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RESEARCH

Early-stage performance evaluation of Sanolife® MIC in enhancing shrimp larval development and reducing pathogenic *Vibrio* under hatchery conditions

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Abstract

Probiotics represent a sustainable alternative to antibiotics in shrimp aquaculture, potentially improving water quality, enhancing nutrient availability, and controlling pathogenic bacteria. Sanolife® MIC (S-MIC), a commercial probiotic formulation developed specifically for marine invertebrate culture, shows potential benefits, but its optimal dosage across different larval developmental phases remained undetermined. This 23-day study evaluated how various concentrations of Sanolife® MIC affect Pacific whiteleg shrimp, *Penaeus vannamei*, larval development, growth performance, and microbial composition from nauplii to postlarval stage 15 (PL-15). The experimental design tested three different S-MIC concentrations (1, 5, and 30 g/m^3/day) applied from nauplii to PL-15. Development parameters including growth rate, weight gain, morphological development, stress resistance, and microbial presence were evaluated. Results demonstrated that S-MIC supplementation at 1 g/m^3/day significantly accelerated larval development to PL-1 stage. The highest concentration (5 and 30 g/m^3/day) significantly improved larval weight at PL-6 compared to lower concentrations. Additionally, all S-MIC-treated groups exhibited significantly reduced *Vibrio* counts in both culture water and shrimp larvae. This study demonstrates that S-MIC supplementation provides concentration-specific benefits: 1 g/m^3/day for accelerated early development and 5–30 g/m^3/day for enhanced growth and robust *Vibrio* control in commercial shrimp hatcheries. These findings provide promising preliminary evidence for the

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Highlights
- Sanolife® MIC probiotic at 1 g/m^3/day significantly accelerates *Penaeus vannamei* development to postlarval stage 1, improving hatchery production efficiency.
- The highest probiotic concentration (30 g/m^3/day) significantly improves larval weight at PL-6 stage compared to lower concentrations, with 30 g/m^3/day promoting more uniform growth by PL-15.
- All tested probiotic concentrations significantly reduce *Vibrio* bacterial counts in both culture water and shrimp larvae, potentially decreasing disease risk in commercial hatcheries.
- Probiotic supplementation shows no adverse effects on survival rates, stress resistance, or hepatopancreas health, confirming its safety throughout larval development stages.

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strategic application of Sanolife® MIC in larval production while highlighting the need for further studies to elucidate underlying physiological mechanisms.

Keywords *Penaeus vannamei* · *Bacillus* probiotics · *Vibrio* control · Shrimp larviculture · Aquaculture disease prevention

Introduction

Probiotics have gained significant attention in aquaculture as sustainable alternatives to antibiotics for disease prevention and growth enhancement. The wide expansion of their usage lies especially in the capacity these live bacteria have for improving water quality by assimilating organic matter and reducing excess nutrients in the environment, thereby minimizing water pollution (Amenyogbe 2023). Additionally, probiotics help maintain a balanced and healthy microbial community in the gut (Ghosh 2023). Due to their potential for inhibiting the growth of pathogenic bacteria, probiotics may reduce the risk of infections in aquaculture systems (Knipe et al. 2021). Ultimately, they support better survival, growth rates, and overall well-being of shrimp (Mathan Muthu et al. 2024).

Especially during larval development, which is considered one of the most critical periods in the production cycle, shrimp have higher susceptibility to changes in environmental conditions, feed quality, and bacterial infections. These are strong causes of poor larval development, mass mortalities, and losses (Xue et al. 2016; Peñalosa-Martinell et al. 2020). Moreover, considering disease outbreaks caused by bacteria, vibriosis remains a constant threat to the shrimp production industry worldwide (Kumar et al. 2016; Abdel-Latif et al. 2022). In this context, and in view of all the key aspects mentioned above, probiotics have great potential to maintain both environmental and shrimp conditions at standards that promote optimal larval development.

A diversity of bacterial species has been used as probiotics for aquaculture (Van Hai 2015); however, *Bacillus* species possess specific characteristics which make them better probiotic candidates (Kuebutornye et al. 2019). For instance, they can produce antimicrobial compounds that are active against many microbes. Additionally, *Bacillus* species are proven to be non-toxic and non-pathogenic. The fact that they are spore-forming bacteria represents an advantage in terms of survival under different conditions and longer shelf-life (Abriouel et al. 2011; Kavitha et al. 2018).

Sanolife® MIC (S-MIC) (INVE Technologies) is a unique proprietary commercial probiotic formulation of three *Bacillus* strains (*B. subtilis*, *B. licheniformis*, and *B. pumilus*; min. 5×10^{10} cfu/g) designed specifically for marine invertebrate culture. Its application produces several benefits, such as gut colonization, inhibition of or competition with *Vibrios*, resulting in fewer potential pathogens, and enhanced stimulation of the animal's immune systems (Muñoz et al. 2014). While this latter study clearly demonstrated improved survival rates in Pacific whiteleg shrimp, *Penaeus vannamei*, from nauplii through PL-3, and showed that continued probiotic treatment through PL-15 increased both overall postlarvae production and animal size, further research is needed to determine the effectiveness of S-MIC at various concentrations during larval shrimp development. Supporting these findings, Silva et al. (2013) demonstrated that *Bacillus* species probiotics (Sanolife® MIC) enhanced survival rates and weight gain during early larval development (zoea and mysis phases) when applied through direct water supplementation. Their research showed that while probiotic treatment effectively

reduced pathogenic *Vibrio* bacterial loads in both culture water and shrimp tissue during larval stages, contributing to improved biosecurity, the effects on growth performance and survival were less pronounced in the postlarval phase.

This study, therefore, aimed to evaluate the effects of three different S-MIC concentrations on *P. vannamei*, larval development, focusing on growth parameters, health indicators, and microbial control from nauplii to postlarvae (PL) stage 15.

This study represents the first step in a phased evaluation of Sanolife® MIC across different stages of *P. vannamei* production. The present trial focused specifically on operational performance during the early larval phase, recognizing the constraints of working within commercial hatchery conditions.

Materials and methods

Experimental design

The experiment was conducted in twelve 300 L tanks ($73 \times 81 \times 55$ cm^3, salinity 30 ppt), each stocked with 50,000 nauplii (acquired from an undisclosed commercial supplier in Phuket province, Thailand). Four treatment groups were established with three replicates per treatment: (1) control (T1)—no probiotic addition; (2) treatment 2 (T2)—S-MIC at 1 g/m^3/day; (3) treatment 3 (T3)—S-MIC at 5 g/m^3/day; and (4) treatment 4 (T4)—S-MIC at 30 g/m^3/day. S-MIC was applied to the rearing water 1 day before stocking and maintained throughout the experimental period.

S-MIC at 1 g/m^3/day represents the manufacturer's recommended daily dosage for routine application following nauplii stocking. A concentration of 5 g/m^3/day is commonly employed within the industry during challenge conditions, particularly when managing *Vibrio* outbreaks. The inclusion of a third concentration at 30 g/m^3/day was designed to evaluate its efficacy based on anecdotal reports from commercial hatcheries in Latin America regarding its use under severe challenging conditions.

Water quality management

Water parameters were maintained within optimal ranges: temperature 28–30 °C, dissolved oxygen > 4 mg/L (YSI Pro20 Handheld for DO/Temp**,** USA), pH 7.8–8.3 (Para® test kits, Thailand), alkalinity 170–180 ppm (Para® test kits, Thailand), and salinity at 30 ppt (refractometer, Atago, Japan). Total ammonia nitrogen (TAN) thresholds were stage-specific: < 0.5 ppm for zoea, < 1 ppm for mysis stages, < 3 ppm for PL1-5, and < 8 ppm for PL6-15. Nitrite and nitrate levels were maintained below 0.5 ppm and 10 ppm, respectively. Water parameters were measured daily using the salicylate-hypochlorite method for TAN determination (Bower and Holm-Hansen 1980) and standard methods for nitrite and nitrate analysis (Strickland and Parsons 1972). Water was exchanged daily at different rates depending on the developmental stage of the shrimp; 10% water exchange at zoea 3, 20% water exchange during mysis 1 to PL6, 40% water exchange during PL7 to PL9, and 50% water exchange during PL10-PL15 (see Supplementary materials S1).

Table 1 Determination of growth performance parameters in *Penaeus vannamei* postlarval stages PL-1, PL-6, and PL-15 following treatment with Sanolife® MIC

PL stage		
PL-1	PL-6	PL-15
% PL-1 ($n=100$)	Weight (0.5 g dry weight)	Weight (1 g dry weight)
	Deformity ($n=50$)	% Survival rate
	Muscle/gut ratio ($n=100$)	Stress test ($n=100$)
		Hepatopancreas health ($n=50$)

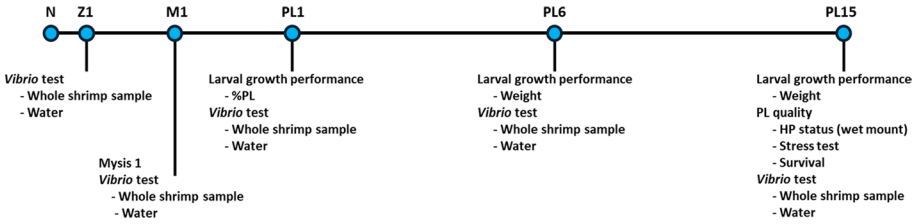


Fig. 1 Sampling protocol to determine the development of *Penaeus vannamei* larvae from nauplius through to postlarvae stage 15. Abbreviations: CV, coefficient of variation; HP, hepatopancreas; MGR, muscle-to-gut ratio; PL, postlarvae

Feeding protocol

Feed types and feeding schedules were adjusted throughout the 23-day study (i.e., zoea to PL15) according to developmental stages following standard commercial hatchery protocols (see Supplementary materials S2). All treatment groups received identical feeding regimens throughout the experiment.

Sample collection and analysis

Larval development was evaluated at PL-1, PL-6, and PL-15 stages (see Table 1 and Fig. 1). The percentage of larvae reaching PL-1 stage was recorded on days 9–11. Shrimp development was assessed at three critical postlarval time points: PL-1, PL-6, and PL-15. Postlarval stage 1 represents a key metamorphic transition from mysis 3, characterized by the development of functional pleopods and a shift from vertical swimming behavior to active forward swimming. PL-15 represents the standard commercial stage at which postlarvae are typically sold by hatcheries and stocked into growout ponds. PL-6 was selected as an intermediate assessment point based on rostral spine development, as larval shrimp are conventionally staged by the sequential appearance of rostral spines (approximately one spine every 3 days). When the second rostral spine is fully developed at PL-6, this stage therefore provides an appropriate intermediate time point for evaluating postlarval performance and development.

Larvae were classified as PL-1 based on morphological characteristics observed under a stereomicroscope at × 4 magnification, including active forward swimming behavior and

the development of the rostrum and pleopods. Weight measurements were conducted at PL-6 and PL-15 stages by recording wet weights of 0.5 g samples at PL-6 and 1 g samples at PL-15. These developmental stages were selected for weight analysis because they provide more accurate and reliable weight data compared to earlier larval stages.

- Morphological deformities were assessed by microscopic examination using a compound microscope (Olympus CX23) at × 4 and × 10 magnification. Observations included malformations of the rostrum, pleopods, or abdominal segment alignment and were recorded using a standardized scoring system adapted from Racotta et al. (2003).
- The muscle-to-gut ratio (MGR) was also determined microscopically using Pixit Pro software (Lanoptik Technologies, Guangzhou, China), based on measurements from 100 larvae per group (Racotta et al. 2003).
- Hepatopancreatic health was evaluated by examining oil droplet concentration under × 4 magnification, based on the count of visible lipid vacuoles in hepatopancreas squashes of PL-15 larvae.
- Stress tolerance, as a measure of PL quality, was assessed at the PL-15 stage using a salinity stress test, in which larvae were transferred from 30 ppt to freshwater (0 ppt) for 30 min. Survival rates are recorded as indicators of physiological robustness (Racotta et al. 2003).
- *Vibrio* populations were monitored using thiosulfate–citrate–bile salts–sucrose agar (TCBS) agar in both rearing water and shrimp larvae samples collected at zoea, mysis I, PL-1, PL-6, and PL-15 stages ($n = 3$ per replicate) using the methods detailed in Vandenberghe et al. (1999). The inoculated TCBS plates were incubated at 30 °C for 16 h before counting total *Vibrio* colonies.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS software. The normality (Shapiro–Wilk test) and homogeneity (Levene's test) of variance were assessed ($p > 0.05$) prior to conducting ANOVA. Tukey's range test was applied for multiple comparisons between treatment means, with significance established at $p \leq 0.05$.

Ethics statement

This experiment was approved by the Animal Ethics Committee of Rajamangala University of Technology Tawan-Ok, Thailand (RMUTTO-AUC-2–2025-004).

Results

Water quality parameters

All water quality parameters remained within acceptable ranges throughout the experimental period. Temperature, pH, dissolved oxygen, and alkalinity were maintained at optimal levels for larval development across all treatment groups. TAN, nitrite, and nitrate concentrations remained below threshold levels in all groups and stages, except for slightly elevated TAN during the mysis stage in the T4 group (30 g/m^3/day) (see Supplementary materials S3-S4).

Table 2 The number of *Penaeus vannamei* postlarvae PL stage 1 (%) during the supplementation with Sanolife® MIC in the rearing water. Superscript letters indicate significant differences at p-value < 0.05

Group	%PL-1 (mean ± SD)		
	DOC9	DOC10	DOC11
T1	11.00 ± 1.32^{a}	69.33 ± 1.53^{bc}	100.00 ± 0.00
T2	17.00 ± 0.87^{b}	74.00 ± 1.80^{c}	100.00 ± 0.00
T3	16.00 ± 3.04^{ab}	65.00 ± 3.91^{ab}	100.00 ± 0.00
T4	15.33 ± 2.47^{ab}	62.67 ± 2.25^{a}	100.00 ± 0.00
p-value	0.035	0.003	

Abbreviations: *DOC* day of culture

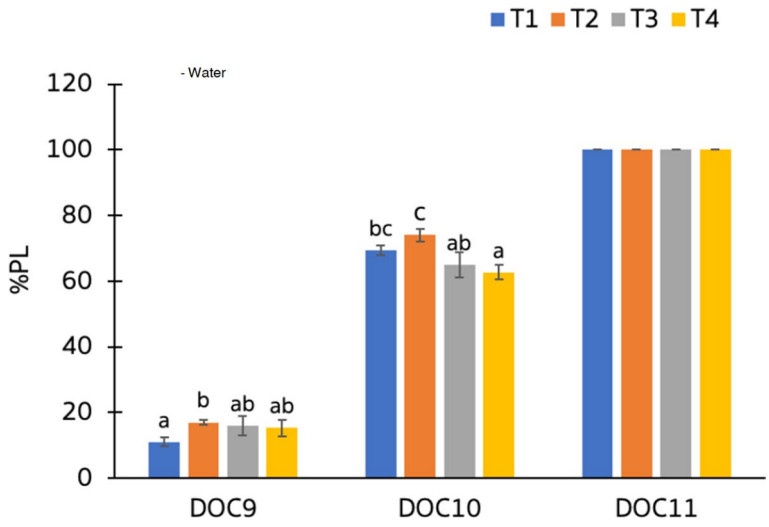


Fig. 2 Comparisons of the percentage of *Penaeus vannamei* postlarvae at the PL-1 stage (%PL-1) in rearing water supplemented with varying concentrations of Sanolife® MIC, compared to the control group without S-MIC supplementation. DOC, day of culture

Larval development

The progression to PL-1 stage was significantly enhanced by S-MIC supplementation (see Table 2 and Fig. 2). On day 9, the control group (T1) showed $11.00 \pm 1.32\%$ of larvae reaching PL-1, while S-MIC-treated groups demonstrated higher PL-1 percentages: $17.00 \pm 0.87\%$ in T2, $16.00 \pm 3.04\%$ in T3, and $15.33 \pm 2.47\%$ in T4. The difference was statistically significant between T1 and T2 ($p = 0.035$). This trend continued on day 10, with T2 maintaining significantly higher PL-1 percentages compared to T1. By day 11, all treatment groups had completed the transition to PL-1 stage.

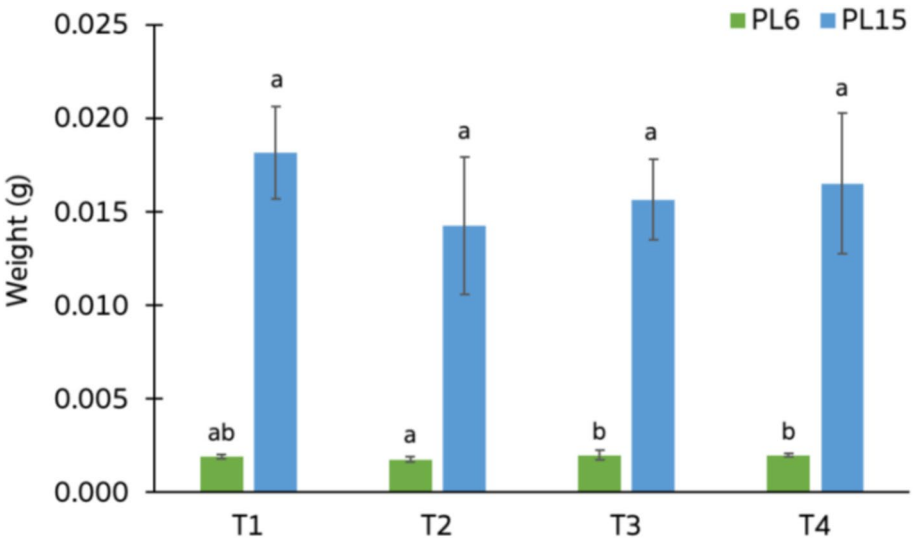


Fig. 3 Comparisons of the average weight of *Penaeus vannamei* postlarvae at the PL-6 and PL-15 stages in rearing water supplemented with varying concentrations of Sanolife® MIC, compared to the control group without S-MIC supplementation. T1, control (i.e., no probiotic addition); T2, S-MIC at 1 g/m^3/day; T3, S-MIC at 5 g/m^3/day; T4, S-MIC at 30 g/m^3/day

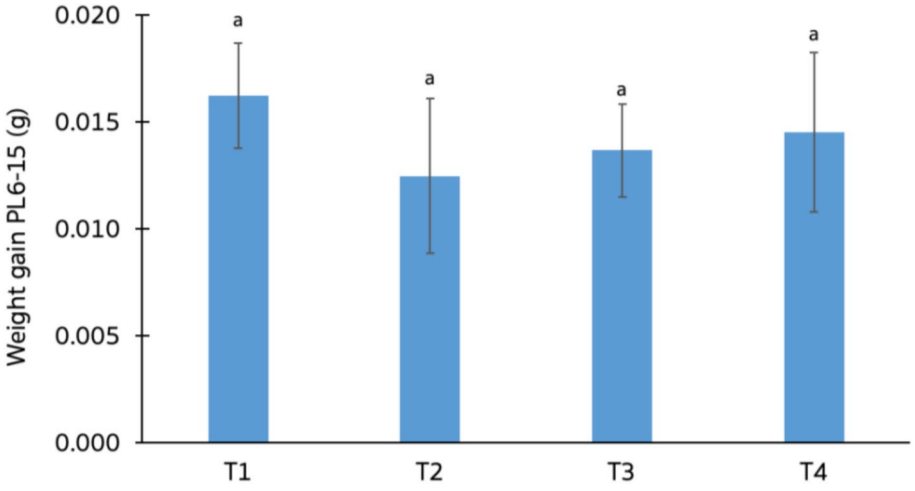


Fig. 4 Comparisons of the average weight gain at the *Penaeus vannamei* postlarval PL-15 stage in rearing water supplemented with varying concentrations of Sanolife® MIC, compared to the control group without S-MIC supplementation. T1, control (i.e., no probiotic addition); T2, S-MIC at 1 g/m^3/day; T3, S-MIC at 5 g/m^3/day; T4, S-MIC at 30 g/m^3/day

Growth performance

Regarding weight, there was no significant difference in the weight of PL-6 among S-MIC supplemented groups and the control group ($p > 0.05$). However, PL-6 larvae

Table 3 Summary of the average weight (g) of *Penaeus vannamei* postlarvae at the PL-6 and the mean weight gain from PL-6 to PL-15 stages of larval development. Superscript letters indicate significant differences at p-value < 0.05

	Weight	Weight gain
Group	(g; mean ± SD)	(g; mean ± SD)
	PL-6	PL-6–PL-15
T1	0.0019 ± 0.0001^{ab}	0.016 ± 0.002^{a}
T2	0.0018 ± 0.0001^{a}	0.012 ± 0.004^{a}
T3	0.0020 ± 0.0003^{b}	0.014 ± 0.002^{a}
T4	0.0020 ± 0.0001^{b}	0.015 ± 0.004^{a}
p-value	0.033	0.088

in the T4 group were significantly heavier than those in the T2 group, indicating a positive effect of higher S-MIC concentrations on early postlarval growth (Figs. 3 and 4). Although there was a significant difference in weight between groups at PL-15 ($p = 0.033$), no significant difference in weight gain was detected ($p = 0.088$) (see Table 3 and Fig. 4).

Morphological and physiological parameters

No deformities were observed in any treatment group at PL-6, indicating that S-MIC supplementation did not adversely affect morphological development. Similarly, no significant differences were detected in muscle-to-gut ratio among all groups, suggesting comparable digestive system development.

Survival rates at PL-15 ranged from 85.70 ± 3.94% in the control group to approximately 86–87% in S-MIC-treated groups, with no statistically significant differences between treatments. All groups exhibited high tolerance to salinity stress, with post-stress survival rates

Table 4 Summary of the survival rate and stress test results at the *Penaeus vannamei* postlarval PL-15 stage of development. Superscript letters indicate significant differences at p-value < 0.05

Group	Mean ± SD	
	Survival rate at PL-15 (%)	Survival rate after stress test (%)
T1	85.70 ± 3.94^{a}	98.00 ± 3.46^{a}
T2	86.00 ± 3.61^{a}	99.33 ± 1.00^{a}
T3	87.00 ± 4.36^{a}	99.33 ± 1.00^{a}
T4	86.67 ± 4.16^{a}	99.56 ± 0.88^{a}
p-value	0.978	0.314

Table 5 Summary of the oil droplet concentrations in the hepatopancreas at the *Penaeus vannamei* postlarval PL-15 stage of development. Superscript letters indicate significant differences at p-value < 0.05

Group	Oil droplet concentrations (%; mean ± SD)
T1	98.0 ± 1.73^{a}
T2	98.6 ± 1.51^{a}
T3	99.3 ± 0.70^{a}
T4	99.4 ± 0.53^{a}
p-value	0.514

Table 6 Summary of the total *Vibrio* count in the culture water during *Penaeus vannamei* larval development. Superscript letters indicate significant differences at p-value <0.05

Group	Total *Vibrio* count (CFU/mL; mean ± SD)				
	Zoea	M1	PL1	PL6	PL15
T1	$5.33 \pm 0.38 \times 10^{3\,a}$	$7.00 \pm 0.96 \times 10^{5\,a}$	$5.67 \pm 0.87 \times 10^{5\,a}$	$8.78 \pm 0.15 \times 10^{5\,a}$	$5.94 \pm 0.01 \times 10^{5\,a}$
T2	$3.62 \pm 0.38 \times 10^{3\,b}$	$5.55 \pm 0.22 \times 10^{5\,b}$	$2.78 \pm 0.48 \times 10^{5\,b}$	$5.00 \pm 0.76 \times 10^{5\,b}$	$2.78 \pm 0.19 \times 10^{5\,b}$
T3	$3.89 \pm 0.44 \times 10^{3\,b}$	$5.12 \pm 0.10 \times 10^{5\,b}$	$2.56 \pm 0.01 \times 10^{5\,b}$	$5.33 \pm 0.76 \times 10^{5\,b}$	$1.44 \pm 0.01 \times 10^{5\,c}$
T4	$3.88 \pm 0.27 \times 10^{3\,b}$	$5.46 \pm 0.17 \times 10^{5\,b}$	$2.56 \pm 0.84 \times 10^{5\,b}$	$5.33 \pm 0.67 \times 10^{5\,b}$	$1.28 \pm 0.01 \times 10^{5\,c}$
p-value	0.002	0.008	0.001	0.001	0.001

Table 7 Summary of the total *Vibrio* count in *Penaeus vannamei* larvae during their development. Superscript letters indicate significant differences at p-value <0.05

Group	Total *Vibrio* count (CFU/g; mean ± SD)				
	Zoea	M1	PL1	PL6	PL15
T1	$1.02 \pm 0.01 \times 10^{5\,a}$	$1.27 \pm 0.01 \times 10^{5\,a}$	$3.02 \pm 0.25 \times 10^{4\,a}$	$2.43 \pm 0.10 \times 10^{4\,a}$	$5.17 \pm 0.33 \times 10^{3\,a}$
T2	$3.36 \pm 0.48 \times 10^{4\,b}$	$8.56 \pm 0.26 \times 10^{4\,b}$	$1.12 \pm 0.18 \times 10^{4\,b}$	$8.67 \pm 0.44 \times 10^{3\,b}$	$1.44 \pm 0.26 \times 10^{3\,b}$
T3	$2.57 \pm 0.38 \times 10^{3\,b}$	$6.36 \pm 0.01 \times 10^{4\,b}$	$7.72 \pm 0.39 \times 10^{3\,bc}$	$9.11 \pm 0.75 \times 10^{3\,b}$	$1.72 \pm 0.19 \times 10^{3\,b}$
T4	$2.27 \pm 0.37 \times 10^{3\,b}$	$2.15 \pm 0.01 \times 10^{4\,c}$	$6.56 \pm 0.48 \times 10^{3\,c}$	$9.94 \pm 0.75 \times 10^{3\,b}$	$1.56 \pm 0.26 \times 10^{3\,b}$
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

between 98.0 and 99.5%, indicating that probiotic supplementation did not significantly impact stress resistance (Table 4).

Oil droplet concentrations in the hepatopancreas were similar across all treatment groups (98.0–99.4%), suggesting comparable nutritional status and metabolic processing regardless of probiotic supplementation (Table 5).

Microbial control

All S-MIC-supplemented groups exhibited lower total *Vibrio* counts in both culture water (Table 6) and larval samples (Table 7) compared to the control group across all developmental stages. The inhibitory effect on *Vibrio* counts was consistently observed in the culture water of all stages, particularly at the PL-15 stage, where S-MIC supplementation (T2-T4) resulted in *Vibrio* counts that were 2.1–4.6 times lower than those in the control (T1) ($p<0.05$). Similar reductions were observed in shrimp larvae across all stages. In larval samples, significant differences were detected at the mysis I and PL-1 stages. At mysis I, the T4 group showed

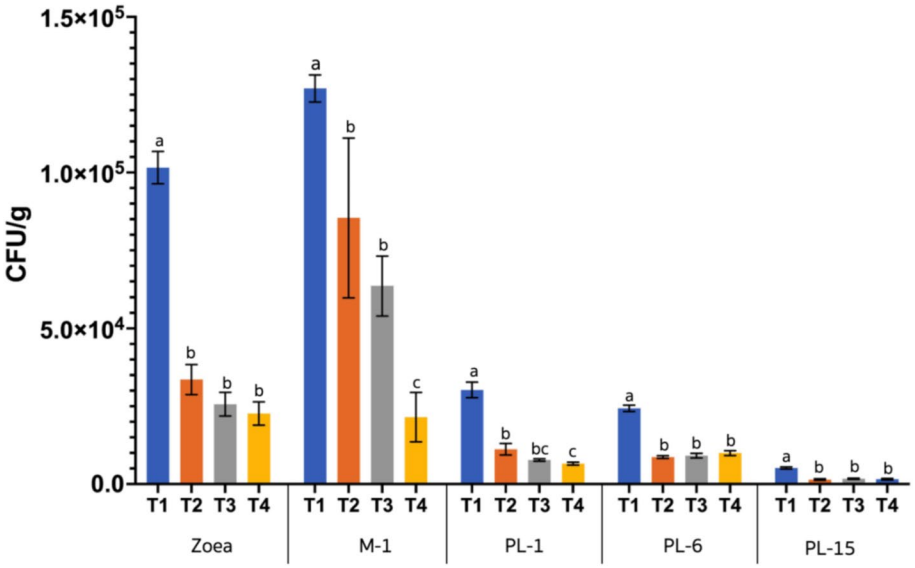


Fig. 5 Comparisons of total *Vibrio* counts in *Penaeus vannamei* larval shrimp reared in culture water supplemented with varying concentrations of Sanolife® MIC, compared to the control group without S-MIC supplementation, during larval development. T1, control (i.e., no probiotic addition); T2, S-MIC at 1 g/m^3/day; T3, S-MIC at 5 g/m^3/day; T4, S-MIC at 30 g/m^3/day

Table 8 Summary of all the parameters measured during the *Penaeus vannamei* larval development

Parameters	Results
% PL-1	On day 10, T2 showed a significantly higher percentage of PL1 stage, compared to T1
Weight at PL6 stage	Both T3 and T4 revealed significantly higher weights compared to T1
Survival rate (%)	No significant differences in survival rate were observed among the tested groups
Total *Vibrio* count (in water and shrimp)	S-MIC groups (T2–T4) showed a significantly lower total *Vibrio* count compared to the control group (without S-MIC)
Deformity	No significant differences in deformity were observed among the tested groups
Muscle/gut ratio	No significant differences in the muscle-to-gut ratio were found among the tested groups
Quantity of oil droplets in the hepatopancreas	No significant differences in the quantity of oil droplets in HP were observed

significantly lower *Vibrio* counts than T2 and T3, while at PL-1, both T3 and T4 demonstrated significantly lower counts compared to T1 (Table 7, Fig. 5).

Discussion

This study employed a comprehensive assessment approach, evaluating multiple interconnected parameters of larval health and development (Table 8) to provide a holistic understanding of S-MIC probiotic effects. The results demonstrated that S-MIC probiotic supplementation positively influences several aspects of shrimp larval development and health, with distinct dose-dependent effects across different developmental parameters. These findings provide important insights into optimal probiotic application strategies for commercial shrimp hatcheries.

The accelerated progression to PL-1 stage observed in the T2 group (1 g/m^3/day) suggests that even low concentrations of S-MIC can enhance early developmental transitions. This finding has significant practical implications for commercial hatcheries, as faster development to postlarval stages can improve production efficiency and reduce the time larvae spend in vulnerable early stages. The mechanism underlying this accelerated development may involve enhanced nutrient availability through probiotic-mediated improvements in water quality and microbial balance, as suggested by previous studies demonstrating that *Bacillus*-based probiotics can enhance digestive enzyme production and nutrient absorption (Ghosh 2023).

Beyond nutrient availability enhancement, well-documented mechanisms of *Bacillus*-based probiotics likely contributed to the observed developmental and microbial control benefits in this study. *Bacillus* species are known producers of diverse antimicrobial compounds, including bacteriocins, lipopeptides, and organic acids, which can directly inhibit pathogenic bacteria while promoting beneficial microbial communities (Zokaeifar et al. 2012; Simons et al. 2020). Additionally, *Bacillus* probiotics stimulate both innate and adaptive immune responses in crustaceans, enhancing phagocytic activity, phenoloxidase activity, and antimicrobial peptide production (Rengpipat et al. 2000; Verschuere et al. 2000). These immune-stimulating properties, combined with antimicrobial compound production, provide a comprehensive explanation for the consistent *Vibrio* count reductions observed across all treatment groups and developmental stages. The enhanced immune status may also contribute to the improved stress tolerance and overall larval quality observed in this study, as immunocompetent larvae are better equipped to handle environmental challenges and pathogenic encounters.

The growth performance results revealed interesting dose-dependent patterns that warrant detailed interpretation. At PL-6, larvae in the T4 group (30 g/m^3/day) were significantly heavier than those in the T2 group (1 g/m^3/day), but not significantly different from T3 (5 g/m^3/day) or the control group. This specific pattern suggests a threshold effect where moderate to high probiotic concentrations (5–30 g/m^3/day) provide growth benefits, while very low concentrations (1 g/m^3/day) may be insufficient to stimulate significant weight gain during early post-larval stages. The lack of significant differences between T4 and the control group, despite T4 showing higher mean weights, likely reflects the high variability typical in larval weight measurements and the relatively small sample sizes for weight determination.

Interestingly, these weight differences were not maintained through PL-15, suggesting that the growth advantage conferred by higher probiotic concentrations may be temporary or that compensatory growth occurs in non-treated groups over time. This pattern is consistent with findings from Silva et al. (2013), who observed that probiotic effects on growth performance were more pronounced during early larval stages (zoea and mysis phases) than in later post-larval phases. The early growth advantage, while temporary, may still

contribute to overall production efficiency by improving larval uniformity and reducing size variation within cohorts.

The comprehensive quality assessment parameters employed in this study represent industry-standard methodologies for evaluating postlarval fitness and commercial viability. The absence of morphological deformities across all treatment groups demonstrates that S-MIC supplementation does not adversely affect normal developmental processes, which is crucial for commercial application.

The muscle-to-gut ratio (MGR) analysis provides critical insights into postlarval nutritional status and development. MGR serves as a fundamental morphological indicator for postlarval quality assessment in commercial aquaculture, measuring the proportional relationship between ventral muscle width and intestinal tract width at the sixth abdominal segment. This parameter directly indicates nutritional status, muscle development, and overall health condition, providing predictive value for survival and growth performance in growout systems (Saurabh et al. 2006). The lack of significant differences in MGR among treatment groups suggests that while S-MIC supplementation enhances certain growth parameters, it does not fundamentally alter the balance between muscle and digestive system development. This finding indicates that probiotic supplementation maintains normal physiological proportions while potentially enhancing overall larval condition.

The assessment of lipid droplets within R-cells of the hepatopancreas provides valuable insights into postlarval nutritional status and metabolic health. High lipid droplet content correlates with improved survival rates and growth performance under growout conditions, making this assessment valuable for predicting aquaculture success (Cervellione et al. 2017). The similar oil droplet concentrations observed across all treatment groups (98.0–99.4%) suggest that S-MIC supplementation does not significantly alter lipid metabolism or energy storage patterns in the hepatopancreas. This consistency indicates that probiotic treatment maintains normal metabolic function while potentially providing benefits through other mechanisms.

The salinity stress testing results provide important insights into postlarval quality and commercial viability. This methodology has become the most widely adopted standard practice for evaluating postlarval quality, robustness, and pond-stocking fitness among commercial producers and hatcheries globally. The test involves transferring PL15 postlarvae from 30 ppt seawater to freshwater (0 ppt) for 30 min, with survival rates of 75% or higher indicating good postlarval quality (Samocha et al. 1998; Pattanavivat et al. 2006; Jory 2016).

All treatment groups exhibited exceptionally high stress tolerance, with post-stress survival rates between 98.0 and 99.5%, well above the industry standard of 80–100% for high-quality postlarvae. These results indicate that S-MIC supplementation does not compromise the physiological robustness of postlarvae under acute stress conditions. The predictive value of salinity stress testing for stocking success has been demonstrated through correlation analysis, where survival was positively correlated with postlarval length (Álvarez et al. 2004). The consistently high survival rates across all groups suggest that larvae from all treatments would be suitable for pond stocking, with probiotic supplementation providing additional benefits without compromising stress resistance.

Perhaps the most consistent and significant effect of S-MIC supplementation was the reduction in *Vibrio* counts in both culture water and larval samples across all developmental stages. This finding represents a critical advantage for commercial aquaculture, as *Vibrio* species are common opportunistic pathogens that pose constant threats to shrimp production worldwide (Kumar et al. 2016; Abdel-Latif et al. 2022). The dose-dependent reduction in *Vibrio* populations suggests that S-MIC effectively modulates the microbial

environment through multiple mechanisms, potentially including competitive exclusion, production of antimicrobial compounds, or enhancement of the host's immune response.

The particularly pronounced *Vibrio* reduction at the PL-15 stage, where S-MIC supplementation resulted in counts 2.1–4.6 times lower than the control, has important implications for disease prevention during the critical transition from hatchery to growout systems. This reduction in pathogenic bacterial load could significantly decrease the risk of vibriosis outbreaks, which remain a major cause of economic losses in shrimp aquaculture.

The substantial *Vibrio* reductions achieved through S-MIC supplementation have direct implications for managing critical diseases that plague shrimp hatcheries worldwide. Acute hepatopancreatic necrosis disease (AHPND), caused by *V. parahaemolyticus* strains carrying the pVA1 plasmid, has devastated shrimp production across Asia and Latin America, with mortality rates often exceeding 70% in affected farms (Tran et al. 2013; Shinn et al. 2018; Kumar et al. 2020). Translucent post larvae disease (TPD), caused by a *V. parahaemolyticus* strain carrying a thermolabile hemolysin gene, similarly causes devastating mortality rates of 80–100% within 24–48 h in *P. vannamei* shrimp post larvae and has emerged as a major threat to aquaculture operations in China and Vietnam since 2020 (Zou et al. 2020; Shinn et al. 2024). The 2.1–4.6 fold reduction in *Vibrio* counts observed at PL-15 stage could significantly reduce the probability of vibriosis (i.e., AHPND, TPD) transmission from hatcheries to growout facilities, as lower initial pathogen loads correlate with reduced disease incidence in production settings.

Similarly, classical vibriosis caused by *V. harveyi*, *V. alginolyticus*, and other *Vibrio* species remains a persistent challenge in hatchery operations, particularly during stressful periods such as metamorphosis and transportation (Austin & Zhang 2006; Flegel 2012; Intriago et al. 2024). The consistent *Vibrio* suppression achieved across all developmental stages suggests that S-MIC supplementation could serve as a preventive strategy, reducing the microbial reservoir of potential pathogens before larvae reach critical vulnerability windows. In production-scale applications, this prophylactic approach could reduce the reliance on therapeutic antibiotics, supporting sustainable aquaculture practices while maintaining biosecurity standards. The dose-dependent nature of *Vibrio* control also provides hatchery managers with flexibility to adjust probiotic concentrations based on historical disease pressure and risk assessment, allowing for targeted intervention strategies during high-risk periods or in facilities with recurring vibriosis challenges.

These findings complement research by Patil et al. (2021), who demonstrated that S-MIC application significantly alters gut microbial communities in *Penaeus indicus* larvae, with their 16S rRNA-based analysis revealing higher microbial richness in probiotic-treated groups. Interestingly, while our study showed reduced overall *Vibrio* counts using culture-dependent methods, Patil et al. observed a higher relative abundance of *Vibrio* in their probiotic-treated group using DNA sequencing techniques. This apparent contradiction may reflect methodological differences between culture-based and sequencing approaches, differences in species-specific responses (*P. vannamei* versus *P. indicus*), or changes in community structure where certain *Vibrio* species decline while others increase proportionally within the overall gut microbiome.

The results of this study support the implementation of S-MIC supplementation in commercial shrimp hatcheries, with optimal application strategies varying by production objective. For hatcheries prioritizing rapid larval development and production efficiency, the 1 g/m^3/day concentration appears most effective for accelerating progression to PL-1 stage. For operations focusing on early growth enhancement and microbial control, concentrations of 5–30 g/m^3/day provide additional benefits in terms of larval weight and *Vibrio* reduction.

The safety profile demonstrated across all tested concentrations is particularly important for commercial adoption, as it indicates that S-MIC supplementation can be implemented without risk of adverse effects on survival, stress resistance, or metabolic health. The consistent microbial control benefits across all concentrations provide additional value through reduced disease risk and improved biosecurity.

While this study demonstrated improvements in key production parameters, future research should investigate the underlying mechanisms of probiotic action, including immune response modulation, digestive enzyme activity, and detailed histological analysis. Additionally, economic analyses of probiotic supplementation in large-scale production settings would provide valuable insights into the cost–benefit relationships of different application strategies.

Conclusion

Sanolife® S-MIC probiotic supplementation demonstrates concentration-specific benefits for enhancing *P. vannamei* larval development. Lower concentrations (1 g/m^3/day) effectively accelerate development to PL-1 stage, improving production efficiency in commercial hatcheries. Higher concentrations (5–30 g/m^3/day) enhance early growth parameters at PL-6 stage, with the highest concentration (30 g/m^3/day) showing particular effectiveness for weight improvement. Most importantly, all tested concentrations (1–30 g/m^3/day) significantly reduce *Vibrio* populations in both culture water and larval samples, providing consistent microbial control benefits throughout larval development. These findings support the strategic use of S-MIC as an effective probiotic supplement in commercial shrimp hatcheries, with application strategies tailored to specific production objectives: 1 g/m^3/day for accelerated development and 5–30 g/m^3/day for enhanced growth and robust microbial control. The demonstrated safety profile across all tested concentrations, with no adverse effects on survival, stress resistance, or physiological health, confirms the suitability of S-MIC for routine hatchery operations. Future research should investigate the mechanisms underlying these concentration-specific effects and evaluate the economic benefits of targeted probiotic supplementation strategies in large-scale production settings.

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Author contribution Barbara Hostins conceived the study and designed the experimental trials; Siriwan Khidprasert, Chanadda Kasamechotchung, and Rungkarn Suebsing performed the experiments; Siriwan Khidprasert, Chanadda Kasamechotchung, Rungkarn Suebsing, and Kallaya Sritunyalucksana analyzed the data; Andrew P. Shinn, Ratchakorn Wongwaradechkul, Barbara Hostins, Olivier Decamp, Siriwan Khidprasert, and Chanadda Kasamechotchung drafted the manuscript; all authors critically revised the manuscript. [PJA1]LE: As per special instruction, full names should be used in presenting the "Author Contribution." Hence, the statement has been modified. Please check if correct; otherwise, kindly amend.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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