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Temperature effect on gonads of freshwater fish *Cyprinus carpio*

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Abstract

Development of ovary and oocyte along with male gonad testis of the freshwater fishes was observed .When rearing 51 day-old *Cyprinius carpio* at elevated sub-lethal temperatures to (control), 28 and 34°C (experimental) was exposed no death was observed, but the ovarian dimensions differed significantly (F=5.25cm, DF=3, P < 0.05) as manually measured by image software of contrast phage microscope between the 27°C and 30°C and between 32°C treatments. Observation indicated that numbers of germ cells per ovarian cross-section seemed to be more numerous and dense in 27°C treatment than in either the germ *>30*°C or 32°C treatments were less dense. Testicular cross-section areas were significantly (T=5.11cm, DF=3, P>0.05), different among all groups at the conclusion of the temperature treatments at 27 °C and 30 °C

Key words : Temperature (28 to 32 °C), ovary, testis, MBB-stain

INTRODUCTION

To meet the needs of the public, hatcheries are required to produce large quantities of fish which can result in a smaller than desired fish size [1]. Current technologies to enhance somatic growth in fish require either chemical means or long-term maintenance of brood stocks [2]. Heat treatment has been shown to eliminate or reduce gonad growth in some species of fish [3].

This technology may have the ability to reduce or eliminate sexual development and activity in fish [3], thereby, redirecting the energy used for reproduction into somatic growth, A few studies have been undertaken to examine the feasibility of this technology. For example, exposure of juvenile large mouth bass to elevated sub-lethal temperatures (32°C) effectively reduced gonadal germ cell numbers in both males and females [4]. Likewise, exposure of *Cyprinius carpio* to 32°C caused germ cell degeneration in both sexes and interfered with spermatogenesis in the males [4]. For prolonged periods at high temperatures caused sterility in individuals of both sexes.

The purpose of this study was to determine the effects of heat on fork length, body weight, gonad development, and oocyte development along with male gonad of the freshwater fishes, when rearing 51 day-old *Cyprinius carpio* at elevated sub-lethal temperatures of 27 (control),30 and 32°C.

MATERIALS AND METHODS

Cyprinius carpio of the Imperial strain were obtained from nearby Aurangabad freshwater body named as Vadgao-lake.

Experimental Design and Sampling

Glass aquaria (15 liters) were randomly assigned a temperature treatment of 27 (Control).Thermostat-controlled heater (model number HL-50 electronic heater) and aeration. Upon arrival to the laboratory, *Cyprinius carpio* were placed within a single aquarium at 27°C to facilitate feeding and cleaning. They were kept in the aquarium until day 19 post hatch and were then distributed in groups of 10 fish to each aquarium until all aquaria were received 10

Individuals at 21 days post-hatch, the water temperature was raised in tank therefore, the last target temperature (32°C) was obtained during day by adjusting temperature adjustment, when the fry were 23 days post-hatch. All temperature treatments were considered to have started at this point. Two fish from each aquarium were sampled prior to the onset of the temperature treatment and combined with 18 fish that were left over following tank assignments.

These 20 fishes were used to establish condition (body weight, length, and gonad stage) at the onset of treatment. Five additional fish were randomly sampled from each tank after 14 days of temperature treatment for preliminary assessment of their condition halfway through the treatment. After completion of the experimental exposure period of 28 days and 14 (age at completion of treatment 51 days post-hatch), 10 fish were randomly sampled from each tank to assess their final condition All fish were anesthetized in 1 g/L (chloroform) prior to processing Fork lengths and weights were recorded for each fish. The gonads of each fish was dissected and placed in Bouin's solution and subsequently processed for histological examination. Routine Maintenance and Feeding aquaria were covered with a hard plastic sheet to help prevent evaporation or contamination of water. The source water for aquaria was de-chlorinated underground water. Water quality measurements were made each morning and evening. Parameters included dissolved oxygen, salinity, conductivity, and pH, Unionized ammonia, levels were recorded each morning and water temperatures were recorded twice daily to ensure tanks were maintained within of their target temperature After 3 days of treatment, the feeding protocol was changed to twice daily using a mixture of size 1000ml and 120ml feed in equal portions After 8 days of treatment, feed size was changed to 500 ml respectively and remained at this size through the completion of the experiment. Thirty minutes after feeding, uneaten food was removed by siphoning with a small volume of water (the siphoned water was immediately replaced).

Data Analysis

The data were analyzed using a completely randomized design in which aquaria were randomly assigned a temperature. Fish weight, fork length, gonad cross-section area of both males and females, and cross-section area of the largest germ cell (oocyte) in females, were analyzed using the statistic software package [5]. Measurements for each parameter were averaged for all fish within an aquarium to obtain a tank value. Data was first tested for homogenous variances. If variances were not homogenous, data were then log-transformed and then tested again for homogeneity. Next an all effects test (F- test) was mean ($P< 0.05$) and then means were separated using Duncan's Multiple Range Test.

Collagen concentration: collagen concentration was estimated by Lowry's modified method.

RESULTS

Ovarian and oocyte cross-section area

Cell aggregations originating from the distal ends of putative ovaries signaled that the process of early sexual differentiation had already begun at onset of temperature treatment (Fig. 2.3), Due to heterogeneous variances for ovarian cross-section areas, the data were log-transformed (which satisfied homogeneity). The ovarian dimensions differed significantly ($F=4.26$, $DF=2.9$, $P < 0.05$) between the 27°C and 30°C and between 32°C treatments but not between the 27°C and 30°C treatments (Fig. 2.4-2 7). Oocyte cross section-areas differed significantly ($F=4. 26$. $DF=2.9$, $P < 0.05$) among all treatments (Fig 2.8) Cursory observation indicated that numbers of germ cells per ovarian cross-section seemed to be more numerous in the 27°C treatment than in either the *>30*°C or 32°C treatments and the treatment seemed to contain more than the 34ºC treatment In fact, gonad sections from 52% of the females from the latter treatment were completely devoid of germ cell.

Testicular cross-section area:

Gonad sex differentiation had not begun in the males at onset of temperature treatment (Fig 2.9), the testicular cross-section areas were significantly ($F=4.26$, $DF=2.9$, $P> 0. 05$), different among all groups at the conclusion of the temperature treatments (Fig 2.10-2)

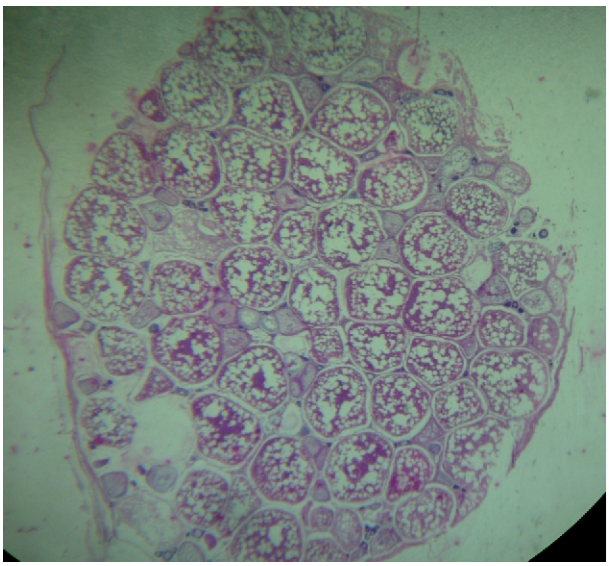

Figure 1. Ovary showing follicles (control)

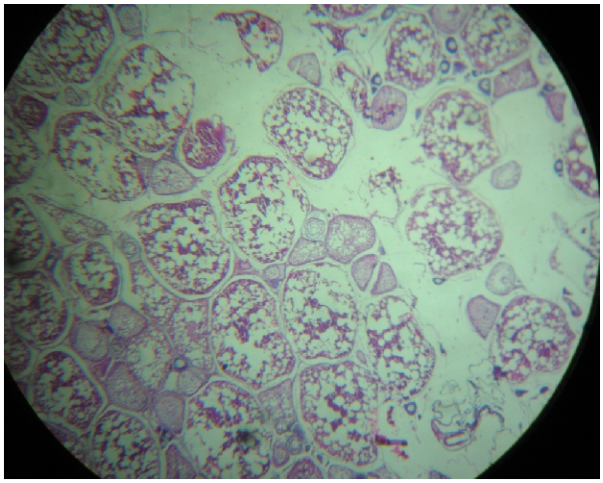

Figure 2. Ovary showing affected follicles at 36 ºC

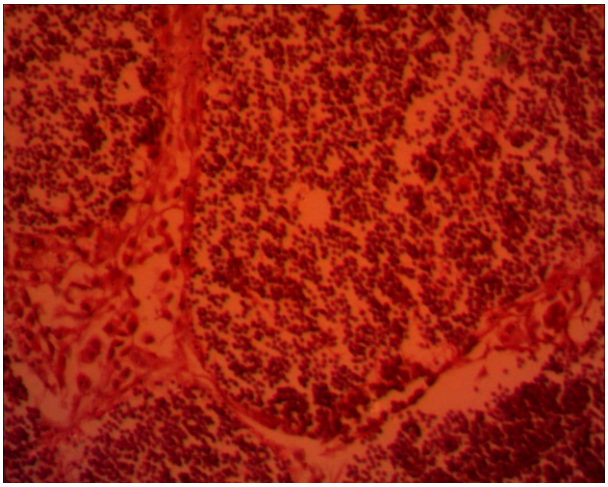

Figure 3. Testicular cross-section area (normal)

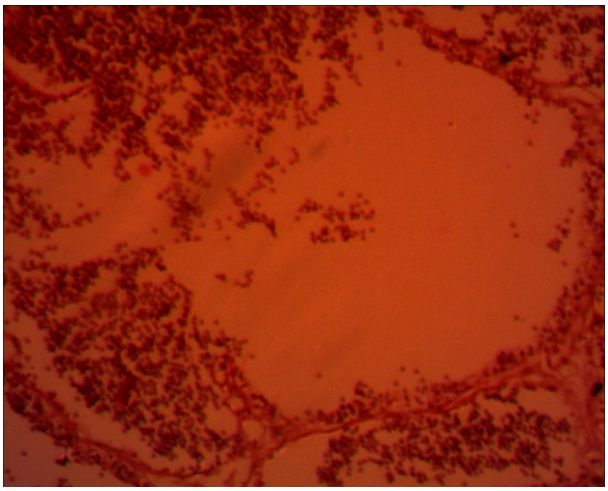

Figure 4. Testicular cross-section area (treated)

Collagen Deposition:

Presence of collagen deposition (fibrosis) within the extra-cellular matrix of the gonad was not detected in any samples observed after a cursory inspection of a total of 10 samples from each treatment (males and females combined) stained for this measurement. A more carefully analysis of two males and two females from each replicate (total of 8 males and 8 females per

treatment) yielded the same conclusion.

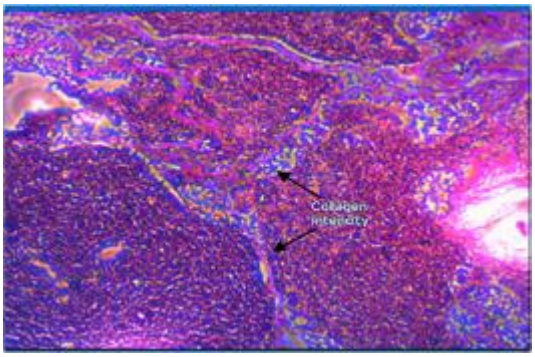

Figure 5. Collagen activity

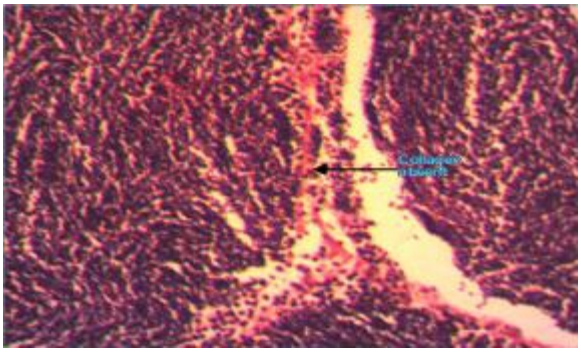

Figure 6. Absent of collagen at connective tissues

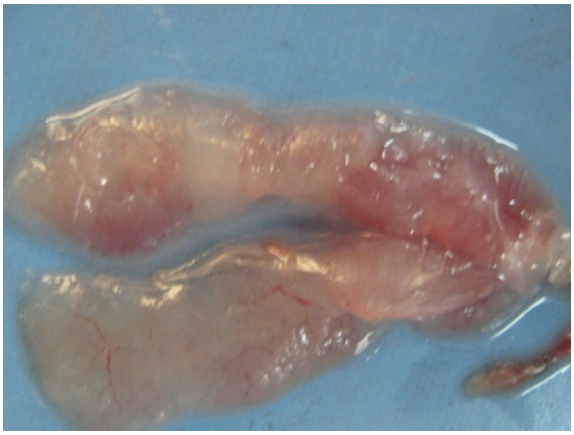

Photo 3. Affected ovary by temperature at 36 ºC

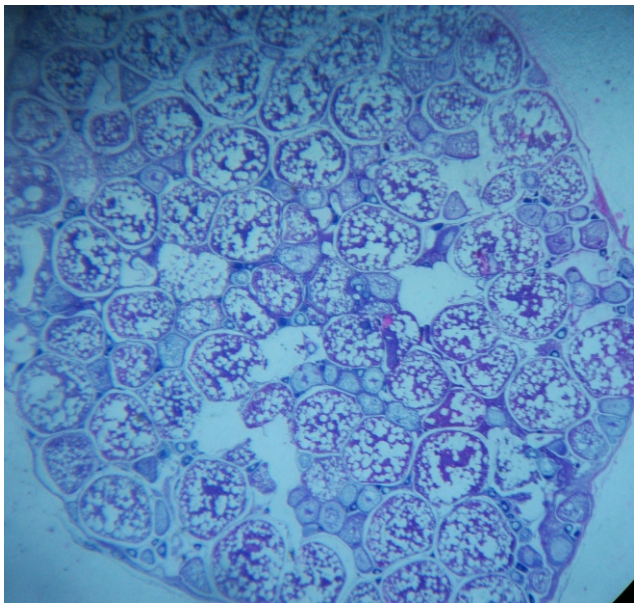

Figure 7. Collagen activities in female ovary (control)

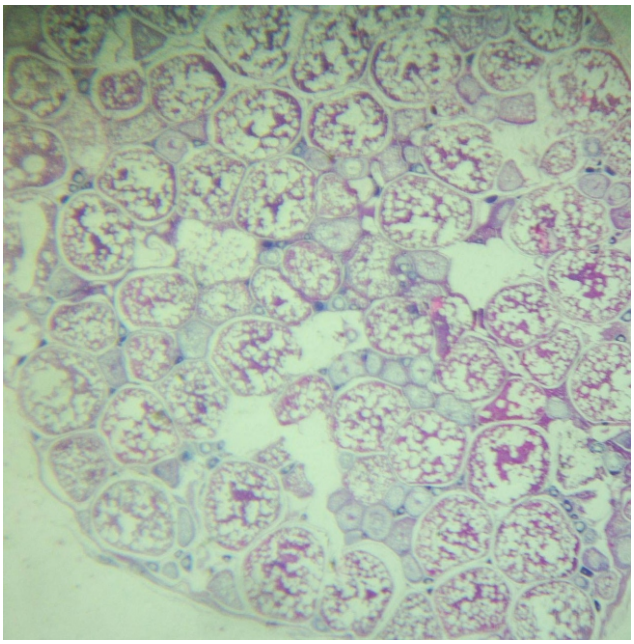

Figure 8. Absent of collagen at connective tissues at 36 ºC (treated)

DISCUSSION

Exposure to 51day-old freshwater fish resulted in reduced Oocyte numbers and ovarian area (size), testicular area (size), and fork length and body weight In Fish exposed to 32ºC the effects were not as pronounced but oocyte number in ovaries, testicular size, and body weight also were reduced In most teleosts including channel catfish [5], gonad sex differentiation occurs much earlier in females than males [3]. In the present study, it was noted that putative females had started the process of ovarian differentiation at the time that heat treatment was initiated, 23 days after hatching This finding is in general agreement with [5] , who found that female *Cyprinius carpio* such result was also found in channel catfish begin ovarian differentiation 19-21 days post-fertilization, whereas testicular differentiation in males occurs about 90-102 days post-fertilization. The effect of high temperature on the females varied between the 28 and 32°C treatments Exposure to 32°C caused a reduction in oocyte number and development. In other result oocytes number were reduced at 36ºC. However, the magnitude of these effects was not high enough to be reflected in a significant decrease in ovarian size compared to fish in the control treatment (27°C), The effects of the 32ºC treatment were more profound. This treatment significantly decreased ovarian size as well as effectively reduced oocyte numbers and development. Further, 52% percent of females exposed to 34°C were devoid of germ cells in the histological sections observed. This latter observation indicates that at least half the females from the 32°C treatment may have become sterile. In mammals, heat-dependent germ cell loss has been observed only in males [6]. However, studies with fishes have reported germ cell loss in females indicating that female gem cells in fishes are also heat sensitive [3].High temperature treatment for these species), were devoid of gonad germ cells after I to 5 weeks of exposure [3];[4] noted increased oocyte

degeneration (pyknotic-condensed cells or necrosis-enlarged and swollen cells) with no significant difference between treated and control ovarian size in largemouth in females (age-6-weeks post fertilization) but did find a significant difference in ovarian size as well as extensive oocyte degeneration in largemouth bass females (age-6-weeks post fertilization) subjected to continuous 32°C heat treatment for seventeen weeks [4] further noted that female *Cyprinius carpio* freshwater fish had an increasing occurrence of degenerative gonad germ cells after an exposure of four weeks to 30 and 32ºC temperatures with no significant decrease in ovarian size. Degeneration within germ cells (eosinophilic or pyknotic nuclei and/or eosinophilic cytoplasm) was noted relatively early after the onset of exposure to 29°C in *O.*Bonariensis [6],but pronounced cell loss (severe to complete germ cell loss) only became apparent after a prolonged exposure of 84 [6] and 90-130 days [3] . In these studies, the gonads were found to be smaller in the exposed fish due to germ cell [3]; [6], noted that during a post-exposure recovery period, the remaining germ cells were able to proliferate and repopulated the gonads. These observations indicate that not all germ cells were eliminated by heat treatment and that no apparent effects occurred in the somatic cells needed to support germ cell development [6]. These observations also suggest that heat-dependent gonad impairment may be reversible [3]; [6], Spermatogenesis is sensitive to high temperature and can be significantly impaired by heat in main species [3]. Germ cell deficiency was observed in 85 7% of Post-hatchery males reared at 27°C treated at 32°C had a lower testicular area than control males after seventeen weeks exposure [4] and similar results occurred in yearling male channel catfish exposed for four weeks to 28ºC and 32°C [4] reported that heat treatment blocked spermatogenesis in the toad animal significantly reducing the number of primary and secondary spermatocytes .A reduction in germ cells numbers in decreased testicular weight, was shown in adult male mice six days after induced cryptorchidism [7]. Humans, ranging from 6 hours to 20 years after birth, with undecided testes, showed germ-cell deficiency. Germ cell deficiency remained constant and no germ cell regeneration was observed regardless of later stages of maturity [8]. In the present study, testicular size was significantly affected in the 30 and 32°C treatment. Development of testes in the 30°C treatment was notably inhibited compared to the control (27°C), but a much larger inhibition occurred in males from the 32°C treatment.

This study found no increase in fibrosis within the extra-cellular matrix of freshwater *Cyprinius carpio* fish gonads after exposure to 30 or 34°C temperatures for four weeks.

CONCLUSION

As now there is threat of rise in temperature in universe due to global warming, present study is looking in to this matter and has worried about fishes which are very sensitive towards temperatures also. Temperature in control is life saving and good in reproduction but beyond limit, temperature will causes damage to fishes on reproduction and chemical composition in fishes. This study supports previous work done by others workers in relation to temperature.

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