EFFECT OF LIVE FISH FEED: *Artemia* sp. ON EARLY GROWTH STAGES OF GRASS CARP (*Ctenopharyngodon idella)* IN AQUARIUM CULTURE

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A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Fish Biology and Aquaculture (Course Code-Zoo 653)

Submitted to:

Department of Zoology

Institute of Science and Technology

Amrit Campus

Thamel, Kathmandu

Nepal

August, 2021

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

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RECOMMENDATION

This is to recommend that the thesis titled **"EFFECT OF LIVE FISH FEED: *Artemia* sp. ON EARLY STAGE GROWTH OF GRASS CARP (*Ctenopharyngodon idella*) IN AQUARIUM CULTURE"** has been carried out by **Suyatra Ghimire** for the partial fulfillment of Master's Degree of Science in Zoology with special paper **Fish Biology and Aquaculture (Course code: Zoo-653)**. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions. I recommend that the thesis be accepted for partial fulfilment of the requirements for the Degree of Master of Science in Zoology.

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LETTER OF APPROVAL

On the recommendation of supervisor **Dr. Ganesh Bahadur Thapa,** this thesis submitted by **Suyatra Ghimire** titled **"EFFECT OF LIVE FISH FEED: *Artemia* sp. ON EARLY STAGE GROWTH OF GRASS CARP (*Ctenopharyngodon idella*) IN AQUARIUM CULTURE"** is approved for the examination and submitted to Tribhuvan University in partial fulfillment of requirements for Master's Degree of Science in Zoology with special paper in Fish Biology and Aquaculture (Course code: Zoo-653).

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Suyatra Ghimire** titled **"EFFECT OF LIVE FISH FEED: *Artemia* sp. ON EARLY STAGE GROWTH OF GRASS CARP (*Ctenopharyngodon idella*) IN AQUARIUM CULTURE"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture (Course code: Zoo-653).

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ACKNOWLEDGEMENTS

I want to thank the Department of Zoology, Amrit Campus for providing me an opportunity to conduct this research. I would like to heartily thank my thesis supervisor Dr. Ganesh Bahadur Thapa for supervising the research. I extend my gratitude towards Associate Prof. Mrs. Prabha Chitrakar for assisting in laboratory with the research and helping with the manuscript. My further gratitude goes to Central Fisheries Promotion and Conservation Centre (CFPCC) Balaju, Kathmandu, Nepal for providing their laboratory and other assistance in carrying out this research even during the COVID-19 pandemic.

I want to acknowledge the help and support provided by officials of Central Fisheries Promotion and Conservation Centre (CFPCC) Balaju, Kathmandu, Nepal, especially, Mr. Balaram Acharya, for supervising the study. My obligation goes to Mr. Pramod Rijal for granting permission to carry out the research in CFPCC's lab. I would further like to thank other staffs of CFPCC; Mr. Subhash Kumar Jha, Mr. Prem Khatri, Mr. Babin Adhikari and Mrs. Laxmi Shrestha for their academic, physical and moral support. My immense thanks to Mrs. Sita Chaudhary for helping with the setup and regular cleaning of the laboratory. I would also like to thank Mr. Sulav Shrestha for his assistance during the research.

My gratitude goes to Amrit Campus, Department of Zoology and its staffs, especially towards Mrs. Renuka Shrestha and Maan Bahadur Gole, Dr. Rakshya Thapa and Mrs. Babita Maharjan, Coordinator of Zoology for their support. I would further like to show my appreciation towards University Grants Commission (UGC), Sano Thimi, Bhaktapur for providing graduate research grant without which it would not have been possible to carry out and complete the research. I am forever grateful for that support from UGC.

Furthermore, my gratitude goes to Mr. Jash Hang Limbu for his help in analyzing the research data and proofreading the manuscript.

Last but not the least, I am forever thankful towards my parents and family for their perpetual help and support in my every endeavors. I am highly obliged to all the individuals who have directly or indirectly contributed their valuable suggestions, guidance and coordination in the completion of my thesis research work.

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LIST OF ABBREVIATIONS

GDP	Gross Domestic Product
FAO	Food and Agriculture Organization
Mm	Millimeter
Cm	Centimeter
Kg	Kilogram
M	Meter
g/gm	Gram
L	Liter
Ml	Milliliter
°C	Degree Celsius
°F	Degree Fahrenheit
hr(s)	hour(s)
Ppt	parts per thousand
CFU	Colony Forming Unit
TDS	Total Dissolved Solids
DO	Dissolved Oxygen
D1	Diet 1
D2	Diet 2
D3	Diet 3
DAH	Days after hatching
WG	Weight Gain
Wt	Mean Final weight
Wi	Mean Initial Weight
IW	Initial Weight
FW	Final Weight
TWG	Total Weight Gain
MWG	Mean Weight Gain
TL	Total Length
FW	Final Weight
SGR	Specific Growth Rate
FI	Feed Intake

FCR	Feed Conversion Ratio
FGR	Food Gain Ratio
FCE	Feed Conversion Efficiency
HIS	Hepatosomatic Index
PI	Protein Intake
PER	Protein Efficiency Ratio
PPV	Productive Protein Values
MWG	Mean Weight Gain
MLG	Mean Length Gain
SR%	Survival Percentage
SR	Survival Rate
ANOVA	Analysis of Variance
NAL	National Agricultural Library
UGC	University Grants Commission
USDA	United States Department of Agriculture
CFPCC	Central Fisheries Promotion and Conservation Centre
CABI	Centre for Agriculture and Bioscience International

ABSTRACT

Brine shrimp (*Artemia* sp.) has been a popular live fish feed in many countries for a long time. However, use of *Artemia* nauplii in earlier stage growth of fish in Nepal had not been properly carried out. So, growth of Grass carp (*Ctenopharyngodon idella*) larvae 7 DAH was tested on three diets; control diet D1 (*Artemia* nauplii plus hen's egg yolk), D2 (egg yolk) and D3 (*Artemia* nauplii), with three replicates each from 5/10/2021 to 6/21/2021. Diet was provided once a day at 4 pm at 5% their body weight. The physicochemical parameters (pH, DO, TDS, Conductivity, Salinity, and atmospheric pressure) were measured every day and the tanks were set at a temperature of 25 ˚C with aquarium heater. The weight of larvae was measured every 7 days. At the end of the study on 42nd day, larvae were dissected and their hepatosomatic index (HSI) was measured. A significant difference ($p<0.05$) among the groups in experiment was seen in feed conversion ratio (FCR), % weight gain, survival percentage, and protein intake. Highest growth, hepatosomatic index (HSI), protein efficiency ratio (PER) and survival was recorded in the group fed diet D2 (egg yolk) and least was observed in the group fed diet D3 (*Artemia*).

Keywords: Brine shrimp, live feed, carp, larval growth, hepatosomatic index

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1. INTRODUCTION

Fish are aquatic poikilotherms and like other vertebrates, they have embryonic, juvenile, adult and aged phases in their lifecycle (Dutta, 1994). The size and growth of fish are controlled by the ecological opportunities available to them in terms of food, nutrition, temperature, photoperiod and sexual rhythms (Parker & Larkin, 1959). The period of growth in fish starts as soon as the young fish begins to feed and continues throughout its life though the growth pattern changes after sexual maturity (Dutta, 1994).

The growth of a fish whether in length or in weight results from the consumption of food, its assimilation and conversion into an organism's body. Knowledge of fish growth is of vital importance for obtaining high yield of fish (Woodhead, 1985). The rate of growth varies from species to species and sometimes it varies even among species. Fish show a considerable variation in their growth rate and age of sexual maturity (Tesch, 1971). Maximum growth rate is found just before attaining sexual maturity (Parker & Larkin, 1959). Availability of food and oxygen, intensity of light, population density, temperature range, etc. play an important role in growth as these factors affect metabolism and food consumption in fish (Parker & Larkin, 1959). It is possible to achieve better growth of a species through manipulation of nutritional requirements and monitoring the ration quality and quantity based on the energy contents of the ingredients of the feed (Dutta, 1994).

1.1 Aquaculture in Nepal

Aquaculture in Nepal is basically new and small scale. During 2003/2004, aquaculture contributed to 2% of the total GDP of Nepal from the agriculture sector (FAO, 2021). Freshwater fisheries are mostly dominated by capture of indigenous fish species from river, lakes and paddy fields. Carp polyculture in ponds is by far the most common and viable aquaculture production system adopted in Nepal and the majority of pond fish production takes place in the southern terai where 94% of the fish ponds are located (FAO, 2021). Fish farming has traditionally been practiced by a few tribal groups in Nepal such as Tharu, Majhi, Malaha, Dunuwar, Kewat, Bote, Musahar, Mukhiya, Darai, Kumal, Dangar, Jalari Rai and other poverty-laden tribes are traditionally involved in capture fishery for their livelihood and food sources (Gurung et al., 2005; Dahal et al., 2013). Aquaculture in Nepal started in the mid-1940s on a small scale with pond culture of indigenous major carp seeds from India (FAO, 2021). The country's fish production has not been able to meet local demand despite a rapid growth in fish farming. However, aquaculture in Nepal is continuously progressing and the number of fishers are increasing for fish farming in rivers, lakes, swamps, gholes, irrigated fields, and ponds. Different systems of fish farming techniques like: monoculture, polyculture, cage culture, pen culture, race way culture, integrated fish farming such as rice-fish farming, horticulture-fish farming, livestock-fish farming, pig-fish farming, duck-fish farming, poultry-fish farming and so on are also being carried out.

1.2 Larval rearing

Larval rearing is a critical period for the successful culture of fish (Mahfuj et al., 2012), particularly in respect to cyprinids (Von Oertzen, 1985). During early stages of growth, the larvae fish rely on the yolk sac for nutritional requirements (Kolkvoski, 2001). Sub optimal nourishment of the fish larvae due to inadequate diets is a major cause of poor survival (Rottmann et al., 1991). In cyprinids such as *Leociscus* species (Kwiatkowski et al., 2008), *Scardinius erythrophthalmus* (Wolnicki et al., 2009), *Aspius aspius* and *Chondrostoma nasus* (Kujawa et al., 2010), *Rutilus frisii kutum* (Falahatkar et al., 2011) and *Cyprinus carpio* (Mahfuj et al., 2012), larval growth and survival rates were strongly and differently affected by both the type of diet given and the age when food was introduced.

An improper weaning diet could impair or delay the development of the gastrointestinal tract causing chronic stress leading to physiological malfunctions or even death (Cahu & Zambonino-Infante, 1994). In general, protein and other dietary requirements are typically lower for herbivorous and omnivorous than they are for carnivorous fish but generally higher for smaller as well as early life stage fish. As fish grow larger, their requirements usually decrease (Craig et al., 2017). At the onset of exogenous feeding, live feeds such as brine shrimp (*Artemia* nauplii), yeast, zooplankton and unicellular algae are more appropriate because the fish have difficulty assimilating dry prepared diets (Verreth & Tongeren, 1989).

Live feeds are feeds which consist of living organism that can be active or inactive. They are palatable to fish and allows for better digestibility (Kolkovski, 2001). The importance of using combined live feed with formulated diets lies mainly in supplying a more suitable and well balanced diet to fish larvae in a digestive form (Adekunle & Joyce, 2014). Despite considerable progress in the development of formulated larval feeds and the adaptation of various techniques to reduce the quantities required, for instance, through improved zoo-techniques, feeding regimes and feeding practices; the worldwide use of live feed in the hatchery rearing of most fish and shellfish species is still essential and is expected to remain so in the future (Dhont et al., 2013).

The *Artemia* is said to be a good diet that can produce quality larvae for some cultured fish species but the high cost of import is prohibitive for most farmers in developing nations and cheaper options could be a better strategy (Olurin & Oluwo, 2010). However, the difficulties of handling zooplankton as live prey food and its seasonal dependence makes *Artemia* a better and adequate feed to start external feeding for many fish larvae (Carral et al., 2006). The growth-promoting potential of live food has been shown for grass carp (*Ctenopharyngodon idella*) and big head carp (*Hypophthalmichthys nobilis*) where fish fed three live food diets (*Artemia*, rotifers and nematodes) grew better than those fed two commercial dry diets (Rottmann et al., 1991).

1.3 Grass carp

Grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) is a native Chinese freshwater fish that has been introduced to about 40 other countries, including Nepal, for aquaculture where various production systems are currently used including semi-intensive and intensive culture ponds, and pens and cages in open waters. After silver carp, grass carp currently has the largest production in freshwater aquaculture globally (FAO, 2021). *Ctenopharyngodon idella*, the Grass Carp, is one of the largest members of the family Cyprinidae and is the only member of the genus *Ctenopharyngodon* (Shireman & Smith, 1983; Chilton & Muoneke, 1992).

Ctenopharyngodon idella (Valenciennes 1844) is characterized by a wide, scaleless head; sub-terminal or terminal mouth with simple lips, absence of barbels, slightly protracted upper jaw and a very short snout, 38-47 moderate to large scales along with a completed lateral line, and 2 rows (2,4/5,4 or 2,5/4,2) of laterally compressed, sharply serrated pharyngeal teeth with prominent parallel grooves (Shireman & Smith, 1983). Its length is less than, or equal to, its eye diameter and its postorbital length is more than half its head length (Page & Burr, 1991; Eccles, 1992; Opuszynski & Shireman, 1995). A typical grass carp has an elongated body dominated by greenish sheen color, a slightly bluish gray back, light gray fins, and a grayish white belly. However, the coloration varies and may be related to habitat, food and/or genetic variation (Shireman & Smith, 1983).

1.4 *Artemia*

Artemia is an aquatic crustacean also known as brine shrimp. *Artemia* populations are found worldwide in inland saltwater lakes, but not in oceans. *Artemia* are able to avoid cohabiting with most types of predators, such as fish, by their ability to live in waters of very high salinity up to 25% (Daintith, 1996). The ability of the *Artemia* to produce dormant eggs, known as cysts, has led to extensive use of *Artemia* in aquaculture. The cysts may be stored indefinitely and hatched on demand to provide a convenient form of live feed for larval fish and crustaceans (Daintith, 1996).

Figure 1.4: Digital inversion of bright-field image of 28 day old Artemia (by Piper J, 2018)

Being extremely osmotolerant, brine shrimp survive in environments with salinities ranging between approximately 10 and 340 g/L with diverse ionic composition and temperature regimes; in general, the lower salinity threshold of its occurrence is determined by the salinity tolerance of its predators in the area, and abundant *Artemia* populations are consequentially only found at salinities elevated enough to eliminate (nearly) all predators or food competitors. *Artemia* is exceptionally adapted to such extreme environments, due to its unique osmoregulatory capacity and its capacity to synthesize highly efficient hemoglobin (Clegg & Trotman, 2002).

1.4.1 *Artemia* use in aquaculture

Fish farm owners search for a cost-effective, easy to use, and available food that is preferred by the fish. From cysts, brine shrimp nauplii can readily be used to feed fish and crustacean larvae just after one-day incubation. Instar I (the nauplii that just hatched and with large yolk reserves in their body) and instar II nauplii (the nauplii after first moult and with functional digestive tracts) are more widely used in aquaculture, because they are easy for operation, rich in nutrients, and small, which makes them suitable for feeding fish and crustacean larvae live or after drying.

In their first stage of development, *Artemia* do not feed but consume their own energy reserves stored in the cyst (Sorgeloos et al., 2001). Wild brine shrimp eat microscopic planktonic algae. Cultured brine shrimp can also be fed particulate foods including yeast, wheat flour, soybean powder or egg yolk (Schumann, 1997).

Although hatchery process of brine shrimp are relative simple and easy to operate, a series of factors need to be controlled and monitored to make optimal use of the cysts. The critical factors are light, temperature, salinity, oxygen level, pH and cyst density, which vary between different brine shrimp strains (Vanhaecke & Sorgeloos, 1983). Hatching quality can be described by hatching efficiency (number of nauplii per gram of cysts), hatching percentage or hatching synchrony (time between first and last hatching cysts).

After hatching, and prior to feeding them to the fish or crustacean larvae, brine shrimp nauplii should be separated from the hatching wastes. After switching off the aeration in the hatching tank, cyst shells will float and nauplii will concentrate at the bottom of the tank. The nauplii are further concentrated in a concentrator rinse and separated from the cysts. The enrichment process, if needed, generally occurs after the nauplii develop a digestive tract (Daintith, 1996).

1.5 Fish growth

The conventional method to study the growth in fish is to measure the length and weight of the body of an individual or a group of individuals at a definite interval of time either (Dutta, 1994). The relationship between length and weight provides an index frequently used by fishery biologists to quantify the state of wellbeing of a fish. In addition to morphometric criteria, several other methods are used to study the growth rates in fishes like Glycine Uptake by Scales, Hepatosomatic Index and RNA: DNA Ratio (Dutta, 1994).

Hepatosomatic Index (HSI) is used as a measure to find out the energy reserve of an animal, especially, fish (NAL Thesaurus Staff, 2021). Hepatosomatic index or ratio of liver weight to total weight of the fish is also a useful indicator of change in growth rate. In *Phoxinus phoxinus* kept under controlled conditions and fed rations, the specific growth rate is highly correlated with the hepatosomatic index (Cui & Wootton, 1988). Hepatosomatic index is an important indicator of growth in fish as liver is particularly sensitive to various nutritional conditions in larvae (Fontagne et al., 1998). In larval fish, liver volume can be increased by a factor of two when feeding carbohydrate-rich diets (Szlaminska et al., 1991) and decreased by one-half when starved, also reducing the size of hepatocyte nuclei (Fontagne et al., 1998). Hepatocyte volume can also be increased by several nutritional disorders, for instance by essential fatty acid deficiency (Watanabe et al., 1989).

In this research, a comparison is made of the relative growths obtained for larvae of grass carp during the first forty-two days after active larval feeding commences, using live feed and hen egg-yolk diet.

1.6 Objectives of the study

1.6.1 General objective

To study the growth of early stages of grass carp in different feeds.

1.6.2 Specific objectives

To understand the survival rate of grass carp larvae fed diets of live feed (*Artemia* sp.) and egg yolk in laboratory conditions

To know the growth performances like total weight gain (TWG), specific growth rate (SGR) and feed conversion ratio (FCR) of grass carp larvae

To study the hepato-somatic index (HSI) of grass carp larvae

1.7 Significance of the research

In the national development policy, agriculture development has the highest priority in the fifth five-year plan. Nepal Government has recognized the importance of aquaculture in improving the nutrition of people through increasing production of fish in a relatively short period compared with other sectors of animal husbandry. National policy has given considerable priority to the development of fish culture and this proposed research addresses that part to improve aquaculture and fish production. The present research is significant in aquaculture as recently the fish farmers in Nepal have become more attracted towards feeding live fish during larval stage of fish to enhance growth. However, due to expensive brine shrimp cysts that need to be imported, potential of its use had not been tested before.

1.8 Limitations of the study

This work is conducted as the partial fulfillment of Master's Degree in Zoology as the course credit according to Tribhuvan University. The major limitation in the present study was the expensive cost of materials required which was alleviated after receiving thesis research grant from University Grants Commission, Sano Thimi, Bhaktapur.

Further, due to the situation of global pandemic resulting from COVID-19 and unavailability of access to the laboratory of Amrit Campus, the research had to be carried out at the lab of CFPCC after being granted permission from the head of Central Fisheries Promotion and Conservation Center (CFPCC), Balaju, Kathmandu, Nepal. Further, commuting to the laboratory every day during research period amidst the rigorous lockdown was a limiting factor. However, with the pass provided by CFPCC, the work was able to be carried out in the lab of CFPCC with hassle-free commute.

Despite these technical challenges, this work was conducted with the help of enthusiastic, co-operating and helpful thesis supervisors, officers and other staffs of CFPCC which provided clean energy and crystal clear vision for the completion of task.

2. LITERATURE REVIEW

a. Enriched *Artemia* nauplii diet

El-Sayed et al. (2021) studied the application of enriched *Cyclops abyssorum divergens* (EC) with mixed algal diet compared to *Artemia franciscana* (EA) for improving larval growth and body composition of *Dicentrarchus labrax*. Specific growth rates (SGR) and survival percentages (S %) of *D. labrax* were higher in sea bass larvae fed with EC compared to the lower values in EA. The study concluded that *C. abyssorum divergens* is the best promising candidate for Sea bass larvae-culture in comparison to *Artemia*.

Prusinska et al. (2020) fed *Artemia* sp. nauplii enriched with PUFAs to barbel larvae (*Barbus barbus*) and studied the effect on their growth and survival rate, blood composition, alimentary tract histological structure and body chemical composition. The growth indices and feed utilization indices were found to improve and the intestinal enterocytes area was found to increase, which helped fish to improve the absorption of nutrients and to improve the fatty acid profile and EPA/DHA ratio in the fish body. The study thus concluded that the use of bio-encapsulated *Artemia* enriched with PUFAs in barbel rearing may be a method of improving the rearing effectiveness in the early stages of the fish.

Radhakrishnan et al. (2020) evaluated the influence of bio-flocculated algae on the growth, digestive enzyme activity and microflora of freshwater fish *Catla catla*. The experimental fish were fed with *Artemia franciscana* enriched with flocculated algae for 60 days. A control group was fed with unenriched *A. franciscana*. This treatment group also had a better growth performance with a higher average body length and weight (8.7 ± 0.3 cm, 5.83 ± 0.9 g) and survival % (98 ± 1.02). High protease (7.8 µmg/protein−1) and lipase (2.56 µmg/protein−1) activity were also found in the enriched *A. franciscana*-fed fish group. Comparatively, higher protein, lipid and PUFA/HUFA contents were also reported in this treatment group. The study found that flocculated algae-enriched *A. franciscana* has a positive impact on gut microflora, growth parameters and survival as compared to the unenriched group. This study thus inferred that a bio-flocculated algae-incorporated *Artemia* diet is a preferable method for larval rearing aquaculture.

Ahmadifard et al. (2018) evaluated the impacts of long-term enriched *Artemia* with *Bacillus subtilis* on growth performance, reproduction, intestinal microflora, and resistance to *Aeromonas hydrophila* of ornamental fish *Poecilia latipinn*. Maximum protein and fat contents were observed in fish fed with Bacillus-enriched *Artemia*; however, no significant difference was found between control and unenriched *Artemia* groups ($P > 0.05$). The study concluded that *B. subtilis* with a concentration of 1×105 CFU mL^{-1} during the period of *Artemia* culturing can improve the reproductive parameters, intestinal microflora, and resistance to pathogenic bacteria of *Poecilia latipinna* compared to unenriched *Artemia* and/or control diet.

Jamali et al. (2018) conducted an experiment on the effects of feeding on a commercial diet and lecithin-enriched (EN) *Artemia franciscana* nauplii for improving co-feeding strategies of Neotropical green terror cichlid (*Aequidens rivulatus*) larvae. Growth performance of fish fed 10 EN and 5 EN had significantly higher values of total weight (120.67, 120.31 mg), %WG (584.48, 580.50%) and SGR (7.69, 7.67%) respectively ($p < 0.05$) but fish fed 25 EN had significantly higher FCE (190.4%), PER (3.95) and NPU (202.5), in comparison with other groups. The result concluded that feeding regimes of 10 EN and 5 EN could improve survival and growth performance of Neotropical green terror cichlid, *A. rivulatus* larvae.

Yang (2017) used newly hatched *Artemia* nauplii, enriched with 4 levels (0, 400, 800, and 1 600 mg/L) of L-methionine for 16 hrs as starter food for rearing larvae. The body length and body weight of the fish were determined at the 7th, 14th and 21st days, respectively. In this experiment, the first feeding larvae of bighead carp (*Aristichthys nobilis*) fed with 800 mg/L L-methionine enriched *Artemia* nauplii showed the best growth performance.

Javad et al. (2011) added Bacillus strains additives to Common carp (*Cyprinus carpio*) and Grass carp (*Ctenopharyngodon idella*) larvae's food and studied its effect on their growth. Result of this experiment showed that enrichment had positive effect on growth rate but each species of *Artemia* had specific result. Comparing treatment with control showed that enriched *Artemia* caused positive growth rate and there was significant difference between them ($P<0.05$).The highest growth rate was related to common carp larvae fed by enriched nauplii of *A. parthenogeneca*. Those treatment that were fed with enriched nauplii of *A. parthenogeneca* had significant difference with the others (P<0.05). In grass carp, those fed with *A. parthenogeneca* nauplii and *A. fransiscana* had highest growth rate than the others in control treatment.

Mai et al. (2009) co-fed live feed and inert diet from first-feeding to evaluate the effects of *Artemia* on lipid digestibility and retention in Senegalese sole (*Solea senegalensis*) larvae. *Solea senegalensis* larvae were reared on a standard live feed regime (ST) and co-feeding regime with inert diet (Art R). Trials using sole larvae fed with *Artemia* enriched with two different lipid emulsions were performed at 9 and 17 days after hatching (DAH) to study lipid utilization. Co-feeding did not affect sole survival rates (ST 59.1±15.9%; Art R69.56±9.3%), but was reflected in significantly smaller final weight at 16 DAH (ST 0.71±0.20; Art R 0.48±0.14 mg). At 9 DAH lipid digestibility was equal among treatments and higher than 90%, while at 17 DAH it was higher in ST treatment (around 73%) compared to the Art R group (around 66%). Lipid retention efficiency at 9 DAH was higher in the Art R treatment, reaching values of 50%, while these values almost duplicated at 17 DAH, ranging up to 80% in both treatments without significant differences. The results from this experiment showed that co-feeding of live feed and inert diet from first-feeding in Senegalese sole has a toll in terms of growth and lipid digestibility but does not seem to compromise lipid metabolic utilization.

Olsen et al. (1999) studied the influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. In a first feeding experiment, halibut larvae were offered either short term enriched (ST) 1-day old *Artemia franciscana* or *A. franciscana* of successively increasing size (ST) 2, 3 and 4-day old juveniles from day 0 to day 60. No differences, either in growth (approximately 6.5% daily weight increase, DWI or in survival 25%) between the two treatments were observed, but both were satisfactory. Feeding increasing sizes of *A. franciscana* increased the number of completely pigmented and metamorphosed larvae from 4 to 20%. Juvenile *A. franciscana* may therefore be a better live feed than ST *A. franciscana* for halibut larvae.

b. Normal *Artemia* nauplii diet

Aruho et al. (2020) evaluated the growth and survival of Ripon barbel (*Barbus altianalis*) larvae and juveniles by feeding five experimental diets of live prey (*Moina* and *Artemia nauplii*), microdiet (57 % Crude Protein), decapsulated *Artemia* cysts and combination (*Moina + microdiet*). In 15 day old larvae, combination diet and decapsulated *Artemia* performed better than microdiet, *Moina* and *Artemia* nauplii in that order.

Hamre et al. (2020) conducted an experiment on Atlantic halibut by feeding on-grown *Artemia*; hypothesizing that it would mitigate the halibut's slow growth during the late larval stages and inferior juvenile quality due to pigmentation errors and incomplete eye migration during metamorphosis. The research concluded that the *Artemia* nauplii probably covers the nutrient requirements of Atlantic halibut larvae since the final weight of Atlantic halibut postlarvae was similar, and 90% of the juveniles had complete eye migration.

Abe at al. (2019) evaluated the productive growth performance of Amazon ornamental fish *Nannostomus beckfordi* larvae submitted to different stocking densities and *Artemia* nauplii feeding management in captivity conditions. At the end of 15 days, the survival and productive performance such as total length (TL), final weight (FW), specific development rate (SDR), specific growth rate (SGR) was determined. The study concluded that during the initial stage exogenous feeding of *Nannostomus beckfordi*, stocking density of 20 larvae per liter fed with 100 *Artemia* nauplii per larvae is recommended.

Zainiyah et al. (2019) studied the effect of giving cake artificial feed and natural feed (*Artemia* nauplii) on the survival rate, and growth of Common carp *(Cyprinus carpio)* larva in an Installation of Freshwater Culture in Punten, Batu. The results of the Cake artificial feeding on the larval Common carp showed there to be no significant difference in comparison with the administration of natural feed (*Artemia* sp.). The effect produced by the Cake artificial feeding on the growth of the larval carp was that SGR was 0.22±0.005 gr /day, FCR was 1.7±0,00 and the survival rate (SR) was 45±0,00 percent. The study recommended that cake is not to be given to the larval Common carp as an alternative to natural feed.

Adekunle and Joyce (2014) carried out an experiment to determine whether live feed (*Artemia*) and formulated imported feed (Coppens) or the combination of the two feeds at different proportions will efficiently enhanced the growth and survivability of hybrid (*H. bidorsalis* x *H. longifilis)* hatchlings. The result showed that live food (D**1**) (100% *Artemia*) was best utilized for growth and feed utilization by the Hybrid (*H. bidorsalis* x *H. longifilis)* catfish fry. Fry fed imported formulated diet (D**2**) (100% Coppens) showed significantly ($p<0.05$) lowest growth rate and survivability compared to fry fed D**3**-D**5** (different ratios of live and formulated diets).

Sahandi et al. (2012) studied the effects of direct inoculation of Bacillus on growth performance of two carp species reared in fish tanks and fed with *Artemia* sp. The results of the study demonstrated that the common carp larvae have different ability in exploitation of various *Artemia* nauplii than grass carp larvae and administration of *Bacillus circulans* and *B. licheniformis* via direct inoculation to rearing tanks resulted significant higher growth and feeding performance in comparison with non-inoculation treatments.

Chepkirui-Boit et al. (2011) studied the growth performance, survival, feed utilization and nutrient utilization of African catfish (*Clarias gariepinus*) larvae co-fed *Artemia* and a micro-diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning for the culture period of 21 days. Larvae co-fed using 50% *Artemia* and 50% formulated dry diet resulted in significantly ($P < 0.05$) better growth performance, food gain ratio (FGR), protein efficiency ratio (PER) and productive protein values (PPV) than other treatments. Better survival of over 90% was obtained in larvae weaned using 50% *Artemia* and 50% dry diet, while abrupt weaning using 100% dry diets resulted in lower survival (<75%).

Jafaryan et al. (2011) analyzed the effect of adding probiotics into the rearing tanks of grass carp larvae that were fed *Artemia urmiana*, *Artemia fransiscana* and *Artemia parthenogenetica* nauplii**.** In probiotic trials, the combination of *Bacillus circulans* and *Bacillus licheniformis* was also added to rearing tanks of grass carp at 1×10 CFU/L. Final body weight, SGR, thermal growth coefficient, daily growth coefficient and relative gain rate were all affected by the addition of probiotic *Bacillus* spp. ($P<0.05$). The highest growth parameters were obtained in the group fed *A. parthenogenetica* plus probiotic bacteria, while the group fed *A. urmiana* plus probiotic bacteria had significantly ($P<0.05$) lower growth parameters compared with the other treatments.

Olurin and Oluwo (2010) investigated the effects of three diets (decapsulated *Artemia*, live Daphnia spp., and commercial starter diet) on the growth and survival of *Clarias gariepinus* in the laboratory for seven days using a completely randomized block design. The highest growth values were obtained in larvae fed decapsulated *Artemia* ($p<0.05$), while the survival rate was similar in fish fed decapsulated *Artemia* and live daphnia. It is concluded that feeds of animal origin are more suitable for first feeding of *C. gariepinus* larvae than inert diets.

Celada et al. (2008) reared Tench (*Tinca tinca* L.) larvae on live feed (*Artemia*) and on two transition schedules from live to dry diets. In experiment 1, *Artemia* nauplii were the sole food where high survival rates (between 79.5% and 95.5%) were obtained. Growth was faster as nauplii amounts were greater; the highest growth rate (11.00), weight (265.5 mg) and Fulton's coefficient (1.40) were obtained when fish were fed in excess once a day. At all stages, growth values were significantly higher from feeding nauplii in excess as the sole food, but the required nauplii quantity was six times higher than the amount supplied to the animals fed the dry diet which did not seem labor and/or cost effective.

Kaiser et al. (2003) compared artificial and natural foods and their combinations in the rearing of goldfish, *Carassius auratus*. This study investigated the use of different types of live food and combinations of live food and dry food in a series of four rearing experiments. The replacement of *Artemia* by *Daphnia* at day 10 appeared feasible, as growth and survival were not significantly affected. However, goldfish fed decapsulated *Artemia* cysts grew better than fish fed live *Artemia*.

Fontagne et al. (1998) observed histological changes induced by dietary phospholipids in intestine and liver of common carp larvae. Larvae were fed for 6 to 8 days after start-feeding on semi-purified diets containing peanut oil and supplemented with or without different PL fractions enriched in phosphatidylcholine (PC) or phosphatidylinositol (PI). A group of larvae was also fed *Artemia* nauplii. Diet supplementation with PC from hen egg yolk or from soybean prevented the intestinal steatosis and resulted in larger liver volume and larger hepatocyte volume. Larvae fed *Artemia* showed the same features as larvae fed PC-enriched diets.

Kaiser and Rouhani (1998) evaluated the growth of juvenile *Synodontis petricola* fed on a formulated diet, *Artemia* or *Spirulina* and combinations thereof. Two age groups of juvenile *Synodontis petricola* (Group I, 0.5 years and Group II, 1.5 years) were reared on each of five diets: formulated diet; *Artemia*; *Spirulina*; formulated diet plus *Artemia*; and formulated diet plus *Spirulina*. There was no significant difference in percent survival between the fish fed on different diets within each of the two groups but the younger fish (0.5 years) had a significantly lower survival percentage (90.3%) than those in Group II (95.3%). Fish in Group I fed on *Spirulina* alone grew slower than those fed only on formulated dry feed, whereas the growth rates of fish fed on other diets, or diet combinations, did not differ from each other in both age groups.

Rottmann et al. (1991) compared three live foods and two dry diets for intensive culture of grass carp and bighead carp larvae. Grass carp *(Ctenopharyngodon idella)* and bighead carp *(Hypophthalmichthys nobilis)* larvae fed freshwater rotifers *(Brachionus rubens)* were consistently longer and heavier at the end of the 3-week feeding trials than those fed the other foods tested. The final length of grass carp fed nematodes *(Panagrellus* sp.) was the lowest of the live foods tested, however, the length of bighead carp fed *Panagrellus* was not significantly different from those fed brine shrimp nauplii *Artemia salina.* Growth of grass carp fed the two commercial dry diets (Ewos Larvstart and Fry Feed Kyowa A) was less than those fed the three live foods.

Bryant and Matty (1980) studied the optimization of *Artemia* feeding rate for common carp larvae. Carp (Cyprinus carpio) larvae were fed on measured numbers of *Artemia* nauplii, and daily growth of the larvae was monitored for a period of 10 days in order to determine the effect of varying feeding levels. A 34% reduction in specific growth rate was observed over the experimental period. At a temperature of 24˚C, carp larvae were found to require 200-250% of their body weight of *Artemia* nauplii per day for optimal growth and food conversion during the first five days of feeding, reducing to lOO-12O%/day over the following five days.

3. MATERIALS AND METHODS

3.1 Materials required

1. *Artemia* cysts
2. Sodium Chloride
3. Sodium Carbonate
4. Bleaching powder
5. Chlorine
6. Sodium thiosulphate
7. Dried *Spirulina*
8. Hen's egg
9. *Artemia* Hatching Kit
10. Petri dish
11. Weighing machine
12. Aquaria (12 tanks)
13. Aquaria cover for each tank
14. Heater for each tank
15. Aerators
16. Aquarium filter
17. Multi-parameter kit
18. Thermometer
19. Egg Boiler
20. Measuring cylinder
21. Sedgewick Rafter Counter
22. 150 micron screen
23. Electronic Microscope
24. Beaker
25. Pipette

3.2 Research center

The present study is conducted at Central Fisheries Promotion and Conservation Centre (CFPCC), Balaju, Kathmandu, Nepal, which is one of the technical directorates of Department of Agriculture mandated for aquaculture sector. Under this directorate there are three farms/centers at different agro-ecological zones; Fisheries Human Resource Development and Technology Validation Centre, Janakpur; Natural Water Resource Fisheries Promotion and Conservation Centre, Makawanpur; and Fisheries Pure Line Conservation and Promotion Resource Centre, Rupandehi.

3.3 Fish Sources and Maintenance

Around 400 grass carp larvae of 7 days were procured from the pond (Pond 2) of Central Fisheries Promotion and Conservation Centre (CFPCC), Balaju, Kathmandu, Nepal. The larvae were adapted to laboratory conditions before the experiment for 3 days and fed control diet (D1). On the fourth day, 120 larvae (10 day old) of average weight 0.03 gm and average length of 1.25 cm were selected at random and subjected to the experiment. Remaining larvae were kept in stocking tank at the temperature of 25˚C

3.4 General classification of the fish used

General classification of Grass Carp according to CABI (2021):

Phylum: Chordata

Subphylum: Vertebrata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Genus: *Ctenopharyngodon*

Species: *Ctenopharyngodon idella*

Figure 3.4: Grass Carp *(Ctenopharyngodon idella)* (Picture Source: Liu and He, 1992)

3.5 Experimental design and set-up

Twelve rectangular glass aquaria were kept in indoor culture conditions. Glass aquaria were filled with dechlorinated water and covered with glass cover to prevent the escape of fish. Adequate aeration was provided in each aquarium with the help of air stones. Water filter and aquarium heater were also fixed in each aquarium. A total of one hundred and twenty grass carp larvae (1.43±0.03g) were distributed randomly at the rate of 10 fish per aquarium (30"×24"×8") into 12 glass aquaria equally with triple replicates i.e., each experimental tank had three more tanks with the same treatments provided as replicates (3 treatments×4).

The decapsulated *Artemia* cysts (3gm/l) were incubated at 25^0C for 24 hrs at a salinity of 35 ppt/l. Constant florescent light was supplied at an intensity of approximately 2,000 lux. The hatching container was vigorously aerated to keep the cysts in suspension and exposed to light. The hatched nauplii were extracted from the container by filtering with a 150 micron sieve and washing. Then the filtered nauplii were put onto a beaker with 100 ml distilled water. The contents of the beaker was mixed well while drawing a 1-ml sample with a pipet for counting.

The nauplii were counted in Sedgewick Rafter Counter under a microscope and the hatch rate of *Artemia* cysts was calculated. After calculation, the nauplii were fed as diet (D3) to the larvae in culture.

3.6 De-capsulation of *Artemia* cysts

The de-capsulation solution was prepared containing tap water, bleaching powder and sodium carbonate. Firstly, bleaching powder was dissolved in tap water for 10 minutes with the help of aerators. Once settled, sodium carbonate was added. The mixture was left aside for 4 hours and only the supernatant was used for de-capsulation process.

Firstly, *Artemia* cysts were hydrated in tap water and the mixture was aerated to keep all the cysts in suspension. After one hour of hydration, the cysts were filtered on a 150 micron screen and washed with tap water. The cysts were then re-suspended in a beaker with the de-capsulation solution which has been cooled to a temperature of 15–20°C. The suspension was stirred continuously with aerators in order to keep all the cysts in suspension. The temperature was checked continuously to avoid a temperature increase above 40°C. After 15 minutes of de-capsulation process, the de-capsulated cysts were filtered off on a 150 micron screen and thoroughly washed with tap water until there was no more smell of chlorine. The next step comprised of re-suspending the de-capsulated cysts in freshwater. After sedimentation of de-capsulated cysts to the bottom of the container, floating materials (non-decapsulated cysts, transparent membranes, plumes, etc.) were removed.

3.6.1 Incubation for hatching

The de-capsulated cysts were incubated in optimal hatching conditions in the *Artemia* hatching kit. Two liters of tap water was poured into the hatching kit and 70 gm of salt (35 ppt) was added to make it a brine solution. Aquarium heater of 100 watt and two aerator stones for continuous air supply were fixed into the hatching kit. Six grams of the de-capsulated *Artemia* cysts (3gm/l) were incubated at 25^0C for 24 hrs at a salinity of 35 ppt. Constant florescent light was supplied from above at an intensity of approximately 2,000 lux. The hatching container was vigorously aerated to keep the cysts in suspension and exposed to light.

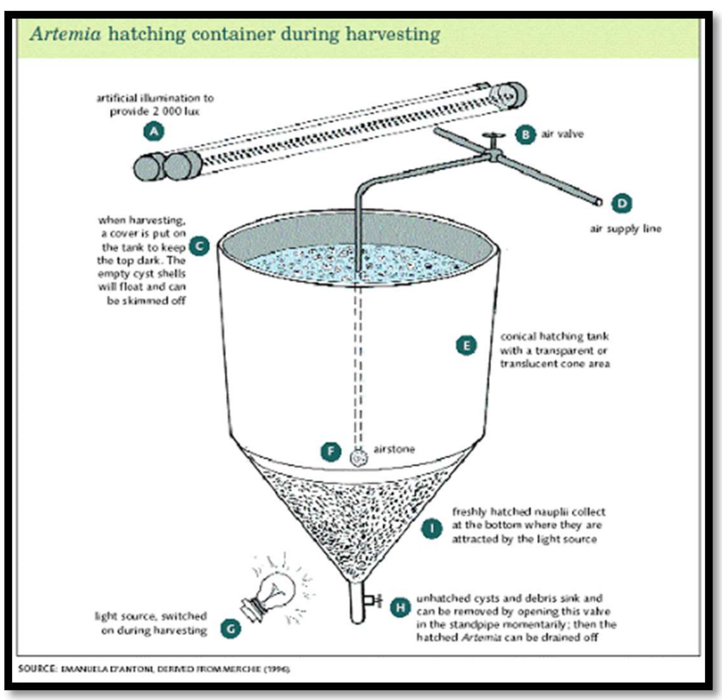

Figure 3.6: Artemia hatching process (Source: FAO, 2021)

3.6.2 Administration to cultured larvae

After 24 hrs, the hatched nauplii were extracted from the container by filtering with a 150 micron screen. The filtered nauplii were put into a beaker with 100 ml distilled water and 1ml of the mixture was extracted onto the Sedgewick Rafter Counter. The number of nauplii in 1 ml of the mixture was counted, the total number of nauplii to be administered to the fish larvae in culture was calculated, and finally provided accordingly.

Table 3.1: Number of *Artemia* provided as diet (D1) to grass carp larvae in Tank 1 (i, ii, iii, and iv)

Week	**Number of nauplii provided per tank**	**Weight (gm)**
1	250	6.75
2	250	6.75
3	400	10.8
4	400	10.8
5	500	13.5
6	500	13.5
Total feed provided		62.1

Table 3.2: Number of *Artemia* nauplii provided as diet (D3) to grass carp larvae in Tank 3 (i, ii, iii, and iv)

Week	**Number of nauplii provided per tank**	**Weight (gm)**
1	500	13.5
2	500	13.5
3	800	21.6
4	800	21.6
5	1000	27
6	1000	27
Total feed provided		124.2

Table 3.3: Crude content of *Artemia* used in culture (according to the packaging label)

S.N.	**Content**	**Percentage**
1.	Protein	57 %
2.	Fat	18 %
3.	Moisture	< 8.5 %
4.	Amino acid	0
5.	Unsaturated fatty acid	0
6.	Calcium	0
7.	Magnesium	0
8.	Potassium	0
9.	Iron	0

3.7 Culture period and conditions

Grass carp (*Ctenopharyngodon idella*) larvae were acclimatized for 3 days and fed control diet (D1). For indoor culture lab, dissolved oxygen was maintained with the help of aerators. Fecal matter was siphoned every afternoon at 4 pm before providing feed. Aquarium filter was adjusted in each tank and two third of water was replenished twice every week at a three days' interval. Dechlorinated tap water was used in the experiment. Water quality parameters like pH, Total Dissolved Solids (TDS), temperature (maintained at 25°C with heater), conductivity, resistivity, salinity and dissolved oxygen along with atmospheric pressure of the tanks were monitored daily for 42 days.

3.8 Feeding and duration of experiment

The total time period of experiment was 42 days. Fish larvae were fed with three different diets; D1: control diet of egg yolk (2.5% of body weight of larvae) and *Artemia* nauplii (Table 3.1) and D2: egg yolk; at the rate of 5% of the body weight of fish larvae and D3: *Artemia* nauplii based on number of nauplii per tank. Feed was provided every day at 4 pm. Fish larvae in each tank were weighed every 7 days to monitor growth and to adjust the feeding rate.

3.9 Sample collection and examination

Sampling was done every seven days, and during specific sampling, the total weight (g) of fish larvae in each tank was recorded using a digital balance. For Hepatosomatic Index (HSI), 5 fish from each tank were randomly selected on the 42nd day of the experiment and sacrificed to collect liver. Liver from the selected 5 fish were weighed together after proper dissection. On the same day, individual fish length (cm) and weight (g) increment were recorded using a steel measuring scale and digital balance.

3.10 Analysis Procedure

a. *Artemia*

Average density, animal density and survival of *Artemia* was calculated using the following formulae:

Average density= sum of individuals in all squares/number of squares

$$\text{Animal density} = \frac{\text{Number of individual species in the plot}}{\text{Total number of square x size of one square}}$$

$$\text{Survival} = \frac{\text{Percentage of living}}{\text{Animal density}}$$

From the animal density the rearing success of the *Artemia* culture was evaluated and expressed as percentage survival.

b. Grass carp larvae

Mean Weight Gain (MWG), Specific Growth Rate (SGR), Percentage Weight Gain (WG%), Protein Efficiency Ratio (PER), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), and Protein Intake (PI) were calculated at the end of experiment using following equations according to Adekunle and Joyce (2014):

Mean Weight Gain (MWG) = Mean final body weight – mean initial body weight

$$\text{Specific Growth Rate (SGR) per day} = \frac{(\ln Wt - \ln Wi)}{T} \times 100$$

Where T is the culture period in days, Wt and Wi were the mean final and initial weights (g) respectively.

$$\text{Weight gain \% (WG \%)} = \frac{\text{Final body weight - Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Hepato-somatic index (HSI)}: \frac{\text{Liver weight}}{\text{Whole-body weight}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Weight of feed provided (g)}}{\text{Weight gain of fish (g)}}$$

$$\text{Food conversion efficiency (FCE)} = \frac{\text{Body weight gain (g)}}{\text{Feed given (g)}} \times 100$$

Feed Intake (FI) = 5% body weight of fish per day.

Protein Intake (PI) = Feed Intake (FI) x % protein in diet,

Where percentage of protein in egg is 15.86% (USDA, 2021) and *Artemia* naupli is 57% .

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Total weight gain of fish (g)}}{\text{Protein Intake (PI) (g)}}$$

$$\text{Survival rate (SR \%)} = \frac{\text{Number of fish survived}}{\text{Number of fish leased}} \times 100$$

3.11 Statistical Analysis

Data analysis was done using R software (Oksanen et al., 2019) and Microsoft Excel 2013. The significant difference between treatments was determined using a one-way Analysis of Variance (ANOVA) and the significance level was at $p < 0.05$. All data presented in figures and tables were set up using Microsoft Excel 2013.

4. RESULTS

4.1 Survival and hatch rate of *Artemia*

Number of cysts in 1 gm= 1,80,000

Percentage of living= 4.4%

Animal density= 0.8

Survival= 7,920 individuals

From this experiment, it is found that the hatch rate of Chinese *Artemia* cysts is only 4.4% which is extremely low as normally the hatch rate of other *Artemia* cysts is more than 50%. Survival rate of the *Artemia* was only 7,920 individuals.

4.2 Growth performance

Mean Weight Gain (MWG) was found to be highest (2.62) in larvae fed with diet D2 followed by control diet (D1) at 1.62 and least MWG of 0.81 was seen in larvae fed with diet D3. Similarly, (Table 4.1) the Specific Growth Rate (SGR) was highest among larvae fed diet D2 followed by D1 and D3 at 7.57, 6.33 and 5.01 respectively. The Mean Length Gain (MLG) was also observed to be high among larvae fed diet D2 followed by control diet and lowest in diet D3 (1.925, 0.95, 0.575). There was no significant difference ($p>0.05$) observed in MWG, SGR and MLG among the experimental groups.

Table 4.1: Weight of fish larvae in experimental tanks during experiment

Tank	**1.i**	**1.ii**	**1.iii**	**1.iv**	**2.i**	**2.ii**	**2.iii**	**2.iv**	**3.i**	**3.ii**	**3.iii**	**3.iv**
IW	0.113	0.115	0.12	0.117	0.111	0.112	0.113	0.118	0.109	0.113	0.117	0.113
FW	1.59	1.86	2.46	1.05	3.14	2.52	2.58	2.72	0.95	0.87	0.94	0.97

IW= Initial Weight, FW= Final Weight

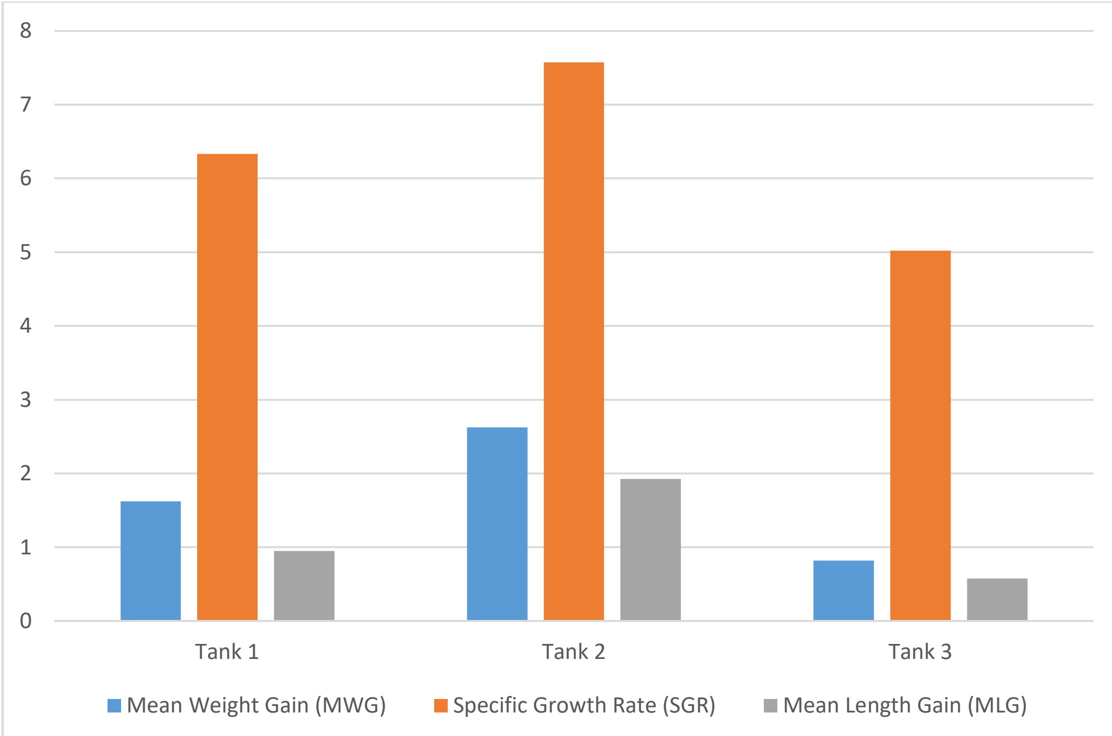

Figure 4.1: Growth comparison among three experimental tank groups

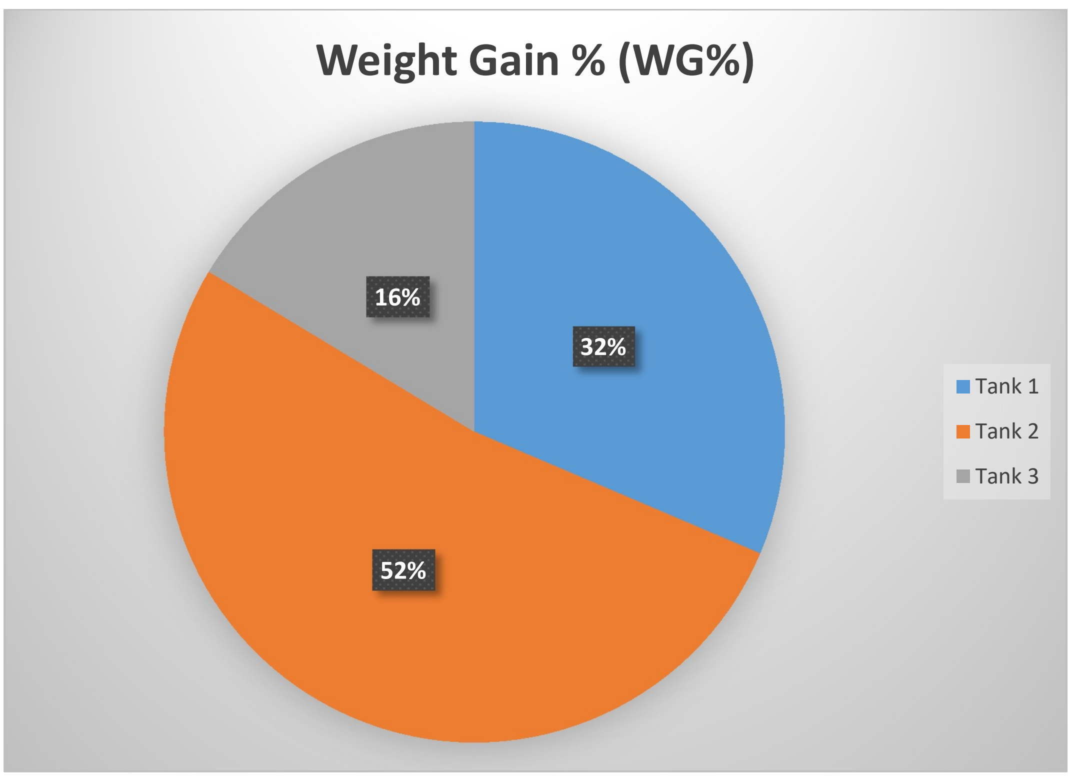

Figure 4.2: Weight gain in the three experimental tanks

4.2.1 Weight Gain (%)

The mean percentage weight gain (WG%) was higher, i.e., 2316.77 in group 2, while it was 1392.97 and 725.81 in group 1 and 3 that were fed control diet (D1) of *Artemia* nauplii plus egg yolk, and diet (D3) of *Artemia* nauplii respectively. Highest percentage of weight gain was seen among the larvae of group 2 and least was in group 3 among the three groups (Figure 4.2). There was a significant ($p<0.05$) difference in WG% among the experimental groups.

4.3 Hepato-Somatic Index

The average Hepato-Somatic Index (HSI) for the grass carp larvae of the three experimental tanks calculated on the 42nd day of the experiment were 10.37, 16.95 and 3.18 respectively for diet D1, D2 and D3. The highest HSI was correlated to the fish larvae fed diet D2 and least was in diet D3 as shown in Figure 4.3. However, there was no significant difference ($p>0.05$) in HSI among the experimental groups.

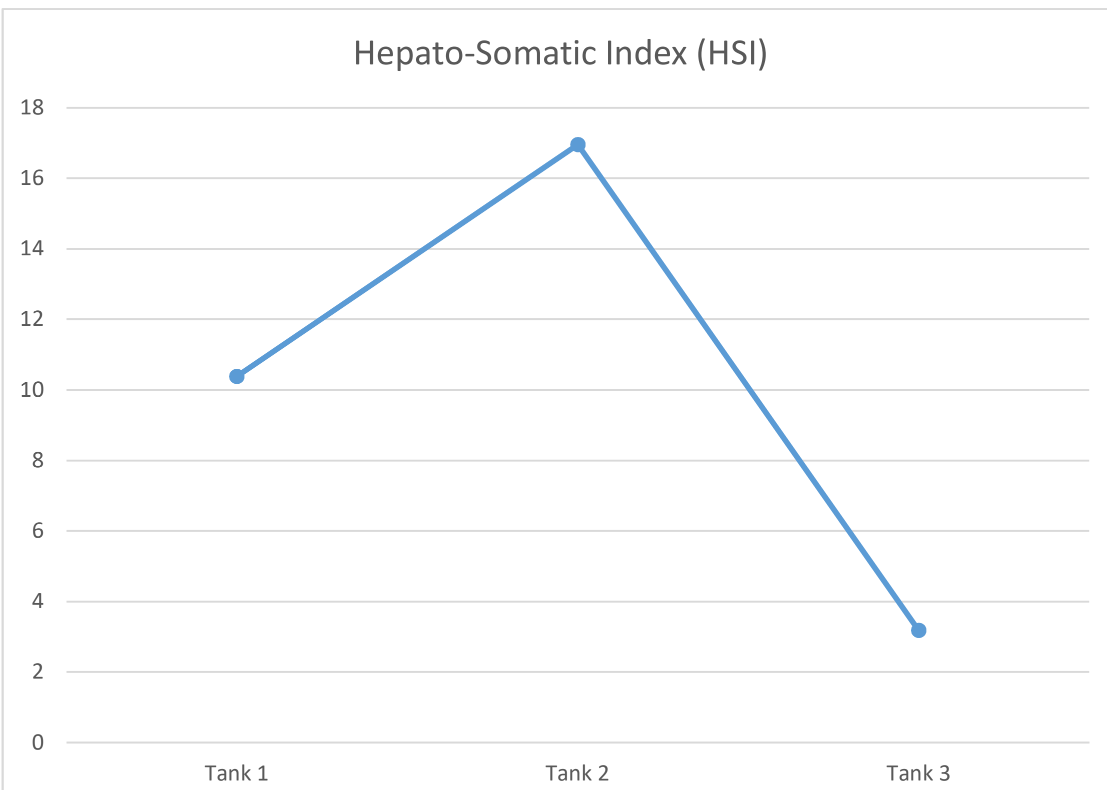

Figure 4.3: Hepato-Somatic Index of fish larvae in experimental tanks

4.4 Feed utilization

Feed utilization among the experiments was calculated using FI (Feed Intake), FCR (Feed Conversion Ratio) and FCE (Food Conversion Efficiency) as shown in Figure 4.4. The highest amount of feed (FI) was provided to the experimental group 3 followed by 1 and 2. However, FCE was highest in group 2 (614.62) and least in group 3 (0.65). The reverse trend in FCR is always an indicator for fish growth and it was minimum at 0.16 in experimental group 2 that were fed diet D2. FCR was highest in group 3 at 151.89. There was no significant difference ($p>0.05$) in FI and FCE among the experiments but a significant difference ($p<0.05$) among the three experiments was observed for FCR.

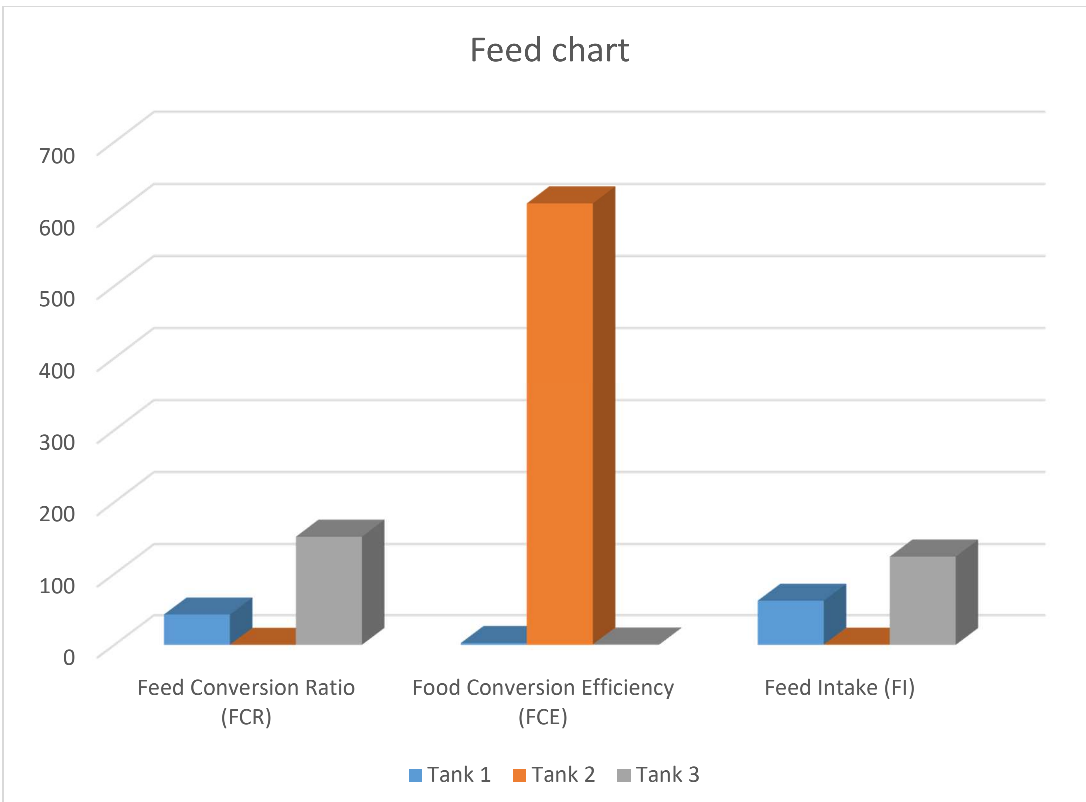

Figure 4.4: Feed utilization chart

4.5 Protein Intake

Protein intake in group 3 was highest throughout the experiment averaging at 7079.4 followed by group 1 at 3547.34 and least in group 2 at 6.76 as shown in Figure 4.5. There was a significant difference ($p<0.05$) in PI among the experimental groups.

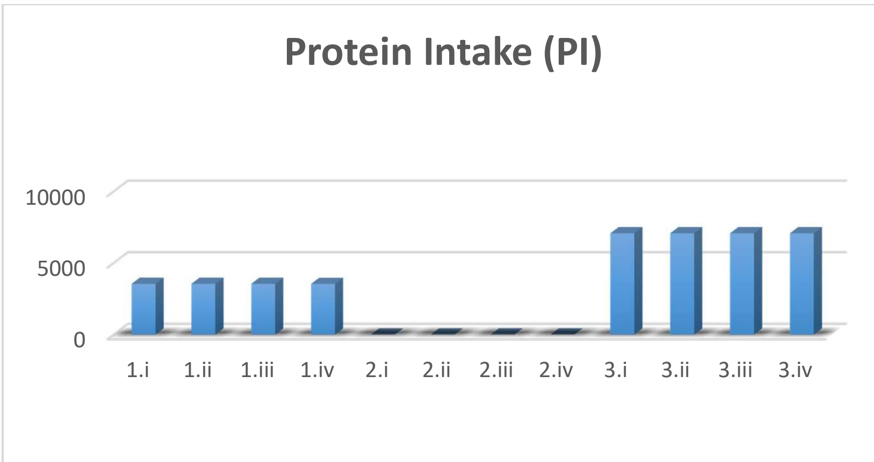

Figure 4.5: Protein Intake by grass carp larvae in experimental tanks

4.6 Protein Efficiency Ratio (PER)

The PER for experimental groups 1, 2 and 3 was 4.57E-05, 0.387 and 1.13E-05 respectively where the highest ratio was in group 2 (Figure 4.6). From this, it was clear that highest protein efficiency was seen among the fish larvae that were fed egg yolk diet compared to the other two experimental groups. There was a significant difference ($p<0.05$) in PER among the experimental groups.

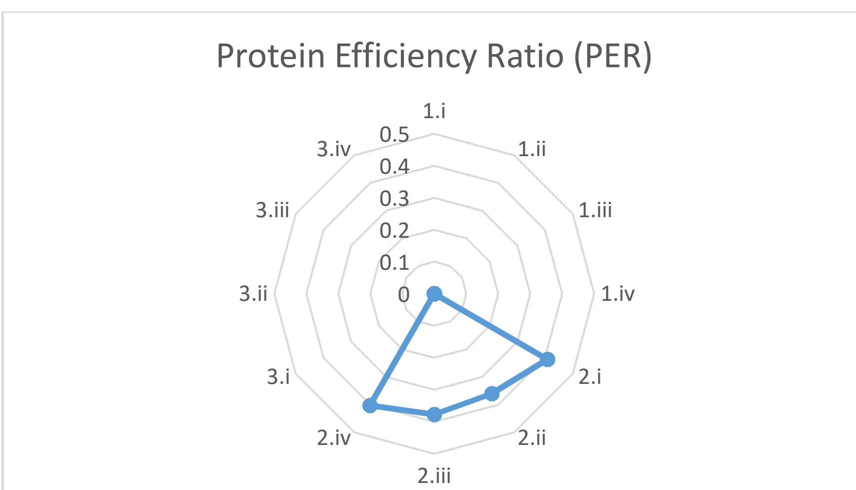

Figure 4.6: Protein Efficiency Ratio among the experimental tanks

4.7 Survival percentage

The survival rates (SR%) of fish fluctuated among the experimental groups and showed significant difference ($P<0.05$) among all the treatments. Meanwhile, high survival was recorded only in D1 and D2 diet-fed groups and the survival rate in percentage was 100, 90.9, 100 and 52.63 in Tank 1 (i, ii, iii and iv); 83.33, 100, 100 and 45.45 in Tank 2(i, ii, iii and iv); and 55.55, 52.63, 71.42 and 62.5 in Tank 3 (i, ii, iii and iv) respectively (Figure 4.7). The lowest survival among the experimental groups was seen in Tank 2.iv.

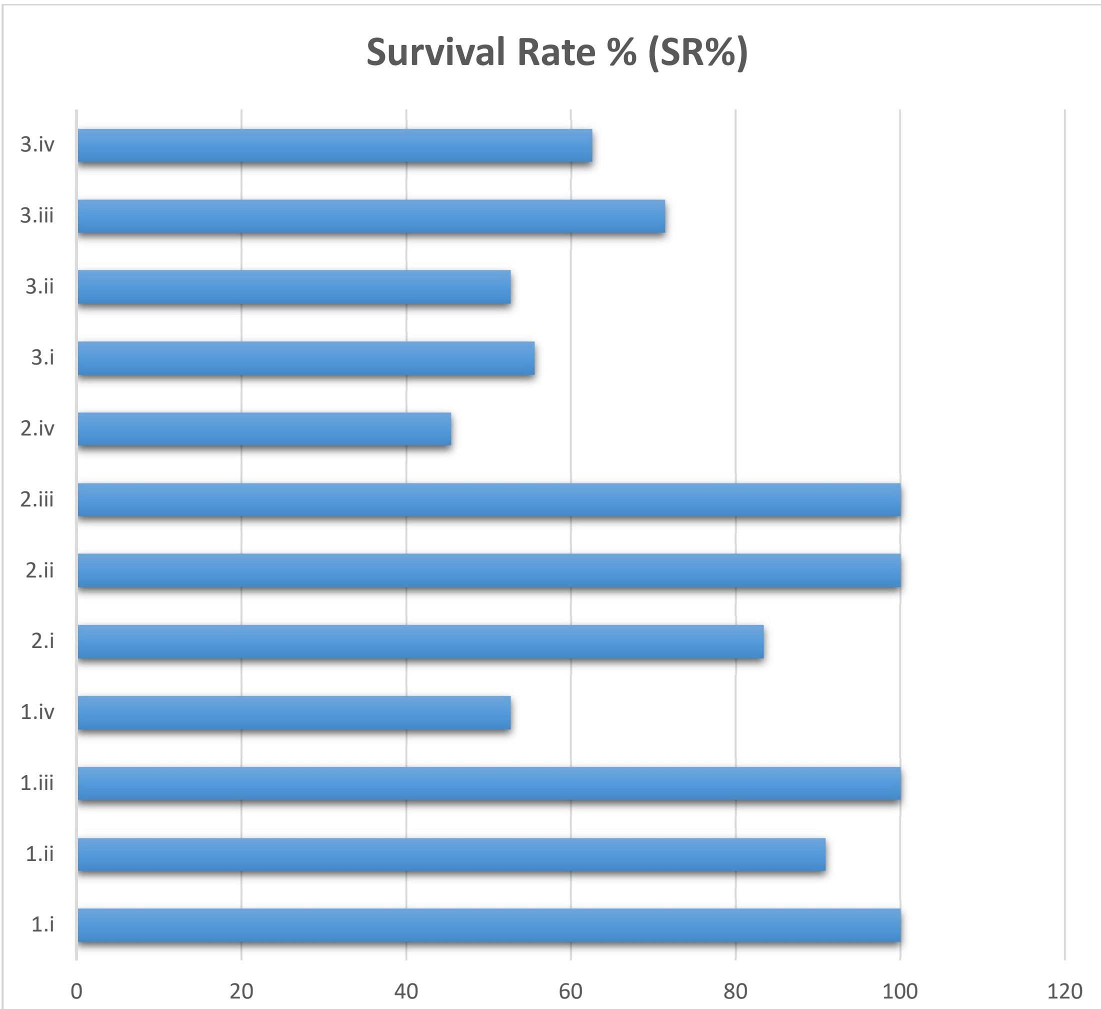

Figure 4.7: Survival rate of fish in experimental tanks

4.8 Comparison of different indices

Comparing all the indices used to evaluate the effect of diets D1, D2 and D3 in experimental groups 1, 2 and 3, it was found that only WG%, FCR, PI, PER and SR% showed a significant difference ($p<0.05$). The average value of growth indices recorded in experimental groups 1, 2 and 3 are shown in Table 4.2 whereas the average value of growth indices calculated in the experiment is shown in Figure 4.8.

Table 4.2: Average value of growth indices in experimental tanks

Growth Indices	Tank 1	Tank 2	Tank 3
MWG	1.62375	2.6265	0.8195
SGR	6.334	7.572	5.01975
MLG	0.95	1.925	0.575
WG%	1392.973	2316.77	725.8175
HSI	10.3775	16.95	3.185
FCR	42.76625	0.1625	151.8925
FCE	2.60975	614.6225	0.6595
FI	62.23425	0.42675	124.2
PI	3547.348	6.76775	7079.4
PER	4.57E-05	0.387	1.13E-05
SR	85.8825	82.195	60.525

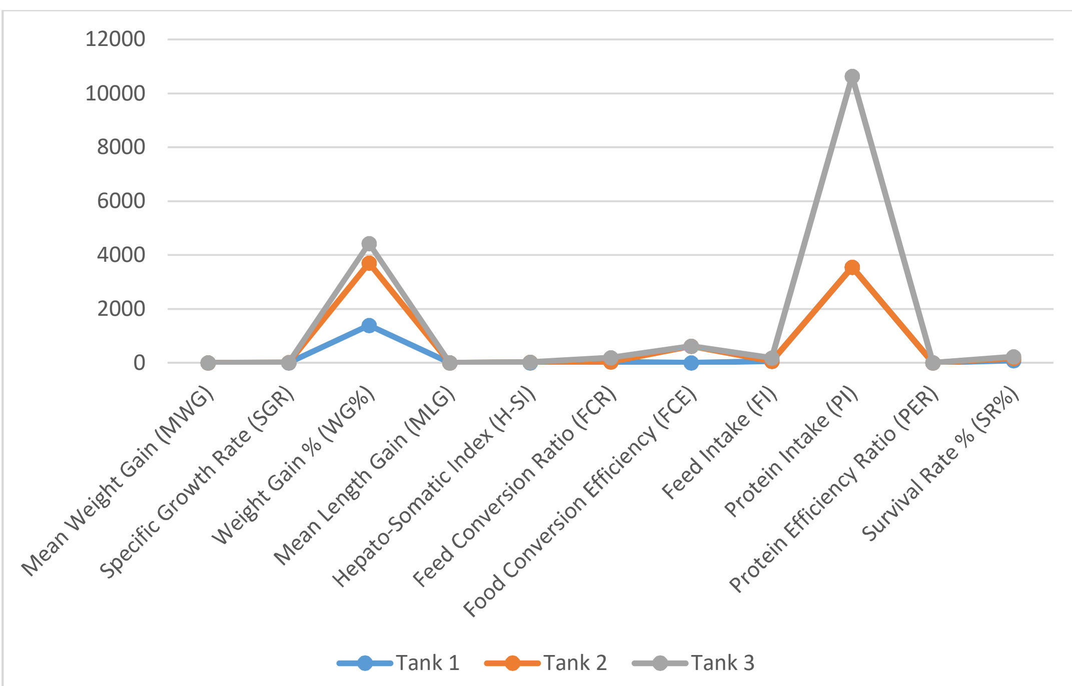

Figure 4.8: Average growth indices in experimental groups

4.9 Physico-chemical parameters

A total of seven water quality parameters were monitored throughout the experiment viz. pH, DO, Conductivity, TDS, salinity, Temperature and Atmospheric Pressure. There was no significant difference ($p>0.05$) in any of the physicochemical parameters among the experimental groups and the average value of every parameter except Conductivity was found to be almost similar in all the tanks as shown in Figure 4.9. This denoted that there was negligible impact of diet on the water quality and also ensured consistency throughout the experimental groups.

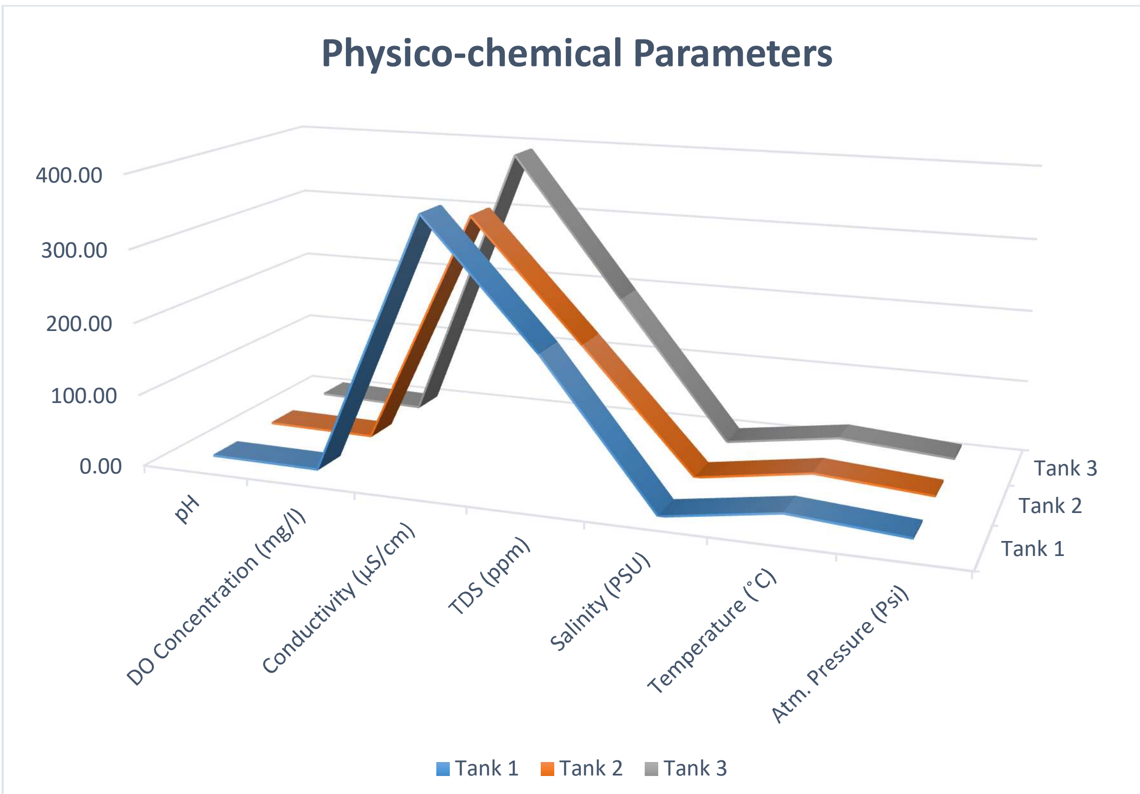

Figure 4.9: Water quality parameters in experimental groups

5. DISCUSSION

Use of the brine shrimp *Artemia* in aquaculture, especially for initial growth stages of fish has been extensively studied in many countries. The brine shrimp is a popular natural feed for fish larvae and aquarium fish because of long term storage capacity of deactivated cysts, availability throughout the year and its relatively easy hatching process. However, its usage in Nepal has not been properly carried out because of having to import the cysts from abroad and its high cost.

In this experiment, the hatch rate of Chinese *Artemia* cysts was found to be only 4.4% and the survival rate was just 7,920 individuals which is extremely low as Ozdemir (2018) compared the hatching rate of *Artemia* cysts from California, Utah, China and Vietnam and in that experiment, China's cysts had 38±2% hatch rate at salinity of 35 ppt. This could be due to various reasons like low quality of cysts imported, unfavorable laboratory conditions, etc. Thus, using expensive *Artemia* as live feed for fish in aquaculture seemed to be neither cost nor labor-effective in terms of Nepal.

Meanwhile, the Mean Weight Gain (MWG) of *Ctenopharyngodon idella* larvae in this research was found to be highest in larvae fed with egg yolk diet followed by control diet of egg yolk plus *Artemia* nauplii and least in diet of only *Artemia* nauplii. The mean percentage weight gain (WG%) was also higher among larvae in group 2 that were fed diet of hen's egg yolk. Highest percentage of weight gain was seen among the larvae of group 2 and least was recorded in group 3. A significant ($p<0.05$) difference was observed in WG% among the experimental groups. Similarly, SGR and MLG were also observed to be highest among larvae fed egg yolk diet compared to control diet and *Artemia* nauplii but there was no significant difference ($p>0.05$) observed in MWG, SGR and MLG among the experimental groups.

Rottmann et al., (1991) found a similar result of improper growth of grass carp *(Ctenopharyngodon idella)* larvae that were fed brine shrimp nauplii (*Artemia salina*) but higher growth in length and weight was seen in those fed with freshwater rotifers *(Brachionus rubens)*. El-Sayed et al., (2021) also found a similar result for Sea bass (*Dicentrarchus labrax*) larvae as growth rates and survival percentages of larvae fed with enriched *Cyclops abyssorum divergens* was higher compared to those fed with *Artemia franciscana*.

However, Chepkirui-Boit et al., (2011) found that African catfish (*Clarias gariepinus*) larvae co-fed using 50% *Artemia* and 50% formulated dry diet resulted in significantly ($P < 0.05$) better growth performance, FGR, PER, productive protein values (PPV) and better survival of over 90% than other treatments.

On the other hand, Kaiser et al., (2003) found that growth and survival of *Carassius auratus* was highest in fish fed decapsulated *Artemia* compared to *Artemia* nauplii. Olurin and Oluwo (2010) recorded highest growth values in *Clarias gariepinus* larvae fed decapsulated *Artemia* ($p<0.05$) compared to inert diets and live *Artemia*. Celada et al., (2008) also found high survival rates (between 79.5% and 95.5%) and significantly higher growth values in Tench (*Tinca tinca* L.) larvae that were fed *Artemia* compared to dry diets, but the required nauplii quantity was six times higher than the amount of dry diet supplied.

The average Hepato-Somatic Index (HSI) for the grass carp larvae of the three experimental tanks in the present study was calculated on the 42^{nd} day of the experiment and the highest HSI was correlated to the fish larvae fed diet D2 of egg yolk and least was seen in diet D3 of *Artemia* nauplii. Fontagne et al., (1998) observed analogous histological changes among common carp larvae that were fed *Artemia* nauplii as that of larvae that were fed phosphatidylcholine enriched diets of hen egg yolk or soybean.

Most of the studies (Olsen et al., 1999; Mai et al., 2009; Javad et al., 2011; Yang, 2017; Ahmadifard et al., 2018; Jamali et al., 2018; Radhakrishnan et al., 2019; Prusinska et al., 2020; El-Sayed et al., 2021) have shown higher growth of different fish larvae during their early growth stages by feeding short-term or long term enriched *Artemia* nauplii compared to unenriched *Artemia* nauplii. Jafaryan et al., (2011) recorded higher growth in grass carp larvae when fed with *A. parthenogenetica* nauplii and probiotic bacteria. Sahandi et al., (2012) fed Bacillus inoculated *Artemia* to common carp larvae and grass carp larvae and found significant higher growth and feeding performance in comparison with non-inoculation treatments.

Use of Bacillus inoculated *Artemia* in feeding larval stages of fish thus might have a positive impact on growth compared to non-inoculated ones. This could be attributed to the high protein content of Artemia which might be broken down and digested by the probiotics used to enrich the nauplii. Thus, use of unenriched *Artemia* nauplii for feeding grass carp larvae might be more harmful to the fish due to difficulty in digesting the high protein content. Hence, use of inoculated nauplii or locally available feed or other rotifers can be better suited for aquaculture in Nepal.

6. CONCLUSION

In conclusion, a significant difference ($p<0.05$) among the groups in experiment was seen in feed conversion ratio (FCR), % weight gain, survival percentage, protein efficiency ratio and protein intake. Highest growth in weight and length of grass carp larvae, hepatosomatic index (HSI), protein efficiency ratio (PER) and survival was recorded in the group that was fed hen's egg yolk.

The feed conversion ratio (FCR) is an appropriate way to judge the acceptability and suitability of feed for fish. The information of FCR on locally available ingredients may provide the basis to develop acceptable fish feed. Feed conversion ratio (FCR) in this experiment was lowest for egg yolk diet and thus its suitability for grass carp larvae was higher compared to other groups.

Finally, the hepatosomatic index (HSI) is the main factor for fish growth, and in this experiment, egg-yolk diet increased the liver weight of fish compared to control group and group fed *Artemia* nauplii. Thus, egg yolk diet would be better for fish farmers to produce healthy fish.

It can thus be concluded that using expensive *Artemia* as live feed for fish in aquaculture is not cost-effective in terms of our Nepal. Other live feed such as planktons or rotifers could be used instead or artificial supplementary feed could be the better option. In a developing country like Nepal, use of *Artemia* by farmers to enhance the growth of fish larvae is extremely expensive and would not yield greater profit and locally available feed would be the best way.

7. REFERENCES

Abe, H. A., Dias, J. A. R., Sousa, N. D. C., Couto, M. V. S. D., Reis, R. G. A., Paixão, P. E. G., & Fujimoto, R. Y. (2019). Growth of Amazon ornamental fish *Nannostomus beckfordi* larvae (Steindachner, 1876) submitted to different stocking densities and feeding management in captivity conditions. *Aquaculture Research*, *50*(8), 2276-2280.

Adekunle, A. I., & Joyce, A. O. B. (2014). Effect of partial and total replacement of live feed (*Artemia*) with formulated diets in early stage growth of hybrid catfish (*Heterobranchus bidorsalis x Heterobranchus longifilis*) fry. *International Journal of Fisheries and Aquatic Studies, 1*(3), 143-146.

Ahmadifard, N., Aminlooi, V. R., Tukmechi, A., & Agh, N. (2019). Evaluation of the impacts of long-term enriched *Artemia* with *Bacillus subtilis* on growth performance, reproduction, intestinal microflora, and resistance to *Aeromonas hydrophila* of ornamental fish *Poecilia latipinna*. *Probiotics and antimicrobial Proteins*, *11*(3), 957-965.

Aruho, C., Walakira, J.K., Owori-Wadunde, A., Nuwamanya, E., Bugenyi, F., Sserwadda, M., Rutaisire, J. & Borski, R.J. (2020). Growth and survival of Ripon barbel (*Barbus altianalis*) larvae and juveniles fed five experimental diets in captivity. *Aquaculture Reports*, *18*, 100441.

Bryant, P. L., & Matty, A. J. (1980). Optimisation of *Artemia* feeding rate for carp larvae (*Cyprinus carpio* L.). *Aquaculture*, *21*(3), 203-212.

CABI, 2021. *Ctenopharyngodon idella*. In: Invasive Species Compendium. Wallingford, UK: Centre for Agriculture and Bioscience International (CABI). www.cabi.org/isc

Cahu, C., & Infante, J. Z. (2001). Substitution of live food by formulated diets in marine fish larvae. *Aquaculture*, *200*(1-2), 161-180.

Carral, J. M., Celada, J. D., Sa´ez-Royuela, M., Rodrı´guez, R., Aguilera, A., & Melendre, P. M. (2006). Effects of four destocking procedures on hatching rate and further survival and growth of larvae in the tench (*Tinca tinca* L.). *Aquaculture Research*, 37(6), 632–636.

Celada, J. D., Aguilera, A., Carral, J. M., Sáez-Royuela, M., & Melendre, P. M. (2008). Rearing tench (*Tinca tinca* L.) larvae on live feed (*Artemia*) and on two transition schedules from live to dry diets. *Journal of Applied Ichthyology*, *24*(5), 595-600.

Chepkirui-Boit, V., Ngugi, C. C., Bowman, J., Oyoo-Okoth, E., Rasowo, J., Mugo-Bundi, J., & Cherop, L. (2011). Growth performance, survival, feed utilization and nutrient utilization of African catfish (*Clarias gariepinus*) larvae co-fed *Artemia* and a micro-diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. *Aquaculture Nutrition*, *17*(2), e82-e89.

Chilton, E. W., & Muoneke, M. I. (1992). Biology and management of grass carp (*Ctenopharyngodon idella*, Cyprinidae) for vegetation control: a North American perspective. *Reviews in fish biology and fisheries*, *2*(4), 283-320.

Clegg, J. S., & Trotman, C. N. (2002). Physiological and biochemical aspects of *Artemia* ecology. In *Artemia: Basic and applied biology* (129-170). Springer, Dordrecht.

Connor, J.V., R.P. Gallagher & M.F. Chantry. (1980). Larval evidence for natural reproduction of the grass carp (*Ctenopharyngodon idella*) in the lower Mississippi River. In Proceedings of the Fourth Annual Larval Fish Conference. *Edited by:* L.A. Fuiman. Ann Arbor, MI. US Fish and Wildlife Service (Biological Service Program, FWS/OBS-80/43). 1-19.

Craig, S., Helfrich, L. A., Kuhn, D., & Schwarz, M. H. (2017). Understanding fish nutrition, feeds, and feeding.

Criel, R. J., & Macrae, H. T. (2002). "*Artemia* morphology and structure". In T. J. Abatzopoulos; J. A. Breardmore; J. S. Clegg & P. Sorgerloos (eds.). *Artemia*: *Basic and Applied Biology. Kluwer Academic Publishers*. 1–33. ISBN 978-1-4020-0746-0.

Cui, Y., & Wootton, R. J. (1988). Effects of ration, temperature and body size on the body composition, energy content and condition of the minnow, *Phoxinus phoxinus* (L.). *Journal of Fish Biology*, *32*(5), 749-764.

Dabrowski, K., & Miller, M. (2018). Contested paradigm in raising zebrafish (*Danio rerio*). *Zebrafish. 15*, 295–309.

Dahal, K. R., Sharma, C. M., and Gupta, R. K. (2013). Threats on fishery resources and fishers livelihood due to riverbed extraction in Tinau River. *Nepal Journal of Sustainable Environmental Research. 2*(1), 111.

Daintith M. (1996). Rotifers and *Artemia* for Marine Aquaculture: a Training Guide. University of Tasmania.

Dhont, J., & Sorgeloos, P. (2002). Applications of *Artemia*. In *Artemia: Basic and applied biology* (251-277). Springer, Dordrecht.

Dhont, J., Dierckens, K., Støttrup, J., Van Stappen, G., Wille, M., & Sorgeloos, P. (2013). Rotifers, *Artemia* and copepods as live feeds for fish larvae in aquaculture. In *Advances in aquaculture hatchery technology* (157-202). Woodhead Publishing.

Dutta, H. (1994). Growth in fishes. *Gerontology*, *40*(2-4), 97-112.

Eccles, D. H. (1992). FAO species identification sheets for fishery purposes. Field guide to the freshwater fishes of Tanzania. FAO, Rome. 145.

El-Sayed, H. S., Ghonim, A. Z., El-Khodary, G. M., El-Sheikh, M. A., & Khairy, H. M. (2021). Application of enriched *Cyclops abyssorum divergens* with mixed algal diet compared to *Artemia franciscana* for improving larval growth and body composition of *Dicentrarchus labrax*. *Aquaculture Reports*, *20*, 100715.

Escaffre, A. M, & Bergot, P. (1986). Quantitative morphology of the liver of rainbow trout (*Salmo gairdnerii*) fry from large or small eggs: incidence of the date of the first meal. *Archiv für Hydrobiologie*, *107* (3), 331-348.

Falahatkar, B., Mohammadi, H., & Noveirian, H. (2011). Effects of different starter diets on growth indices of Caspian Kutum, *Rutilus frisii kutum* larvae. *Iranian Journal of Fisheries Sciences*, *11*, 28–36.

FAO, 2021. Fisheries and aquaculture resources. Cultured Aquatic Species Information Programme. *Ctenopharyngodon idella* (Valenciennes, 1844). Retrieved on 15th June, 2021.

Fischer, Z. & Lyakhnovich, V. P. (1973). Biology and energetics of grass carp (*Ctenopharyngodon idella*). *Polish Archives of Hydrobiology 20(4)*, 309-318

Fontagne, S., Geurden, I., Escaffre, A. M., & Bergot, P. (1998). Histological changes induced by dietary phospholipids in intestine and liver of common carp (*Cyprinus carpio* L.) larvae. *Aquaculture*, *161*(1-4), 213-223.

Gurung, T. B., Wagle, S. K., Bista, J. D., Joshi, P. L., Batajoo, R., Adhikari, P., & Rai, A. K. (2005). Participatory fisheries management for livelihood improvement of fishers in Phewa Lake, Pokhara, Nepal. *Himalayan Journal of Sciences*, *3*(5), 47-52.

Hamre, K., Erstad, B., de Kok, J., Norberg, B., & Harboe, T. (2020). Change in nutrient composition of Artemia grown for 3–4 days and effects of feeding on-grown Artemia on performance of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae. *Aquaculture Nutrition*, *26*(5), 1542-1554.

Hickman C. P. (1967). Biology of Invertebrates. St. Louis, Missouri: C. V. Mosby.

https://agclass.nal.usda.gov (2021). Hepatosomatic Index. National Agricultural Library's Agricultural Thesaurus and Glossary, US Department of Agriculture. Retrieved on June 11, 2021.

Jafaryan, H., Soltani, M., Noferesti, H., & Ebrahimi, P. (2011). Effect of adding probiotics into the rearing tanks of grass carp (*Ctenopharyngodon idella*) for the exploitation of *Artemia urmiana, Artemia fransiscana* and *Artemia parthenogenetica* Nauplii. *Iranian Journal of Veterinary Medicine*, *5*(3), 157-161.

Jamali, H., Ahmadifard, N., Noori, F., Agh, N., & Gisbert, E. (2018). Improving co-feeding strategies for Neotropical green terror cichlid (*Aequidens rivulatus*) larvae with lecithin-enriched *Artemia franciscana* nauplii: Effects on survival, growth performance and body composition. *Aquaculture Research*, *49*(12), 3909-3918.

Javad, S., Hadi, J. H., Mehdi, D., & Hossien, A. (2011). Bacillus strains additives to common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*) larvae's food and different effect of them on growth parameters. *Mediterranean Aquaculture and European Aquaculture Society*. Rhodes, Greece.

Kaiser, H., & Rouhani, Q. (1998). Growth of juvenile *Synodontis petricola* (family: Mochokidae) fed on a formulated diet, *Artemia* or *Spirulina* and combinations thereof. *Aquarium Sciences and Conservation*, *2*(3), 117-123.

Kaiser, H., Endemann, F., & Paulet, T. G. (2003). A comparison of artificial and natural foods and their combinations in the rearing of goldfish, *Carassius auratus* (L.). *Aquaculture Research*, *34*(11), 943-950.

Keith, P. & J. Allardi. (2001). *Atlas des poissons d'eau douce de France*. Collection *Patrimoines naturels*, 47: 387. Paris

Kolkovski, S. (2001). Digestive enzyme in fish larvae and juveniles- implications and application to formulated diets. *Aquaculture,* 181-201.

Kujawa, R., Kucharczyk, D., Mamcarz, A., Jamróz, M., Kwiatkowski, M., Targońska, K., & Żarski, D. (2010). Impact of supplementing natural feed with dry diets on the growth and survival of larval asp, *Aspius aspius* (L.), and nase, *Chondrostoma nasus* (L.). *Fisheries & Aquatic Life*, *18*(1), 13-23.

Kwiatkowski, M., Zarski, D., Kucharczyk, D., Kupren, K., Jamroz, M., Targonska, K., Krejszeff, S., Hakuc-Blazowska Kujawa, R., & Mamcarz, A. (2008). Influence of feeding natural and formulated diets on chosen rheophilic cyprinid larvae. *Fisheries & Aquatic Life*, *16*(4), 383-396.

Lavens, P., & Sorgeloos, P. (2000). The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture*, *181*(3-4), 397-403

Lavens, P., De Meulemeester, A., & Sorgeloos, P. (1987). Evaluation of mono-and mixed diets as food for intensive *Artemia* culture. *Artemia* research and its applications, 3, 309-317.

Liu, J., & He, B. (1992). Cultivation of the Chinese Freshwater Fishes (3rd edition). *Science Press*, 1992. 750. Beijing.

Lubzens, E., Zmora, O., Stottrup, J., & McEvoy, L. (2003). Production and nutritional value of rotifers. *Live feeds in marine aquaculture*, 300-303.

Mahfuj, M. S., Hossain, M. A., & Sarower, M. G. (2012). Effect of different feeds on larval development and survival of ornamental koi carp, *Cyprinus carpio* (Linnaeus, 1758) larvae in laboratory condition. *Journal of the Bangladesh Agricultural University*, *10*(452-2016-35574), 179-183.

Mai, M. G., Engrola, S., Morais, S., Portella, M. C., Verani, J. R., Dinis, M. T., & Conceicao, L. E. (2009). Co-feeding of live feed and inert diet from first-feeding affects *Artemia* lipid digestibility and retention in Senegalese sole (*Solea senegalensis*) larvae. *Aquaculture*, *296*(3-4), 284-291.

NAL Thesaurus Staff. 2021. National Agricultural Library. Agricultural Thesaurus and Glossary. Accessed on 9 July, 2021.

Nico, L., & Fuller, P. (2001). *Ctenopharyngodon idella* (Valenciennes 1984) nonindigenous aquatic species. Non-indigenous species. *Florida Caribbean Science Center*. *Biological Resources Division.*

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., Ohara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H. (2019). Vegan: Community ecology Package. R package version 2.3-1. Available from: http://CRAN.R-project. org/package=vegan

Olsen, A. I., Attramadal, Y., Jensen, A., & Olsen, Y. (1999). Influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. *Aquaculture*, *179*(1-4), 475-487.

Olurin, K. B., & Oluwo, A. B. (2010). Growth and survival of African catfish (*Clarias gariepinus*) larvae fed decapsulated *Artemia*, live *Daphnia*, or commercial starter diet. *The Israeli Journal of Aquaculture - Bamidgeh 62(1), 50-55*

Opuszynski, K. and J.V. Shireman. 1995. Herbivorous fishes: culture and use for weed management. In cooperation with James E. Weaver, Director of the United States Fish and Wildlife Service's National Fisheries Research Center. CRC Press, Boca Raton, Florida.

Özdemir, K. Y. (2018). Detection of Egg Diameters and Hatching Rates of California, China, Utah, and Vietnam Originated *Artemia* sp. in Different Temperatures and Salinity. *Alinteri Journal of Agriculture Science*, *33*(2), 141-144.

Page, L. M., & Burr, B. M. (1991). A field guide to freshwater fishes. *Houghton Mifflin Company*, Boston.

Parker, R. R., & Larkin, P. A. (1959). A concept of growth in fishes. *Journal of the Fisheries Board of Canada*, *16*(5), 721-745.

Piper, J. (2018). *Artemia*: A Model Specimen for Educational Microscopy Projects in Biological and Ecological Fields. Cambridge University Press. *Microscopy Today*, *26*(4), 12-19.

Prusińska, M., Nowosad, J., Jarmołowicz, S., Mikiewicz, M., Duda, A., Wiszniewski, G., & Kucharczyk, D. (2020). Effect of feeding barbel larvae (*Barbus barbus* (L, 1758)) *Artemia* sp. nauplii enriched with PUFAs on their growth and survival rate, blood composition, alimentary tract histological structure and body chemical composition. *Aquaculture Reports*, *18*, 100492.

Radhakrishnan, K. D., Velayudhannair, K., & Schmidt, B. V. (2020). Effects of bio-flocculated algae on the growth, digestive enzyme activity and microflora of freshwater fish *Catla catla* (Hamilton 1922). *Aquaculture Research*, *51*(11), 4533-4540.

Rottmann, R. W., Shireman, J. V., & Lincoln, E. P. (1991). Comparison of three live foods and two dry diets for intensive culture of grass carp and bighead carp larvae. *Aquaculture*, *96*(3-4), 269-280.

Sahandi, J., Jafariyan, H., Dehghan, M., Adineh, H., & Shohreh, P. (2012). Direct inoculation of Bacillus to rearing fish tanks effect on growth performance of two carp species fed with *Artemia* sp. *World Applied Sciences Journal*, *20*(5), 687-690.

Schumann, K. (1997). *Artemia* (brine shrimp) FAQ 1.1. *Portland State University Available:*http://web.cecs.pdx.edu/~davidr/discus/articles/Artemia.html (accessed on 15th June, 2021).

Shireman, J. V., & Smith, C. R. (1983). *Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844)* (No. 135). Food & Agriculture Organization.

Sorgeloos, P., Dhert, P., & Candreva, P. (2001). Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture*, *200*(1-2), 147-159.

Szlaminska, M., Escaffre, A. M., Charlon, N., & Bergot, P. (1991, June). Preliminary data on semisynthetic diets for goldfish (*Carassius auratus* L.) larvae. In *4.* French National Research Institute for Agriculture, Food, and Environment. *International Symposium Fish nutrition and feeding* (No. 61).

Szlaminska, M., Escaffre, A.M, & Bergot, P. (1991). Utilization of dietary pre-gelatinized starch by common carp (*Cyprinus carpio* L.) larvae. *Journal of Animal Physiology and Animal Nutrition*, *65* (1-5), 65-71.

Tesch, F. W. (1971). Age and growth. *Methods for assessment of fish production in fresh waters*. Oxford, Blackwell, 98-130.

Tyson, G. E., & Sullivan, M. L. (1980). Scanning electron microscopy of the frontal knobs of the male brine shrimp. *Transactions of the American Microscopical Society. 99* (2): 167–172.

United States Department of Agriculture, Agricultural Research Service. Extracted on June 13th, 2021. https://fdc.nal.usda.gov/index.html

Vanhaecke, P., & Sorgeloos, P. (1983). International study on *Artemia*: XIX. Hatching data for ten commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept. *Aquaculture*, *30*(1-4), 43-52.

Verreth, J. & Van Tongeren, M. (1989) Weaning time in *Clarias gariepinus* (Burchell) larvae. *Aquaculture, 83*, 81‑88.

Von Oertzen, J. A. (1985). Resistance and capacity adaptation of juvenile silver carp, *Hypophthalmichthys molitrix* (Val.), to temperature and salinity. *Aquaculture*, *44*(4), 321-332.

Watanabe, T., Thongrod, S., Takeuchi, T., Satoh, S., Kubota, S. S., Fujimaki, Y., & Cho, C. Y. (1989). Effect of dietary n-6 and n-3 fatty acids on growth, fatty acid composition and histological changes of white fish *Coregonus lavaretus maraena*. *Nippon Suisan Gakkaishi*, *55*(11), 1977-1982.

Wolnicki, J., Sikorska, J., & Kamiński, R. (2009). Response of larval and juvenile Rudd *Scardinius erythrophthalmus* (L.) to different diets under controlled conditions. *Czech Journal of Animal Science*, *54*(7), 331-337.

Woodhead, A. D. (1985). Feral Fishes in Lints FA (ed): Non-Mammalian Models for Research on Aging. *Interdisciplinary Top Gerontology, Basel, Karger, 21*, 21-50.

Yang, Y., Chen, Y., Lin, W., Wang, Q., Luo, S., Gan, X., & Zhang, D. (2017). Effects of L-methionine enriched *Artemia* nauplii on growth, amino acids composition and enzyme activities of the first feeding larvae of bighead carp (*Aristichthys nobilis*). *Journal of Northwest A & F University-Natural Science Edition*, *45*(1), 14-27.

Zainiyah, I. P., Satyantini, W. H., & Sahidu, A. M. (2019). The effect of giving cake artificial feed on the survival rate, and growth of Common carp (*Cyprinus carpio*) larva in an Installation of Freshwater Culture (IBAT) in Punten, Batu. In *IOP Conference Series: Earth and Environmental Science*. *236* (1), 012070. IOP Publishing.

APPENDIX-I

PHOTO PLATE

Figure 1

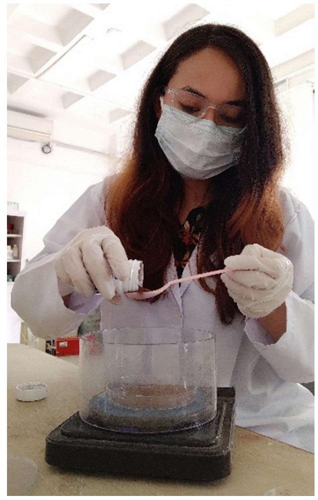

Figure 2

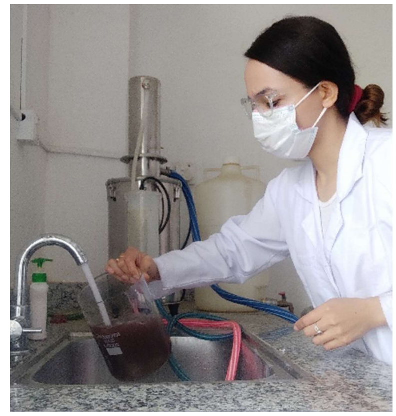

Figure 3

Figure 4

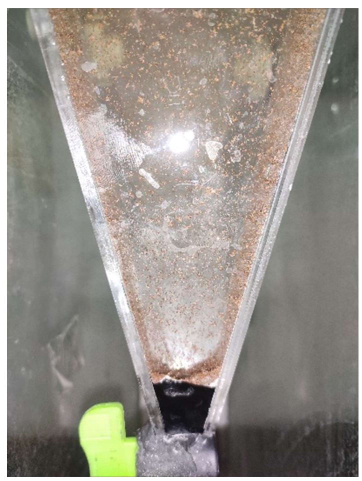

Figure 5

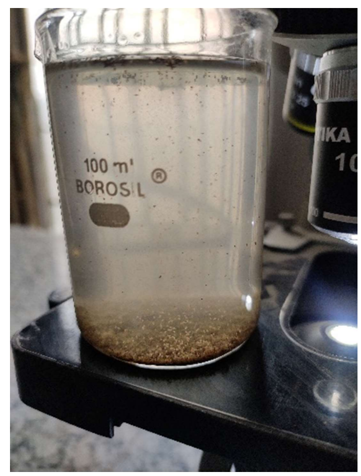

Figure 6

Figure 7

Figure 8

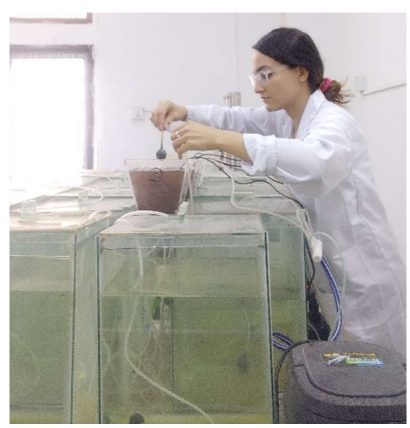

Figure 9

Figure 10

Figure 11

Figure 12

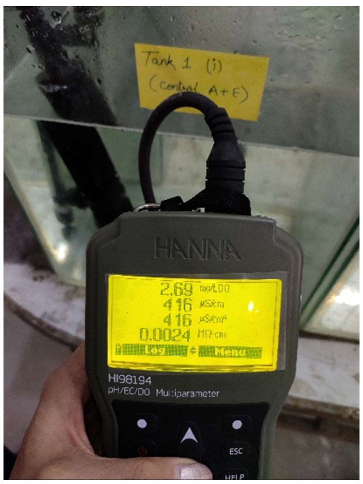

Figure 13

Figure 14

Figure 15

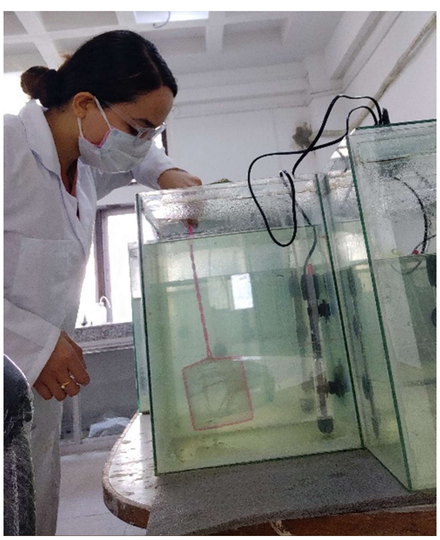

Figure 16

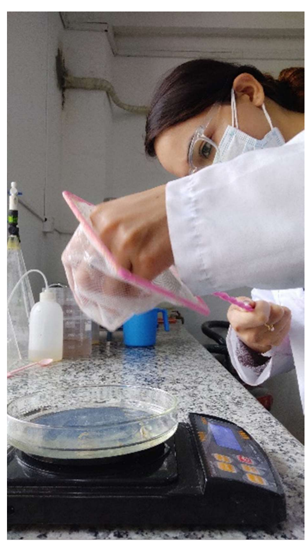

Figure 17

Figure 18

Figure 19

Figure 20

Figure 21

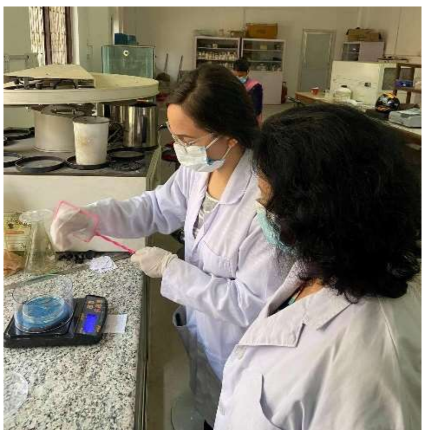
Figure 22


Figure 23


Figure 24

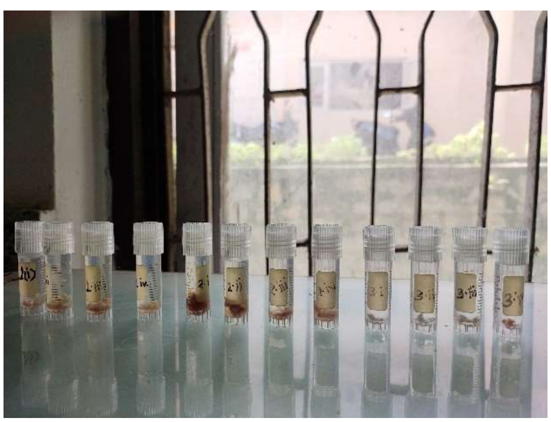
Figure 25


Figure 26

List of figures in photoplate

Figure number	Description of figure
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Figure 2	Measuring Artemia cysts in a digital balance
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APPENDIX-II

TABLES

Table 1: Total weight of fish larvae fed different diets during the study period

Total Weight of fish in gm (10 fish larvae) on different dates (2021)								
Tank	**Diet**	**5/10**	**5/17**	**5/24**	**5/31**	**6/7**	**6/14**	**6/21**
1.i	**D1 (Artemia + Egg)**	0.113	0.14	0.36	0.74	0.93	0.99	1.59
1.ii		0.115	0.57	0.67	0.69	0.81	1.17	1.86
1.iii		0.12	0.28	0.44	0.86	1	1.68	2.46
1.iv		0.117	0.21	0.42	0.51	0.69	0.99	1.05
2.i	**D2 (Egg)**	0.111	0.55	0.65	0.98	1.57	2.32	3.14
2.ii		0.112	0.41	0.64	1.27	1.65	1.82	2.52
2.iii		0.113	0.36	0.64	1.21	1.54	1.82	2.58
2.iv		0.118	0.54	0.45	1.28	1.48	1.58	2.72
3.i	**D3 (Artemia)**	0.109	0.16	0.36	0.48	0.61	0.81	0.95
3.ii		0.113	0.11	0.33	0.55	0.75	0.85	0.87
3.iii		0.117	0.17	0.31	0.49	0.61	0.91	0.94
3.iv		0.113	0.37	0.41	0.52	0.45	0.61	0.97

Table 2: Average length of grass carp larvae on initial and final day of the experiment

Average length of fish (in cm)		
Date	**5/10/2021**	**6/21/2021**
Tank 1.i	1.3	2.2
Tank 1.ii	1.3	2.3
Tank 1.iii	1.1	2.1
Tank 1.iv	1.4	2.3
Tank 2.i	1.1	3.2
Tank 2.ii	1.2	3.1
Tank 2.iii	1.2	3.1
Tank 2.iv	1.4	3.2
Tank 3.i	1.3	1.9
Tank 3.ii	1.2	1.9
Tank 3.iii	1.2	1.7
Tank 3.iv	1.4	1.9

Table 3: Average value of water quality parameters in Tank 1.i

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.33	8.42	8.49	8.47	8.44	8.62
DO (mg/l)	3.43	2.87	3.70	4.15	3.88	4.45
Conductivity (µS/cm)	408.67	308.50	351.40	366.83	388.83	456.67
TDS (ppm)	204.50	155.17	169.20	183.00	194.17	228.83
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 4: Average value of water quality parameters in Tank 1.ii

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.33	8.45	8.50	8.44	8.40	8.51
DO (mg/l)	3.03	3.01	77.91	4.20	3.83	4.75
Conductivity (µS/cm)	386.67	316.67	312.60	346.50	377.83	426.67
TDS (ppm)	193.67	157.83	156.00	173.17	188.17	528.53
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 5: Average value of water quality parameters in Tank 1.iii

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.45	8.47	8.41	8.37	8.38	8.49
DO (mg/l)	3.20	3.08	3.89	4.26	4.10	4.78
Conductivity (µS/cm)	375.83	329.17	351.00	367.50	363.00	418.83
TDS (ppm)	189.00	164.50	175.40	183.83	181.67	208.83
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 6: Average value of water quality parameters in Tank 1.iv

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.37	8.47	8.38	8.33	8.38	8.42
DO (mg/l)	3.28	3.09	3.89	4.26	4.09	4.80
Conductivity (µS/cm)	378.50	317.83	364.40	304.18	334.67	391.00
TDS (ppm)	190.00	158.67	181.60	185.17	167.50	195.83
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 7: Average value of water quality parameters in Tank 2.i

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.26	8.44	8.51	8.39	8.26	8.27
DO (mg/l)	3.30	3.03	4.11	4.35	55.29	4.27
Conductivity (µS/cm)	396.67	308.00	323.00	335.00	359.17	345.83
TDS (ppm)	199.33	154.50	161.60	167.17	179.50	172.50
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 8: Average value of water quality parameters in Tank 2.ii

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.37	8.45	8.45	8.34	8.32	8.30
DO (mg/l)	3.08	3.05	3.85	4.28	4.00	4.59
Conductivity (µS/cm)	303.07	309.67	343.20	362.33	311.33	319.17
TDS (ppm)	187.33	154.33	172.40	180.17	155.83	159.50
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 9: Average value of water quality parameters in Tank 2.iii

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.39	8.46	8.49	8.38	8.32	8.26
DO (mg/l)	3.27	3.13	4.07	4.43	4.28	88.63
Conductivity (µS/cm)	367.17	299.00	308.80	343.67	315.00	287.17
TDS (ppm)	183.17	148.83	155.00	172.17	157.17	143.33
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 10: Average value of water quality parameters in Tank 2.iv

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.35	8.45	8.52	8.36	8.28	8.28
DO (mg/l)	2.98	3.01	4.06	4.16	3.90	4.66
Conductivity (µS/cm)	378.33	331.17	310.00	338.33	351.67	352.83
TDS (ppm)	189.33	165.67	155.20	169.00	175.67	176.50
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 11: Average value of water quality parameters in Tank 3.i

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.44	8.44	8.43	8.33	8.37	8.41
DO (mg/l)	3.34	2.93	3.92	4.28	4.10	4.76
Conductivity (µS/cm)	336.50	326.00	329.80	358.83	423.67	494.33
TDS (ppm)	169.17	163.33	165.20	179.83	189.83	247.17
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 12: Average value of water quality parameters in Tank 3.ii

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.40	8.44	8.46	8.32	8.32	8.38
DO (mg/l)	3.46	3.06	3.88	4.13	4.07	4.62
Conductivity (µS/cm)	360.83	318.33	335.80	381.33	426.50	542.00
TDS (ppm)	181.33	159.00	167.80	190.67	214.33	270.83
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 13: Average value of water quality parameters in Tank 3.iii

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.38	8.42	8.40	8.29	8.32	8.38
DO (mg/l)	3.25	2.92	3.80	4.32	4.00	4.35
Conductivity (µS/cm)	350.67	335.67	351.60	371.67	405.17	504.83
TDS (ppm)	175.17	168.00	175.80	188.50	202.67	252.67
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

Table 14: Average value of water quality parameters in Tank 3.iv

Physico-chemical parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
pH	8.37	8.41	8.33	8.26	8.28	8.28
DO (mg/l)	2.86	2.77	3.67	4.19	3.98	4.57
Conductivity (µS/cm)	358.33	357.50	385.20	416.83	422.17	524.17
TDS (ppm)	178.83	178.50	192.40	208.67	211.33	262.33
Ammonia	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2	> 0.2

List of tables in Appendix-II