Does phytoplankton play a role in the nutrition of the larvae of the prawn, *Macrobrachium rosenbergii* (De Man) ?

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Abstract

Two schools of thought exist concerning the optimum conditions for rearing larvae of the giant freshwater prawn, *Macrobrachium rosenbergii*, one in which larvae are reared in phytoplankton-rich (or "green") water and the other in which clear water is used. Although phytoplankton cells have been observed in the gut of the larvae, the ability of larvae to digest and assimilate algal cells has not been demonstrated. Several workers have shown that lipids can provide up to 95% of energy requirements of prawn larvae and it seems likely, therefore, that any nutritional role of the phytoplankton would be reflected in their lipid metabolism. This was investigated by comparing the lipid composition of larvae reared in green and in clear water, particular attention being given to fatty acid composition. No significant differences were found and it was concluded that phytoplankton contributed little to larval energy metabolism.

Introduction

Two types of rearing systems have been used for the larvae of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man). In the "green-water" technique, larvae are reared in phytoplankton-rich water, whereas in the "clear-water" technique, no phytoplankton are added to the water. The "green-water" system, which many workers claim improves larval growth rates (e.g. Manzi et al., 1977), was first described by Fujimura (1966) and Fujimura and Okamota (1972). This system utilises well-aerated, static water tanks which are supplemented with dense phytoplankton cultures. Cell density is about 750 000 to 1 500 000 cells·mℓ^{-1} and *Chlorella* spp. generally dominate (New and Singholka, 1985; New, 1990). In contrast to this, the larval culture method developed by the AQUACOP team (AQUACOP, 1977; 1979) and favoured by New (1990) and Daniels et al. (1992) utilises a closed, recirculating "clear-water" system where water quality is maintained with aeration and biofilters.

When used in cultures of molluscan larvae, phytoplankton cells are probably filtered by the larvae and used directly as food. In the case of crustacean larvae, however, the role of the phytoplankton is less obvious. Various advantages of phytoplankton supplementation in cultures of penaeid prawns have been reported (Cook and Murphy, 1969; Mock and Murphy, 1971; Meyers, 1971), and Emmerson (1980) stated that larval development and growth of *Penaeus indicus* was positively correlated to the ingestion rate of algal cells. Fujimura and Okamoto (1972) reported that in larviculture of *M. rosenbergii*, algal supplementation resulted in better survival rates and faster larval growth.

In larval culture systems where algal supplementation is used, the precise role of the algae has not been conclusively demonstrated. Although Maddox and Manzi (1976) were able to show that the algal cells were actually ingested by the larvae and Cohen et al. (1976) concluded that algal supplements enhanced growth, they were not able to demonstrate that the larvae derived any direct nutritional benefit from them. They suggested that algae could enhance growth indirectly by removing toxic metabolites, such as ammonia, from the rearing tanks. Maddox and Manzi (1976) could not, however, demonstrate any correlation between the presence of algae and the levels of nitrate, nitrite or ammonia.

Several workers have investigated the possibility that algae could contribute indirectly to prawn nutrition by serving as food for the *Artemia* nauplii which form the main food item for cultured prawn larvae. The nutritional value of *Artemia* nauplii has been shown to be determined partly by their content of n-3 highly unsaturated fatty acids (HUFA) (Watanabe et al., 1978, 1980; Leger et al. 1986; Navarro and Amat, 1992). Lavens et al. (1989) found that it was possible to manipulate the fatty acid profiles of *Artemia* cysts and nauplii by means of dry diets administered to the adults. Equally important was the finding of Navarro and Amat (1992) who were able to demonstrate that the fatty acid profiles of *Artemia* cysts were affected by the phytoplankton upon which the parent adults had been fed.

The objective of the present study was to determine whether the presence of phytoplankton in the culture medium of *Macrobrachium* larvae affected their growth, particular attention being given to the lipid and fatty acid metabolism of the larvae.

Materials and methods

Larval rearing

Eggs and larvae were obtained from the Camaron Hatchery in Mauritius where the normal practice is to rear larvae in the "green-water" system (Thompson, 1980). Adult female prawns were transferred from fresh-water broodstock ponds into brackish water tanks where maturation and hatching were induced. After hatching, the larvae were transferred to 24 m^3 rearing tanks where they went through the 11 larval stages, finally metamorphosing into post-larvae (PL) after about 45 d. Classification of the larval stages was according to the system described by Ling (1969). Initial concentration of larvae in the rearing tanks was about 40 animals per litre and with the enormous number of larvae contained in each tank, removal of larvae for analysis had no significant affect on stocking density. There were no significant differences in the survival rates of larvae in any of the rearing tanks. Water was kept at a salinity of 12 to 15 mg·ℓ^{-1}, a pH of 7,5 to 8 and a temperature of 27 to 30°C.

For the purpose of this study, 2 of the 24 m^3 larval tanks were enriched with phytoplankton (Cultures 1 and 2), whilst in the third (Culture 3) the larvae were reared in phytoplankton-free, clear

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water. As the objective of this study was to compare larvae reared in phytoplankton-rich water with those reared in clear water in genuine farm conditions, the green water used was the natural green water used in the hatchery tanks. Thus, it contained a large selection of different phytoplankton species but, because species-specific differences in the phytoplankton were not relevant to this study, no attempt was made to accurately identify the phytoplankton species. It was noted, however, that in both cultures, *Chlorella* spp. dominated.

In all tanks, freshly hatched *Artemia* nauplii were added as food for the larvae, the number of Artemia being adjusted daily to ensure that all were consumed within 24 h. From Day 2 onwards, feeding was supplemented with small amounts of shredded tuna. Larvae were examined daily to determine whether phytoplankton cells had been ingested. Samples of larvae at each larval stage, from each tank, were removed for analysis on the first day that the particular stage appeared.

Biochemical analyses

Samples of 100 eggs from each of 5 adult females were washed in distilled water and dried overnight. Each of the five batches of eggs was weighed separately. One hundred larvae from each larval stage in each tank, were washed in distilled water, dried overnight and individually weighed. Additional triplicate samples of 100 eggs or larvae were washed in distilled water and freeze-dried and about 2 to 3 mg of the freeze-dried material was used for lipid and fatty acid analysis.

Lipids were extracted under nitrogen using the method of Bligh and Dyer (1959). Total and neutral lipid content was determined using the method described by Holland and Hannant (1973).

For fatty acid determination, the total lipid fraction was methylated to produce fatty acid methyl esters using Boran fluoride-methanol (Morrison and Smith, 1964). All operations were carried out under nitrogen. Gas chromatographic analysis was performed on a Hewlett Packard 5710A gas chromatograph and a HP 18740 capillary column control equipped with flame ionisation detector and operated with temperature programming from 90 to 250°C. Fatty acid methyl esters were identified by comparison with retention times of reference standards and peak areas were quantified using a Hewlett Packard 3352A data processor. It was not the objective of this study to characterise differences in fatty acid compositions of different phytoplankton species, but merely to investigate any possible correlation between the mean fatty acid composition of the whole phytoplankton culture and that of the larvae. Thus, the mean fatty acid compositions of the algal mixture of cultures 1 and 2 were determined, using the same methods as outlined above, on samples removed from the culture tanks at the beginning and at the end of the larval culture cycles. All fatty acid values are presented as percentages of the total fatty acid fraction. Mean values presented from replicate samples had coefficients of variance equal to or less than 5%.

To determine how long it took larvae in each treatment to metamorphose to the post-larval stage, 100 larvae from each tank were examined daily and the larval stage of each determined. The number of days taken to reach a stage where greater than 95% of the larvae had reached the PL stage was used as an indication of relative growth rates in the tanks.

All biochemical analyses were carried out on triplicate samples and, where appropriate, one-way analysis of variance (ANOVA) was used to determine significant differences between treatments.

Results

Phytoplankton cells were observed in the gut of the larvae from the LS2 stage onwards which coincided with the onset of feeding on Artemia nauplii.

The mean dry mass of eggs and of larvae, grown in the 3 culture conditions, is shown in Fig. 1. There was considerable reduction in mass between the eggs and the Stage 1 larvae, the eggs having a mean dry mass of 30,1 (± 2,1) µg whilst first stage larvae weighed

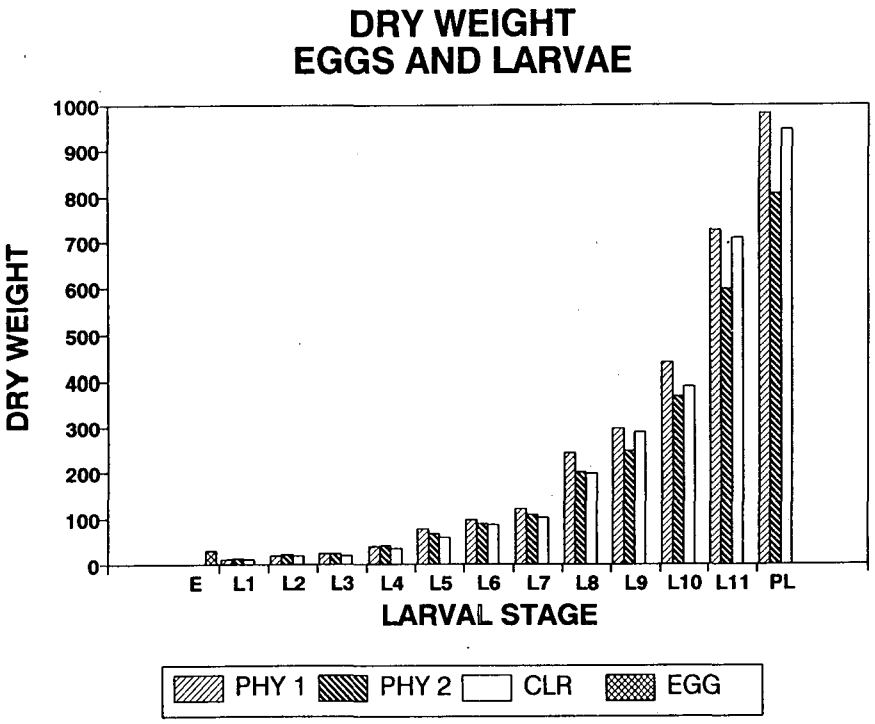

Figure 1
The mean dry mass of eggs (µg) and of larvae grown in the 3 conditions

only about 10,3 (± 0,9) µg. Although there were no significant differences in the mass attained by LS1 to LS10 larvae reared in the 3 culture conditions (ANOVA, p>0,05), there were significant differences between the dry masses of L11 larvae and PL grown in the 3 conditions (ANOVA, p < 0,05). In all 3 tanks, larvae metamorphosed into post-larvae after 45 to 46 d, there being no significant difference between treatments.

Figure 2 shows the total lipid content of the eggs and larval stages as a percentage of the total dry mass. The mean lipid content of eggs, just prior to hatching, was 14,6%, of LS1 larvae 19,0%, of LS2 larvae 13,9% and of LS3 larvae 7,9%. Lipid content increased to about 11,5% at the LS4 stage and then remained fairly constant at about 9% throughout larval development. The 3 larval cultures produced significant differences in total lipid content at stages LS5, LS6 and LS8 (ANOVA, p<0,05) but no significant differences were detected at any other stage.

A similar result was obtained for neutral lipids (Fig. 3). Neutral lipid content was gradually reduced over the first 3 larval stages but then remained relatively constant for the rest of larval development. Significant differences between the cultures were noted at stages LS5 and LS6 only (ANOVA, p<0,05).

Table 1 shows the fatty acid profiles (expressed as a percentage of the total fatty acid fraction) of the algae used in Cultures 1 and 2 and of the LS1 and post-larvae obtained from all 3 cultures. As expected, since the algal cultures were intentionally taken from different outdoor sources and contained a different array of algal species, the fatty acid profiles of the 2 algal cultures were quite different. Whilst differences in the saturated and mono-unsaturated acids were not very marked, the major differences occurred in the polyunsaturated acids. The 16:4 acids were absent from Culture 1 but made up 4,2% of the fatty acids in Culture 2. The 18:2n6 acids formed 12,25% in Culture 1 but only 5,17% in Culture 2 whilst the 20:5n3 acids formed only 0,51% in Culture 1 but 4,36% in Culture 2.

A number of interesting trends in fatty acid composition occurred during the development of the larvae. Taken as an average over the 3 larval cultures, total saturated acids decreased from about 41% in LS1 larvae to about 33% in PL. This decrease was accounted for particularly by the 14:0 and 16:0 acids. The 18:0 acids, on the other hand, increased from 7,5% in LS1 to about 10% in PL. The content of mono-unsaturated fatty acids remained fairly constant, at about 38%, throughout larval development. Polyunsaturated acid composition displayed a trend opposite to that of the saturated acids, increasing during larval development from about 20% at LS1 to about 29% at PL. This was particularly noticeable in the linolenic acids, 20:5n3 and 22:6n3. Although similar trends were shown in all 3 cultures, the polyunsaturated acids did seem to be more variable between cultures than the other fatty acids.

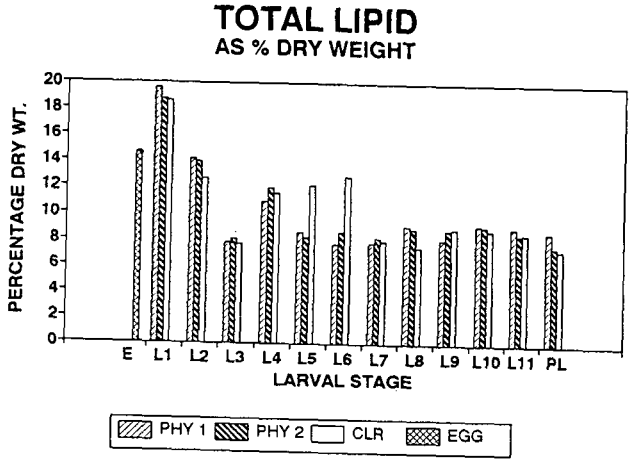

Figure 2
The total lipid content of eggs just prior to hatching and of larvae grown in the 3 conditions

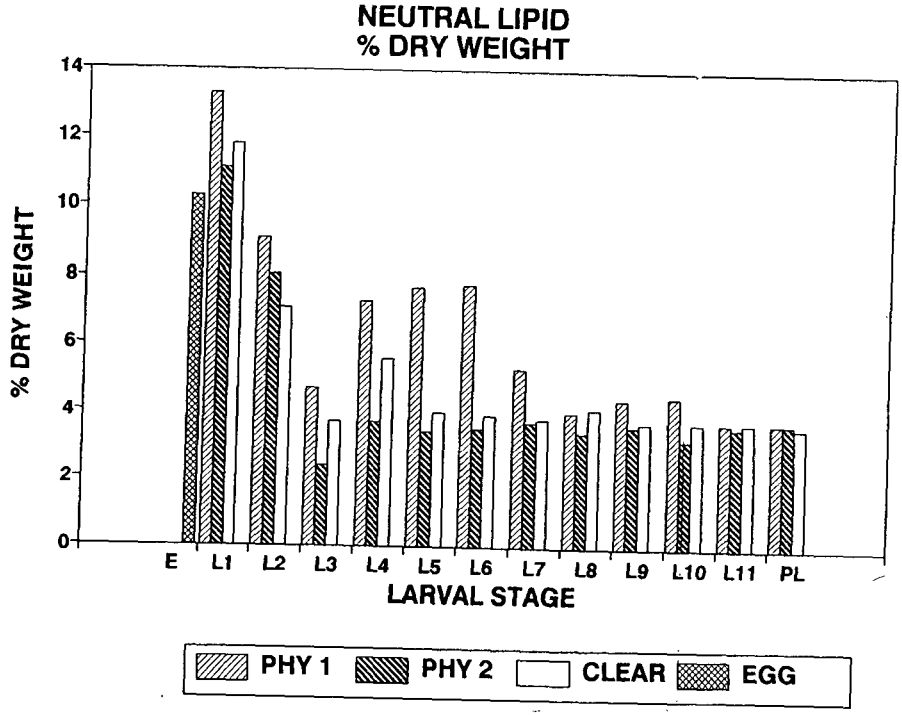

Figure 3
The neutral lipid content of eggs just prior to hatching and of larvae grown in the 3 conditions

TABLE 1
FATTY ACID PROFILES (% OF TOTAL FATTY ACID FRACTION) OF ALGAE IN CULTURES C1 AND C2 AND OF LS1 AND PL OBTAINED FROM THE 3 CULTURES

	Phytoplankton C1			Phytoplankton C2			Clearwater C3		
Fatty acid	**Algae**	**LS1**	**PL**	**Algae**	**LS1**	**PL**	**No Algae**	**LS1**	**PL**
C12:0	0,39	0,25	-	0,32	0,52	-	-	0,45	0,07
C13:0	-	-	-	-	-	-	-	-	-
C14:0	1,01	4,69	1,47	-	3,51	0,76	-	4,56	1,04
C15:0	-	0,7	0,72	-	0,57	-	-	0,53	1,26
C16:0	33,15	29,9	17,61	34,37	23,2	14,53	-	29,46	18,97
C17:0	0,38	1,25	2,32	2,3	0,71	2,22	-	0,77	2,86
C18:0	3,68	7,3	10,88	3,82	8,55	12,3	-	7,26	10,52
C19:0	-	0,14	-	-	-	-	-	0,11	-
C20:0	-	0,47	0,17	-	0,64	0,38	-	-	0,35
C22:0	0,7	-	-	0,8	0,47	0,26	-	-	0,84
C24:0	-	-	-	-	0,36	-	-	0,25	0,72
Total Sat	39,31	44,7	33,17	42,61	38,53	30,45	-	43,39	35,63
C14:1	-	-	-	-	-	-	-	-	-
C16:1n7+n9	1,49	7,02	2,91	-	6,48	3,57	-	6,6	2,51
C18:1n9	35,15	22,38	20,11	33,07	23,93	23,9	-	21,85	26,09
C18:1n7	2,67	5,86	9,95	2,77	5,84	7,85	-	5,93	8,37
C20:1	1,27	1,27	1,21	1,89	1,5	1,5	-	1,71	1,14
C22:1n11	0,41	1,06	1,78	0,43	0,69	1,3	-	1,07	0,41
C22:1n9	-	0,09	-	-	-	-	-	-	-
C22:1n7	-	0,05	-	-	-	-	-	-	-
C24:1	-	-	-	-	0,06	-	-	-	-
Total mono-unsat	41,4	37,73	35,96	37,16	38,44	38,12	-	37,16	38,52
C16:4n1	-	0,16	0,31	4,2	-	-	-	0,16	0,58
C16:3	1,73	0,14	-	-	-	-	-	-	-
C16:2	-	-	-	-	-	-	-	-	-
C18:4n3	4,43	0,15	0,57	4,54	-	0,76	-	0,5	-
C18:3n6	-	-	-	-	-	-	-	0,63	-
C18:2n6	12,25	5,13	3,7	5,17	6,31	3,46	-	5,05	2,94
C20:4n6	-	1,64	2,55	-	3,79	2,94	-	1,82	2,39
C20:5n3	0,51	5,4	11,99	4,36	7,14	11,21	-	5,73	10,81
C20:4n3	-	0,15	0,31	-	-	-	-	0,15	-
C21:5	-	0,09	-	-	-	0,4	-	-	0,25
C22:6n3	-	4,26	11,17	-	5,37	11,13	-	4,62	9,12
C22:5n3	0,4	0,36	0,26	-	0,74	0,42	-	0,64	0,32
Total polyunsat	19,32	17,84	30,86	18,27	23,35	30,32	-	19,4	26,41

Discussion

Dry mass did not differ significantly over larval stages LS1 to LS10 in the 3 cultures. Although there were significant differences in the mass attained by larvae in the 3 cultures at the LS11 and PL stages, these differences could not be attributed to the presence of phytoplankton because the mass of larvae in the clear-water culture was midway between that of the 2 phytoplankton cultures.

Although there was a significant mass loss associated with hatching of the eggs, it appears that little material was lost as lipids as the percentage total lipids and neutral lipids increased between the egg and LS1 stage. Total lipid and neutral lipid content fell sharply between LS1 and LS3 stages but began to increase again at LS4. Many workers have demonstrated the importance of lipids in egg and larval metabolism of marine organisms (Holland, 1978; Webb and Chu, 1983) and, as Moller (1978) showed that LS1 larvae do not feed, it seems likely that the decrease noted resulted from lipid utilisation at this stage. Although the larvae began feeding at the LS2 stage, lipid levels began to rise only at the LS4 stage. Baissac and Cook (1980) demonstrated the presence of lipid filled droplets in the carapace of LS1 larvae. These droplets disappeared by the end of the LS2 stage and they suggested that this represented utilisation of stored yolk lipids which could provide up to 95% of the energy requirement of the larvae.

Although significant differences between total and neutral lipid levels from the 3 larval cultures were noted for some of the larval stages, this could not be attributed to the presence of phytoplankton because in some cases levels were higher in phytoplankton cultures whilst in others they were higher in clear water. By the time the larvae reached the PL stage, no significant differences in lipid levels could be detected between the cultures.

Certain fatty acids, particularly the 20:5n3 and 22:6n3 acids, are known to be essential for marine animals (Bell et al., 1985; Watanabe, 1988) and Lavens et al. (1989) found that variations in the highly unsaturated fatty acid (HUFA) contents of *Artemia* cysts and nauplii could be due to variations in composition of their micro-algal diets. Navarro and Amat (1992) fed *Artemia* adults on 2 different algal diets and found that the cysts and nauplii produced by them varied in their HUFA compositions. In the present study, the fatty acid compositions of the 2 green-water algal cultures differed significantly but these differences were not convincingly reflected in the larvae grown in them, even though it was demonstrated that the algal cells were ingested by the larvae. For example, although 16:4n1 acids were absent from the C1 culture and made up 4,2% of the fatty acids in C2, the levels of these acids were similar in all 3 of the larval groups. In the case of the 18:2n6 acids there did appear to be a slight indication that the phytoplankton affected the fatty acid composition of the larvae. In C1, these acids formed 12,25% of the total fatty acids of the phytoplankton whilst in C2 they formed 5,17%. Significant differences were found in the content of 18:2n6 acids in PL from C1 (3,70%), C2 (3,46%) and C3 (2,94%) and it seems possible that these differences could result from different levels in the phytoplankton cultures.

The long-chain 20:5n3 and 22:6n3 acids have been reported to be essential for normal growth and development in the prawn *Penaeus japonicus* (Jones et al., 1979; Kanazawa et al., 1977) and in *M. rosenbergii* (Sandifer and Joseph, 1976). The level of 20:5n3 in C1 phytoplankton was 0,51% and in C2 phytoplankton was 4,36%. Levels in the larvae from all 3 cultures were very variable but averaged about 10 to 11%, showing no differences between the cultures. The 22:6n3 acids were absent from both phytoplankton cultures but were found in fairly high levels (9 to 11%) in all 3 sets of larvae.

The results of this study indicate that, although minor differences occurred in fatty acid content of larvae grown in different phytoplankton cultures, the presence of phytoplankton had little affect on the lipid or fatty acid metabolism of *Macrobrachium* larvae. Even though phytoplankton cells were ingested by the prawn larvae and could also have been consumed by the *Artemia* in the larval tanks, there was no evidence of a direct nutritional benefit to the prawn larvae. If, as some prawn farmers have suggested, green water is of benefit in the rearing of larval prawns, it is possible that such benefit may operate, as suggested by Cohen et al. (1976), through the removal of potentially toxic wastes, but the present results indicate that any such benefit is not reflected in the lipid metabolism of the larvae.

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