Identification of Long and Short-Term Reared Masu Salmon with Quantified Scale Characteristics

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Masu salmon (*Oncorhynchus masou*) are important to coastal commercial fisheries in northern Japan. Enhancement programs include a combination of release of fry in the spring (short-term rearing: ST) and release as juveniles in the fall, or as smolts the following spring (both long-term rearing: LT). In recent years, the number of LT fish released has increased and it has been practically and financially difficult to mark all of the releases. A simple and accurate method is needed to identify LT and ST fish in the overall numbers of returning adult masu salmon, to evaluate the success of alternative rearing and release strategies. Accordingly, a modification of the assignment rule developed to identify wild and hatchery steelhead stocks in Lake Michigan was applied to masu salmon. Scales of 1,129 adult masu salmon, which returned to the Shiribetsu River in 1989, were examined and various numerical scale characteristics were compared between the LT and ST fish groups. Significant differences in scale characteristics were found between LT and ST fish groups. Two scale variables were found that provided an acceptable level of discrimination. However, the fish could not be discriminated individually, due to an overlap in the distribution of scale variables. Accordingly, further examination is still required to find an effective scale characteristic that can detect individual differences.

INTRODUCTION

Masu salmon (*Oncorhynchus masou*) spend one year in the ocean following seaward migration of juveniles from natal rearing habitat and migrate back to their natal river in the succeeding spring (Machidori and Kato 1984; Kato 1991). Natural production of masu salmon is limited due to shortage of suitable spawning and rearing habitat. To increase production of masu salmon the national and prefectural governments have been carrying out programs of enhancement. These programs consist of either short-term (ST) rearing of fry that are released in the spring or longer-term (LT) strategies involving rearing of juveniles for release before winter or for release as smolts in the succeeding spring (Mayama 1991, 1992; Ohkuma and Nomura 1991). Releasing fry in the spring was the most common approach prior to introduction of the LT methods. In recent years releases of LT fish have become so large that it has been practically and financially difficult to mark all of these fish. A simple and accurate method is needed to identify LT and ST fish among the returning adult masu salmon so fishery contributions can be estimated to evaluate release strategy. Accordingly, I attempted to apply and modify the assignment rule developed by Seelbach and Whelan (1988) to identify wild and hatchery steelhead stocks in Lake Michigan.

MATERIALS AND METHODS

Scales were collected from 3,209 fish out of the 3,731 adult masu salmon that returned to the Mena River, a branch of the Shiribetsu River, in 1989 (Fig. 1). The 1989 return was larger than the 1988 or 1990 return. Scales were removed from the preferred location on the body, a few rows above the lateral line between adipose fin and posterior end of dorsal fin. Scale measurements were taken using vinyl acetate plate impressions magnified 100 times under micro projector. Regenerated scales and age 2.1 fish were excluded and 1,129 age 1.1 fish were examined. Among the scales examined, 701 were from fish (marked: MK) that had been marked and released as 0+ juveniles (right ventral fin clip) in autumn of 1987, or marked and released as 1+ smolts (left ventral fin clip, both LT fish) in the spring of 1988 (Table 1). 428 scales (no-marked: NM) were from unmarked survivors of the 827,000 fry released in the spring of 1987 (ST).

Fig. 1 Locations of the hatchery and trapping site, from which masu salmon were obtained, on the Mena River.

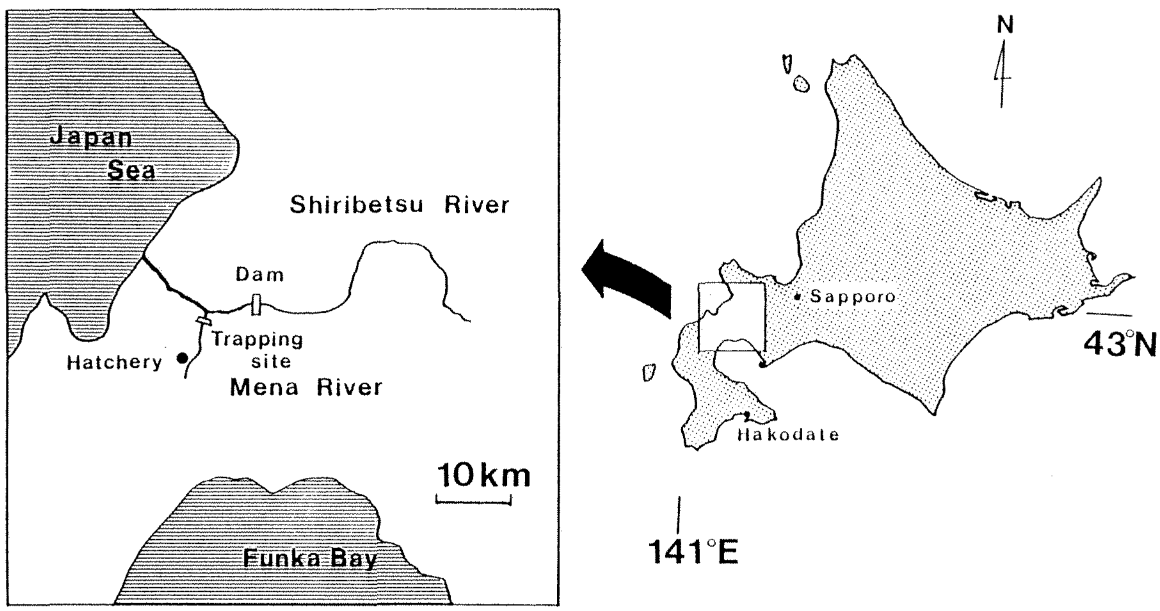


Table 1. Rearing and release history of adult masu salmon returned in 1989 as age 1.1 fish.

Year & Season	1986 brood year group		
1985 Autumn			
1986 Spring			
Autumn	F e r t i l i z a t i o n		
	release		
1987 Spring	827,000		
	F		
Autumn	↓	release 97,000	
	↓	0+ J (Rv cut)	
	↓	↓	release 158,000
1988 Spring	↓	↓	1+ S (Lv cut:
	↓	↓	143,000)
Autumn	↓	↓	↓
1989 Spring	▼	▼	▼
Autumn	return as age 1.1 fish		

F: fry, J: juvenile, S; smolt, Lv: left ventral fin, Rv: right ventral fin

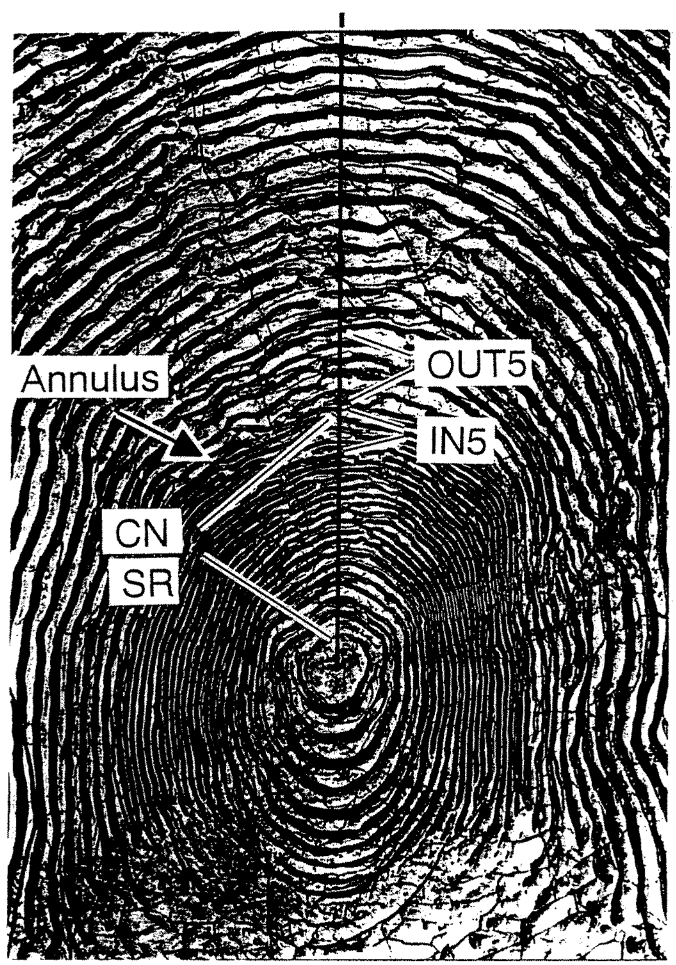
The stock of masu salmon in this Mena River system is maintained primarily by artificial propagation. The fish that escape through the weir may spawn; however, there were few fry from natural spawning found in sampling in the river prior to the fry release in the spring of 1987. Therefore, almost all NM fish examined in this study were considered to be ST fish. After reviewing the records from the 1987 release of age 1+ smolts, it was determined that 15,000 out of 158,000 LT smolts released were not marked.

The following five scale characteristics were measured or counted along the longest axis of freshwater growth zone (Fig. 2): scale radius (SR) from the first circuli to the freshwater annulus, number of circuli (CN) in the extent within the width of five intercirculus spaces inside and outside annulus (IN5 and OUT5, respectively), and ratio between IN5 and OUT5 expressed as OUT5/IN5 (R5). The ratio R5 was a reciprocal number described as band 23 in Seelbach and Whelan (1988).

Statistical significance was examined using 2 tailed t-test for difference between female and male fish, and Mann-Whitney U-test and Smirnov-Kolmogorov 2-sample test were used to examine the difference between NM and MK fish groups.

For identifying LT and ST fish using R5 value solely, two different threshold levels were selected and calculated. The first value was calculated from the average value of R5 of NM and MK fish in 1989, expressed as the point midway between each average (rule A). The other level was also expressed as the midpoint midway of each average of NM and MK fish, but the source for calculation includes fish sampled in 1980-1982, 1988, and 1989 (rule B). Total number of samples used to calculate the threshold value of rule B was 1,334 NM and 1,064 MK fish. A discriminant function analysis using SR and R5 variables was used as rule C (Arima and Ishimura 1987). Measurements of SR and R5 from 1,129 samples collected in 1989 were used for estimating the discriminant function used in this study. In addition, identification using visual examination of scales with aid of a micro projector (rule D) was included in a comparison of accuracy among the different rules. Each scale sample was read three to

Fig. 2 Measured and counted scale characteristics for analysis in this study.



five times. Scale samples were read in random order in an attempt to avoid biasing classification between readings. Scales were classified to either ST or LT groups based on results from three out of five readings. Accuracy was calculated by fish groups (i.e. NM and MK), respectively, and expressed as error rate (%) as follows:

Error rate = Nf/Nt x 100

where Nf is number of misidentified fish, and Nt is number of fish belonging to each group.

RESULTS

No significant differences were found in the five scale variables between female and male MK fish, but OUT5 and R5 showed significant difference for NM fish at $P<0.05$ and $p<0.01$ (Table 2). In contrast, all five variables showed highly significant difference between NM and MK fish ($p<0.001$) when sexes were combined.

The distribution of R5 for MK fish is peaked and skewed to the right while the distribution of NM fish is more evenly distributed (Figure 3). Error rate by the rule A shows 25.5% and 16.1% for NM(ST) fish and MK (=LT) fish, respectively. Rule B shows similar results to rule A with slightly lower rate for MK and higher rate for NM fish. Rule C, using a linear discriminant function combining SR and R5 showed lower rate than rule A and B, and error rate by scale reading by eye shows lowest result in all rules for MK fish though the rate for NM fish is almost same as rule C. The tendency of higher error rates on NM(ST) fish using all rules was observed. This might be due to 15,000 LT juvenile released without marking (Table 1). Distribution patterns of R5 of MK fish, and of NM fish, identified as LT or ST fish by scale reading are shown in Figure 4 and 5, respectively. Though the fish identified as ST of MK fish were very rare, R5 of the fish identified as LT of NM fish were mainly distributed in lower range. Figure 6 shows the distribution pattern of R5 of NM-ST and MK-LT fish excluding mis-read samples. To reduce the effect of not marked LT juvenile from the error rates listed in Table 3, number of LT fish among no mark fish (Na) were estimated from Table 1 and the following equation:

Na=607 x (158,000-143,000)/143,000,

where 607 is the number of Lv-cut LT fish among total of 701 LT fish specimen. The rest of 94 fish were Rv-cut LT fish. Then, Na was estimated as 64. This means 64 fish among 428 NM fish are LT, but no-mark fish. The revised rates are listed in Table 4, and these rates in NM fish by derived from rule A and B decreased to almost half. Also those rates by rule C and D decreased to one-third to one-fourth, though that of MK fish did not show clear decrease.

Table 2. Averages and the results of statistical examination of the scale characteristics of masu salmon. Scale characteristics are described in figure 2. NS denotes no significance (p>0.05); asterisks denote significance at p<=0.05*, p<=0.01, or p<=0.001***. Standard deviations are listed in parentheses.**

Group	N	Scale characteristics					Test
		CN	SR (mm)	IN5 (mm)	OUT5 (mm)	R5	
NM fish							
Female	322	15.14 (3.035)	0.355 (0.077)	0.097 (0.018)	0.188 (0.031)	1.991 (0.426)	
Male	106	15.34 (3.101)	0.361 (0.077)	0.099 (0.017)	0.180 (0.030)	1.863 (0.393)	A
Result		NS	NS	NS	*	**	
MK fish							
Female	526	16.00 (2.408)	0.438 (0.075)	0.124 (0.017)	0.172 (0.031)	1.406 (0.287)	A
Male	175	16.18 (2.050)	0.438 (0.063)	0.125 (0.019)	0.168 (0.031)	1.378 (0.320)	
Result		NS	NS	NS	NS	NS	
							B
NM fish	428	15.19 (3.049)	0.356 (0.077)	0.098 (0.017)	0.186 (0.031)	1.959 (0.421)	
MK fish	701	16.04 (2.324)	0.438 (0.073)	0.124 (0.018)	0.171 (0.031)	1.399 (0.296)	
Result		***	***	***	***	***	

A: two-tailed t—test
B: Mann-Whitney's U—test and Kolmogorov—Smirnov's 2—sample test.

Fig. 3 Frequency distribution of R5 of NM and MK masu salmon returned in 1989.

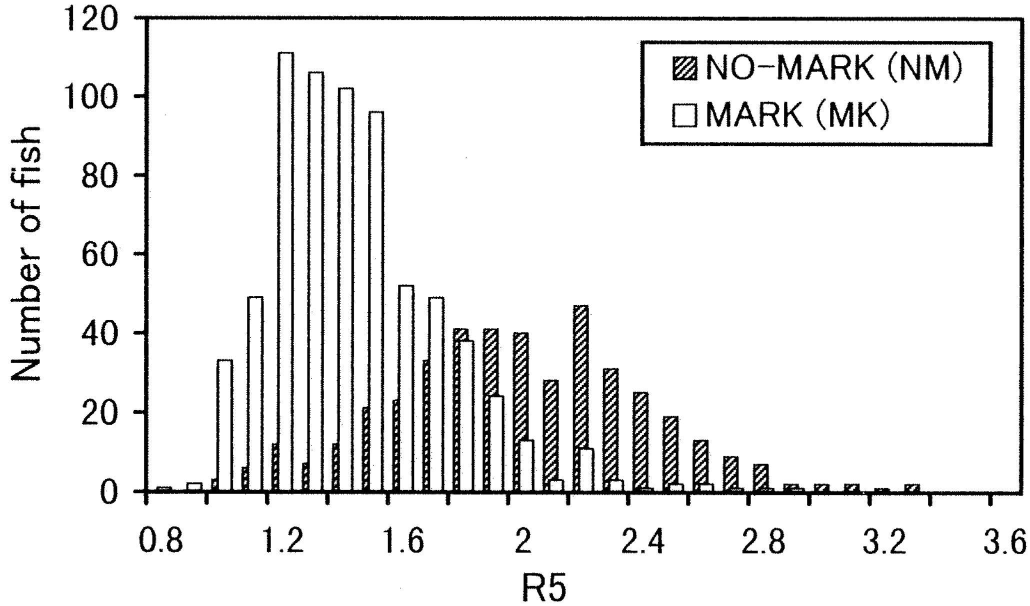


Fig. 4 Frequency distribution of R5 of MK fish identified as ST or LT by scale reading.

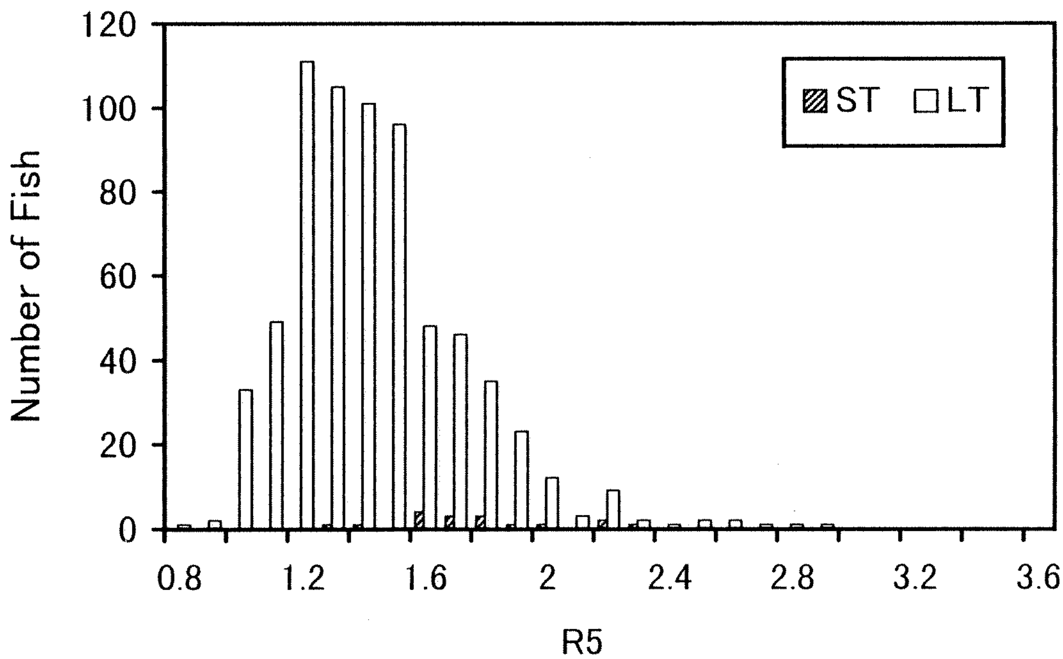


Fig. 5 Frequency distribution of R5 of NM fish identified as LT or ST by scale reading.

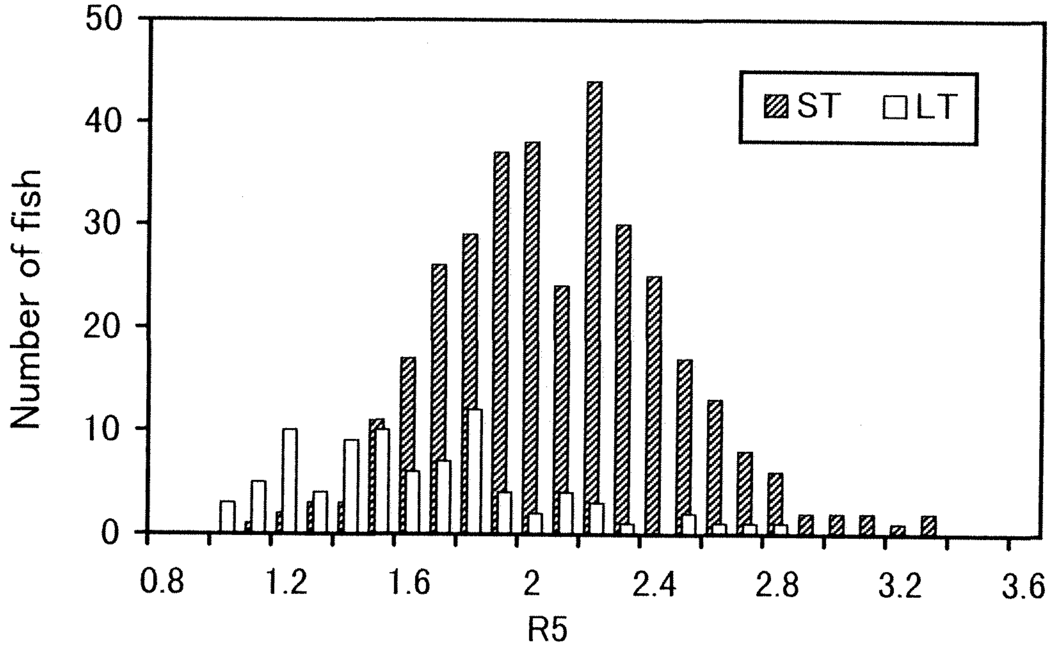


Fig. 6 Distribution of R5 of NM fish identified as ST, and MK fish identified as LT.

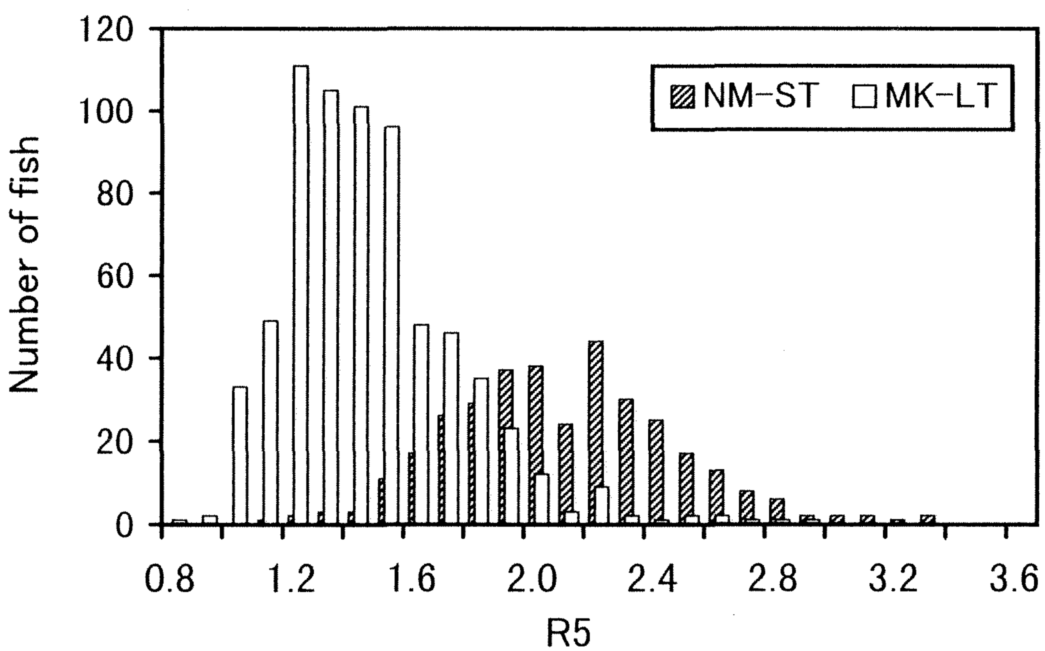


Table 3. Comparison of the error rates of identification of masu adults returned in 1989, using various decision rules.

	NM(ST)	MK	mean %	Decision rule
Error rate %				
	25.5	16.1	20.8	A)
	28.0	13.8	20.9	B)
	19.2	13.4	16.3	C)
	19.9	2.4	11.2	D)
Number of fish	428	701	-	

A) identified using midpoint of average R5 of NM (ST) and MK (LT) fish of the year (threshold value t=1.6786)
B) identified using midpoint of average R5 of 1,334 NM (ST) and 1,064 MK (LT) fish. (t=1.7080)
C) identified using the following discriminant calculated with SR and R5 of 1,129 age 1.1 fish returned in 1989:
discriminant: 14.467 SR-4.521 R5+1.845>0 ..... defined as LT fish
14.467 SR-4.521 R5+1.845<0 ..... defined as ST fish
D) results from scale reading by eye.

Error rate (%) was calculated with following formula: Nf/Nt•100,
for marked (MK) fish, Nf: number of MK fish defined as ST fish, Nt: number of MK fish,
for no-mark (NM) fish, Nf: number of NM fish defined as LT fish, Nt: number of NM fish

Table 4. Revised error rates of identification. Initial rates are listed in table 3.

	Revised error rate %			
Rule	NM(ST)	MK	Mean	Total
A)	12.4	14.8	13.6	
B)	15.3	12.7	14.0	
C)	4.9	12.2	8.6	
D)	5.8	2.2	4.0	
Initial N	428	701		1,129
Revised N	364	765		1,129

DISCUSSION

Seelbach and Whelan (1988) stated that scale variables that are utilized must have following criteria: 1) be stable through years, 2) show clear difference between ST and LT fish, and 3) show little difference among neighboring rivers. The ratio (R5) between five intercirculus spaces inside and outside annulus met these criteria. In addition, it is necessary for accurate classification that scale variables have low standard deviation and wide differences in means between the two fish groups (i.e. ST and LT). Though all five characteristics examined in this study showed significant difference between NM and MK fish at $p<0.001$, both CN and OUT5 were not satisfactory for utilization due to their smaller difference in means and large standard deviations. The ratio between the remainder of averages and the mean of the standard deviation showed that the ratio of R5 ranked as top at 1.56, followed by IN5 at 1.49, and SR at 1.09. Those of CN and OUT5 were less than 0.5. From these reasons, it was ascertained that R5 was a satisfactory characteristics for discrimination, as Seelbach and Whelan (1988) stated.

It is necessary to calculate a threshold value for separating two groups using R5. In this study, a point midway between the averages was chosen as the value for rule A and B. As the threshold value for rule A was calculated from the data obtained in 1989 only, the results were considered to be biased due to the presence of un-marked LT fish in ST fish group. Thus, the alternate value was calculated using data obtained in 1980-82, 1988, and 1989. LT fish were not present in the 1980 and 1981 samples. Though the sampled fish in 1982 included LT and ST, the LT fish of the year were fully marked and thus they could be classified completely. The fish in 1988 contained no-marked LT fish, as in 1989. As the threshold value for separation calculated using these data were slightly larger than that of rule A, the results of rule A and B were somewhat different in each group, but showed scarce difference in the mean value.

The author tried to improve the accuracy of identification using two variables in a discriminant function analysis. Discriminant analysis requires that variables are not correlated. Because the variables R5, IN5 and OUT5 were highly correlated because they were derived from OUT5/IN5, their use was not appropriate. Out of the two remaining characteristics, CN was omitted due to its low difference between groups and high standard deviation. Thus, the discriminant function was calculated with R5 and SR using the data of 1989. Although this method showed high accuracy, results might be affected by variable growth of fish rearing in natural conditions. Though it might be possible to control fish growth in artificial rearing conditions, it is quite reasonable that fish growth under natural river conditions may vary among years, contributing to high variability in scale growth increments (i.e., the scale radius from focus to freshwater annulus) (Ohkuma 1988). These classification rules were calculated based on standards collected in the same year the mixed stock samples were collected. Differences in scale measurements between years could bias ST and LT classifications in other years. The possibility of using scale measurements from smolts to classify fish as adults might be investigated.

Lastly, identification by scale reading (rule D) was compared with these objective methods based on the quantified scale data, shown above. The accuracy by rule D was nearly 100% for MK fish, and close to the accuracy for NM fish using rule C. Thus, scale reading by trained observers can be used to discriminate fish with unexpectedly high accuracy.

The error rates of NM fish were higher than that of MK fish (Table 3) for all classification rules examined. These differences were thought to be due to the 15,000 no-marked LT smolts among total of 158,000 released 1+ LT smolts, as stated before. The error rates were revised assuming some unmarked fish in the LT group, and those error rates of the NM fish decreased nearly to those of MK fish except for rule C (Table 4). The mean of the revised error rates were comparable to those observed by Seelbach and Whelan (1988). For scale patterns to be viable, the accuracy should be the same as that by scale reading. For such improvement, other techniques using other scale characteristics, such as identification from classifying patterns of changes of intercirculus width by circulus, or measuring and counting scale characteristics along another axis, should be developed. However, with progress on the techniques for producing smolts under artificial condition, more natural-like smolt might be produced in the near future. If that is the case, the methods for identification examined and discussed in this report might be abandoned at that time.

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