.7
Sporozoan parasite in secondary lamellae	0	0	0	0	0	0	0	0	0	1.7	0	1.7
Acute exudate	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Olfactory sac												
Focal mononuclear cell infiltration	40.0	48.3	7.7	96.0	58.3	31.7	3.3	93.3	66.7	15.0	0	81.7

[a] Brain tissue was processed and examined for all specimens, and there was no evidence of pathological lesions.

[b] I--recognizable (least severe); II--intermediate; III--severe.

III--severe (a composite summary for all hatcheries is presented in Table 9).

At or near release, Dworshak NFH steelhead were characterized by much lower incidences of gill parasites in 1980 than in 1978 (1.7 vs 28.3%). Incidence rates in eye lesions and other lesions of the gills were approximately the same. There was a marked decrease in miscellaneous gill lesions between the first sampling in March and the final release in late April (Table 9), which was apparently due to a decrease in the incidence of telangiectasis (basically capillary lesions) of the secondary lamellae (Table 8). There was a marked decline in severity of olfactory sac Lesions (Table 8) as the sampling season progressed. Some of the decreases in incidence of tissue lesions may have been the result of a change from recycled to single pass water.

Hatchery records (Table 2) indicate that in the early culture phases (1979) there were some problems with nitrogen saturation, "whitespot" disease, "Ich," and epistylis. Mortality was estimated at 35% before ponding, but only 5% between ponding and release.

Summary

Serial sampling of the Dworshak NFH steelhead in 1980 indicated that the general health of the fish was excellent, and that most of these fish were smolting during the second release (24 April). General health and physical appearance of the fish at release time was markedly improved over 1978. This may be a reflection of the decision to switch from recycled to flow-through water supplies in March. Generally Low temperatures after that (Figure 1) were probably a major factor in' the smolt quality. We estimate that over 70% of the fish were smolting when the test group was

Table 9.--Summary of lesion incidence (% of samples) and average intensity (scale: 1 to 3) for 9 of the 10 hatcheries sampled in 1980.[a]

Stock	Sample No.	Date	Eye muscle	Olfactory sac	Gill-lymphoid	Gill-epithelial	Gill-miscellaneous lesions
Tucannon Steelhead	#3	4 April	(30%)/0.3	(35%)/0.5	(73%)/0.9	(73%)/1.4	17%
	#5A	7 May	(80%)/0.8	(97%)/1.2	(97%)/1.3	(98%)/1.6	12%
	#7	12 June	(70%)/0.7	(98%)/1.6	(100%)/1.7	(97%)/1.6	0
Dworshak Steelhead	#1	4 March	(68%)/0.7	(87%)/1.4	(85%)/1.1	(85%)/1.3	63%
	#3	1 April	(78%)/0.9	(93%)/1.3	(98%)/1.8	(100%)/2.7	23%
	#5	29 April	(47%)/0.5	(83%)/1.0	(88%)/1.3	(95%)/1.7	13%
Big Creek Fall chinook	#1	8 April	(10%)/0.1	(2%)/0.0	(17%)/0.2	(62%)/0.8	2%
	#2	23 April	(18%)/0.2	(62%)/0.6	(37%)/0.4	(68%)/0.8	10%
	#3	6 May	(40%)/0.4	(75%)/1.0	(97%)/1.3	(100%)/1.5	2%
	#4	13 May	(38%)/0.4	(93%)/1.0	(92%)/1.2	(95%)/1.5	2%
Spring Creek Fall chinook	#1	10 March	(18%)/0.2	(63%)/0.7	(75%)/0.9	(80%)/1.1	8%
	#2	10 April	(7%)/0.1	(50%)/0.6	(27%)/0.3	(72%)/0.9	27%
	#3	8 May	(22%)/0.2	(95%)/1.1	(97%)/1.3	(100%)/1.5	2%
Leavenworth Spring chinook	#1	3 March	(33%)/0.3	(97%)/1.0	(100%)/1.3	(98%)/1.7	
	#3	31 March	(38%)/0.4	(90%)/1.1	(98%)/1.2	(98%)/1.7	
	#5	28 April	(38%)/0.4	(48%)/0.6	(72%)/1.0	(73%)/0.8	
Carson Spring chinook	#1	4 March	(82%)/0.8	(88%)/1.0	(85%)/1.5	(93%)/1.3	5%
	#2	4 April	(42%)/0.4	(90%)/1.0	(75%)/0.9	(60%)/0.7	12%
	#3	17 April	(60%)/0.6	(85%)/1.0	(72%)/0.9	(25%)/0.2	3%
	#4	12 May	(42%)/0.4	(93%)/1.0	(90%)/1.2	(53%)/0.7	3%
Kooskia Spring chinook	#1	5 March	(15%)/0.2	(80%)/0.8	(87%)/1.0	(87%)/1.2	15%
	#3	2 April	(33%)/0.3	(90%)/1.0	(67%)/0.8	(73%)/0.9	7%
	#5	30 April	(43%)/0.4	(92%)/1.0	(52%)/0.7	(68%)/0.8	20%
Rapid River Spring chinook	#1	6 March	(43%)/0.4	(83%)/0.8	(97%)/1.2	(100%)/1.7	4%
	#2	20 March	(55%)/0.6	(92%)/1.1	(92%)/1.2	(83%)/1.1	4%
	#3	3 April	(59%)/0.6	(97%)/1.1	(93%)/1.1	(82%)/1.5	8%
Willard Coho salmon	#1	5 March	(63%)/0.4	(98%)/1.2	(97%)/1.1	(75%)/0.9	22%
	#2	2 April	(47%)/0.5	(100%)/1.1	(85%)/1.0	(45%)/0.5	17%
	#3	20 May	(78%)/0.8	(97%)/1.1	(98%)/1.6	(97%)/1.2	0

[a] Brain tissue was processed and examined for all specimens except 7597 and 7598 in the Tucannon steelhead #3. Only 2 specimens showed any pathology: (Carson spring chinook #3) 8,506 had a Class II pyogranulomatous inflammation, and 8516 had a Class III retrobulbar granulomatous inflammation.

released (5th period). Gill Na^+-K^+ ATPase values could only be significantly correlated to fish size during the 4th and 5th periods prior to release. There were no significant correlations of gill Na^+-K^+ ATPase and other measured parameters.

There were no apparent deviations of plasma electrolytes from the expected values throughout the sampling season, with the exception of plasma K^+, which was frequently Lower than reported in the Literature.

Compilation of 1978 and 1980 data suggests that generally high mean hematocrit and hemoglobin values in northwest steelhead stocks may reflect a normal hematological condition for these anadromous strains of rainbow trout. Profiles conducted in 1980 provide excellent significant negative correlations of Hct and Hb values with water temperature, but no correlations with possible smolt indices such as gill Na^+-K^+ ATPase or plasma electrolytes. Very few of the Dworshak NFH fish had "borderline Low" hematological values. MCHC Levels may have reflected some changes in water quality. Although IFAT tests of kidney tissue smears from 180 specimens (total) did reveal the presence of BKD organisms, incidence of the disease was very Low.

An analysis of the veterinary pathologist's data on examinations of 60 fish from each of three sampling periods indicates a general decrease in the incidence of tissue Lesions throughout the season.

In general, the stock appeared to be in excellent health at the time of release of the control and test groups, and they were beginning smoltification.

Tucannon Hatchery (WDG)

General

1980 was the third year in succession that steelhead trout from the Washington Department of Game (WDG) Hatchery at Tucannon were used for the homing studies. In 1978 and 1979, we collected gill Na^+-K^+ ATPase profiles, but data for the other parameters were only collected at the time of release (briefly summarized in Table 10).

Complicating factors in 1980 prevented us from collecting the standard 60 fish samples/period, and adjustments were made to suit the circumstances.

Early in the season, WDG graded steelhead for the homing studies into four size groups, one group for each circular pond. Three of the groups were sequential test releases, and the fourth served as a control. Since there were only 17,000 to 22,000 steelhead/pond, and sampling time was limited, we restricted our sample sizes in the first part of the season to 20 fish/pond in the three test ponds-, and Na^+-K^+ ATPase and plasma electrolyte data were derived from 2-fish rather than 3-fish pools. The control pond was sampled for Na^+-K^+ ATPase only at release, as these fish were approximately the same size as the smallest release group. The releases, as reported here, are:

Release	Reporting group no.	Location	Size
Early	Group I	Pond 1	(Largest fish)
Normal	Group II	Pond 4	(Next largest fish)
Late	Group III	Pond 2	(Smallest fish)
Control (released into the Walla Walla River)	Group IV	Pond 5	(Small fish)

Table 10.--A summary of health and smoltification index data for Tucannon Hatchery steelhead at the approximate times of release in 1978 and 1979.

Condition	1978 Mean	1978 S.D.	1979 Mean	1979 S.D.
Hematological Data				
Hematocrits	48.6%	± 6.8	53.0	± 7.7
Hemoglobins	9.9	± 1.5	9.2	± 1.3
Plasma Electrolytes (mEq/l)				
Na^+	159.5	± 9.5	140.7	±11.3
Cl^-	131.6	± 6.5	127.0	± 8.7
K^+	2.4	± 2.6	2.9	± 1.7
Gill Na^+-K^+ ATP (μ moles pi/mg protein hour)				
5/08/78	18.2	± 5.0	[5/08/79] 25.9	± 9.0
5/22/78	11.7	± 4.0		
BKD - IFAT				
(% of fish with BKD bacteria present in the kidney)	21.6	---	1.7	---

Because of the diversity of sizes in the test release groups, each pond is reported as a separate entity. Histopathology and BKD (IFAT) surveys are summarized for all groups. Water temperatures were taken from one pond for each sampling period, and the seasonal profile for the four release groups is presented in Figure 6. Ambient water temperatures were below 12°C for all release groups except Group III, which was not released until 12 June.

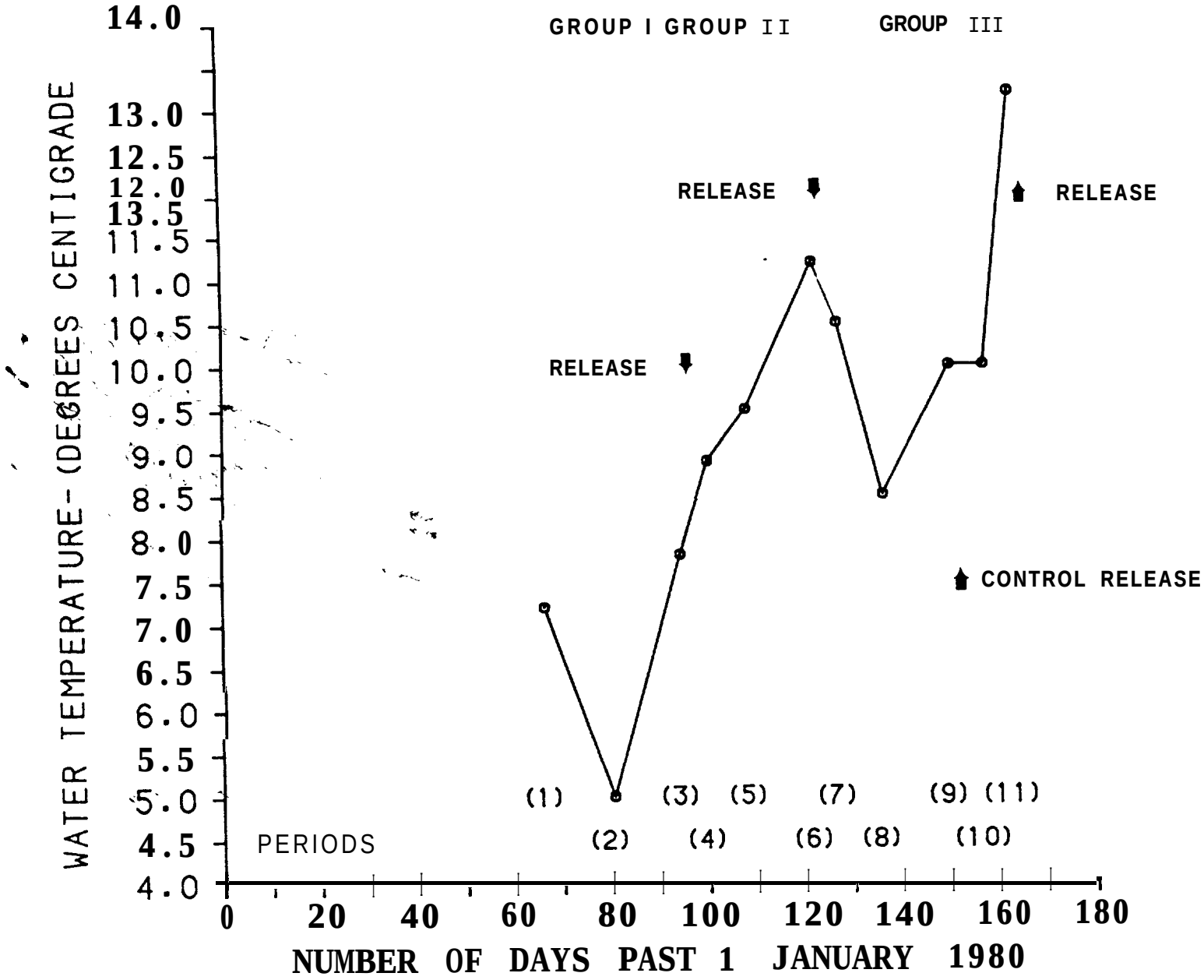
Figure 7 is a composite graph of the mean fork Lengths of all four groups during the sampling period. The average size of Group IV (sampled twice) was much smaller at release than the others.

Profiles of average gill Na^+-K^+ ATPase activities for all four groups are presented in Figure 8. Note that the first two groups were released as activities were ascending, and the third (Late) Group (III) was released about 40 days past a small peak. Group IV, released into the Walla Walla River, had very Low gill Na^+-K^+ ATPase values. None of the Na^+-K^+ ATPase samples collected the first week in May were as high as those in 1978 or 1979 during a comparable period (Table 10). The 1980 stock was from Chelan Hatchery, whereas in 1978 and 1979 the stocks were from Skamania Hatchery.

Summaries of data collected for Tucannon steelhead throughout the 1980 season from all four ponds are presented in Tables 11, 12, 13, and 14.

Group I (Pond 1, Table 11)

Gill Na^+-K^+ ATPase.--The gill Na^+-K^+ ATPase profiles presented for homogenous populations of Tucannon steelhead in 1978 and 1979 (Skamania Hatchery stock) indicated that peak average values occurred in the first 10 days in May (Novotny and Zaugg 1979, 1981). In 1980, there was a sharp



	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
DATES (1980)	MAR 7	MAR 21	APR 4	APR 10	APR 18	MAY 2	MAY 7	MAY 16	MAY 30	JUNE 6	JUNE 12

Figure 6.--Water temperatures measured at Tucannon Hatchery during the 1980 sampling period.

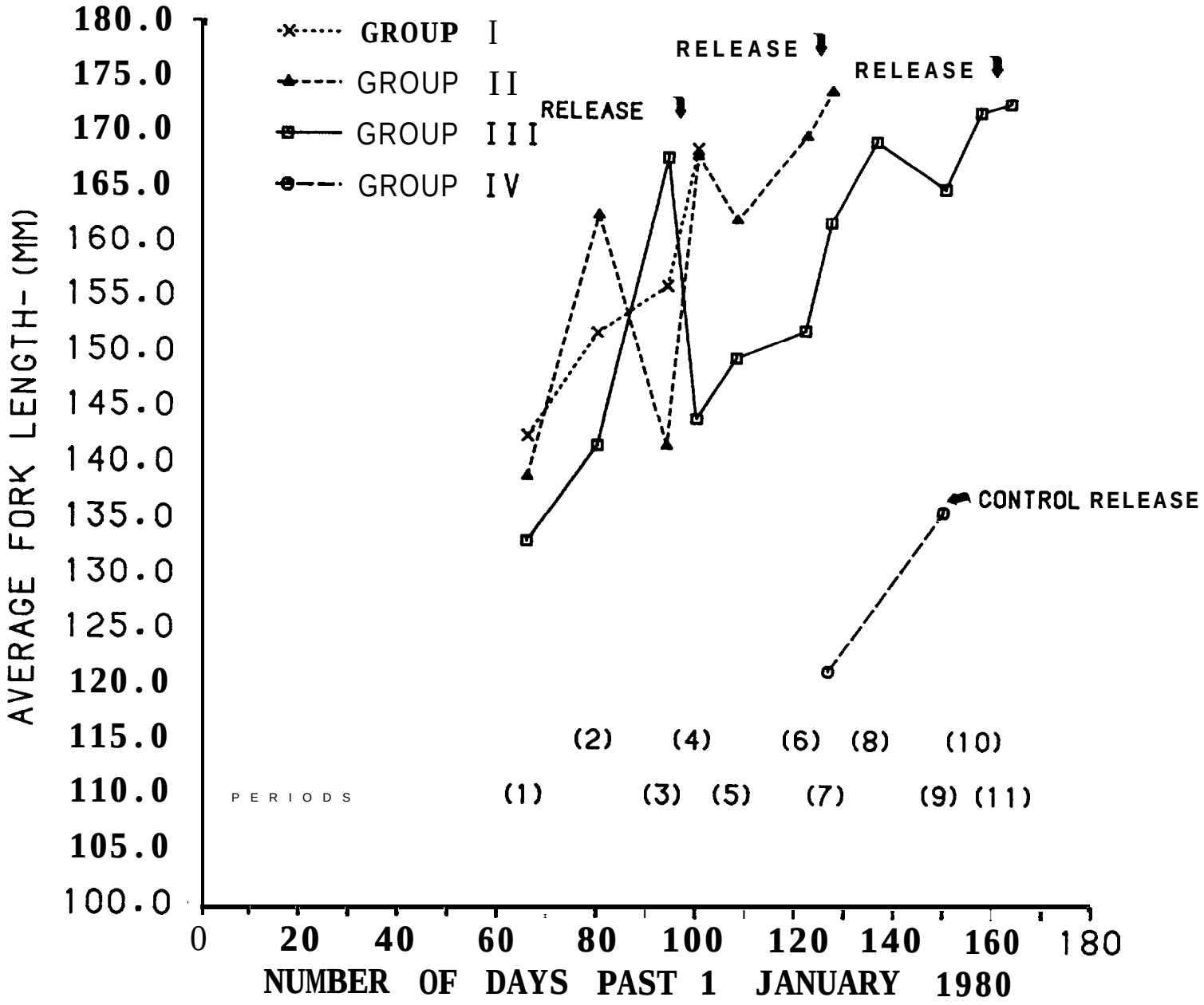


Figure 7.--Average fork lengths of steelhead sampled from the four Tucannon Hatchery ponds during spring 1980.

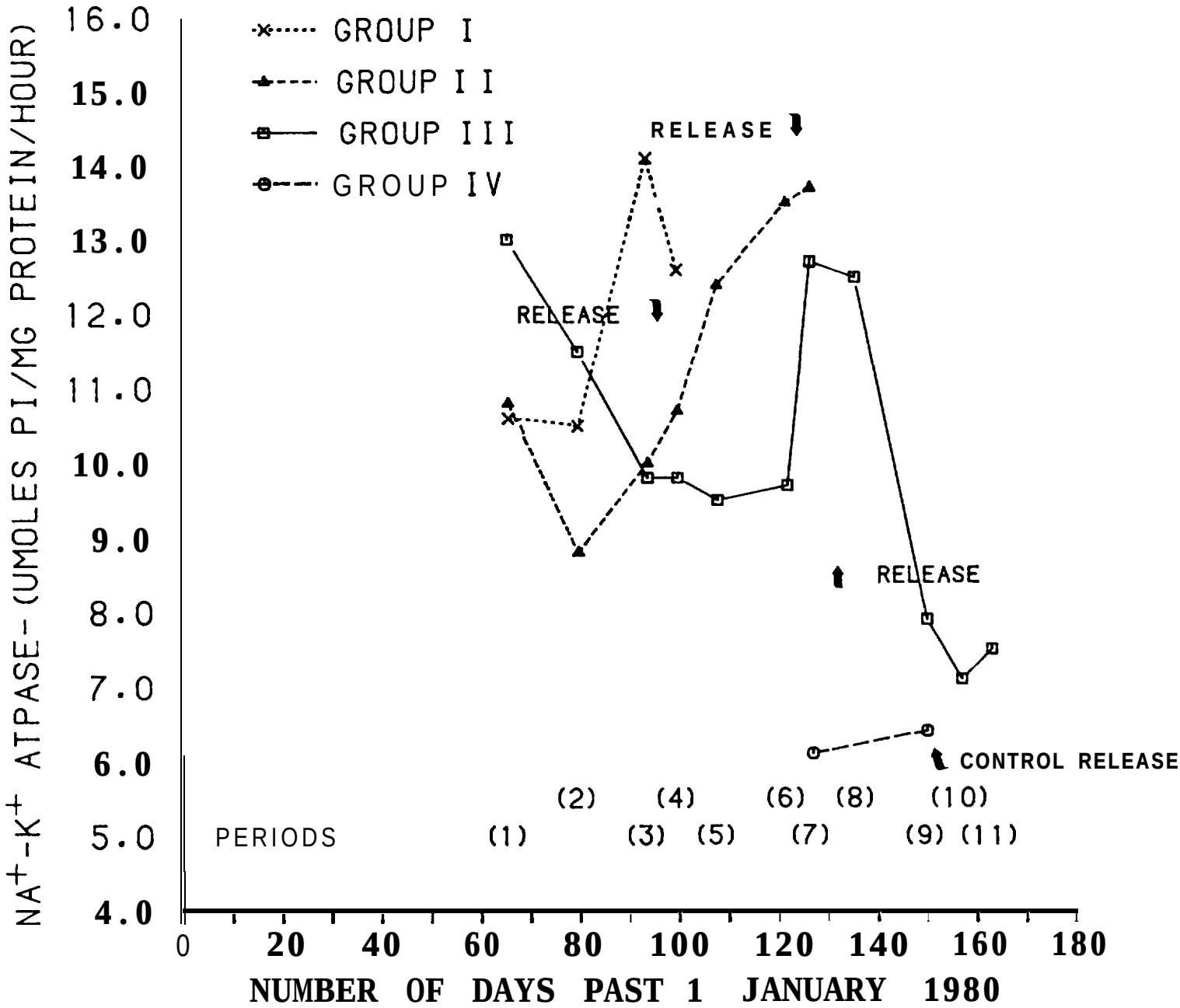


Figure 8.--Average gill Na^+-K^+ ATPase values for four release groups of Tucannon Hatchery steelhead during spring 1980.

Table 11.--Summary data for the spring (1980) sampling of Tucannon Hatchery steelhead, with means, standard deviations ( ), and ranges. Group I, Pond 1, first release, n = 20.

Item	Period 1	2	3	4
Date	7 March	21 March	4 April	10 April
Days>Jal[a]	66	80	94	100
Temp. °C[b]	7.2	5.0	7.8	8.9
Avg. Fk Ln[c]	142.4	151.8	156.1	168.6
	(10.7)	(13.4)	(11.0)	(8.7)
(Range)	124-162	118-176	125-172	153-192
Avg. ATP Fk Ln[d]	142.4	151.8	156.1	168.6
	(10.7)	(13.4)	(11.0)	(8.7)
(Range)	124-162	118-176	125-172	153-192
Avg. ATP[e]	10.6	10.5	14.1	12.6
	(1.3)	(1.2)	(1.9)	(2.2)
(Range)	8.0-12.6	8.5-13.2	10.8-17.5	9.2-16.0
Avg. Hct[f]	45.0	49.4	46.9	51.3
	(4.6)	(6.2)	(3.7)	(4.2)
(Range)	35-55	40-62	42-56	45-60
Avg. Hb[g]	8.9	8.3	10.0	---
	(0.9)	(1.3)	(0.7)	---
(Range)	8.0-10.7	5.7-10.7	8.4-11.3	---
Avg. MCHC[h]	19.5	16.8	21.4	---
	(1.8)	(1.6)	(2.3)	---
(Range)	16.9-23.8	13.6-19.4	16.8-26.2	---
Avg. Na^+[i]	137.3	144.0	160.7	---
	(5.3)	(8.3)	(3.5)	---
(Range)	131-146	129-155	154-167	---
Avg. K^+[j]	0.41	0.46	1.05	---
	(0.08)	(0.25)	(0.12)	---
(Range)	0.36-0.50	0.23-0.73	0.80-1.17	---
Avg. Cl^-[k]	117.6	139.4	130.4	---
	(12.0)	(6.2)	(8.8)	---
(Range)	92-130	127-148	113-144	---
Na^+/Cl^-[l]	1.11	1.03	1.24	---
	(0.03)	(0.06)	(0.07)	---
(Range)	1.06-1.15	0.96-1.18	1.36-1.36	---

a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C.) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour.)
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl^-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period, averaged.

Table 12.--Summary data for the spring (1980) sampling of Tucannon Hatchery steelhead, with means, standard deviations ( ), and ranges. Group II, second release. n = 20.

Date	Period 1 7 March	2 21 March	3 4 April	4 10 April	5 18 April	6 2 May	7 7 May
Days>Jal a/	66	80	94	1no	108	12%	127
Temp.°C b/	7.2	5.0	7.8	R.9	9.5	11.2	10.5
Avg. Fk Ln c/	138.8	162.6	141.6	168.0	162.2	169.8	173.9
	(17.1)	(5.7)	(8.8)	(14.6)	(15.0)	(17.9)	(12.7)
(Range)	107-162	154-173	122-160	134-195	120-185	117-192	139-196
Avg. ATP F k Ln d/	138.8	162.6	141.6	168.0	162.2	169.8	173.9
	(17.1)	(5.7)	(8.8)	(14.6)	(15.0)	(17.9)	(12.7)
(Range)	107-162	154-173	122-160	134-195	120-185	117-192	139-196
Avg. ATP e/	10.8	8.8	10.0	10.7	12.4	13.5	13.7
	(2.4)	(1.8)	(1.7)	(2.2)	(1.8)	(3.9)	(3.5)
(Range)	X.1-16.5	6.1-11 5	6.3-12.1	9.8-12.5	9.7-1 5.7	7.6-18.8	9.0-21.0
Avg. Hc t f/	49.5	43.6	4h.2	46.5	45.7	54.n	48.6
	(5.6)	(6.1)	(5.1)	(2.9)	(5.6)	(5.9)	(5.1)
(Range)	40-63	33-57	39-57	41-53	37-60	40-68	39-59
Avg. Hb g/	9.5	7.4	9.4	---	7.7	8.8	---
	(0.90)	(0.92)	(1.00)	---	(0.85)	(0.97)	
(Range)	7.7-11.7	6.3-10.0	7.7-11.6	---	6.3-9.0	7.1-10.6	
Avg. MCHC h/	19.2	17.1	20.0	---	Ih.9	16.4	
	(1.5)	(1.2)	(3.2)	---	(1.1)	(1.6)	---
(Range)	15.9-21.6	14.8-19.1	14.5-29.2	---	14.5-20.7	14.0-19.3	---
Avg. Na^+ i/	135.n	146.1	152.3	---	142.3	140.1	
	(8.0)	(6.0)	(9.1)	---	(11.7)	(14.4)	
(Range)	123-143	137-154	140-164	---	109-160	88-158	
Avg. K^+ j/	0.8	0.45	1.11	---	n.64	n.59	---
	(0.29)	(0.15)	(0.50)	---	(0.26)	(0.31)	---
(Range)	0.56-1.50	0.24-0.79	0.63-2.00	---	0.34-1.16	0.29-1.50	---
Avg. Cl^- k/	110.5	135.4	122.0	---	139.2	137.4	
	(12.9)	(6.3)	(7.3)	---	(8.8)	(12.3)	
(Range)	89-126	121-141	104-131	---	118-155	86-157	---
Na^+/Cl^- l/	1.23	1.08	1.25	---	1.03	1.02	
	(0.14)	(0.04)	(0.09)	---	(0.07)	(0.08)	
(Range)	1.08-1.54	1.04-1.16	1.10-1.40	---	0.92-1.16	0.89-1.17	

a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C.) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour.)
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins For that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium For that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl-: The average plasma chloride For that period (in meq/l).
l/ Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period, averaged.

Table 13.--Summary data for the spring (1980) sampling of Tucannon steelhead, with means, standard deviations ( ), and ranges. Group III: Pond 2; third release; n = 20 (periods 1-4), n = 30 (periods 5-10); and n = 60 (period 1).

Item	Period 1	2	3	4	5	6	7	8	9	10	11
Date	7 March	21 March	4 April	10 April	18 April	2 May	7 May	6 May	30 Hey	6 June	12 June
Days>Jal[a]	66	80	94	1on	108	122	127	136	150	157	163
Temp. °C[b]	7.2	5.n	7.R	A.9	9.5	11.2	10.5	R.5	10.0	10.0	17.2
Avg. Fk Ln[c]	133.0	141.6	167.8	144.0	149.5	152.1	162.0	169.3	165.1	172.0	172.8
	(12.8)	(10.7)	(10.4)	(15.4)	(9.1)	(17.0)	(15.9)	(9.9)	(17.9)	(12.2)	(16.7)
(Range)	110-156	125-167	145-184	111-169	134-172	112-179	108-182	143-189	127-195	146-199	126-208
Avg. ATP Fk Ln[d]	133.0	141.6	167.8	i44.n	149.5	152.1	162.0	169.3	1h5.1	172.0	173.4
	(12.8)	(10.7)	(10.4)	(15.4)	(9.1)	(17.0)	(15.9)	(9.9)	(17.9)	(12.2)	(19.1)
(Range)	110-156	125-167	145-184	111-169	134-172	112-179	108-182	143-189	127-195	146-199	126-208
Avg. ATP[e]	13.0	11.5	9.8	9.R	9.5	9.7	12.7	12.5	7.9	7.1	7.5
	(1.5)	(1.8)	(1.7)	(1.2)	(1.5)	(2.7)	(2.9)	(4.4)	(1.7)	(0.5)	(2.1)
(Range)	10.2-14.8	8.3-15.3	7.1-12.9	7.9-11.5	7.1-11.9	6.7-13.6	7.9-16.1	8.0-23.3	5.8-10.5	6.3-7.7	3.6-14.9
Avg. Hct[f]	47.7	46.6	46.3	46.9	46.9	53.2	54.4	54.7	---	51.R	54.7
	(6.6)	(4.R)	(4.1)	(4.5)	(6.7)	(7.0)	(5.5)	(7.2)	---	(6.3)	(5.9)
(Range)	35-62	40-56	41-57	40-55	27-59	36-66	43-64	41-70	---	41-69	41-75
Avg. Hb[g]	9.2	7.9	9.6	---	7.7	8.9	--	9.4		9.6	9.7
	(1.2)	(0.7)	(0.9)	---	(1.2)	(1.2)		(1.6)	---	(1.1)	(1.4)
(Range)	7.0-11.7	6.3-9.0	8.1-12.3		4.0-9.7	6.5-11.6		6.7-12.3	---	80-12.7	7.0-16.9
Avg. MCHC[h]	19.4	17.0	20.8	---	16.5	16.7		17.8	---	IR.4	17.8
	(1.6)	(1.8)	(2.4)	---	(1.3)	(1.5)	---	(3.7)	---	(1.4)	(1.5)
(Range)	17.5-22.4	14.3-20.8	17.6-28.0	---	14.5-19.4	14.1-19.6	---	13.3-26.8	---	150-165	14.3-22.9
Avg. Na^+[i]	137.4	147.3	158.9	---	148.3	141.9		14R.0	---	160.0	148.2
	(11.3)	(8.0)	(5.0)	---	(6.5)	(11.5)	---	(3.6)	---	(2.9)	(16.4)
(Range)	117-154	140-165	150-165	---	138-163	117-158	---	145-152	---	150-165	130-164
Avg. K^+[j]	0.59	0.56	1.79	---	0.76	0.52	---		---	0.72	0.97
	(n.29)	(0.11)	(0.83)	---	(0.51)	(0.15)		---	---	(0.38)	(0.65)
(Range)	0.31-1.10	0.41-0.68	1.07-3.95	---	0.36-2.15	0.39-0.72	---	---	---	0.34-1.59	0.35-2.62
Avg. Cl^-[k]	124.n	139.4	128.5	---	131.3	144.h	---	127.R	---	12A.h	125.2
	(9.2)	(8.1)	(4.9)	---	(6.0)	(9.0)	---	(6.7)	---	(6.2)	(1h.6)
(Range)	113-140	126-155	120-134	---	119-141	124-159	---	108-137	---	114-138	92-169
Na^+/Cl^-[l]	1.10	1.05	1.24	---	1.13	6,97	---	1.13	---	1.26	1.21
	(0.09)	(0.05)	(0.05)	---	(0.7)	(0.07)		(0.01)	---	(11.07)	(0.13)
(Range)	0.97-1.22	0.98-1.14	1.16-1.34	- - -	1.01-1.25	0.80-1.15	- - -	1.12-1.13	---	1.11-1.41	0.93-1.4

[a] Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
[b] Temp.-°C: Water temperature (in degrees C.) measured for that period.
[c] Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
[d] Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
[e] Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour.)
[f] Avg. Hct: The average hematocrits for that period (% packed cells).
[g] Avg. Hb: The average hemoglobins For that period (in g/dl).
[h] Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
[i] Avg. Na^+: The average plasma sodium for that period (in meq/l).
[j] Avg. K^+: The average plasma potassium for that period (in meq/l).
[k] Avg. Cl^-: The average plasma chloride for that period (in meq/l).
[l] Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period. averaged.

Table 14.--Summary data for the spring (1980) sampling of Tucannon Hatchery steelhead, with means. standard deviations ( ), and ranges. Group IV, Pond 5, Control (Walla Walla River), n = 30.

Item	Period 7	Period 9
Date	7 May	30 May
Days>Jal[a]	127	150
Temp. °C[b]	10.5	10.0
Avg. Fk Ln[c]	121.4	135.7
	(11.9)	(14.7)
(Range)	97-146	113-164
Avg. ATP Fk Ln[d]	121.4	135.7
	(1 1.9)	(14.7)
(Range)	79-146	113-164
Avg. ATP[e]	6.1	6.4
	(0.9)	(3.1)
(Range)	5.0-8.2	1 .,)-1 2.4
Avg. Hct[f]	---	53.9
	---	(6.2)
(Range)	---	39-66
Avg. Hb[g]	---	8.7
	---	(1.1)
(Range)	---	6.0-11.7
Avg. MCHC[h]		16.3
	---	(1.6)
(Range)	---	12.8-20.5
Avg. Na^+[i]	---	147.0
	---	(4.6)
(Range)	---	141-151
Avg. K^+[j]	---	---
	---	---
(Range)	---	
Avg. Cl^-[k]	---	136.0
	---	(8.9)
(Range)	---	113-155
Na^+/Cl^-[l]	---	1.12
	---	(0.14)
(Range)	---	1.03-1.34

a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C.) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour.)
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl^-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period, averaged.

increase in the average gill Na+-K ATPase activity early in the season (Figure 8). The drop in activity (at release) from the previous sampling period probably does not represent a true decline since there is no statistical difference in the two values, and peak Na+-K+ ATPase activities for steelhead normally occur in May.

There were no significant correlations in this Group (I) between average gill Na+-K+ ATPase values and average fork Lengths, plasma electrolytes, hematocrits, hemoglobins, or water temperatures during the sampling periods, nor were there any correlations between Na+-K+ ATPase values and other parameters measured during any one sampling period.

Plasma electrolytes.--Average plasma Na+ and C1- in Group I reached peak Levels equal to or exceeding those measured in May of 1978 and 1979 (Tables 10 and 11). Average plasma Na+ and C1- values diverged sharply as gill Na+-K+ ATPase activities began to rise (Table 11).

Average plasma K values of fish in Group I (Table 11) never reached the 1978 and 1979 Levels (Table 10), and were below the minimum expected and reported values of other researchers. Although rising rapidly at the time of release, plasma K+ Levels for Groups II and III barely peaked at the minimum expected Level of 1.5 mEq/l (Tables 12 and 13). All mean plasma K+ values followed a trend (similar to that of the Dworshak NFH steelhead) of peaking in April, followed by a general decline.

Hematology.--There were no indications of hematologicaL deficiencies in any of the Group I fish examined (Table 11). There were fluctuations in average hematocrit and hemoglobin levels, with sharp increases prior to release (Table 11).

Comments.--A sample of fish taken from Pond 1 on 8 April ($\bar{x}$ FkLn = 158.8 mm; n = 33), just before transfer of the entire lot to hauling trucks for transportation and release, indicated a significant size differential (P = 0.007) from the subsample collected on 10 April ($\bar{x}$ FkLn = 168.6 mm; n = 20).

Group II (Pond 4, Table 12)

Gill Na+-K+ ATPase.--Group II steelhead were released 7 May, approximately the same time as 1978 and 1979 test fish. The Na+-K+ ATPase profile paralleled that of Group I, (Figure 8), but perhaps due to the different stock of fish, the level of peak activity was considerably Lower than the activities measured at the same time in 1978 and 1979 (Table 10). The apparent growth of this stock was good, and average size of the samples was larger than any other group at release (Figure 7). Although no fish were held over from this pond for further monitoring of Na+-K+ ATPase, it would appear that the release was made near the peak of activity (Figure 8).

There was a significant positive correlation between average gill Na+-K+ ATPase values and water temperature during the periodic sampling of Group II (Figure 9). Average gill Na+-K+ ATPase could not be significantly correlated with averages of other parameters measured.

Correlations between gill Na+-K+ ATPase values of Group II fish and average fork lengths for each Na+-K+ ATPase value generally increased as spring progressed, but were not highly significant until the sixth period, 5 days prior to release (Table 15).

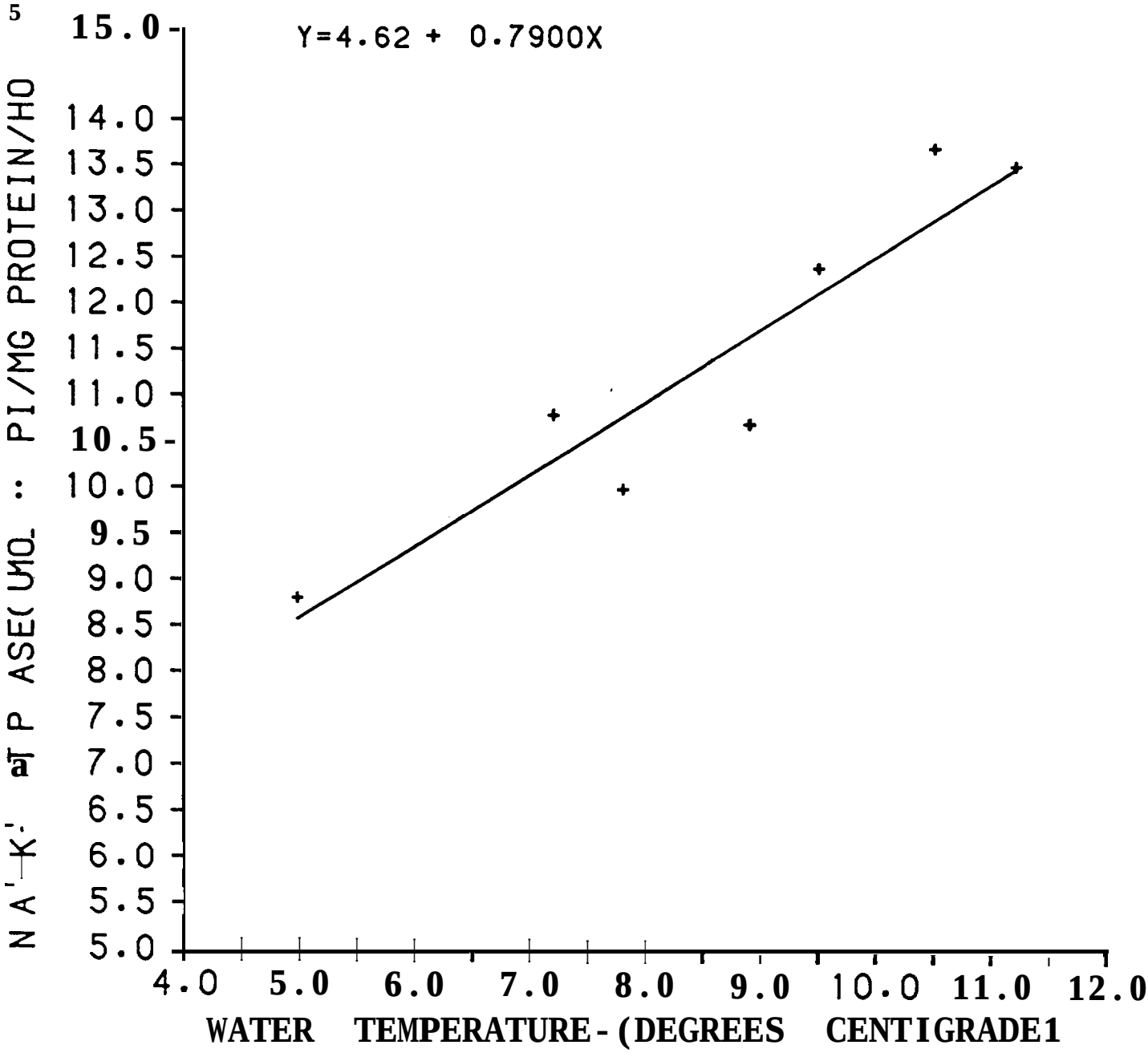


Figure 9.--Regression of average gill Na^+-K^+ATPase values of steelhead in Pond 4 (second release group) on water temperature at Tucannon Hatchery during spring 1980. r = 0.938; P = 0.002.

Table 15.--Correlation coefficients between Gill Na+-K+ ATPase values for each sampling period, and average fork length of Tucannon Hatchery steelhead used to provide gill samples in 1980 from Group II (Pond 4).

Item	Period 1	2	3	4	5	6	7
Correlation coefficient (DF=8)	0.44	-0.09	-0.19	0.13	0.69[a]	0.80[b]	0.45

[a] P<0.02

[b] P<0.01

If smolting was well developed between the sixth and seventh periods, and we use the sixth period as an approximate index, gill Na+-K+ ATPase values of 15 u moles Pi/mg protein/hour or more would represent 50% of the samples collected, and would probably be a high index of smoltification. The regression curve in Figure 10 suggests that we could expect fish >175 mm to have Na+-K+ ATPase values >15.

On the basis of this information, approximately 50% of the Group II steelhead would have been smolting between the sixth period and release (Figure 11).

Plasma electrolytes.--Data for Group II (Table 12) suggest that average plasma Na+ and Cl' values were well within the suggested ranges, but that some individual values were below the expected low in some periods. Mean plasma K+ levels were (as in the Dworshak NFH steelhead) erratic, Lower than the expected low range, and lower than in 1978 or 1979 (Table 10). Mean plasma Na reached an apparent peak early in April and mean plasma Cl' reached a minimum decline at the same time (Table 12).

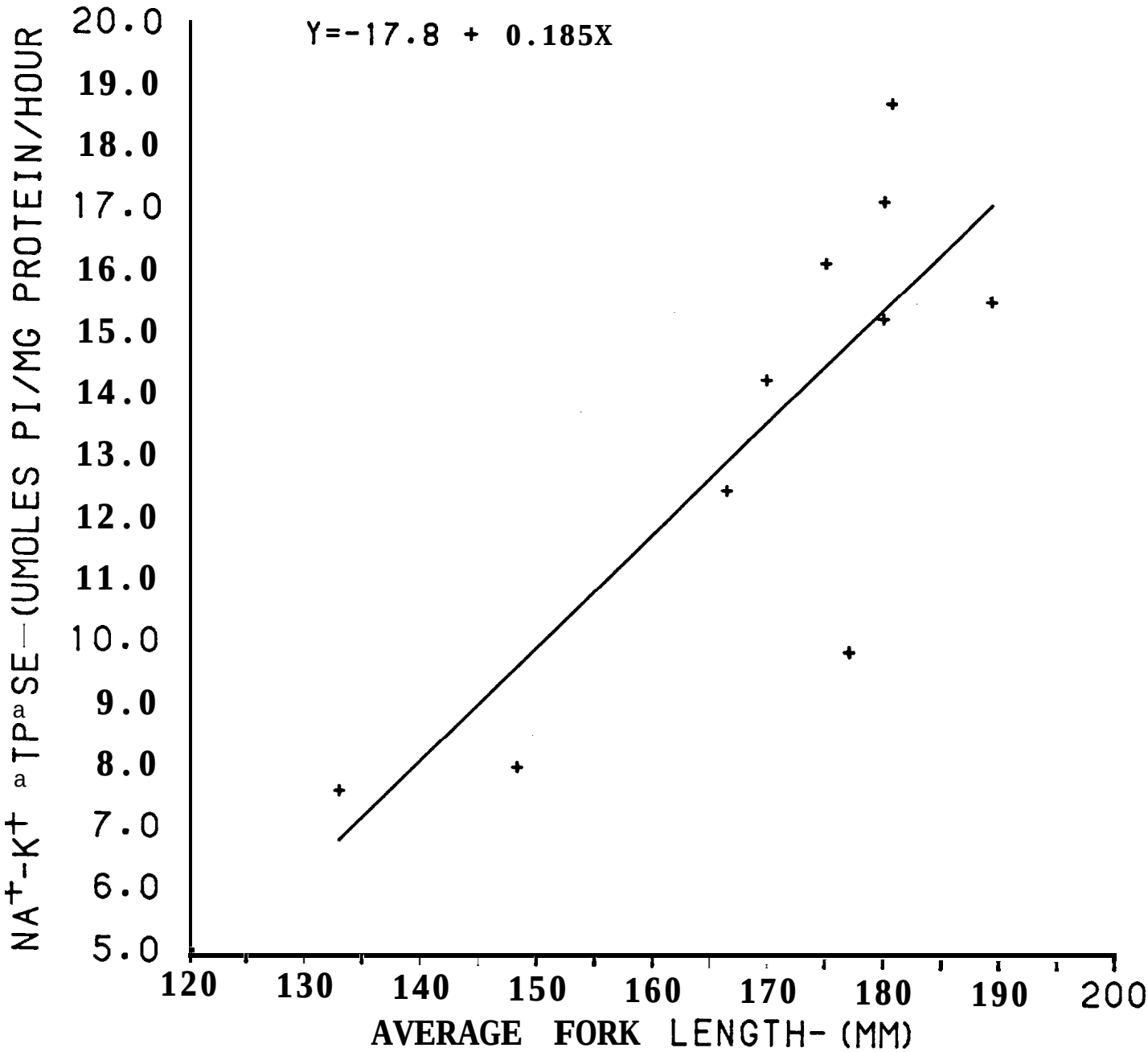


Figure .O.--Regression of average gill Na^+-K^+ATPase activity on average fork length of steelhead (Group II) in the sixth sampling period (3 fish pools for each Na^+-K^+ATPase analysis), 2 May 1980 (Tucannon Hatchery). r = 0.800; P < 0.05.

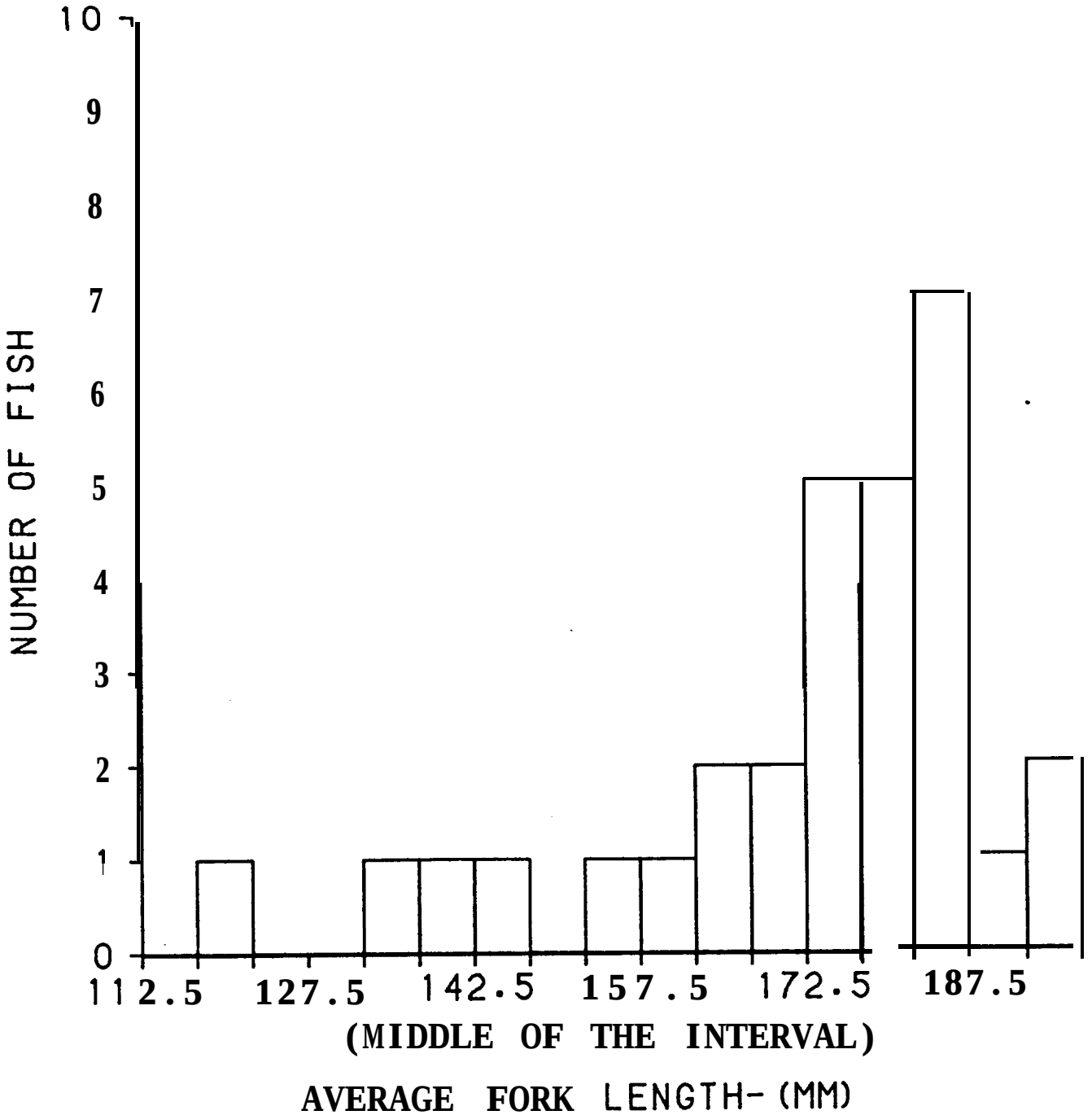


Figure 11.--Histogram of length-frequencies of Group IL Tucannon Hatchery steelhead sampled in the sixth period.

These high and low peaks also occurred at the same time for Group I (Table 11).

Plasma electrolytes of Group II could not be correlated with water' temperature or Na^+-K^+ ATPase activity, but mean plasma chlorides were significantly correlated with mean fork Lengths (Figure 12), and mean plasma K^+ values were significantly correlated with MCHC (Figure 13). Both plasma K^+ and MCHC can be stress indicators.

Hematology.--There were no indications of average hematological deficiencies in the Group 11 steelhead. Some of the individual hematocrits and hemoglobins were much higher than any expected values (Table 12), although not much different from samples collected in 1978 and 1979 (Table 10). Both hematocrits and hemoglobins rose and fell at the same times throughout the season, with hematocrits reaching a peak just prior to release (Table 12). There were no significant correlations between average hematological data and other factors measured, with the exception of average fork length, which was inversely related to MCHC (Figure 14). There was no significant correlation between average hematocrit and average hemoglobin values. However, in four of five sample periods in which hemoglobin was measured, individual hematocrit and hemoglobin values were significantly correlated. MCHC averages of steelhead in Pond 4 (Group II) paralleled those of the-first release (Group I) and then followed a trend similar to that of the Dworshak NFH steelhead (Tables 3, 11, and 12).

Comments.--A sample of 149 fish from Pond 4 was taken for Length and weight measurement during transfer to transportation vehicles on the day of release. The average fork length was 170.2 mm (±17.2), which was not

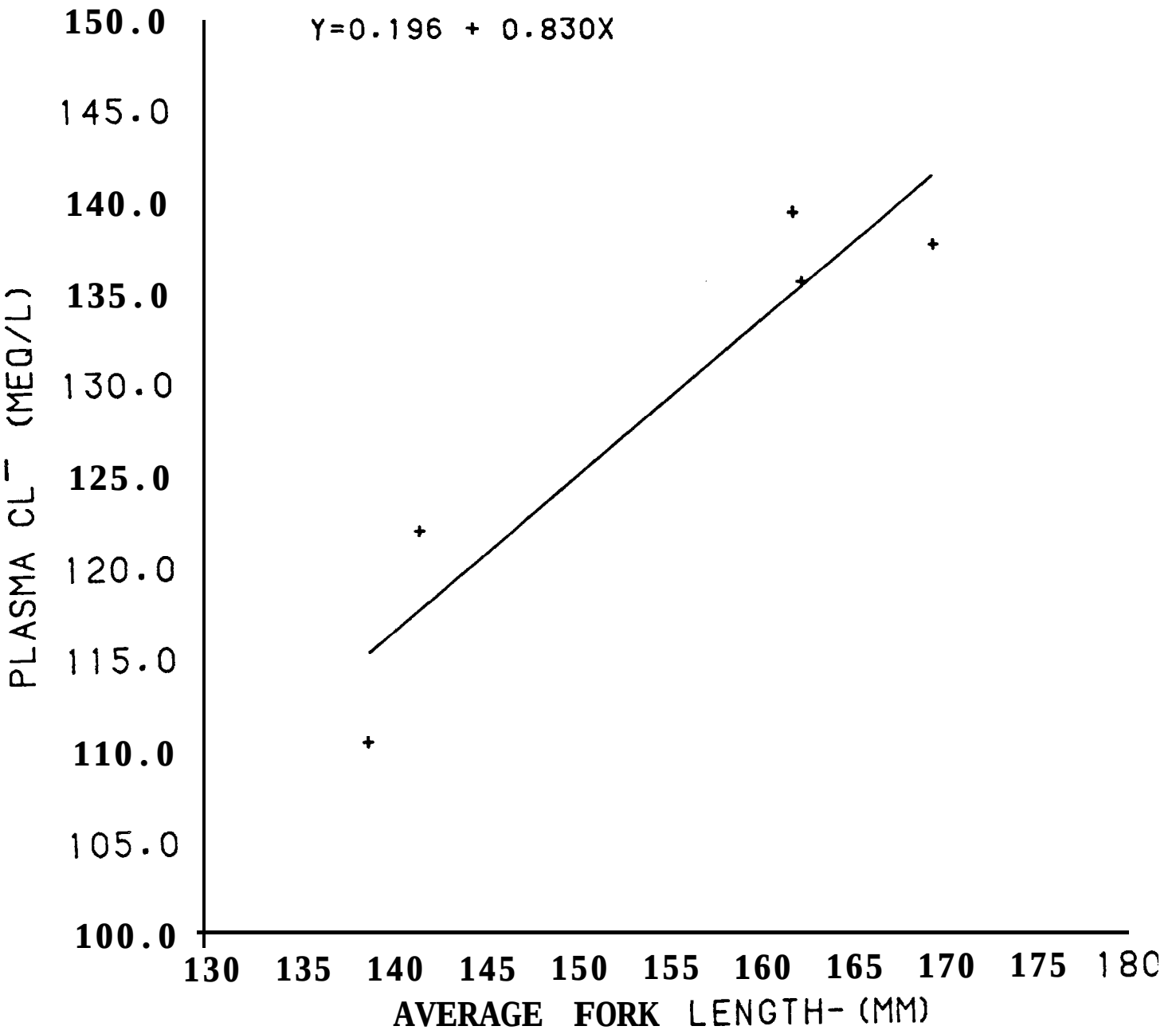


Figure 12.--Regression of average plasma Cl^- values of steelhead in Pond 4 (Group II) on average fork lengths at Tucannon Hatchery, spring 1980. $r = 0.936$; $P < 0.02$.

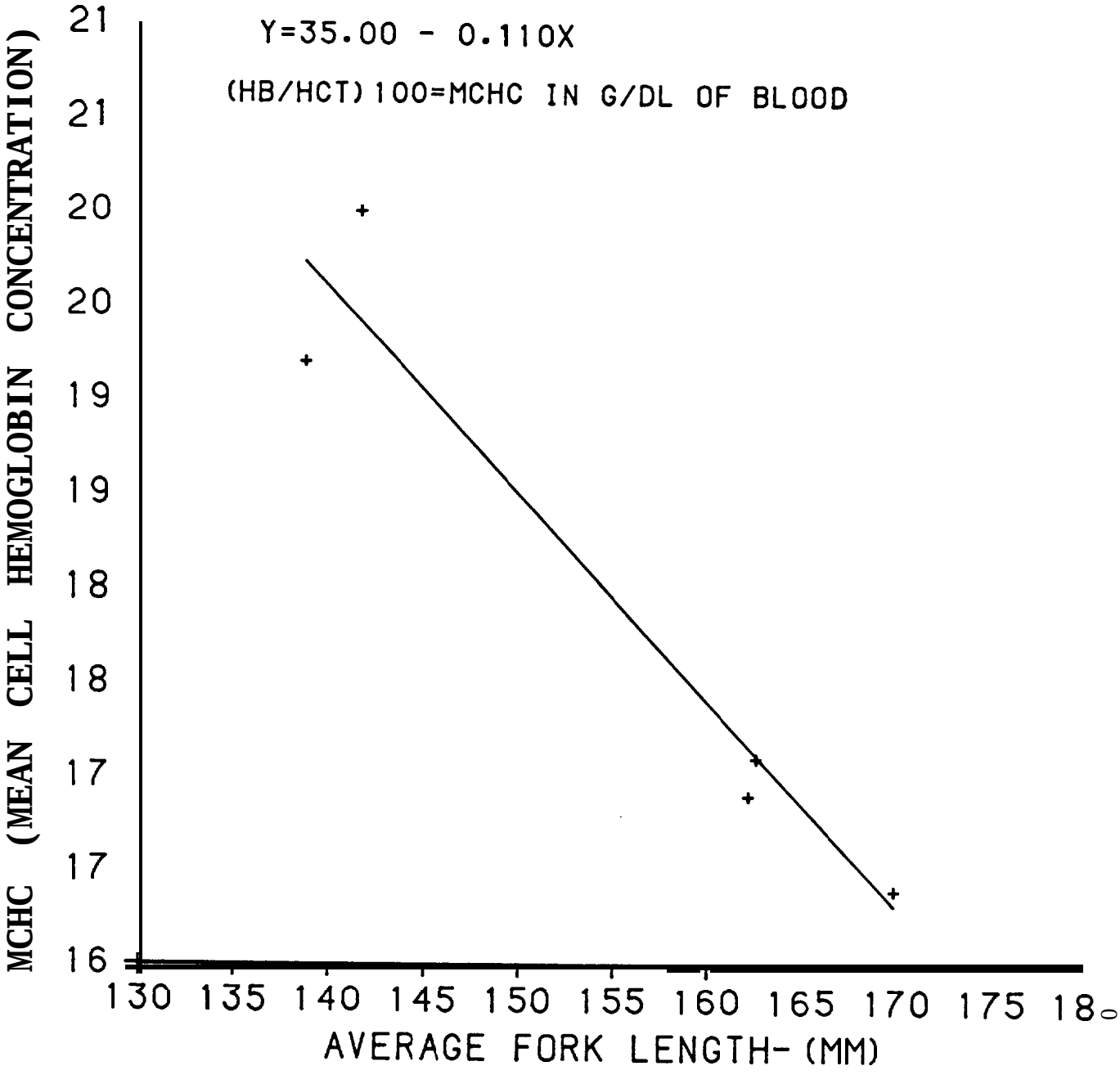


Figure 14.--Regression of average mean cell hemoglobin concentrations (MCHC) on average fork lengths of Tucannon Hatchery steelhead in Pond 4 (Group II) during spring 1980. $r = -0.965$; $P < 0.01$.

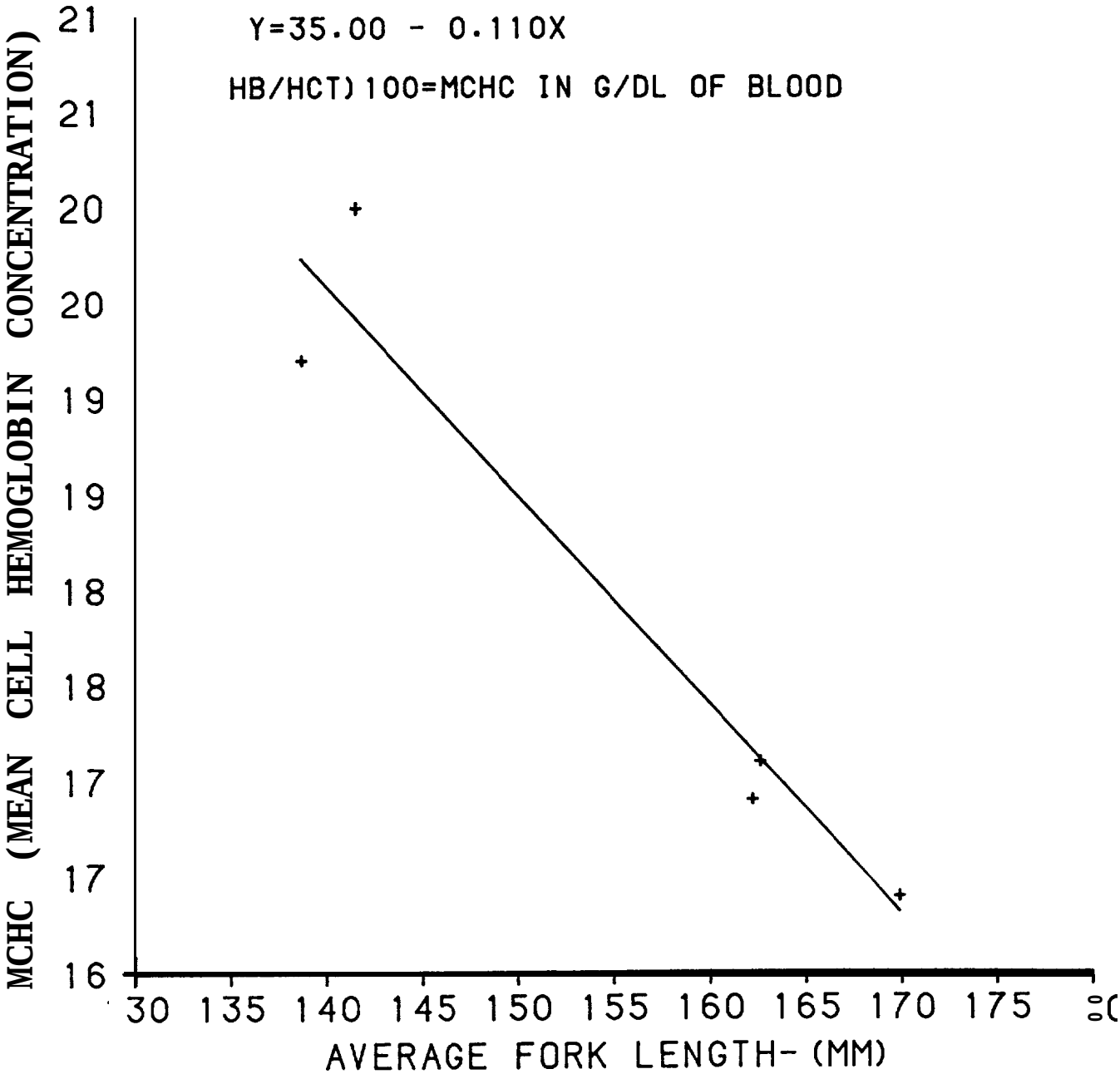


Figure 14 —Regression of average mean cell hemoglobin concentrations (MCHC) on average fork lengths of Tucannon Hatchery steelhead in Pond 4 (Group II) during spring 1980. $r = -0.965$; $P < 0.01$.

significantly different from mean fork lengths of samples collected for the health index (Table 12). The average weight was 54.9 g.

Group III (Pond 2, Table 13)

Gill Na^+-K^+ ATPase.--The Group III steelhead were released on 12 June. The Na^+-K^+ ATPase profile was parallel to and similar to that of Groups I and II early in the season, and shifted forward in time (Figure 8). However, the peak of the average Na^+-K^+ ATPase values occurred at the same time and with almost the same intensity as' the Group II steelhead on 8 May. This lasted for only 1 week and was followed by a steep decline. When the Group III steelhead were released, the average Na^+-K^+ ATPase value was at its lowest level. It is interesting to note that the maximum deviations in Na^+-K^+ ATPase activity in each group occurred at the peaks (Figure 8). Average fork lengths of Group III fish sampled during the last week of peak Na^+-K^+ ATPase activity (16 May) were not different from average fork lengths of Group I (10 April) or Group 11 fish (7 May) at release, and the average size of Group III at release (12 June) was almost identical to Group II (7 May) at release (Figure 7). The additional month of rearing time for Group III did not improve growth, and may have resulted in a post-smolt condition and reluctance to migrate.

If gill Na^+-K^+ ATPase activity can be considered one of the criteria for smolt indexing, then Group III fish were probably smolting during the week that the Group II fish were released. Note that elevated Na^+-K^+ ATPase activity in both Groups II and III had a high frequency of occurrence on 7 May, and Group III had a low frequency of occurrence by 12 June (Figure 15).

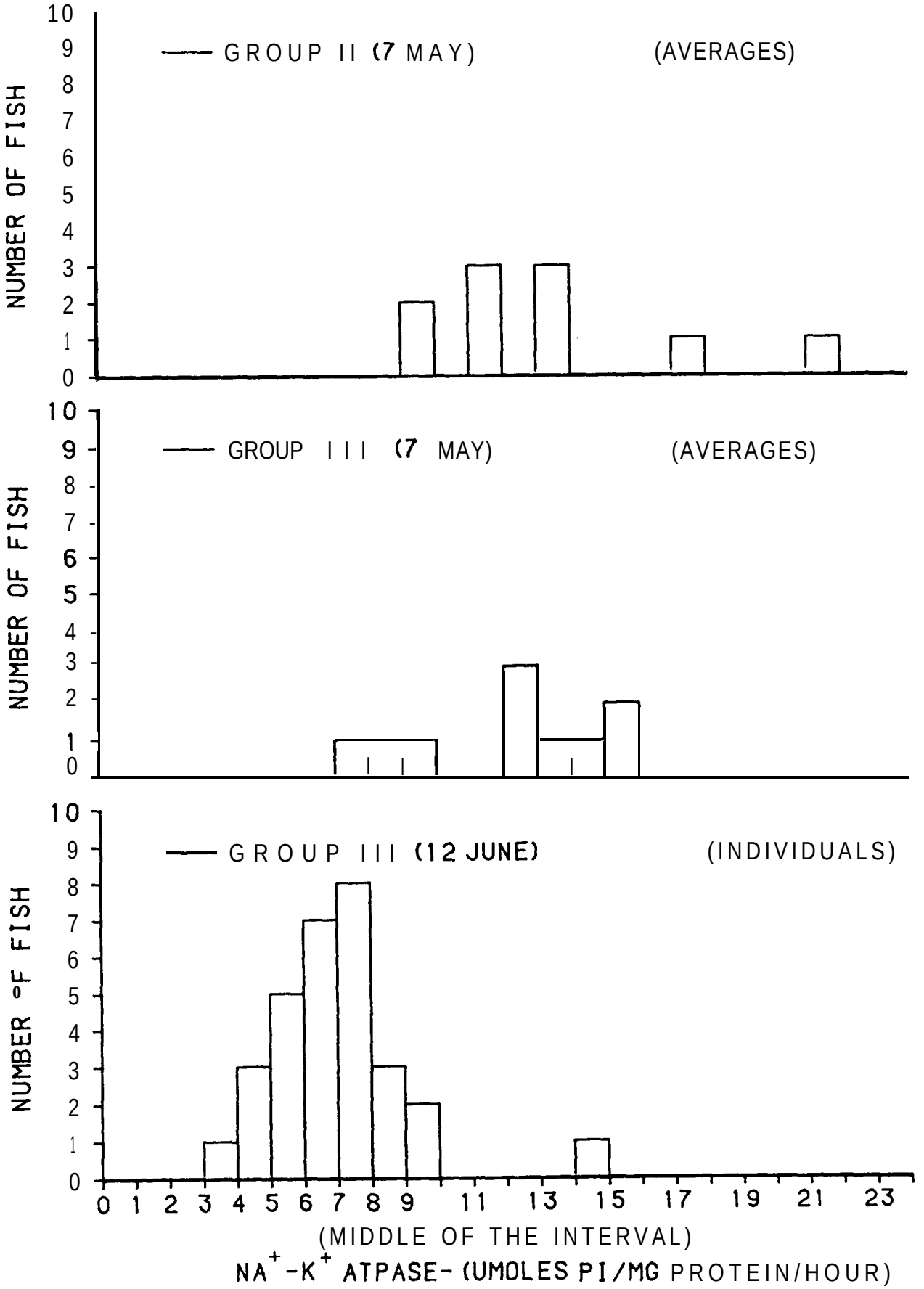


Figure 15.--Frequency histograms of average gill Na^+-K^+ ATPase values from Group II and Group III Tucannon Hatchery steelhead collected 7 May 1980, and individual Na -K ATPase values from Group III fish collected 12 June.

We could find no significant correlations between average gill Na^+-K^+ ATPase values and averages of other factors measured throughout the sampling periods (including water temperature). However, like Group II steelhead, there was a progressive increase throughout the spring in correlations between average Na+-K+ ATPase values and corresponding fork lengths for any one period (Table 16). Furthermore, these correlation coefficients reached the most significant levels on 2 May for both Groups II and III, and from that point on declined rapidly. This would indicate that on 12 June, a depressed Na+-K+ ATPase average for the population was not caused by the influence of larger post-smolted fish, and that all size groups had entered a post-smolt condition.

Plasma electrolytes. Data (Table 13) for Group III (Pond 2) suggests that average plasma Na+ and C1- were also (as with Group II) within expected values, but that some individual samples fell below the expected Levels in certain periods. Non-hemolyzed average plasma K+ values were (again) Lower than expected. Mean plasma Na+ and Cl- reached their first maximum divergence on 4 April (Table 13), which was also the time of maximum divergence for the first two groups. There was a maximum convergence 1 month later (2 weeks later than Group II), followed by another (greater) maximum divergence in early June. The mean plasma K+ and Na+/Cl- ratios followed the same cyclical pattern as Group II.

Mean plasma electrolyte values for the Group III steelhead could not be correlated with water temperature or mean Na+-K+ ATPase values. There was a positive correlation between average plasma Na+ values and average fork lengths for Group III (Figure 16); whereas in Group II, it was the average Cl- that was correlated to size. This suggests a time or

Table 16.--Correlation coefficients between Gill Na+-K+ ATPase values for each sampling period, and average fork lengths of Tucannon Hatchery steelhead used to provide gill samples in 1980 from Group III (Pond 2).

Item	Period 1	2	3	4	5	6	7	8	9	10	11
Correlation coefficient (DF=8)	0.26	0.45	0.43	0.03	0.45	0.87a/	0.79b/	0.32	0.64c/	0.37	0.30

a/ P<0.OOi

b/ P<0.Ol

c/ P<0.05

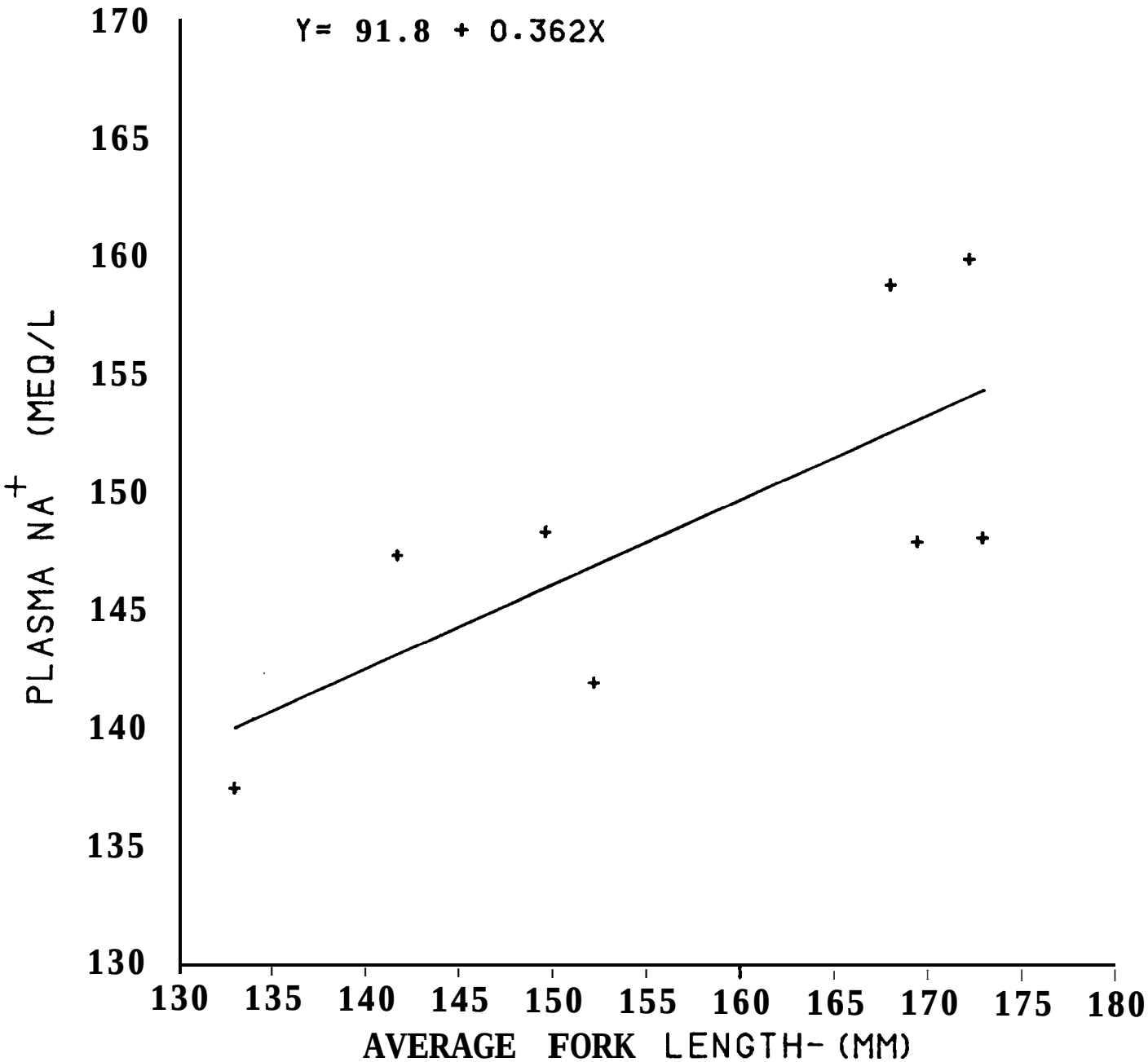


Figure 16.--Regression of average plasma Na^+ on average fork lengths for Pond 2 (Group III) Tucannon Hatchery steelhead; spring 1980. $r = 0.723$; $P < 0.05$.

seasonal dependence on these correlative factors. Conversely, MCHC and plasma K+ were again positively correlated (Figure 17) as in Group II, throughout the season, suggesting that these values are reflections of any factors that might induce stress.

The combination of a sharp decline in gill Na+-K+ ATPase activity, elevation in water temperatures, and elevated plasma K+ and MCHC values (indicating increased levels of stress), at the time Group III fish were released warrants rating the probable emigration and eventual return (as adults) much lower than Group II.

Hematology.--There were no indications of average hematological deficiencies in Group III steelhead. However, there was a small number of fish with either slightly depressed hematocrit values or values much higher than previously reported (Table 13). Average hematocrit and hemoglobin values as well as the mean cell hemoglobin concentrations rose and fell in much the same cyclical pattern, and at the same times as those in Group II. There were no significant correlations between the average hematocrit and hemoglobin levels, but in five out of eight samples the individual values were significantly correlated. In Group III steelhead, there was a significant positive correlation between average hematocrits and water temperature (Figure 18). This is inverse to the relationship found in Dworshak NFH steelhead (Figure 5), and suggests that changes in basic hematology in healthy hatchery steelhead may be a reflection of many factors.

Comments.--A subsample of 108 fish from Group III (Pond 2) were measured separately by sampling during transfer to hauling trucks on 12

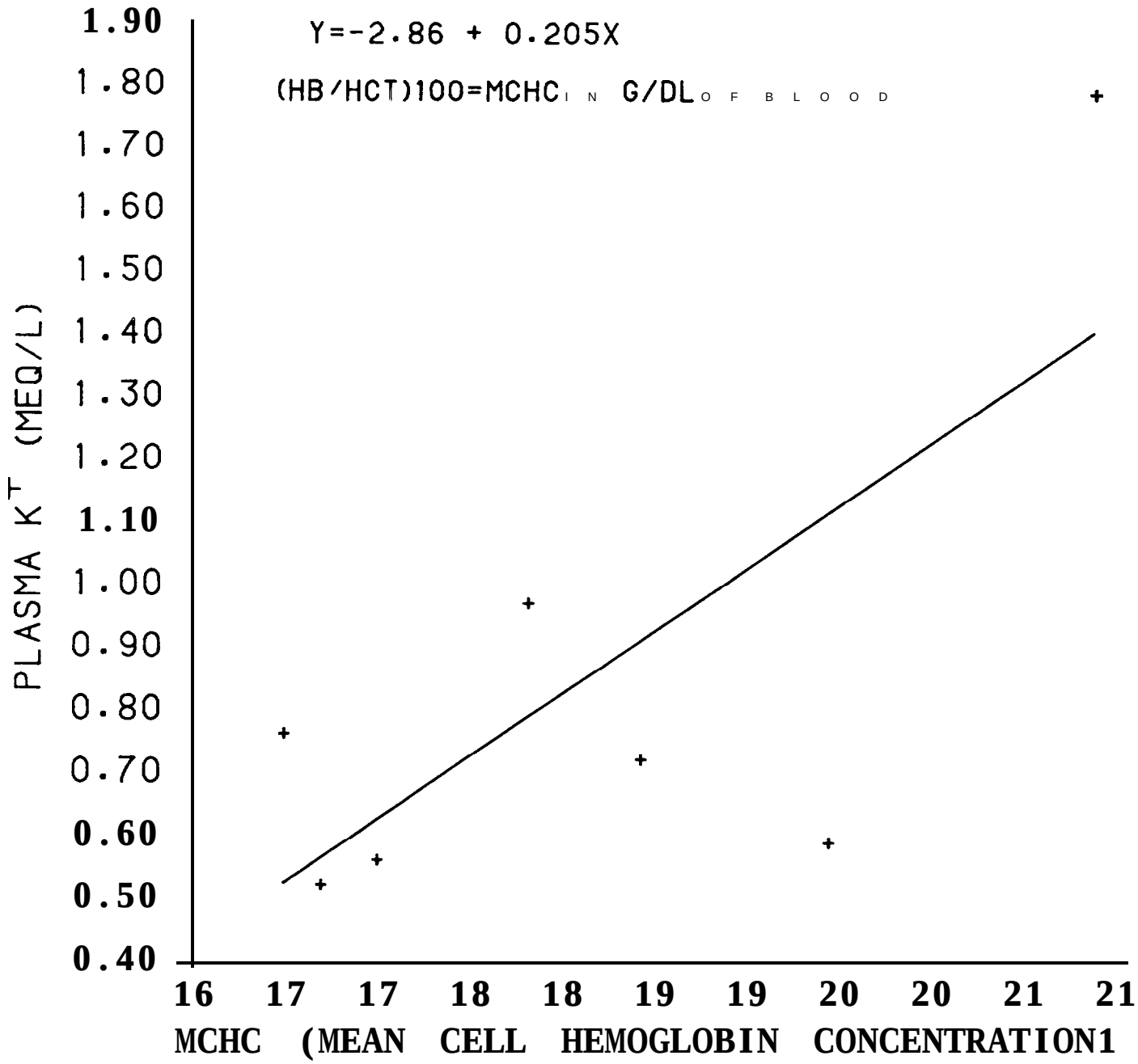


Figure 17.--Regression of average plasma K+ on average mean cell hemoglobin concentrations (MCHC) for Pond 2 (Group III) Tucannon Hatchery steelhead, spring 1980. r =).726; P < 0.05.

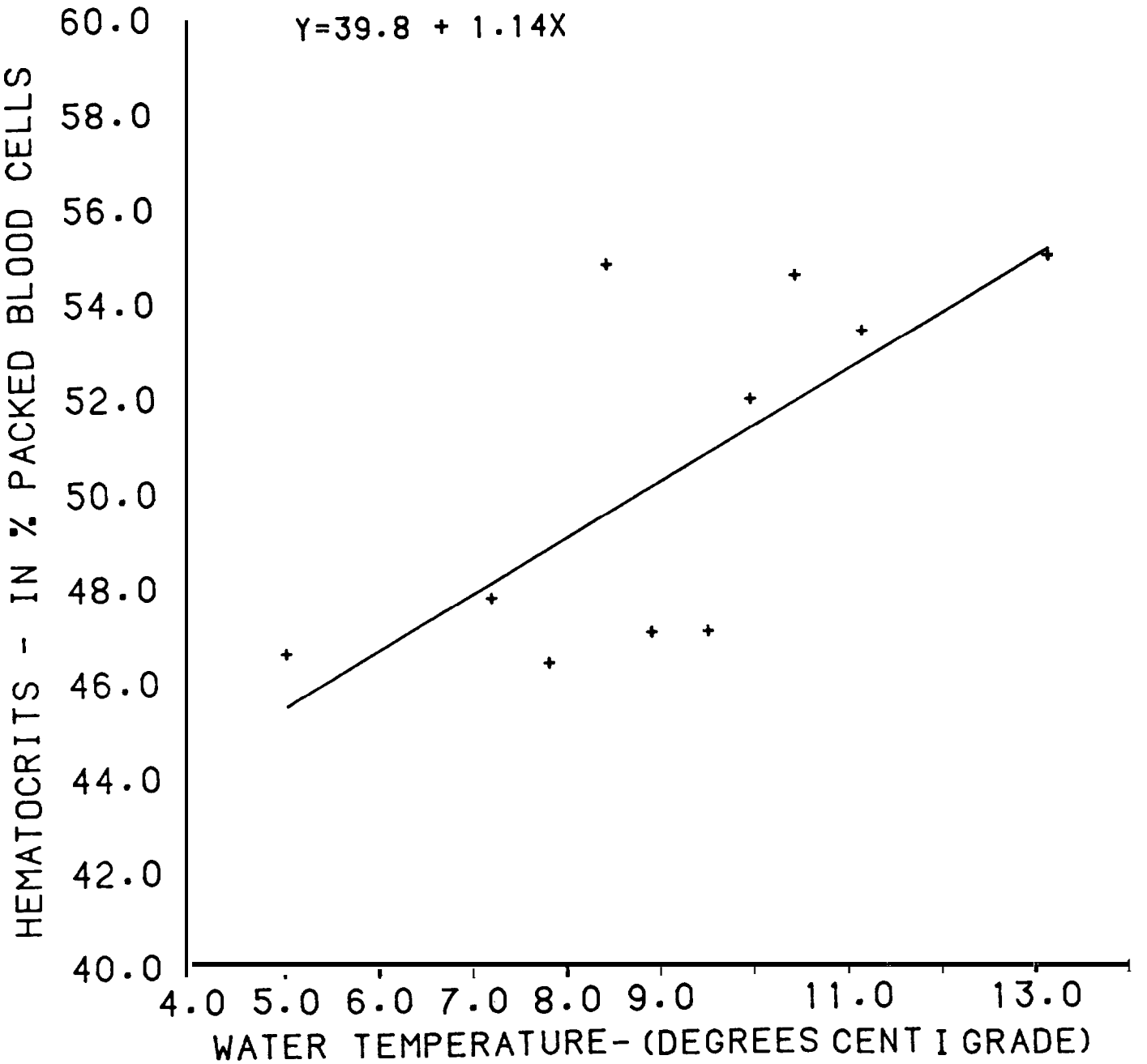


Figure 18.--Regression of average hematocrit values of Pond 2 (Group III) steelhead on the Tucannon Hatchery water temperatures during spring 1980. $r = 0.698$; $P < 0.05$.

June. The average fork length was 169.2 mm (±17.7 mm), which was not significantly different from the samples taken for health surveys on the same day (Table 13).

Group IV (Pond 5, Table 14)

Time constraints did not allow sampling this group as frequently as desired. However, summary data for Group IV (Table 14), and Figures 7 and 8 indicate that at release time (30 May), these fish were significantly smaller than any other group and had the lowest average gill Na^+-K^+ ATPase values. Other parameters measured were within expected ranges. However, the data collected indicate that this Group (IV) would not be expected to emigrate or to return from the sea as well as Group II.

Summary of the Tucannon Steelhead

IFAT-BKD.--Limitations in sampling the usual 60 fish from each pond on a serial basis at the Tucannon Hatchery do not provide a good statistical base for estimating the incidence of BKD infection in the populations. Therefore, the data are presented here primarily to indicate that the incidence and intensity of infection were low (Table 17).

The greatest incidence occurred in Group III (late release) steelhead (21.7%), but the intensity of infection was still low, at between 1 and 7 (more frequently 1 to 2) organisms/l50 microscopic fields (mf) examined. Therefore, BKD would not be expected to affect differential survival of these groups.

Histopathology.--Detailed summaries of the pathological conditions observed in each pond are presented in Tables 18, 19, and 20. For

Table 17.--Rate (%) and intensity of infection of BKD organisms found in the anterior and/or posterior kidneys of Tucannon Hatchery steelhead by IFAT during the 1980 sampling periods.

	Period		
	3	7	11
Date	4 April	7 May	12 June
	% of samples infected		
Group I (Pond 1)	0.0% (Released) (N=20)	---	---
Group II (Pond 4)	20.4% (N=20)	6.7% (Released) (N=30)	---
Group III (Pond 2)	5.0% (N=20) ---	3.3% (N=30) ---	21.7% (N=60) ---
Pooled	25.4% (N=60)	10.0% (N=60)	21.7% (N=60)
Range of intensity (as no. of organisms/ 150 microscopic fields)	1 to 15	1 to 2	1 to 7

Table 18.--Pathological conditions observed in 1980 Tucannon Hatchery steelhead and their percentage of incidence[a] (Group I, Pond 1, n = 20) (3rd period--released 4 April).

Organ and pathology	Incidence (%) Severity[b] I	II	III	Total
Eye				
Skeletal muscle lesions	35.0	0	0	35.0
Retrobulbar fat lesions	0	5.0	0	5.0
Gills				
Increased numbers of lymphocytes	65.0	20.0	0	85.0
Epithelial cell formation	40.0	45.0	10.0	95.0
Olfactory sac				
Focal mononuclear cell infiltration	20.0	20.0	0	40.0

a/ Brain tissue was processed and examined for all specimens, and there was no evidence of any pathology.

b/ I-recognizable (least severe); II-intermediate; III-severe.

Table 19.--Pathological conditions observed in 1980 Tucannon Hatchery steelhead and their percentage of incidence [a] (Group II; Pond 4; 3rd period--released 4 April, n = 20; 7th period--released 7 May n = 30).

Organ and pathology	Incidence (%)							
	Severity[b]				Severity			
	I	II	III	Total	I	II	III	Total
Eye								
Skeletal muscle lesions	25.0	0	0	25.0	80.0	0	0	80.0
Gills								
Increased numbers of lympocytes	50.0	5.0	0	55.0	60.0	40.0	0	100.0
Epithelial cell formation	40.0	50.0	0	90.0	23.3	63.3	13.4	100.0
Vascular telangiectasis of secondary lamellae	10.0	0	0		23.3	0	0	23.3
Bacteria present	0	0	0		3.3	0	0	3.3
Olfactory sac								
Focal mononuclear cell infiltration	25.0	5.0	0	30.0	56.7	40.0	0	96.7
Pyogranulomatous inflammation	5.0	0	0	5.0	0	0	0	0

[a] Brain tissue was processed and examined for all specimens, and there was no evidence of any pathology.

[b] I-recognizable (least severe); II-intermediate; III-severe.

Table 20. Pathological conditions observed in 1980 Tucannon Hatchery steelhead and their percentage of incidence a/ (Group III; Pond 2; 3rd period--released 4 April, n = 20; 7th period--released 7 May, n = 30; 11th period--released 12 June, n = 60).

Organ and pathology	Incidence (%)											
	Severity b/				Severity				Severity			
	I	II	III	Total	I	II	III	Total	I	II	III	Total
Eye												
Skeletal muscle lesions	25.0	10.0	0	25.0	80.0	0	0	80.0	70.0	0	0	70.0
Gills												
Increased numbers of lympocytes	45.0	30.0	0	75.0	76.7	16.7	0	93.4	30.0	68.3	0	98.3
Epithelial cell formation	35.0	50.0	5.0	90.0	56.7	36.7	0	93.4	43.3	46.7	6.7	96.7
Lymphatic telangiectasis of secondary lamellae	0	5.0	0	5.0	6.7	0	0	6.7	0	0	0	0
Vascular telangiectasis of secondary lamellae	5.0	5.0	0	10.0	0	0	0	0	0	0	0	0
Acute focal hemmorhage	15.0	5.0	0	20.0	0	0	0	0	0	0	0	0
Olfactory sac												
Focal mononuclear cell infiltration	30.0	10.0	0	40.0	76.7	23.3	0	100.0	40.0	58.3	0	98.3

a/ Brain tissue was processed and examined for all specimens, and there was no evidence of any pathology.

b/ I-recognizable (least severe); II-intermediate; III-severe.

comparative purposes, these data are joined and summarized in Table 9 with data from the other hatcheries.

Data collected for Tucannon Hatchery steelhead for each pond indicate comparable tissue pathology for each of the three sample periods. Sample sizes varied (for each pond) between periods as fish were released.

In the first sample (3rd period; 4 April), there appeared to be little variation in the types or frequency of occurrence (Table 18) of pathology found in steelhead from either pond. In the second sample (7th period; 7 May), pathological conditions in the remaining Groups (II and III) were comparable (Tables 19 and 20).

Comparing combined data for the sampling periods (Table 9) shows striking increases in the frequency and intensity of pathological conditions of the eye and olfactory sac, and recognizable increases in gill pathology after the first sampling (third period).

Since 1980 was the only year that serial samples were collected, comparisons of tissue pathology between years can only be made for the first week in May (Table 21). The frequency of occurrence in olfactory sac pathology doubled between 1979 and 1980, and it is remarkable that the entire incidence in 1979 was due to ciliated protozoan parasites whereas none were found in 1980. The frequency of eye lesions varied greatly for all 3 years, and gill pathology markedly increased after 1978.

Table 21.--A summarized comparison of the frequency of occurence (%) of tissue pathology observed in Tucannon Hatchery steelhead the first week in May of 1978, 1979, and 1980.

Tissue	Year 1978	1979	1980
Eye	57	2	80
Olfactory sac	a/	54	97
Gill			
Lymphoid	23	100	97
Epithelial	32	100	98

a/ Not available.

Hatchery records.--Information provided by the hatchery (Table 2), indicated that Formalin and malachite green (MG) were used to control periodic parasitic infestations. Our own field notes indicate that on 18 April 1980, a 24-hour 25 ppm Fromalin drip treatment was started on Group II fish, followed shortly thereafter by a treatment with MG. Hyamine was used at 2 ppm for 20, 40, and 60 minutes on 29 and 31 May and 1, 7, and 8 June 1980 to control a light myxobacterial infection of the gills (Group III). Group III was also fed a TM-50 diet from 2 June to 12 June 1980.

Genreal comments.--Group IV, smallest of the Tucannon steelhead in 1980, averaged 35 to 40 mm shorter than any other group at release time (Figure 7), and was below normal in average gill NA+-K+ ATPase values (Figure 8). Although other data for this group are not available, the below normal size and Low average Na+-K+ ATPase values indicate that

survival and returns from this group will be low. Field notes indicated that fish over 150 mm in fork Length appeared smolted.

Our field notes of visual observations indicate that most fish in Groups II and III below 170 mm in fork length were still parred in mid-April, and by the first week in May, Group II was smolting rapidly, with Group III about 1 week later. By mid-May, steelehad greater than 163 mm (±3 mm) in fork length in Group II appeared smolted.

Key physiological indicators of migratory readiness, gill Na+-K+ ATPase and plasma Na+ and Cl^-, were in the smolt indicating segments of their respective profiles between the first and third weeks in May for Group III fish. By the time of release, these indications had disappeared, and there was a sharp increase in possible stress indicators, plasma K^+ and MCHC. Although the incidence of tissue patholgoy did not increase between May and early June, the requirements of additional therapeutic treatments could increase the stress factors for Group III. The analyzed data indicate that Group III fish were probably in a post-smolt condition (regardless of size) by 12 June, and that all of these factors will have a negative influence on their survival and return. Correlations between fish size and gill Na^+-K^+ ATPase at the time of release were poor, and no prognosis on percentages of expected migration should be made.

Group II fish represented the normal early May release (as in 1978 and 1979), which, in 1980, occurred at the probable peak activity of one of the main smolting indicators, gill Na^+-K^+ ATPase (Figure 8), but about 3 weeks after the maximum deviation of plasma Na^+ and CL' (Table 12). Stress indicators (plasma K^+ and MCHC) were low at release and good positive correlations between gill Na^+-K^+ ATPase and fork lengths just

prior to release suggest that at least 50% of the group were in a good state of migratory preparedness.

There were no correlations for the earliest Group (!) released between gill Na^+-K^+ ATPase and other parameters measured that would aid in prognosis of comparative survival or expected smolt indexing, but it is quite clear that their physiological condition was much different than Group III fish at release. In Group III (12 June), mean Na^+-K^+ ATPase activity (Figure 8) and variance (Table 13) was low, indicating that regardless of size, the fish were in a post-smolt condition. However, in Group I, at release (10 April), the mean Na^+-K^+ ATPase was elevated (Figure 8), and the variance was large (Table 11). The lack of correlation between fish size and Na^+-K^+ ATPase suggests that smolting was occuring regardless of size. We indicated that over 80% of the normal release Group (II) samples (Figures 10 and 11) and 90% of Group I samples (Figure 19) had Na^+-K^+ ATPase values greater than 10 at the time of release (7 May). Furthermore, plasma Na^+ had reached maximum and plasma Cl^- had reached minimum values at the time of release (Group I, Table 11). The incidence of any tissue pathology was lowest in Group I at time of release (Tables 9 and la), and average size was quite close to that of other major release groups (Figure 7). All of these factors combined suggest that the ear&y release group may have had a fair migratory development.

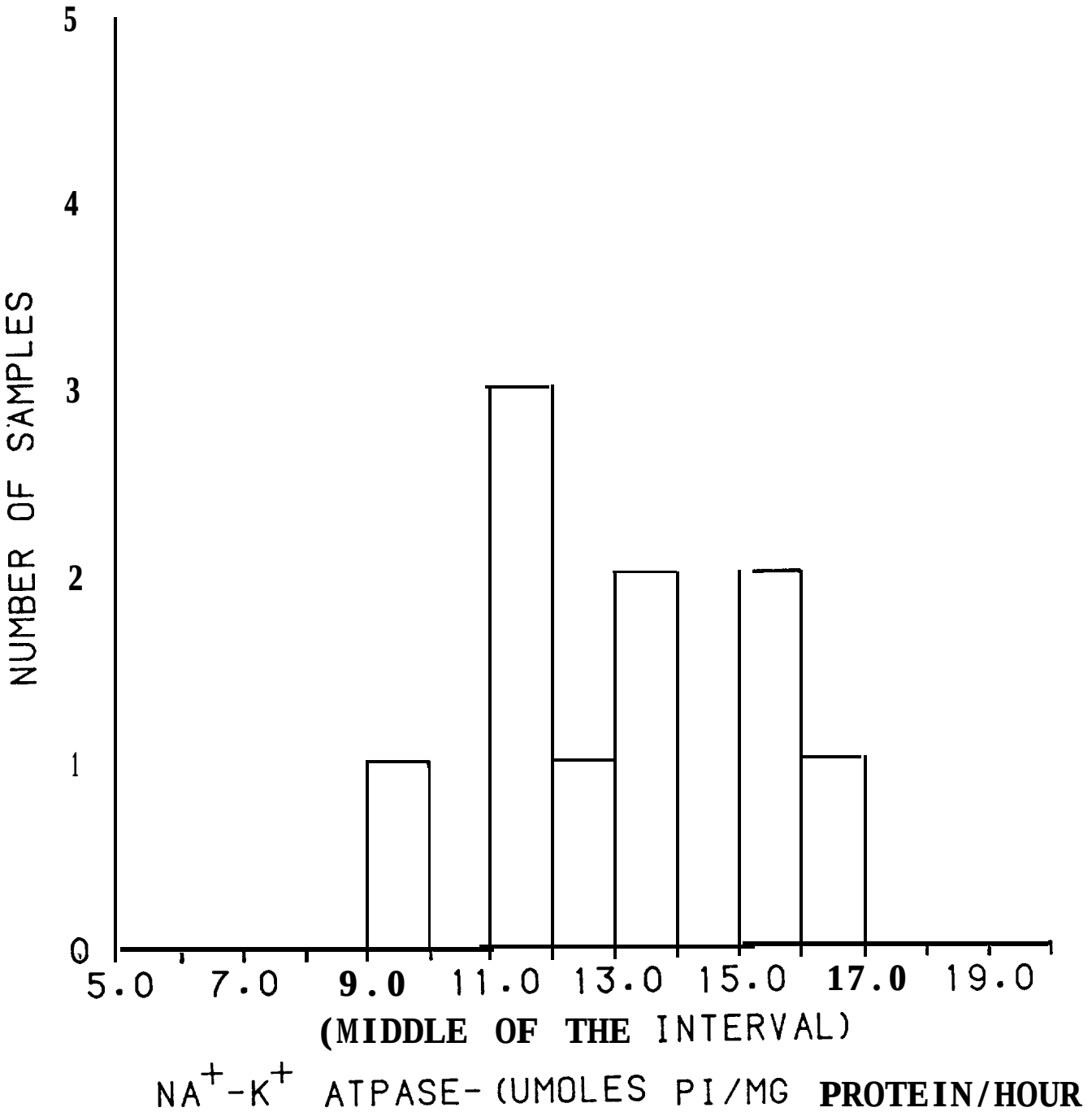


Figure 19.--Frequency histogram of the average gill Na^+-K^+ ATPase values of Group I Tucannon Hatchery steelhead at the time of release (10 April 1980).

RESULTS AND DISCUSSION OF SPRING CHINOOK SALMON SURVEYS

Leavenworth National Fish Hatchery

General

Two sampling trips to Leavenworth NFH were made in late 1979 (Table 22) primarily to test procedures and to sample during a fall marking test. We began collecting full data in March of 1980, and sampling continued into early June. Until early April, the fall-mark group was cultured in colder (Icicle Creek) water. For example, on the first trip in March, water temperature was 3.0°C for the fall-mark group and 6.1°C in the other ponds.

There were 16 different tagged groups that were released under varying conditions between 17 April and 1 May 1982, the bulk of which were released between 24 April and 1 May. Data collected primarily from one normal pond (#17) and the fall-mark group in Late 1979 are presented in Table 22.

Sampling period days are presented with 1 January 1980 as Day +l and 31 December 1979 as Day -1 to simplify computer programming, and are presented in Table 22 along with the calendar dates for each sampling period. In the fifth period (23 April), samples were collected from Pond 17 and other test groups primarily for Na+-K+ ATPase analysis, and these data are presented elsewhere. Sample sizes were reduced to 30 fish/test group in this period.

Figure 20 shows the water temperatures measured at the hatchery at each sampling period in 1980. Temperatures between November 1979 and March 1980 are not shown.

Leavenworth NFH spring chinook salmon were not used for homing experiments in 1978 or 1979, but they were included in a smolt evaluation

Table 22.--Summary data for 1978 brood spring chinook salmon samples collected at Leavenworth National Hatchery in late 1979 and in the spring of 1980, with means, standard deviations ( ), and ranges. Sample size = 60 except for the 5th Period (n=30).

Item	Period A	B	1	2	3	4	5	6	7	8	9
Date	1 Nov 79	14 Nov 79	3 Mar 80	17 Mar 80	31 Mar 80	14 Apr 80	23 Apr 80	28 Apr 80	12 May H0	29 May 80	17 June 80
Days>Jal[a]	-61	-47	63	76	90	104	113	118	132	149	168
Temp. °C[b]	3.0	2.1	6.1	4.5	4.0	6.8	6.1	5.5	6.0	8.0	9.5
Avg. F-K Ln[c]	110.3	112.5	124.8	123.7	136.2	130.4	129.1	132.7	133.4	137.7	140.3
	(10.8)	(11.9)	(16.1)	(12.0)	(18.1)	(12.8)	(8.7)	(13.2)	(10.6)	(15.1)	(10.7)
(Range)	92-141	85-145	98-1 72	97-1 52	106-188	109-164	112-152	106-166	108-162	118-183	126-176
Avg. ATP Fk Ln[d]	---	---	124.4	124.7	135.2	124.3	129. 1	134.6	134.0	138.7	142.4
	---	---	(8.1)	(6.8)	(7.5)	(6.4)	(6.0)	(13.0)	(10.5)	(13.7)	(9.4)
(Range)	---	---	117.0-143.3	117.3-133.7	127.3-150.3	114.7-132.7	121.3-140.7	119.7-164.3	115.0-146.7	121.3-164.0	132.3-156.3
Avg. ATP[e]	---	---	7.0	7.2	10.6	9.3	7.7	12.5	13.7	13.2	10.1
	---	---	(1.3)	(1.2)	(1.4)	(1.1)	(1.4)	(2.2)	(1.6)	(3.1)	(3.3)
(Range)	---	---	5.4-9.4	5.6-9.9	8.3-1 2.6	7.8-11.6	5.7-10.6	9.2-16.3	11.5-15.8	7.3-19.4	4.6-15.1
Avg. Hct[f]	40.2	47.8	46.5	43.6	39.8	41.1	39.6	40.6	34.2	33.5	30.5
	(4.8)	(6.0)	(6.7)	(6.6)	(8.1)	(8.3)	(5.7)	(10.0)	(8.9)	(8.1)	(7.9)
(Range)	31-51	37-71	27-60	24-59	17-56	15-60	25-50	19-64	16-54	13-56	8-48
Avg. Hb[g]	6.5	7.5	6.6	6.5	6.8	6.1		5.9	5.5	5.2	5.0
	(0.6)	(0.8)	(1.1)	(1.5)	(1.6)	(1.4)	---	(1.4)	(1.6)	(1.6)	(1.7)
(Range)	5.0-7.7	4.7-9.0	3.7-9.3	2.0-9.7	2.9-9.7	1.7-9.0	---	3.2-8.7	2.3-8.7	1.0-10.0	1.3-8.3
Avg. MCHC[h]	16.4	15.9	14.2	14.9	17.0	14.9	---	14.7	16.2	15.4	16.1
	(1.6)	(1.8)	(1.1)	(3.5)	(1.9)	(1.8)	---	(1.8)	(2.3)	(3.2)	(2.6)
(Range)	13.4-19.7	9.5-20.0	11.1-16.6	4.4-30.3	13.0-21.1	9.1-18.2	---	10.8-18.8	11.0-21.2	6.7-25.6	8.9-22.4
Avg. Na^+[i]			150.8	146.5	146.6	154.6	---	155.2	155.6	143.4	136.8
			(11.9)	(14.3)	(12.9)	(4.9)	---	(8.1)	(7.7)	(11.0)	(10.9)
(Range)	---	---	132-180	117-169	119-167	144-1 65	---	134-1 65	140-185	85-J 59	104-156
Avg. K^+[j]	---	---	0.65	0.69	0.56	1.25	---	0.88	0.47	0.96	1.99
	---	---	(0.60)	(0.41)	(0.26)	(0.77)	---	(0.66)	(0.25)	(0.88)	(1.23)
(Range)	---	---	0.32-2.7	0.40-2.3	0.33-1.24	0.47-2.85	---	0.20-2.70	0.21-1.06	0.15-4.04	0.21-4.8
Avg. Cl^-[k]	---	---	133.6	136.8	134.0	131.4		134.5	129.1	118.7	130.4
	---	---	(12.8)	(10.3)	(15.6)	(6.0)	---	(7.4)	(11.8)	(10.3)	(9.4)
(Range)	---	---	122-166	124-1 55	106-158	120-1 44	---	15.0-145.0	103-169	87-1 35	112-155
Na^+/Cl^-[l]	---	---	1.13	1.08	1.10	1.18		1.16	1.21	1.23	1.05
	---	---	(0.09)	(0.12)	(0.10)	(0.06)		(0.03)	(0.10)	(0.10)	(0.10)
(Range)	---	---	1.02-1.34	0.79-1.28	0.83-1.25	1.05-1.29		1.10-1.21	1.07-1.56	1.02-1.61	0.74-1.29

a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C.) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour.)
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Ave. Cl-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period, averaged.

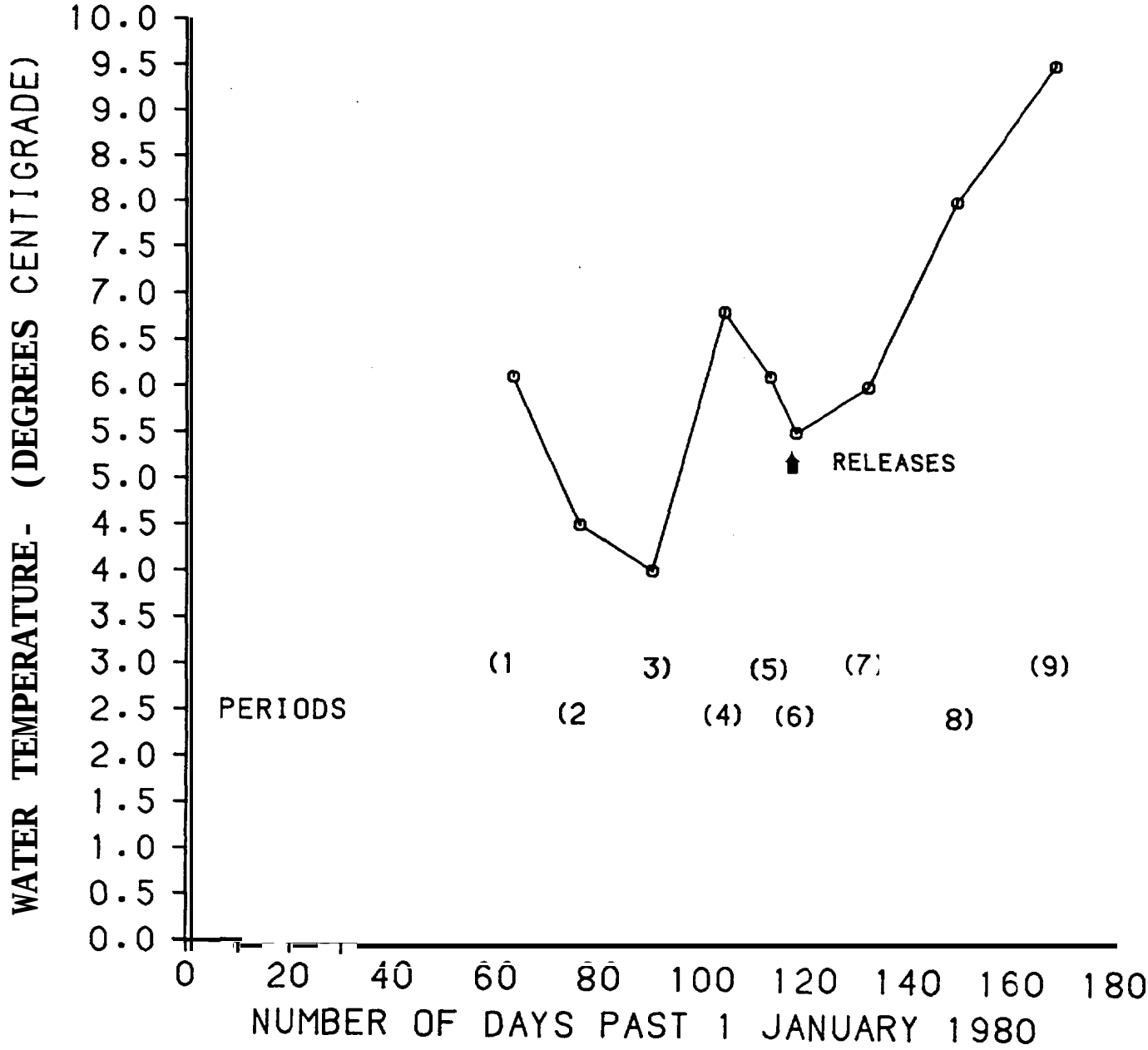


Figure 20.--Water temperatures of the outdoor raceways at Leavenworth NFH during spring 1980.

study (Anon 1979; Prentice et al. 1980). Mean fork lengths of samples collected from the various test groups released during the fifth period (23 April) ranged from 121.9 to 134.6 mm (Table 23). There was a significant difference (P = 0.02) between mean length of the fall-mark group (II) and the controls (III) in 1980 (Table 23). Mean lengths of samples collected at release were 138 and 139 mm in 1978 (25 April) and 131 mm in 1979 (24 April).

Gill Na^+-K^+ ATPase

In 1978, gill Na^+-K^+ ATPase values peaked in early April, stabilized through April, and began a gradual decline in early May (Figure 21, Anon 1979). The profile in 1979 was almost identical to that of 1980 with the exception of a temporary decline in 1980 the first 3 weeks in April (Figure 21). The range of values was similar for the 3 years. In 1980, the releases were ongoing during the period of maximum Na^+-K^+ ATPase rise (Figure 21). Manipulations of water temperatures (by mixing Icicle River water and well water) and/or changes in ponding procedures may have had some effect on the Na^+-K^+ ATPase profiles between years.

The summary gill Na^+-K^+ ATPase data collected shows substantial differences in mean values among the various groups at the time of release (Table 23). There was a significant difference (P = 0.006) between mean Na^+-K^+ ATPase values of Group III (considered controls), and means of Group I (by-pass channel Group); between Groups III and II, the fall-mark group (P = 0.013); and between Groups III and IV, the channel control (P = 0.0000).

We also examined relationships between mean fork lengths of pooled fish used for Na^+-K^+ ATPase analysis and gill Na^+-K^+ ATPase values

Table 23.--A summary of basic data collected from representative samples of various release groups of Leavenworth Hatchery spring chinook salmon (23 April 1980).

Parameter	Representative Group I	II	III	IV
Fork length				
Means	130.0	121.9	129.1	134.6
S.D.	(13.1)	(14.2)	(8.7)	(14.6)
Range	113-168	102-156	112-152	116-180
N	(30)	(30)	(30)	(30)
Hematocrits				
Means	41.4	31.1	39.6	34.2
S.D.	(6.4)	(9.1)	(5.7)	(7.1)
Range	23-50	3-43	25-50	13-46
N	(29)	(26)	(28)	(28)
Gill Na^+-K^+ ATPase				
Means	10.3	9.7	7.7	11.1
S.D.	(2.3)	(1.8)	(1.4)	(1.8)
Range	7.4-15.9	7.3-13.7	5.7-10.6	8.8-15.7
N	(10)	(10)	(10)	(10)

I = Typical bypass channel test group.
II = Fall-mark group (Pond 46).
III = Normal (Pond 17)
IV = Channel control group.

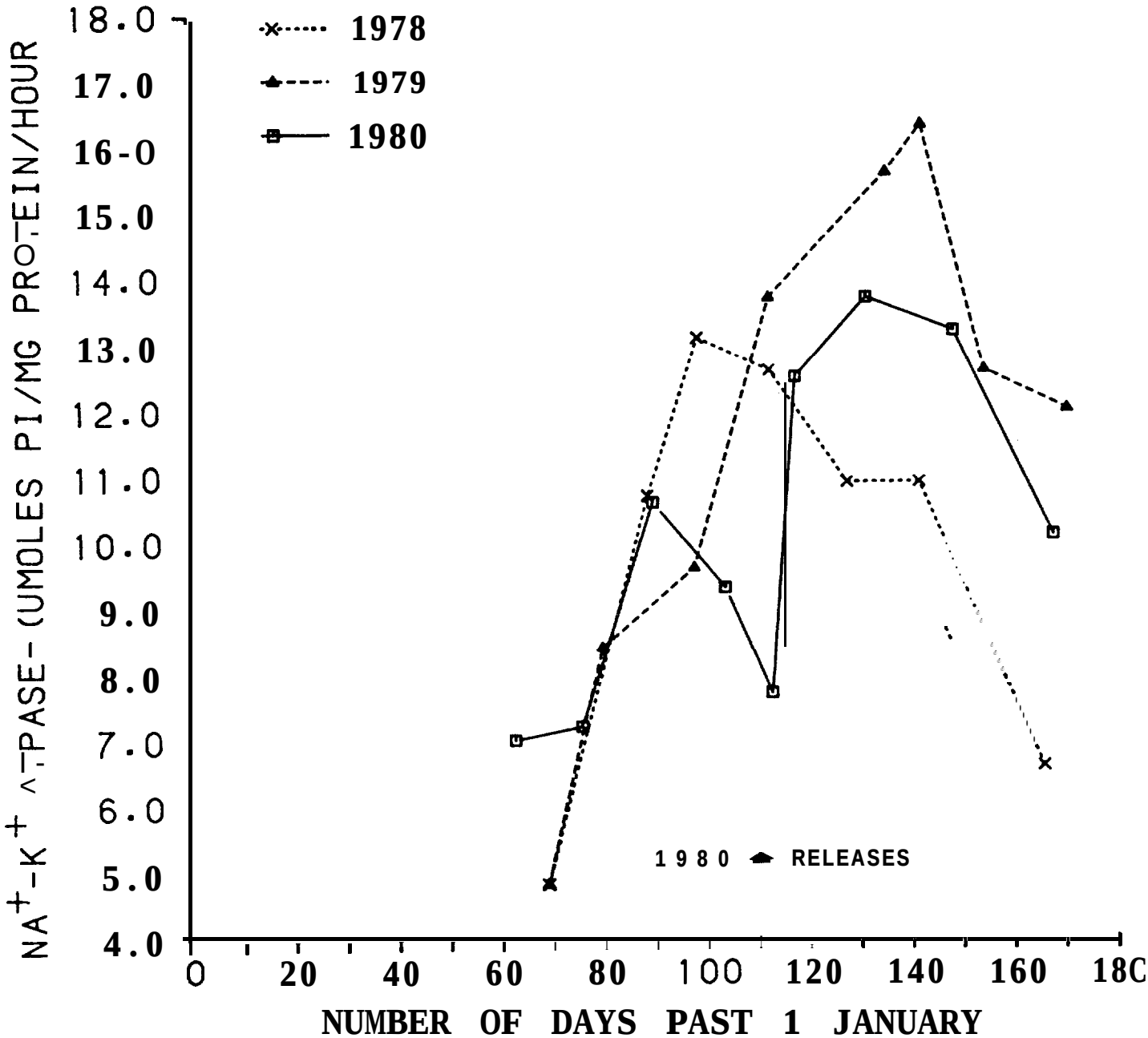


Figure 21.--Average gill Na^+-K^+ATPase values for the Leavenworth NFH spring chinook salmon during the 1978-1979 smolt and 1980 homing studies.

at each sampling as a possible source of group differences in Na^+-K^+ ATPase values. However, the only significant correlations were in the control fish (Pond 17), which occurred during periods of maximum gill Na^+-K^+ ATPase increase, 23 April to 28 April (Table 24). Length data for the fish sampled for Na^+-K^+ ATPase are shown in Table 22 and profiled in Figure 22.

There was a significant correlation ($P < 0.05$) between the mean fork length of fish collected for Na^+-K^+ ATPase analysis in Pond 17 (control) and the mean gill Na^+-K^+ ATPase during the 1980 sampling period (Figure 23). Since there was also a significant correlation between the mean fork lengths of pooled fish and measured Na^+-K^+ ATPase values during the April 23-28 period in normal fish (Pond 17) (Table 24), and this period was one of maximum Na^+-K^+ ATPase increase, the data in Figure 21 suggest that fish with Na^+-K^+ ATPase values of 10.5 or more should have been smolting at the time of release. A regression analysis of samples from the fifth and sixth sampling periods are shown in Figure 24. Since most of the fish were released between 23 April and 28 April, a theoretical regression line of gill Na^+-K^+ ATPase and fork lengths typifying this period has been drawn in. It suggests that any fish $\geq$ 132 mm (Na^+-K^+ ATPase of 10.5) should have been smolting. The percentage of fish with gill Na^+-K^+ ATPase $\geq$ 10.5 increased from 10 to 78%, and the percentage of fish $\geq$ 132 mm increased from 37 to 45% between 23 April and 28 April. On the basis of this information, it would be reasonable to assume that between 40 and 50% of the normal (control) release groups were probably smolting at the time of release. Although fork lengths and gill Na^+-K^+ATPase values in the other marked release

Table 24.--Correlation coefficients for gill Na^+-K^+ ATPase, fork length, and hematological parameters for 1980 Leavenworth Hatchery spring chinook salmon.

	Sampling 1980 program											
					23 April group							
Parameters	3/3	3/17	3/31	4/14	I	II	III	IV	4/28	5/12	5/29	6/17
Ln x Hct	-0.070	0.123	-0.195	0.299	0.150	-0.615	0.363	0.306	-0.309	0.002	-0.051	-0.121
D.F.	58	58	57	58	27	24	26	26	58	57	58	58
P				<0.05		<0.001			<0.05			
Hct x Hb	0.891	0.416	0.876	0.868	-				0.878	0.882	0.761	0.894
D.F.	58	57	57	58	-				58	57	58	58
P	<0.001	<0.005	<0.001	co.001	-				<0.001	<0.001	<0.001	<0.001
Ln x Hb	0.073	0.364	0.032	n.417	-	-	-		0.163	0.160	n.053	-0.135
D.F.	58	57	57	58	-	-	-		58	58	58	58
P		<0.01		<0.005								
Ln x MCHC	0.310	0.264	0.456	0.335					0.342	0.364	0.128	-0.061
D.F.	58	57	57	58					58	57	58	58
P	<0.05		<0.001	<0.02					<0.02	<0.01		
Mfl x ATP	0.295	0.197	0.109	0.527	-0.254	-0.323	0.700	0.346	0.729	-0.345	-0.475	0.384
D.F.	a	a	a	8	a	a	8	a	8	8	a	a
P							<0.05		<0.02			
MHct x ATP	-0.020	0.497	0.112	0.072	-0.010	0.211	0.453	0.146	-0.471	0.496	0.045	-0.524
0.7.	a	8	8	8	a	8	8	8	A	8	a	8
P												
MHb x ATP	0.156	0.285	0.095	0.290	-	-	-		-0.325	0.212	0.298	-0.606
D.F.	8	a	a	8	-	-	-		8	8	8	a
P												
MCHC x ATP	0.334	-0.054	-0.020	0.483	-	-	-		0.501	-0.415	0.364	-0.573
D.F.	a	8	a	8	-	-	-		8	8	8	a
P												

Nomenclature:

Fk Ln = fork length
Hct = hematocrit
Hb = hemoglobin
MCHC = mean cell hemoglobin concentration
Mfl = mean fork lengths for the 3 fish pools used for ATPase
MHct = mean hematocrits for the 3 fish pools used for ATPase
MHb = mean hemoglobins for the 3 fish used for ATPase
MCHC x ATP = average mean cell hemoglobin concentration for the 3 fish pools used for ATPase
ATP = Na^+-K^+ fill ATPase value for each pool of 3 Fish
D.F. = degrees of freedom
P = probability

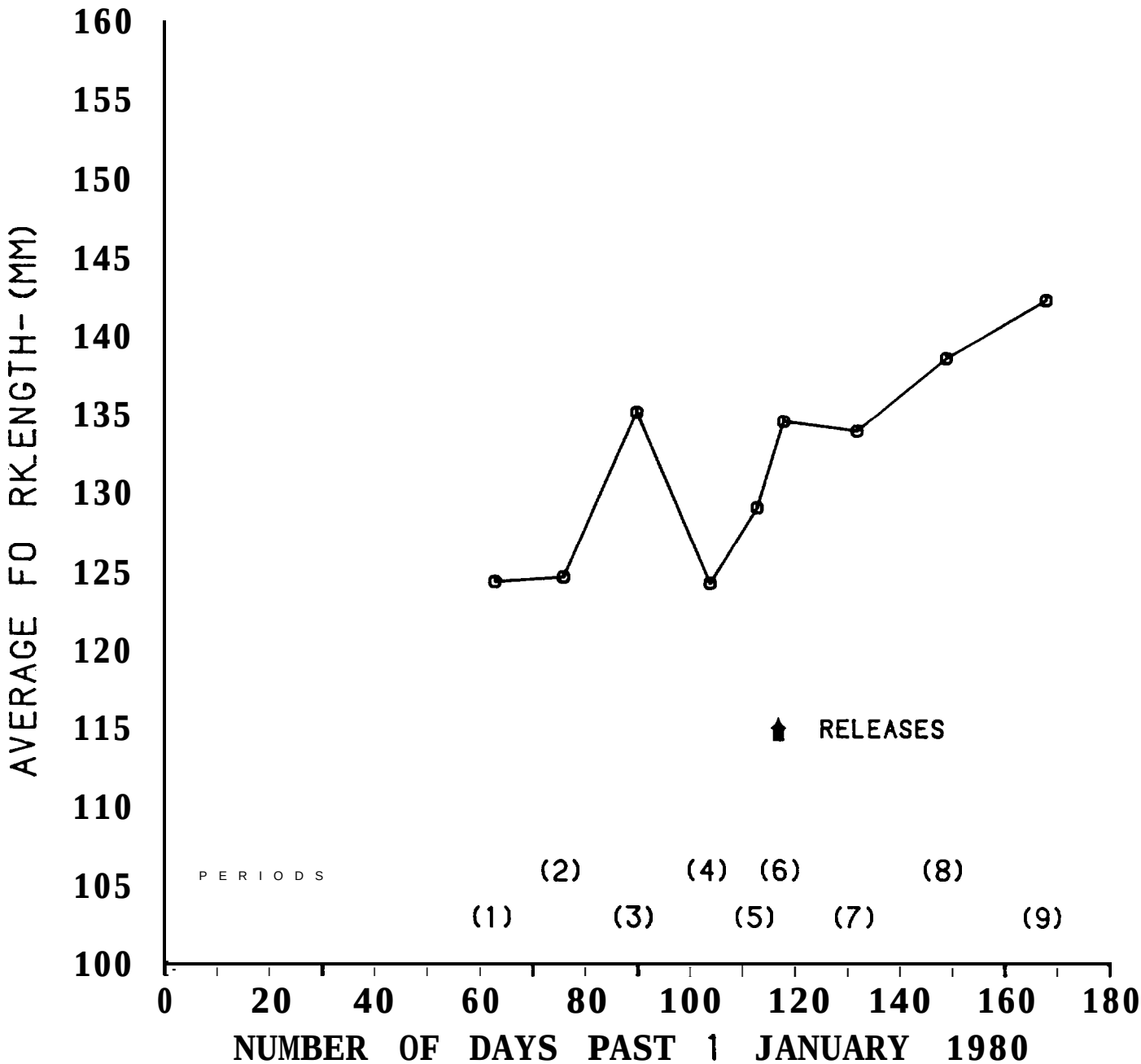


Figure 22.--Mean fork lengths of the spring chinook salmon from Leavenworth NFH used for gill Na^+-K^+ ATPase analyses in spring 1980.

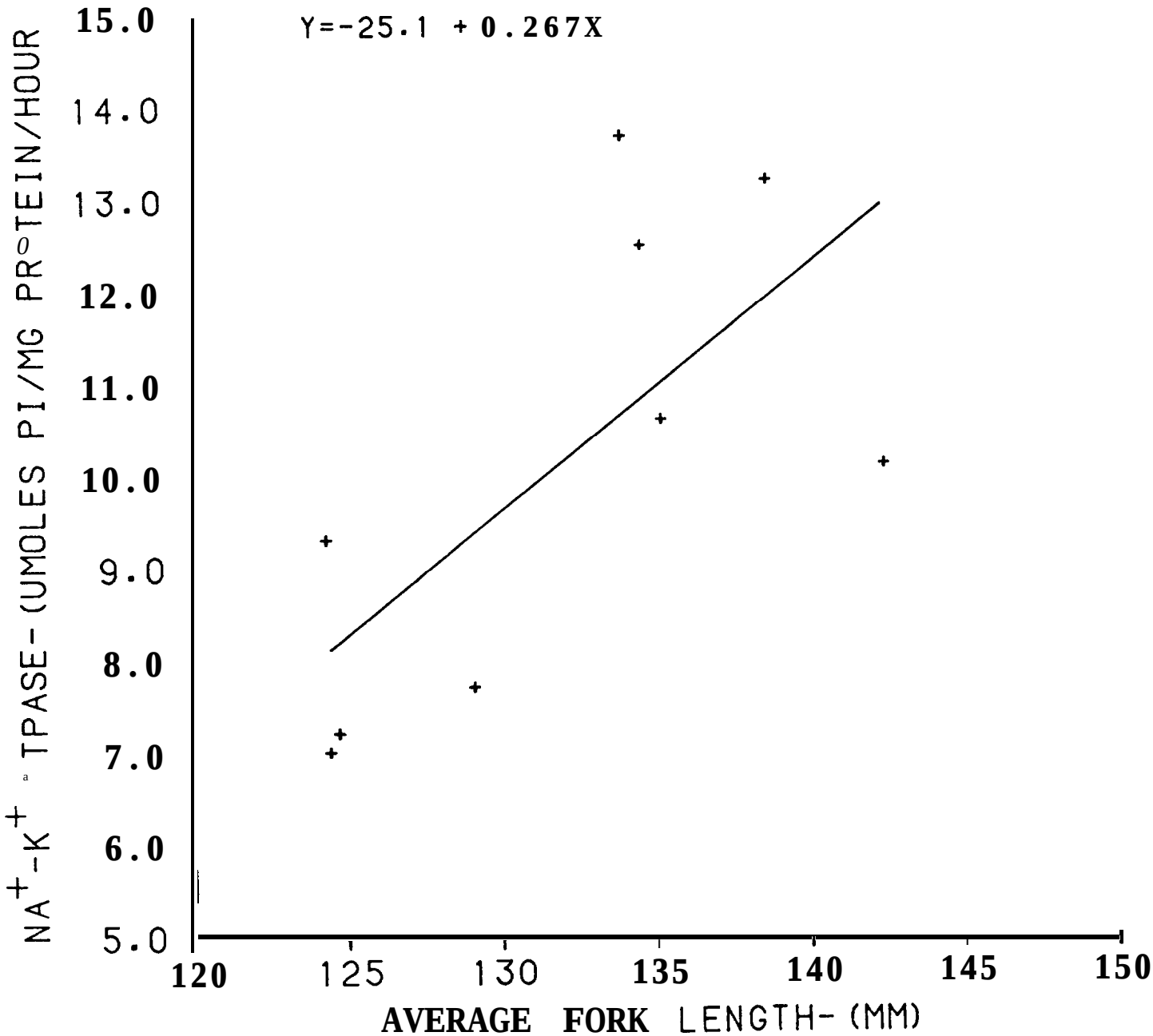


Figure 23.--Regression of the average gill Na^+-K^+ ATPase values on average fork lengths of fish pooled for Na^+-K^+ ATPase analysis (Leavenworth NFH spring chinook salmon - spring 1980). r = Q.689; P < 0.05.

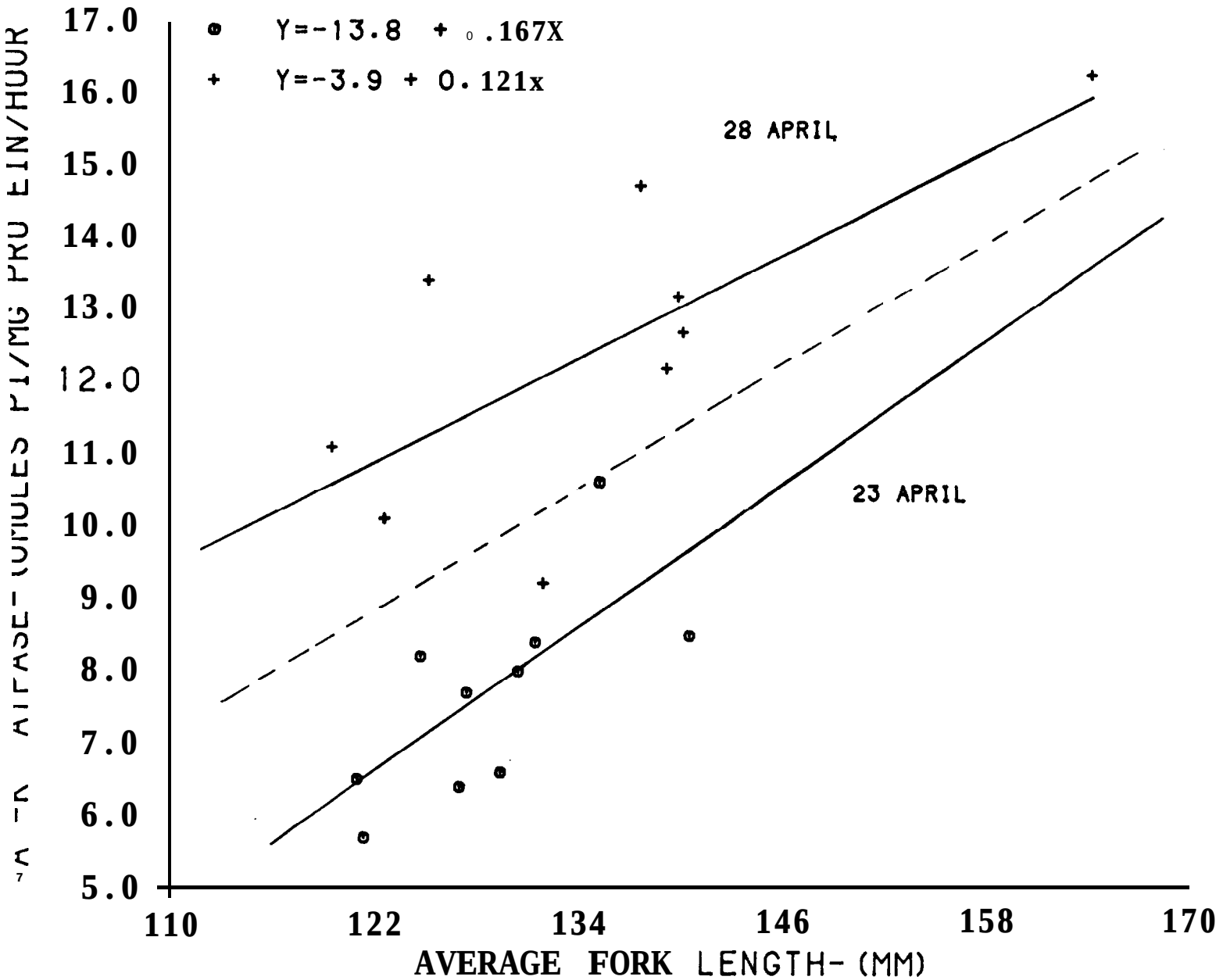


Figure 24.--Regression of gill Na^+-K^+ATPase values on 23 April and 28 April 1980 on average fork lengths of spring chinook salmon pooled (3 fish/pool) for Na^+-K^+ATPase analysis at Leavenworth NFH. $r = 0.699$; $P < 0.05$ for 23 April. $r = 0.729$; $P < 0.05$ for 28 April. A theoretical line (------) has been drawn in for a period halfway between 23 April and 28 April.

groups could not be correlated, Na+-K+ ATPase values $\bar{>}$ io.5 ranged from 30 to 50%. On the basis of this information, we suggest that there may have been differences in the migratory readiness of the normal (Pond 17) and other marked groups.

Plasma Electrolytes

There are few published data on normal plasma electrolyte levels in hatchery chinook salmon. Table 25 is a summary of the mean plasma $Na,^{+}$ Cl^{-}, and K^{+} values from chinook salmon that we examined previously. The exceptionally high K^{+} values in the Kalama Hatchery spring chinook salmon may be due to hemolysis that occurred after sampling.

Mean plasma electrolyte values for Leavenworth NFH spring chinook salmon sampled in the spring of 1980 were generally within expected ranges throughout the season and at the time of release (Table 22). Average plasma Na^{+} reached peak levels at the time of release and declined rapidly from mid-May on (Table 22). Average plasma Cl^{-} values were somewhat more erratic, but followed the same general decline as mean plasma Na^{+}. Maximum deviation of plasma Na^{+} and Cl^{-} (Na^{+}/Cl^{-} ratio) occurred just prior to and after the release period (Table 22).

Significant correlations between individual plasma Na^{+} and CL' occurred frequently throughout the sampling periods (Table 26), and were highly significant ($P < 0.001$) between the sixth and eighth periods (28 April through 29 May). There were significant negative correlations between the plasma Cl' and the Na^{+}/Cl^{-} ratios (Table 27) throughout the sampling season (with one exception; the sixth period - 28 April), and they were most highly significant ($P < 0.001$) throughout May. This is a

Table 25.--Mean values of plasma Na^+, CL-, and K^+ from other samplings of hatchery chinook salmon.

Sample	Millequivalents/1		
	Na+	C1-	K+
1978 Kalama Falls Hatchery spring chinook salmon (at release)	137	116	11.9[a]
1978 Kooskia Hatchery spring chinook salmon (at release)	114	104	---
1978 Leavenworth Hatchery spring chinook salmon (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
1979 Carson Hatchery spring chinook salmon (at release)	146	134	3.7

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

Table 26.--Correlation coefficients between plasma Na+ and C1- for each sampling period in 1980 for Leavenworth Hatchery spring chinook salmon.

Item	Period 1	2	3	4	6	7	8	9
Correlation coefficients	0.64[a]	0.10	0.62[a]	0.03	0.91[a]	0.55[a]	0.49[a]	0.18

[a] Significant (P <0.05)

Table 27.--Correlation coefficients between plasma Cl^- values and individual Na^+/Cl^- ratios for each sampling period in 1980 for Leavenworth Hatchery spring chinook salmon.

Item	Period 1	2	3	4	6	7	8	9
Correlation coefficients	-0.60[a]	-0.55[a]	-0.70[a]	-0.82[a]	-0.21	-0.81[a]	-0.82[a]	-0.59[a]

[a] Significant (P <0.05)

reflection of a rapid decline in plasma CL-, and coincides with a period of peak gill Na^+-K^+ATPase activity (Table 22).

Mean plasma K^+ values (Table 22) were frequently Lower than reported values that we have available (Table 25), but again this may be a reflection of our hemolysis ranking program and/or stresses induced by transporting in 1978 and 1979. Mean plasma K^+ values peaked just prior to release and then declined until mid-May. Increases in mean plasma K^+ after mid-May may be due to holding the fish past the normal time of migration and a reflection of smolting stresses and increasing water temperatures. Plasma K^+ was the only electrolyte that we could significantly correlate with water temperature (Figure 25).

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 28).

Mean values of fall and spring chinook salmon sampled in 1978 and 1979, with one exception, ranged from: (1) hematocrits--34.9 to 59.4% and (2) hemoglobins--5.2 to 8.9 g Hb/dL blood. The one exception was a group of yearling fall chinook salmon in 1979 at Willard NFH with mean hematocrits of 26.5% and mean hemoglobins of 3.2 g Hb/dL blood. Over 56% of the samples from these fish were classified as positive for bacterial kidney disease (BKD). Hematocrit values below 28% in Pacific salmon may be indicative of a number of health problems and should signal a cautionary warning.

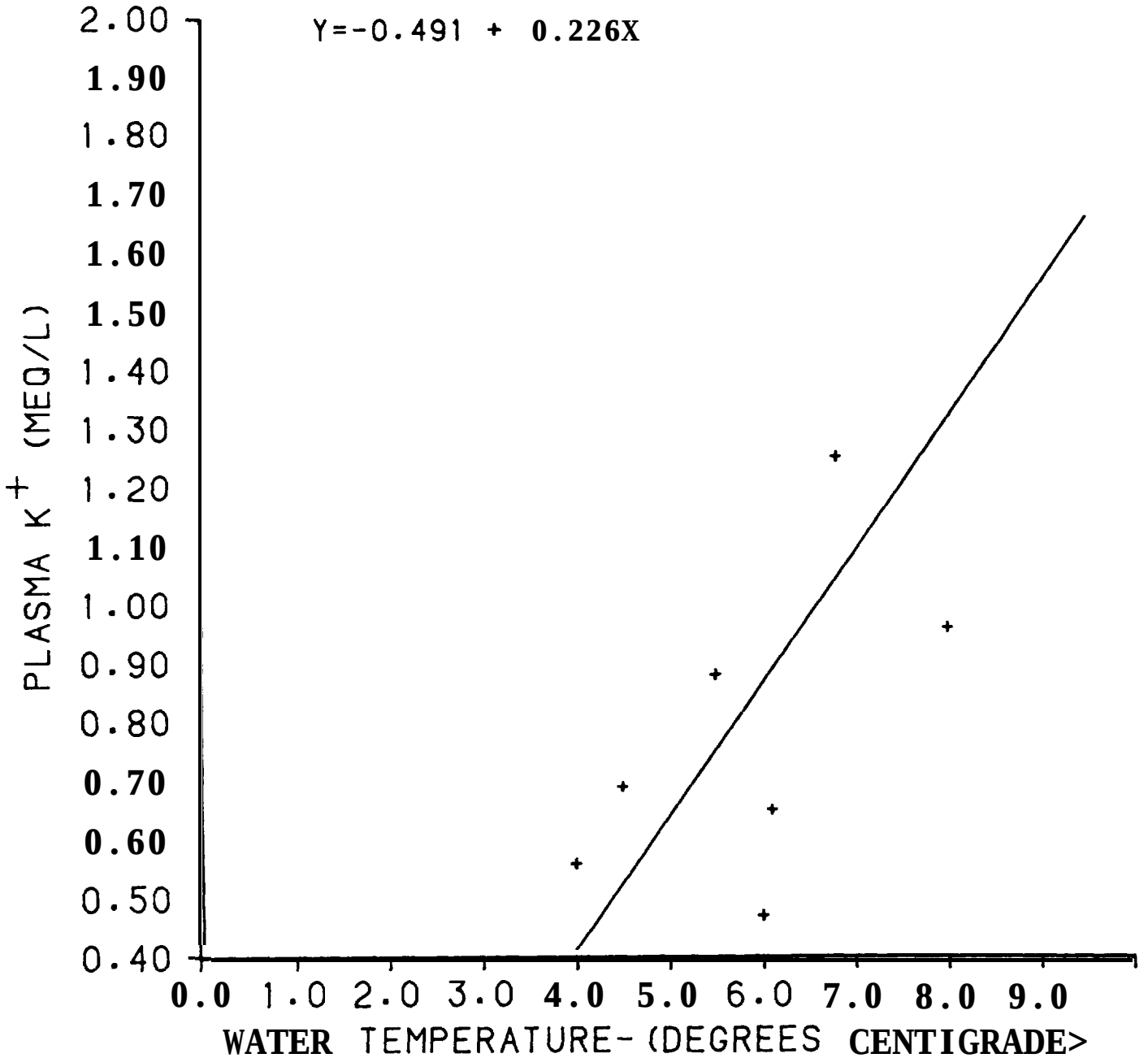


Figure 25.--Regression of the average plasma K^+ on water temperature for Leavenworth NFH spring chinook salmon during spring 1980. $r = 0.821$; $P < 0.02$.

Table 28.--Hematocrit and hemoglobin values of fall chinook salmon cultured for 4 weeks at four water temperatures. Average weights of the fish were 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

Profiles of mean hematocrit and hemoglobin values are compared to a profile of the water temperatures (Figure 26). Note that there was a general decline of both hematocrits and hemoglobins throughout the spring of 1980. This is the same pattern noted in Dworshak steelhead (Table 3), but opposite to that of the Tucannon steelhead (Table 13). Negative correlations of hematocrits and hemoglobins to water temperature were as highly significant for Leavenworth spring chinook salmon as positive correlations in smaller fall chinook salmon found by Banks et al. (1971) cultured under controlled temperature conditions (Figures 27 and 28). Although hematocrit values, gill Na+-K+ ATPase, and fork lengths frequently could not be correlated on any specific sampling period, there were significant correlations between mean values for each period throughout the season (Table 24). However, these may still be coincidental seasonal adjustments of populations, and mean fork Length, hematocrit, and Na+-K+ ATPase may not be directly dependent upon each other.

Correlations between individual hematocrit and hemoglobin values were highly significant in Leavenworth NFH spring chinook salmon in both winter and spring (Table 24). This correlation was also highly significant for the mean hematocrit and hemoglobin values (Figure 29). Data indicate that hematocrit data alone could be *used* to accurately predict hemoglobin values for Large sample sizes.

Mean cell hemoglobin concentrations (MCHC) of Leavenworth NFH spring chinook salmon peaked in Late March, and were at a Low Level throughout April, a probable indication that the fish were not stressed at the time of release (Table 22). Average MCHC values could not be correlated with other stress or smolting indicators.

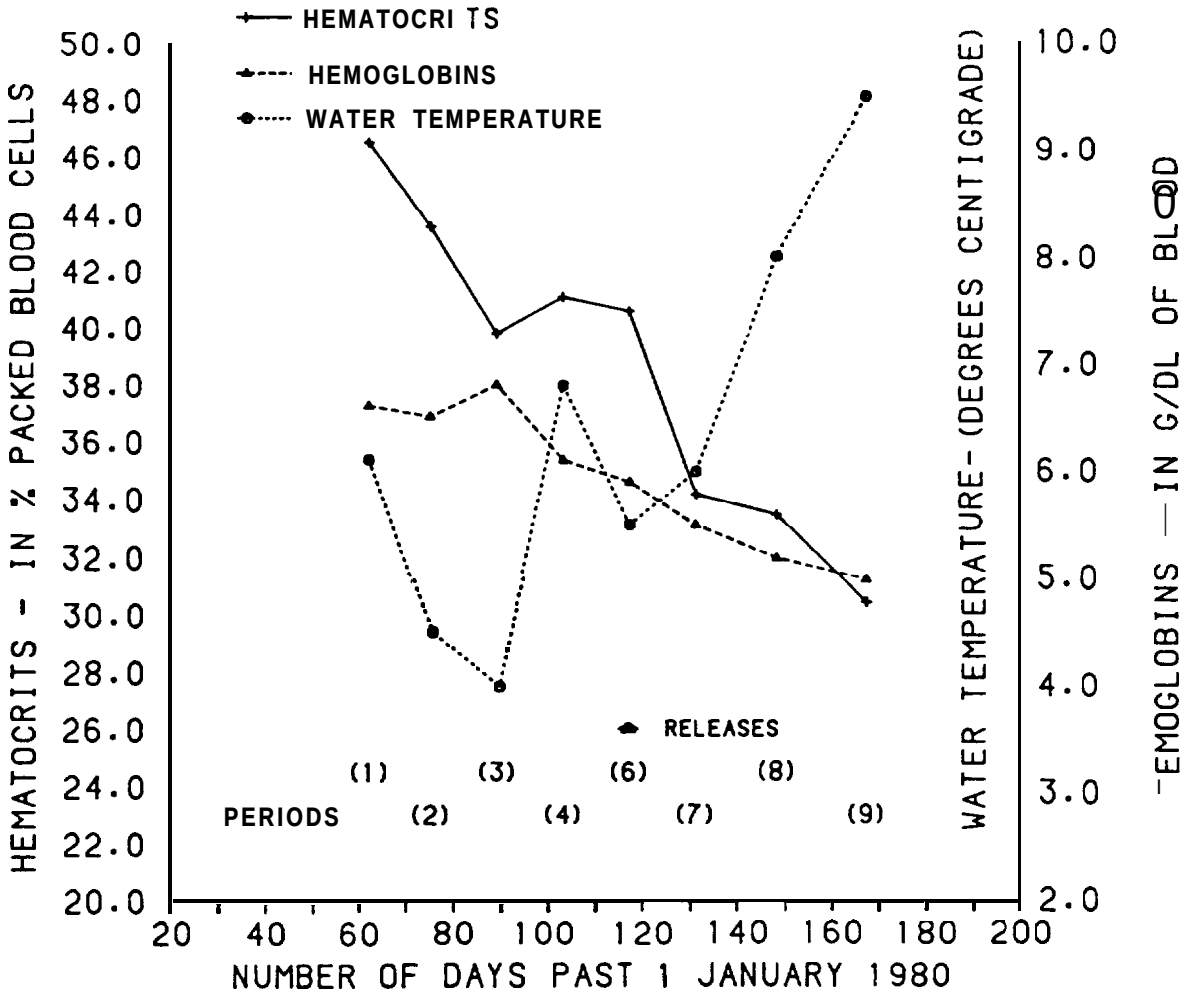


Figure 26.--Average hematocrit and hemoglobin values and raceway water temperatures for Leavenworth NFH spring chinook salmon during spring 1980.

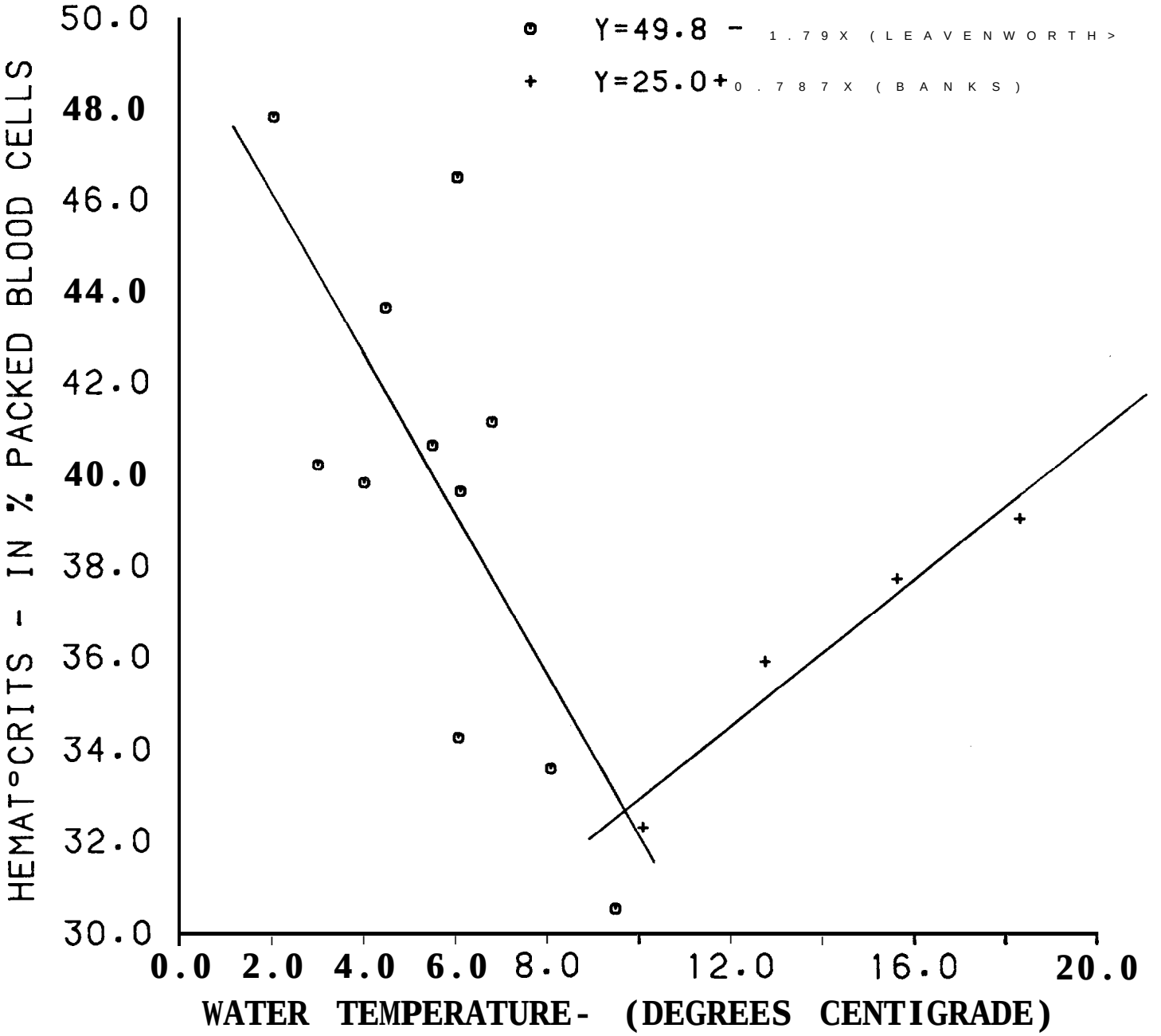


Figure 27.--A comparison of the regressions of mean hematocrit values of Leavenworth NFH spring chinook salmon on Leavenworth NFH water temperatures during spring 1980, and regression of mean hematocrit values of fall chinook salmon cultured at different water temperatures by Banks et al. (1971). $r = 0.720$ ($P < 0.02$) Leavenworth NFH spring chinook salmon, 1980. $r = 0.971$ ($P < 0.05$) Banks et al. (1971) fall chinook salmon.

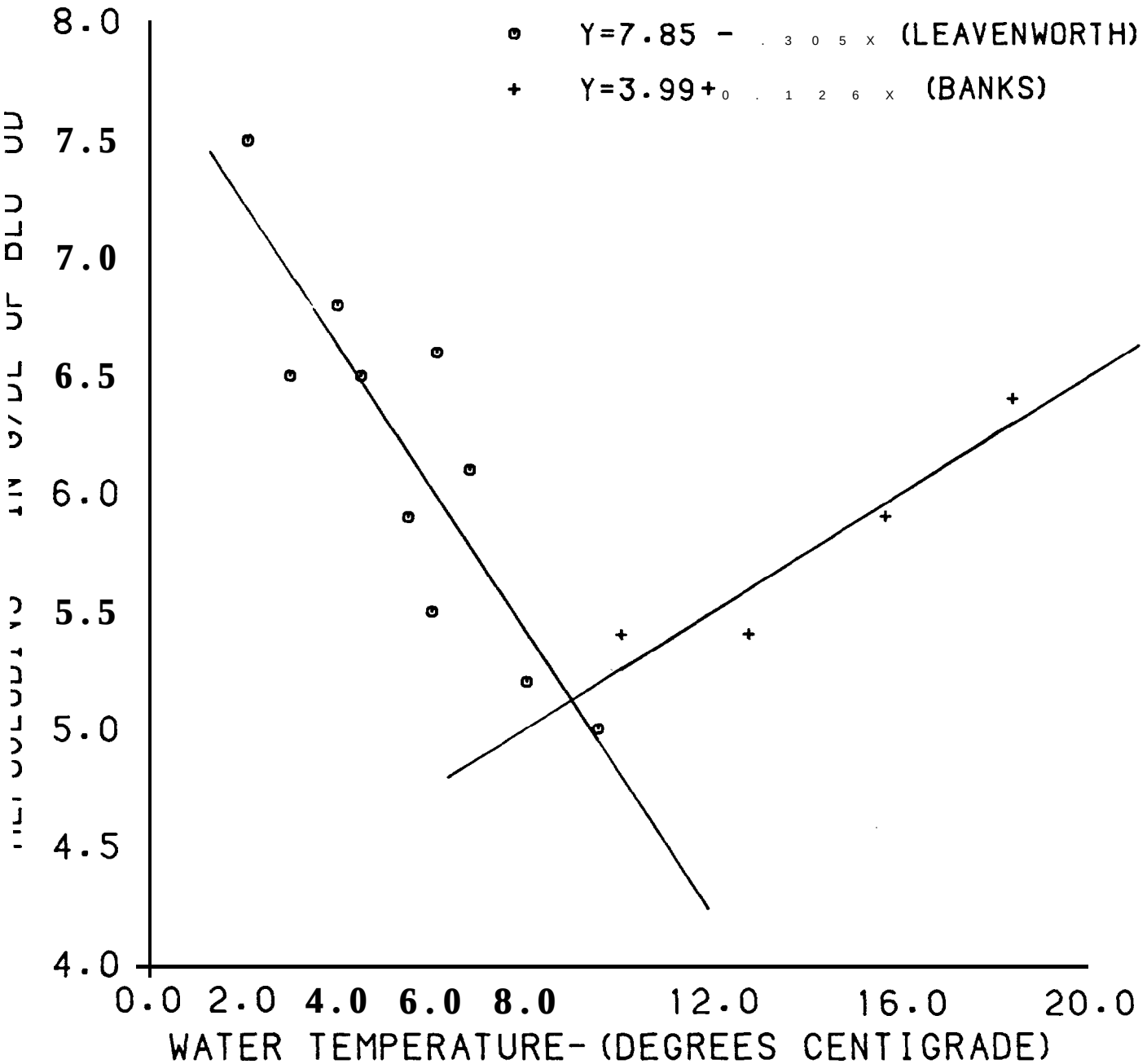


Figure 28.--A comparison of the regressions of mean hemoglobin values of Leavenworth NFH spring chinook salmon on Leavenworth NFH water temperatures during spring 1980, and regression of mean hemoglobin values of fall chinook salmon cultured at different water temperatures by Banks et al. (1971). $r = 0.885$ ($P < 0.001$) Leavenworth NFH spring chinook salmon, 1980. $r = 0.946$ ($P < 0.10$) Banks et al. (1971) fall chinook salmon.

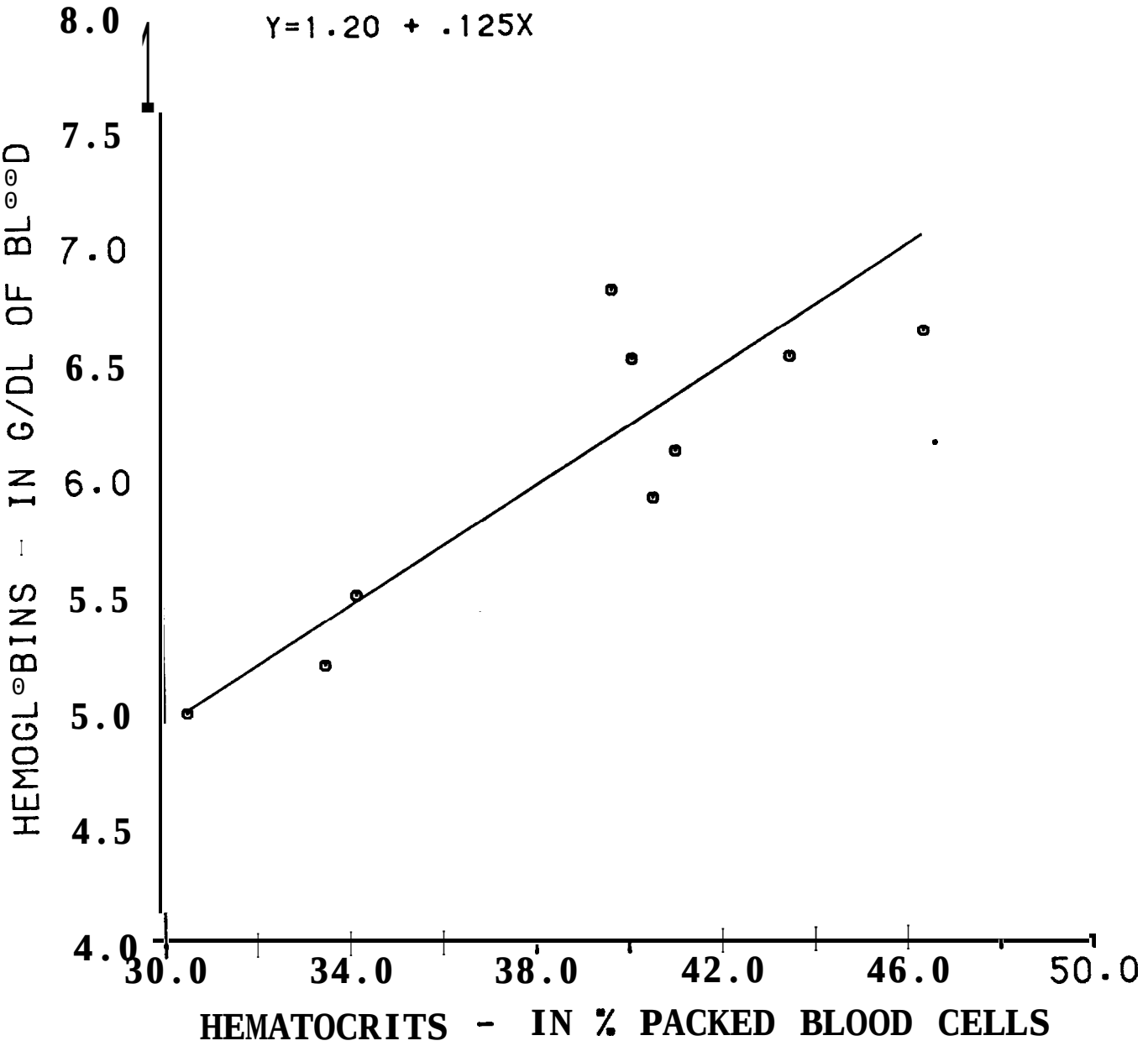


Figure 29.--Regression of average hemoglobin values on average hematocrit values of the Leavenworth NFH spring chinook salmon during spring 1980. $r = 0.903$; $P < 0.001$.

IFAT-BKD

Specimens of Leavenworth NFH spring chinook salmon from the first, third, and sixth sampling periods of 1980 were examined for the presence of BKD organisms by the Indirect Fluorescent Antibody Test (IFAT).

The data in Table 29 indicate a major increase (from 2 to 80%) in the percentage of samples positive for BKD in March followed by a decrease just after release in late April. Although the incidence of BKD was high, the intensity of infection ranged from very light to light, and it appears that BKD is not a serious threat to this stock. Our field notes indicate that of approximately 700 fish that were examined internally at Leavenworth NFH in late 1979 and early 1980, only 10 (approximately 1.4%) had BKD type lesions visible to the naked eye. We did note that 6.7% of the fall-marked fish sampled on 23 April (release) had BKD type lesions in the kidneys.

Table 29.--Incidence of BKD organisms and relative intensity in the kidneys of Leavenworth Hatchery spring chinook salmon sampled in 1980.

Period	Date	% of samples positive for BKD				Range of intensity (no. of organisms/l50 microscopic fields)
		Anterior	Posterior	Both	Total	
1	3 March	1.7	0	0	1.7	4
3	31 March	53.3	61.7	35.0	80.0	1 to 34
6	28 April	28.6	53.6	17.9	66.1	1 to 16

Histopathology

A detailed summary of the pathological conditions is presented in Table 30. For comparative purposes, these data are further summarized with data from other hatcheries in Table 9.

Table 30.--Pathological conditions observed in Leavenworth Hatchery spring chinook salmon in 1980 and their percentage of incidence.[a]

Organ and pathology	Incidence (%)											
	Period 1 (severity)[b]				Period 3 (severity)				Period 6 (severity)			
	I	II	III	Total	I	II	III	Total	I	II	III	Total
Eye												
skeletal muscle lesions	32.2	0	0	32.2	36.7	0	0	36.7	38.3	0	0	38.3
retobulbar granulomatous inflammation	0	0	0	0	0	0	0	0	0	0	1.7	1.7
Gills												
increased numbers of lympocytes	74.5	27.1	0	100.0	81.7	16.7	0	98.3	48.3	20.0	1.7	70.0
epithelial cell formation	13.6	18.6	1.7	33.8	28.3	70.0	1.7	98.3	65.0	8.3	0	73.3
lymphatic telangiectasis of secondary lamellae	25.4	32.2	6.8	64.4	0	0	0	0	0	0	0	0
vascular telangiectasis of secondary lammelae	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Olfactory sac												
focal mononuclear cell infiltration	88.1	6.8	0	94.9	80.0	11.7	0	91.7	38.3	10.0	0	48.3
acute focal hemmorhage	0	0	0	0	0	0	0	0	1.7	0	0	1.7

a/ Brain tissue was processed and examined for all specimens, and there was no evidence of any pathology.

b/ I-recognizable (least severe); II-intermediate; III-severe.

At or near release, Leavenworth NFH spring chinook salmon were characterized by marked decreases in pathological conditions of gill and olfactory sac tissues (Table 30). Types and severity of Lesions encountered were Low, and no evidence of parasitic activity was found in either the gills or olfactory sac.

In addition to microscopic examination of tissue by the veterinary pathologist we made notes in the field of any macroscopic Lesions observed in the various organs from the 700 or more fish examined. One precocious male was noted, and it showed symptoms of nephrocalcinosis; eight fish were noted to have clinical symptoms of BKD (exophthalmos, kidney Lesions); one Lesion of the spleen; one Lesion of the Liver; two fish with ascites, and one with Lesions of the posterior air bladder. This is roughly 2% of the total samples examined.

Summary

Serial sampling of Leavenworth NFH spring chinook salmon in Late 1979 and early 1980 indicated that general fish health was excellent, and may have actually improved (over March) by the time of release in Late April. Water temperatures at this time had declined temporarily (Figure 20), which would have reduced handling stresses. A comparison of gill Na+-K+ ATPase curves between 1980 and two other years indicated that a typical pattern developed, and the bulk of the fish were released on an actively rising profile (Figure 21). Comparisons made at the time of release demonstrated that mean hematocrits were significantly Lower in the fall mark group and channel control groups than in fish from Pond 17, and that mean gill Na+-K+ ATPase values were significantly higher for all other release groups tested when compared to Pond 17, the normal groups (Table

23). Since Pond 17 fish were used to determine the seasonal Na^+-K^+ ATPase profile, the conclusion is that all test groups were at or rapidly approaching peak Na+-K+ ATPase Levels. Significant correlations between gill Na+-K+ ATPase and mean fork lengths enabled us to estimate that at Least 40 to 50% of the samples were probably smolting during the release period. Smolting activity probably continued at a high rate in the released fish. A peak Na^+-K^+ ATPase activity coincided with rapidly declining plasma CL' and mean hematocrit values 'after 28 April and increasing plasma K^+ and MCHC in fish held after release (Table 22). These events also coincided with a sharp increase in water temperature. ALthough holding the fish slightly longer might have produced more smolts, it might have been with some risk as indicated by increased stress indicator values (K^+, MCHC).

Histopathology and BKD analyses indicate that the incidence of combined Lesions of any importance with severities that would call for a pessimistic prognosis are probably Less than 10%. Reduced incidences of lesions to the eyes and olfactory sacs and the absence of parasites at the time of release were also noted.

Carson National Fish Hatchery

General

We sampled spring chinook salmon from a fall marking test at Carson NFH in Late 1979 (Table 31), then collected full data from March until the fish were released between 12 and 15 May 1980. During the sampling time, the fish were cultured in well water at temperatures of 6° to 7°C (Table 31). No fish were held over for post-release sampling.

Sampling period days are presented with 1 January 1980 as Day +1 and 31 December as Day -1 to simplify computer programming and are presented in Table 31 with the calendar dates for each sampling period.

The fork Lengths of the fish used in the sampling surveys averaged 132 mm in 1978, 127 mm in 1979, and 117 mm in 1980 at the times of release in early May.

Gill Na^+-K^+ ATPase

The gill Na^+-K^+ ATPase profile in 1980 was characterized by a gradual increase between the first two periods, followed by a sharp increase in early April, and probably near maximal activity at the time of release (Figure 30). Na^+-K^+ ATPase activities were not followed beyond release, but comparison with the profiles collected in 1978, 1979, and 1980 (Figure 30) suggests that enzyme performance was spatially and quantitatively repeatable, which substantiates the probability that in 1980 maximum activity was reached.

There were no significant correlations between gill Na^+-K^+ ATPase values in any one period and the average fork Lengths of fish used for the pooled gill samples. In the first period there was negative correlation ($r = 0.814$; $P < 0.002$) between gill Na^+-K^+ ATPase and plasma K^+; and

Table 31.--Summary data for spring chinook salmon samples collected at Carson National Hatchery in late 1979 and in the spring of 1980, with means, standard deviations ( ), and ranges. Sample size = 60, released 12 May 80.

Item	Period A	1	2	3	4
Date	13 Dec 79	4 Mar 80	4 Apr 80	17 Apr 80	12 May 80
Days>Jal a/	-19	64	94	107	132
Temp.°C b/	6.7	6.7	6.7	6.7	6.7
Avg. Fk Ln c/	93.4	102.6	109.5	112.5	116.7
	(5.7)	(6.9)	(7.4)	(8.2)	(9.5)
(Range)	79-112	93-130	90-126	98-137	90-135
Avg. ATP FK Ln d/	---	103.8	110.4	112.0	116.4
	---	(4.6)	(7.2)	(7.8)	(10.1)
(Range)	---	99-130	96-122	100-130	98-135
Avg. ATP e/	---	9.5	12.5	18.6	20.4
	---	(1.7)	(2.5)	(3.0)	(4.1)
(Range)	---	6.7-11.7	8.4-16.0	14.3-24.2	13.3-27.6
Avg. Hct f/	36.4	38.8	47.3	53.3	56.7
	(4.7)	(4.1)	(4.9)	(6.4)	(8.9)
(Range)	26-48	28-48	32-55	30-65	30-72
Avg. Hb g/	6.5	---	6.5	7.2	8.2
	(1.0)	---	(1.1)	(1.4)	(1.6)
(Range)	4.3-8.7	---	3.6-8.8	2.7-12.5	2.4-10.6
Avg. MCHC h/	17.9	---	14.1	13.5	14.4
	(2.3)	---	(3.1)	(1.9)	(2.0)
(Range)	11.6-23.1	---	7.2-26.2	9.0-23.1	8.0-21.2
Avg. Na^+ i/	---	180.4	144.6	151.5	143.7
	---	(17.4)	(7.1)	(7.8)	(23.1)
(Range)	---	160-255	130-154	141-167	89-200
Avg. K^+ j/	---	2.16	1.03	2.32	1.86
	---	(0.94)	(0.60)	(1.00)	(1.40)
	---	1.05-4.60	0.55-3.35	0.82-3.98	0.57-6.80
Avg. Cl^- k/	---	132.3	136.7	128.6	109.5
	---	(9.1)	(10.2)	(12.0)	---
(Range)	---	103-145	118-154	111-147	109-110(n=2)
Na^+/Cl^- l/	---	1.38	1.07	1.18	---
	---	(0.19)	(0.09)	(0.10)	---
(Range)	---	1.17-2.07	0.94-1.26	1.02-1.34	---

a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average for lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. ATP: The average gill ATPase levels for that period. (Na^+-K^+ activity in μ moles Pi/mg protein/hour.
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl^-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratio of the plasma sodium to chlorides for that period, averaged.

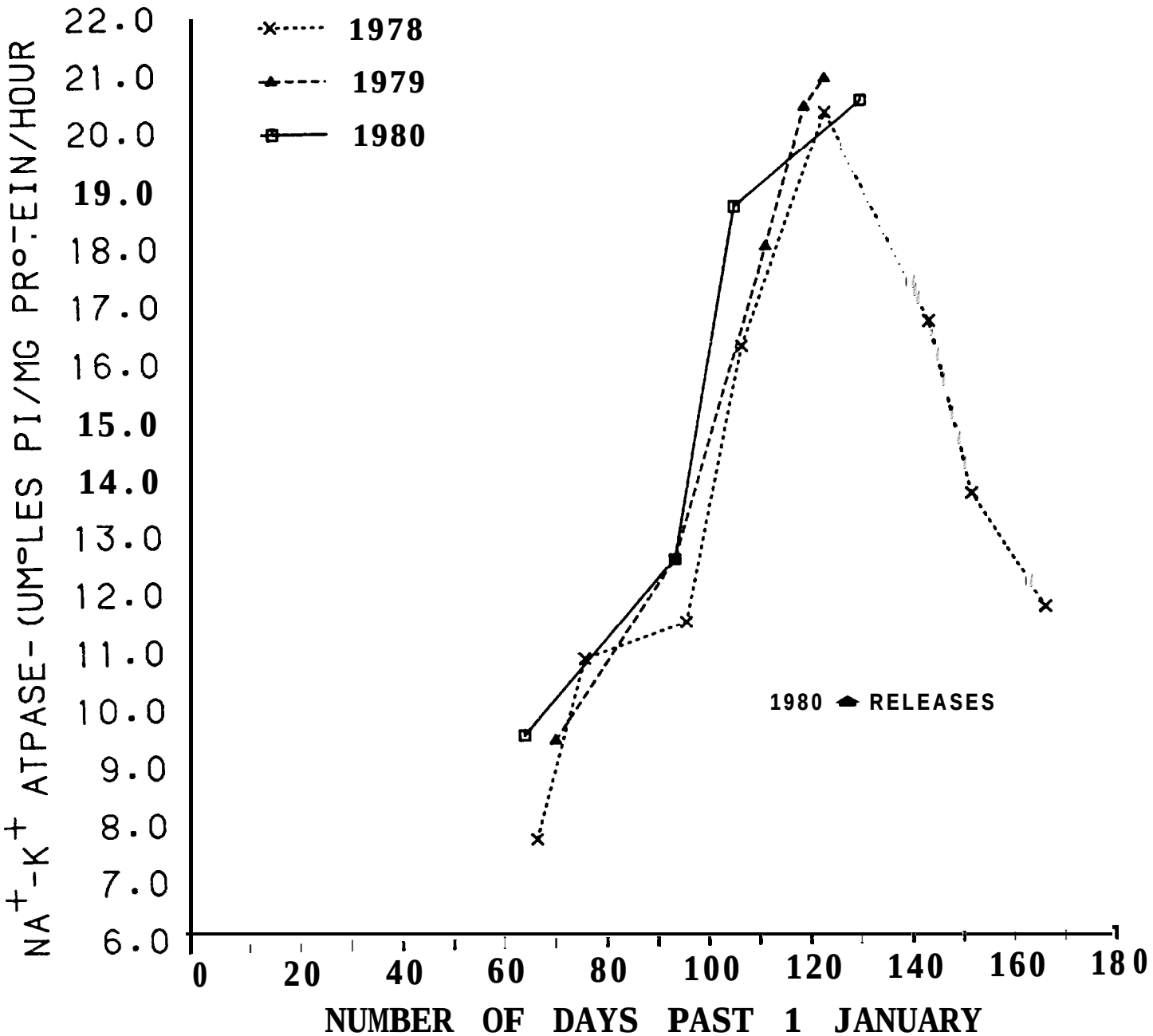


Figure 30.--Average gill Na^+-K^+ ATPase values for Carson NFH spring chinook salmon during 1978, 1979, and 1980.

in the third period there was a positive between gill Na^+-K^+ ATPase and plasma Cl^- ($r = 0.685$; $P < 0.05$). In the fourth period (at release), there was a positive correlation between gill Na^+-K^+ ATPase and hematocrit values ($r = 0.646$; $P < 0.05$). However, there was no consistency in any of these correlations between periods.

Trends of mean data for the entire sampling period were different. Mean fork lengths, gill Na^+-K^+ ATPase values, hematocrits, and hemoglobins all increased almost linearly throughout the sampling season (Figure 31), and there was a positive correlation between mean gill Na^+-K^+ ATPase and mean hematocrit values ($r = 0.975$; $P < 0.05$). As mean gill Na^+-K^+ ATPase values increased, mean plasma Na^+ and CL' values decreased (Table 31). However, there were no significant negative correlations between mean gill Na^+-K^+ ATPase values and plasma electrolytes.

At release, the mean gill Na^+-K^+ ATPase activity was much higher for spring chinook salmon from Carson NFH (20.4) than those from Leavenworth NFH (12.5), and the rate of increase in activity during the month of April was greater for the Carson NFH fish. The percentage of Carson NFH fish with gill Na^+-K^+ ATPase values ≥ 20 went from 40% in the third period (17 April) to 60% in the fourth (12 May), indicating that most of the rise in gill Na^+-K^+ ATPase had taken place (the highest value in the second period, 4 April, was 16.0). Year to year consistency of the profiles (possibly influenced by constant water temperature) plus this quantitative index indicate that at Least 50% of the fish were smolting at the time of release.

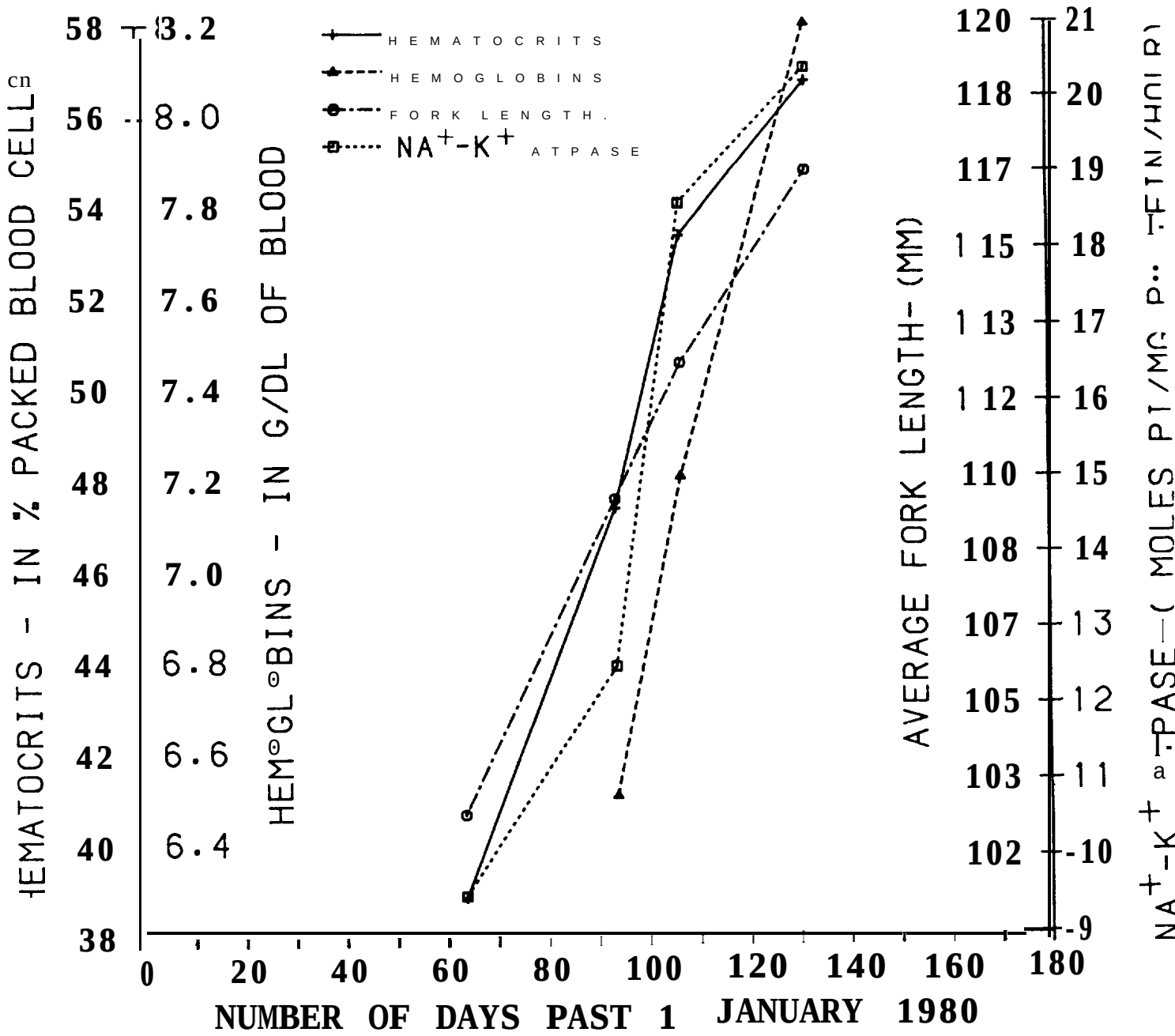


Figure 31.--Average fork lengths, gill Na^+-K^+ ATPase values, hematocrits, and hemoglobins of the Carson NFH spring chinook salmon during spring 1980.

Plasma Electrolytes

Mean plasma electrolyte values (Table 31) were within expected ranges at the time of release (Table 25). Plasma Na^+ was characterized by higher than expected values in early March followed by a sharp decline and leveling off in the expected range near release (Table 31). Plasma Cl^- values declined to their lowest level at the time of release, which coincided with the peak gill Na^+-K^+ ATPase values (Table 31). This further corroborates evidence of good smolting activity at release.

The stress indicators, plasma K^+ and the MCHC, did not show sharp increases at release, and mean values were within normal ranges (Table 31). Comparison of plasma K^+ profiles between Carson and Leavenworth NFH spring chinook salmon shows a similarity in relative changes and timing (Figure 32), and suggests that holding the Carson fish beyond the proposed release dates might have induced a post-smolt stress.

Table 32.--Hematocrit and hemoglobin values for Carson Hatchery spring chinook salmon at the time of release: 1978-1980.

Year	Hematocrits Mean	Hematocrits S.D.	Hemoglobins Mean	Hemoglobins S.D.
1978	44.6	± 11.9	6.9	± 1.6
1979	36.1	± 10.1	5.2	± 1.8
1980	56.7	± 8.9	8.2	± 1.6

Hematology

Mean hematocrit and hemoglobin values of Carson NFH spring chinook salmon were much higher at the time of release in 1980 than in previous years, and were the highest encountered in chinook salmon thus far (Tables 31 and 32).

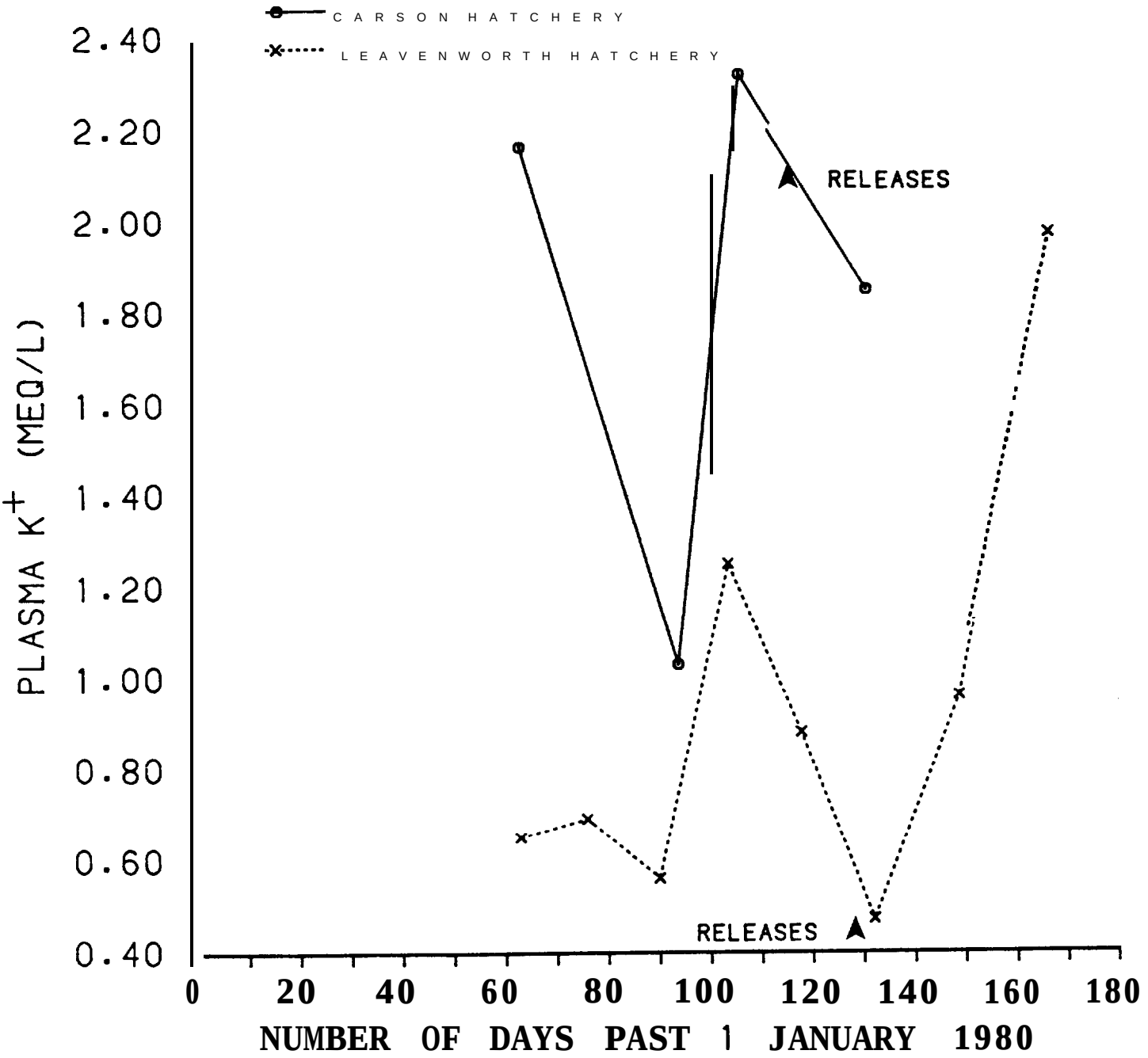


Figure 32.--Average plasma K^+ values for Carson and Leavenworth NFH spring chinook salmon during spring 1980.

Seasonal mean hematocrit and hemoglobin values of the Carson NFH fish (Table 31) are almost the reverse of those from Leavenworth NFH (Table 22). Not only was the trend in Carson NFH fish one of steady increases, starting from a low in December 1979 (Table 31), but all of the values exceeded those of 1978 and 1979 as early as April. There were positive correlations between mean fork lengths and mean hematocrits ($r = 0.993$; $P < 0.05$), mean fork lengths and mean hemoglobins ($r = 1.000$; $P < 0.05$), and mean gill Na^+-K^+ ATPase and mean hematocrits ($r = 0.975$; $P < 0.05$). Note that there were no individual hematocrit values below 30% at the time of release.

These data are also unique in that they represent major changes in hematology in a stable water temperature. Unfortunately, 1980 profiles were not continued for post-release fish, as the significant correlations of means for lengths, hematocrits, and gill Na^+-K^+ ATPase values may prove to be of value for this stock of fish in future years. In Figure 33, we show that the mean gill Na^+-K^+ ATPase could be predicted from the mean hematocrits (in 1980). Since the average growth would (presumably) continue an upward trend even for post-smolt fish, if the mean Na^+-K^+ ATPase and hematocrit value declined after smolting, it might be possible to predict smolting (in this stock) from profiles of mean hematocrit data.

IFAT-BKD

Specimens of Carson NFH spring chinook salmon from the first, second, and fourth sampling periods of 1980 were examined for the presence of BKD organisms by IFAT.

At release, the percentage of fish in the sample population with identified BKD organisms was between that of 1978 and 1979 (Table 33).

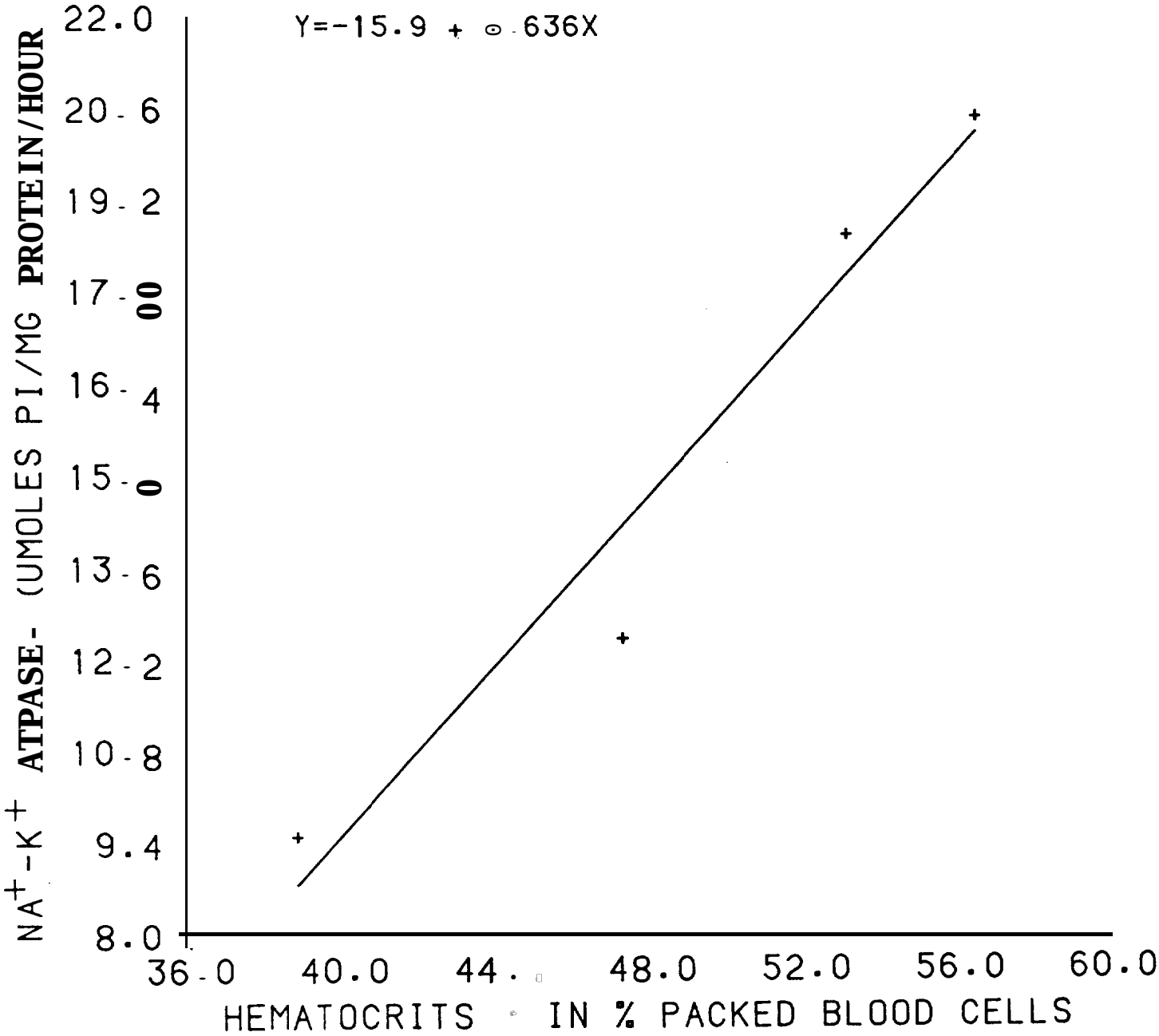


Figure 33. -Regression of the mean gill Na^+-K^+ ATPase values on mean hematocrits of Carson NFH spring chinook salmon during spring 1980. r = 0.7 6; P < 0.05.

Table 33.--The indicence of BKD organisms in juvenile Carson Hatchery spring chinook salmon from 1978-80 as determined by IFAT.

Date	% of fish with BKD bacteria in the kidneys			
	Anterior kidney	Posterior kidney	Both kidneys	Total
1978 (at release)	--	--	--	50.8 (light to moderate)
1979 (at release)	--	--	--	33.3[a]
1980				
3 March	5.0	5.0	0	10.0 (light)
4 April	8.3	1.7	0	10.0 (light)
12 May (release)	6.3	6.3	26.6	31.9[b]

a/ 25% of the infected fish were classed as "severe" in 1979.

b/ Only 4% of the infected fish were classed as "severe" in 1980.

However, note that there were six times as many fish in 1979 with class III (severe) infections as in 1980. The significance of this is confirmed by the excellent hematological record in 1980 fish.

After release in 1980, IFAT-BKD tests were conducted on an additional 25 Carson NFH fish to determine if BKD could possibly affect the sensory organs. We compared tissue smears of anterior and posterior kidney, hind gut, brain, behind (and within) the eye, and the olfactory nare and found that 3 out of 25 (12%) had BKD organisms in posterior kidney ranging from 3 to-35 organisms/l50 microscopic fields (m.f.), and 10 out of 25 (40%) had BKD organisms in the olfactory nare ranging from 1 to 420/150 m.f.

Histopathology

A summary of the pathological conditions observed is presented in Table 34. For comparative purposes, these data are further summarized with data from the other hatcheries in Table 9. The main characteristics of this histopathological profile of the Carson NFH were a decrease in lesions of the eyes and increases in gill lesions. There was a pronounced decrease in gill lesions of intermediate (class II) severity from the earliest part of the season, which may have resulted from early disease treatment. Lesions of the olfactory nares were consistently high throughout the four sampling periods and were almost four times the rate of 1979 (Novotny and Zaugg 1981) and almost double the rate of Leavenworth NFH spring chinook salmon. Although the pathologist's report for the 1979 studies (Novotny and Zaugg 1981) and our IFAT-BKD studies in 1980 suggest that BKD organisms may be associated with olfactory sac pathology, we have not completely confirmed this.

Table 34.--Pathological conditons observed in 1980 Carson Hatchery spring chinook salmon and their percentage of incidence.[a]

Organ and pathology	Incidence (%)															
	Period 1 (severity)[b]				Period 2 (severity)				Period 3 (severity)				Period 4 (severity)			
	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total
Eye																
Skeletal muscle lesions	82.8	0	0	82.8	41.7	0	0	41.7	60.0	0	0	60.0	41.7	0	0	41.7
Retrobulbar fat lesions	0	0	0	0	0	0	0	0	1.7	0	0	1.7	1.7	0	0	1.7
Acute focal hemmorhage	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Retrobulbar granulomatous inflammation	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Retrobulbar pyogranulomatous inflammation	0	0	0	0	0	0	0	0	0	1.7	0	1.7	0	1.7	1.7	3.4
Gills																
Increased numbers of lymphocytes	43.1	52.2	0	98.2	63.3	11.7	0	75.0	53.3	18.3	0	71.7	78.3	15.0	0	93.3
Epithelial cell formation	65.5	31.0	0	96.6	53.3	6.7	0	60.0	23.3	1.7	0	25.0	46.7	6.7	0	53.3
Vascular telangiectasis of secondary lamellae	5.2	0	0	5.2	a.3	0	0	8.3	3.3	0	0	3.3	0	0	0	0
Ciliated protozoan parasite	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Ol factory sac																
Focal mononuclear cell infiltration	09.7	5.2	0	94.8	85.0	5.0	0	90.0	75.0	10.0	0	85.0	93.3	5.0	0	98.3
Pyogranulomatous inflammation	3.4	1.7	0	5.2	0	1.7	0	1.7	1.7	0	3.3	5.0	1.7	1.7	1.7	5.1

a/ Brain tissue was processed and examined for all specimens, and there was no evidence of any pathology.

b/ I-recognizable (least severe); I I-intermediate; III-severe.

Summary

Serial sampling of 1978 brood Carson NFH spring chinook salmon in late 1979 and early 1980 indicated that general health of these fish was good, and that at release, there was clinical evidence of improvements over the fish released in 1979. Gill Na^+-K^+ ATPase profiles have been consistent in timing and level of activity for 3 years (Figure 30), and all of the evidence indicates that in 1980 the fish were released at maximum gill Na^+-K^+ ATPase activity, and that at least 50% of the fish were smolting. The average size at release was the smallest of the 3 years.

In spite of a constant 6.6°C water temperature, mean hematocrits paralled mean gill Na^+-K^+ ATPase values throughout the season Statistically significant correlations suggest that mean hematocrit data may be a useful tool at this hatchery for predicting smoltification in spring chinook salmon, should this relationship prove consistent from year to year.

There were no Low hematocrit or hemoglobin levels in the 1980 samples, and no incidence of severe (Class III) BKD. Means and ranges of the collected hematological data were above reported levels in some periods (Tables 28 and 31). An analysis of variance proved that there were highly significant differences in the mean hematocrits between periods ($F = 116$; $F_{0.0005, 4,240} = 5.20$).

Although histopathology data indicated increases in the incidences of eye and olfactory sac tissue lesions in 1980 as compared to 1979, there were no increases in severity. A separate IFAT-BKC test indicated that BKD organisms could be found in smears from the olfactory sacs of 10 out of 25 fish examined (40%).

Although we have presented an optimistic scenario for the Carson NFH spring chinook salmon released in 1980, one cautious comment must be made regarding the high hematocrits observed. In a general introductory statement, we pointed out that high hematocrits may be the result of dehydration. However, there is nothing in the Literature to indicate what actual levels of hematocrit might be expected as indicators of the first stages of dehydration. Figure 34 clearly shows a major split in the hematocrit levels between winter and late spring. At the time of release, over 80% of the fish had hematocrits of 50% or more, and 30% of the fish had hematocrits of 60% or more.

As of now, we have no reasonable explanation for high hematocrits in the spring of 1980.

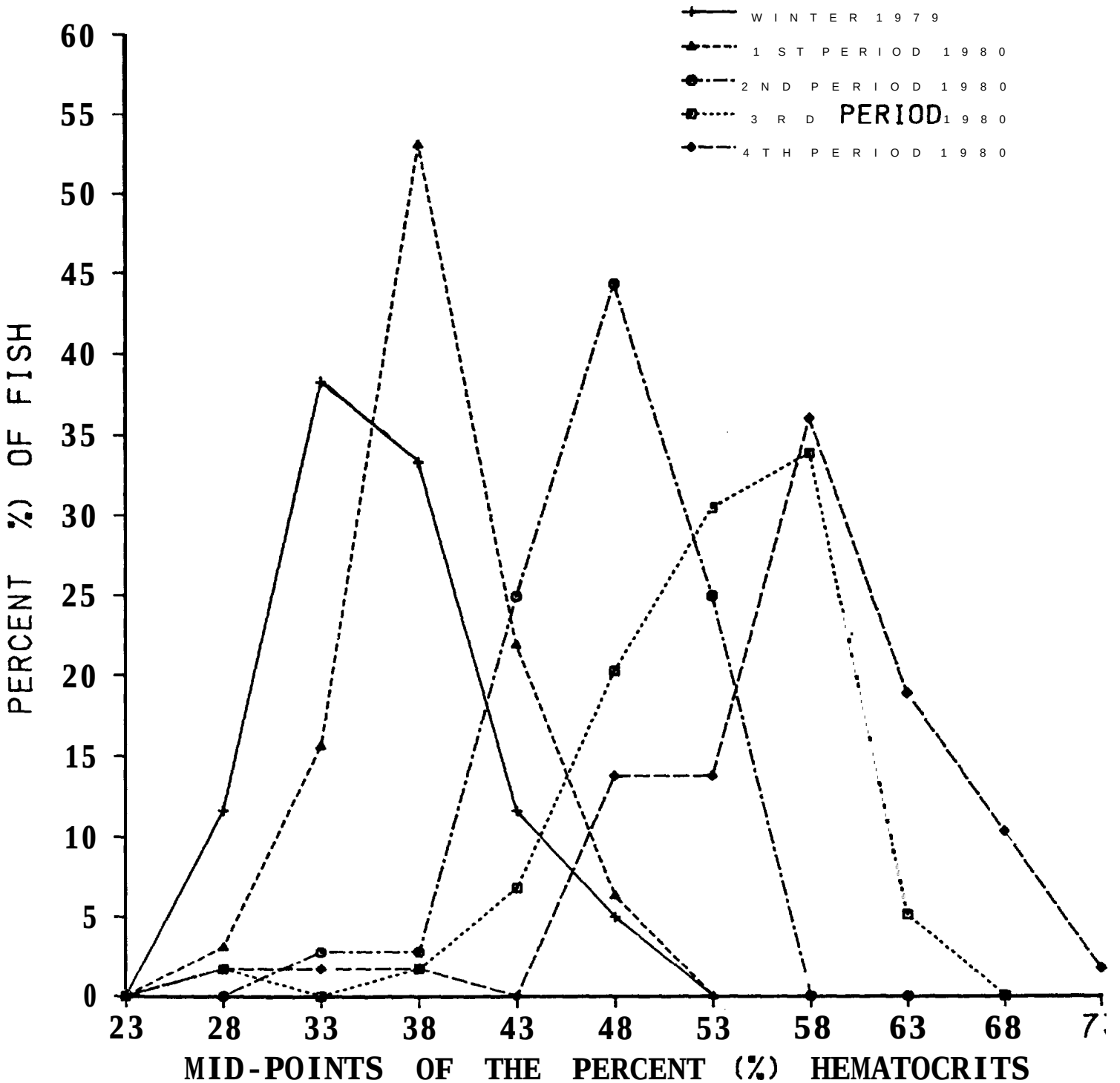


Figure 34.--Frequency of all measured hematocrits (expressed as % of fish) of Carson NFH spring chinook salmon released in 1980. Hematocrits were combined by units of 5 and are shown as the mid-points.

Kooskia National Fish Hatchery

General

Spring chinook salmon were sampled from Raceway 10 at Kooskia NFH in early March, and from Raceway 7 in April. Sampling continued beyond release (14-16 April) until early in June. Data are summarized and presented in Table 35, where periods 5, 6, and 7 represent post-release fish held over in Raceway 12.

Sampling periods are presented as days past 1 January 1980. Plasma electrolytes (Table 35) represent pooled data from 6, 3, or single fish, depending on fish size at the time of sampling.

Figure 35 shows water temperatures as measured in the raceways, and Table 35 shows average fork lengths of the Kooskia NFH fish sampled in 1980. The average size of fish taken at the time of release in 1978 (24 April) was 125 mm compared to 118 mm in 1980.

Gill Na^+-K^+ ATPase

In 1980, the gill Na^+-K^+ ATPase profile of Kooskia NFH fish was characterized by a rapid increase in mean values and standard deviations between 1 April and 15 May, followed by a very sharp decline (Figure 36). Peak Na^+-K^+ ATPase activity obviously occurred 4 weeks after release. Mean Na^+-K^+ ATPase activity at the time of release in 1978 (24 April) was 18.1; whereas in 1980 it did not reach this magnitude until approximately 7 May (Figure 36). Note that the maximum single gill Na^+-K^+ ATPase value found at release was less than the lowest single gill Na^+-K^+ ATPase value during the peak of activity (Table 35). Although there were significant positive correlations between fork lengths and gill Na^+-K^+ ATPase activity during the third and fifth periods,

Table 35.--Summary data for spring chinook salmon samples collected at Kooskia National Hatchery in the spring of 1980, with means. standard deviations ( ), and ranges. Sample size = 60.

Item	Period 1	2	3	4	5	6	7
Date	5 March	19 March	2 April	16 April	30 April	14 May	5 June
Days>Jal[a]	65	78	92	106	120	134	155
Temp.-°C[b]	3.5	4.0	5.0	5.0	6.5	8.9	7.5
Avg. Fk Ln[c]	108.8	112.9	116.8	118.0	123.2	133.2	137.4
	(7.5)	(7.0)	(7.9)	(8.7)	(7.5)	(10.2)	(8.1)
(Range)	89-126	101-131	90-133	100-140	105-141	121-198	123-175
Avg. ATP Fk Ln[d]	108.5	13.5	115.7	117.2	122.9	132.9	137.1
	(3.0)	(6.7)	(5.7)	(7.1)	(5.5)	(6.2)	(5.5)
(Range)	104.0-1 12.7	104.3-124.3	106.3-122.3	105.7-130.0	112.7-130.3	125.3-141.0	125.0-144.0
Avg. ATP[e]	7.2	7.9	7.03	11.3	15.4	19.7	11.6
	(0.8)	(0.6)	(0.9)	(1.6)	(2.8)	(3.8)	(1.9)
(Range)	5.6-8.3	7.1-8.6	6.2-8.6	8.1-13.5	10.4-19.4	16.2-28.3	9.0-14.5
Avg. Hct[f]	42.4	43.6	40.0	45.2	48.5	47.4	37.8
	(4.2)	(4.5)	(6.5)	(6.0)	(8.5)	(8.6)	(5.9)
(Range)	33-57	30-56	18-53	32-57	27-b 3	20-61	22-48
Avg. Hb[g]	6.6	7.3	6.8	7.1	6.6	7.0	6.3
	(0.8)	(0.7)	(1.1)	(1.0)	(0.9)	(1.3)	(1.1)
(Range)	3.7-8.7	5.7-9.0	2.9-9.4	4.3-9.0	3.2-8.1	2.3-9.0	3.7-8.7
Avg. MCHC[h]	15.7	16.8	17.3	15.8	13.9	14.8	16.7
	(1.6)	(1.5)	(16.1)	(1.9)	(1.6)	(1.4)	(2.6)
(Range)	10.0-20.3	13.0-19.8	2.3-22.3	10.8-20.9	11.7-18.2	11.4-17.8	10.4-25.6
Avg. Na^+[i]	146.5	148.3	152.6	156.5	157.0	133.3	144.1
	(7.2)	(9.0)	(10.6)	(4.8)	(7.6)	(11.7)	(9.0)
(Range)	133.0-157.0	141-165	128-170	150-163	138-167	112-163	123-162
Avg. K^+[j]	0.65	1.18	0.62	1.53	0.70	0.81	1.26
	(0.26)	(0.78)	(0.36)	(0.72)	(0.31)	(0.50)	(1.11)
(Range)	0.32-1.18	0.44-2.65	0.32-1.46	0.58-2.60	0.31-1.27	0.26-1.82	0.24-6.1
Avg. Cl^-[k]	153.6	125.9	129.0	124.1	134.8	118.1	129.6
	(9.7)	(4.3)	(6.9)	(5.4)	(8.5)	(10.2)	(11.4)
(Range)	137-174	121-133	118-137	113-131	110-155	93-141	100-156
Na^+/Cl^-[l]	0.96	1.18	1.19	1.26	1.17	1.18	1.12
	(0.07)	(0.07)	(0.10)	(0.03)	(0.05)	(1.0)	(0.10)
(Range)	0.82-1.05	1.07-1.29	1.00-1.33	1.23-1.33	1.07-1.25	0.91-1.39	0.88-1.40

a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C.) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in µmoles Pi/mg protein/hour.)
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period, averaged.

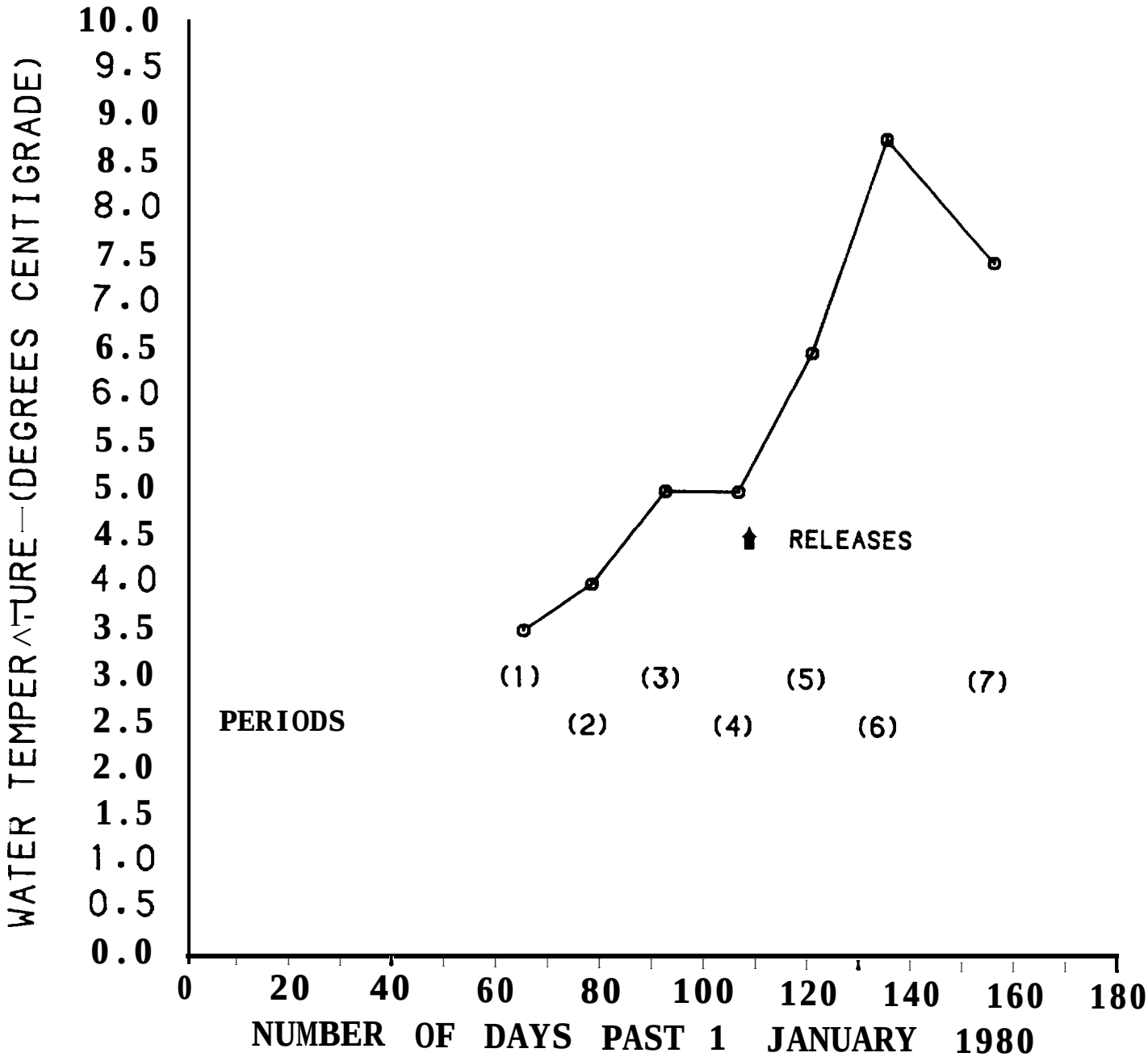


Figure 35.--Water temperatures measured in the raceways at Kooskia NFH during spring 1980.

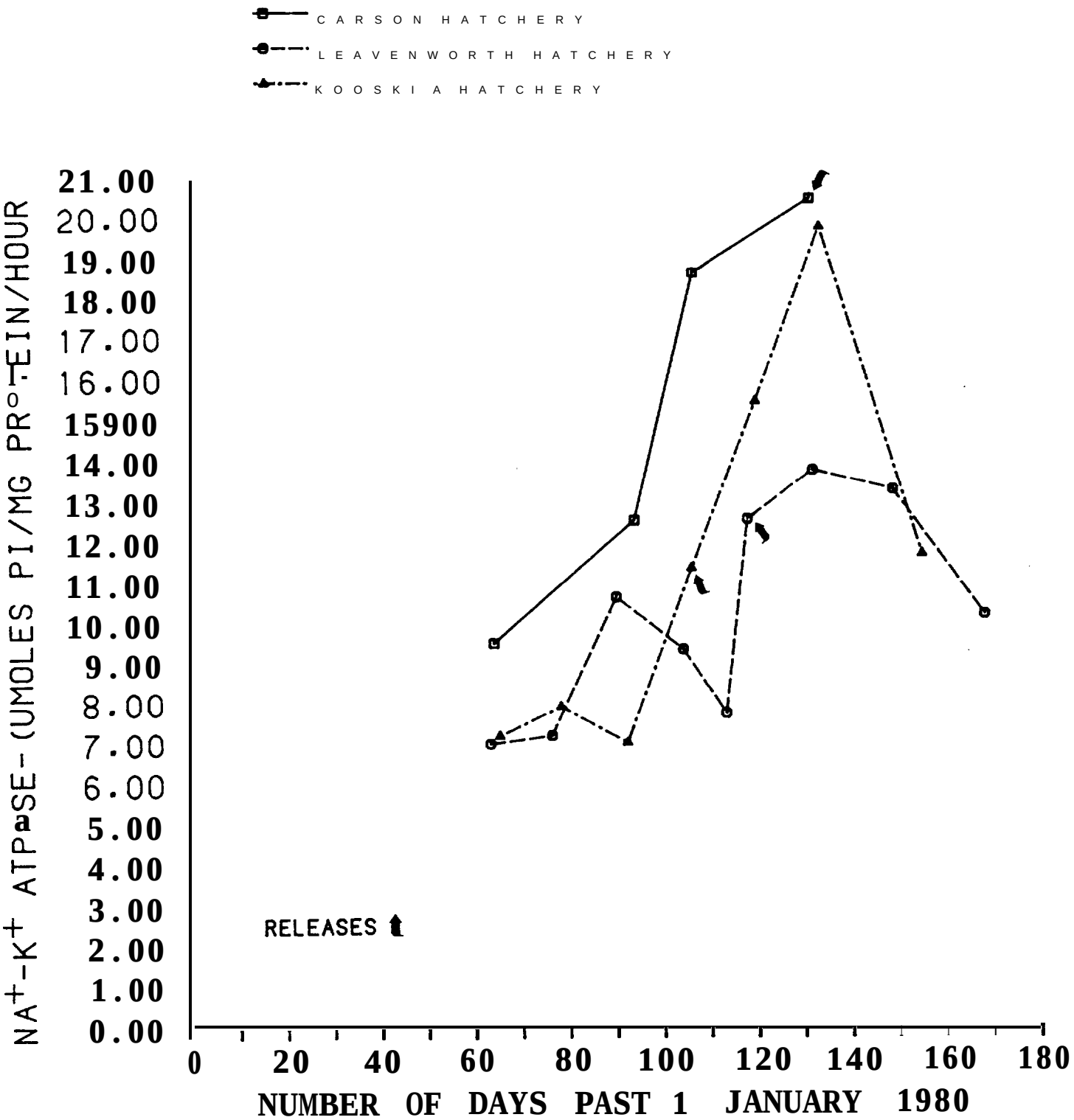


Figure 36.--Mean gill Na^+-K^+ ATPase values of Carson, Leavenworth, and Kooskia NFH spring chinook salmon during spring 1980.

this was not the case at the time of release (fourth period). There was no correlation between mean fork Lengths and mean gill Na^+-K^+ ATPase values. There was a significant positive correlation between mean gill Na^+-K^+ ATPase and the water temperature (Figure 37). However, this is probably coincidental, as the Na^+-K^+ ATPase profile normally includes a post-smolting decline, regardless of water temperature.

On the basis of the above information, especially with ranges of gill Na^+-K^+ ATPase values encountered, it appears that Kooskia NFH fish were still in a pre-smolting condition at the time of release.

Plasma Electrolytes

Mean plasma K^+ values (Table 35) were within expected ranges (Table 25), and the mean plasma Na^+ and Cl^- values were frequently at the upper end of the expected values. Mean Cl^- values in the first period were 14.6% higher than the highest mean Cl^- reported in Table 26. Mean plasma Na^+ and CL- values were much higher at the time of release in mid-April 1980 (Table 35). Both plasma Na^+ and CL- peaked on 30 April (5th period) and then declined rapidly to minimum levels at the same time gill Na^+-K^+ ATPase reached peak value (Table 35). Profiles of plasma Na^+ and Cl^- levels in Kooskia NFH spring chinook salmon were similar to those of Carson and Leavenworth NFH fish (Figures 38 and 39).

Profiles of stress indicators plasma K^+ and the MCHC, indicated nimimals 2 weeks after release (Figure 40). A comparison of the profiles of mean plasma K^+ and MCHC values for spring chinook salmon from Carson, Leavenworth, and Kooskia NFH indicates distinct similarities, and that the spring low points of both indicators occurred about the first week in May (Figures 41 and 42). These data also suggest that Kooskia NFH fish may have been released too soon.

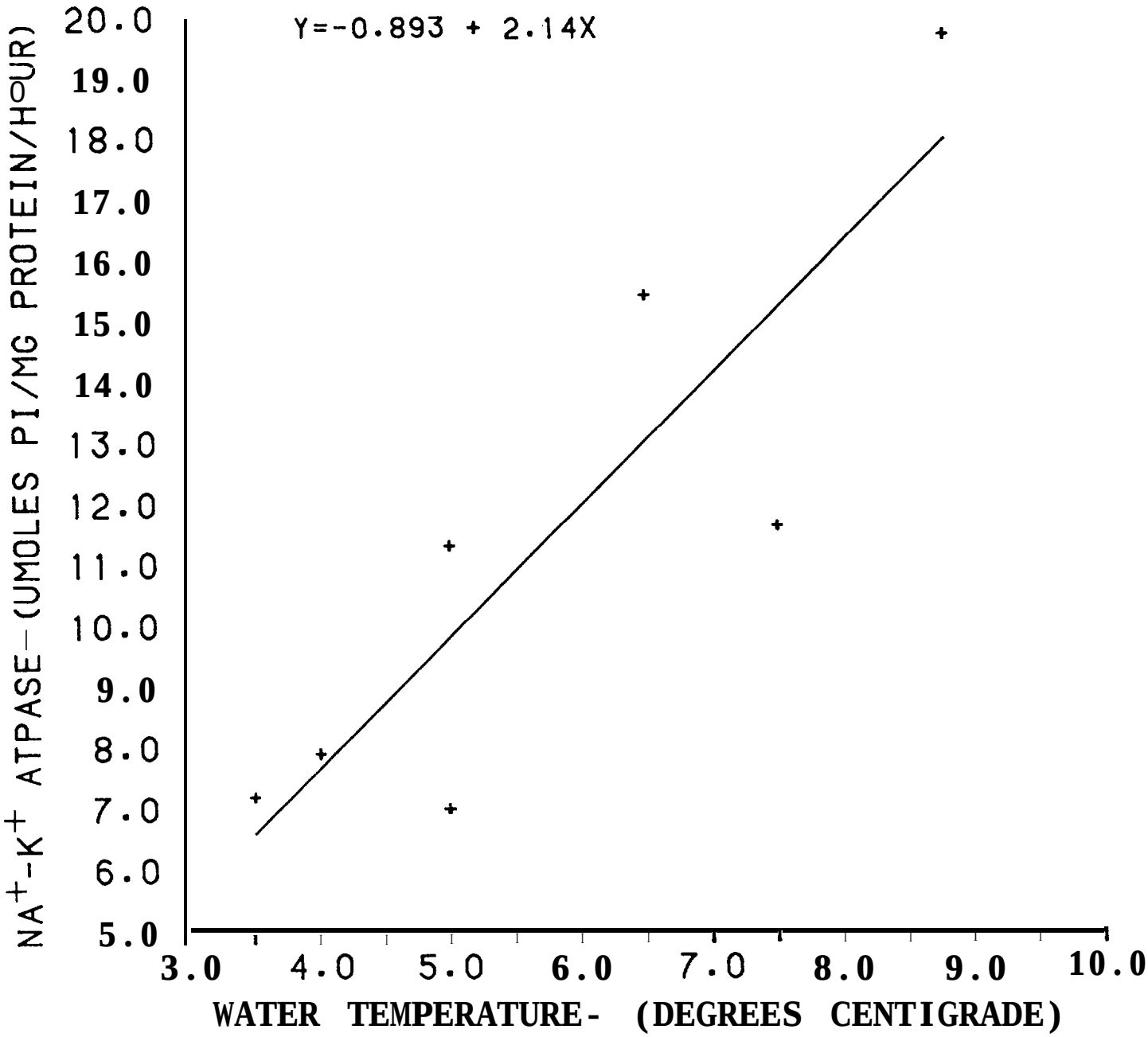


Figure 7.--Regression of average gill Na^+-K^+ATPase values of the Kooskia NFH spring chinook salmon on water temperature during spring 1980. r = 0.873 (P = 0.02).

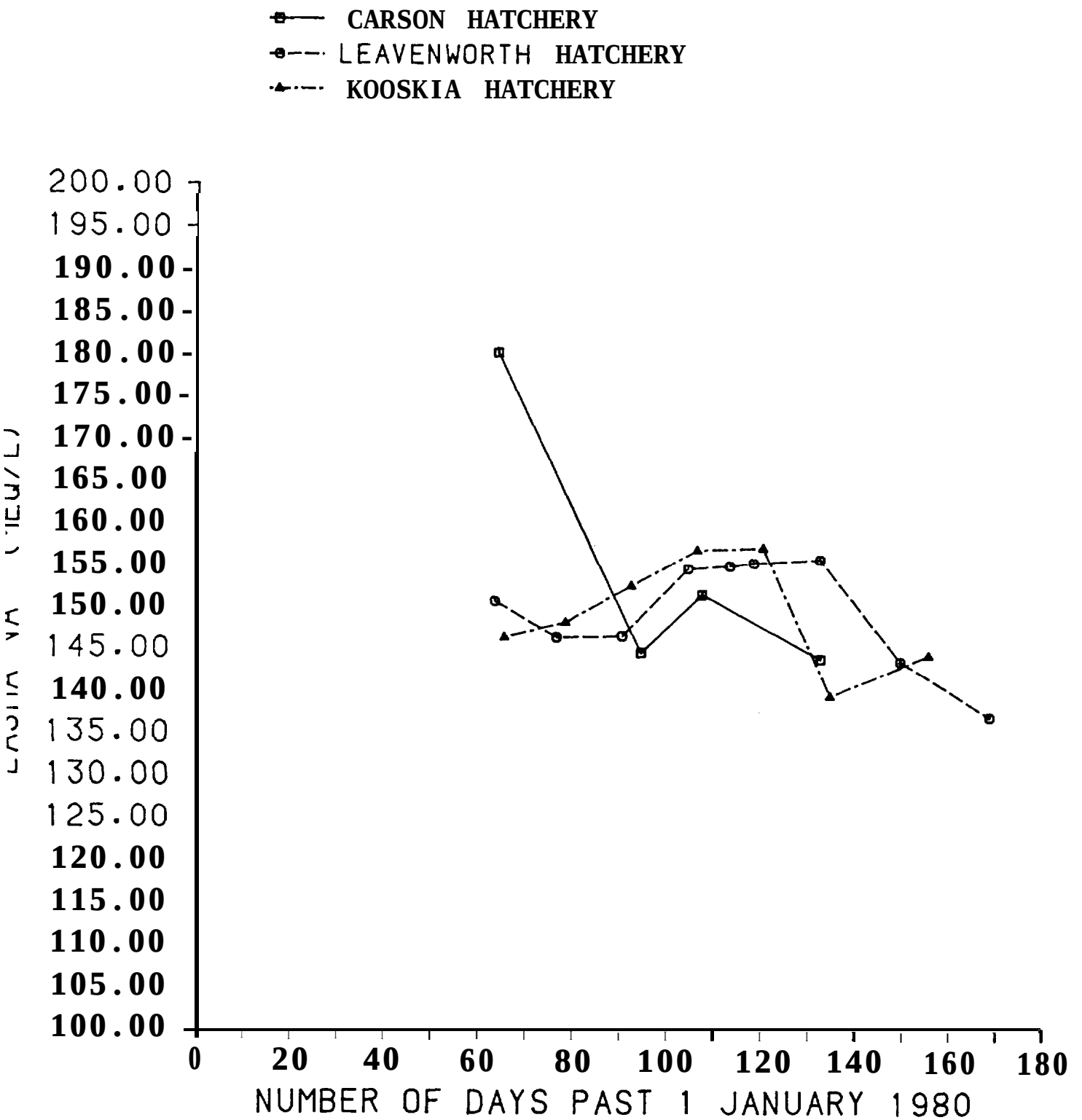


Figure 38.--Average plasma Na^+ values for Carson, Leavenworth, and Kooskia NFH spring chinook salmon during spring 1980.

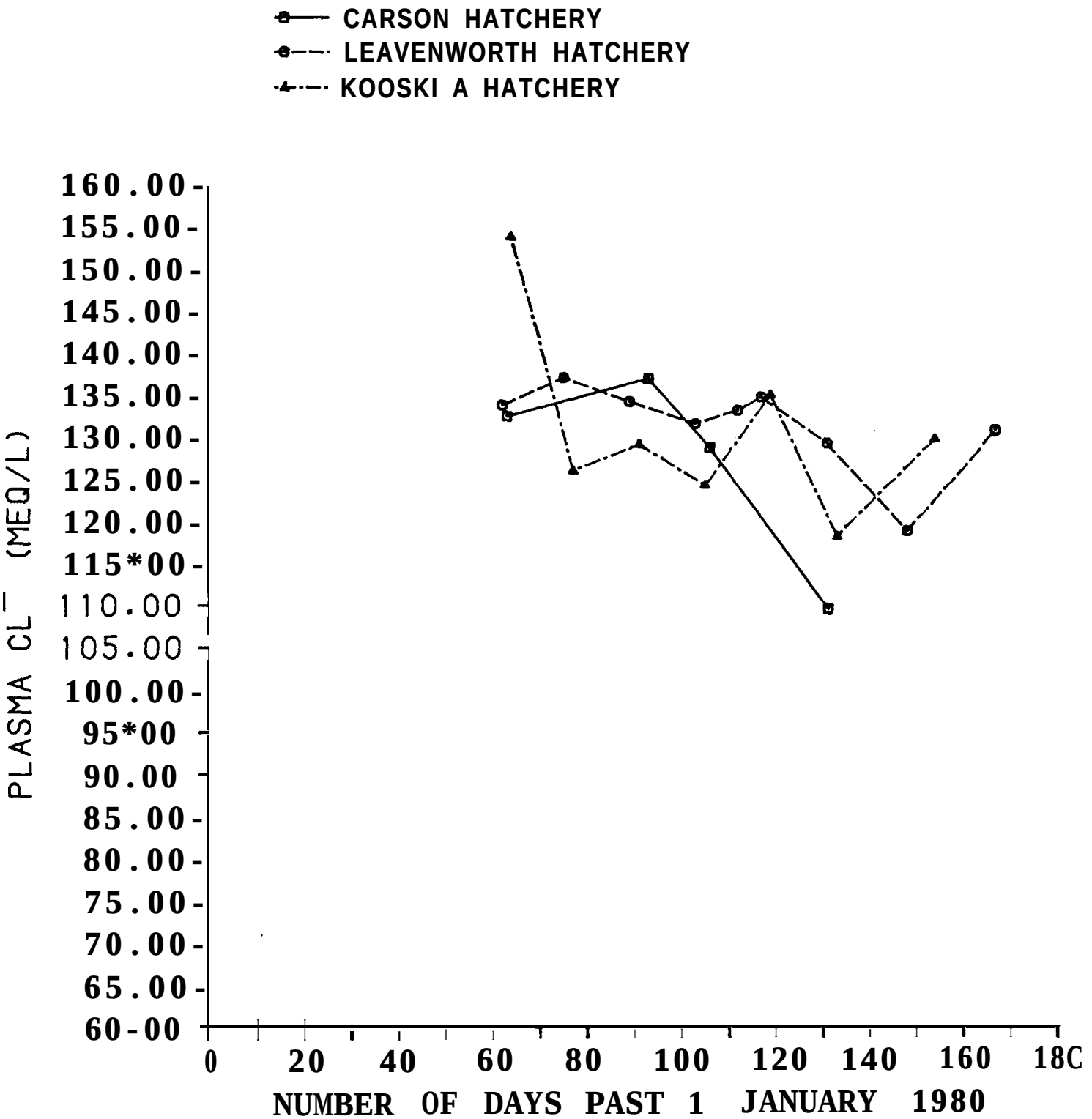


Figure 39.--Average plasma Cl^- values for Carson, Leavenworth, and Kooskia NFH spring chinook salmon during spring 1980.

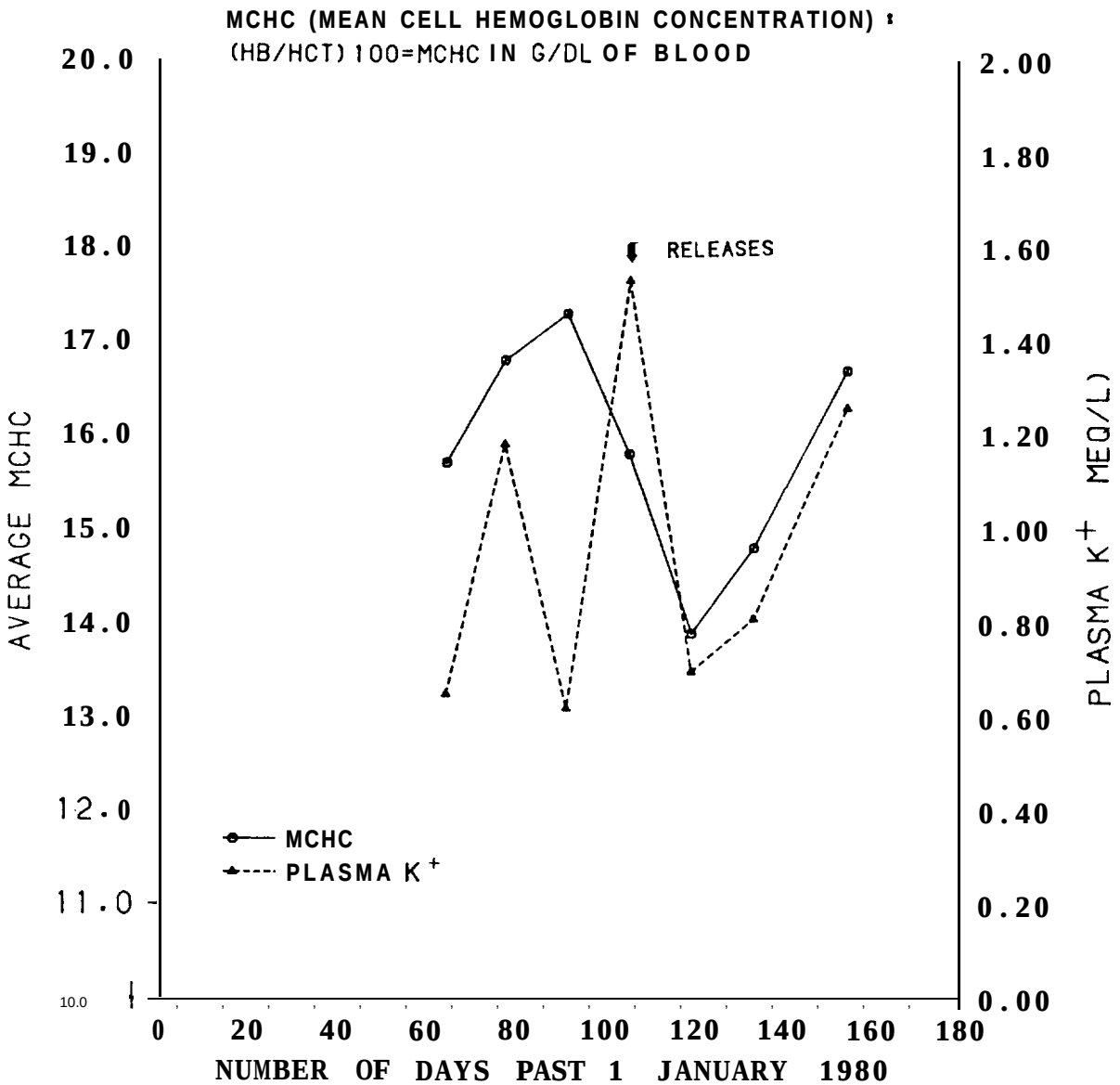


Figure 40.--Average plasma K^+ and mean cell hemoglobin concentration (MCHC) values for the Kooskia NFH spring chinook salmon during spring 1980.

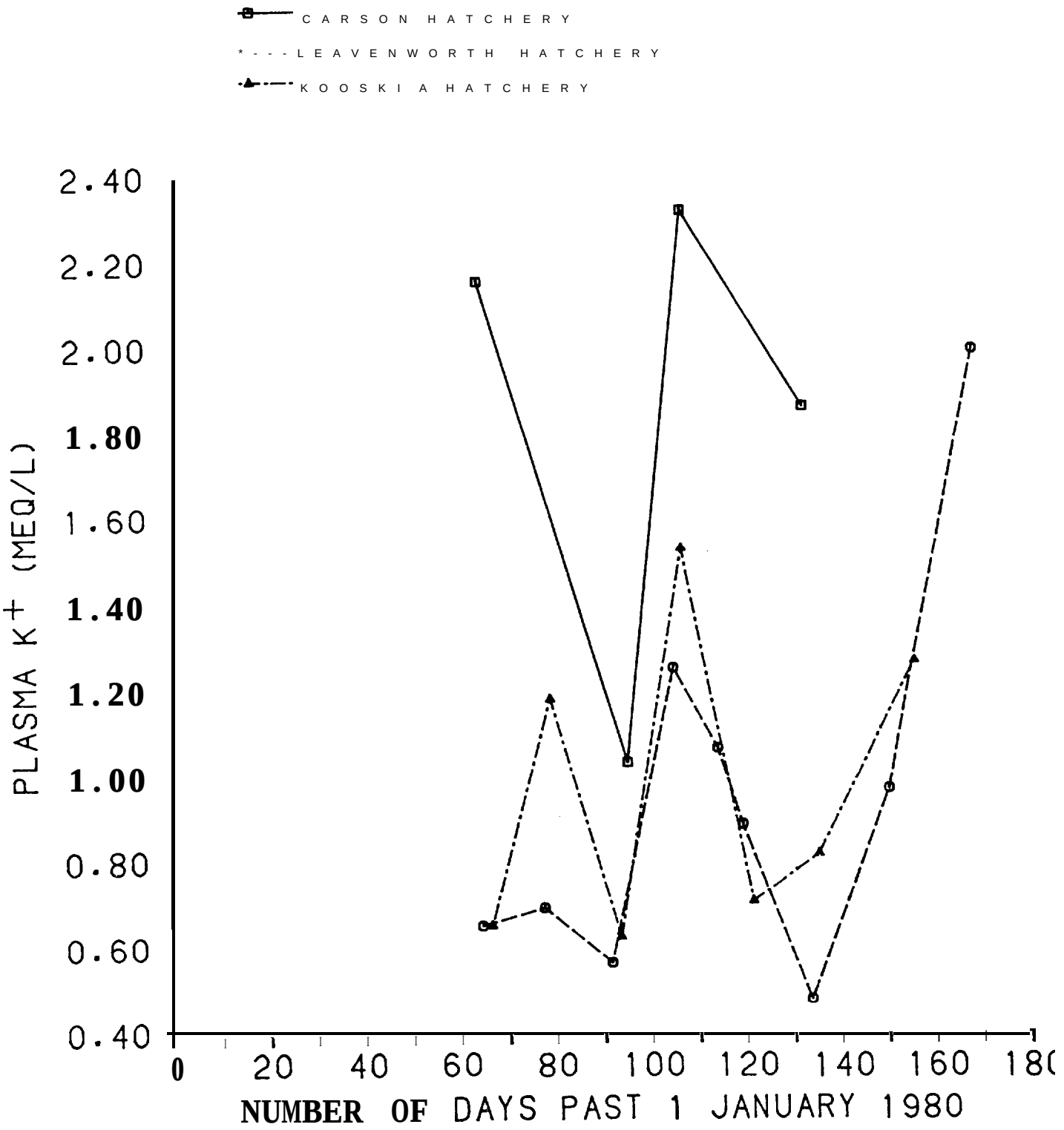


Figure 41.--Average plasma K^+ values for Carson, Leavenworth, and Kooskia NFH spring chinook salmon during spring 1980.

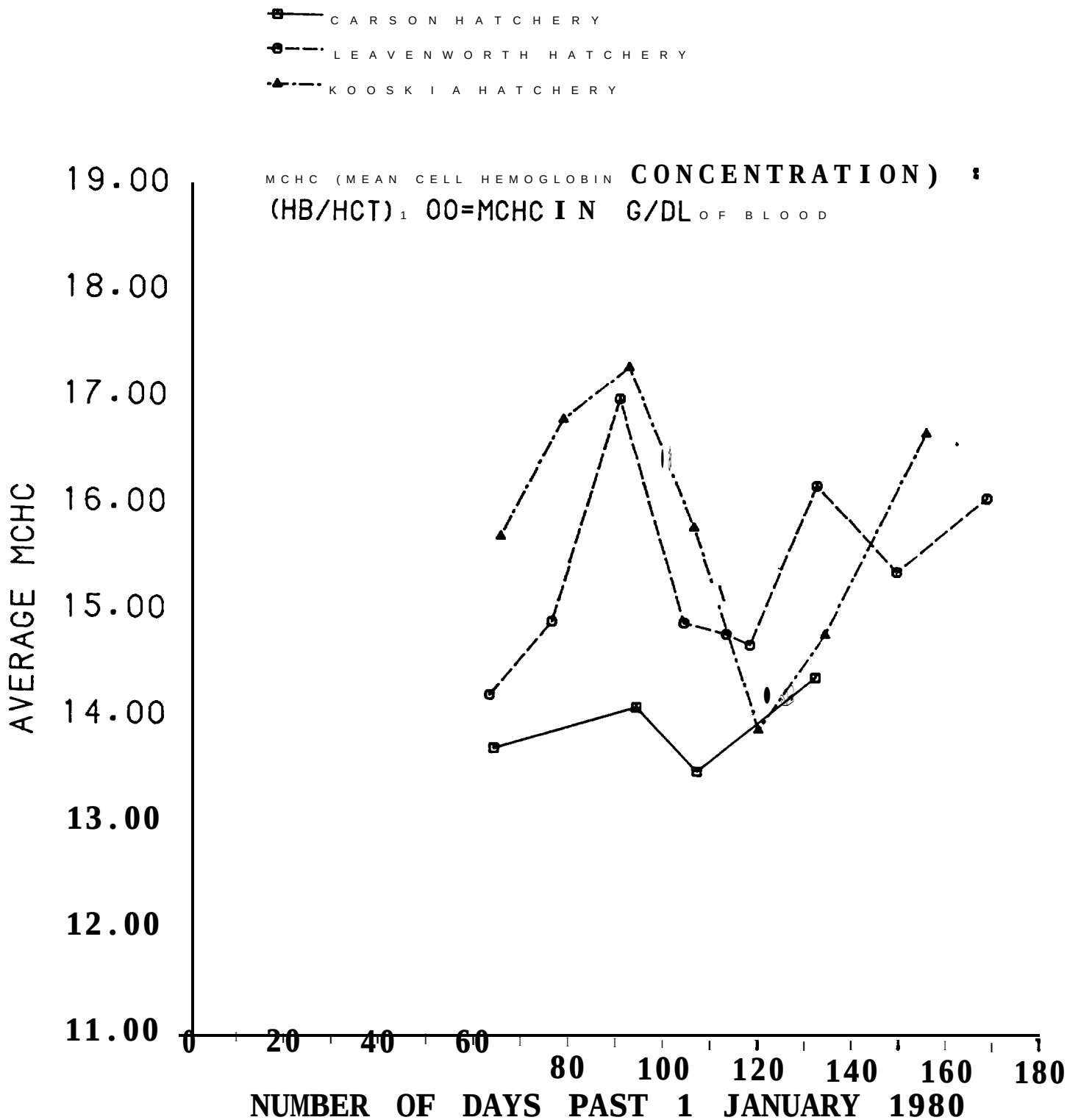


Figure 42.--Average mean cell hemoglobin concentrations (MCHC) for the Carson, Leavenworth, and Kooskia NFH spring chinook salmon during spring 1980.

Hematology'

Mean hematocrits of the Kooskia NFH fish were 39.1% (± 8.2) and mean hemoglobins were 6.6 g/dl blood (± 1.9) at release in 1978. Mean values were slightly higher at release in 1980 (fourth period), and the ranges were all within acceptable levels (Table 35). Steep declines in mean hematocrit and hemoglobin values did not occur until over 6 weeks post-release, and about 2 weeks after the maximum Na^+-K^+ ATPase activity (Table 35). In general, the data indicate that prior to, during, and' after release, only a small percentage of fish showed any evidence of borderline hematology. Only 1.3% of the total fish sampled between early March (first period) and release (fourth period) had hematocrits Less than 30%. This compares with about 8.3% for fish sampled at release in 1979. Less than 4% of the post-release fish held at the hatchery for the remainder of the sampling season had Low hematocrits (< 30%). There were no high hematocrits)> 60%) for the first four periods, and about 6.7% in post-release fish that coincided with the peak of the Na^+-K^+ ATPase activity. About 2.5% of the pre-release and 13.3% of the post-release fish had slightly elevated hematocrits (≥ 55%). There were highly significant correlations of individual hematocrits and hemoglobins ($P < 0.001$) in all seven periods, and percentages of below normal hemoglobins followed the same trends as hematocrits. There were no significant correlations between mean hematocrits and mean hemoglobins, nor between mean Hct, Hb, MCHC, and gill Na^+-K^+ ATPase.

IFAT-BKD

Specimens of Kooskia NFH spring chinook salmon from the first, third, and fifth sampling periods of 1980 were examined for the presence of BKD

organisms by the IFAT (fish were released mid-way between the third and fifth periods). The total incidence of BKD for 1980 appears to be down substantially from 1978 (Table 36), and there is considerable variation in the percentage of fish with light intensity infections throughout the sampling period. Some of this may be due to sampling from different ponds. Data classifying fish with "severe" intensity of infection may be most meaningful. The IFAT-BKD tests indicate that approximately 2% of the samples were in this category in two of three sample periods (Table 36). Close observations were made of internal organs during all seven sampling periods for any gross pathology, especially BKD type lesions. There was an average of 2.4% of the fish with observed BKD type lesions for the seven periods of sampling in 1980, ranging from 0 to 5.0%. Using these data, a minimum estimate of 2 to 5% early mortality directly attributed to BKD, could be expected.

Histopathology

A summary of pathological conditions observed is presented in Table 37. For comparative purposes, these data are further summarized with data from the other hatcheries in Table 9.

Compared to 1978 (Novotny and Zaugg 1979), Kooskia NFH fish were characterized by an increased incidence of eye lesions and both decreases and increases in gill lesions (Figure 43). As in Carson NFH spring chinook salmon, there was a pronounced decrease in certain gill lesions of intermediate (Class IL) severity as the season progressed (Table 37), and the incidence of lesions of the olfactory sac was persistently high throughout the sampling period.

Table 36.--The incidence of BKD organisms in juvenile Kooskia Hatchery spring chinook salmon in 1978 and 1980 as determined by IFAT.

Date	% of fish with BKD bacteria in the kidney				
	Anterior kidney	Posterior kidney	Both kidneys	Totals	
1978 (at release)	11.7	8.3	70.0	90.0	
5 March 1980	13.0	14.8	44.4	72.2	(25.9 moderate 1.9 severe)[a]
2 April 1980	1.8	0.0	1.8	3.5%	(100 light)[b]
30 April 1980	16.1	12.5	23.2	51.8	(12.5 moderate 1.8 severe)[c]

a/ moderate = 10-99 organisms/l50 microscopic fields.

b/ light = l-9 organisms/l50 microscopic fields.

c/ severe = $\geq$ 100 organisms/l50 microscopic fields.

Table 37.--Pathological conditions observed in 1980 Kooskia Hatchery spring chinook salmon and their percentage of incidence.[a]

Organ and athology	Incidence (%) Period 1 (severity)[b] I	II	III	total	Period 3 (severity I	II	III	total	Period 5 (severity) I	II	III	total
Eye												
celetal iscle esions	15.0	0	0	15.0	33.0	0	0	33.0	45.0	0	0	45.0
Gills												
icreased imbers of mphocytes	81.7	5.0	0	86.7	56.7	6.7	0	63.3	40.0	10.0	1.7	51.7
ithelial cell ormation	50.0	35.0	0	85.0	55.0	18.3	0	78.3	63.3	5.0	0	68.3
iscular elangiectasis secondary imellae	15.0	0	0	15.0	31.7	0	0	31.7	20.0	0	0	20.0
Olfactory sac												
cal nonuclear cell ifiltration	76.7	3.3	0	80.0	83.3	6.7	0	90.0	81.7	8.3	1.7	91.7

Brain tissue was processed and examined for all specimens, and there was no evidence of y pathology.

I-recognizable (least severe); II-intermediate; III-severe.

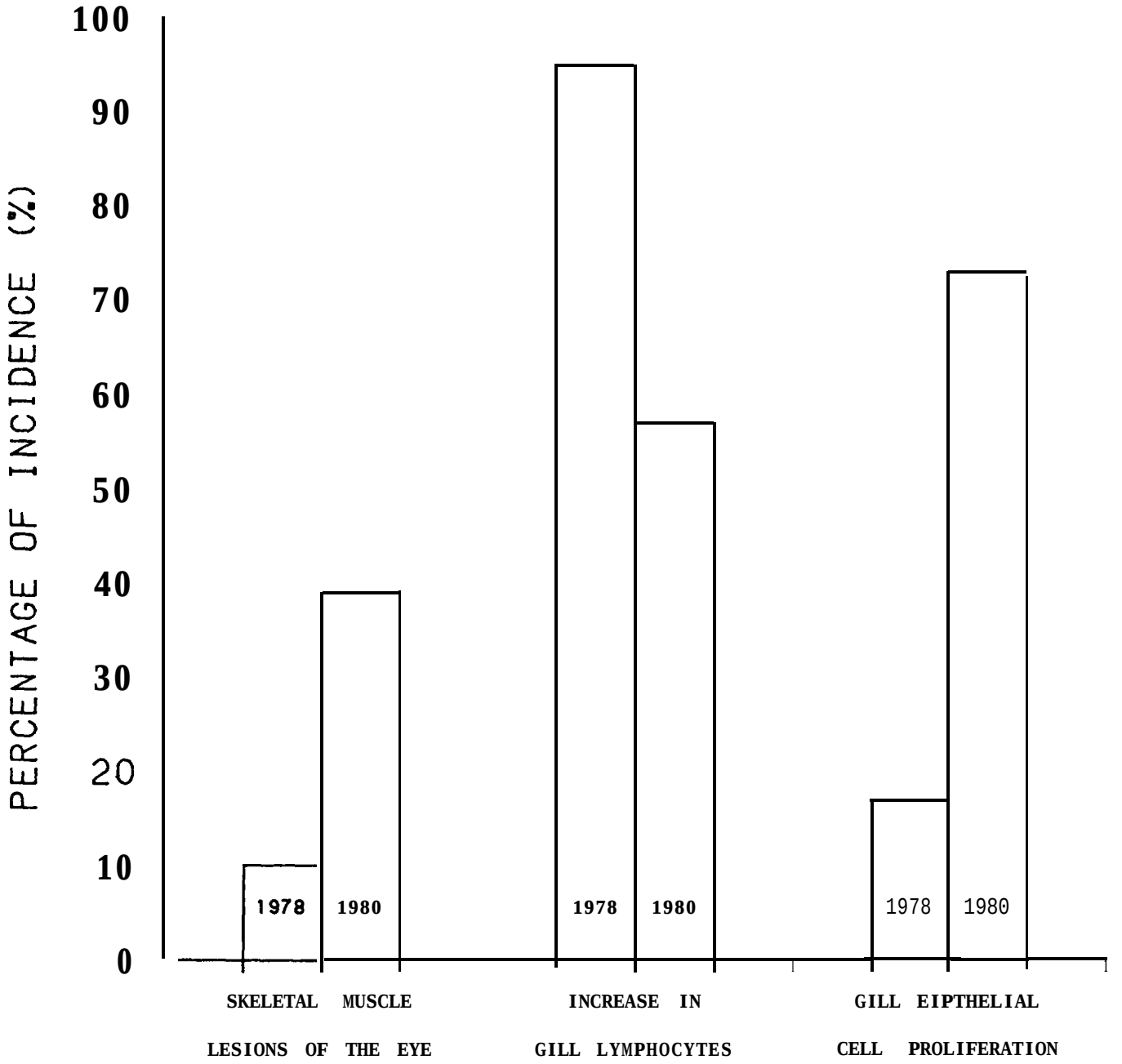


Figure 43.--Changes in the percentage of incidence in certain tissue pathology of Kooskia NFH spring chinook salmon in 1978 and 1980.

Summary

Serial sampling of Kooskia NFH spring chinook salmon in 1980 indicated that general health of the fish was good throughout the sampling season, and that clinical evidence of any disease related problems was low and probably reduced from that of 1978. Field notes indicate that a group of Kooskia NFH fish originating from Carson NFH were released from Kooskia NFH 1 week before the main releases, and there were undocumented reports that this early (Carson NFH origin) release group was suffering from anemia and a high incidence of obvious BKD Lesions. Our data suggest that early mortalities directly attributable to BKD in the normal release groups should be approximately 2 to 5%.

Mean hematocrits of Carson, Leavenworth, and Kooskia NFH spring chinook salmon data suggest that throughout the season, Kooskia NFH fish were approximately between the other two, presenting a normal, healthy appraisal (Figure 44).

However, it would appear from gill Na^+-K^+ ATPase and plasma electrolyte data, that the fish may have been released before a high percentage of the population s molted. A comparison of gill Na^+-K^+ ATPase data from the same three hatcheries (Figure 36) shows a remarkable coincidence in the profiles and presents sufficient data in itself to indicate that holding Kooskia NFH fish until at Least 1 May would have been the best choice.

Since none of the samples had adequately elevated Na^+-K^+ ATPase levels at release, we must conclude that the percentage of smolting was very low, and no estimates can be given.

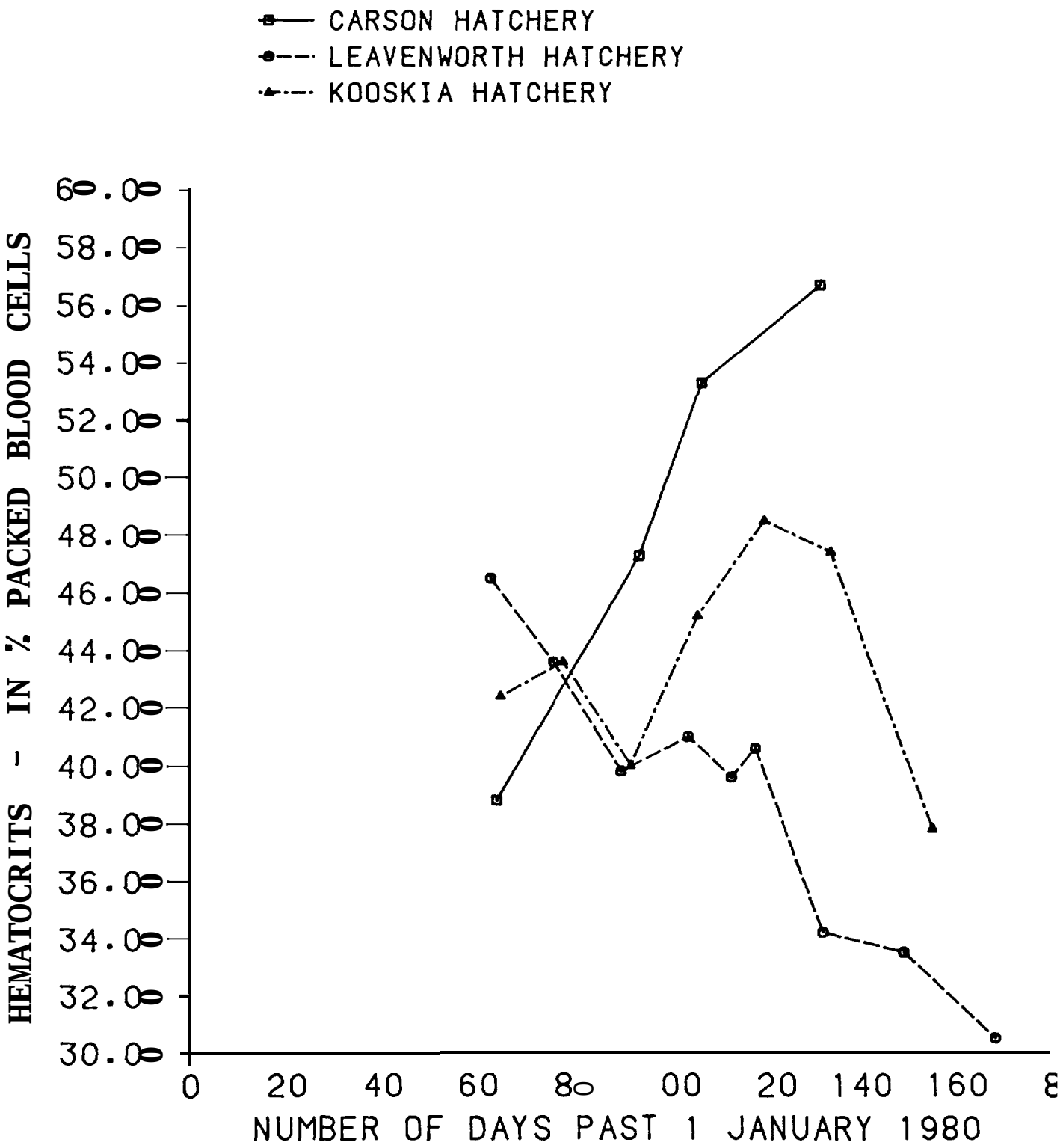


Figure 44.--Average hematocrit values for Carson, Leavenworth, and Kooskia NFH spring chinook salmon during spring 1980.

Rapid River Hatchery (IDFG)

General

Sampling of spring chinook salmon from the earthen rearing ponds at The Idaho Fish and Game Department's (IDFG) Rapid River Hatchery began in early March and continued into mid-May at 2-week intervals, for a total of six periods. The fish were allowed to migrate volitionally. Migration began in early April, and from 17 April (fourth period) post-release fish were held in a temporary circular tank at the hatchery. The main ponds were drained about 1 May for annual maintenance, and at this time any residual fish were flushed out.

Sampling from the ponds without stressing fish presented a problem, as physical conditions were not suitable for crowding. Sampling was accomplished by dropping a Lift-net below an automatic feeder in the middle of the pond, hand broadcasting food over the net, and then rapidly raising the net. Usually over 500 fish could be trapped, and from this sample approximately 20 fish were dipped out for destructive analysis. This was repeated three times during the course of the day.

Field notes indicate that erythromycin phosphate was injected into Rapid River Hatchery adults prior to spawning and also to treat eggs at fertilization to reduce the vertical transmission of BKD.

Plasma samples were pooled by groups of three fish (along with the gill samples for Na+-K+ ATPase analysis) until Periods 5-6, when individual plasmas were used.

Data from six sampling periods are presented in Table 38. The peak of emigration was estimated to be 10 April, and the samples from Periods 4-6

Table 38.--Summary data for the spring (1980) sampling of Rapid River Hatchery spring chinook salmon with means, standard deviations ( ), and ranges. Sample size = 60.

	Period 1	2	3 (peak of emigration)	4	5	6
Date	6 Mar 80	21 Mar 80	3 Apr 80	17 Apr 80	1 May 80	15 May 80
Days>Jan 1 a/	66	79	93	107	121	134
Temp. °C b/	3.5	4.0	5.0	6.0	1.5	8.5
Avg. Fk Ln c/	126.6	130.4	135.7	133.2	136.5	143.7
	(5.0)	(7.4)	(3.7)	(9.5)	(9.1)	(4.9)
(Range)	116.7-135.0	116.3-142.3	130.7-141.3	116.7-147.7	122.3-152.3	136.7-152.0
Avg. ATP Fk Ln d/	126.6	130.4	135.7	133.2	136.5	143.7
	(5.0)	(7.4)	(3.7)	(9.5)	(9.1)	(4.9)
(Range)	116.7-135.0	116.3-142.3	130.7-141.3	116.7-147.7	122.3-152.3	136.7-152.0
Avg. ATP e/	8.4	9.5	12.6	10.8	1u.9	7.9
	(1.4)	(1.0)	(3.6)	(2.0)	(1.9)	(2.8)
(Range)	6.0-10.1	8.1-10.9	8.6-20.2	7.5-14.0	7.8-14.0	5.1-13.5
Avg. Hct f/	40.0	41.8	38.2	42.2	44.5	49.6
	(6.9)	(6.0)	(5.9)	(7.6)	(8.6)	(7.4)
(Range)	9-52	26-53	25-55	18-60	21-65	35-65
Avg. Hb g/	4.8	5.2	5.9	5.9	5.9	7.4
	(1.1)	(1.0)	(1.1)	(1.5)	(1.1)	(1.2)
(Range)	1.0-7.0	2.0-7.3	2.6-8.1	0.7-9.0	3.5-8.1	5.0-10.7
Avg. MCHC h/	12.0	12.5	15.7	14.0	13.4	15.3
	(1.4)	(1.9)	(3.1)	(3.0)	(3.0)	(2.7)
(Range)	8.8-15.6	7.0-17.8	7.6-26.0	3.71-19.6	8.0-23.4	10.4-20.9
Avg. Na^+ i/	15Y.b	146.2	161.7	125.2	130.9	116.0
	(10.2)	(16.9)	(4.0)	(17.6)	(16.3)	(20.0)
(Range)	136-182	112-173	152-167	104-160	83-155	69-165
Avg. K^+ j/	0.86	1.02	0.68	0.59	u.51	0.61
	(0.61)	(0.40)	(0.32)	(0.42)	(0.36)	(0.50)
(Range)	0.37-2.75	0.47-1.86	0.34-1.44	0.19-1.70	0.23-2.00	0.24-1.95
Avg. Cl^- k/	135.4	133.3	133.0	86.9	113.0	93.1
	(10.1)	(11.8)	(4.6)	(12.1)	(21.5)	(13.1)
(Range)	122-158	107-151	123-139	70-119	60-163	63-111
Na^+/Cl^- l/	1.18	1.10	1.22	1.41	1.18	1.24
	(0.10)	(0.13)	(0.03)	(0.09)	(0.15)	(0.10)
(Range)	1.03-1.41	0.86-1.31	1.16-1.29	1.28-1.57	0.85-1.75	1.02-1.54

a/ Days>Jan: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp. -°C: Water temperature (in degrees C.) measured for that period.
c/ Avg. Fk Ln: The average tork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour.)
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium For that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl^-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period, averaged.

(Table 38) were collected from fish held back in circular tanks. Periods 5-6 could be considered "post-emigration."

Field notes indicate that Dr. G.W. Klontz[4/] suspected some epistylus infestations in early March, and we noted a small number of "graybacks" during the second sampling period. By the fourth period, very few parred fish and no BKD lesions were noted. The fish looked good and the scales were loose.

Figure 45 is a profile of water temperatures in the ponds at Rapid River Hatchery as measured during the sampling periods. Figure 46 plots average fork lengths of Rapid River hatchery fish as measured from our sampling. The apparent decline in growth and growth rate after the third period may be an artifact resulting from larger, smolting fish emigrating from the pond first.

Gill Na+-K+ ATPase

There is difficulty in following trends of gill Na+-K+ ATPase (or any of the other-parameters) in Rapid River Hatchery spring chinook salmon because fish were allowed to migrate volitionally, and the only totally captive population from which samples could be taken was not established until the fourth period. Figure 47 profiles the Na+-K+ ATPase values. Note that the curve increases rapidly and then declines between the third and fourth periods. If a major migration of smolting fish had occurred, the emigrating individuals probably had the highest Na+-K+ ATPase values. In the third period, 30% of the Na+-K+ ATPase samples were

[4/] Professor of fisheries, University of Idaho, Moscow, Idaho.

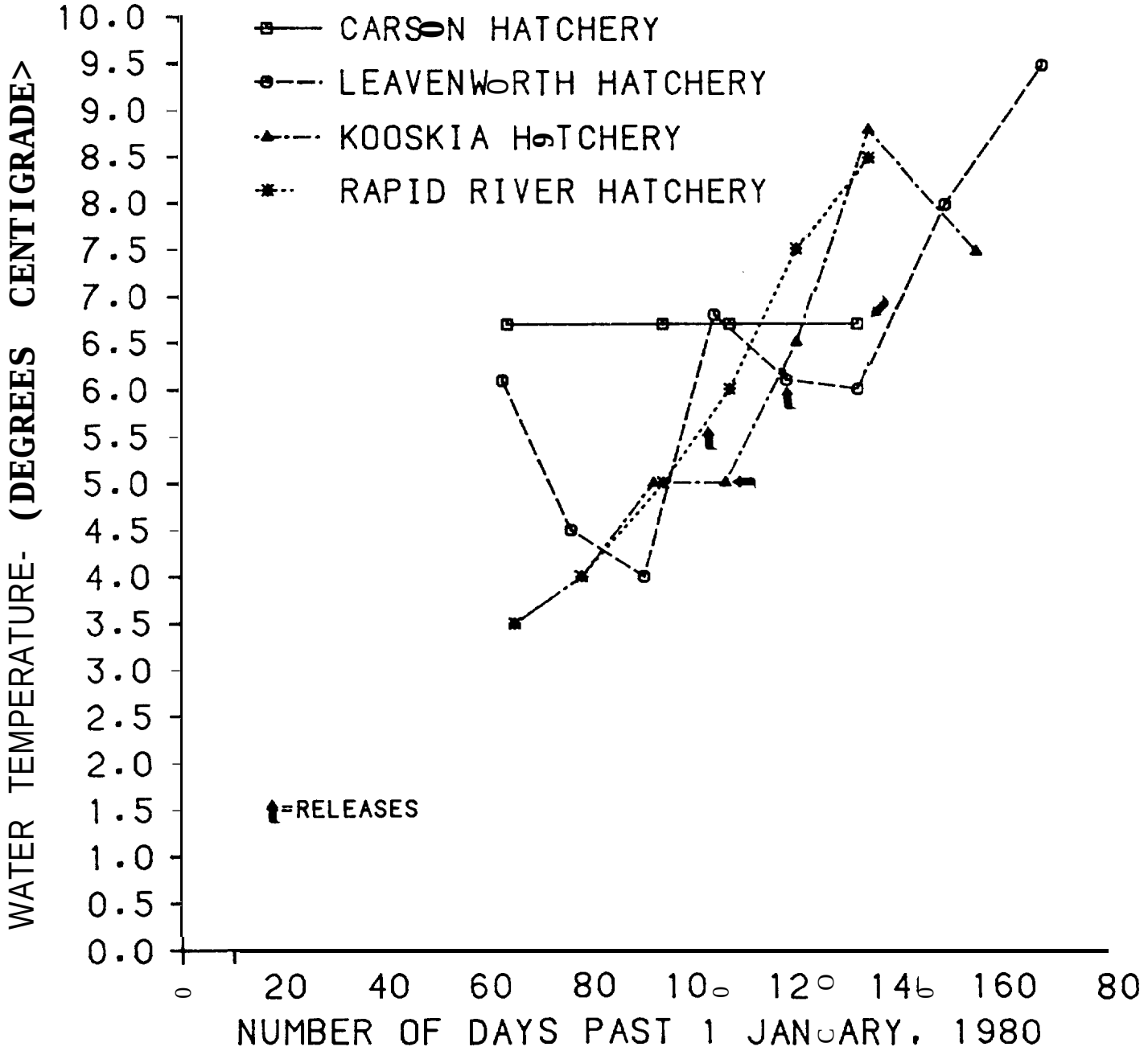


Figure 45.--Water temperatures measured at Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery during spring 1980.

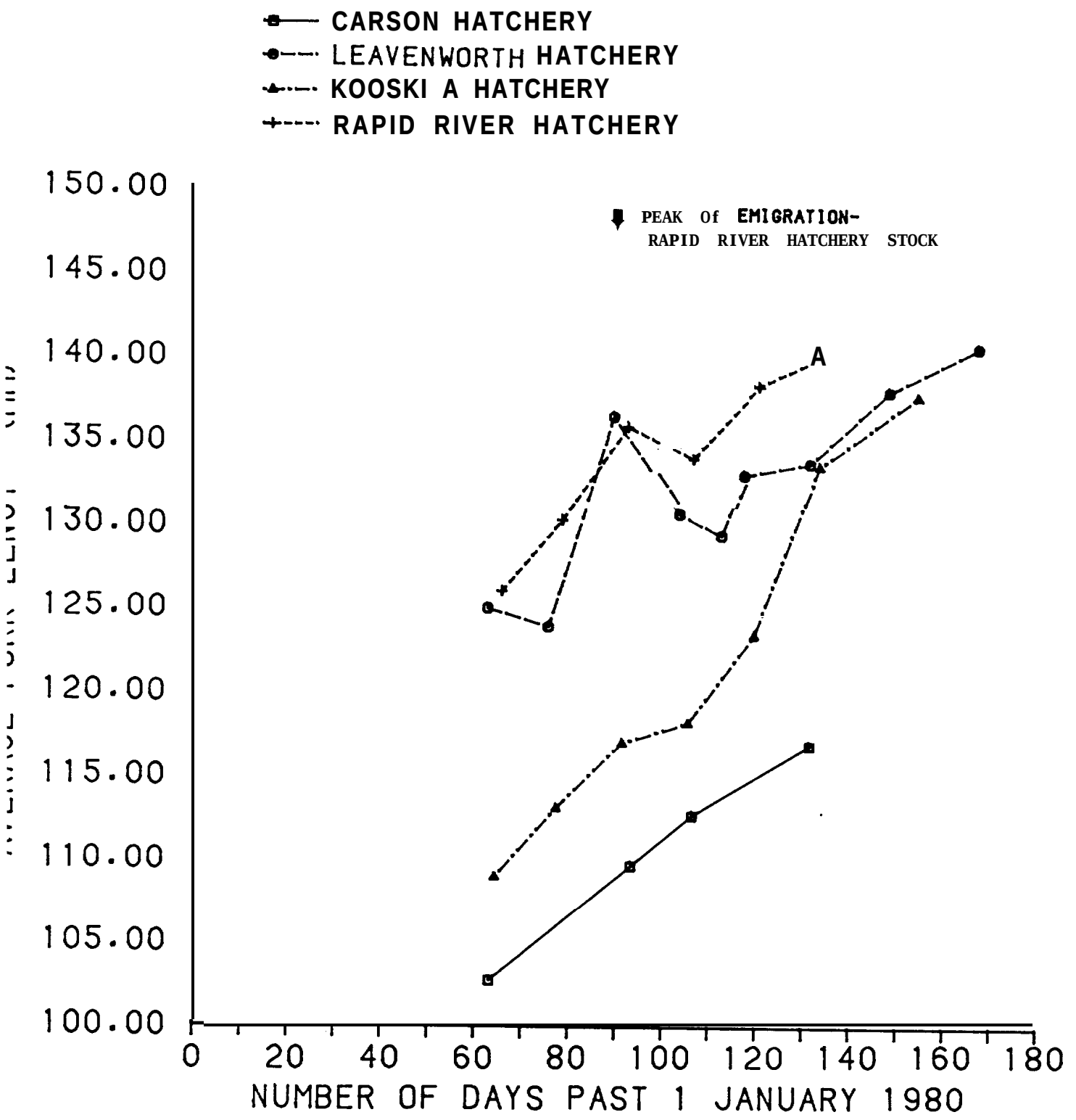


Figure 46.--Mean fork lengths of spring chinook salmon sampled from Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery during spring 1980.

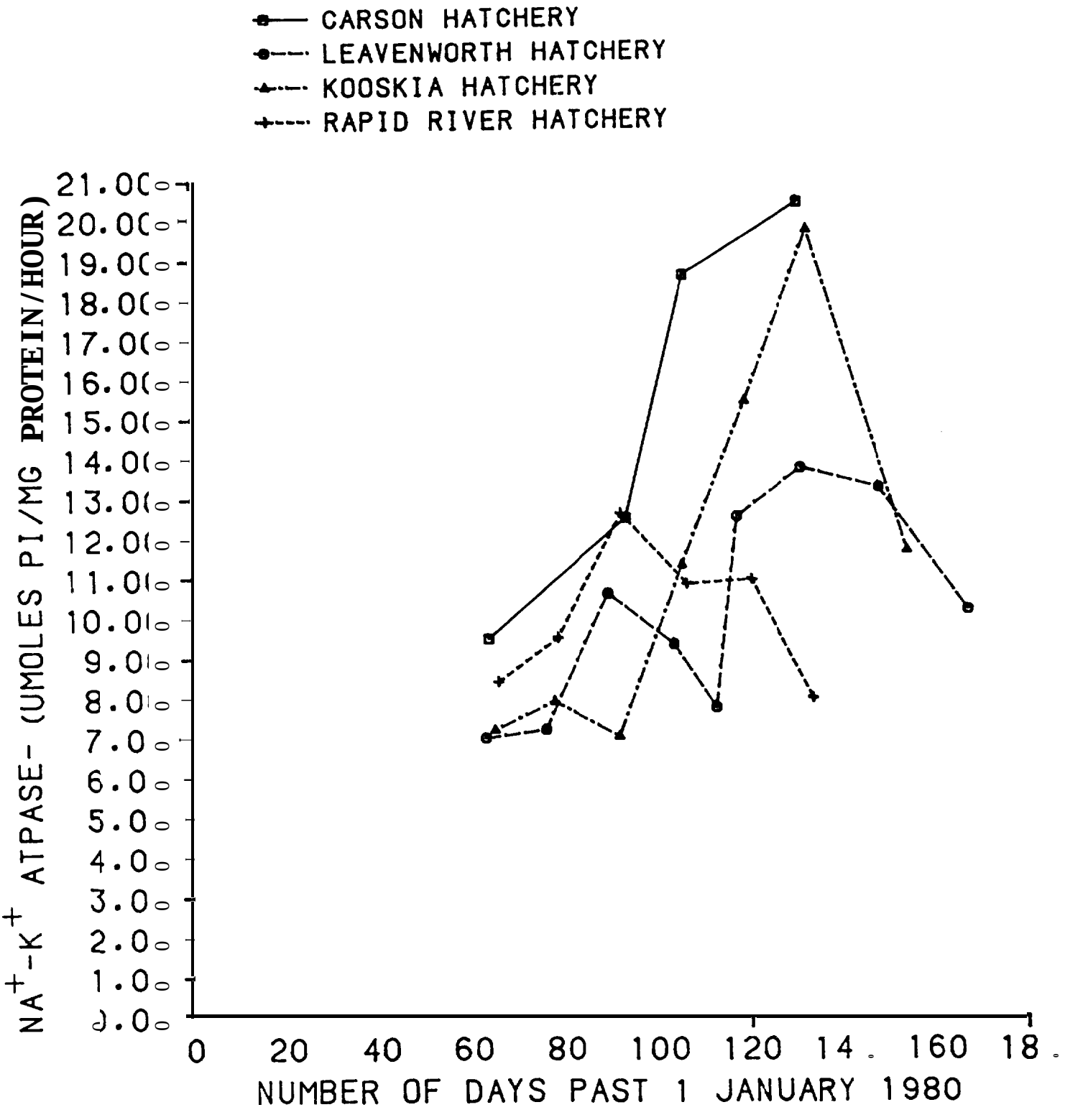


Figure 47. Mean gill Na^+-K^+ ATPase values of Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery spring chinook salmon during sprig 1980.

greater than 15.0 u moles Pi/mg protein/hour. In the fourth period, none of the samples was greater than 14.0 u Pi/mg protein/hour. There were no positive correlations between mean gill Na+-K+ ATPase values or other parameters during the sampling season, or during individual periods. From the profile presented (Figure 47), there definitely appears to be a post-smolting decline in Na+-K+ ATPase values after the fifth period which may have been reduced by a change in environments. A "best guess" would be that at least 30% of the fish sampled were smolting at the beginning of the migration period (3), and that this percentage increased during the next 2 weeks.

Plasma Electrolytes

Mean plasma Na^+ values (Table 38) were occassionally higher than the expected (Table 25), and the mean plasma Cl^- and K+ values were frequently lower. Mean chloride values on two occasions, for example, ranged from 10.5 to 16.3% lower than the Lowest Cl^- values reported in Table 25, and on four occasions mean plasma K^+ values ranged from 16 to 37.5% Lower than the lowest values reported in Table 25.

Mean plasma Na^+ and Cl^- Levels declined rapidly during the peak of migration (Figures 48 and 49), and mean plasma K+ concentrations were well into a decline at the same time (Figure 50). The mean Na^+/Cl^- ratio (which is, again, a measure of the maximum divergence of plasma Na^+ and CL-) increased sharply to a peak value at the same time (Table 38). The key stress indicators (elevated plasma K^+ amd MCHC values) had peaked and were in a sharp decline during this major migration period (Figures 50 and 51).

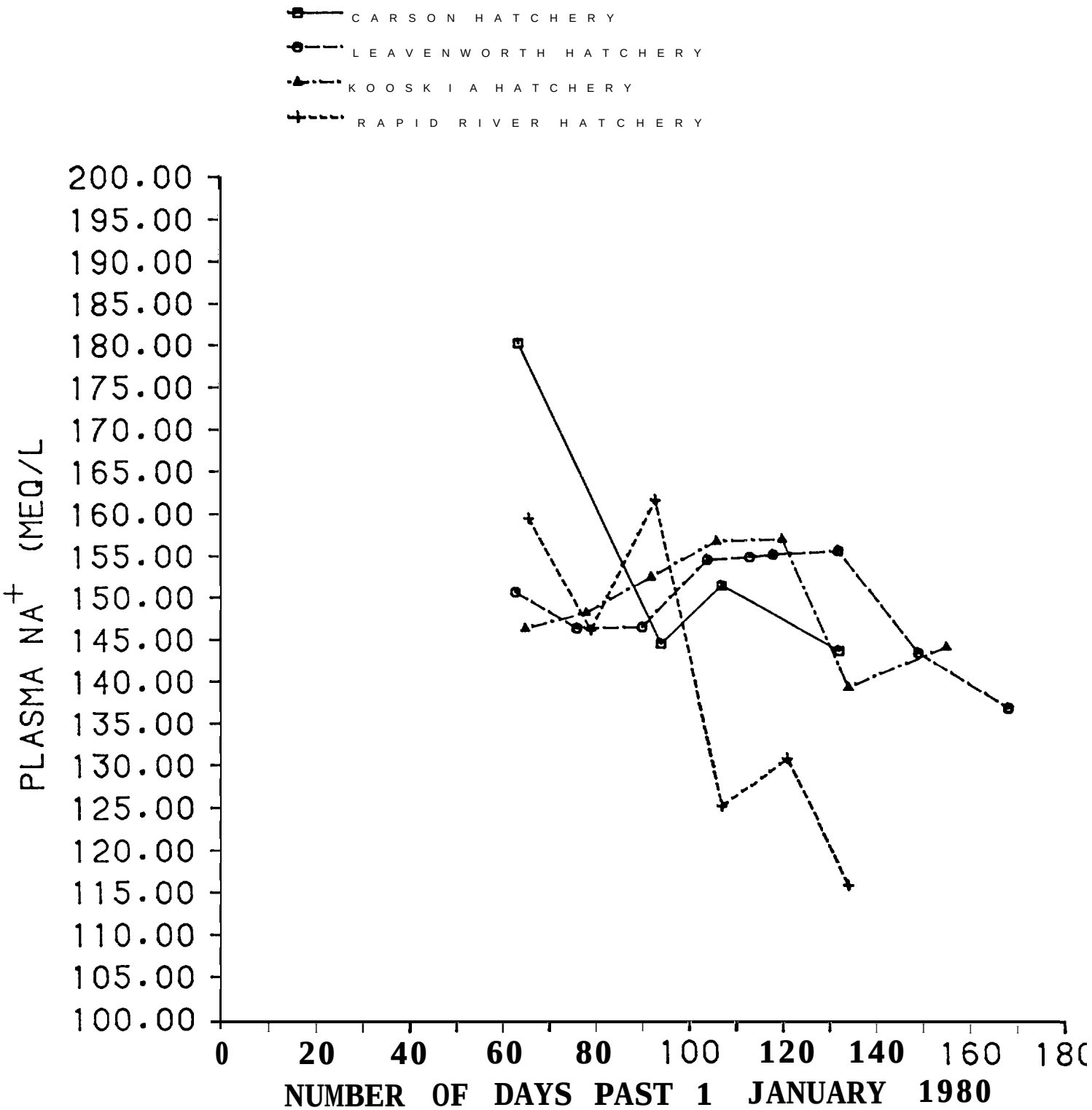


Figure 48.--Mean plasma Na^+ values of Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery spring chinook salmon during spring 1980.

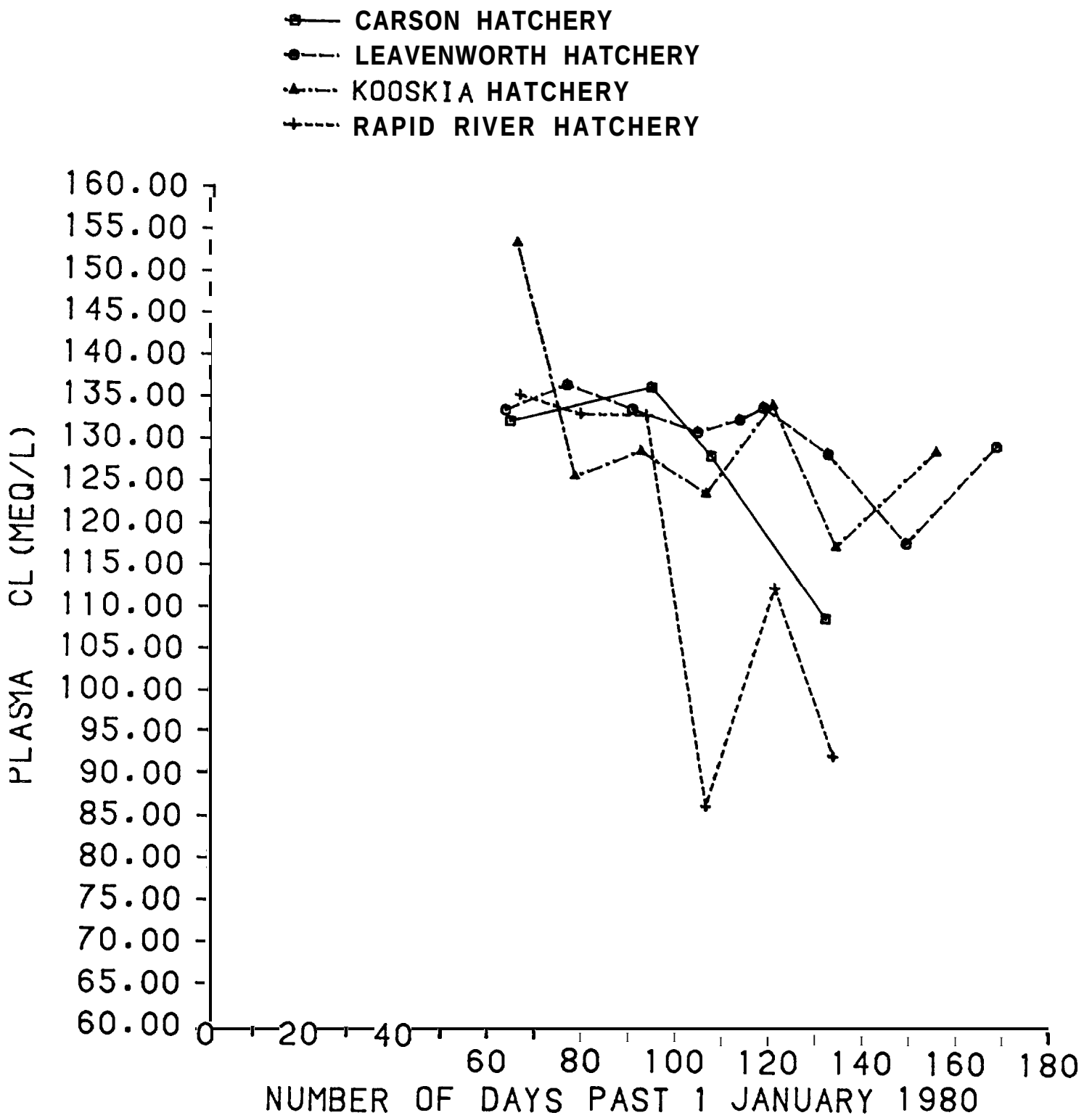


Figure 49.--Mean plasma Cl^- values of Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery spring chinook salmon during spring 1980.

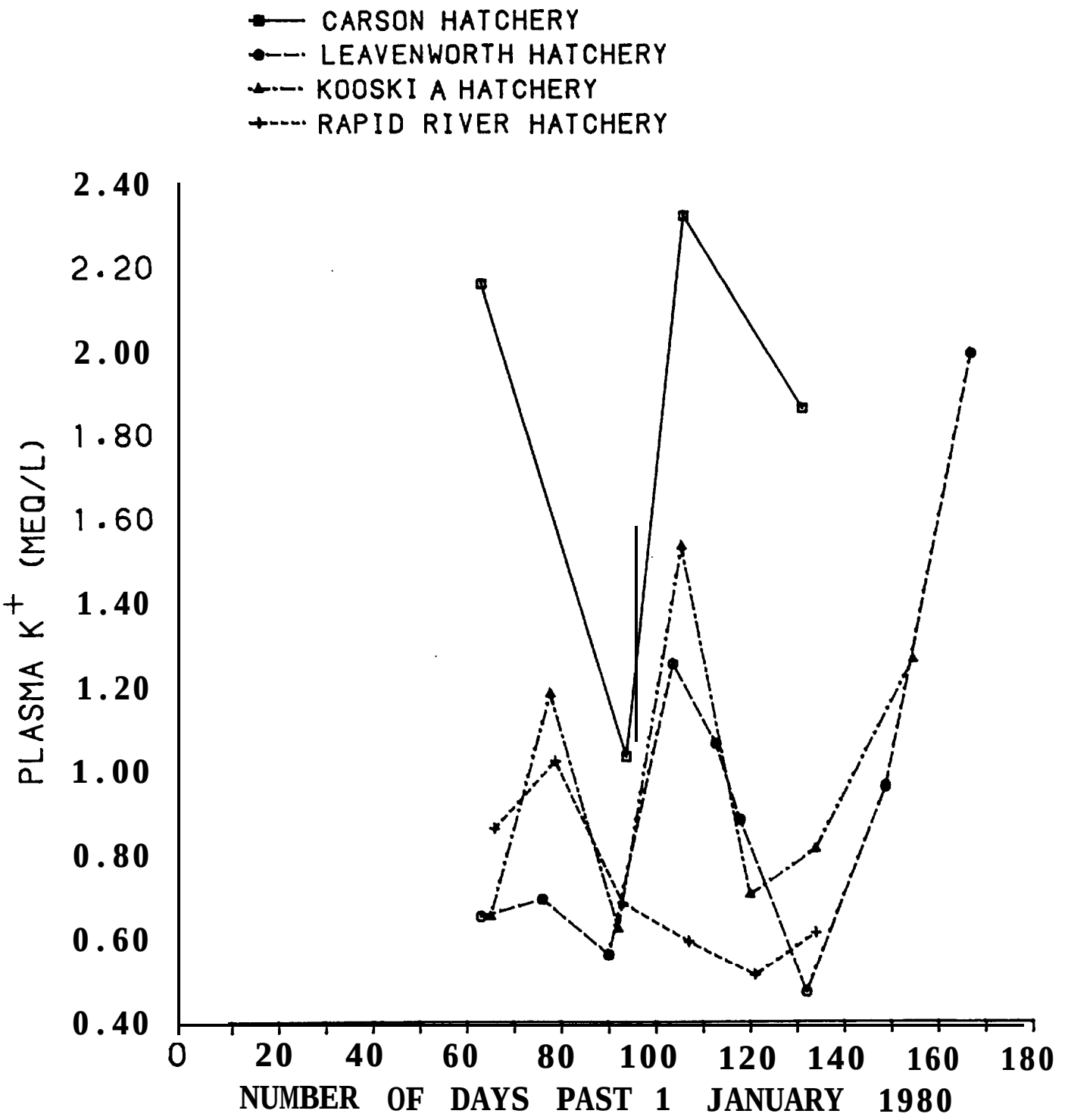


Figure 50.--Mean plasma K^+ values of Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery spring chinook salmon during spring 1980.

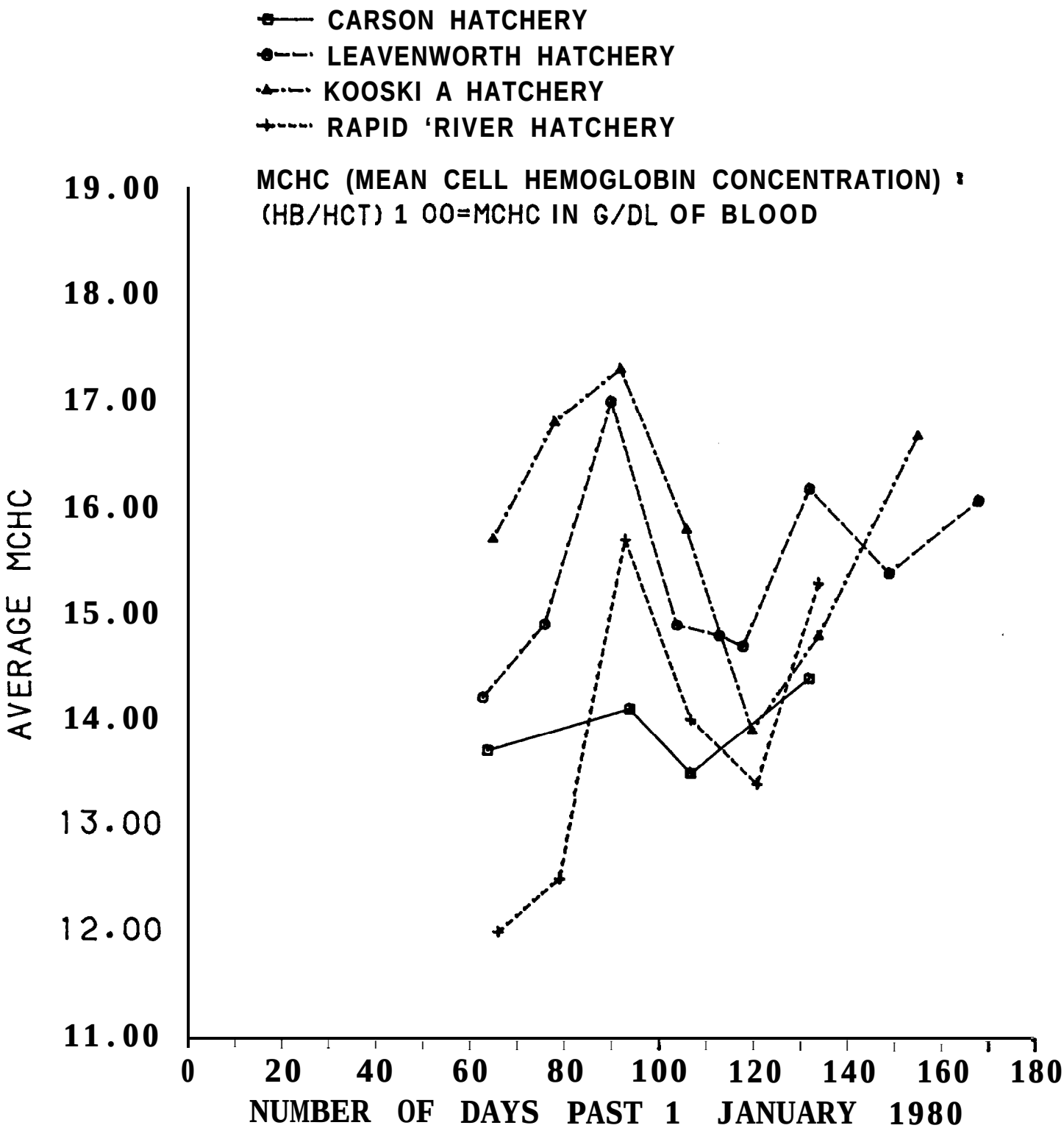


Figure 51 .--Average mean cell hemoglobin concentrations (MCHC) of Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery spring chinook salmon during spring 1980.

There were no correLations between the average gill Na^+-K^+ ATPase value and any of the average plasma electrolytes. There was a significant positive correlation between mean plasma Cl.' and mean plasma Na^+ values (Figure 52), a significant negative correlation between mean plasma Na^+ and water temperatures (Figure 53), and between mean plasma Na+ and mean hematocrits (Figure 54).

There were no significant correlations between individual plasma Na+ and Cl^- values until the third period ($r = 0.615$; $P < 0.005$), early in April. Correlations of Na+ and Cl- from that point increased, and ranged from $r =$ 0.8 to 0.9 ($P < 0.001$). Correlations between individual plasma K+ and the Na+ and CL- values were significant only between the third and fourth periods ($r = 0.6$-0.7; $P < 0.01$-0.02).

Hematology

Mean hematocrits and hemoglobins of Rapid River Hatchery spring chinook salmon were all within normal ranges throughout the 1980 spring sampling season (Table 38) and showed a general increase as the season progressed (Figures 55 and 56). There *were* significant positive correlations between the hatchery water temperature and mean hematocrits ($r = 0.840$; $P < 0.05$) and mean hemoglobins ($r = 0.882$; $P < 0.02$) suggesting that these parameters *are* positively influenced by water temperature in this stock (Figures 57 and 58).

There was no significant correlation between mean hematocrit and hemoglobin values. IndividuaL hematocrits and hemoglobins were all positively correlated throughout the sampLing season (Table 39). Although mean plasma Na^+ values and mean hematocrits were significantly correlated (Figure 54), these relationships could not be shown for individual fish.

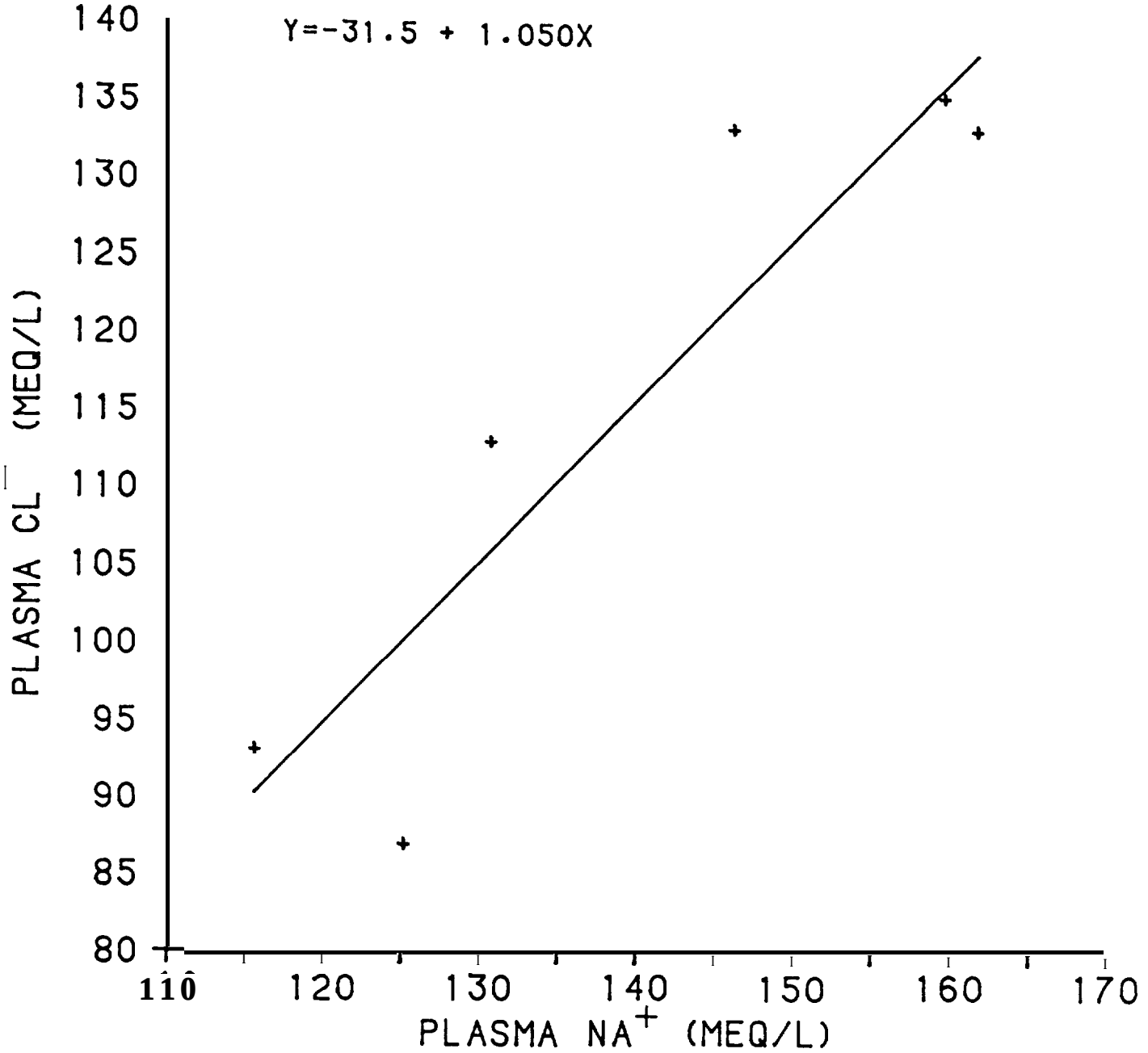


Figure 52.--Regression of mean plasma Cl^- values on mean plasma Na^+ values of Rapid River Hatchery spring chinook salmon during spring 1980. $r = 0.915$; $P < 0.02$.

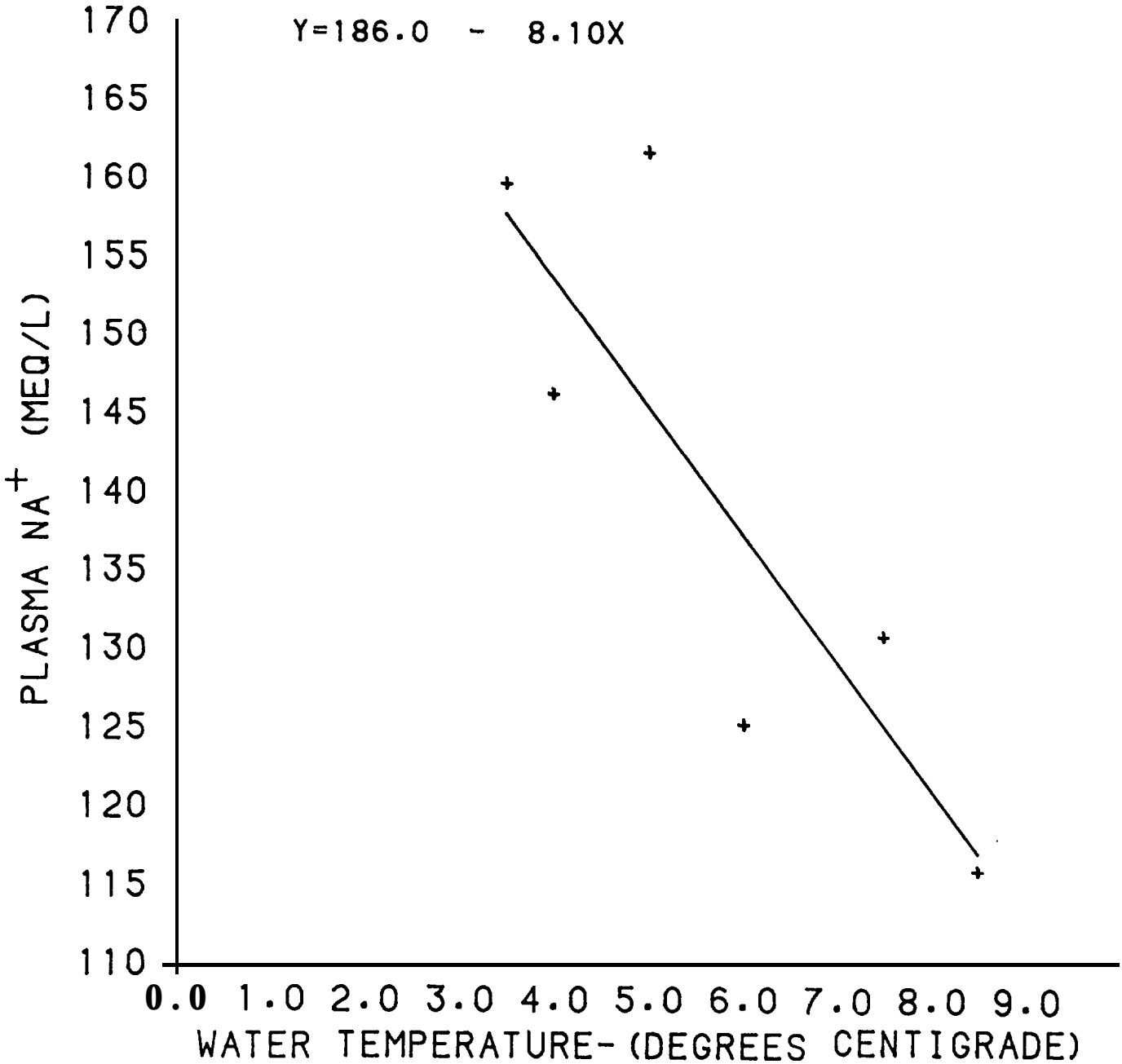


Figure 53.--Regression of mean plasma Na^+ values of Rapid River Hatchery spring chinook salmon on water temperature during spring 1980. $r = -0.847$; $P < 0.05$.

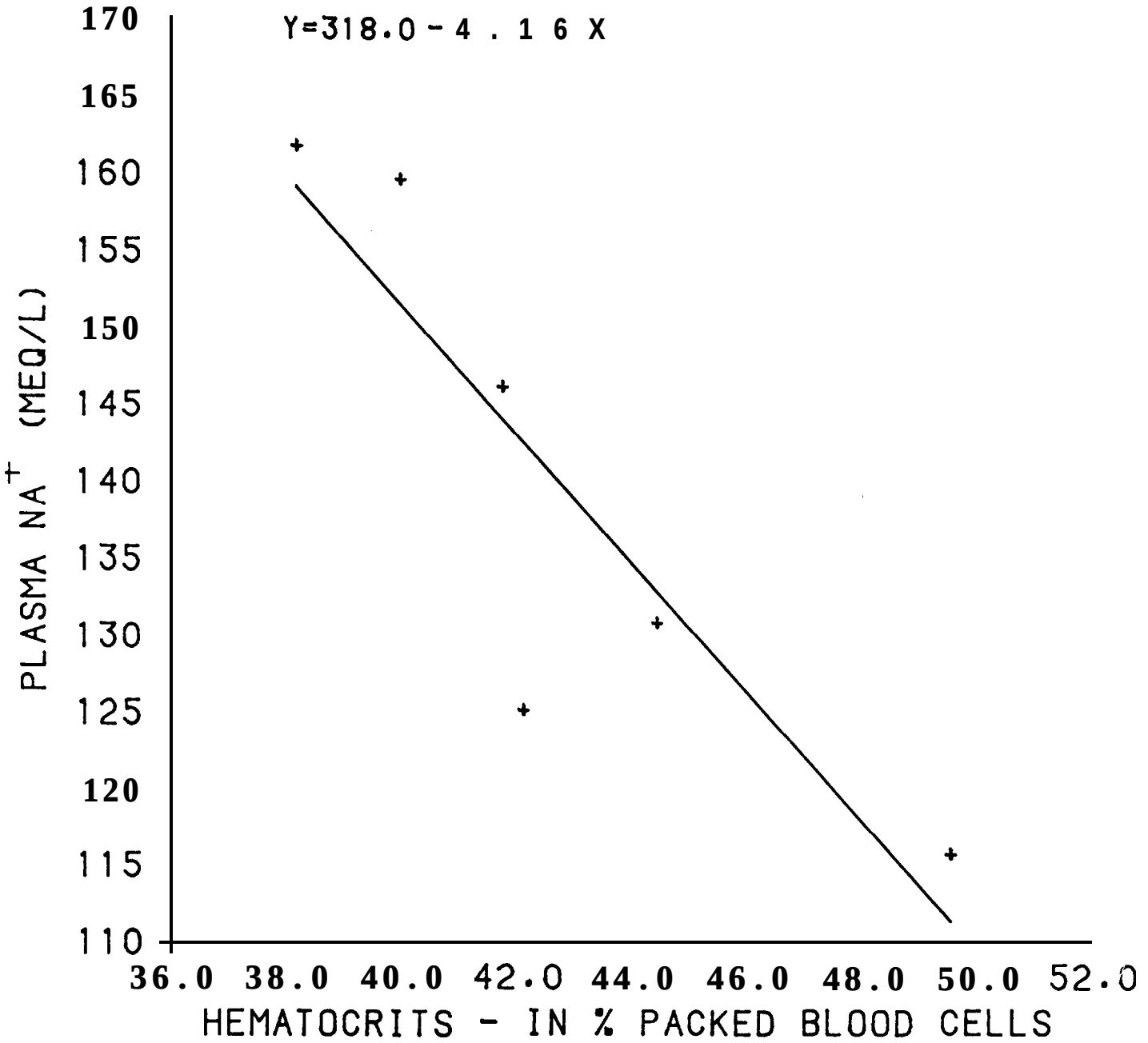


Figure 54.--Regression of mean plasma Na^+ values on mean hematocrits of Rapid River Hatchery spring chinook salmon during spring 1980. $r = -0.882$; $P < 0.02$.

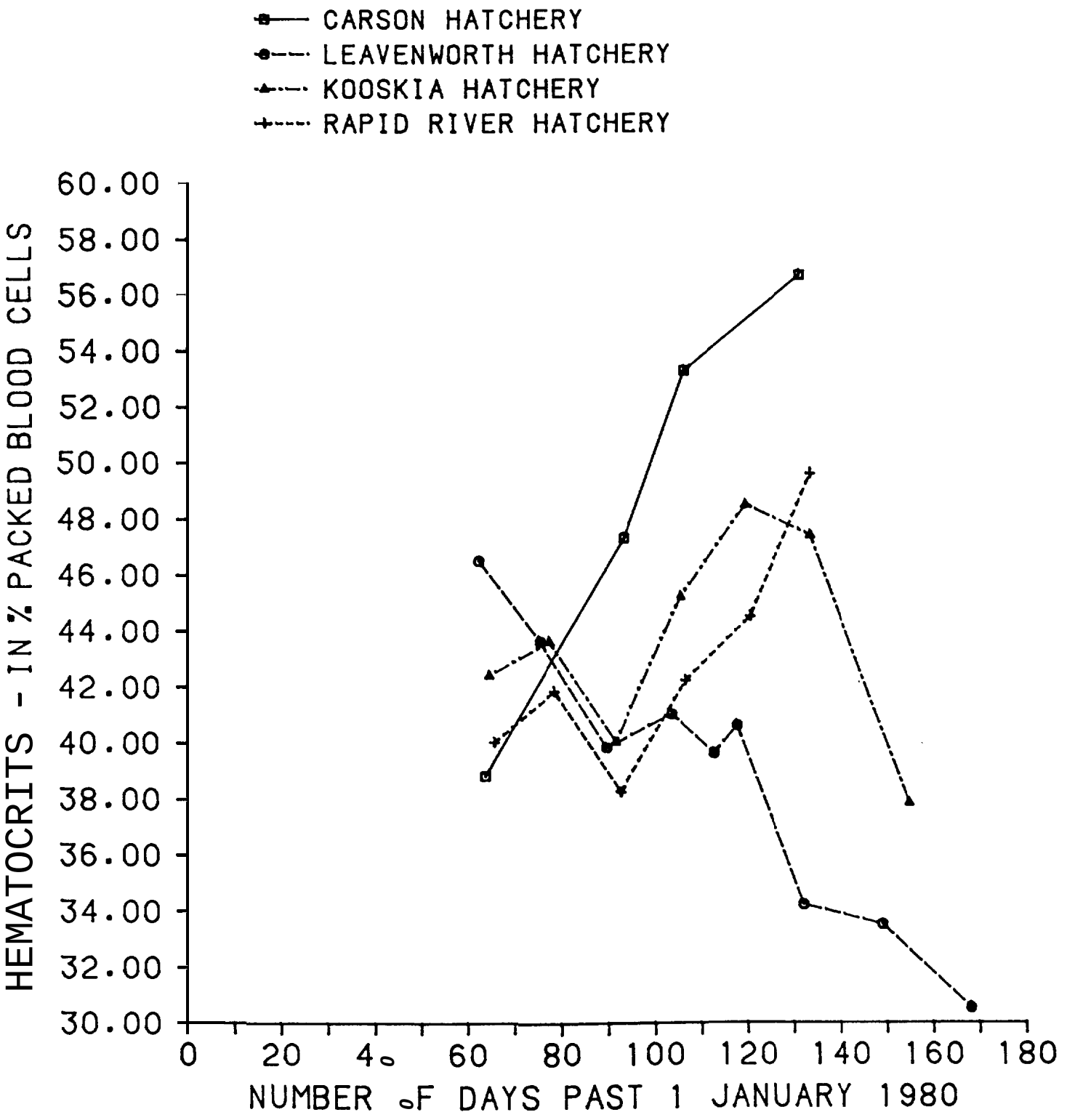


Figure 55.--Mean hematocrit values of Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery spring chinook salmon during spring 1980.

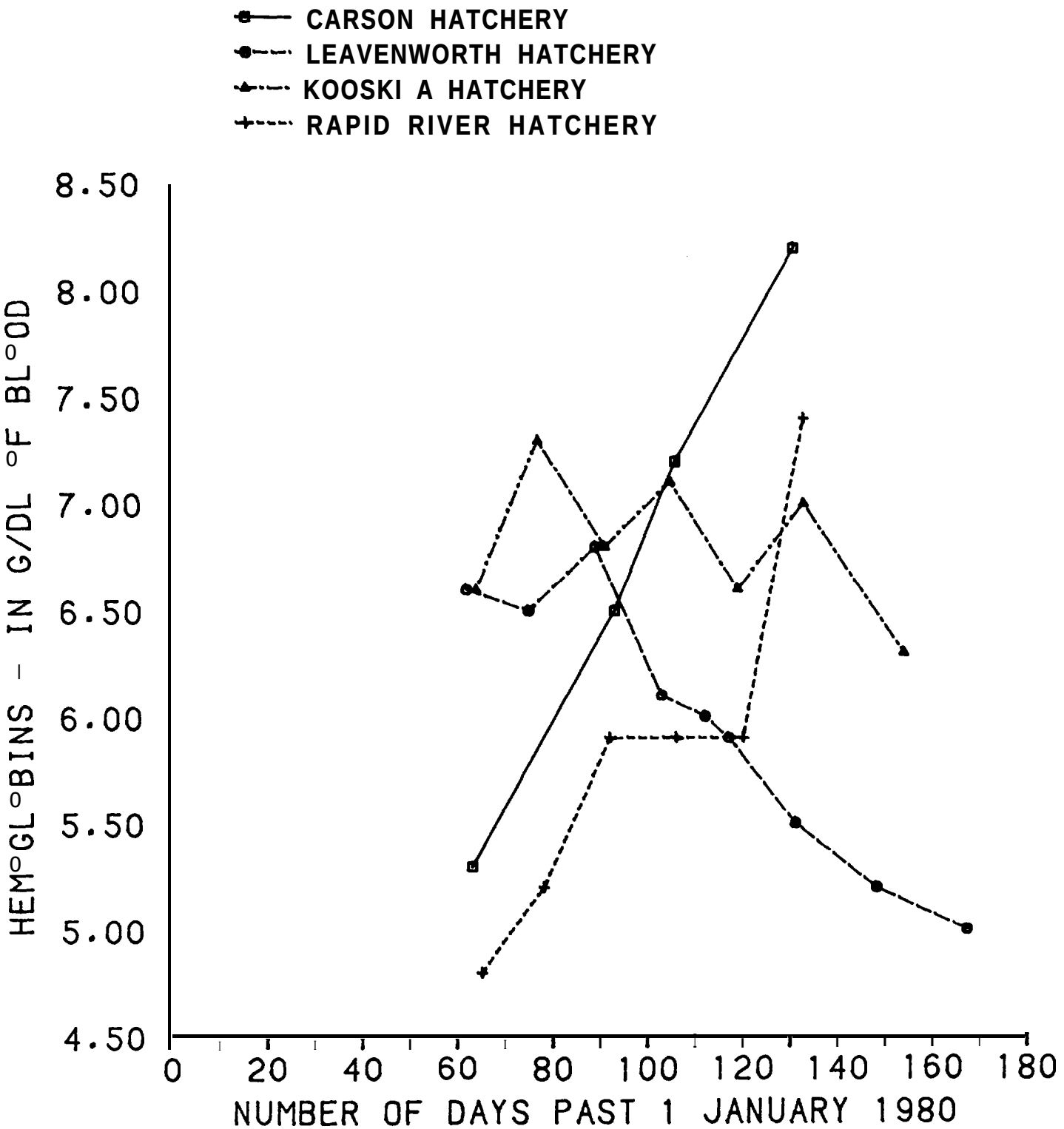


Figure 56.--Mean hemoglobin values of Carson, Leavenworth, and Kooskia NFH and Rapid River Hatchery spring chinook salmon during spring 1980.

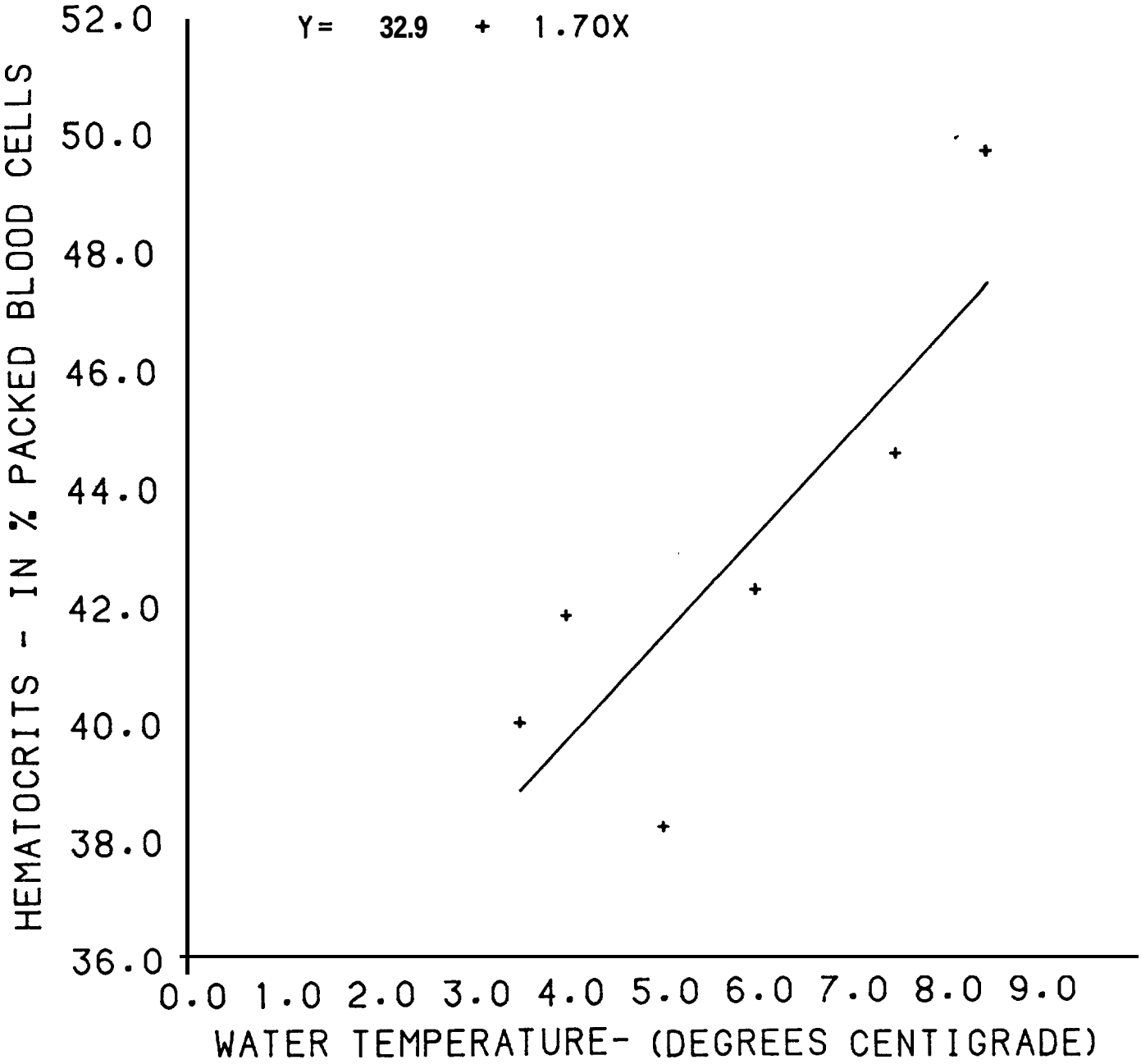


Figure 57.--Regression of mean hematocrit values of Rapid River Hatchery spring chinook salmon on Rapid River Hatchery water temperatures during spring 1980. $r = 0.840$; $P < 0.05$.

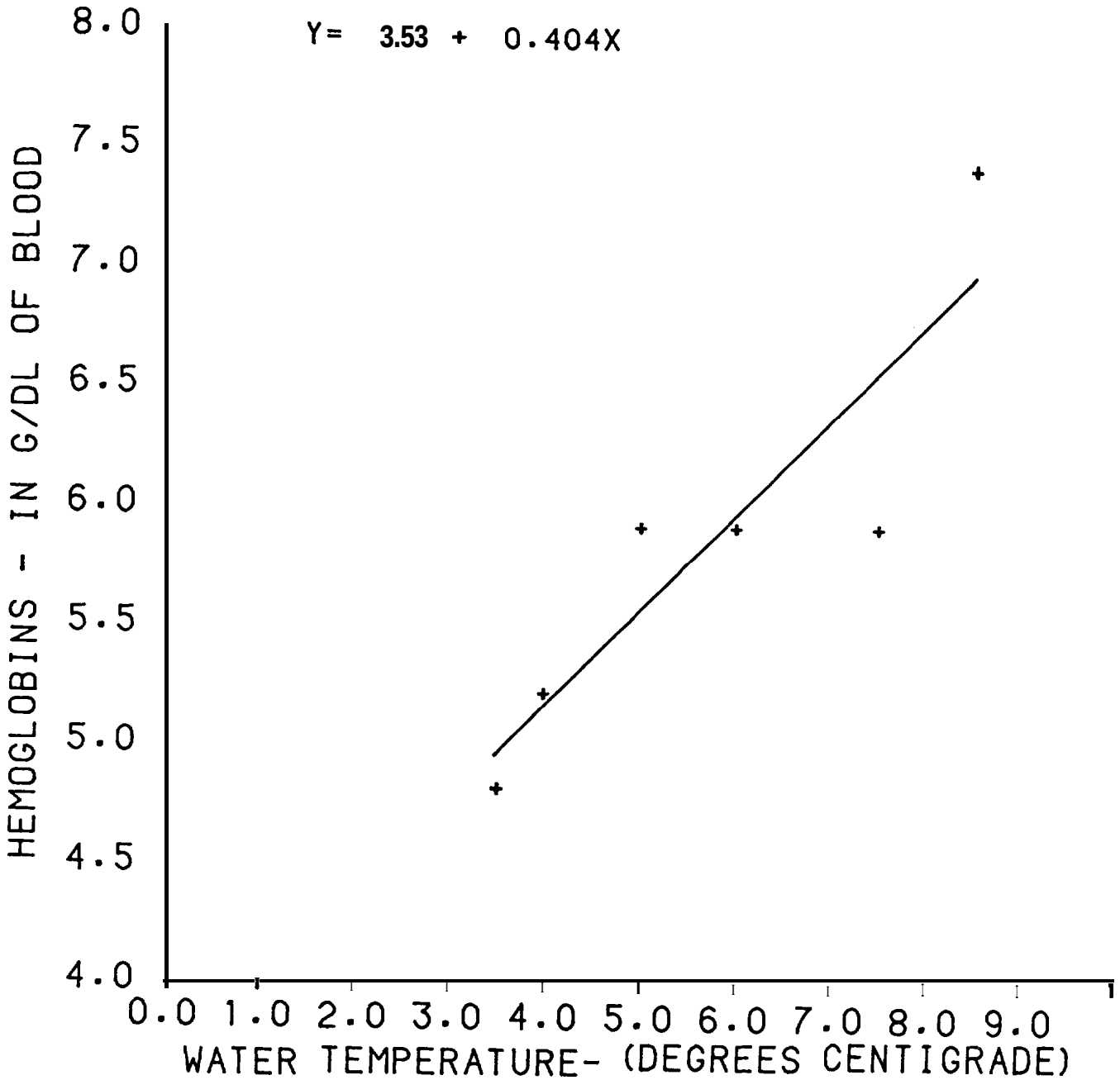


Figure 58.--Regression of mean hemoglobin values of Rapid River Hatchery spring chinook salmon on Rapid River Hatchery water temperatures during spring 1980. $r = 0.882$; $P < 0.02$.

Table 39.--Significant correlaiton coefficients for individual hematocrits and hemoglobins of Rapid River Hatchery spring chinook salmon during the 1980 sampling season.

Item	Period 1	2	3	4	5	6
Correlation coefficient	0.865	0.751	0.479	0.680	0.547	0.334
Degrees of freedom	58	58	58	55	53	50
P	<0.00l	<0.00l	<0.00l	<0.001	<0.00l	<0,02

The incidence of abnormaLly low and high hematocrit values was quite low during the migration period, and remained so until the last sampling period (Table 40).

Table 40.--Incidence of Low and high hematocrit values in Rapid River Hatchery spring chinook salmon during the 1980 sampling season (expressed as precentage of the samples tested).

Item	Period 1	2	3	4	5	6
Hematocrits						
<30	5.[illegible]	3.3	5.0	5.2	5.5	0
>55	0	0	0	5.2	9.0	25.5

Average mean celL hemoglobin concentrations (stress indicators) peaked prior to migration and declined throughout the migration periods (Figure 51).

On the basis of the information collected and evaluated, the general health index as expressed by hematological values examined was quite good throughout the season, and especially so at the time of emigration.

Table 41.--The incidence of BKD organisms in juvenile Rapid River Hatchery spring chinook salmon during the 1980 sampling season, as determined by IFAT-BKD.

Period	Percent of fish with BKD bacteria in the kidneys				
	Anterior kidney	Posterior kidney	Both kidneys	Totals	Comments on intensity[a]
#l					
6 March	3.3	1.7	3.3	8.3	1.7 moderate 1.7 severe
#4					
17 April	0	1.7	0	1.7	All light
#5					
1 May	10.0	10.0	5.0	25.0	1.7 moderate

a/ Light = l-9 organisms/l50 microscopic fields
Moderate = 10-99 organisms/l50 microscopic fields
Severe = > 100 organisms/l50 microscopic fields

IFAT-BKD

Specimens of Rapid River Hatchery spring chinook salmon from the first, fourth, and fifth sampling periods of 198 were examined for the presence of BKD organisms by the LFAT (major migration was between the third and fourth periods).

The incidence of BKD was very low throughout the periods of pond sampling (Table 41), and the intensity of infection was classified parimarily as light (1 to 9 BKD organisms/l50 microscopic fields). Three fish (5%) were observed with gross BKD type lesions during the first sampling trip in March and none thereafter. This incidence averages 0.8% for the six sampling trips with 95% confidence Limits to a 1.8% incidence. The microscopically (IFAT-BKD) observed frequency of 1.7% during the major migration (Table 41) has an upper 95% confidence limit of 5.0%.

The resuLts of detection methods indicate that treatment of the adult parent stock and eggs with erythromycin phosphate was quite successful for the 1980 migrants. Increase in incidence in the "hold-over" fish may have been due to the stresses of confinement.

Histopathology

A summary of the pathological conditions observed is presented in Table 42. For comparative purposes, these data are further submitted with data from other hatcheries in Table 9.

Samples for a histopatholgical profile were collected during the first three periods, the last (3 April) representing the beginning of migration. ALL samples came from ponds, none were from the confined holding area.

Lesions of the gills and olfactory sac were consistently high throughout the sampling periods, and there was a persistently high

Table 42.--Pathological conditions observed in 1980 Rapid River Hatchery spring chinook salmon and their percentage of incidence.[a/]

Organ and pathology	Incidence(%) Period 1 (severity)[b/] I	II	III	total	Period 2 (severity) I	II	III	total	Period 3 (severity) I	II	III	total
Eye												
skeletal muscle lesions	43.3	0	0	43.3	55.0	0	0	55.0	59.3	0	0	59.3
retrobulbar fat lesions	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Gills												
increased numbers of lymphocytes	83.3	13.3	0	96.6	63.3	8.3	0	71.6	78.0	13.6	0	91.5
epithelial cell formation	31.7	65.0	1.7	98.4	58.3	23.3	0	81.6	50.9	47.5	0	98.4
vascular telangiectasis of secondary lamellae	3.3	0	0	3.3	3.3	0	0	3.3	8.5	0	0	8.5
Olfactory sac												
focal mononuclear cell infiltration	76.7	6.7	0	83.40	76.7	13.3	0	90.0	0	79.7	17.0	96.7

a/ Brain tissue was processed and examined for all specimens and there was no evidence of any pathology.

b/ I-recognizable (least severe); II-intermediate; III-severe.

proportion of fish with Lesions of intermediate severity. However, summarized comparative data presented in Table 9 indicate that the severity and incidence were only slightly higher than those of other spring chinook salmon stocks in the study.

Summary

Serial sampling of Rapid River Hatchery spring chinook salmon in 1980 indicated that general fish health was good from the early part of the season through release. The incidence of BKD as documented by indirect fluorescent antibody analysis of random samples was very Low, probably indicating that the erythromycin phosphate program for the prevention of the vertical transmission of BKD was successful.

At release, Rapid River Hatchery fish were the largest of the four spring chinook salmon stocks in the study (Figure 46). Profiles of water temperatures were comparable to Kooskia and Leavenworth NFH (Figure 45), but the peak of the Na+-K+ ATPase profile (Figure 47) occurred much earlier (this may have been due to the abrupt changes in environment). In contrast, the mean gill Na^+-K^+ ATPase values of the Carson NFH stock (at Carson NFH) peaked at the same time as the Leavenworth and Kooskia NFH fish (Figure 47), in spite of a constant temperature at Carson NFH (Figure 45). Leavenworth and Kooskia NFH stocks are basically from Carson NFH transplants.

Average hematocri ts and hemoglobins were comparable to those of Leavenworth NFH spring chinook salmon at release, but lower than Carson or Kooskia NFH fish (Figures 55 and 56). Profiles of average mean cell hemoglobin concentrations of all four spring chinook salmon hatchery stocks in the study indicate that they were all comparable, and that MCHC of the

Rapid River Hatchery stock were rapidly declining during outmigration (Figure 51). Mean plasma Na+ values declined much more rapidly than those of the other spring chinook salmon stocks studied and were the lowest of the four at release (Figure 48). Mean plasma Cl* values had the most abrupt changes of any of the stocks as evidenced by an extremely sharp decline during migration (Figure 49). A comparison of average plasma K+ profiles for the spring chinook salmon stocks (Figure 50) indicates a declining curve throughout the season for Rapid River Hatchery fish, with the lowest points during outmigration., These lower stress indicator values (MCHC and plasma K+> may be from the use of pond culture at Rapid River Hatchery.

All of the data indicate that Rapid River Hatchery stocks were in excellent condition at the time of outmigration.

RESULTS AND DISCUSSION OF FALL CHINOOK SALMON SURVEYS

Spring Creek National Fish Hatchery

General

Fall chinook salmon were sampled from a number of ponds at Spring Creek NFH in 1980, both for homing and smoltification studies. The analyzed data collected from both of these studies are presented in this report. Summarized data for fish released in the homing study are presented in Table 43. Sample sizes for these data are 60 fish/sampling period, and sample sizes for the data collected in other ponds are 30 fish. Individual weights were measured at Spring Creek NFH, and these data are also presented.

Figure 59 shows the average Lengths of the fish sampled from homing study ponds and those sampled throughout the season from other ponds. There were no indications of differences in mean sizes of fish between ponds, and data collected from all normal production ponds typically represent homing study fish. Average weights of production pond fish are shown in Figure 60.

Gill Na+-K+ ATPase

The gill Na+-K+ ATPase profile in 1980 was characterized by a maximum decline at the release time of the homing study and hatchery evaluation fish (Figure 61). This was followed by a rapid increase to a maximum level that occurred about 40 days later in fish held past this release period (Figure 61). In contrast, the gill Na+-K+ ATPase profile in 1979 indicated that the peak of activity occurred at normal release time in early May (Figure 62). Although the level of peak activity

Table 43.--Summary data for the spring (1980) sampling of Spring Creek Hatchery fall chinook used in homing tests, with means, standard deviations ( ), and ranges. Sample size = 60.

Item	Period 1	Period 2	Period 3
Date	10 March 1980	10 April 1980	8 May 1980
Days>Jal[a]	70	100	128
Temp.-C°[b]		7.7 - 11.1°C	
Avg. Fk Ln[c]	74.2	79.2	92.1
	(2.9)	(4.5)	(5.3)
(Range)	68-81	70-90	79-103
Avg. ATP Fk Ln[d]	73.2	80.3	93.0
	(2.5)	(4.5)	(4.9)
(Range)	68-79	71-90	79-102
Avg. ATP[e]	18.2	16.2	9.8
	(2.5)	(1.3)	(2.2)
(Range)	14.6-22.3	13.9-17.6	6.7-13.9
Avg. Hct[f]	48.5	55.2	55.1
	(3.3)	(5.4)	(4.2)
(Range)	41-60	45-68	42-63
Avg. Hb[g]	7.0	8.4	8.8
	(0.6)	(1.02)	(0.8)
(Range)	5.4-8.1	5.7-12.0	6.7-10.9
Avg. MCHC[h]	14.4	15.3	16.0
	(1.5)	(2.4)	(1.4)
(Range)	11.0-19.3	9.0-21.0	13.5-21.0
Avg. Na^+[i]	---	133.6	142.9
		(6.9)	(12.9)
(Range)		120-142	110-153
Avg. K^+[j]	---	4.4	5.1
		(2.7)	(1.8)
(Range)		1.0-7.3	2.0-7.0
Avg. Cl^-[k]	---	118.5	125.7
		(3.5)	(7.6)
(Range)		116-121	121-139
Na^+/Cl^-[l]	---	---	---

[a] Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
[b] Temp.-°C: Water temperature (in degrees C.) measured for that period.
[c] Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
[d] Avg. ATP Fk Ln: The average fork lengths of fish used for the gill ATPase measurements for that period.
[e] Avg. Atp: The average gill ATPase levels for that period. (Na^+-K^+ ATPase activity in μ moles Pi/mg protein/hour.)
[f] Avg. Hct: The average hematocrits for that period (% packed cells).
[g] Avg. Hb: The average hemoglobins for that period (in g/dl).
[h] Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
[i] Avg. Na^+: The average plasma sodium for that period (in meq/l).
[j] Avg. K^+: The average plasma potassium for that period (in meq/l).
[k] Avg. Cl^-: The average plasma chloride for that period (in meq/l).
[l] Na^+/Cl^-: The ratios of the plasma sodium to chlorides for that period, averaged.

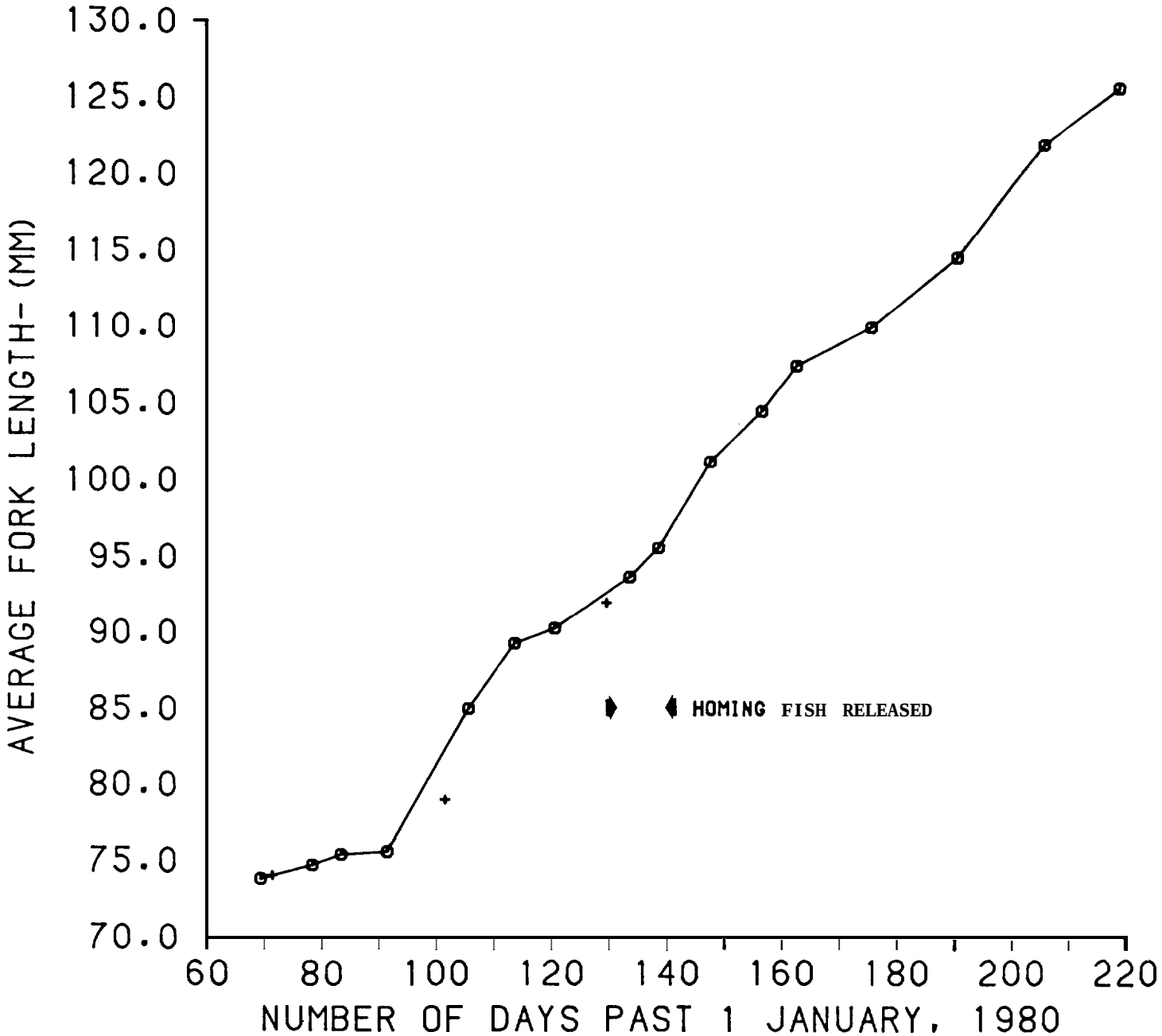


Figure 59.--Mean fork lengths of Spring Creek NFH fall chinook salmon during spring 1980. + = homing study fish (N = 60). ⊙ = fish from other ponds (N = 30).

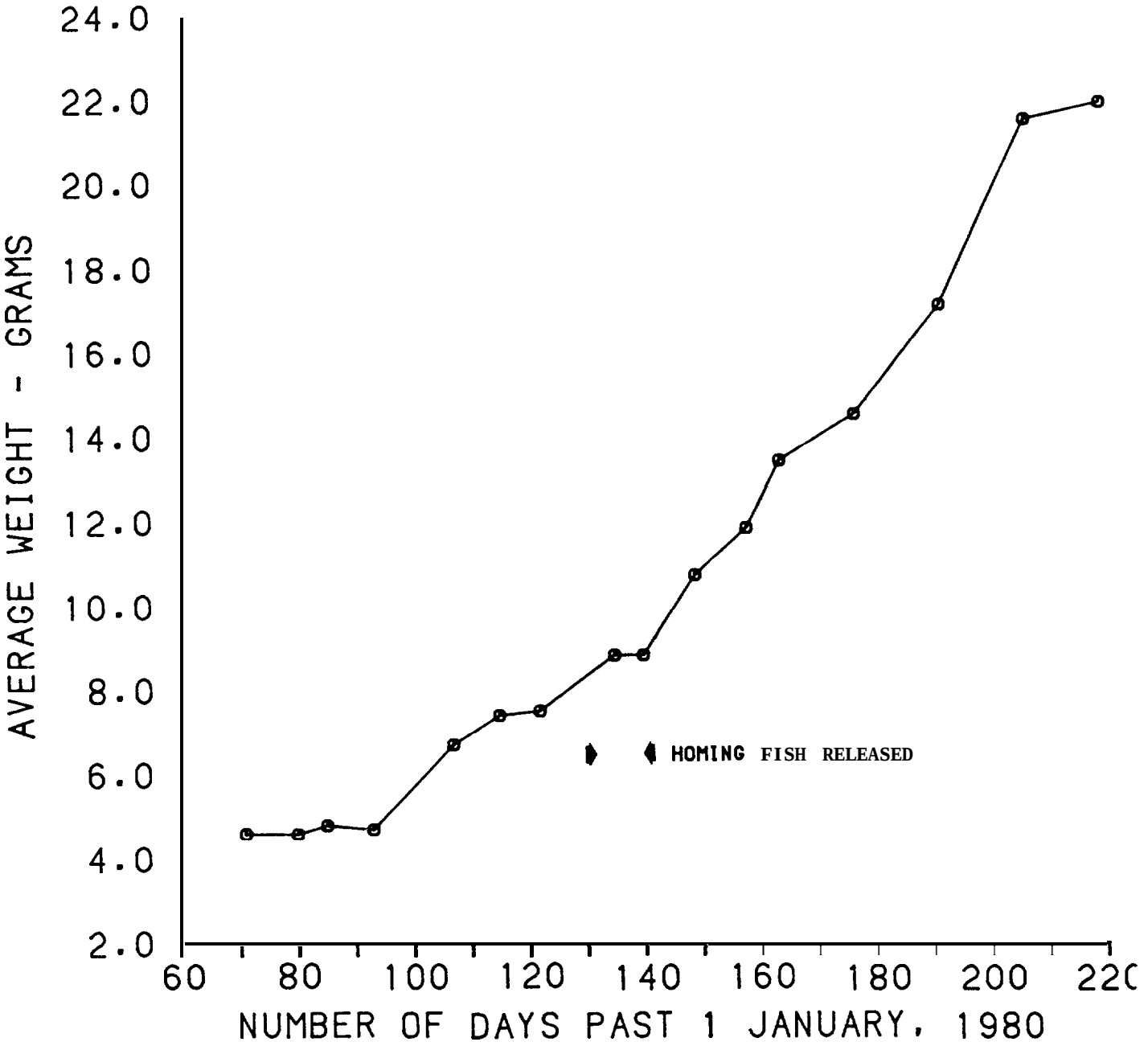


Figure 60.--Mean weights of Spring Creek NFH fall chinook salmon during spring 1980.

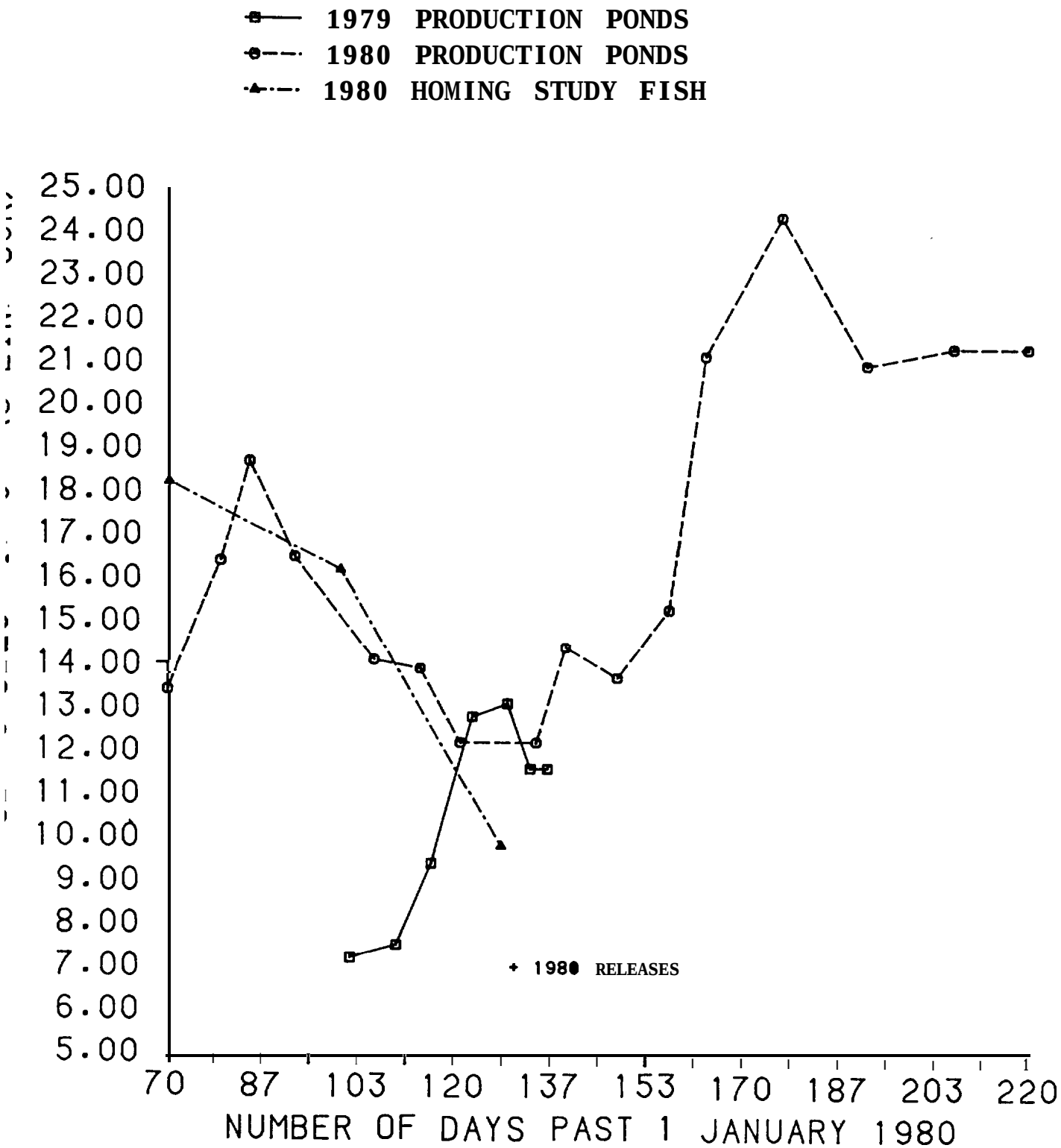


Figure 61 .--A comparison of mean gill Na^+-K^+ ATPase values of Spring Creek NFH fall chinook salmon during spring 1979 and spring 1980.

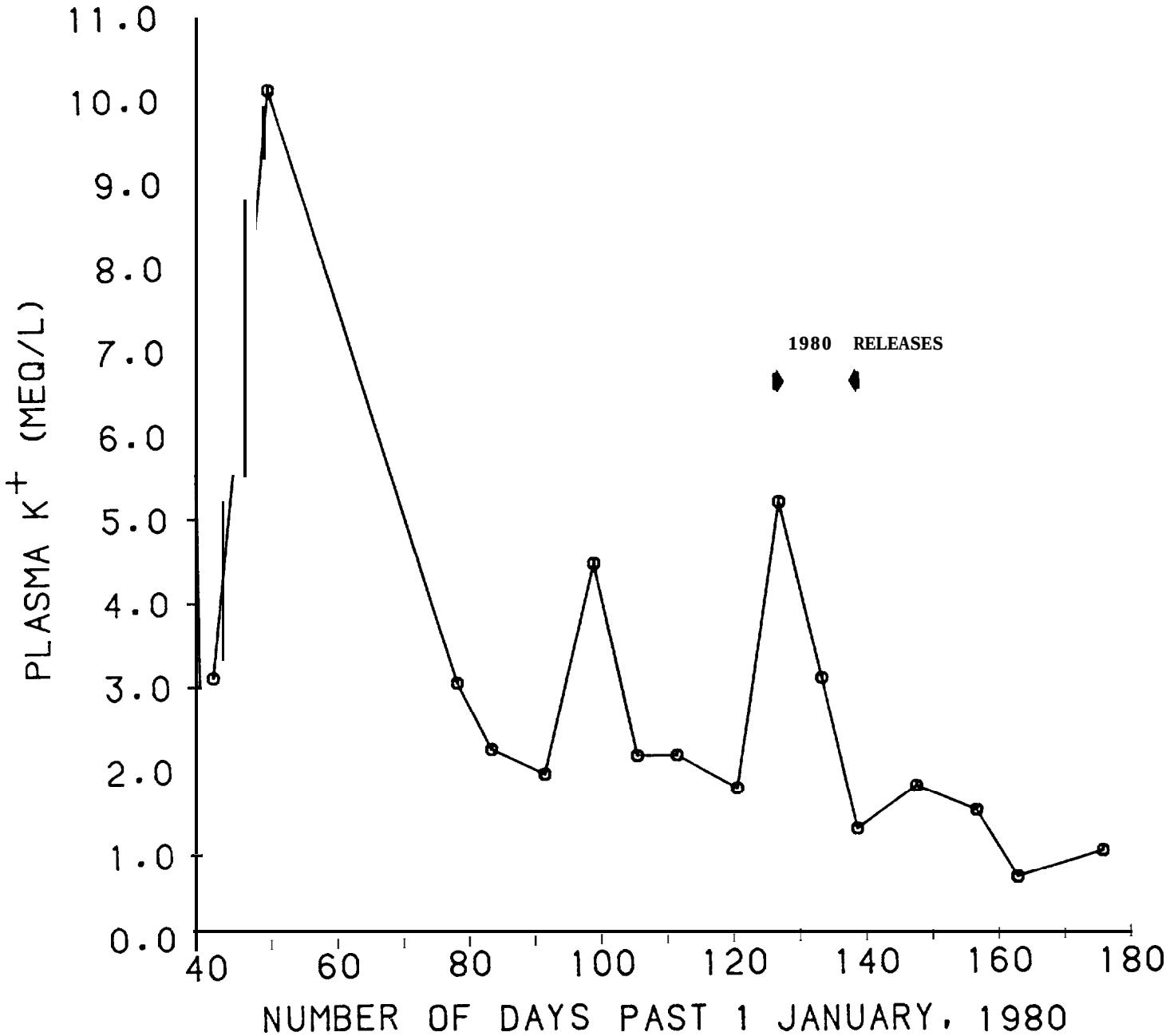


Figure 62.--Average plasma K^+ values for Spring Creek NFH fall chinook salmon during spring 1980.

was not as high in 1979 as in 1980, the rates of change were approximately the same. The difference in timing between the 1979 and 1980 peak activities is about 50 days (Figure 61), and this might influence survival.

Plasma Electrolytes

Mean plasma electrolytes as measured in homing study fish (Table 43) were generally within ranges reported for spring chinook salmon (Table 25), with the exception of K^+ values which were much higher than expected and could not be attributed to hemolysis. A profile of mean plasma K^+ as measured in production pond fish is presented in Figure 62. There are three periods of mean plasma K^+ levels that are sharply elevated above the norm, with a major peak occurring right at the time of release. This may be an indication of physiological stress at a critical time.

Mean plasma Na^+ values of production pond fish were variable early in the season and then generally rose in a cyclic pattern beginning in Late March (Figure 63). There was a sharp increase in plasma Na^+ at the time of release, followed by a sharp decline at the end of the release period. Beginning in early May, these peak increases in plasma Na^+ occurred at regular 14-day intervals.

Mean plasma Cl^- values of the production pond fish were also quite variable (Figure 63). Plasma Cl^- values had declined to an intermediate low level at the time of release.

Mean plasma Na^+ and Cl^- levels in the Spring Creek NFH fall chinook salmon were generally in the same range as a typical yearling spring chinook salmon such as those from Leavenworth NFH. Differences in

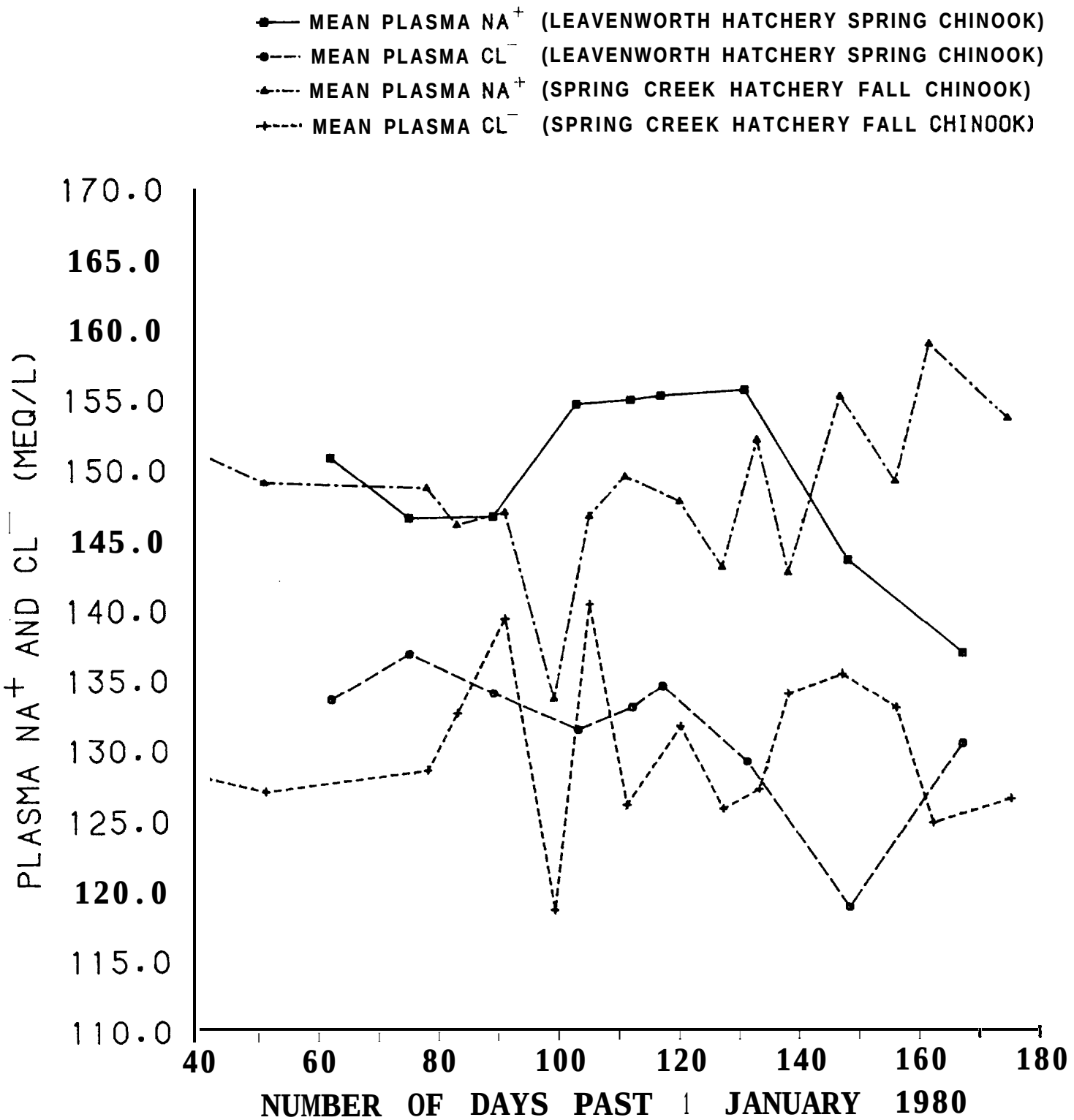


Figure 63.--Average plasma Na^+ and Cl^- values for Leavenworth NFH spring chinook salmon and Spring Creek NFH fall chinook salmon during spring 1980.

the profiles may reflect differences between yearling (spring chinook salmon) and 0-age (fall chinook salmon) fish (Figure 63).

Hematology

Blood was sampled only three times during the 1980 studies at Spring Creek NFH for hematological analysis. There were no individual samples with lower than normal values (Table 43). In the first period (10 March), 3.3% of the samples had hematocrits of 55% or more, and by 10 April, this figure had increased to 46.7%, and at release to 58.3%. There were no significant correlations between individual hematocrits and hemoglobins until the third (release) period ($r = 0.532$ for 53 d.f.; $P < 0.001$), which is unusual. Mean MCHC and hemoglobin values increased throughout the season (Table 43). There is no obvious explanation for the percentage of fish with high hematocrits, MCHCs, or hemoglobins in April-May, except that these values and the generally increasing mean plasma Na^+ (Figure 63) may indicate hemoconcentration resulting from a reduction in tissue water volume.

IFAT-BKD

Prepared specimens of Spring Creek NFH fall chinook salmon from the first, second, and third sampling periods of 1980 were examined for the presence of BKD organisms by the IFAT. However, data are only presented here for the second (10 April) period because of some technical difficulties in preparation of slides from the other periods.

BKD organisms were found in 23.7% of the anterior kidney smears, 11.9% of the posterior kidney smears, and 22.0% in both anterior and posterior kidney. The total number positive for BKD was 57.6%.

Infected fish were classified as 73.6% Light (1.9 orgnaisms/150 m.f.), and 26.4% intermediate (10.99 organisms/l50 m.f.) intensity. The upper limit was 35 organisms/l50 m.f., and even though the incidence of infection was relatively high, no samples were observed with severe infections. Consequently, BKD may not be a serious problem in this stock.

Histopathology

A summary of the pathological conditions observed is presented in Table 44. For comparative purposes, these data are further summarized with data from the other hatcheries in Table 9.

The histopathological profile of Spring Creek NFH fall chinook salmon showed pronounced increases in numbers of Lymphocytes in the gills and Lesions of the olfactory sac by the time of release. Some of these high levels were comparable to spring chinook salmon 1 year older (i.e.,Carson NFH, Table 34).

Summary

Continued sampling of homing study and production fall chinook salmon from Spring Creek NFH in 1980 indicated a shift in the Na^+-K^+ ATPase profile from previous years. Fish released in 1980 for the homing study were in the trough of a Na^+-K^+ ATPase profile, which may have some effect on survival.

There was also evidence from hematological and plasma Na^+ data that indicated a majority of the fish (at release) had excessively high hematocrits and plasma Na^+ values, and may have been under some type of stress. Although we cannot quantify the effects of apparent deviations in these parameters from the norm, they should be taken into consideration.

Table 44.--Pathological conditions observed in 1980 Srping Creek Hatcher fall chinook and their percentage of incidence.[a]

Organ and pathology	Incidence (%)											
	Period 1 (severity)[b]				Period 2 (severity)				Period 3 (severity)			
	I	II	III	total	I	II	III	total	I	II	III	total
Eye												
skeletal muscle lesions	18.3	0	0	18.3	6.8	0	0	6.8	21.7	0	0	21.7
Gills												
increased numbers of lymphocytes	65.0	10.0	0	75.0	25.4	1.7	0	27.1	56.7	43.3	0	100.0
vascular telangiectasis of secondary lamellae	8.3	0	0	8.3	22.0	5.1	0	27.1	1.7	0	0	1.7
Olfactory sac												
focal mononuclear cell infiltration	56.7	6.7	0	63.4	42.4	8.5	0	50.9	93.3	6.7	0	100.0

[a] Brain tissue was processed and examined for all specimens, and there was no evidence of any pathology.

[b] I-recognizable (lease severe); II-intermediate; III-severe.

Big Creek Hatchery (ODFW)

General

Fall chinook salmon were sampled from the homing study fish in ponds at the Oregon Department of Fish and Wildlife's (ODFW) Big Creek Hatchery from 8 April until 13 May 1980, from a production pond on 22 May 1980, and from production fish held over in tanks from 23 May to 7 July 1980. Summarized data for fish released in the homing study are presented in Table 45. Sample sizes for these data are 60 fish/sampling period, and the sample sizes for data collecteed from fish in other ponds and tanks are 30 fish/sampling period. We were not able to collect adequate samples for plasma electrolyte analysis from the fish at Big Creek Hatchery. Water temperatures during the April-May period were quite stable (Table 45).

Although Spring Creek NFH and Big Creek Hatchery fall chinook salmon were released at approximately the same time, Spring Creek NFH fish were (on the average) over 10 mm longer. Mean sample weights of Big Creek Hatchery production fish were 6.8 g on 22 May 1980 (Table 45), and samples from production ponds at Spring Creek NFH averaged 9.0 g (Figure 60) at the same time.

Gill Na^+-K^+ ATPase

The gill Na^+-K^+ ATPase profile of the Big Creek Hatchery fall chinook salmon was characterized by rapidly increasing activity during the release period (Figure 64). Note that the shape of the profile of the Big Creek Hatchery fish is similar to that of fish from Spring Creek NFH (Figure 64) but begins to increase rapidly much earlier in the spring. Big Creek Hatchery fish were released at a favorable time of rapidly rising gill Na+-K+ ATPase values.

Table 45.--Summary data for the spring (1980) sampling of Big Creek Hatchery fall chinook salmon used in the homing tests, with means, standard deviations (), and ranges. Sample size = 60.

Item	Period 1	2	3	4
Date	7 Mar 80	21 Mar 80	4 Apr 80	10 Apr 80
Days>Jal[a/]	98	113	126	133
Temp. °C[b/]	9.3	10.3	9.4	9.4
Avg. Fk Ln[c/]	59.9	67.7	76.0	81.3
	(4.2)	(4.3)	(5.4)	(4.2)
(Range)	46-66	60-76	67-88	69-92
Avg. ATP Fk Ln[d/]	58.8	68.0	76.4	81.7
	(4.6)	(4.6)	(5.1)	(4.8)
(Range)	46-65	60-76	67-85	69-92
Avg. ATP[e/]	--	12.0	14.7	61.6
	--	(2.4)	(5.1)	(1.4)
(Range)	--	8.3-15.7	10.3-27.7	15.1-19.7
Avg. Hct[f/]	47.2	53.1	52.7	53.3
	(4.7)	(5.2)	(4.2)	(7.0)
(Range)	38-55	41-61	46-64	32-75
Avg. Hb[g/]	6.0	6.7	7.1	7.2
	(1.0)	(0.5)	(0.6)	(0.8)
(Range)	3.8-7.5	5.8-7.8	6.3-8.3	4.5-8.5
Avg. MCHC[h/]	--	12.7	13.6	13.7
	--	(1.3)	(0.8)	(2.1)
(Range)	--	11.1-15.4	11.6-15.3	7.6-20.5
Avg. Na^+[i/]	--	--	--	--
	--	--	--	--
(Range)	--	--	--	--
Avg. K^+[j/]	--	--	--	--
	--	--	--	--
(Range)	--	--	--	--
Avg. Cl^-[k/]	--	--	--	--
	--	--	--	--
(Range)	--	--	--	--
Na^+/Cl^-[l/]	--	--	--	--
	--	--	--	--
(Range)	--	--	--	--

a/ Days>Jal: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average for lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. ATP: The average gill ATPase levels for that period. (Na^+-K^+ activity in μ moles Pi/mg protein/hour.
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl^-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratio of the plasma sodium to chlorides for that period, averaged.

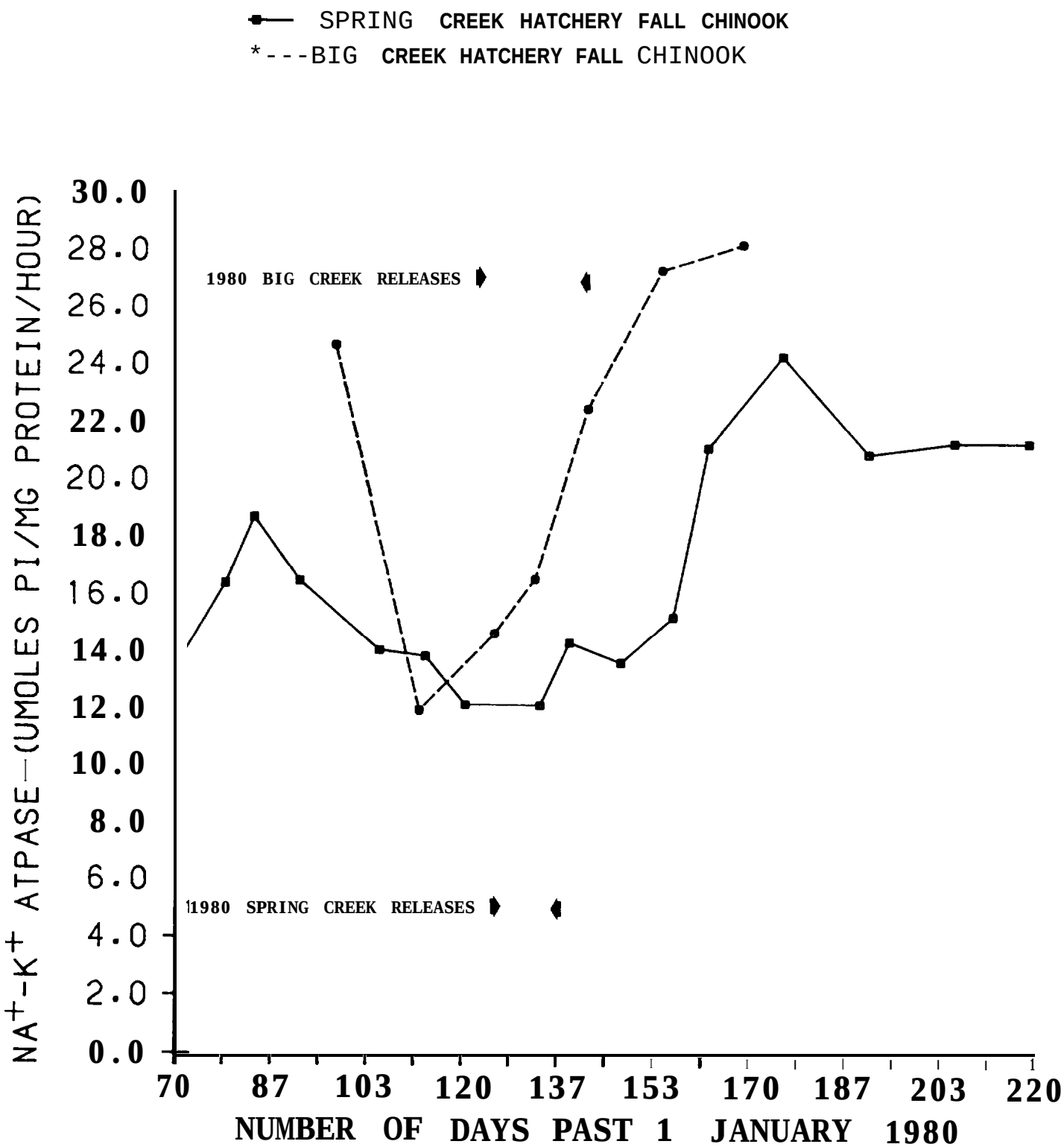


Figure 64.--A comparison of mean gill Na^+-K^+ATPase values of Big Creek and Spring Creek NFH fall chinook salmon during spring 1980.

Gill Na^+-K^+ ATPase values could only be significantly correlated with fork lengths in the second sampling period ($P < 0.020$, $r = 0.717$, 8 d.f.) (Table 45).

Hematology

Blood was sampled four times during the studies at Big Creek Hatchery for hematological analysis. Early in the season, samples had to be pooled to obtain sufficient quantities for analysis as fish were small and volumes of blood that could be obtained were limited. There were no samples with Lower than expected values (Table 45). The percentage of samples with hematocrits of 55% or more from the first through the fourth sampling periods were 10, 40, 30, and 40% respectively. This was generally less than that of Spring Creek NFH fish, but still considerably high. The only significant correlations between hematocrits and hemoglobins occurred in the third period ($r = 0.745$ for 26 d.f., $P < 0.001$). Mean hematocrits and hemoglobins increased sharply during April, and mean hemoglobin and MCHC values rose progressively throughout the season (Table 45). Whether high MCHC values in fall chinook salmon during the period of smoltification are related to smolting stresses or other factors is not possible to judge at this time.

IFAT-BKD

Prepared specimens of the Big Creek Hatchery fall chinook salmon from the second and fourth sampling periods of 1981 were examined for the presence of bacterial kidney disease organisms by the IFAT. Only 5% of the samples in either period were positive for BKD, and all of the intensities were classified as Light.

Histopathology

A summary of the pathological conditions observed is presented in Table 46. For comparative purposes, these data are further summarized with data from other hatcheries in Table 9.

The histopahtological profile of Big Creek Hatchery fish indicates that there were sharp increases in the frequency and severity of pathological conditions of the gills and olfactory sac by the third period, and that these conditions were not reduced by the fourth (release) period. In some cases these conditions were more severe than in Spring Creek Hatchery fish (Table 44).

Summary

The sampling of fall chinook salmon from Big Creek Hatchery in 1980 indicated that the fish were about 12% smaller than Spring Creek NFH fall chinook salmon at the time of release. Hematological data indicate a relatively high percentage of fish with high hematocrits at the time of release, which could indicate some degree of hemoconcentration. Levels of BKD were extremely Low and Light in intensity, which should indicate a Low probability of mortality from this disease.

There were sharp increases in frequency and severity of certain pathological conditions of the gill and olfactory sac during the sampling season. Although we have no way, at present, of quantitatively relating these conditions to survival, this information should be kept in mind when assessing returning adults.

Table 46.--Pathological conditions observed in 1980 Big Creek Hatcher) spring chinook salmon and their percentage of incidence. [a]

Organ & pathology	Incidence (%)															
	Period 1 (Severity)[b]				Period 2 (Severity)				Period 3 (Severity)				Period 4 (Severity)			
	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total
Eye																
Skeletal muscle lesions	20.3	0	0	28.3	18.3	0	0	18.3	20.0	0	0	20.0	38.3	0	0	38.3
Gills																
Increased numbers of lymphocytes	13.3	3.3	0	16.6	33.3	3.3	0	36.6	63.3	33.3	0	96.6	66.7	23.3	0	90.0
Epithelial cell formation	45.0	16.6	0	61.6	a.3	1.7	0		48.3	40.9	3.3	91.6	36.7	58.3	0	95.0
Vascular telangiectasis of secondary lamellae	1.7	0	0	1.:	0	0	0	0	0	0	0	0	0	0	0	0
Olfactory sac																
Focal mononuclear cell infiltration	1.7	0	0	1.7	58.3	1.7	0	60.3	46.7	26.7	0	73.4	71.7	23.3	0	95.0

[a] Brain tissue was processed and examined for all s[eco,ems/ and there was no evidence pf any pathology.

[b] I-recognizable (least severe) ' OO-intermediate; III-severe.

Hagerman National Fish Hatchery

It was not possible to collect extensive samples from fall chinook salmon reared at Hagerman NFH Hatchery because of distances involved. Samples were collecxted for gill Na^+-K^+ ATPase analysis and shipped to the NMFS laboratory at Willard for analysis. Two groups of fish were transported from Hagerman NFH and released; one on 3 June and the other on 5 June 1980.

Mean fork lengths of fish sampled at the hatchery are shown in Figure 65. On the average, Hagerman NFH fall chinook salmon were 21% larger than Big Creek Hatchery fish and 10% larger than the Spring Creek NFH fish at the time of release.

A profile of gill Na^+-K^+ ATPase activity is presented in Figure 66. Apparent peak activity was reached in early May and was sustained for at Least 1 month. This activity level was the highest in fall chinook salmon studied and may be the result of warm water temperatures in the hatchery (approximately 14°C). The abrupt rise in Na^+-K^+ ATPase activity indicates that the fish were probably physiologically prepared for emigration in early May and perhaps should have been released sooner.

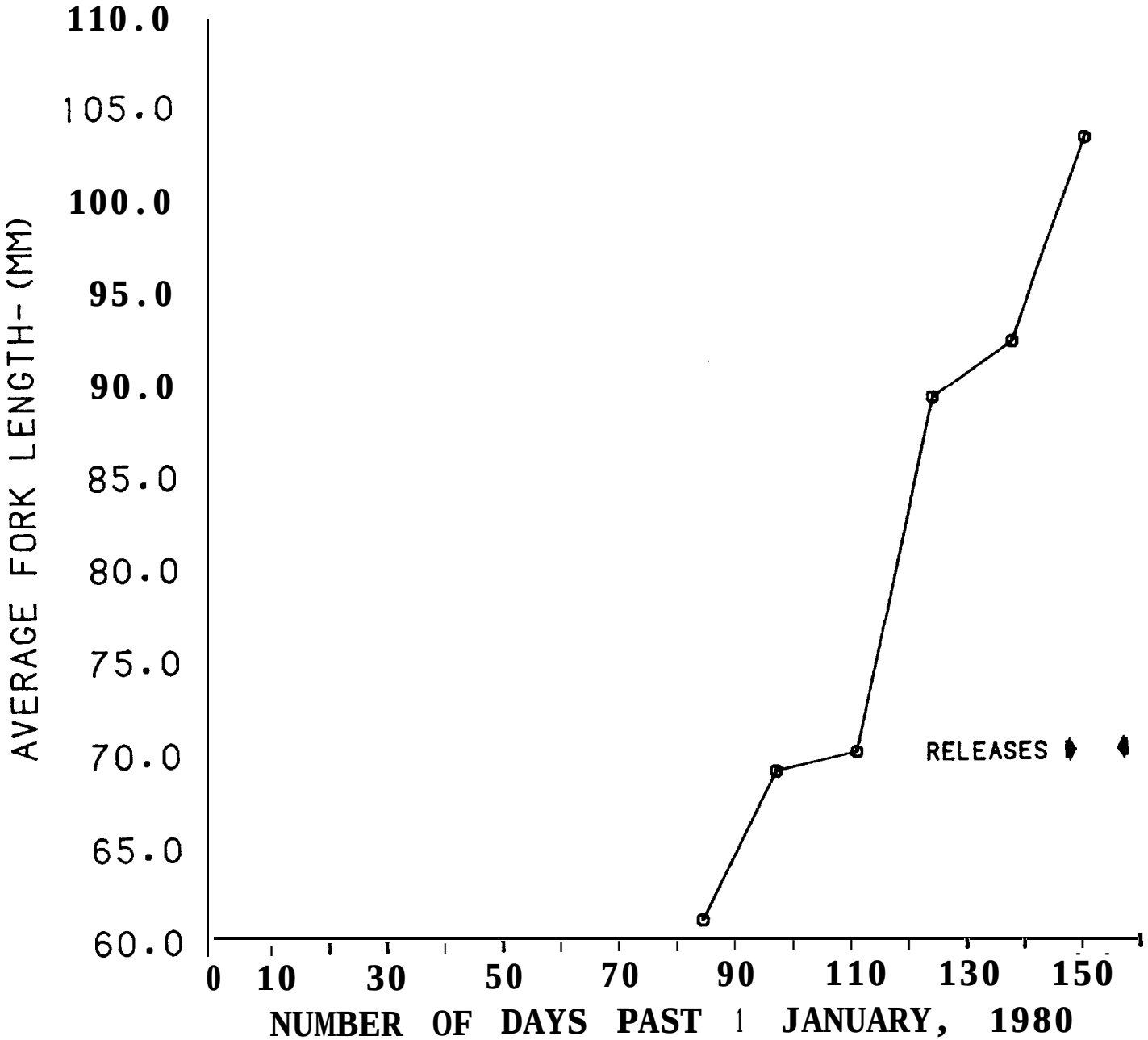


Figure 65.--Mean fork lengths of Hagerman NFH fall chinook salmon sampled during spring 1980 for gill Na^+-K^+ ATPase analysis.

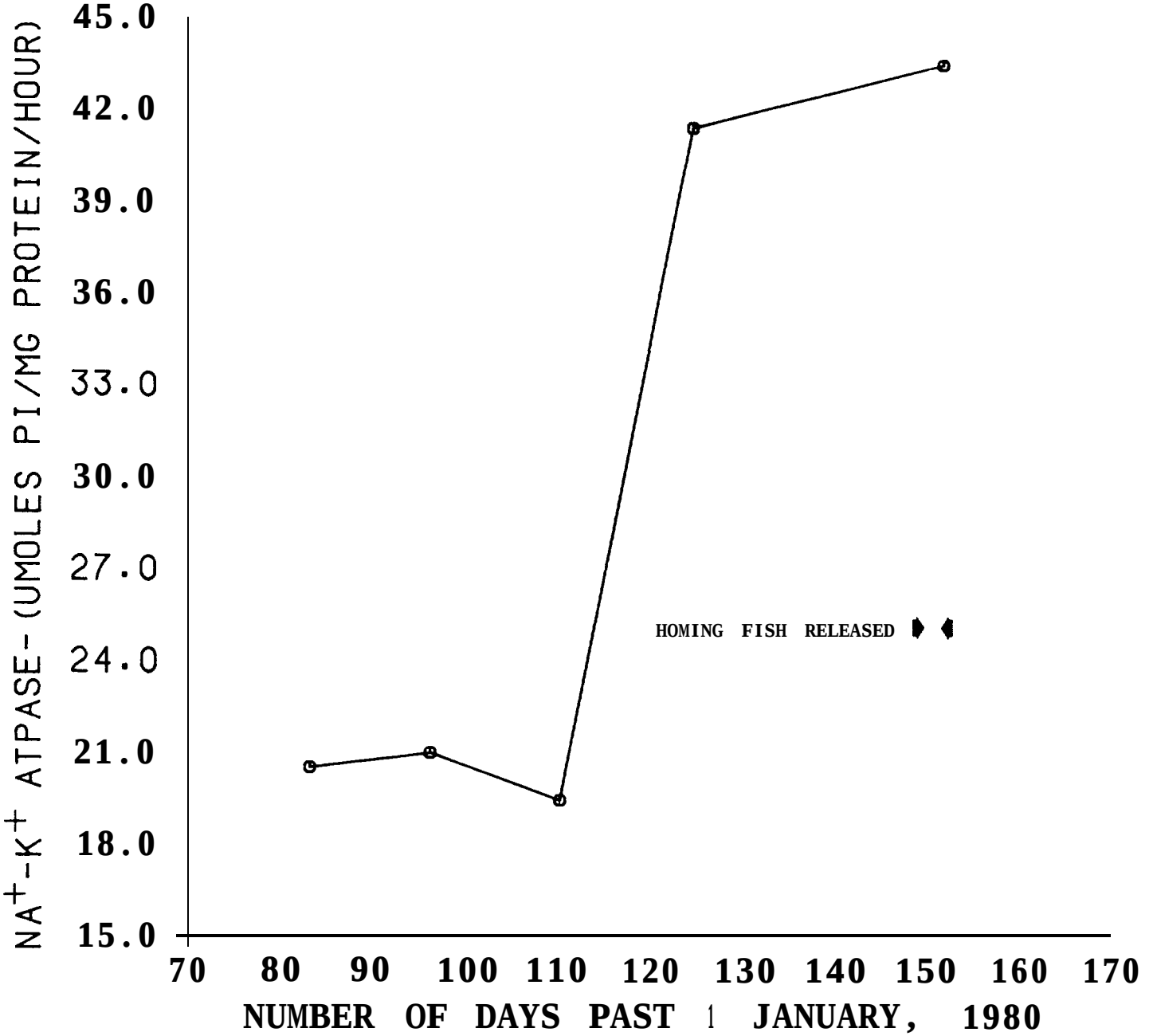


Figure 66.--Mean gill Na^+-K^+ ATPase activity of Hagerman NFH fall chinook salmon sampled during spring 1980.

RESULTS AND DISCUSSION OF COHO SALMON SURVEY

Willard National Fish Hatchery

General

The only coho salmon that were involved in the 1980 homing studies were from the Willard NFH. Complete sampling was conducted at the Willard NFH on three occasions prior to release and these data are summarized in Table 47. Individual fork lengths, weights, and pooled gill samples for Na^+-K^+ ATPase analysis were collected more frequently throughout the season, and representative samples of the population were held in tanks at the hatchery after the 24 May releases for post-release Na^+-K^+ ATPase analysis. Figure 67 is a profile of water temperatures at the hatchery during the sampling periods. Note that temperatures were actually declining during the release period.

Figure 68 is a profile of average fork lengths of all fish measured during the 1980 sampling season at Willard NFH, and Figure 69 is a profile of average weights of fish measured during gill collecting sampling periods. Dips in mean length and weight are probably due to sampling errors.

Gill Na^+-K^+ ATPase

The gill Na^+-K^+ ATPase profile in 1980 was characterized by a rapid increase in activity followed by a similar decline. This peak was a pronounced height within several days of release (Figure 70). The 1980 profile was almost identical to that found in the sampling of Willard NFH coho salmon in 1978 (Figure 70), and on the basis of this information, the timing for release at peak activity was excellent in 1980.

Table 47.--Summary data for the spring (1980) sampling of Willard Hatchery coho salmon with means, standard deviations (), and ranges. Sample size = 60 (released 24 May 1980).

Item	Period 1	Period 2	Period 3
Date	5 Mar 80	2 Apr 80	20 May 80
Days>Jan1[a]	65	92	141
Temp. °C[b]	4.7	5.3	6.9
Avg. Fk Ln[c]	104.0	114.9	126.1
	(10.0)	(11.2)	(8.0)
(Range)	78-120	92-149	103-140
Avg. ATP Fk Ln[d]	102.9	116.3	126.0
	(10.2)	(10.3)	(7.5)
(Range)	82-118	92-142	126-138
Avg. ATP[e]	8.8	10.5	17.5
	(1.8)	(1.9)	(5.5)
(Range)	5.3-11.2	6.8-13.4	10.3-25.5
Avg. Hct[f]	40.3	46.3	57.6
	(5.2)	(7.6)	(11.6)
(Range)	29-57	29-76	24-89
Avg. Hb[g]	5.1	6.6	7.0
	(0.7)	(1.5)	(1.4)
(Range)	3.2-7.2	4.0-12.0	2.3-9.3
Avg. MCHC[h]	12.9	14.3	12.3
	(2.0)	(2.7)	(2.0)
(Range)	6.5-16.5	8.3-25.7	8.7-18.6
Avg. Na^+[i]	161.4	153.2	151.3
	(16.7)	(6.7)	(15.9)
(Range)	116-178	141-162	96-198
Avg. K^+[j]	2.92	5.30	7.09
	(0.87)	(2.92)	(2.66)
(Range)	1.28-4.28	1.92-13.50	2.58-13.50
Avg. Cl^-[k]	129.1	131.6	138.4
	(13.8)	(6.8)	(12.7)
(Range)	98-151	112-141	111-171
Na^+/Cl^-[l]	1.25	1.16	1.11
	(1.10)	(0.05)	(0.10)
(Range)	1.05-1.43	1.08-1.26	0.82-1.27

a/ Days>Jan1: The number of days post 1 January 1980 that the sampling period represents.
b/ Temp.-°C: Water temperature (in degrees C) measured for that period.
c/ Avg. Fk Ln: The average fork length (in millimeters) of all fish measured for that period.
d/ Avg. ATP Fk Ln: The average for lengths of fish used for the gill ATPase measurements for that period.
e/ Avg. ATP: The average gill ATPase levels for that period. (Na^+-K^+ activity in μ moles Pi/mg protein/hour.
f/ Avg. Hct: The average hematocrits for that period (% packed cells).
g/ Avg. Hb: The average hemoglobins for that period (in g/dl).
h/ Avg. MCHC: The mean corpuscular hemoglobin concentrations (Hb/Hct x 100) averaged for that period.
i/ Avg. Na^+: The average plasma sodium for that period (in meq/l).
j/ Avg. K^+: The average plasma potassium for that period (in meq/l).
k/ Avg. Cl^-: The average plasma chloride for that period (in meq/l).
l/ Na^+/Cl^-: The ratio of the plasma sodium to chlorides for that period, averaged.

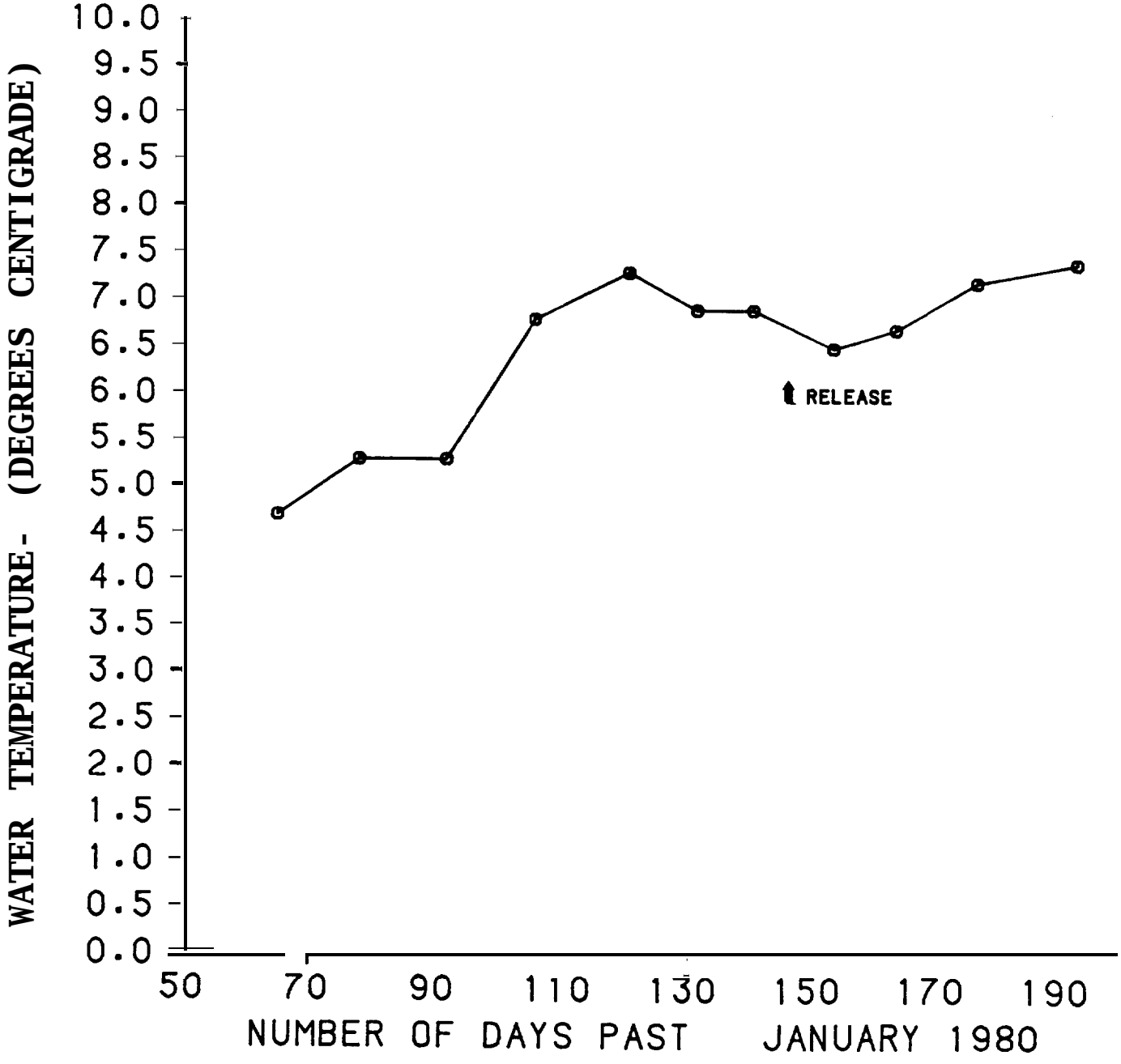


Figure 67.--Water temperatures at Willard NFH during spring 1980.

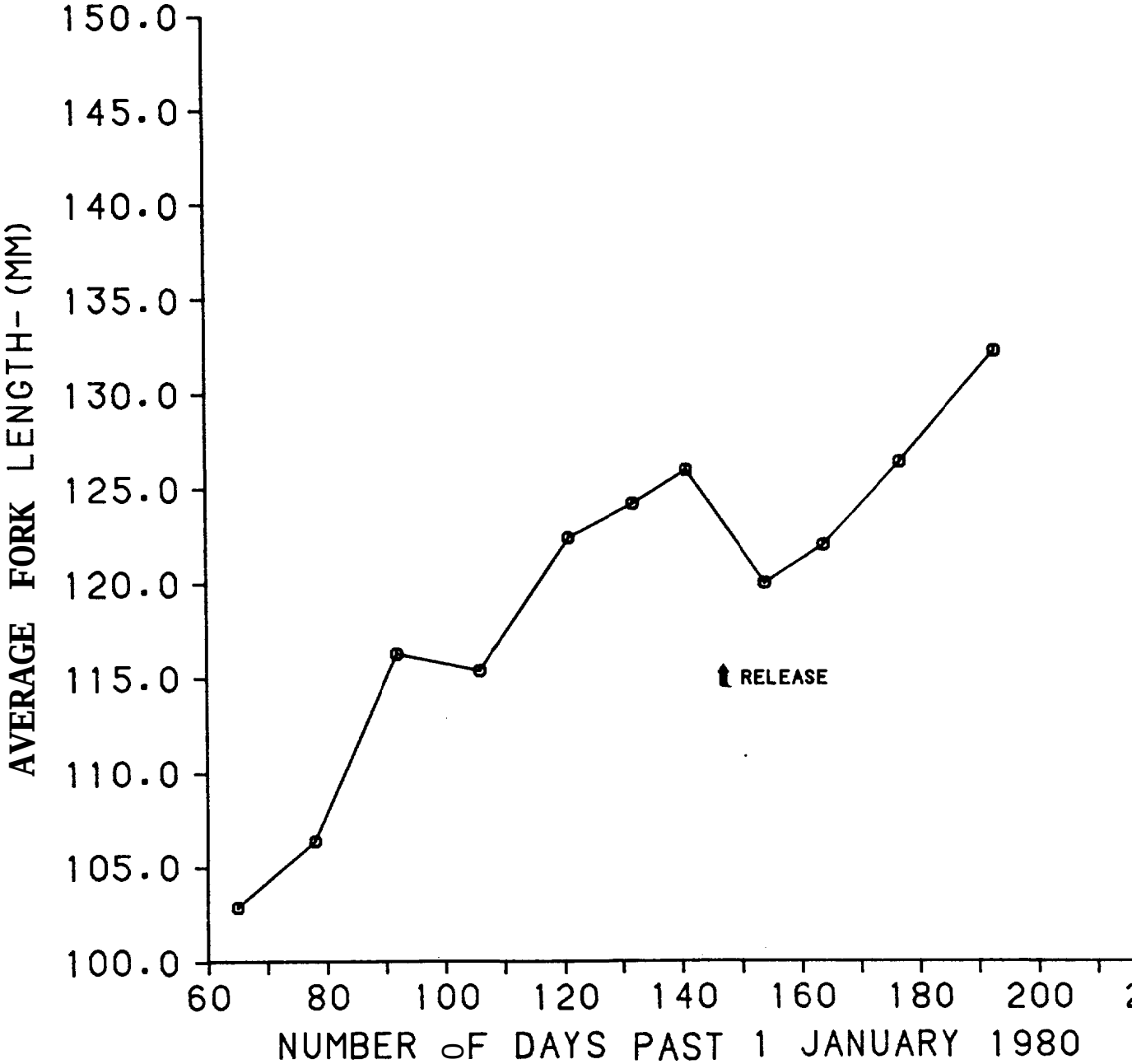


Figure 68.--Mean fork lengths of Willamette NFH coho salmon sampled during spring 1980.

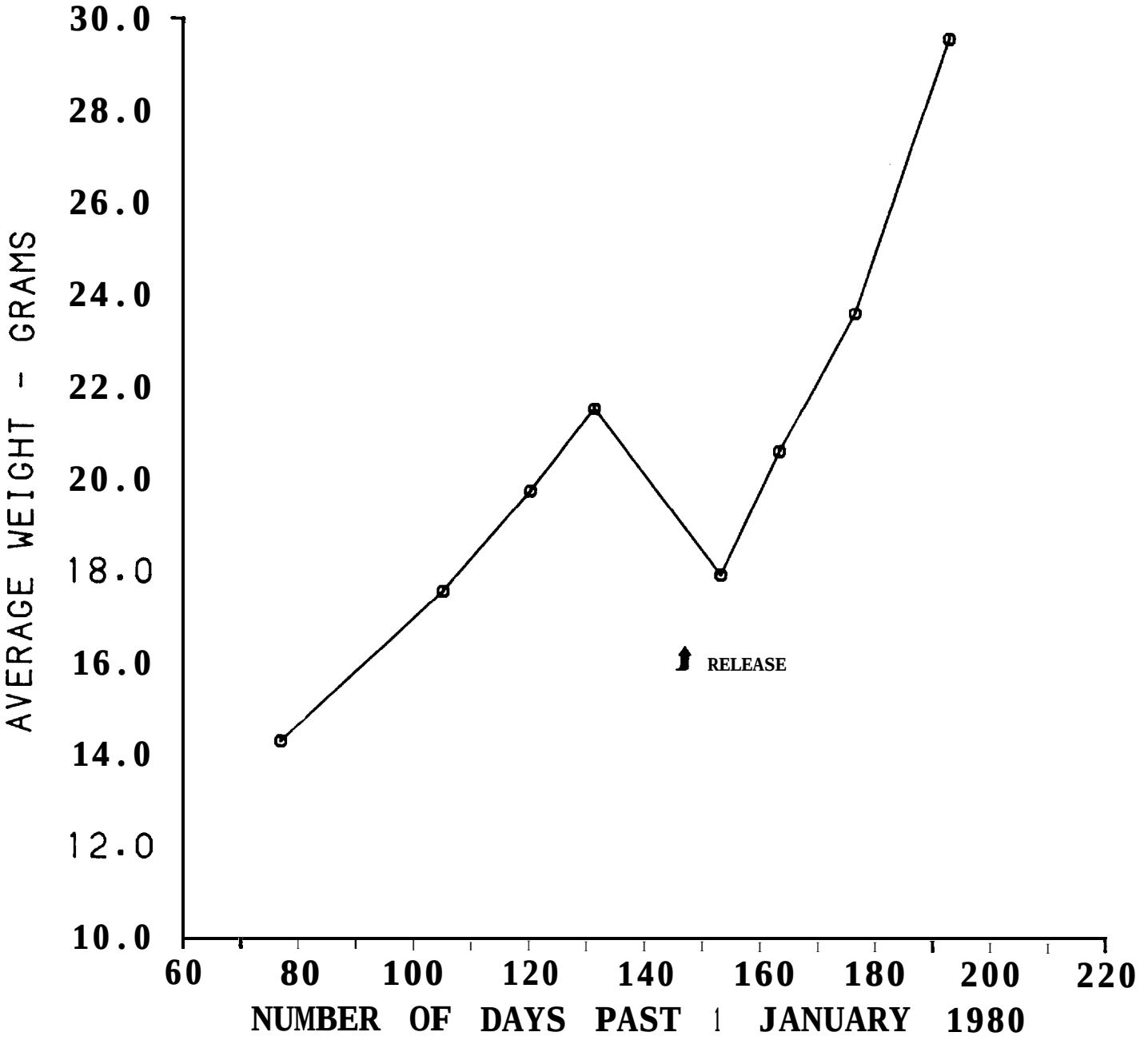


Figure 69.--Mean weights of Willard NFH coho salmon sampled during spring 1980.

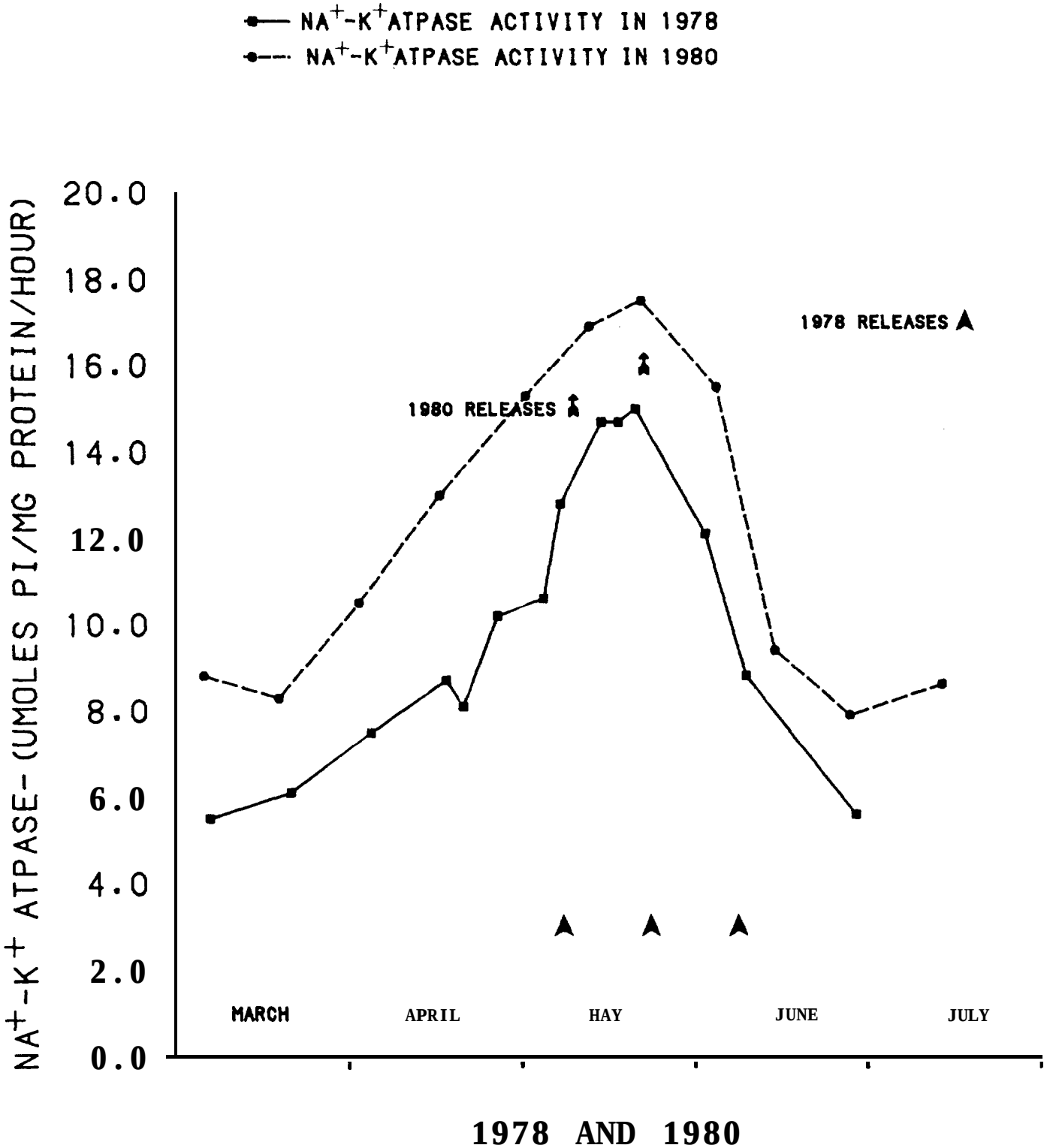


Figure 70.--Mean gill Na^+-K^+ ATPase activity of Willard NFH coho salmon sampled during spring 1978 and 1980.

There were no significant correlations between pooled sample fork lengths and Na^+-K^+ ATPase in any of the sample periods. There was a positive correlation ($r = 0.739$; $P < 0.020$ for 8 d.f.) between gill Na^+-K^+ ATPase and pooled plasma Cl^- in the 5 March sampling period, which is in contrast to the theory that plasma Cl^- declines with the onset of smoltification. There was a negative correlation ($r = 0.847$; $P < 0.005$ for 6 d.f.) between gill Na^+-K^+ ATPase and pooled plasma K^+ in the 20 May sampling period, and a positive correlation ($r = 0.667$; $P < 0.05$ for 7 d.f.) between gill Na^+-K^+ ATPase and pooled hematocrits in the 20 May period. There do not appear to be any consistencies with these correlations, and therefore they may not be relevant.

In Figure 71, the percentage of samples with Na^+-K^+ ATPase values $\geq$ 15 begins to rise rapidly by mid-April (30%). This percentage increases to over 60 by the time of release (mid-May) and is sustained through early June. Before mid-April, none of the fish had Na^+-K^+ ATPase values over 15 u moles Pi/mg protein/hour. On the basis of this information, it would appear that 50-60% of the fish were smolting at release.

Plasma Electrolytes

Miles and Smith (1968) reported on plasma electrolytes in hatchery coho salmon. Spring samples of fish in fresh water averaging 12.5 g were as follows:

	Mean (mEq/l)	Range (mEq/l)
Na^+	146.7	130.0-168.0
Cl^-	117.3	90.0-132.6
K^+	8.6	1.8- 19.0

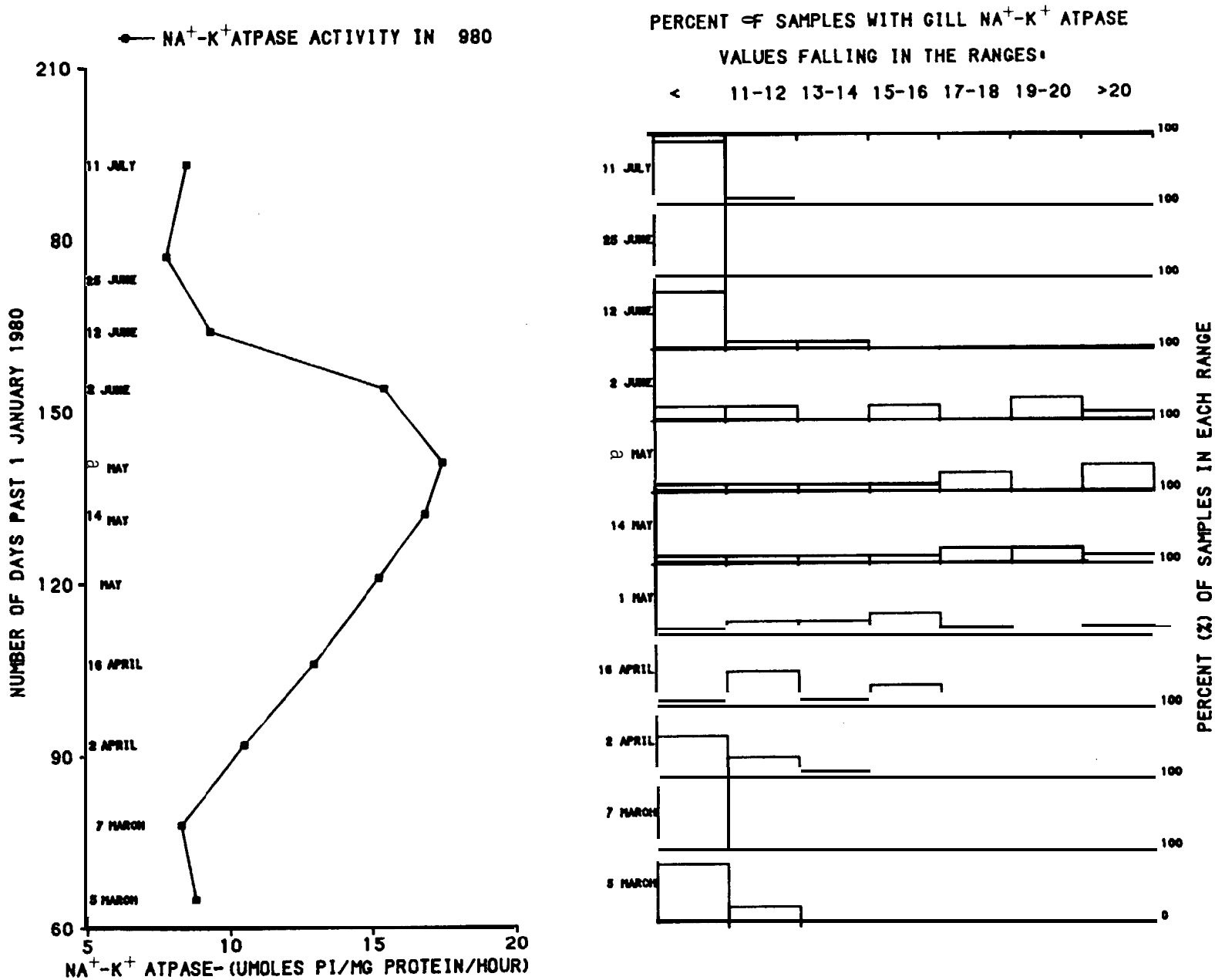


Figure 71. Frequency of gill Na^+-K^+ ATPase values of Willard NFH coho salmon samples throughout spring 1980. A profile of the mean values is shown for reference

Our own studies of Columbia Kiver Hatchery coho salmon in 1978 resulted in the following ranges of mean values for plasma electrolytes:

	mEq 1
Na^+	140.8 - 170.0
Cl^-	113.4 - 140.8
K^+	5.7 - 8.4

Mean plasma electrolyte values (Table 47) were within the expected values or the values found in Columbia River hatchery coho salmon in 1978. Both minimum and maximum plasma Na^+ values were beyond those reported by Miles and Smith (1968), as were the maximum plasma Cl^- values. Apparently plasma K^+ values are normally higher in coho salmon than in chinook salmon.

Profiles of mean plasma electrolyte values for the three major sampling periods are shown in Figure 72, and compared here with mean gill Na^+-K^+ ATPase values collected at the same times. There is actually a significant positive correlation ($r = 0.997$; $P < 0.05$ for 1 d.f.) between the mean Na^+-K^+ ATPase and the mean plasma Cl^- values. But, this trend of increasing plasma Cl^- and decreasing plasma Na^+ as the fish approach smolting would not appear to be typical. The gradual increases in plasma K^+ values could be expected with increased stresses from growth and the onset of smolting. There was no evidence of sharply elevated plasma K^+ values at the time of release that would indicate serious stress problems. The median value (6.4 mEq/l) was below the mean (7.1 mEq/l), and only 12% of the samples had values of 10 mEq/l or higher. As mentioned previously, there was a highly significant negative correlation between the gill Na^+-K+ ATPase and plasma K^+ at the time of release, and this correlation is shown in Figure 73. If the high Na^+-K^+ ATPase

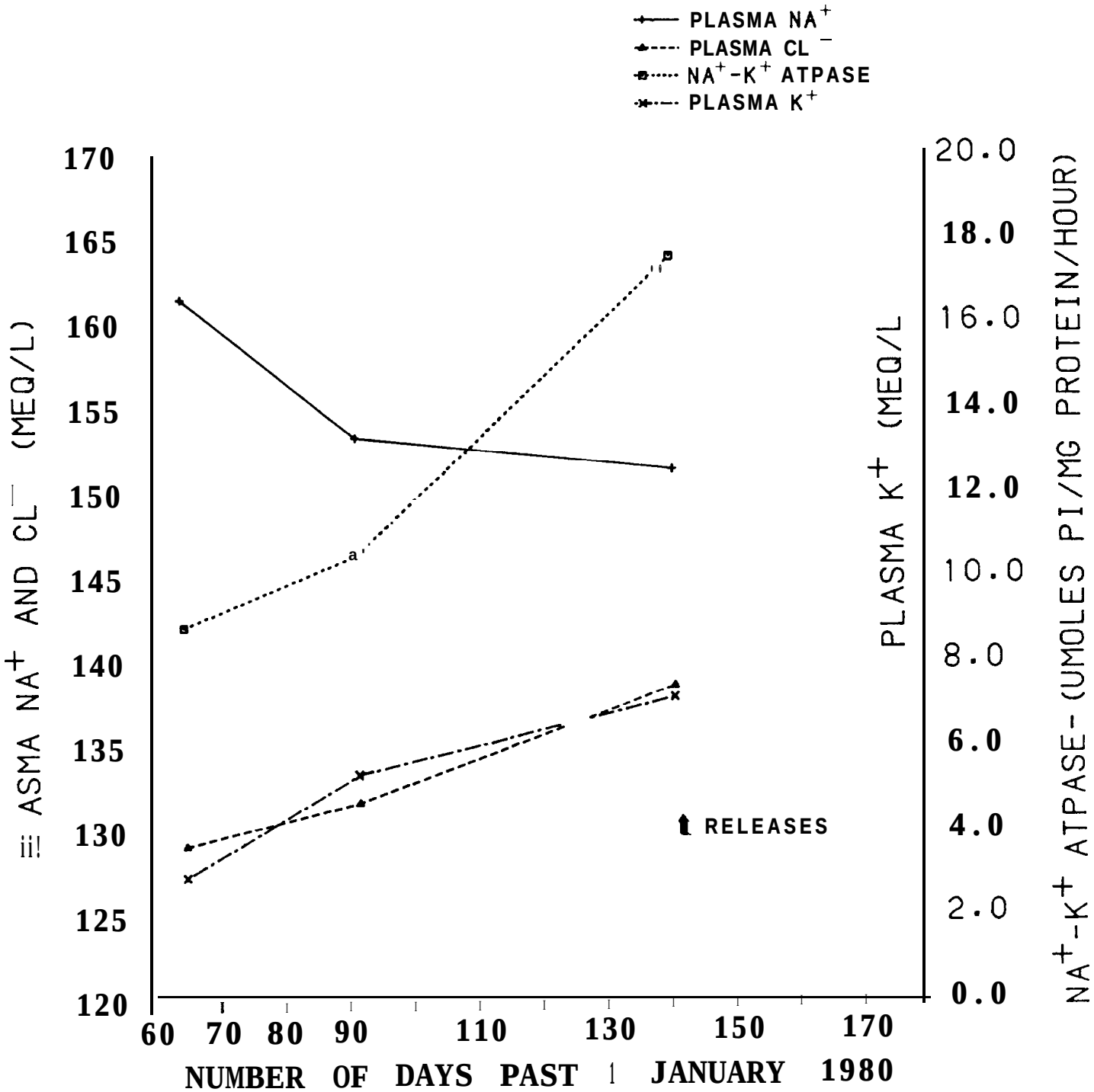


Figure 72. --Mean plasma Na^+, Cl^-, and K^+ values in the Willard NFH coho salmon during spring 1980 are compared with mean gill Na^+-K^+ATPase activity.

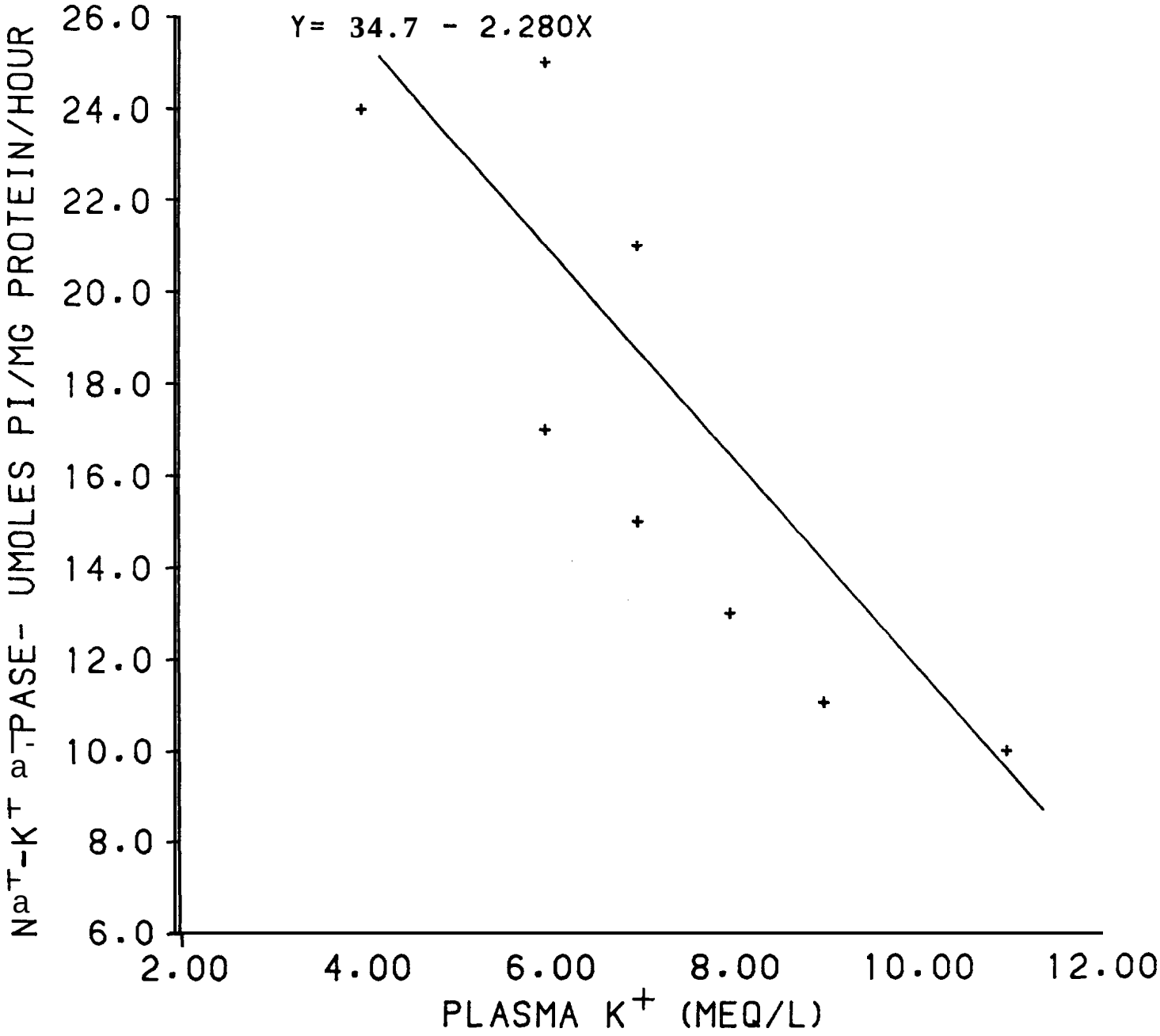


Figure 73.--Regression of pooled gill Na^+-K^+ ATPase activity on pooled plasma K^+ values of Willard NFH coho salmon at the time of release (24 May 1980). $r = -0.847$; $P < 0.005$ (for 6 d.f.).

values are indicative of true smolting activity, any stresses related to this are not indicated by classic plasma K^+ responses.

Hematology

Wedemeyer and Chatterton (1971) list normal expected values (for coho salmon) in fresh water of 32.5 to 52.5% for hematocrits and 6.5 to 9.9 g/100 ml of blood for hemoglobins. Summarized data for the Willard NFH coho salmon in 1980 are presented in Table 47.

In 1978, there were three marked release groups of Willard NFH coho salmon. The first two were within ± 6 days of the 1980 release group.

Figure 74 compares hemoglobin profiles of 1978 and 1980 Willard NFH coho salmon from our samplings. Note that at the times of release, means and standard deviations for both years were quite similar. However, when we compare profiles of hematocrits, there was a substantial difference (Figure 75), and mean values at the time of release in 1980 were 26% higher than peak mean value in 1978. Some of this differential may be due to transport stresses in 1978. We indicated that between 9 and 18% of the fish in the first two release samples in 1978 had hematocrits above the expected high values published by Wedemeyer and Chatterton (1971). In 1980, 68% of the samples had hematocrits above the expected value and even the mean was above the expected high. We also indicated (in 1978) that between 6 and 10% of the first two release groups had hematocrits below the expected low value. In 1980, only 3.4% of the fish had low hematocrits at release, which indicates that only a small number of fish could be classed as having a low health index. This large proportion of fish with high hematocrits at release time may be an indication of extreme water loss associated with smoltification. As was previously mentioned, there was a significant

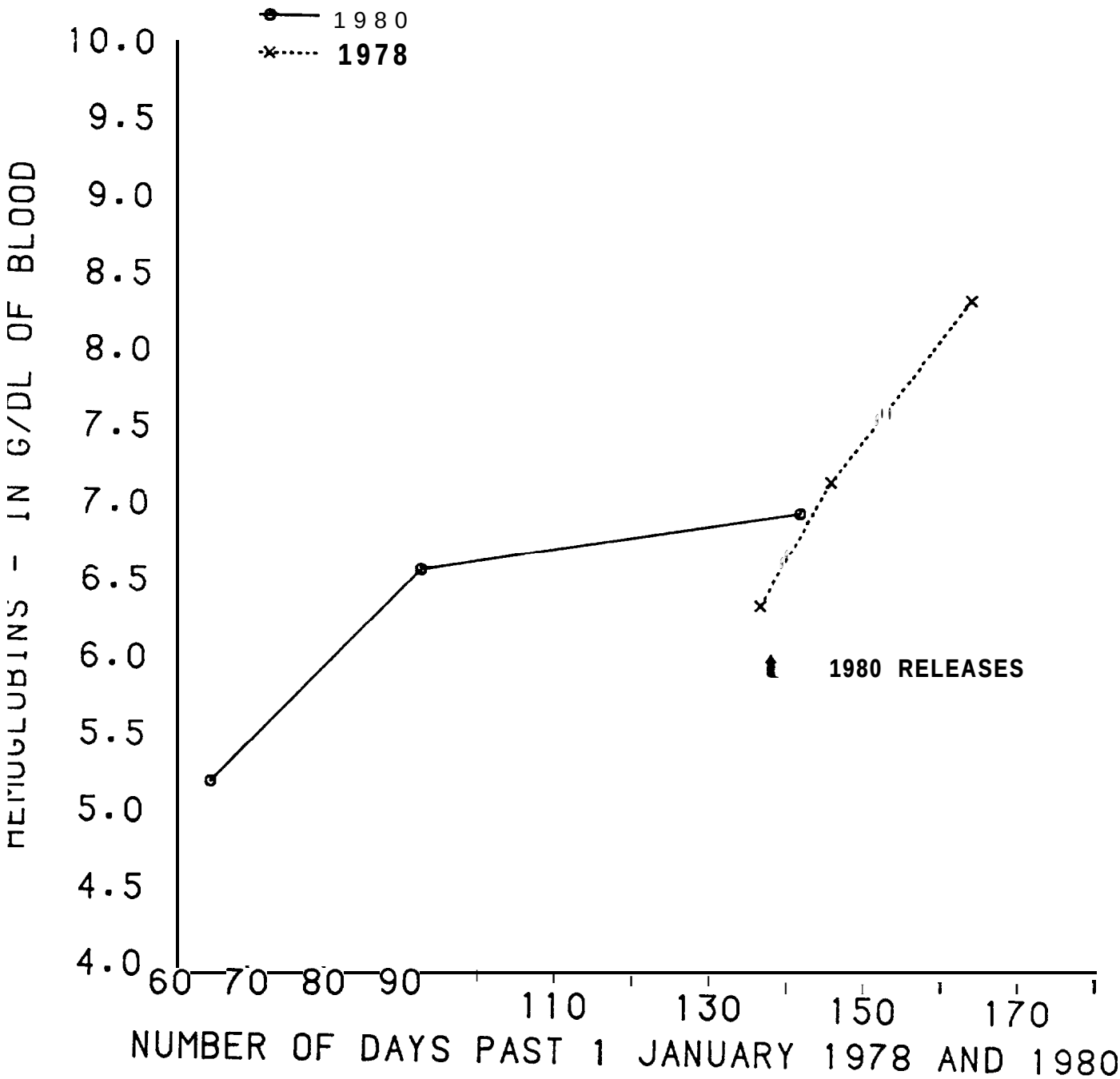


Figure 74.--Mean hemoglobin values of the Willard NFH coho salmon during the spring of 1978 and 1980.

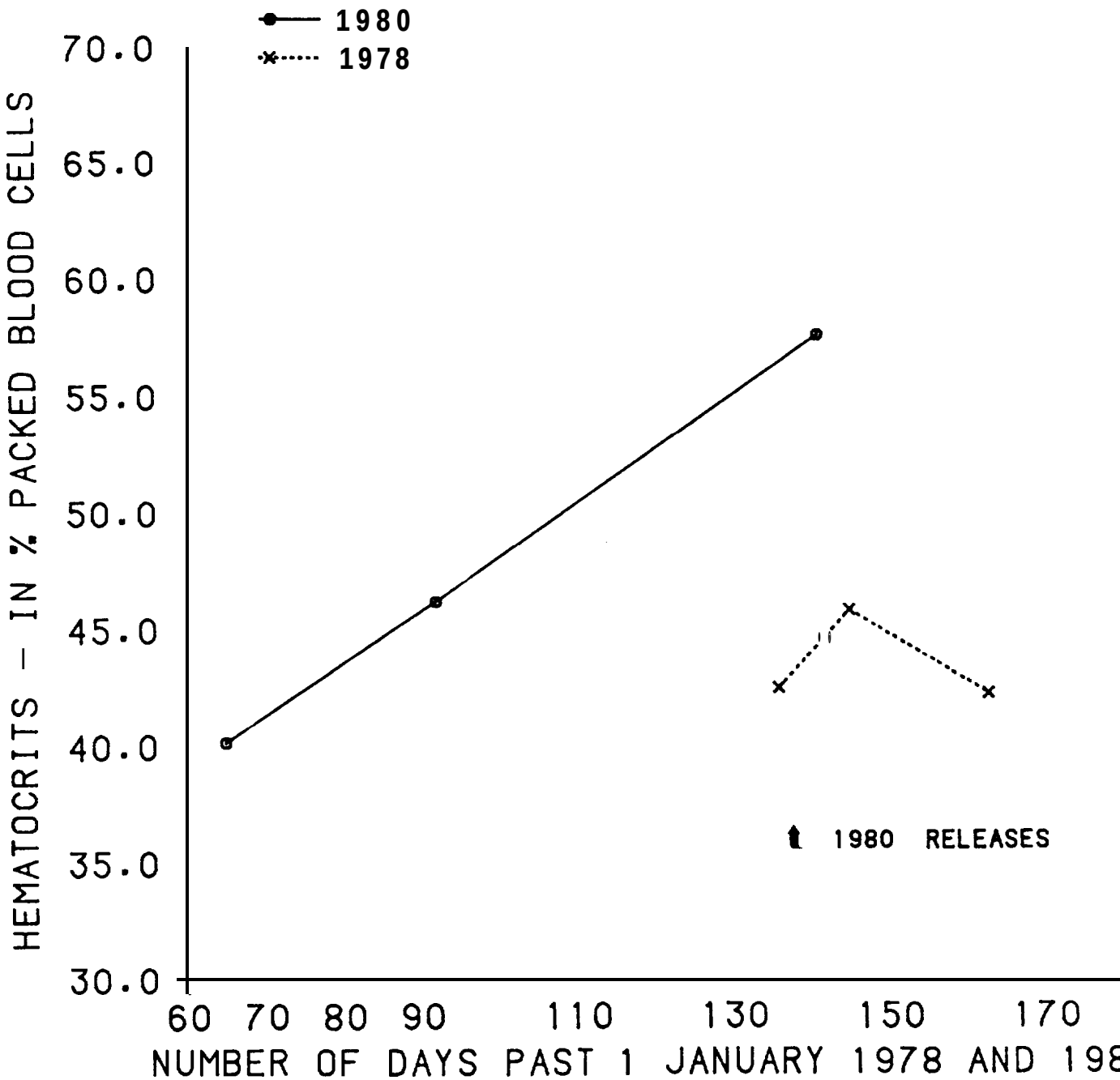


Figure 75.--Mean hematocrit values of the Willard NFH coho salmon during the spring of 1978 and 1980.

positive correlation between pooled hematocrit and gill Na^+-K^+ ATPase values at the time of release in 1980 (Figure 76). Furthermore, there were' no samples with hematocrits above the expected high value (52.5%) on 5 March 1980 and only 17% on 2 April.

The mean cell hemoglobin concentration (MCHC) had actually declined by the time of release, which is another indication that the fish were probably not stressed. However, even though high hematocrit values are generally not considered as indications of stress, the possibility of extreme hemoconcentration may be cause for concern. As the fish enter seawater, there is a further reduction in body water from osmosis.

IFAT-BKD

Specimens of Willard NFH coho salmon from the 3 March and 5 May sampling periods were examined for the presence of BKD organisms by the IFAT.

Only four specimens (6.7%) were found positive for BKD on 3 March, all in the posterior kidney, and all of very Light intensity. Only one specimen (1.7%) was found positive for BKD on 5 May, and this was of moderate intensity (posterior kidney only). This is a Low rate of infection in comparison to the 15-16% of samples positive for BKD at comparable release times in 1978. These data, plus the absence of any samples with extremely Low hematocrits suggest an improved state of health over the 1978 releases.

Histopathology

A summary of pathological conditions observed is presented in Table 48. For comparative purposes, these data are further summarized with data from other hatcheries in Table 9.

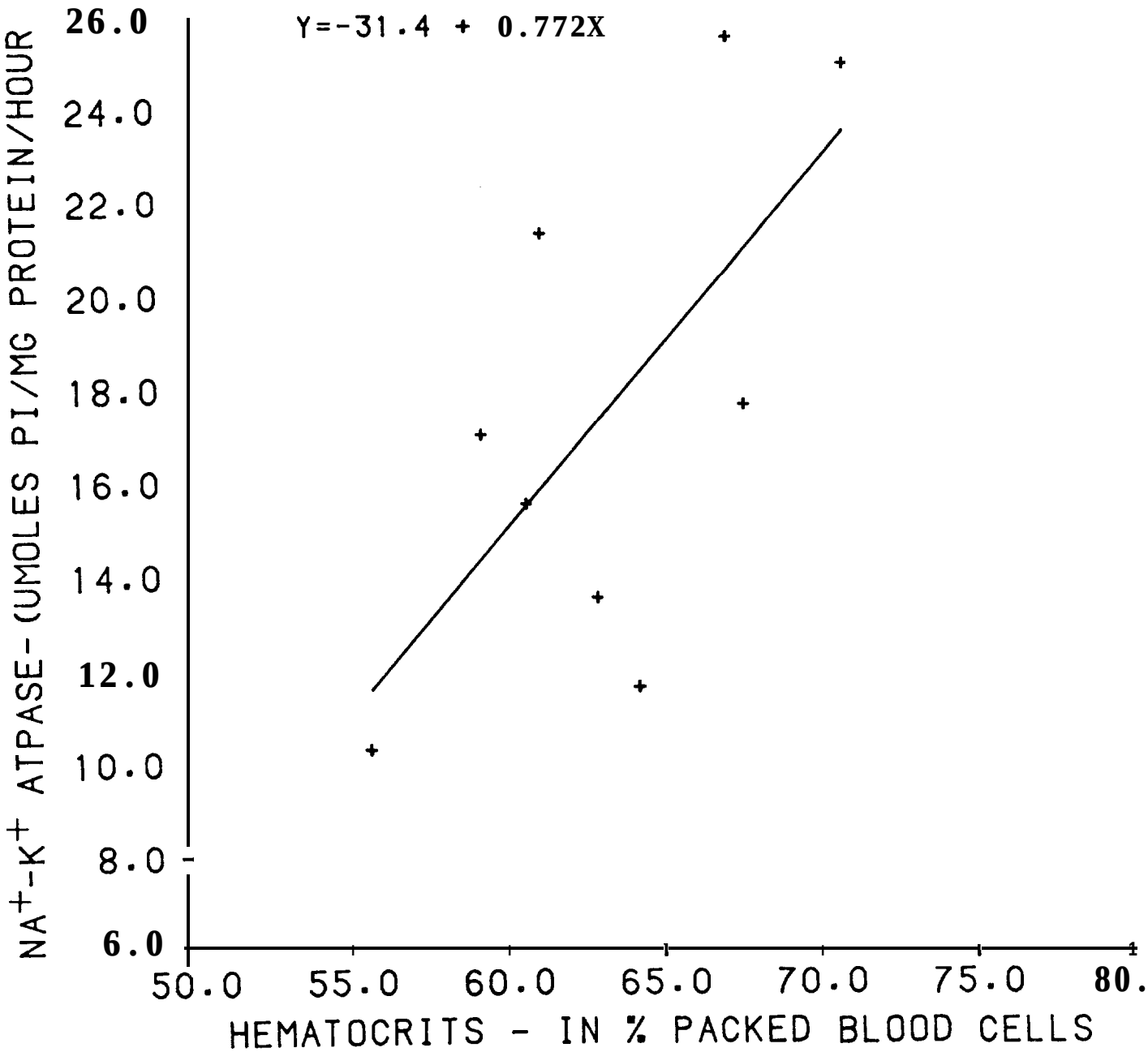


Figure 76.--Regression of pooled gill Na^+-K^+ATPase activity on pooled hematocrit values of Willard NFH coho salmon at the time of release (24 May 1980). $r = 0.667$; $P < 0.05$ (for 7 d.f.).

Table 48.--Pathological conditions observed in 1980 Willard Hatchery coho salmon and their percentage of incidence.[a]

Organ and pathology	Incidence (%) Period 1 (severity)[b] I	II	III	total	Period 2 (severity) I	II	III	total	Period 3 (severity) I	II	III	total
Eye												
skeletal muscle lesions	63.3	0	0	63.3	46.7	0	0	46.7	78.3	0	0	78.3
Gills												
increased numbers of lymphocytes	80.0	16.7	0	96.7	66.7	16.7	0	83.3	41.7	58.3	0	100.0
epithelial cell formation	63.3	11.7	0	75.0	41.7	3.3	0	45.0	70.0	28.3	0	98.3
lymphatic telangiectasis of secondary	1.7	0	0	1.7	10.0	1.7	0	11.7	0	0	0	0
vascular telangiectasis of secondary lamellae	21.7	1.7	0	23.4	6.7	0	0	6.7	3.3	0	0	3.3
Olfactory sac												
focal mononuclear cell infiltration	78.3	20.0	0	98.3	95.0	5.0	0	100.0	93.3	5.0	0	98.3

a/ Brain tissue was processed and examined for all specimens, and there was no evidence of any pathology.

b/ I-recognizable (least severe); II-intermediate; III-severe.

In 1980, there were pronounced increases in pathological conditions of the eyes and gills between the first and last (at release) sampling periods. There was a doubling and tripling of intermediate (Class II) severity in several pathological conditions of the gills. By comparison, in 1978 (at a comparable release time), 40% of the fish had lesions of the skeletal muscle of the eye, 22% had increased numbers of lymphocytes in the gills, and 45% showed evidence of epithelial cell formation in the gills. There was never more than 3.5% of the fish with intermediate (Class II) severity of any Lesion in 1978. Lesions of the olfactory sac were consistently high in 1980.

Summary

The serial sampling of Willard NFH coho salmon in 1980 indicated that gill Na^+-K^+ ATPase profiles were spatially identical to those collected in 1978, and that releases were in accord with a timely peak. High Na^+-K^+ ATPase values were seen in 50-60% of the samples at the time of release.

The general health of the fish was good, with very Little evidence of latent BKD or clinical evidence of anemia or border-line anemia. A very high proportion (68%) of the samples had excessively high hematocrits at release, possibly due to hemoconcentration. This could have posed problems if the fish reached the high salinities in the ocean in this state.

Increases in the frequency and severity of certain Lesions of the eyes and gills at the time of release are difficult to evaluate as a possible detriment to the overall health picture at this time.

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APPENDIX A

APPENDIX A

A quick reference chart to determine the calendar date (for year 1980) from the number of days past 1 January 1980 (days>Jal).

Number of days pas t 1 January 1980	Calendar date
5	5 January
10	10 January
15	15 January
20	20 January
25	25 January
30	30 January
35	4 February
40	9 February
45	14 February
50	19 February
55	24 February
60	29 February
65	5 March
70	10 March
75	15 March
80	20 March
85	25 March
90	30 March
95	4 April
100	9 April
105	14 April
110	19 April
115	24 April
120	29 April
125	4 May
130	9 May
135	14 May
140	19 May
145	24 May
150	29 May
155	3 June
160	8 June
165	13 June
170	18 June
175	23 June
180	28 June

Number of days past 1 January 1980	Calendar date
185	3 July
190	8 July
195	13 July
200	18 July
205	23 July
210	28 July
215	3 August
220	7 August
225	12 August
230	17 August
235	22 August
240	27 August