ON THE EFFECTIVENESS OF SPERMATOZOA OF THE PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) AT VARYING DISTANCES FROM POINT OF DISPERSAL

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INTRODUCTION

The suggestion often has been made that, in the natural spawning of salmon, the ratio of males to females in the escapement need not even approximate 1 : 1; that a relatively few males can achieve successful and efficient fertilization of the eggs of a great many females. This could be accomplished in only one of, or a combination of, two ways: (1) By each male giving service to two or more females, fertilizing the eggs of each in turn, or (2) by the spermatozoa of spawning males being disseminated throughout the stream waters in such a manner that the eggs of all females in the stream will be fertilized, even though each female does not have a male in attendance during her spawning act.

It is unlikely that dual or multiple service by one male played any significant part in the perpetuation of virgin salmon populations, since existing data indicate that, on the average, the two sexes are produced in approximately equal numbers; with no imposed selectivity in mortality rates the sexes would have remained fairly equal in numbers, and there would have been little reason or opportunity for one male to give service to more than one female. With present-day fishing intensities and low-population levels, the sex ratio may vary from year to year in any stream, but seldom (if ever) is the effect of selective fishing accurately measured, and there is a reasonable doubt that the natural production of the sexes is as varied as may be indicated by weir counts or other methods of sampling within the river systems. Foerster (1931: 123) stated, "A shortage of males would seem to represent either one of Nature's weaknesses or a faulty system of regulation of commercial fishing." It is probable that when there is a significant superiority in numbers of either sex in the escapement, the inequality is caused by selective fishing.

Rutter (1902 : 134), in a study of the quinnat (king) salmon of the Sacramento River, reported that there was no evidence that "one sex is produced more than the other." Barnaby (1944: 277) found that with the red salmon of the Karluk River, Kodiak Island, Alaska, the females predominated in the escapement each year from 1923 to 1933, on the average constituting 56.1 percent of the fish in the river. He recognized that the gill-net fishery a few miles from the river mouth was selective, taking more males than females, but he did not believe this factor accounted for the discrepancy in the sex ratio of salmon found in the river, and he offered no explanation for the phenomenon.

At McClinton Creek, a tributary to Masset Inlet, Queen Charlotte Islands, the pink salmon spawn only in even-numbered years. Pritchard (1939: 238), presented counts of the sex ratios obtained at the weir for the cycle years 1930–38. These varied, but the over-all average showed only a slight predominance of females, which constituted 50.6 percent of the escapements. The effect of the commercial fishery on the ratios was not discussed.

In a study of the chinook (king) salmon of the Willamette River, Mattson (1948: 24) found that at the egg-taking station on the McKenzie River, a tributary to the Willamette, 51.3 percent of the fish were females. There was no discussion of the possible effect of commercial fishing.

At the United States Fish and Wildlife Service biological station, Little Port Walter, Baranof Island, Alaska, upstream migration counts have been obtained each year since 1934. During those years the sex ratios varied annually at the Sashin Creek weir, with no over-all significant advantage by either sex. The largest escapements obtained there were in 1941 and 1942. In the former year,

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the escapement was 84,304, of which 46.2 percent were males; the following year, the escapement increased to 92,085, with 55.4 percent being males. The average for the 2 years was 51.1 percent males. It was believed that the commercial fishery had little effect on the spawning migration, since the area immediately surrounding Little Port Walter was relatively free from fishing effort.

Regardless of the sex ratio which obtains on the spawning grounds, pairing or mating of the sexes almost always occurs, and observations made by the author have indicated that with the various species of salmon there is a definite tendency toward monogamy. This tendency has been noted with salmon which, because of peculiar markings, could be identified throughout their courtship, nest building, and spawning activities. In each case, at the termination of spawning the male appeared to be in such a spent physical condition as to be incapable of attending another female, and he was observed on or near the nest until his death, which occurred soon after spawning. In these cases, there appeared to be little differential in the mortality rates of the two sexes. While it is probable that a male occasionally will attend more than one female, either because of the early death of the first mate, or because he has been driven from her by a stronger and more pugnacious male, the effectiveness of multiplicity of service must be demonstrated conclusively before it can be considered an important factor in salmon propagation under natural conditions.

It is not to be assumed that the attendance of a member of the opposite sex is a stimulus necessary to spawning responses. Rutter (1902: 135) stated, "the presence of the other sex is not necessary to excite either to spawning efforts. I have seen the female spawning alone at Battle Creek fishery, and other persons have reported similar observations at other places. In September 1900, I saw a male spawning alone near Sims, the female having been killed by a sportsman in order to get trout bait. Like observations are reported by other persons." The author has observed both pink- and red-salmon females extruding eggs over a redd when no male was in attendance.

Eggs deposited by these lone females could be fertilized only by spermatozoa which drift down to them from males spawning farther upstream. Dissemination of spermatozoa does occur, and was noted by Schultz (1935: 74) in a study of the spawning habits of landlocked sockeye. He stated, "Milt was plainly visible near the conclusion of the spawning act, which lasted about 2 seconds. The milt formed a dense, small, white cloud about 3 inches wide and 4 inches long, which was carried downstream by the current." When intensive spawning is taking place in any stream, great numbers of spermatozoa must be carried downstream and disseminated by the current. The degree of fertilization of eggs deposited by unattended females will be established by the density of the population of spermatozoa, and by the length of time during which the individual cells retain their vitality.

Rutter (1902: 73) reported that spermatozoa of king salmon retained their vitality for from 3 to 5 minutes, although the fertilization achieved under laboratory conditions fell from 88 percent at 30 seconds, to 38 percent at 1 minute, and to 4 percent at 1.5 minutes. Gray (1920: 309) stated that with the spermatozoa of trout (species?) "the period of motility is extremely short, namely 1½–2½ minutes," and he further stated that this period of motility was shortened markedly when the spermatozoa were placed in water.

The union of the spermatozoa and ova must take place very quickly under natural spawning conditions, since the rate of fertilization with normal, paired spawning appears to be very high. Hobbs (1937), in a study of the quinnat salmon, brown and rainbow trout, found that not more than 3 percent of the eggs extruded by the female failed to lodge in the prepared redds, and that lack of fertilization within the redds did not exceed 3.3 percent. White (1930) studying the eastern brook trout (*S. fontinalis*) found that through natural spawning, up to 79 percent fertilization was attained, while in his study of the quinnat salmon, Rutter (1902) conducted a series of experiments and concluded that "these various experiments indicate a high percentage of fertilization, probably over 80 percent."

Whenever a significant number of females spawn unattended, the success of spawning will be affected unless fertilization by free-drifting spermatozoa is as efficient as that obtained with natural, paired spawning. In order to test the degree of fertilization attained by these disseminated spermatozoa, a series of experiments were begun at Little Port Walter in the fall of 1941. As a preliminary step, a standard hatchery trough was set up, and a supply of water introduced. During the experiments the temperatures of the trough water were the same, or nearly the same, as those existing in Sashin Creek. The

tests were begun on September 15, by which time 34,145 pink salmon had been counted through the weir, with most of these fish being engaged in nest-building or spawning activities. Since only 3,700 feet of the stream are available to the salmon, and the average width is somewhat less than 60 feet, this number of fish constituted a large and concentrated (about 1.4 fish per square yard) spawning population; conditions therefore appeared favorable for the tests.

EXPERIMENTS IN 1941

In the late afternoon of September 15, a number of pink salmon of each sex were obtained from the lower reaches of the stream. Only completely ripe fish were selected, and all were killed and bled immediately. The abdominal cavity of the females was opened with a short knife as the eggs were needed, and three screen trays were partly filled in turn. As each tray was laden with eggs, another tray was placed over the top as a lid, and the trays were submerged in the creek at a point above the influence of salt water, yet below the majority of spawning fish. Each tray was rotated slowly for 5 minutes in such a manner as to assure complete circulation of water through the egg mass. In this way any active spermatozoa in the waters would have had ample opportunity to come in contact with and fertilize the ova. After 5 minutes' exposure, each tray was placed immediately in the upper end of the trough and secured there. A second group of eggs, estimated to be equal in numbers to those used in the above trial, were fertilized by standard hatchery methods and placed in the lower end of the trough; these constituted the control.

By November 1 all viable eggs appeared to be eyed out, but the experiment was continued until November 25, at which time cold weather threatened to freeze the pipe line. The trays were removed, the eggs examined and counted. It was assumed that fertilization had not been attained with any of the eggs which had failed to eye out, or which had been removed as dead during the course of the experiment. The counts, calculated volumetrically, showed that originally there had been 102,300 eggs, divided almost equally between the test and control groups. By sample counts it was determined that in the control group a fertilization of 62.8 percent had been achieved. A detailed study of the eggs in the test group showed that none of the ova had been fertilized.

EXPERIMENTS IN 1942

While the 1941 tests were enlightening, they by no means were conclusive, and further tests were made in 1942. Standard wire hatchery baskets were obtained, and two troughs were set up several days before the tests were to begin; the water supply and method of introduction were the same as those used the preceding year. This experiment was begun on the afternoon of September 12, a cloudy day, with the air temperature at 53° F. Although the level of the creek had been raised slightly by rains the preceding night, the water was not perceptibly discolored; conditions were normal. The temperature of the creek water was 55° F., while that in the troughs was 53° F.

A characteristic section of the stream was chosen for the first group of 1942 tests, near the place at which the work had been done in 1941. The water here was approximately 6 inches deep throughout; the bottom was composed of small and medium rubble. The current was moderate, with a velocity of 40 feet per minute.

At the upper end of the chosen section a marker stake was driven into the gravel, while downstream from it, alined with the current, five other stakes were driven at 1, 2, 4, 10, and 20 feet, respectively, from the marker stake. When this had been done, several ripe pink salmon, both males and females, were netted from the holding pool below the weir. The males were held in large buckets; the females were immediately killed, bled, and their eggs transferred to wire baskets. When enough eggs had been obtained for the first basket, four men, each carrying a ripe male salmon, stepped into the stream and began stripping or milking the fish so that the four streams of milt entered the water near to, and even with, the starting stake. A white cloud of milt was carried downstream and, although there was considerable lateral diffusion, the concentration of milt was such that the cloudiness of the water was distinct and dense at a distance of more than 50 feet.

Immediately this milking of male salmon had begun, the basket of eggs was immersed in the stream waters directly in the cloud of milt, the upper edge of the basket even with the 1-foot stake. The basket was rotated gently for a period of 3 minutes, during which time the flow of milt was maintained; spent male salmon were replaced as the necessity arose. At the end of 3 minutes the

eggs were washed in clear water and transferred to one of the hatchery troughs.

New fish were used for each of the succeeding tests, the performance being repeated at each of the remaining stakes, namely, 2, 4, 10, and 20 feet from the point at which the milt was ejected into the water. Later, one basket of eggs was fertilized by standard hatchery methods; this group constituted the control. The troughs then were covered with previously prepared lids.

Although the 1941 experiment had showed no fertilization among the eggs submerged in free-running creek water during the day, it was decided to repeat the tests in the dark of night. Since the extrusion of eggs and milt in natural spawning seldom is observed, it was thought possible that with pink salmon, spawning might take place principally at night, and that spermatozoa would be disseminated throughout the creek waters during the night hours, whereas none might be present in the daytime.

On the dark, cloudy night of September 22, the second group of 1942 tests was begun. Several ripe females were captured, killed, bled, and stripped into a wire basket at 10 p. m. The basket of eggs was submerged immediately into the creek waters at the usual working place. After 5 minutes of gentle rotation in the water, the basket was removed and placed in one of the hatchery troughs. A second group of eggs was treated in a like manner at midnight. At 10 p. m. the air temperature was 46° F., at midnight 44° F. From the former until the latter hour, the temperatures of both the stream and the trough waters remained constant at 53° F. At this time the stream level was normal, the waters clear. The weir count of pink salmon stood at 78,055, with most of these fish engaged in some phase of spawning activity, and well dispersed throughout the stream.

Standard hatchery procedures were followed in caring for the eggs during the course of the experiment, which was terminated on November 3 because of cold weather. The baskets were removed from the toughs, and counts of eggs in each basket determined volumetrically. As before, it was assumed that eggs which had failed to eye out, and eggs that died during the experiment never had been fertilized. All eggs were counted as fertilized when the eyespot could be seen, even though the larvae might be dead at the time of inspection. The number of fertile eggs in each basket was determined by actual count; the results are presented in table 1.

The attempt to achieve fertilization of ova by free-drifting spermatozoa (second group of tests) was completely unsuccessful; a detailed study of the eggs used in these tests yielded only negative results. The escapement was greater in 1942 than in the preceding year, and it is doubtful whether other pink-salmon streams in the Territory often reach the spawning-intensity which existed in Sashin Creek at either time. It, therefore, seems improbable that for this type of fertilization, more propitious conditions often (or ever) occur.

TABLE 1.—*Results of experiments made on pink salmon at Little Port Walter, Alaska, in 1942 to test the relationship between the effectiveness of spermatozoa and distance from point of dispersal*

Distance (in feet) of introduction of sperm to eggs	Number of eggs fertilized	Percentage of eggs fertilized	Number of eggs not fertilized	Percentage of eggs not fertilized
1	10,193	78.7	2,757	21.3
2	8,482	66.6	4,258	33.4
4	6,794	58.8	4,756	41.2
10	2,343	17.9	10,747	82.1
20	504	4.8	10,021	95.2
Control	9,822	86.4	1,553	13.6
Free-exposed, 10 p. m.	0	.0	13,510	100.0
Free-exposed, midnight	0	.0	14,350	100.0

It has been suggested that failure of fertilization by this free-exposure method might be the result of frightening the spawning fish while performing the necessary work in the stream. This is highly improbable. Observations made over a period of years have indicated that salmon engaged in spawning activities (whether actually spawning or nest-building) are not disturbed easily when approached quietly from downstream. These precautions were taken during the tests of both years, while in the night operations of 1942 no lights were used at or near the scene of activity. The females from which eggs were obtained were captured well away from the test location, and only one man entered the stream so as to minimize possible disturbance. In neither year was there any evidence of an interruption of the normal activities of nearby salmon.

Table 1 shows that a fertilization of 86.4 percent was achieved with the control group. The differential between this figure and the one obtained in the control group in 1941 is unexplained, but to the present discussion it is unimportant. Among the baskets of eggs exposed to spermatozoa artificially introduced to the stream waters (first group of tests), the rate of fertilization decreased from 78.8 percent at a distance of 1 foot, to 4.8 percent at a distance of 20 feet. The relationship of degree of fertilization

to time and distance is curvilinear, and is shown by the dotted line in figure 1, which is an exponential curve fitted by the method of least squares.

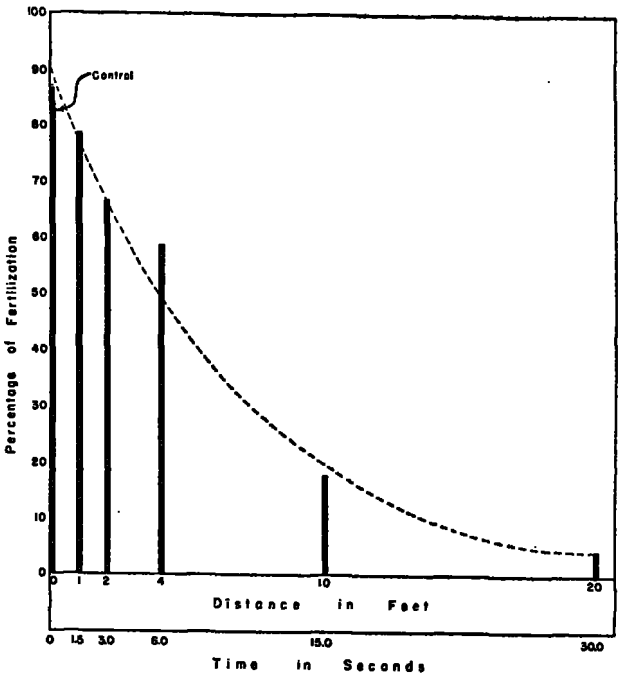

FIGURE 1.—The percentage of fertilization achieved in the various baskets is shown on the ordinate axis. On the axis of abscissa, O represents the point of dispersal of the spermatozoa, the distance to the baskets being designated in feet. Below is shown the number of seconds during which the spermatozoa were exposed to the water before coming in contact with the eggs in the baskets. The data from table 1 were used to produce the smoothed curve, dotted in the figure. The equation was: Log (percentage of fertilization) = 1.96 − 0.065 (distance in feet). The calculated standard error of estimate was 5.1 percent.

It is believed that the decrease in fertilization at the greater distances was caused not by dispersion of the spermatozoa but by their loss of vitality induced by extended exposure to the water. The lateral dispersion was considerable (the vertical dispersion having been limited by the shallowness of the water), but the density of the cloud of milt was such that even at 20 feet the eggs submerged in the basket almost were obscured from view. It is believed that many spermatozoa came into contact with each egg in the baskets, but that their vitality was so lowered that a union of the gametes was impossible. Certainly the density of the milt was greater at all distances than is found under natural conditions; no general cloudiness of the water from this source has been observed by the author or reported in the literature.

CONCLUSIONS

A consideration of the results obtained in these experiments makes it apparent that spermatozoa disseminated throughout the stream waters by spawning males will not achieve fertilization of eggs being deposited farther downstream, and that the eggs of lone-spawning females remain almost or completely unfertilized. For efficient fertilization of eggs each female must be attended by a male during her spawning acts. It remains to be demonstrated whether dual or multiple service by males can offset the difficulties imposed by the males being outnumbered greatly by the females. Until such demonstration, conclusively, can be made it will be advisable to regulate the fishery in such a manner that the ratio of males to females is approximately 1 : 1 in each spawning escapement.

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