Effects of carbon sources on mud crab (*Scylla paramamosain*) larval rearing using biofloc technology

[1]Chau T. Tao, [2]Tran T. P. Lan, [1]Ho N. Truong, [1]Ly V. Khanh, [1]Tran N. Hai, [1]Nguyen V. Hoa

[1] College of Aquaculture and Fisheries, Can Tho University, Can Tho City, Vietnam; [2] Tra Vinh University, Vinh Long Province, Vietnam. Corresponding author: N. V. Hoa, nvhoa@ctu.edu.vn

Abstract. This study evaluated the effects of different carbon sources on the larval rearing of the mud crab (*Scylla paramamosain*) using biofloc technology. The experiment comprised five treatments, three replicates each with different carbon sources: (i) no carbon addition (control), (ii) granulated sugar, (iii) rice flour, (iv) wheat flour and (v) corn starch. Experimental larvae at the Zoea 1 stage were stocked in 250-L tanks at a density of 200 ind L^{-1}. Carbon sources were supplemented daily, starting from the Zoea 3 stage at a C:N ratio of 20. The results indicated that total ammonia nitrogen, nitrite, total bacterial density and *Vibrio* counts were significantly lower in carbon-supplemented biofloc treatments when compared to the control ($p < 0.05$). Conversely, larval stage index, growth in length, survival rate and crab 1 yield were significantly higher in biofloc treatments ($p < 0.05$). The rice flour treatment achieved the highest survival rate (9.89±0.17%) and Crab 1 yield (19,772±337 ind m^{-3}), showing significant superiority over all other treatments ($p < 0.05$). Therefore, rice flour is recommended as the most suitable carbon source for biofloc-based mud crab larval rearing.

Keywords: larval metamorphosis, mud crab larvae, rice flour, survival rate.

Introduction. Biofloc technology (BFT) is recognised as a highly effective biological solution to enhance sustainability in modern aquaculture. Numerous studies have demonstrated that biofloc not only improves water quality through the absorption and conversion of toxic nitrogenous compounds but also provides a rich natural food source containing proteins, lipids, vitamins, minerals and bioactive compounds for culture organisms (Crab et al., 2012). Furthermore, the microbial community that develops in biofloc systems can outcompete pathogenic bacteria, stimulate host immune responses and enhance resistance against environmental stressors (De Schryver et al., 2008; Avnimelech, 2012; Khanjani et al., 2023). Consequently, BFT has been widely adopted in nursery and grow-out systems for various penaeid shrimp and other aquatic species.

Mud crab hatchery seed production continues to face major constraints, notably low and inconsistent larval survival rates. As reported by Quinitio & Parado-Estepa (2008), mud crab (*Scylla paramamosain*) larval survival from zoea to megalopa in commercial hatcheries typically ranges between 5 and 10%. A field survey of 34 mud crab hatcheries across the Mekong Delta, Vietnam, revealed an average crab 1 survival rate of only 4.6% (Hai et al., 2018). This low performance is primarily attributed to unstable water quality, elevated bacterial loads and a lack of optimised nutritional regimes. To improve efficiency and stability in mud crab seed production, the precise synchronisation of culture systems, environmental conditions, nutritional management and rigorous biosecurity protocols must be maintained (Hai et al., 2017).

Given these ongoing hatchery challenges, the application of BFT represents an innovative strategy capable of simultaneously addressing water quality and nutritional requirements in mud crab larval culture tanks. The efficiency of a biofloc system depends strongly on the organic carbon source utilised, as carbon input dictates heterotrophic bacterial proliferation, floc structure and nutritional composition (Crab et al., 2012). Therefore, evaluating different carbon sources is essential to optimise biofloc quality, stabilise the rearing environment and improve survival during mud crab larval development. The present study evaluated the effects of different carbon sources on the larval rearing of the mud crab using BFT, with the aim of identifying the optimal carbon source for sustainable hatchery seed production.

Material and Method

Location and the period of study. The experiment was conducted from June to July, 2026 at Crustacean Hatchery of the College of Aquaculture and Fisheries, Can Tho University.

Water preparation and broodstock management. Seawater with a salinity of 28 g L^{-1} was treated with chlorine at a dose of 50 g m^{-3}, followed by vigorous aeration until chlorine residues were completely removed. Alkalinity was adjusted to 120 mg $CaCO_3$ L^{-1} using sodium bicarbonate ($NaHCO_3$) according to Khanh et al. (2015), and heavy metals were reduced using 10 g m^{-3} ethylenediaminetetraacetic acid. The treated seawater was filtered through a 0.1 µm micro-cartridge filter into storage tanks before being utilised for broodstock conditioning and larval rearing.

Wild-caught gravid female mud crabs were transported in styrofoam boxes (35 cm x 50 cm x 35 cm) containing three female crabs each (weighing 400-500 g per crab); aeration was provided during transport, and the crabs were not fed. Transportation to the Crustacean Hatchery of the College of Aquaculture and Fisheries, Can Tho University, Vietnam lasted 4-5 hours. Crabs were maintained in spawning tanks until hatching. Egg-bearing crabs were reared in 120-liter plastic tanks; the water was completely changed daily, and the crabs were fed blood cockles once a day. Upon hatching, healthy zoea 1 larvae displaying strong positive phototaxis and active swimming were selected for stocking. Larvae were disinfected with a 200 mg L^{-1} formalin bath for 30 seconds prior to distribution into experimental tanks.

Carbon sources and biofloc preparation. Four carbon sources were evaluated: Bien Hoa granulated cane sugar (55.5% carbon (C)), Tai Ky wheat flour (74.4% C), Vinh Thuan rice flour (84.2% C), and Vinh Thuan corn starch (87.9% C). Biofloc inocula were prepared by mixing each carbon source with warm water (60°C) at a 1:3 ratio, stirring thoroughly and pre-fermenting for 48 hours prior to tank addition. Carbon addition rates were calculated based on the crude protein content of artificial feeds and *Artemia* nauplii supplied during each larval stage, according to the stoichiometric formula of Avnimelech (2015).

Experimental design and operation. The experiment comprised five treatments arranged in a completely randomised design (Figure 1) with three replicates per treatment at a target C:N ratio of 20 (Hosain et al., 2021). Daily carbon addition was initiated at the zoea 3 stage (Tao et al., 2019) as follows: control: no carbon addition; treatment 1: granulated cane sugar; treatment 2: rice flour; treatment 3: wheat flour; treatment 4: corn starch.

Larvae were stocked into 250-L fibreglass tanks at an initial density of 200 ind L^{-1}. Continuous aeration was provided to maintain biofloc particles in suspension. Water was not exchanged at any point during the culture period. Larval behaviour and health status were observed daily. Zoea 1 and zoea 2 larvae were fed Vinh Chau *Artemia* umbrellas four times daily (00:00, 06:00, 12:00, 18:00 h) at a rate of 1-2 g m^{-3} application^{-1}. From zoea 3 to zoea 5, larvae received an artificial diet (Larviva PL200) four times daily (03:00, 09:00, 15:00, 21:00 h) at 1-2 g m^{-3} application^{-1} and DHA SELCO-enriched *Artemia* nauplii (1 g per 1,000,000 nauplii) four times daily at 2-3 g m^{-3} application^{-1}. From megalopa to crab 1, larvae were fed Larviva PL300 (2-3 g m^{-3}) and DHA SELCO-enriched *Artemia* (3-4 g m^{-3}). Upon reaching the megalopa stage, three net sheets (30 cm × 50 cm, mesh size 1 mm) were placed in each tank as substrates to minimise cannibalism.

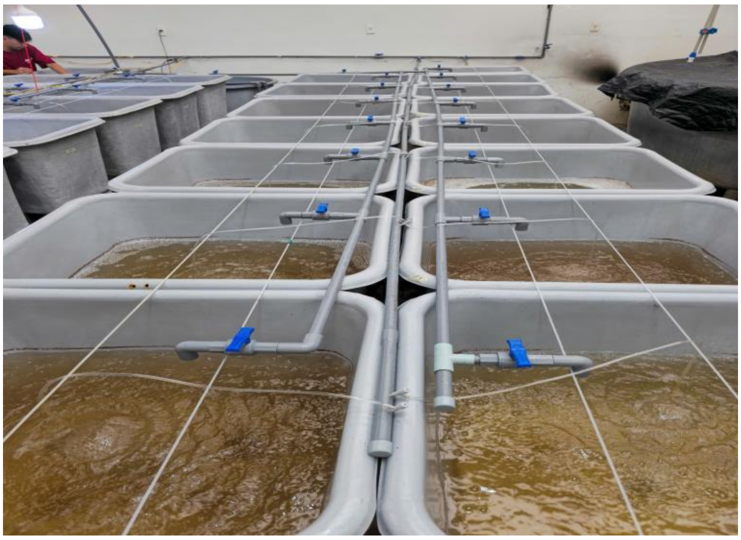

Figure 1. Experimental system.

Data collection and analytical procedures. Water temperature and pH were measured twice daily (morning and afternoon) using a digital pH/temperature meter. Dissolved oxygen (DO) was measured every 3 days. Alkalinity, total ammonia nitrogen (TAN) and nitrite (NO_2^-) were analysed every 3 days using standard spectrophotometric methods (APHA, 2005). Biofloc volume (FV, mL L^{-1}) was measured at the zoea 5, megalopa and crab 1 stages using Imhoff cones after 30 minutes of settling. Floc dimensions (length and width) were measured for 10 randomly selected floc particles per tank under a microscope equipped with a calibrated ocular micrometer.

Bacterial counts (total heterotrophic bacteria and *Vibrio* spp.) in water were quantified weekly (days 7, 14 and 21) and in crab 1 tissue at harvest. Total bacterial counts were enumerated on nutrient agar containing 1.5% NaCl (NA), while *Vibrio* counts were determined on thiosulfate citrate bile salts sucrose (TCBS) agar (Huys, 2002).

Larval metamorphosis stage index (LSI) was evaluated every 3 days by inspecting 10 larvae per tank using the following formula:

$$LSI = \Sigma(ni \times Si) / \Sigma ni$$

where: Si is the stage index and ni is the number of larvae at stage Si. Larval total length (zoea 1 to megalopa) and carapace width (crab 1) were measured for 10 individuals per tank under a micrometer-calibrated microscope. At trial completion (day 21), overall survival rate (%) and crab 1 yield (ind m^{-3}) were calculated.

Statistical analysis. Data were expressed as mean±standard deviation. All data were subjected to one-way analysis of variance (ANOVA) using SPSS version 22.0. Duncan's multiple range test was applied for the post-hoc comparison of treatment means at a significance level of $p < 0.05$.

Results and Discussion

Water quality parameters. Water quality parameters remained stable across all experimental treatments throughout the larval rearing trial (Table 1). Temperature ranged from 29.3 to 30.7°C, pH from 8.21 to 8.26, DO from 6.79 to 6.89 mg L^{-1}, and alkalinity from 121.2 to 122.7 mg $CaCO_3$ L^{-1}, with no significant differences among treatments ($p > 0.05$). These parameter ranges strictly comply with optimal requirements for mud crab larval culture reported by Ganesh et al. (2015) (temperature 27-32°C, pH 7.5-8.5, DO > 4.0 mg L^{-1}) and Khanh et al. (2015) (alkalinity 80-120 mg $CaCO_3$ L^{-1}). Similar environmental stability in biofloc systems was documented by Kasan et al. (2022) for *Scylla olivacea* larvae.

Concentrations of TAN and NO_2^- ranged from 0.43 to 0.95 mg L^{-1} and from 0.15 to 0.36 mg L^{-1}, respectively. The control treatment exhibited significantly higher TAN and

NO_2^- levels than all biofloc treatments ($p < 0.05$). Among the carbon treatments, rice flour maintained the lowest TAN (0.43±0.05 mg L^{-1}) and NO_2^- (0.15±0.01 mg L^{-1}) values. These findings align with the results of Tao et al. (2017), who demonstrated that carbohydrate supplementation significantly reduced TAN and NO_2^- concentrations in penaeid larval rearing through heterotrophic assimilation. Using ozone during mud crab larval rearing, Bac & Ut (2021) recorded TAN and NO_2^- levels of 1.46 mg L^{-1} and 0.22 mg L^{-1}, respectively. Meanwhile, in a study by Khoa (2018) using probiotics (10^6 CFU mL^{-1}), TAN values ranged from 0.16 to 0.35 mg L^{-1} and NO_2^- levels from 0.07 to 0.42 mg L^{-1}. Thus, the TAN and NO_2^- values recorded in the present study were higher than those recorded for the probiotic-based method but lower than those for the ozone-based process. According to Nghia et al. (2007), TAN and NO_2^- levels for mud crab larval culture should not exceed 1.0 mg L^{-1} and 2.99 mg L^{-1}, respectively. Thus, biofloc treatments successfully maintained nitrogenous metabolites well within safe physiological thresholds.

Table 1

Water quality parameters in experimental treatments

Parameter	*Control*	*Cane sugar*	*Rice flour*	*Wheat flour*	*Corn starch*
Temp. (°C) morning	29.4±0.06^a	29.4±0.04^a	29.3±0.02^a	29.5±0.11^a	29.4±0.04^a
Temp. (°C) afternoon	30.7±0.09^a	30.7±0.02^a	30.5±0.12^a	30.6±0.11^a	30.6±0.09^a
pH morning	8.21±0.02^a	8.24±0.04^a	8.21±0.01^a	8.26±0.03^a	8.26±0.03^a
pH afternoon	8.26±0.01^a	8.24±0.01^a	8.26±0.01^a	8.26±0.02^a	8.24±0.03^a
DO (mg L^{-1})	6.79±0.07^a	6.81±0.06^a	6.89±0.02^a	6.84±0.06^a	6.87±0.03^a
Alkalinity (mg $CaCO_3$ L^{-1})	121.2±0.65^a	121.6±0.65^a	122.7±1.29^a	121.6±0.65^a	122.3±0.65^a
TAN (mg L^{-1})	0.95±0.07^b	0.50±0.10^a	0.43±0.05^a	0.45±0.03^a	0.53±0.06^a
NO_2^- (mg L^{-1})	0.36±0.01^d	0.18±0.01^{bc}	0.15±0.01^a	0.16±0.01^{ab}	0.19±0.01^c

Note: Values with different superscript letters in the same row are significantly different ($p < 0.05$).

***Biofloc characteristics*.** Biofloc parameters for the control treatment were the lowest and differed significantly from the other treatments ($p < 0.05$). Observations regarding biofloc formation indicated minimal development in the control treatment due to the absence of an added carbon source. At the zoea 5 stage, biofloc was virtually absent (volume: 0.04 mL L^{-1}). While biofloc formation was observed during the megalopa and crab 1 stages, the levels remained very low (0.15 and 0.40 mL L^{-1}, respectively). Additionally, biofloc particles in the control treatment exhibited the smallest length and width (Table 2). Statistically significant differences ($p < 0.05$) in biofloc formation were observed among the treatments supplemented with different carbon sources. Specifically, the rice flour treatment yielded the highest biofloc values, followed by wheat flour and corn starch, while granulated sugar resulted in the lowest values (Table 2).

Table 2

Biofloc volume, length and width across developmental stages

Parameter	*Control*	*Cane sugar*	*Rice flour*	*Wheat flour*	*Corn starch*
Volume (mL L^{-1}) - zoea 5	0.04±0.02^a	0.16±0.01^b	0.22±0.02^c	0.19±0.01^{bc}	0.18±0.01^{bc}
Volume (mL L^{-1}) - megalopa	0.15±0.02^a	0.52±0.05^b	0.58±0.02^b	0.54±0.01^b	0.55±0.03^b
Volume (mL L^{-1}) - crab 1	0.40±0.07^a	1.14±0.05^b	1.37±0.13^c	1.34±0.02^{bc}	1.41±0.07^c
Length (mm) - zoea 5	0.08±0.01^a	0.19±0.01^b	0.23±0.01^e	0.22±0.01^d	0.20±0.01^c
Length (mm) - megalopa	0.12±0.01^a	0.22±0.01^b	0.25±0.01^c	0.24±0.01^c	0.22±0.01^b
Length (mm) - crab 1	0.15±0.01^a	0.27±0.01^b	0.31±0.01^d	0.29±0.01^{cd}	0.28±0.01^{bc}
Width (mm) - zoea 5	0.04±0.01^a	0.12±0.01^b	0.15±0.01^d	0.14±0.01^c	0.13±0.01^b
Width (mm) - megalopa	0.05±0.01^a	0.14±0.01^b	0.17±0.01^d	0.16±0.01^c	0.15±0.01^b
Width (mm) - crab 1	0.09±0.01^a	0.23±0.01^b	0.26±0.01^d	0.25±0.01^c	0.23±0.01^b

Note: Values with different superscript letters in the same row are significantly different ($p < 0.05$).

Biofloc volume, length and width increased progressively from the zoea 5 to megalopa and crab 1 stages. The treatment supplemented with granulated sugar exhibited the lowest biofloc volumes across the zoea 5, megalopa and crab 1 stages (0.16, 0.52 and 1.14 mL L^{-1}, respectively), differing significantly from the other treatments ($p < 0.05$). No statistically significant differences in biofloc volume were observed among the rice flour, wheat flour and corn starch treatments ($p > 0.05$). Biofloc particle length increased with the developmental stages of the crab larvae (Table 2). The rice flour treatment yielded the greatest biofloc particle length, followed by wheat flour, corn starch and granulated sugar. At the zoea 5 stage, the biofloc particle length in the rice flour supplement treatment was the largest and statistically significantly different ($p < 0.05$) compared to the other treatments, while in the megalopa and crab 1 stages the biofloc particle length of the rice flour treatment did not differ significantly from that of the wheat flour treatment ($p > 0.05$), it differed significantly from the corn starch and granulated sugar treatments ($p < 0.05$). Regarding biofloc particle width, significant differences were observed among the treatments across the zoea 5, megalopa and crab 1 stages ($p < 0.05$). Floc width was greatest with rice flour, followed by wheat flour, corn starch and granulated sugar (Table 2).

In a study on rearing black tiger shrimp (*Penaeus monodon*) larvae at various densities using BFT, Tao et al. (2019) observed that after 23 days, biofloc volume across treatments ranged from 1.53 to 4.33 mL L^{-1}, with particle lengths of approximately 0.26-0.44 mm and widths of 0.15-0.23 mm. Investigating the rearing of whiteleg shrimp (*Litopenaeus vannamei*) larvae at different densities in a biofloc system, Tao et al. (2021) reported that biofloc volume in the treatments increased progressively over successive sampling events. Similarly, Hai et al. (2019) studied the rearing of giant freshwater prawn (*Macrobrachium rosenbergii*) larvae in biofloc and recorded initial biofloc volumes of 0.10-0.50 mL L^{-1}, which rose to a range of 0.35-1.60 mL L^{-1} by the end of the experiment; the average biofloc particle length was 0.54±0.62 mm, and the width ranged from 0.4 to 0.5 mm. According to Avnimelech (2012), biofloc particle sizes ranging from 0.1 to 5 mm are sufficiently large for ingestion by the cultured species. Thus, the biofloc parameters observed in this experiment are consistent with previous studies on crustacean larval rearing.

Bacterial dynamics. Total bacterial density in the water consistently increased throughout the mud crab larval rearing period (Table 3). Throughout the rearing process, the control treatment exhibited significantly higher ($p < 0.05$) total bacterial densities in the water ($4.5\text{-}6.3\times10^4$ CFU mL^{-1}) and in the larvae (4.6×10^4 CFU mL^{-1}) when compared to the biofloc treatments. Regarding the biofloc treatments, bacterial densities in the granulated sugar (3.5×10^4 CFU mL^{-1}) and corn starch (4.2×10^4 CFU mL^{-1}) groups were significantly higher ($p < 0.05$) than those in the rice flour and wheat flour groups (2.4×10^4 CFU mL^{-1} and 2.5×10^4 CFU mL^{-1}, respectively) during the first 7 days. In subsequent sampling events (days 14 and 21), total bacterial densities in the water for the granulated sugar, rice flour and wheat flour treatments were comparable, showing no statistically significant differences ($p > 0.05$). However, bacterial density in the corn starch treatment remained high, differing significantly from the other biofloc treatments ($p < 0.05$).

Table 3

Total heterotrophic bacterial density in water and crab 1 tissue

Sample/Day	*Control*	*Cane sugar*	*Rice flour*	*Wheat flour*	*Corn starch*
Water/Day 7 (10^4 CFU mL^{-1})	$4.5±0.21^c$	$3.5±0.21^b$	$2.4±0.18^a$	$2.5±0.17^a$	$4.2±0.21^c$
Water/Day 14 (10^4 CFU mL^{-1})	$5.9±0.72^c$	$3.7±0.26^a$	$3.2±0.22^a$	$3.8±0.43^a$	$5.3±0.34^b$
Water/Day 21 (10^4 CFU mL^{-1})	$6.3±0.17^b$	$4.3±0.23^a$	$4.2±0.21^a$	$4.7±0.30^a$	$5.6±0.31^b$
Crab 1 tissue (10^4 CFU g^{-1})	$4.6±0.32^b$	$3.6±0.40^a$	$3.3±0.31^a$	$3.4±0.16^a$	$3.6±0.34^a$

Note: Values with different superscript letters in the same row are significantly different ($p < 0.05$).

This indicates that during the initial phase (7 days), significant differences ($p < 0.05$) in total bacterial density were observed in the water among the carbon-supplemented treatments. Notably, this was likely due to the carbohydrates in granulated sugar and corn starch degrading more rapidly than those in rice flour and wheat flour. Consequently, faster cellular biomass synthesis occurred in these treatments, leading to a rapid increase in bacterial numbers. However, the carbon supply from these substrates stabilised over time, causing the differences in bacterial density among the treatments to narrow.

An increase in bacterial counts towards the end of the experiment was also observed, consistent with a study by Hai et al. (2019) on the rearing of giant freshwater prawn larvae at various densities using BFT. According to Vu et al. (2025), a total bacterial density of up to 16.38×10^4 CFU mL^{-1} did not affect mud crab larvae. However, in a study by Tao et al. (2018) on rearing black tiger shrimp larvae using BFT with molasses supplementation at different stages, total bacterial counts reached 12.50×10^4 CFU mL^{-1} in the water and 23.88×10^4 CFU g^{-1} in the shrimp, yet the larvae and post-larvae still developed well.

The results of the present study indicated that *Vibrio* bacterial counts in the water and in crabs (stage 1) were significantly lower than total bacterial counts; however, both followed a similar trend of increasing towards the end of the experiment. During the initial 7–14 days, *Vibrio* density was highest in the control treatment, differing significantly from the other treatments ($p < 0.05$), whereas no significant differences were observed among the biofloc rearing treatments ($p > 0.05$). By the end of the experiment (21 days), significant differences in *Vibrio* bacterial counts were observed among all treatments ($p < 0.05$). Overall, the rice flour treatment showed the lowest count, followed by wheat flour and granulated sugar, while the control treatment exhibited the highest count (Table 4). Similarly, at the crab 1 stage, the control treatment recorded the highest *Vibrio* count (0.63×10^3 CFU mL^{-1}), differing significantly from the other treatments ($p < 0.05$). Among the biofloc treatments, the rice flour treatment yielded the lowest value (0.39×10^3 CFU mL^{-1}). Although this did not differ significantly from the wheat flour and granulated sugar treatments ($p > 0.05$), it differed significantly from the corn starch treatment ($p < 0.05$).

Table 4

Vibrio spp. density in water and crab 1 tissue

Sample/Day	*Control*	*Cane sugar*	*Rice flour*	*Wheat flour*	*Corn starch*
Water/Day 7 (10^3 CFU mL^{-1})	0.11 ± 0.15^{b}	0.09 ± 0.07^{ab}	0.08 ± 0.06^{a}	0.07 ± 0.07^{a}	0.08 ± 0.09^{a}
Water/Day 14 (10^3 CFU mL^{-1})	0.28 ± 0.46^{b}	0.13 ± 0.29^{a}	0.11 ± 0.11^{a}	0.13 ± 0.07^{a}	0.14 ± 0.09^{a}
Water/Day 21 (10^3 CFU mL^{-1})	0.62 ± 0.31^{d}	0.37 ± 0.20^{b}	0.29 ± 0.15^{a}	0.35 ± 0.30^{ab}	0.44 ± 0.02^{c}
Crab 1 tissue (10^3 CFU g^{-1})	0.63 ± 0.15^{c}	0.44 ± 0.20^{ab}	0.39 ± 0.20^{a}	0.41 ± 0.15^{a}	0.48 ± 0.25^{b}

Note: Values with different superscript letters in the same row are significantly different ($p < 0.05$).

In a study on the biofloc-based rearing of Pacific white shrimp larvae, Tao et al. (2021) reported *Vibrio* bacterial densities ranging from 0.77×10^3 to 1.43×10^3 CFU mL^{-1}. In experiments comparing different rearing protocols for mud crabs, Bac & Ut (2021) recorded total bacterial and *Vibrio* spp. densities of 0.86×10^4 CFU mL^{-1} and 0.16×10^4 CFU mL^{-1}, respectively, under ozone treatment - values that differed significantly ($p < 0.05$) from those in the antibiotic treatment. A study on biofloc-based Pacific white shrimp rearing has shown that larger floc volumes correlate with lower *Vibrio* densities, although bacterial density consistently increased towards the end of the experiment (Tao et al. 2017). Overall, *Vibrio* counts in biofloc-based mud crab larval rearing remained consistently lower than those in the control group and in previously studied rearing protocols.

LSI. Between days 3 and 12, the larval metamorphosis process (LSI) showed no significant differences among the treatments ($p > 0.05$). However, from day 15 onwards, differences in larval metamorphosis began to emerge ($p < 0.05$). The treatment supplemented with rice flour exhibited the highest LSI value (4.67±0.06), while the control treatment showed the lowest (4.37±0.12), representing a significant difference ($p < 0.05$) (Table 5).

Table 5

Larval stage index (LSI) across sampling intervals

Culture day	*Control*	*Cane sugar*	*Rice flour*	*Wheat flour*	*Corn starch*
Day 3	1.83±0.06^{a}	1.87±0.06^{a}	1.83±0.15^{a}	1.90±0.10^{a}	1.83±0.06^{a}
Day 6	2.67±0.06^{a}	2.70±0.10^{a}	2.70±0.10^{a}	2.60±0.10^{a}	2.63±0.06^{a}
Day 9	3.53±0.06^{a}	3.60±0.10^{a}	3.63±0.06^{a}	3.60±0.10^{a}	3.63±0.06^{a}
Day 12	4.07±0.06^{a}	4.33±0.06^{a}	4.30±0.10^{a}	4.33±0.21^{a}	4.27±0.06^{a}
Day 15	4.37±0.12^{a}	4.5±0.06^{ab}	4.67±0.06^{b}	4.57±0.12^{ab}	4.50±0.10^{ab}
Day 18	5.53±0.06^{a}	5.67±0.06^{ab}	5.93±0.06^{d}	5.77±0.06^{bc}	5.83±0.06^{cd}
Day 21	5.83±0.12^{a}	6.10±0.10^{bc}	6.33±0.06^{c}	6.20±0.10^{bc}	6.07±0.06^{ab}

Note: Values with different superscript letters in the same row are significantly different ($p < 0.05$).

Throughout the rearing process, crab larvae in treatments supplemented with rice flour and wheat flour tended to undergo metamorphosis more rapidly and synchronously. Metamorphosis indices at the zoea 5, megalopa (after 15-17 days of rearing) and crab 1 (after 21 days of rearing) stages were significantly higher than in the other treatments ($p < 0.05$). Notably, these results reflect improvements in environmental quality (TAN and NO_2^- levels; Table 1), nutrient provision from the biofloc feed source (Table 2) and reduced bacterial density in the culture medium (Tables 3 and 4), all of which contributed to enhanced prey capture, growth and metamorphosis in the larvae. Research by Ut et al. (2007) noted that maintaining good rearing tank water quality and limiting the proliferation of parasites and bacteria facilitates faster and more synchronous moulting in mud crab larvae. Similar observations were made in experiments using ozone treatment, where crab larvae tended to moult rapidly and synchronously because ozone improved water quality and inhibited the growth of parasites and bacteria in the tanks (Bac & Ut, 2021). In a study on density reduction for mud crab larvae at various stages, Hai & Viet (2017) reported that the larval metamorphosis index after 3 days of rearing ranged from 1.60 to 1.78. In a study on density reduction for mud crab larvae at various stages, Hai & Viet (2017) reported that the larval metamorphosis index after 3 days of rearing ranged from 1.60 to 1.78, by day 21 of the rearing period, the average LSI across treatments ranged from 6.98 to 7.00. Thus, the results indicate that while the LSI values were higher during the initial 3-day stage (1.83-1.90), the values at the 21-day stage (5.83-6.33) were lower than those reported in the low-density crab rearing study by Hai & Viet (2017). Overall, the use of biofloc contributed to relatively high LSI values in the mud crab larvae.

Larval growth in length. Larval length did not differ significantly among the treatments from the zoea 1 to zoea 3 stages ($p > 0.05$). From the zoea 4 stage onwards, larval length was greatest in the treatment supplemented with rice flour and lowest in the control group, with the difference being statistically significant ($p < 0.05$). At the zoea 4 stage, crab length did not differ significantly ($p > 0.05$) from the wheat flour treatment but differed significantly from the other treatments; by the crab 1 stage, the maximum carapace width in the rice flour-supplemented treatment differed significantly ($p < 0.05$) from the control but did not differ significantly ($p > 0.05$) from the other treatments (Table 6).

According to Quinitio et al. (2015), enhancing efficiency and stability in mud crab seed production requires consistent rearing environmental conditions that meet the specific nutritional needs of each developmental stage. The results of this study demonstrate that carbon supplementation to generate biofloc during mud crab larval rearing effectively meets these integrated requirements. Data indicate that biofloc treatments yielded better results for environmental parameters, bacterial density and larval growth (in length) than the control; the rice flour supplementation treatment produced the best growth. As reported by Hai et al. (2017), mud crab larval sizes at stages zoea 1, 2, 3, 4, 5 and megalopa were 1.65, 2.18, 2.70, 3.54, 4.50 and 4.01 mm, respectively, with a range of 2.0-3.0 mm for the crab 1 stage. Thus, the growth in length of the crab larvae in this study is consistent with previous research findings.

Table 6

Larval total length and crab 1 carapace width (CW)

Stage	*Control*	*Cane sugar*	*Rice flour*	*Wheat flour*	*Corn starch*
Zoea 1 (mm)	1.32±0.01^{a}	1.32±0.01^{a}	1.31±0.01^{a}	1.32±0.01^{a}	1.31±0.01^{a}
Zoea 2 (mm)	1.87±0.01^{a}	1.88±0.01^{a}	1.88±0.02^{a}	1.88±0.01^{a}	1.88±0.01^{a}
Zoea 3 (mm)	2.45±0.01^{a}	2.45±0.01^{a}	2.46±0.01^{a}	2.45±0.01^{a}	2.46±0.01^{a}
Zoea 4 (mm)	3.26±0.02^{a}	3.31±0.02^{a}	3.37±0.02^{b}	3.31±0.03^{ab}	3.30±0.04^{a}
Zoea 5 (mm)	4.20±0.03^{a}	4.33±0.07^{ab}	4.44±0.06^{b}	4.40±0.07^{b}	4.39±0.01^{b}
Megalopa (mm)	3.99±0.04^{a}	4.16±0.02^{b}	4.28±0.05^{c}	4.22±0.05^{bc}	4.19±0.01^{bc}
Crab 1 CW (mm)	2.92±0.05^{a}	3.02±0.11^{ab}	3.15±0.05^{b}	3.06±0.08^{ab}	3.01±0.05^{ab}

Note: Values with different superscript letters in the same row are significantly different ($p < 0.05$).

Survival rate and crab 1 yield. The results for survival rate and productivity (Table 7) indicate that the rice flour treatment achieved the best results (9.89±0.17% and 19,772±337 ind m^{-3}, respectively), followed by wheat flour, cane sugar and corn starch, while the treatment without carbon supplementation (control) yielded the lowest figures. Overall, the biofloc rearing treatments demonstrated higher survival rates and productivity compared to the control, with statistically significant differences ($p < 0.05$).

Table 7

Survival rate and crab 1 yield in experimental treatments

Performance Index	*Control*	*Cane sugar*	*Rice flour*	*Wheat flour*	*Corn starch*
Survival rate (%)	5.59±0.29^{a}	8.08±0.25^{bc}	9.89±0.17^{d}	8.28±0.58^{c}	7.24±0.47^{b}
Crab 1 yield (ind m^{-3})	11,172±575^{a}	16,168±502^{bc}	19,772±337^{d}	16,557±1,161^{c}	14,478±942^{b}

Note: Values with different superscript letters in the same row are significantly different ($p < 0.05$).

Conclusions. The application of BFT significantly improved water quality, suppressed *Vibrio* spp. accumulation and enhanced larval metamorphosis, growth, survival and production yield in mud crab larval culture compared to the non-biofloc control. Among the four carbon sources evaluated, rice flour demonstrated the best performance, yielding the highest crab 1 survival rate (9.89±0.17%) and production yield (19,772±337 ind m^{-3}). Therefore, pre-fermented rice flour applied at a C:N ratio of 20 from the zoea 3 stage is highly recommended as the optimal carbon source for commercial mud crab hatchery seed production.

Authors Contributions. CTT was responsible for designing the experiment and collecting data; TTPL and HNT monitored the experiment and collected data; LVK and TNH discussed and proposed methods for data processing; NVH discussed the data, summary of responses, wrote the draft, and corected/revised the paper according to the reviewer's feedback.

Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Funding. This study was funded by the research project 'Establishment of high-quality mud crab (*Scylla paramamosain*) seed supply for soft-shell crab, double-shelled crab, and gravid female crab farming models in Ca Mau Province'.

References

APHA (2005). Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, 1288 pp.

Avnimelech, Y. (2012). Biofloc technology - a practical guide book. 2nd edition. The World Aquaculture Society, Baton Rouge, Louisiana, United States, 272 pp.

Avnimelech, Y. (2015) Biofloc technology - a practical guide book. 3rd edition. The World Aquaculture Society, Baton Rouge, Louisiana, United States, 258 pp.

Bac, N. V., & Ut, V. N. (2021). [Rearing mud crab (*Scylla paramamosain*) larvae with different practice protocols]. *Can Tho University Journal of Science, 57*(2), 169-176. DOI: 10.22144/ctu.jvn.2021.051 [in Vietnamese]

Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture, 356-357*, 351-356. https://doi.org/10.1016/j.aquaculture.2012.04.046

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bio-flocs technology: the added value for aquaculture. *Aquaculture, 277*(3-4), 125-137. https://doi.org/10.1016/j.aquaculture.2008.02.019

Ganesh, K., Raj, Y. C. T. S., Perumal, S., Srinivasan, P., & Sethuramalingam, A. (2015). Breeding, larval rearing and farming of mangrove crab, *Scylla serrata* (Forskal, 1775). In: Advances in marine and brackishwater aquaculture. Perumal, S., Thirunavukkarasu, A. R., & Pachiappan, P. (eds), Springer, New Delhi, pp. 163-172. https://doi.org/10.1007/978-81-322-2271-2_14

Hai, T. N., & Viet, L. Q. (2017). [Study on the density reduction at different stages of rearing mud crab *Scylla paramamosain*]. *Can Tho University Journal of Science, 48*, 42-48. DOI: 10.22144/ctu.jvn.2017.615 [in Vietnamese]

Hai, T. N., Tao, C. T., & Phuong, N. T. (2017). [Seed production and farming techniques of crustaceans]. Can Tho University Publishing House, Can Tho, Vietnam, 210 pp. [in Vietnamese]

Hai, T. N., Vinh, P. Q., & Viet, L. Q. (2018). [Technical and financial aspects of mud crab hatcheries in the Mekong Delta]. *Can Tho University Journal of Science 54 (Special Issue: Aquaculture 1),* 169-175. DOI: 10.22144/ctu.jsi.2018.022 [in Vietnamese]

Hai, T. N., An, C. M., Day, P. V., & Tao, C. T. (2019). [Rearing larvae of freshwater prawn (*Macrobrachium rosenbergii*) by biofloc technology at different stocking densities]. *Can Tho University Journal of Science, 55*(2B), 79-87. DOI: 10.22144/ctu.jvn.2019.050 [in Vietnamese]

Hosain, M. E., Amin, S. M. N., Kamarudin, M. S., Arshad, A., & Romano, N. (2021). Effects of C-N ratio on growth, survival and proximate composition of *Macrobrachium rosenbergii* post larvae reared under a corn starch based zero - exchange brackish water biofloc system. *Aquaculture Research, 52*(7), 3015–3025. https://doi.org/10.1111/are.15146

Huys, G. (2002). Preservation of bacteria using commercial cryopreservation systems. Standard Operation Procedure, Asia Resist, 35 pp.

Kasan, N. A., Manan, H., Lal, M. T. M., Rahim, A. I. A., Kamaruzzan, A. S., Ishak, A. N., & Ikhwanuddin, M. (2022). A novel study on the effect of rapid biofloc as pellet feed on the survival rate and water quality of mud crab, *Scylla olivacea* culture. *Journal of Sustainability Science and Management, 17*(2), 46-54. http://doi.org/10.46754/jssm.2022.02.005

Khanh, L. V., Tao, C. T., Hai, T. N., & Son, V. N. (2015). [Effect of alkalinity on metamorphic and survival rate of mud crab larvae (*Scylla paramamosain*)]. *Can Tho University Journal of Science, 38*(1), 61-65. https://ctujsvn.ctu.edu.vn/index.php/ctujsvn/article/view/2108 [in Vietnamese]

Khanjani, M. H., da Silva, L. O. B., Fóes, G. K., Vieira, F. N., Poli, M. A., Santos, M., & Emerenciano, M. G. C. (2023). Synbiotics and aquamimicry as alternative microbial-based approaches in intensive shrimp farming and biofloc: novel disruptive techniques or complementary management tools? A scientific-based overview. *Aquaculture, 567*, 739273. https://doi.org/10.1016/j.aquaculture.2023.739273

Khoa, T. N. D. (2018). [Effect of probiotic (*Bacillus subtilis*) on water quality, survival rate and digestive enzyme activities of mud crab larvae (*Scylla paramamosain*)]. *Can Tho University Journal of Science 54(Special Issue: Aquaculture 1)*, 1-8. DOI: 10.22144/ctu.jsi.2018.001 [in Vietnamese]

Nghia, T. T., Wille, M., Binh, T. C., Thanh, H. P., Danh, N. V., & Sorgeloos, P. (2007). Improved techniques for rearing mud crab *Scylla paramamosain* (Estampador 1949) larvae. *Aquaculture Research, 38*(14), 1539-1553. https://doi.org/10.1111/j.1365-2109.2007.01814.x

Quinitio, E. T., & Parado-Estepa, D. F. (2008). Biology and hatchery of mud crabs *Scylla* spp. 2nd edition. Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC), Tigbauan, Iloilo, Philippines, 44 pp.

Quinitio, E. T., Parado-Estepa, F. D., Huervana, J. J., & Burlas, M. R. (2015). Updates on the seed production of mud crab. In: Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia: Challenges in Responsible Production of Aquatic Species: Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia 2014 (RESA). Romana-Eguia, M. R. R., Parado-Estepa, F. D., Salayo, N. D., & Lebata-Ramos, M. J. H. (eds), Tigbauan, Iloilo, Philippines: Aquaculture Dept., Southeast Asian Fisheries Development Center, pp. 207-212.

Tao, C. T., Hai, T. N., & Khanh, L. V. (2017). [Effects of C/N ratios on growth and survival of black tiger shrimp (*Penaeus monodon*) larvae and postlarvae reared in biofloc system]. *Can Tho University Journal of Science, 49*, 64-71. DOI: 10.22144/ctu.jvn.2017.023 [in Vietnamese]

Tao, C. T., Hai, T. N., & Khanh, L. V. (2018). [Larval rearing of tiger shrimp (*Penaeus monodon*) applying biofloc technology with carbohydrate added at different stages]. *Can Tho University Journal of Science, 54*(1), 27-34. DOI: 10.22144/ctu.jsi.2018.004 [in Vietnamese]

Tao, C. T., Khanh, L. V., Hai, T. N., Viet, L. Q., An, C. M., Toan, P. V., Nghi, D. H., & Viet, H. V. (2019). [Rearing larvae of the black tiger shrimp (*Penaneus monodon*) by biofloc technology at different stocking density]. *Can Tho University Journal of Science, 55*(4), 64-71. DOI: 10.22144/ctu.jvn.2019.109 [in Vietnamese]

Tao, C. T., Hai, T. N., Terahara, T., & Hoa, N. V. (2021). Influence of stocking density on survival and growth of larval and postlarval white leg shrimp (*Litopenaeus vannamei* Boone, 1931) applied biofloc technology. *AACL Bioflux, 14*(3), 1801-1810.

Ut, V. N., Le Vay, L., Nghia, T. T., & Hanh, T. T. H. (2007). Development of nursery culture techniques for the mud crab *Scylla paramamosain* (Estampador). *Aquaculture Research, 38*(14), 1563-1568. https://doi.org/10.1111/j.1365-2109.2006.01608.x

Vu, L. H., Tuyen, C. B., & Bac, N. V. (2025). [Effects of probiotic supplementation in rearing mud crab larvae (*Scylla paramamosain* estampador, 1949)]. *The Journal of Agriculture and Development, 24*(5), 26-37. DOI: 10.52997/jad.3.01.2025 [in Vietnamese]

Received: 09 July 2026. Accepted: 10 August 2026. Published online: 08 September 2026.

Authors:
Chau Tai Tao (CTT), College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Cantho City, Vietnam, e-mail: cttao@ctu.edu.vn
Tran Thi Phuong Lan (TTPL), Tra Vinh University, 126 Nguyen Thien Thanh Street, 890000 Vinh Long Province, Vietnam, e-mail: lanP0624001@gstudent.ctu.edu.vn
Ho Nhut Truong (HNT), College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Cantho City, Vietnam, e-mail: truongm5024005@gstudent.ctu.edu.vn
Ly Van Khanh (LVK), College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Cantho City, Vietnam, e-mail: lvkhanh@ctu.edu.vn
Tran Ngoc Hai (TNH), College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Cantho City, Vietnam, e-mail: tnhai@ctu.edu.vn
Nguyen Van Hoa (NVH), College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Cantho City, Vietnam, e-mail: nvhoa@ctu.edu.vn

How to cite this article:
Tao, C. T., Lan, T. T. P., Truong, H. N., Khanh, L. V., Hai, T. N., & Hoa, N. V. (2026). Effects of carbon sources on mud crab (*Scylla paramamosain)* larval rearing using biofloc technology. *AACL Bioflux, 19*(5), 2095-2104.