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Utilization of *Daphnia longispina* as supplementary food for rearing *Marsupenaeus japonicus* post larvae

M. H. Mona, M. M. El-Gamal, F. A. Abdel Razek[1] and M. F. Nour Eldeen*[1]
Zoology Department, Faculty of Science, Tanta University, Egypt.
[1]National Institute of Oceanography and Fisheries, Alexandria, Egypt.

*Correspondence e-mail: m_fouad_y@yahoo.com

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Original Article

Abstract

The present work aims to use *Daphnia longispina* as a live food for *Marsupenaeus japonicus* post larva. The survival and growth rates as well as biochemical composition of the post larvae were determined. The results showed that the mixed food (*Daphnia* + artificial diet) gave the highest survival ratio when fed to the post larvae of *M. japonicus*, and its survival reached 90 % during the period of 30 days rearing experiments. The results also showed that the mixed food (*Daphnia*+ artificial diet) gave the best length and weight comparable to the other regimes followed by the artificial diet. By looking into the biochemical composition of post larvae, it was observed that the carbohydrate content in PL fed on Daphnia was 14.7 mg/dl though Daphnia samples had high amount of carbohydrate (24.57 mg/dl). Total protein recorded its highest value in the post larvae fed on *Daphnia* + artificial diet. The effect of food types on the proximate composition of lipid showed considerable amount of it in the specimens of *Daphnia*, while this amount was not assembled in PL fed on it. The highest total lipid was recorded with PL fed on mixed food (Daphnia + artificial diet). However, the post larvae fed on artificial diet had only half the amount of lipid compared to the mixed food. The results indicated that although *Daphnia* was not proved to be used as a lonely food regime but it gives a promising noticeable result when mixed with artificial diet.

Keywords: *Daphnia longispina, Marsupenaeus japonicus, post larvae, supplementary food.*

Introduction

The suitability of wild live food organisms as food in the larval rearing was indicated earlier (Alam *et al.,* 1993; Altaff *et al.*, 2002). Lack of suitable live feed is a major deterrent in the rearing of marine prawn larvae (Vyas *et al.*, 1986). Even though the trend to develop artificial feed is fast replacing the live feed, some outstanding advantage of live feed cannot be overlooked due to its high calorific value, greater digestibility and good conversion efficiency (Neelakantan *et al.*, 1988). Although, Artemia nauplii and rotifers are the common live food organisms mass cultured for hatchery use, there is a growing interest for production of cladocera (Sivakumar, 2005; Altaff and Mehraj, 2010) due to the high cost and non availability of good quality Artemia cyst on a large scale. Two important genera, Daphnia and Moina, have been established as important live food in culturing penaeid larvae (Norman *et al.*, 1979; Sargent *et al.*, 1999). They convert phytoplankton, bacteria and fungi and decompose organic matter into animal tissue that can be used by larger animals (Porter and Orcutt, 1983).

Many studies were dedicated to penaeid shrimp nutrition, given the nutritional requirements of *Marsupenaeus japonicus* (Deshimaru and Kuroki, 1974; New, 1980; Guillaume *et al.*, 1987). The prawn has been shown to necessitate adequate levels of proteins, lipids, carbohydrates, minerals and vitamins. The variety of optimum protein levels for prawns is different among species (Aquacop, 1977; Colvin and Brand, 1977; Bages and Sloane, 1981; Kanazawa *et al.*, 1981). Deshimaru and Yone (1978) have pointed out that the prawn, *M. japonicus*, requires 52-57% protein for optimum growth and food efficiency. The content of essential amino acids (EAA) and balance of amino acids could be related to the nutritive value of proteins used (Hew and Cuzon, 1982). Daphnia have a protein content of around 50% on dry weight and a fat content of 20-27% for adults (Munirasu *et al.*, 2016). As a result *Daphnia* frequently used as a live food source in the freshwater larviculture (Shil *et al.,* 2013). However, *Daphnia* being is a freshwater species, it is not a suitable prey organism for marine organisms, because of its low content of essential fatty acids (Lavens and Sorgeloos, 1996). Live prey organisms like *Daphnia* can be bio-encapsulated with a variety of enrichment products to manipulate their content in certain nutrients (Coutteau and Sorgeloos, 1997).

Brett *et al.* (2006) demonstrated that diet has a dominating impact on fatty acids content of *Daphnia* but irrespective of diets, Daphnia also retain some internally consistent features of their fatty acids profiles. Several field studies of freshwater zooplankton have failed to observe dietary impacts even for those taxa which show clear responses in laboratory studies. Brett *et al.* (2006) attributed that these differences are due to the fact that some zooplankton *viz. Daphnia* are very fast growing and relatively lean, so it only takes a short period of time to replace their lipid reserves with new dietary lipids. *Daphnia* spp. that consume saturated fatty acids rich cyanobacteria tend to have only about half as much of these fatty acid as their diet (Muller-Navarra, 2006) which revealed that enrichment is not a complete treatment method to enhance their nutritional value, however, it is better to introduce the living organisms mixed with the appropriate artificial diet to meet the nutritional requirements of the larvae.

So the study was aimed to examine the effect of *Daphnia longispina* alone (with different numbers) and its mixture with artificial diet on feed acceptability, survival and growth rate of the first feeding larvae of *M. japonicas* with a view to develop a larval rearing protocol.

Material and Methods

Feeding experiments were carried out using the post larvae of *M. japonicus* (PL23). The experiment was divided into five treatments with different food regimes each one with one replicate and carried out for a period of 30 days. The first treatment received 10 *Daphnia*, the second 20 *Daphnia*, the third 30 *Daphnia* (for each PL/meal/three times/day), the fourth artificial diet 10 gm plus 20 *Daphnia* (mixed diet/meal/three times/day) and the fifth treatment artificial diet only (10 gm/meal/three times/day) as control.

Glass aquaria (25 x 25 x 50 cm) were stocked at density 25 shrimp larvae each, and were provided with sufficient aeration. A total of 250 post larvae of *M. japonicus* were used in all treatments. Throughout the experimental period, each aquarium was partially cleaned daily and completely cleaned each week with water exchange. Water quality parameters were detected weekly and weight, length and survival rate were calculated for thirty days.

Composition of shrimp larvae artificial diet prepared locally and the percentage of its components are illustrated in Table 1.

Sample analysis

Proximate composition of *D. longispina* and post larvae of *M. japonicus* : After the experimental time (30 days), samples of the post larvae and Daphnia were analysed for their content of crude protein, total lipids and carbohydrates.

Protein content : Samples of post larvae were heated with sulphuric acid, potassium sulphate was added to increase the boiling point of the medium (from 337 °C to 373 °C). Chemical

Table 1. Composition of shrimp artificial diet formulated for experimental trial.

Composition of shrimp artificial diet formulated locally and the percentage of components	
Components	Dry Weight (gm/kilogram)
Fish powder	430
Shrimp Powder	250
Wheat Flour	127
Starch	100
Yeast	5
A mixture of vitamins and salts	52
Cholesterol	5
Dryer Meat of Gandofli	10
Dryer Meat of Red worm	5
Cod Liver oil	40
Calcium phosphate	3
Total	1000gm

The coasts of one kilogram from this composition about 30 pounds decomposition of the sample was completed when the initially very dark-coloured medium became clear and colourless.

The solution was distilled with a small quantity of sodium hydroxide, which converted the ammonium salt to ammonia. The amount of ammonia was dipped into a solution of boric acid. The remainder of the acid was titrated with a sodium carbonate solution by way of a methyl orange pH indicator (Kjeldhal, 1883).

Total lipids: Lipid extracted and analysed according to Soxhlet principle (AOAC, 1995).

Carbohydrates: This group of nutrients *e.g.*, sugars, starch were estimated as the difference between the sum of the other constituents and the original dry weight of the sample.

Results

Water analysis

Water quality of the experimental aquaria such as Temperature; pH and dissolved oxygen ranged from 29 to 320C, 7.5 to 8.5 and 6 to 6.5 mg/L respectively during the study period and is shown in (Table 2). Nearly all the parameters were constant along the experimental period except for slight change in the salinity (0.03g/L) due to evaporation and that was adjusted by adding distilled water to reach to appropriate salinity.

Table 2. Water quality of the experimental aquaria throughout the experimental period (Each values= Mean± SD for three determinations).

Treatments	Different Treatments				
Parameters	Aquarium 1	Aquarium 2	Aquarium 3	Aquarium 4	Control
Water temperature °C	31.75±0.71	31.15±0.21	31.15±0.27	31.15±0.55	31.15±0.21
pH values	8.7±0.05	8.55±0.06	8.6±0.05	8.5±0.08	8.5±0.05
Dissolved oxygen (mg/l)	4.5±0.31	4.65±0.42	5±0.4	4.75±0.47	4.5±0.4
Salinity (g/L)	41.45±0.79	41±0.31	40±0.77	38.65±0.64	40±0.6

Aquarium (1): each Pl feed on 10 *Daphnia* (three times/day), Aquarium (2): Pl feed on 20 *Daphnia* (three times/day), Aquarium (3): Pl feed on 30 *Daphnia* (three times/day), Aquarium (4): Pl feed on mixture of (20 *Daphnia* + 10 gm artificial diet) (three times/day) and Control: Pl feed on artificial diet only (10 gm/three times/day).

Survival rate

Survival rate due to the different food regimes for post larvae of *M. japonicus* is illustrated in Fig. 1. From the beginning of the experiment (after 10 days), the mixed food (Daphnia + artificial diet) gave high survival ratio till the end of the experiment (after 30 days), and it reached 90 %. The other food regimes recorded low survival rates (15-20%). One way ANOVA showed highly significant differences (P<0.001) between the mixed food and the other food. Related to the number of Daphnia, analysis showed that no significant differences due to the number of Daphnia (P> 0.01).

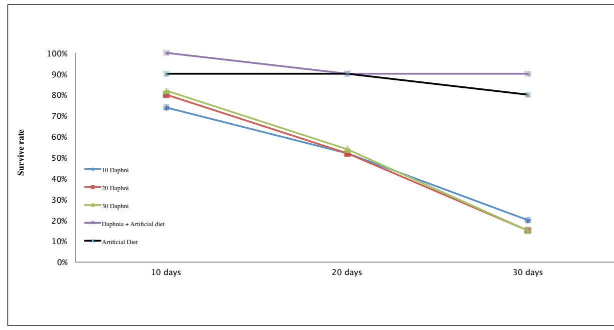

Fig. 1. Survival rate of post larvae of *M. japonicas* fed on different food regime

The effect of food regimes on the growth rate (length and weight)

Five food regimes were manipulated in the feeding of post larvae of *M. japonicus*. They were: *Daphnia* with different quantities (10, 20 and 30), 20 *Daphnia* + 10gm artificial diet /meal/three times/day) and artificial diet alone (10gm/diet).

The effect of the previous food regimes on the length of the post larvae along 30 days are represented in Fig. 2.

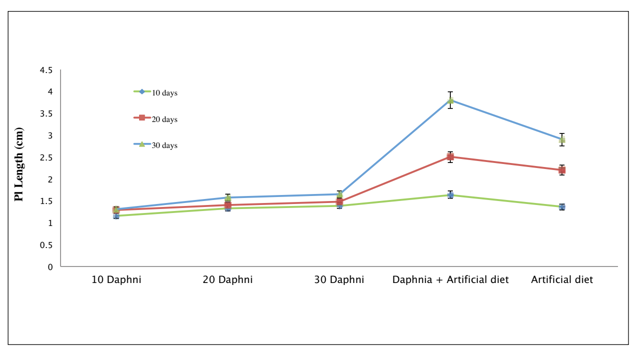

Fig. 2. The effect of different food regimes on the length of post larvae of *M. japonicas* during 30 days of experiment

Along the experiment regardless to the time (10 or 20 or 30 days), the mixed food gave the best length comparable to the other feeding followed by the artificial diet.

Two way ANOVA showed significant differences due to the type of food (P<0.00) and duration (along the 30 days). On calculating the Δ growth length (length gained at specific period) of post larvae of *M. japonicus* due to different food regimes, the results emphasized the above finding, that the mixed food gave the best growth (2.84 cm) as mention in Fig. 3 followed by artificial food (2.36 cm).

Also the Δ growth between PL feed with 20 *Daphnia* was the same as those fed on 30 ones (≈ 0.52-0.6 cm) while those fed

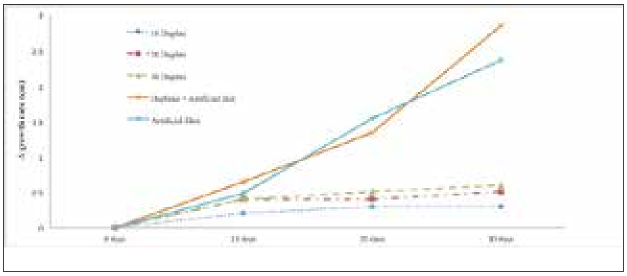

Fig. 3. Δ Growth length of post larvae of *M. japonicus* due to different food regime.

on 10 *Daphnia*/meal three times attained only Δ growth of 0.23 cm after 30 days.

The weight of PL due to the different food regimes is shown in Fig. 4. At the beginning of the experiment, there was no differences in weight between PL fed on 10 *Daphnia*/meal and those fed on artificial diet (0.13 and 0.17 gm respectively), the differences were started with PL fed on 20 and 30 *Daphnia*/meal (0.195, 0.325gm respectively) after 10 days.

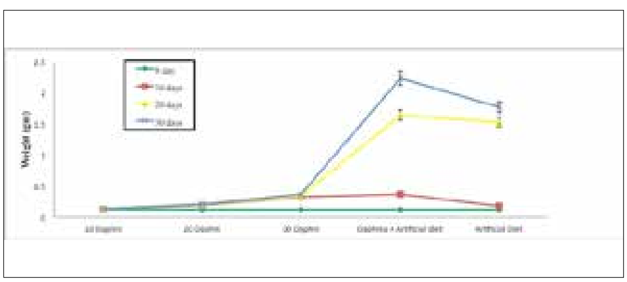

Fig. 4. The effect of different food regime on the weight of post larvae of *M. japonicus* along 30 days

The significant differences in weight started with the two food regimes that were artificial diet+ Daphnia and artificial diet only. The highest weight was recorded with PL fed on 20 Daphnia + 10 gm artificial diet/ meal; it attained 18 times its original weight followed by those fed on artificial diet (15 times its original weight). In PL fed with 30 *Daphnia*/meal the weight increased only to three times its original weight.

The proximate composition:

The carbohydrate composition of PL fed on different food regimes is represented in Fig. 5 In spite of the high amount of carbohydrate in *Daphnia* samples (24.57 mg/dl), its value in PL fed on it equal only 14.7 mg/dl. As general, there were no significant differences in carbohydrate composition in all PL fed on other investigated food regimes (*Daphnia* alone, *D.* with artificial diet or artificial diet only).

Total protein was 0.35 g/dl in *D. longispina* and recorded its highest value in post larvae fed on *Daphnia* + artificial diet

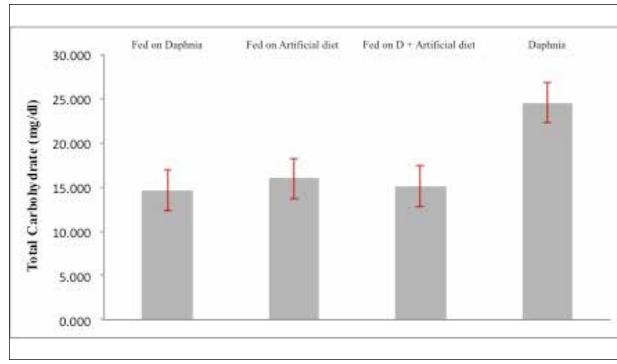

Fig. 5. Total carbohydrate in post larvae of *M. japonicus* fed on different food regimes after 30 days and in live Daphnia

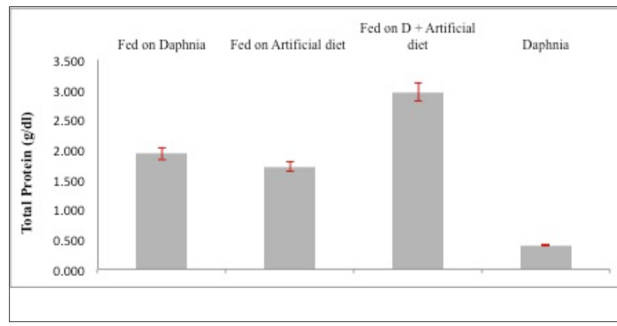

Fig. 6. Total protein in post larvae of *M. japonicus* fed on different food regimes after 30 days and in live Daphnia

(2.95 g/dl). While there were no significant differences (P> 0.05) in PL fed on *Daphnia* or artificial diet alone (1.9 and 1.7 g/dl respectively) as postulated in Fig. 6.

The effect of food type on the proximate composition of lipid is recognized in Fig. 7. The analysis showed considerable amount of lipid in the specimens of *Daphnia* (266.4 mg/dl), while this amount was not assembled in PL fed on it because it recorded 54.4 mg/dl in its PL. The highest total lipid was recorded with PL fed on both types of food (mixed food), it was 438 mg/dl. PL fed on artificial diet only had the half amount of lipid comparable to the mixed food. One Way ANOVA analysis showed significant differences in lipid contents between larvae fed on mixed food and the other type of food regimes (P< 0.001).

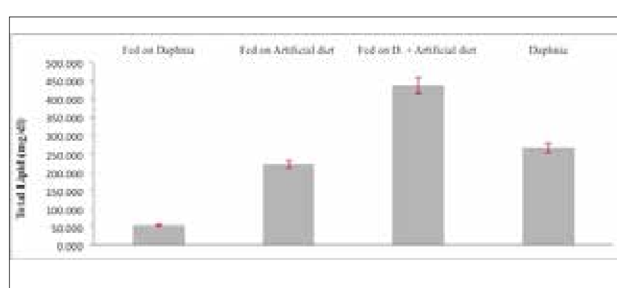

Fig. 7. Total lipid in post larvae of *M. japonicus* fed on different food regimes after 30 days and in live Daphnia

Discussion

The present study investigated the impact of different food regimes on the prawn *M. japonicas* post larvae. The study included the effect of live feed *D. longispina* (with different numbers), artificial diet, and mix between the two food types on the survival, growth rate and proximate composition of prawn larvae. Although live diets represent advantages such as high digestibility and stability in the water, the rotifers, *Artemia* and *Daphnia* may lack essential nutrients for marine organisms (Leger *et al.*, 1986; Lavens and Sorgeloos, 1996; Dhert *et al.*, 2001). In this context the development of nutritionally complete artificial diet that can partially substitute or added to live feed in co-feeding regimes (Teshima *et al.*, 2000) is gaining importance. Complete replacement of live foods with formulated diets through all the larval stage has also been demonstrated (Jones *et al.*, 1979; Kurmaly *et al.*, 1989; LeVay *et al.*, 1993), while survival can be equivalent to that of larvae reared on live foods, but growth and development were slower (D'Abramo *et al.,* 2006).

Normally in quantifying the feed to be supplied in the aquaculture systems the biomass is considered, therefore successful larviculture is dependent on the addition of known quantities of live feeds (i.e. microalgae or Daphnia) to the PL-rearing tanks. In the present work 10, 20 and 30 Daphnia were applied/ meal, three times/ day. Murphy and Cook (1969) provided frozen cladoceran Moina as food for the post larvae (20 nos/ larvae per day). Murphy found the suitability of this number with the PL, but in the present work (live *Daphnia*), Murphy's number was not enough and didn't match the requirement of the herein postlarvae.

The survival rates of PL dependent on *Daphnia* alone were significantly low when compared with those depended on *Daphnia* plus artificial food. With the same line, Lovett and Felder (1990) confirmed this finding; they postulated that freshwater Daphnia are not frequently used as a separate feed for *M. japonicus*. May be because the post-metamorphic life (post larval phase) represent a critical period during which high rates of mortality are encountered. This critical period has been related to changes in digestive enzyme which are unable to digest sufficient amounts of full-length proteins and longer peptides in the feed of different penaeid species (Dall, 1992; O'Brien, 1994; Rodriguez *et al.*, 1994; Aquacop, 1977).

Rodriguez *et al.* (1994) obtained significantly higher survival when they fed *M. japonicus* larvae with algae (*Chaetoceros gracilis*) throughout the larval stages together with *Artemia*, as opposed to larvae fed with only the *alga* or *Artemia*.

In the present study the higher survival rate observed in PL fed with *Daphnia* plus artificial diet is in agreement with studies reporting nutritional benefits when complementing live feed with artificial diets to manipulate the nutritional composition of live feed, which improves the overall response of shrimp with respect to live food alone (Leger *et al.*, 1985; Coutteau *et al.,* 1997; Calderon *et al.*, 2004). Also co-feeding *Artemia* with the inert diets significantly improved survival compared to use of *Artemia* alone (Gamboa-Delgado and Levay, 2009).

The higher growth rate observed in *M. japonicas* post larvae fed on mixed diet contained both *Daphnia* and artificial diet as compared to shrimp fed only *Daphnia* indicated that the artificial diet supplied shrimp with essential nutrients not found in *Daphnia*. Brett *et al.* (2006) demonstrate that diet has a dominating impact on Daphnia fatty acids composition but that irrespective to diet, *Daphnia* retain some internally consistent features of their profiles. With the same line Muller- Navarra (2006) showed that *Daphnia* spp. that consume saturated fatty acids rich in cyanobacteria tend to have only about half as much of these fatty acid as their diet and correlated this to the fact that *Daphnia* are very fast growing and relatively lean so it only takes a short period of time to replace their lipid reserves with new dietary lipids. This means whatever *Daphnia* is enriched or not, it is not enough for larvae feeding. Other advantages in food combination was if *Daphnia* was supplied in an un-enriched form, the artificial diet may have supplied nutrients that were scarce or not found in *Daphnia* thus promoting higher survival and growth rate. The previous suggestion is coincided with Han *et al.* (2001) that when un-enriched *Artemia* is added in co- feeding regimes of shrimp larvae it increases the benefits.

The lower growth rate in larvae fed with artificial diet only confirmed the results of other studies that show that artificial diets were less digestible due to the deficiency of enzymes in the early stages of shrimp as in the post larvae (Leger *et al.*, 1986).

The fatty acid composition of the larvae fed mixed diet showed the role of *Daphnia* to enable the PL to benefits from the lipid in the artificial diet. Larvae fed with *Daphnia* alone cannot attain the quantity of lipid scored in *Daphnia* itself. Teshima (1978) found that crustaceans as well as fish have a requirement for specific fatty acids which has also been confirmed by metabolic studies using radioactive traces. Studies on EFA (essential fatty acids) requirements for crustaceans have suggested that the nutritive value of lipids for prawn is related to the types and contents of EFA. Kanazawa *et al.* (1977) have pointed out that the superior dietary value was obtained with marine lipids containing ω-3HUFA such as Pollack liver oil and short necked clam oil.

Results from the present study indicated that feeding with *D. longispina* accompanied by co-feeding (artificial diet) regimes represents a good strategy as survival, growth and viability indicating a higher ingestion or assimilation efficiency for post larvae stage of *M. japonicas.*

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