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# Algal Diet Impact on Reproductive Performance in Freshwater Copepods: A Case Study

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## ABSTRACT

*Aquaculture is the most significant growth component of the Indian fisheries industry interest in freshwater fish culture has dramatically increased in the commercial fishery. The main bottleneck for fry production in these fishes is associated with larval feeding. It is well accepted that, many copepods are a valuable source of food for fish larval rearing although they are not often used in aquaculture industry. There has been no experimentation to measure the effect of algal diets for the aquaculture of copepods. Therefore, experiments were conducted with local copepod (M. hyalinus) under lab conditions to effect of algal food type on population density, fecundity and egg hatching succession. We used different algal diets for the present study and the experiment was lasted for 12 days at optimum temperature and salinity. The triplicates were made for each treatment. The result showed significant effect on egg production and hatching rate on small copepods as short generation time and high reproductive potential make the use of this copepod as promising as live feed in aquaculture practices.*

***Key words:*** *Algae, copepod, reproductive performance, live food, biochemical, population density*

## INTRODUCTION

The hatched fish larva requires a source of live food very soon after the onset of exogenous feeding (Schipp 2001). It is notable that artemia and rotifers does not always promote optimal larval growth since these live preys may contain an inadequate fatty acid profile and inappropriate size (Sargent *et al*. 1999; Olivotto *et al*. 2003). The identification of alternative food sources revealed that copepods have potential in freshwater aquaculture. 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 larval stages in the fishes. Copepods for aquaculture purposes have been fed a variety of microalgae species.

These microalgae have been fed alone or combined. Each microalga has a different nutritional content and cell size and little is known about the nutritional requirements of copepods. The effects of various dietary microalgae have on culture parameters have been evaluated with many copepods including *Acartia tonsa* (Støttrup *et al*. 1999), *Gladioferens imparipes* (Payne and Rippingale 2000), *Pseudodiaptomous euryhalinus* (Cruz, *et al*. 2009) and *Pseudodiaptomus pelagicus* (Ohs, *et al*. 2010).

In India works related to experimental biology of copepod is very meager and single study is available as to the optimum microalgal diet conditions for the culture of copepods (Rajkumar 2006). Identification of an optimal microalgal diet, which is easy to culture the copepod, is paramount to the successful culture of fishes in aquaculture. The present study examined which microalgae species is suitable to increase the survival, population density and fecundity of selected species population in culture medium under laboratory conditions.

## MATERIALS AND METHODS

### STUDY AREA:

Algal species stock cultures were obtained from local ponds and cultured at 25 °C, normal salinity and 14 L: 10 D light regimes and fertilized with f/2 medium (Guillard and Ryther 1962). The algae were harvested during the log phase (approx. 35,000 cells/mL) for feeding to the copepods. The Copepod samples were collected from the local pond waters near University Deapartment of Jai Prakash University, Chapra during early in the morning using plankton net with 158 m mesh.

### SAMPLE COLLECTION METHODOLOGY:

The collected samples immediately transported to laboratory and thoroughly rinsed to reduce the contamination from other zooplankters. The desired copepod species were identified under microscope using the standard key (Kasturirangan 1963). Thereafter, 100 individuals of each species were isolated and stocked separately in a fiberglass tank of 10 litre capacity with gentle aeration. The water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained in the ranges of 26-30$^0$C; 28 – 32%; 7.5 - 8.5; 5.0 - 7.5 ml/l respectively (during rearing period) fed with a daily ration of algae in the concentration of 20,000 cells/ml. The copepod cultures were harvested at every 12 days by gentle siphoning. Finally the adult male and female copepods were used to restart stock culture. The observations were made for copepod survival and population density under controlled laboratory conditions. Samples of 1ml was taken and counted for the different stages of copepods for average results.

The effect of algal type on copepod survival were determined by experimentation on ten individuals maintained separately in glass jars for each feed. They were transferred daily to a new jar with freshly filtered pond water and feed. The daily mortality was recorded carefully. The experimental sets were maintained at 28 ± 2$^0$C and 30 ± 1 ‰ till the death of all animals. The separate experiment was maintained to examine the effect of algal type on population density, egg production and hatching success of selected copepod species. All experiments were carried out using 1000 mL glass bowl that were placed under similar culture conditions as described for the stock cultures. The feeding rations of the algal diets used in this experiment were 20,000 cells/ml as described by Knuckey, *et al*. (2005). At the beginning of the population density experiment, 10 adult *P. parvus* were stocked into each triplicate culture glass bowl. Adult copepods were separated from the stock culture by draining culture water through a 180 m sieve. The copepods caught on the mesh were then immediately placed in a petri dish with a small amount of pond water. After 12 days, all the contents of each bottle were drained through a 38 m sieve and the total number of nauplii, copepodites and adults of selected species retained were fixed in 5 % formalin solution. The counting of copepod nauplii, copepodites and adults were made using a Sedgewick Rafter counter and a high-powered microscope.

For the egg production and hatching experiment, the male and females of copepods were isolated and kept in beakers containing filtered pond water and incubated for 24 hours. Thereafter, the egg were drained onto a 38 m sieve, which were then rinsed and placed in fresh water for 2.5 min to kill all post-egg-stage copepods. The eggs were then returned to pond water and the number of eggs per replicate was counted under a high powered microscope before being placed in 2 ml of water in sealed specimen containers for 48 hours incubation under identical conditions. In last, the number of un-hatched eggs per replicate was counted and the hatching rate (%) was subsequently determined.

## RESULTS AND OBSERVATION

The algal diets used in this study showed difference in biochemical constituents and accordingly might affect on metabolism, biology and population dynamics of copepods in freshwater aquaculture (Table 1).

The survival of *M. hyalinus* recorded in the present study was not significantly different among the diets treatments. The survival rate was 90% for about 3-9 days 62% observed

on 15th day and 22% on 18th day with *Spirulina* diet indicating its high value for freshwater copepods. There, *Chlorella* also showed higher survival rate, but *Azolla* and *Scendesmus* resulted the poor survival during study period (Fig1).

**Table 1:** Biochemical composition of algal diets used in experimentation for reproductive performance in *M. hyalinus* under laboratory conditions

| Algal sample | Protein (mg/g) | Carbohydrate (mg/l) | Lipid (mg/g) |
|---|---|---|---|
| Spirulina | 1438±312 | 1399±260 | 829±308 |
| Chlorella | 318±16 | 1720±70 | 822±29 |
| Azolla | 964±73 | 2458±327 | 944±63 |
| Scendesmus | 172±64 | 1350±28 | 640±21 |

A significant difference in egg production was detected within each diet treatment. The maximum egg production of 37.33 ± 2.21 eggs/female/day was achieved with *Spirulina* feed which was more than three times higher than that of the *Scendesmus.* The mean percentage of egg production was significantly different among the diet treatments The highest mean hatching rate (35.56 ± 1.40) was recorded for *Spirulina* and the lowest hatching rate (7.23± 0.51) noted at *Scendesmus* fed copepod (Table 2 and Figure 2).
The population density of *M. hyalinus* was significantly affected from different algal diets during 12 day experimentation. Mean population density of this copepod showed maximum population with *Spirulina* somewhat followed by *Chlorella* diet, whereas poor increment in population was observed in copepods fed with other two algal diets (Figure 3). The population dynamics for copepod life stages were also in agreement with population density during the study period (Figure 4).

**Fig. 1:** Survival of *M. hyalinus* after hattching in diffferent medium of algal diets

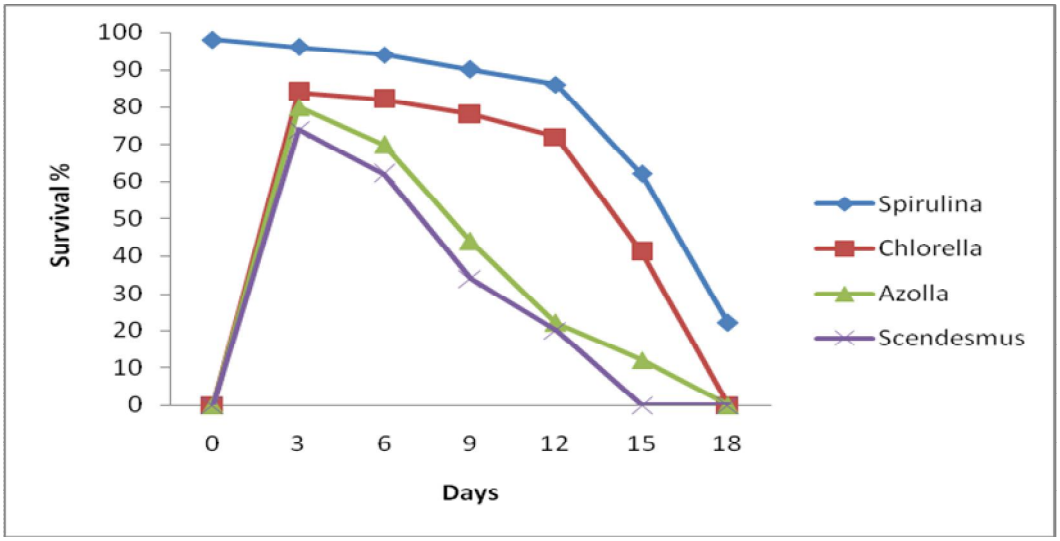



**Table 2:** Mean±SD of egg production, hatching rate and percentage egg hatching for *M.hyalinus* fed the dietary algae in culture medium under laboratory conditions

| Algal diet | Egg production rate (Eggs/female/day) | Hatching rate | Hatching percentage |
|---|---|---|---|
| Spirulina | 37.33±2.21 | 35.56±1.40 | 94.62±2.72 |
| Azolla | 26.21±1.62 | 23.20±1.42 | 86.58±0.54 |
| Chlorella | 19.66±0.52 | 14.00±0.96 | 61.24±3.43 |
| Scendesmus | 12.31±1.15 | 07.23±0.51 | 49.52±4.13 |

**Fig. 2:** Production rate % of *M. hyalinus* with different algal diets

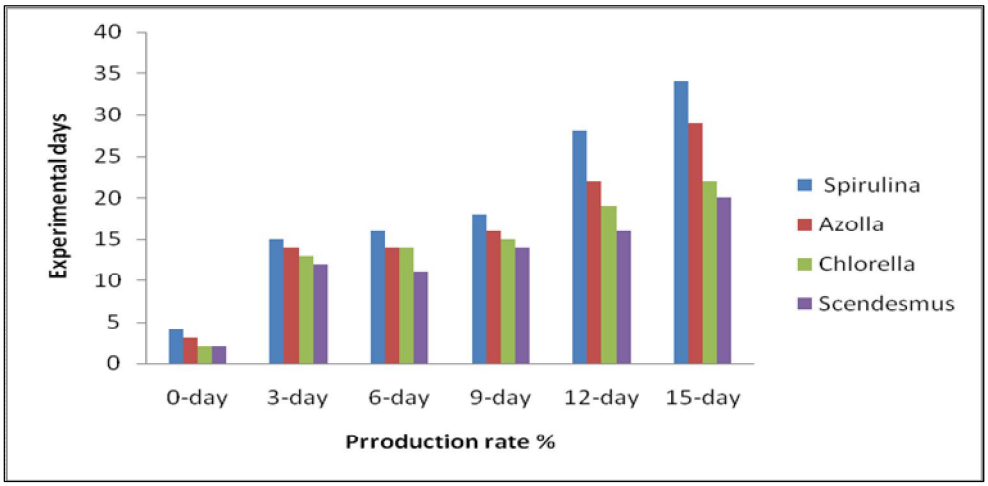



**Fig. 3:** Mean total population of *M. hyalinus* cultured for 12 days on algal diets under laboratory conditions

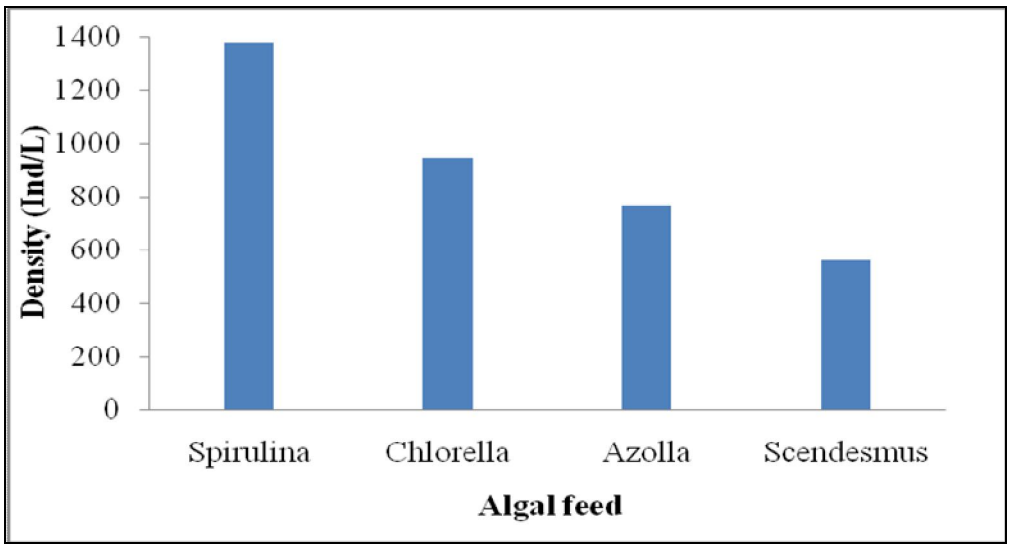
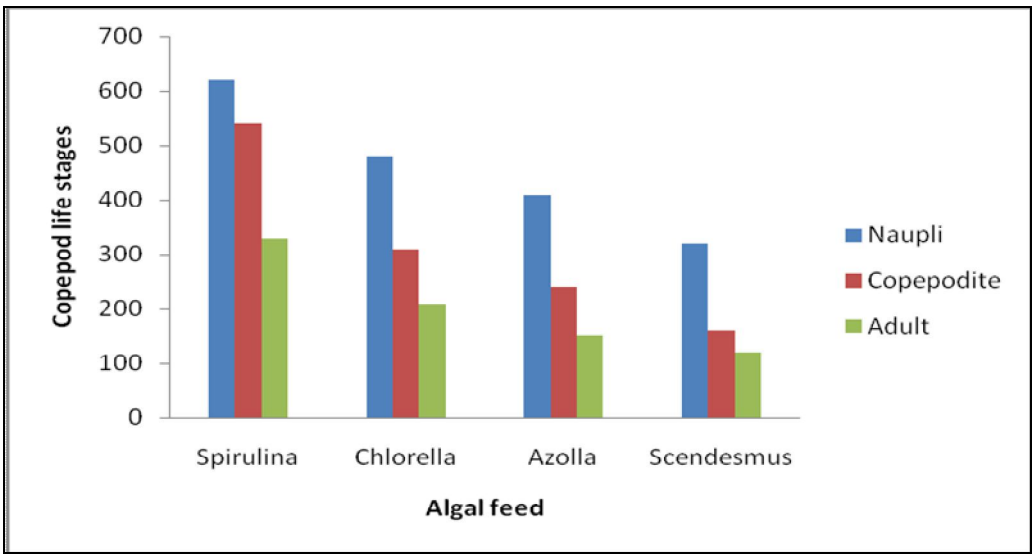



**Fig. 4:** Mean no. of life-stages cultured for 12 days on algal diets under lab conditions



Most notably, the number of nauplii and copepodites was markedly higher for the *Spirulina* followed by *Chlorella* diet treatment whereas the nauplii production was very low for the *Scendesmus* in the present study (Figure 4).

## DISCUSSION

The aquaculture industry develops the need for higher density cultures of small prey items suitable for freshwater fishes. In these circumstances, the culture of copepods such as the *M. hyalinus* of the present study may be enviable.

The present result showed that the first-rate survival was observed in copepod fed with the diet of *Spirulina* may be due to the favorable size of the prey (Shrivastava, *et al*. 1999). copepods had a less survival in *Scedesmus* feed may be due to the less consuming capability of copepods on filamentous algae and also the mouth parts of copepods are not facilitating the capture of larger food organisms which is similar to earlier reports of (Perumal, *et al*. 2000).

The findings of this study have shown clear effects of diets on those parameters related to the productivity of copepods. Egg production is one of the principal factors determining population density in copepods, and it has been linked to the quality of diet (Uye 1981). Meanwhile, the highest egg hatching rate (95.52%) was obtained from the diet of *Spirulina* and *Chlorella*. It has been reported that *Chlorella* and *Isochrysis* have been clearly successful as a food for rearing copepod species (Cruz, *et al*. 2009). In our study *Chlorella* gave second highest population density than other diets. Similarly Lee, *et al*. (2006) suggested that the *Tetraselmis* sp. and *Isochrysis* sp. diets could be the most favorable diet for mass culture of *P. nana.* The present study indicated that *Scendesmus* results to low density and hatching rate is in agreement that larger algal species may not prepare copepod due to the less consuming capability (McKinnon *et al*. 2003).

## CONCLUSION

It is worth noting that, this study was conducted at laboratory scale over relatively short period of time. However, it clearly served the purpose of identifying the optimal microalgal diets for culturing copepod *M. hyalinus* which are likely to be applicable in larger-scale cultures. The use of this zooplankton as fish-food is best than other natural and artificial diets.

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