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Evaluation of the marine copepod *Oithona rigida* Giesbrecht as live feed for larviculture of Asian seabass *Lates calcarifer* Bloch with special reference to nutritional value

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ABSTRACT

The present study compared the growth and survival of 14-day old larvae of Asian seabass, *Lates calcarifer* fed on copepod *Oithona rigida*. The 21 days larval rearing experiment showed that copepod significantly improves growth and survival of seabass larvae. On termination of the experiment, length and weight of the copepod fed larvae were significantly higher than that of seabass larvae fed on *Brachionus plicatilis* and *Artemia* nauplii. The results clearly indicate that the superior n-3 HUFA (highly unsaturated fatty acids) content as well as the nutritional composition of *O. rigida* can support the fish larvae for appreciable growth and survival.

Keywords: *Artemia, Brachionus plicatilis,* Copepod, *Lates calcarifer, Oithona rigida*

Introduction

World's total fishery production has been reported as 142.3 million t in 2008 (FAO, 2010), which included all aquatic organisms used for human consumption and other commercial activities. This great and chief protein source for human beings has been virtually supported by small food organisms present in the aquatic ecosystems. Among these, copepods deserve unique consideration, in new of the fact that all the aquatic higher organisms consistently require these smaller organisms at least during their early life stages. Copepods have been used to rear the larvae and fry of finfish and shellfish (Luis *et al.*, 2010). The larvae fed with copepods exhibited better growth and survival than those fed with artificial diets (Drillet *et al.,* 2011). Copepods are a valuable source of protein, amino acids, lipids, fatty acids and enzymes (Perumal *et al.,* 2009; Ananth and Santhanam, 2011; Ananthi *et al.,* 2011). *Artemia* and rotifers have been widely used as initial live feed for rearing fish larvae for the last many decades. But they are inherently nutritionally inadequate, particularly in essential fatty acids such as 20:5n-3 and 22:6n-3 (Stottrup *et al.*, 1998). Malnutrition is one of the major responsible factors for failure in larval growth and survival (Luis *et al.,* 2010). Hence, the present attempt was made on larviculture of Asian seabass *L. calcarifer* using copepod *Oithona rigida* as a starter feed and the results were compared with traditional live feeds such as *Brachionus plicatilis* and *Artemia* nauplii.

Materials and methods

Growth trials

Fourteen days old larvae of seabass, *Lates calcarifer* were procured from the Central Institute of Brackishwater Aquaculture (CIBA), Chennai. The larvae were acclimatised in the laboratory prior to the experiment. Larval rearing experiment was conducted in triplicate using three different live feeds such as rotifer, *B. plicatilis, Artemia* nauplii and copepod *O. rigida* in the density ranges of 5000-10,000, 4000-6000 and 2000-3000 individuals per litre respectively. Hundred larvae were stocked in 100 l FRP tanks filled with filtered seawater with vigorous aeration. Daily 50% of water was exchanged with fresh filtered seawater. The larvae were collected, measured and weighed at weekly intervals for 3 weeks. Water quality parameters such as temperature, dissolved oxygen and salinity were monitored daily. The water temperature and salinity were measured using standard centigrade thermometer and hand refractometer (ERMA, Japan) respectively. The dissolved oxygen concentration of water was estimated by Winkler's method (Strickland and Parsons, 1972). The rearing experiments lasted 21 days, with 12:12 h light and dark cycle being maintained. Daily food was given thrice a day at 06:00, 13:00 and 19:30 hrs. The total length (TL) and wet weight (WW) of the larvae were measured and recorded at the beginning and the end of the experiments for evaluating the influence of different

live-feeds. Proximate composition of both diets and fish larvae were estimated at the initial stage and final stage of the experiment. Growth of larvae was determined by seine sampling from various points of each tank using a 158 µm scoop net. The excess moisture was removed using tissue paper and the length and weight measurements of the larvae were thaken within a few seconds, to avoid larval mortality. The mortality if any was monitored and dead larvae were collected by gentle siphoning and preserved at -40 °C. Survival of larvae was determined by subtracting the total number of individuals harvested from total number of larvae stocked in each tank.

Copepod culture

Copepods were collected from the Vellar Estuary using plankton net (158 µm) during early morning hours (05:00 hrs). Repeated samplings were made in order to have maximum number of copepods. During the time of sampling the salinity of the Vellar Estuary water was recorded as 33 ‰. The collected samples were immediately transported to the laboratory and thoroughly rinsed to reduce the contamination from other zooplankters. The copepods were screened to isolate the size fraction containing predominantly adult copepods and later stage copepodids of *O. rigida*. The isolation was achieved by a first coarse screening through a 500 µm mesh to remove the fish and prawn larvae. Then the samples were rinsed for 2 h in a zooplankton washer (Schipp *et al.,* 1999) fitted with a 190 µm mesh screen to remove rotifers, copepods and barnacle nauplii. After rinsing, the adult and later stage copepodids of *O. rigida* were gently picked up using fine capillary tube and brush under the stereo-zoom microscope. The isolated *O. rigida* were inoculated initially in 50 ml glass beaker containing filtered seawater. Before stocking, *O. rigida* was acclimatised slowly to laboratory condition by adding water collected from Vellar Estuary from where the copepods were collected. Then the stock culture of *O. rigida* was transferred to 7 l plastic jars filled with filtered seawater. Pure culture of *O. rigida* was obtained only after 2 months of culture maintaining proper water quality and by providing required amount of microalgal diet. Later, the copepods were subcultured into rectangular, flat-bottomed fibreglass tank (70 cm x 50 cm x 30 cm size and 6 mm thick; outside blue and inner white tanks) filled with 100 l filtered seawater provided with vigorous aeration. Partial shade was provided in the tanks to prevent excessive evaporation. Water quality parameters *viz.,* temperature, salinity, pH and dissolved oxygen were maintained in the ranges of 28 – 32 °C; 30 - 34 ‰; 7 - 8.5 and 5 - 7.5 ml l^{-1} respectively. The copepods were cultured with mixed microalgae *viz., Chlorella marina*, *Isochrysis galbana*, and *Dunaliella* sp. in the ratio of 2:2:2 (20,000: 20,000: 20,000 cells ml^{-1} of each algal species). During the culture, copepods were provided with 12:12 h natural light: dark condition. Daily 20% of water was exchanged with fresh filtered seawater. The waste and other debris were removed by gentle siphoning using sterilised silicon tube. Copepods were harvested everyday by gentle siphoning as well as by phototactic isolation and were used to feed the larvae. For analysis of proximate composition, the copepods were concentrated in a sieve (54 µm), washed in distilled water and stored at -80 °C.

Culture of rotifer and Artemia

The stock culture of rotifer, *B. plicatilis* was obtained from CIBA, Chennai. In our laboratory, rotifer culture was initiated at an initial density of 50 nos. ml^{-1} fed with three algal species *viz*., *C. marina*, *I. galbana*, and *Dunaliella* sp. (20,000 cells ml^{-1} of each algal species) in 100 l FRP tanks with vigorous aeration. The water temperature and salinity were maintained in the ranges of 26 - 27 °C and 25-26‰ respectively. After 7 days of culture duration, rotifer density reached over 500 nos. ml^{-1}. Rotifers were harvested using 50 µm net. After repeated cleaning with fresh seawater, the rotifer was given as feed to fish larvae. Likewise, *Artemia* cysts (RED JUNGLE BRAND, Ocean Star International, Inc. USA) were cultured separately at 32 ‰ salinity at 29 °C with vigorous aeration and illumination to feed the fish larvae.

Microalgal culture

Stock cultures of marine microalgae *Chlorella marina, Isochrysis galbana* and *Dunaliella* sp. were grown in 250 as well as 500 ml conical flasks containing filtered seawater at temperature and salinity ranges of 23-25 °C and 28-30 ‰ under12:12 h light and dark cycle with 5000 lux illumination and fertilized with f/2 medium (Guillard, 1972). The algae were harvested during the exponential phase for feeding the rotifers and copepods. Outdoor algal culture was maintained at 100 l FRP tanks inoculated with 2 l of algal culture using commercial fertilizer *viz*., ammonium sulphate, urea and super phosphate in the ratio of 10:1:1 (10 g ammonium sulphate: 1g urea: 1g super phosphate per litre of filtered seawater) with vigorous aeration and natural illumination.

Estimation of proximate composition

The moisture, total protein, carbohydrate, total lipids and ash contents in live feeds and fish larvae were estimated following standard methods (Rajendran,1973; Raymont *et al.,*1964; Dubois *et al.,* 1956; Folch *et al.,* 1956; AOAC,1995) . Protein and carbohydrate levels were estimated using UV-Spectrophotometer. Amino acids were estimated by digesting known amounts of samples with 6 N HCl at 110 °C for 22 h followed by dissolving the samples in 2 ml sample diluents. Acid hydrolysate was dried using

speed vacuum concentrator and the sample was filtered. Amino acids were determined in an automatic amino acid analyser (Shimatzu-High Performance Liquid Chromatography LC 4A). Twenty microliters of the filtered derived amino acid sample was injected into a single column and analysed using sodium buffer system (Yamamoto *et al.,* 1994). Fluorescent detector (FLD 6A) using O-phthalaldehyde fluorescent reagent quantitatively detected the amino acids. By comparing their retention time (Rt) with the standard amino acids run at identical conditions, the amino acids were identified. For estimation of fatty acids, 400 mg of samples were homogenised with chloroform: methanol (2:1 v/v) mixture and they were extracted using the method of Bligh and Dyer (1959). After fat extraction, samples were esterified with 1% H_2SO_4 and fatty acid methyl esters were prepared following AOAC (1995). Identification and quantification of fatty acids were done using a Gas Chromatograph (Hewlett Packard 5890 Model). GC was performed under the following conditions:

Column:	DECS (Diethylene glycol succinate) column
Temperature:	180 °C (isothermal)
Injection port temperature:	200 °C
Detector temperature:	230 °C
Carrier gas:	Nitrogen
Sample volume:	2 µl
Detector:	FID (Flame Ionisation Detector)

Statistical analysis

The results obtained were statistically analysed using one way Analysis of variance (ANOVA) test.

Results

Copepod culture

Copepod culture system produced an average of 1760.06 nauplii l^{-1}, 658.8 copepodids l^{-1} and 412.46 adults l^{-1} on the 12th day. The maximum average density of *O. rigida* was recorded as 3679.3 nauplii l^{-1}, 2016.3 copepodids l^{-1} and 1832 adults l^{-1} on 10, 12 and 12th day(s) of culture respectively. For the entire culture period (2 months), the total mean production recorded was 34276.29 l^{-1}, comprising 21120.79 nauplii, 8205.6 copepodids and 4949.9 adults l^{-1}.

Proximate composition of live feeds

Copepod, *O. rigida* showed greater variations in protein content with 69.24 %. The lipid content was 15.36 % and the moisture and ash contents were 83.07 % and 3.96 % respectively. The moisture, protein, carbohydrate, lipid and ash contents of *B. plicatilis* recorded were 85.22%, 62.84%, 13.36%, 18.87% and 4.89% respectively. Proximate composition of laboratory hatched *Artemia* nauplii were 83.45% (moisture), 59.21% (protein), 18.48% (lipid), 16.31% (carbohydrate) and 5.94% (ash) (Table 1).

Table 1. Biochemical composition (% mean ± SD) of live feeds

Live feed organisms	Moisture	Protein	Lipid	Carbohydrate	Ash
B. plicatilis	85.22 ± 0.053	62.84 ± 0.052	13.36 ± 0.208	18.87 ± 0.236	4.89 ± 0.088
Artemia nauplii	83.45 ± 0.398	59.21 ± 0.087	18.48 ± 0.047	16.31 ± 0.076	5.94 ± 0.036
O. rigida	83.07 ± 0.075	69.24 ± 0.070	15.36 ± 0.103	11.44 ± 0.141	3.962 ± 0.081

The total amino acid content of *B. plicatilis* and *Artemia* nauplii recorded were 56.22 and 50.64% respectively. Among these, glutamic acid, aspartic acid, lysine, alanine and glycine were found to dominate. The total amino acid content estimated for *O. rigida* was 63.50% which is comparatively higher than that of rotifer and *Artemia* nauplii. Among the total amino acids, the glutamic acid, valine, alanine, aspartic acid, leucine and lysine were predominant components and were recorded at levels of 9.19; 5.23; 7.84; 6.93; 8.26 and 6.41% respectively (Table 2).

Table 2. Amino acid composition (%) of live feeds

Amino acids	*B. plicatilis*	*Artemia* nauplii	*O. rigida*
Arginine	3.206	2.38	4.24
Histidine	2.612	0.98	2.69
Isoleucine	3.260	2.63	0.88
Leucine	4.090	5.12	8.26
Lysine	6.124	5.95	6.41
Methionine	1.214	0.92	2.16
Phenylalanine	3.226	2.10	1.04
Threonine	2.250	2.07	1.52
Cystine	-	-	0.061
Valine	4.229	1.83	5.23
Total EAA	30.211	23.98	32.49
Alanine	3.208	2.56	7.84
Aspartic acid	5.016	4.18	6.93
Glutamic acid	7.147	7.84	9.19
Glycine	4.608	4.84	2.16
Serine	2.941	3.12	3.28
Tyrosine	3.091	4.12	3.39
Total NEAA	26.011	26.66	31.01
Total	56.22	50.64	63.50

The total fatty acid content of *B. plicatilis* and *Artemia* nauplii were 82.01and 86.40 % respectively. Fatty acids such as palmitic acid (20.01%), palmitoleic acid (12.68 %) and octadecanoic acid (10.30 %) were found to be higher in *B. plicatilis*. In *Artemia* nauplii, linoleic acid (20.2 %), octadecanoic acid (14.2 %) and palmitic acid (12.7 %) were dominant. The levels of highly unsaturated fatty acids *viz*., EPA and DHA of *B. plicatilis* and *Artemia* nauplii were 6.31; 0.20; 4.0 and 2.9% respectively. However, *O. rigida* showed the maximum fatty acid level of 98.05% with the predominance of palmitic acid (16:0), myristic acid (14:0) and stearic acid (18:0) at 24.58, 12.47 and 10.96% respectively. The percentages EPA and DHA in *O. rigida* were comparatively higher than that of rotifer and *Artemia* with 7.81 and 9.49 % respectively. The percentage of polyunsaturated fatty acids (PUFA) such as linoleic acid, arachidonic acid and linolenic acid were found as 0.64, 1.81 and 0.59 % respectively, and the content of the principal monoenoic fatty acid *i.e.*, oleic acid was 3.83 % (Table 3).

Table 3. Fatty acid composition (% mean ± SD) of live feeds

Fatty acids	*B. plicatilis*	*Artemia* nauplii	*O. rigida*
12:0	-	-	2.29 ± 0.53
14:0	4.26 ± 0.20	1.53 ± 0.32	12.47 ± 0.99
14:1	-	0.46 ± 0.99	-
16:0	20.01 ± 0.38	12.70 ± 0.38	24.58 ± 0.39
16:1	12.68 ± 0.153	5.8 ± 2.07	7.36 ± 0.36
17:0	_	0.61 ± 0.24	3.78 ± 0.23
18:0	5.20 ± 0.811	4.76 ± 0.17	10.96 ± 0.46
18:1	10.30 ± 0.037	14.2 ± 0.74	6.97 ± 0.50
18:1n-9	_	8.6 ± 0.41	3.83 ± 1.00
18:2n-6	6.51 ± 0.115	_	0.64 ± 0.77
18:3n-6	0.57 ± 0.125	_	0.38 ± 0.26
18:3n-3	0.16 ± 0.282	20.2 ± 0.22	0.59 ± 0.07
18:4n-3	0.16 ± 0.052	2.2 ± 1.14	0.73 ± 0.35
20:0	-	0.63 ± 0.11	-
20:1	3.07 ± 0.221	0.82 ± 0.15	1.22 ± 0.67
20:2	-	0.17 ± 0.19	-
20:2n-6	0.21 ± 0.069	_	0.29 ± 0.03
20:4n-6	3.72 ± 0.063	1.1 ± 0.27	1.81 ± 0.25
20:4n-3	0.55 ± 0.145	_	0.21 ± 0.22
20:5n-3	6.31 ± 0.125	4.0 ± 0.17	7.81 ± 0.15
21:0	-	_	0.14 ± 1.33
22:0	0.12 ± 0.090	_	0.38 ± 0.18
22:1	0.58 ± 0.047	_	0.14 ± 0.46
22:5n-6	_	_	0.33 ± 0.22
22:5n-3	4.14 ± 0.105	_	0.81 ± 0.16
22:6n-3	0.20 ± 0.490	2.9 ± 0.35	9.49 ± 0.09
24:0	0.26 ± 0.060	0.71 ± 0.10	0.84 ± 0.18
Sum (n3)	11.52	29.3	19.64
Sum (n6)	11.01	1.1	3.45
Total	82.01	86.4	98.05

Larval growth and survival

Faster growth rate and maximum survival of seabass larvae were noticed when fed on *O. rigida* as compared to *B. plicatilis* and *Artemia* nauplii. The final average length and weight of *L. calcarifer* larvae fed on copepod was found to be as high as 32.69±0.83 mm and 92.18±2.09 mg respectively. Similarly the mean survival rate was also comparatively higher in *O. rigida* fed fish larvae (72.64±0.269 %) which was far better than *B. plicatilis* (36.0±4.32 %) and *Artemia* nauplii fed larvae (28.66±5.73 %). The final mean length and weight gain of seabass larvae fed on rotifer and *Artemia* nauplii were 23.57±0.12 mm; 58.73±1.76 mg and 18.82±0.12 mm; 52.69±2.61 mg respectively (Table 4). The growth and survival of *L. calcarifer* larvae fed with *B. plicatilis* and *O. rigida* were significantly higer ($p<0.005$).

Proximate composition of seabass larvae

The proximate composition of the early *L. calcarifer* larvae fed with different live-feeds was given in Table 5. The carbohydrate and moisture content was found to be high in *Artemia* nauplii fed larvae than *B. plicatilis* and *O. rigida* fed larvae. The maximum amino acid content of 71.72 % in dry matter was obtained in *O. rigida* fed larvae (Table 6) with predominanance of glutamic acid (13.7 %),

Table 4. Growth and survival of seabass *L. calcarifer* larvae fed with various live feeds

Feeding regimes	Initial day		7th day			14th day			21st day		
	Length (mm)	Weight (mg)	Length (mm)	Weight (mg)	Survival (%)	Length (mm)	Weight (mg)	Survival (%)	Length (mm)	Weight (mg)	Survival (%)
B. plicatilis fed larvae	6.03± 0.15	8.43± 0.32	11.56± 0.30	18.56± 0.03	81.49± 0.22	17.86± 0.40	41.26± 0.008	52.51± 0.25	23.57± 0.12	58.73± 1.76	36.0± 4.32
Artemia fed larvae	6.03± 0.15	8.43± 0.32	9.84± 0.28	12.55± 0.16	72.61± 0.93	13.66± 0.16	30.70± 0.16	45.19± 0.34	18.82± 0.12	52.69± 2.61	28.66± 5.73
O. rigida fed larvae	6.03± 0.15	8.43± 0.32	15.30± 0.67	23.76± 0.12	89.43± 0.21	24.46± 0.24	59.63± 0.16	77.30± 0.76	32.69± 0.83	92.18± 2.09	72.64± 0.269

Table 5. Biochemical composition (%) of seabass larvae

Feeding regimes	Moisture	Protein	Lipid	Carbohydrate	Ash
Initial larvae	84.25 ± 0 .134	62.99 ± 0.149	11.45± 0.201	13.59 ± 0.553	11.88 ± 0.232
B. plicatilis fed larvae	82.50 ± 0.169	65.64 ± 0.141	12.35± 0.367	11.92 ± 0.186	10.09 ± 0.063
Artemia fed larvae	89.34 ± 0.242	66.27 ± 0.180	11.12± 0.192	12.0 ± 0.226	10.61 ± 0.362
O. rigida fed larvae	78.74 ± 0.153	68.80 ± 0.451	13.17± 0.137	9.02 ± 0.167	9.01 ± 0.115

alanine (8.72 %) and aspartic acid (8.31%). The *B. plicatilis* fed larvae contained 66.65 % of total amino acids followed by *Artemia* nauplii fed larvae with 57.49 % (Table 6). Among the total fatty acids analysed, palmitic acid (21.86%), oleic acid (10.97%), heptadecanoic acid (10.14%) and palmitoleic acid (8.42 %) were found to be maximum in rotifer fed larvae (Table 7). The *Artemia* nauplii fed fish larvae contained more palmitic acid (29.42 %), linoleic acid (12.15 %), oleic acid (14.62 %) and heptadecanoic acid (9.92 %). The fatty acids such as myristoleic acid (26.04 %), oleic acid (11.62 %) and palmitic acid (10.92 %) were higher in copepod fed larvae. The EPA (10.21%) and DHA (11.08 %) levels were found to be more in *O. rigida* fed fish larvae than the *B. plicatilis* (4.89 % and 0.37 %) and *Artemia* fed larvae (5.20 and 1.76 %). Compared to EPA, the DHA concentration in *O. rigida* fed larvae were several folds higher than *B. plicatilis* fed fish larvae. In *Artemia* fed larvae, the EPA and DHA contents were lower than the copepod fed larvae.

Table 6. Amino acid composition (%) of seabass larvae

Amino acid	Initial	*B. plicatilis* fed larvae	*Artemia* fed larvae	*O. rigida* fed larvae
Arginine	3.25	3.07	6.44	3.04
Histidine	2.41	2.04	2.70	2.13
Isoleucine	2.73	2.72	1.92	2.90
Leucine	5.71	5.42	4.89	5.91
Lysine	5.76	7.39	5.14	5.64
Methionine	1.77	1.85	2.23	1.83
Phenylalanine	2.35	0.33	2.65	2.44
Threonine	3.16	3.11	2.22	3.41
Cystine	0.36	0.01	0.01	0.17
Valine	2.9	2.85	3.09	2.99
Total EAA	30.40	28.79	31.29	30.46
Alanine	7.21	7.63	5.36	8.72
Aspartic acid	7.23	7.47	6.00	8.31
Glutamic acid	11.60	11.61	8.14	13.70
Glycine	5.28	0.48	3.17	5.24
Serine	3.10	3.09	2.21	3.14
Tyrosine	2.03	7.58	1.32	2.15
Total NEAA	36.45	37.86	26.2	41.26
Total	66.87	66.65	57.49	71.72

Table 7. Fatty acid composition (%) of seabass larvae

Amino acid	Initial	*B. plicatilis* fed larvae	*Artemia* fed larvae	*O. rigida* fed larvae
12:0	-	-	-	0.72
14:0	0.14	-	-	3.0
15:0	-	-	-	0.16
16:0	9.76	21.86	29.42	10.92
17:0	6.89	10.14	9.92	5.82
18:0	2.1	5.48	-	6.40
20:0	-	-	-	0.002
21:0	0.64	3.94	-	-
22:0	-	0.14	-	-
24:0	0.001	0.28	0.02	0.0028
14:1	-	-	-	26.04
16:1	4.03	8.42	-	-
18:1	0.21	-	2.88	5.16
18:1-n 9	4.51	10.97	14.62	11.62
18:3-n 3	-	-	-	0.60
18:3-n 6	0.26	0.18	12.15	0.50
18:2-n 6	6.64	6.91	-	-
20:2-n 6	-	-	-	0.033
20:4-n 6	1.24	2.56	-	0.183
20:4-n 5	-	0.86	-	0.0200
20:5-n 3	2.01	4.89	5.20	10.21
22:6-n 3	1.52	0.37	1.76	11.08
22:1	1.04	-	5.98	0.442
Total	40.99	77.0	81.95	92.91

Discussion

In the present study, maximum survival and growth were recorded in copepod fed fish larvae compared to rotifer and *Artemia* fed larvae. This could be attributed to the superior nutritional profile of the copepod as reported by Doi *et al.* (1997). The length, weight and survival were all significantly lower in those larvae reared with *B. plicatilis* and *Artemia* nauplii compared to copepod fed ones. The changes in length and weight were evident in rotifer and *Artemia* fed larvae only from 3rd and 4th day respectively. The slow growth observed in the initial stages of the larvae might be due to their small mouth size which was not suitable to capture the larger prey such as rotifer and *Artemia* (Kayano, 1988). It is well known that the copepod *O. rigida*

provides wide size ranges (Nauplii-I to Copepodite-V) suiting to the mouth size of the fish larvae resulting in appreciable length and weight gain in copepod fed larvae as reported by Rajkumar and Vasagam (2006). Further, the slow growth noticed in rotifer fed fish larvae could also be attributed to the fact that the larvae neeed to spend more energy in search of prey which has a tendency to stick to the sides of the culture tanks (Laurence, 1977).

Assessment of biochemical composition of live feeds and live feed fed fish larvae is important for better understanding in feed development. In the present observation, protein, essential amino acids and fatty acids especially HUFA contents of both copepod and copepod fed fish larvae were comparatively higher than that of rotifer and *Artemia* nauplii fed fish larvae. The protein was found to be the major biochemical component in the cultured copepod, *O. rigida*. It formed the major fraction compared with lipid and carbohydrate, indicating the usefulness as energy reserve. The observed variations in the protein content might be due to the fact that it is utilised as a metabolic substrate (Nageswara Rao and Krupanidhi, 2001). The lipid content of copepod was slightly higher than that of carbohydrate and lower than that of protein. The continuous supply of phytoplankton would render lipid reserve unnecessary, which might account for the low content as reported by Perumal *et al.* (2009). The variations in the lipid content could be attributed to its storage and utilisation during starved period when it serves as an effective energy reserve (Nageswara Rao and Krupanidhi, 2001). The present results showed that proportion of essential amino acids was higher in *O. rigida* than *B. plicatilis* and *Artemia* nauplii. Amino acid content of *O. rigida* indicated adequate levels recommended for the fish larvae as reported earlier by Watanabe *et al*. (1983).

The fatty acid content was comparatively higher in *O. rigida* than *B. plicatilis* and *Artemia* nauplii. It is understood that the copepod, *O. rigida* can produce large amounts of EPA and DHA whcih might be due to the desaturating ability of *O. rigida* as agreed by Toledo *et al*. (1999) who reported more quantity and quality of n-3 highly unsaturated fatty acids (n-3 HUFA) in *Pseudodiaptomus* and *Acartia* sp. which is about 2 to 3 times higher than rotifers. The fatty acid content of copepod may be influenced by the type of algal species used as feed for the copepod (Nanton and Castell, 1999). In the present investigation, the DHA content of *O. rigida* was higher than EPA content as found in rotifer and *Artemia* (Evjemo and Olsen, 1997).

It is inferred that the growth and survival of seabass larvae were improved with nutritionally superior copepod with rich pigmentation as agreed by Santhanam *et al.* (2004) and Ananthi *et al.,* (2011). It is well known that, the nutritional value of live feed, particularly the quantity and quality of n-3 highly unsaturated fatty acids (n-3 HUFA) are known to affect the growth and survival of seabass larvae. In the present study, growth and survival of *L .calcarifer* larvae were higher with copepod, *O. rigida* diet owing to availability of more amounts of EHA (20:5 n-3) and DHA (22:6n-3) which resulted in enhanced growth and survival in fish larvae as observed by Payne and Rippingale (2000). The observed low growth and survival in rotifer and *Artemia* nauplii fed seabass larvae could only be a reflection of the inadequate nutritional value. Similar observations were also reported by earlier workers (McKinnon *et al.,* 2003; Rajkumar and Vasagam, 2006; Olivotto *et al.,* 2008). In fact, copepods clearly outperform in terms of meeting fish larval HUFA requirements (Van der Meeren *et al.,* 2008).

Copepods as initial feed in fish larviculture is more advantageous as reported by several workers in various fish species. Heath and Moore (1997) and Ananthi *et al.* (2011) opined that the superior nutritive value of copepod resulted in better pigmentation and development in the fish larvae. Kraul *et al*. (1993) noticed improved resistance in the larval mahi mahi when fed with copepod, *Euterpina acutifrons*. Furthermore, copepod nauplii can impart an important role in larval digestion due to its source of exogenous digestive enzyme (Munilla Moran *et al*., 1990). Doi *et al*. (1997) observed high feeding performance and growth in red snapper, *Lutjanus argentimaculatus* when fed with nauplii of *Acartia sinjiensis* than when fed with *Brachionus*. Nanton and Castell (1998) observed significant higher growth in haddock larvae fed with copepods than those fed with rotifers. Stottrup and Norsker (1997) noticed that use of *Tisbe* sp. nauplii as a supplement to a basic rotifer diet can enhance the feeding rates as well as growth and survival of the first feeding turbot larvae. Payne *et al*. (1998) studied the larval rearing of pipefish and observed higher growth and 99% survival with copepod diet. Similarly, Barlow *et al*. (1993) reported maximum growth and survival in larvae of barramundi (*L. calcarifer*) reared in ponds with rich zooplankton where the fish grew from 10 to 40 mm total length. Santhanam *et al.* (2004) noticed increased growth and survival in tiger shrimp *P. monodon* fed with copepod *O. rigida* than those fed with *Artemia* nauplii and rotifer. The results of the present study clearly indicate that the marine copepod *O. rigida* can be considered as a promising and nutritionally superior alternative live feed to replace the existing nutritionally doubtful live feeds such as *B. plicatilis* and *Artemia* nauplii. Furthermore, it was evident that seabass larvae could actively feed on the copepod *O. rigida*. The present study concluded that the copepod *O. rigida* can be considered as a potential live feed for rearing of seabass *L. calcarifer* larvae.

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