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Improvement of water quality, survivality, growth performance, and proximate composition of *Penaeus monodon* postlarvae through immobilizing *Tetraselmis chuii*

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ABSTRACT

In this study, an efficient, simple and eco-friendly biological method, immobilized *Tetraselmis chuii* beads (TetraBeads) were tested for its efficacy in maintaining water quality to improve the survival and growth of *Penaeus monodon* postlarvae. Results showed that the tanks, treated with TetraBeads, were able to significantly ($p < 0.05$) sustain TAN and $\text{NO}_2\text{-N}$ concentration below 0.1 mg/L. Shrimp PLs in the tanks, treated with TetraBeads beads, had significantly higher ($p < 0.05$) survival rate ($85 \pm 0.6\%$) and specific growth rate ($16 \pm 0.8\%$) and higher survival ($76 \pm 4.8\%$) when subjected to stress test compared to other treatments. In addition, shrimp PLs cultured in TetraBeads tanks had the highest protein (47.9 ± 0.2) and lipid (28.6 ± 0.3) content compared to control tanks (43.3 ± 0.5 ; 26.1 ± 0.6). This study illustrated the beneficial effects of TetraBeads in improving water quality, growth and survival of shrimp larvae culture in shrimp hatchery.

Keywords: TetraBeads, shrimp postlarvae, water quality, growth, survival

1. Introduction

Aquaculture is one amongst the quickest growing animal food-producing sectors around the world, with yearly increment averaging 6.1 % for 2002-2012 (FAO, 2014a). Significant increases in the demand for fish and seafood throughout the world aquaculture has grown rapidly over the last couple of decades (Banerjee et al., 2015). In many parts of the world, especially in Southeast Asia, *Penaeus monodon* (Tiger shrimp) is the most widely cultivated penaeid shrimp (FAO, 2014b). In the hatcheries, shrimp larvae are cultured from nauplii to postlarvae stage 16 and after which postlarvae are transferred to the grow-out ponds. During the nauplii to zoea stage, the amount of feed given is small, and it does not contribute to ammonia and nitrite accumulation, which is harmful to shrimp culture (Banerjee et al., 2010). Ammonia and nitrite

start to accumulate in the water column from the PL1 stage due to the high stocking density, feeding of artificial feed containing high protein and faeces (Banerjee et al., 2010). This accretion leads to high concentrations of ammonia and nitrite, which is a constraint in the shrimp post-larval rearing system (El-Sayed 2021). High concentration of nitrogenous compounds often causes deterioration of water quality in the hatchery system. Therefore, frequent water exchanges have been practiced in the most of the hatcheries to maintain good water quality in hatchery system (Saraswathy et al., 2015). Discharge of a large amount of wastewater from a hatchery is very laborious and expensive (Thompson et al., 2002), and increases the risk of introducing disease. Also, wastewater from hatcheries and grow-out ponds may cause eutrophication in rivers and coastal waters (Chislock et al., 2013).

Marine microalgal, *Tetraselmis chuii* is flagellated chlorophytes with rapid growth rate, and can stand a broad range of temperatures and pH values (Khatoon et al., 2014). *Tetraselmis* sp. is a widely used species in aquaculture as it contains adequate amounts of protein, lipid, carbohydrate and fatty acids which are essential for the cultured organisms (Khatoon et al., 2017). This species also serves as a rich source of a variety of bioactive compounds, such as vitamin E, carotenoids, phenolic compounds, and terpenes, which have antioxidant, anticancer and antimicrobial properties (Ismail et al., 2016). In addition, natural antioxidants found in microalgae may be used to increase the cell protection against oxidative damage, being a promising alternative to the carcinogenic synthetic antioxidants (Abdu Rahman et al., 2017).

Aquaculture wastewater can be treated through biological, chemical and physical method (Turcios and Papenbrock 2014). Recently, the use of microalgae for wastewater management has become a common choice for treatment (Wollmann et al., 2019; Abdel-Hameed and Hammouda, 2007), and reusing in intensive aquaculture systems. Microalgae have been used in many

researches to improve water quality as they utilize the nitrogenous waste and convert it to energy for their own use (Chopin et al., 2012; Khatoon et al., 2016).

Immobilizing microalgae is a common approach in numerous bioremediation applications (Soo et al., 2017). Sodium alginate is the most commonly used polymer matrix for microalgae immobilization (Moreno-Garrido, 2008). The immobilized microalgae have been exploited for removing nutrient in wastewater and have great potential to be used in marine aquaculture wastewater treatment (de-Bashan and Bashan 2010). Effluents from aquaculture are rich in solids and dissolved nutrients, mainly in the form of inorganic nitrogen and phosphorus (Bui et al., 2012). Effluents from aquaculture industry should be treated before discharge into its adjacent aquatic ecosystem to avoid eutrophication and water contamination. Hence, cultivation of immobilized microalgae in aquaculture water offers the advantages of nutrients removal from water as microalgae grow; the immobilization also solves the restriction of harvesting microalgae from the treatment system at the same time (Pires et al., 2013). Immobilization also enhances nutrient removal (Mallick, 2002; Jiménez-Pérez et al., 2004). Previous studies had shown in the immobilized *Chlorella vulgaris* in carrageenan and alginate (Soo et al., 2017) and *Scenedesmus* sp. cells in chitosan (Fierro et al., 2008) to treat primary domestic wastewater. Immobilization in alginate beads of the microalgae protects the microalgae when they perform wastewater treatment. The beads act as a physical barrier between the two environments. This maintains the treatment agents within the bead and keeps the native wastewater organisms out, thus allowing uninterrupted tertiary wastewater treatment (Sergio et al., 2012).

Immobilization of *Tetraselmis* sp. had shown positive results in the reduction of p -chlorophenol a toxic compound in the aqueous environment and on the other hand, is expected to improve aquaculture wastewater as well. The selected microalgal cells will be able to reduce

nitrogenous compounds and improve water quality in shrimp hatchery systems without having to exchange water during the culture of postlarvae. The use of immobilized *Tetraselmis chuii* beads in marine shrimp larval rearing system has not been tested previously. Therefore, the aim of this study is to investigate the effectiveness of using *Tetraselmis chuii* beads to maintain low levels of ammonia and nitrite and as well as improve growth and survival of *P. monodon* postlarvae in the hatchery system without water exchange.

2. Materials and methods

2.1. Preparation of Tetrabeads

Tetraselmis chuii were obtained from the Laboratory of live feed culture, Institute of Tropical Aquaculture, Universiti Malaysia Terengganu. 2% of the initial concentration in 300 ml Conway media (Tompkins et al., 1995) was cultured indoor with controlled pH (7.5), temperature (25°C), lighting (13.5 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), aeration and mixing. For beads with microalgae, 10^3 cells/ mL of *T. chuii* in 3% of alginate solution was dropped using titration method into 4% strontium chloride solution. For beads without algae that act as a control, no microalgae cells were added. The beads were kept in the strontium chloride solution for a period of 45 minutes to 1 hour to allow complete hardening. Later on, the beads were washed several times with filtered and sterilized seawater to remove any traces of strontium chloride (Mallick 2002). After that, the beads were stored in an algal Conway medium at 4°C in the dark for further use.

There were three treatments, i.e., 1) TetraBeads (2 beads/ml) (bead count based on Erna et al., 2013), 2) beads without *Tetraselmis chuii* cells, 3) 50% water exchange on alternate days (no algae and beads), while in control tank no water exchange and no beads were applied. Twelve rectangular glass aquarium (45×30×30 cm) were used as culture tanks, where each of them

contained 3 L clean filtered (5 µm filter bag) and chlorinated seawater. *Penaeus monodon* postlarvae stage 1 (PL1) were obtained from a commercial hatchery, and then stocked into the tanks at a density of 50 PL1/L. All the postlarvae were collected from a definite age class that hatched from a single brood stock and stocked on the same day. Constant aeration was provided to each tank using an air compressor. The tanks were maintained under a 12 hour light: 12 hour dark cycle. Shrimps in all the tanks were fed four times a day (6 hour interval) with a pellet type penaeid shrimp feed (Feed No 1–3, Higashimaru, Japan) and Artemia (Golden Dolphin, Malaysia). The feeds were supplied based on feed manufacturers' instructions. The experiment was conducted for a period of 14 days. TetraBeads were replaced every 3 days in the PL culture tanks.

2.2. Physical and chemical analyses

Temperature, salinity, pH, and dissolved oxygen in the culture tanks were measured daily using YSI 30 salinity/conductivity/temperature meter, ORION portable pH meter, and YSI 52 dissolved oxygen meter. Total ammonia nitrogen (TAN) and NO₂–N were also analyzed in every alternate day following the method of Parsons et al. (1984).

2.3. Proximate composition analysis

Proximate analysis of shrimp PLs from different treatments were accomplished at the end of the experiment.

Protein was analysed according to Lowry et al. (1951). For every sample, 5-6 mg of freeze dried shrimp PLs were taken and made into 25 mL solution by mixing with distilled water. From the 25 mL of sample prepared, 0.5 mL was taken from each sample for protein analysis. Prior to that, Reactive 1 (1 % Potassium sodium tartarate tartrate) and Reactive 2 (2 g of Sodium carbonate in 100 mL of 0.1 N NaOH) were prepared. Mixed reagent was prepared by adding 1 mL of Reactive 1 to 50 mL of Reactive. Then, 0.5 mL of sample was added with 0.5 mL of 1 N

Sodium hydroxide and it was kept in 100 °C water bath for 5 minutes. It was then cooled in a water bath and 2.5 mL of the prepared mixed reagent was added 10 minutes after cooling. The mixed solution was added with 0.5 mL of Folin reagent and was kept in dark places for 30 minutes. The reading of the mixed solution was taken with spectrophotometer (UV-1601, Shimadzu) at the wavelength of 750 nm.

The lipid analysis was conducted by the sulphuric acid-charring method based on Marsh and Weinstein (1966), following the carbonization method using tripalmitin as the standard after extracting lipids according to the method of Bligh and Dyer (1959). The samples were extracted from 4.5 mL of chloroform: methanol (1:2 concentration) and it was then centrifuged at 10 000 rpm for 10 minutes. After separating the supernatant from the biomass, 1.5 mL of chloroform and 1.5 mL of distilled water was added and the sample was centrifuged again to facilitate the separation of two phases. After centrifugation, the polar phase was removed with a pipette and evaporated under vacuum with a water-bath at 35 °C. The dry-residue was solubilized in 1 mL chloroform. Then, 3 aliquots of 200 µL each was taken from this solution and transferred into test tubes and the solvent was evaporated again. When completely dried, 2 mL of concentrated sulphuric acid was added. The carbonization process was carried out at 200 °C for 15 minutes, then the tubes were cooled and 3 mL of water was added into each tubes. The optical density was measured at 375 nm.

Carbohydrate analysis was conducted based on the method of Dubois et al. (1956). For each sample, 5-6 mg was taken and made into 25 mL solution by mixing with distilled water. Prior to analysis, 5 % phenol solution and concentrated sulphuric acid was prepared. Samples were

analysed by adding 1 mL of 5 % phenolic solution and 5 mL of concentrated sulphuric acid. The optical density was measured at 488 nm in a spectrophotometer (Shimadzu UV-1601, Japan).

2.4. Biological parameters analysis

The specific growth rate (SGR) was calculated according to Ricker (1990) based on dry weight of PLs. At the end of the experiment, survival, and reverse salinity stress test (commonly practiced by commercial hatcheries to distinguish between healthy and weak PLs) were calculated. For the stress test, shrimp PLs were transferred from the culture tanks (30 ppt salinity) to freshwater tanks (0 ppt), and survival was recorded after 2 h.

2.5. Statistical analysis

The collected data were analyzed using one-way analysis of variance (ANOVA). Significant differences amongst treatments were determined using Duncan's multiple range test at 0.05 level. Normality and heteroscedasticity of the data were checked prior to the analysis. Statistical analyses were accomplished using the SPSS (IBM statistical software, v.26.0).

3. Results and discussion

3.1. Water quality parameters

Throughout the experiment there were no significant difference ($p > 0.05$) in physicochemical parameters such as dissolved oxygen, pH, salinity, and water temperature among the different treatments and control, all these physicochemical parameters were within the normal range for shrimp culture (Table 1).

Table 1

Mean values $\pm$ standard error of temperature, dissolved oxygen, salinity and pH range in treated and control tanks.

Treatment tanks	Parameters			
	Temperature ($^{\circ}\text{C}$)	DO (mg/L)	pH	Salinity (ppt)
TetraBeads	28.01 ± 0.01	6.00 ± 0.10	7.20 – 8.1	29.00 ± 0.02
Beads W/O <i>Tetraselmis</i>	28.20 ± 0.03	6.20 ± 0.12	7.10 – 7.9	30.00 ± 0.01
50% water exchange	29.10 ± 0.02	6.10 ± 0.10	7.00 – 8.2	29.80 ± 0.05
Control	29.90 ± 0.04	6.60 ± 0.01	7.50 – 8.0	30.00 ± 0.07

Total ammonia nitrogen (TAN) and nitrite nitrogen ($\text{NO}_2\text{-N}$) concentrations (mg/L) were significantly reduced ($p < 0.05$) from initial concentrations of 1.5 ± 0.02 and 1.0 ± 0.02 , respectively. Significant differences in TAN and $\text{NO}_2\text{-N}$ were also observed in the tanks containing TetraBeads (0.10 ± 0.01 and 0.09 ± 0.02 , respectively) compared to beads without *Tetraselmis* cells (2.0 ± 0.01 and 1.7 ± 0.03 , respectively), 50% water exchange (0.55 ± 0.02 and 1.68 ± 0.01 , respectively) and the control (3.8 ± 0.04 and 1.99 ± 0.03 , respectively). TAN and $\text{NO}_2\text{-N}$ concentration were observed at low concentration in the TetraBeads tanks throughout the 14 days culture periods, while TAN and $\text{NO}_2\text{-N}$ concentrations gradually increased in the control tanks (Fig. 1, 2).

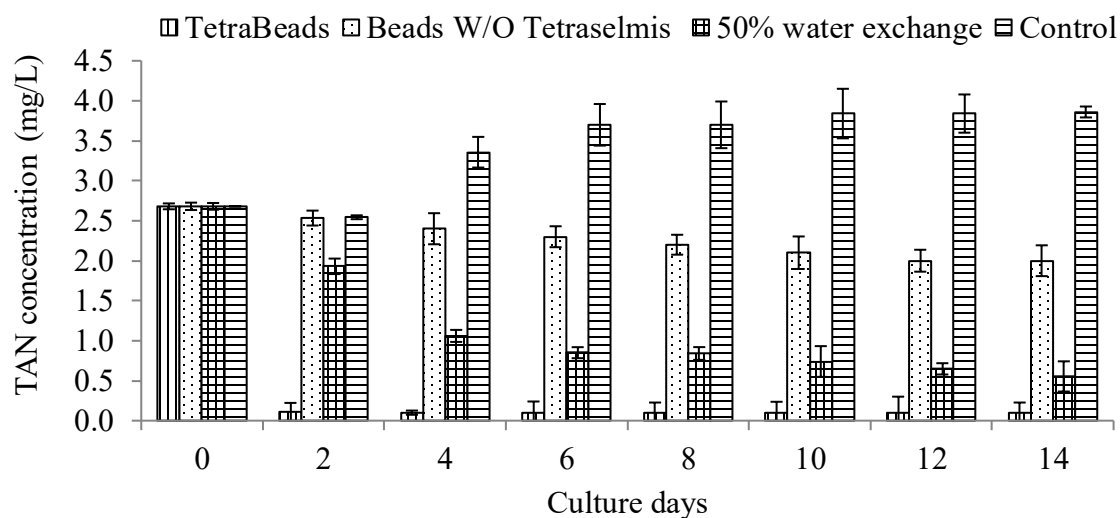


Fig. 1. Total ammonia nitrogen (TAN) concentration (mg/L) in treated and control tanks during the experimental period. Vertical bars indicated standard error of the means.

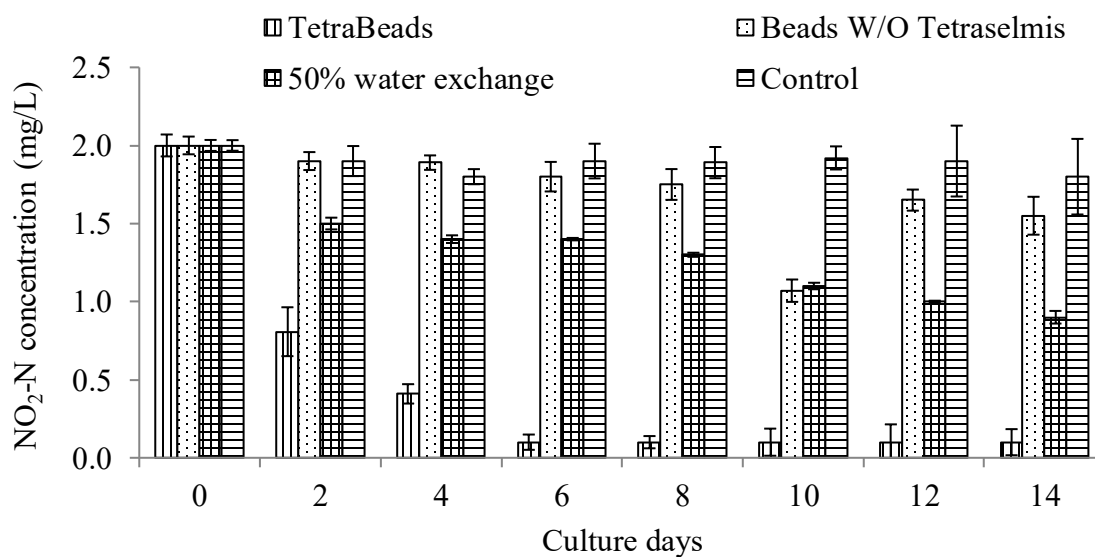


Fig. 2. Nitrite nitrogen ($\text{NO}_2\text{-N}$) concentration (mg/L) in treated and control tanks during the experimental period. Vertical bars indicated standard error of the means.

Nitrogen and phosphorus uptake by the green microalgae *Scenedesmus quadricauda* revealed that the maximum removal percentage of 1 mg/L ammonia and phosphate solutions reached up

to 100% and 86%, respectively, after 4 days using free microalgae but higher removal efficiency can be achieved by properly increasing cell density in beads (Sukačová and Červený, 2017). However, free floating microalgae are difficult to harvest, for this reason immobilized microalgae is used. One of the benefits of cultivating microalgae in alginate beads is to prevent it from being grazed upon by zooplankton (Soo et al., 2017). By immobilizing the microalgae, it keeps the cells in place, thus solving the harvest problem (Soo et al., 2017). It also helps to facilitates easier operation and separation (Mallick and Rai, 1993) during culture of any aquatic organisms.

At present, the most commonly used polymer matrix for microalgae immobilization is sodium alginates which do not suffer extreme physical and/or chemical changes in the process of immobilization. It has shown the excellent permeability, low or null toxicity and high transparency of alginate matrix which produce a suitable environment for immobilized microalgae (Zhang et al., 2012). Microalgae beads can be harvested effortlessly and further processed to obtain the microalgae inside by dissolving the beads. Furthermore, it can be processed into powdered form for fish feed. Besides that, the supernatant obtained from the microalgae beads can be reused in the land base agriculture as fertilizers.

Ammonium is one of the most preferable chemical forms of nitrogen that can be readily absorbed by almost all microalgae species (Wang et al., 2010; Perez-Garcia et al., 2011). *Scenedesmus* sp. remove ammonium (NH_4^+) up to 90 - 93% within four hours (Kaparapu and Geddada, 2016; Pham and Bui, 2020) from waste water; whereas, *Scenedesmus obliquus*, immobilised within 2% calcium alginate, showed that ammonium remediation was not linked to NH_4^+ concentration with minimum residual concentrations of <0.001 mg/L (Whitton et al., 2018). The alginate immobilised *C. sorokiniana* represents a promising tool for the removal of nitrogen from drinking water sources (Petrović and Simonic, 2015) as ammonium is absorbed

directly into the cells and then transformed into amino acids, less energy is required for its uptake than for nitrate.

In our experiment, initial TAN concentration was same in all treatment as all the experiments were started at the same time and fed by the same feed. Only after two days later from the experimental set up significant reduction of TAN was occurred by TetraBeads (from initial concentrations of 1.5 ± 0.02 to reduce at 0.10 ± 0.01) and almost remained at this same lower level throughout the 14 days culture periods as it is well established that NH_4^+ rather than NO_3^- is preferentially consumed by algae (Sanz-Luque, and Chamizo-Ampudia, 2015).

Since the removal efficiencies on nitrate are usually lower than those on ammonium, green microalgae such as *Chlorella* and *Scenedesmus* proved preference for ammonium to any other form of nitrogen present in wastewater (Ruiz-Marin et al., 2010; Delgadillo-Mirquez et al., 2016). Two treatments using i) cyanobacterium coated substrate, ii) mixed diatoms coated substrate carried out *Penaeus monodon* postlarvae (PL) in a shrimp hatchery showed that cyanobacteria coated substrate was more effective in reducing $\text{NO}_2\text{-N}$ than the mixed diatoms (Khatoon et al., 2007). It is well established that NH_4^+ rather than NO_3^- is preferentially consumed by algae. When both forms are present in the wastewater there is minimal consumption of NO_3^- until most of the NH_4^+ has been removed (Silva et al., 2015). Sanz-Luque et al. (2015) explain that high NH_4^+ concentrations will inhibit nitrate reduction (NR) activity, and hence lower NO_3^- uptake. In this study, reduction of $\text{NO}_2\text{-N}$ concentration by TetraBeads was significantly higher (from initial concentrations of 1.0 ± 0.02 to reduce at 0.09 ± 0.02) followed by 50% water exchange tank because along with ammonium microalgae can consume nitrogen from a variety of nitrogen sources, including ammonium, nitrate, nitrite, and urea (Wang et al., 2010; Perez-Garcia et al., 2011). As like as other microalgae *Chlorella* sp., *Scenedesmus* sp., *Oscillatoria* sp., our result significantly confirmed that *Tetraselmis* species may

be a promising candidate for removal of TAN and $\text{NO}_2\text{-N}$ from aquaculture waste water and extremely correlated with the different previous study. Moreover, in our experiment tanks with beads without *Tetraselmis* cells also showed some TAN assimilation and reduced the TAN at a certain level (2.0 ± 0.01) as it was also reported that ammonium ions could interact with the carboxyl groups of the alginate beads (without microalgae) and chemically bound to it, which could also be reflected in the reduction of ammonium as they infiltrated continually through alginate into the cells (Ruiz-Marin et al., 2010). But beads without *Tetraselmis* cells could not reduce $\text{NO}_2\text{-N}$ at a greater level because alginate beads assimilate TAN at a higher level but $\text{NO}_2\text{-N}$ assimilation is very low (1.5 ± 0.03).

In case of tanks with 50% water exchange, all kinds of leached out nutrients removed together with the discharged water which result in a great reduction of nutrient in waste water. Following TetraBeads, 50% water exchange tank showed the lower TAN and $\text{NO}_2\text{-N}$ (0.55 ± 0.02 , 1.68 ± 0.01 , respectively) concentration as in every alternative day 50% tank water was discharged by addition of new water. Along with the discharged water it contained some nutrients released by uneaten feed and feces which consequence in a significant reduction of TAN, $\text{NO}_2\text{-N}$ and other nutrients level. Consequently, there are strict legislations on their discharge in environment without proper treatment to prevent environmental damage because it can cause eutrophication and toxicity in aquatic environments (Chislock et al., 2013). On the other hand conventional biological, chemical and physical treatment methods that separate nutrients from waste water but that is more expensive and uneconomical (Christenson and Sims, 2011).

But in case of control tank, continuous increase of TAN and $\text{NO}_2\text{-N}$ (3.8 ± 0.04 and 1.99 ± 0.03 , respectively) concentration occurred as there were neither assimilation system like microalgae or beads nor water exchange system. Throughout the 14 days experiment period it

showed the highest TAN and NO₂-N concentration due to the continuous assimilation of waste like uneaten feed and feces resulting in a continuously produced TAN during the culture period (Erna et al., 2013).

3.2. Survival and growth of shrimp postlarvae

Shrimp PLs cultured in the tanks containing TetraBeads showed significantly ($p < 0.05$) higher survival ($85 \pm 0.6\%$) followed by beads without *Tetraselmis* cells ($66 \pm 0.3\%$), 50% water exchange ($71 \pm 0.5\%$), and the control ($60 \pm 0.4\%$) (Fig. 3a). Significantly ($p < 0.05$) higher growth rates ($16 \pm 0.8\%$) were found in shrimp PLs cultured in TetraBeads containing tanks compared to beads without *Tetraselmis* cells ($12 \pm 0.5\%$), 50% water exchange ($13 \pm 0.3\%$), and control ($10 \pm 0.9\%$) (Fig. 3b). In addition, reverse salinity stress test showed that PLs cultured in the tanks containing TetraBeads had also significantly higher survival rate ($76 \pm 4.8\%$) followed by beads without *Tetraselmis* cells ($60 \pm 1.0\%$), 50% water exchange ($65 \pm 3.5\%$), and the control ($50 \pm 3.2\%$) (Fig. 3c).

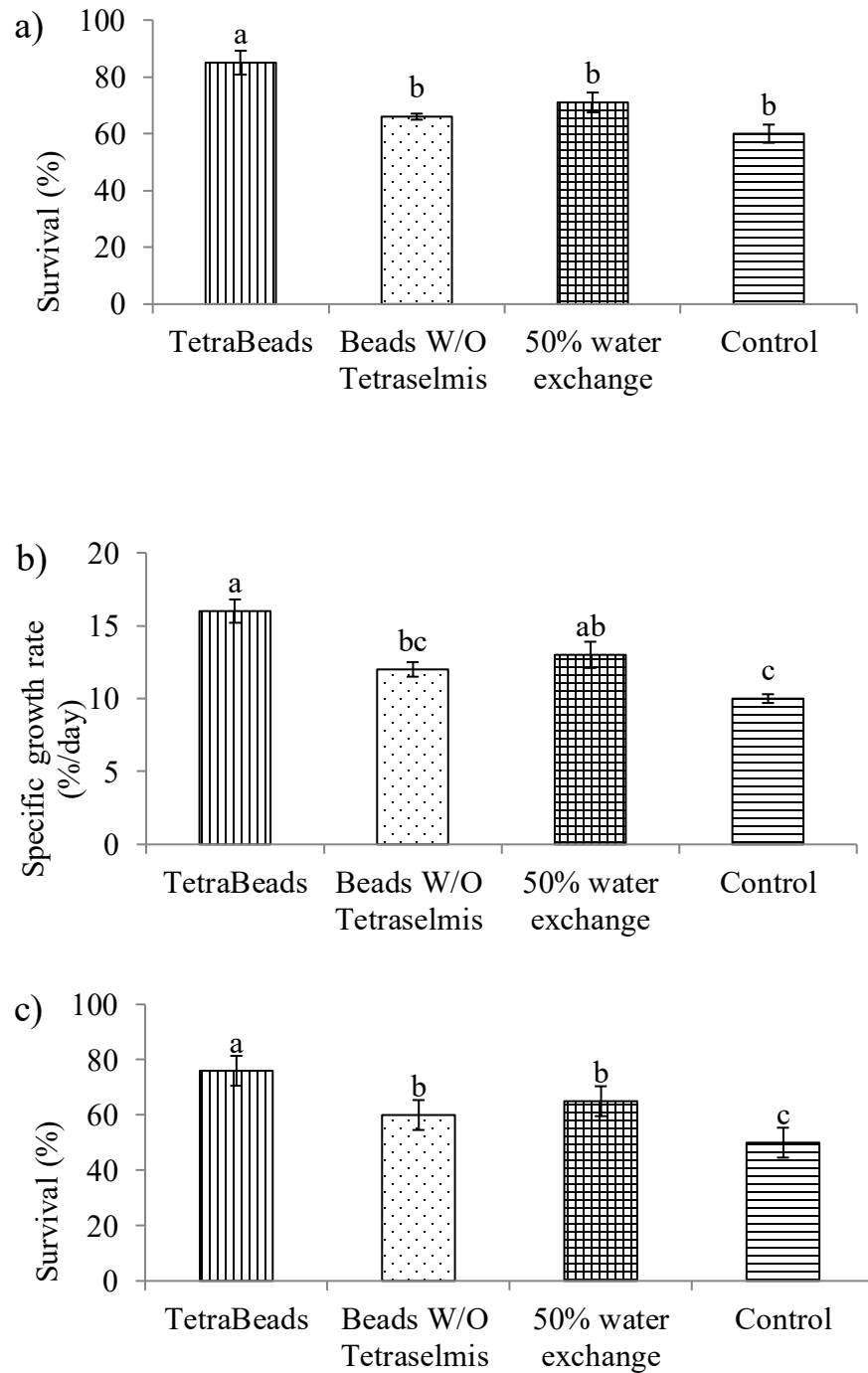


Fig. 3. Survival (a), specific growth rate (SGR; $n = 100$ PL) (b), and survival after the reverse salinity stress test ($n = 100$ PL) (c) in the treatment and control tanks after a culture period of 14 days. Values are mean $\pm$ SE.

As ammonia and nitrite can be lethal to aquatic organisms when present in a significant amount, so they can have detrimental effects on PL of shrimp in a closed culture system (Ciji and Akhtar 2019) and therefore they are required to be removed from the culture water for better growth and survival of the cultured PLs. If not they will cause reduced feed intake, retarded growth, susceptibility to diseases and poor survival of the cultured organisms (Manju et al., 2009). The results of this study indicates that the immobilized microalgae beads (TetraBeads) used here is efficient in maintaining low levels of TAN and $\text{NO}_2\text{-N}$ and that there is therefore has the potential to increase the survival of PLs, produce healthier shrimp and simplify the larval culture technique as no water exchange is required. In the experiment, the survival rate of shrimp PLs in tanks containing the immobilized TetraBeads was significantly higher followed by beads W/O *Tetraselmis* cells, 50% water exchange and control, respectively, probably due to the low concentration of TAN and $\text{NO}_2\text{-N}$. There was a minimum significant variation of TAN concentration between the tanks with 50% water exchange and the tanks treated with immobilized TetraBeads. However, it was quite entrancing to observe that PLs survival rate in tanks with 50% water exchange was relatively lower than PLs in tanks treated with immobilized TetraBeads. In the tanks with 50% water exchange, the shrimp PLs might be subsequently stressed due to the sudden environmental changes caused by the frequent water exchange and that might have made the differences on survival rate. Physicochemical factors such as pH, temperature, dissolved oxygen, etc. vary, shrimps become vulnerable to stress, bringing about diseases like red color, soft shell, tail rot and black gill (Alam et al., 2007). High stocking density and excessive use of feed lower water quality that contributes to fret and diseases among shrimp in intensive farming systems (Abdullah et al., 2019). The shrimp can be stressed if the environmental condition changes instantaneously resulting from water replacement in small culture ponds or tanks. It was also interesting to note that PL mortality also highly occurred in

control tanks associated with high TAN concentration. As there was no water exchange or TetraBeads to uptake ammonia, the TAN concentration was high in both control tank and tank with beads W/O *Tetraselmis* cells resulting in a low survival rate of the cultured PLs.

In the present study, significantly highest specific growth rates (SGR) were found in shrimp PLs cultured in TetraBeads containing tanks compared to beads W/O *Tetraselmis* cells, 50% water exchange, and control. In addition, a reverse salinity stress test was done to perceive the survival strength of shrimp PLs among the four treatments. PLs cultured in tanks containing TetraBeads had significantly higher survival rate compared with the other three treatments. This might be because of better water quality condition and minimum stress in PLs at the tank of TetraBeads compared to other treatments.

3.3. Proximate composition

Proximate composition of shrimp PL varied significantly ($p < 0.05$) among the treatments as well as differed from the control (Table 2). Shrimp PLs reared in the TetraBeads tanks had the highest ($p < 0.05$) amount of protein and lipid but the lowest amount of carbohydrate (Table 2). In addition, protein content was also significantly higher ($p < 0.05$) in shrimp PLs cultured in the tanks of beads without *Tetraselmis* and 50% water exchange than the control (Table 2). On the other hand, there were no significant difference ($p > 0.05$) of lipid content of PLs among the tanks with beads without *Tetraselmis* cells, 50% water and the control (Table 2). The highest carbohydrate was recorded in the PLs cultured with 50% water exchange (Table 2).

Table 2

Mean values of the proximate composition of *Penaeus monodon* postlarvae (PL14) in the treatment and control tanks.

Proximate Composition (% dry weight)	Treatments			
	TetraBeads	Beads without <i>Tetraselmis</i>	50% water exchange	Control
Protein	47.9 ± 0.2 ^a	45.3 ± 0.5 ^b	45.6 ± 0.3 ^b	43.3 ± 0.5 ^c
Lipid	28.6 ± 0.3 ^a	26.2 ± 0.0 ^b	27.2 ± 0.6 ^b	26.1 ± 0.6 ^b
Carbohydrate	22.2 ± 0.1 ^c	23.5 ± 0.3 ^{bc}	25.0 ± 0.8 ^a	24.6 ± 0.3 ^{ab}

Many studies have reported that total ammonia nitrogen originates more toxic stress to shrimp in early stages (Barajas et al., 2006; Luo et al., 2016) followed by nitrite, and nitrate (Luo et al., 2016). Kim et al. (2005) referred that total ammonia nitrogen, nitrite and nitrate nitrogen cause oxygen deficiency in water body. In culture system, when concentration of ammonia and nitrite nitrogen concentration exceeded the optimum level, fish faces obstacles for respiration properly due to lack of oxygen. That also causes disturbance in case of feed intake and appropriately feed utilization. This occurrence ultimately hampers fish growth and proximate quality. In addition, in aquaculture *Tetraselmis* sp. is widely used due to its contained adequate amounts of protein, lipid, carbohydrate and fatty acids which are essential for the cultured organisms (Abdu Rahman et al., 2017).

In the present study, the percentage of protein, lipid, and carbohydrate are significantly higher in PLs reared in tanks containing TetraBeads compared to other treatments. Possible reason might be the water, because TAN and NO₂-N concentrations were comparatively low in tanks containing TetraBeads than 50% water exchange, beads W/O *Tetraselmis* cells, and control. Due to low concentration of ammonia and nitrite nitrogen, feed utilization was maximum that promoted the increasing proximate quality of PLs reared in TetraBeads tanks. On

the other hand, in control treatment TAN and NO₂-N concentrations were the highest that outcomes in lower protein concentration of shrimp PLs compared to the rest three treatments.

4. Conclusion

Water quality has been considered to be an important factor that influences the growth and survival of shrimp postlarvae. The results of the present study suggest that *Tetraselmis chuii* immobilized in alginate is efficient in maintaining low levels of TAN and NO₂-N in postlarvae rearing tank and therefore no necessity to change the water throughout the culture period. In addition, immobilized *T. chuii* beads also have the potential to increase the survival of PLs, produce healthier shrimp and simplify the larval culture technique. These findings may encourage aquaculturist to consider immobilized *Tetraselmis chuii* as a viable alternative biological method to maintain low level of nitrogenous compounds in aquaculture system. Further research should be carried out using this microalgal beads in a commercial hatchery system.

CRedit authorship contribution statement

Helena Khatoon: Conceptualization; Project administration; Critical review & editing and submission. Kwan Penz Penz, Sanjoy Banerjee, Anisul Islam Mahmud, Mohammad Redwanur Rahman, Sohel Mian: Methodology; Data curation; original draft. Tashrif Mahmud Minhaz and Shahadat Hossain: Formatting manuscript; Statistical analysis; Review and editing.

Declaration of competing interest

The authors declare that there is no known competing interest among the authors that may appear in the work reported in this manuscript.

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