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Studies on some aspects of reproductive biology of Shovelnose catfish, *Arius subrostratus* (Valenciennes, 1840) from Cochin estuary, India

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Abstract
This study describes some aspects of reproductive biology and breeding cycle of an estuarine catfish *Arius subrostratus* from Cochin estuary, India. For this study monthly sampling was conducted from Cochin estuary (76º 9’25” E - 76º 24’28” E and 9º 47’31” N - 10 º 12’N) during April 2011 to March 2013 period. The minimum size at first maturity of *A. subrostratus* during the study period was 235 mm. The higher Gonadosomatic index occurred during October to January was an indication of spawning season of the species. Fecundity varied from 11 to 34 eggs during the period and ova diameters were ranged from 0.022 mm to 14.5 mm. The sex ratio calculated was 1: 1.1. From the ANOVA result GSI was significant at 5 % level between different months. This study revealed that *A. subrostratus* from Cochin estuary showed breeding season during Northeast monsoon period.

Keywords: *Arius subrostratus,* Cochin estuary, Size at first maturity, Sex ratio

Introduction
Majority of catfish stocks were seriously depleted due to overexploitation and degradation of ecosystems. In order to improve catfish stocks we must take management plans to restrict overfishing during the breeding periods. Knowledge on reproductive biology of fishes helped us to understand its breeding periods, spawning season and reproductive behaviour [1]. Information on related aspects such as ecological conditions which leads to the organization of maturity and breeding activity in males and females, size at first maturity, breeding migration, sex ratios, sexual dimorphism, fecundity, etc. are having enormous applications for the conservation and management of fish stocks.

Arius subrostratus is an ariid catfish included under the family Ariidae and the order siluriformes. This species is distributed in the Indo- west pacific regions including India, Pakistan, Indonesia, Thailand and Philippines [2]. It occurs in estuaries, lagoons, tidal rivers, marine water and some mangrove area and is a benthic invertebrate feeder. They have three pairs of barbels around mouth, maxillary pair usually not reaching to orbit, but sometimes extending just to a little distance behind the eye. A number of researchers have studied various aspects of biology of catfishes worldwide. Reproductive biology of a catfish *Clarias gariepinus* in the lake Awassa, Ethiopia [3],reproductive parameters of *Heterobranchus longifilis* known as African catfish in Belgium [4], feeding ecology of bagrid catfish, *Mystus tengara* from a wetland in Bangladesh [5] were the major studies done on catfishes. An investigation on the literature of catfishes especially *Arius subrostratus* proved that, there was very little information available on the biology particularly in the Indian context. Particularly there were no previous studies conducted on the reproductive aspects of *Arius subrostratus* from Cochin area. Thus this study would suggest some management measures for the conservation of the species from Cochin estuary.

Materials and Methods
1. Study site and fish collection
Fish specimens were collected on a monthly basis from Cochin estuary (76º 9’25” E - 76º 24’28” E and 9º 47’31” N - 10 º 12’N) from April 2011 to March 2013 period. About 30 to 50 fishes were randomly collected using gill nets of 50 – 60 mesh size. Triplicate samplings were carried out each month from different landing centres of Cochin estuary.

The samples consisted of a total of 878 specimens of *Arius subrostratus* ranging in size from 12.5 to 34.5 cm in total length (TL) and 16 to 410 g in weight. The freshly caught specimens were immediately soaked in ice box and brought to laboratory and analysed in 1-2 hour period. Data on total length, weight, and appearance of gonads were noted from fresh specimens. Then gonads and guts were dissected out and length, weight and sex were noted down and preserved in 4% formalin for further studies.

2. Fish analysis

Size at first maturity was calculated for males and females separately by grouping them into 20 mm groups. Length at which 50% of the fishes that attained first maturity was estimated. The gonadosomatic index (GSI) was determined using the formula, GSI= GW * 100/ BW, [6] where, GW = Gonad weight in gram; BW = Gutted body weight in gram. Sex of the specimens was established by macroscopic examination of the gonads after dissection. The sex ratio (number of females for every male) according to different size group and different months were studied using chi – square (X^2) test [7]. The regression analysis and test of significance of ‘r’ values so obtained were estimated as per standard procedures. Fecundity in fishes is usually determined by the number of mature ova in the ovary. The matured ova in the mature ovaries were taken to estimate the fecundity of each individual. Being very big each ovum was separated by a needle. Multiple regression analysis of the reproductive parameters viz, total length and fecundity in *Arius subrostratus* were calculated to find out the correlation between the parameters. Significance of GSI with, sex, month and body parameters were tested by one way ANOVA [7, 8, 9]. Ova diameter study or measurement of intra ovarian eggs was carried out from the preserved ovaries. Each ovum was separated from the ovaries for the measurements. Large ova were measured by the help of a vernier dial caliper and smaller ones were measured with ocular micrometer.

Results

From the present findings, size at first maturity in male was 24 – 25 cm length group (mean 24.5) and in female it was 22 – 23 cm length group (mean 22.5) (Fig1). The percentage composition of fishes in different maturity stages of each year are presented in the Tables 1 and 2. For the first year (April 2011 – March 2012) immature males and immature females appeared in most of the months. During August to November a continuous appearance of mature females were occurred. In the second year mature females occurred during the months of April to November. Second year showed major percentage of immature stages from April 2012 to September 2012.Spawning season of *A. subrostratus* was determined by the monthly gonadosomatic ratio (Table 3). In the first year higher GSI occurred during the month of October to January, and the second year it was during September to November. Lowest GSI occurred in May to August during the study period. May 2011 to July 2011 showed a decline in GSI with an increase from August. Higher GSI was observed in the month January 2012. From February onwards a slight decline in GSI were observed. ANOVA result of GSI showed that it was significant at 5 % level between month ($F= 2.785$, $P < 0.005$). It also showed significant variation between both sexes ($F = 19.888$, $P < 0.001$).

Here the fecundity of *A. subrostratus* ranged from 11 to 34 (Fig 2). Low fecundity was observed in the length group 20 – 24 cm, whereas higher fecundity occurred in the Length group of 27 – 28 cm (Table 4). Ova diameters of *A. subrostratus* were ranged from 0.022 mm to 14.5 mm (Fig 3). The regression relationship between fecundity and some of the morphometric parameters such as total length ($R^2 = 0.908$) and total weight ($R^2 = 0.918$) was linear and the equations were $y = 3.143x - 3.009$ and $y = 1.065x - 0.777$ respectively (Figs 4a & b). Relationship between fecundity and gonad weight also showed linear relationship and the equation depicted as $y = 0.075x + 1.051$ ($R^2= 0.913$) (Fig 4c). There were significant relationship between fecundity and these body parameters. Highest correlation ($R^2 = 0.918$) occurred between fecundity and total length.

Among the 856 specimens examined for the two year period 404 males and 452 females have been observed. The average sex ratio of males to females during the two year study period was 1: 1.1. For the first year 478 specimens were observed and among them 217 males and 261 females were examined. The sex ratio of males to females during the first year was 0.83. Females showed preponderance over males in most of the months. While during the months July ($X^2 = 0.55$), August ($X^2 = 0.4$), September ($X^2 = 0.38$) and February ($X^2 = 0.4$) males showed preponderance over females. Chi square test showed significant variation from the expected ratio of 1: 1 in these months at 5 % level. Whereas, during the second year 191 males and 187 females have been obtained and the sex ratio was 0.98. Chi square test in the second year, December ($X^2 = 6.545$; $P < 0.05$) and February ($X^2 = 4.481$; $P < 0.05$) showed significant at 5 % level. Overall sex ratio for the entire study period did not differ significantly from the expected 1:1 ratio (Table 4). Length wise distribution of sex ratio showed preponderance of females over the male population in most of the length groups (Table 5).

Table 1: Percentage composition of fishes in different maturity stages of *A. subrostratus* from Cochin Estuary during April 2011 to March 2012

Apr. 11			**May. 11**			**Jun. 11**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	31	21		24	30		25	25
I	48.39	9.52	I	45.84	56.67	I	60	36
II	32.26	47.62	II	37.5	13.33	II	20	44
III	19.35	4.76	III	8.33	6.67	III	12	4
IV	0	38.1	IV	0	0	IV	8	0
Spent	0	0	Spent	8.33	23.33	Spent	0	16
Jul. 11			**Aug.11**			**Sept. 11**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	25	20		22	18		23	19
I	17	60	I	54.55	50	I	43.48	10.53
II	4	10	II	18.18	16.67	II	13.04	36.84
III	4	25	III	4.55	22.22	III	39.13	15.79

IV	0	5	IV	18.18	11.11	IV	4.35	36.84
Spent	0	0	Spent	4.55	0	Spent	0	0
Oct. 11			**Nov. 11**			**Dec. 11**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	17	23		19	21		15	20
I	76.47	13.04	I	36.84	4.76	I	60	35
II	23.53	21.74	II	42.11	19.05	II	13.33	5
III	0	13.04	III	0	19.05	III	6.67	10
IV	0	34.78	IV	15.79	38.09	IV	20	45
Spent	0	17.39	Spent	5.26	19.05	Spent	0	5
Jan. 12			**Feb. 12**			**Mar. 12**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	9	28		22	18		7	18
I	22.22	21.43	I	36.36	16.67	I	57.13	0
II	33.34	10.71	II	22.73	33.33	II	14.29	16.67
III	22.22	10.71	III	18.18	22.22	III	14.29	22.22
IV	22.22	50	IV	22.73	22.22	IV	14.29	50
Spent	0	7.15	Spent	0	5.56	Spent	0	11.11

Table 2: Percentage composition of fishes in different maturity stages of *A. subrostratus* from Cochin Estuary during April 2012 to March 2013

Apr. 12			**May. 12**			**Jun. 12**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	16	23		19	25		24	17
I	56.25	13.04	I	47.37	28	I	50	29.42
II	31.25	43.48	II	31.58	28	II	25	23.53
III	6.25	4.35	III	5.26	4	III	8.33	11.76
IV	6.25	26.09	IV	10.53	8	IV	0	11.76
Spent	0	13.04	Spent	5.26	32	Spent	16.67	23.53
Jul. 12			**Aug.12**			**Sept. 12**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	24	16		13	21		9	17
I	41.67	18.75	I	76.93	38.1	I	0	0
II	20.83	18.75	II	15.38	23.81	II	22.22	0
III	12.5	18.75	III	0	9.52	III	22.22	23.53
IV	8.33	31.25	IV	0	23.81	IV	55.56	64.71
Spent	16.67	12.5	Spent	7.69	4.76	Spent	0	11.76
Oct. 12			**Nov. 12**			**Dec. 12**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	9	16		14	16		17	5
I	22.22	6.25	I	57.14	25	I	47.06	100
II	33.33	6.25	II	21.43	12.5	II	17.65	0
III	22.22	0	III	14.29	6.25	III	11.76	0
IV	11.11	68.75	IV	0	37.5	IV	0	0
Spent	11.11	18.75	Spent	7.14	18.75	Spent	23.53	0
Jan. 13			**Feb. 13**			**Mar. 13**		
	M	**F**		**M**	**F**		**M**	**F**
No of fishes	9	11		19	8		13	17
I	44.44	36.36	I	52.63	25	I	61.55	52.94
II	11.11	18.18	II	31.58	50	II	7.69	23.53
III	33.33	9.09	III	0	0	III	15.38	5.88
IV	11.11	27.27	IV	5.26	25	IV	0	0
Spent	0	9.09	Spent	10.53	0	Spent	15.38	17.65

Table 3: Mean gonadosomatic index (GSI) of different months for *A. subrostratus* from Cochin estuary during April 2011 to March 2013

Month (First year)	**GSR**	**Month (Second year)**	**GSR**
Apr-11	2.5557	Apr-12	2.3519
May-11	0.7785	May-12	0.9184
Jun-11	0.4157	Jun-12	1.1801
Jul-11	0.8719	Jul-12	1.8616
Aug-11	1.5577	Aug-12	1.8884
Sep-11	2.4392	Sep-12	5.8857
Oct-11	4.7425	Oct-12	9.3689
Nov-11	4.3341	Nov-12	2.3712
Dec-11	5.3775	Dec-12	1.1746
Jan-12	8.4380	Jan-13	3.4857
Feb-12	1.5191	Feb-13	1.2401
Mar-12	4.8781	Mar-13	4.9809

Table 4: Monthly distribution of sex ratio and Chi square test in *A. subrostratus* from Cochin estuary during April 2011 to March 2013

Month	Male	Female	Sex ratio (M: F)	Chi- square (X^2)	Level of significance
Apr-11	9	21	0.43	4.8 *	P < 0.05
May-11	24	30	0.8	0.66	P > 0.05
Jun-11	25	25	1	0	P > 0.05
Jul-11	25	20	1.25	0.55	P > 0.05
Aug-11	22	18	1.22	0.4	P > 0.05
Sep-11	23	19	1.21	0.38	P > 0.05
Oct-11	17	23	0.74	0.9	P > 0.05
Nov-11	19	21	0.9	0.1	P > 0.05
Dec-11	15	20	0.75	0.714	P > 0.05
Jan-12	9	28	0.32	9.75 **	P < 0.05
Feb-12	22	18	1.22	0.4	P > 0.05
Mar-12	7	18	0.39	4.84 *	P < 0.05
Apr-12	16	23	0.7	1.256	P > 0.05
May-12	19	25	0.76	0.818	P > 0.05
Jun-12	24	17	1.41	1.195	P > 0.05
Jul-12	25	15	1.67	2.5	P > 0.05
Aug-12	13	21	0.62	1.882	P > 0.05
Sep-12	9	17	0.53	2.461	P > 0.05
Oct-12	9	16	0.56	1.96	P > 0.05
Nov-12	14	16	0.88	0.133	P > 0.05
Dec-12	17	5	3.4	6.545 *	P < 0.05
Jan-13	9	11	0.82	0.2	P > 0.05
Feb-13	19	8	2.38	4.481 *	P < 0.05
Mar-13	13	17	0.76	0.533	P > 0.05

**= significant at 1% level; * = significant at 5 % level

Table 5: Length wise distribution of sex ratio and Chi – square test in *A. subrostratus* during April 2011 to March 2013

Length group	Male	Female	Sex ratio (M:F)	Chi- square (X^2)	Level of Significance	D.F
13 - 15	0	3	0.00	3.00	P > 0.05	1
15 - 17	4	7	0.57	0.82	P > 0.05	1
17 - 19	25	23	1.09	0.08	P > 0.05	1
19 -21	79	88	0.90	0.49	P > 0.05	1
21 -23	155	176	0.88	1.33	P > 0.05	1
23 -25	111	104	1.07	0.23	P > 0.05	1
25 -27	35	32	1.09	0.13	P > 0.05	1
27 -29	8	12	0.67	0.80	P > 0.05	1
29 -31	4	3	1.33	0.14	P > 0.05	1
31 - 33	3	1	3.00	1.00	P > 0.05	1
33 -35	2	2	1.00	0.00	P > 0.05	1
35 -37	0	1	0.00	1.00	P > 0.05	1

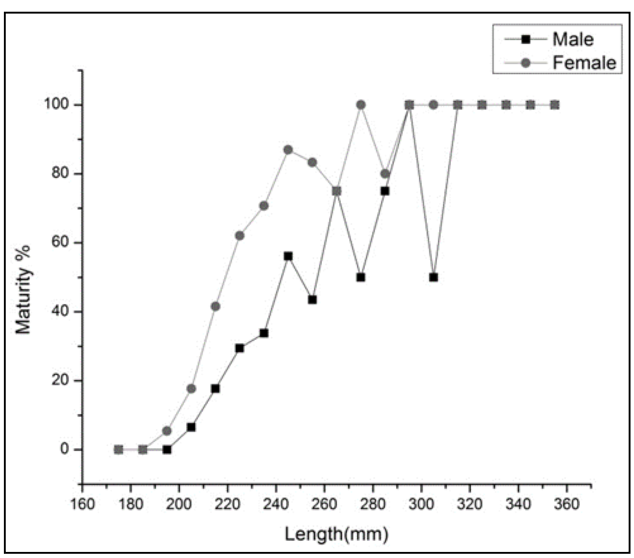

Fig 1: Length at first maturity of *A. subrostratus* from Cochin estuary during April 2011 to March 2013

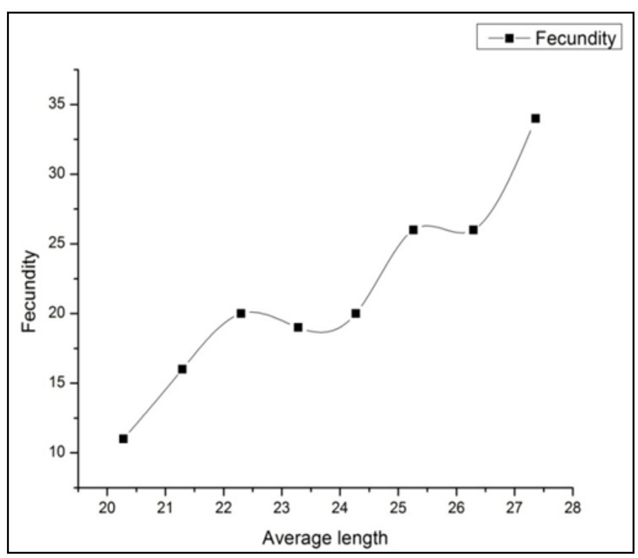

Fig 2: Fecundity of *A. subrostratus* from Cochin estuary based on length group (cm)

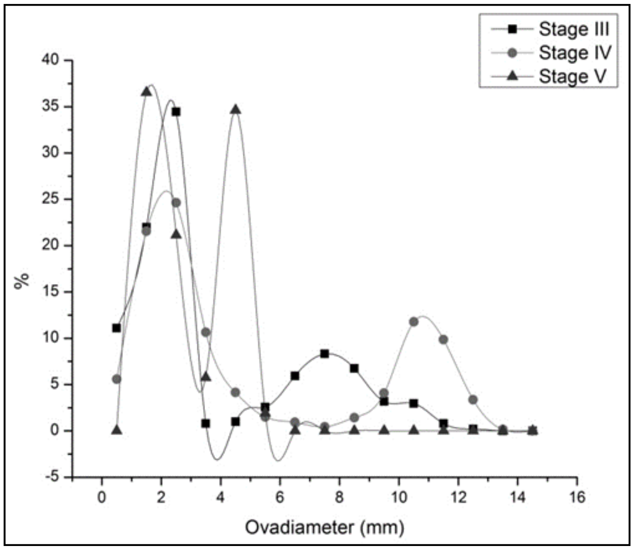

Fig 3: Size frequency distribution of ova of *A. subrostratus* in different stages of maturity from Cochin estuary during April 2011 to March 2013

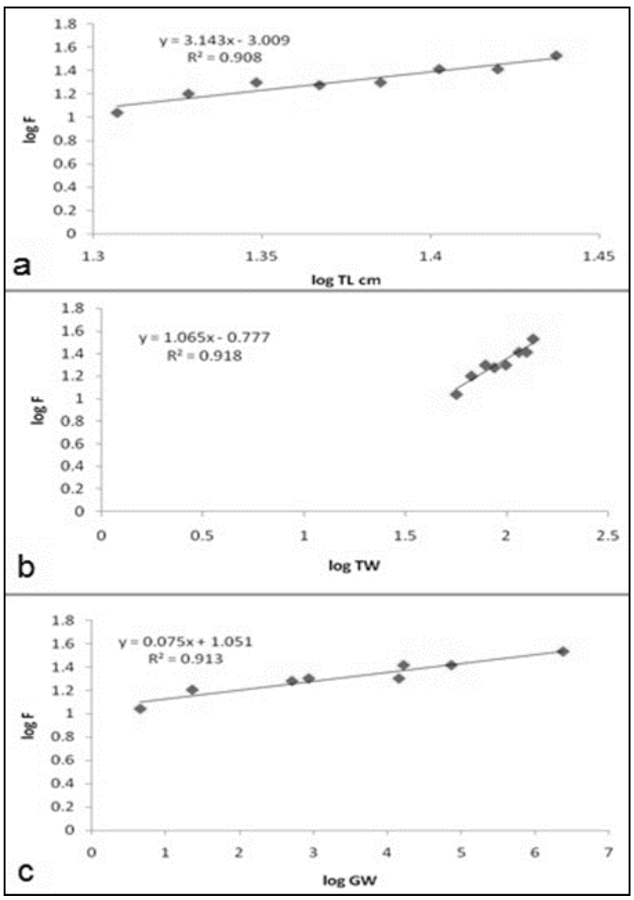

Fig 4 a: Relationship between total length and fecundity of *A. subrostratus* from Cochin estuary; b: Relationship between total weight and fecundity of *A. subrostratus* from Cochin estuary; c: Relationship between gonad weight and fecundity of *A. subrostratus* from Cochin estuary

Discussion

As in most teleosts, the gonads in the males and females of *Arius subrostratus* were paired, elongated structure lying on side of the air bladder ventral to kidneys [10]. Information on the size of maturation is crucial for avoiding over exploitation of immature juveniles and ensuring the spawning of the individual fishes at least once in life. Here the size at first maturity noted in male was 24 – 25 cm length group and in female it was 22 – 23 cm length group. Minimum size at first maturity in *Arius jella* from Mumbai water was 28 cm [11].Determination of maturity of fishes allows the fishery biologist to predict reproductive capacity of fishes in a stock [12]. Nikolski (1963) [13] explained length at maturity of a fish varied in relation to the food availability. Awareness of size at first maturity helps us to avoid overexploitation of juvenile fishes [14].

Gonadosomatic index (GSI) was used for estimating the spawning season. The high gonadosomatic index during the spawning season is an indication of egg production of the fishes [15]. *Psectrogaster rhomboids* from Northeastern Brazil exhibited high GSI during rainy season is an indication of spawning period [16]. Here the mean monthly value of GSI increased during the study period from September to January. Hence the northeast monsoon period showed higher GSI during the study period. It can be concluded that the spawning activity of the *Arius subrostratus* may have started during the late monsoon months. In Indian subcontinent there are two types of monsoon, southwest and northeast monsoon. In south India the south west monsoon start in the month of June and end at August. While northeast monsoon period in South India starts by the month of October and last up to January [17]. Therefore *A. subrostratus* from Cochin estuary showed breeding season during Northeast monsoon period.

Fecundity is the number of eggs produced by an individual fish in its life time [18]. The fishes are either high fecund fishes or low fecund fishes. Fecundity of Ariid catfishes is very low and with large sized eggs or ova of about 13 mm in size [19]. The estimated fecundity in *A. subrostratus* ranged from 11 to 34 eggs with an average of 22 eggs per fish. The low fecundity in *A. caelatus* (44 -81 eggs) and *O.militaris* (27 -61 eggs) might be associated with parental care [20]. Similar conclusion was made by Dan (1977) [21] based on low fecundity in *A. tenuispinis* (29 -82). Muthiah and Rao (1985) [22] estimated highest fecundity of a related species *A. dussumieri* as 207 eggs (average 190 eggs). Reduction in fecundity may be an adjustment of female to survive in the condition with insufficient nutrients [23, 24]. Hence the nutrients are recycled for their somatic growth other than sexual maturation.

In *Arius subrostratus*, ova diameter ranged from 0.022 mm to 14.5 mm, that included mature and immature oocytes. This species showed large eggs of nearly one centimetre in diameter. Largest egg diameter is generally exhibited by salmonids and marine catfishes (ariids) with about 10 mm and 15 mm respectively. The young ones of fishes with large egg size takes more time to reach their adulthood as the re absorption of their yolk required more time [25]. Fecundity is often correlated with length, weight and age of fish and also with the length and weight and that of ovary. The relationship between the body parameters and fecundity vary in different species. Correlation between fecundity and body parameter observed here was linear.

From the present investigation it can be assumed that, *A. subrostratus* is a multiple spawner. In multiple spawning fishes different sized eggs are seen in the same ovary [26]. Multiple spawning fishes produce small number of large eggs and their spawning that lasted for an extended period. While the total spawner breeds over a small period with numerous small eggs [18]. *Arius subrostratus* from the present findings exhibited a prolonged spawning period. Occurrence of a single peak of gonadosomatic index during a year denoted single spawning season of the species. During the present study batches of eggs were present in a single ovary.

The ideal sex ratio in mature population is close to 1:1 [27]. Here the ratio between males and females was 1: 1.1. While during the months July, August, September, and February males showed preponderance over females. Chi square test results of the study reveals that the ratio of males to females was not significantly different from 1:1 in most of the months. The sex ratio in relation to the different size groups of some *Arius sp*. showed that the number of adult males at higher length declined and were missing after a certain length, indicating the possible oral gestation mortality among adult males in the species [20]. Dan (1977) [21] also reported absence of males and excess of females at higher length groups and suggested possibility of oral gestation mortality in adult males of *Arius tenuispinis*. In addition male parent starved during the oral incubation period. They did not take any food during that time because off carrying eggs in their buccal cavity. Hence the chances of mortality due to starvation were higher [20].

The size at first maturity allows us to conserve the species by fixing suitable criterion for minimum size of a fish for its exploitation. In the present study, breeding season of the species occurs during the northeast monsoon period (September - November) when the Gonadosomatic ratio was very high. The restricted capture of the fishes only above the size at maturity of the species from such water bodies will help in the conservation of the fish. The minimum size at first maturity of *Arius subrostratus* was 235 mm the length at which the species is mature to be harvested.

Conclusion

A. subrostratus is a multiple batch spawner that contained at least two types of oocyte in a single ovary. The size at first maturity allows us to conserve the species by fixing suitable criterion for minimum size of a fish for its exploitation. In the present study, breeding season of the species occurs during the northeast monsoon period (September - November). The restricted capture of the fishes only above the size at maturity of the species from such water bodies will help in the conservation of the fish. The minimum size at first maturity of *Arius subrostratus* was 235 mm (minimum legal size (MLS), the length at which the species is mature to be harvested.

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