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Suitability of the copepod, *Acartia clausi* as a live feed for Seabass larvae (*Lates calcarifer* Bloch): Compared to traditional live-food organisms with special emphasis on the nutritional value

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Abstract

Though artificial propagation of Asian seabass *Lates calcarifer* (Bloch) in captivity through induced breeding techniques is standardized under Indian conditions, larval and nursery rearing techniques including suitable nursery feeds have to be standardized to obtain better survival and growth. Feeding experiments in triplicate were conducted to evaluate the suitability of the marine copepod *Acartia clausi* as live prey for fourteen day-old seabass larvae (6.53 ± 0.06 mm; 8.58 ± 0.33 mg) and compared with the traditional live prey, rotifers and *Artemia* nauplii. While *A. clausi* and rotifers were mass produced using algae *Isochrysis galbana*, *Chaetoceros affinis* and *Chlorella marina*, *Artemia* nauplii were produced using cysts. Nutritional quality of cultured copepods was evaluated based on the proximate composition, amino acid and fatty acid composition, and compared with that of rotifers and *Artemia* nauplii. Proximate composition varied significantly ($P < 0.05$) among the different live feeds. *A. clausi* showed higher protein (63.12%) and lipid (16.65%) content than *Artemia* nauplii and rotifers. Total essential amino acids content was 2% lower in *A. clausi* compared to that in *Artemia* nauplii. Fatty acid profiles of the live feed organisms showed that *A. clausi* is a rich source of $n-3$ fatty acids. The total $n-3$ fatty acid content of *A. clausi* was 33.94%. Length, weight overall weight gain and survivorship were significantly ($P < 0.05$) different among the dietary treatments, and weight gain was comparatively higher in *A. clausi* fed larvae. Survival of seabass larvae fed *A. clausi* was obtained highest as 58.13% against the lower values of 39.93% and 41.62% in larvae fed rotifer and *Artemia* nauplii respectively. Final carcass composition of the larvae of *L. calcarifer* fed different live-food organisms showed significant differences ($P < 0.05$) among the dietary treatments. The fatty acid composition of the dietary treatments was reflected to a certain extent in the fatty acid composition of the seabass larvae. The present investigation revealed the nutritional value of calanoid copepod and thus underlining its usefulness as a suitable live-food organism for rearing larvae of the commercially valuable Asian seabass.
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Keywords: Seabass; *Lates calcarifer*; Live feed; Copepod; *Acartia clausi*; Growth

1. Introduction

The production of very small, rapidly developing and highly vulnerable larvae remains a bottleneck in the commercially successful culture of many marine fish species (Shields et al., 1999). More specifically, nutrition

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is considered to be one of the most important issues in production of marine fish larvae (Koven et al., 2001). Nutritional compounds such as $n-3$ fatty acids, essential amino acids (EAA) and protein content of live feeds are critical factors for the survival and optimal growth of larval finfish and shellfishes (Howell and Tzoumas, 1991; Evjemo and Olsen, 1997; Payne and Rippingale, 2000; Hernandez Molejon and Alvarez-Lajonchere, 2003; Kanazawa, 2003). Highly unsaturated fatty acids (HUFA) including eicosapentaenoic acid (EPA, 20:5 $n-3$), and docosahexaenoic acid (DHA, 22:6 $n-3$) play an important role in the development of the brain and central nervous system, maintaining cell membrane structure and function, stress tolerance, and proper development and functioning of neural and visual systems in fish (Sargent et al., 1997). Larval fish also require at least 10 EAA in its diet (Wilson, 2003) for optimal production, growth, feed conversion and carcass quality potential.

Marine fish larval rearing is usually carried out under intensive conditions and larvae are fed on cultured traditional live feeds such as rotifers and *Artemia* nauplii but unfortunately these organisms are naturally deficient in HUFAs. Thus, it is essential to enrich these live feeds with essential fatty acids before offering them to the larvae (Sargent et al., 1997; Stottrup, 2000; Olivotto et al., 2006). Many studies have used different algal species that are high in $n-3$ highly unsaturated fatty acid content to enhance the nutritional quality of live feeds (Lombardi and Wangersky, 1995). The nutritional value newly hatched *Artemia* nauplii varies considerably with the origin of the cysts, and the principal factor affecting the nutritional value is the fatty acid composition of nauplii lipids (Webster and Lovell, 1990). In addition to this, the potential crisis is the declining yield of *Artemia* cysts from the Great Salt Lake, the main source of cysts (Lavens and Sorgeloos, 2000).

The marine copepods are considered to be "nutritionally superior live feeds" for commercially important cultivable species, as they are a valuable source of protein, lipid (especially HUFA, 20:5 $n-3$ and 22:6 $n-3$), carbohydrates and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval survival, growth, digestion and metamorphosis (Stottrup, 2000; Hernandez Molejon and Alvarez-Lajonchere, 2003; Kleppel et al., 2005). Copepods are known to have greater digestibility (Schipp et al., 1999) and a relatively high weight specific caloric content (Sun and Fleeger, 1995). In addition, the growth stages of calanoids from first nauplius to adult, provide a broad spectrum of prey sizes (80 to >900 μm in length and 3–5 μg in dry weight). This makes them suitable prey for a similarly broad range of developing fish sizes (Schipp et al., 1999). For example, a calanoid copepod (*Acartia* sp.) has been successfully fed to red snapper (*Lutjanus argentimaculatus*) for which rotifers are too large (Schipp et al., 1999). Conversely, Kahan et al. (1982) suggest that *Artemia* may be too large for larvae with especially small gapes. In addition to their nutritional and physical superiority as live feed, copepods are also highly suitable for culture because of their eurythermal and euryhaline characteristics, which allow them to tolerate wide environmental fluctuations (Carli et al., 1995).

Acartia clausi, a copious calanoid copepod in tropical waters (especially in India) is found to be nutritionally rich and could be successfully mass cultured (Schipp et al., 1999; Payne and Rippingale, 2000; Rajkumar et al., 2004). Despite the fact that the suitability of copepods as live feed for marine fish larvae is now well established, their use in aquaculture remain sporadic, specifically in Indian waters.

Asian seabass *Lates calcarifer* (Bloch), one of the most suitable finfish for brackish water farming in earthern ponds and in floating net cages has contributed around 20,066 metric tonnes annually to the total world aquaculture production of 3.6 million tonnes (Tucker et al., 2002; Thirunavukkarasu et al., 2004). It is a fast-growing fish cultured extensively in Southeast Asian countries and the Indo-Pacific region (Thirunavukkarasu et al., 2004). Artificial propagation of seabass in captivity through induced breeding techniques is standardized under Indian conditions (Thirunavukkarasu et al., 2001). Larval and nursery rearing techniques for seabass, including suitable nursery feeds, have to be standardized to obtain better survival and growth (Kailasam et al., 2001, 2002). Seabass fry is a highly carnivorous and voracious feeder and development of (shooters) fast-growing individuals during the larval phase drastically reduces the survival rate through cannibalism (Kailasam et al., 2002).

In this back drop, the aim of this study was to examine the use of *A. clausi* as live prey for seabass (*L. calcarifer*) and compare the performance of the copepod as live prey with that of the traditional live prey, rotifers and *Artemia* nauplii.

2. Materials and methods

2.1. Algal culture

Pure strains of the algae *Isochrysis galbana* (Prymnesiophyceae), *Chaetoceros affinis* (Bacillariophyceae) and *Chlorella marina* (Chlorophyceae) were obtained from the Central Institute of Brackishwater Aquaculture (ICAR), Chennai and Central Marine Fisheries

Research Institute (ICAR), Cochin. All the species of algae were grown at 28 °C, 30‰ salinity, and 14 l: 10 D light regimes and fertilized with f/2 medium (Guillard and Ryther, 1962). The algae were harvested during the log phase (approx. 30,000 cells/mL) for feeding to the rotifers and copepods.

2.2. Copepod culture

Zooplankton samples were collected using 158-μm mesh size plankton nets (0.35 m diameter opening) from the Pichavaram mangrove (lat. 11° 29′ N, long. 79° 46′ E) waters early in the morning during the full moon phase. The samples were immediately transported to the laboratory and thoroughly rinsed to reduce contamination by unwanted organisms. After collection, the zooplankton was screened to isolate the size fraction containing predominantly adult copepods and later-stage copepodids of *A. clausi*. This 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 used to remove rotifers and nauplii of copepods and barnacles. After rinsing, the remaining adult copepods and larger copepodids were used to start the culture.

A stock culture of *A. clausi* was maintained in a rectangular, flat-bottomed fiberglass tank (550 mm dia., 850 mm height) filled with 200 L UV filtered seawater that was vigorously aerated. Rearing containers were covered with nylon cloth to prevent excessive evaporation. Seawater was filtered through a membrane filter (pore size > 1 μm). Contamination of the rearing tank was reduced by daily water changes. Temperature, salinity, pH and dissolved oxygen were maintained at between 28 and 32 °C, 30 and 34‰, 7 and 8.5 and 5 and 6.8 mL L^{-1}, respectively, during the rearing period. The copepods were cultured and fed equal quantities of *I. galbana*, *C. affinis* and *C. marina* in a ratio of 3:3:3 (30,000:30,000:30,000 cells/mL of each algal species). The stock cultures were harvested every thirteenth day by gentle siphoning, and the components of the culture tank were transferred to a zooplankton washer and then rinsed for 1 h with seawater from the reservoir. Adult and copepodids collected in the zooplankton washer are used to restart the stock culture tanks or are added to copepod mass culture tanks. In a latter occasion, the adults and the late-stage copepodids were then concentrated in a sieve (54 μm), washed in filtered seawater and immediately frozen and kept under liquid nitrogen at −20 °C before freeze-drying and further analysis.

2.3. Rotifer and Artemia culture

The rotifer, *Brachionus plicatilis,* was cultivated at 28 °C in 250 L conical glass fibre tanks at 32‰ salinity, and aerated to secure mixing and supply oxygen. The animals were fed algae (*I. galbana*, *C. affinis* and *C. marina*) daily, and once a week with 35 g bakers yeast (wet weight per volume 35 g/tank). The cultures were run semi continuously at high and low dilution rates (32‰ and 12% of the culture volume was replaced per day, respectively) to obtain fast and slow growth of the rotifer cultures. The culture density was 200–250/mL at the time of harvest. The harvested rotifers were washed and put directly into the larval rearing tanks. Rotifer samples for chemical analysis were harvested on a nylon net (70-μm mesh size) approximately 1 week after dilution.

Artemia nauplii cysts, available commercially (Red Top™ Brand, from Ocean Star International, Inc., Snowville, UT 84336 USA), were decapsulated and hatched according to standard procedures (Treece, 2000). The cultures were continuously illuminated and vigorously aerated to maintain oxygen (O_2) > 5.2 mg O_2 L^{-1}. Samples of *Artemia* nauplii were taken for biochemical composition analysis and amino acid and fatty acids profiles by collecting them on a 64-μm mesh nylon sieve. The *Artemia* nauplii were washed in filtered freshwater and immediately frozen and kept under liquid nitrogen at −20 °C before further analysis.

2.4. Growth trial

Feeding experiments with fourteen day-old seabass larvae (6.53 ± 0.06 mm; 8.58 ± 0.33 mg) were performed

Table 1
Biochemical composition (% dry matter basis) of different cultured live feed-organisms

Live feed organisms	Moisture*	Protein	Lipid	Carbohydrate	Ash
Rotifer	84.92±0.53^{b}	61.48±0.64^{ab}	13.20±0.38^{a}	14.30±0.51^{c}	4.34±0.10^{a}
Artemia nauplii	83.88±0.33^{a}	60.19±1.31^{a}	16.57±0.50^{b}	11.97±0.40^{b}	7.01±0.29^{c}
Acartia clausi	83.25±0.31^{a}	63.12±0.88^{b}	16.65±0.24^{b}	11.04±0.45^{a}	5.09±0.19^{b}

Means of triplicate in the same column sharing different superscripts are significantly different ($P < 0.05$).
*Wet matter basis.

Table 2
Amino acid composition (amino acid % in protein) of different cultured live feed organisms

Amino acids	Rotifer	*Artemia* nauplii	*Acartia clausi*
Arginine	7.08	7.21	8.57
Histidine	1.61	3.14	3.50
Isoleucine	5.01	5.08	2.17
Leucine	9.12	8.81	9.92
Lysine	10.29	10.20	11.04
Methionine	2.92	1.98	1.98
Phenylalanine	4.69	5.26	1.05
Threonine	3.41	3.94	2.50
Cystine	–	–	0.07
Valine	7.92	6.74	9.57
Total EAA	52.05	52.36	50.37
Alanine	7.18	7.86	11.12
Asparctic acid	9.21	9.20	9.57
Glutamic acid	13.72	10.45	11.10
Glycine	6.92	6.31	4.25
Serine	5.18	5.94	6.37
Tyrosine	3.92	5.29	6.31
Total NEAA	46.13	45.05	48.72
Total	98.18	97.41	99.09

in triplicate using three different live feeds (rotifer, *Artemia* nauplii and copepod). The fish larvae were obtained from the fish hatchery of Central Institute of Brackishwater aquaculture, Muttukkadu, Chennai. The larvae were carefully transported in sealed plastic bags containing seawater and inflated with oxygen. They were acclimatized to the laboratory tank conditions for 2 days prior to the experiment. Growth trials lasted 21 days and were performed in fiberglass tanks painted on the inside with royal blue epoxy paint the larval rearing tanks (70 cm × 50 cm × 30 cm and 6 mm thick). They held approximately 100 L of seawater. Outflows were positioned 5 cm below the top of each tank. Aeration was by means of air stones (two per tank). Fish larvae were stocked at the rate of 10 larvae/L. The fish were exposed to continuous light, a water flow rate of 2–3 L min^{-1} and fed either *A. clausi*, *B. plicatilis* or *Artemia* nauplii. To avoid starvation and further cannibalism the live feed density in larval rearing tanks was kept high. This varied between 6000–10,000, 3500–5500 and 1350–1960 ind.L^{1} for rotifers, *Artemia* nauplii and copepods respectively. Filtered seawater was used for 50% water exchange daily before feeding. Larvae were fed three times a day, at approximately 06:30, 13:00 and 19:30. Water quality parameters such as temperature, salinity, pH and the concentration of dissolved oxygen in the water were measured once a week following the method of Strickland and Parsons (1972). Every 7 days fish larvae were sampled to examine changes in the weight and length. Length of larvae was measured from the tip of the lower mandible to the end of the caudal fin. Samples of larvae were removed and measured after anaesthetizing in 100 ppm tricane methane sulphonate (MS 222). Dead larvae removed from the tanks were also measured, but only those that showed no signs of shrinkage due to decay. As mortality reduced the number surviving, later measurements were made *in situ* using calipers and a scale graduated in mm attached to a glass rod. The number of

Table 3
Fatty acid profiles of different cultured live feed organisms[a]

Fatty acids	Rotifer	*Artemia* nauplii	*Acartia clausi*
12:00	0.58	0.20	0.15
14:00	4.46	3.89	5.82
14:01	0.96	1.58	0.88
15:00	1.15	1.26	1.95
16:00	18.35	21.33	12.99
16:01	11.33	6.26	4.63
17:01	–	–	6.80
18:00	4.79	10.84	13.01
18:01	10.33	11.05	2.47
18:1 $n-9$	9.54	6.53	8.64
18:2 $n-6$	5.67	–	2.08
18:3 $n-6$	0.62	–	–
18:3 $n-3$	5.18	11.72	8.25
18:4 $n-3$	1.91	2.08	2.01
20:00	–	0.62	–
20:01	3.03	5.89	2.04
20:02	–	0.20	–
20:2 $n-6$	0.21	–	1.71
20:4 $n-6$	3.64	2.49	–
20:4 $n-3$	1.62	1.02	1.02
20:3 $n-3$	5.22	–	2.49
20:5 $n-3$	6.27	6.02	9.95
21:00	–	–	0.11
22:00	0.10	–	0.37
22:01	0.60	–	0.13
22:5 $n-6$	–	–	0.31
22:5 $n-3$	2.98	–	0.85
22:6 $n-3$	0.21	2.70	9.37
Saturates[b]	29.43	38.14	34.40
Monounsaturates[c]	35.79	31.31	25.59
PUFA and HUFA[d]	33.53	26.23	38.04
Total $n-3$[e]	23.39	23.54	33.94
Total $n-6$[f]	10.14	2.49	4.10

– not detected.

[a] Fatty acid values (percentage of total fatty acid methyl esters) were adjusted to express a percent of the total area identified in the chromatograms, unidentified peaks were not considered in the computations.

[b] Saturates: 12:0, 14:0, 16:0, 18:0, 20:0, 22:0.

[c] Monounsaturates: 14:1, 16:1, 18:1, 20:1, 22:1.

[d] PUFA and HUFA: 16:2, 16:3, 16:4, 18:2 $n-6$, 18:3 $n-6$, 18:3 $n-3$, 18:4 $n-3$, 20:2 $n-6$, 20: 3 $n-6$, 20:4 $n-6$, 20:3 $n-3$, 20:5 $n-3$, 22:2, 22:3, 22:4, 22:5 $n-3$, 22:6 $n-3$.

[e] Total $n-3$: 18:3 $n-3$, 18:4 $n-3$, 20:3 $n-3$, 20:5 $n-3$, 22:5 $n-3$, 22:6 $n-3$.

[f] Total $n-6$: 18:2 $n-6$, 18:3 $n-6$, 20:2 $n-6$, 20:3 $n-6$, 20:4 $n-6$.

Table 4
Growth and survival of seabass, *Lates calcarifer* fed different live-food organisms

Feeding regimes	First day		7th day			14th day			21st day			Overall weight gain (mg)
	Length (mm)	Weight (mg)	Length (mm)	Weight (mg)	Survival (%)	Length (mm)	Weight (mg)	Survival (%)	Length (mm)	Weight (mg)	Survival (%)	
Rotifer	6.53 ± 0.06[a]	8.68 ± 0.21[a]	12.60 ± 0.20[b]	19.49 ± 0.29[b]	72.17 ± 2.97[a]	19.90 ± 0.53[b]	38.72 ± 1.96[b]	60.90 ± 1.97[ab]	26.90 ± 0.62[b]	56.07 ± 2.72[b]	39.93 ± 3.59[a]	47.39 ± 2.69[b]
Artemia nauplii	6.57 ± 0.06[a]	8.58 ± 0.33[a]	11.00 ± 0.36[a]	14.19 ± 0.34[a]	66.70 ± 4.42[b]	16.90 ± 0.30[a]	30.39 ± 1.69[a]	58.42 ± 3.30[a]	20.93 ± 0.42[a]	40.78 ± 1.52[a]	41.62 ± 2.41[a]	32.20 ± 1.83[a]
Acartia clausi	6.57 ± 0.06[a]	8.75 ± 0.02[a]	15.00 ± 0.26[c]	22.36 ± 0.40[c]	75.47 ± 10.20[a]	24.50 ± 1.40[c]	48.95 ± 4.49[c]	70.13 ± 7.37[b]	34.90 ± 0.70[c]	76.37 ± 4.04[c]	58.13 ± 2.48[b]	67.62 ± 4.03[c]

Means in the same column sharing different superscripts are significantly different ($P<0.05$).

dead larvae was registered daily and was taken into account for survival calculation. At the end of the feeding trials, all of the seabass were chill-killed and stored frozen at −20 °C for subsequent determination of whole body composition. Earlier samples of seabass from the initial population were collected and stored frozen for a similar evaluation. Wet and dry weight of the larvae was measured, depending on the larval size, in an analytical balance with 0.01 mg accuracy (Sartorius, Germany).

2.5. Biochemical analysis

Ash content was determined gravimetrically by burning in a Muffle furnace at 550 °C for 6 h. Standard method of the Association of Official Analytical Chemists (AOAC, 1990) was followed for estimation of crude protein content (AOAC 955.04). The carbohydrate content of the samples was determined by phenol-sulphuric acid reagent at 490 nm using D-glucose as a standard (Dubois et al., 1956). Total lipid content was determined gravimetrically after extraction in Chloroform/methanol (2:1 v/v) (Folch et al., 1957).

The protein bound amino acids were separated from free amino acids by using 6% TCA solution (Finn et al., 1995). Amino acids were analysed after sealed tube hydrolysis with 6 N HCL for 22 h at 110 °C (Finlayson, 1964). After hydrolysis, the acid was evaporated in a vacuum oven and the sample was kept in a NaOH desiccator to remove traces of acid. The residue was brought into 1 mL of sample diluent (pH 2.20). Amino acids were analysed using the Shimadzu HPLC model LC-10A (Shimadzu Corp., Japan). Separation of amino acids was performed in a column (Shimpack ISC-07/S 1504 Na) packed with a strongly acidic Na^+ type cation exchange resin (Styrene-divinyl benzene copolymer with sulfinic group) under gradient elution. The amino acids were detected and quantified using a fluorescent detector (FLD-6A) after post column derivitization with *O*-pthaladehyde and 2-mercaptoethanol. Amino acid standard solution (Sigma–Aldrich Inc., USA) for fluorescent detection was used as an external standard. For every ten-sample injection, one standard run was carried out. Units for amino acids were converted from *n* moles to µg units by multiplying the molecular weight × 1000.

Fatty acids were saponified and methylated using 2% NaOH in methanol, 14% BF_3/methanol and heptane. The fatty acid methyl esters (FAME) were determined on a Hewlett Packard HP 5890 gas chromatograph equipped with a flame ionization detector. The sample was injected at 190 °C onto a J and W Scientific DB23 fused silica capillary column (30 m × 0.25 mm i.d., 0.25-µm film thicknesses) with hydrogen as the carrier gas. The column was operated isothermally at an oven temperature of 180 °C and a detector temperature of 210 °C.

Table 5
Comparison of final carcass (% dry matter basis) composition of seabass, *Lates calcarifer* fed different live-food organisms

Live feed organisms	Moisture*	Protein	Lipid	Carbohydrate	Ash
Initial	83.82 ± 1.17[a]	61.97 ± 0.59[a]	10.83 ± 0.24[a]	11.52 ± 0.46[c]	10.54 ± 0.59[b]
Rotifer	83.20 ± 1.26[a]	62.09 ± 0.73[a]	13.61 ± 0.41[b]	9.62 ± 0.20[ab]	10.48 ± 0.11[ab]
Artemia nauplii	84.93 ± 0.41[a]	60.84 ± 0.85[a]	16.55 ± 0.26[c]	9.74 ± 0.23[b]	10.05 ± 0.10[ab]
Acartia clausi	83.59 ± 1.14[a]	60.92 ± 0.42[a]	16.41 ± 0.50[c]	9.12 ± 0.09[a]	9.86 ± 0.27[a]

Means of triplicates in the same column sharing different superscripts are significantly different ($P<0.05$).
*Wet matter basis.

Fatty acids were identified by comparing with authentic standards.

2.6. Statistical analysis

Dietary treatments were compared by one way ANOVA. Duncan's multiple range test (Duncan, 1955) was applied to ascertain any significant differences between treatment means. All the above mentioned statistical analyses were performed using SPSS statistical software (Ver. 11.5 for Windows, SPSS, Chicago, IL, USA). Limits of significance for all critical ranges were set at $P<0.05$.

3. Results

Biochemical composition of the live feeds, on a wet and dry weight basis, is presented in Table 1. Biochemical constituents like protein, lipid and carbohydrates varied significantly ($P<0.05$) among the different live feeds. Compared to *Artemia* nauplii and rotifers, the copepod *A. clausi* showed higher protein (63.12%) and lipid (16.65%) content.

Amino acid and fatty acid composition of the three different cultured live feed organisms are presented in Tables 2 and 3, respectively. Total essential amino acids content was 2% lower in *A. clausi* compared to that in *Artemia* nauplii. Fatty acid profiles of the live feed organisms showed that *A. clausi* is a rich source of PUFAs, HUFAs and $n-3$ fatty acids. The total $n-3$ fatty acid content of *A. clausi* was 33.94%.

3.1. Growth performance and survival

The growth trials were conducted without interruption or disease problems. The water quality parameters across all experiments were: salinity, 28–32‰; temperature, 28–30 °C; dissolved oxygen, 6.5–7.5 mL/L; and pH, 7.5–8.5. Growth and survival of *L. calcarifer* larvae fed with different live-food organisms are given in Table 4. Seabass larvae fed the copepod *A. clausi* grew faster compared to larvae fed rotifers and *Artemia* nauplii. The weight gain was also comparatively higher in *A. clausi* fed larvae (67.62 mg on Day 21). Length, weight and overall weight gain were significant ($P<0.05$) among the dietary treatments. Right through from beginning, the larvae fed *A. clausi* had the highest survival rate of 75.47%, 70.13% and 58.13% on day 7, 14 and 21 respectively. On days 7 and 14 the larvae reared on *Artemia* nauplii had the lowest survival rate of 66.70% and 58.42% respectively. However on day 21st, survival was lowest as 39.93% in rotifer fed fish larvae.

3.2. Carcass composition

The biochemical composition of the early larvae of *L. calcarifer* fed different live-food organisms showed significant differences ($P<0.05$) among the dietary treatments. Complete data is presented in Table 5.

The fatty acid composition of *L. calcarifer* larvae fed rotifers, *Artemia* nauplii, or *A. clausi* is shown in Table 6. The fatty acid composition of the dietary treatments was

Table 6
Fatty acids composition of seabass, *Lates calcarifer* fed different live-food organisms [a]

Fatty acids	Initial	Rotifer	*Artemia* nauplii	*Acartia clausi*
12:00	–	1.23	–	0.70
14:00	0.13	2.85	3.17	3.21
14:01	–	0.32	–	10.07
15:00	–	1.8	1.28	1.34
16:00	32.73	26.78	27.97	22.29
16:1 $n-7$	8.01	9.28	2.14	2.38
17:00	15.26	9.84	9.03	3.51
17:01	–	–	–	0.73
18:00	8.4	5.14	6.67	10.16
18:01	5.23	–	–	4.07
18:1 $n-7$	0.95	6.27	6.64	2.99
18:1 $n-9$	9.49	14.39	13.37	13.58
18:02	–	–	1.78	1.15
18:2 $n-6$	6.61	3.57	–	–
18:3 $n-3$	–	–	–	0.60
18:3 $n-6$	0.27	2.15	3.13	1.51
19:00	0.35	0.41	0.2	–
19:01	–	0.55	0.22	0.66
20:00	–	0.24	0.15	0.00
20:2 $n-6$	–	–	–	0.03
20:4 $n-5$	0.21	0.77	0.26	0.35
20:4 $n-6$	1.25	3.48	2.3	3.88
20:5 $n-3$	5.04	6.15	9.98	8.17
21:00	0.62	2.85	–	–
22:00	–	0.15	–	–
22:01	1.01	–	4.58	0.45
22:6 $n-3$	1.51	0.35	1.81	5.03
Saturates [b]	57.49	51.29	48.47	41.21
Monounsa [c]	24.69	30.81	26.95	34.93
PUFA [d]	14.89	16.47	19.26	20.72
Total $n-3$ [e]	6.55	6.5	11.79	13.80
Total $n-6$ [f]	8.13	9.2	5.43	5.42

– not detected.

[a] Fatty acid values (percentage of total fatty acid methyl esters) were adjusted to express a percent of the total area identified in the chromatograms, unidentified peaks were not considered in the computations.

[b] Saturates: 12:0, 14:0, 16:0, 18:0, 20:0, 22:0.

[c] Monounsaturates: 14:1, 16:1, 18:1, 20:1, 22:1.

[d] PUFA and HUFA: 16:2, 16:3, 16:4, 18:2 $n-6$, 18:3 $n-6$, 18:3 $n-3$, 18:4 $n-3$, 20:2 $n-6$, 20: 3 $n-6$, 20:4 $n-6$, 20:3 $n-3$, 20:5 $n-3$, 22:2, 22:3, 22:4, 22:5 $n-3$, 22:6 $n-3$.

[e] Total $n-3$: 18:3 $n-3$, 18:4 $n-3$, 20:3 $n-3$, 20:5 $n-3$, 22:5 $n-3$, 22:6 $n-3$.

[f] Total $n-6$: 18:2 $n-6$, 18:3 $n-6$, 20:2 $n-6$, 20:3 $n-6$, 20:4 $n-6$.

reflected to a certain extent in the fatty acid composition of the seabass larvae. For instance, arachidonic acid (20:4 $n-6$, AA), EPA and DHA were always significantly higher in seabass fed copepods as live feed.

4. Discussion

In the present study, the levels of moisture and carbohydrate content were higher, and protein, lipid and ash content were lower in the rotifer than in *A. clausi*. The lower protein content of *B. plicatilis* is comparable to earlier findings by Millamena et al. (1990) in *B. calyciflorus*. The protein in *Artemia* nauplii was comparatively lower in value than in *B. plicatilis* and *A. clausi*. The lipid content was slightly higher in *Artemia* nauplii than in *A. clausi* (Evjemo and Olsen, 1997). Variations in the lipid content can also be attributed to its storage and utilization during starvation periods, when it serves as an effective energy reserve for the organism (Goswami et al., 2000). Carli et al. (1992) reported low levels of protein and carbohydrate in rotifers cultured in a yeast medium. Fluctuations in the biochemical composition of rotifers observed in the present study could thus be related to the type of culture medium used. The protein formed the major fraction compared with lipids and carbohydrates, indicating its usefulness as an energy reserve for zooplankton having low lipid content (Maruthanayagam and Subramanian, 1999).

The differences in amino acid content among the three live feeds are species specific and they can be related to their physiological differences (Safiullah, 2001). Ogino (1963) stated that the various types of natural zooplankton are valuable protein sources, based on their amino acid composition. Values for amino acids in *A. clausi* are comparable with the values reported earlier for wild copepods (Goswami et al., 2000).

The present results showed that the proportion of essential amino acids is higher in *Artemia* nauplii than in *B. plicatilis* and *A. clausi* studied. However, difference in EAA among the three feed organisms was not much, and the EAA content of *A. clausi* was found to be at adequate level relative to what has been recommended in general for various fish and shellfish (Wilson, 2003). A balanced dietary amino acid profile increases the amino acid retention and may improve growth and nitrogen utilization (Aragão et al., 2004).

The observed essential fatty acid content was higher in *A. clausi* compared with the two other food organisms in the present study. Similar findings have been reported for the harpacticoid copepods *Tigriopus* sp. (Watanabe et al., 1978) and *Tisbe* sp. (Norsker and Stottrup, 1994; Nanton and Castell, 1998). In the present study, the DHA content of *A. clausi* was several times higher than in *B. plicatilis* and *Artemia* nauplii, which was similar to earlier observations by Toledo et al. (1999). According to Toledo et al. (1999), the quantity and quality of $n-3$ HUFAs in *Pseudodiaptomus* sp. and *Acartia* sp. is about 2 to 3 times higher than that of rotifers. The higher EPA and DHA content of the cultured copepods in the present study may be attributed to the dietary influences of the feed algae. The dietary influence on the lipid production and fatty acid content in copepods has also been documented by Stottrup and Jensen (1990) and Nanton and Castell (1999). The EPA and DHA content of different stages of copepods have been found to be superior to enriched *Artemia* (Stottrup et al., 1999). *Artemia* and rotifers have no (or only a limited) ability to bioconvert shorter chain $n-3$ PUFA into the longer chain EFA, 20:5 $n-3$ and 22:6 $n-3$ (Howell and Tzoumas, 1991). The calanoid copepod, *A. clausi*, might be able to synthesize significant amounts of 20:5 $n-3$ and 22:6 $n-3$, as has been reported by Nanton and Castell (1998) for the harpacticoid copepod, *Tisbe* sp. However it is not definite, hence this study failed to measure the fatty acid content of the algae which were given as feed to the copepod.

In the present study, the growth and survival of early stages of larvae of seabass *L. calcarifer* vis-a-vis live-food organisms, i.e., rotifers, *Artemia* and copepods, were examined. Growth and survival of *L. calcarifer* larvae were comparatively higher with a copepod diet than with rotifers or *Artemia* nauplii. The small gape of young larvae is a factor which limits the prey size. So the improved survival of marine finfish larvae fed copepod nauplii is accepted to be the result of better ingestion (Luizi et al., 1999) of copepod nauplii that are a smaller size (~ 100 Am) than rotifers and *Artemia*.

The suitability of copepods as live feed for marine fish larvae is well established in fishes such as red-spotted grouper (Doi et al., 1997), mahi mahi (Kraul et al., 1993), haddock (Nanton and Castell, 1998), turbot (Stottrup and Norsker, 1997), grouper (Sunyoto et al., 1995; Nagano et al., 2000) and pipe fish (Payne et al., 1998). However their use in aquaculture is uncommon due to lack of a suitable mass culture technique. More recently, Stottrup (2000) studied the nutritive value and live-food suitability of marine copepods for fish larvae and discussed the advantages of using copepods in larviculture.

The length and weight were significantly lower in seabass larvae reared on *Artemia* nauplii. In addition, survival of seabass reared with *Artemia* nauplii showed greater variation when compared with that of seabass reared on copepods. The observed lower growth rate in

rotifer fed animals might be because the larvae were fed with unscreened *B. plicatilis* and thus had to spend more energy in search of smaller prey. The growth and survival of the fish larvae is also determined by the variety of physical and biological conditions of water used in larval rearing. In the present experiment those conditions were maintained optimum and even among the dietary treatments. The observed difference in growth and survival of seabass larvae in the present study could be attributed to the differences in the nutritional value of live foods used. The nutritional value of the food, particularly the quantity and quality of $n-3$ HUFAs, has been shown to affect the growth and survival of seabass larvae (Rajkumar, 2003). Payne and Rippingale (2000) concluded that the low gains in length and weight in the sea horse, *Hippocampus subelongatus*, fed *Artemia* nauplii were due to its low content of EPA and DHA.

Investigations have indicated that DHA is necessary for the development of the nervous system, including the brain and retina in larval fishes (Mourente and Tocher, 1992). Estevez and Kanazawa (1996) demonstrated that the DHA content of the brain and retina in Japanese flounder (*Paralichthys olivaceus*) was lower in unpigmented fish than in pigmented fish. Dover sole (*Solea solea*) fed copepod rich in DHA resulted in increased pigmentation of the fish (Heath and Moore, 1997). However in the present study no observations were made on variation in pigmentation among dietary treatments.

Providing copepods in the early larval diet increased their stress resistance (Kraul et al., 1993) and decreased malpigmentation (Mc Evoy et al., 1998). Kraul et al. (1992) concluded that mahi mahi larvae fed with copepods survived well under stressful conditions. Munilla-Moran et al. (1990) suggest that the copepod *Eurytemora hirundoides* plays an important role in larval digestion as a source of exogenous digestive enzymes. Prey density is also reported to have a major effect on feeding behaviour and growth of fish larvae (Kailasam et al., 2002; Olivotto et al., 2006). Barlow et al. (1993) reported that the feeding rate of seabass larvae increased when zooplankton density was increased. In the present study, at most care was taken to maintain the prey density in the larval rearing tanks.

A plausible explanation for the recorded low growth and survival in seabass larvae fed rotifers and *Artemia* nauplii is that these prey organisms might provide inadequate nutrition for the larvae in terms of lipid and protein (Watanabe et al., 1978). Though the copepod *A. clausi* is poorer in EAA content, they are comparatively richer in protein and lipid constituents than *B. plicatilis* and *Artemia* nauplii, which could explain the improved growth and survival observed in *L. calcarifer* larvae. Dietary amino acids (both essential and non essential) obtained from hydrolysis of protein are important in larval fish, partly due to their high growth rates that implies high protein synthesis rates and partly since amino acids are important sources of energy for these early stages (Rønnestad et al., 1999; Aragão et al., 2004).

Observations of the scientist elsewhere (Payne and Rippingale, 2000; Stottrup, 2000) and experiences of the present study recognize the difficulties in providing a laboratory cultured copepod as a live feed to fish or crustacean larvae. The real achievement will be the maintenance of stock culture on a continuous basis. Schipp et al. (1999) developed a cost effective system which offers controlled hatchery production of *Acartia* spp. In the present study, stock culture was maintained only for 3 months with tedious effort. Because, this study have not used any high tech water filtration system or rearing facilities. On maintaining the stock culture of copepod, more attention was paid on water quality, feeding and thinning out of matured adults. With taking account these basics, in future copepod culture could be scaled up for commercial operations.

The present investigation revealed the nutritional value of calanoid copepod, *A. clausi,* compared with rotifers and *Artemia* nauplii, and thus, underlining its usefulness as a suitable live-food organism larval rearing of highly commercial *L. calcarifer.*

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