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Comparison of the reproductive biology of two stocks of Indian subcontinental *Mugil cephalus* (Linnaeus, 1758) with special reference to reproductive isolation and philopatry

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The study of reproductive biology is a prerequisite for fishery management and conservation. The current study aimed to compile all available information on the reproductive biology of the grey mullet, *Mugil cephalus* from two geographical regions of India, Cochin backwaters (west coast; n = 362) and Pulicat (east coast; n = 223) southern India to decipher the possible differences in reproductive and biological attributes between the geographical groups. The LWR showed isometric growth (b = 3.08) for females on the west coast and positive allometric growth (b = 3.338) on the east coast, whereas the males showed negative allometric growth on both coasts. The sex ratio (male: female) recorded in this study was 1:1.2 on the west coast and was well balanced. On the east coast, the sex ratio was 1: 2.09 and deviated significantly from the expected 1:1 ratio. The gonadal morphology and developmental pattern were the same for both groups of *M. cephalus*. The length at maturity values of both males (L_{50} = 349.3 mm TL on the west coast and 375.8 mm TL on the east coast) and female (L_{50} = 437.6 mm TL in the west coast and 394.9 mm TL in the east coast) showed a significant difference between two groups. Seasonal distribution of GSI and maturity stages suggested that the spawning period of *M. cephalus* was between May and July coinciding with the onset of the southwest monsoon on the west coast while on the east coast it was between December and January months during the north-east monsoon. The reproductive isolation and philopatry in west and east coast *M. cephalus* groups are discussed based on the observations on the spatio-temporal distribution of fishes in the sampling areas.

[**Keywords**: East coast, *Mugil cephalus*, Philopatry, Reproductive biology, Reproductive isolation, West coast]

Introduction

In the Indian brackishwater aquaculture scenario, the sustainability of shrimp is questioned due to the emergence of the white spot syndrome virus and recently the sector appreciates the necessity of sustainability and diversification. The flathead grey mullet, *Mugil cephalus* is an important brackishwater candidate species for diversification owing to its herbivorous nature[1], and farming of lower trophic level species is the most important strategy for sustainable aquaculture development[2] given its low reliance on animal protein as feed. The grey mullet also provides high valued mullet roe, popularly referred to as grey gold, (US $ 100 per kg), which has elevated the culinary status of mullet in many parts of the world[3].

Despite its huge demand in aquaculture, captive breeding technology, and hatchery production of grey mullet is severely constrained due to the bottlenecks in the captive maturity, short seasonal spawning window[3], the existence of cryptic species[3,4], and reproductively isolated populations. At present, though the hatchery production of the species was standardized in many countries[5,6], most of the hatchery production is limited to the season.

M. cephalus is one of the prime aquaculture species in India and has been cultivating in traditional tide fed systems[7] along with other mullets and shrimps. The distribution range of species in India is restricted to the peninsular region which has a diverse climatic pattern. Although there were a lot of scattered studies on reproductive biology and hormonal maturation of this species in India[8-11], the hatchery seed production technology was not standardized yet. Currently, farming of grey mullet is a fishery-based–aquaculture that exclusively relies on the wild fry. As the sustainability of this form of farming is questionable, the development of full-fledged aquaculture using hatchery-produced seed has been the goal of farmers and aquaculturists. Further, *M. cephalus* is considered a potential indicator of global warming with increasing evidence on declining fisheries and aquaculture due to climate change impacts[12]. Against this backdrop, the reproductive biology of two breeding groups of *M. cephalus* in peninsular India (west coast and east coast) was compared for the first time to decipher the differences in reproductive biology parameters between the groups and to establish the effects of indigenous photo-thermal rhythm and ecology on reproduction and life-history traits of the species. This study will strengthen the knowledge on the biology of this species which will help conservation and management programs in future.

Materials and Methods

Study area and timelines

Live specimens of *M. cephalus* were collected from Vypin and Cochin bar mouth in the Cochin backwaters (9°40’12” and 10°10’46” N, 76°09’52” and 76°23’57” E), Kerala (west coast) and from Pulicat (13°33’34.19” N and 80°10’17.40” E), Tamil Nadu (east coast) during the period May 2015 to April 2017 (Fig. 1). Surface temperature and salinity of the sampling sites were measured using a mercury thermometer and refractometer (Atago, U.S.A), respectively.

Length-weight relationship

The total length (TL) and standard length (SL) of the fish were measured to the nearest millimeter (mm), and the total weight (W) to the nearest gram (g). The length-weight relationship was calculated by the equation, $W = aL^b$, where W is the total weight (g), L is the total length (cm), *a* is the intercept and *b* is the coefficient of growth or allometric coefficient[13]. Deviation from the isometric growth (b = 3) was tested by student’s *t*-test[14] as given in the formula:

$$t_s = (b-3)/sb$$

Where, ‘t_s’ is the student’s ‘*t*’ statistic, ‘*b*’ is the slope/allometric coefficient and ‘*sb*’ is the standard error of ‘b’.

$$sb = \sqrt{\frac{[(sW/sL) - b^2]}{(n-2)}}$$

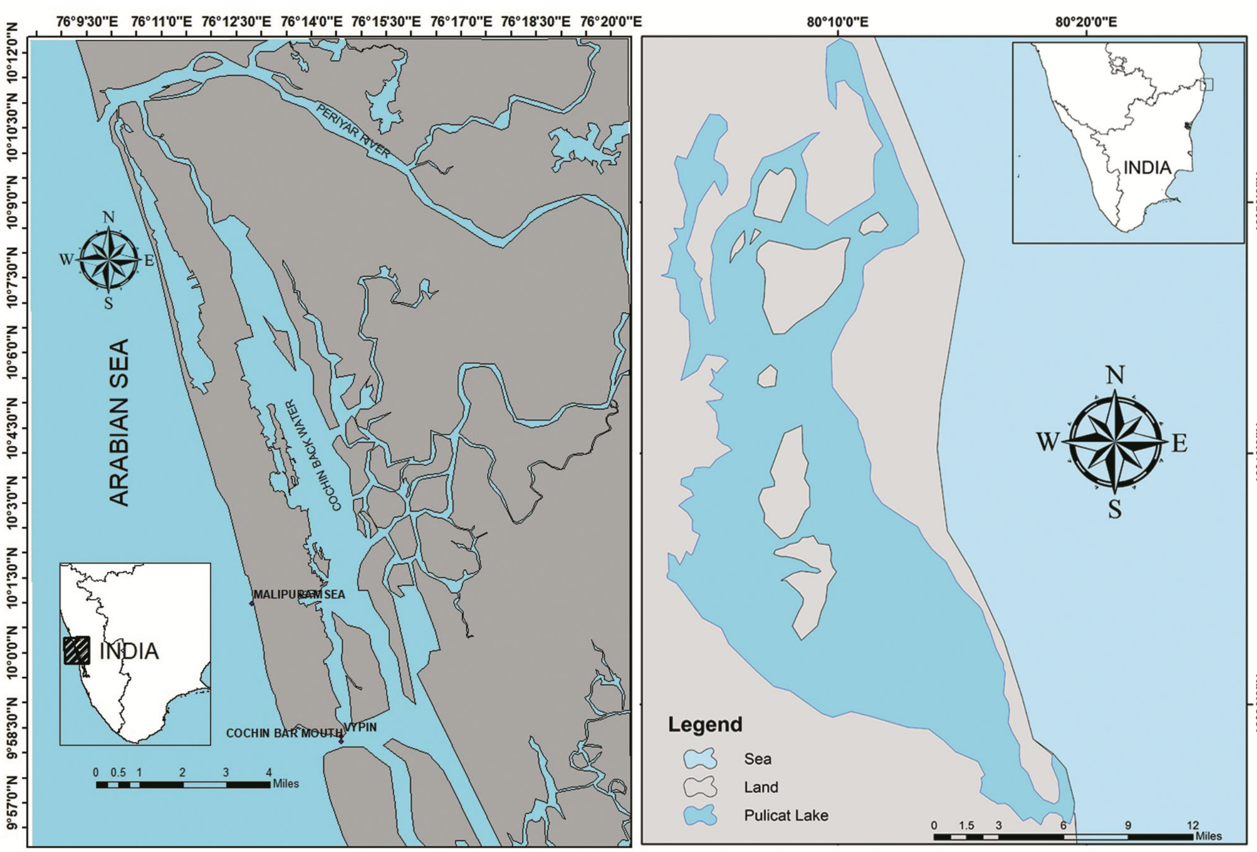

Fig. 1 — Layout of the sampling sites; Cochin backwaters (left) and Pulicat (right)

Where, sW and sL are the variances of the log-transformed weight and length respectively, and 'n' is the sample size.

Reproductive biology

Fishes were dissected and the sex was identified based on the visual observation of gonadal characteristics. The sex ratio was subsequently calculated by dividing the number of females with the number of males and Yates correction was applied to the data and a chi-square (χ^2) test was performed to test whether the sex ratio observed was significantly different from the expected ratio of 1:1[ref. 15]. The ovary weight was recorded to the nearest 0.01 g and the monthly Gonadosomatic Index (GSI) was calculated according to formula[16], GSI = gonad weight/ total body weight x 100. The maturity stages of both females and males were assigned to a five-point scale[17] with some modifications based on visual characteristics of gonads such as shape, size, color, and percentage of space occupied in the abdominal cavity.

For determining the length at first maturity (L_{50}) for males and females, the percentage frequency of immature and mature fishes was used to group the fish into a 1 cm length group, then the maturity curves were fitted to estimate length at first maturity (L_{50})[18]. A total of 62 mature gonads were taken for the fecundity analysis (west coast = 32 and east coast = 30) and the sub-samples (n = 5) of oocytes weighing 0.1 g were taken from each mature ovary and were enumerated using a stereo-microscope (Nikon SMZ25, Japan). Absolute fecundity (F) was found out by gravimetric method, counting the ova number in a known weight of the sample and estimated according to the equation,

F = Ovary weight/sample weight * no of mature eggs in the sample

Relative fecundity (RF) was estimated by dividing absolute fecundity (F) by the total weight of the fish (W). Values are expressed as absolute/relative fecundity ± SE. To determine the diameter and the size-frequency profiles of the oocyte at different maturity stages, a representative portion of the ovary was weighed and preserved in 10 % formalin. The diameter was then measured (n = 100) and its percentage frequency was found out. The spawning season of *M. cephalus* is determined based on the analysis of gonadal maturation stages and GSI of the samples in different months.

Results

Length-weight relationship (LWR)

Samples of length < 25 cm TL were not included in the present study as they could not be distinguished as male or female and considered as juveniles. A total of 362 individuals from the west coast (Cochin backwaters) of length range 29.6 – 64 cm TL and 223 individuals from the east coast (Pulicat) of length range 30.1 – 48.2 cm TL were collected for the study. The length-weight range and allometric coefficients of fishes reported from both coasts are shown in Table 1 and the student's *t*-test revealed that female fishes on the west coast followed an isometric growth pattern (b = 3.081, t_s = 0.969, P > 0.05) while the females on east coast exhibited positive allometric growth (b = 3.322, t_s = 26.29, P < 0.05). The allometric coefficients of males on west coast and east coast were 2.876 (t_s = 1.672, P < 0.05) and 2.606 (t_s = 2.057, P < 0.05), respectively. On both coasts, males followed a negative allometric growth pattern (Table 1).

Sex ratio

Among 362 individuals collected from the west coast, the total number of males and females were 46 % and 54 %, respectively and along the east coast (n = 223) the total number of male and females were 32 % and 67 %, respectively (Table 2). The overall sex ratio (M: F) was 1:1.2 on the west coast and was well

Table 1 — Descriptive statistics and length-weight parameters for both males and females of *M. cephalus* from the Cochin backwaters (west) and Pulicat (east)

Location/ Gender	n	W (g), Mean ± SE (Min-Max)	TL (cm), Mean ± SE (Min-Max)	Regression parameters		r^2	*t*-test	Growth
				a	b			
Male (west)	163	458.30 ± 9.21(249 – 974)	36.55 ± 0.25 (29.6 – 46)	0.14	2.876	0.88	1.672	A^-
Female (west)	199	1040.43 ± 29.28 (368 – 2464)	47.42 ± 0.48 (34.5 – 64)	0.007	3.081	0.89	0.969	I
Male (east)	72	435.61 ± 11.13 (239 – 677)	36.19 ± 0.30 (30.1- 42.3)	0.036	2.606	0.82	2.057	A^-
Female (east)	151	829.29 ± 23.20 (446 – 1492)	42.68 ± 0.31 (35.2 – 48.2)	0.003	3.322	0.792	26.29	A^+

N = sample size, W = body weight (g), TL = total length (cm), SE = standard error, Min = minimum, Max = maximum, a = intercept, b = allometric growth coefficient, r^2 = coefficient of determination, I = isometric growth, A^- = negative allometric growth, and A^+ = positive allometric growth

Table 2 — Chi-square test of sex ratios of *M. cephalus* from west coast (Cochin backwaters) and east coast (Pulicat) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Month	West coast				East coast			
	Males	Females	Sex ratio (M/F)	χ^2	Males	Females	Sex ratio(M/F)	χ^2
May-15	14	31	1:2.21	5.69*	2	3	1:1.5	0
June	8	17	1:2.12	2.56	1	4	1:4	0.8
July	7	9	1:1.28	0.06	2	1	1:0.5	0
August	2	5	1:2.5	0.57	1	1	1:1	0.5
September	3	3	1:1	0.17	0	5	0	3.2
October	5	2	1:0.4	0.57	2	8	1:4	2.5
November	6	5	1:0.83	0	2	3	1:1.5	0
December	5	6	1:1.2	0	0	0	0	0
Jan-16	1	1	1:1	0.5	17	30	1:1.76	3.06
February	4	6	1:1.5	0.1	4	9	1:2.25	1.23
March	3	2	1:0.66	0	2	5	1:2.5	0.57
April	2	4	1:2	0.17	1	1	1:1	0.5
May	15	26	1:1.73	2.43	1	3	1:3	0.25
June	19	30	1:1.57	2.04	3	2	1:0.66	0
July	18	15	1:0.83	0.12	2	3	1:1.5	0
August	6	0	0	4.16*	2	9	1:4.5	3.27
September	5	4	1:0.8	0	1	4	1:4	0.8
October	8	3	1:0.37	1.45	1	5	1:5	1.5
November	2	4	1:2	0.17	5	9	1:1.8	0.64
December	6	2	1:0.33	0.13	11	23	1:2.09	3.55
Jan-17	7	3	1:0.43	0.9	6	18	1:3	5.04*
February	9	3	1:0.33	2.08	1	2	1:2	0
March	6	3	1:0.5	0.44	1	1	1:1	0.5
April	2	15	1:7.5	8.47**	4	2	1:0.5	0.16
Total	163	199	1:1.22	3.38	72	151	1:2.09	28.07***

balanced ($\chi^2 = 3.38$, $P > 0.05$). On the east coast, sex ratio was 1:2.09 and deviate significantly from the expected ratio 1:1 ($\chi^2 = 28.07$, $P < 0.001$) indicating preponderance of females (Table 2). Monthly sex ratio showed a deviation from 1:1 sex ratio during April ($\chi^2 = 8.47$, $P < 0.01$) and May ($\chi^2 = 5.69$, $P < 0.05$) in 2017 on west coast and in January 2017 on east coast ($\chi^2 = 5.04$, $P < 0.01$).

Gonadal maturity stages and oocyte diameters

The ovary and testis are classified into five stages; immature, maturing, mature, advanced mature/oozing and spent (Fig. 2 & Table 3) and there were no morphological differences between gonads of *M. cephalus* of both coasts. In the immature ovary (stage I), the oocyte diameter ranged from 50 to 110 µm whereas, in the maturing stage (stage II), the diameter of oocytes increased up to 180 µm. Two distinct batches of oocytes were detected in the matured ovary (stage III); a clutch of developed oocytes of size range 180-650 µm and a batch of immature oocytes (30-120 µm). In the spent stage, immature oocytes of 30-170 µm formed the single major group, Post Ovulatory Follicles (POF) and a few residual ripe eggs (750-800 µm) were also seen. On the east coast, females with

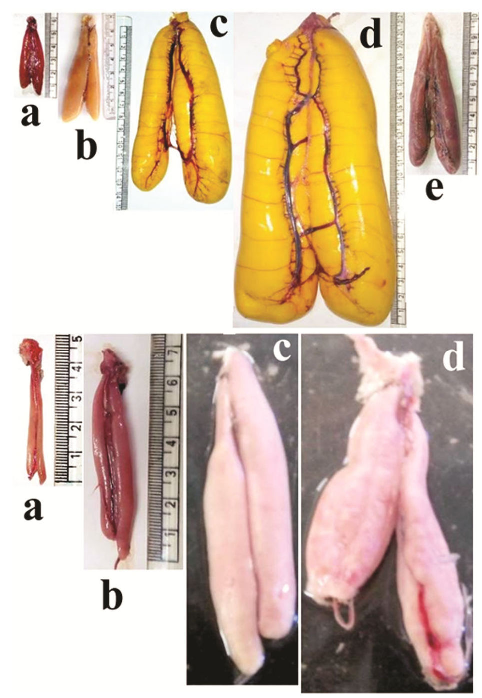

Fig. 2 — Representative images of various maturity stages of female (above) and male (below) gonadal stages. a) Immature stage b) Developing stage c) Mature stage d) advanced mature ovary of GSI 26.53 (above) and oozing testis of total length 12 cm (below), and e) Spent ovary

Table 3 — Macroscopic characteristics of maturity stages of ovary and testis of *Mugil cephalus* and GSI is expressed as mean ± SD

Maturity stages	Macroscopic observation/Number of fishes from the west coast (n_W) and east coast (n_E)	
	Ovary	Testis
Immature (Stage 1)	Small, translucent, pinkish in colour, no blood vessels visible, length – 5-7 cm and occupies one-fourth of the cavity GSI = 0.45±0.21 (n_W = 68, n_E = 60)	Thin, threadlike, semi-transparent, slightly reddish, length – 5-7 cm and occupy half of the body cavity. GSI = 0.026±0.012 (n_W = 91, n_E = 23)
Maturing/ Recovering spent (Stage 2)	Slightly yellowish, more cylindrical compared to stage 1, length - 7- 11 cm and developing oocytes were visible to the naked eye occupies half of the body cavity GSI = 2.07 ±1.26 (n_W = 13, n_E = 28)	More flattened and wider than stage 1, appears opaque and slightly reddish in colour, length 6 –9 cm and unequal lobes were common GSI = 0.037 ± 032 (n_W = 6, n_E = 9)
Mature (Stage 3)	Yellowish, massive, granular, length – 12-21 cm ovaries occupy three-fourth of or full body cavity Blood irrigation is evident. GSI = 12.26 ± 4.89 (n_W = 91, n_E = 37)	Creamy white, length 9-11cm and occupies almost three-fourth of the body cavity, vas deferens was seen for each lobe GSI = 0.987 ± 0.345 (n_W = 20, n_E = 12)
Advanced mature/ oozing (Stage 4)	Yellowish, massive, turgid, granular ovaries of length – 18 – 23 cm, occupy the full body cavity. GSI = 23.57 ± 3.69 (n_W = 0, n_E = 15)	Creamy white, more turgid and milt oozes out on slight pressure on the abdomen; the size and shape were the same as stage 3 GSI = 1.134 ± 0.275 (n_W = 32, n_E = 20)
Spent (Stage 5)	Shrunken, bloodshot, flaccid, purple in color, length – 8-10 cm and occupies almost half of the body cavity GSI = 2.56 ± 1.34 (n_W = 27, n_E = 11)	Wrinkles or folds found on the surface, flaccid, occupy almost two-third of the body cavity and little milt came out on a considerable amount of pressure on the abdomen. GSI = 0.114 ± 0.034 (n_W = 14, n_E = 8)

stage IV (advanced mature) were available and the highest oocyte diameter observed was 697 µm, while on the west coast, the largest oocyte diameter recorded was 620 µm (Fig. 3).

Length at first maturity (L_{50})

On the west coast, length at first maturity (L_{50}) was found out to be 349.3 mm TL for males and 437.6 mm TL for females. On the east coast, L_{50} for the male is 375.8 mm TL, and that for the female is 394.9 mm TL. The grey mullet females on the east coast matured earlier than the females on the west coast ($t = 9.95$, $df = 103$, $P < 0.05$; Fig. 4) and showed a significantly lower L_{50} value. On the contrary, the males on the east coast exhibited a significantly higher L_{50} value ($t = 3.62$, $df = 79$, $P < 0.05$; Fig. 4).

Fecundity

Absolute fecundity of matured grey mullets from Cochin backwaters varied from 0.42 to 3.42 million eggs with an average fecundity of 1.80±0.16 million eggs and the relative fecundity varied from 525 to 2168 eggs with an average of 1256±82 eggs per gram body weight. On the east coast (Pulicat), the absolute fecundity varied from 0.85 million to 2.61 million eggs with an average fecundity of 1.73±0.11 million

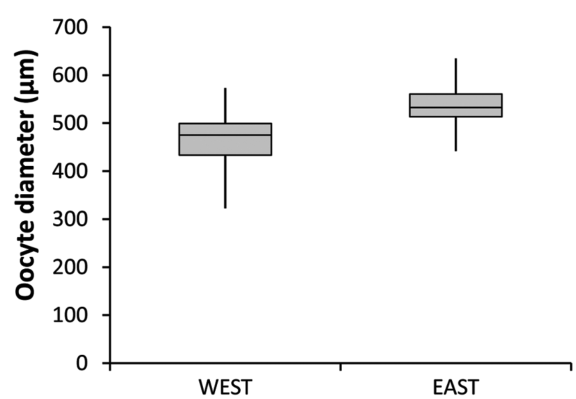

Fig. 3 — Box plot of maximum oocyte diameter range reported from Cochin backwaters (west) and Pulicat (east)

eggs, and the relative fecundity varied from 1084 to 1746 eggs with an average of 1314±91 eggs per gram body weight.

Spawning season

For the inference of spawning season, monthly variation in GSI and gonadal maturity stages of both females and males were analyzed. Along the west coast, the average GSI of female fishes increased significantly during May (8.93±1.96) and June (8.32±1.87), and the lowest average GSI value was

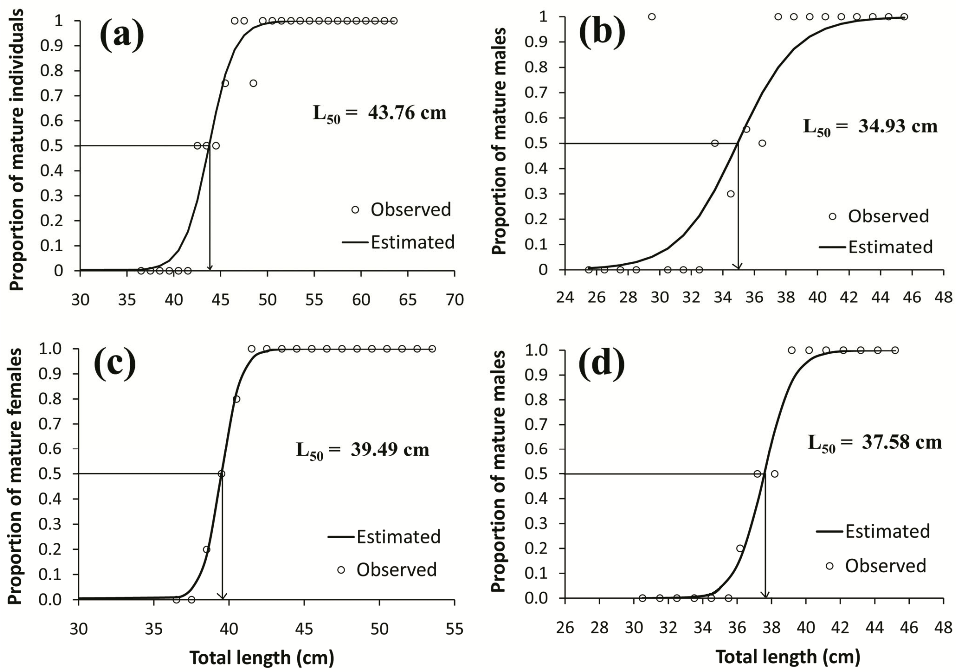

Fig. 4 — Length at first maturity (L_{50}) for *Mugil cephalus*: (a) female, and (b) male from Cochin backwaters (west); and (c) female, and (d) male from Pulicat (east)

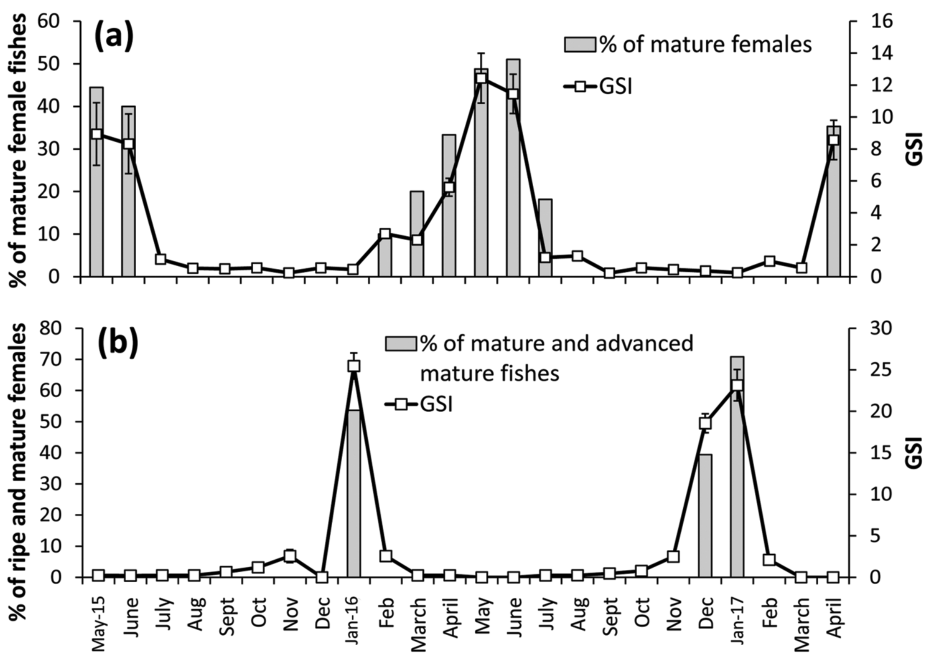

Fig. 5 — Monthly distribution of mature females (%) and GSI of *Mugil cephalus* from Cochin backwaters (a) and Pulicat (b)

observed in August (1.10±0.27; Fig. 5) which indicates that *M. cephalus* spawns between May and July in Cochin backwaters. This observation was supported by the availability of mature and spent fishes in dip net catches from May to July (Fig. 5). Female fishes with spent gonads started appearing in the catches as early as the 2nd week of May and reached a peak in July. Besides, a higher average male GSI was recorded between May (0.95±0.12) and June (1.04±0.084) months on the west coast. The highest female and male GSI recorded in the present study were 21.06 and 1.24, respectively.

Along the east coast, average female GSI values were higher in the months of December (18.58±1.14) and January (25.46±1.57) and later decreased significantly in the subsequent months (Fig. 5). Similarly, higher average GSI values were observed for male during December (0.65±0.11) and January (0.89±0.08), which confirms that *M. cephalus* spawns during December – January on the east coast. Samples could not be collected in December 2015 due to the natural calamity at the Chennai coast. The advanced mature fishes and spent fishes were available in plenty in January 2015 and during December - January months in 2016. On the east coast, the highest female and male GSI were recorded 26.53 and 0.98, respectively.

Discussion

Mugil cephalus is a commercially important brackishwater finfish, with a narrow spawning window on both coasts; on the west coast, it extends from May to July, while on the east coast it spawns during December-January. This finding agrees with the earlier reports that *M. cephalus* spawning window consists of 3-5 months in temperate and sub-tropical countries[3]. The spawning season of *M. cephalus* on both coasts timed differently, but coincided with monsoon rains, agreeing with previous reports[8,19]. The duration of the spawning season and the migration of grey mullet were affected by rainfall patterns as a shift in the breeding season was observed in line with the monsoon rains in 2016 on the west coast.

The temperature, salinity, and day length profiles of both coasts are different during the breeding season. On the west coast, breeding season starts in May when the surface temperature and salinity values are high (32±0.05 ºC, 34.5±0.08 ppt, Fig. 6). The temperature and salinity values drop (27±0.21 ºC, 10.71±1.37 ppt, Fig. 6) at the end of the breeding season (July) due to the ingress of rainwater to sampling sites during the south-west monsoon. On the contrary, lower temperature and salinity conditions (26.14±0.06 ºC, 8.6±1.21 ppt, Fig. 6) were reported throughout breeding season of the grey mullet on the east coast. Similarly, the day length on the west coast (12 hours 38 minutes) is higher than that of the east coast (11 hours 21 minutes) during the grey mullet breeding season (Fig. 6). As the temperature, salinity, and day length profiles are different on both the coasts; it is difficult to conclude the effect of these factors on oocyte development and spawning. The temperature and day length values reported during the spawning season of *M. cephalus* on both coasts were not in accordance with the previous report *i.e.*, the combination of short photoperiod (6L/18D) and low temperature (21 °C) is essential for *M. cephalus* oocyte development and maturation[20]. The possible explanation to this ambiguity is that the seasonal changes in the temperature and photoperiod are minimal in the tropical climate and the nutrient

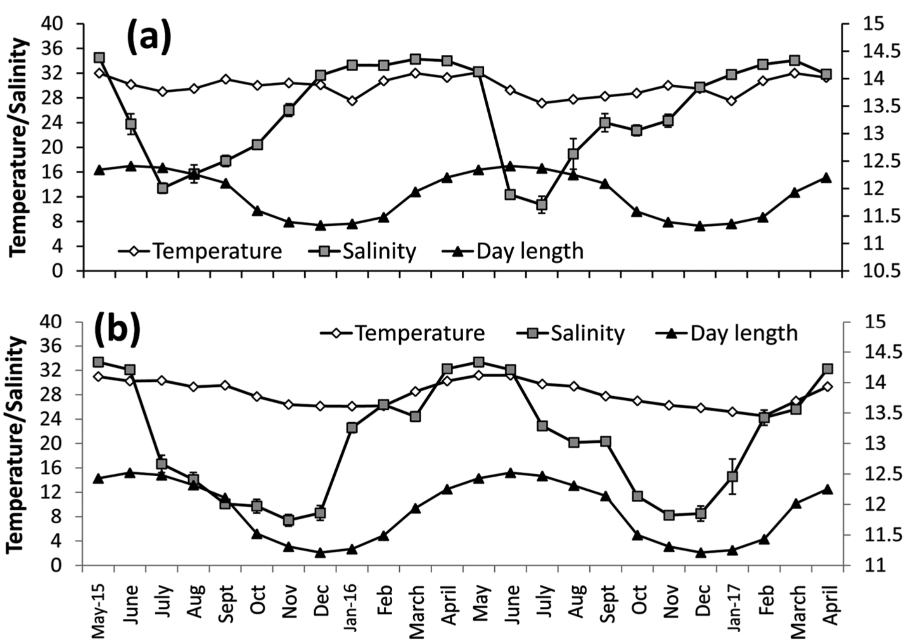

Fig. 6 — Monthly distribution of temperature, salinity, and day length of Cochin backwaters (a) and Pulicat (b)

dynamics in the aquatic habitats are affected by seasonal changes in wind and rainfall[21]. The monsoon showers bring high dissolved oxygen[10], high turbidity, and high nutrient content on both the coasts. Hence, the spawning of *M. cephalus* on both the coasts was timed in such a way that the larvae were accessible to nutrient-rich feeding grounds formed after monsoon rains. The availability of *M. cephalus* fry of size range 16-20 mm in Cochin bar mouth during mid-June to August supports this observation and reported earlier[10]. So there exists spatio-temporal isolation between these groups of *M. cephalus* during the breeding season due to the climatic differences of west and east coasts of India.

The sampling site, *i.e*. the Cochin bar mouth connects the Arabian Sea and Vembanad Lake (Fig. 1). Mature *M. cephalus* were caught in larger numbers in May and June from Cochin bar mouth most likely when they were aggregating at the bar mouth for seaward migration. The absence of ripe females from sampling sites and the availability of spent fishes from the adjoining Malipuram Sea (Fig. 1) during the monsoon season also supports the seaward spawning migration of *M. cephalus*. The eggs of *M. cephalus* are not known to survive salinities < 28 ppt for more than 24 hrs[22] and salinity levels in the sampling sites decreased drastically during monsoon; these conditions further favours the seaward migration for spawning; agreeing with the reports of the availability of mature fishes from the surf area at Beypore, Calicut[19]. The availability of spent fishes from May to July and the schools of *M. cephalus* fry of size range 16-20 mm in the Cochin bar mouth during mid-June to August confirms the 'homecoming' or philopatric nature of *M. cephalus*, as reported earlier by many authors[3,23,24].

The fecundity and length range of the fecund fishes recorded in this study was higher than reports from Goan waters, west coast of India (0.04 to 0.5 million, 140 to 310 mm)[25,26]. Earlier studies from Pulicat (0.43 and 4.71 million)[27] and Krishna estuarine regions (0.4 to 5.2 million)[28] reported a similar fecundity, but the reported length and weight of the fecund fishes were smaller than that of the current study. The length at first maturity (L_{50}) values for both male and female *M. cephalus* between two geographical regions under study were significantly different and also different compared to earlier reports from Krishna estuarine region (16 cm)[28] and Goan waters (31 cm)[26]. This indicates that the climatic conditions and physicochemical parameters of the geographic location affect the gametogenesis and gonadal development in fishes. Further, the description of *M. cephalus* as a species complex based on reproductive biology, genetics, and systematics[3] supports this assumption.

The present study recorded a female allometric coefficient of 3.081 (isometric growth) on the west coast and 3.322 (positive allometric growth) on the east coast which are slightly higher compared to the earlier reports from Vellar estuary (2.8586)[29] and Krishna estuarine regions (2.74)[30] of southeast coast of India. However, the allometric coefficient is known to differ with sex, stage of maturity, and food habits of the fish[31,32]. Males exhibited negative allometric growth on both the coasts (b = 2.876, 2.606) and agreed with earlier reports from Krishna estuarine region (b = 2.66)[30] and from Vellar estuary (2.7658)[29]. The sex ratio (Male: females) of *M. cephalus* from Cochin backwaters was 1:1.2 and showed slight domination of females, like the reports from the Pacific coast of Mexico[33] (male: female 0.88:1) and Lagos lagoon[34] (1:1.09). Earlier reports on sex ratio from Pulicat (1.56:1)[27], Krishna estuarine region (1.99:1)[28], and Goan waters (1.77:1)[26] showed the preponderance of males, while in this study (sex ratio, 1: 2.09) female outnumbered the males; however, the chances of differential fishing or the selectivity of gear towards the bigger female fishes cannot be overlooked.

The maximum GSI recorded in the present study was 21.06 on the west coast and 26.53 on the east coast and agreed with earlier reports[19,23,35]. The maximum oocyte size reported from both places also differs. On the west coast, the maximum oocyte size reported was 620 µm which agrees with the previous studies from Calicut during the breeding season[19], but from Pulicat, the largest oocyte diameter recorded in the present study was 697 µm and it is the first report in this regard. The maximum GSI reported for males in the west coast is concurrent with earlier study[9]; however, the male GSI values differ between two geographical regions under study. These differences in GSI with region among the same species could be attributed to temperature[36] and feed availability[37].

The reported breeding season for Indian *M. cephalus* is different along the west and east coasts (Fig. 7). The breeding season of *M. cephalus* in Goa is reported to be from October to December[26], June to July in Calicut[19], and December to January in the

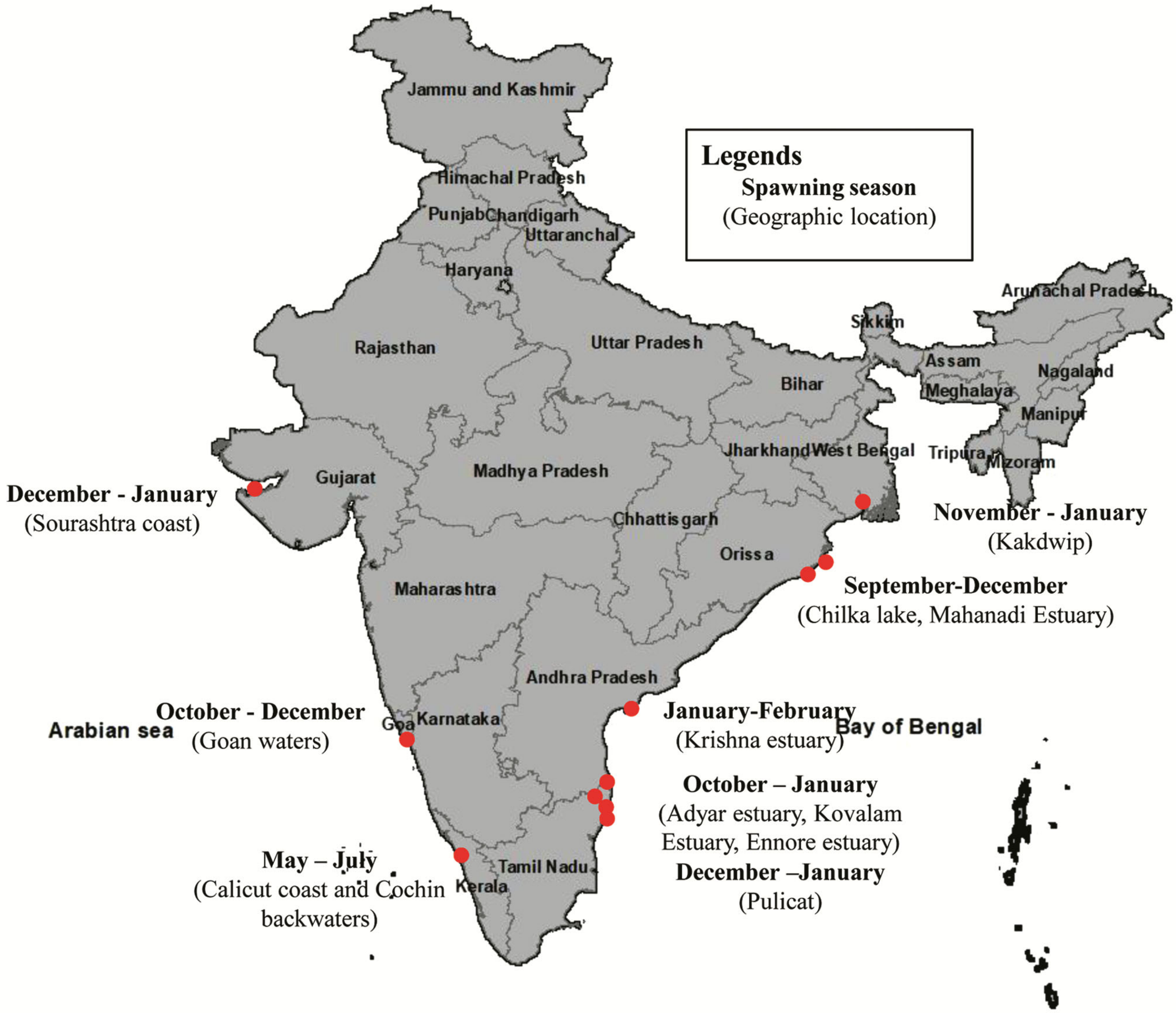

Fig. 7 — *Mugil cephalus* breeding seasons reported across India

Sourashtra coast (Gujarat, personal communication). On the east coast, October – January is the breeding season of grey mullet in Adyar estuary and Kovalam Backwaters[8]. An extended spawning season from October to May was reported from Ennore estuary[38]. Earlier work from Pulicat waters recorded an extended grey mullet breeding season from September to February[27]. In Mahanadi estuary[39] and Chilka lake[40], the breeding season of *M. cephalus* is during September-December. In Krishna estuary, the grey mullet breeding season is between January and February[28]. In west Bengal (Kakdwip, Personal communication), mature grey mullet fishes were available during November – January months. The availability of young ones of *M. cephalus* in different regions of India follows the spatio-temporal differences with respect to the spawning location and season[41]. In Goa and Gujarat (West coast), the breeding season of *M. cephalus* is during the winter months though the SW monsoon season (June – August) dominates along this coast. Similarly, in Kayamkulam lake (Southwest coast) 100 km south of our sampling site on the west coast, the breeding season of *M. cephalus* was during the winter months (October – January)[41]. Detailed studies on eco-biology are therefore required to establish the relationship between abiotic factors and spawning of grey mullet in these regions.

The different grey mullet spawning seasons reported across India indicates that *M. cephalus* is flexible in adapting its eco-biology relative to the specific geographic regions they inhabit, leading to the formation of locally adapted stocks[3]. Hence, we postulate that the spatial and temporal isolation of the species during the spawning season along with philopatry may limit or prevent the mixing of geographical groups; and can create an independent evolutionary lineage, possibly a cryptic species complex with a high level of morphological similarity, as reported from Taiwan[42]. However, more studies are required to establish the existence of cryptic species complex in mullet groups across the Indian subcontinent, though the observations of difference in length at first maturity and spawning season establish a clear demarcation among available *M. cephalus* groups. Failure to the identification of cryptic species may lead to incorrect inferences, which will have implications in the development of captive breeding program and the conservation strategies.

Conclusion

The present study envisages the reproductive factors and the environmental inducers required for the successful recruitment and sustenance of the grey mullet on two geographical regions of India. The spawning window of *M. cephalus* on two geographical regions under study was narrow and timed differently. The reproductive isolation by climatic differences on the east and west coast of India along with philopatry makes the indigenous stocks apparent resident species with different season window for spawning. The observations of difference in length at first maturity and spawning season reported across India establish a clear demarcation among available *M. cephalus* groups; however, more studies are required to decipher different *M. cephalus* stocks available in India and its characteristics. The information generated through this study could be used for developing appropriate conservation measures to avoid exploitation of *M. cephalus* during the spawning season and will be helpful for the stock assessment studies of the species.

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Ethical statement

The dead fish specimens were collected from the traditional fishermen at Cochin backwaters and Pulicat Lake. No animals were stressed or killed during the research and the study was carried out following the current animal welfare laws in India. The provisions of the Govt. of India's Wildlife Protection Act of 1972 are not applicable for the experiments on *M. cephalus*, as this fish is not endangered.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Author contributions

MUR, KS, VR, and ST helped in designing the study, collecting and analyzing the data; and MK, CPB, and KKV helped in analyzing data and drafting the paper.

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