

Discrimination among Populations of Sockeye Salmon Fry with Fourier Analysis of Otolith Banding Patterns Formed during Incubation

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Abstract.—We used otolith banding patterns formed during incubation to discriminate among hatchery- and wild-incubated fry of sockeye salmon *Oncorhynchus nerka* from Tustumena Lake, Alaska. Fourier analysis of otolith luminance profiles was used to describe banding patterns; the amplitudes of individual Fourier harmonics were discriminant variables. Correct classification of otoliths to either hatchery or wild origin was 83.1% (cross-validation) and 72.7% (test data) with the use of quadratic discriminant function analysis on 10 Fourier amplitudes. Overall classification rates among the six test groups (one hatchery and five wild groups) were 46.5% (cross-validation) and 39.3% (test data) with the use of linear discriminant function analysis on 16 Fourier amplitudes. Although classification rates for wild-incubated fry from any one site never exceeded 67% (cross-validation) or 60% (test data), location-specific information was evident for all groups because the probability of classifying an individual to its true incubation location was significantly greater than chance. Results indicate phenotypic differences in otolith microstructure among incubation sites separated by less than 10 km. Analysis of otolith luminance profiles is a potentially useful technique for discriminating among and between various populations of hatchery and wild fish.

The stock concept in management of salmonid populations has been in use since the latter part of the 19th century. Because the life histories of Pacific salmon *Oncorhynchus* spp. include a high degree of fidelity to discrete spawning areas (Ricker 1972; Blair and Quinn 1991), populations may be locally adapted to their specific spawning, incubation, and rearing environments (Taylor 1991). Genetically discrete stocks of Pacific salmon spawning within the same drainage occur in pink *O. gorbuscha*, sockeye *O. nerka*, chinook *O. tshawytscha*, and chum salmon *O. keta* (Wilmot and Burger 1985; Adams et al. 1994; Wilmot et al. 1994; Smoker et al., in press). Also, when genetic evidence is unavailable or inconclusive, there is ecological and behavioral evidence to suggest that locally adapted stocks may co exist within relatively small (<10 km) ranges (Holland-Bartels et al. 1994). Individuals from several potentially discrete spawning populations often rear in a common environment throughout much of their life. For example, early- and late-run sockeye salmon may spawn in different environments (tributary versus lake shorelines); however, their young rear in a common lake environment (Burgner 1991). Therefore, studies of population dynamics during the

freshwater phase require some means of discriminating individual populations.

Fishery biologists have used a host of techniques to separate fish populations. Artificial marking includes fin clipping, branding, and coded-wire-tagging (Jewell and Hager 1972; Nielsen 1992); chemical marking of calcified structures (Mulligan et al. 1987; Yamada and Mulligan 1990; Hendricks et al. 1991); and genetic markers (Ihseen et al. 1981; Lane et al. 1990; Seeb et al. 1990; Gharrett et al., in press). To circumvent the assumption that marked and unmarked individuals behave similarly, naturally occurring patterns in scale patterns have been used to separate Pacific salmon (Rowland 1969; Cook and Lord 1977; Cook 1982; Cross et al. 1987). However, this method is limited when populations are of close geographic origin or when potential discriminating factors are fixed before scale formation, as might be the case for fry incubated in tributaries, lake shorelines, or hatcheries.

Otoliths provide a tool for study of the origin of fish populations through their elemental composition (Rieman et al. 1994) and microstructure (Pannella 1971). Although increment deposition may be tied to an endocrine-driven, endogenous circadian rhythm (Campana and Neilson 1985), factors such as water temperature, photoperiod, and feeding frequency may modify or mask the effects of diurnal rhythms (Marshall and Parker 1982; Neilson and Geen 1984). Stress and life his-

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tory events such as hatching, first feeding, and migration from freshwater to salt water, can be recorded in otoliths (Marshall and Parker 1982; Volk et al. 1984; Neilson et al. 1985a; Brothers 1990; Paragamian et al. 1992; Hendricks et al. 1994). However, for them to be valuable for stock identification of sockeye salmon and other Pacific salmonids, otoliths must form sufficiently early and be capable of recording incubation environments. Such evidence does exist for salmonid otoliths (Neilson et al. 1985b; Brothers 1990; Volk et al. 1990), which form by the time embryos reach the eyed egg stage. In addition, temperature alone has been shown to strongly influence otolith banding during incubation (Volk et al. 1990).

Naturally occurring differences in otolith structure formed during incubation and larval stages have been reported for a wide range of species such as between summer and winter steelhead (anadromous form of rainbow trout *O. mykiss*) (McKern et al. 1974) and between steelhead and resident rainbow trout (Rybock et al. 1975). However, Neilson et al. (1985b) found within-group variability in nucleus size that limited their ability to use this measure to separate steelhead and resident rainbow trout. This disparity occurred despite the fact that young were incubated under controlled conditions, in which group variability should have been minimized. Ambiguity in defining the border of the nucleus, in defining check marks, and in establishing reference points and transects introduce error into increment counts and dimension measures (Wilson and Larkin 1982; Currens et al. 1988; Neilson 1992). We propose a new method that permits examination of variation and components of the overall banding pattern and reduces the subjectivity introduced by artificial definitions of reference points, transects, and increment boundaries.

Fourier analysis allows for the decomposition of complex periodic functions into discrete sub-components. Fourier shape analysis has been used to discriminate fish stocks by scale (Jarvis et al. 1978; Riley and Carline 1982) and otolith shape (Bird et al. 1986; Castonguay et al. 1991; Campana and Casselman 1993; Friedland and Reddin 1994). However, otolith shape at the time of collection illuminates little about differences that occurred during incubation. In contrast, the banding pattern laid down during incubation remains unchanged by subsequent life history events (Campana and Neilson 1985). It is this banding pattern of dark and light intensities (luminance values) across an otolith transect (luminance profile) that can be rep-

resented by a complex periodic function described with Fourier analysis. Therefore, Fourier analysis of luminance profiles may permit discrimination among fish that experience different incubation environments.

The Alaska Department of Fish and Game (ADFG) began a program in 1976 that released hatchery-incubated emergent sockeye salmon fry (fed for several weeks) into Alaska's Tustumena Lake. Eggs obtained from adults returning to spawn each year in two of the lake's tributaries (Bear and Glacier Flats creeks), were incubated at nearby Crooked Creek Hatchery (Figure 1) and released directly into the lake (Kyle 1992). Because fry that incubated at the hatchery and within the lake's drainage experienced different thermal regimes (Figure 2), we hypothesized that these differences would produce varying otolith banding patterns and hence, provide a way to discriminate populations. This could help in future studies of the competitive interactions between hatchery- and wild-incubated young and in discriminating wild fry originating from the various spawning areas in the drainage.

The purpose of this study was to determine the feasibility of using otolith microstructure, as described by Fourier analysis, to discriminate among various sockeye salmon populations rearing within a glacially turbid lake system in Alaska. Specific objectives were (1) to develop standardized techniques for the measurement of otolith microstructure characteristics, (2) to test for differences in the characteristics among sockeye salmon fry originating from various locations in the Tustumena Lake drainage, and (3) to test discriminant models based on banding characteristics.

Study Site

Tustumena Lake is within the Kasilof River watershed, south-central Alaska, and is included within the Kenai National Wildlife Refuge (Figure 1). The 40-km-long, 8-km-wide lake is the largest (about 295 km²) on the Kenai Peninsula and has mean and maximum depths of 124 m and 320 m. The lake is glacially turbid (about 50 nephelometric turbidity units; light penetration, <2 m) and oligotrophic (total phosphorus averages 3.7 µg/L during May–October) due to meltwater from Tustumena Glacier (Kyle 1992).

Sockeye and chinook salmon are the most commercially and recreationally important Pacific salmon species that occur in the system. The system supplies up to 20% (2 million fish) of Cook Inlet's total annual sockeye salmon harvest; esti-

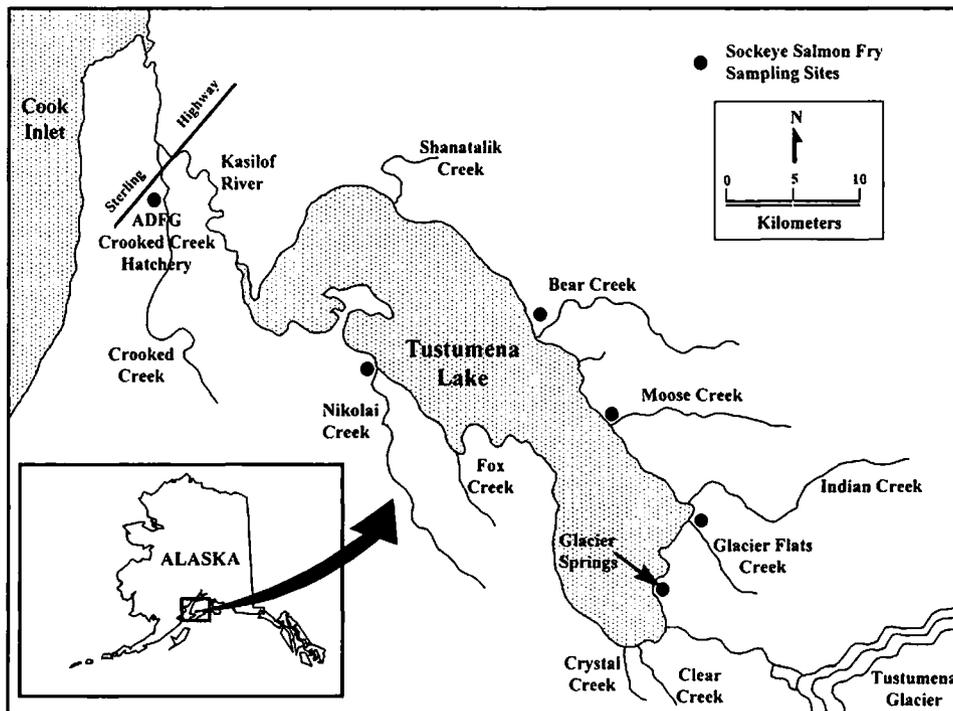


FIGURE 1.—Sampling sites used in analysis of otolith banding patterns of sockeye salmon fry from Tustumena Lake, Alaska.

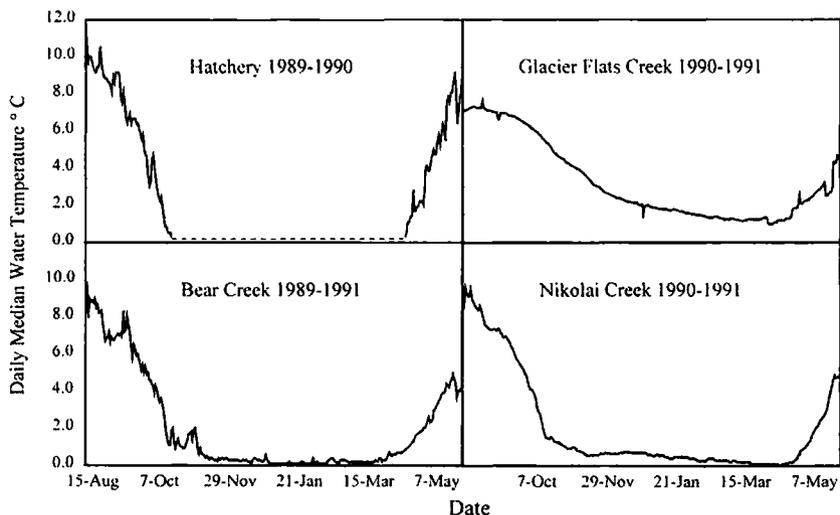


FIGURE 2.—Daily median water temperatures (°C) for Crooked Creek Hatchery, and Bear, Glacier Flats, and Nikolai creeks, Tustumena Lake drainage, Alaska, for various brood years (e.g., 1989: fry spawned during 1989 emerged and migrated into the Lake during spring 1990). Hatchery temperatures not monitored from mid-October to early April.

mated exploitation rates of the lake's sockeye salmon in the commercial fishery range from 50% to 85% (Kyle 1992). About one-third of all sockeye salmon in the system spawn along the lake-shore (Burger et al. 1995); 96% of the remaining spawners are distributed in four tributaries (Bear, Glacier Flats, Moose, and Nikolai creeks; Kyle 1992). Annual hatchery releases have ranged from 400,000 to 17,050,000 fry, and the annual stocking level has been 6,000,000 fry since 1988. Hatchery-incubated fish average about 26% (1981–1990) of the estimated smolt outmigration (Kyle 1992).

Methods

Sample collection and selection.—Six groups of sockeye salmon fry were collected and preserved in ethyl alcohol (>80%; Butler 1992) during 1992: hatchery fish, the shoreline spawning group at Glacier Springs and fish from Bear, Glacier Flats, Moose, and Nikolai creeks (Figure 1). Fry from tributaries were collected as they migrated from incubation areas. At each of Bear, Glacier Flats, and Nikolai creeks, we preserved a sample of fry ($N = 100$) every 7 d from 22 April to 2 June and took an additional sample during peak migration. Moose Creek was sampled three times during the same time period. Collection of fry from the shoreline sample was more problematic. We beach seined regularly where Burger et al. (1995) had documented sockeye salmon spawning activity but collected no more than 50 fry. However, on 2 June several hundred fry were dipnetted as they migrated from clear water springs (Glacier Springs) along the lake shore (Figure 1). These fish constitute our shoreline-incubated sample. Hatchery fry ($N \geq 100$) were dipnetted from hatchery raceways on each of three sampling dates (3, 14, and 15 June). In addition to the fry preserved in alcohol, samples from all locations were preserved in 10% formalin for length and weight measurements and for examination of stomach contents.

When fry from multiple dates were available, subsamples were taken by selecting the peak migration date and then randomly selecting one to two dates before and after the peak date. We selected fry from all three of the hatchery sampling dates. From each of the dates used, 50 fry were randomly selected, except that the Glacier Springs sample included 100 fry randomly sampled on a single date.

Otolith preparation.—Our morphological terminology follows Pannella (1980). We used the largest of the three pairs of otoliths, the sagittae. (Hereafter, our use of the word otolith refers to the

sagittae.) Fry were measured (nearest 0.1 mm, fork length), and otoliths were removed and mounted proximal (sulcus) side down on microscope slides with a thermoplastic glue (Secor et al. 1992) and polished to the primordial zone on the sagittal plane with a lapidary wheel and 1.0- μm and 0.05- μm alumina paste. The polishing process was repeated on the distal side of each otolith.

Feature extraction.—We examined otolith banding features with a transmitted-light microscope, video camera, microcomputer-based digital image analysis system, and Optimas (Bioscan, Inc., Seattle) image analysis software (Finn 1995). The microscope illumination level was held constant for all samples. This process provided a digitized image with luminance intensity measured on a grey scale of black (0) to white (255).

All measurements were taken along transects in the posterior dorsal quadrant of each sagitta (Figure 3), the zone with the greatest growth and best increment definition (Pannella 1980; Marshall and Parker 1982; Wilson and Larkin 1982; Campana and Neilson 1985). To allow application of our technique to older fish, we developed a protocol for the placement and length of measurement transects that was not based on reference points (e.g., rostrum, postrostrum) that can change with fish or otolith growth. In addition, our technique placed transects so that they would include the majority of the otolith formed during incubation, but would not to include areas formed after fry migrate into common lake-rearing environments. We used a random sample of 67 wild-incubated fry otoliths from the four tributary groups to define this zone. Otoliths were measured from the most posterior primordia to the otolith edge along three transects at 40, 60, and 80° angles off a reference line running from the rostrum through the most posterior primordia ($N = 201$; mean = 230.0 μm ; range = 169.5–309.0 μm ; SD = 32.6 μm). Based on these data, we selected a distance of 160 μm for the end point of transects starting at a central primordia, a distance including 55–96% of the zone formed during incubation (0.95 probability) with a probability of less than 0.016 of including otolith banding formed after migration into the lake.

For examination of banding patterns, we selected a portion of the posterior quadrat that included both a distinct primordium and clear banding, and we subjectively eliminated areas with cracks, scratches, and excessive polishing. Transects were then placed perpendicular to the otolith bands. However, before collecting data from transects, the contrast between light and dark bands

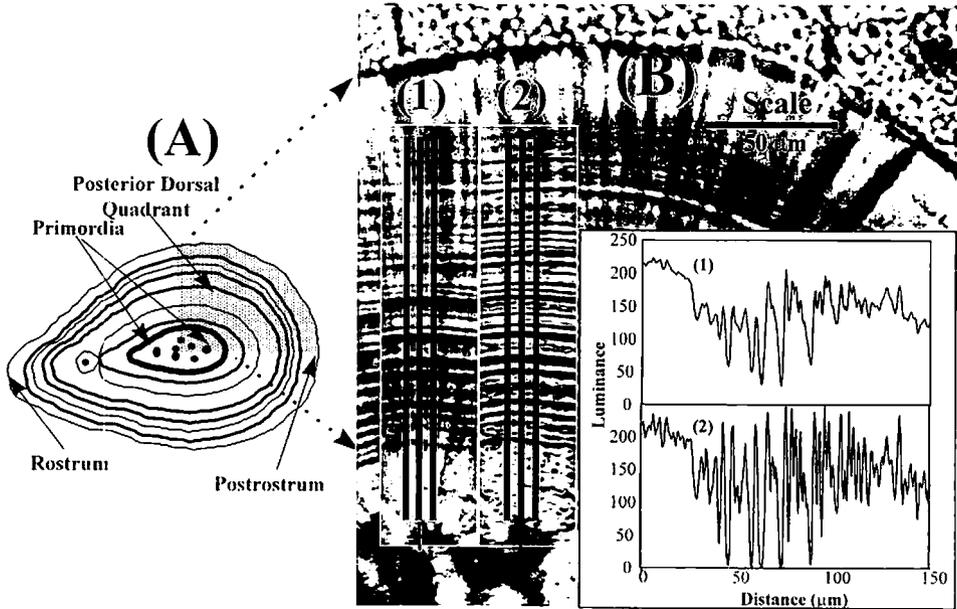


FIGURE 3.—(A) Distal perspective of sockeye salmon fry otolith with major features indicated. All luminance profiles were measured in the stippled area (posterior dorsal quadrant). (B) Otolith image and inserted line graphs show three 150-µm transects, banding pattern, and luminance profiles (1) prior to application of convolution edge detection filter and (2) after filtering.

was enhanced by applying a 3×3 edge detection convolution mask (Figure 3; Gonzalez and Wintz 1987). Then, the primordium was selected as a reference for transect positions. To avoid including the primordium, we began the center transect 10 µm above the start point, resulting in a 150-µm section analyzed for luminance patterns. The other two transects were positioned 5 µm to the left and right of the center transect (Figure 3). Because the algorithm we used for our analyses (described below) required a data series whose N was of an even power (e.g., 64, 128, 256, 512), we selected a data series of 256 measurements along our 150-µm transect, resulting in luminance values measured every 0.586 µm along each of the three transects. Each luminance value was averaged over a width of five pixels (about 3 µm) according to procedures described by Finn (1995). Thus, each otolith had three 150-µm, 256-value luminance transects extracted to spreadsheet data files. In order to integrate the luminance data from the three transects, the luminance values (L) were averaged over the three transects ($i = 1-3$) at each of the $j = 1 \dots 256$ intervals as follows:

$$\bar{L}_j = \sum_{i=1}^3 L_{ij}/3.$$

This resulted in one average luminance profile per

otolith. Average luminance values were then standardized to have a mean = 0 and standard deviation = 1 by subtracting the transect mean luminance and dividing by the transect standard deviation.

Fourier transformation.—The average luminance profiles were transformed into a Fourier series with a fast Fourier transformation (FFT) algorithm (Gonzalez and Wintz 1987; Microsoft Excel 4.0, Microsoft Inc., Redmond, Washington), which decomposed the series into component cosine functions. Cosines are additive such that the luminance value at any given point can be described as

$$L_x = A_0 + \sum_{i=1}^{\infty} A_i \cos(\theta_i - \phi_i); \quad (1)$$

- L_x = luminance value at point x along the transect;
- A_0 = amplitude of the 0th harmonic (amplitude associated with mean luminance);
- A_i = amplitude of the i th harmonic;
- θ_i = polar angle of the i th harmonic; and
- ϕ_i = phase angle of the i th harmonic.

Equation (1) was adapted from Fourier shape analysis (Jarvis et al. 1978) in which the shape is

defined by radii from a centroid by using polar angle coordinates. It can easily describe the shape of a luminance profile if the profile is considered to be an unrolled-shape perimeter and the coordinates along the x -axis are considered distances along a transect, rather than degrees around a polar plot (Jarvis et al. 1978). The luminance profile will be exactly described by a summation of $N = 256$ harmonics at each point of the transect. However, there were only $N/2 = 256/2 = 128$ unique harmonics. The portion of the pattern accounted for by individual or subset harmonics was determined by setting all other harmonics to zero and performing an inverse FFT.

In practice, software algorithms produced a complex number for each point along the luminance profile. This complex number was of the form

$$z = x + yi,$$

where x = a real number; and yi = an imaginary number. Then the amplitude for a given harmonic is defined as

$$A_i = |z| = \sqrt{x^2 + y^2}.$$

The variance of each harmonic is given by

$$V_k = A_k^2/2.$$

As these variances are additive (Jarvis et al. 1978), the proportion of the total variation accounted for by individual and subsets of harmonics is calculated as

$$C_k = A_k^2 / \sum_{k=0}^{N/2} A_k^2.$$

The individual amplitudes were used as variables in statistical analyses. Although the phase angle (ϕ) contains shape information, ϕ is distributed in a circular manner and is often bimodal (Campana and Casselman 1993). Therefore, there are no means to transform ϕ to approximate normality.

Statistical analysis.—Although univariate normality does not insure multivariate normality, tests for multivariate normality are limited (Johnson and Wichern 1988). Therefore to evaluate departures from univariate normality, Lilliefor's test was applied to individual amplitudes (Daniel 1990; SYSTAT 1992). The distributions of the untransformed amplitude variables were all nonnormal (Lilliefor's test; $D_{\max} > 0.059$; $P < 0.001$). Transformations were selected from the family known as Box-Cox power transformations (Sokal and

Rohlf 1981; Johnson and Wichern 1988). The general form of the transformation is

$$X' = (X^\lambda - 1)/\lambda \quad \text{for } \lambda \neq 0;$$

$$X' = \log_e(X) \quad \text{for } \lambda = 0.$$

The "best" value of λ is that value which maximizes the log-likelihood function, L (Sokal and Rohlf 1981). Lambda was evaluated separately for each of the 128 amplitudes over a range of 0.10–1.00 in increments of 0.05. After Box-Cox power transformation, only 6 of the 128 amplitudes were significantly nonnormal ($P < 0.05$). Therefore, all analyses were performed with the above transformation. In contrast, other transformations were less effective: the square root transformation left 43% of the amplitudes significantly nonnormal, and the natural log transformation, 99%.

Data sets were developed to (1) test for differences between left and right otoliths, (2) test for differences between readers, (3) estimate discriminant functions (learning data), and (4) test discriminant functions (test data). Luminance profiles were recorded on 1,203 otoliths. Of these, 427 pairs (left and right from the same fish) were available for testing differences between left and right otolith luminance profiles. To test for consistency between observers, a random sample of 50 otoliths was independently remeasured by a second observer.

Randomized block analysis of variance (ANOVA) on individual amplitudes indicated little difference between left and right otoliths; only 13 (10.2%) of the 128 amplitudes were significantly different ($P \leq 0.05$) between left and right otoliths. Therefore, we felt justified in the random use of either left or right otoliths in learning and test data sets. When paired otoliths were present, left or right otolith values were randomly deleted. This procedure resulted in a data set of 776 luminance profiles that was subdivided into learning and test data sets. For the test data set, a random sample of 25 luminance profiles was taken from each of the six incubation groups (total = 150). The remaining 626 observations made up the learning data set.

We attempted to classify individuals into $m = 2$ groups (hatchery versus wild) and $m = 6$ groups (hatchery, Bear Creek, Glacier Flats Creek, Glacier Springs, Moose Creek, and Nikolai Creek). Linear (LDF) and quadratic (QDF) discriminant functions were used to develop classification rules with the learning data set (SAS Institute 1989b;

Huberty 1994). The LDF provide optimal discrimination rules under the assumptions of multivariate normality and equality among group covariance matrices (homoscedasticity). The QDF classification rules assume multivariate normality; however, the assumption of homoscedasticity is relaxed (Huberty 1994). The assumption of homoscedasticity was tested with Bartlett's log-likelihood ratio (SAS Institute 1989a; McLachlan 1992). Although the form of the data provided guidance for selection of statistical techniques (McLachlan 1992; Huberty 1994), the success (classification rate) of the rule in assigning individuals to the correct group is the most important criteria for population separation. Classification rates were estimated with the learning data with cross-validation (also known as the Lachenbruch's "holdout" or "leave-one-out" technique; Johnson and Wichern 1988; McLachlan 1992) and by applying discriminant rules to the test data.

A problem with Fourier analysis of luminance values is the large number of variables (128) available for the discriminant model. A general rule is to restrict the number of discriminant variables to $p \leq N_i/3$, where N_i = the sample size of the smallest group (Williams and Titus 1988). As a starting point, we used two methods to select initial subsets of variables. The first method, forward-backward stepwise LDF (SAS Institute 1989b) was used to select variable subsets for LDF model development. The second method (ANOVA) provided initial variable subsets for QDF model development. Univariate ANOVA (SAS Institute 1989a) was done on individual amplitudes to test for significant differences among groups. Amplitudes that were highly significantly different ($P \leq 0.01$) were included in the initial QDF models.

Starting with reduced sets of p amplitudes, refinement was done by running PROC DISCRIM (SAS Institute 1989a) on all combinations of $p - 1$ amplitude sets and examining the estimated classification (cross-validation) rates. This process was continued until the $p = 1$ amplitude model was reached. We used SAS programs to calculate the total cross-validation classification rate as the average of the rates realized by the individual groups (SAS Institute 1989a). After initial model selection, total classification rates were calculated as the total (all groups combined) number of otoliths correctly classified divided by the total sample size. The three models that resulted in the highest classification rates were used for classification of the test data set. Pairwise comparisons of classification rates were done with McNemar's test for

related samples (Daniel 1990; Huberty 1994). The overall experimentwise error rate was set at $\alpha = 0.1$ (Daniel 1990). Then the individual comparison significance level was $\alpha' = 0.1/[k(k-1)]$, where k is the total number of pairwise comparisons. To determine whether the classification rates were greater than could be expected by chance, the observed number (o_g) classified to the correct origin was compared to the expected number (e_g). The expected number, calculated under assumptions of equal and independent probabilities of classification, was $e_g = 1/m \cdot n_g$, where n_g = the number of individuals whose true origin was location g , and m = the number of possible origins. The test statistic was

$$z = \frac{(o_g - e_g)}{\sqrt{e_g(n_g - e_g)/n_g}}$$

Under the null hypothesis (H_0), $o_g - e_g = 0$, z has a standard normal distribution (Huberty 1994). Chi-square tests of independence were used to determine whether the proportions of correctly classified otoliths were significantly different among locations and sample dates (Daniel 1990).

Results

Otolith Samples

We extracted and polished otoliths from 776 sockeye salmon fry (Table 1). The difference between the actual sample size and the target sample size (100 for Glacier Springs and 50 for all other site and date combinations) reflects the loss of otoliths during extraction, breakage, and excessive polishing. The loss of otoliths ranged from 4% to 32% (Table 1).

Hatchery fry (mean = 29.3 mm) were significantly larger ($t = 10.78$, $P \leq 0.001$) than wild fry (mean = 27.6 mm). Mean lengths were significantly different among the six groups (ANOVA; $F = 48.12$, $df = 5$, $N = 769$, $P \leq 0.001$). Pairwise Tukey comparisons indicated that the hatchery and Glacier Springs fry were similar and larger than fry from other locations (Figure 4). This difference was expected as hatchery fry were all sampled in June and most had been fed for several weeks. The Glacier Springs fry were also sampled later than most other wild fry. Pairwise comparisons among the other wild fry groups indicated that Moose Creek fry were significantly smaller ($P \leq 0.001$; Figure 4) than other wild groups. Little feeding, as indexed by the percentage of stomachs containing food items, was observed in fry from Bear (0.2%), Moose (0.0%), and Nikolai (0.7%) creeks

TABLE 1.—Sample location, date and fork lengths (mm) of preserved Tustumena Lake, Alaska, sockeye salmon fry used in otolith pattern analysis.

Location and date	Fork length		N
	Mean (range)	SE	
Crooked Creek Hatchery			
3 Jun	28.3 (26.1 – 31.7)	1.13	40
14 Jun	30.1 (27.4 – 34.5)	1.60	47
15 Jun	29.3 (25.7 – 33.8)	1.73	48
Bear Creek			
20 Apr	27.8 (24.8 – 29.3)	0.93	47
18 May	27.4 (25.2 – 29.2)	0.89	48
21 May	27.3 (25.0 – 29.8)	0.98	38
2 Jun	27.3 (24.5 – 28.9)	1.01	49
Glacier Flats Creek			
22 Apr	26.9 (24.7 – 31.6)	1.48	34
27 Apr	27.1 (25.8 – 29.1)	0.82	34
4 May	27.3 (25.4 – 32.9)	1.55	49
29 May	28.9 (24.7 – 33.7)	2.47	47
Glacier Springs			
2 Jun	28.8 (25.3 – 31.4)	1.27	85
Moose Creek			
29 Apr	26.5 (22.1 – 29.7)	1.87	37
22 May	26.9 (23.5 – 28.9)	1.12	40
Nikolai Creek			
27 Apr	27.4 (24.4 – 29.1)	0.97	44
4 May	28.1 (26.4 – 29.7)	0.76	49
25 May	27.3 (25.0 – 29.4)	1.05	40

(J. Finn, unpublished data). On the other hand, 33.4% of the Glacier Flats Creek and 86.0% of the Glacier Springs fry stomachs contained food (predominantly chironomid larvae, pupae, and adults).

Amplitudes were not significantly affected by observer. Significant differences between observer measurements were found for only 3 (2.4%) of the 128 amplitude comparisons (randomized block ANOVA; $P \leq 0.05$) between the two observers. Although not a rigorous test (i.e., the results from only two observers were compared), the method used for feature extraction was repeatable and can be performed with limited instruction.

Hatchery versus Wild Sockeye Salmon

Comparisons of amplitude between hatchery and wild sockeye salmon resulted in significant differences (ANOVA; $P \leq 0.01$) for 30 (23.4%) amplitudes. Although amplitudes within the hatchery data set were neither consistently higher nor lower than amplitudes in the wild samples, the largest differences were seen in amplitudes 20–28 (Figure 5). Stepwise discriminant analysis initially selected 29 amplitudes and of those, 18 (62.1%) were significantly different in the previous ANOVA. These subsets of amplitudes (30 from ANOVA and 29 from stepwise discrimination) formed

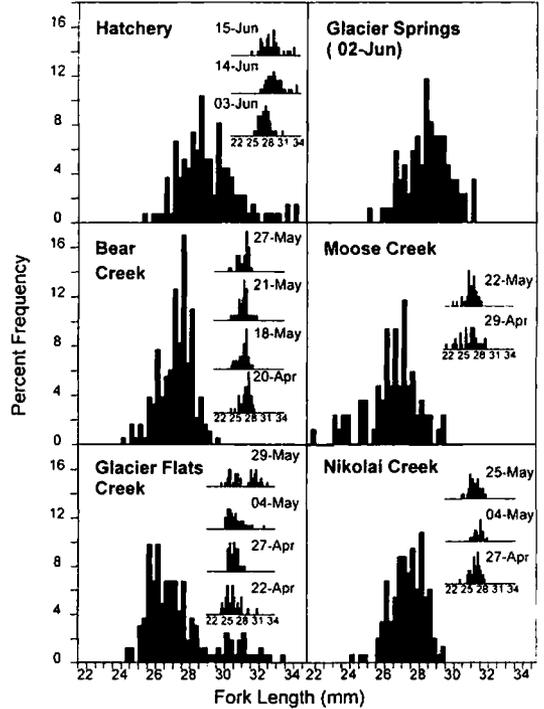


FIGURE 4.—Percent frequency distributions of fork lengths of preserved sockeye salmon fry used in otolith pattern analysis, Tustumena Lake, Alaska, 1992. Insert histograms are distributions by sample date for each location.

the starting points for looping procedures to select more parsimonious subsets of amplitudes.

Linear discriminant analysis.—During the looping process on the 29 amplitudes selected by stepwise discriminant analysis, the total average cross-validation classification rates ranged from 0.646 to 0.861 for models with $p = 29$ through 1 (Figure 6A). The LDF models that resulted in the three highest total average classification rates included 20, 24, and 26 amplitudes (Table 2). The LDF classification rates based on cross-validation were 84% or better for both hatchery and wild otoliths. However, when LDF was used to classify the test data, the classification rates were less than 75.0% (Table 2). The best classification of test data was 60.0% (hatchery) and 77.6% (wild) with the 24-amplitude model. None of the pairwise comparisons between the 20-, 24-, and 26-amplitude models were significant (McNemar's test; $|z| \leq 0.080$, $P > 0.02$) for both cross-validation and test data classification rates. The assumption of equality of covariances was rejected (Bartlett's log-likelihood ratio; $P \leq 0.001$) for all three LDF models.

Quadratic discriminant analysis.—Starting with

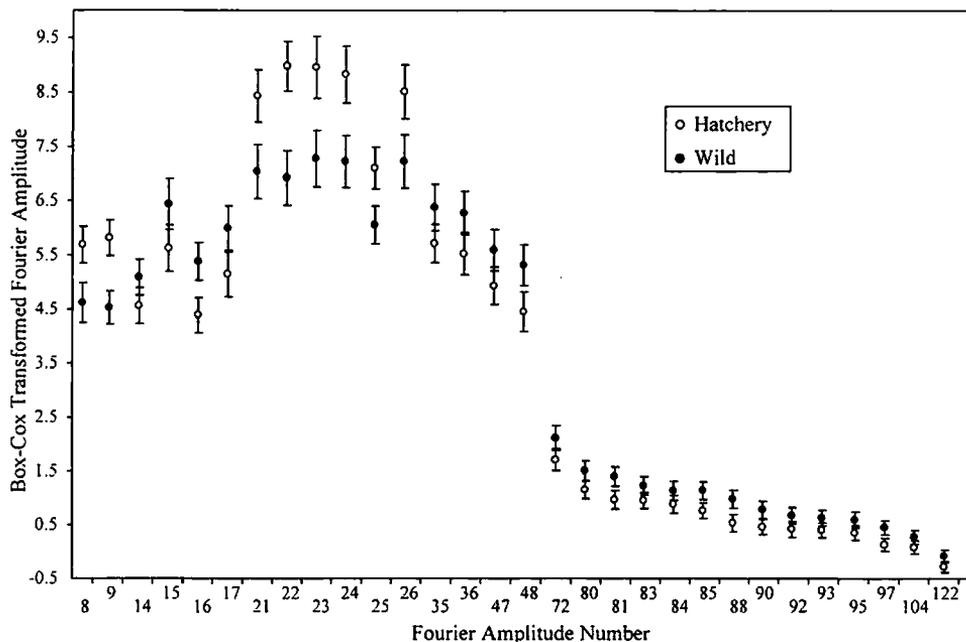


FIGURE 5.—Mean and 95% confidence intervals for 30 highly significant (randomized block analysis of variance: $P < 0.01$) Box-Cox-transformed Fourier amplitudes from hatchery (open circles) and wild (solid circles) sockeye salmon otoliths, Tustumena Lake, Alaska. The wild group included otoliths from fry from Bear Creek, Glacier Flats Creek, Glacier Springs, Nikolai Creek, and Moose Creek.

the 30 significantly different amplitudes (ANOVA; $P \leq 0.01$), classification rates for the hatchery and wild otoliths did not converge until the total number of amplitudes in the model was reduced to 12 (Figure 6B). The QDF models that resulted in the three highest total average cross-validation classification rates included 10, 11, and 13 amplitudes (Figure 6B; Table 2). Although the 13-amplitude model provided the highest cross-validation rate, the test data classification rates of the hatchery and wild otoliths differed by 24.8%. Use of QDF discrimination was supported by rejection of the assumption of equality of covariance matrices for the three models (Bartlett's log-likelihood ratio; $P \leq 0.001$). None of the pairwise comparisons of QDF model cross-validation and test data classification rates were significant (McNemar's test; $|z| \leq 1.567$, $P \geq 0.058$).

LDF and QDF model comparisons.—Both LDF and QDF resulted in three apparently equivocal models (based on total classification rates). In addition, no significant differences were found among the pairwise comparisons of LDF to QDF cross-validation (McNemar's test; $|z| < 1.56$, $P > 0.059$) and test data classification rates (McNemar's test; $|z| < 0.17$, $P > 0.43$). As the assumption of homoscedasticity was apparently vi-

olated for the LDF models, further analyses were done on QDF models. Of the QDF models, we selected the 10-amplitude (QDF10) model as the most parsimonious because it resulted in nearly equal classification rates for both hatchery and wild otoliths (Table 2).

When group mean amplitudes were used, the proportions of the total hatchery and wild luminance profiles explained by the Fourier harmonics associated with the QDF10 model were 0.152 and 0.120. Therefore, the classification rates that were realized with the QDF10 model were based on approximately 12.0–15.2% of the total variation of the luminance profiles. The proportions of otoliths from individual wild locations that were classified to hatchery origin ranged from 0.127 to 0.201 (Figure 7). These proportions were not significantly different ($\chi^2 = 3.39$, $df = 4$, $P > 0.495$). Therefore, it appeared that none of the wild groups were disproportionately misclassified to hatchery origin.

To determine whether sample date affected classification, we examined the proportions of wild otoliths classified to hatchery and wild origin for individual locations by sample date. There was no indication that date affected the classification of otoliths from Bear Creek ($\chi^2 = 4.44$, $df = 3$, $P =$

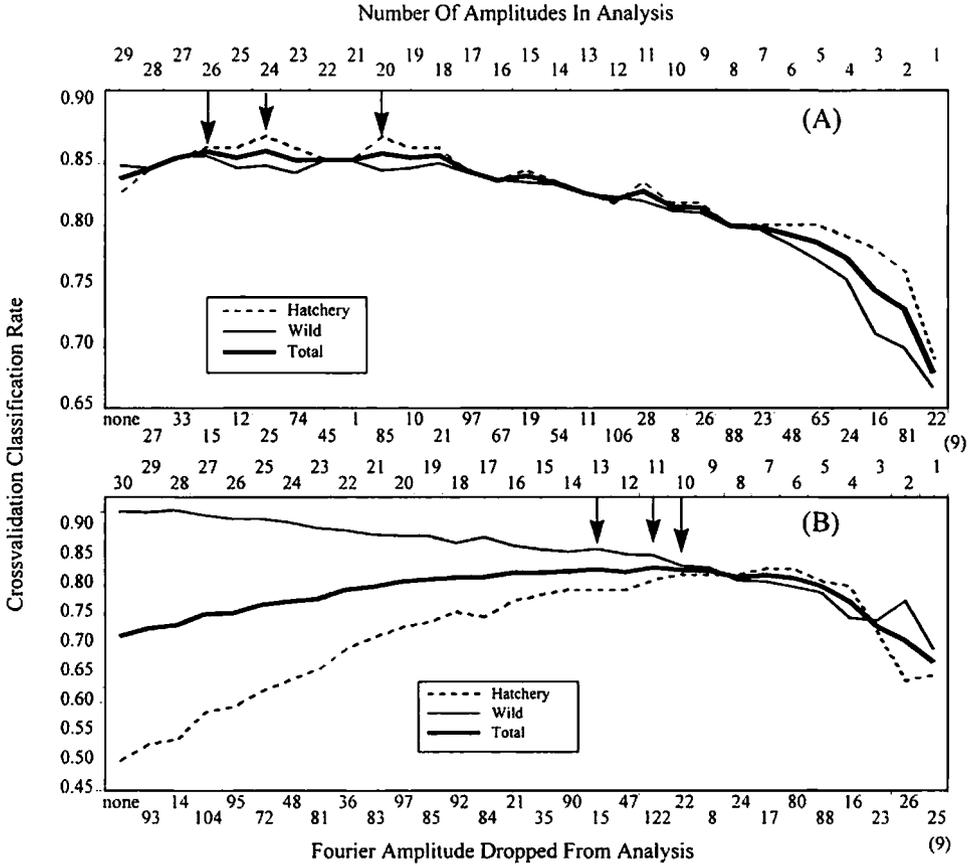


FIGURE 6.—Cross-validation classification rates (proportion correctly classified) for (A) linear (LDF) and (B) quadratic (QDF) discriminant function analyses on Box-Cox-transformed Fourier amplitudes from hatchery and wild sockeye salmon fry, Tustumena Lake, Alaska, 1992. Initial LDF model included 29 amplitudes selected by stepwise discriminant analysis. Initial QDF model included 30 amplitudes found to be significantly different (analysis of variance: $P < 0.01$) between hatchery and wild fry otoliths. At each step an additional amplitude was dropped (lower x-axes). Upper x-axes indicate number of amplitudes used at each step. Number in parentheses (lower axes, extreme right) indicates amplitude retained in final model. Arrows (upper x-axes) indicate models resulting in three highest classification rates.

0.217), Moose Creek ($\chi^2 = 0.716$, $df = 1$, $P = 0.398$), and Nikolai Creek ($\chi^2 = 0.355$, $df = 2$, $P = 0.837$). Although Glacier Flats otoliths showed more variation over the sample dates, the differences were not significant ($\chi^2 = 6.45$, $df = 3$, $P = 0.092$).

The average profile of the hatchery-standardized luminance values appeared to have more pronounced banding than the wild profile, particularly in the first 60–70 μm of the transect (Figure 8A). When luminance profiles were reconstructed by using only the Fourier harmonics associated with the 10 amplitudes in the QDF10 model, this trend was accentuated (Figure 8B). Fluctuating hatchery temperatures and practices such as cleaning, application of fungicides, and artificial light cycles

may have contributed to the distinct hatchery banding pattern.

Six-Group Classification

When the amplitudes were tested for differences among the six groups (i.e., hatchery, Bear Creek, Glacier Flats Creek, Glacier Springs, Moose Creek, and Nikolai Creek), 90 (70.3%) were significantly different (ANOVA, $P \leq 0.01$). Stepwise discriminant analysis initially selected 43 amplitudes. The starting points for the looping procedures were the 43 amplitudes selected by stepwise discrimination and the 51 most significantly different amplitudes. We chose 51 (minimum group [Moose Creek] size - 1 = 52 - 1) of the 90 significant amplitudes to avoid linear dependence

TABLE 2.—Number (percent in parentheses) of Tustumena Lake hatchery and wild sockeye salmon fry otoliths correctly classified by linear (LDF) and quadratic discriminant function (QDF) analysis based on Box–Cox-transformed Fourier amplitudes. Actual number of hatchery and wild otoliths were 110 and 516 (cross-validation) and 25 and 125 (test data).

Model (number of amplitudes) ^a	Cross-validation for:			Test data for:		
	Hatchery	Wild	All	Hatchery	Wild	All
LDF models						
20	96 (87.3)	436 (84.5)	532 (85.0)	14 (56.0)	96 (76.8)	110 (73.3)
24	96 (87.3)	438 (84.9)	534 (85.3)	15 (60.0)	97 (77.6)	112 (74.7)
26	95 (86.4)	442 (85.7)	537 (85.8)	15 (60.0)	95 (76.0)	110 (73.3)
QDF models						
10	90 (81.8)	430 (83.3)	520 (83.1)	19 (76.0)	90 (72.0)	109 (72.7)
11	89 (80.9)	439 (85.1)	528 (84.3)	16 (64.0)	99 (79.2)	115 (76.7)
13	87 (79.1)	445 (86.2)	532 (85.0)	13 (52.0)	96 (76.8)	109 (72.7)

^a Amplitudes used in each model.

10: 8, 9, 16, 17, 23, 24, 25, 26, 80, 88

11: 8, 9, 16, 17, 22, 23, 24, 25, 26, 80, 88

13: 8, 9, 16, 17, 22, 23, 24, 25, 26, 47, 80, 88, 122

20: 8, 9, 10, 11, 16, 19, 21, 22, 23, 24, 26, 28, 48, 54, 65, 67, 81, 85, 88, 97, 106

24: 1, 8, 9, 10, 11, 16, 19, 21, 22, 23, 24, 26, 28, 45, 48, 54, 65, 67, 74, 81, 85, 88, 97, 106

26: 1, 8, 9, 10, 11, 12, 16, 19, 21, 22, 23, 24, 25, 26, 28, 45, 48, 54, 65, 67, 74, 81, 85, 88, 97, 106

among the discriminant variables (i.e., singularity of the covariance matrices; Johnson and Wichern 1988). As the minimum group size was 52, the goal was to determine whether models using $p \leq 52/3 = 17$ amplitudes had potential for discriminating among all six groups.

Linear discriminant analysis.—Total average cross-validation classification rates ranged from 0.247 to 0.550. No distinct peak was evident, and the general trend was a decline from a total rate of 0.533 to 0.443 from the 43- through the 7-amplitude models (Figure 9A). The total classification rate declined rapidly with fewer than 7 amplitudes. Hatchery, Glacier Springs, and Nikolai Creek otoliths were correctly classified at rates over 0.50 for all but the $p < 5$ models (Figure 9a). Otoliths from Bear, Glacier Flats, and Moose creeks classified lower than the total rate for all but the $p < 5$ amplitude models.

When the restriction of $p \leq 17$ amplitudes was considered, the three highest total LDF cross-validation rates occurred with the 14- (45.7%), 16- (46.6%), and 17-amplitude (46.2%) models (Table 3). Covariance matrices were significantly different (Bartlett's log-likelihood ratio; $P \leq 0.017$) for the 16- and 17-amplitude models, but were not significantly different ($P = 0.102$) for the 14-amplitude model. Ranges for cross-validation classification rates for individual locations were 59.1–60.0% for hatchery, 34.4–36.3% for Bear Creek, 33.8–36.7% for Glacier Flats Creek, 63.3–66.7% for Glacier Springs, 42.3–46.2% for Moose Creek, and 51.8–53.7% for Nikolai Creek

(Table 3). Total classification rates for test data were 36.0–39.3%, and individual location classifications ranged from 12.0 to 64.0% (Table 3). None of the pairwise comparisons indicated significant differences in the cross-validation (McNemar's test; $|z| \leq 0.84$, $P \geq 0.20$) or test data classification rates (McNemar's test; $|z| \leq 1.50$, $P \geq 0.06$).

Quadratic discriminant analysis.—Total average cross-validation classification rates appeared to be relatively stable through the 40- to 10-amplitude models (Figure 9b), ranging from 0.243 to 0.473. Increases in the classification rate were primarily due to improvement in the rates for Glacier Springs and Moose Creek.

The three highest classifications occurred with the 11- (44.7%), 14- (46.2%), and 15-amplitude (45.5%) models (Table 3). The covariance matrices were significantly different for all three models (Bartlett's log-likelihood ratio; $P < 0.001$). Ranges for cross-validation rates for individual locations were 45.4: 54.6% for hatchery, 45.9–47.8% for Bear Creek, 41.0–42.4% for Glacier Flats Creek, 50.0–58.3% for Glacier Springs, 25.0–28.8% for Moose Creek, and 47.2–50.0% for Nikolai Creek (Table 3). Test data total classification ranged from 34.7% to 40.7%, and ranges for individual location maximum classifications were 44.0–48.0% for hatchery, 28.0% for Bear Creek, 40.0–48.0% for Glacier Flats Creek, 36.0–48.0% for Glacier Springs, 16.0–28.0% for Moose Creek, and 36.0–44.0% for Nikolai Creek (Table 3). Comparisons among the three models did not result in

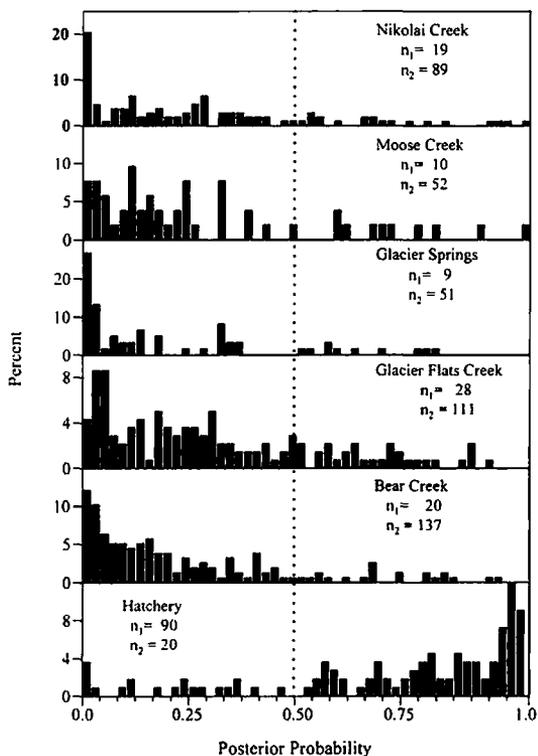


FIGURE 7.—Distributions (based on cross-validation of posterior probabilities) of otoliths classified to hatchery origin for six groups of sockeye salmon fry from Tustumena Lake, Alaska. Probabilities were based on quadratic discriminant analysis that used, Box-Cox-transformed Fourier amplitudes 8, 9, 16, 17, 22, 23, 24, 25, 26, 80, and 88. Labels indicate true origin, n_1 = number classified as hatchery; n_2 = number classified as wild. Observations falling to right of vertical dashed line (0.5 probability level) were classified as hatchery-origin fish.

an obvious “best” model. Total cross-validation (McNemar’s test; $|z| \leq 0.805$, $P \geq 0.21$) and test data ($|z| \leq 1.67$, $P \geq 0.047$) classification rates demonstrated no significant differences.

LDF and QDF model comparisons.—Both LDF and QDF resulted in three equivocal models. Of the LDF models, we selected the 16-amplitude model (LDF16) for comparison because it resulted in maximum cross-validation and test data classification rates (Table 3). Of the QDF models, the 14-amplitude model (QDF14) resulted in the highest cross-validation classification but the lowest test data classification (Table 3). On the other hand, the 11-amplitude model (QDF11) resulted in the lowest cross-validation classification but the highest test data classification. When the LDF16 model was compared with the QDF models there were

no significant differences in the cross-validation (McNemar’s test; $|z| < 0.77$, $P > 0.22$) or test data (McNemar’s test; $|z| < 1.02$, $P > 0.15$) classification rates. Huberty (1994) suggested that a linear classification may provide greater across-sample stability when the sample-to-discriminant-variable ratio (N/p) is small or moderate, although no guidance was given for the definition of small. Huberty (1994) cautioned that such generalizations were based on the $m = 2$ group case. Given the apparent equality of the classification success of the LDF16 and QDF models and the potential for higher stability of the linear rule, we chose the LDF16 model for further examination.

Given that there were six groups, and under the assumption of equal probability of classification, the probability of an individual being assigned to any one location was 1/6. Although the classification rates for $m = 6$ groups were considerably lower than for $m = 2$ (i.e., hatchery-wild classification), cross-validation classifications of individuals to their true location occurred at a higher probability than would be expected by chance ($z > 4.9$, $P \leq 0.0001$; Figure 10A). Test data classifications of otoliths were significantly greater than chance for hatchery ($z > 5.81$, $P \leq 0.0001$), Glacier Springs ($z > 5.81$, $P \leq 0.0001$), and Nikolai Creek ($z > 4.74$, $P \leq 0.0001$) (Figure 10B). Bear Creek ($z = 1.52$, $P > 0.064$), Glacier Flats Creek ($z = -0.63$, $P > 0.266$), and Moose Creek ($z = 0.98$, $P > 0.163$) test data classifications were not significant (Figure 10B). Therefore, the otolith banding patterns as measured by the selected Fourier amplitudes all contained some degree of location-specific information.

Discussion

Our analyses of otolith banding patterns represent a first attempt to use Fourier analysis to describe discriminant variables based on luminance profiles. The Fourier amplitudes provide continuous variables for statistical discriminant analysis, and our methods of feature extraction were repeatable and robust. High correct classification rates (cross-validation 83%, test data 73%) were achieved between hatchery and wild sockeye salmon with quadratic discriminant analysis that used 10 Box-Cox-transformed Fourier amplitudes (Table 2). We were pleased with our efforts to discriminate among various wild fry ($m = 6$ group), even though overall classification rates were below 50% (Table 3), because we did find that site-spe-

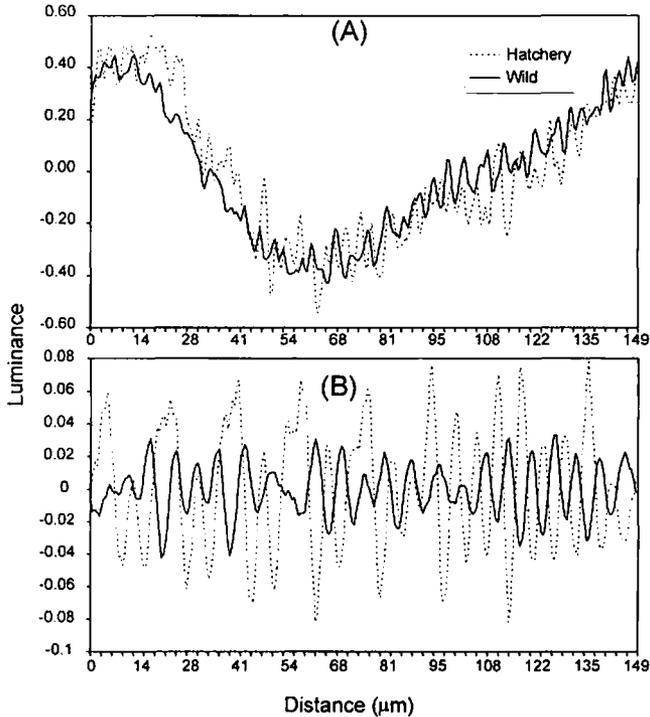


FIGURE 8.—(A) Mean standardized luminance profiles from Tustumena Lake, Alaska, hatchery and wild sockeye salmon fry otoliths and (B) reconstruction of mean standardized hatchery and wild sockeye salmon fry otolith luminance profiles based on Fourier harmonics associated with amplitudes (8, 9, 16, 17, 23, 24, 25, 26, 80, and 88) used in quadratic discriminant analysis. The wild group included otoliths from fry from Bear Creek, Glacier Flats Creek, Glacier Springs, Nikolai Creek, and Moose Creek.

cific information was indeed contained within the banding patterns.

In almost all cases, test data classifications were lower than the cross-validation results. Although intuitively the test data assessments should provide the most realistic estimate of true classification rates, test data results can be highly variable with small sample sizes (Huberty 1994). Therefore, we think that test data results should be viewed as a conservative measure of classification success, particularly in our $m = 6$ group assessment (individual group sample sizes = 25).

Otolith banding patterns formed during incubation have several desirable qualities for stock discrimination. For sockeye salmon, it is relatively easy to collect progeny of known-origin spawners as they migrate from incubation to rearing environments. Also, subsequent growth and environmental conditions should not affect banding patterns formed during incubation (Campana and Neilson 1985). Unlike these banding patterns, the use of otolith or shape analysis to discriminate among wild populations is limited because highest

levels of correct classification seem to be associated with growth rate differences or temporal and spatial separation of populations (Casselman et al. 1981; Capana and Casselman 1993). For example, otolith shape analysis of populations of Atlantic salmon *Salmo salar* from the United States, Canada, Ireland, and Britain resulted in highest correct classification rates (84–91%) between continents and lowest among samples analyzed from within either North America (62–69%) or Europe (64–73%). Campana and Casselman (1993), who found reasonable classification rates among populations of Atlantic cod *Gadus morhua* of different growth rates, but poor classification when similar growth rates existed, suggest that population discrimination based on otolith shape depends not only on differential growth rates, but on consistency of the environment over the lifetime of fish in a population. Therefore, if our purpose is to determine growth or survival differences among comingled groups, characteristics formed during incubation, such as banding patterns, have a higher potential for utility than otolith shape characteristics.

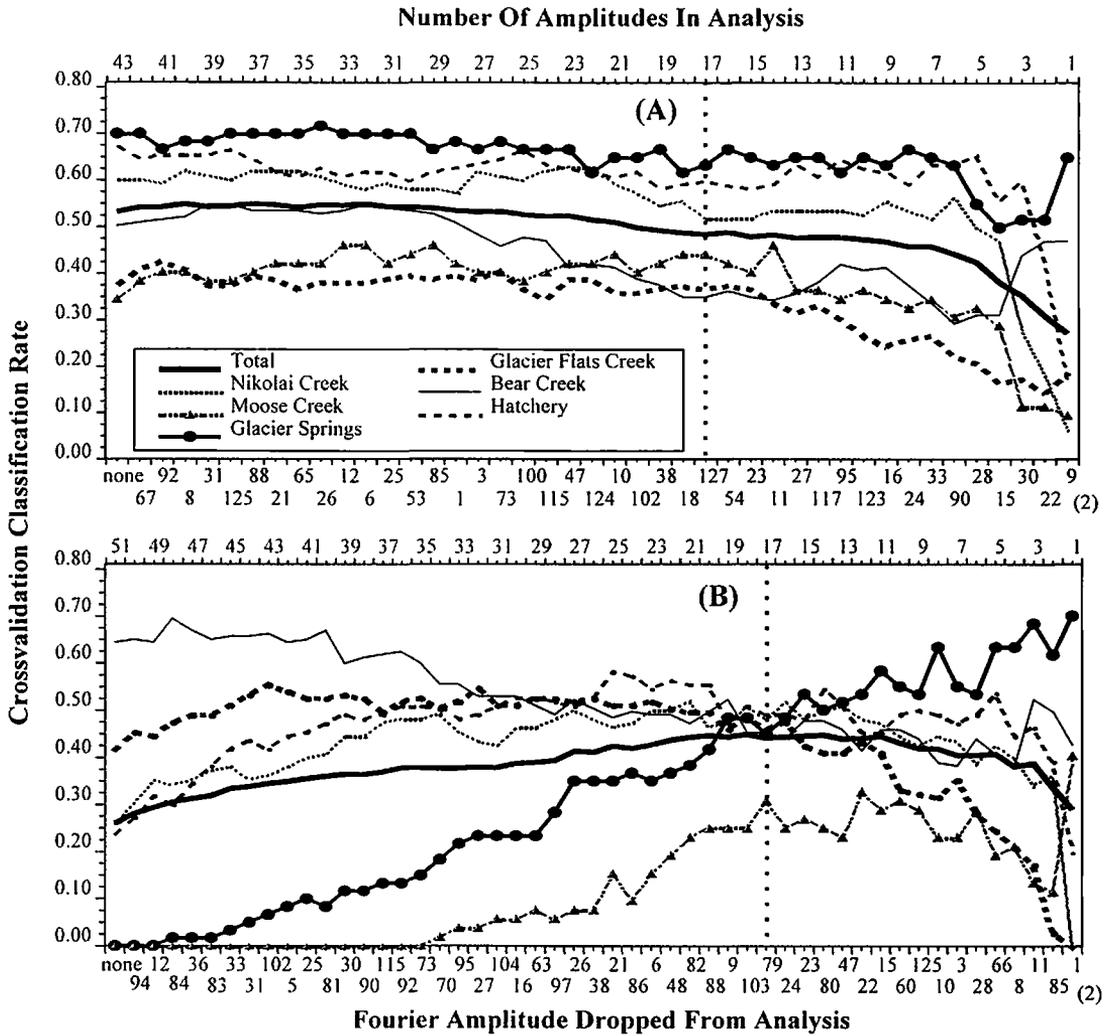


FIGURE 9.—Cross-validation classification rates (proportion correctly classified) for (A) linear (LDF) and (B) quadratic (QDF) discriminant function analysis on Box-Cox-transformed amplitudes from six groups of sockeye salmon fry otoliths, Tustumena Lake, Alaska, 1992. Initial LDF model included 43 amplitudes selected by stepwise discriminant analysis. Initial QDF model included 51 amplitudes found to be significantly different (analysis of variance: $P < 0.01$) between hatchery and wild fry otoliths. At each analysis an additional amplitude was dropped (lower x-axes). Upper x-axes indicate number of amplitudes used for each analysis. Number in parentheses (lower axes, extreme right) indicates amplitude retained in final model. Vertical dashed line indicates the $n/3 = 17$ variable model.

Few studies are available to compare our hatchery versus wild otolith classification rates. Although induced thermal banding results in 100% marking (Volk et al. 1990), we are unaware of findings that demonstrate the rate at which induced marks are recognized from admixtures of hatchery and wild otoliths. Using oxytetracycline (OTC) validation, Paragamian et al. (1992) determined that the presence of hatch and check marks and increment counts allow researchers to distinguish between hatchery and wild kokanee (a nonanad-

romous form of sockeye salmon). However, no classification rate for hatchery otoliths not marked with OTC was reported. Hendricks et al. (1994) used hatch and stocking checks as well as increment counts to achieve a total classification rate of 89% for hatchery and wild American shad *Alosa sapidissima*. These classifications were based on the ability of trained observers to recognize hatchery versus wild patterns and not on statistical classification.

Although we were unable to confidently sepa-

TABLE 3.—Linear (LDF) and quadratic discriminant function (QDF) cross-validation and test data numbers (percent) of Tustumena Lake, Alaska, sockeye salmon otoliths correctly classified to true location for hatchery and five wild groups based on Box-Cox-transformed Fourier amplitudes. (Actual numbers are the true numbers of individuals from a given location.)

Number of amplitudes ^{a,b}	Hatchery	Bear Creek	Glacier Flats	Glacier Springs	Moose Creek	Nikolai Creek	Total
LDF Cross-validation classification							
14	65 (59.09)	54 (34.39)	47 (33.81)	38 (63.33)	24 (46.15)	58 (53.70)	286 (45.69)
16	65 (59.09)	57 (36.31)	52 (37.41)	40 (66.67)	22 (42.31)	56 (51.85)	292 (46.65)
17	66 (60.00)	55 (35.03)	51 (36.69)	38 (63.33)	23 (44.23)	56 (51.85)	289 (46.17)
Actual number	110	157	139	60	52	108	626
LDF test data classification							
14	13 (52.00)	5 (20.00)	3 (12.00)	14 (56.00)	5 (20.00)	14 (56.00)	54 (36.00)
16	15 (60.00)	7 (28.00)	3 (12.00)	15 (60.00)	6 (24.00)	13 (52.00)	59 (39.33)
17	15 (60.00)	6 (24.00)	3 (12.00)	16 (64.00)	6 (24.00)	12 (48.00)	58 (38.67)
Actual number	25	25	25	25	25	25	150
QDF cross-validation classification							
11	50 (45.45)	72 (45.86)	57 (41.01)	35 (58.33)	15 (28.85)	51 (47.22)	280 (44.73)
14	60 (54.55)	75 (47.77)	57 (41.01)	30 (50.00)	13 (25.00)	54 (50.00)	289 (46.17)
15	53 (48.18)	75 (47.77)	59 (42.45)	32 (53.33)	14 (26.92)	52 (48.15)	285 (45.53)
Actual number	110	157	139	60	52	108	626
QDF test data classification							
11	12 (48.00)	7 (28.00)	12 (48.00)	12 (48.00)	7 (28.00)	11 (44.00)	61 (40.67)
14	11 (44.00)	7 (28.00)	10 (40.00)	11 (44.00)	4 (16.00)	9 (36.00)	52 (34.67)
15	12 (48.00)	7 (28.00)	12 (48.00)	9 (36.00)	4 (16.00)	10 (40.00)	54 (36.00)
Actual number	25	25	25	25	25	25	150

^a Amplitudes used in LDL models.

14: 2, 9, 15, 16, 22, 24, 27, 28, 30, 33, 90, 95, 117, 123

16: 2, 9, 11, 15, 16, 22, 23, 24, 27, 28, 30, 33, 90, 95, 117, 123

17: 2, 9, 11, 15, 16, 22, 23, 24, 27, 28, 30, 33, 54, 90, 95, 117, 123

^b Amplitudes used in QDF models.

11: 1, 2, 3, 8, 10, 11, 28, 60, 66, 85, 125

14: 1, 2, 3, 8, 10, 11, 15, 22, 28, 47, 60, 66, 85, 125

15: 1, 2, 3, 8, 10, 11, 15, 22, 28, 47, 60, 66, 80, 85, 125

rate the various wild groups of sockeye salmon in our study ($m = 6$), we found that site-specific information was available within the otolith microstructure formed during incubation. For all wild samples, the cross-validation probability of an individual being classified to its true incubation location was significantly greater than chance (Figure 10A). These are differences among incubation sites that are less than 10 km apart, a result that suggests we may eventually be able to separate stocks after they have migrated into the common rearing environment. Unlike our otolith method, pattern analysis of scales has been restricted to discriminating freshwater origins of Pacific salmonid populations on a relatively broad geographic scale (Rowland 1969; Cook and Lord 1977; Cook 1982; Cross et al. 1987), because scales (unlike otoliths) form after emergence from incubation environments. In addition to our otolith method, however, emerging genetic and behavioral studies show the existence or potential for within-

drainage diversity (Allendorf and Waples 1995; Burger et al. 1995).

Temporal, spatial, and genetic differences in the distributions of sockeye salmon spawning within the Tustumena Lake drainage have been documented. Spawning occurs over a period longer than 30 d, and adults entering lake tributaries spawn significantly earlier than sockeye salmon spawning along the lake's shoreline or outlet (Burger et al. 1995). These differences appear to have a genetic basis (C. Burger, unpublished data). Other differences include the distances over which spawning occurs in Tustumena Lake tributaries (Nikolai Creek, >20 km; Bear and Moose creeks, >10 km; Glacier Flats Creek, <4 km) and the variation suggested by significant differences in fry lengths (Figure 4), degree of development (yolk sac absorption), and frequency of feeding (Finn 1995).

The ecological differences among sockeye salmon that spawn and incubate in the Tustumena

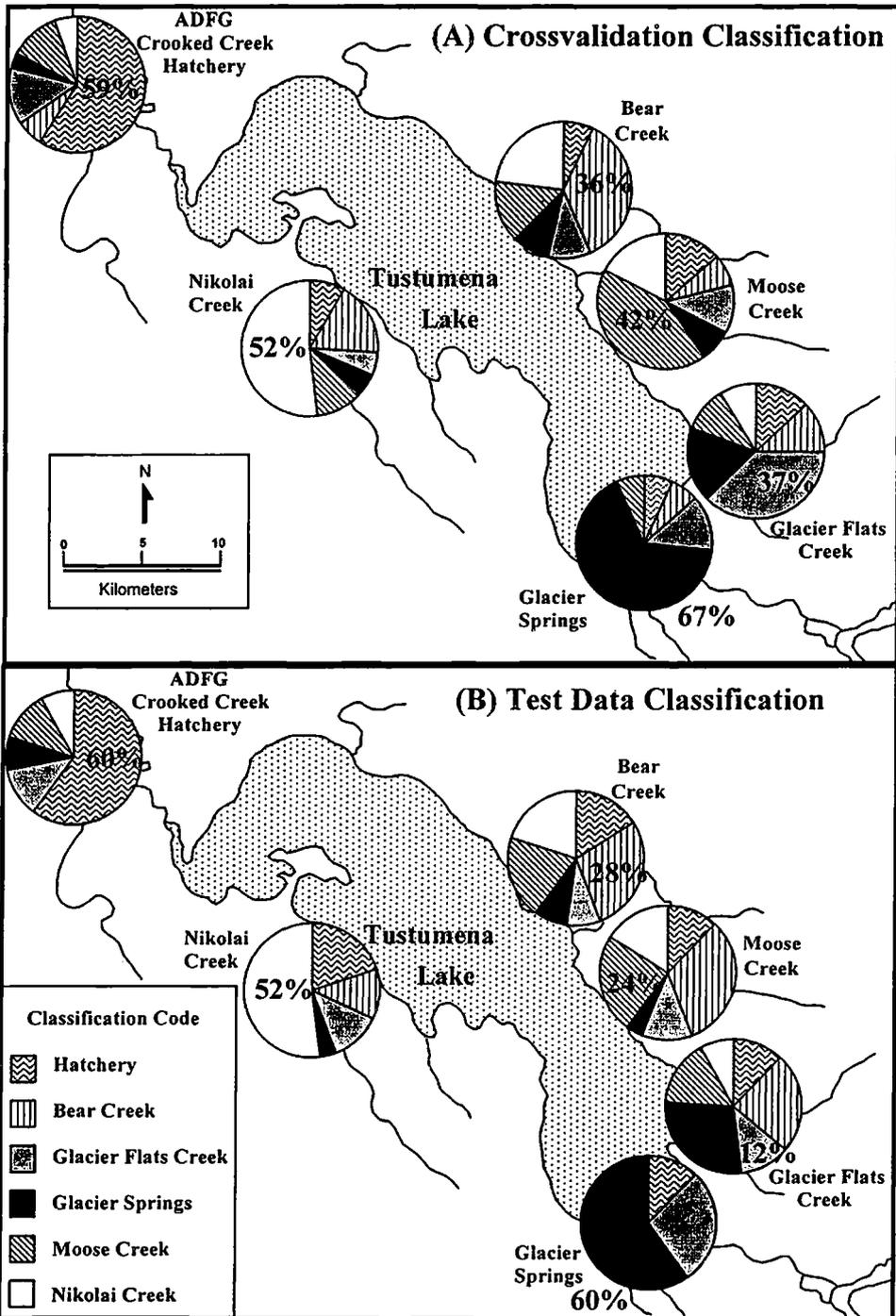


FIGURE 10.—(A) Cross-validation and (B) test data classification results for luminance amplitudes of sockeye salmon fry otoliths from five incubation locations in the Tustumena Lake drainage and from Crooked Creek Hatchery, Alaska. Classifications were based on linear discriminant analysis that used 16 Box-Cox-transformed Fourier amplitudes. Each pie diagram represents 100% of otoliths for a given location. Pie sections are percentages classified to various locations. Percentage value listed with each pie diagram is the percentage classified to true location.

Lake drainage are undoubtedly a factor in the classification rates we achieved with Fourier analysis. Although our best classification rate occurred between groups incubating in substantially different environments (hatchery versus wild), we achieved rates among wild groups that exceeded those expected by chance alone and that appear to reflect site-specific information. The temporal and spatial differences exhibited by Tustumena Lake sockeye salmon are small in comparison with those observed in other parts of the species' range where spawning and fry outmigration can be quite protracted (Gard et al. 1987; Burgner 1991). It should be noted that our study is limited to a single, geologically young drainage that is thought to be undergoing population differentiation (Burger et al. 1995).

An area for improvement in our approach is in the selection of variables. The looping procedure we used did not include all possible subsets of amplitudes. Once an amplitude was removed (e.g., deleted at the $p = 15$ amplitudes step) it was not reevaluated at smaller p subsets. The plots of classification rates that were generated during the looping procedures did not result in clearly defined maxima. Indeed, discrimination among both $m = 2$ and $m = 6$ groups resulted in equivocal models. Further work is necessary to develop and apply algorithms that will evaluate all possible subsets of p amplitudes to improve the discriminant capabilities of the present method (Huberty 1994), not only to improve discrimination, but to allow researchers to concentrate on amplitude subsets that meet variable-to-sample ratio criteria (Williams and Titus 1988). Furthermore we have applied only two very similar discrimination techniques (LDF and QDF) to these data.

Although our method for transect placement and length was developed to provide a means for standardization that was independent of ambiguous reference points, the use of a standard length transect may have introduced some degree of confounding variability. If differential development rates exist, then one would expect that the period of time covered by a standard length transect will vary from otolith to otolith. We are not able at this time to estimate what sort of effect this may have had on our results. It should also be noted that our analysis was limited to otoliths from young-of-the-year fry. Although otolith banding patterns artificially induced during incubation are identifiable in adult otoliths (Volk 1990), it is as yet unproved whether the level of otolith band discrimination we used on fry would be feasible with adult fish

otoliths. It is also not known what factors other than temperature (e.g., genetics) may influence the banding patterns we observed.

Our study represents the first use of Fourier analysis to describe differences in otolith banding patterns. Although our method needs refinement to determine the final degree to which the various wild sockeye salmon fry within Tustumena Lake can be separated, the present study indicates the potential to discriminate phenotypic differences in sockeye salmon otoliths that develop during incubation. With further research and application of image analysis, the examination of otolith banding patterns formed during incubation could provide discrimination that would allow within-drainage estimation of population parameters (e.g., mortality and growth functions) for various groups of hatchery-produced and wild sockeye salmon fry during their freshwater residence.

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