# Live Fish Food Organisms As Alternative to the Conventional Fish Feed for New Born Fishes

*M.Sc. Thesis Submitted to Midnapore City College for the Partial Fulfilment of the Degree of Master of Science in Zoology*


**Submitted by**

**Akash Mandal, Nihar Bag, Nilanjan Jana, Prince Bera, Priyanka Maity, Samarpita Karak, Sayani Mahapatra, Soumyadip Chakraborty, Sumit Ghosh.**

**Under supervision of**

**DR. MONJIT PAUL**

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**Department of Biological Sciences**




**MIDNAPORE CITY COLLEGE**

**Kuturiya, P.O. Bhadutala,**

**Paschim Medinipur, Pin-721129**

**West Bengal, India**

**2023**

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West Bengal, India

**2023**

# MIDNAPORE CITY COLLEGE

Ref. No. - MCC/DIR-CER(PG)/07/23-8281(1) Date: 12-07-2023

## Certificate

This is to certify that the project report entitled "Live Fish Food Organisms as Alternative to the Conventional Fish Feed for New Born Fishes" submitted by AKASH MANDAL, ROLL NO. - PG/VUWGP-29/ZOO-IVS NO-002; NIHAR BAG, ROLL NO.- PG/VUWGP-29/ZOO-IVS NO-0024; NILANJAN JANA, ROLL NO.- PG/VUWGP29/ZOO-IVS NO-025; PRINCE BERA, ROLL NO.- PG/VUWGP-29/ZOO-IVS NO-026; PRIYANKA MAITY, ROLL NO.- PG/VUWGP29/ZOO-IVS NO-027; SAMARPITA KARAK, ROLL NO.- PG/VUWGP-29/ZOO-IVS NO- 036, SAYANI MAHAPATRA, ROLL NO.- PG/VUWGP-29/ZOO-IVS, NO-039; SOUMYADIP CHAKRABORTY, ROLL NO.- PG/VUWGP-29/ZOO-IVS, NO- 050; SUMIT GHOSH, ROLL NO.- PG/VUWGP-29/ZOO-IVS, NO- 055; to the Midnapore City College, Midnapore, and West Bengal, India during the year of 2023 in partial fulfilment for the award of the degree of **M.Sc. in Fisheries Science** is a bonafide record of project work carried out by her under my supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

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# Declaration

I do hereby declare that the present Master thesis entitled "Live Fish Food Organisms As Alternative to the Conventional Fish Feed for New Born Fishes" embodies the original research work carried out by me in the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Dr. Monjit Paul, Assistant Professor, Department of Biological Sciences, Midnapore City College, Paschim Medinipur. No part thereof has been submitted for any degree or diploma in any University.

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This project report entitled "Live Fish Food Organisms as Alternative to the Conventional Fish Feed for New Born Fishes" by Akash Mandal, Nihar Bag, Nilanjan Jana, Prince Bera, Priyanka Maity, Samarpita Karak, Sayani Mahapatra, Soumyadip Chakraborty, Sumit Ghosh is approved for the degree of Master of Science (Zoology).

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Akash Mandal, Nihar Bag, Nilanjan Jana, Prince Bera, Priyanka Maity, Samarpita Karak, Sayani Mahapatra, Soumyadip Chakraborty, Sumit Ghosh.

# Abstract

This thesis aims to explore the potential of live food organisms like *Daphnia magna*, a freshwater zooplankton organism, as a sustainable alternative to *Artemia nauplii* for feeding fish larvae. *Artemia nauplii* has been the traditional live food source for fish larvae in aquaculture due to its high nutritional value and availability. However, concerns regarding the sustainability, cost, and environmental impact 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 proximate composition of *Artemia nauplii* and *Daphnia magna*. 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 highest values (5.44 %, 5.65 %, and 5.80%) in specific 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 feed, fish, aquaculture, larviculture, copepods, *Daphnia magna*, *Artemia nauplii.*

# List of Tables

# List of Figures

# List of Graphs

# Table of Contents

**DEDICATED TO MY PARENTS**

# Chapter – 1: Introduction

# 1. Introduction:

Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplankton are generally eaten by zooplankton. Thus, phytoplankton forms the basis of the food chain. Live foods are able to swim in the water columns and are constantly available to fish and shellfish larvae are likely to stimulate a larval feeding response. In an aquatic ecosystem, these live food organisms constitute the most valuable resource for aquaculture. Most of the fish and shellfish larvae in nature feed on small phytoplankton and zooplankton. However, natural fish food organisms are usually not abundant in clear pond water but are abundant in ponds having greenish water. The green color indicates the presence of phytoplankton and other natural food organisms. In the natural food web, zooplankton constitutes a major part of the diet for marine fish larvae and it is generally believed that copepods can meet the nutritional requirements of fish larvae.

For the past three decades, aquaculture has gained importance and many marine and estuarine cultivable species of finfish and shellfish have been domesticated and their captive development of broodstock, breeding, and spawning technology has been well established. As a result of this biotechnological development, commercial-scale production of seeds of many fish and shellfish in hatcheries is practiced all over the world (Lavens and Sargeloos, 1996). A large quantum of larvae of mollusks, shrimps, and fish are reared and farmed leading to the production of animal protein (Lavens and Sargeloos, 1996). The larval rearing under hatchery conditions is carried out following species-specific procedures due to variations in their larval size, small, fragile nature, the extent of receptors, and digestive system development. For commercial-level seed production appropriate technology pertains to aseptic conditions, maintenance of water quality, and feeding strategies need to be adopted. In this regard, larval nutrition in general and that of the sensitive first-feeding larvae in particular, has become one of the major bottlenecks which prevent the full commercialization of many cultivable finfish and shellfish species (Stottrup and McEvoy, 2003). Different species of finfish and shellfish show a preference for different feeding and breeding grounds in natural water bodies but all the fishes require protein-rich live food for their better growth, efficient breeding, and survival (Stottrup and McEvoy, 2003). The larval development of finfish and shellfish show species-specific variations for example, in the case of shrimp species the developing larvae have to pass through naupliar, zoeal, and mysis stages leading to post-larvae. During the course of development, the larva follows first an herbivorous filter feeding and then a carnivorous with predatory behavior (Bengtson, 2003). While summarising the feeding habits of larvae, Lavens,

and Sorgeloos (1996) reported that the mouth size of the larvae at the first feeding stage is one of the factors which mechanically restrict the size of the food particles which can be ingested. The mouth size is correlated with body size, which in turn is influenced by egg diameter and the period of endogenous feeding. The developmental status of the digestive system of the first-feeding larvae also indicates the ability of the larvae to digest the ingested food. It is well established that the live food organisms meet all the necessary criteria for the small larvae of all commercially important fishes. Further, for food to be ingested by a larva, it first has to be detected, and hence the degree of development of the functional sense organs such as the eyes, olfactory organs, taste buds, and lateral line system is crucial. The occurrence of mostly cones in the retina of the eyes of fish larvae provides poor visibility, while the eyes of juvenile fish contain rods with higher concentrations of visual pigments which provide better vision. Live food organisms usually have much better contrast than artificial feeds and generally have a triggering effect by their continuous movement, allowing an enhanced perception by the feeding larva. Likewise, the swimming activity of live food organisms generally assure a good distribution of food items in the water column, which facilitate frequent encounters with the developing larvae.

Artificial larval feeds are no match to live food organisms in terms of acceptance, nutrition, and other factors. The feeding habit of fishes in natural water bodies is different among the species but all the fishes require protein-rich live food for their better growth, efficient breeding, and survival. Advances in live food enrichment techniques have helped to boost the importance and potential of live food organisms in the raising of larval aquatic species. The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food for feeding fish larvae, fry, and fingerlings. The availability of large quantities of live foods organisms such as marine rotifer (*Brachionus plicatilis* and *Brachionus rotundiformis*) and *Artemia nauplii* meet the different stages of fry. A common procedure during the culture of both larvae of fish and prawns is to add microalgae (i.e., “green water”) to intensive culture systems together with the zooplankton prey, which has become popular practice these days. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids and hence are commonly known as “living capsules of nutrition”. Providing appropriate live food at the proper time plays a major role in achieving maximum growth and survival of the young ones of finfish and shellfish. To achieve maximum production and profitability, the nutritional components of natural foods must be identified and quantified.

The nutritional status of live food organisms can improve through various techniques of enrichment and bio encapsulation.

It is obviously agreed that the production of live food organisms continues to be a very important first step in the intensification of aquaculture, both horizontally as well as vertically.

# Chapter 2: Literature Review

## 2. Literature Review:

Commercial hatcheries are much more cost-effective and their outputs much more predictable than ever before, we need to admit that the methods applied are still very empirical. The early pioneers in the larviculture of fish had to look for a suitable and practical substitute for natural plankton, taking into account nutritional quality as well as production cost in the selection process. Over the years a limited number of algal species, the rotifers, and the brine shrimp *Artemia nauplii* Have become the live feeds used on a worldwide scale in industrial farming (Begum et al. 2013). In nature fish larvae feed on a broad spectrum of zooplankton, providing the larvae a complete and balanced diet (Dhert et al.1995). But the high cost of *Artemia nauplii* cyst has led the aquacultures to search for alternative suitable zooplanktons such as *Daphnia magna* and *Moina sp.* Rotifers and infusoria and termites, bloodworms could be easily reared on a large scale (Begum et al. 2013).

According to Abowei et al., (2011), the aim of this experiment is to analyze the growth of larvae feeds with earthworms, and termite meals so as to provide information that will help in incorporating any of these non-conventional animals as fish feed during the feed formulation by fish nutritionist and fish farmers who may want to use them as on feed ingredients.

Another side, done by Das et al., (2012), Artificial larval feeds are no match to live food organisms in terms of acceptance, nutrition, and other factors. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, and fatty acids. It is obviously agreed that the production of live food organisms continues to be a very important first intensification of aquaculture, both horizontally as well as verticality.

According to Lim et al., (2003), many of the modern larviculture technologies used in food fish hatcheries could be adapted for application in freshwater ornamental fish production. Some of the possible applications have been reported in Dhert et al., (1997). This paper focuses the recent developments and discusses the applications of the freshwater rotifers, *Artemia nauplii,* and grows *Artemia nauplii* in the freshwater ornamental fish culture based on the studies conducted in Singapore.

According to Abowei et al., (2011), live-feed organisms include zooplanktons. These are the rotifers, copepods, Cladocera's, and other larval and adult forms of some invertebrates. The type of fish feed determines fish production. The use of live feed in fish culture has received tremendous attention in countries where fish culture is well developed. For example, in

Malaysia, feeding with rotifers, start from the second day of post-hatch and lasts for one week (Houlihan et al., 2001). Feeding of larvae with *Daphnia magna*, a Cladocera was also successful in Hong Kong. Cultured Monia (Cladocera) in combination with rotifer is used effectively in Singapore. The most widely cultured feed is rotifers because of their abundance in any water body. Monia and Daphnia magna species are also widely cultured (Robert,1979). According to Abowei et al., (2011), essential or indispensable amino acids (EAAS) cannot be synthesized by fish but are needed for growth and tissue culture. The fish meal contains complete EAAS but it is expensive. Nonconventional fees resources (NCFRS) are credited for being non-competitive in terms of human consumption, very cheap to purchase, by-products or waste products from agriculture, farm-made feeds, and processing industries, these include all types of feedstuffs from animals (silkworm, termite, earthworm). Generally, fish diets tend to be very high in protein. As the growth rate decreases and fish age, protein levels in the diet are decreased accordingly.

Cladocerans are generally called 'water fleas. Cladocera is an order of sub-class - Branchiopoda and class - Crustacea of the phylum - Arthropoda. Two cladocerans, namely *Daphnia magna* and *Moina* are important as live food. *Daphnia magna* is found in freshwater ponds, tanks, and lakes, all over the world. It swims by rapid jerks of the two large antennules. *Daphnia magna* contains a broad spectrum of digestive enzymes such as proteases, peptidases, amylase, lipase, and even cellulase which serve as exoenzymes in the gut of fish and prawns. Being larger in size than *Moina,* it serves as live food for advanced stages of fish. *Moina* is primarily inhabitants of temporary ponds or ditches. It is smaller in size (0.5 to 2 mm) than *Daphnia magna* containing 70% more protein and therefore, goes well as a replacement for *Artemia nauplii* in aqua hatcheries. *Moina* has also been extensively utilized as live food in many hatcheries and in the maintenance and culture of aquarium fishes of commercial importance. Cladocerans have the advantage of high reproduction rates, wide temperature tolerance, and the ability to thrive on phytoplankton and organic wastes. The nutritional content of *Daphnia magna* varies considerably depending on their age and the type of food they are receiving. The protein content of *Daphnia magna* usually averages 50% of the dry weight. Adults normally have a higher fat content than juveniles. The total amount of fat per dry weight is 20-27% for adult females and 4-6% for juveniles. In ornamental fish culture, *Moina* used to be the most common live food organism for feeding young fish larvae (*Martin et al*., 2006).

According to Neri et al., (2020), traditional live feeds used in fish culture have been lacking essential fatty acids, particularly, docosahexaenoic acid (DHA) which is an essential fatty acid required for fish embryonic development (Cahu et al.,2003; Aragao et al.,2007). Fish fry production has always depended on the seed production of *Artemia nauplii* to provide necessary nutrition. Thus, a study on alternative live feed for fish fry production is needed (Uppanunchai et al., 2015). *Moina macrocopa,* a Cladocera, has been gaining attention as a substitute for *Artemia nauplii* in the post-larvae production of freshwater fish fry (Alam et al., 1993; Qin and Culver 1996) due to *M. macrocopa* adaptability to the changes in culture condition and predation (Kushniryk et al., 2015; Manklinniam et al, 2018). For *Cladocera* to be efficient as a feed for larval fish, nutrient enrichment is necessary. Food along with ambient culture temperature (ferhadian et al., 2012), is an important factor for Cladocera culture because their biochemical composition changes accordingly with their diet (Olsen et al.,1997).

According to Das et al., (2012); Infusoria being small in size, they are soft-bodied and nutritionally very rich and therefore, serve ideally as a starter diet for the early stages of fish larvae. In the early development stages of fish larvae, infusoria or small live organisms are indispensable (Zableckis, 1998).

According to Das et al., (2012), Tubifex makes an ideally suited diet for brooders of various ornamental fishes. Among the natural food organisms, the red worm (Tubifex tubifex) is one of the best candidates owing to its short generation time, occurrence in a vast range of habitats, and tolerance to a wide spectrum of environmental variables (Kaster, 1980).

Mahmut et al., (2003) reported the percentage crude protein, lipid, ash, and moisture content of tubifex were 11.02±0.58, 2.14±0.06, 1.83±0.16 and 18.78±0.83 respectively. Total fatty acid content was 7.28 mg/100 mg dry weight and ù-3 (C18:3n-3 and C20:5n-3) and ù-6 (C18:2n-6c and C20:4n-6) fatty acids composed 18%, 22% of the total, respectively. The most abundant amino acids (amino acid g/100 g protein) were lysine (6.54±0.12), leucine (6.52±0.13) followed by arginine (5.39±0.04), valine (4.92±0.09), threonine (4.81±0.09), phenylalanine (4.36±0.09), isoleucine (4.31±0.08), tyrosine (2.74±0.07), histidine (2.67±0.03) and methionine (1.82±0.04). The total carotenoid level present in Tubifex is 15.02±0.80 mg/kg.

According to Alfico et al., (2022), Insects are the most diverse group of animals, and a natural food source for fish, especially for carnivorous and omnivorous fish, as these fish species need relatively high amounts of proteins in their diets (Noga-les-M´erida et al., 2019;

Tran et al., 2015; van Huis, 2020). Insect meal production is developing rapidly in China, Europe, North America, Australia, and Southeast Asian countries. insect species have already been evaluated for an alternative protein source in aquafeeds (Henry et al., 2015; Noga-les-M´ erida et al., 2019). These insect species have been analyzed for nutritional composition, including contents of crude protein, amino acids, fat content, fatty acid profiles, and minerals (Sanchez-Muros et al., 2014).

# Chapter 3: Aims and Objectives

# 3. Aims and Objectives:

The success of aquaculture depends on healthy cultured stock. A disease-free healthy stock can be maintained by feeding live food to the cultured stock along with the supplemented artificial feed. The current study will focus on the identification and production of the live fish feed organisms (both the conventional and non-conventional) to –

1. Easy and cost-effective way to culture live feed.
2. Comparative analysis of two feeds, Cultured *Daphnia magna* in a cost-effective way, and commercial *Artemia nauplii*.
3. The project would release the burden of the fishermen to purchase costly live feed for aquaculture.

# Chapter 4: Materials and Methods

# 4. Materials and Methods:

## 4.1. Material Required:

1. Plankton collection nets
2. Glass aquarium (3 numbers) – 24 inches ×12 inches ×12 inches.
3. Compound Microscope (Stereo microscope-MS24).
4. Dissecting Microscope (Mag Master-201).
5. Fixative (70% ethanol).
6. Glass Slides and Coverslips.

## 4.2. Methods:

### 4.2.1. Collection and Identification of Different Live Feeds:

Different live feeds like copepods and insect larvae will be collected from different ponds in the West Midnapore District of West Bengal. They will be identified according to the prescribed protocol.

### 4.2.2. Culture of Live Food Organisms:

For the pure culture of *Daphnia magna*, stock culture needs to be developed. In order to start stock culture, collection of *Daphnia magna is* done from freshwater ponds and tanks with the help of a scoop net having 250 to 500-micron mesh. After collection, the content of the net is placed in a plastic bucket and brought to the laboratory. The sample is then diluted by adding clear freshwater and examined under a microscope. *Daphnia magna* was collected with the help of a fine dropper. Each *Daphnia magna* so picked up is inoculated in a 20 ml glass tube containing 10 ml of filtered water. 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 4 to 5 days, this cultured Daphnia magna are used as inoculums in mass culture tanks.

In mass culture, the culture tanks are treated with groundnut oil cake (75 ppm), single super phosphate (20 ppm), and urea (8 ppm). After fertilization, the tank is inoculated with *Chlorella* or mixed phytoplankton. When algal blooms are developed within 3 to 4 days,

*Daphnia magna* is inoculated @ 40 to 50 nos./liter depending on the availability of stock culture. *Daphnia magna* multiples rapidly, feeding on phytoplankton blooms, bacteria, and small particles of groundnut oilcake. It attains a peak density of 20,000 to 25,000 nos. /liter in 5 to 7 days after inoculation. After attaining peak density, it is regularly harvested to feed the larval stages. As a result of *Daphnia magna* multiplication and reduction of nutritional status of water, *Chlorella* concentration declines. In order to maintain optimum *Chlorella* concentration, partial water exchange from the tank bottom and re-fertilization with groundnut oil cake (75 ppm) is done at an interval of 4 to 5 days after the commencement of the first harvesting of *Daphnia magna*.

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

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

Where, T = total number of zooplankton counted,
A = area of the grid in $mm^2$

N = Number of grids counted,

1000 = area of counting chambers in $mm^2$

## 4.2.3. Monitoring of the Media

The water temperature (°C) of the culture media will be recorded by using a mercury thermometer and pH was detected with the help of a pH meter before sampling started at 10.00 a.m. once every 3 days, to identify the best media and best condition of the culture to maximize the production.

## 4.2.4. Experimental Fish

The freshwater angel fish (*Pterophyllum scalare)* was selected and artificially spawned in the hatchery with ovaprim injected intramuscularly in a single dose of 0.5ml/kg fish weight. The growth and survival 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.

### 4.2.5. 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 Department of Fisheries, Midnapore City College.

### 4.2.6. Feeding of Fish Larvae

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

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

**Table – 1:** Different groupings and treatment methods were adopted in feeding the fry for 21 days.

| Group | Treatment |
|---|---|
| **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 experiment was conducted for 21 days in each group of tanks with two different live feeds. The measurements of the total length and weight of the juveniles were performed both at the beginning (100 individuals) and at the end of the experiment (all individuals). The number of surviving juveniles in each tank was counted at the end of the experiment. The specific growth rate (SGR, %/day) and survival rate 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 is the time in days (t=21 days).

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

### 4.2.7. Analysis of Proximate Composition of *Daphnia magna* and *Artemia nauplii*

The proximate composition of commercial trout feed and D. magna is shown in Table 1. Moisture contents were detected with an automatic moisture analyzer (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 determined according to Standard Association of Official Analytical Chemists (AOAC) methods (AOAC 2000b).

### 4.2.8. Statistical Analysis

The statistical analysis of different physicochemical parameters will be carried out by using one-way ANOVA and any difference at a 5% level of significance by using the statistical package of SPSS- 16(SYSTA, USA) to express the results.

# Chapter 5: Results

# 5. Results:

**Table – 2:** 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*, on the other hand, had lower carbohydrate content (12.50%) than *Artemia nauplii* (14.61%). The moisture and ash content is 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.*

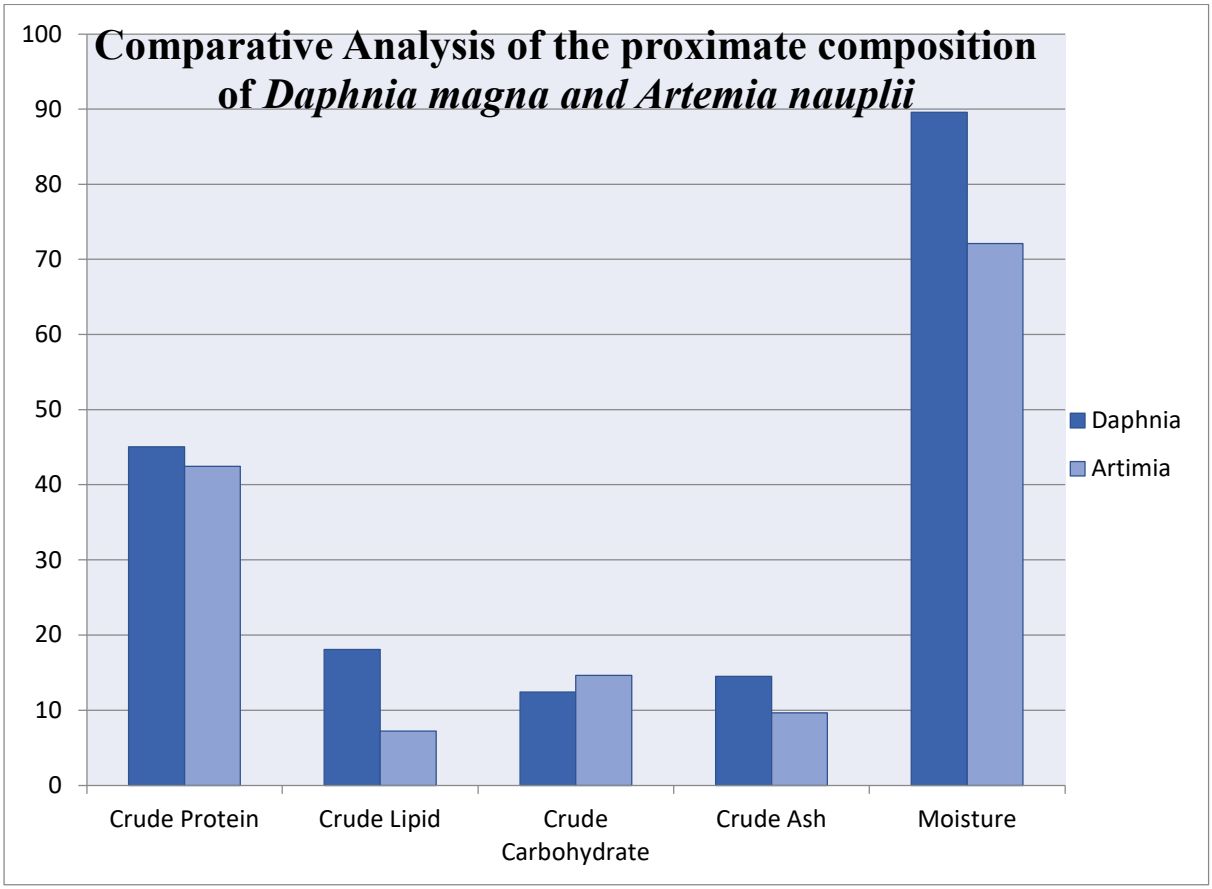


**Table – 3:** Comparative Analysis of *Daphnia magna* and *Artemia nauplii* as food.

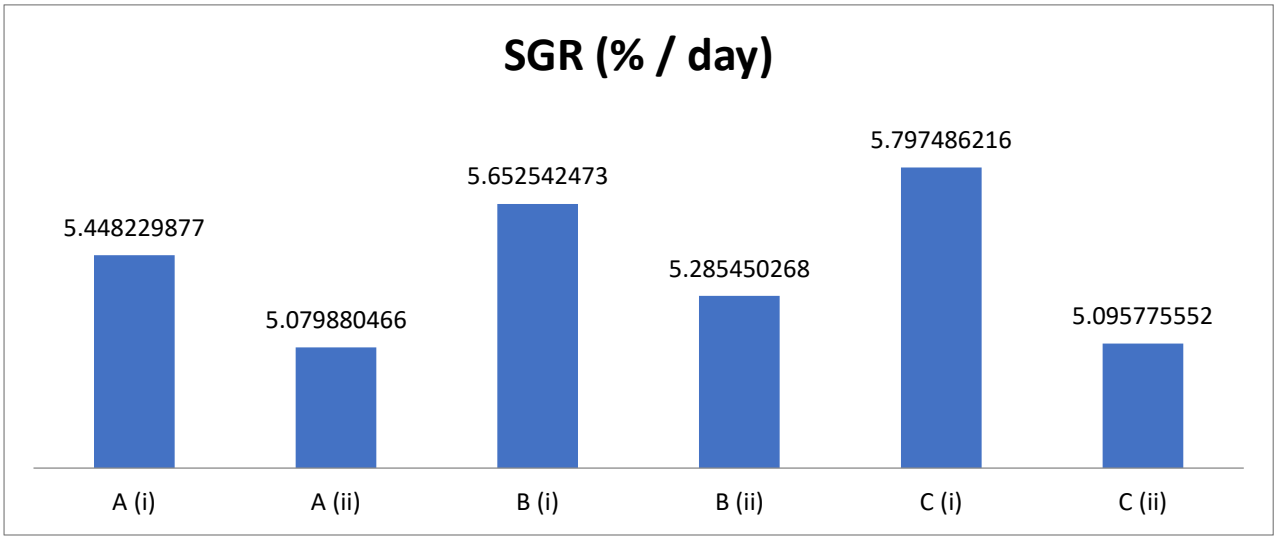
| Group | | Total Number of Fry (Initial) | Total Number of Fry (Final) | Initial Mean Weight of the Juveniles (mg) (W0) | Final Mean Weight of the Juveniles (Wt.) | % of the increase in weight. | SGR (%/day) | Survival Rate |
|---|---|---|---|---|---|---|---|---|
| **A** | A (i) | 100 | 89 | 60.378 | 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 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.*

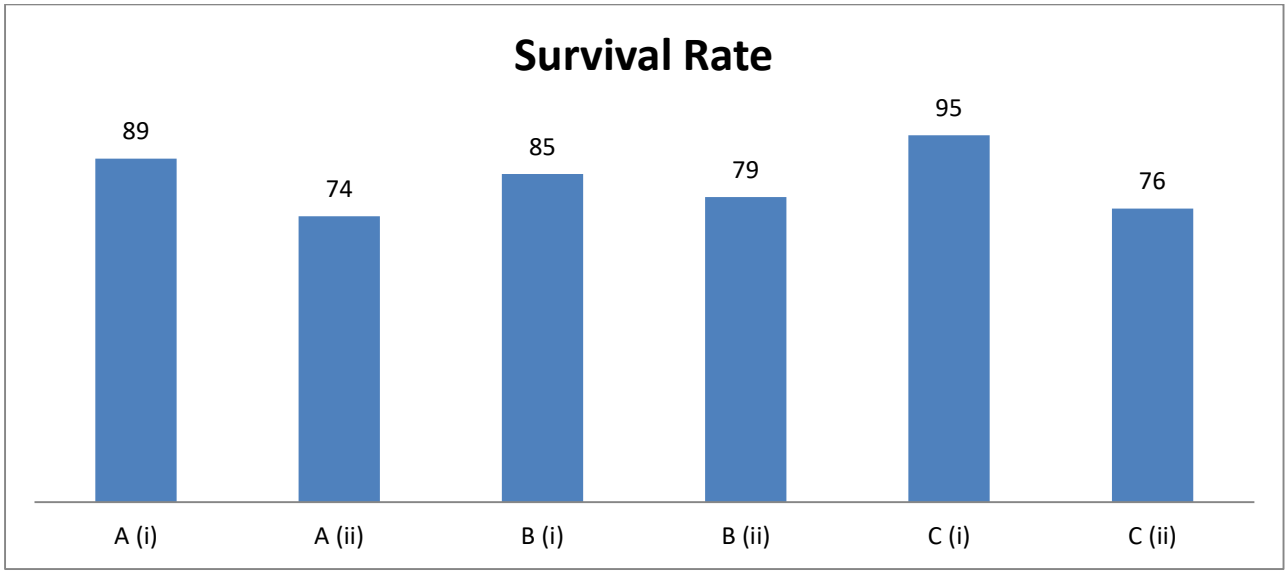
The increase in body weight percentage was recorded higher in *Daphnia magna* treatment (184.24%-210.11%) compared to *Artemia nauplii* treatment (170.57%-181.33%).

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. On the other hand, the specific growth rate was recorded 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 fed 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 Cost of Production**

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

**Table – 4:** Production 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 | Cost of production/ 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 cost of production of *Daphnia magna* as food per gram is much less compared to that of *Artemia nauplii.*

# PLATE-I

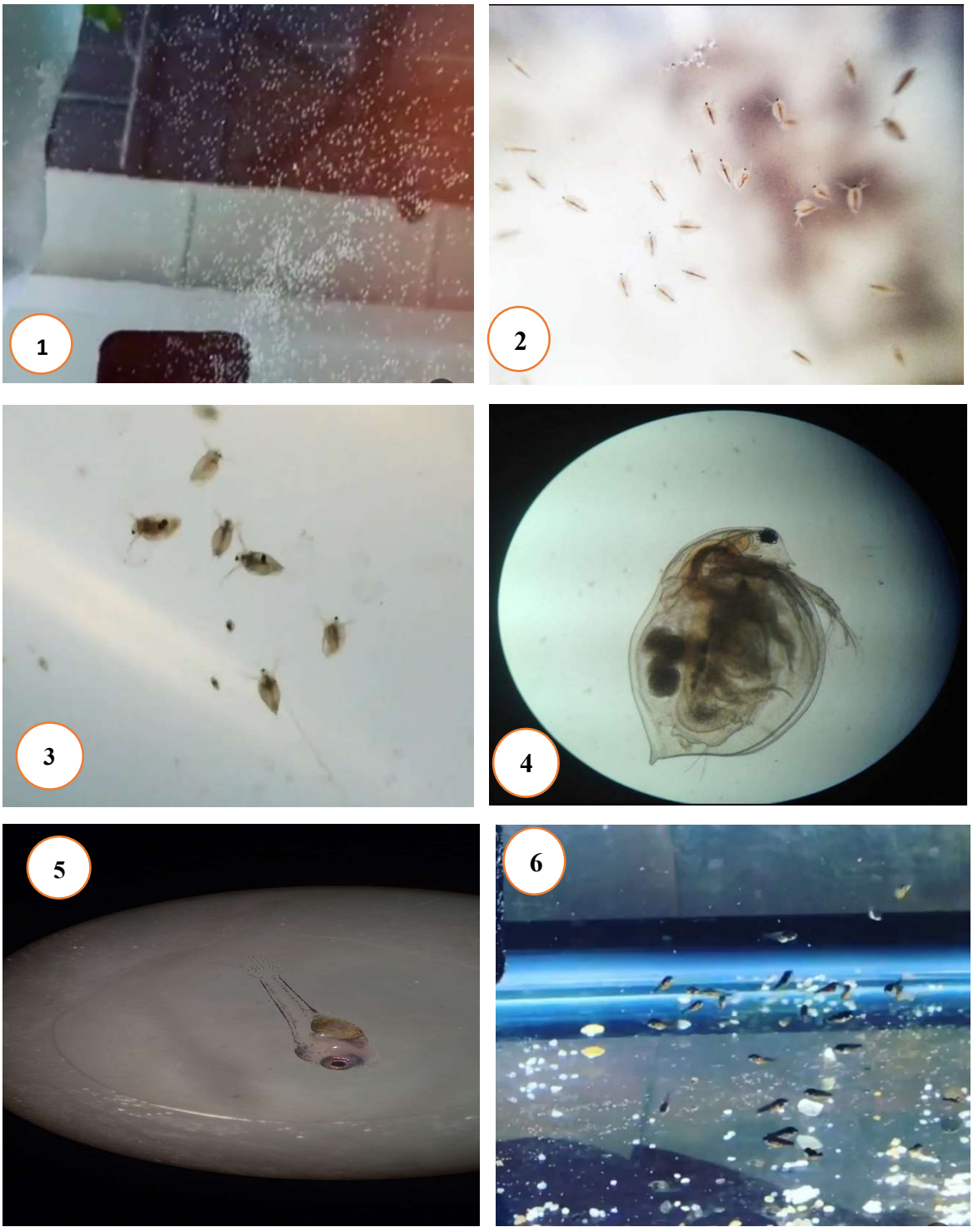
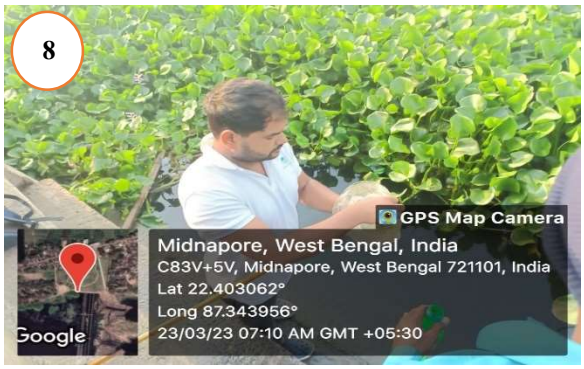



**Figure-1:** Culture of *Daphnia magna.* **Fig-2:** Culture of *Daphnia magna.* **Fig-3:** Microscopic view of *Daphnia magna* (100x) **Fig-4:** Microscopic view of *Daphnia magna* (400x) **Fig-5:** Microscopic view of Juvenile Angel Fish (400x) **Fig-6:** *Daphnia magna* feeding of Juvenile Angel Fish.

# PLATE-II



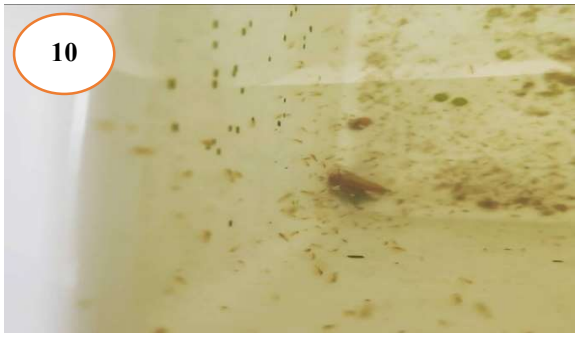





**Figure-7:** Location of sample collection.

**Figure-8:** Collection of Samples.

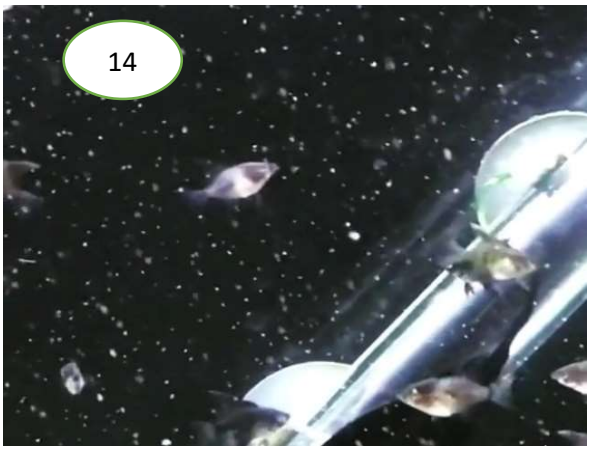
**Figure-9:** Collect the sample.

**Figure-10:** Culture of samples.

**Figure-11:** Observed the sample Species.

**Figure-12:** *Daphnia magna* (400X).

# PLATE-III



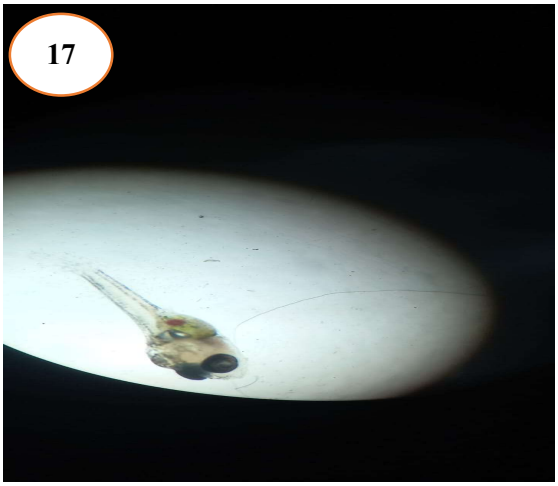





**Figure -13:** *Artemia nauplii.* (400X) **Figure -14:** Feeding of *Artemia nauplii* in Angle Fish. **Figure -15:** Angel Fish feeding. **Figure -16:** Feeding of *Artemia nauplii* in Angle Fish. **Figure -17:** Microscopic view of Juvenile Angel Fish (400x).

**Figure -18:** Growth of Angel Fish.

# Chapter 6: Discussion

# 6. Discussion:

The growth performance of *Pterophyllum scalare* at the end of 21 days feeding trial reveals specific growth rate was slightly higher in *Daphnia magna*-fed larvae compared with the *Artemia nauplii*-fed larvae. The weight was also slightly better in the *Daphnia magna*-fed larvae, compared to the *Artemia nauplii*-fed larvae. The growth of fish is generally believed to be a function of the crude protein level in the diet which was higher in *Daphnia magna*. This could be attributed to the fact that the fish larvae may not require more costly imported *Artemia nauplii* feed for better growth rate and survival 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 not more 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* 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 attributed to the proper management of larvae and the level of acceptability of the two experimental foods by the fish. The *Daphnia magna* is a common live food organism used for the rearing of marine fish larvae. Some authors (Walford et al., 1993; Kolkovski et al., 1993; Reza et al., 2013) 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 *Artemia nauplii* or zooplankton in the larviculture of fish allowed excellent survival rates.

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

Daphnia magna contains a broad spectrum of digestive enzymes such as proteases, peptidases, amylase, lipase, and even cellulase which serve as exoenzymes in the gut of fish and prawns. Being larger in size than *Moina*, it serves as live food for advanced stages of fish. Moina is primarily inhabitants of temporary ponds or ditches. It is smaller in size (0.5 to 2 mm) than *Daphnia magna* containing more protein and therefore, goes well as a replacement for *Artemia nauplii* in aqua hatcheries. *Daphnia magna* has also been extensively utilized as live food in many hatcheries and in the maintenance 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 source of DHA, which helps the fish to make immune resistance against gill and water fouling problems (Jafaryan et al., 2009 and Reza et al., 2013)

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

# Chapter 7: Conclusion

# 7. Conclusion:

The high cost of *Artemia nauplii* cysts has increased fish production costs and cheaper alternative diets with similar nutritional or better quality are needed to maintain the cost competitiveness of the fish in the global market. The industrial development of aquaculture has been hampered by the lack of suitable live feeds for feeding the fish at their various production stages. Here an attempt has been made to make aware of the recent developments in the applications of several live food organisms in the intensive culture of fish and shellfish. The availability of on-grown live food would not only offer farmers and exporters a better alternative option for feeding their fish but more importantly, the possibility of enhancing the fish performance and quality through bio encapsulation. The current study highlighted that the production of *Daphnia magna* is very easy in a home environment, they grow rapidly, and have better alternatives in terms of survival rate and specific growth rate of the fish larvae in comparison with *Artemia nauplii*. Considering several factors, live feed like *Daphnia magna* remains the most practical solution for larval rearing for aquaculture species. However, it is not easy to maintain a steady supply of adequate quantities of *Daphnia magna* at appropriate times in intensive culture systems other than commercial culture. However, the live feed also acts as a carrier of diseases to the larvae of fish and shellfish, therefore, maintenance of hygiene is very important during their production. The nutritional status of the live feed organisms needs to be summarised for feeding different larval stages of fish and shellfish. Therefore, more research thrust should be given on suitability of many of the available live food organisms.

# Chapter 8: Future Prospect

# 8. Future Prospect:

1. Nutritional Analysis and Optimization: Future research can focus on conducting comprehensive nutritional analyses of *Daphnia magna* as a live food source for fish larvae. This would involve determining the precise nutritional composition of *Daphnia magna*, including protein, lipid, carbohydrate, vitamin, and mineral content. Additionally, investigations into the impact of different cultural conditions, such as diet and environmental factors, on the nutritional value of *Daphnia magna* can be explored. These studies will help optimize *Daphnia magna* culture techniques to maximize its nutritional benefits for fish larvae.

2. Evaluation of *Daphnia magna* Species Diversity: *Daphnia magna* species are diverse, and their nutritional composition and suitability as live food for fish larvae may vary. Future studies can evaluate different *Daphnia magna* species to determine their nutritional profiles and their effectiveness in supporting the growth and development of various fish species. This research can provide valuable insights into selecting the most appropriate *Daphnia magna* species for specific fish larvae, considering their nutritional requirements.

3. Culturing Techniques and Optimization: Further research can focus on developing and optimizing efficient culturing techniques for *Daphnia magna.* Investigations can be carried out to determine the ideal culture conditions, including temperature, light intensity, pH levels, and nutrient concentrations, for high-quality *Daphnia magna* production. Additionally, exploring innovative culture systems such as recirculating aquaculture systems (RAS) or integrated multi-trophic aquaculture (IMTA) can help improve *Daphnia magna* yield and minimize environmental impacts.

4. Prey-predator Interactions: Understanding the interactions between *Daphnia magna* and fish larvae is crucial for the successful implementation of *Daphnia magna* as live food. Future studies can investigate the prey-predator relationship between *Daphnia magna* and fish larvae, including factors such as prey size, predator preference, and feeding behavior. This research will aid in determining optimal feeding regimes and methods to enhance the consumption of *Daphnia magna* by fish larvae, leading to improved larval growth and survival rates.

5. Commercial Feasibility and Scaling-up: Evaluating the commercial feasibility and scalability of using *Daphnia magna* as live food for fish larvae is essential for its practical implementation in the aquaculture industry. Future research can explore the economic viability, production costs, and market potential of large-scale *Daphnia magna* culture systems. Additionally, assessing the environmental sustainability and potential for integration with existing aquaculture operations will provide valuable insights into the practicality of utilizing *Daphnia magna* as a live food source.

6. Impacts on Fish Larvae Performance and Health: In-depth studies can be conducted to assess the overall performance, health, and disease resistance of fish larvae fed with *Daphnia magna* compared to other commonly used live food sources. By monitoring growth rates, survival rates, and physiological parameters, researchers can determine the long-term effects of *Daphnia magna*-based diets on larval development and overall fish health. Understanding these impacts will help establish *Daphnia magna* as a reliable and effective live food option for fish larvae.

7. Environmental Benefits and Ecosystem Integration: Examining the potential environmental benefits of using *Daphnia magna* as live food is essential in sustainable aquaculture practices. Future research can investigate the ecological interactions between *Daphnia magna* and the surrounding aquatic ecosystem, assessing its role in nutrient cycling, water quality improvement, and the mitigation of harmful algal blooms. Understanding these ecosystem services will provide a holistic perspective on the use of *Daphnia magna* as live food and its potential contributions to environmental sustainability.

8. Comparative Nutritional Analysis: Future research can focus on conducting a comprehensive nutritional analysis comparing *Daphnia magna* and *Artemia nauplii* as live food sources for fish larvae. This analysis should include an examination of the protein, lipid, carbohydrate, vitamin, and mineral content of both organisms. Understanding the nutritional composition of *Daphnia magna* in comparison to *Artemia nauplii* will provide valuable insights into its suitability as a viable alternative and help determine the specific nutritional requirements of different fish larvae species.
9. Optimal Feeding Regimes: Further investigations can be conducted to determine the optimal feeding regimes using *Daphnia magna* as a replacement for *Artemia nauplii* in fish larval rearing. This research can include studies on feeding frequencies, feeding

rates, and the impact of different *Daphnia magna* concentrations on larval growth, survival, and development. These findings will contribute to establishing effective feeding protocols that maximize the benefits of *Daphnia magna* as a live food source for fish larvae.

10. Culturing Techniques and Scalability: Research efforts can focus on the development and optimization of culturing techniques for *Daphnia magna* to meet the demand as a viable alternative to *Artemia nauplii*. This includes investigating culture conditions, such as temperature, light, nutrient levels, and water quality parameters, to ensure high-quality *Daphnia magna* production on a commercial scale. Additionally, exploring innovative culture systems, such as biofloc technology or aquaponics, can provide sustainable and scalable solutions for *Daphnia magna* production.
11. Environmental Impact Assessment: Assessing the environmental impact of utilizing *Daphnia magna* as a replacement for *Artemia nauplii* is crucial. Future research can investigate the ecological consequences of large-scale *Daphnia magna* culture, including its impact on water quality, nutrient cycling, and potential interactions with the surrounding ecosystem. Understanding the environmental implications will help ensure that the use of *Daphnia magna* as a live food alternative maintains sustainability and minimizes adverse effects on the aquatic environment.
12. Larval Health and Performance: In-depth studies can be conducted to evaluate the impact of *Daphnia magna*-based diets on the health and performance of fish larvae. This research can include assessing growth rates, survival rates, stress response, immune system function, and overall larval well-being when fed with *Daphnia magna* compared to *Artemia nauplii*. Understanding the effects of *Daphnia magna* on larval health will contribute to the establishment of effective feeding strategies that optimize larval growth and development.
13. Economic Feasibility: Examining the economic feasibility of utilizing *Daphnia magna* as a live food alternative to *Artemia nauplii* is essential for practical implementation. Future research can focus on assessing the production costs, market potential, and economic viability of large-scale *Daphnia magna* culture systems. Additionally, comparative cost-benefit analyses between *Daphnia magna* and *Artemia nauplii* can provide insights into the economic advantages of using *Daphnia magna* as a cost-effective alternative feed source for fish larvae.
14. Stakeholder Acceptance and Adoption: Understanding the perspectives and acceptance of stakeholders, including fish farmers, hatchery operators, and aquaculture industry

players, is crucial for the successful adoption of *Daphnia magna* as a live food alternative to *Artemia nauplii*. Future research can explore the attitudes, perceptions, and barriers to adoption among key stakeholders, facilitating the development of strategies to promote the widespread acceptance and implementation of *Daphnia magna*-based feeding practices in the aquaculture industry.

By focusing on these future research areas, the thesis on *Daphnia magna* as live food for fish larvae can contribute to the development of sustainable aquaculture practices, enhance fish larval rearing techniques, and address the growing demand for nutritious and environmentally friendly feed sources in the aquaculture industry.

# Chapter 9: References

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