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A STUDY OF THE LIFE HISTORY OF BRAZILIAN SARDINE, *Sardinella brasiliensis*. III. DEVELOPMENT OF SARDINE LARVAE*

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SYNOPSIS

Larvae and juveniles of *S. brasiliensis* (Steindachner, 1879), ranging from 6.4 mm to 35.5 mm, were identified from plankton samples taken in waters off the southern Brazilian coast from 1969 through 1971. Changes in the pattern of pigmentation, body proportions and formation of fin rays are described. During transformation stage a considerable advancement of the dorsal and anal fins was observed. Changes in body proportions are pronounced at the size of 19 mm. Complete ossification of all fin rays is attained at the size of 20 mm, but ossification of the ventral scutes is delayed and completed only at the size of 30 mm. Ossification of the vertebral column was completed at the size of about 16 mm.

INTRODUCTION

The purpose of this paper is to describe the larval stages of the Brazilian sardine, *Sardinella brasiliensis* and compare them with the larval stages of closely related species of the family Clupeidae.

The systematic status of the Brazilian sardine is still problematic. Whitehead (1967) examined the type specimens of *Sardinella spp.* and suggested the occurrence of two species in Brazilian waters. He confirmed a difference on gill raker counts and cheek depth between *S. brasiliensis* and *S. aurita*. In 1970 he stated that "descriptions of the types of *brasiliensis*, *anchovia* and *aurita* reveals only small differences which, although not totally vindicating

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Regan's synonymizing of the three, at least suggest that no Western Atlantic study can afford to ignore the Eastern Atlantic, Mediterranean and Pacific forms of *S. aurita*".

We have not sufficient data to resolve this problem presently. It is impossible to separate the larvae into two groups using gill raker counts or cheek depth. Therefore the author used the scientific name, *Sardinella brasiliensis* (Steindachner, 1879) to the common sardine for the southern Brazilian waters.

Fage (1920) described, for the first time, the eggs and larvae of the Mediterranean sardine, *S. aurita*. Using the caudal skeleton, Hollister (1936) compared the juveniles of *S. anchovia* to that of *Harengula* and *Opisthonema*. Nair (1959) studied the eggs and larvae of the Indian sardine *Sardinella longiceps*. Conand & Faggeti (1971) studied and compared the larvae of two African species of *Sardinella: S. aurita* and *S. eba* and more recently, Houde & Fore (1973) made a key for identification of the eggs and larvae of Clupeids from the Gulf of Mexico.

MATERIAL AND METHODS

The material used in this study was collected with a plankton net and a beam trawl net, in southern Brazilian waters (from Lat. 22° S to 30° S) by the R/V "Prof. W. Besnard" and "Emília" of the Instituto Oceanográfico da Universidade de São Paulo from 1969 through 1971. The sampling method was the same as described in a previous paper (Matsuura, 1971). The plankton samples were fixed and preserved in a solution of 10 percent formalin.

The specimens for morphological studies were cleared and stained with alizarin red, according to the method of Clothier (1950). Linear regressions were computed for all measurements and only those showing allometric growth are presented. To show the variation of body proportions during development, the body proportions in percent were plotted against standard length using data from the linear regressions. Before the anal fin formation the distance from the tip of snout to the anus was applied to the preanal distance. In larger specimens the origin of the anal fin base appears closely near the anus.

Developmental stage of larvae is based on Mansueti & Hardy (1967). The terminology to supporting bones of the caudal fin is that of Nybelin (1963) as modified by Monod (1967). Details of measurement and terminology were described in a previous paper (Matsuura, 1974).

Dorsal and anal fin counts were made including the unbranched rudimentary soft rays. The last two soft rays were counted as one when they were connected at the base.

RESULTS

PIGMENTATION - The development of *S. brasiliensis* larvae is shown in Figure 1.

The larvae of *S. brasiliensis* are transparent and scarcely pigmented. The pigmentation varies during development and also among individuals.

At the length of 6.5 mm (Fig. 1*a*) the specimen shows two large melanophores at the end of the alimentary canal. A series of melanophores appear along the ventral side of alimentary canal in the antero-pyloric region. A patch of melanophores appears on the ventral margin near the termination of notochord, which later can be observed on the lower lobe of the caudal fin.

At the length of 10.1 mm (Fig. 1*b*) the specimen shows a series of melanophores along the dorsal side of the intestine. Two melanophores are found on the antero and postero-ventral part of the shoulder girdle. A large melanophore is observed on the base of the pectoral fin. Up to 19 mm length, the pigmentation pattern does not show any pronounced variation and at the length of 21.4 mm the larvae show more abundant pigment cells on dorso-lateral side of the body and on the head region. There are also some melanophores on the base of the anal fin.

The author failed to find any distinct pigment pattern for the species which could distinguish it from other clupeid larvae. However when the larvae of *S. brasiliensis* are compared to that of *Harengula* species, the former does not show any pigmentation on the tip of the urostyle (or notochord in small specimens) whereas the larvae of the later have a patch of melanophores on it during early larval stage.

BODY PROPORTIONS - Regression analysis were made using morphometric data. The regression lines of preanal and prepyloric distances to standard length show an inflection at the size of 16 mm (Fig. 2). The regression lines of predorsal distance, head length, and body depth to standard length show an inflection at the size of about 19 mm (Fig. 2). The regressions of snout length and eye diameter to head length and caudal peduncle depth to body depth, proved to be linear. Regression data are shown in Table I.

A change of proportions of body parts to standard length in percent is shown in Figure 3.

In the size between 6 mm and 16 mm, a change of body proportions is isometric. At the length of 16 mm, a radical decrease of preanal distance and increase of prepyloric distance are observed. This is, in part, originated with a shortening of the intestine (distance between the pyloric caecum and the anus). At the length of 16 mm the intestine measures about 7.7 mm whereas at the length of 22 mm it is only 6.5 mm.

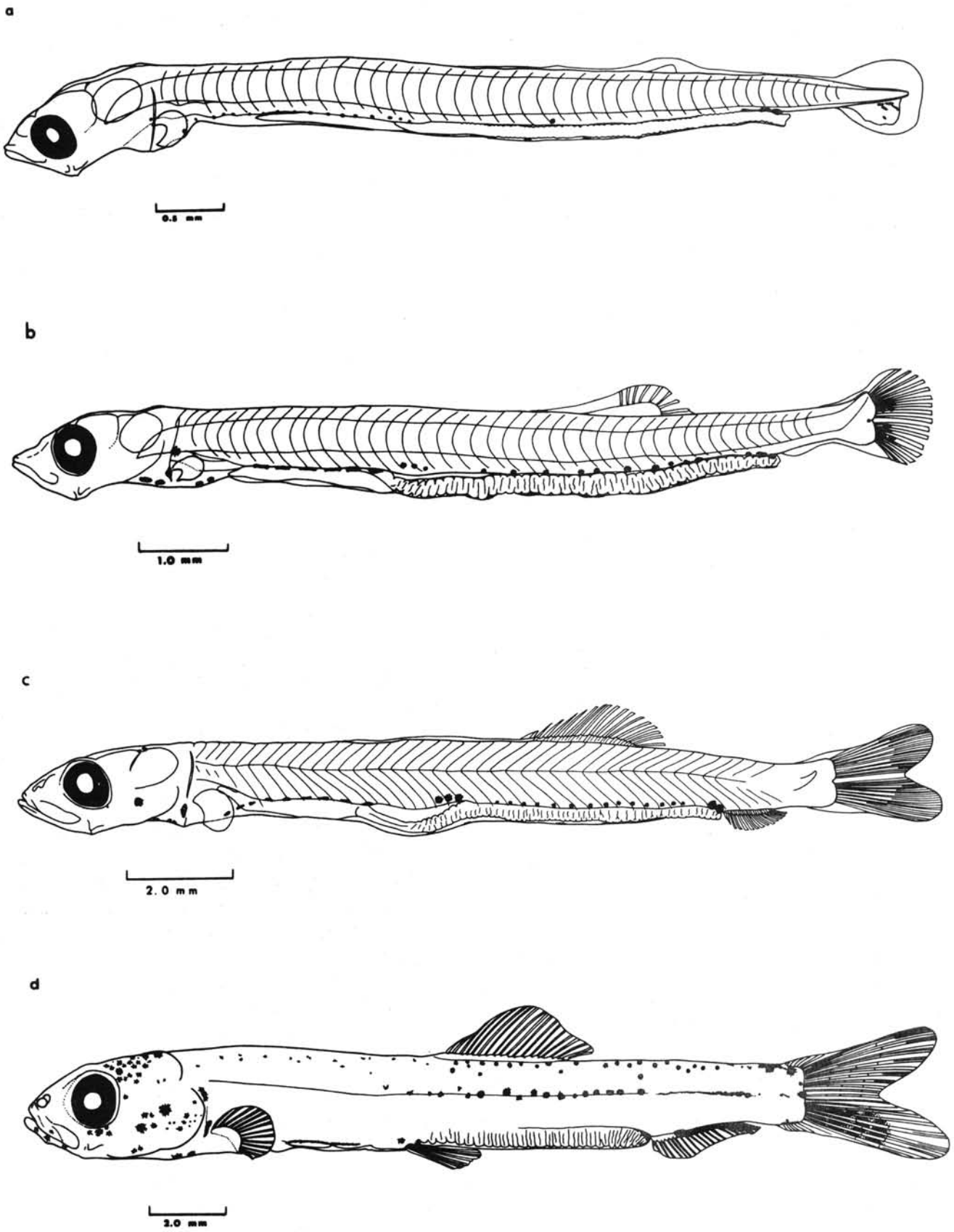

Fig. 1 – Development of the larvae of *S. brasiliensis*. a) 6.5 mm, b) 10.1 mm, c) 15.5 mm, d) 21.4 mm, (standard length).

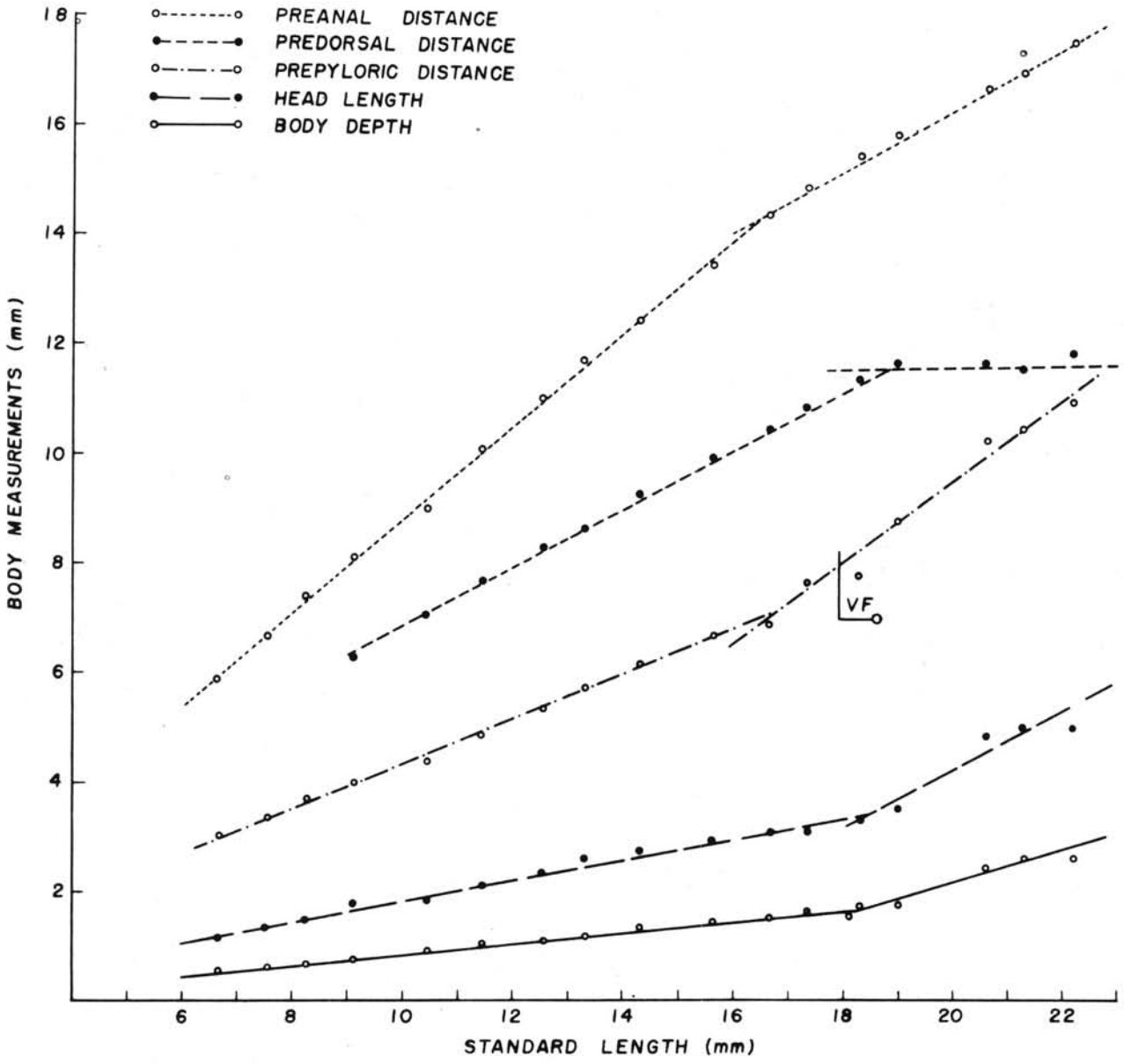

Fig. 2 – Regressions of body measurements to standard length.

Proportion of the predorsal distance decreases constantly up to 19 mm length, then it decreases more radically. The decrease can be said, in other words, as foreward advancement of dorsal fin. Ahlstrom (personal communication) informed me that the forward advancement of dorsal fin in clupeiform fishes, elopids, albulids and Chanos was observed during transformation stage and that the apparent forward advancement during larval stage (up to 19 mm in this case) is resulting from taking measurements on partially formed fin bases.

Gosline (1971) considered the forward advancement of dorsal fin as an attribute of ancestral teleosteans. He suggested that the forward advancement of the dorsal fin in lower teleosteans is derived from a change of function of dorsal fin during larval through juvenile stages.

TABLE I - Regression data for *S. brasiliensis* at the larval stage

Dependent variables (y)	Specimen size range (mm)	n	a	b	s^2	F
Body depth*	6-19	63	-0.13987	0.10039	0.00672	1134
do	19-23	12	-3.56127	0.28745	0.02612	23
Head length*	6-19	63	-0.10249	0.19305	0.01303	2161
do	19-23	12	-5.80737	0.50491	0.10621	17
Predorsal distance*	8-19	36	1.44463	0.54331	0.03090	1923
Preanal distance*	6-16	57	0.25868	0.85353	0.02870	12025
do	16-23	17	5.08858	0.56289	0.04358	418
Prepyloric distance*	6-16	57	0.27826	0.40693	0.01918	4090
Preventral distance*	16-23	17	-5.60018	0.75843	0.09071	364
Eye diameter**	6-23	74	0.00767	0.26033	0.00237	3070
Snout length**	6-23	74	-0.09903	0.28246	0.00415	2064
Caudal peduncle***	6-23	74	-0.14807	0.63378	0.00260	4860

Independent variables (x):
* = standard length
** = head length
*** = body depth

n = total number of specimens
a = y-intercept of regression line
b = slope of regression line
s^2 = mean square deviation
F = variance ratio

A change of all body proportions at the size of about 19 mm was also observed. This size coincided with the end of the ossification of fin rays except pectoral which complete ossification at a length of about 20 mm. These facts demonstrate that the change of body proportions at 19 mm may be considered as the end of the larval stage. After this size up the transformation stage comes.

ADVANCEMENT OF DORSAL AND ANAL FINS - The forward advancement was observed not only in the proportions of predorsal and preanal distances, but also in positions of fin bases expressed in myomere counts.

Figure 4 shows the advancement of dorsal and anal fin bases observed through myomere counts after fin ray formation. The forward advancement of the origin of the dorsal fin is more radical than that of the anal fin. At the length of 15.4 mm the origin of the dorsal fin coincides with the 26th myomere and at the length of 22 mm with 20th. The origin of the anal fin coincides with the 39th myomere at the length of 15.4 mm and with 34th at 22 mm.

The importance of the number of postdorsal-preanal myomeres for the identification of clupeid larvae has been stressed by Houde & Fore (1973). The larvae of *S. brasiliensis* shows the postdorsal-preanal myomere counts between 7 and 4.

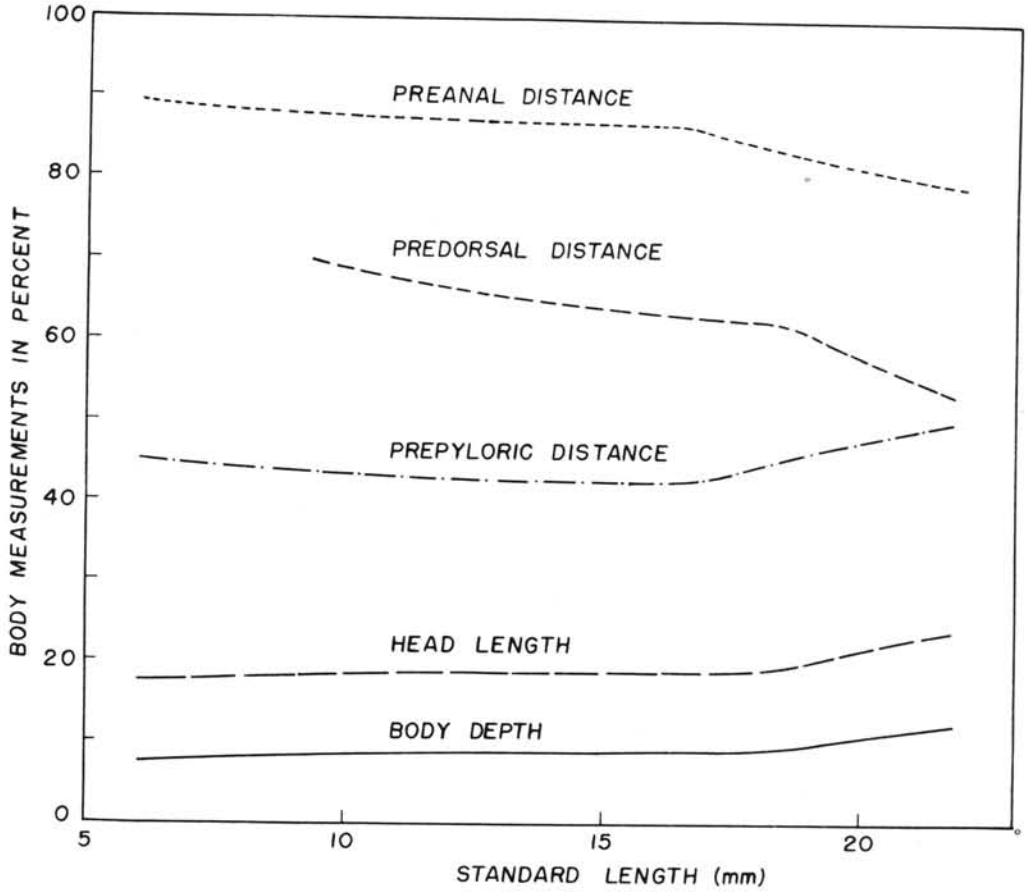

Fig. 3 - Regressions of body measurements, expressed as a percent of standard length, on standard length. The curve is fitted from the regression data of Table I.

SEQUENCE OF OSSIFICATION - The sequence of ossification of *S. brasiliensis* larvae is shown in Figure 5. The sequence is shown as an attempt to present the exact point of fin formation, a character useful in the identification of sardine larvae.

After the absorption of the yolk sac, the early larvae have a membranous pectoral fins. The ossification of fin rays is first observed in the caudal fin rays at 7 mm, proceeding the formation of the urostyle and hypurals. Complete ossification of principal caudal fin rays is reached at 10.5 mm and the completion of procurrent ray ossification is about 20.0 mm. The sequence of ossification is the following: caudal fin, dorsal fin, anal fin, ventral fin and pectoral fin, which completed the ossification of last ray at 20 mm.

The ossification of ventral scutes starts at the length of 22 mm. The anterior part finishes at 26 mm and the posterior part completes at 30 mm.

The 23rd centra of the vertebra is the first to form at the length of 12 mm and then the ossification proceeds anteriad and posteriad. Some individuals complete the full number of vertebrae at the length of about 13.8 mm, but the completion of it for all specimens is considered to finish at the

length of 16 mm (Fig. 6). The ossification of haemal and neural spines starts at the length of 15 mm.

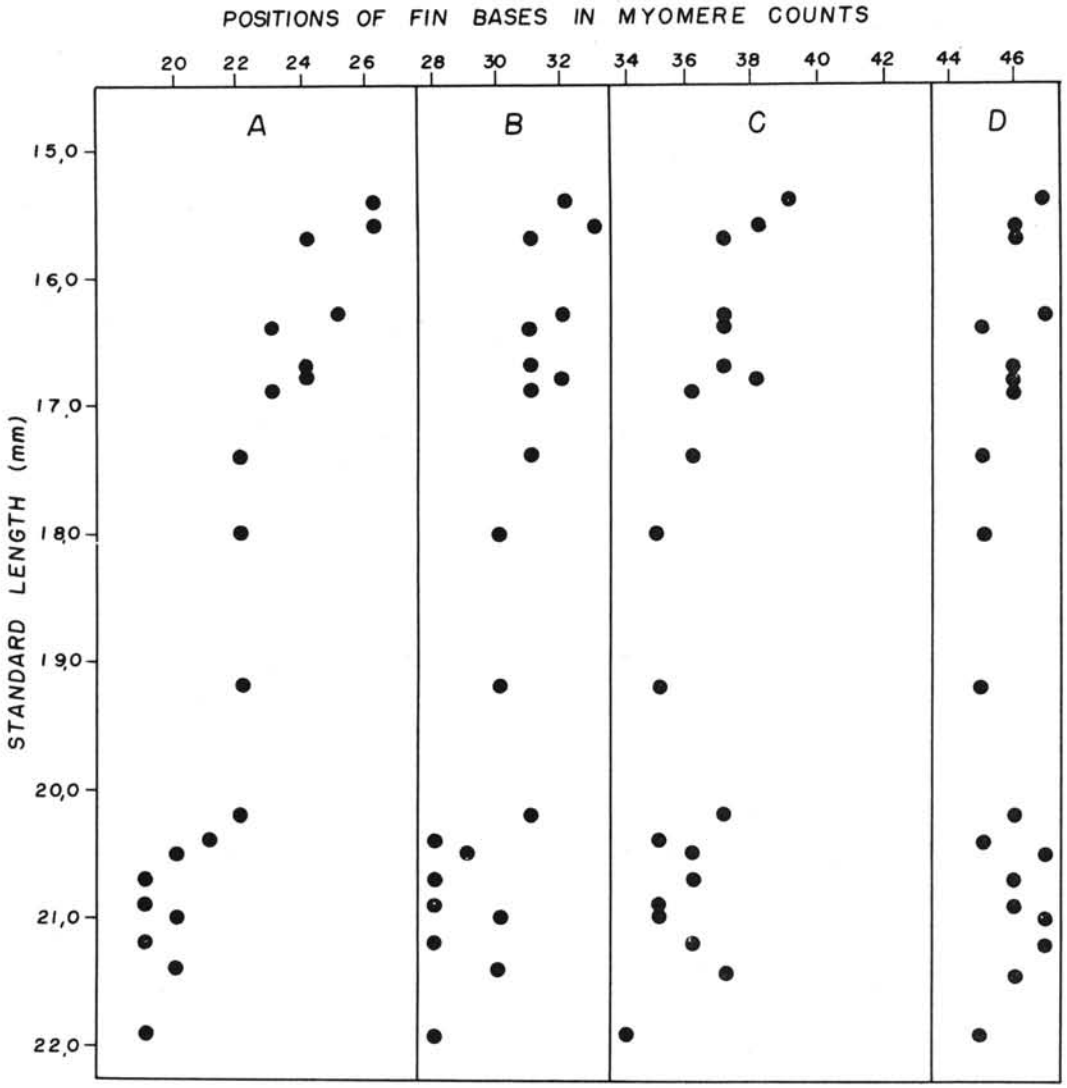

Fig. 4 - Change of the positions of fin bases in myomere counts during growth. (A - the origin of the dorsal fin, B - the posterior insertion of the dorsal fin, C - the origin of the anal fin, and D - total myomere counts).

MERISTIC CHARACTERS - Table II shows the meristic data of the sardine larvae and juveniles. The abdominal vertebrae is 25 or 26 and the caudal vertebrae ranges from 20 to 22. Total vertebral count is from 45 to 48.

Dorsal fin rays is 18 or 19 and the anal fin rays ranges from 16 to 19. These characters can be used to distinguish the larvae of *S. brasiliensis* from the larvae of *Opisthonema oglinum* which has a similar pigment pattern. The later has a larger number of fin rays, i.e. 18-22 for the dorsal fin and 20-25 for the anal fin.

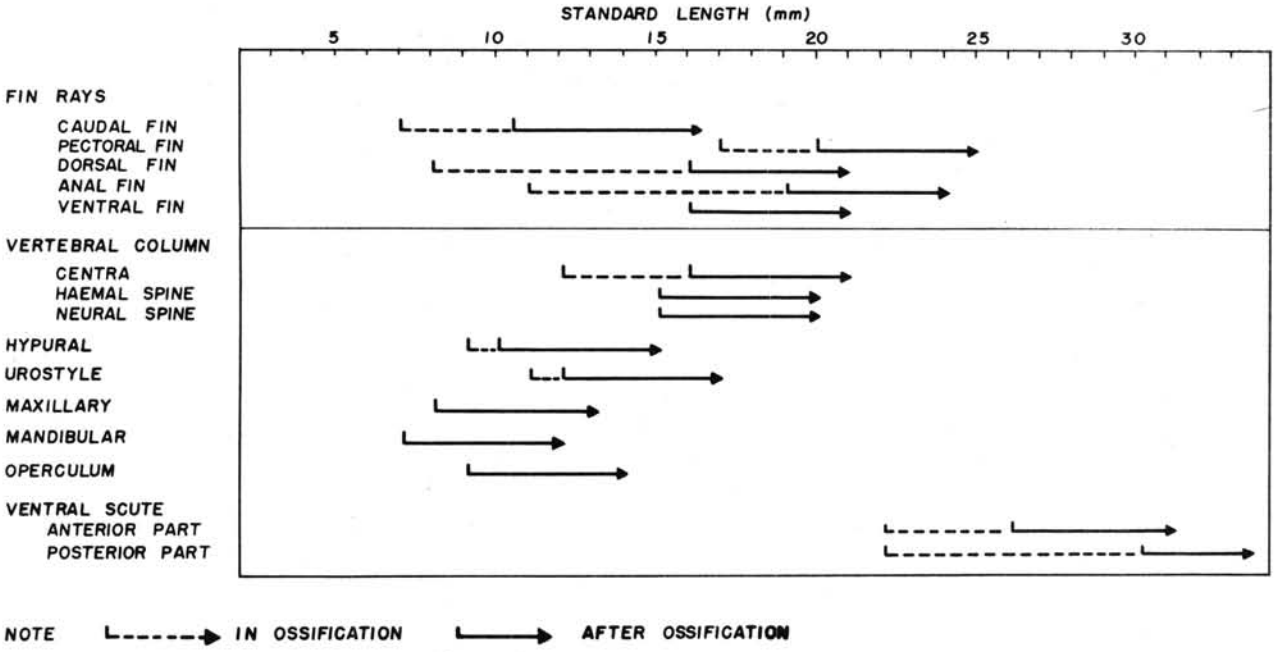

Fig. 5 - Diagramatic summary of the sequence of ossification of basic meristic structures in the larvae of *S. brasiliensis*.

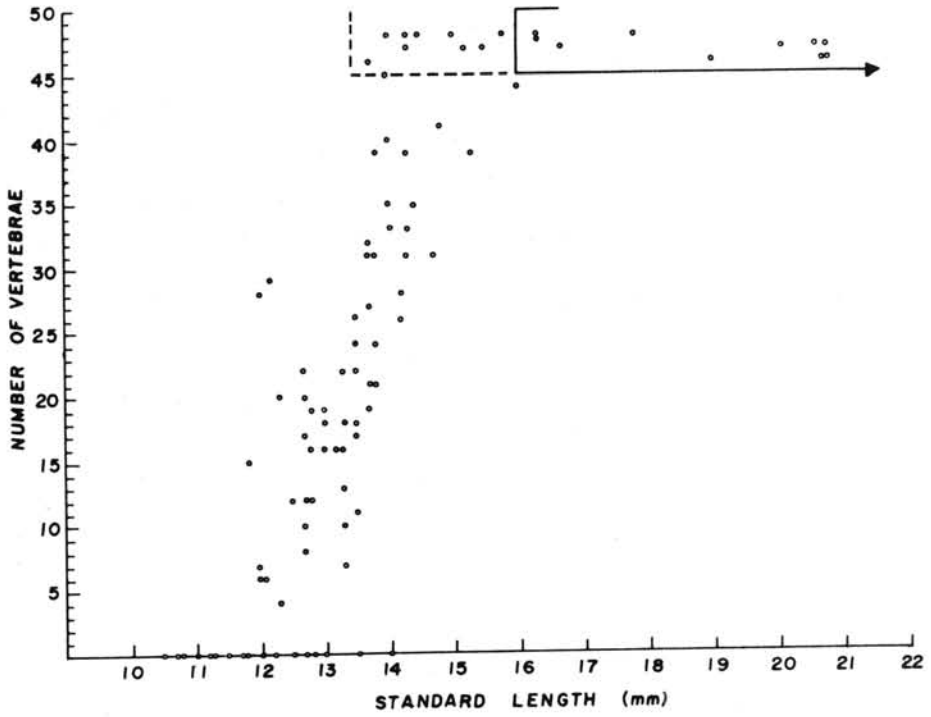

Fig. 6 - Diagram of number of vertebrae ossified in the larvae of *S. brasiliensis* between 10 and 22 mm (standard length).

The caudal fin has 10 principal rays in the upper lobe and 9 in the lower lobe. Six procurrent rays in the ventral side and seven rays in the dorsal side of the caudal fin are observed.

TABLE II - Meristic counts of *S. brasiliensis* larvae and juveniles

	n	Range	$\bar{x}$	s
Dorsal fin ray	42	18-19	18.54	0.5036
Pectoral fin ray	38	15-17	15.97	0.6360
Anal fin ray	40	16-19	17.80	0.7578
Caudal fin ray*	41	19	19.00	0.0000
Ventral scute:				
Anterior	19	19-20	19.11	0.3152
Posterior	11	15-16	15.27	0.4670
Vertebrae	47	45-48	46.68	0.7831

* = principal ray
n = number of specimens
$\bar{x}$ = average of meristic counts
s = standard deviation

OSTEOLOGY OF THE CAUDAL SKELETON AND MAXILLARY BONE - Figure 7 shows the development of the caudal skeleton. The hypurals are the first to ossify in the caudal skeleton at the length of about 9 mm. Then the last three ural centra form a urostyle at about 11 mm.

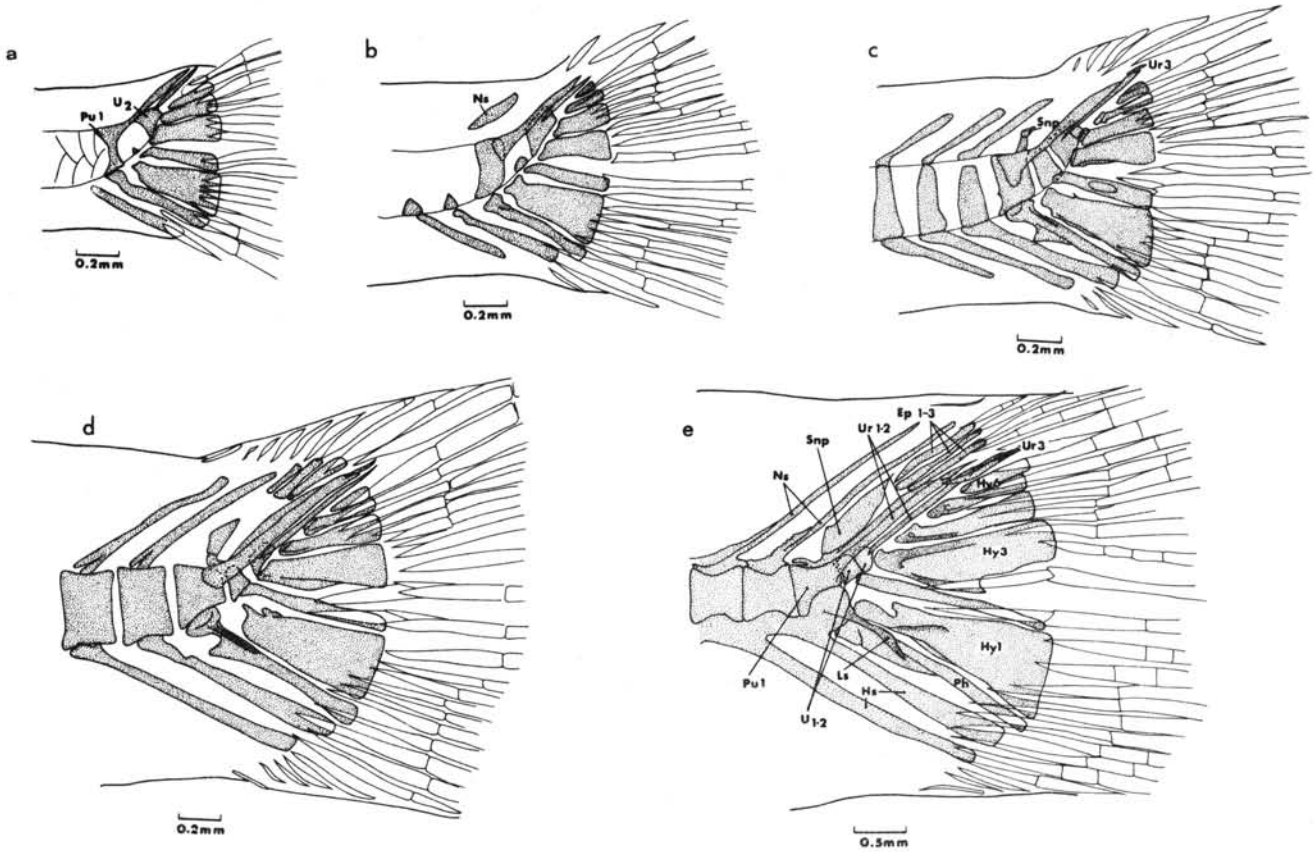

Fig. 7 - Caudal skeleton of *S. brasiliensis* larvae. a) 13.3 mm, b) 15.7 mm, c) 19.3 mm, d) 21.4 mm, e) 35.5 mm (standard length). $Hy_{1\sim6}$=Hypurals, $Ep_{1\sim3}$=Epurals, $U_{1\sim2}$=Ural vertebrae, Pu_1=1st Preural vertebra, Hs=Haemal spine, Ns=Neural spine, $Ur_{1\sim3}$=Uroneurals, Snp=Specialized neural process, Ph=Parhypural, Ls=Lateral spine.

At 13.3 mm, parhypural, hypurals and uroneurals are formed (Fig. 7*a*). At 15.7 mm (Fig. 7*b*), the uppermost 6th hypural appears. Some neural and haemal spines start to ossify in the caudal region. The lateral spine of the parhypural also appears at this size. At 19.3 mm (Fig. 7*c*), the specialized neural process appears and the third uroneural appears on the tip of the second uroneural. The lateral spine of the parhypural develops well at this size. At 21.4 mm (Fig. 7*d*), three epurals appear on the dorsal side of the uroneurals. The first hypural lacks the basal connection with the urostyle but a comblike process at the base is present. This comblike process was mentioned by Hollister (1936) as a peculiarity of the *Sardinella* species, and can be used to distinguish them from *Opisthonema oglinum*. The sharp point of the ventral distal edge of the third hypural (the fourth of Hollister) is visible only at the size of 35.5 mm. According to Hollister this characteristic is absent in *Opisthonema*. At 35.5 mm (Fig. 7*e*), considered as the juvenile stage, the specimen completes the ossification of the caudal skeleton. The specialized neural process develops upward and almost reaches the underside of the epurals. The last three ural centra fuse and form the urostyle.

At the length of 19.3 mm, the bases of the middle two rays of the caudal fin start to show an elongation. At 35.5 mm the bases of the two middle rays reach up to one fourth of the second hypural.

Figure 8 shows the development of the maxillary bone. In the oral region the ossification of the mandible starts at the length of 7 mm. The ossifi-

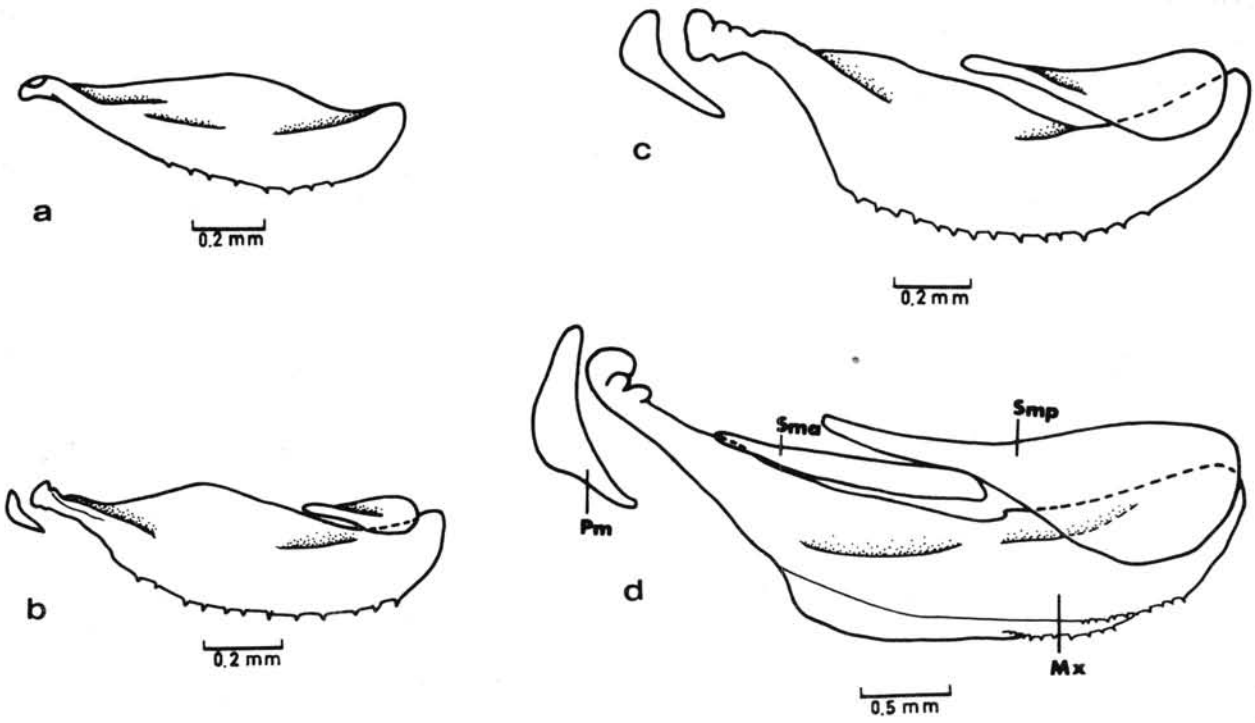

Fig. 8 – Development of the maxillary and associated bones of *S. brasiliensis* larvae. a) 15.7 mm, b) 19.3 mm, c) 21.4 mm, d) 35.5 mm (standard length). Pm=Premaxilla, Sma=Anterior supramaxilla, Smp=Posterior supramaxilla, Mx=Maxilla.

cation of the maxillary bones starts at about 8 mm. At this size only the maxilla is formed and bears some denticulations at the ventral edge. At 19.3 mm (Fig. 8*b*), the premaxilla and the posterior supramaxilla appear. At 35.5 mm (Fig. 8*d*), the anterior supramaxilla appears and the two rows of denticulations appears on the postero-ventral side of the maxilla. The hypomaxilla, characteristic of the *Harengula* species (Berry, 1964), does not appear in *Sardinella*. A space between the premaxilla and maxilla is occupied by unossified connective tissue.

RESUMO

O presente trabalho é parte do projeto SOL e tem por objetivo descrever a morfologia das larvas da sardinha verdadeira, *Sardinella brasiliensis* (=*S. aurita*), com o intuito de determinar um padrão que possibilite a identificação das mesmas. São feitas considerações sobre a morfologia das larvas de outros clupeídeos existentes na região e ressaltadas as diferenças com relação as larvas de *S. brasiliensis*. Os resultados, posteriormente, serão usados como base para o estudo quantitativo da abundância de larvas de sardinha.

O material foi coletado na costa sul do Brasil de 1969 a 1971, com uma rede de plâncton do tipo cônico-cilíndrico.

Durante o desenvolvimento das larvas, foi observado um consideravel deslocamento das bases das nadadeiras dorsal e anal, para uma posição mais anterior. Com o tamanho de 19 mm (comprimento padrão), ocorre uma mudança geral, consideravel, nas proporções corporais.

A ossificação de todos os raios das nadadeiras completa-se quando a larva atinge 20 mm, mas a ossificação dos escudos ventrais só se completa quando ela atinge 30 mm de comprimento. A ossificação das vertebras completa-se a 16 mm de comprimento.

O tamanho de 19 mm foi considerado como o fim do estágio larval, e, após este comprimento, consideramos a larva no estágio prejuvenil (=transformal).

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