Brief Communication

# Comparative study on growth performance of mixed and monocultures of select marine microalgae

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Microalgae forms an integral component of live feed for hatchery production of commercially valuable fish and shell fish. Microalgae has it application for maintaining green water rearing systems for larval rearing of finfishes as well as they form direct feed for the zooplankton component in the food chain and also for shellfish culture. Commonly used marine microalgae includes green algae (*Chlorella* sp., *Nannochloropsis* sp., and *Tetraselmis* sp.), diatoms (*Chaetoceros* sp. and *Thalassiosira* sp.) and flagellates (*Isochrysis* sp.). In general each microalgae species is being cultured separately and fed to the targeted species of culture. In this practice, the major drawback is that individual algae species may not be able to satisfy the nutritional requirement of the cultured finfish / shellfish larvae. At present, mixed cultures of microalgae is gaining importance as an important culture strategy to increase the production of microalgae. Mixed algal diets are regularly used in bivalve hatcheries as well as in marine finfish hatcheries. It is opined that in the mixed cultures, the growth characteristics, biochemical compositions, nutritional compositions and other growth factors are different from those of monocultures.

*Nannochloropsis* and *Isochrysis* are widely used in aquaculture industries as they are comprised of nutritional values suitable for rearing of marine animal larvae. *Nannochloropsis oculata* (Yellow-green algae) of 2-4 µm size, rich in EPA has practical application for rotifer culture, water conditioner in finfish

hatcheries, reef tanks for feeding corals and other filter feeders. *Isochrysis galbana* (a golden brown flagellate) of 3-6 µm rich in DHA has practical application for the culture of rotifers, copepods, brine shrimp, oysters, clams, mussels and scallops also for enrichment of zooplankton. Usually monocultures of *Isochrysis* and *Nannochloropsis* in combination is used for the zooplankton production as well as green water culture system maintenance during larval rearing of marine finfishes in mariculture hatchery. It is also reported that microalgae, *Nannochloropsis oculata* and *Isochrysis galbana* in 3:1 ratio at 1 × 105 cells /ml was found to be suitable for larval rearing of Indian pompano (Ranjan *et al*, 2018, 2022). With this information background, comparative growth performance of microalgae monoculture as well as mixed culture was conducted at the mariculture hatchery of ICAR- CMFRI, Visakhapatnam. Stock cultures of the two species of commercial important marine microalgae, the green algae *Nannochloropsis* sp., and flagellate *Isochrysis* sp., were maintained at the centre as part of the research programme on seed production of marine finfish under various mariculture projects.

Culture of microalgae were carried out in laboratory conditions,

1) monoculture of *Nannochloropsis* sp.,
2) monoculture of *Isochrysis* sp., and
3) mixed culture of both species in different ratio.

All cultures were started with equal inoculums (20%) of respective species while the inoculum of mixed culture consisted of different ratio of inoculum of *Nannochloropsis* and *Isochrysis* (3:1; 1:1; 1:3 respectively) along with monocultures of both *Nannochloropsis* and *Isochrysis*. Microalgae were cultured in enriched seawater (Conway medium, 32 ppt salinity) in 3 L conical flasks (borosil) using Conway medium without silicates (AQUACOP,1984), grown in a temperature-controlled room (190C) under continuous illumination with cool white fluorescent lamps at a light intensity of ahout 2,600 lux, for 168 hours (7 days). The experiment was performed with four replicates and followed a completely randomized design.

The growth rate and total lipid content were highest in mixed culture ratio of 1:3 with Nannochloropsis and Isochrysis respectively. Therefore, this study indicates that mixed culture of microalgae as an alternative option for commercial microalgal production to obtain higher production.

During the experiment, algal cell count of the monocultures were estimated by counting the microalgal cells every morning (24 hours) using an Improved Neubauer Haemocytometer (Superior Co., Berlin, Germany) under the microscope.The *Nannochloropsis* cell count was estimated by counting the non motile cells based on size and colour, wheres Lugols solution was added to the sample of *Isochrysis* culture. Microalgal cell count of mixed cultures were estimated by counting both the species in the mixed culture sample with the addition of Lugol's solution and the *Nannochloropsis* cells separately without the addition of Lugol's solution. The *Isochrysis* count was estimated by subtracting the *Nannochloropsis* cell count from the total cell count of the mixed culture sample. Growth rate of the culture was estimated by the following equation

$$K = \frac{ln\ N_1 - ln\ N_0}{T}$$

Where $N_1$, is the microalgal cell count at time "$t$", $N_0$ is the initial cell count at time "$0$" and $T$ is the time (day).

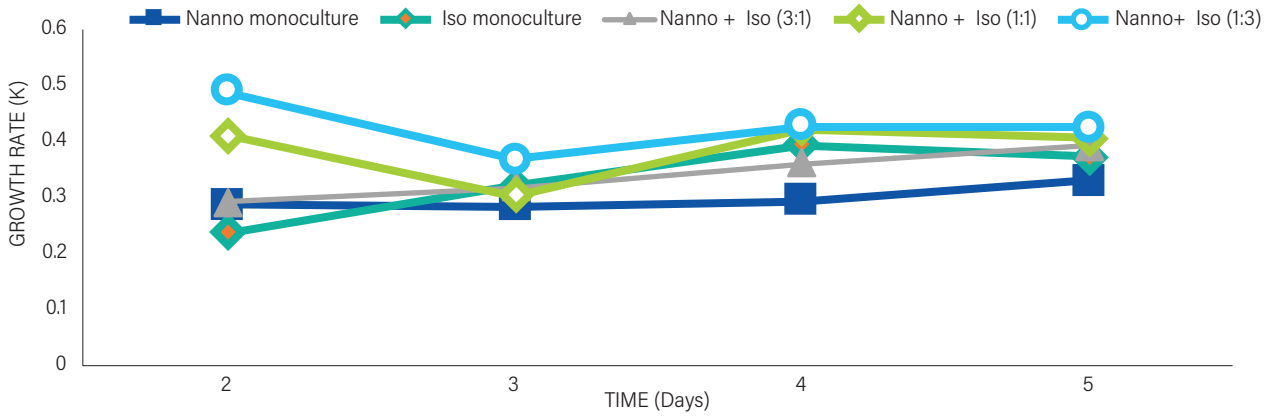


Fig. 1. Growth rate of *Nannochloropsis* sp. and *Isochrysis* sp. in monoculture and mixed culture of both species under laboratory conditions.

The growth rate of mixed cultures were better compared to monocultures of both *Nannochloropsis* and *Isochrysis*. There was exponential growth phase for all cultures from 3rd to 4th day. From 4th day onwards there was declining growth rate in cultures except monoculture of *Nannochloropsis* sp. and mixed culture of *Nannochloropsis* and *Isochrysis* at 3:1 ratio. Over all highest growth rate was observed in the mixed culture of *Nannochloropsis* and *Isochrysis* at 1:3 ratio from the beginning and it reached stationary phase on 5th day with growth rate of 0.426 ± 0.034 $day^{-1}$.

For lipid analysis, algal pellet (1 gm) was harvested by centrifuging microalgal sample for 10 minutes at 3000 rpm. To the pellets, 20 mL of chloroform: methanol ( 2:1 ratio) was added and the homogenized thoroughly with hand held homogenizer followed by vortexing for 30 seconds resulting in two layers, the upper methanol and bottom chloroform with lipid layer. The upper layer of methanol was collected separately and extraction process was repeated. The chloroform layers were combined and dried by rotor evaporator to evaporate the chloroform. The resultant lipid was weighed and expressed as lipid content per ml of microalgal culture (Folch *et al*, 1957).

Analysis of lipid content from all cultures resulted that mixed culture of Isochrysis and Nannochloropsis at 1: 3 ratio could perform better with lipid content of 9.32 ± 0.77 % followed by monoculture of Isochrysis (6.59 ± 0.22) at the end of the culture period.

## References


Folch *et* al., 1957. *J. Bio.Chem*., 226, 497-509

Ranjan *et al*., 2022. *Aquaculture Research*, 00, 1–11.

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