Pakistan J. Zool., vol. 43 (4), pp. 739-745, 2011.

Serum Cholesterol Level During Vitellogenesis of Teleost Fish, *Cyprinus carpio*

Zarina Abassi, S. A. Shaikh and Javid Abbassi
Department of Zoology, University of Sindh, Jamshoro, Pakistan

Abstract.- An attempt was made to study the levels of cholesterol during the vitellogenesis in the ovary of the fish *Cyprinus carpio*. At least 6 female fish were collected weekly from nearby hatchery during the period February to August transferred to the laboratory live and sacrificed for taking the ovaries for histological examinations. Blood sample (5 ml) was collected from each fish for cholesterol estimation. The vitellogenesis and the gradual increase in serum cholesterol starts (65.50±2.0 mg/100ml) in the month of February and reaches its maximum (98.50±4.0 mg/100ml), in mid of March when the ovaries were gravid and ready to spawn. The decline in the serum cholesterol was noted in late March *i.e.* (75.7±2.0 mg/100ml), which continued throughout the month of April. The gradual Increase in serum cholesterol was again noted in early May and reaches to the maximum (93.50± 5.9 mg/100ml). In early August, the ovaries were again gravid and contained mature follicles which were ready for spawning. The decline in serum cholesterol was also noted as the spawning was over in mid August. The results suggest that the fish may spawn second time if the environmental conditions are conducive.

Key words: Vitellogenesis, cholesterol estimation, ovary, teleost fish, *Cyprinus carpio.*

INTRODUCTION

The process of oocyte development in teleosts has been categorized as synchronous, group-synchronous and asynchronous types (Wallace and Selman, 1981). In the Pacific herring, a clutch of oocytes at the cortical alveolus stage appeared from June, developed to yolk globule stages in October, and was distinguished from residual non-vitellogenic oocytes. Such a manner of oocyte development, also found in Atlantic herring *Clupea harengus* (Bowers and Holliday, 1961) belongs to the group-synchronous oocyte development type. The vitelogenic oocytes in each fish synchronously shifted to the migratory nucleus stage in early March, and were ovulated in early April. Ovulation occurs simultaneously in this species in captivity and for other herring species (Bowers and Holliday, 1961).

The maturity of the ovary in Pacific herring has been classified in to eight phases by exterior observation of the ovary (Hay, 1985) which was modified from the classification in Atlantic herring (Bowers and Holliday, 1961). In the said study, ovarian maturity of Scorpio was classified based on the histological observation, reflecting a more accurate physiological status of the ovary. According to classification, non-vitellogenic stages were collected in to a single maturity and in the stages close to maturation, migratory nucleus stages were separated from vitellogenic maturity. The patterns of oocyte growth in teleosts can be divided in to three basic types depending on the range of developmental stages of oocytes present in ovaries during maturation, and the spawning frequency in each single reproductive season. These patterns are: (i) Synchronous ovarian growth where there is only one clutch of oocytes in the ovary and the fish spawns once in a life time, (ii) Group synchronous ovarian growth where at least two clutches of oocytes are present in the ovary and spawning occurs once per season and (iii) Multiple group synchronous or asynchronous ovarian growth where several clutches of oocytes are found in the ovary at once and there is multiple spawning over a reproductive season (Wallace *et al.*, 1987; Pankhurst, 1998; De Vlaming 1983; Bye,1984; Weddle and Burr, 1991; McEvoy and Mc Evoy,1992). Previous studies on wild population of the Australian Pleuronectid *Rhombosolea tapirina* Gunther (green back flounder) have shown that there is multiple group synchronous ovarian development (Barnett and Pankhurst, 1999) and the capacity for multiple spawning. Vitellogenesis is a

* Corresponding author: shamsshaikh4@gmail.com
0030-9923/2011/0004-0739 $ 8.00/0

particularly significant period of reproductive development and is characterized by rapid growth of oocytes and may be responsible for accumulation of upto 90% of the final egg size (Tyler *et al*., 2000).

Little information is available about the serum level of triglyceride and cholesterol ester, the kind and composition of the circulating lipoproteins, or the relationship between these parameters and sex, age or reproductive state. Although the hepatic lipid unloading during vitellogenesis has been well documented in sharks (Templeman, 1944) contradictory data are found in the literature for the cholesterol level of some shark species (Vastesaeger and Gillot, 1965; Mills and Taylaur, 1971). *Cyprinus carpio*, commonly known as Gulfam is available in the fresh water. Earlier studies regarding the ovaries maturation indicate that spawning in the Volga and Leral River begins in late April and ends in May at water temperature 15-22°C. Because of intermittent / batch spawning, the breeding season may last for some 60-70 days. Common carp in the Atrek River spawns from mid March (at 12-14°C) until early April. The spawning season in Malyl Kyzylagach Bay and Kara River starts in early March with a peak in late March-early April and ends in late May.The fish spawn, once a year but not a single study has been published which indicate that the fish can spawn twice as the maturation of the ovarian follicles takes place during June to August after the commencement of fish breeding in March (Memon and Shaikh, 2003).

The objective of the present study was to investigate the maturation of the ovaries of *Cyprinus carpio* during the period of February-August (maturation / breeding periods).

MATERIALS AND METHODS

Mature female specimens of live *Cyprinus carpio* were collected from Maqbool Aijaz Hatchery, Thatta. The fish were cleaned with dry cloth and weighed. In order to collect the serum for cholesterol estimation, the gills were cleaned of water with the help of soft tissue papers. By giving cut to the gills the blood drained out with pressure from which 10 ml blood was collected in glass vials. The vials were sealed and brought to the laboratory for cholesterol estimation. Both the ovaries of all the fish were separated out and weighed. Few pieces from both of ovaries were taken and immediately fixed in the Altman's fixative (Gatenby *et al.*, 1950).

The gonads were processed for the histological examination, 6-8 µm thick sections were cut and stained with haemotoxylin and eosin.

Blood collected from fish was allowed to clot, centrifuged at 3000 rpm for 10 min and serum was used for the analysis of cholesterol by CHOD-PAP method (Deeg and Ziegenhorn, 1983).

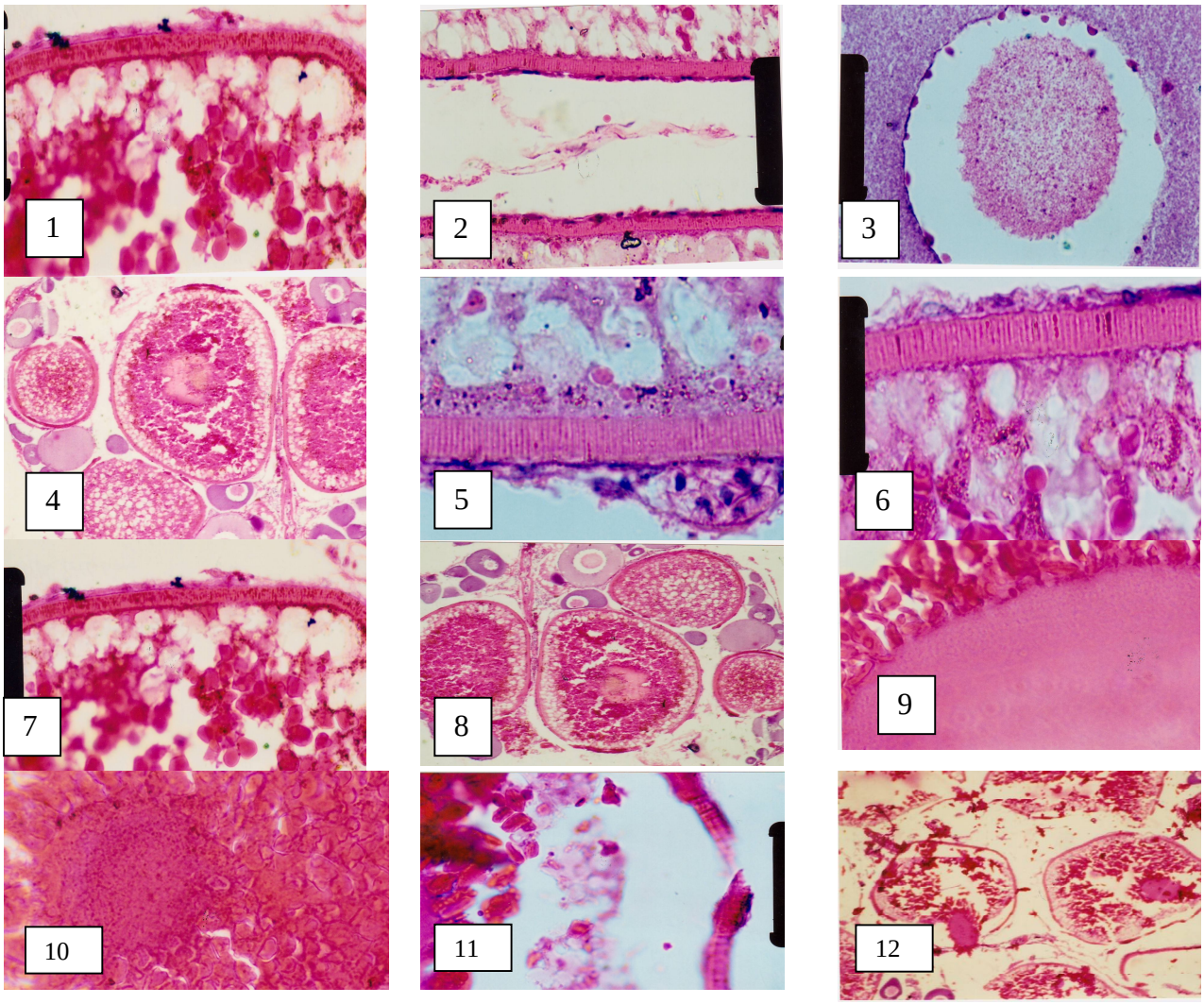
RESULTS

Gonadosomatic index (GSI)

Table I shows that recrudescence of the ovaries started in the month of February with mean GSI of 18.12±1.2. Vitellogenesis started in the mid April and oocytes matured in the month of May with GSI of 22.14±1.5. The GSI dropped after first breeding and reached the lowest level 6.36±1.6 in late July. The GSI again started increasing in early August and reached 26.92±2.0 in the second week of the August.

Histological examination

The histological examination of the sections of the ovaries reveal that the ovaries of the fishes collected with month of early February were undergoing process of maturation. Cortical granules were located at the periphery and few yolk granules were also found scattered around the nuclear membrane (Fig.1). Zona radiata has not yet striatated. A row of granulosa cells and few theca cells are also presents (Fig.2). Though the nuclear membrane is intact, the numbers of nucleoli have been decreased (Fig.3) which is the sign of progression towards maturation. Figure 4 shows an advanced state of ovary of the fish collected in early May. Here theca and granulosa cells have been reduced in size. However cross striations with zona radiata have become more prominent (Fig. 5). The cortical yolk vesicle and yolk granules have also been reduced in number which indicates the maturation of oocytes in the final stage (Fig. 6). After first breeding zona radiata lacking striations, no theca cells, no granulosa cells exist (Fig.7). Recovery was noted in late June. The ovaries contained few maturing oocytes including many



Figs. 1-12. Cross sections of the ovaries of fish showing different stages of maturation. 1, Cortical granules (cg) were located at the periphery and few yolk granules (yg) found scattered around the nuclear membrane; 2, Zona radiata has not yet striatated. A row of granulosa cells (gc) and few theca cells (tc) are also present; 3, Number of nucleoli (nl) which is the sign of progressing towards maturation; 4, shows an advance stage of ovary in early May, with reduced theca (tc) and granulosa cells (gc); 5, Shows cross striation (cs) with prominent zona radiata (zr); 6, Reduce number of cortical yolk vesicle (yv) and yolk granules (yg) which indicates the maturation of oocytes in the final stages; 7, After first breeding zona radiata (zr) lacking striations, no theca cells, no granulosa cells exist; 8, Recovery in late June .The ovaries contain few maturing oocytes including many other follicles in different stages; 9, The ovarian follicles were fully matured and have developed migratory filaments (mf) around oocytes during mid July to late July; 10, The oocyte lacking nuclear membrane and displaced from the center and moving towards periphery for the purpose of ovulation; 11, The oocyte / ovum have migrated to the periphery and ruptured the follicular wall as shown in Figure 12; The other follicles in the same ovarian section show that the oocyte (ocy) is migrating towards periphery. The condition of ovary indicates that the fish is gravid and is in spawning phase.

other follicles in different stages (Fig. 8). Maturing follicles in the ovaries of fish collected in early July shows the sign of maturation by having striated zona radiata outer to that granulosa cells and theca cells are present but scattered (Fig. 9). During mid July to late July the ovarian follicles were fully mature and have developed migratory filaments around oocytes (Fig.10).

Table 1.- Gonadosomatic index (GSI) of the maturation state of *Cyprinus carpio* during first (Feb-May) and second spawning (June-August).

Date	Body wt. (g)	Overian wt (g)	Gonadosomatic index (GSI)	Total cholesterol (TC) mg /100 ml
First spawning				
10-02	1600 ± 20.0	290 ± 13.0	18.12 ± 1.2	65.50 ± 2.0
28-02	1500 ± 32.2	270 ± 15.0	18.00 ± 1.9	70.50 ± 3.0
15-03	1500 ± 30.0	245 ± 10.0	16.33 ± 1.8	98.50 ± 4.0
30-03	1400 ± 10.0	240 ± 11.0	17.14 ± 1.5	75.20 ± 5.0
15-04	1300 ± 20.5	260 ± 12.2	20.00 ± 1.2	65.00 ± 2.0
30-04	1300 ± 30.3	270 ± 14.1	20.76 ± 1.7	65.00 ± 2.0
15-05	1400 ± 40.5	300 ± 3.1	21.42 ± 1.6	65.20 ± 4.0
30-05	1400 ± 20.3	310 ± 15.5	22.14 ± 1.5	70.50 ± 2.0
Second spawning				
27-06	1900± 30.0	378.4± 15.5	19.91± 1.8	75.70 ± 2.0
04-07	1800± 20.3	259.4± 10.5	14.41± 2.0	78.50 ± 5.0
11-07	1600± 40.1	225.0± 12.3	14.00± 1.5	82.20 ± 6.0
18-07	1300± 10.0	205.0± 18.3	15.70± 1.9	85.40 ± 3.0
25-07	1100± 20.0	70.0± 12.3	06.36± 1.6	87.50 ± 2.0
01-08	1200± 30.3	220.0± 10.1	18.33± 1.2	93.50 ± 5.9
08-08	1300± 30.5	350.0± 14.5	26.92± 2.0	75.20 ± 2.0
22-08	1300± 20.5	200.0± 20.5	15.38± 1.9	65.30 ± 3.0

The oocyte lacking nuclear membrane and having displaced from the center and moving towards periphery for the purpose of ovulation (Fig.11). The oocyte/ova have migrated to the periphery and ruptured the follicular wall. The other follicles in the same ovarian section shows that the oocyte is migrating towards periphery. The condition of ovary indicates that the fish is gravid and in spawning phase (Fig.12).

Cholesterol level in blood

The cholesterol level in February was 65.5±3.0 mg%. It started increasing as the vitellogenesis was in progress during this period. It reached 98.5±2.0 mg% in mid March but then decreased gradually to 70±5.0 mg% (Table I). It started increasing again gradually in early June and reached 93.5±2.0 mg% in early August. When the oocyte completely developed the levels dropped 75.20±2.0 and ovaries were full of matured follicles and the GSI was highest 26.92±2.0 in early August.

DISCUSSION

In order to determined the earlier reports about the breeding patterns of the fish, *C. carpio*, the present study was undertaken with the special emphasis on the breeding cycle from the month of February through July till August when breeding of the fish was completely over. The GSI levels as reported in earlier studies reached the peak first in late March and breeding took place somewhere in April and persisted till the end of May. The breeding took place in early March. Present findings though as per data are not in agreement with the past studies but the maturation patterns are almost identical as the recrudescence of the ovarian follicles starts in the same season. However, breeding of fish can be prolonged or delayed depending upon the climatic conditions (Temperature and photoperiod) that has been reported earlier (Lam, 1983; Bye, 1987; DeVlaming, 1972, 1974; Memon and Shaikh, 2003).

The first indication of final oocyte maturation (FOM) in striped bass is the fusion and coalescence of the lipid droplets (Bayless, 1972; King *et al*., 1994) which occurs simultaneously with Germinal vesicle (GV) migration to the peripheral cytoplasm, and resulted in the oocytes becoming more translucent. The GV never -++became truly peripheral, as observed in other species, in the sense of being congential to the cell wall (Goetz, 1983; Murayama *et al*., 1994; Mylonas *et al*., 1995). Migration of the GV in most fishes occurred after the completion of vitellogenesis (Wallace and

Selman, 1981; Goetz, 1983; Jalabert *et al*., 1991). Clearing fixatives are often used in order to identify this GV migration in oocytes that have completed vitellogenesis (Crim and Glebe, 1990; Stoeckel and Neves, 1992).

Vitellogenic oocytes appeared in the ovaries in March developed rapidly from April to May, suggesting that vitellogenesis start in March and progressing actively between April and May. Histological changes in the ovary of several species of groupers have been shown to correspond well with the changes in GSI in *C. carpio*. The GSI of red grouper *E. morio* collected from the central west Florida shelf, increased rapidly in March, and maturing oocytes appeared at the same time. Moreover, the GSI of the honeycomb grouper *E. merra* increased from April through June, synchronizing with oocyte development (Lee *et al.*, 2002). Although the vitellogenic and spawning seasons of groupers are different from species to species, the pattern of gonadal development and the composition of oocytes are similar in all species. This trend is also observed in red grouper (Johnson *et al*., 1998), honeycomb grouper (Lee *et al*., 2002) and white grouper *E. aeneus* (Hassin *et al*., 1997)

Immediately after the first spawning that took place during the month of May, the GSI decreased gradually from 22.14% to 6.36% in the end of July. Abrupt increase of GSI from 18.33 to 26.92 in early August when the ovaries of contained matured follicles or migrating oocytes indicate that *Cyprinus carpio* can spawn more than once depending upon the environmental conditions. In support of this many workers have also reported such type of findings in teleosts and named such fishes as multiple spawners or group spawners (Papadopal, 1968; Vollestad and L'Abee-Lund,1987; Kestemont and Philippart 1991, Jafri,1990; Wootton 1979; Hellawell, 1972). With regard to the histomorphological studies in the present finding about maturation of oocyts after the first spawning with a very little quiescent phase has also been reported by others (Hunter *et al.*, 1985; Deniel *et al*., 1989; Weddle and Burr, 1991; Barberi *et al*., 1994; Rinchard and Kestemont, 1996). With regard to the change in the serum cholesterol in the *Cyprinus carpio*, our results reveal that the levels of cholesterol increased in the serum during votellogenesis/prespawning phase and dropped when the oocyte finally matured and ready to spawn. Previous data in the literature (Larsson and Fange, 1977; Nelson and Shore, 1974; De Vlaming *et al.*, 1984) indicates such increase in serum cholesterol during pre-spawning vitellogenesis and the gametogenesis, respectively. The presence of a wide range of vitellogenic oocytes in individual ovaries, and the coexistence of vitellogenic oocytes and oocytes under going full maturation in some ovaries confirm that the oocyte developmental pattern in *Cyprinus carpio* is conditional multiple group synchrony as like earlier findings (Barnett and Pankhurst, 1999; Poortenaar and Pankhurst, 2000). According to the studies undertaken it has been well established that the fish, *Cyprinus carpio* can be listed in group of spawning fishes.

REFERENCES

BARBIERI, L.R., CHITTENDEN JR, M.E. AND LOWERRE-BARBIERI, S.K., 1994. Maturity, spawning, and ovarian cycle of Atlantic croaker, *Micropogonias undulates*, in the Chesapeake Bay and adjacent coastal waters. *Fishery Bull.*, **92:** 671-685.

BARNETT, C.W. AND PANKHURST, N.W., 1999. Reproductive biology and endocrinology of greenback flounder *Rhombosolea tapirina* (Gunther 1862). *Mar. Freshw. Res*., **50:** 35-42**.**

BAYLESS, J.D., 1972. *Artificial propagation and hybridization of striped bass,* Morone saxatilis *(Walbaum).* South Carolina Wildlife and Marine Resources Department, Columbia.

BOWERS, A.B. AND HOLLIDAY, F.G.T., 1961. Histological changes in the gonad associated with the reproductive cycle of the herring (*Culpea harengus* L.). *Mar. Res. Dept. Agric. Fish Scotland*, **5:** 1-16**.**

BYE, V.J., 1984. The role of environmental factors in the timing of reproductive cycles. In: *Fish reproduction: Strategies and tactics* (eds. G.W. Potts and R.J. Wootton). Academic Press. London, pp. 187-206.

BYE, V. J., 1987. Environmental management of marine fish reproduction in Europe. In: *Proceedings of the third international symposium on reproductive physiology of fish* (eds. D. R. Idler, L. W. Crim and J. M. Walsh), St. John's Newfoundland, Canada, pp. 289-298.

CRIM, L. W. AND GLEBE, B.D., 1990. Reproduction. In: *Methods for fish biology* (eds. C.B. Schreck and P.B. Moyle), American Fisheries Society. Bethesda, Maryland, pp. 529-553.

DEEG, R. AND ZIEGENHORN, J., 1983. Kinetic enzymatic method for automated determination of total cholesterol

in serum. *Clin. Chem*., **29**: 1798-1802.

DENIEL, C., LE BLANE, C. AND RODRIGUEZ, A., 1989. Comparative study of sexual cycles, oogenesis and spawning of two Soleidae, *Solea lascaris* (Risso, 1810) and *Solea impar* (Bennet, 1831), on the western coast of Brittany. *J. Fish Biol*., **35**: 49-58.

DE VLAMING, V., 1972. Environment control of teleost reproductive cycles: A brief review. *J. Fish Biol*., **4**: 131-140.

DE VLAMING, V., 1974. In: *Control of sex in fishes* (ed. C. B. Schreck), Virginia Polytechnic Institute and State University, Blacksburg. pp. 13-83.

DE VLAMING, V., 1983. Oocyte development patterns and hormonal involements among teleosts. In: *Control processes in fish physiology* (eds. J.C. Rankin, T.J. Pitcher and R.T. Duggan), Croom Helm, London, pp. 176-199.

DE VLAMING, V., FITZGERALD, R., DELAHUNTY, G. AND CECH, J.J., 1984. Dynamics of oocyte development and related changes in serum estradiol-yolk precursor and lipid levels in the teleosten fish *Leptocottus armatus*. *Comp. Endocrinol*., **70**: 1-8.

GATENBY, J.B. AND BEAMS, H.W., 1950. *The Microtomists's Vade-Mecum*. J. and A. Churchill, London.

GOETZ, F.W., 1983. Hormonal control of oocyte final maturation and ovulation in fishes. In: *Fish physiology*, (eds. W.S. Hoare, D.J. Randall and E.M. Donation), Volume IX, Part B pp. 117-170. Academic Press, London.

HASSIN, S., MON BRISON, D., HANIN, Y., ELIZUR, A., ZOHAR, Y. AND POPPER, D.M., 1997. Domestication of the white grouper *Epinephelus aeneus* 1. Growth and reproduction. *Aquaculture*, **156:** 305-316.

HAY, D.E., 1985. Reproductive biology of Pacific herring (*Clupea harengus pallasi*). *Can. J. Fish. aquat. Sci*., **42:** 111-126.

HELLAWELL, J.M., 1972. The growth, reproduction and food of the roach *Rutilus rutilus* (L.), of the River Lugg, Herefordshire. *J. Fish Biol*., **4**: 469-486.

HUNTER, J.R., LO, N. C. H. AND LEONG, R.J.H., 1985. Batch fecundity in batch spawning fishes. In: *An egg production method for estimating spawning biomass of Pelagic fish: Application to the Northern Anchory*, Engraulis mordax (ed. R. Lasker). NOAA Technical Report No. NMFS-**36:** 66-77.

JAFRI, S.I.H., 1990. Gametogenesisin roach, *Rutilus rutilus* (L.) (Cyprinidae: Teleostei). *Pakistan J. Zool*., **22**: 361-377.

JALABERT, B., FOSTIER, A., BRETON, B. AND WEIL, C., 1991. Oocyte maturation in vertebrates. In: *Vertebrate endocrinology: Fundamentals and biomedical implication Volume 4, Part A, Reproduction* (eds. P.K.T. Pang and M.P. Schreibman), Academic Press, San Diego, California, pp. 23-90.

JOHNSON, A.K., THOMAS, P. AND WILSON Jr. R.R., 1998. Seasonal cycles of *Nephelus morio*, a protogynous grouper in the eastern Gulf of Mexico. *J. Fish Biol*., **52:** 502-518.

KESTEMONT, P. AND PHILIPPART, J.C., 1991. Considerations sur la croissance ovocytaire chez les poisons a ovogenese synchrone et asynchrone. *Belgian J. Zool*., **121**: 87-98.

KING, W.V., THOMAS, P., HARRELL, R. M., HODSON, R.G. AND SULLIVAN, C.V., 1994. Plasma levels of gonadal steroids during final oocyte maturation of striped bass *Morone saxatilis* L. *Gen. Comp. Endocrinol.*, 95, 178-191.

LAM, T.J., 1983. Environmental influences on gonadal activity in fish. In: *Fish physiology* (eds. W. S. Hour, D. J. Randall and E. M. Donaldson). Vol. 9B, pp. 65-115. Academic Press, New York.

LARSSON, A. AND FANGE, R., 1977. Cholesterol and free fatty acids (FFA) in the blood of marine fish. *Comp. Biochem. Physiol*., **57B**: 191-196.

LEE, Y.D., PARK, S.H., TAKEMURA, A. AND TAKANO, K., 2002. Histological observations of seasonal reproductive and linar-related spawning cycles in the female honeycomb grouper *Epinephelus merra* in Okinawa waters. *Fish Sci*. **68:** 872-877.

McEVOY, L.A. AND McEVOY, J., 1992. Multiple spawning in several commercial fish species and its consequences for fisheries management, cultivation and experimentation. *J. Fish Biol*., **41**, (Suppl. B): 125-136.

MEMON, B. F. AND SHAIKH, S.A., 2003. Maturational changes in gonads in relation to pituitary gland of a teleost fish, *Cyprinus carpio. Proc. Pakistan Congr. Zool*., **23:** 49-65**.**

MILLS, G. L. AND TAYLAUR, C.F., 1971. The distribution and composition of serum lipoprotein.in eighteen animals. *Comp. Biochem*., **40B:** 489-501.

MURAYAMA, T., SHIRASHI, M. AND AOKI, I., 1994. Changes in ovarian development and plasma levels of sex steroid hormones in the wild female Japanese sardine (*Sardinops melanostictus*) during spawning period. *J. Fish Biol*., **45**: 235-245.

MYLONAS, C.C., ZOHAR, Y., RICHARDSON, B. M. AND MINKINNEN, S. P., 1995. Induced spawning of wild American shad (*Alosa sapidissima*) usingsustained administration of gonadotropin-releasing hormone analog (GnRHa). *J. World aquac. Soc*., **26**: 240-251.

NELSON, G.J. AND SHORE, V.G., 1974. Characterization of tile serum high density lipo and apolipoproteins of pink salmon. *J. Biochem*., **249.** 536-542.

PANKHURST, N.W., 1998. Reproduction. In: *Biology of farmed fish* (eds. K. Black, and A.D. Iickering), Sheffield Academic Press. Sheffield, pp. 1-26.

PAPADOPOL, M., 1968. Contribution a letude de la biologie de lablette *Alburnus alburnus* L. dans le bassin inferieur

du Danube. *Acta Rev. Nat. Mus. Nat. Slovakia, Bratislava,* **14:** 113-134.

POORTENAAR, C. W. AND PANKHURST, N.W., 2000. Effect of luteinising hormone-releasing hormone analogue and human chronic gonadotropin on ovulation, plasma and ovarian levels of gonadal steroids in greenback flounder *Rhombosolea tapirina*. *J. World aquacult. Soc.,* **31**: 175-185.

RINCHARD, J. AND KESTEMONT, P., 1996. Comparative study of reproductive biology in single-and-multiple-spawner cyprinid fish. I. Morphological and histological features. *J. Fish Biol*., **49:** 883-894.

STOECKEL, J. N. AND NEVES, R.J., 1992. Comparison of methods for viewing the germinal vesicle in fish oocytes. *Progr. Fish-Cultur.*, **54:** 115-118.

TEMPLEMAN, W., 1944. The life history of the spiny dog fish Squalus acanthias and the vitamin A values of dog fish liver oil. *Res. Bull. Fish*., **15:** 74-102**.**

TYLER, C.R., SANTOS, E.M. AND PRAT, F., 2000. Unscrambling the egg-cellular, biochemical, molecular and endocrine advances in oogenesis. In: *Proceedings of the 6th International symposium on the reproductive physiology of fish* (eds. B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson and S.O. Stefansson). John Greig A/S. Bergen, pp. 273-280

VASTESAEGER, M.M. AND GILLOT, P.H., 1965. Histoire naturelle des maladies degeneratives des arteres dans le regne animal. In: *Actualites Cardiovascularies Medico-Chirurgicales* (eds. R. Froment, A. Goniss amd P. Michaud), Masson et Cie. Paris, pp. 26-51.

VOLLESTAD, L.A. AND L'ABEE-LUND, J.H., 1987. Reproductive biology of stream-spawning roach, *Rutilus rutilus*. *Environ. Biol. Fishes*, **18**: 219-227.

WALLACE, R.A., 1985. Vitellogenesis and oocyte growth in vertebrates. In: *Biology*, Vol. 1, (ed. I.W. Browder). Plenum Publishing, New York, pp. 127-177.

WALLACE, R.A. AND SELMAN, K., 1981. Cellular and dynamic aspects of oocyte growth in teleosts. *Am. Zool*., **21:** 325-343**.**

WALLACE, R.A., SELMAN, K., GREELEY, M. S. JR., BEGOVAC, P. C., LIN, Y. W. P. AND MCPHERSON, R., 1987. Current status of oocyte growth. In: *Proceedings of the 3rd International symposium on the reproductive physiology of fish* (eds. D.R. Idler, L.W. Crim and L.W. Walsh), Memorial University of Newfoundland, St. Johns, pp. 167-177.

WEDDLE, G. K. AND BURR, B.M., 1991. Fecundity and dynamics of multiple spawning in darters: an in-stream study of *Etheostoma rafinesquei*. *Copeia*, **1991**: 419-433.

WOOTTON, R.J., 1979. Energy costs of egg production and environmental determinants of fecundity in teleost fishes. *Symp. zool. Soc. London*, **44**: 133-159.

(Received 2 April 2010, revised 6 October 2010)