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Effects of feeding (ω-3) HUFA-enriched *Artemia* during a progressively increasing period on the larviculture of freshwater prawns

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Larvae of the freshwater prawn (*Macrobrachium rosenbergii*) were reared to post-larvae in a closed recirculation system. Six treatments were designed where freshly-hatched *Artemia* nauplii were first given to the larvae for 3, 7, 11, 15, 17 and 25 days according to the treatment. After that (ω-3) HUFA-enriched *Artemia* were given until the 28th day at which time the test was terminated and evaluated. The requirement for (ω-3) Highly Unsaturated Fatty Acids (HUFA) for the larval stages of *M. rosenbergii* was confirmed by this experiment. Moreover, the longer the period of feeding on (ω-3) HUFA-enriched *Artemia* nauplii, the better the results in terms of growth, metamorphosis rate, survival and stress resistance.

KEYWORDS: Freshwater prawn (*Macrobrachium rosenbergii*), Larviculture, Lipid enrichment, Growth and survival rate, Stress resistance

INTRODUCTION

The need for (ω-3) Highly Unsaturated Fatty Acids (HUFAs) has clearly been demonstrated for the larval stages of the freshwater prawn *Macrobrachium rosenbergii* (Devresse *et al.*, 1990). For the complete larval cycle, these needs have been estimated at a daily dose of maximum 35 mg g^{-1} food dry weight. This addition of HUFA significantly improved the rearing cycle in terms of growth, survival, rate of metamorphosis as well as in terms of stress resistance (salinity shock).

It has not been clearly established however, how these HUFA improve larval development although some valid knowledge for other species could apply (Bell *et al.*, 1986; Hagve, 1988; Sargent *et al.*, 1989). These HUFA could have a more specific influence on some definite stages of larval development, such as the first stages after hatching, the beginning of exogenous feeding, and the metamorphosis into the post-larval stage. In order to better assess the needs for (ω-3) HUFA in the course of larval development, an experiment was designed in which different groups of larvae were fed enriched *Artemia* over different periods.

MATERIALS AND METHODS

Macrobrachium broodstock were imported from Thailand, reared in a recirculation system and fed with shrimp pellets (Aqualim SA, France) which had been coated at 5% by

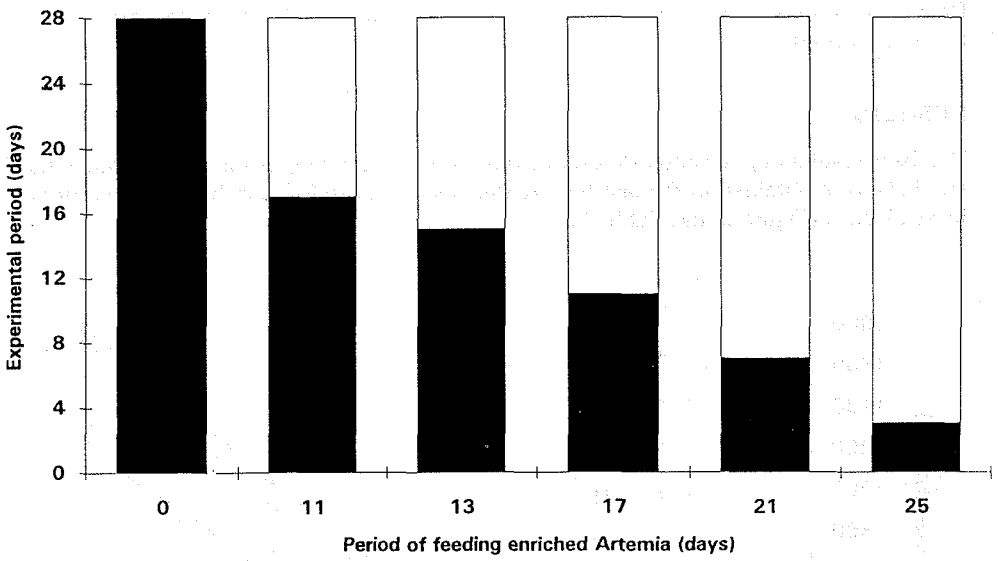

FIG. 1. Schematic outline of experimental treatments (open bars=period of feeding enriched *Artemia*)

weight with the maturation booster MARILA (INVE Aquaculture NV, Belgium). Hatched larvae were transferred into 35 l closed recirculation systems (Léger *et al.*, 1989), each holding a culture volume of 10 l. The larvae were reared at a density of 50 larvae l^{-1} at 28 (1) °C in 12‰ salinity seawater diluted with deionized water. Twice a day a diet of *Artemia* nauplii of Great Salt Lake (Utah, USA) origin was added to achieve a food density of 10 nauplii ml^{-1}. Each morning, the remaining *Artemia* were removed by means of a small filter installed in the recirculation aquarium under the water inlet from the culture cylinder (Léger *et al.*, 1989b).

Six different treatments were tested in triplicate (schematic outline in Fig. 1). Larvae were first fed freshly-hatched *Artemia*, eventually followed by (ω-3) HUFA-enriched *Artemia*, using the (ω-3) HUFA booster SELCO® (INVE Aquaculture NV, Belgium) following the procedures described by Léger *et al.* (1987). The feeding period with non-enriched *Artemia* varied from 3 to 28 days according to the treatment chosen. Larvae were then fed enriched *Artemia* until the end of the experiment (day 28).

Total length (from the tip of the rostrum to the end of the telson, n=50) and dry weight (50 pooled larvae) were recorded every week. At the end of the experiment, survival for both post-larvae and non-metamorphosed larvae, rate of metamorphosis and resistance of the postlarvae to stress were assessed. Stress resistance was measured by recording the rate of mortality resulting from a 1 h exposure in water of 65‰ (Devresse *et al.*, 1990). For statistical analysis, survival data were transformed by ARCSIN and weight data by logarithm. Fatty acid methyl ester analyses were performed on 30 *Macrobrachium* post-larvae harvested on day 28 and pooled per treatment (Léger *et al.*, 1989a).

Upon completion of the sampling analysis on day 28, the remaining larvae were transferred to separate aquaria and were offered the same diet as they received before.

Daily inspections were made to determine the moment at which all larvae had metamorphosed.

RESULTS

The best results (significantly different from the other treatments for most parameters studied) were obtained in the last two treatments, i.e. those fed enriched *Artemia* for at least 21 days (Figure 2 and Table 1).

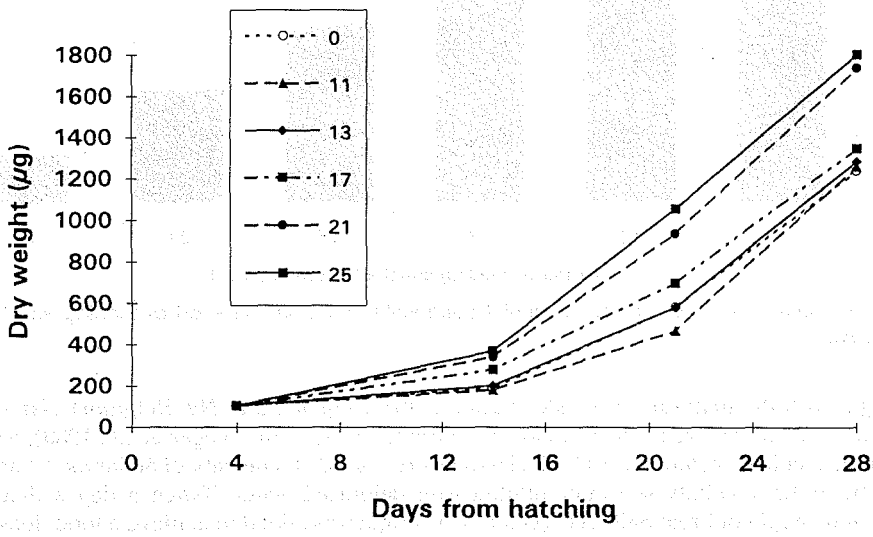

FIG. 2. Larval growth in *Macrobrachium rosenbergii* fed (ω-3) HUFA-enriched *Artemia* for different time periods (0 to 25 days).

TABLE 1. Final length, dry weight, survival, stress resistance and metamorphosis data for *Macrobrachium* larvae sampled on day 28

Treatments (number of days receiving enriched *Artemia*)	Postlarval length (mm)	Postlarval dry weight (mg)	Total survival (larvae + postlarvae) (%)	Postlarval survival to stress (%)	Percent post-larvae	Metamorphosis data: start–end (days after hatching)	Metamorphosis data: period (days)
0	10.20^{ab*}	1.34^{a}	65.3^{a}	8.9^{a}	60.3^{a}	26–40	14
11	9.83^{a}	1.33^{a}	55.4^{a}	8.9^{a}	62.8^{a}	26–38	12
13	10.27^{ab}	1.32^{a}	62.0^{a}	8.9^{a}	71.5^{ab}	24–36	12
17	10.55^{bc}	1.38^{a}	62.1^{a}	13.3^{a}	80.1^{abc}	23–34	11
21	11.19^{cd}	1.76^{b}	68.4^{a}	15.5^{a}	90.5^{bc}	22–30	8
25	11.57^{d}	1.82^{b}	72.3^{a}	27.7^{b}	94.4^{c}	21–29	8

*Means with the same superscript do not differ significantly ($p < 0.05$).

TABLE 2. Mean fatty acid profiles (mg g^{-1} total body dry weight) of *Macrobrachium* postlarvae sampled on day 28

Treatments (number of days receiving enriched *Artemia*)	18:3 (ω-3)	20:5 (ω-3)	22:6 (ω-3)	total (ω-3) HUFA (≥22:3(ω-3))	total lipid (mg g^{-1})
0	0.5	3.6	0.8	4.6	14.43
11	0.6	8.3	3.3	12.3	11.64
13	0.3	10.1	3.9	14.6	12.30
17	0.5	12.5	4.1	16.9	12.77
21	0.4	10.2	4.2	15.2	11.35
25	0.5	11.3	4.9	17.2	11.14

The survival rate at the end of the experiment was not very high which is probably due to the sub-optimal conditions which were caused by the type of rearing system used. Due to high variability among replicates, statistical conclusions on survival data were limited. Nevertheless, the highest values coincided with treatments which were offered enriched *Artemia* for a longer period.

The same trend applied for stress resistance although in this case only the last treatment differed significantly from the others, i.e. feeding (ω-3) HUFA-enriched *Artemia* from the beginning of the larval cycle. Different resistance capacity to osmotic shock appeared clearly between treatments which were fed enriched *Artemia* for 21 and 25 days although growth rates were similar.

For the 21 and 25 day enrichment treatments, metamorphosis was nearly completed on day 28 when final sampling took place (94.4% and 90.5% rate respectively). It was significantly less complete in other treatments, i.e. post-larval count was merely 60.3% in the non-enriched *Artemia* treatment. After day 28, larvae fed non-enriched *Artemia* completed their metamorphosis only on day 40. In general, the longer the enriched *Artemia* feeding period was, the sooner first metamorphosis was noticed and the shorter it took.

There was an increase in the amount of (ω-3) HUFAs in postlarval tissue with time of feeding on enriched *Artemia* (Table 2). This relation was however not linearly proportional, as can be seen from Figure 3. After an 11 day period of feeding on enriched *Artemia*, the total amount of HUFA in the postlarvae reached 12.3 mg g^{-1}. This amount increased to 17.2 mg g^{-1} in the treatment receiving enriched *Artemia* for 25 days. The 20:5 (ω-3) and 22:6 (ω-3) fatty acids showed a similar change to that of total unsaturated fatty acids.

DISCUSSION

This study aimed to better assess the needs for (ω-3) HUFAs in *M. rosenbergii* larval culturing. The experiment tried to verify the needs for (ω-3) HUFAs during the first part of the larval cycle. A similar experiment should be considered to study the needs during the last part of the larval cycle and during final metamorphosis. One could also verify the effect of feeding enriched *Artemia* during the first days or weeks, eventually followed by a regime consisting of non-enriched nauplii.

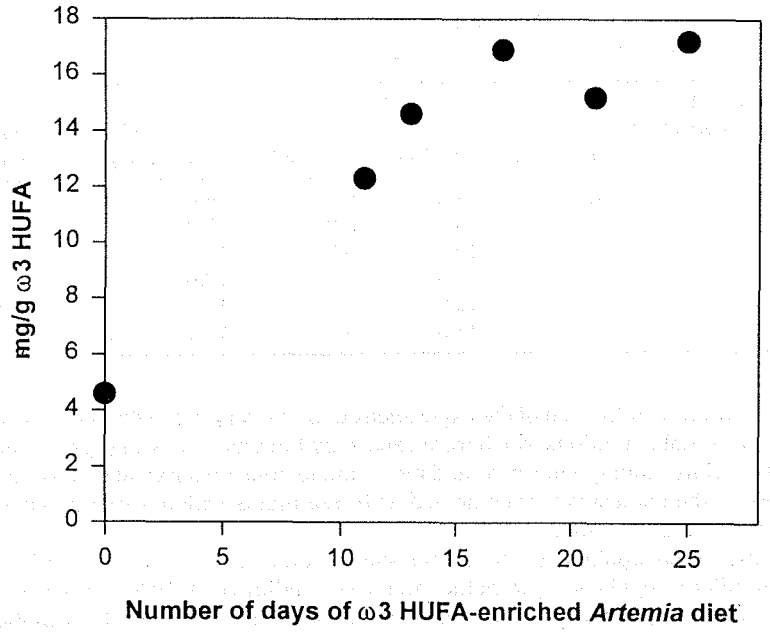

FIG 3. (ω-3) HUFA levels in prawn postlarvae sampled on day 28 as a function of different dietary regimes of (ω-3) HUFA-enriched *Artemia*.

The present experiment has not proven a specific need for HUFA in the first larval stages but rather a constant need throughout the cycle, i.e. a progressive improvement of the results was obtained when the feeding period on enriched *Artemia* was extended.

It was interesting to note that the results obtained in this experiment were inferior to those obtained under similar conditions by previous workers (Buzzi, 1989: Devresse *et al.*, 1990). Our belief is that the difference was due to the fact that in the present study all larvae were fed non-enriched *Artemia* for the first 3 days, whereas in the other experiments enriched *Artemia* were fed from the very beginning. This seems to prove that best larviculture outputs are assured when an (ω-3) HUFA-enriched diet is offered throughout the larval culture period, and, as suggested by Sandifer and Joseph (1976) and Reigh and Stickney (1989), eventually the post-larval period as well.

When considering both the biological and the analytical results, it appears that a shorter enrichment feeding period does not make up for earlier deficiencies even though some (ω-3) HUFA amounts are close to the maximum recorded amounts e.g. treatment 17 days: 16.9 mg g^{-1}, treatment 21 days: 15.2 mg g^{-1}, treatment 25 days, 17.2 mg g^{-1}. The highest recorded levels in this experiment coincide with previous recorded results and suggest that the maximum (ω-3) HUFA level in the post-larval *M. rosenbergii* is in the order of 16–18 mg g^{-1} dry weight. Also interesting to note is that the rate of accumulation is rather slow as more than 3 weeks was necessary to reach the highest levels. This is valuable to an understanding of the kinetics of (ω-3) HUFAs in cellular membrane turnover. Similar to what has been observed in other animals (Holman, 1978), the higher

lipid content (in its hepatopancreas ?) in the control treatment could be symptomatic of a deficiency in an essential lipid compound.

Finally, the more practical consequences of this experiment are doubtlessly worth reiterating as they confirm earlier findings with *M. rosenbergii* larvae (Devresse *et al.*, 1990), i.e. an optimum concentration of about 35 mg g^{-1} (ω-3) HUFA in the larval diet throughout the larval cycle (and maybe even later on) results in a faster larval growth, an earlier and shorter metamorphosis as well as a better resistance to adverse conditions (osmotic shock in this study).

CONCLUSIONS

This study provides further confirmation that for optimal survival, growth, rate of metamorphosis and stress resistance larval *Macrobrachium rosenbergii* require minimum concentrations of 35 mg g^{-1} of (ω-3) highly unsaturated fatty acids in their larval diet.

ACKNOWLEDGEMENTS

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