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REPLACEMENT OF *ARTEMIA NAUPLII* WITH DIFFERENT ALTERNATIVE DIETS FOR LARVAL STAGE DEVELOPMENT AND SURVIVAL OF GIANT FRESH WATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN)

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Abstract: The present study was conducted on *Macrobrachium rosenbergii* (de Man) larvae to estimate the effectiveness of different diets to replace *Artemia nauplii* in the feeding system. The study included two experiments performed at pilot scale in 12–l tanks using a recirculating system. Larval stocking density was 100 l–1. After 10 days of feeding by *Artemia nauplii*, different diets included wet and dry diets (Egg custard, Artemia flakes and Higashi Maru No-3 feed) and de-capsulated Artemia cysts, were tested to replace *Artemia nauplii*. In a control treatment using only de-capsulated Artemia cysts throughout the complete larval rearing was also included. The results showed that feeding larvae exclusively de-capsulated cysts for the complete rearing cycle was not suitable. When gradually replacing up to 50% of the *Artemia nauplii* ratio with wet or dry diets, good results in terms of growth, survival and quality of the larvae were obtained, similar to the control treatment receiving only *Artemia nauplii*. However, immediately replacing 50% of the *Artemia nauplii* ratio with artificial diets negatively affected larval development. Addition of artificial feed could start from larval stage VI, with about 25% of the *Artemia nauplii* replaced with artificial diet. Subsequently, the addition ratio could be increased up to 50% from stage VIII to postlarvae stage. Artificial diets should be provided in different particle size ranges based on the acceptance of larvae, larval stage, gradually increasing from 250 to 1000 µm from stage VI to postlarvae stage. The results obtained in the present work may also helpful for the replacement for different diets for economical aspects of the hatchery operations.

Keywords: *Macrobrachium rosenbergii*, Artemia, Larval Rearing, Artificial Diet, Survival

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 (New 1995). 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 however not producing up to their installed capacity due various constraints. For production of scampi, *Artemia nauplii* are used as a live food source in the larviculture. Several authors demonstrated that *Artemia nauplii* are enough to produce *M. rosenbergii* post larvae (Devresse *et al*., 1990; Lavens *et al*., 2000). However, others showed that *Artemia nauplii* do not completely fulfill the nutritional requirements of scampi larvae during the last larval stages and therefore they recommend the use of supplemental diets like egg custard and pelleted feeds (New, 1995; Valenti and Daniels, 2000). Leger *et al*. (1987) showed that decapsulated *Artemia* embryos have 30–50% more energy than newly–hatched nauplii (Instar I). Bengtson, *et al*., (1991) reported that decapsulated *Artemia* cysts have a higher energy and nutritional value than live *Artemia nauplii*.

Sorgeloos *et al*. (1977) also suggested the use of decapsulated cysts as a direct source for fish and crustacean larvae. Subsequent studies demonstrated those decapsulated cysts are a good feed similar to freshly hatched *Artemia nauplii* for the larvae of marine shrimps *Penaeus monodon* (Mock, *et al*., (1980) and freshwater prawn and *M.rosenbergii* (Bruggeman, *et al*., 1980). *Artemia nauplii* have proven to be successful as live food for raising the larvae of many crustacean species, inherent problems, such as the potential introduction of pathogens into the culture system or the high costs of labor and equipment required for preparation. Sorgeloos *et al*. (1983) noticed that the nutritional quality and physical properties of *Artemia nauplii* depend on the source and time of harvest of cysts. Most of the hatcheries are dependent on imported *Artemia* cysts (O.S.I brand: from U.S.A). Murthy *et al*. (2008) observed that the complete dependence on *Artemia* as feed not only makes hatchery operations expensive, but also unsustainable. New (1990) noticed that the dependence on *Artemia* is also a major constraint in the expansion of *M. rosenbergii* hatcheries and also farming. Hence, there is a need for replacement of *Artemia* and to look for

acceptable alternative diets to reduce the cost of prawn larval rearing.

Several types of alternative diets are being investigated as either supplement or partial or complete replacement for *Artemia nauplii* in prawn hatcheries (Ohs, 1995). Wan (1999) developed several semi–purified spray–dried diets and evaluated their performance with larval farms of freshwater prawn *Macrobrachium rosenbergii*. Larvae of prawn consumed the diets, but growth and survival were significantly less than that of pure *Artemia*–fed larvae Ohs (1995). However, Kovalenko *et al*., (2002) reported that larval growth of freshwater prawn fed on micro bound diet was 90% of that achieved for larvae fed newly–hatched nauplii of *Artemia*. No significant difference was observed between the survivals of the larvae fed with the micro bound diet and *Artemia*–fed.

Several studies are also investigated on supplementation of *Artemia* with prepared feed in prawn larval rearing (Sick and Beaty, 1975; Corbin *et al*., 1983). However no standard substitute for *Artemia* has been developed for freshwater prawn hatcheries. According to Barros and Valenti (2003 a) proposes an ingestion rate model of *Artemia nauplii* for *M. rosenbergii* larvae based on the individual ingestion rate and prey density. However, this equation indicated that later larval stages of prawn larvae do not an adequately prey on *Artemia nauplii* and that there is a necessity for proceeding supplementary diet from stage IX onwards. Several studies confirm that the best timing to introduce formulated feeds in the feeding schedule is a very important step in the larval life cycle. Aquacop (1983) and Daniels *et al*. (1992) recommend diet supplementation from stages V–VI. Barros and Valenti, (2003 b) reported supplementation should start from stage VII onwards. The development of the larval digestive tract and the increase in enzyme activity from stage VI onwards (Jones *et al*., 1993; Kamarudin, 1992; Kamarudin *et al*., 1994; Kumlu and Jones, 1995) explains the acceptance of inert diets, since digestion process become thoroughly functional. In the present study a series of experiments were conducted to find out the feeding schedule and use of formulated larval diets to supplement or partial replacement of *Artemia nauplii* for rearing of *M. rosenbergii* larvae.

MATERILAS AND METHODS

The present experiment was conducted in experimental hatchery located in the Department of Zoology and Aquaculture, Acharya Nagarjuna University, and Andhra Pradesh, South India and conducted two experiments on feeding of *M. rosenbergii*. Dark brown or dark grey colored eggs brooders were collected from culture ponds at Bhimavaram, Andhra Pradesh, India and are acclimated to the hatchery conditions for egg incubation. 48h after hatching, larvae were collected gently and stocked into the experimental tanks for rearing.

Experimental design:

In experiment- 1, seven treatments originated from the combination of different diets (*Artemia nauplii*, decapsulated *Artemia* cysts, two commercial dry diets [*Artemia* flakes and Higashi maru No-3 feed] and a wet egg custard diet (Table.1). Experiment-1 was conducted in three replicates per treatment in 10–L cylindro–conical rearing tanks. Three separate recirculation systems were installed, with one replicate of each treatment assigned to each system. Each recirculation system consisted of 100–L cylindro–conical reservoir tank and a 60–l overhead tank along with filter chamber. Water was continuously pumped from reservoir tank to the overhead tank and then forced back through the bottom of the rearing tanks by gravity. An outlet screen (150 µm) at the surface of the rearing tank led the water back to the biological filter tank and at the same time retained the larvae and *Artemia* within the rearing tank. The filter screen was cleaned daily to avoid water overflow. Water with a salinity of 12 ppt was maintained through mixing of de-ionized fresh water and natural seawater (from Bay of Bengal Sea). Aeration in the rearing tanks and filter tanks maintained the oxygen level above 5.5 to 6 ppm. Other water parameters like ammonia, nitrite and nitrate were always below 0.1, 0.03 and 0.05 ppm respectively, while pH varied from 7.8 to 8.2. The waste and leftover food in rearing tanks were removed every morning and evening before feeding by cleaning and siphoning. The same amount of prepared water was replaced into the system to keep the water volume constant. Every day 30% of water was exchanged from rearing tanks. Florescent lamp was used on the surface of water to maintain light 12h per day at 800–1000 lux intensity. Larvae were stocked at an initial density of 50 larvae L^{-1}. Experiment - 2 consisted of four treatments in these one is control and other three treatments are used for experiment. In three treatments 25–50% of the *Artemia nauplii* ration was replaced with different artificial diets based on the larval stage of the animals. 100 % of *Artemia nauplii* were fed in the control (Table-2). Experiment 2 was performed in 10–L cylindro–conical rearing tanks with three replicates per treatment at initial larval density also maintained at 50 L^{-1} using the same system as described in experiment 1.

Preparation of diet and feeding:

The two experiments of larval rearing of *M. rosenbergii* larvae were fed different diets including *Artemia salina* nauplii (Great Salt Lake strain, O.S.I brand, great salt lake Uttah, USA); a wet egg custard–like diet following the formulation of Hien *et al*., (2002) and two kinds of commercial shrimp larval diets (1) Brine Shrimp Flakes (Ocean Star International, Inc. USA) and (2) Higashi Maru No-3 (Hegashi Maru

company, Taiwan). The formulation of the wet diet and the proximate composition of the three different substitution diets are presented in Table-3. *Artemia nauplii* were hatched according to standard techniques following Van Stappen (1996) *Artemia nauplii* were collected as instar I stage and kept in a refrigerator at 4-6 °C with gentle aeration in order to maintain instar I stage nauplii for feeding throughout the day. Decapsulated *Artemia* cysts used in the experiment - 1 were prepared following Tunsutapanich (1979). The ingredients of the wet diet (egg custard) were weighed and blended. The resulting mixture was placed in a pan and cooked in a water bath to pudding consistency. After cooling, it was cut into small pieces and kept in a freezer for use the next 1–2 weeks. Before being fed to the larvae, the pieces were made into smaller particles, which were then sieved with different mesh screens to obtain three size classes of 250–500, 500–750 and 750–1000 µm for feeding based on the larval stages VI–VII, VII–IX and X–XII respectively.

The Brine Shrimp Flake diet was also sieved into different size classes using mesh screens to obtain the desired sizes for feeding. The No-3 feed had a particle size from 150–500 µm and could directly be fed to the larvae. All supplemental or substitution diets were fed to the larvae from day 10 after hatching onwards (about larval stages V–VI). The artificial diets were fed several times daily following the feeding schemes given in Tables-1 and 2. The different substitution and supplementation treatments were based on a standard *Artemia* ration of 6, 8 and 10 *Artemia nauplii* ml^{-1} day^{-1} for the periods from day 1–7; day 8–15 and day 16–PL stage respectively. The amount of formulated feeds given was based on visual observation of the larval tanks upon feeding. Special care was taken not to overfeed, as this may cause degradation of the water quality.

Evaluation of growth parameters:

Larval stage index (LSI) was determined following Maddox and Manzi (1976) to assess larval development from day 10 and 15. From the larval rearing tank at least 40 larvae were sampled from each treatment and the average larval stage determined. The larval stage was recorded based on the description by Uno and Kwon (1969). The duration of the rearing cycle (days) was determined for each rearing tank. For this the duration from larval stocking up to the time 90% of the larvae in the rearing tank have metamorphosed into postlarvae and the survival was recorded. At the same time the final larval survival in each treatment was recorded. Larval quality was also assessed by the following procedure subjected to a total ammonia nitrogen (TAN) toxicity test following the procedure described by Cavalli *et al*. (2000). The test was performed on postlarvae in a series of 5–l glass cones at 29±1 °C. A group of 40 animals from each treatment were exposed during 24h to four increasing concentrations of total ammonia and a control (no ammonia added) and for the above treatment pH of the test solution was adjusted at 7.8–8.2. Mortality was estimated based on the mean lethal concentrations for 50% of the population (24h–LC_{50}).

Statistical analysis on larval growth:

Larval stage index(LSI); duration of rearing cycle; survival and ammonia toxicity data were analyzed by analysis of variance (one–way ANOVA) and, if significant differences were found ($P<0.05$), the least significant differences (Weller–Duncan) test was applied for comparison.

RESULTS

Experiment-1:

In experiment -1, larval development rate was estimated in terms of larval stage index showed significant differences between treatments. At day 10, three different groups had formed based on larval stage index ($P<0.05$). In the treatment 50A+50D and 100D the lowest performance was observed. In contrast the fastest growth was found for treatments 100A, 75A+F and 75A+E. Treatments 50A+F and 50A+E showed intermediate (middle) development rates.

The 15^{th} day of the experiment, the larval development rate in treatment 10°C was significantly lower compared to all others treatments ($P<0.05$). The treatment 50A+50D had a significantly higher larval survival index than the treatment 100D but lower than treatment 75A+E (Fig-1). Significant differences were observed in larval survival at the end of rearing cycle. Three different groups could be distinguished. The lowest survival (38%) was observed in the treatments 100D and 50A+F. The highest survival (50–55%) was observed in the treatments 100A, 75A+F and 75A+E. Intermediate values around 42% were found in the treatments 50A+50D and 50A+E (Fig-2). Considering the duration of the rearing cycle, an opposite trend as for survival was noted. Larvae in the treatments 75A+F and 75A+W needed around 21–23 days of rearing to reach the postlarvae stage, which was significantly shorter than for treatments 50A+50D and 100C, in which the duration of the rearing cycle was extended up to 25–27 days (Fig-2). The results of the ammonia stress test showed differences in post larval tolerance (LC50) ($P<0.05$). The group containing treatments 100C and 75A+F presented the lowest values (130–132 mg L^{-1} TAN), intermediate tolerance levels were found in treatments 50A+50D and 50A+E (161–164 mg l^{-1} TAN), while the highest tolerance was found in treatments 75A+F and 75A+E (174– 178 mg l^{-1} TAN) (Fig-3). In general, the treatments 100A, 75A+E and 75A+F showed the best overall results in term of larval development, survival and larval quality. While the treatments 100D and 50N+F showed the lowest results.

Experiment-2:

The larvae in the different treatments showed the same development rate (P>0.05) in a day 10 of the rearing period. Larval development rate in treatments 100A and A+E became significantly higher compared to treatment A+H (P<0.05) by day 15 of the rearing cycle (Fig-4). Larval survival results at the end of the experiment revealed a significantly higher survival in treatments 100A and A+E (58–60%) compared to treatment A+H, which had a survival of only 44% (P<0.05). Evaluation of the duration of rearing cycle showed that larvae in the treatment A+E completed the rearing cycle in 25 days, which was significantly shorter than in the treatments A+F and A+H which needed 26 and 28 days respectively (Fig-5). Post larval tolerance to total ammonia was significantly higher in treatments 100A and A+E (200 and 220 mg l–1 TAN respectively), compared to treatment A+H for which the LC50 was only 152 mg l–1 TAN (P<0.05) (Fig-6). In general, the treatments 100A and A+E showed better results in terms of larval development, survival, rearing and larval quality compared to treatment A+H.

Table No.1: Different diets and feeding schedules used in experiment-1

Treatment	Feeding schedule												
	Day 1-8		Day 8- PL										
	7. 00 h	18.00 h	7.00 h	9.00 h	10.00 h	11.00 h	12.00 h	13.00 h	14.00 h	15.00 h	16.00 h	17.00 h	18.00 h
100 A	60 A	60 A	60 A	-	-	-	-	-	-	-	-	60 A	-
50 A+50 D	60 A	60 A	60 D	-	-	-	-	-	-	-		60 A	-
100 D	60 A	60 A	60 A	-	-	-	-	-	-	-	-	60 A	---
75 A + F	60 A	60 A	30 A	-	-	F	-	F	-	F	-	-	60 A
5 A + E	60 A	60 A	30 A	-	-	E	-	E	-	E	-	-	60 A
50 A + F	60 A	60 A	F	F	-	-	F	-	F	-	F	-	60 A
50 A + E	60 A	60 A	E	E	-	-	E	-	E	-	E		60 A

A: *Artemia nauplii*; D: De-capsulated *Artemia* cyst F: Brine shrimp *Artemia* Flakes; E: Wet egg custard. Values represent the percentage of the standard daily *Artemia nauplii*/cysts ration, which constitutes 6, 8, 10 Artemia cysts mn-1 for day 1-8; day 8-16 and day 16- PL stages respectively.

Table No.2: Different artificial diets and feeding schedules used to supplement or Substitute *Artemia nauplii* in experiment -2

Treatment	Larval stage	Feeding schedule				
		7. 00 h	10.00 h	12.00 h	14.00 h	18.00 h
control (1) 100 A	I- PL	60 A	-	-	-	60 A
		Replaced *Artemia nauplii*				
(2) A + E	I - VI	60 A	-	-	-	60 A
(3) A + F	VI- VIII	30 A	-	-	-	30 A
(4) A + H	IX - PL	-	-	-	-	60 A

A: *Artemia nauplii*; F: Brine shrimp *Artemia* Flakes; E: Wet egg custard. H: Hegashi Maru No-3. Values represent the percentage of the standard daily *Artemia nauplii*/cysts ration, which constitutes 6, 8, 10 *Artemia* cysts mn-1 for day 1-8; day 8-16 and day 16- PL stages respectively.

Table No- 3: Formulation of the wet diet and proximate composition of the three formulated diets

Formulation of Egg custard (%)		Composition (% dry weight)			
			Wet weight	Flakes*	Higashi. No-3*
Milk powder	53.8	Protein	49.2	53	58
Chicken egg yolk	41.7	Lipid	26.5	9	9
Cod liver oil	3.5	Ash	5.9	4	15
Soya been (Lecithin)	2.0	Minerals	6.5	2	3
vitamin B complex	400mg kg-1	Fiber	0.4	2	4
Vitamin C	200 mgkg-1	Moisture	58.5	9	9

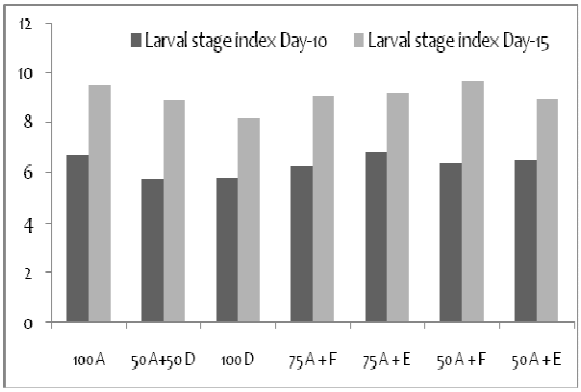

Figure No-1: Larval stage index at day 10 and 15 of *M. rosenbergii* larvae reared according to different feeding schedules in experiment -1

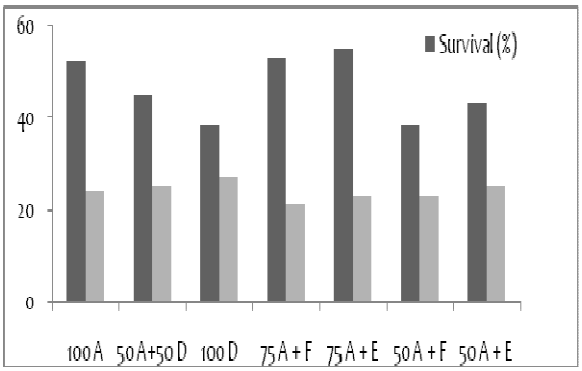

Figure No-2: Survival and duration of the rearing cycle of *M. rosenbergii* larvae reared in different feeding schedules in experiment -1

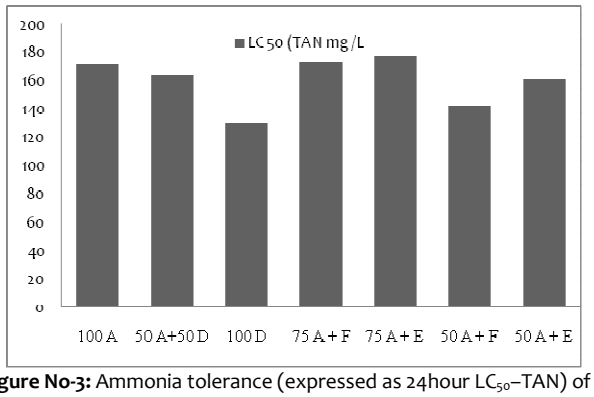

Figure No-3: Ammonia tolerance (expressed as 24hour LC_{50}–TAN) of *M. rosenbergii* larvae reared according to different feeding schedules in experiment -1

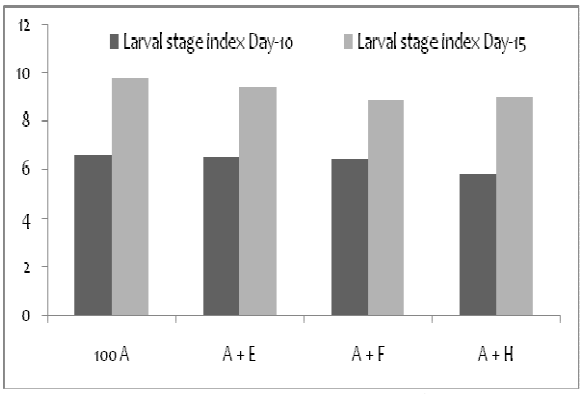

Figure No-4: Larval stage index at day 10 and 15 of *M. rosenbergii* larvae reared according to different feeding schedules in experiment -2

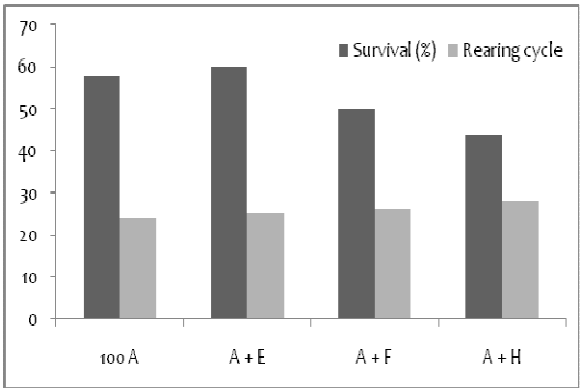

Figure No-5: Survival and rearing cycle of *M. rosenbergii* larvae reared according to different feeding schedules in the experiment -2

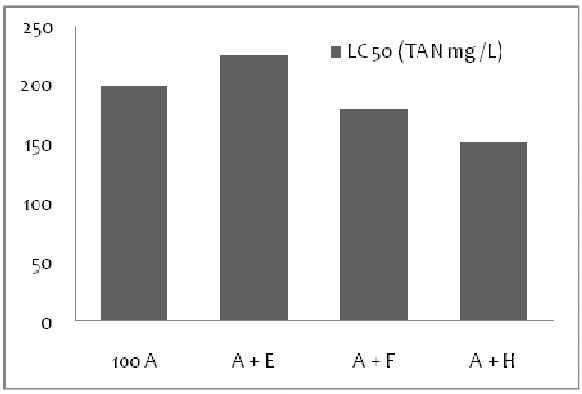

Figure No-6: Ammonia tolerance (expressed as 24hour LC50–TAN) of *M. rosenbergii* larvae reared according to different feeding schedules in experiment -2

DISCUSSION

The results of present study showed that the larval development, survival, duration of the rearing cycle and larval quality. This treatment was distributed in to three distinct groups. 25 % of the *Artemia* ratio was replaced by egg custard or dry diets in the best group. There is no effect in growth of the larvae when the replacement of a part of the live food in the feeding schedule. Larval survival and larval quality was reduced when 50% of the live feed was replaced from day 8 onwards. Especially, the use of an exclusive diet of decapsulated *Artemia* cysts seemed not appropriate for *M. rosenbergii* larval development. Although *Artemia* cysts are reported to contain higher energy and nutrient levels than *Artemia nauplii* (Sorgeloos *et al*., 1977; Bengtson, *et al*., 1991; Leger *et al*., 1987). Lavens and Sorgeloos (1996) were observed that they rapidly sink to the bottom upon feeding, thus reducing their availability for the larvae to feed upon in the water column. This behavior of prawn larvae is rather to swim in the upper part of the water column or at the water surface. Increasing aeration in the rearing containers may keep these particles better in suspension, however the increased turbulence may make it more difficult for the larvae to capture and ingest the prey. Decapods larvae do not specifically orientate towards a food source, they depend on chance encounter to capture food (Mooler, 1978; Meyers and Hagood, 1984; Kurmaly *et al*., 1989). Barros and Valenti, (2003 a) concluded that *Artemia* cysts have a round shape, which may be difficult for the larvae to capture and hold on to during eating. In contrast, the mobility of *Artemia nauplii* allows its permanence in the water column, thus, increasing the chances of encounter. Normal usage decapsulated cysts, which have a narrow size range from 220–260 µm. Tackaert *et al*. (1987) also noted that *Artemia* appropriate for all larval stages during development.

Barros and Valenti, (2003 b) reported that the live food supplementation should start from stage VII onwards, using food particles increasing from 250 to 1170 µm. Therefore, the dimension of decapsulated cysts was appropriate only for VII and VIII stages of *M. rosenbergii* larvae. In experiment – 1, replacing *Artemia nauplii* by artificial diets at a constant ratio of 50% from larval stage VI–VII onwards it gave negatively affected larval survival, but did not affect larval growth. This may be explaining by the sudden reduction of live feed in these treatments. In these treatments live feed was supplied only one time per day in the evening, and subsequently the live feed density during the day time was very low. In the early period of rearing, the larvae may not have been adapted to non–living feed, probably resulting in low survival due to increased cannibalism. Indeed in experiment -2, the larvae were more gradually weaned from *Artemia* onto formulated feeds. By this better results were obtained in this treatment. So that it is recommended to replace only 25% of the *Artemia* ratio at the starting period of the rearing period to allow the larvae to adapt to the new added diet. Subsequently, the weaning ration may be increased up to 50%, spread over several feedings per day. The replacement diets need to be offered with increasing particle sizes in function of the larval stage. Hegashi Maru No-3 feed, which had a rather narrow particle size range of 150–500 µm showed lower results compared to the egg custard and flake diets in the treatment. Although the No-3 feed contained a higher protein level than the other diets, the narrow particle size range may have been a disadvantage for later *M.*

rosenbergii larval stages. In contrast, the wet (egg custard and flake diet could easily be sieved into the desired particle sizes using sieves with different mesh sizes and it will be suitable to intake of the particles which is suitable to larvae for feed.

In the present study, artificial diets were supplied from day 8 or 10 (stage VI–VII) onwards. It was noticed that the larvae readily accepted the inert feeds. In this respect, the wet diet seemed to be more attractive to the larvae than the dry diets because of its different ingredients'. Barros and Valenti, (2003 a) observed that the larvae only accepted inert feed from stage VII onwards and suggested that the live feed could totally be replaced with wet or dry diets from stages VII and IX onwards respectively. Murthy *et al*, (2008) explained that using wet diets which contain shrimp and clam meat fed to larvae in combination with *Artemia nauplii* showed larval survival of 40% in 150–l rearing tanks. Islam *et al*., (2000) proved that freshwater prawn larvae reared in a recirculation system with 140–L rearing tanks fed *Artemia nauplii* supplemented with egg custard obtained a survival of 30%, which was higher than larvae fed exclusive *Artemia* (only 12%). However, Kamarudin *et al*., (1994) reported that the use of artificial diets containing various ratios of cod liver and corn oil to replace 25-100% of the standard *Artemia nauplii* ration from stage III to XI. The results showed that there were no significant differences in survival between the substitution treatments and the control treatment fed exclusively *Artemia nauplii*. In the present study, a gradual replacement of up to 50% of the *Artemia nauplii* ration with wet and dry diets showed similar compared to a 100% *Artemia* control in terms of larval development, survival and larval quality. However, performance was impaired when the *Artemia* diet was abruptly replaced at a constant rate of 50% from day 8 or 10 onwards. In practice production efficiency depends on the production cost, which is based on the feed source and cost, labor cost, etc. The results obtained in the present work may helpful for the replacement for different diets and also larval feeding habitats. This is also helpful for economical aspects of the hatchery operations.

CONCLUSION

The present study showed that the importance of *Artemia* during early stages and artificial feed during growing time. There were no significant differences were observed in survival between the treatments and the control treatment fed exclusively *Artemia nauplii*. A continuing replacement of up to 50% of the *Artemia nauplii* ratio with wet and dry diets showed similar compared to a 100% *Artemia* control in terms of larval development, survival and larval quality but in production point of view it give better results by usage of *Artemia* along with artificial diets. However, performance was impaired when the *Artemia* diet was abruptly replaced at a constant rate of 50% from day 8 or 10 onwards. This study also indicates the economic viabilities of hatchery operations.

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