A PRELIMINARY STUDY OF THE EFFECTS OF THREE FERTILIZER COMBINATION TYPES ON THE PROLIFERATION RATE OF CULTURED MIXED DIATOMS*

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Chemical concentration is one of the principal factors governing the rate of propagation of diatoms, in addition to light and temperature (Needham, *et al.*, 1941; Shigueno, 1974). Consequently, several studies focused primarily toward the development of culture media for the maintenance of food organism to support aquaculture. Most of the pioneering work employed Miguel's A and B media for pure diatom culture (Miguel, 1897; Allen and Nelson, 1910 in Needham, *et al.*, 1941). Miguel's A and B solutions were modified by adding garden soil extract to them, to stimulate effects on diatom proliferation rates (Gran, 1931, 1932, and 1933 in Needham, *et al.*, 1941). A further modification of Miguel's medium was the "NH" Synthetic Culture media in the pure culture of *Skeletonema costatum* in Galveston Texas Marine Biological Laboratory (Anonymous, 1970). Another medium used for the maintenance of stock of 25 species of diatoms in the culture of *Penaeus dourarum* was the Guillard F medium (Tabb, 1972). Other media formulated for marine plankton culture was the Sach, the Eerdschreiber, the Schreiber, the Umebayashi and the Provasoli's solutions (Shirota, 1970). Seawater diluted with secondary treated sewage effluent was also used to provide excellent enrichment for the maintenance of natural populations of marine phytoplankton in continuous culture (Dunstan and Menzel, 1971).

Unfortunately, in the Philippines, studies along these lines have been few. Most of the studies on marine and freshwater phytoplankton are taxonomic in nature (see review by Gonzales, 1961).

Evidently, there is a need of investigation regarding cultured phytoplankton proliferation rates. Accordingly, a study of phytoplankton proliferation rates was undertaken. It aims at the control of the

diatom biomass, which would mean manipulating such important environmental parameters as light, temperature, and concentration of chemicals to obtain the desired diatom blooms. Results of this study will help to provide a continuous supply of diatoms to act as primary food for the zoea of *penaeus monodon* Fabricius (Sugpo).

Owing to inavailability of equipment, certain parameters such as light intensity, total alkalinity and optimum basic productivity could not be measured. In addition, because of restrictions imposed by time and manpower, the less desirable single-sample analysis was followed. These must be considered as the greatest limitations of this work.

Materials and Methods

Tanks. Four adjacent tanks, protected from direct sunlight by Coralex roofing, were filled with nine tons of filtered seawater. Tank 1 (no treatment) was the control and Tanks 2 and 3 and 4 were experimental. The fertilizer combinations used are presented in Tab. 1. Fertilizers were applied to Tanks 2 and 4 only at the start of the experiment; to Tank 3, up to the fourth day. All culture tanks were aerated at 15 psi throughout the culturing period of 7 days.

Chemical Parameters. Determination of chemical factors was made daily. Salinity was measured with a salinometer; water temperature, with a mercury thermometer; and pH, with a portable pH meter. D.O. was measured after the Winkler method and NO_2 concentration was determined by using a spectrophotometer (Strickland and Parsons, 1972).

Phytoplankton. Five hundred (500) ml samples were collected daily from each tank after the water had been thoroughly agitated. From each sample, 250 ml was set aside for live plankton qualitative analysis and 250 ml was preserved with Lugol's solution (as specified by Vollenweider, 1971) for quantitative analysis. One ml subsample of the Lugol preserved and agitated sample was mounted in a Sedgwick-Rafter counting chamber, in which 10 fields (equivalent to 1 mm^3) were examined after a 15-minute settling period, in transects made at 100X (Dr. David G. Frey, personal communication). Numerical counting was made twice by using a Swift inverted microscope. Phytoplankton densi-

Table 1. Fertilizer combinations used.

Concrete Tank No.	Fertilizer combinations and dosage in gm ton^{-1}								
	Inorganic					Organic			
	KNO_3	$NaNO_3$	NaH_2PO_4	K_2HPO_4	Na_2SiO_3	$Clewat_{32}$	Fe Cl_2	Clam Juice	Combination Type
1	0.0	0	0	0	0	0	0	0	—
2	5.5	0	0.83	0	0.55	1.7	0	0	A
3	0	2.0	0	0.2	0	0.2	0.2	0	B
4	5.5	0	0.83	0	0.55	1.7	0	0.55	C

ty was calculated after the following equation:

$$N = 1000 \times \frac{m}{n}$$

Where: N — Number of cells cm^{-3}
m — total number of diatoms in n fields
n — number of fields counted
1000 — number of 1 mm^3 in 1 ml of a Sedgwick-Rafter counting chamber where the chamber volume is 50mm x 20mm x 1mm.

Results

Physical and Chemical Parameters. For the experimental set, Tank 2 averaged 0.06°C higher than Tank 3 and 0.18°C highter than Tank 4. Tank 3 averaged 0.12°C higher than Tank 4 at 0900 hrs. At 1500 hrs., Tank 4 averaged 0.2°C higher than Tank 2 and 0.6°C higher than Tank 3, but the latter averaged 0.14°C higher than Tank 2. However, Tanks 2, 3 and 4 together averaged 0.08°C and 0.18°C less than the control at 0900 and 1500 hrs., respectively (Tab. 2). Generally, water in the culture tanks was isothermal during the experiment. Temperature in Tank 1 ranged from 27.5 to 29.6°C and in Tanks 2, 3 and 4 from 27.0 to 29.6°C. Salinity in Tank 1 ranged from 27.0 to 31.1°/oo whereas in Tanks 2, 3 and 4, it ranged from 26.4 to 29.5°/oo (Tab. 3).

Table 2 — Average water temperature (°C) in control and experimental tanks.

TANK NO.	CT_1	CT_2	CT_3	CT_4
Time Sampled				
0900 hrs.	28.41	28.41	28.35	28.23
1500 hrs.	28.34	28.05	28.19	28.25

Table 3. – Ranges of physical and chemical parameters measured.

Concrete Tank No.	Time Sampled	Ranges				
		H_2O temperature (^oC)	Salinity %	pH	D.O. (ppm)	NO_2 (ppm)
1	0900	27.5–29.6	27.0–31.1	8.12–8.25	6.1–7.3	–
	1500	27.0–29.4	27.1–28.6	8.12–8.23	4.6–7.1	0.00–0.05
2	0900	27.8–29.6	27.1–28.6	8.1 –8.28	6.3–7.9	–
	1500	27.0–29.0	27.1–28.8	8.1 –8.4	5.0–7.5	0.05–0.30
3	0900	27.5–29.3	26.4–29.5	8.1–8.25	6.1–8.3	–
	1500	27.0–29.0	27.8–29.5	8.09–8.29	3.2–7.2	0.05–0.10
4	0900	27.5–29.3	27.7–29.5	8.10–8.20	6.0–8.5	–
	1500	27.0–29.0	26.9–28.9	8.05–8.25	6.0–7.3	0.50–0.5

8.05–8.25

NO_2 was minimal in the control whereas an increase was noted on the 4th day in Tanks 2 and 4 and on the 7th day in Tank 3. The highest NO_2 value was 0.5 ppm obtained in Tank 4 on the 6th day.

Water in all tanks was characteristically alkaline with pH ranging from 8.05 to 8.29. Dissolved oxygen increased generally on the 4th day; its observed ranges were from 6.1 to 7.3 ppm in the control and 6.0 to 8.5 ppm in the experimental tanks at 0900 hrs. At 1500 hrs., D.O. ranged from 4.6 to 7.1 ppm in the control and from 3.2 to 8.1 in the experimental tanks. (Results of daily analysis shown in Fig. 1)

Phytoplankton. A total of 25 species of phytoplankton representing two classes, Dinophyceae and Bacillariophyceae, were found in all tanks (Table 4). The Bacillariophyceae as a group was, on the average, two times higher in concentration in the experimental tanks than in the control. They constitute 100% and 98.9% of the total diatom population in the control and experimental tanks respectively. The dominant diatoms, i.e., the five prevalent species by cell count, are *Chaetoceros* sp.$_1$ (socialis), *Thalassiosira* sp.$_1$ (hyalina?), *Navicula* sp., *Licmophora abbreviata,* and *Cylindrotheca closterium.* Succession of these phytoplankters is shown in Fig. 3A to E.

Table 4. — Species of phytoplankton observed.

Dinophyceae:

Ceratium sp.
Peridinium sp.
Prorocentrum sp.
Cylindrotheca closterium
Diploneis sp.
Leptocylindrus danicus
Licmophora abbreviata

Bacillariophyceae:

Amphiprora sp
Chaetoceros sp.$_1$ (socialis?)
Chaetoceros sp.$_2$ (affinis?)
Chaetoceros sp.$_3$ (didymus?)
Chaetoceros sp.$_4$ (distans?)
Chaetoceros sp.$_5$ (teres?)
Climacosphenia sp
Coscinodiscus sp.
Melosira sp.
Navicula sp.
Nitzschia sp.$_1$
Nitzschia sp.$_2$ (seriata?)
Nitzschia sp.$_3$ (sigma?)
Pleurosigma sp.
Skeletonema costatum
Thalassiosira sp.$_1$ (hyalina?)
Thalassiosira sp.$_2$
Thalassiothrix sp.

Table 5. — Average cell concentration cm^{-3}

	Tanks							
Classes	CT_1	CT_2	CT_3	CT_4	CT_1	CT_2	CT_3	CT_4
Bacillariophyceae	100	2043.75	303.13	1618.75	309.38	3393.75	506.75	4028.13
Dynophyceae	0	0	6.25	6.25	0	56.25	12.5	0
Total	100	2043.75	309.38	1625.00	309.38	3450.00	518.75	4028.13
Time Sampled	0900 hrs.				1500 hrs.			

Fertilizer Effects. Results of 3 types of fertilizer combinations are shown in Fig. 2 and Table 5. Type A gave the highest average diatom count at 0900 hrs and Type A, at 1500 hrs; Type C ranked second at 0900 hrs and Type A, at 1500 hrs; Type B, the least for both sampling hrs. The highest counts of diatoms were obtained on the 5th day (Tank 1 and 3), 7th day (Tank 4) for both 0900 and 1500 hrs; 7th and 6th day (Tank 2) for 0900 and 1500 hrs, respectively.

Discusssion

Dissolved oxygen, pH and water temperature analyses were carried out to keep track of the photosynthetic activity in the culture tanks. Phytoplankton cell count was used as index to proliferation rates. When diatom proliferation or photosynthetic activity is high in the presence of a sufficient supply of nutrients and light, the level of D.O. and Ph increases. Because change in the level of illumination is of primary importance in determining relative photosynthetic and respiration rates, sampling was done both at 0900 and 1500 hrs.

The experimental results at 1500 hrs. show that an increase in phytoplankton cell count seemed to coincide with an increase in D.O. Dissolved oxygen fluctuation was minimal; however, a decrease in D.O. coincided with the decrease in diatom cell counts on the third day and an increase in D.O. on the fourth day coincided also with an increase in diatom cell counts. As the diatom cell count in the experimental tank increased from the fourth day to the seventh day, D.O. should have increased, too. What was observed, however, was that D.O. in the experimental setup generally remained steady at 6.5 to 7.5 ppm, which may be attributed to the aeration system employed. Aeration helps in maintaining saturation of D.O. (Prokesova, 1962). The rate of respiration of diatoms at night was also a factor in the decrease of D.O. saturation, although such D.O. value would increase again in the morning when conditions favor photosynthetic activity.

Diatoms probably greatly influence the pH of seawater. Within the range of 7.8-8.8, pH does not act as a limiting factor to diatoms (Lucas and Hutchinson, 1927 in Cupp, 1943). Old cultures tend to become acidic. A relatively low pH indicates liberation of CO_2, assimilation of O_2 and a high rate of respiration; high pH indicates a high rate of

photosynthesis, which causes absorption of CO_2, thus making the water a bit alkaline. Results of the present experiment, however, showed that diatom cell counts dropped on the 3rd day while pH increased at 1500 hrs. This contradicts the common direct proportionality between pH and rate of photosynthesis. It is probable that this contravening phenomenon was due to a low CO_2 concentration since the low cell count on the preceding day also involved minimal respiration capability. Evaporation of CO_2 through aeration may also have enhanced this condition in the tanks.

On the fourth day, diatom counts increased with a corresponding rise in pH values. On the fifth day, however, the pH dropped once more, but the diatom count continued to increase. This may have been due to the respiration rate of diatoms at night. However, drop in pH did not involve great values since diatom takes in CO_2 during the proliferation process at daytime. Diatom proliferation was further enhanced by the warm temperature at 1500 hrs. Corresponding increment in both pH and diatom count was observed in the experimental set on the 7th day. At 0900 hrs. pH values increased in correspondence with the increase in diatom cell count; decrease in diatom cell count corresponds to a decrease in pH, although pH tended to level off from the fourth to the 7th day in Tanks 2 and 3. This is probably correlated with the release of CO_2 gas to the atmosphere through aeration and through agitation at the time of sampling.

In the sea, salinity, *per se,* has little direct effect upon production. Diatoms are tolerant of wide variations in salinity as well as in temperature (Cupp, 1943). However, the combined effect of temperature and salinity influences the viscosity, density, and vertical stability of water, which in turn tends to affect vertical circulation. In this way, temperature and salinity may indirectly favor or hinder the flotation of marine diatoms and affect the presence of adequate food supply for them. In the experimental setup, salinity fluctuation seemed to have no pronounced effect on diatom population peaks. Fluctuations in salinity values could have been due to differences in the degree of water evaporation.

Increase in solar radiation is commonly associated with increase in phytoplankton production (see review by Gonzales, 1961). Observa-

tions in the experimental setup showed that even subdued sunlight (because of the roofing) plus fertilization was still effective in stimulating diatom "bloom" on the fifth day in Tanks 2 and 3, the sixth day in Tank 3, and the seventh day in Tank 4. Further, although the water temperature tended to decrease, the residual warmth was enough to favor photosynthesis. The water temperatures, ranging from 27-29.5°C in the experiment, could be considered within the high temperature range which favor photosynthesis. Thus, the increased diatom cell counts or "bloom" within that temperature range in the experimental tanks substantiates further the previous finding (Morris and Glover, 1974) that growth of diatoms at higher temperature increases the cellular content of chlorophyll which all diatoms contain. This would mean that the maximum rate of photosynthesis for a given number of cells attained at higher temperature is greater than the maximum rate at lower temperature. This pattern, however, is not true with respect to *C. closterium.* Thus, the considerably lower population count of this species and other benthic diatoms observed in the experiment, agrees with the observations of Morris and Clover (1974) that the photosynthetic rate of *Nitzschia (Cylindrotheca) Closterium* declines most rapidly during growth at higher temperature (24°C). Further research on this aspect in the tropics would be interesting, if one considers the sinking of benthic species such as *C. closterium. Chaetoceros* sp. and *Thalassiosira* $sp._1$, on the other hand, grew comparatively well at high temperature range in the experimental setup, especially in Tank 2.

It appeared that the occurrence of diatom species was governed by the competition scheme for certain resources. It is known that competition for nutrients as well as light plays a role in phytoplankton succession. The nutrient requirement of phytoplankton is not only provided by the elements, C, N, P and Si, but also by Vitamins and trace metals, which are possible determinants of algal succession (see Lehman, *et al.,* 1975). As regards this observation, it is not possible to point out exactly which nutrient would be a limiting factor to the growth of cultured mixed diatoms since nutrients were introduced into the culture tanks without considering the initial nutrient composition already present in the filtered sea water. Besides, nutrient composition was not monitored throughout the culture period. Clewat 32 was intro-

duced into the culture to supply trace elements which were assumed absent. Continuous predominance of *Chaetoceros* sp.$_1$ throughout the culture period may be due to its ability to remain suspended in the medium, which gives it more exposure to light and nutrients than the benthic species.

Specific composition of phytoplankton seemed to be altered by the availability of nutrients in both the control and experimental tanks. For instance, Bacillariophyceae had only four species in Tank 1, which did not receive any treatment, 20 species in Tank 2 with Fertilizer A, 18 species in Tank 3 with Fertilizer B, and 10 species in Tank 4 with Fertilizer C. Although Fertilizer C brought about the highest average diatom cell count, the overall efficiency of Fertilizer A in bringing about high yield was very evident from the daily high diatom cell count in Tanks treated with Fertilizer A. This condition is ideal for the sustained daily food requirement of sugpo larvae. It is also possible that the high daily diatom count in Tank 2 may have something to do with the compatibility of Fertilizer A with seawater, resulting in the production of a suitable nutrient.

The NO_2 concentration building up on the fourth day until the last day, particularly in the experimental set, was due to the bacterial action on dead diatoms that accumulated on the tank bottom. The NO_2 concentration instead of ammonia was used as an index to pollution in the culture tanks because this was easily and quickly analyzed. In summary, the data presented illustrate that D.O., pH and NO_2 concentration increase as diatom cell count increases. However, these effects could be modified by aeration employed in culture tanks and by the control of light intensity. Nutrient-additive combination types and warm water temperature ranges would tend to increase diatom proliferation rates and population speciation affecting diatom class composition. This is shown in the cell counts and succession stages of diatom species in the experimental tanks, particularly Tank 2 with Type A fertilizer combination. Salinity fluctuation has no effect on marine diatom proliferation rate.

Implications and Recommendations

1. Type A fertilizer combination could be used in mass mixed diatom culture, as the results of the experiment suggest, in order to support hatchery operation for *Sugpo* culture.

2. Replication of the experiment should include measurement of light intensity and other meteorological parameters. Analysis of initial nutrient concentration in filtered sea water, should, if possible, be done prior to broadcasting additives. It is further suggested that future work on this aspect should be done on a one-month basis to better keep track of diatom-population succession and oscillations.

3. The problem of NO_2 formation should be minimized, at least by improving the aeration system or tank construction. It should be noted that a NO_2 concentration of 0.5 ppm does not limit zoea larvae of sugpo as can be inferred from empirical data of experiments at MSU-IFRD Sugpo Hatchery. Thus, feeding cultured diatom with a NO_2 concentration of O.5 ppm or lower into sugpo culture tanks would still be suitable for the zoea larvae.

4. Hatchery findings suggest that *Licmophora abbreviata, Leptocylindrus danicus,* and *Streptotheca Thamensis* are among the potentially harmful diatom species to Sugpo zoea. These species seep into the culture medium despite filtration of sea water. To eliminate the incidence of these species, it is suggested that pure cultures of only suitable species be obtained before the infusion of Type A fertilizer. In addition, research on the most effective filtration system of sea water should be undertaken or that sterilization of sea water used as medium be considered before any culture of food organisms should commence.

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