World Applied Sciences Journal 35 (8): 1400-1406, 2017
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2017.1400.1406

Effects of Water Temperature on Egg Development and Hatching of Marble Goby *Oxyeleotris marmoratus* in Saline Water

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Abstract: The present study was designed to assess the egg development and hatching of *Oxyeleotris marmoratus* incubated at 24, 26, 28, 30 and 32°C in 10 psu water until hatching. The eggs were obtained through induced spawning and fertilization was considered as 0 hour after spawning. Egg developmental stages were monitored by sampling eggs in different temperatures each interval changing time. Egg development accelerated as temperature increased. The quadratic equation provided good fits for the relationship between temperature and egg development with r^2 values greater than 0.95. Egg mortality was high during epiboly for all treatments. Total egg mortality and larval deformity were significantly higher (P<0.05) at 24 and 26°C. Hatching period decreased as temperature increased. Shortest hatching was 30 hours at 32°C while longest hatching was 120 hours at 24°C. Eggs incubated at 24°C developed abnormally and all died eventually. As total hatching rates assessed across the range of temperature and fitted with a second-order polynomial, 28 to 32°C was optimal for hatching. Based on the results, higher temperature could be manipulated to hasten the egg hatching and decreased larval deformation in *O. marmoratus* seed production.

Key words: Deformed Larvae · Embryo Development · Embryonic Mortality · Hatching Success · *Oxyeleotris marmoratus* · Water Temperature

INTRODUCTION

Marble goby *Oxyeleotris marmoratus* (Bleeker 1852) is widely distributed in Southeast Asia and known as marble goby in English, “Soon Hock” in Chinese, “Pla bu” in Thai, “ikan ketutu” and “ikan malas” in Malay [1-3]. It is a freshwater fish with commercial importance in Southeast Asia owing to its taste and high protein value, which is being considered as a first grade fish that cost at US$50-60/kg [2, 3]. The culture generally relies heavily on seed collection from the wild but it has been decreased drastically due to overfishing [5, 6]. Therefore, seed production of *O. marmoratus* has been developed to fulfil the market demand and to protect the wild resources. Some studies were carried out to investigate the culture method [7], reproductive cycle [8], genetic population study [9], rearing conditions [10] and growth and feeding performances [11]. However, the seed production is still inconsistent and limited information is available particularly for the early life stages of *O. marmoratus*.

O. marmoratus seeds have been reported to be successfully produced in 10 psu water even though it is a freshwater species [12]. The abiotic factors in water are found to be act directly to the fish growth and survival [13]. Other than salinity, temperature is one of the most decisive factors of all the environmental conditions affecting the early life stages [14]. Eggs and larvae are the most vulnerable stages in fish development, especially susceptible to temperature changes [15, 16]. Previous studies showed that even a temperature change of 2°C were lethal to eggs, larvae and adult freshwater fishes in tropical region [17-19]. Thus, the effects of temperature must be considered when developing fish culture, which essentially aims to produce seeds constantly. Therefore, proper management of water quality parameters is necessary for successful incubation of egg. There is no empirical study available for the different water temperature at early life stage of *O. marmoratus*. Thus, in an attempt to improve seed production techniques,

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experiment was carried out to determine the optimum temperature for egg development and hatching of *O. marmoratus*.

MATERIALS AND METHODS

Egg Collection and Hatching: For egg collection, a female with 285 g and a male with 300 g in body weight were chosen. The female were injected intra-peritoneally with 1, 000 IU/kg human chorionic gonadotropin [20]. Three days after the injection, approximately 20, 000 eggs were spontaneously spawned.The research was an experimental research using complete random design with five treatments of temperature (A: 26±0.5°C; B: 28±0.5°C; C: 30±0.5°C; D: 32±0.5°C) and three repetitions. Research container was transparent tanks (length, width, height; 18 x 26 x 17 cm), placed into five different temperatures of incubation tanks (120 x 75 x 45 cm) with thermo-regulated heaters and coolers (AZ-251X Iwaki Co., Ltd, Japan). Salinity of incubation water was adjusted to 10 psu by mixing filtered seawater with dechlorinated tap water and measured using a hand refractometer (H-50, ATAGO, Japan). One hundred of egg were gently placed in a 9 cm diameter of glass Petri dishes and put into all the transparent tanks in each incubation treatment. Continuous aeration at 250 mL/min was provided in each treatment.

Data Collection: Hourly eggs were collected to monitor the different stages of egg development and larval, then photographed (Canon IXY 600F) for descriptive and photographic documentation. Ages of eggs or larvae were measured in hours starting from spawning time and defined as 0 hour after spawning (hAS). Egg development was differentiated into four major developmental periods I: cell cleavage (the beginning of fertilization and formed the zygote), II: epiboly (the beginning of gastrulation), III: organogenesis (formation of organ) and IV: organogenesis-growth (the development of organ). Opaque eggs are considered dead. Dead eggs were removed and egg survivals were counted. Effects of temperature on egg hatching and early larval survival were evaluated for the following criteria: egg mortality rates = dead eggs/ total eggs x 100%; total hatching rates – hatched eggs/ total eggs x 100%; deformation rates - deformed larvae/ total hatched larvae x 100%; hatching period – the duration of total hatching each temperature treatment. The data then were using SPSS Statistics 15.0 software (IBM Corp., New York, USA).

RESULTS AND DISCUSSION

Egg Development: The fertilized eggs were amber in colour, translucent and attached to the spawning substrate with a bundle of adhesive filaments at the basal end of egg membrane. The fertilized eggs were measured at 1.84±0.03 and 0.64±0.01 mm in length and width (mean±SD, n = 20), respectively and weighed 1, 682 eggs/g. Figure 1 shows the following stages of egg developments of *O. marmoratus*. The egg developments were accelerated with an increase in incubation temperature (Figure 2). The quadratic equation $y = a + bT + cT^2$ showed an accurate fitted for each development stages was summarised in Table 1. The quadratic equation has been widely used to compare the egg development rates among the species and related species [21-23]. Based on this study, the effect of increasing the incubation temperature from 24 to 32°C reduced the incubation time were precisely defined at each stage with $r > 0.95$.

Egg Mortality: There was a significant effect of the incubation temperature on total egg mortality indicating a difference in the egg mortality between the groups (ANOVA, $F_{4, 10} = 533.8$, Tukey's HSD test, $P<0.001$) (Figure 3b). The total egg mortality rates were 87.7±3.8, 22.3±2.5, 13.3±3.2, 17.0±4.6 and 11.7±2.9%, at 24 to 32°C, respectively. Total egg mortality was significantly higher at 24°C than 28, 30 and 32°C. There was a significant interaction between incubation temperature and egg developmental period I to IV (ANOVA, Tukey's HSD test, $P<0.05$) (Figure 3a). The egg mortality rates were significantly higher at 24°C (3.7±2.3, 11.3±1.5, 6.2±3.3 and 4.1±1.1%, respectively) than 30 and 32°C in developmental periods I to IV. The highest egg mortality was observed during developmental period II, epiboly in all experimental treatment, with 11.3±1.5, 6.3±1.5%, 5.0±0.6, 3.3±1.5, 1.7±0.6% at 24 to 32 °C, respectively.

During epiboly, morphogenetic cell movement occurred and allowed the physical restructure as the blastomere cells spreading over the yolk. High mortality commonly occurs during this late blastula and early gastrula due to the high sensitivity to external stress or disturbance, for instance mechanical pressure [24, 25], oxygen deficiency [26, 27] and inappropriate temperature [28, 29]. Low temperature at the early gastrula stage could cause the delay in epiboly of periblast [30]. This is consistent to the results in this study, as the egg mortality of *O. marmoratus* was particularly high during epiboly stage in all temperature treatments. Eggs died at

Table 1: Values of constants a, b and c for the quadratic equation $y = a + bT + cT^2$, where y is the time for 50% of *O. marmoratus* eggs developed to reach stages F, H and K, T (°C) is the incubation temperature. The correlation coefficient (r^2) and level of significance (*P*) of the models are also indicated

Stage	Temperature range (°C)	A	b	c	r^2	*P*
Blastula stage (F)	24-32	95.13	-5.9488	0.0971	0.9809	<0.05
Embryo formation (H)	24-32	150.21	-9.2957	0.1523	0.9809	<0.05
Tail separation from yolk sac (K)	24-32	207.19	-12.651	0.2053	0.9586	<0.05

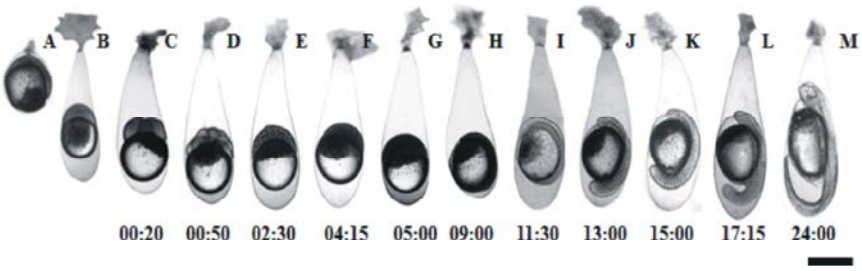

Fig. 1: Photomicrographs shows the egg development of *O. marmoratus* at 28°C in 10 psu water. Four embryo developmental periods: I, cell cleavage, formation of the blastodisc (B-E); II, epiboly, blastodisc spreading over yolk (F-G); III, organogenesis, formation internal organs (H-K) and IV, organogenesis growth, frequent twitching observed and hatching occurred (L-M). (A) unfertilized egg, (B) fertilized egg, (C) 2-celled stage, (D) 16-celled stage, (E) morula stage, (F) blastula stage, (G) gastrula stage, (H) embryo formation, (I) Kupffer's vesicle appeared, (J) tail separated from the yolk sac, (K) head formed, (L) embryo commenced moving, (M) hatching started. The time showed at each egg developmental stage was first observed. Scale, 500 µm.

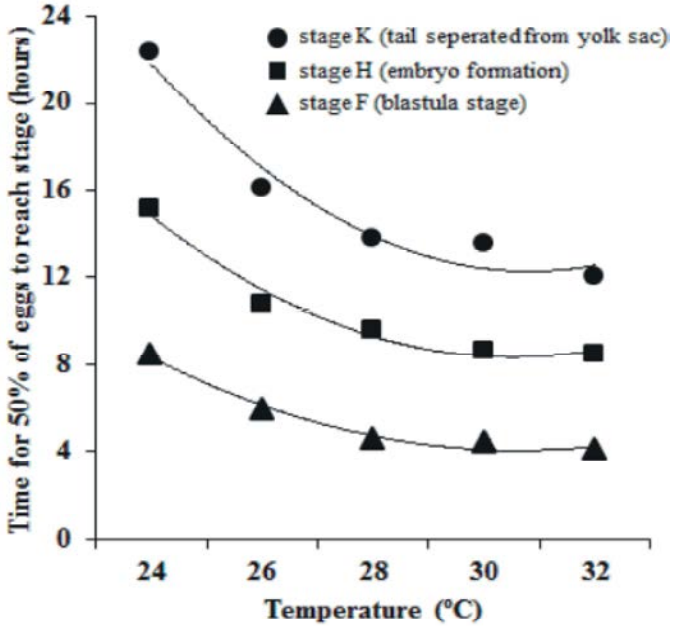

Fig. 2: Time taken for 50% of the eggs developed to reach three stages at different temperatures (24 to 32°C). The curved are fitted according to the quadratic equation $y = a + bT + cT^2$ described in Table 1

low temperature as the eggs were torn physically in the cell migration process and at the margin between the germ rings or at the embryo shield [31, 32]. This observation has provided important information on the probability of collection or transportation of *O. marmoratus* eggs for production.

Hatching Period, Normal Hatching and Deformation Rates: High hatching rate and first hatching were observed earlier at higher temperature. First hatching commenced were observed at 60, 24, 24, 19 and 18 hAS (Figure 4) at 24 to 32°C, respectively. Hatching period showe negatively correlated with incubation temperature

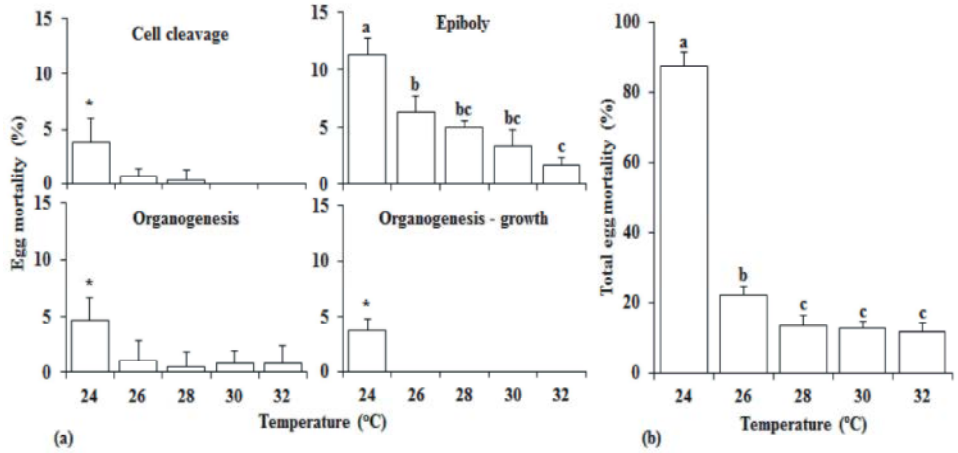

Fig. 3: Effect of incubation temperature on the egg mortality of *O. marmoratus*. (a) Egg mortality during four developmental periods (ANOVA, $P<0.05$); (b) Total egg mortality (ANOVA, $F_{4, 10} = 533.8$, Tukey test, $P = 0.001$). Different letters and asterisk above each bar indicate significant difference between treatments

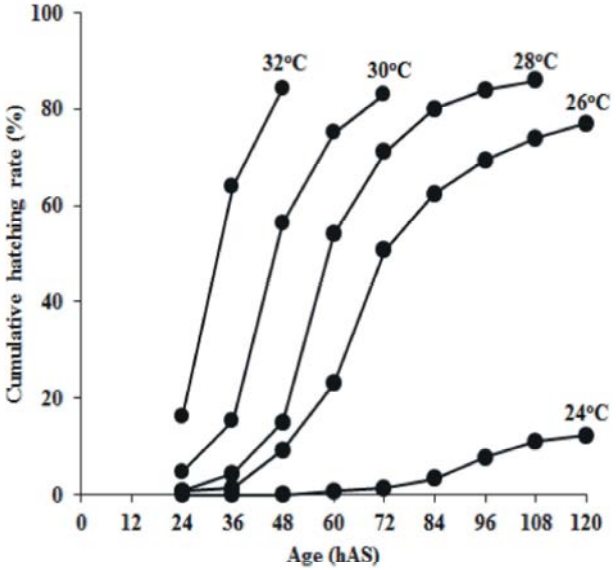

Fig. 4: Cumulative hatching rates of *O. marmoratus* in 10 psu water at different temperatures

with shortest hatching period at 32°C (120 hours) and longest at 24°C (30 hours).The incubation temperature significantly affected the total hatching rate (ANOVA, $F_{4, 10} = 120.3$, Tukey's HSD test, $P = 0.001$) (Figure 5a). Egg hatching occurred within 24 to 32°C but more than 80% of the eggs died at 24°C. Total hatching rates at 24, 26, 28, 30 and 32°C with 12.3±3.8, 77.0±2.6, 86.7±3.2, 83.0±4.6 and 84.3±6.0%, respectively. Hatching success was significantly low ($P<0.05$) at 24°C than higher temperature. When total hatching rates were assessed across the range of temperature (24 to 32°C) and fitted with a second-order polynomial, 28 to 32°C was observed to be optimal for hatching (Figure 5a).

There was no normal hatching observed at 24°C. The normal hatching rates were 68.0±2.6, 78.7±2.1, 76.0±2.6 and 81.3±5.5% at 26 to 32°C, respectively. Normal hatching was significantly low ($P<0.05$) at 26°C. To assess the productivity of larvae in different temperatures, the deformation rates were calculated as percentages of the initial number of total eggs. All larvae hatched at 24°C were deformed with bent body at hatching and eventually died on the tank bottom (Figure 5b). The deformation rates of hatched larvae were 12.3±3.8, 27.4±5.8, 9.2±2.1, 8.0±2.9 and 3.5±1.1% at 24 to 32°C, respectively. Deformity of hatched larvae was significantly high ($P<0.05$) at 24°C, followed by 26°C.

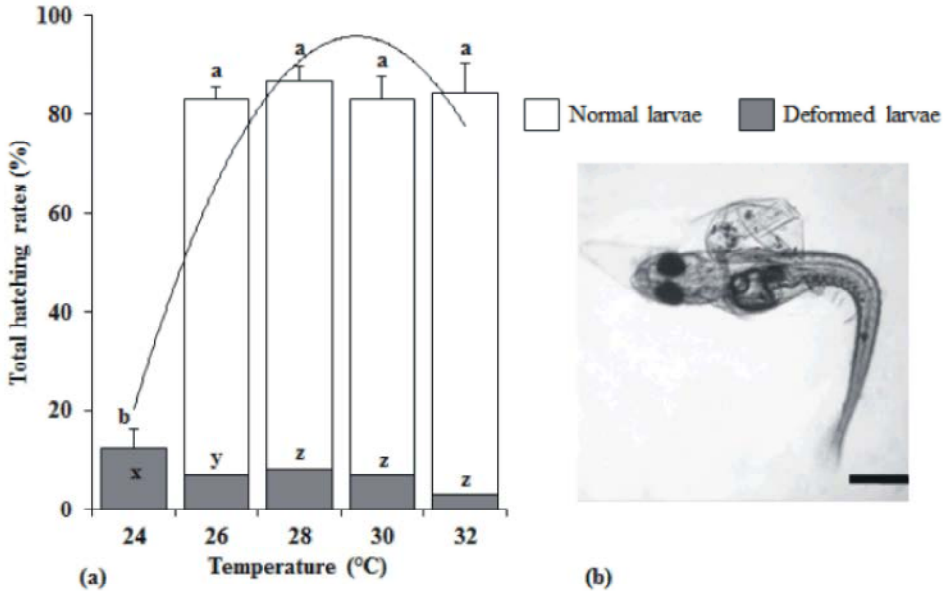

Fig. 5: Total hatching rates for eggs incubated at different temperatures (24 to 32 °C), calculated as a percentage of eggs fertilized. (a) Normal and deformation rates of hatched larvae. Different letters above each bar indicate significant difference between treatments (ANOVA, $F_{4, 10} = 120.3$, Tukey test, $P = 0.001$). The curve was a second-order polynomial ($y = -10.443x^2 + 77.057x - 46.44$, $R^2 = 0.8674$); (b) Deformed larva at hatching. Scale bar, 0.5 mm

Previous studies showed that the thermal tolerance and optimal incubation temperature were influenced by the temperature that experienced by brood fish at spawning site. In pollack *Pollachius Pollachius*, the number of spawns collected per female and viable eggs were significantly lower at 12°C compared to 8 and 10°C proposed12°C to be close to the temperature reproduction upper limit in this species [33]. In sole *Soleo senegalensis*, significantly positive correlation was observed between egg fertilization and the spawning temperature suggested that temperature could be controlled for their captive reproduction [34].In turbot *Scophthalmus maximus*, their spawning time was adapted to the optimum temperature that ranged during 16 to 18°C for egg development [35]. Natufrom 22 to 28°CNatural habitat of *O. marmoratus* was recorded ranged from 22 to 28°C (tropical coordinator, 23°N to 18°S) [3, 36]. In this study, eggs of *O. marmoratus* was observed to hatch into normal larvae at temperature ranged from 26 to 32°C, so the spawning temperature of *O. marmoratus* could be considered above 24°C and temperature range from 26 to 32°C defined suitable for their egg incubation.

Lower temperature has been reported to retard the egg development rate and vice versa [14, 37]. As shown in the common carp *Cyprinus capio,* time to hatch was 96 hours at 24°C and 80 hours at 30°C [18]. Egg hatching of Indian major carps occurred about 15 to 18 hours after fertilization at temperature ranging from 26 to 31°C [38]. The modified temperature 11 to 14°C for the embryonic development of dace *Leuciscus leuciscus* took from 9.5 to 22.5 days [39]. In this study, hatching period of *O. marmoratus* decreased from 120 hours at 24°C to 30 hours at 32°C and was consistent with the widely observed phenomena in many other fishes [14, 18, 38, 39]. Fish body shape and most of the meristic characters were significantly affected by the environmental temperature during their early life stages [40]. Low temperature during egg incubation has been shown to cause the skeletal disorders and impaired growth of sole *Senegalese sole* [41], not developed eyes and misshapen tails in larvae ofAlaska pollock *Theragra chalcogramma* [42] and embryo deformation in dace *L. leuciscus* [39]. Hatching of clown loach *Chromobotia macracanthus* observed to occur at 22 to 30°C and deformed hatched larvae were observed at lower temperature 22°C [43]. In the study of ruffe *Gymnocephalus cernuus*, incubation at 11 to 21°C allowed normal development and hatching but failed to hatch successfully when incubated at lower temperature at 6°C [44]. This is consistent to the results of *O. marmoratus*, incubation temperature at 24°C showed extremes under tolerance, which most of the eggs died and all hatched larvae had a crippled shape due to an abnormal vertebral axis or a bent tail. Meanwhile, the egg morphological development of *O. marmoratus* at 26 to

32°C was normal. Newly hatched larvae at 30 and 32°C hatched earlier and shorter hatching period lead to significantly lower deformity in hatching larvae compared to others. Thus, higher temperature could be manipulated to hasten the egg hatching and decreased deformation of hatched larvae in *O. marmoratus* seed production.

Totally, lower survival of *O. marmoratus*eggs during early development at 24°C suggested that egg incubation at or below this temperature is not suitable for this species. Although there was no significant difference in total hatching rates and egg mortality among the three higher temperatures, trend towards lower total hatching rates and higher egg mortality existed at the cooler temperature of 24 and 26°C. Therefore, based on the results, the optimal temperature for incubating *O. marmoratus* eggs is about 28 to 32°C.

ACKNOWLEDGMENTS

The authors would like to express sincere gratitude to the former manager Prof. Dr Senoo Shigeharu and the all staffs of the fish hatchery Universiti Malaysia Sabah particularly Mr. Norazmi Osman for their advisors and technical supports. The authors also would like to express gratitude to the late Prof. Dr Shigeru Miyashita, Prof. Dr Keitaro Kato, the Global COE Program International education and research centre program at Kindai University and Universiti Sultan Zainal Abidin (UniSZA) for their encouragement and financial supports.

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