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Original Article

Gonad developmental cycle of *Puntius filamentosus*

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Abstract

In the present reprobiological study on *Puntius filamentosus* the spawning months of November and April coincided with North-East Monsoon (November) and South-West Monsoon (April) in India. The gross morphology of *P. filamentosus* showed 6 ovarian stages. The histological appearance of the ovaries showed ten stages (oogonia to atretic oocytes). The testes of *Puntius filamentosus* showed five morphological and histological stages each. The progression gonadal cycles of *P. filamentosus* collectively revealed that the maturation of males coincided with the maturation of females during the spawning period.
Key words: *Puntius filamentosus*, gross morphology, Gonad developmental cycle

Introduction

Gonadal development was monitored on the basis of microscopic and macroscopic appearance (Wang *et al*., 2003). Gonadal maturation represents cyclic morphological changes, in which the male and female gonads undergo to attain full growth and ripeness (Ha and Kinsie (1996). The development of the piscine gonads has been described in terms of stages of maturity (Treasurer, 1990; Ha and Kinzie, 1996). Fish ontogeny is usually divided into different stages. They are larval, pre-reproductive, reproductive and post-reproductive periods (Rass, 1989 and Balon, 1984). The pre-reproductive period is characterized by the formation and maturation of oocytes. This is followed by ovulation and fertilization. Fish ontogeny was influenced largely by environmental factors (Alm, 1959). In freshwater teleosts there was an increase in length, volume and weight of the ovary through somatic growth (Sunder, 1986; Misra, 1994). The number of ovarian stages varies from 5 to 8 in different teleosts species.

Gross morphological changes in the were studied in a number of freshwater fish species viz., Barbus longiceps (Stoumboudi *et al*., 1993), B. scdateri (Encina and Lorencio, 1997), Labeo bata (Siddique *et al*., 1976), Cichla maculus (Chellappa *et al*., 2003), Labeo dussumieri (Madhusoodana Karup, 1994), Labeo fimbriatus (Bhatnagar, 1972), Cynopoecilus melanotaenia (Arenzon *et al*., 1999), Brycinus longipinnis (Ikomi and Sikoki, 2003), Kaiwainus eqcula (Yoneda *et al*., 2002), Xiphias gladius (Wang *et al*., 2003), Barbus stigma (Sathyanaesan, 1962).

The testes of Indian freshwater teleosts undergo cyclic changes in both morphology and histology (Joshi and Joshi, 1989). Grier and Lo Nostro (2000) recently observed changes in the gonadal germinal epithelium and stressed its importance in the reproductive cycle of teleosts such as its use in establishing reproduction classes in males. Based on this observation, annual changes in the testicular germinal epithelium appear to be applicable to a wide variety of fish species (Brown Peterson *et al*., 2002).

The testicular germinal epithelium is composed of somatic and germ cell components (Burgoes *et al*., 1970). In the males, the germinal epithelium includes of the sertoli cells and all of the germ cells involved in sperm production like spermatogonia, spermatocytes, spermatids and sperm (Grier and Lo Nostro, 2000).

Morphological classification of maturity stages of gonads

Males and females of *P. filamentosus* were collected twice in a month for 2 years (May 2003 to April 2005). The standard (SL) and total length (TL) of the fishes were measured. Total length was measured from the tip of the lower jaw to the tip of the caudal fin. The length measurements were taken to the

nearest millimeter and weight to the nearest milligram. Gonads were usually removed from the fish within a few hours of capture and their sex and stage of reproductive maturity were determined using a macroscopic staging system. The criterion for the characterization of the maturity stage was based on color, texture, size, shape and extent of occupancy of gonads in the body cavity (Mc Bride *et al.,* 2002).

Size frequency distribution of oocytes

The spawning periodicity was monitored based on ova-diameter studies (Ramanathan and Natarajan, 1979). The size of the intra-ovarian oocytes was determined by measuring the diameter of oocytes contained in single cross section taken from the central part of the ovary under a microscope with an ocular micrometer, at a magnification of 450X for small follicles and 100X for larger follicles and the number of oocytes in each size class was expressed as a percentage of the total number of oocytes. Average oocyte diameter was determined for each growth stage. As the eggs were uniformly spherical, egg size was measured at the maximum diameter along two axes. A total of 100-150 oocyte measurements were made from five ovaries and the mean diameter was calculated for each month.

Gross morphology

The development of the ovary was closely associated with the somatic growth of the fish. The gonadosomatic index of *P. filamentosus* increased with the progressive development of the ovaries in females until the gonads were ripe, and the index then declined sharply in spent and recovering spent fish. The increase in weight of the ovaries of *Puntius filamentosus* from immature to the ripe condition through well defined intermediate stages of maturation and ripening is illustrated in Fig.1.

Seasonal changes in the gonadal condition were distinctly obvious in *P. filamentosus*. The morphological appearance of the ovaries allowed six maturity phases for female to be identified. The characteristics features of each stage are described in Table -1. The distinctive features of gross ovarian development stages are given below.

Gross maturity stages

1. Immature

Immature ovary of *P. filamentosus* were small, thread like, conical shaped. They were translucent and occupied only a small portion of the abdominal cavity. The oocytes could be identified with naked eye. Immature ovary was found from end of December to March and May to June (Table 1).

2. Early maturing

The early maturing ovary of *P. filamentosus* was translucent, elongated and triangular in transverse section. The ovary was cream colored. Many whitish granular oocytes were distinctly visible. The ovary occupied nearly one-fourth of the abdominal cavity and this type of ovary was found from February to March and June to August (Table 1).

3. Late maturing

The late maturing ovary of *P. filamentosus* was larger in size and fully yolked with ripening ova. The ova were spherical and ivory yellow in color. The eggs were visible to the naked eye. The ripening was found in August to October and April to June (Table 1).

4. Mature

The mature ovaries of *P. filamentosus* were larger in size. Eggs were completely rounded. The ovary occupied 2/3 of the body cavity. The ovary was fully packed with large yellowish intra-ovarian eggs. The eggs were visible through granular surface of abdomen wall from outside. Such ovaries were noticeable in November and April (Table-1).

5. Spent

The spent ovaries were loose, shrunken and empty of mature ova with few scattered atretic oocytes. Some residual oocytes were transparent and visible. This type of ovary in *Puntius filamentosus* was found during the May and December (Table-1).

6. Recovery spent

In this stage, ovary of *P. filamentosus* was small, grey in color with certain residual oocytes. A number of immature and mature eggs were still present. Moreover, the developing oocytes were not visible. It was found in January and June (Table-1).

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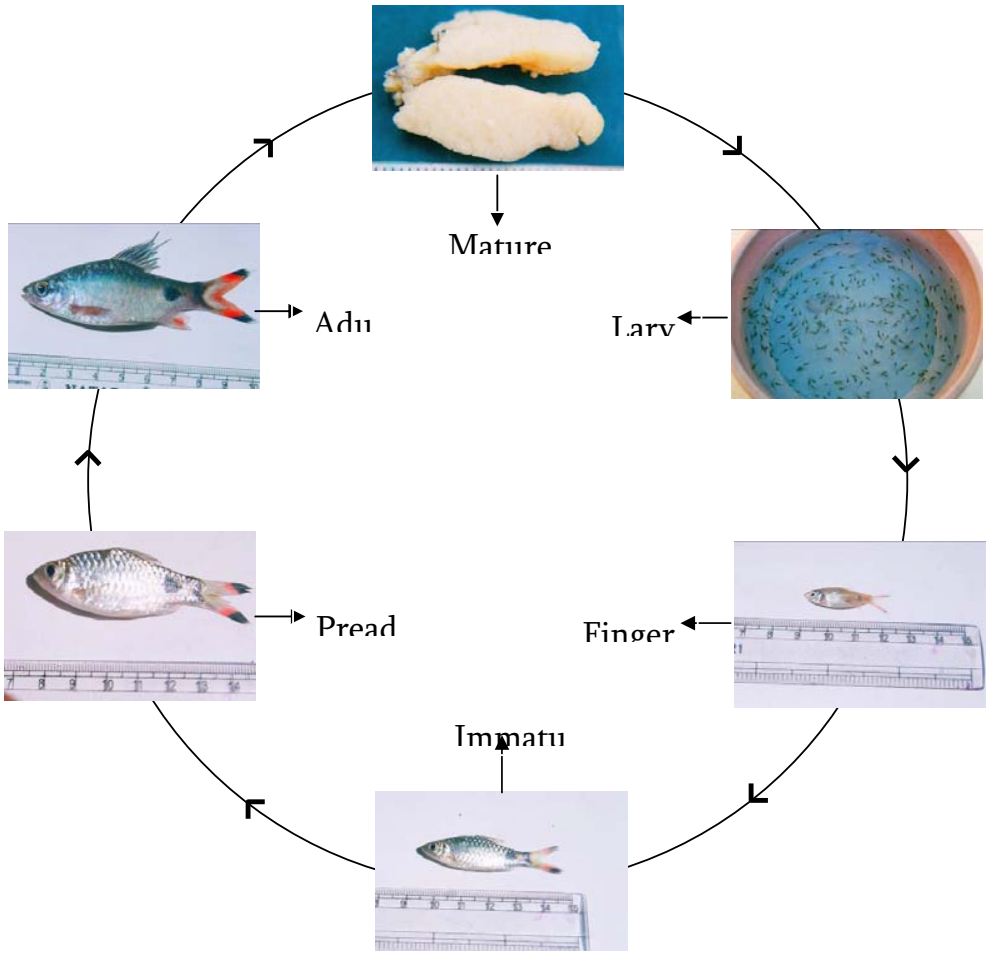

Fig.1: Life stages of ornamental fish *Puntius filamentosus*

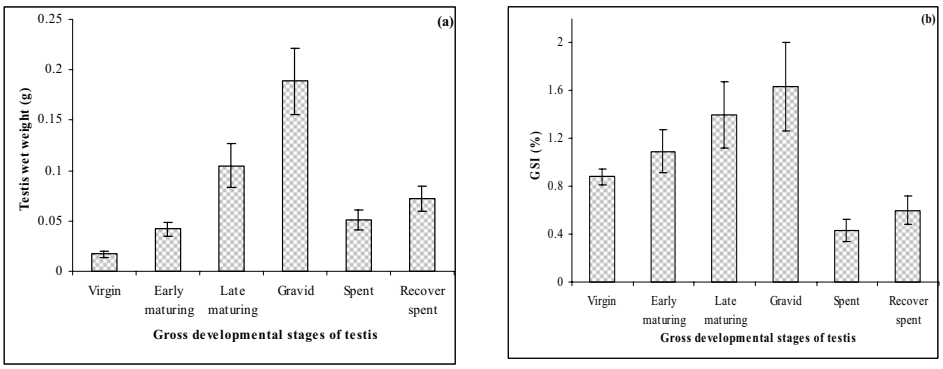

Fig.2: *P. filamentosus*: Relationship of the ovary weight (a) and gonadosomatic index (b) with different stages of maturation

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Table - 1. *P. filamentosus*: Different maturity stages of ovary in female fishes.

Maturity phases	Total length (cm)	Live weight (g)	Ovary length (cm)	Ovary weight (g)	GSI (%)		Color	Gross macroscopic feature
					Range	Mean		
Immature	3.5 – 5.0	1.71 – 3.65	1.8 – 2.7	0.02 – 0.143	1.12 – 3.69	2.65	Transparent/ Colorless	Gonads are very small, thread like, cannot be identify with naked eye
Early maturing	4.7 – 6.8	3.18 – 5.75	2.3 – 5.2	0.125 – 0.265	3.52 – 4.52	4.08	Whitish colored	The gonad becomes translucent and elongated. The eggs are visible with magnifying glass
Late maturing	6.3 – 8.7	5.04 – 8.78	4.9 – 6.6	0.191 – 0.484	3.71 – 5.45	4.96	Yellowish white	Gonads become opaque; eggs are visible to the naked eye. Yolk formation starts.
Mature	8.1 – 11.8	7.12 – 21.0	6.1 – 8.4	0.389 – 1.714	5.17 – 10.71	8.85	Yellowish red	Larger in size, eggs completely rounded, gonads occupy 2/3 of the body cavity. Ovaries visible through translucent body wall of abdomen from outside
Spent	8.2 – 11.3	7.43 – 16.76	4.7 – 7.8	0.112 – 0.196	1.58 – 2.47	2.09	Grey colored	Ovary loses weight, translucent, ovaries are empty bag
Recover spent	7.8 – 11.4	7.08 – 15.45	4.3 – 7.3	0.104 – 0.208	1.43 – 2.74	2.28	Whitish grey	Ovaries small, number of immature and mature eggs still present, developing oocytes were not visible

Table - 2. *P. filamentosus*: Gross anatomical features of testicular maturity phases.

Maturity phase	Total length (cm)	Live weight (g)	Testis length (cm)	Testis weight (g)	GSI (%)		Color	Grass macroscopic feature
					Range	Mean		
Immature	Upto 4.8	1.62 – 3.42	1.7 – 2.8	0.009 – 0.038	0.56 – 1.14	0.72	Colorless/ pale	Testes are very fine, elongate, thread-like
Early maturing	4.4 – 6.5	3.08 – 5.14	2.2 – 4.9	0.033 – 0.081	0.86 – 1.52	1.21	Dull white and creamy white	Testes opaque and extend about two-third of abdominal cavity, right testes slightly longer than left one
Late maturing	5.8 – 8.2	4.92 – 8.23	4.3 – 6.9	0.069 – 0.128	1.37 – 1.58	1.34	Milky white	Testes are thickened and enlarged. The milt does not ooze out by pressure on the abdomen
Mature	7.6 – 11.6	7.04 – 18.35	6.2 – 8.1	0.104 – 0.182	1.35 – 2.08	1.79	Yellowish white	Testes are elongate and bulged. On pressing the abdomen the milt oozes out.
Spent	7.5 – 11.2	7.11 – 16.36	4.4 – 7.2	0.051 – 0.089	0.74 – 1.07	0.82	Whitish grey and soft	Shrunken, uniformly thin and tubular

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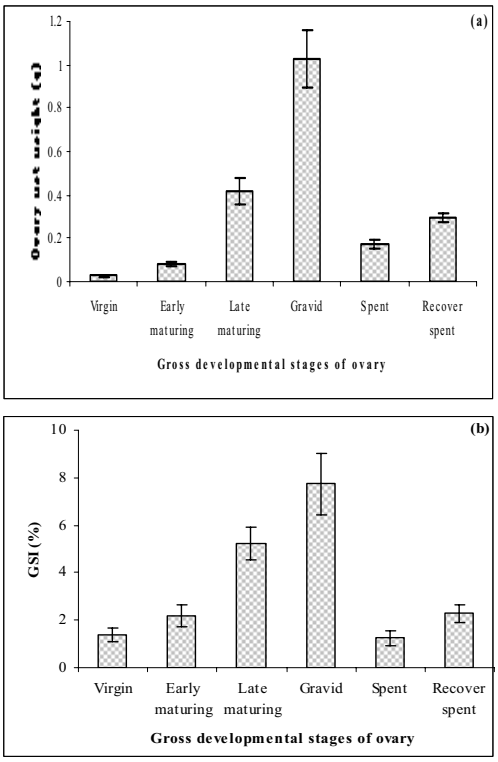

Fig. 3: *P. filamentosus*: Relationship of the testis weight (a) and gonadosomatic index (b) with different stages of maturation

Percentage frequency of ovarian maturity stages

The percentage frequency of various stages of ovarian maturation in different size groups of female *P. filamentosus* during different seasons is shown in Figure 3. During the resting periods the total length groups 3.50 cm to 5cm 86% was immature and in the spawning period the total length group 9.51 cm and above were 100% matured.

Annual testicular cycle

Testes in *Puntius filamentosus* are paired secular bodies situated on either side, ventral to the kidneys along the posterior region of the abdominal cavity. Immature testes are two dull white, thin and elongate bodies extend from the region of liver to the rectum. At maturity, the testes are long, tubular flat ribbon like structures with a maximum length of 8.1 cm and weight of 0.182g. Sperm ducts open separately close to the cloacal aperture, which is an elongate, oval shaped slit.

The gonadosomatic index (GSI) increased with progressive development of the testes in males until they became ripe, and the index then declined sharply in spent fish. The increase in weight of the testes from the immature condition to the ripe condition through various stages of maturation is illustrated in Fig-2. The testes of *Puntius filamentosus* exhibited marked seasonal changes and the annual testicular cycle was divided in to five stages. The gross morphological features of each stage are presented in Table -2.

1. Immature

The testes were thin, slender, thread like, translucent and flesh colored. They extended to about half the length of visceral cavity. The resting phases of testes were found from January, June and July (Table -2).

2. Early maturing

The testes were dull white in color, and showed gradual increase in their volume and weight, and became opaque. They extended to about two third the length of abdominal cavity. This stage of testes was observed in August, September and February (Table 2).

3. Late maturing

The testes were milky white in color, uniformly thickened and enlarged, extending to about three-fourth the length of the visceral cavity. It was found during late March and September. They were greatly increased in size and weight (Table 2).

4. Gravid

The testes were milky whitish, long and flat, ribbon like, and narrower behind. They were found extended to the entire length of the visceral cavity. It was characterized by the onset of spermiation (milt release) on applying slight pressure on the abdomen, it was found during November and April (Table 2).

5. Spent

Testes became uniformly thin, slender, flesh colored, shrunken and tubular; it was found in December and May. Their size and weight became reduced considerably (Table 2).

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Percentage frequency of testicular maturity stages

In *P. filamentosus* the percentage frequency of various maturity stages of testicular cycle as a function of size during different seasons of the year is shown in Figure 15. During the resting period the total length group 3.5-5.0 cm occurred 100% immature and in the pre-spawning and spawning period the total length group 9.5 cm and above occurred 100% is mature stages.

Discussion

Ovarian cycle

The development process of the ovary in *Puntius filamentosus* is similar to that of many teleosts fishes (Sundarabarathy *et al.,* 2004: Encina and Lorencio, 1997; Stoumboudi *etal.,* 1993; Dixit and Agarwal, 1974). The annual cycle and the seasonal pattern were common among juveniles and adult fish, suggested that the seasonal variations encountered were more related to reproductive cycle (Hansen *etal.,* 2001). The assessment of the breeding period in *P. filamentosus* was based on the observation of gonadal maturation as in many other fishes like *Barbus longiceps* (Stoumboudi *etal.,* 1993), *Barbus sclateri* (Encina and Lorencio, 1997) and *Puntius dukai* (Joshi and Joshi, 1989)

The gonadal development and reproductive strategy have been described in many teleosts fish species in an effort to understand the time course and energetic consequences of reproductive efforts. Oocyte growth followed a similar pattern in most teleosts (Arockiaraj *et al.,* 2004). The histological observation of the monthly ovarian samples of *Puntius filamentosus* revealed a biannual monsoon breeding pattern. The major breeding seasons of *P. filamentosus* in November and April were corresponded with to North-East and South-West monsoons. Similar breeding pattern was recorded in *Puntius aurulius* (Arunachalam and Sankaranarayanan, 1998). The ovaries were first classified based on macroscopic features. The spawning stage was characterized by the presence of active follicular epithelium with maturing and mature oocytes (Narahara, 1983). Six ovarian development stages of *P. filamentosus* such as virgin, early maturing, late maturing, gravid, spent and finally recovering spent as reported in *Kaiwarinus equila* (Yoneda *et al.,* 2002) were highly correlated to the GSI. The increased in GSI with advanced developmental stages of ovary also reported in *Puntius titteya* (Sundarabarthy *et al.,* 2004), *Puntius dukai* (Joshi and Joshi, 1989) and *Barbus longiceps* (Stoumboudi *et al.,* 1993).

During the annual ovarian cycle, *Puntius filamentosus* showed a maximum GSI value prior to spawning in November and also during April. This revealed suggested that the fish would shed its eggs all at once with a very short spawning period as reported in three *Barbus* species (De Silva *et al.,* 1985). The highest increase in ovarian weight did not represent the initiation of the spawning season. Spawning occurred later, when the oocytes released through ovulation and was represented actually by the decrease of ovarian weight and subsequently by the decrease of GSI in females. In *Puntius filamentosus* the spawning was represented by a decrease in ovarian weight as in *Leptocephalus thermalis* (Maxwel Samuel, 2001) and *Fundulus diaphanous* (Chippett Jamie, 2003).

The maturation of oocytes seemed to proceed without interruption was indicated by the high frequency of occurrence of primary oocytes in the early stages of gonadal maturation, and their lesser frequency in the gravid ovaries. The formation of primary oocytes from oogonia occurs over a limited period in the early development of the gonads (Babiker and Ibrahim, 1979). In November and April when the ovaries are at the peak of maturation i.e., gravid stage of maturity, the fish spawns and the spawning burst depends upon the availability of appropriate environmental stimuli (Wijeyaratne,1994). Therefore environmental factors play important role in the developing patterns of the reproductive cycle (Wang *et al.,* 2001). Spent ovaries were found in *P. filamentosus* in beginning of the month of December and May. In *P. filamentosus* the gravid stage was found in November and April. The recovery of spent ovary was characterized by the production of new oocytes after spawning (Babiker and Ibrahim, 1979). These oocytes newly arose from the germinal epithelium (Aravindan and Padmanaban, 1972).

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Ova size frequency distribution

Monthly record of the oocytes in *Puntius filamentosus* showed the presence of numerous mature pre-ovulatory follicles in spawning period especially in ripe ovaries. The study of ova diameter has been used as an important tool in assessing the spawning periodicity of fishes by Sundarabarathy *et al.,* (2004). The histological study along with the assessment of size-frequency distribution of oocytes in *P. filamentosus* indicated that this species had a group synchronous pattern of oocytes development with mature ova of 632-686 µm and 628-681 µm in diameter at spawning peak (November and April) as reported in *Puntius titteya* (Sundarabarathy *et al.,* 2004), *Capoeta tetrazona* (Tamaru *et al.,* 2001), *B. longiceps* (Stoumboudi *et al.,* 1993). As a result, the females of *P. filamentosus* probably produce only a single clutch during a spawning event, in the major spawning season of November and April. The same pattern of spawning was also observed in *Periophthalmus barbus* (King and Udo, 2001). In October and March, a distinct unimodel distribution of mature ova was noticeable in the gravid ovaries of *Puntius filamentosus*. But in November and April, the batch of mature ova was lacking in *P. filamentosus*. It was evident that each individual of *Puntius filamentosus* spawns twice in a breeding season, as found in *Puntius sophore* (Dixit and Agarwal, 1974) and *Periophthalmus barbus* (King and Udo, 2001).

According to De Silva *et al.,* (1985) the spawning pattern of *P. filamentosus* is related to the rainfall patterns of their natural habitats which may bring about changes in water quality parameters specifically dissolved solids, water currents and water depth. A simultaneous maturation of all ova destined to be spawned within the spawning season in *Puntius filamentosus* was similar to that found in *P. mahecola* (Selvaraj, 1998).

Testicular cycle

Testicular histology of freshwater teleosts have been described by Brown Peterson *et al.,* (2002), Maxwel Samuel (2001) and Murphy (2007). The entire testis was identical in histological pattern as reported for *Brycinus longipinnis* (Ikomi and Sikoki, 1999). The development of the testes of *Puntius filamentosus* in the present study was similar to that of *Puntius vittatus* (Murphy, 2007) and *Puntius titteya* (Sundarabarathy *et al.,* 2004), with testes weight was increased in November and April. Among teleosts, the spermatogenic activity commenced at various months of the year (Taylor *etal.*, 1998).

Puntius filamentosus also exhibited discontinuous spermatogenic cycle and pass through the successive stages of growth, maturation, activation, depletion and rest during the annual cycle. The spermatogenic activity was started in September and beginning of March, progressing through October and end of March to full maturity into spermatozoa at the time of November and April. Spermiation took place in November and thereafter spermatogenesis slowed down considerably and almost ceased by the December. Again spermatogenesis started in March and ended in April. The testes remained quiescent and passed through a period of rest from December to February and May to August. In general, the testes of fishes showed an intensive phase of spermatogenic activity followed by a period of spermatogenic rest. However, in some freshwater fishes, spermatogenesis began as soon as the spermiation was terminated, and then continued throughout the year (Ahsan, 1966). Most of the Indian freshwater teleosts attained maturity and bred during monsoon season (Encina and Lorencio,1997). In *Puntius filamentosus* also spawning was occurred during the monsoon period.

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