

Fishery and reproductive biology of the spotted sardinella *Amblygaster sirm* (Walbaum, 1972) exploited along the southern coast of India

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Abstract

The study focused on the fishery and biological aspects of the spotted sardinella *Amblygaster sirm* (Walbaum, 1972) exploited along the southern coast of India from 2015 to 2020. The summary of the fishery reveals noticeable annual fluctuations in landing, with peak landings reported from November to March along the coast. The study included fish with total length (TL) ranging from 149 to 208 mm in males and 152 to 217 mm in females. The peak in gonadosomatic Index (GSI) in January indicates its spawning season, and L_{m50} was estimated at 172 mm TL in females. The sex ratio (F: M) was determined at 1:1.2, with males dominating most months. Fecundity ranged from 11632 to 43200 eggs per spawning. Five stages of maturity in *A. sirm* were identified through external analysis of gonads. Seven histological ovarian stages of *A. sirm* were recorded, providing a primary reference for future studies. These findings aim to assist fishery managers and policymakers in adopting effective management practices for the sustainable harvesting of this resource.

Introduction

Comprehending the fishery and reproductive biology of a species is a fundamental aspect of providing scientific guidance for fisheries management (Mariskha and Abdulgani, 2012; Al-Marzouqi *et al.*, 2015). Small pelagic species in particular exhibit distinctive biological characteristics, including rapid growth, short longevity, high natural mortality, shoaling behaviour, high fecundity and significant recruitment fluctuations. Given that many small pelagic species undertake migration, conducting stock assessments once every three years is crucial for understanding their dynamics (Devaraj and Martosubroto, 1997). This enables us to understand the stock state and estimates of many commercially valuable fish species. Understanding aspects of the reproductive biology of a species, including the spawning season, spawning area, age-at-maturity, fecundity and spawning frequency is crucial for effective species preservation and

management. These factors serve as key parameters for fisheries managers in establishing catch and size limits. The study of ova diameter offers valuable insights into reproductive developments and the spawning season of an organism. Analysis of morphological development of the ovaries differs from histological analysis due to the inaccuracy in the description of ovarian development (Jayasankar and Alagarwami, 1994; Santos *et al.*, 2005; El-Halfawy *et al.*, 2007; Ferreira *et al.*, 2012). Ova maturation stages are related to oogenesis (Jayasankar and Alagarwami, 1994; Abdallah and Cruz-Landim, 2003; Abdallah and Cruz-Landim, 2003; Rocha and Rocha, 2006; Le Menn, *et al.*, 2007; Aytekin and Yuze, 2008; Lefler *et al.*, 2008; Kimaro, 2011; Ferreira *et al.*, 2012;). The stages of oocyte development encompass oogonia, perinuclear oocyte, cortical alveoli oocyte, vitellogenic oocyte, mature oocyte, and atresia (Aytekin and Yuze, 2008; Ferreira *et al.*, 2012; Hismayasari *et al.*, 2015). Histological studies, widely employed in

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various biological phenomena such as fish reproduction, play a vital role in predicting new and effective methods for enhancing broodstock efficiency, increasing fish production, and ultimately improving stock management efficiency. Histological studies serve a crucial role in determining the peak period of spawning, assessing and exploiting fish populations, as well as sorting out the biological characteristics and life cycle of a species (Hosseinzadeh *et al.*, 1980; Eigdery, 1981)

A. sirm, a lesser sardine, is annually landed during the post-monsoon period and is esteemed for its popularity and taste among local communities along the south-west coast of India. The species is mainly distributed along the southern Kerala and Tamil Nadu coasts. Occasional occurrences have been reported from other coasts of India. Despite its significance, there is limited information available on the biology of *A. sirm* (Ronquillo, 1960; Chacko and Gnanamekalai, 1963; Gnanamekalai, 1963a, b; Lazarus, 1973; 1987a, b; Bennet *et al.*, 1986; Veerappan *et al.*, 1997). Lazarus (1990) studied the breeding biology of *A. sirm* along the Vizhinjam coast, and Veerappan *et al.* (1997) conducted a study along Parangipettai on the south-east coast of India. To formulate effective strategies for the conservation of small pelagic fisheries, a critical examination of the biological attributes of these fishes and the socio-economic aspects of fisheries rendering them vulnerable is essential (James, 2010). This study focused on examining the fishery and biological traits of *A. sirm*, including maturation and spawning, sex ratio, L_m 50, fecundity, and histology of female gonads. This comprehensive investigation aims to contribute to eco-friendly, rational exploitation, and sustainable management of fisheries along the southern coast of India.

Materials and methods

The fishery of *A. sirm* from 2015 to 2020 along the Indian coast was elucidated based on information collected from the Fisheries Resources Assessment Division of ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi. The data were estimated

using the Multistage Stratified Random Sampling design (Srinath *et al.*, 2005). Biological samples were collected from various landing centres along the southern coast of Kerala and Tamil Nadu from 2015 to 2019, where commercial catches are mostly landed by boat seines and shore seines. The geographical locations of the sample collection sites for the biological studies are depicted in Fig. 1.

A total of 3860 fish, ranging in size from 57 to 217 mm, were collected for the study. The fishes were dissected on the day of sampling, and gonadal examination facilitated the determination of sex and assigning stages of maturity. Gonads were preserved in 5% neutral buffered formalin (NBF) for subsequent analysis. The gonadosomatic Index (GSI) was calculated following Strum's (1978) formula: $GSI = \text{Weight of gonad} / \text{Weight of fish} \times 100$. The growth analysis of oocytes involved measuring ova diameters, a method introduced by Thompson (1915) and subsequently employed by researchers like Clark (1934), Hickling and Rutenberg (1936) and De Jong (1940). A small ovary portion was extracted and placed on a glass slide, and the ova carefully separated using a needle. Water was added to prevent ova desiccation, and a cover glass was applied. Oocyte development from one maturity stage to another was observed using a Zeiss primostar microscope with Zen software. The length at first maturity (L_m 50) was determined using the logistic curve outlined by King (1995). Fecundity was calculated by employing the gravimetric method (Lagler *et al.*, 1967; Das, 1977; Hunter *et al.*, 1989). The formula used for fecundity calculation was:

$$\text{Fecundity} = \frac{\text{Number of ova in the subsample of ovary}}{\text{Weight of the subsample of the ovary}} \times 100$$

Histological procedures were conducted following the methods outlined by Kiernan (2008) and Kerr (2010). Ovaries preserved in 5% NBF were taken and cut into approximately 2 mm-sized pieces. Subsamples were washed overnight and dried using filter paper. The tissue samples were dehydrated in an ascending alcohol series, cleared in chloroform and infiltrated in molten paraffin wax (melting point 60°C) and tissue blocks were prepared. The paraffin

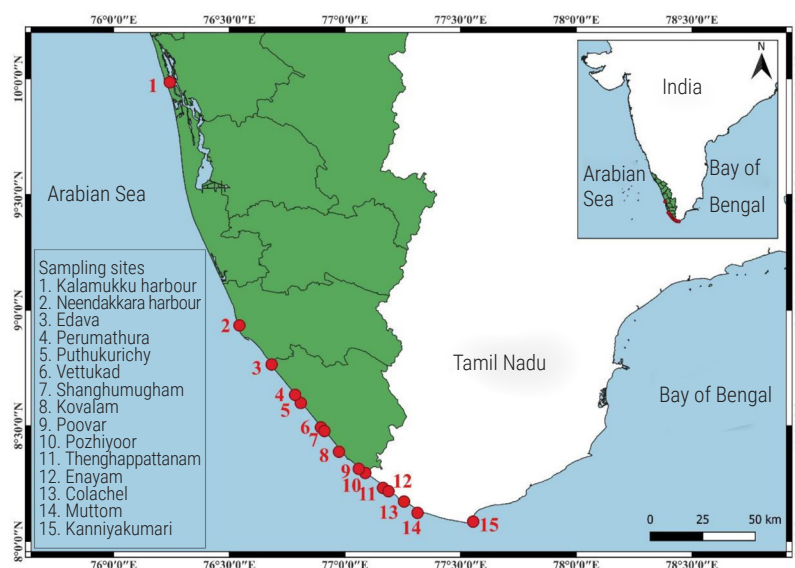


Fig. 1. Map showing the sample collection sites for the biological studies of *A. sirm*

blocks were sectioned at 6 µm thickness using a rotary microtome, tissue sections were spread on glass slides and then subjected to routine Haematoxylin and Eosin (H&E) staining (Kiernan, 2008). Four to six replicates were prepared for each gonad sample and observed in a Zeiss Primostar microscope with Zen software, to identify the maturity stage of ova and digital photomicrographs were recorded.

Results and discussion

Fishery of *A. sirm*

The spotted sardinella, *A. sirm*, holds significant importance in the Indian fisheries, particularly along the southern coast. The annual average landing of *A. sirm* along the Indian coast during 2015-2020 was estimated at 2352 t. Throughout the study period, a discernible pattern of annual fluctuations was observed, with notable increases in 2016 and 2019, while 2017, 2018, and 2020 witnessed a declining trend (Fig. 2). Peak landings were observed during the post and pre-monsoon months, typically spanning approximately five months in a year, with the main landing period from November to March. A diverse array of fishing gears, including boat seine, gillnet, trawl net, ring seine and shore seine were employed in the *A. sirm* fishery. In terms of regional distribution, Kerala and Tamil Nadu are the prominent states reporting higher landings of *A. sirm* in India. In Kerala, an in-depth examination of landings from 2015 to 2020 reveals a peak in 2019, with 3862.3 t. The principal gear utilised is the outboard boatseine, closely followed by the mechanised purse seine. In Tamil Nadu, Tuticorin recorded the highest landing of *A. sirm*, reporting a catch of 1679.3 t in 2020 and 904.5 t in 2018. The primary gear employed in Tamil Nadu is the outboard gillnet. The fishing season typically spans from November to March.

Sex ratio

During the peak months of examination, a total of 1870 males and 1720 females, out of 3860 specimens (with 270 indeterminates), were scrutinised to study the sex ratio. The observed sex ratio was 1:1.2, indicating a dominance of males in most months. This finding is in contrast with the report of Athukoorala *et al.* (2015), where the estimated sex ratio differed significantly. Similarly, Jayasuriya (1989) reported an abundance of females over males. In contrast, the present study reveals male dominance in most months, with females prevailing in January. These variations in sex ratios across studies underscore the dynamic nature of fish populations and the

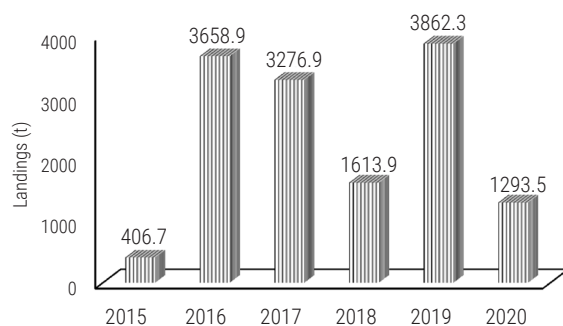


Fig. 2. Landings of *A. sirm* from the Indian waters (2015-2020)

potential influence of environmental factors on their reproductive patterns.

Gonadosomatic index

The gonadosomatic Index (GSI) values for male and female *A. sirm* individuals ranged from 0.27 to 4.0 and 0.31 to 5.05, respectively. A detailed month-wise analysis of GSI in females revealed a notable peak in January, with a mean value of 2.92, which strongly indicates the principal spawning period for *A. sirm*, suggesting a concentrated reproductive activity during this month (Fig. 3). The consistently higher GSI values observed in females compared to males is attributed to the substantial weight of the eggs they carry. Consequently, GSI serves as a reliable indicator of the spawning season, with the results emphasising a distinct peak in January, aligning with the reproductive cycle of *A. sirm*. This information underscores the significance of GSI in elucidating the reproductive patterns of *A. sirm*. Lazarus (1990), observed the monthly trends in the gonadosomatic index and obtained relatively high values for both males and females in January and February, indicating the spawning season from December to February, from the Vizhinjam coast, which agrees with the current study. Athukoorala *et al.* (2015) reported higher GSI values in females, with spawning season from May to July. Conand (1991) from New Caledonia observed that the spawning season of *A. sirm* is from October to December, before the hot, rainy season and Jayasuriya (1989) found that the spawning occurred twice a year, during April-May and August-September in Sri Lankan waters. Milton *et al.* (1994) in Australia reported protracted spawning in *A. sirm* with periods of intense spawning activity from August to October and also during May-June. Based on the presence of more than two distinct batches of ova in the ovaries, Lazarus (1990) suggested that the fish spawns twice in a season and the spawning season extended from December to February off Vizhinjam. Veerappan *et al.* (1997) also observed two distinct batches of ova indicating that the spawning occurs twice a year *i.e.*, February to April and September to November, along the Parangipettai coast. Month-wise assessment of GSI of *A. sirm* in Andaman waters by Monalisha *et al.* (2016) indicated higher values in females and spawning seasons to be January-March and October.

Length at first maturity (L_{m50})

The percentage occurrence of mature females steadily increases with length. From the maturity curve, it was seen that 50% of the females attained maturity at 172 mm (Fig. 4). L_{m50} is of much importance in resource assessment studies, particularly in assessing the exploitation of fish. Veerappan *et al.* (1997),

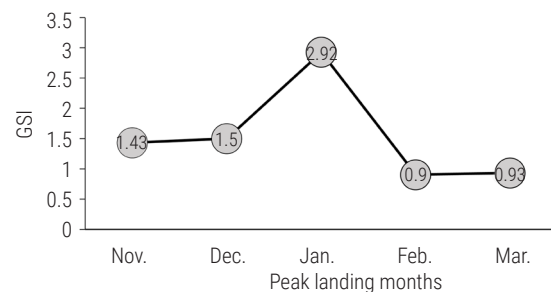
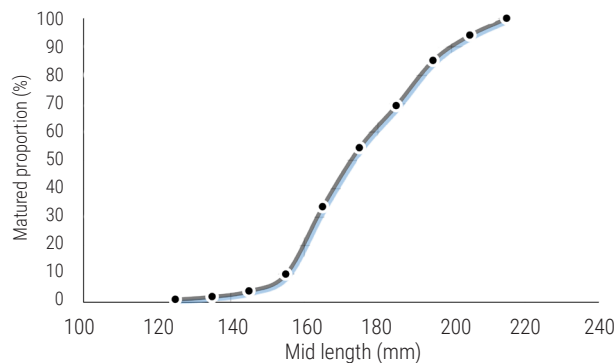


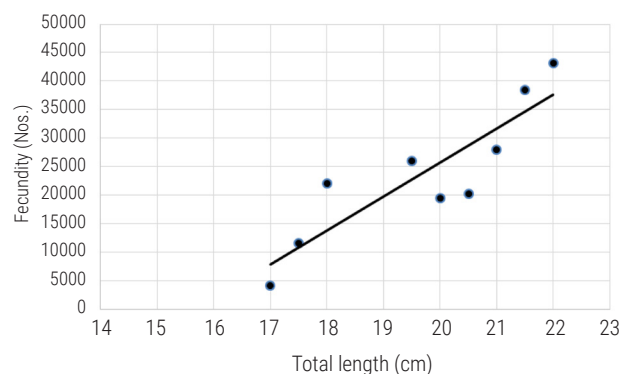
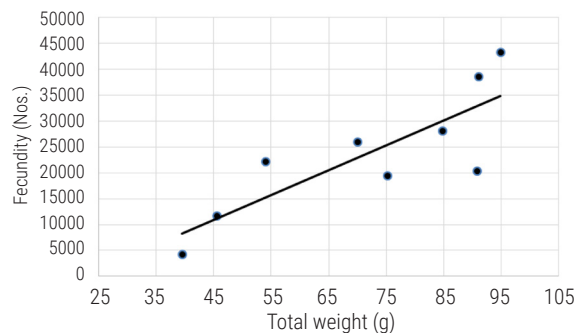
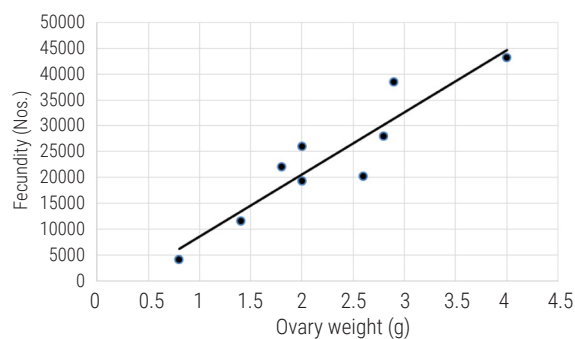
Fig. 3. Gonadosomatic Index for females of *A. sirm*

Fig. 4. Length at first maturity of *A. sirm*

from the south-east coast of India, estimated L_{m50} in males and females of *A. sirm* at 150 and 160 mm respectively. L_{m50} guides the establishment of size limits for fishing, ensuring that individuals have the opportunity to reproduce before being harvested, thereby contributing to sustainable fisheries. Additionally, it provides insights into the reproductive potential of a population, aiding in accurate stock assessments and informing fisheries regulations, such as minimum size limits.

Fecundity

Fish fecundity studies play a pivotal role in fisheries biology and management by offering crucial insights into the reproductive capabilities and sustainability of fish populations. The batch fecundity of *A. sirm* estimated based on mature ovaries of fishes (175 -217 mm TL and 31.7 -94.9 g TW) ranged from 11632 to 26200 eggs and the individuals spawn more than two times in a season. The fecundity of the fish increases with increase in ovary weight and total length. The graph indicates a linear relationship between fecundity and total length (Fig. 5), total weight (Fig. 6) and ovary weight (Fig. 7). Lazarus (1990) reported that the fecundity of *A. sirm* varies from 21,050 to 1,35,900 eggs and Milton *et al.* (1994) observed a mean of 20,000 eggs per batch and individuals probably spawn more than one batch of eggs (Conand, 1988). Veerappan *et al.* (1997) found the fecundity ranged from 21,800 to 1,24,800 eggs. The current study observed that fecundity varied among individuals of the same size. Variations in fecundity may be due to several factors such as the abundance of food and age (Hanson and Wickwire, 1967; Winter, 1971).

Fig. 5. Relationship between total length (cm) and fecundity in *A. sirm*Fig. 6. Relationship between total weight (g) and fecundity in *A. sirm*Fig. 7. Relationship between ovary weight (g) and fecundity in *A. sirm*

Maturity stages of *A. sirm*

The assessment of fish maturity stages is a comprehensive process that integrates both external and internal indicators to gauge the reproductive development of individuals. In the present study valuable insights are derived from the macroscopic examination of gonads, where variations in size, colour and texture serve as key markers for different maturity stages. Gonads were classified into five stages viz., Immature, maturing, mature, gravid, and spent (Table 1) based on the visual observation of gonads (Fig. 8). The ova diameter measurement conducted on an immature ovary revealed ova sizes up to a maximum of 200 μ m, whereas in maturing ovaries, the ova size ranged up to 450 μ m. In fully matured ovaries, the ova size surpassed 450 μ m, with the highest recorded diameter reaching approximately 720.261 μ m. The progression of ova diameter development and the percentage occurrence in immature, maturing, and matured ovaries is visually represented in Fig. 9. This depiction, combining ova diameter measurements and macroscopic observations, facilitated the identification of five distinct stages of maturity in the current study. It is noteworthy that previous research by Veerappan *et al.* (1997) documented seven maturity stages, aligning with the ICES scale proposed by Lovren and Wood (1937). In contrast, Athukoorala *et al.* (2015) reported five stages of maturity for the same species. These findings emphasise the variability in maturity classification methodologies across studies, highlighting the importance of considering multiple factors for a comprehensive understanding of the reproductive development in *A. sirm*.

Histological examination facilitating microscopic analysis of gonadal tissues, offered information about cellular structures that

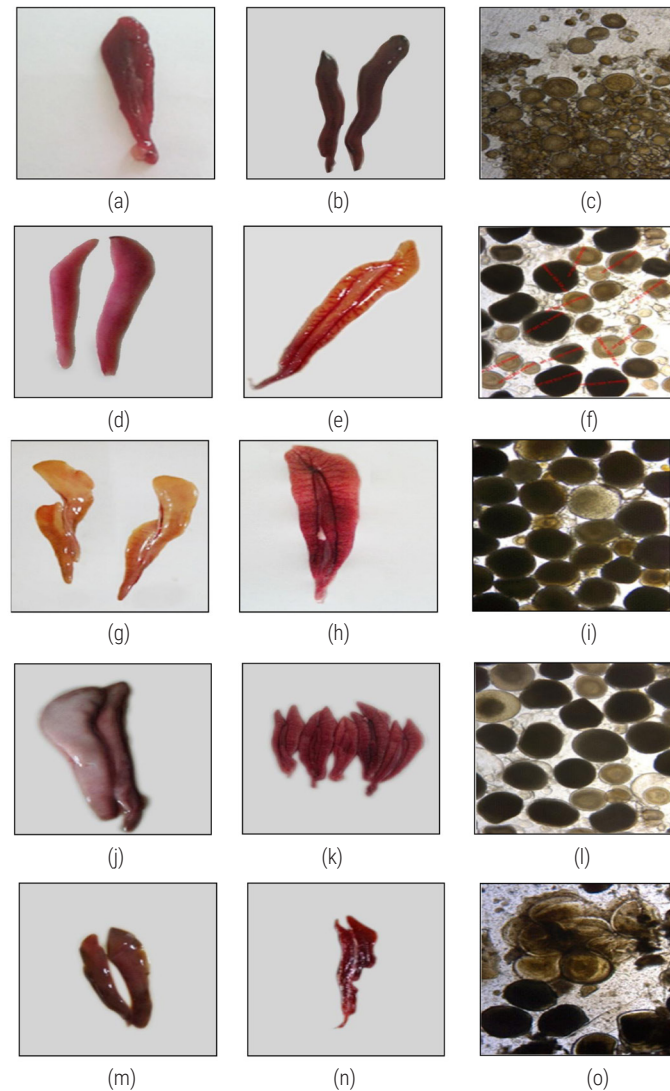


Fig. 8. Macroscopic observation of gonads of *A. sirm* in different maturity stages. (Ia - Immature testis, Ib - Immature ovary, Ic - Immature ova, IIa - Maturing testis, IIb - Maturing ovary, IIc - Maturing ova, IIIa - Matured testis, IIIb - Matured ovary, IIIc - Mature ova, IVa - Gravid testis, IVb - Gravid ovary, IVc - Gravid ova, Va- Spent testis, Vb- Spent ovary, Vc- Spent ova)

Table 1. Maturity stages of *A. sirm*

Stage	State	Description
I	Immature	Sexual organs are very small and situated close to the vertebral column. Testis/ovary translucent. Eggs are not visible to the naked eye.
II	Maturing	Occupy about $\frac{1}{2}$ to $\frac{3}{4}$ of the body cavity. Eggs are visible to the naked eye as whitish granular material.
III	Mature	Testis reddish or creamy, Ovary bright yellow or orange. Eggs are discernible and opaque, and the testis and ovary occupy about $\frac{2}{3}$ to $\frac{3}{4}$ of the body cavity.
IV	Gravid	Gonads extend to the full body cavity length. Testis white. Ovary orange-yellow, fully vascular. Drops of milt ooze under pressure. Eggs are completely round, some already translucent and ripe.
V	Spent	The testis/ovary occupies $\frac{1}{2}$ or slightly more of the body cavity. Bloodshot/ flabby/limp/ flattened / gelatinous/ shriveled. A few eggs are in a state of respiration.

aids in the precise assessment of fish maturity. Histological studies detailed the development of oögonia and was classified as follows;

Stage I (Oögonia stage): Oögonia proliferate by mitosis to become primary oocytes. These are the smallest oocyte cells, which reside within the ovarian germinal epithelium, usually in comparatively low

numbers. They are characterised by a relatively large nucleus with a small or inapparent nucleolus and minimal cytoplasm (Fig. 10a).

Stage II (Chromatin-nucleolus stage): Oögonia are slightly larger and are formed when an oögonium becomes surrounded by follicle cells. Compared to an oögonium, the chromatin nucleolar oocyte

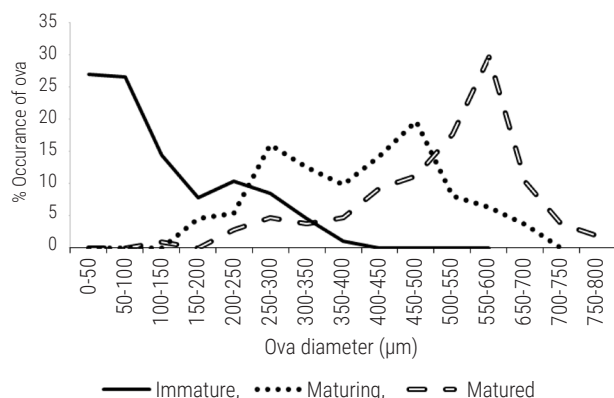


Fig. 9. Percentage distribution of ova diameter (µm) in immature, maturing, and mature ovary of *A. sirm*

has a relatively large nucleus with a single prominent nucleolus and more cytoplasm, which is slightly denser and finely granular (Fig. 10b).

Stage III (Perinucleolus phase): With the increasing oocyte growth, the nucleus (germinal vesicle) increases in size, and multiple nucleoli appear, generally at the periphery of the nucleus. The cytoplasm stains uniformly dark at this stage (Fig. 10c).

Stage IV (Cortical alveolar oocytes): At this stage, the oocyte is larger than perinucleolar oocytes. The appearance of cortical alveoli (yolk vesicles) within the ooplasm was seen in the oocyte. The cortical alveoli do not provide nourishment for the embryo as it is not yolk. The chorion becomes distinctly evident at this phase (Fig. 10d).

Stage V (Early vitellogenic oocytes): Cells are larger than cortical alveolar oocytes and characterised by the centralised appearance of spherical, eosinophilic, vitellogenic yolk granules/globules (Fig. 10e).

Stage VI (Late vitellogenic oocytes): Nucleus begins to migrate towards the periphery of the cell, and these cells are characterised

by an increased accumulation of vitellogenic granules that displace the cortical alveolar material to the periphery of the cytoplasm (Fig. 10f).

Stage VII (Maturation): Nucleus has migrated towards the periphery of the cell and is almost invisible and the cell has become larger and more hydrated (Fig. 10 g, h, i).

Stage VII b (Spent): The ovaries become flaccid due to the ovulation of mature eggs (Fig. 10j).

Histological examination of *A. sirm* ovaries has not been reported earlier, and hence the present study attempted histological examination of the ovary to identify the development of oocytes. Seven stages of ovarian development were assessed based on the histological examination of the ovarian tissue of *A. sirm*. Many authors have documented the ovarian histology of fish (Hismayasari *et al.*, 2015; Acharya *et al.*, 2015; Behera, 2012; Sinlapachai *et al.*, 2017). In a study conducted by Rohit *et al.* (2018), histological examination of oil sardine revealed five distinct developmental stages, aligning closely with the findings of the current study. Understanding ovarian development stages is pivotal in sorting out the reproductive biology of the species. Immature ovaries (Stage I-II) are characterised by soft, translucent features, while maturing ovaries (Stage III-IV) exhibit evolving oocytes with lipid and yolk granules. Mature/ripe ovaries (Stage V-VI) showcase golden-coloured ova. The identification of partially spent (Stage VIIa) and fully spent ovaries (Stage VIIb) signifies post-spawning states. Zaki *et al.* (2012) and Abderrazik *et al.* (2019) conducted histological studies on *S. longiceps* and *Sardina pilchardus*, respectively, revealing distinct stages of ovarian development. Zaki *et al.* (2012) identified four stages viz., immature ovaries with irregular, yolk-devoid ova, maturing ovaries with accumulating yolk, mature ovaries containing opaque, fully yolke, larger eggs and spent ovaries with disintegrating ova. Abderrazik *et al.* (2019) applied a comprehensive approach to assess sexual maturity in *S. pilchardus*, analysing external gonadal conditions and elucidating the oogenesis process, from primordial germ cell transformation to fertilisable egg formation. The comparative examination highlights

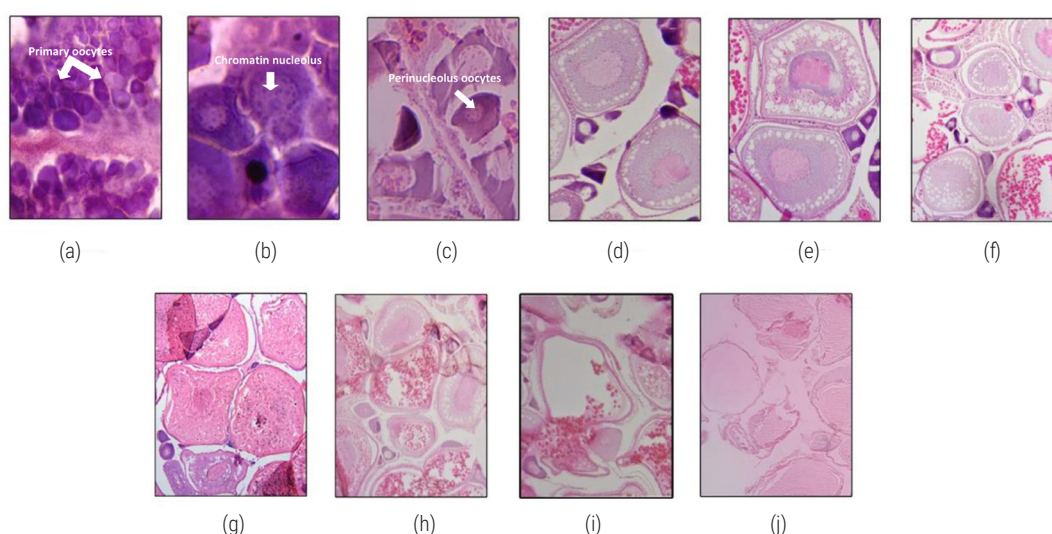


Fig. 10. Photomicrographs of histological sections of ovaries of *A. sirm* at different developing stages. (a) Oogonia stage; (b) Chromatin-nucleolus stage; (c) Perinucleolus phase; (d) Cortical alveolar oocytes; (e) Early vitellogenic oocytes; (f) Late vitellogenic oocytes; (g) Early maturation; (h) Late maturation; (i) Spawning and (j) Spent phase

species-specific variations in ovarian development strategies and contributes to a broader understanding of teleost reproductive biology. Sajan (2015) reported ten stages of ovary development in *Sahyadria denisonii* while Acharya *et al.* (2015) observed five stages of gonad development in *Leiognathus splendens* from Ratnagiri Coast. Brown-Peterson *et al.* (2011) suggested revision of traditional concepts of gonad classification with incorporation of new histological techniques like plastic embedding, selective staining, and histo-chemistry; which were explored to define the stages of development in fish gonads. Only the classification based on cytology systems of gonadal development can provide the most accurate description of transformations in the gonads. The present study reflects the variation in maturity stages which were analysed macroscopically and histologically. Histological studies help to determine the peak period of spawning and life cycle of a species (Hosseinzadeh *et al.*, 1980; Eigdery, 1981)

Accurate assessment of oocyte stages and ovarian phases is essential for precise fish reproduction data (Heins and Brown-Peterson, 2022). Identifying ovarian recrudescence onset is key to understanding when fishes become reproductively active and reach sexual maturity. Recognising the regressing phase defines the end of reproductive season, which is crucial given its sensitivity to environmental variations (Stevenson and Bryant, 2000; Krabbenhoft *et al.*, 2014; Martin, 2014). Elucidation of oocyte stages ensures reliable data for broader reproductive ecology studies, enhancing our nuanced understanding of fish reproductive dynamics (Heins and Brown-Peterson, 2022).

Small pelagics are important sources of income as well as protein source for coastal communities. The inclination towards variable environmental parameters always reflects the annual or biannual fluctuations in fish landings. *A. sirm* too depicts dynamic landings along the Indian coast and for such dynamic groups, continuous assessment of stock and biological examination is mandatory to sustain the fishery. Studies conducted on the fishery and biological attributes of *A. sirm* are very few which are pertinent to decades ago. Considerable gaps exist in the assessment of biology and exploitation of *A. sirm* in the different regions of the Indian coast. Histological examination of *A. sirm* ovary in the present study forms a primary reference for future researchers. The information on the fishery and biological aspects of *A. sirm* in the present study will assist fishery managers as well as policymakers in the effective management and sustainable harvesting of the resource.

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