Journal of Applied and Natural Science
14(4), 1435 - 1440 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

A study on the feeding of shrimp larvae of *Macrobrachium nipponense* on algae *in vitro*

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Article Info
https://doi.org/10.31018/jans.v14i4.3855
Received: August 10, 2022
Revised: November 30, 2022
Accepted: December 5, 2022

How to Cite
Al-Maliky, T. H. Y. *et al.* (2022). A study on the feeding of shrimp larvae of *Macrobrachium nipponense* on algae *in vitro*. *Journal of Applied and Natural Science*, 14(4), 1435 - 1440. https://doi.org/10.31018/jans.v14i4.3855

Abstract
Experiments were carried out for the propagation and rearing of *Macrobrachium nipponense* and its feeding on algae, with the aim of determining the density, survival, and growth of larvae *in vitro*. Hatched larvae of zoae were reared at a density of 50 zoea/L with algae mixture: *Chlorella vulgaris, Scenedesmus* sp., *Pediastrium* sp., *Microctinium* sp., *Navicula* sp., *Ulothrix* sp., *Cyclotella* sp., *Daitoma* sp. at three concentrations of 0.5=A, 1.0=B, 1.5=C x 10^5 cell/ml, and the survival % rates of zoea larvae were 45.00±5.00, 53.33±7.64, 50.00±5.00 respectively. Then, three densities: 25=A, 50=B, 75=C zoea/L were tested by feeding them with the best concentration of 1.0×10^5 cell/ml for 10 days, with the result being survival % rates were 50.00±5.00, 51.67±7.64, 31.67±7.64, respectively. After that, stage post-larvae were reared at a density of (50=A,100=B, 150=C) Pl/pond and fed with a concentration of 1.0 x 10^5 cell/ml of the algae mixture for 28 days, which resulted in survival % rates of 48.33±7.64, 40.00±5.00, 33.33±7.64, and this stage, weight was 50.67±2.08, 50.00±2.00, 40.33±2.52mg respectively. The results of the analysis of survival rates for different densities of zoea larvae found significant differences ($P < 0.05$) between density C and density of both A and B, of which there were no significant differences ($P > 0.05$) between them. There were no significant differences ($p > 0.05$) in the survival rates of zoea in different concentrations of the selected algae. Also, there were no significant differences ($p > 0.05$) in the survival rates and weight rate of post-larvae when fed on algae (B).

Keywords: Aquaculture, Density, Live food, *Macrobrachium nipponense*

INTRODUCTION

There are more than 220 species globally of the genus *Macrobrachium*, the most important of which are economic, such as *Macrobrachium rosenbergii, M. nipponese, M. americanum*, *M. carcinus, M. spinipes* and *M. tenellum* due to their large size, and their production can depend on culture (Yan *et al*., 2001; Gomez *et al.* 2008; Yamasaki-Granados *et al.,* 2013; Al-Maliky, 2013, Lober, 2015). The shrimps *M. nipponese* were bred in the nineties of the last century within the Southeast Asian region, especially in China, which is the most important producing country for this shrimp. It is distinguished by its rapid formation of useful mass in breeding ponds. Its resistance to harsh conditions, especially (such as extreme cold) in its initial stages of life, made it the best compared to competing for shrimp species in the same region. (Food and Agriculture Organization, 2014, Sun *et al.,* 2016). Breeding local species is of great importance, as it provides a good income when cultivated, and the seed produced in the laboratory can be placed in sites where shrimp disappear due to overfishing, pollution or the destruction of their nursery places with market supply and demand (Vega-Villasante *et al.,* 2011).

Success *in vitro* larval production is the main goal before production or conservation purposes can be pursued seriously. Field and laboratory studies are necessary to specifically determine ideal larval conditions, nutritional requirements and water quality. Previous studies have found that *Macrobrachium* larvae need live, fresh food to survive, especially *Artemia* nauplii or fresh ingredients, such as fish meat or egg custard (Arojo and Valenti, 2007). Lober (2015) Studied the

hatchery of Australian shrimp, *M. spinipes*, rearing and brooding larvae and juveniles using different concentrations of green algae with larval feeding. Mohebbi ***et al.*** (2016) studied the growth and survival of *Artemia ormiana* and its feeding on many microalgae. The best was *Dunaliella tertiolecta* for the growth and survival of Artemia.

Santanumurti ***et al.*** (2022) tested the efficacy of microalgae (*Nannochloropsis* sp., *Tetraselmis* sp. and *Dunaliella* sp.) on white shrimp (*Litopenaeus vannamei*) and their culture water.The world's production of aquaculture is estimated at 114.5 million tons, with an estimated value of 263.6 billion dollars in 2018, of which freshwater species constitute 51.3 million tons, and freshwater prawns constitute about 5% of it (Food and Agriculture Organization, 2020).

One of the recent local studies on the breeding of river shrimp in Iraq is the study by Al-Maliky ***et al.*** (2021) through probiotic technology in breeding two types of shrimp, *Metapenaeus affinis* and *M. nipponense* in the laboratory. The results were encouraging through high levels of growth and survival and a lack of both water and fodder use. The present study aimed to determine the best stocking density for hatched zoea larvae and post-larvae and to prefer feeding those larvae to the optimum concentration of algae selected from the environment, thus obtaining higher survival rates for the hatched larvae *in vitro*.

MATERIALS AND METHODS

Egg-bearing females

The egg-bearing females of *M. nipponense* were brought from the Mashhab area (30º38'34'N 47º41'22'E) in Basrah. Iraq and placed in a laboratory with plastic tanks with a capacity of one litre tank (15 L) equipped with aerators. After 15 to 20 days, the shrimp zoea larvae were hatched.

Larval rearing

After the second day, the larvae fed on the yolk sac were distributed to the ponds (15 L) at the rate of three replicates, for each of which there were three troughs. The optimal concentration of the algae mixture was determined by testing three concentrations (0.5=A, 1.0=B, 1.5=C) × 10^5 cell/ml of the algae mixture selected (*Chlorella vulgaris, Scenedesmus* sp*., Pediastrium* sp., *Microctinium* sp., *Navicula* sp., *Ulothrix* sp., *Cyclotella* sp., *Daitoma* sp) for a 50 zoea/L feed twice daily (Simon,1978; Ullman ***et al.,*** 2017). Next, the optimum density of zoea larvae was determined using three different densities of zoea larvae (25=A, 50=B and 75=C) /L (D'Abramo ***et al.***, 2006; Yamasaki-Granados ***et al.***, 2013). And fed on the second concentration of used algae, were distributed in 9 ponds up to 10 days of age. Then in the post-larvae=Pl phase (10 mg), three densities of post larvae (50=A, 100=B and 150=C) Pl/15L(pond) were fed at the same concentration of algae (1.0= B) × 10^5 cell/ml for 28 days. The sensitive scale was used to measure the weight. Survival rates for zoea larvae and post-larvae were calculated based on those presented by Esparza-Leal et al. (2010).

Environmental conditions

Some of the environmental conditions of the experimental tanks were determined in the aquaculture laboratory of the Marine Science Center (Fig. 1). It included ponds of egg-bearing females (A), an outer pond of algae (B) of both zoea larvae and post-larvae and indoor ponds for larval rearing (C).

Algae

Zoea larvae of *M. nipponense* were fed based on algae growth in a fibreglass tank located in the culture station of the Marine Science Center- University of Basrah. One liter volume of water was filtered by filter papers from the source from which it was brought. Then these filter papers were transferred with a volume of 2 liters of distilled water and the algae species were examined under the light microscope after preparing the samples. Algae species were identified on microscopic slides. After diagnosis, it was transferred to a pre-prepared beaker (2 L) equipped with continuous aeration to be a tank. Algae were isolated and diagnosed *in vitro* based on both Stein (1973) and Belcher and Swale (1976).

Statistical analysis

The results were statistically analyzed using (SPSS). The studied factors were tested using the least significant difference (LSD) and under the significance level of 0.05.

RESULTS AND DISCUSSION

A study has been made on the possibility of intensive farming for shrimp, including the most prominent practical applications and progress made from modern shrimp farming methods, including rationing material expenses and reducing water use (Emerenciano ***et al.*** 2022).

Fig. 1 shows the direction from the source of female egg-bearing shrimp and until they passed their incubation in the laboratory, until they released the larvae, rearing the zoea and the corresponding post larvae. The direction of the source of the algae and purification in the laboratory= *In vitro*, thus completing the process of practical tests (indoor ponds for larval rearing= Test aquarium) to determine the best density of zoea and post larvae and the best concentration of the mixture of algae was used for feeding the larvae.

Lober (2015) reported how freshwater prawns hatch and mature mothers are collected from the environment and placed in tanks of 4-5:1 females to males, respec-

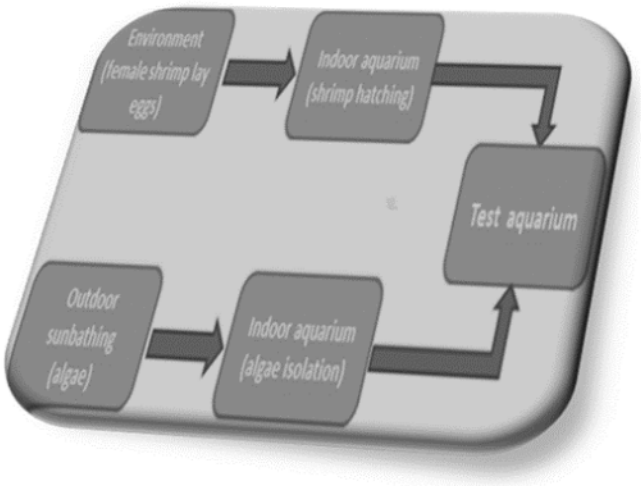

Fig. 1. *Showing the scheme of the experimental application of feeding larvae M. nipponense to live food (algae) in vitro*

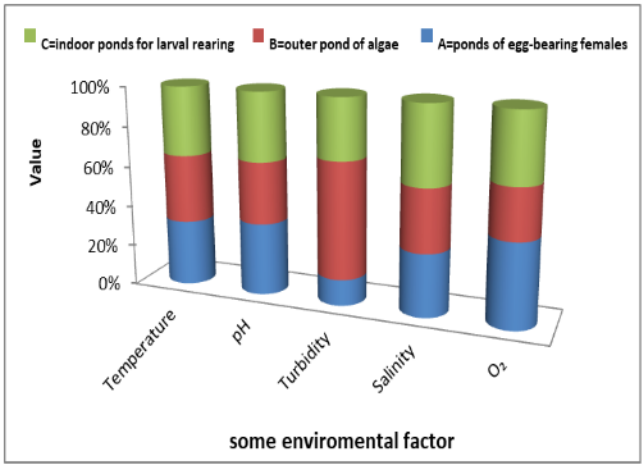

Fig. 2. *Some of the ecological conditions in: A) ponds of egg-bearing females M. nipponense , B) outer pond of algae , C) indoor ponds for larval rearing*

tively, equipped with calculated lighting periods and fed on fresh, high-protein food, produced zoea larvae, and nurtured them to the post-larvae. The present study observed the similarity of temperature and salinity. In contrast, the difference was observed in turbidity and dissolved oxygen because the algae source was more turbid. Therefore the percentage of dissolved oxygen decreased to increase its consumption, while the internal ponds showed high oxygen due to ventilation devices. Turbidity was high in the algae source due to the high density of algae and, therefore, the lack of oxygen. The laboratory basins had low turbidity and natural conditions because they were in a controlled environment. The turbidity of area where the females collected eggs was of relatively high turbidity, which is evidence of the abundance of nutrients from live food from animal and plant plankton (Fig. 2).

There was an improvement in some environmental factors in shrimp ponds with the presence of algae, which agrees with the study by Santanumurti et al. (2022). They mentioned that microalgae density of 10×10^4~80×10^4 cell/ml positively affects the water quality of ponds *Litopenaeus vannamei* by reducing sediments, nitrates and phosphates, thus improving water quality and increasing the growth and survival. They reported that the presence of microalgae in ponds maintained the water quality from the appropriate environmental conditions. Hence, the temperature was between 23-25 ºC, pH between 8-9, salinity between 25-30 ppt, and the appropriate light intensity. The algae species used positively affected the growth and survival of zoea and post-larvae, which is a good step towards starting the idea of creating an incubator for shrimp because algae were of great importance and were specific to the early stages of shrimp life. The algae used in the present study consisted of eight species, five green algae (Chlorophyceae): *Chlorella, Scenedesmus, Pediastrium, Microctinium* and *Ulothrix* and three diatomaceous algae (Bacillariophyceae): *Navicula, Cyclotella* and *Daitoma*. They thus generated good nutrients from those algae as food for the larvae in the zoea and post-larvae stages. These two conditions were of paramount importance in the success of any shrimp farm that aimed to obtain an economic re-

Table 1. Genera of algae and their densities (100 thousand cell/ml) used for feeding shrimp larvae *M. nipponense* (Mean ±SD)

Algae	Densities (Mix algae concentration:cell/ml)		
	A=0.5×10⁵	**B=1.0×10⁵**	**C=1.5×10⁵**
Chlorella vulgaris	14933± 404	29833 ± 764	45500 ± 2291
Scenedesmus sp.	10067 ± 404	20167 ± 764	29500 ± 2291
Pediastrium sp.	7967 ± 252	15833 ± 1756	23833 ± 1756
Microctinium sp.	4133 ± 322	8167 ± 1756	12167 ± 1756
Navicula sp.	4100 ± 265	8167 ± 624	12750 ± 750
Ulothrix sp.	4033± 252	8000 ± 500	11833 ± 1756
Cyclotella sp.	3900 ± 265	8000 ± 500	11250 ± 750
Daitoma sp.	2867 ± 322	5833 ± 624	9167 ± 1756

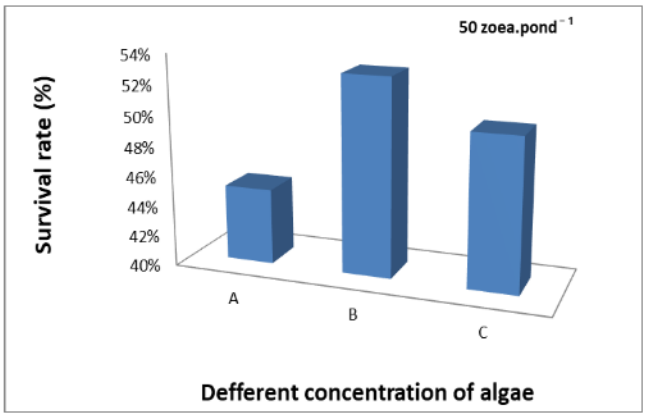

Fig. 3. *Survival rates of zoea larvae of M. nipponense fed on three concentrations (0.5=A, 1.0=B, 1.5=C) x 10⁵ cell/ml from a mixture of algae during 10 days*

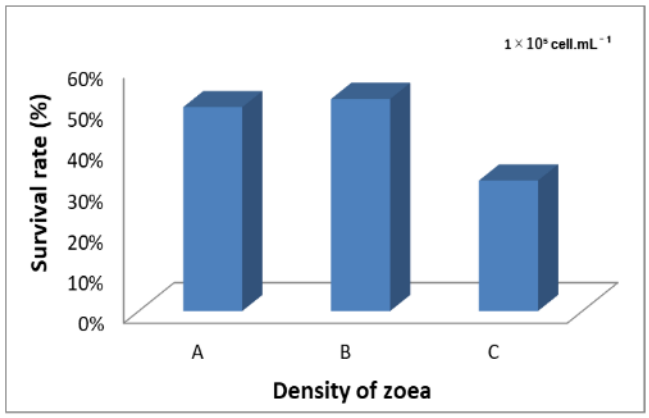

Fig. 4. *Survival ratios of densities (25=A, 50=B, 75=C) zoea/L of M. nipponense fed on 1.0×10⁵ cell/ml. a mixture of algae during10 days*

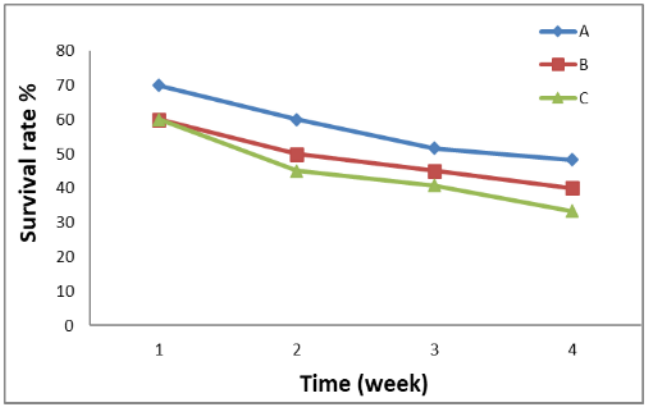

Fig. 5. *Survival ratios of densities (50=A,100=B, 150=C) Pl/15L of M. nipponense fed on 1.0 x10⁵ cell/ml of algae mixture for 28 days*

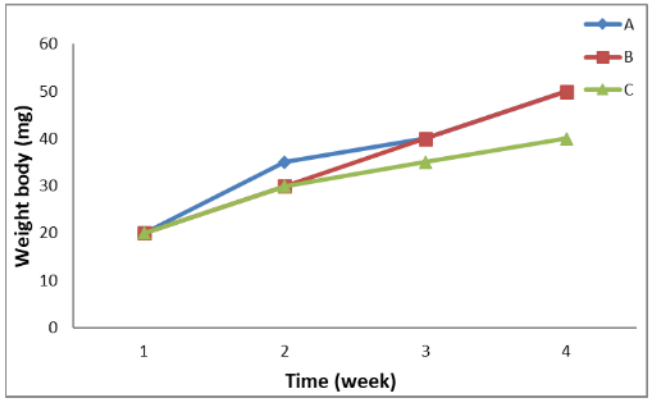

Fig. 6. *Weight rates of densities (50=A, 100=B, 150=C) Pl/15L of M. nipponense fed on 1.0 x 10⁵ cell/ml of algae mixture for 28 days*

turn (Table 1). The percentages of green algae were the highest in the total vegetative for the water content.

Three concentrations of the algal mixture (*Chlorella vulgaris, Scenedesmus* sp., *Pediastrium* sp., *Microctinium* sp., *Navicula* sp., *Ulothrix* sp., *Cyclotella* sp., *Daitoma* sp.) were used: (A), (B) and (C) (Fig. 3). The results of larvae tested at 50 zoea/L showed that the second density was the best followed by the third and the first ones. This gives the impression of an encouraging fact that the different micro-algae under the present study was influential for survival rates during the first ten days. The results showed that the second concentration (50=B) was the best in larval survival rates (Fig. 4), which is consistent with the Iraqi environment, which indicated that the double densities of algae concentrations were the best for larvae. The most important determinants of larvae shrimp production are larval feeding which starts with live microalgae, and nutritional supplementation. The search for practical application, cost reduction, and limiting the spread of pathogens has led to the development of new types of diets that link live food and food. However, using microalgae remains essential for shrimp larval immune response, pigmentation, and animal husbandry performance, as well as maintaining water quality and bacterial control of the system. Therefore, much research has been conducted to evaluate the productive performance of larval and post-larvae of shrimp feeding on microalgae treated as a supplement to microalgae (Moraes ***et al.,*** 2022).

Larval survival rates decreased weekly (Fig. 5), which was normal for most early-stage shrimps. The present study noted that the highest survival rate was with the lowest densities of the post larvae. This indicated the effect of density on survival rates and related to competition for food and oxygen. Therefore the lower the density, the higher the ability of the post larvae to obtain food and oxygen and vice versa, so it was noted in the development of various modern techniques, especially for intensive farming. The study indicated that the weight of the post-larvae increased during the study period, as the weights reached 50.67 and 50.00 mg. The percentages of weight rates were close to the different densities during the first rearing period, while they were close only between the two densities 50 and 100 Pl/15L, during the last period of the experiment,

and this may be due to the effect of the lower density of the post larvae. Therefore they grew faster on the selected algal concentration compared to their weak growth during the higher density of the need of these post larvae to higher concentrations of algae and ideal conditions for intensive culture to achieve a decrease in oxygen levels and a lack of nutrients and an increase in ammonia these and other effects worked to reduce the weight gain of post-larvae comparison (Fig. 6). The present finding agrees with Okutsu ***et al.*** (2020) when compared with the breeding of macro larvae raised in fresh water and another in fish pond water and noticed that the survival rates of those larvae (20.5%) raised in the pond were better than those raised in freshwater (1.8%). This may be due to the containment of fish pond water on natural food from plant and animal plankton.

Conclusion

The present study concluded that the survival rates of the zoea stage in shrimp *M. nipponense* were close to 50% during the different concentrations of algae. The rearing for the larval stage of zoae between 25 to 50 zoae /L was the best. While during rearing for the post-larvae stage, the most appropriate density was 50 Pl/15 L. These results motivated to benefit from this shrimp during its different life stages, either by continuing to raise it to reach the market size or using its first life stage as live food in aquaculture.

ACKNOWLEDGEMENTS

We extend our thanks and appreciation to my ancient university, Basra University, from all researchers who helped me complete our search.

Conflict of interest

The authors declare that they have no conflict of interest.

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