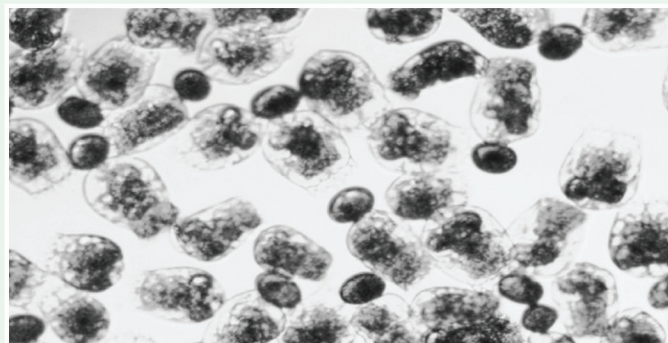




Rotifer cultured on *Nannochloropsis oculata* concentrate for marine finfish larval rearing

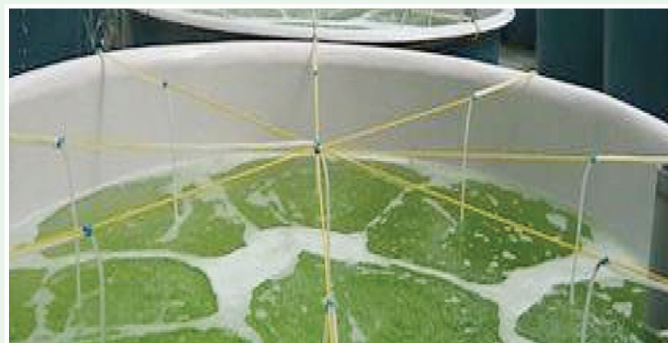
Nanno concentrate as inoculum for *Nannochloropsis oculata* culture:

The preserved nanno-concentrates (0 to 20% of glycerol in chilling) are diluted with sterilized sea water to obtain a cell count of 1×10^6 /ml. A 10% of inoculum is added into 10 ml of culture medium containing Conway medium. The culture is maintained at optimum environmental condition for the stock culture development. It is concluded that at the end of third day of inoculation, the cell count reached 12×10^6 cells / ml in culture and the same stock is used for further development.

The glycerol preserved nanno concentrate is diluted and is also used as direct inoculum to prepare intermediate culture.

Nanno concentrate for Green water system in larval rearing

To maintain green water system for marine finfish larval rearing, an approximately 3.5g of nanno-concentrate is added into 1 cubic meter water volume, and this maintained the nanno concentration of 1 lakh nanno cells / ml in suspended condition, which also maintained the water quality during the period of larval rearing.



Green water rearing system (*Nannochloropsis oculata*) for finfish larval rearing

Economics for production of *Nannochloropsis oculata* concentrate

Product development is dependent on the economic viability of the product and therefore, the cost of production for producing 1 kg of nanno-concentrate was calculated. The cost (fixed and variable) involved is presented in the table.

Cost (₹) of producing 1kg *Nannochloropsis oculata* concentrate

Item	Quantity	Cost (₹)
Depreciation:	A	-
	B	-
Inoculum (@ ₹ 360/100ml)	1.0ml	3.6
Culture (Conway) medium: (@ ₹ 0.27/ml)	480ml	129.6
Gas: CO ₂	-	50
Electricity @ ₹ 4/unit	115.71	462.84
Labour @ ₹ 300/day	(1.5day)	450
Total operating cost (₹)		1158.47

Depreciation

A. Equipments (₹37.96): Dry oven (0.28) + Weighing balance (0.85) + Air blower (5.26) + Autoclave (1.03) + Centrifuge (23.97) + Air condition (6.57).

B. Culture material (₹24.47): 250ml conical flask (2.56) + 3L conical flask (6.10) + 20 L Carboy (1.02) + Air hose (6.58) + Air stone (4.11) + 20watt florescent lights (4.10).

Electricity (kW) - Dry oven (1.0) + Air Blower (0.21) + Autoclave (3.0) + Centrifuge (96.0) + Air condition (9.6) + Distillation (0.15) + Florescent light (5.75) = 115.71 @ ₹ 4.0/kW.

Consultancy Services offered by CMFRI:

Live feed (microalgae, rotifers, copepods and *Artemia*, etc) culture technology for marine finfishes and shell fishes.

Brood stock development and seed production technology for selected marine finfishes

Cage culture technology for marine finfishes

Pond culture technology for marine finfishes

RAS design, construction and operation

RAS based broodstock development and nursery rearing of marine finfishes

Human resource development in live feed culture, marine finfish seed production and marine finfish culture (cage, pond and RAS)

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NANNOCHLOROPSIS OCULATA CONCENTRATE (NANN CON)



**VISAKHAPATNAM REGIONAL
CENTRE CENTRAL MARINE
FISHERIES RESEARCH INSTITUTE**

Indian Council of Agricultural Research
Ministry of Agriculture, Govt of India



Introduction

Nannochloropsis oculata is a marine micro algae (Eustigmatophyte) playing an important role in seed production of marine finfishes. It is a small sized algae (2-5µm) having fast multiplication rate and rich in Chlorophyll a, Astaxanthin, Zeaxanthin, and Canthaxanthin. The algae are used directly and indirectly in marine finfish hatchery for green water larval rearing system and rotifer culture, respectively. An intermediate cultured algae are mainly used in larval rearing and mass cultured algae are used for live zooplankton (rotifer) culture. *Nannochloropsis oculata*, being a temperate species, mass production of microalgae in outdoor culture systems is difficult during summer months, but larval rearing for most of the finfishes are peak at this period. Difficulties in algal culture during summer months are one of the bottlenecks in the year-round finfish larval production.

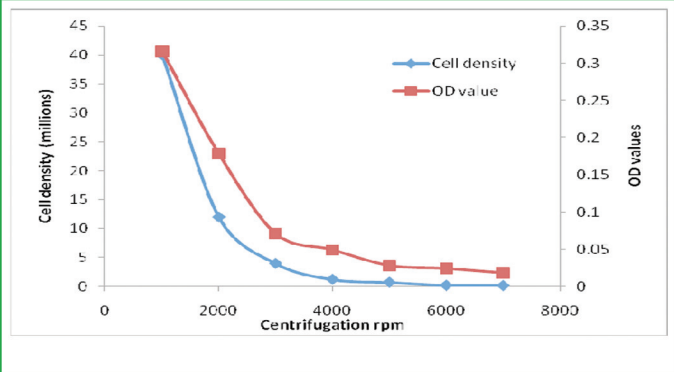
Microalgal concentrate is an alternative approach to ensure all-time availability of sufficient quantities of micro algae for larval rearing and zooplankton culture. Micro-algal concentrates are prepared and stored with added preservatives and this could be used at the time of requirements.

Different methods used to prepare algal concentrates include 1) coagulation, 2) flocculation, 3) flotation, 4) centrifugation and 5) filtration, etc. Among all, centrifugation method is proved to be the most efficient method with >90% harvesting efficiency. Centrifuged micro-algal concentrates remains with same shape and nutritional contents as that of fresh cultured microalgae. Therefore, the cells harvested using the technique can very well be used for green-water larval culture, rotifer culture and even as inoculums for further *Nannochloropsis* culture.

Importantly, at optimum time and speed, the shape of cells is maintained by the centrifugation method, which helps the cells to maintain its viability during storage.



Intermediate culture of *Nannochloropsis oculata* for centrifugation



Count and optical density of *Nannochloropsis oculata* cells in supernatant after centrifugation at different rpm.

Major advantage of *Nannochloropsis oculata* concentrate over commercial products is that the cells in the concentrate are viable (more than 80%) even after five months of storage in glycerol under chilled conditions. This cell can be used as inoculum for scaling up the algal culture. Additionally, the cells of the prepared concentrate remains suspended in water column for longer time as like fresh cultured nanno-cells, which helps to maintain the water quality in rotifer culture and larval culture tanks based on its use. However, in many of the commercially available nanno concentrates viable cells are not maintained and most of the cells settled slowly, which quickly degraded the water quality in the culture environment.

Steps involved in preparation of *Nannochloropsis oculata* concentrate

- 1. Seawater treatment
- 2. Preparation of intermediate culture of *Nannochloropsis oculata*
- 3. Cell harvest by centrifugation
- 4. Cell preservation and viability

Seawater treatment: Sea water drawn from sea is filtered mechanically through sand filter, followed by UV filtration and finally treated with ozone for complete sterilization. A residual ozone concentration of 0.1 - 2.0 mgL⁻¹ for a period of 1 - 30 minutes, is required to be maintained for complete disinfection. It is advisable to maintain nil ozone in the sea water before the inoculation of *Nannochloropsis oculata*.

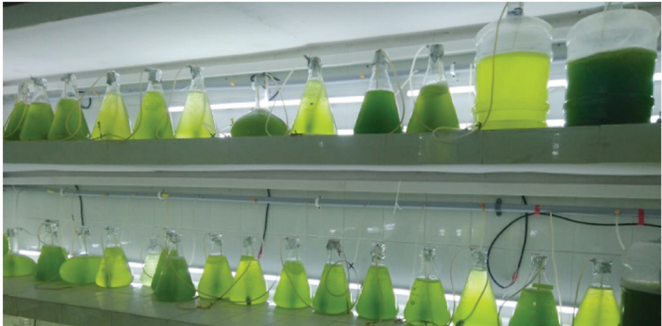
Preparation of intermediate culture of *Nannochloropsis oculata*:

Culture medium: ‘Conway’ or ‘Walne’s medium is used for the preparation of culture medium for the indoor culture of *Nannochloropsis oculata*.

Culture environment: The optimum environmental parameters for *Nannochloropsis oculata* culture include tempera

ture: 18-24°C; salinity: 20-24 gL⁻¹; light intensity: 2,500-5,000 lux; photoperiod: 24hrs and pH: 8.0-8.5.

Inoculation of the culture: Stock culture of microalgae (10%) is inoculated into seawater with culture medium (Conway). The culture is maintained for 3days with required environmental parameters with optimum aeration. The culture in growing phase / log phase is selected for the preparation of *Nannochloropsis oculata* concentrate by centrifugation. At this time, cell count should reach 30-40 million / ml if culture is healthy. Additionally, the cell count could be enhanced further upto 80 million / ml if the culture is supported with pure CO₂.

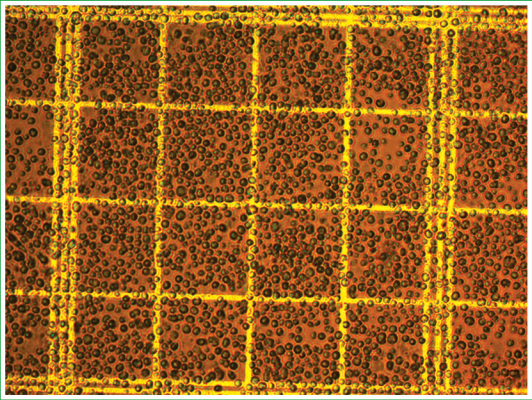


Stock and Intermediate culture of *Nannochloropsis oculata*

Preparation of *Nannochloropsis oculata* concentrate: Industrial centrifuge, which holds more volume of culture, is used for preparation of the concentrate. While preparation, the culture is transferred into centrifugation bottle. The culture is centrifuged at the maximum speed of 4000 rpm for 30 min. The supernatant is decanted and the precipitated concentrate is collected with the help of spatula or any other means without damaging the cells. This method can accumulate a cell count of an approximately 30 billions / ml from centrifugation of 300 lits of *Nannochloropsis oculata* culture.



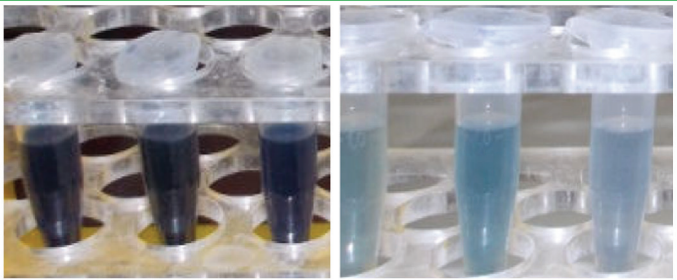
Prepared *Nannochloropsis oculata* concentrate



Concentrated *Nannochloropsis oculata* cells after dilution (20X)

Preservation in glycerol: The *Nannochloropsis oculata* concentrate with 5 – 20% inclusion of glycerol is amenable to preservation by both, chilling and freezing. Glycerol at 10% inclusion performs better with chilling and 20% inclusion performs well with freezing.

Cell viability test: Viability of the harvested and preserved cells are tested using ‘Evans Blue’ stain. Ruptured cells appear blue, since Evans Blue solution diffuses into the protoplasm region and stain the cell blue.



Diluted *Nannochloropsis oculata* cells from concentrate with Evan’s Blue stain and after staining

Application of Nanno concentrates in fish hatcheries:

The persevered *Nannochloropsis* concentrate is effeciently used as feed for rotifer, algal inoculum and green water larval rearing systems.

Nanno concentrate as feed for Rotifer culture:

The rotifer (*Brachionus plicatilis*) when cultured using nanno concentrate (preserved in 10% glycerol) as feed resulted in a maximum of 1040 no of rotifers / ml at the concentration 3x10⁶ nanno-cells / ml in rotifer culture.