Informational Leaflet 27

SIZE OF PINK SALMON DOWNSTREAM MIGRANTS

By:

W. L. Sheridan
and
W. H. Noerenberg
Alaska Department of Fish and Game

May 7, 1963

STATE OF ALASKA
WILLIAM A. EGAN - GOVERNOR

DEPARTMENT OF
FISH AND GAME
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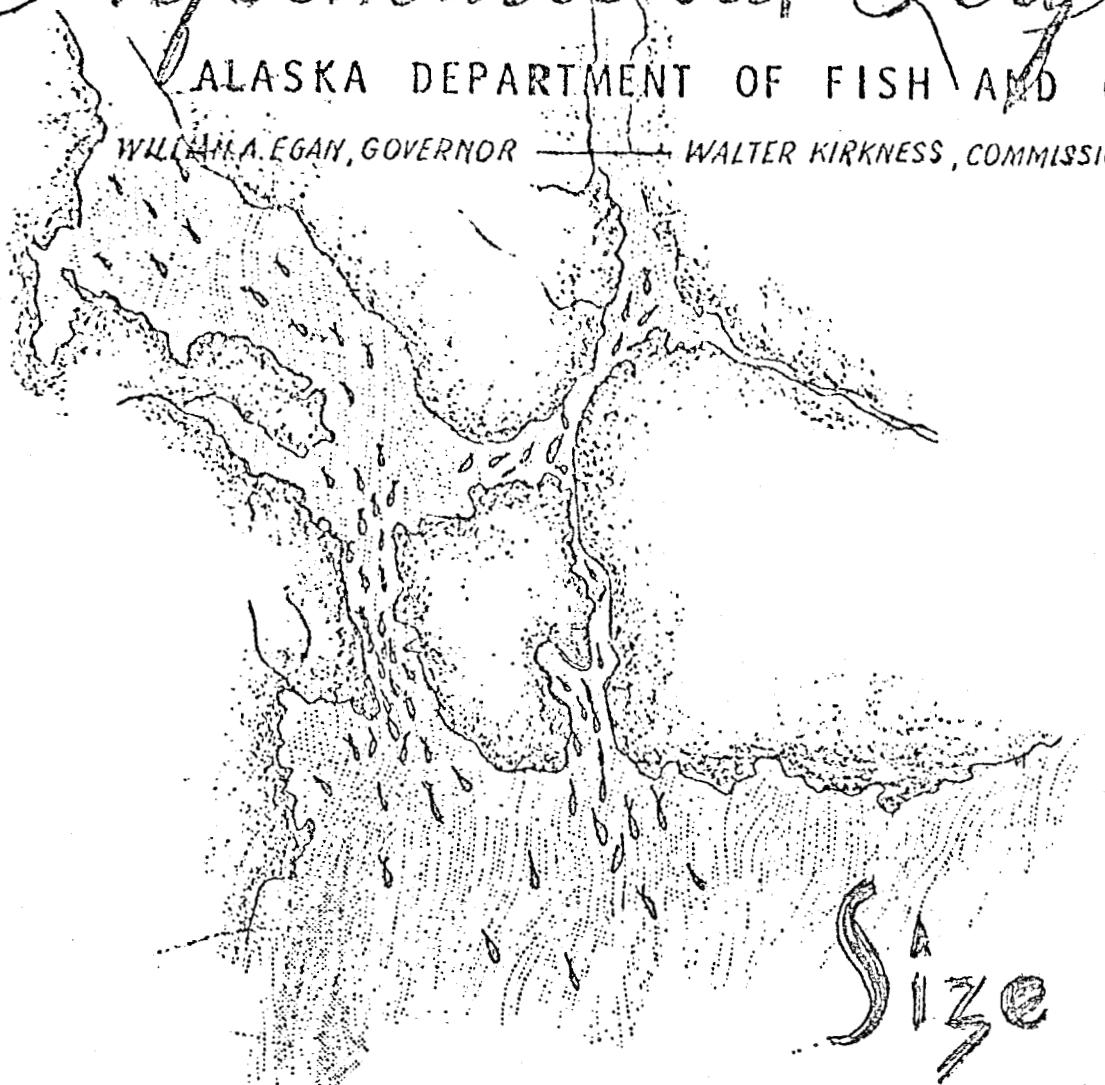

Size of PINK SALMON DOWNSTREAM MIGRANTS

By W. L. SHERIDAN

W. H. NOERENBERG

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OBSERVATIONS ON SIZE OF PINK SALMON DOWNSTREAM MIGRANTS

INTRODUCTION

Studies of Alaska pink salmon *(Oncorhynchus gorbuscha)* conducted by the Fisheries Research Institute of the University of Washington from 1949 through 1954 showed[1] that downstream migrant pink salmon fry varied in length from one stream to another. Skud (1955) demonstrated a length-weight difference between early and late pink salmon fry migrants at Sashin Creek, Little Port Walter. McNeil[2], also working at Sashin Creek, found that the average size of pink salmon fry increased from the beginning to the middle of the run, after which there was a sudden and sharp decline in average size.

Research conducted at Oregon State University[3] on the influence of dissolved oxygen and seepage velocity, on survival and growth of salmonid fishes, shows that larger, more robust fry result from higher dissolved oxygen concentrations during the incubation period.

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[1] Data are on file at Fisheries Research Institute.

[2] McNeil, William J. Pink Salmon studies at Little Port Walter, March 10 - June 30, 1962. Manuscript Report 62-10, Fish and Wildlife Service, Bureau of Commercial Fisheries, September, 1962, 24 pages.

[3] (a) Phillips, R.W. and H.C. Campbell. The embryonic survival of coho salmon and steelhead trout, as influenced by some environmental conditions in gravel beds. Oregon State Game Commission, Res. Div. O.S.U., Corvallis. 17 pages, processed.

(b) Warren, Charles E. 1959. The influence of dissolved oxygen upon the survival, development, growth, activity, and movements of freshwater fishes. Prog. Rpt., U.S. Public Health Service, Grant No. RG - 4352.

(c) Silver, Stuart S. 1960. The influence of water velocity and dissolved oxygen on the development of salmonid embryos. M.S. thesis, O.S.U., Corvallis.

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Because of the possibility of a relationship between robustness of pink salmon fry at time of migration and subsequent survival, Alaska Department of Fish and Game biologists started observations of size of pink salmon downstream migrants in 1961. Objectives were as follows:

1. To assess temporal and spatial variability between size of pink salmon downstream migrants. This meant sampling and measuring fry over the entire migratory period in a number of streams.

2. To determine if size differences between different stocks or races were due to environment or heredity, or both.

3. To attempt to correlate robustness of pink salmon outmigrants with subsequent survival.

Although these objectives were not fully attained, two years sampling gave interesting indications of variation in size of pink salmon fry within one stream and between streams. These results, along with a discussion of methods, should be of interest to others working on similar problems.

METHODS

In 1961 and 1962, pink salmon fry were collected in downstream migrant traps from Big Kitoi Creek on Afognak Island and from several streams in Prince William Sound. In 1962, freshly fertilized pink salmon eggs were sent from Prince William Sound to Kodiak and incubated simultaneously with pink salmon eggs from Big Kitoi Creek fish. Weight and volume of alevins from the two lots were compared.

Pink salmon fry were weighed (after blotting with paper towels) to the nearest hundredth gram on a Mettler balance. Length was measured by placing each specimen on a piece of 10 x 10 to 1 cm graph paper with the end of the snout on zero. A pin prick was made with a probe at the base of the caudal fin. Measurements were made under slight magnification.

Volume was ascertained by placing lots of 10 or 20 fry in a plastic overflow flask and catching overflow in a graduated cylinder. The overflow flask contained 70 percent iso-propyl alcohol to reduce surface tension and to allow more accurate displacement. As a test of the method, seventeen consecutive measurements were made with 80 (at a time) glass laboratory beads (80 beads were roughly equal in volume to 10 pink salmon fry). Measurements ranged from 2.40 to 2.80 ml. The mean was 2.64 ml, variance 0.000824 and 95% confidence limits for the mean value were 2.64 ± 0.046 ml.

Since a major source of error in either weighing or determining volume of pink salmon fry is the differential adhesion of moisture to the bodies, standard blotting procedure is necessary.

RESULTS

Variation in size of pink salmon fry migrants

Differences in size of pink salmon fry at time of migration can be attributed to a number of factors. Skud (1955) lists several environmental, physiological, and genetic factors that might account for length and weight differences between early and late fry migrants at Sashin Creek, Little Port Walter. However, the importance of each factor has yet to be established. One of the more intriguing possibilities is that suggested by results of work at Oregon State University[3a] and b. This work suggests that if environmental conditions (mainly available dissolved oxygen and seepage velocity) vary considerably from one part of a stream to another, both production and size of fry may also vary.

The amount of interchange between surface stream and intragravel water depends, to a large extent, upon the permeability of stream bed materials (Vaux 1962). Therefore, more highly permeable upstream areas might produce more and larger fry than less permeable spawning areas (indications of this are appearing at Sashin Creek -- see McNeil[2]). If true, (and it is also suggested by Wickett, 1958) this means that habitat improvement in the form of removing fine materials from spawning gravels will not only increase production of fry, but it will also produce larger fry. The desirability for larger, more robust fry lies in the possibility that such fry, being more vigorous, will be better able to cope with their estuarine environment; hence, more of them will live, especially under adverse estuarine conditions.

Variability in size within streams

Length, weight, and volume measurements were obtained from pink salmon fry in Big Kitoi Creek on Afognak Island in 1961, three or four different times during the period of downstream migration. In addition, the catch on one night was divided by hourly intervals. Results are given in Table 1.

There is little difference between the various mean values and between n values of the length-weight relationship. However, when weights (column 5, Table 1) are subjected to analysis of variance, the F value is significant at the 95 and 99 percent levels (Table 2).

Skud (1955) and McNeil[2] indicate a size difference in pink salmon fry in one stream during one season. Noerenberg (1963) also implies a size difference in the same stream between seasons. There may also be a size difference between pink salmon fry in one stream migrating at different times during one night.

Therefore, it is essential that the possibility of size variation within one night and between nights is taken into account when sampling programs are designed. For example, to overcome variation in size during one night the entire night's catch can be thoroughly mixed before a sample is drawn.

Of the three measurements (length, weight, and volume), length appears to be the least accurate, most difficult to standardize and most time consuming.

Table 1. Mean length, condition factor (K) wt., length-wt. relationship ($w=aL^n$) and correlation coefficient for regression of weight on length of pink salmon fry captured at hourly intervals, night of May 13-14, Big Kitoi Creek, 1961.

Date	N	Mean Length in mm	Condition factor	Mean Weight in grs.	Length-weight relationship	r Value
May 13-14						
8-10 p.m.	50	31.96	0.6911	0.2322	$\log w = -5.392 + 3.160 \log l$	0.845
10-11 p.m.	50	32.00	0.7167	0.2302	$\log w = -4.992 + 2.893 \log l$	0.762
11-1	50	31.38	0.6820	0.2132	$\log w = -5.096 + 2.954 \log l$	0.861
1-3 a.m.	47	32.54	0.6660	0.2330	$\log w = -5.292 + 3.077 \log l$	0.895

Table 2. Analysis of variance for weights of pink salmon fry captured at hourly intervals on night of May 13-14, 1961, Big Kitoi Creek.

	S.S.	d. f.	M. S.	F.
Means	0.011	3	0.004666	3.933**
Within	0.229	193	0.0011865	
Total	0.243	196		

**Significant at 1% level.

The tail becomes curled or split in specimens preserved in formalin for any length of time and the body is usually curled and must be flattened. In addition, there is often only a few millimeters difference between the smallest and largest specimen in the sample. As far as we know, no method has yet been devised to accurately measure pink salmon fry which overcomes error involved in change in body shape due to manipulation during measurement.

If length measurements must be taken, they should be obtained from fresh specimens or from specimens preserved in a known concentration of formalin for a known time. We know that preservation in formalin changes the body characteristics of fish, and pink salmon fry are no exception. For instance, length and weight measurements were obtained from 50 pink salmon fry collected on May 10, 1961. The sample was preserved in 1:9 formalin and remeasured 12 days later. Results appear in Table 3. Average decrease in length was 1.7 mm and decrease in weight 0.019 grams, 5.1 percent, and 7.4 percent, respectively.

To compare sizes of pink salmon fry within one stream and from stream to stream, a simple yet accurate method should be used. Skud (1955) used the standard length-weight formula $W = al^n$ to determine size differences between fry migrating at different times from Sashin Creek, Little Port Walter. Use of this method of analysis is not only cumbersome, but it also appears to be incorrect.

We see little value in using the exponential formula $W = al^n$ to describe the length-weight relationship of pink salmon fry. These fish are all the same age, have just emerged from streambed gravels, have not yet begun to feed, and are barely separated in length and weight. Hence, the familiar exponential curve has not yet been realized. To find out if the logarithmic ($W = al^n$) or the linear equation ($Y = a+bx$) furnished a better description of the length-weight relationship, the same (Kitoi) data were analyzed both ways. Magnitude of the "deviation from regression" mean square was examined in each instance. If the mean square from logged data is appreciably larger than the same mean square from the arithmetic scale, then the latter is most efficient. Since these are two independent variances, their ratio will be applicable to an F test. Results of this test (Table 4) show that the arithmetic scale and linear equation, $y = a+bx$, best describes the length-weight relationship of pink salmon fry (length frequency distribution and increase of weight with length of Big Kitoi pink salmon fry are shown in Figure 1). The r value for logged data is 0.975 and for arithmetic scale it is 0.991.

Condition factor($K = \frac{Wa}{L3}$) is another way of comparing pink salmon fry. Condition factors were calculated for pink salmon fry from Big Kitoi Creek (Figure 2), but enough samples were not obtained to detect a seasonal trend. However, it does appear (Figure 3) that fry in the most prevalent size groups have a higher condition factor than the extremes at either end of the length frequency distribution curve.

In summary, all evidence indicates variation in size between pink salmon fry from the same stream. This variability must be taken into account when designing sampling programs. Secondly, because of the large numbers of migrants that must be processed, fast, simple and accurate methods of measurement should be used. Lastly, methods of measurement should be standardized because measurements are being collected by more than one individual and by more than one agency.

Table 3. Ninety-five percent confidence limits for mean lengths and weights of 50 pink salmon fry measured while fresh, then preserved in 1:9 formalin for 12 days and remeasured.

	Mean length in mm			Mean weight in grams		
Fresh	32.45	m	33.19	0.2502	m	0.2730
1:9 Formalin	30.81	m	31.51	0.2306	m	0.2538

Table 4. Analysis of variance of length-weight data from 360 pink salmon downstream migrants, Big Kitoi Creek, 1961 (weights are empirical averages of all weights falling in each millimeter class interval --metric system).

Logged data	Arithmetic data
$\Sigma xy = 0.02956$	$\Sigma xy = 0.122$
$\Sigma x^2 = 0.01046$	$\Sigma x^2 = 0.600$
$\Sigma y^2 = 0.085723$	$\Sigma y^2 = 0.0252$

Logged data

	d. f.	S. S.	M. S.
Regression on X	1	0.083536	0.083536
Deviation from regression	7	0.002187	0.0003124
Total	8	0.085723	

Arithmetic data

	d. f.	S. S.	M. S.
Regression on X	1	0.024807	0.024807
Deviation from regression	7	0.0004	0.000057
Total	8	0.0252	

$$F = \frac{0.0003124}{0.000057} = 5.48 \qquad F_{.05,7,7} = 3.79$$

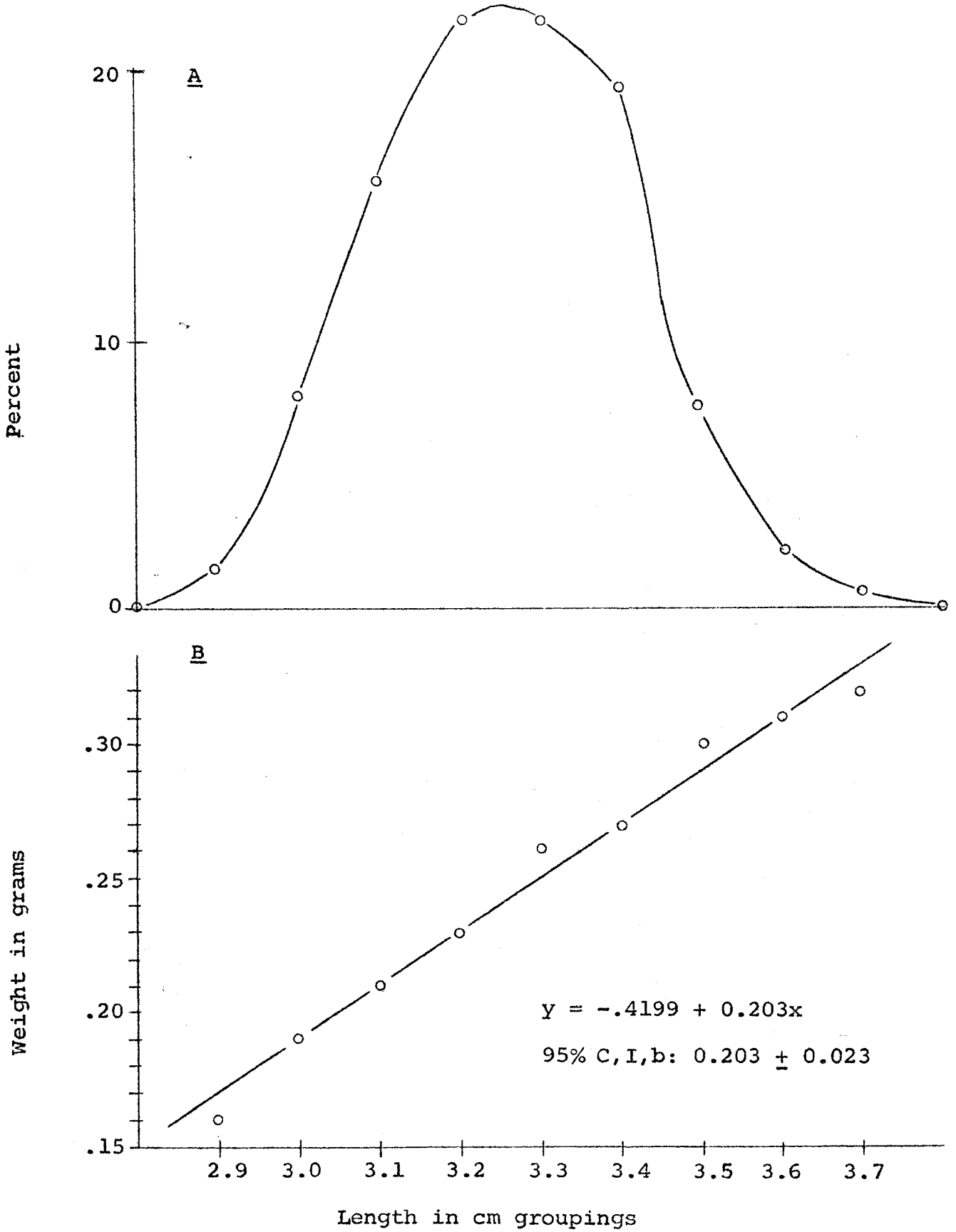

Figure 1. A. Length frequency distribution of 360 pink salmon downstream migrants - Big Kitoi Creek, 1961 (snout to base of caudal fin).

B. Averages of empirical weights for given lengths of 360 pink salmon downstream migrants, Big Kitoi Creek, 1961.

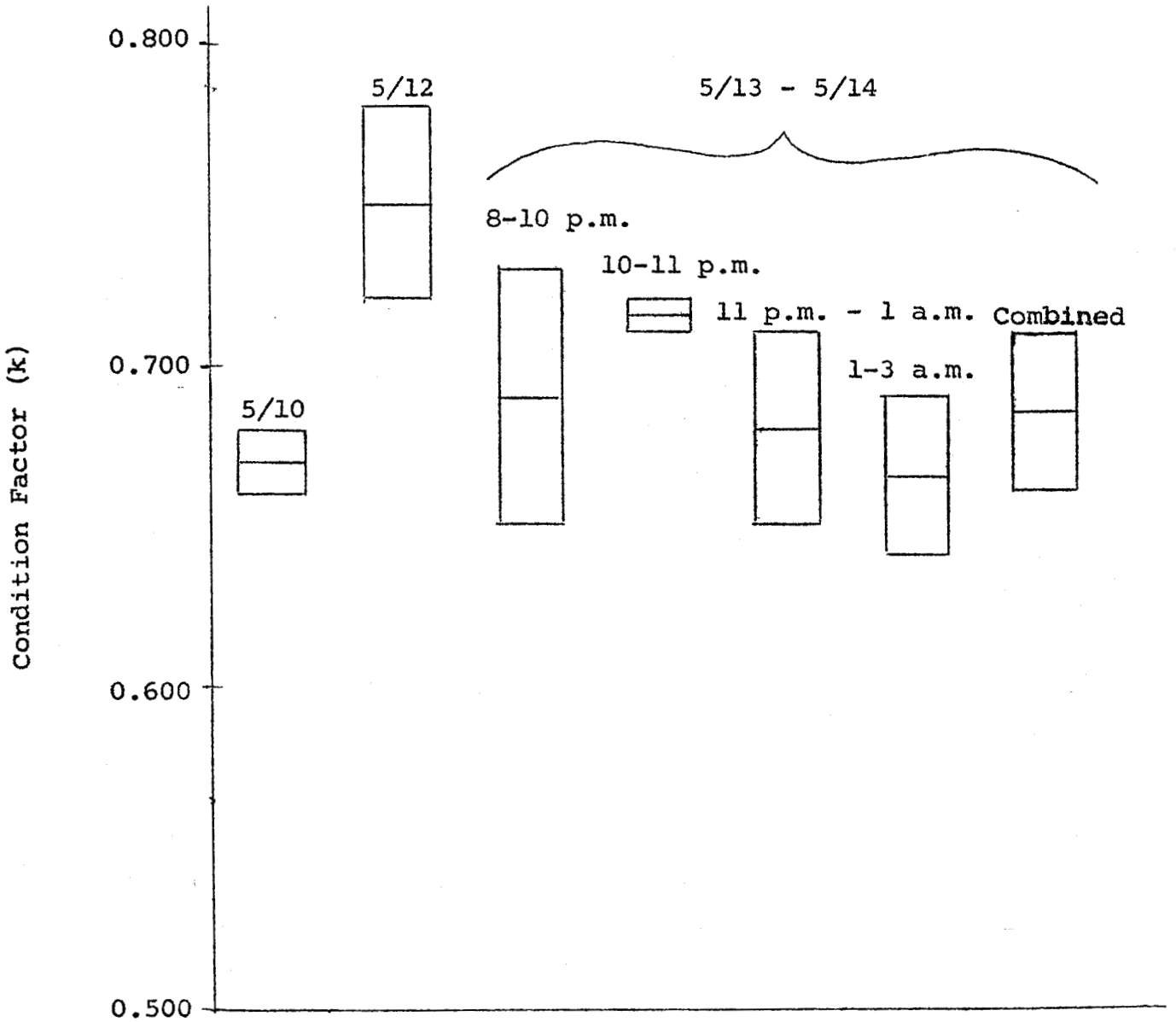

Figure 2. Ninety-five percent confidence limits for mean condition factor (k) for different samples of pink salmon downstream migrant fry captured in Big Kitoi Creek 1961.

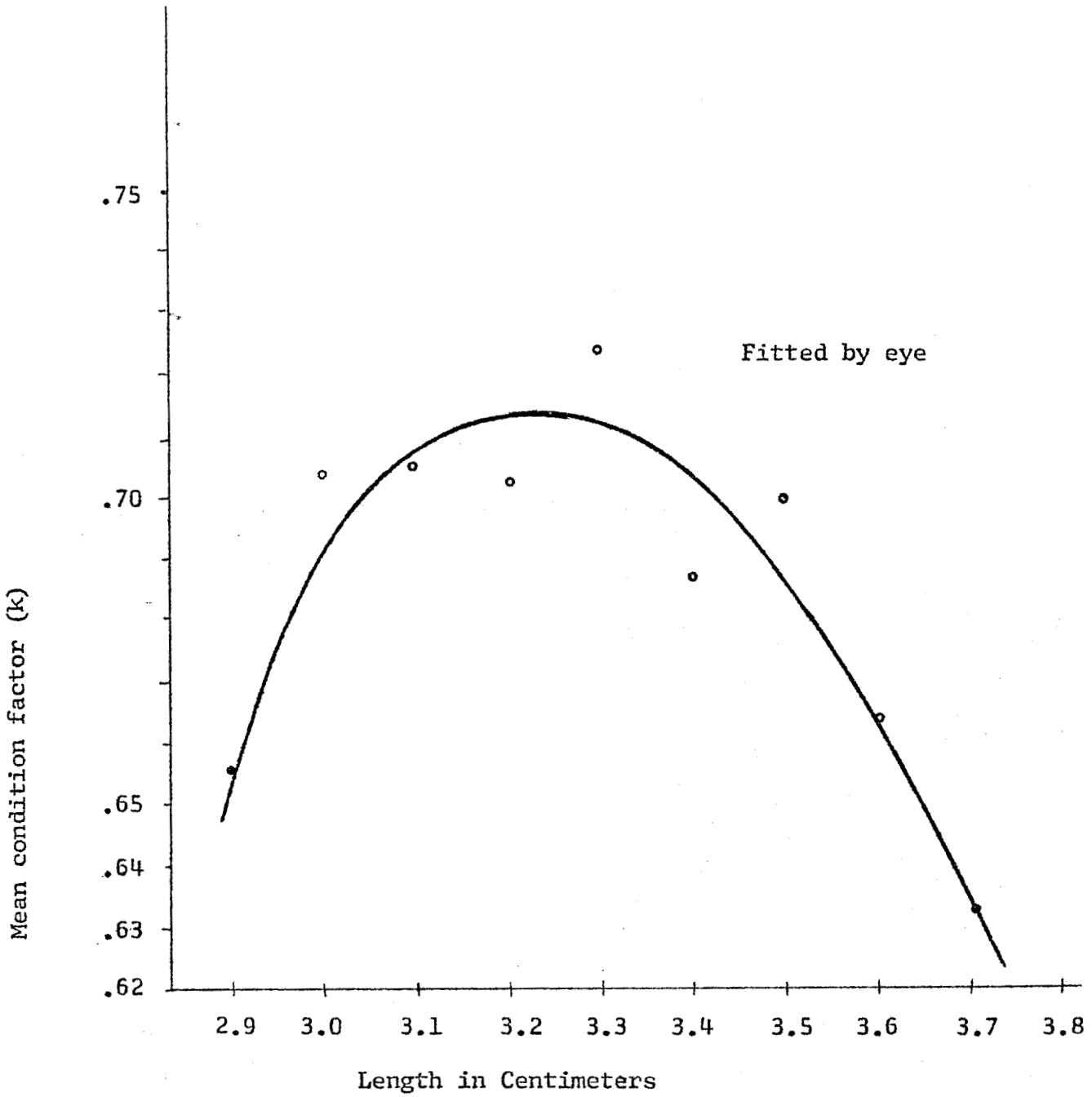

Figure 3. Relationship of condition factor (k) to length of pink salmon downstream migrant fry, Big Kitoi Creek, 1961.

Variability in size between areas

Results of measurement of stream-reared fry.---Length measurements from 9 samples of pink salmon fry collected in Prince William Sound in the spring of 1961 were compared with length measurements from pink salmon fry from Big Kitoi Creek (Figure 4).

To make the statistical test, Sheep Bay samples were combined (N=125) and compared with combined Big Kitoi Creek samples (N=367). Sample means (Big Kitoi 32.69 mm, Sheep Bay 30.13 mm) are significantly different ($t=16.08$, $p=<0.001$).

Samples from both areas were then tested for difference of means of volume. Results are shown in Table 5. Again, Prince William Sound pink salmon fry are significantly smaller than Big Kitoi pink salmon fry.

Downstream migrants collected in the spring of 1962, when subjected to similar analysis, do not show the same clearcut size difference. Preserved samples of pink salmon fry migrants were sent from Prince William Sound to Kodiak and remeasured, along with preserved samples of Big Kitoi migrants. First ten fry at a time, then 20 fry at a time were weighed and volumetric displacement ascertained. Ninety-five percent confidence limits for means are shown in Figure 5. Several things are apparent. First, there is marked variability between samples; secondly, there is less spread when migrants are measured by 10's than when measured by 20's (degrees of freedom of course, vary here from 9 to 19); thirdly, the trend is the same, whether by weight or volume, by 10's or 20's; and when measured by 10's there is no essential difference between weight measurements and volume measurements; lastly, because of variability within one stream, no distinct difference between Big Kitoi and Sheep Bay migrants is apparent. Fry in two samples, Big Kitoi 4/30 and 5/8, are, however, larger than any other fry.

If there was a distinct difference in size between pink salmon fry from the two areas in 1962, it may not appear because of limitations in the data or because measurement of volumetric displacement technique was not completely standardized. An indication of the need for standardization of measurement techniques appears in an analysis of measurement data by two different observers from the two areas before and after preservation in formalin. Figure 6A shows the change in volume and weight of three samples of Big Kitoi fry measured fresh and remeasured about one month later after preservation in formalin. Figure 6B shows the change in volume of fry from Sheep Bay first measured fresh by the observer in Prince William Sound and then (after preservation in formalin) remeasured about a month later by the observer at Kitoi Bay Research Station.

This comparison (Figures 6A and B) shows a distinct decrease in both weight and volume of the Big Kitoi fry measured before and after preservation by the same observer. On the other hand, fry in three of the Sheep Bay samples increased in volume (these fry were not weighed in fresh condition), fry in one sample decreased in volume, and fry in another sample remained essentially the same. There was a decrease in all other instances where fry were measured by the same observer before and after preservation. Therefore, the failure of the Sheep Bay samples to decrease in a similar way when measured by different observers implies a difference in measurement technique between observers.

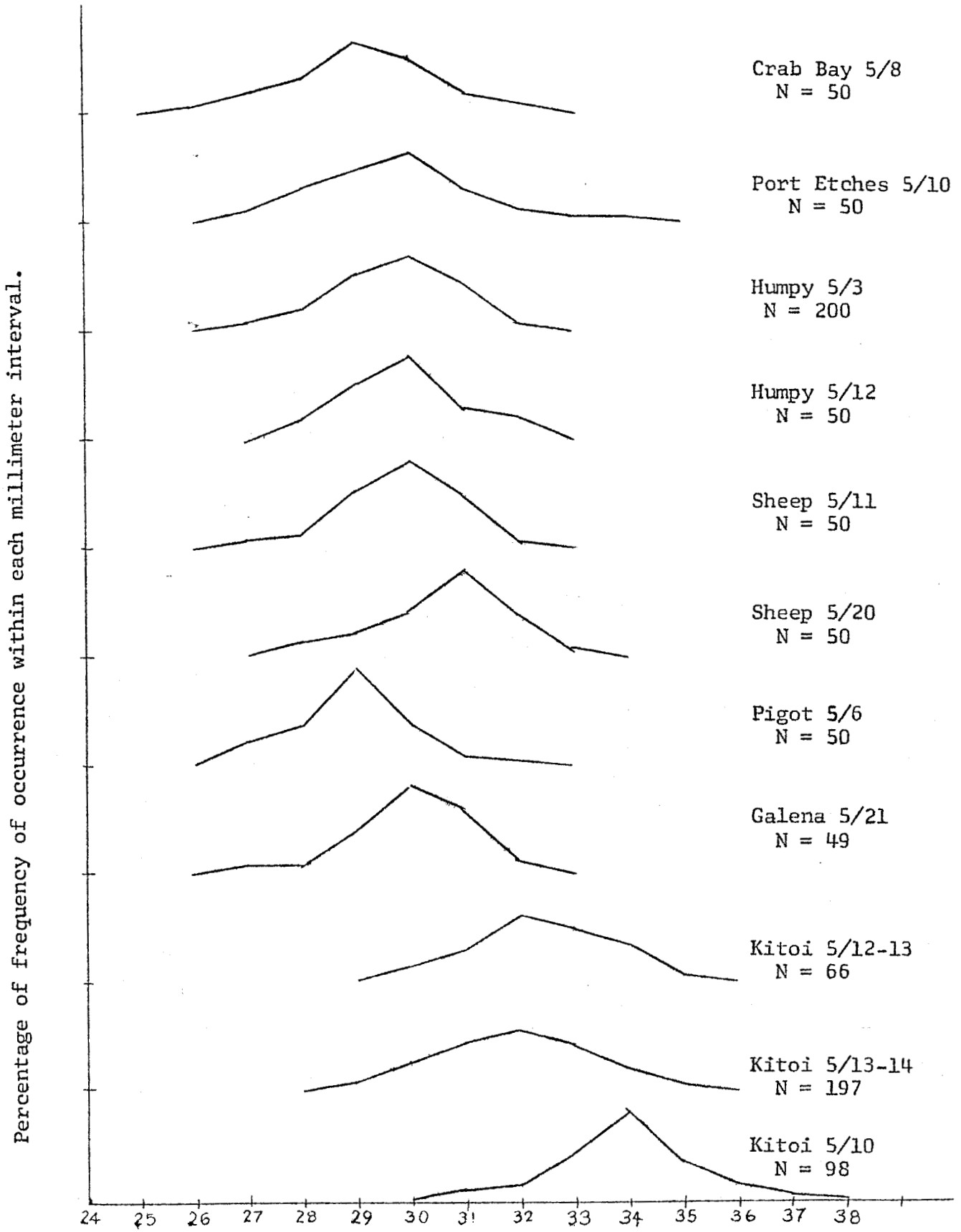

Figure 4. Length frequency distribution of pink salmon downstream migrant fry - Prince William Sound and Kitoi Bay, 1961.

Table 5. Volumetric displacement, samples of pink salmon migrants from Kitoi and Sheep Bay.

	Mean displacement of 20 fry	S^2	t	P
Kitoi Bay	5.33 ml.	0.06793	11.53	<0.001
Sheep Bay	4.47 ml.	0.09896		

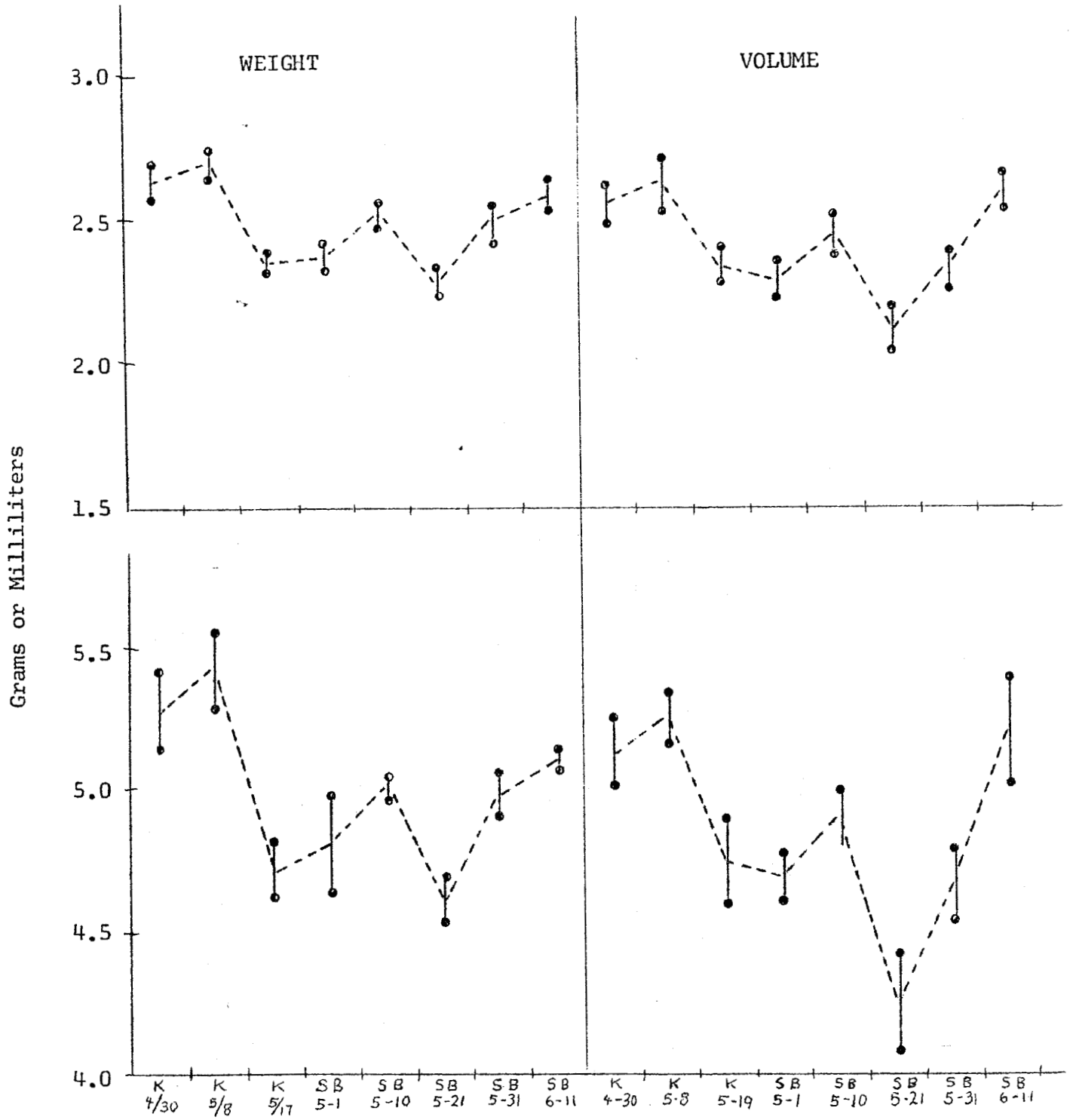

Figure 5. Ninety-five percent confidence interval for mean values of samples of pink salmon fry measured by increments of 10 (above) and 20 (below). (K refers to Kitoi, SB to Sheep Bay) 1962.

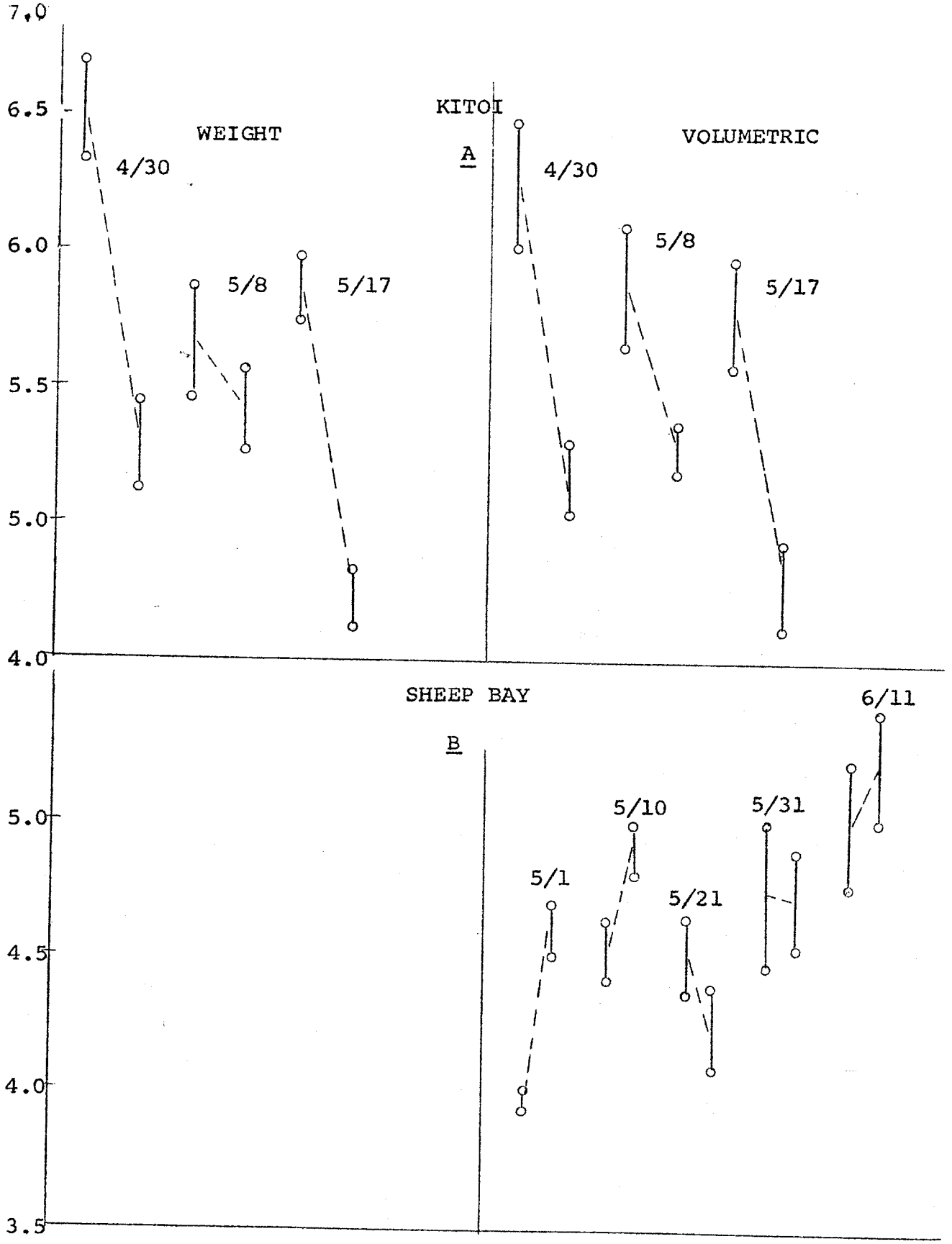

Figure 6. A. Change in volume and weight of 3 samples of Kitoi fry measured while fresh and after preservation in formalin (same observer).

B. Change in volume of 5 samples of Sheep Bay fry (different observers).

Although standardization of techniques was attempted, it is obvious that the volumetric method of measurement should have been tested to determine (1) differences between observers and (2) the ability of one observer to obtain consistent results from repeated measurements of the same sample. It was not possible at the time for observers in the two areas to work together until acceptable standards were developed.

One last point of interest in Figure 6B is the increase in size of Sheep Bay fry as the season progresses. This is not in agreement with Skud (1955) who reported in Sashin Creek a decrease in size of fry with time, or with McNeil[2] who observed (also in Sashin Creek) that average size of fry increased up to the mid-point of the downstream migration, after which a sudden and sharp decline in average size occurred.

Results of measurements of hatchery reared fry

A preliminary step was taken to determine if size differences would appear between pink salmon fry from Prince William Sound and from Afognak Island, if eggs were incubated under identical conditions.

Eggs were taken from six ripe pink salmon females in Sheep Bay Stream #11, in Prince William Sound and fertilized with milt from three different males at 1:15 to 1:30 p.m. on September 19, 1961. Fertilized eggs were allowed to water harden 40 minutes, then 500-600 eggs were placed in each of four thermos bottles. At 5:00 p.m. on September 19, eggs taken to Cordova by skiff were stored overnight in a cool room. At 8:00 a.m., September 20, a few small ice cubes were added to each thermos; thermoses were packed and given to Pacific Northern Airlines at 11:00 a.m., September 20. The eggs arrived at Kodiak at 10:00 a.m., September 21. Eggs from two thermoses were placed in the Alaska Department of Fish and Game hatchery at the Kodiak Naval Base at 11:00 a.m.; those from other two thermoses were placed in the Alaska Department of Fish and Game Kitoi Bay hatchery at 2:00 p.m.

Eggs were also taken from several pink salmon females and fertilized with milt from three males from Big Kitoi Creek. These eggs were placed in modified Roberson hatchery trays with the eggs from Sheep Bay at 4:00 p.m., September 21.

Eggs from both Sheep Bay Stream and Big Kitoi Creek were divided into two trays (total 4). A Roberson incubator of this type contains 10 trays. Assignment of lots of eggs to any particular tray position was random.

The pink salmon eggs were incubated until May 31, 1962 when fry were removed from the trays and samples of 200 fry from Big Kitoi and of 200 fry from Sheep Bay lots were weighed and volume ascertained. The reason for termination of the experiment at this particular time was the fear that an increased silt load in the hatchery water supply (caused by construction activities) would smother alevins. At the 1300-1400 temperature units at which fry were measured, the yolk sac slit was still discernible. Results of these measurements showed a significant size difference between pink salmon fry from Sheep Bay and pink salmon fry from Big Kitoi Creek (Table 6, Figure 7).

Table 6. Nine-five percent confidence limits, and t values for mean weight and volume of 200 pink salmon fry from Prince William Sound and 200 pink salmon fry from Kitoi Bay, Afognak Island (measured in increments of 10 and increments of 20).

95% C.L. by 20's-weight	$3.621 < M < 3.807$	$4.290 < M < 4.491$
95% C.L. by 20's-volume	$3.473 < M < 3.797$	$4.362 < M < 4.664$
95% C.L. by 10's-weight	$1.811 < M < 1.903$	$2.150 < M < 2.241$
95% C.L. by 10's-volume	$1.758 < M < 1.877$	$2.187 < M < 2.326$

t, weight by 10's	11.00	95% C.L. for differences between means 0.3385 ± 0.0622
t, volume by 10's	10.02	
P 0.01		0.4390 ± 0.0885

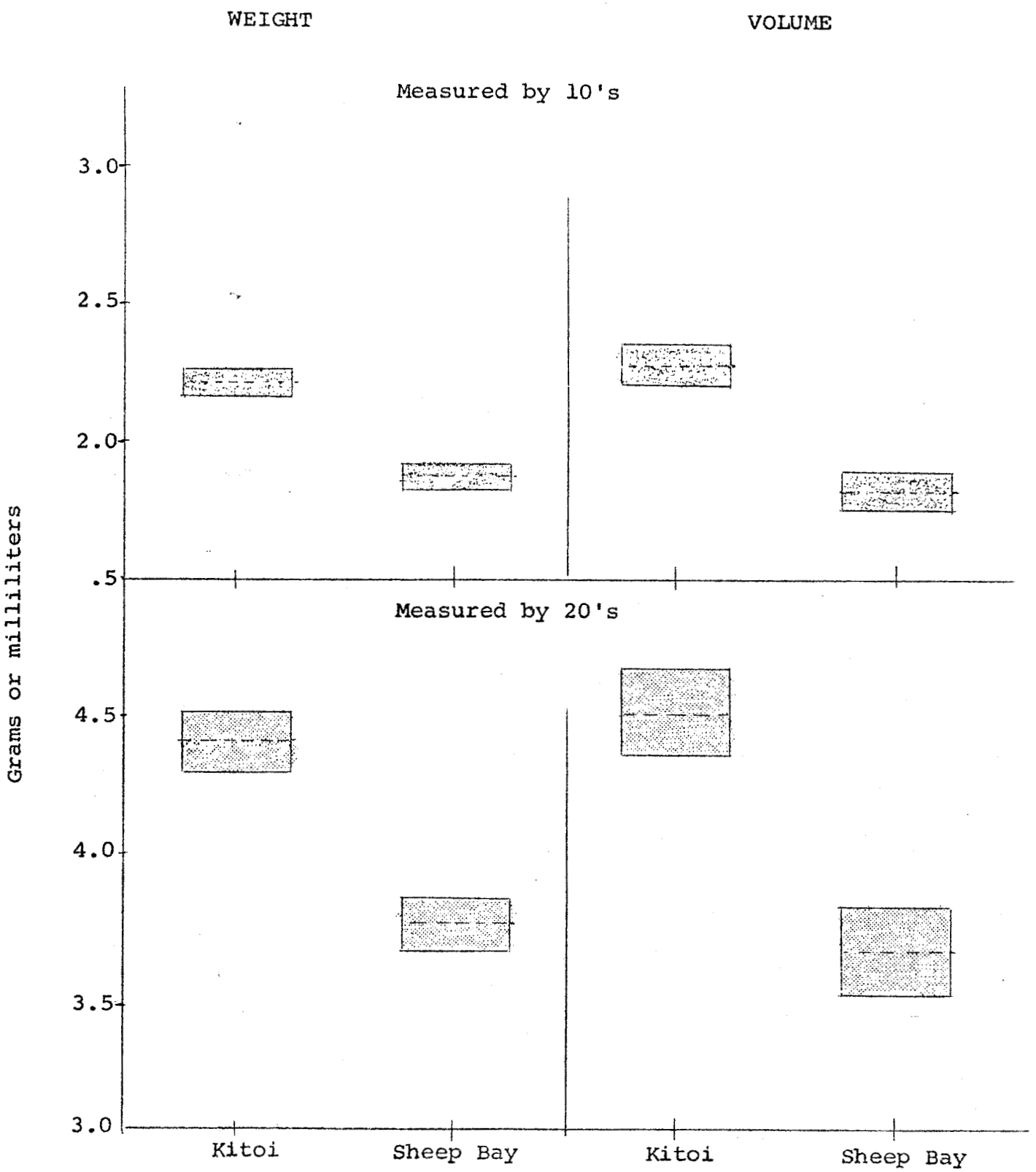

Figure 7. Ninety-five percent confidence limits for mean weights and volumes of pink salmon fry from Prince William Sound and Afognak Island, 1962 (sample size, 200, measured in lots of 10 and 20).

In addition to being statistically significant, the size difference between the two lots of fry raised under identical condition was apparent from gross visual observation. There can be no doubt that (1) the Sheep Bay embryos, given the same number of temperature units, did not develop at as high a rate as the Big Kitoi embryos, or (2) the Sheep Bay embryos were at essentially the same stage of development, but were never intended to be as large at any specific developmental stage as the Big Kitoi embryos. A physiological examination might have revealed additional differences.

DISCUSSION AND CONCLUSIONS

Our discussion and conclusions are as follows:

1. From results of our work and results of other work (cited) we conclude that pink salmon fry vary in size within one stream and from one stream to another. Specific reasons for size differences may be postulated but are as yet not really known. Pink salmon fry may vary in size because of environmental opportunity (this is indicated by the Oregon State University work)[3]. Some embryos may develop under superior conditions of available dissolved oxygen and seepage velocities, other embryos may develop under a more favorable temperature regimen as a result of timing of egg deposition. Or these factors may be, and probably are, interrelated. In addition, certain races may be better endowed genetically. These races would be stronger than others and better able to survive under any given set of environmental conditions.

2. Are size differences between different stocks or races due to environment or heredity, or both? When pink salmon eggs from a stream in Prince William Sound were incubated with pink salmon eggs from a stream on Afognak Island under identical conditions of dissolved oxygen and temperature, resulting fry from Afognak Island were significantly larger at a developmental stage of 1300-1400 temperature units. However, although this is an interesting fact, it does not answer the questions. It does not mean that all fry over the season were larger. It only means that eggs taken from specific pink salmon at a specific time resulted in larger fry from the Afognak Island stock.

3. There is a distinct possibility that a correlation exists between estuarine survival of pink salmon and their size (or robustness) when they enter the estuary. There is also a distinct possibility that robust fry are produced in a good environment and that smaller, weaker fry are produced in a poor environment (strictly aside from genetic characteristics). If true, this means that an improvement of the environment (increase in permeability and stability of streambed gravels) will not only increase net production, but will also increase quality of the embryo thereby enhancing estuarine survival. Since the highest mortality rate occurs in the early life of the pink salmon, any increase in estuarine survival means an increase in numbers of returning fish.

4. What do we need in the way of research to demonstrate some of these points?

a. We need first to develop the association between robustness and dissolved oxygen levels, seepage velocity and timing of egg deposition and sequential temperatures during development in one stream and in the laboratory. (Field work on this phase has been started at the Fish and Wildlife Service, Little Port Walter Research Station).

b. Next we need to demonstrate meristic differences between pink salmon fry from different streams and different areas and ascribe these size differences to their specific cause or causes.

c. Lastly, we need to correlate robustness of pink salmon downstream migrant fry with estuarine survival. To do this we must know the specific effects of estuarine temperatures, salinity, and food availability.

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