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Comparative field evaluation of high-saline probiotics on *Artemia franciscana* cyst yield in earthen ponds across seasonal production cycles

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Original Article

Abstract

Artemia culture systems accumulate organic waste from uneaten feed, moults, and faeces, increasing organic load and ammonia levels, deteriorating water quality. Unlike most aquaculture systems, water exchange is impractical in *Artemia* farming due to the risk of stock escape. Therefore, alternative strategies such as probiotics are used as a sustainable solution for water-quality management. While most commercial probiotics are developed for low-salinity shrimp and fish culture (0–35 ppt), their suitability for hyper-saline *Artemia* systems remains largely untested. This study evaluated two commercial probiotics: Treatment 1 (TR1), Probiotic X (a *Bacillus*-based consortium), and Treatment 2 (TR2), Probiotic Y (*Marinobacter* sp.), over two production cycles-winter (Jan–Apr) and summer (Jun–Oct). Performance was assessed using daily cyst release patterns, reproductive phase duration, total wet cyst yield, and unit area production (kg/ha/crop). Probiotic application significantly prolonged the reproductive phase under hyper-saline conditions. In winter, cyst production in control ponds ceased by 61 days of culture (DOC), whereas probiotic-treated ponds continued until 90 DOC. In summer, control ponds stopped cyst production by 68 DOC, while treated ponds extended cyst release up to 129 DOC. Unit area production followed similar trends; TR1 increased production by 114% (winter) and 304% (summer) over the control, while TR2 achieved increases of 70% and 126%, respectively. Although probiotic Y is more halo-tolerant than probiotic X, probiotic X consistently outperformed probiotic Y and untreated controls, demonstrating that *Bacillus*-based probiotics effectively mitigate water-quality deterioration and enhance cyst production in large-scale, hyper-saline *Artemia* culture systems where water exchange is limited.

Keywords: *Artemia culture, cyst yield, high-saline probiotics, water quality management*

Introduction

Aquaculture, the world's fastest-growing food production sector, continues to expand to meet the rising demand for high-quality protein. However, the industry faces the challenge of sustainable species diversification. While the diversity of aquatic organisms offers vast opportunities for expansion (Duarte *et al*., 2007), persistent constraints such as high larval mortalities limit the realisation of this potential. One of the most critical bottlenecks in larviculture is the consistent supply of high-quality *Artemia* cysts, which are an indispensable live feed during the early life stages of many fish and crustaceans. Consequently, *Artemia* cysts have become a pivotal commodity in the global aquaculture sector. Traditionally, *Artemia* cysts have been harvested from natural hypersaline lakes, such as the Great Salt Lake (USA) and others in Russia, China, and Kazakhstan. However, these natural resources are increasingly threatened by climate change, environmental fluctuations, and over-exploitation, raising concern about long-term supply stability (Wurtsbaugh and Gliwicz, 2001). To reduce dependence on wild harvests, controlled pond-based *Artemia* production systems, especially in solar saltworks, have been developed as an alternative. Since 2007, MPEDA–Rajiv Gandhi Centre for Aquaculture (RGCA) has been demonstrating the feasibility of *Artemia* culture in solar salt pans and trained coastal communities to encourage domestic production and reduce reliance on imports. India, one of the leading countries in shrimp aquaculture, consumes over 300 tonnes of *Artemia* cysts annually, valued at around ₹300 crore (Press Review, 2019), with most imports coming from the USA and China. Shrimp hatcheries typically require 3–5 kg of cysts to produce one million post-larvae, highlighting their economic importance. Regarding the industrial growth, domestic cyst production is still limited despite initiatives by the MPEDA-RGCA. Low and variable yields (about 100 kg/ha/crop) highlight the urgent need to improve cyst productivity per unit area through better pond management.

Successful cyst production depends on optimal water quality and stable pond conditions. A continuous supply of microalgae and supplementary feed is essential to support *Artemia franciscana* growth; however, this daily input steadily increases the accumulation of organic matter in pond sediments (Jimenez-Montealegre, 2001). This leads to sludge formation, anaerobic conditions, and the release of toxic compounds such as ammonia, nitrite, and hydrogen sulfide, which adversely affect survival, growth, and reproductive output (Avnimelech and Zohar, 1986; Boyd, 1995). Water exchange, though common in aquaculture, is impractical in *Artemia* ponds due to stock loss and operational constraints. *Artemia* are continuous filter feeders capable of consuming particles ≤50 μm. To ensure adequate feeding, chain raking is routinely practised in the pond bottom to resuspend settled particles, yet this practice also increases turbidity and organic decomposition. Therefore, science-based management approaches, such as probiotics, are essential to reduce organic load, improve water quality, and maintain a healthy pond environment that fosters optimal productivity of *A. franciscana*.

The decomposition of accumulated organic matter directly affects water quality, survival, and productivity in culture systems. Probiotics improve water quality by biodegrading uneaten feed and faeces (Tuan *et al*., 2013; Hura *et al*., 2018), accelerating mineralisation, producing extracellular enzymes, and reducing biological oxygen demand, thereby improving overall pond conditions (Wang *et al.,* 2008; Pennafirme *et al*., 2015). Beyond water improvement, probiotics enhance growth, reproduction, survival, and disease resistance in *A. franciscana* (Marques *et al*., 2006; Mathivanan *et al*., 2011; Thao *et al*., 2015). The study by Mahdhi *et al.* (2012) shows that halophilic *Bacillus* strains retain probiotic properties under high salinity and prolonged starvation, inhibit pathogenic *Vibrio* species, and improve *Artemia* survival under stress. As *Artemia* ponds are hyper-saline and nutrient-diluted environments, such salt-tolerant strains are particularly valuable in *Artemia* farming. Hence, regular probiotic application represents a preventive and sustainable approach to manage pond ecology, reducing organic load and stabilising microbial balance, which ultimately results in healthier *Artemia* populations and improved cyst yields. Among candidate probiotics, *Bacillus* sp. are widely used in aquaculture due to their spore-forming ability, which ensures stress resistance, heat tolerance, and long shelf life (Kuebutornye *et al*., 2019; Ghosh, 2025). They enhance feed utilisation, growth, stress tolerance, antioxidant and immune responses, water quality, reproduction, and disease resistance (Ghosh, 2025). *Marinobacter* sp., halophilic and non-pathogenic bacteria, also show probiotic potential because of strong antimicrobial activity (Jeganathan *et al.,* 2013) and efficient nitrogen removal in saline systems (Wang *et al.,* 2025). While *Bacillus* sp. tolerates 0–50 ppt salinity, *Marinobacter* sp. can withstand a much broader range of 0–180 ppt (Guo *et al*., 2025). Mahdhi *et al.* (2012) demonstrated the effect of probiotics on survival, stability, and disease protection under laboratory conditions, but did not replicate complex pond environments such as organic matter build-up, salinity fluctuations, stratification, anaerobic sludge formation, or assess cyst production and field performance across genera.

Most commercial probiotics are predominantly formulated for low-salinity aquaculture systems such as fish and shrimp ponds, highlighting the need to identify strains effective under high-salinity *Artemia* culture conditions. Therefore, the objective of the present study was to compare the efficacy of halophilic and halotolerant probiotics, *Marinobacter* sp. and *Bacillus* sp., in enhancing cyst productivity in *Artemia* culture systems. The findings demonstrated that the *Bacillus* sp. were more effective in maintaining pond conditions and enhancing cyst yield.

Material and methods

Experimental design

The experiment was conducted to evaluate the effect of probiotic application on pond performance and *Artemia* cyst production during two seasons of the year, *i.e*. winter (January–April) and summer (June – October). The study was conducted using three treatments, TR1, TR2 and Control. In TR1, ponds were treated with a *Bacillus*-based consortium (*Bacillus coagulans* and *Bacillus polymyxa*). TR2 ponds were treated with *Marinobacter* sp., and a control group (C) without probiotic addition served as the baseline for comparison.

Experimental ponds and pond preparation

The study was carried out at the RGCA *Artemia* Demonstration Farm, Uppoor, Ramanathapuram District, Tamil Nadu, India (9° 62′ 56.7′′ N, 78° 94′ 63.4′′ E) between June 2024 and April 2025. The experiment included 3 sections of the farm: BC section (Control), D section (TR1) and E section (TR2), each with a uniform water spread area of 1.62 ha to ensure comparability. The experimental ponds measured 0.09 ha in the BC section, 0.18 ha in the D and E sections. These ponds were originally constructed in 2015 and have been consistently used for *Artemia* cyst production over the decade. Before the experiment, all ponds were dried thoroughly, and shell lime was applied at a rate of 1,000 kg/ha. Each pond was then filled with bore water having a salinity range of 60–75 ppt, maintaining a depth of 30–40 cm (Lavens and Sorgeloos, 1996). Water quality parameters were measured before stocking to ensure uniform initial conditions (Fig. 1).

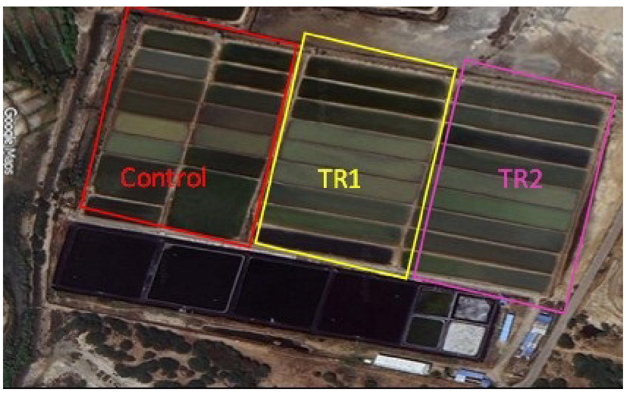

Fig. 1. Experimental ponds at RGCA *Artemia* demonstration farm, Uppoor

Probiotic selection

The choice of probiotic strains for this study was based on their proven efficacy in high-salinity aquaculture systems and their tolerance to extreme salinity levels under laboratory conditions. For Treatment 1 (TR1), a commercial probiotic X, a consortium of *B. coagulans* ($1x10^{10}$ cfu/g) and *B. polymyxa* ($1.2x10^{10}$ cfu/g) was used. These strains were selected based on their demonstrated role in maintaining water quality in high-saline shrimp culture ponds, as reported through farmer testimonials and prior field applications. Their ability to decompose organic matter and stabilise pond ecosystems made them suitable candidates for experimental evaluation. For Treatment 2 (TR2), another commercial probiotic Y, which has *Marinobacter* sp. ($3x10^{9}$ cfu/g), was used. This strain was originally isolated from solar salt farm environments and was found to exhibit an exceptional salinity tolerance ranging from 0 to 180 ppt during *in vitro* screening. Its halotolerance and adaptability to hypersaline ecosystems justified its selection as a probiotic treatment in this study. The control group (C) consisted of ponds without probiotic application, serving as a baseline for comparison.

Application of probiotics

The probiotic treatments were initiated on Day 0 of culture (DOC 0), one day before stocking, to condition the pond water and establish a beneficial microbial environment that enhances nauplii survival. For both treatment groups, the probiotics were applied once per week throughout the culture period. The initial dose was 2500 g/ha, followed by a maintenance dose of 1250 g/ha in subsequent applications until the end of the crop cycle. The probiotic X was mixed with pond water and applied to the culture ponds at 16:00 hours, whereas the microbial formulations of probiotic Y were activated by suspending the powder in molasses and incubating the mixture for 18–24 hours in aerated containers. The activated (probiotic Y) suspension was then broadcast into the ponds at 09:00 hours (as per the directions of the manufacturers). Immediately after application, raking was performed to ensure an even distribution of the microbial suspension throughout the pond water column.

Stocking and pond management

Artemia cysts were incubated at a density of 2 g L^{-1} in well-aerated cylindro-conical FRP tanks for 16–24 hours (Rajamani, 1998). Depending on the hatching synchrony, instar I nauplii were harvested in single or multiple batches to maximise survival potential. The nauplii were carefully counted and distributed equally to all the experimental ponds, ensuring a uniform stocking density of 80-100 nauplii/l. Upon inoculation, mixed microalgae (green algae and diatoms) were supplied to all ponds continuously at a flow rate equivalent to 5% of pond water volume (Rahman and Sorgeloos, 2023). In addition, supplementary feed was supplied to experimental ponds once per day based on the population density as required (Van Stappen *et al.,* 2024). The detailed feeding protocol is given in Table 1. After the maturation, cyst production was visually observed in the downwind corner in all experimental ponds, as *Artemia* cysts floated in the high saline water. From the onset of cyst and nauplii production (F1 generation), supplementary feed was provided to support sustained *Artemia* biomass growth and cyst yield.

Assessment of experimental parameters

Water quality parameters such as salinity, pH, and temperature were measured daily using handheld equipment; alkalinity and ammonia were measured by API kit (Mars Fishcare North America, Inc, USA) on a weekly basis to ensure that optimal environmental conditions for *A. franciscana* growth and reproduction were maintained throughout the culture period. Wet cysts were collected daily from each experimental pond

Table 1. Feeding protocol followed throughout the culture period (Van Stappen *et al.*, 2024)

DOC	Micro algae	Supplementary feed
1 to 20	Mixed microalgae dominantly with diatoms	Nil
20 to the end of culture	Mixed microalgae dominantly with diatoms	Supernatant of a mixture of fermented rice bran, ragi flour and molasses

using a 100 µm scoop net, cleaned and separated in 250 ppt saline water, weighed using a weighing balance and recorded.

Results

The treatment pond's water quality parameters, such as temperature, salinity, pH, alkalinity, nitrite and ammonia, were measured before the day of inoculation, and optimum parameters were maintained throughout the culture period. The application of commercial probiotics in *Artemia* culture ponds resulted in substantial improvements in *Artemia* cyst production compared to the untreated control ponds. The performance of the two commercial probiotics, probiotic X (*Bacillus*-based consortium) and probiotic Y (*Marinobacter* sp), was evaluated across two production cycles, winter crop (January – April) and summer crop (June – October). Considerable variation was observed in both total wet cyst yield and unit area production (kg/ha/crop).

Daily wet cyst yield

Upon inoculation in earthen ponds, *A. franciscana* reaches maturity, and F1 generation (both cyst/ nauplii) production commences. In the winter crop, cyst production commenced on DOC 13 in the control ponds (BC sections) and on DOC 16 in the treatment ponds (D and E sections). In contrast, during the summer crop, control ponds yielded cysts as early as DOC 08, while the treatment ponds produced cysts by DOC 10. The earlier onset of cyst production in the summer crop may be attributed to elevated ambient temperatures, which enhance the metabolic rate and reproductive activity of *A. franciscana*, thereby accelerating cyst formation. As the days of culture (DOC) progressed, the population steadily increased, resulting in a corresponding increase in cyst production. During the initial phase, cyst production was comparable across all ponds; however, a clear difference emerged as the culture advanced. In the control ponds, cyst production was low as DOC increased, whereas both probiotic-treated ponds maintained active production for an extended period. Daily cyst production trends for the winter and summer seasons are illustrated in Fig. 2 and 3, respectively.

In the winter crop, cyst production in the control ponds naturally ceased by 62 DOC, while both probiotic-treated

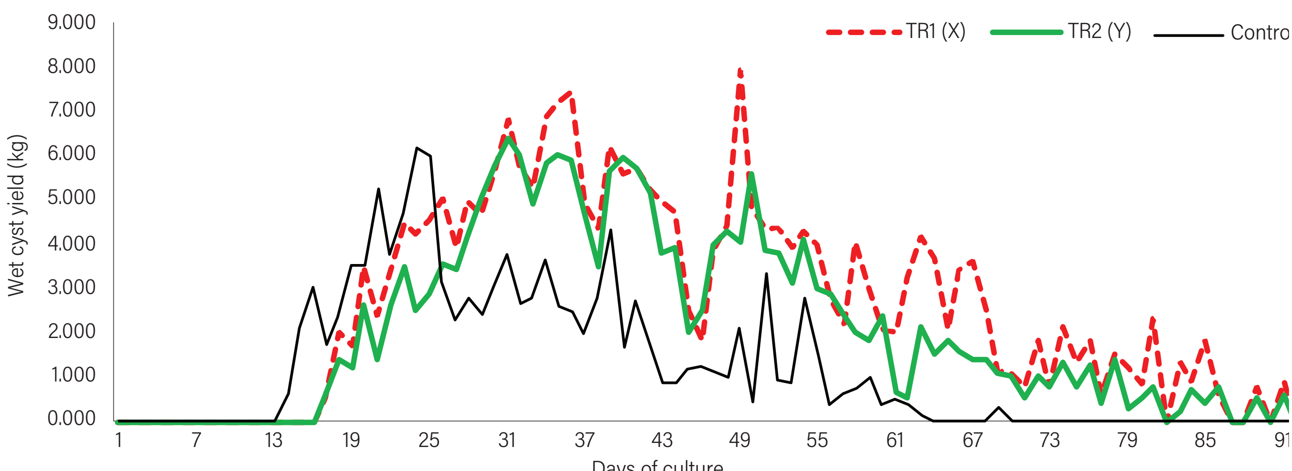

Fig. 2. Daily wet cyst production trends during the winter season

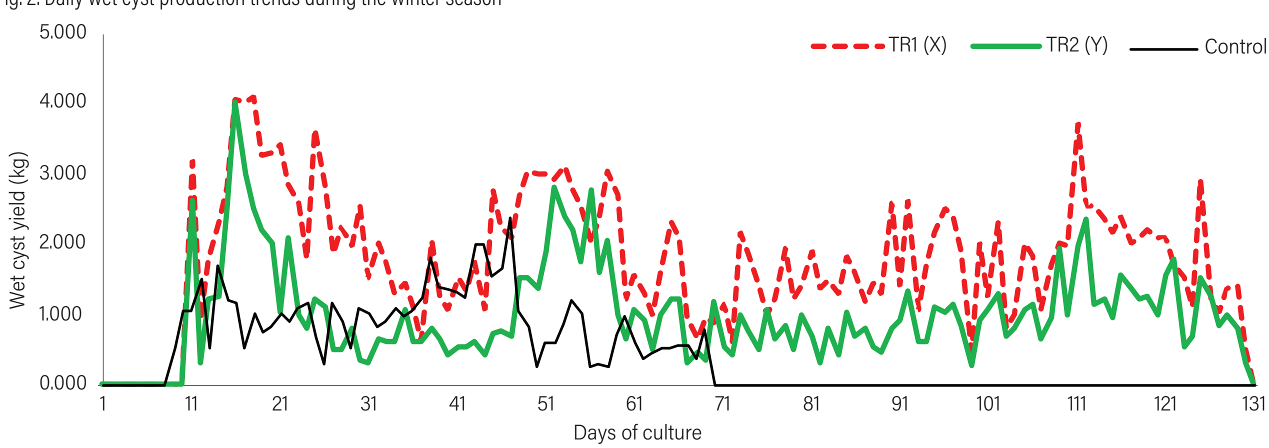

Fig. 3. Daily wet cyst production trends during the summer season

ponds continued producing cysts until 90 DOC. During the summer season, the control ponds ceased cyst production by 68 DOC, in contrast to the probiotic-treated ponds, which sustained production up to 129 DOC. This clearly demonstrates the positive impact of probiotic application in prolonging the reproductive phase of *A. franciscana* under hyper-saline pond conditions.

Unit area production

Unit area cyst production for the treatments and control is summarised in Table 2. During the winter season, TR1 (probiotic X) recorded the highest wet cyst yield of 240.520 kg, with a unit area yield of 148.470 kg/ha/crop. TR2 (probiotic Y) ponds produced 190.955 kg of wet cysts, corresponding to 117.870 kg/ha/crop. The control ponds recorded the lowest wet cyst yield of 112.19 kg, equivalent to 69.25 kg/ha/crop. TR1 (probiotic X) resulted in a 114% increase, while TR2 (probiotic Y) achieved a 70% improvement over the control.

During the summer season, TR1 (probiotic X) yielded the highest wet cyst yield of 232.615 kg, corresponding to 143.580 kg/ha/crop. TR2 (probiotic Y) ponds produced 130.330 kg or 80.450 kg/ha/crop wet cysts, while the control ponds recorded the lowest cyst yield of 57.635 kg, equivalent to 35.570 kg/ha/crop. Relative to the control, TR1 (probiotic X) delivered a remarkable 304% increase, whereas TR2 (Probiotic Y) showed a 126% improvement.

Table 2. Unit area production of the experimental farm during the winter and summer seasons of the year

Unit area cyst production (kg ha^{-1} $crop^{-1}$)	TR1	TR2	Control
Winter (January – April)	148.470	117.870	69.250
Summer (June – October)	143.580	80.450	35.570

Overall, the winter crop outperformed the summer crop, consistent with typical *A. franciscana* production patterns. Across both seasons, TR1 (probiotic X) consistently delivered the highest cyst yield, outperforming both TR2 (probiotic Y) and the untreated control. The differences in unit area productivity among treatments were substantial, clearly indicating the beneficial role of *Bacillus*-based probiotic application on *A. franciscana* cyst production. Unit area production trends for the winter and summer seasons are illustrated in Fig.4.

Environmental parameters

Water quality parameters such as salinity, pH, and temperature were recorded daily. Other parameters, such as alkalinity and ammonia, were observed weekly. The average values in all experimental ponds are tabulated in Table 3.

The water temperature in all experimental ponds during the winter season ranged from 29-32 °C, while in summer it increased to 32-36 °C. This temperature variation showed a clear relationship with cyst yield. During winter, the consistently lower temperature supported higher cyst production, recording 240.52 kg in TR1, 190.955 kg in TR2, and 112.19 kg in the control ponds. However, the culture could not be extended

Table 3. Water quality parameters in experimental ponds during the winter and summer

HO Parameters	Winter			Summer		
	Control	TR1	TR2	Control	TR1	TR2
Temperature (°C)	31±0.75	31±0.95	31±0.81	32±0.53	33±0.84	33±1.03
pH	8.25±0.05	8.21±0.07	8.06±0.05	8.46±0.13	8.5±0.24	8.53±0.25
Salinity (ppt)	78±5.15	73±6.08	75±7.19	74±2.42	72±3.33	69±2.79
Alkalinity (mg/L)	100.87±6.16	91.25±5.23	97.49 ±6.98	113.87 ±5.16	92.54±6.06	98.69±5.96
Ammonia (mg/L)	0.806±0.09	0.172±0.05	0.258±0.03	0.921±0.06	0.137±0.08	0.278±0.09

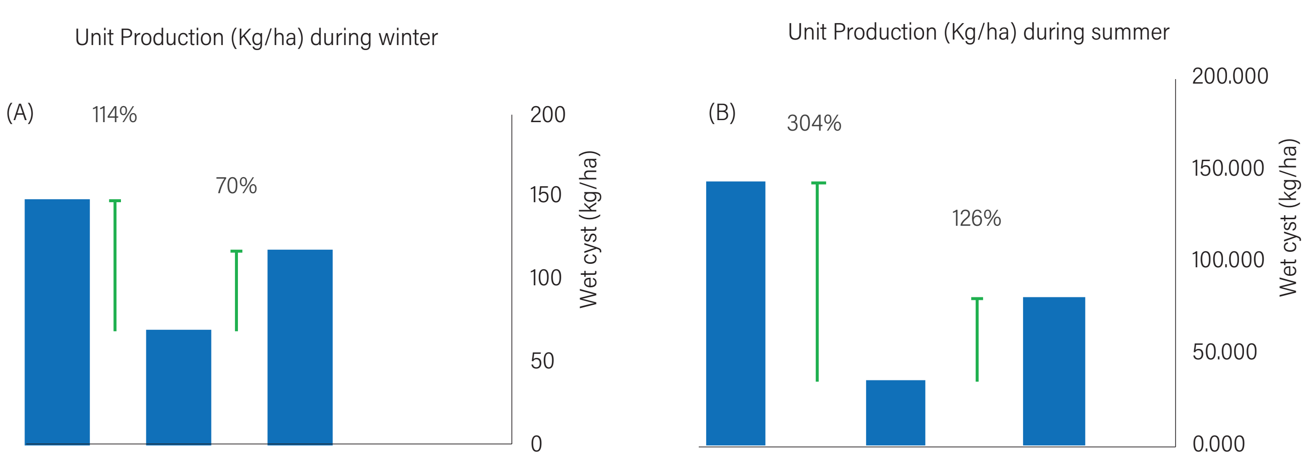

Fig. 4. Unit area production trends for the (A) winter and (B) summer seasons

beyond 90 days, as *A. franciscana* initially acclimated to lower temperatures (around 29 °C) and conditions exceeding 32 °C during that season were not favourable for its growth.

In contrast, summer cultures produced comparatively lower cyst yields, 232.615 kg in TR1, 130.330 kg in TR2, and 57.635 kg in the control. The initial inoculation temperature during this season was between 35-36 °C, and the culture duration extended up to 129 days within a temperature range of 32–36 °C. Even a slight increase in temperature was reflected in reduced cyst output, indicating the sensitivity of *A. franciscana* production to thermal fluctuations. The temperature profile throughout the culture is graphically represented in Fig. 5.

The pH values remained within favourable limits for *A. franciscana* culture, ranging from 8.0–8.25 during winter and 8.4–8.5 during summer. Salinity fluctuated between 60–75 ppt in winter and 60–85 ppt in summer. Alkalinity was consistently lower in TR1 ponds compared to TR2 and the control in both seasons (TR1 < TR2 < Control), which is attributed to the ability of the applied bacterial species to lower alkalinity through acid production during organic matter decomposition and nitrogen transformation. Ammonia levels were markedly lower in TR1 and TR2 ponds than in the control throughout both seasons. This reduction can be linked to the activity of *Bacillus* sp. and *Marinobacter* sp., which facilitate organic matter degradation along with nitrification and denitrification processes, thereby effectively minimising ammonia accumulation in the culture environment.

Discussion

Artemia culture systems tend to accumulate considerable organic waste due to uneaten feed particles, moults, and faecal matter. In addition, raking, a routine daily practice in *Artemia* ponds, causes vertical mixing of bottom sediments into the water column, further contributing to increased organic load. As a result, high concentrations of organic

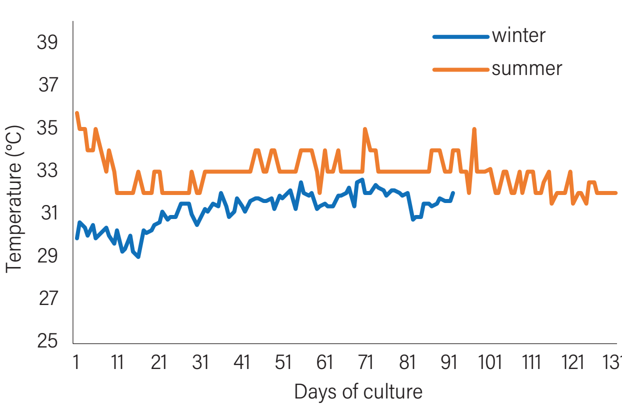

Fig. 5. Graphical representation of temperature fluctuations during winter and summer

matter, nitrogen, and ammonia ultimately deteriorate the pond water quality of the *Artemia* ponds due to decomposition of organic matter. These poor water conditions negatively affect the survival and reproductive ability of the *A. franciscana*. To maintain suitable environmental conditions, various water-quality improvement methods have been developed for aquaculture. Among these, frequent water exchange is the widely used method for controlling metabolites in aquaculture (Crab *et al*., 2007; Martins *et al*., 2010; Jahangiri and Esteban, 2018). However, this method is not feasible for the *A. franciscana* culture. Consequently, the use of probiotics becomes a practical and effective alternative for improving pond water quality. Probiotics not only improve water quality (Jafaryan *et al,* 2011) but also offer other benefits to cultured animals (Zhao *et al*., 2009). Since water quality plays a key role in the growth and overall well-being of aquatic species, probiotic supplementation can help create more favourable culture conditions (Hura *et al*., 2018; Tuan *et al*., 2013). Reflecting their global importance, the probiotic ingredients market size was valued at USD 7.10 billion in 2024 and is projected to grow to USD 22.65 billion by 2034 (Swar, 2025).

A number of probiotic species have been evaluated for their effectiveness in aquaculture. Because *Artemia* is cultured in high saline environments, the selected bacterial strains must perform efficiently under halophilic conditions. In the present study, application of commercial probiotics significantly enhanced *A. franciscana* cyst production compared to the control ponds, emphasising the importance of microbial management in hyper-saline culture systems. The superior performance of the *Bacillus*-based consortium highlights its effectiveness in improving pond conditions, regulating organic matter, and promoting enhanced reproductive output. *Bacillus* sp. are well known for producing extracellular enzymes, degrading accumulated organic matter, rapidly colonising pond environments, stabilising pond micro-biota and reducing harmful metabolites such as ammonia, nitrite, and sulphides (Sedhiqi *et al*., 2025). The consistent improvement observed in TR1 indicates that *Bacillus* sp. effectively minimised bottom sludge formation, thereby supporting *A. franciscana* survival and cyst yield. Previous studies also confirmed the benefits of *Bacillus* in aquaculture systems. *Bacillus* consortium, including *B. polymyxa,* significantly reduced total ammonia nitrogen in carp pond effluents at an optimal dose of 10^8 CFU/l as an optimal amount of inoculum, while *Bacillus* supplementation at 1×10^6 CFU/l improved growth performance in grass carp through enhanced water quality or direct health benefits (Jafaryan *et al*., 2011). Verschuere *et al.* (2000) also noted that *Bacillus* sp efficiently outcompete other microbes due to their better nutrient utilisation.

The performance of probiotics was evaluated across two production cycles in winter (January-April) and summer (June-October) to assess the seasonal effectiveness under field conditions. Probiotic application significantly extended the reproductive period of the *A. franciscana* by maintaining favourable water quality parameters. As a result, cyst production persisted for a longer duration in probiotic-treated ponds, whereas production in control ponds declined and ceased earlier in both seasons due to progressive deterioration of water quality. Seasonal variation also influenced productivity. Cyst production started earlier in the summer crop compared to the winter crop. This earlier onset of cyst production during the summer crop may be attributed to elevated ambient temperatures, which enhance the metabolic rate and reproductive activity of *A. franciscana*, thereby accelerating cyst formation, as mentioned by Rahman and Jeyalakshmi (2009), that the production of cysts and biomass is directly related to the environmental temperature and salinity, supporting the observed seasonal differences in reproductive performance.

In the present study, probiotics-treated ponds exhibited better water quality than untreated control ponds, particularly in terms of ammonia. This improvement can be attributed to the nitrification capability of *Bacillus* sp. and *Marinobacter* sp., which convert nitrogenous waste generated during culture into less harmful forms. The TR1 ponds treated with a *Bacillus-based* consortium showed more effective ammonia reduction, resulting in higher cyst yield compared to control ponds. These findings align with those of Thao *et al.* (2015), who reported that the application of *Lactobacillus* and *Bacillus* lowered TAN levels and subsequently improved *A. franciscana* productivity, characterised by higher growth rates, mating rates, survival, and fecundity. Additionally, Thao and Nguyen (2014) reported that the application of *Bacillus* sp. in *A. franciscana* culture medium significantly improved fecundity and survival rates. Previous works, including those performed on laboratory scale (Mahdhi *et al.*, 2012; Thao and Nguyen, 2014; Thao *et al,* 2015), have also demonstrated that *Bacillus* can help to enhance survival, growth rate and fecundity, but viability or performance experiments were not conducted under commercial pond conditions. The current findings refine this understanding through the measurement of real production outcomes, a phenomenon that is rarely reported in the existing literature. The higher cyst yield of *Bacillus*-treated ponds at all times provides important field-based evidence for the practical application of *Bacillus* probiotics in *A. franciscana* operations.

Although *Bacillus* treatment resulted in the highest cyst production, the contribution of *Marinobacter* sp. remains noteworthy. Hu *et al.* (2021) described *Marinobacter* sp. as efficient degraders of organic pollutants, supporting nitrogen and phosphorus removal and thereby improving overall water quality. Similarly, Han *et al.* (2023) reported reduced ammonia levels in *Marinobacter-treated* systems. Correspondingly, the present study also recorded lower ammonia concentrations in ponds treated with *Marinobacter* sp., reaffirming its beneficial role in nitrogen regulation within *Artemia* culture ponds. Despite its halophilic adaptability and increasing recognition for wastewater treatment (Cheng *et al.*, 2025), the performance of *Marinobacter* in *Artemia* ponds was relatively lower than that of the *Bacillus*-based probiotic X. Nevertheless, *Marinobacter* treatment still resulted in improved cyst yields relative to the control. This comparatively lower performance may be attributed to slower growth kinetics of *Marinobacter*, as observed in laboratory conditions and possible competition with native halophilic microbiota. However, the 70% improvement over the control indicates that *Marinobacter* contributes positively to water quality and *A. franciscana* reproduction, albeit less efficiently than *Bacillus* sp.

The lowest yield recorded in control ponds highlights the challenges associated with *A. franciscana* culture in unmanaged microbial environments. Organic matter build-up and accumulation of toxic intermediates likely resulted in lower survival and diminished reproductive performance. Since *Artemia* reproduction is strongly influenced by environmental stress, the absence of microbial regulation in control ponds likely contributed to the reduced cyst yields.

Conclusion

Overall, the study confirms that effective microbial management plays a pivotal role in maximising cyst yield in *Artemia* production systems. The findings suggest that integrating effective probiotics, especially *Bacillus*-based formulations, into *Artemia* production systems can significantly increase cyst yield per unit area. This has significant implications for regions like India, where demand for *Artemia* cysts continues to rise, and domestic production remains insufficient. This study demonstrates that *Artemia* cyst productivity can be enhanced through the combined application of suitable probiotics during the culture period, offering a pathway toward more sustainable and efficient production. Although previous studies have highlighted the use of probiotics and their positive effect in improving survival and growth performance, they lack field testing. Therefore, the current work bridges the gaps between laboratory and field by providing a first-of-its-kind field-level, industrially salinised, multi-probiotic comparison directed to augmenting the productivity of *Artemia* cysts in order to improve both scientific knowledge and application-driven aquaculture output.

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Author contributions

Conceptualisation: MS; Methodology: MS; Data Collection: NVR; Data Analysis: MS, AAM; Writing original draft: AAM, MS, GS; Writing Review and Editing: MK, AAR; Supervision: SK, MS, MK.

Conflicts of interest

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Data availability

The data are available and can be requested from the corresponding author.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ the collection of sensitive samples/ protected environments.

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References

Avnimelech, Y. and G. Zohar. 1986. The effect of local anaerobic conditions on growth retardation in aquaculture systems. *Aquaculture*, 58 (3–4): 167–174.

Boyd, C. E. 1995. Bottom soils, sediment, and pond aquaculture. Springer, New York, 348 pp.

Cheng, L., P. Zhu, H. Ke, S. Hou, Q. Luo and X. Lu. 2025. Metabolic versatility of *Marinobacter* and its biotechnological potential. *Biotechnol. Adv.,* 83: 108650. *https://doi.org/10.1016/j.biotechadv.2025.108650*

Crab, R., Y. Avnimelech, T. Defoirdt, P. Bossier and W. Verstraete. 2007. Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270: 1–14.

Duarte, C. M., N. Marba and M. Holmer. 2007. Rapid domestication of marine species. *Science*, 316 (5823): 382–383.

Ghosh, T. 2025. Recent advances in the probiotic application of *Bacillus* as a potential candidate in the sustainable development of aquaculture. *Aquaculture*, 594 (7): 1–18.

Guo, M., K. Liu, H. Wang, Y. Song, Y. Li, W. Zhang, J. Gao and M. Liao. 2025. The aerobic denitrification characteristics of a halophilic *Marinobacter* sp. strain and its application in a full-scale fly ash-washing wastewater treatment plant. *Microorganisms*, 13 (6): 1274.

Han, F., C. Zhao, W. Zhang, T. Jiao, Z. Zhang and W. Zhou. 2023. Responses of halophilic microbial communities to changes in salt composition: Comparison between autotrophic nitrification and heterotrophic ammonia assimilation biosystems. *Bioresour. Technol.*, 386: 129500.

Hu, J., J. Yan, L. Wu, Y. Bao, D. Yu and J. Li. 2021. Simultaneous nitrification and denitrification of hypersaline wastewater by a robust bacterium *Halomonas salifodinae* from a repeated-batch acclimation. *Bioresour. Technol.*, 341: 125818.

Hura, T., K. Zafar, J. R. Borana, J. Prasad and J. Iqbal. 2018. Effect of commercial probiotic *Bacillus megaterium* on water quality in composite culture of major carps. *Int. J. Curr. Agric. Sci.*, 8: 268–273.

Jafaryan, H., M. Soltani, H. Noferesti and P. Ebrahimi. 2011. Effect of adding probiotics into the rearing tanks of grass carp (*Ctenopharyngodon idella*) for the exploitation of *Artemia urmiana, A. franciscana* and *A. parthenogenetica* nauplii. *Int. J. Vet. Res.*, 3: 125–128.

Jahangiri, L. and M. Á. Esteban. 2018. Administration of probiotics in the water in finfish aquaculture systems: A review. *Fishes*, 3 (3): 30–33.

Jeganathan, P., K. M. Rajasekaran and N. K. Asha Devi. 2013. Antimicrobial activity and characterization of marine bacteria. *Indian J. Pharm. Biol. Res.*, 1 (4): 38–44.

Jimenez-Montealegre, R. 2001. Nitrogen transformation and fluxes in fish ponds: A modelling approach. *Fish Culture and Fisheries*, Wageningen University, The Netherlands, 185 pp.

Kuebutornye, F. K. A., E. D. Abarike and Y. Lu. 2019. A review on the application of *Bacillus* as probiotics in aquaculture. *Fish Shellfish Immunol.*, 87: 820–828.

Lavens, P. and P. Sorgeloos (Eds.). 1996. Manual on the production and use of live food for aquaculture. *FAO Fish. Tech. Pap.* No. 361, FAO, Rome. 295 pp.

Mahdhi, A., M. Á. Esteban, Z. Hmila, K. Bekir, F. Kamoun, A. Bakhrouf and B. Krifi. 2012. Survival and retention of the probiotic properties of *Bacillus* sp. strains under marine stress starvation conditions and their potential use as a probiotic in *Artemia* culture. *Aquac. Res.*, 43 (13): 1980–1988.

Marques, A., J. Dhont and P. Sorgeloos. 2006. The use of probiotics in aquaculture. *Rev. Fish. Sci.*, 14 (1–2): 1–16.

Martins, C. I. M., E. H. Eding, M. C. J. Verdegem, L. T. N. Heinsbroek, O. Schneider, J. P. Blancheton, E. R. d'Orbcastel and J. A. J. Verreth. 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquac. Eng.*, 43: 83–93.

Mathivanan, R., P. Selvaraj and S. Subramanian. 2011. Probiotics in aquaculture: A review. *J. Appl. Biosci.*, 47: 2361–2366.

Pennafirme, S., I. C. B. Lima, J. A. P. Bitencourt, M. Crapez and R. T. Lopes. 2015. Organic matter biodegradation by bacterial consortium under metal stress. In: Chamy, R., Rosenkranz, F. and Soler, L. (Eds.), *Biodegradation and bioremediation of polluted systems*. InTech.

Press Review. 2019. Press review: 16 to 30 September 2019. https://www.tresor.economie.gouv.fr/Articles/456619cc-8a00-4813-8cb7-9c674327595e/files/22ba4654-06ec-4a0f-9d95-9a079f436235

Rahman, A. A. and R. Jeyalakshmi. 2009. Integration of *Artemia* in Indian salt works – economic opportunities. In: *Proc. 2nd Int. Conf. Ecol. Importance of Solar Saltworks (CEISSA 2009)*, Merida, Yucatan, Mexico. https://ceissa.eu/wp-content/uploads/2025/06/0a8a861d6d3c78b4488752e2d07c0663.pdf

Rahman, M. M. and P. Sorgeloos. 2023. Standard operating procedure for *Artemia* pond culture. WorldFish, *Artemia*4 Bangladesh Program. 16 pp. https://digitalarchive.worldfishcenter.org/server/api/core/bitstreams/79c3334e-181f-4954-be6a-d256bdaa9921/content

Rajamani, M. 1998. *Artemia* culture and cyst production. In: *Proc. Workshop National Aquaculture Week*. Aquaculture Foundation of India: p. 86–90.

Sediqi, S., M. T. Omid, A. B. Hejran, A. Q. Baseer, M. Hassan, B. Sawicka, D. Skiba and P. Niazi. 2025. An investigation of the role of *Bacillus subtilis* in water purification and organic matter decomposition. *Cognizance J. Multidiscip. Stud.*, 5 (8): 529–543.

Swar, V. 2025. Probiotic ingredients market growing with rising health awareness and innovation. *Food Beverages Ingredients*, Insight code: 5532.

Thao, N. T. T. and N. T. Nguyen. 2014. Effects of probiotic supplementation methods on the growth and reproduction of *Artemia franciscana* Vinh Chau. *Can Tho Univ. J. Sci.*, 32: 94–99.

Thao, N. T. T., N. T. B. Trang and P. T. T. Ngan. 2015. Combined effects of salinity and *Bacillus subtilis* supplementation on the growth and reproduction of *Artemia franciscana*. *Can Tho Univ. J. Sci.*, 39: 118–127.

Tuan, T. N., P. M. Duc and K. Hatai. 2013. Overview of the use of probiotics in aquaculture. *Int. J. Res. Fish. Aquac.*, 3 (3): 89–97.

Van Stappen, G., P. Sorgeloos and G. Rombaut. 2024. Manual on *Artemia* production and use. Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/cc7873en

Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete. 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiol. Mol. Biol. Rev.*, 64: 655-671.

Wang, Y. B., Z. Q. Tian, J. T. Yao and W. Li. 2008. Effect of probiotics *Bacillus subtilis* and *Bacillus licheniformis* on growth performance and digestive enzyme activity of tilapia (*Oreochromis niloticus*). *Aquac. Res.*, 39 (2): 170-173.

Wang, F. R., S. Y. Feng, S. Liang, W. Y. Du, L. Q. Wang, Y. W. Zhang, J. Y. Ren, S. Gao, Y. J. Zhu and Y. T. Cong. 2025. Rapid and efficient nitrogen removal by a novel heterotrophic nitrification–aerobic denitrification bacterium *Marinobacterium maritimum* 5-JS in aquaculture wastewater. *Environ. Res.*, 276: 121500.

Wurtsbaugh, W. A. and Z. M. Gliwicz. 2001. Limnological control of brine shrimp population dynamics and cyst production in the Great Salt Lake, Utah. *Hydrobiologia*, 466: 119–132.

Zhao, S., N. Hu, Z. Chen, B. Zhao and Y. Liang. 2009. Bioremediation of reclaimed wastewater used as landscape water by using the denitrifying bacterium *Bacillus cereus*. *Bull. Environ. Contam. Toxicol.*, 83: 337–340.