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Comparative Elevation in Water Temperature Induces Somatic Growth and Rapid Proliferation of Gonadal Germ Cells in Three Species of Carp

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Abstract

The eastern Himalayan region, hot spot for fish biodiversity, is characterized by extreme climate and low water temperature. The poor somatic growth and delayed gonadal maturation in carp due to low temperature add considerably to the expense of producing fish from aquaculture. This study examines the response (somatic growth and gonadal maturation) of three carp species (*Hypopthalmichthys molitrix*, *Cirrhinus mrigala* and *Labeo bata*) to low and elevated water temperature. The effectiveness of the water temperature on fishes was assessed by weight gain, gonadal index (GSI), histology, and germ cell count. This study revealed a significant increase in body weight and gonadal index in all the three species from warm water at the end of one year culture period. Further, at the end of 12 months culture period, males from all the three species from greenhouse pond appeared to have prominent cysts of spermatogonia and other active spermatogenic stages and females have large cysts of oogonia interspread with oocytes at various stages of follicular development, a condition not observed in any fish species from the low water temperature. These results suggesting that elevation in water temperature, greenhouse as in this case, in fish ponds of eastern Himalayan region could tremendously benefit the aquaculture in terms of growth enhancement and brood stock development in considerably short period.

Keywords: Temperature, germ cell, gonad, carp, eastern Himalaya.

Introduction

The sustainability of natural fishery resources largely depends on continuous self recruitment of young ones into the ecosystem thereby striking a balance in the population stock (Pukazhenthi *et al.*, 2006). On the other hands, aquaculture of commercially important fish species required periodical stocking of healthy seeds captured from nature and/or produced by induced breeding in captivity. Often nutritional rich formulated diets are preferred to raise a brood stock in confinement for seed production (Watanabe, 1985). Although, it immensely helps in augmenting somatic growth in short period, the gonadal maturation progress in comparatively slower pace (Jhingran and Pullin, 1985). This obviously adds considerably to the cost of rising brood stock for commercially important fish species those takes years together to attain first sexual maturity. This is particularly true for teleost fishes originating and/or introduced to the temperate climatic zone for aquaculture, like in this case, the eastern Himalayan region where environmental temperature remain low throughout the year (Dash *et al.*, 2007).

The water temperature is known to be a fundamental physical regulatory factor in the lives of teleost fishes and affects growth and reproductive process from gamete development and maturation to larval and juvenile development and survival (Pankhurst and Munday, 2011). With increasing latitude, characterized by greater amplitude of seasonal variation and low water temperature, the gonadal maturation in fish often gets delayed (Pankhurst and Porter, 2003). Probably, this being the reason that many of the fishes originating from mid or higher latitude region depicts delayed sexual maturation by one or two years compared to their peers in lower latitude (Prosser and Heath, 1991; Jhingran and Pullin, 1985). It is true that, increase in water temperature, although not beyond the optimal range, for any fish species can influence metabolic activities and somatic growth (Reynolds and Casterlin, 1979). For instance, Indian major carp (IMC) grow more quickly at the temperature range of 28-35°C and reach the first sexual maturity at the age of 1.5~2 year (Jhingran and Pullin, 1985). However, their growth attributes and reproductive maturity gets

delayed by additional one year approximately while grown in comparative cool water 15~20°C (Srivastava and Chowdhary, 1979; Bhatt and Bujarbaruah, 2011). These observations have significant implications for seed production and aquaculture in temperate and/or sub-temperate regions those are characterized by extreme climate including low water temperature. It is believed that low water temperature in the eastern Himalayan region is partly responsible for poor fish productions, although region is bestowed with enormous natural resources (Bhatt and Bujarbaruah, 2011; Majhi and Das, 2013). In the present study, attempt was made to study the effects of elevated water temperature, induced by greenhouse in this case, on somatic growth and gonadal germ cell proliferation and differentiation (a proxy for evaluating maturation status) in three carp species viz. silver carp *Hypopthalmichthys molitrix*, mrigal *Cirrhinus mrigala* and bata *Labeo bata*. The results obtained in this study have the practical implication for aquaculture and brood stock development of important fish species in considerably short period in eastern Himalayan region and cooler environmental condition prevailing elsewhere.

Materials and Methods

Experimental Setup and Pond Preparation

Ponds (n=2) of 0.04 ha (20×20 m^2) were selected for the experiment. A greenhouse was constructed over a pond with locally available bamboos and ultraviolet plastic sheets (thickness: 15 mm), whereas the other one kept open, at the fish farm facility of ICAR (Indian Council of Agricultural Research) Research Complex for Northeastern Hill Region, Eastern Himalaya, India (21.5° to 29.5° North latitude and 85.5° to 97.5° East longitude). Thus, the ponds were named as `greenhouse pond` and `open pond`.

Both the ponds were applied with 10 mg L^{-1} chlorine (bleaching powder with 20% available chlorine) after dewatering to the lowest possible levels. Dead fishes, if any, were removed by manual and repeated netting. The ponds were kept as such for fourteen days for complete dechlorination and then filled up to 1.5 m depth, with water filtered through fine mesh net. Subsequently, the water depth was maintained through fortnight compensation of about 5–6 cm of seepage and evaporation loss. Each pond was fertilized with basal dose of 240 kg raw cow dung one week prior to stocking, followed by fortnight application of 500 kg cow dung, 10 kg urea and 15 kg single super phosphate (Jena and Das, 2011). The ponds were applied with lime ($CaCO_3$) at 100 kg ha^{-1} $month^{-1}$ at monthly intervals after third months of culture to maintain the water pH of 7.5 to 8.5. The experiment was conducted during September 2011 to August 2012.

Experimental Animals, Rearing Protocol and Water Quality

Six months old silver carp *H. molitrix* (mean body weight±SD; 22.45±3.49 g), bata *L. bata* (19.32±2.54 g) and mrigal *C. mrigala* (21.08±2.75 g) produced in the hatchery facility of ICAR Research Complex for Northeastern Hill Region, Eastern Himalaya, India were stocked at combined density of 7500 numbers ha^{-1}, keeping the stocking composition at 33.33% each species. Fishes were fed with conventional mixture of mustard oil cake and rice bran (1:1w/w) in dough form at 5% of biomass per day during the study period. Quantity of feed was adjusted based on the mean fish biomass in each pond, estimated after monthly sampling with dragnets of suitable mesh sizes and considering an assumed survival of 90%. Water temperature in open and greenhouse pond was recorded every day between $10:^{00}$-$12:^{00}$ hr using a digital thermometer (Thermo Scientific, USA). However, for analyzing important physico-chemical parameters, water samples were collected monthly between $8:^{00}$–$9:^{00}$ hr and analyzed following standard methods (APHA, 2005).

Growth and Histological Analysis of the Fish Gonads

Fishes were sampled at one-month intervals for assessment of growth and biomass. The mean body weight of each species was recorded from randomly drawn samples (n=15) and weight gain was calculated. For gonadal studies, each carp species (n=5 per sex) were randomly sampled from greenhouse and open pond at beginning (0 day) and 12 months past rearing. Fish were sacrificed by an overdose of anesthesia MS-222 (Tricaine methanesulfanate, Sigma, St. Louis, MO) and their body weight was recorded. The gonads were excised, macroscopically examined, and weighed to the nearest 0.01 g. The middle portion of the right and left gonads from each fish were then immersed in Bouin's fixative for 24 hours and preserved in 70% ethanol. Gonads were processed for light microscopical examination following routine histological procedures up to sectioning at a thickness of 5 µm and staining with hematoxylin-eosin. About 150-200 serial histological sections from each fish were examined under a light microscope (Olympus BX40, Japan) at magnifications between 10-100X. The germ cell profile of each specimen was classified following the published information (Ito *et al.*, 2003) with minor modifications (Table 1). Digital images taken from five representative histological sections of the right and left lobes of the gonad of each individual were used for determination of the number of spermatogonia or oogonia per section and the cross-section area of the gonad using the Image-Pro Plus software ver. 4.0 (Media Cybernetics, Silver Spring, USA).

Table 1. Histological criteria for classification of fish gonads (from Ito *et al.*, 2003, with modifications)

Category	Males	Females
I	Prominent cysts of spermatogonia and other active spermatogenic stages	Prominent cysts of oogonia interspersed with oocytes at various stages of follicular development
II	Only cysts of spermatogonia and spermatocyte; efferent ducts may or not contain spermatozoa	Cysts of oogonia and fewer oocytes at the stage of maturing
III	Cysts of spermatogonia are few and small	Only few small cysts of oogonia

Blood Plasma Glucose Assay

Blood sample were collected monthly from three carp species (each species; n=3) from open and greenhouse pond following standard protocol (Heming, 1989). Briefly, the fishes were sacrificed described previously and blood was collected by cutting the caudal peduncle. The samples were left at room temperature for one hour and then stored at 4°C overnight. The blood was centrifuged at 3000 rpm for 10 min for the collection of serum. The aliquots of serum were used for glucose analysis. The serum sample (20 µl) was added to 2000 µl glucose reagent in a test tube. The content was mixed and incubated for 10 minutes at 37°C. A quantity (20 µl) of glucose standard solution was also mixed with 2000 µl of glucose reagent and incubated for 10 minutes at 37°C. Here the plasma glucose was determined by enzymatic oxidation caused by glucose oxidase. The enzyme glucose oxidase is extracted from the growth medium of *Aspergillus niger*. Glucose oxidase catalyse the oxidation of Beta D- glucose present in the plasma to D glucono-1, 5-lactone with the formation of hydrogen peroxide; the lactone is then slowly hydrolysed to D-gluconic acid. The hydrogen peroxide produced is then broken down to oxygen and water by a peroxidase enzyme. Oxygen then react with an oxygen acceptor such as ortho toluidine that convert to a colored compound, which we can be measured against the reagent blank in a UV spectrophotometer (Thermo Scientific, NC 28803, USA) at 505 nm.

Statistical Analysis

The statistical significance of the differences in recorded parameters between the group was analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test using Graphpad Prism ver. 4.00 (Graphpad Software, San Diego, California, USA). Data are presented as mean ± standard deviation (SD) and differences between groups were considered as statistically significant at $P<0.05$.

Results

Water Temperature and Fish Growth

The water temperature recorded in open and greenhouse pond during the study period is presented in Figure 1. The record revealed that, the average water temperature difference between the open and greenhouse ponds during the 12 month long culture period was 2°C (minimum; September) to 5°C (maximum; January). The difference between the pond was more prominent during winter months (November to January) as water temperature in open pond remain low (below 15°C), contrary the greenhouse pond water temperature touch 20°C. Further, there was a significant gain in body weight in all the three fish species cultured under the greenhouse pond condition contrary to their peers in open pond (Figure 2). In general, among the three representative carp tested in this study, the growth performance was comparatively better in *H. molitrix* followed by *L. bata* and *C. mrigala*, irrespective of the rearing condition. However, in greenhouse pond, *H. molitrix* attain a maximum weight of 757±20.43 g followed by *L. bata* (546±17.1 g) and *C. mrigala* (511±21.31 g) over their counterparts from open pond (*H. molitrix*; 355±15.98 g), *L. bata* (342.5±13.17 g) and *C. mrigala* (272.5±17.19 g). These results confirm that elevated water temperature, induced by greenhouse in this case, augments fish growth significantly ($P<0.05$).

Water Temperature and Gonadal Development

The Gonado Somatic Index (GSI) of all the three fish species, irrespective of sex, from both greenhouse and open pond increased steadily between 0 day and 12 months (Figure 3). However, the increases were much more pronounced in the fishes from greenhouse pond than open pond. For instance, the GSI on 0 day in *H. molitrix* was 0.79±0.19% (♂) and 0.55±0.18% (♀). Although GSI in the species has markedly increased to 1.58±0.22% (♂) and 1.91±0.49% (♀) in open pond, further significant increase was recorded in greenhouse group (♂: 4.6±0.37%; ♀: 5.56±0.85%, $P<0.05$).

The microscopic examination of testes at the end of 12 month culture period revealed that the spermatogonia cells have proliferated and significantly increased in number in all three species from warm water group. For example, in *H. molitrix* (♂) recovered from greenhouse pond counted to poses 249±7 numbers of spermatogonia cell per 500 µm^2 gondal area compared to its peer from open pond (132±15 cells/500 µm^2, $P<0.05$). The similar trend was also observed in other two species (Figure 4). Further, the histological section revealed that 100 %

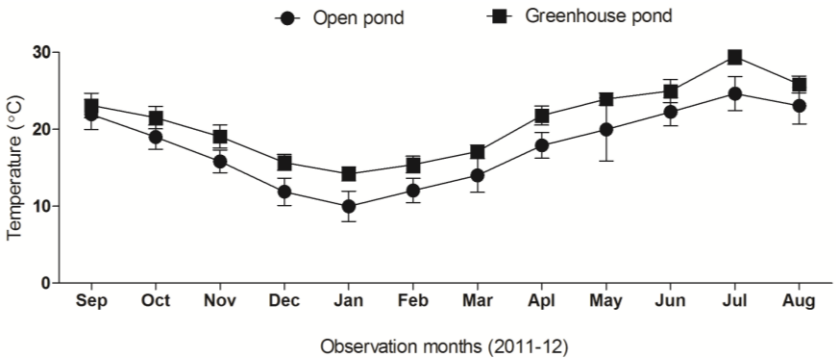

Figure 1. Trends of monthly water temperature recorded in open (●) and greenhouse (■) pond during the study period. There was mean difference of 2 (minimum; September) ~5°C (maximum; January, July) between the two ponds during the 12 months long culture period. Data presented as means ± standard deviation.

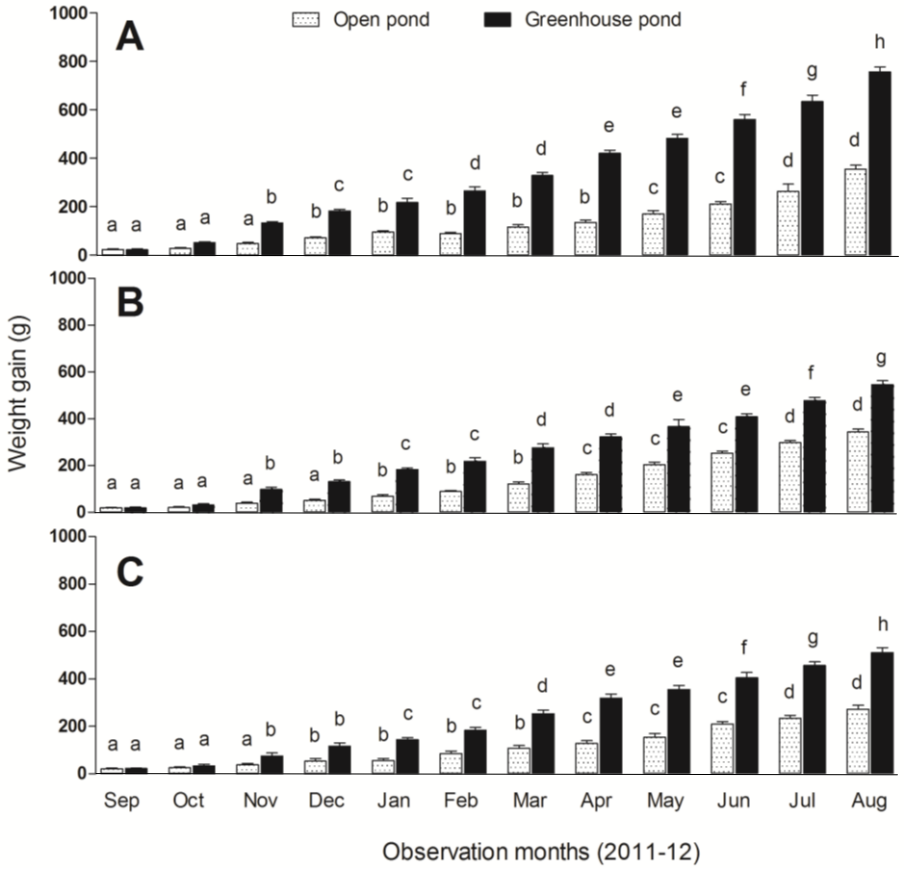

Figure 2. Changes in the body weight of three species of carp *Hypopthalmichthys molitrix* (A), *Labeo bata* (B) and *Cirrhinus mrigala* (C) from open and greenhouse pond during 12 months culture period. There was significant weight gain in fishes recovered from greenhouse pond (elevated water temperature) compared to open pond (low temperature). Columns with different letters vary significantly for a species (Tukey's multiple comparison test, $P<0.05$). Data presented as means ± standard deviation.

of *H. molitrix* and 80 and 60% of *L. bata* and *C. mrigala*, respectively represented characteristic of a functionally mature testes consisting all the stages of spermatogenesis (Batlouni *et al.*, 2009) (Tables 1 and 2; Figure 5). The remaining 20% of *L. bata* and 40% of *C. mrigala* represented presence of only cysts of spermatogonia and spermatocyte but the efferent ducts did not contain spermatozoa. Contrary, the spermatogonia cell population in the testes of fishes from open pond remains comparatively low. The histological observation revealed that testes of all the three species had only cysts of spermatogonia and/or spermatocytes, but lacked all other spermatogenic stages. Similarly, all the histological section examined from the ovaries of three species suggests that oogonia population significantly increased in fishes from greenhouse pond. For instance, in *L. bata* ovary recovered from greenhouse pond had 46±5 numbers

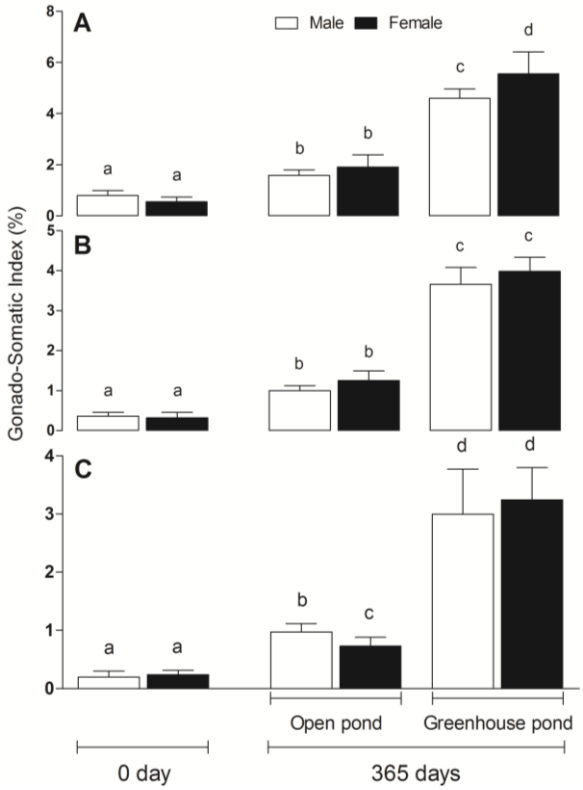

Figure 3. Changes in the gonado-somatic index of three species of carp *Hypopthalmichthys molitrix* (A), *Labeo bata* (B) and *Cirrhinus mrigala* (C) from open and greenhouse pond between 0 and 365 days. Columns with different letters vary significantly (Tukey's multiple comparison test, P<0.05). Data presented as means ± standard deviation.

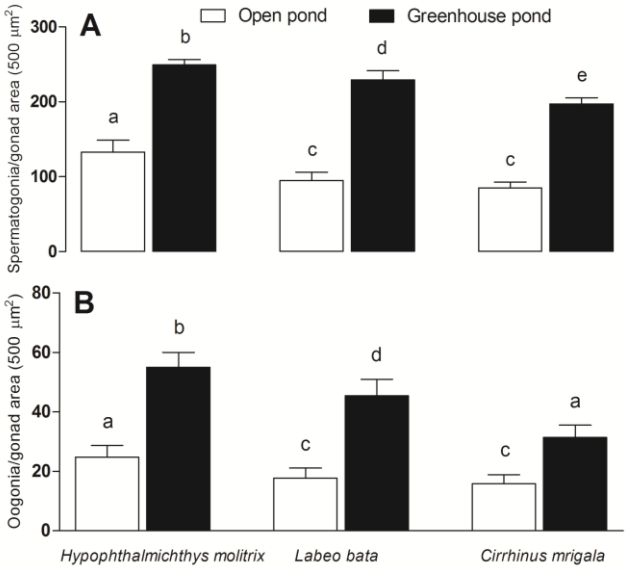

Figure 4. Changes in the number of spermatogonia and oogonia per unit area of gonadal cross section in three species of carp *Hypopthalmichthys molitrix*, *Labeo bata* and *Cirrhinus mrigala* from open and greenhouse pond; male (A) and female (B). The gonial cells in the gonads of three fish species derived from greenhouse pond have rapidly proliferated and increased in number at the end of 12 months culture period compared to fishes from open pond. Columns with different letters vary significantly (Tukey's multiple comparison test, P<0.05). Data presented as means ± standard deviation.

of oogonia cell per 500 µm^2 over its counterpart from open pond (17±3 cells/500 µm^2, P<0.05). The similar trend was evident in other two species (Figure 4). Among the 5 ovary examined from each species, all *H. molitrix* but 80, 40% of *L. bata* and *C. mrigala*, respectively had prominent cysts of oogonia interspersed with maturing oocytes. The remaining 20 and 60% of *L. bata* and *C. mrigala* had ovary

Table 2. Frequency of individuals per category of histological appearance of the testes and ovaries in *Hypopthalmicthys molitrix*, *Labeo bata* and *Cirrhinus mrigala* after 12 months culture in greenhouse and open ponds for germ cell proliferation.

Treatments	Fish species	Sex	Number of fish		
			Histological category		
			I	II	III
Greenhouse pond	*Hypopthalmicthys molitrix*	♂	5	-	-
		♀	4	1	-
	Labeo bata	♂	4	1	-
		♀	3	2	-
	Cirrhinus mrigala	♂	3	2	-
		♀	2	3	-
Open pond	*Hypopthalmicthys molitrix*	♂	-	4	1
		♀	-	1	4
	Labeo bata	♂	-	3	2
		♀	-	-	5
	Cirrhinus mrigala	♂	-	-	5
		♀	-	-	5

Histological categories are described in Table 1.

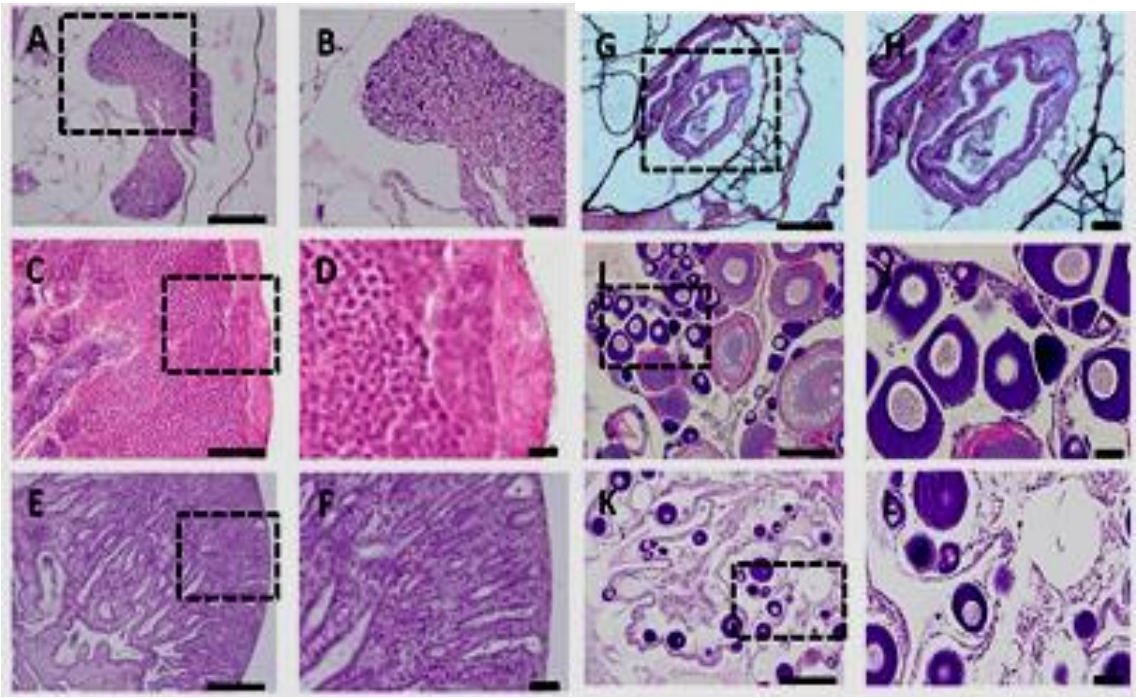

Figure 5. Histological appearance of the fish gonads from open and greenhouse pond at the beginning and end of 12 months culture period (haematoxylin and eosin). Panels on the right are high magnifications of insets inside the left panels. A, B) Immature testis of *Labeo bata* at the begging of experiment showing small spermatogonia cells at the peripheral region. C, D) Testis of *Labeo bata* recovered from greenhouse pond after 12 months culture period showing a thick germinal epithelium and accumulation of spermatozoa in the lumen of the efferent ducts, which indicate active spermatogenesis. E, F) Testis of *Labeo bata* recovered at the same time from open pond showing small cysts of spermatogonia in the periphery of the gonad (arrows), spermatocytes and absence of other types of germ cells. G, H) Immature ovary of *Cirrhinus mrigala* at the begging of experiment showing isolated small oocytes. I, J) Ovary of *Cirrhinus mrigala* recovered after 12 months culture period from greenhouse pond showing oocytes at advance stage of development (arrows). K, L) Ovary of *Cirrhinus mrigala* from open pond at the same time showing the presence of few oocytes at comparatively early stage of development (arrow). Scale bars indicate 100μm (A, C, E, G andI) and 20μm (B, D, F, H and J).

containing fewer oocyte at the stage of maturing. On the other hand, females from open pond were mostly seen to contain only few small cysts of oogonia but lack all other active oogenic stages, suggesting warm water regulate the proliferation of gonadal germ cells and augment maturation (Figure 5).

Water Temperature and Fish Stress

The monthly blood plasma glucose level, an indicator for stress, in three carp species from both open and greenhouse pond is presented in Figure 6. The study recorded increased level of plasma glucose

in animals from open pond, touching the maximum during winter months (November to February). The order of average (12 months) plasma glucose level in the three species from open pond are *C. mrigala* (7.47±0.93 $mmol^{-1}$) >*L. bata* (6.55±1.05 $mmol^{-1}$) >*H. molitrix* (5.61±0.7 $mmol^{-1}$) and greenhouse pond are *H. molitrix* (2.65±0.89 $mmol^{-1}$) <*L. bata* (3.16±0.98 $mmol^{-1}$) <*C. mrigala* (4.96±1.01 $mmol^{-1}$). These results indicate that, fishes from open pond encounter comparatively more stress due to low water temperature prevails in nature.

Discussions

The significant weight gain in all the three carp species cultured at comparative elevated water temperature, and advancement in their gonadal maturation in considerably short time over their counterparts from open pond confirm that; 1) water temperature is indispensable abiotic factor in the lives of teleost fish and immensely affects growth and reproduction, 2) manipulation of this factor lone could significantly benefit the aquaculture sector, especially in eastern Himalayan region where water temperature remain low throughout the year and pose hindrance to fisheries activities, as most of the commercially important fish species fail to attain good growth in captivity. Overall, the results obtained in this study suggest that, greenhouse based fish farming could be a viable option in the eastern Himalayan region for growth enhancement in commercially important fish species and development of brood stock in considerably short period thereby cutting significantly cost of raising broods for seed production.

The study recorded approximately 10 % mortality at lower temperature (open pond) during the winter months (November-February), mostly in *L. bata* and *C. mrigala* (P>0.05; results not shown). Although no pathology condition of any kind were observed in those dead animals and the water quality parameters of the two ponds were within the acceptable range for aquaculture throughout the study period (Figure 7) (Boyd, 1982), the mortality was largely suspected to be due to low temperature stress (Figure 6; Beitinger *et al.*, 2000). This conclusion was also burn out due to the fact that, glucose level in blood plasma of *L. bata* and *C. mrigala* recovered from open pond increased significantly oppose to the fishes from greenhouse pond. Often during winter month severe mortality are reported in the fish culture pond of eastern Himalayan region of India (Bhatt and Bujarbaruah, 2011; Das *et al.*, 2012) largely due to

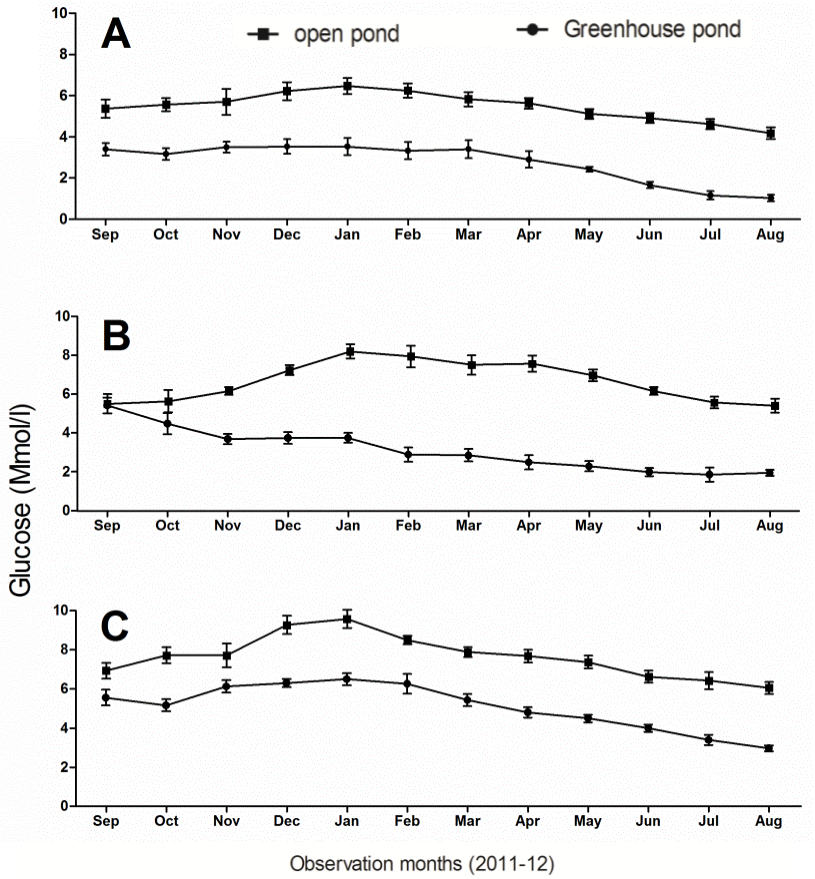

Figure 6. Monthly changes in blood plasma glucose level in three species of carp *Hypopthalmichthys molitrix* (A), *Labeo bata* (B) and *Cirrhinus mrigala* (C) from open (■) and greenhouse (●) pond during 12 months culture period. There was remarkable increase in plasma glucose level in all the three carp species recovered from open pond. Data presented as means ± standard deviation.

sharp fall in water temperature in ponds and tanks <15°C and remain low for months together (Dash *et al.*, 2007; Xu *et al.*, 2009). In such prevailing climatic conditions, preventing the animals from coldwater-induced stress always pose a grave challenge to the fish farmers. The results obtained in this study suggest, mortality in cultivable fish species due to low water temperature could be partly overcome by rearing the animals under greenhouse ponds.

There was significant increase in somatic weight of three carp species in both the culture system. However, weight gain was much more prominent in fishes recovered from greenhouse pond, especially after 6 months from the beginning of experiment. Usually teleost fish attains better growth and effectively utilize the feed when reared at comparatively higher temperature ranges versus the lower range (Gadowaski and Caddell, 1991). Numerous research findings across the fish species suggest that, at elevated temperature the activities of digestive enzymes increases and facilitate the digestion of the nutrients, resulting in good growth (Ray *et al.*, 2010; Beitinger *et al.*, 2000; Quintana *et al.*, 2004). Confirming the previous reports, this study observed that slight increase in water temperature, although not beyond the tolerance limits, help the carp in efficient utilization of feed and results in better growth. This observation has significant implication in aquaculture sector of eastern Himalayan region, particularly in boosting the fish production. However, it is believed that growth in carps could be further accelerated by combining the beneficial effects of warm water and nutritionally rich balance diet. This strategy would make the aquaculture more profitable in eastern Himalayan region. Future study should investigate the possibility of growth augmentation in native fish species of the region by cultivating them under greenhouse system. This might increase the commercial importance of some fish species like chocolate mahseer *Neolissochilus hexagonolepis* that is nutritionally superior to many cultivable fish species (Sarma *et al.*, 2013) but do not attract the

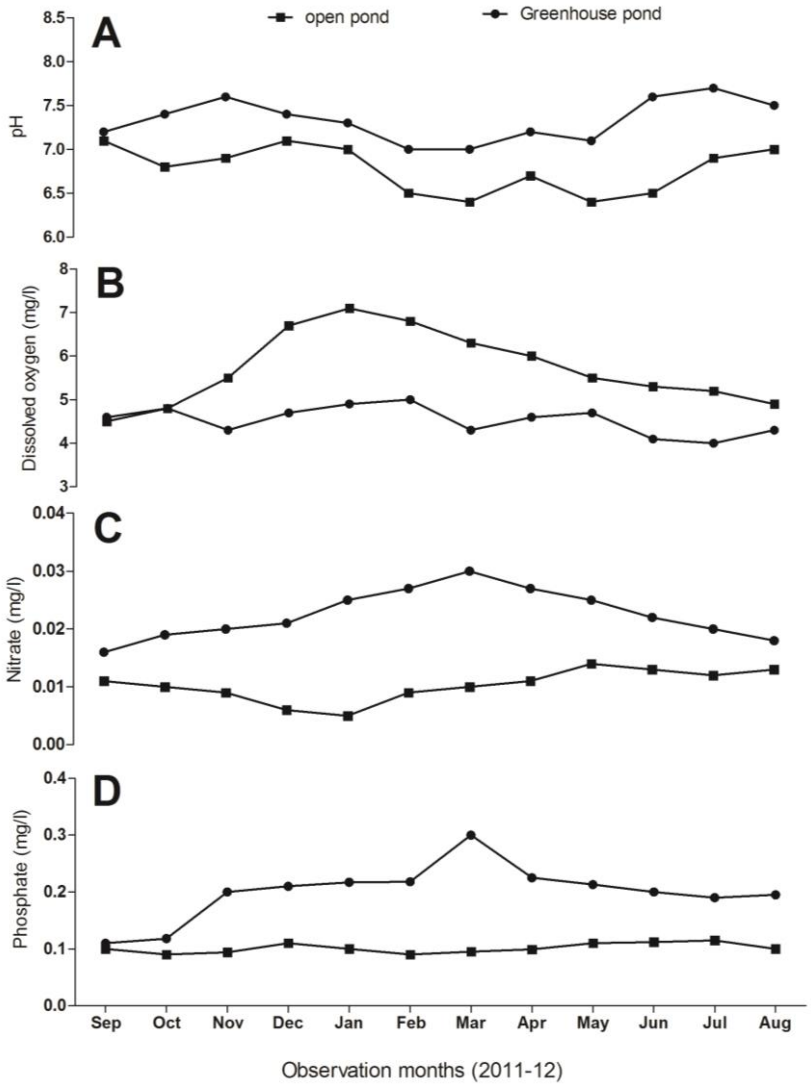

Figure 7. Monthly variation of important water quality parameters (A: pH; B: Dissolved oxygen; C: Nitrate; D: Phosphate) in open (●) and greenhouse (■) pond during 12 months culture period. Data presented as means of replications (n=2).

consumer due to small size and poor body weight gain in wild.

In general, the Gonado-somatic index steadily increased in all the three carp species reared under both open and greenhouse ponds between 0 and 365 days. However, the increase was more prominent in elevated water temperature induced by greenhouse. For instance, at the end of this study, the GSI value in *C. mrigala* males and females had reached 3.0±0.77% and 3.25±0.55%, respectively at greenhouse pond than their peers at open pond (males, 0.97±0.14%; females, 0.72±0.14%; $P<0.05$). The similar trend was also recorded in other two species. Usually the fishes from open pond at mid hill condition (>1000 m msl) of eastern Himalayan region takes additional 1.5∼2 years to match the GSI value obtained in this study for *C. mrigala* from greenhouse pond (unpublished data of Majhi SK; also see Jhingran and Pullin, 1985; Dash *et al.,* 2007). Such phenomenal delay in attaining first sexual maturity in IMCs and minor carps add considerably to the expense to raise brood stock in captivity for seed production. In this context, GSI results obtained from three species in this case suggest that, the gonadal index, a proxy of gonadal maturation, could be significantly augmented in commercially important fish species from temperate region under greenhouse based culture system. Nevertheless, the future study should aim at further shortening the maturation time in carp by combining the benefits of synthetic hormones, nutrition enrichment through diets and elevated water temperature.

In this study, comparative elevation in water temperature (2∼5°C) caused rapid proliferation and differentiation of germ cells in the testes and ovaries of all the three carp species after one year culture period. The noticeable changes observed included the appearance of all the stages of spermatogenesis between spermatogonia and spermatozoa cells in males and enlargement of the ovary, hypertrophy of the ovigerous lamella, large cyst of oogonia population with cortical alveoli oocytes and spread of mature ovum in females. Quintana and colleagues also recorded a similar degree of gonadal maturation in *Brachyhypopomus pinnicaudatus*, a freshwater fish, after exposure to 28ºC and noted that the level of gonadal maturation was equivalent to that observed in wild animals during summer (Quintana *et al.,* 2004). They concluded that such phenomenal progress in gonadal integrity represents a natural response to elevated temperature during the annual thermal (and reproductive) cycle. In this context, the germ cell profile seen in the three fish species after 12 months at elevated water temperature probably represents also a response to "continuous summer" that ultimately resulted in rapid proliferation and differentiation of gonadal cell. This must be explored in other commercially important fish species because of the obvious advantages of non-chemical methods. Further, judging by the frequency of animals with possession of different types of germ cells in the gonadal sections during histological observation and cell counting, males of all the three species were found to be heat-sensitive (Ito *et al.,* 2003; Batlouni *et al.,* 2009) and germ cells (GCs) were rapidly proliferated and differentiated than females. Although animals from open pond (low temperature) also exhibited GCs proliferations, but at comparatively slower pace. This indicates that, gonadal germ cells proliferation and differentiation in teleost fish is directly linked with the surrounding water temperature.

Conclusion

The Indian carps are widely cultured fish species in the eastern Himalayan region, although they originate from tropical environment. This is probably due to thorough knowledge available in the public domain on the species account starting from brood stock development, breeding to culture. The results of this study confirm that, carp culture in eastern Himalayan environment could be viable and the gonadal maturation could be augmented to attain first sexual maturity in 1.5∼2 years by culturing them in greenhouse pond, oppose to 3∼4 years in nature. Although this study used conventional feedstuff to feed the animals during the study period, further investigation should be done to observe the changes in weight gain and reproductive indices by feeding the animals with nutritionally rich formulated diets.

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