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"DAPHNIA CULTURE AND EVALUATING THE ROLE OF DAPHNIA IN MOLLY FISH GROWTH AND DEVELOPMENT"

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ABSTRACT :

Daphnia is a small planktonic crustacean, *Daphnia* were cultured in fresh water Borosil jar (~3 Lit of water capacity) using a diet rich in yeast and algae, and using it as high-quality live food for molly fish (*poecilia sphenops*) *Daphnia* belong to the Cladocera, whose bodies are enclosed by an uncalcified shell, known as the carapace. It has a double wall, between which hemolymph flows and which is part of the body cavity. The carapace is largely made of chitin, a polysaccharide. Cladocere have up to 10 pairs of appendages, which are (from front to back) antennules, antennae (the second antennae, used for swimming); maxillae; and mandibles; followed by 5 or 6 limbs on the trunk. The limb forms an apparatus for feeding and respiration. At the end of the abdomen is a pair of claws.

INTRODUCTION :

Mostly, the younger form of fish – larva, hatchlings, fry, and advanced fry- requires live feed than the adult fish. In general, young ones of many fish consume mixture of algae, Cladocerans, copepods, rotifers, debris and other organisms. Among them, Clodocerans- a smaller version of crustacean- is highly preferred group by the fresh water fish – Indian major carps, Chinese carps, tilapia, ornamental fish, etc. There are about 420 species of cladocerans have been found around the world, however, the most commonly cultured species for the feeding of early stage of finfishes and shellfishes is daphnia. It is found in tropics to the arctic, especially in the smaller ponds to larger freshwater lakes. There are 50 species *Daphnia* are reported worldwide, but out of only six of them normally occur in tropical areas.

Daphnia is an inevitable live feed used in freshwater aquaculture. Due to its general appearance and irregular behaviour, it is called by the name of "WATER FLEA". It is small crustacean which sizes around 0.2-3.0 mm long. The impotence of commercial feed at early larval culture makes *Daphnia* as potent live feed in many freshwater fish hatcheries. Therefore, the insemination of *Daphnia* culture and their nutritional make up could help the hatchery people to perform well in the near future.

IDENTIFICATION OF *DAPHNIA:*

- It has a large head with rostrum.
- Thorax and abdomen covered by the carapace.
- The abdomen which is bent down ward is free of appendages.
- In cephalic appendages, second maxilla is absent.
- Paired eyes are fused in one.
- Five pairs of leaflike feet present on thorax.

The nutritional value of *Daphnia* depends strongly on the chemical composition of their food source. However, since *Daphnia* is a freshwater species, it is not a suitable prey organism for marine organisms.

Daphnia are planktonic crustaceans that belongs to Phyllopoda (sometimes called branchiopoda), which are characterized by flattened leaf-like legs used to produce a water current for the filtering apparatus. Within the branchiopods, *Daphnia* belong to the Cladocera, whose bodies are enclosed by an uncalcified shell, known as the carapace. It has a double wall, between which hemolymph flows and which is part of the body cavity. The carapace is largely made of chitin, a polysaccharide. Cladocere have up to 10 pairs of appendages, which are (from front to back) antennules, antennae (the second antennae, used for swimming); maxillae; and

mandibles; followed by 5 or 6 limbs on the trunk. The limb forms an apparatus for feeding and respiration. At the end of the abdomen is a pair of claws. *Daphnia* feed on small, suspended particles in the water. They are suspension feeders (filter feeders). The food is gathered with the help of a filtering apparatus, consisting of the phylopods, which are flattened leaf-like legs that produce a water current. As the current flows anterior to posterior, the *Daphnia* collect particles that are transferred into the food groove by special setae. Although the feeding apparatus is so efficient that even bacteria can be collected, the food is usually made up of planktonic algae. Green algae are among the best food, and most laboratory experiments are done with either

Scenedesmus or Chlamydomonas, both of which are easy to culture in monoclonal chemostats. *Daphnia* usually consume particles from around 1m up to 50 m, although particles of up to 70 m in diameter may be found in the gut content of large individuals.
Daphnia have an open blood circulation. The heart is located dorsally and anterior from the brood chamber. At 20^0C, it beats about 200 times per minute, slowing down at lower temperatures. Blood cells are easily visible through the transparent body as they flow rapidly through the body cavity.

MATERIALS

1. Plastic container or aquarium
2. Air stone or filter
3. Live *Daphnia* starter
4. pH test
5. Active yeast
6. Silk cloth

PROCEDURE

PHYSICAL AND CHEMICAL REQUIREMENTS: *Daphnia* appear in high concentrations in pools, ponds, lakes, ditches, slow-moving streams, and swamps where organic material is decomposing. They become especially abundant in temporary water bodies, which provide them with suitable conditions for only a brief period.
pH and ammonia- A pH between 6.5 and 9.5 is acceptable, with the optimum

pH Test

being between 7.2 and 8.5. ammonia is generally highly toxic to all organisms, even in small amounts, but in alkaline conditions, the toxicity is radical.

Oxygen: *Daphnia* are generally tolerated of poor water quality, and dissolved oxygen varies from almost zero to supersaturation. Like the brine shrimp, their ability to survive in an oxygen poor environment is in their ability synthesize hemoglobin may be promoted by high temperatures, and a high population. Also, like brine shrimp, *Daphnia* are not tolerant of fine air bubbles. A slow aeration is needed with *Daphnia* as a large bubble column will strip the *Daphnia* out and kill them. I have found

Dissolved oxygen test

that bio-foam filters are ideal for aerating *Daphnia* culture, and the removal of larger particles from the water is added bonus.

SALINITY: *Daphnia* are typically freshwater organisms and there are no marine species of the *Daphnia* genus. 99% of Cladocerans are found in freshwater, and the remaining few species have been observed in salinities of 1.5 to 3.0 ppt are common in pond cultures in the orient. Fill a container with chlorine-free water. Use a 20gallon plastic tub or aquarium to house your *Daphnia* cultures. *Daphnia* will die in tap water because of chlorine that it often contains. If you have an existing aquarium, use the water from the aquarium instead. We can also use distilled water. Its easier to monitor and watch your *Daphnia* if you use a glass aquarium.

Treat chlorinated water with de-chlorinated. we can purchase de-chlorinator in online or at a pet shop. We must follow the instructions on the back of the packaging and add the appropriate amount of chemical to the water. Let the solution sit in the water for 30 min, then we can proceed to next step.

Install a bubbler line or sponge filter in the tank. Don't use an air stone or mechanical filter, as small bubbles can cause buoyancy problems in daphnia, and mechanical filters can crush them. Use the lowest air setting to keep the water relatively calm.

Aim for the pH level between 6.5 to 8.5. if the pH level of your water is too high or low, boil it to give it a neutral pH level. We can also add distilled water to it.

Keep the water temperature between 18-22°C. *Daphnia* culture prefers cooler climates, so leave them in a cooler place like a basement or garage. If you are keeping the *Daphnia* cultures outside, make sure it doesn't go above 22°C or it will prevent the *Daphnia* from reproducing. Consider setting up more than one culture. Having multiple tanks with different cultures will ensure that if one culture die you'll have a backup. Set up to three different tanks to house your *Daphnia* cultures.

Purchasing the *Daphnia* online or a pet store. If you buy your *Daphnia* online, they will usually be shipped to you within a day or two. Be careful when opening the packaging so that you don't accidentally puncture the bags that they come in. we can start a culture with around 30- 40 *Daphnia* per container. The water that the *Daphnia* comes in will be yellow.

Submerge the bag into the water. Place the container or bag that the *Daphnia* arrived in into the water for around 30 min so that they can become acclimated to the temperature of the water. Pour the *Daphnia* into the water. Open up the bag and the container that the *Daphnia* came in and carefully pour them into the container.

Change the 10-20% of water every week. Remember that you shouldn't use your regular tap water when changing the water because it contains chlorine. You can use the old water from the existing aquarium. Drain 20-30% of the water from the container and replace it with the new water.

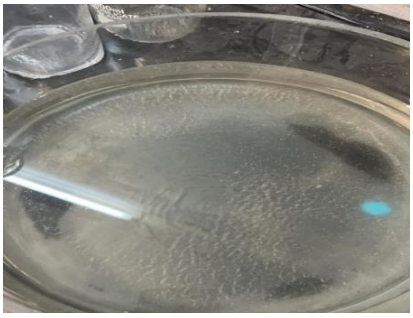
Feed the *Daphnia* one to three pinches of active yeast per day. When we add yeast to the water, it will become cloudy.

Feeding *Daphnia* with yeast

Examine the clarity of the water the next day. The water should be clear the next day. If it's cloudy, it's an indication that you're feeding them too much. Lower the amount of yeast you feed the daphnia. If the water clears up before 24 hours is over, it means that you need to feed *Daphnia* more. Adjust the food levels until they eat all of the yeast over a 24hour period.

Monitor the health of your *Daphnia* cultures every day. *Daphnia* communicates can die off from temperature changes, overfeeding, or overcrowding. Avoid changing their environment drastically and monitor the tank to make sure you are feeding them enough. If you notice that *Daphnia* are becoming over crowded, harvest them more regularly.

Harvest ¼ of the *Daphnia* after a week and a half. If your culture is strong, you can harvest ¼ of the *Daphnia* population. Use a net that has net large enough to let the baby *Daphnia* slip through, but small enough to catch the adults. Examine the *Daphnia* population before harvesting. If you notice they aren't reproducing as fast as usual, delay the harvesting so their numbers can rise.



Staining Daphnia

A healthy *Daphnia* culture can be harvested daily or every other day once it's stable depending on the size of the population.
Daphnia are generally quite tolerant of poor water quality. They live in water where the amount of dissolved oxygen varies from almost zero to supersaturation.
The ability to survive in oxygen-poor environment is due to their capacity to synthesize hemoglobin. Hemoglobin formation is dependent on the level of dissolved oxygen in the water. The production of hemoglobin may also be caused by high temperature and high population density.
Daphnia are resistant to extremes in temperature and easily withstand a daily variation of (5°- 31°C) their optimum temperature is (24°-31°C). The high temperature tolerance of *Daphnia* is of great advantage for both the commercial fish farmers in the southern us and hobbysists culturing live food at home.

FOOD REQUIREMENTS:

Daphnia a feed on various groups of bacteria, yeast, phytoplankton, and detritus(decaying organic matter). Bacterial and fungal cells rank high in food value. Populations of *Daphnia* grow most rapidly in the presence of adequate amounts of bacterial and yeast cells as well as phytoplankton. *Daphnia* are one of the few zooplankton which can utilize the blue-green algae microcystis aeruginosa. Both plant and animal detritus depends on its origin and diminishes with the age of detritus.

FEEDING OR FERTILIZING:

The initial fertilization rates provided only a starting point and will probably need to be adjusted depending on individual culture conditions.
The quantity of fertilizer materials should be added initially for each 100 gallons (397L) of water. Additional feed or fertilizer, approximately 50%-100% of the initial amount, should be added about 5 days later.
Yeast: 0.3-0.5 ounces (8.5-14.2g) of baker's yeast, and 0.5 ounces (14.2g) of ammonia nitrate.

Feeding mollies with *Daphnia*

INOCULATING:

Use pure live cultures to inoculate. Avoid using animals for inoculation from poor or declining cultures, cultures producing resting eggs, or cultures containing predators of fish larvae or fry. Inoculate with approximately 100 daphnia/gallon(25/L). although a culture can theoretically be started with a single female, always use an adequate number to develop a harvestable population quickly. If fewer are used, the population in the culture will increase more slowly, therefore, the initial quantity of fertilizer or food should be reduced to prevent over-feeding. A greater number is used for inoculation reduces the time to harvesting and lessens the chance of contamination by competitors.
Cultures are usually inoculated 24 hours or more after fertilization. However, when yeast is used, *Daphnia* can be added to the culture after few hours of aeriation, assuming good water quality and proper temperature. This is because the yeast cells are immediately available. The small amount of phytoplankton present in the water and digestive tract of *Daphnia* used to inoculate the culture is usually sufficient to initiate a phytoplankton bloom.
In their natural pond habitats, *Daphnia* feed on algae, bacterial, flora, and other tiny planktonscreatures even smaller than themselves. In your tank, though, you will feed them active dry yeast.
Daphnia reproduce every 8 days. *Daphnia* are really good at exponential math. It only takes 8 days for a baby *Daphnia* to grow to maturity and begin breeding. Each *Daphnia* has ten babies. If you have 100 *Daphnia* today, you'll have 1000 *Daphnia* in a week. A week after that you'll have 10,000 daphnia. In a month you could go from 100 to 100,000 daphnia. Their life cycle is only couple of months.

REPRODUCTION AND LIFE CYCLE OF DAPHNIA

Daphnia has the capability to reproduce in both sexually and asexually. However, parthenogenesis reproduction is very common among Cladocerens.
Life cycle of *Daphnia* during the growth season is characterized by its asexual mode of reproduction. A female produces parthenogenic eggs after every adult molt show female with parthenogenetic eggs. These eggs are placed in the brood chamber, which is located dorsally beneath the carapace and which

is closed by the abdominal processes. Development of eggs is direct. At 20°C, the embryo's hatch from the eggs after about 1 day but remain in the brood chamber, the young *Daphnia* are released by the mother through ventral flexion of the post- abdomen. The newborn look more or less like the adult daphnia, except the brood chamber is not yet developed. In most species, a juvenile *Daphnia* passes through four to six juvenile before it becomes. Produces eggs for the first time. The age at which the first eggs are deposited into the brood chamber is around 5-10 days at 20°C, but this may take longer under poor feeding conditions. Clutch sizes vary among species, from 1 t0 2 eggs in small species such as D. cucullate to more than 100 in large species such as D. magna.

Life cycle of a cyclic parthenogenetic daphnia. This depicts the sexual and the asexual life cycle of a daphnia. During the parthenogenetic cycle, females produce diploid eggs that develop directly into daughters. The same female may produce diploid asexual eggs that develop into sons. Male production is under environmental control. Furtherfemale may produce haploid eggs that require fertilization by males. These eggs are then enclosed in a protective shell and need to undergo a diapause before female offspring will hatch from them.

Daphnia Observing developmental stage of Daphnia

Although in a typical growth season *Daphnia* produce diploid(2n) eggs that develop directly and without a resting phase, a different type of egg is produced for resting . these resting eggs are encapsulated in a protective, saddle-like structure called an ephippium and which is usually strongly melanized and contains 2 large eggs, 1 from each ovary. It is not uncommon, however, to find ephippia with only 1 egg, or none at all.

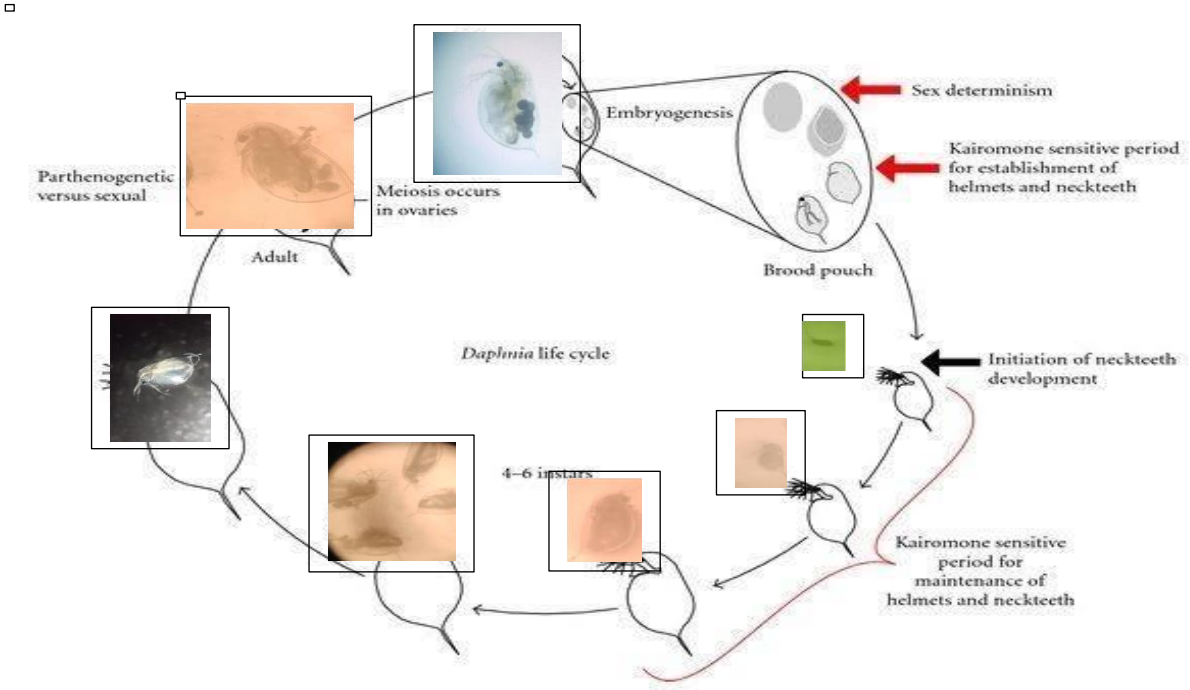
RESULT AND DISCUSSION

The number of *Daphnia* decreased since 1st day of culture. The first day was the adaptation stage for *Daphnia* to grow. Adaptation phase in culture media started from Day 0 to 3. Declamation of population occurred linearly until all of the population died. All *Daphnia* from quail treatment died on 8th day, while those the other treatment died on the 10th or 11th day. This condition was in accordance with deckaestacker et al. who reported 9-11 days as the generation time of daphnia. Life span of *Daphnia* from being in brood chamber to adulthood and death depends on the species and environment condition. With their habitat limited to standing water bodies, *Daphnia* populations are strongly subdivided. Gene flow is usually moderate, allowing populations to evolve rapidly in line with local environment conditions.

The long-lasting resting stages of *Daphnia* provide an even more compelling demonstration of the high evolutionary potential of these populations. While the majority of resting stages usually hatch during favourable conditions for the planktonic phase, some eggs may remain unhatched, though viable. With time, sediment buries these eggs, archiving them in layered sediments of the past.

Daphnia have been shown to adapt locally to a long and growing list of environmental factors, including heavy metal pollution, predators, high temperature, habitat stability, photoperiod, water salinity, UV light.

- *Daphnia* used as food source for molly fishes. The molly fishes adopted the feed conversion.
- *Daphnia* cultured under the optimum temperature(24°Cto28°C) and pH (6.5 to 8.5), and dissolved oxygen 5mg/L.
- Molly feed on *Daphnia* showed a significant resultin growth of fishes.
- Feed on daphnia, the molly fish growth rate increased with increased feed availability.
- Ensuring proper environment conditions for fish is one of the major problems in rearing fish larvae in controlled condition. Salinity in molly breeding is certainly one of these conditions.
- *Daphnia* can be seen with naked eye. But, in microscope- *Daphnia* has a discreate antennae and a bivalve carapace that encloses all or most of the trunk and abdomen.
- The improved growth rates of molly's fed *Daphnia* suggest that *Daphnia* provide high nutritional value, possibly due to their rich protein content and balanced micronutrients.
- *Daphnia* cultures were largely free from contamination due to strict aseptic techniques.
- Molly's on the commercial food diet also grow but at a slightly less vigorous active and colouration compared to while fed *daphnia.*
- The colouration in molly fishes are very dark while feeding *daphnia*.
- Molly fishes not only fed on *Daphnia* they also fed pellets, but comparatively they mostly feed on daphnia.



S NO	PARAMETERS	MEASURING DEVICES	OBTAINED RANGE	MAINTAINED RANGE
1	pH	PH Meter	6.5 to 8.5	7.0 to 7.5
2	Dissolved Oxygen	Dissolved oxygen meter	2-5mg/litre	2mg/litre
3	Temperature	Thermometer	18-24^{0}C	22^{0}C
4	Hardness	Rock well hardness texture	250 mg/litre	250-300mg/lit

CONCLUSION

I hear by conclude that,by feeding mollies with daphnia the growth rate is enhanced. To evaluate the growth rate of molly fishes (poecilia sphenops) when fed with daphnia as a primary Food source. Over the course of experiment, molly fishes exhibited a significant increase in growth rate When daphnia was provided as their diet compared to Daphnia culture has emerged as a vital component of aquaculture and freshwater ecology, offering a nutritious &healthy for aquatic animals. Through optimization of culture conditions, nutrition, and feeding strategies, Daphnia growth and nutritional value can be significantly enhanced. Effective disease management and genetic selection programs can further improve the sustainability and scalability

of Daphnia culture. As research continues to advance, Daphnia culture is poised to play an increasingly important role in supporting the growth of aquaculture and promoting freshwater ecosystem health. Future studies should focus on scaling up Daphnia culture, exploring novel applications, and addressing the challenges of commercialization to fully realize its potential.

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