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Laboratory Culture Of Selected Freshwater Zooplankton Species

Mohammad Mudassir Attar[1]*, Prachi Patil[2], Rashmi Joshi[3], Vineetkumar Patil[4]

[1*,2,3,4]*Department of Zoology, Karnataka State Akkamahadevi Women University, Torvi, Vijayapura-586104, Karnataka, India*

***Corresponding author:** Mohammad Mudassir Attar*
**Department of Zoology, Karnataka State Akkamahadevi Women University, Torvi, Vijayapura-586104, Karnataka, India; Email: mohammedmuddassirattar@gmail.com*

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Abstract

The present study was undertaken to standardize culture methods for native freshwater zooplankton species under captive conditions, to achieve this the selected species of zooplankton available in the local freshwater bodies were collected and bred under laboratory conditions. The species selected for culture were individuals from rotifer group, cyclops & *Lecane inermis*. The cultures were designed in two different methods viz polyculture, this group consisted of crustaceans like cyclops, rotifers like Branchionus sps, protozoans like Paramecium, another culture was monoculture, in which two species of zooplankton viz *Eucyclop serrulatus* and *Lecane inermis* were maintained separately. The polycultures were maintained in two sets one being fed with yeast and another with bacterial culture(curd). The replica maintained on yeast showed good results in comparison to the one fed with bacterial culture, care was taken to maintain the appropriate temperature and humidity of 27± 2°c and 75% respectively in the culture rooms. Along with this monoculture of *Eucyclop serrulatus* was undertaken this species grew well when fed with bacterial culture comparatively when fed with yeast. Further monoculture of *Lecane inermis* was subjected to two different volumes (200ml and 2000ml), and were fed with algae for 20days, it was noted that higher volume of water aided in the reproductive success and it was high in 2000ml compared to the other volume. Lecane species are recently been used in Waste Water Treatment (WWT) process and their culture in laboratory is crucial for commercial supply of these species. The zooplankton are proven to be the best live feed in aquaculture practices, so this work was an attempt to culture them under captivity and supply for commercial aquaculture needs hence emphasizing the entrepreneurship opportunities in this sector as well.

Keywords: zooplankton, captive culture, laboratory culture, aquaculture, Lecane inermis, Eucyclop serrulatus, entrepreneurship.

Introduction:

Zooplankton (zoo-animal; planktons –wanderers) are the diverse group and are heterotrophic in nature which consumes phytoplankton, restores the nutrients through metabolism and transfers the energy [1]. Majority of them are microscopic, some are unicellular, some are multicellular forms and their size ranges from, microns

to millimeter or more [2]. Size variation leads to change in morphological features and taxonomic position [2]. Based on the sizes zooplankton are classified into – micro-zooplankton (2-200µm), meso- zooplankton (200µm-2mm), macro-zooplankton (2 -20 mm) and mega-zooplankton (> 20 mm) [5]. The important role of zooplankton in food webs provide importance to ecosystems – fish, consumers, phytoplankton and other preys [4]. The fishes breed in such areas where planktonic organism is many in number so that their young ones can get sufficient amount of food for their survival and growth [2]. Planktonic organisms have the ability to concentrate radio isotopes and also can act as indicator of certain pollutants, by which it is important for the study of marine environmental science [2]. Zooplankton is a decent indicator of variationsin water quality as it is strongly affected by environmental conditions and responds quickly tochange in physical and chemical conditions.

Aquaculture is considered to be the trending sector. For survival, fishes need live food. Live food is available in markets but are quite costly. Hence to reduce the production costs the alternative can be culturing these zooplankton. The live food used in aquaculture is Cyclops, Daphnia, etc., which can be cultured under low costs. Cyclops is rich in proteins, vitamins, carbohydrates and lipids which is suitable for fish [29].

The three groups form important food source for fish larvae but more specifically copepods have potential to reproduce in large-scale. Regular and close monitoring of samples is essential. Samples can be taken on a slide and observations are done on the basis of their growth, composition of life stages and also their health status [30].

Water quality also plays important role in mass culture of copepods water quality determines the health of copepod culture. Though copepods can tolerate salinity and temperature but stillfluctuation of environmental parameters can affect the culture. The water taken for the cultureshould be chlorine treated, de-chlorinated so that the growth ratio increases. The culture should be kept in 60% Shady area, Feeding also plays an important role for maintaining stable culture of copepods the food should be chosen in such a way that it has its suitable particle size and is completely digestible[32].

Sieves Suitable sizes are essential for handling copepods in culture. Sieves of suitable sizes are essential for handling copepods in culture. Sieves can be purchased outside or self- prepared. On alternate days the water should be cleaned so that contamination does not occur.[35]

Copepods have high amount of DHA. They also have a rich source of Polar lipids that can be easily digestible for fish larvae copepods are having rich source of carotenoid astaxanthin precursor to vitamin A and other digestive enzymes [36]. High level of astaxanthin can reduce oxidative stress which keeps copepods grow healthy.

Now a days, excessive growth of filamentous bacteria presents serious issue in waste water treatment plants. For control of huge growth of filamentous bacteria, rotifers (*Lecane inermis*) can be used. Activated sludge is one of the common techniques which is used for waste water treatment management. This contains a diverse population rate. The microorganisms present in sludge are bacteria and fungi which are decomposers. If the bacterial growth exceeds very much then their exist serious issue – the bulking and foaming of activated sludge [12].

Lecane inermis is considered as one of the best zooplankton which is useful for Waste Water Treatment (WWT). They are the most fecund rotifers with populations dominated by amitic females which produce average of 20 eggs during their lifetime of 9 days. To have a successful growth of *Lecane* sps feeding them is very crucial and has to be fed very accurately. They feed on bacteria, algae. *Lecane inermis* has already been recognized as a potential control agent.

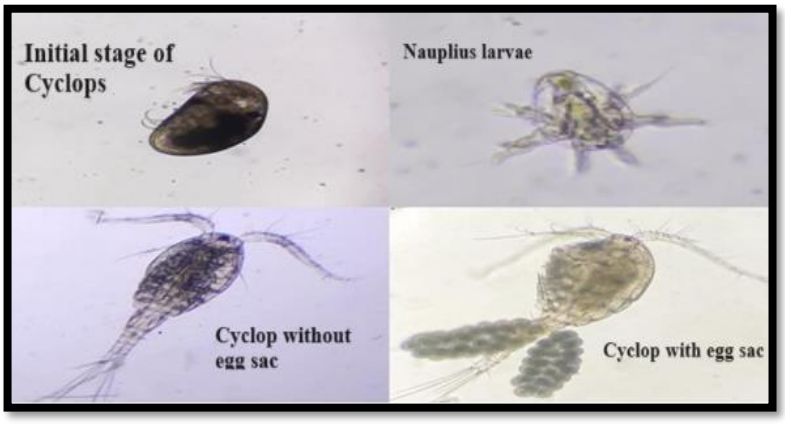

Fig 1: *Different stage of cyclops and an Nauplius larvae*

Fig 2: Lecane inermis

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Materials and Methods:

Zooplankton includes different methods for collection such as collecting the samples in water bottles, collecting through indigenous plankton net. The success rate of collection mainly depends upon the time of collection, water depth of the collected area and the season in which it is collected. There are different areas and methods for sample collection which are as follows-

2.1 Collection area:

Water samples were collected from different Seasonal water bodies from different monument places of Vijayapur District, Karnataka at specific time duration. The areas are as follows:

a. Sangeet Mahal[Latitude- 16.8278 N, Longitude-75.6575 E]
b. Bara Kaman [Latitude – 16.8303 N, Longitude – 75.7183 E]
c. Lake near Shri Laxmi Narsimha temple[Latitude – 16.8249 N, Longitude- 75.7154 E]
d. Bhutnal [Latitude – 16.8852 N, Longitude- 75.7095 E]
e. Fertilized pond from university campus [Latitude-16.8307° N, Longitude-75.6405° E]
f. Gagan Mahal [Latitude- 17.4080 N, Longitude-78.4802 E]

Fig 3: *The ponds as seen while collection of water samples*

Plankton net:

Plankton net was designed and used for collecting samples in standing water bodies. Plankton exists everywhere such as in Perennial as well as seasonal water bodies but are relatively low concentration in most places, for that reason plankton net is suitable for collecting where the population of the plankton is high.

Procedure of designing the plankton net:
2 liter water bottle was chosen to design plankton net. Along with the water bottle, nylon cloth was taken which contains minute fine pores. Cut the bottle into three parts. On the lower edge of the middle part, punch three evenly spaced holes. Snip a small hole in the lower edge. Put the top of the bottle through the hole. Tap to secure. Tape the other end to the second bottle section. Make a tow line with 3 x 90 cm lengths of string. Collect plankton lower vertically and drag along. Empty the sample into the container.

Fig 4: *Indigenous plankton net*

Bottles:
All the water sample after collecting it with plankton net will be stored in bottles. The bottles chosen for storing must be clean and dry, free from the dust particles and should be kept according to its suitable temperature.

Fig 5: *Collected water samples stored in plastic bottles*

The most common fixative and preserving agent is Formaldehyde as it is the cheapest fixative, the zooplankton samples can be stored for a number of years. The other fixatives which are infrequently used are ethanol, picric acid, acetic acid, etc. For proper fixation, analytic grade formalin should be used instead of commercial formalin. This formaldehyde is a clear and colorless solution. The concentrated formalin should be diluted with potable water to avoid undesirable osmotic effects. The dilution is in the ratio of 1 part of formalin and 10 parts of potable water. The pH of the fixative should be 8.

Fig 6: *Fixative reagent*

Maintenance:
Maintenance of zooplankton samples would be better if it is preserved in the well-ventilated room at optimum temperature. These samples should be kept in the glass chambers. Good qualities of the labels should be stuck to the containers on which the collectors name should be added along with Date and Day, Time of the sample collected should also be mentioned. This information is required for reference for further analysis. The culture was maintained using aerator for proper supply of oxygen.

Identification:
The study of the identification of zooplankton is carried out by using a microscope. The microscopes used in our study were: Binocular microscope of the QUASMO brand star 3 model and a Trinocular microscope of QUASMO brand star 7 Elite. The identification was done under higher magnification(40x) and lower magnification (10x). The observation was done by taking water sample on the slide. To that water sample, few drops of Formaldehyde was added to fix the zooplankton so that identification will be easier rather than identifying in live condition due to their fast and vigorous movement. But while capturing Lecane species

Polyculture of zooplankton (Algae v/s Yeast)
a. Inoculation of the polyculture:
Culturing of the freshwater zooplankton was maintained in two equal sized Polystyrene glass chambers and two litters of chlorine free potable water was inoculated in two different chambers and zooplankton such as rotifers, copepods etc were selectively inoculated in the chamber and was kept for culture process this was maintained with aerator hence to balance the oxygen in the culture media, the food was provided alternatively with particular time duration**.**

b. Feed preparation:
During the culture period, one culture was fed with algae and the other culture was fed with yeast. Algae was collected from nearby water bodies and the Baker's yeast (Blue bird) was commercially purchased and used for this study.

Fig 7: *Polyculture of Algae v/s Yeast*

c. Maintenance of polyculture:
The feed was provided on alternative days and on regular basis the sample was observed in Binocular and Trinocular microscopes checking the population growth of zooplankton. When there was decrease in water level of the culture due to changes in temperature, addition of de-chlorinated water was carried out.

Monoculture of Cyclops:
a. Collection of Cyclops:
The water sample collection is an important step to run monoculture of cyclops species. The water sample was collected from a small lake near "Shri Laxmi Narsimha Temple", Vijayapura District of Karnataka. The collection was done in late evening as maximum availability of zooplankton is observed during that time of the day. The cyclops was collected by using plankton net. By using the plankton net maximum number of zooplankton can be collected.

b. Isolation of cyclops:
Isolation is an important step which was carried out in a laboratory. The selected live cyclops were carefully isolated using nylon cloth. The collected water sample was passed through nylon cloth which contains small fine pores. The cyclops can be seen through naked eyes which is useful for culture purpose.

Fig 8 *– Monoculture of Eucyclop serralutus*

c. Inoculation of cyclops :
The isolated cyclops species were inoculated to petri plates containing de-chlorinated water for this 400ml of two glass jars were selected. Fresh de-chlorinated or chlorine free water was poured to the glass jars. In each jar 5ml of water sample containing 20 individuals were inoculated.

d. Taxonomical identification of cyclops:
For taxonomical identification, live Cyclops was taken on the slide. It was fixed by using Formaldehyde(0.5%) solution, the Cyclops was observed under Trinocular microscope. By observing its morphology i.e., size, claws, appendages it was found that the species was *Eucyclop serrulatus*, identification was done by using zooplankton manual[2] and also by using Google lenses.

e. Culture vessels and water:
Choosing the culture vessel is also important. For culturing, two transparent glass jars were taken. The culture was kept open to air so that proper aeration can take place. The water was changed once in four days to obtain good results.

f. Feed for the culture:
To run the good culture, we should provide feed to Cyclop.One culture was fed with Baker's yeast(Blue bird) and other culture was fed with curd containing Bacteria which has rich amount of phosphorous and calcium loaded with riboflavin, vitamin B12 and pantothenic acid. The growth rate was checked and analysed. The feed was provided on alternate days.

g. Culture of *Eucyclop serrulatus*:
The culture was kept for 40 days. On daily basis water sample was taken and analysed for population increase. Culture was kept in 60% shady area.

Monoculture of *Lecane inermis:*

a. **Observation of *Lecane inermis:***
In the collected water sample from Bhutnal FRIC in Vijayapura District of Karnataka, it was observed that the species is rotifer. This species was continuously observed and was chosen for monoculture. The sample was collected early morning in the month of June. The collection time should be suitable when lower temperature is observed for best results. The collection was done by using plankton net.

b. **Isolation of *Lecane inermis:***
As they are not visible with naked eyes, care should be taken that while inoculation no other species of zooplankton enter. For isolation, a clean slide was taken and water sample was taken from which only Lecane species were made separate. The observed sample were transferred to the petri plate containing de-chlorinated water which was used for monoculture. Likewise 20 individuals were separated in isolation step.

Fig 9 *– Monoculture of Lecane inermis*

c. Taxonomical identification of *Lecane inermis:*

For taxonomical identification, the live sample was taken on slide. It was observed and fixed by using formaldehyde solution. The Lecane were observed under Trinocular microscope. By observing its size and morphological characters it was found that the species was *Lecane inermis* which is a rotifer. Its identification was done by using zooplankton hand manual[2] and also Google lens.

d. Culture vessels:

The experiment which was designed based on choosing different water column. One culture was kept in 2000ml chamber and the other was kept in 200ml of glass chamber. Observation was done based on higher reproduction rate. The culture was kept open to air so that proper aeration can take place.

e. Feed for culture:

To run the successful culture, feed is an important step. Feeding is necessary for the better growth of the species. The feed chosen for the culture was algae. To both the culture same feed was fed. Fresh algae was collected from University campus near library lake. On alternate days the feed was given to the culture.

f. **Culture of *Lecane inermis:***

The culture was kept for 35 days. On the daily basis the water sample was taken and analyzed for population growth. The culture was kept under 60% shady areas.

Results:

Our study is based on culture of zooplankton. Culture was fed with different feeds such as Baker's yeast, algae and bacteria and observed their growth rate and survivability. All the Zooplankton are selective feeders. They select food based on particle size, configuration, chemical nature of food and also their feeding behaviour. Usually the particle size of a food chosen by zooplankton vary species to species. Generally larger the animal, larger is the particle size where as a smaller choose a minute food particle[15].

Sl.no	Population growth	Yeast	Algae	Bacteria
1.	Rotifers	+	+	_
2.	Copepods	+	+	+
3.	Cladocerans	_	_	_
4.	Others	+	+	_

Table no 1: *Population growth of different zooplankton as seen in yeast, algae and curd*

The selective feeding was done to individual cultures. The culture was carried for 2 months, observed continuously and fed on alternative days.
The three cultures which was set to run were:

a) Zooplankton Fed with Yeast v/s algae:

The maximum growth of population of zooplankton was observed in Yeast fed culture. The plankton were active and growing rate was also high. In case of algae fed culture, the plankton activities were comparatively low as that of yeast fed culture.

FEED	READING 1 No of individuals	READING 2 No of individuals
ALGAE	90	100
YEAST	113	121

Table no 2: *Table showing no of zooplankton counted on two different intervals during the culture period*

b) Monoculture of cyclops:

The monoculture of *Eucyclops serrulatus* was carried out in 400 ml glass jars. It was fed with two different feeds that is yeast and bacteria. The high population growth was observed in curd fed culture containing bacteria. Cyclops were highly active in curd fed culture and were reproducing in higher rate compared to that of yeast fed culture.

FEED	READING 1	Reading 2
YEAST	47	55
CURD (BACTERIA)	51	63

Table no 3: *Table showing no of Eucyclop serrulatus counted on two different days*

c) Monoculture of *Lecane inermis*:

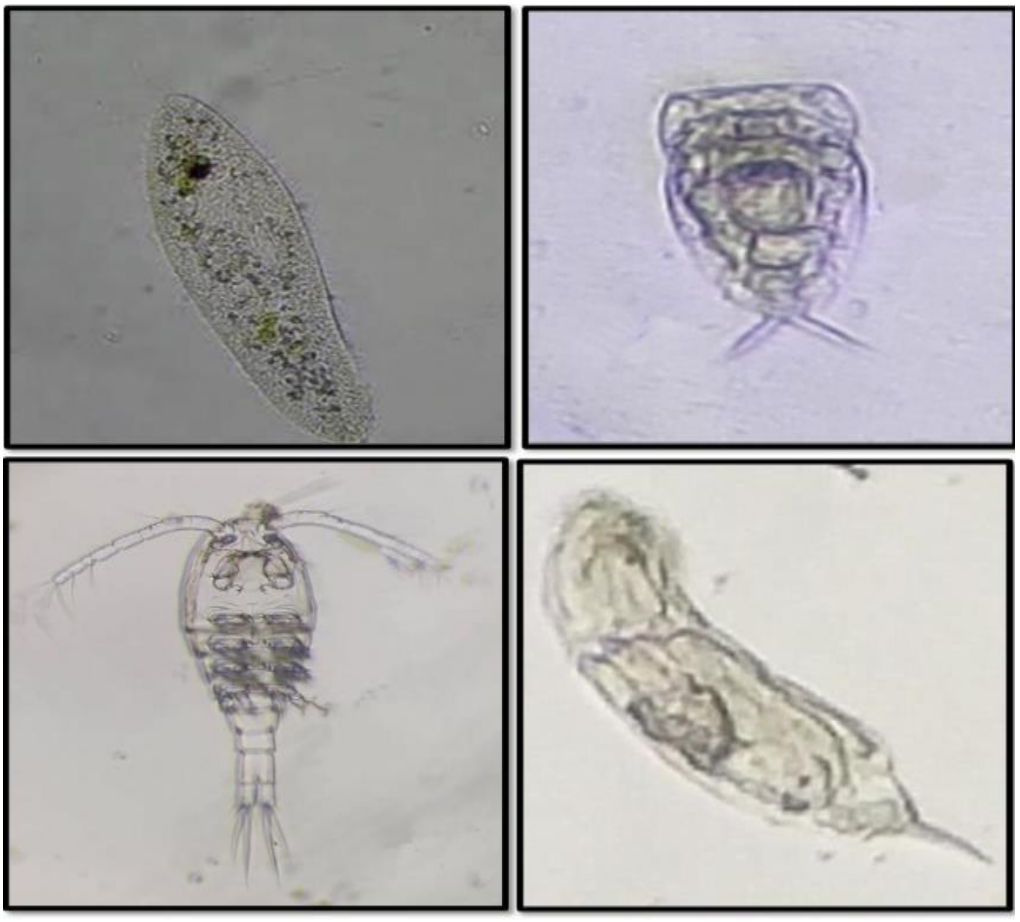
Monoculture of *lecane inermis* was carried out in two different sized glass jars. One was of 2000 ml capacity jar and other was 200 ml capacity jar by doing so the cultures were maintained in different water column length. The high rate of growth was observed in 2000 ml glass jar which had more surface area compared to that of smaller 200ml glass jar whose surface area was lesser.

FEED	WATER COLUMN	READING 1	READING 2
ALGAE	200ML	37	48
ALGAE	2000ML	42	56

Table no 4: *Table showing no of Lecane inermis species counted on two different days*

YEAST FED CULTURE:

Fig 10 - *Diversity of different species of zooplankton fed with yeast at lower magnification*



Culture at higher magnification

Fig 11 -Above left: *Paramecium caudatam*, Above right- *Lecane pyriformis*Below left- *Eucyclop serrulatus*, -Below right -*Brachionus forficula*

MONOCULTURE OF CYCLOPS

Fig 12: Above -*Eucyclop serrulatus fed with bacteria,* Below -*Eucyclop serrulatus fed with yeast*

MONOCULTURE OF Lecane inermis

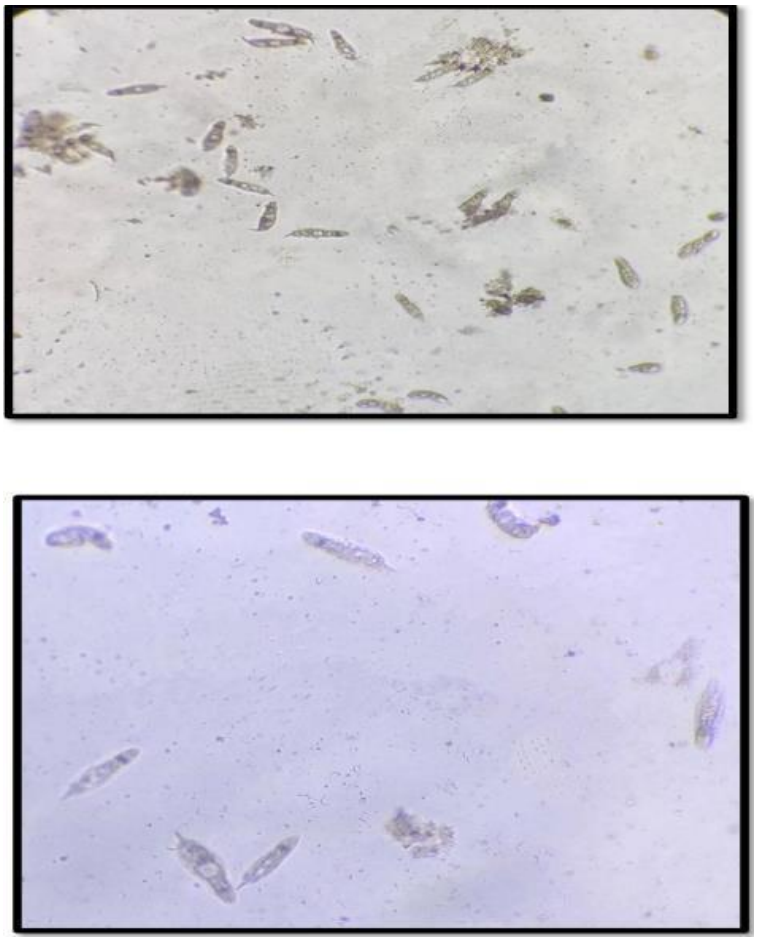


***Fig 13*:** Above– *Lecane inermis* cultured in 2000 ml glass jar Below– *Lecane inermis* cultured in 200 ml glass jar

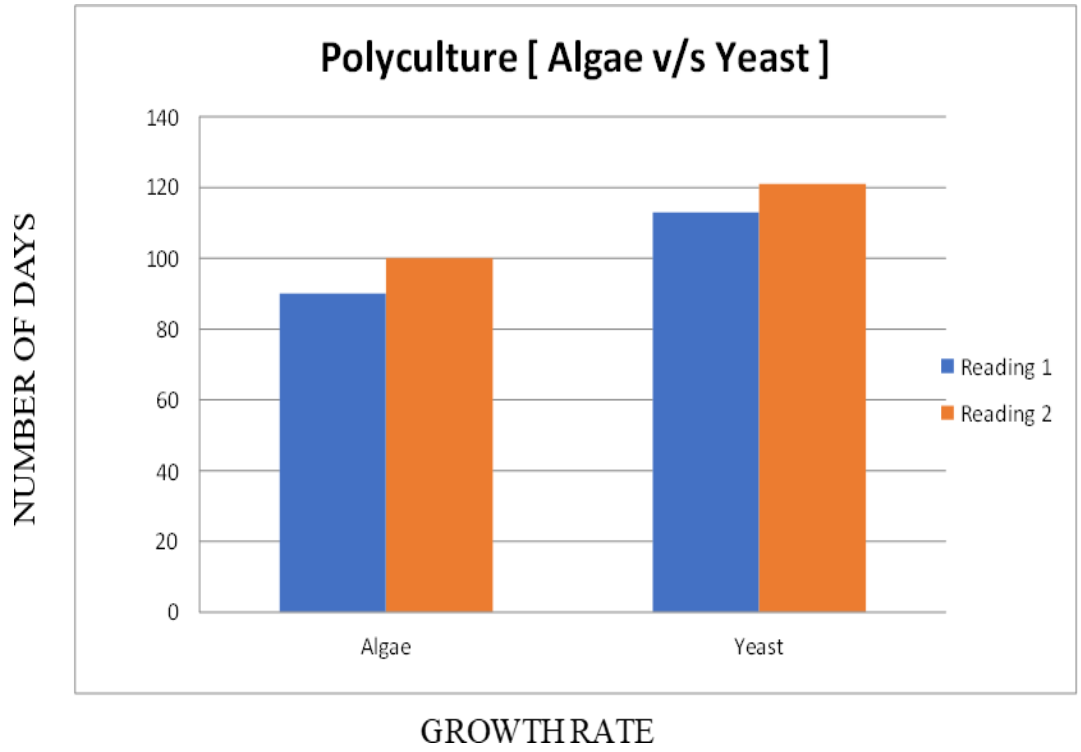


***Fig 14*:** Graph showing differential growth rate of zooplankton in Polyculture [Algae v/s Yeast]

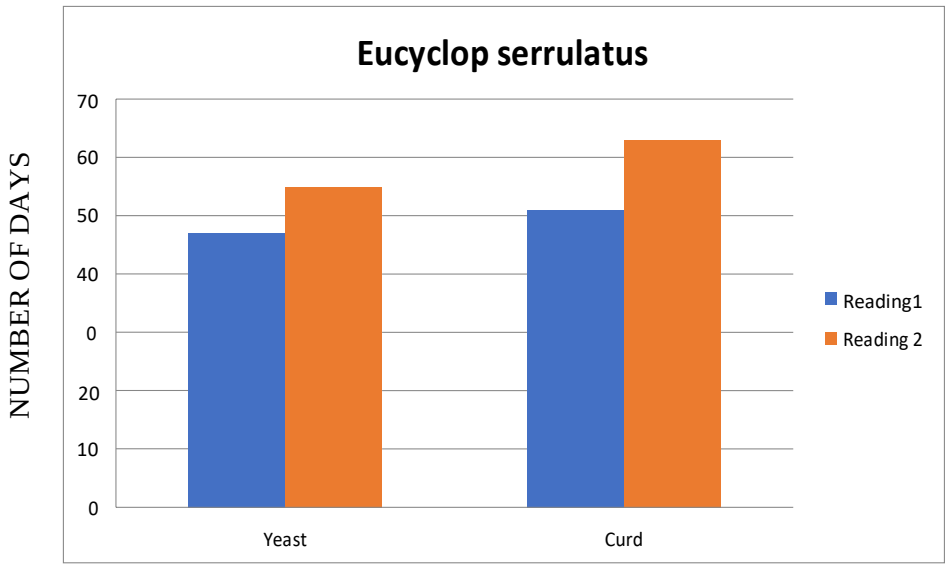
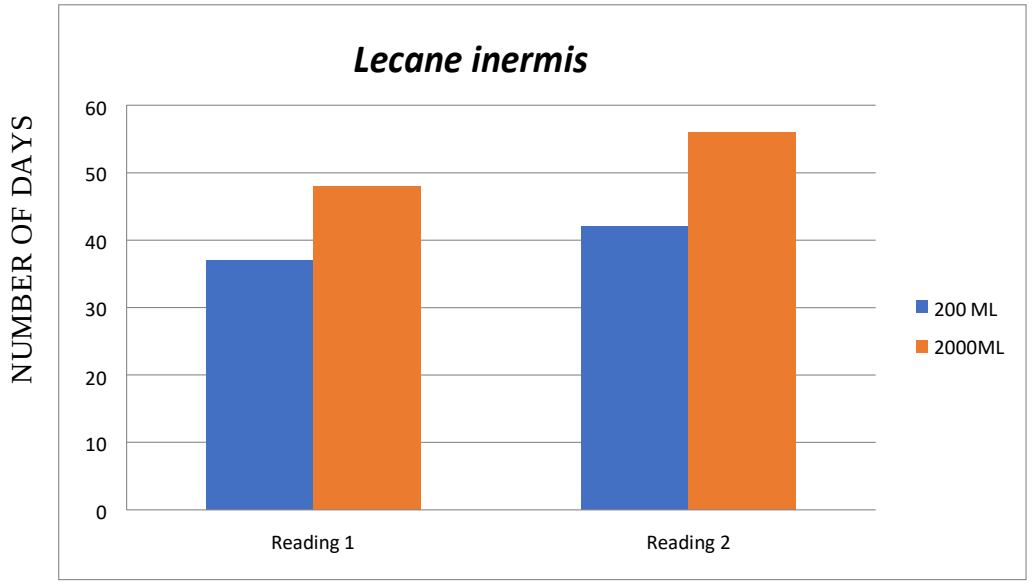


***Fig 15*:** Graph showing Differential growth rate of a *Eucyclop serrulatus* in differently fed cultures



GROWTH RATE

***Fig 16*:** Graph showing differential growth rate of *Lecane inermis* varying in water column length.

Discussion:

Freshwater zooplankton are one of the oldest communities of earth that shows the adaptations to varying ecological conditions. The profusion and distribution of zooplankton depends upon the various ecological factors. Temperature plays an important role where the nature and pattern of fluctuation in population density of zooplankton are dependent on the temperature the ecological factors like light, pH, dissolved oxygen, and other various factors of the water body [14].

The life in aquatic systems is rich in micro and macro fauna. In the water bodies zooplankton are considered as ecological indicators [14]. The Zooplankton animals that feed on small food particles are called microphages where as large particle are called as macrophages [17]. Seasonal changes can affect grazing which are highly

variable and dependent on species composition of zooplankton community, available food supply, temperature and many other environmental factors [15]. Temperature can influence biological functions such as grazing rates of organisms and filtering rates [15].

Zooplankton plays a key role in the pelagic food web by controlling phytoplankton production and shaping pelagic ecosystems. In addition, because of their critical role as food source for larval and juvenile fish, the dynamics of zooplankton populations have a significant influence on recruitment to fish stock [16].

In the present study of zooplankton copepods were dominant in the lake near "Sri Laxmi Narasimha Temple which is followed by rotifers collected in FRIC, Bhutnal and there were no cladocerans found in that season. Most of the zooplankton are measured as a live food for larval forms which are commercially important food of freshwater fishes and prawns [14]. Live food provides high amount of exogenous enzymes and it also helps to enhance chemoreception and visual stimuli of fishes [16].

In the present study, the nutritional requirements of the zooplankton was analyzed and the particular feed for the particular culture was fed. Yeast (vitamin B, thiamine, riboflavin, niacin and B12), algae (calcium, iron, vitamin A, C, and K), Curd(phosphorous, calcium, riboflavin and pantothenic acid) containing bacteria was chosen as feed and fed for successful result. These feeds contain high nutritional value.

Zooplankton have both Predator and Prey organisms [16]. The organisms modify their feeding habitats in response to prey availability and presence of predators with connection to populations of both predators and prey. The assimilation efficiency depends on growth of the organism and its feeding behaviour. Biomass and energy are the terms that determines growth of the zooplankton[17].

Rotifers have 2000 species in the freshwater ecosystem. They consist of a distinct mouth, which is mainly used for filter feeding and for locomotion also. Rotifers serve as a food source for the larger aquatic animals and also for other zooplankton [23]. They have a complete digestive tract. Most of the rotifers are free living. They have the capacity to swim or walk by anchoring their feet. The rotifers reproduce both asexually and sexually, it all depends upon the environmental conditions. Cryptobiosis allows the rotifers to survive in extremely high conditions[24].

Rotifers are multicellular organisms. Rotifers are recognized as animals, even though they are small as protists. These rotifers contain thousands of cells that belong to multicellular animals. Rotifers are one of the strongest of all animals. Some species have unique characteristics that belong to the roofers such as at any point of their life cycle, they can be completely dried out and they can live happily in a dormant state before they rehydrate again. [25]. Rotifers also contribute decomposition of soil and organic matter. Most species belonging to the rotifers are cosmopolitan[26]. Rotifers in the wild have little economic significance to humans. They have economic significance such as many species of the rotifers are cultured as a food source for aquariums and are also cultured filter-feeding invertebrates and fish fry. Rotifers are also used as a biological pollution indicator [27]. Rotifers act as a link between the microbial community and high trophic levels[28]. Rotifers are majorly used in aquaculture and are popularly known as animalcules. These are one of the important group of live food organisms which is used in aqua hatcheries. Brachionus is one of the most known species in rotifers which have been observed during the investigation of the study. This species serves as an ideal starter diet for early larval stages for many fishes and prawn species which are found in both marines as well as freshwater [29].

Copepods are the active swimmers and these will undergo diurnal migration. During the present investigation, Cyclops were dominant among the copepods [18]. There are about 13000 known species which are free living marine forms that occur throughout the world's oceans [19]. Copepods rule the world, at least whenever it comes to oceans and estuaries. Copepods are an important link between phytoplankton and fish in marine food webs [20]. The smaller copepods generally dominate both in the terms of abundance as well as biomass and copepods are important grazers in low chlorophyll environments, the relative size of primary consumers is expected to be smaller and microbial components are dominant [21]. Cyclop is a planktonic species of copepod found throughout the world, except in Australia. It is a deep-water species that is found throughout the year with a peak that is more abundant that occurs in May and June [22]. Cyclops are the most important part of the ecological systems where they are the natural prey of large fry, small fishes, and other aquatic organsisms. That include hydras and white mosquitos' larvae [21].

The monogonant rotifer *Lecane inermis* which is commonly known as a discretional parthenogen. Unexpectedly, among all the rotifers lecane will be isolated from wastewater treatment plants (WWTP) among the colony of lecane only one will be capable of sexual reproduction. The reproductive mode of lecane was examined. The population growth rate of parthenogenetic lecane by asexual will be significantly higher than that of the sexual one. The mean number of females will be greater in asexual than in sexual mode [22]. The final result of the study suggests that culturing of the lecane can be selected for inoculation in activated sludge in waste water treatment plants [22].

Growing a mixed group of species tend to be more effective production for the fishes compared to the average monoculture technique [40]. The culturing method also depends on the season and the temperature [41]. In this study, we followed the Polyculture technique which was set up during the rainy season in the month of June. Care should be taken that it is kept in a dark and shady area so that it is set free from sunlight. As per the observations, temperature fluctuation may result in a decreased production of zooplankton. To avoid it, an aerator was placed and regular feeding was carried along. This is an easy technique that can be followed to produce more species which acts as live feed for fishes.

The benefits of culturing the zooplankton is they serve as an intermediary species in the food chain. The flavour and texture of fish are also improved with zooplankton as feed [42].
Zooplankton help food chains and webs because they are the base of the food chain for a lot of animals. Many animals rely on them to get food. Zoopankton cannot produce their own food. They are relied onto millions of algae, otherwise the algae will grow out of control. Zooplankton comprises many microscopic and macroscopic animals represented by almost all the major taxa of the kingdom Animalia. Zooplankton are ubiquitous. Zooplankton play a pivotal role in the aquatic food web, having the potential to affect Water transparency, levels of suspended algae(phytoplankton), and the fishes. Many economically important fish depend on diet of zooplankton during some stages in their life cycle. Zooplankton are used as one of the bio-indicators for accessing aquatic ecosystem health. The zooplankton are more varied as compared to phytoplankton, their variability in aquatic ecosystem is influenced mainly by patchiness, diurnal vertical migration and seasons.

Conclusion:

Zooplankton are considered to be the source of life of aquatic organisms especially for larval stages. They play an important role in food chain and acts as food for higher organisms in aquatic life due to their ability of supplying nutrients. In the trophic level, the interactions between predator and prey are also affected which may result in degradation and dys- functioning of ecosystem. Different feeds such as yeast, algae and bacteria are important for feeding zooplankton. These feeds contain rich nutritional value and enhance the growth rate of the species. The variety in the feed has given different results proving that the nutritional requirements are different for different group of zooplankton. Hence, by culturing and preserving the zooplankton species, it can help us to supply them as and when needed. Culture of zooplankton helps in aquaculture and specifically monoculture of Cyclop helps to provide selective nutrition to fishes and Lecane helps in Waste Water Treatment Plants (WWTP).

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