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Original Article

Evaluation of Using Decapsulated *Artemia* Cysts and *Artemia* Nauplii or Commercial Feed as Starter Feed for Freshwater Prawn *Macrobrachium rosenbergii* Larvae

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Abstract:

The present study was carried out to evaluate the effect of decapsulated *Artemia* cysts as direct food for *Macrobrachium rosenbergii* larvae on the growth and survival rate. *Macrobrachium rosenbergii* larvae (48 hour after hatching) weighted about 0.03 mg were reared (50 larvae/L) in cylindro-conical rearing fiberglass tanks (10 L) under controlled conditions. The larvae were fed on Commercial diet 46% crude protein (T_1), DE capsulated *Artemia cysts* (T_2), *Artemia* naupli (T_3) and DE capsulated cysts & *Artemia* naupli at constant ratio (1:1) (T_4). Three replicates for each treatment. Water parameters in all tanks were recorded. After 21-days rearing period, feeding of the larvae with commercial diet resulted in a significantly ($P \leq 0.05$) lower average weight (0.18 mg), mean length gain (4.80 mm), weight gain (0.15mg), K factor (0.16) and SGR (8.53%/d) compared to the other groups. Group T2 (fed with DE capsulated *Artemia* cysts) resulted in good survival (87%) of larvae, which was not significantly different from T4 (fed with 1:1 ,86.4%) and T3 (fed with *Artemia* nauplii ,86%). These results indicated that the use of decapsulated *Artemia* cysts (low price) appear to be a suitable feed for exogenous feeding in *M. rosenbergii* larvae. However, *Artemia* nauplii are more expensive than some commercial feeds.

Keywords*: Freshwater prawn, Macrobrachium rosenbergii, Artemia cysts, Growth, Survival %.*

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Introduction

Freshwater prawn culture has great potential for rural aquaculture, generating considerable employment and income for rural and poor people. Freshwater prawn farming is environmentally sustainable, since it is practiced at low stocking density (Lavens et al., 2000). The success on the commercial production of *Macrobrachium rosenbergii* post larvae and seeds of other crustacean decapods depends on the efficient use of available Decapsulated *Artemia* cysts have been successfully fed to fish and crustacean larvae (El-Dakaer, 1994). As decapsulated embryos have more energy content than newly hatched nauplii (Magurran, 2005), they are potentially more nutritious for feeding. Murthy et al.,(2008). The advantages of decapsulation include disinfection of the cysts, improved hatching of the cysts into nauplii, higher energy content of the nauplii, and no risk of fish larvae suffering from gut obstruction due to the ingestion of the cyst shell (JavaheriBaboli et al., 2012a). Expensive. To solution of these problems, the use good alternative live foods to *Artemia* nauplii become very important. The small particle size of cysts (200–250m) is suitable for successful rearing of larval stages of aquatic organisms and, after chemically removing of shell, decapsulated cysts can be handled as an inert diet, they are food sources (Barros and Valenti, 2003). A majority of seed used for farming of *M. rosenbergii* comes from hatcheries (Murthy *et al*., 2004; Phuong *et al*., 2006). Existing hatcheries in the country are not producing up to their installed capacity due to various constraints. The *Artemia* is widely used as a commonly used prey for many larval organisms (Hung et al., 2002). The daily production of *Artemia* nauplii is difficult, require dedicated facilities and disinfected and do not leach nutrients (Harzevili et al. 2003). After the decapsulation process, the cysts can be used readily (fresh cysts) or dehydrated in brine solution for storage (brine cysts), or subjected to a drying process for longer term storage (dried cysts) (González et al., 2009).Poor quality of *Artemia* cysts might represent a potential alternative to *Artemia* nauplii. The outer layer of the *Artemia* cyst is non-digestible by predator organisms, but this outer layer can be quickly removed with hypochlorite treatment, using a procedure called decapsulation. The aim of the current study was to evaluate the suitability of using decapsulated *Artemia* cysts, *Artemia* nauplii or constant ratio (1:1) decapsulated *Artemia* cysts & *Artemia* nauplii in comparison with commercial diet for *M. rosenbergii* larvae during their early stage and its effects on growth, survival rate and condition factor (K).

Materials and Methods

The present study was carried out at Fish Research Center (FRC), Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Prawn stocking and management:

Larvae of *M. rosenbergii* were presented from Private fish farm Elkantra, Ismailia, Egypt during (2014) with mean initial weight of 0.03±0.01 mg/larvae and initial length of 3±0.11 mm. They were stocked 48 hour after hatching at an initial density of 50larvae/L in cylindro-conical rearing fiberglass tanks containing 10 L of water. Water salinity was kept at about 12 ppt (Barros and Valenti, 2003) through mixing of freshwater and natural seawater. About

30% of water was exchanged daily from rearing tanks to keep the water volume.

Treatments and Feeding

Four feeding treatments were tested: (1) commercial diet (46%) crude protein, (2) Decapsulated *Artemia* cysts, (3) freshly hatched *Artemia* nauplii, and (4) Decapsulated *Artemia* cysts & freshly hatched *Artemia* nauplii(1:1). Larvae of *M. rosenbergi* were fed a commercial diet (Table 1) contained 46% crude protein according to NRC (1993), at rate of 20% of total stocking biomass. This starter feed (0.3-0.5 mm) was ground and sieved to a small particle size. Larvae were fed three times daily by hand (Wessels and Horstgen-Schwark, 2007). The diet was stored at (4ºC) during the experimental duration to avoid the nutrients deterioration. Fresh *Artemia* nauplii were obtained from hatching *Artemia* cysts of Port Said (local source of Egypt) according to Lavens and Sorgeloos (1996) and Murthy et al. (2012). The cysts used for preparation of decapsulated cysts were of low hatchability (Wadi El-Natrun). The cysts were decapsulated according to the technique described by Bengtson et al. (1991) and Lim et al. (2002). This process involved hydration of the cysts, removal of the shell with an alkaline hypochlorite solution, washing and deactivation of the hypochlorite. After decapsulation, the cysts were dehydrated in a saturated brine solution for storage (Vanhaecke et al., 1990).

Table 1. Composition and proximate analysis of the commercial diet

Ingredients	%
Fish meal[1]	60.9
Fish oil	5.8
Gelatinized starch [2]	29.8
Vitamins premix [3]	1.0
Minerals premix [4]	1.0
Choline chloride (50%)	0.5
Lignin sulphate	1.0
Total	**100**
Proximate analysis (% DM):	
Dry matter	95.5
Crude protein	46
Crude fat	11.7
Crude fiber	4.3
Crude Ash	8.4
Nitrogen free extract (NFE)	29.6
Total	**100**

1- Triple Nine, Denmark (CP: 78.6% DM; GL: 9.8% DM).

2- C-Gel Instant-12016, Cerestar, Mechelen, Belgium.

3- Vitamins (mg kg−1 diet): retinol, 18,000 (IU kg−1 diet); calciferol, 2000 (IU kg−1 diet); alpha tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400 (Pfizer).

4- Minerals (mg kg−1 diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.40 (g kg_{-1} diet); dibasic calcium phosphate, 5.9 (g kg−1 diet) (Pfizer).

* The proximate analysis of diet was performed according to AOAC (1990).

Nauplii and decapsulated cysts or constant ratio (1:1) from nauplii and decapsulated supply for larvae per day was increased during the experiment, (ration of 6, 8 and 10 *Artemia nauplii* or cysts/ml/day) during the first, second and third week, respectively according to Shailender et al. (2013), food was supplied manually at two times a day (8.30 and 17.30 hrs).

Water quality measurements

During rearing period (21 days, according to Petkam and Moodie (2001) the water parameters temperature, pH, dissolved oxygen and ammonia were monitored. Dissolved oxygen and water temperature measured using oxygen temperature meter (YSI model L 57) and were monitored daily throughout the experimental period. Water pH was tested daily at late of afternoon using pH meter (model 56, NR 87BB 203). Water salinity was determined using the conductivity meter. Ammonia was determined weekly intervals according to standard methods (Boyd, 1998). Florescent lamp was used on the surface of water to maintain light 12 h per day at 800–1000 lux intensity. The waste and leftover feed in rearing tanks were removed every morning and evening before feeding by cleaning and siphoning. There were three replicates per treatment.

Growth parameters

Growth parameters (length and weight) were measured on days 7, 14 and 21 of the experimental period. Larvae length was measured with a binocular microscope equipped with an ocular micrometer. For length measurements, 10 larvae were randomly collected from each tank. The individual weight was determined by means of precise balance (to the nearest 0.1 mg).

The body weight gain was expressed as

$WG\ (mg) = W_t - W_0$.

Specific growth rate (SGR) was expressed as

$SGR\ (\%/day) = 100\ (\ln W_t - \ln W_0)/t$

Where W_t is the mean final weight, W_0 is the mean initial weight and t is the duration of experiment (days).

Survival of the larvae was calculated by counting the larvae in the tank at days 7, 14 and 21 and was expressed as

$SR\ (\%) = (Ni \times 100)/N_0$, where N_0 is number of larvae initially stocked, Ni is number of larvae at the end and t is the duration of experiment (days), according to Harvell et al. (1990).

Fulton's coefficient (K) was used to determine the fish condition factor (K), since $K = 100\ (W_t/TL^3)$, where TL is total length at the end; according to Barnabe (1994).

Statistical analysis

The obtained data were analyzed by one-way ANOVA test (SAS, 2000). Means were compared by Duncan's new multiple rang test Dancan (1955).

Results and Discussion

Water quality

From the tabulated data (Table 2) it is obvious that the water temperature, pH, dissolved oxygen and ammonia in different treatments varied from 25.2 to 25.8°C, 7.1-7.5, 5.5- 6.3 mg/l and 0.04 to 0.05 mg/L respectively. All physico-chemical parameters of water in this study were found within the range of requirement for the growth of *M. rosenbergii* larvae (Barros and Valenti, 2003; Tidwell et al., 2004; Phuong et al., 2006; Murthy et al., 2012; Montchowui et al., 2012).

Growth performance and survival %

Growth performance of *M. rosenbergii* larvae were fed four different diets are shown in Table (3). After 21 days of feeding, the mean weights and weight gain of the larvae fed on freshly hatched *Artemia* nauplii, decapsulated cysts and constant ratio (1:1) were not significantly different

(P>0.05) from each other. However, a better growth (in terms of final weight 1.19 mg and weight gain 1.16mg) in larvae fed on *Artemia* nauplii was observed throughout the period of the experiment. While, the larvae fed on the commercial diet had the significantly (P≤0.05) lowest final weight (0.18 mg) and weight gain (0.15 mg) on day 21, respectively. These findings agree with those obtained by (El-Sherif, 2001; Lim et al., 2002; Vanhaecke et al., 2007; Shailander et al., 2013). Also, Kaiser et al. (2003); Policar et al. (2007) and Murthy et al. (2012) observed that, slow growth and high mortality of larvae fed on artificial diets may be related to the absence of a stomach and low digestive capacity at the beginning of their development.

However, decapsulated cysts have a good potential for application in freshwater prawn (*M. rosenbergii*) culture. Due to their smaller diameter (about 200 um), decapsulated cysts may be used for feeding larvae that cannot feed directly on *Artemia* nauplii (Fleig et al., 2001). The cysts contain an average of 30% more energy than newly hatched *Artemia* nauplii (Lim et al., 2003).

Table 2. Water quality recorded in different experimental tanks (values are means ±SD*)*

Item	Water temperature	pH	DO (mg/L)	Ammonia (mg/L)
Commercial diet	25.2 ±0.62	7.1 ±0.08	5.5 ±0.07	0.05±0.01
Artemia nauplii	25.7 ±0.44	7.3 ±0.06	6.3 ±0.11	0..04±0.02
Decapsulated cyst	25.8 ±0.46	7.5 ±0.10	6.2 ±0.08	0.04±0.01
Decapsulated cyst + *Artemia* nauplii (1:1)	25.7 ±0.46	7.4 ±0.10	6.1 ±0.08	0.04±0.01

Table 3. Growth performance of freshwater prawn (*M. rosenbergii*) larvae reared in tanks for 21 days under different diets (mean±SE)

Treatment*		Diets** No.		
	T1	T2	T3	T4
Initial weigh (mg)	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01
Final weigh (mg)	0.18±0.89^{b}	1.12±0.94^{a}	1.19±1.06^{a}	1.16±0.92^{a}
Weight gain (mg)	0.15±0.5^{b}	1.09±0.70^{a}	1.16±0.90^{a}	1.13±0.90^{a}
SGR (%/day)	8.53±0.11^{b}	17.24±0.13^{a}	17.53±0.15^{a}	17.40±0.15^{a}
Survival %	43.0±3.20^{b}	87.0±5.70^{a}	86.0±6.3^{a}	86.4±6.40^{a}
Initial length (mm)	3.00±0.15	3.00±0.15	3.00±0.15	3.00±0.15
Final length (mm)	4.80±0.69^{b}	6.86±1.52^{a}	7.25±1.81^{a}	7.15±1.76^{a}
Condition factor (K)	0.16±0.01^{c}	0.35±0.04^{a}	0.31±0.05^{b}	0.32±0.03^{b}

* Means with the same letter in each row are not significantly different (P≤0.05)

**T1, T2, T3 and T4 mean Commercial diet, decapsulated cysts, *Arteemia* nauplii and decapsulated cyst+ *Artemia* nauplii (1:1)

The results indicated that the highest value of SGR (17.53%) was obtained at the end of the experiment with larval rearing on *Artemia* nauplii. Statistical analysis showed no significant (P>0.5) difference among the treatments fed on decapsulated cysts (17.24%), *Artamia* nauplii (17.53%) or constant ratio (1:1) (17.40%). The lower SGR value (8.53%) was obtained at the larval

treatment that fed on the commercial diet. This is in agreement with the finding of Wolnicki, (2005); Gonzalez et al. (2009); JavaheriBaboli et al. (2012b) and Celada et al. (2013) demonstrated that, the larvae reared on *Artemia* nauplii and decapsulated cysts had the highest SGR than that of commercial diet. It is significant that the contact time between food and digestive enzymes in such young fish is very short. Data in Table (3) showed that the final weight of larvae fed freshly hatched *Atemia nauplii*, decapsulated cysts and constant ratio (1:1) were not significantly (P>0.5) different from each other. However, a significantly better total length (7.25 mm) in larvae fed *Artemia* nauplii was observed at the end of the experiment. Both diets of decapsulated cysts, *Artemia* nauplii and constant ratio (1:1) produced significantly faster growth (in terms of total length) than that of commercial diet. At the end of the experiment, the larvae fed on the commercial diet had the lowest mean length (4.8 mm). Similar results were obtained by Hung et al. (2002); Barros and Valenti (2003) and Murthy et al. (2012). Also, El-Sherif (2001) found that better performance of prawn post-larvae fed on decapsulated cysts appeared to be related to retention of the nutritional value after rehydration in water, thus leaching very low levels of soluble protein and carbohydrates in comparison with the artificial diets. On the other hand, JavaheriBaboli et al. (2012_b) demonstrated that for the fry of the guppy fry, the performance in terms of wet weight, dry weight, length, specific growth ratio, length gain, percentage weight gain and increase body weight of fish fed dried decapsulatd *Artemia urmiana* cysts was better than to those fed decapsulatd *Artemia urmiana* cysts, *Artemia urmiana* nauplii and artificial feed feeds. This is mainly due to the dried decapsulated cyst has a high floating capacity and sink slowly to the bottom of culture tank. Similar results were observed with common barbel larvae, *B. barbus* (Policar et al., 2007). It seems that mouth size, mouth position and superior have a major role in this case. Although, cyst has a higher nutritional value than dry cyst but guppy fry fed cyst had a better performance growth.

Data presented in (Table, 3), no significant (P>0.5) difference was observed in larval survival % throughout the experiment period among the treatments fed decapsulated cysts (87%), *Artemia* nauplii (86%) and constant ratio (1:1) (86.4%). The survival rate of the larvae fed on commercial diet was significantly lower (43%) compared with the other treatment groups. This is in agreement with the finding of El-Sherif (2001); Barros and Valenti (2003); Kaiser et al. (2003); Murthy et al. (2012). Hung *et al.*, (2002) observed high survival rates for Asian catfish (*Pangasius bocourti*) larvae fed on decapsulated cysts. On the other hand, JavaheriBaboli *et al*. (2012b) found that the guppy fry fed dried decapsulated *Artemia* cysts and *Artemia nauplii* displayed consistently higher survival (92±1.3 and 90±3.2, respectively) than those fed decapsulated *Artemia* cysts and artificial feeds (83±1.2 and 80±1.6, respectively). The results of condition factor (K) presented in (Table, 3) showed that the greatest (P≤0.05) larvae condition factor (0.35) was obtained at the end of the experiment with the larvae fed decapsulated cysts, while the lowest condition factor (0.16) was recorded with the larvae fed commercial diet. A similar finding was also reported by El-Sherif (2001); Murthy *et al.* (2008); Shailender et al. (2013).

CONCLUSION

The results of the present study demonstrate that decapsulated *Artemia* cysts (which have poor hatching quality

and low price) appear to be a suitable live food for rearing the early developmental stage of *M. rosenbergii* larvae. This is also an economic issue, since *Artemia* nauplii are more expensive than some commercial feeds.

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