A METHOD OF MEASURING MORTALITY OF PINK SALMON EGGS AND LARVAE

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ABSTRACT

A method of estimating total mortality of salmon eggs and larvae from time of spawning to time of sampling is described. The method provides a measure of mortality, regardless of cause, and is unaffected by the disappearance of dead eggs or larvae.

Total mortality rate is calculated from estimates of abundance and fecundity of parent female spawners (potential egg deposition) and abundance of live eggs and larvae in spawning beds at the time of sampling. A hydraulic sampler, developed to collect samples of eggs and larvae, is described.

Two sources of bias are considered in mortality rates calculated from percentages of dead eggs and larvae in samples: (1) eggs not initially buried by spawners, and (2) eggs and larvae disappearing from the spawning bed. Neither source of bias exists where mortality is calculated from estimated potential egg deposition and abundance of live eggs and larvae at time of sampling.

The efficiency of estimating abundance of eggs and larvae is affected by their tendency to be clustered. Clustering causes the sample variance to increase with the sample mean, and confidence limits of estimates of abundance may be widely separated. The use of a logarithmic transformation improved the efficiency of estimates in certain instances.

A chi-square test based on the premise that a change in the total mortality level would be associated with a change in frequency of occurrence of dead or live eggs and larvae in samples is described. This test was useful in identifying periods when significant mortality occurred, especially where confidence limits of total mortality percentages were widely separated.

Pink salmon (*Oncorhynchus gorbuscha*) is the most abundant of the Pacific salmons and in most years provide a larger commercial catch than all other salmon species. In the eastern Pacific, pink salmon are commercially important from Bristol Bay, Alaska, to Puget Sound, Washington. They support a major fishery in southeastern Alaska where there are about 1,100 spawning streams (Martin, 1959).

Spawning female pink salmon bury their eggs in gravel beds of coastal streams and die soon after spawning. Their eggs are deposited in summer or autumn and hatch within 60 to 90 days. The larvae usually remain in the streambed over winter and the fry emerge and migrate immediately to salt water the following spring. In most instances fry do not feed in fresh water.

There is evidence that fry production is ultimately limited by the area available for spawning. Ricker (1954) and Neave (1958) note that escapements of large numbers of spawners do not necessarily result in large yields of fry. Their findings indicate that the yield of fry approaches an asymptote (and may even decline at high spawning densities) with increased density of spawners. Ricker points out that optimum escapement would most likely occur at a level intermediate between low and high density of spawners.

Determining optimum escapement will depend

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ultimately on a thorough understanding of factors limiting fry production. Total fresh-water mortality of pink salmon has been measured in McClinton, Hook Nose, and Morrison Creeks, British Columbia (Pritchard, 1948; Neave, 1953; Hunter, 1959) and in Sashin Creek, southeastern Alaska (Davidson and Hutchinson, 1942; Hutchinson and Shuman, 1942; Merrell, 1962). Mortalities in those streams ranged from 76 to over 99 percent.

Periods in the fresh-water life of pink salmon during which major mortalities occur have not yet been sufficiently elucidated. Variable mortalities occur among adults before spawning, among eggs and larvae, and among fry. The period beginning with egg deposition and terminating with emergence of fry typically encompasses 80 to 90 percent of the total fresh-water life of pink salmon, and during most of this period the developing embryos and larvae are immobilized and concealed by the surrounding solids of the streambed. While buried in the streambed, eggs and larvae are subjected to numerous stresses imposed by the environment that may cause death.

The measurement of mortality in spawning beds is complicated in many instances by the disappearance of eggs and larvae from decomposition, scavenging, predation, and bed movement. Where disappearance of eggs and larvae has taken place, the percentages of dead eggs and larvae collected in samples will underestimate the true total mortality percentage. There is a need, then, to devise methods of estimating changes in density of eggs and larvae in spawning beds so that estimates of total mortality can be calculated. This paper describes procedures that can be employed to measure total mortality percentage of pink salmon eggs and larvae from egg deposition to any selected time during the period of streambed residence.

Studies on environmental factors causing mortality of pink salmon eggs and larvae were initiated by the Fisheries Research Institute, University of Washington, in 1948. These early studies were undertaken in streams near Ketchikan, Alaska, with financial support from the Alaska salmon industry. Industry-supported studies were discontinued after 1955, and in 1956 Saltonstall-Kennedy Act funds were provided by the Bureau of Commercial Fisheries to study the effects of logging on pink salmon. Mortality studies were continued as part of this program. The study streams were Harris River and Indian and Twelvemile Creeks, located on Prince of Wales Island about 40 miles west of Ketchikan.

Three important problems were considered in the development of methods used to measure periodically egg and larval mortality. First, equipment and methods for obtaining samples of eggs and larvae representative of the populations sampled were developed and tested. Second, equations for calculating total mortality percentage from the time of spawning to the time of sampling were formulated. Third, statistical methods to test for significant changes in total mortality percentage were tried.

COLLECTING EGGS AND LARVAE

Several workers have examined samples of eggs and larvae collected from spawning beds and have calculated the mortality percentage from the proportion of dead eggs and larvae in samples (Hobbs, 1937 and 1940; Cameron, 1940; Briggs, 1953; Hatch, 1957; Hunter, 1959; Mathisen, 1962). This method introduces bias into calculated total mortality percentages if parent females have failed to deposit eggs or eggs and larvae have disappeared from the spawning bed. To overcome these sources of bias, periodic estimates of abundance of eggs and larvae must be obtained. A method for calculating total mortality percentage from estimated density of live eggs and larvae was used.

Density of eggs and larvae in the spawning bed was estimated by collecting specimens from small discrete quadrats or circular plots (sampling units) ranging from 2 to 4 square feet in area. An estimate of the average number of eggs and larvae per square foot of spawning bed was obtained by sampling a number of units located randomly within a spawning bed. The accuracy of this estimate was influenced largely by the thoroughness with which eggs and larvae were collected from within a sampling unit without losing any, yet excluding eggs and larvae adjacent to the sampling unit. To meet the requirements of the sampling scheme special equipment for collecting eggs and larvae was developed. The apparatus, called a hydraulic sampler, was first tried in 1956.

DESCRIPTION OF THE HYDRAULIC SAMPLER

The hydraulic sampler utilizes suction created by passing water at high velocity through an annular aperture to mix air with water. The air and water mixture is injected into the streambed to free eggs and larvae from bottom materials. The device is patterned after equipment used by Mathisen (1962). The major difference is that Mathisen excavated both gravel and eggs with the suction created.

Basic components of the hydraulic sampler are a 7,200-gallon per hour centrifugal water pump and a cone assembly that creates a mixture of air and water. Water is delivered by the pump to the cone through flexible rubber hose of 1½-inch diameter. The mixture of air and water from the cone assembly is injected into the streambed through a nozzle (fig. 1).

A jacket between the terminal openings of the cone assembly receives water from the pump which is forced under pressure from the jacket at high speed through an annular aperture on the inside of the tube. Water flowing through the aperture creates a suction at the opening opposite the direction of flow, and water and air mix beyond the aperture as they flow toward the nozzle. The size of the aperture can be adjusted to regulate the quantity of air in the mixture. A disassembled cone is shown in figure 2. Drawings of the cone assembly are shown in figure 3.

OPERATION OF THE HYDRAULIC SAMPLER

Eggs and larvae were flushed from the streambed by rising air bubbles and water currents and were collected in a net attached to the downstream side of a metal frame outlining the sampling unit (fig. 4). Square frames enclosing 4 square feet and circular frames enclosing 2 square feet were tried. The circular frame is recommended because it has the smallest perimeter for a given area, which minimizes the number of eggs and larvae removed from outside the boundary of the sampling unit and not collected.

Penetration of the nozzle into the streambed was greatly facilitated by the flow of water and air from the nozzle. The depth of penetration depended largely upon size composition of bottom materials, being least where large cobbles were present. In mixtures of fine gravel and sand it was possible to penetrate readily more than 24 inches.

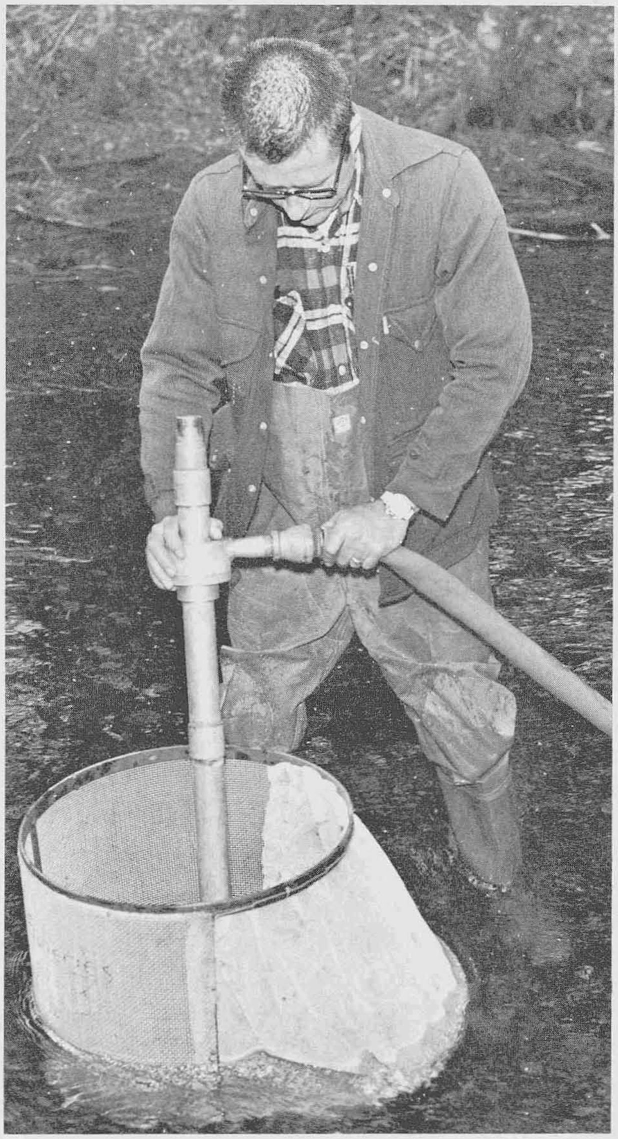

FIGURE 1.—Collecting pink salmon eggs or larvae with hydraulic sampler.

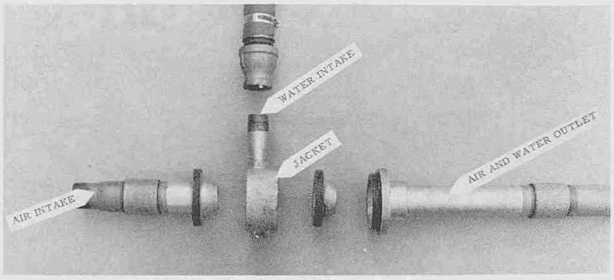

FIGURE 2.—Disassembled cone assembly of hydraulic sampler.

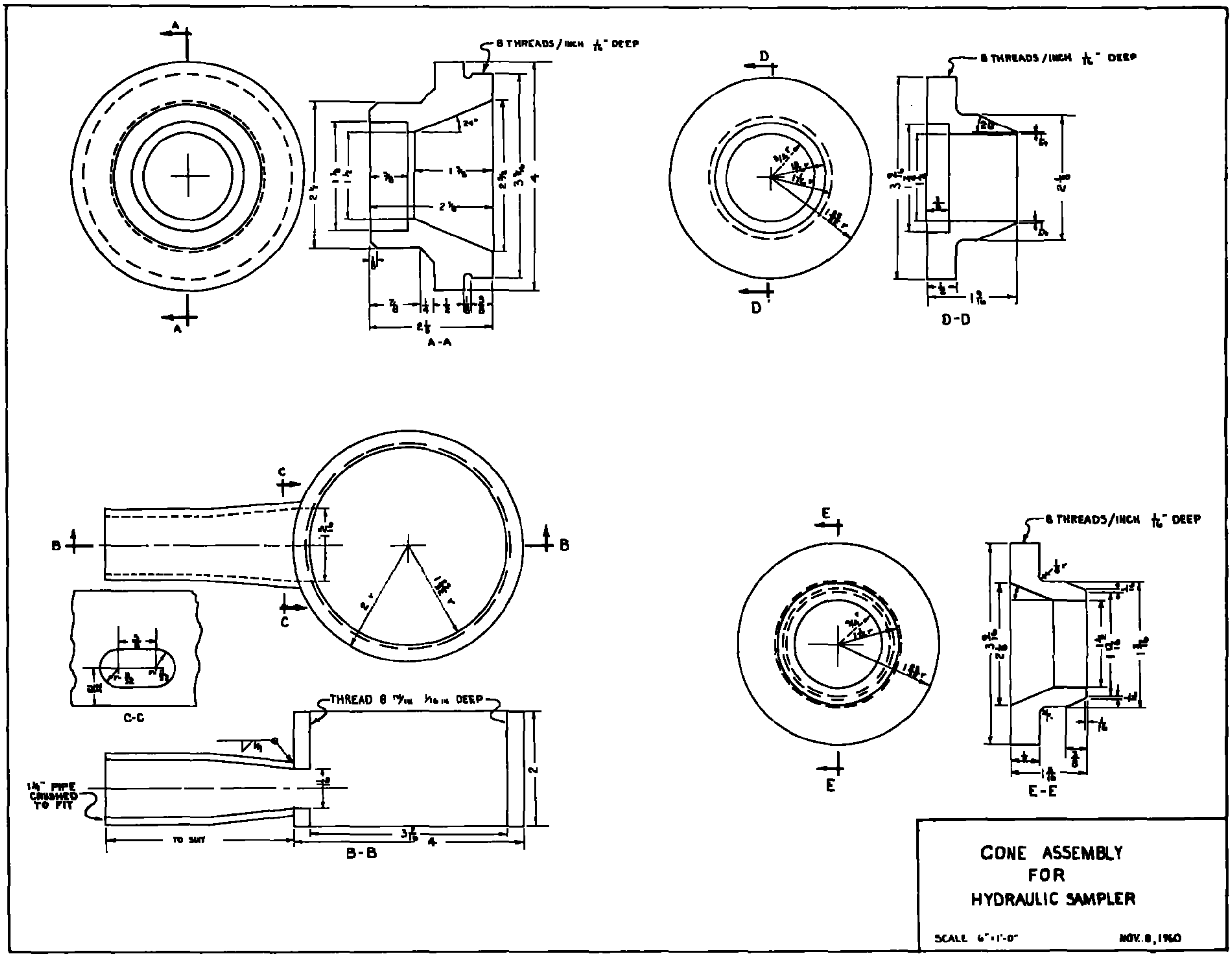

FIGURE 3.—Machine drawing of cone assembly of hydraulic sampler.

In this study, each sampling unit was sampled by two persons. As soon as the first person felt he had removed all eggs and larvae from within a sampling unit, the second person attempted to remove any that might have been missed.

PERFORMANCE OF THE HYDRAULIC SAMPLER

When a streambed is excavated with a shovel, it is difficult to collect eggs and larvae from one sampling unit without collecting specimens from adjacent units. Difficulties occur because stream bottom materials containing eggs and larvae shift horizontally when a hole is dug. With the hydraulic sampler, heavier materials were not transported away, and very little horizontal shift of the streambed occurred. Furthermore, the action of air and water currents was restricted to an area adjacent to the nozzle orifice, and the disturbance of the streambed beyond the boundaries of the sampling unit was minimized.

The percentage of eggs and larvae recovered from a sampling unit with the hydraulic sampler was estimated from results of two tests. First, after completion of the initial sampling by two persons, sampling units were resampled by a third person in an attempt to remove additional specimens. In the opinion of those doing the work, sufficient effort was expended in resampling to remove virtually all specimens within the boundaries of sampling units. In tests of this type, the two persons doing the initial sampling were not advised that the resampling was to be done. Second, known numbers of preserved eggs were buried. The persons doing the sampling were not advised either of the number or of the exact location of preserved eggs buried within a

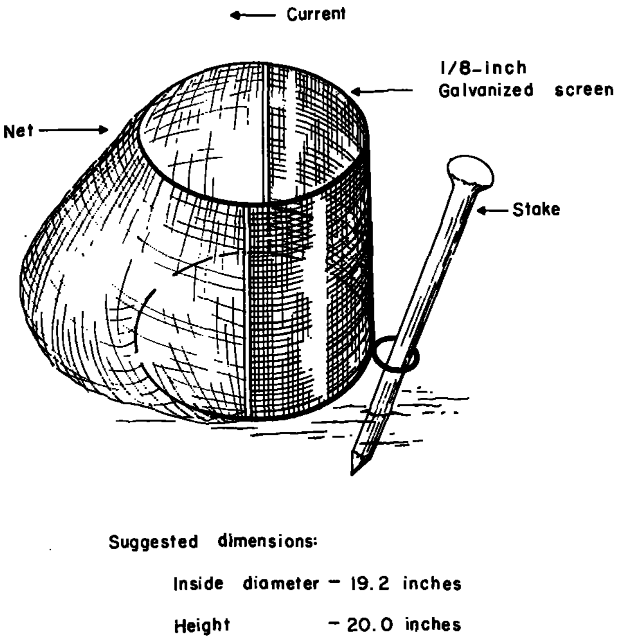

FIGURE 4.—Circular frame and collection net used with hydraulic sampler.

sampling unit. In tests of this type, no resampling was done to recover eggs not recovered by two persons sampling.

The mean percentage of eggs recovered after two persons had sampled was about the same for both types of tests—93 percent. Results of tests where the initial effort by two persons was followed by resampling by a third person are given in table 1. Table 2 gives results of tests where known numbers of preserved eggs were buried and recovered by two persons sampling.

TABLE 1.—*Number and percentage of naturally spawned eggs collected by two persons sampling followed by resampling*

Collected by two persons sampling	Collected by a third person resampling	Collected by two persons sampling
Number	*Number*	*Percent*
30	5	85.7
327	----------	100.0
108	7	93.9
85	1	98.8
249	5	98.0
235	126	65.1
634	12	98.1
521	35	93.7
45	----------	100.0
203	6	97.1
Mean	----------	93.0
Standard deviation	----------	10.7

To estimate the density of salmon eggs and larvae in a spawning bed, a recovery percentage of 90 percent was assumed. Furthermore, because a high percentage of eggs present in the test sampling units was collected, it was concluded that the possibility of introducing significant bias to estimates of abundance through an error in the assumed recovery percentage was minimized.

BROKEN SHELLS

Hobbs (1937), Cameron (1940), and Mathisen (1962) noted the presence of clean broken shells among eggs recovered from the gravel. The shells apparently originated from eggs damaged during excavation. Each author eliminated clean broken shells from his analysis because it was not possible to determine if these eggs were dead or alive when damaged.

TABLE 2.—*Number and percentage of buried preserved eggs collected by two persons sampling*

Buried	Recovered by two persons sampling	Recovered by two persons sampling
Number	*Number*	*Percent*
200	175	87.5
200	195	97.5
200	174	87.0
200	183	91.5
582	552	94.8
10	10	100.0
50	50	100.0
150	129	86.0
200	171	85.5
200	191	95.5
173	159	91.9
80	77	96.2
140	136	97.1
19	17	89.5
380	354	93.2
40	39	97.5
200	194	97.0
60	57	95.0
120	116	96.7
100	83	83.0
Mean	----------	93.1
Standard deviation	----------	5.1

We found that fewer eggs were broken by the hydraulic sampler than by a shovel. Comparisons were made between eggs collected by the two methods on Indian Creek in November 1956 (table 3). Observations were given equal weight for this comparison. Since clean broken shells averaged only 0.24 percent of the total eggs per sample, they are not included in our mortality estimates.

MEASURING MORTALITY

The percentage of eggs and larvae dying from the time of spawning to the time of sampling can be calculated from estimates of potential egg deposition and abundance of live eggs and larvae in the spawning bed at the time of sampling. It will be shown how total mortality percentage calculated in this manner is unbiased either by failure of females to deposit or bury their eggs or by disappearance of eggs and larvae from the spawning bed. Eggs that are not deposited may be retained in the coelom of the female and, may be consumed by predators, or may drift free of the spawning bed. Factors causing eggs and larvae to disappear include consumption by predators or scavengers, decomposition, superimposition of redds, and erosion of spawning beds by floods, ice, or debris.

TABLE 3.—*Number and percentage of broken shells in samples collected with a shovel compared with those collected with the hydraulic sampler, Indian Creek, November 1956*

Shovel			Hydraulic sampler		
Collected	Broken	Broken	Collected	Broken	Broken
Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
276	6	2.17	115	1	0.87
260	15	5.77	608		
585	8	1.37	368		
493	16	3.25	128		
24			779	3	.39
602	5	.83	447		
329	2	.61	149		
223	1	.45	13		
157	3	1.91	221	1	.45
240	4	1.67	445	4	.90
372	4	1.08	2,105	4	.19
723	27	3.73	893	1	.11
438	16	3.65	872		
679	7	1.03	1,125	11	.98
773	3	.39	341	2	.59
521	12	2.30	103		
			234		
			295		
			1,080	1	.09
Mean		1.89			.24

The notations listed below will be used in the discussions that follow:

D = Actual number of dead eggs and larvae in a spawning bed at time of sampling.

A = Actual number of live eggs and larvae in a spawning bed at time of sampling.

$P = D + A$.

E = Potential number of eggs available for deposition in a spawning bed.

x = Fraction of E surviving to time of sampling.

y = Fraction of E not deposited in the spawning bed or lost from the spawning bed prior to sampling.

$1 \geq x + y$.

M_r = Mortality percentage calculated from the proportions of dead eggs and larvae collected in samples.

M_t = Calculated total mortality percentage from time of spawning to time of sampling.

BIAS IN CALCULATED MORTALITY LEVELS

An estimate of mortality level commonly reported in the literature is the ratio of number of dead to total number of eggs and larvae collected in k samples from a spawning bed, i.e.

$$M_r=\frac{1}{k}\left(\sum_{i=1}^{k}\left(\frac{\text{live}}{\text{live}+\text{dead}}\right)i\right) \tag{1}$$

If it is assumed that live and dead eggs and larvae are collected in the same ratio as they occur in the total population, then the expected value of M_r is

$$\frac{D}{P}=1-\frac{A}{P} \tag{2}$$

Substituting

$$P=E-yE \text{ and}$$
$$A=xE$$

gives

$$M_r=1-\frac{x}{1-y} \tag{3}$$

where y is the bias introduced by disappearance of eggs and larvae and by failure of eggs to be deposited.

An estimate of total mortality level (M_t) which is not biased by y may be obtained from estimates of the actual population of live eggs and larvae in the spawning bed (A) and the potential egg deposition (E) in the following manner:

$$M_t=1-\frac{A}{E}=1-x \tag{4}$$

If all eggs are deposited in the spawning bed, and if there are no subsequent losses of eggs or larvae from the spawning bed, the value of y in equation (3) is zero; and equations (3) and (4) are identical. In this case, the observer can calculate the total mortality percentage by the two methods and select the one that is most efficient.

The calculation of total mortality level (M_t) based on E and A, equation (4), requires estimates of potential egg deposition and the number of live specimens in the spawning area at the time of sampling. Consideration will be given to certain important problems in obtaining estimates of E and A, but considerable additional research will be required before the methods of estimating mortality described in this paper become fully developed.

ESTIMATION OF POTENTIAL EGG DEPOSITION

An estimate of potential egg deposition can best be obtained from weir counts, but in some instances it may be desirable or necessary to estimate numbers of females spawning within defined stream sections or in streams where the construction of a weir is not feasible. The present study was conducted in intertidal spawning beds, making it impractical to construct weirs. The number of female salmon spawning in a study area was determined from observation. The method is illustrated for Harris River in 1959 as follows:

Daily counts of the number of female pink salmon spawning in the sampling area were obtained (fig. 5). Daily observations were made of 43 tagged pink salmon females to obtain an estimate of the average time each spawning female occupied the spawning ground. In 1959, tagged female pink salmon occupied the spawning bed an average of 10.35 days (range: 4 to 20 days), and it was assumed that untagged females occupied the spawning bed an equal time. To estimate the total number of females spawning within the sampling area, the area under the curve (fig. 5) was divided by the average number of days each female occupied the spawning bed. The estimate was

$$\frac{15{,}850 \text{ female-days}}{10.35 \text{ days}}=1{,}531 \text{ female pink salmon.}$$

The average fecundity of pink salmon that spawned in McClinton Creek, northern British Columbia (Foerster and Pritchard, 1941), was used as an estimate of fecundity, since adequate data on fecundity of Harris River pink salmon were not available. For the years 1930–40, McClinton Creek pink salmon averaged 1,733 eggs per female. An average of 1,700 eggs per Harris River female was assumed. The potential egg deposition (E) within the Harris River sampling area in 1959 was thus estimated to be

$$1{,}531\times 1{,}700=2.6 \text{ million.}$$

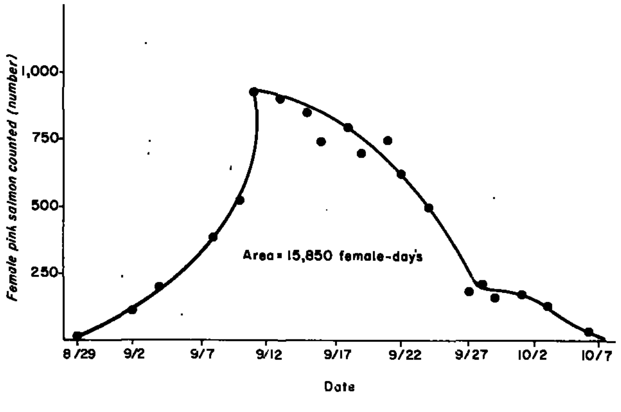

FIGURE 5.—Number of female pink salmon counted within the Harris River sampling area in 1959.

The use of tagged females to obtain estimates of the average time female pink salmon occupied the spawning bed involved the assumption that tagged females lost no tags. It was assumed also that they behaved in a manner similar to and suffered the same mortality as untagged females, that is, tagged and untagged females reacted the same while on the spawning bed.

Although no attempt was made to measure variance in estimates of potential egg deposition, it is a random variable subject to at least two sources of sampling variability—(1) the estimated number of females spawning and (2) their estimated average fecundity. In the future, studies should be undertaken to evaluate the variance in estimates of each statistic. It would be more satisfactory, however, to conduct mortality studies in streams equipped with weirs for enumeration of adults, making it possible to minimize such sources of variation.

CALCULATION OF TOTAL MORTALITY LEVEL

A confidence interval estimate of total mortality level is calculated from the double inequality.

$$1-\frac{\bar{a}}{E'}<M_t<1-\frac{\underline{a}}{E'} \qquad (5)$$

The values $\bar{a}$ and $\underline{a}$ are the upper and lower confidence limits of the estimated number of live eggs and larvae per square foot of spawning bed, and E' is the expected number per square foot. Values of $\bar{a}$ and $\underline{a}$ can be calculated with the t-distribution and the standard error of the mean obtained either from arithmetic counts of live eggs and larvae or from transformed values.

Double inequality (5) is derived in the following manner:

$$\text{Let survival to time } t=\frac{A_t}{E}=1-M_t. \qquad (6)$$

Let the confidence interval estimate of the number of live eggs and larvae per square foot within a sampling area be represented by the double inequality

$$\underline{a}<A_t<\bar{a} \qquad (7)$$

Dividing (7) by the potential egg deposition, E, gives

$$\frac{\underline{a}}{E}<1-M_t<\frac{\bar{a}}{E} \qquad (8)$$

which is equivalent to

$$1-\frac{\bar{a}}{E}<M_t<1-\frac{\underline{a}}{E} \qquad (9)$$

For calculating total mortality, the value E' is substituted for E in double inequality (9). E' is E adjusted to account for the percentage of specimens actually collected within a sampling unit. Tests to determine the percentage collected (tables 1 and 2) suggested that $E' \approx 9/10\ E$. This relationship was used to calculate values of E'.

The method of calculating M_t is illustrated with data from Harris River samples collected October 6, 1959. A 90-percent confidence interval estimate of mean number of live eggs per square foot was calculated from the arithmetic counts, giving the following estimate of their abundance:

$$16.0 \text{ eggs/sq. ft.} < A < 35.0 \text{ eggs/sq. ft.}$$

The expected number of eggs per square foot (E') was calculated from the estimated total potential egg deposition, the size of the sampling area (62,640 sq. ft.), and the estimated percentage recovered, i.e.,

$$E'=\left(\frac{9}{10}\right)\left(\frac{2{,}600{,}000 \text{ eggs}}{62{,}640 \text{ sq. ft.}}\right)=37.4 \text{ eggs/sq. ft.}$$

Using inequality (5) the estimated total mortality was

$$1-\frac{35.0}{37.4}<M_t<1-\frac{16.0}{37.4} \text{ or } 6.4\%<M_t<57.2\%.$$

The estimated mean mortality was 31.6 percent. For the same sample, M_r was estimated to be only 5 percent.

COMPARING SAMPLES FOR SIGNIFICANT DIFFERENCES

It is not always possible to determine by inspecting confidence limits alone if calculated mean total mortality levels (M_t) differ significantly. The use of confidence limits to detect significant differences implies that assumptions of analysis of variance are satisfied. Furthermore, it is difficult to assign exact levels of significance to confidence limits. This is due in part to the reduced efficiency of confidence-interval estimates of abundance calculated from arithmetic counts because the distribution of eggs and larvae within the streambed is nonrandom.

Arithmetic counts for calculating confidence-interval estimates of abundance are not always most efficient, but are not too likely to be seriously biased. It should be noted, however, that use of the t-distribution for setting confidence limits requires that sample means from a given population be normally distributed. This assumption can be satisfied for highly skewed population distributions if the sample size is large.

In the discussions that follow, the use of a logarithmic transformation to improve the efficiency of confidence-interval estimates of abundance will be considered. Also described are the uses of single binomial parameters and live-dead ratios to detect changes in total mortality level.

DISTRIBUTION OF EGGS AND LARVAE

The distribution of eggs and larvae within a spawning bed influences the choice of statistical methods to test for sufficient and significant differences among calculated total mortality percentages. Because spawning salmon deposit their eggs in egg pits, eggs are initially clustered. Furthermore, evidence from field sampling indicates that occurrence of mortality does not materially affect the tendency of surviving eggs and larvae to be clustered.

When organisms tend to become clustered, the variance commonly increases as their mean abundance increases. The observed relationship between the mean number of live eggs and larvae collected per square foot of spawning bed and the sample variance is given in table 4. Samples were collected in autumn and winter and were from populations suffering light to heavy mortality. The data from table 4 are plotted in figure 6 to show graphically the relation between mean and variance.

The negative binomial distribution has frequently been fitted to counts of organisms where mean and variance were directly related. Bliss (1953) described several models of the negative binomial. One was derived to describe insect larvae hatching from eggs laid in masses, a condition possibly similar to that found among salmonid eggs and larvae.

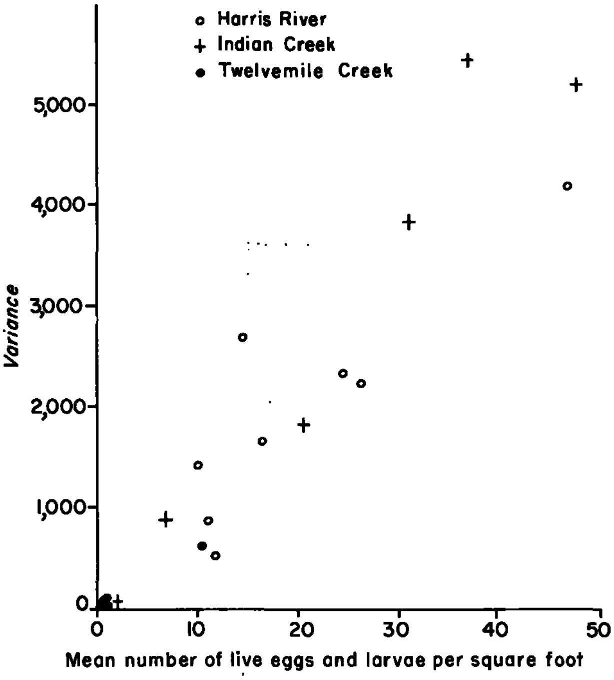

FIGURE 6.—Relation of mean to variance of live eggs and larvae collected per square foot of spawning bed in three southeastern Alaska streams.

TABLE 4.—*Means and variances of number of live eggs and larvae collected per square foot of spawning bed in three southeastern Alaska streams*

Stream and year class	Collection date	Size of sampling unit (sq.ft.)[1]	Number of sampling units	Mean	Variance	Variance mean
Harris River:						
1957	Apr. 1, 1958	4	74	1.1	41.2	37.5
1958	Sept. 18, 1958	4	34	10.7	878.9	82.1
1958	Nov. 15, 1958	4	32	14.6	2,688.8	184.2
1958	Apr. 5, 1959	4	66	9.9	1,408.7	142.3
1959	Oct. 6, 1959	4	74	24.6	2,338.1	95.0
1959	Oct. 20, 1959	4	43	26.2	2,229.3	85.1
1959	Nov. 16, 1959	4	20	16.3	1,633.0	100.2
1959	Feb. 26, 1960	4	69	11.4	512.2	44.9
1960	Sept. 27, 1960	2	56	47.2	4,157.5	88.1
Indian Creek:						
1958	Nov. 15, 1958	4	50	20.5	1,832.4	89.4
1958	Mar. 28, 1959	4	91	6.8	905.1	133.1
1959	Oct. 10, 1959	4	75	31.0	3,845.0	124.0
1959	Nov. 10, 1959	4	41	47.8	5,192.3	108.6
1959	Feb. 29, 1960	4	70	2.1	70.4	33.5
1960	Sept. 22, 1960	2	89	36.9	5,424.5	147.0
Twelvemile Creek:						
1958	Nov. 30, 1958	4	29	.4	4.2	10.5
1958	Dec. 28, 1958	4	32	.1	.1	1.0
1958	Mar. 24, 1959	4	47	.1	.5	5.0
1959	Oct. 27, 1959	4	77	1.0	68.3	68.3
1959	Feb. 21, 1960	4	56	.04	.07	1.8
1960	Sept. 30, 1960	2	50	10.3	636.1	61.8

[1] Sampling units of 4 square feet were quadrats and sampling units of 2 square feet were circles.

The negative binomial is a unimodal distribution; but biomodal or multimodal distributions may result also from contagion, as, for example Neyman's contagious distributions which have been studied with regard to their modality and other properties (Beall and Rescia, 1953; Beall, 1954). However, Beall (1954) concluded that, regardless of the form of the distribution, if contagion occurred the empirical relationship between population variance (θ^2) and mean (μ) was

$$\theta^2=\mu+\beta\mu^2 \tag{10}$$

The β term in equation (10) is a constant. Its value depends upon the degree of contagion—with β increasing with increasing degree of contagion.

Curves of equation (10) are drawn in figure 7 for $\beta=3$ and $\beta=30$.

The variance and mean of samples of live eggs and larvae collected from Harris River are shown in figure 7 as open circles. The plotted points indicated that values of β associated with samples of live eggs and larvae collected in Harris River fell mostly between $\beta=3$ and $\beta=30$. Values of β were also calculated for the Harris River samples from the expected frequency of zero observations in a negative binomial distribution (see Anscombe, 1949, and Bliss, 1953) and values $\beta \geqq 2.9$ were obtained by this method for the samples plotted in figure 7.

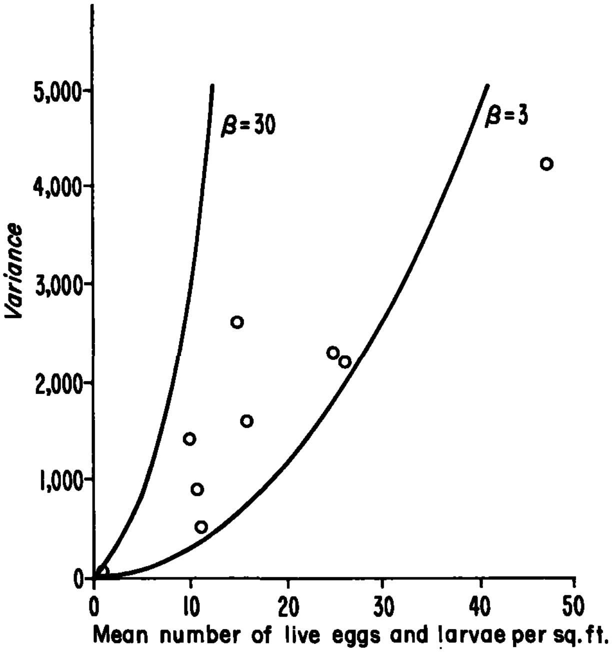

FIGURE 7.—Curves of $\theta^2=\mu+\beta\mu^2$ for $\beta=3$ and $\beta=30$. Values of variance and mean for samples collected in Harris River are plotted as open circles.

TRANSFORMATION OF COUNTS

A transformation was sought to render variance independent of the mean and to increase efficiency of confidence interval estimates of abundance. Where variance and mean are related according to equation (10), the arc hyperbolic sine transformation is appropriate; but for larger values of β, the transformed values vary almost as the log[1] of the number of live eggs and larvae in a sample (Beall, 1942). Furthermore, Barnes (1952) showed that the logarithmic was equally as applicable as the more complex arc hyperbolic sine transformation where values of β were large.

Jones (1956) and Taft (1960) used the logarithmic transformation to set confidence limits to estimates of abundance of bottom fish and sardine eggs because mean and variance were directly related. Since field sampling data suggested that the logarithmic transformation was also applicable for pink salmon eggs and larvae, the transformation

$$b_i = \log\ (n_i + \beta) \tag{11}$$

was tried. In equation (11), n_i is the number of live eggs and larvae per square foot at the i$^{\text{th}}$ point and b_i is the transformed variate. A value of β was calculated for each sample from the expected frequency of zero observations in a negative binomial distribution. The technique is described by Anscombe (1949) and Bliss (1953). It requires an iterative solution of the equation

$$\frac{1}{\beta}\log\ (1+\beta\bar{n}) = \log\left(\frac{k}{k_o}\right), \tag{12}$$

where k is the total number of observations (sampling units), k_0 is the number of zero observations, and $\bar{n}$ is the sample arithmetic mean.

For samples where $\beta > 3.0$, the logarithmic transformation (equation 11) helped render the variance independent of the mean (see table 5) and improved the efficiency of confidence interval estimates (table 6). However, for samples where $\beta < 3$, there was no apparent improvement in efficiency, and confidence-interval estimates had a significant bias when compared with the arithmetic mean (table 6).

TABLE 5.—*Comparison of mean and variance of untransformed and log-transformed counts of number of live eggs and larvae per square foot collected in Harris River samples where $\beta > 3.0$*

Date of collection	Arithmetic values (N_i)		Log-transformed values ($b_i = \log\ (n_i+\beta)$)	
	Mean	Variance	Mean	Variance
1958				
Apr. 1	1.1	41.2	2.100	<0.001
Sept. 18	10.7	878.9	.985	.131
Nov. 15	14.6	2,688.8	1.479	.058
Dec. 20	3.7	193.6	1.185	.029
1959				
Apr. 5	9.9	1,408.7	1.576	.032
Oct. 6	24.6	2,338.1	1.057	.292
Oct. 20	26.2	2,229.3	1.130	.284
Nov. 16	16.3	1,633.0	.949	.231
1960				
Feb. 26	11.4	512.2	1.040	.143
1961				
Mar. 7	6.6	203.2	.909	.099

TABLE 6.—*Ninety-percent confidence interval estimates of mean number of live eggs and larvae per square foot in Harris River calculated from untransformed and log-transformed data*

Year class and collection date	Arithmetic mean	Confidence interval estimate of mean	
		Untransformed	Log-transformed
1957			
April 1, 1958	1.1	$0<\mu<2.3$	$0<\mu<2.2$
1958			
September 18, 1958	10.7	$2.1<\mu<19.3$	$4.5<\mu<11.2$
November 15, 1958	14.6	$0<\mu<30.1$	$4.8<\mu<16.6$
December 20, 1958	3.7	$0<\mu<9.1$	$0.6<\mu<6.2$
April 5, 1959	9.9	$2.3<\mu<17.5$	$4.4<\mu<11.3$
1959			
October 6, 1959	25.6	$16.0<\mu<35.2$	$14.6<\mu<26.6$
October 20, 1959	26.2	$14.1<\mu<38.2$	$15.6<\mu<33.9$
November 16, 1959	16.3	$0.7<\mu<31.9$	$6.1<\mu<20.6$
February 26, 1960	11.4	$6.9<\mu<15.9$	$7.2<\mu<12.8$
1960			
September 27, 1960	47.2	$32.8<\mu<62.6$	*$64.3<\mu<131.9$
December 1, 1960	27.0	$18.6<\mu<35.4$	*$28.4<\mu<52.7$
March 7, 1961	6.6	$3.8<\mu<9.4$	$4.2<\mu<7.2$

*Value of $\beta<3.0$.

To set confidence limits to estimates of abundance of eggs and larvae with log-transformed data it is first necessary to correct mean log values so that the arithmetic mean will result from the antilog. A correction term is required because the mean of log-transformed data is a geometric rather than an arithmetic mean (Ricker, 1958: ch. 11). Jones (1956) gives the correction term and develops the method used here to calculate confidence limits with log-transformed counts. The equation used to obtain an arithmetic mean ($\bar{n}$) from the log-transformed counts is

$$n = \text{antilog}\ (\bar{b} + 1.1518\ s_b^2) - \beta \tag{13}$$

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[1] In this paper the term "log" refers to the logarithm to the base 10.

where $\bar{b}$ is the logarithmic mean value and s_b^2 is the sample variance of the log-transformed counts. It is necessary to subtract β to correct for its addition to counts before making the transformation (equation 11).

Improvement in efficiency of confidence interval estimates of total mortality percentage calculated with log-transformed data can be shown with the October 6, 1959, Harris River sample considered previously. Ninety-percent confidence limits of M_t set with arithmetic counts were

$$6.4\% < M_t < 57.2\%,$$

and the corresponding confidence limits set with log-transformed counts were

$$28.9\% < M_t < 61.0\%.$$

TESTS OF PROPORTIONATE FREQUENCIES

Tests of proportionate frequencies are based on the premise that the proportion of points within a spawning bed occupied by eggs and larvae varies with the total mortality level. If no change in mortality occurs, the following conditions will have been satisfied:

1. There will have been no decrease in the fraction of points populated by eggs or larvae ($Pt1=Pt2=Pt3=\ldots\ldots\ldots$).
2. There will have been no decrease in the fraction of points populated by live eggs or larvae ($At1=At2=At3=\ldots\ldots\ldots$).
3. There will have been no increase in the fraction of points populated by dead eggs or larvae ($Dt1=Dt2=Dt3=\ldots\ldots\ldots$).

Each condition was tested separately with chi square. This was done by classifying each point sampled in accordance with the presence or absence of eggs or larvae. In the examples to be given, points containing three or less eggs and larvae per square foot were counted with zeros; hence, the following three conditions were tested:

1. Presence or absence of three or less total eggs and larvae per square foot.
2. Presence or absence of three or less live eggs and larvae per square foot.
3. Presence or absence of three or less dead eggs and larvae per square foot.

One purpose of the classification scheme was to classify jointly all points containing zero or few specimens. The selection of three or less specimens per square foot for joint classification was arbitrary, however, and the possibility that other criteria for the joint classification of points might improve the tests should not be ignored.

Each of the three conditions was tested by a method described by Bliss and Calhoun (1954: p. 36) and Snedecor (1956: pp. 227–230). Data from the 1959 year class in Harris River are used to illustrate the method in table 7. There was no significant increase in the fraction of points containing three or less total or three or less live eggs and larvae per square foot ($x^2=0.85$ and 1.30). There was a significant decrease, however, in the fraction of points containing three or less dead eggs and larvae per square foot ($x^2=11.16$).

TABLE 7.—*Tests of independence among samples containing three or less eggs and larvae per square foot (Harris River, 1959 year class)*

Date of sampling	Number of points sampled	Three or less total		Three or less live		Three or less dead	
		k_o	p_o	k_o	p_o	k_o	p_o
1959							
October 6	74	44	0.5946	47	0.6351	72	0.9730
October 20	43	25	.5814	26	.6047	38	.8837
November 16	20	13	.6500	14	.7000	18	.9000
1960							
February 26	69	45	.6522	48	.6957	55	.7971

$X^2=\dfrac{\Sigma k_o p_o-\bar{p}_o\Sigma k_o}{\bar{p}_o(1-\bar{p}_o)}$, where $\bar{p}_o$=mean of p_o values

X^2 for total = 0.85 (3 degrees of freedom)
X^2 for live = 1.30 (3 degrees of freedom)
X^2 for dead = 11.16 (3 degrees of freedom)

These results suggested that the disappearance of eggs and larvae of the 1959 year class may not have been a major factor in this instance; but the increased fraction of points containing dead eggs and larvae indicated that a significant mortality had occurred nevertheless. The next problem was to determine the periods during which mortality levels had changed. This was done by first setting confidence limits to the number of points containing three or less dead eggs and larvae per square foot (k_o). Where the sample size was adequate, the normal approximation of the binomial distribution was used to set confidence limits. In the present study 90-percent confidence limits of k_o were obtained from the roots of equation (14), i.e.:

$$(\bar{k}_o, \underline{k}_o)=kp_o\pm 1.645\,[kp_o\,(1-p_o)]^{1/2}. \qquad (14)$$

Confidence limits of the fraction of points containing three or less eggs and larvae per square foot (p_0) corresponding to k_0 were obtained by dividing the limits of k_0 by the number of points sampled. Values of k_0 and p_0 for samples of the 1959 year class in Harris River obtained October 6 and 20 and February 26 are given in table 8. Confidence limits were not set to the November 16 sample because of its small size. Inspection of confidence limits of p_0 (table 8) shows that a significant mortality occurred between October 6 and 20 and between October 20 and February 26. Since mortality in these instances was detected with remains of dead eggs and larvae, it was concluded that factors not associated with direct removal of affected specimens from the spawning bed were responsible. If this conclusion is correct then estimates of mortality percentage based on live-dead ratios (M_r) should also indicate that a mortality occurred between October 6 and 20 and between October 20 and February 26. It will be shown shortly that these results are verified by estimates of M_r.

MORTALITY CALCULATED FROM LIVE-DEAD RATIOS

Although estimates of mortality based on ratios of dead eggs and larvae collected in samples (equation 1) are sometimes biased, they do give important evidence of mortality caused by factors not associated with direct removal of affected specimens from spawning beds. Because numbers of eggs and larvae collected from sampling units vary, the necessity of weighting samples for binomial variation before calculating M_r must be considered.

TABLE 8.—*Mean and 90-percent confidence limits of estimated number of points with 3 or less dead eggs and larvae per square foot, Harris River, 1959 year class*

Date of sample	Points sampled	Points containing 3 or less dead specimens per square foot			
		Points ($\underline{k}_0$)		Fraction of points ($\underline{p}_0$)	
		Mean	90-percent confidence limits	Mean	90-percent confidence limits
1959	*Number*		*Number*		
October 6	74	72	±3.2	0.97	±.04
October 20	43	38	±3.5	.88	±.08
1960					
February 26	69	55	±5.5	.80	±.08

Cochran (1943) has shown that the proper weight applied to each observed fraction of mortality depends on the relative amounts of binomial and extraneous variation in samples. This question was considered for eggs and larvae collected from Harris River and Twelvemile and Indian Creeks (McNeil, 1962), and it was found that equal weight should be given to sampling units from which 10 or more specimens were collected.

Returning to the Harris River 1959 year class example, we find that estimates of M_r support the conclusion that mortality occurred between October 6 and 20 and between October 20 and February 26 (refer to estimates of p_o, table 8). For the three samples considered, estimates of M_r and 90-percent confidence limits of the mean were:

	Percent
Oct. 6, 1959	$M_r = 5 \pm 5$
Oct. 20, 1959	$M_r = 12 \pm 11$
Feb. 26, 1960	$M_r = 27 \pm 11$

DISCUSSION AND CONCLUSIONS

The manner in which total mortality estimated from percentages of dead eggs and larvae collected in samples is biased by disappearance of dead eggs and larvae from the population has been shown algebraically. Estimates of mortality based on live-dead ratios ignore completely mortality that is caused by factors inherently associated with the direct removal of affected eggs and larvae from the population. Decomposition and scavenging also introduce bias to mortality estimates based on the percentage of dead eggs and larvae in samples.

Mortality from all causes occurring over any period of the fresh-water life of pink salmon can be assessed from estimates of abundance. By this method, total fresh-water mortality of pink salmon has been determined in a number of streams by enumerating the adults entering, by estimating their average fecundity, and by enumerating the fry departing. Such data have shown rather conclusively that 75 to 99 percent of total deaths within a population commonly occur in fresh water. An important problem requiring solution is to determine the periods of fresh-water residence during which major changes in the mortality rate occur. Solution of this problem is prerequisite to the identification of the major mortality-causing factors.

With the hydraulic sampler, it is possible to estimate quantitatively the abundance of pink salmon embryos and larvae in spawning beds. Results of unpublished studies conducted by the

Bureau of Commercial Fisheries in Sashin Creek, southeastern Alaska, have shown consistent good agreement between the total fresh-water mortality rate of pink and chum (*O. keta*) salmon estimates by sampling with the hydraulic sampler just prior to emergence of fry and by enumerating fry at a weir.

In Sashin Creek potential egg deposition for the stream as a whole is calculated from the number of adult females enumerated at the weir and their average fecundity. Under some circumstances, however, it may be desirable or necessary to estimate spawning densities within defined segments of the spawning ground. A method of estimating density of females spawning by visual foot survey censuses has been described, but the technique requires further evaluation to determine variability and bias in estimates. The Bureau of Commercial Fisheries is making comparisons between the number of females spawning (estimated by censuses taken on foot surveys) and the number counted at the weir in Sashin Creek. Preliminary unpublished results of these comparisons are not inconsistent with the hypothesis that estimates from foot surveys are essentially unbiased estimates of the true population at spawning densities similar to those observed in Harris River and Indian and Twelvemile Creeks.

Estimates of egg and larval abundance determined from sampling spawning beds are not always as efficient as an investigator might desire. High variability in estimates of abundance and mortality is due primarily to clustering of eggs and larvae in spawning beds. Clustering causes the variance and mean to increase dependently; and confidence limits of estimates of abundance may be widely separated, even with relatively intensive sampling. There is no evidence of reduced tendency of larvae to be clustered, suggesting little if any horizontal scattering of young pink salmon within spawning beds.

Three statistics useful for evaluating mortality in spawning beds have been considered. Total mortality (M_t) is least sensitive as a measure of change in the mortality rate, but it is most useful in determining the magnitude of mortality from all causes. The single binomial parameters of presence or absence of live or dead eggs and larvae at sampling points (p_o) are helpful in two ways. They provide a basis for identifying the periods during which total mortality increased significantly and for determining whether or not such mortality was associated with disappearance of eggs or larvae from the spawning bed. Mortality estimated from live-dead ratios (M_r) is a useful statistic for evaluating mortality caused by factors not associated with the direct removal of dead eggs and larvae from the spawning bed. M_r is biased by the disappearance of dead eggs and larvae from decomposition and scavenging, however. By sampling spawning beds randomly with the hydraulic sampler, the field data can be evaluated by the three independent methods described.

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LITERATURE CITED

ANSCOMBE, F. J.
1949. The statistical analysis of insect counts based on the negative binomial distribution. Biometrics, vol. 5, No. 2, pp. 165–173.

BARNES, H.
1952. The use of transformations in marine biological statistics. Conseil Permanent International pour l'Exploration de la Mer, Journal du Conseil, vol. 18, No. 1, pp. 61–71.

BEALL, GEOFFREY.
1942. The transformation of data from entomological field experiments so that the analysis of variance becomes applicable. Biometrika, vol. 32, pp. 243–262.
1954. Data on binomial and near binomial distribution. *In* O. Kempthorne, T. A. Bancroft, J. W. Cowen, and J. L. Lush (editors), Statistics and mathematics in biology, pp. 295–302. The Iowa State College Press, Ames.

BEALL, GEOFFREY, and RICHARD R. RESCIA.
1953. A generalization on Neyman's contagious distributions. Biometrics, vol. 9, No. 3, pp. 354–386.

BLISS, C. I.
1953. Fitting the negative binomial distribution to biological data. Biometrics, vol. 9, No. 2, pp. 176–200.

BLISS, C. I., AND D. W. CALHOUN.
1954. An outline of biometry. Yale Co-operative Corporation, New Haven, Connecticut, 272 pp. [Processed.]

BRIGGS, JOHN C.
1953. The behavior and reproduction of salmonid fishes in a small coastal stream. California Department of Fish and Game, Fish Bulletin No. 94, 62 pp.

CAMERON, W. M.
1940. The efficiency of fertilization in the natural spawning of Pacific salmon. Fisheries Research Board of Canada, Progress Reports of the Pacific Biological Station and Pacific Fisheries Experimental Station, No. 45, pp. 23–24.

COCHRAN, WILLIAM G.
1943. Analysis of variance for percentages based on unequal numbers. American Statistical Association Journal, vol. 38, pp. 287–301.

DAVIDSON, F. A., AND S. J. HUTCHINSON.
1942. Natural reproduction of pink salmon studied at Little Port Walter, Alaska. Ecology, vol. 23, No. 2, pp. 234–235.

FOERSTER, RUSSELL E., AND ANDREW L. PRITCHARD.
1941. Observations on the relation of egg content to total length and weight in the sockeye salmon (*Oncorhynchus nerka*) and the pink salmon (*O. gorbuscha*). Transactions of the Royal Society of Canada, vol. 35, section 5, pp. 51–60.

HATCH, RICHARD W.
1957. Success of natural spawning of rainbow trout in the Finger Lakes region of New York. New York Fish and Game Journal, vol. 4, No. 1, pp. 69–87.

HOBBS, DERISLEY F.
1937. Natural reproduction of quinnat salmon, brown and rainbow trout in certain New Zealand waters. New Zealand—Marine Department, Fisheries Bulletin No. 6, 104 pp.
1940. Natural reproduction of trout in New Zealand and its relation to density of populations. New Zealand—Marine Department, Fisheries Bulletin No. 8, 93 pp.

HUNTER, J. G.
1959. Survival and production of pink and chum salmon in a coastal stream. Journal of the Fisheries Research Board of Canada, vol. 16, No. 6, pp. 835–886.

HUTCHINSON, S. J., and R. F. SHUMAN.
1942. Reproduction of pink salmon at Little Port Walter, 1941–42. Pacific Fisherman, vol. 40, December 1942, pp. 29–31.

JONES, R.
1956. The analysis of trawl haul statistics with particular reference to the estimation of survival rates. Conseil Permanent International pour l'Exploration de la Mer, Rapports et Procès-Verbaux des Réunions, vol. 140, part 1, pp. 30–39.

MARTIN, JOHN W. (editor)
1959. Stream catalog of eastern section of Ketchikan Management District of Southeastern Alaska U.S. Fish and Wildlife Service, Special Scientific Report—Fisheries No. 305, 379 pp.

MATHISEN, OLE A.
1962. The effect of altered sex ratios on the spawning of red salmon. *In* Ted S. Y. Koo (editor), Studies of Alaska red salmon, pp. 137–246. University of Washington, Seattle, Publications in Fisheries, vol. 1.

MCNEIL, WILLIAM JOHN.
1962. Mortality of pink and chum salmon eggs and larvae in Southeast Alaska streams. Ph. D. Thesis, University of Washington, Seattle, 270 pp.

MERRELL, T. R., JR.
1962. Freshwater survival of pink salmon at Sashin Creek, Alaska. *In* N. J. Wilimovsky, editor, Symposium on pink salmon, pp. 59–72. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver.

NEAVE, FERRIS.
1953. Principles affecting the size of pink and chum salmon populations in British Columbia. Journal of the Fisheries Research Board of Canada, vol. 9, No. 9, pp. 450–491.
1958. Stream ecology and production of anadromous fish. *In* P. A. Larkin (editor), The investigation of fish-power problems, pp. 43–48, Institute of Fisheries, University of British Columbia, Vancouver, H. R. MacMillan Lectures in Fisheries.

PRITCHARD, A. L.
1948. Efficiency of natural propagation of the pink salmon, (*Oncorhynchus gorbuscha*) in McClinton Creek, Masset Inlet, B.C. Journal of the Fisheries Research Board of Canada, vol. 7, No. 5, pp. 224–236.

RICKER. W. E.
1954. Stock and recruitment. Journal of the Fisheries Research Board of Canada, vol. 11, No. 5, pp. 559–623.
1958. Handbook of computations for biological statistics of fish populations. Fisheries Research Board of Canada, Bulletin No. 119, 300 pp.

SNEDECOR, GEORGE A.
1956. Statistical methods, 5th ed. Iowa State College Press, Ames, 534 pp.

TAFT, BRUCE, A.
1960. A statistical study of the estimation of abundance of sardine (*Sardinops caerulea*) eggs. Limnology and Oceanography, vol. 5, No. 3, pp. 245–264.