Int Aquat Res (2024) 16:257–269
https://doi.org/10.22034/iar.2024.1999915.1541

ORIGINAL RESEARCH

Evaluation of hatching mixed probiotics effects on water quality, larval development, thermal, hyposalinity shocks of kuruma shrimp, *Penaeus japonicus* (Bate 1888)

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Received: 27 October 2023 / Accepted: 01 September 2024 / Published online: 10 September 2024

Abstract This research aimed to investigate how different doses of hatchery probiotic affected water quality, thermal shock, hyposalinity stress tolerance, growth, survival, and metamorphosis rate of kuruma shrimp (*Penaeus japonicus*) larvae. The probiotic was added to the water in three concentrations: 2 (T1), 3 (T2), and 4 (T3) gm/m3 respectively, as well as a control group (T0) (without probiotic); then the diets were fed to triplicate groups of shrimps. Obtained results pointed out that the probiotic treatments significantly improved growth, metamorphosis, and survival rate of the various shrimp larval stages. Furthermore, there was a favorable effect on shrimp larvae tolerance to temperature shock and hyposalinity stress. Probiotics reduced the quantities of ammonia and nitrite in water while increasing the pH level. Therefore, application of probiotic had beneficial effects on the kuruma shrimp larviculture as well as the water quality parameters.

Keywords Growth . Hyposalinity shock . Metamorphosis rate . *Penaeus japonicus* . Probiotic survival rate . Thermal shock resistance . Water quality

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Introduction

Aquaculture industry has grown in recent decades, causing environmental pollution, and decreasing production of many cultured organisms. Several countries are experiencing massive losses in aquaculture production, primarily as a result of microbial diseases (viruses or bacteria) (Hendam et al. 2023). Furthermore, non-methodological management practices such as overfeeding, high stocking densities, unsuitable fishing practices, and water pollution increase the likelihood of disease outbreaks in aquaculture industries (Eissa et al. 2023; Abdelmeguid et al. 2024). Antibiotics are commonly used in fish farming sectors to prevent pathogens, adverse environmental conditions, and disease outbreaks (Ghori et al. 2022). However, long-term antibiotic use is not a safe option due to the emergence of antibiotic-resistant bacteria, which have a negative impact on the aquaculture sector as well as the environment and consumers (Eissa et al. 2023). Consequently, more attention was paid to the use of probiotics as a cost-effective and environmentally friendly bioremediation alternative to aid in the development of sustainable aquaculture (Zibiene and Zibas 2019; Eissa et al. 2024). They emerged as a suitable substitute for synthetic chemicals (e.g., antibiotics), which are famous for their harmful effect on microflora, especially the natural beneficial bacteria (Mamun et al. 2019).

Administration of probiotics in the environments of aquatic culture helps in pathogens' control, enhance the response to feed intake, tolerance to environmental stress, survival, disease resistance, activities of digestive enzymes, physiological conditions, and growth rate, while their intake decreases production costs of farmed species and improving water quality (Bachruddin et al. 2018; Wanna et al. 2021; Dighiesh et al. 2024). Moreover, probiotics modulate gut microbiota, and improve the spawning and hatching rate without any side effect internally (Huynh et al. 2017) in aquaculture systems (Eissa et al. 2022a,b). Using probiotics is essential to increase performance of of the cultured aquatic animals with no adverse effects on the end user (Muhammadar et al. 2018). Successful aquaculture production required suitable indices of water quality (Radwan et al. 2023). Therefore, there is a growing interest in the probiotics' practice to enhance water quality by creating a balanced microflora in water and decreasing the load of pathogenic bacteria (Sunitha and Krishna 2016). This also involves manipulation of the water microbes to improve organic matter mineralization and removal of waste compounds that are undesirable (Adorian et al. 2019) by decomposition or direct uptake of the toxic material or organic matter from the water (Aalimahmoudi and Bavarsad 2015). Much research has illustrated that using probiotics can be effective for different fishes (Pasala et al. 2018; Tabassum et al. 2021) and shrimp (Garcia-Bernal et al. 2018; Lante et al. 2021).

The kuruma shrimp, *Penaeus japonicus* (Bate 1888) is widely distributed in many countries. Despite the increasing development in the shrimp's global aquaculture, the culture sector faced different problems that caused economic losses (Seibert and Pinto 2012) due to pollution and diseases which led to the shrimp mortality in hatcheries, especially at the early stages of larval development (Kumar et al. 2016). The administration of probiotics has been used efficiently in many countries to control many disorders of shrimp and other aquatic animals (Newaj-Fyzul et al. 2014). Therefore, the overall goal of most studies was to set up and estimate new techniques to improve growth performance and to increase the productivity of shrimp, in particular the larval stages, through modulating their water ecosystem or nutritional regimes. Nevertheless, the application of probiotics in the aquaculture sector is still a new approach as few studies discussed their effect on shrimp developmental stages and larvicultural practices. Hence, the objective of the present study was to evaluate the influence of a commercial hatchery probiotic AquaStar® on the water quality, thermal shock resistance, hyposalinity stress resistance, body length, survival rate, and the speed of metamorphosis of the larvae of the kuruma shrimp (*Penaeus japonicus*).

Materials and methods

The current investigation was executed at the Mariculture Research Center (MRC), Faculty of Environmental Agricultural Sciences, Suez Canal University, Arish, North Sinai Governorate, Egypt.

Broodstock and experimental shrimp larval stages

P. japonicus broodstock was collected from Abo-Qir Port, Alexandria, Egypt. The broodstock was transported and spawned in MRC. Different shrimp larval stages, including nauplius (N_1-N_6), zoea (Z_1-Z_3),

mysis (M_1-M_3), and post-larvae (PL_1- PL_{15}), were stocked in experimental vessels in triplicates at a density of 280 larvae/triplicate (840 larvae /treatment). During the experimental period, larvae were fed with microalgae, rotifers, *Artemia* sp. and artificial feed until the PL_{15} stage (Table 1).

Experimental design

Water was sterilized with chlorine for 24 hours, then filtered before the beginning of the experiment. The experimental plastic vessels (7 Liters) were filled up with sterilized water (5 Liters). They were maintained under constant aeration by using air blowers. The commercial probiotic AquaStar® Hatchery (BIOMIN) was applied into the water in triplicates with three different concentrations; 2 (T_1), 3 (T_2), and 4 (T_3) gm/m^3, in addition to the control group (T_0) (without probiotic). The probiotic used in this study contained spores of *Bacillus subtilis, Enterococcus faecium, Pediococcus acidilactici, and Lactobacillus reuteri*. The total concentration of bacteria in T_1, T_2 and T_3 was 2 x 10^9 CFU g^{-1}, 3 x 10^9 CFU g^{-1} and 4 x 10^9 CFU g^{-1}, respectively.

Water quality parameters

Water temperature and dissolved oxygen (DO) were recorded twice/day (06:00 h and 16:00 h) using a thermometer and an oxygen meter, respectively. While other water quality parameters were recorded daily during the experimental period. The temperature (°C) was maintained at 26 ± 1°C, salinity was 33–35 ppt, turbidity was 25 ± 1 NTU, and the total bacterial count was NIL cell/ml. Water was free of chlorine residual (NIL mg/l Cl_2), and kept with continuous aeration to maintain the DO at 5.8–6.3 mg/l. The pH was measured daily using a pH meter. In addition, the NH_4-N (ammonium nitrogen) and NO_2-N (nitrite) were measured according to APHA (2000).

Measurements of survival rate and growth assays

After hatching, the larvae were counted immediately at stage N_1 (nauplius), and 280 larvae were placed in

Table 1 Live food program of kuruma shrimp (*Penaeus japonicus*) larvae reared in different probiotic levels was prepared by Eissa et al. (2021)

Day	Larval Stages	Food type				
		Live food				Artificial feeding g/m^3**
		Algae (1000 cells /ml)		Rotifer(Animal /ml)	*Artemia* sp. (Animal /ml)	
		Diatoms	*Tetraselmis* sp.			
1	N1	-	-	-	-	-
2	N6	-	-	-	-	-
3	Z1	30	-	-	-	-
4	Z2	50	5	-	-	-
5	Z3	80	10	5	0.5	-
6	M1	100	10	10	0.5	-
7	M2	100	5	5	1	-
8	M3	100	2	-	1	-
9	PL1	50	2	-	2	0.2
10	PL2	30	1	-	2	0.3
11	PL3	20	-	-	3	0.3
12	PL4	15	-	-	3	0.4
13	PL5	15	-	-	4	0.5
14	PL6	10	-	-	4	0.5
15	PL7	5	-	-	5	0.7
16	PL8	3	-	-	5	0.7
17	PL9	3	-	-	6	0.8
18	PL10	2	-	-	7	1.0
19	PL11	2	-	-	8	1.0
20	PL12	-	-	-	7	1.5
21	PL13	-	-	-	6	1.5
22	PL14	-	-	-	5	2.0
23	PL15	-	-	-	4	2.0

* N= Nauplius, Z= Zoea, M = Mysis, and PL= Postlarvae.
** INVE Aquaculture Company.

each experimental vessel. At the end of the experiment, the survival rate percentage was calculated from nauplius to postlarvae$_{15}$ for each treatment according to the following equation:

Survival rate % = (Final number of larvae / initial number of larvae) x100

The increase in body length was recorded for each experimental treatment when all larvae reached the end of each larval stage. Samples of 5 larvae per experimental vessel were selected randomly and measured individually to determine the length (mm) by an electronic microscope. In addition, samples were collected continuously and examined to follow the rate of larval development in the different treatments (the speed of metamorphosis through the growth stages) and to calculate the duration of each stage until reaching the PL_{15} stage.

Survival rate by imposing thermal and saline shock

Thermal shock

At the end of the experimental period, 20 postlarvae were collected from each treatment then transferred simultaneously from the rearing vessels that had a temperature of 26 ± 1°C to 1 L aquariums containing saltwater at 13°C under constant aeration. They were kept in these conditions for one hour to induce a thermal shock response. Then, they were concurrently moved back to the experimental vessels, and the larval survival was monitored for 48hrs after thermal shock (Pontinha et al. 2018). It is worth mentioning that during the trial of thermal shock, the larvae did not feed. The saltwater used during the thermal shock was similar to that used in the hatching and rearing phases maintaining the same salinity (33–35 ppt).

Salinity shock

The hyposalinity stress resistance was monitored by transferring 50 individuals of PL_{15} from each treatment in triplicates with salinity corresponding to 35 ppt to 1 L freshwater (3 ppt) provided with gentle aeration for 30 min. Then, they were back to 35 ppt seawater for another 30 min. Mortality and survival rates were calculated every 15 min until the test ended. The freshwater was prepared before the test to assure evaporation of any potential chlorine residue from the water before shrimp transfer for two days. Postlarvae were not fed during the salinity stress test.

Statistical Analysis

All parameters were expressed as means ± standard errors. The statistical analysis was carried out using the one-way analysis of variance (ANOVA) to determine whether there are any significant differences between the means of the various measured parameters using the SPSS software (version 25). Data were expressed as mean and standard errors. All data were analyzed using one-way ANOVA and differences between means were tested using at $p < 0.05$ according to Duncan test, using SPSS software (version 25).

Results

Assessment of water quality parameters

The differences in NH_4 concentrations between the control and the other treatments were illustrated in Table (2). There was a significant decrease in NH_4 concentration ($p<0.001$) in T_1, T_2 and T_3 (0.0259-0.1838, 0.0222-0.1582, and 0.0190-0.1377 mgL^{-1}) respectively, compared to the control group (0.0336 -0.2174 mg/l) in the different larval stages from Z_1 to PL_{15} of kuruma shrimp. Nitrite exhibited the same pattern in its variations (Table 3). The control (T_0) recorded the highest values that ranged from 0.0069 to 0.0207 mgL^{-1}, and the minimum values were recorded in T_3 and varied from 0.0032 to 0.0105 mgL^{-1} for the different larval stages. There was a significant variation in nitrite concentration among the probiotic treatments ($p< 0.001$).

There is a slight increase in pH mean values in T_0, T_1, T_2, and T_3, respectively. However, these values fall

within the optimum range (Table 4). The maximum mean values of the pH measurements were detected in T3 (7.53±0.037 to 7.86±0.015) from Z_1 to PL_{15} stages of kuruma shrimp and were significantly different ($p< 0.001$).

Table 2 The effect of using probiotic on NH_4–N concentrations (mg/l) (means ± standard errors) of the larval rearing water of the kuruma shrimp (*Penaeus japonicus*)

Stages	Treatments			
	T0	T1	T2	T3
		Zoea		
Z1	0.0336±0.00018 [a]	0.0259±0.0003 [b]	0.0222±0.00029 [c]	0.0190±0.00012 [d]
Z2	0.0343±0.00024 [a]	0.0270±0.0003 [b]	0.0229±0.0001[c]	0.0193±0.0002[d]
Z3	0.0360±0.0003 [a]	0.0286±0.0002[b]	0.0237±0.0001 [c]	0.0209±0.00008 [d]
		Mysis		
M1	0.0364±0.0004[a]	0.0313±0.0002 [b]	0.0244±0.00015 [c]	0.0224±0.0002[d]
M2	0.0373±0.0005 [a]	0.0315±0.0002 [b]	0.0253±0.0003[c]	0.0252±0.00028[c]
M3	0.0395±0.0012 [a]	0.0316±0.0003[b]	0.0275±0.0014[c]	0.0290±0.00023[c]
		Postlarvae		
PL1	0.0461±0.0011 [a]	0.0324±0.0009 [b]	0.0295±0.0004[c]	0.0297±0.00035 [c]
PL5	0.0837±0.001 [a]	0.0703±0.0008[b]	0.0640±0.0005 [c]	0.0592±0.0007 [d]
PL10	0.1383±0.0007 [a]	0.1175±0.004[b]	0.1054±0.003[c]	0.0936±0.0004 [d]
PL15	0.2174±0.0018 [a]	0.1838±0.0019 [b]	0.1582±0.0012 [c]	0.1377±0.002 [d]

Means within the same row with different superscript letters are significantly different at $P < 0.05$.
* Z= Zoea, M = Mysis, and PL= Postlarvae.

Table 3 The effect of using probiotic on NO_2 concentrations (mg/l) (means ± standard errors) of the larval rearing water of the kuruma shrimp (*Penaeus japonicus*)

Stages	Treatments			
	T0	T1	T2	T3
		Zoea		
Z1	0.0069±0.0007 [a]	0.0047±0.00013 [b]	0.0040±0.00015 [bc]	0.0032±0.0001[c]
Z2	0.0071±0.00068 [a]	0.0048±0.00003 [b]	0.0042±0.00012 [bc]	0.0034±0.00003[c]
Z3	0.0073±0.0005 [a]	0.0053±0.0001[b]	0.0043±0.00009[c]	0.0036±0.0001 [c]
		Mysis		
M1	0.0081±0.0008 [a]	0.0055±.00009[b]	0.0045±0.00008 [bc]	0.00397±0.00014 [c]
M2	0.0085±.0.001 [a]	0.0058±.00008[b]	0.0050±0.00015 [bc]	0.0041±0.0002 [c]
M3	0.0094±0.0011 [a]	0.0063±0.00012 [b]	0.0055±0.00014 [bc]	0.0043±0.00015 [c]
		Postlarvae		
PL1	0.0104±0.001 [a]	0.0074±0.00014 [b]	0.0068±0.0002 [bc]	0.0055±0.00011 [c]
PL5	0.0134±0.0005[a]	0.0106±0.001 [b]	0.0087±0.0004 [c]	0.0074±0.00036 [c]
PL10	0.0164±0.0006 [a]	0.0128±0.0005 [b]	0.0107±0.00048 [c]	0.0094±0.0003 [c]
PL15	0.0207±0.0004 [a]	0.0163±0.0007 [b]	0.0125±0.001[c]	0.0105±0.0006 [c]

Means within the same row with different superscript letters are significantly different at $P < 0.05$.
* Z= Zoea, M = Mysis, and PL= Postlarvae.

Table 4 The effect of using probiotic on the pH concentrations (means ± standard errors) of the larval rearing water of the kuruma shrimp (*Penaeus japonicus*)

Stages	Treatments			
	T0	T1	T2	T3
		Zoea		
Z1	7.50±0.015 [a]	7.50±0.01 [a]	7.52±0.01 [a]	7.53±0.02 [a]
Z2	7.53±0.017 [a]	7.55±0.015 [a]	7.56±0.006 [a]	7.56±0.015 [a]
Z3	7.56±0.015 [b]	7.58±0.02 [b]	7.62±0.02 [ab]	7.65±0.0153 [a]
		Mysis		
M1	7.59±0.02[b]	7.62±.0.015 [b]	7.63±0.012 [b]	7.69±0.02 [a]
M2	7.62±0.01 [b]	7.623±0.007 [b]	7.64±.0.017 [b]	7.70±0.015 [a]
M3	7.62±0.0153[c]	7.66±0.021[bc]	7.68±0.01[ab]	7.72±0.02 [a]
		Postlarvae		
PL1	7.70±0.012 [c]	7.73±0.015[bc]	7.76±0.02 [b]	7.82±0.01 [a]
PL5	7.683±0.009[d]	7.723±0.012[c]	7.77±0.011[b]	7.84±0.006 [a]
PL10	7.71±0.003[c]	7.733±0.007 [c]	7.78±0.005 [b]	7.85±0.02 [a]
PL15	7.72±0.008 [d]	7.78±0.003 [c]	7.81±0.006 [b]	7.86±0.008[a]

Means within the same row with different superscript letters are significantly different at $P < 0.05$.
* Z= Zoea, M = Mysis, and PL= Postlarvae

Survival rate and growth assays of shrimp larvae

The present study indicated that the average survival rates of larvae were 83.4, 86.4, 87.8, 75.2, 80.2 and 82.4% in T_1, T_2, and T_3, respectively, and 77.4 and 65.4% in the control group regarding PL_1 and PL_{15}, respectively (Table 5). Concerning T_1, T_2, and T_3, their larval survival rates were increased by 6, 9, and 10.4% in PL_1 and by 9.8, 14.8 and 17% in PL_{15} respectively, more than their corresponding in the untreated group (Figure 1).

Table 6 describes the impact of commercial probiotic AquaStar® on the growth performance of the

Table 5 The effect of using probiotic on the survival rate% (means ± standard errors) of the kuruma shrimp (*Penaeus japonicus*) larvae

Parameters	Treatments			
	T0	T1	T2	T3
N1-N6	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
PL1	77.4±1.54[c]	83.4±0.93 [b]	86.4±1.33 [ab]	87.8±1.16 [a]
PL15	65.4±2.01[c]	75.2±1.07[b]	80.2±0.917 [a]	82.4±1.47 [a]

Means within the same row with different superscript letters are significantly different at P < 0.05.
* N= Nauplius, Z= Zoea, M = Mysis, and PL= Postlarvae.

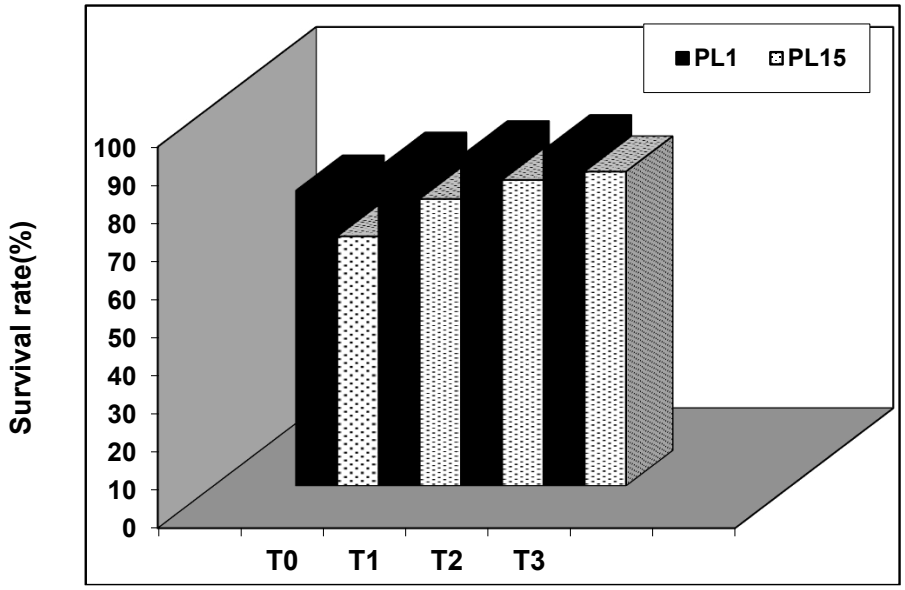

Fig. 1. Changes in the survival rate of the kuruma shrimp (*Penaeus japonicus*) larvae in the different treatments

Table 6 Effect of using probiotic on the body length (mm) (means ± standard errors) of different larval stages of the kuruma shrimp (*Penaeus japonicus)*

Stages	Treatments			
	T0	T1	T2	T3
N	0.32±0.00[a]	0.32±0.00[a]	0.32±0.00[a]	0.32±0.00[a]
		Zoea		
Z1	0.90±0.0058[a]	0.91±0.006[a]	0.91±0.01[a]	0.91±0.01[a]
Z2	1.58±0.01 [b]	1.61±0.0057 [a]	1.62±0.006 [a]	1.62±0.01[a]
Z3	2.25±0.006[b]	2.33±.015 [a]	2.33±0.01 [a]	2.34±0.0058 [a]
		Mysis		
M1	2.85±0.01 [c]	2.97±.0.006 [b]	3.15±0.01 [a]	3.18±0.01[a]
M2	3.35 ±0.01 [c]	3.55±0.02 [b]	3.77±0.006[a]	3.80±0.0058[a]
M3	4.15±0.01 [c]	4.45±0.0058 [b]	4.55±0.015 [a]	4.58±0.012 [a]
		Postlarvae		
PL1	4.88±0.006 [d]	5.09±0.011 [c]	5.15±0.015 [b]	5.20±0.01 [a]
PL5	5.88±0.043[d]	6.27±0.038 [c]	6.45±0.026 [b]	6.59±0.035 [a]
PL10	7.75±0.035[d]	8.21±0.04 [c]	8.40±0.043 [b]	8.57±0.038 [a]
PL15	10.03±0.104 [d]	10.61±0.043[c]	11.12±0.06[b]	11.70±0.08 [a]

Means within the same row with different superscript letters are significantly different at P < 0.05.
* N= Nauplius, Z= Zoea, M = Mysis, and PL= Postlarvae.

studied PL shrimp. The length of larvae (from Z_1 to PL_{15}) fluctuated between 0.32 to 11.70 mm and increased by 9.71, 10.29, 10.80 and 11.38 mm in T_0, T_1, T_2, and T_3, respectively (Table 6).

Differences in the metamorphosis rates of larval stages are shown in Table 7. There was a significant decrease in the metamorphosis rates ($p<0.001$, 0.01, 0.05) in T_1, T_2 and T_3 (45.15-23.40, 37.00-23.00, and35.00-22.45 h, respectively) compared to the control group (50.15±1.5 - 24.05±0.36 h). The data indicated that the highest rate was obtained in T_0 (11.5 days) and the lowest was detected in T_3 (9.4 days) among the different larval stages. The metamorphosis rate was decreased by 18.3% in T_3 compared with the control from N_1 to PL_1.

Status of survival rate by thermal and saline shock

The different concentration of commercial probiotic AquaStar® had a positive effect on larval survival performance against thermal shock. Results indicated that the mortality rate was higher in untreated or control group by applying thermal shock (Figure 2). 33% was found to be the lowest significant ($p<0.001$) mortality against the thermal shock observed in T3 (4 gm/m^3) followed by T2 (3 gm/m^3) and T1 (2 gm/m^3). The present study investigated the performance of survival rate or mortality in the four treatments including control against the saline shock. Figure 3. exhibited that there was no significant ($p<0.001$) difference in

Table 7 The effect of using probiotic on the metamorphosis rate (Hours) (means ± standard errors) of different larval stages of the kuruma shrimp (*Penaeus japonicus*)

Stages	Treatments			
	T0	T1	T2	T3
N	50.15±0.85 [a]	45.15±0.83[b]	37.00±0.33[c]	35.00±0.29 [c]
		Zoea		
Z1	44.15±0.93 [a]	39.00±1.28 [b]	34.45±0.29 [c]	34.00±0.3 [c]
Z2	43.05±1.54 [a]	39.10±1.11 [a]	33.10±0.9[b]	33.00±0.92 [b]
Z3	42.30±1.03 [a]	38.00±0.29[b]	32.00±0.72[c]	31.00±0.57 [c]
		Mysis		
M1	24.05±0.21 [a]	24.00±0.19 [ab]	23.30±0.12 [b]	23.30±0.115 [b]
M2	24.02±0.017 [a]	23.45±0.21 [b]	23.28±0.107 [b]	23.04±0.111 [b]
M3	24.00±0.185 [a]	23.20±0.25[ab]	23.00±0.01 [b]	23.00±0.33 [b]
		Postlarvae		
PL1	24.05±0.21 [a]	23.40±0.06[ab]	23.00±0.212 [b]	22.45±0.29 [b]

Means within the same row with different superscript letters are significantly different at $P < 0.05$.
* N= Nauplius, Z= Zoea, M = Mysis, and PL= Postlarvae.

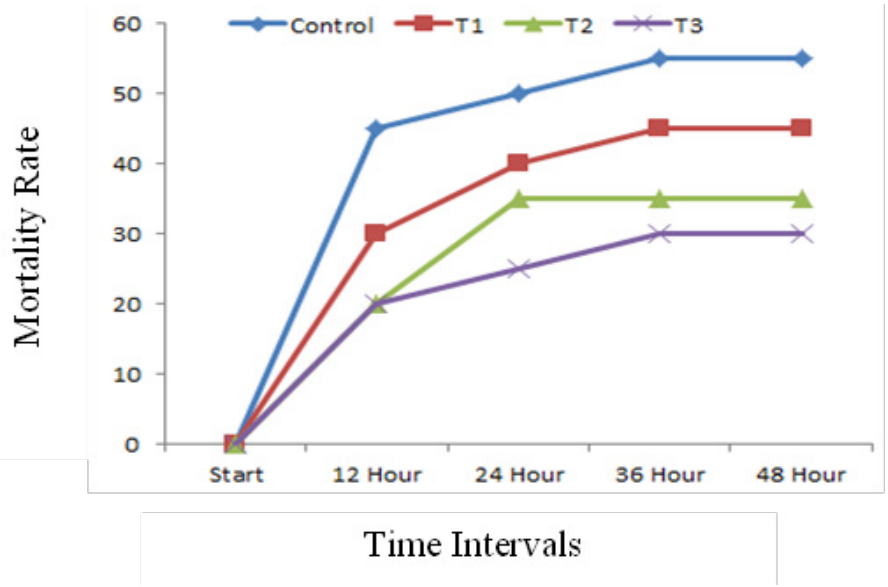

Fig. 2. Changes in the morality rate of the kuruma shrimp (*Penaeus japonicus*) postlarvae after thermal shock at different time intervals

survival performance against saline shock between the probiotic treatments of T3 (4 gm/m^3) and T2 (3 gm/m^3). However, the significant ($p<0.001$) difference was observed in T2 (2 gm/m^3) and control. After 30 min of exposure to fresh water, mortality of PL15 in the control group was higher than their corresponding in T1, T2, and T3. At the end of the test, about 2/3 of PL15 in the control group had died, whereas the mortality rate of PL15 in probiotic treatments was 46, 40, and 38.33 %, respectively.

Discussion

Ammonium (NH_4) and nitrite (NO_2) originate from the waste excretions and unconsumed food in aquatic systems. The increase in their concentration causes enormous economic loss (Su et al. 2016). They cause negative impacts on immunity, physiology, growth, and survival of animals (Jahangiri and Esteban 2018; Fadel et al.2024). Hence, they are critical parameters of water quality that must be kept at the optimal level (Padmavathi et al. 2012). The present study proved that the concentration of ammonia nitrogen, nitrite and pH were influenced during the rearing period. The reduction in ammonium and nitrite concentrations may be due to the presence of nitrifying bacteria in the used probiotic (Ghosh et al. 2016). These bacteria help in the conversion of ammonia to nitrite then to nitrate (Mamun et al. 2019). The present results are in accordance with the study of Nimrat et al. (2012) on shrimp larvae. Several studies reported that the nitrogenous wastes are mineralized through nitrification and/or denitrification leading to the reduction of ammonium and nitrite levels by the help of probiotics, which leads eventually to improvement of water quality (Xie et al. 2013).

The hydrogen ion concentration (pH) is an essential parameter in the aquatic environment since it affects the animal's metabolism and many other physiological processes. The accumulation of dead algae, residual feed and excreta changes the pH concentration. In the optimum pH range, ammonia is not affected, which maintains the highest values of growth performance and production in the culture (Ramanathan et al. 2005). At low pH, the nitrite toxicity increases (Kannupandi et al. 2002) causing growth retardation and a decrease in the capacity of oxygen uptake (Ciji and Akhtar 2020). While at high pH, water exchange may represent the best solution for the recovery of the appropriate pH levels (Boyd 2001). These results agreed with the findings of Hossain et al. (2013). The high values of pH may be attributed to the presence of the probiotic, which helped in the maintenance of the desired level of pH (Soundarapandian and Babu 2010). This was supported by Laloo et al. (2007) who reported that probiotics are used to clean water because they have a role in the conversion of organic matter to carbon dioxide (CO_2). Under the effect of the photosynthetic activity, CO_2 (whether free CO_2 or CO_2 bicarbonate) is consumed by algae. This leads to the reduction of CO_2 levels and the increase in levels of carbonate (Padmavathi et al. 2012). It is well known that the

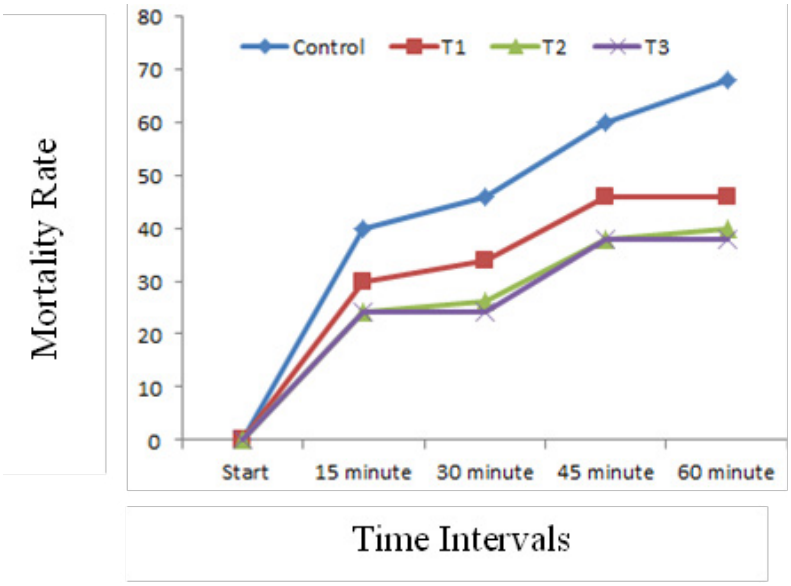

Fig. 3. Changes in the morality rate of the kuruma shrimp (*Penaeus japonicus*) postlarvae after salinity shock at different time intervals

increase in the pH of water is attributed to carbonates hydrolysis (Pasala et al. 2018).

Water parameters were found more suitable in the probiotic treatments than in the control group, which is harmonious with the work of Sunitha and Krishna (2016). Pacheco-Vega et al. (2018) reported that probiotics are considered as a new remediation technique for the improvement of water quality in the aquaculture sector. Moreover, Liu and Han (2004) explained that the direct administration of probiotics into the water has many benefits, including the ability to control water quality through pathogenic biocontrol and bio-cleaning. The parameters of water quality measured in the current investigation were within the acceptable range for the larval shrimp culture (Silva et al. 2012). Adequate water quality parameters are a vital factor for the preservation of the optimum growth performance and survival rate of the kuruma shrimp larvae (Aalimahmoudi and Bavarsad 2015).

The survival trend of the present study was $T_0<T_1<T_2< T_3$, meaning the different concentration of commercial probiotic AquaStar® had a positive effect of the survival performance. The obtained results were similar to the studies of Garcia-Bernal et al. (2018) and Toledo et al. (2019). The high survival rate found in the present study may be due to maintaining good water quality and reducing aquatic diseases by the use of probiotics (Hossain et al. 2013).

The survival rate of the present study exhibited the order: $T_0<T_1<T_2< T_3$, this means that different concentration of commercial probiotic AquaStar® had a positive effect of the survival performance. The obtained results were similar to the studies of Garcia-Bernal et al. (2018) and Toledo et al. (2019). The high survival rate found in the present study may be due to maintenance of good water quality and reducing aquatic diseases by using probiotics (Hossain et al. 2013).

The survival rate, body length, and metamorphosis rate were improved in the probiotic treatments (T_1, T_2, and T_3) compared with the control group (T_0). There were significant differences in all parameters among treatments and the control group in the different larval stages. These results coincided with the findings of (Bachruddin et al. 2018). The largest length was obtained in T_3, and the smallest was found in T_0 for the different larval stages, which confirmed the beneficial role of probiotics. The characteristics of these probiotics enable aquatic animals to enhance the efficiency of food assimilation and improve growth (Zibiene and Zibas 2019). Moreover, the increase in growth may be due to probiotics which contribute in the development of zooplankton (Sunitha and Padmavathi 2013) which in turn maximizes another form of nutrients leading to enrichment of food supply for the cultured organisms **(**Ludwig 1999).

Rate of Metamorphosis in the studied shrimp pointed out to the impact of probiotics on its larval development. Similar results were documented by Wang et al. (2020) and Eissa et al. (2022a,c). The probiotic enrichment technique has led to increased body length, metamorphosis rate, and survival rate (from zoea to postlarvae) in the shrimp larviculture. These findings coincided with the results of Nair (2016) and Sandhya et al. (2020). For most parameters, the best results were obtained in T_3 treatment with a concentration of 4 gm/m^3 of the commercial water AquaStar® Hatchery probiotic. The beneficial effect of probiotic may be due to the activity of its bacterial components comprising *Bacillus subtilis, Enterococcus faecium, Pediococcus acidilactici, and Lactobacillus reuteri*.

Previous studies reported that addition of the probiotic, particularly the *Bacillus* bacteria, can help in the detoxification of the harmful constituents of feed **(**Adeoye et al. 2016; Liu et al. 2017) enhances the inhibition of pathogenic bacteria (Huys et al. 2001) and secretes many exoenzymes, which play a vital role in the breakdown of many carbohydrates, proteins and lipids into smaller units (Ninawe and Selvin 2009). These processes may contribute to the improvement of digestion and the increase in food absorption, which in turn, enhance the growth, survival, and metamorphosis of the shrimp larvae (Wang et al. 2020; Jamali et al. 2015). It has been established that using probiotics in the early larval stages can modulate the microflora of the gut since enzyme levels are low and the digestive tract is not completely developed (Rahiman et al. 2010). In aquaculture, addition of probiotics to water could also improve and purify the farming environment (Ringo 2020).

From the results of the current investigation, it was noted that probiotic treatments had lower morality rates than the control one in PL_{15} stage after thermal shock. At the termination of the trial, the maximum value was recorded in the control group (55.0%), and the minimum values were obtained in T3 (30%) in PL_{15}, where the probiotic had positive impact on the resistance of the shrimp larvae to thermal shock. These findings disagreed with the study of Liu et al. (2010) in shrimp larvae and consistent with the study of Coelho et al. (2023).

The decrease in survival rate following thermal shock could be related to functional changes in the cell membranes, structural damage of proteins, and other biomolecules (Pruitt 1990). Furthermore, the thermal shock could cause hypoxia and re-oxygenation conditions in the animals by decreasing their metabolism and physiological activities during the cold conditions (13°C). The production of reactive oxygen species (ROS) is the result of these hypoxia and re-oxygenation conditions in the organism, which causes oxidative damage, cell weakness, and lipid peroxidation (Schleder et al. 2017). Shrimp resistance to thermal shock and the enhancement of its survival rate observed in the current work may depend on many physiological responses caused by addition of the applied probiotic. Tomanek (2008) reported that the aquatic animals' responses to thermal shock differ according to the environmental stability or the presence of several variables.

Environmental stresses are one of the most suitable methods to evaluate the quality and resistance of shrimp larvae in the Penaeoidea family **(**Racotta et al. 2004). In hyposaline (freshwater) stress, the low mortality rate in T3 group showed high tolerance compared to the other treatments. Our results were in agreement with the results of Louis et al. (2018) and Najmi et al. (2018). The improvement of survival rates and tolerance to fresh water in the probiotic treatments may be due to the effect of probiotics and the general improvement of the shrimp's health status **(**Najmi et al. 2018) or may attributed to the effective role of probiotic in the regulation of shrimp's physiological response which resulted in acclimation to the critical environmental stressors (Lignot et al. 2000).

The postlarvae of shrimp are exposed to many environmental stressors like variations in temperature and salinity during transport from hatcheries to shrimp farms. Hence, the use of the AquaStar® Hatchery probiotic throughout the farming practice is essential to help shrimp to tolerate such environmental stressors in the different rearing systems. In experiments of resistance against stress, probiotic enhanced larval resistance against stressors and increased their lifespan (Louis et al. 2018)

Conclusion

The present study demonstrated that inclusion of the commercial probiotic AquaStar® in water played a significant role in increasing the resistance of the *P. japonicus* larvae to thermal shock and salinity stress. Moreover, this probiotic enhanced larval growth performance and survival rate by maintaining suitable parameters of water quality (especially pH, ammonium and nitrite). Hence, addition of probiotics to the shrimp hatchery phases and their sustainable use throughout its culture is important to increase water purification and maximize larval development in aquaculture.

Funding Not Applicable

Ethics approval The experimental setup and fish handling procedure were approved by the Research Ethical Committee of Faculty of Fish Resources, Suez University, Egypt.

Author contributions All authors took part in the title suggestion, proposal development and writing processes. El-Sayed Hemdan Eissa, Moaheda E. H. Eissa, Nadia N.B. Abd El-Hamed and Hagar Sedeek Dighiesh carried out the lab works, data organization, and analysis. Sara F. Ghanem and Nadia N.B. Abd El-Hamed prepared the manuscript. Finally, the manuscript was reviewed by Mohammad Bodrul Munir, Ammar AL-Farga, Muhammad Nur Syafaat, and Zulhisyam Abdul Karia and subsequently some changes were made in response to their feedback. Furthermore, all authors are responsible for the accuracy and approval of the final manuscript.

Data availability The data of this article can be obtained upon request.

Competing interests The authors declare that there is no conflict of interest

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