

The combined effects of salinity and temperature on the egg hatching rate, incubation time, and survival until protozoal stages of *Metapenaeus monoceros* (Fabricius) (Decapoda: Penaeidae)

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Abstract: The combined effects of 3 different temperatures (24, 28, and 32 °C) and salinity levels (35, 40, and 45 g L⁻¹) on the egg hatching rate, incubation time, larval activity, and survival rate until protozoal (PZ1) stage of *Metapenaeus monoceros* (Fabricius) were investigated under controlled laboratory conditions. The average fertilization rate was 97.25% following spawning. The eggs were stocked in 2-L glass larval culture flasks and received 1 of 9 salinity and temperature combinations in 3 replicates. The eggs hatched in all treatments. Temperature, salinity, and their interaction had significant influences on the hatching rate of the eggs ($P < 0.05$). The hatching rate decreased as salinity increased and was lowest in all temperature levels at a salinity of 45 g L⁻¹. With regard to the hatching rate, the best combinations were obtained at 35 g L⁻¹ and 32 °C (91.67%) and at 35 g L⁻¹ and 28 °C (89.17%). The incubation time was shorter at 32 °C (11.2 h) than at 28 °C (14 h) or 24 °C (17.2 h). The development rate from the naupliar stage to the protozoal stage was best at a salinity level of 35 g L⁻¹ combined with temperatures of 28 °C and 32 °C. Larval activity was also found to be best at 28 °C and 32 °C at 35 g L⁻¹ and 40 g L⁻¹, as compared to that at 24 °C in all salinity levels.

Key words: *Metapenaeus monoceros*, shrimp, eggs, hatching rate, salinity, temperature

Tuzluluk ve sıcaklığın *Metapenaeus monoceros* (Fabricius) (Decapoda: Penaeidae) yumurtalarının açılma oranı, açılma süresi ve protozoal döneme kadar yaşama oranı üzerine etkileri

Özet: Üç farklı su sıcaklığı (24, 28 ve 32 °C) ve tuzluluğunun (35, 40 ve 45 g L⁻¹) *Metapenaeus monoceros* yumurtalarının açılma oranı, ve süresi, elde edilen larvaların protozoal döneme kadarki yaşama oranı ve larval aktivite üzerine etkileri laboratuvar koşullarında araştırılmıştır. Yumurtlamayı takiben ortalama döllülük oranı % 97,25 olarak bulunmuştur. Yumurtalar 2 L kapasiteli larval kültür kaplarına dokuz salinite ve sıcaklık kombinasyonunu oluşturacak şekilde 3 tekerrürlü olarak stoklanmıştır. Tüm muamelelerde yumurtalar açılmıştır. Yumurtaların açılma oranı üzerine su sıcaklığı, tuzluluk ve her ikisinin birlikte önemli etkileri olmuştur ($P < 0,05$). Açılma oranı tuzluluğun artışına bağlı olarak azalmış, 45 g L⁻¹ tuzlulukta ve tüm sıcaklık düzeylerinde en düşük olmuştur. Açılma oranı bakımından en iyi 35 g L⁻¹, 32 °C (% 91,67) ve 35 g L⁻¹, 28 °C (% 89,17) kombinasyonu olarak bulunmuştur. İnkübasyon süresi 32 °C'de (11,2 saat) 28 °C (14 saat) ve 24 °C'den daha kısa bulunmuştur. Naupli döneminden protozoal döneme geçiş oranı en iyi 35 g L⁻¹ tuzlulukta 28 °C ve 32 °C su sıcaklıklarında olmuştur. Larval aktivitenin 24 °C su sıcaklığının tüm tuzluluk sevipleri ile karşılaştırıldığında, 35-40 g L⁻¹ tuzluluk ve 28-32 °C su sıcaklıklarında daha iyi olduğu belirlenmiştir.

Anahtar sözcükler: *Metapenaeus monoceros*, karides, yumurta, açılma oranı, tuzluluk, sıcaklık

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Introduction

The environmental parameters associated with optimum incubation conditions of eggs are important for the success of a hatchery. Salinity and temperature are 2 of the most important environmental factors affecting the hatching rate and hatching time of eggs and the larval survival rate. In nature, the spawning, egg hatching, and larval stages of penaeid shrimps generally occur in oceanic waters, where salinity and temperature do not fluctuate considerably. It is commonly accepted that penaeid eggs hatch between 12 and 17 h at oceanic salinities and temperatures of 25–28 °C (Lester and Pante, 1992). However, little is known about the combined effects of temperature and salinity on the hatching rate and hatching time of penaeid eggs. The optimal temperature and salinity combination for egg incubation is species-specific (Preston, 1985) and have to be determined for each species. *Metapenaeus monoceros* migrated from the Indo-Pacific region to the Mediterranean Sea through the Suez Canal, suggesting that the species was able to adapt from stable oceanic temperatures to the fluctuations of the Mediterranean basin (Kumlu et al., 1999).

There is little information on the optimal salinity and temperature from egg incubation to the protozoal (PZ1) stage for penaeids, and this information is available for only a few species. Courties (1976) reported that *Metapenaeus* spp. eggs hatch in 0.35–0.79 days at 30 °C and 22.5 °C, respectively, but no information was given about salinity and its interaction with temperature. Adequate egg incubation temperatures for *Penaeus monodon* range between 26 °C and 29 °C (Hillier, 1984; Primavera, 1985). The latter researcher recorded that an increase in temperature from 23 °C to 28 °C or 33 °C significantly decreased the incubation period and had no effect on hatching rates. Primavera (1985) also reported high hatching rates at a salinity level of 33 g L⁻¹ and temperatures between 23 and 33 °C, but found weak larval activity after hatching at 23 g L⁻¹, regardless of the experimental temperatures. The eggs of *P. indicus* and *P. semisulcatus* exhibited retarded development at salinity levels of 20–25 g L⁻¹ and even burst at 10–15 g L⁻¹ (Tseng and Cheng, 1981; Primavera, 1985). Nisa and Ahmed (2000) found that the best egg hatching rate of *Penaeus merguensis*, *P. penicillatus*, *Metapenaeus affinis*, and *Parapenaeus*

stylifera occurred at 35 g L⁻¹, that the hatching rate decreased gradually with declining salinity, and that no hatching occurred at 20–25 g L⁻¹ for *Parapenaeus stylifera*. The hatching rate of eggs from eyestalk-ablated females of *P. semisulcatus* was reported to be 90.3% at 22.4–27.5 °C (Browdy and Samocha, 1986), compared to 87% for *Penaeus merguensis* at 33 °C and 35 g L⁻¹ (Zacharia and Kakati, 2004). More recently, Aktaş et al. (2004) studied the combined effects of temperature and salinity on the hatching success of *P. semisulcatus* and reported that the highest hatching percentage was obtained at 24 °C and 40 g L⁻¹. The authors reported the lowest hatching rates at 30 g L⁻¹ and 28 °C or 32 °C and, following hatching, poor larval activity at 30 g L⁻¹, regardless of the experimental temperature.

To date, only one study has determined the effects of salinity on the development of *M. monoceros* from the PZ1 stage to the postlarval stage. There is no information available on the optimal salinity and temperature during egg incubation and nauplii production of this species. A few commercial farms and research centers in Turkey cultivate this species on a small scale. A better understanding of the combined effects of temperature and salinity on the hatching rate, incubation time of eggs, larval activity, and survival rate of this penaeid shrimp is important for optimal hatchery production (Kumlu et al., 2000). Therefore, the primary aim of this study was to determine the best salinity and temperature combination and to define adequate conditions for optimal larval production of *M. monoceros*.

Materials and methods

This study was undertaken at the Marine Research Station of the Faculty of Fisheries, Mustafa Kemal University, in Kale Village (36°17'29.98"N, 35°47'4.40"E) in İskenderun, Hatay, Turkey. The eggs were obtained from females caught in the fourth gonadal stage in İskenderun Bay, in the northeastern Mediterranean Sea (36°22'35.39"N, 35°44'15.10"E and 36°38'23.08"N, 36°3'0.57"E), by commercial trawl operation in the early morning during July 2008. Females (35.75 ± 2.21 g, 132.13 ± 7.15 mm in total length) were transferred to a 150-L plastic tank in oxygenated sea water and taken to our station by boat within approximately 2 h. Upon arrival, they

spawned in a 100-L tank at 38 g L⁻¹ salinity and 28 °C at 2230 hours on the same day. The fertilization rate and egg quality were determined under an inverted microscope at 4× (CKX31, Olympus). Ten minutes after the spawning, the eggs taken from 2 females were pooled and concentrated onto a 100-µm sieve, and the eggs were acclimated to experimental test salinities (35, 40, and 45 g L⁻¹) and temperatures (24, 28, and 32 °C) by decreasing or increasing the salinity and temperature every 15 min at rates of 2 g L⁻¹ and 1 °C, respectively. Salinity was adjusted by adding either fresh water (well water) that was prepared and aerated before use or sea salt (Instant Ocean, USA), as described by Kumlu et al. (2000, 2001). The eggs were then stocked in 2-L round-bottom glass flasks in 3 replicates at a density of 100 eggs L⁻¹.

Salinity and temperature were measured with a digital salinometer (YSI 30 salinometer, YSI, USA). Temperatures for each treatment were maintained in 3 thermostatically controlled fiberglass water baths (± 0.5 °C). Moderate aeration (approximately 4 bubbles s⁻¹) was maintained through a silicon rubber tube with a glass rod at the tip.

Hatching time (incubation time) was determined when 50% of the eggs were hatched in each flask. When the hatching finished, the nauplii and dead eggs in each flask were concentrated using a 100-µm mesh and counted to determine the hatching rate. All of the nauplii were transferred again to the flasks to evaluate the larval activity and to develop

until the PZ1 stage. In practice, positive photo-taxi is considered to be an indicator of larval quality in penaeid shrimps.

Larval activity was determined as the response to a light source (5-W Xenon) in a given time (1 min). Therefore, a 5-W power flashlight was used to attract the larvae (Aktas et al., 2004). At the end of the nauplius stage, in the PZ1 stage, each flask was concentrated using a 100-µm mesh. All larvae were then counted with the loop to determine the hatching and survival rates up to the PZ1 stage.

Statistical analysis

The hatching and survival rates of nauplii to the PZ1 stage were analyzed using 2-way ANOVA. Significant difference was determined at a 0.05 probability level using Tukey's test after the normality and homogeneity of the data were checked using the Minitab (Windows Release 10.0, PA, USA) statistical package (Pelosi and Sandifer, 1995).

Results

A total of 42,500 eggs from 2 spawning females were obtained. The average fertilization rate was 97.25% following spawning.

Hatching rate

Temperature, salinity, and their interaction had significant influences on the hatching rate of eggs, but salinity was the most important factor (Table).

Table. The hatching rate, hatching time, larval activity, and survival rate at PZ1 of *M. monoceros* (Fabricius) eggs incubated at different salinity levels and temperatures.

Temp. (°C)	Salinity (g L ⁻¹)	Hatching rate* (%)	Hatching time (h)	Larval activity	Protozoa* (%)
24	35	87.33 ^a ± 1.97	18	Average	81.50 ^a ± 2.00
	40	85.00 ^a ± 1.44	18	Average	80.16 ^a ± 1.44
	45	0.00 ^b ± 0.00	--	--	0.00 ^b ± 0.00
28	35	89.17 ^a ± 2.21	13.5	Good	88.63 ^a ± 4.10
	40	83.33 ^a ± 4.41	13.5	Good	79.16 ^a ± 10.23
	45	35.83 ^c ± 3.63	13.5	Poor	21.00 ^c ± 7.05
32	35	91.67 ^a ± 2.21	11.2	Good	89.81 ^a ± 3.81
	40	80.00 ^a ± 2.89	11.2	Good	75.33 ^a ± 7.30
	45	0.67 ^b ± 0.44	11.2	Poor	0.00 ^b ± 0.00

*Each value is a mean ± SD (n = 3); means with different superscripts were found to be significantly different from each other (P < 0.05).

Eggs incubated at 35 g L⁻¹ had a higher mean hatching rate (89.39%) than those incubated at 40 and 45 g L⁻¹ (82.73%, 12.17%; $P < 0.05$). The hatching rate was also higher at 28 °C than at the lowest and highest temperatures, 24 °C and 32 °C. The highest hatching rate was obtained at 32 °C and 35 g L⁻¹ (91.67%), followed by 28 °C and 35 g L⁻¹ (89.17%) (Table). The lowest hatching rate occurred at 45 g L⁻¹ in all temperature levels ($P < 0.05$).

Results show that successful incubation of *M. monoceros* eggs can take place over a wide range of temperatures and salinity levels, but salinity appears to have a more profound effect than temperature.

Hatching time

Incubation time was mainly affected by temperature and was shortest at 32 °C (11.2 h), followed by 28 °C (13.5 h) and 24 °C (18 h) (Table).

Larval activity

Larval activity was poor at a salinity level of 45 g L⁻¹, regardless of the temperature, and was highest at 35-40 g L⁻¹ at temperatures of 28 °C and 32 °C. The activity of larvae obtained at 24 °C was average (Table).

Survival to the PZ1 stage

The results, summarized in the Table, show that temperature, salinity, and their interaction had an important influence on survival to the PZ1 stage. Although metamorphosis from the naupliar stage through the first PZ1 stage was successful at all salinity and temperature levels, the larvae grown at the highest salinity level (45 g L⁻¹) had a lower survival rate (7%) than those grown at lower salinities (35 g L⁻¹ and 40 g L⁻¹), which had survival rates of 86.65 % and 78.22%, respectively ($P < 0.05$). The highest larval survival up to PZ1 occurred at 28 °C, regardless of the salinity level, and the second best survival rate was obtained at 32 °C. Taking the survival data into account, the best temperature and salinity combination to the PZ1 stage of *M. monoceros* was 35 g L⁻¹ at 32 °C and 28 °C.

Discussion

This study shows that salinity is a more important parameter than temperature, as it affects the hatching rate, larval activity, and survival of *M. monoceros*. Eggs incubated at 45 g L⁻¹ had the lowest hatching

rate. The best hatching success was obtained at the lowest salinity (35 g L⁻¹). Nisa and Ahmed (2000) studied the effects of 6 different salinities on the hatching success of 4 penaeid shrimps, *M. affinis*, *P. stylifera*, *F. merguensis*, and *F. penicillatus*. They reported that hatching success was highest at 35 g L⁻¹ for all of the species studied. Zacharia and Kakati (2004) also found hatching and survival rates to be highest at 33 °C and 35 g L⁻¹ for *F. merguensis*. In a study by Kumlu et al. (2001), a salinity level of 35-40 g L⁻¹ promoted the survival, growth, and development of *M. monoceros* throughout the larval culture period. Browdy and Samocha (1986), Aktaş and Kumlu (1999), Kumlu et al. (1999), and Aktaş et al. (2004) reported that *P. semisulcatus* eggs incubated at 35-40 g L⁻¹ had good hatching success, supporting the findings of the present study on *M. monoceros*. The present study results and those reported in the literature indicate that the optimal egg incubation salinity for *M. monoceros* is about 35-40 g L⁻¹.

Hatching time was mainly influenced by temperature in the current study. Preston (1985) reported that there is a relationship between temperature and salinity, and both hatching time and success can be further modified by the temperature and salinity during spawning. This suggests acclimation during maturation for *P. plebejus*, *Metapenaeus macleayi*, and *M. bennettiae*. Aktas et al. (2004) reported a similar finding for *P. semisulcatus*. Regardless of the salinity levels tested in our study, hatching occurred in 18 h at 24 °C, while hatching occurred at 13.5 h and 11.2 h at 28 °C and 32 °C, respectively. Overall, the hatching time recorded in this study was lower than that recorded for *Penaeus* eggs by Primavera (1985), Dall et al. (1990), Lester and Pante (1992), and Aktaş et al. (2004).

The spawning and larval stages of penaeid shrimps occur offshore at oceanic salinities and stable temperatures. Hence, poor hatching rates, retarded larval development, and poor larval activity are generally expected in extreme salinities and temperatures that vary from normal oceanic levels (Lester and Pante, 1992). In the current study, larval survival to the first PZ1 stage was highest at salinity levels of 35 g L⁻¹ and 40 g L⁻¹ and at temperatures of 28 °C and 32 °C. Survival was shown to be adversely affected by high salinity (45 g L⁻¹). Kumlu et al. (2001)

reported that a salinity level of 40 g L⁻¹ promotes the survival, growth, and development of *M. monoceros* larvae from PZ1 to the PL1 culture period. This study also supports the finding that 40 g L⁻¹ salinity is suitable for the incubation of eggs and survival from nauplii to the PZ1 stage.

Based on the results of the present study, the optimal salinity and temperature levels for egg incubation and survival, larval activity, and larval development until the PZ1 stage seem to be 35-40 g

L⁻¹ and 28-32 °C for *M. monoceros* in the northeastern Mediterranean Sea.

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