

In: Nitrogen and Rice. Symposium held at the  
International Rice Research Institute, Los Baños,  
Philippines, 18-21 Sept. 1978. - Manila, International  
Rice Research Institute, 1979.

# Ecology of blue-green algae in paddy fields

P. A. ROGER and P. A. REYNAUD

Climatic, physicochemical, and biotic factors as well as cultural practices, affecting qualitative and quantitative variations in the algal community in rice paddies are reviewed.

A survey of ecological studies concerning the development of the algal biomass and variation in its  $N_2$  fixing activity during the cultivation cycle of rice yielded some results from well-defined situations, but the scarcity of similar studies slows research progress in these areas.

BLUE-GREEN ALGAE (BGA), which taxonomically are midway between prokaryotic and eukaryotic organisms, are capable of both photosynthesis and  $N_2$  fixation in aerobic conditions. Such *trophic independence* in regard to carbon and N, combined with a great adaptability to variations in edaphic factors, permits BGA to be ubiquitous and at the same time gives them a unique potential to contribute to productivity in a variety of agricultural and ecological situations (Fogg et al 1973).

Several factors, including the high temperature requirements of rice, nutrient availability, reducing conditions in soil, and ability of BGA to withstand desiccation, favor the growth of BGA in paddy fields. The growth of BGA contributes significantly to spontaneous fertility of paddy soils (Singh 1961, Venkataraman 1972).

Because there are about 100 million hectares of paddy fields in the world, considerable research is focused on the use of BGA in agriculture. A survey of the literature shows the abundance of research on the physiology of  $N_2$ -fixing BGA and the practice of algalization and its effects on rice yields (Venkataraman 1972). On the other hand, ecological studies emphasizing qualitative algal composition or algal succession in various geographical situations, or, more rarely, in certain physicochemical parameters are scarce and generally descriptive.

To our knowledge, only four reports on quantitative variations of algal flora during a crop cycle have been published (Table 1).

23 DEC. 1980

Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), Boite Postale 1386, Dakar, Senegal.  
The assistance of Dr. Donald Taylor, ORSTOM, Dakar, in verifying the English of our manuscript is gratefully acknowledged.

O. R. S. I. O. M.

Collection de Références

n° 10. 108 Bio Jols

Table 1. Partial list of the main ecological studies<sup>a</sup> on BGA in paddy fields.

Author	Geographic location of the study	Floristic inventory	Qualitative study	Quantitative study	ARA measurement
Al-Kaisi 1976	Iraq	+	+	-	-
Gupta, A. B. 1966	India	+	+	-	-
Okuda, A., and Yamaguchi, H. 1952	Japan	+	+	-	-
Pantastico, J. B., and Suayan Z. A. 1974	Philippines	+	+	-	-
Renaut, J., and Sasson, A. 1970	Morocco	+	-	-	-
Reynaud, P., and Roger, P. 1978b	Senegal	+	+	+	-
Roger, P., and Reynaud, P. 1976-77	Senegal	-	+	+	-
Singh, R. N. 1961	India	+	+	-	-
Traoré et al 1978	Mali	-	+	+	+
Watanabe, A., and Yamamoto, Y. 1971	Tropics	+	-	-	-
Watanabe, I. et al 1977	Philippines	-	-	+	+

<sup>a</sup> + = studied, - = not studied.

## METHODOLOGY

Ecological studies of BGA in submerged soils are limited by problems in methodology, primarily in estimating algal biomasses qualitatively and quantitatively, and in measuring algal N<sub>2</sub>-fixing activity (NFA). In addition, problems in sampling techniques in relation to spatial distribution of BGA increase the inaccuracy of quantitative measurements.

### Methods of estimating algal abundance

Algal abundance is determined by three principal methods: plating techniques, measurement of pigments, and direct observation. None, however, is completely satisfactory (Fogg et al 1973).

*Dilution techniques and plating.* Plating techniques are advantageous in providing qualitative and quantitative results simultaneously; however, the accuracy of the counts depends on the reliability of the particular dilution method. Petersen (1932) pointed out that the dilution is not reliable for fila-

mentous forms because even with vigorous shaking, filaments are difficult to separate into individual cells. It is also possible that small-celled forms, such as *Anabaena*, which are easily separated, may give inflated figures of abundance (Pandey 1965).

Plating techniques can be greatly improved by:

1. using selective media that permit enumeration of algae classified as  $N_2$ -fixing, prokaryotic, and eukaryotic (Reynaud and Roger 1977); and
2. determining the mean volume of each *count unit* (cell, filament, or colony, according to species) by directly examining the first dilution and multiplying the results by the corresponding *volume unit*.

An improved method, in which algae counts are expressed in terms of biomasses (Roger and Reynaud 1976), is more accurate but is time-consuming and extremely tedious.

*Pigment analysis.* Pigments are extracted in acetone. Chlorophyll concentration characterizes the total algal biomass, and myxoxanthin characterizes the BGA biomass (Singh 1961).

Unfortunately, this method does not indicate the composition of the algal flora and, in most instances, is not suitable for field material because colored organic material, such as humic acids, may also be extracted in acetone and affect the results (Fogg et al 1973).

*Direct microscopic examination.* Direct microscopic examination can be achieved with either ordinary light or fluorescence microscopy (Tchan 1953, Drew and Anderson 1977). This method, generally used for qualitative measurements, was used by Saito and Watanabe (1978) for quantitative evaluation of algal biomass of floodwater or surface soil. Floodwater was filtered on membrane filters and gelatinized soil suspension was smeared on glass slides.

### Nitrogen-fixing activity measurements

The advantages and disadvantages of the acetylene ( $C_2H_2$ )-reduction technique, now widely used, have been reviewed by Hardy et al (1973). Problems concerning aquatic  $C_2H_2$ -reduction techniques were reviewed by Flett et al (1976) and improvements proposed.

Although this method is excellent for comparative studies, users who attempt to obtain quantitative data in situ should realize the possible associated problems and extrapolate the results to quantity of N fixed per ha and per cycle of cultivation with caution (Fogg et al 1973). However, estimates of algal NFA, by this method, unlike measurements of rhizospheric NFA, are relatively reliable when the measurements are brief and the problems of gas diffusion are negligible.

### Distributional ecology of algae and acetylene reduction activity sampling

The validity and accuracy of algal enumerations and of in situ  $C_2H_2$  reduction activity (ARA) measurements depend principally on density of sampling. The density of sampling for a given degree of accuracy varies with the distribution

law of the variable. This law was determined for algae and in situ ARA measurements in paddy field in Senegal (Roger et al 1977, Roger and Reynaud 1978) through a study of the correlation between means and variances of groups of replicate measurements. The algae and ARA had an approximately log-normal distribution (logarithms of numbers were normally distributed).

The first implication of the results was that the confidence interval and parametric statistical variables (i.e. *t* variable of Student-Fischer) must be calculated using only the logarithms of algal enumerations or ARA measurements. The confidence interval so calculated was disymmetrical. Its lower limit was generally a little less than those incorrectly calculated using the *t* variable of Student-Fischer; the upper limit was generally higher. The disymmetry increased as the number of replicates was reduced and was markedly strong with less than five replicates.

The second implication of the distribution of algae and ARA concerned the density of sampling. For a given accuracy, more replicates were needed when distribution was log-normal than when it was normal.

As an example, the mean value of *Anabaena* biomasses based upon 40 samples of 10 cores each, taken in a 2,500 m<sup>2</sup> paddy, had a confidence interval of +32% and -27% of the mean. Such an evaluation made on three selective media required 1,800 petri dishes.

These difficulties may explain the scarcity of quantitative ecological studies of the algal flora in paddy fields.

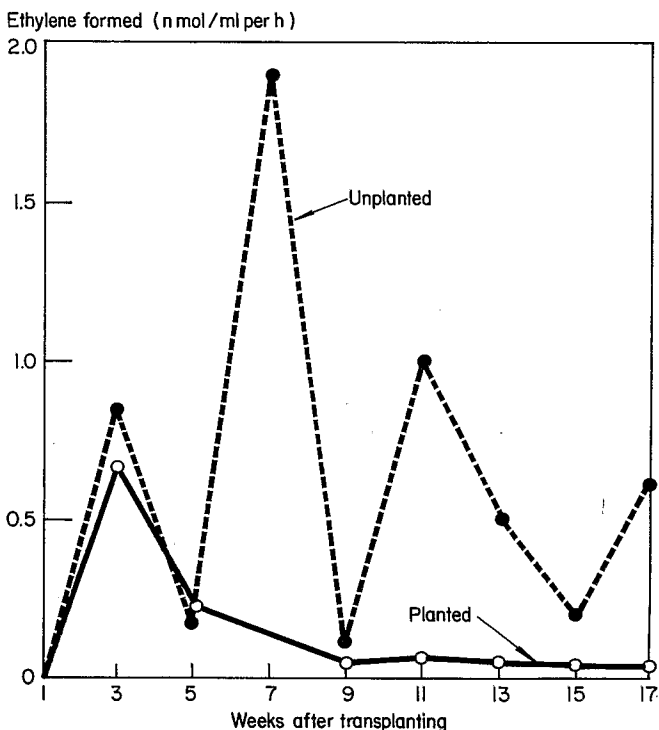
#### GROWTH, COMPOSITION, AND NITROGEN-FIXING ACTIVITY OF THE ALGAL COMMUNITY

The algal community is less affected by chemical properties of the soil than by climate and the composition of the floodwater.

##### **Climatic factors**

*Light.* Algae, as phototrophic microorganisms, are restricted to the photic zone and are usually located in the upper 0.5 cm of the soil. In submerged soils, light availability depends on the season and latitude, the cloud cover, the plant cover, and the turbidity of the water. In paddies, algae occur especially as a surface scum, as a bloom, or as crust-forming aggregates at the soil-water interface. During daylight, vertical migration of algae occurs in the water in relation to oxygen production by photosynthesis.

Light tolerance differs among species and may be roughly correlated with taxonomic groups. Whitford (1960) indicated that many green algae are adapted to high light conditions (high-light species) and red algae are low-light species; diatoms and chrysophyceae seem more indifferent to light. BGA are generally sensitive to high light intensities (Reynaud and Roger 1978a) and may be regarded as low-light species (Brown and Richardson 1968). Therefore, because algae have different light-adaptation abilities, light may have a selective effect



1. Variations of algal ARA in paddy water during cultivation cycles at IRRI; cropped and noncropped fields during the wet season (Yoshida and Ancajas 1973).

on the floral composition.

After the rewetting of paddy soils in Senegal, diatoms and unicellular green algae develop first. BGA develop when the plant cover is sufficiently dense to protect them from high light intensities (higher than 80,000 lx at 1300 h). The N<sub>2</sub>-fixing algal biomass and the density of the plant cover were positively correlated (Roger and Reynaud 1977). While studying algal flora in a monsoon zone where light intensities were not as high, Gupta (1966) observed no such succession, and BGA developed from the beginning of the cultivation cycle.

The influence of high light intensities on algal successions was confirmed by Reynaud and Roger (1978b), who incubated submerged unplanted soil under screens to permit the transmission of 100, 60, 22, and 7% of incident sunlight. After 1 month of incubation, the ARA was highest in the most heavily shaded soil, and green algae and diatoms were dominant in the soil placed in full sunlight.

Deficiency of light may also act as a limiting factor. In the Philippines, during the wet season when light was moderate, ARA was higher in bare soil than in planted soil (Yoshida and Ancajas 1973) (Fig. 1a).

*Desiccation and rewetting.* Soil algae, especially BGA, have a high capacity to withstand desiccation. Resistance to desiccation increases with the dryness of the biotope (Hess 1962) and can be related to the floristic composition of desiccated soils. Materasi and Balloni (1965) found that  $N_2$ -fixing forms comprised about 30% of the algal flora in a paddy field in Italy, where the dry period was relatively short. In Senegal, where the dry season lasts about 8 months, Roger and Reynaud (1976) observed that spores of heterocystous BGA constituted more than 95% of potential flora at the end of the dry period and that eukaryotic algae were present primarily because of their introduction in irrigation water.

In Uttar Pradesh, India, a large number of chlorophyceae occur in low-lying fields where the moist habitat apparently is suitable for their growth; BGA, being more resistant to drier conditions, occur in larger numbers in paddy fields at higher elevations (Pandey 1965).

Species that form globose or maculiform mucilaginous colonies lose water slowly. Several authors have indicated that *Cylindrospermum* sp. becomes dominant and can develop profusely at the end of the cultivation cycle as the soil dries (Pandey 1965, Reynaud and Roger 1978b, Traoré et al 1978).

Alternation of periods of desiccation and submersion may also influence quality and quantity of the algal biomass. In paddy soils in Japan, Okuda and Yamaguchi (1952) reported that algae were more abundant when the soil was waterlogged throughout the year than when it was waterlogged during rice growth only.

A peak of carbon and N mineralization occurs during rewetting of desiccated soils; the high concentration of mineral N may decrease the relative competitiveness of  $N_2$ -fixing forms, and the high concentration of carbon dioxide may favor the growth of green algae (Shapiro 1973).

*Temperature.* The optimal temperature for the growth of BGA is about 35°C, higher than that of eukaryotic algae (Sorokin 1959). Temperature extreme permitting their growth is, therefore, beyond the range within which rice grows; thus, rarely is temperature a limiting factor for BGA in paddy fields.

However, Subrammangan et al (1965), in both paddy and pot experiments, observed a setback to the growth of algae during the cold season in India. In temperate or sahelian zones during the dry season, a lower temperature at the beginning of the cultivation cycle may inhibit BGA growth and favor eukaryotic algae (Roger and Reynaud 1976).

On the other hand, in dryland rice cultivation, under a weak plant cover, a high temperature in the middle of the day may inhibit algal NFA. Jones (1977) observed such an inhibition in a soil planted to Kikuyu grass.

On wet soil, high temperatures at the soil surface have little effect on the survival of BGA, whose resistance to temperature increases with desiccation (Glade 1914).

*Other factors.* In paddies, rain may increase water turbidity, limiting available light to such an extent that the NFA is significantly decreased. On non-

submerged soils, buffeting rains may mix algae and the top layer of the soil, and nearly completely inhibit the algal NFA (Traoré et al 1978).

Wind may cause algae to accumulate on one side of the field, decreasing light availability or, on the contrary, cause dense algal masses to disperse and form colonies.

### Physicochemical factors

*pH.* pH is important in determining algal flora composition, but it is difficult to distinguish the effects of pH that are due directly to hydrogen ion concentration from those that are due to other chemical factors such as solubility of trace elements (Fogg et al 1973).

As pointed out by Tiffany (1951), the dominant algal species in acidic and alkaline soils often differ, i.e. the growth of chlorophyceae is favored by low pH values and that of BGA by higher values (Pandey 1965). In laboratory cultures, the optimal pH for growth of BGA seems to range from 7.5 to 10.0 and the lower limit is about 6.5 to 7.0 (Holm-Hansen 1968).

In natural conditions most BGA grow in environments that are neutral to alkaline; a few species occur in habitats with a pH between 5.0 to 6.0 (Fogg 1956).

However, Okuda and Yamaguchi (1956) demonstrated that in submerged acidic soils, the supernatant water, in which most algae grow, always has a higher pH than the soil itself. They observed a positive correlation between the water pH and the occurrence of BGA in paddies in Japan.

Garcia et al (1973) found a highly significant positive correlation between the pH of paddy soils (4.0 to 6.8) and the number of spores of  $N_2$ -fixing BGA in the soil during the dry season. A pH of 5.0 after 1 month rewetting and submersion was the lower limit compatible with the presence of  $N_2$ -fixing BGA. In the same soils during crop growth, Roger and Reynaud (1977) observed a positive correlation between the soil pH (5.0 to 6.9) and the  $N_2$ -fixing biomass. This relationship was conspicuous only in samples homogenous for stage of rice development, fertilization, and plant cover density.

*Oxidation-reduction level of the soil.* A number of unicellular and homocystous BGA reduce  $C_2H_2$  under microaerophilic conditions (Stewart et al 1977). In paddies where deoxygenated conditions occur and where nonheterocystous algae are dominant,  $N_2$ -fixation by these algae may be important.

*Phosphorus.* The phosphorus requirement for optimal algal growth differs considerably among species when no other external factor is limiting (Kuhl 1974). Rhode (1948) differentiated three main groups of freshwater algae according to the ability to tolerate phosphorus below, around, and above 20  $\mu\text{g/l}$ . However, no conclusion could be made about the relationship between phosphorus requirements and taxonomic groups.

The phosphorus levels within algal cells may fluctuate widely depending on whether or not the algae are growing under phosphorus-limited conditions. BGA assimilate more phosphorus than they require and store the excess as

polyphosphates, which can be used under phosphorus-deficiency conditions (Batterton and Van Baalen 1968).

Stewart et al (1970) reported that phosphorus-deficient cultures had low ARA and the addition of phosphorus induced a rapid stimulation of the  $N_2$ ase activity, often within 15 to 30 minutes. This response could be used as a bioassay (Stewart and Alexander 1971).

Many limnologists consider phosphorus the primary limiting nutrient for algae growth in most bodies of water (Woldendorp 1972). In paddies, phosphorus-supplying manure enhances algae growth and ARA. Okuda and Yamaguchi (1952) incubated 117 submerged soils in a greenhouse and noted that BGA growth seemed closely related to the available phosphorus content of the soil. Algal growth in moist soils was poor at 0 to 5 ppm phosphorus, but vigorous above 6 ppm.

According to Fogg et al (1973) the growth of  $N_2$ -fixing BGA in paddies is most commonly limited by low pH and phosphorus deficiency.

*Carbon.* In general, limnologists have paid little attention to the possibility that carbon supply may be the controlling factor in algae growth. This hypothesis, opposed by *phosphate advocates*, is supported by the demonstrated stimulatory effect of carbon dioxide on algal growth under natural conditions (Woldendorp 1972). De and Sulaiman (1950) observed that  $N_2$ -fixation by algae in the absence of a crop is stimulated considerably by passing air containing 2% carbon dioxide over the surface water. They concluded that the stimulatory effect of the crop was largely due to the increased supply of carbon dioxide evolved as a result of plant respiration and root decomposition.

Algae can obtain carbon for synthesizing new cells from a variety of organic and inorganic sources, and they exhibit heterotrophic or autotrophic metabolism.

Obligate photoautotrophy occurs in many different classes, including chlorophyta, diatoms, and cyanophyta. Although some algae exhibit heterotrophic growth and some require organic compounds to photosynthesize, it appears that when carbon dioxide and light are adequate, the photosynthetic mechanism becomes predominant (Goldman et al 1972).

Dissolved inorganic carbon in floodwater occurs as a  $CO_2 \rightleftharpoons HCO_3^- \rightleftharpoons CO_3^{2-}$  equilibrium system. This system is largely affected by pH changes resulting from algal growth. The extraction of aqueous carbon dioxide by the algal biomass at a higher rate than it can be replaced (through atmospheric carbon dioxide diffusion, respiration, fermentations, and solid carbonates equilibria) leads to an increased pH level that can affect algal growth in a number of ways, including solubility of essential elements (e.g. molybdenum, iron metabolic effects, and shift of the equilibrium system, so that  $HCO_3^-$  and even  $CO_3^{2-}$  predominate (Goldman et al 1972).

For a number of algae, carbon dioxide is the only carbon compound that can support growth (Raven 1974). Direct utilization of  $HCO_3^-$  and even  $CO_3^{2-}$  by the other algae, including BGA, is controversial and King (1970) assumed



that under natural conditions, algal populations consume only carbon dioxide and no bicarbonate.

Shapiro (1973) confirmed this hypothesis in situ by manipulating pH, nutrient concentration, and amount of free carbon dioxide in isolated lake zones. As summarized by Reynolds and Walsby (1975), the simultaneous addition of nutrients and free carbon dioxide result in a large dominance of green algae; lowering the pH had a similar effect. At higher pH values, free carbon dioxide has a lower concentration; after the addition of nutrients, BGA predominate.

Whether or not King's hypothesis is correct, it appears that BGA competently extract dissolved inorganic carbon at high pH values, and that high carbon dioxide production, after soil rewetting or organic material incorporation, may favor the growth of green algae.

*Nitrogen.* Although algae require more N than phosphorus, it is generally assumed that in natural conditions, N is not the primary nutrient limiting the growth of non-N<sub>2</sub>-fixing algae, but is more likely to be a limiting element in eutrophic waters than in oligotrophic waters (Woldendorp 1972).

Among the products of the N cycle, nitrite sometimes occurs but at low concentrations (0–3 ppm) (De and Sarkar 1936), and gaseous products of denitrification do not accumulate in the soil. Therefore, the principal sources of N correspond to the main forms of N fertilizers. Little is known about the competition between N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing forms as affected by the nature and the concentration of inorganic N.

However, the selective action and inhibitory effect of nitrogenous fertilizers on NFA have been demonstrated in many experiments. Okuda and Yamaguchi (1952) observed algal flora monthly from April 1944 to November 1945, in soils of lysimeters treated with different N-fertilizers. N<sub>2</sub>-fixing forms (*Anabaena*) became abundant only in the unfertilized control. In soils treated with ammonium sulfate, only green algae appeared dominant. In soils treated with calcium cyanamide, three green algae and one *Euglena* sp. became abundant.

The addition of farmyard manure and ammonium sulfate induced a diversification of the dominant species, with five green algae and four eukaryotic nongreen algae.

After ammonium sulfate treatments, Subramanyan et al (1965) also observed that *Spirogyra* sp. and *Euglena* sp. were so abundant that rice farmers had to intertill their crop to prevent the algae from smothering the rice plants.

In pot experiments, Yoshida et al (1973) observed that N fertilizer increased algal growth but that generally there were more BGA in pots without N fertilizer. N<sub>2</sub> fixation decreased markedly with the addition of 200 kg N/ha; it was reduced as much as 72% by ammonium sulfate and as much as 98% by ammonium chloride; it was completely inhibited by the addition of 400 kg N/ha.

In situ ARA measurements confirm the inhibitory effects of N fertilizers on algal NFA. However, the role of mineral N as the main factor inducing

the development of a  $N_2$ -fixing bloom, or, on the contrary, the dominance of other forms, does not appear to be well established.

Concurrent utilization of ammonium, nitrate, and  $N_2$  by algae was demonstrated in a freshwater algal bloom by Dugdale and Dugdale (1965) and, as indicated by Mague (1977),  $N_2$  fixation may not be a last resort but may supplement other sources of combined N.

In stagnant paddy water, within mixed algal masses, combined N may diffuse at a slower rate than the readily available dissolved  $N_2$ , and a local depletion of combined N may favor  $N_2$  fixation. Thus, the competitiveness of  $N_2$ -fixing algae in N-limited conditions may not be as clear-cut and limited as it was first thought.

*Calcium.* A calcium requirement for maximum growth has been shown for some green algae, BGA, and diatoms (Healey 1973). In general BGA require more calcium for growth on  $N_2$  than on combined nitrogen (Fay 1962, doctoral thesis, University of London) and the addition of calcium carbonate to paddy soils enhances both growth of the algae and N fixation (Okuda and Yamaguchi 1952).

It is also uncertain whether the beneficial effect of liming paddy soils is due solely to the increased availability of calcium or to the increase in soil pH, which occurs simultaneously (Fogg et al 1973).

*Molybdenum.* Because of its function in nitrate reductase and  $N_2$ ase, molybdenum is required by all algae obtaining N through either process.

The amount of molybdenum required by *Anabaena cylindrica* for  $N_2$  fixation exceeds that for nitrate reduction (Wolfe 1954). In paddy fields molybdenum is also likely to be a limiting factor for NFA (Okuda et al 1962). Subramanyan et al (1965) suggested the addition of sodium molybdate (0.25 kg/ha) to soil to improve  $N_2$ -fixing algae growth.

*Other elements.* Many other elements are required for optimal growth of algae but their ecological implication as limiting factors or as factors affecting the composition of the algal community in paddies has not been demonstrated.

### **Agricultural factors**

*Crop and tillage practices.* The crop, per se, has a positive effect on soil algae which appear to be more abundant in cultivated than in uncultivated soils (Tiffany 1951). There is also a positive effect in the root zone of rice fields (Shtina et al 1964).

However, cultural practices disturbing the biotope may affect algal composition or activity; for example, after plowing the recolonization of non-submerged soil by diatoms took about 2 months (Lund 1945).

In paddies, the disturbing effects of tillage consist mainly of the incorporation of algae (or spores) in the soil and dispersion of soil clay into the submersion water, which considerably decreases available light.

After a superficial incorporation of algae into the soil, motile forms such as *Oscillatoria* and *Pseudanabaena* are probably more adapted to the recolo-

nization of the submersion water, as shown by Moss (1977) in a marine habitat.

*Fertilization.* The nature and the quantity of fertilizers as well as techniques of fertilization may have a considerable influence on subterranean algal flora. In Senegal paddies fertilizers were incorporated into the soil at the beginning of the cultivation cycle but were not spread over the surface of the soil to prevent a dense initial growth of algae that may cause young plants to lodge.

The addition of organic matter to soil temporarily depressed the algal population especially during the active decomposition of the organic matter, after which, however, algae grew extensively.

It appears that heterotrophic NFA is promoted by NPK fertilizers whereas phototrophic NFA is predominant in unfertilized soils (Watanabe et al 1977).

In comparing the effects of different doses of ammonium sulfate and triple superphosphate, Marathe (1963) observed that ammonium sulfate increased the number of algal species and the addition of phosphates yielded the largest number of species.

In Senegal, the study of the qualitative and quantitative composition of algal flora in 30 paddies differing in geographic location, stages of rice growth, and fertilizer treatment, indicated that N and phosphorus fertilization had a positive effect on both total algal biomass and potential  $N_2$ -fixing biomass. However, it had a negative effect on the relative potential  $N_2$ -fixing algal biomass expressed in terms of percentage of the total biomass (Roger and Reynaud 1977).

*Pesticides.* Da Silva et al (1975) identified two types of algal response—an increase or a distinct decrease in activity—following an initial period of depression after a treatment with pesticides. Cullimore and McCann (1977) noted an overall reduction in cell number in  $N_2$ -fixing algae treated with 2,4-D, trifluralin, MCPA, and TCA. Some pesticides have a selective effect on algae; pentachlorophenol used at a concentration of 100 ppm suppressed green algae without any deleterious effect on BGA (Watanabe 1967).

However, some pesticidal compounds severely limited the  $N_2$ -fixing capacities of BGA, thereby affecting the overall N economy of the soil (Da Silva et al 1975).

*Algalization.* Inoculation of paddies with BGA may significantly increase ARA and rice yield (Venkataraman 1961, 1972). Venkataraman (this volume) gives precise information on the response of rice plants to algal inoculation.

### Biotic factors

*Antagonism.* Many algae release substances that inhibit either their own growth or the growth of other species, or both (Fogg 1962, Hellebust 1974). *Nostoc punctiforme* produces autotoxic substances (Harder 1917); *Microcystis* sp. produces substances that inhibit the growth of other algae, such as *Chlamydomonas*, *Haematococcus*, *Navicula*, and *Cryptomonas* (Vance 1965).

In a filter-culture system that allowed only an exchange of media, *Chlamydomonas globose* was inhibited by *Chlorococcum ellipsoideum* (Kroes 1971).

But the large difference in cell densities between culture conditions and those found in nature, even in a bloom, made ecological extrapolation rather uncertain (Hellebust 1974).

Growth inhibiting and promoting substances may play important roles in the succession of species in aquatic ecosystems, but direct evidence for BGA in natural environments is lacking (Fogg et al 1973).

*Pathogens and predators.* Pathogens or predators—cyanophages, bacteria, fungi,—and fish grazing on algae may cause the decline of an algal bloom.

Cyanophages have a much more specific host range than the algicidal Myxobacteria (Shilo 1970); the cyanophage D<sub>1</sub> isolated by Daft et al (1970) was tested for susceptibility on 51 algae strains, of which 38 were resistant.

Considering the widespread occurrence of algal viruses (Safferman and Morris 1963, Padan et al 1967) and the specific host ranges of the individual strains, it is possible that in natural situations algal pathogens may be important in determining algal successions and disappearance.

Parasitic or saprophytic bacteria may also cause the rapid disintegration of large algal biomasses (Daft and Stewart 1971). Lytic bacteria may cause BGA cell lysis within 2–10 hours. Frequently bacteria caused the decomposition of BGA vegetative cells although they did not affect heterocysts and spores.

Parasitic fungi have a host range limited within a single species and some may even be confined to a specific structure as a heterocyst or a resting spore (Canter 1972). Their seasonal variations are directly correlated with the abundance of the algae on which they occur but their ecological role is not known.

Daphnids may proliferate in paddy fields and devour the whole algal population within 1 or 2 weeks (Venkataraman 1961). Their action can be prevented with pesticides (Watanabe 1967); seasonal drying may also help control their proliferation (Watanabe and Kiyohara 1960).

Some fish such as *Tilapia nilotica* that may develop profusely in some paddy waters are known to ingest great quantities of BGA (Moriarty et al 1973) as do some chironomid larvae (Martinez et al 1977).

### **Algal biomasses in paddy fields**

Probably because of the above-mentioned technological difficulties, quantitative evaluations of algal biomasses in terms of kg/ha are scarce. In cultivated soils in the USSR, the estimates stated are 40–50 kg/ha—double that amount under favorable circumstances—and as much as 500 kg/ha, or even 1,400 kg/ha, in *takyrs*, the unique clay plains where BGA are the dominant vegetation (Forest 1965).

In paddy soils in Senegal, Roger and Reynaud (1977) measured algal biomasses from a few kg to 6 t (fresh weight)/ha, depending on the stage of rice development, soil fertilization, and physicochemical properties of soil. In the Philippines, Watanabe et al (1977) observed a maximum value of about 24

t (fresh wt)/ha in nonfertilized soil during the dry season.

The values mentioned above are comparable with those obtained in laboratory cultures. Myers et al (1951) obtained a maximal cell density of 55 g(dry wt)/l in very shallow cultures of *Chlorella* sp. (0.5 cm). A maximum value of 13.6 g/l was obtained by Milner (1953) at a depth of 1.5 cm of medium. The results, expressed as g/m<sup>2</sup>, are similar. Milner (1953) assumed that the mass of cells per unit area would not increase beyond a value that is probably determined by mutual shading of the cells.

Extrapolation of the results indicates a maximal biomass of 2.75 t (dry wt)/ha; assuming a water content of 90 to 95% in algae, this value corresponds to 27–54 t (fresh wt)/ha.

### Quantitative variations during the cultivation cycle

In paddies in Senegal, maximal biomass developed between tillering and panicle initiation (Roger and Reynaud 1977). In dryland fields in India, a similar evolution was observed by Gupta (1966); in wetland fields, however, the density of the biomass was maximal a little later than in Senegal.

In the Philippines, algal density during the dry season was highest just after heading of the rice crop (Watanabe et al 1977). Thus, a roughly bell-shaped distribution of the total algal biomass along the cultivation cycle seemed most frequent. During the wet season, however, development was maximum after harvesting (Watanabe et al 1977), probably because of an increase in light availability.

### Qualitative variations of the algal community

Qualitative and quantitative variations in the algal community in paddies have been studied in India (Gupta 1966), in the Philippines (Pantastico and Suayan 1974), and in Senegal (Roger and Reynaud 1976, 1977; Reynaud and Roger 1978b).

A qualitative study of algae in the Allahabad district, India, (Gupta 1966) describes the evolution of the algal flora in dryland rice fields planted to an early rice variety and in wetland fields planted to a late variety.

In the dryland fields, the algae began to grow 1 month after the first monsoon rain; the algal community was poor in both quality and quantity and was dominated by *Spirogyra* sp. associated with *Anabaena* sp. During the second third of the cultivation cycle, the algal biomass became abundant with an important growth of *Anabaena* sp. During the last third of the cycle more species were observed and the N<sub>2</sub>-fixing forms were well represented by *Anabaena* sp., *Scytonema* sp., and *Nostoc* sp.

In the wetland fields, fresh water remained muddy 2 months after the beginning of the monsoon and no algae grew. *Anabaena* was the first species to develop, about 2 weeks after transplanting. During the next 2 months, an abundant mixed algal flora was observed. N<sub>2</sub>-fixing forms (*Scytonema* sp., *Aulosira* sp., *Nostoc* sp.) were associated with *Chara* and filamentous green

algae. At the end of the cycle, non-N<sub>2</sub>-fixing forms were disintegrating and *Scytonema* was dominant.

In both soils, N<sub>2</sub>-fixing forms were present in the algal community from the beginning and became abundant in the second half of the cycle.

The main differences between these two types of rice paddies were:

- a later growth of algae in wetland soil,
- a well-marked dominance of BGA in dryland soils, and
- a mixed community of eukaryotic and prokaryotic algae in wetland soils.

Gupta (1966) concluded that the difference in growth between wetland and dryland fields was associated with ecological conditions, and the overall succession was controlled chiefly by seasonal variations.

In the Philippines, Pantastico and Suayan (1973) observed that diatoms predominated during land preparation for transplanting, followed by green algae as the crop grew. BGA predominated just before and during the harvest.

Paddy soils in Senegal are acidic, with an average pH value of 5.0 at the beginning of rice cultivation and 6.2 after 2 months of submersion. The rainy season is short (15 July–15 November) and rice fields are dry the rest of the year. High light intensities, reaching 70,000–80,000 lx, occur throughout the year. The qualitative and quantitative compositions of total and N<sub>2</sub>-fixing algal flora were studied first during the cultivation cycle in a paddy in the north of Senegal (Roger and Reynaud 1976) and then in 40 paddy fields differing in geographic location, stage of rice growth, and fertilizer treatment (Table 2).

The similar results of the two studies can be the basis of a scheme for algal successions. During the early part of the cultivation cycle (planting to tillering), the algal biomass increased and consisted mainly of diatoms and unicellular green algae. From tillering to panicle initiation, the algal biomass reached its highest values, and filamentous green algae and non-N<sub>2</sub>-fixing BGA were dominant. After panicle initiation the total biomass decreased. If the plant cover was sufficiently dense, heterocystous and homocystous BGA became dominant but if the cover was thin, filamentous green algae and homocystous

Table 2. Algal biomass composition in relation to rice development (40 paddy soils studied) (Reynaud and Roger 1978b).

Stage of rice development	Dominant flora			N <sub>2</sub> -fixing algae			
	Nature	% of total biomass			% of total biomass		
		Mean value	Max. value	Min. value	Mean value	Max. value	Min. value
Tillering	Diatoms, unicellular green algae	73	99	49	2	4	0.1
Panicle initiation	Filamentous green algae	89	93	86	3	9	0.1
	Non-heterocystous blue green algae						
Heading to maturity (Weak plant cover)	Filamentous green algae	70	91	62	8	14	0.2
	Non-heterocystous blue-green algae						
Heading to maturity (Dense plant cover)	Blue-green algae	71	99	16	38	99	13

BGA remained dominant.

The following interpretation of algal flora variations was proposed. At the beginning of the cultivation cycle, paddy soils were characterized by:

- a low pH, which favored the development of chlorophyceae but not that of BGA,
- an absence of plant cover and a corresponding high light intensity at the air-water interface that was also favorable to the development of chlorophyceae and diatoms but unfavorable to BGA,
- the presence of mineral N caused by the organic N mineralization after soil remoistening or from manure on fertilized soils, and
- a high level of carbon dioxide, caused by soil remoistening, which was favorable to green algae.

During the cultivation cycle, a decrease in light intensity related to rice growth, and to N level, and an increase in pH value favored BGA growth. The nonevolution of algal flora composition under a weak plant cover indicated the important role of light in regulating algal composition.

It is clear that the proposed interpretation of algal successions is incomplete and that other factors of nutrition and competition affect the sequence of the algae groups. The previously described algal succession should be considered characteristic of a definite geographic zone, and of unusual conditions of rice cultivation (acidic mangrove soils, high light intensities, and a climate at the limit of aridity).

#### **Turnover of algal biomass material**

The elemental nutrients fixed by the algal biomass are released either through exudation or through microbial decomposition after the cells die.

The production of a great variety of extracellular substances by algae is now well established. Carbon is released as carbohydrates: the amounts exuded may represent 7 to 50% of the photoassimilated carbon (Fogg et al 1965). Amino acids and peptides are common in algal filtrates but, in most cases, represent only a small fraction of the total extracellular material. BGA, on the other hand, liberate large portions of their assimilated nitrogenous substances into the medium, between 20 and 40% according to Fogg et al (1973). Taha and El Refai (1962) have reported that *Nostoc commune* produces nitrate and nitrite that can make up a third of the extracellular N.

The release of organic substances often plays an important role in aquatic food chains (Hellebust 1974) and probably also in algal successions. More important, however, is the quantity of the nutrients released through microbial decomposition after the death of algal biomasses. Some algae are decomposed in 2-3 days but others withstand microbial digestion for more than 4 weeks (Gunnison and Alexander 1975).

The susceptibility to decomposition and the amount of nutrients released depend on

- the physiological state of the algae,

- the composition of the associated microflora (Watanabe and Kiyohara 1960),
- the suitability of the cell wall as a substrate for microorganisms, and
- the relative biodegradabilities of specific components of the algal walls.

Laboratory experiments have demonstrated that more than half of the nutrients contained in an algal biomass can be regenerated in less than 1 month with the aid of microbial degradation (Verhoff and De Pinto 1977).

In paddies the death of the algal biomass is most frequently associated with soil desiccation at the end of the cultivation cycle. BGA, therefore, show a partially delayed manuring effect; successive inoculations have a cumulative effect (Watanabe 1965) and a significant residual effect (Subramanyan et al 1965).

### ALGAL NITROGEN-FIXING ACTIVITY IN PADDY FIELDS

#### Daily variations in algal nitrogen-fixing activity

A review of literature reveals four general forms of diurnal curves in the ARA of BGA:

1. asymmetrical curves with a maximum in the morning and a low decreasing activity in the afternoon (Alimagno and Yoshida 1977),
2. curves with two maxima, one in the morning and another in the afternoon (Renaut et al 1975, Traoré et al 1978),
3. asymmetrical curves with a maximum in the afternoon (Balandreau et al 1974, Alimagno and Yoshida 1977), and
4. symmetrical curves according to variations of incident light with a slight delay (Reynaud and Roger 1978b).

These four types of curves were also observed with samples of an *Anabaena* bloom placed under screens that permitted the passage of 100, 60, 22, and 7%, respectively, of the incident sunlight. The maximum light intensity in the day was about 90,000 lx (Reynaud and Roger 1978b). These results indicate that light seems to be an important factor, if not the most important factor in regulating diurnal variations of ARA.

The observed curves may be explained as

- Curves of the first form seem artificial. They were obtained only with cultures or algal masses, not adapted to high light intensities, placed under direct sunlight. (In such conditions a bleaching of algae occurs in stirred diluted cultures.)
- Curves of the second form result from a transitory inhibitory effect of high light intensity in the middle of the day.
- Curves of the third form are obtained with optimal light intensities.
- Curves of the fourth form occur with limited light intensity because of cloudy weather or a dense plant cover, or both.

Factors other than light may induce diurnal variations of ARA. In a water-logged soil, high temperature in the middle of the day had an inhibitory effect



(Jones 1977). In submerged paddy fields, the floodwater is a relatively good temperature buffer and inhibitory temperatures probably do not occur frequently.

If King's hypothesis (see the preceding section on carbon dioxide) is correct, available carbon dioxide may be depleted at noon because of high photosynthetic activity corresponding to pH values higher than 8.2, but such a depletion and its influence on ARA were not demonstrated.

Under some conditions BGA can fix N in the dark (see Fay 1976). In situ measurements by Alimagno and Yoshida (1977) confirmed this nonnegligible activity.

### Variations of algal NFA during the cultivation cycle

The most important works concerning variations in algal NFA during the cultivation cycle are those at the International Rice Research Institute (IRRI).

Yoshida and Ancajas (1973) compared algal NFA in planted and nonplanted flooded soils during the wet and the dry seasons by measuring the ARA of the floodwater. No fertilizers were applied. During the wet season, ARA was higher in nonplanted fields; its sharp variations correlated with fluctuations in algal growth. In planted fields ARA reached its maximum 3 weeks after transplanting, then it decreased from the third to the ninth week, and finally remained low. The kinetics of ARA was related to limiting light intensities; the deficiency increasing with the density of the plant cover.

This hypothesis was confirmed with measurements made during the dry season. Under an increased light intensity, there was no difference between planted and nonplanted soils and an approximately bell-shaped curve with a peak around the ninth week was observed. The estimated amount of N fixed, using the theoretical conversion factor, was 3 kg/ha in a planted flooded field and 11 kg/ha in a nonplanted flooded field during the wet season, and 15 kg/ha in either planted or nonplanted fields during the dry season.

At IRRI, in situ ARA assays were also conducted. Algal ARA showed two peaks, a small one at the beginning of the crop season and a larger one near or after harvest (Watanabe et al 1977).

In some areas of the Philippines where N fertilizers were not applied, as much as 30 kg N/ha was fixed by BGA in one cropping season. Alimagno and Yoshida (1977) compared ARA during a cultivation cycle in a NP (42.4 kg-4 kg) fertilized paddy and in an unfertilized paddy. Samples covered with black cloth exhibited no ARA, suggesting that BGA were the principal N<sub>2</sub>-fixing agents in these fields. In both cases maximum NFA occurred about 40 days after transplanting. A much higher NFA was estimated in the nonfertilized field (18-33 kg N/ha per cropping season) than in the fertilized one (2.3-5.7 kg N/ha per cropping season).

Seasonal changes in algal ARA, determined with field assays at IRRI, are discussed by Watanabe and Cholitzkol (this volume).

A study of algal ARA variation during the 1976 and 1977 rainy seasons in

an upland nonfertilized rice field in Mali (Traoré et al 1978) indicated that maximal ARA may occur at both the start and end of the cultivation cycle. The estimated great amounts of  $N_2$  fixed (50–80 kg N/ha per cultivation cycle) agree with a high density of  $N_2$ -fixing algae ( $2.2 \times 10^6$  cells/g dry soil).

From the results it appears that a peak of algal ARA may occur any time during the cultivation cycle. The reports of results are too scarce, however, to deduce rules governing relative algal NFA variations during the cultivation cycle. However, a predominant effect of light intensity in relation to the season and the plant cover is clear and an inhibitory effect of N fertilization is incontestable.

The estimated amounts of  $N_2$  fixed vary from a few to many kilograms and the value of 30 kg N/ha per crop indicated by Watanabe et al (1977) seems to constitute a satisfactory reference value when edaphic factors are favorable for BGA growth.

### CONCLUSION

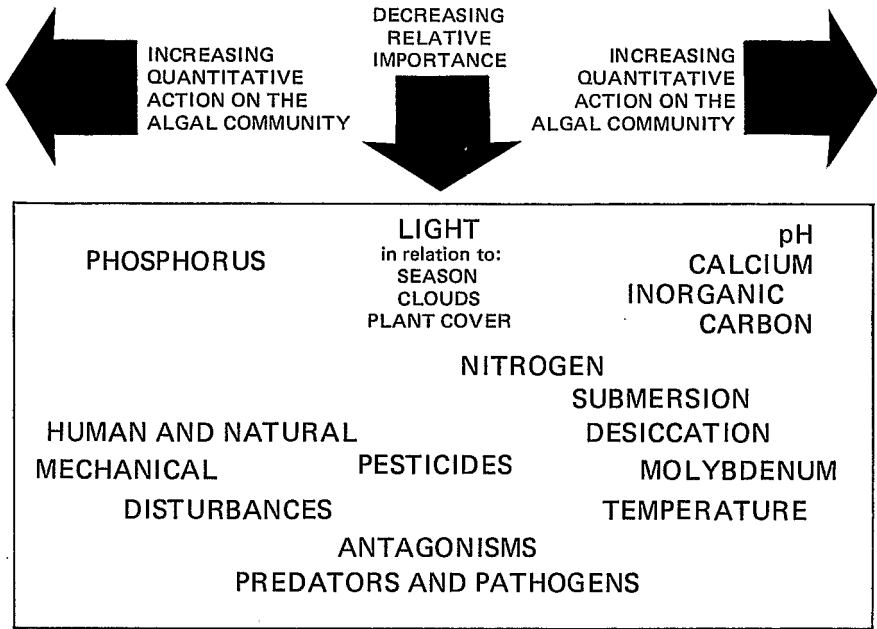
Ecological studies concerning qualitative and quantitative variations of the algal community in paddy soils are scarce and are limited by research methodology problems. However, numerous observations on fields and experimental plots, complemented with limnological studies in fresh water, identify major factors affecting changes in the algal community in paddy fields.

By their action on the algal biomass, it is possible to distinguish among the factors that affect the variations those that are quantitative, qualitative, and both quantitative and qualitative. An assay of hierarchization and classification is presented in Figure 2. When light is not a limiting factor, climatic and physicochemical factors (light, pH, water regime, temperature) seem to affect the algal composition more markedly than the absolute value of the total algal biomass.

Among the trophic factors, phosphorus is probably the most important nutrient determining the luxuriance of the algal community and, partially, its composition. Nitrogen, in conditions unfavorable for BGA growth, may determine the whole algal productivity but, more frequently, it acts as a selective factor, as other trophic factors do. Depending on their selectivity, pesticides and biotic factors (predators and antagonisms) may influence both the quantity and the composition of the algal biomass.

Reported variations in algal biomasses and NFA during the cultivation cycle of rice should be considered limited examples for well-defined situations; they do not permit generalizations. According to these observations, BGA may become dominant anytime during the cultivation cycle, exhibiting one or several peaks of ARA.

A low pH and a low level of available phosphorus have been found to limit BGA growth in paddy soils, and the absence of conspicuous growth is far more likely to be caused by unfavorable growth conditions than by their



2. Assay of classification and hierarchization of the main factors affecting the ecology of the algal community in paddy fields.

absence. The comparison between the low algal biomasses observed in acidic phosphorus-deficient paddies in Senegal (max value = 6 t/ha), and the high value observed in the neutral paddies in the Philippines (26 t/ha) illustrate the predominant role of pH and phosphorus when both factors are favorable. The differences observed between the dry- and wet-season in the Philippines indicate that light intensity, in relation to clouds and plant cover, seems to determine BGA growth.

Further observation and experimentation are needed to determine the mechanisms of algal successions and the influence of biotic factors. Thus, since methods for estimating the quantity and quality of algal biomasses and their ARA are not completely satisfactory, they should be used critically with a precise knowledge of the validity and accuracy of the measurements.

As pointed out by Vollenweider (1974), many biologists restrict their knowledge to Gaussian distributions, variances calculation, and parametrical tests suitable only with random distributions. Because densities of algae and ARA measured in situ generally have a log-normal distribution, the data must be transformed before statistical exploitation; the data must be sufficient and a suitable experimental design should be used to allow statistical evaluations.

## LITERATURE CITED

- ALIMAGNO, B. V., and T. YOSHIDA. 1977. In situ acetylene-ethylene assay of biological nitrogen fixation in lowland rice soils. *Plant Soil* 47:239-244.
- AL-KAISL, K. A. 1976. Contribution of the algal flora of the rice fields of Southeastern Iraq. *Nova Hedwigia* 27:813-827.
- BALANDREAU, J., C. MILLIER, and Y. DOMMERGUES. 1974. Diurnal variations of nitrogenase activity in the field. *Appl. Microbiol.* 27:662-665.
- BATTERTON, J. C., and C. VAN BAALEN. 1968. Phosphorus deficiency and phosphate uptake in the blue-green algae *Anacystis nidulans*. *Can. J. Microbiol.* 14:341-348.
- BROWN, T. E., and F. L. RICHARDSON. 1968. The effect of growth environment on the physiology of algae, light intensity. *J. Phycol.* 4:38-54.
- CANTER, H. M. 1972. A guide to the fungi occurring on planktonic blue-green algae. Pages 145-158 in T. V. Desikachary, ed. *Taxonomy and biology of blue-green algae*. University of Madras, India.
- CULLIMORE, D. R., and A. E. MCCANN. 1977. Influence of four herbicides on the algal flora of a prairie soil. *Plant Soil* 46:499-510.
- DAFT, M. J., J. BEGG, and W. D. P. STEWART. 1970. A virus of blue-green algae from fresh water habitats in Scotland. *New Phytol.* 69:1029-1038.
- DAFT, M. J., and W. D. P. STEWART. 1971. Bacterial pathogens of freshwater blue-green algae. *New Phytol.* 70:819-829.
- DA SILVA, E. J., L. E. HENRICKSSON, and E. HENRICKSSON. 1975. Effect of pesticides on blue-green algae and nitrogen fixation. *Arch. Environ. Contam. Toxicol.* 3:193-204.
- DE, P. K., and S. N. SARKAR. 1936. Transformation of nitrate in waterlogged soil. *Soil Sci.* 42:143-155.
- DE, P. K., and M. SULAIMAN. 1950. Fixation of nitrogen in rice soils by algae as influenced by crop, CO<sub>2</sub>, and inorganic substances. *Soil Sci.* 70:137-151.
- DREW, E. A., and J. R. ANDERSON. 1977. Studies on the survival of algae added to chemical treated soils: 1) Methodology. *Soil Biol. Biochem.* 9:207-215.
- DUGDALE, V. A., and R. C. DUGDALE. 1965. Nitrogen metabolism in lakes: III. Tracer studies of the assimilation of inorganic nitrogen sources. *Limnol. Oceanogr.* 10:53-57.
- FAY, P. 1976. Factors influencing dark nitrogen fixation in a blue-green alga. *Appl. Environ. Microbiol.* 31:376-379.
- FLETT, R. J., R. D. HAMILTON, and N. E. R. CAMPBELL. 1976. Aquatic acetylene-reduction - techniques: solution to several problems. *Can. J. Microbiol.* 21:43-51.
- FOGG, G. E. 1956. The comparative physiology and biochemistry of the blue-green algae. *Bacteriol. Rev.* 20:148-165.
- FOGG, G. E. 1962. Extracellular products. Pages 475-489 in R. A. Lewin, ed. *Physiology and biochemistry of algae*. Academic Press, New York and London.
- FOGG, G. E., C. NALEWAJKO, and W. D. WATT. 1965. Extracellular products of phytoplankton photosynthesis. *Proc. R. Soc. London, Ser. B*, 162:517-534.
- FOGG, G. E., W. D. P. STEWART, P. FAY, and A. E. WALSBY. 1973. *The blue-green algae*. Academic Press, London and New York. 459 p.
- FOREST, H. S. 1965. The soil algal community: II. Soviet soil studies. *J. Phycol.* 1:164-171.
- GARCIA, J. L., M. RAIMBAULT, V. JACQ, G. RINAUDO, and P. ROGER. 1973. Activités microbiennes dans les sols de rizière du Sénégal: relations avec les caractéristiques physico-chimiques et influence de la rhizosphère. *Rev. Ecol. Biol. Sol.* 11:169-185.
- GLADE, R. 1914. Zur Kenntnis der Gattung *Cylindrospermum*. *Beitr. Biol. Pfl.* 12:295-344.
- GOLDMAN, J. C., D. B. PORCELLA, E. J. MIDDLEBROOKS, and D. E. TOERIEN. 1972. The effect of carbon on algal growth—its relationship to eutrophication. *Water Res.* 6:637-679.
- GUNNISON, D., and M. ALEXANDER. 1975. Resistance and susceptibility of algae to decomposition by natural microbial communities. *Limnol. Oceanogr.* 20:64-70.
- GUPTA, A. B. 1966. Algal flora and its importance in the economy of rice fields. *Hydrobiologia* 28:213-222.
- HARDER, R. 1917. Ernährungs physiologische Untersuchungen an Cyanophyceen, hauptsächlich dem endophytischen. *Nostoc punctiforme*. *Z. Bot.* 9:145-245.
- HARDY, R. W. F., R. C. BURNS, and R. D. HOLSTEN. 1973. Application of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem.* 5:47-81.

- HEALEY, F. P. 1973. Inorganic nutrient uptake and deficiency in algae. *C. R. C. Crit. Rev. Microbiol.* 3:69-113.
- HELLEBUST, J. A. 1974. Extracellular products. Pages 838-863 in W. D. P. Stewart, ed. *Algal physiology and biochemistry*. Bot. Monogr., Vol. 10. Blackwell, New York.
- HESS, U. 1962. Über die hydratabhängige Entwicklung und die Austrocknungsresistenz von Cyanophyceen. *Arch. Mikrobiol.* 44:189-218.
- HOLM-HANSEN, O. 1968. Ecology, physiology and biochemistry of blue-green algae. *Annu. Rev. Microbiol.* 22:47-70.
- JONES, K. 1977. The effects of light intensity on acetylene reduction by blue-green algal mats in subtropical grassland. *New Phytol.* 78:427-431.
- KING, O. L. 1970. The role of carbon in eutrophication. *J. Water Pollut. Control Fed.* 42:2035-2051.
- KROES, H. W. 1971. Growth interactions between *Chlamydomonas globosa* Snow and *Chlorococcum ellipsoideum* Deason & Bold under different experimental conditions with special attention to the role of pH. *Limnol. Oceanogr.* 16:869-879.
- KUHL, A. 1974. Phosphorus. Pages 636-654 in W. D. P. Stewart, ed. *Algal physiology and biochemistry*. Bot. Monogr., Vol 10. Blackwell, New York.
- LUND, J. W. G. 1945. Observations on soil algae. I. The ecology, size and taxonomy of British soil diatoms. *New Phytol.* 44:196-219.
- MAGUE, T. M. 1977. Ecological aspects of dinitrogen fixation by blue-green algae. Pages 85-140 in R. W. F. Hardy and A. H. Gibson, eds. *A treatise on dinitrogen fixation*. J. Wiley and Sons, New York.
- MARATHE, K. W. 1963. A study of the effect of fertilizers on the subterranean algal flora of paddy field soils from Karjat. *J. Univ. Bombay (India)* 31:1-10.
- MARTINEZ, M. R., C. L. Evangelista, and J. B. Pantastico. 1977. *Nostoc commune* Vauch. as a potential fertilizer in rice-fish culture: a preliminary study. *Philipp. J. Crop. Sci.* 2-4:252-255.
- MATERASI, R., and W. BALLONI. 1965. Quelques observations sur la presence de microorganismes autotrophes fixateurs d'azote dans les rizie-res. *Ann. Inst. Pasteur* 109:218-223.
- MILNER, H. W. 1953. Outdoor mass-culture units: rocking tray. Pages 108-113 in J.S. Burney, ed. *Algal culture from laboratory to pilot plant*. Carnegie Institution of Washington, Publication 600.
- MORIARTY, D. J. W., J. P. E. C. DARLINGTON, I. G. DUNN, C. M. MORIARTY, and M. P. TELVIN. 1973. Feeding and grazing in Lake George, Uganda. *Proc. R. Soc. London, Ser. B*, 184:299-319.
- MOSS, B. 1977. Adaptations of epipelic and epipsammic freshwater algae. *Oecologia* 28:103-108.
- MYERS, J., J. N. PHILIPS, and J. R. GRANHAM. 1951. On the mass culture of algae. *Plant Physiol.* 26:539-548.
- OKUDA, A., and M. YAMAGUCHI. 1952. Algae and atmospheric nitrogen fixation in paddy soils. II: relation between the growth of blue-green algae and physical or chemical properties of soil and effect of soil treatments and inoculation on the nitrogen fixation. *Mem. Res. Inst. Food Sci.* 4:1-11.
- OKUDA, A., and M. YAMAGUCHI. 1956. Nitrogen-fixing microorganisms in paddy soils. II: distribution of blue-green algae in paddy soils and the relationship between the growth of them and soil properties. *Soil Plant Food* 2:4-7.
- OKUDA, A., M. YAMAGUCHI, and I. NIOH. 1962. Nitrogen-fixing microorganisms in paddy soils. X. Effect of molybdenum on the growth and the nitrogen assimilation of *Tolypothrix tenuis*. *Soil Sci. Plant Nutr. (Tokyo)* 8:35-39.
- PADAN, E., M. SHILO, and N. KISLEV. 1967. Isolation of "Cyanophages" from freshwater ponds and their interaction with *Plectonema boryanum*. *Virology* 32:234-246.
- PANDEY, D. C. 1965. A study of the algae from paddy soils of Ballia and Ghazipur districts of Uttar Pradesh, India. I. Cultural and ecological considerations. *Nova Hedwigia* 9:299-334.
- PANTASTICO, J. B., and Z. A. SUAYAN. 1974. Algal succession in the ricefields of College and Bay, Laguna. *Philipp. Agric.* 57:313-326.
- PETERSEN, J. B. 1932. The algal vegetation of Hammer Bakkar. *Bot. Tidsskr.* 42:1-48.
- RAVEN, J. A. 1974. Carbon dioxide fixation. Pages 434-455 in W. D. P. Stewart, ed. *Algal physiology and biochemistry*. Bot. Monogr., Vol. 10. Blackwell, New York.
- RENAUT, J., and A. SASSON. 1970. Les Cyanophycées du Maroc. étude préliminaire de quelques biotopes de la région de Rabat. *Bull. Soc. Sci. Natl. Phys. Maroc* 50(1, 2):37-52.
- RENAUT, J., A. SASSON, H. W. PEARSON, and W. D. P. STEWART. 1975. Nitrogen-fixing algae in Morocco. Pages 229-246 in W. D. P. STEWART, ed. *Nitrogen fixation by free-living microorganisms*. Cambridge University Press, Cambridge.

- REYNAUD, P., and P. ROGER. 1977. Milieux sélectifs pour la numération des algues encaryotes, — procaryotes et fixatrices d'azote. *Rev. Ecol. Biol. Sol* (14) 3:421-428.
- REYNAUD, P. A., and P. A. ROGER. 1978a. Les hautes intensités lumineuses, facteur limitant l'activité fixatrice spécifique des Cyanobactéries in situ. *C. R. Acad. Sci.* (in press)
- REYNAUD, P. A., and P. A. ROGER 1978b. N<sub>2</sub>-fixing algal biomass in Senegal rice fields. *Ecol. Bull.* — Stockholm 26:148-157.
- REYNOLDS, C. S., and A. E. WALSBY. 1975. Water-blooms. *Biol. Rev.* 50:437-481.
- RHODE, W. 1948. Environmental requirements of freshwater plankton algae. *Symb. Bot. Ups.* — 10:1-49.
- ROGER, P., and P. REYNAUD. 1976. Dynamique de la population algale au cours d'un cycle de culture dans une rizière sahélienne. *Rev. Ecol. Biol. Sol.* 13(4):545-560.
- ROGER, P. A., P. A. REYNAUD, G. E. RINAUDO, P. E. DUCERF, and T. M. TRAORÉ. 1977. Mise en — évidence de la distribution log normale de l'activité réductrice d'acétylène in situ. *Cah. ORSTOM, Ser. Biol.*, 12:133-140.
- ROGER, P., and P. REYNAUD. 1977. La biomasse algale dans les rizières du Sénégal; importance relative des Cyanophycées fixatrices de N<sub>2</sub>. *Rev. Ecol. Biol. Sol* 14(4):519-530.
- ROGER, P., and P. REYNAUD. 1978. La numération des algues en sol submergé; loi de distribution — et problèmes d'échantillonnage. *Rev. Ecol. Biol. Sol* 15(2):229-244.
- SAFFERMAN, R. S., and M. E. MORRIS. 1963. Algal virus: isolation. *Science New York* 140:679-680.
- SAITO, M., and I. WATANABE. 1978. Organic matter production in rice field flood water. *Soil Sci.* — *Plant Nutr.* (Tokyo) 24. (in press)
- SHAPIRO, J. 1973. Blue-green algae: why they become dominant. *Science* 179:382-384.
- SHILO, M. 1970. Lysis of blue-green algae by Myxobacter. *J. Bacteriol.* 104:453-461.
- ŠHTINA, E. A., L. A. BAIRAMOVA, G. N. PERMINOVA, and A. N. TRETYAKOVA. 1964. The interaction between soil algae and higher plants. *Trans. 8th Int. Congr. Soil Sci.* III. 44:953-958.
- SINGH, R. N. 1961. Role of blue-green algae in nitrogen economy of Indian agriculture. *Indian* — *Council of Agricultural Research.* New Delhi. 175 p.
- SOROKIN, C. 1959. Tabular comparative data for the low and high temperature strain of *Chlorella*. *Nature* (London) 184:613-614.
- STEWART, W. D. P., G. P. FITZGERALD, and R. H. BURRIS. 1970. Acetylene reduction assay for — determination of phosphorus availability in Wisconsin Lakes. *Proc. Natl. Acad. Sci. U.S.A.* 66:1104-1111.
- STEWART, W. D. P., and G. ALEXANDER. 1971. Phosphorus availability and nitrogenase activity in aquatic blue-green algae. *Freshwater Biol.* 1:389-404.
- STEWART, W. D. P., P. ROWELL, and S. K. APTE. 1977. Cellular physiology and the ecology of — N<sub>2</sub>-fixing BGA. Pages 267-307 in W. E. Newton, J. R. Postgate, and C. Rodriguez-Barrueco, eds. *Recent developments in nitrogen fixation.* Academic Press, London.
- SUBRAMANYAN, R., L. L. RELWANI, and G. B. MANNA. 1965. Fertility build-up of rice field soils by blue-green algae. *Proc. Indian Acad. Sci., Sect. B*, 62:252-277.
- TAHA, E. E. M., and A. E. EL REFAI. 1962. Physiological and biochemical studies on the nitrogen- — fixing blue-green algae: 1) on the nature of cellular and extracellular substances formed by *Nostoc commune*. *Arch. Mikrobiol.* 41:307-312.
- TCHAN, Y. T. 1953. Study of soil algae. I. Fluorescence microscopy for the study of soil algae. *Proc. Linn. Soc. N.S.W.* 77:265-269.
- TIFFANY, L. M. 1951. Ecology of fresh water algae. Pages 293-311 in G. M. SMITH, ed. *Manual of* — *phycology* *Chronocia Botanica.* Waltham, Massachusetts.
- TRAORÉ, T. M., P. A. ROGER, P. A. REYNAUD, and A. SASSON. 1978. Etude de la fixation de N<sub>2</sub> par les cyanobactéries dans une rizière du Mali. *Cah. ORSTOM, Ser. Biol.* 13-2 (in press).
- VANCE, B. D. 1965. Composition and succession of Cyanophycean water blooms. *J. Physiol.* — 1:81-86.
- VENKATARAMAN, G. S. 1961. The role of blue-green algae in agriculture. *Sci. Cult.* 27:9-13.
- VENKATARAMAN, G. S. 1972. Algal biofertilizers and rice cultivation. *Today and Tomorrow's* — *Printers & Publs.* Faridabad (Haryana). 75 p.
- VERHOFF, F. H., and J. V. DE PINTO. 1977. Modeling and experimentation related to bacterial mediated degradation of algae and its effects on nutrient regeneration in lakes. Pages 213-229 in *Development in industrial microbiology*, Vol. 18, Society of Industrial Microbiologists.
- VOLLENWEIDER, R. A. 1974. A manual on methods for measuring primary production in aquatic — environments. *I.B.P. Handbook* 12. Blackwell Sc. Publications. 225 p.
- WATANABE, A., and T. KIYOHARA. 1960. Decomposition of blue-green algae as affected by the action of soil bacteria. *J. Gen. Appl. Microbiol.* 5(4):175-179.

- WATANABE, A. 1965. Studies on blue-green algae as green manure in Japan. Proc. Natl. Acad. Sci. India 35:361-369.
- WATANABE, A. 1967. The blue-green algae as the nitrogen fixators. Int. Congr. for Microbiol. Moscou 66. Symp. C.: 77-86.
- WATANABE, A., and Y. YAMAMOTO. 1971. Algal nitrogen fixation in the tropics. Plant Soil, Spec. Vol.: 403-413.
- WATANABE, I., K. K. LEE, B. V. ALIMAGNO, M. SATO, D. C. DEL ROSARIO, and M. R. DE GUZMAN. 1977. Biological N<sub>2</sub>-fixation in paddy field studied by in situ acetylene-reduction assays. IRR I Res. Pap. Series 3. 16 p.
- WHITFORD, L. A. 1960. Ecological distribution of fresh water algae. Pages 2-10 in C. A. Tryon, Jr. and R. T. Hartman, eds. The ecology of algae; a symposium held at the Pymatung Laboratory of Field Biology on June 18 and 19, 1959. University of Pittsburg.
- WOLDENDORP, J. W. 1972. Nutrients limiting algal growth. Stickstoff 15:16-25.
- WOLFE, M. 1954. The effect of molybdenum upon the nitrogen metabolism of *Anabaena cylindrica*. I. A study of the molybdenum requirement of nitrogen fixation and for nitrate and ammonium assimilation. Ann. Bot. N.S. 18:299-308.
- YOSHIDA, T., and R. R. ANCAJAS. 1973. Nitrogen-fixing activity in upland and flooded rice fields. Soil Sci. Soc. Am., Proc. 37(1):42-46.
- YOSHIDA, T., R. A. RONCAL, and E. M. BAUTISTA. 1973. Atmospheric nitrogen fixation by photosynthetic microorganisms in a submerged Philippine soil. Soil Sci. Plant Nutr. (Tokyo) 19:117-123.

## DISCUSSION

ROY: You mentioned that blue-green algae differ in N<sub>2</sub>-fixing capacity and in their capability to thrive in different soil fertility conditions. Is there a species that can be used by rice farmers in Bangladesh who need only 30-40 kg N/ha for an average crop but do not have the cash to buy fertilizer N?

Reynaud: A super N<sub>2</sub>-fixing strain is not found everywhere. It is best to select and use N<sub>2</sub>-fixing strains from a specific area that are adapted to the area's ecological problems.

ROY: You mentioned that low pH and low phosphorus are limiting factors for growth of blue-green algae in paddies. We know that the pH of paddy soils is generally higher than that of dryland soils. Phosphorus availability is also higher in the reduced soil of the paddy. What are the critical values of pH and phosphorus for optimum blue-green algae growth in paddy soils?

Reynaud: Data on axenic strains show that pH 7 is optimum for N<sub>2</sub> fixation. An in situ pH higher than 7 favors blue-green algae growth but is not favorable to NFA. As noted in our paper, blue-green algae growth seemed closely related to available phosphorus content, and was vigorous above 6 ppm.

V. P. SINGH: What water management practices will enhance algal growth and its contribution to N<sub>2</sub> fixation in an irrigated rice field?

Reynaud: Field observations are that there is an algal succession and the algal biomass is greater with than without fertilizer use. Thus, to increase N<sub>2</sub>-fixing algal growth, management is suggested to provide a low level of fertilizers when seeding followed by abundant broadcast fertilizers when conditions are good for blue-green algae growth.

CRASWELL: It is observed at IRR I that soil treated with fertilizers placed below the soil surface develop blue-green algae, but soils treated with broadcast fertilizers develop green algae. Thus, improved fertilizer practices may promote increased algal N<sub>2</sub> fixation.

Reynaud: Fertilizers placed below the soil surface reach the surface when conditions are more favorable for blue-green algae, i.e. increased pH and vegetative cover. When fertilizers are broadcast at the start of the crop cycle other algal forms are promoted by conditions of low pH and no vegetative cover.

KULASOORIYA: It is difficult to study the ecology of algal populations in a natural ecosystem. What methods do you use?

Reynaud: Our methods, in summary, are: sampling, homogenization, dilution, incubation, enumeration, and biomass evaluation. We have reported development of each in previous publications. Accuracy was determined at each step and it was about 100% for the total method.

HONG: It would be beneficial to have algae capable of using the ammoniacal N that exists in paddy floodwater. They would help reduce loss of N via runoff and volatilization.

Reynaud: In some tropical sandy soils the floodwater picks up ammoniacal N as soon as it becomes soluble. It would be interesting to recover that N with algae, whether  $N_2$  fixers or not. To select efficient algae will require in vitro screening to determine tolerance for the ammoniac rather than capacity for N assimilation.

OH: What are the possible management practices to keep pH and phosphorus levels of stagnant water at an optimum for algae?

Reynaud: Our observations were in rice fields with minimal fertilizer and ecological preoccupation. Study of transformation and stabilization in a complex biotope are future objectives.

PONNAMPERUMA: Both you and Dr. Stewart stressed the effects of environmental factors on algal  $N_2$  fixation. But neither referred to carbon dioxide as a possible limiting factor. Dr. Mikkelsen reported that the pH of paddy floodwater could be as high as 9.5. If that is correct, the carbon dioxide concentration of the water is about 1,000 times less than that of the atmosphere. What soil management practices do you recommend to increase the carbon dioxide concentration in the algal water?

Reynaud: Calcium carbonate can be added to the water but it sometimes would be required in too large amounts. Another practice would be to incorporate organic matter with the soil but its decomposition produces toxic products, such as phenolic acid, in addition to carbon dioxide.

WADA: What is the best method of estimating algal abundance in rice fields?

Reynaud: A first requirement is a well-conducted sampling. Selection of method depends on what you hope to learn but plating techniques will give you the greatest amount of knowledge.

YOSHIDA: In Senegal you plant rice by direct seeding. That may allow an unfavorable crop effect from algal bloom unless you have good water management.

Reynaud: In extensive rice crop areas farmers use pregerminated seed and fertilizers placed in the soil. With too much algal growth copper sulfate is sometimes added.

KULASOORIYA: You showed a correlation between rice plant cover and heterocystous algal population. How do you explain this? Is it likely that the crop used soil N and the algal colonization was better following that?

Reynaud: With optimum light intensity we noted no heterocystous forms in a fertilized soil with no vegetal cover (Reynaud and Roger 1978b). Thus, on soils without fertilizers we note a positive correlation between  $N_2$  fixing blue-green algae and plant cover (Roger and Reynaud 1977).

STANGEL: It appears that the phosphorus requirement for optimal growth of blue-green algae may be above that required for optimal growth of rice. What are the prospects for finding strains of blue-green algae that will be high  $N_2$  fixers but have a phosphorus requirement no higher than rice?

Reynaud: NFA is limited by phosphorus when pH is optimum. Blue-green algae strains must surely exist that have lower phosphorus requirements than others but we have done no screening for them.