Evaluation of different diets to replace *Artemia* nauplii for larval rearing of giant freshwater prawn (*Macrobrachium rosenbergii*)

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ABSTRACT

A study was conducted on *Macrobrachium rosenbergii* larvae to evaluate the efficiency of different diets to replace *Artemia* nauplii in the feeding scheme. The study included two experiments performed at pilot scale in 12–L tanks using a recirculating system. Larval stocking density was 100 larvae/L. After 7 days of feeding by *Artemia* nauplii, different diets, included wet and dry diets and decapsulated *Artemia* cysts, were tested to replace *Artemia* nauplii. An extra treatment using only decapsulated *Artemia* cysts throughout the complete larval rearing was also included. The results showed that feeding larvae exclusively decapsulated cysts for the complete rearing cycle was not appropriate. When gradually replacing up to 50% of the *Artemia* nauplii ration 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, abruptly replacing 50% of the *Artemia* nauplii ration with artificial diets negatively affected larval development. Weaning could start from larval stage V, with about 25% of the *Artemia* nauplii replaced with artificial diet. Subsequently, the weaning ration could be increased up to 50% from stage IX to postlarva stage. Artificial diets should be provided in different particle size ranges based on the larval stage, gradually increasing from 250 to 1000 μm from stage V to postlarva stage. The results obtained in the present study may aid future research and serve as a baseline for further optimization of feeding strategies in prawn larviculture.

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1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* is a commercially important species in freshwater aquaculture in Vietnam and other Southeast Asian countries. Freshwater prawn farming has been pinpointed as one of the major target species of the aquaculture sector. The Ministry of Fisheries of Vietnam has put forth that the annual production of *M. rosenbergii* must reach 50,000 tons utilizing 50,000 ha by the year 2025. The seed production demand of freshwater prawn will be of sufficient quality and quantity from 2 to 3 billion per year in 2025 to serve farming (GOV, 2018). Freshwater prawn culture has great potential for rural aquaculture, generating considerable employment and income, thereby bringing prosperity to rural poor. Giant freshwater prawn farming is environmentally sustainable, since it is practiced at lower grow–out density (New, 1995). A majority of seed used in grow out 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.

Artemia nauplii are the preferred live food source used in the larviculture of many crustaceans of commercial value. Lavens et al. (2000) demonstrated that *Artemia* nauplii suffice to pro-

duce *M. rosenbergii* postlarvae. However, others showed that *Artemia* nauplii do not completely fulfil the nutritional requirements of larvae during the last larval stages and therefore recommend the use of supplemental diets (Valenti & Daniels, 2000). As a feed source, decapsulated *Artemia* cysts have a higher energy and nutritional value than live *Artemia* nauplii (Bengtson et al., 1991). Leger et al. (1987) showed that decapsulated *Artemia* embryos have 30–50% more energy than newly–hatched nauplii (instar I). Sorgeloos et al. (1977) suggested the use of decapsulated cysts as a direct source for fish and crustacean larvae. Subsequent studies demonstrated that decapsulated cysts are a good feed similar to freshly hatched *Artemia* nauplii for the larvae of marine shrimps and freshwater prawn, such as *Penaeus monodon* (Mock et al., 1980), and *Macrobrachium rosenbergii* (Bruggeman et al., 1980).

Although live food such as *Artemia* nauplii has proven successful for raising the larvae of many species, inherent problems remain such as the potential introduction of pathogens into the culture system or the high costs of labour and equipment required for preparation. In addition, the nutritional quality and physical properties of *Artemia* nauplii are depending on the source and time of harvest of cysts (Sorgeloos et al., 1983). Imported *Artemia* cysts are predominantly used, which are expensive and uncertain in availability. Dependence entirely on *Artemia* as feed not only makes hatchery operations expensive, but also unsustainable (Murthy et al., 2008). The dependence on *Artemia* is also a major constraint in the expansion of *Macrobrachium rosenbergii* hatcheries (New, 1990). Hence, there is a need to look for acceptable alternative diets to replace *Artemia* and reduce the cost of prawn larval rearing. Several alternative foods, both live and inert, are being investigated as either supplement or replacement for *Artemia* nauplii in crustacean hatcheries. Wan (1999) developed several semi–purified spray–dried diets and evaluated their performance with larval striped bass, *Morone saxatilis* and freshwater prawn *Macrobrachium rosenbergii*. Larvae of both species consumed the diets, but growth and survival were significantly less than that of *Artemia*–fed larvae. However, Kovalenko et al. (2002) reported that larval growth of freshwater prawn fed a microbound diet was 90% of that achieved for larvae fed newly–hatched nauplii of *Artemia*. Survival of the larvae fed the microbound diet was not significantly different from that of *Artemia*–fed larvae. Several studies also investigated supplementation of Artemia with prepared feed in prawn larval rearing (Sick & Beaty 1975; Corbin et al., 1983). However, no standard substitute for *Artemia* has been developed for freshwater prawn hatcheries. Barros & Valenti (2003a) developed 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 *Artemia* is not an adequate prey for later larval stages and that there is a necessity for a supplementary diet from stage IX onwards. Several studies indeed confirm this finding, however controversy still exist concerning the best timing to introduce formulated feeds in the feeding schedule. Daniels et al. (1992) recommend diet supplementation from stages V–VI. Barros & Valenti (2003b) reported supplementation should start from stage VII onwards. The development of the larval digestive tract and the increase of enzyme activity from stage VI onwards (Kumlu & Jones, 1995) may explain the acceptance of inert diets, since digestion processes become thoroughly functional. In order to further optimize the feeding schedule for *M. rosenbergii* larval rearing, a series of experiments were performed in the present study to evaluate the use of formulated larval diets to supplement or partially replace *Artemia* nauplii.

2. Materials and Methods

2.1. Experimental animals

Two experiments were conducted at the experimental hatchery of the Faculty of Fisheries, Nong Lam University, Vietnam. *M. rosenbergii* breeders bearing yellow eggs were obtained from culture ponds in Ben Tre province, Southern Vietnam and acclimated to the hatchery conditions for egg incubation. The water quality parameters of the broodstock tanks, photoperiod, and feeding were adjusted in accordance with the recommendations for prawn rearing (New, 2003). In both experiments, the larvae were obtained from several oviparous female breeders to ensure that enough the quality larvae was supplied for the pilot scale experiments. Twenty four hours after hatching, larvae were collected and stocked into the experimental tanks.

2.2. Experimental design

Experiment 1 consisted of seven treatments, which originated from the combination of different diets (*Artemia* nauplii, decapsulated *Artemia* cysts, two commercial dry diets and a wet egg custard diet (Table 1). Experiment 1 was performed in pilot–scale 12–L cylindro–conical rearing tanks with three replicates per treatment. Three separate recirculation systems were installed, with one replicate of each treatment assigned to each system. Each recirculation system consisted of 120–L cylindro–conical reservoir tank connected to a 160–L submerged biological filter and a 60–L overhead tank. Water was continuously pumped from reservoir tank to the overhead tank and then forced back through the bottom of the rearing tanks by gravity at 0.3 L/min. 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 g/L was obtained through mixing deionised water (tap water source) and natural seawater. Aeration in the rearing tanks and filter tanks maintained the oxygen level above 5 mg/L. Ammonia, nitrite and nitrate were always below 0.1, 0.03 and 50 mg/L respectively, while pH varied from 7.8 to 8.2. The waste and uneaten food in rearing tanks were removed every morning before feeding by siphoning. The same amount of prepared water (mixed water) was added into the system to keep the water volume constant. Light was supplied for 12h per day at 800–1000 lx at the water surface. Larvae were stocked at an initial density of 50 larvae/L. Experiment 2 consisted of four treatments. In three treatments 25–50% of the *Artemia* nauplii ration was replaced with different artificial diets based on the larval stage of the animals. A control treatment was fed 100% *Artemia* nauplii (Table 2). Experiment 2 was performed in pilot–scale 12–L cylindro–conical rearing tanks with three replicates per treatment at initial larval density of 50 larvae/L using the same recirculation system and rearing condition as described in experiment 1.

2.3. Diet preparation and feeding

M. rosenbergii larvae in the two experiments were fed different diets including *Artemia franciscana* nauplii (Great Salt Lake strain, Crystal Brand, Ocean Star International, Inc. 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) Gromate (Fantai company, Taiwan). The formulation of the wet diet and the proximate composition of the three different substitution diets are presented in Table 3.

Artemia naupllii 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^{0}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 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, individually wrapped with polyethylene film 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 IV–VI, 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 Gromate 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 8 after hatching onwards (about larval stages V–VI). The artificial diets were fed several times daily following the feeding schemes 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/day 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.

2.4. Evaluation parameters

At day 10 and 15, a larval stage index (LSI) was determined following Maddox and Manzi (1976)

Table 1. Different diets and feeding schedules used in experiment 1

Treatment[1]	Feeding scheme										
	Day 1–7		Day 8–PL								
	7h	17h	7h	9h	10h	11h	12h	13h	14h	15h	17h
100N	50N	50N	50N								50N
50N+50C	50N	50N	50C								50N
100C	50N	50N	50C								50C
75N+F	50N	50N	25N		F		F		F		50N
75N+W	50N	50N	25N		W		W		W		50N
50N+F	50N	50N	F	F		F		F		F	50N
50N+W	50N	50N	W	W		W		W		W	50N

[1]N: *Artemia* nauplii; C: Decapsulated *Artemia* cysts F: Brine Shrimp Flakes; W: Wet diet. Values represent the percentage of the standard daily *Artemia* nauplii/cysts ration, which constitutes 6, 8 and 10 *Artemia* nauplii/cysts/mL for day 1–7; day 8–15 and day 16–PL stage respectively.

Table 2. Different artificial diets and feeding schedules used to supplement or substitute *Artemia* nauplii in experiment 2

Treatment[1]	Larval rearing day	Feeding scheme				
		7h00	10h00	12h00	14h00	17h00
Control treatment (1) 100N	1–PL	50N				50N
Replaced *Artemia* treatments was applied the same feeding regime in below						
(2) N+W; (3) N+F; (4) N+G	1–7	50N				50N
	8–15	25N	*x*	*x*	*x*	50N
	16–PL	*x*	*x*	*x*	*x*	50N

[1]N: *Artemia* nauplii; W: Wet diet; F: Brine Shrimp Flake; G: Gromate; "*x*": time points when artificial diet was fed. Values represent the percentage of the standard daily *Artemia* nauplii ration, which constitutes 6, 8 and 10 *Artemia* nauplii/mL for day 1–7; day 8–15 and day 16–PL stage respectively.

to assess larval development. (LSI was determined during larval stage from 1-11 when has not any PL occurred). For this at least 30 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 had metamorphosed into postlarvae was recorded. At the same time the final larval survival rate in each treatment was recorded. Larvae were also subjected to a total ammonia nitrogen (TAN) toxicity test following the procedure described by Armstrong et al. (1978) in order to assess larval quality.

Where:

$[NH3] = [TAN] / (1 + 10^{[pK–pH]})$

pK = 9.31 at temperature of 28^0C and salinity of 12 g/L.

pH = mean of values measured at the beginning and the end of test.

The test was performed on postlarvae in a series of 1–L glass cones at 28 ± 1^0C. Groups of 30 animals from each treatment were exposed during 24h to 4 increasing concentrations of total ammonia and a control (no ammonia added). As the toxicity of TAN is a function of temperature and pH, the pH of the test solution was adjusted at 7.8–8.0. Based on the mortality rates, the mean lethal concentrations for 50% of the population (24h–LC_{50}) were estimated.

2.5. Statistical analyses

Larval stage index; 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 post hoc comparison. All percentage data were normalized by square root–arcsine, but only non–transformed means are presented.

Table 3. Formulation of the wet diet and proximate composition of the three formulated diets

Formulation of wet diet (%)		Proximate composition of formulated diets (% dry weight)			
			Wet diet	Flakes*	Gromate*
Milk powder	53.8	Protein	48.6±1.2	53	57
Chicken egg yolk	41.7	Lipid	25.5±0.7	9	8
Squid oil	3.0	Ash	5.8±0.1	4	13
Lecithin	1.5	Mineral	6.5±0.1	2	2
Vitamin C	200 mg/kg	Fiber	0.3±0.0	2	4
		Moisture	57.7±2.5	9	9

*Composition based on the product label.

3. Results

3.1. Experiment 1

Larval development rate in terms of larval stage index in experiment 1 showed significant differences between treatments. At day 10, three different groups had formed based on larval stage index ($P < 0.05$). The lowest performance was observed in the treatments 50N+50C and 100C. In contrast to the fastest growth was found for treatments 100N, 75N+F and 75N+W. Treatments 50N+F and 50N+W showed intermediate development rates. At day 15 of the experiment, the larval development rate in treatment 100C was significantly lower compared to all others treatments ($P < 0.05$). The treatment 50N+50C had a significantly higher LSI than the treatment 100C but lower than treatment 75N+W (Figure 1). Larval survival rate at the end of rearing cycle also showed significant differences. Three different groups could be distinguished. The lowest survival (30%) was observed in the treatments 100C and 50N+F. The highest survival (43–45%) was observed in the treatments 100N, 75N+F and 75N+W. Intermediate values around 35% were found in the treatments 50N+50C and 50N+W (Figure 2). Considering the duration of the rearing cycle, an opposite trend as for survival was noted. Larvae in the treatments 75N+F and 75N+W needed around 24–25 days of rearing to reach the postlarval stage, which was significantly shorter than for treatments 50N+50C and 100C, in which the duration of the rearing cycle was extended up to 28–29 days (Figure 2). The results of the ammonia stress test showed differences in postlarval tolerance (LC_{50}) ($P < 0.05$). The group containing treatments 100C and 75N+F presented the lowest values (136–138 mg/L TAN), intermediate tolerance levels were found in treatments 50N+50C and 50N+W (165–168 mg/L TAN), while the highest tolerance was found in treatments 75N+F and 75N+W (185–189 mg/L TAN) (Figure 3). In general, the treatments 100N, 75N+W and 75N+F showed the best overall results in term of larval development, survival and larval quality. While the treatments 100C and 50N+F showed the lowest results.

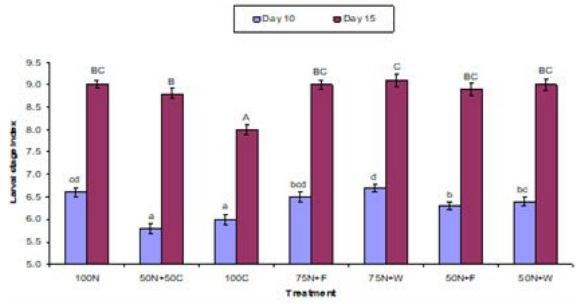

Figure 1. Larval stage index at day 10 and 15 of*M. rosenbergii* larvae reared according to different feeding schedules in experiment 1. Different letters between treatments denote significant differences ($P < 0.05$). For description of treatments refer to Table 1.

3.2. Experiment 2

At day 10 of the rearing period, the larvae in the different treatments showed the same development rate ($P > 0.05$). However, larval development rate in treatments 100N and N+W became significantly higher compared to treatment N+G ($P < 0.05$) by day 15 of the rearing cycle (Figure 4). Survival rate results at the end of the experiment revealed a significantly higher survival in treatments 100N and N+W (53–54%) compared to treatment N+G, which had a survival of only 40% ($P < 0.05$). Evaluation of the

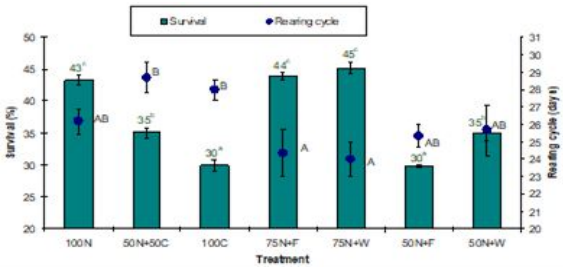

Figure 2. Survival and duration of the rearing cycle of *M. rosenbergii* larvae reared according to different feeding schedules in experiment 1. Different letters between treatments denote significant differences ($P < 0.05$). For treatment descriptions refer to Table 1.

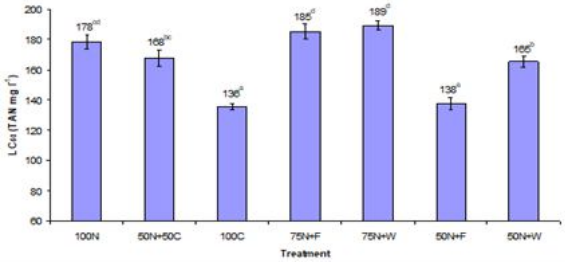

Figure 3. Ammonia tolerance (expressed as 24 hour LC_{50}–TAN) of *M. rosenbergii* larvae reared according to different feeding schedules in experiment 1. Different letters between treatments denote significant differences ($P < 0.05$). For treatment descriptions refer to Table 1.

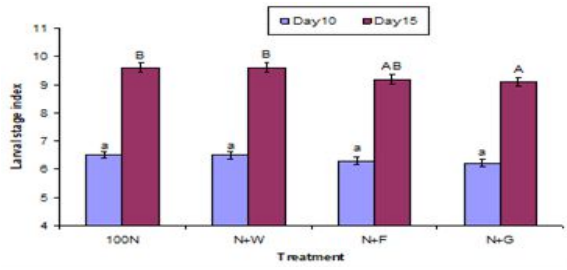

Figure 4. Larval stage index at day 10 and 15 of *M. rosenbergii* larvae reared according to different feeding schedules in experiment 2. Different letters between treatments denote significant differences ($P < 0.05$). For treatment descriptions refer to Table 2 and 3.

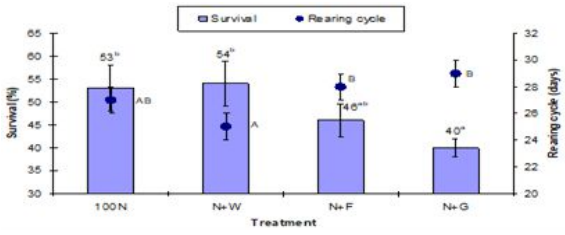

Figure 5. Survival and rearing cycle of *M. rosenbergii* larvae reared according to different feeding schedules in the experiment 2. Different letters between treatments denote significant differences ($P < 0.05$). For treatment descriptions refer to Table 2 and 3.

duration of rearing cycle showed that larvae in the treatment N+W completed the rearing cycle in 25 days, which was significantly shorter than in the treatments N+F and N+G which needed 28 and 29 days respectively (Figure 5). Postlarval tolerance to total ammonia was significantly higher in treatments 100N and N+W (190 and 214 mg/L TAN respectively), compared to treatment N+G for which the LC_{50} was only 145 mg/L TAN ($P < 0.05$) (Figure 6). In general, the treatments 100N and N+W showed better results in terms of larval development, survival, rearing and larval quality compared to treatment N+G.

4. Discussion

In experiment 1, the results of larval development, survival, duration of the rearing cycle and larval quality distributed the treatments into three distinct groups. The best group included the treatments fed exclusively *Artemia* nauplii and the treatments in which around 25% of the *Artemia* ration was replaced with artificial wet or dry diets. Consequently, the replacement of a part of the live food in the feeding schedule did not affect performance of the larvae. However, treatments in which 50% of the live feed was replaced from day 8 onwards reduced survival rate and larval quality. 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; Leger et al., 1987; Bengtson et al., 1991), it was observed that they rapidly sink to the bottom upon feeding, thus reducing their availability for the larvae to feed upon in the water column (Lavens & Sorgeloos, 1996). This while the behavior of prawn larvae is rather to swim in the upper part of the water column or at the water surface. Increasing the aeration in the rearing containers may keep these particles better in suspension, however the increased turbulence may make it

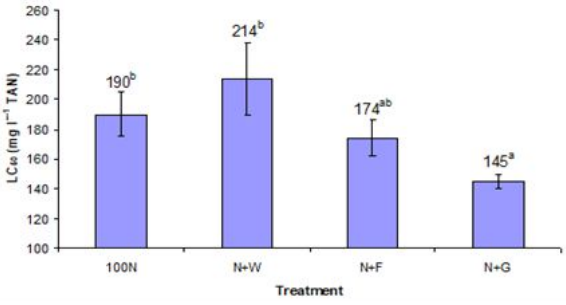

Figure 6. Ammonia tolerance (expressed as 24hour LC_{50}–TAN) of *M. rosenbergii* larvae reared according to different feeding schedules in experiment 2. Different letters between treatments denote significant differences ($P < 0.05$). For treatment descriptions refer to Table 2 and 3.

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 (Kurmaly et al., 1989). In addition, *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 (Barros & Valenti, 2003a). Using exclusively decapsulated cysts, which have a narrow size range (210–260 μm, Tackaert et al., 1987) may also not be appropriate for all larval stages during development. Barros & Valenti (2003a) suggested that live food supplementation should start from stage VII onwards, using food particles increasing from 250 to 1190 μm. Therefore, the dimensions of decapsulated cysts may be appropriate for stage VII and VIII *M. rosenbergii* larvae only.

Replacing *Artemia* nauplii by artificial diets at a constant ratio of 50% from larval stage V–VI onwards (in experiment 1) negatively affected survival rate, but did not affect larval growth. This may be explaining by the drastic and sudden reduction of live feed in these treatments. In these treatments live feed was supplied only one time per day in the evening, and consequently the live feed density during the day time was low. Especially in the early period of weaning, the larvae may not have been adapted yet to non–living feed, probably resulting in low survival due to increased cannibalism. Indeed, when the larvae were more gradually weaned from *Artemia* onto formulated feeds (experiment 2), better results were obtained. Therefore, it is recommended to replace only 25% of the *Artemia* ration at the start of the weaning period to allow the larvae to adapt to the new 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. In this respect, it was found that the Gromate feed, which had a rather narrow particle size range of 150–500 µm showed lower results compared to the wet and flake diets. Although the Gromate 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 and flake diet could easily be sieved into the desired particle sizes using sieves with different mesh sizes.

In the present study, artificial diets were supplied from day 8 (stage V–VI) 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. Barros & Valenti (2003a) stated 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. However, it is necessary to evaluate final survival rates and productivity when applying total substitution of *Artemia* for commercial larviculture. Murthy et al., (2008) suggested that using wet diets which contain shrimp and clam meat fed to larvae in combination with *Artemia* nauplii showed larval survival rates of 40% in 150–l rearing tanks. Islam et al. (2000) reported 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. (2002) studied 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 solely *Artemia* nauplii. In the current 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 qual-

ity. However, performance was impaired when the *Artemia* diet was abruptly replaced at a constant rate of 50% from day 8 onwards. In practice production efficiency depends on the production cost, which is based on the feed source and cost, labour cost, etc., cost–effectiveness may therefore vary from one region to another. Therefore, the feeding strategy in *M. rosenbergii* larviculture cannot be standardized. The results obtained in the present work may however serve as a guideline for practical considerations of feeding strategies.

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