

Effect of Temperatures on the Embryonic Development, Morphometrics and Survival of *Macrobrachium Idella Idella* (Hilgendorf, 1898)

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Abstract

The development of *M. idella idella* eggs incubated at four different temperatures (26, 30, 33 and 36°C). An increase in major axis length was evident at 26, 30, 33 and 36°C. However at 36°C, size variation increased with developmental stages, indicating abnormalities until 192 h, after which total mortality was observed. A distinct change in morphometric parameters (major half axis, minor half axis, area and perimeter) was demonstrated at higher temperatures, irrespective of the developmental duration of eggs. Length of hatched embryos increased with increasing incubation temperatures. Therefore a rapid rate of increase of major half axis and early hatching was observed at 33°C. The larva hatched first in 33°C (241 hrs) then followed by 30°C (265 hrs) and 26°C (302 hrs). In 36°C (182 h) there was total mortality during embryonic development so there was no hatching.

Keywords: *M. idella* Idella; Embryonic development; Temperatures; Effect; Survival

Introduction

The emission of greenhouse gases and carbon dioxide are expected to increase global mean temperature by 1.5-4.5°C over the next half-century [1]. The impact of such a large temperature will affect the biological functions of freshwater and marine fishes and shellfishes, as most of the species are poikilothermic in nature. Thermal tolerance of aquatic animals is also dependent on acclimation temperature and the duration of acclimation [2]. Over the years, attention has focused on the thermal tolerance of embryos and larvae [3] that are more sensitive to temperature changes than adult fishes [4]. The earlier investigation on *Labeo rohita* revealed similar results [5]. The embryos of temperate species are more sensitive to extreme temperatures than embryos of tropical species [6]. In general, thermal limits are narrower for early stages and reduced survival of embryos and juveniles. Whereas it was wider for adults [7]. It is also reported that upper lethal temperatures of embryos, larvae [8] and adults [9] of the freshwater Mozambique tilapia (*Oreochromis mossambica*) varies in the range of 2°C among different life stages. In *M. rosenbergii* and *P. serratus* higher temperature seems to be shortening the incubation period [10]. Hence, the present study was undertaken to assess the effect of low and higher temperatures on the incubation period and embryonic development of edible prawn *M. idella idella*.

Materials and Methods

Experimental animals

Gravid or Berried females (80-90 mm) with opaque, greenish, round or oval in shape fertilized eggs in their brood pouch were used for the present experiment.

Experimental conditions

Twelve newly spawned brooders were stocked; one in each 120L fiber glass tank to assess the effect of incubation temperature on embryonic stages of *M. idella idella*. Acclimation was carried out at one degree per day from water temperature (25°C). Since the experiment was carried out during December to January the normal water temperature was found to be 25-26°C.

Temperature maintenance

The test temperatures (26, 30, 33 and 36°C) were regulated by

using automatic thermostat until hatching of the eggs. Sampling of eggs was carried out once the cleavage was completed, since this early development phase was not easily observed and was considered as the initial period (0 h). Eggs were sampled aseptically by gently removing a bunch of eggs from the brood pouch using sterilized forceps and separated with the help of needle and forceps without damaging the eggs. After each sampling, brooders were given a 1-min prophylactic fungus dip treatment in malachite green (5 mg L⁻¹) before being returned to incubation tanks.

Sampling

In four different temperatures the embryonic stages of two brooders were rarely matched. Therefore, embryos were sampled at several intervals (i.e. 0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288 and 312 h) from each brooder. Organogenesis, developmental changes and physiological processes were recorded under a light microscope. Eggs collected from four sampling points of brood pouch (anterior to posterior) were pooled to minimize sampling error due to position of eggs in brood pouch and assessed the percentage mortality of fully developed embryos (from an aggregate of 12 embryos/brooder) at the onset of hatching. Embryo dimensions (major axis, minor axis, area and perimeter) were measured at 48-h intervals (0, 48, 96, 144, 192h) until total mortality or hatching occurred.

Results

Organogenesis and morphophysiology of eggs

Development of *M. idella idella* eggs incubated at four different

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temperatures is presented in Plate 1. At 0 h, eggs in all the treatments were in similar phase of development (morula stage) and primordial mesodermal and endodermal cells were visible. After 24 h, the blastocyst was visible in all the four-temperature treatments. Embryos were visible after 48 h in all the treatments. However, abnormal embryonic development was evident at 36°C. At 72 h, the primordial compound eye was visible. At 96 h, a considerable increase in the length of the major axis was seen at higher temperatures (30, 33 and 36°C). At 120 h, star shaped and round protoplasmic islands was visible at 33 and 36°C, respectively and heartbeat was discernible at all temperature treatments. The compound eye with a visible optic lobe was visible at higher temperatures (30, 33 and 36°C). At 144 h, star shaped protoplasmic islands appeared at lower temperatures (26 and 30°C). Rudiments of appendages started developing at all acclimation temperatures. At 168 h, paired compound eyes were visible in all treatments. At 192 h, star shaped protoplasmic islands were seen at lower temperatures (26 and 30°C) while at 36°C, protoplasmic islands appeared was degenerated. After 192 h, complete mortality was noticed at 36°C (Plate 1).

At lower temperatures, rudiments of appendages were visible. The primordial digestive canal developed in segments as a dotted line in the posterior region, and appeared to originate from primordial hepatopancreas. The primordial brain was visible at the anterior part of primordial hepatopancreas. The major half axis attained maximum length at the time of hatching. Hatching was initiated by a sudden twitching movement in the posterior region (below the compound eye) by means of the rudimentary antennule. At the time of hatching, telson (or tail) and rudiments of uropod (folded below compound eye) unfolded and the embryonic case was removed from anterior portion by straightening of abdominal segments. Embryos hatched out with a jerky movement and all the anterior appendages in the cephalothoracic region (including walking legs) were started moving vigorously. The larva hatched first in 33°C (241 hrs) then followed by 30°C (292 hrs) and 26°C (340 hrs). In 36°C (192 h) there was total mortality during embryonic development so there was no hatching. Finally, larvae appeared bilaterally symmetrical (zoea 1) (Tables 1-3 and Figure 1). The embryonic development duration decreased with increasing temperatures as indicated by the negative slope in the linear regression equation, $y = -14.909x + 732.15$ and $R^2 = 0.9932$ (Figure 2).

Embryonic morphometry

The data on embryonic morphometry of *M. idella idella* exposed to four incubation temperatures (26, 30, 33 and 36°C) are reported in Table 1. An increase in major axis length was evident at 26, 30, 33 and

S. No.	Incubation temperatures (°C)	Mortality at hatching (%)
1	26	28.75 ± 0.70
2	30	25.0 ± 0.77
3	33	42.33 ± 0.66
4	36	99.00 ± 0.49

Table 1: Percentage mortality of fully developed embryos at different incubation temperatures (mean of 12 values ± SE).

S.No.	Incubation temperatures (°C)	Duration of development
1	26	340.00 ± 0.81
2	30	292.08 ± 0.79
3	33	241.00 ± 0.95
4	36	182.07 ± 0.53

Table 2: Duration of embryonic development with increasing incubation temperatures from morula to eggs hatching (mean of 12 values ± SE).

Parameter	Duration of embryonic development (h)	Acclimation temperatures (°C)			
		26	30	33	36
Major half axis (µm)	0	260.15 ± 3.54	270.3 ± 3.33	275.71 ± 2.63	275.06 ± 3.21
	48	273.26 ± 2.96	276.65 ± 2.64	279.17 ± 1.36	281.61 ± 2.36
	96	279.18 ± 3.08	285.95 ± 1.86	291.11 ± 3.23	289.23 ± 2.15
	144	287.23 ± 2.67	290.60 ± 4.38	298.20 ± 2.21	303.10 ± 3.25
	192	293.12 ± 3.56	303.72 ± 3.25	308.16 ± 2.06	311.69 ± 7.31
	Mean ± SE	278.58 ± 5.72	285.44 ± 5.77	290.47 ± 5.99	292.13 ± 6.75
Minor half axis (µm)	0	224.61 ± 3.18	233.07 ± 4.37	234.06 ± 1.62	235.16 ± 3.02
	48	227.15 ± 2.96	233.5 ± 2.38	235.61 ± 1.68	232.22 ± 2.12
	96	231.38 ± 2.24	235.61 ± 2.52	237.56 ± 3.18	234.81 ± 4.16
	144	234.77 ± 2.52	236.04 ± 1.86	238.11 ± 2.20	239.16 ± 3.69
	192	236.03 ± 2.37	237.30 ± 2.45	238.65 ± 1.85	240.56 ± 3.28
	Mean ± SE	230.79 ± 2.17	235.10 ± 0.79	236.80 ± 0.85	236.38 ± 1.52
Area (x10 ⁵) (µm ²)	0	1.83 ± 0.38	1.98 ± 0.28	2.03 ± 0.23	2.03 ± 0.36
	48	1.95 ± 0.56	2.03 ± 0.18	2.07 ± 0.32	2.05 ± 0.26
	96	2.03 ± 0.28	2.12 ± 0.42	2.18 ± 0.19	2.13 ± 0.19
	144	2.12 ± 0.26	2.16 ± 0.33	2.23 ± 0.16	2.28 ± 0.42
	192	2.17 ± 0.41	2.26 ± 0.27	2.31 ± 0.40	2.35 ± 0.32
	Mean ± SE	2.02 ± 0.07	2.11 ± 0.06	2.16 ± 0.06	2.17 ± 0.08
Perimeter (µm)	0	1522.16 ± 24.36	1580.60 ± 32.21	1600.68 ± 30.12	1602.09 ± 26.62
	48	1571.32 ± 32.41	1601.86 ± 28.06	1616.41 ± 22.02	1613.44 ± 17.24
	96	1603.19 ± 30.21	1637.73 ± 31.68	1660.04 ± 19.83	1645.47 ± 42.33
	144	1639.06 ± 28.25	1653.67 ± 22.63	1684.04 ± 34.22	1702.71 ± 36.23
	192	1661.53 ± 22.32	1698.82 ± 33.72	1717.01 ± 28.43	1734.07 ± 23.54
	Mean ± SE	1599.45 ± 22.5	1634.54 ± 18.8	1655.64 ± 19.5	1659.56 ± 24.6

Table 3: Effect of incubation temperatures (26, 30, 33, 36°C) on morphological changes during the embryonic development of *M. idella idella* eggs until hatching.

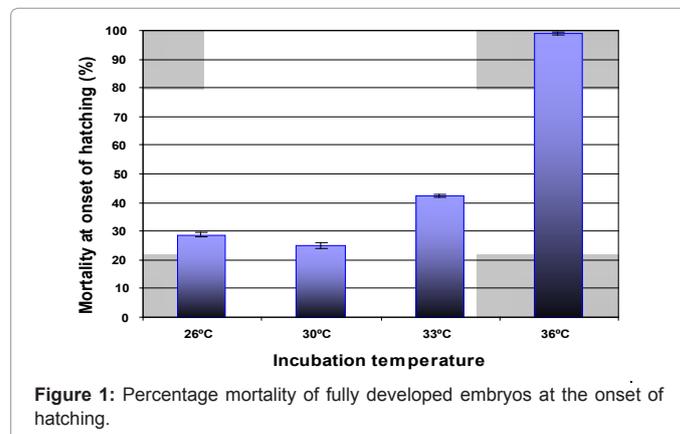


Figure 1: Percentage mortality of fully developed embryos at the onset of hatching.

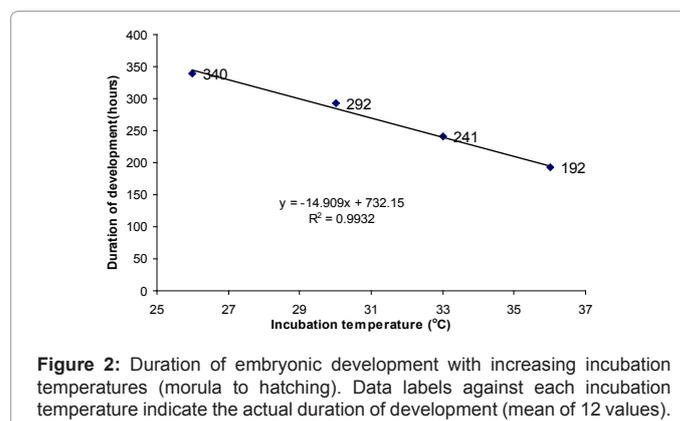


Figure 2: Duration of embryonic development with increasing incubation temperatures (morula to hatching). Data labels against each incubation temperature indicate the actual duration of development (mean of 12 values).

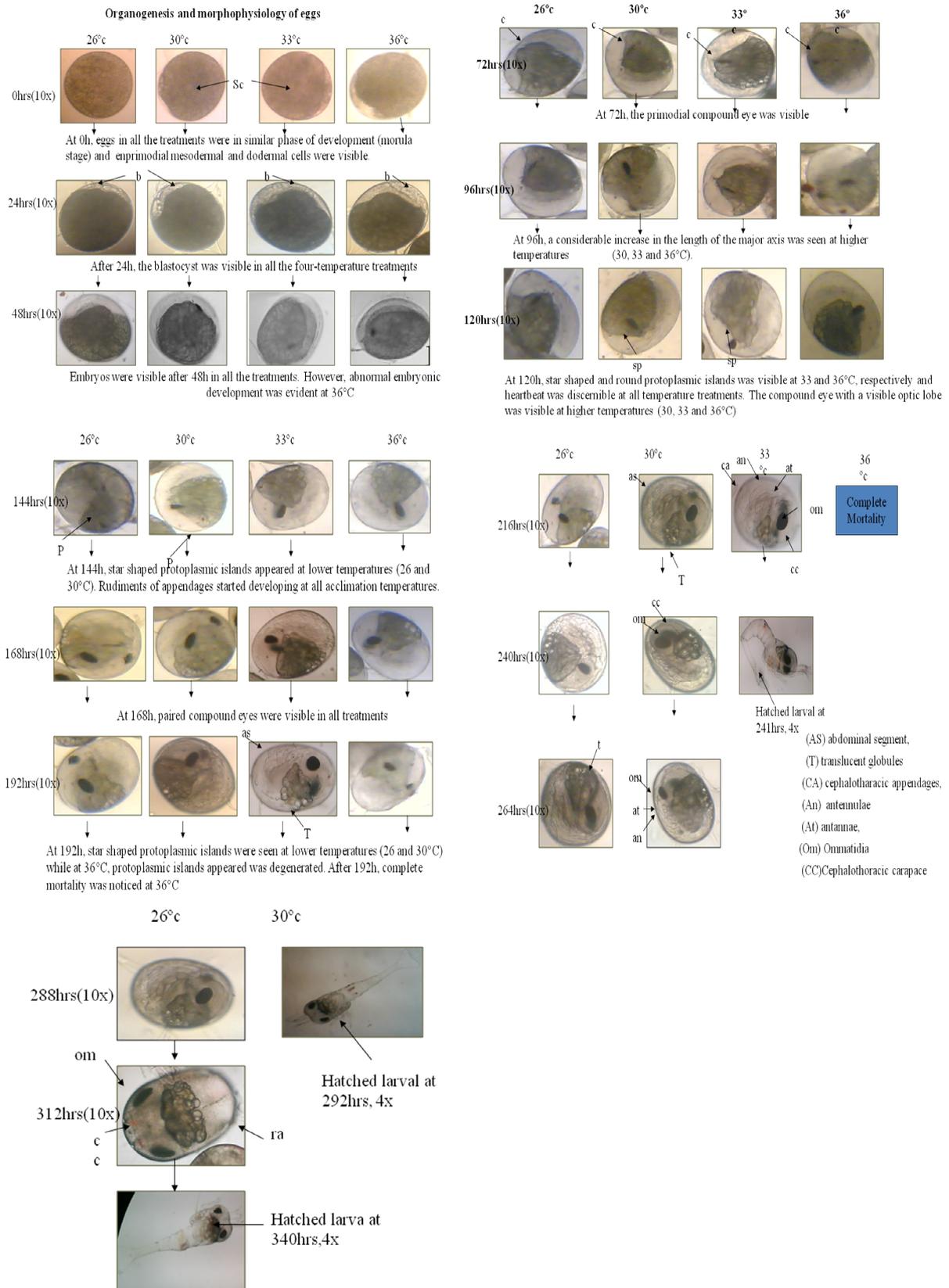


Plate 1: Development of *M. idella idella* eggs incubation at four different temperatures. sc-superficial cleavage, b-blastocyst, c-compound eye, p-protoplasmic island, sp-starshaped protoplasmic island, ra-rudimentary alimentary canal, an-antennule, at-antennae, om-Ommatidia, cc-Cephalothoracic carapace, ca-ephalothoracic appendages, as-abdominal segment.

36°C. However at 36°C, size variation increased with developmental stages, indicating abnormalities until 192 h, after which total mortality was observed. A distinct change in morphometric parameters (major half axis, minor half axis, area and perimeter) was demonstrated at higher temperatures, irrespective of the developmental duration of eggs. Length of hatched embryos increased with increasing incubation temperatures.

Therefore a rapid rate of increase of major half axis and early hatching was observed at 33°C (Plate 1).

Discussion

Prior knowledge on the effects of temperature on cultured aquatic organisms, especially during embryogenesis, is a prerequisite for successful hatchery operation and seed production. Embryonic development is a complex process in which cellular differentiation and proliferation occurs simultaneously but at different rates [11]. Both organogenesis and somatic growth are controlled by enzymatic activities. Embryonic development of ectotherms mainly depends on the differential expression of certain genes and temperature [12] and the rates of their biological functions are critically dependent on environmental temperature. The effect of temperature on developmental rate is direct and development is faster at higher temperatures. However, this increase of developmental rate of embryos at higher temperatures occurs only within tolerable thermal limits [13]. In the present study, organogenesis and physiological responses of *M. idella idella* eggs incubated at different temperatures indicates that higher temperatures increase the rate of embryonic development. Dark brownish structures appearing on the surface of embryos during the development phase of *M. idella idella* are known as protoplasmic islands. Morphology of protoplasmic islands of embryos was specific at different incubation temperatures. Embryos with round, oval or star shaped protoplasmic islands demonstrated normal development until hatching. But at 36°C the protoplasmic island were not clearly visible. Immediately after appearance of degenerated protoplasmic islands, complete mortality was recorded at 36°C. Further isolation and structural analysis of these protoplasmic islands may indicate a functional significance during early development of *M. idella M. idella*. It is evident that heat shock proteins are induced at early developmental phases in response to various physiological stimuli (growth factors, cell differentiation, and hormonal stimulation) and under the influence of temperature [14]. In *M. rosenbergii* also at 36°C complete mortality of embryos was reported once the protoplasmic island was degenerated [15].

Morphometric measurements indicated that an early increase in the length of the major axis of embryos at higher temperatures. Finally, length at hatching was increased with increasing temperatures and the larval major axis was highest at 33°C. However, complete mortality was observed at 36°C after 10 days, which indicates that incubation at 36°C is an upper temperature threshold for *M. idella idella* embryos. Earlier reports on duration of hatching in *Macrobrachium* spp at different temperatures indicates that eggs hatch out in 25 days at 26°C, 20 days at 28-28.5°C and in 17 days at 32°C [16]. In the present study, in *M. idella idella* hatching took first at 33°C (241 hrs) than followed by 30°C (292 hrs) and 26°C (340 hrs). Even though hatching took first at 33°C the survival rate was low when compared with 30 and 26°C. In *M. rosenbergii* the survival was more at 33°C than in 29 and 25°C [15]. In most crustaceans the incubation period is highly dependent on the temperature [17,18]. Incubation periods of Moreton Bay (Australia) population of *S. serrata* are usually in early

spring, at water temperatures of 18-20°C [17]. Water temperatures in estuaries and coastal waters along the north coast of South Africa range between 17-22°C in winter and 23-30°C in summer [19]. Temperature directly influences the developmental rate and development is faster at increasing temperatures. However, this increase of developmental rate of embryos with increase in temperatures occurs only within the acceptable thermal limits [7,13]. In general, thermal limits are narrower for early stages and reduced survival of embryos and juveniles but wider for the adults [7]. Acclimation temperature or thermal history may also affect the temperature tolerance of embryos. In crayfish, time needed for egg development varies with temperature, suggesting the possibility of extending or reducing the incubation period [20]. In crustacean eggs, metabolic rate increases with temperature [21], which affects growth [22], survival [23], and yolk absorption rates [24]. However, high temperature could cause high mortality or serious deformities during egg incubation [25]. Studies on fish [26,27], crustaceans [23,25] and molluscs [28] have shown that there are different approaches to studying the effects of temperature on development. One of the criteria is to measure changes in lipid, protein, and carbohydrate biochemical contents during egg development, which reflect utilization rate [29].

Experiments with *Liocarcinus depurator* showed that a three-fold decrease in development time could occur in successive batches of eggs incubated during the early spring to mid-summer breeding season in one locality [30]. In *L. holsatus* and *Necora puber* hatching success was greater at low temperature-low salinity and high temperature-high salinity combinations. However, *L. holsatus* was relatively more tolerant to the lower range while *N. puber* was more tolerant to the higher range [31]. The better survival of *L. holsatus* eggs and larvae at lower temperatures than that of *N. puber* may reflect field situation; *L. holsatus* produces eggs and larvae earlier and at colder temperatures than does *N. puber* [30,32] noted that within a related group of species there was a direct relationship between egg size and incubation period. In the present study the eggs of the same species (*M. idella idella*) responded differently when exposed to same environmental conditions [33] studied the effect of temperature on egg extrusion rate in *C. quadricarinatus*. They found that egg production was more frequent when mature females were maintained in water over 28°C compared to 25°C and lower. In general the survival and incubation time decreased with increased temperature [34]. As expected, higher temperature caused faster development because it had a direct effect on physiological and biochemical processes [35]. This is a reflection of higher metabolic rate [20] and a decrease in the duration of embryonic or larval development, commonly documented for crustaceans [23]. This effect was previously observed in *C. quadricarinatus* by [34,36] reported approximately 28 days for egg incubation until hatching at 28°C, which is very similar to the present work. The difference in water temperature from 22 to 31°C resulted in 50% shorter development time from egg extrusion to juvenile stage in the work. However, shorter development time in response to higher water temperature is compromised with abnormal development and lower survival [37] found most hatching crayfish reared at extremely high temperatures had deformed limbs and failed to moult normally. In the present study, deformities and abnormal sizes were not considered, although some deformed hatchlings were observed at 33°C that subsequently died. Crustaceans are highly sensitive to environmental changes during ontogeny, and are therefore at higher risk to reach lethal temperature during this period [24,38] report that the consumption rate of total lipid and protein in *Artemia* gradually increases as temperature increases. The higher lipid depletion rate at higher temperature occurs because lipids are the main source of energy during ontogeny of aquatic

organisms [39,40]. Growth and differentiation processes demand large amounts of energy and all metabolic processes are intensified when the temperature is higher [25]. It was also observed that lipid consumption per day intensified at hatching, which could be related to a higher energy production need during this process [41]. For proteins, the consumption rate during embryogenesis might increase as temperature rises [42]. At high temperatures, tissue synthesis is inefficient due to the high cell proliferation rate, and more protein is used as fuel [39]. In the present study, embryonic development was assessed after acclimating the brooders (carrying newly released eggs in the brood chamber) at the rate of 1°C per day to test the temperatures and maintained until hatching, as described by [43]. A negative slope in the linear regression of development time to hatching, indicates a strong inverse relation with incubation temperature.

Our study indicates a direct linear relationship between development rates of *M. idella idella* embryos with incubation temperature. Hence, a direct relation between organogenesis and morphological measurements and development was established. A rapid increase in major axis length observed at 30 and 33°C with a concomitant rate of development and earlier hatching. But when compared to 30°C with 33°C the survival rate is very low. Increase in larval length was observed at higher incubation temperatures indicate that such larvae may develop into dominant prawns. Therefore, incubation temperature may prove vital in producing healthy, high quality prawn seeds for successful prawn farming, by taking advantage of “leap frog pattern” of enhanced growth and overall production. However, this hypothesis needs to be investigated by rearing freshwater prawns from embryos until adult stages at higher temperatures.

In the present study the optimal temperature for incubating *M. idella idella* eggs is recorded at 30°C considering the rate of development and hatching percentage. Total mortality was found at 36°C. High temperatures are related to poor cementing and attachment of eggs [31]. In the case of *M. rosenbergii*, the development and hatching percentage were maximum at 33°C than in 29°C and 99% mortality was recorded at 36°C [15]. Poor hatching percentage and formation of malformed embryos in later stages at 36°C suggest that this rearing temperature is well above the tolerance limit for development of *L. rohita* eggs or may be due to the lack of adequate enzymes involved in hatching [44]. This may be due to adaptive response of *M. idella idella* embryos evolved over the years due to global warming and climatic changes. The complete mortality percentage and gross morphological abnormalities at 36°C suggest that the thermal limit for embryonic development of *M. idella idella* is below 36°C. However, some of the embryos reared at 33°C reached relatively advanced developmental stages in a short time, inspite of low survival rate and gross abnormalities.

From the point of fertilization until hatching, low temperatures retard and high temperatures accelerate embryonic development [45,46]. According to [26] 29-33°C is acceptable for *M. rosenbergii* embryonic development, which is higher than earlier reports of 29-31°C [47]. According to [5] the optimal temperature for incubating *L. rohita* eggs is recorded at 31°C considering the rate of development and hatching percentage, which is higher than earlier reports of *L. rohita* [48]. But in the present experiment, *M. idella idella* at 26-30°C were found to be optimum temperature than other experimental temperature. Overall results suggest that 30°C is the ideal temperature for egg incubation of *M. idella idella* for faster embryonic development, better hatching percentage and least time duration for attaining given ontogenic stages. In *L. rohita* at 26 and 31°C suggests that these temperature ranges are most suitable for incubation but 31°C is the ideal

temperature for egg incubation. A rise in the optimum temperature for embryonic development over the years may be due to continuous warming in the test region along with a gain of adaptive capability and induced thermal tolerance over the years. This hypothesis needs to be tested at the genetic level. These results may be a prelude to effectively utilize the benefits of temperature on better hatching rate and reduced hatchery man-days and ultimately the cost of production in *M. idella idella* hatcheries. However, hatchery seed production of *M. idella idella* is recommended between 26 and 30°C. This study reveals that *M. idella idella* embryos can accommodate climatic changes due to global warming up to 33°C, without hampering the reproduction and embryonic development.

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