

ORIGINAL PAPER

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Effects of nitrogen fertilizer and pesticide management on floodwater ecology in a wetland ricefield

I. Experimental design and dynamics of the photosynthetic aquatic biomass

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Abstract This paper is the first in a series reporting a study on the effects of N fertilizer, green manure, and pesticide applications on the floodwater ecology of a tropical ricefield. Algal, zooplankton, and mollusc populations were monitored during a crop cycle in plots subjected to various methods of N management and different pesticide regimes. This paper presents the experimental design, general features of the statistical methods for data analysis, and the dynamics of algal growth in the floodwater. Transient green algal blooms and increases in floodwater dissolved O₂ concentrations were observed after the broadcast application of N fertilizer 7 days after transplanting. These effects were less pronounced after the second N application 55 days after transplanting. Photosynthetic activity in the floodwater of planted plots decreased as the crop season progressed. Mucilaginous colonial blue-green algae proliferated in the zero-N control and unplanted plots, but were inhibited by the broadcast N fertilizer and green manure incorporation. Deep placement of urea supergranules reduced the negative impact of N fertilizer on blue-green algal populations. There was limited evidence that the application of pesticides promoted the development of blue-green algal populations in the absence of readily available N.

Key words Zooplankton · Cyanobacteria · Molluscs · Fertilizer · Pesticide · Primary production · Ricefields

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Introduction

Traditional wetland rice cultivation has sustained moderate yields for thousands of years without deterioration of the environment (Bray 1986). During recent decades, yields have been increased by crop intensification including the introduction of high-yielding varieties, and the adoption of new technologies including pesticide and chemical fertilizer use.

However, research on rice nutrition has shown that even at the high levels of inorganic fertilizer currently applied in ricefields, most N absorbed by the plant originates from the soil. Available soil N is released by the turnover of a microbial biomass which represents only a few per cent of total soil N (Watanabe et al. 1988). Crop residues, rhizosphere exudates, and algae and aquatic plants in the floodwater contribute nutrients which replenish the microbial biomass. Nutrients accumulating in the photosynthetic aquatic biomass and the detritus layer may be recycled and incorporated into the soil by aquatic invertebrates. Mineralized nutrients in the floodwater can be used immediately by primary producers or translocated into the soil (Roger and Kurihara 1991).

A 65% increase in rice production is required from 1992 to the year 2020 (International Rice Research Institute 1989). Therefore, it is important to understand and predict how factors associated with crop intensification may affect the sustainability of the ricefield agro-ecosystem through its effects on non-target organisms (Roger et al. 1991a). In particular, despite the recognized importance of the floodwater biota for soil fertility (Watanabe et al. 1988) and human health issues (Roger and Bhuiyan 1990), little attention has been paid to the effects of inorganic and organic fertilizers, pesticides and the rice crop itself, on floodwater ecology.

Applications of mineral fertilizers to the floodwater will usually increase primary production and invertebrate consumers. Green manure incorporation produces decomposition products which may affect aquatic invertebrates. The denser rice canopy resulting from fertilizer

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application may also affect floodwater ecology. Agrochemicals may interfere with floodwater invertebrate populations. In a review, Roger et al. (1991a) concluded that pesticide applications in ricefields generally caused a decrease in floodwater invertebrates followed by a proliferation of primary consumers. Recovery of predatory populations is usually slower (Takamura and Yasuno 1986). Collectively, these processes can modify the structure of the aquatic community in ricefields.

The effects of agrochemicals on microorganisms and invertebrates found in ricefields have been mostly tested in the laboratory, often at concentrations much higher than those encountered in farmers' fields (Roger et al. (in press)). A few field experiments have studied the impact of pesticides on specific components of the zooplankton, but records of the dynamics of the major components of the floodwater biota during a crop cycle and under a range of agricultural practices are extremely scarce and limited to temperate ricefields (Kurasawa 1956).

This paper is the first in a series reporting a study on the effects of N fertilizer, green manure, and pesticide applications on the floodwater ecology of a tropical ricefield. Subsequent papers will discuss the dynamics of microcrustaceans, dipteran larvae, and molluscs.

Materials and methods

Experimental design

The study was conducted in 65 experimental plots (4×4 m) at the International Rice Research Institute, Philippines, during the 1990 dry season. The soil in the plots was Maahas silty clay loam (Aquic Tropudalf; pH 7.2; 0.18% N; Grant et al. 1983). The experimental

design consisted of five replicated randomized blocks of 13 treatments, combining N-management practices and pesticide regimes (Table 1).

Rate variables were selected to represent a range of situations either prevailing or likely to occur in farmers' fields in the tropics. The amounts of N fertilizer broadcast were representative of average (55 kg N ha⁻¹) and relatively high (110 kg N ha⁻¹) levels of application by rice-growers. The deep placement of N increases fertilizer use efficiency in flooded soils (Roger et al. 1980). *Azolla* sp. is a green manure that has been traditionally used in China and Vietnam (Lumpkin and Plucknett 1982). Levels and frequencies of pesticide application were established from a survey of 33 farms in the Laguna area (Philippines) which showed that farmers applied between 0.5 and 2.5 kg ha⁻¹ of active ingredient per crop cycle (Roger et al. (in press)).

Rice (IR 72) was transplanted at 25×25 cm spacing. The floodwater depth was maintained at between 5 and 10 cm throughout the crop cycle. All planted and unplanted plots received 30 kg P ha⁻¹ as superphosphate 7 days after transplanting to ensure that P was, at least initially, non-limiting for algal growth.

Broadcast N fertilizer was applied as prilled urea, two-thirds 7 and one-third 55 days after transplanting. When deep-placed, N was applied as 1 g urea supergranules placed 10 cm below the soil surface, at a rate of three for every four rice hills 7 days after transplanting.

Live *Azolla* sp. was inoculated at 0.4 kg biomass m⁻² 3 weeks before transplanting and incorporated before final soil preparation. Superphosphate was applied to the *Azolla* sp. plots three times at 2 kg P ha⁻¹ (5-day intervals).

Carbofuran (Furadan) was applied as granules and butachlor (Machete) was sprayed (Table 1).

Treatment groupings for data analysis

As the experimental design was incomplete, statistical analysis of the data by a single analysis of variance procedure was not possible. Three groupings of selected treatments were used to optimize the analysis.

The effect of N management and pesticides at the low and average levels were tested, by a three-way analysis of variance or a non-

Table 1 Pesticide and N management in experimental ricefields, 1990 dry season. Quantity of pesticide per application (kg active ingredient ha⁻¹) and number of applications are given. Broadcast, prilled urea two-thirds broadcast 7 days after transplanting and one-third 55 days after transplanting; USG, urea supergranules deep-placed 7 days after transplanting; *Azolla* sp. was inoculated

at 0.4 kg biomass m⁻² 3 weeks before transplanting and incorporated before final soil preparation; carbofuran application: A–C, 54 days after transplanting, D–H, 13 and 54 days after transplanting; I–L, 13, 28, 42, 54, and 69 days after transplanting; butachlor application, 3 days after transplanting

	N	Management	Pesticide management				Groupings for statistical analysis		
			Carbofuran		Butachlor		N management and pesticide groups	Pesticide regime at 110 kg Na ha ⁻¹	Planted vs. unplanted
			Rate	No.	Rate	No.			
A	Zero N		0.1	1	0	0	×		×
B	55 kg N ha ⁻¹	Broadcast	0.1	1	0	0	×		
C	55 kg N ha ⁻¹	USG	0.1	1	0	0	×		
D	Zero N		0.3	2	0.35	1	×		
E	55 kg N ha ⁻¹	Broadcast	0.3	2	0.35	1	×		
F	55 kg N ha ⁻¹	USG	0.3	2	0.35	1	×		
G	<i>Azolla</i> sp.		0.1	1	0	0	×		
H	110 kg N ha ⁻¹	Broadcast	0.1	1	0	0	×	×	
I	110 kg N ha ⁻¹	Broadcast	0.3	5	0.35	1		×	
J	<i>Azolla</i> sp.		0.3	2	0.35	1	×		
K	110 kg N ha ⁻¹	Broadcast	0.3	2	0.35	1	×	×	
L	110 kg N ha ⁻¹	Broadcast	0.5	5	0.35	1		×	
M	Fallow		0	0	0	0			×

parametric test, using N management, pesticide, and blocks as the main factors. These treatments were (Table 1): zero N (A and D), 55 kg N ha⁻¹ broadcast (B and E), 55 kg N ha⁻¹ deep-placed (C and F) *Azolla* sp. (G and J), and the two 110 kg N ha⁻¹ split treatments with the lower pesticide application rates (H and K). This grouping is hereafter referred to as the N management and pesticide grouping.

The effect of pesticides in the presence of a high level of N fertilizer was tested by a one-way analysis of variance or a non-parametric test on the 110 kg N ha⁻¹ split treatments (H, I, K and L) using the four pesticide levels as the discriminating factor. This grouping is hereafter referred to as the pesticide regime at 110 kg N ha⁻¹.

Differences between planted and unplanted plots were tested by a one-way analysis of variance or a non-parametric test on the planted treatment (A) and the unplanted treatment (M). This grouping is hereafter referred to as planted/unplanted.

Dynamics of primary production in floodwater

Three measurements of floodwater dissolved O₂ and one of temperature were taken in each plot throughout the crop cycle at mid-day (11.00–14.00 h), when concentrations are likely to be maximal (Saito and Watanabe 1978). Measurements were made with an O₂ meter every 2–3 days, except immediately before and after the addition of fertilizer, when they were recorded daily (total 34 times). Visual assessments of the abundance of large mucilaginous blue-green algal colonies were made whenever dissolved O₂ was recorded using a visual index (Roger et al. 1991b):

0 = 0	
1 = +	6 = 1/3 cover
2 = ++	7 = 1/2 cover
3 = +++	8 = 2/3 cover
4 = ++++	9 = 3/4 cover
5 = 1/4 cover	10 = total cover.

Samples of algae were taken at intervals for microscopic determination of the dominant taxon.

A statistical analysis of dissolved O₂ measurements was performed by three-way analysis of variance (N management and pesticide groupings) and one-way analysis of variance (pesticide regime at 110 kg N ha⁻¹ and planted/unplanted groupings) on non-transformed data. Least significant difference multiple-range tests were used to identify significant differences among the treatments.

Statistical analysis of the incidences of algal abundance was performed by the non-parametric Friedman's two-way rank analysis in the N management and pesticide groupings. Kruskal-Wallis one-way rank analysis was used to test for significant differences among: (1) replicates, (2) pesticide regimes at 110 kg N ha⁻¹, and (3) between planted and unplanted plots. When significant differences were identified among groups of more than two treatments, a Kruskal-Wallis one-way analysis of variance was performed on pairs of treatments to find where the differences occurred. Interactions between N management and pesticide treatments were assessed using a Kruskal-Wallis one-way analysis of variance, analysing different pesticide regimes separately for each N-management treatment.

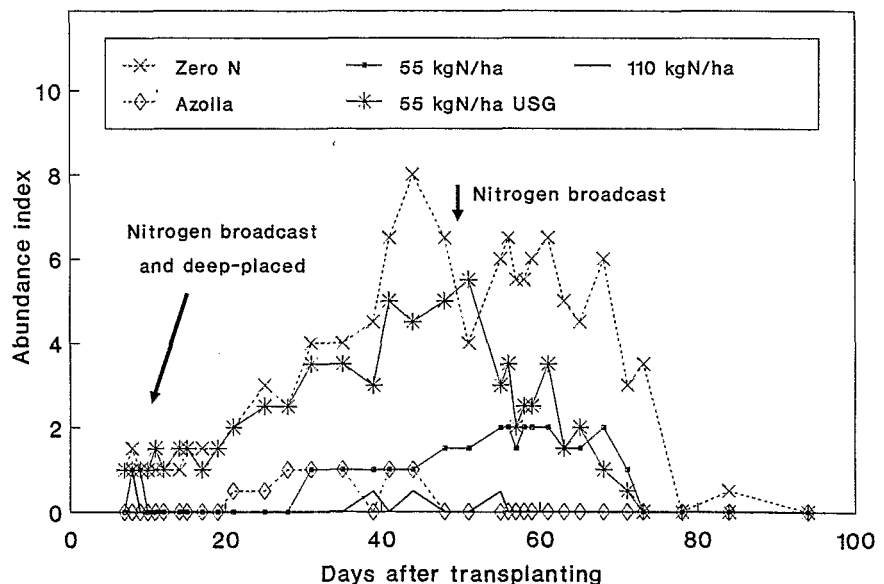
Results

Floodwater algae

Green algae were observed to bloom for 1–5 days soon after N fertilizer was broadcast. This occurred with the first split application and to a lesser extent with the second. For the rest of the crop cycle in these plots and for the whole crop cycle in the other plots, algal populations were consistently dominated by mucilaginous N₂-fixing cyanobacteria belonging to the genera *Aphanothece* and *Nostoc*.

In the absence of N fertilizer, blue-green algae blooms developed from the start of the crop season and reached maximum coverage (frequently over 75%) between 40 and 70 days after transplanting (Fig. 1). From 70 days after transplanting the populations declined rapidly and were virtually absent from 80 days after transplanting onwards. Blue-green algal populations in the *Azolla* sp. and 110 kg N ha⁻¹ treatments were negligible and significantly ($P < 0.05$) lower than the zero-N control throughout most of the crop season. Isolated blue-green algal colonies developed in the 55 kg N ha⁻¹ broadcast treatments and were not significantly ($P > 0.05$) different from the zero-N control from 61 days after transplanting onwards.

Fig. 1 Effects of different N-management treatments on colonial blue-green algae abundance. *USG*, Urea supergranules



The blue-green algal cover in the 55 kg N ha⁻¹ broadcast treatments was significantly ($P < 0.05$) higher than in the 110 kg N ha⁻¹ and *Azolla* sp. treatments, at most times, between 21–68 and 48–71 days after transplanting, respectively. For the majority of the time, between 12 and 41 days after transplanting, the blue-green algal abundance was significantly ($P < 0.05$) lower in the 110 kg N ha⁻¹ treatments than in the plots where *Azolla* sp. was incorporated.

Deep placement of N permitted the development of significantly ($P < 0.03$) larger blue-green algal blooms between 10 and 51 days after transplanting than an equivalent quantity of broadcast N (Fig. 1). From 55 days after transplanting onwards no significant ($P < 0.15$) differences were observed between the broadcast and deep-placed treatments.

In the unplanted treatment, peak colonial blue-green algal coverage was achieved between 50 and 80 days after transplanting; some plots achieved 100% cover (Fig. 2). Population dynamics were similar to those in the planted zero-N control, but modal cover indices were consistently higher (significantly, $P < 0.04$, 78 and 84 days after transplanting).

There was no consistent evidence of significant ($P = 0.05$) relationships between colonial blue-green algal blooms and pesticide regimes across N-management treatments or in the four 110 kg N ha⁻¹ treatments. An independent analysis of N-management treatments under different pesticide regimes showed that increased pesticide application rates stimulated the development of blue-green algal blooms under conditions of zero N and deep-placed N (Figs. 3, 4). In the absence of N fertilizer, blue-green algal blooms developed more rapidly where carbofuran was applied twice at 0.3 kg a.i. (active ingredient) ha⁻¹ than in the lower input treatment, but the abundance maxima were similar. When N was deep-placed, population development was faster and maximum cover

was increased in the higher pesticide treatment (Fig. 4). Despite the consistency of these differences, they were mostly not significant ($P > 0.05$).

Floodwater dissolved O₂

After the application of N fertilizer (7 days after transplanting) mid-day dissolved O₂ concentrations were significantly ($P < 0.01$) higher in the broadcast-treatments than the zero-N control and *Azolla* sp. plots; dissolved O₂ increased from 10 to 20 mg l⁻¹ (Fig. 5). Significantly ($P < 0.01$) higher dissolved O₂ levels were maintained in the 55 and 110 kg N ha⁻¹ treatments until 19 and 21 days after transplanting, respectively. With the exception of a significant ($P < 0.01$) but transitory rise in dissolved O₂ after the second application of N 55 days after transplanting, this parameter was higher in the zero-N control than in other treatments for the remainder of the crop season. Dissolved O₂ concentrations in the *Azolla* sp. treatments were relatively low throughout the crop season.

In spite of considerable short-term fluctuations, seasonal trends in dissolved O₂ were evident across all treatments (Fig. 5). Generally, dissolved O₂ decreased as the crop season progressed; concentrations declined from 10–20 mg l⁻¹ (9 days after transplanting) to 6–8 mg l⁻¹ (94 days after transplanting). Mid-season dissolved O₂ peaks were observed between 42–45 and 55–63 days after transplanting.

Floodwater dissolved O₂ was significantly ($P = 0.05$) affected by the method of fertilizer application (Fig. 5). When N was deep-placed, dissolved O₂ was initially unaffected; however, from 20 days after transplanting, concentrations were consistently (from 36 days after transplanting significantly, $P < 0.01$) higher than the broadcast N treatment until the second split application (55 days after transplanting). Immediately after the second applica-

Fig. 2 Effects of the presence of rice plants on colonial blue-green algal abundance

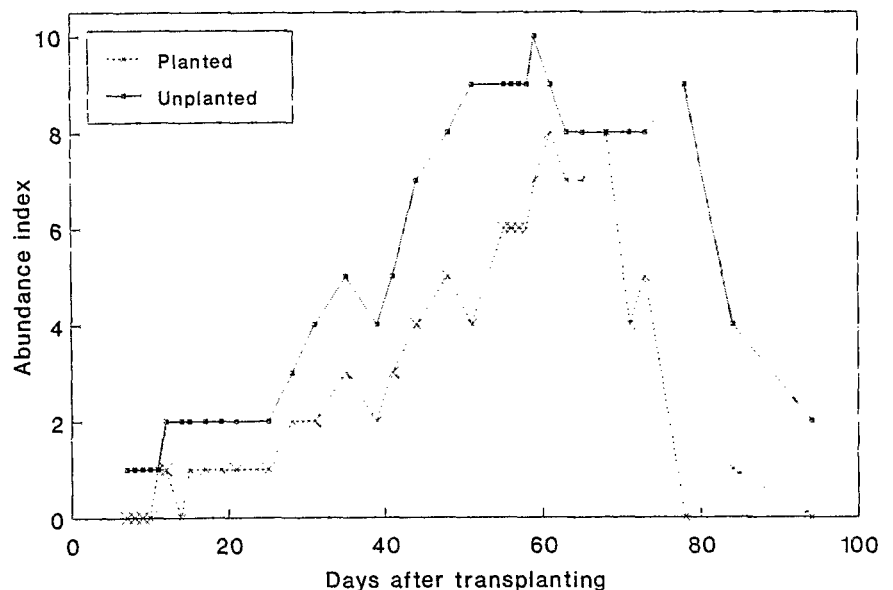


Fig. 3 Effects of two different pesticide treatments on colonial blue-green algal abundance in the absence of N fertilizer

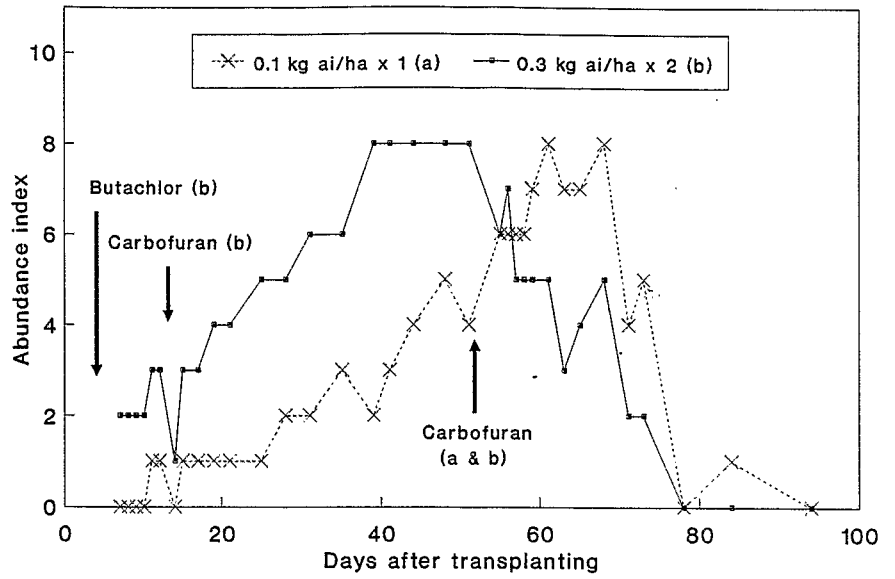


Fig. 4 Effects of two different pesticide treatments on colonial blue-green algal abundance when N fertilizer was deep-placed at 55 kg N ha⁻¹

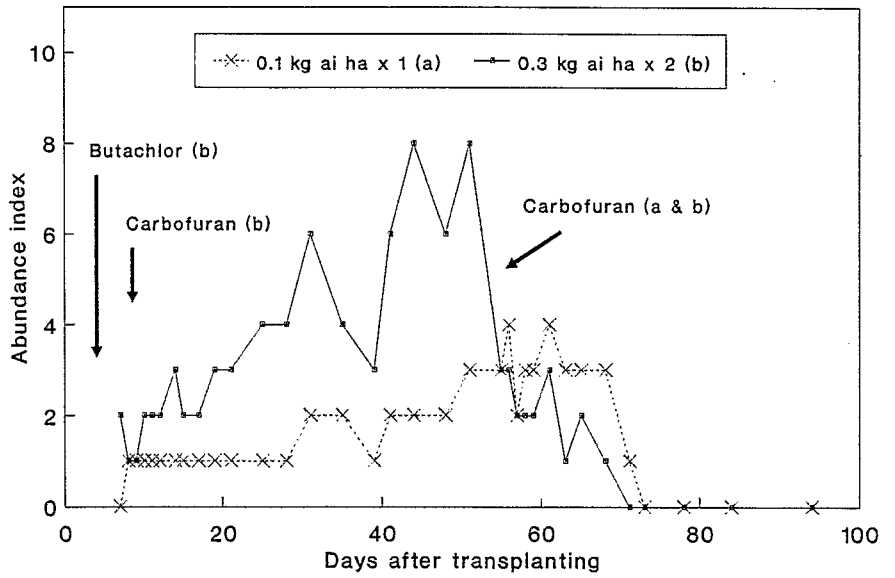
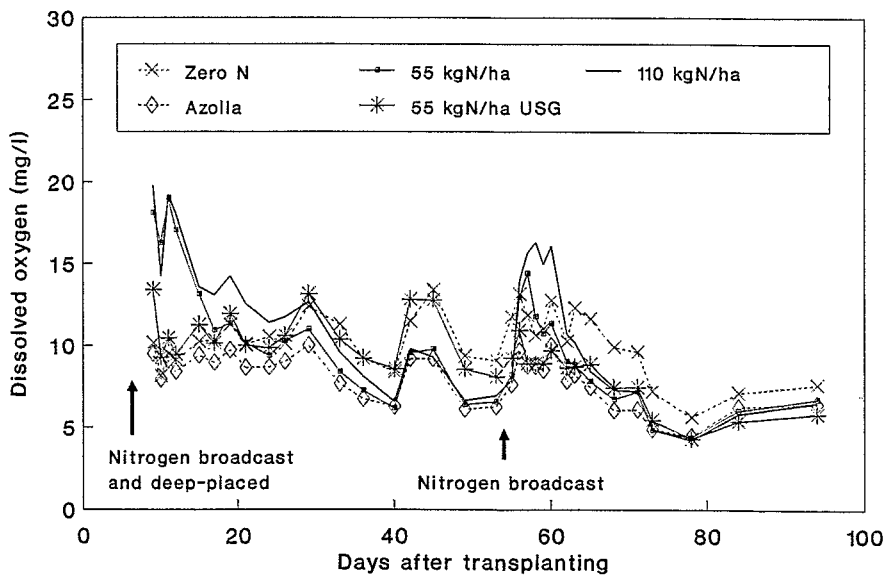


Fig. 5 Effects of different N-management treatments on mid-day floodwater dissolved O₂ concentrations. *USG*, Urea supergranules



tion dissolved O₂ was significantly ($P < 0.01$) higher in the broadcast treatments for several days.

Mid-day floodwater dissolved O₂ concentrations were not affected by the presence of transplanted rice until 55 days after transplanting; thereafter, it was mostly significantly ($P < 0.05$) higher in unplanted plots (Fig. 6). In unplanted plots it increased over the crop season and stabilized towards the end.

Across N-management treatments, dissolved O₂ levels were significantly ($P < 0.05$) higher 15–24, 49, 55–56, and 65 days after transplanting, when pesticide application rates were higher (Fig. 7).

Under different pesticide regimes in the 110 kg N ha⁻¹ broadcast treatments, dissolved O₂ levels were similar throughout the crop season (Fig. 8). Some significant ($P < 0.05$) differences were observed but trends were in-

consistent. By 45 days after transplanting the dissolved O₂ concentrations were significantly ($P = 0.01$) higher when pesticide application rates were highest, but later in the season, 60 and 63 days after transplanting, the position was reversed ($P < 0.05$).

Significant ($P < 0.02$) interactions between pesticide regimes and N-management treatments on floodwater dissolved O₂ occurred 15, 42–49, and 55 days after transplanting. The interactions were assessed by constructing mean plots for the different N-management treatments at the two pesticide levels. They showed that dissolved O₂ levels were significantly higher in the zero-N treatment at the higher pesticide level. By 15, 42, and 49 days after transplanting they were also significantly higher in the deep-placed N treatment at the higher pesticide level.

Fig. 6 Effects of the presence of rice plants on mid-day floodwater dissolved O₂ concentrations

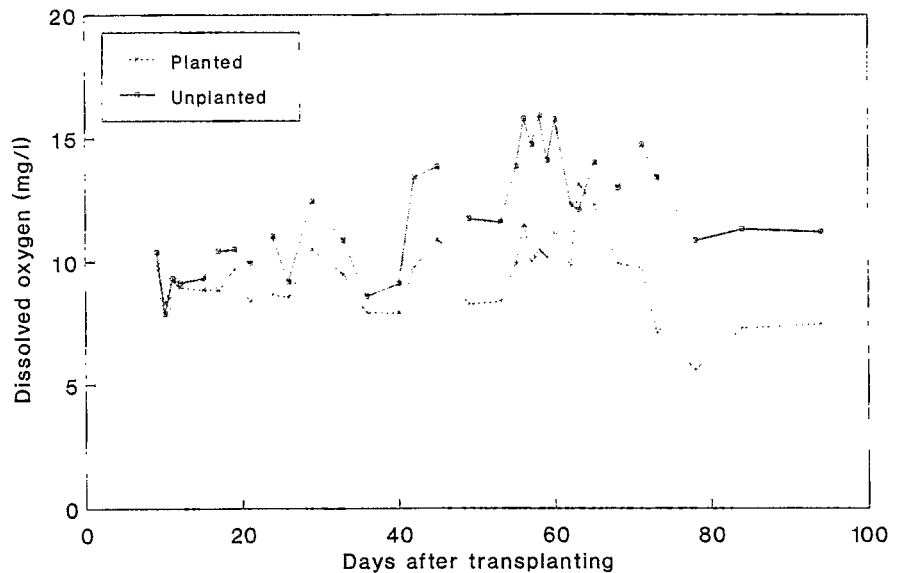
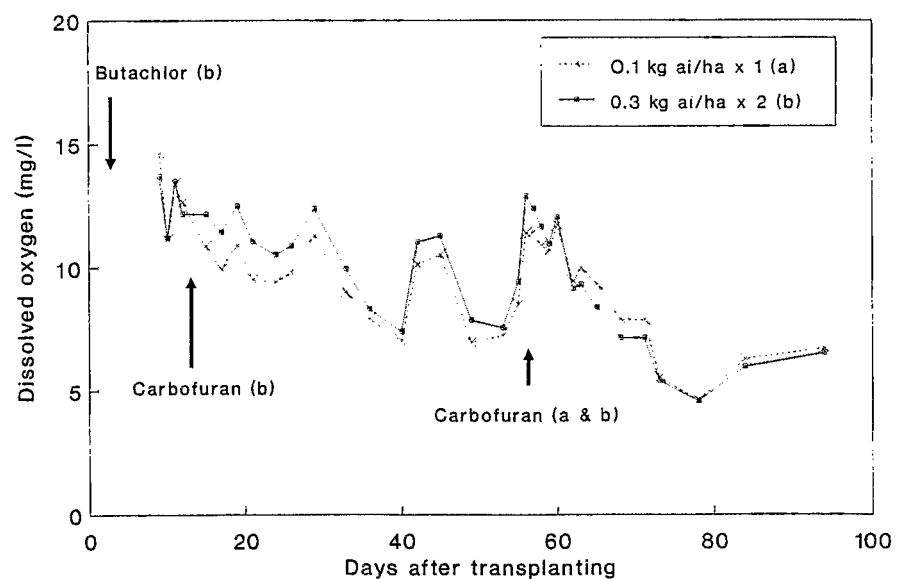


Fig. 7 Effects of two different rates of pesticide application on mid-day floodwater dissolved O₂ concentrations



Floodwater temperature

Mid-day floodwater temperatures (Fig. 9) fluctuated from a minimum of 26°C to a maximum of 40°C (seasonal average 35°C).

Discussion

The increases in floodwater dissolved O_2 within days of the broadcast fertilizer applications were almost certainly due to increased primary productivity. Algal blooms, particularly green algae, immediately after the application of mineral N fertilizer have been reported previously (Saito and Watanabe 1978; Roger et al. 1980). The reduced effect of the second application could be a consequence of

(1) the reduction in the quantity of N applied, compared with the first split; (2) faster absorption of N by the rice plant, which has developed a root mat at the soil-water interface; (3) the absence of a complementary addition of P; (4) a reduction in the availability of native nutrients as they are used by the rice crop; (5) the effect of increased shading as the rice canopy closes; and/or (6) an established population of grazing invertebrates.

The virtual absence of colonial blue-green algae where N was broadcast may have been a consequence of reduced competitiveness relative to the faster growing green algae. Furthermore, invertebrate grazers, which developed to exploit the green algae, may have suppressed the build-up of blue-green algal blooms before they could form mucilaginous colonies. The adverse effects of N fertilizer on N_2 -fixing blue-green algal populations, relative to other algal, are well documented (Roger and Kulasooriya 1980;

Fig. 8 Effects of four different rates of pesticide application on mid-day floodwater dissolved O_2 concentrations when N fertilizer was applied at 110 kg N ha^{-1}

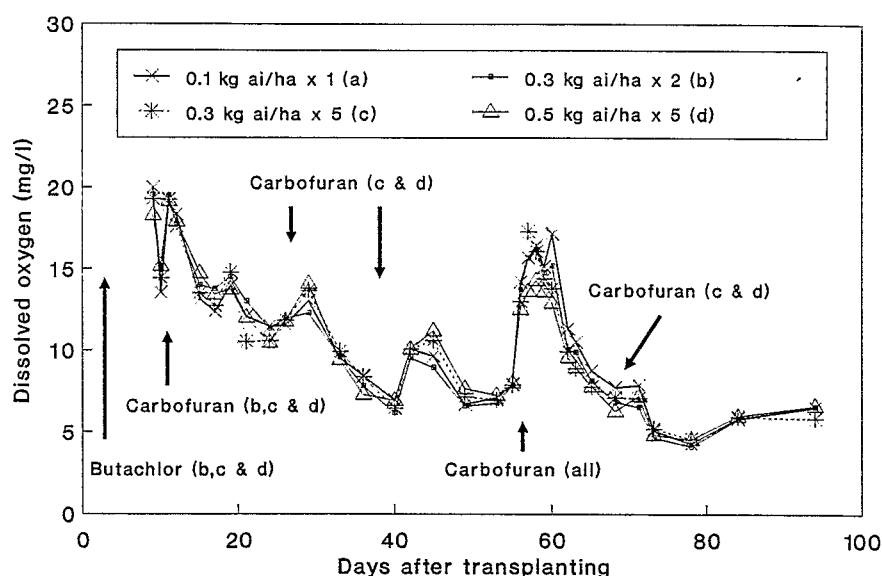
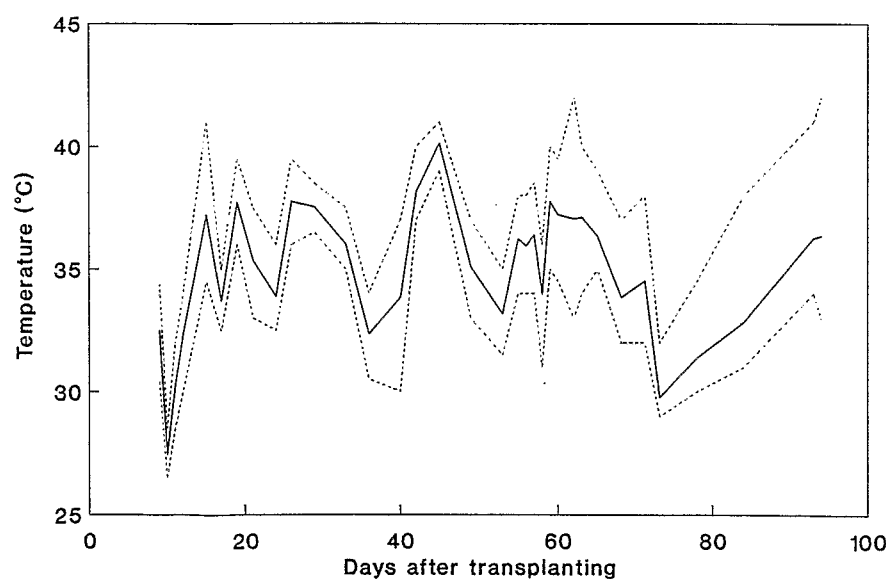


Fig. 9 Mean, minimum, and maximum mid-day floodwater temperatures in the experimental ricefields



Roger et al. 1980). In the absence of readily available N, blue-green algal blooms developed. These blooms were persistent because they were dominated by mucilaginous species which, once established, are not readily palatable to many aquatic invertebrate grazers (Grant et al. 1985). Photosynthetic activity by the blue-green algal explains the elevated dissolved O₂ concentrations in the zero-N control and unplanted plots towards the middle and end of the crop cycle.

Floodwater primary productivity, as indicated by the mid-day dissolved O₂ concentrations and blue-green algal abundance, appeared to be lower when *Azolla* sp. was incorporated than in control treatments. This may be attributed to the combined effects of the O₂ demand by the decomposing *Azolla* sp. and possibly the release of phytotoxic substances during the decomposition process.

When N is deep-placed its availability in the floodwater is reduced (Savant and De Datta 1980), inhibiting the development of green algal blooms early in the crop cycle. Relative increases in floodwater dissolved O₂ later in the crop season can be attributed to the proliferation of colonial blue-green algal. The development of significant N₂-fixing blue-green algal blooms in treatments where N fertilizer was deep-placed relative to broadcast treatments has been reported previously (Roger et al. 1980).

Higher floodwater dissolved O₂ concentrations and colonial blue-green algae cover in unplanted plots, and the tendency of both to decline in planted plots, indicated that floodwater primary productivity was dependent on crop development. This was probably a consequence of nutrient uptake by the rice plants and the reduced light intensity as the canopy closed. Shading has been identified as one of the most important factors limiting aquatic photosynthesis in ricefields (Saito and Watanabe 1978; Heckman 1979).

Mid-season peaks in the concentration of dissolved O₂ in the floodwater can probably be explained by ambient climatic conditions, indicated by the increases in floodwater temperature that occurred concurrently. The relatively higher increase in broadcast N treatments 55 days after transplanting could be explained by transitory algal blooms which developed in response to the second split application of N.

The dissolved O₂ levels provided little evidence of pesticide effects on primary production in the floodwater. When colonial blue-green algal bloomed in planted plots (control and deep-placed N) they were more prolific at higher pesticide application rates. The mechanism of growth promotion is difficult to ascertain; it may be a result of direct stimulation or an indirect effect. Invertebrate population densities were consistently lower at the higher pesticide level when the above differences developed (Simpson et al. 1993) and perhaps individual feeding rates were lowered. Insecticide-induced reductions in invertebrate grazing pressure accelerate the development of algal blooms in ricefields (Grant et al. 1983, 1985, 1986). When pesticide application rates were low, late-developing blue-green algal blooms were more dense in the zero-N control than in the deep-placed N treatment. This

can be explained by the higher canopy density in the latter, which increased shading and reduced the floodwaters' photosynthetic potential.

In their bibliographic review, Roger et al. (in press) listed several pesticides that exhibited a preferential inhibitory effect on green algal (BHC, PCP, Symetryne, and algaedyn) or were harmless to blue-green algae while they decreased algal grazer populations (parathion, methyl parathion, and Phorate); both groups resulted in the promotion of blue-green algal growth when applied.

The observed impacts of N management and pesticide applications on the floodwaters' photosynthetic aquatic biomass have important agronomic implications for the ecosystem. High photosynthetic activity reduces the dissolved CO₂ concentration in the floodwater, which causes an increase in pH (Mikkelsen et al. 1978). When pH rises, NH₃ volatilization increases (De Datta 1981). When primary productivity is high, the plant respiratory O₂ demand will be correspondingly high, and this could create anoxic conditions in the floodwater at night (Saito and Watanabe 1978). The implications of this for organisms dependent on dissolved O₂ are obvious. Nutrients immobilized in the photosynthetic aquatic biomass are temporarily unavailable to rice plants, but are conserved within the system when they may otherwise have been lost (Vlek and Craswell 1979). Populations of aquatic invertebrates in ricefields are controlled directly or indirectly by the composition and quantity of the photosynthetic aquatic biomass. Their relationships with this biomass will be discussed in a subsequent paper.

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