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Follicular development status and profile of 17β estradiol and cortisol levels during spawning cycle in *Oreochromis mossambicus* (Peters)

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ABSTRACT

In this study, the duration of different phases of the ovarian cycle, the pattern of follicular development and profile of serum levels of 17β estradiol (E_2) and cortisol were determined in female tilapia, *Oreochromis mossambicus*. Five oocyte developmental stages, namely chromatin-nucleolous, perinucleolar, cortical alveolar, vitellogenic and early maturation stages were identified. The mean number of stage I (previtellogenic) follicles showed continuous proliferation irrespective of phases of the ovarian cycle, while the stage II (previtellogenic) follicles increased significantly ($p<0.05$) until the end of vitellogenic phase (day 18) compared to previtellogenic phase (day 6), followed by a significant decrease at the end of prespawning phase (day 23), and a significant increase during the postspawning phase (day 26) ($p<0.05$). The mean number of stage III (previtellogenic) follicles decreased significantly on day 6 compared to day 1, but increased significantly later through the postspawning phase except for a short decline during prespawning phase. The recruitment of stage IV (vitellogenic) follicles occurred between days 13-18; however their number declined significantly ($p<0.05$) by the end of prespawning phase compared to vitellogenic phase. The progressive increase in the mean number of stage V (early mature) follicles were noticed only prior to spawning. Furthermore, despite high gonadosomatic and hepatosomatic indices, the serum E_2 levels were significantly lower at the end of prespawning phase compared to vitellogenic phase, whereas the serum cortisol levels rose at the end of vitellogenic phase and remained significantly high until postspawning phase compared to previtellogenic phase ($p<0.05$). These results are helpful to understand the roles of hormonal and environmental factors in regulation of ovarian activity during the spawning cycle in a continuously breeding fish such as *O. mossambicus*.

Keywords: Cortisol, Estradiol, Follicular development, Mouth brooding, Spawning cycle, Tilapia

Introduction

Recrudescence of gonadal activity and spawning are the two important events in the reproductive cycle of fish. While majority of teleosts exhibit some form of seasonal breeding, a few are continuous breeders (Tokarz, 1978). In seasonally breeding fish, the oogonial proliferation and follicular development coincide with the annual cycle (Wallace and Selman, 1981). In continuously breeding teleosts such as *Periophthalmus chrysospilos* and *Periophthalmus vulgaris* (Eggert, 1931), no cyclicity of oogenesis is observed. The information on the pattern of oogenesis in fish which exhibit continuous series of short cycles throughout the year is very much limited (Tokarz, 1978; Tyler and Sumpter, 1996), and the hormonal mechanisms controlling oogonial proliferation, recruitment of oocytes and spawning are not completely understood (Lubzens *et al*., 2010). Understanding the reproductive biology of a species is much important while selecting the species for fish culture (Van Oordt *et al.,* 1987). The tilapia *Oreochromis mossambicus* is a cichlid fish, which shows short ovarian cycles throughout the year. The mouthbrooding *O. mossambicus* exhibits ovarian cycle for 40-42 days, whereas in non-mouthbrooding conditions the ovarian cycle lasts 25 days (Smith and Haley, 1988). Although the duration of ovarian cycle is known, the timing of previtellogenic, vitellogenic and prespawning phases is not clearly understood in this fish. Unlike seasonally breeding fish, the exact phase of the ovarian cycle cannot be determined on the basis of external morphology or climatic conditions in *O. mossambicus*. In the present investigation, the pattern of follicular development in relation to serum 17β estradiol (E_2) and cortisol levels at different time points of the spawning cycle was studied to determine the duration of the different phases such as previtellogenic, vitellogenic, prespawning and postspawning phases in the stripped female *O. mossambicus*.

Materials and methods

Adult female *O. mossambicus* were collected from ponds in Dharwad District (longitude 75° 01' E, latitude 15° 27' 'N), Karnataka and were acclimatised in small freshwater tanks measuring 3×3×3 ft under natural photoperiod. From this stock, mouth brooding fish weighing 25-35 g were selected and the eggs were manually removed by inverting their heads in a bucket containing water. The day of egg removal was considered as the day 1 of the ovarian cycle. A group of five fish was sacrificed on the day 1. The remaining stripped females (n=25) were maintained in five separate 70 l outdoor glass aquaria (size, 36×12×18 inches; L×W×H) at a sex ratio of five females: two sexually mature males per aquarium. The fish were fed everyday with commercially available food pellets during the period of acclimatisation and experimentation. The fish were exposed to natural photoperiod (11.33 ± 0.03 h) every day. Each aquarium containing aquatic plants was fitted with aerators to ensure the supply of oxygen. The mean water temperature (30.88±0.62 ^{0}C) was calculated, considering the average of three readings recorded per day during the period of experimentation. Recirculation of water was done once in every three days, and prior to each recirculation, the pH and dissolved oxygen levels were recorded (average dissolved oxygen, 9.18 ± 0.03 mg l^{-1}; and pH, 9.36 ± 0.04). The female fish (n=5 each) were sacrificed on days 6, 12, 18, 23 and 26 during the phase of the spawning cycle.

Histology

At the time of autopsy, the weights of the body, ovary and the liver were recorded to calculate the gonadosomatic index (GSI = 100 × weight of the ovary/body weight) and hepatosomatic index (HSI = 100 × weight of the liver/ body weight). The ovaries were fixed in Bouin's fluid and processed for histological studies. Serial paraffin sections of 5 µm thickness were cut and stained with haematoxylin and eosin and the follicles belonging to different categories were counted. The criteria for classification of follicles are given in Table1. The stage I, II, III, IV and V follicles were counted in 9th, 25th, 60th, 120th, and 300th sections of the ovary respectively. The stage I follicles were counted under 10X objective, whereas the stage II - V follicles were counted under 4X objective lens.

Enzyme-linked Immuno assay (EIA) for serum estradiol (E_2) and cortisol

Prior to sacrificing the fish, blood was collected, serum separated and stored at -20 °C until the 17β-estradiol (E2) and cortisol levels were estimated,. The serum E_2 levels were measured by EIA using the kits as per the procedure supplied by Syntron Bioresearch Inc, USA, whereas the serum cortisol levels were estimated using the kits supplied by IBL International GMBH, Germany. The intra and inter assay variations were below 6% for both E_2 and cortisol.

Statistical analysis

The mean values of different parameters were compared by one way ANOVA and post hoc Tukey's multiple comparison procedure using SigmaStat 3.5 software. Significant differences for all comparisons were considered at $p < 0.05$ level.

Results and discussion

The overall duration of the ovarian cycle of stripped female *O. mossambicus* observed in the present study is in conformity with that reported by Smith and Haley (1988). However, the study of follicular developmental status at different time points revealed the duration of different phases such as previtellogenic, vitellogenic, prespawning and postspawning (mouth brooding) phases and the timing of recruitment of follicles for higher category during ovarian cycle in *O. mossambicus*. While the previtellogenic phase was extended for about 12 days after stripping, the vitellogenic and prespawning phases were observed between days 13-18 and 19-23 respectively. The postspawning phase was characterised by the mouthbrooding behaviour that lasted for about 5-7 days in *O. mossambicus*. Further, the mean GSI and HSI (Fig. 1) did not show significant variation during previtellogenic, vitellogenic and postspawning phases, whereas both increased significantly ($p<0.05$) during prespawning phase. Since determination of phase of ovarian cycle based on morphology or season is not possible in this continuously breeding fish, the

Table 1. Classification of the ovarian follicles in *O. mossambicus*

Stage of follicular development	Morphological appearance	Size (mm)	Nature of follicle
I	Chromatin nucleolus stage	0.01 - 0.04	Previtellogenic
II	Perinucleolar stage	0.05 - 0.14	Previtellogenic
III	Cortical-alveolar stage	0.15 - 0.34	Previtellogenic
IV	Vitellogenic stage	0.35 - 0.80	Vitellogenic
V	Early maturation stage	>0.80	Vitellogenic

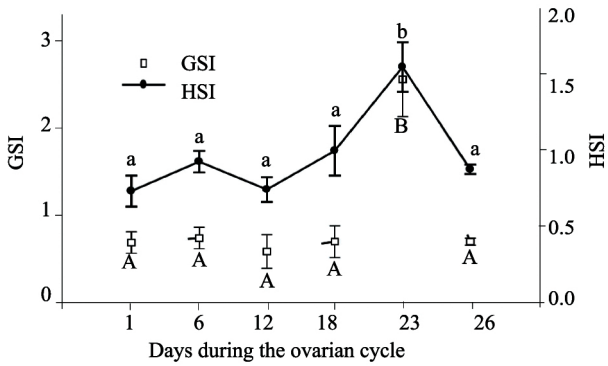

Fig. 1. Gonadosomatic index (GSI) and hepatosomatic index (HSI) during different phases of the ovarian cycle in *O. mossambicus*. (Groups with same superscripts (uppercase and lowercase letters for GSI and HSI respectively) are not significantly different, whereas groups with different superscripts are significantly ($p < 0.05$) different. Values are means ± S.E.)

data provided in the present study is useful in designing experiments on role of different factors in regulation of follicular development in fish.

The ovaries of *O. mossambicus* were paired elongated structures like that of majority of Indian freshwater fishes. The right and left ovaries were connected to each other at their posterior ends. The ovarian follicles were divided into five types on the basis of their morphology. The stage I follicles with chromatin nucleolus (Fig. 2A and H), stage II follicles with perinucleoli (Fig. 2A and E), stage III follicles with cortical alveoli (Fig. 2A and F) and stage IV follicles with yolk granules (Fig. 2A and G) were found in all the phases of the ovarian cycle, whereas the stage V follicles with larger yolk granules (Fig. 2D and H) were found only in prespawning phase. The ovary was asynchronous type and showed numerous ovigerous cords that contained oogonia (Fig. 2E). The advanced follicles were found away from the ovigerous cords, whereas the small, previtellogenic follicles were seen near the base.

The ovarian cycle in fish includes a period of primary oocyte growth, followed by vitellogenesis and then final maturation and ovulation of oocytes (Guraya, 1986). Although Smith and Haley (1987) reported an increase in the young oocytes during the first seven days after spawning in *O. mossambicus*, pattern of different follicular developmental stages was not clear in that study. The results of the present study showed that the progressive increase in the mean number of stage I follicles occur from day 1-18 and a significant decrease ($p<0.05$) at the end of prespawning and spawning phases (Fig. 3A) in stripped female *O. mossambicus*. This pattern is similar to that reported in the seasonally breeding fish,

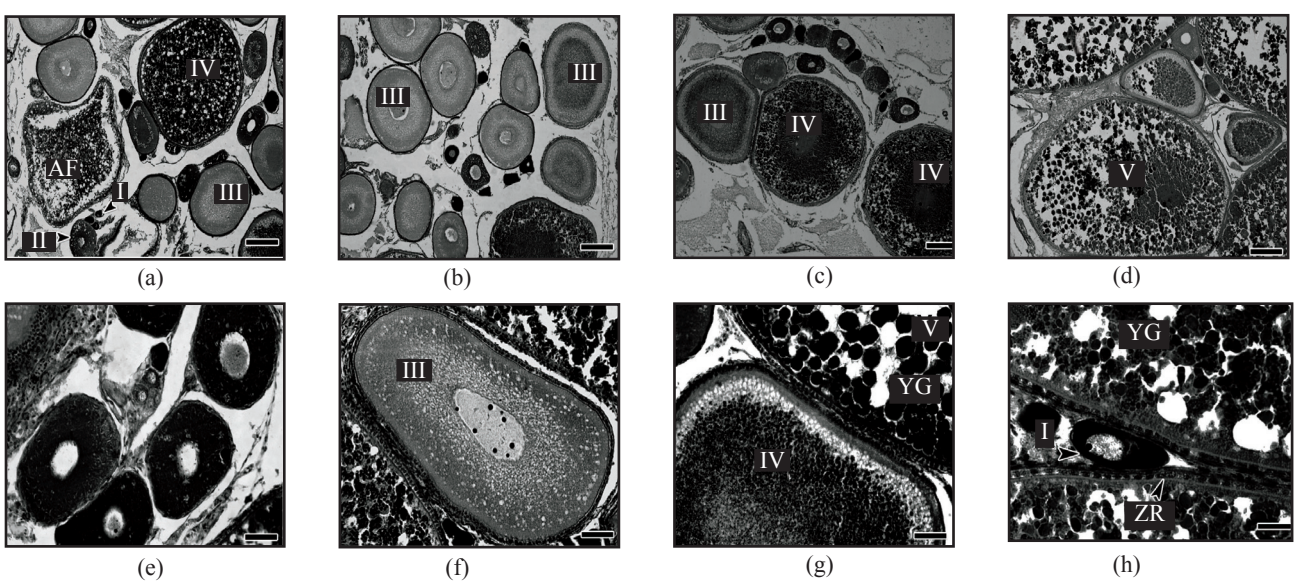

Fig. 2. A-D: Cross sections of the ovary of *O. mossambicus* showing follicles belonging to different categories (Stage I, II, III, IV and V) during different phases of the spawning cycle. (Note the presence of previtellogenic follicles and few atretic follicles on day 1 (A), mostly previtellogenic follicles on day 12 (B), vitellogenic follicles on day 18 (C) and early maturation follicles (stage V) on day 23 (D). Bar, A-D = 100 µm; Haematoxylin and Eosin). E-H: Cross sections of the ovary showing oogonia (OG) in the ovigerous cord, and stage II follicles with perinucleoli (PN, E), stage III follicle with cortical alveoli (CA, F), stage IV, V and I follicles (G and H). (Note the large yolk granules YG and yolk vacuoles (YV) in early mature follicles of stage V and chromatin - nucleolus in stage I follicle (H). Bar, E-G = 25 µm, H =10 µm; Haematoxylin and Eosin). GR- granules, N- nucleus, NU- nucleolus, T - thecal layer, ZR - zona radiata

Carassius auratus, *Catastomus commersoni*, *Mystus tenagra* and *Pleuronectes platessa* (Tokarz, 1978), which show peak in oogonial proliferation during postspawning phase. It differs from the fish, *Oryzias latipes* (Yamamoto, 1962) and *Rhodeus ocellatus* (Yamamoto and Shirai, 1962), which show greater oogonial proliferation during the spawning phase. It also differs from the other teleosts, *P. chrysospilos* and *P. vulgaris* (Eggert, 1931), which sporadically spawn throughout the year, but show no cyclic pattern in oogenesis.

Vitellogenic follicular growth in fish is characterised by the accumulation of vitellogenin in the oocytes resulting in the appearance of large yolky granules as in other nonmammalian vertebrates. In the present study, the previtellogenic follicles like stage II and III follicles showed a significant ($p<0.05$) decrease in their number at the beginning of previtellogenic phase compared to day 1, followed by a gradual increase until day 18, and a decrease on day 23 and again increased during postspawning phase (Fig. 3B and C). Further, although the vitellogenic follicular growth occurred between day 12-18 (Fig. 3D), it reached maximum only on day 23 as shown by a significant ($p<0.05$) increase in the number of stage IV follicles on day 18 (Fig. 3D) followed by the appearance of stage V follicles on day 23 (Fig. 3E). This finding clearly indicates that the conversion of stage IV to stage V follicles occurs during prespawning phase.

Follicular atresia in the fish ovary occurs commonly during prespawning, spawning and postspawning periods (Saidapur, 1978). Although atresia of previtellogenic follicles is relatively of rare occurrence in majority of the teleosts, most of the previtellogenic follicles in different stages were affected by atresia in the garfish *Xenentodon cancila* (Guraya, 1986). In the present study, the absence of atresia in stage I follicles throughout the ovarian cycle suggests that these are completely converted to the advanced stages. A significantly lower mean number of stage II, III and IV follicles is correlated with the high atresia on day 6 (Fig. 4). Therefore it is very likely that the unspent follicles belonging to these categories through postspawning to previtellogenic phase of the previous cycle are not recruited to the vitellogenic phase of the next cycle. The progressive increase in the number of stage I, II and III follicles during previtellogenic phase suggests that new stream of stage III follicles contribute to the increased number of stage IV follicles during vitellogenic phase in *O. mossambicus*. This supports the concept that oocytes are renewed in the ovary after expulsion of the ripe eggs in most of the fish (Tokarz, 1978). However, in some salmonoids such as the brook trout, *Salvelinus fontinalis* (Tam *et al.,* 1986), the young oocytes recruited in the previous year did continue on with their development in the following year.

Implication of estrogen in the stimulation of vitellogenin secretion is known in fish (Kwon *et al.,* 1993). Elevated levels of plasma E_2 is correlated with vitellogenic phase followed by decrease during final stages of maturation of the follicles in the catfish *Heteropneustes fossilis* (Lamba *et al.,* 1983), the pacu *Piaractus mesopotamicus* (Gazola *et al.,* 1996), the spiny damsel fish *Acanthochromis polyacanthus* (Pankhurst

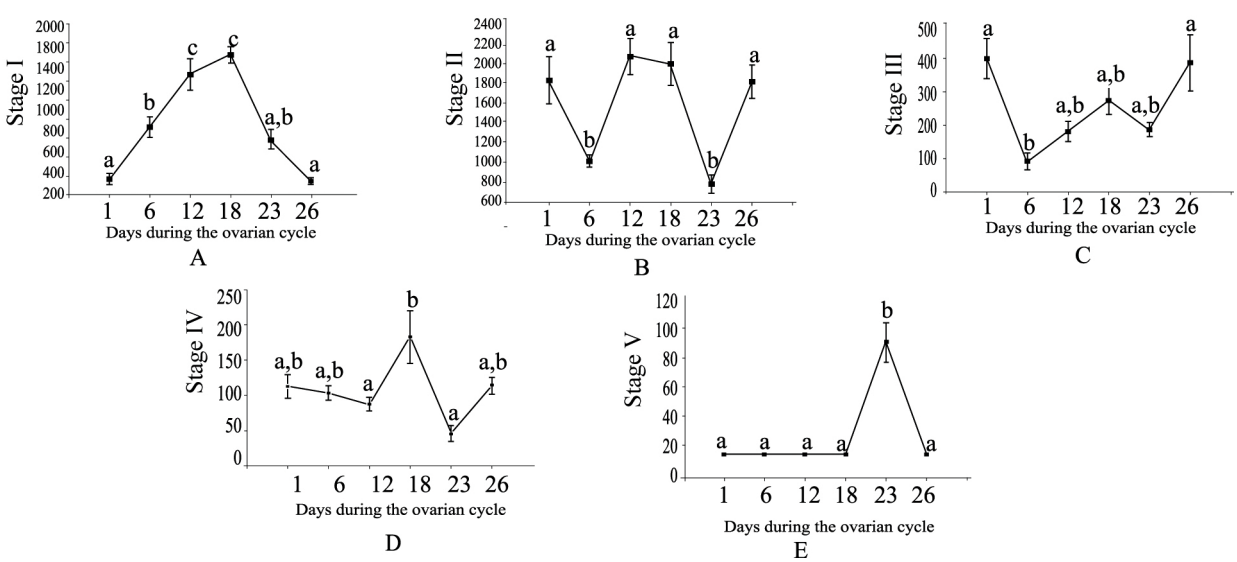

Fig. 3. Pattern of follicular development during the spawning cycle in *O. mossambicus*. A - Stage I, B - Stage II, C - Stage - III, D - Stage IV, E - Stage V. Note the presence of stage V follicles only during prespawning phase. Groups with same superscripts are not significantly different, whereas groups with different superscripts are significantly ($p < 0.05$) different. Values are means±S.E

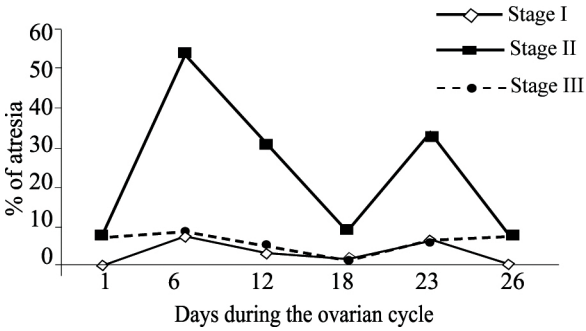

Fig. 4. Percentage of follicular atresia during different phases of the ovarian cycle in *O. mossambicus*. Note the high percentage of atresia particularly in stage III follicles on day 6 followed by day 23

et al., 1999), the swordfish *Xiphias gladius* (Corriero *et al.,* 2004) and the shark *Cephaloscyllium laticeps* (Awruch *et al.,* 2008). In the present study, the serum E_2 levels were significantly higher during vitellogenic phase compared to previtellogenic phase and dropped to low levels prior to spawning (Fig. 5A). It is believed that decrease in E_2 levels prior to spawning stimulate GTH surge (Donaldson and Hunter, 1983) and LH acts as switch between production of E_2 and maturation inducing steroid in fish (Lubzens *et al.,* 2010), which is required for oocytes maturation and ovulation. Hence, it appears that drop in serum levels of E_2 is a prerequisite for spawning in *O. mossambicus* as observed in seasonal breeders (Lamba *et al.,* 1983; Gazola *et al.,* 1996; Pankhurst *et al.,* 1999; Corriero *et al.,* 2004; Awruch *et al.,* 2008).

Hypothalamo-hypophysial-gonadal (HPG) axis is influenced by hyopothalamo-pituitary-interrenal (HPI) axis hormones in fish (Flik *et al.,* 2006; Schreck, 2010). The principal stress hormone, cortisol is known to modulate reproduction in fish (Mommsen *et al.,* 1999). High levels of plasma cortisol were observed during spawning period in the killifish, *Fundulus heteroclitus* (Bradford and Taylor, 1987), the pacu *P. mesopotamicus* (Gazola *et al.,* 1996), the mullet *Liza ramada* (Mousa and Mousa, 2005) and the Pacific salmon *Oncorhynchus mason* (Westring *et al.,* 2008). In white sucker *Catastomus commersoni*, although the plasma levels of cortisol were high prior to spawning, a significant ($p < 0.05$) decrease was observed at the time of ovulation (Scott *et al.*, 1984). These studies suggest a role for cortisol during the process of vitellogenesis and follicular maturation. In contrast, plasma cortisol levels remained low during vitellogenic and oocyte maturation stage, but increased 3-fold during postovulatory period in female rainbow trout *Salmo gairdneri* (Bry, 1985). In the present study, significant ($p < 0.05$) elevation in the serum cortisol levels is observed during vitellogenic, prespawning and postspawning phases compared to the previtellogenic phase (day 12, Fig. 5B) suggesting a role for cortisol during vitellogenic follicular growth and maturation phases in *O. mossambicus* as in majority of the seasonal breeders.

The results of the present study are useful in determining influence of different factors on ovarian activity, which has implication in fish culture. The present

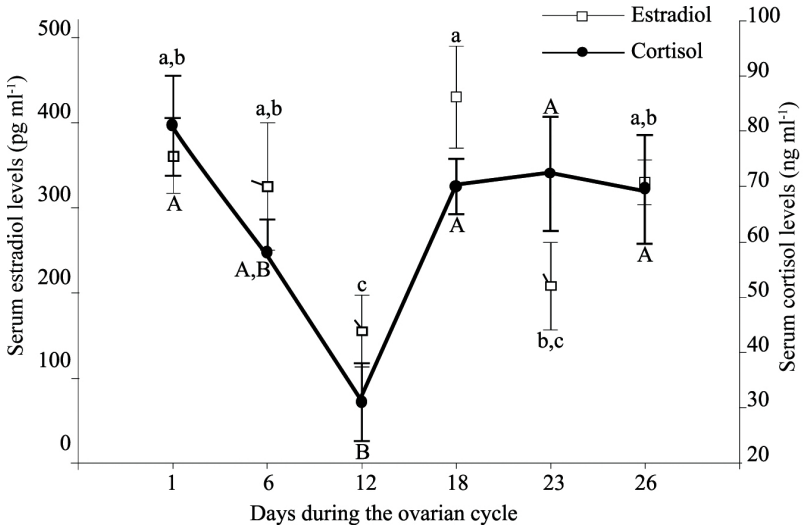

Fig. 5. Profile of serum E_2 and cortisol levels during the ovarian cycle in *O. mossambicus*. Note the increase in the serum E_2 levels during vitellogenic phase and subsequent decrease prior to spawning, whereas the serum cortisol levels are significantly lower only on day 12 compared to other phases. Groups with same superscripts (lowercase and uppercase letters for serum estradiol and cortisol levels respectively) are not significantly different, Groups with different superscripts are significantly ($p < 0.05$) different. Values are mean ± S.E.

study gains importance as majority of the fish fail to spawn in captivity, and the reproductive success depends on many factors including environment.

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References

Awruch, C. A., Pankhurst, N. W., Frusher, S. D. and Stevens, J. D. 2008. Endocrine and morphological correlates of reproduction in the draughtboard shark *Cephaloscyllium laticeps* (Elasmobranchii: Scyliorchinide). *J. Exp. Zool. Ecol. Genet. Physiol.,* 309A: 184-197.

Bry, C. 1985. Plasma cortisol levels of female rainbow trout (*Salmo gairdneri*) at the end of the reproductive cycle: relationship with oocyte stages. *Gen. Comp. Endocrinol*., 57: 47-52.

Bradford, C. S. and Taylor, M. H. 1987. Semilunar changes in estradiol and cortisol coincident with gonadal maturation and spawning in the killifish *Fundulus heteroclitus*. *Gen. Comp. Endocrinol.,* 66: 71-78.

Corriero, A., Acone, F., Desantis, S., Zubani, D., Deflorio, M., Ventriglia, G., Bridges, C. R., Labate, M., Palmieri, G., McAllister, B. G., Kime, D. E. and De Metrio, G. 2004. Histological and immuno-histochemical investigation on ovarian development and plasma estradiol levels in the swordfish (*Xiphias gladius* L.). *Eur. J. Histochem*., 48: 413-22.

Eggert, B. 1931. Die geschlechts organe der Gobiiformes and Blenniformes. *Z. Wiss. Zool.,* 189: 249-558.

Donaldson, E. M. and Hunter, G. A. 1983. Induced final maturation, ovulation and spermiation in cultured fish. In: Hoar, W. S, Randall D. J. and Donaldson, E. M (Eds.), *Fish physiology*, Vol. IX B, Academic press, New York, p. 351-403.

Flik, G., Klaren, P. H. M., Van den Burg, E. H., Metz, J. R. and Huising, M. O. 2006. CRF and stress in fish. *Gen. Comp. Endocrinol.,* 146: 36–44.

Gazola, R., Borella, M., Donaldson, E. M., Val-Sella, M.V., Sukumasavin, N., Fava-de-Moraes, F. and Bernardino, G. 1996. Plasma steroid and corticosteroid levels in wn the rainbow trout, *Oncorhynchus mykiss*. *Comp. Biochem. Physiol.,* 104B: 381–396.

Lamba, V. J., Goswami, S. V. and Sundararaj, B. I. 1983. Circannual and circadian variations in plasma levels of steroids (cortisol, estradiol-17 beta, estrone, and testosterone) correlated with the annual gonadal cycle in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.,* 50: 205-225.

Lubzens, E., Young, G., Bobe, J. and Cerda, J. 2010. Oogenesis in teleosts: how fish eggs are formed. *Gen. Comp. Endocrinol*., 165: 367-389.

Mommsen, T. P., Vijayan, M. M. and Moon, T. W. 1999. Cortisol in teleosts: dynamics, mechanisms of action and metabolic regulation. *Rev. Fish. Biol. Fish.*, 9: 211-268.

Mousa, S. A. and Mousa, M. A. 2005. Involvement of corticotropin-releasing factor and adrenocorticotropic hormone in the ovarian maturation, seawater acclimation, and induced spawning of *Liza ramada*. *Gen. Comp. Endocrinol.,* 146: 167-179.

Pankhurst, N. W., Hilder, P. I. and Pankhurst, P. M. 1999. Reproductive condition and behaviour in relation to plasma levels of gonadal steroids in the spiny damselfish *Acanthochromis polyacanthus*. *Gen. Comp. Endocrinol.,* 115: 53-69.

Saidapur, S. K. 1978. Follicular atresia in the ovaries of nonmammalian vertebrates. *Int. Rev. Cytol.,* 54: 225-244.

Schreck, C. B. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. *Gen. Comp. Endocrinol.,* 165: 549-556.

Scott, A. P., MacKenzie, D. S. and Stacey, N. E. 1984. Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni* steroid hormones. *Gen. Comp. Endocrinol.,* 56: 349-359.

Smith, C. J. and Haley, S. R. 1987. Evidence of steroidogenesis in postovulatory follicles of the tilapia, *Oreochromis mossambicus*. *Cell. Tissue. Res*., 247: 675-687.

Smith C. J. and Haley, S. R. 1988. Steroid profiles of the female Tilapia, *Oreochromis mossambicus*, and correlation with oocyte growth and mouth brooding behaviour. *Gen. Comp. Endocrinol.,* 69: 88-98.

Tam, W. H., Roy, R. J. J. and Makaran, R. 1986. Ovarian cycle and plasma concentrations of estrogen and

vitellogenin in brook trout (*Salvelinus fontinalis*, Mitchill) . *Can. J. Zool.,* 64: 744-751.

Tokarz, R. R. 1978. Oogonial proliferation, oogenesis and folliculogenesis in non-mammalian vertebrates. In: Jones, R. E. (Ed.), *The vertebrate ovary*. Plenum Press, New York, p. 145–179.

Tyler, C. R. and Sumpter, J. P. 1996. Oocyte growth and development in teleosts. *Rev. Fish. Biol. Fish.,* 6: 287-318.

Van Oordt, P. G. W. J., Peute, J., Van Den Hurk, R. and Viveen, W. J. A. R. 1987. Annual correlative changes in gonads and pituitary gonadotropes of feral African catfish, *Clarius gariepinus. Aquaculture*, 63: 27-41.

Wallace, R. A. and Selman, K. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. *Am. Zool.,* 21: 325–343.

Westring, C. G., Ando, H., Kitahashi, T., Bhandari, R. K., Ueda, H., Urano, A., Dores, R. M., Sher, A. A. and Danielson, P. B. 2008. Seasonal changes in CRF-I and urotensin I transcript levels in masu salmon: correlation with cortisol secretion during spawning. *Gen. Comp. Endocrinol.,* 155: 126-140.

Yamamoto, K. and Shirai, K. 1962. Origin of the yearly crop of eggs in the bitterling, *Rhodeus ocellatus*. *Annot. Zool. Jpn.,* 35: 218-222.

Yamamoto, K. 1962. Origin of the yearly crop of eggs in the medaka, *Oryzias latipes*. *Annot. Zool. Jpn.,* 35: 156-161.

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