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Comparative growth of seven species of micro-algae in artificial and natural media

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Abstract

Results of an experiment to compare the growth of seven species of microalgae in natural seawater and an artificial medium are presented. Artificial medium was found to support better growth of algae under identical culture conditions. Based on the findings, the artificial medium was suggested as ideal for maintenance of stock cultures in the shrimp hatcheries along the east coast where temporal variations in hydrological conditions are well known.

Artificial seawater media are widely used for culture of marine organisms all over the world. Artificial seawater mixes are commercially available in countries where marine organisms are extensively grown in aquariums. The utility of such medium in giant freshwater prawn hatchery operations is also well documented (Reddy *et al,* 1991, Qureshi *et al,* 1993).

Growth in micro-algal culture is commonly measured by taking dry weight, packed cell volume or optical density (Sorokin, 1973). Considering the difficulties in obtaining accurate measures of dry weight and packed cell volume, optical density was chosen as an index of growth in the present experiment. To assure dependability of turbidity measurements, it was suggested that they could be checked with some other index of growth (Sorokin, 1973). As an index of increasing cell concentration and physiological state of majority of the cells in the culture medium, a secondary criterion, the Chlorophyll pigments, were selected for measurement.

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Material and methods

Twelve kg of clean dry common salt was dissolved in 50 l of groundwater and 240 ml of additive solution was added to it. The final salinity was brought to about 140 ppt by adding water/salt. The solution was filtered through a fine meshed net and kept for aging for a fortnight under aeration. Aeration was stopped for a day and the clear solution was decanted/siphoned out, discarding the settled sediments at the bottom. The aged solution was filtered through a bio-filter and again kept under aeration for a fortnight (This stock solution can be kept for longer duration with intermittent aeration). A portion of the solution was diluted to the

required salinity, kept for a week under aeration, filtered through bio-filter and kept again under aeration for a week before using it for experiments. Initially a refractometer was used for adjusting the salinity of artificial medium to equal that of natural seawater. Final estimation of salinity was made by standard titrimetric methods (Strickland and Parsons, 1968).

Before the starting of the experiment, stock culture of seven species of micro-algae namely *Chaetoceros sp., Isochrysis sp., Dicrateria sp., Tetraselmis sp., Nannochloropsis sp., Chlorella sp.,* and *Chryptochrysis sp.* were maintained under similar conditions of the experiment. A set of eight culture flasks was filled with 1.5 litre of natural seawater of known salinity and pH, sterilized and enriched with Conway medium. A second set of eight culture flasks was filled with 1.5 litre of artificial medium (adjusted to the salinity and pH of natural seawater), sterilized and enriched with Conway medium. Three ml each of the stock culture inoculum of seven species of algae was transferred aseptically into 7 pairs of culture flasks (one of the pair containing natural and the other artificial media) and kept under constant lighting without aeration but with periodic agitation. The eighth pair was kept as blank. A light meter (*Lutron Lx* 102) was used for measuring the light intensity and pH meter (*Entech pH Sean -2)* was used for checking the pH.

From each of the 7x2 culture flask, a little over 10ml of the sample was aseptically drawn from the third day to 24th day to measure optical density and plant pigments. Transmittance (%) was measured at three wave lengths namely 438nm, 540nm, and 678nm as suggested in Sorokin (1973) using a spectrophotometer (*Systronic 106).* Optical density (OD) was calculated as $\log_{10} (I_0/I)$ where I_0 and I are the transmittance of the blank and sample respectively. Difference in the OD_s was obtained by subtracting the OD of natural medium from the OD of artificial medium and their corresponding mean and standard deviations are worked out. From 4th day to 24th day, the 10 ml of the culture sample after transmittance measurement was filtered through a Whatman GF/D glass fibre filter paper for estimation of chlorophylls. Pigments extracted in 90 percent acetone were estimated by spectrophotometry (*ECIL/GS 866* C) as per procedure given in Parsons *et al* (1984).

Results and discussion

Basic conditions

The salinity of the natural seawater was 29.13‰ and that of artificial medium was 29.51‰. The pH of the experimental medium was adjusted to 8.0 in both the media. The mean illuminations at the two racks were slightly different, the first one having 1534.2 lx and the second one receiving 1413.5 lx. Therefore, the pair of flasks containing the same species was kept on the same rack to avoid variations in growth due to difference in illumination. The temperature in the culture room was maintained in the range of 24° C to 27° C throughout experiment.

Optical density

The trend of optical density (OD) for five of the seven species used in this experiment showed clear difference between the two culture media. Compared to the natural seawater medium, artificial media in general showed a higher OD after an initial lag period indicating higher cell concentration of micro-algae. This difference in ODs remained significant till the end of the experimental period. The differences in ODs were not much significant for two species namely *Nannochloropsis* sp. and *Chlorella* sp.

In the case of *Chaetoceros* sp., the difference in ODs at all the three wave lengths became evident from about 8th day of the culture experiment, became prominent from 11th day and exhibited a slight increasing trend till the 22nd day (Fig.1a). For *Isochrysis* sp., the difference in ODs at all the three wave lengths started showing up around 10th day of the culture experiment, became prominent from 11th day and showed a marginal increasing trend till the 23rd day (Fig. 1b). As in the case of *Isochrysis* sp., in *Dicrateria* sp. also the difference in ODs at 438 nm and 540 nm started showing up around 10th day of the culture experiment, became prominent from 11th day and remained more or less same till the 23rd day (Fig.1c). The difference in OD at 678 nm started showing significant difference only around 13th day. However, the magnitude of the difference at all the three wavelengths, was not as high as in the case of *Chaetoceros* sp. and *Isochrysis* sp. In the case of *Tetraselmis* sp., the difference in ODs was evident from around 4th day itself (Fig.1d). A rapid increase in OD till the 7th day and a subsequent decrease was observed in the case of artificial media at all the three wavelengths. The difference in ODs exhibited a slight increasing trend till the 24th day. In the case of *Nannochloropsis* sp., the differences in ODs was not much significant at 540nm and 678nm though a little difference was evident during the last week of the experiment. At 438 nm, however, the difference in ODs was much significant on 12th day which became less significant subsequently (Fig 1e). In the case of *Chlorella* sp., the differences in ODs was as not much significant and fluctuated most often because of the plots crossing over (Fig.1f). In the case of *Chryptochrysis* sp., the difference in ODs became prominent at around 9th day and the magnitude of difference was much lesser, more or less similar to that of *Dicrateria* sp.

The mean difference in ODs at all the three wave lengths for all the seven species was positive indicating an overall higher cell concentration in the artificial medium (Table-1). the hgihest mean differences on OD were recorded in the case of *Chaetoceros* sp., and *Isochrysis* sp., fo llowed by *Tetraselmis* sp. The mean difference in OD, though positive, was mush less in the case of the other four species.

Plant pigments

Unlike the optical density, the concentration of chlorophylls *a*, *b* and *c* showed wide fluctuations in all the species during

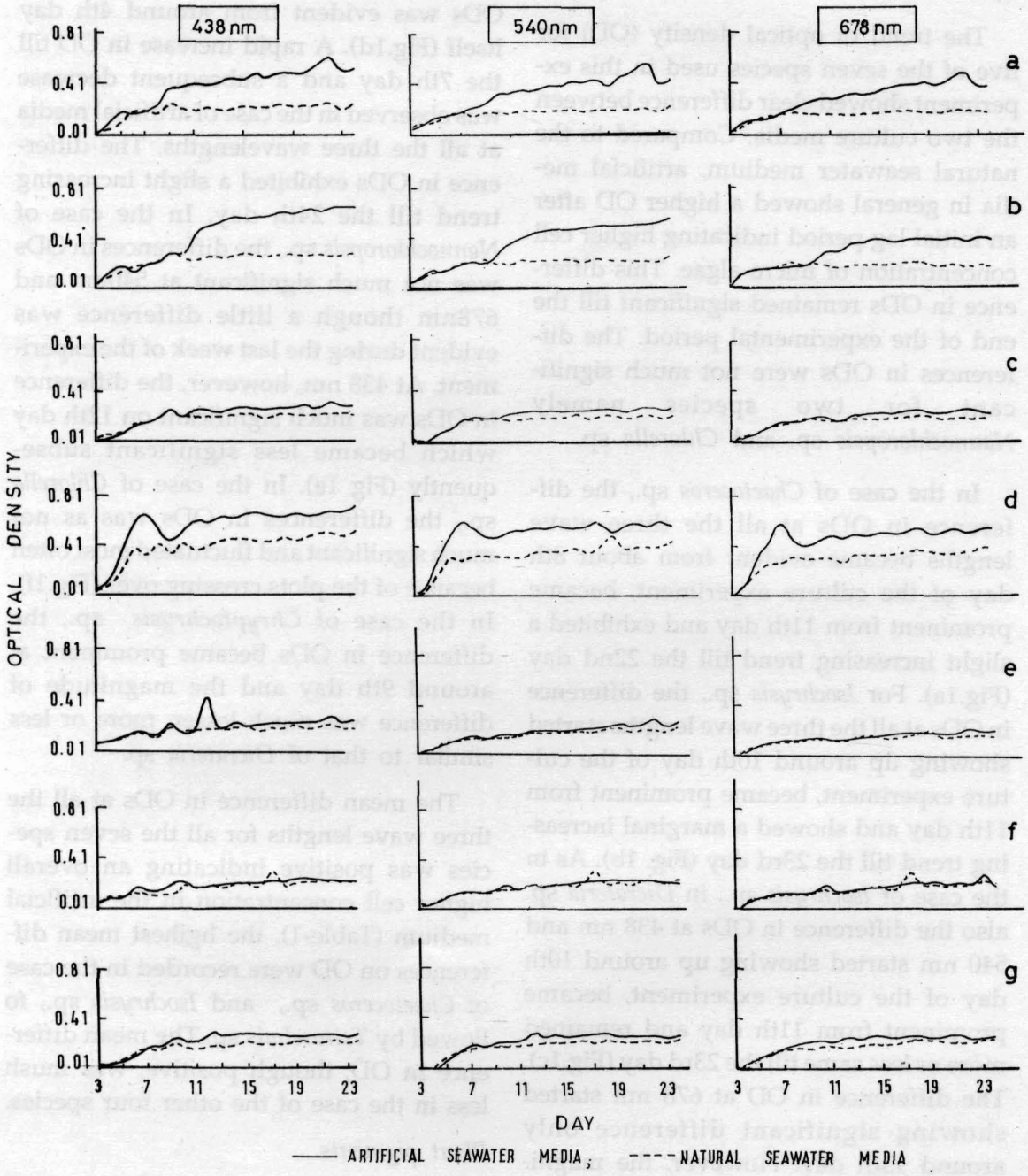

Fig. 1. *The daily variation in Optical Density in the cultures in artificial medium and natural seawater medium w.r.t seven species of micro-algae (a) Chaetoceros sp., (b) Isochrysis sp., (c) Dicrateria sp., (d) Tetraselmis sp., (e) Nannochloropsis sp.,(f) Chlorella sp., and (g)Chryptochrysis sp. during the experimental period.*

the experimental period. A general declining trend of chlorophylls was visible in some cases such as *Chaetoceros sp., Isochrysis* sp. *Dicrateria* sp. and *Tetraselmis sp.* In the case of *Chaetoceros* sp., the higher values of all pigments were recorded during 7th and 11th day in both the culture media (Fig,2a). In the case of *Isochrysis* sp. chlorophylls *a* and *c* peaked during 7th to 11th day whereas chlorophyll *b* peaked during 3rd day (Fig.2b). In the case of *Dicrateria* sp., the peak in chlorophylls was recorded on 6th day in the case of artificial medium and around 10th day and 16th day in the case of natural medium (Fig.2c). Higher values of chlorophyll were recorded in the initial 6th to 14th day in the case of *Tetraselmis* sp. The values in natural medium fell below undetectable level around 8th day whereas a peak in chlorophyll *c* was observed in the artificial medium around 7th day (Fig.2d). Except for a peak in chlorophyll *c* during 22nd day in artificial medium, the pigment values fluctuated within narrow amplitude without definite trend in the case of *Nannochloropsis* sp. (Fig.2e). Two peaks, one during 4th to 6th day and another during 8th to 12th day, were observed in the case of *Chlorella* sp.(Fig.2f). A similar trend, though with shorter peaks, was observed in the case of *Chryptochrysis* sp. (Fig.2g)

By virtue of the fluctuations in the values of chlorophylls due to factors beyond control, drawing direct relations between plant pigment concentration and optical density was not possible. However, the mean difference in concentration of three chlorophylls for all the seven species was positive indicating an overall higher pigment concentration in the artificial medium (Table 1). The highest mean difference in chlorophyll *a* was recorded in the case of *Tetraselmis* sp. followed by *Isochrysis* sp. and *Dicrateria* sp. In the case of chlorophyll *b*, the mean difference was highest in the case of *Isochrysis* sp. followed by *Tetraselmis* sp. and *Dicrateria* sp. The mean difference of chloropphyll *c* concentration was highest in the case of *Dicrateria* sp. followed by *Tetraselmis* sp. and *Nannochloropsis sp.*

It could be noted that variability as evident from the standard deviations of mean difference in pigments was very high, especially in the case *Dicrateria* sp., *Chlorella* sp., *Isochrysis* sp. and *Nannochloropsis* sp. Whereas the variability in the mean difference in optical density was comparatively less. The use of plant pigments as an index of growth is thus not as reliable as the optical density under the present conditions of experiment, though the overall mean difference in pigments was positiive.

Conclusions

The seasonal variations in hydrological features along the east coast have repercussions on the various activities where natural seawater is being made use. Shrimp hatcheries all along the coast are faced with problems associated with the changes in the quality of seawater taken in from near-shore areas. Severe problems in the micro-algal culture system such as failures of the development of starter

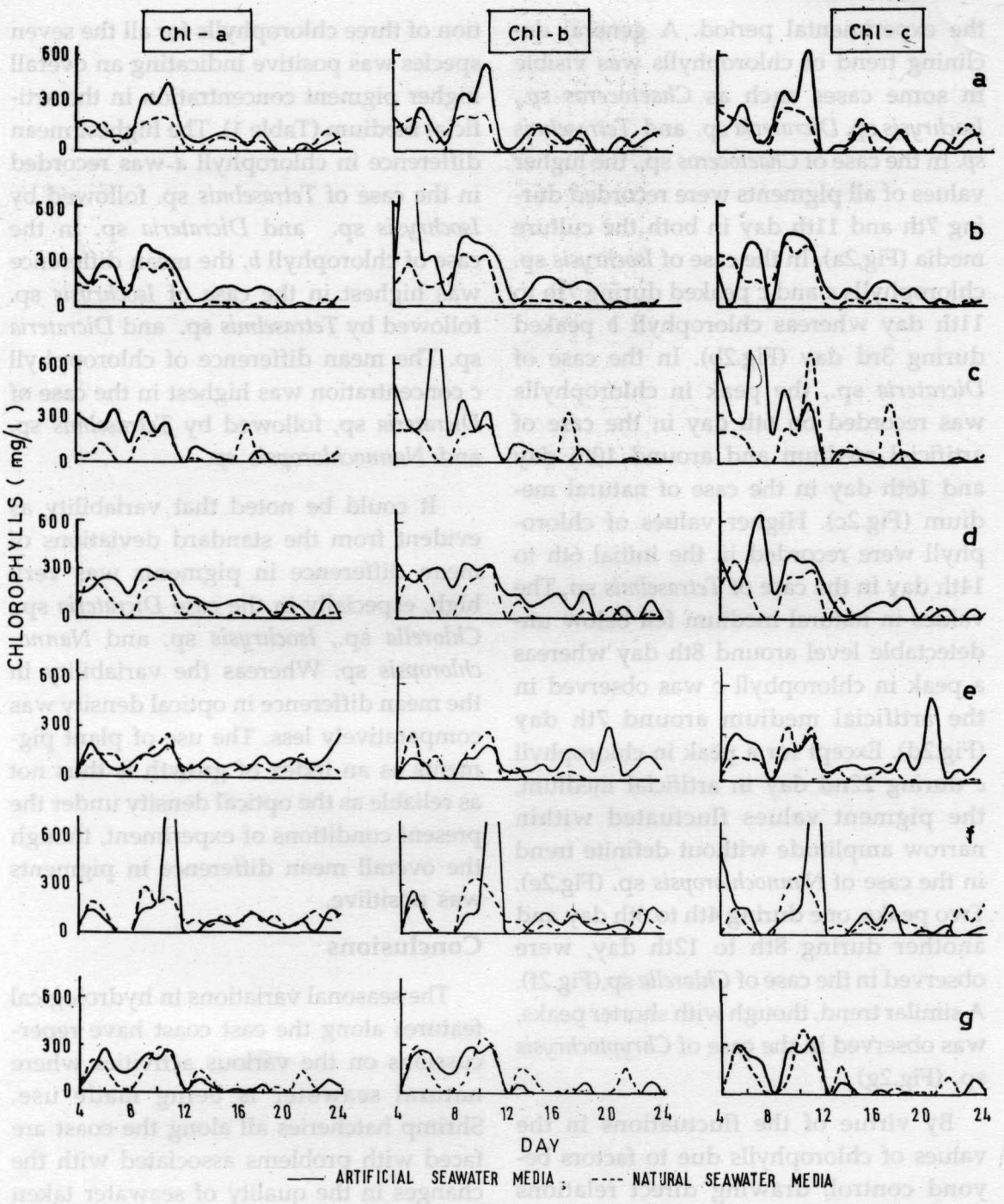

Fig. 2. *The daily variation in Chlorophylls in the cultures in artificial medium and natural seawater medium w.r.t seven species of micro-algae (a) Chaetoceros sp., (b) Isochrysis sp., (c) Dicrateria sp., (d) Tetraselmis sp., (e) Nannochloropsis sp., (f) Chlorella sp., and (g) Chryptochrysis sp. during the experimental period.*

Table.1 *The averages and standard deviations of difference (Artificial media - Natural media) in optical density and chlorophylls with respect to the seven species for the experiment period.*

Species		Optical Density*			Chlorophylls (mgl^{-1})*		
		438nm	540 nm	678nm	Chl-*a*	Chl-*b*	Chl-*c*
Chaetoceros sp.	*Average*	0.18	0.15	0.13	27.96	35.40	23.66
	Std.dev.	0.12	0..11	0.1	73.05	92.55	121.06
Isochrysis sp.	*Average*	0.20	0.16	0.12	57.61	122.95	35.58
	Std.dev	0.16	0.13	0.1	57.91	227.38	103.73
Dicrateria sp.	*Average*	0.04	0.04	0.03	53.10	73.26	80.50
	Std.dev	0.03	0.02	0.02	110.44	204.44	301.07
Tetraselmis sp.	*Average*	0.17	0.16	0.16	58.43	74.43	74.29
	Std.dev	0.06	0.07	0.07	64.90	94.25	149.10
Nannochloropsis sp.	*Average*	0.03	0.02	0.02	23.89	40.81	65.50
	Std.dev	0.05	0.02	0.01	72.44	108.52	125.18
Chlorella sp.	*Average*	0.01	0.01	0.01	10.58	17.05	39.11
	Std.dev	0.03	0.02	0.02	164.71	206.20	261.75
Chryptochrysis sp.	*Average*	0.05	0.04	0.03	12.58	10.58	20.13
	Std.dev	0.03	0.02	0.02	62.36	83.10	84.97

*Number (days) of observations - Optical Density :22; Chlorophylls:21

culture, abrupt crashes of the culture, non-development of culture to the required concentration etc. are reported. These problems critically handicap the smooth running of the hatchery system. In the light of the above facts the need for a cheap and easy-to-make alternative medium, which could sustain stable growth of micro-algae, is severely felt.

The results of the present experiment show that the artificial medium used in the present experiment is quite superior to the natural seawater medium in supporting the micro-algal cultures. The better growth performance of the three most commonly used micro-algal species namely *Chaetoceros sp., Isochrysis sp.* and *Tetraselmis sp.* is a point worth noting. Therefore, this artificial medium could be suggested as suitable for maintenance of stock cultures of micro-algae in shrimp hatcheries along the east coast of India.

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