OBSERVATIONS ON SCALE PATTERNS AND GROWTH OF THE PACIFIC SARDINE REARED IN THE LABORATORY

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ABSTRACT

Scale patterns and growth of Pacific sardine (*Sardinops caerulea*) were studied with laboratory-reared fish held for 24 months. All scales examined after the fourth month had accessory marks. The first accessory mark was formed in August to October and the second accessory mark, in May to August. The accessory marks were indistinguishable from annuli that formed in November to March.

Five possible causal factors of mark formation were investigated—temperature, salinity, gonad index, condition factor, and relative growth rate. Growth rate showed the best correlation. The accessory mark was formed during a period of change in growth rate and the annulus during a period of relatively constant growth rate.

An estimate of the body length-scale radius relation indicated that scales first increase in size at a body length of 33 mm.

Growth in length was rapid from the start of the experiment to the fourth month, after which the increase was gradual. The average instantaneous rate of growth was about 0.47/month during the first 4 months and about 0.03/month thereafter.

It was concluded that the abrupt increase in L_1 that was recorded in the 1940's for the sardine population was probably caused by errors in aging, owing to a change in scale readers and scale-reading criteria. The ages of fish age II and older were probably underestimated by the recent scale readers.

In the course of aging Pacific sardine (*Sardinops caerulea*) from scales, the senior author observed, from comparison of back-calculated lengths with growth curves, that accessory marks or false annuli occurred frequently and were easily mistaken for true annuli. This was contrary to the findings of Walford and Mosher (1943:8-9), who reported that the annulus was "present on all normal scales of an individual" whereas an accessory mark was only rarely present on "all the scales of an individual." Since misidentifying an accessory mark as an annulus can affect the estimated age of a fish, a laboratory experiment was conducted to determine (1) the frequency of occurrence of accessory marks, (2) the time of accessory mark formation, (3) possible factors that may cause accessory mark formation, (4) the time of annulus formation, and (5) the seasonal pattern of sardine growth. The experiment was initiated in May and was terminated approximately 24 months later. Results from the first 12 months of the experiment were reported by Kimura (1970), who showed that an accessory mark was present on scales of 4.5- to 5.0-month-old fish. This report is a more comprehensive presentation of results from the entire experiment.

METHODS

REARING

The collection of sardine eggs and the hatching and rearing of the young in the laboratory for the first 12 months were described by Kimura (1970). The general procedures, including those used after the 12th month, are briefly reviewed as follows. In May 1968, sardine larvae were hatched from eggs collected in plankton tows off San Diego, Calif., and the larvae were held in a

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polyethylene bag, which was suspended in a 4.6-m diameter pool (13.2 kliter). The pool also contained about 15 northern anchovy (*Engraulis mordax*) larvae. A 1,500-w mercury lamp that was suspended about 1 m above the bag provided illumination and a source of heat. The larvae were fed daily with plankton collected in Mission Bay, Calif., and live *Artemia*. On the 29th day the larvae were released into the pool. Feeding continued with plankton and live *Artemia* up to the 45th day, after which frozen *Artemia* and dry trout food were substituted. The daily ration was about 10% of the estimated total weight of fish alive on a given day, based on the average weight of fish sampled in the previous month.

On the 31st day the density of sardine larvae was noticeably reduced. Since few dead larvae were found during the previous days, the anchovies (average length 66 mm) were suspected of feeding on the sardines (average length 24 mm). Analysis of stomach contents of three anchovies revealed that they were indeed feeding on the sardines.[2]

During the first few months of the experiment, water was added to the pool only periodically to remove surface scum. But beginning on the 61st day, seawater was circulated continuously through the pool at an initial rate of about 19 liter/min. This rate was increased on the 180th day to about 34 liter/min.

SAMPLING

Fish were sampled and sacrificed at various intervals during most of the duration of the experiment. About 7-17 larvae were sampled daily from the polyethylene bag during the first month. During the second month 6-26 larvae were sampled primarily on a weekly basis. Starting with the third month and continuing through the 12th month, about 20-24 fish/month were sampled. After the 12th month, the sampling rate was reduced to 11-14 fish/month until an unusually large mortality from unknown causes reduced the population to 12 fish in the 16th month. Two more samples were taken thereafter; in one sample four fish were measured for length and returned to the pool. After the 24th month, the seven surviving fish were sampled and the experiment was terminated.

Standard length, total weight, gonad weight, and six scales removed from the body area at the tip of the pectoral fin (see Walford and Mosher, 1943: 4) were obtained from most samples.

Water temperature was recorded daily on a thermograph. The monthly mean temperature ranged from 15.3° to 25.0°C and was generally 2.9°C higher than the monthly mean surface temperature recorded off the Scripps pier, La Jolla (Kimura, 1970), site of the water intake for the experimental pool[3] (see Lasker and Vlymen, 1969).

Salinity measurements were made by daily titrations from surface water sampled off Scripps pier. The mean values ranged from 33.09 to 34.38‰. It was assumed that water salinity was the same off the Scripps pier and in the experimental pool.

SCALE MEASUREMENTS

Scales were placed between two glass slides and viewed on a scale projector that magnified them 30 times. All marks that appeared to be annuli were recorded and the widths from focus to mark and focus to margin in the anterior field were measured.

ANALYSIS

Gonad weight was expressed as a percentage of total weight. This was designated a gonad index, or relative measure of sexual development.

The relative "fatness" of a fish was estimated

[2] To our knowledge there have not been previous reports on predation of sardine larvae by anchovies. The significance of our observation is that predation by anchovies may have contributed to the decline of the Pacific sardine population. Our anchovies were hatched from eggs and were 44 days older than the sardines. This difference in age (size) between the species is also found in the wild; the northern anchovy has a peak spawning period during January-March and the sardine, during April-June (Ahlstrom, 1966). The young of both species coexist in the California Current; thus it is conceivable that predation by anchovies is an important source of sardine natural mortality.

[3] The experimental pool was located at the Southwest Fisheries Center, about 1 km from the Scripps pier.

TABLE 1.—Results of aging of 205 known age, laboratory-reared sardines from scales.

Age (months)	Total number of fish	Total number of marks	Actual		Estimated				Percent disagreement
			Number with:		Number with:				
			0 mark	1 mark	0 mark	1 mark	2 marks	3 marks	
5	20	1	20	0	20	0	0	0	0
6	20	1	20	0	20	0	0	0	0
10	15	2	0	15	0	12	3	0	20.0
11	21	2	0	21	0	10	11	0	52.4
12	24	2	0	24	0	18	6	0	25.0
13	11	3	0	11	0	3	8	0	72.7
14	12	3	0	12	0	2	6	4	83.3
15	14	3	0	14	0	0	12	2	100.0
16	64	3	0	64	0	4	50	10	93.7
18	4	3	0	4	0	0	3	1	100.0

TABLE 2.—Frequency and percent (in parentheses) of Pacific sardines with various number of scale marks, as determined from scale reading. (Sardines were reared in the laboratory for 24 months.)

Age (months)	Number of fish	No mark	Accessory mark I	Annulus I	Accessory mark II	Annulus II
1	3	3 (100)				
2	8	8 (100)				
3	17	17 (100)				
4	9	4 (44)	5 (56)			
5	20		20 (100)			
6	20		20 (100)			
7	20		20 (100)	10 (50)		
8	20		20 (100)	13 (65)		
9	20		20 (100)	18 (90)		
10	20		20 (100)	20 (100)		
11	21		21 (100)	21 (100)		
12	24		24 (100)	24 (100)		
13	11		11 (100)	11 (100)	4 (40)	
14	12		12 (100)	12 (100)	6 (60)	
15	14		14 (100)	14 (100)	10 (100)	
16	64		64 (100)	64 (100)	64 (100)	
18	4		4 (100)	4 (100)	4 (100)	
24	7		7 (100)	7 (100)	7 (100)	7 (100)

by a condition factor (MacGregor, 1959). The factor (K) is calculated as a ratio of the weight in grams (W) to the cubic power of length in millimeters (L): $K = (W/L^3) \times 10^7$.

Back-calculated lengths from scale measurements were derived by two methods. Method one was by direct proportion, $L_m = (s_m/s_r)L$, where L_m = back-calculated length at time of mark (m) formation, s_m = scale width from focus to mark m, s_r = scale width from focus to margin, and L = length at sampling. Method two was also by direct proportion but with a correction factor (c) for body length when scales first increase in size in the Pacific sardine. The method uses the equation $L_m = c + (s_m/s_r)(L - c)$.

ACCESSORY MARKS AND ANNULI

FREQUENCY OF OCCURRENCE

An annulus is generally believed to form at annual intervals, usually as the result of a slowing down in growth such as occurs in winter for temperate species. An accessory mark, on the other hand, is believed to occur, if at all, at irregular intervals (Walford and Moser, 1943), the causes of which are unknown.

We examined the scales of the laboratory-reared fish and were unable to distinguish between the two types of marks. To test if other scale readers similarly could not distinguish accessory marks from annuli as we did, a series of scales from the laboratory-reared fish was mixed with scales collected from wild fish and three experienced scale readers read the scales (Table 1). The percent error was as large as 100%, which indicates that accessory marks can indeed be easily misidentified as annuli.

TIME OF FORMATION

As many as four marks were observed on some scales. The percentage of fish with various numbers of marks was tabulated for each sample (Table 2). The results show that the first mark was formed from August to October (4-5 months old), the second from November to March (6-10 months old), the third from May to August (13-15 months old), and the fourth sometime after November (18 months old) but probably before January (20 months old). This January date for the fourth mark was deduced from two scales collected in late December from the bottom of the pool, but the data are not given in Table 2. The two scales had four distinct marks, but it is not known whether the scales were from one or two fish. The bottom of the pool was cleaned daily.

Based on the above criteria of an annulus and accessory mark, the second and fourth marks are annuli, and the first and third marks are accessory marks. The interval between annuli was about 12 months, and that between accessory marks was only 9 months.

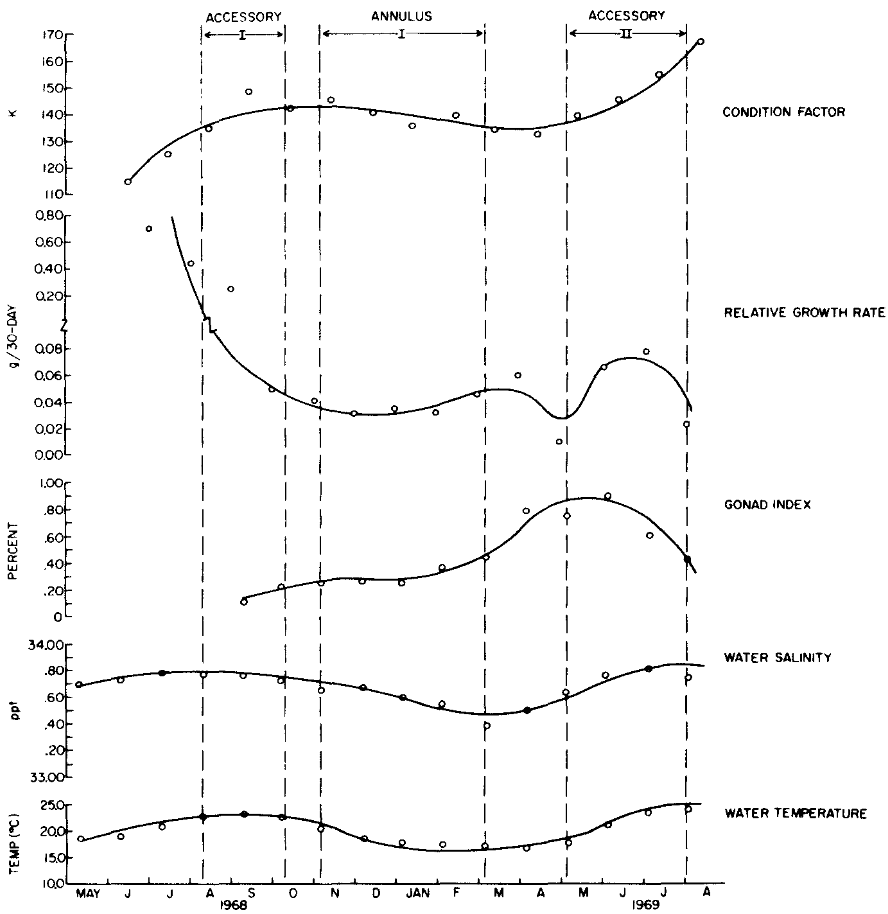

FIGURE 1.—Four possible factors that may be associated with mark formation on scales of Pacific sardines. Periods of mark formation are delineated by vertical lines.

POSSIBLE FACTORS AFFECTING MARK FORMATION

Water temperature and salinity, gonad index, condition factor, and growth rate were analyzed to determine whether they affected mark formation (Figure 1). Growth rate appeared to be best correlated with mark formation.

Kimura (1970) used monthly growth increments[4] for the first year to show that formation of the accessory mark was associated with maximum growth, and formation of the annulus was associated with the onset of rapid growth. His choice of growth increments for his analysis was not ideal because the magnitude of an increment is dependent on the size of the fish. We therefore chose to use the instantaneous rate of growth, $g = (\ln L_t - \ln L_0)/(t - t_0)$, Ricker (1958) on a 30-day basis to analyze the data from the entire experiment. The results (Figure 1) show that the accessory marks formed during periods of change in growth rate, whereas the annulus formed during a period of relatively constant growth rate.

GROWTH

BODY LENGTH-SCALE RADIUS RELATION

A body length-scale radius relation was fitted by least squares to data from 283 fish. A straight line of the form $Y = 32.856 + 9.030X$, where Y = standard length and X = scale radius, was calculated (Figure 2). The intercept of the line, or 32.856, is an estimated body length when scales first increase in size in the Pacific sardine. This estimate is probably too high, because scales with several circuli were observed on 26- to 30-mm long fish.

Landa (1953) reported 12 positive intercept values (68-191 mm) and 1 negative value (—102 mm) for body length-scale radius relations of fish caught by the commercial fishery in the 1940's. Compared to our estimate, his estimates are considerably larger. One reason for the difference is Landa used data from large fish (mean lengths of 186-228 mm), whereas we used data from small fish (mean length of 115 mm). This suggests that a relation over the entire size range of sardines may be nonlinear, although it is linear over a short segment of the curve. The parameters of a linear relation could hence vary, depending on the segment of the curve being examined.

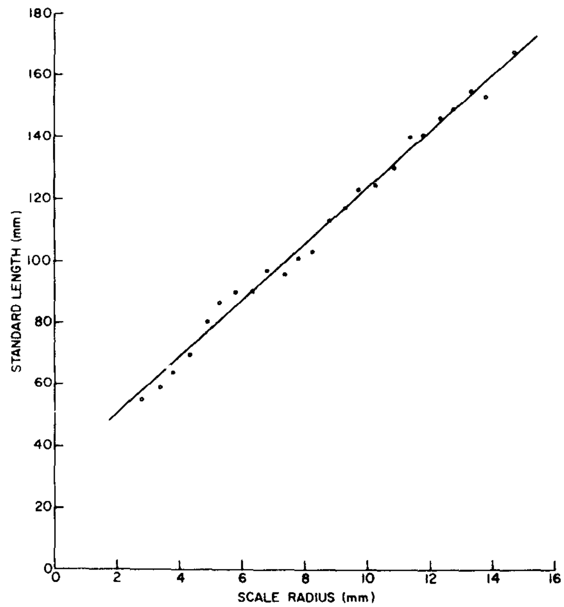

FIGURE 2.—Body length-scale radius relation for 283 laboratory-reared Pacific sardines.

WEIGHT-LENGTH RELATION

Data from 326 fish were used to estimate the weight-length relation. The relation (Figure 3) appears to underestimate the average weight of fish greater than 135 mm long. This is probably because the weight-length relation was based on data from individual fish whereas the data points in Figure 3 represent average weights for 5-mm groupings of lengths.

Clark (1928) estimated a weight-length relation for sardines landed at San Pedro, Calif., in the 1920's. Her estimate was compared to ours

[4] In Kimura's Figure 3 the notations for weight and length increments are mislabeled. The increments are not percentages but absolute values.

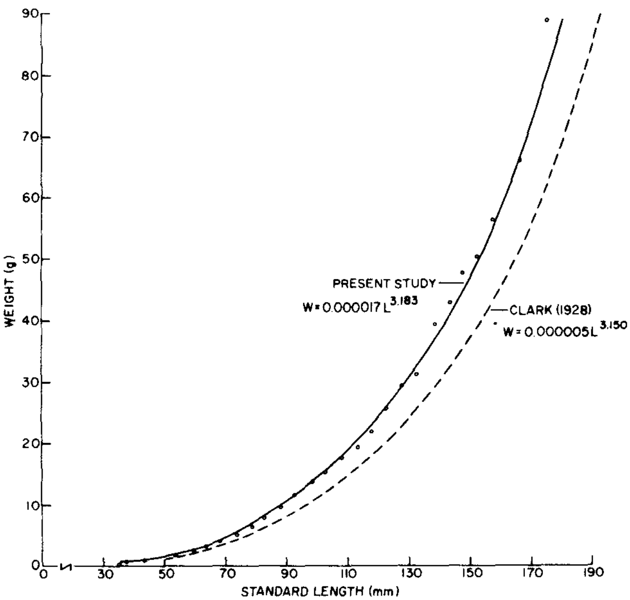

FIGURE 3.—Weight-length relation for the Pacific sardine.

(Figure 3) and found to be significantly different based on analysis of covariance ($F = 18.02$, df $= 1, 237$). The laboratory-reared fish were appreciably heavier for a given length than sardines caught in the 1920's. This may be attributed to several causes, among them difference in diet, in amount of exercise, and in the range of sizes sampled.

SEASONAL GROWTH PATTERN

Growth of the Pacific sardine has been well documented by several investigators (e.g., Walford and Mosher, 1943; Phillips, 1948; Felin, 1954; Clark and Marr, 1955). Most of the studies have concentrated on estimating growth based on scale readings of fish caught by the commercial fishery.

Another method of estimating growth is by rearing experiments. Although we recognize the limitation of laboratory vs. natural conditions, we believe that estimates of growth of laboratory-reared sardines can indicate the general trend in growth in the wild. We therefore estimated growth of our laboratory-reared sardines.

As shown in Figure 4, growth in length was rapid from the start of the experiment to the fourth month, after which the increase was more gradual. The average instantaneous rate of growth was about 0.47/month during the first 4 months and about 0.03/month during the fifth to 24th month. In contrast, growth in weight increased somewhat exponentially during two phases: during the first 4 months and again during the fifth to 14th month (Figure 5).

Walford and Mosher (1943) reported the standard lengths of juvenile sardines caught in monthly samples in the late 1930's. Although the date of birth, and hence the exact age, of

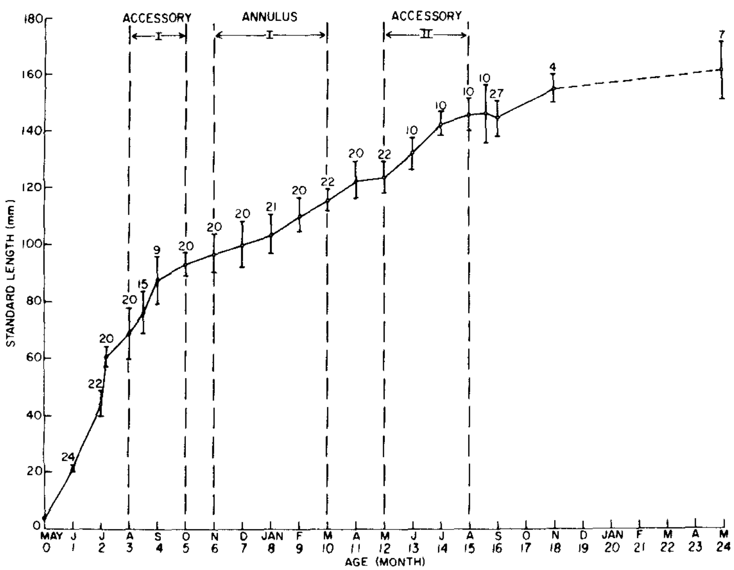

FIGURE 4.—Growth in length of Pacific sardines reared in the laboratory for 24 months. Mean length is represented by a circle, and one standard deviation is shown on each side of the mean. The sample size is also indicated. The first accessory mark occurred in August-October, and the second in May-August. The first annulus formed in November-March, and the second apparently in December.

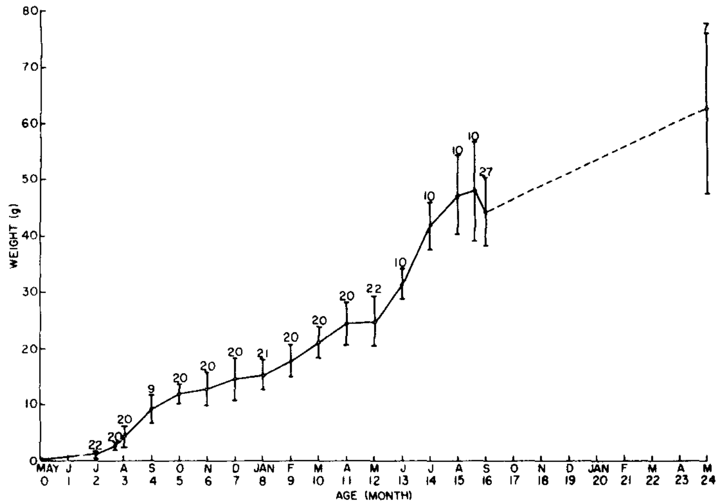

FIGURE 5.—Growth in weight of Pacific sardines reared in the laboratory for 24 months. Mean weight is represented by a circle, and one standard deviation is shown on each side of the mean. The sample size is also indicated.

their fish was not known, we compared their data for the 1937 and 1938 year classes with ours (Figure 6). The results indicate that although growth of the 1937 year class was fast, growth was similar in fish caught in the 1930's and in the laboratory-reared fish.

Marr (1960) presented data on the average length at time of first annulus formation (L_1) and showed that there was a sharp change in L_1 to a higher level with the 1944 year class landed at San Pedro. Using his data, we calculated separate estimates of average L_1: one for the 1934-43 year classes, and another for the 1944-57 year classes. The estimates are 101.3 and 131.5 mm, respectively. Compared with our estimate of 103.0 mm, growth of the 1934-43 year classes was almost identical to that for the laboratory fish, and growth for the 1944-57 year classes appears to have been faster than that for the laboratory fish. This faster growth may be an artifact and is discussed in a later section.

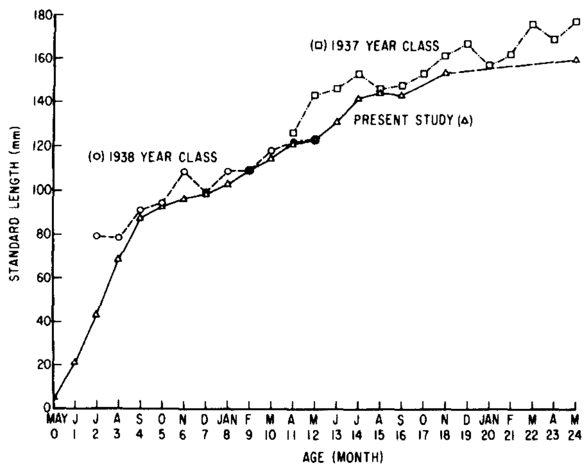

FIGURE 6.—Comparison of growth of juvenile sardines caught in the 1930's with growth of laboratory-reared sardines. Data for the 1937 and 1938 year classes are from Walford and Mosher (1943).

BACK-CALCULATED LENGTHS

Back-calculated lengths were computed for two samples, taken in September (16th month) and May (24th month). We reasoned that the back-calculated lengths would give an independent estimate of the time of mark formation, if growth is assumed to follow that shown in Figure 4. The average back-calculated lengths based on two methods are shown in Table 3. The first method gave lower estimates than the second. Both methods, however, gave back-calculated lengths that were reasonably similar to average lengths of samples collected during the period when the marks formed.

TABLE 3.—Back-calculated length of sardines sampled on two dates. The two methods of back-calculating length are discussed in detail in the text; in general, method 1 is by direct proportion, and method 2 is by direct proportion with a correction factor for the body length-scale radius relation.

Sampling date	Method	Average length (mm) at:					Sample size
		Accessory mark I	Annulus I	Accessory Mark II	Annulus II	Margin	
September	1	71.5	99.9	135.6	--	143.3	27
	2	88.9	110.6	137.8	--	143.3	27
May	1	75.1	104.7	137.4	153.1	160.3	7
	2	93.6	116.8	142.3	155.0	160.3	7

DISCUSSION

MARK FORMATION ON SCALES

Walford and Mosher (1943) indicated that on sardine scales an accessory mark was distinguishable from an annulus by its finer sculpturing and its rare occurrence on all scales of an individual. The results of our study on sardines reared in the laboratory showed that accessory marks occurred on all scales examined after the fourth month, and they were generally indistinguishable from annuli. But for some scales in which the accessory mark was distinguishable from an annulus, the identifiable characteristic was the fine sculpturing mentioned by Walford and Mosher.

Interruptions in the growth pattern are generally assumed to form marks on scales (e.g., see Van Oosten, 1957). It is also widely assumed that the driving mechanism behind mark formation is temperature, through the influence of a fish's metabolism (Brown, 1957). Our results indicate that mark formation is unrelated to temperature, but appears to be related to growth rate. Hogman (1968) obtained somewhat similar results in his experiments with coregonids. He found that formation of marks was closely related to growth and somewhat related to temperature. However, Hogman indicated that light period may be the primary driving mechanism. Bilton and Robins (1971) found that mark formation was correlated with increase in food supply, but not with resumption of feeding after starvation in sockeye salmon. Their experiments with light period proved inconclusive. It thus appears that mark formation in fishes is probably related to growth, although the actual driving mechanism(s) have so far not been clearly identified.

ERROR IN AGING

Scales are routinely used to age and study growth of the Pacific sardine. Our results indicate that extreme caution must be exercised in aging because of the presence of accessory marks on scales. Furthermore, since the annulus is formed during the winter, the actual age of a fish at time of first annulus formation may vary depending on its date of hatching. In our study the first annulus was laid down after the sixth month for fish hatched in May. May is the middle of the heavy spawning season for the Pacific sardine, but the season extends from March to October (Kramer and Smith, 1971).

Kimura (1970)[5] conducted an experiment to test the consistency of early and recent scale readers in aging sardines from scales. He found that the abrupt increase in L_1, as reportedly observed in the 1940's by Marr (1960) for sardines landed at San Pedro, may have actually been caused by a change in scale readers and in criteria used in reading scales. We compared our average length at annulus formation with the average L_1 of fish aged by the early ($L_1 = 101.3$ mm) and recent ($L_1 = 131.5$ mm) scale readers, and discovered that the early readers probably aged sardines correctly, whereas the recent readers probably underestimated the age of age II

[5] Kimura, M. 1970. Possible errors in locating the first scale annulus and in estimating the length of Pacific sardines. Manuscript filed at National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, CA 92037.

and older fish. If this was the case, and assuming that growth has not appreciably changed, then the number of fish that was incorrectly aged as age I by recent scale readers was at most equal to the number of fish correctly aged as age I. This is deduced from the fact that the presumably overestimated L_1 of 131.5 mm is about midway between our L_1 of 103 mm and L_2 (=length at time of second annulus formation) of about 160 mm.

Back-calculated lengths for Pacific sardines (Marr, 1960) have been based on Method 1. A statistical test of the intercept of our body length-scale radius relation (Figure 2) indicated that the intercept is not significantly different from zero ($t = 1.831$, df $= 282$). Although this indicates that Method 1 is acceptable, the back-calculated lengths at first annulus are appreciably and significantly different between Method 1 and Method 2 (Table 3). It is therefore advisable that Method 2 be used since it is the better procedure (Ricker, 1969).

We conclude from these results that a bias may be present in published accounts of the age composition of the Pacific sardine catch, which in turn may have affected studies on the population dynamics of the Pacific sardine. We recommend that the method of aging Pacific sardines be reevaluated, perhaps with the consideration of modifying the scale method so that the scale reader is made aware of the length of fish being aged or even utilizing other hard parts for aging, and that appropriate steps be taken to eliminate aging errors in the historic records on age composition of the catch. We realize that this task will not be easy, but it may be worthwhile because of the frequent use of the records to analyze fisheries hypotheses.

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