Scale and Otolith Patterns Prove Growth History of Pacific Salmon

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Scale and otolith patterns are frequently used for age determination, growth estimation, and stock identification of Pacific salmon. However, little information is available about their formation mechanism such as circulus, check in the scale, and growth increment in the otolith. I clarified the formation mechanisms of scale and otolith patterns by rearing experiments using juvenile sockeye and chum salmon. Circulus spacing was positively correlated with somatic growth in juvenile chum salmon. A check was formed soon after release from a hatchery in juvenile chum salmon and at the time of tagging in juvenile sockeye salmon. Growth increments were produced in otolith on a daily basis. An allometric equation was fitted to the relationship between fish length and otolith length. These results indicate that growth history of individual fish can be back-calculated from scale and otolith patterns. Circulus spacing is also useful as an indicator of somatic growth. Scale and otolith patterns are influenced by somatic growth rate and by environmental conditions. From this, scale and otolith patterns are useful for the estimation of fish growth through the life history.

INTRODUCTION

The assessment of growth condition provides valuable information for monitoring salmonid stocks. Ocean growth is a determinant of the biomass of salmonid stocks inhabiting Pacific Ocean. Somatic growth would also influence the population dynamics of Pacific salmon. Mortality of slower growing fish is generally higher than for faster growing fish (Healey 1982; Holty et al. 1990). Density-dependent growth reduction was suggested in some populations of chum salmon (Ishida et al. 1993). Somatic growth rate is strongly influenced by environmental conditions (see Brett 1979). Growth estimation may also provide information on the environmental condition of salmonid stocks.

Growth estimation has been performed by sequential sampling, mark-recapture, scale and otolith readings in fisheries studies (Ricker 1976). Especially, scale or otolith readings have been routinely performed during age determination. Age determination and growth estimation have been performed using annual rings or growth checks in scales or otoliths. Back-calculation technique has been used in growth estimation from otoliths or scales. It is necessary in back-calculation to validate the formation periodicity of growth check and to establish the relationship between scale/otolith size and fish size (Campana and Neilson 1985). However, little information is available for formation mechanism of scale and otolith characteristics.

Scales of teleost fish are calcified tissues formed of calcium phosphate, and are embedded in the connective tissue of the skin (Yamada and Mugiya 1988). The upper surface of the scale is characterized by a series of ridges. Scale formation by the osteoblasts proceeds around the periphery of the scale. The scale ridge grows in the intercellular space formed by two overlapping osteoblasts on the scale surface (Yamada 1971). The overlapping of the osteoblasts is brought about in the course of transformation of marginal round osteoblasts into inner flattened ones. The concentric scale ridges form the surface structure of scale, which are called the circuli. The annulus or growth check on salmonid scale are observed as the band of narrowly spaced circuli and/or the discontinuously formed circulus (Kobayashi 1961). Many investigators have considered that the annulus forms in the winter, when the fish experiences low growth rate in cold water.

Otoliths are crystalline (calcium carbonate) structures within the inner ear of teleost fish. They are acellular and grow in a manner similar to molluscan shell formation rather than by ossification that occurs in the scale and other hard parts of fish. Microstructure of otoliths consist of translucent incremental and opaque discontinuous zones (Campana and Neilson 1985). An incremental and a discontinuous zone is usually formed over 24 hours (Campana and Neilson 1985). The formation
periodicity of growth increment consisting of two zones has been validated by rearing and sequential sacrifice, otolith marking experiments, estimation of hatching dates, and so on (Geffen 1987).

To assess usefulness of scale and otolith patterns for growth estimation, I examined the formation mechanisms of scale and otolith increments by rearing experiments using chum and sockeye salmon.

**MATERIALS AND METHODS**

**Rearing Experiments in Different Environmental Conditions**

In the fall of 1993, eggs and sperm were stripped from chum salmon returning to the Chitose River. Eggs fertilized by the dry method were incubated in about 8°C water. From emergence to reaching about 0.5g in body weight, juveniles were reared in tanks and fed dry pellets in 8°C water for one or two weeks. Six hundred juveniles were sacrificed and their body weight were measured to calculate feeding rates. Six sets of 30 juveniles were measured for fork length and observed as to whether scales were formed. Another 6 sets of 200 juveniles were reared for 2 weeks in different sets of environmental conditions: 8, 10°C in water temperature \( \times 70, 85, 100\% \) of the maximum feeding rate for a given water temperature and fish size (Suzuki et al. 1994). At the end of the rearing period, 30 juveniles were measured for fork length, and scales were collected from the body area near the lateral line between dorsal and anal fins.

The number of scale circuli, scale radius to the outermost circulus of the scale, and scale radius of the focus were counted and measured along the longest axis. Average circulus spacing was estimated by the equation:

\[
\text{circulus spacing (\( \mu m \))} = \frac{\text{scale radius to the outermost circulus (\( \mu m \))}}{\text{scale radius of the focus (\( \mu m \))}} \times (\text{number of circuli - 1}).
\]

Somatic growth was calculated as the difference between initial mean fork length in each subgroup and individual fork length at the end of rearing period, because initial individual fork length was not available in this analysis. The analysis of variance (ANOVA) was used to detect the effects of water temperature and feeding rate on somatic growth. The analysis of covariance (ANCOVA) was used to detect the effects of two variables on circulus spacing.

**Field Mark-Recapture Experiment**

Juvenile chum salmon were marked by clipping both ventral fins from March 24 to April 5, 1993. Marked juveniles were collected on March 29 and April 5 in Shou River Hatchery in Shou River which discharges into Toyama Bay in Japan Sea. Juveniles were released from the hatchery on April 5, 1993. These fish were recaptured in coastal waters off northern Honshu in Japan Sea. After fixation in 10% formalin, I measured fork length and collected scales.

Radii and circulus spacings of scales mounted in glycerin-gelatin were measured along the longest axis. The change in circulus spacing was analyzed by determining the correlation between circulus spacing and circulus number from the center of the scale focus to each circulus. To detect the check, circulus spacings were smoothed by calculating a moving average.

**Individual Tagging Experiment**

In the fall of 1991, eggs and sperm were stripped from anadromous sockeye salmon returning to the Bibi River and from nonanadromous sockeye in Lake Shikotsu. Eggs fertilized by the dry method were incubated in about 8°C water. After emergence, juveniles were reared in tanks and fed dry pellets. For measurement of individual growth, Passive Integrated Transponder (PTT) tags were injected into the peritoneal cavities of 152 juveniles after measurement of fork length on October 8, 1992. These juveniles were transported to Chitose Hatchery and reared in a circular tank for 214 days under a natural photoperiod.

On May 10, 1993, juveniles were measured (fork length in millimeters), and their scales were collected. Collected scales were mounted on a glass slide in glycerin-gelatin. Scale radius and circulus spacing were measured in micrometers along the longest axis of scale. A check was found by calculating the moving average of circulus spacing (Fukuwaka and Kaeriyama 1994). Scale radius at check formation was equal to the sum of the circulus spacing from the center of the focus to the check. In this paper, I considered the check as a very narrowly spaced and/or discontinuously formed circulus.

For the relationship between fork length and scale characters since scale formation, we observed early growth of scale in 43 juvenile collected at Lake Shikotsu Hatchery on May 17, 26, and June 24, 1994. To use in back-calculation, I measured scale radius and fork length for 5 juveniles (age 0.0) with no circuli evident, collected at Lake Shikotsu Hatchery. To estimate individual fork length at the check formation, I used the biological intercept backcalculation (Belding, 1934; Campana, 1990; Ricker, 1992):

\[
(S_E - S_o) / (L_E - L_o) = (S_C - S_o) / (L_C - L_o)
\]

where \( S_E, S_o, \) and \( S_C \) were scale radii at the end of the experiment, at squamation, and at check formation,
respectively, and $L_E$, $L_o$, and $L_c$ were fork lengths at the end of the experiment, at squamation, and at check formation, respectively.

Otolith Marking Experiment

On October 25, 1993, chum salmon eggs were stripped and fertilized and then incubated in 8°C water. Twenty embryos, alevins, or fry were collected at 0, 10, 20, 30, 60, 90, 120, and 150 days after fertilization. The fish were fixed in 10% buffered formalin for one day and then stained with alizarin red S so that their otoliths could be easily observed. For the otolith growth of successive stages, chum salmon juveniles were also collected off Yamagata, Japan Sea in March 22, April 7 and 21, 1994. These fish were fixed in 10% buffered formalin for 3 or 4 days and then preserved in 70% ethanol. Total or fork lengths taken on the fish were in millimeters. Sagittae were dissected from all specimens under a stereo microscope. The long axis of each sagitta was measured in millimeters. To describe the relative growth of otolith, I used an allometric equation:

$$O = O_0 + a \cdot (L - L_0)^b$$

where $O$ is sagitta length (mm); $L$ is body length (mm); $O_0$ is sagitta length at hatching; $L_0$ is body length at hatching; $a$ and $b$ are constants.

To investigate increment periodicity, a mark was induced on the otoliths of 40 juvenile chum salmon by immersing the fish in 10 ppm alizarin complexone (ALC) for one day on May 17, 1994 (Tsukamoto 1988). Each fish was also marked a second time; ten were marked 7 days after receiving their first exposure to ALC, and other sets of ten fish were marked at 14, 21, and 28 days. Seven days after the fish had been marked for the second time, they were sacrificed and fixed in 70% ethanol. Sagittae from each fish were ground and polished to produce thin sections for microscopic observation. A fluorescent microscope was used to count the number of growth increments between the two ALC marks.

RESULTS

Scale Growth in Chum Salmon Juveniles

Mean fork lengths at the start of rearing period were not significantly different among 6 sets of juveniles (ANOVA, $P > 0.05$; Table 1). Fourteen juveniles did not form the second circulus in their scales at the end of rearing period, so that they were rejected in these analyses. Somatic growth of chum salmon juveniles was affected by rearing water temperature but not by feeding level (Table 2). Circulus spacing was positively correlated with

<table>
<thead>
<tr>
<th>Water temp. (°C)</th>
<th>Feeding level (%)</th>
<th>Fork length at the start (mm)</th>
<th>F. L. at the end (mm)</th>
<th>Scale radius (μm)</th>
<th>Number of circuli</th>
<th>Circulus spacing (μm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>70</td>
<td>43.0 (1.91)</td>
<td>48.2 (2.30)</td>
<td>170 (20.7)</td>
<td>2.28 (0.458)</td>
<td>40.6 (9.06)</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>85</td>
<td>42.9 (1.98)</td>
<td>48.3 (2.25)</td>
<td>164 (23.8)</td>
<td>2.29 (0.460)</td>
<td>41.6 (6.82)</td>
<td>28</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>41.7 (2.61)</td>
<td>47.4 (2.71)</td>
<td>159 (19.0)</td>
<td>2.08 (0.277)</td>
<td>36.3 (5.50)</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>42.9 (1.85)</td>
<td>49.6 (2.37)</td>
<td>183 (20.5)</td>
<td>2.80 (0.484)</td>
<td>41.3 (5.64)</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>85</td>
<td>41.8 (2.50)</td>
<td>48.5 (2.78)</td>
<td>180 (26.9)</td>
<td>2.60 (0.563)</td>
<td>40.4 (6.69)</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>41.7 (3.08)</td>
<td>49.5 (2.83)</td>
<td>187 (22.6)</td>
<td>2.57 (0.504)</td>
<td>38.5 (6.94)</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 2. ANOVA table of somatic growth on water temperature and feeding level in the rearing experiment of juvenile chum salmon.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature</td>
<td>1</td>
<td>158</td>
<td>24.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feeding level</td>
<td>2</td>
<td>1.80</td>
<td>0.138</td>
<td>0.871</td>
</tr>
<tr>
<td>Water temperature</td>
<td>2</td>
<td>28.1</td>
<td>2.15</td>
<td>0.120</td>
</tr>
<tr>
<td>by feeding level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>1044</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
somatic growth \((r = 0.244, \ P < 0.01; \text{Fig. 1})\). Circulus spacing was affected by feeding level from ANCOVA with somatic growth as the covariate (Table 3).

**Fig. 1** Relationship between somatic growth and circulus spacing of juvenile chum salmon. The line indicates the regression equation: circulus spacing \((\mu m) = 0.683'\) somatic growth \((mm) + 35.6 (r = 0.244, n = 166, \ P < 0.01)\).

**Check Formation in Chum Salmon Juvenile**

Circulus number of chum salmon juvenile ranged from 3 to 8 at the date of release from Shou River Hatchery. Circulus spacings of juveniles decreased simply as circulus number increased during the rearing period in the hatchery (Fig. 2). Eight fishes were recaptured in coastal waters after more than 14 days after release. A check was observed in each of their scales (Fig. 3).

**Table 3.** ANCOVA table of circulus spacing on water temperature and feeding level with somatic growth as the covariate.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>F-ratio</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic growth</td>
<td>1</td>
<td>600</td>
<td>11.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Water temperature</td>
<td>1</td>
<td>31.9</td>
<td>0.595</td>
<td>0.442</td>
</tr>
<tr>
<td>Feeding level</td>
<td>2</td>
<td>501</td>
<td>4.67</td>
<td>0.011</td>
</tr>
<tr>
<td>Water temperature by feeding level</td>
<td>2</td>
<td>52.0</td>
<td>0.299</td>
<td>0.742</td>
</tr>
<tr>
<td>Error</td>
<td>159</td>
<td>8523</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2** Circulus spacings of juvenile chum salmon reared in the Shou River Hatchery (from Fukuwaka and Kaeriyama 1994). Vertical bars indicate 95% confidence limits.

**Fig. 3** Changes in circulus spacings of 8 juvenile chum salmon recaptured in coastal waters (from Fukuwaka and Kaeriyama 1994). Solid and broken lines indicate non-smoothed and smoothed (by moving average) circulus spacings, respectively.

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The number of circuli from the focus to the check (3 - 7) was consistent with the number of circuli at release (3 - 8). The scales of these fish showed the following pattern: circulus spacing decreased continuously during the rearing period in the hatchery, the check was formed immediately after release, and circulus spacing increased in the early ocean life period (Fig. 3).

**Check Formation in Sockeye Salmon Juvenile**

A common relationship between fork length and scale radius at check formation (the mid-sized group in Fig. 4) and the relationship between fork length and scale radius (the small and large fishes in Fig. 4) is evident. Back-calculated fork length at check formation was consistent with fork length at tagging (Fig. 5). The regression equation was fork length at check formation (mm) = 1.04 · fork length at tagging (mm) - 5.89 (r = 0.759, n = 152, P < 0.001).

**Otolith Growth in Chum Salmon Juvenile**

Sagittae were first observed in developing chum salmon 60 days after fertilization (at hatching). At this time the mean total body length was 19.5 mm (0.75 SD) and the sagitta had a mean longitudinal length of 0.312 mm (0.0256 SD). The allometric equation:

\[
\text{sagitta length (mm)} = 0.312 + 0.0359 \times (\text{body length (mm)} - 19.5)^{0.790}
\]

was fitted the relationship between body length and sagitta length (Fig. 6).
DISCUSSION

Somatic Growth and Scale Circulus Spacing

The relationship between somatic growth and circulus spacing is positive but weak in Pacific salmon (Fig. 1; Fisher and Pearcy 1990; Fukuwaka and Kaeriyama 1997). A linear relationship between circulus spacing and somatic growth rate was useful in estimating individual growth rate in an experimental population of tilapia (Doyle et al. 1987), but in coho salmon it was useful only for comparing somatic growth rate between groups of juveniles (Fisher and Pearcy 1990). Healey (1982) suggested the size-selective mortality of chum salmon juvenile from the scale analysis. Fukuwaka and Kaeriyama (1997) indicated that circulus spacing was useful for comparing mean growths among groups of juvenile sockeye salmon, but less useful to estimate individual growth. Circulus spacing was also influenced by environmental conditions (Table 3). Circulus spacing may be used as an indicator of mean growths in the same condition of environmental factors, but cannot be used in the calculation of individual growth.

Scale Annulus Formation

The annulus is observed in salmonid scales as a band of closely spaced circuli and/or discontinuously formed circuli (Kobayashi 1961). The narrowly spaced and discontinuously formed circuli is considered as the check in this paper. A check was formed at release in chum salmon juveniles (Fig. 3) and at tagging in sockeye salmon juveniles (Fig. 4 and 5). The check of tagged sockeye salmon juvenile is likely formed as a result of the stress of tagging and transporting procedures and the change in growth rate in the different rearing conditions. The check of recaptured chum salmon juvenile is likely formed as a result of large changes in growth rate induced by the drastic change of their environment. Marked juveniles migrating into the sea immediately after release encounter changes in the environment from the hatchery to the sea. The check on the scales of marked juveniles would be formed immediately after their release. The check formation was not affected by additional handling at fin-clipping because release checks were also observed on the scales of non-marked juveniles caught in the coastal waters.

A check is likely formed at the time of large and discontinuous changes of somatic growth rate and environmental factors. Bilton (1975) suggested from rearing experiments for sockeye salmon that the check formed as a result of increased growth. He stated that the checks were formed as a result of changes in physical and non-physical factors affecting circulus formation, such as water temperature, food, and light. Suzuki and Kaeriyama (1990) also indicated that a check formed at the time when the feeding level increased.

Circuli spacing was positively correlated with somatic growth, and the band of closely spaced and continuously formed circulus appears to be formed during the period of slow growth (Fig. 1; Bilton 1975; Fukuwaka and Kaeriyama 1997). Scale growth would be stopped when fish growth is stopped. Suzuki and Kaeriyama (1990) observed that scale growth was stopped during the starvation period in their rearing experiment of sockeye salmon.

Thus an annulus defined as the band of closely spaced circuli and/or discontinuously formed circuli should be formed during the period of slow growth occurring before or after the period of stopped growth in winter, and would be completed in the short duration at the start of growing period from spring to fall. Bilton and Ludwig (1965) concluded from the observation of scales from fishes collected in winter that the annulus was formed in January in sockeye, in late December in pink, and in February or March in chum salmon. However, Vedensky (1954) stated that in pink salmon a band of narrowly spaced circuli was formed in early spring when the fish had low assimilation rates and low feeding rates after and before the no-growth period in winter. He also suggested that the annulus would be formed in early
spring after the period of stopped growth in winter.

Back-calculation using the scale annuli would be an evaluation of fish sizes in winter and early spring. Ricker (1976) pointed out that a weakness of back-calculation method for Pacific salmon was that the information on the timing of annulus formation was scanty and contradictory between different authors. A comparison of field observations between the timing of annulus formation and the seasonal growth pattern would be necessary for the assessment of somatic growth of Pacific salmon in the ocean by means of back-calculation to be fully satisfactory.

Somatic Growth and Otolith Growth

Daily growth increment of otolith has been used to estimate fish growth by back-calculation (for example, Wilson and Larkin 1982). The formation periodicity of growth increment was validated in the otolith of chum salmon juvenile. Volk et al. (1984) also observed the production of daily increments in chum salmon juveniles when they were held in sea water tanks. However, non-daily increment production was observed in juvenile pink salmon that had been freely migrating and feeding in an Alaskan estuary (Volk et al. 1995). Consequently, when studies are undertaken to evaluate early marine growth of chum salmon or other juvenile salmonids it will be necessary to conduct field evaluations of increment production by using otolith marking and recapture experiments.

The relationship between otolith length and fish length was found to be allometric during the alevin and juvenile stages in chum salmon. Hence it is not possible to simply use a proportional back-calculation method to predict fish size based on otolith size. Fitzhugh and Rice (1995) described the relationship between otolith size and fish size by an allometric equation. Other non-linear relationships between otolith length and fish length are often observed during early life in many fishes. For example, otolith - fish size relationship have been described by using a polynomial equation (Mugiya and Tanaka 1992). Moreover, this relationship appears to change with ontogenetic stages in early life (Hare and Cowen 1995). In their review, for instance, Campana and Neilson (1985) state that a curvilinear relationship is characteristic during larval phases and this usually changes into a linear one during later juvenile stages.

Allometric back-calculation may be used to estimate somatic growth rate of chum salmon juvenile. However, it is necessary in back-calculation to validate the formation periodicity of growth increment and to establish the relationship between otolith size and fish size (Campana and Neilson 1985). I validated the daily growth increment and established the relationship between otolith size and fish size of juvenile chum salmon. Volk et al. (1984) used another method to estimate somatic growth of chum salmon. They found that the width of daily growth increment was positively correlated with the instantaneous growth rate in body weight (Volk et al. 1984).

CONCLUSIONS

Scale patterns formed by circuli, growth checks, and annuli are related to somatic growth of chum and sockeye salmon. Circulus spacing was positively correlated with somatic growth, and might be useful as an indicator of somatic growth. A check was formed after the time of large change in somatic growth. Therefore, an annulus would be formed in a short duration of slow growth after the period of stopped growth. Scale radius at annulus formation would indicate fish size at winter or early spring when the fish began to grow in the ocean. Otolith pattern formed by growth increment also shows somatic growth of juvenile chum salmon. Somatic growth of juvenile chum salmon would be estimated by the back-calculation technique or the regression analysis using the width of otolith daily growth increment.

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