Summary

Banded gourami, *Trichogaster fasciata* (Bloch and Schneider, 1801), an ecologically important fish of West Bengal reside nearby water bodies of human inhabitant which is connected to agricultural runoff or from industrial and household/urban sewage that are always contaminated with mercury. It is also important for maintaining rural health to control mosquito transmitted diseases like Malaria, Dengu, Chikungunya etc. due to feeding on mosquito larvae. No statistical data is available in relation to the population dynamics, stock of status of this species; but unavailability in the market indicates that the population of this species is declining rapidly. Therefore, there is a growing demand to conserve and proper management of this fish in Indian subcontinent. Conservation and management measures require information on fundamental biology of banded gourami; particularly sound information is required on reproductive biology of this species. The present study is designed to provide some new information about the hormonal regulation on ovarian steroidogenesis during vitellogenesis to final oocyte maturation and the hormonal regulation of oocyte maturation in this fish.

Any pollutant exerts its action on organisms or populations by affecting their normal endocrine function as well as reproduction. Mercury is one of the key pollutants responsible for the degradation of natural aquatic ecosystems. Among the different forms of mercury that exist in the environment, mercuric chloride ($HgCl_2$) is one of the dominant pollutant for freshwater environments as it is used as an ingredient in antiseptics, disinfectants and preservatives, insecticides, batteries and in metallurgical and photographic operations. Pollutant may exert their action on organisms or populations by affecting their normal endocrine function as well as reproduction. Thus, the present study tried to understand the effect of $HgCl_2$ on reproductive function and to decipher the molecular mechanism of Hg-induced reproductive impairments of female *Trichogaster fasciata*.

Present study described the ovarian development of *T. fasciata* histologically throughout the year in relation to the seasonal variations of gonadosomatic index (GSI); follicle stimulating hormone (FSH) and luteinizing hormone (LH); three key steroids for folluculogenesis and maturation i.e. testosterone (T), 17β-estradiol (E_2) and 17α20βdihydroxy4pregnen3one (17,20β-P). In addition to that, we reports about the expression pattern of *cyp19a1a*, localization of aromatase and SF-1in different developmental stages of ovary to find out their participation in the folliculogenesis process of this fish species. Furthermore, this study highlighted about the

potential role of mercury as EDC by assessing mercury-induced reproductive toxicity in *T. fasciata*. For this, both *in vivo* and *in vitro* experiments were conducted to elucidate the effect of mercury on follicular development, *in vivo* steroidogenesis and *in vitro* production of E_2 and testosterone (T) by gonadotropin-induced ovarian follicles of *T. fasciata*. Lastly, present study performed *in vitro* experiments to elucidate the molecular mechanism of mercury-induced reproductive impairments by analyzing aromatase gene expression and steroidogenic factor 1 (SF-1) level.

The present study records relatively higher level of FSH till the ovary reaches in late vitellogenic stage confirms that FSH regulates the early folliculogenesis of the ovary, whereas LH peak was observed in the postvitellogenic stage, which indicates that maturation and ovulation were controlled by LH. This study characterizes seven different maturity stages of ovary which is correlated with the monthly variation of GSI, gonadotropin, steroids and is one of the indicators of the spawning periodicity of the teleost. The GSI value of increased from the month of April and reaches in a single peak in the month of August. This value decreased abruptly in the month of October, which indicates the onset of spawning in that month. Co-appearance of post-GVBD stage oocytes and single GSI peak in the month of September indicates the single spawning season of *T. fasciata*.

This study document also the seasonal cycles of steroid levels T, E_2, 17,20β-P in plasma and gonads as they relate to gonadal development and reproductive behavior in natural population of *T. fasciata*. Results clearly show that in the month of January and February, when the ovary of female *T. fasciata* was very small and contained mostly immature stage follicles; circulatory levels of E_2 and T were although detectable, but very low and 17,20β-P was non-detectable. From March, concentrations of E_2 and T in plasma began to reach their highest value in May-June, coinciding with the prepondance of vitellogenic follicles in the ovary. After July, plasma E_2 and T levels decrease slightly than the vitellogenic stage. Ovary in this period contained mostly postvitellogenic follicles, with centrally located GV. This is the period of final oocyte maturations and 17,20β-P reaches its maximum level. In the month of September onwards, E_2 and T levels decreased and 17,20β-P was not detectable. The pattern of circulatory gonadotropins and other steroids like E_2, T and 17,20β-P levels in *T. fasciata* indicates that, a shift in the production of estrogenic to progestational steroid may occurs and 17,20β-P may be the potent maturation steroid of this fish.

We identified tentatively five steroid hormones in the plasma and ovary at different stages of reproductive cycle in *T. fasciata* by HPLC. During immature stage we identified T and E_2 in plasma and T, E_2 and 17α-OH-P in ovary. During vitellogenic stage we obtained T, E_2, 17α-OH-P and P levels in the plasma and or ovary. 17,20β-P was detected only during postvitellogenic stage both in ovary and plasma along with other steroids. The HPLC analysis of three steroids, namely E_2, T, and 17,20β-P correlates very much with the assay of these steroids by RIA.

Present study shows that aromatase becomes highly active in the vitellogenic stage; in postvitellogenic and post-GVBD stages this activity becomes quiescent, and in spawning stage it was inactivated again just like the preparatory stage. Localization of Steroidogenic factor 1 (SF-1) does not correlate with the aromatase expression and localization. SF-1 is found in the vitellogenic, postvitellogenic and also in post-GVBD stages. This result supports the idea that in vitellogenic stage, SF-1 regulates the *cyp19a1a* expression and E_2 synthesis which in turn regulated by the FSH; whereas SF-1 activity may be regulated by both FSH and LH in *T. fasciata.*

Present study reports about the *in vitro* steroidogenic effects of heterologous gonadotropins; FSH, LH and HCG on the ovarian follicles of *T. fasciata* at different developmental stages. Less amount of T and E_2 production by the maturing stage follicles and comparatively greater stimulatory action of FSH indicates that during early stage of folliculogenesis in *T. fasciata*, FSH have a regulatory role on steroidogenesis. The equipotent activity of FSH, LH and HCG at vitellogenic stage follicle in the production of T and E_2 indicate that during this stage of development both FSH and LH and equally active. Our results clearly indicate that steroidogenesis in postvitellogenic follicles is regulated by LH and HCG, not by FSH. In post-GVBD stage, the responsiveness of oocytes to any gonadotropin (FSH, LH and HCG) is lower down. Our result also indicates a shift in steroidogenesis from estrogenic to progestetional steroid from vitellogenic to postvitellogenic stage and high production of 17,20β-P during final oocyte maturation indicates its involvement in oocyte maturation. We observe that T, E_2 and 17,20β-P production in response to HCG was inhibited by 1-octanol and 1-heptanol indicating that homologous and heterologous gap junction among follicular cells (granulosa and theca cells) is associated with the action of HCG to produce 17,20β-P. Cycloheximide and actinomycin-D were shown to block HCG-induced 17,20β-P production in *T. fasciata* oocytes. It

means production is 20β-HSD enzyme production is necessary for the production of 17,20β-P. The present work provides evidence that enzymatic step catalyzed by cholesterol side-chain cleavage enzyme is essential for HCG-stimulated T, E_2 and 17,20β-P by the ovarian tissue of *T. fasciata*. By employing trilostane in the incubation of ovarian follicles, it is clear that HCG stimulated the synthesis of 17,20β-P is catalyzed by 3β-HSD which is essential for the biochemical and physiological responses of *T. fasciata* postvitellogenic follicle.

In this study, $HgCl_2$ was used as a source of Hg to determine the effect of Hg contamination on fish reproduction by assessing its impacts on follicular development, steroid biosynthesis and aromatase activity in the banded gourami, *T. fasciata*. The results indicated that environmentally relevant concentrations of $HgCl_2$ (50 μg/L) act as endocrine disrupting chemicals (EDC) by inhibiting folliculogenesis, oocyte maturation and normal steroidogenic production. In the present study, we observed that both gonadal development and GSI values were inhibited by $HgCl_2$ exposure and that this inhibitory effect is a temporary phenomenon because if $HgCl_2$-treated fish are cultured in normal water, they recover their normal reproductive potentiality immediately. Induction of GVBD by HCG in postvitellogenic follicles of *T. fasciata* was inhibited by actinomycin D suggest that oocyte maturation induced by HCG required synthesis of mRNA. Inhibition of GVBD induced by HCG in the presence of cycloheximide suggests the requirement of translation of mRNA to protein for mediating the actions of HCG. This translation blockage indicates the inhibition of induction of MPF or protein required for the activation of MPF. Results of the present study using n-alkanol gap junction uncouplers indicate the requirement of functional gap junctions for gonadotropin-induced GVBD in such fish. Employing a specific inhibitor of 3β-HSD, in the incubation mixture it is evident that HCG-induced GVBD was blocked by this inhibitor. This observation clearly indicates that HCG induced 17,20β-P production through Δ5-Δ4 pathway.

In the present work, the results of $HgCl_2$ exposure on aromatase activity and gene expression clearly indicate that $HgCl_2$ acts as an EDC. The present study reports on the role of $HgCl_2$ as an EDC by blocking gonadal development, P450 aromatase gene expression, steroidogenesis and SF-1 level. Interestingly, these activities of $HgCl_2$ are reversible since removal of mercury pollution from the aquatic body is able to regenerate the reproductive potentiality of teleost fish. Thus, release of mercurial compounds in aquatic body should be controlled to conserved fish diversity as well as a healthy ecosystem. Evidence obtained in *T.*

fasciata oocyte in our study suggests that $HgCl_2$ played an inhibitory role on PI3 kinase activity which is essential for HCG-induced oocyte maturation. The present study also indicates that MAP kinase activation is unaffected by the $HgCl_2$ for the activation of cdc2 and induction of GVBD in HCG-induced oocyte maturation.