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ERRATA

Citations of Taubert and Cole (1977) should read:

Taubert and Coble (1977)

on pages 1, 2, 11, 15, 17, and 18.

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CHIGNIK SOCKEYE STUDIES:

DAILY GROWTH PATTERNS OF SOCKEYE SALMON OTOLITHS

by

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Final Report (supplement)

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ABSTRACT

Experiments in which sockeye salmon (*Oncorhynchus nerka*) were reared showed that daily rings are produced on the otoliths of fry fed *ad libitum* at temperatures higher than approximately 5°C. Subdaily rings were present on some otoliths, but they are faint and this distinguishes them from daily rings. Weekly patterns were also identified.

Significant linear relationships between the radius of the otoliths and the fork length of the fish were found which were affected by brief periods of starvation.

INTRODUCTION

Age determination of adult sockeye salmon (*Oncorhynchus nerka*) from the Chignik lakes, Alaska, based on the seasonal growth patterns of scales, has proven difficult (Burgner and Marshall 1974; Marshall and Burgner 1977). The problem of interpretation is restricted to the freshwater zone. Scales of Chignik sockeye salmon may contain a weak first annulus (Roos 1960;[1] Narver 1966; Marshall 1977) and/or a smolt migration check which may be either a true or false annulus (Koo 1962; Mosher 1968; Marshall 1977). These patterns often lead to uncertain interpretations and result in considerable variability in age readings between biologists and in multiple age readings by the same biologist (Marshall and Burgner 1977). The use of otoliths[2] to resolve discrepancies has provided little relief because weak, poorly defined freshwater annuli are often present on these structures as well (Burgner and Marshall 1974).

Reports by Pannella (1971, 1974), Brothers et al. (1976), Taubert and Cole (1977), and Struhsaker and Uchiyama (1976) show that the otoliths of many fish grow in diurnal cycles which result in the formation of a concentric ring pattern. The number of rings is a function

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[1] Roos, John F. 1960. Life history of red salmon (*Oncorhynchus nerka* (Walbaum)) at Chignik, Alaska. Univ. Washington Fish. Res. Inst. 56 pp. [Unpublished manuscript.]

[2] Otolith refers to the sagittae, the largest of the three otoliths.

of the age in days, except that under simulated winter conditions, ring formation ceases and an annulus is formed when growth resumes (Taubert and Cole 1977). We believed that if daily rings are present on sockeye salmon otoliths and the factors governing their formation could be elucidated, then a method for validating age readings from seasonal patterns evident on scales could be developed.

In this paper we report on the periodicity of ring formation on sockeye salmon otoliths under conditions where temperature and diet varied separately and together.

METHODS AND MATERIALS

Experimental Design

Sockeye salmon eggs of the Lake Washington stock were incubated in drip trays at the University of Washington's fish hatchery. Eggs were dissected during the incubation period to determine when otoliths first formed. Alevins hatching within a 24-hr period were isolated into separate trays. At the swim-up stage four experimental groups, each containing approximately 200 fish which hatched on the same day, were placed in 55-gal rearing barrels. All groups were fed regularly for 3 weeks. Feces and/or surplus food were removed regularly.

Four experimental lots were defined as follows: 1) ambient-fed (AF) fish held at ambient water temperature and fed to excess; 2) ambient-starved (AS) held at ambient temperature but starved for 2 weeks; 3) cool-fed (CF) held at reduced water temperature for 3 weeks and fed to excess; and 4) cool-starved (CS) held at reduced water temperature without food for 3 weeks. The AF group served as a control. After an exposure to reduced water temperature and/or starvation the environment in each tank was returned to the ambient-fed state. Samples of 20 fish were taken from each lot at the beginning and end of the experiment, prior to starvation, when food was returned, and periodically as water temperature varied.

Sample Preparation

Sacrificed fish were frozen and later measured for length (TS-FT) and weight. Both sagittae were removed and affixed to a microscope slide with Canada balsam. Care was taken to ensure that the medial surface was down, and that the plane of the lateral surface was horizontal to the glass slide.

Otoliths were ground to the maximum diameter of the sagittal plane on 600 grit wet sandpaper, etched for 5-10 seconds with 10% HCl, thoroughly rinsed with water, and set aside to dry. A gummed notebook paper reinforcer was affixed to the slide around each otolith pair. Euparal® was dripped into each of these shallow wells and the wells were sealed with a glass cover slip. This method is similar to that of Ralston (1974). The procedure from removing the sagittae to preparation for viewing took about 10 min per pair of otoliths.

We found that one to several broad bands are laid down on the otoliths at the time of hatching and we used the outermost of these hatch marks as a reference point to make ring counts and radial measurements. This pattern is discussed in greater detail in the Results section. Counts of the concentric rings were usually made in the posterior-dorsal quadrant and it was necessary to focus the microscope up and down through the otolith in order to observe all rings present. Two counts were made from the most readable of the two otoliths; we used their mean value in subsequent analysis. Measurements of the otolith radius were made from the outer edge of the hatch ring to the margin in the posterior-dorsal quadrant about 20° from the anterior-posterior axis. Measurements were made with an ocular micrometer at 200 x.

RESULTS

Growth

The mean lengths, weights, and linear growth rates between sampling dates are provided in Table 1 and illustrated in Figs. 1 and 2. There were no significant differences in the length ($P = 0.24$) or weight ($P = 0.64$) of the four groups at the outset of the experiment. The control group (AF) exhibited approximately linear growth in length and exponential growth in weight during the test. Fish fed but held briefly at reduced water temperatures (CF) showed a significant slowing of growth in terms of both length and weight during the period of reduced water temperature, but growth accelerated immediately following this period. Fish starved for 2 weeks (AS) exhibited continued but very reduced growth in length during the period of starvation, and weight remained constant. Following the period of starvation, growth in weight was more evident than was growth in length. Fish held at reduced temperatures and starved (CS) also showed very reduced but continued growth in length while weight remained constant. Following the period of starvation and reduced temperatures, body growth also accelerated in this group.

Table 1. Mean lengths, weights, and linear daily growth rates.

Group	Julian date (D)	Mean length (L)	Change in mean length (Δ L)	Δ L Day	Mean weight (W)	Change in mean weight (Δ W)	Δ W Day
Ambient-fed	93	30.7			0.22		
			2.7	0.39		.11	0.016
	100	33.4			0.33		
			3.3	0.24		.18	0.014
	114	36.7			0.51		
			5.7	0.52		0.27	0.025
	125	42.4			0.78		
			3.6	0.51		0.42	0.060
	132	46.0			1.20		
Ambient-starved	93	30.0			0.22		
			2.4	0.34		0.09	0.013
	100	32.4			0.31		
			1.6	0.11		weight constant	
	114	34.0			0.31		
			1.1	0.10		0.08	0.007
	125	35.1			0.39		
			1.0	0.33		0.06	0.020
	132	36.1			0.45		
Cool-fed	93	31.1			0.24		
			1.5	0.24		.07	.010
	100	32.6			0.31		
			3.0	0.214		.12	.009
	114	35.6			0.43		
			2.0	0.143		.05	.007
	121	37.6			0.48		
			0.5	0.125		.02	.005
	125	38.1			0.50		
			2.2	0.314		.20	.029
	132	40.3			0.70		
Cool-starved	93	30.6			0.22		
			2.9	0.41		0.10	0.006
	100	33.5			0.32		
			0.8	0.06			
	114	34.3			0.31		
			0.3	0.04		weight constant	
	121	34.6			0.32		
			0.1	0.03		0.01	0.003
	125	34.7			0.33		
			1.7	0.24		0.13	0.019
	132	36.4			0.46		

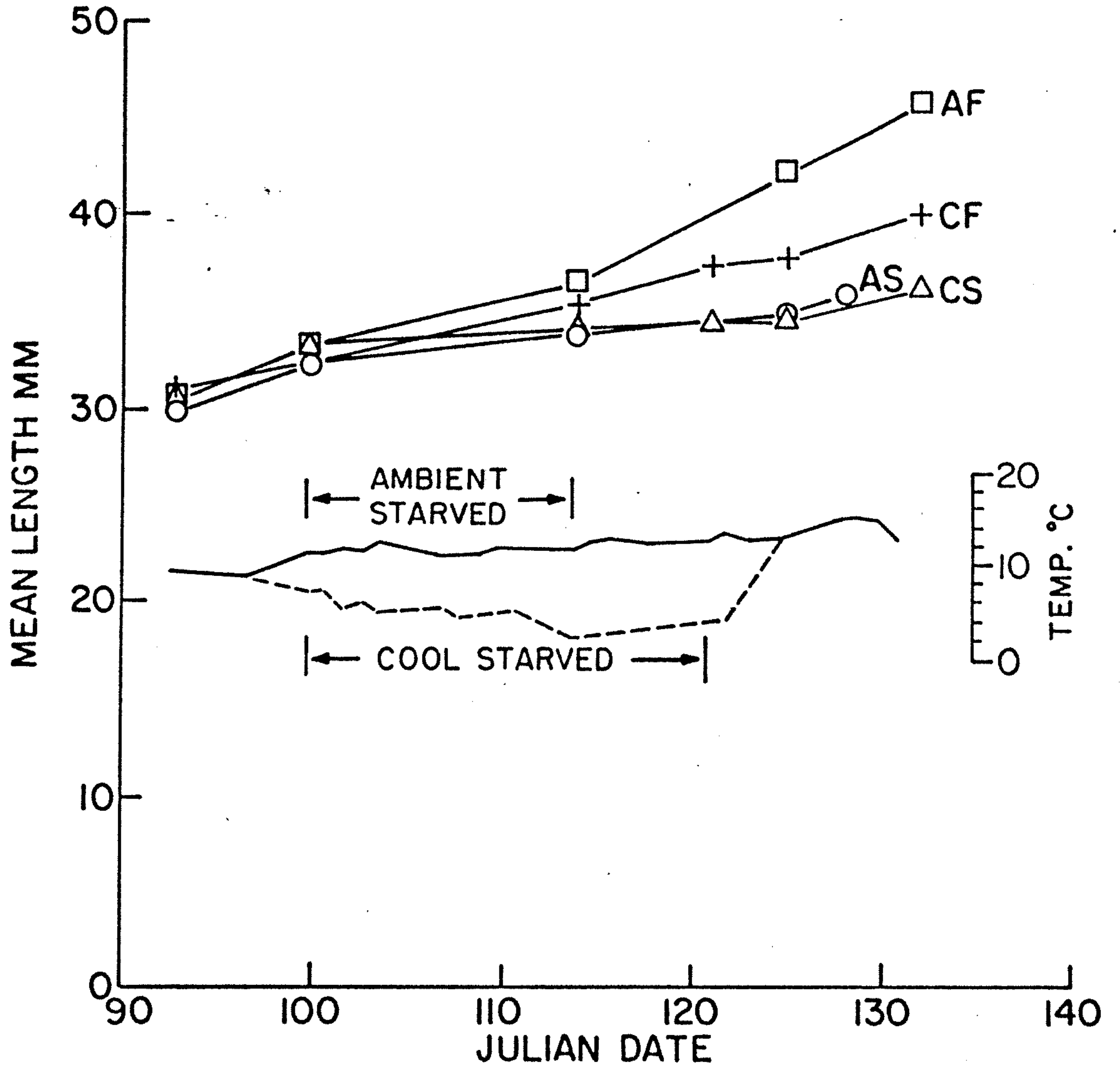

Fig. 1. Growth trajectories of experimental groups expressed as lengths.

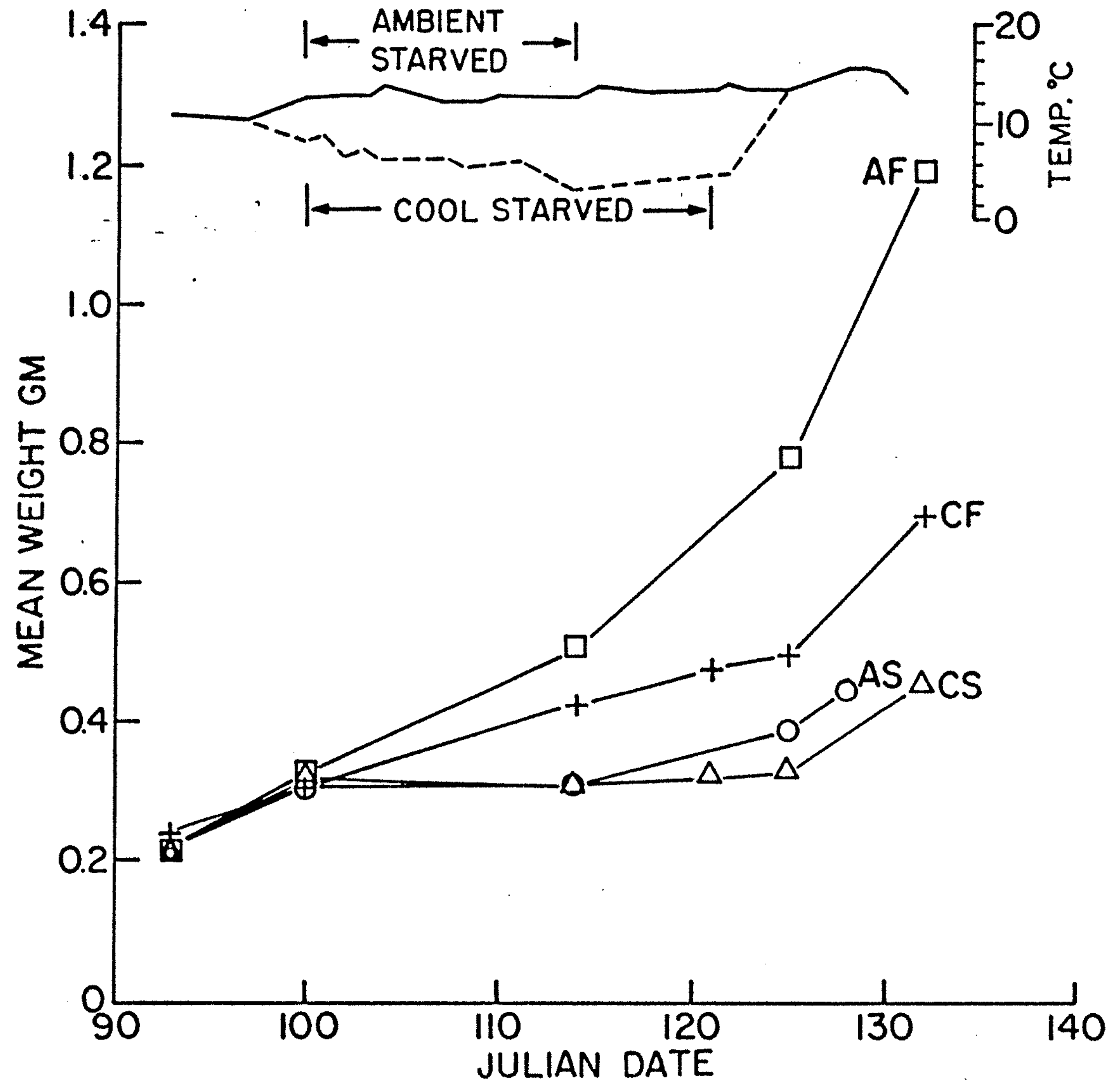

Fig. 2. Growth trajectories of experimental groups expressed as weights.

Daily Patterns

Periodic dissection of eggs during the period of incubation produced otoliths from embryos 12 days before the first eggs hatched, and a concentric ring pattern was apparent on the otoliths at that time. Otoliths from alevins that had recently hatched showed one to several broad bands encompassing the prehatch rings (Fig. 3).

The mean number of rings counted on otoliths from the control group (AF) was highly correlated with the number of days since hatching (Fig. 4). A regression of count data on age showed that the slope was not significantly different from 1 ($P < 0.001$) and that the intercept was not significantly different from 0 ($P < 0.001$). The mean number of rings counted on otoliths from the AS group was also highly correlated with number of days since hatching. Regression analysis of the count data on age also showed that the slope was not significantly different from 1 ($P < 0.001$) and that the intercept was not significantly different from 0 ($P < .05$).

Ring counts were highly correlated with age on samples collected from the CF group until water temperatures fell to 4°C on day 114 (age = 75 days). The decrease in mean ring count from 74.4 ± 0.5 to 73.8 ± 1.4 from day 114 to day 121 (age 75 and 82 respectively) was not significant; likewise the increase to 76.5 ± 2.2 on day 125 (age = 86) was not significant. The mean count of 83.9 ± 2.2 on day 132 at age 93 clearly shows a resumption of ring formation. The difference of -9.1 days between the mean ring count and true age corresponded with a 9-day period when water temperatures were below 5°C.

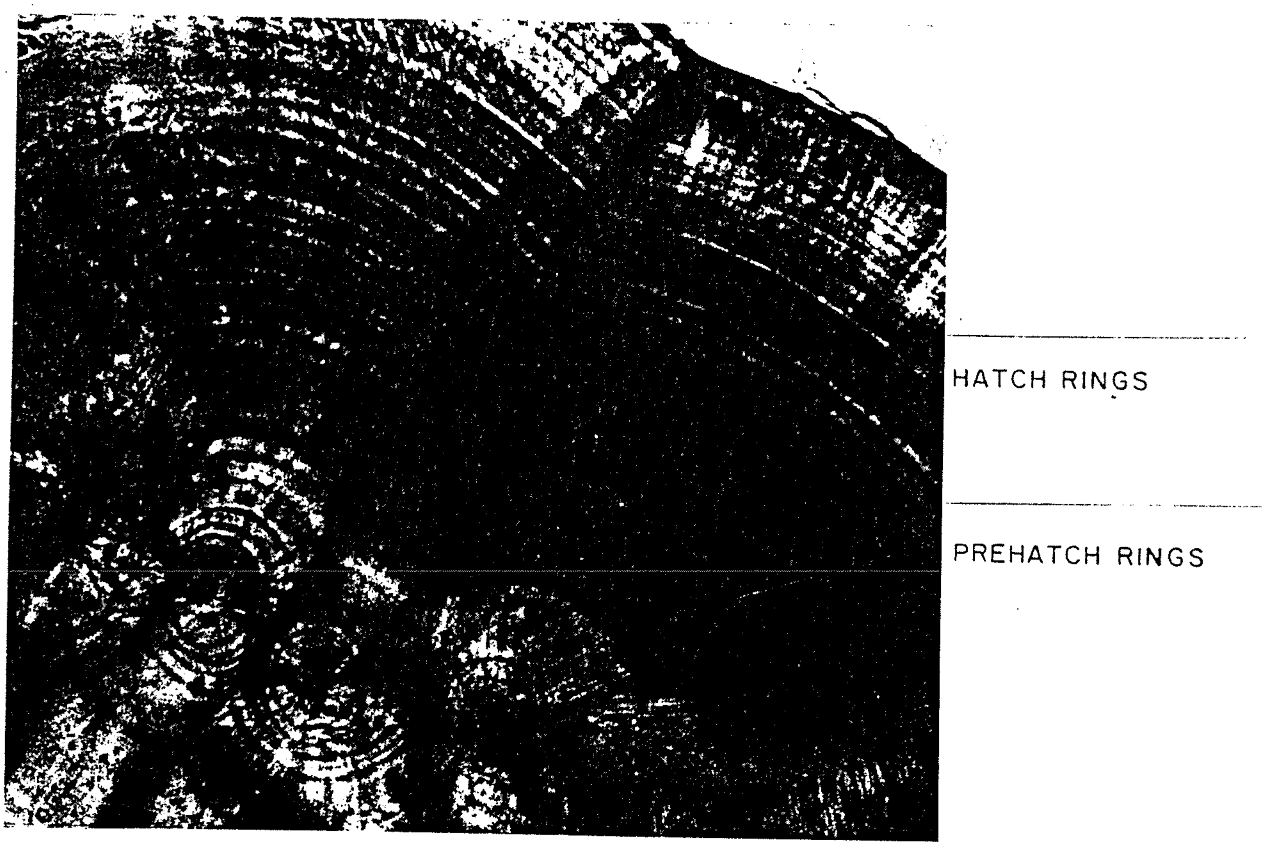

Fig. 3. Prehatch and hatch rings on the otolith of a 34-day-old sockeye.

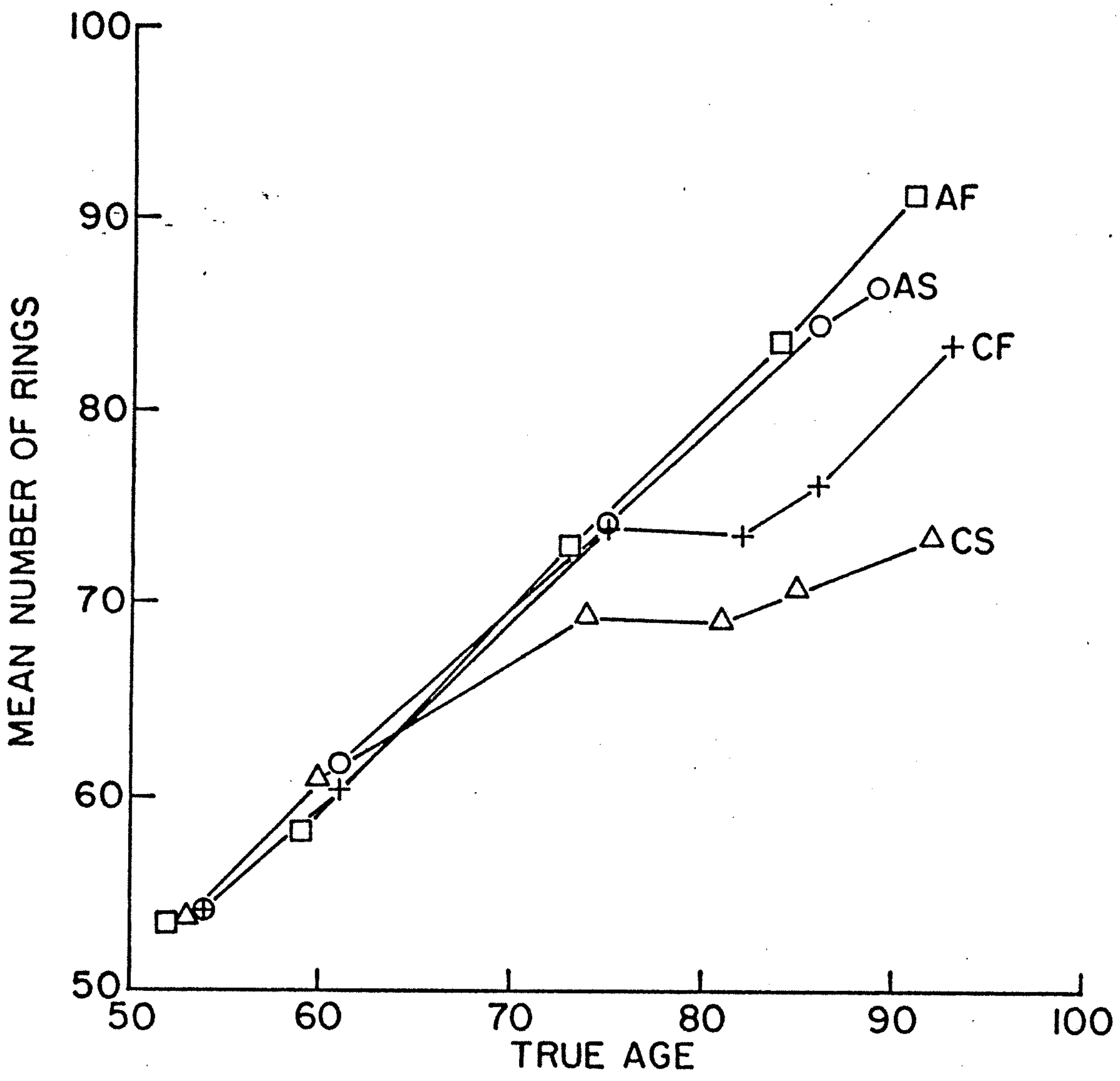

Fig. 4. Mean numbers of rings as a function of age in days post-hatching, by experimental group.

Mean ring counts were highly correlated with age on the CS group through day 100 (age = 60) at which time the period of starvation began and water temperatures began to drop. On day 114 the mean ring count of 69.5 ± 0.6 was 4.4 less than the true age of 74. Samples collected at the end of the period of starvation on day 121 (age = 81) showed a difference of -11.7 between mean ring count and true age. Subtracting a previously noted difference of 4.4 days between true age and ring count on day 114 the remaining difference of -7.3 days corresponded very closely with the 7 days between the dates the two samples were collected indicating that no rings were formed during the period. On day 125, 4 days after the period of starvation had ended and water temperatures were being elevated, an increase, though not significant, in ring count was noted. On day 132, the difference between mean ring count and true age was -18.2. This represents an increase in the difference between mean ring counts and age on samples collected both at the end of the period of starvation (-11.7) and on samples taken 4 days following the period of starvation (-13.9). It appears that ring formation did not begin in this group after control conditions were resumed.

Other Periodic Patterns

Occasionally, the number of rings counted was approximately twice the true age of the fish. The photograph in Fig. 5 is from a 13-day-old sockeye and 26 rings may be counted. We agree with Taubert and Cole (1977) that it is generally possible to distinguish such patterns because subdaily rings are poorly defined; the difference may be seen by comparing Fig. 5 with Fig. 6.

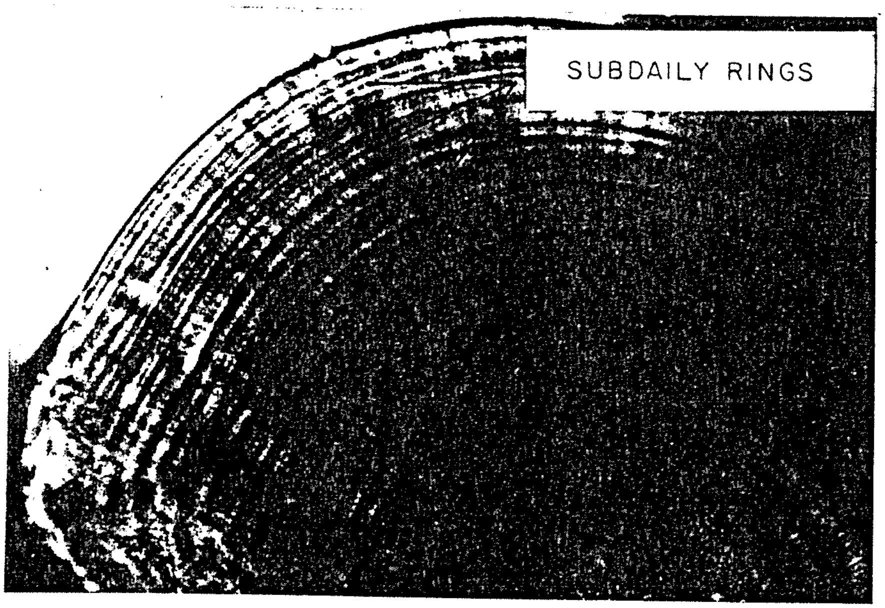

Fig. 5. Subdaily rings on the otolith of a 13-day-old sockeye.

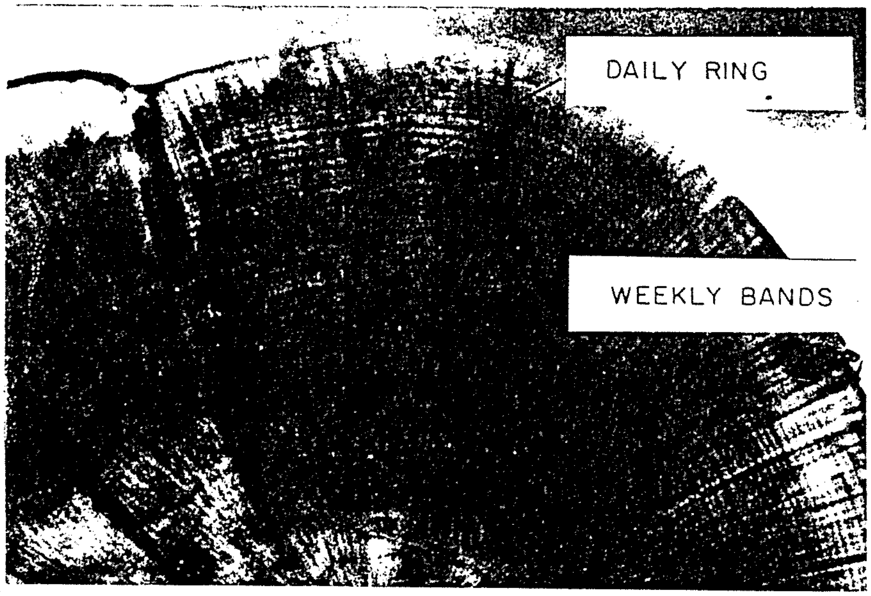

Fig. 6. Daily rings and weekly bands on the otolith of a 59-day-old sockeye.

Broad bands were observed on most otoliths and their periodicity closely coincides with weekly intervals (Fig. 6). In some otoliths this pattern was made conspicuous by a cyclic narrowing in the spacing between adjoining daily rings.

Relationship of Otolith Radius and Body Length

In Fig. 7 we plot the mean otolith radius versus mean length for each of the experimental groups. We fitted functional regression lines (Ricker 1973) to the data of each group. Our estimated 95% confidence intervals for the slopes of each of these lines indicated that: 1) slopes calculated for groups AF and CF were not significantly different; 2) slopes calculated for groups CS and AS were not significantly different; and 3) there was a significant difference in slopes between those fed continuously (AF and CF) and those briefly starved (AS and CS).

DISCUSSION

Effects of Temperature and Starvation on Ring Counts

Ring counts from otoliths of the AF group clearly demonstrate a daily periodicity for sockeye salmon fed *ad libitum* at temperatures in the range 10-12°C.

A 2-week period of starvation at water temperatures in the range 10-12°C did not halt ring production. During this period of starvation, stored energy reserves were apparently used to sustain hard tissue growth, since fork length and otolith radius increased while weight remained constant during this period. Because ring production continued in the absence of food, ring periodicity is not a function of foraging periodicity. It may, however, be related to the existence

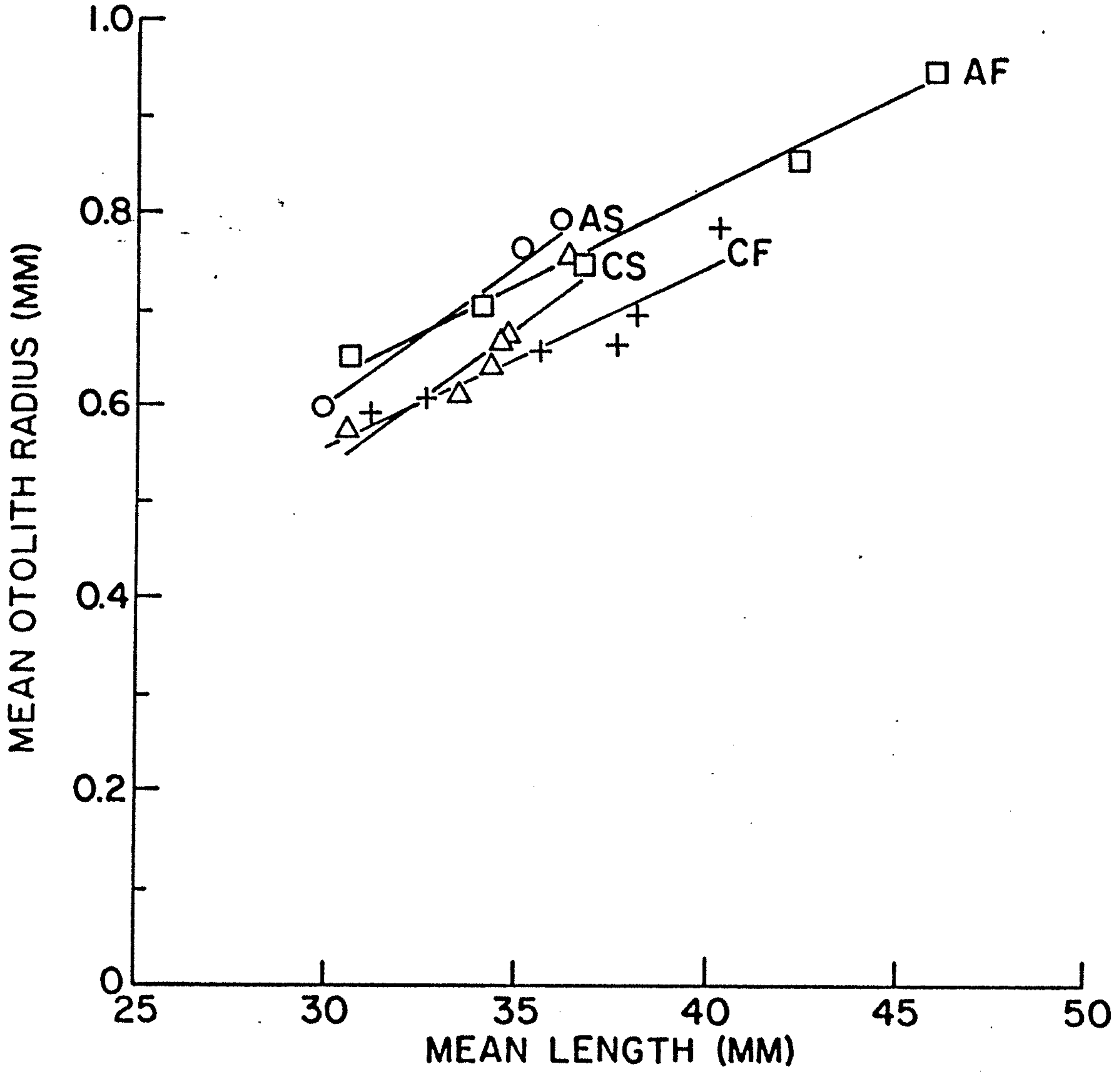

Fig. 7. Functional relationship between mean otolith radius and mean length, by experimental group.

of an internal diurnal "clock" entrained by a 24-hr light-dark cycle as reported by Taubert and Cole (1977) for the Mozambique mouthbrooder (*Tilapia mossambica*). These considerations led us to hypothesize that ring production would cease in sockeye starved at near optimal temperatures when body reserves were exhausted.

While ring production appears to be directed by an internal diurnal "clock" (Taubert and Cole 1977), its expression in sockeye salmon requires temperatures above about 5°C. This conclusion stems from the cessation of ring production below this temperature and the continued growth in both length and weight that was observed for the CF group. Taubert and Cole (1977) believed that cessation of daily ring formation in bluegill (*Lepomis macrochirus*) was a result of cessation of fish growth. Our data show that for sockeye salmon this is not the case.

A compounding effect of reduced water temperatures and starvation was evident for the CS group. It is unclear to us why daily ring production did not resume in this group when water temperatures were elevated and feeding was resumed.

Liew (1974) and Taubert and Cole (1977) characterized an annulus as a deeply etched groove or band following the contour of the otolith. In Fig. 8 we show a freshwater annulus on an otolith from a sockeye salmon. We did not see such marks on the otoliths from either the CF or CS groups, and believe that an annulus did not form because of the short duration of the period of reduced water temperatures.

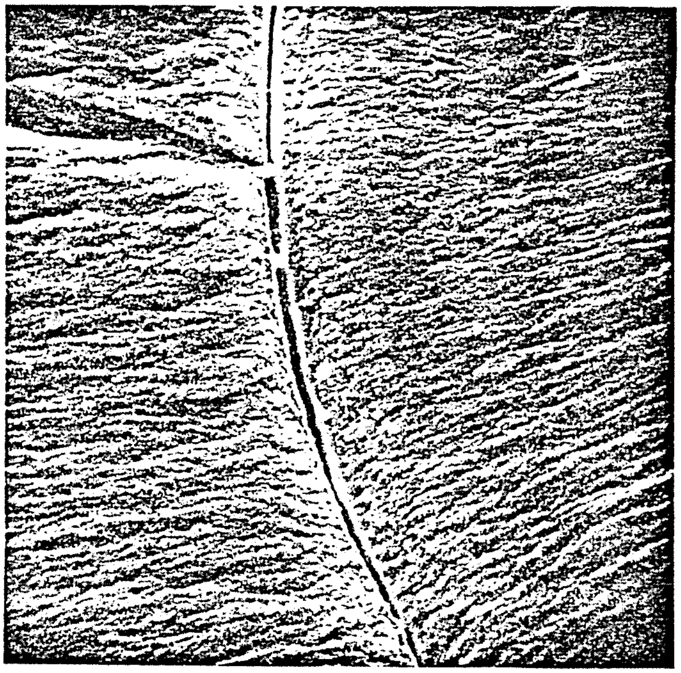

Fig. 8. Scanning electron micrograph of a freshwater annulus on the otolith from a mature sockeye salmon (2050 x).

Otolith Radius - Body Length Allometry

Our data show that the allometry of otolith radius to body length is subject to environmental conditions. It is our hypothesis that the increase in slope for starved fish relative to fed fish represents only a short-term response to starvation. Long-term rearing experiments will be required to clarify this question. It is becoming apparent, however, that such relationships may be used in conjunction with daily ring counts to reconstruct detailed growth histories of individual fish (Struhsaker and Uchiyama 1976; Taubert and Cole 1977) and the uses of such a tool in ecological research are clearly far-reaching.

We have shown that sockeye salmon fry experiencing sustained growth above 5°C may be aged on a daily basis. This information coupled with the identification of growth checks and reasonably precise estimates of the average growing season will allow interpretation of such checks as either true or false annuli. Such results will then facilitate proper interpretation of ambiguous scale patterns.

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