Evaluation of the role of *Bacillus subtilis* on life performance of *Artemia franciscana*

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Abstract. The present study evaluated the role of *Bacillus subtilis* (BC24.4) isolated from the Vinh Chau salt fields on the growth and reproduction of *Artemia franciscana*. The study comprised two experiments. Experiment 1 tested the effects of various bacterial densities (treatments), where all environmental factors were within the appropriate range for *A. franciscana* development. The treatments using *B. subtilis* were able to control total ammonia nitrogen (TAN) and nitrite concentrations, as well as *Vibrio* density. The results indicate that *A. franciscana* cultured with *B. subtilis* at a density of 10^8 CFU mL^{-1} was optimal, corresponding to a survival rate of 78.26±4.88%, a mean body length of 6.97±0.09 mm, an average reproductive time of 25.57±3.52 days, a mean female lifespan of 46.13±2.71 days, an average male lifespan of 38.57±5.55 days, a total embryo number of 985.67±202.35, and a mean female fecundity of 146.22±31.41 embryos/brood. Experiment 2 applied several different C:N ratios. The results indicate that a C:N ratio of 5:1 was optimal, corresponding to a survival rate of 88.22±3.37%, a mean body length of 8.01±0.18 mm, a female lifespan of 41.47±1.25 days, a male lifespan of 34.43±4.45 days, a mean total embryo number of 932.97±117.56, and a female fecundity of 155.95±52.84 embryos/brood. Both experiments demonstrated a higher rate of nauplii reproduction than cyst reproduction females. The research results provide a basis for the application of *B. subtilis* in *Artemia* culture, which can contribute to increasing productivity.

Key Words: *Artemia franciscana*, *Bacillus subtilis*, growth, reproduction.

Introduction. In Vietnam, *Artemia franciscana* is cultured in the Vinh Chau-Soc Trang salt fields, providing a large annual supply of *Artemia* cysts (Hoa et al 2007; Hoa & Van 2018). In recent years, climate events such as unseasonal rains have affected *Artemia* farming activities, destabilizing the output of *Artemia* cysts (Hoa et al 2022).

Beneficial microorganisms serve a crucial role in transforming organic matter in the aquaculture environment. Bacteria have been widely applied to improve the culture conditions and increase disease resistance in farmed species. *Bacillus* is a genus of bacteria commonly used to improve the quality of water and soil in aquaculture ponds. This group of bacteria exhibits the characteristics of a wide distribution, high heat resistance, rapid growth in liquid culture media, and the easy formation of heat-resistant spores. Additionally, *Bacillus* not only secretes antibacterial compounds (e.g., bacteriocins and antibacterial peptides) to increase its ability to compete with pathogenic bacteria but also produces many types of extracellular enzymes (e.g., amylase, protease, and cellulase), which promote organic matter decomposition and mineralization in aquaculture ponds (Soltani et al 2019; Kamilya & Devi 2022).

However, to date, *Artemia* farming in salt fields has not effectively utilized beneficial microorganisms. Therefore, the isolation and selection of *Bacillus* sp. strains for application in the *Artemia* farming process, especially at low salinity values (i.e. less than 80‰ as conventional *Artemia* cultivation), may contribute to increasing the productivity and yield of *Artemia* at Vinh Chau salt fields (Soc Trang, Vietnam).

The present study was conducted to evaluate the role of isolated *Bacillus subtilis* on the growth and reproduction of *A. franciscana*. The results can provide a basis for the application of *B. subtilis* biological products in *Artemia* aquaculture, thereby contributing to environmental improvement and increased productivity, which can support the sustainable development of *Artemia* farming.

Material and Method

Research materials. *A. franciscana* (Vinh Chau strain) was provided by the College of Aquaculture and Fisheries, Can Tho University. The studied *B. subtilis* strain (BC24.4) is a beneficial bacterium that was isolated from *Artemia* ponds in the Vinh Chau area, Soc Trang, Vietnam (Tam et al 2025). The culture used water from a high-salinity source (80-100‰) taken from the Vinh Chau salt fields and combined with tap water to achieve a salinity of 60‰.

Experimental setup. Experiment 1, that lasted during April to May 2025, was conducted using laboratory-reared *A. franciscana*. The experiment comprised five treatments, each with three replicates, as follows: control 1 (no *B. subtilis* addition), control 2 (using commercial *B. subtilis*), and three treatments with *B. subtilis* (BC24.4) at densities of 10^7 CFU mL^{-1}, 10^8 CFU mL^{-1}, and 10^9 CFU mL^{-1} respectively.

For raising *A. franciscana* in the laboratory (Hoa et al 2022), cysts that hatched after 24 hours were placed in 30 L plastic buckets at a stocking density of 200 nauplii L^{-1}. The experimental system was equipped with continuous lighting and aeration, the temperature was maintained at a stable 26-28°C, and the alkalinity was maintained above 90 mg $CaCO_3$ L^{-1} during the experiment. *A. franciscana* were fed four times per day (7:00 a.m., 11:00 a.m., 2:00 p.m., and 5:00 p.m.). During the first two days, *A. franciscana* were provided with fresh algae *Chaetoceros calcitrans* at a density of 6.74 x 10^6 cells mL^{-1}. From the third day, the *A. franciscana* were switched to feeding on processed food (30% protein, 9% lipid), as described by Han (2015). The diet was based on the standard diet for one individual, calculated by dry weight (Hoa 1993) and adjusted according to individual needs. The dry food was weighed, then mixed with water and sieved through a 50 µm mesh to form a feeding solution. When a pairing rate greater than 70% was observed in the breeding population, we randomly captured 30 pairs for each treatment and raised each pair separately (in falcon tubes) to monitor reproductive indicators. In each tube, dead males were replaced by another male from the same population. If the female died, monitoring of the individual pair ended (Hoa et al 2022).

Experiment 2, conducted during June to July 2025, used the results of Experiment 1 to arrange six treatments, each with three replications, as follows: control 1 (no *B. subtilis* addition); control 2 (using commercial *B. subtilis*); and four treatments with *B. subtilis* (BC24.4) at 10^8 CFU mL^{-1} with C:N ratios of 5:1, 10:1, 15:1, and 20:1 respectively. Molasses (40% carbon content) was used as an organic carbon source to adjust the C:N ratio for each treatment on the fifth day, with periodic adjustment performed every three days (Hoa et al 2022). The amount of molasses was calculated according to the total ammonia nitrogen (TAN) content measured in the culture medium.

Monitoring indicators during the experiment. Several environmental parameters were measured. Temperature and pH were measured twice per day using a HANNA meter. Dissolved oxygen was also measured twice per day (7:00 and 14:00) using a Handy Polaris 2 meter. The alkalinity, TAN, and nitrite levels were checked every five days via a colorimetric method using a SERA test kit (Heinsberg, Germany). The colony counting method was used to determine the density of *B. subtilis* (Dung 1983), the density of *Vibrio* bacteria, and the total bacterial count (Baumann et al 1980), with samples taken every five days.

Survival rate and length were determined on the seventh and fourteenth days of the experiment. Length (mm) was determined by randomly sampling 30 individuals in each treatment, fixing the *A. franciscana* in Lugol's solution, and then measuring the length from the top of the head to the end of the tail under an electron microscope:

$$L\ (mm) = (1/10) \times (A/\gamma)$$

where: A is the number of lines measured on the glass slide; γ is the magnification of the objective lens.

Survival rate was determined by dividing the number of surviving animals by the total initial number:

$$\text{Survival rate (\%)} = (N_t/N_0) \times 100$$

where: N_t is the total number of *A. franciscana* counted; N_o is the total initial number of *A. franciscana.*

Life cycle indicators (Van et al 2011): pre-reproductive time, reproductive time, post-reproductive time, and the lifespans of females and males.

Reproductive indicators (Van et al 2011): the total number of embryos (cyst and nauplii) born to a female over the complete life cycle, the total number of cysts per female, the total number of nauplii per female, the number of broods per female over the life cycle, the reproductive cycle (the time between two births) of a female, fecundity (the number of embryos) born from a female in a single litter, the cyst reproduction rate (the total number of cysts/total number of embryos) per female over the life cycle (%), and the nauplii reproduction rate (total number of nauplii/total number of embryos) per female over the entire life cycle (%).

Data processing*.* The data were analyzed for differences between treatments using one-way ANOVA, followed by Tukey's test. Statistical analyses were performed using SPSS 21.0. A p-level < 0.05 was considered significant.

Results

Effect of B. subtilis on the life activities of A. franciscana

Water environmental factors. During the experiment, the average morning temperature was 28.17°C, while the afternoon temperature was 29.12°C. These temperatures fall within the suitable range for *A. franciscana* growth. The pH value did not fluctuate considerably during the experiment, being maintained between morning and afternoon at 8.18 and 8.04, respectively. The dissolved oxygen monitoring results indicate that the treatments had stable oxygen concentrations, fluctuating between morning and afternoon, at 7.25 and 7.02 mg mL^{-1}, respectively.

Alkalinity in the treatments was 125.3-274.5 mg mL^{-1} and tended to increase toward the end of the experiment. The treatment using *B. subtilis* had lower alkalinity than the treatment not using *B. subtilis*. TAN content in the experiments using *B. subtilis* strain BC24.4 tended to decrease toward the end of the experiment, with a range of 0.14-0.33 mg mL^{-1}, which is lower than the control treatments (0.48-0.63 mg mL^{-1}). Nitrite content in the experiment exhibited clear fluctuations, with Control 1 and Control 2 ranging from 0.13 to 0.2 mg mL^{-1}. The treatments using *B. subtilis* (BC24.4) did not record any nitrite content.

The results of the variation in *B. subtilis* bacterial density are presented in Figure 1. The density of *B. subtilis* bacteria in the treatments using biological products reached a level greater than 10^7 CFU mL^{-1} and exhibited a statistically significant difference ($p < 0.05$) when compared to Control 1. *Vibrio* density in the Control 1 and Control 2 treatments was greater than 10^1 CFU mL^{-1} and was significantly different ($p < 0.05$) when compared to the remaining treatments. The total bacterial density in the water was quite high, exceeding 10^8 CFU mL^{-1}, and there was a statistically significant difference ($p < 0.05$) between the treatments (Figure 2).

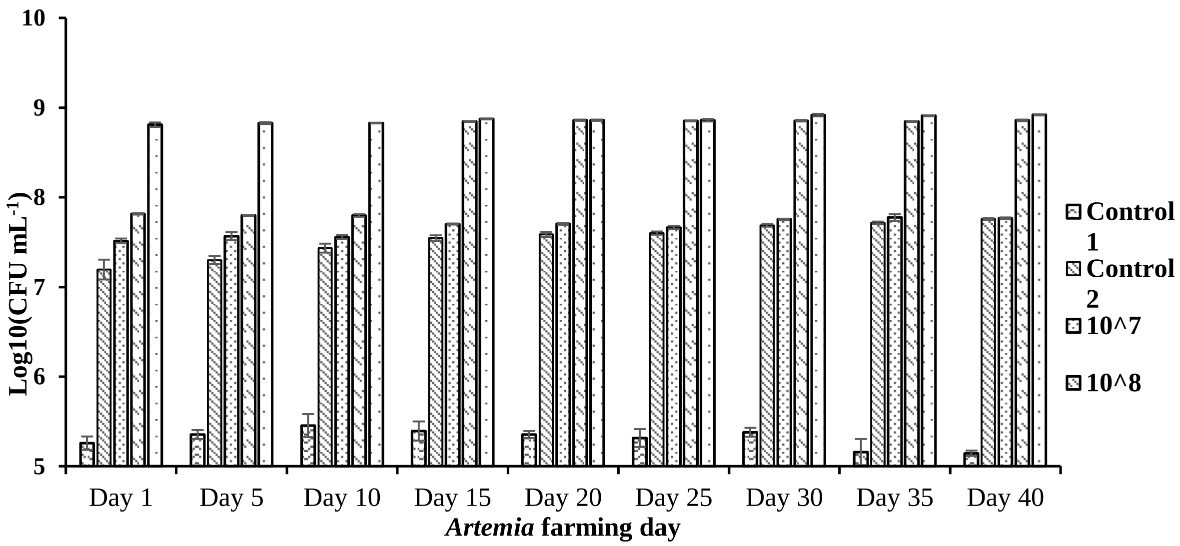

Figure 1. Fluctuations in *B. subtilis* density during *A. franciscana* culture in Experiment 1.

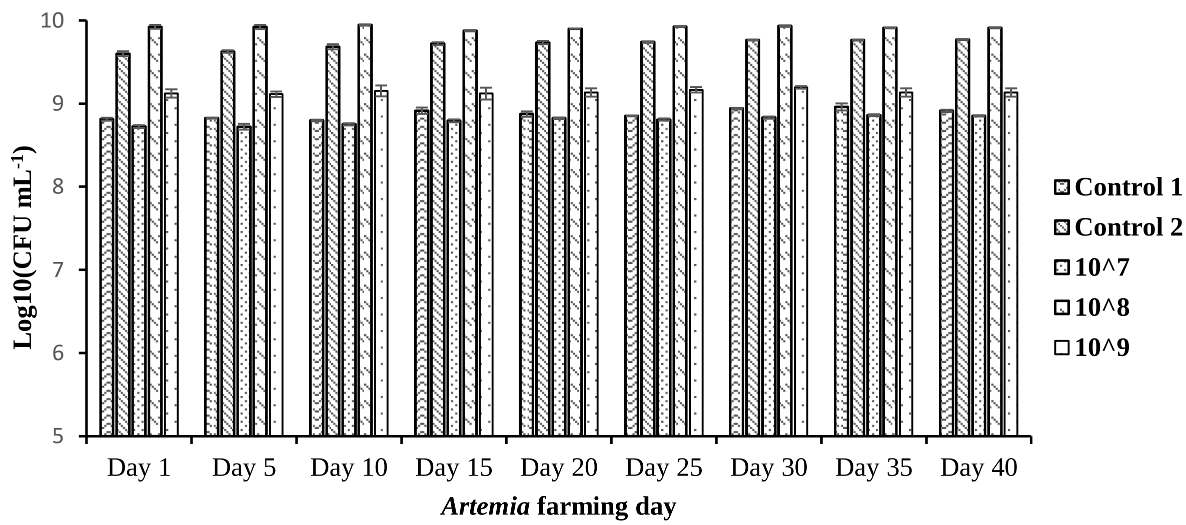

Figure 2. Fluctuations in total bacterial density during *A. franciscana* culture in Experiment 1.

Biological parameters of A. franciscana

Survival rate and body length. After 14 days of rearing, the survival rate of *A. franciscana* supplemented with *B. subtilis* was significantly higher ($p < 0.05$) when compared to Control 1 (61.72±2.76%). The survival rate was highest in the experiment supplemented with *B. subtilis* at a density of 10^8 CFU mL^{-1} (day 14 = 78.26±4.88%) (Table 1).

Table 1

Survival rate and length *of A. franciscana* on days 7 and 14 in Experiment 1

Treatment	*Survival rate (%)*		*Length (mm)*	
	Day 7	*Day 14*	*Day 7*	*Day 14*
Control 1	82.28±2.37^a	61.72±2.76^a	1.42±0.11^a	6.42±0.70^a
Control 2	85.56±3.99^{ab}	67.64±3.84^{ab}	1.52±0.01^a	6.55±0.15^a
10^7	87.93±1.31^{ab}	75.73±6.13^b	1.53±0.08^a	6.61±0.40^a
10^8	89.39±2.12^b	78.26±4.88^b	1.56±0.05^a	6.97±0.09^a
10^9	87.40±1.57^{ab}	75.96±7.04^b	1.52±0.03^a	6.89±0.21^a

Values represent the mean±standard deviation, with different superscript letters in the same column indicating a significant difference ($p < 0.05$).

The body length of *A. franciscana* did not differ among the treatments on days 7 or 14 of culture; however, each treatment was supplemented with biological products containing *B. subtilis. A. franciscana* grew rapidly from day 7 to 14, reaching the maximum length by day 4 (day 1 mean = 1.56±0.05, and day 14 mean = 6.97±0.09 mm) (Table 1).

Life cycle indicators of A. franciscana. The results presented in Table 2 indicate that the pre-reproductive time ranged from 18.03±1.38 to 20.07±1.57 days. In the treatments supplemented with *B. subtilis* at densities of 10^8 CFU mL^{-1} and 10^9 CFU mL^{-1}, the shortest times were 18.27±1.36 and 18.03±1.38 days, respectively, and the difference was statistically significant when compared to the remaining treatments.

Reproductive time ranged from 19.73±5.74 to 25.57±3.52 days. The highest value was observed in Treatment 4 at 25.57±3.52 days. This result is similar to the value reported by Hoa et al (2022) of 24.97±10.31 days when *A. franciscana* were raised at a salinity of 60‰.

The lifespans of females and males in the treatments showed significant differences, ranging from 41.73 to 46.13 days and from 32.83 to 38.57 days, respectively. The highest value was observed in the treatment using *B. subtilis* at a density of 10^8 CFU mL^{-1}, with females reaching 46.13 days and males reaching 38.57 days.

Table 2

A. franciscana life cycle indicators in Experiment 1

Treatment	*Pre-natal period (days)*	*Reproduction time (days)*	*Post-reproductive period (day)*	*Female lifespan (day)*	*Male lifespan (day)*
Control 1	$19.47±2.05^{b}$	$19.73±5.74^{a}$	$2.53±1.36^{ab}$	$41.73±5.06^{a}$	$32.83±6.25^{a}$
Control 2	$19.17±1.66^{ab}$	$22.63±5.42^{ab}$	$2.13±0.51^{a}$	$43.93±5.16^{abc}$	$35.37±6.95^{ab}$
10^7	$20.07±1.57^{b}$	$20.17±5.34^{a}$	$2.73±1.53^{ab}$	$42.97±5.09^{ab}$	$36.40±4.67^{ab}$
10^8	$18.27±1.36^{a}$	$25.57±3.52^{b}$	$2.30±0.75^{ab}$	$46.13±2.71^{c}$	$38.57±5.55^{b}$
10^9	$18.03±1.38^{a}$	$24.73±3.13^{b}$	$3.17±1.62^{b}$	$45.93±2.48^{bc}$	$37.63±6.73^{b}$

Values represent mean±standard deviation, with different superscript letters in the same column indicating a significant difference ($p < 0.05$).

Reproductive indicators of A. franciscana. The results presented in Table 3 indicate that the total number of embryos per female in the treatments ranged from 630.87±213.59 to 985.67±202.35. The highest number was in Treatment 4, and the difference was significant when compared to the remaining treatments ($p < 0.05$). *A. franciscana* tended to give nauplii reproduction more than cysts reproduction; the number of embryos as cysts was highest in Treatment 5 with 371.03±139.41 embryos/female, while the number of embryos as nauplii was highest in Treatment 4 with 629.63±160.34 embryos/female. The control treatment without *B. subtilis* supplementation had the lowest number of embryos in the form of cysts, 244.40±107.99 embryos/female and 386.47±184.37 embryos/female, respectively. The cyst reproduction rate in all treatments was always lower than the rate of nauplii reproduction. In Treatment 4, the rate of cyst reproduction was the lowest at 35.32±13.99%, yet the rate of nauplii reproduction was the highest at 64.68±13.99%.

The number of broods in the treatments using *B. subtilis* was higher than in the control treatment without *B. subtilis*, and the difference was statistically significant ($p < 0.05$). For the treatment using *B. subtilis* at a density ≥ 10^8 CFU mL^{-1}, *A. franciscana* tended to spawn more times in treatments 4 and 5, with 6.83 and 7.47 broods, respectively. The reproductive cycle of *A. franciscana* ranged from 3.43 to 4.07 days. In the control treatment without *B. subtilis* supplementation, the time between two spawning events of a female was the longest at 4.07 days. The fecundity in Treatment 4 was the highest, at 146.22±31.41 embryos/brood, and was significantly different from the remaining treatments. The lowest value was observed in the control treatment without *B. subtilis* at 116.60±23.06 embryos/brood.

Table 3

Reproductive indicators *of A. franciscana* in Experiment 1

Treatment	*Total number of embryos (cyst and nauplii)*	*Total number of cysts/female*	*Total number of nauplii/female*	*Number of broods*	*Reproductive cycle*	*Fecundity*	*Cyst reproduction rate (%)*	*Nauplii reproduction rate (%)*
Control 1	630.87±213.59[a]	244.40±107.99[a]	386.47±184.37[a]	5.47±1.72[a]	4.07±0.74[c]	116.60±23.06[a]	40.30±15.55[a]	59.70±15.55[a]
Control 2	784.33±245.29[ab]	325.43±120.35[ab]	458.90±204.33[ab]	6.03±1.83[ab]	3.93±0.69[ab]	131.85±24.73[abc]	43.24±13.16[a]	56.76±13.16[a]
3 (10^7)	788.43±227.11[b]	327.93±109.13[ab]	460.50±199.06[ab]	5.90±1.95[ab]	3.96±0.93[c]	139.79±34.02[bc]	42.87±12.25[a]	57.13±12.25[a]
4 (10^8)	985.67±202.35[c]	356.03±184.07[b]	629.63±160.34[c]	6.83±1.18[bc]	3.63±0.56[ab]	146.22±31.41[c]	35.32±13.99[a]	64.68±13.99[a]
5 (10^9)	914.67±195.84[bc]	371.03±139.41[b]	543.63±227.85[bc]	7.47±1.17[c]	3.43±0.77[a]	122.95±19.91[ab]	42.04±17.67[a]	57.96±17.67[a]

Values represent mean±standard deviation, with different superscript letters in the same column indicating a significant difference ($p<0.05$).

Evaluation of the effect of B. subtilis at different C:N ratios on the life activities of A. franciscana

Water environmental factors. The average morning temperature was 28.19°C and the average afternoon temperature was 29.09°C. The pH fluctuated between morning (8.16) and afternoon (8.01). The dissolved oxygen content was greater than 7 mg mL^{-1}, and the alkalinity of the treatments ranged from 129.7 to 171.0 mg mL^{-1}. For the treatments using *B. subtilis* and added molasses, the TAN content ranged from 0.14 to 0.33 mg mL^{-1}, lower than the control treatment (0.42-0.61 mg mL^{-1}). The same trend was observed for the nitrite content in the two control treatments, where it ranged from 0.14 to 0.20 mg mL^{-1}.

The results for the variation in the density of *B. subtilis* are presented in Figure 3. In the experiments using *B. subtilis* at 10^8 CFU mL^{-1} and added molasses, *B. subtilis* density was always greater than 10^8 CFU mL^{-1} and was significantly different from Control 1 and Control 2, with a slight increase toward the end of the experiment. The highest density occurred in the experiments with C:N ratios of 5:1 and 10:1. *Vibrio* density in treatments 1 and 2 was greater than 10^2 CFU mL^{-1} and 10^1 CFU mL^{-1}, respectively, and was significantly different from the remaining treatments. The total bacterial density in water was quite high (above 10^9 CFU mL^{-1}) for the treatments using bacteria and significantly different from the control treatments (Figure 4).

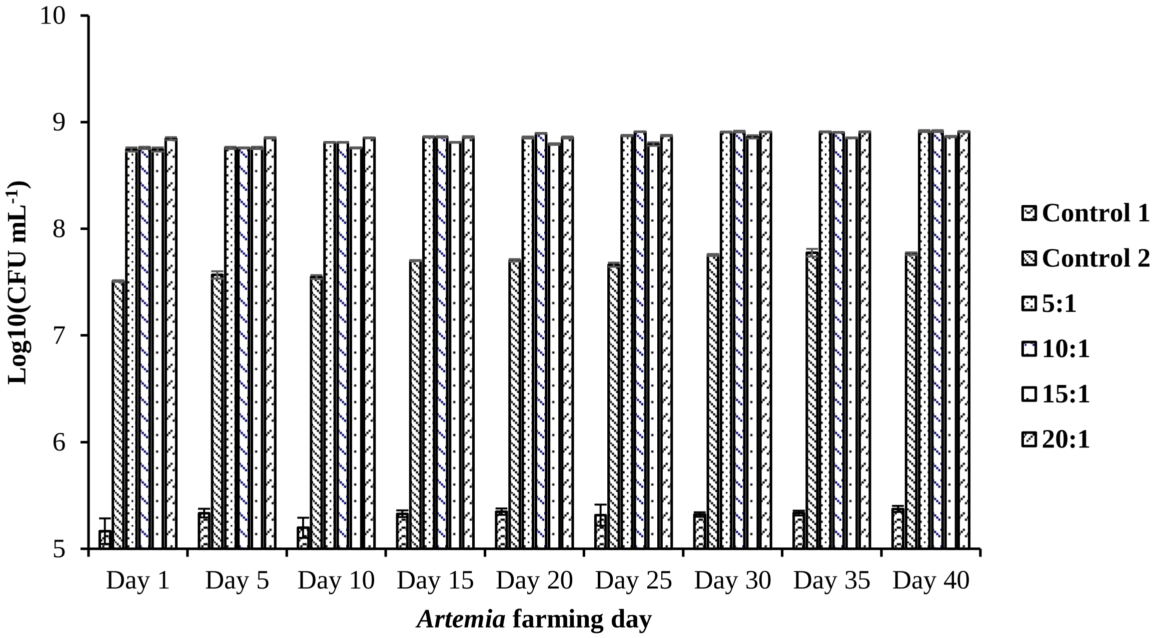

Figure 3. Fluctuations in *B. subtilis* density during *A. franciscana* culture in Experiment 2.

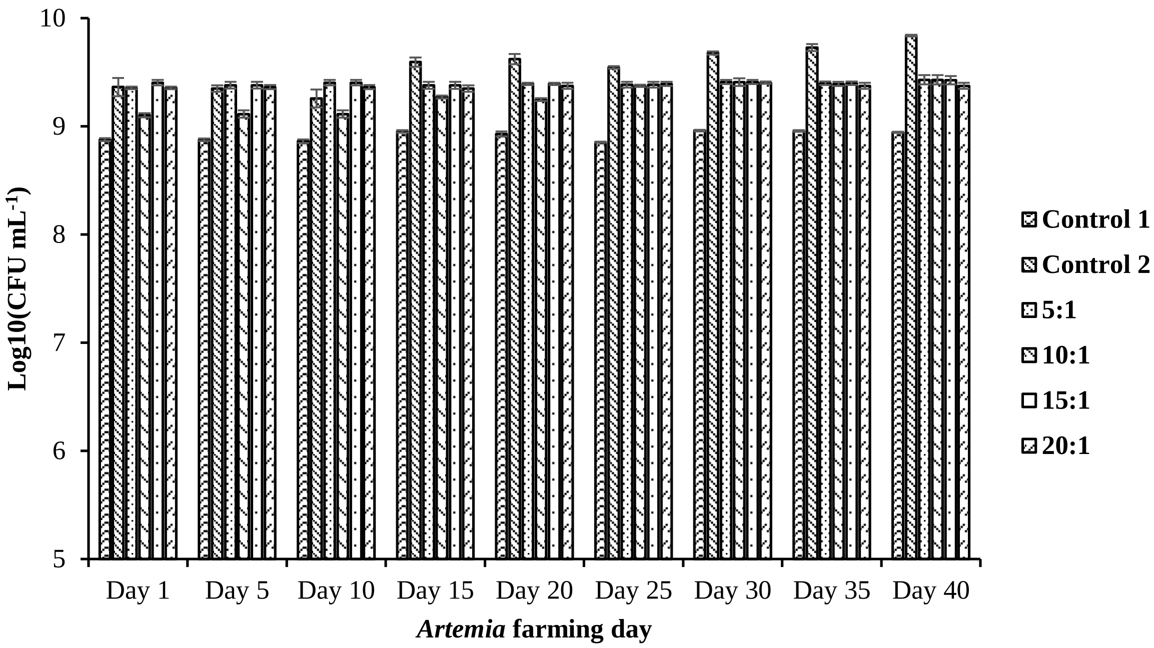

Figure 4. Fluctuations in total bacterial density during *A. franciscana* culture in Experiment 2.

Biological parameters of A. franciscana

Survival rate and body length of A. franciscana. The survival rate and body length of *A. franciscana* in Experiment 2 were higher than in Experiment 1 (Table 4). On the 14th day of rearing, the treatments using *B. subtilis* yielded higher survival rates than those without *B. subtilis*, and the difference was statistically significant ($p < 0.05$). In treatment 3 (C:N ratio = 5:1), the highest survival rate was 88.22±3.37%, and the greatest body length was 8.01±0.18 mm, observed on the 14th day of rearing.

Table 4

Survival rate and body length *of A. franciscana* on days 7 and 14 in Experiment 2

Treatment	*Survival rate (%)*		*Length (mm)*	
	Day 7	*Day 14*	*Day 7*	*Day 14*
Control 1	89.20±0.83[a]	75.67±1.20[a]	1.82±0.09[b]	7.27±0.22[a]
Control 2	92.75±2.65[a]	82.70±0.70[ab]	1.82±0.03[b]	7.62±0.13[ab]
5:1	93.29±1.10[a]	88.22±3.37[b]	1.81±0.09[b]	8.01±0.18[b]
10:1	92.61±1.78[a]	88.13±3.21[b]	1.81±0.09[b]	7.99±0.10[b]
15:1	91.58±1.49[a]	78.71±4.99[a]	1.70±0.05[ab]	7.91±0.32[b]
20:1	93.65±2.79[a]	78.35±4.69[a]	1.60±0.02[a]	7.83±0.20[ab]

Values represent mean±standard deviation, with different superscript letters in the same column indicating a significant difference ($p < 0.05$).

Life cycle indicators of A. franciscana. The life cycle indicators did not differ between the treatments (Table 5). However, compared to the optimal treatment in Experiment 1, the lifespan of females was approximately five days less. The longest lifespan was observed in treatment 3 (C:N ratio = 5:1), at 41.47 days in females and 34.43 days in males.

Table 5

A. franciscana life cycle indicators in Experiment 2

Treatment	*Pre-natal period (days)*	*Reproduction time (day)*	*Post-reproductive period (day)*	*Female lifespan (day)*	*Male lifespan (day)*
Control 1	17.97±1.56^{a}	20.73±2.39^{a}	1.97±1.59^{a}	40.67±1.27^{ab}	31.97±6.18^{a}
Control 2	17.83±1.44^{a}	20.80±1.94^{a}	1.83±1.49^{a}	40.47±1.66^{a}	33.23±4.77^{a}
5:1	18.20±2.22^{a}	20.53±2.52^{a}	2.73±1.23^{a}	41.47±1.25^{b}	34.43±4.45^{a}
10:1	18.50±2.15^{a}	20.23±2.78^{a}	2.00±2.05^{a}	40.73±1.26^{ab}	33.43±4.17^{a}
15:1	18.37±1.29^{a}	20.73±1.64^{a}	1.73±1.11^{a}	40.83±1.18^{ab}	33.23±5.58^{a}
20:1	18.07±1.57^{a}	21.13±1.74^{a}	1.77±1.25^{a}	40.97±1.19^{ab}	33.37±6.18^{a}

Values (mean±standard deviation) with different superscript letters in the same column are significantly different ($p < 0.05$).

Reproductive indicators of *A. franciscana*. The total number of embryos in the treatments using *B. subtilis* was always higher than in the control treatments not using *B. subtilis*, and the difference was significant (Table 6). The highest value was observed in Treatment 3 with 932.97±117.56 embryos/female. Additionally, *A. franciscana* tended to give nauplii reproduction more than cyst reproduction, as in Experiment 1. The lowest total number of cyst/female was observed in Treatment 1 (229.27±111.21 cysts), and the highest total number of offspring/female was in Treatment 3 with 657.97±105.08 individuals. In terms of percentage, the proportion of females giving nauplii reproduction was higher than the proportion of females cysts reproduction. The number of broods and the length of the reproductive cycle were similar to those in Experiment 1, at 6.47-7.50 broods and 3.70-4.03 days, respectively. In treatment 3 (C:N ratio = 5:1), the shortest reproductive cycle was 3.70±0.75 days, and the highest fecundity was 155.95±52.84 embryos/brood.

Table 6

Reproductive indicators *of A. franciscana* in Experiment 2

T	*Total number of embryos (cyst and nauplii)*	*Total number of cysts/ female*	*Total number of nauplii/ female*	*Number of broods*	*RC*	*Fecundity*	*CRR (%)*	*NRR (%)*
Control 1	809.83± 120.23^{a}	229.27± 111.21^{a}	580.57± 48.99^{ab}	6.60± 1.90^{a}	4.03± 0.85^{a}	129.63± 30.20^{a}	27.15± 9.79^{a}	72.85± 9.79^{b}
Control 2	876.20± 116.31^{ab}	302.97± 105.55^{b}	573.23± 71.24^{a}	6.47± 2.03^{a}	4.00± 0.74^{a}	150.39± 58.29^{a}	33.97± 8.18^{b}	66.03± 8.18^{a}
5:1	932.97± 117.56^{b}	275.00± 76.34^{ab}	657.97± 105.08^{c}	6.70± 2.49^{a}	3.70± 0.75^{a}	155.95± 52.84^{a}	29.43± 7.39^{ab}	70.57± 7.39^{ab}
10:1	886.80± 78.92^{ab}	260.73± 76.55^{ab}	626.07± 78.11^{abc}	7.00± 2.36^{a}	3.83± 0.83^{a}	141.18± 51.36^{a}	29.25± 7.51^{ab}	70.75± 7.51^{ab}
15:1	907.93± 119.63^{b}	268.20± 105.65^{ab}	639.73± 97.39^{bc}	7.50± 2.42^{a}	3.73± 0.79^{a}	131.47± 38.04^{a}	29.05± 9.08^{ab}	70.95± 9.08^{ab}
20:1	879.97± 100.59^{ab}	242.03± 101.76^{ab}	637.93± 89.10^{bc}	7.07± 1.84^{a}	3.77± 0.68^{a}	131.95± 33.39^{a}	27.09± 9.19^{a}	72.91± 9.19^{b}

Values represent mean±standard deviation, with different superscript characters in the same column indicating a significant difference ($p < 0.05$); T = treatment; RC = reproductive cycle; CRR = cyst reproduction rate; NRR = nauplii reproduction rate.

Discussion

Evaluation of the role of B. subtilis in the life activities of A. franciscana

Water environmental factors. According to Hoa et al (2007), if the temperature is too low (≤ 20°C), *Artemia* may grow slowly and may not survive. In contrast, if the temperature is too high (> 36°C), mass death can occur, as well as reduced reproductive ability and slow population recovery. *A. franciscana* Vinh Chau strain can grow well from 22 to 35^{o}C and at a pH range of 7 to 9. Therefore, the temperature and pH range during the experiment were suitable for *A. franciscana*. According to Briggs & Funge-Smith (1994), water sources with a pH ranging from 7.5 to 8.5 are optimal for bacterial growth.

The dissolved oxygen values obtained in this study were all higher than the minimum required level, indicating that the aquatic environment maintained sufficient aerobic conditions for the respiration of the cultured organisms, as well as for the effective decomposition of organic matter. According to Hoa & Nam (2018), the *A. franciscana* strain widely stocked in Soc Trang-Bac Lieu grows well under dissolved oxygen ≥ 2 mg mL^{-1}. The optimal dissolved oxygen content for most aquatic species is 5 mg mL^{-1} or higher (Boyd 1998).

The alkalinity in the experimental treatments was similar to that reported by Hoa et al (2022) in a study on the use of selected *Bacillus* sp. for treating the bottom sludge in *Artemia* ponds. The results showed that the alkalinity during *Artemia* farming at a salinity of 60‰ from day 1 to 15 ranged from 95.5 to 173 mg mL^{-1} at *Bacillus* sp. densities of 10^7 and 10^9 CFU mL^{-1}. Wurts & Durborow (1992) found that in low-alkalinity environments, pH was more susceptible to fluctuations than in high-alkalinity environments. This buffering capacity can result in less fluctuation in pH.

The TAN content in the experiments was similar to the value reported in the study of Ngan & Ngoc (2014). In the bacterial supplementation experiment, the level of TAN was relatively stable because the bacteria effectively decomposed the organic matter that accumulated during the rearing process. According to Te (2003), heterotrophic bacteria can decompose organic matter remaining in the rearing pond.

Thao et al (2015) showed that in treatments with added *B. subtilis*, the nitrite content was relatively stable during the experiment at 0.5 mg mL^{-1}. In the treatments without *B. subtilis*, the nitrite content fluctuated. According to Thao & Tam (2013), adding *B. subtilis* to the *Artemia* culture environment reduced both nitrite and ammonia. The enzymes of *B. subtilis* are effective in decomposing proteins, carbohydrates, and lipids of animal or plant origin into simpler forms that are more easily absorbed by other microorganisms or filter feeders in the water body (Sonnenschein et al 1993). The above results indicate that the addition of the isolated *B. subtilis* significantly improved the nitrite content of the *A. franciscana* culture environment.

The density of *B. subtilis* in the present study was higher than in the study of Ngan & Ngoc (2014). The authors also noted that experiments supplemented with bacteria every 4 days revealed that the density of *Bacillus* remained relatively stable throughout the experiment. In the experiment using *B. subtilis* at ≥ 10^8 CFU mL^{-1}, no *Vibrio* were detected from day 15 to the end of the experiment. This suggests that *B. subtilis* can control *Vibrio* better than commercial microorganisms. This result is consistent with Moriaty's (1998) suggestion that *Bacillus* can control *Vibrio.* Rengpipat & Rukpratanporn (1998) suggested that using *Bacillus* spores as a biological control can reduce the incidence of *Vibrio* disease in aquaculture systems.

The addition of *B. subtilis* at a density ≥ 10^8 CFU mL^{-1} increased the total bacterial count in the culture medium. In particular, treatment 4 had the optimal *B. subtilis* density of 10^8 CFU mL^{-1}, which not only created a clear difference in total bacteria when compared to the control but also outperformed commercial microorganisms and eliminated the *Vibrio* bacteria group. Research by Hastings & Nealson (1981) suggested that *Bacillus* can produce antibacterial substances that can kill *Vibrio harveyi.* The *Bacillus* genus also helps improve the development of *Artemia* larvae resistant to *Vibrio alginolyticus* bacteria (Mahdhi et al 2010).

Biological parameters of A. franciscana

<u>*Survival rate and body length of A. franciscana*</u>. The survival rate of *A. franciscana* in this study was higher than that reported by Thao & Tam (2013) for the same species. Experiments supplemented with *Bacillus* achieved a survival rate of 47.5 to 57.5% after 15 days of culture. The addition of *Bacillus* can help *Artemia* limit pathogenic bacteria (Gomez-Gil et al 1998; Verschuere et al 1999). Therefore, raising *Artemia* supplemented with *Bacillus* sp. bacteria will increase the survival rate and biomass of *Artemia*, thereby increasing productivity during the culture process (Ngan & Ngoc 2014).

In the experiments using *B. subtilis*, *A. franciscana* grew rapidly from day 7 to 14. Thao et al (2015) showed that *Artemia* growth gradually increased in the salinity range from 30 to 90‰, with the addition of *B. subtilis* to the *Artemia* culture medium yielding better growth in this species.

Life cycle indicators of A. franciscana. The pre-spawning period in the study of Van et al (2011) was 18.7-20.4 days. The use of *B. subtilis* at a density ≥ 10^8 CFU mL^{-1} in *Artemia* culture can prolong the spawning period of *Artemia* due to *B. subtilis* increasing the available nutrients via the transformation of organic matter during the rearing process (Thao & Ngoan 2014). Research by Hoa et al (2022) showed that when raising *Artemia* at a salinity of 60‰, the lifespan of females in this experiment was 46.2 days, whereas the lifespan of males was shorter at 31.5 days.

Reproductive indicators of A. franciscana. The results for the total number of embryos per female showed that in the treatment supplemented with *Bacillus*, females formed ovaries more rapidly when compared to the control treatment without bacteria. According to Ngan & Ngoc (2014), when adding biological products containing *Bacillus* bacteria during *Artemia* culture, the *Artemia* produced a greater number of embryos when compared to the control treatment. When *Bacillus* bacteria were added to the culture medium, they were able to use food particles as a substrate for development, and when *Artemia* consumed food particles containing bacteria, this promoted their growth and reproduction (Thao et al 2015).

In this experiment, *A. franciscana* tended to give nauplii reproduction rather than cyst reproduction. According to previous studies, the reproductive mode of *A. franciscana* (cysts or nauplii reproduction) is influenced by factors such as the stocking density, temperature, salinity, food source, and environmental stress conditions (Lavens & Sorgeloos 1996). In favorable environments, *Artemia* tend to give nauplii reproduction to optimize growth and shorten the reproductive cycle, while under unfavorable conditions, they often switch to reproducing cysts to ensure the long-term survival of the species (Browne & Wanigasekera 2000). According to Thao & Ngoan (2014), the simultaneous addition of bacteria to algae and the culture medium may have resulted in a higher feed content, more efficient food digestion, and thus a richer source of nutrients for *Artemia* than in other treatments. Additionally, according to previous studies, the optimal salinity range for *Artemia* to reproduce cysts is from 80 to 120‰ (Hoa et al 2007). Thus, a salinity of 60‰ may be more suitable for *Artemia* to produce nauplii (Van Stappen 1996). In conditions of abundant food and a favorable environment, *Artemia* will tend to lay more pupae than cysts.

The number of broods was higher in the treatments using *B. subtilis*. The number of broods is related to the reproductive period and lifespan of *Artemia*. According to Van et al (2011), under good breeding conditions, healthy *Artemia* will have a longer lifespan, with the opportunity to produce more offspring than those with a short lifespan. The reproductive cycle of *A. franciscana* in this study was similar to that observed by Hoa et al (2022). When raising *A. franciscana* at a salinity of 60‰, the reproductive cycle was 3.5 days. Notably, this reproductive performance was greater than in the study by Thao & Ngoan (2014). The reproductive performance of *A. franciscana* in an experiment supplemented with biological products (two types of bacteria, *B. subtilis* and *Lactobacillus acidophilus*, at a density of 10^8 CFU mg^{-1}) into the environment was the highest (126±0.30 embryos/female). Adding biological products directly to the culture environment enhances the fecundity of the first spawning of *Artemia* (Thao & Ngoan 2014).

The results of the present study indicate that the optimal density of *B. subtilis* (BC24.4) is 10^8 CFU mL^{-1}; hence, this value was applied in the next experiment.

Evaluation of the role of B. subtilis with different C:N ratios on the life activity of A. franciscana

Water environmental factors. Most of the environmental factors (temperature, pH, and dissolved oxygen) were within the suitable range for the growth of *A. franciscana* Vinh Chau strain, except that the alkalinity of the treatments was lower than in Experiment 1.

The TAN content results were consistent with the conclusion by Thao et al (2016) that in *Artemia* culture, when the density of *Bacillus* increases, the ability to handle TAN content increases, and the conversion from nitrite to nitrate occurs in cultures supplemented with *Bacillus* sp. under conditions of high salinity. Our results demonstrated that using isolated *Bacillus* sp. at a density of 10^8 CFU mL^{-1}, combined with molasses to control the C:N ratio, improved environmental quality by controlling the TAN and nitrite contents. This is a meaningful result for aquaculture systems or wastewater treatment plants with high concentrations of ammonium and nitrite.

The results also showed that a high density of *B. subtilis* was capable of controlling *Vibrio* during *A. franciscana* culture. *Bacillus* can decompose organic matter, thereby improving the quality of the soil and water environment while increasing the survival rate of *Artemia* (Ngan & Ngoc 2014; Thao et al 2016). In the experiments using *B. subtilis* and adding molasses three times per day, *Vibrio* bacteria did not appear from day 15 until the end of the experiment. This result was similar to that of Experiment 1. According to Moriarty (1998), biological products are effective in preventing luminescent *Vibrio* bacteria based on competition between bacterial species and the various antibiotic compounds produced by *Bacillus*. *Bacillus* species (*B. subtilis*, *B. cereus*, and *B. coagulans*) help defend *Artemia* larvae against pathogenic *Vibrio alginolyticus* (Mahdhi et al 2010). Madhi et al (2011) studied the effectiveness of three *Bacillus* strains (*B. subtilis*, *B. cereus*, and *B. coagulans*) on the growth of *Artemia* and the ability to inhibit the harmful *Vibrio alginolyticus*, noting that the composition of 32% *B. subtilis* and 68% *B. cereus* achieved the best results.

Using *B. subtilis* at 10^8 CFU mL^{-1} and adding molasses can help maintain a high total bacterial density throughout the culture process. The *Bacillus* genus not only adapts well to harsh environmental conditions (e.g., high salinity in *Artemia* ponds) but can also inhibit pathogenic bacteria such as *Vibrio parahaemolyticus*, a significant threat in aquaculture (Ngan et al 2021). *B. subtilis* can even survive and grow at a salinity of 90‰ (Thao et al 2015).

Biological parameters of A. franciscana

<u>*Survival rate and body length of A. franciscana*</u>*.* In Experiment 2, the survival rate and body length *of A. franciscana* were both higher than in Experiment 1. In treatment 3 (C:N ratio = 5:1), the highest survival rate was 88.22±3.37%, and the greatest body length was 8.01±0.18 mm on day 14 of culture. This survival rate was similar to the 86.0±13.3% reported in the study of Toi & Van (2018); however, the shortest body length was 4.78±0.63 mm under the same C:N condition of 5:1. This shows that the use of biological products containing *B. subtilis* combined with molasses affected the growth of *A. franciscana* during the culture process. According to Toi et al (2013), heterotrophic bacteria are stimulated to grow with molasses as a supplementary food for *Artemia*, but the addition did not improve the survival rate of *Artemia*. Research by Hoa et al (2022) also showed that the addition of molasses had a significant impact on the body length of *Artemia,* particularly during the second week when bacterial growth was substantial. Additionally, the addition of processed feed provided a sufficient amount of food for *Artemia* to grow.

<u>*Life cycle indicators of A. franciscana*</u>*.* The results of the life cycle indicators showed that using biological products containing *B. subtilis*, combined with an appropriate molasses dosage, helped *A. franciscana* reproduce, even though the reproductive period was shorter than in Experiment 1. This was demonstrated by the highest fecundity of 155.95±52.84 embryos/brood being observed in treatment 3, in comparison to the optimal treatment in Experiment 1, which exhibited 146.22±31.41 embryos/brood.

Reproductive indicators of A. franciscana. The results showed that the total number of embryos in the treatments using *B. subtilis* was always higher than in the control treatment without *B. subtilis*, and that *A. franciscana* tended to give nauplii more than lay cysts. According to Toi & Van (2018), in their carbon-supplemented treatment, female *Artemia* tended to give birth to more nauplii than in the treatments not supplemented with carbon. The large number of nauplii in the carbon-supplemented treatments was due to the bacteria growing and using nitrogen sources in the water to reproduce, thereby improving the rearing environment (Hari et al 2006). Under favorable conditions, *Artemia* tend to give birth to nauplii (Van Stappen 1996). The use of *B. subtilis* with molasses affected females' time between spawnings and the number of embryos per brood. When using molasses to adjust the C:N ratio in the *Artemia* culture process with a salinity of 60‰ and a C:N ratio of 5:1, the nauplii and cyst reproduction of *Artemia* were always higher than at other C:N ratios (10:1; 15:1; 20:1) (Hoa et al 2022).

The results of Experiment 2 indicate that the addition of *B. subtilis* at 10^8 CFU mL^{-1} combined with a C:N ratio of 5:1 is optimal for raising *A. franciscana* at a salinity of 60‰.

Conclusions and recommendations. The present study evaluated the role of *Bacillus subtilis* bacteria isolated from the Vinh Chau salt fields on the life activities of *Artemia franciscana*. The results showed that a *B. subtilis* density of 10^8 CFU mL^{-1} with a C:N ratio of 5:1 was optimal for raising *A. franciscana* at a salinity of 60‰. The environmental factors were all within the appropriate range for the development of *A. franciscana* and the ability to control TAN, nitrite content, and *Vibrio*. The indicators recorded for *A. franciscana,* including survival rate, body length, reproductive time, female and male lifespans, total number of embryos, and fecundity, all reached their highest values, with the rate of live birth being higher than that of cyst laying. The results obtained provide a basis for the application of *B. subtilis* in *Artemia* farming, thereby contributing to an improved farming environment and increased productivity.

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References

Baumann P., Baumann L., Bang S. S., Woolkalis M. J., 1980 Evaluation of the taxonomy of *Vibrio*, *Beneckea*, and *Photobacterium*: abolition of the genus *Beneckea*. Current Microbiology 4:127-132.

Boyd C. E., 1998 Water quality for pond aquaculture. Research and Development Series, No. 43, 37 pp.

Briggs M. R. P., Funge-Smith S. J., 1994 A nutrient budget of some intensive marine ponds in Thailand. Aquaculture Fisheries Management 25(8):789-811.

Browne R. A., Wanigasekera G., 2000 Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. Journal of Experimental Marine Biology and Ecology 244(1):29-44.

Dung N. L., 1983 [Microbiology practice]. Hanoi University and Vocational High School Publishing House, X, 368 pp. [in Vietnamese]

Gomez-Gil B., Herrera-Vega M. A., Abreu-Grobois F. A., Roque A., 1998 Bioencapsulation of two different *Vibrio* species in nauplii of the brine shrimp (*Artemia franciscana*). Applied and Environmental Microbiology 64:2318-2322.

Han D. T. M., 2015 [Effects of different protein and lipid contents in feed on survival, growth and reproduction *of Artemia franciscana* Vinh Chau]. MSc thesis, Can Tho University, 62 pp. [in Vietnamese]

Hari B., Kurup B. M., Varghese J. T., Schrama J. W., Verdegem M. C. J., 2006 The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 252(2-4):248-263.
Hastings J. W., Nealson K. H., 1981 The symbiotic luminous bacteria. In: The Prokaryotes. Starr M. P., Stolp H., Truper H. G., Balows A., Schlegel H. G. (eds), Springer-Verlag, New York, pp. 1332-1345.
Hoa N. V., 1993 Effect of environmental conditions on the quantitative feed of the brine shrimp *A. franciscana* (Kellogg). MSc thesis, Ghent University, Ghent, Belgium, 58 pp.
Hoa N. V., Ba N. V., Phuong T. V., Van N. T. H., Thong L. V., Han D. T. M., 2022 [Research on the process of cultivating *Artemia* at low salinity using an improved nutritional regime in Vinh Chau Town, Soc Trang Province]. Soc Trang Provincial Science and Technology Project, 326 pp. [in Vietnamese]
Hoa N. V., Nam T. N. H., 2018 [Application of bio-floc technology for *Artemia* farming]. Agricultural Publishing House, 158 pp. [in Vietnamese]
Hoa N. V., Van N. T. H., 2018 [Principles of *Artemia* culture in salt fields]. Agricultural Publishing House, 205 pp. [in Vietnamese]
Hoa N. V., Van N. T. H., Anh N. T. H., Ngan P. T. T., Toi H. T., Le T. H., 2007 [*Artemia* research and application in aquaculture]. Agricultural Publishing House, 134 pp. [in Vietnamese]
Kamilya D., Devi W. M., 2022 *Bacillus* probiotics and bioremediation: an aquaculture perspective. In: Bacilli in agrobiotechnology: plant stress tolerance, bioremediation, and bioprospecting. Islam M. T., Rahman M., Pandey P. (eds), Springer Cham, pp. 335-347.
Lavens P., Sorgeloos P., 1996 Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper No. 361, FAO, Rome, 295 pp.
Mahdhi A., Harbi B., Esteban M. A., Chaieb K., Kamoun F., Bakhrouf A., 2010 Using mixture design to construct consortia of potential probiotic *Bacillus* strains to protect gnotobiotic *Artemia* against pathogenic *Vibrio*. Biocontrol Science and Technology 20(9):983-996.
Mahdhi A., Kamoun F., Bakhrouf, A., 2011 Inhibitory activity and adhesive ability of potential probiotic *Bacillus* species to confer protection for *Artemia* gnotobiotic culture against pathogenic *Vibrio* spp. Turkish Journal of Veterinary and Animal Sciences 35(4):227-233.
Moriarty D. J. W., 1998 Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164(1-4):351-358.
Ngan P. T. T., Ngoc T. S., 2014 [Effect of selected *Bacillus* sp. mixture on growth of *Artemia franciscana*]. Can Tho University Journal of Science. Special Issue: Aquaculture 2014(2):184-191. [in Vietnamese]
Ngan P. T. T., Phu T. Q., Hai V. H., Tu P. T. C., Toi H. T., Giang H. T., Ut V.N., 2021 [The importance and application of probiotics in aquaculture in the Mekong Delta]. Project VN14-P6 JICA, Agricultural Publishing House, 150 pp. [in Vietnamese]
Rengpipat S., Rukpratanporn S., 1998 Probiotics in aquaculture: a case study of probiotics for larvae of the black tiger shrimp (*Penaeus monodon*). In: Book of Abstracts of the Fifth Asian Fisheries Forum - International Conference on Fisheries and Food Security Beyond the Year 2000. Asian Fisheries Society, Chiang Mai, Thailand, 193 pp.
Soltani M., Ghosh K., Hoseinifar S. H., Kumar V., Lymbery A. J., Roy S., Ringø E., 2019 Genus *Bacillus*, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. Reviews in Fisheries Science and Aquaculture 27(3):331-379.
Sonnenschein A. L., Losick R., Hoch J. A., 1993 *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology and molecular genetics. American Society for Microbiology, Washington, DC, 987 pp.
Tam L. T., Hai V. H., Hoa N. V., Giang H. T., 2025 [Isolation and selection of native *Bacillus* spp. strains capable of decomposing organic matter from *Artemia* ponds]. Vietnam Journal of Agricultural Science 23:1364-1373. [In Vietnamese].

Te B. Q., 2003 [Diseases of cultured shrimp and prevention and treatment measures]. Agricultural Publishing House, Hanoi, 112 pp. [in Vietnamese]
Thao N. T. T., Tam M. L., 2013 [Effects of glucose and probiotic supplementation on growth and reproduction of *Artemia franciscana*]. Can Tho University Journal of Science. Part B: Agriculture, Aquaculture and Biotechnology 29:96-103. [In Vietnamese].
Thao N. T. T., Ngoan N. T., 2014 [Effects of biological product supplementation methods on growth and reproduction of *Artemia franciscana* Vinh Chau]. Can Tho University Journal of Science. Part B: Agriculture, Aquaculture and Biotechnology 32:94-99. [in Vietnamese]
Thao N. T. T., Ngan P. T. T., Trang N. T. B., 2015 [Combined effects of salinity and *Bacillus subtilis* supplementation on the growth and reproduction of *Artemia franciscana*]. Can Tho University Journal of Science. Part B: Agriculture, Aquaculture and Biotechnology 39:118-127. [in Vietnamese]
Thao N. T. T., Diem V. T. K., Phuong N. T., Ngan P. T. T., 2016 [Growth and influence of *Bacillus amyloliquefaciens* in *Artemia* culture environment at high salinity]. Can Tho University Journal of Science. Part B: Agriculture, Aquaculture and Biotechnology 45:101-111. [in Vietnamese]
Toi H. T., Van N. T. H., 2018 [Effects of C/N ratio and diet on growth and biomass yield *of Artemia franciscana* under laboratory conditions]. Can Tho University Journal of Science. Part B: Agriculture, Aquaculture and Biotechnology 54:90-97. [in Vietnamese]
Toi H. T., Boeckx P., Sorgeloos P., Bossier P., Van Stappen G., 2013 Bacteria contribute to *Artemia* nutrition in algae-limited conditions: a laboratory study. Aquaculture 388-391:1-7.
Van Stappen G., 1996 Introduction, biology and ecology of *Artemia*. In: Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper No. 361, Lavens P., Sorgeloos P. (eds), FAO, Rome, pp. 101-170.
Van N. T. H., Han D. T. M., Hoa N. V., 2011 [Effects of salinity on growth and reproduction of two *Artemia strains* San Francisco Bay (SFB_VC) and Great Salt Lake (GSL)]. In: [Proceedings of the 4th Aquaculture Science Conference], January 26, 2011, Can Tho, Can Tho University Publishing House, pp. 126-136. [in Vietnamese]
Verschuere L., Rombaut G., Huys G., Dhont J., Sorgeloos P., Verstraete W., 1999 Microbial control of the culture of *Artemia* juveniles through pre-emptive colonization by selected bacterial strains. Applied and Environmental Microbiology 65(6):2527-2533.
Wurts W. A., Durborow R. M., 1992 Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. Southern Regional Aquaculture Center Publication No. 464, 4 pp.

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