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Gonadal Assessment of Picnic Sea Bream *Acanthopagrus berda* (Forsskål 1775), a Potential Aquaculture Candidate for Indian Waters

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Abstract

Gonadal assessment of *Acanthopagrus berda* (Forsskål 1775), a commercially and recreationally important fish from Indian waters, was studied by collecting 250 fishes from the Korapuzha estuary, Calicut, Kerala using cast net during December 2015 to January 2016. External morphology of the gonads reveals that *A. berda* is bisexual (with ovo-testis) in nature with the ovarian lobe in the mid-dorsal region of the abdominal cavity and the testicular lobe as a band along the ventro-lateral wall with a major portion running along the extreme posterior region of the gonad. Males were dominant in lower length classes (17–23 cm) while females dominated in upper length classes (24–43 cm), confirming protandrous hermaphroditism in the species. Gonado-somatic index (GSI) was significantly higher ($P < 0.05$) for the females. Males and transitional groups were showing almost similar GSI. External morphological and histological evaluation of the gonads of *A. berda* during the 2-month study revealed the presence of different developmental stages such as matured testis with oozing milt, an intermediate gonad structure with an anterior thin ovary-like structure and a posterior thick testis-like morphology indicating a transitional ovo-testis, maturing ovary and matured ovary. The simultaneous availability of milt oozing males and matured females from the wild indicates the opportunity for development of captive breeding, seed production and hatchery technology for this important commercial food fish.

Keywords: *Acanthopagrus berda*, gonadal development, gonado-somatic index, protandrous hermaphroditism, sea bream

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Introduction

In India, the development of hatchery technology for commercial seed production of marine finfishes is still in its infancy. At present, Indian mariculture is limited to a few species of shrimps, lobsters, molluscs (oysters and mussels), seaweeds and finfishes (cobia, pompano, sea bass and grouper) (Gopakumar 2016). India is not a leading producer in mariculture due to lack of attention to research on developing seed production methods for high value finfishes suited for sea farming. In many Asian countries with similar climate and natural endowments as India, revenue from mariculture production makes a significant contribution to national economies (Joseph and Ignatius 2016). Species diversification is needed to enhance Indian mariculture production through the culture of high value species to augment seafood production in the country. Sparidae (Order Perciformes), commonly known as porgies or sea breams, are mainly marine coastal fish of high economic value which are exploited and farmed for human consumption, as well as for recreational purposes (Basurco et al. 2011). Their exceptional edible qualities (Yennawar and Tudu 2012) and appearance have ensured high market acceptance and intense commercial fishing efforts on their stocks (Cowden 1995). The picnic sea bream *Acanthopagrus berda* (Forsskål 1775), commonly known as Goldsilk sea bream, is a fairly small euryhaline, estuary-dependent sea bream (Begg 1978; van der Elst 1988; Randall et al. 1997) with a wide distribution throughout the tropical Indo-West Pacific region (Munro 1949; Smith and Heemstra 1986), from South Africa to India and extending to Japan, the East Indies and Northern Australia (Garratt 1993; Iwatsuki and Heemstra 2010).

Acanthopagrus berda is a marine fish native to the Indian Ocean. It is considered an important commercial sparid fish (Kasahara 1957) because of its recreational value (James et al. 2003), excellent meat quality (Anonymous 2012), market acceptance, high economic value, ability to tolerate wide variations in salinity and temperature (Rahim et al. 2017), resistance to disease, easy adaptation to captive conditions, and fast growth rate (Samuel and Mathews 1987). *Acanthopagrus berda* cultured in brackish water ponds in Pakistan have shown a high weight gain (to 2,665 g) and specific growth rate (2.76 ± 0.5 % day⁻¹) (Rahim et al. 2017). Due to its good quality meat, *A. berda* is extremely popular with consumers as a source of protein (Anonymous 2012). The species is mainly exploited by artisanal fishers both by cast net and hook and line along the Indian coasts (FAO 1984) and, since the flesh quality is excellent, it is sold fresh in Indian markets at Rs. 450–500 per kg. It has the potential to attract commercial interest in the near future and therefore development of aquaculture techniques for the species is important. Even though artificial spawning of *A. berda* (formerly known as *Mylio berda*) has been attempted in Hong Kong (Mok 1985), wild larvae and juveniles harvested from estuaries were mainly used for restocking and rearing for many years. This species is also considered as a prime candidate for ranching in estuaries in order to enhance wild stocks and recreational fishing (Taylor et al. 2005). Development of seed production technology is the first step towards the commercial aquaculture of a candidate species and reproductive biology studies are considered as important prerequisites for developing successful breeding protocols.

Members of the Sparidae family express protandrous, protogynous and simultaneous or rudimentary type of hermaphroditism and this diverse array of reproductive strategies caught the attention of reproductive biologists many decades ago (D' Ancona 1941; Atz 1964; Buxon and Garratt 1990).

Detailed studies on the gonadal development of this important hermaphroditic fish are scanty, even though Tobin et al. (1997) carried out a reproductive biology study of this species in tropical North-eastern Australia using tag release-recapture study and reported protandrous sex change in them. Thus, the aim of the present study is to elaborate the reproductive biology of *A. berda*, a potential candidate collected from tropical Indian waters, in order to initiate broodstock development in captivity for induced spawning and seed production.

Material and Methods

Fish

The picnic sea bream *A. berda* (Fig. 1) used for this study were collected from Korapuzha estuary, Kerala, India by cast net. The study was conducted by collecting 250 fishes from December 2015 to January 2016. The taxonomic identification of the species was confirmed using FAO species identification sheets (FAO 1984). The total length and weight of the fish were measured shortly after collecting from the landing centre. Body weight were measured using a top loading electronic balance with an accuracy of 0.001 g and the length was measured using a wooden scale with an accuracy of 1 mm.



Fig. 1. Adult *Acanthopagrus berda* specimen collected from Korapuzha estuary, Calicut, India

Gonad examination

The fishes were cut open and the morphology of the gonad was observed for sex determination. The gonads were removed, weighed and examined macroscopically.

Gonado-somatic Index

Gonado-somatic Index (GSI) was calculated according to Charan et al. (2014) using the following formula:

$$\text{GSI} = \frac{\text{Total weight of gonad (g)} \times 100}{\text{Total weight of fish (g)}}$$

Gonad histology

After evaluating the GSI, gonads of all the fish, samples were fixed in 10% neutral buffered formalin. Small portions of the gonad from the posterior, middle and anterior parts were cut separately and subsequently processed for histology (Gabe 1976). All the tissues were dehydrated in gradient solutions of ethanol and embedded in paraffin. The embedded gonads were sectioned (5 µm) and rehydrated in ethanol in a stepwise manner. The sections were stained with Harris haematoxylin and eosin in compliance with the accepted procedures. The histological changes during maturation of the gonads were evaluated according to Abou-Seedo et al. (2003a).

Measurement of oocyte diameter

The ovarian oocyte diameter was measured using Zeiss Axio Lab A1 light microscope at magnifications of x10 and x40. The ovaries of five fish from each developmental phase were examined, and in each ovary, cell diameters were measured for 50 randomly selected oocytes and their mean and standard deviation were analysed.

Sex ratio

The proportion of the two sexes relative to one another was used to calculate the sex ratio.

Statistical analysis

Statistical analysis of the data was carried out with Statistical Package for the Social Sciences (SPSS) 16.0 version and the difference in GSI between groups was tested by one-way analysis of variance followed by Duncan's multiple range test.

Results

Morphology of gonad

The gonad of *A. berda* is observed as a paired organ suspended from the dorsal side of the peritoneal cavity by a thin mesorchium. The gonadal lobes are fused at the posterior end, free anteriorly, and the gonads are cylindrical in shape. The gonads collected from all the specimens indicated the presence of both ovarian and testicular tissue.

External morphologic evaluation of the gonads of *A. berda* samples during December and January revealed the presence of maturing testis (Fig. 2a), matured testis with oozing milt (Fig. 2b), an intermediate gonad structure with an anterior thin ovarian-like structure and a posterior thick testis-like morphology which is assumed to be transitional ovo-testis (Fig. 2c), maturing ovary (Fig. 2e) and matured ovary (Fig. 2f).



(a) Maturing testis

(b) Matured milt oozing testis

(c) Ovo-testis at early transitional stage



(d) Ovo-testis at late transitional stage

(e) Maturing ovary

(f) Matured ovary

Fig. 2. Morphology of different reproductively distinguished groups of *Acanthopagrus berda*

In the ripe ovary of *A. berda*, branches of ovarian blood vessels arise from the main vessel and form a network enclosing the ovary; on maturity the blood vessels dilate to enhance the blood circulation in the ovary (Fig. 2f). The maturing females had well-vascularised ovaries, orange in colour, with a slightly granular appearance (Fig. 2e).

Initially the ventral testicular portion of the gonad undergoes intensive spermatogenesis and functions as a mature testis (Fig. 2b). During the early period of the transitional stage, the testicular region gradually reduces and the ovarian part advances in development until it reaches full maturity (Fig. 2c and 2d). Later the ventral testicular part of the gonad regresses and the dorsal part develops into a young ovary (still remaining bisexual) with a dominant ovarian part and a small testicular portion (Fig. 2e). During the second reproductive phase, the testicular portion completely regresses and the ovarian part of the gonad develops completely (Fig. 2f).

Histology of gonad

The histological changes during different maturity stages of *A. berda*, were evaluated in the present study. Histological observations of different sections of the gonads collected during December and January months revealed the presence of maturing testis, maturing ovary, functional male dominant ovo-testis, female dominant ovo-testis and ovo-testis at the transitional stage.

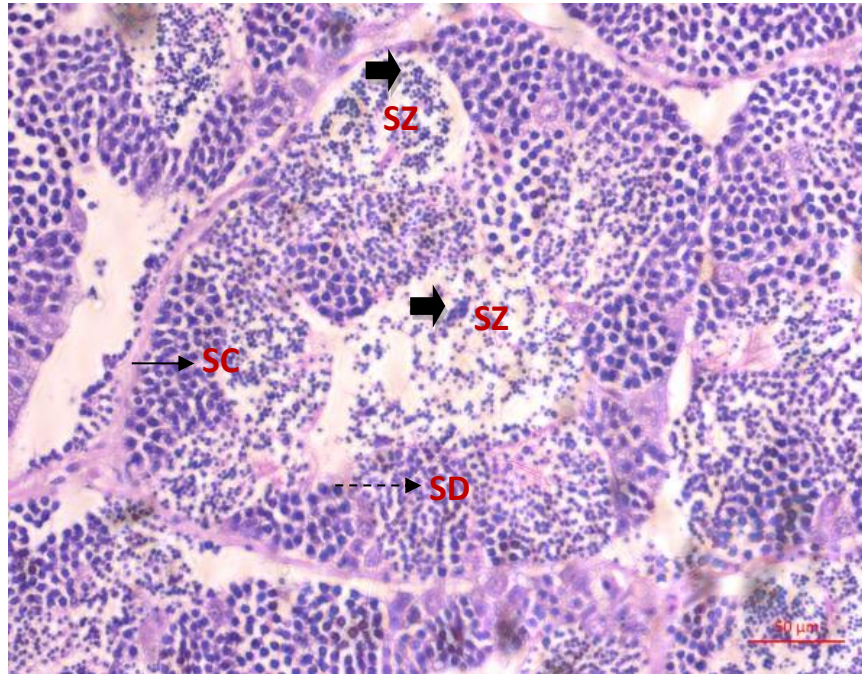


Fig. 3. Transverse section of a testis of an actively maturing *Acanthopagrus berda* male showing enlarged seminiferous tubules irregular in shape and primarily filled with mature spermatozoa (SZ-thick arrow). The spermatozoa found in the lumen of the lobules were free. The spermatozoa (SD-dotted arrow) and spermatocytes (SC-thin arrow) were observed at the periphery of the seminiferous tubule.

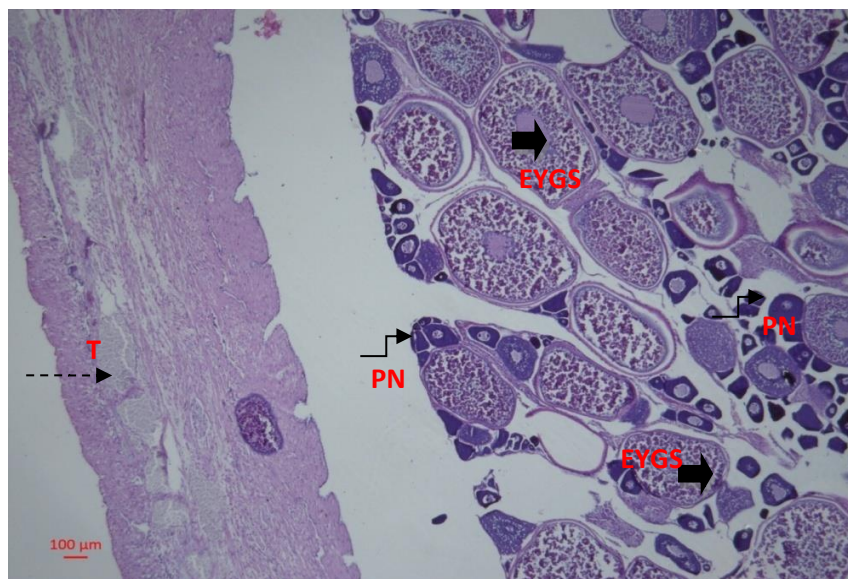
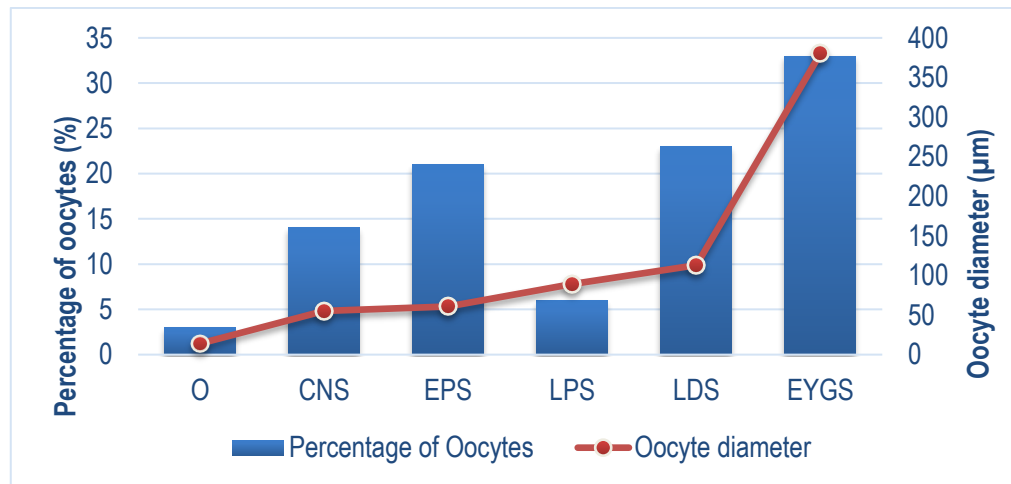


Fig. 4a. Transverse section of a maturing ovary of an active female: presence of majority of maturing oocytes with perinucleus stage (PN-thin arrow) and early yolk globule stage (EYGS-thick arrow); and testicular tissue at the periphery (T-dotted arrow)

Active maturing testis (Fig. 3) of *A. berda* were observed with spermatids and spermatocytes, and active maturing ovaries (Fig. 4a) had maturing oocytes with yolk vesicles and yolk granules in the vitellogenic stages. Microscopically, lipid vesicles and yolk granule oocytes ($230 \pm 81.97 \mu\text{m}$) (Table 1) are found dominant at this stage with a few oocytes in the perinuclear stages (Fig. 4b).



O- Oogonia, CNS- Chromatin nucleus stage, EPS- Early perinucleus stage, LPS- Late perinucleus stage, LDS- Lipid droplet stage, EYGS- Early yolk globule stage

Fig. 4b. Different stages of oocytes in maturing ovary of *Acanthopagrus berda*

Functional ovo-testis of an active male (Fig. 5) was identified by the presence of the testicular region characterised by spermatids and spermatozoa and the ovarian

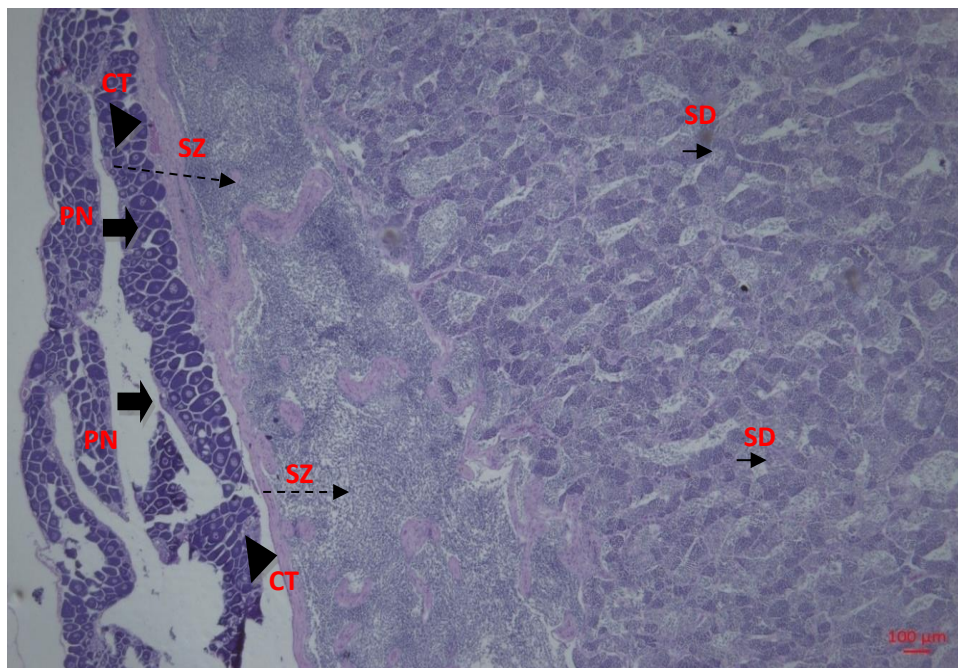


Fig. 5. Transverse section of an ovo-testis of an active *Acanthopagrus berda* male: the testicular region is characterised by the presence of spermatids (SD-thin arrow) and spermatozoa (SZ-dotted arrow) and the ovarian region by the presence of immature oocytes at the perinuclear stage (PN-thick arrow). Both the regions are separated by a thin layer of connective tissue (CT-arrow head).

Ovo-testis of a functional female (Fig. 6a) was distinguished by the presence of mature oocytes with vitellogenic stages and thin testicular tissue at the periphery. The mature ovary develops numerous advanced vitellogenic oocytes ($460 \pm 71.83 \mu\text{m}$) (Table 1) with a few early and late perinuclear oocytes and lipid vesicle oocytes (Fig. 6b).

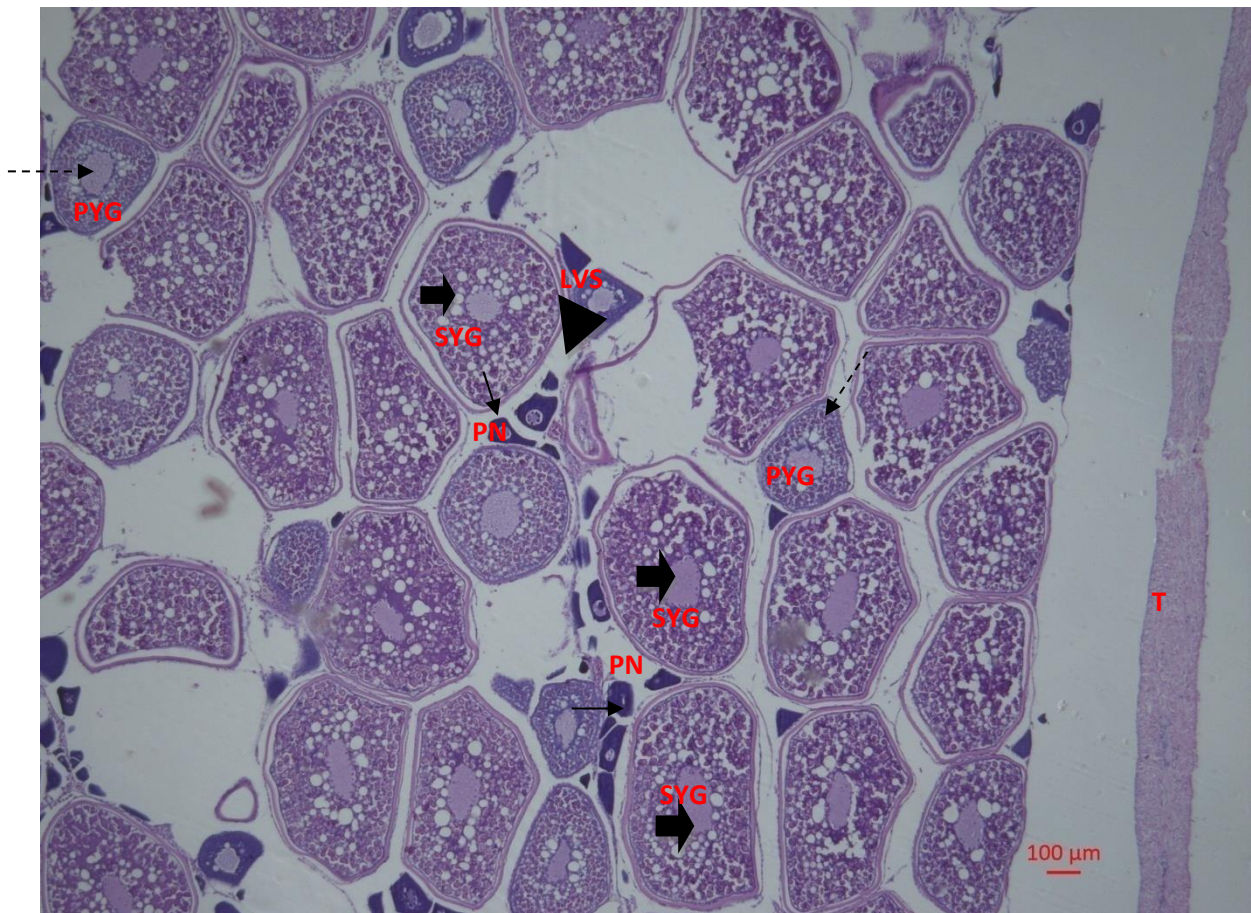
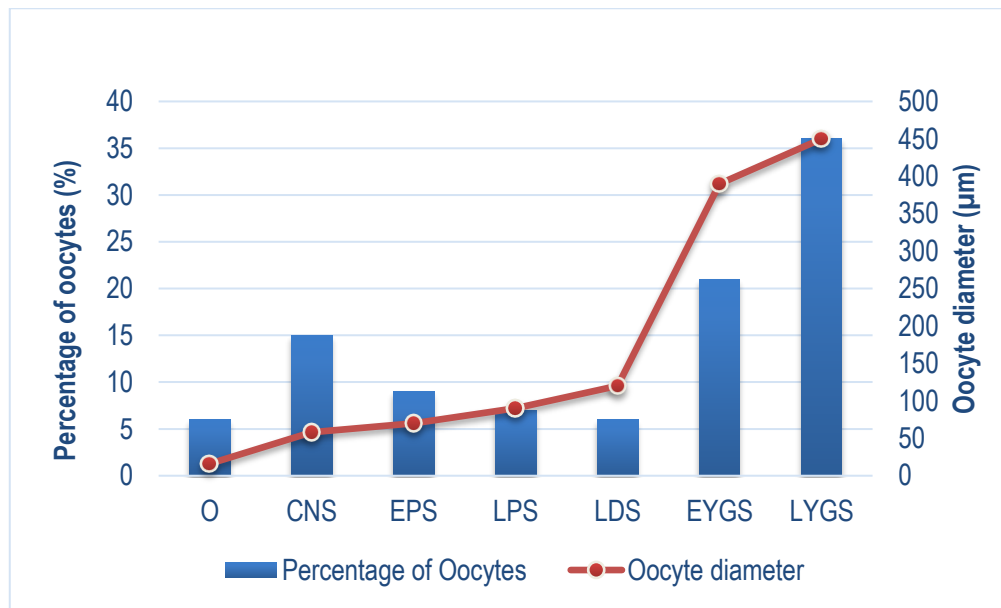


Fig. 6a. Transverse section of an ovo-testis of an active *Acanthopagrus berda* female: presence of the majority of mature oocytes at primary yolk granule stage (PYG-dotted arrow) and secondary yolk granule stage (SYG-thick arrow) and a few oocytes at perinuclear stages (PN-thin arrow) and lipid vesicle stage (LVS- arrow head) and thin testicular tissue at the periphery (T).

Table 1. Oocyte size at different stages of ovary development in *Acanthopagrus berda*

Stages of oocytes	Oocyte diameter (μm)	
	Maturing ovary	Matured ovary
Oogonia	14 \pm 8.24	16 \pm 10.12
Chromatin nucleus stage	55 \pm 15.41	58 \pm 18.08
Early perinucleus stage	61 \pm 13.47	70 \pm 17.51
Late perinucleus stage	85 \pm 11.93	90 \pm 17.02
Lipid droplet stage	113 \pm 13.19	120 \pm 18.16
Early yolk globule stage	230 \pm 81.97	390 \pm 57.27
Late yolk globule stage	-	460 \pm 71.83



O- Oogonia, CNS- Chromatin nucleus stage, EPS- Early perinucleus stage, LPS- Late perinucleus stage, LDS- Lipid droplet stage, EYGS- Early yolk globule stage, LYGS- Late yolk globule stage

Fig. 6b. Different stages of oocytes in matured ovary of *Acanthopagrus berda*

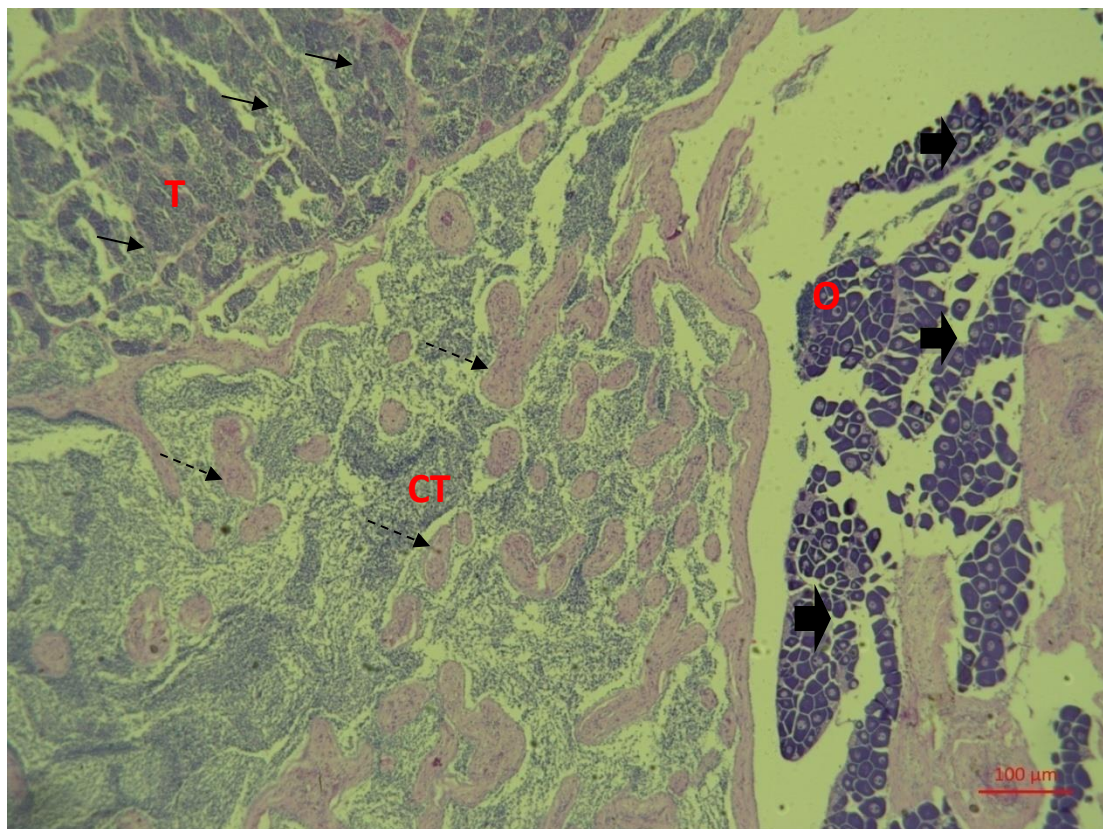


Fig.7. Inactive ovo-testis of *Acanthopagrus berda* at an early transitional stage from male to female with ovarian region (O-big arrow) and vast testicular region (T-small arrow). The transitional phase is characterised by a large mass of connective tissue (CT-dotted arrow) and the degeneration of the testicular region

Inactive ovo-testis at an early transitional stage from male to female (Fig. 7) could be observed in the collected sample with a narrow ovarian region at perinuclear stage, vast testicular region while the transitional phase is characterised by a typical large mass of connective tissue and the degeneration of the testicular region. An inactive ovo-testis at a late transitional stage from male to female (Fig. 8) was identified with oocyte development in lobes, a rudimentary testicular region. An inactive ovo-testis at a late transitional stage from male to female (Fig. 8) was identified with oocyte development in lobes, a rudimentary testicular region with a large mass of connective tissue and vascular tissues.

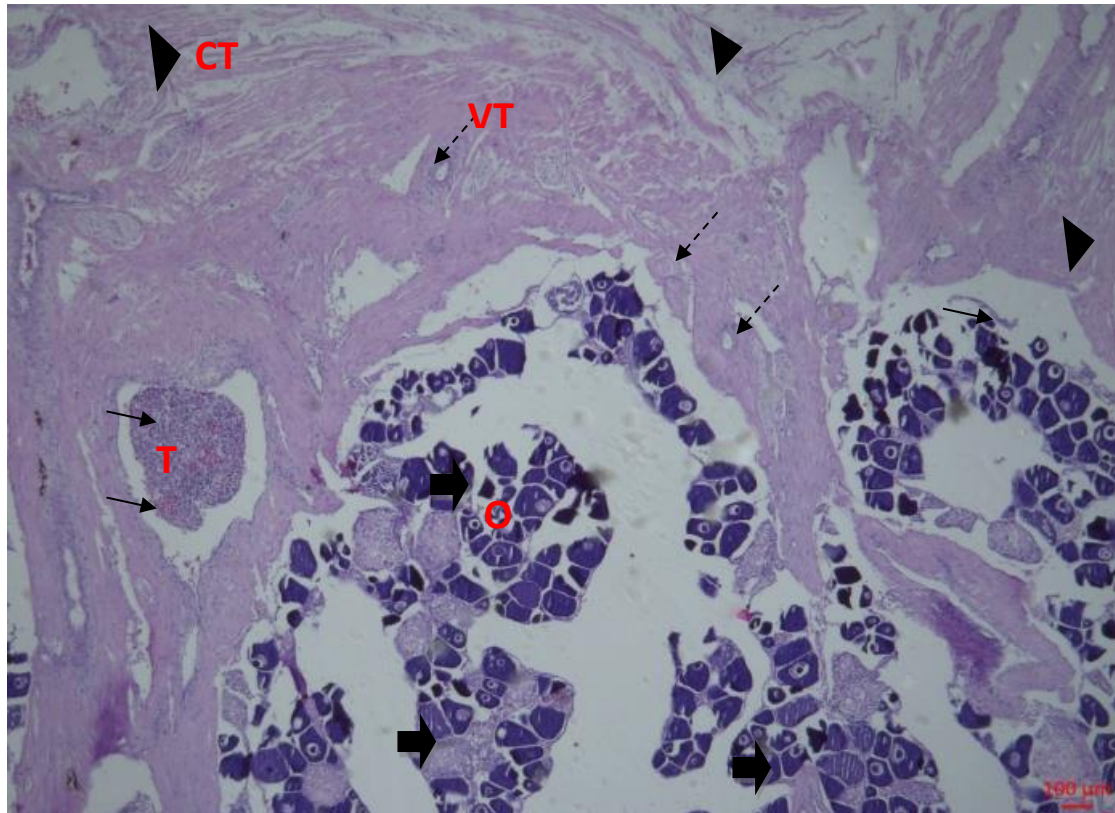


Fig. 8. Inactive ovo-testis of *Acanthopagrus berda* at a late transitional stage from male to female with oocyte development in lobes (O-big arrow), and rudimentary testicular region (T-small arrow). The transitional phase is characterised by the large mass of connective tissue (CT-arrow head) and vascular tissues (VT-dotted arrows).

Table 2. Maturation indices of different reproductively distinguished groups of *Acanthopagrus berda*

Sex	Number of samples	Body length range (cm)	Average Body Weight (g)	Average Gonad Weight (g)	GSI
Male	110	17.5-34.6	283.4± 91.80 ^b	3.603± 2.75 ^b	1.12 ±0.64 ^b
Female	131	17.8-43.2	553.3±312.46 ^a	11.96 ± 15.33 ^a	2.05 ± 1.91 ^a
Transitional	9	18.7-27.1	250.2±72.83 ^b	2.00 ± 0.33 ^b	0.98 ± 0.12 ^b

In each column mean ± SE followed by different letters were found to differ at 0.05 probability level by Duncan's multiple range test.

Male, female and transitional individuals were classified according to the histological observations. The GSI was found to be significantly higher ($P<0.05$) for the females compared to males and transitional groups which were having almost similar GSI (Table 2).

Similarly, average body weight (g) and average gonad weight (g) were also highest in females. In the present study, male fishes were dominant in smaller length classes (17–23 cm) while females dominated in the larger size group (24–43 cm) (Table 3). According to the data females are found to be more abundant in the catch than males and transitional individuals, where overall sex-ratio can be considered as 1:1.21.

Table 3. Percentage occurrence of different reproductively distinguished groups of *Acanthopagrus berda* at various length classes

Total Length (cm)	Male		Female		Transitional		Total
	N	%	N	%	N	%	N
17	4	80	1	20	0	0	5
18	3	60	0	0	2	40	5
19	4	57.142	1	14.285	2	28.571	7
20	7	70	3	30	0	0	10
21	9	52.941	7	41.176	1	5.882	17
22	15	68.181	6	27.272	1	4.545	22
23	10	58.823	6	35.294	1	5.882	17
24	15	57.692	11	58.307	0	0	26
25	7	46.666	7	46.666	1	6.666	15
26	7	41.176	10	58.823	0	0	17
27	9	36	15	60	1	4	25
28	6	60	4	40	0	0	10
29	4	26.666	11	73.333	0	0	15
30	4	28.571	10	71.428	0	0	14
31	2	16.666	10	83.333	0	0	12
32	2	25	6	75	0	0	8
33	1	20	4	80	0	0	5
34	1	12.5	7	87.5	0	0	8
35	0	0	3	100	0	0	3
36	0	0	2	100	0	0	2
37	0	0	2	100	0	0	2
38	0	0	1	100	0	0	1
39	0	0	1	100	0	0	1
40	0	0	0	0	0	0	0
41	0	0	1	100	0	0	1
42	0	0	1	100	0	0	1
43	0	0	1	100	0	0	1
	110		131		9		250

*N-number of individuals of different reproductively distinguished groups of *A. berda*

Discussion

Gonads of *A. berda* examined in the present study were bisexual (ovo-testis) in nature with the ovarian lobe in the mid-dorsal region of the abdominal cavity and the testicular lobe as a band along the ventro-lateral wall with a major portion running along the extreme posterior region of the gonad. D'Ancona (1949) and Besseau and Brusle-Sicard (1995) explained the bisexuality in the sparidae gonad as a combination of both ovary and testis separated by a connective tissue.

Buxton and Garrat (1990) reported both gonochorism and hermaphroditism in sparidae and concluded that the presence of ovotestis is a pre-adaptation for sex change in which reproductive success is size related. Sparidae possess a wide range of reproductive strategies (Abou-Seedo et al. 2003a; Hesp and Potter, 2003; Hughes et al. 2008). The structural pattern of gonads of hermaphroditic fishes varies according to their taxonomic group as well as the type of hermaphroditism exhibited by them.

The co-existence of both male and female tissues in the gonad apparently indicates that *A. berda* are hermaphroditic. *Acanthopagrus berda* initially undergoes juvenile hermaphroditism, developing an ovo-testis. According to Zohar et al. (1978) the ovotestis in protandrous hermaphrodites are formed very early during gonadal ontogeny. In the present study during the initial reproductive phase the ventral testicular portion of the gonad undergoes intensive spermatogenesis and functions as a mature testis; during the early period of transitional stage, the testicular region gradually reduces in size and the ovarian part undergoes rapid development and becomes dominant.

During the second reproductive phase, the testicular portion regresses completely and the ovarian part of the gonad develops completely. According to Sadovy and Shapiro (1987) the gonad of transitional individuals shows degeneration of the germinal tissue of the primary sex accompanied by proliferation of the tissue of the opposite sex. A similar pattern of gonad development has been described in blackhead sea bream, *Acanthopagrus schlegeli* (Bleeker 1854) (Lee et al. 2001, 2002; Du et al. 2005). *Acanthopagrus berda* was reported as a protandric hermaphrodite in Australian waters by Tobin et al. (1997).

The presence of the ovo-testis structure of the sparid gonad actually complicates attempts to identify the transitional ovo-testis structure (Yeung and Chan 1987). Buxton and Garrat (1990) reviewed various reproductive styles of sparidae and stated that many of the reports detailing sex change lack clarity of terminology and are based on superficial observation. Even though histological changes in hermaphrodites are a complicated process, the information is important for understanding the mechanism in reproductive progression. Identification of sex change in *A. berda* is important as the sex change process may have serious implications for their management and general survival of the species. The confirmation of the hermaphroditic nature of the species is possible only by histological observations (Sadovy and Shapiro 1987).

Histological observations of different sections of the gonads of *A. berda* revealed the presence of a maturing testis, a maturing ovary, a functional male dominant ovo-testis, a female dominant ovo-testis and an ovo-testis at the transitional stage which confirms the protandrous sex change in *A. berda* collected from tropical Indian waters. Lipid vesicles and yolk granule oocytes ($230 \pm 81.97 \mu\text{m}$) are found dominant in the active maturing ovary with a few oocytes in perinuclear stages. Abu-Seedo et al. (2003b) also described similar observations in *Acanthopagrus latus* (Houttuyn 1782).

Gonado-somatic index was found to be significantly higher ($P < 0.05$) for the females compared to male and intersex groups. GSI values increase with increase in mean body weight and length during spermatogenesis and gametogenesis in fishes. Bhatta et al. (2012) reported that body size growth and gonadal development are interconnected. In the case of *A. berda*, the dominant occurrence of male and intersex fishes in smaller length classes might have resulted in a lower GSI compared to females.

In the present study, male fishes were dominant in smaller length classes (17–23 cm) while females dominated in the larger size group (24–43 cm). This observation was similar to that of Tobin et al. (1997) with a strong bimodality in the length and age frequency distributions of *A. berda*, with exclusive male dominance of smaller length classes and female dominance of larger length classes. Abou-Seedo et al. (2003b) also reported male dominance in the smaller size group (22.3–24.2 cm) and female dominance in the larger size group (24.3–26.2 cm) of *A. latus*, a phenomenon related to the protandrous hermaphroditism exhibited by the species.

Conclusion

The present investigation revealed that all stages of reproductive development were observed in the population of *A. berda* at a given point of time since the samples were collected within a short span of time (2 months). Moreover, the simultaneous availability of milt oozing males and matured females from the wild indicates a greater opportunity for development of captive breeding, seed production and hatchery technology for this important economic food fish.

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