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Effect of temperature on incubation period and hatching of neon tetra *Paracheirodon innesi* eggs

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Abstract: **In the commercial development of freshwater ornamental fish culture, provision of appropriate temperature for egg incubation and further development of the fish at various stages is an important aspect in successful attainment of seed production. Little work has been done on the breeding of *Paracheirodon innesi*. Since, this species is very temperature sensitive, the study was conducted to determine the effect of different temperatures on breeding behaviour, hatchability and larval rearing of *P. innesi*. Broodstock were fed three times a day with bloodworms, tubifex and cladocerans at 4% body weight. Artificially fertilized eggs of neon tetra *P. innesi* obtained after spawning of cultured broodstock were incubated at temperatures of 18, 20, 22, 24 and 26^{0}C in triplicates. The results showed that the optimal temperature for neon tetra embryonic development ranged from 22 to 26^{0}C. Highest (70 ± 1.9 %) mean hatching percentage of eggs was observed at 26^{0}C, whereas, the lowest (52.2 ± 4.8%) was observed at 18^{0}C ($P < 0.05$). The incubation period varied inversely with temperature. Eggs took 38 - 40 hours for hatching at 18^{0}C while it took only 24 - 26 hours at 26^{0}C ($P< 0.05$). Based on the results of the present experiment, the temperature range of 24 to 26 ^{0}C can be recommended as optimum temperature for artificial propagation, larval rearing and higher survival of *P. innesi*.**

Keywords: **neon tetra, Paracheirodon innesi, temperature, embryonic development, hatching success.**

1. INTRODUCTION

Ornamental fish keeping is the second most popular hobby in the world after photography and ornamental fishes are the most popular pets liked by people in the world. An estimated one billion ornamental fish are exported annually (Dykman, 2012). The world export value in 2010 was over US$ 350 million and since 1985 the value of international trade in exports of ornamentals has been increasing at an average growth rate of approximately 14% per year. The main exporting countries includes Singapore, Malaysia and Thailand. Brazil and Columbia are also significant exporters. The main importing countries include Germany, Japan, Singapore, the US and the UK. The neon tetra is the most widespread and colourful aquarium fish among the tetras. They are frequently mentioned as aristocrats of the small aquarium. It is also termed as the jewel in the aquarium tank. Their colour is magnificent and a school of neon tetras competing about catching beams of sunlight is quite a sight. Compared with the guppy *Poecilia reticulata*, the neon tetra has become one of the most popular ornamental fish kept in household. During a single month, an average of 1.8 million of neon tetras, with an estimated value of US$ 175000 were imported by the United States for the aquarium trade (Chapman *et al.*, 1997).

The neon tetra is described as 40 mm long, luminously coloured freshwater fish with a dark olive-green back over a silver-white abdomen. The fish is characterized by a shimmering blue-green lateral body stripe that covers from the head to the base of the adipose fin. A broad, shining red stripe begins at the middle of the body and extends posteriorly to the base of the caudal fin. The fins are transparent and colourless (Myers, 1936). There are three main sister species of neon tetra *P. innesi* (Myers, 1936), *P. axelrodi* (Schultz, 1956) and *P. simulans* (Gery, 1963).

An essential step in the successful culture of any species is to understand the optimal environmental conditions for egg incubation. Temperature is one of the most decisive environmental variables affecting embryonic development in fish eggs (Bra¨nna¨s, 1987; Beacham and Murray, 1990; Baynes and Howell, 1996; Bermudes and Ritar, 1999; Kamler, 2002). Captive breeding and seed production of neon tetras required more intensive investigations in scientific lines for better growth and survival. Yet breeding protocol of neon tetra has not been understood scientifically. Temperature differentiation studies on this species are essential to assess its temperature limits so that we can manipulate the environmental conditions to improve its breeding conditions, hatching rate, and growth of larvae thereby enhancing its ornamental value in the market.

The present investigation is a part of a larger study of effects of extrinsic factors on the hatching success and survival of neon tetra eggs. The result of this study will be useful in improving the production in hatcheries.

2. MATERIALS AND METHODS

2.1 Fertilised eggs collection

Spawning tanks were prepared and kept ready for conducting breeding experiments. Brooders were introduced at 1:1 ratio into the spawning tanks. All 18 tanks were covered from three sides, to provide darkness in the tanks, with brown paper except the front side, which was covered later. The temperature of water in tanks, which was the main independent variable, was maintained at 18^0C, 20^0C, 22^0C, 24^0C and 26^0C. The temperature below 22^0C was maintained by using an aquarium chiller (RESUN® MINI-200) and temperature above 22^0C was maintained by using thermostat heater (AMAZON® XL-700). Spawning tanks of size 1.5ft × 0.75ft × 1.0ft of 30 liter capacity, filled with 15-L water in each, were supplied with aeration. In each aquaria, two nylon brushes were placed (30-cm long × 11 cm in diameter) that served as a spawning substrate. After the tanks were ready, mature fish were selected by body conformation and were not fed for 24 h before each spawning trial. The females were introduced into the breeding tank in the afternoon for acclimatization and males during the night. Normally the spawning occurred within 1-3 days, and the brooders, which did not respond were replaced with another. Tanks were checked three times a day whether spawning took place or not. Immediately after eggs are laid, the brooder pair was removed from the tank. For calculating the hatching percentage, 30 fretilised eggs were randomly distributed in all tanks, when fertilized eggs were at the stage of middle blastula, dead and physically damaged eggs were removed using a wide-mouth pipette and only developing fertilized eggs were placed into experimental units. For calculating incubation period (time required to hatch after fertilization**)**, observations were recorded at every one hour interval up to hatchlings were hatched out and data was recorded for each treatment.

2.2 Conditions of incubation

Experiments were conducted in glass tank equipped with thermoregulators and immersion heaters or coolers. Experimental temperatures of incubation were 18, 20, 22, 24 and 26^0C, There were three replicates for each of the 5 treatments. Eggs were transferred and counted using a wide-mouth pipette. Experimental incubation units consisted of 30L capacity glass tank filled with 15L sterilized freshwater. Eggs were stocked at a number of 30 eggs per tank. All temperature gradients were adjusted at an appropriate rate from initial temperature of 22 ^{0}C to their final temperatures within 2 hours. Eggs were incubated statically in the tanks under darkness created by black paper surrounding the tanks from all sides. Fifty percent of the incubation water in each tank was replaced daily with new sterilized freshwater.

2.3. Data collection

For all replicates, mortalities were removed and counted each day, until all the larvae were comes out from the egg. At the same time, three eggs were sampled every day from each replicate using a wide-mouthed pipette and were observed quickly through a dissecting microscope for determination of the developmental stage. Sampled eggs were returned to their respective incubation units. Total hatch rate was determined as the percentage of stocked embryos that hatched, regardless of viability.

Incubation period was calculated as

$$IP = H_{50} - FT$$

Where, IP = incubation period

H_{50} = time in hours for 50% hatching

FT = time of fertilization

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Duration of hatching was calculated as

$HD = H_{50} - H_1$

Where, HD = hatching duration

H_{50} = time at which 50% hatchlings come out

H_1 = time at which 1st hatchling come out

Newly hatched larvae were removed and held in static containers for a further 24 h to observe their continued normal development and immediate post-hatch viability. Total mortality rate represented the combined percentage of dead eggs, abnormal fry, and dead and moribund larvae.

2.4. Statistical analysis

All data (total hatch rate, viability of newly hatched larvae 24 h post-hatch, total mortality rate, time to 50% hatch) were presented as mean ± Standard Error of Mean (S.E.M.). The influence of temperature on above indices was analyzed by One Way Analysis of Variance (ANOVA). Statistical significance was established at α=0.05

3. RESULTS

3.1. Total hatch rate

The optimum percentage of healthy eggs (70±1.92 %) was obtained at temperature of 26 ^{0}C followed by 24 ^{0}C (68.33±5.0 %), 22^0 (67.78±1.11), 20^0 (58.33±2.15) and minimum (52.2±8.3 %) at 18 ^{0}C (P<0.05). Eggs incubated at 18 ^{0}C showed mortality rate of 48 percent, hence unhealthy eggs which turned opaque were siphoned out (figure 1).

3.2. Viability of newly hatched larvae 24 h post-hatch

Viability of newly hatched larvae 24 h post-hatch at 18, 20, 22, 24 and 26 ^{0}C were 92.7%, 94.6%, 97.3%, 99.3% and 99.3%, respectively. Viability of newly hatched larvae 24 h post-hatch was significantly lower (P<0.05) at 18 ^{0}C than at 20, 22, 24 and 26 ^{0}C, while there was no significant difference (P<0.05) among 20, 22, 24 and 26 ^{0}C treatments (figure 2).

3.3. Total survival rate

Total mortality rates at 18, 20, 22, 24 and 26 ^{0}C were 44.44± 5.87%, 41.66 ± 4.19%, 82.22 ± 4.44%, 83.33 ± 10.00 and 93.33 ± 6.66%, respectively. Total survival rate was significantly higher (P<0.05) at 26 ^{0}C than at the other three temperatures, but not significantly different (P<0.05) at 18, and 20 ^{0}C. (Fig. 3)

3.4. Rate of embryonic development

Rate of embryonic development to hatch was accelerated with increase in incubation temperature. The times taken for 50% of the embryos to hatch at 18, 20, 22, 24 and 26 ^{0}C were 38.67 ± 0.66, 32.00 ± 0.81, 30.00 ± 1.15, 29.00 ± 1.0 and 24.67 ± 0.66 hours (P<0.05), respectively. There were significant differences in time to 50% hatch among all temperatures of this experiment.

4. DISCUSSION

Water temperature plays an important role in the lives of fishes and is considered an ecological resource, similar to food, habitat and access to mates, which can influence individual fitness, Magnuson *et al*., (1979). When temperature varies away from the optimal for an organism, this may act as a stressor and impair physiological and behavioural activities (Fry, 1947; Beyers and Rice, 2002; Donaldson *et al*., 2008).

Within a viable range, incubation temperature strongly affects the rate of embryonic development of fish. Generally, lower temperature retards the rate of embryonic development and higher temperature accelerates it. In the present study it was observed that hatching rate was directly related to the temperature as maximum percentage (70.0 ± 1.92) for hatching was observed at 26 ^{0}C and minimum at 18 ^{0}Cand was consistent with the widely observed phenomena in many other fishes (Marangos et al., 1986; Pepin, 1991; Blaxter, 1992; Mihelakakis and Kitajima, 1994; Hart and Purser,1995; Hart et al., 1996; Hamel et al., 1997b; Mihelakakis and Yoshimatsu, 1998; Hansen and Falk-Petersen, 2001; Kamler, 2002). A strong, inverse relationship between the time needed for fish to develop within the egg envelope and ambient water temperature has been reported as early as the 1890s, Dannevig (1895) and this relationship has been studied quantitatively by a number of authors (Apslein 1909, Johansen and Krogh 1914, Blaxter 1956, Ignatyeva 1974, Ryland and Nichols 1975, Russell 1976, Martin and Drewery 1978, Hoestlandt and Devienne 1980, Jones 1978, Thompson *et al.* 1981).

In present study it is observed that the incubation period varied inversely with the temperature as results showed that maximum time for hatching was observed at 18 ^{0}C and minimum time at 26 ^{0}C is similar to that of Lasker (1964), Swift (1995), Hubbs *et al.*, (1969), Edsall (1970), florez (1972), Kmaldeep and Toor (1980), Jungwirth and Winkler (1984) and Rana (1990). Incubation period was found to be apparently higher in *P. innesi*. At 18 ^{0}C it was 39 hours; at 26 ^{0}C it was 25 hrs. With increase in the incubation period decreased to only 25 hours.

Blaxter (1969) stated that the time for hatching is both a specifically and environmentally controlled character with temperature and oxygen supply exerting a considerable effect. Most studies have been conducted with freshwater species, particularly salmonids, in which a precise knowledge of the influence of temperature (and temperature fluctuations) on hatching time can be used in the context of commercial farming and stocking ventures (Hayes 1949, Alderdice and Velsen 1978, Crisp 1981). Moreover, in present study the sensitivity of neon tetra eggs to thermal stress, measured as percentage of hatching, indicates that 26 ^{0}C is the most optimum incubation temperature at which hatching percentage was 90%. The range of temperature under which satisfactory (more than 60%) hatching occurred was between 22 ^{0}C and 24 ^{0}C, whereas, the percentage of hatching at 18 ^{0}C and control was very low (52 and 54% respectively).

The present study can be supported by the findings of Hyojin *et al*, (2011) which showed that there was variation in the hatching rate among the different lots of Japanese's eel eggs (*Anguilla japonica*), there was a clear pattern of hatching rate being higher at 22 ^{0}C and 25 ^{0}C than at 19 ^{0}C and 28 ^{0}C, and of no hatching eggs at 16 ^{0}C and 31 ^{0}C. Hatching was highest at 25 ^{0}C in all three lots of eggs. This indicates that the optimal temperature for hatching of these eggs were in the range of 22 – 25 ^{0}C and probably somewhere close to 25 ^{0}C. Chang *et al*., (2004) also found that the optimum temperature for egg incubation was 24 – 26 ^{0}C. A study on the effect of water temperature on larval deformity of the Japanese eel, suggested that 25 – 28 ^{0}C was the most suitable for reducing deformity rates in eggs and pre-feeding larvae, Okamura *et al.*, (2007). Another study of A. japonica eggs and embryos found that 24 – 26 ^{0}C was the optimal temperature, Kurokawa *et al.*, (2008). Therefore, the findings of the present study are similar with the results of previous studies.

Dube and Reddy (1992) found that the incubation period varied inversely with temperature, percentage of hatching showed a curvilinear relationship with temperature, high hatching rates were recovered at temperature ranges of 24 – 30 ^{0}C, the best being 27 ^{0}C for *L. rohita*.

Cole and Haring (1999) found that in serape tetra once spawning has been completed the eggs hatch in about 24 hours and look like slivers of glass clinging to the spawning substrate.A 30-50% reduction of fertilization percentage and an increase in deformities percentage were reported in cod when water temperature exceeded 10°C in the spawning tank, Van der Meeren and Ivannikov (2001).

Chapman *et al*, (1998) recorded the incubation period of embryo for *P. innesi* at 25 ^{0}C as 24 – 26 hours. A similar observation was also reported in the related lake white fish *Coregonus clupeaformis*, Davis and Behmer, (1980). In this case, it seems that a rise in temperature stimulates enzyme secretion, which solubilizes the egg envelope faster at higher temperatures than at lower temperatures, bringing about earlier hatching (Davis and Behman 1980). Similar results were obtained for the hatching period by many authors, According to Axelrod and Shaw (1967) the eggs hatch after two days at 25 ^{0}C, while Richter (1991) and Tsilinszky (in Noznov 1986) observed that the eggs of neon tetra hatched after 20 hours at 26 – 27 ^{0}C and Roloff (in Noznov 1986) reported that the neon tetra eggs hatch after 24 - 36 hours at 26^{0}C.

5. SUMMARY

In summary, our results showed that the optimal temperature for incubating neon tetra eggs ranged from 22– 26 ^{0}C. This temperature range results better hatching rate and less incubation period.

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APPENDICES - A

Figures -

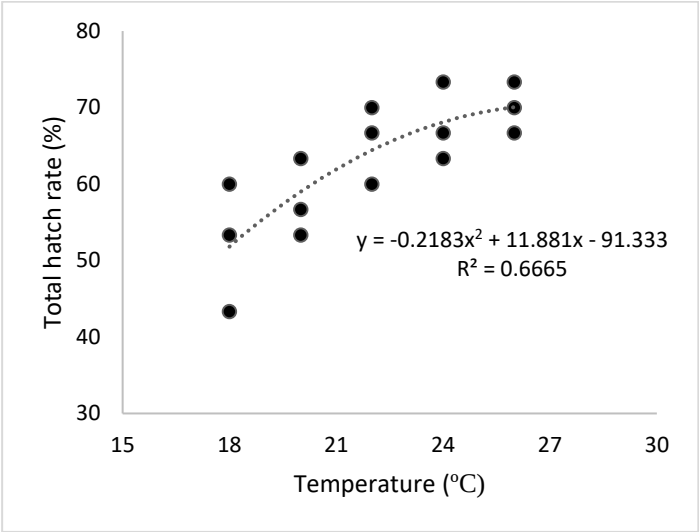

Fig. 1. Total hatch rate for eggs incubated at different temperatures, calculated as a percentage of eggs fertilized. The curve was a second-order polynomial.

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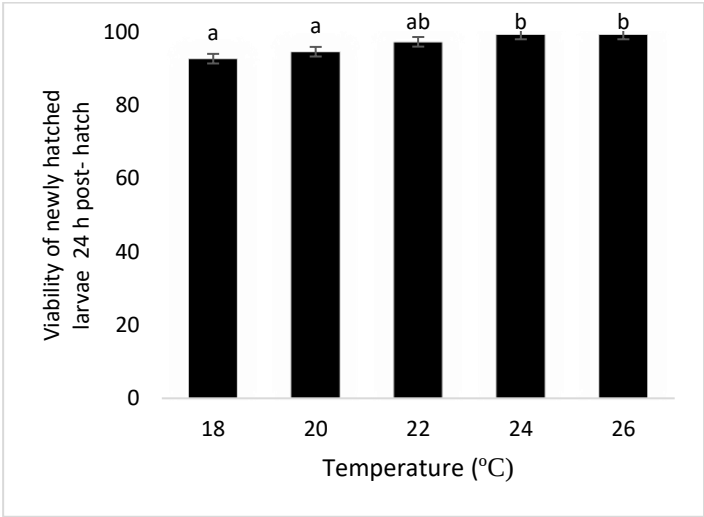

Fig. 2. Viability of newly hatched larvae incubated at different temperatures. Vertical bars represent one standard error. Bars with different superscripts denote significant difference at P<0.05 (ANOVA).

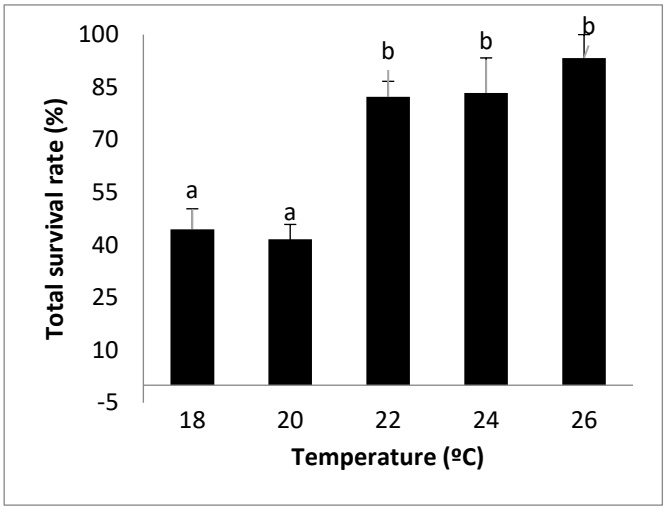

Fig. 3. Total mortality rate of obscure puffer embryos incubated at different temperatures. Vertical bars represent one standard error. Bars with different superscripts denote significant difference at P<0.05 (ANOVA).

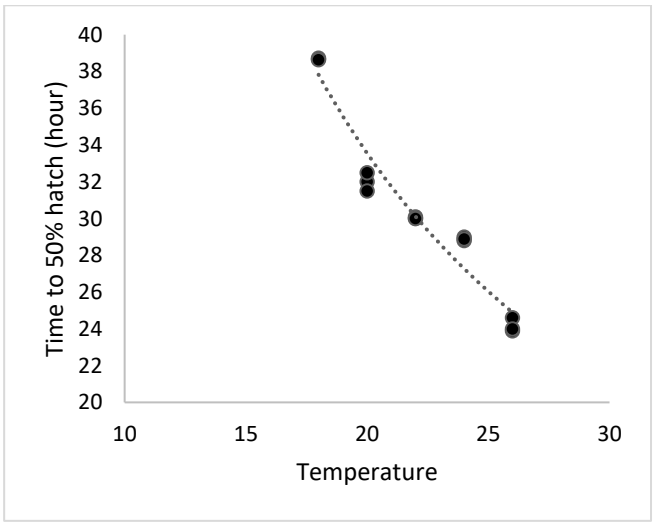

Fig. 4. Relationship between incubation temperature and time to 50% hatch for neon tetra embryos. The curves fitted according to the exponential equation.