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Impact of Temperature Variations on Breeding Behavior of *Cirrhinus mrigala* during Induced Spawning

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SAK designed and conducted the research trial. MA supervised the research work. DS and SI helped in data collection. SS and UA assisted in manuscript write-up. SA did the statistical analysis of data. FA and MSH reviewed the manuscript.

Key words
***Cirrhinus mrigala*, Induce spawning, Ovaprim, Temperature variation, Embryonic development**

ABSTRACT

Cirrhinus mrigala naturally breeds in rivers and streams during monsoon season but in ahatchery, it can be induced to spawn by the administration of ovaprim or its analogues. However, the conditions of the induced environment vary from place to place as well as the dose of ovaprim. The present investigation is focused on possible impact of different temperatures on the induced spawning behavior of *C. mrigala*. The factors investigated include the ovulation time, embryonic and larval development, and yolk sac absorption induced at 4 different water temperatures viz. 26, 29, 32 and 34 ºC. *C. mrigala* displayed the maximum release of eggs, fertilization and hatching rates at 29 ºC. The maximum number of eggs were observed in 26ºC whereas the lowest were obtained in 34ºC. 85020.48). However, the temperature exceeding 30ºC not only increased the cell growth but significantly decreased the hatching time. On the contrary, temperature exceeding 32ºC caused denaturation of all eggs and larvae that resulted in reduced hatching rate. Microscopic examination revealed that eggs were more influenced by high temperature at early stages of development. Impacts of slight change in water temperature were observed from Blastodisc to morula stage. After gastrula stage, temperature effects were evident but not critical. This was a first documented report on effects of temperature on breeding performance and embryonic development of *C. mrigala* from Pakistan.

INTRODUCTION

Cirrhinus mrigala is one of the most important Indian major carps and integral component of present semi-intensive fish polyculture system (Beyers and Rice, 2002). It is benthic feeder targeting detritus and left over food items from other surface feeders. It does not breed in captivity therefore it is induced to spawn by variety of fresh and synthetic hormones.

The induced breeding in hatcheries assisted in mass production of quality carp seed under controlled condition and assured timely supply of stocking material for culture farms (Mohapatra *et al*., 2016). Induced breeding is based on the principles of manipulating hormonal or environmental factors for stimulation of reproduction in fishes (Marimuthu *et al*., 2000). Use of exogenous hormones is an effective way to induce reproductive maturation and produce fertilized eggs (Mylonas *et al*., 2010). Naturally it breeds during monsoon when temperature and other environmental conditions are favorable (Donaldson *et al*., 2008). Artificially it is induced to spawn by ovaprim dose which is a synthetic hormone registered with Syndel Laboratories, Canada (Syndel International Inc, 2003). It is the most common and effective synthetic inducing agent used extensively at fish hatcheries for commercial production of variety of fish species. The first introduced synthetic hormone was ovaprim-C and has set good results with dosages for females ranging from 0.5-0.7 ml/kg^{-1} for spawning, fertilization and hatching of Indian and Chinese carps (Afzal *et al*., 2008).

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Due to poikilothermic nature of fish, water temperature has always a key role in its life whether it is growth or breeding (Mills and Hurley, 1990). When temperature deviates from the normal, it stresses the fish and impairs physiological and behavioral activities (Beyers and Rice, 2002; Donaldson *et al*., 2008). For larval fishes, water temperature can influence growth rates (Leggett and Deblois., 1994), swimming performance and predator avoidance (Leggett and Deblois., 1994; Pepin, 1991), as well as metabolic rate (Blaxter, 1991) and cellular function (Somero and Hofmann, 1997). Large, abrupt changes in temperature can induce significant larval mortality which in turn has profound impacts on year-class strength (Planque and Fredou, 1999). Temperature effects can be positive or negative. Temperature induces growth, maturity, and decreases egg hatching rate if in acceptable range; if violates, it then disrupts its whole physiology and kills the fish consequently affecting recruitment (Pepin, 1991; Marimuthu *et al*., 2000). However, fish species which are capable of tolerating wide temperature ranges (eurythermal) behave quite differently and temperature effects are less prominent (Marimuthu *et al*., 2000).

Present study was therefore, conducted to explore the optimum breeding temperature with maximum output in terms of release of eggs, rate of fertilization, hatching, embryological stages and survival of fry produced in *C. mrigala* in hatchery conditions.

MATERIALS AND METHODS

Breeding trials

Healthy and mature fish were collected from pond complex of Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences Ravi Campus Pattoki, Punjab, Pakistan, and transferred to hatchery in brood stock holding tanks for conditioning. Four breeding trials were conducted at different temperatures during whole breeding seasons. Total of 36 brooders (24 males and 12 females) used in this study were divided into combination of two males and one female fish in each circular tank. Fish was induced to breed at four different temperatures viz. 26, 29, 32 and 34ºC. Other water quality parameters like dissolved oxygen, electrical conductivity and pH were also recorded (Table I). Prior to hormone administration fish were weighed and then the hormone dosage was calculated following standard injection rate of 0.4 ml/kg body weight to female and 0.2 ml to male. The males and females were released into respective circular spawning tanks maintained at temperatures of 26 ºC, 29 ºC, 32 ºC, and 34 ºC at different times of peak spawning season. During spawning process water flow was maintained at the rate of 23 liter/min to facilitate ovaprim best performance in inducing ovulation in female and release of sperms in male. In 26ºC group female fish started to release eggs after 8 h and 35 min in 26 ºC group, after 9½ h in 29 ºC group, after 9 h and 38 min in 10 h and 16 min in 34 ºC group after ovaprim injection.

Counting of eggs and hatchlings

Hatching rate was calculated from the number of eggs in a brood and the number of larvae hatched out (based on the method of Soundarapandian, 2008). The number of live ones was estimated volumetrically by taking 50 ml samples (Yen and Bart, 2008).

Examination of developmental stages of eggs

Eggs were collected at different time intervals from the incubation tank and each stage was comparatively observed to identify effects of varying temperatures. Egg diameter and yolk mass were measured with the help of eye piece micrometer.

Statistical analysis

Data were analyzed by using one-way ANOVA technique (F test, $P \leq 0.05$) to assess the impact of temperature (26, 29, 32 and 34 ºC) on reproduction, fecundity and hatching of *C. mrigala.* Significant treatment means were separated through Duncan's Multiple Range test with the help of SAS software (version 9.1). Following mathematical model was applied:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where, Y_{ij} is observation of dependent variable recorded on i^{th} treatment group; μ is population mean; τ_i is effect of i^{th} treatment group ($i = 1, 2, 3, 4$) and ε_{ij} is residual effect of j^{th} observation on i^{th} treatment group NID ~ 0, σ^2.

RESULTS AND DISCUSSION

Water quality parameters

The study indicated slight change in water quality parameters like dissolved oxygen, electrical conductivity and pH with increase in temperature; however, this change was not influential on breeding and larval development. Therefore, a non-significant influence of these parameters was observed on ovulation, fertilization and hatchability of *Cirrhinus mrigala*. These results were in line with a study conducted on Lake Malawi Tilapia (Chambo), *Oreochromis karongae* in which dissolved oxygen, electrical conductivity, pH and ammonia did not significantly influenced hatching period and the decrease in hatching period was attributed to temperature increase (Valeta *et al*., 2013). So, the temperature is an important factor determining egg and larval development as it influences metabolic rate (Blaxter, 1991; Kamler, 2008)

and cellular function (Somero and Hofmann, 1997).

Effects of temperature on fecundity

During current study, the fish released eggs after 9.79 h of ovaprim administration in T_1, 9.59 h in T_2, 10.70 h in T_3, and 11.32 h in T_4 (Table II) which means that fishes kept at 29ºC took the minimum time to ovulate and when compared it with T_1, T_3, and T_4 (Table II). Brooders in T_4 took maximum time (11 h 17 min) to ovulate. These observations were in close agreement with those of Nandeesha *et al*. (1990). Egg releasing time directly corresponded to rise in temperature but the opposite was true when temperature reduced from 29 ºC. Similar results were reported at temperature range of 29-32 ºC in which spawning was observed after a latency period of 5-6 h (Mohapatra *et al*., 2018). Maximum fecundity was observed in 29 ºC group, whereas minimum was observed in at 34 ºC; moreover, a slight variation in fecundity at 26 ºC and T_3 (31 ºC) was also noted. Current findings reveal that 29 ºC is an optimum temperature for maximum effectiveness of ovaprim and subsequent release of eggs which is evident by the number of eggs released which

Table I. Some physicochemical quality parameters of water used in the experimental work.

Parameters	T1 (26°C)	T2 (29°C)	T3 (31°C)	T4 (34°C)
Temperature (ºC)	26.04±0.06^a	28.99±0.09^b	30.96±0.08^c	33.91±0.05^d
Dissolved oxygen (mg/L)	5.40 ± 0.01	5.42 ± 0.01	5.39 ± 0.01	5.41 ± 0.01
Electrical conductivity (mS/cm)	2.54 ± 0.05	2.42 ± 0.04	2.51 ± 0.05	2.52 ± 0.04
pH	7.53 ± 0.04	7.53 ± 0.17	7.62 ± 0.11	7.63 ± 0.11

Superscripts on different values within rows differ significantly at $p \leq 0.05$

Table II. Effect of different water temperatures on different breading parameters and egg development stages of *Cirrhinus mrigala* during induced spawning.

Parameter	Temperature ºC			
	26°C	29°C	31°C	34°C
Egg releasing time (h)	9.79±0.06^c	9.59±0.04^d	10.70±0.04^b	11.32±0.07^a
Eggs released kg $^{-1}$	96225.38±117.11^c	110676.78±268.59^a	97959.94±184.05^b	85020.48±115.00^d
Eggs fertilized	81821.68±148.95^b	100293.23±206.40^a	78292.93±76.62^c	63720.99±196.44^d
Fertilized eggs (%)	85.98±0.48^b	91.58±1.07^a	79.39±0.79^c	75.49±1.11^d
Hatchlings produced	71051.60±206.09^b	91538.00±117.17^a	63906.42±227.74^c	40461.90±106.96^d
Eggs hatched (%)	86.86±0.11^b	91.30±0.48^a	79.98±0.49^c	63.48± 0.57^d
Fry obtained after yolk sac absorption	63957.51±105.57^b	86743.90±105.71^a	57739.49±69.85^c	35739.23±156.89^d
Survival rate	77.64±0.45^b	86.95±0.35^a	73.26±0.75^c	56.04±0.81^d
Egg development stage				
Blastodisc stage	20.00±0.07^a	17.02±0.07^b	13.89±0.12^c	11.92±0.12^d
2 cell stage	27.90±0.10^a	25.94±0.09^b	21.97±0.08^c	19.89±0.18^d
4 cell stage	38.02±0.12^a	33.96±0.10^b	28.04±0.09^c	26.12±0.12^d
8 cell stage	44.86±0.08^a	42.00±0.16^b	37.09±0.07^c	34.95±0.08^d
16 cell stage	57.85±0.05^a	51.11±0.09^b	44.86±0.13^c	42.14±0.10^d
32 cell stage	82.05±0.06^a	70.00±0.20^b	63.92±0.20^c	57.95±0.08^d
Morula stage Early	133.07±0.14^a	117.97±0.12^b	107.15±0.03^c	97.03±0.18^d
Mid	149.81±0.11^a	130.08±0.14^b	118.12±0.08^c	107.19±0.14^d
Morula stage Late	219.97±0.13^a	208.89±0.09^b	179.01±0.07^c	165.92±0.12^d
Early	261.97±0.12^a	250.04±0.17^b	237.09±0.15^c	229.10±0.23^d
Gastrula stage Mid	362.88±0.11^a	340.90±0.10^b	321.94±0.09^c	282.15±0.13^d
Late	425.96±0.12^a	409.03±0.16^b	379.08±0.07^c	360.02±0.04^d
Neurula stage Early (head formation)	508.92±0.16^a	473.05±0.11^b	449.23±0.05^c	419.22±0.10^d
Late (optic bud formation)	570.95±0.11^a	533.97±0.20^b	503.09±0.08^c	463.35±0.14^d

Superscripts on different values within rows differ significantly ar $P \leq 0.05$

was the highest number among all the treatments (Table II). These findings are very close to those of El-Gamal (2009) on breeding of common carp in which temperatures higher than optimum proportionately decreased fecundity. The Maximum number of eggs were observed in 29ºC group whereas the lowest were obtained in 34ºC. Similar findings were observed by Herzig and Winkler (1986) where maximum number of eggs (98503 eggs) were at 29 ºC and the lowest number of eggs (1937) were obtained at 34 ºC. These findings validate that exceeding temperature beyond optimum limit affect the eggs released.

Effects of temperature on fertilization rate

Current findings on *C. mrigala* further confirm that the highest fertilization rate was observed at 29 ºC and the lowest eggs got fertilized at 34 ºC (91.58 vs. 75.49%). Similarly, number of hatchlings produced were lower in 34ºC group while the higher were reported in 26ºC (91.3 vs. 63.48%). Similar fertilization rate of 95-100% and 90-98% hatching success was observed in *Labeo rohita* at a water temperature of 28-31 ºC using hormone Ovatide (Pandey *et al*., 2002). Fry recovered after yolk sac absorption followed the same pattern as observed in egg fertilization rate; however, spawn production was found to be lower as compared to the results by Mohapatra *et al*. (2018) in which spawning fecundity was found to be 0.13-0.182 and 0.125-0.158 million egg/kg female body weight of *Labeo rohita* and *C. mrigala*, respectively. Similar results were observed in case of fertilization in which fertilized eggs (%) during spawning was found to be 90-100% for both species (Mohapatra *et al*., 2018). Spawn production per kg female body weight was found to be lower as compared to Mohapatra *et al*. (2016) in which spawn production per kg female body weight was found to be 0.109-0.14 million eggs/kg for *C. mrigal*a and spawn survival (%) from fertilized egg ranged 87.3- 94.74% in *C. mrigala*. Previously it has been observed that abrupt fluctuations in temperature negatively affected growth rates of larval fishes (Leskelä and Kucharczyk, 1995), metabolic rate (Blaxter, 1991) and cellular functions (Somero and Hofmann, 1997). These findings are quite in line with findings in current study which further confirmed that increase in temperature beyond optimum limit is not favorable to the developing egg. For the reasons it is evident from our finding and can also be suggested that 29 ºC is the optimum temperature for induced breeding of *C. mrigala* in Punjab Pakistan. Any sort of fluctuations in terms of increase or decrease in temperature pose a noteworthy impact on egg release, fertilization percentage and hatching of eggs while though it is slightly in contradiction to the findings of Das *et al*. (2006) who reported the highest hatching rate at 31 ºC in *Labeo rohita* which might be due to species differences.

Effects of temperature on early stages of development

Table II also shows the effect of temperature on the early developmental stages specifically up till morula stage. Microscopic examination of egg development stages revealed denaturation in egg contents when temperature exceeded beyond optimal limit. Previous studies have demonstrated that temperature has direct effects on incubation of eggs (Kamler *et al*., 1998; Rechulicz *et al*., 2002). In present study, eggs incubated at 29ºC showed normal development pattern while increase in temperature to some extent fasten the embryonic development. Similar findings confirm our results that embryonic development responds positively during optimum temperature and if temperature deviates from the normal then it shows abnormal developments and sometimes resulting in even death of egg and/or hatchling by Kamler (2008) and Bermudes and Ritar (1999). Faster multiplication of cells decreased hatching time but decreased hatching rate indicating that 32-34 ºC is not favorable to normal embryonic development.

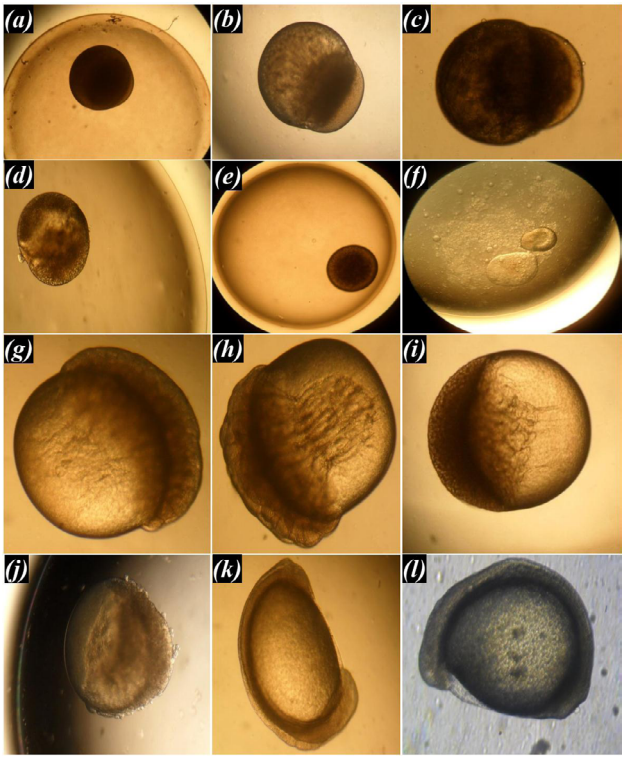

Fig. 1. Temperature dependent developmental stages of *Cirrhinus mrigala.* **(a)** blastodisc stage observed at 26 ºC, **(b)** blastodisc stage observed at 29 ºC, **(c)** blastodisc stage observed at 32 ºC, **(d)** blastodisc stage observed at 34 ºC, **(e)** fertilized egg at 29 ºC, **(f)** denatured fertilized egg at 34 ºC, **(g)** morula stage at 26 ºC, **(h)** morula stage at 29 ºC, **(i)** late morula stage at 29 ºC, **(j)** late morula stage at 34 ºC, **(k)** yolk plug stage at 29 ºC, **(l)** yolk plug stage at 32 ºC.

Eggs kept at 27-29 ºC appeared more robust with healthier hatchlings than those kept at 32-34 ºC. It was observed that at high temperature time difference between different stages was reduced and a gap of 25 to 30 min was observed between some stages (Fig. 1). Higher temperatures also increased rupturing of the egg shells that sometimes led to premature hatching and consequently death of hatchlings which was very much evident in that batch where temperature remained over 32 ºC, and lot of larvae perished at different stages of development even when healthy larvae were transferred to this temperature they could not flourish and succumbed (Fig. 2). Effect of different temperatures was not limited to fertilization and hatching but when hatched out larvae were measured larvae developed at 29 ºC significantly longer to those grown up at 26 or 32 ºC.

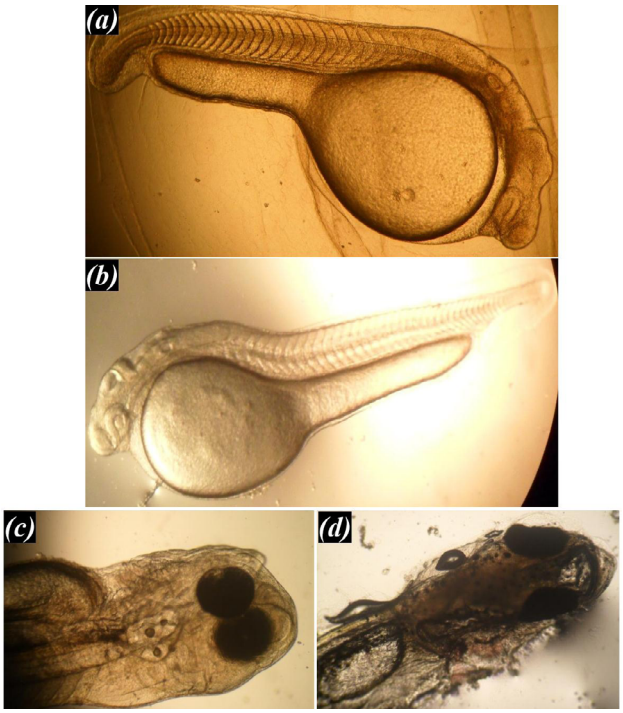

Fig. 2. Process of eggshell thinning in *Cirrhinus mrigala* **(a)** premature hatchling, **(b)** normal hatchling, **(c)** normal fry reared at 29 ºC, **(d)** denatured fry reared at 34 ºC.

CONCLUSION

Temperature has a great influence on fish breeding. Increase in temperature gradually enhances the breeding performance and embryonic development up to optimum value. In our study Exposure of eggs of *C. mrigala* to temperature exceeding 32ºC during early stages of development resulted in an abnormal development of the eggs. High temperature beyond optimum limit resulted in egg shell thinning and premature hatching of eggs which perished before the absorption of yolk sac. This study could provide useful insights into the role of temperature in induced spawning of fishes.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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