AN ANALYSIS OF THE FEATURES OF *SARDINELLA GIBBOSA* (BLEEKER) SCALES, WITH SPECIAL REFERENCE TO THE PROBLEM OF AGE DETERMINATION

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INTRODUCTION

Five species of *Sardinella* have been recorded in East African waters (Losse, 1968), *S. gibbosa* and *S. albella* (Valenciennes) being the most common in inshore waters of the Tanzanian coast. This investigation on the scales of *S. gibbosa* was a product of an attempt to find out whether there was any possibility of reading from the scale of the age of this species of sardine from its scales.

In temperate waters where the seasons are well marked, many of the piscine structures, e.g. scales, otoliths, vertebrae, dorsal spines, pectoral spines and opercular bone, show well defined differences in their growth. The seasonal growth zones in scales and otoliths have provided a relatively easy and quick way of assessing the absolute ages of commercially important fishes such as herring, cod, plaice, and salmon with a satisfactory degree of accuracy. Beside these growth rings which are formed as a result of the accelerated and retarded growth processes during spring and winter respectively, "the scales of many fishes show spawning rings and marks which are the result of the cessation of feeding and exhaustion during the spawning period", (Nikolsky, 1963, page 194). Therefore if the fish spawns once a year and at the same time leaves a spawning ring on its scales, the number of these rings can be used to read the age of the fish since it first started spawning.

The biology of East African *S. gibbosa* and other local sardines is very poorly known. For example, it is not known at what length or at what age the local *S. gibbosa* attains sexual maturity. Its maturation and spawning habits are also not known. For the Indian *S. gibbosa*, Nair (1959) mentions that Chacko (1946) found *S. gibbosa* to attain sexual maturity in the Palk Bay and Gulf of Manar regions when it reaches a length of 14 cm. whereas Sekharan (1955; quoted from Nair's paper) "showed that the fish below 8.5 cm. are immature and a proportionate increase of mature fish was observed in the higher size groups, with most of the sardines of the 10.7 cm. size group being mature." Sekharan also concluded that maturity is attained at this size at the end of first year. Ganapati and Rao (1957; quoted from Nair's paper) working on *S. gibbosa* of Lawson's Bay, Waltair, found that the species attains maturity at an average length of 11 to 12 cm. As for the maturation and spawning habits of this species of sardine, Dharmamba (1959) showed that *S. gibbosa* of Lawson's Bay has "a single spawning season a year" and "spawning is restricted to a definite short period with individuals spawning only once in each season." Losse (unpublished data) says that, in the Zanzibar Channel, the spawning season of *S. gibbosa* extends from April to October, with the smaller fish being first spawners and spawning early in the year (April/May) and the largest spawning in August to October, after the main spawning peak of June/July. From Losse's observation, it is perhaps justifiable to say that the local *S. gibbosa* has a single spawning season a year and that generally, individuals belonging to different size groups have a restricted spawning period at different times during the spawning season. Therefore if *S. gibbosa* spawns once a year, it is worth investigating whether the annuli seen on its scales result from the spawning activity of the fish or are caused by other unknown factor/facortors.

At first it was found necessary to investigate and ascertain the nature of other conspicuous features of the scale, namely the *striae* and the fissures, so as to eliminate any possibility that they may be associated in some way with spawning.

MATERIAL AND METHOD

For all examinations, the specimens were obtained from a local, Japanese-built, sardine-fishing vessel operating the Japanese stick-held dipnet, the "*Bouk-ami*". Material for histological examination was fixed in Allen's B15 (1500 ml. saturated aqueous picric acid + 500 ml. 40 per cent formalin + 100 ml. glacial acetic acid + 40 gm. urea + 30 gm. chromic acid) immediately after the fish were caught. Fixation was allowed to take place for about twenty-four hours and the fixed material was then washed thoroughly with tap water. Pieces of the skin with a little underlying muscle were then cut—each about 2 cm. long, 3 cm. deep and 2 to 3 mm. wide—and processed as follows:—
Dehydration to 70 per cent alcohol—decalcified in a solution of 5 per cent HNO_3 in 70 per cent ethanol for 12 hours—dehydrated to 100 per cent alcohol—left overnight in 8μ methyl benzoate/celloidin solution—blocked in wax and sectioned at 8μ thickness. The sections were stained with Masson's trichrome method, using iron Haematoxylin, Xylidine ponceau—acid fuchsin mixture and light green solution.

Scales for whole mount examination were first stained with carmine and mounted with Canada Balsam, but this method proved faulty as some of the features were masked with the stain and the mounting fluid. The method was abandoned in preference to mounting the scales dry between two glass slides taped at the ends. The latter method was also proved useful for the Northern achovy, *Engraulis mordax* and the Pacific sardine *Sardinops caerulea* (Girard) (Miller, 1955).

THE SCALE

Examination of *S. gibbosa* scale (fig. 1) shows several features whose nature has received a sparse and inadequate mention in the literature:

(i) *The circuli or striae:—*

These are the very fine transverse lines that run from dorsal to ventral* sides of the scale, nearly parallel to each other. Sometimes, one or a few may end abruptly or one *stria* may fuse with another. In longitudinal section (fig. 2) the striae can be seen to form very fine ridges in the bony layer. The posterior region of the scale which is not embedded in the scale pocket lacks the surface sculpture of *striae;* instead there are a number of round or oval perforations and growth patterns unique to this region. The posterior margin of the scale is crenulate.

As to the function of these *striae*, Van Oosten (1957) says "they are probably functioning in anchoring the scale in its pocket". He makes no suggestion as to how this may be achieved. Kerr (1952) makes the following comment about the longitudinal calcified ridges of *Amia calva* L. scale: "Possibly along with anchoring strands of connective tissue from the rear of the scale, they serve to maintain the position of the scale, to allow flexibility under normal circumstances and yet to permit the tearing out of a scale by a predator without excessive damage to the skin. Such a general explanation might apply also to the concentric striations of the teleost scale . . . . " In the light of modern studies on the reflectivity in silvery teleosts (Denton & Nicol, 1966) one may also postulate that the *striae* may play a part in the visibility of the fish. For instance, it would be relevant to investigate the light transmitting properties of the striated and non-striated parts of the scale, and also the relationship of the reflecting layers (stratum argenteum and layers of

* The lateral sides of the scale are referred to as the dorsal and ventral with respect to the scale's orientation on the body of the fish.

orientated reflecting platelets) to the two distinct regions of the scale. All these suggestions need to be subjected to the rigours of experiments, before the functions of the *striae* can be fully understood.

(ii) *The posterior continuous fissure and the dorsal and ventral fissures:—*
These fissures, the dorsals, ventrals, and the posterior are represented by thick lines in fig. 1, and labelled dvf and pf in fig. 2. No work to my knowledge exists on the nature and formation of these fissures. Photographs of the scale of *Caspialosa kessleri* Grimm, (in Nikolasky's *The Ecology of Fishes*, 1963, page 194) and *Engraulis mordax*, (in Miller's 1955 paper) show structures which appear to be similar but not identical to the fissures under consideration; no mention of these structures is made in the text.

Eighty scales from twenty-five individuals of *S. gibbosa* were removed from the lateral body wall, in the region behind the dorsal fin, about 1/3 to 1/2 from the dorsal profile of the body. All of them showed one general pattern; a posterior continuous fissure and several dorsal and ventral fissures (fig. 1). Often, the scales have an equal number of the dorsal and ventral fissures which are symmetrically arranged. For example, of the 80 scales examined, 43 had an equal number of the dorsal and ventral fissures and the remaining 37 had an unequal number of dorsal—ventral fissures. In another fish, 43 scales were removed from a single scale row (hatched line, fig. 5), beginning with the scale behind the humeral spot and ending with the one preceding the

Table 1, showing the number of dorsal and ventral fissures of 43 scales removed from a single scale row, (hatched line, fig. 5)

Scale	Number of fissures	Length of scale row occupied by consecutive five scales. All distances measured from humeral spot.	Scale	Number of fissures	Length of scale row occupied by consecutive five scales. All distances measured from humeral spot.
	Dorsal-Ventral			Dorsal-Ventral	
1	2—2	0—1cm	21	5—5	4.85-6.2cm
2	1—1		22	6—5	
3	3—3		23	5—4	
4	5—3		24	6—4	
5	4—3		25	6—5	
6	3—4	1—2.2cm	26	5—5	6.2-7.4cm
7	3—3		27	6—5	
8	4—3		28	6—5	
9	4—3		29	7—6	
10	5—3		30	7—5	
11	4—3	2.2-3.65cm	31	7—5	7.4-8.5cm
12	5—3		32	7—6	
13	4—3		33	5—5	
14	5—3		34	5—5	
15	4—4		35	5—6	
16	5—5	3.65-4.85cm	36	6—4	8.5-9.75cm
17	4—4		37	6—6	
18	3—5		38	5—4	
19	6—4		39	7—7	
20	6—4		40	6—4	
			41	5—5	9.75-10.55cm
			42	5—3	
			43	4—4	

TABLE 2

Number of annuli	Length of fish in mm
0	117(1), 118(1), 119(1), 123(1), 125(2), 126(3), 127(1), 128(1), and 131(1).
1	118(1), 122(1)

Table 3 gives the result of annulus reading of samples of fish taken during the months of January, March, April, and May, 1969. For convenience, the individual lengths of fish have been omitted and instead the fish have been put into 5mm size groups.

irregular scales of the caudal peduncle. Again all of them showed the basic pattern—a posterior continuous fissure and several dorsal and ventral fissures. Of the 43 scales, 15 had an equal number of the dorsal—ventral fissures, while the remaining 28 had an unequal number of dorsal—ventral fissures, there being not more than 2 extra fissures on either the dorsal or ventral side of the scale (see Table 1). Scales from the caudal peduncle, from the region immediately behind the opercle and from the dorsal profile showed an irregular pattern of these fissures.

After examination of all these scales, it became apparent that these fissures had nothing to do with spawning, simply because spawning marks are left as circular scars rather than transverse marks. Further, longitudinal sections of the scale (fig. 2) revealed that the fissures were filled with a connective tissue material that was staining green, just like the walls of the scale pocket, suggesting that the fissures were probably passages for strands of connective tissue that helped to hold the scale in its pocket. Perhaps they also serve to increase the scale's flexibility.

An explanation as to how these fissures may be formed was sought by studying the scale in relation to its surrounding features. The imbrication of the scales (fig. 3, 4) seemed to be a possible cause for the fissures. Fig. 4 shows the pattern of the anterior edges of the scale pockets, and it is from these anterior edges that the dorsal walls of the scale pockets emerge from the body wall. Considering the scale M (fig. 4), it seems that as this scale grows outwards and overlaps part of the anterior edges, l_1, l_2, r_1, r_2, (shown by thick lines in fig. 4) of the scale pockets flanking it dorsally and ventrally, connective tissue from l_1, l_2, r_1, and r_2, grows through scale M which overlies l_1, l_2, r_1, and r_2. This explanation is based on the observation that the only structure on the body wall that can be likened with the form (i.e. orientation, thickness and length) of the dorsal and ventral fissures are the overlain portions of the anterior edges of the scale pockets. (see fig. 4).

However, there are several difficulties involved in this hypothesis. Firstly, a scale usually overlies the portions of anterior edges of *two* pockets on its dorsal and ventral sides, whereas the number of dorsal and ventral fissures found on a scale varies from two to eight on either side. That is, a scale may have two dorsals and two ventrals or four dorsals and five ventrals or eight dorsals and six ventrals and so on.

Secondly, although the formation of dorsal and ventral fissures may be explained by considering the bordering scale pockets, the formation of the posterior continuous fissure does not seem to be explicable on the same basis.

Thirdly, it is difficult to explain the pattern of fissures of the scale of *E. mordax* (assuming that the lines and splits seen in the photographs of *E. mordax* scales are similar to the fissures under consideration) or of the caudal peduncle scales of *S. gibbosa* by the growth—imbrication theory.

TABLE 3

MONTH AND NUMBER OF FISH EXAMINED	NUMBER OF ANNULI	SIZE GROUPS (LENGTH IN MM.)									
		86-90	91-95	96-100	101-105	106-110	111-115	116-120	121-125	126-130	131 -
JANUARY, 1969	0	0	6	10	1	1					
24 fish	1-doubtful	1		1		1					
	1-clear		1	1	1						
MARCH, 1969	0	1	2	2	3	4	1	0	1	4	
25 fish	1-clear							1		3	
	1-clear, 2nd doubtful								1		
	2-clear								1	1	
APRIL, 1969	0				1	4	2	6	4	2	
	1-doubtful					1		1		1	
25 fish	1-clear							1		1	
	1-clear, 2nd doubtful										1
MAY, 1969	0						4	2	2	1	
	1-doubtful									2	2
25 fish	1-clear									3	3
	1-clear, 2nd doubtful									1	
2-clear										4	
	2-doubtful									1	

Whitear (pers. comm.) points out that the fissures might correspond to the canals penetrating the scales of more primitive actinopterygians which usually carry blood capillaries and nerves. Further histochemistry is needed to ascertain the presence of these structures in the fissures of *S. gibbosa.*

Thus the problem of explaining the formation of these fissures still remains unsolved and a solution could perhaps emerge by studying the development of these scales.

Before leaving the fissures, it may be mentioned that Losse (1966, 1968) refers to these fissures as *striae,* whereas Van Oosten (1957) defines *striae* as "the relieved[1*] surface ridges that are continuous and homogenous with the bony layer of the scale". As mentioned earlier, the *striae* are the very fine and numerous transverse lines that are seen in a whole mount of the scale and in L. S. as fine ridges in the bony layer. Van Oosten (1957) does not mention the fissures[2*] although they are of importance in classification of the Clupeidae (Losse, 1966, 1968).

(iii) *Annuli*

In fig. 1, these are shown by the two narrow and light coloured bands that are concentric with most of the periphery. Miller (1955) refers to these rings in *E. mordax* scales as "typical clupeoid annual checks". He uses the definition of an annulus as given for the Pacific sardine, *Sardinops caerulea* (Giaard), by Walford & Mosher (1943b) as a basis for annulus interpretation in *E. mordax*. It seems that this definition which is as follows, also applies to annulus recognition in *S. gibbosa.*

"An annulus is concentric with the margin of the scale. It is not always a sharp or unbroken line; nor are the segments of an interrupted annulus always perfectly cocircular (if the shape of a scale may be called circular in this discussion). But the course of an annulus, continuous or broken as it may be, can usually be traced, by careful scrutiny if necessary, entirely around the sculptured part of the scale from left hand to right hand margins. Sometimes they can be followed even around the unsculptured part. Annuli are clearly separated from each other and do not ordinarily meet at any point. *If an annulus has formed it is present in all the normal scales of an individual*", (quoted from Miller, 1955).

TABLE 4

Month	Percentage composition of fish in the sample with different number of annuli					
	0-annulus	1-doubtful	1-clear	1-clear 2nd doubtful	2-clear	2-doubtful
January 1969	75	12.5	12.5	0	0	0
March 1969	72	0	16	4	8	0
April 1969	76	12	8	4	0	0
May 1969	36	16	24	4	16	4

*1 = relief. His usage is not correct. Surface ridges would have been sufficient.

*2 = unless his *radii,* defined as "open channels cut completely through the bony surface", are equivalent to what I have called fissures.

It may be mentioned that the annual growth rings on scales of temperate fishes such as salmon and trout are also called annuli. The annuli in these fishes are formed by the circuli being closer together at certain times of the year than at others. De Bont (1967) mentions that Holden (1955) described the annuli of *Tilapia variabilis* Boulenger and *T. esculenta* Graham of Lake Victoria as rings which are formed by irregular circuli.

In *S. gibbosa*, the annulus is formed by the *striae* being either interrupted, indented or bent in the annular region and the spaces between the *striae* are "lighter" in the annular region compared to the rest of the striated region of the scale.

In order to find out whether the Walford and Mosher definition of an annulus also holds for *S. gibbosa* scales, three individuals of the sardine were examined in detail for annuli marks on their scales. 259 scales were removed from regions 1 to 12 (fig. 5) of a 125 mm. Standard Length (S. L.) fish, and nearly all of them had no annulus. In a second individual of 132 mm. S. L., 151 scales were removed from regions 1 to 12, and nearly 90 per cent of the scales showed a clear formation of a single annulus. In the third specimen 129 mm. S. L., 153 scales from regions 1 to 12 were examined and 70 per cent of the scales showed a clear formation of two annuli.

Fourteen other individuals (a very small part of the catch taken on the night of 26 November, 1968) were examined for annulus marks. In these cases about 12 scales only were removed from regions 5 and 8 (fig. 5) of every fish and the scales were then examined for annuli. Table 2 shows the result obtained; the figures in parentheses give the number of fish in that size group.

Table 4 gives the percentage of fish in the sample with different number of annuli for the same months viz. January, March, April and May 1969.

Table 5 has been included to show that it is also possible to read the number of annuli from *S. albella* scales. Sixteen individuals were obtained from a mixed subsample of about a thousand fish, the latter being part of the catch taken on the night of 17 February, 69.

TABLE 5

Number of annuli	Length of fish in mm.
0 1 doubtful	72(1), 91(1), 98(1), 99(3), 101(1), 102(1) 103(2). 104(1)
1 clear	105(1)
1 clear, 2nd doubtful	97(1), 129(1)
2 clear	102(1), 103(1).

Fig.1

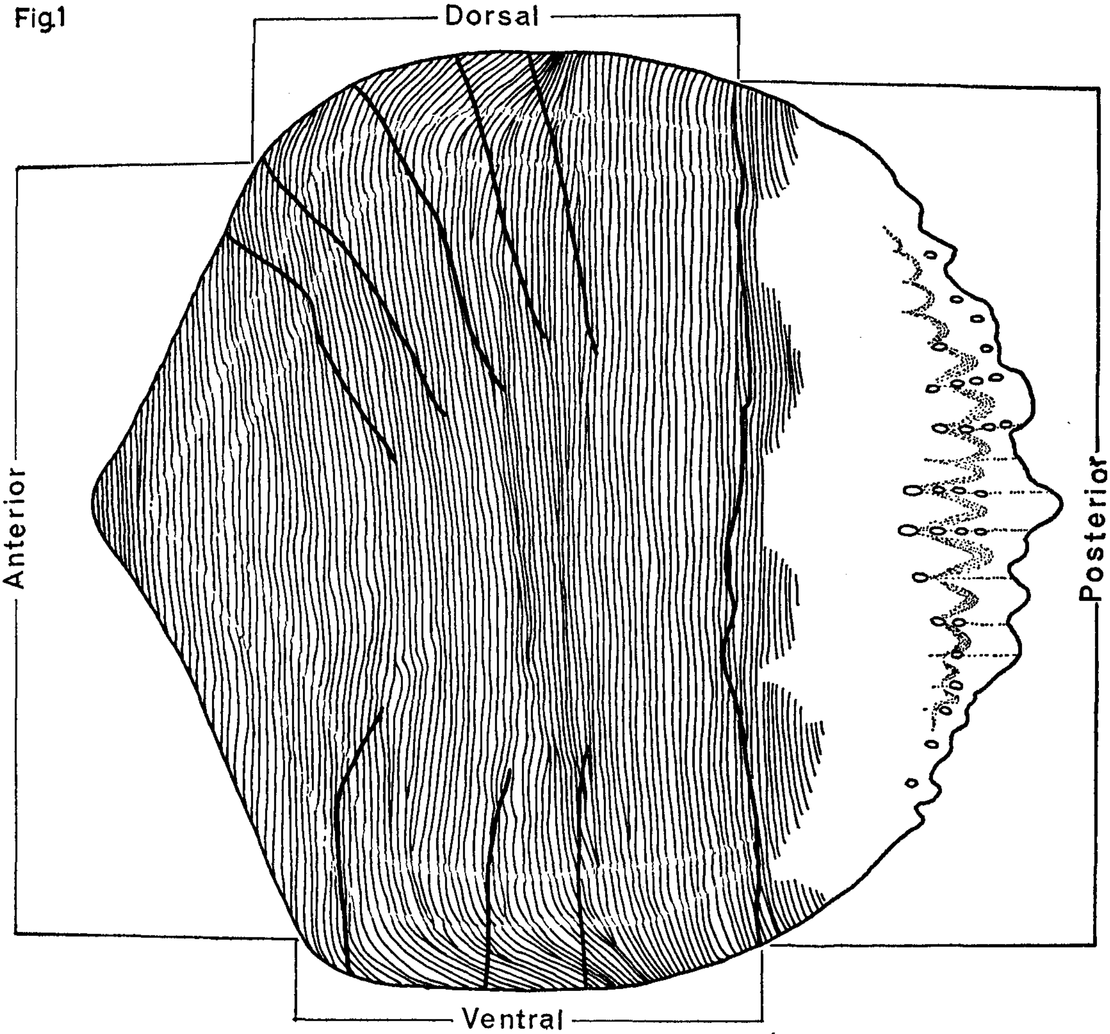

Fig. 1—Surface view of *S. gibbosa* scale. The directions indicate the orientation of the scale relative to the orientation of the fish. Fissures are shown in thick lines and the two annuli by the two light incomplete circular bands. × 30.

Fig. 2

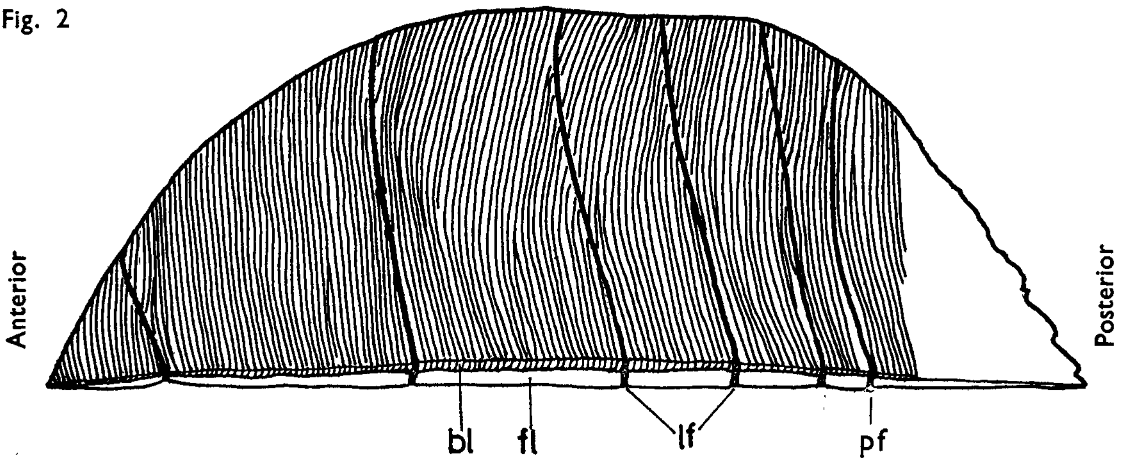

Fig. 2—Longitudinal section of *S. gibbosa* scale, showing the posterior fissure and one set of the lateral fissures. Key: pf—posterior fissure; lf—lateral fissures; bl—bony layer of the scale; fl—fibrillary layer of the scale. × 60.

Fig. 3

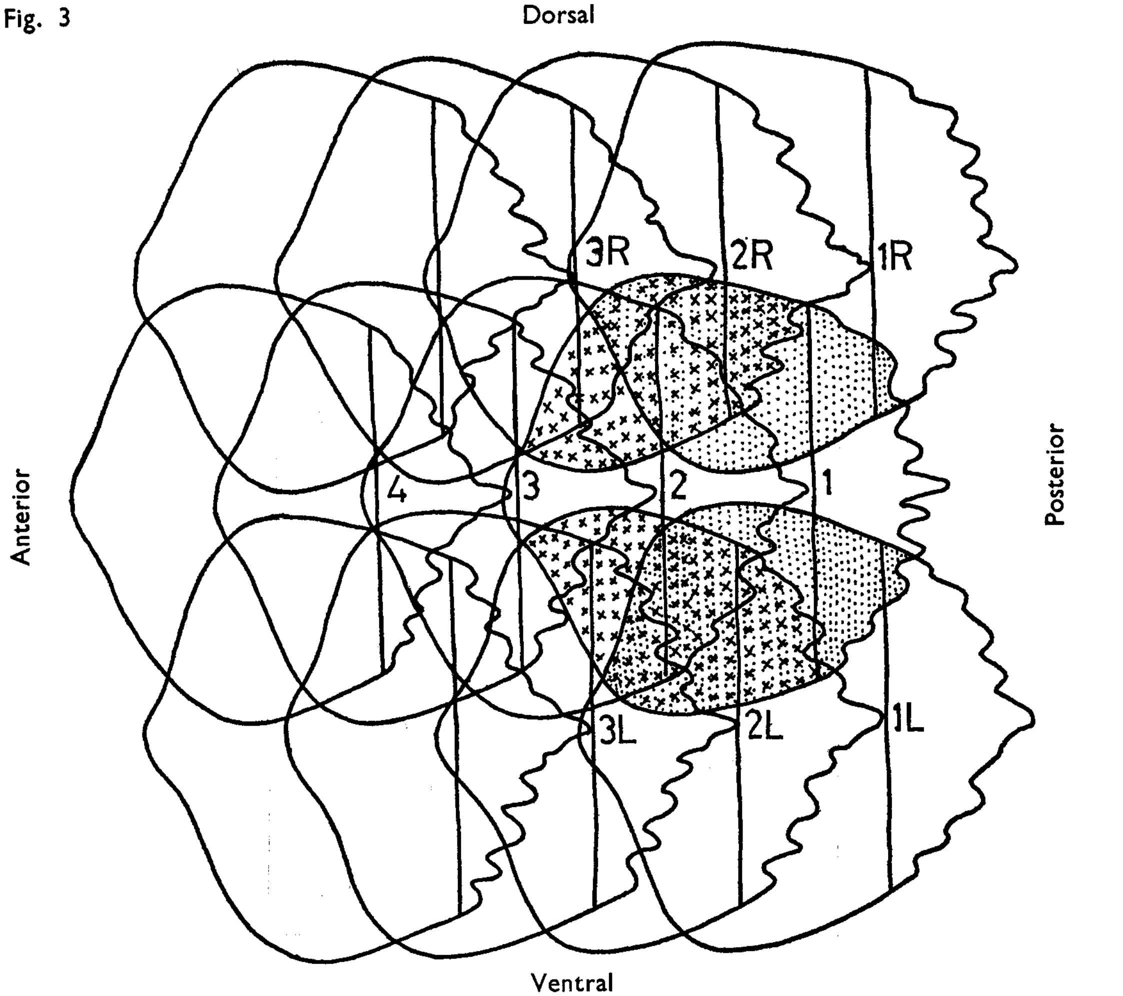

Fig. 3—Diagrammetic representation of the surface view of the arrangement of the scales from the middle of the lateral body wall of the fish. Scale 1 overlaps scale 1 R and 1 L (dotted areas). Scale 1 itself is overlapped by 2 R and 2 L (areas with crosses), the latter two scales being overlapped by scale 2 which is itself overlapped by 3 R and 3 L and so on.

Fig. 4.

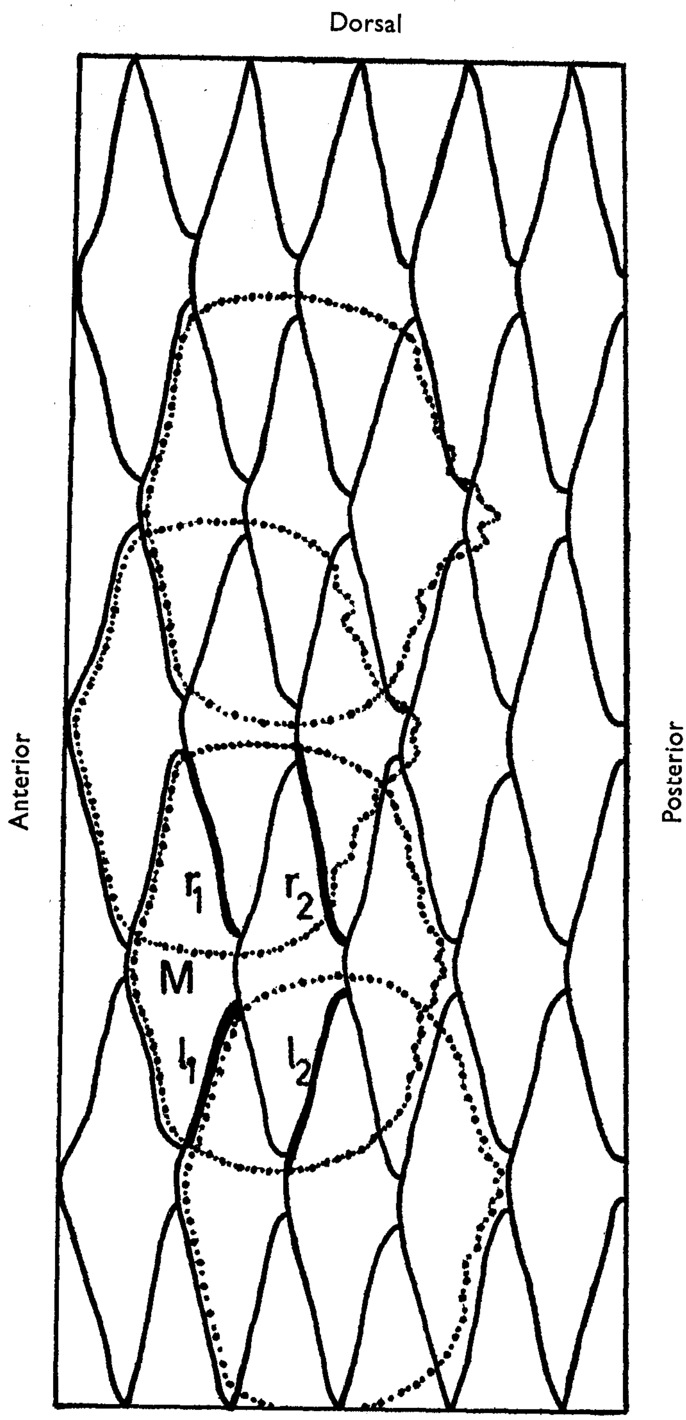

Fig. 4—Diagrammatic representation of the surface view of the anterior edges of scale pockets from middle of the lateral body wall of the fish. Four scales are shown with dotted outline. For l_1, l_2, r_1, r_2, and M, see text.

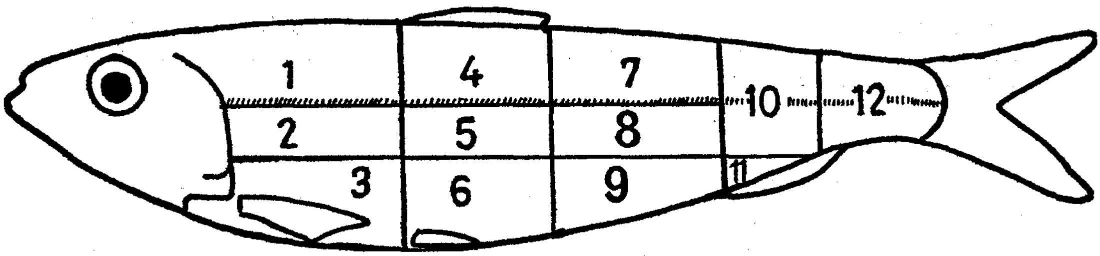

Fig. 5

Fig. 5—Division of the lateral body wall into twelve regions from which scales were removed for annuli studies. The hatched lines represents the scale row from which 43 scales were removed for fissure investigation. × 1.

This preliminary investigation on the annuli gives the following indications:—

1. The definition of an annulus as given by Walford and Mosher for *S. caerulea* can also be used to identify annuli in *S. gibbosa* and *S. albella.*

2. In *S. gibbosa* fish showing no annulus varied in length from 86 mm. to 131 mm., a range which nearly overlaps that of those showing one annulus (Table 3). On the other hand, the same table shows that fish with two annuli on their scales have so far been found only in the higher size groups, none of them being less than 123 mm. in length.

3. For the months of January, March, April and May, 1969, the percentage of fish with no annulus is higher than of those with one annulus and the proportion of the latter is greater than of those with two annuli (doubtful cases have been omitted). Until the causation of the annulus is established, it will be perhaps hazardous to extract any information from these figures.

4. If annuli on *S. gibbosa* scales are yearly registered marks, then the few year-groups (?) observed probably fits in with the other properties, viz. early maturity and annual spawning, of fish with a short life cycle (Nikolasky, 1963, p. 217).

Several workers have tried to investigate what induces the formation of annuli in some other fishes. For *E. mordax,* Miller (1955) says "that these rings are not spawning checks is shown by the presence of an annulus on young immature fish and by the presence of new rings already forming on the scales of most fish collected just before and during the spring spawning." Further, he establishes that these checks result from the retarded growth during late summer and fall, whereas most of the spawning is confined to winter, spring and early summer, the latter two periods being also the time when maximum growth of the fish and its scales takes place.

Newell (1957) mentions that F. Talbot found that "in the fishes of East African waters, a zoning is often present in the scales of many species. In *Lethrinus nebulosus* (Forskal) a check in scale growth has been found in September, the month of minimum temperatures".

On the other hand, Van Someren's work (1950; quoted from De Bont, 1967) on rainbow trout, *Salmo gairdneri,* living in the rivers of the foothills of Mount Kenya, showed that the annuli are spawning marks. In *Tilapia macrochir* Boulenger, De Bont (1950; quoted from his 1967 paper) found no correlation between spawning and the laying of annuli on the fish's scale.

For *S. gibbosa* work is in progress to find out whether annuli are spawning rings or whether they develop as a result of some other cause of unknown periodicity. Only when this latter point is cleared, would it be possible to say whether or not annuli can be used as age indicators. For the moment, it seems that they are probably the best structures yet available as tentative indicators of age.

SUMMARY

1. Scales of *S. gibbosa* have been investigated in an attempt to find an ageing method for the fish from these structures.

2. The nature of the *striae*, posterior, dorsal and ventral fissures is ascertained. A suggestion is made as to how the fissures may be formed, although several difficulties are involved in the hypothesis.

3. Annuli of the clupeoid type have been observed and the validity of their use in age determination is the subject of further investigation.

ACKNOWLEDGEMENTS

I am greatly indebted to Dr. Mary Whitear, University College, London, for her assistance in going through the manuscript and in making quite a number of useful comments. I also wish to express my thanks to Mr. G. F. Losse for having allowed me to use some of his unpublished biological data, Prof. I. Griffiths for many helpful suggestions and for lending me literature.

I am grateful to the Research and Publications Committee, University College, Dar es Salaam, for giving me a grant to pursue the work on the local sardines.

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(Received 8th July, 1969)