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CYCLIC CHANGES IN GONADAL MATURATION AND HISTOLOGICAL OBSERVATIONS OF THREATENED FRESHWATER CATFISH "NARIKELIRU" *MYSTUS MONTANUS* (JERDON, 1849)

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Background. Usage of biosciences in increasing fish production needs to have a proper understanding and knowledge of endocrine physiology of fish reproduction. The most suitable method of determining the reproductive cycle in fishes is to observe seasonal development changes in the gonads. The present paper describes morphological changes in gonads of *Mystus montanus*.

Materials and Methods. The individuals of *M. montanus* were sampled monthly in captive condition throughout one year to determine the changes occurring in gonadal histology and reproductive status. The stages of gonadal maturation and the seasonal changes in the proportion of oocyte development within the ovaries were noticed and maturity stages were assessed by microscopic and also macroscopic observations.

Results. The peak spawning period of *Mystus montanus* was noticed during November–January in male and October–December in female. The size at first maturity was 10–11 cm (8–12 g) in male and 13–14 cm (14–16 g) in female, respectively. *M. montanus* spawned only once in a year with the onset of north-east monsoon. The gonadosomatic index (GSI) ranged from 2.8 to 8.5 in males and from 4 to 16 in females and their condition factor (CF) was 4–6 and 6–9, respectively.

Conclusion. The sequence of gonadal maturation in *M. montanus* is morphologically and histologically divided into five stages viz. immature, maturing, mature, matured, and spent or rest.

Key words: gonadal maturation, histology, freshwater catfish, *Mystus montanus.*

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INTRODUCTION

The spawning biomass is employed routinely in stock assessments of fishes as indicators of reproductive potential. For proper management of a fishery, a thorough study of maturation cycles and depletion of gonads is important, since such a study is aimed in understanding and predicting the annual changes of the population (Thorpe et al. 1990, Jobling et al. 2002, Tomkiewicz et al. 2003, Shein et al. 2004). The reproductive cycles in teleost occurs during a particular phase; some breed once in a year as annual breeders and others as monsoon breeders (Zuckerman 1962).

Cyclic changes in the gonads (ovaries and testes) have been examined in a few species viz. *Channa marulius* (Hamilton, 1822)(cf. Parameswaran and Murugesan 1976), *C. punctata* (Bloch, 1793)(cf. Srivastav and Srivastav 1998), *C. striata* (Bloch, 1793)(cf. Haniffa et al. 2000), "*Clarias lazera*" = *Clarias gariepinus* (Burchell, 1822)(cf. Richter and van den Hurk 1982), *C. macrocephalus* Günther, 1864 (cf. Mollah 1986), *C. batrachus* (Linnaeus, 1758)(cf. Fagbenro et al. 1992), "*M. nemurus*" = *Hemibagrus nemurus* (Valenciennes, 1840)(cf. Khan et al. 1990), *Leiognathus brevirostris* (Valenciennes, 1835)(cf. Jayawardane and Dayaratne 1998), *Nemipterus randalli* Russell, 1986 (cf. Rao 2003) and *Micropogonias furnieri* (Desmarest, 1823)(cf. Vicentini and Araújo 2003). The purpose of the present study was to observe the changes in gonadal maturation and their histological differentiation of the threatened freshwater catfish *Mystus montanus.*

MATERIALS AND METHOD

Fingerlings of *M. montanus* (88 males and 114 females) (5.5 ± 0.4 cm; 3.8 ± 0.38 g) were collected from the Tambaraparani River (irrigation canal) by a cast net during February 2000, transported to the Centre for Aquaculture Research and Extension (CARE), and acclimatized in cement tanks (3 × 1 × 1 m) for a week period by feeding finely chopped chicken intestine ad libitum. They were introduced into the culture pond (8.3 × 7.6 × 3 m) supplied with bore-well water (dissolved oxygen 5.8 mg · l^{-1}, temperature 29 ± 2°C, and pH 6.8–7.2) and the experiment was conducted between April 2000 and March 2001. During the experimental period they were fed formulated diets following Haniffa et al. (1999) (60% chicken intestine, 17% ground nut oil cake, 11% rice bran, 10% tapioca, and 2% vitamin and mineral mixture). The test individuals were sampled monthly (5–10 individuals) by a drag net. The growth performance values were estimated and the feeding level was adjusted accordingly. The fish were raised up to adult stage.

During monthly sampling the fish were taken out and the gonads were dissected and weighed. The gonadal maturation was assessed by microscopic and also macroscopic observations. Gonadosomatic index (GSI) and condition factor (CF) have been calculated following Billard et al. (1993).

Histological observations were made in every maturity season and / or stages during sampling. Small pieces of ovary (6–8 mm) and testicular follicle (4–6 mm) were fixed in Bouin's solution for 48 h and subsequently processed for histology following Degani (1994). Each piece of tissue, embedded in paraffin wax was sectioned at 5 mm and stained either with Ehrlich haematoxylin or Heidenhain's iron haematoxylin and counter stained with eosin.

RESULTS

At maturity the range of total length of the fish was 10–11 cm in males and 13–14 cm in females while the range of body weight was 8–12 g and 14–16 g, respectively. Cyclic changes studied in relation to different maturity stages and the following maturity stages have been observed seasonally (Table 1).

The male and female fish showed weight changes in the gonads, corresponding to the three gametogenic stages (pre-spawning, spawning, and post spawning). In the pre-spawning period there was a gradual increase in the gonadosomatic index (GSI) (2.9 ± 0.3 and 3.9 ± 0.5), which showed a marked, increase (9.7 ± 1.2 and 15.8 ± 2.1) reaching a peak during the spawning period and a gradual decrease (4.5 ± 0.9 and 4.3 ± 0.5) in the post-spawning period in males and females respectively. Their respective CF increased gradually during the pre-spawning period (6.1 ± 0.2 and 6.4 ± 0.3) and decreased thereafter (5.8 ± 0.3 and 4.9 ± 0.4) during the spawning season and again it automatically increased when the gonads entered the atresia stage. So there was a linear relationship between GSI and CF, when GSI increased CF decreased and when CF decreased—GSI increased accordingly (Figs. 1, 2).

Table 1

Stages of maturity at different periods in *M. montanus*

Stage No.	Stage	Period	
		Male	Female
I	Pre-spawning (immature)	April–May	March–May
II	Pre-spawning (maturing or rebuilding)	June–August	June–July
III	Pre-spawning (mature)	September–October	August–September
IV	Spawning (fully matured or ripe)	November–January	October–December
V	Post-spawning (spent or resting)	February–March	January–February

The gradual changes in the weight of the testes were not uniform and did not coincide with the peaks of the female fish. The weight of the testes gradually increased in the months of June through August reaching a peak in October and remained as such up to January, and then began to decrease till March, after which it again increased till May. Whereas the weight of the ovary increased gradually during the months of June–July reaching a peak in October–December and then decreased till February and after which it again increased till May. The five maturity stages of gonads of the male and female *M. montanus* determined (Fig. 3a, b) based on the morphology of gonads are presented in the following Table 2.

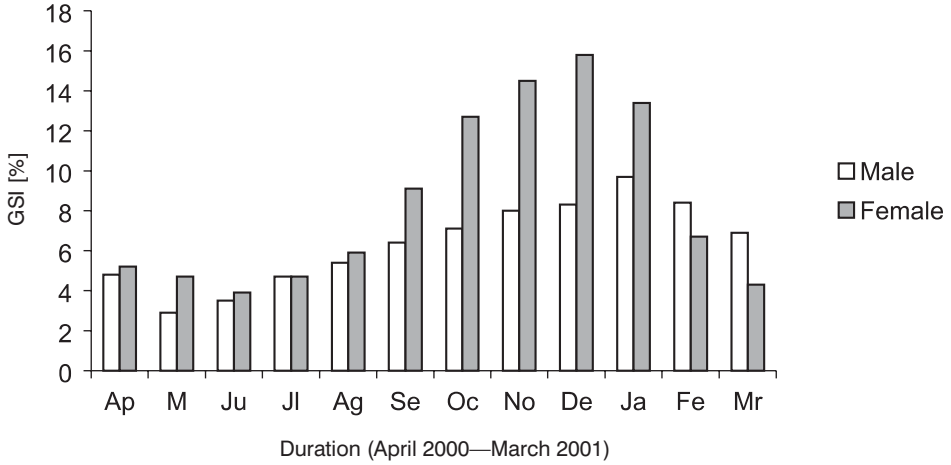

Fig 1. Seasonal changes of average gonadosomatic index (GSI) for males and females of *M. montanus*

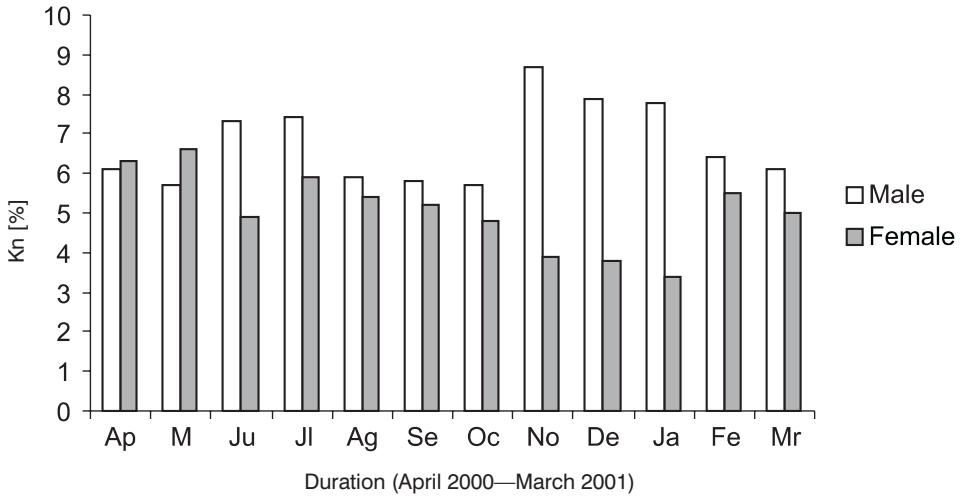

Fig 2. Seasonal changes of average condition factor (kn) for males and females of *M. montanus*

Table 2

Gonadal condition of different maturity stages of male and female *M. montanus*

Spawning period	Maturity stage	Gonadal condition	
		Male	Female
Pre-spawning	I (immature)	Testes very fine, colourless, elongate, thread-like; left slightly longer than right one	Right and left ovaries more or less equal in length and size; colourless to whitish; eggs very minute, distinct only under microscope (Fig. 3c)
Pre-spawning	II (maturing or rebuilding)	Reddish-white or creamy-white; left slightly longer than right one	Ovary considerably larger; white or yellowish-white; maturing eggs visible through wall under microscope; left ovary longer than right one (Fig. 3d)
Pre-spawning	III (mature)	Stages II and III cannot be distinguished clearly; both stages more or less similar	Yellowish-white; shorter than fully mature ovary; differs from fully mature one in its colour (quite yellow in case of fully mature ovary) (Fig. 3g)
Spawning	IV (fully matured or ripe)	Testes are elongate, bulged, and dark-reddish to yellowish or creamy-white plus additional red spots	Yellowish to yellow-white; ovaries visible through translucent body wall of abdomen from outside; vagina becoming dark-pink; one or two ripe eggs remaining in oviduct (Fig. 3f)
Post-spawning	V (spent or resting)	White; red spots still visible; left testis longer than right one; testes seem to be dorsoventrally flattened	Slightly shorter than fully mature one; a number of immature and a few mature yellow eggs still remaining in nearly empty bag; bag transparent and yellow eggs visible from outside (Fig. 3g)

Table 3

Histological differentiation of the gonadal condition of male and female *M. montanus*

Spawning period	Maturity stage	Histological differentiation	
		Male	Female
Pre-spawning	I (immature)	Nuclear diameter 5.7–2.7 μm; nucleus of sperm atogonium with centrally-located nucleolus (occasionally with two nucleoli) (Fig. 4a)	Large, intensely stained nucleus; cytoplasm containing few primary oocytes; oocytes surrounded by follicular epithelium (20–40 μm in diameter) (Fig. 5a)
Pre-spawning	II (maturing or rebuilding)	Spermatogonia to primary spermatocytes, 3.9–4.8 μm in diameter; nuclei 2.5–2.7 μm in diameter; nucleus staining purple in H + E (Fig. 4b)	Chromatide threads visible; oocytes growing rapidly; follicular epithelium still visible; ger minal vesicle more or less oval; nuclei attached to inner border of nuclear membrane (42–65 μm) (Fig. 5b)
Pre-spawning	III (mature)	Primary- to secondary spermatocytes; nuclear diameter 2.6–3.5 μm; dense chromatin usually dispersing along threads throughout nucleus (Fig. 4c)	Secondary growth phase of oocytes; germinal reside oval and lobulated; nuclei enlarging during vacuolization of cytoplasm; membranes of theca externa and interna and inner layer of zona pellucida or radiata visible (75–102 μm) (Fig. 5c)
Spawning	IV (fully matured or ripe)	Nucleus (0.4–2.1 μm in diameter) staining deeply with H + E; finely formed spermatids (Fig. 4d)	Completely mature eggs; follicular tissue much reduced; germinal vesicle still oval; nucleoli reduced in number (Fig. 4d)
Post-spawning	V (spent or resting)	Spermatozoa darkly -stained; kidney-shaped nuclei (1.1–1.4 μm in diameter); 1obules mainly packed with mature spermatozoa, each having a distinct tail (Fig. 4e)	Fully mature eggs; showing few advanced oocytes and undergoing atresia; ovary appearing very slender with prominent ovocoel (Fig. 5e)

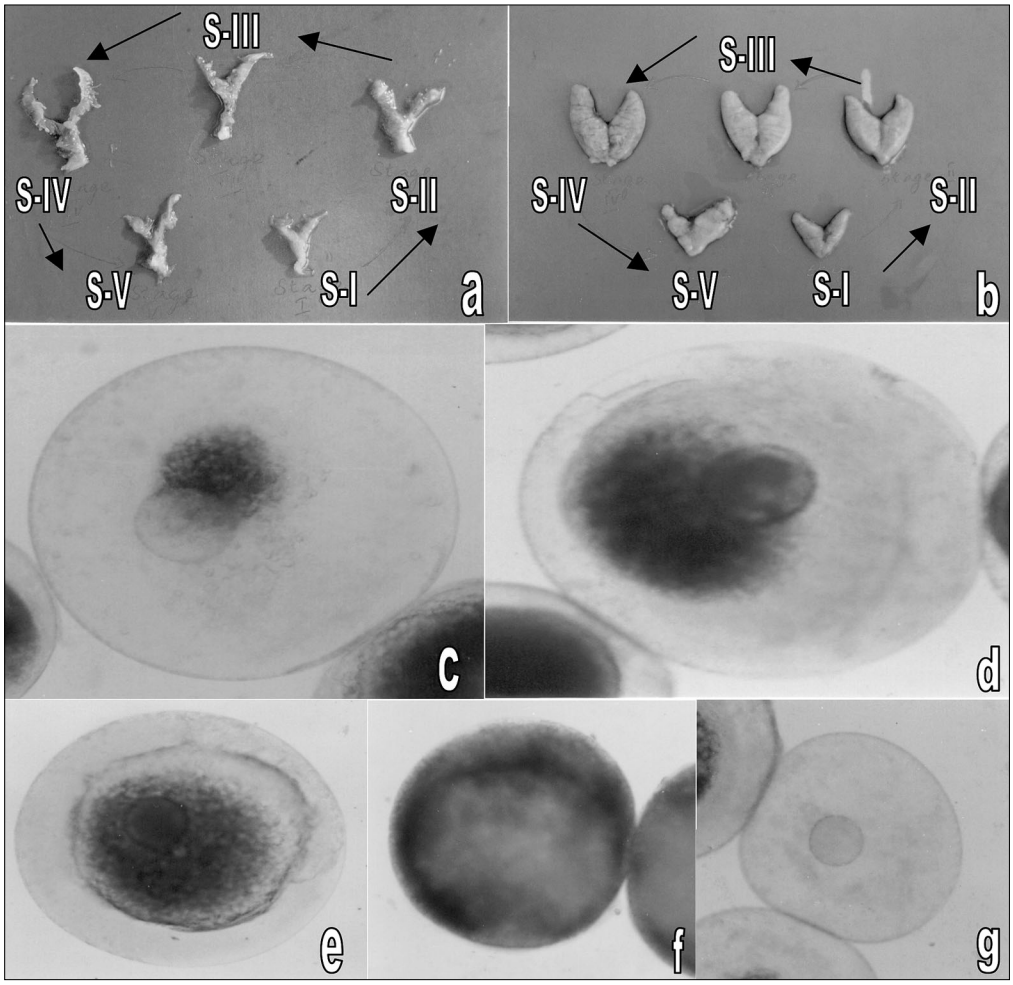

Fig. 3a–g. *Mystus montanus*; aspects of reproduction. **Fig 3a.** Five sequential maturity stages (S-I–S-V) of testis. **Fig. 3b.** Five sequential maturity stages (S-I–S-V) of ovary. **Fig. 3c–g.** Observation of nuclear position in egg during five different maturity stages of ovary (4×). **Fig. 3c.** Immature ovum (S-I). **Fig. 3d.** maturing or rebuilding ovum (S-II). **Fig. 3e.** Mature ovum (S-III) (early). **Fig. 3f.** Fully-mature or ripe ovum (S-IV). **Fig. 3g.** Spent or resting ovum (S-V)

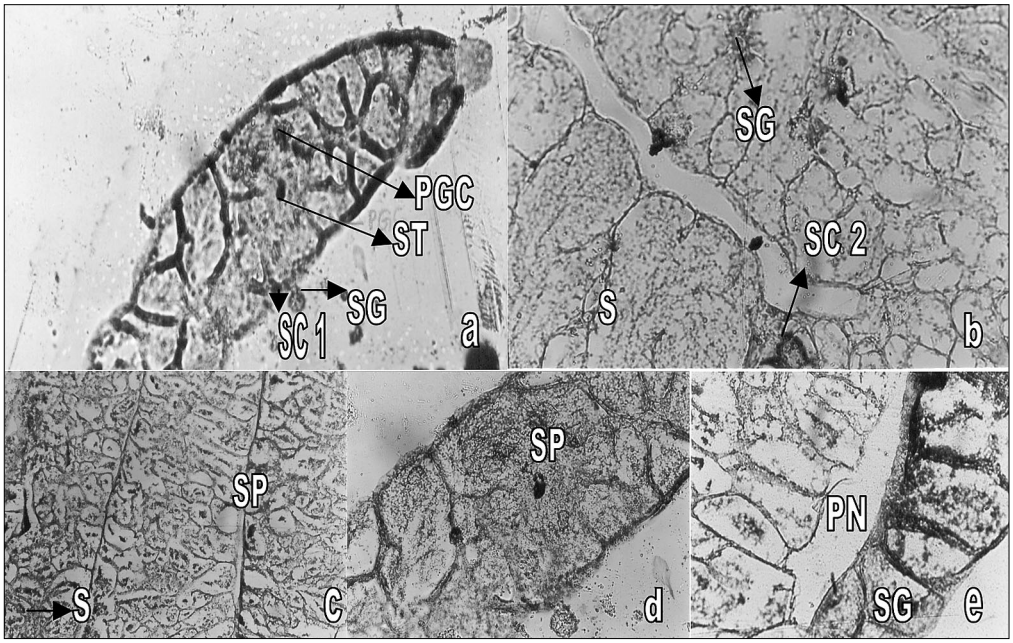

Fig. 4a–e. Histological appearance of testis maturation in *M. montanus.* **Fig. 4a.** immature testis (S-I) (4×). **Fig. 4b.** Mature or rebuilding testis (S-II) (10×). **Fig. 4c.** Mature testis (S-III) (early) (10×). **Fig. 4d.** Fully-mature or ripe testis (S-IV) (10×). **Fig. 4e.** Spent or resting testis (S-V) (10×); PGC, primary germ cells; SG, spermatogonia; SC 1, primary spermatocyte; SC 2, secondary spermatocyte; ST, spermatid; SP, spermatozoa; PN, pycnotic nets of degenerating cells

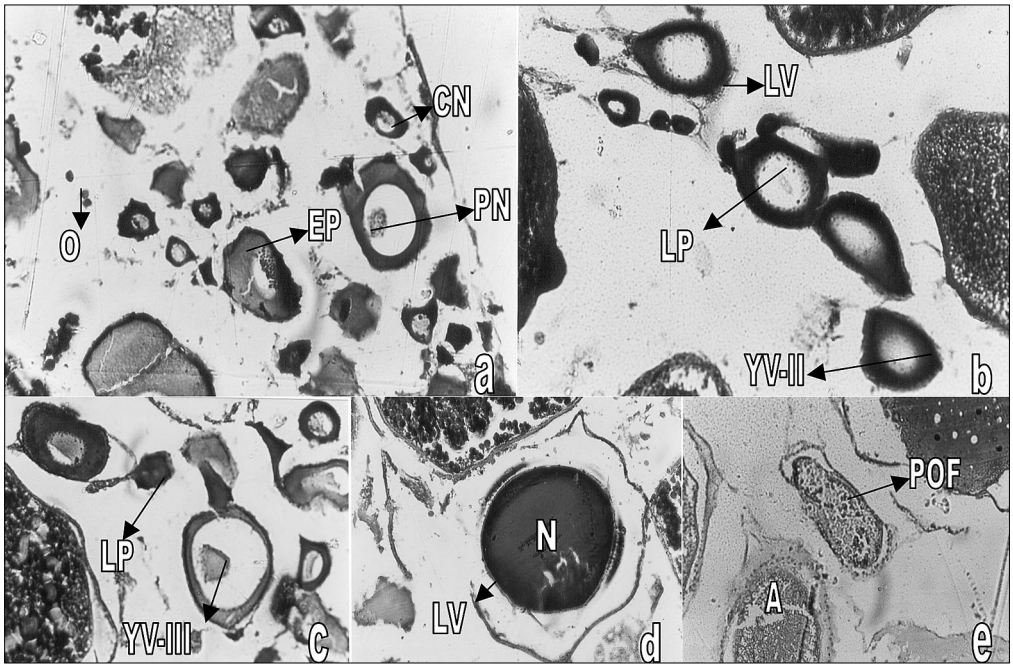

Fig. 5a–e. Histological appearance of ovary maturation in *M. montanus.* **Fig. 5a.** Immature ovary (S-I) (4×). **Fig. 5b.** Mature- or rebuilding ovary (S-II) (10×). **Fig. 5c.** Mature ovary (S-III) (early) (10×). **Fig. 5d.** Fully-mature or ripe ovary (S-IV) (10×). **Fig. 5e.** Spent or resting ovary (S-V) (10×); O, oogonia; CN, chromatin nucleus; PN, peri-nucleus; EP, early peri-nucleus; LV, lipid vesicle; LP, late peri-nucleus; YV-II, yolk vesicle II; YV-III, yolk vesicle III; POF, post ovulatory follicle; A, atresia

Histological observations of gonad

Primary germ cells occurred in the intertitial tissue as well as in the wall of the resting lobules. They occurred in small number throughout the year, but were most prominent after the breeding season when they divided mitotically to form nests of oogonia. Each of these large and relative inconspicuous cells showed light staining often with peripheral chromatin and each with a single nucleolus (Table 3).

Each testis was enclosed by a thick tunica albuginea. The testis was attached to the dorsal body wall by the connective tissue mesorchium. Each testis was composed of numerous thin walled lobules. Within the lobules, cells in various stages of spermatogenesis were present in discrete nests of cells, each nest consisting of cells at the same stage of development. During the breeding seasons the lobules became greatly distended with spermatids and spermatozoa (Table 2).

DISCUSSION

The highest values of CF and GSI were due to the active somatic energy accumulation and the lowest CF and GSI due to the somatic energy depletion (Encina and Lorencio 1997). The variation probably due to the fact that the changes in energy are usually more than the seasonal weight variations as was found by Scott et al. 1980

Photoperiod was also affect the CF and GSI as reported by Hansen et al. (2001) in *Gadus morhua* Linnaeus, 1758. Cambray and Bruton (1984) pointed out that the similarity in the CF and GSI, annual cycle and the fact that the seasonal pattern common among juveniles and adult fish, suggest that the seasonal variations encountered were more related to variations in food availability than to the reproductive cycle. However some divergence registered in the seasonal pattern among juvenile and adult fish suggest that gonadal development and spawning action could also affect the somatic energy storage in adult fish. CF and GSI values were relative more stable over the year for the juveniles than for the adult fish. On the other hand CF and GSI values showed a parallel pattern throughout all the annual cycle in juveniles.

Gonadal development and reproductive strategy have been described in many teleost fish species in an effort to understand the time course and energetic consequences of reproductive effort. Oocyte growth follows a similar general pattern in most teleosts (Maddock and Burton 1999, Knuckey and Sivakumaran 2001). In the present investigation, five different maturity stages of gonads have been described. Similar observations were made in "*Gobioides rubicundus*" = *Odontamblyopus rubicundus* (Hamilton, 1822) by Kader et al. (1988). In *O. rubicundus* the spawning season differed from *M. montanus*. *O. rubicundus* mainly breeds in late January to early February and late June to early October. In the present investigation, *M. montanus* breeds from October to December.

Those fish that undergo gonadal maturation during periods of lower food availability such as *Hippoglossoides platessoides* (Fabricius, 1780) and *Pleuronectes platessa* Linnaeus, 1758 are thought to utilize somatic energy reserves, particularly

protein for reproductive growth (Roff 1982, Sivakumaran et al. 2003). Vitellogenesis is one of the most important reproductive phenomenons in egg-laying animals. Vitellogenin is synthesized in the liver under hormonal influence and is deposited in growing oocytes as yolk protein, which serves as building and energy material after fertilization during embryogenesis till hatchlings start feeding.

Histology revealed the reabsorption of both tertiary yolk stage oocytes and the other second growth phase oocytes. This resolves some conflicting reports found in earlier studies (Palmer et al. 1995). Daiber (1953) reported three distinct size classes of oocytes and concluded that the largest were released during spawning, the middle size were released the next year, and the smallest were released in subsequent year.

The post ovulatory follicles degenerate rapidly, making it difficult to assess the percentage of oocytes spawned unless samples are collected daily (Hunter and Macewicz (1985). Also the elastic nature of ovarian tissue allows the ovary to contract as oocytes are spawned so that the remaining oocytes are still closely grouped, making oocytes loss less apparent. It is also difficult to determine whether the observed atresia represented premature reabsorption of oocytes that could have been released later or it was part of the natural tissue resorption process after cessation of spawning. However, the fact that atresia was observed while GSI was still high and that atretic oocytes were found in ovaries that also displayed germinal vesicle maturation suggest that atresia may decrease the reproductive potential of *M. montanus.*

The testes maintained more weight from October to January and these four months may be considered to be the peak spawning season of the male *M. montanus.* The attainment of early maturation and maintenance of more weight for a longer period by the testes probably facilitate and ensure successful fertilization. Htun-Han (1978) and Nash (1982) also observed earlier maturation and maintenance of more weight by the testes for longer periods than the ovaries in *Limanda limanda* (Linnaeus, 1758) and *Lesueurigobius friesii* (Malm, 1874), respectively. The existence of spawning season in *M. montanus* has been established through the present histological study. Similar observations were made in *C. macrocephalus* by Mollah and Tan (1982) and Mollah (1986).

Davis (1977) also reported the presence of dense connective tissue and occasional blood vessels in the tunica albuginea covering the testis of an Australian catfish *Tandanus tandanus* (Mitchell, 1838) similar to those observed in *M. montanus.* The interlobular space is occupied by blood vessels, nerve fibres (Gresik et al. 1973), fibroblast cells, collagen fibres and other cell types, which vary in different species. In *M. montanus,* the interlobular space is occupied by blood vessels and smooth muscles. It is possible that the same species may not possess all the cell types presented in other fish species. It was Okuzawa et al (1989) who suggested that elastic tissue might be responsible for contraction of the testis and discharge of sperm although he was unable to demonstrate the presence of elastic fibres. In "*Eucalia inconstans*" = *Culaea inconstans* (Kirtland, 1841)*,* however, elastic fibres were found in the walls of the testis and were especially visible after spawning (Ruby and McMillan 1970).

The cyst wall consists one or more cells which appear to play a role similar to that of Sertoli cells of tetrapod and indeed they have been called Sertoli cells by some scientists (King et al. 1995). The Sertoli cells surround cysts of germ cells in *M. montanus* and therefore, were mostly seen in the immature testes. The seminiferous tubules (ampullae) are composed of many spore sacs. These spore sacs are separated by a thin layer of follicular cells (Sertoli cells) in each spore sac there is plenty of synchronously developing germ cells (spermatocytes and spermatids). After formation of sperms, spore sacs disintegrate and sperm enter the cavity of seminiferous tubules secreted by testis forming the so-called milt (Srivastav and Srivastav 1998).

Development of sperm passes through 3 stages i.e. multiplication stage, growth stage, and maturation stage (Shein et al. 2004). Just as the ovary, the testicular maturity can be judged by visual observations by morphological and histological observations (Rath 2000). However the stages can be classified as stage I (resting phase), stage II (late immature phase), stage III (maturity phase), stage IV (mature phase), and stage V (spent phase).

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