

Biochemical Profile of *Heliodiaptomus viduus*, *Sinodiaptomus (Rhinediaptomus) indicus*, and *Mesocyclops aspericornis* and their Dietary Evaluation for Postlarvae of *Macrobrachium rosenbergii*

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Safiullah Aman and Kareem Altaff (2004) Biochemical profile of *Heliodiaptomus viduus*, *Sinodiaptomus (Rhinediaptomus) indicus*, and *Mesocyclops aspericornis* and their dietary evaluation for postlarvae of *Macrobrachium rosenbergii*. *Zoological Studies* 43(2): 267-275. The biochemical profiles of a calanoid, *Sinodiaptomus (Rhinediaptomus) indicus*, and a cyclopoid, *Mesocyclops aspericornis*, from a natural pond were studied for a period of 1 yr. In *S. (R.) indicus*, moisture, protein, lipid, carbohydrate, ash, and amino acid contents were 81.1%, 68.1%, 8.9%, 19.11%, 3.2%, and 56.2%, respectively; while in *M. aspericornis*, the values of these parameters were 82.4%, 69.0%, 12.4%, 13.97%, 4.5%, and 62.9%, respectively. Fatty acid content was higher in *M. aspericornis* (102.38%) than in *S. (R.) indicus* (42.87%). Variations were observed with regard to biochemical components in different seasons. The biochemical profile of *S. (R.) indicus* and *M. aspericornis* cultured in medium fertilized with an equal mixture of yeast (Y), poultry waste (PW), cotton seed cake (CSC), gingly cake (GIN), and ground nut cake (GNC), was comparable with that of copepods from natural sources. Higher values of the biochemical profile, survival, and growth were recorded in postlarvae of *M. rosenbergii* fed on a mixture of *S. (R.) indicus* and *M. aspericornis* than those fed on individual copepods or on *Artemia nauplii*. The amino acid content of postlarvae of *M. rosenbergii* fed on *M. aspericornis* was higher (71.83%) than that of postlarvae fed on *S. (R.) indicus* (46.15%) and a mixture of *M. aspericornis* and *S. (R.) indicus* (34.48%). The nutritional importance of copepods in aquaculture is discussed. <http://www.sinica.edu.tw/zool/zoolstud/43.2/267.pdf>

Key words: Freshwater copepods, Live food, Feeding experiments.

Copepods constitute an important component of the food chain in aquatic systems. The nutritional quality of copepods is accepted to be highly satisfactory for larvae of prawn and finfish species. Biochemical studies have shown that copepods are rich in proteins, lipids, essential amino acids (EAAs), and essential fatty acids (EFAs) which can provide enhanced reproduction of broodstock, augmented growth, immune stimulation, and color enhancement in prawns and fishes (Watanabe et al. 1983, Altaff and Chandran 1989, Safiullah 2001).

Copepods show wide occurrence from wild sources throughout the year (Rajendran 1973, Dharani 1998). However, fluctuations in quality

and quantity and drawbacks of collecting methods are major problems for their commercial utilization. In view of the growing needs for the production of large quantities of larval shrimp/fish for aquacultural practices, the culture of copepods has been attempted (Stöttrup et al. 1986, Vilela 1992, Tawfiq et al. 1997, Zehra 2000). Copepods constitute important live food in the rearing of larvae of fishes (Hussain and Higuchi 1980, Kraul et al. 1991, Toledo et al. 1997, Doi et al. 1997). Many authors have reported the utilization of copepods from wild and cultured sources for higher yields of prawns in ponds (Anderson et al. 1987, D'Abramo and Sheen 1991, Collins 1999). Studies on the comparative biochemistry of different copepods and

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their utilization as food for the freshwater prawn, *M. rosenbergii*, postlarvae is limited. Therefore, the current study focused on variations in the biochemical composition of laboratory-cultured copepods and copepods from natural sources during different seasons as well as the biochemical composition of *M. rosenbergii* postlarvae fed on these copepods.

MATERIALS AND METHODS

Collection and identification of copepods from wild sources

Copepods were collected from Nanganallur Pond, Chennai, from Jan. to Dec. 1998, using a plankton net (150- μ m mesh size) by towing at a depth of 1 m. Collections were made between 06:00 and 07:00, and samples were transported to a wet laboratory. Copepods were sorted from the sample with the help of a 200~500 μ m mesh filter and were maintained in concrete tanks. Identification was carried out following the taxonomic descriptions of Edmondson (1959), Rajendran (1973), Rangareddy (1994), and Dussart and Defaye (1995).

Culture of copepods

Mass culture of copepods was achieved using different organic ingredients like yeast (Y), cotton seed cake (CSC), gingly cake (GIN), ground nut cake (GNC), and poultry waste (PW) in equal proportions prepared at 250 ppm. This medium was added to 500 L of aerated, filtered fresh water. Laboratory-reared copepods of *M. aspericornis* and *S. (R.) indicus* were separately inoculated (25 individuals L⁻¹) on the 3rd day and then harvested after 13 d. A continuous culture was maintained by fertilizing the culture tanks for 45 d.

Feeding experiments

Macrobrachium rosenbergii postlarvae were individually reared with *S. (R.) indicus* and *M. aspericornis* separately and with their combination in equal weight from a wild source as test feed and with *Artemia nauplii* (GSL strain) as the control feed. Triplicate experiments were conducted for 45 d in circular concrete tanks of 50-L capacity (45 x 40 cm), and 300 acclimatized healthy postlarvae were randomly introduced into each tank. The total length and weight of postlarvae were mea-

sured.

Formalin-treated (5 ppm) control and test feed was given 3 times a day (08:00, 14:00, and 20:00) at the rate of 10% body weight of *M. rosenbergii* postlarvae. During the experimental period, the parameters of pH, salinity, and temperature were recorded, and 50% of the water was exchanged daily.

Biochemical analysis

Copepods from cultured and natural sources as well as pre- and post-experimental *M. rosenbergii* postlarvae were weighed, and the moisture content was determined (AOAC 1995). Lyophilized samples were analyzed for protein by estimating the nitrogen content by the micro-Kjeldahl method (Hawk 1954). The amino acid composition was determined using an automatic analyzer (Shimadzu, high performance liquid chromatography LC 4A) using a single column and a sodium buffer system (Yamamoto et al. 1994). The lipid content was estimated following the modified procedure of Bligh and Dyer (1959). Methyl esters of fatty acid mixtures were quantified using gas chromatography (Hewlett Packard model 5890) following AOAC (1995) procedures. The carbohydrate content was determined by phenol sulfuric acid reagent (Dubois et al. 1956), and the ash content was determined at 550°C (AOAC 1995).

Statistical analysis

Data on the biochemical composition of wild and laboratory-cultured copepods as well as experimental animals were analyzed statistically using one-way of analysis of variance (ANOVA) and Duncan's multiple range (DMR) test (Snedecor and Cochran 1980).

RESULTS

Copepods of Nanganallur Pond include *Heliodiaptomus viduus*, *S. (R.) indicus*, and *M. aspericornis*, and the results indicated that the latter 2 species were more dominant than the former species. The biochemical composition of *M. aspericornis* and *S. (R.) indicus* from Jan.~Dec. 1998 showed variations (Figs. 1, 2). The protein content was found to be highest in the cyclopoid during the months of Aug., Sept. Oct., and Feb. (71.5%~74.7%), low concentrations of protein were recorded during January (62.36% \pm 1.69%)

and June ($62.65\% \pm 5.86\%$), and moderate levels were observed during other months ($68.27\% \sim 69.0\%$). ANOVA showed that recorded protein values significantly differed during Oct. and Apr. compared to other months ($p < 0.05$). Lipid contents were low ($7.5\% \sim 8.83\%$) in the cyclopoid during Apr., May, Aug., Nov., and Dec. (Fig. 1). The highest lipid levels ($17.33\% \sim 20.76\%$) were recorded during Jan., June, and July, whereas moderate levels were recorded during Feb., Mar., and Oct. ($9.47\% \sim 11.32\%$). However, one-way ANOVA showed that these values did not significantly differ ($p > 0.05$). Nitrogen free-extract levels were low ($2.65\% \sim 4.12\%$) during Aug. to Oct. and high ($13.01\% \sim 18.65\%$) in Dec., Apr., and May, but during other months they were moderate ($7.40\% \sim 11.16\%$). ANOVA also indicated that the nitrogen free-extract values were significant ($p < 0.05$). The ash content was lowest (1.40%) in May, and highest (8.10%) in Oct. Overall, ANOVA for ash levels was significant. Values for fiber and moisture did not significantly differ.

Protein values of *S. (R.) indicus* were low in Jan. ($63.03\% \pm 2.51\%$), high in Sept. ($71.11\% \pm 5.71\%$), and moderate during other months ($66.47\% \sim 69.96\%$) (Fig. 2). However, these values

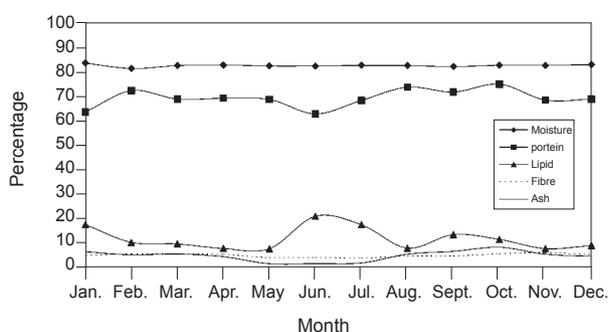


Fig. 1. Seasonal variations in biochemical composition of *M. aspericornis* during 1998.

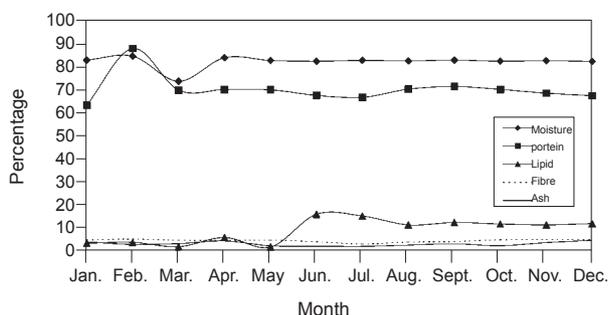


Fig. 2. Seasonal variations in biochemical composition of *S.(R.) indicus* during 1998.

did not significantly differ ($p > 0.05$). Moderate lipid levels ($11.36\% \pm 4.38\%$) were observed from Aug. to Dec., whereas during June and July, higher lipid levels ($15.95\% \pm 4.54\%$ and $15.19\% \pm 0.97\%$, respectively) were recorded. The low levels of lipids ($1.80\% \sim 5.94\%$) during Jan. to May significantly differed from values in other months ($p < 0.05$). The nitrogen free-extract levels during Jan. to May ($15.16\% \sim 24.54\%$) were double those of June to Dec. ($9.15\% \sim 13.12\%$). ANOVA also indicated that the nitrogen free extract during June to Dec. did not significantly differ, but values were significant in the remaining months at the $p < 0.05$ level. Both the ash ($2.18\% \sim 2.33\%$) and fiber contents ($3.94\% \sim 4.10\%$) were low during May, June, and July, but higher levels of ash ($3.73\% \sim 4.70\%$) and fiber ($5.0\% \sim 5.1\%$) were recorded during Nov. and Dec. (Fig. 2). *M. aspericornis* contained a higher amino acid proportion as protein (62.88%) and AA% as dry matter (43.66%) than *S. (R.) indicus*, in which AA% as protein and AA% as dry matter were 56.21% and 39.43% , respectively (Table 1). Similarly, the fatty acid content was higher (102.38 mg g^{-1}) in *M. aspericornis* compared to *S. (R.) indicus* (42.87 mg.g^{-1}) (Table 2). The levels of tridecanoic acid, palmitic acid, stearic acid, arachidonic acid, cis linoleic acid, and arachidonic acid were also higher in *M. aspericornis* than in *S. (R.) indicus*. However, levels of capric acid and oleic acid were higher in *S. (R.) indicus* (at 13.55 and 2.68 mg g^{-1} , respectively).

A higher biomass of copepods was obtained after 21 d of culture. The maximum populations of *M. aspericornis* and *S. (R.) indicus* recorded were 625 and 250 individuals ($\text{ind.} \text{ L}^{-1}$), respectively. Cultured *S. (R.) indicus* contained $65.14\% \pm 0.98\%$ of protein, $6.47\% \pm 0.51\%$ of lipids, and $23.14\% \pm 1.97\%$ of carbohydrates; in the case of *M. aspericornis*, the protein, lipid, carbohydrate, and ash contents were found to be $64.48\% \pm 1.21\%$, $6.27\% \pm 1.25\%$, $24.70\% \pm 0.7\%$, and $2.91\% \pm 1.23\%$, respectively (Table 3). *M. aspericornis* showed a quantitatively higher amino acid content than the calanoid *S. (R.) indicus*. The AA% as dry matter and AA% as protein of *M. aspericornis* were almost 3 times higher (26.01% and 45.34%) than those of *S. (R.) indicus* (9.87% and 17.22%), respectively (Table 1). The levels of methionine, arginine, and lysine were found to be higher in *M. aspericornis* (0.936% , 2.28% , and 1.95%) than in *S. (R.) indicus* (0.19% , 0.65% , and 0.67%), respectively.

Postlarvae of *M. rosenbergii* fed with a mixture of cyclopoids and calanoids showed signifi-

Although these organisms are locally available, very few investigations have attempted to determine their nutritional quality and culture aspects due to the ready availability of *Artemia* cysts. The present study reveals the occurrence of 3 species of copepods, *M. aspericornis*, *S. (R.) indicus*, and

H. viduus. However, the density of zooplankton in these ponds was found to be identical with that of other tropical ponds (Battish 1992, Venkataraman 1992). Results of biochemical profiles of monthly samples of calanoids indicate variations in all components except moisture and fiber contents. The lipid content of cyclopoids was high (17.33%~20.77%) during Jan. and July 1998 and low (7.51%~8.83%) during Apr., May, Aug., and Nov. 1998. These results differ from those of Shamsudin (1994) who recorded a high lipid content in Sept. and a low content during the monsoon. The lower lipid content may be due to high caloric expenditures during spring and winter to maintain body temperature. The most important element of a viable diet is protein, and the biological value of dietary protein depends on its EAA composition (Kanazawa and Teshima 1981). Levels of phenylalanine of *S. (R.) indicus* (2.65%) and *M. aspericornis* (2.37%) may be sufficient for protein synthesis and other physiological functions, a finding which supports results of an earlier study by Nose (1979).

Fatty acids play a vital role in maintaining structural and functional integrity of fish/prawn cell membranes. Zooplankton contain high levels of arachidonic acid which help in the growth and survival of larvae as documented by Bell et al. (1995) and Sargent et al. (1995). The cislinolic acid content of *M. aspericornis* was found to be high. Chanmugam et al. (1983) also reported high linoleic acid contents in freshwater species. Fatty acid analysis indicated that *M. aspericornis* has higher total saturated (61.24 mg g⁻¹) and unsaturated fatty acid (41.15 mg g⁻¹) contents than does *S. (R.) indicus*. Fatty acids like heptadecanoic acid, caprylic acid, and capric acid were not recorded in *M. aspericornis*, while *S. (R.) indicus* lacked arachidonic and behenic acids.

The maximum population of *S. (R.) indicus* recorded was 250 ind. L⁻¹ in medium containing an

Table 2. Fatty acid composition of live food organisms from a wild source (mg g⁻¹ of lipid)

Fatty acid	<i>Sinodiaptomus (R.) indicus</i>	<i>Mesocyclops aspericornis</i>
Saturated		
Hepatonic acid (7:0)	-	-
Caprylic acid (8:0)	-	-
Nonanoic acid (9:0)	-	1.64
Capric acid (10:0)	13.55	-
Undecanoic acid (11:0)	0.05	5.43
Lauric acid (12:0)	0.32	0.15
Tridecanoic acid (13:0)	0.32	14.46
Myresticacid (14:0)	0.74	7.00
Pentadecanoic acid (15:0)	0.03	0.09
Palmitic acid (16:0)	20.91	19.51
Heptadecanoic acid (17:0)	0.22	-
Stearic acid (18:0)	0.16	2.77
Nondecanoic acid (19:0)	-	-
Arachidonic acid (20:0)	3.74	10.09
Heneicosanoic acid (21:0)	-	-
Behenic acid (22:0)	-	0.11
Tricosanoic acid (23:0)	-	-
Lignoceric acid (21:0)	-	-
Total	40.04	61.25
Unsaturated		
Palmitoleic acid (16:1)	-	-
Oleic acid (18:1 n-9)	2.68	2.42
Cis linoleic acid (18:2 n-6)	0.15	4.18
Linolenic acid (18:3 n-3)	-	-
Arachidonic acid (20:4 n-3)	-	34.55
Total	2.83	41.15
Total (saturated + unsaturated)	42.87	102.40

Table 3. Biochemical compositions of live food organisms from wild and cultured sources

Live food organisms	Wild source		Cultured source	
	<i>Sinodiaptomus (R.) indicus</i>	<i>Mesocyclops aspericornis</i>	<i>S. (R.) indicus</i>	<i>M. aspericornis</i>
Moisture (% wet weight)	83.23 ± 1.23	82.36 ± 0.00	80.97 ± 2.90	81.16 ± 2.00
Protein (%)	68.17 ± 0.82	71.01 ± 1.07	65.19 ± 0.98	64.49 ± 1.21
Lipid (%)	10.76 ± 1.72	11.82 ± 0.59	6.47 ± 0.51	6.20 ± 1.25
Carbohydrate (%)	19.11 ± 0.54	13.97 ± 0.90	23.14 ± 1.97	24.70 ± 0.70
Ash (%)	3.01 ± 0.91	4.70 ± 0.91	3.54 ± 1.30	2.91 ± 1.23

equal mixture of Y: PW: CSC: GIN: GNC at 250 ppm; a similar density was recorded by Dharani (1998). Similarly, *M. aspericornis* (625 ind. L⁻¹) was also recorded in this medium. Zehra (2000) found oil cake-fertilized medium to be suitable for raising maximum populations of *M. aspericornis*.

The calanoids and cyclopoids of both laboratory-cultured and wild sources showed similar bio-

chemical compositions. Likewise, earlier reports on biochemical compositions of copepods support the present findings (Watanabe et al. 1983, Altaff and Chandran 1989, Dong et al. 1993). The levels of some of the EAAs such as methionine and histidine were low in copepods (0.33% and 0.24%, respectively), similar results to those reported by Sorgeloss and Lavens (1996).

M. aspericornis and *S. (R.) indicus* met the requirements of *M. rosenbergii* larvae with regard to fatty acid content as live food organisms; in particular, palmitic acid in both *M. aspericornis* (19.50 mg g⁻¹) and *S. (R.) indicus* (20.01 mg g⁻¹) was utilized by *M. rosenbergii* larvae to synthesize saturated and unsaturated fatty acids as reported by Kanazawa et al. (1979) and Kattner et al. (1981).

Feeding experiments with *M. rosenbergii* larvae using a mixture of *S. (R.) indicus* and *M. aspericornis* showed better survival and growth than with individual copepods or even with *Artemia* nauplii. This may have been due to the higher levels of required EFAs (n - 3 and n - 6 families) in the mixed *M. aspericornis* and *S. (R.) indicus* diet as documented by earlier studies (Fujita 1979, Coyle et al. 1996, Tidwell et al. 1997). *Artemia* nauplii-fed on animals showed lower growth (79.08 ± 1.34 mg) than those fed on other food types (Fig. 3),

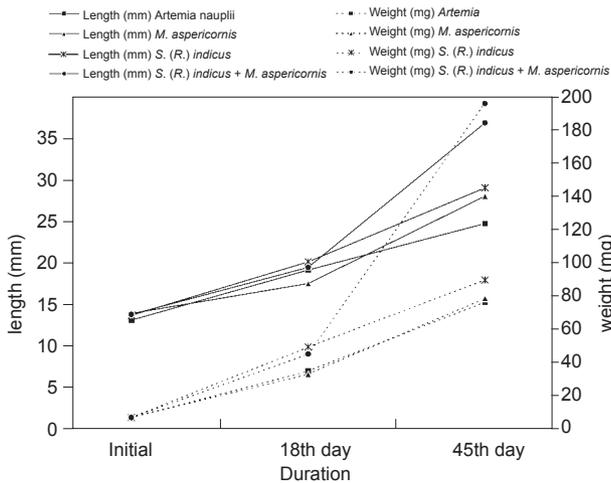


Fig. 3. Length and weight of *M. rosenbergii* post larvae fed on wild live food organisms.

Table 4. Amino acid composition (% as dry matter and % as protein) of *M. rosenbergii* postlarvae fed on live food organisms from wild and cultured sources (g 100 g⁻¹ of crude protein)

Amino acid	Initial		Cyclopoid (<i>M. aspericornis</i>)		Calanoid (<i>S. (R.) indicus</i>)		Copepods (<i>M. aspericornis</i> + <i>S. (R.) indicus</i>)	
	AA % dry matter	AA % as protein	AA % dry matter	AA % as protein	AA % dry matter	AA % as protein	AA % dry matter	AA % as protein
Arg	2.35	3.95	2.65	4.63	1.91	3.07	1.64	2.01
His	0.49	0.83	0.53	0.92	0.44	0.7	0.56	0.69
Ilu	1.57	2.64	1.76	3.08	1.08	1.73	0.92	1.19
Leu	2.68	4.51	2.77	4.84	2.29	3.68	1.86	2.28
Lys	2.44	4.1	4	7	3.36	5.39	3.51	4.3
Met	1.14	1.92	1.57	2.73	1.16	1.87	0.74	0.91
Phe	1.52	2.55	2.4	4.2	1.33	2.14	1.59	1.95
Thr	1.51	2.55	1.63	2.84	1.26	2.02	1.17	1.44
Val	1.97	3.32	2.35	4.11	1.57	2.52	1.16	1.43
Total EAA	15.67	26.37	19.66	34.35	14.4	23.12	13.15	16.2
Ala	2.36	4.27	3.15	5.5	2.41	3.87	2.51	3.08
Asp	3.05	5.14	3.55	6.22	2.36	3.79	2.28	2.79
Glu	5.6	9.43	6.38	7.14	4.32	6.92	4.13	5.1
Gly	2.27	3.82	3.21	5.61	2.74	4.38	2.81	3.44
Ser	1.6	2.7	1.77	3.09	1.48	2.37	1.36	1.67
Tyr	1.53	2.57	3.4	5.94	1.07	1.72	1.85	2.27
Total NEAA	16.41	27.93	21.46	33.5	14.38	23.05	14.94	18.35
Total	32.08	54.3	41.12	67.85	28.78	46.17	28.09	34.55

which is in accordance with results of an earlier study by Kitajima (1978). Decreased growth of postlarvae may be due to the reduced nutritional value of *Artemia* nauplii to *M. rosenbergii* larvae. The mixture of cyclopoids and calanoids supplies all required nutrients, as evidenced by their biochemical and amino acid contents. Many earlier investigations also recorded higher survival and weight gain in postlarvae of *M. rosenbergii* when fed on zooplankton from a wild source than an artificial diet (Brown et al. 1992, Collins 1999, Paulraj and Altaff 1999). Rearing of early larvae of groupers with copepodid nauplii as food showed improved growth and survival (Doi et al. 1997). Further, Toledo et al. (1999) indicated that *Epinephelus coioides* showed a higher preference for copepod nauplii than for rotifers. Higher survival and faster growth of larvae of *E. coioides* were reported when they were fed on nauplii of

calanoid copepods such as *Pseudodiaptomus annandalei* and *Acartia testuoidensis* along with rotifers (Toledo et al. 1999).

Furthermore, these copepods feed on algae, which contain large amounts of free amino acids, and it is believed that amino acid release could be responsible for trypsin stimulation in postlarvae (Admiral et al. 1986). Although higher AA% as protein (71.83%) was recorded in *M. rosenbergii* which fed on *M. aspericornis*, the weight gain was less in postlarvae (81.34 ± 1.99 mg). This may have been due to the excess free amino acids causing a depressive effect leading to a reduction in weight of the animal as reported by Ravi and Devaraj (1991) in *Catla catla*.

Lower levels of lysine and arginine in *S. (R.) indicus* (3.36% and 1.07%) than in *M. aspericornis* may have been responsible for the reduction in survival rate of *M. rosenbergii* fed on *M. aspericornis* collected from a wild source; similar observations were also reported by Keembiychetty and Galtin (1992). A reduction in growth rate with increasing levels of methionine in *M. aspericornis* indicates a depressive effect of excess free methionine, which was also reported earlier (Ravi and Devraj 1991).

Analysis of the biochemical composition of copepods (from cultured and wild sources) and results of feeding experiments suggest that copepods can serve as good live food for postlarvae of *M. rosenbergii*. If these copepods are used instead of prepared feed (egg custard/artificial pellet feed) in *M. rosenbergii* hatcheries from the postlarvae stage (PL 1) to the stocking stage (PL 15~12), then seed quality can be improved.

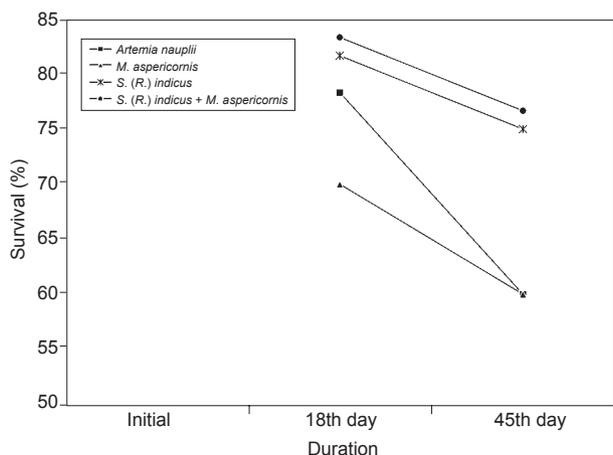


Fig. 4. Survival of of *M. rosenbergii* post larvae fed on wild live food organisms.

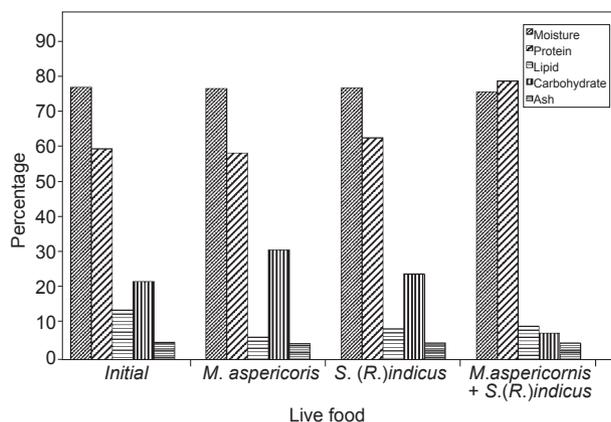


Fig. 5. Biochemical composition of *M. rosenbergii* post larvae fed on wild live food organisms.

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