INTERNATIONAL PACIFIC SALMON
FISHERIES COMMISSION

PROGRESS REPORT

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NEW WESTMINSTER, B.C.
CANADA
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INTERNATIONAL PACIFIC SALMON
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in the Fraser River System

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FOREWORD

This program was made possible with the cooperation of Canada Department of Fisheries and the Environment, and the University of Washington. Each individual involved represented a specific area of expertise. Dr. Strasdine was responsible for the bacteriology of the migrating and spawning fish, Dr. Tsuyuki was responsible for the enzymology, Mr. McBride was responsible for the histology, Mr. Fagerlund was responsible for the cortisol determinations, and Dr. E. Ordal was responsible for the bacteria counts in the river and the on-site examinations of moribund fish, including the use of phase contrast microscopy.

ABSTRACT

The 1973 Horsefly sockeye population had an overall prespawning mortality of 27.1%, the lowest mortality since 1957 for this dominant cycle. In distinct contrast with other years, there was no significant difference in the mortality between the first, central, and late segments. The fresh water temperatures encountered by the 1973 Horsefly sockeye were above average in the Fraser River at Hell's Gate, but below average at the spawning grounds, and the timing of the run was later than average for this cycle.

The various parameters measured indicated that there were very few differences between the first and central fish sampled both in salt water and upon arrival at the spawning grounds. The plasma cortisol levels were higher among the first fish, and serological studies indicated the first fish had encountered a fish pathogen Aeromonas liquifaciens after passing Lummi Island. The histopathology and diagnostic enzymology indicated no significant differences in the pathology of the gill tissue from early and central fish in salt water, but examination of gill tissue on arrival at the spawning grounds indicated a higher concentration of foreign materials in the gills of first fish. Large fusiform shaped bacteria were present, varying in concentrations on both moribund and spawning fish. Similar bacteria have been reported to be associated with mortalities of hatchery reared sockeye, pink and coho salmon fry.

It is concluded that the cool water temperatures during spawning and late timing of the migration apparently were favorable factors which limited the prespawning mortality in 1973.

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INTRODUCTION

Significant prespawning losses have been documented from Fraser River sockeye populations since the mid-1940's. It has been calculated that prespawning losses during the ten year period of 1961-1970 represented a potential production loss of 40 million pounds of sockeye (Williams 1976). The International Pacific Salmon Fisheries Commission began investigating diseases of feral populations in 1963 (Pacha 1964) when 565,000 females died before spawning. Since this time the investigations have continued and expanded to include various disciplines involved either directly or indirectly with prespawning mortality (D. J. Colgrove and J. W. Wood 1966; G. S. Colgrove 1966; Williams 1973; J. S. Wood 1965).

Generally the prespawning mortality has been highest among sockeye that arrive first on the spawning ground and often the central and last segments have little or no prespawning mortality. In 1964, Colgrove (1966) examined histological changes accompanying maturation in both first and central Chilko sockeye and found the different segments of the run to be comparable. However, this run had a small prespawning mortality of only 2%. In 1969 the first, central and last segments of the Horsefly run were examined at the spawning grounds for a variety of parameters in order to distinguish possible differences between segments. There were indications that inherent differences may exist between the first and central segments of a run (Williams 1973). In 1971 an intensive investigation was undertaken to examine the first and central segments of the Chilko population during their spawning migration from salt water to spawning and death for factors associated with prespawning mortality. This investigation led to several observations of possible differences between the first and central fish, indicating the need for further research. Therefore in 1973 the International Pacific Salmon Fisheries Commission, with the cooperation of the then Fisheries Research Board of Canada, Vancouver Technological Station and the University of Washington, conducted an intensive investigation of the Horsefly population.

Historically the Horsefly population has had significant prespawning mortalities on the dominant cycle dating back to 1953. The only exception to this was the 1957 population (Williams 1973). In most years of high prespawning mortality, more first segment fish died unspawned than central or last segment fish. The investigation of the 1973 Horsefly population was an expansion of the 1971 Chilko program (Williams 1977) and was designed to examine a variety of parameters from both the first and central segments of the population during various phases of their spawning migration.

METHODS

Fish Capture

Three sample locations were chosen for the 1973 study. A total of 115 fish were captured during two excursions approximately 12 miles off the west coast of Vancouver Island near Tofino (FIGURE 1). There were 2 female, 2 male first segment and 12 female, 15 male central Horsefly sockeye identified from these samples on the basis of length and scale information. In addition, there were 3 female, 6 male first segment and 13 female, 12 male central Late Stuart fish identified. Late Stuart is the other major sockeye run migrating at a similar time which usually has little prespawning mortality.

The fish captured at Lummi Island totaled 221 in three fishing periods. Commercial reef net gear was used to capture the fish. There were 12 female, 13 male first segment Horsefly and 13 female, 10 male first segment Stuart fish captured during July 20-22. Fourteen female, 18 male central Horsefly and 14 female, 8 male central Stuart fish were captured during July 27-28, and 3 female, 15 male central Horsefly and 7 female, 4 male central Stuart were sampled August 3 at Lummi Island.

Upon arrival at the spawning grounds, fish sampled from the first and central segments were seined from the Horsefly River near the townsite of Horsefly. Twenty female, 18 male first segment fish and 24 female, 14 male central fish were collected on August 15 and 28 respectively (TABLE 1).

TABLE 1 - Summary of fish capture.

Date	Segment of Population	Location	Number of Horsefly Fish		Number of Stuart Fish	
			♂	♀	♂	♀
July 13	First	Tofino	2	2	6	3
July 12-21	Central	Tofino	15	12	12	13
July 20-22	First	Lummi	13	12	10	13
July 27-28	Central	Lummi	18	14	8	14
August 3	Central	Lummi	15	3	4	7
August 15	First Arrival	Horsefly	18	20	-	-
August 28	Central Arrival	Horsefly	14	24	-	-

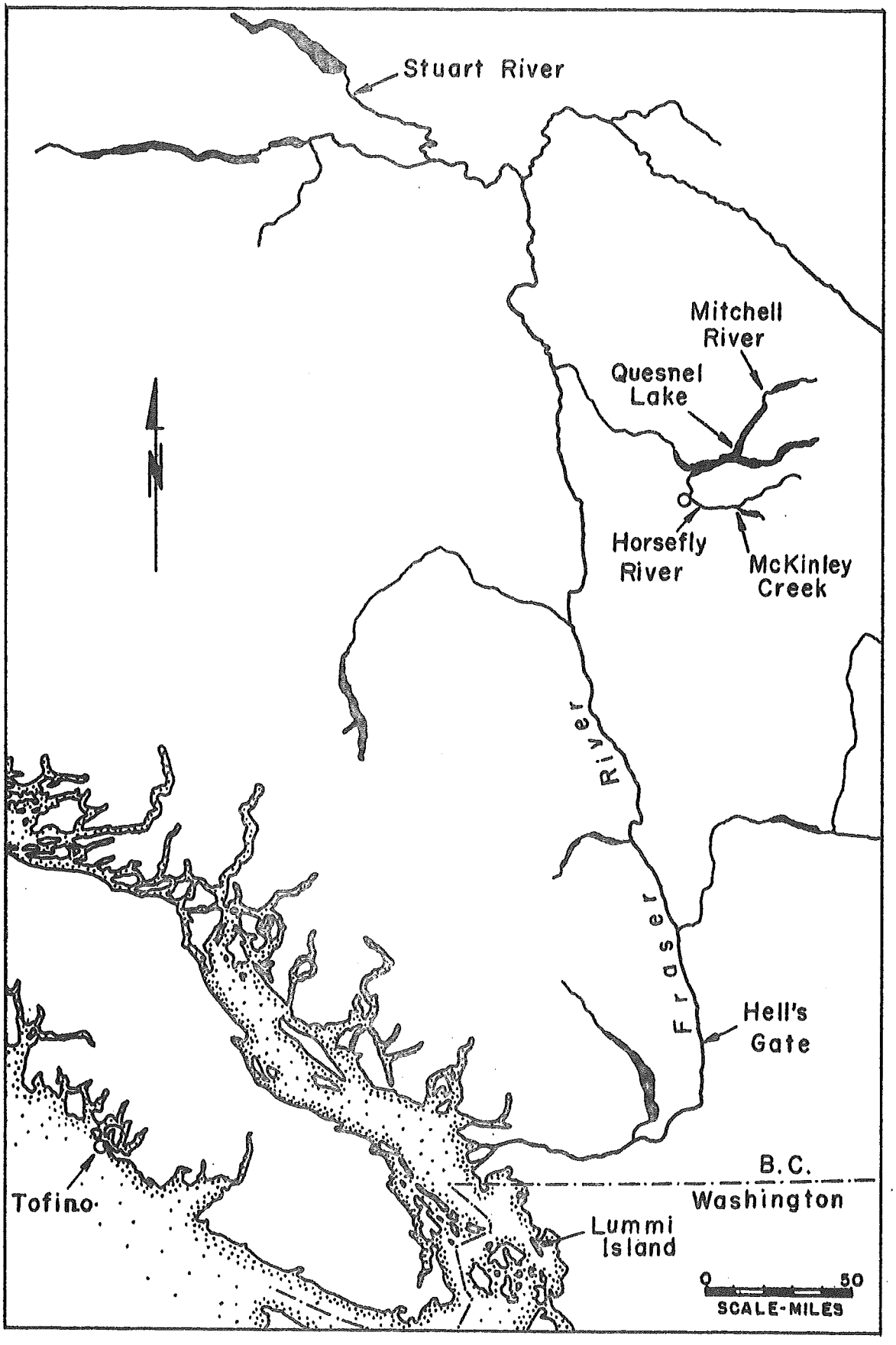


FIGURE 1 - Map showing areas of fish capture and spawning.

Sampling and Analytical Techniques

Each fish upon capture was immediately tagged and this number was used for identification of all samples collected. Blood samples were taken from each fish within 5 minutes after initiation of capture to avoid the effects of stress on the plasma cortisol concentrations (Fagerlund 1967). Whole blood was drawn from the caudal vein of each fish with heparinized 10 ml Vacutainer tubes. A hematocrit sample was taken and plasma was then separated from the whole blood sample and frozen on dry ice for further processing in the laboratory.

Plasma samples were utilized for diagnostic enzymology, agglutinating antibody tests, and cortisol determinations.

Previously described methods were used for all enzyme activity measurements as follows: serum glutamic-oxalecetic aminotransferase and serum glutamic-pyruvic aminotransferase (SGPT) (Sigma Technical Bulletin No. 410-UV, 1967); adenosine triphosphatase (ATP-ase) (Post and Sen 1968); carbonic anhydrase (Wilbur and Anderson 1948); lactic dehydrogenase (Aewe and Fromm 1962, Tsuyuki and Williscroft 1973).

The agglutinating antibody tests provide a rapid, although presumptive, test for determining if a host is infected by or has come in contact with an infectious agent (Krantz, et al. 1964; Schachte and Mora 1973).

For the preparation of formalized antigen, isolates were cultured in 50 ml tryptone medium (pH 7.2) containing three percent yeast extract and one percent Tween 80. Cells were collected by centrifugation (5000 x g at 4° C for 15 min.), washed and resuspended in saline to one-tenth original growth volume (ca 1 x 10^{10} cells/ml). Ten percent formalin was added to a final concentration of 0.2% and the tubes incubated 48 h at 30° C, removed and stored at 4° C.

Slide agglutination tests were performed with 24 h plate cultures (preliminary screening) or with formalized cell suspensions (kidney isolates 701 and 705). Cultures were mixed with one drop of saline on a Kline agglutination slide, one drop of test serum added, mixed again, and read after one minute had elapsed. Plasma cortisol concentrations were determined with a competitive protein binding technique (Fagerlund 1970).

After blood sampling, the fish were placed in a live box until they could be sampled for histology and bacteriology. This reduced the time out of water for each fish to periods of less than 5 minutes, which minimized the risk of autolysis. The fish were immobilized by severing the spinal cord just behind the head and both gill and kidney were sampled for bacteria. Sealed "Culturette" tubes containing modified Stuart's transport media were used to swab gill tissue of

moribund fish. Swabs of kidney tissue were obtained after aseptic removal of the overlying peritoneal membrane. Swab tubes were sealed, packed in ice and transported to the laboratory for subsequent culture. Swabs were streaked directly onto trypticase-soy agar, incubated at 25° C and representative isolates checked for purity before storing in trypticase-soy agar deeps. Then samples of liver, kidney, interrenal, stomach, gill (2nd arch), gonad, and skin were excised and immediately placed in Bouin-Sublimate fixative. Pituitary glands were fixed in toto.

The methods for decalcification, embedding, tissue sectioning and staining have been described (McBride and van Overbeeke 1971; van Overbeeke and McBride 1971). Brown and Hopps (1973) gram staining procedure was used for selective identification of bacteria in the tissue sections.

Due to the heterogenous appearance of the interrenal tissue during sexual maturation, a semi-quantitative method developed in a previous study with this species (van Overbeeke and McBride 1971) was used to define the histological activity of this tissue. Each fish was assigned a degree of activity according to the following index:

1. tissue generally consisting of groups of densely packed small cells with basophil cytoplasm, but occasional small groups of hypertrophied cells present,
2. considerable cellular hypertrophy involving 10 to 30 percent of the tissue,
3. hypertrophy involving slightly more than half of the tissue,
4. hypertrophy general, but small groups of quiescent cells still present,
5. tissue uniformly hypertrophied.

Prior to sampling for histopathological examination, the fish were measured and weighed. The fork and eye socket hyperal plate lengths were determined. The total weight was taken and then, after sampling for histology, the weights of eviscerated body and gonads were recorded. The gonads were preserved and the fecundity of each sample was determined in the laboratory.

In addition, moribund and healthy sockeye were examined on the spawning grounds during spawning. Gill tissue was examined with phase contrast microscopy and swabs of gill and kidney tissue were cultured for bacteria.

Spawner Enumeration

The size of the spawning population was determined by a standard mark-recapture program (Schaefer 1951). A total of 5,299 tags were applied to the sockeye in proportion to the numbers of fish migrating past the town of Horsefly,

and these fish were released (FIGURE 2). Tag and sex ratios were obtained from the recovery of 72,279 dead sockeye, enabling an estimate of the total and female sockeye populations.

During the course of the dead recovery, 1,371 tagged and 7,653 untagged sockeye were examined for success of spawning.

DESCRIPTION OF THE 1973 POPULATION

Size, Timing and Escapement

The dominant cycle sockeye run to the Quesnel Lake system in 1973 produced a total run of 1,750,000 fish. The Horsefly fish passed Tofino from approximately July 10 to August 15 with the peak on July 26. The run passed Lummi Island from approximately July 15 to August 15, with the peak on August 1 (FIGURE 3).

Escapement to the Horsefly area spawning grounds was 253,384 with 139,579 female sockeye. Over 90% of the spawners occupied the Upper Horsefly spawning grounds. A total of 24,673 spawners escaped to the Mitchell River including 13,817 females (TABLE 2, FIGURE 2).

TABLE 2 - Distribution of sockeye spawners in Quesnel Lake system, 1973.

Area	♀ Spawners	Total Spawners
Lower Horsefly River below Woodjam	4,751	8,669
Upper Horsefly River	125,734	229,609
Lower McKinley Creek (McKinley Lake to Horsefly River)	6,588	10,944
Upper McKinley Creek (above McKinley Lake)	2,506	4,162
	139,579	253,384
Mitchell River	13,817	24,673

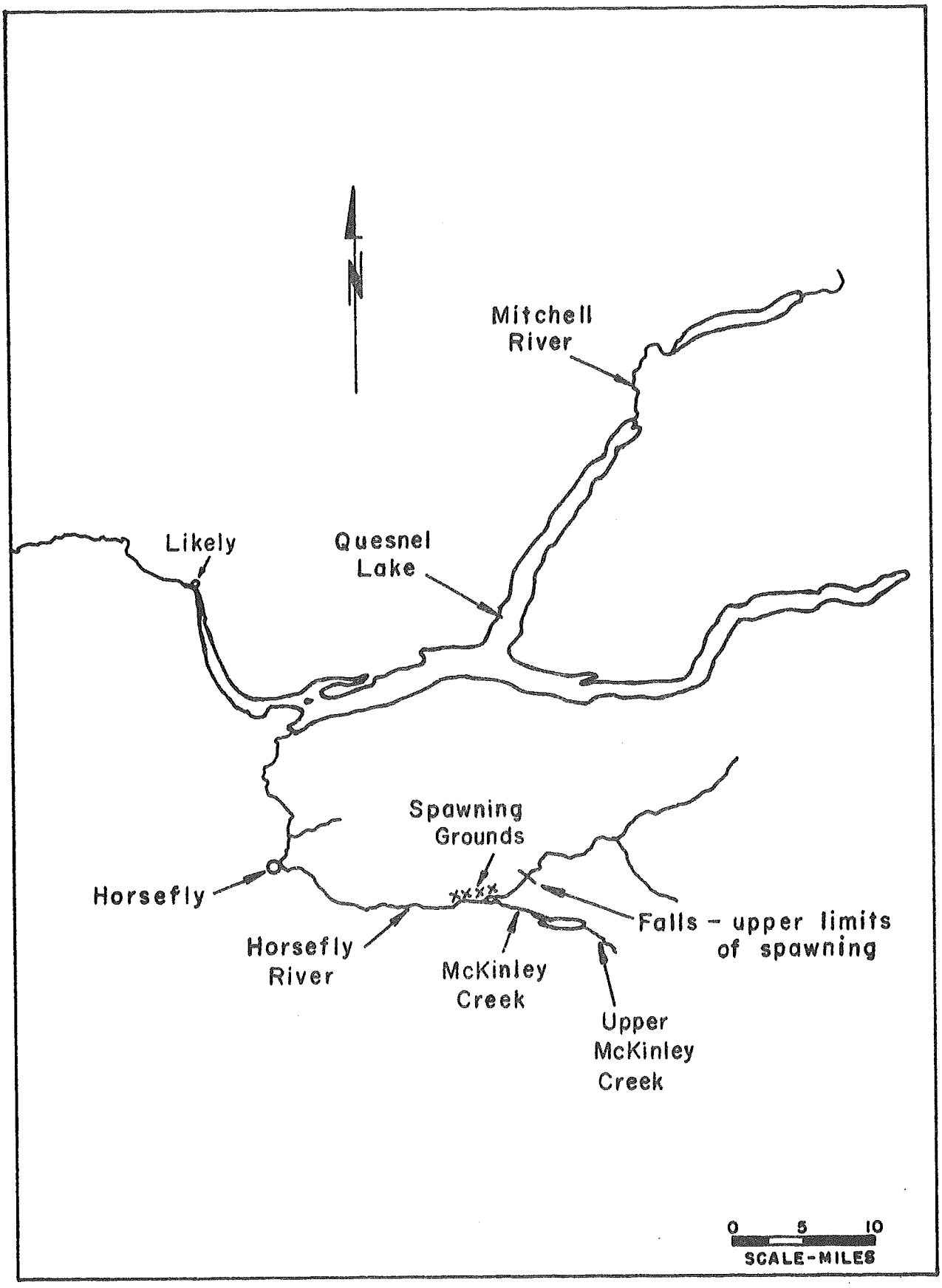
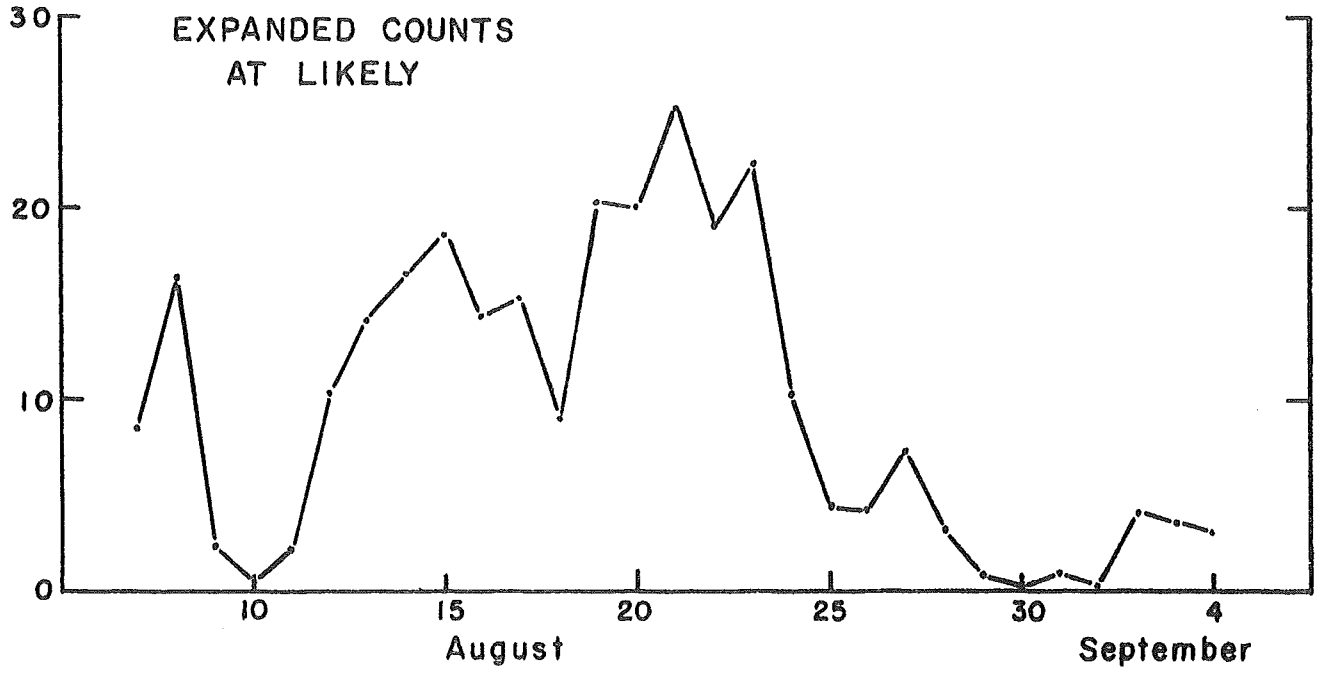
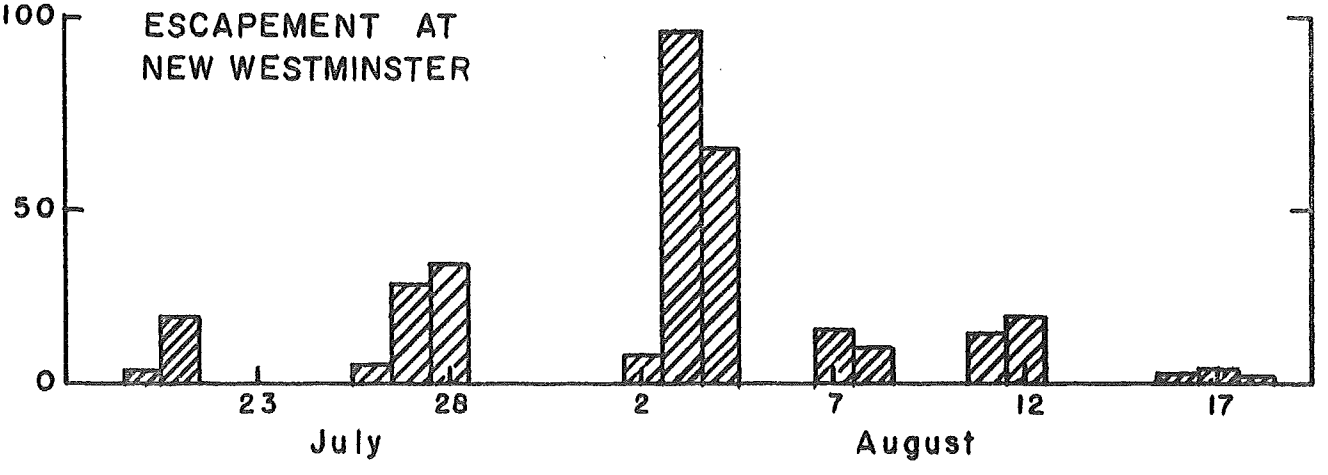


FIGURE 2 - Map showing sockeye spawning grounds of the Horsefly-Quesnel area.





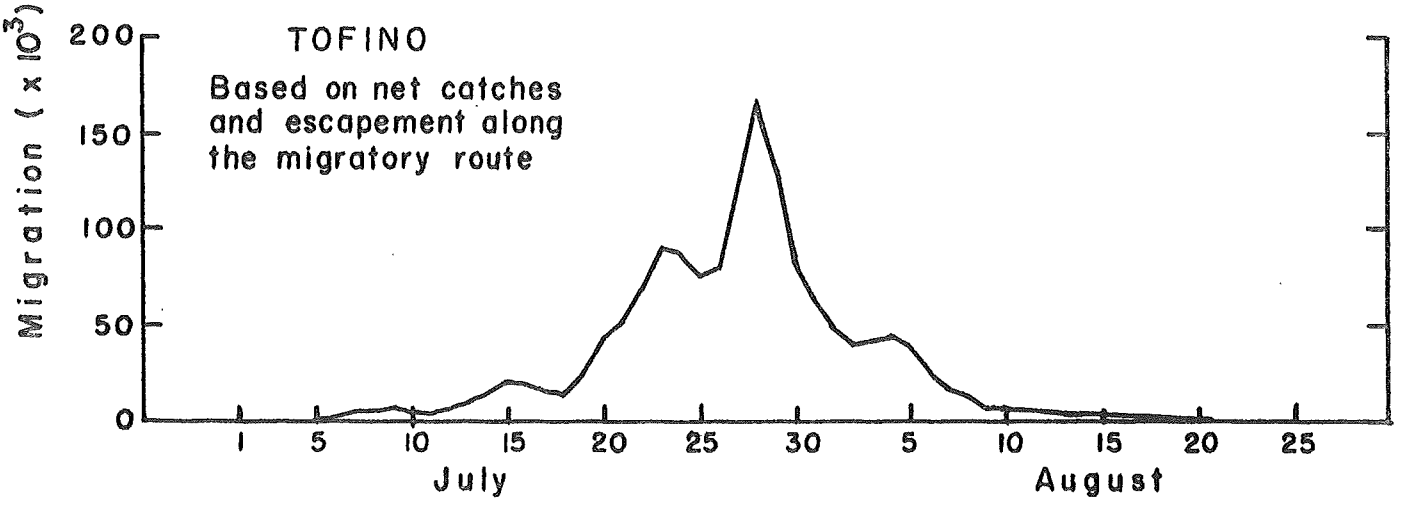


FIGURE 3 - Migration timing of the 1973 Horsefly sockeye population at Likely, New Westminster and Tofino, B.C.

The first fish arrived on the spawning grounds prior to August 14, with all of the fish on the spawning grounds by September 15. The mid-date of arrival at the spawning grounds was August 22, or 4 days later than the average for the dominant cycles 1945-1969 (TABLE 3).

TABLE 3 - Mid-date of arrival of sockeye at the Horsefly spawning grounds.

Year	Date
1945	August 15
1949	August 17
1953	August 15
1957	August 26
1961	August 17
1965	August 17
1969	August 19
Average	August 18
1973	August 22

The peak of spawning was from August 29 to September 2. Fish arriving at the spawning grounds prior to August 20 were classed as first segment, those arriving from August 20-28 were classed as central, and those arriving after August 28 were classed as late segment. There was a small but distinct group of fish which was recognizable prior to the first segment, both at New Westminster and Likely. These fish were not distinct at the Horsefly townsite and probably migrated into the Horsefly River with the first segment fish (FIGURE 4).

Water Temperatures

Water temperatures in the Horsefly River above McKinley Creek in the main spawning area were 2.3° C below average (TABLE 4), with an average daily maximum temperature during arrival of 13.3° C for early, 13.9° C for central, and 12.5° C for late fish, giving a mean daily maximum temperature of 13.1° C during arrival. The maximum water temperatures averaged 14.1° C during peak of spawning. These temperatures were cooler than in the previous dominant cycles 1953-1969.

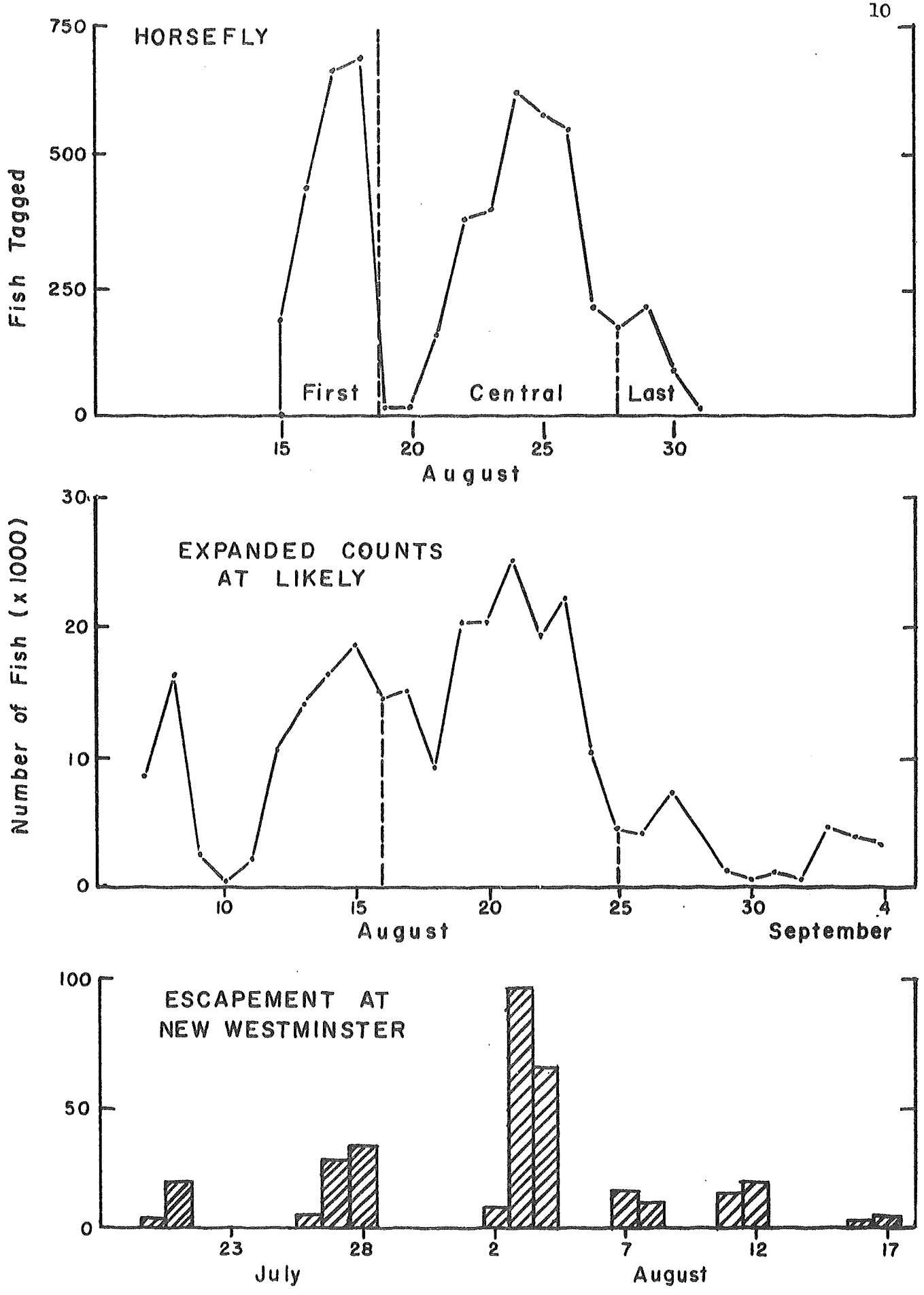


FIGURE 4 - Migration, timing and division of tagged fish in first, central and last segments of the Horsefly population.

TABLE 4 - Average daily maximum temperatures for Horsefly River above McKinley Creek during arrival of fish.

Year	Temperature °C
1953	16.2
1957	14.0
1961	17.5
1965	15.9
1969	13.5
Average	15.4
1973	13.1

Temperatures in the Fraser River at Hell's Gate during the period of migration of the 1973 Horsefly run were 0.7° C above the average for the cycle years 1949-1969 (TABLE 5).

TABLE 5 - Average daily maximum temperature of Fraser River at Hell's Gate during Horsefly sockeye migration.

Year	Temperature °C
1949	15.7
1953	16.9
1957	16.8
1961	19.3
1965	17.9
1969	17.3
Average	17.3
1973	18.0

Prespawning Mortality

The prespawning mortality was calculated as previously reported (Williams 1973). The loss of spawners in the Upper Horsefly River, based on fresh dead tagged females weighted to number of sockeye arriving at the spawning grounds, averaged 27.3% for the first segment, 21.3% for the central segment, and 11.8% for the last segment, with a mean mortality of 22.52%. Based on fresh dead untagged weighted to number arriving, the mortality was 27.7% in the first segment, 27.03% in the central fish, and 26.9% in the last segment, with an overall mean of 27.11% (TABLE 6). The first 737 fish recovered had a mortality of 43.4%. This is consistent with previous years, where the first fish had the highest mortality. These fish may have been from the early part of the first segment at Likely.

TABLE 6 - Percent unspawned sockeye based on fresh dead tagged and untagged females weighted for daily numbers of spawners arriving at the spawning grounds.

TAGGED ♀ FRESH DEAD			UNTAGGED ♀ FRESH DEAD		
Segment	Number Recovered	Weighted % Unspawned	Segment	Number Recovered	Weighted % Unspawned
First	39	27.3	First	2,360	27.7
Central	33	21.3	Central	3,366	27.3
Last	18	11.8	Late	1,854	26.9
Average		22.5			27.1

The mortalities in McKinley Creek are based on samples of untagged females only because of the scarcity of tags available. The mean percent unspawned in Lower McKinley Creek was 33.1%. Upper McKinley Creek above the lake was only sampled once and that sample indicated a mortality of only 6.7%, significantly lower than any individual sample taken on the Lower McKinley Creek or Horsefly River. The mean percent unspawned for the Mitchell River was 19.1% based on untagged females recovered on three sample dates (TABLE 7).

TABLE 7 - Percent sockeye unspawned in McKinley Creek and Mitchell River based on fresh dead untagged females.

Location	Date	No. Sampled	% Unspawned
Upper McKinley	September 9	239	6.70
McKinley	Aug 24-Sept 20	1,040	33.1
Mitchell	Sept 13-22	374	19.1

Life Span

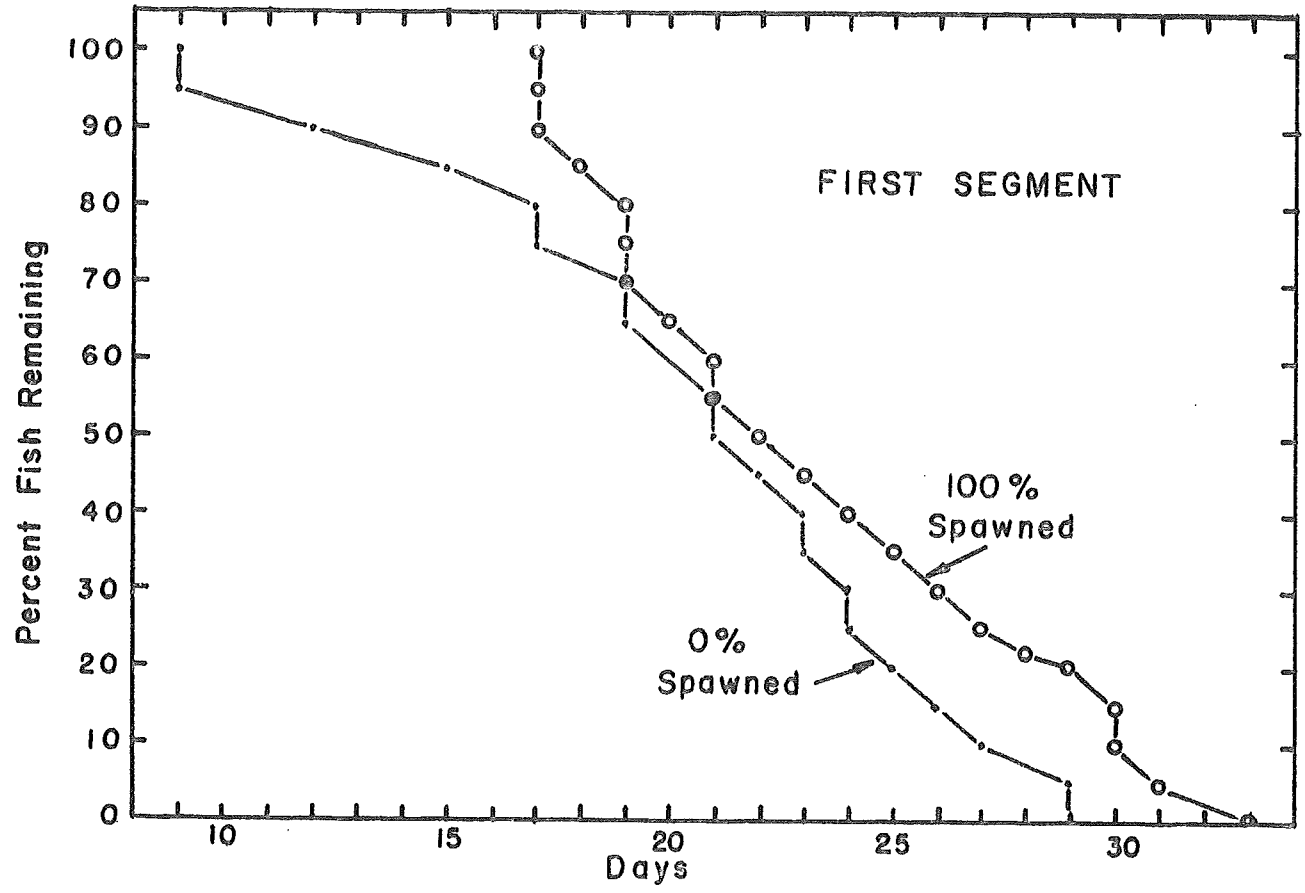
The average life span of tagged female sockeye after arrival on the spawning grounds ranged from a maximum of 32 days for a first segment fish to a minimum of 2 days for a late segment fish (TABLE 8). The life span of unspawned, partially spawned, and spawned out fish showed a different trend for each segment. The first segment fish which spawned successfully lived an average of 1.8 days longer than the unspawned fish, while the unspawned fish from the central segment lived an average of 1.1 days longer than the spawned out fish (FIGURE 5). There was a trend towards a shorter life span as the later fish moved onto the spawning grounds. The first segment fish had a mean life span of 19.3 days compared to a mean of 17.2 days for the central fish and 13.3 days for the late fish. The comparable data for the 1969 run were 17.6, 15.4, and 13.8 days (Williams 1973).

TABLE 8 - Life span of tagged female sockeye after arrival on the Horsefly spawning grounds.

Segment	0% Spawned		50% Spawned		100% Spawned		Mean Days
	Mean Days	Range	Mean Days	Range	Mean Days	Range	Total Fish
First August 15-19	17.9	9-25	19.0	13-26	19.7	13-32	19.3
Central August 20-28	18.2	9-25	16.6	12-24	17.1	9-26	17.2
Late August 29-31	12.0	-	10.8	2-17	13.8	10-20	13.3

Physical Measurements

There appeared to be no consistent differences between first and central segment Horsefly fish in either length or weight. The sample of central fish from the spawning grounds tended to be both shorter and lighter than the other groups. The dry egg weight was similar in samples taken at Tofino and Lummi, and showed a substantial increase upon arrival at the spawning grounds (FIGURE 6). There was no significant difference between the egg weight of early and central fish either in salt water or at the spawning grounds. There was a significant relation ($p = .001$) between egg size and sockeye size at Tofino and Lummi, but this relation was not significant on the spawning grounds (FIGURE 7). The fecundity of the fish sampled at Lummi Island tended to be greater than for similar sized fish at Tofino or the spawning grounds (TABLE 9).



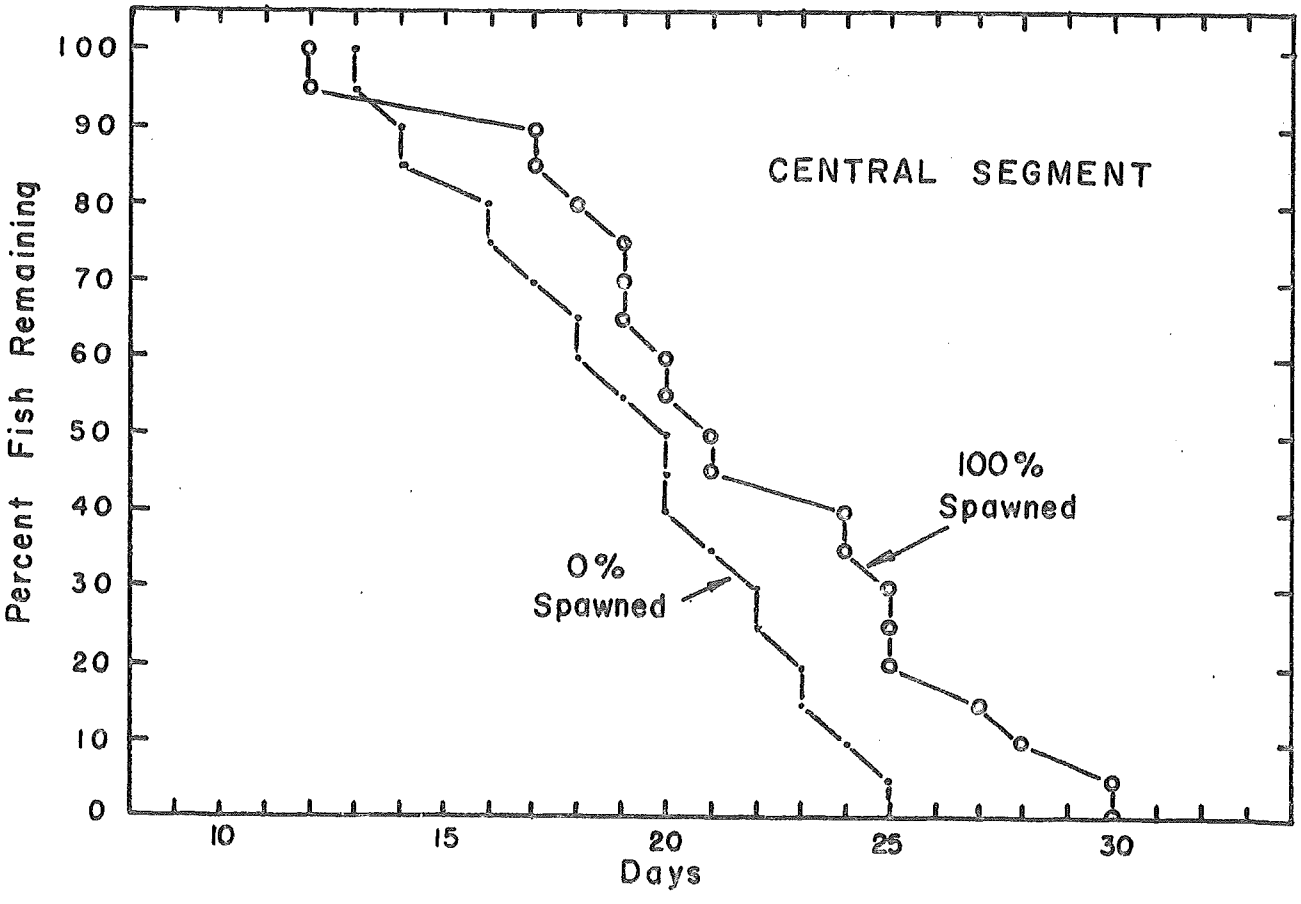


FIGURE 5 - Life span of first and central female Horsefly sockeye, spawned and unspawned.

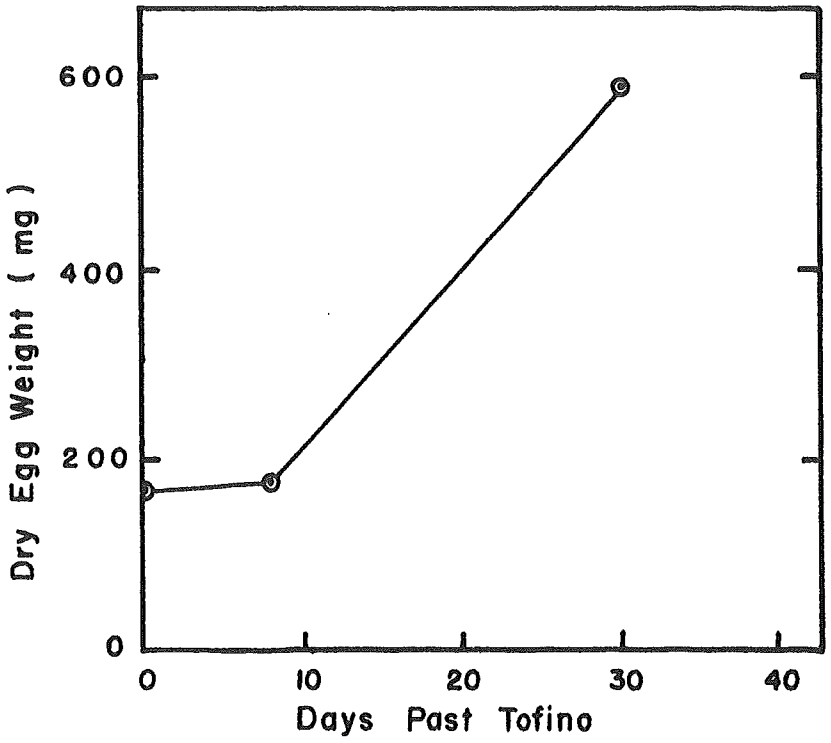


FIGURE 6 - Dry egg weight of Horsefly sockeye versus days after passing Tofino.

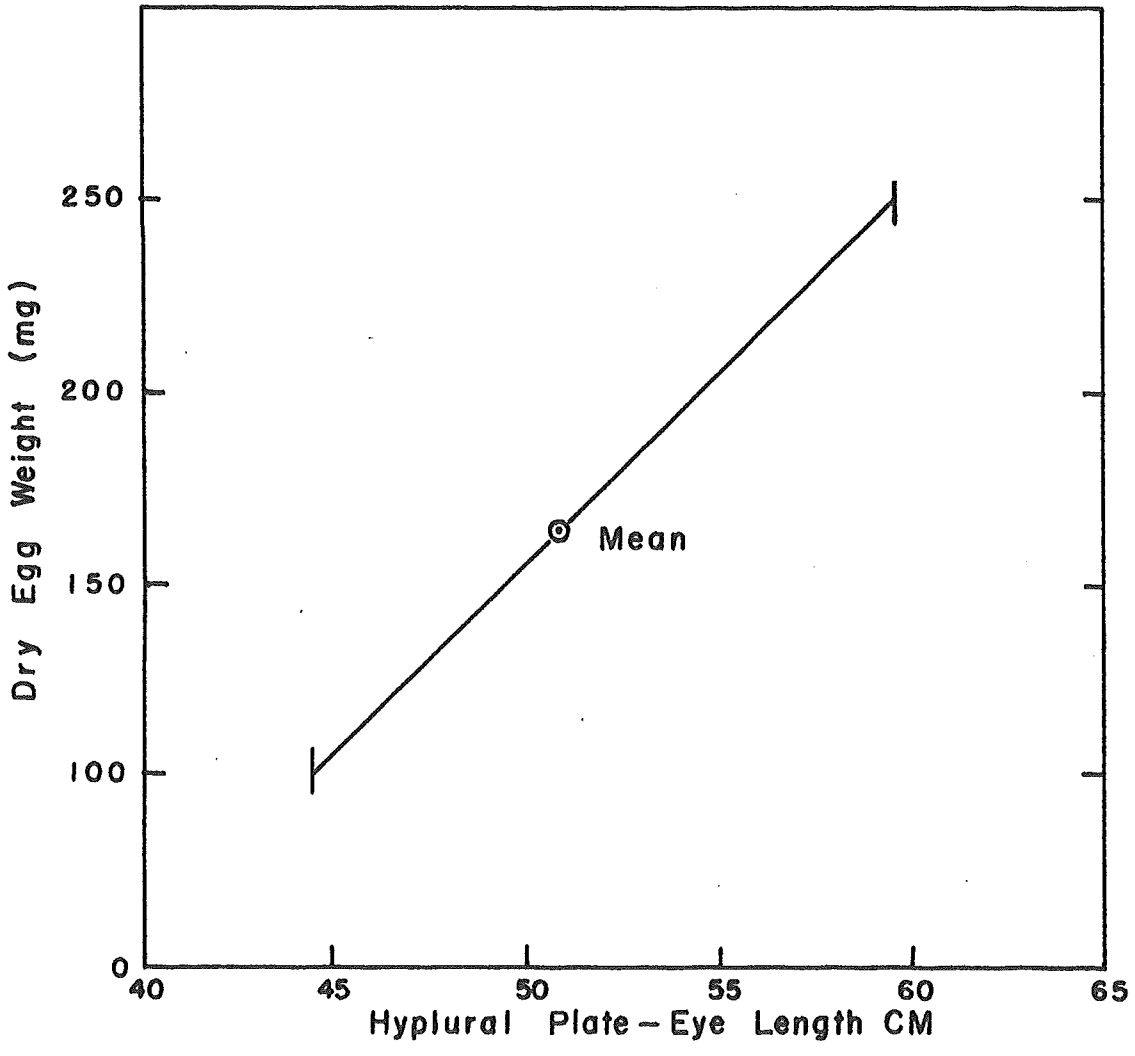


FIGURE 7 - Regression of size of female sockeye sampled at Tofino versus weight of egg.

TABLE 9 - Physical measurements of 1973 Horsefly female sockeye.

Sample Location	Date	Segment	Fork Length cm	Hypural Length cm	Total Weight gm	Body Weight gm	Fecund - ity	Egg Weight mg
Tofino	July 13	First	61.1 (.4)	51.9 (.1)	ND	ND	4,126	176 (.5)
Tofino	July 19-21	Central	60.2 (3.8)	51.3 (3.1)	ND	ND	3,377 (151)	164 (67)
Lummi	July 21	First	60.4 (2.8)	51.0 (3.3)	2,802 (390)	2,548 (332)	3,604 (319)	170 (48)
Lummi	July 27-28	Central	60.6 (.9)	50.4 (.8)	2,795 (225)	2,519 (146)	3,724 (266)	167 (52)
Lummi	August 3	Central	61.1 (.2)	51.4 (.6)	2,940 (125)	2,683 (125)	3,870 (402)	221 (16)
Horsefly	August 15	First	60.1 (2.09)	51.1 (1.88)	2,445 (251)	2,085 (232)	3,435 (341)	580 (70)
Horsefly	August 28	Central	58.5 (2.36)	49.8 (1.97)	2,209 (318)	1,835 (249)	3,194 (779)	493 (69)

( ) Standard Deviation

Body Constituents

The body constituents for the Horsefly sockeye did not differ significantly between segments of the run at the same location. The first group at Tofino had a lower percent lipid than the central group, however only 2 fish were sampled. Percent water content increased from the salt water to arrival on the spawning grounds similarly to increases reported previously for the 1971 Chilko fish (Williams 1977) (TABLE 10).

The percent lipids in general decreased in a linear trend with time. The mean of both first and central fish combined was 9.15% at Tofino, 6.41% at Lummi, and 2.68% upon arrival at the spawning grounds (FIGURE 8). These values are similar to data reported for the 1971 Chilko fish (Williams 1977).

TABLE 10 - Lipid and water content of Horsefly female sockeye.

Sample Location	Sample Date	Segment	No.	Percent Lipid	Percent Water	Percent Constit -uents Remaining (protein primarily)
Tofino	July 13	First	2	6.62 (.07)	70.5 (.4)	23.42 (.42)
Tofino	July 19-21	Central	11	9.62 (2.5)	68.4 (2.2)	22.12 (1.42)
Lummi	July 21	First	12	6.22 (.86)	69.4 (1.53)	24.31 (1.49)
Lummi	July 27-28	Central	14	6.88 (1.1)	68.7 (2.5)	24.47 (2.01)
Lummi	August 3	Central	3	5.02 (.11)	70.8 (.95)	24.18 (0.85)
Horsefly	August 15	First	18	2.93 (.78)	76.4 (1.06)	20.64 (.86)
Horsefly	August 28	Central	23	2.49 (.71)	78.4 (1.00)	19.09 (1.14)

( ) Standard Deviation

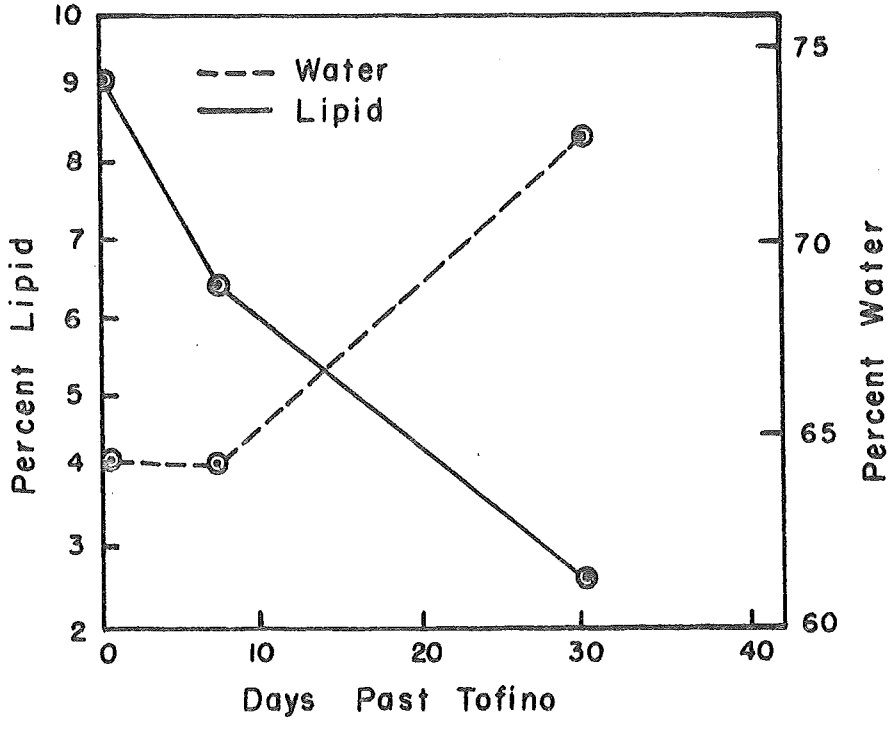


FIGURE 8 - Relationship of percent lipid and percent water during maturation of Horsefly sockeye.

DIAGNOSTIC ENZYMOLOGY

The activity of enzymes released into the bloodstream by damage to specific tissues is being used increasingly for clinical diagnosis in humans (Goodley 1970). In teleost fish the techniques have not been used to any great extent. It has been reported that juvenile rainbow trout injected with infectious hematopoietic necrosis virus showed an increase in specific lactate dehydrogenase (LDH) activity early in the infection, while no change was observed in fish infected with infectious pancreatic necrosis virus, Vibrio anguillarum, Aeromonas salmonicida, and redmouth bacterium (Amend 1974). Serum glutamic-oxalacetic aminotransferase (SGOT) activity increased in sockeye salmon with bacterial kidney disease or following injection of the hepatic poisons, bromobenzene and carbon tetrachloride (Bell 1968). Therefore changes in the activities of enzymes during the spawning migration of sockeye salmon to the Horsefly River were examined with a view towards developing a method for diagnosing specific tissue damage in salmon.

Of 30 random plasma samples analyzed for ATP-ase, including 8 from the spawning ground, only trace activities were observed. No significant difference was found in samples from different locations.

Twenty-five plasma samples including 12 from the Horsefly River were analyzed for SGPT. Only trace activity was observed with no obvious differences among samples from the various locations.

The activity of carbonic anhydrase showed no difference in 6 plasma samples from Tofino and 6 from the Horsefly River and the analysis in blood was not continued. The gill tissue showed a 12% increase from first segment to last segment fish sampled at Horsefly, and an 18% increase from the first segment to last segment fish sampled at Tofino. Since the change in activities was not large, no further attempt was made to relate these changes at Tofino to authentic Horsefly sockeye.

The results of lactic dehydrogenase activity in the plasma of fish are shown in TABLE 11. For samples from Tofino and Lummi Island, only those definitely established as belonging to the Horsefly stock were included. In the first and last segment samples, the plasma LDH was lower at Lummi and higher at Horsefly River than at Tofino. The LDH activity in the gill tissue likewise increased between Tofino and Horsefly River, both in the first and last segments (TABLE 12).

TABLE 11 - Average LDH activity (units/ml of plasma), Horsefly sockeye.

Segment	Tofino	Lummi	Horsefly River
First	-	1.01 (16)	-
First	1.19 (4)	0.59 (23)	1.26 (51)
Last	1.18 (16)	0.77 (12)	1.52 (39)

( ) Number of fish sampled.

TABLE 12 - Average LDH activity (units/g) of gill tissue, Horsefly sockeye.

Segment	Tofino	Lummi	Horsefly River
First	12.56 (5)	-	15.94 (5)
Last	9.02 (5)	-	17.40 (5)

( ) Number of fish sampled.

The comparative SGOT activity of the plasma from samples of authentic Horsefly River populations at different points along the spawning migration path is shown in TABLE 13. In both the first and last segments, the activity decreased slightly at Lummi and increased dramatically in fish captured at the spawning ground at Horsefly River. It is noted that the activity of fish from the last segment is a little higher than fish from the first segment at all locations.

TABLE 13 - Average SGOT activity (O.D. units/ml plasma).

Segment	Tofino	Lummi	Horsefly River
First	-	147.8 (16)	-
First	177.8 (4)	157.5 (23)	626.3 (52)
Last	196.2 (17)	176.5 (12)	733.4 (40)

( ) Number of fish sampled.

Compared to changes in the plasma, the gill tissue shows very little change from Tofino to Horsefly River, indicating no obvious damage in this tissue (TABLE 14).

TABLE 14 - Average GOT activity (O.D. units/g gill tissue).

Segment	Tofino	Lummi	Horsefly River
First	6,850 (10)	-	6,870 (10)
Last	8,203 (10)	-	7,522 (8)

( ) Number of fish sampled.

HISTOLOGY

The structural alterations that occur in the various tissues and organs of the sexually maturing sockeye salmon have been documented in great detail (Colgrove 1966; McBride & van Overbeeke 1969; Robertson & Wexler 1959, 1960). To avoid undue repetition of published material, the results of this study have been summarized very briefly in TABLES 15 and 16. Also, in order that any deviation from the recorded pattern can be readily identified, a separate column listing the typical pathological changes associated with sexual maturation in the sockeye salmon has been included with each table.

Comparison of the pathology of Horsefly sockeye from the three segments of the population brings out the following principal points.

1. At Lummi Island the fish from the central portion of the run show a higher adrenal activity index than those from the first segment (TABLE 15),
2. Distinct deviations in structure between different segments of the race at either Tofino or Lummi Island were not noted in any of the other tissues examined (TABLE 16),
3. The histological structure of all of the tissues examined at these two collection points is in agreement with that recorded previously for sockeye salmon,
4. The infestation of the gill region by non-bacterial foreign matter was recorded as extensive in the first segment fish on the spawning area, but moderate to light in the sockeye from the central segment,
5. The prevalence of bacteria in the gill area was more marked in the fish from the central portion than in the first fish upon arrival at the spawning grounds (TABLE 16).

TABLE 15 - Histophysiological state of the adrenal in Horsefly sockeye sampled at different points along the migration route.

Sample Collection Site	Segment of Race	Frequency Distribution of Adrenal Activity Index in Individual Fish[a]					Activity Index for Pacific Salmon[b]
		1	2	3	4	5	
West Coast Vancouver Island (Tofino)	First	3					1
	Central	15	1				
Lummi Island	First	18	1				1 or 2
	Central		44				
Spawning Ground	First				1	27	5
	Central					34	

[a] For outline describing activity index, see text under heading Sampling.

[b] Estimated values based on fish of comparable stage of sexual maturity. Data for different segments of run not available (Robertson and Wexler 1959, 1961; McBride and van Overbeeke 1969).

The gill structure of the fish collected upon arrival at the spawning grounds reflected the pathology recorded for this tissue in maturing Pacific salmon in general. In the first fish the changes were relatively minor, and for the most part involved a visible but not marked hyperplasia of the epithelium. Occasional swelling of the individual lamella was noted, but hemorrhages were rarely recorded. The alterations in the central segment of the run were more conspicuous. Hyperplasia and hypertrophy of the lamella epithelium was pronounced. Fusion of the lamella was not uncommon, as were hemorrhages, which often appeared to involve a considerable portion of the filaments. Examination of these specimen for the presence of bacteria revealed small numbers in a few of the early fish but dense concentrations in a majority of the salmon from the central portion of the migration. The gram negative rods were located not in the tissue per se, but adhering along the periphery of the lamella epithelium (FIGURE 9). Bacteria were not observed in any of the gill specimens collected at either Tofino or Lummi Island, nor in any of the other tissues examined.

TABLE 16 - Comparison of histopathological changes in the different segments of the Horsefly sockeye race and maturing sockeye salmon.

Organ	HORSEFLY SOCKEYE Sample Site	Segment of Run	No. of Fish	Histopathology	PACIFIC SALMON Histopathology[a]
Skin, Liver Kidney, Stomach Pituitary, Gill, Testis	Vancouver Is. (Rogino)	Segments combined[b]	19	Sea green condition	Sea green condition
				Early Spermatogenesis	Early Spermatogenesis
Liver, Kidney Stomach, Gill Skin	Lummi Is.	Segments combined[b]	63	Sea green condition Slight Hyperplasia	Sea green condition Slight Hyperplasia
Testis				Early Spermatogenesis	Early Spermatogenesis
Pituitary				Increase in numbers of Basophils	Increase in numbers of Basophils
Skin	Spawning Ground	First	28	Hyperplasia and Hypertrophy	Hyperplasia and Hypertrophy
Liver				Degeneration	Degeneration
Kidney				Degeneration	Degeneration
Stomach				Atrophy and Degeneration	Atrophy and Degeneration
Testis				Advanced Spermatogenesis, a few ripe	Advanced Spermatogenesis, a few ripe
Pituitary				Large numbers of Basophils, degeneration	Large numbers of Basophils, degeneration
Gill				Degeneration, few bacteria but heavy infestation of foreign bodies.	Degneration, bacterial or fungus infections common
Skin	Spawning Ground	Central	35	Hyperplasia and Hypertrophy	Hyperplasia and Hypertrophy
Liver				Degeneration	Degeneration
Kidney				Degeneration	Degeneration
Stomach				Degeneration	Degeneration
Testis				Advanced Spermatogenesis, majority ripe	Advanced Spermatogenesis, or ripe
Pituitary				Degeneration, large numbers of Basophils	Degeneration, large numbers of Basophils
Gill				Degeneration abundant, numbers of foreign bodies few	Degeneration, bacterial or fungal infections common

[a] Based on data for Pacific salmon in a comparable stage of maturity (Robertson and Wexler 1960, McBride and van Overbeeke 1969, 1971).

[b] As differences between the segments were not noted, the results have been pooled.

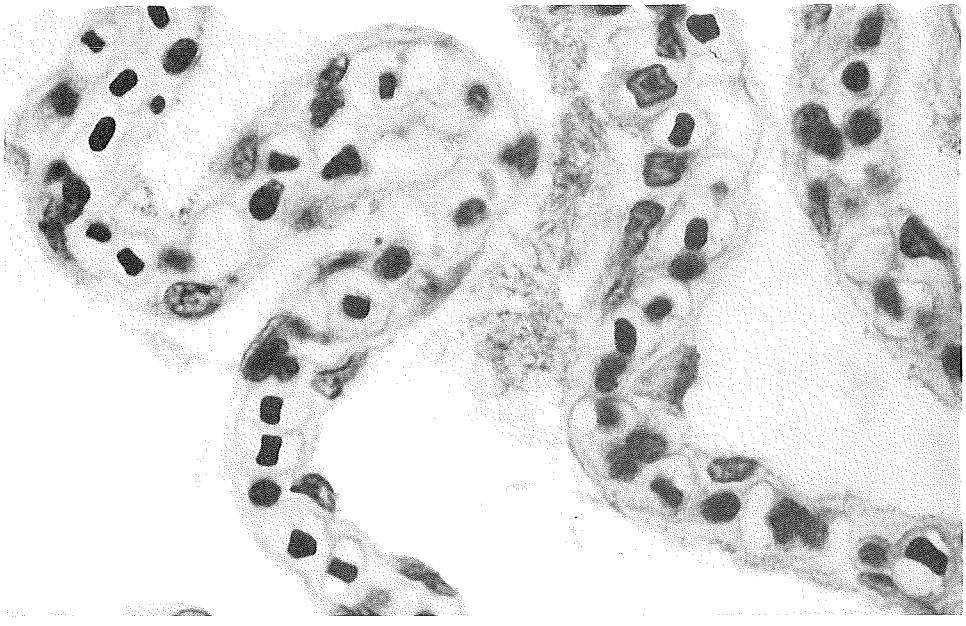


FIGURE 9 - Gram-negative bacteria adhering to gill lamella of Horsefly sockeye.

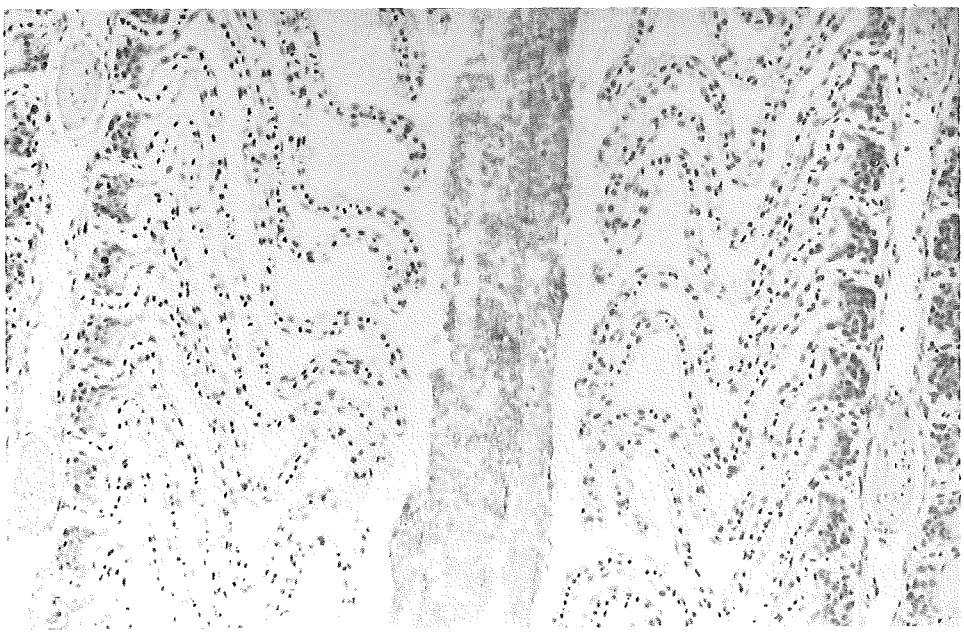


FIGURE 10 - Unidentified mass lying adjacent to gill lamellae of Horsefly sockeye.

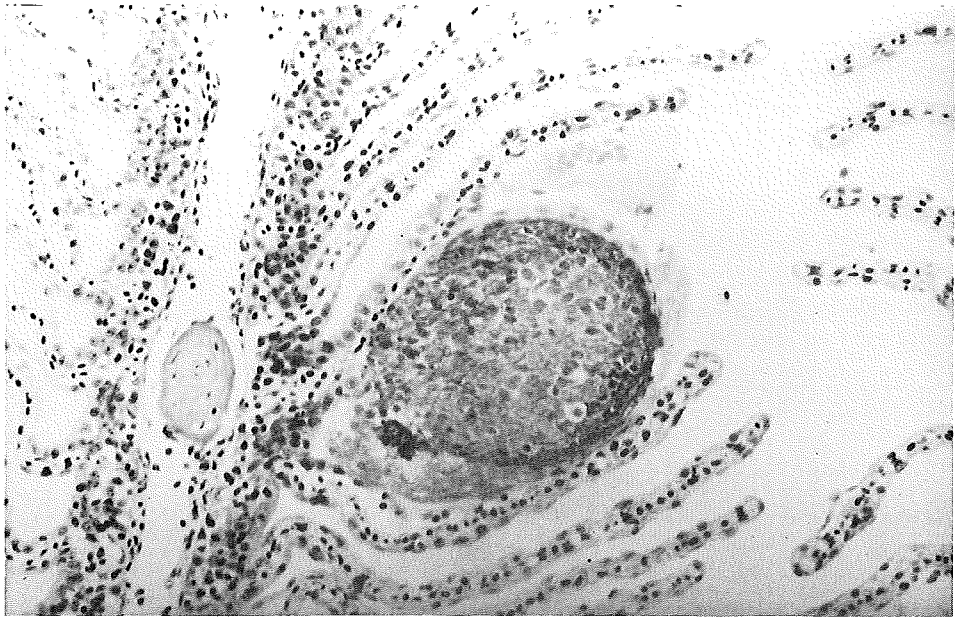


FIGURE 11 - Unidentified material embedded in gill tissue of Horsefly sockeye.

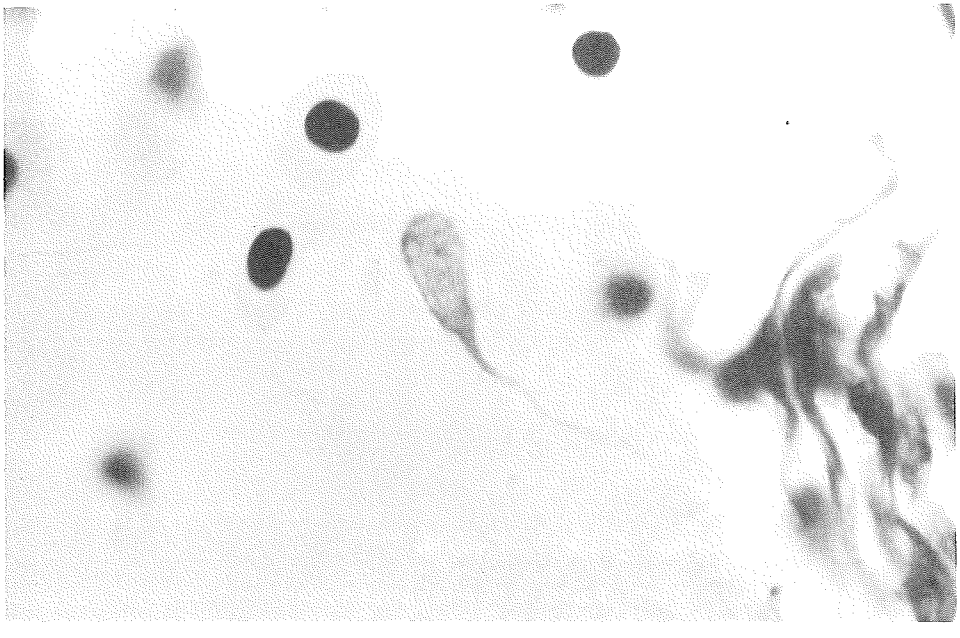


FIGURE 12 - Individual protozoa found in association with unidentified mass.

The first conspicuous deviation between the different segments of the run concerned the relative abundance of non-bacterial foreign matter in the gills of the fish sampled upon arrival at the spawning grounds. While the infestation was recorded in every fish examined from both the first and central segments of the run, it was visibly more extensive in the first fish. As shown in FIGURE 10, the material was found in scattered masses between, or closely adjacent to, the lamella. Instances of the material being embedded in the tissue itself were rarely noted (FIGURE 11). The foreign mass consisted of large numbers of uniformly circular or oval bodies, 10 to 15 μ in diameter, which lacked any discernible internal structure. The material was relatively refractory to dyes. With the various staining procedures used in this study, the foreign structures showed no clear differentiation, rather the bodies displayed a uniform brownish-gray coloration.

Initially the mass was thought to be either sloughed tissue or decaying blood clots. The apparent absence, however, of any internal cellular detail makes such an identification untenable, particularly as globose masses of blood cells trapped between the lamella, as well as detached pieces of epithelium, were noted and readily identified on the basis of structure. The presence of a few discrete protozoa (FIGURE 12) in or close to these masses, suggested the possibility that the material might be aggregates of spores.

Another possibility is that the foreign mass consists of algae. Certainly the shape of the foreign bodies, their size and presence in such large numbers, is consistent with what one would expect from an algae infestation.

BACTERIOLOGY-SEROLOGY

One method of determining if a host is infected by, or has come in contact with, an infectious agent is to examine the serum of the host for the presence of agglutinating antibody active against the suspected agent. Although the method is only presumptive, it does offer both rapidity and wide applicability when large numbers of samples must be screened.

Apart from a small number of fish at Horsefly River which showed lesions on their gills, all the fish examined at the three locations along the migration route appeared to be in remarkably good condition. Neither external nor internal lesions were observed on fish either on or below the spawning grounds, including those fish observed to be in a moribund state.

Preliminary screening of sera samples for the presence of agglutinating antibody was performed using the ten isolates recovered from the sockeye kidneys. Of those isolates tested, only two gave a positive agglutinating response, both of which were isolated from the kidneys of moribund females. Formalized cell suspensions of each were prepared and all sera samples obtained at each location checked for agglutinating antibody activity against the two isolates. These results are tabulated in TABLE 17.

TABLE 17 - Percent of sera samples from first and central segments showing agglutinating antibody activity against kidney isolates.

Location	Stage of Run	Number Tested	Number Positive	Percent Positive
Tofino	First	4	0	-
	Central	25	0	-
Lummi	First	23	1	4
	Central	18	0	-
Horsefly River	First	34	20	59
	Central	30	3	10

Of the twenty sera samples from the first segment of the Horsefly run showing a positive response, 12 were described as strongly positive and 8 as "probable". The single positive serum sample obtained from a first fish at Horsefly River was also described as "probable". In view of the broad specificity inherent in the slide agglutination technique, the single positive serum obtained from the fish at Lummi Island can probably be discounted as a "false" positive.

The data clearly indicate that the first segment of migrating Horsefly salmon were subjected to a heavy incidence of infection by the isolated organism(s) and that infection occurred sometime after these fish left the sampling site near Lummi Island.

To define more precisely the nature of the suspected etiological agent, the two isolates were subjected to a series of biochemical tests. The presumptive identification scheme described by Bullock (1961) was employed for this purpose. Both isolates reacted identically to the tests selected.

Morphologically, young cells (16 h) were short, Gram-negative, non acid-fast, motile (monotrichous) rods. Both acid and gas were produced in glucose media and H_2S was formed in triple-sugar iron agar. Positive tests for cytochrome oxidase activity, starch hydrolysis and 2 C, 3-butanediol production were also obtained. Strong beta-hemalysis was evident on blood agar medium. On the basis of the above tests, the organism was tentatively identified as Aeromonas liquefaciens. The isolate was shown to be sensitive to erythromycin (15 mcg), terramycin (5 mcg), furodantin/macrodantin (199 mcg), and sulfamerazine (1 mg); moderately sensitive to streptomycin (100 mcg), elkosin (1 mg) and kanamycin (30 mcg); and resistant to the cephalothin (30 mcg), methacillin (5 mcg), penicillin (10 units) and ampicillin (10 mcg).

Bacteriological sampling of the water from the lower end of the main spawning grounds indicated that 70 cells of Flexibacter columnaris per ml were present in the Horsefly River during peak of spawning. This is much lower than 1965 when an average of 150 cells per ml were reported (Colgrove and Wood 1966). Even though an appreciable number of fish must have been shedding F. columnaris cells, examinations of the fish using phase-contrast microscopy as well as cultures of gill tissue, indicated no appreciable damage to the gills due to F. columnaris.

Although F. columnaris was relatively scarce on the gill tissue of the 1973 Horsefly sockeye, there was a heavy infestation of large fusiform shaped bacteria (FIGURE 13) present on each moribund unspawned fish examined, while the spawning fish had degrees of infestation ranging from heavy to very light (FIGURE 14). These bacteria resemble those reported to have been associated with high mortalities of sockeye, pink and coho reared in fresh water at the Rosewall Creek hatchery, Vancouver Island, British Columbia (Hoskins 1976). Attempts to culture the organism were unsuccessful, therefore identification and pathogenicity of this organism could not be established.

The average hematocrits (% Packed Cell Values PCV) appeared to increase from Tofino to Lummi Island for both the Horsefly and Stuart fish. The first segment Horsefly females had an average % PCV of 41.8 while the central segment had a % PCV of 38.8. Females from both the first and central segments of the Stuart run had average % PCV of 43.5. The PCV values determined from samples taken at Lummi Island indicated an increase of about 7% PCV for Stuart fish and up to 14.8% PCV for the central and 17.7% for the first segment Horsefly fish. Samples taken from Horsefly fish on the spawning grounds indicated a drop to % PCV levels similar to those recorded at Tofino (TABLE 18).

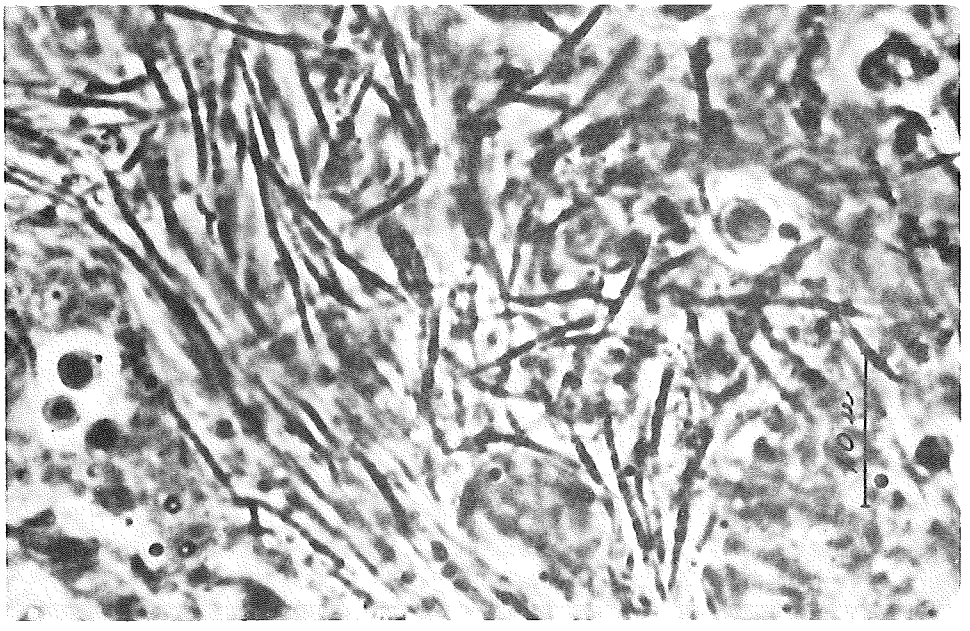


FIGURE 13 - Heavy concentration of fusiform shaped bacteria found in gills of moribund Horsefly sockeye.

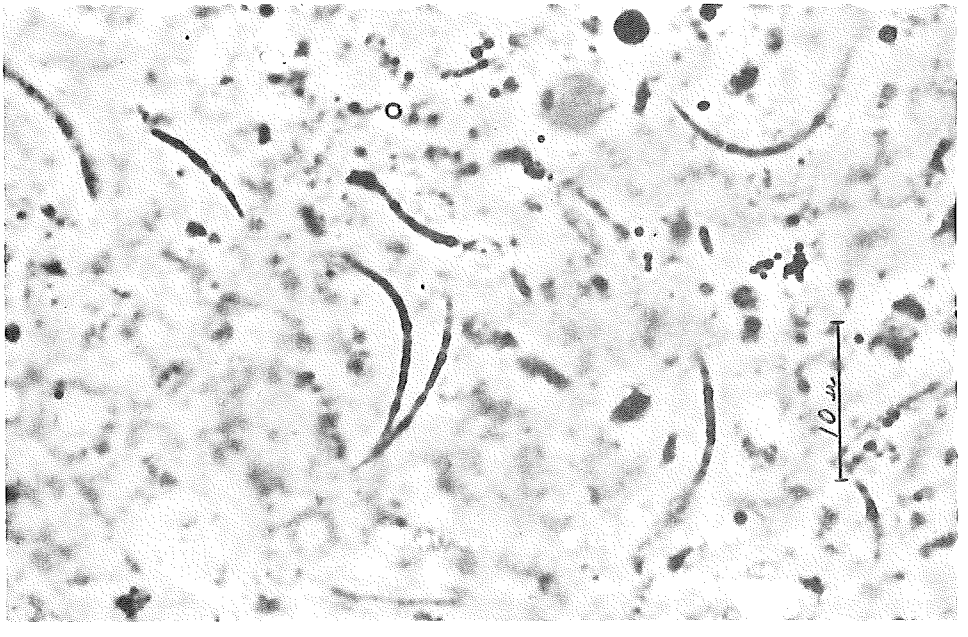


FIGURE 14 - Light concentration of fusiform shaped bacteria found in gills of Horsefly sockeye.

TABLE 18 - Hematocrits (% Packed Cell Value) of blood samples taken from Horsefly and Stuart female sockeye.

Location	Segment	Horsefly ♀ % PCV	Horsefly ♀ S.D.	Stuart ♀ % PCV	Stuart ♀ S.D.
Tofino	First	41.8	8.8	43.5	1.3
	Central	38.8	4.1	43.5	4.7
Lummi	First	59.5	4.2	51.0	7.4
	Central	53.6	5.1	50.2	4.8
Spawning Grounds	First	40.8	4.3	N.A.	
	Central	37.6	4.6	N.A.	

The mean cortisol concentration for all first segment Horsefly females was 14.6 $\pm$ 11.3 (S.D.) and for all central segment females, 8.4 $\pm$ 9.9 (TABLE 19). When cortisol values were grouped according to location, mean cortisol concentrations in first segment fish were higher than in central fish at all three locations (TABLE 20). An analysis of variance of means of all six groups (both central Lummi groups combined) showed that there was a highly significant difference (Prob. = 0.00051) between the first segment and the central segment fish. The difference between locations was significant (Prob. = 0.012), but the interaction between segment and location was not significant (Prob. = 0.069).

TABLE 19 - Mean and standard deviation of cortisol concentration in all first segment migrants compared with all central segment migrants of the Horsefly run (3 locations) and Stuart Lake run (2 locations).

Segment of Run		Horsefly ♀	Stuart L. ♀
Early	Mean	14.6	5.6
	S.D.	11.3	3.7
	N.	26	7
Central	Mean	8.4	8.7
	S.D.	9.9	10.7
	N.	49	28

TABLE 20 - Mean and standard deviation of plasma cortisol concentrations of groups of sockeye salmon of the Horsefly and Stuart Lake runs. (Number of fish in brackets.)

Location	Date	Segment of Run	Horsefly ♀	Stuart L. ♀
Tofino	July 13	First	34.7 ± 2.0 (2)	5.7 ± 5.7 (3)
Tofino	July 19-21	Central	10.6 + 16.6 (11)	11.3 + 12.3 (11)
Lummi Is.	July 20	First	14.8 + 16.5 (7)	5.5 + 2.4 (4)
Lummi Is.	July 27	Central	9.8 + 12.0 (11)	6.9 + 7.8 (11)
Lummi Is.	Aug. 3	Central	1.8 + 2.6 (3)	7.3 + 12.8 (6)
Horsefly R.		First	12.2 + 6.4 (17)	- -
Horsefly R.		Central	7.6 + 3.4 (24)	- -

Three female early migrants of the Stuart Lake run were caught at Tofino. Contrary to fish of the Horsefly run, the mean cortisol concentration of these fish (5.7 ± 5.7 ug/100 ml) was considerably lower than that of central fish (11.3 ± 12.3 ug/100 ml) (TABLE 20). At Lummi Island the mean of first fish was not appreciably different from that of central fish.

An analysis of variance of all 5 groups of the females of the Stuart Lake run revealed no significant difference between groups or between locations.

DISCUSSION

This program was designed to compare the first and central segments of the population based on the premise that in most years the first fish suffer a significantly larger prespawning mortality than the central or last fish. The last fish were not examined due to the difficulty of obtaining samples. The 1973 prespawning mortality of the first segment Horsefly sockeye was virtually identical to the mortality of the central fish (TABLE 6). In addition, the last segment of the untagged female sample indicated a mortality very similar to the first and central segment. The tagged females indicated a lower mortality, however there were only 18 late fish recovered with tags, whereas the untagged sample was based on 1,854 fish. In either case, the 1973 populations within the Quesnel Lake system

had the lowest prespawning mortality since 1957 (TABLE 21), with 27.1% (based on untagged fish) of the fish in the Horsefly River unspawned while the average for the last six cycles was 40.7%. Therefore it was not surprising that few differences were detected between the first segment and central segment fish.

TABLE 21 - Average percent unspawned females based on recovery of the untagged females from Horsefly River, McKinley Creek and Mitchell River, 1949-1973.

Year	Horsefly	McKinley Creek	Mitchell
1949	12.1	N.A.	0
1953	29.6	N.A.	23.4
1957	5.0[e]	5.0[e]	5.0[e]
1961	62.2	65.0	60.0
1965	46.8	47.0	41.9
1969	48.7	66.2	50.3
Average 1957-1969	40.7	45.8	39.3
1973	27.1	25.64	19.1

[e] Estimate.

The SGOT data indicated a slight increase among the last segment Horsefly sockeye, but a substantial increase was recorded between salt water and arrival on the spawning grounds (TABLE 13). This large increase could well reflect a basic change in the body reserves utilized for energy production during spawning migration. As sockeye salmon do not feed during spawning migration, energy for swimming is drawn from reserves of fat, carbohydrates, and muscle proteins (Bilinski 1974). Muscle proteins become the dominant source of energy during the later stages of spawning migration. As glutamic oxalacetic aminotransferase is centrally involved in the production of energy from amino acids, it is not surprising that its activity increases during the later stages of migration. However, the increase could also reflect escapement of the enzyme into the bloodstream as a result of tissue damage through disease or other forms of destruction. In any case, the magnitude of the change would indicate that this enzyme may have some promise as a diagnostic tool.

The only difference between segments of the run that could be detected in the histopathological examinations related to the presence of large masses of non-cellular material among the first segment upon arrival at the spawning grounds. These masses were much more infrequent in the gills of the central fish. The possible involvement of this material in the prespawning mortality is uncertain, particularly since the material was not detected in previous investigations of Horsefly spawners when even larger mortality occurred.

In the previous study of the Chilko sockeye, the gills of a majority of the first segment migrants examined at Lummi Island showed a detachment of the lamella epithelial layer. Furthermore, the blood cortisol concentration of the females indicated that they were in a state of stress. Clearly, the results in the present investigation do not corroborate the earlier findings in regard to the gill tissue. While one might attribute the derangement of the delicate gill lamella epithelium to the rather vigorous effects of paraffin embedding, a response that is commonly noted with this tissue, this would not account for the increase in cortisol levels.

The role of the adrenal in the sexual maturation of sockeye salmon is a complex one. Recent studies have demonstrated that this gland is responsive to external stressors, and also may be directly affected by the hormones involved with sexual maturation. Hane et al. (1966) suggested that the hyperadrenocorticism noted in ripening salmon is a direct response to the gonadal hormones rather than an effect of the metabolic demands related to gonad development. These investigators demonstrated a progressive lessening in the response of the adrenocortical tissue to external stressors with increasing sexual maturity. In the sexually ripe salmon, little if any additional output of the cortical cells was noted. In studies with gonadectomized sockeye salmon, as reported by Fagerlund and Donaldson (1969) and van Overbeeke and McBride (1971), both androgenically and estrogenically treated fish displayed characteristics of adrenal hyperfunction. In the present study the histological structure of the adrenal closely parallels that recorded for sockeye salmon in general (TABLE 16).

Another interesting difference between segments of the 1973 Horsefly run was the results of the serological study which indicated that fish from the first segment of the run were subjected to a heavy incidence of infection by A. liquefaciens after these fish left the Lummi Island sampling site. A. liquefaciens is a recognized fresh water fish pathogen causing hemorrhagic septicemia in fish (Bullock and Conroy 1971). The disease is known to take many forms ranging from an acute and rapidly fatal septicemia with few obvious gross symptoms, to a more chronic form with development of blisters, abscesses, etc. Bullock (1961) describes

a latent form in which bacteria may be isolated from internal organs, blood and peritoneum, but without visible external or internal signs of the disease. Under hatchery conditions, removal of only a few scales, crowding or rough handling can all contribute to infection.

The hematocrits of both the Horsefly and Stuart females indicated an increased PCV at Lummi Island when compared to samples taken at Tofino and on the spawning grounds. The Horsefly fish showed a much higher increase than the Stuart fish, especially among the first segment fish, with an average percent PCV of 59.5 compared to 41.8 at Tofino and 40.8 on the spawning grounds.

The decrease in the percent PCV of samples taken on the spawning grounds compared to samples taken at Lummi Island is similar to data collected on the 1971 Chilko population. Samples from fish on the Chilko spawning grounds indicated a decrease of 6.6% PCV for the central and 10.6% PCV for the first segment Chilko fish when compared to samples obtained at Lummi Island (Williams 1977). However, the first segment 1973 Horsefly fish did have a much higher percent PCV than either the first segment 1971 Chilko or the 1973 Stuart fish samples from the first segment.

The cortisol data indicated that there were significant differences between the first segment and central segment Horsefly female sockeye. In the comparison of female sockeye from the Horsefly and Stuart Lake runs, the female of the Horsefly run are distinct in that the first fish had plasma cortisol concentrations which were significantly higher than those of central fish.

Plasma cortisol concentrations proportional to the severity of handling stresses have been demonstrated in Pacific salmon (Fagerlund 1967). Therefore, it may be assumed that the increased plasma cortisol concentrations in the first Horsefly migrants are an indication that these fish were subjected to an increase in stress. Whether this stress is caused by factors in the environment or whether the stress is caused by endogenous factors could not be determined by this investigation. The indicated increase in stress as measured by cortisol levels, in combination with the stress of the activity on the spawning ground, may accelerate the process of deterioration and thus may be one of the determining factors in the death prior to spawning.

In contrast, the Stuart Lake females showed no significant differences between first and central segments. This population seldom has any significant prespawning mortalities, with only 3.7% of the females dying unspawned in 1973.

The evidence collected to date indicates that there is no association of spawner density and magnitude of prespawning mortalities within the Horsefly system (Williams 1973).

Examination of the fish on the spawning grounds with phase contrast microscopy indicated very large numbers of fusiform shaped bacteria present on the gills of moribund and spawning sockeye. Similar bacteria have been associated with large fish mortalities in a hatchery (Hoskins 1976). Since 1973 it has been observed associated with large mortalities of pink salmon being raised in fresh water at the Commission's Sweltzer Creek laboratory. These mortalities all have taken place in 6°-12° C water. To date the organism has not been cultured, therefore identity and pathogenicity are not known. The fact that it is present in large numbers on the gills of dying salmon in hatchery conditions as well as in the Horsefly River suggests that the fusiform shaped bacteria may have been a factor in the 1973 prespawning mortality.

Previous reports have suggested a relationship between water temperature at Hell's Gate during migration of the Horsefly sockeye, and timing of the migration and the magnitude of the prespawning mortality (Williams 1973, IPSFC 1974). High temperatures and early timing suggest large mortalities, while low temperatures and late timing suggest low mortalities. Therefore, while the high temperatures at Hell's Gate suggested a high prespawning mortality, the effect of late timing of the run suggests a below average mortality. In addition, the daily maximum Horsefly River temperatures over the spawning period were below the level of 14° C described by Colgrove and Wood (1966) as the maximum temperature desirable if mortalities from F. columnaris are to be kept to a minimum. The combination of the late timing of the Horsefly run, plus favorable spawning ground temperatures during spawning, probably contributed to a mortality below the average of other cycles.

A perplexing aspect of the investigation is the low mortality (6.7%) of Upper McKinley sockeye, which migrate with the first segment of the Horsefly sockeye. While only one sample of 239 fish was examined, the mortality is lower than any single sample taken from the Horsefly system. The reasons for the lower mortality of this population are unknown at this time.

SUMMARY AND CONCLUSIONS

In summary, the first segment Horsefly fish in 1973 had an elevated cortisol level and an elevated hematocrit level at Lummi Island, they apparently had encountered a heavy infestation of A. liquifaciens, and they had a large number of indefinable masses in the gill tissue upon arrival at the spawning grounds. F. columnaris cells were present in the Horsefly River water during spawning in 1973, although at a level much lower than that detected in 1965. Fusiform shaped bacteria were present in large numbers on the gill surface of the sockeye from both first and central segments. The potential for a serious prespawning mortality did exist in 1973, but the late timing of the population and below average spawning ground temperatures apparently were favorable factors which limited the mortality.

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