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REPRODUCTIVE CHARACTERISTICS AND GERM CELL STATUS OF INDIAN MAJOR CARP, *LABEO ROHITA* REARED IN ELEVATED WATER TEMPERATURE REGIME

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Rearing water temperature and fluctuations in it has a profound effect on survival and gonadal development of fish. Reproduction in fish, compared with other physiological processes, only occurs in a bounded temperature range; therefore, small changes in water temperature could significantly affect this process. Here we analysed the effects of different rearing water temperatures (28 °C, 30 °C, 32 °C, 34 °C and 36 °C) and a cytotoxic drug (busulfan) on germ cell status and maturation in Indian major carp, *Labeo rohita.* The effectiveness of the treatment was assessed by gonadosomatic index, histology and dye uptake of GC. Thermo-chemical treatments were given either: as elevated water temperature alone (0.69±0.09) or in combination with busulfan that showed a low GSI value (0.49±0.26) as compared to control (0.88±0.009). Gonadal morphology visibly shrunk after the thermochemical treatments. Similarly, the gonadal histology confirmed that the GC depletion took place when the rohu were reared at elevated temperature along with the application of a cytotoxic drug busulfan (40 mg/kg). According to the deteriorating reproductive responses of the fish by temperature fluctuations, it is plausible that changes may affect aquaculture production and affecting future populations of fish, so new strategies for amelioration should be anticipated.

INTRODUCTION

Reproduction in fish is influenced by different abiotic and biotic factors. Compared with other physiological processes, reproduction occurs in a specific temperature range (Pörtner and Farrell, 2008), thus small changes in water temperature could significantly affect this process (Van der Kraak and Pankhurst, 1997; Zięba *et al*., 2010; Zucchetta *et al*., 2012). Temperature is a critical physical factor in the lives of fish that is directly related to the control of all fish reproductive processes from gamete development, maturation, spawning to larval and juvenile development and survival (Sponaugle and Cowen, 1996; Pauly and Pullin, 1988; Ito *et al*., 2008; Pankhurst and Munday, 2011). Temperature plays a crucial role in regulating reproductive cycle in many fish, particularly in carps (Davies *et al*., 1986). However, these optimal temperature regimes vary from species to species. Teleost fish like carps prefer a temperature range of 24 ºC

to 30 ºC for their growth and reproduction (FAO, 1989). In many parts of the Indian sub-continent, maximum surface water temperature in summer months (April to July) rises above 38 ºC. This has been a usual scenario for the last decade in the eastern state of Odisha, India. Rapid and high fluctuating temperature influence fish reproduction as maturation process of gonad of carps commences during February-March when the temperature gradually increases and completes prior to onset of monsoon in May-June. Under these compelling temperature regimes, what happens to the gonadal status of cultured carp is neither known clearly nor reported by other researchers.

Moreover, there is scanty literature available about the gonadal growth, maturation and reproduction under elevated temperature for Indian major carps (Dash *et al.*, 2009). Elevated water temperature has been found to cause gonadal degeneration in fish, including the partial or complete loss of germinal elements that might impair fertility and reproductive performance (Strüssmann *et al*., 1998; Ito *et al*., 2008). Fish being cold blooded animal is affected by the temperature of the surrounding water which influences the body temperature, growth rate, food consumption, reproduction and other body functions. Germ cells are the building blocks of future gametes which proliferate under optimal conditions of environment. Strüssmann *et al*., (1998) reported the occurrence of GC-deficient fish among groups exposed to high temperatures during gonadal sex differentiation. Germ cell depletion is believed to be one of the major factors that are responsible for gonadal sterility and infertility in fish. It has been reported that maturation of carp broodstock is affected by elevated temperature and also this has been a continued observation by the authors who state this phenomena (germ cell depletion/ non-attainment of maturity in carps) occurs when water temperature rises beyond 34 °C.

This study was conducted to ascertain our hypothesis that the gonadal development and maturity of carps is affected by thermo-chemical parameters. Here, an attempt has been made to establish how the elevated water temperature and a cytotoxic drug affects the proliferation/depletion of germ cells in Indian major carp rohu, *Labeo rohita*. A cytotoxic drug is used in this study to compare the effect of elevated water temperature on germ cells, as many reports on different fish species showed that busulfan suppress spermatogenesis and gonad sterilization, such as the Nile tilapia *Oreochromis niloticus* (Lacerda, *et al.,* 2010), the Patagonian pejerrey *Odontesthes hatcheri* (Majhi, *et al.,* 2009a), the zebrafish *Danio rerio* (Nóbrega, *et al.,* 2010).

Indian major carps (IMCs) are a group of tropical fish that belong to the family cyprinidae, which contributes most to the aquaculture production in India and widely found and cultured in the Indian sub-continent that includes three major species viz. catla, *Catla catla*, rohu, *Labeo rohita* and mrigal, *Cirrhinus mrigala*. Hence, *L. rohita* was taken for this study as a representative of Indian major carps. This is widely cultured in the freshwater systems of the Indian sub-continent due to its high economic value and consumer preference.

MATERIALS AND METHODS

Tank setting and experimental fish rearing

Adult fish *Labeo rohita* (mean body weight of males 400.6 ± 1.44 g and 400.2 ± 0.86 g of females) were collected from 0.2 ha brood rearing earthen ponds and kept for acclimatization for two weeks in a cemented tank of 5100 L capacity (3.4 m L × 1.5 m B × 1.0 m H) at 28°C water temperature prior to the thermo-chemical treatments. The stocking density was maintained at the rate of 1.0 kg/m^3 in each tank for the entire experimental period of 28 days.

At every one week of interval, samples were taken from each tank for gonadosomatic index, histology, germ cell localization using marker dyes and confocal microscopy. To avoid experimental error, the dimensions of all the tanks (nine numbers in each group) were kept same, covered with polyethylene sheets and fitted with 45 W fluorescence lamps with electronic timers for regulating the duration of illumination in different tanks. Fish were reared at 28 °C, 30 °C, 32 °C, 34 °C and 36 °C under a 14-hour light and 10-hour dark photoperiod. The temperature of the water was modulated using two electric heaters (capacity 300 W) (RS Electrical, Zhongshan RISHENG Electrical Product Co. Ltd., China) with thermostat control and filters were placed in each tank along with aerators to maintain the water quality. The physico-chemical parameters of rearing water were tested at weekly intervals following standard methods described in APHA, 1998 (Table 1) (Clesceri, 1998). Fish were fed twice a day till satiation using a commercial pelleted diet (Abis Exports India Pvt. Ltd., Rajnandgaon, India).

Table 1 : Physico-chemical parameters (mean values and SE) in *Labeo rohita* rearing tanks during the experimental period. Values are represented for 1st, 3rd and 7th week showing the status of rearing water. (DO - Dissolved oxygen)

Parameters	pH	DO	CO_2	Alkalinity	NH_3
			1st Week		
Tank 1	7 ± 0.11	4.5 ± 0.04	NIL	178±0.09	0.006±0.004
Tank 2	7.2 ± 0.04	4.4 ± 0.04	NIL	159 ± 0.14	0.003±0.001
Tank 3	7 ± 0.12	4.8 ± 0.04	NIL	169 ± 0.07	0.008±0.002
Tank 4	7.2 ± 0.04	4.8 ± 0.2	NIL	158±0.9	0.008±0.001
Tank 5	7 ± 0.04	5.7 ± 0.04	NIL	162±0.16	0.004±0.002
Tank 6	7 ± 0.05	4.4 ± 0.004	NIL	165±0.04	0.005±0.003
Tank 7	7.2 ± 0.03	4.5 ± 0.007	NIL	170±0.09	0.004±0.002
Tank 8	7.2 ± 0.07	4.5 ± 0.007	NIL	172±0.07	0.007±0.004
Tank 9	7.1 ± 0.03	4.7 ± 0.009	NIL	160±0.09	0.008±0.005

Parameters	pH	DO	CO_2	Alkalinity	NH_3
3rd Week					
Tank 1	7.3±0.09	4.5±0.04	NIL	170±0.03	0.006±0.001
Tank 2	7±0.02	4.8±0.02	NIL	180 ±0.07	0.008±0.0002
Tank 3	7.5±0.04	4.8±0.07	NIL	176±0.09	0.007±0.001
Tank 4	7.4±0.09	5.8±0.02	NIL	156±0.09	0.004±0.002
Tank 5	7.2±0.09	5.6±0.07	NIL	149±0.02	0.0043±0.00
Tank 6	7±0.05	4.6±0.007	NIL	168±0.07	0.005±0.003
Tank 7	7.2±0.007	4.7±0.09	NIL	181±0.15	0.0043±0.001
Tank 8	7.3±0.007	5.1±0.09	NIL	179±0.07	0.004±0.001
Tank 9	7±0.03	5.5±0.01	NIL	175±0.04	0.005±0.001
7th Week					
Tank 1	7 ± 0.02	4.5 ± 0.07	NIL	162 ± 0.1	0.007 ± 0.004
Tank 2	7.1 ± 0.04	4.6 ± 0.05	NIL	180 ± 0.04	0.005 ± 0.003
Tank 3	7 ± 0.07	5.2 ± 0.10	NIL	170 ± 0.07	0.006 ± 0.04
Tank 4	7.2 ± 0.05	5.0 ± 0.02	NIL	183 ± 0.07	0.005 ± 0.01
Tank 5	7.1 ± 0.04	5.6 ± 0.02	NIL	180 ± 0.05	0.008 ± 0.001
Tank 6	7 ± 03	4.8 ± 0.004	NIL	165 ± 0.04	0.006 ± 0.004
Tank 7	7.1 ± 0.02	4.7 ± 0.004	NIL	179 ± 0.09	0.005 ± 0.003
Tank 8	7 ± 0.03	5.1 ± 0.02	NIL	180 ± 0.08	0.005 ± 0.003
Tank 9	7.2 ± 0.02	5.27 ± 0.04	NIL	182 ± 0.07	0.043 ± 0.001

Thermo-chemical treatments

First group of male and female fish were reared in water temperature regimes of 28 °C, 30 °C, 32 °C, 34 °C, and 36 °C only, the second group received busulfan dosage of 40 mg/kg and reared at 28 °C temperature and third group of fish received a combination of busulfan (40 mg/ kg) and elevated water temperature (34 °C). Ten numbers of male and female fish were used in each of the experimental groups. Each treatment was performed in replicate tanks except the controls. Busulfan dose was prepared by dissolving it in dimethyl sulfoxide (DMSO) and further diluting it with freshwater fish Ringer solution to avoid precipitation and maintained at 30 °C following the methods described by Wenzhi *et al*., 2011. Busulfan was intra-peritoneally administrated in two doses (1st week 20 mg/kg and then 40 mg/kg) to fish that were anesthetized using 200 ppm 2-phenoxyethanol (MP Biomedicals, Inc. Ohio 44139). Control group reared at 28 °C received the vehicle DMSO (Merck Limited, Mumbai) only.

GSI and histological analysis

For GSI and histological observation, each time two fish were humanely sacrificed at 0, 7, 14, 21 and 28 days. GSI was calculated using the formula ($GSI = \frac{\text{Gonad weight}}{\text{Bodyweight}} \times 100$). For histology, middle portion of the right and left lobes of the gonads from the sampled fish were taken after dissection. The gonad samples (1-1.5 mm thickness) were immersed in Bouin's fixative for 24 hour and 5µm thick sections were cut using a mechanical microtome (WESWOX Optik Rotary Microtome, Ambala Cantt, India) and stained using haematoxylin and eosin (Merck, India Ltd). Gonads were processed for examination with light microscopy using routine histological procedures (Luna, 1968).

Isolation and gradient separation of testicular germ cells

Testis tissue were collected under sterile conditions, cut in small pieces (~ 2 mm^3), rinsed in phosphate buffered saline (PBS), kept in Leibovitz (L-15) medium (Sigma Aldrich, St.Louis, MO, USA) and enzymatically digested with trypsin (Sigma Aldrich,St.Louis MO,USA). Thereafter, germ cell isolation was done by percoll (MP Biomedicals, LLC, France) gradient centrifugation. This involved centrifuging testicular cells for 10 min (800 g) at 25 °C, resulting in three bands. The phase containing the largest cells (germ cells) was harvested, rinsed and subjected to a cell viability test by trypan blue (0.4 %) dye exclusion assay. The protocol described by Lacerda *et al*., 2006 was followed to obtain rohu germ cells.

Enumeration and labelling of germ cells

To detect the germ cell population before and after treatment, fluorescent cell linker mini kit of PKH 26 and PKH 67 (Sigma- Aldrich Inc. CA, USA) were used. Approximately 10 million cells were suspended in 0.4 mL of diluent C (an iso-osmotic aqueous solution provided with the dye) in which PKH was diluted to a ratio of 4 µL of dye: 0.4 mL of diluent C. The diluted dye was then incubated with the cells (final concentration, 10 µmol /L) for 5 min. The cells were centrifuged at 100×g for 5 min, washed two times, suspended again in L-15 and stored in ice until use. The stained and unstained germ cells were tagged with the fluorescent membrane dye PKH 26 and PKH 67 and observed under a fluorescent microscope at an excitation wavelength of 551 nm and 490 nm.

Statistical analysis

All qualitative data are presented descriptively, whereas quantitative data were tested statistically using ANOVA (analysis of variance). Student's t-test was used to determine the significant differences between the treatments. Statistical analysis was performed using SPSS 18.0 for Windows **7**. Differences between groups were considered as statistically significant at $P < 0.05$.

RESULTS

The tolerance limit of rohu to elevated water temperature was recorded at different temperature regimes and shown in Fig. 1. None of the fish held in experimental tanks in control group

(28 °C) died or showed symptoms of stress during the experiment but highest mortality (100 %) was recorded at 36 °C. It was noticed that temperature tolerance capacity of fish decreased with increasing temperature beyond 34 °C and a significantly low survival was noticed at 34 °C and beyond this water temperature. After 14 days of rearing marked differences were clearly evident in the survival pattern.

After 28 days of thermo-chemical exposure it was seen morphologically that the gonad size of rohu shrunk (Fig. 2B) significantly, compared to the control (Fig. 2A) which was further ascertained by lower GSI (0.49 ± 0.26 and 1.78 ± 0.99). The GSI value decreased steadily with busulfan administration (40 mg/kg) also. GSI of rohu male (0.88 ± 0.009) and female (3.30 ± 0.11) in the control group indicated healthy and well developed gonad. GSI of male and females in the treated group-I (elevated temperature only) were (0.69 ± 0.09 and 2.50 ± 0.32) and similarly, in the treated group-II (only busulfan administration) it was 0.52 ± 0.15 and 1.81 ± 0.84 for males and females respectively. Significantly lower GSI of males and females (0.49 ± 0.26 and 1.78 ± 0.99) were recorded from treated group-III that received a combination of elevated temperature with busulfan administration. The gonadosomatic index (GSI) of all groups decreased steadily especially the group-III that received a combination treatment of elevated temperature and busulfan administration at 28 days in females (Fig. 3A) and males (Fig. 3B).

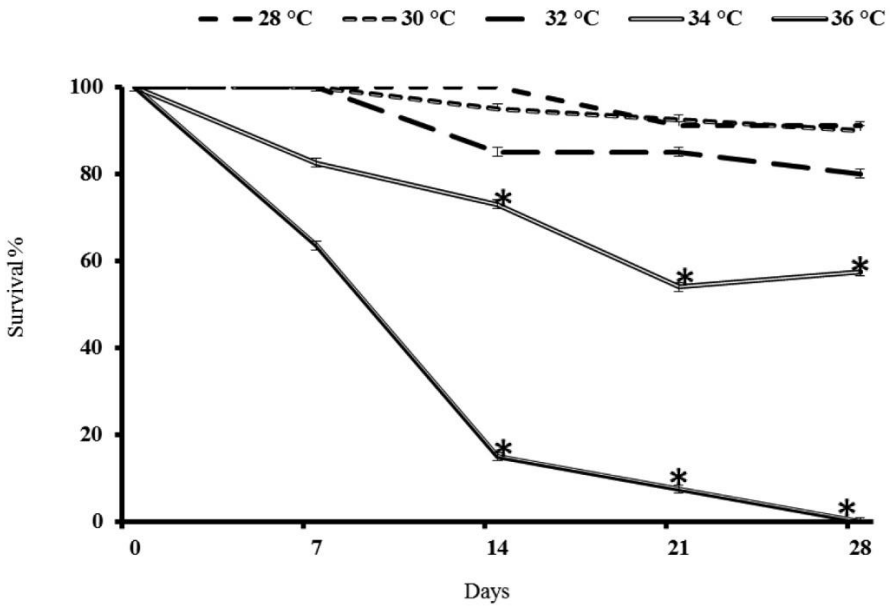

Fig. 1. Effect of temperature on survival of adult rohu at different rearing water temperatures for a period of 28 days experimental period. Data shown as mean ± S.E.M (vertical bars) (n=10 of each sex). Asterisks indicate significant values.

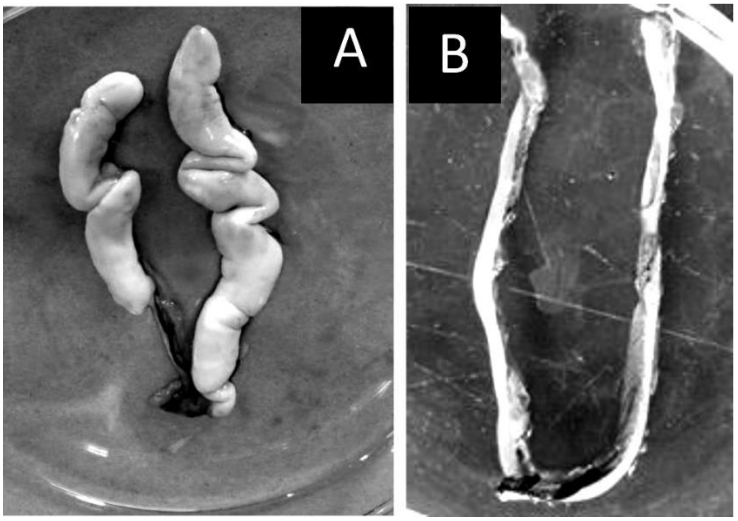

Fig. 2. Changes in the testis morphology of *Labeo rohita* before (A) and after (B) busulfan treatment in combination with that were elevated ambient water temperature (> 34 °C).

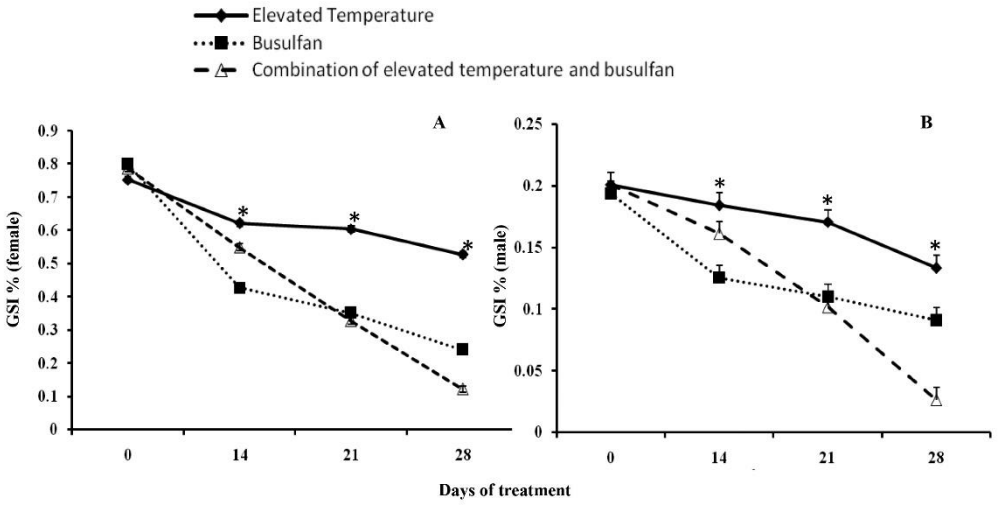

Fig. 3. Effect of elevated rearing water temperature 34˚C, busulfan treatment 40 mg/kg and a combination of both 34˚C water temperature and busulfan treatment 40 mg/kg on the GSI of *Labeo rohita*, (A: female and B: male). Data shown as mean ± SEM (vertical bars), n= 10 of each sex. Asterisks indicate significant values between treatments.

Gonadal histology

The histological analysis of treated and control fish gonads after 7 days of treatment showed active oogenesis with different stages of oocytes, perinuclear oocytes, cortical alveolus oocytes observed. After 14 days of treatment there were prominent cysts of oogonia with decrease of primary oocytes. At the end of 28 days of treatment it was observed that the number of atretic oocytes increased with concomitant decrease of cortical alveoli and vitellogenic oocytes in treated females (Fig. 4). In treated males, initially there was active spermatogenesis within the lobules, as the days of treatment progressed, gradual decreases of spermatogenic cysts were observed. After the completion of 28 days of treatment, reduced number of spermatogonia cells was visible that seemed to lack the capacity for initiation of spermatogenesis (Fig. 5).

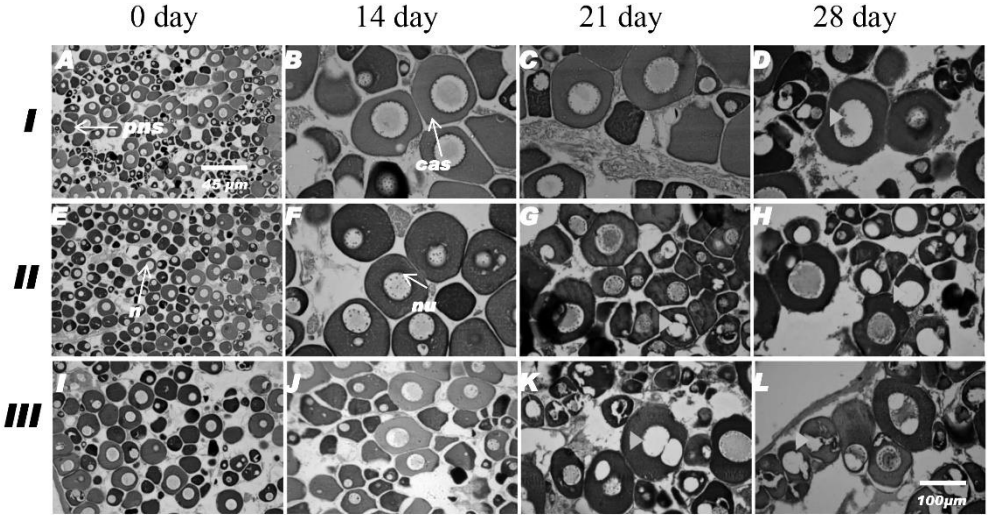

Fig. 4. Histological changes in the ovary subjected to (I) Elevated water temperature (34 °C), (II) Intraperitoneal busulfan administration (40 mg/kg) and (III) Combination of elevated temperature with 40 mg/kg busulfan dose. A, E, I: Ovary occupies mostly with primary oocutes of various classes at the start of the treatment experiment (0 days); B, F, J: showing absence of prominent cysts of oogonia after 14 days of treatment represented by arrow heads; C, G, K: showing absence of oogonia and other types of GCs after 21 days; D, H, L: degeneration of oogonial cells with atretic oocytes after 28 days indicated by arrow head; perinuclear oocytes (pns) or immature oocyte, cortical alveolus oocytes (cas) nucleolus (nu).

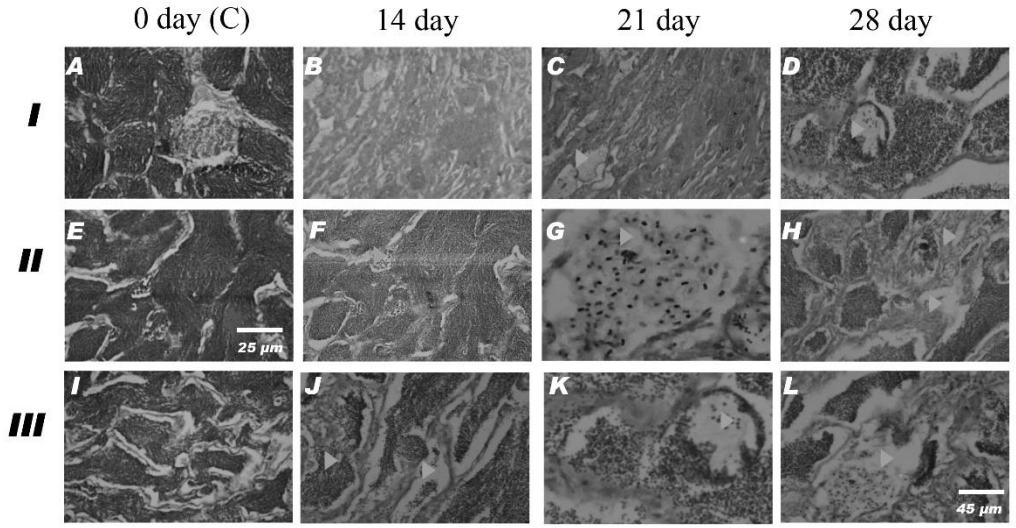

Fig. 5. Histological changes in the testes of males subjected to (I) elevated water temperature (34 °C), (II) Intraperitoneal busulfan administration (40 mg/kg) and (III) combination of elevated temperature with 40 mg/kg busulfan dose. A, E, I; active spermatogenesis within the lobules at the start of experiment (0 days). B, F, J; absence of spermatogenic cysts after 14 days of treatments indicated by arrowhead; C, G, K; absence of spermatogonia after 21 days indicated by arrow head; D, H, L; absence of GCs after 28 days indicated by arrow head.

Germ cell labelling

The study implies that the GCs can be dyed with florescent dye without compromising cell viability. After 2 h staining, it was observed that most (90%) of the germ cells have taken-up both the dyes (PKH 26 and PKH 67). Similar uptake and retention of PKH 26 and PKH 67 dye was observed after one week time (Fig. 6). However, some cells (nearly 5%) showed less fluorescent intensity. This may be due to the fact that many of the cells are in dividing stage. The fish reared in elevated temperature and in combination with busulfan showed less number of GCs as evident from dye uptake studies.

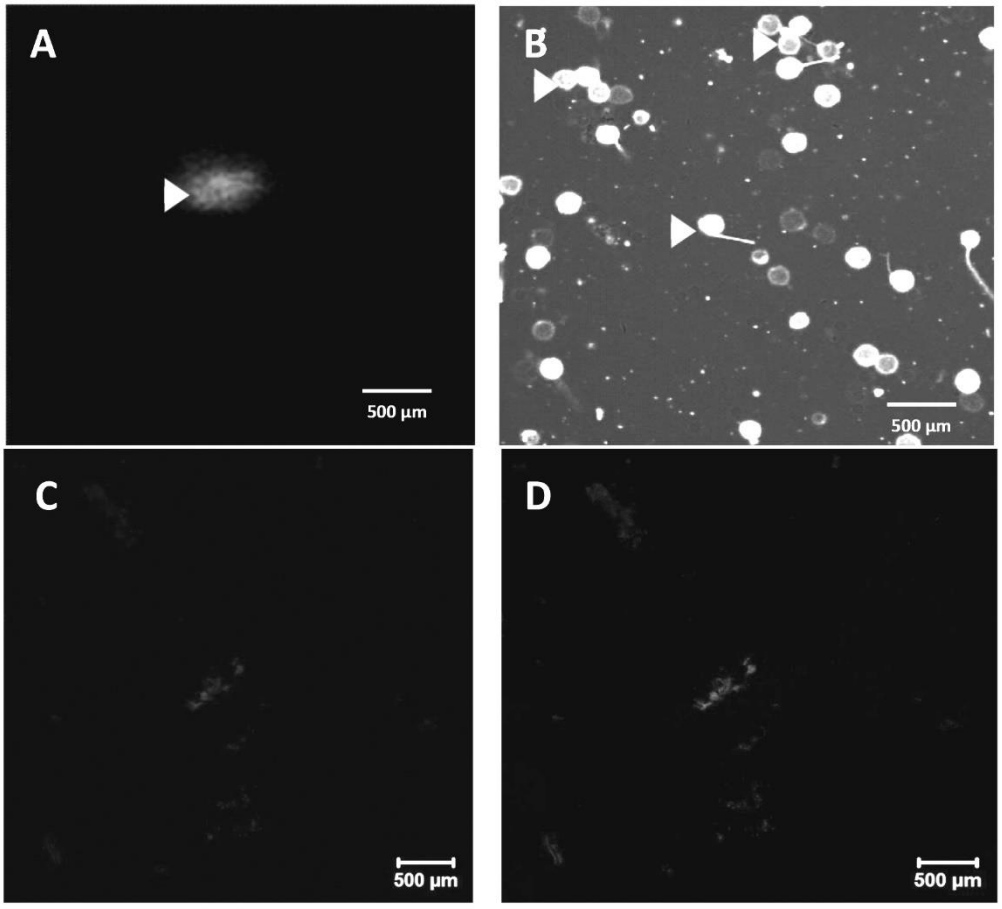

Fig. 6. Proliferative and depletion status of isolated germ cells observed under confocal microscope A: red color showing florescent PKH 26 labelled germ cells in the control; B: green color showing PKH 67 labelled germ cells at different stages of development (represented by arrow head) in control; C and D: poor expression of PKH 26 and PKH 67 in the treated fish (reared in elevated water and administered with busulfan).

DISCUSSION

Temperature is one of the most pervasive environmental factors that influence physiology and ecology of aquatic organisms including fish. Fish reproduction is likely to be affected by increasing and decreasing water temperatures arising from climate change, which has the capacity to affect endocrine function that may either advance or retard gametogenesis and maturation. The present study revealed that elevated water temperature and a cytotoxic drug

(busulfan) affected the reproductive characteristics and germ cell proliferation of rohu, *Labeo rohita*. Elevated rearing water temperature (>34 °C) beyond their thermal threshold resulted in impairment in germ cell proliferation as seen from GSI and histology. The thermal threshold of adult rohu has been reported by Das *et al.*, 2004; 2005; 2006. This study is relevant in the present context of global warming that predicts the water temperature to rise (Wohlschlag *et al*., 1968; Franklin *et al*., 1995; Schmidt- Nielsen, 1997) that may affect the aquatic fauna in terms of their physiology and reproduction. Here, it was observed that elevated rearing water temperature (>34 °C) has a fatal effect on their survival. The thermal limit of rohu has been reported to be 35 °C (Chatterjee *et al*., 2004). Our studies are also in agreement with their findings and further give more insight into the reproductive status of carps reared at elevated water temperature that were not reported before in carps. These findings are important because water temperatures above 34°C impair fish physiology (Das *et al*., 2004). In temperate teleosts such as pejerrey, the increase of water temperature during summer signals the end of reproductive episodes (Pankhurst and Porter, 2003). Here experimental data showed that rohu has a temperature tolerance limit of 36 °C as no fish survived beyond this temperature. It was observed that rohu has certain thermal tolerance range beyond which it has adverse effects on physiological, metabolic and reproductive activities. Further, it was investigated whether this rearing water temperature rise is affecting the gonadal status and reproductive ability of carps. This becomes more important when the problem of global warming (temperature rise) is believed to affect the food production sector including aquaculture.

Germ cell depletion is believed to be one of the major factors that are responsible for gonadal sterility and infertility in fish. Here, GC status of rohu was assessed when they were reared in elevated water temperature along with a cytotoxic drug busulfan that is known to destroy endogenous germ cells (Brinster and Zimmermann, 1994; Lacerda *et al*., 2006). These thermo-chemical treatments showed that depletion of endogenous germ cells of rohu took place as evident from shrunken gonad and lower GSI. Fish reared in elevated water temperature alone (GSI 0.69±0.09) and in combination with busulfan showed low GSI value (0.49±0.26) as compared to control (GSI 0.88±0.009). Similar results of germ cell depletion by warm water temperature and busulfan have been reported in other teleost species such as Pattagonian pejerrey (*Odontesthes hatcheri*) (Majhi *et al*., 2009 a, b).

Treated fish exposed to thermo-chemical treatments showed increased number of atretic oocytes with concomitant decrease of cortical alveoli and vitellogenic oocytes in females and absence of spermatogenic cysts within the lobules with complete depletion of GCs in males. These observations indicates that somatic cells (such as sertoli and Leydig cells in males and follicular cell in females) that support the proliferation and development of germ cells, were not critically affected by the thermo-chemical treatments at this doses and duration. Similar results were reported in *Odentesthes bonariensis* reared in higher water temperature (Ito *et al*., 2008; Soria *et al*., 2008). It is worth to mention that pejerrey requires an optimum range of water temperature of 5 °C to 25 °C for its growth and propagation (Majhi *et al*., 2009b). In other species,

study on exposure to higher temperature resulted in degeneracy of sertoli and germ cells in the seminiferous tubules of rat (Strüssmann *et al*., 1998) and in Table 2 some studies on other animals have been discussed for reference. Here in rohu, the effect of elevated temperature was shown to be negatively affecting germ cell proliferation but sertoli cells remained unaffected. Similarly, busulfan, a known cytotoxic drug was used to deplete the GC content and compare the same with the temperature treatments. Moreover, it is not known at what dosage it can make the fish completely GC deficient. The toxicity and sensitivity of busulfan is reported to be varying from species to species and requires a specific dose for each animals *viz*. pigs and goats (7.5 mg/ kg), mice (10-50 mg/ kg), pejerrey (30-40 mg/ kg) (Honaramooz *et al*., 2005; Majhi *et al*., 2009a; Wang *et al*., 2010) which resulted in complete removal of germ cells without any lethal effect. Here, fish administered with 40 mg/ kg busulfan showed no mortality but high mortality was recorded when applied in combination with elevated rearing water temperature beyond 34 °C.

Table. 2 : A comparative account of rohu (*Labeo rohita*) germ cell depletion studies with other vertebrates with special focus on endogenous GC depletion and spermatogenesis.

Species	Treatment	Effects	Year	Reference
Mice (Male)	Busulfan	Depletion of spermatogonial germ cell	1994	Brinster *et al.*
Swiss nude Mice	Busulfan	Depletion of endogenous germ cell in testes	1999	Ogawa *et al.*
Tilapia	6-n-Propyl-2-Thiouracil (PTU)	Loss of germ cells with increase of dose	2002	Matta *et al.*
Mice	Ionizing radiation	Depletion of seminiferous epithelium of host mice	2002	Creemers *et al.*
Pig	Busulfan	Suppression of endogenous spermatogenesis	2005	Honaramooz *et al.*
Goat	Radiation	Testicular irradiation results in reduction of endogenous germ cell population	2005	Honaramooz *et al.*
Dog	Irradiation	Depletion of endogenous spermatogenesis	2008	Kim *et al* .
Nile-tilapia (*Oreochromis niloticus*)	Busulfan with Elevated temperature	Depletion of endogenous germ cell	2006	Lacerda *et al.*

Species	Treatment	Effects	Year	Reference
Patagonia pejerrey (*Odontesthes hatcheri*)	Busulfan and high temperature	Incipient gonadal degeneration and germ cell loss	2009	Majhi *et al.*
Chicken	Busulfan	Reduction of number of endogenous primordial germ cells (PGCs) in embryonic gonads and hatchability.	2010	Nakamura *et al.*
Cat	Busulfan with local X-ray radiation	Depleting endogenous spermatogenesis	2012	Silva *et al.*
Chicken	Busulfan	Depletion of endogenous germ cells in embryonic gonads	2013	Lee *et al.*
Rohu (*Labeo rohita*)	Busulfan and elevated temperature	Depletion of endogenous germ cells	2015	Present study

In this study the increase in rearing water temperature consistent with climate change predictions shown to affect the gonadosomatic index of rohu, due to depletion of germ cells. To our knowledge, the present study provides the first hand evidence that germ cells proliferation in carps is temperature sensitive**.** As can be inferred from this study for *Labeo rohita*, germ cell depletion occurs when exposed to elevated water temperature. To ascertain the germ cell status in the treated fish fluorescent dye (PKH 26 and 67) was used and the effectiveness of thermo-chemical treatment in the gonad assessed. The proliferative cell were dyed and shown positive fluorescence under a confocal microscope. It was verified from this study that elevated rearing water temperature beyond their thermal limit (>36 °C) grossly affected the gonadal maturation process as evident from the depleted GC content. In conclusion, the results of this study indicate that elevated water temperature affects germ cell proliferation and gonadal status in carps as temperature fluctuation (>34 °C) adversely affected reproductive characteristics of carps that suggests that climate change is an additional stressor to fish populations (brood stock). The potential importance of water temperature-induced reproductive dysfunctions must not be underestimated as freshwater fish constitute the largest harvestable natural food resource and production of freshwater fishes has been dominated by carps including Indian major carps (71.9%, 24.2 million tonnes, in 2010) (FAO, 2012). According to the deteriorating reproductive responses of the fish to temperature fluctuations, it is plausible that changes may affect aquaculture production and affecting future populations of fish, so new strategies for amelioration should be anticipated.

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