Daphnia magna (Straus, 1820)– an alternative live fish food for *Pterophyllum scalare* and its commercialization

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Abstract:

Daphnia magna, a freshwater zooplankton organism, is a sustainable alternative to *Artemia nauplii* for feeding fish larvae. *Artemia nauplii* have been used as a traditional live food source for juvenile fishes in aquaculture due to their high nutritional value and availability. However, concerns regarding the sustainability, cost, and environmental influence of *Artemia nauplii* cultivation have prompted the search for alternative live-food sources. *Daphnia magna*, with its inherent nutritional composition, ease of cultivation, and potential for mass production, holds promise as a viable alternative. Though there were slight differences in the adjacent alignment of *Daphnia magna* and *Artemia nauplii.* The relative examination of data on two individual live feeds showed that at the end of the 21-day experiment, that means survival rate of the juveniles of *Pterophyllum scalare* was high in *Daphnia magna* as compared to *Artemia nauplii* as a food source. Survival rates were achieved 85%-95% in three experiments with *Daphnia magna* as food compared to 74%-79% with *Artemia nauplii*. The highest values (5.44 %, 5.65 %, and 5.80%) in particular growth rates were also obtained in three different experiments where the *Daphnia magna* is used as fed. The cost of production of *Daphnia magna* as food per gram is much less compared to that of *Artemia nauplii*.

Keywords: Live fish food, *Daphnia magna*, *Artemia nauplii*, larval feed, larviculture, copepods

Introduction:

For the past three decades, aquaculture has gained importance and different species of shellfish and finfish have gained commercial importance, thus their captive development of broodstock, breeding, and reproduction is well established.

Live-food organisms encompass required essential nutrients such as carbohydrates, proteins and amino acids, lipid and fatty acids, vitamins, and minerals; hence the live-food organisms are usually recognized as “living capsules of nutrition”. But the high cost of *Artemia nauplii* cyst has led the aquacultures to examine for alternative suitable zooplanktons such as *Daphnia magna* sp., *Moina* sp., Rotifers, infusoria, termites and bloodworms could be easily reared on large scale. Artificial larval feeds are no match to live organisms in rapports of acceptance, nutrition, and other factors. The habits of feeding among different species though different in nature but of them require high-protein live food for better growth performance, competent breeding, and survival.

Providing suitable live food at the proper time plays a key part in reaching the supreme growth and endurance of the juvenile stages of both the shellfish and finfish. Likewise, the swimming movement of live-food organisms usually assures a good distribution of food items in the water column, which facilitate frequent encounters with the developing larvae. To succeed in extreme profitability and production, the nutritional components of natural foods must be identified and quantified. The production of live-food organisms endures being a very significant key phase in the strengthening of aquaculture activity.

Materials and Methods:

Culture of Live-food Organisms:

The pure culture of *Daphnia magna*, stock culture needs to progress. In order to start stock culture, collection of *Daphnia magna is* done from freshwater ponds and tanks with the support of a scoop net having 250 to 500-micron mesh. Feeding of *Daphnia magna* is done with yeast @ 200 ppm or *Chlorella* at a cell density of 10×106 cells/ml. Each gravid *Daphnia magna* produces 8 to 10 offspring in about 24 hours. Dilution of the test tube culture is done daily through several 100 ml beakers. The volume is increased to 1 to 2-liter beakers or jars. Feeding is continued in a similar manner as in the test tube culture. After 6 days, this cultured Daphnia magna are used as inoculums in mass culture tanks.

In mass culture, the culture tank is prepared with urea (10 ppm), single super phosphate (25 ppm), and oil cake (groundnut) (70 ppm). After manuring, *Chlorella* was added to the tank. When *Chlorella* is developed after 5 to 6 days, *Daphnia magna* is added @ 50 to 60 nos./liter from the stock culture. *Daphnia magna* multiples quickly, nourishing on phytoplankton. It reaches an ultimate density of 20,000 to 25,000 nos. /liter in 6 to 7 days after immunization. After attaining peak density, it is frequently collected to feed in the larval stages. As a consequence of *Daphnia magna* growth, the concentration of *Chlorella* declines. In sequence to maintain the optimum density of *Chlorella*, 5% water exchange from the bottom of the tank and additional groundnut oil cake @ 70 ppm is done at an interval of 7 days, after the commencement of the first collecting of *Daphnia magna*.

The population of *Daphnia magna* was recorded by using the Sedgewick-Rafter counter cell (50 mm × 20 mm × 1 mm). Zooplankton number (no./ml) will be calculated according to the formula outlined by Boyd and Lichktoppler (1979):

Number of Zooplankton/ml = $\frac{T\times 1000}{A\times N\times Vol.of\ concentrate\ in \frac{ml}{Vol.of\ teh\ Sample}}$

Where, 1000 = area of counting chambers in mm^2

T = total number of zooplankton counted,

N = Number of grids counted,

A = area of the grid in mm^2

Experimental Fish:

The freshwater angel fish (*Pterophyllum scalare)* was selected and artificially spawned in the hatchery with ovaprim injected intramuscularly in a solitary dose of 0.5ml/kg fish weight. The growth and endurance rate of the larvae of *Pterophyllum scalare* were monitored with two different diets of *Daphnia magna*, a common zooplankton, cultured in the laboratory, and Artemia nauplii, a common live feed used in commercial aquaculture. *Artemia nauplii* are much more costly than cultured *Daphnia magna*, increasing the burden of commercial aquaculture.

Collection and Preparation of Experimental Foods:

The *Daphnia magna* used for this study were collected daily with a zooplankton net from a cultured tank set up at the Laboratory of Fishery Science, Department of Biological Sciences. This zooplankton was confirmed to be *Daphnia magna* through the selective culture method and when examined under a microscope with the aid of an FAO plankton identification sheet in the fisheries laboratory prior to the commencement of the experiment. The proximate analysis of *Daphnia magna* was carried out in a laboratory at the Department of Biological Sciences, Midnapore City College of West Midnapore in West Bengal, India.

A tin of Vacuum-packed *Artemia nauplii* cysts (PRO 80) was purchased from a local supplier and hatched in the hatchery unit of the Fisheries Laboratory, Midnapore City College.

Feeding of Fish Larvae:

Six glass aquarium tanks (24″ ×12″ ×12″) each containing 25 liters of water from a borehole supplied with aeration devices were used for the nourishing experiment of the juvenile fish. The tanks were covered with mosquito nets to prevent insect predators from entering.

The six tanks were assembled into three A, B, and C [i.e., two tanks per group; A (i-ii), B (i-ii), and C (i-ii)]. Each group was stocked with 100 fries and nourished with *Daphnia magna* and *Artemia nauplii* twice daily for 21 days in the following manner.

Table – 1: Different groups and treatments were adopted in feeding the fry for 21 days.

Groups	**Treatments**
A (i)	*Daphnia magna* as feed.
A (ii)	*Artemia nauplii* as feed.
B (i)	*Daphnia magna* as feed.
B (ii)	*Artemia nauplii* as feed.
C (i)	*Daphnia magna* as feed.
C (ii)	*Artemia nauplii* as feed.

The research was directed for 21 days in each group of tanks with two different live feeds. The measurements of the weight and total length of the juveniles were performed both at the beginning (100 individuals) and at the end of the research (all individuals). The number of surviving juveniles in individual tanks was counted at the end of the research. The survival rate and specific growth rate (SGR, %/day) were calculated as follows:

$$SGR\ (\%\ /day)\frac{100(lnW_t\ -\ lnW_0)}{t}$$

$W_{t.}$ and W_0 are the final and initial mean weights of juveniles, respectively, and t means time in days (t=21 days).

$$Survival\ rate\ (\%) = \frac{final\ number\ of\ fish}{initial\ number\ of\ fish} \times 100$$

Analysis of Proximate Composition of *Daphnia magna* and *Artemia nauplii:*

The proximate composition of commercial trout feed and *Daphnia magna* is shown in Table 1. Moisture contents were detected with an automatic moisture analysis (AND MX-50). The crude protein contents according to the Kjeldahl method (Nx6, 25) (AOAC 2000a), crude lipid contents by Bligh and Dyer (1959)'s method, and crude ash contents according to (Lovell, 1981) were done. Crude fiber content was considered according to Standard Association of Official Analytical Chemists (AOAC) methods (AOAC 2000b).

Results:

Table – 1: The Proximate Composition of *Daphnia magna* and *Artemia nauplii*

Parameters	*Daphnia magna*	*Artemia nauplii*
Crude Protein	45.06	42.44
Crude Lipid	18.09	7.21
Crude Carbohydrate	12.40	14.61
Crude Ash	14.50	9.65
Moisture	89.56	72.12

There were slight differences in the proximate composition of *Artemia nauplii* and *Daphnia magna*. The crude protein and lipid contents of *Artemia nauplii* (42.44%, 7.21%, and 14.61%) were lower than that of *Daphnia magna* whereas, *Daphnia magna*, Alternatively, had lower carbohydrate content (12.50%) than *Artemia nauplii* (14.61%). The ash and moisture content are also higher in *Daphnia magna* (89.05% and 14.50% respectively) than in *Artemia nauplii* (72.12% and 9.65%).

Graph – 1: Comparative Analysis of the proximate composition of *Daphnia magna* and *Artemia nauplii*.

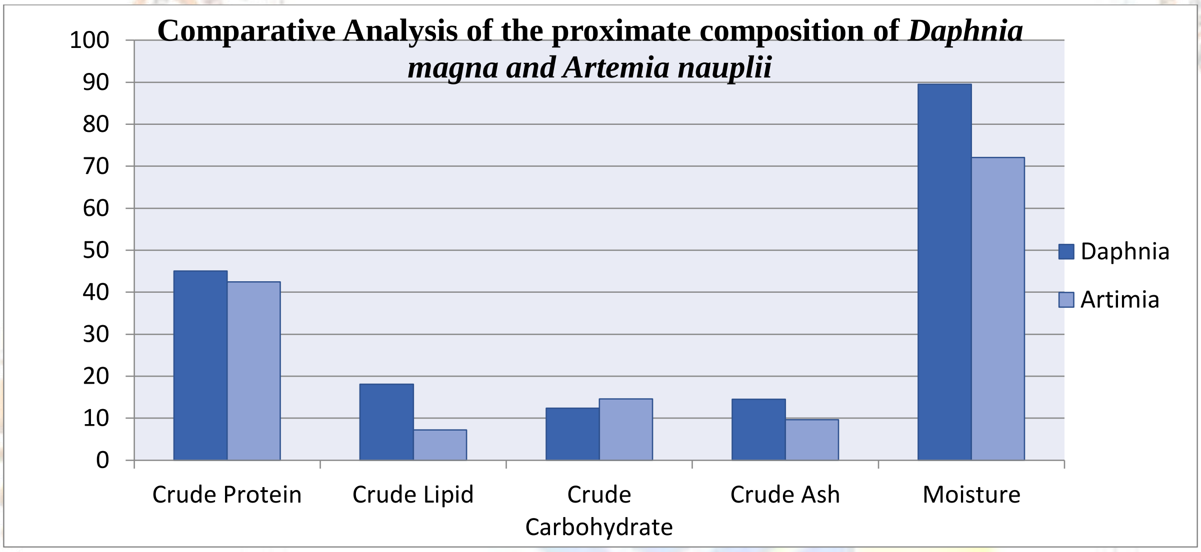


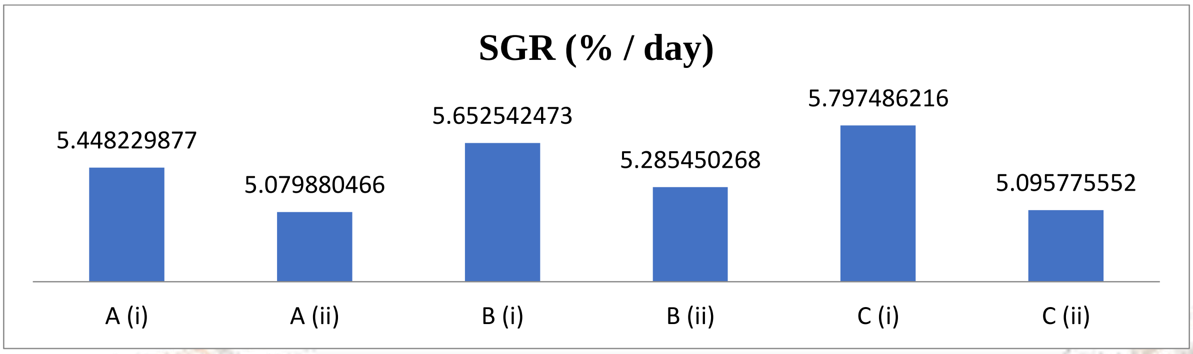
Table – 2: Comparative Analysis of *Daphnia magna* and *Artemia nauplii* as Food.

Group		Total Number of Fry (Initial)	Total Number of Fry (Final)	Initial Means Weight of the Juveniles (mg) (W0)	Final Means Weight of the Juveniles (Wt.)	% of the increase in weight.	SGR (%/day)	Survival Rate
A	A (i)	100	89	60.378	189.569	213.97032	5.44823	89
	A (ii)	100	74	60.378	175.458	190.59922	5.07988	74
B	B (i)	100	85	56.215	184.236	227.73459	5.65254	85
	B (ii)	100	79	56.215	170.567	203.41902	5.28545	79
C	C (i)	100	95	62.189	210.114	237.86361	5.79749	95
	C (ii)	100	76	62.189	181.325	191.57086	5.09578	76

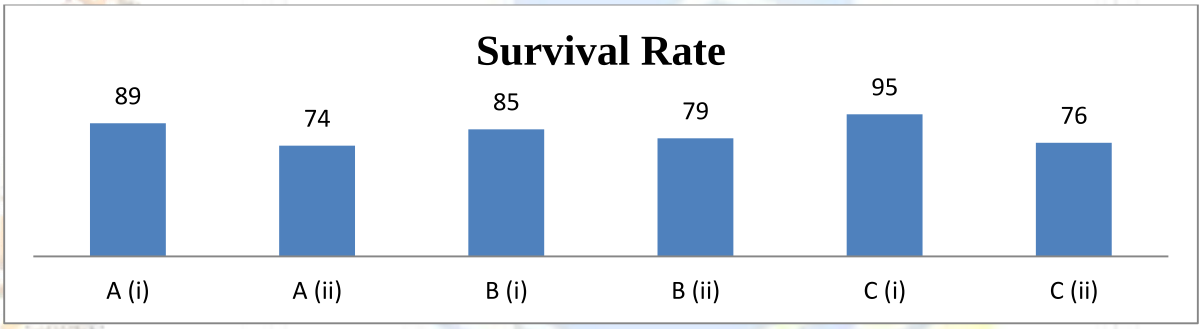
The comparative analysis of data on two different live feeds showed that at the end of the 21-days experiment, the mean survival rate of the juveniles of *Pterophyllum scalare* was high in *Daphnia magna* as compared to *Artemia nauplii* as a food source. Survival rates were achieved 85%-95% in three experiments with *Daphnia magna* as food compared to 74%-79% with *Artemia nauplii.*

The growth in body weight percentage was recorded higher in *Daphnia magna* treatment (184.24%-210.11%) compared to *Artemia nauplii* treatment (170.57%-181.33%). The highest values (5.44 %, 5.65 %, and 5.80%) in specific growth rates were obtained in three different experiments where the *Daphnia magna* is used as fed. Alternatively, the specific growth rate was recorded as low in *Artemia nauplii-treated* juveniles compared to *Daphnia magna* (5.08%, 5.28%, and 5.09%).

Graph – 2: Comparative Analysis Graph of Specific Growth Rate (%/day) of *Pterophyllum scalare* larvae feed with *Daphnia magna* {A (i), B (i) and C (i)} and *Artemia nauplii* {A (ii), B (ii) and C (ii)} as food.



Graph – 3: Comparative Analysis Graph of (Survival Rate) of *Pterophyllum scalare* larvae fed with *Daphnia magna* {A (i), B (i) and C (i)} and *Artemia nauplii* {A (ii), B (ii) and C (ii)} as food.



The Production of cost

The production of cost of *Daphnia magna* and *Artemia nauplii* per gram feed is shown in Table – 4:

Table – 4: Production of cost of *Daphnia magna* and *Artemia nauplii*:

Live Feed Used	Total Amount of the Feed Purchased/Produced (Grams)	Cost of the Cyst (Rs.)	Hatching of Cyst	Culture of Feed	Total	Production of cost/ gram of feed
Artemia nauplii	100	₹ 2,500.00	₹ 250.00	₹ 0.00	₹ 2,750.00	₹ 27.50
Daphnia magna	721	₹ 0.00	₹ 0.00	₹ 322.00	₹ 322.00	₹ 0.45

The production of cost of *Daphnia magna* as food per gram is much less compared to the *Artemia nauplii.*

PLATE

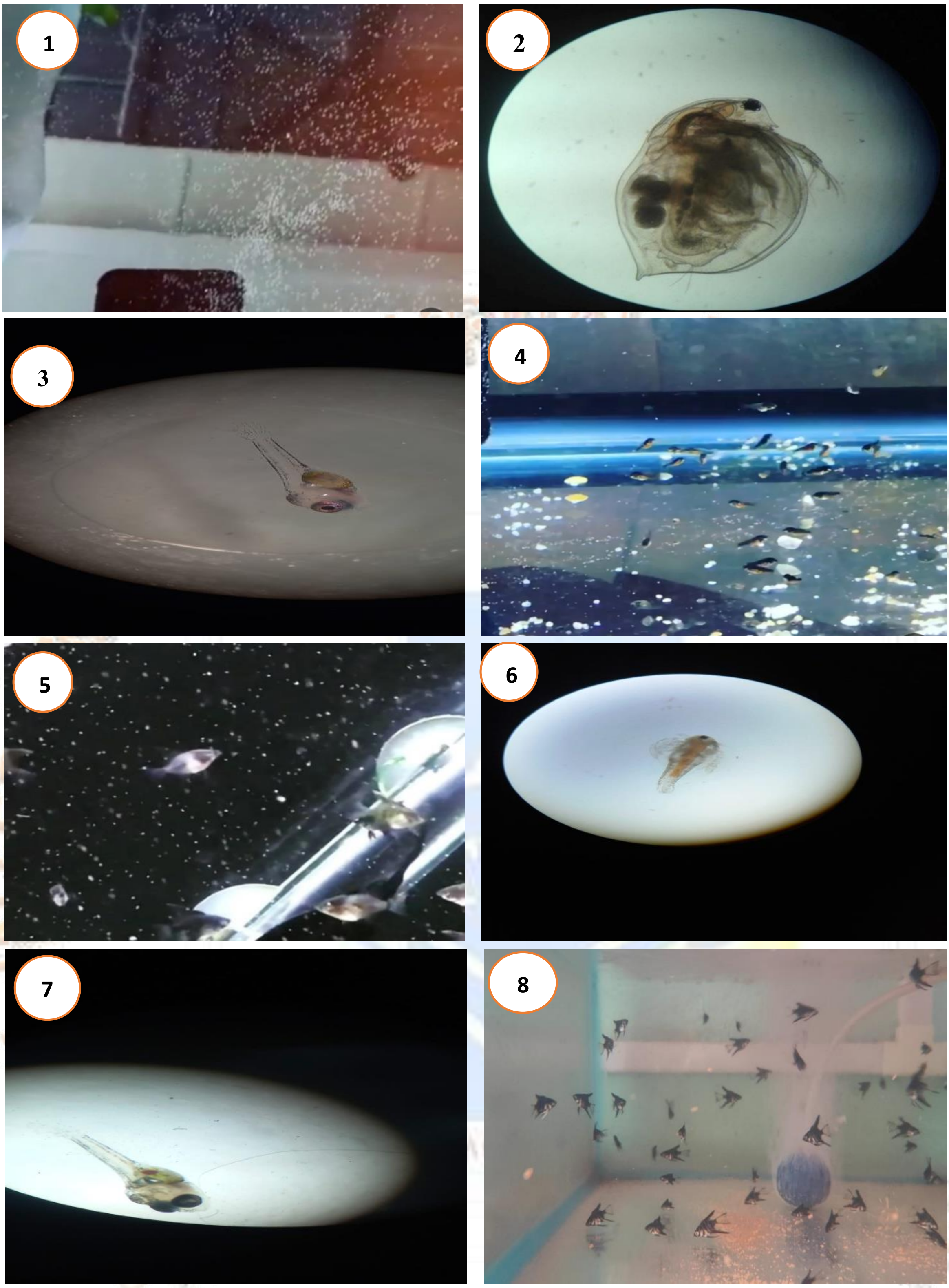


Figure-1: *Daphnia magna* culture. **Fig-2:** Microscopic view of *Daphnia magna sp.* (400x) **Fig-3:** Microscopic view of *Daphnia magna* feeding of Fish fry (Angle). (400X) **Fig-4**: *Daphnia magna* feeding of Angel Fish fry. **Fig-5:** *Artemia nauplii* culture**. Fig-6:** Microscopic view of *Artemia nauplii* (400X) **Fig-7:** Microscopic view of *Artemia nauplii* feeding of Angel Fish fry. (400X) **Fig-8:** Feeding of Juvenile Angel Fish.

Discussion:

The *Pterophyllum scalare* growth performance at the end of the 21-day feeding trial reveals specific growth rate was slightly higher in *Daphnia magna*-fed larvae related to the *Artemia nauplii*-fed larvae. The weight was also slightly better in the *Daphnia magna*-fed larvae, related to the *Artemia nauplii*-fed larvae. The growth of fish is generally believed to be a function of the level of crude protein in the diet which was higher in *Daphnia magna*. This could be qualified to the fact that the larval fish may not require more costly imported *Artemia nauplii* feed for better survival rate and growth rate because the farmers may easily culture *Daphnia magna* on a large scale to substitute *Artemia nauplii* to raise the new-born fishes. The production cost is also much lower than *Artemia nauplii.*

This assertion is in line with the work of those who stated that the proximate composition of fry feed should consist of 40% protein; and also (National Research Council, 2000) recommended dietary requirement of less than 45% crude protein for fish larvae. It was reported that the efficient growth of fish depends on feeding the best possible diets at levels not exceeding the dietary needs (*Aderolu et al*., 2010). The moisture content of *Daphnia magna* (89.56%) is higher than that of *Artemia nauplii* (72.12%). This could mean better digestibility for *Daphnia magna* by the fish larvae. However, the higher carbohydrate content in *Artemia nauplii* (14.61%) compared to that of *Daphnia magna* did not give any extra advantage. The relatively long-life span of *Daphnia magna* in water as observed during the study, coupled with the fact that *Daphnia magna* is freshwater zooplankton, makes it more available and acceptable to the freshwater fish larvae in sub-continent environment larvae which also dwell naturally in freshwater compared with *Artemia nauplii sp.* which are saltwater zooplankton (*Robison et al*., 2001).

Survival of larvae was recorded higher in *Daphnia magna*-fed. The generally high survival rate, however, could be qualified to the proper management of larvae and the level of acceptability of the two experimental foods by the fish. *Daphnia magna* is a common live-food organism applied for the rearing of marine fish larvae. Some authors (*Walford et al*., 1993; *Reza et al*., 2013; *Kolkovski et al.,* 1993) have suggested that fish larvae initially have a low endogenous increase in the digestion of food. The result obtained (*Reza et al*., 2013) showed that the use of zooplankton or *Artemia nauplii* in fish larviculture allowed excellent survival rates.

Cladocerans are generally called ‘water fleas. Cladocera is an order of sub-class -

Daphnia magna comprises several digestive enzymes like proteases, peptidases, amylase, lipase, and cellulase which act as exoenzymes in the gut of fish. Being larger in size than *Moina*, it serves as live food for advanced stages of fish. *Daphnia magna* contains added protein and consequently, is a good replacement for *Artemia nauplii* in aqua hatcheries. *Daphnia magna* has also been extensively utilized as a live-food source in various hatcheries and in the care and culture of aquarium fishes of commercial importance (*Martin et al.,* 2006).

Daphnia magna has the advantage of high reproduction rates, wide temperature tolerance, and the ability to thrive on phytoplankton and organic wastes. This enrichment of food with *Daphnia magna* is accomplished with a basis of DHA, which helps the fish to make immune resistance against gill and water fouling problems (*Reza et al.,* 2013; *Jafaryan et al.,* 2009)

The research of the current study highlighted that both the survival rate and growth performance were higher among the most cost-effective *Daphnia magna*-fed larvae than *Artemia nauplii*-fed. These results were in accordance with previous findings of (*Jafaryan et al*., 2009 and *Reza et al*., 2013).

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