ON THE MATURITY STAGES OF INDIAN OIL-SARDINE, *SARDINELLA LONGICEPS* VAL., WITH NOTES ON INCIDENCE OF ATRETIC FOLLICLES IN ADVANCED OVARIES

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Introduction

Workers engaged in research on the maturity and spawning conditions of fish have prepared different types of maturity scale according to the peculiarities presented by different fishes studied by them. Except for a few remarks about the external appearance of ovaries belonging to stages V to VII by Devanesan (1942), there is no detailed account of the different stages of maturity for *Sardinella longiceps*. Nair (1959) has adopted the maturity scale prepared by the International Council for the Exploration of the Sea for investigations on oil-sardine. However, when studies on the maturity and spawning habits of oil-sardine were taken up by the author, the absence of a detailed classification was keenly felt and since the International key was found to be not adequate enough to distinguish the different stages, it was considered necessary to prepare a detailed scale for the guidance of workers both in the laboratory and the field so that confusion in the interpretation and assignment of the stages of maturity can be avoided and a uniformity in eporting achieved. Moreover, it is seen from a perusal of literature on maturity stages of fishes in general (*vide infra*) that workers have only drawn up the maturity scales more as a guide for their detailed investigations on the biology of the fish in general or its reproductive cycle in particular rather than as a detailed study on the key itself. As a comprehensive study involving various factors like general external appearance, the extent of gonads in relation to length of the body cavity, length and weight of gonads, gonad: body weight ratio, maximum and modal size of the ova and their appearance under microscope has not been attempted so far, it is the purpose of this paper to present one such study. Although the histology of the gonads would enhance the value of such a study, it is felt that the following contribution may be of interest to those engaged in similar studies and be of some help in designing the maturity scale in other fishes.

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Previous Work on the Maturity Stages in General

The following resume is by no means an exhaustive one but only illustrative to show that no standard classification has been followed and how, according to the convenience of various workers and the peculiarities exhibited by different fish, the systems of classification of maturity stages are diversified. Since the publication of Hjort's scale of maturity for herring by the I.C.E.S. (quoted by Lovern and Wood, 1937), different workers have adopted different systems of classification while describing the various stages of maturity with the result the descriptions are not immediately comparable. Even in the number of stages, there is no uniformity, for, at one extreme, workers like Matsui (1950) in *Katsuwonus pelamis* and Bagenal (1957) in *Hippoglossoides platessoides* have recognised only four stages, at the other extreme, Clark (1934) has drawn up as many as twelve stages for *Sardinops caerulea*. In between, we have ten stages for *Sardinops ocellata* (Davies, 1956), eight stages for *Neoplatycephalus macrodon* (Fairbridge, 1951) and *Reporhamphus melanochir* (Ling, 1958), seven stages for the Australian clupeoids including *Sardinops neopilchardus* (Blackburn, 1941 & 1950), *Gadus merlangus* (Bowers, 1954; Gokhale, 1957) and Philippine tunas (Bu¯ag, 1956) and five stages for *Gadus merlangus* (Bull, 1928), *Euthynnus alleteratus* (Desylva and Rathjen, 1961), and yellowfin tuna and skipjack (Orange, 1961). In these studies, while some of the workers have used the Roman numerals, certain others have favoured the Arabic type. While all the workers have retained the word *stage*, the only exception appears to come from Matsui (*loc. cit.*) who has used the word *grade* instead. While some investigators like Powles (1958) have described the stages as immature, ripening, etc., without assigning any number to the stages, Davies (*loc. cit.*) is of opinion that the use of symbols like *i*, *a*, etc., to represent inactive, active and other nature of the gonad was far more satisfactory than numbers. On the other hand, we have the example of Clark (*loc. cit.*) who has used capital alphabets from A to L instead of numbers, to designate twelve stages. A few workers have also subdivided certain stages. Thus, Bull (*loc. cit.*) considers the spent as Stage II-A, whereas Stages II-A and III-A in the studies of Bu¯ag (*loc. cit.*) refer to intermediate stages between Stages II and III and III and IV respectively. The latter has also created Stage VII-A to accommodate the completely spent ovaries, while stage VII represents partially spent ovaries. Similarly, Orange (*loc. cit.*) bifurcates Stage V into A and B to accommodate recently spawned and post-spawning conditions. Holt (1959) in his recommendations on *Rastrelliger* research in the Indo-Pacific region has drawn up a five-stage key with three sub-divisions under Stage IV. In almost all these studies,

the factors that have been taken into consideration are the colour, the general appearance of gonads and their extent in the body cavity. Only Clark (*loc. cit.*) has based her classification on the modal sizes of ova. Bunag (*loc. cit.*) is, however, of the opinion that external characters are not always dependable and sometimes misleading also and hence has applied the largest ova diameter method, wherein the upper size limit of the largest size group of ova is taken as the deciding factor. Mention may be made here of Graham's (1924) classification with colour as the criterion and the works of Hickling (1930) in hake, Chrzan (1949) in Baltic cod and Qasim (1957) on *Blennius pholis* which have included the weight of the gonads or gonad: body weight ratio also in the descriptions of maturity stages.

Similarly, among the Indian works also there is diversity in the classification of fish gonads. While generally the trend of opinion is to follow the I.C.E.S. scale as far as possible with suitable modifications in the descriptive part of the different stages as seen from the studies of Seshappa and Bhimachar (1955) on Malabar Sole, Pradhan and Palekar (1956) on Indian Mackerel and Dharmamba (1959) on six clupeoids, there are deviations also in cutting short the number of stages, for, while Prabhu (1955) in *Trichiurus haumela* has found it convenient to have five stages, Tandon (1961) in *Seleroides leptolepis* has drawn up six stages. Nayak (1959) recognises only four stages in the case of *Polydactylus indicus* whereas Karekar and Bal (1960) following the lines of Clark (*op. cit.*) have drawn up 14 stages for the ovaries of the same fish. However, the latter have indicated how these stages can be conveniently condensed to correspond to the standard seven stages of I.C.E.S. scale. Except for them and to a certain extent Dharmamba (*loc. cit.*) who have taken into consideration the diameter range and modal size of ova, others have based their classification on the general appearance, colour and extent of gonads in the body cavity. There appears to be no work wherein the weight of the gonads or the gonad: body weight ratio or the gonad length has been taken into account.

Methods

In the following account all the descriptions relate to fresh material only except the modal sizes of ova which refer to 2% formalin preserved material. The range of quantitative values and the averages mentioned in Tables I and II for male and female respectively relate to the entire period of investigation from 1959 to 1962. The length of body cavity is taken as the distance from the lower tip of the heart to the anal opening and the gonad: body weight ratio is referred to as percentage relative weight.

Description of Maturity Stages

Male

Stage I (Immature).—The testes are extremely small, often recognised only as translucent filamentous strands in the early stages. Later, when easily visible, they are small, opaque, pinky-white, leaf-like structures with a fairly long vas deferens, which get easily snapped when the testes are removed. There is very little asymmetrical development and, if any, the left testis is slightly longer. The testes with the vas deferens occupy roughly 50% of the length of the body cavity or occasionally slightly more, measuring a maximum overall length of about 35 mm, of which the testicular portion may form 10 to 15 mm only. The gonad weight ranges up to 0·2 gm, but it is usually less than that. Their relative weight to body weight is normally below 0·8%. This stage is encountered during the whole of oil-sardine

Table I. *Range in values of various factors with the averages in brackets for different stages of maturity (male) in* Sardinella longiceps

Stage	No. of fish	Total length (mm)	Length of body cavity (mm)	Length of testes (mm)	Weight of testes (gm)	Relative weight %
I	35	127-149	47-59	23-35 (30)	0·03-0·22 (0·10)	0·15- 0·92 (0·64)
II *a*	14	131-161	49-61	26-39 (37)	0·24-0·48 (0·39)	1·03- 1·67 (1·42)
II *b*	84	152-188	58-74	29-38 (35)	0·08-0·29 (0·15)	0·20- 0·68 (0·51)
III	17	144-185	53-73	36-51 (41)	0·90-2·10 (1·23)	2·47- 4·24 (2·91)
IV	139	124-189	47-74	40-70 (59)	2·52-5·14 (3·74)	5·10- 7·20 (5·56)
V	70	142-202	51-78	50-82 (68)	6·21-8·56 (7·33)	10·33-14·47 (12·52)
VI	22	167-190	64-74	73-84 (76)	9·10-12·82 (10·13)	15·67-20·88 (17·34)
VII *a*	34	147-188	56-73	40-60 (49)	1·24-2·62 (2·02)	2·52- 4·66 (3·64)
VII *b*	21	159-189	60-74	30-45 (41)	0·22-1·10 (0·48)	0·43- 2·07 (1·34)

fishery season from September to March. The maximum values of weight given above are obtained during the end of the season.

Stage II a (*Developing Virgin*).—The testes are thicker and more elongated. They are opaque, pink or white in colour, with vas deferens reduced but thread-like. Asymmetrical development has set in and the left testis is almost always longer. The testes with the ducts extend to 50–60% of the body cavity and measure 30 to 40 mm and the testicular portion measures about 25 to 30 mm. Their weight ranges from 0·2 to 0·5 gm, but it is usually around 0·4 gm. The relative weight varies from 1·0 to 1·7%. Fish in this stage are found during April and May.

Stage II b (*Spent-resting*).—The testes are pinkish or brownish-white in colour but different from the previous stage in having shrunken and wrinkled appearance when viewed against light, showing that the organs are not compactly filled with germ cells. The left testis is usually longer. The vas deferens is a much wider duct than the thread-like passage of the previous stage and is covered by the lower halves of the testes which are narrower and extend almost to the posterior end of the body cavity. With the degree of opacity varying, there are a few patches of semi-opaque regions. The organs fill up 50 to 55% of the body cavity and measure almost the same length as the previous stage, *i.e.*, 30 to 40 mm, but their absolute and relative weight are much less. While the former ranges up to 0·29 gm with an average of about 0·15 gm, the latter is about 0·5%. This stage is met with during September to April.

Stage III (*Maturing*).—The testes are well developed and thickened, white in colour. Vas deferens, being filled with spermatogonia, are reduced and measure less than 15 mm. The left testis is distinctly longer and this condition persists in the subsequent stages also. The gonads varying in length from 36 to 50 mm occupy 70 to 75% of the body cavity. Their weight ranges up to 2·0 gm but it is usually around 1·0 gm forming 2·5 to 4·0% as relative weight. The occurrence of this stage is very brief, usually restricted to late May—early June.

Stage IV (*Maturing*).—Quite massive and creamy-white are the testes with vas deferens hidden under them. The organs, while extending to the entire breadth of the body cavity, occupy 85 to 90% of the body cavity length, measuring 40 to 70 mm. Their absolute weight ranges from 2·5 to 5·0 gm, but in majority of cases it is around 3·5 gm. Their relative weight varies from 5·0 to 7·0%. Fish of this stage are common during June and July, but occasionally may be obtained in August also.

Stage V (*Mature*).—The testes are opaque white in colour, soft, more extensive than Stage IV and occupy the entire length of the body cavity but very often even more with the result their anterior ends tend to fold down along the ventral body wall. On a little pressure internally at the posterior end, spermatic fluid oozes out. Their usual length is 65 to 70 mm but can attain even 80 mm sometimes. Their absolute weight ranges from 6·0 to 8·0 gm, with an average around 7.0 gm. The relative weight works up to 10·0 to 14·0%. The fish in this stage are found in July, August, September months and very rarely in October.

Stage VI (*Running*).—The testes are very extensive, white in colour and fill the entire space of body cavity displacing the intestines to a fraction of space. Not only the anterior tips are folded down along the ventral body wall but even the outer margins extending along the sides of the body wall curve towards the middle so much so very often an insertion along the mid-ventral line cuts through the outer edges of the testes. Under a slight pressure externally on the flanks of the fish or even while handling, milt extrudes out. The organs always measure more than 70 mm and weigh from 9·0 to 13·0 gm with an average around 10·0 gm and the relative weight ranges between 15 and 20%. The fish in this stage are very rarely encountered in the landings but, if available, July to October is the period of their occurrence.

Stage VII a (*Partially Spent*).—The testes are meat-coloured, a bit leathery in texture, shrunken with wrinkles and semi-opaque spaces visible when viewed against light. Measuring 40 to 60 mm, they occupy 70 to 80% of the body cavity and weigh usually 2·0 gm but may range from 1·0 to 2·5 gm, which forms 2·5 to 4·5% as relative weight. This stage is encountered during July to September.

Stage VII b (*Spent*).—The testes are deep flesh-coloured, shrunken, flat, strap-like, shrivelled with translucent patchy regions. They occupy 50 to 60% of the body cavity and measure 30 to 45 mm. Their weight is around 0·5 gm, forming about 1·5% as relative weight. This stage is encountered from August to early November.

The general extent of the testes in relation to the body cavity for the above stages is shown in Fig. 1.

Female

Stage I (*Immature*).—The ovaries are soft cylindrical and almost translucent, pink or flesh-coloured. Sometimes due to post-mortem changes

they appear purple in colour. The surface of the ovary is smooth with no distinct blood vessels. There is very little asymmetry in the size of the ovaries. The oviduct is fairly long and completely transparent with the result, the ovarian bodies look like detached stubs, short and plump. The entire length of the ovaries with their ducts occupies about or slightly more

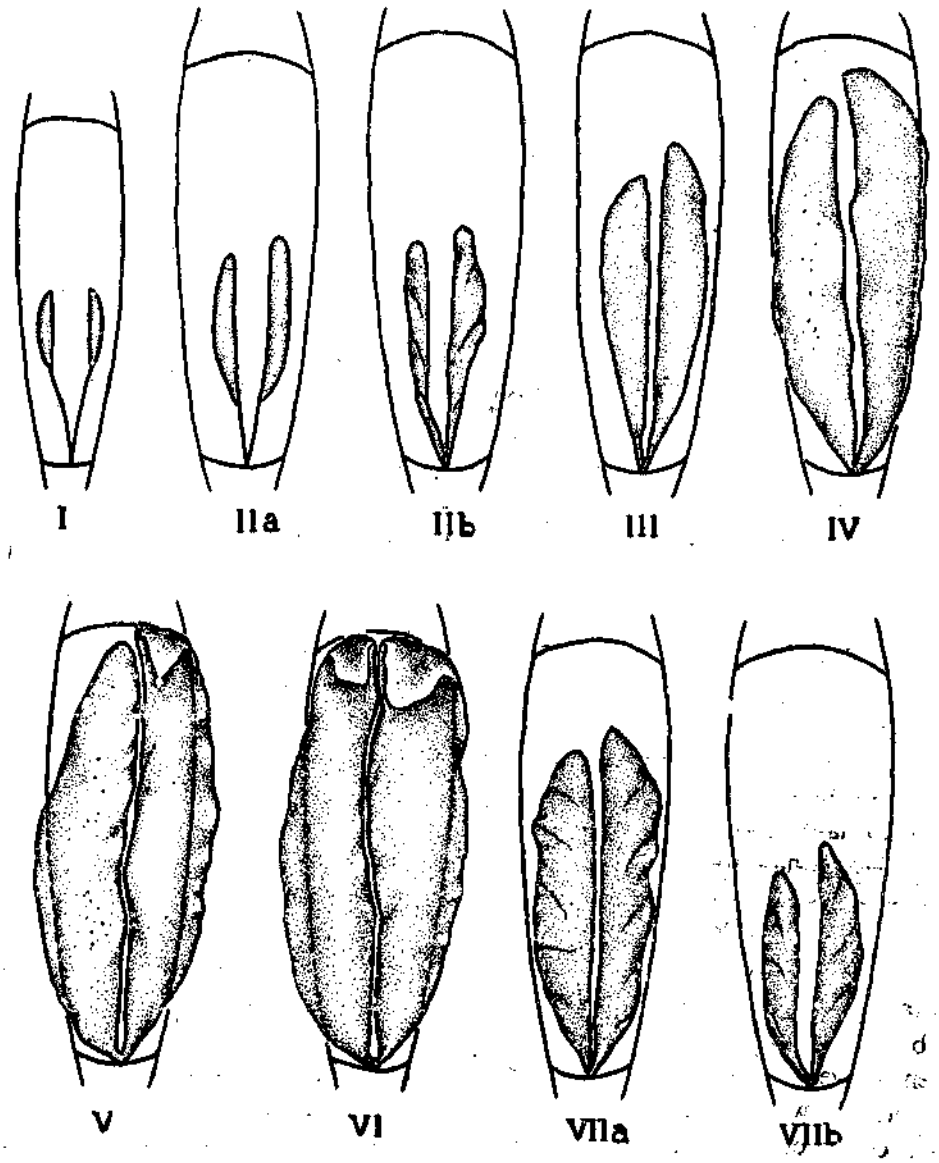

FIG. 1. Diagrammatic representation of different maturity stages of male oil-sardine.

than 50% of the body cavity and measures up to about 35 mm of which the length of the ovary alone ranges from 10 to 25 mm. Their absolute weight may be about or below 0·25 gm forming a maximum of 0·8% as relative weight. The ovaries are compactly filled with oocytes, not visible to naked eye. The oocytes are yolkless and transparent and measure up to 0·13 mm with the majority of them in the size range of 0·07 to 0·09 mm. Fish in this stage form the mainstay of commercial fishery from September to April but abundant during October to December.

Stage II a (*Developing Virgin*).—Cylindrical, soft, translucent ovaries pink or flesh-coloured. Asymmetry is not quite distinct yet. Oviducts, thin and thread-like, are a little reduced in length and not more than 10 mm. The overall length of the gonads ranges from 30 to 35 mm forming 55 to 60% of body cavity. The ovaries alone measure about 20 to 25 mm in length and usually weigh around 0·4 gm, but may range from 0·3 to 0·6 gm. The relative weight is 1·0 to 1·5%. Majority of the ova are transparent with signs of yolk formation in some, which are mostly semi-opaque but sometimes fully opaque with or without translucent periphery. However, even these do not appear as distinct grains to be easily recognised with naked eye. Maximum diameter of ova recorded is 0·30 mm with a large number of ova ranging in size from 0·15 to 0·18 mm. This stage is seen only for a short time during May.

Stage II b (*Spent-resting*).—The ovaries are dark-red or brownish-red or deep flesh-coloured, having a collapsed and flattened appearance. External surface is wrinkled. The tunica is thicker and the oviducts much wider and shorter than in the previous stage. The length of the organs is 30 to 45 mm occupying 55 to 60% of the body cavity. Their weight is commonly around 0·2 gm, forming less than 0·5% as relative weight. Occasionally, late spawners resting in January-February period may record a maximum gonad weight of about 0·4 to 0·5 gm with their relative weight of about 0·8%. Majority of ova are transparent, not visible to naked eye and measure 0·07 to 0·11 mm. A few scattered opaque ova may be present without transparent periphery and measure up to 0·15 mm. This stage is characteristically distinguished by the presence of clots of blood cells appearing as brownish masses in between the oocytes. Moreover a portion of ovary when viewed under microscope shows small empty spaces between groups of oocytes, whereas in developing virgins, the oocytes overlap each other, being compactly arranged on the lamellae with no spaces in between. This stage is obtained during November to April.

TABLE II. *Range in values of various factors with the averages in brackets for different stages of maturity (female) in* Sardinella longiceps

Stage	No. of fish	Total length (mm)	Length of body cavity (mm)	Length of ovaries (mm)	Weight of ovaries (gm)	Relative weight (%)
I	52	123-147	46-58	25-33 (30)	0·09- 0·24 (0·17)	0·30- 0·83 (0·70)
II *a*	17	134-159	50-60	30-37 (35)	0·25- 0·55 (0·39)	0·90- 1·52 (1·35)
II *b*	80	157-206	60-82	32-44 (37)	0·22- 0·48 (0·27)	0·25- 0·83 (0·44)
III	15	137-181	50-71	35-50 (43)	0·78- 1·47 (0·92)	2·21- 4·23 (2·82)
IV	164	131-188	49-74	46-65 (54)	2·12- 4·46 (3·34)	4·48- 7·13 (5·32)
V	19	162-191	61-75	65-82 (67)	5·51- 7·32 (6·48)	9·20-12·79 (10·65)
VI	9	173-190	64-74	72-84 (74)	8·24-11·76 (8·90)	13·27-16·79 (14·50)
VII *a'*	59	158-185	58-73	40-60 (47)	1·47- 2·85 (2·12)	2·54- 4·79 (3·82)
VII *b*	24	161-188	61-74	33-47 (38)	0·33- 0·97 (0·45)	0·65- 1·54 (1·23)

Stage III (*Maturing*).—The ovaries are turgid, opaque and yellow in colour with granular appearance. Development of blood vessels is perceptible. The oviducts are very much reduced. Usually there is asymmetrical development in the size of the ovaries, either gonad longer than the other. This condition persists in the subsequent stages also. The ovaries occupy about 65 to 70% of the body cavity and measure from 35 to 50 mm. Their weight ranges from 0·8 to 1·5 gm, but usually it is below 1·0 gm. The relative weight may accordingly vary from 2 to 4%. The maximum diameter of ova recorded is 0·54 mm. The size frequency distribution of ova normally shows one distinct mode of maturing ova around 0·35 to 0·40 mm, which are opaque with translucent periphery and visible to naked eye. In these ovaries, some semi-opaque ova with yolk deposition around the centre measure 0·15 to 0·17 mm and form a good percentage. Sometimes, ovaries advanced a little further but not entered into Stage IV show two modes of maturing ova, an advanced one at 0·42 to 0·47 mm and a minor one around

0·22 to 0·27 mm. Both sets of ova are opaque and are provided with translucent periphery. This stage is of very short duration limited to late May to early June.

Stage IV (*Maturing*).—The ovaries are compact, vascular with conspicuous blood vessels on the tunica and bright yellow in colour. Oviducts are not quite distinct. The organs almost extend to the entire body cavity forming 80 to 90% of the latter's length, with its own length varying from 45 to 65 mm. They weigh about 2·0 to 4·5 gm with an average of 3·0 gm, forming 4·5 to 7·0% as relative weight. The largest ova may measure about 0·67 mm and the size frequency polygons show two distinct modes, one anywhere between 0.51 and 0·57 mm and the other around 0·27 to 0·34 mm. The former are completely opaque, while the latter are provided with translucent periphery. June to August is the period of availability of fish in this stage, either in a typical condition as described above or a bit advanced further but not belonging to Stage V.

Stage V (*Mature*).—The ovaries are orange-yellow in colour, fully vascular with prominent blood vessels ramifying on the surface. Tunica is very thin and bursts at slight pressure. Ovaries are very often more than the length of the body cavity with the anterior tips taking a loop down. Their normal length is 65 to 70 mm, but according to the size of fish may even extend up to 80 mm. Their average weight is 6·5 gm with a range of 5·5 to 7·5 gm. Relative weight ranges from 9 to 13%. Maximum diameter of ova observed is 0·82 mm. The distribution of ova shows two groups, an advanced mature one anywhere between 0·62 and 0·67 mm. and a maturing group around 0·35 to 0·47 mm. The ova of former group present varying appearances; some are completely opaque, some provided with narrow or wide transparent periphery, some vacuolated, some partly transparent and a few fully. Completely transparent ova have one big oil globule or two-three smaller droplets of oil globule which measure from 0·054 to 0·109 mm and the other partly transparent and vacuolated ova have a number droplets of oil globule. The ova of less advanced mode are fully opaque. This stage is available during July to September but fish in this stage are very rarely encountered in the landings.

Stage VI (*Running*).—The ovaries appear as a sort of cream-coloured cellophane bags filled with boiled sago. At a slight prick, a gelatinous mass of transparent ova flows out, the tunica being so thin. Ova can be extruded on slight pressure externally on the flanks of the fish or even while handling. The ovaries measure more than 70 mm and fill the entire space of abdomina

cavity displacing the intestine to a narrow space in between the two ovaries. They may weigh from 8·0 to 12·0 gm, but ordinarily around 9·0 gm, forming 13 to 17% as relative weight. The largest ova are transparent and jelly-like reaching a maximum diameter of 1·2 mm, but the majority of these range from 0·80 to 0·91 mm in diameter with one or two oil globules very rarely cleaved into three which measure 0·091 to 0·127 mm. There is a significant number of medium-sized ova forming another distinct mode between 0·46 and 0·51 mm, which are completely opaque or provided with transparent periphery. Very rarely fish in this stage are obtained but when available they are seen during July to October.

Stage VII a (*Partially Spent*).—The ovaries are dark red in colour either throughout the length or only at the posterior half. They are a bit flaccid and collapsed with slight wrinkles on the surface. The ovarian lamellae are clearly seen as book leaves especially at the posterior region indicating that the lamellae are not compactly filled with maturing ova and that some have been shed. The ruptured blood capillaries produce blood clots which appear as brownish or reddish masses. Blood capillaries penetrate deeply into the interior. Although the ovaries are shrunk in volume and reduced in breadth, they extend to 70 to 80% of the body cavity measuring 40 to 60 mm. Their weight ranges from 1·5 to 3·0 gm but is usually around 2·0 gm, amounting to 2·5 to 5·0% as relative weight. The maximum diameter of ova is about 0·60 mm and the frequency distribution shows only one distinct mode anywhere within 0·35 to 0·51 mm. These ova are completely opaque but a few of these perhaps in the process of resorption are translucent with greyish yolk in the centre within which may be found a few droplets of oil-globule. Fish in this stage are encountered during July–September period.

Stage VII b (*Spent*).—The ovaries are elongated, honey-coloured, bloodshot, flabby, limp and gelatinous with wrinkles on surface due to collapsed condition. The tunica is leathery and the wide oviduct is now recognizable. The ovaries measure about 30 to 45 mm and occupy 55 to 60% of the body cavity. They almost always weigh around 0·5 gm with a maximum limit of 1·0 gm, forming 0·6 to 1·5% as relative weight. Recently, spent fish have remnants of mature ova, measuring a maximum diameter of 0·47 mm, as resorbing and disintegrating opaque structures. The blood cells from ruptured capillaries appear as reddish clots. Sometimes, there may be a few scattered droplets of oil globule. The resorbing eggs are sometimes translucent in appearance, with the yolk in the form of small spherules, light grey or brownish in colour with many droplets of oil

globule clustering around the centre. These ova form a small mode in the size frequency distribution around 0·27 to 0·31 mm. At a later stage, a few blood-coloured or brownish masses are seen which represent the unspawned ova broken down and covered up by the blood cells. In this state, the ovaries appear deep flesh-coloured. The rest are all immature transparent oocytes less than 0·25 mm in diameter. This stage is encountered from August to early November.

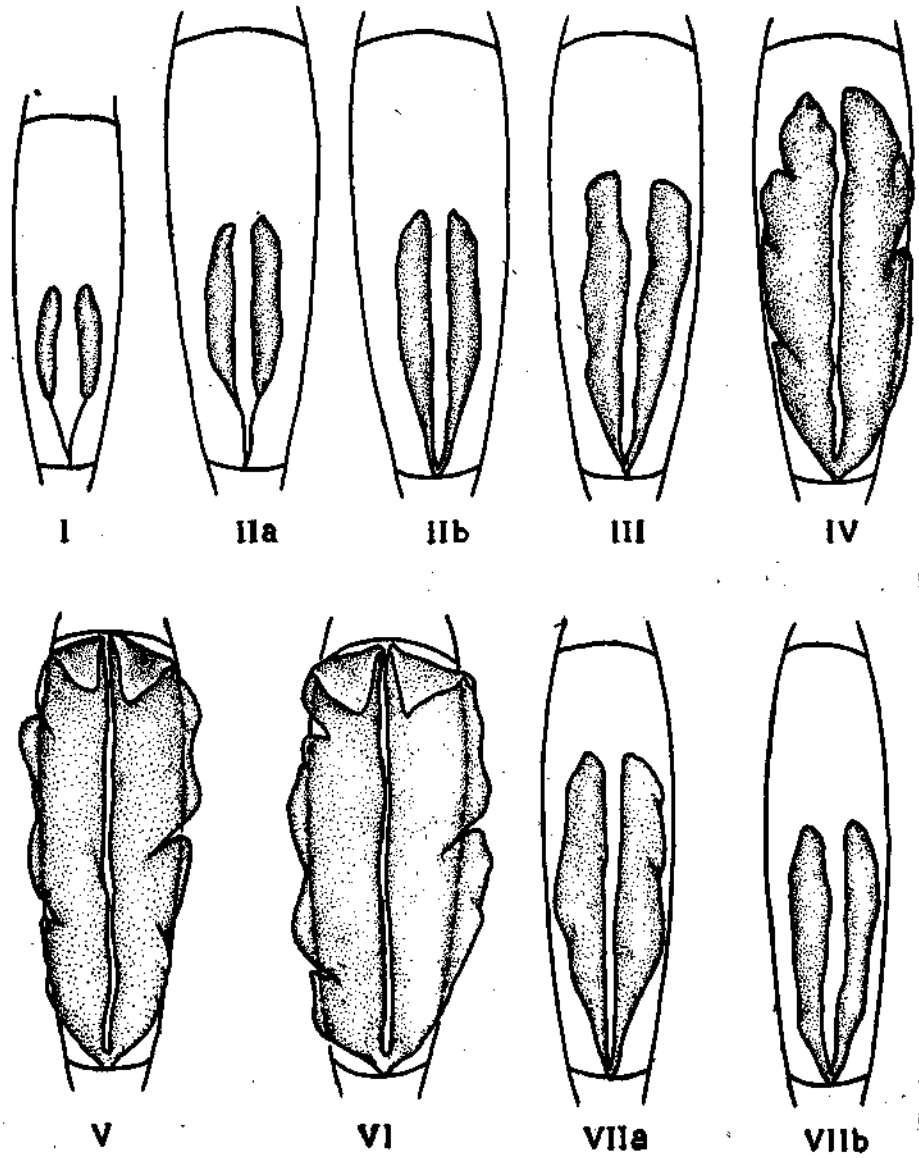

FIG. 2. Diagrammatic representation of different maturity stages of female oil-sardine.

The general extent of ovaries in relation to the body cavity for the above stages is shown in Fig. 2.

The characteristic features of external appearance and extent of gonads in relation to the body cavity are presented in Table III as a field key with the International key for herring for comparison.

Atretic Follicles in Advanced Ovaries

An interesting feature seen during the study of mature ovaries, especially, in July and August, was incidence of vascular hypertrophy and follicular breakdown as the ovaries progress from Stages IV to V. In these ovaries, the bright yellow colouration of Stage IV is lost, replaced by a diffused red colouration. Sometimes, dark reddish patches are seen on the surface of the ovary. These changes in colouration manifest themselves only on the ventral side of the ovary, while the dorsal side attached to the wall of the body cavity remains yellow. Macroscopically, through tunica the lamellae appear loosely packed with a few empty spaces between groups of opaque ova but in fact, these spaces are filled up with immature ooëytes and fully or partially transparent large ova. Even the oil globules, as glistening refringent yellow bodies, can be seen scattered beneath the tunica. Under the microscope, along with large opaque ova measuring 0·55 to 0·64 mm, a good number of larger ova are seen free in the lumen of the ovary presenting various appearances. Some are partly transparent with granular yolk and droplets of oil globule clustering around the centre. Some are fully transparent but shrunken and shrivelled or in the process of disintegration, releasing the oil globules. A few empty egg shells are also noticed. These disintegrating ruptured ova normally measure within a range of 0·55 to 0·73 mm, but instances are found even in larger ova up to 1·09 mm. In a few cases, there has been only vascular hypertrophy but without any incidence of follicular breakdown. In the size frequency distribution of ova of these ovaries, two modes are present, the more advanced group belonging to 0·49 to 0·61 mm size range and the minor mode comprising ova from 0·25 to 0·34 mm. The significance of this phenomenon is discussed in the subsequent section.

General Remarks

When any classification of maturity stages is devised, as far as possible, it must try to serve as an index to the spawning period of the fish, especially if it is of seasonal character. The yolk deposition in the oocytes is an indication

TABLE III. *Field key for stages of sexual maturity of* Sardinella longiceps *with I.C.E.S. maturity scale for comparison*

Stage	OIL-SARDINE		HERRING
	External appearance and extent of gonads		
	Male	Female	Male and Female
I	Often recognised as translucent filamentous strands. When easily visible, pinky-white in colour, leaf-like with long vas deferens. With the duct, organs occupy about half the length of body cavity.	Ovaries soft, cylindrical, almost translucent, pink or flesh-coloured. Oviduct fairly long and completely transparent with the result ovarian bodies appear as detached stubs, short and plump. With the duct, organs extend to about half of body cavity.	Sexual organs small, breadth about 2–3 mm. Clear wine or amber-coloured. No eggs visible to naked eye.
II *a*	Opaque, pink or white. Vas deferens reduced and thread-like. With the duct, extend to slightly more than half the length of body cavity.	Soft cylindrical, pink or flesh-coloured. Oviducts thin and thread-like and reduced to not more than 10 mm. With the duct, occupy slightly more than half of body cavity. Ova not visible to naked eye.	Diameter nearly 1 cm, length slightly more than half the length of body cavity. Eggs small unyolked-not visible to naked eye. (Developing immature and recovering spents).
II *b*	Pink or brownish-white, tapering posteriorly, shrunken and wrinkled. Vas deferens wider than II *a*. Degree of opacity varies at different regions with semi-opaque patches. Organs occupy about half or slightly more than half of body cavity length.	Dark red or brownish-red or deep flesh-coloured with collapsed and flattened appearance and tapering posteriorly. Surface wrinkled, tunica thicker. Oviducts wider but shorter. Extend to slightly more than half of body cavity length.	
III	Thickened testes, white. Vas deferens not more than 15 mm. Occupy about three-quarters of body cavity.	Turgid, opaque and yellow with granular appearance. Oviducts very much reduced. Extend to more than half the length of body cavity but less than three-quarters. Ova visible to naked eye.	Organs occupying about half of the body cavity. Eggs amber coloured, yolked.

IV	Quite massive, creamy-white with vas deferens hidden under testes. More than three quarters of body cavity.	Compact, vascular, bright yellow ovaries. Oviducts not distinct. Occupy more than three-quarters of body cavity.	Organs occupying two-thirds of the body cavity. No large transparent eggs.
V	Opaque white, soft and slightly more than the body cavity with their anterior tips folding down. Under some pressure internally at the posterior end, spermatic fluid oozes out.	Orange yellow and fully vascular. Tunica being thin bursts at slight pressure. Slightly more than the length of body cavity with the anterior tips curling down.	Organs filling body cavity. Some large transparent eggs may be present.
VI	Very extensive, white in colour and fill the entire space of body cavity displacing the intestine to a fraction of space. Both the anterior tips and the outer edges curve towards interior. Milt extrudes while handling or under slight pressure externally on the flanks of the fish.	Appear as cream-coloured cellophane bag filled with boiled sago At a slight prick, gelatinous mass of transparent ova flows out. Ova may be extruded under slight pressure externally or even while handling the fish. More than the length of body cavity and more extensive displacing the intestine to a small space in between the organs.	Spawning in progress or just imminent. Large transparent eggs.
VII *a*	Meat-coloured, slightly shrunken with wrinkles on the surface and semi-opaque regions. Leathery in texture. Occupy about three-quarters of body cavity.	Dark red either throughout or only at the posterior half. A bit flaccid, shrunken with wrinkles on the tunica. Ovarian lamellae clearly seen as book leaves. Occupy about three-quarters of body cavity.	..
VII *b*	Deep flesh-coloured, flat, strap-like, shrivelled with translucent patches. Occupy about half the length of body cavity or slightly more.	Elongated, honey-coloured, bloodshot, flabby, limp, flattened and gelatinous with wrinkles on the surface. Tunica leathery. Wide oviducts now discernible. Occupy slightly more than half of body cavity.	Spent. Organs bloodshot. Large residual eggs.

that the process of ripening has been initiated and the interval between this condition and full maturity, when transparency sets in the ova, shows the growth of ovary through certain stages. The time taken at each of these intermediate stages would then throw light on the rate of ripening of sexual products, which is important in understanding the reproductive cycle of the fish. But in a four or five stage key it would be difficult to clearly understand the period of maturing stage, for, then, Stages II to IV of a seven-stage key will have to be condensed under one stage and this will obliterate the time element. On the other hand, a scale of larger number of stages, although it may carry greater accuracy and finer details, may be not only cumbersome, but may be of use only in the laboratory and may not be applicable in field studies. Neither is it advisable to have two different keys with different numerical notations for laboratory and field. The seven stages recommended by I.C.E.S. appear to be under the circumstances ideal in satisfying both the requirements of the laboratory and the field and hence, a seven-stage scale has been prepared in this study with subdivisions under Stages II and VII.

As can be seen from Table III, there is a sort of ambiguity in the description of Stages II and III in the I.C.E.S. maturity scale. While the gonad length of Stage II is "slightly more than half the length of body cavity", organs belonging to still advanced stage, namely, Stage III, "occupy about half of body cavity" (Lovern and Wood, 1937). It is not clear whether for Stage III, the space of body cavity is taken into consideration or only its length. Perhaps, the ambiguity might have resulted because of clubbing together both the virgin developing and spent recovering state under Stage II, as workers in this field have very often done, due to practical difficulties in distinguishing the two conditions. Hence an attempt has been made in this study to distinguish the differences between the above said two conditions and classify them as subdivisions of Stage II.

Hornell and Nayudu (1924) have found during September and October a considerable percentage of males and females with gonads varying from half to three-quarters the mature size and were inclined to doubt them as partially spent. Devanesan (*op. cit.*) has also come across during the above months, fish which were completely spent and also in various stages of being spent and found difficulty in distinguishing half-spent testes from immature testes, half-developed. However, Nair (*op. cit.*) has not encountered during his studies any partially spent fish. Hickling and Rutenberg (1936) have observed that in the case of fractional spawning some other method of recording the stages should be used in view of difficulties in following the

International key. Hence in the present classification, the partially spent fish are assigned to a subdivision of Stage VII, *i.e.*, VII *a* in the lines of earlier workers who have put such a state of maturity as a penultimate stage before the completely spent condition. Thus, Fairbridge (*op. cit.*) in the case of *Neoplatycephalus macrodon* has put such instances under Stage VII with Stage VIII as spent. Bunag (*op. cit.*) in his study on Philippine tunas, classifies them as Stage VII, with Stage VII—A as completely spent. In his recommendations for a five-stage key for *Rastrelliger*, Holt (*op. cit.*) places the partially spent fish under Stage IV *c* and the completely spent fish under the next, Stage V.

Although Devanesan (*op. cit.*) has stated that his efforts to classify the maturity stages were parallel to the studies on herring, his classification and descriptions of Stages V to VII in *S. longiceps* are a bit confusing and misleading, as shown below:—

"*Stage V*: Ovary reddish with small opaque eggs, one-third or quarter of the full size.

Stage V (a): Ovary yellowish with small eggs, one-third or quarter of the full size.

Stage V (b): Ovary partly yellowish and/or partly pinkish with slightly larger eggs showing a tendency to become translucent.

Stage VI (a): Ovary large, pinkish, nearly fills the visceral cavity and reaches into thorasic region. . . .Almost all the eggs are large and transparent. Some loose free ova are also present.

Stage VII: Spent ones. The ovary is a cylindrical tube, reddish with a few ova."

It appears from the above descriptions that ovaries which belong to, perhaps, much earlier stages are placed in Stages V and V *a*. Even the stage V *b* appears to be rather Stage IV than a typical Stage V of the classification given in the preceding pages. Further, it is not clear why a fully mature ovary is designated as VI *a* with no further subdivisions to follow. It is possible that since his observations were based on collections made during September and October only, certain amount of confusion between Stages IV, VII *a* and VII *b*, has resulted in the above classification, for even according to him, ovaries during these months were "in various stages of being spent".

As to the nature and cause of atresia in advanced ovaries, a few possibilities may be examined here. The question of adolescence setting in these

ovaries as reported in the case of hake (Hickling, *op. cit.*) and Norway pout (Gokhale, *op. cit.*) remains to be studied but it is unlikely that a tropical fish matures to advanced state, but does not spawn, especially, when the life span is of short duration. The possibility that this may be due to post-mortem changes because of quicker decomposition of the alimentary tract lying in contact with the ventral side of the ovary, which in turn affects immediately the ventral surface of the latter and hence the marked red colouration only on that particular side, does not appear very convincing since all the ovaries of the same sample did not exhibit this phenomenon and even very fresh material examined immediately after they were caught in the near-shore area presented this feature. If it is only due to proximity to the digestive system that the ventral side starts decomposing earlier, then, after some time lapse, it should set in throughout the organs, which was not found to be so. It is also doubtful whether it is a simple case of follicular breakdown as reported by Gokhale (*loc. cit.*) wherein it involves only the breaking of oolemma and the phagocytosis of the cytoplasm, for, according to him, the blood cells do not take any part in this atresia, whereas the distinguishing feature of the present phenomenon is vascular hypertrophy and rupture. Another possibility is that the fish, which are heading for the spawning grounds, have started developing ripe ova and entered into Stage V, but when they are caught at that moment, all these ripe ova get ruptured due to shock, for, it has been shown that such disturbances can affect the ovo-follicular system (Bretschneider and Duyvene de Wit, 1947). The presence of a few partly as well as fully transparent ova around the external oviducal opening in most of these fishes appears to be an indication in support of this possibility. It appears also likely that the disturbance may be physiological—a setback in the spawning rhythm of the fish due to some unfavourable conditions not providing the required stimulus for the act. A doubt may arise whether these are residual eggs after spawning. The process of resorption of ova in both pre-ovulation and post-ovulation phases is roughly the same but while in the former case only a few of the larger eggs are involved in this breakdown, not affecting the bimodal distribution of yolked ova in the frequency polygon, in the latter they represent the real residual eggs of that group of ova which has been shed, thus leaving a picture of unimodal appearance of the secondary group only.* There are other differences also such as the general appearance of the ovaries, their absolute and relative weight.

* Antony Raja, B. T. 1967 .. Some aspects of spawning biology of Indian oil-sardine, *Sardinella longiceps* Valenciennes. *Indian J. Fsh.*, **11** (1) A, 45–120 (1964).

Atresia of follicles prior to ovulation has been recognised by a number of workers in adult teleost ovaries. While some investigators have looked upon such structures as inert degenerating masses, consisting of follicular cells, leucocytes and disintegrating ova and termed them as simple *corpora atretica*, others have emphasised functional aspect and, comparing them to *corpora lutea*, suggest that, perhaps similar structures with variations in the degree of development are present in almost all teleost ovaries. Such a debatable question has been critically reviewed by Atz in the monograph on "The Physiology of the Pituitory Gland of Fishes" by Pickford and Atz (1957) and after analysing both the viewpoints from histological, experimental and endocrinological evidences, he remarks that the latter contention about occurrence of functional *corpus luteum* has already caused enough confusion and appears premature with the available evidences. Hence, it may not be possible to throw any further light on this phenomenon until a more searching and detailed work is attempted on oil-sardine ovaries. This interesting feature is herein reported for the benefit of workers in this line, who may be inclined to classify them as partially spent, whereas it is only a transitory phenomenon from Stage IV to VI in an unspawned ovary, as is evident from the ova frequency distribution, and for record purposes may be termed as Stage IV.

Summary

Detailed descriptions of seven stages of sexual maturity with two subdivisions under Stage II to distinguish the virgin developing and spent-resting conditions and two other subdivisions for Stage VII to differentiate between the partially spent and completely spent nature, both for male and female of *Sardinella longiceps*, are given along with a field key. Factors like external appearance, extent of gonads in relation to body cavity length, length and weight of gonads, gonad: body weight ratio, maximum and modal size of ova and their general appearance under microscope have been taken into consideration.

Incidence of vascular hypertrophy and follicular breakdown in advanced ovaries is reported and the nature and cause of this phenomenon are examined.

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