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Effects of temperature and salinity on growth and reproduction of the freshwater prawn, *Macrobrachium rosenbergii* (Crustacea- Decapoda) in Egypt.

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ABSTRACT
The effect of different levels of temperature (24 ºC, 29 ºC and 34 ºC) and salinity (8 ‰ and 16 ‰) compared to dechlorinated tap water (0 ‰) as control group on growth and reproduction performance of female *Macrobrachium rosenbergii* was studied under controlled laboratory conditions. Juvenile prawns of 0.21± 0.021g and 3.1±0.208 cm were reared at these conditions for 8 months. The results revealed that growth of the prawn was increased as temperature increased from 24 to 29 ºC then the growth declined at the highest temperature (34 ºC). Also as salinity increased from 0 to 16 ‰, growth of females decreased at all temperatures tested. The highest total length (16.2cm) and total weight (40.53g) were obtained at a combination of 29ºC – 0 ‰. Under the above conditions, the weight of eggs increased significantly up to 5.75g, while the lowest weight (0.20g) was obtained at 34 ºC-16 ‰. The incubation period was significantly affected by temperature. The longest mean period (23 days) was recorded at the lowest tested temperature (24 ºC) with all salinities, while the shortest one (17 days) was reported at 34 ºC at both 8 and 16 ‰. The hatching rate was increased (ranging from 57.3 to 82.3 %) as salinity increased at all treatments. The highest hatching rate (82.3%) was observed at (29 ºC - 8 ‰), while the lowest rate was found at 16 ‰ with all temperatures tested. The number of accumulative berried females decreased as salinity increased from freshwater to 16 ‰. While the highest number was observed at 29 ºC -0 ‰ and the lowest one at 34 ºC-16 ‰. It was clearly found that optimum level of both temperature and salinity for growth, reproduction and hatching success of this species was 29 ºC-0 ‰ and 29 ºC-8‰.

1. INTRODUCTION

Macrobrachium rosenbergii is known to be the largest freshwater prawn, since it is commonly recognized as the giant river prawn. It migrates from a region of lower to higher salinity during its breeding season.

Macrobrachium species has many favorable characters for artificial prawn culture (John, 2009).

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It can tolerate wide ranges of temperature (14-35 ºC) and salinity (0-25 ppt) (New, 1995).

Gravid females migrate across saline gradients to estuaries, where eggs hatch and larvae develop (Ismael and New, 2000). In an earlier study, Singh (1980) demonstrated that prawns were able to grow in salinity up to 17 gL^{-1} with highest growth achieved at salinity between 0 and 2 gL^{-1}. On the other hand, Smith *et al*. (1982) studied the growth of *M. rosenbergii* and found little difference in growth rate up to 10gL^{-1}. In prawn hatcheries, berried females are commonly transferred from freshwater to brackish water to improve their eggs hatching rates. Law *et al.* (2002) reported that the egg hatching rate improved when the females were held in 12gL^{-1}. New (2005) similarly suggested that hatchability could increase if the females were held at low level salinity (5gL^{-1}). A recent study carried out by Yen and Bart (2008) demonstrated that females of *M. rosenbergii* reared at lower salinity of 0 and 6gL^{-1} produced larger number of larvae compared to 12 and 18 gL^{-1}, and the number of larvae produced was inversely related to the salinity levels.

The successful establishment of a species in a given habitat depends on its ability to adapt to the ambient environment (Charmantier, 1998). Salinity and temperature are the most important abiotic factors affecting growth and survival of aquatic organisms (Kinne, 1963&1964). They strongly affect the hatching rate of eggs and the survival of the resulting larvae of *Penaeus* shrimp (Preston, 1985). The influence of temperature on Crustacae depends on the thermal range of the species, geographical distribution, acclimatization response and physiological and behavioral adaptations (Espina *et al.* 1993 and Gutierrez-Yurrita, 2000). The effect of temperature on gonadal development and spawning of freshwater crayfish at different temperatures was studied by Osalde *et al*. (2004) who found that the gonadosomatic and maturation indices were significantly different between 16ºC and 21 and 26ºC. The effect of temperature on reproduction and spawning was studied on some crustacean species such as *Penaeus merguiensis* (Hoang *et al.*, 2002), the Japanese spiny lobster *Panulirus japonicus* (Matsuda *et al*. 2002), and the crayfish *Procambarus llamasi* (Osald *et al*. 2004).

Meanwhile, numerous studies were focused on the effect of a single environmental variable on growth, survival and reproduction of crustaceans such as Jayalakshmy and Natarajan (1996) who investigated the effect of salinity on *M. idella*; Soundrapandian (2008)studied the effect of salinity on *M. malcolmsonii*; Law *et al*. (2002) and Chen and Chen (2003) studied the influence of pH on *M. rosenbergii*; Yen and Bart, (2008) examined the effect of salinity on *M. rosenbergii* but relatively few workers studied the interrelationship of two or more factors that influence aquatic organism. Hill (1974); Lee and Fielder (1982); Vljayan and Diwan (1995); Ponce-Palafox *et al*. (1997) and Zacharia and Kakati (2004) examined the effect of temperature and salinity on *Scylla serrata*, *M. australiense*, *Penaeus indicus, Litopenaeus vannamei* and *Penaeus merguiensis*.

The effect of temperature and salinity on reproduction of the present prawn has important implications for increasing its seed production. Therefore, the present study examined reproductive performance of *M. rosenbergii* females in response to the combined effect of temperature and salinity under laboratory conditions.

2. MATERIALS AND METHODS

Juveniles of freshwater prawn *M. rosenbergii* belonging to the same brood (total weight 0.21 ±0.021 g and total length 3.1±0.208cm) were purchased from Mariut Fish Farming Company at Alexandria(El-Amria region). This study was conducted at the Invertebrate Laboratory, Fish Research Station belonging to National Institute of Oceanography and Fisheries, El-Qanater El-Khayria, Egypt.

2.1. Experimental design

Animals were divided into three groups, each was held at one of the constant temperatures 24, 29, 34 ºC. Each of the three groups was divided into three sub groups treated with different salinity levels 0 ‰ (dechlorinated tap water), 8 ‰, and 16 ‰. Prawns were allowed to acclimate to the selected treatments of temperature and salinity for one week prior to the experiment start. Animals were fed twice a day (10:00 and 17:00 h.) on a pelletized shrimp feed (40% protein) based on visual observation of leftover feed and fecal matter to be removed daily from each container (plastic tanks with a diameter of 70 cm). Observation took place over 8 months (from September 2009 to April 2010). Water temperature was adjusted daily by using 300-W thermostat-controlled immersion heater. Salinity was obtained by mixing de-chlorinated tap water and raw salt and adjusted to the desired levels salinity by using a salinity-conductivity-temperature Meter (YSI Model 33).

Maturing females were counted and observed daily for the presence of eggs and change in egg color. Five females from each treatment with gray-black eggs (24-48 before hatch) were selected and individually transferred into glass aquaria (20x30x50 cm) with continuous aeration and having the same temperature and salinity conditions. Total length of females was measured from the rostrum to the end of telson using a Vernier caliper and the weight was recorded before and after egg hatching by electronic digital balance (Model MR-220), the difference was equal to the weight of eggs.

Hatching rate was calculated from the number of eggs in a brood and the number of larvae hatched out (Soundarapandian**,** 2008). Egg numbers were determined by taking a sample of eggs using a forceps and were weighed after the eggs were wiped by using a paper towel and counted then the total number of eggs was calculated (Das *et al.*, 1996). The egg incubation period was determined as the number of days from spawning to hatching time.

Newly hatched larvae were siphoned into a plastic bucket and were gently stirred in a circular motion. Dead (immotile) larvae accumulating at the center of the bucket were siphoned. Aeration was applied to uniformly distribute the larvae in the water column. The number of live ones was estimated volumetrically by taking 50 ml samples (Yen and Bart, 2008).

2.2. Statistical analysis

Data were analyzed by using a two-way ANOVA (F test, $P<0.05$) to asses the effect of temperature (24, 29 and 34 ºC) and salinity (0 ‰, 8 ‰ and 16 ‰) on growth and reproduction of used animals. If significant difference was indicated at the 0.05 level, then Scheffer's test was used to compare treatments (Scheffer's, 1943).

3. RESULTS AND DISCUSSION

Temperature and salinity are extremely important parameters affecting growth and reproduction of the freshwater prawn, *Macrobrachium rosenbergii***.** The total length, weight and reproduction performance of *M. rosenbergii* were summarized in Table (1). All specimens that were held at freshwater (dechlorinated tap water) and 24 – 29 ºC did not show any significant difference in total length, carapace length and weight of females except at 34 ºC where they decreased significantly. The highest total length (16.2 cm) and total weight (40.53 g,) were observed at combination of 29 ºC- 0 ‰, followed by combination of 24 ºC-0 ‰. The lowest values (8.53 cm total length and 6.03 g total weight) were found at 34 ºC -16 ‰. The growth rate was significantly affected (P<0.05) due to temperature and salinity interaction. Our results showed that growth of the present prawn increased as temperature increased from 24 to 29 ºC, while at 34 ºC, growth declined to its lowest value at all salinity levels. This may be due to the increased calorific intake at higher temperature. Firkins and Holdich (1993) reported similar results and stated that growth of crayfish decline at 34 ºC, might result from increased metabolic demands approaching the calorific intake, leaving little energy for growth, despite animals being fed to excess. This fact confirms the suggestion that high temperature to a certain point increases the molting frequency and growth of the penaeid shrimp (Staples and Heales, 1991; O Brien, 1994 and Parado-Estepa, 1998). The present study revealed that growth of females decreased as salinity increased from 0 ‰ to 16 ‰ which agrees with Goodwin and Hanson (1975) who indicated that juvenile *M. rosenbergii* grows more rapidly in fresh water or slightly brackish water (<5 ‰) when compared to more brackish water of up to 15 ‰. Additionally, Vljayan and Diwan (1995) reported that the optimal levels of temperature and salinity which gave the fast molt with highest growth increment of *Penaeis indicus* were 31 ºC and 15 ‰. Furthermore, Jane and Goldman (1978) reported that growth of juvenile freshwater crayfish, *Pacifastacus leniusculus* decreased with increasing salinity while daily food consumption decreases sharply with salinity. Higher final weight of *M. rosenbergii* females at lower salinity may be due to the fact that prawns takes in more water at ecdysis than in higher salinities and this results in size increase (Yen and Bart, 2008).

The weight of eggs of female was influenced significantly (P<0.05) where the highest weight was 5.75 g at a combination of 29 ºC- 0 ‰, and the lowest value (0.2 g) was obtained at (34 ºC-16‰). Consequently, the highest number of eggs (98503 eggs) and number of Eggs Per Female (NEPF), 2430.4 were achieved at 29 ºC-0 ‰, followed by 24 ºC -0 ‰ and the lowest number of eggs (1937) and NEPF (321.2) were obtained at 34 ºC-16 ‰. This change was highly significant (P<0.05). In this respect, Dube and Portelance (1992) recorded that warm temperature is an effective mean and a preponderant factor in accelerating ovarian maturation and promoting egg laying among the crayfish, *Orconectes limosus.*

The incubation period of the female eggs was significantly affected by temperature (P<0.05). According to Wear (1974); Heasman and Fielder (1983); Choy (1991); Zeng *et al*. (1991) and Arshad *et al* (2006) temperature is one of the most important factors regulating egg development for several crustacean species. The longest mean incubation periods (23, 22 and 20.33 days) were recorded at 24 ºC with different salinity levels, while the highest temperature (34ºC) resulted in the shortest incubation period (17, 17.33, 18 days) at all salinity levels (P<0.05) and this may be due to the fact that lower temperature merely slow down the rate of egg development, while as temperature increases ovarian maturation and egg laying increase (Dube and Porlelance, 1992). This finding is in agreement with the results obtained by Arshad *et al.* (2006) who stated that the egg incubation period of blue swimming crab, *Portunus pelagicus* decreased exponentially from 8.33 to 6.67 days with increasing temperature from 28 ºC to 32 ºC.

On the other hand, Soundarapandian (2008) reported that the incubation period of *M. malcolmsonii* was 14 days in freshwater but it decreased to 11 days with the addition of brackish water of 7 ‰ salinity.

Table 1: Growth and reproduction performance of *M. rosenbergii* females at different temperature and salinity levels (means ±SD).

Temp. (°C)	Salinity (‰)	Final T.L (cm)	C.L. (cm)	Final Wt. (g)	Wt. of eggs (g)	No. of eggs	NEPF	Incubation period (days)	No of hatched larvae	Hatching rate%
24	0	14.6±1.002^{a}	7.17±0.635^{a}	33.1±2.346^{b}	4.37±0.702^{b}	37500±4272^{b}	1132.9±50.18^{b}	23±1.732^{a}	17063±1583^{a}	45.5±10.133^{c}
	8	12.33±1.65^{a}	6.23±0.874^{a}	18.53±3.137^{a}	1.79±0.200^{a}	17850±1884.5^{b}	963.3±96.806^{b}	20.33±2.31^{a}	9104 ±965.2^{b}	51± 8.581^{c}
	16	11.33±1.422^{a}	5.6±1.572^{a}	14.2±1.900^{a}	1.12±0.253^{a}	10900±2885.2^{a}	767.6±109.6^{a}	22±1.000^{a}	4382±841.4^{a}	40.2±11.63^{b}
29	0	16.2±0.819^{a}	8±0.3000^{a}	40.53±3.707^{a}	5.75±1.545^{a}	98503±4190.2^{a}	2430.4±128.9^{a}	19±2.52^{b}	72104±1739.5^{b}	73.2±9.673^{b}
	8	13.03±1.03^{a}	6.4±0.458^{a}	23.2±2.696^{a}	2.31±0.409^{a}	25995.3±3077.1^{a}	1120.5±112.23^{a}	20±2.11^{a}	21399±1398.9^{a}	82.3±14.86^{a}
	16	11.9±0.361^{a}	5.9±0.173^{a}	16.9±2.007a	1.55±0.394^{a}	14700.3±2058.1^{a}	869.8±48.510^{a}	20±1.00^{b}	9658 ±758.98^{b}	65.73±17.501^{a}
34	0	11.23±2.011^{b}	5.53±0.987^{b}	16.93±1.76^{c}	1.12±0.116^{c}	8716±1243.7^{c}	514.8±24.408^{c}	18±1.00^{b}	5700±574.77^{c}	65.43±17.089^{b}
	8	9.03±1.662^{b}	4.5±0.764^{b}	6.17±0.764^{b}	0.31±0.074^{b}	2351.3±492^{c}	381.1±31.470^{c}	17.33±1.528^{a}	1682±322.93^{b}	71.53±13.553^{b}
	16	8.53±0.503^{b}	4.23±0.643^{a}	6.03±0.643^{b}	0.20±0.087^{b}	1937±203.6^{b}	321.2±55.846^{c}	17±1.350^{c}	1109±192.6^{b}	57.3±11.510^{a}

Means bearing different letters are significantly different from one group to another (P<0.05).

T.L =Total length at the end of study (cm)

C.l. =carapace length (cm)

W t= Final weight (g)

NEPF = Number of eggs per female

In the present study, the highest hatching rates (82.3, 73.2 %) were recorded at both 0 ‰ and 8 ‰ salinity levels under 29 ºC, respectively, while the lowest one (40.2%) was obtained at 24 ºC -16 ‰. Therefore, salinity at a certain limit increases the hatching rate due to the absorption of salts, which results in more internal pressure that enhances rupture of the egg membrane. Soundarapandian (2008) obtained a similar result where he reported that hatching percentage of *M. malcolmsonii* increases when the berried females were reared in 7 ‰ salinity than in freshwater. Ling (1969) found that the presence of lower small amount of brackish water (4-6 ‰) provides a better media for hatching of *M. rosenbergii* eggs. Katre and Pandian (1972) reported that the egg of *M. idea* is able to pick up salts from brackish water than from freshwater.

Table (2) indicates that the number of accumulative berried females decreased as salinity increased from freshwater to 16 ‰. It is evident that higher salinity delays maturation for about two months, while higher temperature accelerates maturation. The highest number of berried females (52females) was observed at 29 ºC- 0 ‰, while the lowest number (5 females) was obtained at 34 ºC-16 ‰. This confirms the observation of Yen and Bart (2008) that salinity delays maturation of *M. rosenbergii*, while the number of berried females (with gray eggs) decreased with increasing salinity from 143 at 0gL^{-1} to 59 at 18gL^{-1}. Moreover, Jayalakshmy and Natarajan (1996) held *Macrobrachium idella* at 15 gL^{-1} salinity and observed that any post molt attempt to mate was largely unsuccessful and fertilization of eggs did not take place.

Table 2: number of berried females over 8 month period of breeding females at different treatments

Temp. (ºC)	Salinity (‰)	2009 Sep	2009 Oct	2009 Nove	2009 Dec	2010 Jan	2010 Feb	2010 Mar	2010 April	Total
24	0	0	0	0	0	4	7	6	9	26
	8	0	0	0	0	4	5	3	6	18
	16	0	0	0	0	0	0	5	1	6
29	0	0	0	7	5	8	11	9	12	52
	8	0	0	3	3	5	7	10	7	35
	16	0	0	0	0	0	0	2	5	7
34	0	0	0	5	3	8	8	6	3	33
	8	0	0	0	0	7	3	5	2	17
	16	0	0	0	0	0	0	1	4	5

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