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ORIGINAL RESEARCH

Interaction between probiotic bacteria and endemic microalgae in Japanese oyster larvae (*Crassostrea gigas)*

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Abstract Oysters are among the most widely cultivated aquatic species due to their economic and ecological importance. However, hatchery production of Japanese oyster (*Crassostrea gigas*) faces critical challenges, particularly high larval mortality caused by opportunistic bacterial infections and suboptimal feeding practices. In recent years, microalgae and probiotics have emerged as promising tools to improve larval performance and disease resistance, but their combined application remains insufficiently explored. This study evaluated the effects of native microalgae and the probiotic strain *Lacticaseibacillus plantarum* 69Cr on the survival of oyster larvae under controlled and pathogen-challenged conditions. The best performance was observed in the co-culture treatment of *Schizochytrium sp*. and *Lpb. plantarum*, both at a concentration of 10^4 colony forming units per milliliter (CFU mL^{-1}), which resulted in a 37.5% survival rate in the absence of the pathogen and 18.75% under challenge with *Vibrio parahaemolyticus*. These findings indicate a positive synergistic interaction that enhances larval resistance and overall viability. However, more than 50% of larvae showed early digestive evacuation in treatments with *Schizochytrium sp.*, suggesting limitations in its digestibility during early larval stages. Despite this, the use of native probiotics and microalgae presents a sustainable alternative to reduce antibiotic use in hatchery systems. By incorporating functionally compatible microbial strains into feeding protocols, hatcheries may improve survival rates, reduce costs associated with auxiliary algal cultures, and minimize pathogen outbreaks. This study contributes novel insights into probiotic–microalgae interactions and supports the integration of locally adapted microbial tools in bivalve aquaculture as part of a broader strategy to enhance sustainability and productivity.

Keywords Effective dose . *Lpb. Plantarum* . *V. parahaemolyticus* . Microalgae

Introduction

Oysters lead global seafood production due to their wide distribution, rapid growth, and adaptability to diverse environmental conditions, making them a key component of aquaculture systems (FAO 2024). Among them, *Crassostrea gigas* has become the most widely cultivated species, supported by standardized hatchery protocols and strong market demand. Despite technological advances, hatcheries still face signif-

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icant bottlenecks during the larval stages, where mortality rates remain high due to opportunistic bacterial infections and inadequate nutritional regimes (Dubert et al. 2017; Takyi et al. 2023). In this context, recent strategies have focused on enhancing larval health and performance through the application of beneficial bacteria (probiotics) and improvement of microalgae-based diets. Microalgae serve not only as a primary nutritional source for bivalve larvae, but also as functional components that can modulate microbial interactions in the rearing environment. Species adapted to regional ecosystems commonly referred to as endemic microalgae may offer enhanced performance under local conditions, improving digestibility, tolerance to abiotic stressors, and interaction with native microbiota (Pacheco-Vega et al. 2015). Furthermore, several endemic species have demonstrated a capacity to produce bioactive compounds, such as essential fatty acids or polyunsaturated aldehydes, with antibacterial or immunostimulatory properties (Desbois et al. 2009; Ribalet et al. 2008).

At the same time, the use of probiotics live microorganisms that confer benefits to the host when administered in adequate amounts has shown promise in improving survival and controlling pathogens in aquaculture species. Their effectiveness depends on multiple factors, including the method of application, compatibility with the host, and stability within the aquatic system (Savin-Amador et al. 2021). When applied alongside microalgae, these bacteria may find a supportive biochemical environment that facilitates their persistence and interaction with the host's microbiota, particularly during the early stages of development (Liu et al. 2024; Hua and Li 2024). Despite this potential, *in vivo* studies addressing the interaction between native microalgae and probiotic strains in bivalve larviculture are scarce. Most research to date has focused on commercial species and bacterial isolates, leaving the local microbial diversity underutilized. Harnessing the potential of regionally adapted strains may contribute to more stable, effective, and environmentally compatible rearing systems.

Given the susceptibility of *C. gigas* larvae to *Vibrio* spp. infections and its well-established use in hatchery systems, this species provides a suitable model for evaluating microbial supplementation strategies. Its physiological characteristics, sensitivity to environmental conditions, and predictable developmental patterns allow for robust comparisons across experimental treatments (Helm et al. 2006; Madison et al. 2022).

Therefore, this study aimed to evaluate the *in vivo* effects of selected endemic microalgae and the probiotic strain *Lacticaseibacillus plantarum* 69Cr on the survival and physiological condition of *C. gigas* larvae under challenge with *Vibrio parahaemolyticus*. The novelty of this work lies in the combined use of native microalgae and probiotic bacteria from the same region as functional agents to enhance larval performance, reduce pathogen impact, and support antibiotic-free hatchery practices.

Materials and methods

Reactivation of probiotic bacterial strains

Probiotic strains were obtained from the culture collection of the Laboratory of Food Science and Technology (LABCyTA) at the Autonomous University of Baja California Sur (UABCS, by its Spanish acronym). The pathogenic strain (*Vibrio parahaemolyticus*) was acquired from the American Type Culture Collection (ATCC) (Table 1). Reactivation of lactic acid bacteria was performed by cross streaking on Man, Rogosa, and Sharpe (MRS) agar, followed by incubation under anaerobic conditions at 30 °C for 48 hours. Representative colonies were selected and subcultured in 4.5 mL of MRS broth in duplicate, incubated at 30 °C for 12–18 hours. *V. parahaemolyticus* was reactivated on trypticase soy agar (TSA), followed by inoculation at 1% (v/v) in 4.5 mL of trypticase soy broth (TSB), and incubated at 30 °C for 12–18 hours until reaching exponential growth phase.

Table 1 Bacterial strains used in the study and their corresponding codes

Strain code	Scientific name
69Cr	*Lactiplantibacillus plantarum*
101Cc	*Lactiplantibacillus fermentum*
V. parahaemolyticus	*Vibrio parahaemolyticus*

Laboratory culture of *Crassostrea gigas* larvae

Larvae were produced following standard hatchery protocols (Li et al. 2024). Reproductive procedures were conducted at LABCyTA–UABCS. For Experiments 1 and 2, 2-day-old larvae were transferred using sterile pipettes to 3 L containers containing 2,000 mL of filtered and sterilized seawater, at a density of 8 larvae mL^{-1}. For Experiment 3, larvae were cultured in twelve 20 L tanks at 15 larvae mL^{-1} according to Helm et al. (2006).

Cultivation of microalgae strains used in larval feeding trials

The microalgae strains (Table 2) were provided by the Aquaculture Laboratory in Pichilingue, UABCS, and included *Isochrysis galbana, Chaetoceros calcitrans, Grammatophora* sp., *Navicula* sp., and *Schizochytrium* sp. Cultures were maintained in 500 mL Erlenmeyer flasks with F/2 nutrient medium (Guillard 1975) under controlled temperature and continuous light, with conditions optimized for the requirements of each species. Water quality parameters were monitored daily using a portable multiparameter probe (HI98107, Hanna Instruments, USA). Salinity was maintained at 30 ± 1 PSU and pH between 7.8 and 8.2, in accordance with optimal growth conditions according to Helm et al. (2006).

Experiment 1: Determination of optimal microalgae feeding dose for *Crassostrea gigas* larvae

A 21-day bioassay was conducted to evaluate three concentrations (10^3, 10^4, and 10^5 cells mL^{-1}) of five microalgae species, including three endemic strains and two controls (*I. galbana and C. calcitrans*) (Table 2), aiming to determine the most effective feeding dose for *C. gigas* larvae. Each treatment was performed in four replicates using 1.5 mL tubes with sterilized seawater at 8 larvae mL^{-1}. Cultures were maintained at 25 °C, with seawater renewal every three days.

Experiment 2: Quantification of bacteria-microalgae interaction

To evaluate the interaction between microalgae and probiotic bacteria, 5 mL tubes containing sterilized seawater were inoculated with *Lpb. plantarum* 69Cr at 1×10^4 CFU mL^{-1} (Savin-Amador et al. 2021) and each microalga at 1×10^5 cells mL^{-1}. Viable bacterial counts (expressed as CFU mL^{-1}, determined by serial dilution and plate counting on TSA) and microalgae cell concentrations (cells mL^{-1}, measured using a Neubauer chamber under light microscopy) were recorded every 3 hours over a 24-hour period. Control tubes containing only microalgae were included under identical experimental conditions.

Experiment 3: Evaluation of larval survival in co-cultures of microalgae and probiotic bacteria

This experiment evaluated larval survival using microalgae-probiotic combinations. Each treatment (Table 3) included one microalga at the optimal dose (as determined in Experiment 1) and *Lpb. plantarum* 69Cr

Table 2 Microalgae concentrations used in larval feeding bioassays

Microalgae	Low CFU mL^{-1}	Medium CFU mL^{-1}	High CFU/mL^{-1}
Isochrysis galbana	$1x10^3$	$1x10^4$	$1x10^5$
Chaetoceros calcitrans	$1x10^3$	$1x10^4$	$1x10^5$
Grammatophora sp.	$1x10^3$	$1x10^4$	$1x10^5$
Navicula sp.	$1x10^3$	$1x10^4$	$1x10^5$
Schizochytrium sp.	$1x10^3$	$1x10^4$	$1x10^5$

Table 3 Concentrations of microalgae, probiotic bacteria, and *C. gigas* larvae used in co-culture experiments

Microalgae	Concentration	Probiotic bacteria concentration	Larvae
I. galbana	$1x10^4$	$1x10^4$	8 mL^{-1}
C. calcitrans	$1x10^4$	$1x10^4$	8 mL^{-1}
Grammatophora sp.	$1x10^4$	$1x10^4$	8 mL^{-1}
Navicula sp.	$1x10^4$	$1x10^4$	8 mL^{-1}
Schizochytrium sp.	$1x10^4$	$1x10^4$	8 mL^{-1}

at 1×10^4 CFU mL^{-1} (Savin-Amador et al. 2025). Sterilized seawater was dispensed into 1.5 mL tubes, each containing an initial density of 8 larvae per mL^{-1}, for 21 days. Larval survival, gut condition, and algae-bacteria interactions were monitored throughout the trial. .

Experiment 4: *In Vivo* challenge of larvae with *Vibrio parahaemolyticus* following co-culture treatment

A pilot-scale *in vivo* challenge was performed using 100 L tanks, each stocked at a density of 8 larvae mL^{-1}. Each treatment was conducted in triplicate (n = 3) using independent tanks to ensure experimental reliability. This experiment aimed to assess the protective effect of the co-culture of *Schizochytrium* sp. and *Lacticaseibacillus plantarum* 69Cr against *V. parahaemolyticus* (Table 4). Larvae were fed daily and exposed to different treatments: microalgae alone, probiotic alone, pathogen alone, and combinations thereof. The probiotic was added on day 1 and after each water exchange. On day 3, the challenge was initiated using a cell suspension at Lethal Dose (LD_{50}) (1×10^5 CFU mL^{-1}) of *V. parahaemolyticus* (Savin-Amador et al. 2025). Larvae were observed every three days using a stereomicroscope (Labomed CxL, 10×), and digestive condition was assessed as full, half full, or empty according to Carreño et al. (2012) (Table 5). Survival was recorded throughout the 15-day trial.

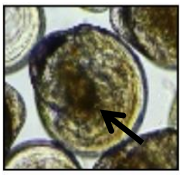
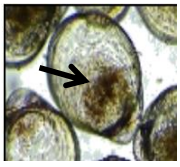
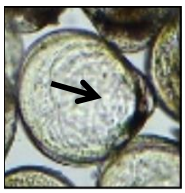
Statistical analysis

Data were tested for homogeneity of variance (Bartlett test) and normality (D'Agostino-Pearson test) at $\alpha = 0.05$. One-way ANOVA was used to compare response variables, including larval survival, CFU mL^{-1},

Table 4 Experimental design of larval exposure to microalgae, probiotics, and *Vibrio parahaemolyticus*

Treatment	Larvae	Microalgae	Probiotic bacteria	Pathogenic bacteria	Probiotic bacteria + pathogen
T1	8/mL	*I. galbana*			
T2	8/mL	*Schizochytrium* sp			
T3	8/mL	*Schizochytrium* sp	Effective dose		
T4	8/mL	*Schizochytrium* sp		Median lethal dose LD_{50} $1x10^5$	
T5	8 /mL	*Schizochytrium* sp			Effective dose Median lethal dose DL_{50} $1x10^5$

Table 5 Classification of digestive condition in *C. gigas* larvae based on visual appearance of the digestive gland. Arrows black indicate the position and relative fullness of the digestive gland as observed under stereomicroscopy (Carreño et al. 2012).

Larvae condition status	Description	Figure
Full	Digestive gland easily distinguishable, dark brown to dark yellow.	
Half full	Digestive gland distinguishable, light brown to light yellow.	
Empty	Transparent digestive gland, difficult to differentiate from the rest of the organs.	

microalgae concentration, and larval gut condition. Significant differences were identified using the LSD (Least Significant Difference) post-hoc test (Sokal 1980). Percentage data were arcsine-transformed prior to analysis. All statistical tests were conducted using GraphPad Prism 9.0.2 (GraphPad Software, San Diego, CA, USA).

Results

Estimation of optimal microalgae dose for oyster larval feeding

Figure 1 shows the survival percentage of oyster larvae over a 21 day period when fed with different concentrations (10^3, 10^4, and 10^5 cells mL^{-1}) of endemic microalgae strains (Grammatophora sp., Navicula sp., and Schizochytrium sp.). By day 15, complete mortality was observed in larvae fed with *Grammatophora sp.* at 10^3 cells mL^{-1}. In contrast, the highest survival at day 21 was recorded in the group fed with *Schizochytrium sp.* at 10^4 cells mL^{-1} (22%), followed by *Schizochytrium sp.* at 10^5 cells mL^{-1} (19%). A moderate survival rate (13%) was observed in groups fed with S*chizochytrium sp.* 10^3, Navicula sp. 10^5, *Grammatophora sp.* 10^4, and the control treatment. The lowest survival rates were recorded in larvae fed with *Navicula sp.* at 10^3 and 10^4 cells mL^{-1} (6.5%) and with *Grammatophora sp*. at 10^5 cells mL^{-1} (9.7%). Statistical analysis revealed that survival differences among treatments were statistically significant ($p < 0.05$), indicating that both microalgae species and cell concentration affected larval survival.

Assessment of probiotic growth in co-culture with microalgae and oyster larvae

Figure 2 and Table 6 present the quantification of colony forming units (CFU mL^{-1}) for the strains *Lpb. fermentum* 101Cc, *Lpb. plantarum* 69Cr, and *V. parahaemolyticus* over a 24 hour incubation period. Samples were taken every 3 hours from triplicate cultures and plated on selective media: MRS agar for Lpb. strains and TSA for *V. parahaemolyticus*. Colony counts were performed following serial dilution and incubation under appropriate conditions (30 °C, 48 h for Lpb. *spp*.; 30 °C, 24 h for Vibrio). The maximum growth was derived from the highest CFU value observed among the time points, with *Lpb. plantarum* 69Cr reaching 260 CFU mL^{-1} at 18 hours, *V. parahaemolyticus* 10^8 CFU mL^{-1} at 15 hours, and *Lpb. fermentum* 101Cc 60 CFU mL^{-1} at 12 hours. Statistical analysis confirmed that the differences in maximum growth among the three strains were statistically significant ($p < 0.05$).

Figure 3 shows the bacterial quantification in co-culture with different endemic microalgae. The highest average CFU mL^{-1} was observed in the treatment *I. galbana* / *V. parahaemolyticus* 10^5, with 50 CFU mL^{-1} at 18 hours. This was followed by *Schizochytrium sp*. / *Lpb. plantarum* 69Cr 10^4, which reached 39 CFU mL^{-1} at the same time point. The lowest CFU values were recorded in the treatment *Grammatophora sp*. / *Lpb. fermentum* 101Cc 10^4. Statistical analysis indicated that the differences in bacterial counts among treatments were statistically significant ($p < 0.05$).

Survival of oyster larvae exposed to selected microalgae and probiotic bacteria

Figure 4 shows the survival rates of oyster larvae after 21 days of exposure to different co-cultures of selected endemic microalgae and the probiotic strain *Lpb. plantarum* 69Cr (10^4 CFU/mL). The highest survival was observed in the treatment with *Schizochytrium sp*. (10^4 cells/mL) + *Lpb. plantarum* 69Cr, reaching 18.75%. This was followed by *Grammatophora sp*. + *Lpb. plantarum* 69Cr with 15.63%, *Isochrysis galbana* (control) + *Lpb. plantarum* 69Cr with 9.38%, and *Navicula sp*. + *Lpb. plantarum* 69Cr, which showed the lowest survival at 3.2%. Statistical analysis revealed significant differences in survival rates among treatments ($p < 0.05$).

Pilot-scale *in vivo* challenge of larvae treated with probiotic bacteria and exposed to *Vibrio parahaemolyticus*

Figure 5 shows the survival curves of oyster larvae over a 15-day period under different treatments. The highest survival rate (37.5%) was recorded in the group treated with the co-culture of *Schizochytrium sp*.

(10^4 cells/mL) and *Lpb. plantarum* 69Cr (10^4 CFU/mL), without exposure to *V. parahaemolyticus*. In contrast, survival dropped to 19% when the same co-culture was challenged with *V. parahaemolyticus* (10^5 CFU/mL), while the presence of the pathogen alone resulted in the lowest survival rate, with nearly 0% by day 15. Statistical analysis revealed that these differences in survival rates among treatments were statistically significant ($p < 0.05$).

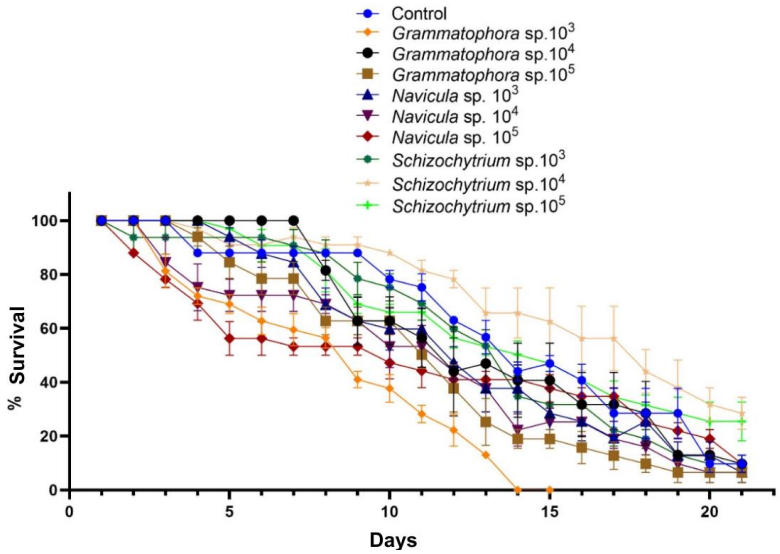


Fig. 1 The line graph indicates the average survival rates of larvae for 21 days, with the different endemic microalgae and different concentrations.

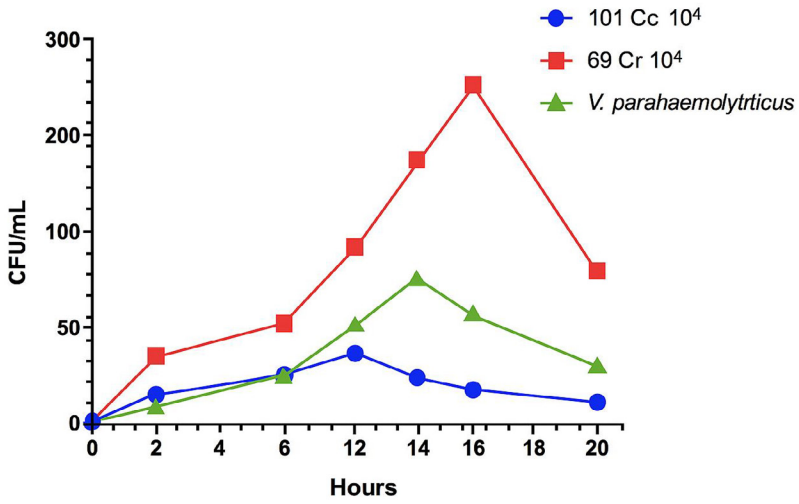


Fig. 2 Quantification of *Lpb. plantarum* 69Cr, *Lpb. fermentum* 101Cc, and *V. parahaemolyticus* in CFU mL^{-1} over a 24 hour period. The line graph displays the growth dynamics of each strain, indicating differences in proliferation rates under the same culture conditions..

Table 6 Colony count of strains *Lpb. fermentum* 101Cc, *Lpb. plantarum* 69Cr, and *V. parahaemolyticus*

Hours	*Lpb. fermentum* 101 Cc 10^4(CFU ml^{-1})	*Lpb. plantarum* 69 Cr 10^4 (CFU ml^{-1})	*V. parahaemolyticus* 10^5 (CFU ml^{-1})
0	$3x10^2$	$6x10^3$	$1x10^2$
3	$3x10^2$	$8.4x10^2$	$4x10^2$
9	$6x10^3$	$1.4x10^3$	$6x10^2$
12	$4.5x10^2$	$2.1x10^3$	$1.08x10^3$
15	$1.5x10^2$	$2.6x10^3$	$7.9x10^2$
18	2x10	$1.25x10^3$	$4x10^2$
24	$3x10^2$	$6x10^3$	$1x10^2$

Statistical analysis (multiple comparison test) conducted on day 15 showed that there were no statistically significant differences ($p > 0.05$) between treatments combining different microalgae with *Lpb. plan-*

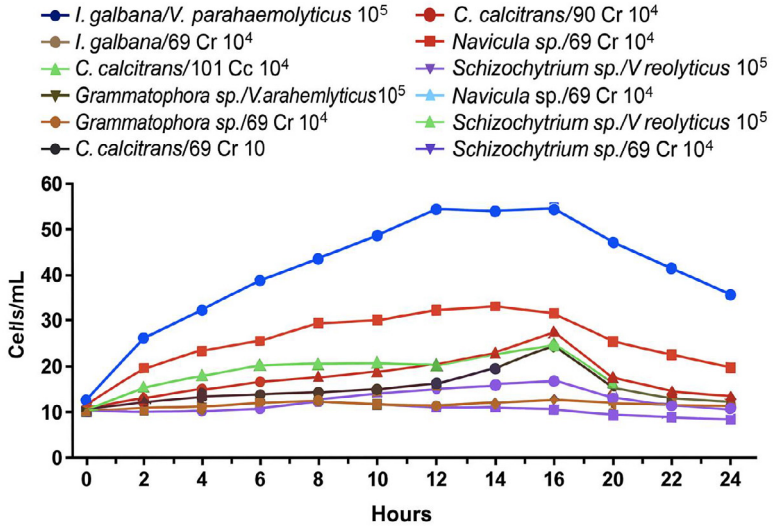


Fig. 3 Cell growth dynamics (cells/mL) of various endemic microalgae species (*Isochrysis galbana*, *Grammatophora* sp., *Chaetoceros calcitrans*, *Navicula* sp., and *Schizochytrium* sp.) exposed to the pathogen *Vibrio parahaemolyticus* (10^5 CFU/mL) and two probiotic strains (69 Cr and 101 Cc at 10^4 CFU/mL), over a 24 hour incubation period. The lines represent changes in cell concentration under different experimental treatments.

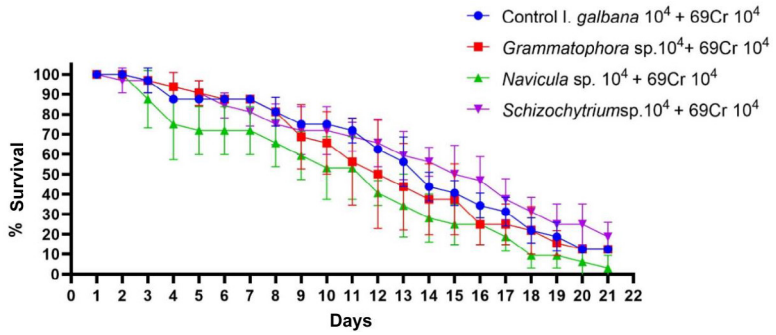


Fig. 4 The line graph indicates the averages of larval survival for 21 days, with the endemic microalgae at the concentration of 10^4 and *Lpb. plantarum* 69Cr at the concentration of 10^4.

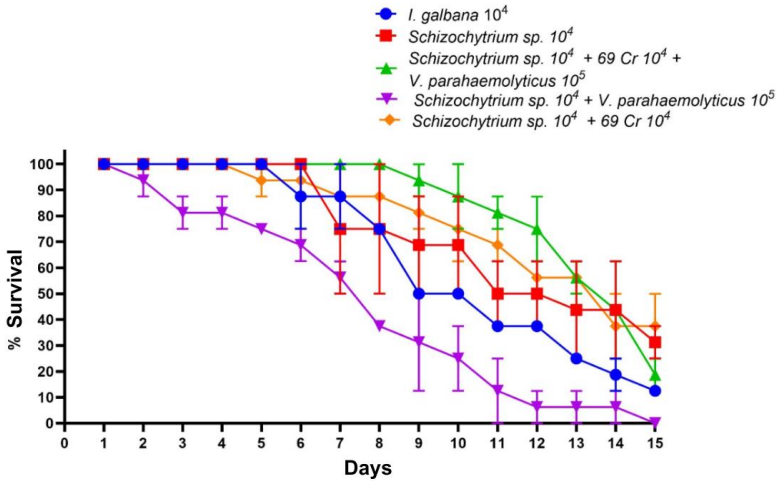


Fig. 5 The line graph indicates the averages of larval survival for 15 days, with the endemic microalgae *Schizochytrium* sp. at a concentration of 10^4 and the probiotic strain *Lpb. plantarum* 69Cr at a concentration of 10^4, challenged with the pathogen *V. parahaemolyticus* at a concentration of 10^5.

tarum 69Cr (10^4 CFU/mL) in the presence of *V. parahaemolyticus* (10^5 CFU/mL), as illustrated in Figure 6.

To assess larval feeding activity, gut condition was monitored under a microscope throughout the experimental period. In the treatment with *I. galbana* (10^4 cells/mL), a trend toward 50% of larvae exhibiting empty digestive tracts was observed starting on day 9. For *Schizochytrium* sp. (10^4 cells/mL), this occurred on day 11. In the co-culture of *Schizochytrium sp. + Lpb. plantarum* 69Cr (both at 10^4 CFU/mL), more than 50% of larvae appeared empty as early as day 7. Similarly, in treatments with *Schizochytrium* sp. + *V. parahaemolyticus* (10^5 CFU/mL), and *Schizochytrium sp. + Lpb. plantarum* 69Cr + *V. parahaemolyticus*, over 50% of the larvae showed empty guts by day 7 (Figure 7). Although a progressive trend toward partial or complete gut emptiness was observed in all treatments, statistical analysis revealed no significant differences between groups ($p > 0.05$).

Discussion

Microalgae are fundamental in the nutrition of bivalves due to their appropriate size for digestion and their richness in essential fatty acids such as DHA and EPA, which these organisms cannot synthesize (Helm et al. 2006; Marquez et al. 2019; Pronker et al. 2008). In this study, endemic microalgae from northwestern México (*Grammatophora* sp., *Navicula sp*., *Schizochytrium* sp.) were used, along with *Isochrysis galbana*. According to Pacheco-Vega et al. (2015), these species exhibit diverse biochemical profiles, with *Grammatophora sp*. standing out for its high DHA and EPA content, in contrast to *Schizochytrium sp*., where EPA was not detected.

The combination of *Schizochytrium* sp. and *Lactiplantibacillus plantarum* 69Cr proved effective in increasing the survival of *C. gigas* larvae challenged with *V. parahaemolyticus*, supporting the use of microal-

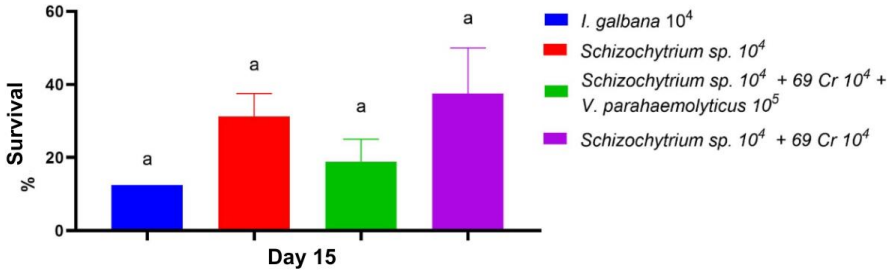


Fig. 6 The bar graph shows the survival of larvae on day 15 with the endemic microalga *Schizochytrium* sp. at a concentration of 10^4 and the probiotic strain *Lpb. plantarum* 69Cr 10^4 and the pathogenic strain *V. parahaemolyticus* at a concentration of 10^5.

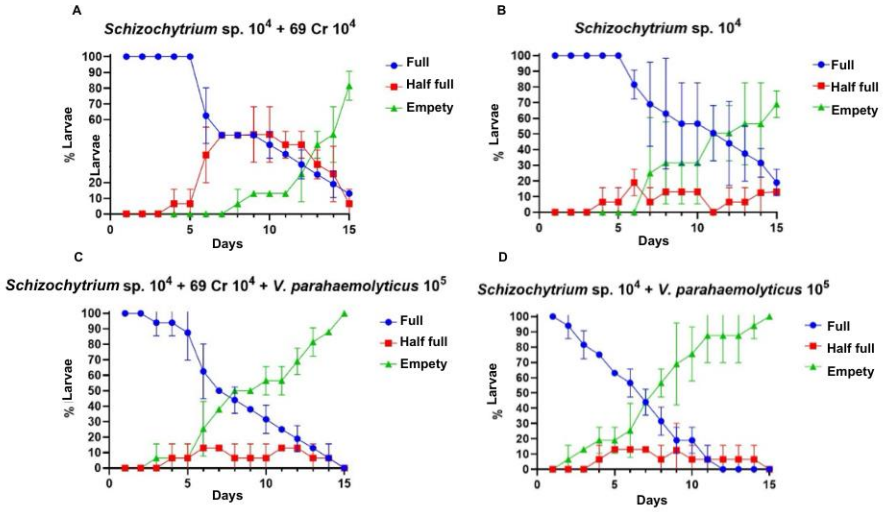


Fig. 7 The different line graphs (A, B, C and D) indicate the average percentage of the condition status (Full, Half-full and Empty) of the larvae for 15 days, with the endemic microalgae *Schizochytrium* sp. at a concentration of 10^4 and the probiotic strain *Lpb. plantarum* 69Cr at a concentration of 10^4, challenged with the pathogen *V. parahaemolyticus* at a concentration of 10^5.

gae–probiotic synergies as a biocontrol strategy. This outcome is consistent with findings by Savin-Amador et al. (2021), who observed significantly higher larval survival when *Lpb. plantarum* was administered alongside *I. galbana* and *C. calcitrans*. Similarly, Amador et al. (2024) confirmed the effectiveness of *Lpb. plantarum* 69Cr against the opportunistic pathogen *Staphylococcus pasteuri*, suggesting a broad protective spectrum. D'Alvise et al. (2012) demonstrated that *Phaeobacter gallaeciensis* can coexist with microalgae such as *Tetraselmis suecica* and *Nannochloropsis oculata* without negatively impacting algal growth, confirming the compatibility of certain beneficial bacteria in mixed cultures. Consistent with these findings, Čanak et al. (2023) reported that dietary supplementation with *Lactiplantibacillus plantarum I* significantly enhances growth performance and reduces pathogen prevalence during the culture of *Argopecten opercularis*. In their study, scallops fed with a combination of microalgae (*Tetraselmis sp., Nannochloropsis* sp*., and Phaeodactylum* sp.) and *Lpb. plantarum I* exhibited a higher meat yield (33.15 ± 2.63%) compared to the control group fed only microalgae (29.66 ± 2.87%). Sánchez-Ortiz et al. (2020) also reported enhanced bacterial growth of *Bacillus* strains co-cultured with *I. galbana* and *C. calcitrans* without compromising algal viability, which may support the persistence of probiotics in larval systems. Complementarily, Pande et al. (2015) documented that the combination of *Bacillus* sp. and microalgae such as *Tetraselmis* and *Chaetoceros muelleri* improved the survival of *Macrobrachium rosenbergii* larvae challenged with *Vibrio campbellii*, although no significant effects on growth were observed.

The use of probiotics in bivalve aquaculture has been widely supported by studies highlighting their benefits for growth, survival, and pathogen resistance. For instance, Bernal et al. (2020) demonstrated that *Streptomyces* spp. strains V4 and RL8 promoted greater growth in *C. gigas* juveniles compared to other treatments. Similarly, Madison et al. (2022) reported that a single early application of a probiotic consortium (B11, D16, and DM14) in *C. gigas* and *Crassostrea sikamea* larvae not only increased survival against *Vibrio coralliilyticus*, but also enhanced growth and metamorphosis, emphasizing that strain combinations are more effective than individual applications. Zheng et al. (2023) further showed that *Bacillus hwajinpoensis* effectively colonizes the digestive tract of *C. gigas* larvae, following entry routes similar to those of *Vibrio alginolyticus*, thereby competing for adhesion sites and significantly reducing both mortality and pathogen load. This competitive exclusion mechanism aligns with the protective effects observed in our study.

Regarding potential candidates, Modak and Gomez-Chiarri (2020) identified *Pseudomonas* strains CA6 and CCH18 as promising probiotics due to their inhibitory activity against *V. parahaemolyticus*, non-hemolytic profiles, antibiotic susceptibility, and efficient growth in seawater. These traits suggest high applicability, especially in combination, as no antagonistic interactions were observed between the strains. Although their results were derived from *in vitro* assays, the authors emphasized the necessity for *in vivo* validation such as conducted in the present study to confirm their efficacy under real aquaculture conditions. Supporting the relevance of *in vivo* trials, Liu et al. (2024) demonstrated that the application of *Lacticaseibacillus plantarum* in the rearing water of *Ruditapes philippinarum* significantly enhanced antioxidant capacity, nonspecific immunity, resistance to *V. parahaemolyticus*, and stability of the intestinal microbiota. These effects were dose-dependent, with optimal responses observed at 1×10^7 CFU L^{-1} (10^4 CFU mL^{-1}), consistent with the concentration used in this investigation. Furthermore, in a complementary probiotic approach, Hua and Li (2024) showed that encapsulated *Lpb. plantarum* 299V (6.00 log CFU g^{-1}) administered to adult oysters significantly reduced *V. parahaemolyticus* and *Salmonella enterica* by 1.00 log within four days compared to control samples ($p < 0.05$). These findings underscore the potential of *L. plantarum* strains in enhancing host resilience and pathogen control across different bivalve species and life stages.

From a nutritional perspective, microencapsulated diets have demonstrated significant advantages over traditional live microalgae. Willer et al. (2020) reported that microcapsule-based diets using *Schizochytrium* improved gonadal development and omega-3 fatty acid (EPA and DHA) accumulation in oyster broodstock. Similarly, Willer and Aldridge (2019) highlighted that such diets reduce production costs and sanitary risks, offering a pathway to minimize antibiotic use in hatcheries. However, in this study, results from monitoring larval gut condition suggest that the nutritional performance of *Schizochytrium* sp. (10^4 cells/mL), both alone and in combination with *Lpb. plantarum* 69Cr, was suboptimal under larval culture conditions. Over 50% of larvae exhibited empty digestive tracts by day 11 in the *Schizochytrium*-only treatment, and as early as day 7 in treatments combined with *Lpb. plantarum* or *V. parahaemolyticus*. Even in the *I. galbana* treatment, massive gut evacuation occurred slightly later (day 9), suggesting that *Schizochytrium*

may have limited digestibility or assimilation in early larval stages compared to traditional live microalgae.

These findings contrast with the nutritional benefits observed in broodstock (Willer et al., 2020), which may be attributed to differences in digestive requirements between ontogenetic stages or the physical presentation of the feed (microencapsulated vs. live cells). Our results indicate that while *Schizochytrium* sp. may contribute valuable nutrients, its exclusive or combined use in larval diets should be carefully evaluated, potentially requiring adjustments in concentration, delivery format, or supplementation with other sources to avoid adverse effects on larval feeding and survival.

Conclusions

This study demonstrates that the combination of *Schizochytrium sp*. 10^4, an endemic microalga from northwestern México, and the probiotic strain *Lacticaseibacillus plantarum* 69Cr 10^4 significantly enhances the survival of *Crassostrea gigas* larvae challenged with *Vibrio parahaemolyticus*. This synergy offers a promising alternative for the development of functional diets and biocontrol strategies in bivalve hatcheries, contributing to the gradual replacement of antibiotics in aquaculture. While *Schizochytrium sp*. provides valuable nutritional compounds, our findings suggest that its digestibility during early larval stages requires optimization, particularly when combined with probiotics or under pathogenic pressure.

From a practical standpoint, the use of native microorganisms as integrated tools for nutrition and health management can reduce the costs associated with auxiliary algal cultures and support more sustainable hatchery operations. Future research should focus on optimizing concentrations, delivery methods, and strain combinations, as well as exploring the complex interactions among larvae, microalgae, probiotics, and associated microbiota. These findings lay the groundwork for designing more efficient, resilient, and antibiotic-free production systems in bivalve aquaculture.

Conflicts of interest The authors report no conflict of interest.

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