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# Some Aspects of Reproductive Biology of the Indegenous Ornamental Fish, *Horadandia brittani* Rema Devi & Menon, 1992 (Pisces: Cyprinidae) From the Western Ghats of India.

Seena Augustine. P.A.\*, T.V. Anna Mercy

**Abstract:** *Horadandia brittani* is a recently reviewed, popular and well recognized ornamental fish in the Western Ghats of India, a biodiversity hot spot. There have been no reports on the reproductive biology of this fish species to date. The present study has been carried out to understand the various aspects of reproductive biology of this species. *H. brittani* was collected from the wetlands of Alleppey district, Kerala, India. 1021 specimens collected during Jan 2013 to Dec 2013 have been used in the study. Size at first sexual maturity, Monthly variation in Sex Ratio, Gonadal maturation cycle, Spawning frequency and spawning season has been studied as a part of this study. Five maturity stages could be identified based on external morphology. Ova diameter distribution was studied microscopically, in the five maturity stages. Sex ratio has been determined as 1:3.625 (female: male), Size at first maturity in the males was 1.3mm TL (1-1.5mm) and in female 1.5mm (1.5-2mm). Ovary showed fractional spawning behavior along with asynchronous development of oocytes. *H. brittani* was found to be a multiple spawner with protracted spawning.

**Key words:** *Horadandia brittani*, Reproductive biology, Sex ratio, Size at first maturity.

## I. INTRODUCTION

*Horadandia brittani* is a very small well recognized cyprinid ornamental fish, termed “glow light carplet,” belonging to the sub family Danioninae [12]. It is native to southern India. It inhabits still or slow moving water, frequenting swamps, rice fields and similar still waters. [30] [23]. A subspecies, *Horadandia atukorali brittani* was described by Devi and Menon in 1992 [24] from Pathiramanal Island, Vembanad Lake, Kerala, but was brought into synonym by Menon in 1993 [17]. But again in 2013 Sudesh Batuwita, Madura Desilva and Udeni Edirisinghe [3] described *H. brittani* as a valid species, distinguished from *H. atukorali* by possessing a smaller eye and having the dorsal fin origin closer to the hypural notch. According to him the species present in Kerala is *H. brittani* and the species present in Sri Lanka is *H. atukorali*. It is locally known as “Chuttichi” in Malayalam. A complete knowledge of the reproductive biology of fishes is essential to understand the reproductive strategy of any given species. Studies on reproductive biology of any species is also very essential for assessing commercial potentialities of its stock, life history, culture practices and actual management and conservation of its fishery.

## II. MATERIAL AND METHODS

Samples of *H. brittani* have been collected monthly from 2013 January to 2013 December from wetlands near Changarampadam paddy field, at Ezhupunna south, Alappuzha District, Kerala (Latitude- 9° 49'24"N, Longitude- 76°18'36"E). 1021 specimens of *H. brittani* have been collected during the entire period of study for studying different aspects of reproductive biology.

They have been collected randomly by using scoop nets of 0.625mm mesh size from water bodies with water depth ranging from 0.3m to 2m. Depending on the availability, 15 to 30 scoops have been needed for the collection of 30-150 numbers of samples. After collection they have been transferred to 10% formalin and morphometric study have been conducted in the laboratory. For studying the reproductive biology total length (TL), has been taken using Vernier calipers to the nearest 0.01cm and total body weight (BW) in grams has been taken by using digital balance to the nearest 0.001gm. The specimens have been dissected out from the left side and the body cavity and contents have been examined carefully. The type and maturity stages of gonad, the space it occupied inside the body cavity, the texture, shape and color of gonad etc., have been observed. Then the gonads have been dissected out cautiously and the surface moisture of the gonads has been removed using the blotting paper. The weight and length of the gonads have been taken to the nearest of 0.001 gms and 0.01 cms respectively.



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Sex ratio has been estimated by studying the monthly variation in sex ratio. It has been done by counting the total number of two sexes in the monthly collected sample. Chi-square test has been conducted to determine the differences in sex ratio (monthly value and overall value) from the expected ratio of 1:1.

Size at first maturity has been determined by plotting graph between length class and percentage frequency of mature fish. The length at which 50% of fish become mature has been regarded as length at first sexual maturity.

Gonadal maturation cycle has been quantified by macroscopic and microscopic observation of the different maturity stages of gonads. According to Nickolsky [19] male and female gonads have been grouped into different gonadal stages of development. From this observation spawning frequency has been determined.

Spawning season has been determined by monthly evaluation of the gonado somatic index (GSI), mean monthly evaluation of gravid females, and mean monthly ova diameter measurement.

GSI has been measured using the following formula

$$\text{GSI} = \text{Gonadal weight (gm)}/\text{body weight (gm)} * 100$$

Size frequency distribution of intra ovarian oocyte has been studied by taking the ova diameter measurement at different maturity stages for explaining the oocyte development. [16]

### III. RESULT AND DISCUSSION

#### A. Sex ratio

606 specimens have been examined for determining the sex. 157 fishes have been found to be male and 449 fishes have been observed to be female. The average ratio of male to female has been observed to be 1:3.625 from the monthly sample of *H.brittani* (Table 1) Overall females have shown significant ( $P < 0.01$ ) dominance over males on monthly basis except in the month of December and January

Table. 1: Monthly variation of sex ratio in *H. brittani*

Month	No. of Fish	No. of Male fish	% of Male fish	No. of Female fish	% of Female fish	Ratio of male and female
Jan,2013	47	19	40.42	28	59.57	1.47
Feb,2013	58	15	25.86	43	74.14	2.87
Mar,2013	57	13	22.81	44	77.2	3.38
Apr,2013	40	10	25	30	75	3
May,2013	34	6	17.65	28	82.35	4.67
Jun,2013	49	7	14.28	42	85.7	6
Jul,2013	54	12	22.22	42	77.78	3.5
Aug,2013	66	28	37.88	41	62.12	1.46
Sep,2013	56	13	23.21	43	76.79	3.31
Oct,2013	43	8	18.6	35	81.4	4.38
Nov,2013	44	5	11.36	39	88.63	7.8
Dec,2013	58	21	36.21	37	63.79	1.77



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### B. Size at first maturity

First mature males have been observed in the length class of 1.0 -1.5cm length group (63%) and few female (20%) in 1.0 -1.5cm length group have been observed with mature gonads. 50% of the females have been found with mature gonad in the length group 1.5-2.0cm. All males of total length 2.0cm and above and all females of total length 2.5cm and above have been found to be mature. The size/length at first maturity for males and females has been observed to be 1.35cm TL (1.0 -1.5cm) and 1.5cm TL (1.5-2.0cm) respectively. (Fig. 1).

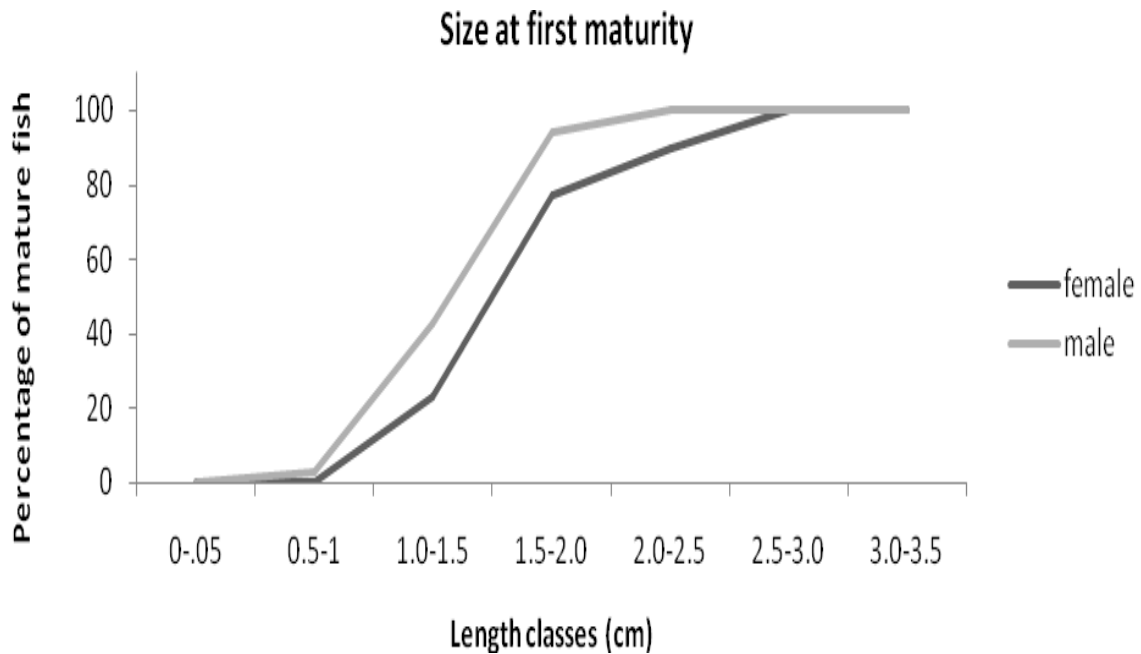


Fig. 1: Graph showing percentage of mature fish in different length classes

### IV. QUANTIFICATION OF MATURITY STAGES

Five maturity stages of gonads of *H. brittani* have been observed based on the macroscopic and microscopic observation of gonad as follows:

**Stage I (Immature):** These are the young individuals that have not yet spawned. The ovaries were transparent and irregular in shape with microscopic ova. A cursory examination under the microscope was required to differentiate ovary from the testis. Microscopically, the oocytes were oval/spherical in shape and completely transparent with a large nucleus. The ova diameter ranged from 16µm to 112 µm. (Fig. 2)

**Stage II (Maturing):** Ovaries generally off white /creamy in colour occupying half to less than three fourth of the body cavity. Ova still invisible to the naked eye. The maximum size of ova was about 240 µm. (Fig. 3)

**Stage III (Mature):** Ovaries became enlarged and pale yellow in colour, occupying three-fourth or more of the body cavity. The maximum size of ova was about 320µm. (Fig. 4)

**Stage IV (Ripe):** Ovaries yellow in colour, very turgid and occupied more than ¾ of the body cavity. Ovarian lobes were quite stout and equal in length. Ova size reached a maximum of 528µm. (Fig. 5)

**Stage V (Spent):** Ovaries at this stage were slightly flaccid, but still retaining a number of residual ripe ova/atretic ova after the spawning. Different stages of ova were found, mostly immature ones with a few ripe ones. The largest ova that could be found in this stage were 400µm. This stage enters the maturation cycle at early ripening stage. (Fig.6).



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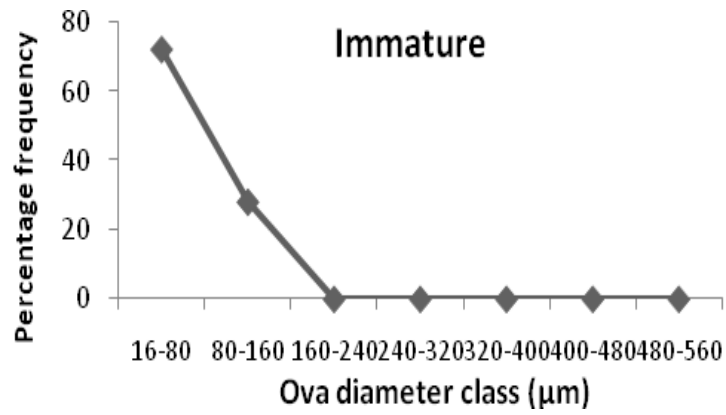


Fig. 2

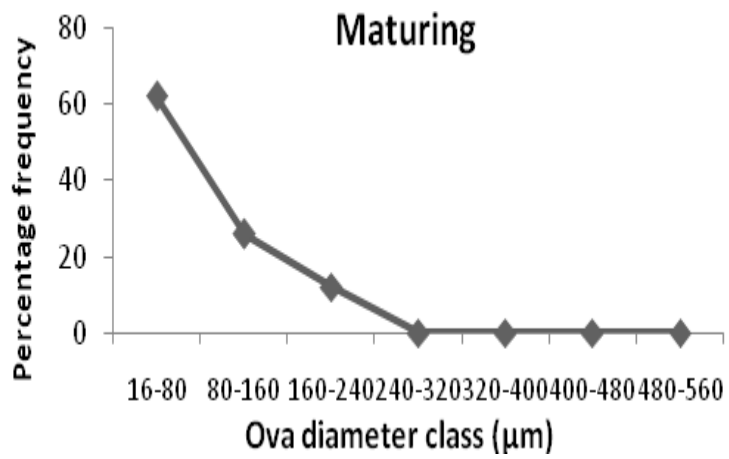


Fig. 3

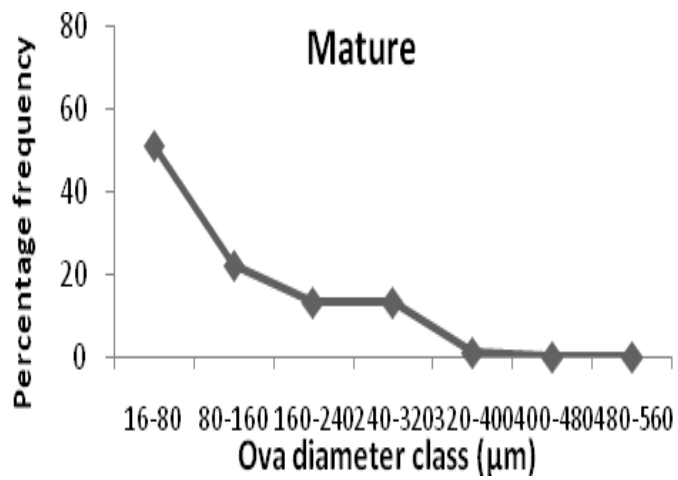


Fig. 4

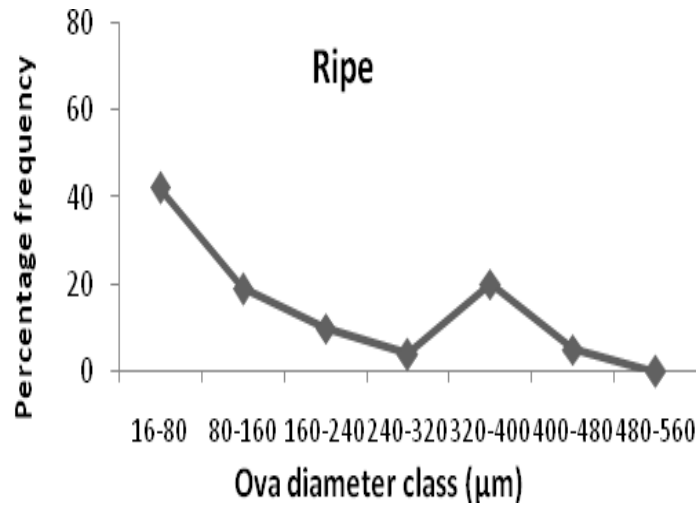


Fig. 5

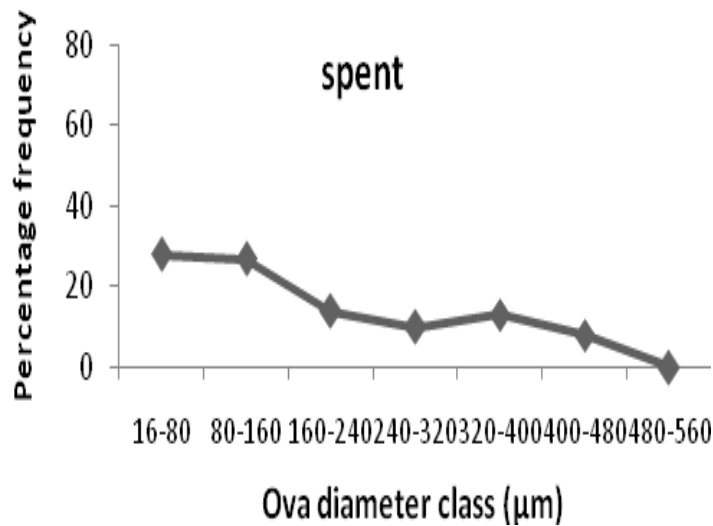


Fig. 6

Fig. 2 to fig. 6 showing ova diameter frequency distribution in Immature fish, Maturing fish, Mature fish, Ripe fish and Spent fish of *H. brittani*

There were four maturity stages of testes have been recognized in male fishes of *H. brittani* as follows:  
*Stage I (Immature)*: Testes small in size, very fine thread like in appearance. Under microscope the testes appeared as undifferentiated tissue.

*Stage II (Mature)*: The testes occupied less than 1/2 of the body cavity. Testes enlarged in size and weight; lobes are creamy white in color.

*Stage III (Ripe)*: The testes occupied about half of the body cavity and the lobes were white and fleshy.

*Stage IV (spent)*: Testes looked flabby, transparent in appearance and occupied about 1/4 of the body cavity.

In January females with Immature gonads (Stage I) have been observed. This stage has been present in female fishes till June. Highest percentage has being observed in January while lowest percentage in June. Occurrence



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of first fish with Stage II (Maturing gonad) gonads has been observed in February and available till August with highest percentage in April and lowest percentage in August. Stage III (Mature gonad) females have been noticed from March to October, highest percentage being observed in September and lowest percentage in March. Stage IV (ripe gonad) fishes have been observed from March to December with highest percentage in November and lowest percentage in March. Spent females (Stage V) have been observed in November, December and January with highest percentage in December and lowest percentage in November (Fig. 7).

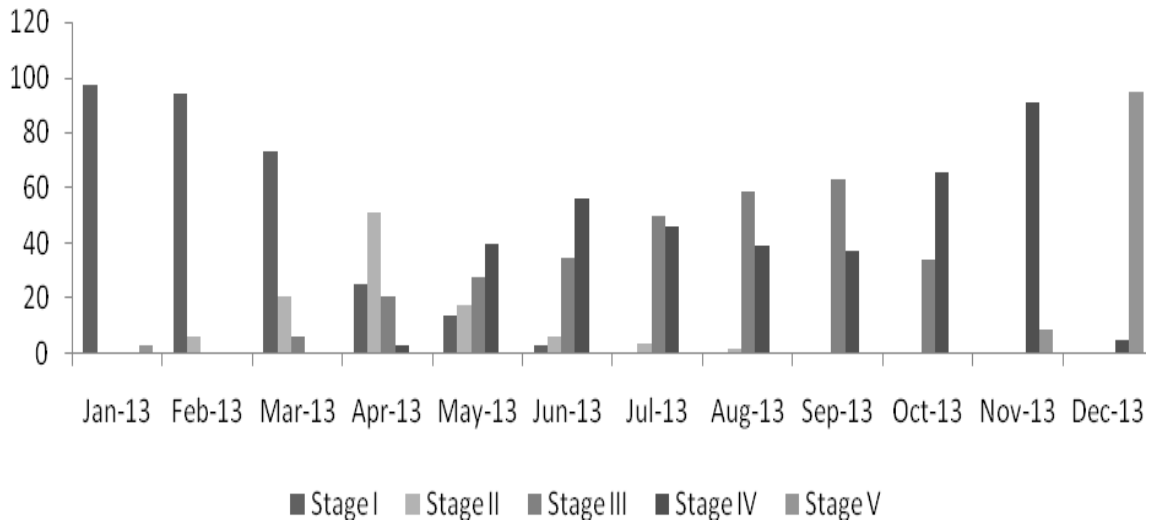


Fig. 7: Monthly percentage of different gonadal maturation stages in female *H. brittani*

Males with immature gonads (Stage I) have been observed in January and available till June, peak percentage being observed in February and lowest percentage in June. Males with Maturing gonads (Stage II) have been observed from March to October; highest percentage being observed in August and lowest percentage being observed in October. Stage III (ripe gonad) males have been observed in April and available till December; highest percentage in November and lowest percentage being in December. Stage VI (spent gonad) males have been found from November to January; highest percentage being in December and lowest percentage being in January.

**Gonado somatic Index (GSI):**

GSI has been observed to be the lowest value in January, in the female fishes of *H. atukorali*. Then it started to increase from February and reached the first peak in June; then it came down in July and continued the trend till September; then again it started to increase from October and reached the second peak (highest peak) in November; then again dropped down in December and reached the minimum value in January (Fig. 8). Being very small the determination of GSI in male fishes of *H. atukorali* is very difficult.

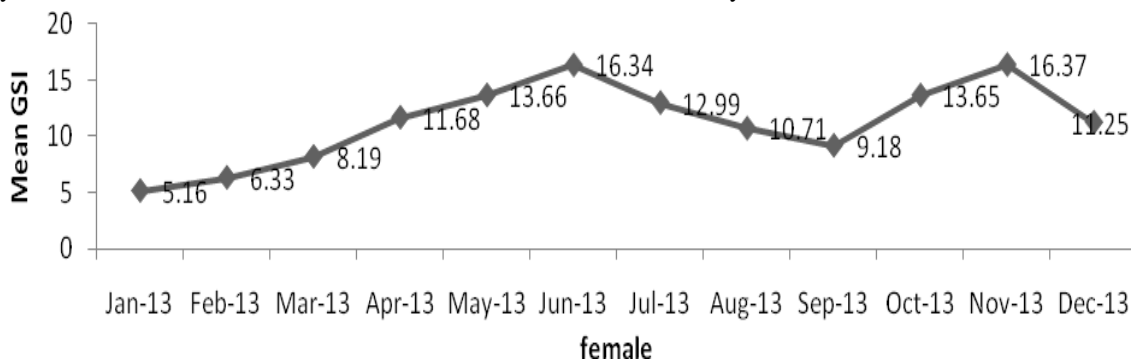


Fig. 8: Monthly variation of Gonado Somatic Index (GSI) of female *H. brittani*



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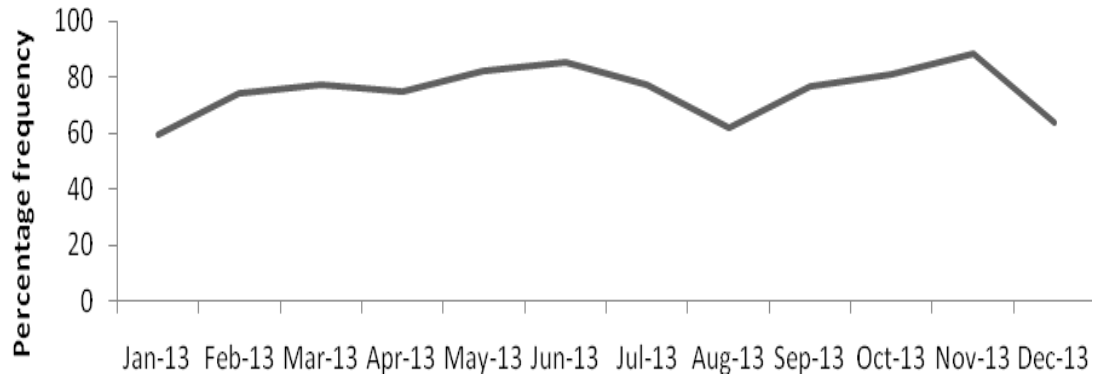
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**Monthly percentage of gravid females:**

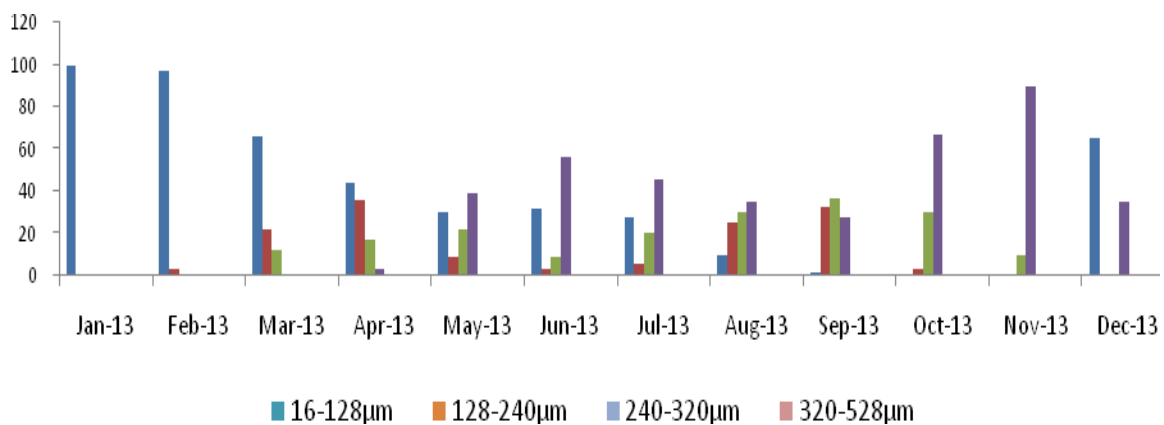
The gravid females have been observed throughout the year in the monthly samples (Fig. 9). Highest percentage has been observed in November and June. In December the percentage of ripe females gradually came down and continued the trend till April. From April onwards there has been an increase in the percentage of gravid females and reached the second peak in June followed by a decreasing trend till August. From September onwards the percentage has been followed an increasing trend and reached the highest peak in November.



**Fig. 9: Monthly variation of Gravid females of *H.brittani***

**Size frequency distribution of intra ovarian oocyte.**

In *H. atukorali*, Size frequency distribution of intra ovarian oocytes has been observed by taking the ova diameter measurements in the five maturity stages of ovary. Ova diameter class frequency has been grouped into four. Immature ova (16µm to 128µm), maturing ova (128 µm -240 µm), mature ova (240 µm -320 µm) and ripe ova (320 µm -528 µm). The percentage occurrence of different ova diameter classes plotted against months (fig. 10) have shown that immature ova (16µm to 240 µm) have been observed from January to September and again in December; with highest percentage being in January and lowest percentage in September. Maturing ova (128 µm -240 µm) have been observed from February to October; with highest percentage being in the month of April and lowest percentage in the month of June. The mature ova (240 µm -320 µm) have been observed from March to November; highest peak in September and lowest percentage in March. The ripe ova (320 µm -528 µm) have been occurred from May to December; with highest percentage being observed in November and lowest percentage in May.



**Fig 10: Monthly percentage frequency of four size groups of intra ovarian ova in *H.brittani***

In January, the monthly average ova diameter has been observed to have the lowest value; then it started to increase gradually from February and reached the first peak in June; then dropped down in July till August and then started to increase from September to reach the second peak in November, which is the highest peak and





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then showed a sharp fall again in December to reach the lowest value in January. Thus the monthly average ova diameter class frequency distribution has been observed to be show two peaks; once in June and the second and highest peak in November. The lowest value has been observed in January.

#### *Spawning frequency in H. brittani*

From the ova diameter frequency distribution of a ripe ovary (Fig. 5), it is observed that there are three batches of eggs representing the immature, ripening and ripe ova stocks. The ova diameter size ranged from 16 $\mu$ m to 128 $\mu$ m for the immature stocks. The ova were transparent without any yolk material. The ripening stock had an ova diameter range of 128  $\mu$ m -320  $\mu$ m. The first yolk vesicle eggs appeared at an ova diameter of 128 $\mu$ m and on reaching a diameter of 320 $\mu$ m the eggs had become ripe and ready to ovulate. Above an ova diameter of 320 $\mu$ m, the eggs constitute the ripe stock.

Ova diameter frequency showed that there has been a large single stock of immature ova and a small stock of ripe ova. An intermediate stock of ripening ova has been present in between these two stocks.

Thus the ripe ovary has been consists of mainly three batches of ova with different stages of maturity. Spawning takes place more than once during a protracted spawning season.

#### V. DISCUSSION

Study on the monthly sex ratio has proved the predominance of female over male in the population and the deviation of sex ratio from the expected ratio of 1:1. Gupta and Banergee, 2013 (11) reported female dominance over male in *Amblypharyngodon mola*. Similar types of observation on the deviation of sex ratio from the expected ratio and the dominance of female over male in the population have been reported in *Rasbora tawarensis* [18]; *Tilapia mariae* [1]; and *Pellonula leonensis* [14]. In contrast, Singh 2007 [26] reported the number of male has been higher than female in *Tor putitora*.

According to Fagade et al, 1984, [7] the reason behind the female predominance over male may be it is a mechanism of population regulation. On the other hand Cooper (1983) [5] suggested that higher metabolic strain of spawning in older males than in older females could has caused mortalities amongst males and has resulted in an excess of females, especially during spawning period in the population. The seasonal variation in the sex ratio observed have been probably because once fertilization of eggs was completed, male possibly emigrates from spawning area go feeding ground.

Size at first maturity: The size and age at first maturity depends on the nature of the environment in which the population of concern lives [4]. In this present study, it is the female that is larger or atleast achieves larger size. This is a kind of reproductive strategy to enhance the existence of the race by increasing the fecundity, directly related to the size of the female fish [15]. Early maturation of males in respect to females earlier has been reported in *Amblypharyngodon chakaiensis*, [2], *A. mola* [11]. Similar works have been reported earlier by many researchers in different fish species and support the observation in the present study.

Study on the monthly GSI values has shown two peaks in a year. Once in November and another in June for both sexes and high GSI values have been observed from April to December. The relationship between GSI value and spawning season has been studied earlier: GSI tends to increase with maturation of gonads, becomes higher during the period of peak maturity and dropped down abruptly thereafter, when the fish becomes spent after gamete extrusion or reabsorption [19], [6]. So monthly GSI value depicts the monthly spawning activity and maturation of gonad, with peak Monthly GSI value at peak spawning season. The two peak of GSI value, once in November and another in June thus depict that *H. brittani* spawns twice a year. GSI values of females have been found to be higher than in male due to greater proportion of body reserves has been allocated to the gonads. *H. atukorali* was reported to spawn during November to January (g). However, the annual dynamics of GSI from the fishes in the wetlands of Kerala observed in this study indicated that *H. brittani* breeds during two seasons, June-July and November-January.

The seasonal changes in the gonad weight also show two peaks, one in June and other in November, supporting the fact that *H. brittani* spawns twice every year, thus belonging to the category 'C' of Prabhu [22], and Karekar





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and Bal [14] or category II of Quasim and Qayyum [23]. Similar two peak spawning period is reported in the *Amblypharyngodon mola* [12], *Puntius sarana subnasutus* [28] and in *Cyprinus carpio* [21].

Early maturation and multiple spawning is an adaptation in unstable environments where adult mortality may be high [4]. *H. brittani* is a tropical barb distributed in the coastal wetlands of Kerala. The multiplicity of modes of ova diameter frequency in the mature ovary of this fish indicates that the fish spawns more than once in a year. Monthly values of GSI, and mean monthly ova diameter showed that the bulk of the ova have been spawned during June and then second batch of ova have spawned in November. Ref [29] described the release of clutches of ovulated eggs in batches in *Chela labuca*, indicating fractional spawning activity in the tropical small cyprinids. Fractional spawning describes the batch release of ovulated eggs [7]. According to [9] Satinfin shiner, *Notropis analostanus* and fathead minnow, *Pimephales promelas* [10] showed batch release of eggs under captivity.

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