

Original Research

Received 9 December 2025

Revised 12 January 2026

Accepted 29 January 2026

Natural Resources for Human Health 2026, Vol. 6 (6): 69–80

KEYWORDS:

Photosynthetic bacteria, *Moina* sp., Siamese fighting fish, Carotenoids, Natural pigments, Sustainable aquaculture, Natural resources.

Natural Carotenoids from Photosynthetic Bacteria: Enriched *Moina* Sp. Enhances Coloration of Siamese Fighting Fish

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ABSTRACT: Siamese fighting fish (*Betta splendens*) cannot synthesize carotenoids and depend on dietary sources for body coloration, while synthetic pigments are costly and raise environmental concerns. This study evaluated *Moina* sp. enriched with photosynthetic bacteria (PSB) as a natural carotenoid carrier and its effects on growth, coloration, survival, and water quality. *Moina* sp. was immersed in a PSB suspension (5.0×10^4 cfu/mL) for 60 min and fed once daily for eight weeks to male red and blue fish (five fish of each color per treatment); control fish received non-enriched *Moina* sp. Enrichment raised the carotenoid content of *Moina* sp. from 2.54 ± 0.22 to 3.38 ± 0.48 mg/g, whereas crude protein (62.90 vs. 65.99%) and crude fat (3.09 vs. 3.67%) did not differ significantly ($p > 0.05$). Weight gain (0.32–0.41 g) and specific growth rate (0.56–0.68%/day) were similar among groups ($p > 0.05$). Survival was 100% in all groups except the red control fish (60%). After eight weeks, redness of blue fish increased by 64% with enriched *Moina* sp. compared with 42% in the control, and greenness increased by 64% versus 56% in red fish and by 24% versus 6% in blue fish. Water quality remained within suitable ranges in both treatments (pH 7.0–8.3; ammonia ≤ 0.03 mg/L; nitrite ≤ 0.19 mg/L). PSB-enriched *Moina* sp. is a natural live feed that enhances coloration and supports survival of *B. splendens* without impairing growth or water quality, offering a natural microbial alternative to synthetic pigments for safer, more sustainable ornamental aquaculture.

1. INTRODUCTION

Betta splendens (Siamese fighting fish) is one of the most popular ornamental fish worldwide, valued for its vivid coloration and fin patterns. *B. splendens* occurs naturally in rice fields and shallow water bodies of Southeast Asia [1]. Its labyrinth organ functions as an air-breathing organ, allowing the fish to thrive in water with low dissolved oxygen [2]. This adaptation, together with its aggressive display behaviour and ease of handling, has made the species a staple of the aquarium trade. Its market value, however, depends mainly on the intensity of its body color [3]. The skin contains chromatophores, which are specialized pigment cells [4]. Among the pigments involved, carotenoids are the most important, as they produce the red and orange hues most appreciated in ornamental markets [5, 6]. Like other fish, *B. splendens* cannot synthesize carotenoids and must obtain them from its diet [7]. The aquaculture sector therefore seeks efficient, sustainable, and cost-effective dietary sources of carotenoids.

Synthetic carotenoids have so far been the main means of enhancing coloration in the ornamental fish industry. These compounds are effective but have several drawbacks [8]. Their high manufacturing costs and potential residual toxicity raise environmental and ecological concerns, and they are often too expensive for small-scale aquaculture enterprises. Consequently, natural carotenoids from plants, fruits, and microorganisms are becoming increasingly popular in the ornamental fish industry because they are regarded as safer and more sustainable. Biodegradable, non-toxic, and usually less costly than synthetics, natural

carotenoids also meet the expectations of environmentally minded consumers [9]. Gupta et al. [10] reported that natural carotenoids can produce coloration comparable to or better than that obtained with synthetic carotenoids. Replacing synthetic colorants with pigments from natural biological resources is also in line with the wider shift toward natural products that are safer for farm workers, consumers and the environment [11]. Natural pigments such as chlorophylls and carotenoids can be obtained from plant biomass by simple, low-cost extraction methods [12]. The remaining challenge is to deliver natural carotenoids to the fish effectively.

The use of live feed organisms, such as *Moina* species, as natural carotenoid carriers is a promising approach. *Moina* sp. is known for its high nutritional value, sensitivity to environmental conditions and short reproductive cycle [13]. These qualities make it a practical and economical live feed for ornamental aquaculture. In addition, *Moina* sp. is a filter feeder that consumes and stores bacteria and phytoplankton, which makes it a suitable carrier of nutrients and pigments. When loaded with carotenoids, *Moina* species can transfer these pigments to fish and improve their body coloration [14]. The alternative live feed, *Artemia* sp., contains higher levels of carotenoids but is much more expensive. The carotenoid content of *Moina* sp. can be increased by enrichment with photosynthetic bacteria (PSB), but this approach remains underutilized in ornamental aquaculture.

PSB are a diverse group of microorganisms that can serve as a sustainable carotenoid source for aquaculture. They are known for producing large amounts of pigments under various culture conditions, for their adaptability, and for their positive environmental effects [15]. Unlike plant sources of carotenoids, PSB can be grown with little resource consumption and are therefore potentially suitable for year-round production in different climates within a relatively small area. Their cell walls are also easily digested, which is an advantage when the cells are fed to filter feeders such as *Moina* species during enrichment. In addition to pigment formation, PSB play an important role in environmental control in aquaculture systems: they use organic waste as a substrate and reduce chemical oxygen demand (COD), thereby helping to preserve water quality [16]. Deterioration of water quality is one of the largest problems that fish farms must deal with. The nutritional enrichment and environmental benefits of PSB are therefore both relevant to environmentally responsible aquaculture practices.

Feeding PSB-enriched *Moina* sp. to ornamental fish may therefore offer a useful innovation for the industry. Previous studies have demonstrated that PSB use can improve growth, coloration, and survival rates in several aquaculture species, including rainbow trout and other commercially important species [17, 18]. Labaiden et al. [19] demonstrated that the addition of PSB to the feed of white shrimp larvae improved survival and coloration. However, the application of PSB-enriched live feed to increase the market value of *B. splendens* has rarely been explored [14, 1]. Such an approach would address broader environmental concerns in the ornamental fish sector while providing a sustainable way of improving the appearance of the fish.

This study therefore investigated the growth performance, coloration, and water quality of Siamese fighting fish fed PSB-enriched *Moina* sp. It also aimed to demonstrate the potential of this live feed as an environmentally friendly alternative to synthetic pigments and as a contribution to sustainable environmental management in aquaculture systems. To this end, *Moina* sp. was enriched with PSB carotenoids, and growth indices, coloration intensity, and water quality parameters were evaluated. The findings are expected to assist fish farmers in developing sustainable aquaculture practices that reduce production costs, improve the health and appearance of fish, and reduce the environmental footprint of fish farming. By valorizing a microbial natural resource as a pigment source, the work also responds to the growing demand for natural, residue-free inputs that protect environmental and human health.

2. MATERIALS AND METHODS

2.1 Experimental design

The experiment compared two independent groups (control and PSB-enriched), each comprising five red and five blue male fish. Fish in the control group were fed *Moina* sp. (at 10 g of fresh weight per day, taken directly from the culture medium without PSB immersion), whereas fish in the enriched group were fed *Moina* sp. (at 10 g of fresh weight per day, having been immersed in PSB for 60 min). The fish were fed once daily (between 09:00 and 10:00 h) for eight weeks.

2.2 *Moina* sp. enrichment

Moina sp. (20 g fresh weight) was immersed in 150 mL of a photosynthetic bacteria (PSB) suspension (5.0×10^4 cfu/mL) obtained from the Aquacultural Technology and Aquatic Resource Management Program, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang. After immersion for 60 min, the *Moina* sp. was filtered, washed with clean

tap water, and fed to the fighting fish. A separate portion was used for proximate analysis following AOAC methods [20] and for determination of carotenoid content following Saejung and Puensungnern [15]. For carotenoid analysis, the *Moina* sp. sample was washed twice with 0.85% NaCl solution. Carotenoids were extracted with methanol and acetone (2:3, v/v) until the extract was clear and colorless. The volume of the pooled extract was recorded and its absorbance was measured using a spectrophotometer at 480 and 770 nm. Total carotenoid content was calculated using the following equation:

$$\text{Total carotenoid content (mg/g)} = (A_{480} - 0.1 \times A_{770}) \times 0.385 \times V / W$$

where A_{480} and A_{770} are the absorbance values of the extract at 480 and 770 nm, respectively; W is the weight of *Moina* sp. (g); and V is the extract volume (mL). Crude protein was analyzed by the Kjeldahl method and crude fat by Soxhlet extraction [20].

2.3 Fish and culture conditions

Twenty male fighting fish (10 blue and 10 red), 2–2.5 cm in length, were purchased from an ornamental fish breeding farm in Nong Chok District, Bangkok, Thailand. Each fish was reared individually in a 22 oz clear PET cup (bottom 9.8 × top 6 × height 15 cm) with a hole drilled in the bottom. The cups were hung in a foam sheet cut to fit the cup mouth so that they floated in a 300 L rectangular plastic container (88 × 118 × 43 cm). Water quality (ammonia, nitrite, dissolved oxygen, and pH) was measured every two weeks throughout the culture period. During the 8-week experiment, 20% of the water in the container was replaced every evening.

2.4 Growth performance

All fish were measured for total length (cm) and weight (g) every 2 weeks. Weight gain (WG), specific growth rate (SGR) and survival rate (SR) were calculated as follows:

$$\text{WG (g)} = \text{final weight} - \text{initial weight}$$

$$\text{SGR (\%/day)} = [\ln \text{ final weight} - \ln \text{ initial weight}] / \text{number of rearing days} \times 100$$

$$\text{SR (\%)} = (\text{number of surviving fish} / \text{initial number of fish}) \times 100$$

2.5 Color measurement

Fish were photographed individually in a 5 × 20 × 15 cm glass tank placed inside a 25 × 37 × 21 cm box with solid white walls and white lighting. Photographs were taken with a phone camera positioned 30 cm from the imaging tank, with the exposure locked to prevent automatic color shift. Imaging was carried out every 2 weeks from the beginning to the end of the 8-week experiment, using the same water throughout [1]. An area of equal size (12.6 mm²) on the caudal peduncle of each fish was analyzed in each image using Color Picker software. Color intensity was expressed as RGB values, with each parameter (red, green and blue) ranging from 0 to 255 [921, 1].

2.6 Ethical approval

This study was approved by the Animal Care and Use Committee of King Mongkut's Institute of Technology Ladkrabang (ACUC-KMITL-RES) under protocol number ACUC-KMITL-RES/2024/001.

2.7 Statistical analysis

Statistical analysis was performed using SPSS for Windows. Data are presented as mean ± SD. The two treatments were compared using independent-samples t-tests, and growth parameters were analyzed under a completely randomized design (CRD). Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Carotenoid enrichment of *Moina* sp. by PSB

PSB are an attractive source of protein because they contain all the essential amino acids required for growth. Photosynthetic microorganisms grown in low-cost natural media are likewise recognized as economical sources of functional proteins [22]. They are autotrophic, easy to digest, harmless, and non-pathogenic, which gives them several advantages as a feed [15]. Because the cells are so small, they can be eaten by many microscopic organisms and serve as a food source for many species [19]. After

60 min of immersion in PSB, the digestive tract of *Moina* sp. showed more intense red pigmentation (Figure 1B) than that of the control (Figure 1A), and the carotenoid content was higher (3.38 mg/g) than in *Moina* sp. not immersed in PSB (2.54 mg/g). PSB immersion also produced a small increase in the protein and lipid contents of *Moina* sp., but the differences were not statistically significant ($p > 0.05$) (Table 1).

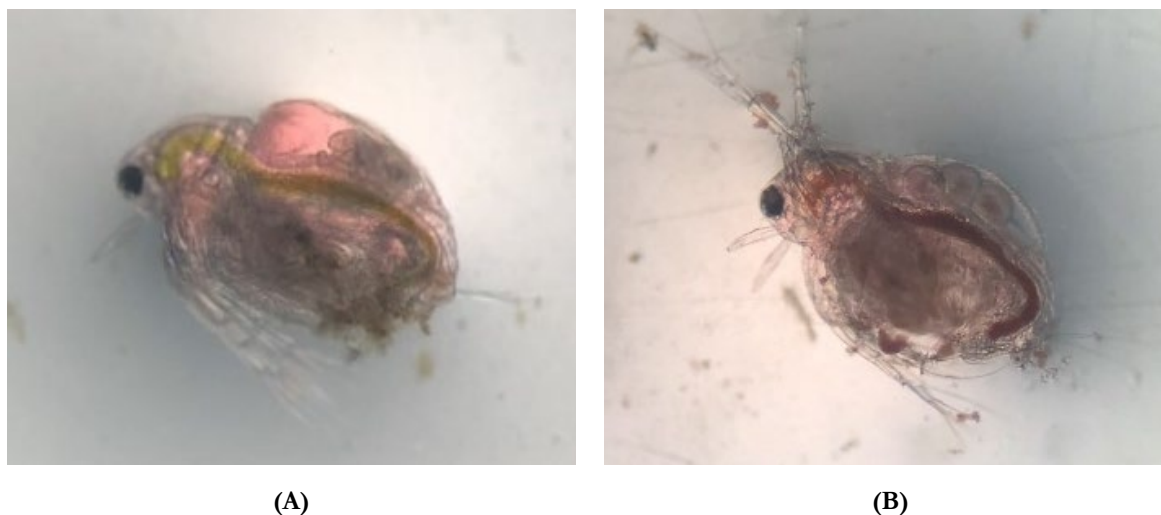


Figure 1. *Moina* sp. without PSB immersion (control) (A) and after immersion in PSB for 60 min (B).

Table 1. Proximate composition and carotenoid content of *Moina* sp. with and without immersion in photosynthetic bacteria (PSB)

Indicator	<i>Moina</i> sp.	
	Control	PSB immersion
Moisture (%)	11.98 ± 1.70	11.95 ± 0.76
Ash (%)	9.84 ± 0.35	9.78 ± 0.72
Crude protein (%)	62.90 ± 6.48	65.99 ± 3.02
Crude fat (%)	3.09 ± 1.29	3.67 ± 1.34
Carotenoid content (mg/g)	2.54 ± 0.22 ^b	3.38 ± 0.48 ^a

Note: Different letters indicate a statistically significant difference by the t-test ($p < 0.05$). Data are presented as mean ± SD.

The increase in carotenoid content after immersion can be explained by the feeding behaviour of *Moina* sp. PSB are producers in the food chain [16], and as a filter feeder *Moina* sp. readily consumes them; this is supported by the visible increase in red pigment in the digestive tract. Labaiden et al. [19] reported that white shrimp (*Litopenaeus vannamei*) larvae fed *Moina* sp. enriched with PSB showed improved growth and survival, while protein and lipid contents did not differ significantly ($p > 0.05$), which is consistent with the present findings. Other research on Siamese fighting fish showed that PSB added to the diet improved survival but not growth, possibly because the amino acid profile of PSB was insufficient for this carnivorous fish. On the other hand, PSB can improve water quality by using waste in the water as a substrate for cell growth [16].

3.2 Effects of PSB-enriched *Moina* sp. on the growth of Siamese fighting fish

PSB-enriched *Moina* sp. has been proposed to improve the growth, health and appearance of Siamese fighting fish while supporting sustainable aquaculture practices, and these benefits may be refined through further work on dose, feeding frequency and PSB strain [28, 12]. In the present study, however, weight gain (g) and specific growth rate (%/day) did not differ significantly among the groups ($p > 0.05$) (Table 2).

Table 2. Growth, weight gain, specific growth rate and survival rate of red and blue Siamese fighting fish fed *Moina* sp. enriched with PSB

Indicator	Control		PSB immersion	
	Red	Blue	Red	Blue
Initial weight (g)	0.77 ± 0.20	0.93 ± 0.29	0.76 ± 0.17	0.80 ± 0.17
Final weight (g)	1.34 ± 0.25	1.14 ± 0.26	1.10 ± 0.15	1.14 ± 0.24
Weight gain (g)	0.41 ± 0.23	0.41 ± 0.22	0.32 ± 0.11	0.35 ± 0.27
Initial length (cm)	4.48 ± 0.73	4.54 ± 0.78	4.54 ± 0.43	4.36 ± 0.43
Final length (cm)	6.06 ± 0.67	5.92 ± 1.00	6.18 ± 0.40	6.67 ± 0.61
Length gain (cm)	1.16 ± 0.86^b	1.28 ± 0.29^{ab}	1.66 ± 0.39^{ab}	2.12 ± 0.75^a
Specific growth rate, SGR (%/day)	0.68 ± 0.34	0.65 ± 0.26	0.56 ± 0.18	0.56 ± 0.39
Survival rate (%)	60 ± 0.55^b	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a

Note: Different letters indicate a statistically significant difference at a confidence level of 95% ($p < 0.05$). Data are presented as mean $\pm$ SD.

Blue fish fed PSB-enriched *Moina* sp. showed the greatest length gain (2.12 ± 0.75 cm), whereas red fish in the control group showed the lowest (1.16 ± 0.86 cm). Mortality occurred only among red fish in the control group, which had a survival rate of 60% compared with 100% in all other groups (Table 2).

3.3 Effects of PSB-enriched *Moina* sp. on the color of Siamese fighting fish

PSB produce pigments within their cells that can be exploited industrially [24, 29] as food and cosmetic colorants or as a vitamin A precursor in animal feed [8]. Moreover, *Moina* sp. can act as a reservoir of carotenoids and other nutrients and transfer them to the next consumer in the food chain, here the fighting fish [14]. This agrees with Kiswara et al. [21], who found that skin color development in *B. splendens* was promoted when *Artemia* was enriched with *Tagetes erecta* flower powder. In the present study, Color Picker software was used to determine the red, green and blue values from photographs of red and blue male fighting fish after eight weeks of feeding (Figures 2 and 3).

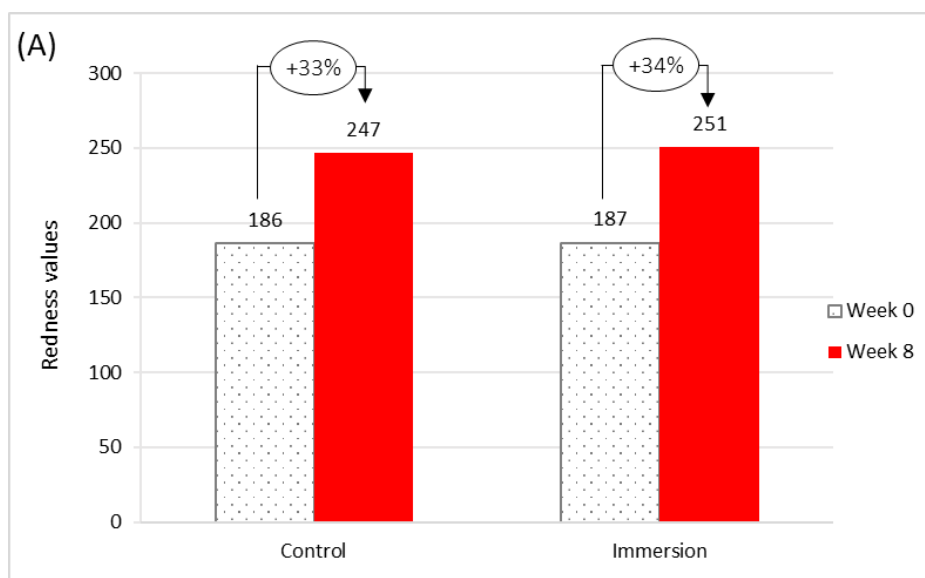


Figure 2. Red male fighting fish fed control *Moina* sp. (A) and PSB-immersed *Moina* sp. (B).



Figure 3. Blue male fighting fish fed control *Moina* sp. (A) and PSB-immersed *Moina* sp. (B).

Fish fed *Moina* sp. immersed in PSB for 60 min showed greater increases in red values than control fish (Figure 4). In blue fish, redness increased by 64% in the enriched group compared with 42% in the control (Figure 4B), whereas in red fish the increase was similar in both groups (34% and 33%, respectively; Figure 4A). Green values also increased more in fish fed PSB-immersed *Moina* sp. than in control fish (Figure 5): by 64% versus 56% in red fish (Figure 5A) and by 24% versus 6% in blue fish (Figure 5B). In contrast, blue values increased more in control fish than in fish fed enriched *Moina* sp. (Figure 6): by 94% versus 45% in red fish (Figure 6A) and by 30% versus 21% in blue fish (Figure 6B)



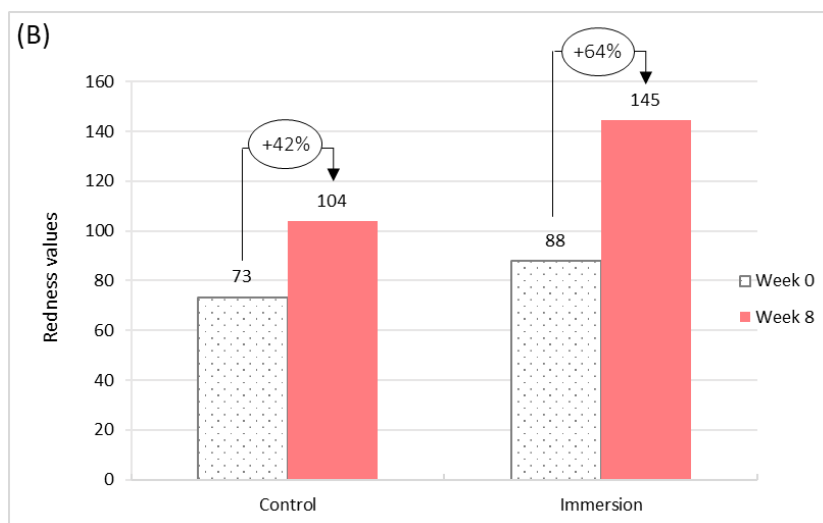


Figure 4. Redness values of red male fighting fish (A) and blue male fighting fish (B) at week 0 and week 8.

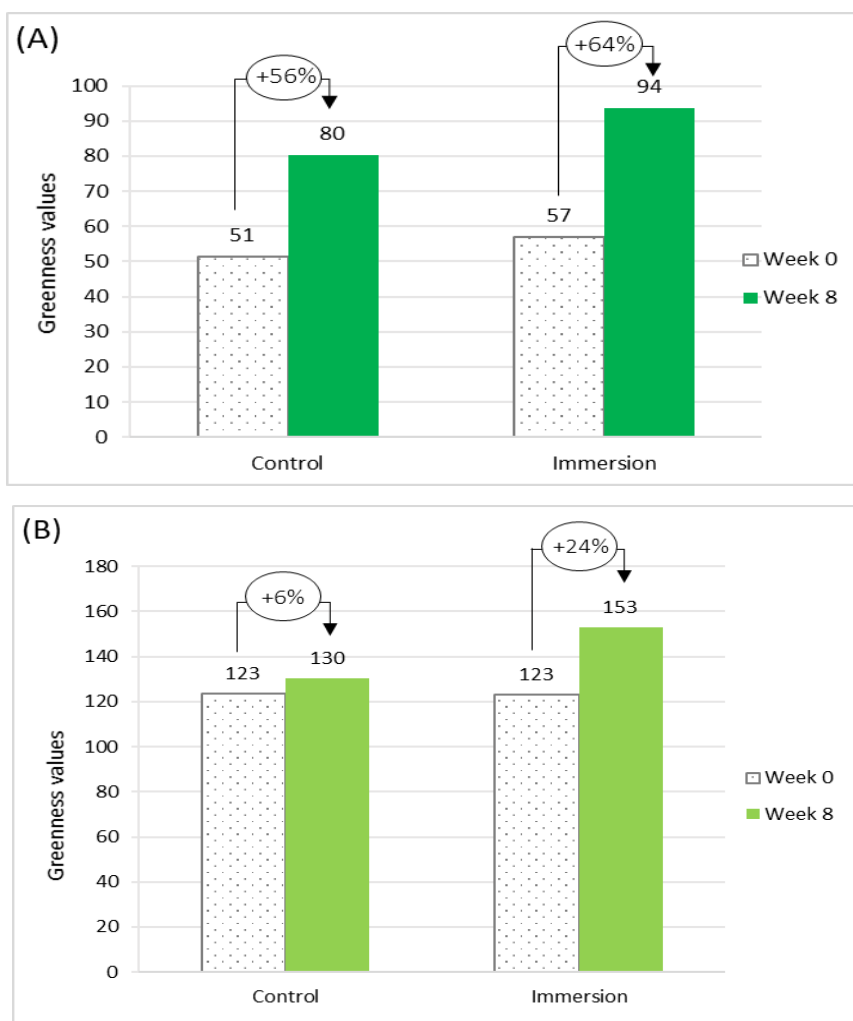


Figure 5. Greenness values of red male fighting fish (A) and blue male fighting fish (B) at week 0 and week 8.

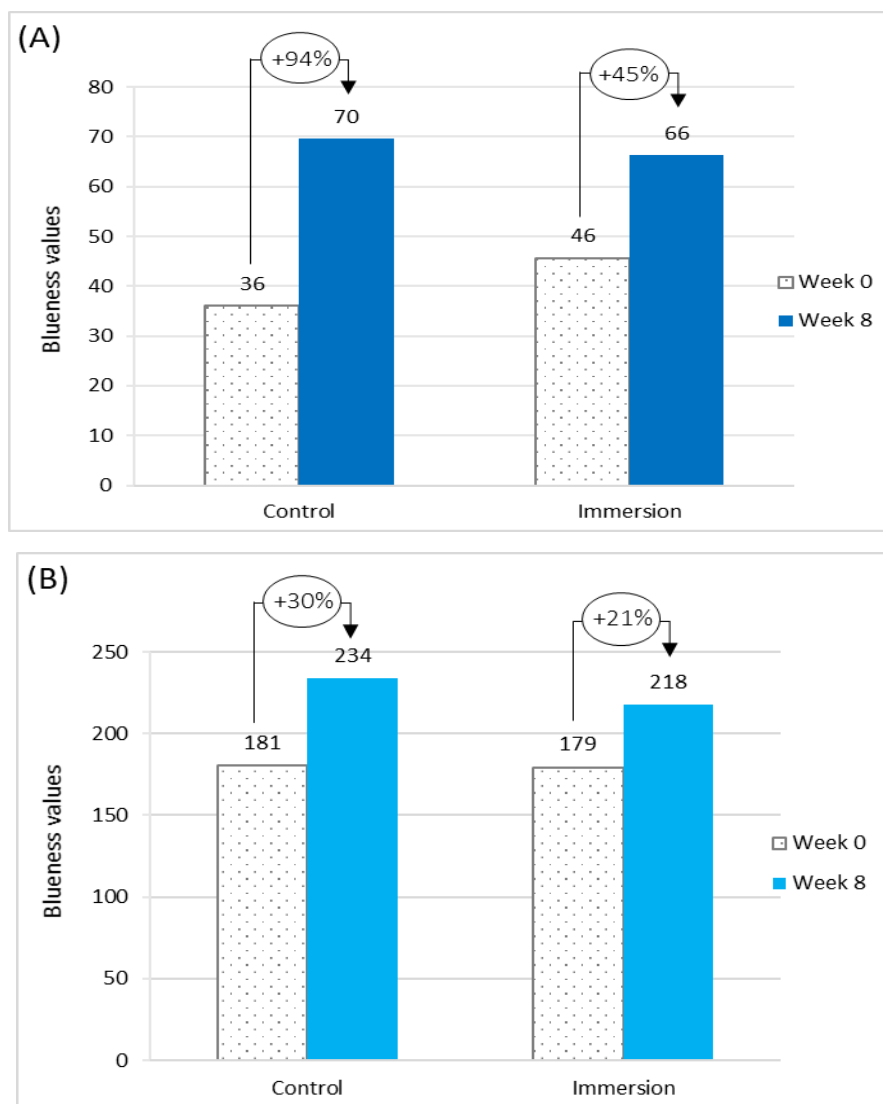


Figure 6. Blueness values of red male fighting fish (A) and blue male fighting fish (B) at week 0 and week 8.

According to Ratananikom et al. [25], fish cannot synthesize carotenoids on their own; the pigments must be obtained from the diet before being stored in the tissues and skin. Accordingly, the red and green color intensity of fighting fish fed PSB-supplemented *Moina* sp. was higher than that of the control group. Wongphonprateep and Pichitkul [9] similarly reported that natural carotenoid supplementation gave coloration close to that obtained with synthetic carotenoid supplements. Carotenoids can also chemically bond to proteins to form carotenoproteins, which can modify their color to purple, blue, and even green [8]; this may explain the changes in green and blue values observed in fish fed PSB-supplemented *Moina* sp.

3.4 Water quality during rearing of fighting fish fed PSB-immersed *Moina* sp.

Water quality during the eight weeks of the study remained suitable for fish culture [26]. pH ranged from 7.0 to 8.3, ammonia from 0.00 to 0.03 mg/L, dissolved oxygen (DO) from 2.50 to 7.87 mg/L, and nitrite from 0.00 to 0.19 mg/L (Table 3). Ammonia and nitrite concentrations in the PSB-immersion treatment were similar to those in the control without PSB immersion [27], so introducing PSB through the food chain did not alter the measured water quality parameters. These results suggest that the presence of PSB in the culture system is not detrimental to aquatic animals. Similarly, Labaiden et al. [19] reported a higher survival rate of white shrimp fed *Moina* sp. and *Chlorella* sp. together with PSB.

Table 3. Water quality during rearing of fighting fish fed *Moina* sp. with and without PSB immersion over 8 weeks

Parameter	Immersion duration	
	0 min (Control)	60 min
pH	7.0 – 8.2	7.0 – 8.3
Dissolved oxygen, DO (mg/L)	2.74 – 7.87	2.50 – 7.72
Temperature (°C)	20.2 – 29.6	20.4 – 29.6
Ammonia (mg/L)	0.00 – 0.03	0.00 – 0.02
Nitrite (mg/L)	0.00 – 0.19	0.00 – 0.17

Note: Values are ranges (minimum – maximum) recorded during the 8-week culture period.

3.5 Implications for natural resource utilization and human health

PSB are a renewable microbial resource that can be cultured on low-cost substrates, and their carotenoids are natural compounds that are also valued in food, feed and cosmetic applications. Delivering these pigments through live feed avoids the use of synthetic colorants in ornamental fish culture, which reduces the handling of chemical additives by farmers and hobbyists and limits pigment residues in effluent discharged to surrounding water bodies. Because PSB use organic waste as a substrate, the same resource also contributes to cleaner culture water. Nutrients in fishpond wastewater can similarly be recovered by other photosynthetic organisms, such as the green alga *Spirogyra*, which removes nutrients while generating useful biomass [18]. Ornamental fish farming is an important source of income for small-scale producers in Thailand, and a low-cost natural color enhancer can raise product value without increasing chemical inputs. The present trial was small, and the effects on fish and water quality should be confirmed at farm scale; the carotenoid profile of the PSB strain should also be characterized to identify the compounds responsible for the color enhancement. Because light and pH strongly influence the biomass production of photosynthetic microorganisms [3], the culture conditions of the PSB should also be optimized before enrichment.

PSB are a renewable microbial resource that has great potential to be used in sustainable ornamental aquaculture. The use of low-cost organic substrates and its production of natural carotenoids makes it an environmentally friendly option to the use of synthetic colourants in ornamental fish production. The addition of PSB to live feeds can improve fish colouration, and reduce the need for chemical pigments and the potential discharge of chemicals to water as well. In addition, the use of organic residues as substrates for the cultivation of microorganisms for biotechnological applications can be related to waste valorisation and resource recovery as part of the global biorefinery strategy, which aims at turning agricultural and industrial wastes into useful bio-energy products [30-33]. Prior research has shown that bamboo waste and cinnamon leaves are promising substrates for biohydrogen production via pretreatment and fermentation methods [30,31] with the microbial fermentation effluent also being a potential substrate for biogas and biomethane recovery based on effluents generated from the microbial fermentation process [32]. Likewise, the ability to utilize low-grade longan for multiple conversions such as for bioethanol and biogas production with high methane as is also a useful example of cascading biomass conversion and paying attention to circular resource utilization [33]. The environmental benefits of PSB-based systems in ornamental aquaculture needs further validation, but the overall sustainability capacity of this technology is confirmed by these findings. The use of nutrient recovery by the photosynthetic organism such as *Spirogyra* also shows the opportunities to increase the management of wastewater and production of valuable biomass [28]. Natural pigments could help in the production of resource efficient ornamental fish for small-scale farmers in Thailand and help them to market their fish at higher prices. But extensive trials at farms are needed to assess pigmentation, fish health, water quality and economics. More in-depth characterization of PSB carotenoids, optimization of light intensity and pH are also suggested to maximize the productivity and cultivation efficiency of the pigments [29].

4. CONCLUSION

PSB-enriched *Moina* sp. can serve as a sustainable live feed for improving the coloration and survival of Siamese fighting fish. Immersion in PSB for 60 min increased the carotenoid content of *Moina* sp. from 2.54 to 3.38 mg/g, indicating that *Moina* sp. is a suitable carrier for transferring PSB carotenoids to fish. Fish fed the enriched *Moina* sp. showed greater increases in red and green color values, with the largest increase in redness in blue fish (64% versus 42% in the control). Weight gain and specific growth rate did not differ significantly between fish fed the enriched and control diets. Water quality parameters (pH, dissolved oxygen, ammonia and nitrite) remained within suitable ranges and were not affected by PSB. Survival was 100% in all groups except the red control fish (60%). PSB-enriched *Moina* sp. is therefore an environmentally acceptable alternative to synthetic pigments for ornamental fish farming. These results illustrate how a microbial natural resource can replace synthetic inputs in ornamental aquaculture in a way that benefits both environmental and human health. Future studies should optimize the duration and concentration of PSB enrichment and test the approach in other aquaculture species.

ACKNOWLEDGEMENTS

The authors thank the Aquacultural Technology and Aquatic Resource Management Program, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, for providing the photosynthetic bacteria and research facilities.

FUNDING

This work was supported by the School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, through the Graduate Study Fee Waiver Scholarship (recipient: Nattasiri Suddoung).

AUTHOR CONTRIBUTIONS

Nattasiri Suddoung: Investigation, Laboratory Analysis, data collection, Data Curation, Formal Analysis.

and writing -first draft.

Nongnuch Laohavisuti: Conceptualization.

Buppha Jongput: Laboratory Analysis.

Uscharee Ruangdej: Data Curation, Formal Analysis, Writing—Review & Editing.

Suwaree Kitikiew: Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

The animal experiment was approved by the Animal Care and Use Committee of King Mongkut's Institute of Technology Ladkrabang (protocol number ACUC-KMITL-RES/2024/001).

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