EFFECT OF LIVE FEED COPEPODS (*MESOCYCLOPS ASPERICORNIS*) ENRICHED WITH SPIRULINA, CHLORELLA AND AZOLLA ON THE GROWTH OF ORNAMENTAL FISH (*BRACHYDANIO RERIO*)

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ABSTRACT: *Zooplanktons are one of the primary foods for the fishes. Advances in live food enrichment technique have helped to boost the importance and potential of live food organisms in the raising of aquatic species. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids. Providing appropriate live food at proper time play a major role in achieving maximum growth and survival of the young ones of finfish and shellfish. Nutritional status of live food organisms can improve through various techniques of enrichment and bioencapsulation. Thus in the present study live feed copepods (Mesocyclops aspericornis) is enriched with spirulina, chlorella and azolla and thieir effects in growth of ornamental fish (Brachydanio rerio) was evaluated. The length of the Brachydanio rerio fed with enriched Mesocyclops aspericornis by spirulina, chlorella, azolla and yeast was found to be 3.74^{c}±0.238cm, 3.81^{c}±0.270cm, 3.12^{c}±0.380cm, 2.91^{c}±0.270cm. The weight of the Brachydanio rerio fed with enriched Mesocyclops aspericornis by spirulina, azolla, chlorella and yeast was found to be 0.56^{c}±0.023g, 0.54^{c}±0.015g, 0.52^{c}±0.019g, 0.49^{c}±0.024g. Thus from the present study, it is confirmed that feeding of nutrient rich phytoplanktons resulted in enriched copepods. Those copepods served as a highly enriched feed for the ornamental fish (B. rerio), indicated by the increased length and weight of the fish and copepods enriched with different phytoplanktons due to its significant growth rate could be given as feed for fishes than the normal feeds.*

Key Words: *Mesocyclops aspericornis, live feed, Brachydanio reri, spirulina, azolla, chlorella, growth rate*

Introduction

In nature zooplanktons are one of the primary foods for the fishes. Energy in pelagic ecosystems flows from phytoplankton to zooplankton through the classical food chain and microbial food webs. Energy flow at the primary producer and herbivore interface is influenced by both the quality and quantity of food available for zooplankton (Brett and Muller, 1997). Algal size, secondary metabolites, digestibility, elemental and biochemical composition have previously been used to explain food quality for zooplankton. Zooplankton growth in nature may depend on the quality of the food available as the phytoplankton community changes. Phytoplankton may stimulate zooplankton development by production of vitamin E (α-tocopherol) and releasing `odour' into water (Rutkowska and Pijinowska, 1999). Feeding rates of zooplankton organisms are mainly dependent on food concentration, food quality and water temperature. Zooplankton is the valuable source of amino acids, fatty acids, minerals and enzymes. Live zooplankton contains enzymes (amylase, protease, exonuclease and esterase), which play important role in larvae nutrition and easily digestible. The live food organisms have a high food value as protein source of fish (Ogino, 1963).

Advances in live food enrichment technique have helped to boost the importance and potential of live food organisms in the raising of aquatic species. An important factor in the success of aquaculture is the availability of suitable food at a suitable time, at a reasonable cost. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1998) and hence are commonly known as “living capsules of nutrition”. Providing appropriate live food at proper time play a major role in achieving maximum growth and survival of the young ones of finfish and shellfish. To achieve maximum production and profitability, the nutritional components of natural foods must be identified and quantified. Nutritional status of live food organisms can improve through various techniques of enrichment and bioencapsulation. It is obviously agreed that the production of live food organisms continues to be a very important first step in intensification of aquaculture, both horizontally as well as vertically (Das *et al.,* 2012).

Copepods are microscopic zooplankton inhabiting both fresh water and marine environment. Copepods are the most important components of aquatic ecosystem and play vital role as primary consumers. They range in size from less than 1mm to more than 5mm. In the aquatic habitat, their small size is compensated by their occurrence in large numbers (Russel-Hunter, 1969). Copepods are the dominant and most abundant secondary producers among marine and fresh water zooplankton and hence are of major ecological significance.They are an excellent food of high nutritional value for zooplanktivorous fish and shrimps. Copepods constitute an important component of the food chain in aquatic systems. They can also be an important source of food for larval, juvenile, and adult fish of many species (Hart, 1990).

Copepod larvae are reported to be more easily and completely digested than either rotifers or Artemia. The movement of copepods and their nauplii triggers the feeding responses in fish larvae. The 'jerking' swimming action of most copepod nauplii and adults is believed to be an important stimulus for initiating feeding by fish larvae (Marcus, 2005). Many copepods have small sizes (<100um) for at least one or more developmental stages that make them suitable as first feeds for small larvae with small mouth gap14. It has been shown by several workers that feeding among copepods was related to chemo receptors; mechanoreceptors and taste of the particular food (Murugesan *et al.,* 2010). Interest in the use of copepods in aquaculture has grown since 1980's. Over the past few years there have been several studies related to copepod culture and the important role that copepods can play as feed for fish larviculture. Fregadolli, (2005) has studied predation of larvae of Brazilian fishes, *Piaractus mesopotamicus* and *Colossoma macropomus* on a cyclopoid copepod *Thermocyclops decipiens* as first feed. Mass culture of zooplanktonic copepods has been the subject of many investigations during recent years. Omori *et al.,* 1996 have stated that twenty species of calanoids, one cyclopoids and nine harpacticoids have been reared under laboratory condition. Only a few species of copepods have been successfully reared at near commercial scale in extensive systems. An extensive analysis of current copepod rearing technologies has been provided by Lee *et al*., (2005). The three main copepod orders, Cyclopoida, Calanoida and Harpacticoida have each been investigated for their suitability as feeds for larval and juvenile fish.

The aim of the present study was to evaluate the effect of live feed organism (*Mesocyclops aspericornis*) on *Brachydanio rerio*. These *Mesocyclops aspericornis* were enriched with different phytoplanktons like spirulina, chlorella, azolla; their growth on fishes and the nutritional qualities were evaluated.

Materials and Methods

Procurement of zooplanktons and phytoplanktons

The live feed copepods (*Mesocyclops aspericornis*) were used for present investigation. The species were collected from the Muthanna Lake, P.N Pudur, Coimbatore, Tamilnadu using 100µm mesh dipnet (Figure 1).

Isolation of copepods from lake water

The water with zooplankton which was collected from the lake was filtered in the 200µm mesh; all the zooplanktons were retained in the net, the water was collected in the trough. Unwanted waste materials are removed by washing under running tap water. Then the mesh containing zooplanktons was placed in a petridish containing water. Using the eyedropper copepods (*Mesocyclops aspericornis*) were isolated, the isolated copepods were put into the beaker containing water.

Cultivation of phytoplanktons

Culturing of C*hlorella vulgaris* and *Spirulina platensis*:

The *Chlorella vulgaris* and *Spirulina platensis* was cultured in Aquarium glass tank (50 l capacity) separately (Figure 2,3). The typical medium used for the mass culture of *Chlorella vulgaris* and *Spirulina platensis* were as follows.

CHEMICALS	QUANTITY (gm)
Potassium nitrate	202.0
Sodium dihydrogen phosphate	310.5
Sodium monohydrogen phosphate	89.0
Magnesium sulphate	246.5
Calcium chloride	14.7

The tank was kept in sunlight for photosynthesis; tank was filled with 40 l of tap water. The chemical ingredients were dissolved in it. After that cow dung were mixed with water and filtered with the

nylon cloth. To the filtrate about 250ml of the pure mother culture of *Chlorella vulgaris* and *Spirulina platensis* inoculums were added to separate tanks and mixed well. Mixing was done twice a day during the culture period for proper distribution of chemicals and for proper aeration. The pH was maintained at 8, no aeration was provided. Several physico-chemical parameters were maintained in culture medium for the proper culture of chlorella, such as pH, temperature and dissolved solids. These parameters were checked every two days interval. Proper agitation and aeration was provided by stirring. It prevents sedimentation and a homogenous exposure of algal cells to light and reduces the nutrient and temperature gradient along depth of culture.

Culturing of *Azolla*

A trough of 20 l capacity was taken to which a sediment layer of 5cm clay soil was poured without any waste material. To that 0.5% of urea was dissolved in 5l of water, along with that 1kg cow dung extract was also added and poured into the trough. This composition was allowed to stand for 2 days. The trough was kept outdoor in direct sunlight. To this medium Azolla mother culture was added. After twenty days the Azolla culture were collected and dried in incubator. The dried Azolla was powdered to fine particles. After every 7 days Azolla biomass was harvested, weighed and dried at 60^{o}c in an oven till constant weights were recorded. Water quality parameters like temperature, pH and dissolved solids are monitored regularly (Figure 3).

Harvesting of phytoplanktons

Harvesting of C*hlorella vulgaris* and *Spirulina platensis*:

The *Spirulina platensis* and C*hlorella vulgaris* were harvested by filtration through meshes having pore size of about 10µm. The harvested spirulina were rinsed very well with distilled water to remove the any chemical load on the biomass carried by the nutrient medium. After the harvested algal biomass must be dewatered by drying operation; to dehydrate biomass are normally done by sun drying. After drying the harvested dried biomass was made into powder and stored in clean, airtight containers. Dried weight measurements were calculated in gram per liter by dried the biomass in an oven at 105^{o}C for two hours. This spirulina powder was used to feed the copepods (*Mesocyclops aspericornis*) along with other two algae.

Harvesting of *Azolla Pinnata*to

The matured Azolla are harvested by using net. The Azolla were dried in shade for three days on metallic sheet until they become crispy while retaining their greenish colouration. The dried leaves were then milled using a hammer mill to produce fine powder, which was then stored in air tight containers for enrichment process. The process is repeated and collected the required amount of Azolla powder.

Enrichment

Mesocyclops aspericornis was enriched with *Spirulina platensis, Chlorella vulgaris* and *Azolla Pinnata*to feed the ornamental fish *Brachydanio rerio,* cultured in laboratory. The 48 hours adult nauplii of copepod *Mesocyclops aspericornis* were fed with each type of food at same concentration 0.5 mg/ml/d. The powdered feeds are taken at 0.5 mg concentration, mixed with distilled water and stirred for 2-3 minutes vigorously. *M. aspericornis* (50/ml) was introduced into 500ml culture flasks containing freshwater and mild aeration was provided. After 6 hours of enrichment *M. aspericornis* (adult nauplii*)* were fed to experimental fishes *Brachydanio rerio* twice a day. Daily observations were done.

Nutritinal analysis of phytoplanktons

Nutritional analysis of Protein, lipid and carbohydrates were determined by the following methods. The basic procedures of the analytical scheme were taken from Lowry et al., (1951) for protein, Folch et al., (1957), for lipids, Roe (1955) for carbohydrates and amino acids (Yamamoto et al., 1994).

Experimental setup

The fishes were divided into 50 numbers in 4 groups:
Experiment 1: ***B. rerio*** fed with *Mesocyclops aspericornis* enriched by Spirulina
Experiment 2: ***B. rerio*** fed with *Mesocyclops aspericornis* enriched by Azolla
Experiment 3: ***B. rerio*** fed with *Mesocyclops aspericornis* enriched by Cholerlla
Experiment 4: ***B. rerio*** fed with *Mesocyclops aspericornis* enriched by yeast

After 60days, *Brachydanio rerio* fishes of each experimental group were subject to the determination of the final length and weight.

Statistical Analysis

As all the tests were performed with triplicates and the results were recorded and expressed in terms of mean ± Standard deviation.

Results

Nutritinal analysis of phytoplanktons

Algal sample	Protein (mg/g)	Carbohydrate (mg/g)	Lipid (mg/g)
Spirulina	1438.65 ± 342.02[a]	1399.1 ± 260.63[c]	829.4 ± 308.99[b]
Chlorella	318.08± 16.22[c]	1720.9 ± 70.71[b]	822.9± 29.01[b]
Azolla	964.58± 73.28[b]	2458.8 ± 487.10[a]	944.2± 63.83[a]

Table 1: Nutritinal analysis of phytoplanktons

The total protein content of spirulina, chlorella and azolla were found to be 1438.65 ± 342.02[a] mg/g, 318.08± 16.22[c] mg/g, 964.58± 73.28[b] mg/g. The carbohydrate content of spirulina, chlorella and azolla were found to be 1399.1 ± 260.63[c] mg/g, 1720.9 ± 70.71[b] mg/g, 2458.8 ± 487.10[a] mg/g and the lipid content were 829.4 ± 308.99[b] mg/g, 822.9± 29.01[b] mg/g, 944.2± 63.83[a] mg/g (Table 1). The protein and carbohydrate content found to be significantly higher on spirulina than chlorella and azolla. Surprisingly, the lipid content was higher in azolla than spirulina and chlorella.

Length of *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by different feeds

Parameters	Exp1	Exp2	Exp3	Control
Initial length (cm)	1.78[a]±0.037	1.84[a]±0.343	1.88[a]±0.033	1.92[a]±0.316
Fish length 30 days (cm)	2.72[b]±0.028	2.78[b]±0.025	2.64[b]±0.030	2.02[b]±0.031
Final length 60 days(cm)	3.74[c]±0.238	3.81[c]±0.270	3.12[c]±0.380	2.91[c]±0.270

Table 2: Length of *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by different feeds

Brachydanio rerio fed with enriched *Mesocyclops aspericornis* by spirulina had length of 1.78[a]±0.037cm initially. After 30 days the length was found to be 2.72[b]±0.028cm and after 60 days the length was 3.74[c]±0.238cm. The length of the *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by azolla was 2.72[b]±0.028cm. After 30 days the length was 2.78[b]±0.025cm and after 60 days the length was 3.81[c]±0.270cm. *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by chlorella had length of 1.88[a]±0.033cm initially. After 30 days the length was found to be 2.64[b]±0.030cm and afer 60 days the length was 3.12[c]±0.380cm. The length of the *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by azolla was 2.72[b]±0.028cm. After 30 days the length was 2.78[b]±0.025cm and after 60 days the length was 3.81[c]±0.270cm. The length of the *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by yeast was 1.92[a]±0.316cm. After 30 days the length was 2.02[b]±0.031cm and after 60 days the length was 2.91[c]±0.270cm (Table 2).

Weight of *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by different feeds

Parameters	Exp1	Exp2	Exp3	Control
Initial weight(g)	0.07[a]±0.033	0.08[a]±0.027	0.06[a]±0.023	0.09[a]±0.031
Fish weight in 30 days(g)	0.22[b]±0.027	0.26[b]±0.036	0.25[b]±0.023	0.21[b]±0.025
Final weight in 60 days (g)	0.56[c]±0.023	0.54[c]±0.015	0.52[c]±0.019	0.49[c]±0.024

Table 3: Weight of *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by different feeds

Brachydanio rerio fed with enriched *Mesocyclops aspericornis* by spirulina had length of 0.07[a]±0.033g initially. After 30 days the length was found to be 0.22[b]±0.027g and after 60 days the length was 0.56[c]±0.023g. The length of the *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by azolla was 0.08[a]±0.027g. After 30 days the length was 0.26[b]±0.036g and after 60 days the length was 0.54[c]±0.015g. *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by chlorella had length of 0.06[a]±0.023g initially. After 30 days the length was found to be 0.25[b]±0.023g and after 60 days the length was 0.52[c]±0.019g. The length of the *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by azolla was 0.09[a]±0.031g. After 30 days the length was 2.78[b]±0.025cm and after 60 days the length was 3.81[c]±0.270cm. The length of the *Brachydanio rerio* fed with enriched *Mesocyclops aspericornis* by yeast was 0.09[a]±0.031g. After 30 days the length was 0.21[b]±0.025g and after 60 days the length was 0.49[c]±0.024g (Table 3).

Discussion

Carbohydrates in the form of polysaccharide glycogen are also an important form of energy storage in zooplankton. These results differ from that of Groeger et al., (1991) who recorded that copepod (*Argyrodiaptomus furcatus*) had carbohydrate levels above 27%, showing that all the diets studied were efficient with the result of this component. In fish, the carbohydrate is present in lower quantities in the

form of glycogen, sugar and their derivatives. It was observed by Santhanam et al., (1990) that, ten to fifty percent of carbohydrates in fish feed enhanced the growth of fish through their protein sparing action. Fishes, in general, utilize dietary carbohydrates poorly. The optimum level of dietary carbohydrate should enhance the maximum growth and feed efficiency (Shiau, 1994). The lipid requirement of any cultivable fish depends largely on its digestibility, quality and amount of essential fatty acids present in the diet. Furthermore, the fatty acids and esters of glycerols are used by fish for long term energy requirements, more particularly during the period of extensive swimming and inadequate food supply. Freshwater fish have high levels of poly unsaturated fatty acids (PUFA) compared to their marine counterpart. Fatty acids play a vital role in maintaining structural and functional integrity of fish cell membranes. Zooplankton contains high level of arachidonic acid and which help in the growth and survival of larvae as documented by Bell et al., (1995). Fishes derive much of their energy from proteins. The growth of fish in terms of muscle formation depends on the high percentage of protein intake. The protein requirements of cultureable fish depend on size, age, stocking density, oxygen supply and the presence of toxicants. Only dietary protein concentration has a significant influence on fish performance. The dietary proteins, such as aminoacids are of primary importance for the effective utilization and for successful growth and the amino acid patterns should meet the requirement of the organism. In the present study, the *Mesocyclops aspericornis* feed with high carbohydrate, protein and lipid content phytoplanktons resulted in significant growth (length and weight) of the *Brachydanio rerio* fishes.

Conclusion

The different phytoplanktons were cultured and used as feed for copepods. The copepods were fed for the ornamental fish (*B. rerio*). Thus from the present study, it is confirmed that feeding of nutrient rich phytoplanktons resulted in enriched copepods. Those copepods served as a highly enriched feed for the ornamental fish (*B. rerio*), indicated by the increased length and weight of the fish. Though copepods are easily digested by the fishes and high in nutrients, copepods enriched with different phytoplanktons could be given as feed for fishes than the normal feeds.

Figure 1: Collection site – Muthanna lake,Coimbatore.

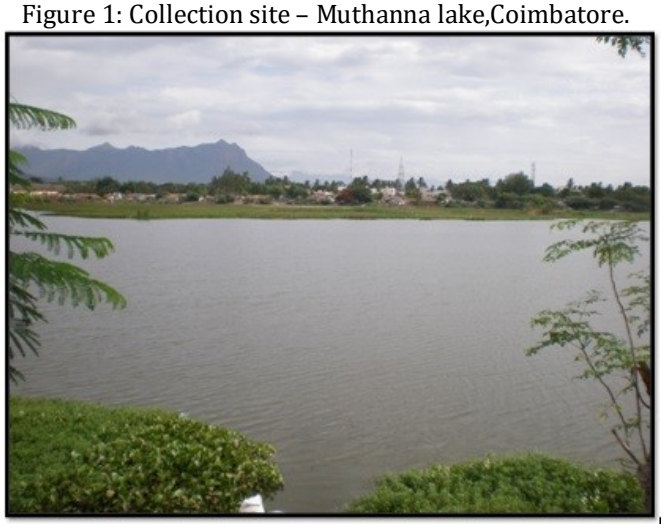


Figure 2: Chlorella culture

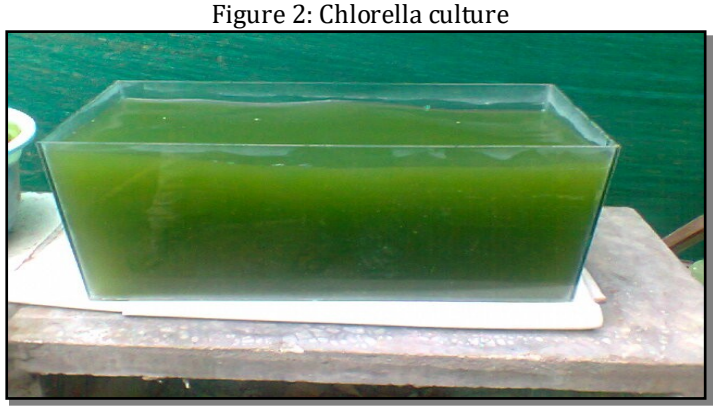
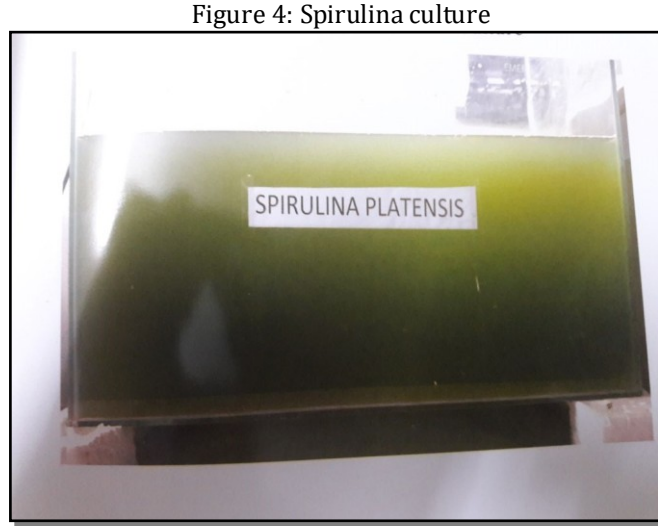


Figure 3:Azolla culture

Figure 4: Spirulina culture



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