A METHOD OF ESTIMATING THE NUMBERS OF SMALL FISH FRY

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MEETING OF THE BIOMETRICS SECTION

The Biometrics Section will participate in the meetings of the American Association for the Advancement of Science, which will be held in Boston, Massachusetts, December 26 to 31, 1946. The Program of the Section, which appears in this issue of *Biometrics*, provides for two sessions of contributed papers in which both members and non-members are invited to take part. Persons desiring to present papers should notify Dr. D. B. DeLury, Box 551, Blacksburg, Virginia.

The headquarters of the Biometrics Section will be at the Statler Hotel and all of its meetings will be held there. The Bellevue, Commonwealth, Lincolnshire, and Parker House Hotels are grouped about the Boston Common and are within convenient walking distance of the Statler.

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Statler, Park Square	3.85 to 5.50	5.50 to 7.70	6.60 to 8.80

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BIOMETRICS SECTION PROGRAM

Held in conjunction with the 113th Annual Meeting of the
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE
Boston, Massachusetts, December 27 - 29, 1946
Headquarters — Statler Hotel

FRIDAY — DECEMBER 27

10:00 A.M.
Hotel Statler
Parlor C

Biometric Methods in Fishery Investigations
(With the Atlantic Fishery Biologists)

Papers: An Application of Multiple Regression Methods to Growth Studies in Fish Populations
Howard A. Shuck, *North Atlantic Fishery Investigations*

A Method of Estimating the Number of Small Fish Fry
W. F. Royce, *North Atlantic Fishery Investigations*

General Discussion of Biometric Aspects of Fish Population Dynamics

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INTRODUCTION

One of the hatchery superintendent's more difficult problems has been the estimation of numbers of fish fry. The delicacy of such fry and the large numbers of them coupled with the primary necessity of keeping them alive, has frequently precluded the possibility of obtaining a count. This problem is especially difficult in the culture of the extremely small fry of such fish as blackback flounder, whitefish, wall-eyed pike, cod, haddock, and pollock. The fact that the numbers involved in the case of marine species may range into the billions adds to the difficulties. The present hatchery technique for estimating the production of such fry depends on estimating the number of eggs placed in the hatching apparatus by more or less accurate volumetric methods. This figure is then corrected by a factor for mortality which may be obtained by a volumetric estimate of dead eggs or a guess related to previous years experience.

The alternative to a total count is a sample. This may be obtained for a tray of fish, a can of fish, or a truck-load of fish, by using modern sampling techniques to obtain a sample, the volume of which is a known fraction of the total, and estimating the total by a simple proportion. In the case of a single container of fish the fry may be distributed evenly throughout the measured quantity of water in that container by stirring and a sample of known volume taken. Then the number of fry in the entire container may be calculated from the proportion that was sampled.

This problem of estimating numbers of fish fry occurred when it became necessary to have accurate estimates of the number of blackback flounder fry stocked in an experiment to determine the effectiveness of such stocking. All the techniques and suggestions in the following paragraphs have been worked out with the cooperation of Mr. Gordon L. Davis, Superintendent of the Wood's Hole station of the Fish and Wildlife Service and apply particularly to blackback flounder fry. It is believed that this same technique with slight modifications could be used for other species.

The author is indebted to Dr. C. I. Bliss of the Connecticut Agricultural Experiment Station and to Mr. S. Lee Crump of the Iowa State College Statistical Laboratory who have reviewed the statistical techniques used in this article. They have suggested certain changes in methods of analysis of variance which will be used in future work. The principal changes suggested include the use of the chi square test where possible and if analysis of variance is used, to transform the data to square roots instead of logarithms. Such changes will not materially alter the results of this analysis and will not influence the suggestions made. Accordingly, this article is presented as a preliminary report without changes in the method of analysis.

METHOD OF SAMPLING

The primary requirement in taking any sample is to make sure each individual in the group or population to be sampled has an equal chance of being included in the sample or subsample.[1] When taking a subsample from a can or hatching box, the contents were stirred thoroughly but not violently enough to harm the fry. Subsamples were taken from a hatching box after stirring with the paddle which is ordinarily used to stir the box every two hours. Subsamples from a shipping (milk) can were taken after stirring with a small dipper. The circular shape of the can made it tempting to stir in a circular manner. This was avoided since the fry in some cases concentrate near the central axis of the can and stirring round and round does not mix them thoroughly. The stirring was done from top to bottom and the contents thoroughly mixed without swirling. After stirring, the subsample was dipped out quickly to prevent the actively swimming fry from avoiding the subsample container or again concentrating.

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[1] In this discussion the name subsample will be applied to the quantity dipped from a container in one instant and the name sample will be applied to the collection of several subsamples obtained from one or more containers.

After obtaining the subsample the actively swimming fry were counted. They were counted alive because the fry may be not only returned to the box or can but may be also distinguished from dead fry which were quite numerous in the case of blackback flounders. These dead fry were recognized because they were distorted with the body twisted in one or more curls. Straight bodied fry generally moved after stimulation even though at first they were so motionless as to appear dead.

Accurate counts of the actively moving fry were obtained by placing them in a double receptacle in which they could be poured single file through a narrow opening from one side to the other. Such a receptacle was constructed for flounder fry (figure 1) of celluloid put together with acetone (Duco Household Cement also works well). The small size of the flounder fry made it necessary to count them under the lowest power of a binocular microscope.

Because the fry can swim fairly actively it was thought possible that they might tend to escape the container used to obtain the subsample. Presumably they would find escape easier from a small container than from a large one into which the water would rush with greater rapidity when quickly dipped. To test this possibility two series of subsamples were taken from 2 hatching boxes (table 1). The subsamples which were dipped with 5, 10, and 25 cc graduates ranged in size from 2.3 cc to 21.8 cc (table 1). The correlation between size of sample and number of fry per cc was only -0.15 in one instance and 0.04 in the other. This is far below the value (0.58) which would indicate any significant tendency for large samples to capture more fry per cc.

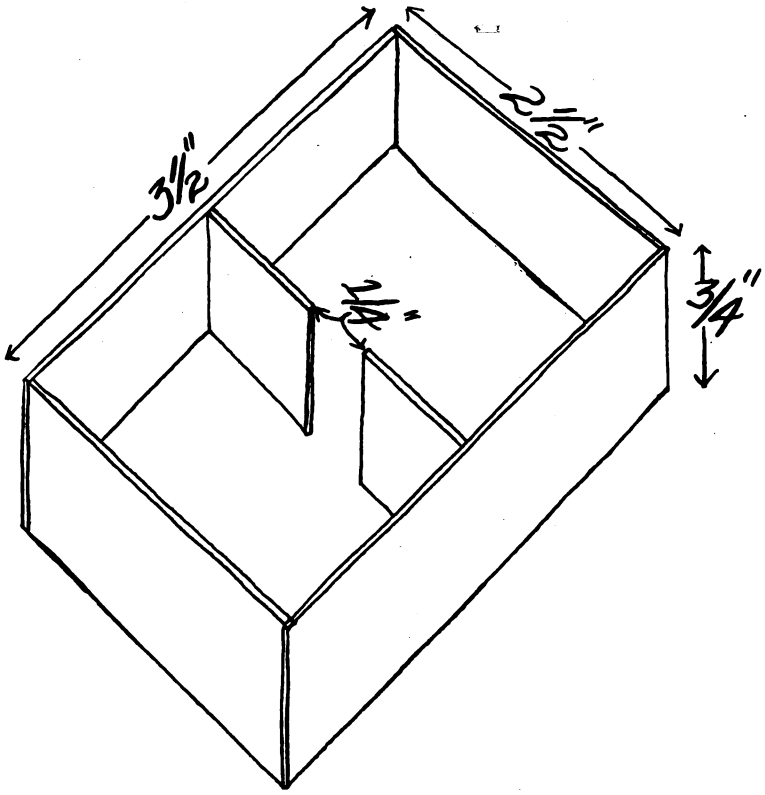

FIGURE 1. A CONTAINER FOR COUNTING SMALL FISH FRY.

TABLE 1.—Samples of Blackback Flounder Fry obtained from hatching boxes

Box B7	3/21/46	Box B9	3/21/46
Size of Subsample in cc	Number of Viable Fry & Eggs	Size of Subsample in cc	Number of Viable Fry & Eggs
2.3	1	2.3	4
2.7	8	2.8	6
3.4	3	3.6	8
4.5	6	4.4	10
4.9	10	4.8	15
7.3	15	7.3	17
9.0	5	7.3	22
9.7	9	9.2	14
10.6	16	14.0	42
16.8	22	16.6	38
19.2	20	20.9	55
19.5	29	21.8	40

Subsamples of this kind which involve counts of an occurence, theoretically should be distributed in a Poisson distribution if they are taken from a homogeneous population. If this is the case, it makes no difference whether many small subsamples or a few large subsamples are taken. However, in sampling fry the populations may vary from container to container; that is, not homogeneous, and many small subsamples will be preferable. Accordingly, we may take as a subsample as small as is convenient. Experience in counting the blackback flounder fry indicated that it required but a few seconds longer to count 20 to 30 fry than it did to count less than 10. However, when the number of fry surpassed 50 they tended to group, which made counting difficult, and more recounts became necessary. Accordingly, it is recommended that the container selected for subsamples should be of a size which will usually catch between 10 and 50 fry. The other principal requirement for the container is that it should hold a uniform quantity of liquid when filled by a quick dip. This will eliminate broad flat dishes. A container similar to a milk sampler, such as is frequently used for sampling plankton, should be ideal.

The number of such subsamples to be taken from a truckload or battery of hatching boxes will depend on time available and accuracy desired. A study of the sampling which was done at Woods Hole on a truckload of 50 cans (table 2) of blackback flounder fry has provided estimates of the reliability of such sampling. The analysis of variance of these data (transformed to logarithms because of the relationship of mean and variance) is presented in table 3. The techniques outlined by Snedecor (1946) and Winsor and Clarke (1940) on the segregation of components of variance have been followed in arriving at the estimates of the accuracy of the various methods of sampling presented in table 4. Very briefly, this method is as follows: The variance attributed to cans (s_c^2) in table 3 was calculated from the mean square for cans by subtracting the mean square for subsamples and dividing by 5, the number of subsamples per can. The subsample variance (s_s^2) remains the same as the mean square since there is only one observation per subsample. From these two components we may estimate the variance of the mean and fiducial limits for different numbers of subsamples taken from different numbers of cans. Since the number of cans which may be sampled is relatively small, it is appropriate to correct the estimate of the can component by the factor:

$$1 - \frac{n}{N}$$

where n is the number of cans sampled and N the total number of cans. The variance of the mean ($s_{\bar{x}}^2$) for a sample of 5 subsamples from each of 5 cans was estimated from the following equation:

$$s_{\bar{x}}^2 = s_c^2/n\,(1 - n/N) + s_s^2/k$$

in which

n = number of cans sampled
N = number of cans in truck
k = number of subsamples taken

TABLE 2.—Sample of Blackback Flounder Fry obtained from a truckload of 50 cans

Can Number	Contents cc	Subsample cc	No. of Viable Fry & Eggs	No. per cc
1	31,600	17.0	22	1.29
		22.5	63	2.80
		21.5	37	1.72
		16.5	30	1.82
		24.5	30	1.22
2	29,300	14.5	14	.97
		15.0	12	.80
		16.0	8	.50
		20.5	8	.39
		19.0	13	.68
3	33,100	24.5	5	.20
		22.0	2	.09
		19.5	2	.10
		22.0	4	.18
		19.5	4	.21
4	31,000	24.0	24	1.00
		25.0	16	.64
		25.0	19	.76
		22.0	17	.77
		20.0	14	.70
5	30,300	12.0	3	.25
		16.5	2	.12
		18.0	5	.28
		13.0	4	.31
		23.0	9	.39

TABLE 3.—Analysis of Variance

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Components
Total	24	3.9620		
Cans	4	3.4846	.8712	.1695
Subsamples	20	.4774	.0239	.0239

TABLE 4.—Estimates of Relative Accuracy of Different Methods of Sampling a Truckload of Small Fish Fry

Sampling Technique	Estimated Variance of the mean in Logarithms	Estimated Lower and Upper Fiducial Limits 1/ if mean is assumed to be 100
5 subsamples from each of 5 out of 50 cans	.0315	44-226
1 subsample from each of 25 out of 50 cans	.00435	74-136
1 subsample from each of 50 out of 50 cans	.000478	90-111
1 subsample from each of 10 out of 10 cans	.00239	80-125

1/ Limits have been calculated from twice the standard error. This indicates that if the calculated mean of the sample is 100 other sample means from the same population will fall between the limits indicated 19 times out of 20.

Substituting in the equation

$$\sigma_{\bar{x}}^2 = .1695/5\ (1-\frac{5}{50}) + .0239/25 = .0315$$

The calculations for the other methods of sampling were carried out similarly by substituting the appropriate numbers.

It is apparent that the greatest accuracy is derived by sampling each can in a truckload. In a truckload of 50 cans one subsample may be taken from each can and from the results the total number of fry may be estimated within about 10 percent 19 times out of 20. It is probably not feasible to attempt to reduce the limits of error any more since to halve them again will require 4 subsamples per can instead of one.

If less than all the cans are sampled, it must be done at random. If 25 out of 50 on a truck are to be sampled, it would be proper to flip a coin to decide between the first 2 cans and take every other can after that (if the cans were arranged at random). Also, if less than the total number of cans are sampled, it should be possible to considerably increase the reliability of the estimate by loading the cans more uniformly. A great difference in the density of the blackback flounder fry in the cans at Woods Hole was evident to the eye when the cans were illuminated properly. It should be possible to fill them much more equally by simple visual comparison under proper lighting. In this case 25 samples from 50 cans might provide estimates within about 15 percent.

METHODS OF CALCULATING NUMBER OF FRY

If the quantity of fry per can is reasonably uniform and estimates of reliability are not wanted, the calculation of the total number in a truckload may be reduced to the simple proportion:

$$\frac{\text{Total volume in cans}}{\text{Total number of fry}} = \frac{\text{Volume of sample}}{\text{Number of fry in sample}}$$

If the standard milk can which is 12 3/4 inches inside diameter is used and it is not filled above the bottom of the neck, the volume of water in cubic inches may be calculated by subtracting 3/8 inches from the depth for the curvature of the bottom and multiplying by 128, the area of a cross section of the can in square inches. For example, if the can contains 15 3/8 inches of water, the volume is $(15\frac{3}{8} - \frac{3}{8}) \times 128$ or 1,920 cubic inches. If a cubic inch sample contains 50 fry, the estimate of the number of fry is 50 x 1,920 or 96,000.

TIME AND EQUIPMENT REQUIRED

Adoption of any sampling procedure will require additional work on the part of the hatchery personnel. The additional amount of time required will be determined to a large extent by the integration of the sampling with the normal operation of preparing a truckload of fish for shipment. As an example of the maximum time required, most of the 25 subsamples enumerated in table 2 were taken by 1 person and and all were counted by the same person in a total time of about 2 hours. The subsamples were taken after the truck was loaded and the cans covered with canvas and considerable extra time was spent uncovering the cans and prying loose the covers. Furthermore, the person doing the counting was doing it for the first time. It is believed that with reasonable organization 50 subsamples can be obtained and counted in 4 man hours. There would be no necessity to hold up the truck. If sufficient small bottles were available, the subsamples could be obtained in a few minutes and counting done later (providing the bottles are kept cool so the fry remain alive). The calculation of an estimate of the total number of fry should require only a few minutes especially if an adding machine is available. In brief, the total time required for estimating the number of fry in a truckload should not differ greatly from the time required to count a sample and weigh up a truckload of trout fingerlings.

The counting of the blackback flounder fry was done under the lowest power of a binocular microscope with a magnification of six diameters. A large reading glass should work equally well since difficulty was experienced with too much magnification and consequent limited field in the microscope.

SUMMARY AND RECOMMENDATIONS

Trial sampling of hatching boxes and shipping cans containing blackback flounder fry has indicated that estimates of the total number of fry in a truckload within about 10 percent may be obtained by taking a small subsample which should contain between 10 and 50 fry from each can. Limited information on time required indicates that, at most, about 4 man hours per truckload will be needed to provide estimates within about 10 percent and less time if less accurate estimates are adequate.

In the event that sampling is adopted by a hatchery, the procedure should be carefully standardized and additional estimates of reliability calculated from the results obtained under actual operating conditions. Once consistent results have been obtained under a carefully standardized procedure, the calculations may be reduced to the simple estimate of total number of fry by the method outlined. The results obtained by the limited sampling which has been done on the flounder fry should not be accepted as final. They merely serve to indicate the practical possibilities of obtaining results of known accuracy with a reasonable expenditure of time.

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