Oogenesis, Hepatosomatic and Gonadosomatic Indexes, and Sex Ratio in Rosy Barb (*Puntius conchonius*)

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Abstract

Seven stages of oogenesis were identified in the rosy barb (*Puntius conchonius*). Oogonia were present throughout the reproductive cycle. A group-synchronous ovary with a possible asynchronous ovarian development was observed. The most important event observed during the developmental stages of oogenesis was the observation of the crystalline yolk (at the maturation stage). Maturation of females was observed at 112 days post hatch. Mean hepatosomatic index (HSI) and gonadosomatic index (GSI) increased with increasing mean body weights and lengths during gametogenesis in females. The relationship was statistically significant ($p< 0.05$). The sex ratio observed in 300 fish was 210:80 (female:male) and the difference was statistically significant ($p<0.05$).

Key Words: *Puntius conchonius*, oogenesis, sex ratio, hepatosomatic index, gonadosomatic index, histology.

Introduction

The rosy barb, *Puntius conchonius,* is a Cypriniform of the family Cyprinidae and a prime candidate for tropical exotic aquarium. The generic name of this species Barbus or Puntius, is still under discussion (De Silva *et al*, 1985).

Reviews of oocyte development in teleost fish have been given by de Vlaming (1983), Guraya (1986, 1994), Selman *et al*. (1993), Bromage and Cumaranatunga (1988), Selman and Wallace (1989); West (1990), and Tyler and Sumpter (1996). The development follows a similar pattern in most species. In cyprinids, Meyyen (1927, 1939; cited in Makeyeva and Yemel'yanova, 1989) introduced three stages of oogenesis: 1- the synaptic path (from oogonia to oocytes in diplotene); 2- slow growth; and 3- rapid growth including maturation of the oocytes. Wallace and Selman (1981) described four developmental stages: primary growth, yolk vesicle (cortical alveoli) formation, true oogenesis and maturation including hydration. Wallace (1985) reported that oogenesis consists of the primary growth phase from oogonia up to maturation, although Bromage and Cumaranatunga (1988) suggested that the term oogenesis should be used only for the transformation of secondary oogonia into the primary oocytes and 7 stages of oocytes development described.

Most of the information available on the rosy barb is based on the observations of aquarists or hobbyists and it is only recently that laboratory studies have begun to broaden our knowledge of rosy barb in terms of oogenesis and reproductive biology. Rosy barb breeds throughout the year under laboratory conditions, with large numbers of eggs shed at spawning intervals as short as 8 days (Varadi and Horvath, 1993; Adam, 1995; Adam *et al*, 1995). The species is easily obtainable and can be propagated inexpensively (Çek *et al*., 1998). It tolerates a wide range of temperatures from 22°C to 28°C and accepts a variety of feeds (zoo-phytoplankton) (Malhotra and Gupta, 1990; Axelrod, 1974). In other closely related species, of genus *Puntius,* not the oogenesis, but the reproductive biology is well documented (De Silva and Kortmulder, 1977; De Silve *et al*., 1985; Chandrasome *et al.,* 1994).

Principal objectives of the present study were: to describe oogenesis by the classification of developing oocytes into different stages using histological examination, to observe and measure gonadosomatic index (GSI), hepatosomatic index (HSI), and sex ratio.

Materials and Methods

This study was performed in recirculating systems, each consisting of four 20 l plastic tanks. Rosy barbs were reared from one batch of eggs in aquaria at 27±1°C, and larvae were stocked at 5 larvae/l. From the ages of 84 up to 112 days (5 weeks), 5 fish from each tank were sampled each week and their body weights and total lengths recorded. Subsequently, they were sacrificed and their sexes were noted. The gonads from the fish sacrificed were individually weighed on a balance to 0.001 mg precision. The gonads were then fixed in Bouin's solution in preparation for histological examination. Immature gonads were embedded in paraffin and mature gonads in Histo-resin, and sections cut at 3-4μ in paraffin or 1-1.5μ in Histo-resin respectively. The

sections were stained with Haematoxylin and eosin, Periodic Acid-Schiff (PAS), and Polychrome, prior to assesment of the stage of gametogenesis. All the slides were examined under 2 light microscopes. All data are presented as mean ± sd. Statistical analysis was conducted according to Zar (1984) and Fowler and Cohen (1987) using the Minitab Statistical Package.

Linear regression analysis was used to determine the relationships between GSI, HIS, body weight, and length of species. Analysis of covariance was used to determine if the regression lines of GSI and HSI versus body weight and length varied significantly throughout oogenesis. GSI and HSI (de Vlaming *et al*., 1982; Delahunty and de Vlaming, 1980) were calculated as follows: GSI = (L+R)×100/BW; HSI = LW×100/BW; where GSI = gonadosomatic index; LW= liver weight (g); L = left ovary weight (g); R = right ovary weight (g); BW = body weight (g).

The ChiSquare (X^2) test was used to test whether the sex ratios observed were significantly different from the expected sex ratios (Fowler and Cohen, 1987).

Results

Oocytes were classified by developmental stage. Classification was based on the histological criteria outlined in Table 1, adapted from Bromage and Cumaranatunga (1988).

Table 1. Phases of Oocyte Growth in *Puntius conchonius,* based on histological criteria.

Primary Growth Phase (PGP)	a) Chromatin nucleolar	a) Oocytes possessed a spherical nucleus, with chromatin-threads and a large nucleolus in the nucleoplasm. The nucleus was surrounded by cytoplasm. At the beginning of this stage oocytes were within a nest and all of them were in the same phase of meiotic prophase. At the end of this stage the oocytes left the nest (Çek *et al*., 1998).
	b) Perinucleolar st	b) The Balbiani bodies occurred in the ovary 45-65 days after hatching. Oocytes were subdivided into 3 phases according to the position of their Balbiani bodies. In the first stage (stage 2a), the Balbiani bodies were located close to the nuclear envelope and stained strongly basophilic. In stage 2b, Balbiani bodies were distributed all over the cytoplasm. Balbiani bodies migrated to the periphery of the oocytes cytoplasm. In the mean time ovarian cavity was formed and the ovary remained continued to curve inwards as it enlarged (Figure 1).
Secondary Growth Phase (SGP), was divided into 3 stages; stages 3, 4 and 5.	Stage 3 Oocytes	Cortical vesicles were detected for the first time. These were usually spherical structures that appeared at random at various depths in the ooplasm. They provided the first evidence for initiation of the secondary growth phase. They appeared usually as empty unstained vacuoles. The cytoplasm close to the nucleus was more compact and more basophilic then the outer layer of oocytes. This stage was first detected at 70 days of age (Çek *et al*., 1998).
	Stage 4 Oocytes	The nucleus consists of many nucleoli and continues to enlarge, becoming very irregular in shape. This stage was detectable at 94 days of age and the number of nucleolivaried between 35-60. The process of vacuolisation was completed by formation of two rows of vacuoles that were not stained. The zona radiata was more conspicuous. (Figures 2 and 3).
	Stage 5 Oocytes	Yolk granules were first detectable only between vacuoles, and later in the cytoplasm free from them (Figure 3). The nucleus showed a significant number of projections into the cytoplasm. The nucleoli were pleomorphic and variable in size (Figures 4 and 5) and the yolk pushed the vacuoles to the periphery of the oocytes and subsequently filled the whole cytoplasm. The development of the eggshell was completed with the zona radiata and chorion. The former underwent changes during ovarian maturation and increased rapidly in size (Figure 5). The development of the zona radiata, from stage 4 to stage 6, is shown in Figure 5, and the formation of the micropyle in Figure 6. The micropyle cell (m), which is thought to subsequently form the micropyle, projected into the follicular epithelium through the vitelline envelope, towards the oocytes (Figure 6). At this stage the nucleus was in the centre of the oocytes. This stage was observed at the age of 15 weeks. From the end of stage 4, through stages 5 and 6, crystalline yolk was clearly detected and gradually changed to non-crystalline yolk (Figures 4 and 7).
Maturation and Hydration Phase	Stages 6 and 7	Stage 6 was distinguished by migration of the nucleus to the animal pole where it remained, but the nuclear membrane disintegrated. The nucleus was smaller in size. The nucleoli were smaller than the previous stage, and hardly distinguishable in the nucleus. The layers of oocytes were thinner then those of stage 5 oocytes. Also during stage 6, an increase in the size of the yolk platelets was observed as they became enlarged as a result of hydration of the oocytes and slowly lost their crystalline structure. However, they did not merge into a homogenous mass of yolk. As a result of these changes, the cytoplasm was displaced into a peripheral rim surrounding the yolk (Figures 7 and 8). At stage 7, the yolk granules enlarged as a result of hydration of the oocytes and lost their crystalline structure but did not merge (Figures 9).

Figure 1. Transverse section of larvae, 65 days after hatching. Arrows show the development of ovarian cavity, (Oc); T, tunica; St_1, stage 1 oocytes; St_{2b}, stage 2b oocytes; LB, lampbrush chrosomes and oocytes at the different developmental stages, (Magnification×400).

Figure 2. A post spawning section of stage 4 oocytes. Oo, oogonia; St_{2a} stage 2a oocytes; St_4 , stage 4 oocytes; Bv, Blood vessel. Th, theca, Zr, zona radiata; G, granulosa, (Magnification×1000).

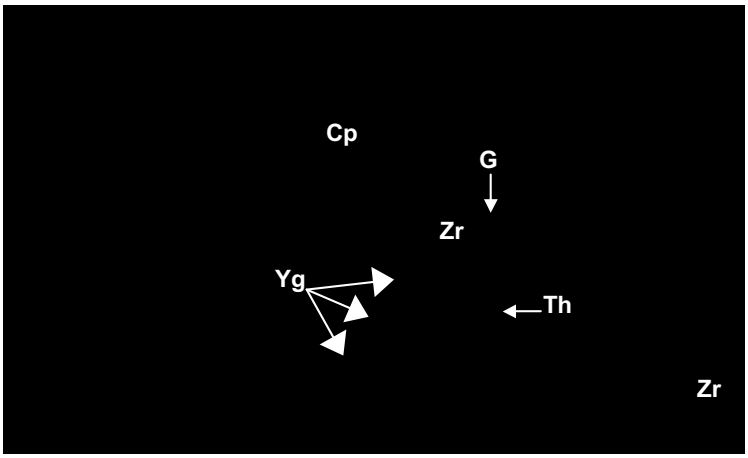

Figure 3. Section of a stage 4 oocyte: arrows show the formation of yolk granules. Cv, cytoplasmic vesicle; Yg, yolk granules; Cp, cytoplasm; Th, theca; Zr, zona radiata; G, granulosa, (Magnification×800).

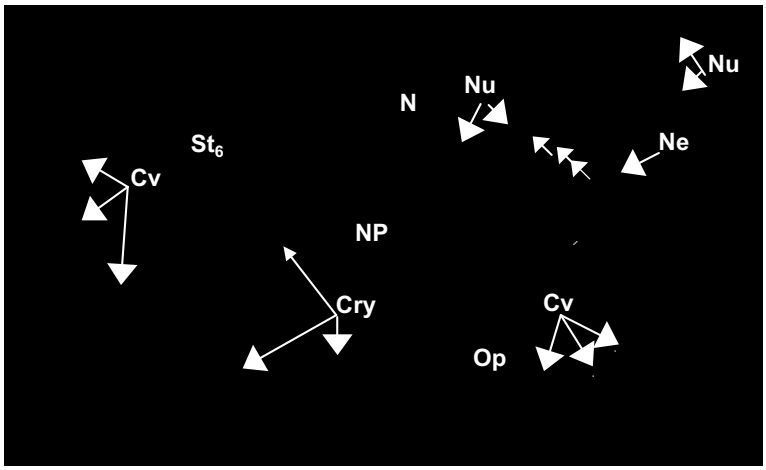

Figure 4. Unlabelled arrows show the movement of nucleoli to the centre of the nucleus (germinal vesicle breaking-down) $St_{6,}$ stage 6 oocytes; Nu, nucleoli; Op, ooplasm; N, nucleus; Ne, nuclear envelope; Cry, crystalline yolk; Cv, cytoplasmic vesicle; Np, nucleoplasm, (Magnification×1000).

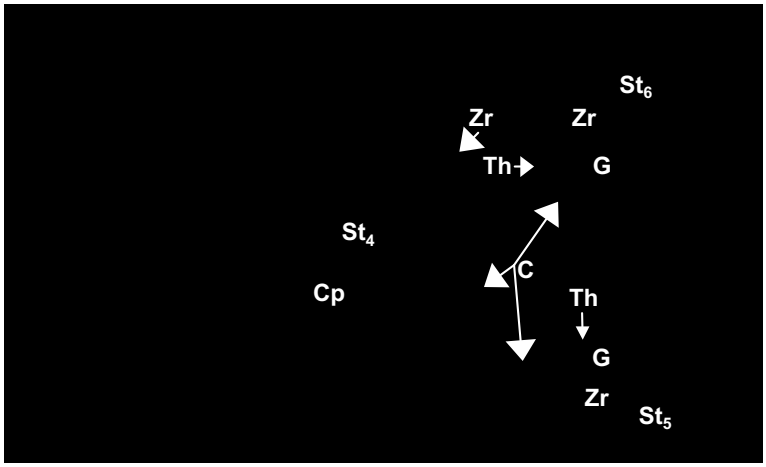

Figure 5. Section of stage 4, 5 and 6 oocytes, showing gradual changes in the zona radiata. C, connective tissue; Cv, cytoplasmic vesicle; Yg, yolk granules; Cp, cytoplasm; Th, theca; Zr, zona radiata; G, granulosa; $St_{4a,}$ stage 4 oocytes; $St_{5,}$ stage 5 oocytes; $St_{6,}$ stage 6 oocytes , (Magnification×1000).

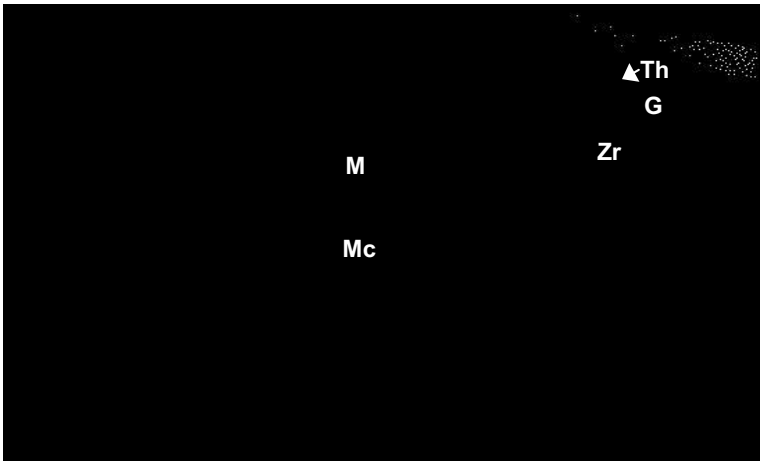

Figure 6. Section of ovary showing a stage 7 oocyte with micropyle. Mc , micropylar cell; M, micropyle; Th, theca; Zr, zona radiata; G, granulosa St_7 , stage 7 oocytes, (Magnification×1000).

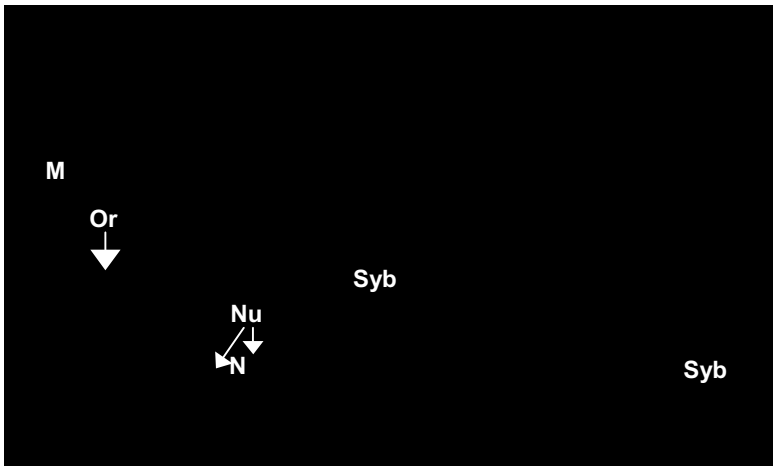

Figure 7. Vitellogenic oocytes of rosy barb showing morphological transformation of crystalline yolk. Or, Ooplasm rim; POF, post ovulatory follicles; N, nucleus; Nu, nucleoli; Syb, spherical yolk body (non crystalline yolk); M, micropyle; Th, theca; G, granulosa; Zr, zona radiata, (Magnification×1000).

Figure 8. Section of stage 4 and 6 oocytes are showing the migration of the nucleus. St_4, stage 4 oocyte; St_6, stage 6 oocyte; Th, theca; G, granulosa; Zr, zona radiata; N, nucleus; Nu, nucleoli; M, micropyle, (Magnification×1000).

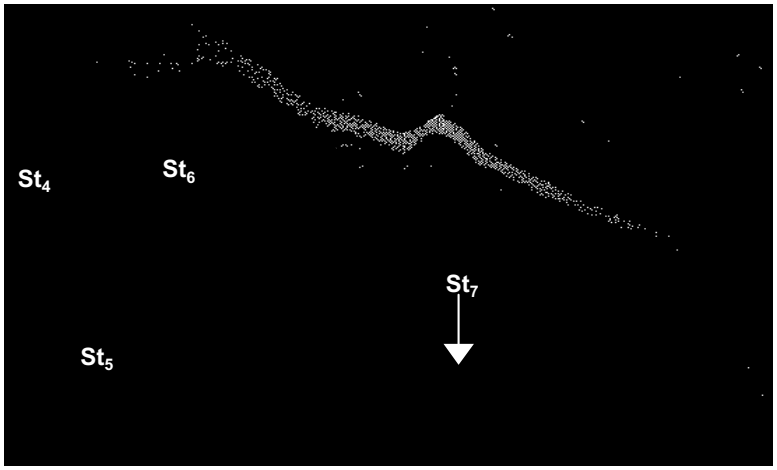

Figure 9. A proportion of oocytes at different developmental stages. Arrow indicates stage 7 oocytes with non-fluid and non-crystalline yolk, (Magnification×1000).

GSI and HSI during gametogenesis

GSI and HSI values in female specimen were plotted against fish weight and length during different stages of gametogenesis. Mean values of GSI and HSI increased with increasing mean body weight and length during gametogenesis in females (Figs. 10, 11 and 12). The relationship was statistically significant (P< 0.05); hence larger fish developed proportionately larger ovaries during gametogenesis (Table 2).

The regression equations for female GSI (FGSI) and HSI (FHSI) with weight and length were:

FGSI = -7.39243+4.3534W (r=0.98; P< 0.05)
FGSI = -42.1129+9.09373L (r=0.98; P< 0.05)
FHSI= -1.22086+1.1663W (r=0.77; p< 0.05)
FHSI= -10.7704+2.4835L (r=0.77; p< 0.05)

where W and L were female weight, length respectively.

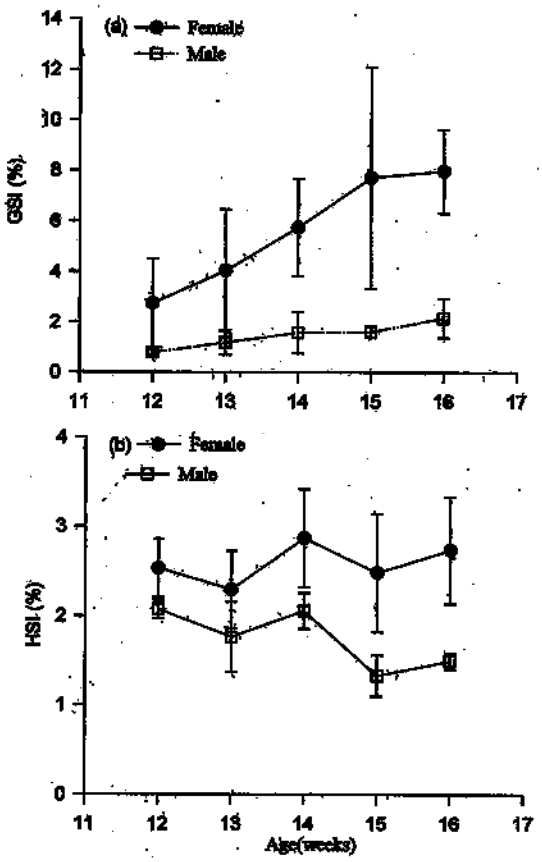

Figure 10. Changes in GSI (a) and HSI (b) during the 12-16 weeks development period, (Mean±sd,n=74)

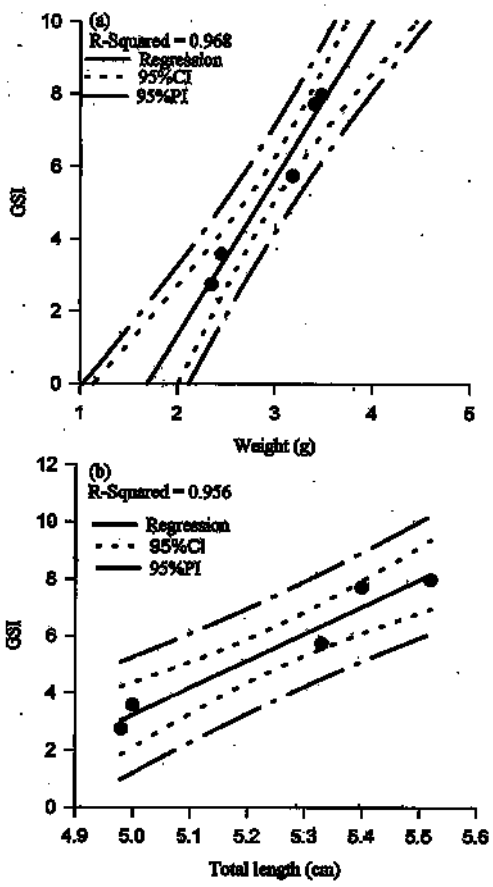

Figure 11. The relationship between GSI and body lenght (a) and weight (b) in female rosy barb during the 12-16 week of development period.

Table 2. Changes in the gonadosomatic index (GSI) and hepatosomatic index (HSI) values during the 12 - 16 weeks development period. (mean ± sd; n= 74 female).

Sampling time (weeks)	12	13	14	15	16
Total length (cm)	4.94±0.191	5.00±0.232	5.33±0.174	5.40±0.396	5.52±0.413
Weight (g)	2.35±0.277	2.45±0.349	3.18±0.321	3.41±0.576	3.48±0.853
Weight of left gonad (g)	0.03±0.013	0.04±0.029	0.08±0.039	0.13±0.066	0.13±0.034
Weight of right gonad (g)	0.03±0.028	0.04±0.031	0.09±0.030	0.10±0.073	0.14±0.042
Liver weight (g)	0.07±0.007	0.07±0.018	0.09±0.023	0.09±0.028	0.09±0.018
GSI (%)	2.74±1.775	3.58±2.426	5.74±1.921	7.71±4.395	7.99±1.666
HSI (%)	0.84±0.326	2.29±0.430	2.86±0.550	2.48±0.663	2.76±0.600

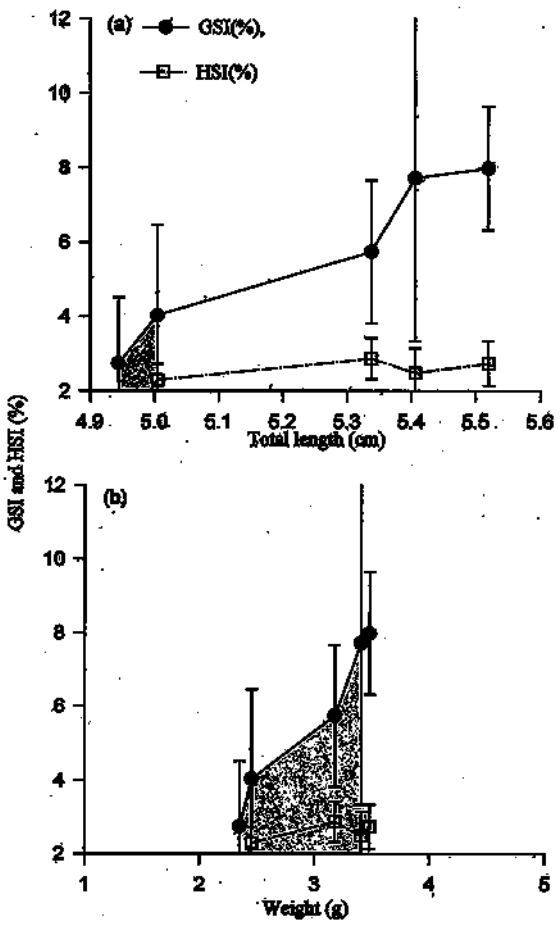

Figure 12. The relationship between GS, HSI and body lenght (a) and weight (b) in female *Puntius conchonius* during the 12-16 week of development period. (Mean±sd, n=74)

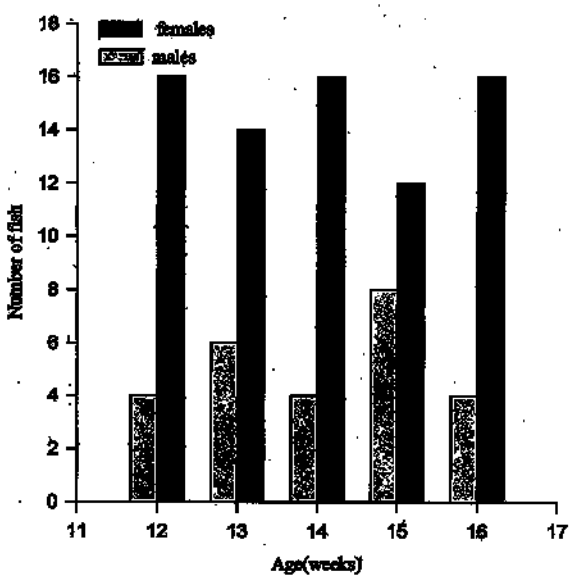

Figure 13. Differences in the number of male and female *Puntius conchonius*, during gametogenesis.

Sex ratio

The sex ratio observed for the rosy barb was slightly different from the expected ratio of 1:1 (female: male). The sex ratio observed in 100 fish taken during weeks 12-16 post-hatch was 74:26 (female: male); this difference was statistically significant ($p<0.05$). In a second sampling group composed of 200 fish, taken during weeks 16-24 post-hatch observed ratio was 146:54 (f: m), and the difference was also significant ($p<0.05$), confirming the first sampling result. (Figure 13).

Discussion

The present study briefly described histological characteristics of developing oocytes of the rosy barb that were assigned to 3 phases (primary growth, secondary growth and maturation including hydration) and 7 stages. The stages of oocyte development were classified on the basis of observations of changes in the nucleus, nucleoli, cytoplasm, and formation of zona radiata.

In rosy barb, as in other teleosts, oogonia proliferated and turned into primary oocytes, which subsequently grew within follicles, formed cortical alveoli, entered vitellogenesis, underwent maturation, and finally was ovulated. During these phases the changes were basically similar to those reported for other teleosts (Gupta, 1975; Cumaranatunga, 1985; Bromage and Cumaranatunga, 1988; Iwamatsu *et al*., 1988; Srisakultiew, 1993). The only notable difference from the above studies was that, at the end of stage 4, crystalline yolk was clearly detected in the oocytes. As the oocytes proceeded through maturation, the crystalline yolk underwent a morphological transformation (size increased, lost their crystalline structures and became spherical in shape), although there was no evidence of yolk granules merging into a homogenous mass in the rosy barb. Thus, yolk in both post-maturational oocytes and ovulated eggs was non-crystalline. This finding was similar to those of Makeyeva and Yamel'yanova (1989) and Selman *et al*. (1993). This process is very important, since it confers on many teleosts eggs their characteristic transparency (Selman *et al*., 1993).

Rosy barbs are multiple spawners (Varadi and Horvath, 1993 and present study) and their ovaries contain oocytes in various sizes and at stages. At the maturation stage, the major part of the ovary was occupied by stage 6 oocytes that comprise a synchronous population of larger oocytes, defined as a clutch. However, a large number of previtellogenic and vitellogenic oocytes (stages 2, 3 and 4) were also detected among the mature oocytes that is a more heterogeneous population from this population clutch were recruited. On the basis of this description the rosy barb might be classified as group synchronous. In contrast, after spawning the ovaries of rosy barb contained a range of oocyte sizes and stages and

oocytes in all stages were present without dominant populations. Thus, rosy barbs' ovaries might also be classified as asynchronous. Confirmation of the types of ovaries present in the rosy barb awaits further studies.

Changes in GSI and HSI followed a similar pattern during gametogenesis. This finding is similar to that reported for other species, e.g (Awaji and Hanyu, 1987; Delahunty and de Vlaming, 1980; Htun-Hun, 1978; Asahina *et al*., 1990). Since total fish length and weight are included in the calculation of GSI and HSI, they present an auto - corelation.

As previously stated, in the present study the sex ratio of *Puntius conchonius* was found to be 74% female and 26% male. This is contradictory to that of Varadi and Horvath (1993), despite the experiments being conducted under the similar conditions and rearing temperatures. The rearing temperatures were 27± 1°C and 25°C for the former and latter, respectively. As the background information on the requirements for sex determination of *Puntius conchonius* is limited, the mechanisms involved in sex determination have yet to be elucidated. Further studies should, therefore, be carried out to determine whether *Puntius conchonius* has a species-specific sex determination mechanism or possesses a major gene determining sex and/or sex chromosomes.

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