4.0. A STUDY ON THE EFFECT OF ZOOPLANKTONS AS LIVE FEED FOR FISH

4.1. INTRODUCTION

Presently more than 300 species of fish are reared world-wide and this number is increasing continually with the development of rearing techniques and improved feed quality (Watanabe et al., 1985) cited in Abi-Ayad and Kestemont, 1994). Improved techniques for marine Food-fish larviculture since the early 1980’s have greatly enhanced the growth and survival of freshwater ornamental fish larvae (Dhert et al., 1997), largely through improved technology regarding live food culture and larval rearing practices. Research developments in larviculture and early rearing technology have allowed 90% of currently marketed freshwater ornamental fish to be cultured (Tlusty, 2002).

Many fresh water ornamental fish farmers have shifted from Moina to the cleaner Artemia nauplii for feeding their young fish. As the nauplii (length of instar-1 Artemia < 0.55 mm) are only half the size of Moina (length <1.20 mm), it is necessary to look for bigger organisms, both to fill in the size gap, and as a substitute of Tubifex for feeding larger fish such as brooders (Dhert et al., 1997).

Availability of live food organisms in sufficient quantities is a major factor in the cultivation of early stages of shell fish and finfish. Only a few live feed organisms have been used in hatcheries (Wheeler, 2006). In aquaculture, an
increasing demand exists for live zooplankton in spite of the availability of Artemia nauplii and rotifers (Ortega-Salas and Reyes-Bustamante, 2006; Cooper, 2006; Rema and Gouveia, 2005). The zooplankton forms ideal food usually in the larval stages of prawns and in early larval stages of fishes (Charles et al., 1984). Zooplankton is the preferred food of fishes, particularly, fry and fingerling stage (Mollah and Tan, 1982). Being a natural food of fish and prawn larvae, zooplankton collected from natural resources are used as diet for the larvae of ornamental fish in many hatcheries (Kiron and Paulraj, 1990).

In larviculture, artificial diet may perform poorly due to poor digestibility and deficiency of growth factors (Jarboe and Grant, 1996). Common carp and Atlantic salmon grew faster when fed on zooplankton than those fed on formulated diets (Dada et al., 2002). Many authors have emphasized zooplankton as live food in general, particularly, Copepods and Cladoceran (Carlos, 1998; Chua and Teng, 1978; Goldan et al., 1997; Velasco et al., 1999; Marian et al., 1982). Nutritional quality of Copepods is reported to have high protein content and a good amino acid profile. The fatty acid composition with regard to HUFA is high in Copepods (Evjemo and Olsen, 1997).

Feeding rates of zooplankton are mainly dependent on food concentration, food quality, and water temperature (Peters and Downing, 1984; Kiorbae et al., 1982). It has been shown by several workers that feeding among Copepods was related to chemoreceptors (Friedman and Strickler, 1975; Paffenhofer et al., 1995) mechanoreceptors (Sterner and Schulz, 1998) and taste of the particular food (Poulet and Marsot, 1978). Feed problems to be encountered while
ontogenic development of larvae is one of the most crucial factors in terms of larval quality, health and feeding. In this regard, *Artemia* sp., is the most appropriate live prey for the larvae of the aquarium fish out of all live prey sources and has been used commonly since 1950s. *Artemia* species which are collected as egg in the nature could be given to the larvae either in the form of Artemia nauplii or as decapsulated cyst. As it has 30-40% more energy content, it can be used directly as food for larval feeding (Bengston *et al*., 1991; Vanhaecke *et al*., 1983).

Microalgae which are at the base of the food chain represent the third largest aquacultured crop in the world (Hanisak, 1998; Anon, 2000). In nature, most fishes and shrimps feed on varied types of natural phytoplanktons and zooplanktons. Farming of marine animals, including both finfish and invertebrates – chiefly crustaceans (shrimps) and mollusks requires microalgae as feed at some point in the life cycle (Jeffrey *et al*., 1994). However microalgae are also used widely to improve the nutritional content of zooplankton live feeds by allowing the zooplankton to fill their digestive systems with microalgae before subsequently being fed to the fish or shrimp larvae. In this “conditioning” strategy the zooplanktons serve as bags of appropriate size that partially digest the algae and stimulate components to the larvae. The green algae *Dunaliella*, produces abundant β-carotene (Wikifors, 2000) (an accessory light harvesting pigment) (Glazer, 1983).
Carotenoids contribute to the health and reproduction in fishes the carotenoids are included in the feed and fed to fishes and shrimps through Artemia nauplii. Many marine fishes produce small pelagic eggs. Larvae hatched from small eggs require a source of live food very soon after the onset of exogenous feeding (Schipp et al., 2001). Most of the commercial species are reared using rotifers and Artemia nauplii since they can be cultured in large quantities at high densities. Survival rate of Heteroclarias fry fed on shell free Artemia, live M. micrura and mixture of both as starter feed. Although shell free Artemia has been reported as good source of food nutrient in fish culture (Sorgeloos and Beard-More, 1980).

Copepods have potential as an alternative food resource in larval fish mariculture that might replace rotifers and Artemia (or) both (Cutts, 2002). Copepods are an important food source for many developing larvae, post larvae and juvenile fish and crustaceans (Sun and Fleeger, 1995). When provided as a first feed, copepod nauplii promote development and improve the survival rate of marine finfish larvae (Toledo et al., 1999).

According to Elangovan and Shim (1997), the comparison of protein requirements between fish species is complex since this can vary according to the size and life stage, diet formulation or farming condition. In Red tailed tinfoil (Barbodes altus), the optimal dietary protein level has been reported to be 41.7% with positive effect on weight gain. The protein source is also an important factor to be considered in the diet formulation. Marine protein sources were more efficiently to induce the weight gain in neon tetra (Paracheirodon innesi) than
diets based on vegetable proteins; however, fish fed with both protein sources diets containing 45% or 55% crude protein showed a better growth performance than 25% crude protein diets (Sealey et al., 2009). The life stage also affects the protein requirements level, for example in juvenile goldfish the protein requirement is lower (29%) than larvae (53%) (Fiogbe and Kestemont, 1995; Lochmann and Phillips, 1994).

According with Sales and Janssens (2003), the lipids are important sources of energy and fatty acids which are essential for normal growth and fish survival. Ling et al. (2006) suggested that the muscle lipid content acts as a source of lipid in the ovary, making it a useful indicator of reproductive performance. Increases in dietary lipid from 8% to 16% with the same protein level, improved the growth performance in swordtail. The muscle lipid content had the same trend as the protein level, with the highest accumulation observed with the highest dietary lipid (Ling et al., 2006).

Hence, present study further investigated the effect of different feed on the growth performance of fry. For the feeding practices workers have used control feeds or combinations of control feeds and live feeds in feeding trials with the larvae of gold fish. In most studies, live foods (e.g., Artemia, copepods) produced better results in terms of growth and survival than inert diets. In the present work three different types of feed used were pelleted, (control) and live feed (Artemia nauplii and copepod).
The major objectives of the present study are

The present study was undertaken

➢ To study the effect of different pH, salinities and temperature on the survival of Copepod and Artemia nauplii.

➢ To study the percentage survival of Cyclops and Artemia in different algae culture.

➢ To estimate the biochemical composition of Cyclops and Artemia nauplii.

➢ To analyze the water quality parameters such as temperature, pH, dissolved oxygen and ammonia during the culture of Goldfish.

➢ To evaluate the differences in the biochemical composition of Gold fish fed Control feed, Artemia nauplii and Cyclops.

➢ To test the effect of three different diets i.e., Artemia nauplii, Cyclops and Control feed on growth responses and survival of gold fish.
4.2. MATERIAL AND METHODS

4.2.1. Sample collection

For the present study the samples were collected from Kovalam saltpan with the help of sterile containers. The collected samples were packed in transparent plastic bottles and marked for identification and brought to the laboratory for further investigation.

4.2.2. Enrichment of sample

The water sample was centrifuged at 4000 rpm for 10 minutes. After centrifugation the sediments were divided into two portions. One portion of sediment was taken in 250 ml conical flask and 200 ml of algal working solution (Conway medium) was added into it. The other portion of the sediment was enriched with Conway medium and sodium meta silicate prepared separately to encourage the growth of Bacillariophyceae. The enriched samples were kept in a stock culture room and provided with 2000 lux of white fluorescent light without aeration. The samples were placed on a shaker, under low motion. The conical flasks were observed every day for colour development. The development of colour was considered as an indicator for the growth of algae.

4.2.3. Isolation of algal cells

From each enriched sample, a little quantity of sample was pipetted out on a slide and kept in microscope for identification. Based on observation, different microalgae present in a sample were isolated by specially made micro tubes and placed in 1 ml transparent tube containing sterilized culture media. These
transparent tubes were kept in a shaker and illuminated with white fluorescent light for inducing the growth of isolated algal cells.

**4.2.4. Isolation of zooplankton**

The live samples collected from the saltpan were examined under a dissection microscope by using a fine dropper or micropipette. The desired zooplankton were picked up into a glass cavity block containing 3ml of filtered sea water, the salinity was adjusted to the field salinity from where the sample was collected.

**4.2.5. Identification of phytoplankton and zooplankton**

The identification of Phytoplankton and Zooplankton were carried out with the help of standard books and identification manuals. The detailed procedure has been given the previous chapter page no 69.

**4.2.6. Isolated microalgae and zooplanktons are**

*Chlorella salina*

- **Kingdom**: Plantae
- **Phylum**: Chlorophyta
- **Class**: Chlorophyceae
- **Order**: Chlorellales
- **Family**: Chlorellaceae
- **Genus**: *Chlorella*
- **Species**: *Salina*
**Chaetoceros sp.**

<table>
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<td>Bacillariophyceae</td>
<td>Centrales</td>
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**Dunaliella salina**

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<td>Chlorophyceae</td>
<td>Volvocales</td>
<td>Dunaliellaceae</td>
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</table>

The zooplankton isolated during the present study are

**Artemia**

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<tr>
<td></td>
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<td>Branchiopoda</td>
<td>Anostraca</td>
<td>Artemiidae</td>
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Cyclops

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<td>Crustacea</td>
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<tr>
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<tr>
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<tr>
<td>Order</td>
<td>Cyclopoida</td>
</tr>
<tr>
<td>Family</td>
<td>Cyclopidae</td>
</tr>
</tbody>
</table>

4.2.7. Stock culture maintenance of microalgae

One litre filtered and heat sterilized seawater, after cooling added with stock solution of conway medium was considered as working solution, now ready to maintain the stock culture. Ten to twenty percentages of growing inoculums were transferred into culture flask and placed in front of the light (1000 lux). After 4-5 days, the culture reached to log phase. Then in 8-10 days, the culture reached the exponential phase.
4.2.8. Stock culture maintenance of Artemia and Cyclops

After isolation, the medium was replaced with fresh algae at every 24 hours interval. The isolated Artemia and Copepod species were transferred to the conical flask and the volume was gradually increased from 5 ml to 100 ml capacity.

4.2.9. Mass culture of marine microalgae

Initially sterilized 250ml conical flask was taken, the conical flask having 200ml of Conway medium was used as culture medium for algae. All the glasswares used were thoroughly washed with concentrated hydrochloric acid and then tap water with soap solution. The flasks were rinsed with distilled water for minimum 5 times. After drying, the glasswares were kept in a hot air oven at 160°C. The filtered seawater was sterilized by autoclaving. After cooling to room temperature, 200 ml of sea water was taken in to each conical flask. Prior to sterilization, salinity and pH was checked by refractometer and pH meter respectively. The filtered sea water was enriched with required quantity of Walne’s medium. Then 20ml of growth phase inoculums was added in to the
culturing medium containing conical flask. Finally, the culture flasks were kept in front of the 1000 lux fluorescent light. The temperature was controlled at 28°C and 30°C.

4.2.10. Mass culture of Artemia and Cyclops

Two large size buckets were taken with 10 litre filtered seawater and the temperature maintained at room temperature 28±1°C. The salt concentration was adjusted by using of refractometer. Two litre inoculum (100 Cyclops /ml) was taken and which was distributed into one bucket and two litre of inoculum (25 Artemia nauplii/ ml) was taken and which was distributed into another one bucket. 1 lakh algal cells/ml of desired algal density was maintained with the help of haemocytometer.

4.2.11. Survival of Artemia and Cyclops at different algae culture

The isolated Artemia and Cyclops were cultured in four different conical flasks containing different algae culture (Chlorella sp., Chaetoceros sp. and Dunaliella sp.). Then the conical flasks were kept in a suitable place. The Artemia and Cyclops were counted at the time interval of one day with the help of wide mouth 1ml glass pipette.

4.2.12. Survival of Artemia and Cyclops at different salinities

The Artemia and Cyclops were cultivated at different salinities such as 10, 20, 30, 40, and 50ppt separately in different conical flasks. The Artemia and Cyclops were counted at the time interval of one day in the conical flask with the help of glass pipette.
4.2.13. Survival of Artemia and Cyclops at different pH

The Artemia and Cyclops were cultivated at different pH such as 6, 7, 8, 9 and 10 separately in different conical flasks. The Artemia and Cyclops were counted at the time interval of one day with the help of glass pipette.

4.2.14. Survival of Artemia and Cyclops at different temperature

The Artemia and Cyclops were cultivated at different temperatures such as 20, 25, 30 and 35°C separately in different conical flasks. The Artemia and Cyclops were counted at the interval of one day with the help of glass pipette.

Experimental Gold fish

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</tr>
<tr>
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<tr>
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<td>Cypriniformes</td>
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<tr>
<td>Family</td>
<td>Cyprinidae</td>
</tr>
<tr>
<td>Genus</td>
<td>Carassius</td>
</tr>
<tr>
<td>Species</td>
<td>C. auratus (Linnaeus, 1758)</td>
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</tbody>
</table>

The goldfish (*Carassius auratus*) is a freshwater fish in the family Cyprinidae of Order Cypriniformes. It was one of the earliest fish to be domesticated, and is one of the most commonly kept aquarium fish.
4.2.15. Collection and maintenance of experimental fish

For the present study, gold fish *Carassius auratus*, a red variety obtained from a local commercial aquarium centre was kept under quarantine conditions for 10 days and then acclimatized to the experimental conditions. During this period, the fish were fed with control diet at 5% of their body weight. The fishes with the same colour and initial weight of 1.63, 1.89 and 1.76gm and initial length of 12.29, 12.24 and 12.18mm were selected. The fishes were starved for two days before taking the initial weight and length in order to evacuate their gut contents.

4.2.16. Larval feeding regime

The fish larvae were divided into 3 different feeding regimes with triplicate tanks. The three feeding regimes were Control diet, Artemia nauplii and Cyclops nauplii.

4.2.17. Cultivating algae

Initially sterilized 250ml conical flask was taken, the conical flask having 200ml of Conway medium was used as culturing medium for algae. All the glass wares used were thoroughly washed with concentrated hydrochloric acid and then tap water with soap solution. The flasks were rinsed with distilled water for minimum 5 times. After drying the glass wares were kept in a hot air oven for at 160°C. The filtered seawater was sterilized by autoclaving, after cooling to room temperature 200 ml of sea water was taken in to each conical flask. Prior to sterilization, salinity and pH was checked by refractometer and pH meter, respectively. The filtered sea water was enriched with required quantity of
Walne’s medium. Then 20ml of growth phase inoculums was added in to culturing medium containing conical flask. Finally, the culture flasks were placed in front of the fluorescent light of 1000 lux light intensity. The temperature was controlled at 28°C and 30°C.

4.2.18. Experimental feed

Newly hatched Artemia nauplii and Cyclops were used as experimental feed.

4.2.19. The culture of Cyclops was carried out by following the method described by Janakiraman and Altaff (2015)

Mass culture of live food organisms was carried out by collecting zooplankton from the saltpan. Species of Cyclops was sieved by different mesh filters (300 µm, 200 µm, and 100µm size) were separated using binocular microscope. These species were raised in laboratory to serve as inoculums for mass culture of zooplankton. Different algae were used for the enrichment of culture medium. Zooplankton species were cultured in 100-litre tanks for 35 days. Each culture tank was filled with 20 litre of filtered water of 17 to 25 ppt salinity. The medium was continuously aerated and inoculum of Cyclops was introduced in the tanks at the rate of 100 ind/l. To determine population density of the cultured species, the culture medium was thoroughly mixed and 1 litre of water samples was drawn from the culture tank.
Sub samples of 100ml and then 10 ml were drawn from these samples and the cultured species were counted under binocular stereoscopic dissection microscope using Sedge-wick Rafter cell. Five sub samples were analyzed to determine the population density of Cyclops.

4.2.20. Production of Artemia

Decapsulation of *Artemia franciscana* was performed according to Sorgeloos *et al.*, (1986). The cysts were kept refrigerated for maximum one week. Cysts were transferred to 10 litre plastic bucket with 30ppt saline water and aerated for 24 hours still the maximum cyst hatched into nauplii. After hatching, the Artemia nauplii were washed in order to remove the hatching debris. The hatched nauplii were moved back to the tanks for enrichment.

4.2.21. Enrichment of Artemia

The hatched nauplii of Artemia were moved back to the tanks for enrichment. The nauplii were allowed for enrichment after 24 hour of hatching. Enrichment was done using the algae of *Dunaliella* sp. and *Chlorella* sp. for 6 hours at a temperature between 25 and 28°C, 34 ppt seawater and with heavy aeration. After 6 hours of enrichment, the Artemia nauplii were washed again before being fed to the fish larvae.
4.2.22. Control feed preparation

The ingredients for control feed are

Fish meal - 46g
Soya bean flour - 15g
Ground nut oil cake - 18g
Wheat flour - 10g
Tapioca powder - 5g
Cod liver oil - 3g
Gelatin - 2g
Vitamin B12 - 1g
BHT (antioxidants) - 0.5g

a. Fish meal

Fish meal was obtained from freshly dried and powdered anchovies. Fish meal carries large quantities of energy per unit weight and is an excellent source of protein, lipids (oils), minerals and vitamins. Fishmeal contains certain compounds that make the feed more acceptable and agreeable to the taste. This property allows for the feed to be ingested rapidly and reduction of nutrient leaching.

b. Soya meal

Among the major plant protein sources, soybean is considered the best protein source in terms of its protein content and amino acid profile. With essential amino acid supplementation, it is a potential partial replacement for fish meal. Levels of incorporation in feeds for prawns are up to 40%. For the present study, soybeans obtained from the commercial market was ground well and powdered and used as a ingredient in shrimp feed.
c. **Ground nut oil cake**

Ground nut oil cake was obtained from the commercial market. It was powdered well and used as an ingredient for the present study. It is a good source of magnesium, sulphur and potassium, vitamins-niacin, pantothenic acid and thiamine.

d. **Wheat bran**

Wheat bran is a good source of energy and is also a good source of phosphorus, potassium, manganese and zinc, niacin, pantothenic acid and biotin. Ground whole wheat flour is widely used in prawn feeds. Gelatinization improves water stability. For the present study ground wheat bran obtained from the commercial market was used as an ingredient in shrimp feed preparation.

e. **Binders**

Binders are essential to provide the desired water stability to the feeds, to prevent disintegration of feeds and leaching of nutrients into the water. Recommended levels of binders are gelatin, collagen, alginates, carrageenan and agar 2.5%. For the present study, gelatin (HIMEDIA) 2% was used as the binder for the preparation of shrimp diet.

f. **Tapioca powder**

It is used as a natural binder and improves water stability of the feed. It is a source of carbohydrate. Tapioca powder purchased from the commercial market was used as an ingredient for the preparation of shrimp diets.
g. **Cod liver oil**

Cod liver oil purchased from the commercial market was used as lipid source. Moreover, it also acted as a lubricant for extruding the feed in the form of pellets.

h. **Vitamins and minerals**

Vitamins are chemically diverse group of organic substances. It was used for the maintenance of normal metabolic and physiological functions, resulting in increased growth and high survival rate of organisms.

Minerals are the important constituents of the structural components of tissues and skeleton in the regulations of osmotic pressure, nerve impulse transmission and in muscle contraction. In the present study, for the feed preparation, ‘supradin’ multivitamin and mineral tablets were used.

i. **Chromic oxide**

Chromic oxide is an inert marker used in the preparation of diet. For the present study, 0.5% of chromic oxide is used for the preparation of fish feeds.

4.2.23. **Preparation of feeds**

Fish feeds should have adequate energy for body maintenance and growth. It is contributed by three major nutrients namely protein, fat and carbohydrate. The feeds should have vitamin and minerals to avoid deficiencies.
The feed ingredients in the above table were weighed and mixed well in a container by adding sufficient quantity of distilled water and then the ingredients were made into dough. The dough was then placed in a container and boiled in a pressure cooker for 20 minutes. After boiling, the dough was taken out of the container and then vitamins and mineral mixture, cod liver oil, chromic oxide, gelatin and the active fraction of \textit{T.chebula} was added to the dough and mixed well.

The dough was then allowed to pass through a pelletizer having perforation diameter of 1.5 mm in the diet. Then the control diet was dried in a hot air oven at a temperature of 40\(^{0}\)C for duration of 15 hours. Then the dried pellets were collected and stored in air tight plastic containers.

\textbf{4.2.24. Experimental work}

The gold fish were collected from the aquarium. The gold fish were cultured for two days to get adapted. After adaptation, the fish were transferred into three different tanks and the tanks were named as F1, F2 and F3. The fish larvae of F1 tank was fed with control diet and the tank of F2 and F3 were fed with Artemia nauplii and Cyclops respectively. About 50\% of water from each tank was partially substituted through pure water at every alternate day before feeding. Appropriate aeration was done to supply adequate oxygen into the tank by motorized aerator. The feces in each tank was removed.
by siphoning and the dead larvae were removed in the morning and in the evening prior to feeding. Sampling was made at every 5 days interval. The larvae were collected from each tank to obtain the length and weight. The weight (g) was taken in an analytical balance and the length (mm) was measured by placing the fry on a transparent petridish placed on a 1 mm graph sheet.

The following formulae were used to determine the different growth parameters.

i. Weight gain of larvae was calculated by the following formula

\[
\text{Weight gain (mm)} = \frac{\text{Average final weight of larvae} - \text{Average initial weight of larvae}}{\text{Experimental period (days)}}
\]

ii. Length gain of larvae was calculated by the following formula

\[
\text{Length gain (mm)} = \frac{\text{Average final length of larvae} - \text{Average initial length of larvae}}{\text{Experimental period (days)}}
\]

iii. Absolute growth rate

\[
\text{AGR (g/body wt/day)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Total number of days}}
\]

iv. Specific growth rate (SGR)

\[
\text{SGR (\%)} = \frac{\text{Final wet weight (g)} - \text{Initial wet weight (g)}}{\text{Experimental period (days)}} \times 100
\]
v. The percentage survival was calculated by using the following formula.

\[
\text{Percentage survival} = \frac{\text{Final number of surviving fish}}{\text{Initial number of fish}} \times 100
\]

4.2.25. Water quality parameters

The water quality parameters were monitored and maintained at an optimum level during the entire experimental duration. Water samples were collected once in 5 days from respective tanks and temperature, pH, dissolved oxygen and ammonia were measured and recorded as per the detailed procedures given below.

a. Temperature

The water temperature of the control tank (F1) and experimental tanks (F2 and F3) were recorded using a Digital Thermometer.

b. pH

pH of water sample was recorded by using an Elico pH meter (LI10) and was calibrated each time using standard buffers.

c. Estimation of dissolved oxygen

Estimation of DO was done by following the standard procedure of Winkler method as described by Strickland and Parsons (1972). The detailed procedure has been given the previous chapter page no 20-22.
d. Estimation of ammonia (NH₃) (Solorzano, 1969)

Principle

Ammonia reacts with phenol and hypochloride at the high pH with sodium nitropruside as a catalyst and result in the formation of Indophenol, a blue coloured compound. The intensity of the colour was proportional to the ammonia concentration. The absorption of which was read at 640 nm spectrofiguremetrically.

Reagents

Phenol alcoholic solution

Phenol alcoholic solution was prepared by dissolved 10g of phenol crystals in 100 ml of 95% ethyl alcohol.

0.5% Sodium nitroprusside

Sodium nitroprusside solution was obtained by dissolving 500 mg sodium nitroprusside in 100 ml of distilled water.

Oxidizing solution

20 g of trisodium citrate and 10g of NaOH were dissolved in 100 ml of dissolved water and to this 25 ml of 1.5 N sodium hypochloride solution was added and used as an oxidizing solution.

Procedure

All the glassware used were cleaned by washing initially with warm dilute hydrochloric acid and rinsed thoroughly with distilled water. In a clean 10 ml test
tube, 5 ml of water sample, 0.2 ml of phenol alcoholic solution. 0.2 ml of 0.5% sodium nitroprusside solution ant 0.5 ml of oxidizing solution was taken. The solution was mixed thoroughly after each addition. Simultaneously 5 ml of distilled water was taken, treated similarly and used as reagent blank. It was kept for 1 hour at room temperature for the colour to develop and then the absorbance was measured at 640 nm in a spectrofiguremeter.

**Standard**

Ammonium chloride 0.3819 g was dissolved in 100 ml of distilled water and was used as the standard.

**Calculation**

\[
\text{Ammonia (mg/l)} = \frac{\text{Con. of standard x OD of unknown sample}}{\text{OD of known sample (standard)}}
\]

**4.2.26. Biochemical analysis**

Biochemical constituents such as protein (Lowry *et al.*, 1951), carbohydrate (Seifter *et al.*, 1950) and lipid (Folch *et al.*, 1957) contents of the muscle and tissues of control and experimental diets (Artemia nauplii and Cyclops) fed fishes were measured individually by following the standard methods.

**4.2.27. Survival of gold fish using different feeds**

The gold fish larvae were fed with different feeds such as control feed, Artemia nauplii and Cyclops separately in different glass tanks. The fish larvae were counted at the interval of 5 days.
4.3. RESULT

Zooplankton being one of the most important components of aquatic ecosystem plays an important role in growth of fish. In this chapter our focus was completely based on the effect of Artemia and Cyclops on the growth of fish. The results obtained during the present study are presented and discussed invariably in this section.

4.3.1. The effect of different pH on the growth of Cyclops and Artemia

The effect of different pH on the growth of Cyclops was also studied. In each pH range, the numbers of Cyclops were continuously observed for 10 days from the day of inoculation. The Cyclops tested for different pH was provided with all the optimum parameters except the pH. In pH 6, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.07, 7.62, 5.31, 3.91, 1.37, 0, 0, 0 and 0 animals/10ml respectively. In pH 7, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 10.23, 13.68, 17.41, 19.57, 16.83, 12.31, 9.06, 7.54 and 7.42 animals/10ml respectively. In pH 8, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 10.57, 12.69, 16.83, 18.57, 14.81, 11.74, 8.96, 7.49 and 7.31 animals/10ml respectively. In pH 9, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 7.01, 6.87, 6.21, 5.07, 4.32, 2.09, 1.47, 1.07 and 1.02 animals/10ml respectively. In pH 10, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 6.24, 4.09, 3.27, 1.04, 0, 0, 0 and 0 animals/10ml respectively (Table 4.1).
The effect of different pH on the growth of Artemia was also studied. In each pH range, the numbers of Artemia were continuously observed for 10 days from the day of inoculation. The Artemia tested for different pH was provided with all the optimum parameters except the pH. In pH 6, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.03, 6.02, 5.36, 4.16, 3.02, 1.46, 0, 0 and 0 animals/10ml respectively. In pH 7, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.92, 9.89, 9.71, 9.52, 9.48, 9.41, 9.39, 9.39 and 9.32 animals/10ml respectively. In pH 8, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.98, 9.91, 9.87, 9.64, 9.55, 9.46, 9.41, 9.36 and 9.31 animals/10ml respectively. In pH 9, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 8.02, 7.10, 6.54, 6.26, 5.82, 5.62, 5.11, 5.03 and 5.01 animals/10ml respectively. In pH 10, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 6.27, 5.14, 3.21, 1.06, 0, 0, 0, 0 and 0 animals/10ml respectively (Table 4.1).

4.3.2. The effect of different salinities on the growth of Cyclops and Artemia

The effect of different salinities on the growth of Cyclops was studied. In each salinity range, the Cyclops numbers were continuously observed for 10 days from the day of inoculation. The Cyclops tested for different salinities were provided with all the optimum parameters except the salinity. In 10ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 12.24, 16.09, 19.09, 24.39, 29.42, 21.03, 17.21, 14.07 and 11.23 animals/10ml respectively. In 20ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 14.87, 19.26, 23.93, 28.86, 32.33, 27.89, 24.54, 21.06 and 19.73 animals/10ml respectively. In 30ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days
were 10.00, 13.28, 17.03, 24.64, 29.32, 34.94, 31.56, 26.48, 22.92 and 19.71 animals/10ml respectively. In 40 ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 12.35, 17.52, 20.21, 24.89, 28.07, 22.31, 18.82, 15.07 and 12.04 animals/10ml respectively. In 50 ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.42, 7.01, 6.58, 6.06, 5.65, 5.04, 4.39, 3.21, and 3.04 animals/10ml respectively (Table-4.2).

The effect of different salinities on the growth of Artemia was studied. In each salinity range, the Artemia numbers were continuously observed for 10 days from the day of inoculation. The Artemia tested for different salinities were provided with all the optimum parameters except the salinity. In 10ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.73, 9.13, 8.24, 7.04, 6.05, 6.01, 5.93, 5.90, and 5.81 animals/10ml respectively. In 20ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.0, 9.86, 9.62, 8.93, 8.02, 8.02, 7.93, 7.09, 7.06, and 7.01 animals/10ml respectively. In 30ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 9.96, 9.95, 9.53, 9.35, 9.29, 9.19, 9.08, 9.08 and 9.01 animals/10ml respectively. In 40ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.94, 9.92, 9.58, 9.44, 9.33, 9.12, 9.12, 9.11, and 9.01 animals/10ml respectively. In 50ppt, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.83, 9.26, 8.73, 7.09, 6.03, 5.89, 5.79, 5.07 and 5.02 animals/10ml respectively (Table 4.2).
4.3.3. The effect of different temperature on the growth of Cyclops and Artemia

The effect of different temperature on the growth of Cyclops was studied. In each temperature range, the Cyclops numbers were continuously observed for 10 days from the day of inoculation. The Cyclops tested for different temperature were provided with all the optimum parameters except the temperature. In 20°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 7.32, 5.48, 4.87, 2.73, 1.63, 0, 0, 0, and 0 animals/10ml respectively. In 25°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 13.81, 19.52, 23.13, 29.13, 26.00, 25.00, 23.83, 22.08 and 19.06 animals/10ml respectively. In 30°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 12.00, 18.74, 24.33, 28.26, 32.10, 29.10, 26.00, 21.00 and 19.00 animals/10ml respectively. In 35°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 8.24, 7.40, 5.25, 3.16, 0, 0, 0 and 0 animals/10ml respectively (Table 4.3).

The effect of different temperature on the growth of Artemia was studied. In each temperature range, the Artemia numbers were continuously observed for 10 days from the day of inoculation. The Artemia tested for different temperature were provided with all the optimum parameters except the temperature. In 20°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 6.20, 5.09, 3.01, 1.08, 0, 0, 0 and 0 animals/10ml respectively. In 25°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.98, 9.93, 9.84, 9.77, 9.71, 9.64, 9.49, 9.31 and 9.24 animals/10ml respectively. In 30°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 9.99,
9.97, 9.91, 9.86, 9.79, 9.71, 9.53, 9.33 and 9.24 animals/10ml respectively. In 35°C, the growth recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 10.00, 7.56, 6.01, 3.02, 2.41, 1.44, 0, 0, 0, 0 and 0 animals/10ml respectively (Table 4.3).

4.3.4. Percentage survival of Cyclops and Artemia at different algae culture

The effect of different algae (Chlorella sp., Chaetoceros sp. and Dunaliella sp.) on the survival rate of Cyclops was studied. In each algal feeding, the Copepod numbers were continuously observed for 10 days from the day of inoculation. The Cyclops were provided with all the optimum parameters. In Chlorella culture, the percentage survival recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 100.00, 99.97, 99.89, 94.03, 90.63, 90.52, 88.59, 86.41, 86.03 and 85.12% respectively. In Chaetoceros culture, the percentage survival recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 100.00, 99.93, 99.82, 98.42, 94.92, 91.84, 90.06, 89.93, 89.74 and 88.82% respectively. In Dunaliella culture, the percentage survival recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 100.00, 99.99, 99.81, 98.67, 98.48, 98.03, 96.92, 96.83, 96.28 and 95.32% respectively (Table 4.4).

The effect of different algae (Chlorella sp., Chaetoceros sp. and Dunaliella sp.) on the survival of Artemia was studied. In each algal feeding, the Artemia numbers were continuously observed for 10 days from the day of inoculation. In Chlorella culture, the percentage survival recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 100.00, 99.66, 99.41, 99.22, 98.33, 98.01, 95.44, 95.21, 94.98 and 92.63% respectively. In Chaetoceros culture, the percentage survival recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 100.00, 99.58, 99.01,
97.64, 91.03, 89.43, 78.57, 71.84, 68.23 and 65.82\% respectively. In *Dunaliella* culture, the percentage survival recorded in 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days were 100.00, 99.62, 99.36, 98.06, 96.23, 90.48, 83.94, 80.52, 77.83 and 74.29\% respectively (Table 4.4).

### 4.3.5. Estimation of biochemical composition from enriched Cyclops and Artemia

Three types of algal feed such as *Chlorella* sp., *Chaetoceros* sp. and *Dunaliella* sp. were fed to the Cyclops for 10 days. After 10 days, the Cyclops were harvested and estimated the biochemical composition of the body. The protein, lipid and carbohydrate level estimated in the wild Cyclops were 62.39, 12.93 and 9.52\% respectively. The protein, lipid and carbohydrate level estimated in Cyclops fed with *Chlorella* algae were 65.68, 13.26 and 10.83\% respectively. The protein, lipid and carbohydrate level estimated in Cyclops fed with *Chaetoceros* algae were 63.27, 14.27 and 9.69\% respectively. The protein, lipid and carbohydrate level estimated in Cyclops fed with *Dunaliella* algae were 64.23, 12.99 and 11.03\% respectively (Table 4.5).

Three types of algal feed such as *Chlorella* sp., *Chaetoceros* sp. and *Dunaliella* sp. were fed to the Artemia for 10 days. After 10 days, the Artemia were harvested and body biochemical composition was estimated. The protein, lipid and carbohydrate level estimated in the wild Artemia were 43.05, 20.54 and 17.94\% respectively. The protein, lipid and carbohydrate level estimated in Artemia fed with *Chlorella* algae were 45.71, 21.47 and 20.83\% respectively. The protein, lipid and carbohydrate level estimated in Artemia fed with
Chaetoceros algae were 45.59, 23.83 and 19.57% respectively. The protein, lipid and carbohydrate level estimated in Artemia fed with *Dunaliella* algae were 46.83, 22.18 and 21.08% respectively (Table 4.5).

4.3.6. Water quality parameters

In the present study, water quality parameters were also checked in control (F1) and experimental tanks (F2-F3) during the culture period. The water quality parameters recorded in the culture tanks are given in Table 4.6.

In control tank (F1), the temperature and pH value recorded during the experimentation ranged between 30°C and 30.5°C and 7.13 and 7.79 respectively. The dissolved oxygen content ranged between 3.327 and 6.127 mg/l. The ammonia concentrations also ranged between 0.492 and 2.317 µg/l respectively.

In experimental tank (F2), the temperature and pH values recorded during the experimentation ranged from 30°C to 30.5°C and 7.19 to 7.89 respectively. The dissolved oxygen content and ammonia content ranged between 3.831 and 6.483 mg/l and 0.592 and 2.057 µg/l respectively.

In experimental tank (F3), the temperature and pH values recorded during the experimentation ranged from 30.0°C to 30.5°C and 7.16 to 7.61 respectively. The dissolved oxygen content was ranged from 3.546 to 6.103 and ammonia content of tank f2 was ranged from 0.521 to 2.173 µg/l.

4.3.7. Length of goldfish larvae fed with dry and live feed once a day

In control fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 12.29, 19.13, 23.29, 26.27 and 29.51 mm length respectively. In
Artemia nauplii fed groups, the length was recorded in 1, 10, 20, 30 and 40 days were 12.24, 19.19, 23.81, 27.08 and 30.57mm. In Cyclops fed groups, the length was recorded in 1, 10, 20, 30 and 40 days were 12.18, 19.11, 23.53, 26.89 and 30.31mm respectively (Table 4.7).

4.3.8. Length of goldfish larvae fed with dry and live feed twice a day

In control fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 12.34, 19.17, 24.69, 26.77 and 30.24 mm. In Artemia nauplii feed fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 12.29, 20.31, 26.83, 30.86 and 33.88 mm. In Cyclops fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 12.31, 19.92, 25.43, 29.42 and 33.76 mm respectively (Table 4.7).

4.3.9. Length of goldfish larvae fed with dry and live feed thrice a day

In control fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 12.34, 19.63, 24.98, 27.92 and 31.26 mm length. In Artemia nauplii feed fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 12.31, 21.68, 27.90, 32.41 and 35.34 mm. In Cyclops fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 12.32, 20.08, 26.48, 30.18 and 35.32 mm respectively (Table 4.7).

4.3.10. Weight of goldfish larvae fed with dry and live feed once a day

In control fed groups, the weight was recorded in 1, 10, 20, 30 and 40th days were 1.63, 1.74, 1.86, 1.99 and 2.11gm. In Artemia nauplii fed groups, the weight was recorded in 1, 10, 20, 30 and 40th days were 1.79, 1.96, 2.15, 2.35
and 2.53gm. In Cyclops fed groups, the weight was recorded in 1, 10, 20, 30 and 40th days were 1.76, 1.87, 2.04, 2.19 and 2.32 gm respectively (Table 4.8).

4.3.11. Weight of goldfish larvae fed with dry and live feed twice a day

In control fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 1.72, 1.96, 2.19, 2.62 and 3.07 gm. In Artemia nauplii fed groups, the weight was recorded in 1, 10, 20, 30 and 40th days were 1.72, 2.22, 2.63, 2.93 and 3.19 mm. In Cyclops fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 1.72, 2.19, 2.56, 2.81 and 3.18 gm respectively (Table 4.8).

4.3.12. Weight of goldfish larvae fed with dry and live feed thrice a day

In control fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 1.72, 1.96, 2.26, 2.73 and 3.12 mm. In Artemia nauplii fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 1.72, 2.16, 2.71, 3.04 and 3.27 mm. In Cyclops fed groups, the length was recorded in 1, 10, 20, 30 and 40th days were 1.72, 2.13, 2.64, 2.99 and 3.24 gm respectively (Table 4.8).

4.3.13. Estimation of biochemical composition of gold fish fed with different feed at five days interval

Table 4.9 provides the data on biochemical composition in the tissues of *Carassius auratus* (Gold fish) fed with control feed, Artemia nauplii and Cyclops for different duration i.e., 1, 10, 20, 30, and 40 days. The tested biochemical constituents [protein, carbohydrate and lipid] showed much variation between control Artemia nauplii and Cyclops diets.
In control feed fed groups, the initial (1day) protein content in the fish tissue was 6.04 mg/g and during experimental period, it ranged from 6.93 mg/g (5days) to 9.37 mg/g (40days). The tissue carbohydrate content of *Carassius auratus* was 4.13 mg/g on 1 day and it ranged from 4.46 to 6.52 mg/g for 5 to 40 days fed experimental fishes. The initial (1day) lipid content was 1.03 mg/g and during experimental period it ranged from 1.19 to 2.63 mg/g.

In Artemia nauplii fed groups, the initial (1day) protein content of fish tissue was 6.43 and during experimental period, it ranged from 7.08 mg/g (5days) to 9.51 mg/g (40days). The carbohydrate content of *C. auratus* was 4.13 mg/g on 1 day and it ranged from 4.53 to 6.86 mg/g for 5 to 40 days fed experimental fishes. The initial (1day) muscle lipid content was 1.05 mg/g and during experimental period it ranged from 1.23 to 2.79 mg/g.

In Cyclops fed groups, the initial (1day) protein content of fish tissue was 6.31 and during experimental period it ranged from 7.04 mg/g (5days) to 9.48 mg/g (40days). The carbohydrate content of *Carassius auratus* was 4.13 mg/g on 1 day and it ranged from 4.49 to 6.73 mg/g wet weight for 5 to 40 days fed experimental fishes. The initial (1day) lipid content was 1.03 mg/g wet weight and during experimental period it ranged from 1.21 to 2.71 mg/g.

4.3.14. Growth responses

The growth responses of control and experiment fishes were studied on 40th day of experimental duration and the data are shown in Table 4.10.

During the experiment, the initial average length of gold fish larvae were 12.29, 12.24 and 12.18 mm respectively in gold fish which were fed with control
feed (C), Artemia nauplii and Cyclops and final average length were 29.51, 30.57 and 30.31 mm respectively in gold fish those fed on control feed, Artemia nauplii and Cyclops. The initial average weight of gold fish larvae were 1.63, 1.89 and 1.76gm and final average weight were 2.11, 2.53 and 2.32gm in gold fish which were fed with control feed (C), Artemia nauplii and Cyclops respectively. The highest length gain of 18.33mm and weight gain of 0.64mm were observed in gold fish which were fed with Artemia nauplii. The absolute growth rates were 0.012, 0.016 and 0.014 gm in gold fish those received control feed, Artemia nauplii and Cyclops respectively. The highest specific growth rate was found to be 1.6 % which was shown by the larvae fed with Artemia nauplii, which was higher when compared to those in Cyclops (1.4) and control feed (1.2).

4.3.15. Survival of gold fish larvae using different feeds

Table 11 provides the data on survival of *Carassius auratus* (Gold fish) fed with control feed, Artemia nauplii and Cyclops for the duration of 40 days. In control fed groups, the survival rate recorded in 1, 10, 20, 30 and 40 days were 100.0, 93.2, 82.1, 78.9 and 69.8% respectively. In Artemia nauplii feed fed groups, the survival rate recorded in 1, 10, 20, 30 and 40 days were 100.0, 98.6, 93.7, 89.1, and 80.3% respectively. In Cyclops fed groups, the survival rate recorded in 1, 10, 20, 30 and 40 days were 100.0, 94.1, 90.3, 84.3, and 71.9 % respectively.
4.4. DISCUSSION

The present study was carried out to find the effect of zooplankton (Artemia and Cyclops) on the growth and survival of fish larvae (Gold fish). Aquaria which are known to have positive impact on human health in psychological and sociological aspects (Brodie and Biley, 1999; Herzog, 2011) have led to the development of a huge sector throughout the world (Livengood and Chapman, 2007). Feed problems to have been countered while ontogenic development of larvae is one of the most crucial factors in terms of larval quality, health and feeding. In this regard, *Artemia sp.* is the most appropriate live prey for the larvae of the aquarium fish out of all live prey sources and has been used commonly since 1950s. *Artemia species* which are collected as egg in the nature could be given to the larvae either in the form of Artemia nauplii or as decapsulated cyst. As it has 30-40% more energy content, it can be used directly as food for larval feeding (Bengston *et al*., 1991; Vanhaecke *et al*., 1983).

Availability of live food organisms in sufficient quantities is a major factor in the cultivation of early stages of shellfish and finfish. Only a few live feed organisms have been used in hatcheries (Kahan, 1982). In aquaculture, an increasing demand exists for live zooplankton in spite of the availability of Artemia nauplii and rotifers (Pourriot, 1986; Versichele *et al*., 1986; Pagano *et al*., 2000). The zooplankton forms ideal food usually in the larval stages of prawns and in early larval stages of fishes (Neelakandan *et al*., 1988). Zooplanktons are the preferred food of fishes, particularly, fry and fingerling stage (Murugan and Moorthy, 1990). Being a natural food of fish and prawn
larvae, zooplankton collected from natural resources are used as diet for the larvae of ornamental fish in many hatcheries (Altaf et al., 2002). Zooplankton have been widely used for rearing fish larval stages, and most studies indicated that the fry performed better when fed live zooplankton than dry artificial diets (Dabrowski and Rusiecki, 1983; Sivakumar, 2005). In larviculture, artificial diet may perform poorly due to poor digestibility and deficiency of growth factors (Lauff and Hofer, 1984). Common carp and Atlantic salmon grew faster when fed on zooplankton than those fed on formulated diets (Holm and Moller, 1984).

Copepods are microscopic zooplankton inhabiting both fresh water and marine environment (Alfred et al., 1973). They are an excellent food of high nutritional value for zooplanktivorous fish and shrimps (Zaleha et al., 2012). Copepods constitute important live food in the rearing of larvae of fishes (Hussain and Higuchi, 1980; Kraul et al., 1991). Nutritional quality of live food in aquaculture is important for survival and growth of larvae (Szyper, 1989). Zooplankton is the valuable source of amino acids, fatty acids, minerals and enzymes (Hertrampf and Piedad-Pascual, 2000). Live zooplankton contains enzymes (amylase, protease, exonuclease and esterase), which play important role in larvae nutrition (Fluchter, 1986) and easily digestible. The live food organisms have a high food value as protein source of fish (Ogino, 1963; Yamamoto, 1994).

The present research work was carried out the growth response of goldfish using different types of feed (dry and live). Study was conducted to investigate the effect of three different feeds on the growth performance and
survival of goldfish larvae. The initial average length of the larvae were 12.29, 12.24 and 12.18 mm and final length were 29.51, 30.57 and 31.31 mm and the initial average weight of the larvae were 1.63, 1.79 and 1.76 g and the final average weight were 2.11, 2.53 and 2.32 g respectively in goldfish those fed with control feed, Artemia nauplii and Cyclops fed once in a day. The highest length gain, weight gain, absolute growth rate and specific growth rate were found to be 18.33 mm, 0.64 g, 0.016% and 1.6% respectively in tank 2 (fed with Artemia nauplii) which is higher than the rest of two treatments (control and Cyclops).

When the gold fish larvae were fed with Control feed, Artemia nauplii and Cyclops twice in a day, the highest average length, weight, length gain and weight gain, were 33.88 mm, 3.19 g, 21.59 mm and 1.47 g respectively in Artemia nauplii fed larvae and highest absolute growth rate and percentage growth of 0.037% and 3.68% were observed by gold fish fed with both (Artemia nauplii and Cyclops).

When the gold fish larvae were fed with Control feed, Artemia nauplii and Cyclops thrice a day, the highest average length, weight, length gain, weight gain, absolute growth rate and percentage of growth were 35.34 mm, 3.27 g, 23.03 mm, 1.55 g, 0.039% and 3.90% and 1.47 g respectively in Artemia nauplii fed larvae.

In intensive rearing of fish larvae, feeding constitutes the major factor, since fish obtain their entire nutritional requirement through the food consumed by them (Pillay, 1990). Various studies highlights the fact that fish and prawn larvae prefer live feed compared to formulated feed (Nose, 1979; Murugesan et
al., 2010; Bakhtiyar et al., 2011). In the present study, it is clearly evident that
gold fish larvae showed increase in growth, survival, SGR, FCR, and protein,
when fed with live feed like Artemia naupli and Cyclops which is in agreement
with the previous reports (Murugesan et al., 2010; Priyadarshini et al., 2011).

In the present study, it was demonstrated that both control feed (artificial pellets) and live feeds were accepted by the larvae of goldfishes as evidenced by their development of late larval stage. These diets supported varying degrees of growth and survival of the fish larvae in a forty-day larvae rearing experiment. Further, results of present study suggest that larvae rearing up to late larval stage are better with live feed than the control (artificial pellets) as evidenced by the higher growth and survival of the larvae of goldfish. With regard to the chemical parameters of the culture system, higher dissolved oxygen was recorded in the rearing system of gold fish larvae with Artemia nauplii as the live feed. Dissolved oxygen levels are improved due to figuresynthesis, Different levels of dissolved oxygen content were reported in fish rearing system of larvae of Cyprinus carpio (8.77mg/L to 10.85mg/L) (Priyadarshini et al., 2011). Generally, cyprinids are capable of tolerating low oxygen levels of 3mg/L (Huet, 1972). The level of dissolved oxygen content in the goldfish larvae rearing system of present study was found between 4.41mg/L and 6.12mg/L. This level of dissolved oxygen is adequate to promote normal growth and development (Jhingran, 1991). It is interesting to note that with similar intensity and duration of aeration, lower dissolved oxygen content in the koi carp tanks might be due to their higher oxygen consumption which is indicative of higher metabolism in the larvae of koi carp than the goldfish (Sivakumar, 2005).
Temperature is also suggested to be optimum for the larval development of freshwater fishes. It is reported that the optimum development of *W. attu* is at a temperature range of 28.5°C–30°C (Jhingran, 1991; Giri *et al.*, 2004). It is quite evident that the duration of larval development of fishes is longer in temperate waters (Pilar *et al.*, 2000; Woods *et al.*, 2003). pH ranges between 7.13 and 7.89 however, the values of pH in the present study are in agreement with the earlier work (Priyadarshini *et al.*, 2011). Thus, successful hatchery production of fish larvae depends on many factors, the most important being the type of rearing system, physical and chemical parameters, and the type of diet used (Sivakumar, 2005). However, the values of ammonia recorded in the present experiments showed low level when compared to previous study (Priyadarshini *et al.*, 2011). Sugiyama and Kawai (1978) reported that higher concentration of dissolved oxygen decreases ammonia level through oxidation (Sugiyama and Kawai, 1978). Nevertheless, they point out that unionized ammonia and nitrate are the most dangerous metabolites for fry development and are very important in the first phases of ontogeny and hence should be maintained at a lower level. In the present study, the level of ammonia in the rearing system of goldfish larvae is lower when compared to the earlier results (Priyadarshini *et al.*, 2011). Hence, the water quality should be maintained at optimum levels for the normal development of fish fry.

In the present study, goldfish larvae fed with three times / day has higher specific growth rate and percentage survival. According to Mondal *et al.* (2007), the growth rate of fish increases within the level of dietary protein till the optimum level is reached. Jana and Chakraborty (1990) suggest that the growth,
reproductive potentials and survival of each species are affected by the nutrient conditions of the culture media. The feeding frequency of 3 times/day was adopted during the present experiment to avoid water fouling and ease of feed provision and other managements. Feeding frequency has direct impact on the growth performance and survival of fry and larvae of *Clarias macrocephalus* (Mollah and Tan, 1982). They found that a feeding frequency of three times every day was best for rearing the fry and larvae of goldfish which is relevant to the present study.
4.5. SUMMARY

The aim of this chapter is to understand the effect of different feeds (Artemia nauplii, Cyclops and Control feed) on the growth responses of gold fish larvae. Artemia nauplii and Cyclops are used as a diet for rearing the larvae of many marine fish species and aquarium fish species. Hatching Artemia from cysts is time-consuming and a hatchery is often required to facilitate continuous production of nauplii. Thus, it would be beneficial to find an alternative diet that would require less labour, time and money to prepare. The important observations made in this study have been given below.

- Among the tested pH, pH 7-8 was better for the maximum growth of Artemia and Cyclops.

- Among the different salinity tested on Cyclops and Artemia, the salinity 20 was better for the growth of Cyclops and salinity 30-40 were found to be the optimum for the growth of Artemia.

- Among the different temperature tested on Cyclops and Artemia, the temperature 25 -30°C were found to be the optimum temperature which help to increase the population in Cyclops and Artemia.

- Among the three algae, Chlorella sp. culture was better for the survival of Cyclops while Artemia had maximum survival in Dunaliella sp.

- After enrichment, the biochemical constituents such as protein, lipid and carbohydrate were found to be highest in Cyclops and Artemia.
The biochemical constituents such as protein, lipid and carbohydrate were found to be high in the gold fish larvae which were fed with Artemia nauplii.

Among the three different feeds, (Control feed, Cyclops and Artemia nauplii) Artemia nauplii were the better one for the growth of gold fish larvae.

When the gold fish larvae were fed with three different feeds such as Control feed, Cyclops and Artemia nauplii at different time intervals per day (once a day, twice a day and thrice a day). The gold fish larvae fed thrice a day was found to be very effective and supported for maximum survival.
5. CONCLUSION

The distribution of phytoplanktons and zooplanktons in the selected salt pans varied based on the topography of the salt pan, salinity of water and seasons. A species which was found dominant in one salt pan was not found to be dominant in another salt pan. The study done on species abundance for different months in two years would help immensely in the utilization of phytoplankton and zooplankton for commercial uses.

Though the salt pans are considered as an extreme environment, it is a storehouse for the production of commercially untapped resource for new products might lead to discovery of new organisms of great importance like carotenoid, pigment and Artemia related feeds.

This study has opened a new avenue for the salt pan. The seasonal variation which run the physico chemical changes in the salt pan environment in turn create diversity among the availability of species of animals and plants which can adopt for very low and high salinity. There are some phytoplanktons and zooplanktons that can survive in both high and low salinity.