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# EXPERIMENTAL EVALUATION OF DIETS FOR CULTURE OF A POTENTIAL LIVE FEED, *Euplotes* Sp. (PROTOZOA, CILIOPHORA)

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**AUTHOR'S CONTRIBUTION**

The sole author designed, analysed, interpreted and prepared the manuscript.

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## ABSTRACT

Ciliate protozoans such as *Euplotes* which are seen among the mass cultures of rotifers have the potential as a live feed for larvae and juveniles of the aquaculture species in hatchery operations. Free living ciliates are potential natural food of many fish larvae because of their smaller size. To obtain the culture of *Euplotes* sp., four diets were tested 1) Microalgae, *Nannochloropsis oculata*, 2) *Isochrysis galbana*, 3) Equal proportion of *N. oculata* and *I. galbana* and 4) Baker's yeast (*Saccharomyces cerevisiae*). The ciliates were inoculated at a concentration of 50 cells $mL^{-1}$. On day 5, *Euplotes* density reached highest in the groups fed the baker's yeast (14600.00 ± 409.88 cells $mL^{-1}$) in comparison with the other three media *N. oculata* (933.3 ± 659.29 cells $mL^{-1}$ ); *I. galbana* + *N. oculata* (966.67 ± 806.64 cells $mL^{-1}$ ) and *I. galbana* (333.33 ± 103.28 cells $mL^{-1}$ ). Six days culture is crucial because most of the marine fish larvae start their feeding by 5-7 days of post hatching. It was observed from the results that Baker's yeast is highly significant ($P$<0.01) diet of *Euplotes* sp. in mass culture.

**Keywords:** Live feed; *Euplotes*; aquaculture; microalgae; bakers yeast.

## 1. INTRODUCTION

In fish hatchery operations, the main challenge is the development at the larval stage. This is the most delicate stage presenting elevated mortality, therefore requires more care, especially at the beginning of exogenous feeding. The type of zooplankton to be fed as a first food to fish larvae, i.e., in the transition from endogenous (yolk reserves) to exogenous feeding, is determined mainly by its nutritional quality. Traditional live prey utilized on aquaculture such as artemia naupli and rotifers cannot be provided to those species that have a smaller size than most larvae, hence they need a smaller food appropriate for their nutritional requirements. This study attains its relevance in the scenario where there is an extreme

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need to increase the culture of such organisms both quantitatively and nutritionally. Ciliates are important as the prey for the first feeding stages of larvae and juveniles of which are ideal live food organisms for their size, swimming pattern and nutritional content [1] and hence are known as "living capsules of nutrition". The first feed required for larval fish is usually determined by their mouth size [2]. Larval stage is very crucial for the optimal development of marine fish [3]. According to Lavens and Sorgeloos, small sized mouth, under developed organs, are the limiting factors in proper food selection for the first feeding period [4].

Selection of appropriate live feed for the first feeding of larvae is important in the case of fishes like salmon alevins which already have developed digestive tract, with functioning enzyme system permitting the digestion of feeds on first feeding. But fishes like Gilthead sea bream (*Sparus aurata*), red snapper (*Lutjanus campechanus*) larvae and most of the other fish larvae lack a functional stomach and possess only a short digestive tract with few functional digestive enzymes at the onset of first feeding. Live food organisms usually have a much better contrast than artificial feeds and generally create a triggering effect in larvae by their continuous movement. This allows an enhanced perception by the first feeding larva and also ensures a good distribution of food items in the water column, which in turn facilitates more availability of food for the developing larvae [4].

Smaller feed items such as ciliated protozoans can be a suitable first food for larval fish. In case of red snapper, *Lutjanus campechanus*, with weak larvae having relatively smaller mouth size, limited yolk reserve, and late development of functional mouthparts, naked ciliates may serve as an important food for fish larvae [5]. Some experiments demonstrated that the high ciliate densities augment larval survival after hatching until 4-6 days, suggesting the importance of ciliates as initial food for larvae [6]. Due to their higher metabolism and growth rates [7] compared to autotrophic, heterotrophic protists have a higher biomass conversion potential [8] and are more nutritionally important as well as qualitatively and quantitatively vital as diets for copepods [9]. Ciliates may be considered as important first feed for fish larvae which are alternative source to copepod nauplii because ciliates are smaller and most abundant than copepod nauplii in coastal waters in addition to being dominant [10].

Ciliates are imperative components of the microbial loop, and are often responsible for consuming the majority of primary production and bacterial production in some habitats [11]. Food for fish larvae is usually determined by mouth size of the fish, its prey size and fish's ability to prey. This proceed to suggest that small prey enriched with nutritional value, such as ciliates and dinoflagellates protozoa would be more suited [12]. Ciliates are capable of converting the lipids obtained from their food to polyunsaturated fatty acids (PUFAs) which proves vital to copepod growth and reproduction. Thus, small sized food, comprising of super small rotifers, copepod nauplii or ciliate protozoa such as *Euplotes* sp will encapsulate the food demands of fish larvae.

*Euplotes* is a single cell animal measuring around 50-75μm in length, that can walk about on surfaces, swim as needed and can engulf relatively large cells. *Euplotes* is a very diversified and cosmopolitan genus, with numerous species frequently observed in both marine and freshwater samples [13,14] *Euplotes* sp. is the only ciliate known to produce a sterol [15] and had a good fatty acid profile containing high proportion of unsaturated fatty acids. Sterols are indispensable for maintaining cell membrane fluidity [16]; thus, they occur in almost all higher organisms. As most of the juvenile fish mouth is ventrally placed, they usually feed on ectoparasites or zooplankton like *Euplotes* sp. [17]. *Euplotes* sp. are viewed as a good source of food for marine larvae of fish like gobies, and have also proved to possess many positive qualities such as those discussed above like the case of size, nutrition, slow rate of movement and auto-digestion.

This study primarily aims to evaluate the effect of different diets in the culture of *Euplotes* sp. and, thus, determine the diet yielding the best growth performance. Further, the potential to make use of *Euplotes* sp. as first food for fish hatcheries is also discussed.

## 2. MATERIALS AND METHODS

The ciliate protozoan *Euplotes* sp. was isolated from rotifer *Brachionus* sp. culture kept at 28 ± 1°C water temperature and 30 ± 2 ppt salinity was sieved through 45 μm filter for rotifers, and the collected culture was filtered through 18μm filter to retain *Euplotes* sp. These were thoroughly washed by passing 3 liters of water through the mesh for the removal of any debris if present. Ciliates were then concentrated and isolated from the mesh into 100 mL beaker with fresh sea water. Individuals mean sizes were nearly about 81μm length and 41μm width.

Growth of *Euplotes* sp. were assessed in a 6- day feeding trial using four different food sources having three replicates each. For which, twelve beakers were

taken and to each beaker 250 mL sea water was added. *Euplotes* sp. was inoculated at a concentration of 50 numbers $mL^{-1}$. Throughout the experiment continuous aeration was provided in all beakers.

Feeding trials for *Euplotes* sp. growth was done with four food sources:

- *Nannochloropsis oculata*
- *Isochrysis galbana*
- Equal proportion of *N. oculata* and *I. galbana*
- Baker's yeast *Saccharomyces cerevisiae*

Each diet was tested with three replicates. For the culture of microalgae *N. oculata* the water was previously chlorinated and de-chlorinated with sodium thiosulphate and the microalgae were filtered using a 5µm mesh. Throughout the trial the microalgal concentration was monitored and kept at $10.8 \pm 0.2 \times 10^6$ cells mL-¹. Microalgae *N. oculata* were cultured in modified Walne's medium under constant light and aeration and then supplied to the protozoan culture. The culture of *I. galbana* was done in Walne's medium. Throughout the trial the concentration of *I. galbana* was kept at a concentration of $10.3 \pm 0.2 \times 10^6$ cells mL-¹. Standard method was followed for the yeast culture preparation. The yeast extract will typically contain all the amino acid necessary for its growth. Also, the concentration of yeast was kept at $2.4 \pm 0.6 \times 10^6$ cells mL-¹. The experimental cultures were kept under 24 hr light, 28 ± 1°C, salinity at 30 ± 2 ppt, constant aeration, and no water renovation was done.

Microalgae and yeast cell densities were determined using hematocytometer, viewed under a Transmission Light Microscope (Leica DM 1000 with camera Leica DFC 290) with magnification 40x - 1000 X. Here 0.5 mL algal medium was diluted with water and the count was taken.

Ciliate density was estimated from a 10 µL sample, which was taken in a slide after fixation with 0.05% formalin and was converted to 1mL. The ciliate protozoan (*Euplotes* sp.) is not always seen in the water column as it prefers to move on the substrate. To ensure reliable counting, each beaker was homogenized with strong aeration prior to sampling. All counting was performed in triplicate.

The diet yielding the best growth performance of *Euplotes* sp. during the trial was determined by using One Way ANOVA in Statistical Package for the Social Sciences (SPSS version 20) using Duncan's Multiple Range Test. The results are expressed as the means ± S.D of the data.

## 3. RESULTS AND DISCUSSION

Population density of *Euplotes* sp. were evaluated using different diet sources in terms of increase in number per ml with three replicates for each day. During the first day of culture, *Isochrysis galbana* had a higher population density with 200±154.92 individuals $mL^{-1}$ followed by *Nannochloropsis oculata* with 166.67±103.28 ind. $mL^{-1}$. Baker's yeast and mixture of *I. galbana* + *N. oculata* kept the same density.

On the second day there was a drastic increase in the population fed with baker's yeast from 133.33±51.64 to 2,100±89.44 ind. $mL^{-1}$. On day 3, there was no significant increase in the population density fed with baker's yeast compared to the second day but were statistically higher than the cell density in the culture fed with *I. galbana*, *N. oculata* and mixture of *I. galbana* + *N. oculata*. A drastic change in the population density was found on the fourth day of the culture. The culture fed with baker's yeast had an exponential population growth (10,433.33±581.59 ind. $mL^{-1}$ ). The growth of population fed with N. oculata (933.3±659.29 ind. $mL^{-1}$) and *I. galbana* + *N. oculata* (966.67±806.64 ind. $mL^{-1}$) had doubled. But culture fed with *I. galbana* had no much progress in their population density (333.33±103.28 ind. $mL^{-1}$).

On the fifth day also the growth rate of the population density in the baker's yeast maintained the same level of growth and reached 14600.00±409.88 ind. $mL^{-1}$, which was the highest population density of *Euplotes* sp. during this trial. But the population density of culture fed with *I. galbana*, *N. oculata* and *I. galbana* + *N. oculata* was much lower than those fed with yeast. On day 6 of trial the population density in the baker's yeast came a bit down to 14,033.33±811.58 ind. $mL^{-1}$. But the other three trials had a slight increase of population density. Thus, the result suggests that the baker's yeast is the best suited feed for the culture of *Euplotes* sp. and the group fed with *I. galbana* did not show any significant growth rate for *Euplotes* sp. Table (1) shows the comparison of population growth of four groups of treatment using One Way ANOVA (Duncan's Multiple Range Test). Four treatment registered significant ($P < 0.01$) difference between treatments in each day of culture except in the first day. In all treatments Baker's yeast showed significantly higher cell growth compared to other treatments, whereas three microalgae treatments do not showed significant difference each other.

Fig. (1) reveals the cell growth in all the four-culture medium during the culture period. It clearly shows the significance of baker's yeast for the cell culture. Even though on first day of the trial it shows very less cell density, remaining days shows an excellent progress. From the fourth day onwards, there is an exponential growth in the cell density. The figure clearly shows that the peak value is attained on the fifth day. But on the next day the cell density has shown a considerable decrease.

Population growth in different treatment except baker's yeast during the culture period is shown in Fig. (2). From the figure, it is difficult to predict the culture medium favorable for the growth of *Euplotes* sp. At the same time, the culture medium unfavorable for the growth of *Euplotes* sp. could be predicted as *I. galbana*, for they showed decreased cell density throughout the trial. From the other two mixtures *N. oculata* shows an increase in the cell density on fourth day of trial. *N. oculata* is highly rich in their nutritive value. So *Euplotes* sp. can be first cultured on baker's yeast, so that the population density of cells can be increased and then be treated with *N. oculata* by which the nutritive value tends to show a considerable rise.

**Table 1. Analysis of variance (One Way ANOVA) comparing four groups of treatment**

| Day of Culture | | Baker's yeast | *N. oculata* | *I. galbana* | *N. oculata* + *I. galbana* | F value |
|---|---|---|---|---|---|---|
| Day 6 | Mean | 14033.33b | 366.67a | 600.00a | 700.00a | 718.611** |
| | ± SD | 811.58 | 206.56 | 558.57 | 709.93 | |
| Day 5 | Mean | 14600.00b | 400.00a | 366.67a | 466.67a | 3457.983** |
| | ± SD | 409.88 | 236.64 | 273.25 | 225.09 | |
| Day 4 | Mean | 10433.33b | 933.33a | 333.33a | 966.67a | 394.015** |
| | ± SD | 581.95 | 659.29 | 103.28 | 806.64 | |
| Day 3 | Mean | 2733.33b | 333.33a | 233.33a | 300.00a | 498.519** |
| | ± SD | 103.28 | 206.56 | 103.28 | 89.44 | |
| Day 2 | Mean | 2100.00b | 366.67a | 200.00a | 233.33a | 217.405** |
| | ± SD | 89.44 | 258.20 | 0.00 | 136.63 | |
| Day 1 | Mean | 133.33a | 166.67a | 200.00a | 133.33a | 0.611 |
| | ± SD | 51.64 | 103.28 | 154.92 | 51.64 | |

*** P < 0.01; a,b – Means with same superscript within each day of culture do not differ each other (Duncan's Multiple Range Test)*

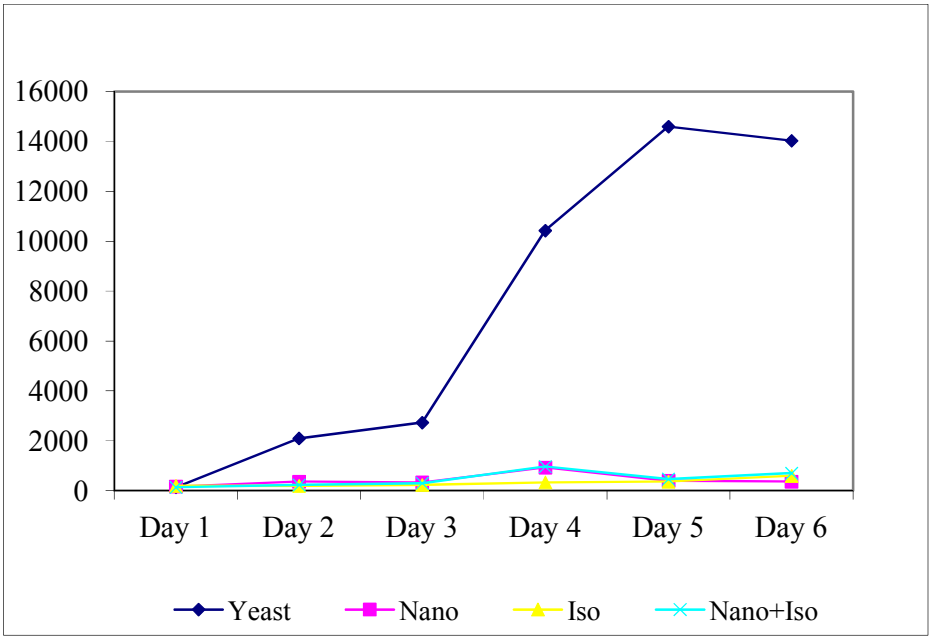


**Fig. 1. Population growth in different treatments during the culture period**

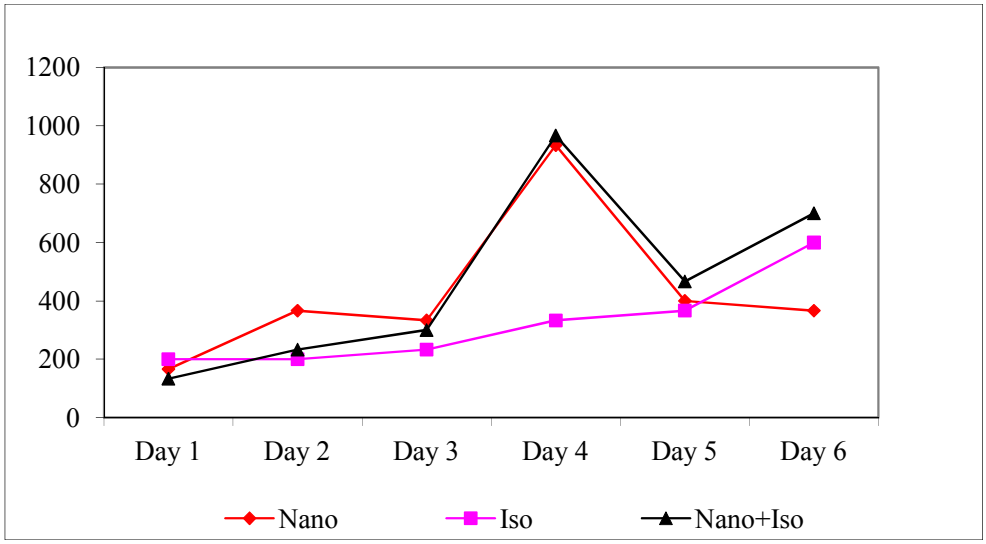


**Fig. 2. Population growth in different treatments except yeast during the culture period**

Live food organisms are very vital for the better survival of fish larvae with small mouth size. Therefore, an appropriate live food like small ciliates (*Euplotes* sp.) must be provided. They are considered to be much more resistant than other live food organisms. From the studies, it is clear that the baker's yeast is the best medium for culture of *Euplotes* sp. than the popular algal feeds like *Isochrysis galbana* and *Nannochloropsis oculata* and their combination. In this case the *Euplotes* sp. is found growing to maximum population on the fifth day of culture. In fin fishes, the fish larvae start its feeding by 5-7 days of post hatching. Until that time, larvae like salmon grow using their yolk [18].

So, by observing the day of larval hatching, the live feed *Euplotes* sp. can be grown to their maximum density for feeding. Yeasts can be mass-produced at relatively low cost. But some reports suggest that the *Euplotes* sp. fed with baker's yeast had less nutritive value compared to microalgal feed. The nutritional value and digestibility of yeast-based diets can be improved by the addition of essential nutrients and the chemical treatment of the yeast cell wall [4]. The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Micro-algae can be considered as a rich source of ascorbic acid [4].

Therefore, enriching the *Euplotes* sp. with microalgae after being grown in baker's yeast (Fig. 3) will result in higher survival of marine fish larvae. This study also indicates that *Euplotes* sp. can survive and multiply using nutritionally rich microalgae like *N. oculata* and *I. galbana* within shorter duration. So, the yeast fed *Euplotes* sp. culture can be highly enriched easily by growing them further in micro-algal media for one day. It is also favorable to culture the *Euplotes* sp. in baker's yeast and then in the mixture of microalgae.

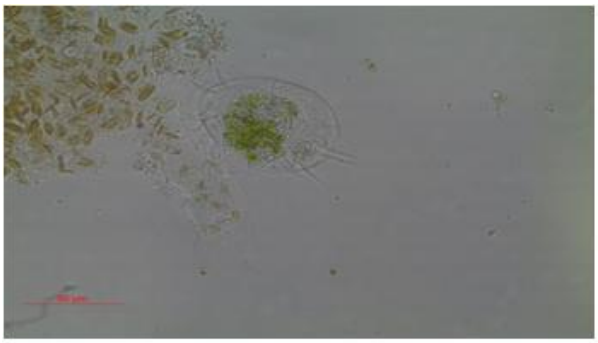

**Fig. 3. Yeast fed *Euplotes* sp. after enrichment with *N. oculata***

The density of *Euplotes* sp. fed with *N. oculata* was found satisfactory. Even with a growth performance lower than the group fed with baker's yeast, *N. oculata* in the culture of *Euplotes* sp. may be interesting when the intention is to feed these organisms to fish larvae, as it is enriched with polyunsaturated fatty acids. They are of nutritional value for marine fish larvae and juvenile stages of molluscs [19]. *I. galbana* is also used as a marine culture feed due to its high content of protein and polyunsaturated fatty acids (PUFAs) [20,21]

Like all the other organisms, culturing *Euplotes* sp. also has advantages and some disadvantages. Some of the advantages are the small size, fast growth, high culture densities, and resistance to high concentrations of ammonia in water. Also, the possible disadvantages may be that these ciliates need to be enriched before supplied to fish larvae, and predation may be hindered by their benthic habit [20,22,23]. Sorgeloos *et. al*. [24] reported a strong correlation between the dietary EPA content and survival, and between DHA and growth of the Asian sea bass larvae. But disadvantages could be overcome by the early said methods like enriching with other supplements like microalgae or any other artificial feeds.

Many live feeds are widely used such as rotifers (*Brachionus* sp.), Artemia nauplii and others which are of no match to smaller and not ideal for fish larvae like red snapper, cleaner goby (*Gobiosoma evelynae*) and groupers with much smaller mouth size. The present study points to the feasibility of intensive culture of the ciliate protozoan *Euplotes* sp. which are very small compared to the popular live food organisms. Soft bodied, microscopic, and nutritionally rich ciliates are more preferred by larval fishes. The culture of *Euplotes* sp. was done in different culture medium like baker's yeast, *Nannochloropsis oculata*, *Isochrysis galbana*, and a combination of *N. oculata* + *I. galbana*. *Euplotes* sp. fed with baker's yeast showed high population density which were significant ($P<0.01$). Growth rate of *Euplotes* sp. fed with baker's yeast were found to be 15 times greater when compared to those fed with other feeds. *Nannochloropsis oculata* and *Isochrysis galbana* are also good food for the survival and multiplication but baker's yeast is proved as the best media for mass production. *Euplotes* sp. can be cultured easily in large scale for commercial hatcheries using relatively cheaper method proved here as using baker's yeast. *Euplotes* sp. thus produced, should be enriched with a suitable medium. *Nannochloropsis oculata* and *Isochrysis galbana* can be used for the final enrichment of the mass culture produced using baker's yeast.

## 4. CONCLUSIONS

The present study demonstrates the feasibility of an intensive culture of the ciliate protozoan *Euplotes* sp., to be used as a live feed in lieu of others, for its growth in a suitable medium. The culture of *Euplotes* sp. was done in different culture medium like baker's yeast, *Nannochloropsis oculata*, *Isochrysis galbana*, and a combination of *N. oculata* + *I. galbana*., but all the other three except baker's yeast medium was not found much efficient. Enhanced biomass growth was obtained by feeding baker's yeast i.e., 15 times greater when compared to those fed with commonly used mediums in rotifer culture. The result suggests that the baker's yeast is the best suited medium for the culture of *Euplotes* sp. as the groups fed with other feed did not show any significant growth rate.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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