International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 5; September 2018; Page No. 01-04

Studies on effect of temperature on fertilization and hatching rate of *Pangasianodon hypophthalmus* (Sauvage, 1878) eggs

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Abstract
Pangasius fish (*P. hypopthalamus*) is an exotic striped catfish, introduced in India during the year 1997. Farming of *P. hypopthalamus* is an important aquaculture Andhra Pradesh. The present study is carried out to determine the effect of temperature on fertilization and hatching rates of *P. hypopthalamus* eggs. This experiment was investigated under controlled temperature hatchery. Stripping was done 6 and 9 hours after hormonal injection with crude pituitary gland extract and Ovatide respectively. The water temperature was controlled with an adjustable electric thermostat heater empowered by a generator while other water quality parameters were kept constant in acceptable ranges. The eggs were mixed with the milt using chicken feather and the fertilized eggs were transferred to hatching pool. The temperature for fertilization and hatching maintained in the range of 24-34ºC with 2ºC intervals. Fertilization and hatchability of the eggs were significantly highest ($p < 0.05$) at 28ºC and 30ºC compared to other tested temperatures. The results also reveal that the hatching time decrease when the temperature increases, which indicates a positive correlation between incubation temperature and embryonic development rate. Fertilization and hatchability of *P. hypopthalamus* eggs are best achieved at temperature range of 28 - 30ºC.

Keywords: *P. hypopthalamus*, fertilization, temperature, hatching, hatching time

1. Introduction
Pangasianodon hypophthalmus is native of Mekong Basin and Chao Phraya River in Thailand, Cambodia and Vietnam. It has been introduced in Singapore, Philippines, Taiwan, Malaysia, China, Myanmar, Bangladesh, Nepal and India. The fish was introduced to India in the State of West Bengal probably in 1997 from Bangladesh where this fish is largely cultured in monoculture as well as polyculture (Mukai, 2011) [7].
Pangasianodon hypophthalmus is the important fresh water aquaculture species in Andhra Pradesh and is popular and profitable after carp culture. In Andhra Pradesh, the culture of P. *hypopthalamus* farms are located in Krishna and West Godavari districts of Andhra Pradesh.West Bengal is major source for *Pangasius* seed production and supply in India. However, its culture is affected by both abiotic and biotic factors.
Sexual cycle related to the environment, Changes in environmental condition will influence the growth, survival and reproduction and egg hatching (Cacot, P., 1999) [3]. Temperature is a fundamental physical regulatory factor in the lives of fishes and this effect is expressed particularly strongly in the control of all reproductive processes from gamete development and maturation, ovulation and spermiation, spawning, embryogenesis and hatching, to larval and juvenile development and survival. In reproductively mature adults, temperature is generally considered to be a secondary due to photoperiod in phasing reproductive seasonality but it has a major role in synchronising the final stages of reproductive maturity, and also in truncating reproductive episodes (reviewed in Pankhurst and Porter 2003) [10]. The effects of temperature can be differentially expressed depending on when in the annual thermal cycle spawning normally occurs, with increasing spring temperatures being required to cue maturation in spring and early summer spawners (Stacey (1984) [16]; Scott and Pankhurst (1992) [13] and Shimizu (2003) [14], but elevated temperatures delaying the onset of maturation and ovulation in autumn-spawning species (reviewed in Pankhurst and King 2010) [10]. Temperature has a similarly important role in the modulation of post-fertilization processes both through its rate-determining effects on embryogenesis and hatching (Pauly and Pullin 1988) [11]. and subsequent larval development (Howell *et al*. 1998) [5], growth (Jobling 1997) [6] and survival (Sponaugle and Cowen 1996) [15].
The present study was conducted to detect the influence of temperature on fertilization and hatching in *P. hypophthalmus.*

2. Materials and methods
Breeding and hatching experiments were carried out at government issued fish seed farms, Penamaluru, Vijayawada. Male and female brooders of *P. hypophthalmus (*Family Pangasiidae) were collected from same center. Adult *Pangassius* fish males and females having age of 3 years, showed signs of sexual maturation and a total of 120 No. of both sex fish; average weight and length of male were 2.4 kg, 53 cm, and female 2.9 kg, 50 cm were used for breeding studies. Brood fish selected visually based on secondary

sexual characteristics, condition and experience.

The six temperature treatments were based on a pilot study that recorded 100% mortality at <21ºC and >39ºC. The treatments here were: 24ºC, 26ºC, 28ºC, 30ºC, 32ºC, 34ºC. For the 24ºC, 26ºC treatment, tanks were set up in a cold room using an air conditioner to maintain the desired temperature. Individuals assigned to higher temperature treatments (28ºC, 30ºC, 32ºC, 34ºC) were acclimated gradually to their individual treatment temperatures before the experiment commenced using thermostats in a stepwise fashion at 2ºC per day until all tanks had reached their target temperatures.

Selected males and females were injected with a crude pituitary gland extracts (CPGE) and Ovatide, a synthetic hormone according to recommended dose and dosage depending upon the physiological status of fishes. Male brooders were also given CPGE at the time of second injection to females, whereas in studies with Ovatide, single recommended dose is given at same time to male and female fish. Injected brooders were kept in cemented breeding tanks of size (3 x 2 x 1 m) with flowing water. Physicochemical parameters of the water during the breeding and hatching experiments were analyzed as per APHA (1998). Fish responded positively and ovulated, later gametes are collected by gentle squeezing of the abdomen. The gametes of ripe male fish (n=1.5) and a female fish (n=1) were combined in a clean, dry container and gentle mixing of both using a chicken feather for a 1-minute period and stickiness of eggs is removed by milk treatment, immediately after fertilization the diameter of egg increased owing to slight swelling and size is 1.12-1.18 mm with a transparent layer. Fertilized and un-fertilized eggs were distinguishable with naked eye. Fertilized egg were transferred to hatching pool.

Highest temperature treatment acclimations began first and in sequence such that all treatments achieved their respective experimental temperatures on the same day. The time interval from transfer fertilized egg in hatching pool to egg just hatching was recorded. Brood fish pond adjacent pond water is used for hatchery operation

Statistical analysis

All the experiments were performed in quintuplicate and results are presented as mean ± SEM (Standard Error of Mean). The results are considered to be significant at $P<0.05$. All statistical analysis was performed in MS-Excel and the graphs were drawn using Graph Pad prism5 software.

3. Results and discussion

Physico-chemical parameters of the water during the breeding experiments were found to be within the optimum range (Table 1).

The influence of temperature on fertilization, hatching rates and hatching time of CPGE and Ovatide injected *P. hypophthalmus* eggs are represented in table 2 and 3 respectively. The graphical representations of these results were also given in figure 1 to figure 3. Results of fertilization rate in CPGE and Ovatide induced fish, at 28ºC and 30ºC treatments showed values higher than 80% and were not significantly different ($p>0.05$) fr**o**m each other, whereas 24, 26, 32ºC and 34ºC treatments showed the lowest percentages ($p<0.05$). Hatchability of the fish eggs at 28ºC and 30ºC were not significantly higher between each other although they were significantly higher than those of 24, 26, 32ºC and 34ºC. The same results are observed in fish group induced by Ovatide. The results also suggest that the difference in temperature as a single parameter could adopt the egg development rate. Incubation temperature leads to a significant difference in time to hatch. The observations reveal that the hatching time decrease when the temperature increases, which indicates a positive correlation between incubation temperature and embryonic development rate.

Table 1: Physico-chemical parameters during the breeding experiments.

S. No.	Parameters	
1	Dissolved Oxygen mg/l	8.4 – 8.6
2	pH	7.2
3	Total Alkalinity mg/l	185
4	Total Hardness mg/l	280
5	Free carbon dioxide	--
6	Iron (mg/l)	0.1-0.2
7	Water temperature ºC	24- 34

Table 2: Effect of temperature on fertilization and hatching rate (%) of CPGE induced *P. hypophthalmus* eggs.

Weight of fish		Crude pituitary gland extract (mg/Kg)		Eggs produced In lacks	Temperature (º C)	% of fertilization	%of hatching	Time taken for hatching (h)
Female	Male	Female	Male					
2.82±0.054	2.42±0.036	5,14	5	4.2±0.179	24	64	62	20±0.360
2.84±0.040	2.38±0.054			4.5±0.089	26	72	70	19±0.434
2.81±0.058	2.39±0.080			4.4±0.134	28	92	84	18±0.481
2.79±0.107	2.42±0.054			4.4±0.268	30	89	88	16±0.420
2.81±0.062	2.40±0.063			4.5±0.179	32	78	72	15±0.540
2.78±0.035	2.41±0.067			4.2±0.268	34	62	58	14±0.250

*Each value is represented as mean ± SEM (n=5); Values are significant at $p<0.05$.

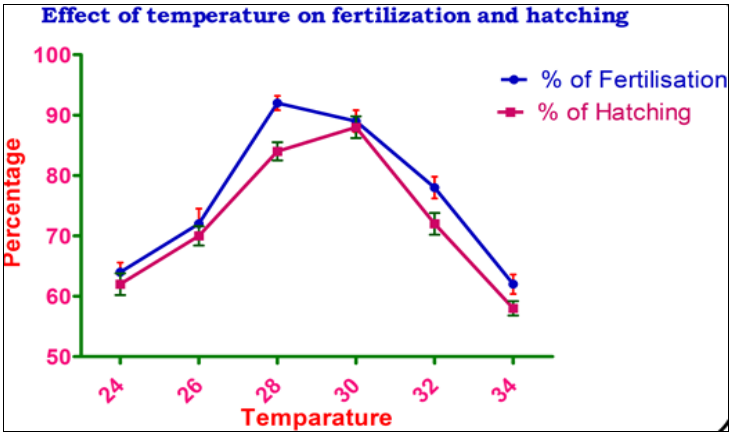

Fig 1: Effect of temperature on fertilization and hatching rate (%) of CPGE induced *P. hypophthalmus* eggs

Table 3: Effect of temperature on fertilization and hatching rate (%) of Ovatide induced *P. hypophthalmus* eggs.

Weight of fish		Ovatide (ml/Kg)		Eggs produced In lacks	Temperature (º C)	% of fertilization	%of hatching	Time taken for hatching (h)
Female	Male	Female	Mle					
2.88±0.035	2.62±0.089	0.2	0.1-0.2	4.0±0.017	24	62	57	20±0.510
2.85±0.053	2.58±0.035			4.2±0.536	26	70	70	18±0.432
2.86±0.035	2.49±0.080			4.3±0.357	28	90	81	17±0.340
2.82±0.107	2.48±0.098			4.3±0.268	30	86	84	16±0.420
2.83±0.107	2.50±0.062			4.3±0.089	32	74	68	15±0.520
2.82±0.089	2.49±0.080			4.1±0.357	34	60	58	14±0.120

*Each value is represented as mean ± SEM (n=5); Values are significant at p<0.05.

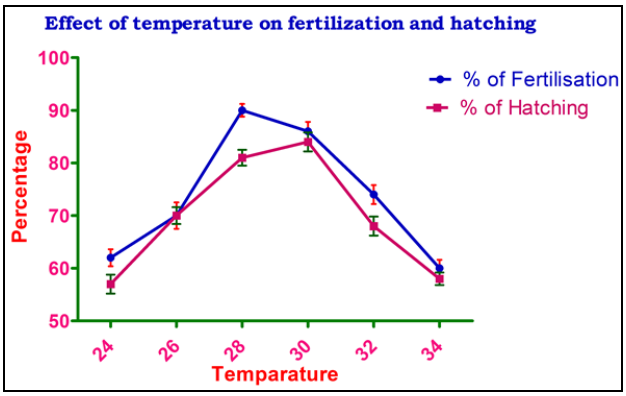

Fig 2: Effect of temperature on fertilization and hatching rate (%) of Ovatide induced *P. hypophthalmus* eggs

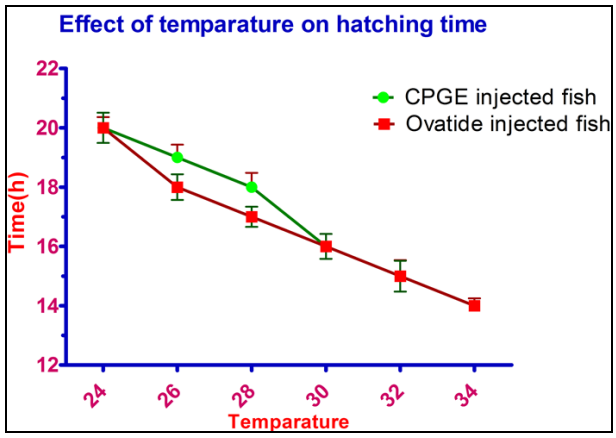

Fig 3: Effect of temperature on hatching time of CPGE and Ovatide induced *P. hypophthalmus* eggs

The study of the effect of temperature on fertilization and hatching rate of *P. hypophthalmus* eggs shows an interesting water temperature range that can be established for the culture of this specie, with some considerations. Fertilization and hatching rates showed values above 80%. Fertilization rate of *P. hypophthalmus* eggs at 28ºC and 30ºC was significantly not different from each other in the various treatments because this selected range of temperature support fertilization of fish eggs as one of the warm water fishes. The results of hatchability also show that temperature treatments of 28 – 30ºC significantly improve the hatchability of the fish eggs as temperature has main effect on hatching of fish eggs. It was reported that growth of larvae increased at the optimum temperature range of 28-30°C and no eggs hatched at 20ºC and 38ºC for *Cyprinus carpio* (El-gamal, 2009) [4]. It is most likely that temperature affects the tolerance level of viable eggs as documented by (El-gamal, 2009) [4]. The optimum range for good hatchability of catfish eggs was proved to be 28-30ºC as this temperature range is reported to be an acceptable water temperature **r**ange for warm water fishes (El-gamal, 2009) [4].

The results in this study indicated that the optimum temperature for hatchability of the eggs lies between 28ºC and 30ºC. Similar results were also obtained by S.A. Okunsebor (2015) [12] in their study of on *Heterobranchus bidorsalis*. The present finding also agrees with the results found for other fish species according to Nwosu and Holzlohnev (2000) [8]. At 24, 26, 32 and 34**º**C, hatchability was found highly reduced indicating that the temperature was very low or very high, influence the development of egg in hatchery. In addition, when temperature becomes super optimal, it influences negatively the efficiency of yolk utilization (Brett JR and

Groves, 1979) [1]. Temperature also affects physiological processes (Brett, 1979) [2] in fish hatchlings.

Temperature influence is the single most important factor that determines growth rates in fish (Brett, 1979) [2]. Above 55-70% survival rate of hatchlings, found at 24-26ºC seems reasonable but in this study 28- 30ºC gave above 80% survival of hatchlings during yolk sac larval stage optimum temperature range of 28-30ºC plays an important role for improving fertilization, hatchability and embryonic development within a limited period. This finding is very important as 40- 45% mortality at this stage can affect directly fish production.

4. Conclusion

Temperature has significant effect on fertilization and hatching rate of *Pangasius hypophthalmus.* Temperature range of 28-30ºC significantly supported fertilization and hatchability rates of *P. hypophthalmus* eggs while 80% survival of hatchlings during yolk sac stage was achieved at 28- 30ºC. High rate of fertilization and hatchability of the Pangasisus eggs was achieved at 28- 30ºC is highly recommendable for fry production.

5. References

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