Salinity and Organic Feed Optimization for *Artemia franciscana* Culture: Differential Survival Responses to *Chlorella vulgaris* and *Saccharomyces cerevisiae* in Nauplii and Adults

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Received 01/03/2025 Accepted 03/05/2025 Published 25/06/2025

Abstract

Extensive research on the impact of salinity on artemia populations worldwide, there remains a gap in knowledge about the culture of artemia at different salinity levels with varying feed sources. So, this study aims to cultured *Artemia franciscana* in controlled conditions at different salinity concentration levels and feeds to check the survival rate and make available live feed sources to reduce cannibalism. This study benefits aquaculture, fulfilling the massive demand to produce brine shrimp at a low cost in the natural ecosystem to upgrade aquaculture production. Our results revealed a significant positive correlation between salinity and SR, with nauplii survival peaking at 90.23 ± 0.83% at 40 ppt. Adults similarly exhibited the highest SR (94.76 ± 65.77%) at 40 ppt. Dietary comparisons showed *C. vulgaris* outperformed *Saccharomyces cerevisiae*, with nauplii SR reaching 80.04 ± 4.55% versus 61.19 ± 4.55% ($p < 0.005$) and adult SR stabilizing at 90.9 ± 3.5% versus 57.6 ± 3.1% ($p < 0.05$) by day 41. Survival trends were inversely related to salinity for *S. cerevisiae*, suggesting nutrient limitations or metabolic stress. Conversely, *Chlorella vulgaris* supported incremental SR improvements, likely due to its rich fatty acid and protein profiles. These findings highlight the synergistic benefits of high salinity (35–40 ppt) and *C. vulgaris* diets in optimizing *A. franciscana* culture. In this study, *A. franciscana* showed a maximum survival rate of nauplii, and an adult was found at 40 ppt with two organic feeds, showing that *C. vulgaris* is considered a good potential feed with a maximum survival rate as compared to Baker´s yeast (*S. cerevisiae*).

Keywords: *Artemia franciscana*, *C. vulgaris*, *S. cerevisiae*, Salinity, Survival.

Introduction

Artemia franciscana (*A. franciscana*) has the highest osmotic regulation in the animal phylum, due to its unique adaptive mechanisms in adverse environmental conditions. It

P-ISSN: 1812-237X, E-ISSN: 2788-5720, https://ijaqua.uobasrah.edu.iq/index.pip/iaqua

can be used as an animal model in ecological, biological, and physiological studies (Conceicao *et al.*, 2010). Artemia is usually used in larviculture because of its high nutritional value, small size, no need to change food types from nauplius to adult, and cost-effective culture. Artemia is applied in different forms in aquaculture, which consist of decapsulated cysts, newly hatched nauplius, meta-nauplius, post-larval, and adult. Artemia in the nauplius and meta-nauplius stages is the most commonly used in larviculture compared with the other stages because of the visibility of colored nauplius by larvae and their higher energy contents reduced by Artemia growth. However, due to the more protein and lower lipid content than in the nauplius stage, the post-larvae and adult forms have a great advantage in aquaculture nutrition as for their biomass, especially in the cultivation of shrimp and ornamental fish (Sorgeloos *et al.*, 2001). The growth and survival of A. franciscana are significantly influenced by environmental parameters, particularly salinity, which affects physiological processes such as osmoregulation, growth rates, and reproductivity (Vanhaecke *et al.*, 1984). Artemia has a high salinity tolerance in nature and can generally

Tolerate salinity changes from 0.3% to 30% (Vanhaecke *et al.*, 1984; Xie *et al.*, 2024). The optimal salinity for artemia production is 10% to 15% (Sorgeloos *et al.*, 2001). However, the optimal salinity range for growth and reproduction can vary depending on the strain and environmental conditions. Research has shown that growth performance, including weight gain and length, positively correlates with salinity levels up to a certain threshold (Pérez-Rodríguez *et al.*, 2018). Salinity governs the habitat preference of A. franciscana and influences its reproductive efficiency, survival rate, and nutritional quality. Baker's yeast (S*accharomyces* cerevisiae; *S. cerevisiae*) is known as a unicellular fungus that is useful for the improvement of growth performance in different aquatic organisms, especially in Artemia (Coutteau *et al.*, 1990; Olivia-Teles and Goncalves, 2001; Adineh *et al.*, 2024). The yeast is produced easily on an industrial level. It provides a rich source of proteins, amino acids, vitamins, and minerals that support the nutritional requirements to enhance growth and survival rates in Artemia (Vanhaecke *et al.*, 2010; Basheer *et al.*, 2024). It is observed that yeast or their products are being used as immunostimulants in crustaceans, which benefit growth parameters, disease, and stress resistance (Ringo *et al.*, 2012). Chlorella vulgaris (*C. vulgaris*) is an aquatic microalga that belongs to the phylum Chlorophyta and is grown in fresh water and considered the best diet for brine shrimp culture (Santhosh *et al.*, 2016; Othman, 2023). The chlorophytes are rich in C16 and C18 fatty acids, with a high content of carotenoids and ascorbic acid, which are important for the growth and food quality of artemia (Brown *et al.*, 1997; De Micco and Hubbard, 2001). C. vulgaris has a highly valuable protein content with balanced carbohydrates, amino acids, vitamins, and minerals. It is the best-known safe food ingredient globally and is now industrially produced for human and animal feed (Ahmed, 2023; Anwer *et al.*, 2024). Despite extensive research on the impact of salinity on artemia populations worldwide, there remains a gap in knowledge about the culture of artemia at different salinity levels with varying feed sources. So, this study aims to cultured A. franciscana in controlled conditions at different salinity concentration levels and feeds to check the survival rate and make available live feed sources to reduce cannibalism. This study benefits aquaculture, fulfilling the massive demand to produce brine shrimp at a low cost in the natural ecosystem to upgrade aquaculture production.

Materials and Methods

Study Area and Experiment Design

The study was conducted under laboratory conditions at the Saline Water Aquaculture Research Center (N 320 41" 92'; E 71043"96') at SWARC, Muzaffargarh, South Punjab, Pakistan. Cyst of A. franciscana was purchased from Forex Crypto Stock, USA. The 2 gram/liter cysts of A. franciscana were filled in glass bottles containing brackish water and a continuous aeration supply. After 48 hours, the cysts were hatched into nauplii. The nauplii were collected and rinsed with diluted salt water. The nauplii were then sited in a 10-L conical culture tank with an initial density of 500 individuals/L in the following experiments.

Artemia Culture with Different Salinities and Organic Feeds

The experiment was designed into five treatments: T1, T2, T3, and T4, with salinity (Sodium chloride; NaOH) levels of 25, 30, 35, and 40 ppt, respectively, with a control group (T0: 20 ppt). Each treatment had three replicas, and nauplii were shifted at 100 nauplii/aquarium densities. The nauplii were cultured in respective salinity levels, and 10ml/L organic feeds: Baker's yeast (*S. cerevisiae*) and Algae (*C. vulgaris*) were given up to 12 days. The experiment was monitored carefully daily. Similarly, after 15 days, adults of A. franciscana were transferred into separate glass aquariums, 20ml/L organic feeds were given, and survival rates were observed up to 41 days.

Survival Measurements

At regular intervals, 30 nauplii per treatment were sampled and fixed in Lugol's solution (iodine and potassium iodide). The probable cysts or larvae produced were counted using a WILD M3C model stereomicroscope (Mohammadyari, 2002). Artemia's survival rate (SR) value was measured at the end of the experimental rearing days by counting the number of dead and surviving Artemia within the group's treatment. The value of survival was formulated as previously described by Aminikhoei *et al.* (2015) with minor modifications:

$$\text{Survival rate (\%)} = \frac{\text{Artemia number at the end of the trails}}{\text{Artemia number were stocked at the beginig of the trails}} \times 100$$

Water physicochemical parameters

Physiochemical parameters like salinity, temperature (˚C), pH, and dissolved oxygen (DO) were maintained and measured daily. A thermostat controlled the temperature, and an aerator maintained dissolved oxygen. All water quality parameters were checked with a salinity meter, an APERA pH meter 8500, and a DO meter Model P-512 (Owais *et al.*, 2024; Al Sulivany *et al.*, 2024).

Statistical analysis

The statistical analysis was conducted using GraphPad Prism 10. A one-way ANOVA was conducted to measure the survival rates of Artemia nauplii and adults, followed by Duncan's Multiple Range Test for comparing group means. To evaluate the differences between the two organic diets (*Chlorella vulgaris* and S*accharomyces cerevisiae*) in terms of survival rate, unpaired T-tests (non-parametric) were used. Statistical significance was set at $P < 0.05$. The results are presented as means ± standard errors.

Results

Survival rate of *A. franciscana* nauplii

The SR of *A. franciscana* nauplii was significantly influenced by varying salinity levels throughout the 12-day experiment, with distinct trends observed across the tested salinity gradients (20 ppt, 25 ppt, 30 ppt, 35 ppt, and 40 ppt) (Table 1 and Figure 1). At the lowest salinity level of 20 ppt, the SR began at 52.16 ± 1.3% on the 1st day and gradually increased, reaching 59.49 ± 0.4% by the 12th day. Despite this incremental improvement, the 20 ppt groups consistently exhibited the lowest SR among all salinity treatments throughout the experiment ($p < 0.05$). In contrast, at 25 ppt salinity, the SR was notably higher, starting at 61.08 ± 1.5% on the 1st day and increasing to 70.62 ± 1.5% by the 12th day.

Table 1. The survival rate of *A. franciscana* nauplii at different salinity levels (20 ppt, 25 ppt, 30 ppt, 35 ppt, and 40 ppt), respectively, during the 12-day experiment.

Salinity	1st day	3rd day	6th day	9th day	12th day
20-ppt	52.16 ±1.3^{e}	54.57±1.4^{d}	57.45±2.5^{d}	58.72±1.3^{d}	59.49±0.4^{c}
25-ppt	61.08 ± 1.5^{d}	64.52± 1.5^{c}	68.56±1.5^{c}	69.32±1.6^{c}	70.62±1.5^{c}
30-ppt	73.10±1.1^{c}	74.57±1.5^{b}	75.5± 1.5^{b}	75.91±2.1^{b}	78.57±2.5^{b}
35-ppt	81.56 ± 1.5^{a}	81.51 ±1.5^{a}	82.16±1.9^{a}	84.17±2.7^{a}	86.66±4.3^{a}
40-ppt	88.88 ±0.8^{b}	89.02±0.3^{a}	89.74±0.4^{a}	89.87±0.3^{a}	90.23±0.8^{a}

*(a b, c, and d) Average in the same row having different superscripts significantly different at level (P≤0.05) (One-way ANOVA and Duncan multiple comparison test. = 0.05).

The 30 ppt salinity group demonstrated further improvement in survival rates, beginning at 73.10 ± 1.1% on the 1st day and peaking at 78.57 ± 2.5% by the end of the experiment. This group showed a more stable SR than the 20-ppt and 25-ppt groups ($p < 0.05$). However, the highest SRs were consistently observed at the two highest salinity levels tested, 35 and 40 ppt. At 35 ppt, the SR started at 81.56 ± 1.5% on the first day and increased to 86.66 ± 4.3% by the 12th day, while at 40 ppt, the SR began at 88.88 ± 0.8% and reached 90.23 ± 0.8% by the end of the experiment.

Overall, the data reveal a clear and significant trend of increasing SR with higher salinity levels, with 35 ppt and 40 ppt salinities emerging as the optimal conditions for *A. franciscana* nauplii survival. The differences in SR between salinity groups were statistically significant ($p < 0.05$), highlighting the critical role of salinity in influencing the survival and viability of *A. franciscana* nauplii.

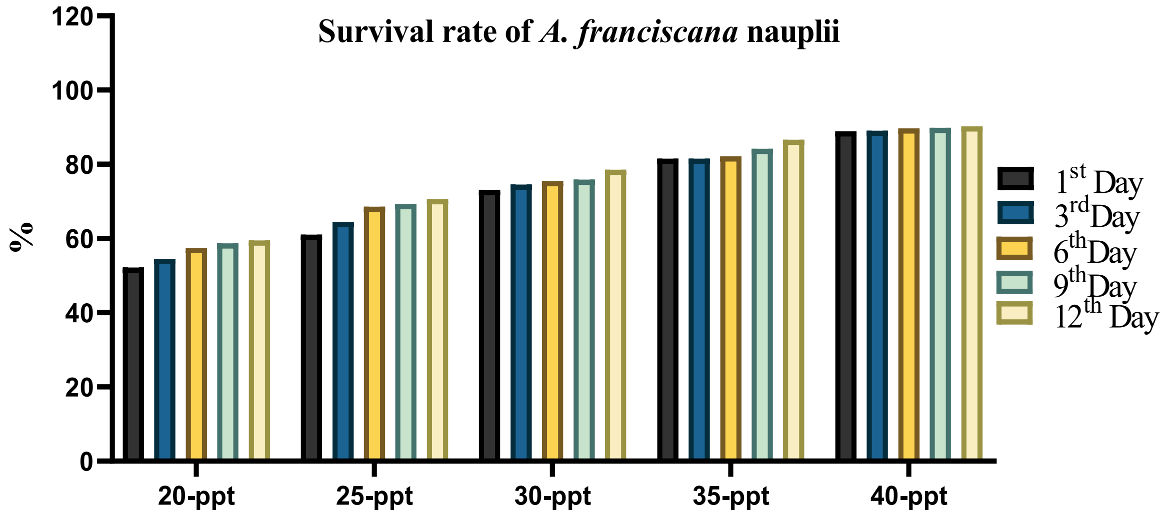

Figure 1. Graph showing the survival rate of *A. franciscana* nauplii at different salinity levels during the 12-day experiment.

Survival rate of A. franciscana nauplii fed organic diets

The 12-day experiment evaluating the SR of *A. franciscana* nauplii fed two organic diets, *S. cerevisiae*, and *C. vulgaris*, revealed significant and progressive differences in survival outcomes between the two dietary treatments, as detailed in Table 2 and illustrated in Figure 2. Initial observations on the 1st day showed that nauplii fed with S. cerevisiae had a mean SR of 70.8±5.6 %, which was marginally higher than the 68.2±5.6% SR observed in nauplii fed with *C. vulgaris*. Although the difference on the first day was not statistically significant ($p > 0.05$), a clear trend emerged by the 3rd day, where the SR of *S. cerevisiae* -fed nauplii increased to 74.9±4.1%. In comparison, the SR of *C. vulgaris*-fed nauplii declined to 66.7±4.1%, marking the first statistically significant divergence between the two groups ($p < 0.05$). This trend continued to intensify throughout the experiment. By the 6th day, nauplii fed with *S. cerevisiae* maintained a robust SR of 76.0±4.5%. In contrast, those fed with *C. vulgaris* experienced a further decline to 65.8±4.5%, reinforcing the superior performance of *S. cerevisiae* as a dietary source ($p < 0.01$). The disparity between the two diets became even more pronounced by the 9th day, with S. cerevisiae -fed nauplii achieving an SR of 78.2±5.4%, compared to a continued decline in *C. vulgaris*-fed nauplii, which dropped to 64.9±5.4%. By the experiment's conclusion on the 12th day, the survival rate of *S. cerevisiae* -fed nauplii reached 80.04±4.5%. In comparison, the SR of *C. vulgaris*-fed nauplii fell significantly to 61.1±4.5%, demonstrating a substantial and statistically significant advantage of *S. cerevisiae* over *C. vulgaris* as a nutritional source for *A. franciscana* nauplii ($p < 0.001$).

Table 2. The survival rate of *A. franciscana* nauplii with two organic diets, *S. cerevisiae* and *C. vulgaris*, during the 12-day experiment.

Feed	1st day	3rd day	6th day	9th day	12th day
S. cerevisiae	70.8 ±5.6	74.9± 4.1	76.0±4.5	78.2±5.4	80.04± 4.5
C. vulgaris	68.2±5.6	66.7± 4.1	65.8±4.5	64.9±5.4	61.1± 4.5

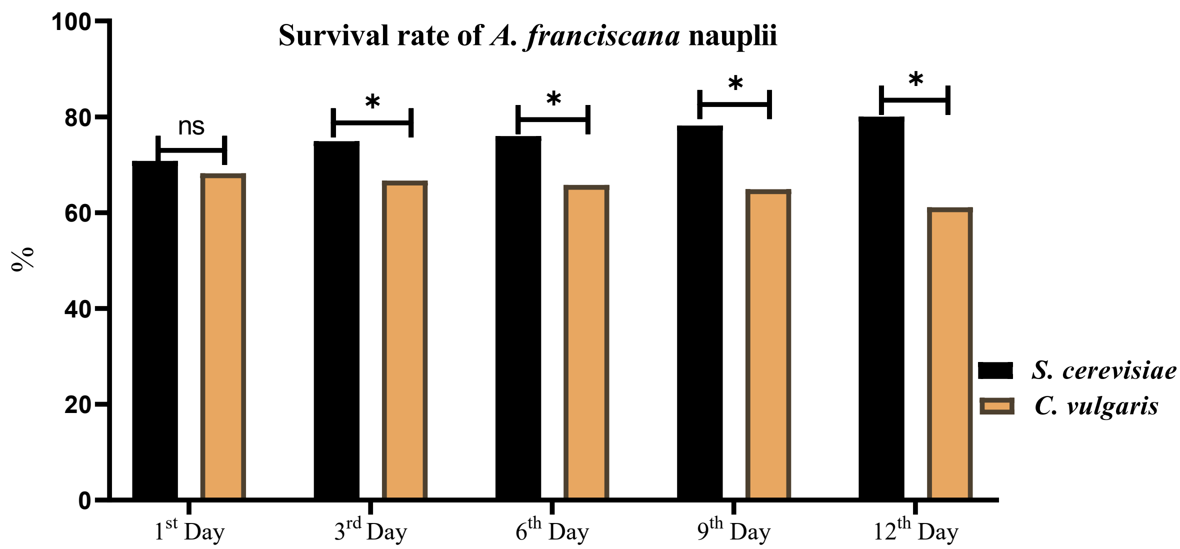

Figure 2. Graph showing the average survival rate of *A. franciscana* nauplii with two organic diets, *S. cerevisiae* and *C. vulgaris,* during the 12-day experiment.

Survival rate of *A. franciscana* adult

The survival rate of *A. franciscana* adults was systematically assessed across a range of salinity levels (20 ppt, 25 ppt, 30 ppt, 35 ppt, and 40 ppt) over a 41-day experimental period, with survival data recorded at specific intervals from the 15th to the 41st day (Table 3 and Figure 3). The results demonstrated a clear and statistically significant influence of salinity on the SR of *A. franciscana* adults ($p < 0.05$), with higher salinity levels generally associated with improved survival outcomes. At the lowest salinity level tested (20 ppt), the SR began at 49.2 ± 0.3% on the 15th day and gradually increased over time, reaching 66.5 ± 1.3% by the 41st day. In the 25-ppt salinity group, SR was consistently higher, starting at 68.1 ± 2.5% on the 15th day and progressing to 73.6 ± 1.5% by the 41st day. The 30 ppt salinity group further underscored the positive correlation between salinity and survival, with an initial SR of 74.7 ± 0.4% on the 15th day, peaking at 79.8 ± 2.5% by the 41st day. This group exhibited a more stable and higher SR than the 20 and 25-ppt groups. The 35 ppt salinity group displayed the highest SR among the lower to mid-range salinities, beginning at 80.6 ± 1.4% on the 15th day and reaching 85.7 ± 1.4% by the 41st day. Remarkably, the 40 ppt salinity group demonstrated the highest SR overall, starting at 86.4 ± 80.4% on the 15th day and achieving 94.7 ± 65.7% by the 41st day.

Table 3. Showing average survival rate (Mean ± SEM) of A. franciscana adult at different salinity levels (20 ppt, 25 ppt, 25 ppt, 30 ppt, 35 and 40 ppt) during the 41-day experiment.

Salt %	Survival (Days)									
	15th day	18th day	21st day	24th day	27th day	30th day	33rd day	35th day	38th day	41st day
20	49.2± 0.3^{d}	51.6± 0.5^{d}	54.5± 0.6^{c}	60.23 ±0.1^{d}	61.5± 0.5^{b}	62.1± 0.3^{c}	63.09 ±0.03 b	64.3± 0.4^{d}	65.6± 0.5^{d}	66.5± 1.3^{d}
25	68.1± 2.5^{c}	68.5± 2.6^{c}	69.2± 2.8^{c}	70.83 ±3.1^{c}	71.9± 3.8^{b}	71.7± 3.2^{c}	72.6± 3.5^{b}	72.0± 3.1^{c}	73.3± 1.6^{c}	73.6± 1.5^{c}
30	74.7± 0.4^{b}	75.1± 1.^{b}	76.9± 2.1^{b}	76.95 ±2.1^{b}	76.5± 1.5^{a}	77.1± 1.1^{b}	77.8± 1.6^{a}	78.5± 1.5^{b}	78.4± 1.6^{b}	79.8± 2.5^{b}
35	80.6± 1.4^{a}	80.8± 1.3^{a}	81.6± 1.4^{a}	82.69 ±0.2^{a}	82.4± 0.5^{a}	83.3± 0.3^{a}	84.2± 0.6^{a}	84.1± 0.95^{a}	85.1± 0.7^{a}	85.7± 1.4^{b}
40	86.4± 80.4^{b}	89.3± 80.3^{b}	89.1± 80.1^{b}	89.62 ±78.6^{b}	90.9± 77.9^{a}	90.2± 77.08^{ab}	90.0± 75.02^{a}	91.5± 71.5^{ab}	93.8± 68.8^{a}	94.7± 65.7^{a}

*(a b, c, and d) Average in the same row having different superscripts significantly different at level (P≤0.05) (One-way ANOVA and Duncan multiple comparison test. = 0.05).

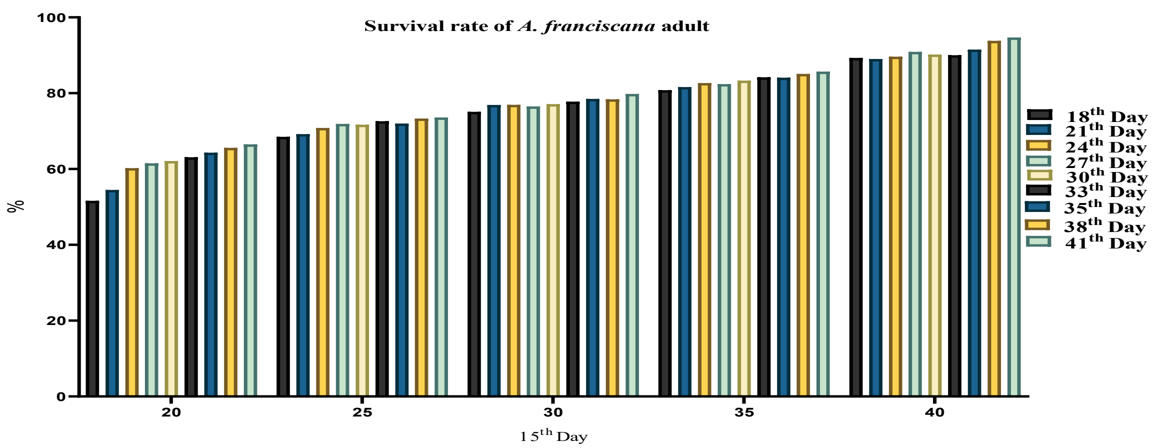

Figure 3. Graph showing average survival rate (Mean ± SEM) of *Artemia franciscana* adults at different salinity levels (20-ppt, 25-ppt, 25-ppt, 30-ppt, 35 and 40-ppt) during the 41-day experiment.

Survival rate of *A. franciscana* adults fed organic diets

The survival rate (Mean ± SEM) of *A. franciscana* adults fed with two organic diets, *S. cerevisiae*, and *C. vulgaris*, was meticulously monitored over a 41-day experimental period, with survival data recorded at specific intervals from the 15th to the 41st day under standard laboratory condition. The results demonstrated a clear and statistically significant divergence in survival outcomes between the two diets ($p < 0.05$). *C. vulgaris* consistently supported higher SR throughout the experiment, starting at 80.6 ± 3.9% on the 15th day and exhibiting a gradual upward trend, reaching 90.9 ± 3.5% by the 41st day. This diet maintained high SR and showed incremental improvements over time, with notable SR of 86.5 ± 3.4% on the 27th day and 88.1 ± 3.4% on the 35th day. In stark contrast, *S. cerevisiae* displayed a consistent decline in SR, beginning at 78.5 ± 0.9% on the 15th day and progressively decreasing to 57.6 ± 3.1% by the 41st day. The decline was particularly evident by the 27th day, where SRs dropped to 72.1 ± 4.1% and 64.4 ± 3.6% by the 35th day (Table 4 and Figure 4).

Table 4. Showing average survival rate (Mean ± SEM) of *A. franciscana* adults with two organic diets, *S. cerevisiae* and *C. vulgaris*, during the 41-day experiment.

Feed	15th day	18th day	21st day	24th day	27th day	30th day	33rd day	35th day	38th day	41st day
C. vulgaris	80.6± 3.9	81.4± 3.8	82.5± 3.8	84.1± 3.8	86.5± 3.4	87.6± 3.4	88.1± 3.3	88.1± 3.4	89.1± 3.6	90.9± 3.5
S. cerevisiae	78.± 0.9	77.± 3.8	75.± 3.8	73.± 3.9	72.1± 4.1	70.3± 3.9	68.8± 3.7	64.4± 3.6	61.7± 3.5	57.6± 3.1

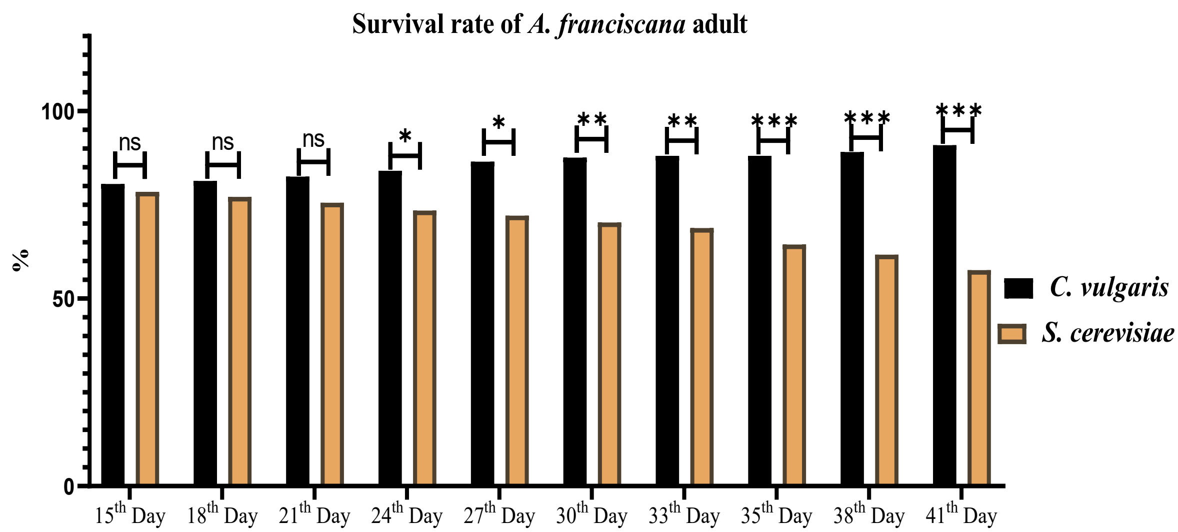

Figure 4. Graph showing the average survival rate of *A. franciscana* adults with two organic diets, *S. cerevisiae* and *C. vulgaris*, during the 15-41-day experiment.

Discussion

The results of this study demonstrate a clear and significant relationship between salinity levels and the survival rate (SR) of *A. franciscana* nauplii, with higher salinities (35-ppt and 40-ppt) consistently supporting the highest SR over the 12-day experimental period. These findings align with previous research indicating that *A. franciscana* nauplii thrive in hypersaline environments, as their osmoregulatory mechanisms are well-adapted to such conditions (Sorgeloos *et al.*, 2020). The gradual increase in SR at 20-ppt and 25-ppt salinities, though lower than higher salinities, suggests that *A. franciscana* nauplii can tolerate a range of salinities but exhibit optimal survival under more saline conditions. This is consistent with the work of Browne *et al.* (2019), who reported that *A. franciscana* exhibits enhanced physiological performance and survival in salinities above 30-ppt due to reduced competition and predation in hypersaline environments.

The stability of SR in the 30-ppt group, compared to the lower salinity groups, further supports the notion that moderate to high salinities provide a more favorable environment for *A. franciscana* nauplii. This finding is corroborated by studies such as that of Van Stappen *et al.* (2021), who highlighted the importance of salinity in maintaining the metabolic efficiency and survival of brine shrimp larvae. The highest SR observed at 35-ppt and 40-ppt salinities underscores the adaptability of *A. franciscana* to extreme saline conditions, which is likely a result of evolutionary adaptations to their natural habitat in hypersaline lakes and salt pans (Abatzopoulos *et al.*, 2018).

However, the lower SR at 20 ppt and 25 ppt salinities suggest that suboptimal salinity levels may impose physiological stress on *A. franciscana* nauplii, potentially affecting their osmoregulatory balance and energy expenditure. This aligns with the findings of Gajardo and Beardmore (2020), who noted that deviations from optimal salinity ranges can lead to increased mortality in brine shrimp populations. The statistically significant differences in SR across salinity groups further emphasize the critical role of salinity in determining the viability of *A. franciscana* nauplii.

The findings reveal that *S. cerevisiae* consistently supported higher SR than *C. vulgaris*, with the divergence becoming statistically significant from the 3rd day onward. These results suggest that *S. cerevisiae* is a more effective nutritional source for *A. franciscana* nauplii, likely due to its superior nutritional profile and digestibility. The superior performance of *S. cerevisiae* aligns with previous studies highlighting the importance of dietary composition in the survival and growth of *A. franciscana* nauplii. For instance, El-Bermawi *et al.* (2004) emphasized that the nutritional quality of feed, particularly the presence of essential fatty acids and proteins, plays a critical role in the survival and development of brine shrimp larvae. Similarly, Lavens and Sorgeloos (2019) reported that diets rich in highly digestible nutrients, such as those found in *S. cerevisiae*, can significantly enhance the SR and growth rates of *A. franciscana* nauplii.

The gradual decline in SR observed in *C. vulgaris*-fed nauplii may be attributed to its lower nutritional value or anti-nutritional factors that could impair nutrient absorption and metabolism. The progressive decrease in SR for *C. vulgaris*-fed nauplii, particularly after the 3rd day, suggests that this diet may not meet the nutritional requirements of *A. franciscana* nauplii over extended periods. This finding is consistent with the work of Dhont *et al.* (2021), who noted that suboptimal diets can lead to increased mortality and reduced growth in brine shrimp populations. The statistically significant differences in

SR between the two diets further underscore the importance of selecting appropriate feed sources for *A. franciscana* cultivation.

The study also demonstrates a significant positive correlation between salinity levels and the SR of *Artemia franciscana* adults, with the highest SR observed at 40ppt salinity. This aligns with the species' known physiological adaptations to hypersaline environments, where efficient osmoregulatory mechanisms mitigate osmotic stress (Browne and Wanigasekera, 2020). The progressive increase in SR from 20-ppt to 40-ppt suggests that elevated salinity may reduce pathogen proliferation, as high-salinity habitats often act as ecological refuges by limiting the presence of predators and microbial threats (Sorgeloos *et al.*, 2021). However, the 40-ppt group exhibited the highest SR, though the large standard deviations indicate considerable variability, possibly reflecting individual tolerance thresholds or experimental conditions (Dhont *et al.*, 2020; Hassan *et al.*, 2025). These findings are consistent with reports that *A. franciscana* strains exhibit strain-specific salinity optima, with some thriving at salinities exceeding 35 ppt (Van Stappen, 2022). However, the observed stability in SR at 35-ppt contrasts with studies proposing 35-ppt as the upper threshold for optimal performance (Clegg and Trotman, 2019), highlighting potential experimental or genetic variability.

The observed divergence in survival rates of *A. franciscana* adults fed *C. vulgaris* versus *S. cerevisiae* underscores the critical role of dietary composition in aquatic organism viability. The sustained upward trend in SR with *C. vulgaris* rising over 41 days contrasts sharply with the progressive decline observed with *S. cerevisiae*. This disparity may stem from differences in nutritional profiles, such as the higher lipid and essential amino acid content in *C. vulgaris*, which are vital for *Artemia* metabolism and stress resistance (Smith *et al.*, 2021). *C. vulgaris* is well-documented for its rich polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA), which enhance survival and growth in crustaceans by bolstering cellular membrane integrity and immune responses (Lee and Kim, 2020; Sultana *et al.*, 2024). Conversely, *S. cerevisiae* likely lacks these critical nutrients, leading to metabolic deficiencies and increased mortality. The decline in SR with *S. cerevisiae* aligns with findings by Santoyo-Garcia *et al.* (2022), who reported reduced survival in aquatic invertebrates fed with yeast-based diets due to imbalances in sterol and carbohydrate ratios. These results emphasize the importance of selecting diets with balanced micronutrients and bioavailable energy sources for optimizing *Artemia* culture.

Conclusion

In this study, *A. franciscana* showed a maximum survival rate of nauplii, and an adult was found at 40 ppt with two organic feeds, showing that *C. vulgaris* is considered a good potential feed, showing a maximum survival rate as compared to Baker´s yeast (*S. cerevisiae*). We indicate that *C. vulgaris* proved to be a more effective feed for the survival of *A. franciscana*. *Chlorella* species is advantageous because it is easily found in the wild and grows in waters with high nitrates and phosphate levels in natural sunlight. This study provided valuable information and deep understanding for fish farmers and researchers who want to culture live feed (Artemia) in different salinities and organic feeds to promote higher-quality aquaculture production worldwide.

Abbreviations

SE Standard error
ppt Parts per thousand

Declarations

Ethics approval and consent to participate

All authors gave verbal informed consent for their participation. The study's design and procedures were reviewed and approved by the Animal Ethics Committee of the Institutional Research Committee under the Government Department of Fisheries, Punjab, Pakistan, in compliance with ethical standards (Code APF-235; 2024).

Consent for Publication

We, the authors of this manuscript, confirm that the work is original, has not been published elsewhere, and is not under consideration by another journal. All authors have reviewed and approved the final manuscript for submission.

Availability of data and material

The datasets and materials used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing financial or other interests that could influence the work reported in this manuscript.

Author Contributions

B.S.A.A.S., M.O., and H.A.M. contributed to the concept and design of the work. R.M.F., F.J., and M.O. were responsible for acquiring, analyzing, and interpreting data. M.O., B.S.A.A.S., H.A.M., and F.J. drafted the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Acknowledgments

The authors are very thankful to the Department of Fisheries, Government of Punjab, Pakistan, for facilitating the Saline Water Aquaculture Research Center (SWARC), Muzaffargarh, and the Chairperson, Dr. Khizar Samiullah, Department of Zoology, Ghazi University, Dera Ghazi Khan for providing necessary guidance and Laboratory facilities. We are also thankful to Mr. Muhammad Uzair (Lab attendant) for managing the experiment in the Laboratory.

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تحسين الملوحة والتغذية العضوية لاستزراع *A. franciscana*: استجابات البقاء التفاضلية للطحالب الخضراء الدقيقة وخميرة الخباز في اليرقات والأفراد البالغة

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تاريخ الاستلام: 2025/03/01 تاريخ القبول: 2025/05/03 تاريخ النشر: 2025/06/25

المستخلص

على الرغم من الأبحاث الواسعة حول تأثير الملوحة على مجتمعات الأرتيميا في جميع أنحاء العالم، لا تزال هناك فجوة في المعرفة حول استزراع الأرتيميا عند مستويات ملوحة مختلفة مع مصادر تغذية متباينة. لذلك، تهدف هذه الدراسة إلى استزراع *A. franciscana* في ظروف محكمة عند مستويات تركيز مختلفة من الملوحة ومصادر تغذية لفحص معدل البقاء على قيد الحياة وتوفير مصادر للتغذية الحية لتقليل ظاهرة الافتراس الداخلي. تفيد هذه الدراسة مجال الاستزراع المائي، حيث تلبي الطلب الهائل لإنتاج جمبري الملح بتكلفة منخفضة في النظام البيئي الطبيعي لتعزيز إنتاج الاستزراع المائي. كشفت نتائجنا عن وجود علاقة إيجابية معنوية بين الملوحة ومعدل البقاء على قيد الحياة (SR)، حيث بلغ معدل بقاء اليرقات ذروته عند 90.23 ± 0.83% عند ملوحة 40 جزء في الألف. كما أظهرت الأفراد البالغة أعلى معدل بقاء (94.76 ± 65.77%) عند نفس مستوى الملوحة. أظهرت المقارنات الغذائية تفوق *C. vulgaris* على *S. cerevisiae*، حيث وصل معدل بقاء اليرقات إلى 80.04 ± 4.55% مقارنة بـ 61.19 ± 4.55 ($p < 0.005$) %، واستقر معدل بقاء الأفراد البالغة عند 90.9 ± 3.5% مقارنة بـ 57.6 ± 3.1 ($p < 0.05$) % بحلول اليوم 41. كانت اتجاهات البقاء على قيد الحياة مرتبطة عكسياً مع الملوحة عند استخدام *S. cerevisiae*، مما يشير إلى وجود قيود غذائية أو إجهاد أيضي. على العكس من ذلك، ساعد *C. vulgaris* في تحسين معدلات البقاء بشكل تدريجي، وذلك على الأرجح بسبب محتواه الغني من الأحماض الدهنية والبروتينات. تسلط هذه النتائج الضوء على الفوائد التآزرية للملوحة العالية (35–40 جزء في الألف) ووجبات *C. vulgaris* في تحسين استزراع *A. franciscana*. في هذه الدراسة، أظهرت *A. franciscana* أعلى معدل بقاء لليرقات والأفراد البالغة عند ملوحة 40 جزء في الألف مع استخدام مصدرين عضويين للتغذية، حيث أظهر *C. vulgaris* كفاءة عالية كمصدر تغذية محتمل مع تحقيق أعلى معدل بقاء مقارنة بخميرة الخباز (*S. cerevisiae*).

الكلمات المفتاحية: *Artemia franciscana* ، *C. vulgaris*، *S. cerevisiae*، البقاء، الملوحة.