

The Growth of Microalgae in Shrimp Hatchery: Impact of Environment on Nutritional Values

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Abstract: India is the second-largest aquaculture producer in the world. Like the largest producer, China, India's Aquaculture is dominated by carp production: about 80% of India's aquaculture production is composed of carps of Indian and Chinese origin. In the last 20 years, carp production has intensified in several parts of India. The challenges ahead include feed cost management which needs to be achieved through optimization of feed ingredients, and increasing farm gate price of fish through efficient post-harvest preservation and marketing techniques. Also species diversification to include higher value fish in farming will pave way for widespread feed-based farming and bring better returns to the farmer. The aim of present research study is to describe the various techniques employed for the production and application of live food organisms as well as their applications in shrimp hatcheries. The natural diet of most cultured species consists of phytoplankton and zooplankton found abundant in natural plankton. For culturing of fish and shrimp, readily and consistently available, practical and performing live diets need to be selected. From the practical viewpoint of the culturists a good diet should be readily available, cost-effective as well as versatile in application. Analysis of the economics of large-scale culture indicates that the total cost of the algae produced will be approximately proportional to the growth area. By using methods mentioned the Culture of Algae are taken, their essential Amino acids are separated and identified. During culture of microalgae environmental parameters like light, Temperature and media effect on nutritional values.

Keywords: production, Amino acids, environmental parameters.s

I. Introduction

Throughout the centuries fish has been an important component of the population's diet in many parts of the world. Fish catches increased rapidly over the past hundred years due to improved technology, which provided more powerful engines and sonar equipment. This led to over fishing and caused a worldwide decrease in wild stocks. As a result, the growth in fish catches stopped some 20 years ago. The need to increase fish production by farming became therefore an urgent matter. The term 'aquaculture' covers all forms of cultivation of aquatic animals and plants in fresh-, brackish- and saltwater. Aquaculture has the same objective as agriculture, namely, to increase the production of food above the level that would be produced naturally. Today, aquaculture is responsible for an ever-increasing share of global aquatic food production, which has increased from 3.9 percent in 1970 to 41.9 percent in 2014. Land, water and climatic conditions are probably the most important natural factors that need to be assessed. When developing a site for fish farming you should consider the effect it may have on the environment. Important natural areas (e.g. fish nursery grounds like mangrove forests) *should not be used for fish farming*. One of the most essential requirements is water availability, in terms of quality and quantity. The type of aquaculture farm and species of animals or plants that you will be able to culture will depend largely on the properties of the site. The risks involved in fish farming should also be stressed. Fish need protein in order to grow and reproduce. This means they can become competitors for products, which could otherwise be used directly for human consumption. Furthermore, the cost of production is fairly high and therefore *fish grown in ponds are not always able to compete financially with fish caught in the wild*. Setting up a fish farm involves high initial investment and high production costs as well as economic risks. Therefore, there are some very important factors a prospective fish farmer should consider before embarking on a fish farming venture. India rank 17th position in world seafood exports. Marine products contribute 1.30% of India's total exports. India is the 8th largest shrimp producer and ranks 2nd largest in aquaculture production in global scenario. The pollution free waters along the 8129 km long Indian coastline, 1.2 million hectares of brackish water area and 5.4 million hectares of fresh water area. India's growing environmental consciousness has been increase awareness in practice of water aquaculture. In India aquaculture constitutes freshwater and brackish water aquaculture; aquaculture is practiced in 9 maritime states of India. Species wise aquaculture production through India (FAO 2008) and brackish water (MPEDA statistics 2009-10) aquaculture. *p. monodon*, *p.indicus* and *L.vannamei* are the two species cultured in brackish water, this forms the bulk of export to EU and other countries. It may be seen from the data that Indian major carps namely *catla*, *rohu*, and *mrigal* are the main species which contribute major share of aquaculture production in the country and the main crustacean species culture in freshwater is *macrobrachium rosenbergii* (fresh water prawn) India has the potential to

achieve 5,00,000 tonnes of aqua-production, by 2015. Various agencies and the industry must work hand-in-hand, to bring 50,000 hectares of new area per year, into production. The need of the hour is to increase the infrastructure, such as ice plants, insulated vehicles and processing capacity. Right now, the major constraints are the lack of inputs such as SPF seeds, power, finance from banks, and the absence of insurance. All these can be solved if aquaculture is granted agricultural status, and by supplying the farmers with 100% SPF seeds, whether Vannamei or Monodon.

II. Fish Nutrition, Health And Reproduction

Fish Nutrition

There are usually two types of food available to the fish: natural and supplementary. Natural fish food consists of phytoplankton, zooplankton, periphyton, water plants, etc. produced in the pond itself. Supplementary fish feed is produced outside the pond and supplied to the fish regularly to further increase the amount of nutrients in the pond.

Natural fish food

The natural fish food in the pond largely consists of phytoplankton. The amount of phytoplankton can be increased by the addition of fertilizer to the pond.

III. Materials And Methods

Micro-Algal culture:

The isolation of required species, identification, preparation of culture media, stock culture maintenance, mass culture, harvest and preservation of the culture.

ISOLATION: Isolation of the micro-algae can be done by any one of the following methods.

- A. By exploiting the Phototactic Movements
- B. By agar plating method
- C. Serial Dilution Culture Technique

This method is widely used in hatcheries mainly for the isolation of phyto flagellates. In this method, 5 dilution steps (the inocula corresponding to 1, 10¹, 10², 10³ and 10⁻⁴ or 4 steps – [0.001, 0.01, 0.1 and 1 ml] are involved for the isolation of the required species. For the serial dilution technique, nearly 40 culture tubes (15 ml) are required. After filtering the seawater through 10 micron sieve, the filtrate has to be inoculated in five series of culture tubes in various concentrations. This has to be kept under sufficient light (1 k lux) with uniform temperature (25°C) conditions. After 15 days, some discolouration can be seen in the culture tubes, due to the growth of micro-algae. Further purification of this culture can be done by sub-culturing it in 50 ml conical flasks and then in 500 ml and one litre conical flasks. Once the culture was full, it was transferred into a 3 or 4 litre culture flask and maintained as stock culture. After the isolation of the required species in culture tubes, it may be sub-cultured again in few 50 ml test tubes.

Stock Culture Maintenance: Stock culture of all the micro-algae was maintained in a special air conditioning room adjacent to the mass culture room. Guillard medium enriched with vitamins is the ideal one suitable to maintain the stock of all the diatoms. The time required for the maximum cell concentration varied depending on the species. However, it was noticed that all the diatoms required 2 days for the completion of growth phases before entering into the stationary phase. In the stationary phase, the micro-algae was kept for a period of 1 month in the stock culture room, under controlled conditions of light and temperature, with or without aeration. When the colour of the culture turned into brown, then the culture enters into the stationary phase.

Mass Culture of Micro-algae: Large-scale culture of micro-algae, are necessary for feeding the rearing larval forms in a hatchery. The containers used for the mass culture of micro-algae are 20 litre capacity carbuoys, 100 litre trash cans, 250 litre or 1 tonne fibre-glass tanks. Fully grown culture from the stock culture room is used as inoculum for the mass culture in these containers. About 100 ml of the inoculum is used for the 2 l conical flasks, 250 ml for the glass carbuoys and 2 litres for the 100 litre trash cans which are properly lighted and aerated. Cells in the growing phase on the 9-10th day were harvested. After estimating the cell concentration and ensuring the quality and using a haemocytometer, the culture is supplied to the hatchery for the rearing operations of the larval organisms,

IV. Algal Production:

Physical and Chemical conditions: The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, salinity and temperature and aeration.

Culture Medium/Nutrients: Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the seawater. Macronutrients include nitrate, phosphate and silicate.

Parameter	Range	Optima
Temperature (0 C)	20-35	24-26
Salinity (g.1-1)	12-40	20-24
Light intensity (lux)	500-4000	2000-3000
PH	7-9	8.2-8.7

Silicate is specifically used for the growth of diatoms which utilize this compound for the production of their external shell. Micronutrients consist of various trace metals and the vitamins like thiamin (B₁), cyanocobalamin (B₁₂) and sometimes biotin. Two enrichment media that have been used extensively and which are suitable for the growth of most algae are the Walne medium and the Guillard's (F/2) medium.

Nutrients	Quantities in seawater/ 1litre
NaNo ₃	75 g
NaH₂PO₄.2H₂O	5 g
Sodium metasilicate (Na ₂ SiO ₃ .5H ₂ O)	30 g
Na ₂ EDTA	4.36 g
COBALTOUS CHLORIDE (CoCl ₂ .6H ₂ O)	0.01g
CuSO ₄ .5H ₂ O	0.01g
Ferric chloride	3.15g
MnCl₂.5H₂O	0.18 g
Na ₂ moO ₄ .2H ₂ O	0.006g
ZnSO ₄ .7H ₂ O	0.022g
Thiamine HCl	0.1 g
Biotin	0.0005 g
B ₁₂	0.0005 g

Light: As with all plants, micro-algae photosynthesize, i.e. they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity, spectral quality and photoperiod need to be considered. Light may be natural or supplied by fluorescent tubes. Too high light intensity may result in photo-inhibition. Moreover, overheating due to both natural and artificial illumination should be avoided.. The duration of artificial illumination should be a minimum of 18 h of light per day.

pH : The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. pH is accomplished by aerating the culture. In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth.

Maintenance of Cultures: Several laboratory techniques are available for isolating individual cells, such as serial dilution culture, successive plating on agar media, and separation using capillary pipettes. Bacteria can be eliminated from the phytoplankton culture by washing or plating in the presence of antibiotics. Stock cultures are kept in test tubes at a light intensity of about 1000 lux and a temperature of 16°C to 19°C. Constant illumination is suitable for the maintenance of diatoms,

Water Treatment: Seawater used for algal culture should be free of microorganisms. Sterilization of the seawater is done either by physical (filtration, autoclaving, pasteurization, UV irradiation) or chemical methods (chlorination, acidification, ozonization) is therefore required. Autoclaving (15 to 45 min. at 120°C and 20 psi, depending on the volume) is mostly applied for sterilizing the culture medium in test tubes, volumetric flasks, and carboys. Volumes greater than 20 l are generally filtered at 1 µm and treated with acid (e.g. hydrochloric acid at pH 3, neutralization after 24 h with sodium carbonate).

Algal Culture Techniques : Algae can be produced using a wide variety of methods

- Indoor/Outdoor. Indoor culture allows control over illumination, temperature, nutrient level, contamination with microorganisms and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.
- Open/Closed. Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.

Quantifying Algal Biomass: There are several ways to evaluate the quantity of algal biomass present in cultures either by counting the number of cells or through determination of volume, optical density or weight. Cells can be counted either with an electronic particle counter or directly under a microscope, using a haemocytometer. Algal cells growth rate is counted after adjusting the environmental parameters like temperature, light. This counting is done by haemocytometer. It is usually performed in slide chamber which has a film depth of 0.2 mm between the counting surface of the slide and the over lying cover slip. The counting surfaces is marked with triple lines into nine large squares, each 1 mm³ in area and subdivided into 16 small squares. The volume of the culture in

the film over lying fine large squares in thus 1mm³ and the count of the cells on fine large squares in this the count per mm³.

Media is also altered in the cultures and their cell count is also done. The media widely used in F/2 media. When F/1 & F/4 media is used change in cell count has be observed in haemocytometer. A relationship between optical density and cellular concentration can be established using a spectrophoto meter. The density of harvested algal cultures generally ranges between 80 and 250 mg of dry weight per liter.

V. Results

Yield per Unit Area: Analysis of the economics of large-scale culture indicates that the total cost of the algae produced will be approximately proportional to the growth area.

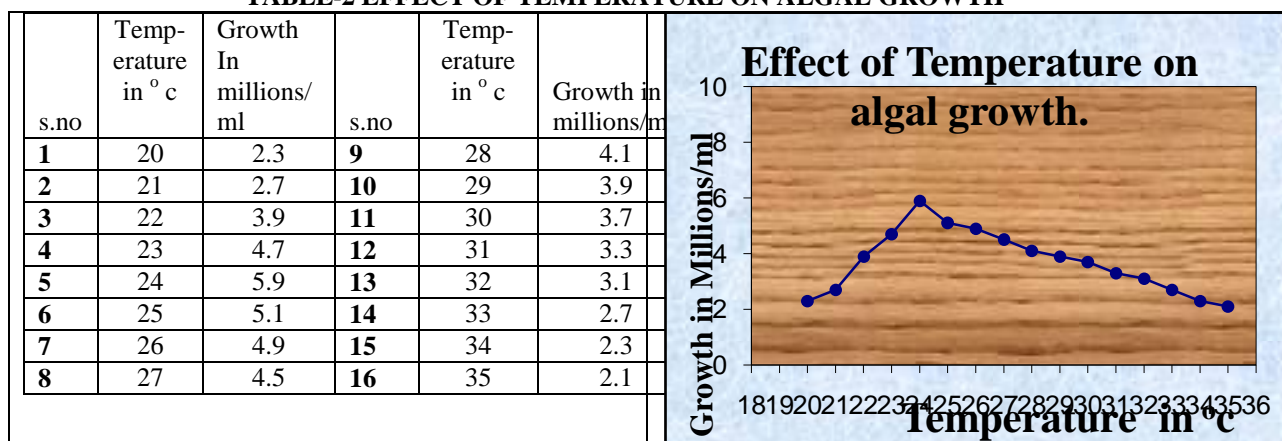
Algal species	Dry weight (pg cell ⁻¹)
Skeletonema costatum	52.2
Chaetoceros Gracilis	30.6

Effect of Environmental parameters on Algae:

By using methods mentioned the Culture of Algae and *Artemia* are taken, their essential Aminoacids are separated and identified. The Aminoacids which were identified are: 1) Iysine, 2) Histidine, 3) Arginine, 4) Threonine, 5) Valine, 6) Methionine, 7) Isoleucine, 8) Leucine, 9) Tyrosine, 10) Phenylalanine.

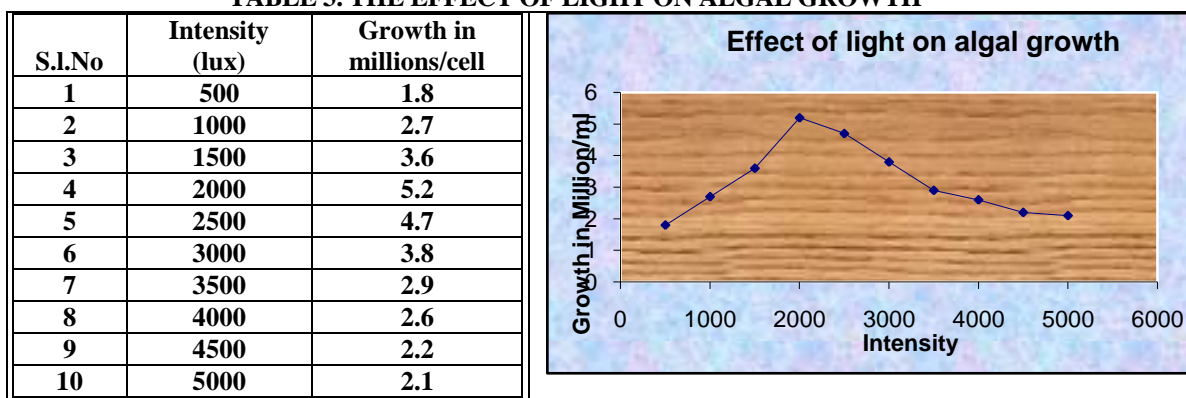
During culture of microalgae environmental parameters like light, temperature and media was altered for every 2 days. After observation against temperature changes from 20°C to 35°C maximum growth was at 25°C i.e., 5.9 millions/ml . The cell growth is minimum at 20°C and 35°C. The results were shown in table 2 and graph. In the similar way light is adjusted from 500 to 5000lux maximum cell count is observed at 2000 lux which is 5.2 millions/ml. Table 2 and graph shows detailed analysis of cell count. At optimum conditions if the media composition changed from F/1 to F/4, cell count is increased from 4.1 and decreased to 3.8 million shown in table 4 and graph.

TABLE-2 EFFECT OF TEMPERATURE ON ALGAL GROWTH



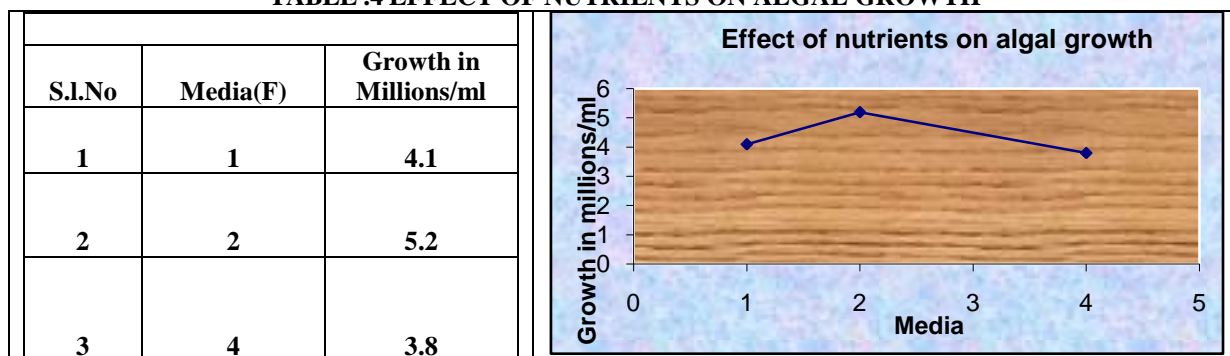
After counting the number of algal cells by haemocytometer at different temperatures, a graph is plotted against temperature (x axis) and growth (y axis). Highest count was found at temperature 24°C .Cell count slightly decreases after optimum temperature. After optimum temperature 30°C there is a rapid decrease of cell count which indicates that temperature has a major impact on the growth of algal cells.

TABLE 3. THE EFFECT OF LIGHT ON ALGAL GROWTH



A standard graph is plotted by taking intensity of x-axis and growth of cells of algae on y-axis. This results in the formation of peak which indicates that maximum growth has been observed upto 5.2 millions at optimum light intensity which is 2000 lux. Minimum growth is observed at 500 lux and 5000 lux.

TABLE .4 EFFECT OF NUTRIENTS ON ALGAL GROWTH



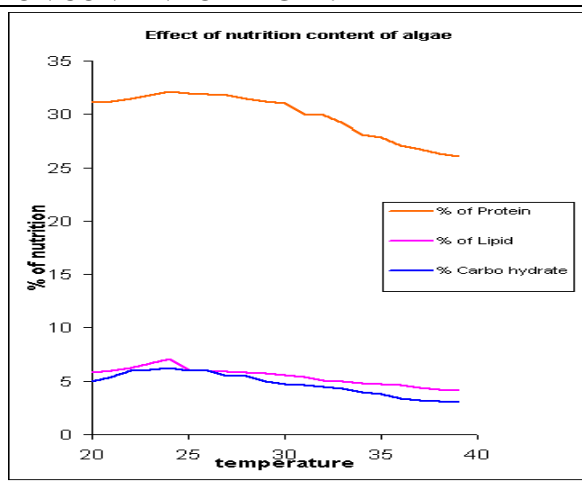
When the algae were cultured in three different F media, change in cell count has been observed. Maximum growth is observed when cultured in F/2 i.e., 5.2 million /ml. A graph is plotted against media and growth indicates that growth is maximum in F/2 media than F/1 media. As F/1 is having high concentration of nutrients, it decreases the growth of microalgae.

NUTRITIONAL VALUE OF MICRO-ALGAE

Effect of Temperature on Nutritional content of Algae: The nutritional value of any algal species depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. After estimating level of protein, lipid, and carbohydrate it is found that proteins are 32%, lipids- 7.1%, and carbohydrate 6.2%, respectively at optimum temperature. This percentage of nutrition value is shown in table and graph. These changes were observed at different temperature ranges from 20 to 39°C. Like temp, when light is changed from 500 to 4000 lux nutrition content was estimated to be more at 2000 lux and minimum at 500 and 4000 lux i.e., protein 2.1%, lipid 7.2% and carbohydrate 7.5% ,this can be shown in table and graph. In the same way media was also changed from F/1 to F/4, it is observed that protein is 34%, lipid 7% carbohydrate 6.2% in F/2 media at optimum conditions. All the above estimations were done by spectrophotometer described in methods. No abrupt changes of protein were noticed in temperature 24°C, but a significant fall in protein was observed at 20°C and 39°C. Carbohydrate and lipid breakdown was more significant at low and high temperaturez

TABLE 5. EFFECT OF NUTRITION CONTENT OF ALGAE.

S.I.No	Temperature in °c	% of Protein	% of Lipid	% of Carb0 hydrate
1	20	31.10	5.80	5.00
2	21	31.20	6.00	5.40
3	22	31.50	6.20	6.00
4	23	31.80	6.70	6.10
5	24	32.10	7.10	6.20
6	25	32.00	6.10	6.00
7	26	31.90	6.00	6.00
8	27	31.80	5.90	5.50



Today live diet which is widely applied in industrial larviculture of fish and shellfish are : Microalgae (chaetoceros, skeletonema etc.)

MICROALGAE

Phytoplankton comprises the base of the food chain in the marine environment. It is an indispensable food source in the commercial rearing of many cultivated species. Algae are further more used to produce mass quantities of zooplankton, which in turn serve as food for the larvae and adult. Algae is used directly with in larval rearing tanks, where they are believed to play a role in stabilizing the water quality, nutrition of the larvae, and microbial control. All algal species may not equally support the growth and survival of a particular filter-feeding animal. Suitable algal species have been selected on the basis of their mass culture potential, cell size, digestibility, and overall food value for the feeding animal. Various techniques have been developed to culture these food species on large scale. However, the controlled production of micro algae is a complex and expensive procedure. In order to reduce and overcome problems and limitations associated with algal cultures, various investigators have attempted to replace algae using artificial diets either as a supplement or as the main food.

Major Classes and Genera of Cultured Algal species : Today, more than 40 different species of micro-algae, isolated in different parts of the world, are cultured as pure strains in intensive systems. These Include species of diatoms, flagellated and chlorococcalean green algae, and filamentous blue-green algae, ranging in size from a few micrometres to more than 100 µm.. The most frequently used species in commercial mariculture operations are the diatoms, *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *C.calcitrans*, the flagellates *Isochrysis galbana*, *Tetraselmis suecica*, *Monochrysis lutheri*, and the chlorococcalean *Chlorella*.

The floating microscopic plants or the phytoplankton are the micro-algae which form the basic food of almost all the animals in aquatic ecosystem. Most phytoplankton organisms are unicellular and are the primary producers of organic matter in aquatic habitats. The scope of micro-algae as a possible source of protein food was recognised by the researches in the middle of the 20th century (Gopinathan, 2000). The success of a hatchery operation depends mainly on providing the required species of micro-algae. The larvae of prawns and fish prefer the diatoms as the basic food. Hence the culture of micro-algae is an essential pre-requisite for the rearing operations of shellfishes and finfishes in a hatchery system.

TABLE 6. EFFECT OF LIGHT ON NUTRITIONAL CONTENT OF ALGAE

S.I.No	light(lux)	% of Protein	% of Lipid	% Carbo hydrate
1	500	31.7	6.6	6.5
2	1000	31.8	6.9	7.3
3	1500	32	7.1	7.1
4	2000	32.1	7.2	7.5
5	2500	32	7	7.3
6	3000	31.9	6.7	7.1
7	3500	31.8	6.6	6.8
8	4000	31.6	6.8	6.5

Different intensities of light i.e., 500 lux to 4000 lux was applied to algal culture. Then the % of nutritional content is estimated by spectrophotometer. Then the data obtained a graph is plotted against light intensity (x axis) and % of nutrition (y axis) the graph indicates that slight variation has been observed than temperature maximum percentage has been found at 2000 lux with 32.1% of proteins, 7.2% of lipids and 7.5% of carbohydrates. The utility of proteins, lipids and carbohydrates is not more than temperature in changes. So percentage of nutritional content was not so effected by light.

EFFECT OF MEDIA ON NUTRITIONAL CONTENT OF ALGAE:

Media of different concentrations i.e., F/1, F/2, F/4 were prepared. Algal culture was done separately in each media. After 2 days its nutritional content was estimated by spectrophotometer. Finally a graph is plotted against media and percentage of nutritional content. .

TABLE 7 EFFECTS OF MEDIA ON NUTRITIONAL CONTENT OF ALGAE.

S.I. No	Media (F)	% of Protein	% of Lipid	% Carbo hydrate
1	1	30.5	6.5	6
2	2	34	7	6.2
3	4	29.5	6.2	5

The graph obtained indicates maximum percentage of protein, lipid and carbohydrate is observed in F/2 media than F1 & F4. This implies that media utilization will also effect the percentage of nutrition in the cells of algae

VI. Discussion

1. Attention was paid on to environmental parameters such as temperature, light, and nutritional suipplement to the algal culture on their counts and multiplication
2. The environmental parameters like temperature light and salinity not only effect the and cell count but also it influences on the synthesis of Aminoacids. The identified Aminoacid are mentioned in results.
3. The optimal temperature for phytoplankton cultures is generally between 20°C and 24°C, Most commonly cultured species of micro-algae tolerate temperatures between 16°C and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 30°C are lethal for a number of species. Algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air- conditioning units. Temperature will influence more not only on cell count of algae but also the percentage of nutritional content i.e., decreased at low and high temperatures

4. Marine phytoplankton is extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water, Salinities of 20-24 g.l⁻¹ have been found to be optimal.
5. The optimum growth for algal species is 2000 lux maintained in indoor cultures of microalgae culture light is an important factor for the growth of algae. It effects the not only cell count but also the percentage of nutrients in the cells when light is altered nutrients compositions are utilized. Carbohydrate is more consumed than lipid and carbohydrates.
6. Nutrients metabolism and cell count identified by alterations of the compositions of media from F/1 to F/4. This results in increased cell count (5.2 millions) than F/1 and F/4 when culturing in F/4 percentage of carbohydrate used is more than lipid and carbohydrate. It was identified in graph which was plotted after 2 days.
7. Micro-algae is an essential food source in the rearing of all stages of marine bivalve molluscs (clams, oysters, scallops), the larval stages of some marine gastropods, larvae of several marine fish species and penaeid shrimp, and zooplankton.
8. The amount of hatched *Artemia* cysts to feed will depend on the density of shrimp in the tanks. Start feeding during mysis, 3. 1 gram of dry cysts of 90% to 95% hatch rate will yield 218,700 Nauplii or approx. 5 cc's. A 50 ton tank will require 50 grams dry weight, which will yield approx. 10,900,000 Nauplii or approx. 250 cc's on the first day and increase of 5-20% per day depending on the consumption to a maximum of 454 grams of dry cysts per day. Divide the total amount into several feedings throughout the day.
9. Algae are added during the non-feeding nauplius stage so that algae are available immediately upon molting into the protozoa stage. Algal species most often used are *Chaetoceros gracilis*, and *Skeletonema costatum*. As feeding preference changes from primarily herbivorous to carnivorous during the mysis stages, the quantity of algae is reduced.

VII. Conclusion

Aquaculture is one of the commercial practices done to culture shellfish, finfish, shrimps, prawns etc. In shrimp hatcheries live feed like Microalgae cultured, which has an major impact on the growth of shrimps. The microalgae are cultured in indoor cultures and outdoor cultures. Its growth can be estimated by haemocytometer. The growth rate on cell count can be used to analyse the amount of algae to be given to hatcheries. When different environmental conditions are altered i.e. temperatures, light, salinity, ph; variations in the cell count and nutritional content has been observed which was shown graphical representation. These parameters also influence the hatching percentage and nutritional value of Algae. From the results obtained it is found that for both Algae are highly influenced by environmental conditions. At optimum conditions maximum cell quality and quantity is observed than at minimum and maximum environmental conditions. The present study shows how the growth of live feed influences the growth of shrimp in hatcheries. If researchers would find better techniques to improve the quality of cell environment, we expect good quality live feed to the aquaculturist.

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