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EFFECT OF SALINITY STRESS ON LONG WHISKERS CATFISH *MYSTUS GULIO* (SILURIFORMES: BAGRIDAE): FROM PERSPECTIVES OF GONADAL HISTOLOGY AND SEX STEROID HORMONES

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ABSTRACT *Mystus gulio* (Hamilton, 1822), a euryhaline fish belonging to the family Bagridae was subjected to increasing salt concentrations of upto 22.27 PSU in experimental tanks within a study period of 21 days with an aim to investigate into the effect of salinity stress on the histology and development of the gonads and synthesis of sex steroid hormones estradiol and testosterone. The GSI (%) was found to decrease in both the male and female fishes with exposure to increasing salt concentrations. The histological sections of the ovary after exposure to salinity stress showed a decline in the number and size of the developing oocytes with structural deterioration of follicles and tissue alterations like the expansion, rupturing and detachment of follicular basement membrane, cytoplasmic vacuolization, hypertrophied follicular epithelium and more number of atretic oocytes. In case of testes there was thinning of the basement membrane resulting in fusion of some tubules, hypertrophy of spermatocytes, degenerative appearance with different degrees of vacuolation, decrease in size of testicular lobules, hemorrhage in the intertubular space, fibrosis and vacuole formations. Low salinity stress upregulated estradiol synthesis but level of testosterone showed a decrease with increasing salt concentrations.

KEYWORDS : Salinity Stress, Histological Alterations, Estradiol, Testosterone

INTRODUCTION

Fishes being aquatic depend largely on the water quality for their physiological performance (Asaduzzaman et al., 2022). They are dependent not only upon internal factors (neuroendocrinological), but also upon certain external (ecological) factors for the control and synchronization of their different activities and functions (Bœuf and Payan 2001). Temperature, salinity, and photoperiod are some of the most important ecological factors affecting aquatic life and salinity being a critical factor affecting the survival rate, distribution, growth, metabolism and reproduction (Smyth and Elliott 2016). The alarming rate of global warming is leading to the increase of saltwater intrusion into freshwater and many freshwater fish are being affected (Kang'ombe and Brown, 2008). This salinity intrusion is now a major concern particularly in the estuarine systems, as further rise in sea level is being projected in the subsequent years (Willis et. al., 2010; Siegert and Pearson, 2021). It is evident that any changes in the optimal dilutions of salinity can impose imbalance on the homeostasis of the fish and the manifestations may be reflected by certain specific physiological responses (Enayati et al., 2013).

The timing and success of reproduction in teleost fishes is directly or indirectly controlled by the environmental factors (Moharram, 2000). Variations in temperature, salinity, stress and photoperiod have been reported to have clear influences on the reproductive activities of fishes (Ashan, 1966; Breton and Billard, 1977; Bye, 1984; de Vlaming, 1972; Lam, 1983; Stacey, 1984). These environmental factors influence the gonadal activity which is mediated through the hypothalamo-pituitary gonadal axis resulting in endocrine fluctuation (Crim, 1982). The optimal range of salinity for growth, survival, and production efficiency is species-specific (Ruscoe et al., 2004). Studies have shown that some species of fish can tolerate higher salinities and some can even survive extended exposure to high levels of salinity of up to 120% (Nordlie et al. 1992; Nordlie and Haney, 1998). In case of euryhaline species, salinity affects growth as the energy is utilized for osmoregulation and is not available for growth (Brett, 1979).

Mystus gulio (Hamilton, 1822) is a small to medium sized species of catfish belonging to the family Bagridae. This teleost is a euryhaline fish, occurring mostly in freshwater. But it has also been found to thrive in backwaters of low salinity (Gupta, 2014; Pandian, 1966). Talwar and Jhingran (1991) have reported that this fish is primarily a brackish water fish that enters and lives in fresh water. It is found in schools of 10-25 individuals in rivers and large streams which has mud or clay substrates. This species is common in India (Jhingran, 1997; Kumar et al., 2018) and has also been reported in the Gangetic estuary, Chilka lake and Kerala brackish waters (Talwar and Jhingran, 1991). It prefers a pH of 6.0 - 7.8 and a temperature range of 20-27°C (68-80.6°F) [Hamilton, 1822] and is a diurnal, oviparous and facultative air-breathing fish (Looby et al., 2023). The juveniles and the adult of this fish feeds on the debris, zooplankton, zoobenthos, other benthic invertebrates, fish eggs and larvae (Siddique, 2007). As a small indigenous fish species (SIS), containing high amount of nutrients, it is commercially a very important food fish as it fetches high market price and is in increasing demand which makes it a desirable candidate species for aquaculture in Southeast Asia (Ross et al., 2003). Being euryhaline, it is well suited for both fresh water and brackish water aquaculture (Siddiky et al., 2015). It is mostly cultured with other euryhaline species, such as *Liza parsia*, *Oreochromis niloticus*, *Penaeus*

monodon and *Rhinomugil corsula* (Abraham, 2014; Hossain et al., 2015). There are also reports of its role in controlling water pollution by consuming aquatic detritus (Siddique, 2007). Although this species has been listed as Least Concern while being assessed for The IUCN Red List of Threatened Species in 2019, the natural population of *Mystus gulio* is known to be fast decreasing in recent years, due to over-exploitation, various ecological changes in the natural habitats and habitat destruction (Hossain et al., 2015; Ng et al., 2019). According to a report by Patra et al. (2005), even in the Ganges–Brahmaputra estuary of the Indian Sunderbans, one of the world's largest mangrove ecosystem, the catch of this species has reduced by 33.6% between 1960 and 2000.

Negative impact on the distribution and reproductive process of fish is expected when there is escalated variation in the amplitude of salinity and temperature gradients. This is a challenge to the habitat-specific physiological adaptation of fish (Paul et al., 2019). With regard to *Mystus gulio*, previous studies have merely focused on the biology and various physiological studies. There have been studies on oxygen consumption of *Mystus gulio* under combined stress of varying salinity and temperature (Paul et al., 2019), habitat salinity and source-induced variation in body shape in *Mystus gulio* (Iqbal et al., 2024), seasonal changes in selected immune response of *Mystus gulio* and *Mystus vittatus* (Sakthivel et al., 2013), survival and growth performance of *Mystus gulio* and other fishes in freshwater, and subsequent low salinities (Dubey et. al., 2022), toxic and sublethal effects of pesticides and ionic metabolism regulation on different freshwater catfish species *Mystus vittatus* and *Mystus keletius* (Arunachalam et al., 1980; Ramya et al., 2023). However, studies on the effect of different levels of water salinity on the reproductive physiology of this commercially important species *Mystus gulio* has not yet been documented. There has been a related study on a different species *Mystus montanus* (Arockiaraj, 2001). This paper reports the effect of salinity stress on the histology of the gonads and sex steroid hormones (estradiol and testosterone) in *Mystus gulio* under experimental conditions.

MATERIALS & METHODS

Experimental set up

Fish (*Mystus gulio*) were transported from the local fish farm. Almost equal sized fishes measuring approximately 9-10 cm were collected. Fish were set randomly in 3 glass aquaria with 36 litres dechlorinated tap-water in each tank and acclimatized to captive conditions for one week by maintaining them in normal conditions (pH 7.7, temperature 27.24°C, dissolved oxygen 4.72 mg/L and a salinity of 1.13 PSU approximately). One tank (Tank 1) was set as control. The other two tanks (Tank 2&3) were set as experimental tanks. At the outset of the trial, there were 10 fishes in each tank. The fishes were fed with formulated diet at 4% of fish total body weight. Each aquarium was facilitated with an aerator for proper oxygen supply and a filtration facility to remove the fecal matter and other debris and keep the water clean. A variable amount of NaCl was added to the water of the experimental tanks per day to increase the salinity of the tanks by 1 PSU per day. The salinity of tank 2 was increased to approximately 15.29 PSU within a span of 14 days and the salinity in tank 3 was increased upto 22.27 PSU within 21 days. The control aquarium was maintained at the salt concentration of normal tap water without adding any salt.

Sampling of fish and water quality parameters

Sampling was performed on the 8th day (salt concentration 9.13 PSU), 14th day (15.29 PSU) and 21st day (22.27 PSU) from the 3 tanks and the fish were sacrificed. Weight of each fish was assessed with a digital electric balance and length measured with a steel scale. The value of temperature, pH, dissolved oxygen and salinity were measured in each tank on a regular basis using a Multi-parameter Probe (Make: Hanna Instruments, Model no.: H198194).

Dissection of gonads and GSI estimation

Gonads were dissected out from fish exposed to salt as well as from the control tank on the same days. The sex of each specimen was identified by visual examination and confirmed after dissection and examination of the gonads following standard protocol. The gonads were weighed using a digital electronic weight balance and the gonadosomatic index (GSI) was calculated by the formula

GSI (%) = Weight of gonad (g) / Weight of fish (g) x 100

Histological study

Sub-samples of gonadal tissues were fixed in Bouin's solution for 48 hrs. The tissues were then dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4 to 6 μm thick were cut from the prepared paraffin blocks using a Microtome. The slides with the tissue section were then processed and stained with Haematoxylin and Eosin (H&E). Dimensions of the oocyte and testicular lobules were measured in micrometer (μm) using an ocular micrometer, the reading of which was standardized with that of a stage micrometer.

Statistical analysis

The datasets for water quality and histological measurements of gonads were small in nature, hence the Shapiro-Wilk Normality tests were conducted using CRAN R software (Version: 4.1.1) for all water variables and gonadal tissues, followed by measurements of their central tendencies.

ELISA analysis of Estradiol and Testosterone

Estradiol and testosterone hormone assay from the samples was done using ELISA kit (Fish Estradiol E2 Elisa Kit, Make: Biospes, Chongqing Biospes Co. Ltd., Catalogue No. BYEK1437 for Estradiol and Fish Testosterone T Elisa Kit, Make: Biospes, Chongqing Biospes Co. Ltd., Catalogue No. BYEK1437 for testosterone) following the manufacturer's recommendations. Each of the samples was analysed in duplicate, across 96-well plates. Hormone concentrations were calculated based on a standard curve.

The assay was based on standard sandwich ELISA technique. For both the hormones (estradiol and testosterone), 50 μl of undiluted samples were added to the well of the assay plate coated with purified anti-E2 antibody and anti-T antibody respectively and incubated at 37°C for 30 min. The solution in each well were then discarded and washed 5 times with diluted wash buffer. 50μl of HRP conjugated anti-E2 antibody (for estradiol) and HRP conjugated anti-T antibody (for testosterone) was then added to each well and incubated at 37°C for 30 min. This was followed by the washing procedure. 50 μl of TMB substrate A was then added to each well followed by 50 μl of TMB substrate B, vortexed and incubated in the dark at 37°C for 15 min. A blue colour developed in the wells. The reaction was stopped by adding 50 μl of Stop Solution into each well. The plate was read at 450 nm using a microplate ELISA Reader. The estradiol and testosterone concentration of the samples were then interpolated from the standard curve. The final reading of the hormone level was averaged according to respective treatments.

RESULTS & DISCUSSION

Water quality variables

Temperature, pH and dissolved oxygen levels were relatively stable during the course of the experiment. A summary of the statistical results of observed water quality in each experimental tank is presented in Table 1. On each occasion of salt treatment in the 21 day (Day 0 + 20 treatment days) study period, water quality variables in control and experimental tanks were monitored every 24 hours using a handheld multiparameter probe. Variables like water

temperature (°C), pH, salinity (in practical salinity unit), % saturation and amount (in mg/L) of dissolved oxygen were measured in triplicate from the subsurface for each tank. Shapiro-Wilk Normality tests were performed for each variable and each tank and subsequent data distribution and analysis were ascertained. The pH levels in control tank (8.02 ± 0.19), experimental tank 2 (8.06 ± 0.04 (SE)) and tank 3 (8.01 ± 0.19) showed an overall range of 7.58 - 8.56. The water temperature was normally distributed in all tanks and ranged between 22.22 – 29.08°C. The salinity remained between 1.06 - 1.39 PSU in the control tank with tap water while in the experimental tanks it was gradually raised from 1.06 PSU to 22.27 PSU by 24 hourly salinity treatments during the study period. The saturation of dissolved oxygen showed normal distribution (75.67 ± 12.68%) and ranged from 48.10 - 96.20 % in the control tank while it was not-normally distributed in experimental tank 2 (80 ± 4.81(SE)%) and tank 3 (77.85 ± 2.88(SE)%) with an overall range of 25.50-90.40 %. The DO concentration (mg/L) were not normally distributed in experimental tank 2 (6.58 ± 0.38(SE)) and tank 3 (5.81 ± 0.23(SE)) with a lowest record of 2.03 mg/L in tank 2. The overall DO ranged from 2.03 - 6.68 for the experimental tanks and from 3.67-7.85 for the normally distributed DO levels (6.044 ± 1.14) in the control tank.

Gonadosomatic index (GSI):

Three stages of maturity of ovary and testes were observed. These included immature, maturing and mature stages. Only in case of ovary, ripe stage was observed in some fish in the control tank after 3 weeks of the treatment process. Five stages of maturity of ovary and testes have been recognized by Gupta and Banerjee (2013) in *Mystus tengara*, which include immature, maturing, mature, ripe and spent. In the present study, in case of immature stages of *Mystus gulio*, the ovaries appeared translucent and colourless with the oocytes appearing irregular in shape, transparent with no yolk. The testes appeared thread like, very narrow, whitish in colour with no testicular lobules visible. In the maturing stages, the ovaries appeared yellowish white in colour with the ova still not prominent, spherical in shape, slightly opaque due to deposition of yolk at the central position. The testes on the other hand appeared thread like, milky white in colour with small testicular lobules. In the mature stage, the ovaries were enlarged and yellow in colour with the ova appearing spherical in shape and completely opaque (except at the periphery) in appearance due to presence of yolk. The size, length and number of testicular lobules also increased in the mature stages. In the ripe ovaries, the ova appeared spherical in shape and opaque due to presence of huge amount of yolk and somewhat reddish yellow in colour. The profiles and dimensions of the oocyte and testicular lobes obtained from the female and male fish at different stages of maturity in the control and experimental tanks after 21 days of treatment were subjected to statistical analysis and stage of oocyte and lobular growth for each group were determined (Table 3). Gonadosomatic index values decreased with increasing salt concentrations in both male and female fish. The highest GSI value of 4.29 was observed only in case of the female reared in the control tank (Table 2). In the experimental tanks, the GSI values decreased from 2.26 to 0.18 in case of female and from 1.82 to 0.23 in case of male. This decrease may be attributed to the increasing salt concentrations and its impact on the gonadal development. The lack of ripe or spent stages may be due the timing of the experiment which was conducted from mid-February to early March, 2024. The spawning season in *Mystus gulio* has been reported to range from March to November (Islam et al., 2008) in the natural conditions and this species has been reported to breed during the monsoon months (Mukherjee et al., 2002). Earlier reports have shown that in both male and female *Mystus gulio*, the GSI increased from March onwards reaching a peak in July followed by a gradual decrease up to December (Islam et al., 2008; Lal et al., 2016).

Histology of the ovary under ambient salinity (control fish):

During the histological analysis of the ovarian sections, the four different oocyte developmental stages (immature, maturing, mature and ripe) were distinguished, based on the shape and changes in the nuclear and cytoplasmic components of the oocytes. The oogenesis process was classified based on the oocyte size and staining, presence of follicular layer, and the distribution of cytoplasmic inclusions. Earlier workers have reported five-eight stages of oogenesis in majority of teleost fishes (Nagahama, 1983; Milton et. al., 2018). In case of *Mystus* sp. five stages have been described by Arockiaraj et al. (2004) in the gonad of *Mystus montanus* and by Gupta and Banerjee (2013) in *Mystus tengara*.

The immature, maturing and mature stages belong to the pre-spawning phase. In the immature stage, the primary oocyte consisted of monolayer follicles with the ovigerous lamellae from the tunica albuginea evident.

The maturing stages consisted of the oogonia with the chromatin nucleolar and perinuclear oocytes growing rapidly and the cortical alveoli start to appear. In the matured stage, late perinuclear oocytes were visible with the presence of primary yolk vesicle. A few secondary yolk vesicle oocytes were also present. The ripe or the spawning stage was dominated by tertiary oocytes and fully mature eggs with yolk globules.

The histological section of the ovary of control fish after 21 days of the experimental duration showed the ovigerous lamellae in the ovary parenchyma with abundant follicles at different stages of development (Fig. 1), embedded in a connective tissue mass. A single layer of follicular cells was seen surrounding each developing oocyte. The early perinucleolar oocytes were mostly immature and polygonal in shape (Fig. 2) whereas the late perinucleolar oocytes were larger in size and varied in shape from polygonal to oval. The oocytes were greatly increased in diameter at this stage ranging from 192.2 - 338.4 μm (Table 3). The yolk vesicles which were first seen at the periphery of the oocyte slowly spread towards the central nucleus. The light pink stained yolk granules were first observed in the outer cortex which gradually increased in number and size. As the yolk granules moved towards the inner cortex, they fused with lipid droplets and appeared deep pink with haematoxylin and eosin staining.

Histology of ovary exposed to salinity:

The histological section of the ovary after exposure to salinity stress showed a decline in the number of developing oocytes. The ovarian follicles were also of much smaller sizes and diameters ranging from 84.59-173.03 μm (Table 3, Fig 3). The ovigerous lamellae in the ovary parenchyma were less distinct with the ovarian follicles scattered far apart. The ovaries also showed tissue alterations like the expansion of the follicular epithelium in vitellogenic oocytes, rupturing and detachment of follicular basement membrane, tissue with cytoplasmic vacuolization, immature oocytes with scattered yolk droplets, hypertrophied follicular epithelium, interstitial proteinaceus fluid deposition and structural deterioration of follicles with early oocytes. The sections showed more number of atretic oocytes and the ovarian follicles separated due to loss of inter-follicular connective tissue.

Histology of the testis under ambient salinity (control fish):

The histology of the testes showed the germinal compartments with the germinal epithelium, composed of the germ cells and the somatic epithelial cells i.e. the Sertoli cells. The germ cells included all the stages of differentiation of the spermatogenic cells i.e. the spermatogonia, spermatocytes, spermatids and spermatozoa. But as in other bony fishes, there were clear differences in the histology from other vertebrates. All germ cells were found to be developing as a synchronous clone within a cystic structure surrounded by Sertoli cells (Billiard, 1986; Grier, 1993). The spermatogonia were found to be

associated in the germinal compartment with the Sertoli cells by processes that joined laterally and completely enveloped the germ cells isolating them from contact with either the basement membrane or with the lobular lumen. The interstitial compartment was found to be composed of connective tissue and Leydig cells.

Testicular histology of the control fish showed the lobules covered by a basement membrane and were distended with different spermatogenic stages of the spermatogenic cells peripherally. The dimensions of the testicular lobules ranged from 139.3-207.2 μm (Table 3). Sertoli cells were found at the luminal side of the basement membrane of the spermatogenic lobules. They occur either solitary, or in close association with the spermatogenic cysts, which they enclose. The germinal cysts were found to be surrounded with the cytoplasmic processes of the Sertoli cells. The testis lobules were separated by thin strands of interstitial connective tissue. This interstitial tissue contained connective tissue cells and Leydig cells. The Leydig cells had regularly shaped round or oval nuclei. In the control testis they occurred solitary or in small clusters (Fig. 4). Apart from the Leydig cells, fibroblasts and smooth muscle cells were also present in the interstitium.

Histology of testes exposed to salinity:

The testis histology of salt treated fishes exhibited several histopathological alterations. There was thinning of the basement membrane of some tubules resulting in fusion of these tubules (Fig 5). Hypertrophy of spermatocytes was noticed. Degenerative appearance of some of the developing spermatogenic cell clusters was observed and the testis showed different degrees of vacuolation. There was a decrease in the dimensions of the testicular lobules (65.36-99.97 μm) as shown in Table 3. Some tubules showed dramatically decreased number of developing spermatogenic cells and in these tubules only sperm cells were noted in the luminal area. There was hemorrhage in the intertubular space, fibrosis and vacuole formations. Larger clusters of Leydig cells were observed.

Effect on the levels of Estradiol and Testosterone:

The levels of the sex steroid hormones in the control and salt treated fish have been presented in Table 4. The level of testosterone showed a gradual decrease in concentration with increasing levels of salinity. This may be interpreted to be due to the decreased testes development with the salinity stress. On the other hand, the level of estradiol showed an increase with the elevation levels of salinity from 9.13 to 15.29 PSU followed by a decline upto 22.27 PSU. This indicates that low salinity stress upregulates estradiol expression although synthesis of testosterone was affected even at this salinity range. Low salinity stress has been reported to induce estradiol synthesis which upregulates vitellogenin expression (Liang et al., 2014). *Mystus gulio* being a euryhaline fish, this increase may help in its adaptation to both fresh water and brackish waters with low salinities in female fish. But exposure to high levels of estrogen can reduce general viability, induce gonadal malformations or feminization of genetic males (Vanden et al, 2003), or even lead to sterilization (Scholz and Kluver, 2009) in fishes as reported earlier. This was also seen in the present study with several tissue alterations in the ovary of the salt exposed fishes.

CONCLUSION

The results of the current experiment showed that increased salinity has a negative effect on the development of the gonads in *Mystus gulio* fish. Although a euryhaline fresh water fish with reports of it entering into brackish water with low salinities (Gupta, 2014; Pandian, 1966; Talwar and Jhingran, 1991), this fish cannot withstand high salt concentrations, can neither be categorized as mesohaline (salinity: 5·0-17·9): nor polyhaline (salinity: 18·0-29·9) or euhaline (salinity: 30·0-39·9) as classified by Whitfield (2015) based on the capability of freshwater fishes to penetrate estuarine zones. Even salinity increase of up to as low as 9 PSU was found to decrease the GSI percentage as compared to the control fish exposed to a salt concentration of 1.13 PSU. In the present study, it was observed that fishes succumbed with further increase of salinity above 9 PSU. Those that survived showed reduced growth with histological alterations in the gonads and showing slower rate of maturity. From the physiological perspective it is very difficult to explain why this freshwater fish is unable to withstand salinity ranges falling within the mesohaline range. A possible explanation may be due to the inability to develop chloride cells in gill filament epithelia and lack of other osmoregulatory adaptations present in other euryhaline fishes which is the key to the penetration of estuarine waters by freshwater taxa (Whitfield, 2015).

The sex steroid hormone estradiol however expressed significantly under low salinity stress with subsequent decrease at higher salt concentrations, but male testosterone synthesis was affected even at lower levels of salinity increase. Further investigations are necessary to understand the physiological restrictions pertaining to limited salinity tolerances in this species of fish.

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Conflict Of Interest

The authors declare that there are no conflicts of interest.

Table 1: Statistical data of the physical parameters of the water in the experimental tanks as measured with the Multiparameter Probe during the entire span of the salt treatment

Parameters	Tank	Shapiro-Wilk Normality Test	Distribution	Mean	SD	Median	SE	Range
pH	Control	p=0.61	Normal	8.02	0.192	8.035	NA	7.58-8.56
	Tank 2	p=0.022	Not Normal	8.01	NA	8.06	0.041	7.71-8.22
	Tank 3	p=0.61	Normal	8.012	0.192	7.995	NA	7.66-8.35
Temperature (°C)	Control	p=0.738	Normal	26.48	1.373	26.61	NA	23.86-29.08
	Tank 2	p=0.406	Normal	25.14	1.19	25.1	NA	22.22-27.06
	Tank 3	p=0.95	Normal	25.62	1.46	25.66	NA	22.23-28.72
Salinity (PSU)	Control	p=0.0016	Not Normal	1.219	NA	1.155	0.024	1.06-1.39
	Tank 2	NA	NA	NA	NA	NA	NA	NA
	Tank 3	NA	NA	NA	NA	NA	NA	NA
DO (%)	Control	p=0.409	Normal	75.67	12.68	78.95	NA	48.10-96.20
	Tank 2	p=0.0024	Not Normal	71.01	NA	80	4.81	25.50-90.40
	Tank 3	p=0.026	Not Normal	71.94	NA	77.85	2.881	43.20-88.90
DO ppm (mg/L)	Control	p=0.378	Normal	6.044	1.14	6.3	NA	3.67-7.85
	Tank 2	p=0.0002	Not Normal	5.65	NA	6.58	0.38	2.03-6.95
	Tank 3	p=0.014	Not Normal	5.47	NA	5.81	0.23	2.82-6.68

Table 2: Morphometric data and Gonadosomatic index (%) of male and female of *Mystus gulio* fish exposed to different salt concentration

S.No.	Concentration of salt in the Experimental Tank	Day of sampling	Average Total length (cm)	Average Standard length (cm)	Average body weight (g)	Average weight of gonad (g)	GSI (%)
	Testis						
1	Control (1.13 PSU)	21st	11.59	9.26	14.00	0.6	4.29
2	9.13 PSU	8th	11.00	9.30	13.28	0.30	2.26
3	15.29 PSU	14th	11.60	8.80	13.31	0.08	0.60
4	22.27 PSU	21st	11.70	8.60	10.94	0.02	0.18
	Ovary						
1	Control (1.13 PSU)	21st	11.19	9.16	13.20	0.4	3.03
2	9.13 PSU	8th	10.30	8.00	11.01	0.20	1.82
3	15.29 PSU	14th	10.20	8.50	9.90	0.08	0.80
4	22.27 PSU	21st	10.50	8.50	8.66	0.02	0.23

Table 3: Results of the effects of salinity on the development of the gonads reared under different salinities (diameter of oocytes and diameter of the testicular lobules have been expressed in μm for ovary and testis respectively)

Fish sample	Salt concentration in PSU	Gonad	Shapiro-Wilk Normality Test	Mean (μm)	Median (μm)	SD (μm)	Range (μm)
Control	1.13	Testis	p=0.37	182.2	192.9	26.62	139.3-207.2
	1.13	Ovary	p=0.401	251.5	246.1	53.67	192.2-338.4
Exposed to salinity	15.29	Testis	p=0.74	79.98	80.75	13.7	65.36-99.97
stress	15.29	Ovary	p=0.004	106.12	92.28	16.87 (SE)	84.59-173.03

Table 4: Results of the effect of salinity stress on the testosterone and estradiol hormone level of experimental fish (*Mystus gulio*)

S.No.	Concentration of salt in the Experimental Tank	Concentration of Testosterone in pg/ml	Concentration of Estradiol in pg/ml
1	Control 1.13 PSU	1542	8.36
2	9.13 PSU	1465	4.27
3	15.29 PSU	1285	12.93
4	22.27 PSU	225	1.8

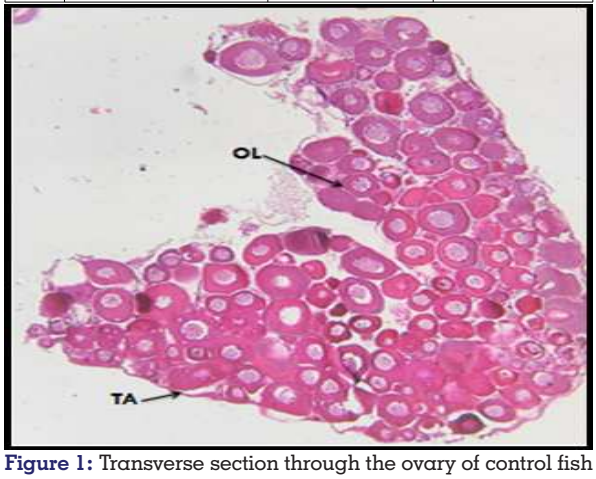


Figure 1: Transverse section through the ovary of control fish showing different stages of oogenesis; Ovegerous lamellae (OL) from tunica albuginea (TA).

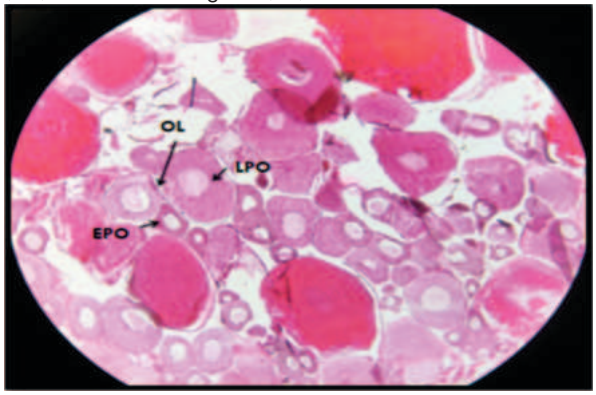
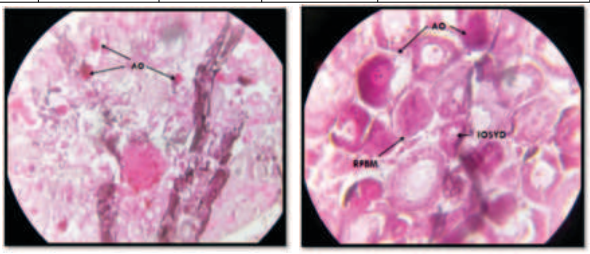
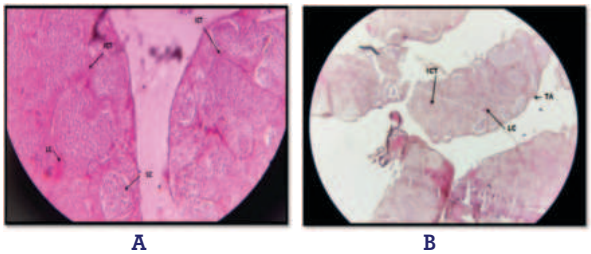


Figure 2. Ovarian section showing the early perinuclear oocytes (EPO) and late perinuclear oocytes (LPO).



A B

Figure 3 A&B: Transverse section through an ovary after salt exposure showing atretic oocytes (AO), rupturing and detachment of follicular basement membrane (RFBM), immature oocytes with scattered yolk droplets (IOSYD) and hypertrophied follicular epithelium.



A B

Figure 4A&B. Transverse section through the testis of control fish showing different stages of spermatogenesis within the cysts. The testis is surrounded by the tunica albuginea (TA). Interstitial tissue contains interstitial connective tissue cells (ITC) and Leydig cells (LC).

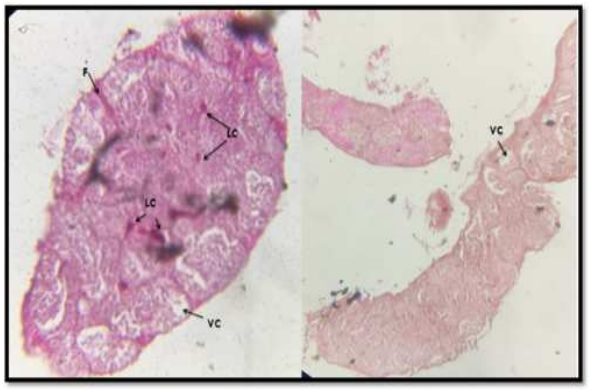


Figure 5. Transverse section through testis after salt exposure showing degenerative appearance of some of the developing spermatogenic cell clusters with different degrees of

vacuolation (VC), fibrosis (F) and larger clusters of Leydig cells (LC).

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