Marine and Life Sciences
E-ISSN: 2687-5802
Journal Homepage: https://dergipark.org.tr/en/pub/marlife
Research Article

The effect of salinity and temperature on egg hatching rate, hatching time and larval activity of *Farfantepenaeus aztecus* (Ives, 1891) (Decapoda: Penaeidae)

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Received: 22.10.2021 Accepted: 25.11.2021

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ABSTRACT

This study was conducted to determine the optimum temperature and salinity for the hatching rate, hatching time of eggs and larval activity of *Farfantepenaeus aztecus* (Ives, 1891). For this purpose, *F. aztecus*, which was caught from nature in the 4th gonad stage and were spawned on the same night in controlled laboratory conditions. The eggs were stocked in 2-l round bottom glass flasks and received one of nine temperature (24, 28 and 32°C) and salinity (30, 35 and 40 ppt) combinations as 50 eggs per liter after determining the fertility rate of the eggs. Although eggs hatched in all salinity and temperature combinations, water temperature, salinity and interaction had significant effects on hatching rate, hatching time and larval activity (P<0.05). Considering only salinity, the best hatching ratio was found at 35 ppt (48.44%), 40 ppt (47.89%) and the lowest hatching rate was found at 30 ppt (34.77%, P<0.05). It was found that the best hatching ratios were at 28°C (52.22%), at 32°C (48.33%) and followed by the eggs incubated 24°C (30.55%, P<0.05). The incubation time was shortened due to the increase in water temperature and changed between 11.40-17.10 hours. It was determined that the activities of the larvae incubated at lowest water temperature (24°C) and at 30 ppt were weaker than those incubated at 28°C and 35- 40 ppt salinities. The results show that 28-32°C water temperature and sea salinity slightly less than the salinity of the Mediterranean Sea (38.5 ppt) are optimal for the incubation eggs and for the production of high quality nauplii of *F. aztecus* found in the Northeastern Mediterranean.

Keywords
Farfantepenaeus aztecus
Eggs
Incubation
Salinity
Temperature
Nauplii

Introduction

The vast majority of penaeid shrimps spawn and larval development take places in full strength seawater in the natural environment that is in environments with constant water temperature and salinity level. It is known that salinity and temperature are two of the most important abiotic factors affecting the survival and growth of penaeid shrimp larvae in aquaculture and natural environment conditions. It has been reported that their ability to withstand basic environmental changes are not fully equipped during the hatching of the eggs laid by the female and larval development. For this reason, the success of the shrimp hatchery in aquaculture is related to keeping the egg incubation and larval rearing conditions close to optimum.

It is widely accepted that penaeids eggs hatch between 12-17 hours at oceanic salinities and temperatures of 25-28°C (Lester and Pante, 1992; Aktaş and Çavdar, 2012). However, the effects of both salinity and temperature on hatching rate, hatching time and larval activity are unknown at the species level. It is reported that the combination of salinity and temperature is specific-specific and should be determined for each species with aquaculture hatchery facilities (Preston, 1985).

Cite this article as
Durmuş Çolakoğlu, D. & Aktaş, M. (2021). The effect of salinity and temperature on egg hatching rate, hatching time and larval activity of *Farfantepenaeus aztecus* (Ives, 1891) (Decapoda: Penaeidae). *Marine and Life Sciences*, 3(2): 88-92.

There are few studies on the effects of water temperature and salinity on the hatching and survival rate of penaeid shrimps eggs and are limited to a few species. For example, *Metapenaeus* spp eggs hatched to 0.35-0.79 days in the water temperature range of 22.5-30°C (Courties, 1976). Primavera (1985) also reported higher hatching rates at a salinity level of 33 g L^{-1} and temperatures between 23 and 33°C, but found poor larval activity after hatching at 23 g L^{-1}, regardless of the experimental temperatures for *P. monodon*. It is reported that the development of *P. semisulcatus* and *P. indicus* eggs started to deteriorate at low salinity levels such as 20-25 ppt, and completely collapsed at 10-15 ppt salinity (Tseng and Cheng, 1981; Primavera, 1985). It was stated that the eggs of *Metapenaeus affinis, Parapenaeus stylifera, Penaeus merguensis* and *Penaeus penicillatus* had the best hatching rate at 35 ppt salinity level and the hatching rate decreased with decreasing salinity, and P. stylifera eggs did not hatch at 20-25 ppt salinity levels (Nisa and Ahmet, 2000). On the other hand, Aktaş et al. (2004) reported that the best hatching rate of *P. semisulcatus* eggs was obtained at 24°C temperature and 40 ppt salinity level. Most recently, the best combination of temperature and salinity for incubation of *Metapenaeus monoceros* eggs has been reported as 35 ppt at 32°C and 35 ppt at 28°C (Aktaş and Çavdar, 2012). These results show that the combined effects of water temperature and salinity were species specific in the early stages.

The crustacean fishery production was around 5,997 million tons and aquaculture production was 9.4 million ton in 2018, the majority of which consisted of penaeid shrimps (FAO, 2020). Penaeid shrimps are one of the most valuable crustaceans in the world. The shrimp species cultured in worldwide belong to the family Penaeidae. Among the species of these family, *Litopenaeus vannamei, Penaeus monodon, Marsupenaeus japonicus, P. merguiensis, P. indicus, P. orientalis, P. semisulcatus, Farfantepenaeus aztecus* and *Metapenaeus ensis* are the important ones.

The presence of brown shrimp, *Farfantepenaeus aztecus* (Ives, 1891) a shrimp species of Atlantic origin were first reported from the Mediterranean in 2010 (Deval et al., 2010). After this date there are reports of Finike, Antalya Bay, Mersin Bay, Iskenderun Bay, Yumurtalık Bight (Gökoğlu and Özvarol, 2013), and then reported from the Aegean Sea (Kapiris et al.,2014). In addition, the presence of *F. aztecus* has also been recorded recently other parts of Mediterranean Sea (Minos et al., 2015). The species having been commercially caught by the anglers throughout the Eastern Mediterranean. In addition, the species also have been reported an important aquaculture species for recreational and sport fishing industry in USA.

Although brown shrimp, *F. aztecus* have been caught in different salinity levels ranging from 1-69 ppt (Simmons 1957; Zein-Eldin and Renaud 1986) in natural environment, but there are any correlations between salinity and spawning, hatching, produced nauplius and growth have been reported. Saoud and Davis (2003) reported that the capacity of *F. aztecus* postlarvae to acclimate to low salinity waters improves after PL-13. Before this age the PLs are not tolerant to low salinity waters. The reproductive performance and larval rearing of *F. aztecus* have been examined (Gandy, 2004). In all previous studies with Penaeid shrimps, the relationship between PL size and salinity was evaluated, and the effects of water temperature and salinity on egg incubation and nauplius periods were not taken into account.

Considering its evaluation as an alternative species for shrimp farming, it is necessary to know the optimal salinity and water temperature on the hatching of eggs, survival rate, hatching time and activities of the obtained larvae in order to produce high quality post larvae and to define adequate conditions for optimal larval production of *F. aztecus.*

Material and Methods

This study was carried out at the Marine fish and Crustacean Research Station of Faculty of Marine Sciences and Technology, Iskenderun Technical University in İskenderun, Hatay, Turkey.

The eggs were obtained from a female with the fourth gonadal stages caught off Iskenderun Bay in the north Eastern Mediterranean Sea (36°33'08.6"N 35°55'10.7"E), by commercial gill net operation. The female (42 g, 179 mm in total length) were transferred to the station with a 150-L plastic tank in oxygenated seawater and upon arrival, the female stocked in an 80-L black spawning tank containing the seawater of 38 g L^{-1} salinity and 27.6°C temperature on the same evening. Oxygen was supplied to the spawning tank by a silicon rubber tube with a moderate aeration. The female spawned at 22:00 on the same day. Egg quality and fertility rate were determined under an inverted microscope at 4× (CKX31, Olympus) in the first 15 minutes following spawning.

After egg count and egg quality were determined, all eggs were concentrated onto a 100-μm sieve and then the eggs were acclimated to the test temperatures (24, 28, and 32°C) and salinities (30, 35, and 40 g L–1) planned for the experiment. Acclimation of the eggs to the experimental temperature and salinity conditions were done by decreasing and increasing of the water temperature and salinity at rates 1°C and 2 g L^{-1} every 15 minutes. Well water and sea salt (Instant Ocean, USA) were used to adjust the salinity. The eggs, whose acclimation was completed, were stocked in 2-liter glass flasks in 3 replicates as 50 pieces per liter. The controlling and maintaining of water temperature, salinity, and establishing other experimental conditions were performed as described by Aktaș et al. (2004), Aktaș and Çavdar (2012). A salinometer YSI 30 (Yellow Springs Instrument Company, Inc. Yellow Springs, Ohio) was used to measure water temperature and salinity, and thermostatic (150-watt, (±0.5°C) aquarium heaters was used to maintain water temperature.

In determining the hatching time of the eggs, the incubation of 50% of the eggs was taken as a criterion. When the eggs incubation finished, the ventilation was stopped for a short time to calculate the number of nauplius produced. Actively swimming nauplius were counted with the naked eye, and dead eggs that did not open and accumulated at the bottom were concentrated using a 100-μm sieve and counted under the microscope.

The following formula was used for determining fertilization rate, hatching rate:

Fertilization rate = Total number of fertilized eggs / Total number of eggs spawned × 100

Hatching rate = Total number of nauplii /Total number of eggs spawned × 100

The determination of larval activity was based on counting the larvae collected towards the light source within 30 seconds and scoring the activity as 1 (poor), 2 (average) and 3 (good) (Aktaș et al., 2004; Aktaș and Çavdar, 2012). In order to determine larval activity, all larvae were transferred to a two-liter, black plastic box with an observation point and light source point. A 5-W power flashlight was used to attract the larvae.

Data were analyzed using the SPSS software (version 17.0; SPSS Chicago,IL,USA). Normality and homogeneity was tested by using Kolmogorov Smirnov and Levene test, respectively. Two-way ANOVA was used to determine the effects of temperature and salinity on the survival rate. A post hoc Duncan's multiple range test was used to determine the differences among the treatments. All statistical analyses were considered significant with P (≤0.05). All means were given with ± standart deviation (SD).

Results

39500 eggs were obtained from a female in a single spawning. The average fertility rate of the eggs was found to be 96%.

Hatching rate

Eggs hatched at all tested temperature and salinity levels. Salinity, temperature and the interaction of both had significant effects on the hatching rate (Table.1). Considering only salinity, the best hatching ratio was found at 35 ppt (48.44%), 40 ppt (47.89%) and the lowest hatching rate was found at 30 ppt (34.77%, P<0.05). When the evaluation was made considering the temperature condition, it was found that the best hatching ratios was at 28°C (52.22%) and at 32°C (48.33%) followed by the eggs incubated 24°C (30.55, P<0.05).

The best hatching combinations were found to be 28°C water temperature with 35-40 ppt salinity level, 32°C water temperature with 35 ppt salinity level." (Table 1).

Incubation time

The eggs hatching time were inversely affected by decreasing temperature (Figure 1) and the eggs incubated at 32°C was the shortest (11.40 h), followed by 28°C (13.40 h) and 24 °C (17.00 h).

Larval activity

According to the larval activity results determined as a result of larval motility and phototaxis reactions of the larvae, it was determined that the activities of the larvae incubated at lowest water temperature (24°C) with 30 ppt were weaker than those incubated at 28°C with 35- 40 ppt salinities level (Table 1).

Discussion

It is accepted that the spawning and larval culture of many shrimp species belonging to the Penaeids take place in ocean-characterized sea water with a salinity of 35 ppt and 25 -28°C water temperature. The present study showed that temperature, salinity and their interaction had significant effect on the hatching rate, incubation time and larval activity of *F. aztecus* eggs incubated different salinity and temperature combinations.

Table 1. Hatching rate (means±s.d., n= 3), Larval activity and hatching time (incubation duration, hours) of *Farfantepenaeus aztecus* eggs incubated in different salinity and temperature combinations. Means with different superscripts are significantly different ($p<0.05$)

Temperature (°C)	Salinity (ppt)	Hatching rate (%)	Hatching time(Incubation duration, hours)	Larval activity
24	30	20,00 ±1,00a	17.00	Poor
	35	36,33 ± 5,50b	17.10	Poor
	40	35,33 ± 4,04b	17.10	Poor
28	30	43,33 ± 8,73c	13.50	Average
	35	52,66 ± 6,50d	13.40	Good
	40	60,66 ± 2,08d	13.50	Good
32	30	41,00 ± 12,16c	11.40	Average
	35	56,33 ± 4,16d	11.50	Good
	40	47,66 ± 8,96d	11.50	Good

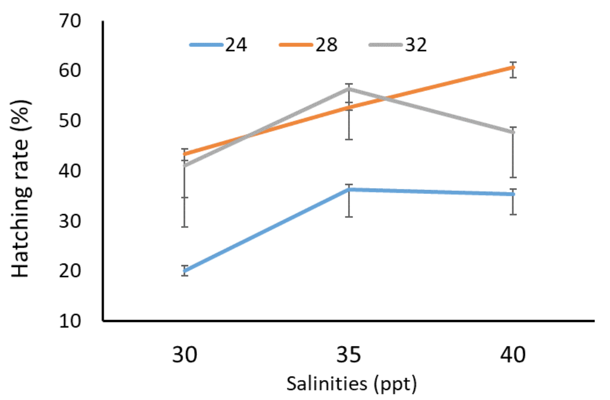

Figure1. Hatching rate (means±s.d., n= 3) of *Farfantepenaeus aztecus* eggs incubated in different salinity and temperature levels

In the current study, the optimum salinity level for egg incubation was found to be 35-40 ppt, and the temperature was found to be 28°C when larval activity is taken into account.

In the literature on egg incubation of marine shrimps, optimal salinity ranges from 30 to 40 ppt and specific to the species. For example, Nisa and Ahmed (2000) were studied six different salinity levels in the incubation of the eggs of four different shrimp species (*M. affinis, P. stylifera, F. merguensis,* and *F. penicillatus*) and the best hatching success was achieved at the 35 ppt level. Zacharia and Kakati (2004) also found hatching and survival rates to be highest at 33°C and 35 ppt for *F. merguiensis*.

Aktaş et al. (2004) suggested a salinity level of 28°C and 35-40 ppt for the incubation of *P. semisulcatus* eggs. Aktaş and Cavdar (2012) stated that the best hatching rate in the incubation of *Metapenaeus monoceros* eggs occurs at 35 ppt salinity level at three different temperature levels (24, 28, 32°C). Our current study shows that it would be appropriate to spawn at a salinity level that it is slightly less salinity than the Mediterranean salinity (38.5 ppt) for spawning of *F. aztecus* obtained from the Mediterranean.

Although the fertility rate of the eggs obtained in our current study was high around 96%, the highest hatching rate was around 60.66%. It is reported that diet composition is a critical factor during maturation, spawning and hatching. Deficiencies of the essential substances produce adverse effect on egg quality and hatching rate (Kjøsrvik et al. 1990; Palacios et al., 1998; Regunathan, 2008). In general, the low hatching rate of eggs in this study at all temperature and salinity levels can be attributed to low egg quality.

The incubation time of the eggs was affected by the water temperature and was completed in the range of 12-17 hours. There are similar results related with hatching time of penaeid shrimp eggs and it is stated that incubation time is shortened depending on the increasing of water temperature (Preston, 1985; Aktaş et al., 2004; Aktaş and Çavdar, 2012).

Spawning, hatching and larval stages of many penaeid shrimp species generally take places in environments with oceanic character and constant water temperature. In this study, prolonged incubation time and low hatching rate were found at 24°C temperature and 30 ppt salinity levels. Lester and Pante (1992) reported that poor hatching rate, retarded larval development and poor larval activity could be expected in extreme salinity and temperature levels. Similar results were found for *P. semisulcatus* (Aktaş et al., 2004) and *M. monoceros* (Aktaş and Çavdar, 2012).

Conclusion

In conclusion, it can be said that sea water with a temperature of 28-32°C and a salinity lower than the salinity of the Mediterranean Sea (38.5 ppt) would be suitable for incubating the eggs and production of high quality post larvae of *F. aztecus* found in the North-eastern Mediterranean.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

Authors contributed equally to this paper.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Acknowledgements

The authors thank Dr. Yavuz Mazlum (Iskenderun Technical University, TURKEY) for his data analysis and valuable suggestions for this manuscript. This study (MSc thesis) was carried out under the supervision of Dr. Mevlüt Aktaş, who is a team member of the TÜBİTAK 1001 project (121O713), within the scope of the preliminary studies realised to carry out biofloc applications for *Farfantepenaeus aztecus* culture.

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