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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of feeding different densities of *Artemia* nauplii on the growth and survival of larvae of the hairy river prawn, *Macrobrachium rude* (Heller, 1862)

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Abstract: The effects of feeding at different densities of *Artemia* nauplii on the growth and survival of *Macrobrachium rude* larvae were explored in this study. Two experiments were carried out. In the first trial, larvae were fed three different feeding densities: 1, 3, and 5 nauplii/mL. In the second experiment, feeding densities of 5, 10, and 15 nauplii/mL were used to determine the maximal feeding density of *Artemia* nauplii for *M. rude* larvae. There were no significant differences statistically in growth rate or survival between larvae fed 1, 3, or 5 nauplii/mL ($P>0.05$). Increased feeding density from 5 to 10 nauplii/mL resulted in growth but drastically decreased survival. Feeding above 10 nauplii/mL decreased both growth rate and survival. According to the results of the study, the optimal feeding density of *M. rude* with *Artemia* nauplii should be between 5 and 10 nauplii/mL. The study suggests further research into determining ideal feeding density at various phases of larval development in order to better understand the individual feeding requirements at each stage.

Keywords: Survival rate, total length, gain in length, water quality, broodstock

INTRODUCTION

Freshwater prawn of the genus *Macrobrachium* are decapods, palemonids and are found on all continents except Europe. *Macrobrachium* is a diverse group of about 240 species, showing great similarity (Makombu et al., 2015). *Macrobrachium* prawn has a very diverse habitat, distributed in rivers, lakes, ponds, irrigation ditches, and aqueducts in subtropical and tropical climates (Valencia and Campos, 2007). Although they spend most of their time in freshwater, the majority of prawn species migrate to brackish water, mainly during the reproductive and larval stages of development, to complete their life cycle (Lima et al., 1997; Barbieri et al., 2016). In general, *Macrobrachium* prawn reach an adult size large enough to be eaten by humans. Prawns are mainly the target of artisanal fishermen and an important source of income in many developing countries, especially in Africa (Nwosu and Wolfi, 2006). About 10 species of *Macrobrachium* prawns have been described from Africa (Makombu et al., 2015). Among the species of interest is the hairy prawn (*M. rude*), which is associated with the economy of small-scale fishermen in Thailand, India, Kenya and Tanzania (Schoonbee et al., 1989). *M. rude* is the most abundant freshwater prawn species in Kenya, accounting for about 11% of all prawns caught by small-scale fishermen (Kimani et al., 2018). It is distributed in two major freshwater rivers in Kenya, namely the Tana and Athi rivers where prawns are harvested by small-scale fishermen from these rivers and sold to local people as an important source of food, employment and livelihood for people in Tana River and Kilifi counties, Kenya (Kimani et al., 2018; Kochey, 2018). *M. rude* presence in both rivers has been reported in most months of the year except March, June, October and November, with a peak in January, April, September and May (Kochey, 2018). Due to overfishing and seasonality of river species, catch rates have plummeted, affecting the incomes of small-scale fishermen and fish-dependent traders (Kimani et al., 2018). It is also reported that female prawns with eggs are more preferred by consumers and are sold by the majority of fishermen (Kochey, 2018). Due to the high local demand, the sustainability of the prawn fishery is threatened and it justifies a sustainable production technique to ensure sufficient supply for the local people. Prawn farming can be a sustainable alternative to prawn fishing in Kenya. Several commercially important species of *Macrobrachium* have been bred in captivity with great success, and in some cases, others are being grown commercially. These include but are not limited to

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M. rosenbergii, De Man, 1879 and *M. nipponesis*, De Haan 1849 (Weimin and Xianping, 2002), *M. australe*, Guérin-Méneville, 1838, *M. lar*, Holthuis 1980 (Williams, 2018), *M. vollenhovenii*, Herklots 1857 (Ndao et al., 2019), *M. olfersii*, Wiegmann 1836 (Barbieri et al., 2016), *M. amazonicum*, Heller 1861 (Soeiro et al., 2016) and *M. malcolmsonii*, Milne-Edwards 1844 (Soeiro et al., 2016 and Nair and Salin, 2012). For *M. rude*, some grow out trials in ponds at different stocking densities have been undertaken in Bangladesh (Awal et al., 2021). However, no breeding initiative for *M. rude* has been done even though it has contributed to the economy of some fishing communities, particularly in Kenya, where it accounts for a large share of the prawn population hence the need for this study. A major challenge in freshwater prawn breeding is feeding the larvae for the first time. The feeding of *Macrobrachium* prawn larvae is generally fraught with serious problems, especially poor survival and growth during the first months of independent life (González et al., 2009, 2011). *Artemia* nauplii remain the most essential food for marine crustacean larvae and fish due to their nutritional quality, digestibility, ability to combine the benefits of live and dry diets, and convenience of dry cysts that can hatch within 24 h (Bardócz et al., 1999; Nkambo et al., 2019; Van Stappen et al., 2020). Although *Artemia* nauplii have been widely used in other freshwater prawn larval culture (Kovalenko et al., 2002; Barros and Valenti, 2003; De Aviz et al., 2018), their use in larval rearing of *M. rude* larvae has not been documented. In addition, optimal feeding density of *Artemia* nauplii is essential for the survival of *M. rude* and avoid overfeeding, which can lead to poor water quality, high costs of tank cleaning and maintenance, and stress on larvae (De Aviz et al., 2018). This study was conducted to determine the optimal feeding density of *Artemia* nauplii for *M. rude* larvae.

MATERIALS AND METHODS

Study Area

The study was conducted at the Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa Center (Figure 1). The station, which is located between 4° 03' 19.29" and 39° 40' 54.53", is served by a well and seawater.

Figure 1. A map of Kenya showing the location of KMFRI's Mombasa station where the study was conducted

Broodstock collection

Marobrachium rude broodstock was obtained from local fisherman fishing with prawn seine nets and traps in the Sabaki River near Sabaki Bridge (3° 8' 55" S, 40° 7' 31" E), Kilifi County. Captured broodstock were kept in the river using pre-established hapa nets and were selected based on egg development stage, health status and appearance. Only healthy broodstock with all appendages intact and bearing eggs were chosen for females. As for the males, only those with blue claws were selected. Selected broodstock was delivered to the KMFRI Mombasa Center for spawning in polyethylene fish packaging bags filled with pure oxygen. The broodstock were acclimated before being placed in aerated circular tanks filled with freshwater. The broodstock were fed commercial feed (Skretting ME-0.5 GR starter 315-630 µm) that contained 40% crude protein. Natural photoperiod (12 h light/12 h dark) and a temperature range of 26-28°C were maintained (Valenti et al., 2010). To induce hatching, gravid females bearing grayish-black eggs were put into individual plastic hatching basins with 5 ppt salty water and kept under continuous aeration until the eggs hatched. When the eggs hatched, the female was removed, and the larvae were gathered with a 100 µm net and put into 12 ppt saline water.

Experimental design

One day after hatching, batches of 100 larvae were collected and put in a 10 L glass aquarium with 5 L of 12 ppt saline water at a stocking density of 20 larvae/L. Two experiments were carried out. The larvae were treated to three feeding densities of *Artemia* nauplii/mL in triplicate for 42 days in the first experiment. *Artemia* nauplii were obtained by decapsulating and hatching *Artemia franciscana* cysts grown in Kenyan artisanal salt ponds at Kadzuhoni (20 58'54" S, 400 08' 37" E) under ideal hatching circumstances according to Lavens and Sorgeloos (1996). After 18 hours of incubation, instar 1 *Artemia* nauplii were gathered using a 100 m net and washed, then concentrated in a 1 L glass beaker with autoclaved saltwater. After thorough mixing, six samples of 250 L were collected from the beaker and counted under a dissecting microscope (Carl Zeiss Microscopy GmbH, Germany) to determine concentration. The beaker containing *Artemia* was kept in a refrigerator at 4 °C. For 42 days, the larvae were fed once a day. In addition, 10 days after larvae stocking, 1 g of commercial feed was added to each tank once a day as a supplement. Every morning, dead larvae and other wastes were drained from the bottom of the aquaria, and 50% of the water was replaced. Based on the results of experiment I, a second experiment was conducted using the same procedure as in experiment I. The feeding densities, however, were reviewed to 5, 10, and 15 nauplii/mL. The second experiment's culture time was 14 days because 100% larval mortality occurred after day 14.

Data collection

A YSI professional plus multi-parameter water probe (Model No. 6050000, YSI Industries, Yellow Springs, OH, USA)

was used to measure water temperature, dissolved oxygen (DO), and pH on a regular basis. Every day, the salinity was measured with a portable refractometer (Extech Instruments RF20, USA). Fortnightly growth sampling was performed by selecting five larvae from each tank, fixing them with 1% Lugol's solution, and measuring the total length (TL) of each larva using a STEMI 305 dissecting microscope equipped with an Axiocam (RS5S) camera (Carl Zeiss Microscopy GmbH, Germany). Active swimming larvae were physically counted for survival using a Petri plate and a micropipette. The following formulas were used to compute the growth parameters below:

i. Specific growth rate (SGR, %) = 100 × [(Ln final length (g) – Ln initial length (g)) / days of the experiment]

ii. Percentage Survival (%) = 100 × (final number of prawns)/ (initial number of prawns)

iii. Length gain (LG) = final length - initial length of larvae at stocking.

Data analysis

Shapiro-Wilk test was used to check the normality of the collected data. Descriptive statistics such as mean growth, survival rate and standard error were calculated on Microsoft Excel spreadsheet (Version 2016) using formulas (i), (ii) and (iii) below. Leven's test and one-way analysis of variance (ANOVA) were used to evaluate the equality of variance and the respective means significant difference between treatments. The Tukey HSD test was used to compare each pair of treatments. All statistical tests were performed using R statistical software (version 4.1.0 for Windows) and considered significant at the 95% confidence interval. The results were presented using tables.

RESULTS

Experiment 1

Water quality

Water quality parameters were monitored during the feeding trial (Table 1). The average temperature ranged from 24.32 to 24.34°C while the salinity ranged from 13.78 to 14.00 g/L. The mean dissolved oxygen (DO) ranged from 4.87 to 5.23 mg/L and the pH ranged from 7.82 to 7.90, respectively. The combined results showed that the water quality parameters amongst the feeding densities had small differences but were not statistically significant at the 95% confidence level (*P>0.05*).

Growth parameters

Table 2 shows the growth parameters during the feeding trial. For all feed administrations, the mean total length rose gradually over the culture period. The final total length was greatest in larvae fed 5 nauplii/mL, followed by 3 nauplii/mL, and lowest in larvae fed 1 nauplii/mL. Overall mean length increase, SGR, and SR were all higher in the 5 nauplii/mL treatment than in the 3 nauplii/mL treatment, with the lowest in the 1 nauplii/mL treatment. A one-way analysis of variance in all parameters examined, however, revealed no significant difference amongst the feed treatments (*P>0.05*).

Table 1. Water quality parameters during larval rearing of *M. rude* fed different feeding densities of *Artemia* nauplii

Parameter	Feeding density			
	1 np/mL	3 np/mL	5 np/mL	*P* value
Temperature (°C)	24.34±0.23[a]	24.32±0.22[a]	24.33±0.22[a]	*P>0.05*
Salinity (ppt)	13.78±0.15[a]	14.00±0.00[a]	14.00±0.00[a]	*P>0.05*
pH	7.82±0.27[a]	7.86±0.25[a]	7.90±0.26[a]	*P>0.05*
Dissolved oxygen (mg/l)	5.23±0.12a	5.05±0.10[a]	4.87±0.11a	*P>0.05*

Values are presented as mean± standard error. All statistical tests were considered significant at *P<0.05*. Superscript letters compare mean values between groups. Different letters in a row represent significant differences between groups.

Table 2. Growth parameters of *M. rude* larvae fed different densities of *Artemia* nauplii

Parameter	Feeding density			
	1 np/mL	3 np/mL	5 np/mL	*P* value
Initial TL (mm)	1.92±0.28[a]	1.92±0.28[a]	1.92±0.28[a]	*P>0.05*
Final TL (mm)	3.95±0.06[a]	4.05±0.14[a]	4.24±0.21[a]	*P>0.05*
Mean TLG (mm)	1.71±0.06[a]	1.81±0.14[a]	2.00±0.21[a]	*P>0.05*
SGR (TL) (%)	1.35±0.04[a]	1.41±0.08[a]	1.51±0.12[a]	*P>0.05*
Survival rate (%)	11.67±0.09[a]	11.67±0.09[a]	18.00±3.51[b]	*P>0.05*

TL=Total length, TLG=Total length gain, SGR=Specific growth rate, SR=Survival rate and np/mL=nauplii of *Artemia* per millilitre. Values are presented as means ± standard error. Superscript letters compare mean values between groups. Different letters in a row show significant differences between groups.

Experiment 2

Water quality

The water quality parameters for experiment 2 are presented in Table 3. The results showed that the water quality parameters did not differ between the feeding densities.

The average temperature ranged from 24.53 to 24.59°C, the salinity ranged from 13.97 to 14.29 g/l. The average dissolved oxygen (DO) content ranged from 4.80 to 4.88 mg/l and the corresponding pH ranged from 7.94 to 8.01.

Growth parameters

Total final length also varied significantly between dietary treatments (*P<0.05*). The highest total final length was observed in the 10 nauplii/mL treatment, followed by the 5 nauplii/mL treatment and the lowest in the 15 nauplii/mL treatment. A similar observation of total final length was also observed for SGR and total length gain (Table 4). Survival varied significantly between different dietary treatments (*P<0.05*). The highest mean survival was observed in the 5 nauplii/mL treatment, while the lowest survival was observed in the 10 and 15 nauplii/mL treatments.

Table 3. Water quality parameters during larval rearing of *M. rude* fed different feeding densities of *Artemia* nauplii

Parameter	Feeding density			
	5 np/mL	10 np/mL	15 np/mL	*P* value
Temperature (°C)	24.59±0.08^{a}	24.58±0.07^{a}	24.53±0.08^{a}	*P>0.05*
Salinity (ppt)	13.97±0.10^{a}	14.29±0.07^{a}	14.18±0.32^{a}	*P>0.05*
pH	8.01±0.20^{a}	7.94±0.18^{a}	7.99±0.19^{a}	*P>0.05*
Dissolved oxygen (mg/l)	4.88±0.18^{a}	4.84±0.12^{a}	4.80±0.07^{a}	*P>0.05*

Values are presented as mean± standard error. All statistical tests were considered significant at *P<0.05*. Superscript letter compares mean values between groups. Different letters in a row show significant differences between groups.

Table 4. Growth parameters of *M. rude* larvae fed different densities of *Artemia* nauplii

Parameter	Feeding density			
	5 np/mL	10 np/mL	15 np/mL	*P* value
Initial TL (mm)	1.03±0.01^{a}	1.03±0.01^{a}	1.03±0.01^{a}	*P>0.05*
Final TL (mm)	1.93±0.03^{a}	2.36±0.18^{b}	1.82±0.05^{a}	*P<0.05*
TLG (mm)	0.90±0.03^{a}	1.33±0.18^{b}	0.80±0.05^{a}	*P<0.05*
SGR (% day^{-1})	4.48±0.09^{a}	5.86±0.56^{b}	4.08±0.21^{a}	*P<0.05*
SR (%)	20.00±1.40^{a}	5.71±0.80^{b}	5.71±0.80^{b}	*P<0.05*

Values are presented as means ± standard error. TL=Total length, TLG=Total length gain, SGR=Specific growth rate, SR=Survival rate and np/mL=nauplii of *Artemia* per millilitre. Superscript letters compare mean values between groups. Different letters in a row show significant differences between groups.

DISCUSSION

Freshwater prawns like many crustaceans undergo a complex life cycle characterized by a planktonic larval phase and a benthic adult phase. The transition from one life style to another is accompanied by morphological changes in internal organs, tissue systems, and most importantly, the gastrointestinal tract (Anger, 2006; Korzelecka-Orkisz et al., 2012). Due to the rapid changes, the larval stage differs from the adult stage in several respects, including behaviour, nutrition, and physiology. Newly hatched larvae are lecithotrophic and are completely dependent on yolk mass to meet nutritional requirements lasting a few days. When external feeding is initiated, the larvae must accumulate enough food reserves to survive through the different metamorphosis stages into juvenile and adult stages (Le Vay and Gamboa-Delgado, 2011).

Therefore, determining the optimal feeding density of *Artemia* nauplii for *M. rude* larvae are essential to maximize growth, development and survival rates. In experiment 1 of the study, three feeding densities of *Artemia* nauplii were compared, namely 1, 3 and 5 nauplii/mL. The results showed that the growth parameters of *M. rude* larvae were not significantly different between the 3 feed densities. The observation depicted a similar intake pattern of *M. rude* larvae treated with feed densities of 1, 3 and 5 nauplii/mL (Table 2). According to Kurmaly (1990), a small percentage of decapod larvae search for food, while the majority depend on chance encounters to capture food. Therefore, increasing *Artemia* densities from 1 to 5 nauplii/mL did not provide a better opportunity to encounter and capture the food by *M. rude* larvae. A similar result has also been reported in other studies. For example, Barros and Valenti (2003) observed similar growth parameters in *M. rosenbergii* larvae fed 2 and 4 *Artemia* nauplii/mL. In experiment 2, *M. rude* larvae survived better at the feeding density of 5 nauplii/mL compared with the feed densities of 10 and 15 nauplii/mL. Overall, a downward trend in survival was observed from 5 nauplii/mL to the highest

feeding density, 15 nauplii/mL. The poor survival at the highest feeding density could be associated with evolution of ammonia from the excess *Artemia* nauplii supplied that remained uneaten in the culture tanks. According to De Aviz et al. (2018), increasing *Artemia* feeding deensities is wastefull and results in increased waste in the culturing tanks in this case nitrogenous waste since Artemia is highly proteinous. Another pattern was observed for growth, where the highest mean total length was observed at a feeding density of 10 nauplii/mL, followed by 5 nauplii/mL with lowest observed in larvae fed 15 nauplii/mL. Increasing the feeding density from 5 to 10 nauplii/mL increased feed intake, as demonstrated by mean total length, total length gain and SGR, but significantly decreased survival. Growth was affected at 15 nauplii/mL, which could be a result of excess feed intake. According to El-Sayed (2002), increased feed density in water may increase feed intake, but high intake of particles may cause feed to flow rapidly through the intestine, resulting in poor digestion and poor assimilation of nutrients hence the poor growth perfomance observed in the prawn larvae fed at high *Artemia* nauplii densities. These results are consistent with the study of De Aviz et al. (2018), who noted that the survival rate of *M. rosenbergii* decreased when *Artemia* nauplii density increased from 5 to 10 and 20 nauplii/mL. In another study, Maciel et al. (2012) observed an increase in feed intake as feed density increased, but this did not lead to an increase in yield.

The results of the present study are in contrast to those reported by Daniel et al. (2019) on the increase in larval growth parameters of Amazon aquarium fish when the *Artemia nauplii* feeding density was increased from 50 to 150 nauplii/mL. The survival rate of *M. rude* larvae in the present study ranged from 6 to 20% in both experiments. This is consistent with the study of Makombu et al. (2014), who observed survival rates of 3 to 9% when rearing *Macrobrachium vollenhovenii* larvae. The survival rates observed in the present study were much lower than those associated with other *Macrobrachium* species. For example, research by Gomes et al. (2014) reported survival rates of 56 to 78% with *Macrobrachium equidens*. In another study, Habib et al. (2014) reported a 64% survival rate with *Macrobrachium rosenbergii* larvae. The survival observed in the present study was also lower when compared to other crustaceans. The study of Rodríguez-Serna et al. (2010) on Mexican crayfish (*Procambarus llamasi*) fed different farm animal feed reported 100% survival whereas Kaldre et al. (2015) observed 89% and 78% survival with marbled crayfish (*P. virginalis*) fed with carp and discus feeds. Amanyazov and Karadal (2023), on the other hand stated 58-75% survival with red swamp crayfish (*P. clarkii*) fed with three different commercial aquarium feeds. The survival of prawn larvae is related to several factors. Brown (2005) associated poor survival with sub-optimal nutritional factors that render larvae incapable of transitioningfrom one developmental stage to another.

In contrast, Armstrong et al. (1976) and Aquacop (1983) linked prawn larval survival to the maintenance of good water quality, the quality and quantity of feed as well as the ability of the larvae to obtain food from the water. In the present study, the water quality parameters monitored (Table 1 and 3) were within the optimal range for prawn larval development. In addition, feed density treatments were also within the recommended range, consistent with previous studies. Therefore, the overall lower survival rates observed in the present study as compared to other studies could be due to handling and other zootechnical challenges (Brown, 2005).

CONCLUSION

In summary, the results of this study have important implications in determining the density of *Artemia* nauplii fed to *M. rude* larvae to optimize larval growth and survival. From the study, it was clear that feeding *Artemia* nauplii at lower densities (1-5 nauplii/mL) gave similar results in terms of growth rate and survival.

The study also demonstrated that increasing the density of *Artemia* nauplii fed to *M. rude* larvae would increase growth parameters, but there was an optimal range of 5 to 10 nauplii/mL. According to the study, increasing the feeding densities beyond 10 nauplii/mL not only leads to wasted feed but also reduces the growth and survival rates of the prawn larvae. The study recommends repeating the same work but with *Artemia* nauplii enriched with highly unsaturated fatty acids (HUFA) to determine if survival could be improved.

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AUTHORSHIP CONTRIBUTIONS

Sheban Mdzomba Hinzano: Conceptualization, data curation, formal analysis, investigation, methodology, software, supervision, validation, visualization, writing original draft, writing review and editing. Morine Mukami Ngarari: Conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, visualization, writing original draft, writing review and editing. Mary Opiyo: Conceptualization, funding acquisition, methodology, project administration, resources, supervision, review and editing. Francis Okalo: Conceptualization, funding acquisition, resources, writing review and editing. Betty Nyonje Mindraa: Conceptualization, project administration, supervision, writing review and editing. David Midumbi: Data curation, investigation, methodology, writing review and editing. Derrick Gitari: Data curation, investigation, methodology, writing review and editing.

CONFLICTS OF INTEREST

The authors state that there is no conflict of interest to declare.

ETHICS APPROVAL

The experiment was carried out in accordance with the Kenya Marine and Fisheries Research Institute (KMFRI) guidelines for animal handling, as registered with the National Commission for Science, Technology, and Innovation (NACOSTI) registration number NACOSTI/2016/05/001, and in accordance with the Prevention of Cruelty to Animals Act 1962, CAP 360 (Revised 2012) of Kenyan laws, and the EU regulation (EC Directive 86/609/EEC).

DATA AVAILABILITY

All relevant data is in the article.

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