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MICRO-ALGAL CULTURE FOR FISH FOOD PRODUCTION

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National Institute for Freshwater
Fisheries Research, New Bussa

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ABSTRACT

The paper defines Micro-algae and states its ecological importance especially in the wild and as a major feed for the mass culture of zooplankton needed to feed fish larvae, fry and fingerlings in fish hatcheries. Methodologies for micro-algal isolation and culture are discussed in relation to nutrient requirements, and culture techniques. The use of the haemacytometer as a handy tool for the counting of micro-algae is also described.

1.0. INTRODUCTION

Micro-algae, otherwise known as phytoplankton, are microscopic free-floating aquatic plants that are responsible for primary production in aquatic ecosystems. In this wise, they occupy the very bottom of the aquatic food chain, providing the food and other basic necessities of life for organisms higher up in the food chain. The micro-algae is the basic and direct source of food for primary consumers such as the different species of micro-crustecean zooplankton. These micro-crustecean zooplankton are in turn fed upon by organisms higher up in the food chain notably fish. Other organisms that depend on micro-algae as direct source of food include bivalves (snails) and shrimps.

A simplified ecological relationship of these organisms could be represented as shown below:

Phytoplankton	Zooplankton	Fish
A	B	C

Ecologically, the population density of the micro-algae (A) is controlled by the amount of nutrient concentration notably, phosphates and nitrates available in the water. On the other hand, the abundance of B is usually dependent on the abundance and quality of A, while the growth, survival and well being of C is among other factors is a measure of the availability and abundance of B. Sketches of some aquacultural species of micro-algae and zooplankton are shown in Figure 1A and 1B respectively.

Micro-algae are cultured for a number of purposes (Darley, 1982). Some of these include:-

(1) As food for humans e.g. Spirulina

(2) For glycerol production e.g. Dunaliella

(3) As a high-priced health food especially in Japan.

(4) For use in a multi-purpose system such as a sewage treatment plant in which an algal-bacterial biomass produced on waste water is used by filter feeding fish or bivalves which are consumable foods by humans, and lastly and most importantly

(5) As food for zooplankton needed to feed fish fry and fingerlings in fish hatcheries. This paper is concerned with this latter purpose.

The availability of suitable micro-algae needed to raise zooplankton has been identified as a major bottleneck in induced breeding operations in fish hatcheries (Fox, 1983; Ovie et al, 1986). This is so because, the microalgae are needed to grow the zooplankton which is the preferred natural food for the young growing fish especially at the fry and fingerling stages. These 'baby' fish have been shown to live and grow better on living natural food especially zooplankton than on non-living or artificially formulated feeds such as bean and groundnut cakes, rice bran etc (Kirk and Howel, 1972).

In general, culturable micro-algae for aquacultural purposes fall into two main groups viz.: the greens (Chlorophyceae), and the diatoms (Bacillariophyceae). The third group, i.e. blue-greens are often not used as many are not only toxic but are difficult to digest because of their characteristic outer coat of mucilage. They are therefore of low nutritional quality.

As stated earlier, to start a zooplankton culture, a suitable micro-algae is a pre-requisite. In developed and advanced aquacultural countries, such micro-algae could easily be obtained from Biological Companies maintaining algal banks. Such micro-algal banks are absent in most developing countries like Nigeria. Thus Ovie et al (1986) identified the first major task in starting a zooplankton culture in developing countries as that of isolating suitable species of the micro-algae from the wild.

This first report therefore, covers the methodology for micro-algae isolation and culture under a hatchery management programme. In general, three important factors which must be considered in micro-algal cultures are nutrient medium, micro-algal source and culture techniques. These, along with methods for micro-algal counts are discussed under various sections in this report.

2.0. MICRO-ALGAL CULTURE

3.1. Nutrient Medium

According to Krauss (1953), the nutrient medium is a dilute chemical environment from which the growing algae obtains its raw materials for structural and protoplasmic synthesis. In this wise, the nutrient medium is to the growing algae, as typical fertilizers such as N.P.K, D.A.P, C.A.N etc are to arable crops such as maize guinea corn, rice, millet etc. Some of the media commonly used in cmicro-algal culture include:

(i) Chu 10 (Chu, 1942)

(ii) Pratt (Pratt, 1940)

(iii) Guillard and Ryther's modified F medium (Guillard and Ryther, 1962)

(iv) Walne's medium (Walne, 1974)

(v) Hirata medium (Hirata, 1977)

The first two nutrient media have been used by the authors to culture Scenedesmus under the Institute's Natural Fish Food Production Programme. The composition of these two nutrient media and that of Guillard and Ryther are reproduced in Tables 1, 2 and 3.

As is evident from the tables, algal culture media are composed of numerous reagent grade chemicals. In general, these chemicals fall into four major groups:

(i) Macro-nutrients such as nitrates, phosphates and silicates

(ii) Micro-nutrients such as chlorides and sulphates

(iii) Trace metals such as zinc and cobalt

(iv) Chelators such as EDTA and Citric Acid. Chelators are typically micro-elements, but their essential function is to keep certain essential trace metals in solution so that they can be available to the growing algae.

Vitamins, especially biotin, thiamine (B_1) and cyanocobalamin (B_{12}) are also commonly used in algal cultures. However, their non-availability should not stop the production process as good results have been

obtained in our laboratory without their inclusion. Fox (1983) also indicated that the use of vitamins is not essential in mass cultivation of all algal species.

2.1.1. Nutrient Stock Solution

A look at Tables 1, 2 and 3 will show that the weight of each chemical needed to constitute a culture medium is very small. Consequently, weighing is not only difficult but also requires a very sensitive weighing balance. In fact, it is impossible to weigh some, given the low sensitivity of weighing balances available. To get around this problem of having to frequently weigh small amounts of reagents, or not being able to weigh some at all, concentrated stock solutions are usually prepared from which small volumes corresponding to the desired concentration are taken. This is done by increasing the weight/litre of the various reagents. An example is given below with the first reagent (Sodium nitrate) in Chu 10 medium (Table 1) where stock solution is prepared at x 100 strength.

Component	Formula	Wt.g/l	Wt.g/l for a 4-litre stock	Wt. g/l for a 4-litre stock at x 100 strength
Sodium nitrate	$NaNO_3$	0.0413	0.1652	16.52

This 16.52g could be conveniently weighed and dissolved in four litres of distilled water to give a x 100 stock solution. This means that for every litre of culture medium, only 10ml of the concentrated stock solution is required. Other reagents are similarly treated, and in this way, frequency and time of weighing are not only reduced, but reagents are handled less often, thus, reducing the potential of contamination.

2.2. Sources of Micro-Algal Species

There are two main sources of obtaining micro algal species needed for aquaculture viz: commercial sources and laboratory isolation of locally available species.

2.2.1. Commercial Sources

As earlier mentioned, desirable species of micro-algae are obtained from Biological Companies maintaining algal banks, or from non-profit sources such as culture collections of individual Algologist. Such orders, usually received as a small ampoule, are then bloomed to larger volumes in the laboratory using appropriate culture nutrient medium.

2.2.2. Isolation of Locally Available Species

Aujero (1981) and Fox (1983) listed a number of techniques for micro-algal isolation from mixed micro-algal population. Some of these techniques which are not described in details by the authors include:-

(i) Capillary pipetting: This involves using small bore-size pipettes to separate large, single-celled spe des from chained species such as members of the blue greens.

(ii) Agar plating: This is a micro-biological technique which involves streaking 1 - 3 drops of the mixed phytoplankton population on a solid agar medium. Such agar medium, enriched with an appropriate nutrient medium allows the growth of the various species of algae present in the mixture. This method was tried by the authors in laboratory but it was not successful because of bacterial infestation. A sterile condition is necessary for this isolation technique.

(iii) Dilution followed by capillary pipetting: This involves continued dilution of the mixed phytoplankton population to a point where the subject species is well separated from any associated contamination. This technique has been shown to work well with motile species such as flagellates.

(iv) Repeated subculture technique: As the name suggestes, this method involves repeated subculturing of mixed phytoplankton population on a liquid nutrient medium. This technique has been successfully used in our laboratory to isolate pure species of Scenedesmus and it is described in detail in the next section.

2.3. Culture Techniques

2.3.1. A Procedure for Micro-algal Isolation Using the Repeated Subculture Technique

(1) Collect water containing mixed phytoplankton population from an appropriate green environment with a clean uncontaminated small plastic container of about 1 litre capacity and bring to the laboratory.

(2) Put a drop of raw water sample on a slide and examine under the microscope. If species present are predominantly blue gree, discard sample and look for another appropriate water source. If the greens or diatoms predominate, proceed to 3.

(3) Dispense appropriate volume of culture medium into culture flasks by using clean uncontaminated pipette.

(4) Using a measuring cylinder, measure 1.5 litres of filtered natural water, or distilled water or aged tap water and add to culture nutrient in flask. Aged tap water is tap water that has been left standing for sometime or aerated to loose its chlorine content. It should be noted that filtered natural water is always recommended for cultures if available.

(5) Measure a certain volume of the raw water sample containing the mixed phytoplankton population and add to the culture flask just enough volume to colour the flask water faintly green (250 ml of sample was used in our isolation).

(6) Aerate and illuminate isolation flasks by using micro-air pumps and fluorescent tubes respectively. (see section on "Some Culture Practices" for details of aeration and illumination).

(7) Observe isolation flasks for 2 - 3 days and at the end of which you remove 200 ml of the culture and inoculate into another isolation flask containing fresh nutrient medium. Discard content of previous flask after microscopic examination of the culture.

(8) Repeat step 7 (i.e. continue the subculturing process until a unialgal culture is obtained).

(9) Bloom mono-species (i.e. unialgal culture) to obtain high concentrations of micro-algae to serve as algal stock for starter cultures.

A diagramatic representation of the repeated subculture technique is shown in Figure 2. The technique is based on the dominance of one species and its ability to gradually eliminate all other species from the population.

Once a desirable species is obtained, it should be bloomed to high densities by using larger culture vessels. In this way, part of it could be used for continuous regular cultures while some of it is stored as stock for starter cultures whenever desired. Furthermore, the growth characteristics of the isolated algae should be investigated immediately for reasons stated in the next section.

2.3.2. Growth Curve Characteristics of Isolated Algae

To the fish breeder, a good knowledge of the growth curve of the isolated algae is very important as this guides the culturist as to the time of utilization of the algal population for the specific purpose for which it is grown.

Fogg (1965) identified four distinct stages associated with algal growth curves (Figs. 3 a and b). The lag phase is the first stage, and this is when the algal population remains temporarily unchaged on addition of the inoculum to the nutrient. The cells at this time increase in size with addition of new protoplasm, preparatory to cell division. The second stage is the exponential phase and this is when the cells divide rapidly and steadily at a constant rate. Given optimal conditions, growth rate is maximal here. The stationery phase is the third stage and this is when the growth rate gradually levels out and the population remains relatively constant due to equilibrium between reproduction rate and death rate. The fourth stage is the death phase and this is when the cells die faster than the rate at which new ones are produced.

Utilization of the algal population such as inoculation with zooplankton, bivalves and shrimps or harvesting for preservation, is usually done at the exponential phase (preferrably before the end of the exponential phase when the cells are still young, viable and actively dividing).

2.3.3. Procedure for Running Growth Curve Experiment

(1) Using a stock culture, bloom algal cells in an appropriate medium for about 2 - 3 days using a two litre culture flask or any other appropriate small culture vessel.

(2) Count algal cells by use of a haemacytometer (see section under algal count for details).

(3) At a known initial desired density (50,000 cells/ml is used in our laboratory) measure a calculated volume of inoculum and use to inoculate culture flask as previously described under isolation.

The volume of inoculum is calculated according to the formula of Escritor and Javellana (1981) in which

$$N = \frac{X}{Y} \times V$$

where N = volume of inoculum

X = initial desired density

Y = algal count at source i.e. count obtained from (2) above

V = volume of culture vessel

Note: The units of volumes must be the same.

(4) Aerate and illuminate cultures as described under "Some Culture Practices".

(5) Take culture sample (2 ml is enough) with a clean uncontaminated pipette every four hours or daily and put into a small vial bottle. Preserve in Lugol (iodine) solution, and label properly stating time or date of taking sample.

(6) Estimate algal density by use of haemacytometer.

(7) Plot algal density as a function of time (hours or days) and identify the various stages as shown in Figures 3a and 3b.

Note: Once the time it takes the culture to get to the exponential phase is known, and the initial desired density is kept constant, the procedure does not need to be repeated everytime inoculation with zooplankton is required. This is so because the procedure is to a large extent reproducible as Ovie et al (1986; 1987; 1988) have found population peaks of 156 x 10^4 cells/ml, 173 x 10^4 cells/ml and 176 x 10^4 cells/ml respectively, for Scenedesmus cells. Periodic check is however, advised.

2.4. Culture Vessels

Culture vessels for algal culture could be conical, cylindrical or rectangular, and the volume of each vessel depends on the magnitude of culture and feeding to be performed.

In our laboratory, small scale cultures are initiated with 2-litre flat bottom flasks and these are used as inoculum for bigger volume cultures such as the 30 - 45-litre glass aquaria. Several of these glass aquaria are then used as inoculum for concrete tanks. The concrete tank should usually be filled below the 1m mark, preferrably between 0.5 - 0.7 m in order to ensure maximum light penetration and ease of manual stirring if mechanical aerators are not available. Fibre glass materials are also commonly used in constructing culture vessels.

2.5. Some Culture Practices

Two very important micro-algal cultural practices worthy of mention are aeration and illumination, and these are discussed here.

2.5.1. Aeration

Aeration is the act of bubbling gaseous air into live cultures. It serves various functions among which are:

(i) Dispersion of dissolved nutrients in solution thus preventing settling of the nutrients.

(ii) Making CO_2 and O_2 readily available to the photosynthesizing algal cells.

(iii) Preventing stratification (i.e. non-uniform distribution) of algal cells.

(iv) Prevention of adherence of the algal cells to the sides of the culture flask thus reducing shading by peripheral cells.

Uhlig (1981) has shown that aeration increases the cell size of organisms. Heisig (1979) compared the effect of aeration of algal and zooplankton development in two ponds in which one was aerated and the other not. It was found that aeration increased algal production and consequently led to increased egg production in Daphnia. The number of egg-bearing females and the number of eggs per egg-bearing females was found to be considerably higher in the aerated than in the unaerated ponds. It is, therefore, highly recommended that cultures should be aerated especially in indoor hatcheries where atmospheric aeration is almost nil.

2.5.2. Types of Aerators

There are various types of micro-air pumps under different trade names that are suitable for aerating small-scale indoor cultures. Some of the aerators we have used in our cultures and are found to be relatively reliable include:

(i) HYFLO model B air pump

(ii) Hagen Elite 802

(iii) WR-A_1 air pump, and

(iv) Golden GMS air pump.

Most of these micro air pumps could be obtained from the following local sources.

(1) OX_4 Aquarium, Abbe Road, Onipanu, P.O. Box 49, E.B. Tel. 820303.

(2) Arroways (Nig) Ltd, 71 Airport Road, Ikeja, Tel. 961278

(3) Aquarium Designers and Manufacturers, 19, Tonode Street, Ikeja, Tel. 964261.

(4) Rock Water Fish Farm, Jos, Plateau State.

Note: All aerators and airline tubes must be located above the water level of the culture to prevent damage to aerators caused by water-back-flow into aerators during power failure.

For large volume aeration such as large indoor and outdoor concrete tanks, more powerful aerators are required. For more details contact, TALON (Nig) Ltd, 12/14, Aerodrome Road, Apapa, Lagos, Tel. 875962.

2.5.2. Illumination

Increase in algal cell number is a direct function of the rate of photosynthetic activity. Photosynthesis requires light energy and in indoor cultures, this light energy is provided by direct illumination with cool, white fluorescent tubes. In our laboratory cultures, illumination is done by using three pairs of 4-ft, 40W cool, white fluorescent tubes. A pair each illuminates the cultures from the sides, while the third pair illuminates the cultures from the top. The side pairs are fitted to wooden platforms while the top pair is usually hung from the ceiling using appropriate nylon rope or iron chain.

3.0. METHODS FOR COUNTING ALGAL CELLS

Algal cultures usually contain high densities of algal cells per unit volume. Consequently, estimating the density of such sample which usually runs into millions of cells/ml is not an easy task. However, various counting techniques have been developed to achieve this purpose. Some of these include:

(i) Use of Coulter counters;

(ii) Use of turbidimeters;

(iii) Spectrophotometric analysis of chlorophyll a; and

(iv) Haemacytometric technique.

The first three methods require a great deal of technological expertise and expensive instruments and do not allow for visual microscopic examination of the culture which is useful in detecting possible contamination. Although, the spectrophotometric method has been used to some extent, the haemacytometric technique is our routine procedure and is hereby described in details. A brief description of the haemacytometer is first given.

3.1. The Haemacytometer

The original use of the haemacytometer is for counting blood cells. However, because of the microscopic size of the micro-algae, this instrument has been adapted to count algal cells as well.

The improved Neubauer haemacytometer is a thick rectangular slide with an H-shaped trough forming two main counting chambers; one on each end of the H-figure. A sketch of the haemacytometer indicating the various parts is shown in Figure 4.

Each main chamber has a total of nine smaller chambers or counting grids. Each grid is 1mm x 1mm x .1mm giving a total volume of $0.1mm^3$. The smaller chambers or grids at the corners of the main chambers are sub-divided into sixteen small squares each with an area of $0.0625\ mm^2$ (i.e. .25 mm), while the center grid is sub-divided into twenty-five squares each with an area of $.04\ mm^2$ (.2 mm x .2 mm).

3.2. Procedure for Use of the Haemacytometer for Algal Count

3.2.1. Preparing sample and haemacytometer

(1) Shake culture flask and quickly withdraw about 2 ml sample with a clean uncontaminated pipette.

(2) Put into a labelled bottle and add a drop or two of Lugol's solution.

(3) Clean haemacytometer and cover slip thoroughly with lens paper or good quality tissue paper wetted with alcohol.

Note: Instrument should be free of grease or lint.

(4) Place cover slip on haemacytometer.

(5) Shake sample bottle very well and quickly obtain a sample from it with a clean pipette, or any other suitable device such as dropper.

(6) Load both chambers of the haemacytometer by placing a fine drop of the sample on the loading port or groove. The sample is quickly drawn into and dispersed into the counting chamber by capillary action.

3.2.2. Counting of Algal cells

(1) Place haemacytometer on stage of inverted microscope or any other suitable compound microscope.

(2) Using an appropriate magnification, adjust microscope until a good and clear view of cells is obtained. If cell density is not too high (< 100 cells/grid) proceed to (3).

(3) Count all cells in 2 or more of the four outer grids as shown in Figure 4, and compute algal density as shown below:

$$\text{Algal cells/ml} = \frac{\text{Total algal count}}{\text{No. of grids counted}} \times 10^4$$

If cell density is high (> 100 cells/grid) proceed to (4).

(4) Count cells in the five small squares (1 - 5, Fig.4) in the center grid E. Repeat count in the second main chamber and compute algal density as shown below:

$$\text{Algal cells/ml} = \frac{\text{Total algal count}}{10 \times 4 \times 10^{-6}}$$

where 10 = the ten squares of the two main chambers; and

4×10^{-6} = the volume of sample over the small square area which is equivalent to 0.004 mm^3 (.2 mm x .2 mm x .1 mm) expressed in cm^3 (ml).

4.0. CONCLUSION

Studies on the culture of micro-algae as feed for zooplankton needed to feed fish fry and fingerlings in hatcheries is very new in developing countries like Nigeria. Live natural fish food such as the zooplankton, has benn shown to be the preferred food of the products of induced breeding in fish hatcheries as the survival rate of these "baby" fish is enhanced by the adequate provision of these natural food. Currently, most fish hatcheries in the world depend mainly on the use of Artemia as a natural feed. Artemia is not only a marine copepod zooplankton, but it is also imported and so needs foreign exchange to procure. This no doubt, will considerably add to the overall cost of hatchery production.

There is therefore, the need to develop requisite methodologies for the mass production of our indigenous freshwater zooplankton as feed for fish fry and fingerlings in fish hatcheries. This report "Micro-algal Production for Fish Food Culture", therefore, represents the first step in achieving this noble scientific goal. It is my hope and desire that this document will serve to stimulate a concerted effort towards research on this subject matter.

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Table 1: Composition of standard Chu No.10 nutrient media

Component	Formula	Wt. g/lit.
Sodium Nitrate	$NaNO_3$	0.0413
Sodium Hydrogen Phosphate	Na_2HPO_4	0.0083
Potassium Chloride	KCL	0.0086
Magnesium Chloride	$MgCL.6H_2O$	0.0309
Sodium Sulphate	Na_2SO_4	0.146
Calcium Chloride	$CaCL_2.2H_2O$	0.0359
Ferric Citrate	$C_6H_5O_7fe.5H_2O$	0.0030
Citric Acid	$C(OH)\ (COOH)\ (CH_2COOH)\ _2H_2O$	0.0030
Sodium Carbonate	Na_2CO_3	0.0030
Sodium silicate	$NaSiO_3$	0.020

Note: Silicate is only required for diatom culture, as it is necessary for the formation of their cell wall.

Table 2: Composition of standard Pratt nutrient medium

Component	Formula	Wt. g/lit
Potassium Nitrate (0.025M)	KNO_3	2.5
Potassium hydrogen Phosphate (0.018M)	KH_2PO_4	3.16
Magnesium sulphate (0.02M)	$MgSO_4.7H_2O$	4.60
Ferrous sulphate (0.00001M)	$FeSO_4.7H_2O$	Pinch
Potassium citrate (0.00001M)	$C_6H_5O_7K.5H_2O$	Pinch

Table 3: Chemical composition of Guillard and Ryther's modified F. Medium

Component	Formula	Wt. (g/lit)
Sodium Nitrate	$NaNO$	.084148
Sodium hydrogen phosphate	$Na\ H_2PO_4H_2O$	.010
Ferric Chloride	$FeCl_3.6H_2O$	.0029
Sodium EDTA	Na_2 EDTA	=.1=
Sodium Silicate	$Na_2Si0_3.9H_2O$	.050
Trace metals:		
Copper sulphate	$CuSO_4.5H_2O$	0009196
Zinc sulphate	$ZnSO_47H_2O$	000044
Cobolt Chloride	$CoCl_2.6H_2O$	00001
Manganous Chloride	$MnMoO_4.3H_2O$	0.0136
Vitamins:		
Thiamin HCL	(Vit. B_1)	0002
Cyanocobalamine	(Vit. B_{12})	000001(i.e 1 ug)
Biotin		000001(i.e 1 ug)

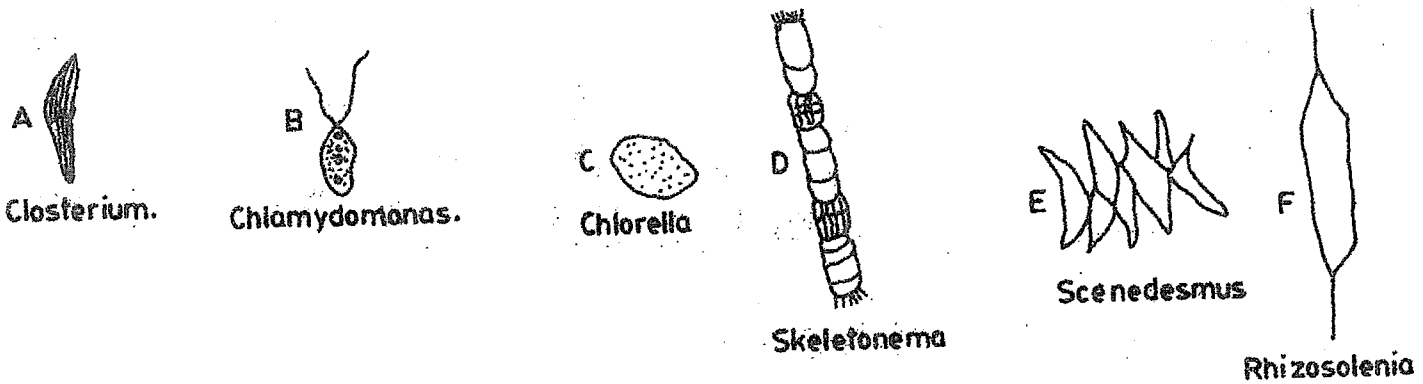


Fig. 1a. Sketches of some common aquacultural micro-algae.

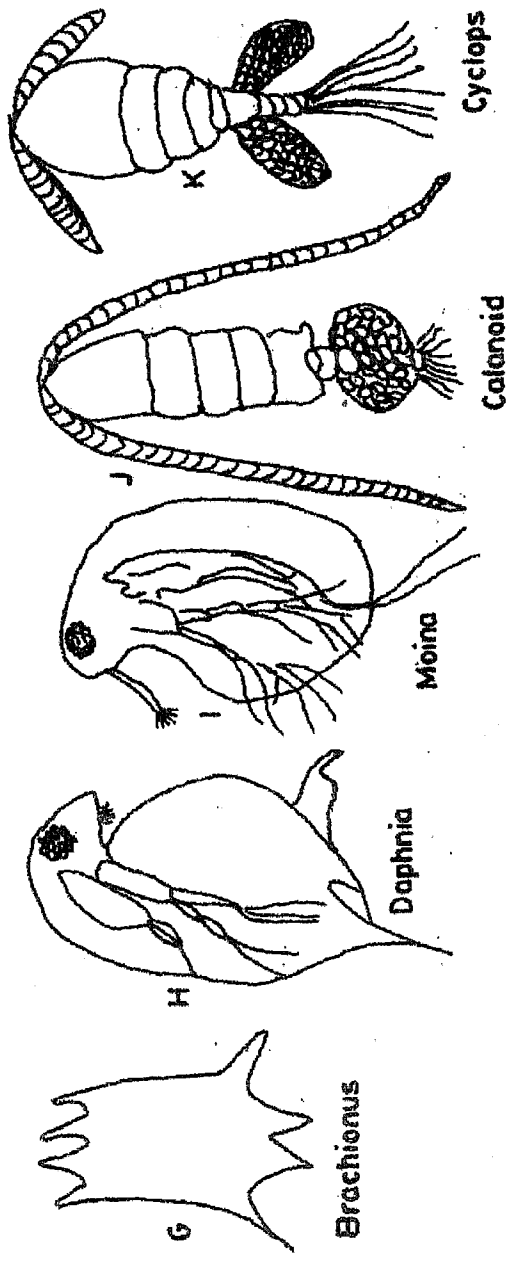
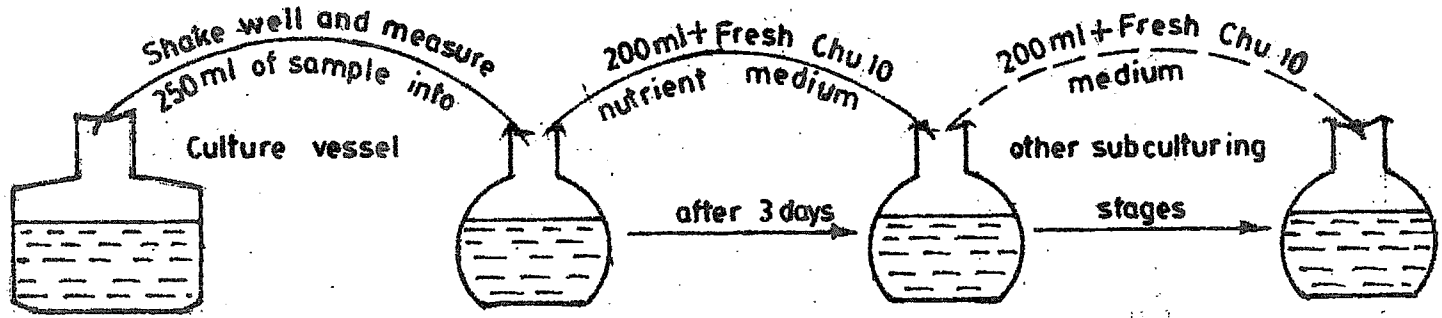


Fig. 1b. Sketches of some zooplankton fish food.



Container containing mixed plankton population from the wild.	1st culture vessel containing 250ml sample+Chu 10 nutrient medium.	2nd culture vessel containing 200ml sample from first culture+ fresh Chu 10 medium.	Final culture containing a unialgal species.

Fig. 2. Stages in the isolation of a unialgal species from a mixed phytoplankton population (Adapted from Ovie et al 1987).

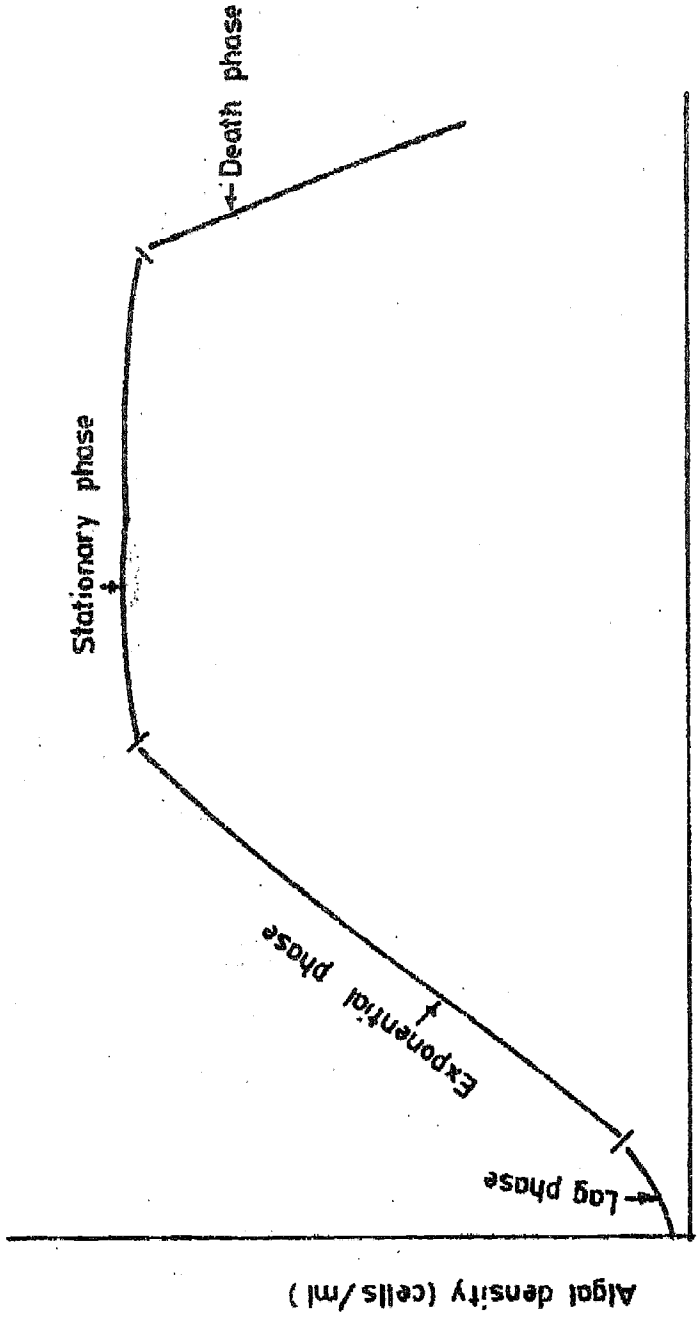


Fig. 3a: Smoothed typical Algal growth curve

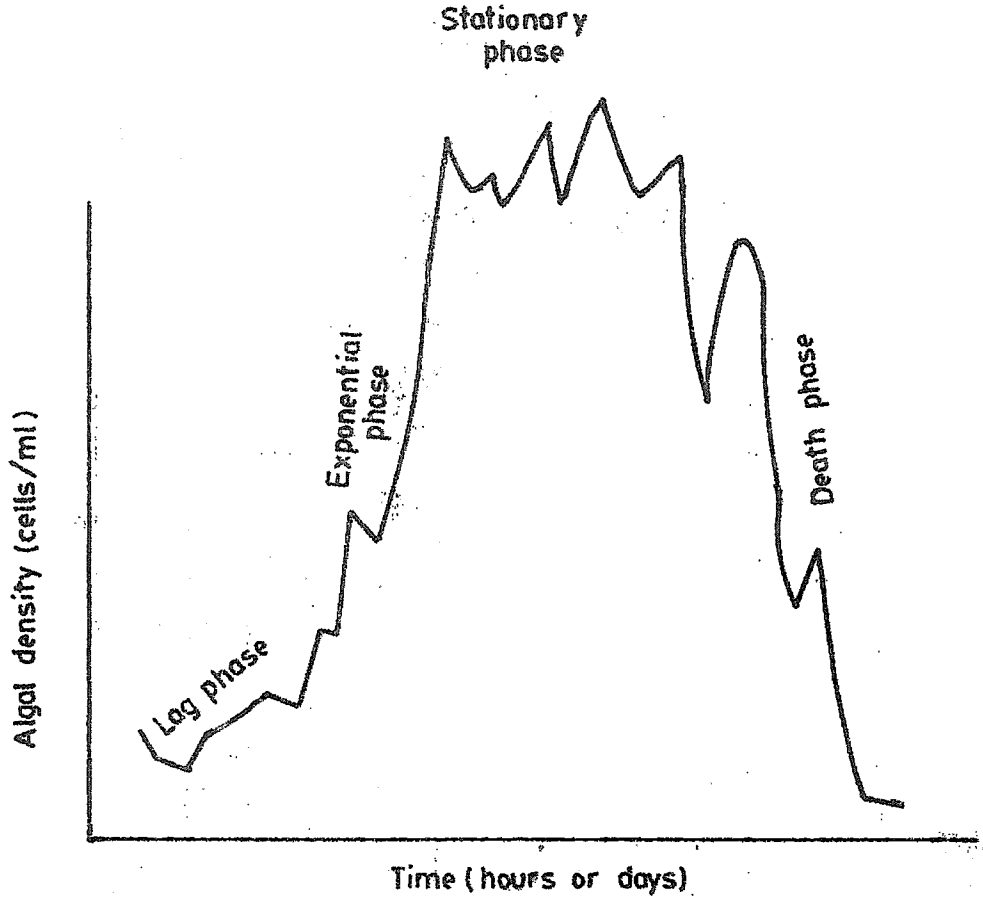


Fig. 3b: Unsmoothed typical Algal growth curve. Adapted from Anderson and Smith (1983).

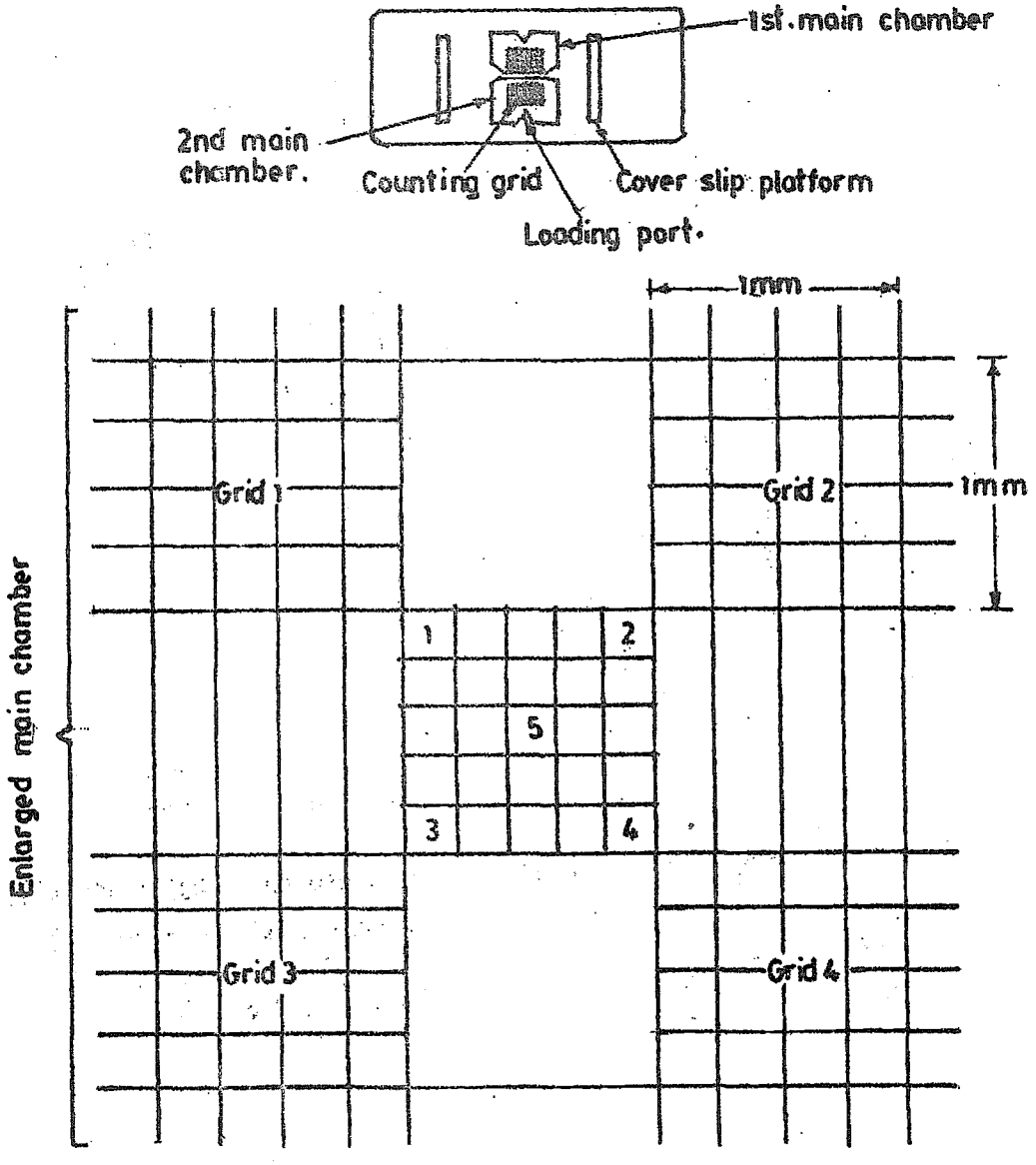


Fig. 4. The new improved Neubauer haemacytometer (above) with one enlarged counting main chamber (below). Adapted from Fox 1983.

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