



# International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2016; 4(6): 05-10

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www.fisheriesjournal.com

Received: 02-09-2016

Accepted: 03-10-2016

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## The effect of different feeds such as *Chlorella vulgaris*, *Azolla pinnata* and yeast on the population growth of *Daphnia magna* commonly found in freshwater systems

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### Abstract

The effect of different concentrations of Phytoplankton (*Chlorella vulgaris*, *Azolla pinnata*, and *Saccharomyces cerevisiae*) for fed with *Daphnia magna* on Survival, population and Growth rate for 20 days, algae cells are harvested, dried, powdered and used as feed and both species are easily maintained in culture. The experimental was E1, E2 and E3 small freshwater Zooplankton organisms (*D. magna*) fed with mix phytoplankton Feed different Concentration (%) 1, 2, 3, 4 and 5. The Experimental *Daphnia magna* on Survival, population and Growth rate Biochemical composition, of the three different species analyzed were significantly ( $p < 0.05$ ) higher in (E1) 4% concentration in *Chlorella vulgaris*, when compared to other experimental groups. The experiment results have shown that the feed chlorella resulted in maximum growth and increase in *Daphnia* population compared to other feeds like *Azolla* and yeast, we conclude that phytoplankton *Chlorella vulgaris* 4% was the best algal concentration food for the *Daphnia magna* culture.

**Keywords:** *Daphnia magna*, phytoplanktons, growth rate, population

### 1. Introduction

Cladocerans are Zooplanktonic organisms belonging to the class Crustacea of phylum Arthropoda. Majority of the Cladocerans occur in fresh water followed by brackish water and marine habitats. These organisms are fleshy and non-thorny and hence are used as forage organisms to feed larvae and post larvae of commercially important fishes and prawns. These Cladocerans are quite popular and inexpensive live food in aqua hatcheries. *Daphnia* are small fresh water crustaceans commonly called "Water fleas". Animals such as *Daphnia*, *Moina* and *Ceriodaphnia* can be easily cultured. Cladocerans respond rapidly to changes in the environment notably food type, quality and quantity, temperature, competition and predation [36, 11, 16]. The most important and commonly affecting factor for Cladocerans is the food concentrations both in the field and under laboratory conditions [22]. However, it is unknown whether the response of planktonic and littoral Cladocerans to increasing food concentrations is similar [29]. Parameters such as survivorship, life span, growth rate provide valuable insight into suitability of the ambient conditions for the zooplanktons [37]. For example in *Daphnia*, the first few broods are important for contributing to the population growth when compared to older class groups which normally experience mortality from predators in nature [22]. They become especially abundant in temporary waters which provide them optimal growth condition for brief period. The upper size range for edible particles varies directly with body length, both between and within species of *Daphnia* [8]. Green algae are commonly encountered food source for *Daphnids* [36]. The brood pouch where the eggs and embryos develop is on the dorsal side of the female. In *Daphnia*, the brood pouch is completely closed. Periodically, *Daphnia* molt or shed their external shell [21]. The reproductive cycle of *Daphnia* has both sexual and asexual phases. In most environments, populations consist exclusively of females that reproduce asexually. The stimuli for the switch from asexual to sexual reproduction in populations of *D. magna* include factors like food deficiency, oxygen starvation, and high population density. *Daphnia* health and reproduction depends on how often they feed, how much energy they must put into finding and locating food Ryther J.H., [3]. Mass production of the fresh water *D. magna* is possible by the use of appropriate algal band supplemented with baker's yeast (*S. cerevisiae*). Since algal cultivation under control conditions in laborious, time consuming and expensive, other recent improvements have been the development of condensed phytoplankton products, such as fresh water chlorella, for *Daphnia* food and the development of high density *Daphnia* mass culture technology using condensed phytoplankton products. Alternative food yeast has been used for mass culture of *Daphnia*. These live feeds usually contain for large fraction of soluble intact proteins [15, 17, 6] and it has been suggested that soluble proteins are more available for larval digestion and absorption than are insoluble proteins [7].

Daphnia have a protein content of around 50% dry weight and a fat content of 20-27% for adults (4-6% for juveniles). As with most live foods, they are what they eat and so vitamin and other formulas are available as food for filter feeders like Daphnia will give them certain food values or an increase in a given fatty acid, for example (H.U.F.A or Highly unsaturated Fatty Acids). Algae are the main sources of highly unsaturated fatty acids (HUFA) for zooplankton. The best foods for culturing Daphnia are algae and yeast. For culturing, the algal feeds such as *A. pinnata*, *C. vulgaris* and the yeast (*S. cerevisiae*) are used. The advantages of algae as a food are that, algal feeds are easy to culture and it is an excellent feed for the growth of Daphnia. Yeast is easy to acquire, and there is a minimum of fuss while preparing it for the culture. Industrial yeast is commonly used in Aquaculture, either alive to feed live food organisms or after processing as a feed ingredient [42]. An important link in food chains is that Cladocerans convert phytoplankton, bacteria, and fungi and decompose organic matter into animal tissue that can be used by larger animals. The aim of the study was to test the effect of different feeds such as Chlorella, *A. pinnata* and Yeast on the population growth of *D. magna* commonly found in Freshwater systems.

## 2. Materials and Methods

### 2.1. Collection of Daphnia

The Daphnids were collected from Muthanna Lake, P.N. Pudur, Coimbatore. The collection was done during early morning hours, because maximum numbers of Daphnia species were available only during morning hours. The Daphnids were collected by using plankton net. The collected plankton water samples were passed through the 100 mm mesh size net. So the larger sized species and dust particles will get deposited on the net. The filtered water contains Daphnia and other small species. This procedure can be repeated for 50 and 20 mm mesh size net. Finally, the Daphnia species were separated alone.

### 2.2. Isolation and Fixative Stain of *D. magna*

Isolation of *D. magna* was based on a methodology of Hoff and Snell [18]. The selected live planktons were carefully isolated by using dissection microscope. The species identification was based on the available taxonomical characteristics using Leica Trinocular microscope. At the time many undesired organisms seen along with plankton were carefully removed using a fine tip Pasteur pipette. After identification, the *D. magna* were carefully isolated from the other Daphnis. The analysis of *D. magna* species was completed within 24 hours after the collection. Lugol is a fixative used for staining of Daphnia. Solution A: Dissolved 50g Potassium iodide and 25g iodine in 100 ml boiling Water. Solution B: 25g Sodium acetate was dissolved in 250ml water. When the Solution A cools, mix the two Solutions and stored in a cool, dark place. 1ml of legal fixative was added to the *D. magna* sample. After proper identification, *D. magna* Specimen were observed under micro scope and photographed.

### 2.3. Taxonomical Identification of *D. magna*

For taxonomical identification of live sample taken were less than 50 µm. It was carefully isolated and observed by using microscope. The sub samples were observed in Legal solution and the species were identified up to genera level with the help of standard book (Stein J.R., (editor), [38]. The taxonomical

characters were monitored as outlined by Benzie, [4]. The Species identification study involves various features such as size, Carapace, Claws, Antennae, Head, Post abdomen, Eye, Rostrum of each Specimen. Ocular micrometer was fixed with compound microscope. The size measurements were made by using ocular micrometer.

### 2.4. Culture Vessels and Water

There is quiet number of culture vessels for *D. magna* production. Culture plates with the volume of 50 ml were used. The water collected from the site of sampling and this water was filtered through a Whitman no 1 filter paper. To this an equal volume of unchlorinated tap water was thoroughly mixed. This medium was used for *D. magna* culture studies [35].

### 2.5. Feed Preparation

During the culture period the *D. magna* were fed with inert feeds such as baker's yeast (*S. cerevisiae*) and fresh water algae (*C. vulgaris* and *A. pinnata*). The Baker's yeast was commercially purchased and used for this study. The yeast was dissolved in 100 ml of distilled water and homogenized. The homogenate was centrifuged for 10 minutes. The clear supernatant was decanted and given freshly to the *D. magna* at a concentration of 1 ml/10<sup>6</sup>. From this stock solution different percent concentration of yeast was prepared.

### 2.6. Culture of *C. vulgaris*

The freshwater green algae were cultured in aquarium tanks. The algae require large amount of inorganic fertilizers such as Ammonium sulphate, Super phosphate and Urea in the, ratio of 10:1:1. These salts were dissolved in ground water. After that, Cow dung was mixed with water and filter with the nylon cloth. The filtrate was put into the culture tank, and mixed well. During the culture period the pH was maintained at 8. No aeration was provided, except for stirring of the culture twice a day. Now these tanks looked brown in colour. After 15 to 20 days, slowly colour was changed into green colour. As a result the algae were developed. This cultured alga was prepared by five different concentrations.

### 2.7. Culture of *A. pinnata*

A trough of 25 liters was taken to which a sediment layer of 3cm clay was made. 20 liters of water was poured into the trough. 0.5% of urea was dissolved in the water along with 1 kg cow dung extract. This composition was allowed to stand for 2 days. The trough was kept outdoor in direct sunlight. To this 10 g of *A. pinnata* powder was added. The Azolla growth was monitored daily. After 20 days the Azolla culture were collected and dried in incubator. The dried Azolla was powdered to fine particles. Five different concentrations were made out of the *A. pinnata* powder in distilled water.

### 2.8. Maintenance of Daphnia Culture

The development of culture system was based on the feeding habits of selected Daphnia. Based on the maximum frequency, *D. magna* were successfully isolated. Then it was placed in cultured plate with 50 ml medium. For the isolation of desired Daphnia species, each specimen was first placed in an embryo cup and then transferred to a small beaker. From this, selected *Daphnia magna* species were transferred to Petri dishes for further culture trials. The Daphnia culture was successfully maintained by keeping the right amount of algae and yeast in the water. If too little algae were fed to the animals when they

are hungry, they start the production of male Daphnia and cyst formation. *D. magna* need a consistent supply of feed in the water, allowing them to graze continuously.

### 2.9. Assessment of Population Growth

Experiments were conducted to know the effect of feed types with different concentrations on population growth rate of *D. magna*. The population growth rate was determined on the second day. The effect of various feed concentration was assessed and checked. They had any effect on the Daphnia reproduction and density in culture and assessed for all the parameters, movement of the Daphnia and number of egg bearing females. The medium and diet were changed every other day and the Daphnia density was estimated by counting the number of animals under Leica Trinocular microscope.

### 2.10. Calculation of Growth Rate in Daphnia Culture

The density and growth rate were determined from the average number of individuals in the five replicates after a period of seven days. The maximum population density attained by the Daphnia were also assessed during the period, the instantaneous growth rate was calculated from the equation [28].

### 2.11. Physico – Chemical Characteristics of Water

The following parameters were analyzed in the season of March for habitat water as well as in tap water, used in the laboratory for *D. magna* Culture. The parameters like pH, Salinity, Hardness, Alkalinity, Fluoride and Chloride concentration were estimated by using water testing kit. The temperature was noted by using thermometer and dissolved oxygen was determined by Winkler's method Strickland and Parsons, using starch indicator [43]. Nutritional analysis was Protein, lipid and carbohydrates were determined by the following methods, protein [23], lipids [13] and carbohydrates [32].

### 3. Results

In the present study, the population growth of *D. magna* reared fewer than three different food types and five different concentrations were observed. Table-1 shows the production rate of *D. magna* fed with chlorella at five different concentrations. The production rate of *D. magna* was increased in the 4% concentration showing a maximum production rate of 5.5. In 5% concentration the production rate was 4.2 and found to be decreased. The *D. magna* fed with *A. pinnata* at five different concentrations of the production rate of increased in the 4% concentration showing a maximum production rate of 5.0. After the production rate was decreased up to 4.0 in 5% concentrations. The *D. magna* fed with yeast at five different concentrations was increased in the 4% concentration showing a maximum production rate of 4.1. After that, there is a slight decrease in the production rate of 3.2 in 5% concentrations. Table 2 shows the data on growth rate (in length) of *D. magna* fed with different concentrations of chlorella for the period of 2 days. During the above treatment, the growth rate of *D. magna* was increased in 4% concentration showing a maximum increase 0.123 mm/day. However, the growth rate was decreased in 5% concentration of chlorella. Statistical analysis of the above experiment showed significant at 5% level except in 1% and 4% concentration. The growth rate (in length) of daphnia magna fed with different concentrations of *A. pinnata* and yeast for the period of 2 days. During above treatment, the growth rate of *D. magna* we increased in 4% concentration showing a

maximum increase 0.112 mm/day and 0.092 mm/day. However, the growth rate was decreased in 5% concentration of *A. pinnata* and yeast. Statistical analysis of the above experiment showed significant at 5% level except in 1% and 4% concentration. The Physico-chemical parameters of natural Daphnia habitat and tap water have been depicted in the temperature were recorded high in habitat water (28.2 °C to 26.7 °C). The pH was higher in tap water (7.5 to 7.4), in the present study, most alkalinity was observed in tap water (182mg/L to 174mg/L). Dissolved oxygen level is high in tap water (6.321mg/L to 5.230mg/L). The hardness was found to be high in habitat water (137 ppm) whereas it decreased in tap water (24.3ppm) the salinity was found higher in habitat water (1.60 to 1.50ppt). Figure 1, shows the various biochemical profiles of protein, carbohydrate, and lipids analyzed in different feeds such as *S. chlorella*, *A. pinnata* and yeast the protein level was high in chlorella (48.7%) when compared to *A. pinnata* (21.4%) and yeast (20.5%). The carbohydrate level was high in *Azolla* (46.9%) when compared to chlorella (23.1%) and yeast (42.8%). The lipid level was high in yeast (9.9%) when compared to *A. pinnata* (6.8%) and chlorella (4.6%).

### 4. Discussion

Daphnia is one of the most popular live feed for aquaculture fishes. It is a frequently used food source in the fresh water larviculture (ie, for different carp sps). They have been used extensively to rear larvae and fry [9, 44]. Daphnia includes several species, the largest of which is *Daphnia magna*, shows high reproductively. *Daphnia magna* is mainly used, alive or preserved as food for fish in aquaculture [12, 34]. These are herbivores which mainly fed on micro algae. The herbivorous zooplankton growth rates are sometimes strongly correlated with the mineral and biochemical composition of the phytoplankton they consume reported that food nutrient content influenced the growth rate of *D. magna* even at very low food levels [27, 39]. The purpose of the present study was to analyze the three different types of feeds on the growth and production rate of *D. magna*. Daphnia species reproduce by cyclical parthenogenesis which is characterized by several parthenogenetic generations alternating with sexual generations. Offspring size and number in Daphnid Cladocerans are remarkably sensitive to food conditions [46, 24, 14]. *D. magna* may grow during its whole life cycle [25], may show a distinct plateau [31] or reproduce its body length in later stages [20]. The results of Vijverberg [45] suggest that there is a strong relationship between body length and the food. *D. magna* feeds mainly on various groups of bacteria, yeast, micro algae (both single-cell algae and colonies of blue-green algae consisting of two or three cells), detritus and dissolved organic matter. In this study, the Daphnia were fed with three different types of feed namely *C. vulgaris*, *A. pinnata* and *S. cerevisiae*. This feed may vary for the dietary components of *D. magna*. Populations of Daphnia grow most rapidly in the presence of adequate amounts of bacterial and yeast cells as well as micro algae cells [10]. The *D. magna* being indeed able to feed on other food sources such as protozoans, bacteria and detritus [30] *C. vulgaris* and *A. pinnata* can be cultured in the laboratory for feeding Daphnia. The industrial Baker's yeast *S. cerevisiae*, is used because it is an inert feed which yield a significant production of Daphnia when these inert feed were used, it shows a moderate growth rate in *Daphnia hyaliana* [19]. The reason why baker's yeast has been used for Daphnia is attributable to the combined supplemental nutrition effects of

other micro algae, bacteria and vitamin B12. The phytoplankton (different microalgal sps) such as chlorella is a rich source of ascorbic acid, but show a considerable variability among the different species and different phase of life cycle [26]. A variety of algal cellular constituents are important determinants of food quality for zooplankton; C, N, P, lipids, essential fatty acids (FA), protein and essential amino acids [40]. The Zooplankton growth and reproduction will depend not only on algal quantity, but also on the algal community composition [5]. *A. pinnata* is an aquatic fern which contains 21.4 crude proteins and contain minerals such as sodium, manganese, and zinc which increase the growth rate. The chemical score index showed the potential of *A. pinnata* meal as a good source of protein [3]. Hence, in the present study the productivity of *Daphnia magna* was enhanced using Baker's yeast, *A. pinnata* and *C. vulgaris* as supplementary food Vijverberg [45] has reported that green algae like *Chlamydomonas* and *Scenedesmus* are good quality food sources for zooplankton, the life-history effects of feeding on filamentous *Cyanobacteria* include increased mortality, reduced body growth, reduced and delayed reproduction [48]. Recently it has been recognized that many high quality algae have a high content of w3- highly unsaturated fatty acids, especially eicosapentaenoic (EPA,20.,5w<sup>3</sup>) and docosahexaenoic acid (DHA,22;6w<sup>3</sup>) [1, 2]. The production and growth rate highly occurred in algae fed *Daphnia*, because they are highly nutritive compared to yeast and *A. pinnata*. The algae (*C. vulgaris*) contain 48.7% protein, carbohydrate-23.1% & lipid- 4.6%. It is used to improve the production and capable of rapid population growth. *Daphnia* population showed a significant increase in moderate concentrations in all the different feed concentration. This is may be due to that the moderate concentrations supply enough nutrients for their reproductive process. The growth rate of *Daphnia* increased with *C. vulgaris* concentration upto fourth but showed a

significant decrease in the fifth concentration. The same phenomenon was noticed in Azolla, in yeast the growth rate was high in fourth and fifth concentrations, reported that several cultivation techniques, including feeding with baker's yeast, different algae artificial diets are used to improve lipid and vitamin content in *Daphnia*. Sterner [41] found that algae high in carbohydrates were of low quality, whereas high protein content was mostly an indicator of good quality [47]. The various biochemical nutrient components such as total protein, total carbohydrates and total lipids were analysed in the different feeds, such as *C. vulgaris*, Azolla and Yeast. The table shows *C. vulgaris* had higher protein when compared to Azolla and yeast.

**5. Summary**

*D. magna* is a valuable live food for the culture of different species of fishes. In the present study, the effect of different foods like *C. vulgaris*, Azolla, and yeast on the population growth of *D. magna* found in the freshwater system were investigated. *Daphnia magna* were introduced into separate culture plates with 50 ml of water. Then the species were nutritionally fed with chlorella, Azolla and yeast for 7 days. *Daphnia* cultures were maintained successfully fed with different feeds in the water for all the times. These experiments were conducted for a period of seven days. After that the production rate and growth rate were measured. Biochemical analysis of protein, carbohydrate and lipids of different feeds were analysed. Physico chemical parameters were analysed for tap water and habitat water (Muthanna Lake). The results were tabulated and graphical representations clearly state the production rate of *Daphnia*. Standard deviation was preformed and indicates the growth rate values. The experiment results have shown that the feed chlorella resulted in maximum growth and increase in *Daphnia* population compared to other feeds like Azolla and yeast.

**Table 1:** The Population level of *Daphnia magna* fed with different concentrations feed (*C. vulgaris*, *A. pinnata* and *S. cerevisiae*)

Feed Concentration (%)	<i>Chlorella vulgaris</i> -E1			<i>Azolla pinnata</i> - E1			<i>Saccharomyces cerevisiae</i> - E1		
	Initial Population level in 50ml	Final Population level in 50ml	<i>Daphnia</i> production Nos/ 50ml/day	Initial Population level in 50ml	Final Population level in 50ml	<i>Daphnia</i> production Nos/ 50ml/day	Initial Population level in 50ml	Final Population level in 50ml	<i>Daphnia</i> production Nos/ 50ml/day
1	10	32	3.1	10	30	2.8	10	28	2.5
2	10	38	4.0	10	36	3.7	10	32	3.1
3	10	43	4.7	10	40	4.2	10	35	3.5
4	10	49	5.5	10	45	5.0	10	39	4.1
5	10	40	4.2	10	38	4.0	10	33	3.2

**Table 2:** The Growth rate of (length in mm) *Daphnia magna* fed with different concentrations of Phytoplankton (*C. vulgaris*, *A. pinnata* and *S. cerevisiae*) for two days

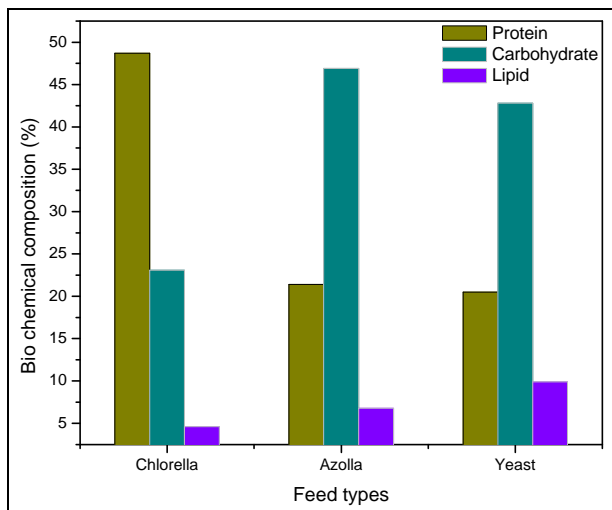
Feed Concentration (%)	<i>Chlorella vulgaris</i> -E1			<i>Azolla pinnata</i> -E2			<i>Saccharomyces cerevisiae</i> -E3		
	Initial mean body length(mm)	Final mean body length(mm)	Growth rate(mm/day)	Initial mean body length(mm)	Final mean body length(mm)	Growth rate(mm/day)	Initial mean body length(mm)	Final mean body length(mm)	Growth rate(mm/day)
1	0.111±0.024	0.133±0.042	0.077	0.111±0.024	0.124±0.027	0.069	0.109±0.024	0.115±0.036	0.060
2	0.112±0.025	0.145±0.045	0.089	0.112±0.025	0.132±0.032	0.077	0.109±0.024	0.124±0.039	0.069
3	0.114±0.025	0.167±0.052	0.110	0.114±0.025	0.154±0.034	0.098	0.111±0.024	0.133±0.042	0.077
4	0.116±0.025	0.181±0.057	0.123	0.116±0.025	0.169±0.037	0.112	0.112±0.025	0.148±0.046	0.092
5	0.109±0.025	0.137±0.043	0.082	0.109±0.025	0.128±0.025	0.073	0.111±0.024	0.141±0.044	0.085

Each observation is mean ± SE of five individuals.

- Denotes percent decrease over control

+ Denotes percent increase over control

Values are significant at 5% level



**Fig 1:** Biochemical composition of different feeds fed to *Daphnia magna*

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