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RESEARCH ARTICLE

Effects of *Thermocyclops decipiens* and *Artemia* Nauplii for Larval Rearing of *Macrobrachium rosenbergii* (De Man, 1879)

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Key words:

M. rosenbergii
Live-feed
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Biochemical profile

Abstract: This study examines the effects of the freshwater cyclopoid *Thermocyclops decipiens* and *Artemia* nauplii on the growth and survival of *Macrobrachium rosenbergii* (De Man, 1879) larvae during the rearing phase. *M. rosenbergii* larvae were divided into three groups and fed exclusively with either *Artemia* nauplii or *T. decipiens*, and a mixed diet (50% *T. decipiens* and 50% *Artemia* nauplii) in triplicate. The results indicated that *M. rosenbergii* larvae reached 90% post larvae (PL) on the 23rd day of the mixed diet feeding regime, followed by 88% and 82% PL on the 24th and 26th days when fed with *Artemia* nauplii and *T. decipiens*, respectively. The highest length and weight of *M. rosenbergii* PL were observed in the mixed diet treatment with 14.37±0.51mm and 0.76±0.04mg, respectively. The specific growth rate and percentage weight gain were significantly ($p < 0.05$) higher in the mixed feeding treatment. However, the survival of larvae was highest (69.89±4.55%) in the *Artemia* nauplii treatment. The larval stage index (LSI) of *M. rosenbergii* larvae fed on different feeding regimes was mixed diet > *Artemia* nauplii > *T. decipiens*. The biochemical constituents of *M. rosenbergii* PL showed that protein concentration was higher in the larvae fed with *T. decipiens*, while carbohydrate and lipid content were also high in mixed feeding regimes. Results indicated that the larval stage index and growth parameters of *M. rosenbergii* larvae were highest in the mixed diet treatment.

Anahtar kelimeler:

M. rosenbergii
Canlı yem
Büyüme parametreleri
Biyokimyasal profil

Dev Tatlısu Karidesi'nin (*Macrobrachium rosenbergii* De Man, 1879) Larval Yetiştiriciliğinde *Thermocyclops decipiens* ve *Artemia* Nauplii'nin Etkileri

Öz: Bu çalışma, tatlısu siklopoidi *Thermocyclops decipiens* ve *Artemia* nauplii'nin *Macrobrachium rosenbergii* (De Man, 1879) larvalarının yetiştirme döneminde büyümesi ve hayatta kalması üzerindeki etkilerini incelemektedir. *M. rosenbergii* larvaları üç gruba ayrıldı ve *Artemia* nauplii, *T. decipiens* ve karma bir diyet (%50 *T. decipiens* ve %50 *Artemia* nauplii) ile 3 tekerrürlü olarak beslendi. Sonuçlar, karma diyetle beslenen *M. rosenbergii* larvalarının, 23. gününde %90 PL aşamasına ulaştıklarını ve bunu sırasıyla 24. günde %88 ile *Artemia* nauplii ve 26. günde %82 ile *T. decipiens* ile beslenen larvaların takip ettiğini göstermiştir. *M. rosenbergii* PL'nin en yüksek boy ve ağırlığı sırasıyla 14.37±0.51mm ve 0.76±0.04mg ile karma diyet uygulamasında gözlendi. Spesifik büyüme oranı ve yüzde ağırlık artışı, karma besleme uygulamasında önemli derecede daha yüksekti ($p < 0.05$). Bununla birlikte, *Artemia* nauplii uygulamasında larvaların yaşama oranı en yüksek (%69,89±4,55) olmuştur. Farklı besleme rejimlerinde beslenen *M. rosenbergii* larvalarının larva evre indeksi (LSI) karma diyet > *Artemia* nauplii > *T. decipiens* şeklinde gerçekleşmiştir. *M. rosenbergii* PL'nin biyokimyasal bileşenleri, *T. decipiens* ile beslenen larvalarda protein konsantrasyonunun daha yüksek olduğunu, karma besleme rejimlerinde karbonhidrat ve lipid içeriğinin de yüksek olduğunu göstermiştir. Sonuçlar, *M. rosenbergii* larvalarının larva evre indeksi ve büyüme parametrelerinin karma diyet uygulamasında en yüksek olduğunu göstermiştir.

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii*, are the commercially important aquaculture species in India. The utilization and nutritional quality of food are critical to the success of prawn larval rearing. (Yufera et al., 1984; Freeman, 1990). Choice of food, on the other hand, is the bottleneck for the larval rearing of prawn larvae (Alam et al., 1991). Food and feeding affect the growth and survival of the early stages of larvae (Alam et al., 1996). Effective *M. rosenbergii* culture is a major issue, particularly the supply

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of food and nutrition to their larvae (Hanson and Goodwin, 1977). *Artemia* nauplii are suitable for early stages of fish, as reported by many authors (Aniello and Singh, 1982; Sandifier et al., 1976; New, 1990), and demand is increasing as a result of the unpredictability of supply and the high cost of their cysts (Bengtson et al., 1991).

The reliance on live food necessitates challenges with adequate food supply and management during the prawn larval phase. (Jones et al., 1993; Dhont et al., 2010; Valenti et al., 2010). *Artemia* nauplii are utilized for *M. rosenbergii* larval rearing, and in such conditions, the production of larvae is high-cost. Other zooplankton have been used as live feed for the early stages of finfish and shellfish (Manickam et al., 2020). Alam et al. (1993) suggested that *Moina micrura* be considered as a supplement to *Artemia* nauplii, and Ling (1969) stated that rotifers, cladocerans, and copepods may be suitable live feed to *M. rosenbergii* larvae. However, zooplankton mass production continuous systems is a major problem (Rasdi et al. 2020). In our study, mass culture *T. decipiens* was used for the rearing of *M. rosenbergii* larvae. Therefore, reliable continuous culture method is adopted to maintain the *T. decipiens* densities during culture.

Suitably prepared live feed plays an important role in the rearing of *M. rosenbergii* larvae. In the last two decades, *M. rosenbergii* has been studied in relation to cultural aspects. Zooplankton contains more nutrients than *Artemia* nauplii (Rajkumar et al., 2004). The nutritional values of zooplankton are evaluated by various authors (Watanabe et al., 1983; Safiullah, 2001; Aman and Altaff, 2004; Manickam et al., 2017). Hence, the present study aims to determine the effects of *T. decipiens* on the growth and survival of *M. rosenbergii* larvae. Our findings have the potential to contribute to the development of improved larval rearing techniques for more efficient hatchery production of *M. rosenbergii.*

Material and Methods

Culture of *T. decipiens*

Zooplankton samples were collected using a bolten silk plankton net (50 µm) from Chetpet Pond, Chennai, India, during the early hours of the day. They were transported to the laboratories immediately. *T. decipiens* was sorted out from the samples using a binocular stereomicroscope. The inoculum of *T. decipiens* (50nos./l) was introduced into the culture tanks (Altaff and Sivakumar, 2003; Sivakumar, 2015). *T. decipiens* were cultured using a combination of chicken manure (150ppm) and mixed algae (*Pennate* sp., *Eurastrum* sp. and *Stephanodiscus* sp.;4.25 x 10^4 cells/ml) in 25 l fiberglass tanks. The cultured species were harvested from the culture tank and fed to the prawn larvae.

Hatching of *Artemia* nauplii

The *Artemia* cysts were hatched out in a cone-shaped culture tank under controlled conditions. The water medium was maintained at 25 ppt salinity, pH 8 and temperature ranged between 28-30 ºC. A luminous bulb placed above the hatching cone provided sufficient heating for hatching. For optimum hatching results, the cysts were illuminated (2000 lux illumination) during the entire incubation period. Constant aeration was provided and a 3-4 ppm oxygen level was maintained. A total of 75 mg cysts were introduced into the hatching medium and incubated for 24 hours. After hatching, instar I was used for the rearing of *M. rosenbergii* larvae.

Preparation of egg custard

One hundred grams of fish meal were blended using a blender. It was passed through a muslin cloth; six whole eggs were added and blended; the mixture was steamed with 250 ml of water until it solidified into custard followed by the other ingredients added as shown in Table 1. The screened egg custard was stored for a few days.

Table 1. Formulation of egg custard

Ingredients	Formula
Fish meal (gm)	100
Skimmed milk (gm)[1]	250
Whole (yolk and white) chicken eggs Nos.)	6
Wheat flour (g)[2]	250
Vitamin C (mg)	250
Vitamins A and D (ml)[3]	2.5
Vitamin B complex (mg)	125
Tetracycline (mg)	250
Calcidol (ml) [4]	10

[1] Amul, India
[2] Wheat flour is a powder made from the grinding of wheat
[3] Vitamin A and D each 1.25ml
[4] Every 5 ml of calcidol contains Calcium Carbonate Eq to Elem.Cal 125 mg Vitamin D3 62.5 IU Elemental Magnesium (as Hydroxide) 10 mg Elemental Manganese (as Sulphate) 0.5 mg Elemental Zinc (as sulphate) 2.5 m Elemental Boron(as sodium borate) 62.5 mcg

Experimental setup

M. rosenbergii, larval rearing experiment was conducted for 26 days at Aqua-Nova Hatcheries, Kanathur, Chennai, India. Water quality parameters were maintained as follows: salinity 12 ppt, temperature 28 - 31ºC and hardness of 60 - 100 ppm.. The culture water was filtered and chlorinated for 3 to 4 days and then dechlorinated before use.

For the experiment, 25 l of 12 ppt dechlorinated water was filled in conical tanks. A total of 300 larvae (1 day after hatching (DAH); 1.90-1.96 mm) were introduced into each tank. The experimental tanks were provided with continuous vigorous aeration except during feeding and cleaning. *M. rosenbergii* larvae were fed with *Artemia* nauplii (Treatment I), *T. decipiens* (Treatment II), and a mixed diet (50% *T. decipiens* and 50% *Artemia* nauplii) (Treatment III) in triplicate. Experiments were conducted when larvae reached the post larvae stage. The feed was broadcast thrice daily at 7.00 hrs, 13.00 hrs, and 18.00 hrs. The number of live-food organisms provided for the different stages of *M. rosenbergii* larvae is as follows (Table 2).

Table 2. *M. rosenbergii* larvae feeding program

Larval Stages	Feeding Regimes		
	Control	Experimental feed	
	Artemia nauplii	*T. decipiens*	Mixed diet
Stage 2 and 3	10-16 individuals/larva	10-16 individuals/larva	10-16 individuals/larva
Stage 4 to 7	20-46 individuals/larva	20-46 individuals/larva	20-46 individuals/larva
Stage 8 to PL	46-60 individuals/larva	46-60 individuals/larva	46-60 individuals/larva

For the larvae in the control and experimental tank, egg custard was provided as supplement feed at 10.30 hrs, and 22.30 hrs. During the experimental period, 40% of the tank water was replenished daily in the morning hours, when excess feed and faecal matter of the larvae were removed.

In addition, 1 ppm tetracycline was administered to each tank once a day. Daily measurements of dissolved oxygen (Lutron DO-5510 Electronic Dissolved Oxygen Meter) and salinity (Dual scale salinity refractometer ATC) were taken. When the larvae reached the post-larval stage, they were gradually acclimatized from brackish water to freshwater to avoid the physiological shock of sudden transfer from brackish water to freshwater.

Biochemical analysis

At the end of the experiments, a total of 10 PL were collected from each tank for biochemical parameters such as protein (Lowry et al., 1951), carbohydrate (Roe, 1955), and lipid (Folch et al., 1957).

Data collection and statistical analysis

M. rosenbergii larvae were sampled at end of the experiments (larvae that reached to PL) to measure length, weight, and survival on 5th, 10th, 15th, 20th, and 26th, day. The total length (TL) was measured using a 30-cm (0.1 mm) ruler. Analytical balances (precision of 0.01 g) were used to record body weight (BW). The larvae's specific growth rate (SGR), percentage weight gain (PWG), survival rate (Dash et al., 2014) and condition factor (Htun-Han, 1978) were calculated using the following equations:

$$SGR = \frac{\ln(\text{final weight of the larvae}) - \log(\text{initial weight of the larvae})}{\text{Experimental periods in days (t)}}$$

where:

SGR % = percentage increase in body weight per larvae per day

$$\text{Percentage weight gain (PWG)} = \frac{\text{Final weight of the larvae} - \text{Initial weight of the larvae}}{\text{Inital weight of the larvae}} \text{ x } 100$$

$$Survival\ (\%) = \frac{\text{Number of live fish counted}}{\text{Number of fish stocked}} \text{x} 100$$

Condition factor

$$Conditionfactor\ (CF) = \frac{\text{Weight of the larvae}}{\text{Length of the larvae}^3} \text{x} 100$$

Larval stage index (LSI) was calculated according to the following formula (Manzi et al., 1977)

$$LSI = (\sum Si\ x\ ni)/N^{-1}$$

where,

S_i is the larval/PL stage (I= 1–12), ni= number of animals in stage S_i and N= total number of animals observed. LSI= ranges varied from 1 to 10.

The experimental data was collected at the end of the experiment and statistically analyzed. Prior to analysis, the data were checked for normality and variance homogeneity using Levine's test. At the end of the experiment, the length and weight of the post-larvae from different feeding regimes were measured. Survival of the larvae was determined on the 5th, 10th, 15th, 20th, and 26th days of experimentation. The data was presented as mean ± standard deviation. Data were subjected to one-way ANOVA followed by Tukey's test to determine which treatments differed from each other ($p<0.05$). Random ANOVA was performed to calculate the survivorship of *M. rosenbergii* larvae (IBM SPSS for Windows, version 21.0. Armonk, NY: IBM Corp).

Results

M. rosenbergii larvae growth parameters are presented in Table 2. The effect of different feeding regimes was significantly different at the end of experimental period ($p < 0.05$). The length and weight of the *M. rosenbergii* larvae were 14.37±0.51 mm and 0.76±0.04 mg respectively, in the mixed diet feeding regimes and it was higher compared to those of other treatments (Table 3). The results of all the twelve stages gradually increased their length and weight linearly. However, during the larval stages I–IV, faster growth and high survival were recorded in *Artemia* nauplii treatments (Fig. 1). Subsequently, larval development was higher than those recorded in the mixed feeding regimes, and also 90% PL stage was reached on 23rd day of the experiment. However, larvae fed exclusively on either *Artemia* nauplii or *T. decipiens* reached 88% and 82% PL on the 24th and 26th

days, respectively. The final length and weight of larvae fed with different feeding regimes were significantly different ($p<0.05$). However, Tukey's test showed that the weight of the larvae fed with *Artemia* nauplii was not significantly different ($p>0.05$) compared to *T. decipiens* treatment. Regression analysis between the length and weight of *M. rosenbergii* larvae showed a positive relationship in three different feeding regimes (Figs 2a-c).

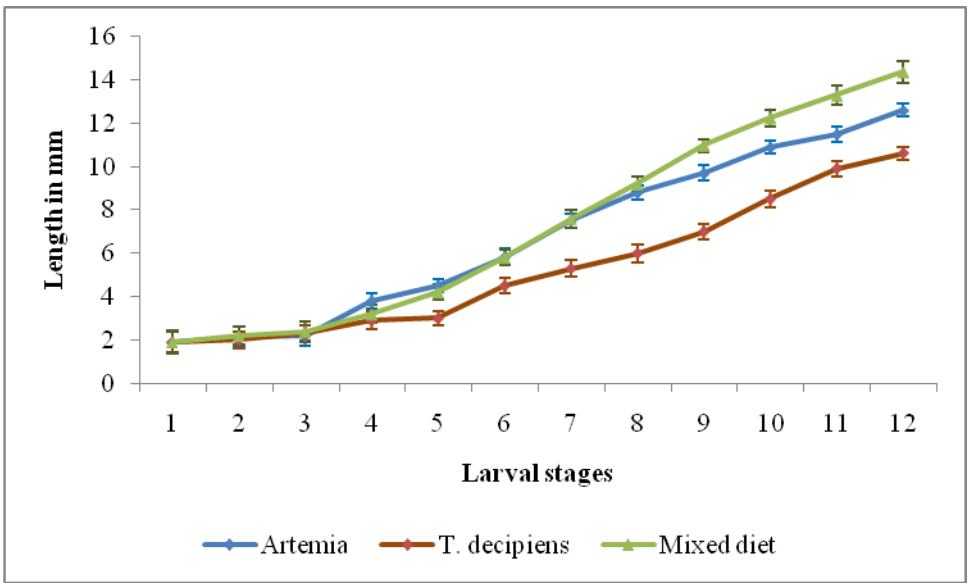

Figure 1. Length of different larval stages of *M. rosenbergii* larvae in different feeding regimes

Table 3. Growth and survival of *M. rosenbergii* larvae with different feeding regimes

	***Artemia* nauplii**	***T. decipiens***	**Mixed diet**	**F**	**P**
Initial length (mm)	1.96 ± 0.53^{a}	1.95 ± 0.05^{a}	1.94 ± 0.04^{a}	0.111	0.897^{NS}
Final length (mm)	12.60 ± 0.30^{a}	10.60 ± 0.30^{b}	14.37 ± 0.51^{c}	72.098	0.00^{*}
Initial weight (mg)	0.14 ± 0.02^{a}	0.13 ± 0.02^{a}	0.14 ± 0.01^{a}	0.056	0.946^{NS}
Final weight (mg)	0.68 ± 0.05^{ab}	0.62 ± 0.04^{b}	0.76 ± 0.04^{a}	9.257	0.02^{*}
SGR (%)	6.51 ± 0.21^{ab}	6.16 ± 0.20^{b}	7.00 ± 0.30^{a}	9.222	0.02^{*}
PWG (%)	409.36 ± 25.44^{ab}	306.75 ± 23.39^{b}	475.56 ± 42.34^{a}	9.058	0.02^{*}
LSI	4.34	3.66	4.94	-	-
CF	1.79 ± 0.08^{a}	1.95 ± 0.07^{b}	1.77 ± 0.03^{a}	7.752	0.02^{*}
Survivorship	χ^2 (9) = 25.901, p = 0.003; F (4,32) = 60.106, p = 0.000				

Values are represented as mean ± SD of triplicate. Letters denote significant differences ($p < 0.05$). – NS: Not Significant

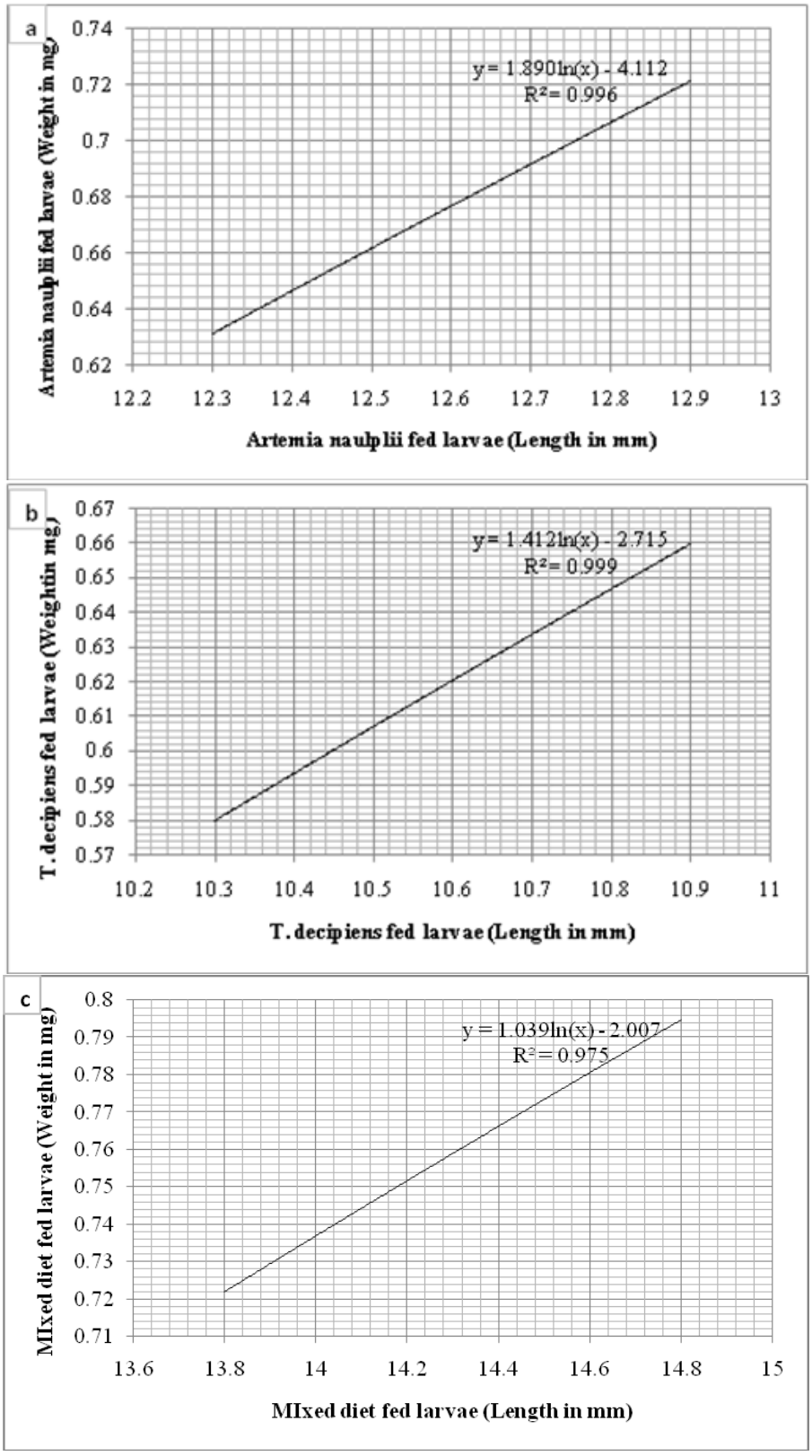

Figure 2. Regression analysis between length and weight of *M. rosenbergii* larvae fed in different feeding regimes

M. rosenbergii larvae had the highest specific growth rate and highest weight gain of 7.00 ± 0.30% and 475.56 ± 42.34%, respectively, in the mixed feeding treatment, followed by *Artemia* nauplii and *T. decipiens* treatments One-way ANOVA for growth parameters showed that their growth rates were significantly different ($p < 0.05$. The LSI of *M. rosenbergii* larvae in different feeding regimes showed the highest index (4.94) in mixed feeding regimes (Table 3). However, up to day 7 (DAH), the LSI was not significantly different between *Artemia* nauplii

(LSI = 3.2) and mixed diet (LSI = 3.6) feeding regimes (Table 4). The CF of all the larvae fed with either *Artemia* nauplii or mixed diet was isometric and their growth linearly increased throughout the experiment. However, larvae fed with *T. decipiens* showed the highest CF values (1.95 ± 0.07) with negative allometric growth. Tukey's test also confirmed that *T. decipiens* fed *M. rosenbergii* larval growth significantly differed from their linear growth compared with *Artemia* nauplii and mixed diet (Table 3).

Table 4. Larval stage index of *M. rosenbergii* larvae fed in different feeding regimes

Stages	Feeding Regimes		
	Artemia	***T. decipiens***	**Mixed diet**
1	1	1	1
2	1.8	1.4	1.8
3	2.7	2.1	2.7
4	3.2	2.8	3.6
5	3.5	3	4
6	4.2	3.6	4.8
7	4.9	4.2	5.6
8	5.6	4.8	6.4
9	5.4	4.5	6.3
10	6	5	7
11	6.6	5.5	7.7
PL	7.2	6	8.4

Survival rates of *M. rosenbergii* in three feeding regimes on various days were recorded as shown in Fig. 3. The highest survival was recorded in *Artemia* nauplii fed larvae, followed by mixed diets and *T. decipiens* fed larvae. The highest survival of 69.89 ± 4.55% was recorded in *Artemia* nauplii fed larvae, followed by mixed feeding regimes (48.11 ± 2.59%) and the lowest survival of 38.00 ± 5.03% was recorded in *T. decipiens* fed larvae on the 26th day. Tukey's test indicated that survival of larvae showed significant differences ($p < 0.05$) among their feeding regimes on the 5th and 26th days. However, no significant differences were observed between *T. decipiens* and mixed diet feeding regimes on the 10th, 15th, and 20th days ($p > 0.05$) (Fig. 3). The random ANOVA for survivorship of *M. rosenbergii* larvae fed with different feeds on various days significantly differed ($p < 0.05$) (Table 3). The general linear model (GLM) for the survival of *M. rosenbergii* showed that observed data was more than predicted values. Based on this calculation, the linear mortality rate increased (Figs 4a-e).

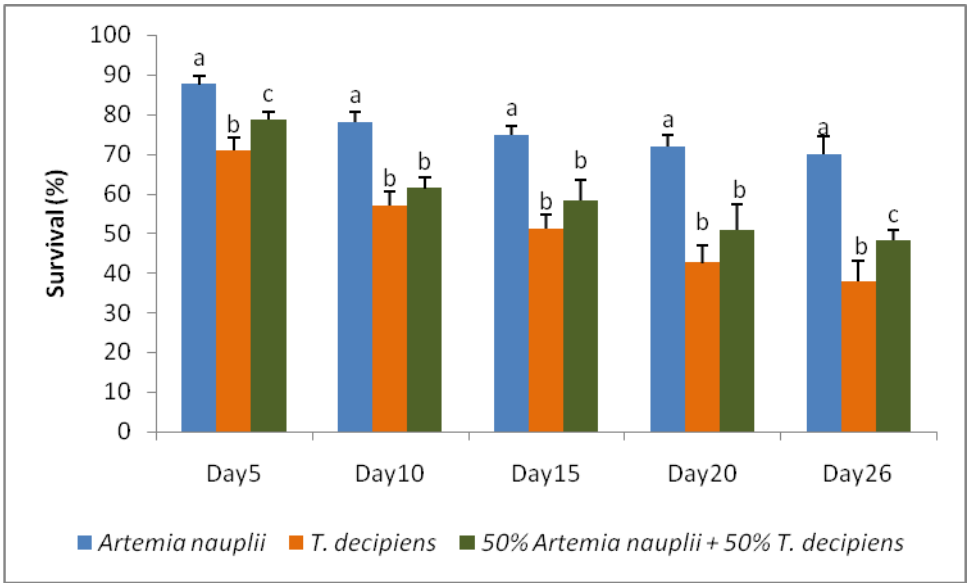

Figure 3. Effect of different feed on survival of *M. rosenbergii* larvae. Values are represented as mean ± SE. Letters denote significant differences ($p < 0.05$).

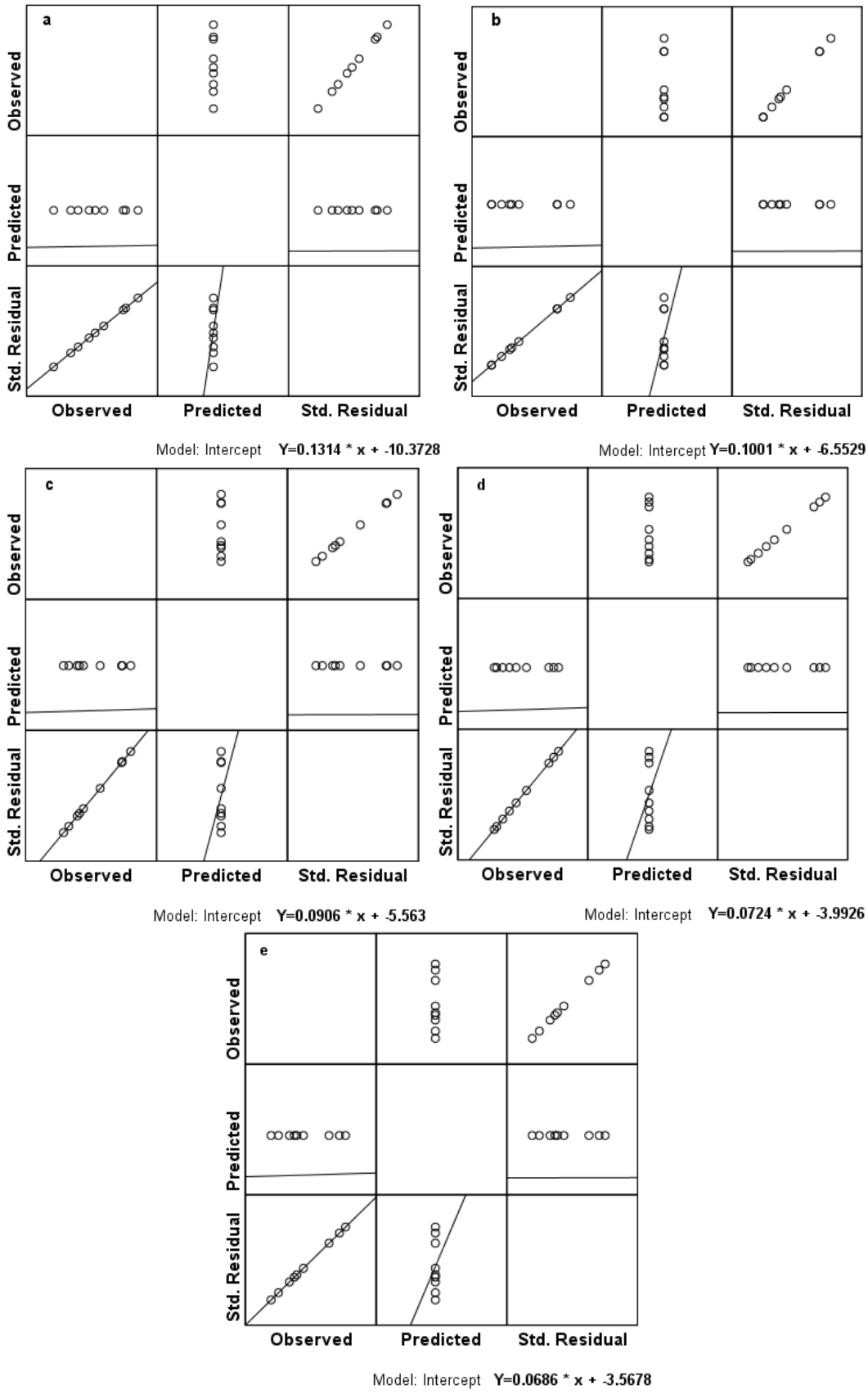

Figure 4. General linear model of survivorship of *M. rosenbergii* larvae fed in different feeding regimes; a. day 5, b. day 10, c. day 15, d. day 20, e. day 26

The biochemical profile of *M. rosenbergii* larvae fed with three different feeding regimes is presented in Table 5. The protein content of larvae fed with *T.decipiens* was higher (47.97 ± 0.35%), but carbohydrate (1.67 ± 0.03%) and lipid (8.03 ± 0.21) levels were higher in the mixed diet. Biochemical constituents of *M. rosenbergii* fed with different diets were significantly different ($p < 0.05$). Tukey's test showed that carbohydrate and lipid contents were not significantly different between *T. decipiens* and mixed diet. Protein content, on the other hand, was significantly higher ($p < 0.05$) in the *Artemia* nauplii fed group than those in other treatments (Table 5).

Table 5. Biochemical composition of *M. rosenbergii* larvae

	Protein (%)	**Carbohydrate (%)**	**Lipid (%)**
Artemia nauplii	43.74 ± 0.58^{a}	1.43 ± 0.06^{a}	7.35 ± 0.13^{a}
T. decipiens	47.97 ± 0.35^{b}	1.65 ± 0.05^{b}	7.14 ± 0.09^{b}
Mixed diet	46.57 ± 0.60^{c}	1.67 ± 0.03^{b}	8.03 ± 0.21^{b}
F	50.914	23.793	29.333
p	0.000^{*}	0.001^{*}	0.001*

Values are represented as mean ± SE. Letters denote significant differences ($p < 0.05$).

Discussion

In the present study, *M. rosenbergii* post larval production was successfully achieved using *Artemia* nauplii, mixed diet, as well as with *T. decipiens* diet. Results of the present study showed higher growth of *M. rosenbergii* larvae fed exclusively with *Artemia* nauplii; however, weight, SGR, and PWG were not significantly different in larvae fed either Artemia or *T. decipiens* ($p > 0.05$). In the *T. decipiens* treatment different life stages such as nauplii, copepodids and adults were introduced into the larval rearing tank as feed. The larger size of the adult *T. decipiens* (850-1100 µm) may have prevented feeding by *M. rosenbergii* larvae. This may be a reason for slower growth observed in *T. decipiens* fed larvae. CF also confirmed the negative allometric growth parameters in larvae fed exclusively with *T. decipiens.* In treatments fed with either *Artemia* nauplii or a mixed diet, isometric growth of the larvae was observed. Similar to the weight of the larvae, SGR and PWG were not significantly ($p > 0.05$) different between *T. decipiens* and *Artemia* nauplii fed larvae. However, SGR and PWG were significantly ($p < 0.05$) higher in the mixed diet than those in other feeding regimes.

The present study indicated higher survival of the *M. rosenbergii* larvae fed with *Artemia* nauplii than other feeds. However, it is worth noting that the total length of *M. rosenbergii* post-larvae fed on a mixed diet was higher but the survival rate was lower than that of *M. rosenbergii* larvae fed on the *Artemia* nauplii diet. Alam et al. (1993) reported that *M. rosenbergii* larvae fed on *Moina* had high mortality. In the present study, larvae fed on a diet of *T. decipiens* had higher mortalities larvae fed on*Artemia* nauplii showed a significantly higher survival rate than those of other feeding regimes. Islam *et al.* (2000) reported a high survival percentage of larvae fed on *Artemia* nauplii with egg custard compared to those fed exclusively on *Artemia* nauplii and rotifers. Manickam et al., (2020) reported that *M. rosenbergii* larvae fed with a mixture of rotifers, cladocera, and copepoda had significantly higher survival and growth than those fed only *Artemia.* Similarly, other researchers reported higher growth and survival when larvae were fed with live feeds (Sunyoto et al., 1995; Aman and Altaff, 2004; Santhanam et al., 2004; Simhachalam et al., 2015).

In the biochemical profile, protein was the major component followed by lipids and carbohydrates in *M. rosenbergii* (Roustaian et al., 2001). In the present study, a similar pattern was found in all the feeding regimes. The biochemical compositions of live feed play a significant role in larval rearing; protein, carbohydrates, and lipids ensure the physiological status of organisms. The zooplankton are a better source of biochemical constituents for aquatic organisms (Tidwell et al., 1997; Manickam et al., 2017). The present study results showed higher biochemical contents in *M. rosenbergii* larvae fed with *T. decipiens.*

From the results of the present study, it can be suggested that *M. rosenbergii* postlarvae can be produced with a mixed diet, which will reduce the cost of live-feed substantially in seed production. Furthermore, the higher total length of the post-larvae fed with a mixed diet indicates superior nutritional status of the developing larvae by the mixed diet compared to a single diet. However, the survival percentage of *M. rosenbergii* was high in *the Artemia* feeding regime. As *M. rosenbergii* is the most intensively cultured species, its seed production using only indigenous live-feed organisms or in combination with *Artemia* nauplii may provide cost-effective culture methods.

Conclusion

The hatchery seed production of *M. rosenbergii* post larvae was possible with a live feed of cyclopoid copepod. A low percentage of survival was recorded with this feed compared to *Artemia* nauplii. However, production of *M.*

rosenbergii post larvae using a mixed diet might result in the cost-effective production of seeds.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Sivakumar K: Conceptualization, Methodology, Investigation, writing- original draft Muthupriya M**:** Investigation, writing – reviewing data curation and editing Altaff K: Supervision, Validation and Formal analysis.

Ethics Approval

The material used in this article is invertebrate species therefore ethics committee approval is not required for this study.

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