Enrichment of *Artemia* Nauplii With Bacteria Grown in High C/N Ratio, Carbon Source-Microalgae Media

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Abstract

The microalgae-bacteria interaction in enriched culture media could affect the growth and nutritional properties of *Artemia*. The addition of carbon sources to the media improves the heterotrophic bacterial population, which in turn interacts with microalgae to improve the health and production of *Artemia*. The aim of this study was to assess the impacts of the microbial consortium developed with different fermented carbon sources and microalgae on *Artemia*. Three different microalgae, *Chlorella* sp./A1, *Nannochloropsis oceanica*/A2 and *Chaetoceros calcitrans*/A3 were mixed with three fermented carbon sources (rice bran/C1, tapioca flour/C2 and molasses/C3) to produce nine treatments (A1C1, A1C2, A1C3, A2C1, A2C2, A2C3, A3C1, A3C2, A3C3) and three controls with microalgae alone (A1, A2, A3). Enrichment was carried out for 24 h with *Artemia* instar I at an initial density of 25 individuals mL^{-1}. Two-way ANOVA revealed that the survival, length, protein and lipid contents of *Artemia* nauplii were significantly affected ($P < 0.05$) by carbon source and species of microalgae. The carbon source–microalgae interaction also significantly affects *Artemia* survival, length, biomass and protein content. This study illustrated that bacteria associated with different carbon sources and microalgae consortia improved *Artemia* growth, survival, protein and lipid content.

Keywords: enrichment, proximate composition, growth, survival, protein, lipid

Introduction

Dietary intake of highly unsaturated fatty acids (HUFA) is essential for growth, development, and stress tolerance because marine species have a limited ability to biosynthesise essential nutrients (Kanazawa et al., 1985; Sargent et al., 1997; Bell et al., 2003; Cahu et al., 2003; Tocher et al., 2008; Glencross, 2009; Guinot et al., 2013). Brine shrimp *Artemia* is one of the most significant live feeds in commercial hatcheries for producing marine fish and shellfish larvae. Due to its simplicity of preparation, availability, and larval attractiveness, *Artemia* is one of the most favoured live-feeds in aquaculture (Kittaka, 1994; Silva et al., 2017), despite its deficiencies in eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Sorgeloos et al., 2001; Conceição et al., 2010). Microalgae, bacteria, oil emulsion, and other substances can enrich *Artemia*, which enhances its biochemical composition and, consequently, its lipid content and fatty acid profile. *Artemia* enrichment with HUFA rich microalgae leads to improving the nutritional value of *Artemia* larvae by influencing the microbial communities in the system (Skjermo and Vadstein, 1993; Leema et al., 2010). Microbial interaction is an important part of microbial communities, where species synergism or competition can determine the success of a species and foster ecological diversity.

Bacteria are good sources of essential nutrients including vitamins, minerals, fatty acids, and amino acids and are believed to remove toxic metabolites from aquaculture systems (Austin, 1988; Verschuere et al., 2000; Toi et al., 2014). Intriago and Jones (1993) and

Nevejan et al. (2018) reported that bacteria may participate in bivalve and *Artemia* digestion by producing extracellular enzymes such as proteases and lipases. More importantly, bacteria might satisfy all nutritional requirements and are capable of being used as sole food source for *Artemia*. (Gorospe and Nakamura, 1996; Verschuere et al., 2000; Tkavc et al., 2011).

Heterotrophic bacteria are widespread scavengers that consume organic carbon produced by other autotrophs, and remineralize a significant amount of organic matter back to CO_2 (Cho and Azam, 1988; Amin et al., 2012). Some bacteria associated with *Artemia* have the ability to eliminate pathogenic or potentially pathogenic bacteria by inhibition or competitive exclusion. (Go´mez-Gil, 1995; Verschuere et al., 2000; Maeda-martı an Lo, 2002; Toi et al., 2014). Thus, the nutritional value of *Artemia* for aquaculture can possibly be improved by bioencapsulating it with bacteria. There are groups of bacteria that have positive impacts on *Artemia* growth and survival, such as *Flavobacterium* and *Aeromonas* while some others might cause negative impacts (Rico-Mora and Voltolina 1995; Maeda-martı and Lo, 2002). A higher C/N ratio can enhance bacterial growth in culture media (Avnimelech, 1999). The C/N ratio in aquaculture systems can be increased by adding carbon sources (Burford et al., 2004; Avnimelech, 2007; Crab et al., 2007; Schneider et al., 2007; Nootong et al., 2011; Toi and Hong Van, 2017). The heterotrophic bacterial population in higher C/N ratio systems degrade organic particles to be available for non-selective filter feeders. Toi et al. (2013) demonstrated successful *Artemia* growth in C/N ratio controlled microalgae limited media. The bacterial population is diverse with carbon sources and microalgae associated with the system.

Therefore, this study aimed to elucidate the effect of different microalgae, carbon sources and the interaction of microalgae–carbon source on the growth, survival, and proximate composition of enriched *Artemia* with bacteria and microalgae.

Materials and Methods

Ethical approval

No live animals were used in this study. Therefore, no Institutional Animal Care and Use Committee (IACUC) approval was required.

Experimental design

The experiment was conducted at the Aquatic Animal Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). The marine microalgae *Chlorella* sp., *Nannochloropsis oceanica* and *Chaetoceros calcitrans* were obtained from the Aquatic Animal Health and the Therapeutics Laboratory at the Institute of Bioscience, UPM.

The seawater was filtered with 1.2 µm filter paper and the salinity was adjusted to 25 ppt for microalgal culture. All three microalgal species were cultured with Walne's medium in a 2-L Erlenmeyer flask with a 1-L culture volume. Light intensity was maintained at 84 µmol m^{-2} s^{-1} with continuous illumination using fluorescent light source. The temperature was maintained at 23.6 ± 0.14 °C and cultures were continuously aerated. Cell density was determined daily by counting cells using a Neubauer haemocytometer (Marienfeld, Germany) under an Olympus microscope (Olympus, Japan) attached to a Nikon compact camera (Nikon, Japan). Microalgae were harvested at the early stationary phase on day seven. The schematic diagram of the experiment on *Artemia* enrichment with bacteria associated with a high C/ N ratio is shown in Figure 1.

The bacteria–microalgae consortium was developed in 2-L Erlenmeyer flasks with microalgae and fermented carbon sources. The experiment was a two-way ANOVA (three replicates) design consisting of nine treatments with three types of microalgae (*Chlorella* sp./A1, *Nannochloropsis oceanica*/A2 and *Chaetoceros calcitrans*/A3) and three types of fermented carbon sources (rice bran/C1, tapioca flour/C2 and molasses C3) in different combinations (Table 1). The control consisted of microalgal species alone without the carbon source. On day 1, the fermented carbon source, ammonium sulphate (10 mg.L^{-1}), and microalgae (7×10^6 cells.mL^{-1} day^{-1}), were added to 500 mL of 25 ppt sea water, and the pH of the culture media was measured. The consortia were allowed to develop over the period of four days before adding the *Artemia*. All the flasks were capped with cotton plugs. The C/N ratio of treatment flasks was maintained at 15:1 by adding the carbon source (Avnimelech, 1999) at 24-h intervals, and on day 4, the enrichment media volume was increased to 1-L. All flasks were exposed to 12 h light:12 h dark photoperiod and continuously aerated.

Temperature, dissolved oxygen, pH and salinity were measured daily using a multi parameter (YSI Model 556, USA). Strickland and Parsons (1972) method was used to determine total ammonia nitrogen (TAN), nitrite-N and nitrate-N in the samples obtained at the end of the enrichment period. Dried *Artemia* cysts were soaked in tap water for 1 h before being decapsulated as described by Sorgeloos et al. (1977) and Marques et al. (2006). To remove any remaining bleach, the decapsulated cysts were thoroughly rinsed with distilled water. Under recommended hatching conditions, cysts were incubated in a 1-L conical tube containing 800 mL saltwater at 32 ppt salinity at 28 °C for 24 h (Sorgeloos et al., 1986; Toi et al., 2013).

On the fourth day, *Artemia* instar I nauplii were added to all experimental flasks at a stocking density of 25 nauplii.mL^{-1}. Enrichment was carried out for 24 h with 12:12 photoperiod (Toi et al., 2013). After 24 h, three 10 mL samples from each replicate were randomly collected and the number of live *Artemia* nauplii were

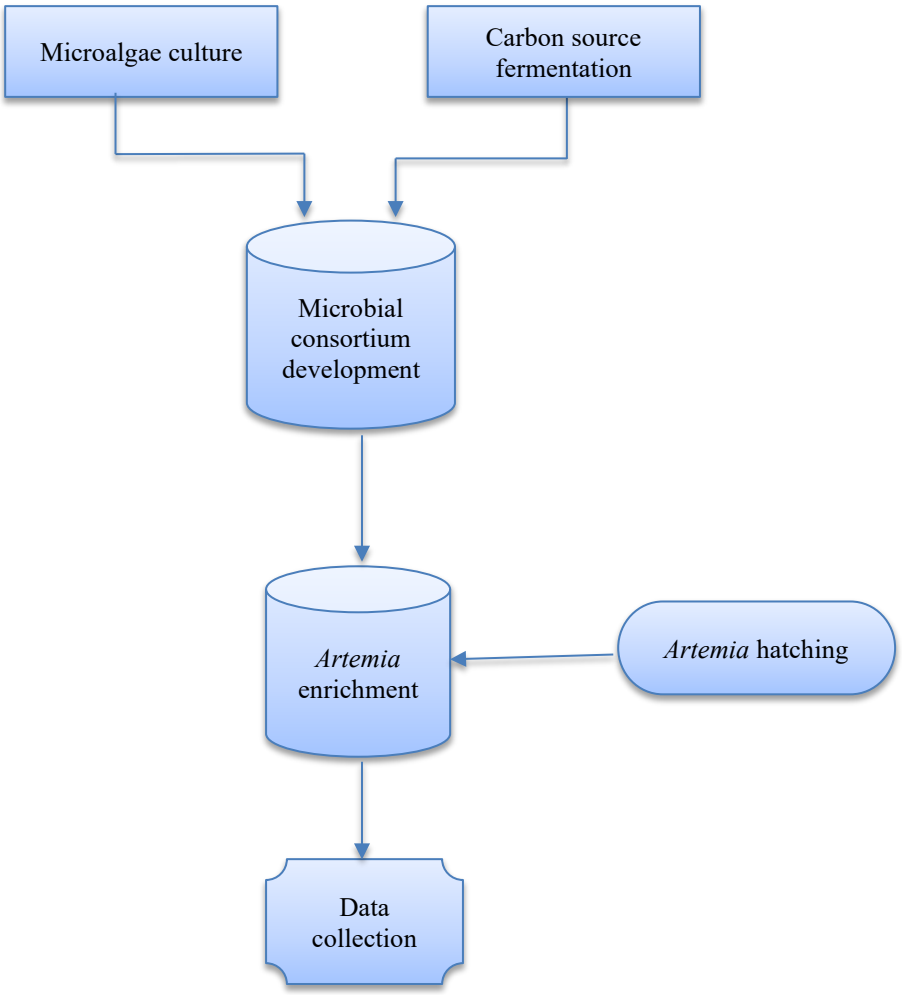

Fig. 1. Schematic diagram of the experiment on *Artemia* enrichment with bacteria associated with high C/ N ratio.

Table 1. Experimental design (two-way ANOVA) with microalgae, A1 - *Chlorella* sp., A2 – *Nannochloropsis oceanica*, A3 – *Chaetoceros calcitrans* together with carbon sources, C1 -rice bran, C2 –tapioca flour, C3-molasses. Each treatment had three replicates.

Treatment	Microalgae/ Carbon source
A1C1	*Chlorella* sp. + rice bran
A1C2	*Chlorella* sp. + tapioca flour
A1C3	*Chlorella* sp. + molasses
A2C1	*Nannochloropsis oceanica* + rice bran
A2C2	*N. oceanica* + tapioca flour
A2C3	*N. oceanica* + molasses
A3C1	*Chaetoceros calcitrans* + rice bran
TA3C2	*C. calcitrans* + tapioca flour
A3C3	*C. calcitrans* + molasses
A1	*Chlorella* sp.
A2	*N. oceanica*
A3	*C. calcitrans*

counted. All the samples were preserved in 5 % Lugol's solution.

The survival of *Artemia* nauplii was calculated by equation:

$$\text{Survival } \% = \frac{N(f)}{N(0)} \times 100$$

Where: *N(o)* is the initial number of *Artemia* nauplii and *N(f)* is the number of *Artemia* nauplii at the end of enrichment period. The individual length of preserved *Artemia* was determined (from the front of the head to the end of the telson) by analysing a photomicrograph. Images of *Artemia* were captured under 10× magnification using an Olympus microscope attached to a Nikon compact camera. The remaining *Artemia* in

enrichment flasks were harvested after 24 h and rinsed thoroughly with de-mineralised water on a sieve to remove waste. Then *Artemia* was freeze-dried to determine the dry weight and proximate compositions. Protein was assayed according to the Bradford method (1976). Total lipids were determined according to Folch et al. (1957) and ash content was measured by drying samples at 550 °C for 5 h. All proximate composition parameters were performed in triplicates.

Statistical analyses

All data analyses were performed using the Statistical Analysis System (SAS 9.4). The main effects were carbon source, microalgae and interaction between carbon source and microalgae (Two-way ANOVA) on the growth, survival and proximate compositions of *Artemia*. The factors with significant differences ($P < 0.05$) were investigated further using the Tukey's post hoc test.

Results

Artemia length differed significantly among microalga types, carbon sources and the microalgae-carbon source interaction was significant. The mean *Artemia* length was significantly ($P < 0.05$) higher in treatments without a carbon source than the other treatments (Fig. 2).

The two-way ANOVA showed that the mean *Artemia* length was significantly greater in the *Chlorella* sp. than the other two microalgae species (Fig. 2).

Mean biomass of *Artemia* also differed significantly among microalgal species (Fig. 3). *Chlorella* sp. exhibited significantly higher *Artemia* biomass in the absence of a carbon source than in A1C1, A3C2, A2C2, A3C3, and the other noncarbon treatments but not A1C1, A3C1, A1C2, A2C2, and A1C3 (Figs. 2, 3). *Artemia* biomass was not significantly different among carbon sources and the microalgae–carbon source interaction was not significant ($P > 0.05$).

Artemia survival differed significantly among microalgae, carbon sources, and the microalgae-carbon sources interactions. Survival was significantly higher for *N. oceanica* (69.7 %) and *C. calcitrans* (68.7 %) than in *Chlorella* sp. (56.2 %). Additionally, the analysis demonstrated that all three carbon sources had a significant effect on *Artemia* survival. Specifically, molasses, tapioca flour, and rice bran exhibited survival rates of 69.3 %, 68.2 %, and 65.1 %, respectively. With respect to all treatments, A3C1 revealed highest survival rate (Fig. 4).

The results of the two-way ANOVA (Table 2) indicate significant effects ($P < 0.05$) of microalgae species on the lipid, protein, and ash contents of *Artemia*. *Chaetoceros calcitrans* showed highest level of protein and lipids in *Artemia*. However, it was not significantly different from *Chlorella* sp. The addition of a carbon source resulted in a significant increase ($P < 0.05$) in lipid content. Rice bran exhibited significantly higher lipid (16.96 %) content. The interaction between microalgae species and carbon source was found to significantly affect protein and moisture content only.

In the study, the microalgae species *C. calcitrans* and rice bran had significantly higher protein and lipid contents, with percentages of 58.41 % and 16.45 % for *C. calcitrans* and 58.68 % and 16.96 % for rice bran, respectively (Table 2). *Chaetoceros calcitrans* exhibited no significant difference from *Chlorella* sp. in both protein and lipid content. In case of microalgae interference, *N. oceanica* and *Chlorella* sp. had significantly higher ash content at 10.63 % and 10.51 %, respectively (Table 2). With respect to carbon source, *Artemia* without a carbon source (10.62 %) showed significantly higher ash content. However, it was not significantly different from rice bran and tapioca flour. Microalgae species did not influence moisture content. Without a carbon source, tapioca flour and rice bran showed significantly higher moisture content. Nevertheless, rice bran was not significantly different from molasses.

The total ammonia nitrogen (TAN) and nitrite-nitrogen were not detected in treatments with carbon source (Table 3). However, the nitrate-nitrogen levels were significantly higher ($P < 0.05$) in treatments with supplemented carbon source (Table 3). The mean initial pH at the time of carbon source introduction across all treatments was 7.62 (Table 3). During the period from carbon source introduction to the end of the *Artemia* enrichment, the pH in all treatments ranged from 7.44 to 7.58. Significantly higher pH was recorded in *Chlorella* treatments than other microalgal species. Meanwhile, considerably higher DO levels were observed in treatments without a carbon source compared to those with carbon source.

Discussion

The findings from the study illustrate the complex interactions between microalgal species and bacteria associated in different carbon sources, and their effects on the growth, survival, and biochemical composition of *Artemia*. These results reveal valuable insights into the dynamics of C/N ratio-controlled systems and the complex relationships between microalgae and bacteria. Firstly, the study demonstrates that *Artemia* length is significantly influenced by the presence or absence of a carbon source, as well as the type of microalgae used. Remarkably, treatments without a carbon source resulted in significantly longer *Artemia* than those with a carbon source.

Furthermore, *Artemia* length and biomass were greater in *Chlorella* sp. than others indicating its potential as an effective source for *Artemia* enrichment. This increase in biomass correlates with

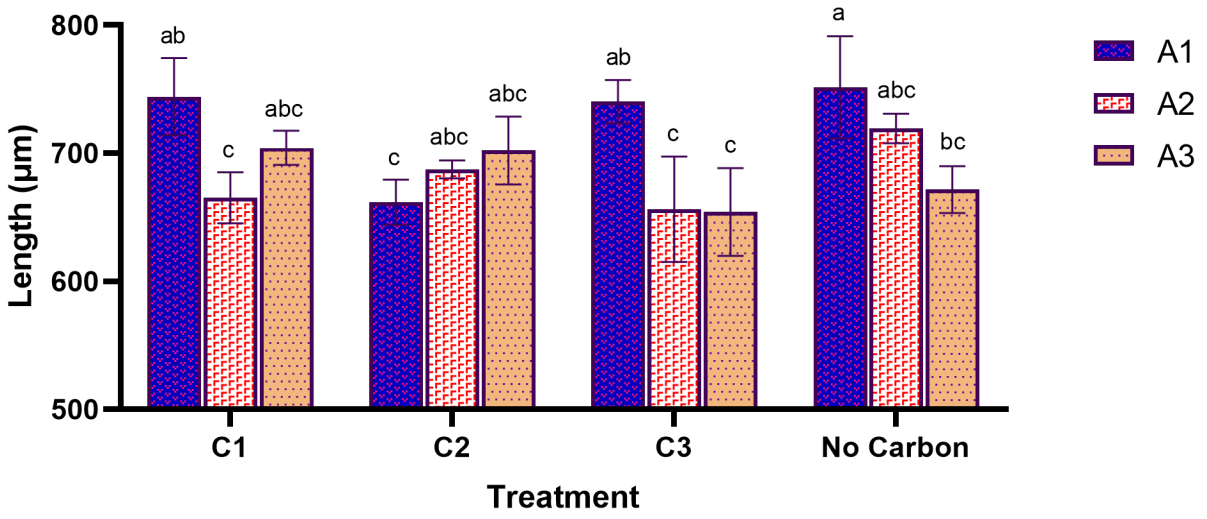

Fig. 2. Effect of microalgae and carbon sources (C1 - rice bran, C2 -tapioca flour, C3-molasses) on *Artemia* length after a 24-h enrichment period (A1 - *Cholrella* sp., A2 – *Nannochloropsis oceanica*, A3 – *Chaetoceros calcitrans*). Different superscripts indicate significant differences ($P < 0.05$) between treatments.

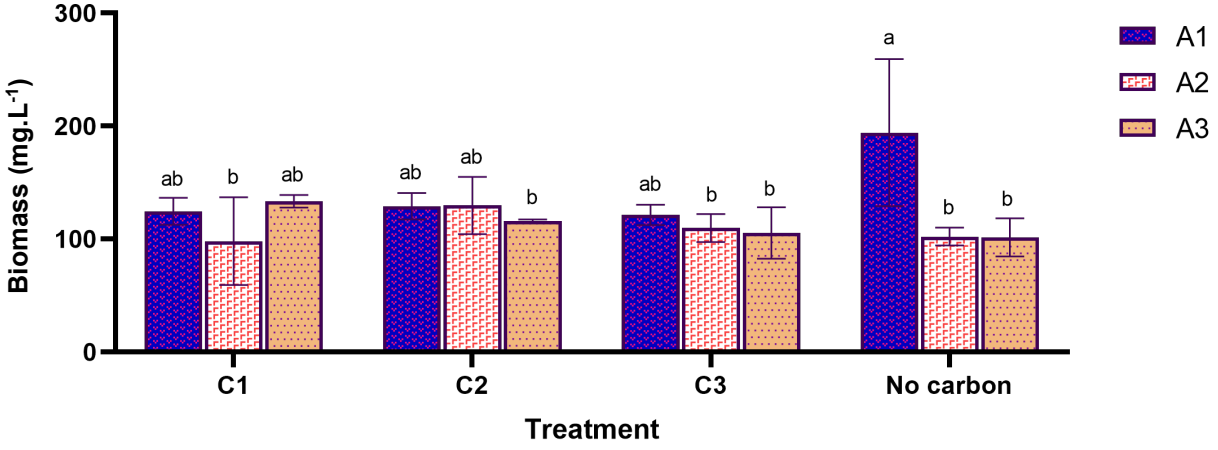

Fig. 3. Effect of microalgae and carbon source (C1 - rice bran, C2 -tapioca flour, C3 - molasses) on *Artemia* biomass after a 24-h enrichment period (A1 - *Cholrella* sp., A2 – *Nannochloropsis oceanica*, A3 – *Chaetoceros calcitrans*). Different superscripts indicate significant differences ($P < 0.05$) between treatments.

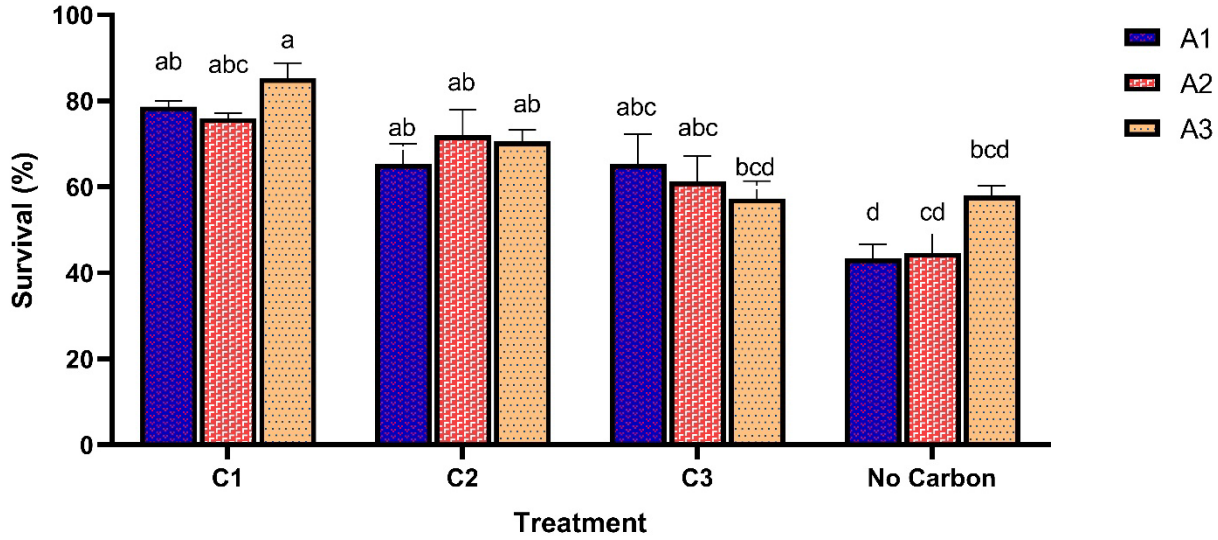

Fig. 4. Effect of microalgae and carbon source (C1 - rice bran, C2 -tapioca flour, C3 - molasses) on *Artemia* survival percentage after a 24-h enrichment period (A1 - *Cholrella* sp., A2 – *Nannochloropsis oceanica*, A3 – *Chaetoceros calcitrans*). Different superscripts indicate significant differences ($P < 0.05$) between treatments.

Table 2. Mean protein, lipid, ash and moisture percentage composition for main factors, results of post hoc Tukey's test (superscript characters) from two-way ANOVAs to test for the effect of different treatments on proximate composition of *Artemia* after 24-h enrichment period.

Treatment	Protein %	Lipid %	Ash %	Moisture %
Microalgae				
Chlorella sp.	58.16^{ab}	16.19^{ab}	10.51^{a}	90.85^{a}
Nannochloropsis oceanica	58.02^{b}	15.79^{b}	10.63^{a}	90.77^{a}
Chaetoceros calcitrans	58.41^{a}	16.45^{a}	10.03^{b}	90.61^{a}
Carbon source				
Rice bran	58.68^{a}	16.96^{a}	10.36^{ab}	90.54^{ab}
Tapioca flour	57.67^{c}	15.74^{b}	10.35^{ab}	90.96^{a}
Molasses	58.07^{b}	15.93^{b}	10.24^{b}	90.51^{b}
No carbon	58.37^{ab}	15.94^{b}	10.62^{a}	90.97^{a}
Significance level				
Microalgae	***	***	***	ns
Carbon source	***	***	***	ns
Microalgae* Carbon source	***	ns	ns	***
Mean	58.19	16.14	10.39	90.74
CV %	0.57	3.09	3.16	0.49

*Means with the same letter are not significantly different at $P > 0.05$ using LSD.
*Percentage protein, lipid and ash contents were determined from dry weights.

Table 3. Water quality parameters observed in different enrichment media.

Treatment	Variable						
	pH	Temperature (°C)	Salinity (ppt)	DO (mg.L^{-1})	TAN (mg.L^{-1})	NO_2-N (mg.L^{-1})	NO_3-N (mg.L^{-1})
A1	7.58 ± 0.01^{a}	25.7 ± 0.6	25.63 ± 0.31	6.57 ± 0.17^{cd}	0.30 ± 0.06^{a}	0.25 ± 0.02^{c}	11.27 ± 0.67^{c}
A2	7.55 ± 0.03^{ab}	26.2 ± 0.4	26.57 ± 0.25	7.32 ± 0.13^{ab}	0.31 ± 0.07^{a}	0.28 ± 0.01^{b}	12.93 ± 0.35^{c}
A3	7.44 ± 0.03^{e}	25.9 ± 0.2	26.13 ± 0.49	7.70 ± 0.20^{a}	0.31 ± 0.03^{a}	0.32 ± 0.03^{a}	12.30 ± 0.46^{c}
A1C1	7.52 ± 0.01^{abcd}	26.1 ± 0.9	26.10 ± 0.69	6.65 ± 0.28^{cd}	ND	ND	23.17 ± 1.60^{b}
A1C2	7.50 ± 0.01^{bcd}	25.8 ± 0.3	26.53 ± 0.55	6.57 ± 0.33^{cd}	ND	ND	26.10 ± 2.36^{ab}
A1C3	7.52 ± 0.05^{abc}	26.4 ± 0.6	27.03 ± 0.21	7.01 ± 0.05^{bc}	ND	ND	25.87 ± 1.34^{ab}
A2C1	7.51 ± 0.02^{bcd}	25.9 ± 0.3	26.83 ± 0.35	6.50 ± 0.23^{cd}	ND	ND	26.27 ± 0.55^{ab}
A2C2	7.48 ± 0.03^{cde}	26.0 ± 0.4	26.13 ± 0.49	6.70 ± 0.2°C^{d}	ND	ND	24.33 ± 0.67^{ab}
A2C3	7.44 ± 0.05^{e}	26.2 ± 0.4	26.40 ± 0.10	6.40 ± 0.12^{d}	ND	ND	25.73 ± 2.78^{ab}
A3C1	7.48 ± 0.01^{de}	26.0 ± 0.7	26.00 ± 0.35	6.72 ± 0.14^{cd}	ND	ND	28.03 ± 0.91^{a}
A3C2	7.54 ± 0.01^{abc}	26.0 ± 0.8	26.40 ± 0.36	6.35 ± 0.09^{d}	ND	ND	28.23 ± 0.91^{a}
A3C3	7.55 ± 0.05^{ab}	26.3 ± 0.4	25.47 ± 0.29	6.59 ± 0.12^{cd}	ND	ND	27.17 ± 0.64^{a}

DO = Dissolved oxygen; TAN = Total ammonia nitrogen; ND = not detected.
Different superscripts indicate significant differences ($P < 0.05$) between treatments.

faster growth, higher survival rates, and shorter moult intervals observed in scalloped spiny lobster *Panulirus homarus* phyllosoma larvae when enriched with *Chorella vulgaris* (Rosowski, 1989; Leema et al., 2010). When *Artemia* are grown in *Chlorella* sp.-based enrichment media, they are exposed to a high concentration of vitamins, including vitamin C, ascorbyl acetate, vitamin E, and alpha tocopherol (Smith et al., 2004). As a result, the *Artemia* larvae receive a nutritional boost, which enhances their immune system and disease resistance. This increased resistance to diseases allows for healthier larvae, leading to a higher biomass of *Artemia*. Emerenciano et al. (2011) observed similar enhanced growth in pink shrimp *Farfantepenaeus paulensis* postlarvae in C/N ratio-controlled culture systems.

The current study shows that aquatic media with a controlled C/N ratio promote *Artemia* growth through the supply of extra nutrients, and bacteria that are also thought to contribute to food breakdown through the release of enzymes (Toi et al., 2014). Bacteria may contribute in bivalve digestion by forming extracellular enzymes such as proteases and lipases (Prieur et al., 1990) and breakdown utilising bacterial enzymes has also been hypothesised for *Artemia* algal cell digestion (Maeda-martı and Lo, 2002; Toi et al., 2013).

In the context of microalgae-bacteria interactions, the study aligns with previous research showing that heterotrophic bacteria and autotrophic microalgae have intricate interactions that are either complimentary or competing, in addition to a range of stimulatory and inhibitory functions (Fuentes et al., 2016; Dauda, 2020). Microalgae excrete dissolved organic carbon (DOC), which serves as a substrate for heterotrophic bacteria. This DOC-mediated interaction plays a crucial role in nutrient cycling and organic matter decomposition within the ecosystem. *Artemia* survival was significantly affected by all three factors. The approach to enriched *Artemia* with microalgae observed a decreased level of the associated pathogenic bacterial population (Makridis et al., 2006; Makridis et al., 2009). By inhibiting the growth of pathogenic bacteria, the antimicrobial substances released by microalgae cells improve *Artemia* survival in enrichment media (Duff and Bruce, 1966; Makridis et al., 2006; Subashchandrabose et al., 2011).

In terms of biochemical composition, microalgal species exerted significant effects on the lipid, protein, and ash contents of *Artemia*. *Chaetoceros calcitrans* emerged as an effective enrichment source, showing higher levels of protein and lipids. Additionally, the addition of a carbon source led to a significant increase in lipid content, underlining the role heterotrophic bacteria in nutrient assimilation and energy storage.

Due to their abundance and functional variation, marine bacteria drive the biogeochemical cycles of a significant number of biologically important elements (Amin et al., 2012). Previous research has shown that carbohydrate supplementation stimulates bacterial growth, which not only improves water quality but also boosts the productivity of target aquaculture animals (Avnimelech, 1999; Hari et al., 2004; Crab et al., 2009; Nootong et al., 2011; Toi et al., 2013). A metagenomics analysis of alga-associated biofilms has shown that genes for lipases, esterases, and vitamin B production are common and functional, suggesting that these genes play important roles in algae-associated bacteria (Krohn-molt et al., 2013; Kouzuma and Watanabe, 2015). Diatoms release transparent extra polymer particles (TEP), an acidic polysaccharide that helps to colonize bacteria in aquatic systems (Passow, 2002). A genomic analysis of bacterial diversity in six non-axenic diatom cultures (*Ditylum*, *Thalassiosira*, *Asterionella*, *Chaetoceros*, *Leptocylindrus*, and *Coscinodiscus*) identified unique bacterial phylotypes linked with each genus and Proteobacteria and Bacteroidetes heterotrophic bacterial phyla observed in each diatom genera (Amin et al., 2012).

Artemia cultured in axenic conditions with dry food exhibited minimal or no survival, whereas the same meal with different microflora resulted in more than 60 % survival (Douillet, 1987). *Artemia* associated with *Flavobacterium* and *Aeromonas* strains positively effects *Artemia* survival while *Vibrio parahaemolyticus* and *V. alginolyticus* have a negative effect (Rico-Mora and Voltolina, 1995). *Artemia* cultured with rice bran demonstrated the highest *Artemia* survival and growth in *Pseudomonas* sp. associated media (Gorospe et al., 1996; Maeda-martı and Lo, 2002). Bacteria species, *Microbacterium* sp. and *Exiguobacterium* sp. serve as probiotics and improve the survival and growth of *Artemia* in cultures.

The total ammonia nitrogen (TAN) and nitrite-N were not detected in C/N ratio-controlled media. Addition of carbon source significantly reduced TAN and nitrite-N content in enrichment media. In aquatic systems, microbial processes such as photosynthesis, mineralisation, nitrification, denitrification, and assimilation by heterotrophic bacteria reduce the TAN content (Avnimelech 1999; Ebeling et al., 2006; Xu et al., 2016; Silva et al., 2017). In the current study, improved levels of heterotrophic bacteria were mainly involved to reduce the TAN and Nitrite-N content in enrichment media. The efficient conversion of ammonia and nitrite into nitrate by oxidising bacteria in the systems resulted in greater concentrations of Nitrate-N in all treatments with a carbon source. The C/N ratio-controlled enrichment media salinity was slightly higher than control treatments, possibly due to evaporation that occurred during the microalgae-bacteria consortium development period. Increased heterotrophic bacterial populations are likely to have resulted in higher respiration rates that increase the carbon dioxide concentration, which in turn resulted in lower DO content and pH in carbon source added treatments (Tacon et al., 2002; Wasielesky et al.,

2006; Wei et al., 2016; Silva et al., 2017; Deng et al., 2018). The favourable pH level within the enrichment media, coupled with reduced levels of TAN and nitrite nitrogen, significantly impacted the survival of *Artemia* in treatments with added carbon sources.

Conclusion

Despite initial expectations that *Artemia* would be lacking in unsaturated fatty acids, the findings revealed a significant rise in lipid content when the C/N ratio was properly regulated through the addition of a carbon source. The manipulation of this ratio appears to have positively influenced the accumulation of lipids in *Artemia*, potentially making it a more promising source of unsaturated fatty acids. Moreover, the addition of carbon sources improved the water quality of the enrichment media, enhancing them for *Artemia* nutrient assimilation and survival. Further studies are recommended to determine the bacterial species diversity in different carbon sources and microalgal mediated systems.

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Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Jayanthi Chandani Mallawaarachchi: Conducted experiments, analysed data, writing manuscript. Fatimah Md Yusoff: Conceptualisation, experimental design, manuscript review. Yam Sim Khaw, Zarirah binti Mohamed Zulperi, Ina Salwany Md Yasin: Formal analysis.

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