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Research Article

Integrated stage-specific management across the production cycle of *Macrobrachium rosenbergii*: effects of broodstock nutrition, larval salinity and biofloc carbon management

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ABSTRACT. This study evaluated stage-specific management strategies across three successive phases of the *Macrobrachium rosenbergii* production cycle: broodstock conditioning, larval rearing, and juvenile grow-out. Each phase was evaluated through an independent experiment testing specific nutritional, environmental, or microbial management factors, while together the three experiments provided a coordinated production framework. Broodstock conditioning compared a commercial control diet with three experimental diets containing 7, 11, and 14% crude lipid. Larvae used in the subsequent larval-rearing experiment were obtained from spawnings produced during the preceding broodstock reproductive phase. Larval rearing evaluated six treatments differing in salinity source, production system, and carbon supplementation strategy. Juvenile grow-out compared recirculating aquaculture systems (RAS) with biofloc technology (BFT) supplemented with either molasses or corn starch. Broodstock fed the 11% lipid diet consistently exhibited numerically superior reproductive performance, although differences among dietary lipid levels were not statistically significant. During larval rearing, seawater-based BFT supplemented with molasses resulted in higher survival and shorter larval development time, whereas brine-based BFT treatments failed to reach the first postlarval stage (PL_1) under the tested conditions. During juvenile grow-out, survival was comparable among treatments; final body weight was higher in RAS than in corn starch-supplemented BFT, whereas molasses-supplemented BFT produced growth comparable to RAS. Biofloc treatments, particularly those supplemented with molasses, also promoted a higher proportion of advanced male morphotypes (orange-claw and blue-claw) while reducing the frequency of small-claw males. Taken together, these findings indicate that dietary lipid levels within the evaluated range adequately supported broodstock reproductive performance. In contrast, larval and juvenile responses were primarily influenced by salinity source and carbon management. Overall, these results support a stage-specific management framework in which nutritional, environmental, and microbial strategies are applied according to the specific requirements of successive developmental stages of *M. rosenbergii*.

Keywords: freshwater prawn; larviculture; heterotrophic systems; recirculating aquaculture system: reproductive conditioning; biofloc technology

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INTRODUCTION

The giant freshwater prawn *Macrobrachium rosenbergii* is one of the most economically important freshwater crustaceans in global aquaculture because it grows rapidly, has a high market value, tolerates a wide range of environments, and is widely accepted by consumers. Global production has increased steadily in recent decades, driven by expanding freshwater aquaculture and growing demand for high-value crustaceans (New et al. 2010, FAO 2026). Despite considerable advances in production technology, hatchery performance remains highly variable among farming systems, and bottlenecks during broodstock conditioning, larval rearing, and juvenile culture continue to limit productivity and profitability (New 2002, Hargreaves 2013, Karplus & Barki 2019).

The *M. rosenbergii* production cycle comprises successive developmental stages with distinct physiological and management requirements. Broodstock nutritional condition affects reproductive performance and offspring production (Cavalli et al. 1999, 2000, Wouters et al. 2001, Song et al. 2024). In contrast, larval rearing requires appropriate ionic and environmental conditions to support osmoregulation, molting, and development (New 2002, Charmantier & Anger 2011, Henry et al. 2012). During juvenile culture, water quality, microbial communities, and carbon availability increasingly shape nutrient recycling, feed utilization, growth, and population structure, particularly in biofloc-based systems (Crab et al. 2010, Emerenciano et al. 2013, Hargreaves 2013, Avnimelech 2015). Although these production stages differ biologically and operationally, most studies have evaluated broodstock nutrition, larval culture, or juvenile production separately, leaving the potential complementarity of stage-specific management strategies insufficiently explored in *M. rosenbergii* (New et al. 2010, Karplus & Barki 2019).

Within this production continuum, larviculture represents one of the principal biological and operational bottlenecks limiting large-scale freshwater prawn production. Although *M. rosenbergii* is euryhaline, its larvae require brackish water (approximately 10-15‰), where appropriate ionic composition is essential for osmoregulation, molting, and successful metamorphosis (Charmantier & Anger 2011, Henry et al. 2012). For inland hatcheries, this requirement often necessitates using either natural seawater or concentrated solar-saltwork brines diluted to the desired salinity. However, these saline sources often differ in their ionic composition, particularly in the relative concentrations of potassium, magnesium, calcium, and sulfate, differences that may increase osmoregulatory costs and compromise larval development despite equivalent salinity values (Millero et al. 2008, De Medeiros et al. 2012, Vicari et al. 2022). Therefore, improving larval performance is essential not only to maximize hatchery survival but also to ensure the production of robust juveniles for subsequent grow-out. Although solar-saltwork brines represent an economically attractive alternative for inland hatcheries, their biological suitability for *M. rosenbergii* larviculture remains insufficiently evaluated under commercial production conditions (Miglio et al. 2021).

Beyond ionic composition, increasing evidence indicates that the microbial environment also plays a fundamental role in hatchery performance. Among available microbial-based approaches, biofloc technology (BFT) has emerged as one of the most promising strategies for improving water quality while enhancing nutrient recycling and generating microbial biomass as a complementary nutritional resource (Crab et al. 2010, Emerenciano et al. 2013, Avnimelech 2015). BFT effectiveness depends largely on appropriate carbon supplementation, which stimulates heterotrophic bacterial growth, promotes inorganic nitrogen assimilation, and helps establish stable microbial communities (Ebeling et al. 2006, Emerenciano et al. 2013). In biofloc systems, the carbon source type is one of the most influential operational variables because it affects microbial growth kinetics, floc formation, nutrient recycling, and the nutritional quality of microbial aggregates (Xu & Pan 2012, Tinh et al. 2021, Li et al. 2023). Readily available carbon sources such as molasses generally promote rapid heterotrophic bacterial proliferation.

In contrast, more complex carbohydrates such as starch require enzymatic hydrolysis before microbes can use them, leading to different microbial dynamics and biofloc characteristics. Although researchers have extensively investigated carbon supplementation during juvenile grow-out, comparatively few studies have examined how carbon-source selection influences larval performance under contrasting saline media or helps maintain continuity between hatchery and nursery production. Addressing this knowledge gap is essential for developing integrated production continuum strategies that maximize production efficiency across successive developmental stages of *M. rosenbergii*.

Accordingly, this study evaluated complementary nutritional, environmental, and microbial strategies across successive phases of the *M. rosenbergii* production cycle. Specifically, we investigated how dietary lipid levels affect broodstock reproductive

performance and compared two saline water sources for larval rearing and development. We evaluated how different organic carbon sources influence biofloc development and juvenile performance. Rather than evaluating these practices as unrelated production interventions, this study examines complementary nutritional, environmental, and microbial management strategies across successive production stages, providing a broader framework for improving culture performance throughout the production cycle.

We hypothesized that optimizing broodstock nutrition, larval rearing conditions, and juvenile microbial management at each stage would improve biological performance within each phase and, collectively, provide a coherent management framework for *M. rosenbergii* culture.

MATERIALS AND METHODS

Experimental overview

The study comprised three independent experiments conducted at successive phases of the *M. rosenbergii* production cycle: broodstock conditioning, larval rearing, and juvenile grow-out (Fig. 1).

Each experiment evaluated stage-specific management factors using its own experimental design and experimental units. Experiment 1 assessed broodstock reproductive performance under different dietary lipid levels. Experiment 2 evaluated larval development, metamorphosis, and survival under contrasting salinity sources, culture systems, and carbon supplementation strategies. Experiment 3 assessed juvenile growth performance, apparent feed conversion ratio (AFCR), survival, and male morphotype distribution under recirculating aquaculture systems (RAS) and BFT conditions with different carbon sources. Although the experiments were conducted within the same production sequence, statistical inference was restricted to each experiment independently. Together, the three experiments were used to evaluate complementary management strategies across successive developmental stages of *M. rosenbergii*. All experiments were conducted at the Laboratory of Culture and Nutrition of Native Crustaceans, Instituto Tecnológico de Boca del Río (TecNM-ITBOCA), Veracruz, Mexico (19°06′N, 96°06′W).

General culture conditions

Unless otherwise specified, all experimental units operated under the same general environmental conditions. Continuous aeration was supplied by a central blower (Sweetwater S31, 1 HP) through independent air diffusers to ensure homogeneous mixing and adequate dissolved oxygen (DO) concentrations. Water temperature, DO, and pH were monitored daily using a calibrated multiparameter probe (YSI 556 MPS, Yellow Springs, USA). Salinity was verified with a handheld refractometer whenever saline water was used. Ammonia, nitrite, nitrate, and alkalinity were measured every 48 h following APHA (2017) standard methods. When operational thresholds established for each experiment were approached, partial water exchanges and/or carbon supplementation were adjusted to maintain target water-quality conditions. Unless otherwise indicated, all experiments were conducted under a 12:12 h light:dark photoperiod, and culture units were protected from direct sunlight and air drafts.

Broodstock conditioning

Experimental design

Broodstock were obtained from a domesticated captive population maintained at the Aquaculture Research Laboratory of the Instituto Tecnológico de Boca del Río (ITBOCA), Mexico. Individuals (average initial weight: 18.7 ± 2.6 g; total length: 10.4 ± 1.1 cm) were acclimated for 15 days under clear-water conditions before being randomly distributed among 12 fiberglass tanks (500 L), with three replicate tanks per dietary treatment and seven prawns per tank (six females and one male). Four dietary treatments were evaluated: a commercial broodstock diet used as the control and three experimental diets formulated to contain 7, 11, and 14% crude lipid, respectively. All experimental diets were formulated to contain approximately 35% crude protein. The conditioning period lasted 60 days.

Culture conditions

Each experimental unit consisted of a 500 L fiberglass tank operating within a semi-closed recirculating aquaculture system equipped with continuous aeration, sponge mechanical filtration, and a 40 L biological filter to maintain water quality. A 20% water exchange was performed every 48 h using dechlorinated freshwater. Water temperature was maintained at 28 ± 1°C, DO remained above 5 mg L^{-1}, and pH was maintained at 8.0 ± 0.1.

Diet formulation and feeding

A commercial broodstock diet served as the control reference, whereas the experimental diets contained 7, 11, and 14% crude lipid, respectively. The ingredient composition of the experimental diets is presented in

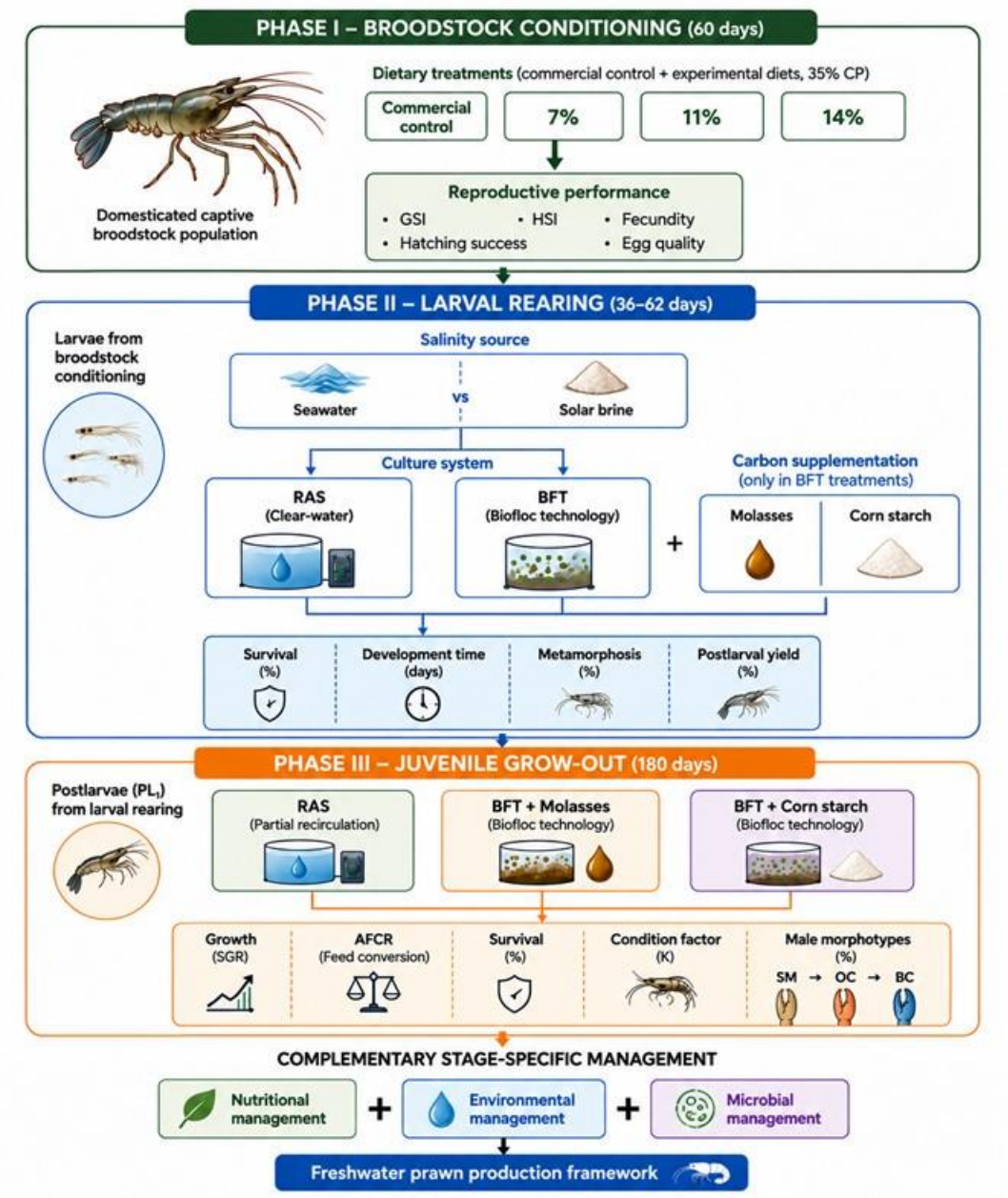


Figure 1. Experimental framework across successive production stages of *Macrobrachium rosenbergii*. CP: crude protein; GSI: gonadosomatic index; HSI: hepatosomatic index; RAS: recirculating aquaculture system; BFT: biofloc technology; SGR: specific growth rate; AFCR: apparent feed conversion ratio; SM: small claw; OC: orange claw; BC: blue claw.

Table 1, and their proximate composition is shown in Table 2.

Fish meal, chicken meal, coconut meal, and soybean meal served as the main protein sources, while fish oil, oleic oil, and soy lecithin provided dietary lipids. All ingredients were ground to <500 µm, thoroughly mixed, pelleted (2 mm), dried at 45°C for 12 h, and stored at 4°C until use. Broodstock were fed twice daily (08:00 and 18:00 h) at 3% of tank biomass per day, following recommendations for freshwater prawn broodstock (Cavalli et al. 2000, Wouters et al. 2001, New et al. 2010). Feeding rates were adjusted biweekly according to biomass estimates, and uneaten feed was removed 30 min after feeding.

Response variables

At the end of the 60-day feeding period, reproductive and physiological variables were evaluated. The gona-

Table 1. Ingredient composition of the experimental broodstock diets.[1]Premix provided per kg diet: vitamin A 10,000 IU; vitamin D_3 2,000 IU; vitamin E 150 mg; vitamin K 10 mg; thiamine 20 mg; riboflavin 25 mg; niacin 100 mg; folic acid 5 mg; Fe 100 mg; Zn 60 mg; Mn 20 mg; Cu 5 mg; Se 0.5 mg. All diets were formulated to be isoproteic (35% crude protein). BHT: butylated hydroxytoluene.

Ingredient (%)	Dietary lipids		
	7%	11%	14%
Fish meal	23.68	24.37	24.37
Chicken meal	10.76	11.70	11.70
Coconut flour	7.78	9.33	9.33
Soybean flour	30.00	35.00	35.00
Corn gluten	17.95	6.42	4.42
Fish oil	0.0	0.86	1.86
Oleic oil	0.33	2.82	3.82
Soy lecithin	1.0	1.0	1.0
Vitamin-mineral premix[1]	3.0	3.0	3.0
Carboxymethyl cellulose	3.0	3.0	3.0
Wheat bran	1.0	1.0	1.0
Vitamin c	1.0	1.0	1.0
Antioxidant (BHT)	0.5	0.5	0.5
	100	100	100

dosomatic index (GSI) and hepatosomatic index (HSI) were calculated as the ratio of gonad or hepatopancreas weight to total body weight, respectively. Fecundity was determined as the number of eggs per female, whereas hatching success was expressed as the percentage of eggs that successfully hatched. Gonads and hepatopancreas from three females per replicate were dissected, weighed, and analyzed for proximate composition (moisture, crude protein, lipid, and ash) according to AOAC (2005) procedures to characterize broodstock physiological condition at the end of the experimental period.

Larval rearing

Experimental design

Larvae produced from the broodstock conditioning phase were subsequently used to evaluate the effects of saline water source and microbial management during larval rearing. A completely randomized design was established to compare six treatment conditions defined by the combination of salinity source, culture system, and carbon supplementation strategy. Because external carbon was applied only in BFT treatments, the experimental design is not a fully orthogonal factorial arrangement. Instead, treatment comparisons were structured to evaluate biologically relevant contrasts among production conditions.

Table 2. Proximate composition (mean ± standard deviation) of the experimental broodstock diets.

Variable	Dietary lipids		
	7%	11%	14%
Crude protein (%)	35.2 ± 0.3	35.1 ± 0.2	35.0 ± 0.4
Crude lipid (%)	7.1 ± 0.1	11.0 ± 0.2	14.2 ± 0.2
Ash (%)	11.8 ± 0.3	11.5 ± 0.2	11.4 ± 0.3
Fiber (%)	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
Gross energy (kJ g^{-1})	18.5 ± 0.2	20.3 ± 0.3	21.8 ± 0.3

Table 3. Experimental treatments used during larval rearing of *Macrobrachium rosenbergii*. BFT: biofloc technology; RAS: recirculating aquaculture system. RN: RAS with natural seawater; RA: RAS with solar-saltwork brine; BMN: BFT with molasses and natural seawater; BMA: BFT with molasses and solar-saltwork brine; BAN: BFT with corn starch and natural seawater; BAA: BFT with corn starch and solar-saltwork brine.

Treatment	Culture system	Salinity source	Carbon source
RN	RAS	Seawater	None
RA	RAS	Solar-saltwork brine	None
BMN	BFT	Seawater	Molasses
BMA	BFT	Solar-saltwork brine	Molasses
BAN	BFT	Seawater	Corn starch
BAA	BFT	Solar-saltwork brine	Corn starch

The six treatment conditions are summarized in Table 3. Each treatment included three replicate tanks. Broodstock originated from the domesticated population maintained at the Aquaculture Research Laboratory of ITBOCA. Larvae used in the larval-rearing experiment were obtained from spawnings produced during the preceding broodstock reproductive phase. A total of 9,000 larvae were assigned to each treatment and stocked at an initial density of 50 larvae L^{-1}.

Saline media preparation

Two saline media were prepared for the larval-rearing experiment. Natural seawater was collected at an initial salinity of 32 from El Canal beach, Alvarado, Veracruz, Mexico. Before use, seawater was disinfected with chlorine at 5 mg L^{-1}, allowed to stand for 24 h, and then vigorously aerated for 48 h to remove residual chlorine. Salinity was subsequently adjusted to 12 with disinfected freshwater. The second saline medium was prepared using commercial solar-saltwork brine (La Purísima®), with an initial salinity >50. For each 60 L experimental unit, we diluted 840 g of brine with previously disinfected freshwater to reach a final salinity of 12. Salinity in both media was verified daily using a calibrated refractometer.

Culture conditions

Larvae were reared in circular plastic tanks with a total capacity of 80 L and a working volume of 60 L. Water temperature was maintained at 29 ± 1°C, DO ≥5 mg L^{-1}, and pH at approximately 8.0. Continuous aeration was supplied to all experimental units.

In the RAS treatments, RN (natural seawater) and RA (solar-saltwork brine), the tanks operated under continuous recirculation and were connected to PVC biological filters (4-inch diameter) containing 500 g of previously washed and disinfected oyster shells as substrate for nitrifying bacteria. A cotton-mesh layer was placed inside each biofilter to retain suspended organic particles. An air-lift system returned water from the biofilters to the culture tanks. Each experimental unit received independent aeration, and screened covers were installed at the biofilter inlets to prevent accidental larval entrainment. Submersible 75-W heaters were used to maintain the required larval-rearing temperature.

The BFT systems consisted of 80 L circular plastic tanks with a working volume of 60 L and continuous bottom aeration. The biofloc systems were matured for 45 days before larval stocking. Each experimental unit was filled with brackish water adjusted to 12‰ according to the corresponding salinity treatment. Molasses or corn starch (Ingredion®) was used as the organic carbon source, whereas finely ground commercial shrimp feed (Silver Cup®, El Pedregal) was used as the nitrogen source. The C:N ratio was maintained at 7:1 through daily carbon supplementation. During maturation, water quality was monitored until stable conditions were reached, with ammonia concentrations < 0.01 mg L^{-1}, which were used as an indicator of system maturity. Larvae were stocked only after these conditions had been established.

Water-quality monitoring

Water quality was monitored in each larval-rearing unit throughout the experimental period. Temperature, pH, and salinity were measured using a portable digital multiparameter meter (Pusokei®). Dissolved oxygen was determined using a YSI 556 MPS meter, and settleable solids were measured using Imhoff cones. Ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations were determined using API® colorimetric kits designed for freshwater and brackish-water aquaria.

Feeding and husbandry

During larval rearing, feeding consisted of a combination of newly hatched *Artemia* nauplii and a laboratory-prepared moist diet. *Artemia* cysts (2 g per culture unit) were incubated for 24 h in 4 L containers at 32‰ salinity under continuous aeration. Newly hatched nauplii were collected using a 300-µm mesh and gradually acclimated to 12‰ before feeding.

The moist diet was prepared as a semi-solid formulation containing fish meal, defatted soybean meal, fresh egg yolk, beef liver, fresh squid, spirulina powder, fish oil, a vitamin-mineral premix, unflavored gelatin, and corn starch. Ingredients were homogenized for 5 min, cooked in a water bath for 120 min until a firm consistency was obtained, and stored at 4°C until use.

Larvae were fed the moist diet at 08:00 and 12:00 h to apparent satiation, distributed uniformly over the tank surface through a mesh strainer. Newly hatched *Artemia* nauplii were supplied at 16:00 h to ensure food availability during the nocturnal period. The amount of moist diet was adjusted according to larval stage and estimated consumption.

Response variables

Larval development was monitored daily until individuals reached the first postlarval stage (PL_1). Five larvae were collected daily from each experimental unit by pipetting and examined under an optical microscope at 10x-40x magnification. Larval stages were identified according to the morphological criteria described by New et al. (2010) for the 12 larval stages of *M. rosenbergii*. Progression to a new larval stage was recorded when ≥85% of the sampled population had reached that stage.

The response variables evaluated were larval survival (%) and developmental duration (days). Developmental duration was defined as the number of days from hatching to the first observation of PL_1. At the end of the larval-rearing period, the total number of postlarvae produced in each experimental unit was estimated using a standardized volumetric method involving tank homogenization, subsampling, counting, and extrapolation to the total culture volume. Larval survival was calculated as the percentage of postlarvae obtained relative to the initial number of larvae stocked.

Juvenile grow-out

Experimental design

Postlarvae produced during the larval rearing phase were subsequently transferred to juvenile grow-out to evaluate the effects of microbial management and carbon source on growth performance and male morphotype development. A completely randomized

design was established using three culture conditions: RAS, BFT with molasses, and BFT with corn starch. Because carbon supplementation was applied only under BFT conditions, the experimental design is not a fully orthogonal factorial arrangement. Consequently, treatment comparisons were based on biologically relevant contrasts among production systems and carbon supplementation strategies.

Each treatment included three replicate tanks (n = 3), for a total of nine experimental units. Juveniles (initial body weight: 1.87 ± 1.01 g; total length: 4.8 ± 0.7 cm) were stocked at a density of 22 ind m^{-2} in 1,200 L geomembrane tanks with a working volume of 1,135 L. Each tank was considered one experimental unit.

Culture conditions

Juveniles were reared in circular geomembrane tanks (1.7 m diameter × 0.5 m height) with a working volume of 1,135 L. Each experimental unit received independent aeration through a bottom diffuser hose connected to a central 10-HP centrifugal blower, maintaining dissolved oxygen above 5 mg L^{-1} throughout the experiment. Forty shelters were provided in each tank to reduce antagonistic interactions. Shelters consisted of 15-cm sections of 1-inch-diameter PVC pipe together with burlap mesh placed on the tank bottom.

The RAS treatment consisted of three experimental units of the same dimensions. Each unit was equipped with a biological filter constructed from 6-inch-diameter PVC pipe and filled with previously cleaned and disinfected oyster shells, which served as filtration medium and substrate for nitrifying bacteria. A cotton-mesh layer was included to retain solids. Water recirculation was maintained by an air-lift system connected to the central blower through ¼-inch air hoses, and each tank received continuous aeration through individual air stones. The same type of shelters used in the BFT treatments was provided in the RAS tanks.

The BFT systems were matured for 45 days before juvenile stocking. Each experimental unit was filled with previously treated freshwater, and biofloc development was initiated using either corn starch (Ingredion®) or commercial molasses as the organic carbon source according to treatment. Finely ground commercial shrimp feed (Silver Cup®, El Pedregal) was used as the nitrogen source. Continuous vigorous aeration was maintained throughout maturation. Ammonia was monitored daily, and the systems were considered mature when concentrations decreased below 0.01 mg L^{-1}. The C:N ratio was maintained at 15:1 by adding the corresponding carbon source daily or every third day as required. Juveniles were stocked after the biofloc systems had reached stable physi-cochemical conditions.

Feeding and husbandry

Juveniles were fed a commercial shrimp diet (Silver Cup®, El Pedregal) containing 35% crude protein and 7% crude lipid. The daily ration was set at 5% of total tank biomass and adjusted every 15 days based on biomass estimates obtained from routine sampling. Feed was supplied once daily at 16:00 and distributed uniformly over the tank surface to ensure all individuals could access it.

Growth was monitored every 15 days by randomly sampling 20 juveniles from each experimental unit. Individual body weight was measured using a balance with a precision of ±0.01 g, and total body length was recorded using an ichthyometer. These measurements were used to monitor growth and adjust feeding rates throughout the experimental period.

Response variables

At the end of the 180-day experimental period, all surviving individuals in each tank were counted to determine survival and were individually weighed and measured. Male prawns were classified into the three recognized morphotypes: small claw (SM), orange claw (OC), and blue claw (BC), following the classification proposed by Sagi & Ra'anan (1990).

The response variables evaluated were final body weight, weight gain, specific growth rate (SGR), apparent feed conversion ratio (AFCR), survival, biomass, total length, condition factor (K), and male morphotype frequency. Survival (%) = 100 × (final number of juveniles / initial number of juveniles); weight gain (%) = 100 × (final weight - initial weight) / initial weight; SGR (% d^{-1}) = 100 × [Ln(final weight) - Ln(initial weight)] / culture duration (d); AFCR = total feed supplied (dry weight) / biomass gain (wet weight); and K = 100 × body weight (g) / total $length^3$ (cm^3).

Statistical analysis

For all experiments, the tank was the experimental unit. Before statistical analyses, we screened data for outliers and tested normality with the Shapiro-Wilk test and for homogeneity of variances using Levene's test. When necessary, data were transformed (log_{10} for continuous variables and arcsine square-root transformation for percentage data) to meet the assumptions of parametric analyses.

In experiment 1, the effects of dietary lipid level were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for multiple comparisons ($\alpha = 0.05$).

In experiments 2 and 3, treatment effects were evaluated using ANOVA followed by *a priori* planned contrasts designed to test biologically relevant hypotheses. For the larval rearing experiment (experiment 2), contrasts compared culture systems (RAS *vs*. BFT) within each salinity source and carbon supplementation strategies within BFT treatments. For the juvenile grow-out experiment (experiment 3), planned contrasts evaluated differences among culture systems (RAS *vs*. BFT) and between the two carbon sources used within BFT (molasses *vs*. corn starch). Because carbon supplementation was evaluated exclusively within BFT treatments, planned contrasts were considered more appropriate than a fully factorial analysis.

Male morphotype frequencies (SM, OC, and BC) were analyzed using chi-square (χ^2) tests to detect overall differences in morphotype distribution among treatments. Additionally, multinomial generalized linear models (GLMs) with a logit link function were fitted to evaluate the effects of treatment on morphotype frequencies. When appropriate, Bonferroni correction was applied to account for multiple comparisons. Statistical significance was accepted at $\alpha = 0.05$. All analyses were performed using Statistica v13.5 (TIBCO Software Inc., Palo Alto, CA, USA).

Ethical statement

All experimental procedures involving animals were conducted in accordance with the institutional guidelines for the care and use of aquatic organisms in research at the Instituto Tecnológico de Boca del Río (TecNM-ITBOCA). Animal handling and sampling procedures were performed to minimize stress and unnecessary suffering throughout the study.

RESULTS

Broodstock conditioning

Reproductive performance

Broodstock reproductive performance was influenced by dietary lipid level after the 60-day conditioning period (Table 4). Females fed the experimental diets (7, 11, and 14% lipid) generally exhibited higher GSI, HSI, fecundity, egg diameter, and hatching success than those receiving the commercial control diet ($P < 0.05$). However, no consistent significant differences were detected among the three experimental lipid levels for most reproductive variables ($P > 0.05$).

Among the experimental diets, females receiving the 11% lipid treatment consistently showed the highest mean values for GSI, HSI, fecundity, egg diameter, and hatching success. Nevertheless, these responses were statistically comparable to those obtained with the 14% lipid diet ($P > 0.05$), indicating that reproductive performance was maintained across the evaluated lipid range. Egg diameter and hatching success were generally greater in females fed the experimental diets than in those fed the commercial control diet ($P < 0.05$).

Embryonic development duration did not differ significantly among dietary treatments ($P > 0.05$), suggesting that lipid inclusion affected reproductive quality rather than the duration of embryogenesis.

Larval rearing

Larval performance

Larval performance differed markedly among treatments according to salinity source, culture system, and carbon supplementation strategy (Fig. 2). Overall, seawater-based treatments consistently outperformed solar-saltwork brine treatments. In contrast, the carbon supplementation response depended on the saline medium.

Developmental duration to the first postlarval stage (PL_1) was shorter under seawater than under solar-saltwork brine in both culture systems. Within seawater-based BFT treatments, larvae receiving molasses supplementation (BMN) reached PL_1 significantly faster than those supplemented with corn starch (BAN) ($P < 0.05$). In contrast, neither brine-based BFT treatment (BMA: BFT with molasses and solar-saltwork brine; and BAA: BFT with corn starch and solar-saltwork brine) completed metamorphosis under the experimental conditions.

Survival followed a similar pattern. Under seawater conditions, larvae cultured in BFT supplemented with molasses exhibited significantly higher survival than those receiving corn starch ($P < 0.05$). Within the RAS treatments, survival was also higher in seawater (RN) than in solar-saltwork brine (RA) ($P < 0.05$). Both brine-based BFT treatments failed to produce postlarvae by the end of the experimental period.

Embryonic development duration did not differ significantly among treatments ($P > 0.05$), indicating that the observed differences were associated with post-hatch rearing conditions rather than embryonic development itself.

Table 4. Reproductive performance (mean ± standard deviation; n = 3 tanks) of *Macrobrachium rosenbergii* broodstock fed diets with different lipid levels. GSI: gonadosomatic index; HSI: hepatosomatic index. Different superscripts within the same column indicate significant differences ($P < 0.05$).

Diet (lipid %)	Female weight (g)	GSI (%)	HSI (%)	Fecundity (eggs female^{-1})	Egg diameter (mm)	Hatching success (%)	Embryonic development (days)
Commercial control	50.5 ± 4.2	6.2 ± 0.8^{b}	3.1 ± 0.4^{b}	23,915 ± 5,859^{b}	0.46 ± 0.02^{b}	72.4 ± 5.3^{b}	15.5 ± 0.0
7	53.1 ± 4.1	6.8 ± 0.6^{ab}	3.5 ± 0.3^{ab}	47,038 ± 4,184^{a}	0.47 ± 0.02^{ab}	80.1 ± 4.9^{ab}	15.5 ± 0.0
11	49.8 ± 4.6	7.4 ± 0.9^{a}	3.9 ± 0.5^{a}	55,525 ± 14,942^{a}	0.48 ± 0.01^{a}	86.3 ± 3.8^{a}	15.5 ± 0.0
14	50.3 ± 0.7	7.1 ± 0.7^{a}	3.8 ± 0.4^{a}	52,892 ± 3,432^{a}	0.47 ± 0.01^{ab}	83.5 ± 4.2^{a}	15.5 ± 0.0

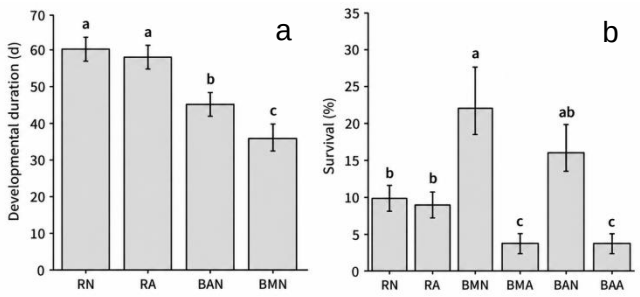


Figure 2. a) Larval development time and b) survival of *Macrobrachium rosenbergii* under contrasting salinity and culture conditions (mean ± standard deviation; n = 3 tanks). Different letters indicate significant differences among treatments ($P < 0.05$). RAS: recirculating aquaculture system; BFT: biofloc technology; RN: RAS with natural seawater; RA: RAS with solar-saltwork brine; BMN: BFT with molasses and natural seawater; BMA: BFT with molasses and solar-saltwork brine; BAN: BFT with corn starch and natural seawater; BAA: BFT with corn starch and solar-saltwork brine.

Water quality and biofloc characteristics

Water quality remained within the recommended ranges for larval culture throughout the experiment (Table 5). Total ammonia nitrogen (TAN), nitrite, and nitrate concentrations remained consistently low across all treatments. Biofloc treatments supplemented with molasses developed greater volumes of settleable solids than those supplemented with corn starch. Overall, water quality and biofloc characteristics differed among treatments in ways consistent with the observed larval performance.

In contrast, biofloc systems operated with solar-saltwork brine exhibited less stable floc development and failed to sustain successful larval metamorphosis under the experimental conditions. Despite maintaining acceptable physicochemical conditions throughout the trial, differences in water quality and biofloc characteristics were consistent with the biological responses observed among treatments.

Juvenile grow-out

Growth performance

Juvenile growth performance differed among culture conditions after the 180-day grow-out period, whereas survival remained comparable among treatments (Table 6). Final body weight was significantly higher in the RAS treatment than in BFT supplemented with corn starch ($P < 0.05$). In contrast, juveniles reared in BFT supplemented with molasses achieved intermediate final weights that did not differ significantly from those observed in RAS ($P > 0.05$).

Morphometric characteristics

Morphometric traits differed between sexes at the end of the grow-out period (Table 7). Females attained greater final body weight and total length than males ($P < 0.05$), whereas males exhibited significantly greater chela length and higher chela length-to-total length (CL:TL) ratios.

Table 5. Water quality and biofloc characteristics (mean ± standard deviation; n = 3 tanks) during larval rearing of *Macrobrachium rosenbergii*. RN: recirculating aquaculture system with natural seawater; RA: recirculating aquaculture system with brine; BAN: biofloc with corn starch and natural seawater; BAA: biofloc with corn starch and brine; BMN: biofloc with molasses and natural seawater; BMA: biofloc with molasses and brine. Values below the analytical detection limit are presented as <0.1 or <1, respectively.

Parameter	RN	RA	BAN	BAA	BMN	BMA	Recommended values (New et al. 2010)
pH	8.2 ± 0.1	8.2 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	6.5-8.5
Temperature (°C)	29.0 ± 0.7	29.0 ± 0.6	29.0 ± 0.5	29.0 ± 0.6	29.0 ± 0.5	29.0 ± 0.4	28-30
Salinity	12.5 ± 0.5	12.5 ± 0.5	12.5 ± 0.5	12.5 ± 0.5	12.5 ± 0.5	12.5 ± 0.5	12-15
Dissolved oxygen (mg L^{-1})	6.9 ± 0.3	7.0 ± 0.3	6.4 ± 0.2	6.3 ± 0.15	6.5 ± 0.2	6.4 ± 0.15	>5
Settleable solids (mL L^{-1})	0.5 ± 0.1	0.5 ± 0.1	7.0 ± 1.0	8.0 ± 0.5	9.5 ± 1.0	9.0 ± 0.5	<25
Ammonia (mg L^{-1})	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Nitrite (mg L^{-1})	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Nitrate (mg L^{-1})	<1	<1	<1	<1	<1	<1	0-100

Table 6. Growth performance (mean ± standard deviation; n = 3 tanks) of juvenile *Macrobrachium rosenbergii* under recirculating aquaculture system (RAS) and biofloc culture. AFCR: apparent feed conversion ratio; SGR: specific growth rate; PGI: individual weight gain; GPD: daily weight gain; CL:TL: chela length to total length ratio. Different superscripts in a row denote significant differences ($P < 0.05$)

Variable	Recirculation	Biofloc-corn starch	Biofloc-molasses
Survival (%)	63.33 ± 7.57	58.67 ± 9.87	56.0 ± 4.0
Final weight (g)	11.63 ± 1.05^{a}	9.38 ± 1.36^{b}	10.49 ± 1.09^{ab}
Weight gain (%)	538.56 ± 83.47	397.03 ± 50.21	458.68 ± 46.92
SGR (% d^{-1})	1.20 ± 0.08	1.03 ± 0.06	1.11 ± 0.05
PGI (g)	9.80 ± 1.14	7.49 ± 1.20	8.60 ± 1.14
GPD (g d^{-1})	0.067 ± 0.006	0.050 ± 0.010	0.057 ± 0.006
AFCR	3.3 ± 0.6	3.7 ± 0.59	3.8 ± 0.35
Biomass (g m^{-2})	369.49 ± 68.48	273.36 ± 44.63	293.80 ± 25.61
Total length (cm)	10.08 ± 1.44^{a}	9.34 ± 1.37^{b}	9.71 ± 1.42^{ab}
Condition factor (K)	1.06 ± 0.01	1.05 ± 0.04	1.07 ± 0.04
Chela length (cm)	5.95 ± 2.92	5.62 ± 3.58	5.20 ± 3.36
CL:TL ratio	0.59 ± 0.26	0.59 ± 0.31	0.53 ± 0.30

Among males, morphometric characteristics also differed according to morphotype (Table 8). Blue-claw (BC) males exhibited the greatest final body weight, total length, carapace length, chela length, and CL:TL ratio, followed by orange-claw (OC) and small-claw (SM) individuals ($P < 0.05$).

Male morphotype distribution

Male morphotype distribution varied among culture conditions (Fig. 3). The RAS treatment had a higher proportion of females. In contrast, biofloc treatments, particularly those supplemented with molasses, had higher frequencies of advanced male morphotypes (OC and BC) together with a lower proportion of SM individuals (Fig. 3a).

Mean body weight within each morphotype followed the expected hierarchical pattern (BC > OC > SM) across all culture conditions (Fig. 3b), indicating that the observed differences among treatments were primarily associated with morphotype frequency rather than with growth within individual morphotypes.

Table 7. Morphometric characteristics (mean ± standard deviation) of male and female *Macrobrachium rosenbergii* juveniles. CL:TL: ratio between chela length and total length. Different superscript letters in the same row indicate significant differences ($P < 0.05$).

Variable	Males	Females
Final weight (g)	9.64 ± 1.40^{b}	10.96 ± 1.62^{a}
Total length (cm)	9.59 ± 1.39^{b}	10.12 ± 1.35^{a}
Carapace length (cm)	2.84 ± 0.36^{b}	2.96 ± 0.37^{a}
Chela length (cm)	4.66 ± 2.97^{a}	1.36 ± 0.48^{b}
CL:TL ratio	0.46 ± 0.27^{a}	0.13 ± 0.05^{b}

DISCUSSION

Broodstock conditioning: dietary lipid requirements for reproductive performance

The present study indicates that dietary lipid supplementation within a physiologically adequate range supports reproductive performance in *M. rosenbergii* broodstock. Females receiving the experimental diets (7-14% lipid) exhibited higher GSI, HSI, fecundity, egg diameter, and hatching success than those fed the commercial control diet. In contrast, no consistent differences were detected among the experimental lipid levels. These findings suggest that reproductive performance depends primarily on achieving an adequate lipid supply rather than maximizing dietary lipid concentration.

This pattern agrees with previous studies showing that moderate dietary lipid inclusion supports ovarian maturation and reproductive performance without a proportional response once nutritional requirements are satisfied (Cavalli et al. 2000, Wouters et al. 2001, Song et al. 2024). Although females fed the 11% lipid diet consistently showed numerically superior reproductive indices, these values did not differ significantly from those obtained with the 14% diet, indicating that both diets provided sufficient lipid availability to sustain reproductive processes. Likewise, the comparable performance observed with the 7% lipid diet suggests that the lower inclusion of fish oil and animal-derived lipid sources did not compromise reproductive capacity under the present conditions.

Dietary lipids play multiple physiological roles during crustacean reproduction. Beyond serving as an energy source for vitellogenesis and embryogenesis, they provide essential structural components for cell membranes and precursors for vitellin synthesis and steroid hormones (Harrison 1990, Cavalli et al. 2000). The higher GSI and HSI values observed in females receiving the experimental diets therefore suggest greater hepatopancreatic activity and more efficient lipid mobilization toward ovarian development, processes regulated by endocrine pathways involving ecdysteroids and methyl farnesoate (Laufer et al. 1998, Sagi & Aflalo 2005). This interpretation also aligns with recent evidence emphasizing the central role of dietary lipids in crustacean ovarian development, where adequate lipid supply supports energetic demands, membrane synthesis, steroidogenic pathways, and vitellogenesis (Dildar et al. 2025).

The larger egg diameter and higher hatching success recorded in the experimental treatments further support the importance of adequate lipid nutrition for egg quality. Larger oocytes generally contain greater yolk reserves, providing sufficient energy for embryonic development and early larval growth (Izquierdo et al. 2001, Wouters et al. 2001). In contrast, dietary treatment did not affect embryonic development time, indicating that lipid supply primarily influenced reproductive quality rather than the duration of embryogenesis.

Collectively, these findings indicate that broodstock diets containing 7-14% lipid adequately supported reproductive performance in *M. rosenbergii* under the present conditions. Rather than identifying a single optimal lipid concentration, the results suggest that maintaining dietary lipid levels within an appropriate physiological range is sufficient to support broodstock reproductive condition and provide high-quality offspring for the subsequent larval phase. More broadly, recent observations in *M. rosenbergii* under Mexican culture conditions have also shown that broodstock reproductive responses are influenced by husbandry factors such as stocking configuration and sex ratio, further emphasizing the importance of stage-specific management during the reproductive phase (Peña-Almaraz et al. 2026).

Larval rearing: interaction between ionic composition and microbial management

The present results indicate that larval performance in *M. rosenbergii* was associated with both the saline medium and carbon-driven microbial management under the evaluated conditions. Larvae reared in seawater-based systems exhibited higher survival and faster development than those cultured in solar-saltwork brine. At the same time, the positive effects of molasses supplementation were evident only under seawater conditions. These findings indicate that microbial management alone cannot compensate for suboptimal ionic conditions during larval development.

Table 8. Morphometric characteristics (mean ± standard deviation) of *Macrobrachium rosenbergii* male morphotypes. CL:TL: ratio between chela length and total length. Different superscript letters in the same row indicate significant differences ($P < 0.05$).

Variable	Small claw (SM)	Orange claw (OC)	Blue claw (BC)
Final weight (g)	7.32 ± 1.32^{c}	10.87 ± 1.42^{b}	17.90 ± 2.63^{a}
Total length (cm)	8.84 ± 1.26^{c}	9.95 ± 1.33^{b}	11.87 ± 1.58^{a}
Carapace length (cm)	2.64 ± 0.31^{c}	2.87 ± 0.34^{b}	3.35 ± 0.45^{a}
Chela length (cm)	2.36 ± 1.17^{c}	4.89 ± 1.74^{b}	8.99 ± 2.63^{a}
CL:TL ratio	0.26 ± 0.12^{c}	0.49 ± 0.18^{b}	0.76 ± 0.20^{a}

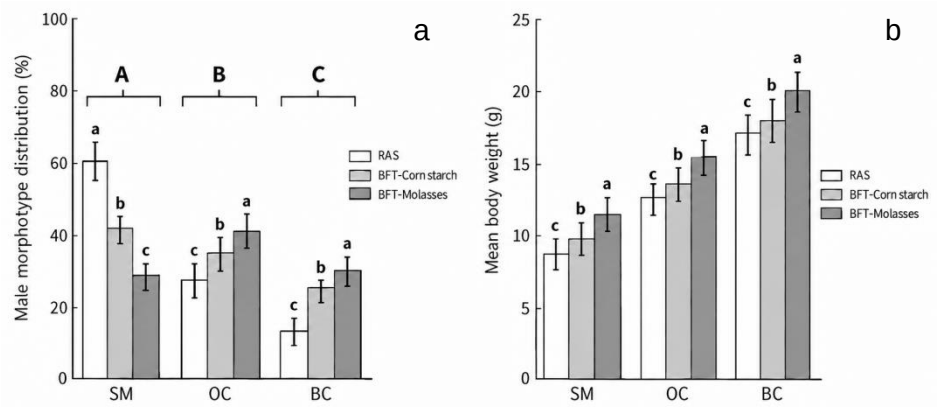


Figure 3. a) Male morphotype distribution and b) body weight by morphotype in juvenile *Macrobrachium rosenbergii*. RAS: recirculating aquaculture systems; BFT: biofloc technology; SM: small claw; OC: orange claw; BC: blue claw. Values are mean ± standard deviation. Uppercase letters indicate significant differences among treatments, and lowercase letters indicate significant differences among morphotypes within treatments ($P < 0.05$).

Although both saline media were adjusted to the same nominal salinity (12), seawater consistently produced superior biological performance. This supports previous studies showing that successful larval development depends more on ionic composition than on salinity per se (Charmantier & Anger 2011, Henry et al. 2012). Because the ionic composition of the saline sources was not analytically determined in the present study, published reference profiles were used only to provide physiological context (Table S1). Differences in essential ions such as Ca^{2+}, Mg^{2+}, and K^{+} have been associated with osmoregulation, molting, and metabolic homeostasis (Millero et al. 2008, De Medeiros et al. 2012, Vicari et al. 2022), providing a plausible explanation for the lower survival and delayed metamorphosis observed in larvae reared with solar-saltwork brine.

The response to carbon supplementation further suggested that BFT performance was influenced by the surrounding physicochemical environment. Under seawater conditions, molasses-supported biofloc systems promoted higher survival and faster larval development than starch-based systems, whereas this advantage disappeared when brine was used as the salinity source. These results suggest that heterotrophic microbial communities can improve larval performance only when environmental conditions adequately support both microbial activity and larval physiology.

Molasses likely enhanced biofloc performance because heterotrophic bacteria rapidly assimilate its readily available carbohydrates, stimulating microbial growth and biofloc formation (Ebeling et al. 2006, Emerenciano et al. 2013). In contrast, bacteria must enzymatically hydrolyze starch before they can use it, resulting in slower microbial development and biofloc formation (Tinh et al. 2021). Recent evidence in *M. rosenbergii* further supports the importance of carbon management in biofloc systems, as different C:N ratios under molasses-based BFT significantly affected growth, survival, feed conversion, and microbial abundance during the nursery phase (Halim et al. 2025). Although inorganic nitrogen concentrations

remained similarly low across treatments, molasses-based systems developed greater volumes of settleable solids, reflecting a greater accumulation of suspended biofloc biomass. This enhanced biofloc development was accompanied by higher larval survival and faster development under natural seawater conditions, suggesting that molasses supplementation benefited larvae primarily through increased microbial biomass and biofloc availability rather than changes in conventional water-quality parameters. Moreover, bioflocs provide an additional nutritional resource through microbial protein, lipids, vitamins, poly-β-hydroxybutyrate (PHB), and other bioactive compounds that may enhance digestive function and stress tolerance during larval development (Crab et al. 2010, Emerenciano et al. 2013).

Despite these microbial benefits, the complete failure of metamorphosis in the brine-based biofloc treatments clearly indicates that biofloc cannot overcome physiological limitations imposed by an unsuitable ionic environment. Thus, optimal larval production appears to require simultaneous optimization of ionic composition and microbial processes, rather than either factor alone. Practically, these findings support using seawater-based systems supplemented with molasses as an effective management strategy to improve larval survival and successful development to the postlarval stage in *M. rosenbergii*.

Juvenile grow-out: carbon management and production performance

Juvenile performance in *M. rosenbergii* was influenced primarily by carbon management rather than by culture system alone. Although survival remained similar across treatments, growth performance differed by the carbon source used in the biofloc systems. Juveniles reared in RAS achieved the highest final body weight. In contrast, those cultured in molasses-based BFT showed comparable growth, while those maintained in starch-based BFT showed significantly lower performance. These findings suggest that treatment differences were more closely tied to microbial management than to the production system itself.

This interpretation aligns with previous studies reporting that BFT benefits depend largely on operational management and the characteristics of the microbial community. Consequently, biofloc should not be regarded as a single production strategy, but rather as a microbial-based process whose effectiveness depends on appropriate management of carbon inputs, nutrient dynamics, and system conditions (Crab et al. 2010, Hargreaves 2013).

The superior performance of molasses-based BFT likely reflects the rapid availability of simple carbohydrates that stimulate heterotrophic bacterial growth and nitrogen assimilation (Ebeling et al. 2006, Emerenciano et al. 2013). By contrast, microbes must enzymatically hydrolyze starch before they can metabolize it (Tinh et al. 2021), delaying bacterial development and potentially reducing nutrient-recycling efficiency. These mechanisms align with the more stable water-quality dynamics observed in the molasses treatments and the associated improvements in juvenile growth.

Bioflocs may also contribute directly to nutrition. Microbial aggregates contain protein, lipids, vitamins, and other bioactive compounds that can supplement formulated feeds and improve nutrient utilization (Anand et al. 2014, Avnimelech, 2015). However, the results indicate that these nutritional benefits alone were insufficient to improve RAS performance consistently. Instead, they appear to complement conventional feeding when microbial communities remain stable and operational conditions are properly maintained.

Although feed conversion tended to be lower in RAS than in the biofloc treatments, this difference may reflect the energetic costs associated with maintaining dense microbial communities and suspended solids, which can partially offset the nutritional contribution of bioflocs (Hargreaves 2013). Therefore, successful BFT implementation depends on balancing nutrient recycling, microbial productivity, and system management rather than maximizing microbial biomass alone.

Culture conditions and male morphotype differentiation

Beyond their effects on growth, the evaluated culture conditions also influenced male morphotype distribution, suggesting that production systems may affect the social structure of juvenile *M. rosenbergii* populations. Biofloc systems supplemented with molasses showed a higher proportion of advanced male morphotypes (OC and BC) and a lower frequency of small-claw males than starch-based systems. These findings indicate that culture conditions may influence not only somatic growth but also the progression of male differentiation.

Male morphotype differentiation in *M. rosenbergii* is a complex process regulated by interactions among nutritional status, social hierarchy, and endocrine signaling, particularly through androgenic gland hormone (AGH), which coordinates male sexual differentiation, somatic growth, and morphotype transition (Sagi & Ra'anan 1990, Sagi & Aflalo 2005).

Under favorable environmental conditions, males progressively develop from the SM morphotype to the OC and finally the dominant BC morphotype, a process closely associated with increased energy availability and social interactions (Ra'anan & Cohen 1985, Karplus & Barki 2019). Recent evidence further shows that size disparity within mixed *M. rosenbergii* populations can strongly influence growth, survival, digestive metabolism, and physiological condition, with smaller individuals showing progressively reduced performance as size heterogeneity increases (Li et al. 2025).

The higher proportion of OC and BC males observed in the molasses-based biofloc systems may therefore reflect improved energetic conditions resulting from more efficient microbial nutrient recycling and the supplementary nutritional contribution of bioflocs. Rather than directly inducing morphotype transition, these conditions likely provided the energetic resources males needed to progress through their natural developmental hierarchy. This interpretation is consistent with the comparable overall growth observed between molasses-based BFT and RAS, while suggesting that differences among systems became more evident in population structure than in average body weight alone.

Beyond nutritional effects, the structural complexity generated by suspended biofloc particles may have influenced behavioral interactions among individuals. More heterogeneous environments may modify encounter frequency, territorial interactions, and dominance establishment, potentially stabilizing social hierarchies and facilitating morphotype progression (Tidwell et al. 1999). Although these behavioral mechanisms were not directly evaluated in the present study, they provide a plausible explanation for the observed differences in morphotype frequency.

Morphometric differences among treatments were generally less pronounced than differences in morphotype frequency. Consequently, the primary response to culture conditions appears to involve changes in the proportion of males reaching advanced morphotypes rather than substantial alterations in the size attained within each morphotype. This distinction suggests that population structure may represent a more sensitive indicator of production-system effects than conventional growth variables alone.

The present study indicates that no single management strategy consistently delivered the best performance across the evaluated production stages of *M. rosenbergii*. Instead, different production stages responded to distinct nutritional and environmental conditions, emphasizing the importance of stage-specific management rather than a universal culture strategy.

During broodstock conditioning, maintaining dietary lipid levels within an appropriate physiological range improved reproductive performance, with no evidence that higher lipid inclusion provided additional benefits. During larval rearing, successful production depended on optimizing ionic composition and microbial management, as seawater-based systems supplemented with molasses consistently produced higher survival and faster larval development. Finally, during juvenile grow-out, biofloc performance depended primarily on carbon management, with molasses-based systems achieving growth comparable to RAS while also promoting a greater proportion of advanced male morphotypes.

Collectively, these findings indicate that different management factors influence biological performance at successive stages of freshwater prawn production through distinct physiological and microbial processes. Conventional production indicators such as growth, survival, and feed conversion describe overall system performance. In contrast, reproductive traits, larval development, and male morphotype distribution provide complementary information regarding physiological condition and population structure. Consequently, evaluating these biological responses together offers a more comprehensive assessment of production strategies than relying solely on traditional performance metrics.

Rather than identifying a universally superior production system, this study supports the idea that freshwater prawn culture can be improved by matching management practices to the physiological requirements of each developmental stage. Together, the three independent experiments provide a practical stage-specific framework for broodstock conditioning, larval rearing, and juvenile grow-out.

CONCLUSIONS

This study shows that different stages of *M. rosenbergii* production respond to distinct nutritional, environmental, and microbial management strategies. The sequential evaluation of broodstock conditioning, larval rearing, and juvenile grow-out showed that each developmental stage responded differently to nutritional, environmental, and microbial management practices.

Broodstock reproductive performance was maintained across dietary lipid levels ranging from 7 to 14%, indicating that achieving an adequate physiological lipid supply is more important than maximizing dietary lipid inclusion. During larval culture, seawater-based systems combined with molasses-supported biofloc consistently produced higher survival and faster larval development, highlighting the importance of simultaneously optimizing ionic composition and microbial processes. During juvenile grow-out, molasses-based biofloc achieved growth comparable to RAS while promoting a greater proportion of advanced male morphotypes, indicating that carbon management influences not only production performance but also population structure.

Collectively, these findings show that reproductive performance, larval development, and juvenile production respond to different physiological and microbial management factors across successive developmental stages. Beyond conventional production indicators such as growth, survival, and feed conversion, reproductive performance, larval development, and male morphotype distribution provide complementary biological information that improves the evaluation of production systems.

Rather than identifying a universally superior culture system, these findings provide useful information for improving stage-specific management of *M. rosenbergii*, with nutritional strategies, salinity source, and carbon management considered according to the physiological requirements of each developmental stage.

Credit author contribution

C.I. Pérez-Rostro: conceptualization, methodology, supervision, project administration, funding acquisition, writing-original draft and writing-review & editing. G. Juárez-Sánchez: investigation, data curation, formal analysis, visualization and writing-original draft. M.P. Hernández-Vergara: methodology, supervision, validation and writing-review & editing. A. Gallardo-Colli: formal analysis, validation and writing-review & editing. All authors read and approved the final version of the manuscript.

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Author note on AI assistance

The authors used ChatGPT (OpenAI, San Francisco, CA, USA) to assist in language editing and consistency checking during manuscript preparation. The authors reviewed and verified all AI-generated suggestions to ensure accuracy, clarity, and compliance with scientific and ethical standards. The authors did not use AI tools for data analysis, interpretation, or decision-making.

Ethical approval

All experimental procedures involving *Macrobrachium rosenbergii* were conducted in accordance with the ethical guidelines for using aquatic organisms established by Tecnológico Nacional de México and complied with national animal welfare regulations for research purposes.

Conflicts of interest

The authors declare that they have no commercial or financial conflicts of interest related to the content of this publication.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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REFERENCES

Anand, P.S., Kohli, M.P.S., Kumar, S., et al. 2014. Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture, 418-419: 108-115. doi: 10.1016/j.aquaculture.2013.09.051

Association of Official Agricultural Chemists (AOAC) International. 2005. Official methods of analysis.

Horwitz, W. & Latimer, G.W. (Eds.). AOAC International, Gaithersburg.

American Public Health Association, American Water Works Association, World Economic Forum (APHA, AWWA & WEF). 2017. Standard methods for the examination of water and wastewater. APHA, Washington, DC.

Avnimelech, Y. 2015. Biofloc technology: A practical guide book. World Aquaculture Society, Baton Rouge.

Barnes, H. 1954. Some tables for the ionic composition of sea water. Journal of Experimental Biology, 31: 582-588. doi: 10.1242/jeb.31.4.582

Cavalli, R.O., Lavens, P. & Sorgeloos, P. 1999. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid compositions. Aquaculture, 179: 387-402. doi: 10.1016/S0044-8486(99)00173-8

Cavalli, R.O., Lavens, P. & Sorgeloos, P. 2000. Broodstock nutrition and maturation diets. In: New, M.B. & Valenti, W.C. (Eds.). Freshwater prawn culture: The farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, pp. 89-111.

Charmantier, G. & Anger, K. 2011. Ontogeny of osmoregulatory patterns in the South American shrimp *Macrobrachium amazonicum*: loss of hypo-regulation in a land-locked population indicates phylogenetic separation from estuarine ancestors. Journal of Experimental Marine Biology and Ecology, 396: 89-98. doi: 10.1016/j.jembe.2010.10.013

Crab, R., Chielens, B., Wille, M., et al. 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquaculture Research, 41: 559-567. doi: 10.1111/j.1365-2109.2009.02353.x

De Medeiros-Rocha, R., Costa, D.F.S., Lucena-Filho, M.A., et al. 2012. Brazilian solar saltworks: ancient uses and future possibilities. Aquatic Biosystems, 8: 8. doi: 10.1186/2046-9063-8-8

Dildar, T., Cui, W. & Ma, H. 2025. Physiology of ovarian development in crustaceans: interactions among hormones, nutrients, and environmental factors from integrated perspectives. Aquaculture Nutrition, 2025: 4900891. doi: 10.1155/anu/4900891.

Ebeling, J.M., Timmons, M.B. & Bisogni, J.J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture, 257: 346-358. doi: 10.1016/j.aquaculture.2006.03.019

Emerenciano, M., Gaxiola, G. & Cuzon, G. 2013. Biofloc technology (BFT): a review for aquaculture application and animal food industry. In: Matovic, M.D. (Ed.). Biomass now: Cultivation and utilization. InTech, Rijeka, pp. 301-328. doi: 10.5772/53902

Food and Agriculture Organization (FAO). 2026. *Macrobrachium rosenbergii*. Cultured aquatic species information program. FAO, Rome. [https://www.fao.org/fishery/en/culturedspecies/macrobrachium_rosenbergii]. Reviewed: April 10, 2026.

Halim, M.A., Aziz, D., Arshad, A., et al. 2025. Effects of C/N ratio for bi-culture of *Litopenaeus vannamei* and *Macrobrachium rosenbergii* in a molasses-based biofloc system. Aquaculture, Fish and Fisheries, 5: e70032. doi: 10.1002/aff2.70032

Hargreaves, J.A. 2013. Biofloc production systems for aquaculture. SRAC Publication No. 4503. Southern Regional Aquaculture Center, Stoneville.

Harrison, K.E. 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. Journal of Shellfish Research, 9: 1-28.

Henry, R.P., Lucu, Č., Onken, H., et al. 2012. Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals. Frontiers in Physiology, 3: 431. doi: 10.3389/fphys.2012.00431

Izquierdo, M.S., Fernández-Palacios, H. & Tacon, A.G.J. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197: 25-42. doi: 10.1016/S0044-8486(01)00581-6

Karplus, I. & Barki, A. 2019. Male morphotypes and alternative mating tactics in freshwater prawns of the genus *Macrobrachium*: a review. Reviews in Aquaculture, 11: 925-940. doi: 10.1111/raq.12273

Laufer, H., Biggers, W.J. & Ahl, J.S. 1998. Stimulation of ovarian maturation in the crayfish *Procambarus clarkii* by methyl farnesoate. General and Comparative Endocrinology, 111: 113-118. doi: 10.1006/gcen.1998.7109

Li, C., Zhang, X., Chen, Y., et al. 2023. Optimized utilization of organic carbon in aquaculture biofloc systems: a review. Fishes, 8: 465. doi: 10.3390/fishes8090465

Li, X., Zhou, H., You, S., et al. 2025. Impact of size disparity on growth performance, digestive metabolism, and antioxidant capacity of *Macrobrachium rosenbergii* under mixed culture conditions. Aquaculture International, 33: 372. doi: 10.1007/s10499-025-02041-5

Miglio, M.C., Zaga, B., Gastelu, J.C., et al. 2021. Survival and metamorphosis of giant river prawn *Macrobra-*

chium rosenbergii larvae in a commercial recirculation system with artificial seawater. Aquaculture Research, 52: 6063-6073. doi: 10.1111/are.15468

Millero, F.J., Feistel, R., Wright, D.G., et al. 2008. The composition of standard seawater and the definition of the reference-composition salinity scale. Deep-Sea Research Part I: Oceanographic Research Papers, 55: 50-72. doi: 10.1016/j.dsr.2007.10.001

New, M.B. 2002. Farming freshwater prawns: A manual for the culture of the giant river prawn *Macrobrachium rosenbergii*. FAO Fisheries Technical Paper No. 428. FAO, Rome.

New, M.B., Valenti, W.C., Tidwell, J.H., et al. 2010. Freshwater prawns: biology and farming. Wiley-Blackwell, Oxford. doi: 10.1002/9781444314649

Peña-Almaraz, O.A., Reta-Mendiola, J.L., Vega-Villasante, F., et al. 2026. Social behavior and reproductive development of the Malaysian prawn (*Macrobrachium rosenbergii*) under different stocking densities and sex ratios in Veracruz, Mexico. Agrociencia, 60: 3487. doi: 10.47163/agrociencia.v60i5.3487

Ra'anan, Z. & Cohen, D. 1985. Ontogeny of social structure and population dynamics in the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man). In: Wenner, A.M. (Ed.). Crustacean issues 3: Factors in adult growth. A.A. Balkema, Rotterdam, pp. 277-311.

Remita, F., Zatout, M., Hacini, M., et al. 2025. Major ionic evolution of Na-Mg-Cl brines in solar saltworks and their processing optimization: a case study from Chott Melrhir Salt Lake, Algeria. Geological Journal, 60: 1825-1839. doi: 10.1002/gj.5232

Sagi, A. & Aflalo, E.D. 2005. The androgenic gland and monosex culture of freshwater prawn *Macrobrachium rosenbergii* (De Man): a biotechnological perspective. Aquaculture Research, 36: 231-237. doi: 10.1111/j.1365-2109.2005.01238.x

Sagi, A. & Ra'anan, Z. 1990. Effect of androgenic gland ablation on morphotypic differentiation and sexual characteristics of male freshwater prawns *Macrobrachium rosenbergii*. General and Comparative Endocrinology, 77: 15-22. doi: 10.1016/0016-6480(90)90201-V

Song, J., Jian, Y., Xie, Y., et al. 2024. The dietary lipid requirement for ovarian maturation and health in female giant river prawn, *Macrobrachium rosenbergii* broodstock. Aquaculture Nutrition, 2024: 7462841. doi: 10.1155/2024/7462841

Tidwell, J.H., Coyle, S.D., Weibel, C., et al. 1999. Effects and interactions of stocking density and added substrate on production and population structure of freshwater prawns, *Macrobrachium rosenbergii*. Journal of the World Aquaculture Society, 30: 174-179. doi: 10.1111/j.1749-7345.1999.tb00864.x

Tinh, N.T., Hai, T.N., Minh, N.V., et al. 2021. Effects of carbohydrate sources on a biofloc nursery system for whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture, 531: 735795. doi: 10.1016/j.aquaculture.2020.735795

Vicari, F., Randazzo, S., López, J., et al. 2022. Mining minerals and critical raw materials from bittern: Understanding metal ions fate in saltwork ponds. Science of the Total Environment, 847: 157544. doi: 10.1016/j.scitotenv.2022.157544

Wouters, R., Lavens, P., Nieto, J., et al. 2001. Penaeid shrimp broodstock nutrition: an updated review on research and development. Aquaculture, 202: 1-21. doi: 10.1016/S0044-8486(01)00570-1

Xu, W.J. & Pan, L.Q. 2012. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C:N ratio in feed. Aquaculture, 356-357: 147-152. doi: 10.1016/j.aquaculture.2012.05.025

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SUPPLEMENTARY MATERIAL

Table S1. Reference ionic composition of seawater and solar-saltwork brine at 12 ‰ (mmol L^{-1}). Values compiled from published sources, including Barnes (1954), Millero et al. (2008), New et al. (2010), De Medeiros et al. (2012), Vicari et al. (2022), and Remita et al. (2025). Values are presented for comparative purposes and were not measured in the present study.

Ion	Seawater (mmol L^{-1})	Brine (mmol L^{-1})
Na^+	157.5	94.5-141.7
K^+	3.3	5.0-10.0
Ca^{2+}	3.4	0.3-1.7
Mg^{2+}	17.9	21.5-35.9
Cl^-	183.6	146.8-201.9
SO_4^{2-}	3.2	4.1-7.9
HCO_3^-	0.8	0.2-0.4