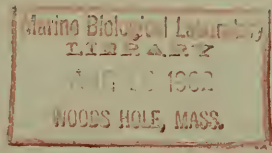


MARKING SOCKEYE SALMON SCALES BY SHORT PERIODS OF STARVATION



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Richard L. Major and Donovan R. Craddock



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ABSTRACT

Experiments conducted at the Leavenworth, Wash., national fish hatchery in 1959 and 1960 demonstrated that the scale pattern of Columbia River sockeye salmon *Oncorhynchus nerka* can be recognizably modified by a short period of starvation. Modification was obtained with little mortality. Starved fish readily resumed feeding; and although they did not make up for lost growth, they regained their robustness and outward vigor.

The tests were the first in a series to determine whether a starvation-marking technique can be developed as a means of separating wild from hatchery-reared sockeye salmon.

INTRODUCTION

Seaward migrations of sockeye salmon *Oncorhynchus nerka*¹ from Lake Wenatchee, Wash., are composed of both wild and hatchery-reared fish. Both types of migrants are progeny of the same parent stock, some of which spawned naturally while others were spawned artificially. Artificially spawned eggs are hatched, and the fish reared to fingerling size at the nearby Leavenworth hatchery operated by the U.S. Fish and Wildlife Service. Hatchery-reared young are planted in the lake in the fall where they are subject to mixing with the wild fish. Young sockeye either migrate seaward in the spring of the second or third year of life or remain in the lake to complete their life cycle in fresh water, in which case they are called "kokanee."

¹Sockeye in the Columbia River are also called "blueback."

The difficulty in separating wild from hatchery-reared fish hampers efforts to evaluate either natural or hatchery production to the migrant stage, or to measure the contribution of either type to the kokanee population. A means is needed, therefore, to readily and accurately distinguish wild from hatchery fish captured in the seaward migration and in the kokanee sport fishery.

Efforts to discover a built-in identification characteristic by the comparison of known wild and hatchery samples² were unsuccessful in 1957 and 1958. In addition to intensively examining the scales, which are known to

²The origin of the samples was known in this case because the hatchery fish were taken directly from the hatchery ponds and the wild sample consisted of fish in their second year that were captured in the lake prior to the planting of the hatchery fish.

reflect the environment of young salmon, we also compared length and weight frequencies, length-weight relationship, and general external appearance.

A second approach to the problem of separating wild from hatchery fish is the artificial marking of the hatchery fish. Because of the large numbers of fish involved (approximately 3 million young sockeye are planted each year) techniques, such as fin clipping, which require handling each fish, are time consuming and expensive.

A review of the literature suggested that "marking" the scales of hatchery fish by a short period of starvation might be a more promising method. Gray and Setna (1931) and Bhatia (1931, 1932) successfully modified the circuli spacing on the scales of rainbow trout (*Salmo irideus* [gairdnerii]) by manipulating the food intake. Circuli are the concentric rings which occur naturally on the scales of many fish including salmon and trout. When growth is fast, circuli are relatively wide-spaced; and when growth is slow, they are more closely set and sometimes broken. Slow winter growth is denoted by series of closely spaced circuli called annuli.

As the first step to determine whether a starvation-marking technique could be developed as a means of separating Lake Wenatchee wild from hatchery-reared fish, we conducted hatchery experiments in 1959 and 1960. The primary objective of these experiments was to determine whether or not a recognizable alteration (mark) could be imprinted on the scales by short periods of starvation. Other objectives were: (1) to measure the mortality caused directly by starvation, (2) assess the effect of starvation on growth, and (3) determine the optimum time and duration of starvation for obtaining the desired modification.

METHODS AND MATERIALS

The experimental procedure was essentially the same each year. Initially, we selected a representative sample of approximately 1,200 fish from an outdoor pond and halved the sample into two lots of 600, one a control lot

for regular feeding and the other a test lot for starvation. Every 2 weeks³ thereafter, we removed 100 fish from the starvation lot, placed them in a separate trough and reintroduced food. At the end of the experiment then, we had fish that had been starved for 0, 2, 4, 6, and 8 weeks (the latter in 1959 only). All lots were sampled approximately every 2 weeks--lengths and weights recorded and scales taken. Mortality was recorded daily.

The 1959 experiment was conducted with fish that (representative of the bulk of the hatchery fish for that and preceding years) had been reared on a wet-type diet. Most of the hatchery fish were changed to a dry diet in 1960, and mainly because of this the tests were repeated to determine whether or not similar results could be obtained with fish fed the new diet.

To determine whether seasonal timing of starvation and recovery periods influenced marking success, we ran three variations of the 2-week starvation period in 1960. We chose the 2-week interval for this purpose because it had produced the best marking success in 1959.

A more detailed description of the methods and materials is presented in the following subsections.

Trough specifications and water supply

The experiments were conducted inside the main hatchery building in cement troughs, 187½ inches by 16½ inches by 16¼ inches, filled to an average water depth of 10 inches. The main source of the hatchery water supply is nearby Icicle Creek, although some well water is occasionally used in the summer for cooling purposes. Water temperature is recorded continually by a thermograph.

Feeding

All feeding was at the prescribed hatchery rate based on the weight of the fish in a trough.

³The 2-week periods described in this report varied from 13 to 18 days. There is no evidence that the variation affected the experimental results.

The feeding schedule was not rigidly maintained during the final week of the 1959 experiment due to the press of the fish planting operation. Care was exerted to avoid a recurrence of this in 1960.

Starvation, as it is referred to in this report, is defined as the cessation of artificial feeding. Although we did not measure the plankton in the water supply, we do not believe that it was of sufficient quantity to affect the results. This belief is supported by previous chemical and plankton data (unpublished).

Sampling

All sampling was done with a quartering net (fig. 1) which was developed, evaluated, and described by Hewitt and Burrows (1948). The operation of the device is as follows: First, the net with one of the four bobinetting bags tied closed is placed flat on the bottom of a half-filled washtub. The population to be sampled is transferred from the trough to the tub and dispersed by three gentle clockwise rotations of the hand after which the net is quickly raised thereby trapping the fish in the closed bag. Fish which fall into the three untied bags remain in the tub. Sample size can be increased by closing additional bags or reduced by repeating the process on the initial sample.

The fish in the samples were anesthetized in a 1/20,000 solution of MS222; fork length

to the nearest millimeter and weight to the nearest gram recorded from each fish; and scales selected from a position just below the insertion of the dorsal fin. To eliminate the possibility that anesthetized fish might influence the experimental results, all samples were removed from the experiment.

Scale analysis

Several scales from each of 10 fish were mounted dry between a pair of glass slides. The ends of the slides were secured with masking tape, and the slides appropriately labeled. Scales were examined microscopically at a magnification of 83 diameters.

Starvation is reflected upon the scales of hatchery-reared sockeye salmon by two or three faint, closely set, and often broken circuli. The recognition of these circuli is dependent on their contrast with the surrounding circuli pattern. Scales from slow growing fish, therefore, do not reflect the effects of starvation as do scales from rapidly growing fish. Most of the experimental fish, typical of hatchery-reared salmon, were rapidly growing fish; consequently, starvation induced an abrupt, hence easily recognized, change. However, even fish that grew rapidly both before and after starvation did not reflect the mark in significant numbers until 2 to 4 weeks after they had resumed feeding. The position of the mark, relative to the center and edge of the scale, is dependent upon the amount of pre-starvation and poststarvation growth. Preliminary observations revealed no apparent difference in the intensity of the marks induced by the various starvation intervals. Figure 2 contains photographs of typical scales from starved and control fish.

Because of the criteria employed to determine whether the scales of an individual fish were marked were entirely visual and somewhat subjective, we felt that personal bias must be reduced. To accomplish this we read all scales without reference to other scales on the slide and without knowing the feeding history of the fish bearing the scales. This was done by assigning a code number to the back of each slide and then changing the slides after each individual reading.



Figure 1. --Quartering net.

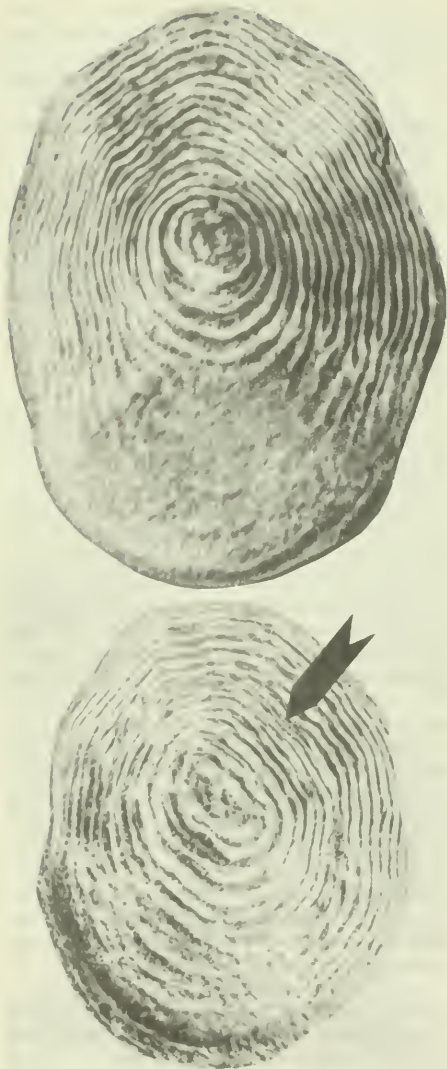


Figure 2.--Typical scales of control (upper) and starved (lower) sockeye salmon. Arrow indicates starvation check.

The results obtained by this method were evaluated statistically. The tests employed and their results are presented in the following section.

Before we could proceed with the analysis of the marking success, it was necessary to validate the use of the visual method for designating scales as either marked or unmarked. To do this, we hypothesized that the number called marked is independent of (1) reader and (2) of different readings by the same reader. Of the 652 scales examined from the 1960 experiment, reader A called 193 marked and 459 unmarked, whereas reader B called 165 marked and 487 unmarked. The total difference between readers was 4.29 percent. Comparison of these results by chi-square revealed no significant difference at the 5-percent level ($\chi^2 = 3.00$ with 1 d.f., $n = 652$). Comparison of two readings of a cross section of the 1959 scales made over 1 year apart by reader A revealed that the overall results were identical, 135 designated as marked and 77 unmarked. On the basis of this evidence, the hypotheses were not rejected, and with reasonable assurance we accept the validity of the results obtained by this method.

Marking success as referred to hereafter in this paper will be the results obtained by the most experienced reader.

Marking success

The results of the 1959 tests (fig. 3) are striking in two respects. First, excellent marking success was obtained in the 2- and 4-week lots and little success achieved in the 6- and 8-week lots. Second, in no case did a starvation mark appear on the scales in significant numbers until the fish had been returned to food for over 2 weeks.

Strikingly similar results were obtained in 1960 (fig. 4). Excellent marking success was obtained in only certain test lots and in no case did marks appear in significant numbers until the starved fish had resumed feeding for over 2 weeks. Marks were detected in 67, 100, and 96 percent of the 4-week test lot after 4, 6, and 8 weeks of resumed feeding in that order. Seventy-one percent of the 6-week test lot exhibited marks after having been returned to food for 6 weeks.

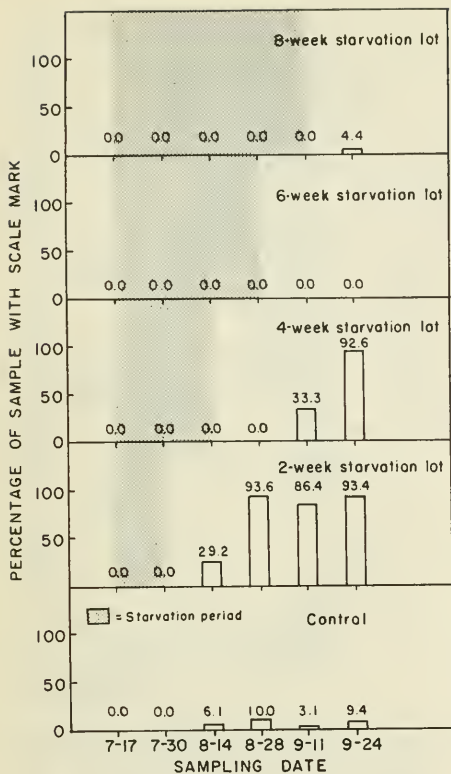


Figure 3.--Results of the 1959 experiment to mark the scales of sockeye salmon fingerlings by short periods of starvation.

In either year, scales of less than 5 percent of the control fish were judged to be marked.

Wide variation is noted in the marking success obtained in the three 2-week lots in 1960. The first of these exhibited 87-, 92-, 100-, and 93-percent marking success after having been back on food for 4, 6, 8, and 10 weeks. Only moderate success is noted in the second 2-week test lot even after 8 weeks of renewed feeding, and little marking success was obtained in the third 2-week lot.

Mortality

Mortality was not a factor in the 1959 experiment. Only one dead fish was noted and

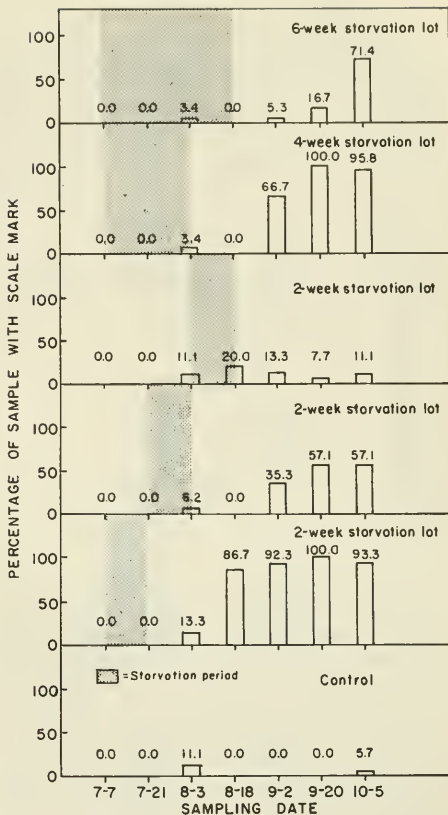


Figure 4.--Results of the 1960 experiment to mark the scales of sockeye salmon fingerlings by short periods of starvation.

that was found on the last day of the experiment in the 8-week test lot. Mortalities occurred in only the starvation lot in 1960, but these mortalities did not become a factor until the 6th week. Feeding was resumed at that time after which mortality reached its peak and then decreased (table 1).

Growth

The growth data of the 1959 experiment are presented in table 2 and figure 5. In addition to mean length and mean weight, condition factor ($\frac{100,000 \text{ mean weight}}{\text{mean length}^3}$) is presented for

TABLE 1
Mortalities in the 1960 6-week
starvation lot by weekly interval

Week	Mortality
1	13
2	0
3	1
4	1
5	4
6	11 (feeding resumed)
7	14
8	7
9	1
10	0
11	0
12	0
13	0
14	0
Total	52

each sample. Condition factor is widely used by fishery biologists as an expression of relative robustness or "degree of well being" of the fish. Condition factor was not calculated for the initial 1959 test lot sample due to an oversight in which lengths were not recorded. In table 2 the initial mean weight of the control lot (3.94 grams, n. = 84) is significantly higher than that of the test lot (3.41 grams, n. = 117). This unexpected difference may be due to (1) size selectivity in the dip net method used to halve the original sample of 1,200 fish, (2) a sampling error, or (3) a recording error. In any case, we do not feel that this discrepancy materially affected the overall experimental results.

With few exceptions, fish gained weight when fed and lost weight when starved in 1959. We initially suspected that two of these exceptions, the weight loss by the fast growing control and 2-week test lots from September 11-24, might be mainly due to the previously described irregular feeding during that period. Subsequent examination of the 1960 data (table 3 and fig. 6) revealed the same type of pattern (a growth slowdown in some lots and an actual loss of weight in others) after September 15

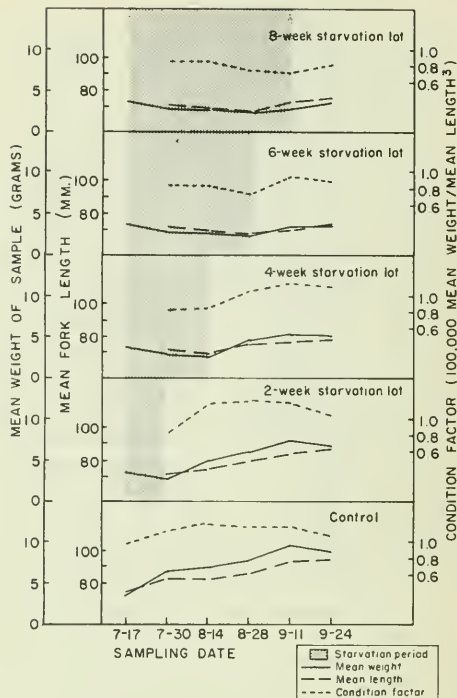


Figure 5.--Growth rates of the various lots in the 1959 experiment to mark the scales of sockeye salmon fingerlings by short periods of starvation.

of that year despite regular feeding. It appears, then, that growth slows naturally after mid-September, particularly in the lots with the higher condition factors. This slowdown in naturally occurring growth may have been aggravated in 1959 by irregular feeding.

Another exception to the general rule that fish gained weight when fed and lost weight when starved was an unexpected gain by the starved fish between August 28 and September 11, 1959. Because the condition factor, unlike mean length and weight of that lot, decreased during that period, it appears that a sampling error was involved, i.e., that the sample somehow contained fish that were longer and heavier but not more robust than those in the previous sample. Evidence indicates that a similar type of sampling error occurred in the

TABLE 2.--Growth statistics of the 1959 experiment to mark sockeye salmon scales by starvation.

Lot and sampling date	Mean weight	Percentage weight gain or loss	Final mean weight	Mean fork length	Condition factor	Sample size
8-week test:	<i>g.</i>		<i>Percent of control</i>	<i>mm.</i>		
July 17	3.41					117
30	2.94	-14		70.83	0.827	24
Aug. 14	2.78	-5		69.18	0.839	33
28	2.30	-21		67.29	0.755	31
Sept. 11	2.83	+23		72.83	0.730	36
24	3.24	+14	37	73.56	0.813	23
6-week test:						
July 17	3.41					117
30	2.94	-14		70.83	0.827	24
Aug. 14	2.78	-5		69.18	0.839	33
28	2.30	-21		67.29	0.755	31
Sept. 11	3.45	+50		71.07	0.959	30
24	3.49	+1	39	73.14	0.893	29
4-week test:						
July 17	3.41					117
30	2.94	-14		69.18	0.839	24
Aug. 14	2.78	-5		67.29	0.755	33
28	4.53	+63		75.30	1.069	30
Sept. 11	5.08	+12		76.40	1.137	30
24	5.09	0	58	77.28	1.102	28
2-week test:						
July 17	3.41					117
30	2.94	-14		69.18	0.839	24
Aug. 14	4.95	+68		74.62	1.192	24
28	5.77	+17		78.13	1.211	31
Sept. 11	7.11	+23		84.29	1.186	24
24	6.87	-3	78	86.69	1.050	45
Control:						
July 17	3.94			73.81	0.980	84
30	6.35	+61		82.24	1.143	54
Aug. 14	6.73	+6		82.00	1.221	33
28	7.54	+12		85.80	1.194	30
Sept. 11	9.49	+26		92.56	1.196	32
24	8.85	-7	100	93.70	1.075	33

third 2-week test lot in 1960. Because we feel that the sampling technique used throughout these experiments is sound if properly conducted, we can only speculate that these occasional errors were due to haste or other inattention.

The most important factor revealed by the 1959 growth data is the definite ability of

starved fish to resume feeding and growing when food is reintroduced. Examination of figure 5 reveals that all test lots reflected a weight gain during the first 2-week period of resumed feeding--the amount of which appears to be inversely related to the duration of starvation, i.e., fish starved a shorter time seemed to rebound more rapidly than did fish that had been starved for a longer

TABLE 3.--Growth statistics of the 1960 experiment to mark sockeye salmon scales by starvation.

Lot and sampling date	Mean weight	Percentage weight gain or loss	Final mean weight	Mean fork length	Condition factor	Sample size
6-week test:	8.			<i>mm.</i>		
July 7	2.75			66.79	0.923	38
21	2.49	-9		67.68	0.803	31
Aug. 3	2.11	-15		66.76	0.709	29
18	2.03	-3		67.42	0.662	26
Sept. 2	2.54	+26		68.74	0.782	19
20	3.80	+50		72.83	0.984	18
Oct. 5	4.13	+9	33	75.29	0.968	14
4-week test:						
July 7	2.75			66.79	0.923	38
21	2.49	-9		67.68	0.803	31
Aug. 3	2.11	-15		66.76	0.709	29
18	3.10	+47		68.75	0.954	12
Sept. 2	4.46	+43		75.11	1.052	18
20	4.96	+11		79.13	1.001	15
Oct. 5	5.84	+17	44	83.38	1.007	24
2-week test-A:						
July 7	2.75			66.79	0.923	38
21	2.49	-9		67.68	0.803	31
Aug. 3	3.42	+37		68.27	1.075	15
18	5.11	+49		77.13	1.114	32
Sept. 2	7.06	+38		85.92	1.113	13
20	8.13	+15		91.17	1.073	12
Oct. 5	8.44	+4	67	94.10	1.013	30
2-week test-B:						
July 7	2.75			66.79	0.923	38
21	4.42	+60		75.76	1.016	21
Aug. 3	3.38	-24		74.38	0.821	16
18	5.09	+51		78.05	1.070	17
Sept. 2	6.80	+34		85.65	1.082	17
20	8.73	+38		93.22	1.078	14
Oct. 5	8.15	-7	65	91.80	1.053	35
2-week test-C:						
July 7	2.75			66.79	0.923	38
21	4.42	+60		75.76	1.016	21
Aug. 3	6.53	+48		84.22	1.093	27
18	5.95	-9		86.60	0.916	10
Sept. 2	5.80	-3		81.40	1.074	15
20	8.77	+51		94.07	1.053	13
Oct. 5	8.10	-7	65	92.44	1.025	27
Control:						
July 7	2.75			66.79	0.923	38
21	4.42	+60		75.76	1.016	21
Aug. 3	6.53	+48		84.22	1.093	27
18	8.25	+26		91.31	1.084	29
Sept. 2	10.80	+31		97.16	1.177	19
20	11.65	+8		99.92	1.168	13
Oct. 5	12.52	+7	100	105.71	1.060	35

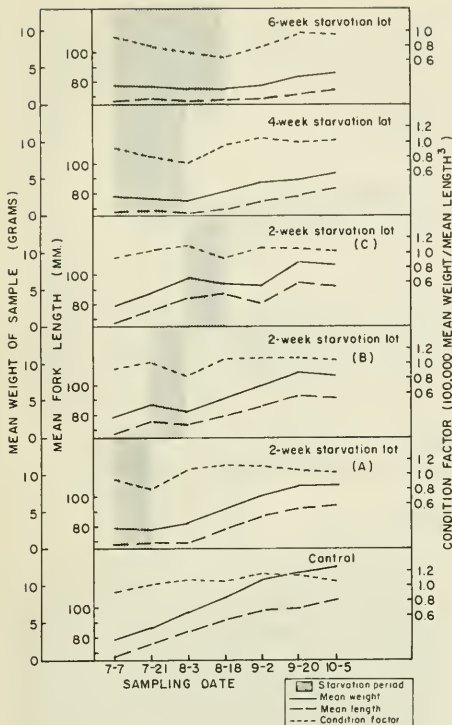


Figure 6.--Growth rates of the various lots in the 1960 experiment to mark the scales of sockeye salmon fingerlings by short periods of starvation.

duration. Although growth was noted in all test lots following reintroduction of food, in no case within the experimental confines did the mean weight of the test fish exceed that of control fish. The amount by which the mean weight of the various test lots lagged behind that of the control fish corresponds directly to the duration of the starvation period. It is noteworthy that at the end of the experiment, the condition factor of the control fish was roughly the same as that of the 2-week test lot and was even exceeded by that of the 4-week test lot.

Remarkable agreement exists between the main growth features of the 1959 and 1960 tests, despite differences in diet, duration of

the experiment, and initial size of the test fish. We have already shown that growth generally slowed after mid-September in both years. The ability of starved fish to resume feeding and growing was also observed in both years as was the final weight lag between the control and test lots. As was the case in 1959, the condition factor of some of the final 1960 test lot samples nearly equaled that of the control sample. Despite the variations in timing of the starvation period, the three 1960 2-week lots exhibited similar mean weights at the end of the experiment (8.44, 8.15, and 8.10 grams for the first, second, and third variations, respectively).

DISCUSSION

In our presentation of the experimental results we have placed particular emphasis on the variability between the various test lots with respect to such salient features as marking success, weight loss during starvation, and the final mean weight and condition factor. When the above items are considered together it is obvious that, of the intervals tested, the optimum duration of starvation is the shortest (2-week) period.

Widely divergent marking success was obtained in the three 1960 2-week test lots even though the lots finished the experiment with approximately the same mean weight. Further examination reveals that for these lots, marking success is inversely related to the timing of the starvation and recovery period, i.e., the first 2-week lots were marked the best, the second period the second best, etc. If, as we assume, starvation was the same in all cases, it is apparent that some other factor(s) associated with the time variable influenced marking success. Among the time (seasonal) associated factors of possible importance in this experiment were (1) water temperature, (2) size of the fish at the onset of starvation, and (3) growth rate. Let us examine the results of the three 2-week test lots in terms of these factors.

The mean daily water temperature during the starvation (fig. 7) period was 59.5, 61.5, and 59.7 degrees for the first, second, and third 2-week test lots, respectively. The

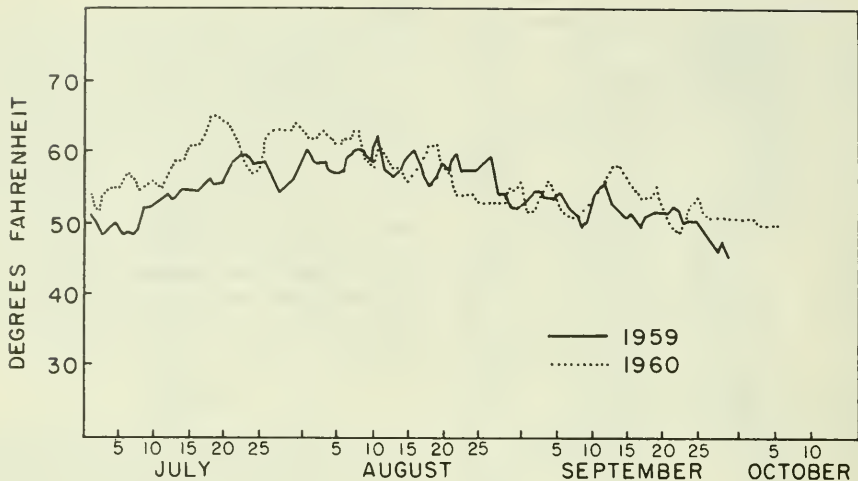


Figure 7.--Mean daily water temperature during the 1959 and 1960 experiments.

means for the corresponding 6-week recovery periods are 58.5, 56.1, and 53.4 in that order. These limited data suggest, then, that marking success, although independent of mean temperature during starvation, is related to mean temperature during recovery.

When we consider another time dependent factor, size of the fish at the onset of starvation, we find that the smaller the fish at the onset of starvation the greater the marking success. No relationship is evident between the size of the fish at this stage and the percentage weight loss during starvation (9, 24, and 9 percent in that order) or between marking success and percentage weight loss.

The 1960 data reveals that the marking success of the three 2-week test lots is related to relative growth of the control fish during the corresponding period (60-, 48-, and 26-percent weight gains by the control fish during the first, second, and third starvation periods in that order). Marking success is also related to the percentage weight gained by the three test lots during their 6-week respective recovery period (184, 158, and 36 percent for the three lots respectively).

Summarizing the discussion on the question of why certain 2-week lots marked better

than others, it appears that marking success is strongly influenced by one or more time (seasonally) related factors such as falling temperature or reduced hours of daylight, hence reduced growth during the recovery period. To obtain optimum results starvation should take place early when the fish are small and their growth rate fast thus allowing the major portion of their recovery period to fall in midsummer when growth is still relatively rapid.

CONCLUSIONS

The experiments described in this paper were the first in a series to determine whether a starvation-marking technique could be developed to mass-mark populations of hatchery-reared salmon. The results of these initial tests have yielded certain conclusions on one hand and point out the need for further experimentation on the other. The conclusions are:

1. Under the proper conditions of timing and duration, a highly recognizable mark can be imprinted on the scales of hatchery-reared sockeye salmon. This can be accomplished with little mortality due directly to starvation.

2. A 2-week interval commencing no later than mid-July appears to be the optimum duration and time for marking success. Timing appears to be important because it allows time for a summer rather than a fall-recovery period. Fish whose recovery period extends into the fall do not mark well.

3. The scale mark does not show in appreciable numbers until the fish have resumed feeding for 2 to 4 weeks.

4. Starved fish readily resume feeding and growing when food is reintroduced. Although they do not make up for lost growth (observation time limited to 10 weeks after starvation), they regain their robustness and outward vigor.

From the experimental results have emerged the following questions:

Could marking success be obtained with a starvation interval of less than 2 weeks? Might not the intensity of the mark be made more distinct by surrounding it with areas of artificially accelerated growth? Could marking success be obtained by starving the fish earlier in their life? Do starved fish ever make up for lost growth? What is the long-range effect of starvation on survival? What is the mark recognition to the migrant stage? To the adult stage? Do wild fish sometimes lay down a false check that might be confused with the starvation mark? Or, will the starvation mark be confused with a true annulus? Large-scale experimentation to answer some of these and other questions associated with the development of a starvation-marking technique is being continued at Leavenworth in 1961.

SUMMARY

1. Wild and hatchery-reared sockeye salmon of the same parent stock are mixed in the seaward migrations from Lake Wenatchee. An economical means is needed for separating the components, thus allowing evaluation of the wild and hatchery production.

2. Experiments were conducted at the Leavenworth hatchery in 1959 and 1960 to

determine if the scales of hatchery-reared fish could be identifiably marked by short periods of starvation. Fish were starved 2, 4, 6, and 8 weeks and returned to food. Lots were sampled every 2 weeks for length, weight, and scales. In 1959 marking success in the 2-week test lots ranged between 87 and 94 percent after the fish had been returned to food for 4 weeks. Excellent marking success was also obtained in the 4-week lot but not until the fish had resumed feeding for at least 6 weeks. Few marks were noted in the 6- or 8-week lots. In 1960, satisfactory marking success was obtained in only the first of the three 2-week lots. The 4-week lot marked well but, as in 1959, not until after 6 weeks of resumed feeding. In either year, control fish seldom were marked.

3. Mortalities (1 in 1959 and 52 in 1960) were confined to the starvation lots. Mortality did not become a factor in 1960 until the fifth and sixth weeks of starvation.

4. Fish generally lost weight during starvation but resumed feeding and growing when returned to food. Although most lots of starved fish rapidly regained their robustness (condition factor often met or exceeded that of control fish), they did not make up for lost weight. Fish starved 2 weeks lagged behind control fish by 22 to 33 percent at the end of the experiment. Growth generally leveled off after September 1.

5. We are unable to pinpoint the exact reason that some 2-week lots marked and others did not. Indications are that timing is important, e.g., starvation should be conducted in early July, thus allowing fish to recover during the summer rather than during the fall months when growth naturally slows.

6. On the basis of these experiments we conclude that under certain conditions a highly identifiable mark can be imprinted on the scales of hatchery-reared sockeye with little mortality. Weight loss due to starvation may be offset at least in part by the fact that the fish, although smaller, have a condition factor comparable to or higher than that of the control fish.

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