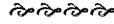


## Digitized Scale and Otolith Microstructures as Correlates of Juvenile Pink Salmon Size

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**Abstract:** The purpose of this study was to determine whether scale or otolith microstructures were strongly related to fish growth. Scales and otoliths were sampled from 231 juvenile pink salmon (*Oncorhynchus gorbuscha*) collected from the marine waters of Southeast Alaska during two periods in 1993 and 1994. A computerized image analysis system was used to measure several periodic and non-periodic scale and otolith microstructures from each specimen. The measurements from each fish were compared with each other and with fish length using non-parametric correlation analysis and parametric regressions. As expected, growth of most scale and otolith microstructures was significantly positively correlated with fish growth. Scales and otoliths also portrayed recent marine growth (growth near the time of capture) more reliably than earlier marine growth (growth more distant from the time of capture). An unexpected result was that the number and width of periodic scale microstructures (circuli) were more strongly related to fish length than were the number and width of periodic otolith microstructures (increments). These results indicate that, at least for pink salmon, there is a tradeoff between the finer temporal resolution available from otolith increments (near daily) and the stronger correlation with fish length available from scale circulus measurements.

### INTRODUCTION

Given approximate proportionality between fish growth and the periodic features in its bony structures, the width of the most recently formed periodic structures should provide a measure of recent growth (Campana and Jones 1992). The use of scale circulus widths as a proxy for fish growth in Pacific salmon (*Oncorhynchus* spp.) has a historical and ongoing precedent; for example, the estimation of variability in natural mortality rates and environmental effects on fish growth (Clutter and Whitesel 1956; Healey 1982; Friedland et al. 1996; Walker et al. 1998). More recently, the collection and interpretation of otolith daily increments has been introduced (e.g., Stevenson and Campana [ed.] 1992). Campana and Neilson (1985) concluded that the width of otolith increments was generally proportional to fish growth. In pink salmon, otolith increment widths have been used as a proxy for growth rates to analyze environmental and experimental effects on fish growth (Mortensen and Carls 1995; Volk et al. 1995).

The availability of microcomputer digitizing technology provides a tool for comparing the proportionality of scale and otolith microstructures in relation to fish length. Microcomputer digitizing archi-

ture allows for the rapid measurement of many features from the microstructure of fish scales and otoliths in addition to the standard periodic microstructures such as scale circuli and otolith increments (Courtney 1997; Courtney and Mortensen in press). Measurements can be obtained from any area or distance of interest and at several different resolutions depending upon the magnification chosen. Once a desired protocol for feature extraction has been established, much of the process can also be automated with image analysis software (Courtney and Mortensen in press). The purpose of this study was to utilize available microcomputer digitizing technology to obtain a variety of periodic and non-periodic scale and otolith microstructure measurements at various resolutions in order to determine which microstructures were most strongly related to fish growth.

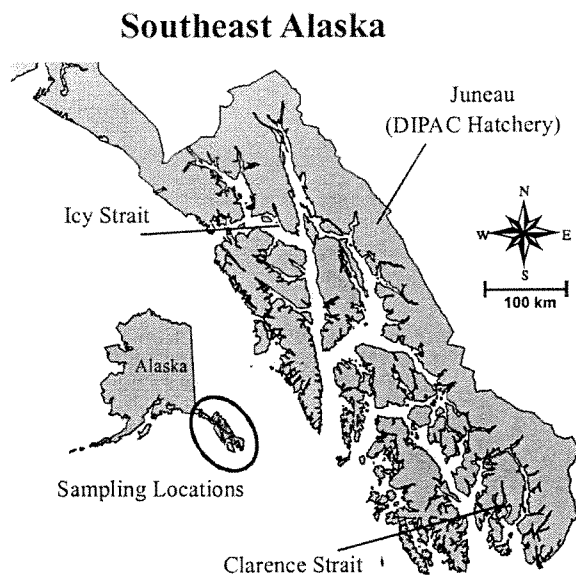
### MATERIALS AND METHODS

#### Field Collections

The National Marine Fisheries Service (NMFS) Auke Bay Laboratory has collected and maintained a time series of juvenile pink salmon scales and otoliths

from the marine waters of Southeast Alaska for the years 1993–1996 (Orsi, NMFS unpublished Cruise Reports 96-05 and 96-08). Juvenile pink salmon (*O. gorbuscha*) used in this study were captured with surface trawl-nets from Clarence Strait and Icy Strait, Alaska in 1993 and 1994 (Fig. 1). Two cruises were conducted each year, the first in early July (early-summer) and the second in early August (late-summer). Each cruise consisted of three to four nights of sampling in Clarence Strait followed by three to four nights of sampling in Icy Strait. In each region, six equally spaced stations were sampled each night along a transect bisecting the strait. Sampling was conducted with a Kodiak FRI pair trawl fished on the surface between the 27.5 m NOAA ship *John N Cobb* and a seine skiff. The surface trawl had a 6 m (horizontal) by 3 m (vertical) opening and was towed at 3.7 km/h (2 kn) for 10 minutes per set. Two sets were made at each station usually perpendicular to the transect line with one set in either direction roughly parallel to shore. Fishing was conducted primarily from dusk to dawn.

Fig. 1. Sampling locations for juvenile pink salmon in Icy Strait and Clarence Strait, Southeast Alaska.



All pink salmon captured were measured for fork length (tip of snout to fork in tail) on an electronic fish measuring board. Up to 50 juveniles per set were sub-sampled for scales and otoliths until as many as 300 fish were sampled from each region for each cruise for each year. A smear of scales was removed from the preferred region, as described by Bilton (1985), of each sub-sampled fish and placed between strips of acetate and stored in individual coin envelopes. The heads of each sub-sampled fish were removed and preserved in vials of 95% ethanol for later otolith retrieval.

## Scale and Otolith Processing

Juvenile pink salmon scales and otoliths selected for this study were further sub-sampled from those collected at sea. Up to 50 scales and otoliths were selected at random from each region (Icy Strait, and Clarence Strait) for each cruise (early-summer and late-summer) for both years (1993 and 1994) for a total of 231 samples.

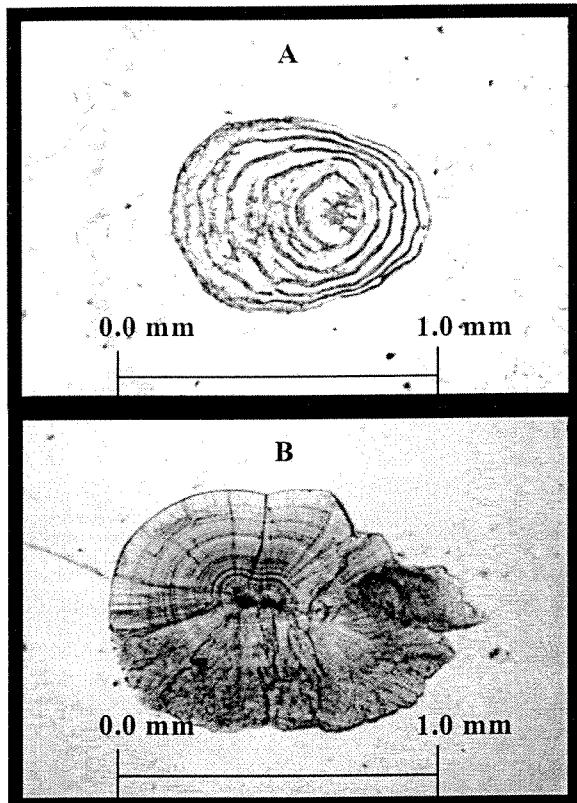
Scale processing generally followed that of Courtney (1997). For each selected fish, a single scale was selected from among the smear of scales collected. The smear of scales was searched under magnification systematically from top to bottom and left to right. The first scale encountered with a clear first circulus (focus), an unbroken scale edge, and clear circuli from the focus to the edge was digitized (Fig. 2A).

The left sagitta otolith of each selected fish was extracted from the head using the guillotine method described by Secor et al. (1992). The otolith was placed sulcus side down on a glass petrographic slide and partially immersed in a drop of thermoplastic cement (thermal resin) in a manner similar to that described by Secor et al. (1992). When the thermal resin hardened, it affixed the otolith to the slide and provided a bulwark around the otolith for grinding and polishing. Many of the pink salmon otoliths exhibited growth patterns that obscured the fine increments and gave the otolith an uncharacteristic shape. This type of otolith growth was consistent with the anomalous compositional pattern described as waterite crystalline mineral structures in the otoliths of other species (Brown and Severin 1999). Before grinding, each mounted otolith was visually inspected under magnification for such anomalous growth. If the otolith exhibited this type of anomalous growth it was rejected. The surface of the otolith was rough ground to the primordium on 1,000 grit wet or dry silicon carbide paper on a rotating lap wheel and polished with 12,000 grit paper. The grinding process was repeated on the second side by reheating the thermal resin and flipping the otolith. Each otolith was ground along a plane extending from the primordium to the outside edge of the dorsal posterior quadrant (e.g., see Fig. 1 from Mortensen and Carls 1995). Care was taken to leave the dorsal posterior edge of the otolith intact and not to grind completely through the primordium (Fig. 2B).

Scale and otolith microstructures were digitized with two software specific computer programs (macros) written for image analysis software (Courtney and Mortensen in press.). One macro digitized scales while the other digitized otoliths. The image analysis software utilized a transmitted-light compound microscope, a video camera, and a personal computer equipped with a 486 processor, a 640 x 480 pixel

frame grabber board, a dual video monitor display and an electronic drawing pad and pen. The frame grabber board converted the black and white video input to a calibrated digital image which was output to the dual video monitor screen while the interactive program was run on the computer monitor screen. Scales were digitized at a magnification of 40x and otoliths at magnifications of 40x, 100x and 400x. The digitized measurements were automatically stored on a spreadsheet for analysis.

**Fig. 2.** A scale (A) and the left sagittal otolith (B) from the same 104.0 (mm) fork length juvenile pink salmon.



### Scale and Otolith Comparisons

Similar scale and otolith microstructures were grouped into two categories: non-periodic, and periodic. Non-periodic scale microstructure measurements included the area ( $\text{mm}^2$ ), focus area ( $\text{mm}^2$ ), major axis (mm) and minor axis (mm). Non-periodic otolith microstructure measurements included the area ( $\text{mm}^2$ ), major axis (mm), minor axis (mm), distance from primordium to marine entry (mm) and distance from marine entry to the outside edge of the otolith (mm). Periodic scale microstructure measurements included the number of circuli, the cumulative distance measured between circuli ( $\mu\text{m}$ ), and the mean distance per circulus ( $\mu\text{m}$ ). Periodic otolith microstructure measurements included the number of

increments, the cumulative distance measured between increments ( $\mu\text{m}$ ), and the mean distance per increment ( $\mu\text{m}$ ).

Two obvious outliers were revealed with scatter plots of all variables regressed against each other in SPLUS statistical software and were removed from the sample set. Many of the scale and otolith microstructure measurements had bimodal distributions as a result of sampling for fish during two periods (early-summer and late-summer) each year. Consequently, non-parametric methods were chosen for correlations of the overall growth of microstructures and fish length. The scale, otolith, and length measurements were compared using Spearman Rank non-parametric correlation analysis conducted with SAS statistical software.

Scale circulus counts, otolith increment counts, and cumulative widths between scale circuli and otolith increments were compared with fish length using parametric straight-line regressions. Scale circulus and otolith increment counts were normalized with the square root transformation as suggested by Zar (1984), for normalizing data consisting of counts. An examination of the regression residuals revealed an apparent increase in the magnitude of residuals with an increase in fish length, so the length data were transformed with the natural log to normalize the error. The cumulative widths between scale circuli and otolith increments were not transformed because their distributions were only slightly bimodal and the large sample size ( $n = 231$ ) was assumed to be of sufficient size to approximate a normal distribution. Standard statistical procedures could not be used to test for differences in the regression  $R^2$  values because scale circulus and otolith increment measurements from the same fish were not independent.

### Validation of Otolith Daily Increments

Thermally-induced hatchery otolith marks (thermal marks) were recovered from several of the fish captured from Icy Strait in 1993. The thermal marks identified the pink salmon as hatchery fish and uniquely identified their hatchery of origin (Hagen et al. 1995; Farley and Munk 1997; Courtney et al. 2000). All of the recovered thermal marks originated from Douglas Island Pink and Chum (DIPAC) hatchery in Juneau, Alaska (Fig. 1). Pink salmon start laying down increments after entering marine water and the entry event can be distinguished on the otolith by a distinct check (Volk et al. 1995). For the purposes of age validation, it was assumed that the dates of marine entry were the same for all DIPAC fish. Sampling in 1993 occurred during two periods, early-summer and late-summer, separated by about 30 days. The difference between the mean number of daily increments counted from each group of fish

(early-summer and late-summer) recovered from DIPAC was compared to the mean number of days between capture for each sampling period. The difference between the mean number of scale circuli counted from each group of fish (early-summer and late-summer) recovered from DIPAC was also compared to the mean number of days between capture for each sampling period. However, it was likely that scale formation did not begin at the same time as the marine entry check for fish recovered from DIPAC because scale formation begins at an indeterminate time following marine entry for pink salmon in south-eastern Alaska.

**RESULTS**

**Otolith Daily Increment Validation**

Sixteen thermally marked DIPAC otoliths were recovered from Icy Strait (Fig. 1): one in 1994, seven from the early-summer sampling period in 1993, and eight from the late-summer sampling period in 1993 (Table 1). The average day of the year of capture was computed for the thermally marked fish recovered from each sampling period in 1993 and the elapsed time between the capture of these two periods was calculated as 33.1 days (Table 1). Increment counts were only obtained from six marked otoliths from the early sampling period and three otoliths from the late sampling period. The difference between the average number of increments counted for each period was 32.2 (Table 1). Therefore, the number of increments added between periods agreed with the number of days between capture for the two groups of marked fish to within one day. The difference between the average number of scale circuli counted for each period was 4.5 (Table 1).

**Table 1.** Mean number of otolith increments, scale circuli, days between capture, and mean fish length of 15 thermally marked juvenile pink salmon recovered in 1993, ± 1 SD. Sample size is given in parentheses.

Period	Otolith increments	Scale circuli	Number of days	Fish length (mm)
Early-summer	47.8 ± 7.4 (6)	7.3 ± 1.1 (7)	181 ± 0.38 (7)	113.3 ± 10.8 (7)
Late-summer	80.0 ± 16.0 (3)	11.8 ± 1.9 (6)	214 ± 1.5 (8)	147.0 ± 16.9 (8)
Difference	32.2	4.5	33.1	33.7

**Scale and Otolith Comparisons**

After the beginning of scale formation, both fish length and the number of otolith increments generally increased with the number of scale circuli (Table 2). Fish length and many of the scale and otolith measurements were bimodal. Consequently, the overall growth comparisons were performed non-parametrically. The majority of the non-parametric

**Table 2.** The average number of otolith increments and mean fish length corresponding to the number of scale circuli for 231 juvenile pink salmon.

Scale circuli	Mean number of increments	Mean length (mm)	Sample size
2	43	79.0	2
3	43	86.0	1
4	53	93.4	13
5	57	96.6	30
6	54	103.8	39
7	56	109.9	31
8	63	121.1	21
9	66	122.3	21
10	71	129.7	22
11	72	137.0	22
12	75	140.9	19
13	86	149.3	6
14	78	154.7	3
15	61	150.0	1

correlations were either significant ( $p \leq 0.05$ ), or highly significant ( $p \leq 0.001$ ; Tables 3, 4, and 5). However, as a result of the large number of correlations performed, the individual correlations had a higher likelihood of being significant by chance alone than was reported by the  $p$ -values. Therefore, the significant correlation coefficients were used only as a relative measure of the strength of each microstructure's correlation to all of the others. Significant correlation coefficients  $|r| \geq 0.7$  were considered very strong, significant correlation coefficients  $0.5 \leq |r| < 0.7$  were considered moderately strong, and significant correlation coefficients  $|r| < 0.5$ , or non-significant correlations were considered poor.

In general, scale microstructure measurements were more highly correlated with each other and with fish length (Table 3) than were otolith microstructure measurements (Table 4), and non-periodic scale and otolith microstructures were more highly correlated with each other (Table 5A) than were periodic scale and otolith microstructures (Table 5B). In particular, most non-periodic scale and otolith microstructure measurements were strongly correlated ( $r \geq 0.7$ ) with each other, with other scale and otolith microstructure measurements, and with fish length (Tables 3, 4, and 5). Exceptions were microstructures representing the early development of scales (focus area) and otoliths (distance from primordium to marine entry), which were poorly ( $|r| < 0.5$ ) correlated with each other, with other scale and otolith measurements, and with fish length (Tables 3, 4, and 5).

Most periodic scale and otolith microstructure measurements were also strongly ( $r \geq 0.7$ ) or moderately ( $0.5 \leq r < 0.7$ ) correlated with each other, with other scale and otolith measurements, and with fish length (Tables 3, 4, and 5). Exceptions were the mean distance per scale circuli and the mean distance

**Table 3.** Scale microstructure measurements and correlation coefficients from 231 juvenile pink salmon.

A. Mean, standard deviation, and coefficient of variation of fish length and digitized scale measurements from 231 fish.

Statistic	Fish length (mm)	Scale area (mm <sup>2</sup> )	Major axis length (mm)	Minor axis length (mm)	Number of circuli	Cumulative circuli width (μm)	Focus area (μm <sup>2</sup> )	Distance per circuli (μm)
Mean	117	0.661	0.972	0.865	7.97	362.2	38.8	47.1
Standard deviation	19.1	0.301	0.231	0.194	2.70	97.6	10.1	7.38
CV (Percent)	16.3	45.5	23.8	22.5	33.9	26.9	26.1	15.6

B. Spearman rank correlation coefficients (*r*) of fish length and digitized scale measurements from 231 fish.

Scale Parameters	Fish length	Scale area	Major axis length	Minor axis length	Number of circuli	Cumulative circuli width	Focus area	Distance per circuli
Fish length <sup>a</sup>	1							
Scale area <sup>a</sup>	0.918**	1						
Major axis length <sup>a</sup>	0.911**	0.989**	1					
Minor axis length <sup>a</sup>	0.900**	0.981**	0.950**	1				
Number of circuli <sup>a</sup>	0.903**	0.912**	0.911**	0.884**	1			
Cumulative circuli width <sup>a</sup>	0.920**	0.948**	0.951**	0.920**	0.931**	1		
Focus area <sup>c</sup>	0.204*	0.276**	0.259**	0.283**	0.063	0.247**	1	
Distance per circuli <sup>c</sup>	-0.491**	-0.458**	-0.451**	-0.447**	-0.697**	-0.410**	0.317**	1

\* Significant correlation,  $p \leq 0.05$ \*\* Highly significant correlation,  $p \leq 0.001$ <sup>a</sup> Relatively strong correlation,  $|r| \geq 0.7$ <sup>b</sup> Moderately strong correlation,  $0.5 \leq |r| < 0.7$ <sup>c</sup> Poor correlation,  $|r| < 0.5$ , or non-significant.**Table 4.** Otolith microstructure measurements and correlation coefficients from 231 juvenile pink salmon.

A. Mean, standard deviation, and coefficient of variation of fish length and digitized otolith measurements from 231 fish.

Statistic	Fish length (mm)	Otolith area (mm <sup>2</sup> )	Major axis length (mm)	Minor axis length (mm)	Marine entry to edge (mm)	Number of increments	Cumulative increment width (μm)	Distance per increment (μm)	Primordia to marine entry (mm)
Mean	117	0.978	1.22	1.12	0.201	62.6	179	2.92	0.217
Standard deviation	19.1	0.253	0.170	0.146	0.0566	16.0	50.0	0.430	0.0425
CV (Percent)	16.3	25.9	14.0	13.0	28.1	25.6	27.9	14.7	19.6

B. Spearman rank correlation coefficients (*r*) of fish length and digitized otolith measurements from 231 fish.

Parameter	Fish length	Otolith area	Major axis length	Minor axis length	Marine entry to edge	Number of increments	Cumulative increment width	Distance per increment	Primordia to marine entry
Fish length	1								
Otolith area <sup>a</sup>	0.889**	1							
Major axis length <sup>a</sup>	0.879**	0.972**	1						
Minor axis length <sup>a</sup>	0.870**	0.966**	0.917**	1					
Marine entry to edge <sup>a</sup>	0.771**	0.782**	0.786**	0.763**	1				
Number of increments <sup>b</sup>	0.537**	0.521**	0.510**	0.500**	0.599**	1			
Cumulative increment width <sup>a,b</sup>	0.701**	0.699**	0.703**	0.678**	0.779**	0.837**	1		
Distance per increment <sup>c</sup>	0.364**	0.401**	0.422**	0.405**	0.426**	-0.112	0.403**	1	
Primordia to marine entry <sup>c</sup>	-0.002	0.024	-0.011	0.020	-0.320**	-0.258**	-0.309**	-0.188*	1

\* Significant correlation,  $p \leq 0.05$ \*\* Highly significant correlation,  $p \leq 0.001$ <sup>a</sup> Relatively strong correlation,  $|r| \geq 0.7$ <sup>b</sup> Moderately strong correlation,  $0.5 \leq |r| < 0.7$ <sup>c</sup> Poor correlation,  $|r| < 0.5$ , or non-significant.

**Table 5.** Correlation coefficients of non-periodic and periodic scale and otolith microstructures.

**A. Spearman rank correlation coefficients (*r*) of non-periodic scale and otolith microstructures.**

Scale parameters	Otolith parameters				
	Otolith area <sup>a,c</sup>	Major axis length <sup>a,c</sup>	Minor axis length <sup>a,c</sup>	Marine entry to edge <sup>a,c</sup>	Primordia to marine entry <sup>c</sup>
Scale area <sup>a,c</sup>	0.854**	0.843**	0.832**	0.731**	-0.004
Major axis length <sup>a,c</sup>	0.847**	0.837**	0.825**	0.726**	-0.016
Minor axis length <sup>a,c</sup>	0.838**	0.824**	0.819**	0.702**	0.008
Focus area <sup>c</sup>	0.158*	0.157*	0.185*	0.109	0.098

**B. Spearman rank correlation coefficients (*r*) of periodic scale and otolith microstructures.**

Scale parameters	Otolith parameters		
	Number of otolith increments <sup>b,c</sup>	Cumulative increment width <sup>a,b,c</sup>	Distance per increment <sup>c</sup>
Number of scale circuli <sup>a,b,c</sup>	0.508**	0.705**	0.421**
Cumulative circuli width <sup>b,c</sup>	0.481**	0.662**	0.391**
Distance per circuli <sup>c</sup>	-0.364**	-0.494**	-0.280**

- \* Significant correlation,  $p \leq 0.05$
- \*\* Highly significant correlation,  $p \leq 0.001$
- <sup>a</sup> Relatively strong correlation,  $|r| \geq 0.7$
- <sup>b</sup> Moderately strong correlation,  $0.5 \leq |r| < 0.7$
- <sup>c</sup> Poor correlation,  $|r| < 0.5$ , or non-significant.

per otolith increment which were poorly correlated ( $|r| < 0.5$ ) with most other scale and otolith measurements, and with fish length (Tables 3, 4, and 5). In addition, the mean distance per scale circuli was strongly negatively correlated ( $r = -0.7$ ) with the number of scale circuli (Table 3).

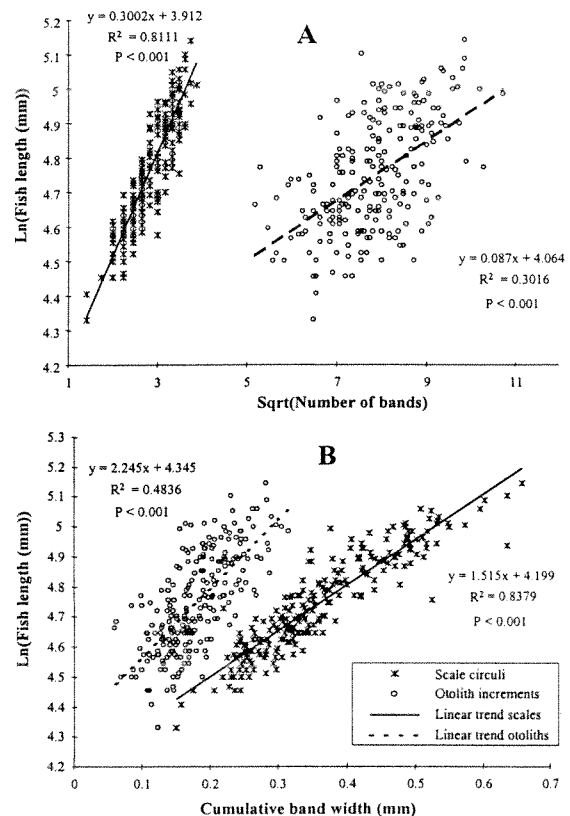
Parametric regression analysis revealed significant linear relationships ( $p < 0.001$ ) for the natural log of fish length with the square root of both the number of scale circuli and the number of otolith increments. The number of scale circuli explained 81 percent of the variation in fish length ( $R^2 = 81\%$ ), while the number of otolith increments explained only 30 percent of the variation in fish length ( $R^2 = 30\%$ ; Fig. 3A). There was a similar relationship for the cumulative width measurements from scales and otoliths regressed upon the natural log of fish length. Both regressions were significant ( $p < 0.001$ ), but scale circulus widths explained a higher proportion ( $R^2 = 84\%$ ) of the variation in fish length than otolith increment widths ( $R^2 = 48\%$ ; Fig. 3B).

**DISCUSSION**

**Scale and Otolith Proportionality**

The purpose of this study was to compare scale and otolith microstructures for estimating growth from periodic and non-periodic hard part measurements. In general, the results of this study agree with previous findings in that the width measurements of periodic scale and otolith microstructures were significantly proportional to fish length (Weisberg 1993;

**Fig. 3.** Parametric regressions of (A), number of scale circuli and number of otolith increments on fish length, and (B), cumulative scale circuli and otolith increment width on fish length.



Fukuwaka 1998). In addition, non-periodic structures representing more recent growth (nearer the time of capture) were more highly correlated with each other, with other scale and otolith microstructures, and with fish length than were structures representing earlier growth (growth more distant from the time of capture).

The authors expected that periodic otolith microstructures (increments) would provide better proxies for fish growth than the periodic structures of scales (circuli) because the resolution of otolith increment deposition is higher (near daily) than that of scale circuli (one circulus forms every four to eight days). However, the widths and number of periodic otolith increments in this study were more poorly correlated with each other, with other otolith and scale microstructures and with fish length than were the widths and number of periodic scale circuli (Tables 3, 4, and 5). In particular, cumulative width measurements of scale circuli explained 84 percent of the variation in fish length ( $R^2 = 84$ ,  $p < 0.001$ ; Fig. 3A), while cumulative width measurements of otolith increments explained only 48 percent of the variation in fish length ( $R^2 = 48$ ,  $p < 0.001$ ; Fig. 3B).

On the one hand, the relatively strong relationships found here between periodic scale microstructures and fish length, may imply that somatic growth in pink salmon is more strongly related to periodic scale microstructures than to periodic otolith microstructures. Campana and Jones (1992) and Neilson (1992) have suggested that backcalculation from otolith increments can suffer from a number of logistical and theoretical constraints, including the uncoupling of somatic and otolith growth and the appearance of nondaily increments. The use of periodic boney structures as a proxy for fish growth are necessarily subject to the same constraints. For salmonids in particular, Wright et al. (1990) described the uncoupling of somatic and otolith growth in some Atlantic salmon (*Salmo salar*) parr. Bradford and Geen (1987) suggested that the conservative nature of otolith growth compared to fish growth in juvenile chinook salmon (*O. tshawytscha*) may preclude very detailed analyses of juvenile chinook growth rates based on otolith microstructure. If backcalculation is used, then there are also those additional problems encountered when backcalculating growth from any periodic boney structure (Campana 1990; Pierce et al. 1996).

On the other hand, the relatively weak proportionality of periodic otolith microstructures to fish length may have resulted from resolution-related effects (Neilson 1992). Scale circuli were spaced more widely apart (mean 47.1  $\mu\text{m}$ ; Table 3) than otolith increments (mean 2.92  $\mu\text{m}$ ; Table 4) and were more clearly defined; consequently, a lower magnification (40x) was required to resolve scale circuli, than to

discriminate between otolith increments (400x), and it was relatively less difficult to detect and measure scale circuli than otolith increments. With the scale digitizing macro used in this study, it was possible to repeatedly detect nearly the same number of circuli along a measurement axis (Courtney and Mortensen in press). With the otolith digitizing macro used in this study it was more difficult to consistently detect the same number of increments along a measurement axis (Courtney and Mortensen in press).

Furthermore, the wide spacing of scale circuli made it possible to view a whole image of a juvenile pink salmon scale at a resolution high enough to discriminate between scale circuli on a single video display. This resulted in the establishment of a consistent measurement axis from one scale to the next from which to extract periodic microstructure measurements.

In contrast, the higher magnification required to discriminate between otolith increments made it impossible to view a whole image of a pink salmon otolith at a high enough resolution to resolve between otolith increments on a single video display. Consequently, multiple high resolution images on the video display were required to extract otolith increment measurements from a single otolith and this made it more difficult to establish a consistent digitizing axis from one otolith to the next (Courtney and Mortensen in press). These results occurred despite the authors' use of the latest image analysis technology and their best efforts to develop an otolith digitizing protocol that was comparable with protocols already established for scale digitizing (Courtney 1997; Courtney and Mortensen in press).

### Scale and Otolith Periodicity

The assumptions underlying the use of periodic feature width measurements as a proxy for instantaneous growth rate are the same as those for general growth backcalculation: (a) the frequency of formation of the periodic feature is constant, and (b) the distance between consecutive features is proportional to fish growth (Campana and Jones 1992). For the purposes of this study it has been assumed that the formation of periodic otolith (increments) and scale (circuli) microstructures was constant, but this is a simplifying assumption at best, and needs further investigation. The daily periodicity of otolith increment formation has been documented in over 30 species (e.g., Campana and Neilson 1985). However, Volk et al. (1995) described the presence of indistinguishable daily and sub-daily increments at magnifications as high as 2000x in the sagitta otoliths of juvenile pink salmon and validated these findings with electron scanning microscopy. A magnification of 400x was used in this study in an attempt to coalesce

the sub-daily bands documented by Volk et al. (1995) into daily bands.

Campana (1992) suggests that 400x is an appropriate magnification for the examination of rapidly growing otoliths with width measurements between increments greater than or equal to one to two micrometers. The average width between otolith increments visible at 400x in this study was 2.9  $\mu\text{m}$  (Table 4). The validated periodicity of pink salmon otolith increment deposition (visible at 400x magnification) in this study was near daily when averaged over a one-month period (Table 1). However, the marked fish were only recovered from two sampling periods and average periodicity does not necessarily imply that the daily periodicity of increment formation was constant. Furthermore, the average periodicity reported here might be inaccurate because of the small sample size (a total of 15 thermally marked individuals with only 3 fish in the smallest sample group), and more individuals need to be examined.

The periodicity of scale circulus formation remains unclear. Scale formation in southeastern Alaska pink salmon is thought to occur as juvenile fish leave the near-shore marine environment. In British Columbia, juvenile pink salmon emigration from the near-shore environment occurs approximately 40 days after marine entry (Parker 1964, 1965, 1968), which is consistent with the number of otolith increments (43) found in this study for fish near scale formation (fish with 2 or 3 circuli; Table 1). In the coastal northern Pacific, juvenile pink salmon captured on the surface in seine and trawl nets between June 23 and September 10 during the years 1962–1965 averaged 60 to 80 mm fork length when scale formation began (Pearson 1966) which is also consistent with the lengths (mean length  $\geq 79.0$  mm) found in this study for fish near scale formation (Table 1). In the coastal northern Pacific, Pearson (1966) found that pink salmon scale formation began on or after June 25 and by September 10 averaged 17.4 circuli. That is, the population gained an average of 17.4 circuli in 77 days or approximately 1 circulus every 4.4 days. In this study, the average periodicity of scale circulus deposition, for pink salmon recovered from DIPAC, was 4.5 circuli over an average of 33.1 days, or one circulus every 7.4 days (Table 1). However, recovered DIPAC hatchery pink salmon, like wild pink salmon in southeastern Alaska, begin scale formation at an indeterminate time after marine entry and it is possible that the 16 hatchery fish recovered in this study did not begin scale formation at the same time.

## CONCLUSIONS

The results of this study agree with previous findings in that the width measurements of periodic

scale and otolith microstructures were significantly proportional ( $p < 0.001$ ) to fish length. As expected, non-periodic microstructures representing recent life history (near the time of capture) or that integrated all of a fish's life history were generally more strongly correlated to fish length than were microstructures representing earlier growth (life history more distant from the time of capture). An unexpected result was that the periodic structures of scales were more strongly related to fish length than were the periodic structures of otoliths. We had assumed that the higher resolution available from periodic otolith microstructures would provide for a stronger relationship between periodic otolith microstructures and fish length. This creates an apparent trade-off between the higher resolution available from periodic otolith microstructures, and the higher proportionality with fish length of periodic scale microstructures. This trade-off should be considered before choosing which structure to use as a proxy for recent growth. In addition, the assumption of constant periodicity in periodic pink salmon scale and otolith microstructures required to obtain these results needs further examination.

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## REFERENCES

- Bilton, H.T. 1985. Variation in scales sampled from different body areas of adult pink salmon. Can. Tech. Report Fish. Aquatic Sci., No. 1360.
- Bradford, M.J., and G.H. Geen. 1987. Size and growth of juvenile chinook salmon back-calculated from otolith growth increments. *In* The age and growth of fish. Edited by R. C. Summerfelt and G. E. Hall. The Iowa State University Press, Ames, Iowa. pp. 453–461
- Brown, R., and K.P. Severin. 1999. Elemental distribution within polymorphic inconnu (*Stenodus leucichthys*) otoliths is affected by crystal structure. *Can. J. Fish. Aquat. Sci.* 56: 1898–1903.
- Campana, S.E. 1990. How reliable are growth back-calculations based on otoliths? *Can. J. Fish. Aquatic Sci.* 47: 2219–2227.
- Campana, S.E. 1992. Measurement and interpretation of the microstructure of fish otoliths. *In* Otolith microstructure examination and analysis. Edited by D. K. Stevenson and S. E. Campana.

- Can. Sp. Pub. Fish. Aquat. Sci. No. 117: 59–71.
- Campana, S.E., and C.M. Jones. 1992. Analysis of otolith microstructure data. *In* Otolith microstructure examination and analysis. *Edited by* D.K. Stevenson and S.E. Campana. Can. Sp. Pub. Fish. Aquat. Sci. No. 117: 73–100.
- Campana, S.E., and J.D. Neilson. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquatic Sci.* 42: 1014–1032.
- Clutter, R.I., and L.E. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. *Int. Pac. Sal. Fish. Comm. Bull.* No. 9.
- Courtney, D.L. 1997. Variability in early marine scale growth of Southeast Alaska pink salmon and its implications for predicting abundance. Masters Thesis, University of Alaska, Fairbanks.
- Courtney, D.L., and D.G. Mortensen. In press. Automated measurements of juvenile pink salmon scales and otoliths. NOAA Technical Memorandum NMFS.
- Courtney, D.L., D.G. Mortensen, J.A. Orsi, and K.M. Munk. 2000. Origin of juvenile Pacific salmon recovered from coastal southeastern Alaska identified by otolith thermal marks and coded wire tags. *Fish. Res.* 46: 267–278.
- Farley, E.V., and K. Munk. 1997. Incidence of thermally marked pink and chum salmon in the coastal waters of the Gulf of Alaska. *Alaska Fish. Res. Bull.* 4: 181–187.
- Friedland, K.D., R.E. Haas, and T.F. Sheehan. 1996. Post-smolt growth, maturation, and survival of two stocks of Atlantic Salmon. *Fish. Bull. (U.S.)*, 94: 654–663.
- Fukuwaka, M. 1998. Scale and otolith patterns prove growth history of Pacific salmon. *N. Pac. Anadr. Fish. Comm. Bull.* No. 1: 190–198.
- Hagen, P., K. Munk, B. Van Alen, and B. White. 1995. Thermal mark technology for inseason fisheries management: a case study. *Alaska Fish. Res. Bull.* 2: 143–155.
- Healey, M.C. 1982. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. *Can. J. Fish. Aquat. Sci.* 39: 952–957.
- Mortensen, D.G., and M.G. Carls. 1995. Effects of crude oil ingestion on growth and microstructure of juvenile pink salmon (*Oncorhynchus gorbuscha*) otoliths. *In* Recent developments in fish otolith research. *Edited by* D. G. Secor, J. M. Dean, and S. E. Campana. Belle W. Baruch Library of Marine Science, No. 19: 197–209.
- Neilsen, J.D. 1992. Sources of error in otolith microstructure examination. *In* Otolith microstructure examination and analysis. *Edited by* D.K. Stevenson and S.E. Campana. Can. Sp. Pub. Fish. Aquat. Sci. No. 117: 115–126.
- Parker, R.R. 1964. Estimation of sea mortality rates for the 1960 brood-year pink salmon of Hook Nose Creek, British Columbia. *J. Fish. Res. Board Can.* 21: 1019–1035.
- Parker, R.R. 1965. Estimation of sea mortality rates for the 1961 brood-year pink salmon of the Bella Coola area, British Columbia. *J. Fish. Res. Board Can.* 22: 1523–1554.
- Parker, R.R. 1968. Marine mortality schedules of pink salmon of the Bella Coola River, central British Columbia. *J. Fish. Res. Board Can.* 25: 757–794.
- Pearson, R.E. 1966. Number of circuli and time of annulus formation on scales of pink salmon (*Oncorhynchus gorbuscha*). *J. Fish. Res. Board Can.* 23: 747–756.
- Pierce, C.L., J.B. Rasmussen, and W.C. Leggett. 1996. Back-calculation of fish length from scales: empirical comparison of proportional methods. *Trans. Am. Fish. Soc.* 125: 889–898.
- Secor, D.H., J.M. Dean, and E.H. Laban. 1992. Otolith removal and preparation for microstructure examination and analysis. *Edited by* D.K. Stevenson and S.E. Campana. Can. Sp. Pub. Fish. Aquat. Sci. No. 117: 19–57.
- Stevenson, D.K., and S.E. Campana [Editors]. 1992. Otolith microstructure examination and analysis. Can. Sp. Pub. Fish. Aquat. Sci. No. 117.
- Volk, E.C., D.G. Mortensen, and A.C. Wertheimer. 1995. Nondaily otolith increments and seasonal changes in growth of a pink salmon (*Oncorhynchus gorbuscha*) population in Auke Bay, Alaska. *In* Recent developments in fish otolith research. *Edited by* D.G. Secor, J.M. Dean, and S.E. Campana. Belle W. Baruch Library of Marine Science, No. 19: 211–226.
- Walker, R.V., K.W. Myers, and S. Ito. 1998. Growth studies from 1956–1995 collections of pink and chum salmon scales in the Central North Pacific Ocean. *N. Pac. Anadr. Fish. Comm. Bull.* No. 1: 54–65.
- Weisberg, S. 1993. Using hard-part increment data to estimate age and environmental effects. *Can. J. Fish. Aquat. Sci.* 50: 1229–1237.
- Wright, P.J., N.B. Metcalfe, and J.E. Thorpe. 1990. Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L: evidence against coupling. *J. Fish Biol.* 36: 241–249.
- Zar, J.H. 1984. Biostatistical analysis. 2nd edition. Prentice Hall, Englewood Cliffs, New Jersey.

