Aquacult Int (2017) 25:393–411
DOI 10.1007/s10499-016-0037-6

Evaluation of suitability of wastewater-grown microalgae (*Picochlorum maculatum*) and copepod (*Oithona rigida*) as live feed for white leg shrimp *Litopenaeus vannamei* post-larvae

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Received: 2 March 2016 / Accepted: 5 July 2016 / Published online: 11 July 2016

Abstract The present work deals with the growth efficiency of *Picochlorum maculatum* and *Oithona rigida* in shrimp-cultured wastewater. In addition, the effects of wastewater (WW)-cultured *P. maculatum* and *O. rigida* on the growth and survival of *Litopenaeus vannamei* post-larvae (PLs) was studied and the results were compared with artificial culture media (ACM)-cultured *P. maculatum* and natural seawater (NSW)-cultured *O. rigida*. The results revealed that the high density obtained in microalgae and low in copepod using wastewater as a medium. Further, shrimp PLs fed with WW-cultured microalgae, and NSW-cultured copepod had specific growth rate and higher survival, but it was not significantly different ($p > 0.05$) from PL fed on ACM-cultured microalgae and WW-cultured copepod, indicate that *P. maculatum* has potential to be used as live feed for the hatchery rearing of *L. vannamei* PLs, in replacing microdiet. Further study is needed on optimization of wastewater-cultured copepod as a live feed to yield maximum growth and survival.

Keywords *Picochlorum maculatum* · *Oithona rigida* · *Litopenaeus vannamei* · Wastewater · Larval rearing · Nutritional composition

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Introduction

Plankton is the natural food item of many marine and freshwater fishes and crustaceans. They have been used extensively to rear larvae and fry (Kibria et al. 1999). Both live (Ananthi et al. 2011) and frozen live feed (Sargent et al. 1997) have been used to rear fish and crustaceans. Earlier studies have shown that the larvae performed better when fed with plankton than when fed with artificial dry or commercial feed (Dabrowski 1984; Dave 1989). As microalgae and copepod protein is of good quality, with amino acid profiles comparable to that of other reference food proteins (Becker 2007), it could be a possible alternative to commercial feeds. In addition, microalgae, which are the source of all photosynthetically fixed carbon in the food web of aquatic animals (Kwak and Zedler 1997), may be an ideal replacement for commercial diet. Some of the workers have successfully reared the fish and shrimp by using microalgal species such as *C. mulleri* and *I. galbana* (Sangha et al. 2000), Spirulina extraction (Palmegiano et al. 2005), *Nanofrustulum* sp., *Tetraselmis* sp. (Kiron et al. 2012), and *I. galbana* (Rohani-Ghadikolaei et al. 2013).

Similarly, marine copepods are considered to be "nutritionally superior" for marine fish and prawn larvae, and they are valuable sources of proteins, lipids, carbohydrates, amino acids, fatty acids and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval digestion and metamorphosis (Yurkowski and Tabachek 1979; Fluchter and Rembold 1986; Munilla-Moran et al. 1990; Pillay 1990; Stottrup 2000). Ananthi et al. (2011) have suggested that the marine copepod (*Oithona rigida*, *M. gracilis* and *Pseudodiaptomus* sp.) enhances the growth, survival and astaxanthin of tiger shrimp *Penaeus monodon* when compared to *Artemia*.

It is well known that the plankton (microalgae and copepod) grow abundantly in the nutrient-rich wastewaters such as shrimp- and fish-cultured wastewater (SCWW and FCWW), but the resource remains unutilized. However, to date, there is no research on the utilization of this resource for secondary aquaculture in Indian context except Rajthilak et al. (2013). On the other hand, commercially available artificial culture media (ACM) is widely utilized for cultivating algae. There are a number of predictable media, such as Walne's or Conway, F/2 media, Miquel's, and Scheiber's, being used for the culture and maintenance of microalgae in research laboratories as well as in fish and shrimp hatcheries (Guillard 1975; Ip et al. 2004). The production of ACM is tedious and often too costly. Therefore, it is essential to evaluate other source as alternative culture medium for culturing microalgae and copepod. This study examined the nutritional composition of wastewater-grown microalgae (*Picochlorum maculatum*) and copepod (*O. rigida*) and evaluated their suitability as live feed for white leg shrimp *Litopenaeus vannamei.* As a first step, in this direction, we have cultured marine microalga *Picochlorum maculatum* and marine copepod *O. rigida* in 90-day-old shrimp-cultured wastewater. Then, the PLs 10 of *L. vannamei* were fed with ACM- and WW-cultured microalgae and NSW- and WW-cultured copepod for 21 days. The length, weight and survival were analyzed every 7 days, and nutritional characters were analyzed initial and final day of the experiment.

Materials and methods

Wastewater collection

The 90-day-old *L. vannamei*-cultured wastewater was collected from Parangipettai, Tamil Nadu, India (11°28′4.18″N; 79°43′45.12″E). The collected wastewater was transported to

laboratory using temperature-controlled ice box and kept undisturbed for the suspended particles to settle down.

Collection and culture of microalgae

Marine microalgae *Picochlorum maculatum* (PSDK01) (Accession number: KJ754560) was isolated from Palk Bay region of Muthukuda coast (9°51′48″N; 79°7′15″E), Tamil Nadu, India and isolated by using agar plating technique (Robert 2005). Indoor algal stock culture was maintained in a special air-conditioned room, and algal culture was made according to Anderson (2005). Stock cultures were kept in 250-ml culture flasks. Seawater was filtered using filter bag (1 μm) and sterilized using autoclave. After cooling, water was transferred to the culture flask plugged with cotton. Vessels used for algal culture was sterilized properly and dried in an oven before use. The Walne medium (Walne 1970) was used for indoor culture. About 10 ml of inoculum in the growing phase was transferred to the culture flasks, and the culture was maintained with 12:12 h light and dark cycle provided with 200 μmol m^{-2} s^{-1} using fluorescent bulbs. After 5–8 days, the maximum exponential phase was obtained. Temperature and salinity was maintained in the range between 23 and 25 °C and 28 and 30 ppt., respectively, for entire culture period. The same light conditions and temperature were maintained for culturing microalgae in wastewater. The wastewater was used as a culture medium instead of Walne medium.

Copepod culture

The copepod *O. rigida* were used from our culture collection. From these, the healthy gravid female of *O. rigida* was picked up by using a fine capillary tube. The isolated copepods were kept overnight in 250-ml beakers containing filtered seawater (1 μm) of ambient salinity (34 psu) with vigorous aeration for starving prior to the experiment, and the same conditions were maintained for culturing copepod in wastewater. NSW and WW copepod were fed one time per day with ACM and WW-grown microalgae, respectively, with average cell concentration (20,000 cells ml^{-1}).

Feeding experiment

In this experiment, twelve rectangular Fiberglass Reinforced Plastics (FRP) tanks (70 cm × 50 cm × 30 cm size and 6 mm thick; outside blue and inner white tanks, 100 L capacity) each containing 50 L clean, filtered (5 μm filter bag) seawater were used. Four treatments were maintained and were fed with (1) artificial culture medium (ACM)-cultured microalgae (2) wastewater (WW)-cultured microalgae (3) natural seawater (NSW)-cultured copepod and (4) wastewater (WW)-cultured copepod with all treatment in triplicates. *Litopenaeus vannamei* PL stage 10 (PL10) were obtained from the Rank Marine Hatchery (Marakkanam, Tamil Nadu, India) and were stocked at a density of 50 PL L^{-1}. An air compressor was used to provide constant aeration to each FRP tanks. The FRP tanks were maintained under a 12- h:12-h light and dark cycle. Shrimp PLs were regularly fed every 8-h interval daily with ACM microalgae (Treatment 1), WW microalgae (Treatment 2), NSW copepod (Treatment 3) and WW copepod (Treatment 4) with densities of microalgae (approximately 1,00,000 cells ml^{-1}) and copepod (8–10 individual's ml^{-1}). NSW and WW copepod *O. rigida* were cultured in 5-L-round-bottom plastic container and fed with ACM and WW microalgae *P. maculatum* (PSDK01), respectively, at 18,000–20,000 cells ml^{-1}. The experiment lasted for 21 days.

Water quality analyses

Temperature, salinity, pH and dissolved oxygen were recorded daily in culture tanks. Temperature was estimated using standard centigrade thermometer. Salinity was estimated by using Hand Refractometer (Erma, Japan). pH was estimated using Elico grip pH meter. Dissolved oxygen, total dissolved phosphorous (TP) and total dissolved nitrogen (TN) were estimated according to Strickland and Parsons (1972). TP and TN were estimated on the final day of the experiment. Daily water exchange was carried out at 30 %, and uneaten food and fecal matter were siphoned out. Ahead of larval rearing, heavy metal (Zn, Cr, Cu, Pb and Cd) concentration in wastewater was estimated by APDC (ammonium pyrolidine dithiocarbamate) and MIBK (methyl isobutyl ketone) extraction method with help of atomic absorption spectrophotometer (1983- 400 HGA 900/AS 800 PerkinElmer) (Brooks et al. 1967).

Morphometric analyses

Shrimp sampling was made every 7 days once for morphometric analyses (Length, weight and survival). Shrimp growth rate (dry weight) was calculated from the body weight (mg) based on the formula derived from Ricker (1979): $G = (W_2 - W_1)/(T_2 - T_1)$, where W_2 and W_1 represent the final and initial weight of the shrimp, respectively, and $(T_2 - T_1)$, the duration of the experimental period. For length (L) analyses, initial length (I_L) of shrimp was subtracted from final length (F_L) with the help of following formula ($L = F_L - I_L$). The survival of the PL was also determined at each sampling. Survival was calculated as per the percentage of shrimp remaining in each tank from the estimated number stocked initially.

Nutritional composition analyses

ACM- and WW-cultured microalgae (*P. maculatum*) were harvested at exponential phase, washed with double distilled water for removing salts from the algae then freeze-dried and used for further analyses. NSW- and WW-cultured copepod (*O. rigida*) were harvested on day 20 based on the life span, freeze-dried and used for analysis. In case of *L. vannamei*, the initial and final concentrations of nutritional compounds of shrimp PLs were analyzed. The moisture, protein, carbohydrate, lipid and ash contents in live feeds and shrimp larvae were estimated following standard methods (Rajendran 1973; Raymont et al. 1964; Dubois et al. 1956; Folch et al. 1956; AOAC 1995). Amino acids composition of live feed and shrimp larvae were analyzed according to Yamamoto et al. (1994) using high-performance liquid chromatography (HPLC). For analyses of fatty acid composition in live feeds and shrimp larvae, 0.4 g of dried samples was homogenized with 2:1 (v/v) combination of chloroform and methanol mixture and they were extracted using the modified method of Bligh and Dyer (1959). Further, extracted samples were esterified with 1 % H_2SO_4, and fatty acid methyl esters (FAME) were estimated according to AOAC (1995) using gas chromatography–mass spectrometry (GC–MS).

Statistical analyses

Data were analyzed using one-way analysis of variance (ANOVA). Significant differences among the different treatments were determined using Duncan multiple range test at 0.05 level of probability. Statistical analyses were accomplished using the Graph Pad Prism (Version 5.0).

Results

Growth of microalgae and copepod in wastewater

Figure 1a illustrates the cell growth rate of *P. maculatum* (PSDK01) in artificial culture medium (ACM) and shrimp-cultured wastewater (WW). The maximum cell growth (0.88 abs) was obtained on the 12th day in wastewater compared to artificial culture medium (0.66 abs). For the entire culture period (2 months), the total mean production of copepod *O. rigida* in NSW was 2,18,835 nos./l. comprising 1,12,345 nauplii, 71,234 copepodids and 35,256 adults (Fig. 1b), whereas in wastewater medium consisting 65,735 nauplii, 49,128 copepodids and 29,982 nos./l adults with a total of 1,44,845 nos./l was found.

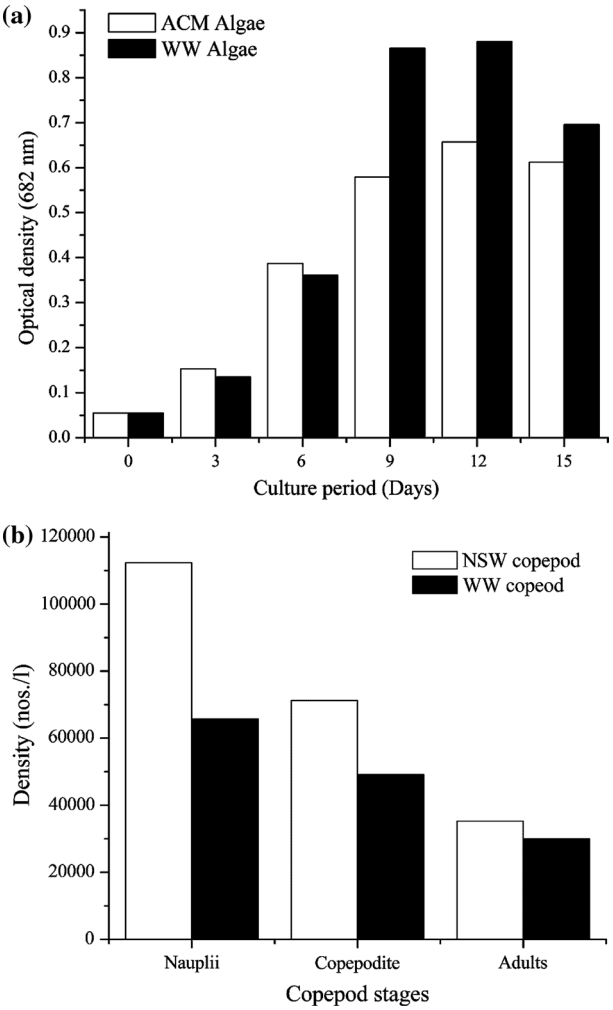

Fig. 1 Culture density of microalgae (**a**) and copepod (**b**)

Nutritional composition of live feeds

Picochlorum maculatum and *O. rigida* were cultured in normal seawater and wastewater. Slight variation was observed in live feed fed with different culture medium and results are as shown in Fig. 2a–e. The moisture, protein, carbohydrate, lipid and ash contents of ACM and WW-cultured *P. maculatum* were 30.29, 58.42, 12.27, 14.64, 4.98 and 31.85 %, 60.57, 10.34, 16.72 and 4.22 %, respectively. From the nutritional point of view, the normal seawater (NSW)- and wastewater (WW)-cultured *O. rigida* had moisture content (83.07

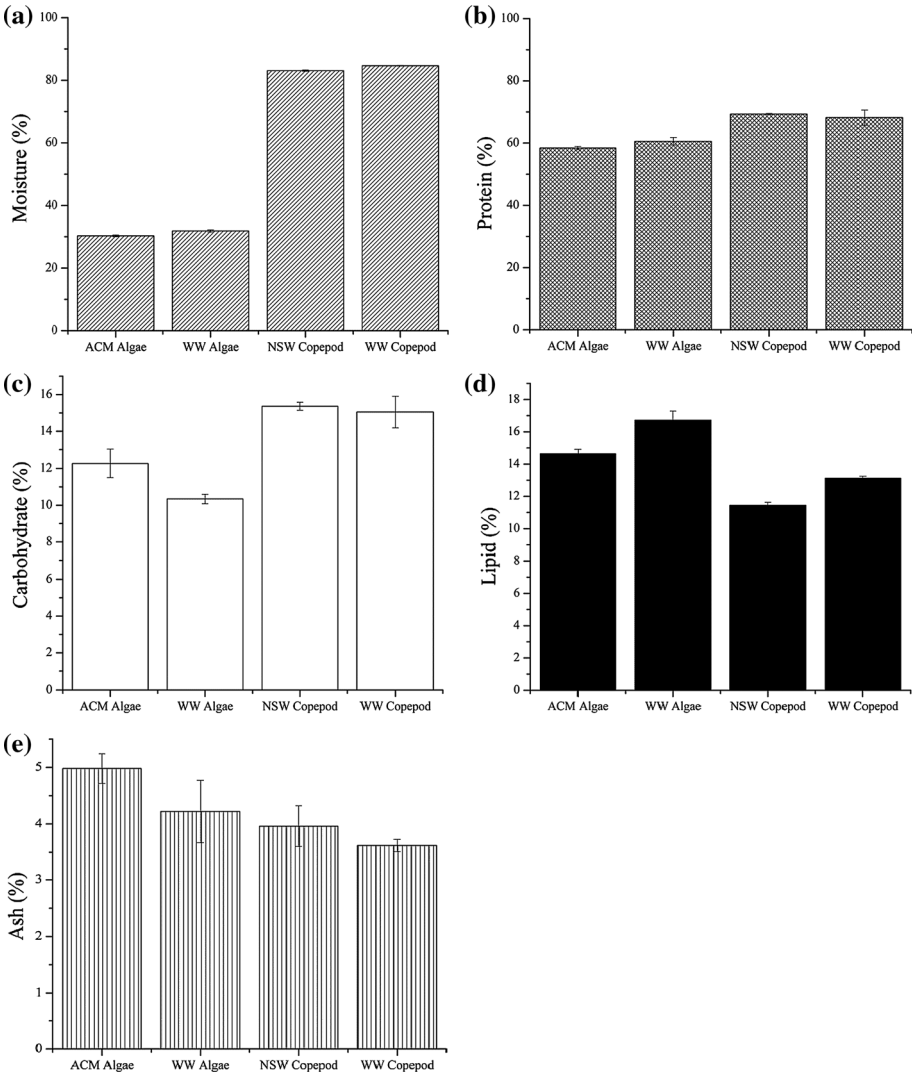

Fig. 2 Nutritional composition of live feeds; moisture (**a**), protein (**b**), carbohydrate (**c**), lipid (**d**) and ash (**e**)

and 84.64 %), protein (69.34 and 68.21 %), carbohydrate (15.36 and 15.05 %), lipid (11.44 and 13.12 %) and ash (3.96 and 3.62 %), respectively. The total amino acid content of ACM and WW-cultured *P. maculatum,* NSW and WW-cultured *O. rigida* were 84.72, 85.36, 99.24 and 94.18 %, respectively. The total amino acid content from WW-cultured *Picochlorum maculatum* was 85.36 % which is comparatively higher than ACM-cultured *P. maculatum* (84.72 %) (Table 1). But while looking copepod, NSW-cultured copepod resulted in higher amino acid content (99.24 %) than WW-cultured copepod (94.18 %). Among these, serine, glycine, valine, threonine, phenylalanine, leucine were prevailing components and were recorded with the percentage of 6.08, 6.34, 5.23, 5.24, 5.77 and 7.5 in WW-cultured microalgae, whereas in NSW-cultured copepod, glutamic acid, aspartic acid, serine, lysine, leucine and arginine were found to be dominant with the percentage of 10.32, 8.79, 7.97, 12.97, 8.94 and 7.94.

The saturated fatty acid (SFA) content of ACM and WW-cultured microalgae and NSW and WW-cultured copepod was 35.72, 42.67, 44.21 and 45.46 %, respectively (Table 2). The recorded MUFA and PUFA contents in ACM algae, WW algae, NSW copepod and WW copepod were 27.85, 25.22, 22.43, 21.48 % and 14.77, 15.51, 31.84, 28.67 %, respectively. Fatty acids such as palmitoleic acid (8.79 and 8.57 %), stearic acid (12.24 and 13.17 %) and linoleic acid (6.64 and 7.48 %) were found dominant in ACM-cultured algae and WW-cultured algae, respectively. In the case of NSW- and WW-cultured copepod myristic acid (10.67 and 11.95 %), palmitic acid (7.41 and 6.0 %), stearic acid (10.96 and 10.21 %) and elaidic acid (7.94 and 8.6 %) were found to be predominant.

Table 1 Amino acid composition of various live feeds

Amino acids	ACM algae	WW algae	NSW copepod	WW copepod
Arginine	6.22	5.6	7.50	5.98
Histidine	1.13	1.18	4.05	3.81
Isoleucine	4.05	3.97	3.41	4.83
Leucine	6.79	7.5	8.94	7.82
Lysine	7.21	6.67	12.97	10.86
Methionine	2.42	2.25	0.52	0.91
Phenylalanine	4.51	5.77	2.57	2.87
Threonine	5.08	5.24	2.63	2.92
Cystine	0.68	0.76	1.6	0.9
Valine	4.91	5.23	8.61	8.19
Alanine	7.98	7.08	10.12	11.18
Aspartic acid	8.87	8.43	8.79	8.13
Glutamic acid	10.26	9.14	10.32	9.19
Glycine	4.97	6.34	4.01	5.29
Serine	5.94	6.08	7.97	6.12
Tyrosine	3.70	4.12	5.23	5.18
Total EAA	43.00	44.17	52.80	49.09
Total NEAA	41.72	41.19	46.44	45.09
Total AA	84.72	85.36	99.24	94.18

Table 2 Fatty acid composition of various live feeds

Fatty acids	ACM algae	WW algae	NSW copepod	WW copepod
12:0	–	–	2.91	3.57
14:0	4.5	4.9	10.67	11.95
14:1	0.9	0.7	0.72	0.46
16:0	13.1	12.8	14.2	19.15
16:1	8.79	8.57	7.41	6
17:0	0.84	5.71	6.78	2.87
17:01	2.49	2.09	–	–
18:0	12.24	13.17	10.96	10.21
18:01	9.43	8.61	5.14	5.62
18:1 n-9	1.12	0.91	7.94	8.6
18:1n9c	3.19	2.68	–	–
18:2n-6	6.64	7.48	1.64	1.52
18:3n-6	–	–	0.32	0.27
18:3n-3	1.92	1.67	7.25	5.62
18:4n-3	–	–	1.73	1.68
20:0	2.13	2.64	0.22	0.13
20:1	1.93	1.66	1.22	0.8
20:2	1.03	0.68	–	0.17
20:2n-6	0.35	0.16	0.29	0.21
20:4n-6	–	–	1.96	1.38
20:4n-3	–	–	0.21	0.18
20:5n-3	0.93	0.92	7.81	7.62
21:0	2.91	3.45	0.14	0.09
22:0	–	–	0.4	0.35
22:1	–	–	0.14	0.11
22:5n-6	–	–	0.33	0.28
22:5n-3	–	–	0.81	0.76
22:6n-3	3.9	4.6	9.49	8.98
24:0	–	–	0.84	0.71
$\sum$ SFA	35.72	42.67	44.21	45.46
$\sum$ MUFA	27.85	25.22	22.43	21.48
$\sum$ PUFA	14.77	15.51	31.84	28.67
Total FA	78.34	83.4	98.48	95.61

Feeding trial of white leg shrimp *L. vannamei* post-larvae with various live feeds

Microalgae

A rapid growth and maximum survival were noticed when *L. vannamei* post-larvae were fed on WW-cultured algae in comparison with ACM-cultured algae (Fig. 3a, c, e). The final average length, weight and survival of post-larvae fed on WW-cultured algae were

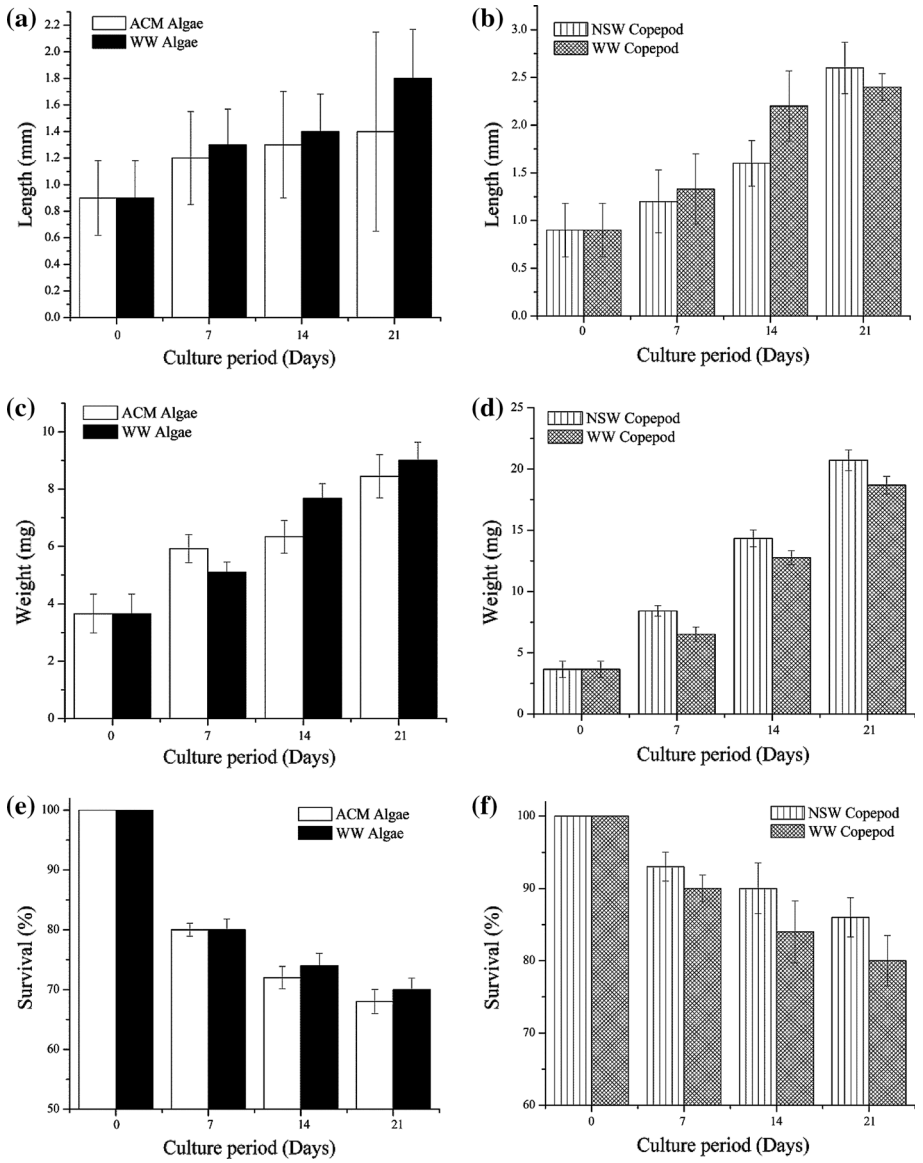

Fig. 3 Morphometric variation of shrimp larvae with reference to different live feeds; microalgae-fed larvae (**a**, **c**, **e**) and copepod-fed larvae (**b**, **d**, **f**)

found to be high as 1.8 ± 0.37 mm, 9.01 ± 0.63 mg and 70 ± 1.95 %, respectively, which was better than ACM-cultured algae (1.4 ± 0.48 mm, 8.45 ± 0.75 mg and 68 ± 2.01 %). The growth and survival of *L. vannamei* post-larvae fed with ACM and WW-cultured algae were significantly different ($p < 0.05$).

Copepod

The slender growth and survival of *L. vannamei* PL was found when feeding with NSW-cultured copepod instead of WW-cultured copepod (Fig. 3b, d, f). Finally, 2.6 ± 0.27 mm of length, 20.27 ± 0.85 mg of weight and 86 ± 2.7 % of survival were found in post-larvae fed on NSW-cultured copepod. The final mean length, weight and survival of post-larvae fed on WW-cultured copepod were 2.4 ± 0.14 mm, 18.68 ± 0.72 mg and 80 ± 3.5 %, respectively. The growth and survival of *L. vannamei* post-larvae fed with NSW and WW-cultured copepod were significantly different ($p < 0.01$).

Nutritional composition of shrimp larvae

P. maculatum-fed post-larvae

The nutritional composition of the *L. vannamei* larvae fed with ACM and WW-cultured *P. maculatum* is shown in Fig. 4a–e. All the nutritional compositions were found to be high in WW-cultured *P. maculatum*-fed larvae than in ACM-cultured *P. maculatum*-fed larvae. The percentage of moisture, protein, carbohydrate, lipid and ash contents in ACM-cultured algae-fed post-larvae were 83.49 ± 0.42, 62.33 ± 0.29, 9.36 ± 0.5, 12.34 ± 0.25 and 3.99 ± 0.27 which were lower than WW-cultured algae-fed post-larvae. and the values are 84.59 ± 0.62, 63.54 ± 0.01, 10.22 ± 0.57, 12.73 ± 0.21 and 4.38 ± 0.41. Among the two algae feed regimes, maximum amino acid content of 55.36 % in dry matter was obtained in ACM-cultured *P. maculatum*-fed larvae than in the WW-cultured *P. maculatum* (52.80 %) (Table 3). Among the amino acids, aspartic acid (7.71 %), valine (2.84 %), methionine (2.2 %), lysine (4.98 %), leucine (4.87 %), isoleucine (2.34 %), histidine (2.85 %) and arginine (3.18 %) were found to be dominant in ACM-cultured *Picochlorum maculatum*-fed larvae. The fatty acids such as myristic acid (3.81 %), heptadecanoic acid (7.85 %) and heneicosanoic acid (3.95 %) were high in ACM algae-fed larvae (Table 4). Myristic acid (8.36 %), palmitic acid (9.89 %), stearic acid (9.53 %), elaidic acid (10.72 %), linolelaidic acid (7.24 %) and eicosapentaenoic acid (4.68 %) were found high in WW algae-fed larvae. Higher concentration of fatty acids have been recorded in WW algae-fed larvae (83.44 %) compared to ACM-fed larvae (77.90 %)..

Oithona rigida-fed larvae

The nutritional compositions of *O. rigida*-fed larvae are shown in Fig. 4. The nutritional composition was found to be high in NSW copepod-fed larvae than in WW copepod-fed larvae except for moisture and lipid. The recorded level of protein, carbohydrate and ash content was found high as 69.34 ± 0.18 %, 15.36 ± 0.22 % and 3.96 ± 0.36, respectively, in NSW copepod-fed larvae. The moisture (84.64 ± 0.0.15 %) and lipid (13.12 ± 0.13 %) were found to be high in WW copepod-fed larvae. A total amino acid contents were found higher in NSW copepod-fed larvae (71.41 %) than WW copepod-fed larvae (66.35 %) (Table 3). Among these, aspartic acid (9.45 %), glycine (4.42 %) and serine (2.52 %) were found to be high in WW copepod-fed larvae. In NSW copepod-fed larvae, higher values of glutamic acid (11.46 %), alanine (9.63 %), valine (3.72 %), lysine (5.78 %), leucine (4.70 %) and arginine (3.34 %) were recorded. Totally, 97.43 % of fatty acids were recorded in NSW copepod-fed larvae consisting of 39.40 % saturated fatty acids, 30.32 % monounsaturated fatty acids and 27.71 % polyunsaturated fatty acids

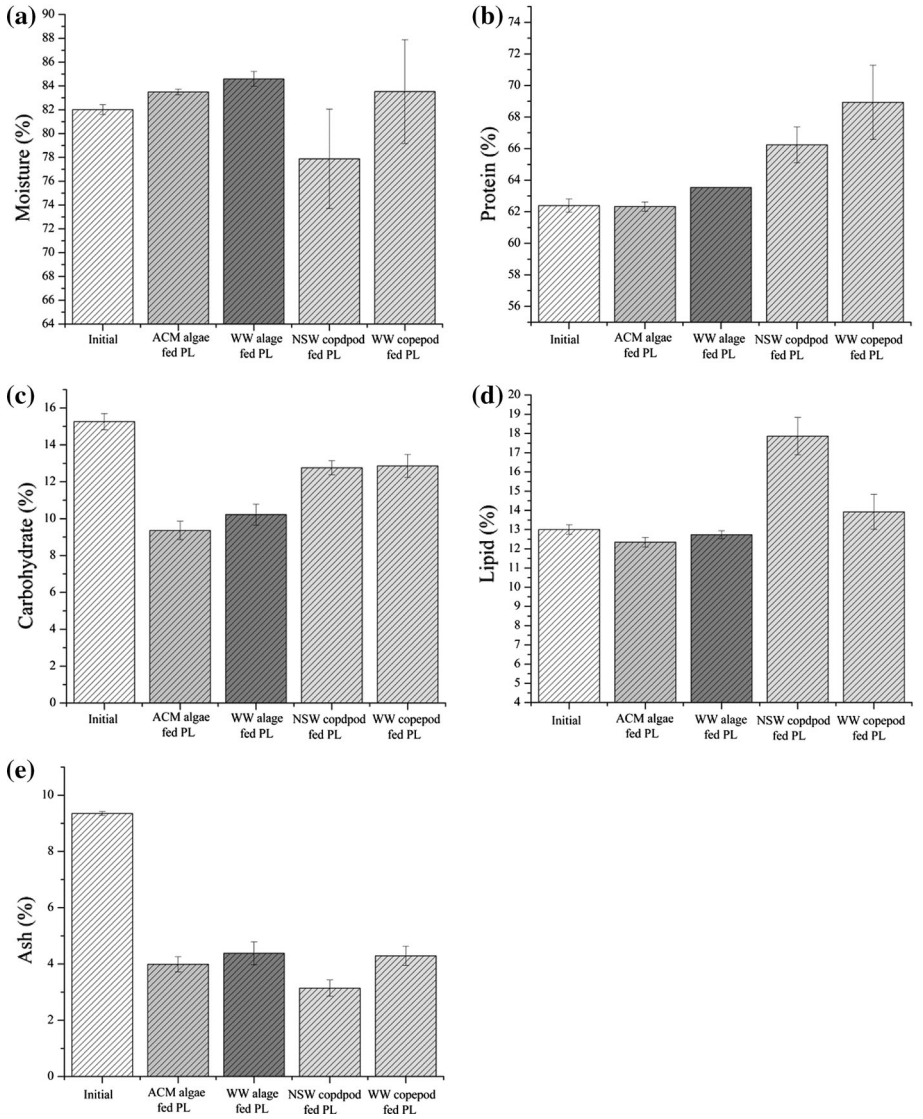

Fig. 4 Nutritional composition of shrimp (*L. vannamei*) larvae with reference to different live feeds: moisture (**a**), protein (**b**), carbohydrate (**c**), lipid (**d**) and ash (**e**)

(Table 4). Among these, myristic acid (8.28 %), palmitoleic acid (5.33 %), stearic acid (9.13 %), elaidic acid (10.54 %), arachidonic acid (7.1 %), eicosapentaenoic acid (7.17 %) and docosahexaenoic acid (9.04) were found higher in NSW copepod-fed larvae. Saturated fatty acids (41.54 %), monounsaturated fatty acids (26.67 %) and polyunsaturated fatty acids (22.69 %) with total of 90.50 % fatty acids were recorded in WW copepod-fed larvae. Palmitic acid (21.66 %), arachidic acid (0.8 %) and behenic acid (0.23 %) were found dominant in WW copepod-fed larvae.

Table 3 Amino acid composition of white leg shrimp *L. vannamei* PL fed on various live feeds

Amino acids	Initial larvae	ACM algae-fed larvae	WW algae-fed larvae	NSW copepod-fed larvae	WW copepod-fed larvae
Arginine	3.12	3.18	3.04	3.34	2.52
Histidine	2.54	2.85	2.31	1.68	1.91
Isoleucine	2.34	2.34	1.56	3.13	2.86
Leucine	4.18	4.87	4.12	4.70	4.19
Lysine	5.16	4.98	3.41	5.78	5.65
Methionine	1.84	2.22	1.23	2.34	2.42
Phenylalanine	2.10	1.94	2.26	2.46	2.43
Threonine	2.64	2.82	3.12	3.87	2.98
Cystine	0.09	0.11	0.9	0.17	–
Valine	2.69	2.84	2.56	3.72	3.63
Alanine	8.98	6.61	7.34	9.63	8.11
Aspartic acid	5.65	7.71	7.43	8.95	9.45
Glutamic acid	9.69	7.16	8.42	11.46	10.33
Glycine	5.13	3.04	2.34	4.31	4.42
Serine	2.89	1.81	1.62	2.41	2.52
Tyrosine	2.68	0.88	1.14	3.46	2.93
Total EAA	26.7	28.15	24.51	31.19	28.59
Total NEAA	35.02	27.21	28.29	40.22	37.76
Total AA	61.72	55.36	52.8	71.41	66.35

Analyses of water quality parameters in larval rearing tanks

No significant differences ($p > 0.05$) were detected in the shrimp larviculture water with regard to pH, salinity, dissolved oxygen, temperature, total phosphorus and total nitrogen among the four treatments (Table 5). The heavy metal concentrations (before experiment) of wastewater are summarized in Table 6.

Discussion

Kovalenko et al. (2002) suggested that the live feed has been treated to be a controlling factor in the commercial culture of shrimp and fish larvae. It is an important factor in the overall production cost as well. Competition in shrimp production in overseas market is rapidly increasing, and it is necessary to find more efficient ways to culture the best quality shrimp. Recently, there has been a great interest in wastewater treatment by using microalgae, after which their biomass can be use not only for biofuels, but also for products that could be processed for food, feed, pharmaceuticals and other high-value chemicals (Kiron et al. 2012; Dinesh Kumar et al. 2014, 2015). According to Wilson (2002) report, amino acid requirements of the crustacean species, the algae and algal products will be able to provide most of the essential amino acids.

Table 4 Fatty acid composition of white leg shrimp *L. vannamei* PL fed on various live feeds

Fatty acids	Initial larvae	ACM algae-fed larvae	WW algae-fed larvae	NSW copepod-fed larvae	WW copepod-fed larvae
12:00	–	–	–	1.3	2.1
14:00	0.12	3.81	3.08	4.58	6.09
14:01	9.82	7.22	8.36	8.28	7.16
16:00	6.93	9.13	9.89	18.76	21.66
16:01	8.95	6.52	7.21	5.33	5.28
17:00	10.35	7.85	7.38	4.42	4.31
17:01	–	–	–	1.23	0.92
18:00	2.89	9.19	9.53	9.13	7.53
20:00	–	1.46	1.32	0.56	0.8
21:00	0.68	3.95	2.52	0.54	–
22:00	–	0.19	0.24	0.18	0.23
18:01	0.25	–	2.88	8.17	5.26
18:1-n 9	4.58	9.02	10.72	10.54	8.97
18:3-n 6	0.31	1.32	1.45	1.96	1.76
18:2-n 6	6.59	7.02	7.24	0.56	0.00
20:4-n 6	1.38	2.63	1.05	7.1	5.06
20:4-n 5	–	0.87	0.85	1.10	0.92
20:5-n 3	3.12	4.41	4.68	7.17	6.34
22:6-n 3	1.61	1.39	2.15	9.04	8.09
22:01	1.03	1.92	2.89	0.78	0.12
$\sum$ SFA	20.97	35.58	33.96	39.40	41.54
$\sum$ MUFA	23.6	22.76	29.17	30.32	26.67
$\sum$ PUFA	14.04	19.56	20.31	27.71	22.29
Total FA	58.61	77.90	83.44	97.43	90.50

Table 5 Water quality parameters measured in *Litopenaeus vannamei* post-larvae reared tanks during 21-day experimental period

Parameters	ACM algae-fed larvae	WW algae-fed larvae	NSW copepod-fed larvae	WW copepod-fed larvae
pH	5.79 ± 0.4^{a}	7.93 ± 0.7^{a}	7.2 ± 0.3^{a}	7.6 ± 0.2^{a}
Salinity (psu)	29.3 ± 0.8^{a}	30.4 ± 0.5^{a}	27.2 ± 0.4^{a}	27.8 ± 0.6^{a}
Temperature (°C)	30.5 ± 0.2^{a}	30.2 ± 0.4^{a}	30.6 ± 0.5^{a}	30.5 ± 0.3^{a}
Dissolved oxygen (mg L^{-1})	5.45 ± 0.6^{a}	5.37 ± 0.3^{a}	5.81 ± 0.7^{a}	5.79 ± 0.3^{a}
Total phosphorus (μmol L^{-1})	0.53 ± 0.12^{a}	0.59 ± 0.14^{a}	0.63 ± 0.21^{a}	0.65 ± 0.23^{a}
Total nitrogen (μmol L^{-1})	19.59 ± 0.8^{a}	19.81 ± 0.9^{a}	18.92 ± 0.3^{a}	18.05 ± 0.5^{a}

Note [a, b, c] Values within a column with different superscripts are significantly different ($P < 0.05$); same superscripts are not significantly different ($P < 0.05$)

Table 6 Heavy metal concentration (mg L^{-1}) of 90-day-*L. vannamei*-cultured wastewater

Metals	Zinc	Chromium	Copper	Lead	Cadmium
Concentration (mg L^{-1})	3.041 ± 0.06	0.008 ± 0.001	2.259 ± 0.009	0.097 ± 0.002	0.031 ± 0.004

Based on the above statements, the present studies were attempted on culture of microalgae (*P. maculatum*) and copepod (*O. rigida*) in shrimp culture wastewater and estimate the live feed suitability in *L. vannamei* post-larvae. A number of studies are available on use of microalgae (Sangha et al. 2000; Zelaya et al. 2007; González-Davis et al. 2012; Kiron et al. 2012; Khatoon et al. 2009, 2013; Rohani-Ghadikolaei et al. 2013) and copepod (Ananthi et al. 2011; Jothiraj 2012; Nandakumar 2015) as feed for shrimp larvae. But there is no study for wastewater-grown microalgae and copepod feeding by shrimp and fish or cultivable organisms especially *P. maculatum* and *O. rigida*. Santhanam (2002), Santhanam et al. (2004), Ananthi (2012), Raju (2012), Santhanam and Perumal (2012), Kathiresan (2013) and Santhanam and Perumal (2013) estimated the live feed suitability of *O. rigida* on *Lates calcarifer*, *P. monodon* and *L. vannamei*. In this concern, this study might be a first report for the live feed suitability of wastewater-grown *P. maculatum* and *O. rigida* on white leg shrimp *L. vannamei*.

As per the early reports, the heavy metal concentration of WW has been found as permissible for larval rearing (FAO 2007). In algal feeding, maximum growth and survival were recorded in WW-cultured algae-fed larvae. This could be attributed to the higher amount of protein, lipid and amino acid contents in the WW-cultured algae. Laurence (1977) advised that the larvae need more energy while culturing in tanks. Feeding ration might affect the larval growth. The length, weight and survival were all significantly lower in ACM-cultured algae-fed larvae when compared to WW-cultured algae-fed larvae. During copepod feeding, maximum growth and survival was found in NSW-cultured copepod-fed larvae compared to WW-cultured copepod-fed larvae. This might be due to culture density of copepod in wastewater and natural seawater. In wastewater, density of copepod was quietly low when compared to the natural seawater. In *O. rigida*, culture density consists of variety of stages such as nauplii, copepodite and adults. Rajkumar and Kumaraguru Vasagam (2006) suggested that the *O. rigida* size changes (Nauplii-I to Copepodite-V) directly ruled in the larval length and weight. The biochemical composition of live feed plays a vital role in their physiological functions, metabolism, nutritive value, energy transfer and secondary production. Mostly, prawn and fish depend on plankton at some stage during their life span, and some feed exclusively on plankton during their entire life cycle (Sumitha 2006).

In the present study, moisture, protein, lipid and total amino acid contents were high in both WW-cultured algae and WW-cultured algae-fed larvae. In the case of fatty acid contents, saturated fatty acids and polyunsaturated fatty acids were found to be high in WW-cultured algae, and monounsaturated fatty acids was high in WW-cultured algae-fed larvae. Moisture, lipid, non-essential amino acids and saturated fatty acids were found to be high in WW-cultured copepod and WW-cultured copepod-fed larvae. In the cultured microalgae and copepod, protein was identified as a major component which played a major role than compared to lipid and carbohydrate and these findings were supported by Santhanam and Perumal (2012).

In the present study, the higher protein content was found in microalgae cultured with WW compared to ACM algae. But the carbohydrate values were shown high in ACM algae than in WW algae. This result was comparable to Luis et al. (2010). They experienced the similar result with aquaculture fertilizer and agricultural fertilizer used for the culture of *Chaetoceros mulleri*. Changes in protein and carbohydrate levels attribute to the alteration in the media composition and nutrient changes in the WW (Gatenby et al. 2003). The level of lipid content was observed in ACM algae (%) than in WW algae (%). Brown et al. (1997) reported that culture condition and growth phase lead to changes in lipid composition of microalgae. Lipid content of microalgae increased with advanced growth as supported by Gatenby et al. (2003). The calorific content of the larvae fed with ACM algae and WW algae showed greater variations. The protein, carbohydrate and lipid values were higher in the larvae fed with WW algae. The higher level of biochemical composition noticed in the larvae might be a reflection of the feed supply (Evjemo et al. 2003; Payne et al. 2001).

Few differences were observed in the amino acid composition of WW algae (85.36 %) and ACM algae (84.72 %). The essential amino acid accounted to 44.17 %, and this level was comparatively higher than the earlier report (Derrien et al. 1998). In our study, glutamic acid was the dominant in all the 16 amino acids recorded in both ACM and WW algae as agreed by Martin-Jezequel et al. (1988), who found a high concentration of aspartic acid and glutamic acid in *Skeletonema costatum.* Depending on the culture conditions and microalgae cultured, the amino acid composition varies from species to species (Brown 1991; Brown et al. 1997; Carbajal Miranda et al. 2005; Moura Junior et al. 2007).

The stearic acid was found to be the most dominant in WW Algae followed by palmitic acid. In the case of ACM algae, palmitic acid was the most dominant and the second dominant was stearic acid as supported by Widianingsih et al. (2013). They found that palmitic acid were dominant in diatom *Nitzchia* sp. The exponential phase of the WW microalgae accumulates high amount of lipids than ACM algae. This might be due to the nitrogen limitation in the medium (Sánchez-Saavedra and Voltolina 2005). Growth and survival of *L. vannamei* larvae were superior with WW algae diet due to more amounts of 20:5 n-3 (EPA) and 22:6n-3 (DHA) as supported by Payne and Rippingale (2000) and Santhanam and Perumal (2013). Due to the low nutritional quality of ACM algae, low survival and growth were found in ACM algae-fed larvae. From this study, it is clearly understood that nutritional value of live feed, in particular the fatty acids such as EPA and DHA and amino acids (glutamic acid), is known to affect the growth and survival of larvae (Stottrup 2000; Evjemo et al. 2003). In the present study, the protein content of the NSW copepod and NSW copepod-fed *L. vannemei* larvae was low compared to WW copepod. The presently observed variation in the protein content might be due to the fact that it is utilized as a metabolic substrate as reported by Nageswara Rao and Krupanidhi (2001); Santhanam and Perumal (2013). But lipid content of the WW copepod showed small variation compared to NSW copepod, and it did not influence the larvae. The larvae fed with WW copepod showed lower lipid content than NSW copepod-fed larvae. The variations in the lipid content are attributed to its storage and utilization during starved period (Vengadeshperumal et al. 2010). Variations in the protein and lipid content of WW copepod observed in the present study is related to the type of feeds used (Rajkumar and Kumarguru Vasagam 2006; Santhanam and Perumal 2013).

The essential amino acids of the WW Copepod showed adequate level of the larvae (Rajkumar and Kumarguru Vasagam 2006; Ananth and Santhanam 2011), but lower than the NSW copepod. Among the amino acids recorded, isoleucine, threonine, alanine and glycine were dominant in the WW copepod. Rajkumar and Kumaraguru Vasagam (2006)

reported dominance of lysine, alanine and glutamic acid in *A. clausi*. Santhanam and Perumal (2013), noticed glutamic acid, leucine, lysine and arginine as most prevailing amino acids in the same species of the present study. The larvae fed with WW copepod showed the dominance of methionine and histidine in *L. vannamei* larvae. The above result was different from Santhanam and Perumal (2013), who reported methionine and histidine as low in *O. rigida*-fed sea bass larvae.

The saturated fatty acids were found to be higher in WW copepod, and it is reflected in the larvae also when compared to the NSW copepod. In the present study, 16:0 (Palmitic acid), 18:0 (Stearic acid), 20:5 (EPA) and 22:6 (DHA) were dominant in both NSW copepod and WW copepod. Similar results were experienced by many authors in various copepods, *Acartia clausi* (Rajkumar and Kumaraguru Vasagam 2006), *O. rigida* (Santhanam and Perumal 2013) and Nanton and Castell (1998) in *Tisbe* sp. The above dominant fatty acids may be synthesized by the copepods or by the feed given. Nanton and Castell (1998) documented the influence of feed on the lipid and fatty acid composition of the copepod *Tisbe* sp. The live feed compatibility with copepods is transferred by several authors Rajkumar and Kumaraguru Vasagam (2006), Santhanam and Perumal (2013) in sea bass larvae, Ananthi et al. (2011) with *Penaeus monodon* larvae, Nandakumar (2014) with ornamental angel fish *Monodactylus argenteus* and Kathiresan (2013) with *L. vannamei*. However, the use of WW-cultured copepod is the first approach. Even though the growth and survival of larvae fed with NSW copepod showed higher values, they did not show much variation. This result showed that the adequate amount of nutrition especially HUFA is provided from WW copepod to the shrimp larvae. The small variation in growth of the larvae observed in this study is due to the nutritional quality of the food provided to the larvae (Payne et al. 2001).

Conclusion

The present study has helped us to understand the potential of the wastewater-cultured microalgae and copepod as replacements for commercial fish feeds of white leg shrimp *L. vannamei*. The two live feeds (*P. maculatum* and *O. rigida*) could be incorporated at levels tested in this study. Some significant effects were noticed on growth and survival as an effect of higher inclusions. Further studies are necessary to confirm the suitability of wastewater-grown microalgae and copepod on larval rearing of other shrimp and fish species.

Acknowledgments Authors are thankful to the Head, Department of Marine Science and authorities of Bharathidasan University, Tiruchirappalli, for the facilities provided. Authors are indebted to Department of Biotechnology, Government of India for Microalgae and Copepods culture facility provided through extramural project (BT/PR 5856/AAQ/3/598/2012). Authors (SDK & SA) thank the Department of Biotechnology, Government of India for fellowship provided.

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