Lipid and Fatty Acid Composition and Energy Partitioning During Embryo Development in the Shrimp *Macrobrachium borellii*

Horacio Heras*, M.R. Gonzalez-Baró, and Ricardo J. Pollero

Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, (1900) La Plata, Argentina

ABSTRACT: Energy partitioning, composition of lipids and fatty acids, and their utilization by embryos were determined in the lecithotrophic shrimp *Macrobrachium borellii* during seven development stages. The biochemical composition at stage I is represented by lipids, proteins, and carbohydrates, with 29.3, 28.7, and 0.2% dry weight, respectively. The former two were identified as the major energy-providing components, contributing 131 and 60 cal/100 mg egg, dry weight, respectively. The overall conversion efficiency (CE) was 45.0% (calculated as percentage of vitelline energy transformed into embryonic tissues). Lipids were the most important energy reserve (CE 39.3%), followed by proteins (CE 57.1%), both being simultaneously utilized during development while carbohydrates were synthesized *de novo* (CE 587.5%). Variation in the lipid class composition of embryos and vitellus showed an accumulation of triacylglycerols (TAG) and phospholipids (PL) up to stage IV, a more active accumulation and selective utilization phase (stages V and VI), and a consumption and *de novo* synthesis period until hatching. Structural lipids (PL and cholesterol) and pigment astaxanthin were selectively conserved in embryos, but TAG, hydrocarbons, and esterified sterols were preferentially depleted. Monounsaturated fatty acids (FA) were the major group in TAG, whereas polyunsaturated FA (PUFA) were the major group in PL after organogenesis. Certain PUFA such as 22:6n-3 and 20:5n-3 were selectively accumulated in PL.

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Physiological energetics encompasses the study of gains and losses of energy and the efficiency of its transformation from the standpoint of the whole organism. Studies on the energetics during embryogenesis in invertebrates are difficult to compare because of the different methodologies employed and also because of the varied life histories of invertebrate species (1–5).

Conversion efficiency (CE) gives a rough estimate of the amount of each constituent used for growth and formation of energy reserves in the embryo and of the amount used for metabolism. In most cases CE is significantly higher for proteins than for lipids, a fact that is consistent with the assumption that during development amino acids are conserved to build up the structure of embryos, while lipids would serve as fuel for active cell division and differentiation (2).

In many aquatic invertebrates such as crustaceans, bivalves, cephalopods, and sea urchins, the amount of proteins and lipids in the egg is higher than carbohydrate, clearly showing that carbohydrate is not the major energy reserve for egg development (2,6–8).

Crustacean oviposited eggs have two compartments, the ooplasm and the vitellus or yolk, which are enclosed by an egg membrane, the chorion. The ooplasm contains mitochondria, cortical granules (glycoproteins), and lipid globules. The yolk is surounded by the vitelline sac which contains water, lipids, proteins, and also minor amounts of carotenoid pigments, carbohydrates, and free amino acids (9,10). Most crustacean eggs store some of the nutrients in the yolk in the form of a complex lipoprotein called lipovitelin or as lipid droplets scattered throughout the cytoplasm. Most studies made on crustacean eggs refer to whole egg composition and show that lipid is the major energy reserve, although there is no mention of species such as *Macrobrachium borellii, in* which the egg has an abbreviated development and hatches into a postlarval stage. There are also no available data on the transfer of yolk into decapod embryos from the biochemical point of view. For our study we selected the decapod *M. borellii,* which is an endemic South American lecithotrophic freshwater shrimp in Argentina, Paraguay, and Uruguay (approximately from 22 to 37°S and 53 to 60°W) (11). Females brood large, yolky eggs during late spring and summer in turbid, temperature water streams (November to February). The small clutches (50–60 eggs) are kept by females attached to the pleopods. Eggs take about 50 d to hatch at 25°C (11). Hatchlings are 5–6 mm long, translucent postlarvae of benthic habits and are omnivorous scavengers.

In the present work, we studied the energy partitioning and the lipid and fatty acid (FA) composition in *M. borellii* eggs from fertilization until hatching in order to identify the nutrient sources of eggs, and how they are utilized by embryos during development.

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*To whom correspondence should be addressed at INIBIOLP, Fac. Medicina, Universidad Nacional de La Plata, Calles 60 y 120, (1900) La Plata, Argentina. E-mail: h-heras@atlas.med.unlp.edu.ar

Abbreviations: BSA, bovine serum albumin; CE, conversion efficiency; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; HP-TLC, high-performance thin-layer chromatography; PL, polar lipid; PUFA, polyunsaturated fatty acid; SM, sphingomyelin; TAG, triacylglycerol; TLC, thin-layer chromatography.

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EXPERIMENTAL PROCEDURES

Sample collection. Eggs from *M. borellii* were sampled from ovigerous females collected during spring and summer (October to February) in the Zapata Stream (20 km southwest of La Plata, Argentina), which has turbid waters with temperatures ranging from 22 to 26°C. The females were taken to the laboratory and kept in dechlorinated tap water at room temperature until the eggs were removed for the experiments. Embryo size and development stages were checked in each egg brood using a stereoscopic microscope (Nikon SMZ-10, Tokyo, Japan). Seven developmental stages were therefore identified using major development events.

Stage I constitutes embryos from four-cell morula to gastrula with a highly viscous vitellus that takes up about 90% of the egg; in Stage II (10–18 d) vitellus fills up to 80% of the egg volume; in Stage III (18–26 d), embryos have incipient eyes visible and the vitellus fills up to 70%. At stage IV (26–35 d) the vitellus mass is divided into four lobules; in Stage V (36–40 d) the cuticle pigmentation becomes evident and embryo is under active organogenesis. At stage VI (40–46 d) blue from hemocyanin becomes visible and the vitellus has almost internalized into the embryo and represents about 20% of the egg volume; organisms in Stage VII (46–50 d) are 4–5 mm long with all the characteristics of postlarvae ready to hatch. In general, vitellus decreases its viscosity along with development.

Wet weight and moisture were obtained from egg masses of each developing stage and analyzed following a method from the International Association of Fish Meal Manufacturers (12). Samples were analyzed in triplicate and each replicate was composed of pools including eggs from 3 or 4 clutches.

Isolation of embryos and vitellus. Eggs were weighed; then egg shells, vitellus, and embryos were separated from each other. Embryos from stage I were virtually impossible to isolate free from vitellus due to its high viscosity and the size of the morula. Therefore, at this stage we studied the whole egg homogenate without the egg shells, which were separated by gentle centrifugation.

Stages II to IV: Embryos were first manually isolated from vitellus by breaking the vitelline sac under a stereoscopic microscope followed by the separation of embryo, egg shell, and vitellus using a Percoll® discontinuous density gradient (Pharmacia LKB, Uppsala, Sweden) with solutions of 100, 50, and 25% Percoll diluted with 75 mM NaCl. Samples were loaded onto the gradient and then centrifuged at 400 × *g* for 10 min (13). Thus, a shell-containing pellet was formed while the embryos were located in the interphase of the two Percoll solutions, and the yolk floated on top of the gradient.

Stage V and VI: Embryos were manually isolated from vitellus under a stereoscopic microscope and washed repeatedly with a solution of 75 mM NaCl. Stage VII embryos have already internalized the vitelline sac and therefore were analyzed as a whole egg homogenate.

Embryos were homogenized in a Potter-type homogenizer (Thomas Scientific, Swedesboro, NJ) using 0.02 M, pH 7.5 Tris-HCl buffer containing 2 mg/mL aprotinin (Trasylol, Mobay Chemical Co., New York). The ratio buffer/sample was kept at 3:1, vol/vol. All samples obtained were frozen at −70°C until analysis.

Protein, lipid, and carbohydrate determination. Proteins were determined by the method of Markwell *et al*. (14) using bovine serum albumin (BSA) as a standard. Samples and BSA were first digested with 1 N NaOH, 5:1 vol/vol, vortexed, and incubated overnight at 37°C.

Lipids were extracted with a chloroform/methanol mixture following the method of Bligh and Dyer (15), and total lipid concentrations were determined gravimetrically. Carbohydrates were determined following the spectrophotometric procedure of van Handel (16).

Lipid analysis. Eighty percent of each sample was employed for lipid analysis. Lipid class analysis was performed by thin-layer chromatography (TLC) on silica gel Chromarods (type S-III) with quantitation by flame-ionization detection using an Iatroscan TH-10, Mark III (Iatron Laboratories Inc., Tokyo, Japan) as described by Parrish and Ackman (17). The separation was conducted with a sequence of three different solvent systems according to Ackman and Heras (18). The first development was carried out for 45 min in hexane/ethyl acetate/diethyl ether/formic acid (91:6:3:1, by vol). Chromarods were dried, partially scanned to determine neutral lipids, and then developed in acetone for 15 min to quantify the carotenoid (astaxanthin) peak. Finally, the Chromarods were developed in chloroform/methanol/formic acid/water (50:30:4:2, by vol) for 60 min and completely scanned to reveal the different phospholipids. Tetracosanol was used as an internal standard, and quantitation was performed with calibration curves of authentic standards run under the same conditions. Lipids were also identified on HP-TLC plates (Merck, Darmstadt, Germany) developed with hexane/diethyl ether/acetic acid (80:20:1.5, by vol) for neutral lipids and chloroform/methanol/acetic acid/water (65:25:4:4, by vol) for polar lipids (PL). Esterified sterols and hydrocarbons coeluted using the first developing solvent system, although a qualitative separation performed on HP-TLC showed that esterified sterols were the major component of the unresolved peak. Standard lipids, iodine vapor, and specific reagents were used to identify lipid classes. Preparative HP-TLC of neutral lipids, as described above, was used to isolate PL and neutral lipids for fatty acid analysis and to isolate an egg carotenoid pigment, which was then employed as a standard for Chromarod calibration.

FA analysis. FA methyl esters from total lipids, triacylgycerols (TAG), and PL, were prepared with BF_3/MeOH according to the method of Morrison and Smith (19). The analyses were performed by gas chromatography using a Shimadzu 9A gas chromatograph (Tokyo, Japan) fitted with an Omegawax 250 fused-silica column, 30 m × 0.25 mm, with 0.25 μm phase (Supelco, Bellefonte, PA). Peaks were identified by comparing the retention times with those from a mixture of standard methyl esters.

Energy conversion factors. We employed the energy conversion factors described by Beningher (3) calculated for aquatic invertebrates: carbohydrates, 4.1 kcal/g or 17.2 kJ/g; proteins, 4.3 kcal/g or 17.9 kJ/g; and lipids, 7.9 kcal/g or 33.0 kJ/g.

Statistical analyses. Data collected from all experiments were analyzed by analysis of variance using Instat v.2.0 (GraphPad Software, San Diego, CA). Whenever significant differences among samples were found, a *post-hoc* Tukey's test was performed to identify the differing means. Results were considered significant at the 5% level.

RESULTS

Dry weight variations. The optimized methodology, employing Percoll gradients, allowed us to separate the vitellus from embryos and egg shells of *M. borellii*. Dry weight of whole eggs showed a steady decrease during development from 0.98 ± 0.05 mg/egg to 0.81 ± 0.08 mg/egg ($P < 0.05$). Morphological changes under the stereoscopic microscope were also evident in the 1.7–2.0 mm eggs, the vitellus being more condensed and reduced in size as the embryo developed, finally occupying about 20% of the egg volume at the last stage.

Energetic changes during development. Analysis of the composition of just-layed-egg vitellus showed that the major nutrients were lipids (29.3% dry weight) followed by proteins (28.7% dry weight) and carbohydrates (0.2% dry weight) (Fig. 1). From the biochemical composition of the developing eggs it was possible to calculate the equivalent calories depicted in Figure 2. The overall CE calculated as the percentage of vitellus total energy transformed into embryonic tissue energy was 45.5%. All reserves displayed significant changes from stages IV to V (Fig. 1), evidencing a net fall in the vitellus and an increase in the embryo content. The CE for lipids was 39.3, 57.1 for proteins, and 587.5% for carbohydrates.

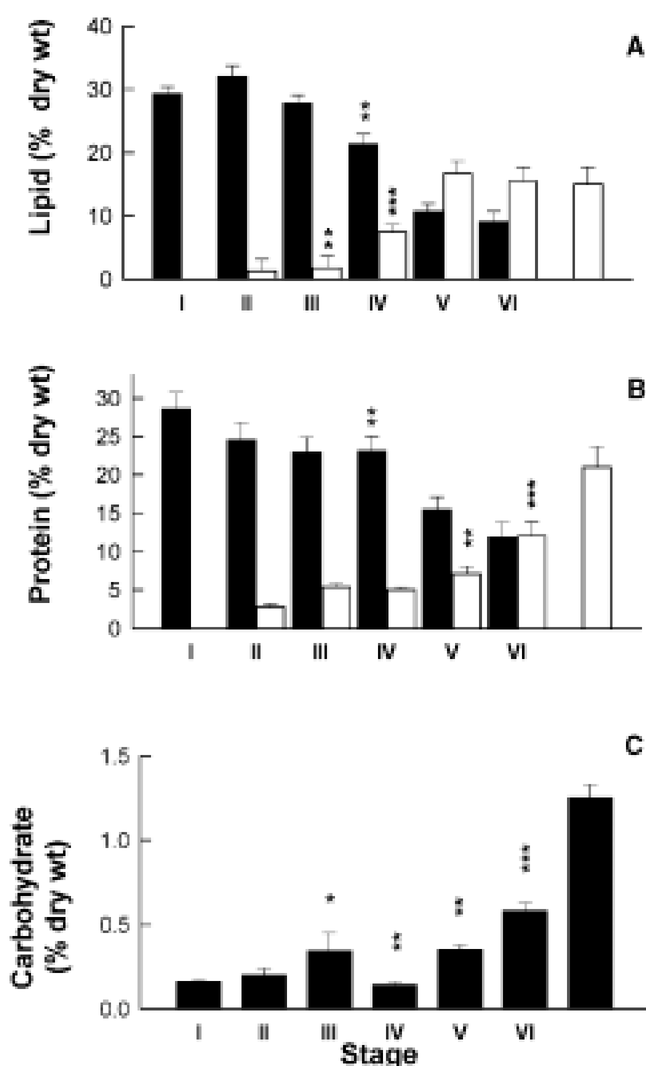

FIG. 1. Changes in (A) lipid, (B) protein, and (C) carbohydrate content in embryos (□) and vitellus (■) during development. Values are expressed as % dry weight of whole egg. Bars with different characters on top are significantly different from the next bar. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Changes in lipid and FA composition along development. In order to determine the role of lipids during development, each lipid class was studied separately in embryos and in yolk. PL and TAG were the most important lipids in vitelline fluid, representing more than 89% w/w at stage I (Table 1). TAG evidenced significant changes between stages IV and V ($P < 0.01$), decreasing to 33% of the whole lipids, a reduction of more than 30% compared to the percentage in the original vitellus total lipid (Table 1). On the other hand, sphingomyelin (SM) increased in the embryos ready to hatch. Phosphatidylcholine was the second-most important lipid during the whole development, changing its relative amount from 14 to 25% by weight but without any defined pattern. Phosphatidylethanolamine also showed significant changes in vitellus between stages IV and V. Therefore, at the end of development, the composition of the vitellus remaining in the hatched postlarvae is particularly enriched in esterified sterols, astaxanthin pigments, and SM at the expense of TAG. The changes observed at stage V yolk were accompanied by an increase of PL in embryos, followed by a significant increase in embryo cholesterol at stage VI (Table 2). The astaxanthin pigment was also taken up by embryos at stages VI and VII, mainly at the end of embryogenesis, accounting for 11% of the total lipids in embryos ready to hatch as postlarvae. Free FA were always found in small amounts in the vitellus as well as in embryos, except in the yolk at stage V where it was 3.3% w/w. Esterified sterols, which cochromatographed with hydrocarbons, did not show any changes in embryos, although they were selectively conserved in vitellus, particularly at the end of development ($P < 0.01$). Nevertheless, they were always minor components.

Regardless of variations according to lipid class or development stage analyzed, the major FA were 16:0, 18:0, 16:1n-7 18:1n-9, 18:1n-7, 18:2n-6 18:3n-3, 20:4n-6, and 20:5n-3 (Table 3). Monounsaturated FA were the major group in the TAG fraction at all stages. Polyunsaturated fatty acids

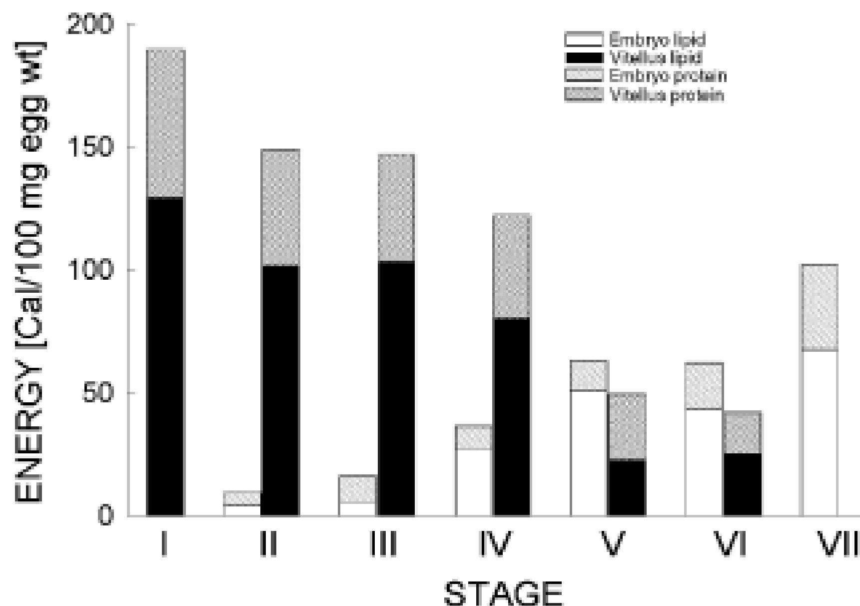

FIG. 2. Total caloric changes in embryos and vitellus during development. Values are expressed as total cal/100 mg egg (wet weight) for each stage, and they were calculated by adding protein and lipid calories. Carbohydrates represented less than 1.5% of the calories, and they were omitted for calculations.

(PUFA), on the other hand, were the main FA group in PL after embryos reached stage V; this increase was paralleled by a decrease in PL saturated and monounsaturated FA. The PUFA increment in PL fraction continued until hatching, reaching a value of 43.6% of total FA. Moreover, some PUFA such as 22:6n-3 (docosahexaenoic acid; DHA) and 20:5n-3 (eicosapentaenoic acid; EPA) were selectively accumulated in PL during development while 18:0 and 16:3 showed a clear association with PL throughout development.

Changes in protein and carbohydrate composition along development. Protein reserves in *M. borellii* yolk represent about 29% dry weight at stage I. Lipid and proteins were catabolized in equal proportion for energy until stage VII, where a much greater proportion of energy appears to have been derived from the catabolism of lipids and a greater proportion of the protein retained for converting into body components (Figs. 1 and 2). Therefore, yolk protein values fell, reaching a minimum of 14% at stage VII. The protein uptake by embryos was rather constant from stage II on, and did not increase until the end of embryogenesis. Embryo protein reached a maximum of 24% dry weight before hatching.

Compared to protein and lipid, the total carbohydrate content was low throughout development and increased strikingly at the end of embryogenesis, but it never exceeded 1.2% dry weight (Fig. 1).

DISCUSSION

Eggs in decapod carideans are surrounded by a chitin membrane that confers impermeability. However, it was interesting to note that *M. borellii* eggs increased their water content with development, probably due to a variation in inside os-

TABLE 1
Lipid Class Composition[a] of Vitellus During *Macrobrachium borellii* Development

Class[b]	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI
SM	6.10 ± 0.42	5.05 ± 0.56	4.03 ± 0.25	3.40 ± 0.27	6.23 ± 0.13*	9.09 ± 0.48*
PC	14.72 ± 0.77	25.00 ± 1.14*	24.94 ± 0.89	21.01 ± 0.53	24.84 ± 1.07*	16.48 ± 0.84*
PE	13.28 ± 1.06	9.90 ± 3.63	13.69 ± 1.00	16.98 ± 0.70	14.13 ± 0.31*	13.59 ± 0.82
ASX	6.42 ± 0.21	6.86 ± 0.65	6.21 ± 0.12	5.72 ± 0.54	11.15 ± 0.23*	12.44 ± 3.95
Cho	4.05 ± 0.23	4.49 ± 0.26	3.65 ± 0.20	3.16 ± 0.07	5.61 ± 0.33*	8.51 ± 0.29*
FFA	Trace	Trace	Trace	0.53 ± 0.18	3.30 ± 0.06*	1.44 ± 0.12
TAG	55.40 ± 2.05	48.63 ± 1.59	47.43 ± 1.18	48.99 ± 1.95	32.95 ± 2.26*	37.38 ± 1.86
HC+ SS	Trace	Trace	Trace	0.21 ± 0.05	1.78 ± 1.41*	1.06 ± 0.45

[a]Values (mg lipid/g egg wet weight) are the mean of triplicate analyses ± SD. *Significant changes compared with the preceding stage ($P < 0.05$).
[b]HC + SS, hydrocarbons + esterified sterols; TAG, triacylglycerols; FFA, free fatty acids; ST, free sterols; ASX, astaxanthin; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; Cho, cholesterol.

TABLE 2
Changes in Lipid Class Composition[a] of *M. borellii* Embryos During Development

	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII
SM	2.65 ± 0.12	3.23 ± 0.06	2.95 ± 0.23	2.39 ± 0.12	3.77 ± 0.40	6.34 ± 0.87*
PC	11.92 ± 1.21	9.04 ± 0.52	8.07 ± 0.45	15.61 ± 1.21*	14.62 ± 1.07	18.12 ± 2.59
PE	9.78 ± 0.52	6.97 ± 0.67	7.70 ± 0.14	12.50 ± 1.52*	11.63 ± 0.66	11.00 ± 1.63
ASX	3.91 ± 0.52	3.38 ± 0.27	4.19 ± 0.72	4.33 ± 0.34	5.73 ± 0.59	11.24 ± 2.86*
Cho	3.53 ±0.25	3.21 ± 0.25	3.38 ± 0.24	3.33 ± 0.24	5.29 ± 0.12*	5.93 ± 0.21
FFA	0.75 ± 0.25	0.66 ± 0.07	0.73 ± 0.13	0.36 ± 0.10	0.54 ± 0.04	0.78 ± 0.33
TAG	66.32 ± 1.25*	72.25 ± 1.21	71.68 ± 1.10	60.39 ± 1.54*	56.09 ± 1.50	45.53 ± 1.17*
HC + SS	1.13 ± 0.15	1.26 ± 0.21	1.30 ± 0.10	1.10 ± 0.10	2.32 ± 0.03*	1.06 ± 0.13

[a]Values (mg lipid/g egg wet weight) are the mean of triplicate analyses ± SD. *Significant changes compared with the preceding stage ($P < 0.05$). For abbreviations see Table 1.

molarity that favored the exchange of water and some other compound responsible for keeping the embryos under optimal conditions. Nevertheless, *M. borellii* is a lecithotrophic shrimp, and therefore once fertilized, the yolk content of each egg wholly supports the development of the embryo and the first postlarval stage until the postlarva starts feeding. This strategy implies that maternal diet modifies the biochemical composition of eggs because there is, to a variable extent, a direct dietary input to oocyte lipid accumulation (20). Therefore, the composition of *M. borellii* eggs was always analyzed using wild gravid females. Yolk composition of just-layed eggs (stage I) showed that the major component was lipid (34.8% dry weight) followed by proteins (33.0%), but only a small amount of carbohydrates was detected (1.5%). High lipid levels are a common feature of other crustacean eggs. Holland (2) reviewed the biochemical composition of several invertebrate eggs, reporting that crustaceans showed a heterogeneous picture, with protein prevailing in some cases whereas lipids were the most important reserves in the majority of crustaceans, with carbohydrates always being a minor component. It is worth pointing out that comparisons among decapod eggs should be made taking into account the different life histories of the group. For example, the prawn *M. idella* has an egg composition in which proteins constitute more than 80% of dry weight (2) and a life cycle that includes very small eggs and feeding larvae. On the other hand, Sarojini *et al.* (21) reported the composition of eggs of *M. kistnensis,* showing that lipids were the most important reserve. Although the three species belong to the genus *Macrobrachium*, their life histories and type of eggs are different. Anger (22) reviewed the importance of lecithotrophy, as compared to feeding, on early larval development.

Regarding the use of the energy sources, we found that lipids, followed by proteins, are the major source of energy available in vitellus for embryogenesis. On the other hand, there was a net carbohydrate synthesis as the mass in the em-

TABLE 3
Major Fatty Acids of Triacylglycerols and Polar Lipids of Whole Eggs During *M. borellii* Embryogenesis[a]

	Stage													
	I		II		III		IV		V		VI		VII	
Fatty acid	TAG	PL	TAG	PL	TAG	PL	TAG	PL	TAG	PL	TAG	PL	TAG	PL
14:0	2.56	2.75	2.54	2.03	2.28	0.97	2.95	1.89	2.35	1.34	2.80	0.84	2.88	0.77
16:0	18.15	19.09	20.80	18.06	19.89	18.57	19.79	18.30	19.32	15.40	18.87	14.07	18.93	14.26
18:0	3.66	4.43	4.03	6.08	3.87	6.07	3.43	5.64	3.46	6.58	3.98	8.19	3.98	9.60
16:1n-7	13.23	13.12	12.72	8.82	12.99	8.80	13.42	11.36	14.09	9.21	11.99	6.68	12.84	7.06
18:1n-9	15.94	15.01	16.22	15.67	15.66	15.47	15.51	15.70	15.28	14.08	15.52	15.12	14.08	10.21
18:1n-7	9.20	6.11	6.34	6.40	7.84	7.06	6.21	5.52	6.43	6.82	7.50	7.47	7.13	7.77
18:2n-6	5.40	5.12	6.15	4.74	6.11	4.12	6.92	4.06	6.33	4.24	6.56	4.74	6.30	4.79
16:3[b]	1.80	5.68	1.55	5.05	1.41	4.80	1.12	4.54	1.33	5.67	1.24	5.82	1.81	5.24
18:3n-3	4.98	6.83	4.44	7.19	6.35	6.53	7.83	5.54	9.84	5.15	10.87	7.38	9.59	7.12
20:4n-6	4.58	4.04	5.08	5.15	4.22	5.16	4.04	5.40	3.96	4.82	3.73	5.23	3.21	5.96
20:5n-3	10.10	8.19	10.35	8.26	9.76	8.98	9.19	10.25	8.37	11.48	8.35	11.80	7.66	11.93
22:6n-3	1.62	1.39	1.56	1.53	1.36	1.62	1.39	1.57	1.50	2.46	1.14	2.07	1.20	2.61
∑ Saturates	26.43	28.51	29.45	27.85	28.24	27.44	29.61	28.44	27.29	25.12	27.64	24.27	28.06	26.85
∑ Monounsaturates	40.36	36.20	37.17	36.10	38.47	36.60	37.10	35.80	37.51	35.45	37.25	32.72	36.25	29.66
∑ Polyunsaturates	33.22	35.27	33.41	36.05	33.24	35.96	33.51	35.81	35.18	39.46	35.16	43.01	35.68	43.58

[a]Values are the mean of duplicate analyses as % w/w. SD have been omitted for clarity but never exceeded 10% of the mean. PL, Phospholipid; for other abbreviations see Table 1.
[b]Identity not established.

bryos exceeds the mass incorporated from the vitellus. Approximately 78% of the vitellus lipid was absorbed by the embryos during development, particularly during organogenesis (stages IV and V), and about 22% was left in hatchlings, suggesting lipids were actively catabolized by embryos for their growth and maintenance. There was a delay in the utilization of vitellus lipids until stage V, which is coincident with observations in a related work where we found at this stage the highest activity of several enzymes involved in the lipid metabolism. In particular, we found higher β-oxidation and TAG-lipase and palmitoyl-CoA ligase activities at stage V (23).

Protein levels showed significant changes along development. Embryos took up 90% of vitellus proteins, which decreased sharply between stages IV and V, similarly to the case for lipids. Subramoniam (24) also found that proteins were used for the growth of *Emerita asiatica* during embryogenesis, and Claybrook (25) reported that during embryonic development of *Palaemon serratus*, yolk proteins were apparently oxidized for energy as well as reincorporated into tissues of the embryo, indicated by a 25% decrease in total protein content of the whole egg.

Interestingly, there was no net increase in embryo protein content until stage VII, just before hatching, suggesting that most proteins incorporated at stages IV and V must have been consumed as energy sources or were converted into other body components. The embryo protein level also showed a trend of increasing between stages III and IV. The major vitellus protein in decapods is vitellin, whose site of biosynthesis has aroused great controversy. A combination of autosynthesis as well as heterosynthesis occurring sequentially in ovaries along with vitellogenesis is the most probable picture (26–29). The ratio of energy supplied by lipids and proteins throughout development was quite constant at around 2, indicating a simultaneous utilization of both.

The calculated protein CE was 57%, comparable to CE values of 50 to 80% that have been observed in other crustaceans that use proteins as the major energy source (2). The protein at stage VII would be all newly synthesized protein and represents 57% of the total protein available in the egg provided from maternal sources.

Stage VII embryos increased their carbohydrate content fivefold compared with stage I. The energy CE was 587%, suggesting an active carbohydrate synthesis throughout development, probably associated with the active shell synthesis that takes place in the late stages of embryogenesis.

We could therefore suggest that absorption of nutrients from vitellus should be divided into two phases. The first one shows a mild uptake up to stage IV, followed by very active uptake and consumption phase from stage V until hatching where carbohydrates, lipids and proteins would be used for organogenesis. At the same time embryos would consume the previously accumulated nutrients, particularly after stage V, together with a *de novo* synthesis of molecules. This is merely a simplification of the real picture, because we are only considering interconversions from the different reserves, but as postlarvae hatch, they become an open system. On the whole, vitellus seems to provide the embryo with both an adequate environment throughout development and a nourishing medium, particularly for late embryogenesis. Proteins provide structural precursors during embryogenesis, and they also contribute to the embryo energetics, mainly supplied by lipids, especially TAG.

FA of PL and neutral lipids presented a similar pattern throughout development, regardless of variation either in lipid class or development stage. Some n-3 PUFA such as DHA and EPA were selectively accumulated in PL during development, while 18:0 and 16:3 showed a clear association with PL. Teshima *et al*. (30), using a double tracer experiment in *Penaeus japonicus,* reported that n-3 FA were partitioned primarily into PL whereas palmitic acid was partitioned into TAG. We have shown that in this direct-development species, PUFA are only preferentially conserved in PL at the end of development, whereas at the begining PUFA are equally distributed in TAG and PL. In the lobster *Homarus americanus*, Sasaki *et al.* (31) found that reserves of egg yolk, especially essential FA, may be depleted during embryogenesis and thus are insufficient to support larval development.

The amount of EPA and DHA is somewhat higher than that expected for a freshwater species and for other invertebrates living in the same area (32) where the n-3/n-6 ratio is below one. Nevertheless, values are similar to those observed in adult *M. borellii* (33). This composition may probably be linked to the fact that the genus *Macrobrachium* has recently colonized the freshwater environment and came from a marine environment where n-3 fatty acids prevail over the n-6 family.

Lipids have the highest energetic yield of all molecules and represent a compact and concentrated form of energy storage. Most aquatic invertebrates have taken advantage of this property, and a high lipid content is a common feature in many marine species (34). This is a common situation for females that need a compact energy store since egg volume imposes body space restrictions. Thus, the use of lipid catabolism as an energy source during embryogenesis may endow the species with some advantages in the unpredictable freshwater environment. Ongoing research in our laboratory has revealed a very active lipid metabolism in these shrimp embryos (23). This is also coincident with the general observation on the life history of marine species that conquered freshwater environments; they frequently present a series of adaptations to this environment, including a tendency to brood protection, low fecundity, large egg size, unusually high lipid content in eggs and larvae, abbreviated larval development, and lecithotrophy (35,36). All of these characteristics were found in *M. borellii* eggs compared to other members of the genus, making this species well-adapted to the nonmarine environment.

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