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PART I

TEMPERATURE CONTROL DURING SOCKEYE SPAWNING PERIOD IN McKINLEY CREEK IN 1969

BY

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PART II

INVESTIGATION OF THE PRESPAWNING MORTALITY OF SOCKEYE IN HORSEFLY RIVER AND McKINLEY CREEK IN 1969

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PART II

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Ву

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ABSTRACT

Serious prespawning mortalities, averaging 34%, have occurred among the Horsefly River sockeye populations in the past six dominant cycle runs. Available evidence implicated the myxobacterium Chondrococcus columnaris as responsible for much of this mortality, and previous research suggested that C. columnaris could be controlled if water temperatures on the spawning grounds were kept below a maximum of 57 to 58°F. A pilot project to control water temperatures at the spawning grounds in McKinley Creek, a tributary of Horsefly River, was completed for the 1969 dominant cycle run. Water temperatures in 1969 were lower than 58°F in Horsefly River during the period of spawning, and were controlled to less than 58°F in the upper part of McKinley Creek for much of the spawning period. Although high virulence strains of C. columnaris were present, they were controlled by the low water temperatures. However, despite cool water temperatures at the spawning grounds, a prespawning loss of 48.7% occurred in Horsefly River and 64.8% in McKinley Creek. Myxobacterium associated with bacterial gill infection were observed in gill tissues, which showed damage typical of this infection.

It is concluded that many factors are involved in the prespawning mortality of Horsefly River sockeye. Temperature in the lower Fraser River during the upriver migration, timing of the run, infections, degree of maturation, and spawning ground temperatures all appear to be involved.

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INVESTIGATION OF THE PRESPAWNING MORTALITY OF SOCKEYE IN HORSEFLY RIVER AND MCKINLEY CREEK IN 1969

INTRODUCTION

Major sockeye salmon (Oncorhynchus nerka) runs to the Fraser River system have been subject to serious prespawning mortalities, at least during the past 25 years, and especially among the early arrivals on the spawning grounds. Losses of the Horsefly River run have been as high as 62% in 1961, and losses of the Chilko River run have been as high as 90% in 1963.

When it became evident that a large loss could be expected at Chilko in 1963, studies were made to determine the cause, with emphasis being given to bacteriology. Since 1963, these investigations have been expanded to include histology and histopathology, bacteriology, virology and some endocrinology. A survey among various sockeye populations during 1968 indicated that known viruses found in Pacific salmon were not present in any of the adult sockeye tested, including numerous samples from moribund unspawned salmon (Williams MS 1972a).

Previous studies in 1963 and 1964 indicated the presence of high virulence strains of the myxobacterium <u>Chondrococcus</u> <u>columnaris</u> at several sockeye spawning grounds in the Fraser River watershed (Pacha MS 1963, MS 1964; Wood 1965).

An association between the incidence of <u>C</u>. <u>columnaris</u> and warm water was demonstrated during these studies, therefore the Commission considered the possibility of lowering water temperature to prevent the outbreak of <u>C</u>. <u>columnaris</u>. Experimental evidence obtained at Horsefly River in 1965 indicated that <u>C</u>. <u>columnaris</u> could be controlled on the spawning grounds by water temperatures averaging 55°F, with maximum daily temperatures not exceeding 57 to 58°F (Colgrove and Wood 1966).

Extensive study of methods to control temperatures at the Horsefly River spawning grounds indicated that temperature control could be obtained by release of cold water from Crooked Lake and McKinley Lake (FIGURE 1), (Internat. Pacific Salmon Fish. Comm. 1966). However, it was desired that verification of the experimental evidence be obtained from actual experience with a full scale operation. The Horsefly River sockeye run spawns principally in Horsefly River near the confluence with McKinley Creek, but part of the run also spawns in McKinley Creek below McKinley Lake. Water

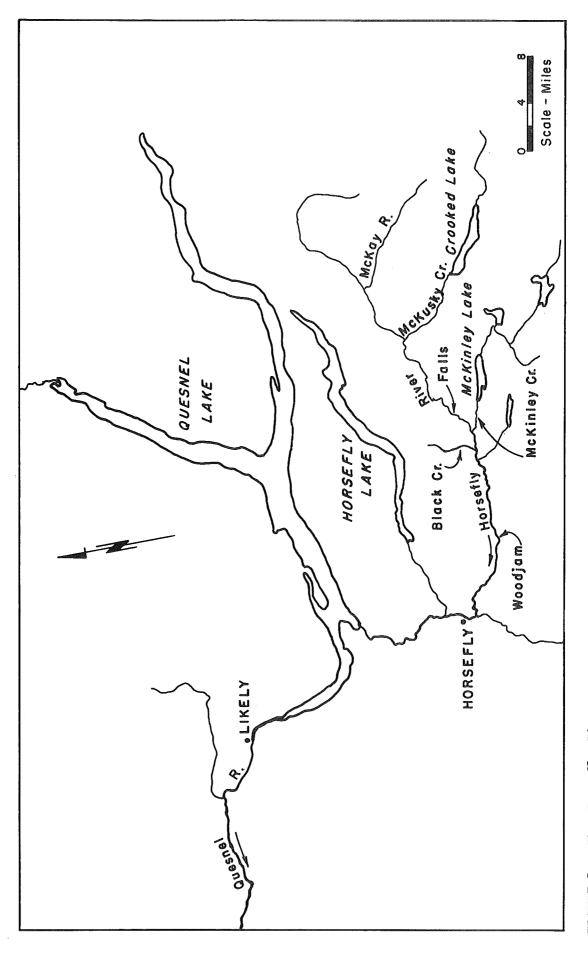


FIGURE 1 - The Horsefly River.

temperatures in this part of McKinley Creek are much warmer than in Horsefly River at the time of spawning because the flow in the creek comes from the warm surface water of McKinley Lake. Sockeye prespawning mortalities in McKinley Creek have been higher than in Horsefly River. Lowering the water temperature in McKinley Creek to the prescribed limits during spawning would allow comparison of the mortality with that in Horsefly River where temperatures would not be controlled, and could be accomplished for about one sixth the cost of lowering temperature in the Horsefly River.

The facilities for controlling temperature in McKinley Creek (Cooper 1972) were constructed for use during the 1969 dominant cycle run to Horsefly River and McKinley Creek. Accompanying this test, detailed investigations were made of success of spawning and the cause of prespawning mortality.

Since a higher prespawning mortality has occurred among sockeye that arrive early on the spawning grounds, early, peak and late fish have been examined for distinguishable differences between these segments of the run. For the 1964 Chilko River sockeye run, Colgrove (1966) found that the histological changes accompanying sexual maturation were entirely comparable for both early and peak segments. This run had a small prespawning mortality of only 2%. To obtain further data on possible differences between the segments of the run, the investigations of the 1969 Horsefly sockeye included measurements of lengths, weights and gonads, and testing of fish stamina. In addition measurements of bacteria in Horsefly River were made below the spawning grounds before and during spawning, to help identify the source of infection.

DESCRIPTION OF 1969 SPAWNING

Size and Timing of Run

The dominant cycle sockeye run to the Horsefly River system in 1969 contained nearly 1.5 million fish. The main part of the run entered Juan de Fuca Strait at about the same date as the runs in 1953 and 1965, and about 5 days earlier than the 1961 run and about 11 days earlier than the 1957 run (FIGURE 2). For the years 1953, 1961, 1965 and 1969 the peak of the run occurred between July 20 and 25, compared to July 28 to 31 in the years 1901, 1907, 1909 and 1913 (Internat. Pacific Salmon Fish. Comm. 1966).

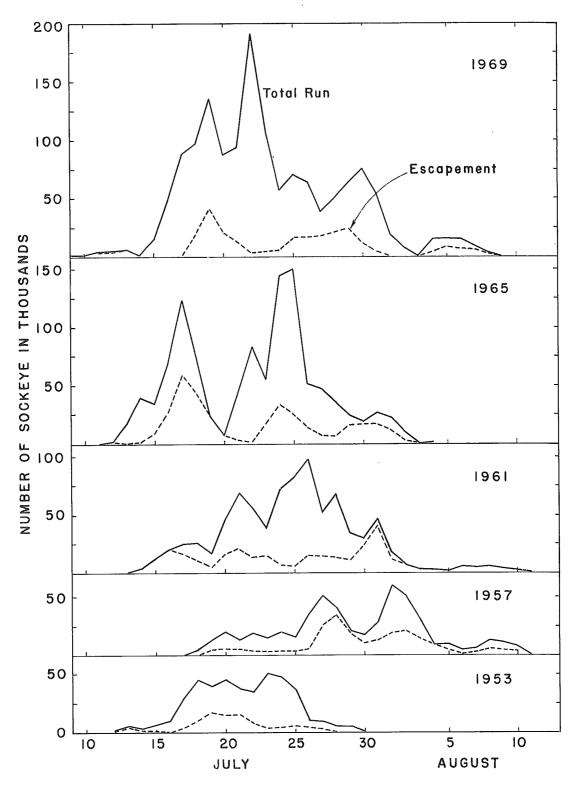


FIGURE 2 - Timing of Horsefly sockeye into Juan de Fuca Strait in dominant cycle years, 1953 to 1969.

The escapement to the spawning grounds in 1969 was obtained principally from segments of the run just before and after the peak, with two smaller groups from the first and last parts of the run. The largest daily escapement was obtained from about the same time as in the 1953 run, 9 days earlier than the 1957 run, 12 days earlier than the 1961 run, and a few days later than the 1965 run. The time of passage of the runs at other points on the migration route can be determined by adding the migration times given in TABLE 1.

TABLE 1 - Horsefly River sockeye migration times from entrance to Juan de Fuca Strait to the spawning grounds and intermediate points.

То		Days of Migration
New Westminster	5	
Hell's Gate	10	
Spawning Ground	25	(except 28 in 1957)

The escapement to the spawning grounds comprised 270,023 fish, of which 224,732 remained in the Horsefly River between the falls and Black Creek, and the balance in other parts of the Horsefly River system (TABLE 2).

TABLE 2 - Distribution of sockeye spawners in Horsefly River system, 1969.

Area	Spawners	Female Spawners
Horsefly River (below Woodjam)	17,110	9,840
Horsefly River (falls to Black Creek)	224,732	131,594
McKinley Creek (McKinley Lake to Horsefly River)	19,512	11,727
McKinley Creek (above McKinley Lake)	8,424	5,063

The daily numbers of sockeye entering Quesnel Lake were determined by sample counts at the town of Likely. These counts, transposed four days to the town of Horsefly and one additional day to the spawning grounds, show that most of the sockeye arrived at the spawning grounds between August 3 and September 3 (FIGURE 3). A few fish could have arrived a week before and after this period. The travel time from Likely to Horsefly and to the spawning grounds was based on observations in previous years, and was substantiated by observations during tagging in 1969. The mid-date of arrival at the spawning grounds was august 19, or two days later than the average for the dominant cycles since 1945, excluding 1957 (TABLE 3). The peak of spawning in Horsefly River occurred from August 27 to September 1, and in McKinley Creek from August 25 to 30. Fish arriving at the spawning grounds prior to August 14 were classified as early, those arriving from August 14 to 24 were classified as central, and those after August 24 were classified as late.

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

Dominant Years	Subdomi	nant Years	
Year	Date	Year	Date
1945	August 15	1946	August 25
1949	August 17	1950	August 23
1953	August 15	1954	August 28
1957	August 26	1958	September 5
1961	August 17	1962	August 28
1965	August 17	1966	August 25
1969	August 19	1970	August 30
Average excluding 1957	August 17	Average	August 28

FIGURE 3 - Arrival timing of the 1969 run to Horsefly River and McKinley Creek.

Water Temperatures

Water temperatures in the Horsefly River above McKinley Creek exceeded 57°F maximum with an average of 59.3°F in the first 12 days of August but were 57°F or less with an average temperature of 55.1°F for the next 19 days. Temperatures were cooler than in the preceding cycle years 1953, 1957, 1961 and 1965 (TABLE 4).

Daily maximum water temperatures in McKinley Creek at its mouth prior to the start of temperature control on August 12 averaged 68.1°F, but were cooler than in 1965 (TABLE 5). During the period of temperature control from August 13 to 31, temperatures in McKinley Creek at the outlet of McKinley Lake were less than 57°F maximum at all times (FIGURE 4) and were an average of 10°F cooler than would have occurred without temperature control (Cooper 1972). Midway down the creek, temperatures exceeded 57°F by 1 to 4°F with an average temperature of 58.7°F in the first seven days of the control period because of the necessity to discharge as much water as possible without excessive use of the available cold water. Starting on August 20, after the lake inflow reduced, a maximum temperature less than 57°F was maintained with an average temperature of 55.1°F for nine days, until the temperature of the cooling water rose above 53°F. It had been estimated that the temperature in McKinley Creek at its mouth could be as high as 62 to 69°F on a clear day depending on flow and date. Because of the cool weather and high discharge, the recorded temperatures did not exceed 61°F.

Temperatures in the Fraser River at Hell's Gate during the period of migration of the Horsefly run in 1969 were warmer than in 1949, 1953 and 1957, but were cooler than in 1961 and 1965 (TABLE 6).

METHODS

The study of the effectiveness of the temperature control and the probable causes of prespawning mortalities in the Horsefly-McKinley system consisted of the following phases:

- 1. The measurement of several physical characteristics including the stamina of adults from the early, peak and late segments;
- 2. The determination of success of spawning of the early, peak and late segments of the spawners in Horsefly River, and in the upper and lower halves of McKinley Creek below the control structure;

TABLE 4 - Daily maximum water temperatures in the Horsefly River above McKinley Creek, F during August 1953, 1957, 1961, 1965 and 1969.

Date	1953*	1957	1961	1965	1969
August 1		magings community on the ray of plantacopeus and an electric and experience of the second	Confidence of the Confidence o	67.5	63.0
2				66.0	62.0
2 3				64.0	61.0
4				61.0	59.0
4 5 6	63.0			60.0	57.0
6	63.5			61.5	55.0
7	62.0			64.0	59.0
8	62.5			63.5	60.0
9	62.5			64.0	60.0
10	61.5			62.5	59.0
11	62.0		66.0*	65.0	59.0
12	62.0		67.0*	62.0	58.0
13	62.5		66.0*	59.0	56.0
14	63.5		66.5*	54.5	57.0
15	62.0		64.5*	58.0	57.0
16	63.5	55.0*	65.0*	59.0	56.0
17	62.5	57.0*	64.0	60.5	_
18	63.0	57.5*	63.0	62.0	PRET
19	62.0	59.0	64.0	64.0	55.0
20	60.5	58.0	66.0	63.5	53.0
21	60.0	58.0	66.0	64.0	53.0
22	59.5	60.0	65.0	63.5	54.0
23	60.0	59.0	63.0	62.0	56.5
24	58.0	59.0	62.0	63.0	56.0
25	57.5	58.0	60.0	61.0	56.0
26	59.0	56.0	61.0	56.5	55.0
27	57.5	56.0	61.0	55.5	54.5
28	59.0	56.0	60.0	51.5	54.0
29		56.0	62.0	50.5	54.0
30		56.0	58.0	52.0	55.0
31		56.0	58.0	54.0	55.0

^{*}estimated

TABLE 5 - Daily maximum water temperatures in McKinley Creek at its mouth, F during August 1965 and 1969.

Date	1965	1969
August 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31	77.0 73.5 72.0 70.0 68.0 74.0 75.5 72.0 72.5 71.5 75.0 67.5 65.0 70.0 72.0 74.0 74.0 74.0 74.0 74.0 74.0 74.0 74.0 74.0 74.0 68.5 74.0 68.5 76.5 66.5 66.5 66.0	71.0 70.0 68.0 68.5 63.0 68.0 68.0 68.0 67.0 61.5 68.0 58.0 58.0 58.0 57.5 55.0 57.0 55.5 56.0 57.0 56.0 59.0
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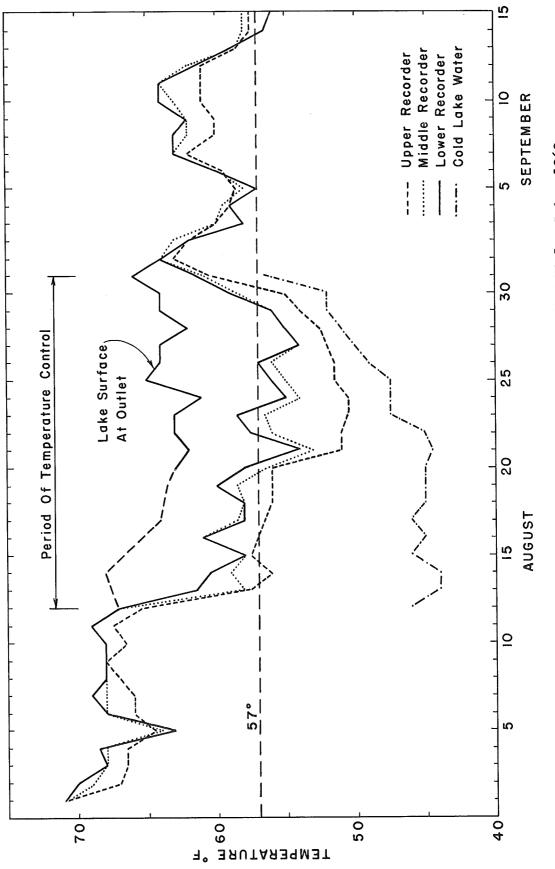


FIGURE 4 - Daily maximum water temperatures in McKinley Creek and McKinley Lake, 1969.

TABLE 6 - Daily maximum water temperatures of the Fraser River at Hell's Gate during the period of migration of Horsefly River sockeye in the years 1949, 1953, 1957, 1961, 1965 and 1969, in F.

^{*}estimated from records at New Westminster.

- 3. The measurement of dissolved oxygen and nitrogen in river water;
- 4. The examination of river water for bacteria; and
- 5. The examination of fish to determine cause of death before spawning.

Physical Measurements

One hundred female sockeye from each segment of the population (early, central and late) were sampled to determine possible differences between segments. The fish were seined from the river near the Horsefly townsite, approximately 19 miles downstream from the main spawning grounds.

Physical measurements collected were fork length, total weight, viscera weight, gonad weight, and eviscerated body weight. All fish were taken by beach seine and measured and weighed within a few minutes after removal from the water. Water was allowed to drain from bodies before measurements were taken and after evisceration the blood was allowed to drain from the body before the eviscerated weight was measured. A condition factor was calculated for each fish using eviscerated body weight and fork length.

Stamina of Adults

The stamina, or prolonged swimming ability, of sockeye approaching spawning was measured in a mobile performance tunnel, described by Williams (MS 1969), which was set up at the sampling site near the town of Horsefly, downstream from the spawning grounds.

Ten adult sockeye from each segment of the run were seined from Horsefly River, placed in a live-box and left for 12 to 24 hr. Fish were then placed individually in the loading basket of the stamina tunnel and left for 1 hr in a water velocity of 0.5 ft/sec. Following this acclimation, the fish was forced into the plexiglass tunnel and electrodes at the downstream end were activated. The velocity was first increased to 1.3 ft/sec for 4 min, then to 2.5 ft/sec for 4 min. Following acclimation at these intermediate velocities, the velocity was increased to the test level of 3.9 ft/sec and the total time the fish remained in the tunnel at 3.9 sec/ft was recorded. After the test the fish were measured and released. A few were tagged upon release and were noticed later passing through the McKinley Creek weir, approximately 19 miles upstream.

Prespawning Mortality

Two principal methods were used to determine the prespawning mortality of female sockeye at Horsefly in 1969.

1. To determine whether females examined in the dead recovery died in their order of arrival, a special tagging program was conducted wherein tags were applied to migrating sockeye at the town of Horsefly, prior to reaching the spawning grounds. Tagging commenced on August 7, although small numbers of very early arrivals had actually passed prior to this. Tagging continued in relative abundance to numbers of fish passing until August 29. Numbered tags larger (18 mm) than usual enumeration tags (14 mm) and of three colors were applied to distinguish early, central and late segments by visual means on the spawning grounds. Upon recovery after death each of these specially tagged sockeye was examined for success of spawning. From the numbered tag, the original date of entry into the spawning grounds and the length of life could be determined. A total of 3,176 female sockeye were tagged in this manner.

The daily percentage prespawning mortality, determined by the date of arrival of each fish, was applied to the calculated daily numbers of fish arriving at the spawning grounds to calculate the daily loss of spawners, from which the mortality for each segment, and the mortality for the entire run was determined. For comparison, the daily percentage prespawning mortality of the tagged fish was also determined in the same manner as for untagged fish, using the date of recovery of each fish.

A temporary picket fence installed at the lower end of McKinley Creek controlled entry of fish and allowed enumeration of the run to McKinley Creek. Tags were applied to 442 females as they entered the creek to determine success of spawning by date of entry. When it was observed that spawners were starting to die in the upper half of McKinley Creek below McKinley Lake, a net was placed across the creek at the middle temperature recorder site to prevent dead fish from drifting downstream and allow determination of success of spawning in the two halves of the stream.

2. During the customary enumeration procedure, daily examinations for 0, 50 and 100% spawned were made of "fresh-dead" untagged females beginning with the first available dead fish and continuing each day until no further fresh-dead females were present. At least 100 fish were examined when more than that number were available. From these data, a daily average success of spawning was calculated. This percentage was then weighted by the number of females recovered each day. The sum of these products divided by the sum of all females recovered yields the percentage success of spawning for the total run, from which the prespawning mortality can be obtained by subtraction. It was also possible to calculate separately the success of spawning of the early, central and late segments of the run.

Life Span

The life span of spawned and unspawned females was determined from the recoveries of fresh-dead females that had been tagged near Horsefly village. To determine the life span on the spawning grounds one day travel time was allowed from the tagging site to the spawning ground.

Dissolved Oxygen and Nitrogen in River Water

Oxygen and nitrogen levels were determined in McKinley Creek at the outlet of the control structure and 2 miles downstream from the lake outlet as well as in the Horsefly River above and below the Horsefly falls. Dissolved oxygen was determined by the Azide modification of the Winkler method (Standard Methods). Nitrogen was determined by a micro-gasometric method (Scholander et al 1955).

Bacterial Investigations of River Water

Water samples for bacterial culturing were taken from McKinley Creek near its mouth, and from Horsefly River and three sites on the Horsefly River at Black Creek, Woodjam and Horsefly village (FIGURE 1). In each case 1 ml of river water was diluted with 9 ml of sterile distilled water. From this dilution 0.1 ml was plated onto dry cytophaga media containing neomycin (5 µg/ml), polymyxin B (10 units/ml), and cyclohexomide (10 µg/ml) in a technique modified after those described for the isolation of myxobacteria

by Fijan (1969) and Carlson and Pacha (1968). Numbers of colonies were counted on the plates after incubation for 72 hr at 65°F. Water sampling began on July 17, several weeks prior to the arrival of the fish at the spawning grounds and continued on a weekly basis until September 1, at the peak of spawning.

Pathology

The 300 female sockeye obtained at the town of Horsefly for physical measurements, described earlier, were also examined for gill or other lesions and the percentage of such lesions were recorded for each segment of the run.

At the spawning grounds, both moribund and fresh-dead sockeye were examined similarly throughout the spawning period. The gills were also examined microscopically, and gill tissues were collected and preserved for subsequent examination after processing at the Sweltzer Creek Research Laboratory. In addition gill tissue smears were cultured for bacteria, using the modified cytophaga media, and the cultures were taken to the University of Washington to be tested for virulence by the contact method (Ordal 1970).

Gill tissues of sockeye from Chilko River, Nadina River and Stellako River where significant mortalities have occurred in recent years, and from Pitt River where mortalities have not occurred, were collected and examined at the laboratory for comparison with the tissues from the Horsefly River sockeye.

RESULTS

Physical Measurements

The physical measurements of female sockeye from three segments of the 1969 run collected at the town of Horsefly are given in TABLE 7. Mean fork length and viscera weight remained relatively constant throughout the run, but average body and gonad weights differed significantly as the run progressed. Early arrivals were heaviest in average eviscerated body weight, central fish weighed 5% less, and the late fish were 11% lighter than early fish. The regressions between weight and length for the three segments are

TABLE 7 - Physical measurements of 1969 Horsefly female sockeye salmon.

	eliteria mandet de Amelika sussilia i Albusso de meliteria i	Philippine visitation international enternational enternat	Fork Average Weight - gm		an kalanda amakaman kalanda ka			
Sample Dates	Segment of Run	No. Sampled	Length cm	Total	Eviscerated Body	Viscera	Gonad	Condition Factor
Aug. 9-12	Early	100	58.9 + 1.8	2,464 <u>+</u> 261	2,137 ± 223	73.6 <u>+</u> 10.8	240 <u>+</u> 43	1.05 + 0.8
Aug.18-20	Central	100	58.7 ± 1.8	2,347 + 266	1,992 ± 224	74.4 ± 10.6	265 <u>+</u> 50	0.98 ± .07
Aug.27-28	Late	100	58.5 ± 2.1	2,189 <u>+</u> 266	1,819 ± 222	72.7 ± 15.5		0.91 ± .08

shown in FIGURE 5. Average gonad weights were lowest in early fish and highest among the late fish. The mean percentage of gonads (gonadosomatic index) increased progressively from early to late segments, although there was considerable overlap in the range of values (TABLE 8).

TABLE 8 - Gonad weights expressed as percentage of eviscerated body weight and total body weight for 1969 Horsefly River female sockeye salmon.

Segment	Mean Per Cent of Eviscerated Weight	Mean Per Cent of Total Weight	Range of Per Cent of Total Weight
Early	11.22	9.72	7.08-12.31
Central	13.27	11.25	8.65-15.68
Late	14.91	12.56	8.74-18.45
(ACTION OF THE PROPERTY OF THE	and the state of t		

Stamina of Adults

Stamina tests showed the early fish had the greatest endurance. They remained at the top test velocity 40% longer than the central fish and over twice as long as the late fish (TABLE 9). Warmer test temperature may have contributed to the higher stamina rating of the early fish but this probably accounted for only a small part of the differences recorded. The data show a good correlation between average endurance time and average condition factor of the early, central and late segments (TABLES 7 and 9).

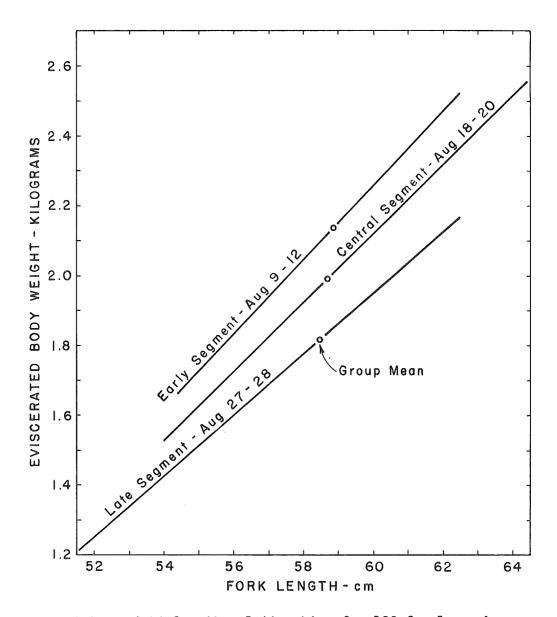


FIGURE 5 - Weight-length relationships for 100 female sockeye from each of the three segments of the 1969 Horsefly River runs sampled at Horsefly approximately 19 miles downstream from the spawning grounds.

TABLE 9 - Length of time adult sockeye salmon females remained in stamina tunnel at 3.9 ft/sec after a standard acclimation regime.

Segment of Run	No. Tested	Average Body Length (cm)	Test Temp. (°F)	Average Time at 3.9 ft/sec
Early (Aug. 13)	12	59•4	58.5	10.5 min
Central (Aug. 22)	13	57.3	56.0	7.5 min
Late (Sept. 2)	10	58.0	56.5	5.1 min

Prespawning Mortality

The prespawning mortality of the spawners in the Horsefly River between the falls and Black Creek averaged 48.5% on the basis of the tagged females and 48.7% on the basis of the untagged females (TABLE 10).

TABLE 10 - Percentage of Horsefly River sockeye unspawned at death at the spawning grounds between the falls and Black Creek in 1969, weighted for daily numbers of spawners arriving at the spawning ground.

	Tagged	Females	Untagg	ed Females
Segment	No. Recovered	Weighted % Unspawned	No. Sampled	Weighted % Unspawned
Early	186	70.5	1,154	68.6
Central	524	43.9	3,034	48.1
Late	53	19.5	933	26.5
Average		48.5		48.7

The prespawning mortality of the spawners in McKinley Creek below McKinley Lake averaged 64.8% on the basis of untagged females (TABLE 11). Only 79 of the tags applied at Horsefly were recovered in McKinley Creek. This is considered too few to give reliable estimates of the prespawning mortality, but the data indicate a mortality of 59.5%.

TABLE 11 - Percentage of sockeye spawners in McKinley Creek unspawned at death in 1969, weighted by daily numbers of dead females pitched.

Segment	No. Sampled	Weighted % Unspawned
Early	589	87.6
Central	925	58.3
Late	195	46.4
Average		64.8

Weighted percentage mortality cannot be determined separately for the upper and lower halves of McKinley Creek. Data from sampling of fresh-dead females indicates the same average mortality in each section of the creek, whereas data from recovery of fish tagged at the mouth of the creek indicate a higher average mortality in the lower half of the creek (TABLE 12).

TABLE 12 - Percentage of sampled sockeye spawners in McKinley Creek unspawned at death in 1969.

	Upper	Half	Lower	Half
Segment	No. Sampled	% Mortality	No. Sampled	% Mortality
		Untagged Fer	nales	
Early	82	86.1	507	86.3
Central	218	59.3	707	55.1
Late	15	23.2	180	39.7
Average		64.6		64.5
		Tagged Fema	ales	
Average	32	53.1	85	65.3

The recoveries of individual fish tagged at the town of Horsefly did not demonstrate chronological order of death after arrival on the spawning grounds, as shown by the wide range in life spans. However, the similarity of average life spans for the early, central and late segments indicates chronological order of death for the composite of each segment. The daily prespawning mortality determined for the tagged fish by date of arrival shows a trend from high mortality for the first arrival, to low mortality for the last arrivals (FIGURE 6). The large deviations of some points are associated with small sample numbers. The data for these same fish, determined by date of recovery, shows a parallel trend, approximately 17 days later. The daily prespawning mortality determined from the untagged fish by date of recovery, shows a trend very close to that of the tagged fish. Thus, it would appear that data obtained from the usual dead recovery, transposed by the average life span, can be used as representative of the prespawning mortality for the fish by date of arrival.

Life Span

The average life span of female sockeye after arrival on the spawning grounds ranged from 17.6 days in the early segment of the run to 13.8 days in the late segment (TABLE 13). For both the early and central segments the life span of completely spawned fish average approximately one day longer than the completely unspawned fish. The numbers of unspawned fish recovered from the late segment were too small to give adequate determination of life span.

TABLE 13 - Average life spans of tagged female sockeye with 0.50 and 100% success of spawning, recovered fresh dead at the Horsefly River spawning grounds, in days after arrival at the spawning grounds.

desirative forestyderous assessment ordered and selectives desirates absorber specimen secures assessment team as	Per Cent	Success of	Spawning	Weighted
Segment of Run	0	50	100	Average
Early	17.0	19.1	17.9	17.6
Aug. 3-13	(76)	(24)	(43)	(143)
Central	14.6	16.4	15.8	15.4
Aug. 14-24	(86)	(26)	(124)	(236)
Late	9.5	13.5	14.2	13.8
Aug. 25 to Sept. 3	(2)	(4)	(23)	(29)

(Numbers of fish recovered shown in parentheses)

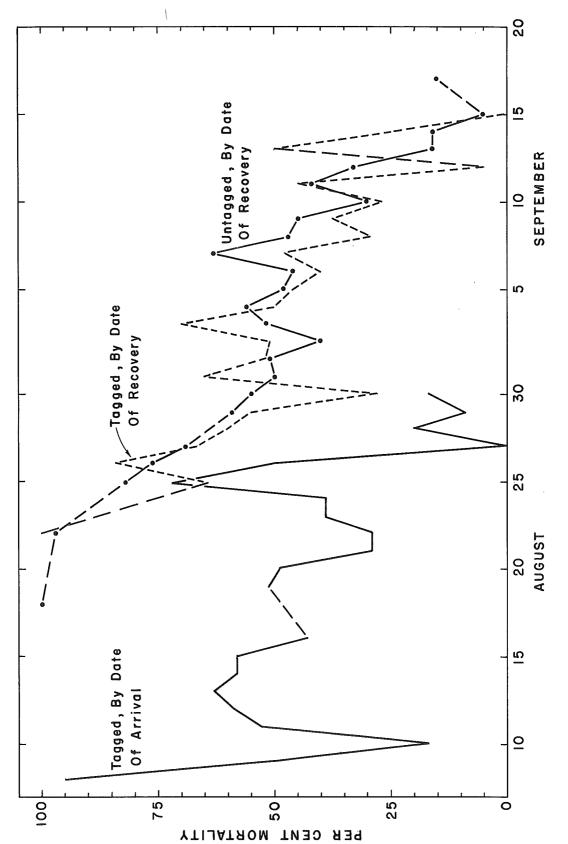


FIGURE 6 - Daily prespanning mortality of tagged and untagged sockeye in Horsefly River, 1969.

The life spans of both spawned and unspawned females range from 12 to 25 days in the early segment and from 6 to 23 days in the central segment (FIGURE 7). In the early segment, the spawned out fish lived from zero to three days longer than the unspawned fish, the largest difference occurring among the fish with life span shorter than average. In the central segment the spawned out fish lived from one half to three days longer than the unspawned fish.

Dissolved Oxygen and Nitrogen in River Water

The measurements of dissolved oxygen and nitrogen measurements showed supersaturation of nitrogen in both McKinley Creek and Horsefly River at the time of arrival of the early segment of the sockeye run.

Nitrogen concentration midway down McKinley Creek ranged from 104 to 106% of saturation, and was 108% of saturation in the Horsefly River below the falls (TABLE 14). The data show no correlation between the dissolved gases and prespawning mortality.

Dissolved oxygen was below saturation in the cold water discharged from the pipeline at the outlet of McKinley Lake, but increased to near saturation midway down the creek as a result of aeration.

TABLE 14 - Per Cent saturation of dissolved oxygen and nitrogen in McKinley Creek and Horsefly River, August 1969.

Page Principal Company of the Principal Company of the State Company of	an a	Temp.	Per Cent	Saturation
Location	Date	F F	Oxygen	Nitrogen
McKinley Lake control structure	Aug. 13	50.1	66.6	101.9
McKinley Creek two miles downstream from control structure	Aug. 13	57.0 59.0	95.8 97.7	104.0 106.1
Horsefly River above falls	Aug. 11	59.0	102.5	102.1
Horsefly River one mile downstream from falls	Aug. 12	58.0	104.9	108.0

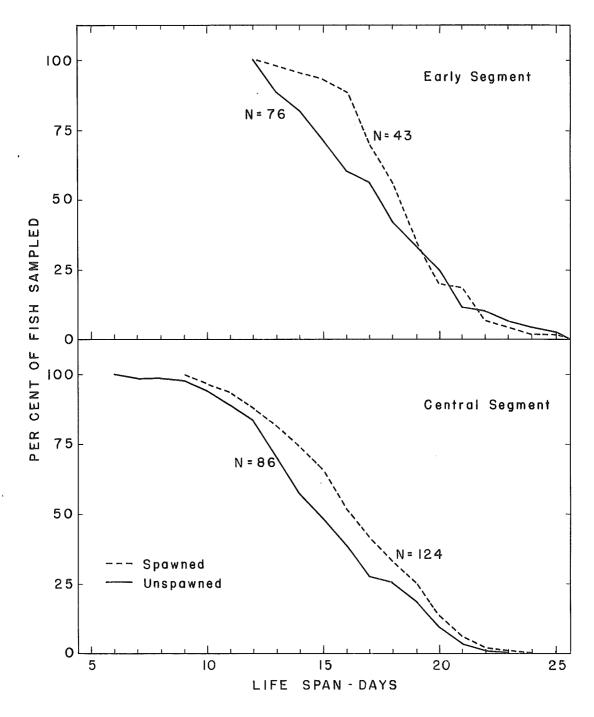


FIGURE 7 - Life spans of early and central segments of sockeye run to Horsefly River, 1969.

Bacteria in River Water

Bacterial culturing of the water on the spawning ground showed a dramatic rise in bacteria count with the arrival of the fish (TABLE 15). The <u>C. columnaris</u> organism apparently did not reach numbers great enough to be recovered from the water samples (Mead 1969), whereas in 1965 cultures were obtained from Horsefly River water which indicated 150 columnaris bacteria per cc of water (Colgrove and Wood 1966). However, in contrast to the 24 tests by Mead, cultures of <u>C. columnaris</u> were isolated from a sample of water obtained from McKinley Creek 2 miles downstream from McKinley Lake and a sample from Horsefly River near the confluence with McKinley Creek (Ordal 1970).

The bacteria that did grow on the media are only an indicator of the increase in total bacteria count since a selective media was used.

TABLE 15 - Bacteria cultured from water of McKinley Creek and Horsefly River, 1969.

	Thousands of Bacteria per cc of Water							
Date Sampled	McKinl Colum.	ey Cr. Other		efly R. ack Cr. Other	Horsef at Wood Colum.	ly R. ljam Br. Other	Horsefl at Tow Colum.	•
July 17	0	0,2	0	0.8-2.0	0	0.7-1.0	0	0.6
Aug. 5	0	0.6-1.0	0	0.5	0	1.2-2.0	0	1.4-30
Aug. 11	0	12-20	0	27-50	0	16-40	0	19-20
Aug. 18	O	50 – 66	0	80	. 0	75-90	0	40-60
Aug. 25	0	120	0	100	0	140	0	110
Sept. 1	0	250	0	Present	0	500	0	110

Pathology

Examination of fish passing the town of Horsefly showed that at least 2% of the early fish had active <u>C. columnaris</u> lesions on their gills. Evidence that viable columnaris can be present in fish with no gross lesions is demonstrated by the positive culture taken from a single apparently "clean" fish. The central and late segment fish had a 5 and 2% incidence of gill lesions respectively, with no additional columnaris detected by culturing "clean" fish (TABLE 16).

TABLE 16 - Incidence of columnaris on female sockeye upon arrival at Horsefly townsite.

ger and Talenton Angeles of New York, the State of Angeles of New York, the State of New Yo	**************************************	No.	Gross	Gill Cultur	es From Fish Without Lesions
Date	Segment	Sampled	% Lesions	No. Sampled	No. Positive for Columnaris
Aug. 12	Early	100	2	20	The second street of the control of the second street of the control of the second street of
Aug. 18	Central	100	5	20	О
Aug. 27	Late	100	2	20	0
Company of the Compan					

At the spawning grounds, the sockeye had a healthy appearance until death occurred. Fresh-dead unspawned females on the spawning grounds showed an incidence of gill lesions ranging from 4.6 to 20% (TABLE 17). All gill lesions which were examined microscopically had viable columnaris organisms present. It is noteworthy that in three cases the culturing method failed to show viable columnaris organisms, but microscopic examination confirmed the presence of C. columnaris. Since two samples of C. columnaris were cultured from gills without lesions, the incidence of columnaris was probably greater than indicated by the observations of lesions. Fish recovered dead from McKinley Creek had an incidence of columnaris lesions ranging from 17.8 to 20% during the August 20 to 29 period with a sharp drop to 4.6% on September 1 (TABLE 17). The adults recovered from Horsefly River had 7.7 to 19% incidence of columnaris lesions with the lowest occurring August 20 and the highest August 25. These lesions did not increase greatly in size from those observed at the Horsefly townsite and a large number of healing or healed lesions were evident on fresh-dead sockeye.

Microscopic gill examination revealed that three organisms were present on moribund fish, the myxobacteria C. columnaris (FIGURE 8), small paired gram-negative bacterium, and a large myxobacteria commonly associated with bacterial gill infection (Ordal 1970). The virulence of the strains of C. columnaris present was tested on 29 cultures of C. columnaris obtained from salmon in Horsefly River and McKinley Creek. It was found that 19 of the 29 cultures of C. columnaris could be characterized as extremely high virulence in that they killed experimental fish in less than 24 hr. The balance of the cultures killed in between 48 and 72 hr, although one culture

TABLE 17 - Incidence of columnaris on fresh dead unspawned female sockeye, McKinley Creek and Horsefly River, 1969.

					Fish with Gross Lesions	ions		
		Total F	Total Fish Examined		% Positive for Columnaris	mnaris	Fish Without Gross Lesions	ross Lesions
Date	River	Number	% With Wimber Gill Lesions	No. Examined	(A) By Microscopic Examination	(B) By Culturing	No. Cultured	$^{\it \%}_{ m Positive}$
Aug. 20	McKinley	27	18.5	2	100	100	2	0
	Horsefly	56	7.7	8	100	100	CI	0
Aug. 23	McKinley	30	20.0	7	100	100	9	0
	Horsefly	55	12.7	Н	100	100	4	0
Aug. 25	McKinley	45	17.8	7	100	75	N	0
	Horsefly	1.47	19.0	М	100	100	CV.	0
Aug. 29	McKinley	50	20.0	α	100	100	9	0
	Horsefly	50	10.0	Н	100	100	Μ	0
Sept. 1	McKinley	65	9.7	П	100	100	9	16.7
	Horsefly	97	15.2	4	100	50	Н	0
		-						



FIGURE 8 - \underline{C} . columnaris forming columns on a sockeye gill x 500.

failed to kill in 96 hr. Efforts to infect test fish from cultures of the large myxobacteria and the paired gram-negative bacteria were not successful. Similar difficulties have been encountered by others trying to reproduce bacterial gill infection under laboratory conditions (Ordal 1970).

Tissues from the gills preserved for histological examination clearly showed massive gill damage in the unspawned moribund or fresh-dead Horsefly and McKinley Creek sockeye, even when lesions and damage were not observed grossly (FIGURES 9 and 10). In contrast, the gills from a spawned out male sockeye from McKinley Creek did not show the damage evident in unspawned fish (FIGURES 11 and 12). Similarly, spawned out fish from Nadina, Pitt, Stellako and Chilko Rivers showed little if any gill damage, whereas unspawned moribund or fresh-dead fish from these areas had gill damage very similar to the Horsefly and McKinley unspawned fish.

The primary damage site was the epidermal tissue of the gill. In a normal gill the epithelial layer is squamated as is shown on gill tissue taken from a sockeye salmon in the estuary (FIGURE 13). As the fish approach and begin spawning the gill tissue normally undergoes some pathological changes with the epithelial tissue generally becoming swollen (FIGURE 14).

The epithelial tissue from the gills of moribund unspawned fish however definitely shows pathological changes advancing beyond the swelling with a general breakdown of the tissue involving epithelial separation, advanced swelling and general necrosis in various stages (FIGURES 15 and 16). This type of damage is typical of that caused by bacterial gill infections.

Because of the evidence of severe gill damage, an experiment was set up during the course of the Horsefly investigation to evaluate the ability of moribund fish to extract oxygen from the environment. Bottled oxygen was bubbled into a 15-gal water-filled container into which moribund fish from McKinley Creek were introduced. Exposure to a superabundance of oxygen resulted in temporary recovery lasting an average of 5 to 15 min after fish were returned to the creek, whereupon fish resumed a moribund state (Mead MS 1969).

In summary, the observations of sockeye at Horsefly River and McKinley Creek in 1969 indicated the presence of <u>C. columnaris</u>, but many of the lesions healed or were healing prior to death of the fish. The healthy appearance of the fish, the abundance of myxobacteria similar to those associated with bacterial gill infection, the evidence of severe gill damage, and the response to oxygen therapy are considered consistent with a diagnosis of bacterial gill infection.

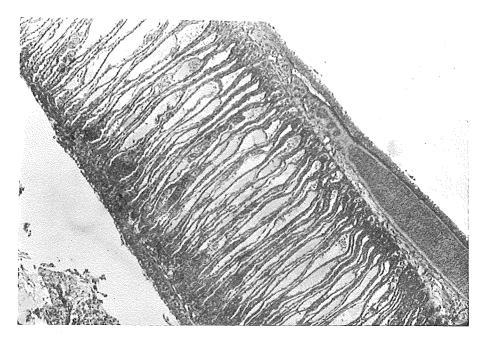


FIGURE 9 - Gill tissue of moribund unspawned McKinley Creek female sockeye with no visible gill lesions \times 50. Severe gill damage to 80 to 90% of gill.

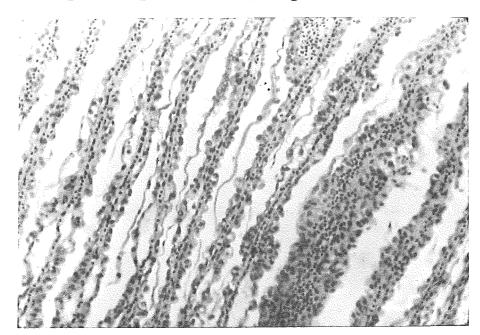


FIGURE 10 - Gill tissue of moribund unspawned McKinley Creek female sockeye with no visible gill lesions x 156. Shows swelling and desquamation of epithelial layer.

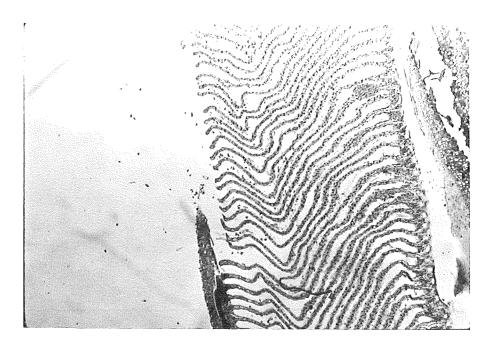


FIGURE 11 - Gill tissue of spawned out male McKinley Creek sockeye with no visible lesions x 50. Gill in relatively good condition.

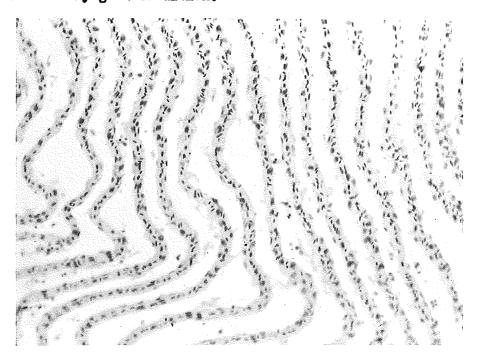


FIGURE 12 - Gill tissue of spawned out male McKinley Creek sockeye with no visible lesions x 156. Gill in relatively good condition.

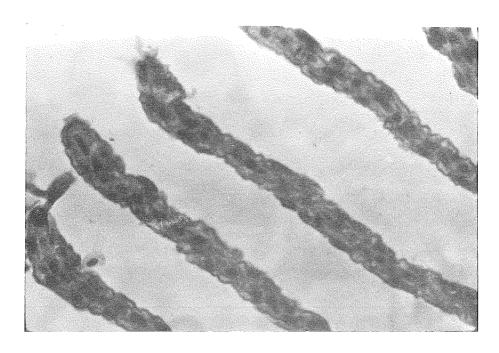


FIGURE 13 - Gill tissue taken from sockeye salmon at Lummi Island \times 500.

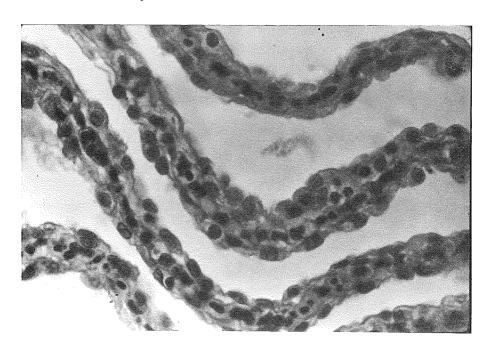


FIGURE 14 - Gill tissue taken from spawned out Pitt River female sockeye x 500. Epithelial cells swollen.

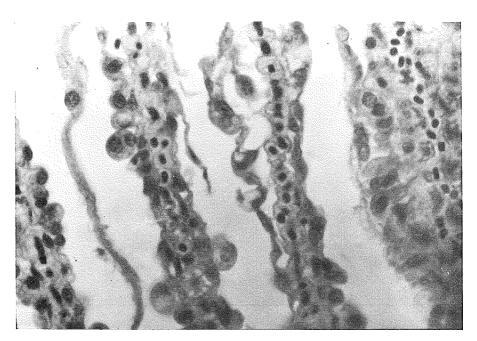


FIGURE 15 - Gill tissue taken from unspawned moribund female McKinley Creek sockeye no visible lesions \times 500. Lamellae showing desquamation and swelling of epithelial cells.

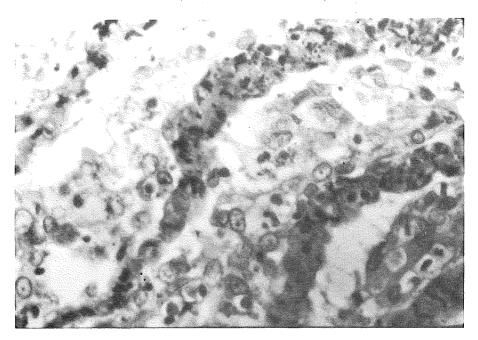


FIGURE 16 - Gill tissue taken from unspawned moribund McKinley Creek female sockeye with columnaris lesions x 500. Tissue is in advanced stages of necrosis.

DISCUSSION

Examination of the 1969 Horsefly sockeye run 19 miles downstream from the spawning ground indicated that at least 2 to 5% of the arriving fish were carrying active columnaris lesions on the gills. In addition, extremely high virulence strains of C. columnaris were present in a significant percentage of the spawning population, and if water temperatures had been warm, catastrophic mortalities due to columnaris infection would undoubtedly have occurred (Ordal 1970). Examination of the fish on the spawning ground failed to reveal any evidence that columnaris was spreading or causing significant mortalities. Many of the columnaris lesions showed signs of healing. These observations confirmed the conclusions from earlier research (Colgrove and Wood 1966) that C. columnaris could be controlled on the spawning grounds by water temperatures averaging 55°F, with maximum daily temperatures not exceeding 57 to 58°F. The temperature control structure on McKinley Creek therefore accomplished the objective of controlling C. columnaris in McKinley Creek, just as the low temperatures that occurred naturally controlled columnaris in Horsefly River.

However, it is evident that \underline{C} . columnaris is not the only factor involved in prespawning mortality, and that the causes of the mortality are complex.

In addition a number of other races of sockeye migrating up the Fraser River at the same time as the Horsefly run had significantly lower prespawning mortalities. In 1969 the Chilko run had a prespawning mortality of 27%, Stellako 10%, Late Nadina 11%, Tachie 9%, and Middle River 4% (Killick MS 1972), compared to 48.5% for the Horsefly run. It is evident, therefore, that racial characteristics and/or spawning ground conditions are prominent factors involved in the mortality.

Water temperatures in the lower Fraser River are implicated in the infection of sockeye during their upriver migration as shown by the apparent relation between prespawning mortality of the dominant cycle Horsefly run and the average daily maximum temperature of the Fraser River at Hell's Gate during the period of sockeye migration (FIGURE 17). The 1957 run is an exception in this relationship, although this run was later than the other cycle years, with timing similar to the pre-1913 run.

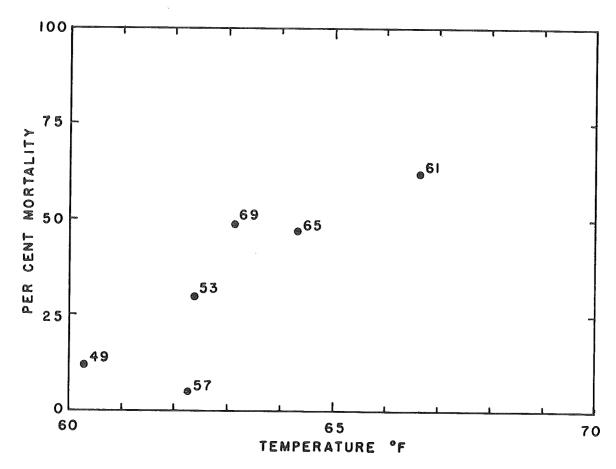


FIGURE 17 - Apparent relation between prespawning mortality of Horsefly River sockeye and average daily maximum water temperatures of the Fraser River at Hell's Gate during the migration of the run.

At the spawning grounds no relationship is indicated between prespawning mortality and the number of spawners or the number of spawners per cfs of river discharge. The prespawning mortality for the sockeye run to Mitchell River, another tributary of Quesnel Lake, has followed a pattern very similar to that for the Horsefly River, but the number of spawners in Mitchell River has been less than 4% of the number in Horsefly River (TABLE 18).

TABLE 18 - Prespawning mortality for sockeye runs to Mitchell River and Horsefly River and the corresponding spawning populations.

garanteen (mark left and garanteen (market (ma	Per Cent Prespaw	ning Mortality	Female Spawners		
Year	Horsefly	Mitchell	Horsefly	Mitchell	
1949	12.1	0	13,220	209	
1953	29.6	23.4	57,838	1,172	
1957	5.0 ^e	(a)	142,700	1,686	
1961	62.2	60.0	179,870	4,027	
1965	46.8	41.9	194,819	2,893	
1969	48.7	(a)	158,367	5 , 363	
Average	34.1				

⁽a) Estimated to be the same as Horsefly River.

The spawners in Mitchell River are spread out over a mile of river in discharges equal to or greater than the Horsefly River with temperature very similar to the Horsefly River above McKinley Creek. In the Horsefly River in 1969, there were three times as many female spawners per cfs than in McKinley Creek, but the mortalities were similar in both areas. Data on spawning ground areas and number of spawners indicates the density of spawners per sq yd of spawning area was about half that in Horsefly River. In the years 1954, 1958 and 1966, the subdominant cycle for the Horsefly run, the prespawning mortalities were 3, 2 and 8% respectively (Killick MS 1972). The numbers of spawners in these years (279, 1,784 and 1,607 respectively) were small, but the fish also were very late in arrival at the spawning ground, similar to 1957 (TABLE 2). However, in 1970, with a run of only

e estimated from observer reports and a sample of 26 tagged fish.

1,350 spawners, the prespawning mortality was 58%, and nearly all the fish showed visible evidence of <u>C. columnaris</u> lesions, including those that spawned successfully. The large prespawning mortality (89%) of sockeye at Chilko River in 1963 was attributed to early timing of the escapement and the excessive number of spawners (Pacha 1964). However the excessive spawners entered the lake and apparently remained there as the "river population density compared with 1960" population which had a 5% mortality (Internat. Pacific Salmon Fish. Comm. daily reports). Also in other years with smaller numbers of spawners, the mortality at Chilko River is not related to population size. It is concluded therefore, that number of spawners per cfs of flow and population size and spatial distribution of fish do not appear to be factors in prespawning mortality.

The overall appearance and behavior of the 1969 Horsefly sockeye spawners was quite different from that seen in 1961 and 1965 during severe outbreaks of C. columnaris. In those years, large numbers of fish appeared lethargic, with little interest in nest digging or pairing. Skin lesions were common, giving the fish an unhealthy appearance. In 1969, the spawners had a healthy appearance and showed signs of active nest digging. However, in addition to C. columnaris, cultures of large gill myxobacteria and an unidentified gram-negative paired bacteria were found. All the moribund unspawned fish examined showed evidence of gill damage with many displaying the large myxobacteria and the gram-negative paired bacteria. These observations are in accord with a diagnosis of bacterial gill infection (Mead 1969).

It has been observed that bacterial gill infections are not unusual for adult salmon in fresh water, and that such infections may be involved in post-spawning death of Pacific salmon (Wood 1965). Wood states that "Although the bacterial gill diseases may be precipitated by the normal physiological degeneration of post-spawning salmon, it is possible that the gill diseases actually kill fish before the degenerative changes per se do. With high fish density and a sizable flora of the involved bacteria, it is also possible that the bacterial gill diseases may kill adults before they spawn." These observations seem to be applicable to the Horsefly River in 1969 where the bacterial count of the river water increased during the period of spawning and was greatest at the end of spawning.

The source of infection with bacterial gill myxobacteria is not known. Wood (1965) presented the hypothesis that scrap fish in the lower Fraser River could be a source of infection with high virulence strains of C. columnaris at temperatures exceeding 60°F and this hypothesis was substantiated by subsequent research (Colgrove and Wood 1966). It appears reasonable to speculate that the infection with other bacterial gill myxobacterium occurs in this area also. Snieszko (1964) places species of Pseudomonas, Aeromanus and Myxobacteria in the category of facultative fish pathogens (Collins 1970). Regarding these bacteria, Snieszko states as follows:

"All these organisms are common in bodies of fresh water inhabited by fishes, and can usually be found on the body surface and intestinal lumen. Under conditions optimal for fishes, seldom, if ever, do these bacteria cause diseases. However, once the balance is disturbed to the detriment of the fish, dramatic epizootics often break out."

Dubos (1955) states: "There are many situations in which the microbe is a constant and ubiquitous component of the environment but causes diseases only when some weakening of the patient by another factor allows infection to proceed unrestrained, at least for awhile. Theories of disease must account for the surprising fact that in any community, a large percentage of healthy and normal individuals continually harbor potentially pathogenic microbes without suffering any symptoms or lesions". Wedemeyer (1970), in a review of the literature dealing with stress and fish diseases, points out that a stressful environment can cause biochemical and physiological changes within a fish which can be ultimately responsible for potentiating microbial infectious processes. Colgrove (1966a) noted that a rise in plasma levels of the adreno-corticosteroids might be expected as the spawning migration nears its termination, in view of the stress of migration and the building of a large mass of gonadal tissue under conditions of starvation. Measurements on the Early Stuart sockeye run in 1965 showed higher cortisol levels at the spawning ground than near the mouth of the Fraser River at Lummi Island, but they also showed higher levels of cortisol among the early segment of the run at Lillooet than among the peak segment. These results may indicate the early segment fish were subject to more stress than the peak segment.

The decline in prespawning mortality from the first fish arriving to the last fish, as previously mentioned, suggests the possibility of a significant difference between early and late fish. Differences were observed in the Horsefly sockeye in 1969, in condition factor life span and per cent gonads. The life span of fish in the early segment of the run was approximately two days longer than for fish in the central segment. As the fish normally do not die until several days after spawning, these small differences in average life span do not seem significant, particularly since the life spans ranged from 6 to 25 days in both segments. The early segment also had a lower percentage of weight as gonads than the central and late segments, as well as a higher condition factor although there was overlap in the range of values for each segment. The Chilko sockeye run in 1964 also showed lowest per cent gonads in the early segment (Colgrove 1966). If fecundity and egg size are the same for each segment, these data would indicate that females that spawned successfully were more mature on arrival than those that arrived at the same time and died without spawning.

From the foregoing considerations it appears that many factors are involved in the prespawning mortality of Horsefly River sockeye. Early timing of the run, temperature of the lower Fraser River during the upriver migration, infections, degree of maturation and spawning ground water temperature all appear to be involved in the mortality.

CONCLUSIONS

- 1. Cool water temperatures in 1969 that occurred naturally in the Horsefly River and which were obtained in McKinley Creek as a result of temperature control, did control <u>C</u>. <u>columnaris</u> in the spawning sockeye. Many visible columnaris lesions healed or were healing prior to death of the fish. The extremely high virulence strains of <u>C</u>. <u>columnaris</u> present probably would have caused catastrophic prespawning mortality if river temperatures had been warm.
- 2. Despite the cool temperatures, a prespawning mortality of 48.5% occurred in the Horsefly River and 64.8% in McKinley Creek. The healthy appearance of the fish and the evidence of gill damage was consistent with a diagnosis of bacterial gill infection. Gill damage due to the infection is believed to have been a factor in the prespawning mortality.

3. Many factors are involved in the prespawning mortality of Horsefly River sockeye. Temperature in the lower Fraser River during the upriver migration, timing of the sockeye run, infections, degree of maturation and spawning ground water temperature all appear to be involved. Further studies will be required to define the relative significance of each of these factors with respect to the mortality.

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