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Identification by Scale Analysis of Farmed Atlantic Salmon Juveniles in Southwestern New Brunswick Rivers

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Abstract.—A procedure was developed to identify whether the natal origin of juvenile Atlantic salmon *Salmo salar* in the Magaguadavic River, New Brunswick, was farmed or wild. Farmed juveniles enter this river as escapees from commercial hatcheries. The discriminant function was developed using measured scale characteristics for the first year of growth, as determined from samples of farmed and wild juvenile Atlantic salmon of known origin. Eight scale characteristics proved to be significant predictors of origin. In a jackknife cross-validation, the discriminant function was 90% accurate in predicting the origin of juvenile Atlantic salmon in the Magaguadavic River. The procedure was then applied to juvenile Atlantic salmon of unknown natal origin sampled from the Magaguadavic and neighboring Waweig and Digdequash rivers, which also support salmon hatcheries. Of the juvenile Atlantic salmon sampled in the Magaguadavic River in 1996, 1997, and 1998, 36, 59, and 43%, respectively, were estimated to be of farmed origin. During 1998, an estimated 9% and 42% of juvenile Atlantic salmon sampled from the Digdequash and Waweig rivers, respectively, were of farmed origin. The study indicated that farmed juvenile Atlantic salmon escaped from hatcheries and occupied suitable habitat in all three rivers.

Farmed Atlantic salmon *Salmo salar* escape into the wild as juveniles and adults. Juvenile salmon escape into rivers and lakes from freshwater hatcheries (Stokesbury and Lacroix 1997; Clifford et al. 1998a). Adult salmon escape into the marine environment from sea cages (Gausen and Moen 1991; Webb et al. 1991; Hansen et al. 1993; Carr et al. 1997).

The escape of farmed salmon into the wild is of concern because farmed salmon may differ both genetically (Cross and King 1983; Youngson et al. 1991; Einum and Fleming 1997) and behaviorally (Einum and Fleming 1997) from their native counterparts. Their presence in the wild may have ecological (Johnsen and Jensen 1991; Einum and Fleming 1997) and genetic (Verspoor and Hammar 1991; McGinnity et al. 1997; Clifford et al. 1998b) impacts on native stocks.

Most identification studies of escaped salmon to date have verified adult escapees from marine grow-out sites (Crozier 1991; Hansen et al. 1993; Crozier 1998). Typically, identification methods have been based on the physical characteristics of the salmon. The extensive time that adult salmon spend in culture alters their morphology and condition, making them easy to distinguish from their wild counterparts (Fleming et al. 1994). In con-

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trast, the limited time that juveniles spend in culture before escape makes them more difficult to discern from native juveniles by external examination (Fleming et al. 1994).

A previous study attempted to distinguish farmed juvenile Atlantic salmon from those of wild origin. Stokesbury and Lacroix (1997) determined natal origin of Atlantic salmon smolts, using a discriminant function based on the number of circuli in the first annual zone and the back-calculated length at age 1. Because this procedure required the presence of the first annulus, however, it could not accurately identify the natal origin of salmon that entered the wild during their first year. Approximately 95% of juveniles contained in commercial hatcheries in New Brunswick are age 0 (Chang 1998); coincidentally and because of their small size, many escapees from commercial hatcheries escape at age 0.

The purpose of the present study was twofold: to develop an identification procedure capable of determining the natal origin of Atlantic salmon at age 0, and to determine the proportion of farmed origin juvenile Atlantic salmon present at sampling sites in the Magaguadavic (1996–1998), Digdequash (1998), and Waweig (1998) rivers. A discriminant function analysis was developed from scale characteristics by using characteristics for the first year of growth, including the area of the focus, the width of each of the first six circuli pairs, and the mean and standard deviation of the width of each of the first six circuli pairs.

Methods

Study site.—The Magaguadavic River in southwestern New Brunswick (Figure 1) was chosen as the site for this study. The mouth of the river is within 10 km of 70% of Canada's East Coast commercial salmon aquaculture grow-out sites (Carr et al. 1997), and the river supports three commercial salmon hatcheries that produce more than 2 million smolts annually for use in commercial aquaculture (Stokesbury and Lacroix 1997). In 1996, between 51.0% and 67.2% of the smolts migrating from the river were juvenile escapees from one or more of the river's three hatcheries (Stokesbury and Lacroix 1997). The Magaguadavic River thus provided a natural study site where juvenile escapees (hatchery origin) and wild (river origin) salmon were both present. The two other rivers examined during this study, the Waweig and Digdequash, are situated close to the Magaguadavic River and also support commercial salmon aquaculture hatcheries (Figure 1). Juvenile escapees had not been reported from these rivers.

Magaguadavic River sampling sites (Figure 1) were chosen to correspond with those used in historical sampling completed by the Atlantic Salmon Federation. The sampling site used in the Digdequash River was chosen because it was approximately 30 km upstream from the only commercial hatchery in the drainage system (Figure 1). The Waweig River sampling site was chosen because it was directly (approximately 0.25 km) downstream from the only commercial hatchery in the system (Figure 1).

Discriminant function analysis.—A linear discriminant function was developed and tested for use in classifying the origin of juvenile Atlantic salmon. The discriminant function was developed from two groups of Atlantic salmon parr, ‘‘known origin farmed’’ fish from two commercial hatcheries on the Magaguadavic River, and ‘‘known origin wild’’ fish from selected sites on the Magaguadavic River and Dennis Stream. Unknown-origin juveniles were sampled in the Magaguadavic River, Digdequash River, and Waweig River.

Samples.—The Magaguadavic River contained three groups of juvenile salmon, namely, those of wild origin, stocked origin, and farmed origin. The wild-origin group could have included wild salmon whose parents were of farmed origin; however, because genetic screening was outside of the scope of this study, we classified these salmon as wild in origin. The Atlantic Salmon Federation, which stocked juvenile salmon (age 1) in the Magaguadavic River (n = 2,767) during 1997 (Carr and Whoriskey 1998), had adipose fin-clipped these juveniles before release. Sampled juvenile salmon that had clipped adipose fins were removed from the data set.

Farmed juveniles were sampled in 1997 (Table 1) at two commercial aquaculture hatcheries operating on the Magaguadavic River system (Figure

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FIGURE 1.—Map of the rivers of southwestern New Brunswick sampled in the study, showing parr sampling sites (circles) and commercial hatchery installations (squares). Known farmed samples were taken at the two southernmost hatcheries on the Magaguadavic River system. Known wild samples were taken from Dennis Stream and sites in the midreaches of the Magaguadavic River.

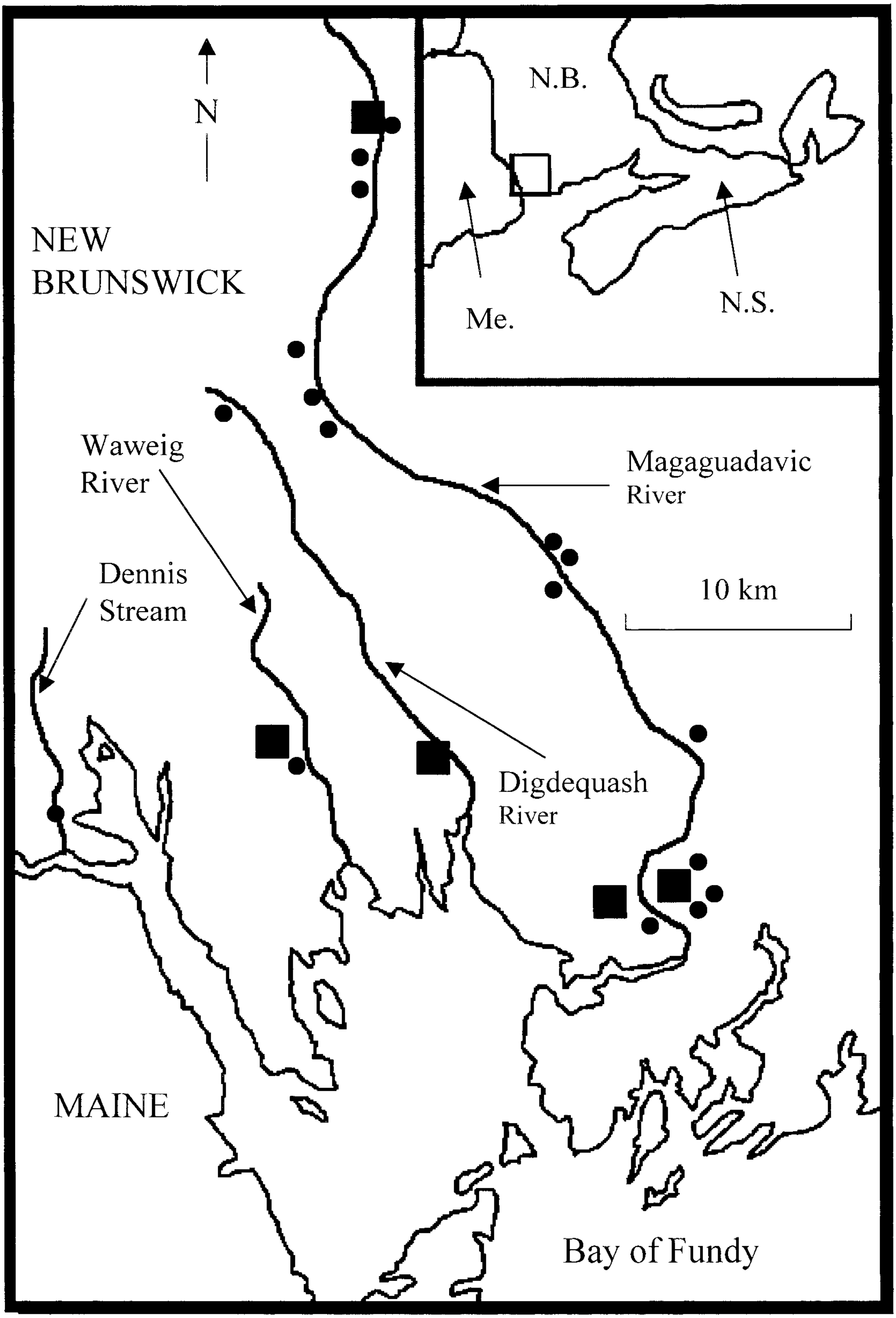
N
N.B.
NEW
BRUNSWICK
Me.
N.S.
Waweig
River
Magaguadavic
River
Dennis
Stream
10 km
Digdequash
River
MAINE
Bay of Fundy

TABLE 1.—Origins of Atlantic salmon juveniles from which scales were used for the discriminant function analysis.

Origin	Sample size	Year collect-ed	River
Known hatchery	67	1997	Magaguadavic River
Known wild	30	1996	Magaguadavic River
Known wild	27	1998	Dennis Stream
Unknown	123	1996	Magaguadavic River
Unknown	143	1997	Magaguadavic River
Unknown	127	1998	Magaguadavic River
Unknown	87	1998	Digdequash River
Unknown	97	1998	Waweig River

1). Farmed fish were sampled at age 1, 12 months after hatching.

The sample of known wild origin consisted partly of 30 Atlantic salmon parr sampled from side streams along the middle reaches of the Magaguadavic River in 1996 (Figure 1). Because of the strong site fidelity of riverine Atlantic salmon parr (Keenleyside 1962; Cunjak 1992), we assumed that parr occupying areas far from hatcheries were of wild origin. Sites for sampling wild-origin parr were selected because they were more than 25 km from any hatchery. They were also located in side streams that had not been stocked recently and that were more than 0.5 km from the main river. In addition, all three areas contained spawning grounds for wild Atlantic salmon, the spawning in 1996 having been documented (Carr et al. 1997). The remainder of the sample of known wild origin ($n = 27$) was taken from the Dennis Stream on 23 July 1998 (Table 1). The Dennis Stream had not been stocked during the past 10 years and had no hatchery on the stream system. Dennis Stream juvenile salmon samples were all assumed to be of wild origin.

Atlantic salmon fingerlings and parr (fork length [FL] $\geq$ 5 cm) were sampled in the Magaguadavic River in 1996 ($n = 123$), 1997 ($n = 143$), and 1998 ($n = 127$). Sampling periods were 4–12 September 1996 and 9–10 September 1997. Juveniles were collected from 13 sites in 1996 and 7 sites in 1997. Juveniles sampled in the wild in 1998 were taken at three sites in the Magaguadavic River and were captured during five periods, approximately monthly, from June to October. Atlantic salmon juveniles of unknown origin were collected, approximately monthly, from June to October 1998 in the Digdequash River ($n = 87$) and Waweig River ($n = 97$).

Sampling procedure.—Electrofishing gear was used to capture juvenile Atlantic salmon in the wild (FL $\geq$ 5 cm). A dip net was used to collect the hatchery salmon. In all cases the salmon, once captured, were handled the same: They were removed from the river or tank, anesthetized with a clove oil solution (Soto and Burhanuddin 1995), weighed, measured (FL), and visually checked for fin clips. Scale samples were taken from an area posterior to the dorsal fin and above the lateral line. The scales were stored on acetate slides as described in Power (1964). The fish were allowed to recover in a bucket of fresh water. They were then redistributed over the sampling site in the wild or returned to the tank of origin in the hatchery.

Scale analysis.—Scales were evaluated by using an image analysis system with Image Tool software. The scales were magnified (210×) in the image. Linear measurements were taken in the optical units of Image Tool (1 optical unit = 0.678 μm). Area measurements of scale focus were in optical units squared.

Linear measurements were made along a line perpendicular to a reference line as described in Schwartzberg and Fryer (1993). The width of each successive pair of circuli for the first six pairs was measured. Area measurements of scale focus were calculated automatically by Image Tool software after the periphery of the focal area was traced.

Statistics.—A discriminant function analysis was conducted by using nine scale measurement variables as predictors of membership in two groups. To develop the discriminant function, independent variables that might be predictors of the farmed or wild origin of juvenile salmon had to be investigated. These predictors were either measured from, or derived from measurements of, scale samples from the two groups of known-origin fish, one from the Magaguadavic River and Dennis Stream (known origin wild), one from the Connors Brothers and Stolt Sea Farms hatcheries (known origin farmed) (Table 1). Groups were therefore farmed-origin and wild-origin juvenile Atlantic salmon.

First, the area of the focus was determined. Focus formation can occur in native Atlantic salmon 25 mm long (G. L. Lacroix, unpublished data) and farmed salmon 28 mm long (R. H. Peterson, Fisheries and Oceans Canada, St. Andrews, New Brunswick, Canada, personal communication). Because of the size difference of the two groups of fish at platelet formation, we expected that the size of the focus would be proportionally bigger in fish of farmed origin than in those of wild-origin.

TABLE 2.—Results of the discriminant function analysis performed on juvenile salmon of unknown origin from the Magaguadavic River in 1996–1998, the Digdequash River in 1998, and the Waweig River in 1998. Except in the last column, numbers in parentheses are standard deviations (SDs).

		Distance (μm)								
Group	*N*	Circuli 1–2	Circuli 2–3	Circuli 3–4	Circuli 4–5	Circuli 5–6	Focal area (μm^2)	Mean distance between the first 6 circuli pairs (μm)	SD of distance between the first 6 circuli pairs (μm)	Predicted no. (%) of farmed fish
Farmed	67	15.75 (2.83)	15.28 (2.28)	13.93 (2.76)	13.83 (2.53)	13.72 (2.60)	5,752 (998)	14.25	2.21	63 (94)
Native	57	12.92 (3.99)	11.05 (3.35)	10.40 (2.83)	10.60 (3.62)	10.78 (3.60)	4,411 (978)	11.29	3.30	2 (4)
Unknown										
Magaguadavic River	393									186 (47)
Digdequash River	87									6 (9)
Waweig River	97									41 (42)

Second, we measured the width of the first six consecutive circuli pairs (e.g., the distance from circuli 1 to circuli 2 = Circuli pair 1). Circuli form regularly: When growth is accelerated, circuli form farther apart; when growth is slowed, circuli form closer together (DeVries and Frie 1996). Accelerated growth rates in hatcheries should result in larger spacing of circuli for farmed fish than for wild fish.

The mean [(circuli pair 1 + circuli pair 2. . . circuli pair 6)/6 = *X*] and standard deviation of the width of the first six circuli pairs were also evaluated for their predictive power. Because growth is accelerated in hatcheries, we expected that the mean width of the first six circuli pairs would be greater in the farmed-origin fish than in wild-origin fish. Further, because growth in hatcheries is controlled, we thought that the standard deviation of the width of the first six circuli of farmed fish would be less than that for the wild-origin fish.

The classification of the ‘‘known origin’’ juveniles into farmed or wild groups provided a measure of the accuracy of the function. We tested the discriminant function in a jackknife cross-validation (Tabachnick and Fidell 1989), using it to determine the source of the ‘‘unknown origin’’ juveniles collected from the Magaguadavic (1996–1998), Digdequash (1998), and Waweig Rivers (1998) and using the variables that were significant ($P < 0.05$) predictors of origin in the development of the function. Farmed or wild origin was predicted for these samples.

Subsequently, to determine if whether differences between the two groups that made up the ‘‘wild’’ origin group were significant, we conducted a logistic regression on the measured variables in juveniles from the Dennis Stream and Magaguadavic River, fish known to be of wild origin.

Results

One discriminant function was calculated ($\chi^2(8) = 137.68$, $P < 0.001$) using the following nine variables: the width of each of the Circuli pairs 1–6; the area of the focus; the mean width of each of the first six circuli pairs; and the standard deviation of the width of the first six circuli pairs (Table 2). The discriminant function accounted for 100% of the between-group variability. Correlation between the predictors and the discriminant function suggested that the best predictors for distinguishing between farmed-origin and wild-origin Atlantic salmon juveniles were the mean width of the first six circuli pairs, the width of Circuli pair 2, the area of the focus, the width of Circuli pair 3, the width of Circuli pair 4, the standard deviation of the width of the first six circuli pairs, the width of Circuli pair 5, and the width of Circuli pair 1 (Table 2). The width of Circuli pair 6 did not load significantly and was dropped from the analysis.

Farmed-origin salmon had a larger focal area, a larger width of the first five consecutive circuli pairs, and a larger mean width for the first five circuli pairs than did the wild-origin group (Table 2). Further, the wild-origin group had a larger standard deviation for the width of the first six circuli pairs than did the hatchery-origin salmon. Using the jackknife cross-validation procedure for the total usable sample of 124 salmon showed that 118 (90%) were classified correctly, compared with 62 (50%) that would be correctly classified by chance alone.

For the ‘‘unknown origin’’ juveniles from the

TABLE 3.—Proportion of juvenile Atlantic salmon from the Magaguadavic River predicted to be of farmed origin by a discriminant function analysis.

Year	Number of sampling sites	*n*	Predicted farmed (%)
1996	13	123	36
1997	7	143	59
1998	3	127	43

Magaguadavic River in 1996–1998, the discriminant function analysis classified 47% as being of farmed origin. In individual years, this was 36% of the juveniles in 1996, 59% in 1997, and 43% in 1998 (Table 3).

From 1996 to 1998, 81% of the juveniles classified as farmed origin were located within 5 km of a hatchery installation (Figure 1; Table 3). However, the correlation between the proportion of farmed escapees in each sample and the distance to the closest upstream hatchery was not significant ($P = 0.12$).

The analysis of juveniles of unknown origin from the Digdequash River, using the discriminant function developed from the Magaguadavic River samples, indicated that only 6 (9%) of the 87 juveniles were of farmed origin. Of the Waweig River ‘‘unknown origin sample,’’ 41 (42%) of 97 juveniles were predicted to be of farmed origin.

Logistic regression indicated no significant difference in the measured variables between the wild Dennis Stream juveniles and the wild Magaguadavic River juveniles ($P < 0.01$).

Discussion

Development of a linear discriminate function to assign juvenile Atlantic salmon on the basis of their natal origin appears to have been successful. The jackknife cross-validation indicated that 90% of the known-origin juveniles were correctly classified as to origin. The method was capable of identifying the farmed or wild origin of juvenile salmon by scale characteristics established in their age-0 year, a time that may precede the establishment of definitive morphological characteristics of cultured origin.

The strength of the focal area and the first five circuli pairs as predictors demonstrates how large the growth difference is between farmed and wild salmon, especially at an early age. The difference in focal area and the spacing of each of the first five circuli pairs was significantly larger for the farmed-origin juveniles than for the wild-origin juveniles (Table 2). However, difference in the spacing of the sixth circuli pair was not significant, perhaps indicating that feeding rates and therefore growth rates in the wild equaled those in captivity by the time the sixth circuli pair was established.

The standard deviation of the first six circuli was greater for the sample known to be of wild origin (3.30 μm) than for the farmed-origin sample (2.21 μm). This was the first time that standard deviation of circuli spacing has been used to classify farmed and wild-origin juvenile salmonids. The strength of standard deviation as a predictor of origin suggests that the growth rate of juvenile salmon in the wild varies much more than their growth rates in the hatchery.

Juvenile escapees enter river systems from hatcheries at age 0. Previous procedures may not have identified some or all of these escapees. Lacroix et al. (1998) identified the number of escapees entering the wild as a key factor in the genetic impact of escapees on wild stocks, noting that spawning of past juvenile escapees may accelerate the loss of the wild stock through genetic introgression. They suggested that the constant intrusion of escapees (as occurred in the Magaguadavic River from 1996 to 1998) gives a false perception of the robustness of a wild population, which may actually be dwindling, and may have important consequences for the management of these rivers.

Although beyond the scope of this study to provide a quantitative estimate of escapees from the hatcheries on the Magaguadavic River, results suggest that significant numbers of juvenile escapees were at large in the Magaguadavic River in 1996 (36%), 1997 (59%), and 1998 (43%). These proportions indicate that juveniles escaped repeatedly each year and their presence was not the result of an isolated incident.

A confounding factor may affect the accuracy in the use of this discriminant function analysis. The procedure used in this study was developed by using juvenile salmon from the Magaguadavic River and Dennis Stream but was used to classify juvenile salmon of unknown origin from the Magaguadavic River and other nearby rivers (Digdequash River and Waweig River). Because smolt age is linked to growth (Symons 1978), this age can be used as an indicator of growth conditions in a river. Smolt ages from rivers in this region are similar. Thus we assumed salmon from adjacent river systems could be examined by criteria established on juvenile salmon from the Magaguadavic River and Dennis Stream with minimal error in identification.

The growth patterns of farmed and wild salmon, which were recorded on their scales, differed significantly. The different developmental forces experienced in the first year of life by these two groups in effect left characteristics that would allow classification of their farmed or wild origin at any age. Using discriminate function analysis based solely on scale characteristics, we were able to predict the origin of juvenile Atlantic salmon 90% of the time. This precision was accomplished by using a procedure that was both economical and had little impact on the wild salmon population. Through the use of this procedure, one may evaluate groups of rivers that produce smolts of the same age, with similar life histories. Through the development of similar models on strategic river systems, overall assessment of the farmed or wild origin of juvenile salmon produced in rivers may be determined in a framework of quantifiable assessment.

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