

ORIGINAL PAPER

Ian C. Simpson · Pierre A. Roger · Roberto Oficial
Ian F. Grant

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

II. Dynamics of microcrustaceans and dipteran larvae

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Abstract Algal, zooplankton, and mollusc populations were monitored over a crop cycle in the floodwater of a tropical ricefield subject to various N-management and pesticide regimes. This paper gives the results on dynamics of microcrustaceans and dipteran larvae. Dominant groups developed in the succession mosquito+chironomid larvae → ostracods → copepods+cladocerans. Populations of ostracods and mosquito+chironomid larvae expanded rapidly after broadcast N applications. Deep placement of N avoided the proliferation of ostracods and dipteran larvae. With the exception of chironomid larvae early in the crop cycle, aquatic invertebrates were generally less abundant in treatments where *Azolla* sp. was incorporated. When considered at the crop cycle level, aquatic invertebrate populations were not significantly affected by applications of butachlor and carbofuran.

Key words Aquatic invertebrates · Ricefields · Fertilizer · Pesticides · Population dynamics

Introduction

This paper presents the dynamics of microcrustaceans and dipteran larvae in a study on the effects of N fertilizer, green manure, and pesticide application on the floodwater ecology of a tropical ricefield. Algal, zooplankton,

and mollusc populations were monitored for a crop cycle in plots subjected to various types of N and pesticide management. Simpson et al. (1993) described the experimental design, statistical methods, and the dynamics of the photosynthetic aquatic biomass.

Aquatic invertebrates in ricefields are derived from contiguous water bodies and span the whole spectrum of freshwater fauna (Fernando et al. 1980). The dominant groups are ostracods, copepods, cladocerans, rotifers, insect larvae, aquatic insects, molluscs, oligochaetes, and nematodes (Heckman 1974; Lim 1980; Grant et al. 1983, 1985, 1986; Roger and Kurihara 1991). They have agricultural and environmental significance as nutrient recyclers, rice pests, pest predators, and vectors of human and animal diseases (Roger et al. 1991).

Grazers and detritivores accelerate the recycling of nutrients in wetland ricefields by consuming the photosynthetic aquatic biomass and detritus, and excreting nutrients (Grant et al. 1983) and more readily decomposable material. However, grazing activities may reduce N fixation by suppressing populations of heterocystous blue-green algae (Grant et al. 1983). Benthic organisms expedite the translocation of nutrients across the soil/floodwater interface.

Some aquatic invertebrates, including certain chironomid larvae, ostracods, and corixids are rice pests (Clement et al. 1977; Barrion and Litsinger 1984). Predatory invertebrates such as Odonata, Coleoptera, and Notonectidae also contribute to nutrient cycling and to the control of some rice pests.

Mosquito larvae can achieve considerable densities in the floodwater, which may have serious health implications for local communities as the adults transmit malaria and encephalitis (Roger and Bhuiyan 1990).

Detrimental effects of pesticides applied in ricefields that have been demonstrated on microcrustacea in laboratory experiments include acute toxicity, alteration of filtration and assimilation rates, and inhibition of growth and egg production. The effective rate of some insecticides on chironomid larvae was lower than concentrations recorded in ricefields (Sato and Yasuno 1979).

I. C. Simpson (✉) · I. F. Grant
Natural Resources Institute, Central Avenue,
Chatham Maritime, Kent ME4 4TB, UK

P. A. Roger
Institut Français de Recherche Scientifique
pour le Développement en Coopération,
213, Rue la Fayette, F-75480 Paris Cedex 10,
France

R. Oficial
International Rice Research Institute,
P.O. Box 933, Manila, Philippines

O.R.S.T.O.M. Fonds Documentaire

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Materials and methods

Experimental design

The experimental design (13 treatments, five replicated randomized blocks) used in the present study was detailed in Simpson et al. (1993).

Enumeration of invertebrate populations

Five cores (71 mm diameter) were inserted in each plot (4×4 m) along an L-shaped transect. Cores were dropped into position from the bund (bank) and pushed into the soil until 2–3 cm remained above water. The enclosed floodwater was sucked into a collection bottle using a vacuum pump. As the water was withdrawn the cylinder was tilted so that the remaining water collected in a peripheral depression. Water collected from the five cores was combined and the total volume recorded.

Samples were poured through a mesh (105 μm). The material retained was water-cleansed and backwashed into a mesh funnel (128 μm) to reduce sample volume. Samples were preserved and stored in 4% formaldehyde. Preserved samples were washed through a series of graded meshes (final mesh 128 μm). Material retained on each mesh was backwashed into separate Petri dishes and organisms enumerated. Invertebrate groups counted were ostracods, copepods, cladocerans, mosquito larvae, chironomid larvae, other aquatic insects, and miscellaneous organisms. No attempt was made to quantify individual species within these taxonomic groups. The method of sampling allowed the determination of population densities on a volumetric or an areal basis. However, as area remains constant whereas floodwater depth varies, the dynamics of population densities are presented in areal terms.

Data analysis

Invertebrate densities were log-normally distributed and transformed for statistical analysis by $Y = \log(X+1)$. A three-way analysis of variance incorporating a least significant difference multiple-range test ($P = 0.05$), was performed on the treatments of the N

Table 1 Range of invertebrate population densities in individual plots regardless of treatment

Invertebrate group	Number m^{-2}		Number l^{-1}	
	Mean	Range	Mean	Range
Ostracoda	6000	0–98 000	200	0–4300
Copepoda	6000	0–40 000	180	0–1700
Cladocera	900	0–33 000	30	0–1100
Mosquito larvae	170	0–7000	10	0–350
Chironomid larvae	600	0–10 000	20	0–700

management and pesticide grouping (Simpson et al. 1993), which included the treatments combining one or two applications of pesticide with zero N, 55 kg N ha^{-1} split or deep-placed, *Azolla* sp., and 110 kg N ha^{-1} split. Interactions between blocks and other factors were removed from the analysis. One-way analysis of variance was performed on (1) the 110 kg N ha^{-1} split treatments, using pesticide levels as the discriminating factor, and (2) planted and unplanted plots.

Aquatic invertebrate populations

Population densities of invertebrate groups for individual plots are summarized in Table 1. Population dynamics, across all planted plots, for each invertebrate group, are shown in Fig. 1. Ostracods and copepods were the most numerous invertebrates. When expressed as percentages of the respective maxima, the populations clearly show succession (Fig. 2). Mosquito and chironomid larvae populations increased rapidly to peaks within 15 days of transplanting. As these populations declined, those of ostracods expanded, and peaked 35 days after transplanting. Towards the end of the crop, densities of copepods and cladocerans increased.

Ostracod populations

Ostracod populations responded positively to the first broadcast application of N fertilizer (Fig. 3). At 110 kg N ha^{-1} , populations

Fig. 1 Population succession of floodwater invertebrates averaged over all planted treatments

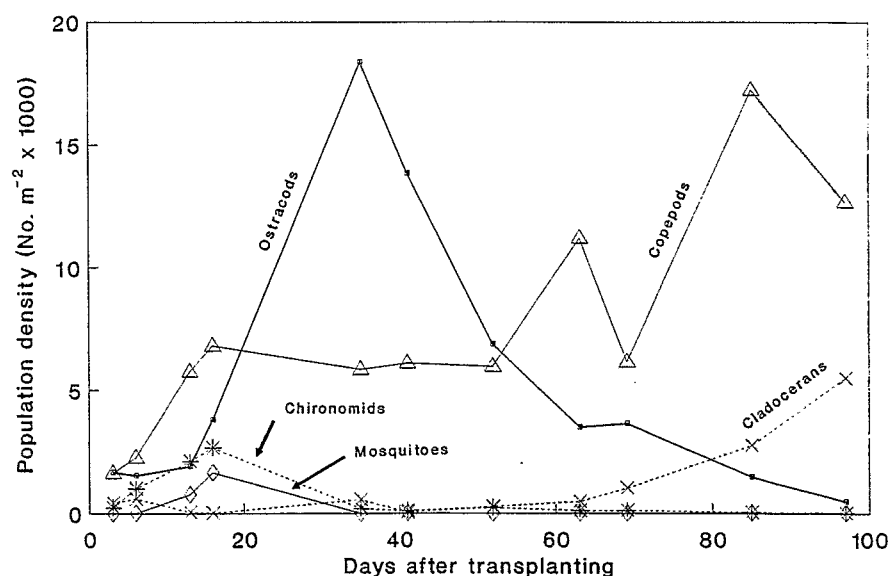
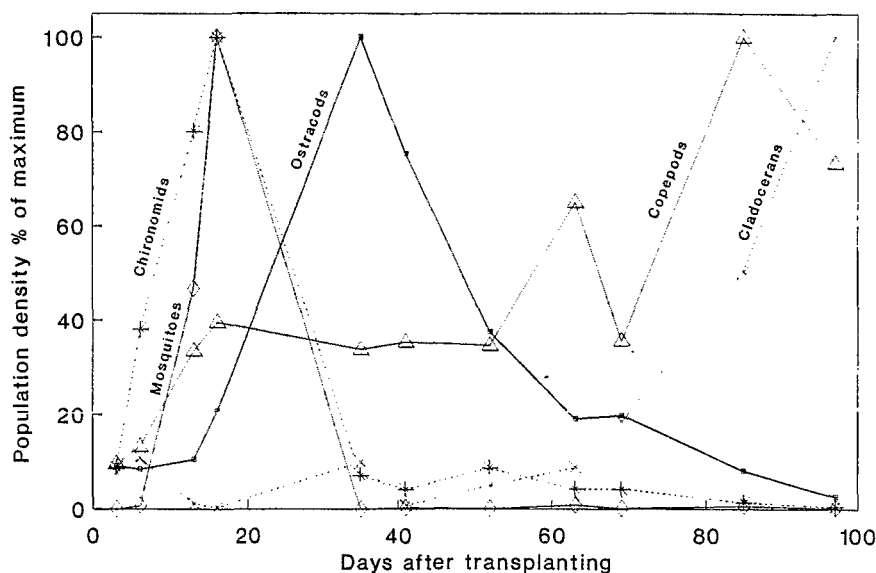


Fig. 2 Population succession of floodwater invertebrates averaged over all planted treatments and presented as percentages of the maximum density



generally remained below 5000 m^{-2} until 16 days after transplanting, after which they increased rapidly to over 40000 m^{-2} by 27 days after transplanting. High population densities were maintained until 45 days after transplanting, when they started to decline rapidly to below 5000 m^{-2} . They did not respond to the second split application of N (55 days after transplanting). Populations in the 55 kg N ha^{-1} treatment displayed similar dynamics, but peak densities were significantly ($P < 0.02$) lower (Fig. 3).

Ostracod densities in the zero-N control treatment fluctuated below a maximum of 5000 m^{-2} throughout crop growth and were significantly ($P < 0.05$) lower than in the treatments where urea was broadcast, especially between 27 and 52 days after transplanting. In *Azolla* sp.-treated plots ostracod dynamics were similar to those in plots treated with 55 kg N ha^{-1} as broadcast urea. Peak ostracod population densities were significantly ($P < 0.05$) denser in *Azolla* sp. plots than in the control and lower than in the plots given 110 kg N ha^{-1} (Fig. 3). No significant ($P > 0.05$) differences were recorded between ostracod dynamics in treatments where 55 kg N ha^{-1} was deep-placed or broadcast.

Ostracod population densities in the unplanted plots expanded from virtually zero to over 6000 m^{-2} by 16 days after transplanting. This density was maintained throughout the remainder of the crop cycle (Fig. 4). Densities were significantly ($P < 0.05$) higher than in the planted zero N control for part of the crop cycle.

Ostracod population densities were significantly ($P < 0.05$) higher where carbofuran was applied once at $0.1\text{ kg a.i. (active ingredient) ha}^{-1}$ than where applied twice at $0.3\text{ kg a.i. ha}^{-1}$ 16, 57, and 69 days after transplanting (Fig. 4), and also 3, 52, 85, and 97 days after transplanting at the $P = 0.10$ level. The timing and magnitude of peak population densities were unaffected by pesticide applications.

At higher levels of fertilization, the pesticide effects were less obvious, but the ostracod density was greater with the lowest pesticide input than with the highest 6, 16, 52, and 69–97 days after transplanting (Fig. 6). Intermediate pesticide levels were rarely significantly ($P > 0.05$) different from each other or the two extremes.

Copepod populations

As the crop season progressed, the copepod abundance increased erratically to densities higher than 12000 m^{-2} (Fig. 3). Differences among treatments were not prominent. Between 41–52 and 41–57 days after transplanting copepods were significantly ($P < 0.04$) more abundant in the 55 kg N ha^{-1} and 110 kg N ha^{-1} than the zero-N

treatment. From 69 days after transplanting onwards, no significant ($P > 0.05$) differences were observed among these three treatments.

Copepod population densities were significantly ($P < 0.01$) lower in treatments where *Azolla* sp. was incorporated than in other N-management treatments 3 days after transplanting. As the growing season progressed, their densities increased and from 35 to 69 days after transplanting they were significantly ($P < 0.05$) higher than in the zero-N control. During this period, copepod densities in the *Azolla* sp. treatment were not significantly ($P > 0.05$) different from other N-management treatments, except 69 days after transplanting when they were more abundant (Fig. 3). Densities were not significantly ($P > 0.05$) affected by the method of fertilizer application (broadcast or deep placed) or the presence of rice plants (Figs. 3, 4).

Copepod densities across N-management treatments were independent of carbofuran at low and intermediate application rates (Fig. 5). Significant ($P < 0.05$) differences were observed among the four pesticide treatments at 110 kg N ha^{-1} , but the trends were inconsistent (Fig. 6). By 45 and 57 days after transplanting copepod densities were significantly ($P < 0.05$) lower in treatments where carbofuran was applied at higher rates 42 and 54 days after transplanting. However, significant ($P = 0.01$) differences observed 72 days after transplanting, after the final application of carbofuran 69 days after transplanting, did not follow a dose-related pattern.

Cladoceran populations

During the first two months of the crop cycle, cladoceran populations rarely exceeded 1000 m^{-2} in the planted treatments (Fig. 3). From 60 days after transplanting, they increased to values higher than 5000 m^{-2} in most treatments.

The only significant ($P < 0.02$) evidence of N-management effects on cladoceran populations was the detrimental impact of *Azolla* sp. incorporation from 3 to 6 days after transplanting (Fig. 3).

From 6 days after transplanting, the density of cladocerans was consistently higher (significantly, $P < 0.02$, 16 and 41 days after transplanting) in unplanted than planted plots (Fig. 4). The population dynamics in planted and unplanted plots were similar, indicating that cladocerans were unaffected by crop development.

Analysis of variance results across all N-management treatments showed that cladocerans were significantly ($P < 0.03$) more abundant in the lowest rate and pesticide treatments from 57 to 85 days after transplanting, from 35 to 85 days after transplanting at $P < 0.10$ (Fig. 5).

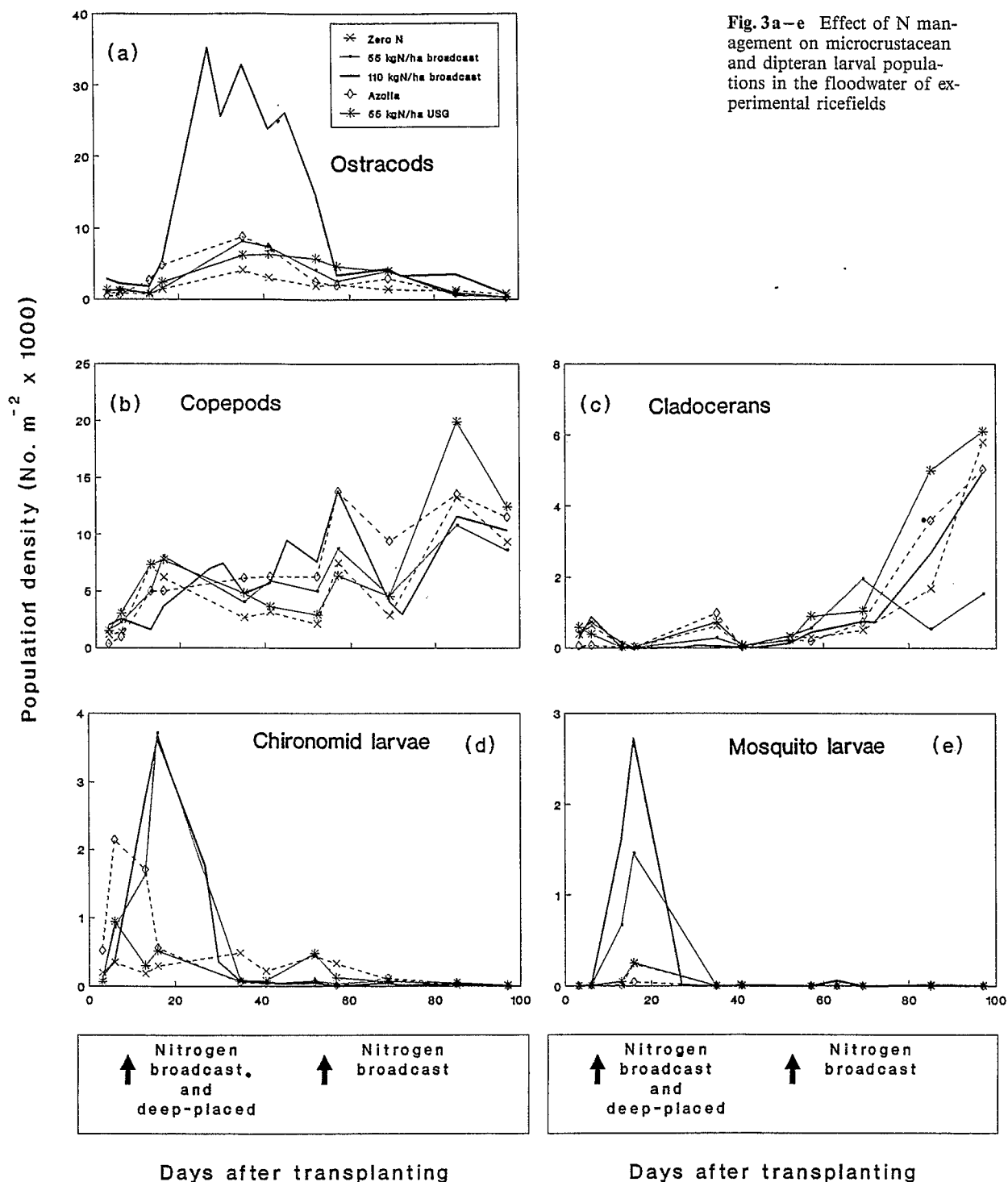


Fig. 3a-e Effect of N management on microcrustacean and dipteran larval populations in the floodwater of experimental ricefields

In the 110 kg N ha⁻¹ treatments cladocerans were virtually absent early in the crop season (Fig. 6). Populations were significantly ($P < 0.04$) denser in the lowest than the highest pesticide treatment from 52 to 85 days after transplanting. The length of time before the populations started to expand was pesticide-dependent. For the majority of plots the last application of carbofuran was 54 days after transplanting. Cladoceran populations in plots supplied with low levels of pesticides (0.1 kg a.i. ha⁻¹ × 1) started to expand on-wardly. Where the applications had been at intermediate rates

(0.3 kg a.i. ha⁻¹ × 2) expansion was delayed until 70 days after transplanting. Populations in plots with higher frequencies of application (0.3 and 0.5 kg a.i. ha⁻¹ × 5) did not start to increase until 85 days after transplanting, the latter plots were not given a final application of carbofuran until 69 days after transplanting. From 85 days after transplanting, population densities continued to rise at all pesticide levels, except for the lowest where they declined markedly.

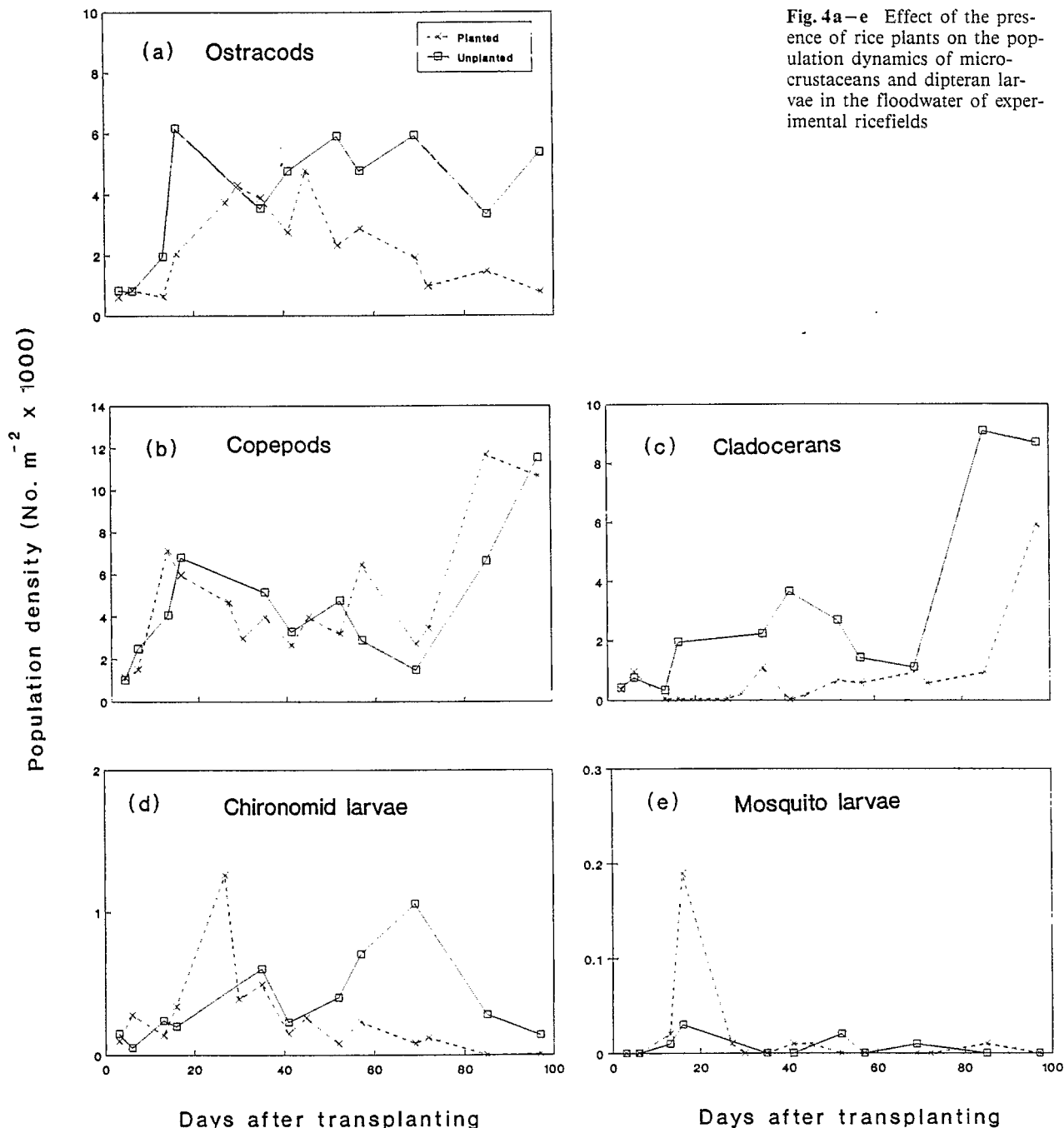


Fig. 4a-e Effect of the presence of rice plants on the population dynamics of microcrustaceans and dipteran larvae in the floodwater of experimental ricefields

Chironomid larval populations

The population dynamics of chironomid larvae were strongly affected by the fertilizer treatments (Fig. 3). When the first split of 55 and 110 kg N ha⁻¹ was broadcast, population densities increased from virtually zero to 300-5000 m⁻² by 16 days after transplanting. This was followed by a rapid decline to original levels by 35 days after transplanting. Densities were significantly ($P < 0.01$) higher in broadcast fertilizer treatments than in the zero-N control from 13 to 16 days after transplanting. After the peak, population densities were similar across treatments, although occasionally they were significantly ($P < 0.05$) higher in the control. Chironomid larval populations did not respond to the second split of broadcast N.

Where *Azolla* sp. was incorporated chironomid larval numbers increased rapidly to densities of over 2000 m⁻² by 6 days after transplanting (Fig. 3). The increase was transitory, and by 16 days after transplanting densities had returned to the original levels where they stayed for the remainder of the crop. Peak populations were significantly ($P < 0.02$) denser in the *Azolla* sp. treatments than in the zero-N control.

Deep placement of N significantly ($P < 0.01$) reduced peaks of chironomid larval abundance compared to the equivalent level broadcast (Fig. 3). From 27 days after transplanting onwards, populations in both treatments were virtually absent.

Chironomid larval populations were influenced by the presence of rice plants and crop development (Fig. 4). Densities were similar

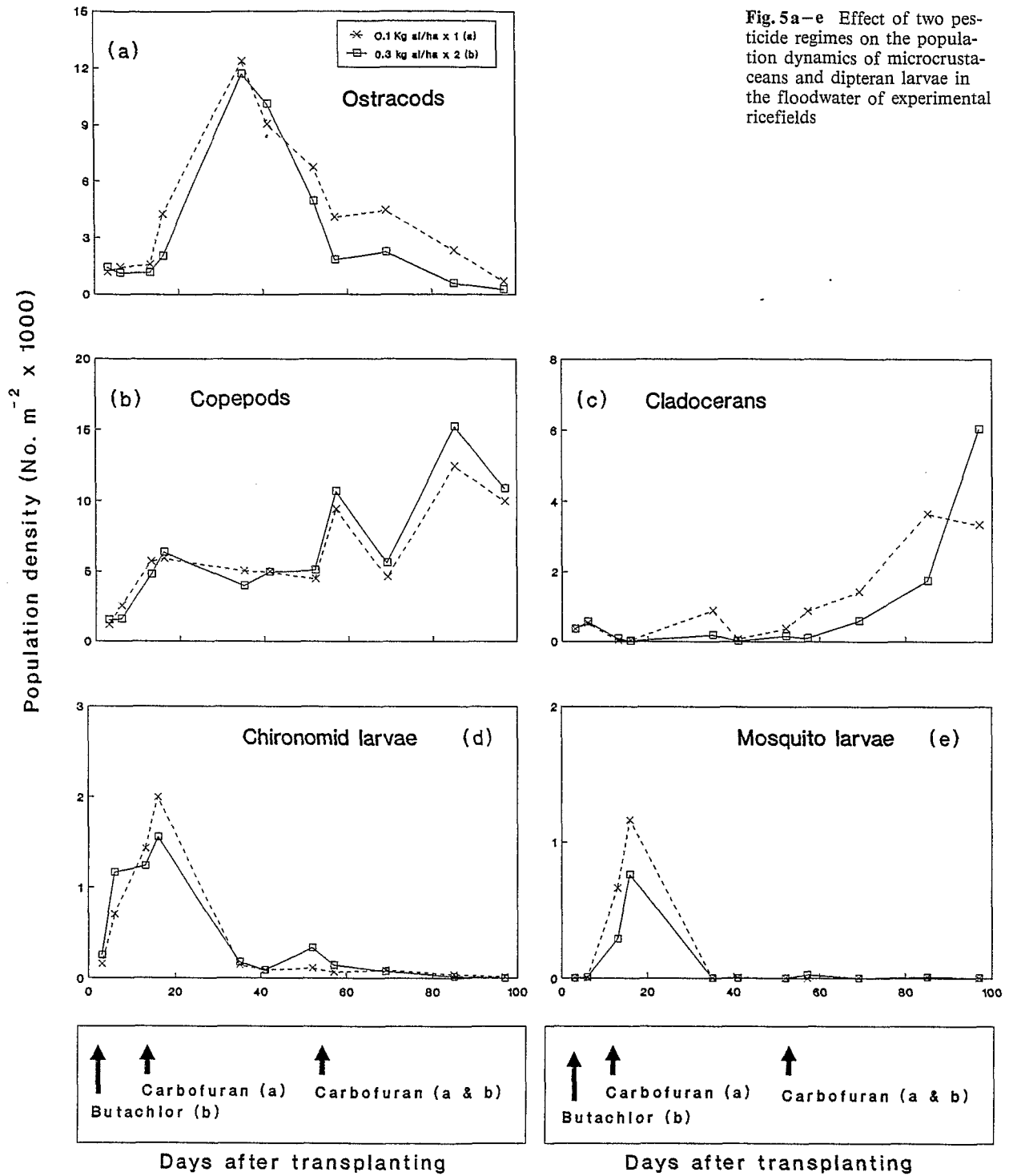


Fig. 5a-e Effect of two pesticide regimes on the population dynamics of microcrustaceans and dipteran larvae in the floodwater of experimental ricefields

to those in the zero-N control plots until 45 days after transplanting, after which they started to diverge, as populations expanded in the unplanted treatment to 1000 m⁻². Chironomid larval densities were significantly ($P < 0.04$) higher in unplanted plots from 69 days after transplanting onwards.

Significant ($P < 0.05$) differences were observed among chironomid larval populations subjected to different pesticide treatments, but they were isolated and did not conform to any logical pattern (Fig. 5, 6).

Mosquito larval populations

Mosquito larval (*Culicidae*) population dynamics were similar to those of the chironomid larvae (Fig. 3). The main differences were a virtual absence after the peaks and extremely low densities throughout the season in the *Azolla* sp. and unplanted plots. The application of 55 kg N ha⁻¹ produced a peak population density intermediate to zero N and 110 kg N ha⁻¹ (Fig. 3). Densities in

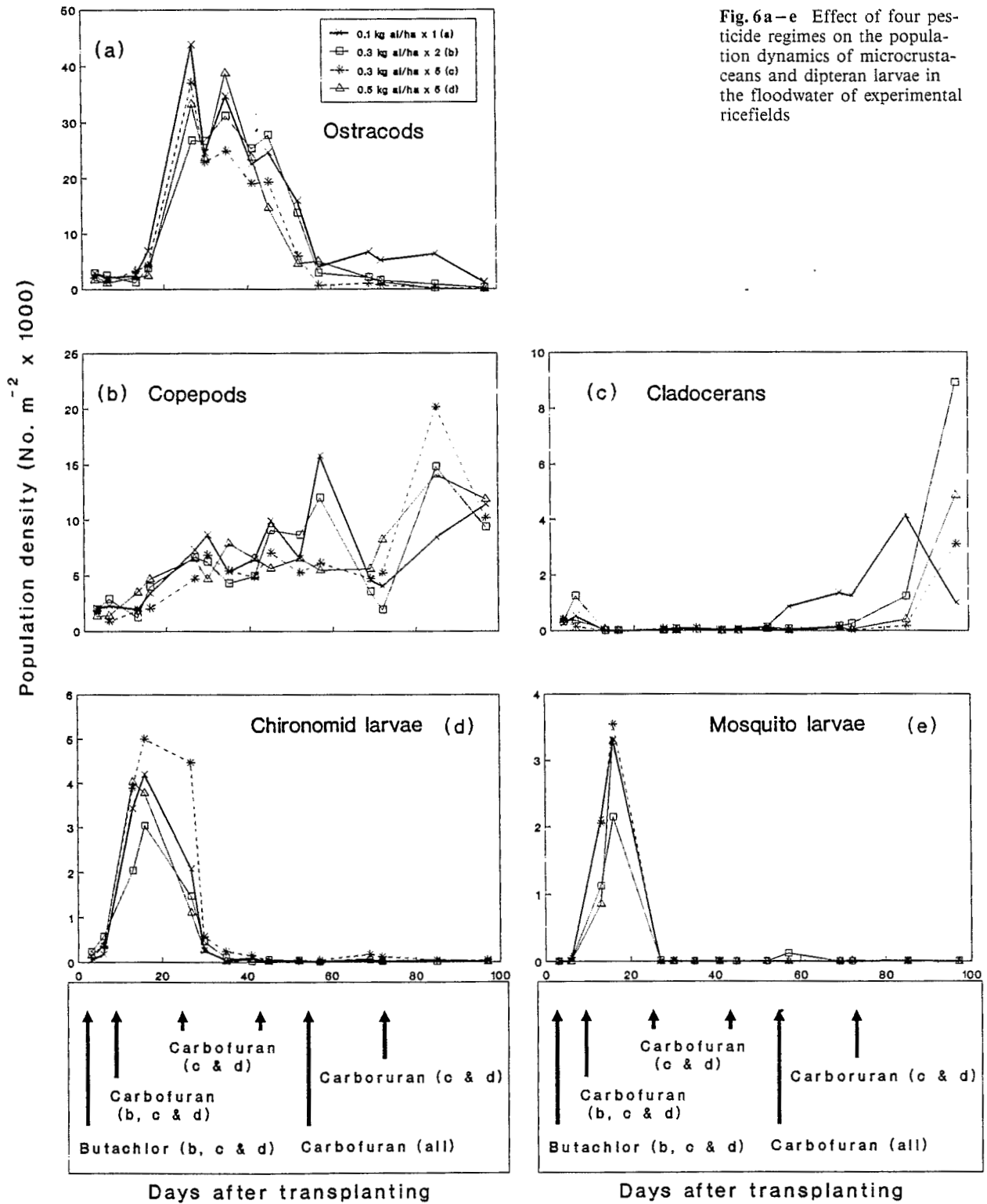


Fig. 6a-e Effect of four pesticide regimes on the population dynamics of microcrustaceans and dipteran larvae in the floodwater of experimental ricefields

plots at both levels of broadcast N were significantly ($P < 0.01$) higher 13 and 16 days after transplanting than the control. At times outside the peaks, populations never exceeded 100 m^{-2} in any treatment.

In treatments where *Azolla* sp. was incorporated, population densities never exceeded 60 m^{-2} and rarely exceeded 25 m^{-2}

(Fig. 3), but they were not significantly ($P > 0.05$) different from the zero-N control. Deep placement of N reduced mosquito larval numbers at the time of maximum abundance (Fig. 3). No significant ($P > 0.30$) differences were recorded between populations in the planted and unplanted plots (Fig. 4). There was no consistent evidence of pesticide impacts on mosquito larvae (Fig. 5, 6).

Discussion

Aquatic invertebrates in the experimental ricefields were affected by N management and, to a lesser extent, pesticide treatment. Fertilizer effects on fauna were probably mediated through the photosynthetic aquatic biomass (Simpson et al. 1993). Pesticide effects may be direct or indirect. Agrochemical inputs in irrigated rice culture have increased dramatically as the system has been intensified. Alterations to the dynamics and structure of the aquatic invertebrate community in ricefields by the adoption of these agricultural practices could have important agro-ecological consequences.

Aquatic invertebrate populations recovered from the floodwater were dominated by species of Ostracoda, Copepoda, Cladocera, Culicidae, and Chironomidae. Population densities quoted previously for these organisms vary considerably between location, stages of crop development, sampling methods, and authors (Table 2). Direct comparisons between published values of floodwater invertebrate population densities are not always possible, because they are expressed in either volumetric (Kurasawa 1956; Kikuchi et al. 1975; Ali 1990) or areal terms (Lim 1980; Grant et al. 1983, 1986; Lim and Wong 1986). In the present investigation, densities were assessed in both ways. Differences were observed between population dynamics expressed in volumetric and spatial terms, but treatment effects were visible with both methods. Population densities reported previously for the various invertebrate groups fall broadly within the ranges recorded in the present experimental ricefields.

The development and successions of aquatic invertebrate populations are controlled by their ability to colonize, interspecific competition for resources and predation, physicochemical parameters, and agricultural practices. The first invertebrate groups to establish significant populations in the broadcast-N treatments were the dipteran larvae. This was probably in response to blooms

of readily palatable unicellular green and blue-green algae (Simpson et al. 1993). Adult mosquitoes selected plots with algal blooms to deposit their eggs. When the measured indicators of primary productivity showed signs of diminishing activity, dipteran larval populations crashed. These dynamics are in agreement with reports by Clement et al. (1977), Mather and That (1984), and data summarized by Roger and Bhuiyan (1990).

The observation that deep placement of N fertilizer inhibited the development of dipteran larval populations is potentially of great importance. This practice could be adopted as part of a vector control programme in areas where disease transmission by mosquitoes represents a public health problem. It is already well established that deep placement of urea supergranules increases the N fertilizer use efficiency, and in this respect there would be no conflict of interest between rice production and vector control.

The occurrence of high chironomid larval population densities following the incorporation of *Azolla* sp. agreed with the observations of Yasumatsu et al. (1979), who reported that chironomid larvae bred in dead organic matter in Korean ricefields and that they were the dominant insect group.

The succession of dipteran larvae by rapidly expanding ostracod populations supports the theory of competition between the two groups suggested by Takamura and Yasuno (1986). Further evidence of competition between chironomid larvae and ostracod populations was recorded in pot experiments at the International Rice Research Institute (I. C. Simpson, unpublished data 1990).

Ostracods quickly colonize the floodwater from eggs in the soil which are resistant to desiccation (Grant et al. 1985). The introduction of significant numbers of grazers in irrigation water was unlikely, as the water came almost directly from a deep well. Accelerated population growth approximately 20 days after transplanting in treatments where N was broadcast was probably in response to increased floodwater primary productivity (Simpson et al. 1993). The rapid decline of this population from 45 days after transplanting was probably because their prolific grazing activities had exhausted their available food. Similar ostracod dynamics were reported by Grant et al. (1983, 1985, 1986). It was suggested that a change in the blue-green algal community structure from palatable non-mucilaginous to unpalatable, buoyant, mucilaginous, colonial forms was responsible for the change in grazer populations from growth to decline (Grant et al. 1985).

As the ostracod abundance declined, population densities of cladocerans and copepods increased. Copepods were numerically dominant throughout most of the crop season and were apparently unaffected by the agricultural practices imposed. Ali (1990) reported that copepods were the numerically dominant microcrustaceans in Malaysian ricefields and that they suffered transitory adverse effects from fertilizer and pesticide applications. In the current investigation, sampling dates may have been too far apart to identify transitory impacts.

Table 2 Population density estimates of crustacean zooplankton and dipteran larvae in ricefields

Crustacean zooplankton		
200–800 l ⁻¹	Japan	Kikuchi et al. (1975)
Cladocerans		
Maximum 300 l ⁻¹	Malaysia	Ali (1990)
<i>Daphnia</i> spp. 198 l ⁻¹	Japan	Kurasawa (1956)
<i>Bosmina</i> spp. 15 l ⁻¹	Japan	Kurasawa (1956)
Copepods		
Maximum 800 l ⁻¹	Malaysia	Ali (1990)
<i>Cyclops</i> sp. 42 l ⁻¹	Japan	Kurasawa (1956)
Ostracods		
300–2400 l ⁻¹	Philippines	Lapis (unpublished data)
0–422 l ⁻¹	Philippines	Grant et al. (1983)
10–20000 m ⁻²	Philippines	Grant et al. (1986) ^a
300–37000 m ⁻²	Malaysia	Lim and Wong (1986)
Dipteran larvae		
Chironomid larvae		
0–22000 m ⁻²	Philippines	Lapis (unpublished data)
8000 m ⁻²	Philippines	Grant et al. (1986) ^a

^a Cited in Grant et al. (1986) but previously unpublished

When cladoceran populations developed towards the end of the crop cycle they were adversely affected by pesticide applications. This is in agreement with Ali (1990), who reported that carbofuran was acutely toxic to cladoceran populations in ricefields.

The implications of the relationship between primary production and invertebrate grazers on soil fertility depends on the type of algae and their stage of development. Wilson et al. (1980) reported that large numbers of microcrustaceans inhibited algal development and reduced N input to the rice system from biological fixation. Reciprocally, high primary productivity encourages grazing populations. Agricultural practices which affect the relationship between primary producers and consumers will alter the periodicity of nutrient recycling.

Numerical dominance does not necessarily reflect the functional importance of an invertebrate group in the ricefield ecosystem. Their body size and activity rates are also important. For example, a sparse population of the relatively large mosquito larvae could ingest an equivalent quantity of algae to that ingested by a dense population of the relatively small cladocerans.

In the present investigation aquatic invertebrates were categorized into broad taxonomic groups. It is probable that successions of individual species occurred within the group trends presented. The assumption in this investigation that species within a group perform similar functions and respond similarly to treatments is incorrect. Successions of individual species could be important and should be investigated.

Previous quantitative studies of zooplankton and benthic invertebrates have almost exclusively been conducted in fertilized fields (Grant et al. 1986). Differences between fertilized and unfertilized plots should be appreciated. Perhaps most notably, pesticide impacts on indicators of floodwater primary productivity were only identified in the absence of broadcast N fertilizer (Simpson et al. 1993).

In the majority of published work where pesticide impacts on aquatic invertebrates have been reported, application rates have been higher than the recommended doses. Rotifer, cladoceran, and copepod populations in Malaysian ricefields were adversely affected when carbofuran was applied at 5.6 kg a.i. ha⁻¹ (Ali 1990). This information is useful in the context of pesticide mis-use and accidental spillages, but where pesticides are used judiciously the findings are of limited value.

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