

*Research article***Hatching and survival performance of *Artemia franciscana* under different salinities****Kola SUNEETHA^{ORCID}, P. PADMAVATHI*^{ORCID}, Darwin CHATLA^{ORCID}**Aquatic Biology Laboratory, Department of Zoology & Aquaculture Acharya Nagarjuna University, Nagarjuna
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Abstract: *Artemia* (brine shrimp) is a suitable food source for a variety of aquatic animal species at all stages of their life cycle. Nauplii, specifically, are crucial for shrimp larviculture in aquaculture systems. During the hatchery phase, to ensure high hatching synchrony and maximize the yield of nauplii, it is important to consider the effects of environmental factors such as salinity. To determine the effect of salinity on the % hatching and survival of *Artemia franciscana*, experiments were conducted with salinities ranging from 5-40 ppt. The results of the experiments showed that the highest hatching rate of $67.00\% \pm 1.53$ was observed at 35 ppt salinity, while the lowest was found at 5 ppt salinity ($33.04\% \pm 4.53$). Similarly, the highest survival rate of $61.33\% \pm 2.33$ was recorded at 35 ppt salinity, and the lowest was recorded at 5 ppt, $27.97\% \pm 1.47$. These findings suggest that to achieve optimal hatching rates, maintaining a salinity level of 30-40 ppt is essential.

Keywords: *Artemia franciscana*, salinity, hatching, survival

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Introduction

Aquaculture is one of the fastest-growing food-producing sectors in the world (Kavitha et al., 2017; Chatla et al., 2020). Providing ideal nutrition for any aquatic species at its initial stage is a crucial task (Kandathil et al., 2020). Since many of these larvae have small mouth gaps, the larval feed must be suitably small for the tiny larvae to swallow (Olivotto et al., 2017). The viability of larval feeds depends not only on their particle size but also on their nutritional composition (de Moraes et al., 2022). Generally, the cultivation of larvae of various aquaculture species is highly dependent on live food (Sandeep et al., 2021). Live feeds provide most of the

essential nutrients to finfish and shellfish larvae, which are required for growth and development (Altaff, 2020). To date, no replacement for tiny plant and animal water creatures (phytoplankton and zooplankton) has been found to match their range of particle sizes and nutritional qualities (Sandeep et al., 2015).

In a hatchery system, the shrimp larval diet consists of a combination of microalgae and the early stages of the phyllopod crustacean *Artemia* as well as dry food available on the market, or manufactured locally. *Artemia* (brine shrimp) is an important type of zooplankton that closely resembles the shrimp species that belong to the phylum Arthropoda

(Thackeray & Beisner, 2024). It has gained much importance in the ever-growing aquaculture industry, as this species is highly suitable as live feed for many cultured species (Gonçalves Junior et al., 2022). *Artemia* is mostly used as a live food along with microalgae for the larval stages of shrimp species (Briski et al., 2008). The supplementation of *Artemia* provides not only primary nutritional requirements but also enzymes and other essential dietary elements for the early stage of fish and crustacean larvae (Sorgeloos et al., 2001).

The global production of *Artemia* cysts oscillates at approximately 3000 - 4000 tonnes per annum. Great Salt Lakes in the USA, Russian saline biotopes, China, and Kazakhstan are the major suppliers of *Artemia* cysts to the world aquaculture industry (Browdy et al., 2017; Litvinenko et al., 2015; Sellami et al., 2020; Camara, 2020). India is one of the major importers of *Artemia* cysts, with a total of approximately 325-345 million tons imported annually (Lucia et al., 2022).

The efficiency and optimal hatching percentage of *Artemia* cysts mainly depend on environmental factors such as temperature and salinity (Gajardo & Beardmore, 2012; Kumar & Babu, 2015; Sharahi & Zarei, 2016; Hasan & Rabbane, 2018; Htun et al., 2019; Choi et al., 2021; Dey et al., 2023). Changes in the salinity of the habitat cause variations in the tolerance limits of these populations (Choi et al., 2021). Hence, understanding the optimal ranges of water salinity for *Artemia* production is of fundamental importance. A greater percentage of *Artemia* cysts hatched to meet nutritional demands in shrimp larval forms can contribute to ensuring hatchery protocols. Hence, the present investigation was carried out to estimate the effect of various salinity ranges on the hatching and survival percentage of *Artemia franciscana*.

Materials and Methods

Experimental design

To carry out the present research, the facilities were acquired from the BKMN shrimp hatchery, which is located in Undavalli (16°50'64" N and 80°57'13" E), Guntur district of Andhra Pradesh, India. Two different experiments were carried out to determine

the effects of salinity tolerance on the percentages of hatching and survival of *A. franciscana*.

Estimation of hatching percentage

To determine the percentage of hatched *Artemia* (%), commercially available *Artemia* cysts of *A. franciscana* (M/s INVE Aquaculture, Belgium) were used. Cylindrical FRP tanks with transparent conical bottoms were used, and vigorous aeration was provided for *Artemia* hatching. Adequate lighting (2000 lux) was provided to the hatching tanks. Different experiments were conducted on the effect of salinity on the percentage of hatched *Artemia* larvae, and salinities ranging from 5-40 ppt were maintained at a temperature of 30°C. The *Artemia* hatching protocol was carried out according to Lavens and Sorgeloos (2000). In brief, the *Artemia* cysts were initially hydrated in fresh water for 1 h before being incubated for hatching, followed by a 15 min decapsulation process using 5.5% sodium hypochlorite and 40% NaOH. The decapsulated cysts were subsequently rinsed with 0.1% sodium thiosulfate and followed by cleaning in fresh seawater. After that, they were incubated at a concentration of 1 g of cysts per liter of seawater for 24 h. After 24 h of incubation, the hatched nauplii were counted using a 1 ml pipette. The percentage of *Artemia* hatched was calculated by using the following formula (Kulasekarapandian, 2003):

$$\% \text{ Hatching (H)} = \frac{N}{C} \times 100 \quad (1)$$

where H = hatching percentage of *Artemia nauplii*; N = number of nauplii obtained; and C = total number of cysts.

Estimation of survival percentage

After hatching, the nauplii were subjected to feeding experiments. To estimate the survival rates of *A. franciscana*, one-day-old nauplius (Instar II) was collected using a 100 µm sieve. They were then rinsed with running water for 5 m and transferred to experimental tanks. The experimental design was randomized with different salinity treatments, each with three replicates. Salinities ranging from 5-40 ppt were used under conical FRP tanks. The nauplii were fed daily with microalgae (*Thalassiosira* sp.) at 1.5×10^4 cells for 7 days. Daily water exchange was

performed at a rate of 15–20% to remove fecal matter and settle flocculated particles. All tanks were equipped with uniform linear aeration tubes to maintain the oxygen concentration ($> 5 \text{ mg L}^{-1}$). At the end of the 7th day of the feeding experiment, the survival of the *A. franciscana* culture was determined by Thangal et al (2021).

$$\text{SR (\%)} = \frac{N_t}{N_o} \times 100 \quad (2)$$

where, SR = survival (%), N_t = the number of live shrimps that survived until the end of the experiment, and N_o = the number of shrimps that were available at the beginning of the experiment.

Statistical analysis

All the values are presented as the mean \pm standard deviation (SD) of three replicate analyses. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was carried out by IBM SPSS software (version 29) at the 0.05 level ($P < 0.05$) of significance.

Results

The hatching performance of *Artemia* at different salinities is shown in Table 1 and Figure 1. Hatching at different salinities resulted in different hatching rates. The maximum hatching percentage of 67.00 ± 1.53 was observed at 35 ppt, whereas the minimum hatching percentage of 33.04 ± 4.53 was found at a salinity of 5 ppt. The mean values of different hatching percentages showed significant differences ($P < 0.05$) among the various salinity ranges (Table 1).

It is well known that salinity has a significant impact on the hatching percentage of *Artemia* nauplii. Vanhaecke and Sorgeloos (1989) suggested that salinity levels between 20 and 30 ppt are optimal for hatching *Artemia* cysts. In a study by Kumar and Babu (2015), the effect of salinity on the hatching percentage of *Artemia* was examined by altering salinity levels from 24 to 35 ppt, and the maximum hatching percentage occurred at 29 ppt. Furthermore, Hasan & Rabbane (2018) reported that the maximum percentage of *Artemia* hatched at a salinity of 30 ppt. Our experiment altered the salinity from 5 to 40 ppt, and the maximum hatching

percentage (67%) occurred at 35 ppt. The present study confirmed a positive correlation between salinity and hatching success, which is consistent with previous research findings (Kumar & Babu, 2015; Sharahi & Zarei, 2016; Hasan & Rabbane, 2018). It is important to note that salinity levels outside the optimal range could negatively affect the hatching rate and overall development of *Artemia* nauplii. Therefore, it is crucial to carefully regulate salinity levels in aquaculture systems to ensure optimal hatching and growth of *Artemia* nauplii.

Table 1. Hatching and survival performance of *Artemia franciscana* under different salinities

Salinity range (%)	Hatching (%)	Survival (%)
5	33.04 ± 4.53^f	27.97 ± 1.47^e
10	42.71 ± 1.46^e	34.30 ± 1.75^d
15	44.33 ± 1.76^e	40.67 ± 1.42^c
20	51.33 ± 2.03^d	44.72 ± 2.44^c
25	55.00 ± 1.73^{cd}	51.93 ± 2.51^b
30	60.00 ± 1.15^{bc}	58.33 ± 2.08^a
35	67.00 ± 1.53^a	61.33 ± 2.33^a
40	62.00 ± 1.73^{ab}	60.65 ± 2.60^a

All the values are the means \pm SD (standard deviation) of three replicate analyses.

Data with different superscript letters in the same column are significantly different ($P < 0.05$).

Similarly, the salinity levels from 5 to 40 ppt were adjusted to estimate the survival of *A. franciscana*. The mean values of survival rates showed significant differences ($P < 0.05$) among the various salinity ranges (Table 1). The results indicated that the highest survival rate of $61.33\% \pm 2.33$ was observed at 35 ppt, while the lowest survival rate of $27.97\% \pm 1.47$ was observed at 5 ppt (Table 1 and Figure 1). Survival rates provide basic information on the tolerance of organisms to environmental conditions (Pörtner and Peck, 2010). The ability of *Artemia* species to thrive under diverse salinity conditions has been well documented in previous research (El-Bermawi et al., 2004; Agh et al., 2008; Aalamifar et al., 2014). Our findings further corroborate the significant influence of salinity on the survival of *A. franciscana*, in line with similar studies by Mali et al. (2023). Notably, our study revealed that *A. franciscana* shows a narrower salinity tolerance range

for survival at higher salinity levels, particularly beyond 25 ppt. These findings contribute to our understanding of the ability of *A. franciscana* to adapt to various salinity levels and highlight the importance of considering environmental factors. These variations in optimal parameters may be influenced by differences in test settings, such as environmental conditions, container size, and gradients in bulk media (Dey et al., 2023).

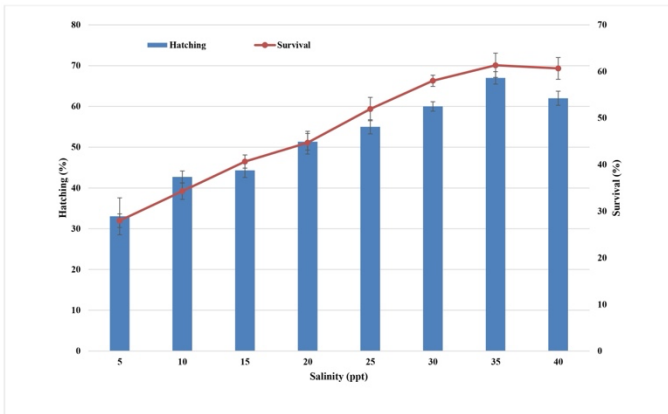


Figure 1. Hatching and survival performance of *Artemia* at different salinities

Conclusion

Based on the current findings, it can be concluded that the percentages of hatching and the survival rate of *A. franciscana* are significantly influenced by salinity. The results indicate that a salinity of 35 ppt leads to the highest hatching and survival rates. These findings provide strong evidence for a direct correlation between environmental factors and the hatching efficiency of *A. franciscana*. Therefore, it is crucial to carefully regulate salinity levels in aquaculture hatchery systems to ensure the optimal hatching and survival of *A. franciscana*.

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Ethical Approval

Not applicable.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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