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Food intake of *Macrobrachium rosenbergii* during larval development

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Abstract

This work investigates the acceptance of different food types and sizes by *Macrobrachium rosenbergii* during each larval stage. Food intake of dry and wet formulated diets of four different size classes (250–425, 425–710, 710–1000 and 1000–1190 μm), as well as *Artemia* nauplii, was determined. Larvae of each zoeal stage were stocked in beakers and fed ad libitum. After 30–45 min, the digestive tract of each larva was observed under a stereomicroscope. Acceptance was evaluated by food intake frequency (FFI). There was no significant interaction ($P<0.05$) between inert diet size and FFI for each larval stage. Therefore, food intake during larval development is independent of food particle size. The ingestion of *Artemia* nauplii was significantly higher by larvae between stages II and VI. Between stages VII and XI, FFI for *Artemia* nauplii and wet diet was similar, while the FFI of the dry diet was similar to live food between stages IX and XI. The wet diet was ingested by more than 50% of the larvae only from stage VII onwards, while the dry diet from stage VIII onwards. These results indicate that larvae could be fed *Artemia* nauplii only until stage VI. Diet supplementation should start from stage VII onwards, using food particles varying from 250 to 1190 μm.

Keywords: Diet; Food intake larvae; *Artemia*; *Macrobrachium*

1. Introduction

The success on the commercial production of *Macrobrachium rosenbergii* postlarvae and seeds of other crustacean decapods depends on the efficient use of available food

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sources (Yúfera et al., 1984; Freeman, 1990). The dependence on live food imposes difficulties to define adequate and economical food management during the larval phase (Jones et al., 1997). Newly hatched *Artemia* nauplii constitute the principal live food used in the larviculture of crustaceans of commercial value. In spite of the advantages presented by this microcrustacean, such as easy handling and high protein content (Emmerson, 1984), the limited number of cyst production sites and the direct relationship between productivity and climate have caused peaks in cyst availability (Lavens et al., 2000). Stock vulnerability, great differences of cyst quality in terms of hatching rate and nutritional composition, in addition to the booming shrimp and fish hatchery industry, have increased cyst prices considerably (Lavens et al., 2000), thus, making food one of the most expensive items for the producers. For this reason, several alternative foods, live and inert, are being investigated as either supplement or replacement for *Artemia* nauplii in crustacean hatcheries (Aniello and Singh, 1982; Meyers and Hagood, 1984; Jones et al., 1987; Lovett and Felder, 1988; Kurmaly et al., 1989; Samocha et al., 1989; Alam et al., 1995a,b; Silva and Rodriguez, 1997).

Some authors believe that *Artemia* nauplii suffice to produce *M. rosenbergii* postlarvae (Devresse et al., 1990; Lavens et al., 2000). However, several others believe that *Artemia* nauplii do not fulfill the nutritional requirements of larvae during the last zoeal stages and therefore recommend the use of supplemental diets (Daniels et al., 1992; Alam et al., 1995b; New, 1995; Valenti et al., 1998; Valenti and Daniels, 2000). While penaeid larvae presented good survival and developmental rates when cultivated with rotifers or micro-encapsulated diets, *M. rosenbergii* larvae did not respond favorably to *Artemia* nauplii replacement (Lavens et al., 2000).

Low efficiency of inert diets has been attributed to the limited knowledge of larva nutritional needs (Jones et al., 1979a,b; Wilckenfeld et al., 1984; Sorgeloos and Léger, 1992). However, the success of formulated diets for crustacean larvae depends on other factors in addition to nutritional value. Before essential nutrients can be completely evaluated, behavioral, mechanical and physiological processes that occur during feeding must be known (Jones et al., 1997). Food should be perceived, captured, accepted and efficiently ingested by the larvae. Thus, particle size, consistency, texture and density of inert food may influence selection and consequently, ingestion by the larvae.

Currently, the majority of commercial *M. rosenbergii* hatcheries utilize newly hatched *Artemia* nauplii during the entire larval cycle in addition to a wet diet (known as egg custard) or dried supplement (Correia et al., 2000; Valenti and Daniels, 2000; Lavens et al., 2000). However, diets and feeding management result from empirical observations, since little is known about the feeding behavior and nutritional needs of larvae (Valenti and Daniels, 2000). Some authors report that particle size of inert food is specific for each larval stage (Aquacop, 1977; Corbin et al., 1983; New and Shingholka, 1985; Daniels et al., 1992; New, 1995), while others verified capture and ingestion of several size particles during all larval stages (Sick and Millikin, 1983; Barros, 1996).

Optimization of feeding efficiency depends both on adequate physical characteristics of the food and establishing specific diets for each larval stage (Valenti and Daniels, 2000). Several feeding schedules are used in *M. rosenbergii* larviculture, depending mainly on the culture system. However, there is a lot of controversy regarding from which stage onwards, supplemental diets should be offered.

Determining the suitable particle size and from which stage onwards inert and live foods are accepted by the larvae are of great importance. Feeding in excess causes organic matter to accumulate, which may trigger proliferation of bacteria and diseases, while underfeeding causes poor growth and enlarges culture cycle (Valenti and Daniels, 2000). In addition, overfeeding augments labor related to tank cleaning and management of water quality and larval health.

This work investigated the acceptance of inert food items and *Artemia* by *M. rosenbergii* during each larval stage. Initially, two inert diets (dry and wet) of different sizes were considered. Secondly, food intake of both diets and *Artemia* nauplii was determined.

2. Material and methods

This work was conducted at Crustacean Biology Laboratory, Aquaculture Center, UNESP-CAUNESP (Jaboticabal, SP, Brazil), in a controlled temperature room. Larvae were obtained from ovigerous *M. rosenbergii* females in reproduction ponds. A larviculture was established to supply larvae at different stages. Newly hatched larvae were stocked in a 120-l cylindrical tank, with conical bottom, equipped with heating and water recirculating system. Initial stocking density was approximately 90 larvae/l and water salinity was kept at about 12%. The main water parameters (temperature, pH, ammonia, nitrite and dissolved oxygen) were monitored and kept within the ranges recommended by Valenti et al. (1998) and Correia et al. (2000). Temperature, ammonia and nitrite were measured daily, while pH, salinity and dissolved oxygen, weekly. The larvae were fed dry and wet (egg custard) diets to excess, at 08:00 and 11:00, and newly hatched *Artemia* nauplii ad libitum (more than 6 nauplii/ml), in the afternoon, throughout the entire cycle.

Frequency of food intake (FFI) of inert and live diets was studied for each larval stage of *M. rosenbergii*, except stage I, since these larvae do not feed (Mooler, 1978; Barros and Valenti, 1997). FFI means the proportion of larvae that ingested a particular feed. Two experiments were conducted. In the first one, four size classes of both dry and wet diets were tested. In the second experiment, FFI of newly hatched *Artemia* nauplii and both diets was investigated. Dry and wet diets presented different formulation, color and consistency. The sequence in which the experiments were carried out followed the larval development cycle. Batches of larvae were randomly sampled from rearing tank as they molted to a new stage to perform every trial. The identification of the larval stages was done according to Uno and Kwon (1969). Larval condition was checked before each trial according to Aquacop (1983). The duration of each experiment was 34 days.

2.1. Inert diet preparation

2.1.1. Dry diet

Dry diet contained more than 90% of dry matter, and presented a dark red color (due to presence of iron oxide) and a rigid consistency. The ingredients used in this diet are common in aquaculture feeds. Corn, wheat, soybean and fishmeal were purchased, while shrimp meal was prepared at Laboratory of Nutrition of Aquatic Organisms, CAUNESP.

Composition and nutritional value of dry diet is shown in Table 1. Proximate analysis was performed according to AOAC (1984).

The ingredients were ground, weighed and homogenized. Iron oxide, 1% dry weight, was added to facilitate the observation of diet ingestion in the digestive tract. Warm water was then added to obtain a cream, which was then oven-dried at 60 °C for 24 h. The dried mixture was ground and sieved using different mesh screens to obtain four size classes: 250–425, 425–710, 710–1000 and 1000–1190 μm.

2.1.2. Wet diet

The wet diet consisted of the egg custard, one of the main foods used to supplement the diet of *M. rosenbergii* larvae (Valenti and Daniels, 2000). Dry matter content is lower than 20% and it presents a yellow color and a soft consistency. Table 1 shows the composition and nutritional value of the wet diet. Proximate analysis was performed according to AOAC (1984).

The ingredients 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 frozen at − 18 °C. Before being

Table 1
Composition and nutritional value (based on 100% dry matter) of dry and wet diet

Composition			
Dry diet		Wet diet	
Ingredients	(%)	Ingredients	(%)
Corn meal	8.0	Homogenized chicken eggs	34.0
Wheat meal	12.0	Mussels	10.0
Soybean meal	22.0	Fish fillet	10.0
Fish meal	27.0	Dried milk	4.0
Shrimp meal	30.0	Wheat flour	2.0
Vitamin and mineral supplement[a]	1.0	Cod liver oil	0.8
		Vitamin and mineral supplement[a]	1.4
		Water	37.8
Total	100	Total	100

Proximate analysis		
	Dry diet (%)	Wet diet (%)
Crude protein	43.42	45.07
Fat	6.14	22.55
Nitrogen-free extract	28.83	23.55
Minerals	11.89	8.83
Original dry matter	90.28	18.29
Gross energy (kcal/kg)	4213.39	4989.20

[a] Agromix AC 50—vitamin and mineral supplement, which contains per kilogram: vitamin A = 176.000 I.U., vitamin D_3 = 40.000 I.U., vitamin E = 500 mg, vitamin K_3 = 36 mg, vitamin B_{12} = 560 mg, niacin = 700 mg, biotin = 3 mg, pantothenic acid = 500 mg, folic acid = 30 mg, choline = 20 mg, iron = 1.100 mg, copper = 300 mg, manganese = 1.800 mg, zinc = 1.200 mg, iodine = 24 mg, selenium = 3 mg, methionine = 20 mg, calcium = 176 mg, phosphorus = 68 g, sodium = 23 g, chlorine = 36 g, B.H.T. = 1 g, q.s.p. = 1.000 g.

fed to the larvae, the pieces were made into smaller particles, which were then sieved with different mesh screens as described earlier for the dry diet.

2.2. Frequency of food intake of dry and wet diets of different sizes

Four size classes (250–425, 425–710, 710–1000, 1000–1190 μm) of both dry and wet diets were tested for larval stages II–XI. An experimental design, $2 \times 4 \times 10$ factorial (diet type × pellet size × larval stage), with three replications, was used.

Five larvae at the same zoeal stage were placed in 100-ml beakers filled with 80 ml of water (62.5 larvae/l) from the larviculture tank, which had been previously filtered using a 125-μm mesh nylon screen. Beakers were placed in boxes subdivided in 12 black compartments. Each beaker was provided with enough aeration to keep the particles in suspension and avoid stressing the larvae. Following a 2-h starvation, larvae were fed the respective dietary treatment ad libitum. After 30 min, the larvae were pipetted out of the beaker and placed in Petri dishes. The digestive tract of each larva was analyzed under a stereomicroscope and the number of larvae with any quantity of feed in the stomachs was recorded. Frequency of food intake (FFI) was then calculated for each replicate as follows:

$$\mathrm{FFI} = (\text{number of larvae that ingested feed}) / (\text{total number of larvae in the beaker})$$

FFI data were arcsine transformed and subjected to a three-way ANOVA, followed by Tukey test. Differences were significant when $P < 0.05$.

2.3. Frequency of food intake of Artemia nauplii, dry and wet diets

Newly hatched *Artemia franciscana* nauplii (Great Salt Lake stock) as well as dry and wet diets of 1000–1190 μm were fed to the larvae. An experimental design, 3×10 factorial (food type × larval stage), with seven replications, was used.

Ten larvae in the same zoeal stage were placed in beakers filled with 500 ml of water (20 larvae/l) from the larviculture tank that had been previously filtered in 125-μm mesh nylon screen. The beakers were wrapped in a black polyethylene film to avoid excess of light and provided with aeration. Following a 2-h starvation, the different diets were supplied ad libitum. Each diet was tested separately using new groups of larvae. After 45 min, larvae were pipetted from the beaker and placed in Petri dishes. The digestive tract of each larva was analyzed under stereomicroscope in order to record the number of larvae that had feed in the stomachs. The frequency of food intake (FFI) of each item for every replicate was calculated as explained before. FFI data were arcsine transformed and subjected to a two-way ANOVA, followed by Tukey test. Differences were significant when $P < 0.05$.

3. Results

Average values of water temperature, salinity and pH in the larviculture tank during the first and second experiments were 28.9 and 30.0 °C, 13.6% and 12.9%, and 8.3 and 7.9,

respectively. Ammonia and nitrite varied between 0.0 and 0.1 mg/l and light intensity was about 200 lx. Dissolved oxygen was close to saturation point. It is assumed that the water in the beakers presented the same characteristics. Survival in the larviculture tank at the end of experiment was estimated at about 50% and larvae presented good condition throughout culture.

3.1. Frequency of food intake for dry and wet diets of different sizes

Fig. 1 shows the frequency of food intake of all four size classes for both dry and wet diets during larval development. A three-way interaction was not observed. However, the

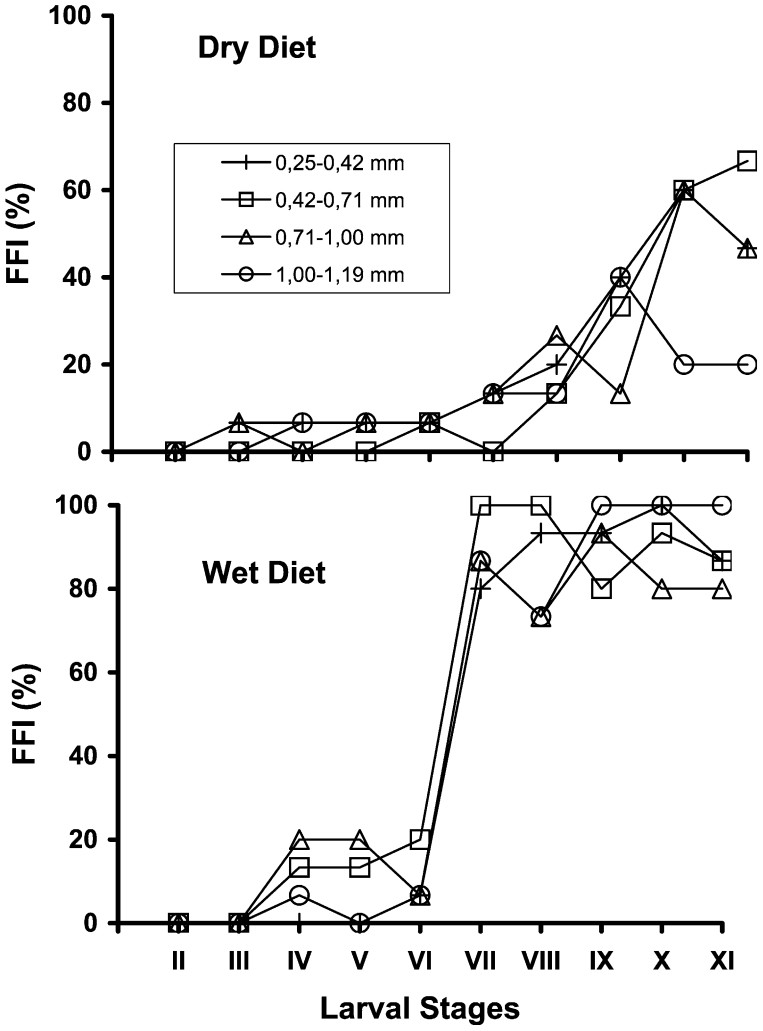

Fig. 1. Frequency of food intake (FFI) with respect to size classes of dry and wet diets for each larval stage.

interaction between diet type and larval stage was statistically significant. Significantly higher acceptance of diets at the end of the larval cycle (VII–XI for the wet diet and IX–XI for the dry diet) was observed. The intake of wet diet increased sharply from stage VII onwards, being ingested by more than 70% of the larvae, while the ingestion of the dry diet was significantly lower. In general, the ingestion of dry diet increased gradually from stage VIII onwards. On the other hand, there was no significant interaction between particle size and diet type and between particle size and larval stage; therefore, wet and dry diets of all sizes were accepted at similar levels by larvae at the same stage of development.

3.2. Frequency of food intake for Artemia nauplii, dry and wet diets

The interaction between food type and larval stage was statistically significant. The ingestion of *Artemia* nauplii varied between 70% and 100%, with no significant differences among larval stages. Between stages II and VI, live-food intake was significantly higher compared to both inert diets. Between stages VII and XI, FFI of *Artemia* nauplii and wet diet was similar, while FFI of the dry diet was similar to live food only between stages IX and XI (Fig. 2).

A similar pattern was observed for food intake of both inert diets along larval development (Fig. 2). During initial larval stages (II–V), similar number of larvae ingested rations, which varied between 15% and 40%. At stage VI, although dry-food intake was significantly lower than wet food, the rate of ingestion for both diets remained between 10% and 40%. From this stage onwards, food intake of both diets increased. Wet-diet intake increased significantly from 35% for stage VI to 70% for stage VII, and about 90% for the following stages (VIII–IX). Dry-diet intake followed a similar pattern. However, only from stage VIII onwards, more than 50% of the larvae ingested the diets. For stages IX, X and XI, values were significantly higher and FFI was between 80% and

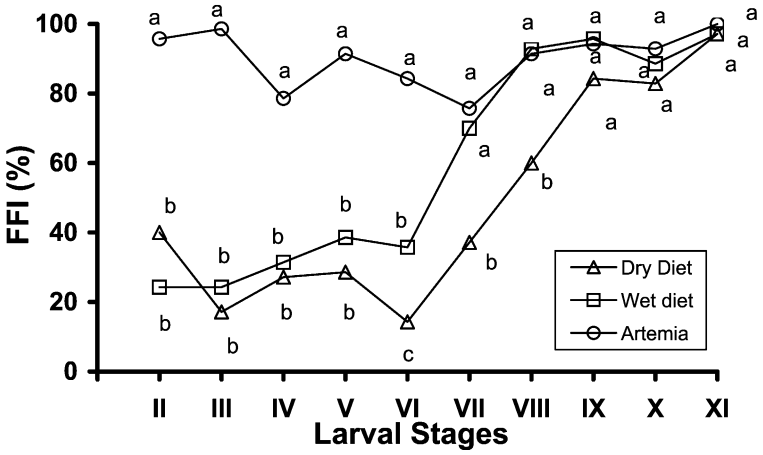

Fig. 2. Frequency of food intake (FFI) of live, inert (dry and wet) diets for each larval stage. Different letters indicate significant differences among food types.

90%. It is similar to values obtained for two other food types (*Artemia* nauplii and wet diet) (Fig. 2).

4. Discussion

M. rosenbergii larvae fed indistinctly on diet of all sizes. Particle size in the 250–1190-μm range may be captured and ingested without any preference. This indicates that the common practice of breaking the food into variable size pellets during larval development (Corbin et al., 1983; New and Shingholka, 1985; Daniels et al., 1992; Alam et al., 1993; Correia and Castro, 1998; Valenti et al., 1998) is not necessary. Larvae are probably able to break food particles using buccal apparatus down to sizes compatible with their ingestion capacity. Studies with other decapod species, such as *Penaeus marginatus* (Gopalakrishnam, 1976), *P. monodon* (Jones et al., 1979a; Kurmaly et al., 1989), *P. indicus* (Emmerson, 1984), *P. kerathurus* (Yúfera et al., 1984), *P. vannamei* and *P. stylirostris* (Jones et al., 1987) and *P. semisulcatus* (Samocha et al., 1989) have shown that several size particles are ingested by larvae. In the present study, the difference in food intake frequency (FFI) between the two diets with the same particle size observed from stage VII onwards (Figs. 1 and 2) indicates that color, consistency or composition of the diet may influence food intake more than particle size.

In the first trial, inert diets were ingested from stages III (dry diet) and IV (wet diet). However, in the second trial food ingestion occurred from stage II and FFI values were comparatively higher. This difference may be attributed to the different methodology applied. The water volume used in the second trial (500 ml) might have caused the particles to disperse more and remain in suspension for longer periods of time, therefore increasing the chances of being captured by the larvae.

The great increase in the FFI of wet diet from stage VII onwards may be explained by changes in the perception ability and/or selectivity of food after capture. This behavior might be due to changing morphophysiological characteristics of the larvae during development. In the beginning (stages II–VI), mechanoreception seems to be the only mechanism used to detect food (Barros and Valenti, 1997). The mobility of *Artemia* nauplii allows its permanence in the water column, thus, increasing the chances of encounter. In addition, larvae in the initial zoeal stages (I–III) present mandible characteristic of carnivorous larvae (Jones et al., 1997). As development occurs, mandibular teeth increase and become rounder, thus, indicating that the larva becomes omnivore (Jones et al., 1997). The development of the digestive tract and increase of enzyme activity from stage VI onwards (Kamarudin et al., 1994; Kumlu and Jones, 1995) may also help to explain the increasing acceptance of inert diets, since digestion processes become thoroughly functional. According to Agard (1999), the beginning of effective feeding occurs at stage V[1] (which corresponds to stage VI described by Uno and Kwon, 1969), when yolk reserves disappear and the digestive tract is completely developed. The increase

[1] Agard (1999) used the description of *M. rosenbergii* larval stages defined by Gomez Diaz and Kasahara (1987), who identified 17 larval stages during development.

in the specific activity of amylase from stages VII–VIII onwards (Kamarudin et al., 1994) indicates the change of larvae feeding habits from carnivore to omnivore, which coincides with the increased ingestion of inert diets observed in the present work. The ability to feed on nonliving food items is ecologically important, not only as a supplemental diet when zooplankton concentration is low, but also to provide a broader biochemical diversity to cover larval nutritional requirements (Harms and Seeger, 1989). Stage VII larvae and onwards are more capable of exploring food resources from the medium and, possibly present increased nutritional demands.

The preference of larvae from stages VI, VII and VIII to ingest wet rather than dry diets might indicate a preference for clear and soft particles during this transition period. Meyers and Hagood (1984) observed that *M. rosenbergii* larvae from stage VI onwards preferred particles of diets with lighter colors. The comparatively lower FFI values for dry diet suggest that larvae may have a higher difficulty in perceiving dark particles and/or handling and ingesting more rigid particles. On the other hand, the wet diet presented physical characteristics similar to *Artemia* nauplii, which is advantageous since artificial diets should have the shape, physical and tactile properties of the natural diet (Dall and Moriarty, 1983). Similar to our results, Pillai and Mohamed (1973) verified that *Artemia* nauplii were quickly accepted by *M. idella* larvae of every stage, while a dry diet was barely accepted and minced meat of shrimp was well accepted from stage IV onwards.

It seems that in the final stages (IX–XI), *M. rosenbergii* larvae fed indistinctly on inert diets and *Artemia*, demonstrating that the animal becomes more generalist and omnivorous, and consumes food according to availability. This increase in the feeding spectrum by more developed larvae may be attributed to higher nutritional needs. Moreover, this could also indicate a higher capability of food perception.

Determining the initial stage at which inert diets are effectively ingested by larvae is very important from a practical and economical viewpoint. Currently, supplemental diets have been introduced at different larval stages. New and Shingholka (1985) recommend egg custard from stage III onwards (about the fourth day of culture), while Carvalho Filho and Mathias (1998) suggest the use of supplementary food from stages IV to V (between the sixth and eighth day of culture). Aquacop (1983) and Daniels et al. (1992) recommend diet supplementation from stages V toVI (between the 8th and 10th day) and Valenti et al. (1998) between stages VI and VII (between the 10th and 14th day). However, the results presented here indicate that only from stage VII onwards the larvae are able to accept inert food items satisfactorily. Therefore, supplying inert food before stage VII results in waste, increases production costs and deteriorates the quality of the water.

Total replacement of live feed by wet and dry diets may be feasible in stages VII and IX, respectively, since FFI was not significantly different for *Artemia* nauplii. Furthermore, *M. rosenbergii* larvae already present digestive and enzymatic systems completely functional at this stage of development (Kamarudin et al., 1994; Kumlu and Jones, 1995; Jones et al., 1997). This may represent a great economy of *Artemia*, which have become scarce and expensive presently. However, it is necessary to evaluate the final results for survival rates and productivity when this feed management is applied in commercial larviculture. A balanced diet should be developed to totally substitute *Artemia*. Labor costs also need to be evaluated since it is deemed to increase as formulated feed should be offered more

frequently, and cleaning of the tanks will also be more labor-intensive. The proportional use of *Artemia* and formulated feeds depends on the cost-effectiveness and is site-specific.

Based on the present results, it is suggested that *M. rosenbergii* larvae should be fed *Artemia* nauplii exclusively up to stage VI. From stage VII to VIII, the diet should be supplemented with wet food (250–1190-μm pellets) and from stage IX onwards, supplemental diet could be either wet or dry food (250–1190-μm pellets), depending on cost and availability. This management should reduce the amount of food used, labor to prepare the ration and clean the tanks, which can represent considerable savings. However, large-scale trials should be carried out to confirm whether results obtained from beakers are valid. Larval behavior may be different in larviculture tank, which is larger and different in shape. On the other hand, the relationship between the feeding schedule and its cost-effectiveness varies geographically. Therefore, feeding strategy in any *M. rosenbergii* larviculture cannot be standardized. Results obtained in the present work may subsidize future research works and serve as a guideline for practical considerations of feeding strategies.

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