Increasing Efficiency in Coho Salmon Scale Sampling

by

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Abstract

The proportion of fish eliminated from scale pattern analyses because of scale regeneration can be 50% or more of an entire sample. The effect of increasing the number of scales collected per fish on estimated regeneration rates was examined for ten populations of coho salmon (*Oncorhynchus kisutch*). Increasing the number of scales collected per fish from 1 to 6 doubled the average proportion of fish possessing at least one non-regenerated or usable scale within its scale sample. Scale regeneration rates were not significantly different between left and right sides of the fish's body ($P>0.05$). However, some scale samples taken from only one side showed significant dependence in the probability of scale regeneration, so that scale sampling should be spread out over as large an area of the fish's body as possible while still controlling for body area induced bias in scale measurements. Simulation analyses indicated that spreading sampling out over one side of the fish's body resulted in the highest proportion of fish with at least one usable scale and eliminated the need to collect samples from both sides. As the body area of scale collection decreased in size, dependence in regeneration of scales increased, and collecting samples from both sides of a fish resulted in more usable scales.

Introduction

Regeneration of scales is a common phenomenon in Pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*Salmo gairdneri*), and occurs after a scale is dislodged from the epidermis and lost due to injury, disease, or stress (Neave 1940). Replacement scales, formed to cover and protect the underlying epidermis, lack the life history information described by the circuli patterns formed prior to removal of the previous scale. As a consequence, regenerated scales have incomplete life history records and cannot be used for age determination or stock identification.

Researchers need to efficiently sample fish that are collected because the sampling of large numbers of fish, particularly in remote locations such as the high seas, is difficult and costly. Small sample sizes can affect the precision of stock proportion estimates obtained by the classification matrix procedure, since variances of estimates are high when standard and unknown samples are less than 100 (INPFC 1987). The proportion of fish eliminated from scale pattern analyses because of scale regeneration can be 50% or more of an entire sample (Bohn and Jensen 1971; Harris et al. 1981; Rogers et al. 1983; INPFC 1987). Coho (*O. kisutch*), chinook (*O. tshawytscha*), and sockeye

salmon (*O. nerka*) and steelhead trout have scale regeneration rates of 27% or greater, while regeneration rates in chum salmon appear to be on the order of 10% or less (Bilton 1984; INPFC 1987; J. Sneva, Washington Department of Fisheries, unpublished data).

For species with high regeneration rates, the proportion of fish possessing at least one non-regenerated or usable scale within its scale sample can probably be increased by collecting more than one scale per fish. However, collecting scales located next to each other should result in scale samples exhibiting a certain degree of dependence in the probability of being regenerated, since phenomena causing regeneration will affect patches of adjacent scales. Thus, if a scale taken from an individual fish is found to be regenerated, the probability that adjacent scales are also regenerated will be high. Therefore, it seems reasonable that scales should be collected from over as large an area of the body as possible in order to minimize any dependence in regeneration rates, while still controlling for variation in scale patterns due to differences between body areas (Knudsen 1985). If there is dependence in the probability of regeneration when scales are collected from one side of a fish, it may be possible to reduce or eliminate dependence by taking samples from both sides of a fish.

In this paper, I examine the effect of increasing the number of scales collected per fish on the estimated regeneration rates of ten populations of coho salmon. Dependence in the probability of regeneration for scales collected from one side of a fish and differences in scale regeneration between left and right sides of the fish are also examined. In addition, simulation analyses are performed to determine whether it is more efficient to spread scale sampling out over one side of a fish or between both sides.

Scale Collections

Spawned-out adult coho salmon carcasses were sampled for scales at seven Puget Sound locations between 1985 and 1987 (Table 1), and acetate impressions of the scales were made (Koo 1962). Six scales were taken from both the left and right sides of each fish (i.e., a total of twelve scales) in the 1986 Skykomish Hatchery and Grizzly and Lewis Creek samples and from the left side only in the other seven samples.

Scale samples were collected from the area of the fish's body described in Figure 1. This area includes the International North Pacific Fisheries Commission's (INPFC) preferred area A and half of the dorsal/anterior INPFC area B (Major et al. 1972). Knudsen (1985) showed that measurements of scales collected from within the body area described in Figure 1 were not significantly different in chinook salmon and are suitable for stock separation analyses, and Clutter and

Whitesel (1956) found that lacustrine circuli counts of scales from within this body area differed by less than 0.3 circuli in sockeye salmon. Although these body areas have not been tested in coho salmon, it seems reasonable to assume the same trend also holds for this species.

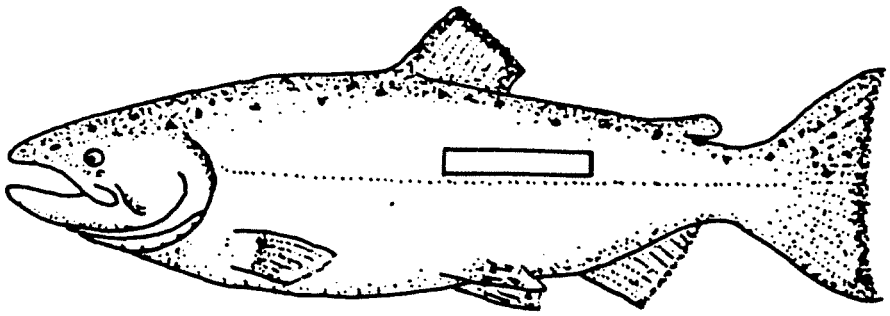

Figure 1. The area of each fish's body sampled for scales is enclosed in a box just dorsal to the lateral line. Sampling was spread out over the approximately 100 scales covering this area in order to reduce any dependence in the probability of scale regeneration.

Scale impressions were viewed with a microfiche reader at 24 and 48 power and characterized as either regenerated or usable based on the size and shape of the central portion of the scale (called the focus). In general, small circular or slightly oblong foci are characteristic of usable scales. Slightly regenerated scales (scales regenerated early in life) are missing small portions of life history information and have irregularly shaped larger foci, while extremely regenerated scales are deformed with large amounts of life history information missing (Neave 1940; Mosher 1968).

Estimates of Regeneration Rates

In this paper, a 'usable fish' is any fish possessing at least one non-regenerated or usable scale within its scale sample, the 'proportion of usable fish' is equal to the number of usable fish divided by the total number of fish sampled, and the 'regeneration rate' is the proportion of fish possessing only regenerated scales within its sample. Estimates of the proportion of usable fish available when one scale per fish is taken were made for each of the ten populations by summing the total number of usable scales and dividing by the total number of scales collected. Estimates of the proportion of usable fish when six scales per individual were collected were made by determining the total number of usable fish and dividing by the total number of fish sampled. The mean overall between-group difference in the proportion of usable fish was calculated in the following manner.

The absolute values of all between-group pairwise differences in the proportion of usable fish were calculated. This was done for each of the two sampling methods, one or six scales collected per fish, and the mean was then determined for each method.

In order to test whether there was dependence in the probability of regeneration of scales collected from one side of a fish, the observed number of fish possessing n regenerated scales ($0 \leq n \leq 6$) out of six was compared to the expected number, assuming independence of events, using a chi-squared test. The expected frequencies were based on a binomial distribution with q equal to the probability of a scale being regenerated and p equal to the probability of the fish being usable when one scale per fish is collected (1-q) and with the number of trials (scales collected per fish) equal to six. If there is dependence between events, then expected values of p^x and q^x ($x>1$) are actually less than observed values. Thus, the tails of the expected frequency distribution of fish, representing fish with large or small numbers of regenerated scales, will underestimate the observed values, and the expected middle frequencies will overestimate the observed values. Expected cell values were pooled so that no expected value equaled less than five (Zar 1984) in order to avoid bias in the chi-square statistic.

In order to test for differences in scale regeneration between left and right sides, the number of usable scales from each side of each fish was calculated (values ranged from 0 to 6) for each of three populations and used in paired-sample t-tests.

Regeneration Results

Estimates of the proportion of usable fish from each stock when one and six scales per fish were collected are given in Table 1. Comparing the overall means for each sampling routine shows that sample sizes can, on average, be doubled by increasing the number of scales taken per fish from 1 to 6 (range of 1.5 to 3.0 times). The average absolute value of all between-group differences in the proportion of usable scales when 1 scale per fish is taken was 18%. This was reduced to 10% when 6 scales per fish were collected. In theory, the proportion of usable fish will increase as the number of scales collected per fish increases to some upper limit determined by the proportion of fish in the population that possess only regenerated scales within the body region sampled.

The observed percentages of fish with n usable scales ($0 \leq n \leq 6$) for each of the ten populations are given in Table 2 and the results of the chi-squared tests are given in Table 3. Six of the ten groups had frequency distributions that were significantly different from expected values given independence of trials ($P<0.01$). Collecting scales over

the body area described in Figure 1 did not eliminate dependence in scale regeneration in all groups. It is likely that the 1986 Skykomish Hatchery sample (P=0.07; N=29) would also have given a significant chi-squared value if the sample size were increased, thus increasing the number of degrees of freedom to more than one by increasing the number of expected cells containing at least five observations.

Table 1. The proportion of fish with at least one usable scale (p=1-q) when 1 and 6 scales are collected per fish. The values of $1-q^6$ represent the expected proportion of fish with at least one usable scale when six scales per fish are sampled given independence of events.

Sample	Proportion of fish with at least one usable scale: One scale per fish	Six scales per fish	$1-q^6$	N
Skykomish Hatchery 1985	0.578	0.977	0.994	85
Skykomish Hatchery 1986	0.638	0.966	0.998	29
Skykomish Hatchery 1987	0.598	0.945	0.996	109
Skagit Hatchery 1985	0.574	0.939	0.994	131
Skagit Hatchery 1987	0.531	0.954	0.989	172
Simpson Hatchery 1987	0.441	0.887	0.969	124
Bingham Creek wild 1987	0.291	0.786	0.873	168
Grizzly Creek wild 1986	0.253	0.759	0.826	29
Lewis Creek wild 1986	0.347	0.875	0.922	24
Deschutes River wild 1985	0.234	0.710	0.798	62
average	0.449	0.880	0.936	

Table 2. Observed frequency distributions of the percentage of fish with n usable scales ($0 \le n \le 6$) when six scales per fish were collected.

Sample	Frequency (%) of fish with n usable scales: n=0	n=1	n=2	n=3	n=4	n=5	n=6
Skykomish Hatchery 1985	2	5	18	27	22	19	7
Skykomish Hatchery 1986	3	10	14	3	24	31	14
Skykomish Hatchery 1987	6	6	14	15	29	18	12
Skagit Hatchery 1985	6	8	17	17	19	21	12
Skagit Hatchery 1987	5	11	21	20	20	16	8
Simpson Hatchery 1987	11	18	26	22	10	10	3
Bingham Creek wild 1987	21	27	23	18	7	4	0
Grizzly Creek wild 1986	24	31	25	14	3	3	0
Lewis Creek wild 1986	13	25	25	25	4	8	0
Deschutes River wild 1985	29	34	15	14	6	2	0

Table 3. Results of chi-squared tests for each of the ten coho salmon samples comparing the expected frequency of regenerated scales per fish to observed values.

Sample	Chi-squared value	df	P
Skykomish Hatchery 1985	3.65	2	0.16
Skykomish Hatchery 1986	3.22	1	0.07
Skykomish Hatchery 1987	12.69	2	<0.01
Skagit Hatchery 1985	43.58	3	<<0.01
Skagit Hatchery 1987	32.51	3	<<0.01
Simpson Hatchery 1987	41.58	4	<<0.01
Bingham Creek wild 1987	20.94	3	<<0.01
Grizzly Creek wild 1986	1.41	2	0.49
Lewis Creek wild 1986	0.69	1	0.41
Deschutes River wild 1985	9.51	2	<0.01

In all ten populations, the expected proportion of fish in the tails of the frequency distribution, representing fish with either all or nearly all usable or regenerated scales, underestimated the observed proportions. Thus, dependence is present at significant levels in six of the samples, and at low levels in the other samples. If one were to estimate, using the binomial distribution and data based on one scale per fish, what proportion of fish in the ten populations would be usable when six scales per fish are collected, the proportion of fish having all regenerated scales would always be underestimated. Consequently, the number of usable fish would always be overestimated. This is demonstrated in Table 1 where the difference in predicted and observed percentages is as high as 9%.

Paired-sample t-tests comparing the number of usable scales in samples taken from the left and right sides of each fish were all non-significant ($P>0.05$). Thus, samples collected from either side of a fish should contain similar numbers of usable fish.

Simulation Analyses

Two simulation analyses were designed to determine whether it is more efficient, in terms of the resulting number of usable fish, to sample scales from a larger (Fig. 1) or a more restricted body area and from one or both sides of the fish. The 1986 Grizzly Creek (one randomly selected fish was removed so that the total number of fish would be even) and Lewis Creek samples were pooled in order to increase the size of the data set analyzed (n=52). The 1986 Skykomish Hatchery sample was not included due to its distinctly different frequency distribution of usable scales (see Table 2).

In the first simulation analysis, sampling of scales was stratified over the body area described in Figure 1. Fish in the pooled data set were randomly divided in half. In the first half, a random sample without replacement of three scale values from the left side and three scale values from the right side ("combined left/right" sampling method) of each fish was selected. Since scale samples were originally collected from over the entire body area in Fig. 1, sampling without replacement results in half of the six sampling sites on each side of the fish being represented. Thus, scale sampling is spread out randomly over six strata on each side of the fish. The number of usable fish was then determined for this half of the pooled data set. For the second half of the pooled data set, the number of usable fish was determined from all six scales from only the left side ("only left" sampling method) and from all six scales from only the right side ("only right" sampling method). The process of dividing the pooled data set into random halves, sampling the first and second halves, and determining the number of usable fish was repeated 100 times.

In the second simulation analysis, each scale value was randomly selected with replacement. Although unlikely, it was now possible for a single scale value to be selected every time within an individual fish, simulating sampling from a very small body area in which dependence is extremely high and all scales are either regenerated or usable. This resulted in sampling being spread over fewer sampling sites on average and therefore over a smaller total area of each fish's body. Once again the original pooled data set was randomly divided in half. Random samples with replacement were selected from the first half of the pooled sample by the "combined left/right" sampling method and from the second half of the pooled sample by the "only left" and "only right" sampling methods. The number of usable fish in each sample was then calculated. As in the first simulation analysis, this process of dividing the pooled data set in half and selecting random samples was repeated 100 times and the mean number of usable fish over the 100 runs determined.

Simulation Results

The mean number of usable fish was calculated for the "combined left/right", "only left", and "only right" sampling methods in each of the simulation analyses. The results of the first simulation analysis show that when scale sampling is spread out over the body area shown in Fig. 1, sampling six scales by the "only left" (mean=21.5 usable fish out of 26) or "only right" (mean=20.8 fish) methods produces as many usable fish as the "combined left/right" method (mean=20.7 fish). Results for the second simulation analysis indicate that when the total area of the body from which scales are collected is restricted, sampling six scales by the "only left" (mean=17.9 fish) or the "only right" (mean=17.7 fish) methods does not yield as many usable fish as the "combined left/right" method (mean=19.6 fish).

However, the number of usable fish was less than the number obtained when sampling was spread out over a greater total area of the fish's body.

Conclusions

Increasing the number of scales collected per fish for coho salmon to six or more should significantly increase the proportion of usable fish within a sample. Increasing baseline and fishery sample sizes will, in turn, increase the precision of classification estimates.

Spreading scale sampling out over as large an area of the body as possible, while still controlling for bias in scale measurements due to differential growth of body areas, decreases dependence in scale samples, yields the greatest number of usable fish, and eliminates the additional effort and time involved in sampling scales from both sides of a fish. However, as the total body area sampled for scales is reduced, dependence in scales will increase. As dependence increases, sampling from both the left and right sides of a fish becomes important and will yield more usable fish than the same number of scales collected from a small area on one side.

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