ALGAL PRODUCTION AND UTILIZATION RELEVANT TO AQUACULTURE IN THE PHILIPPINES

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INTRODUCTION

Phycological researches in support of aquaculture are a recent development in the Philippines. Progress in this area gained momentum with increased efforts to expand fish farming in the Philippines as a means of producing more animal protein. The Aquaculture Department (AQD) of SEAFDEC, having taken the lead in larval rearing of penaeids and economically important fish species, intensified its search for promising algal species as natural feed. Imported and indigenous algal species were screened and tested for use in hatchery and nursery operations. The vital role of microalgae to sustain growth of larvae during critical stages of development was demonstrated.

This paper presents the researches on algal culture and utilization conducted at AQD, SEAFDEC from 1974 to date. Both brackishwater and freshwater species are covered, with emphasis on freshwater algae.

SELECTED ALGAL SPECIES AS NATURAL FEED

Brackishwater Algae

Diatoms species were considered highly acceptable to *Penaeus monodon* at the early zoea stages. For this reason, early attempts at growing natural feed mentioned unidentified diatoms at 20,000 to 50,000 cells/ml for hatchery operations (AQD Annu. Rept. 1974). *Nitzschia, Navicula* and *Thalassiosira* in washings from the seaweed *Sargassum* were also supplied as natural feed in combination with bread yeast.

Mixed diatoms, predominantly *Chaetoceros* sp., were given at 1-5 x 10^3

cells/ml (AQD Annu. Rept. 1975). A new technique of growing natural feed was applied wherein *Chaetoceros* sp. and *Skeletonema* sp. were grown separate from the larval rearing tank (AQD Annu Rept. 1976). *Skeletonema costatum* imported from Japan had a low temperature requirement which limited its use in the Philippines.

Of the naturally occurring diatom species collected from Buyuan Bay, Iloilo, *Chaetoceros calcitrans* was most promising because of its small size (4-5 μm diam.) and stability in culture under different environmental conditions. This indigenous species has proved very effective as natural food.

More recently, algal species other than those belonging to the Bacillariophyceae have been used as natural feed. *Tetraselmis chuii* and two strains of *Isochrysis galbana* were imported from other laboratories. Local strains of *Tetraselmis* sp. and *Dunaliella* sp. were also tested (AQD Annu. Rept. 1980).

Freshwater Algae

Expansion of the research program to freshwater led to the establishment of the Binangonan Research Station. Here, emphasis on hatchery and nursery operations for tilapia and milkfish necessitated algal production. Algae representing different major groups were selected, namely: *Chlorella ellipsoidea,* Chlorophyta; *Chroococcus dispersus,* Cyanophyta; *Navicula notha,* Chrysophyta; and *Euglena elongata,* Euglenophyta.

The algal composition of "green" water usually varies. The predominant species are *Chlorella* spp. and *Scenedesmus* spp. *Ankistrodesmus* sp. and *Nannochloris* are also present in lesser numbers.

CULTURAL METHODS

Brackishwater

Batch cultures of algae, being the simplest, were used during the early attempts at larval rearing of P. monodon (AQD Annu. Rept. 1976). Seawater was enriched with commercial fertilizers (NPK or urea), and naturally occurring algal species were made to "bloom" in rearing tanks. However, problems were encountered when excessive diatom blooms resulted in left-over algae which decayed and polluted the water. Furthermore, fertilizers seemed to be toxic to the larvae. Thus, the procedure was modified so that the algal culture was sand-filtered and the diatom concentrated was pumped into the larval rearing tank (Platon 1978).

More improvements were made later in the algal production system. Stock cultures of different species isolated from Buyuan Bay were main-

tained in the laboratory. *Chaetoceros calcitrans* was among the first algae to be studied extensively and utilized effectively as live, natural food for P. *monodon* larvae. It was established in a culture medium containing macro- and micro-nutrients (Table 1). There were two sources of silicon in the medium, the inorganic salt and "Agrimin" which is a commercially available mixture of micro-nutrients. Growth of *C. calcitrans* in this medium was monitored. When nutrients were replenished daily, growth per day for the first three days was significantly higher (148.7%) than in the control (35.6%) without replenishment (Fig. 1).

Improvements of the basal *Chaetoceros* medium were made later with good results (Platon, 1978). Simplication of culture media for large-scale tank cultures utilized commercial fertilizer (e.g., urea). Silicon was always provided to enhance growth of diatoms.

Table 1. *Chaetoceros* medium

$NaNO_3$	- 0.1 g/l
K_2HPO_4	- 1.0 g/l
$FeCl_4$	- 0.2 mg/1
Na_2SiO_3	- 0.1 mg/l
Vitamins (B_{12} & B_1)	- 1.0 mg/1
Agrimin*	- 1.0 mg/1
Seawater (boiled/filtered)	- 500 ml
Freshwater	- 500 ml

*Agrimin, a brand name: Manganese, 15%, Boron, 5%; Iron, 8%; Calcium, 3%; Zinc, 10%; Molybdenum, 5-10%; Copper, 5-10%; Potassium, 3%; Silicon, 36%.

In Freshwater

Laboratory cultures. Recent experiments were conducted to compare the growth of selected algal species in three types of media: a) organic, b) inorganic, and c) semi-synthetic (Table 2). Inexpensive organic sources of nutrients such as ipil-ipil *{Leucaena leucocephala)* leaf meal extract and duck manure extract were used. Chemical analyses of the three types of media show some differences in the amounts of major and minor elements required for algal growth (Table 3).

Growth rates of *Chroococcus dispersus* in the different types of media were comparable (Fig. 2). However, the lag phase was longest in the organic medium. It took about eleven days for the logarithmic phase to be reached as compared to only seven days in the inorganic and semi-synthetic media. This may be explained in terms of the slow release of nutrients in the organic medium.

Duncan's Multiple Range Test did not show significant differences among the different media tested for *C. dispersus* (Table 4).

Chlorella ellipsoidea showed the best growth rate in semi-synthetic (K= 1.16) and inorganic (K=1.12) media (Table 4). The organic medium was relatively poor for *Chlorella* (Fig. 3).

*Navicula notha** preferred the organic and semi-synthetic media over the inorganic one. With the inorganic medium, the log phase was reached only after 12 days of culture (Fig. 4).

Euglena elongata showed significantly different growth rates based on the type of medium: best in semi-synthetic, moderate in organic, and poor in inorganic (Fig. 5, Table 4).

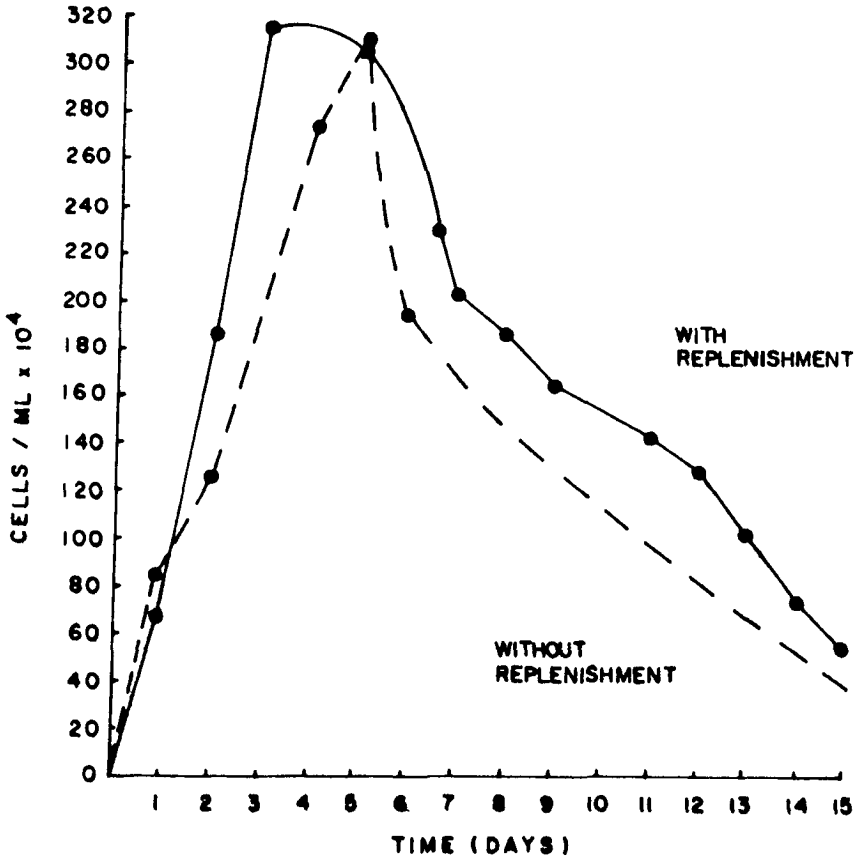

Fig. 1. Growth of *Chaetoceros calcitrans* in *Chaetoceros* medium *(see* Table 1 for composition).

*Verification of identification courtesy of Dr. Milagrosa Martinez, University of the Philippines at Los Banos.

Table 2. Media for growing selected species of freshwater algae

Inorganic Medium

	g/l
$CaNO_2$	.1258
$MgCl_2$	.0664
$MgSO_4$	.0450
KCl	.0191
NaCl	.0812
$NaHPO_4$	.0229
$NaNO_3$	.2573
Na_2SiO_3	.1861
$FeCl_3$	.0003
Micronutrients*	1 ml/l

Organic Medium

Prepare following stocks separately:	*ml stock /l*
- *Ipil-ipil leaf meal extract*	
Grind 500 g ipil-ipil leaves; squeeze through cheese-cloth in 500 ml distilled water; autoclave at 20 psi for 15 min.	10
- *Duck manure extract*	
Pulverize 500 g duck manure; squeeze through cheese-cloth in 500 ml distilled H_2O; autoclave at 20 psi for 15 min.	10
- Agrimin** - 10 g/100 ml	1
- Water	159

Semi-synthetic medium

	ml stock /l
Inorganic medium (without micronutrients)	800
Soil water extract	200
Agrimin**	1

*Composition/100 ml: H_3BO_3, 200 mg; $MnCl_2.H_2O$, 150 mg; $ZnSO_4.7H_2O$, 20 mg; $CuCl_2.5H_2O$, 10 mg; $NaMoO_4$, 1 mg; Hormex, 1 ml.

**For composition, see footnote for Table 1.

Table 3. Chemical analyses of different media (ppm)

Parameters	Organic	Semi-synthetic	Inorganic
Total inorganic N	.096	.051	.283
Orthophosphate	.00017	.0032	.0153
Silica	20	82.5	91.6
Total Hardness ($CaCO_3$)	158	158	-
Calcium	5.58	4.64	30.73
Magnesium	35	35.5	25.5
Sodium	25	180	118.2
Potassium	7.0	28.0	10.02
Manganese	.87	.14	416.4
Iron	.231	.046	.103

Table 4. Mean generation rates (K) of four algae in different media (Figures are means of three replicates)

B. Media	A. Algal Species			
	C. dispersus (A_1)	C. *ellipsoidea* (A_2)	*N. notha* (A_3)	*E. elongata* (A_4)
Organic media (B_1)	0.74^a	0.85^b	0.89^a	0.81^a
Inorganic media (B_2)	0.61^a	1.12^a	0.58^b	0.61^b
Semi-synthetic (B_3)	0.65^a	1.16^a	0.75^a	0.87^a

Means of the same superscript in a column are not significantly different from one another.

ANOVA for species (A), media (B) and AxB are highly significant. The organic medium exerted the same effect on all the species, i.e., comparable growth rates were shown by the four algal species (Table 5). In the inorganic and semi-synthetic media, *Chlorella* showed significantly faster growth rate. In general, the organic and semi-synthetic media proved best for all the algal species representing different major groups (Table 6).

Based on the foregoing, the possibility of growing selected algae singly or in combination in inexpensive media seems to be a promising alternative in the production of natural food for use in aquaculture.

Outdoor tank cultures. A simplified, continues culture technique was followed in producing phytoplankton for fry-to-fingerling production. Marine plywood tanks (1,000 liters capacity) were field with water to a depth of 40 cm only. NPK(14-14-14) was added at 0.1 g/l every three days to sustain algal bloom. Furthermore, one-third of the old culture medium, including algal cells that settled at the bottom, was siphoned out every three days prior to fertilizer application. The same amount of tap water was added as replenishment.

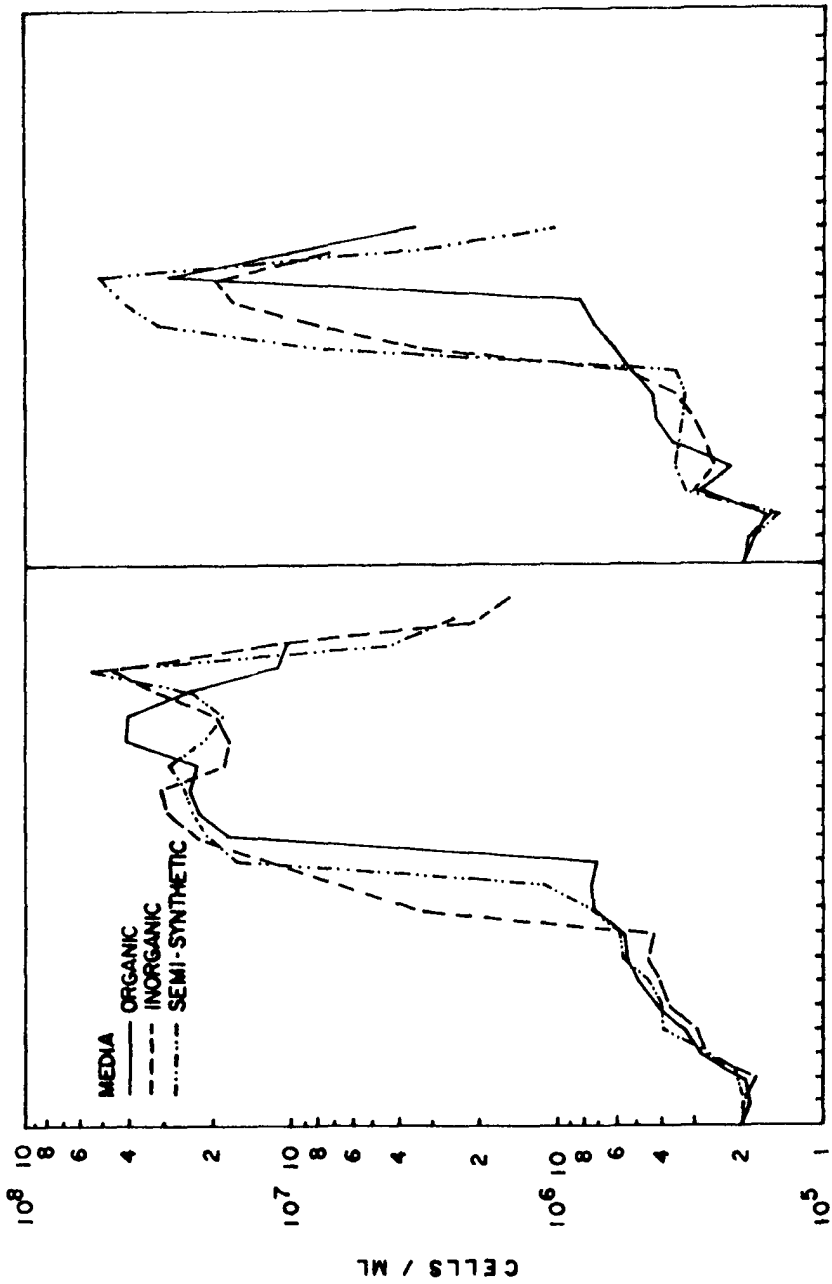

Fig. 2. Growth of *Chroococcus dispersus* in different media.

Fig. 3. Growth of *Chlorella ellipsoidea* in different media.

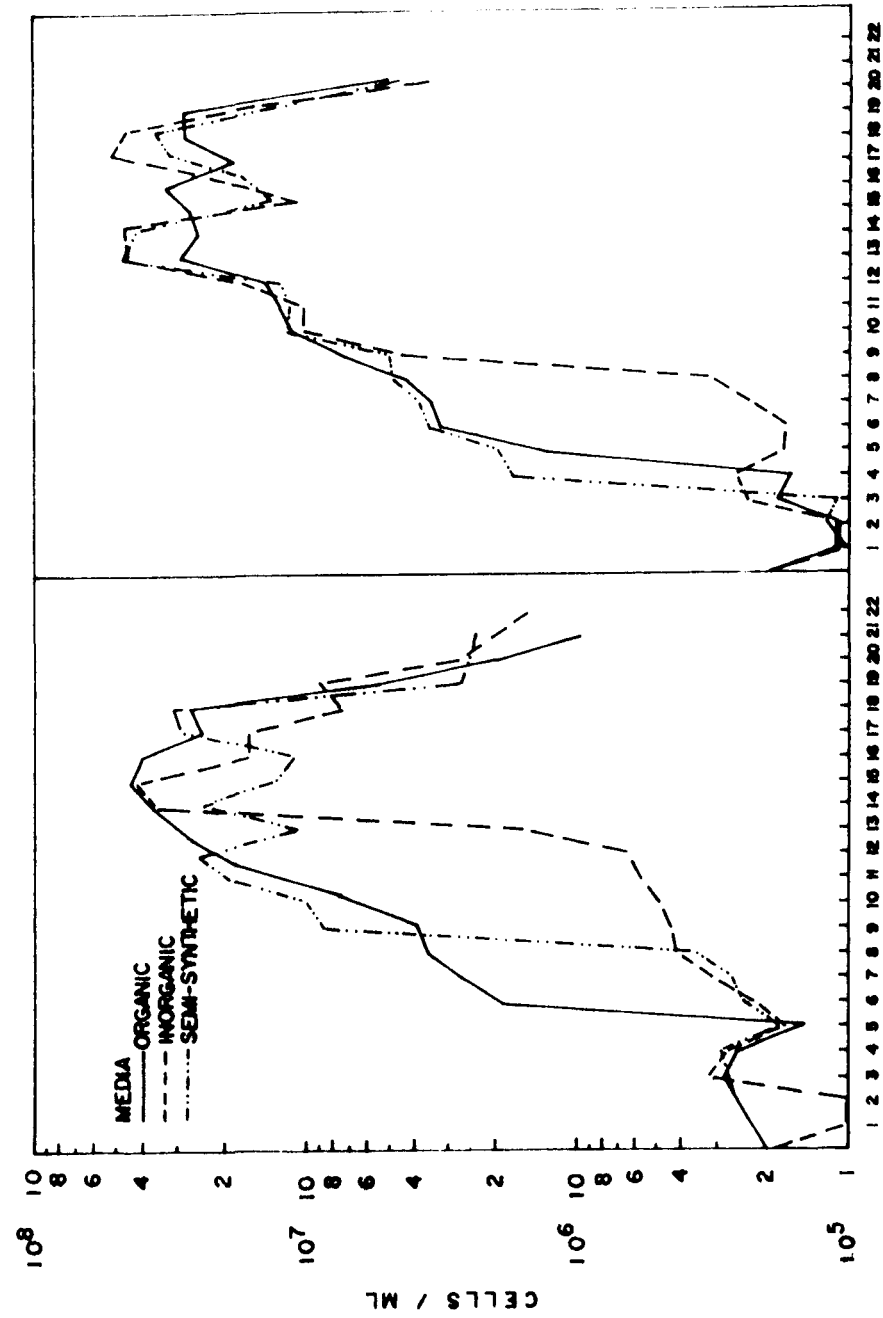

Fig. 4. Growth of *Navicula notha* in different media.

Fig. 5. Growth of *Euglena elongata* in different media.

Table 5. Duncan's Multiple Range tests of growth rates of different algae in different media (Means underlined are not significantly different from one another)

Organic medium			
C. dispersus	*E. elongata*	*C. ellipsoidea*	*N. notha*
.74	.81	.85	.89
Inorganic medium			
N. notha	*C. dispersus*	*E. elongata*	*C. ellipsoidea*
.58	.61	.61	1.12
Semi-synthetic medium			
C. dispersus	*N. notha*	*E. elongata*	*C. ellipsoidea*
.65	.75	.87	1.16

Table 6. Duncan's Multiple Range test for media based on total growth rate means of four species (Means underlined are not significantly different)

Organic (B_1)	Semi-synthetic (B_3)	Inorganic (B_2)
3.29	3.43	2.92

With the method described above, "green" water with a cell density of 150-175 x 10^3 cells/ml was produced. This optimum concentration of algal cells can be maintained up to 60 days with proper management.

UTILIZATION OF MICROALGAE IN AQUACULTURE

Larval Rearing of *P. monodon*

One of the major problems in the operation of P. *monodon* hatcheries is the need for a continuous and adequate supply of the right kind of live food. Reports of high fry mortality triggered the all-out effort to conduct extensive feeding studies and screen promising algal species.

Earlier laboratory studies (AQD Annu. Rept. 1976) used live and frozen *Chaetoceros calcitrans* as feed for up to zoea 3 giving as high as 93% and 98% survival, respectively. Thus, there is the possibility of harvesting and storing diatoms for future use to augment the supply of natural feed during times of scarcity. In the same experiment, diatom consumption was determined by monitoring the cell density of the medium with or without larvae. Results showed average diatom consumption per larva at the zoea stages as follows: zoea 1 = 6,000 cells; zoea 2 = 13,100 cells; and zoea 3 = 14,000 cells (Fig. 6).

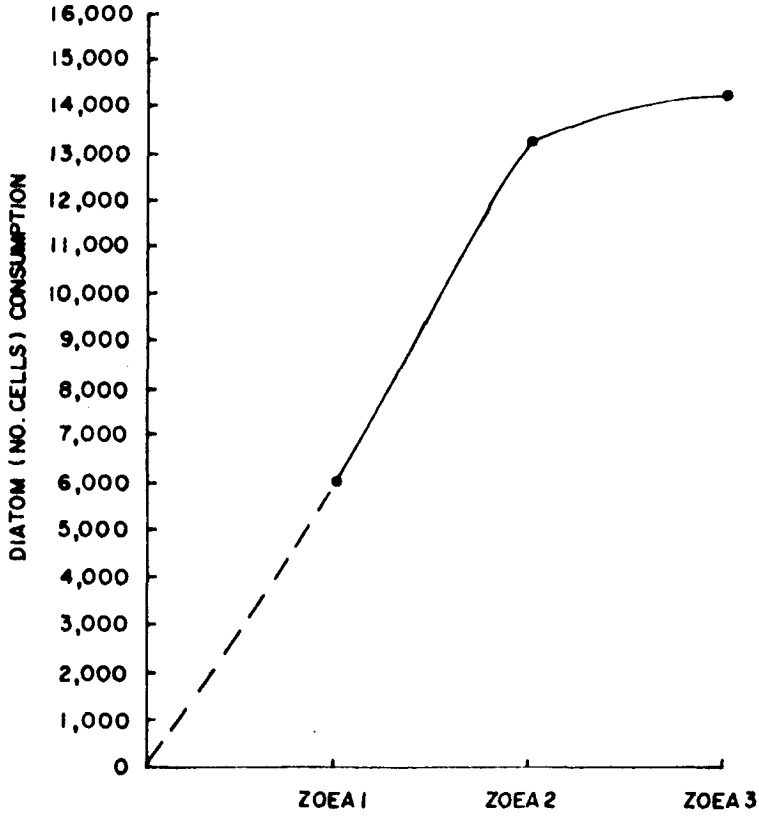

Fig. 6. Estimated algal consumption *(Chaetoceros calcitrans)* of different zoea stages of *P. monodon.*

Later, feeding experiments explored the use of a variety of algal species (AQD Annu. Rept. 1980). Five algal species which include local strains of *Chaetoceros calcitrans* (10 x 104 cells/ml), *Tetraselmis* sp. (5 x 104 cells/ml), *Dunaliella* sp. (5 x 104 cells/ml), two imported strains *of Isochrysis galbana* (7 x 104 cells/ml), and *Skeletonema costatum* (10 x 104 cells/ml) were used as natural feed for P. *monodon* larvae. Highest survival was obtained with C. *calcitrans.*

Sunaz (1980) compared growth and survival of P. *monodon* zoeas given different diatom feeds. Highest mean survival rates were obtained using *Chaetoceros gracilis* (62.90%) and mixed diatoms (60.43%).

Fry to Fingerling Production

Tilapia nilotica. "Green" water consisting of *Nannochloris* sp., *Chlorella* spp. and *Scenedes* spp. was given to *T. nilotica* fry at various concentrations:

a) high density - 150-175 x 10^3 cells/ml; b) moderate density - 90-120 x 10^3 cells/ml; and c) low density - 50-60 x 10^3 cells/ml.

There was a proportionate increase in growth and survival of tilapia fry with increased density of phytoplankton. "High", "moderate" and "low" algal densities gave growth rate of 13.3, 8.9 and 4.7 mg/day, respectively (Table 7). Growth of tilapia fry given limited amounts of phytoplankton was poor and comparable to that given rice bran as feed.

Table 7. Mean weight, survival rate and growth rate of tilapia fry fed phytoplankton at various density levels

Algal density	Weight (g)				Survival rate (%)	Growth rate (g/day)
	0	2nd wk	4th wk	6th wk		
High	0.008	0.164[a]	0.481[a]	0.546[a]	93[a]	0.0133[a]
Moderate	0.008	0.098[b]	0.330[b]	0.363[b]	93[a]	0.0089"
Low	0.008	0.033[c]	0.099[c]	0.192[c]	62[b]	0.0047[c]
Rice bran (control)	0.009	0.024[c]	0.045[c]	0.186[c]	36[c]	0.0045[c]
F values (ANOVA)	·	23.35*	29.04**	14.40**	-	-

Means with the same superscript in a column are not significantly different from one another (DMRT).
*Significant. **Highly significant.

Gut analysis of *T. nilotica* fry grown in various algal concentrations gave an estimate of the relative intake of algal food at various levels of feeding (Fig. 7). Results showed decreasing algal food in the gut of *T. nilotica* with decreasing amount of phytoplankton in the rearing medium.

Milkfish (Chanos chanos). Stage 1 milkfish fry reared in aquaria with different algal species for five days showed high survival in the *Chlorella-Chroococcus-Euglena* combination and *Oscillatoria* alone (Table 8). These algal species were shown to be suitable natural feeds up to ten days of culture for the *Chlorella-Chroococcus-Euglena* combination and up to 15 days for the treatment *Oscillatoria* alone. Older milkfish fry, 20 days in culture (stage IV), showed poor survival in all treatments.

More experiments are being conducted to pursue the preliminary results described above.

CONCLUSION

Accelerated pace in aquaculture to produce fish protein for the people of Southeast Asia calls for support from all disciplines. Phycology, despite its

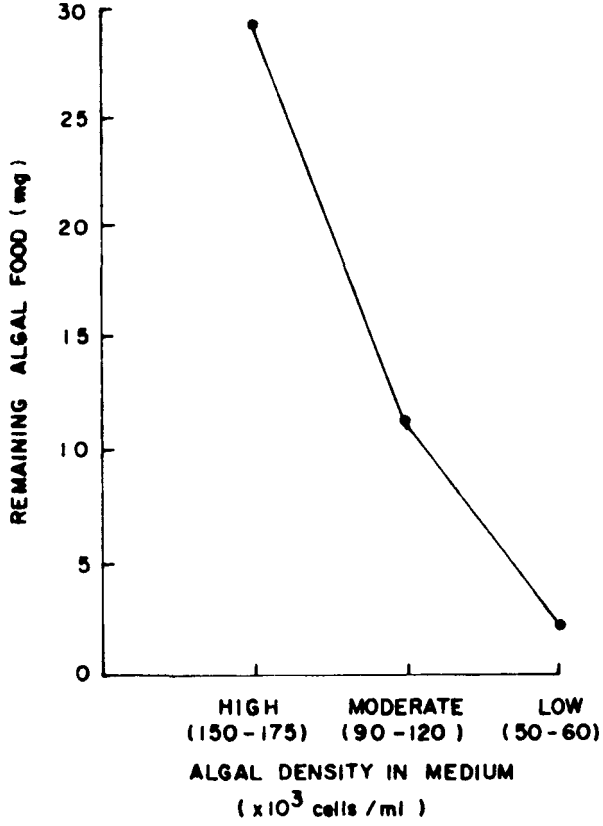

Fig. 7. Remaining algal food after 24 h in gut of *T. nilotica* fingerlings in "green water" with different phytoplankton densities.

Table 8. Mean survival (%) of different stages of milkfish fry given different algal feeds (Figures are averages of three replications)

Algal feed	Stage			
	I (Sept. 3-8)	II (Sept. 9-14)	III (Sept. 16-21)	IV (Sept. 21-27)
Chlorella	41.7	75.0	50.0	8.0
Euglena	75.0	41.7	0	8.0
Oscillatoria	100.0	100.0	100.0	50.0
Navicula	91.7	100.0	75.0	16.7
Chroococcus	41.7	91.7	41.7	8.0
Euglena-Oscillatoria-Navicula	58.0	100.0	66.7	8.0
Chlorella • *Chroococcus* - *Euglena*	100.0	100.0	58.0	50.0
Rice Bran	91.7	91.7	66.7	33.0

being a very basic discipline, is most relevant to fish farming. It is in the area of natural food production where the micro-algae have become very important to sustain high fry and fingerling survival.

There is need to integrate efforts in the culture and utilization of algae for greater impact to fisheries development. Manpower and physical resources should be pooled effectively. Only then can we go beyond the laboratory scale and find application in the field.

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