AQUATIC TOXICOLOGY OF SELECTED RICE INSECTICIDES, WITH SPECIAL REFERENCE TO THEIR EFFECTS ON FISH CULTURE IN WEST JAVA, INDONESIA

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NOTE : Raising fish in wet rice field in
the village of Ciherangpondok,
Bogor, Indonesia

For my wife Fatimah,
and my children
Ari, Dewi and Puri.

I declare that this thesis has been composed by myself and that it embodies the results of my own research. Where appropiate I have acknowledged the nature and extent of work carried out by others in this thesis.

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ABSTRACT

Insecticides are widely used in SE Asian countries to control insect pests in rice, mainly stemborers *(Chilo suppresallis*) and brown plant hoppers (*Nilaparvata lugens*).

The use of highly toxic insecticide compounds, however, is known to cause serious problem for fish culture in wet rice fields in many of these countries, particularly in Indonesia where this practice assumes a tremendous "house hold economic" importance, as well as being an essential part of the nation's inland aqua-culture production system.

The laboratory and field experiments described were carried out to establish whether the application of five selected rice insecticides (*fenobucarb, isoprocarb, buprofezin, diazinon* and *alphamethrin*) would influence the growth and production of common carp fingerlings (*Cyprinus carpio* LINN.) raised in wet rice fields.

Laboratory static toxicity tests revealed that the carbamate insecticides (*fenobucarb* and *isoprocarb*) were the least toxic insecticide compounds. The 96 hour Median Lethal Concentration (i.e the concentration that killed 50% of the test fish in 96 hours exposure period, under specified conditions) of these insecticides were 5.8mgl^{-1} and 5.3mgl^{-1}, respectively. The synthetic pyrethroid insecticide *alphamethrin* was the most toxic insecticide with 96h-LC50 of 0.037mgl^{-1}, while the organophosphate *diazinon* and the thiadiazin *buprofezin* showed intermediate toxicity to common carp (96h-LC50 = 2.3mgl^{-1} and 1.5mgl^{-1}, respectively). A series of five field experiments were consecutively conducted, using 24 specially constructed rice field plots to accomodate the culture of common carp fingerlings for a period of 21 days. A single application of three dose regime, i.e 1/2X, 1X and 2X of the recommended dose rate for insect control was given as treatment in each experiment. The survival of fish in all experiments were not significantly influenced by the insecticide treatment ($P > 0.05$). The growth rate and the production of fish biomass in rice fields treated with *isoprocarb, buprofezin, diazinon* and *alphamethrin*, were also found to be comparable with

those in the untreated control plot ($P > 0.05$). In the rice fields treated with the highest dose rate of *fenobucarb* (1500gha^{-1},AI), the growth and production of fish were significantly lower than those in the untreated control rice fields ($P < 0.05$). Observations on the rice field biota revealed no definite pattern in the temporal changes of the population of zooplankton and macroinvertebrates both in the insecticide treated plots as well as in the untreated control plots. The minimum effects of the insecticide treatment to fish and rice field biota observed in the experiment were presumably due to several factors, mainly because a significant amount of the compounds were adsorbs by the rice field soil and aquatic vegetations and not onto the water, causing less toxicity, followed by the rapid flushing of the chemicals from the rice field system.

The composition of the diet of common carp fingerlings in the rice field was found to be similar with those reported in the natural ponds, consisting mainly of aquatic insects (and their larvae), crustaceans, benthic macroinvertebrates and plant detritus. Based on the results of the present experiments, of the five insecticide compounds tested *diazinon* and *fenobucarb* appeared to produce greater risk to fish when used in rice-fish farming. The use of agrochemicals in rice-fish farming should be carefully managed and controlled, using selected low toxic and non persistent insecticides based on the result of laboratory and field toxicity tests.

CHAPTER 1

GENERAL INTRODUCTION

1.1. THE AQUATIC TOXICOLOGY OF INSECTICIDES

1.1.1. TYPE OF INSECTICIDES

Insecticidal compounds are basically designed to control pest insects, and to persist in the environment for a specific time. Insecticide comprises a greater proportion of pesticides used in the tropical and subtropical countries because of the higher intensity of pests in these climatic condition. Based on their chemical structure, HUTSON and ROBERTS (1985) have divided insecticides into four main types of compound :

(a The organochlorines (OCs).

Insecticide compounds belonging to this group include *DDT* and related compounds (e.g. *methoxychlor*), hexachlorocyclohexane (*HCH,lindane*) and the cyclodienes (e.g.*endrin,aldrin, dieldrin, chlordane* and *heptachlor*). Most organochlorines have high insecticidal activity, low acute mammalian toxicity and residual biological activity. In agriculture these compounds have been extensively used as a wide spectrum insecticides for the control of many insect species. Concern over bio-accumulation has restricted the use of these compounds in developed countries. However, because of their low cost, the organochlorines are still routinely used in developing countries. DDT acts as a nerve poison causing disturbance of the sodium balance of the nerve membrane. All cyclodienes have a characteristically slow-acting effect on insect (BROOKS,1974).

(b) The organophosphates (OPs)

The organophosphates consist of a large number of compounds, including *malathion, parathion, diazinon*, *chlorpyrifos* and *fenitrothion*. The active ingredients of these compounds have high but narrower spectrum of insecticidal activity and with low persistency when compared with the organochlorines. The essential features of the organophosphates is the electrophylic nature of the phosphorus which is partly owing to the polarisation of the P=O bond and partly owing to the electron withdrawal properties of the nitrophenyl group. This electrophylic nature of the phosphorus atom has the ability to phosphoroxylate nucleophiles, including biological nucleophiles, which aided by the properties of the nitropheny substitutes, is a good leaving group in the SN_2 phosphoroxylation reaction. Another important features is the use of the phosphorothionates (P=S compounds) as opposed to phosphate (P=O compounds). The former acts as pro–pesticide, since they do not possess the electrophilic phosphorus atom required for intrinsic reactivity and must be bioactivated by oxidative desulphurization in the insect, soil, plant or mammal before toxic interaction can occur. This requirement for bioactivation offers the opportunity for other metabolic processes to operates and infer selective toxicity, Further, as esters the organophosphates are liable to hydrolysis and to other mechanisms of ester cleavage. Therefore, in practice they are biodegradable and are relatively non–persistent in animals, plants and the environment (DAHM,1970).

It is now generally recognised that the toxic action of the organophosphates upon insect as well as invertebrates is caused by their ability to inhibit acetylcholinesterase in various parts of the nervous system, and thereby disrupt nervous transmission. Until recent years acetylchloninesterase was treated as a single kind of enzyme, however, it was not unusual for these enzymes to occur in multiple molecular form (= "iso–enzymes") (O'BRIEN *et al.*,1974). Poisoning of organophosphates results from toxification by endogenous acetylcholine which builds up following the inhibition of acetylcholinesterase.

(c) The carbamates

This type of insecticide include some 40 insecticidal compounds, most of them could be categorised into three main classes, (i) the aryl N-methyl carbamate, (ii) the dimethyl carbamyl ester of a heterocyclic hydroxy compound, and (iii) N-methylcarbamyl ester of an oxime, or oxime carbamate. The most popular groups include the phenyl-N-methyl carbamate (*isoprocarb or MIPC, bassa or BPMC,* and *bufencarb),* naphthyl carbamate (e.g. *carbaryl*) and benzofuranyl carbamate (*carbofuran*). A greater proportion of the carbamate are systemically active in plants. The mode of action of carbamate insecticide is similar to that of organo-phosphates, i.e. inhibition of acetylcholinesterase. N-methyl carbamates inhibit acetylcholinesterase by means of N-methylcarbamylation of the serine hydroxyl group of the enzyme, analog to the alkylphosphorylation by the organophosphate triester, with the significant difference that the carbamylated enzyme is much less stable than the phosphorylated enzyme. The carbamate is destroyed in the process, with the liberation of a phenol or an oxime. The metabolism of the carbamates in mammals and plants is dominated by hydrolysis to the phenol, oxime (or other hydroxy compounds) and methyl carbamic oxide (which is further metabolised to carbon dioxide and ammonia).

(d) The pyrethrins and synthetic pyrethroids

The photo-labile early synthetic pyrethroids have been developed from the discovery of insecticidal activity in the flowers of chrysanthemum species. These compounds and the natural pyrethrins are not stable and have limited (mostly domestic) use. The new generation of photostable pyrethroids , first introduced in 1976, are suitable for use in field. The compounds, which include *permethrin, cypermethrin, fenvalerate, deltamethrin* and *alphamethrin*, have remarkable residual activity and are effective for the control of insect pests using smaller dose rates and less frequency of application. They are potent neuropoisons, primarily affecting nerve membrane sodium channels by delaying their closing. The most important factor of the pyrethroid insecticides in relation to their metabolism is the ester bonds (HUTSON,1979;CASIDA and RUZO,1980; CHAMBERS,1980). Ester cleavage is very important in limiting the toxicity of the pyrethroids. In mammals their toxicity is generally low, particularly owing to the readily hydrolysed *trans*–isomers. In fish, possessing a low capacity for pyrethroid hydrolysis and relying instead on peripheral hydroxylation and conjugation, their toxicity is rather high.

(e) Other compounds

Other insecticide compounds include organotin compounds, pest control agents (bacteriophages) and growth regulators including pheromones and juvenile hormones and analogues. In general, these compounds, except the organotin, pose less hazard to the aquatic environment (RAND and PETROCELLI,1985).

The insecticides, as a group, have the highest acute mammalian and aquatic toxicity, followed by fungicides and herbicides. They probably have also the greatest potential for adverse environmental impacts, although the wide scale use of some herbicides can have long–range consequences on the ecosystem (VON RUMKER,*et al.*,1975).

1.1.2 FACTORS AFFECTING THE HAZARD OF INSECTICIDES TO AQUATIC ENVIRONMENT.

Since the early times of their use, it has been recognised that many insecticides are also toxic to fish and other non target aquatic organisms. Contamination of the aquatic environment with these chemical compounds and their bioaccumulation through food chain, constitutes a potential ecological hazard to fish (CARSON,1962; MULLA *et.al*,1963; MOORE, 1967; MUIRHEAD–THOMSON,1971). Influence of insecticides on fish may include both lethal effects (death) and sub–lethal effects (e.g. changes in growth, reproduction, pathology, biochemistry, physiology and behaviour) (HOLDEN,1973).

The hazard of insecticide to aquatic ecosystems depends on the chemical and physical properties of the compound, type of formulation, rate and method of application, and characteristics of the receiving water system (RAND and PETROCELLI,1985). Principle properties of chemicals that

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studies by SIEBER and ADAMSON (1977) revealed that fish can also produce a range of bio-transformation reactions, though generally with lower overall activities than mammalian system counterparts.

The rates and routes of bio-transformation were dependent upon species and strain, as well as on the size, age and sex of the individual fish (NAGEL,1983; PEDERSON *et al.*,1976; FORLIN,1980). According to BUHLER and RASMUSSON (1968), bio-transformation in fish appears to be carried out largely in the liver. Bio-transformation products are then eliminated in the urine or via the bile into faeces, or by diffusion through the gills into the surrounding water.

Bio-transformation of the main groups of insecticides in fish has been reviewed by EDWARDS and MILBURN (1985). According to the authors, the overall biotransformation of organochlorine compounds in fish appeared to be complex, and most of the products remain unidentified because of the small amount present. Biotransformation reaction in organophosphate insecticides would lead to activated product, e.g. the oxidation of the P=S group to the P=O derivates, and also to products with negligible or reduced activity in terms of acetylcholinesterase inhibition. GILL (1980) has investigated the in vitro biotransformation of *carbofuran*, a carbamate rice insecticide, in *Trichogaster pectoralis* and found that the major metabolism products in fish was the N–hydroxymethyl derivatives, instead of 3–hydroxy carbofuran, which was found in the mammalian system. The author concluded that the detoxication rates of the insecticide in fish should not, therefore, be extrapolated from laboratory animals, such as rodents. In general the biotransformation products of insecticide in fish are less toxic than the parent compound. However, occasionally the reverse is true. For example, the primary biotransformation product of *carbaryl*, 1–naphthol, is more toxic to marine and freshwater bivalves and fish, than the parent compound (STEWART *et al.*,1967; BUTLER *et al.*,1968; TILAK *et al.*,1980;1981).

1.1.3. TOXICITY OF INSECTICIDE TO FISH

Substantial information on the acute toxicity of insecticides to fish is available, especially since the development of a greater degree of standardisation of toxicity testing methods, during the the past 20–25 years (RAND and PETROCELLI,1985; MURTY,1985). In general, the organochlorine compounds has the greatest potential for adverse effects to fish, specifically the cyclodienes such as *endrin and* endosulfan (GRANT,1976; IYATOMI *et al.*,1958; SCHOETTGER,1970). As a group the organochlorine compound has greater acute toxicity to fish than organophosphate and carbamate compounds (KATZ,1961; PICKERING *et al.*,1962; HENDERSON *et al.*,1959).

Organophosphate compounds have moderate to high acute toxicity to fish (HENDERSON and PICKERING,1958; JOHNSON *et al.*,1980). The phosphorothionates (P=S linkage compounds) have initially low acute toxicity to fish as they need to be bioactivated and converted to their P=O analog before exerting actual toxicity (see Section 1.1.1) (BEDFORD and ROBINSON, 1972). Some organophosphates such as *azinphos-methyl* and *phosdrin*, were found to be high toxic. On the other hand, the organchlorines *BHC* and *heptachlor* were reported to be less toxic to fish (Table 1.1).

Carbamate compounds are generally moderately toxic to fish, except *carbofuran* which has a relatively high fish toxicity. The 96h–LC50 of the commonly used carbamate insecticide *carbaryl* and *carbofuran* to various freshwater fish in North America were found to be 2 to 30$\mu g\,l^{-1}$ and 150

TABLE 1.1. Acute toxicity of some organochlorine and organophosphate insecticides to fish[*]).

INSECTICIDE	Bluegills (*Lepomis macrochirus*)	Rainbow trout (*Salmo gairdneri*)
	96h-LC50 (in ppm)	
Organochlorines		
Toxaphene	0.004	0.008
Endrin	0.006	0.007
Dieldrin	0.008	0.019
Aldrin	0.013	0.036
Chlordane	0.022	0.022
Methoxychlor	0.062	0.020
Lindane	0.062	0.060
Heptachlor	0.190	0.150
BHC	0.790	-
	24h-LC50 (in ppm)	
Organophosphates		
Azinphosmethyl	0.022	0.014
Phosdrin	0.041	0.034
Fonofos	0.045	0.110
Malathion	0.120	0.100
Diazinon	0.052	0.380
Phosphamidon	-	5.00
Methylparathion	5.70	2.70
Dimethoate	28.00	20.00

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[*]) Source : Edwards (1977)

870$\mu g\,l^{-1}$, respectively (JOHNSON and FINLEY,1980). HEJDUK and SVOBODOVA (1980) reported that the 48h–LC50s of the more recent carbamate compounds to three species of fish (*Cyprinus* t*ocarpio, Salmo gairdneri and Poecilia reticulata*) ranged between 1.80$\mu g\,l^{-1}$ and 190.0$\mu g\,l^{-1}$ (Table 1.2)

Pyrethroid insecticides are highly toxic to fish, although they are not persistent in the environment. MAUCK and OLSON (1976) determined the toxicity of natural pyrethrins and five pyrethroids to coho salmon (*Oncorhynchus kisutch*), rainbow trout (*S. gairdneri*), fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), blue gill (*Lepomis macrochirus*) and yellow perch (*Perca flavescens*), and found that the 96h–LC50 for natural pyrethrins ranged between 24.6$\mu g\,l^{-1}$ and 114$\mu g\,l^{-1}$, and those for pyrethroids ranged between 0.110$\mu g\,l^{-1}$ and 1140$\mu g\,l^{-1}$. HANSEL *et al.* (1983) reported variations in the lethal effect of the synthetic pyrethroid AC 227,705, *permethrin* and *fenvalerate* on sheephead minnow (*Cyprinodon variegatus*) in early stage toxicity tests. AC 222,705 was 750 times more toxic than permethrin and 20 times more toxic than fenvalerate. The most sensitively affect

TABLE 1.2. Acute toxicity of some carbamate insecticides to fish [*].

INSECTICIDE	Common carp *(C. carpio)*	Rainbow trout *(S. gairdneri)*	Gyppy *(P. reticulata)*
	48h-LC50 (in ppm)		
Mancozeb	24.0	1.85	2.20
Dioxacarb	25.5	2.70	36.0
Carbofuran	11.0	8.50	3.40
	72h-LC50 (in ppm)		
Benomyl	190.0	1.80	110.0

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[*] Source : Hejduk & Svobodova (1980)

measured in the tests was on the survival and size of fish. Coldwater fish were considered more susceptible to pyrethroid poisoning than warmwater fish species (JOLLY *et al.*,1978; COATS and O'DONNELL-JEFFERY,1979).

Several factors affect the toxicity of insecticides to fish. There is evidence that larger, or older, fish are less susceptible to insecticide than smaller, or younger, fish (BULL *et al.*,1974; PICKERING *et al.*,1962). The eggs and the fry tend to be more tolerant to insecticide poisoning than the 7-14d old fry. Increased tolerance was further observed in 60 to 90d fry and particularly in adults. IYATOMI *et al.*(1958) noted that the 24h–LC50 values of *endrin* to common carp (*C. carpio*) are 19.9ppm for eggs, 10.7 to 4.2ppm for 1d to 4d old (sac) fry and 0.046ppm for 5d to 6d old (floating) fry. Similar result was obtained by HASHIMOTO *et al.* (1982) from their experiment on the susceptibility of common carp to eight pesticide formulations with special reference to growth. Eyed eggs were almost always more tolerant than fry or fingerlings, and floating fry were more susceptible than sac fry to six pesticides tested. However, no noticeable changes was observed in the susceptibility of the experimental fish to five organophosphate compounds tested (*trichlorfon, fenitrothion, phenthoate, diazinon and IBP*) with reference to their growth. The authors concluded that each pesticide has a different age–susceptibility pattern, which was presumably related to their particular chemical structure or their toxicological mode of action.

MACEK and McALLISTER (1970) confirmed the relative susceptibility of twelve species of fish from four families, i.e the Ictaluridae, Cyprinidae, Centrarchidae and Salmonidae to nine pesticides, including three organochlorines (*DDT, lindane,toxaphene*), four organophosphates (*methyl parathion, fenthion,malathion* and *azinphosmethyl*) and two carbamates (*carbaryl* and *mexacarbate*). The variation in species susceptibility was found to be minimal for the organochlorine. Of the families of fish it was noted that the Salmonidae was the most susceptible, the Centrachidae intermediate and the Ictaluridae and Cyprinidae the least susceptible. CLARK et al. (1985) determined the relative sensitivity of six estuarine fishes to the organophosphates *carbophenothion* and *chlorpyriphos ethyl*, and to the pyrethroid *fenvalerate*, and found that the atherinid fishes (*Menidia* spp) were the most sensitive estuarine fish species to the insecticides.

Water quality may influence the toxicity of chemicals to fish. Toxicity of most insecticides, except *DDT*, increases at higher temperature (COPE,1965; MACEK *et al.*,1969; CAIRNS *et al*,1975). Toxicity of endrin to gold fish (*Carassius carassius*) increased with temperature: 48h–LC50 at 4 °C was 0.14µg l^{-1}, at 17°–19°C was 0.004 to 0.008µg l^{-1} and at 27° to 28°C was 0.002µg l^{-1} (IYATOMI *et al.*, 1958). MAUCK and OLSON (1976) reported that temperature affected the biological activity of pyrethrins and pyrethroids to fish. The toxicity of dimethrin, RU–11679 and SBP–1387 was two to three times higher at 12°C than at 22°C. On the other hand, d–trans allethrin and S–bioallethrin were found to be less toxic at 12°C than at 17°C, but more toxic at 22°C than at 17°C. Temperature, directly or indirectly, influences enzyme activity, metabolic rate and certain environmental factor (mainly dissolved oxygen). The higher toxicity of many insecticides at higher temperature can be explained on the basis of increased uptake of the toxicant because of a higher ventilation rate (MACEK *et al.*,1969; CAIRNS *et al.*,1965).

The influence of water hardness on the toxicity of insecticides appears to be limited, unless changes in pH are produced at the same time (PICKERING *et al.*,1962; HENDERSON *et al.*, 1959; INGLIS and DAVIS,1972; JOHNSON and FINLEY,1980). The variation of acute toxicity of both the organochlorines and the organophosphates was found to be small and non significant in water of varying hardness (20–400µg l^{-1}) (HENDERSON *et al.*,1960). The only 96h–LC50s that differed between hard and soft water were those of trichlorfon, for which the LC50 in hard water was less than 1/3 of those in soft water. This was due to a rapid breakdown of the insecticide to more toxic products at the higher pH.

Increased turbidity generally decrease the toxicity of insecticides, due to increased adsorption on organic matter and reduced bioavailability of toxicant in water (WEBER,1972; CAIRNS,1968; FERGUSON,1965). However, the influence of suspended matter on the acute toxicity of insecticide to fish has not been investigated to a great extent (MURTY,1986).

The toxicity of a commercial product of insecticide to fish also depends on the type of formulation used. Oil formulation can be more toxic than emulsifiable concentrate, and granule form was found to be less toxic, presumably due to its slow releasing effect (ALABASTER,1969; PICKERING *et al.*, 1962; HILTIBRAN,1967, PRIYAMVADADEWI *et al.* 1981). Field evidence strongly suggests that emulsifiable concentrate is much more toxic to fish than wettable powder or dust formulation (EDSON *in* GRIST,1975).

Fish may also be subjected to long-term stress arising from exposure to small quantities of sublethal concentrations of insecticide, leading to various subtle effects on fish physiology. This long-term toxicity is more difficult to identify and mainly considered in the case of persistent insecticides and herbicides (HOLDEN,1973). In terms of practical fish husbandry the most important sublethal effects are the effect on feeding and growth, and the effects on the reproduction and early development stages of fish. Fish are reported to avoid food contaminated with subacute level of *parathion*, consequently reducing their food intake (KLEEREKOPER *et al.*,1974). Food intake of young salmon was markedly reduced when *DDT* was incorporated in the diet (BUHLER *et al*,1970). Exposure of insecticide could result in stimulated growth or selective elimination of fish, but could also retard or inhibit growth. Growth of fathead minnows was stimulated by mirex at

$13 \mu g l^{-1}$, but a concentration of $34 \mu g l^{-1}$ had no effect (BUCKLER *et al.*,1981). Similarly, exposure of gold fish (*Carassius auratus*) to endrin induced weight gain (GRANT and MEHRLE,1970). WILDISH and LISTER (1977) administered fenitrothion orally to brook trout, and found no effect on the growth of the fish. CHATTERJEE and KONAR (1984) reported that exposure of diazinon at $0.463 mg l^{-1}$ significantly reduced the growth of *Tilapia mossambica* at pH 6.5 and 7.0.

1.1.4 TOXICITY OF INSECTICIDE TO FISH FOOD ORGANISMS

In assessing the effects of insecticide on fish production, the direct and indirect effects of insecticide on fish food organisms need also to be evaluated (CAIRNS *et al*, 1978). Many aquatic invertebrates, especially cladocera, copepods and insect larvae, form an important source of food for fish which are reported to be more sensitive to insecticide than fish (JOHNSON and FINNEY,1980; HUGHES *et al.*,1980). According to HURLBERT (1975) toxicological data suggest that three generalisations can be made on the effect of insecticides to aquatic biota : (i) phytoplankton are generally more susceptible to organochlorine than to organophosphate compounds, (ii) crustacean zooplankton are generally more susceptible to organophosphate than to organochlorine compounds, and (iii) as a group, insecticides are generally more toxic to crustacean zooplankton than to phytoplankton. The generally greater susceptibility of crustacean zooplankton to organophosphate than to organochlorine insecticides was demonstrated by SANDERS and COPE (1966), who tested 12 organochlorine and 10 organophosphate compounds against *Daphnia pulex*. The results of their study showed that the average 48–EC50 (= concentration required to immobilise 50% of the test animals within 48h) was 148ppb for organochlorine compounds, and 1.7ppb for the organophosphate compounds. The susceptibility of the phytoplankton to the two insecticide groups was shown by the result of screening tests of 17 pesticides to five species of marine phytoplankton conducted by UKELES (1962). Phytoplankton growth was much more inhibited by the organochlorines (*lindane, toxaphene, DDT*) than by the organophosphates (*dipterex, TEPP*). The tests also revealed that brown pigmented phytoplankton (*Monochrysis, Phaedactylum*) consistently proved more susceptible to the insecticides than the green pigmented species (*Protococcus, Chlorella, Danaliella*).

It is known that complete or partial removal of a species from an aquatic ecosystem is usually followed by changes in the prey, competition and other populations with which that species interacts (HURLBERT,1971). Numerous observations confirmed that phytoplankton population could increase as a result of insecticide treatments. RUBER and FERRIGNO (1964) reported increased phytoplankton productivity after treatments of *DDT, endrin* and *fenthion* at the rates used in mosquito control. However, the authors suggested that the increase was due by released of nutrient from dead mosquitoes or other insects killed during the spraying. HURLBERT *et al* (1972) found the treatment of the organophosphate Dursban (*chlorpyriphos ethyl*) in freshwater ponds at 0.5 to 5 times the rate for mosquito control (= 0.056kg.ha^{-1}) at two week intervals, reduced the population of crustacean zooplankton (principally *Moina* and *Cyclops*), which were followed by dramatic increase of phytoplankton population (principally *Anabaena*). Furthermore, IKESHOJI and HURLBERT (1971) reported that a population bloom of a phytoflagellate *Chlorogonium* persisted longer in experimental pools treated with the insecticide *dibutyl cresol* (1.8ppm), than in the untreated control pools.

It would be expected that benthic organisms will generally be exposed to higher concentrations of insecticide residue and for longer periods, than will be organisms of the limnetic zone. This is mainly because most insecticide have low water solubility and have a tendency to be absorbed on suspended matters, on sediments, on aquatic vegetations and other surfaces (see Section 1.1.2). HURLBERT *et al* (1972) in their Dursban experiment additionally revealed that benthic–littoral rotifers (e.g *Lecane, Monostyle, Platyas*) generally decreased after treatment, while planktonic species (e.g *Brachionus, Hexaartha, Filinia*) generally showed population increase. Greater mortality to a benthic–littoral copepode (*Cyclops vernales*) was also recorded as compared to a more planktonic species (*Diaptomus pallidus*).

Secondary effects of pesticide treatments in the form of expansion of benthic food webs and removal of predaceous invertebrates could also occur, and cause elevation in certain benthic invertebrate populations (HURLBERT,1975). The dead plants resulting from a herbicide treatment, for example, constitute a large new food supply for decomposers and for certain annelids, arthropods, snails and other organisms that can feed on plant detritus. Increased oligochaetes and *Chaobarus* population was reported following the treatment of a herbicide *diquat* to control the aquatic weed *Najas* (TATUM and BLACKBURN,1962). Densities of oligochaete worms and chironomid midges were two to ten times greater in Silvex (a herbicide) treated ponds than in the untreated control ponds (HARP and CAMBELL,1964). HYNES (1961) reported that contamination of a stream with *gamma–BHC* was followed by a large population increase of oligochaetes and chironomids, presumably due to mortality of their predators, specifically amphipods (*Gammarus*) and predaceous cranefly larvae (*Dicranota*). Chironomid population in experimental ponds treated with *methoxychlor* (0.01 and 0.04ppm) continuously increased in abundance up to more than ten times at 56 to 84d after treatment, compared to the population in untreated control ponds (KENNEDY *et al.*,1970). According to the authors the resurgence of chironomids and snails in treated ponds was due to the temporarily reduction of their predators by damselfly naiads (Coenagrionidae). WEBB (1967) observed increased oligochaetes population following *toxaphene* treatment of a lake, and concluded that this was attributable to the increase of food supply in the form of dead fish and insects. LARKIN et. al. (1970) also observed increased number of oligochaetes, leeches and snails (*Lymnea*) following *toxaphene* treatment of a lake in British Columbia, and attributed the increase to the availability of fish carcasses.

1.2 THE WET RICE ECOSYSTEM

1.2.1 GENERAL CHARACTERISTICS

Little published information can be found on the wet rice field ecosystem in scientific literatures. HECKMAN (1979) described the ecology of the fauna of a rice field in Northern Thailand, which included the seasonal variation during the dry and wet season. His account is probably the most comprehensive account on the subject of tropical rice field ecology, so far. More recently WHITTON *et al.* (1988) recounted the ecology of deep water rice fields in Bangladesh. Their studies were mainly concerned with the physical and chemical aspects of the ecosystem.

Rice fields form a distinct ecosystem which consist of two main ecological components: the rice plant and the aquatic environment. The latter component is temporary, littoral and heavily populated. Differences between the rainy and dry season can be extreme in certain regions and can also make the aquatic rice field habitat a temporarily one. However, in other climatic regions and in areas where good irrigation systems exist, periods of total dryness do not necessarily occur in the rice fields. Further discussions on the rice field system in the following text refer to this latter type of rice field.

Basically, the rice agronomic practice in wet rice fields involve land preparation, water management, fertiliser application, rice planting, pest and disease control and harvesting of rice. In most tropical Asian countries wet land preparation is employed, i.e the field is plowed wet and thoroughly harrowed until the soil is well puddled. The stubbles, rice straws and weeds are plowed under and thoroughly decayed and distributed in the fields. The fields are then kept flooded until transplanting to minimise the loss of nitrogen released by decomposition of organic matter (weeds and crop residues). In general the water in the field reaches a level of 5cm which is gradually increased to 25cm. In most Southeast Asian countries rice planting is done by transplanting the seedlings from isolated seed beds. The rice seedlings are planted at a distance of 20cm between rows and 15 to 20cm between each plant. Like other agroecosystems from which biomass is continually removed, the rice field relies on fertilisation from some external sources. The nutrient uptake by the rice plant is a function of climate, soil type, the amount of fertilizer applied, the rice variety and the method of fertilizer application. In general, the inorganic and complete NPK fertilisers are used for this purpose. Water management, both irrigation and drainage, is of utmost important in wet rice agronomy, since water is required in different amount at different stages of the rice plant. One to two weeks before harvest the rice field water is gradually drained. Rice yield might be improved by elimination of weed, pest and diseases using pesticides (herbicide, insecticide and fungicide). Weeds are usually removed manually 21d and 45d after rice transplanting. Generally, insecticides are applied when pests occur in the rice plants, but insecticide treatment as a prophylactic measure in seed beds and just before transplanting (often using *carbofuran*) is common.

The rice field aquatic habitat shows large diurnal fluctuation in temperature (24° to 38°C), pH (6.0 to 9.0), dissolved oxygen (low to over–saturation) and carbon dioxide (0 to 10ppm). Suspended solid is usually high due to the application of fertilisers. The main source of dissolved oxygen is photosynthesis (HECKMAN,1979). At sundown a remarkable decrease of dissolved oxygen occurs. During the midday hours dissolved oxygen content is usually high (near saturation value), water pH also increases. Low pH values, however, can be found associated with high biological decomposition rate occurring in the rice fields.

In flooded rice field soils, unique patterns of metabolism are found, which include transformation of nitrogen and sulfur, and biological nitrogen fixation. Flooded rice fields encourage several nitrogen–fixing agents, including free–living blue green algae, nitrogen–fixing heterotrophic bacteria and a symbiotic relation between nitrogen–fixing blue green algae and the water fern (*Azolla*), known as the *Azolla–anabaena* complex (SWAMINATHAN,1984).

1.2.2 THE RICE FIELD FLORA AND FAUNA

A number of studies have been conducted on the fauna of the aquatic system of rice fields, mainly in relation to fish production and epidemiology (YUNUS and LIM,1971; HECKMAN, 1979; ALI,1990). Other studies focuse on the effects of pesticide on plankton and aquatic invertebrates in the rice fields (TAKAMURA and YASUNO,1986; LIM *et al*,1984). FERNANDO (1980) and FERNANDO *et al.* (1980) presented a general introduction on the ecology of the aquatic fauna of rice field, with special reference to the Southeast Asia. According to the authors, the rice field aquatic fauna is derived principally from the original marsh, lake, stream or pond fauna of the area.

In spite of the annual dessication resulted from the wet and dry climatic cycle and the drastic intervention required to prepare the rice planting, the rice field habitat generally supports a diverse fauna and flora (FERNANDO,1979). The distinct condition of the habitat, however, requires the rice field biota to be adapted to extreme physical and chemical fluctuations, leading to the development of a well defined seasonal succession of dominant species (HECKMAN,1979). This is mainly due to the definite preferences of most animals in carrying out their breeding activities in terms of water availability in the rice field. Most of such species are insects belonging to the order of Odonata and Hemiptera, some rice field cladocerans, the chonchostracan, and some of the rotifers, may breed in the rice field without seasonal rhythm. Although the species diversity may be temporarily reduced, rice fields tend to have as rich and diverse fauna as the continuous natural habitats, due to the rapid colonisation of the fauna from surrounding marshes, streams and ponds. Moreover, the decaying vegetation left in the field releases nutrients which help the rapid growth of the rice and other vegetation (FERNANDO , 1980; HECKMAN,1979).

In the wet rice field ecosystem three component communities are recognised : terrestrial, semi aquatic and aquatic. All three communities occupy the same physical location at different times of the year, and are so complex that they cannot be clearly separated. These aquatic communities encompass a rather large number of species which was well described by HECKMAN (1979), based on his study on the ecology of rice fields in Northeastern Thailand. According to the author the dominant taxa in the rice fields included Insecta (106 species), Ciliata (83 species), Rotifera (50 species), Crustacea (34 species) and Pisces (18 species).

Higher plants in the rice field are important in providing substrates for the settlement of other organisms, modifying the environment and contributing to the production of biomass. These rice field flora commonly comprise of littoral plants (*Frimbristylis, Cyperus*), emergent plants (*Marsilea, Echinochloa*), submerged plants (*Ludwigia, Ipomoea*) and floating plants (*Salvinia, Azolla, Pistia, Lemna, Spirodela*). The primary producers comprise of a great number of taxa, showing definite seasonal succession. Chlorophyta, Euglanophyta and Sarcodina are commonly found dominating the algal and protozoan flora of the rice field.

The taxon Rotifera is always well represented in the rice field. The role of these micro–invertebrates in providing initial food for small fish fry is well known, therefore their abundance is of particular importance for maintaining fish population in the rice fields. (TAMAS and HORVARTH, 1976; WOYNAROVOICH and HORVARTH, 1980)

Among the crustaceans, the cladocerans have usually the greatest species diversity in the rice field (LIM *et al.*,1984; IDRIS,1983). These invertebrates and the ostracods are abundant at times, particularly when the water is rich in detritus.
The dipteran larvae Chironomus are sometimes found in large numbers. Many of the Diptera are filterers of microorganisms as larvae. It is believed that predation pressure is an important factors limiting the population development of these insect larvae in rice fields. The majority of the water beetles belong to the Hydrophilidae and the predatory Dysticidae. The life cycle of most beetles has strong seasonal influences. Some of the Odonata seems to make and deposit eggs in the rice field water. Larvae of these insects are always present in the rice fields. (HECKMAN, 1979)

Molluscs have been subjected to a comprehensive systematic investigation in Southeast Asia (BRAND,1974). Gastropods show strong preferences for water of a particular hardness or alkalinity, and are sometimes found in abundance in rice fields because they can survive well in the sediment (HARRISON *et al.*,1970).

The amphibians and reptiles in the rice fields are aquatic or fully terrestrial, and many could be described as semi–aquatic.

Due to the periodic decrease of oxygen, many fish species (such as *Anabas, Clarias,* and *Ophicephalus*) living in the rice fields are air breathers. Several of the fish species are at or near the top of the aquatic food web, and they are generally present an important protein source for humans. (FERNANDO 1980; HECKMAN 1979; ALI 1990)

Effects of tubificid worms on the biological, chemical and physical characteristics of submerged rice field soil and over laying water, have been studied by KIKUCHI and KURIHARA (1977). The authors reported that tubificid worms destroyed the oxidised superficial soil layer by mixing the soil, altered the size composition of soil particles and allowed a free exchange of dissolved substance between soil and the overlaying water. Further, the presence of tubificids decreased the number of aerobes in the soil and increased the number of sulphate–reducing bacteria and the ammonia content of soil.

Finally, the authors suggested that the tubificid worms inhibited nitrification in the submerged rice field soils.

Based on his observations in Indonesian wet rice fields, ARDIWINATA (1957) recounted that the phytoplankton *Myxophyceae* were abundant during the initial period of flooding, followed by the presence of *Desmidiaceae* and a mixture of both groups. Blue green algae appeared before the rice plants and other aquatic vegetations shade the rice field water. *Chlorophyceae* appeared particularly in rice fields which have been irrigated for a long time, together with *Diatomae*. Both algae are important as oxygen producers for the upper as well as the bottom layers of the rice field water. With these algae, many aquatic organisms such as protozoa, rotifers, copepods, cladocerans, ostracods, oligochaetes, chironomids and other insect larvae, could be found in the wet rice field system. Snails, bivalves and aquatic insects could usually be found in a short time after the fields were flooded. Some of the above biota were probably introduced into the rice fields, together with "wild" fish species, from rivers, marshes and irrigation canals. Fish species in the wet rice system consist mainly of the well known predatory species such as snakeheads

(*Ophiocephalus striatus*), climbing perch *(Anabas testudidineus)*, catfish *(Clarias batrachus)*, and other species including java tilapia (*S. mossambicus)*, sepat siam *(Trichogaster pectoralis)*, guppies *(Lebistes reticulatus)* and *Panchak panchak*.

Various aquatic plants are found in the wet rice fields, some of them, such as *Salvinia natans, Pistia stratiotes* and *Marsilia crenata,* often covered the entire water surface and interfer with the cultivation of fish in the system. Others such as *Azolla pinnata, Lemna paucicostata,* and *Spirodela polyrrhiza*, which are also abundant, are not consider harmful to fish culture practice, due to their value as fish feed and fodder.

Planktonic and epiphytic organisms are important natural food for fish fry in the rice fields. Fry of 3cm body length, however, was found in a study of common carp (*Cyprinus carpio*) to feed more on insect larvae, chiefly the red and yellow larvae of *Chironomus, Endochironomus* and other related genera (SCHUSTER,1955b). According to the author, although zoo and phytoplankton are important natural food for common carp, common carp are a bottom feeder, and the benthic macro fauna and flora of the rice fields are of much greater importance than the floating organisms, particularly at the later stage.

1.3 RICE–FISH CULTURE

1.3.1 GENERAL CONSIDERATION

Fish production is intimately connected with rice cultivation in wet rice fields in both the eastern and western hemisphere, probably because the rice farmer has always used indigenous fish found in paddy field to supplement his diet (GRIST, 1967). This is perhaps also part of the reason why production of fish in wet rice fields has been practised world wide for centuries (HICKLING, 1962; COCHE, 1967). The Chinese began rearing fish in wet rice fields as early as 1700 years ago (GU0 QING–HUA, 1986 *in* KANG–MIN, 1988). Rice fish culture was introduced into Southeast Asia from India about 1500 years ago (TAMURA, 1961). In Thailand, the integrated rice and fish cultivation was known more than 200 years ago (PONGSUWANA, 1962). Fish trials of rice–fish culture in Indonesia date back from 1860 (ARDIWINATA, 1957; VINCKE, 1979). The oldest records of rice fish culture in Japan date to around 1844 (TAMURA,1961). In 1933 fish were known to be cultivated in 80% of the rice fields of the northern region of Italy (TONNOLI, 1955). COCHE (1967) also reported that countries like

Madagascar, USA, Hungary, Taiwan, India, Egypt and the Philippines have also developed integrated rice fish farming

SCHUSTER (1955) considered rice–fish culture as an almost ideal method of land use, and described the system as "a contemporaneous production of grain and animal protein on the same piece of land". The author further stated that production of fish in wet rice fields can be a great importance in the economy and nutrition status of the people in the rural areas of Asian countries and other countries where rice is the dietary mainstay. The author's classic account thoroughly discussed all aspects of rice fish culture, and his views are still of much interest today.

Further advantages of rice–fish culture have been recognised by recent investigators who are interested in integrated agriculture and aquaculture farming systems in the rural areas (PULLIN 1986). Fish culture in rice fields requires relatively small capital inputs, have a short payback period, and is a rational, highly valued technology to Asian rice farmers (RUDDLE, 1980). In Indonesia, rice–fish culture yields an average increase in revenue of 28% above rice revenue, and contributes significantly to overall family income from small land holdings (SCHMIDT,1980; DJAJADIREDJA *et al.*,1980). Based on the study in Thailand MIDDENDORP and VERRETH (1986) reported increases in return rates of 20–50% from rice cultivation to rice–fish culture, leaving the benefit/cost ratio constant.

Fish culture can be a valuable addition to management of natural resources in the rice agroecosystem, and provides a number of very practical benefits for the cultivation of wet rice. Reports from different countries in Indo Pacific region (Indonesia, China, Japan) state that the integrated rice–fish culture system can increase rice yield by 4 to 15% (HOFSTEDE and ARDIWINATA,1950; HORA and PILLAY,1962; GRIST,1975; KHOO and TAN,1980). This increase of rice yield in rice–fish farming system is attributable to several direct and indirect influences of fish rearing to rice agronomy, which are as follows :

(a) Fish loosen the rice field soil as a result of their swimming and puddling of the mud in search of food, thereby aerating the soil, enhancing the decomposition of organic matter and promoting the release of nutrients from the soil (SCHUSTER, 1955; KANG-MIN,1988).

(b) Fish reduce the need for fertilizer on rice by increasing the availability of nitrogen, phosphorus, potassium, calcium and magnesium in the rice field water and soil. The excretion of fish and the remains of food, if they are given supplementary feeding, also serve as additional fertilizer in the rice field (SATARI,1962; HORA and PILLAY, 1962).

(c) Fish feed on many insect or their larval stages that are rice pests, mainly stemborers (*Chilo suppressalis* and *Tryporyza incestulas*) and planthoppers (*Nilaparvata leugens, Sigatella fincifera, Nephotettia virescens*), (YIN PI-ZHEN, 1983 *in* KANG-MIN, 1988). Fish cultivation is moreover often the only practical mean to control malaria carrying *Anopheles* mosquitoes, which often breed in wet rice fields (SCHUSTER, 1955).

(d) Growth of weeds in the rice field can be reduced by 30% when the cultivation of fish, particularly a strongly herbivorous fish like tilapias, is employed, and labor costs for weeding can be curtailed (RUDDLE, 1980, KANG-MIN,1988).

The above mentioned merits of rice–fish culture have stimulated rice farmers in Southeast Asian countries to adopt the practice in their rice farming system (KHOO and TAN, 1980). In Indonesia for example, fish culture in rice fields assumed a tremendous household economic importance, as well as being an essential part of the national inland aquaculture production system. Furthermore, rice fish culture has great potential in increasing the efficiency and productivity of rice farming systems, particularly when its benefits are considered within the context of rural development (DJAJADIREDJA *et al.*,1980; SCHMIDT,1980 ; HUISMAN,1984).

1.3.2 RICE–FISH PRODUCTION SYSTEM

1.3.2.1 Captural system

Fish production systems in wet rice fields vary greatly from one region to another, according to climate, water availability, fish species and traditional practices. Basically, rice field fisheries can be grouped into captural and culture systems (RUDDLE,1980).

Captural systema are the simplest type of rice field fishery, in which indigenous stocks are trapped in the flooded fields, usually by means of small sumps to concentrate the fish crop in the lower portion of the rice field. The fish crop from capture systems consists predominantly of predatory fish. According to FERNANDO *et al.* (1979), in Southeast Asia the fish harvest from captural system mainly consists of air breathing species such as Ophicephalidae (snakeheads), Anabantidae (labyrinth fish), Clariidae (catfishes) and Heteropneustidae (stinging catfish). The production of fish from captural system is generally lower than from culture system. The best captural systems have yielded an average of approximately 135 kg.ha^{-1}

(HORA and PILLAY, 1962). ALI (1990) reported a maximum yield of 174.6kg.ha^{-1} obtained from a captural system in Malaysia, comprising mainly of 7 species, principally *Trichogaster pectoralis*, *Clarias macrocephalus* and *Channa striata*. According to MOULTON (1973) fish harvests in capture systems can be improved by introduction of the culture species *T. pectoralis* (sepat siam).

1.3.2.2 Culture system

In the culture system, the main feature is the production of stocking material, i.e eggs, fry, fingerlings or on–growing, and the subsequent rearing of that material in the field until it becomes suitable for consumption or as a market able commodity. The method of rearing varies, principally according to water availability, fish species, rice plant variety and traditional customs (LITTLE and MUIR,1987). The yields depend to a large extent on the species stocked, the culture period, the fertility of soil and water, and the degree of supplemental feeding. Acceptable rice yields (3 to 5 ton.ha^{-1}) and fish yields (200–300 kg.ha^{-1}) have been obtained from rice–fish culture (COCHE,1967; GROVER,1979; RUDDLE,1980).

Both monoculture and polyculture are practised in rice field culture. Monoculture of common carp is prevalent in Indonesian wet rice fields, while polyculture is important in China and the Philippines (KANGMIN,1988; DELA CRUZ *et al.*, 1980). According to SCHUSTER (1955) the best fish culture region of the tropics (between 20° north and south of the equator), lies between 1000 and 1500 meters above sea level, where practically every well–irrigated rice field can be stocked with fish. At lower altitudes, the possibilities for successful cultivation of fish in rice fields are restricted by the occurrence of carnivorous fish, high water temperatures, low oxygen concentration, and the acidity of the rice field water.

The species used in the practice also vary from country to country (TABLE 1.3). COCHE (1967) listed 14 major rice-fish culture species. LITTLE and MUIR (1987) and RUDDLE (1980) updated the number in the list to 28 and 29, respectively. According to the authors, in Southeast Asia the main species cultured in rice field are *Cyprinus carpio, Oreochromis mossambicus, Oreochromis niloticus, Trichogaster pectoralis, Osphronemus gouramy* and *Osteiochilus hasseltii*.

Fish cultivation in wet rice fields requires physical modifications of the rice field in order to accommodate fish for their proper growth and survival, without damaging the prospect of optimum rice yield. These modifications involve strengthening and increasing the height of bunds, and the creation of trenches as refuges for fish to obtain shelter during periods of high temperature or temporary draining of the rice field. The trenches can take various forms (DJAJADIREDJA *et al.*, 1980), but in general do not exceed 10–15% of the total areas of rice field (LITTLE & MUIR,1987).

1.3.3 RICE–FISH SYSTEM AS INTENSIVE NURSERIES IN INDONESIA

Traditional rice–fish culture system in Indonesia has been recognised world wide as successful, productive, diverse, dynamic and unique from both economic as well as ecological perspectives (KHOO and TAN, 1980). The system has been reviewed by numerous authors (SCHUSTER,1955; ARDIWINATA, 1957; HICKLING,1962; COCHE,1967; KHOO and TAN,1980; RUDDLE, 1980,1982; LITTLE and MUIR,1987). In 1985, rice–fish farming in Indonesia employed over 302,000 people who worked on 94,309ha of rice fields and produced 63,218 tonnes of fish. The annual average fish production from rice field in Java amounted to 804.6kg.ha^{-1} (Indonesian Directorate General of Fisheries, 1984). Rice–fish farming system in Indonesia traditionally plays an important role in the supply and distribution of fish seed for further on–growing, and is an essential part of the aquaculture production net work in the rural areas of this country (COSTA–PIERCE,1988). Basically, this rice–fish farming system can be divided into rotational and concurrent cultivation of fish and rice, using three main types of method the details of which can be summarised as follows :

1.3.3.1 The alternate crop method or *palawija*.

In the *palawija* method, fish are reared during the what would otherwise be a fallow period after a single annual crop of rice has been harvested. This is the oldest method of rice fish culture, and has a wide diversity of different stocking and harvesting patterns. The rearing period can vary between 30 and 120 days, depending on the size of fish harvested. The *palawija*, however, were traditionally more oriented toward the production of 125–200g common carp for local consumption. *Palawija* is the most productive rice–fish culture method in Indonesia, as well as in Thailand, Japan and Vietnam (ARDIWINATA,1957; COCHE,1967; MIDDENDORP,1985). The traditional 3 month rearing period *palawija* produces fish yield of 600kg.ha^{-1} in fertile areas, 300kg.ha^{-1} in moderately fertile areas and 100–200kg.ha^{-1} in less fertile areas (ARDIWINATA,1957).

1.3.3.2 The intermediate crop method, or the *panyelang*.

This method offers the cultivation of fish between two rice crops for a period of 30–40 days. Rice farmers practice the *panyelang* system while waiting for rice seedlings to grow in seed beds and be transplanted to rice fields (which generally takes 28–30 days, depending on the variety of rice), or while waiting to get rice seedlings from elsewhere. Due to the shorter rearing period of the *panyelang* method, the rice field is used as nursery, producing carp fry of 3 to 5cm or 5 to 8cm body length, which they sell to pond owners or to other rice farmers for the purpose of stocking. The average yield is 40 to 60kg.ha^{-1} (KHOO and TAN,1980), but fish yields of 75 to 80kg.ha^{-1}, can often be obtained.

1.3.3.3 The concurrent crop method, or the *mina–padi*.

In this method, fish can be cultivated with rice in three successive periods; (i) a few days after rice transplanting , until the first weeding, about 3 weeks later; (ii) a few days after the first weeding until the second weeding, about 3–4 weeks later; and (iii) a few days after the second weeding until the flowering of rice plant, which depending upon the requirement of watering and drying period for the good growth and maturing of rice, may take 3 to 6 weeks. Thus, in the *mina–padi* system, a total fish rearing period of at least 60 days can usually be obtained. The average production, which depends on the rearing period used, varies from 150kg.ha^{-1} to 300kg.ha^{-1}, consisting of fry (3–5cm body length), large fingerling (8–12 body length) or on growing size (60–100g body weight).

1.3.4 CONSTRAINTS AND FUTURE PROSPECT

In many parts of the world interest in producing rice and fish together has declined in recent years. One of the many factors causing this decline is the adoption of modern agrotechnology in rice production (the so-called Green Revolution) in Asia, in which the incorporation of high yielding rice varieties and large amount of pesticides and fertilisers are the main components. High yielding rice varieties have a short to medium growing period (110 days), allowing rice fields to be planted twice a year or even 5 times in two years, which in turn results in shorter period of fish rearing (MOULTON,1973; GROVER,1979; PULLIN,1986; ALI,1990). The use of greater amount of toxic pesticides have been recognised to be hazardous to fish in rice fields and present a serious problem to the development of rice-fish farming system (KOESOEMADINATA,1980, KHOO and TAN, 1980). The effect of excessive use of inorganic fertilisers to fish in rice fields has not been studied in detail.

However, the growing interest of scientists and rural development administrators in integrated farming system has recently stimulated a re–evaluation of the potential of rice field fisheries for low income rural and urban population and ways to deal with problems associated with the farming system (MIDDENDORP and VERRETH,1986; SOLLOWS and TONGPAN,1986).

1.4 THE USE OF INSECTICIDES IN RICE–FISH CULTURE

The introduction of modern high–yielding rice varieties, with shorter growth duration, and factors such as high tillering of varieties, closer spacing, and more intensive use of fertilisers are believed to be responsible for an increase in severity of damage by rice insects. Yield losses due to rice insect pests in Asia are estimated to be about 30%. Despite recent significant advances in the development of insect–resistant rice plant varieties, insecticides remain a common control method for rice pests (CRAMER,1976). Unfortunately, the extensive use of these agrochemicals has created considerable environmental problem (WILLIS and McDOWELL,1982). The adverse effects of pesticides use in agriculture to inland fisheries has been recognised as a worldwide scale problem (FAO,1964). Insecticide application in wet rice field has been reported to caused fish mortalities in several Southeast Asian countries, including Thailand, Indonesia and Malaysia (BOONBRAHM,1975 *in* MIDDENDORP,1985; KOESOEMADINATA,1980;YUNUS and LIM,1971). Since most pest control requires at least 3 to 4 applications of insecticides in one growing season, fish culture in wet rice system can be severely hampered . Furthermore, low level agrochemicals may effect fish feeding habit and reduce the availability of natural food for fish in the rice fields, thereby reducing fish growth and yield (ARUNACHALAM *et al.*,1980; TAKAMURA and YASUNO,1986; MUIRHEAD–THOMSON,1988).

Many rice insecticides, particularly organochlorine compounds, such as endrin, dieldrin, DDT, endosulfan and BHC, are toxic to fish and are persistent in rice field water and soil (MURTY, 1986; EDWARDS, 1977; MOULTON,1973), and thus, rice farmers health may be at risk through consumption of contaminated fish (MEIER *et al* .,1983). Aquaculture within the irrigation system is also at risk from contamination by large scale pest control operation using persistent insecticides (WAUCHOPE,1978, GORBACH *et al.*,1971).

Most agrochemicals are not applied to target areas as a pure material (i.e active ingredient or A.I), but rather in a formulation, i.e. a prepared mixture to give proper result in pest control (VOLKENBURG,1973). This formulation can be emulsifiable concentrate (EC), wettable powder (WP), granulated preparation, dust or aerosol. According to KOEMAN (1974), the can be influenced markedly by the type of formulation employed. Oily formulation can reduce the chance of appreciable runoff, while granular formulation allows gradual release of the chemical into rice field water over a long period, which can be disadvantageous to rice–fish culture. The author further stated that the danger of runoff or leaching of insecticides depend upon their water solubility and rate of application. High solubility generally implies that a chemical will be very mobile in irrigated rice fields. High solubility also gives quick dilution to a level non–toxic to fish.

The use of some insecticides in rice fields, were found to be compatible with fish culture, because of their long stability, low fish toxicity and low effective dose rate for insect pests. According to ESTORES *et al.* (1980), *carbofuran*, a broad spectrum systemic insecticide and nematicide, was found to be readily degradable by hydrolysis and converted to water soluble metabolites, which are the less toxic. Therefore, *carbofuran* can be used safely in rice–fish culture system, provided it is applied at least 7 days before the stocking of fish. Broadcasting the granular form of *carbofuran* directly into fish bearing rice field, however, will cause fish mortalities. The author also reported that *carbofuran* as a water soluble compound is not accumulated in fish or persistent in crops or soils.

Based on the results of field trials, CROSSLAND (1982) and STEPHENSON *et al* (1984) reported that *cypermethrin,* a pyrethroid insecticide, was potentially less harmful than *carbofuran* to fish in wet rice fields.

According to PULLIN (1980) insecticides derived from natural sources, such as seeds from Neem tree (*Azadirachta indica*) were non toxic to fish, and have an encouraging prospect as "rice–fish culture insecticides". SPILLER (1985) also stated that there is a trend towards development of pesticides which are less harmeful to fish, which may encourage future wide spread development of rice–fish farming.

1.5 OBJECTIVES AND JUSTIFICATION OF THE RESEARCH

The extensive use of insecticides to control insect pests in rice is a major constraint to fisheries in wet rice system. One way of dealing with the problem is that the use of highly toxic insecticides to fish and aquatic food organisms for fish, should be allowed to be discouraged. For this purpose the development of selective insecticides for use in rice fish culture should be considered. This will need implementation of an effective and sound toxicity screening procedure in both laboratory and field conditions.

The objectives of the present research are as follows :

(a) to determine the toxicity of five selected rice insecticide formulations to fish in laboratory and under field conditions, to predict the potential impact these insecticides on fish and fisheries production in wet rice ecosystems;

(b) to evaluate the applicability of laboratory based toxicity tests procedures to rice field conditions, ;

(c) to develop an appropriate laboratory and field–based experimental methodology for the assessment of insecticide toxicity to fish, in order to be able to screen " rice–fish culture insecticides" to be used safely in rice–fish farming systems.

TABLE 1.3. Principle fish species cultured or captured in rice fields.

Country	Fish species	Ref
China	*Aristichthys monilis, Carrasius auratus, Ctenopharyngodon idellus, Cyprinus carpio, Hypothalmichthys molitrix.*	1
India	*Anguilla japonica, Catla catla, Channa striata, Chanos chanos, Cirrhina mrigala, Clarias batraacus, C. carpio, Labeo rohita, Lates calcacifer, Mugil sp., Mystus gulio, Ophicephalus striata, Oreochromis mossambica, Osphronemus gourami.*	2
Indonesia	*Anabas testudidineus, C. chanos, C. batrachus, C. carpio, Fluta alba, Helostoma temincki, O. striata, O. mossambicus, O. niloticu, Osphronemus gourami, Osteochillus hasseltii, Puntius gonionotus, Trichogaster pectoralis, Trichogaster trichopterus.*	3
Japan	*Anguilla japonica, Carrasius auratus, Cambarus clarkii, Clarias batrachus, Cyprinus carpio, Helostoma temincki, Trichogaster pectoralis.*	4
Malaysia	*Anabas testudidineus, Clarias batrachus, C.macrocephalus, Ophicephalus striatus, Oreochromis mossambicus, Trichogaster pectoralis, T.trichopterus.*	5

TABLE 1.3 continued

Country	Fish species	Ref
Philippines	*Anabas testudidineus, Helostoma temincki, Ophicephalus striatus, Oreochromis mossambicus, Osphronemus gourami, Trichogaster pectoralis.*	6
Thailand	*Anabas testudidieus, Helostoma temincki, Ophicephalus striatus, Oreochromis mossambicus, Trichogaster pectoralis.*	7

REFERENCES

1. KANG-MIN (1988)
2. JINGHRAN (1985), LITTLE & MUIR (1987)
3. ARDIWINATA (1955), RUDDLE (1988)
4. SINGH *et al.* (1980), YOSHIHIRO *et al.* (1958)
5. SOONG (1954), MOULTON (1973)
6. ARCE (1977), LITTLE & MUIR (1987)
7. PONGSUWANA (1962), LITTLE & MUIR (1987)

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

The study was split into laboratory and field-based experiments. The laboratory study comprised of a series of static toxicity tests, followed by a series of field trials in specially designed rice field plots. The static toxicity tests were performed in the laboratory of the Research Institute of Freshwater Fisheries in Bogor, Indonesia. The main purpose of the toxicity test was to determine the acute (lethal) toxicity of the insecticides to fish. Field trials were conducted on an experimental rice field unit which was set up on mature rice farm land, located at the village of Ciherangpondok, Ciawi, Bogor, Indonesia. The total area of the experimental rice field unit was approximately 1150m^2. The water source was an irrigation channel which was located about 500m from the rice field plots. Most of the land surrounding the experimental site was used for agriculture (mainly rice and vegetables) and family homes.

2.2. INSECTICIDE SAMPLES

The insecticide used in the experiments were commercial products commonly used to control insect pests, such as planthoppers, stemborers and gall midges, in wet rice fields in Southeast Asia. The samples included two carbamates, one organophosphate, one thiadiazin and one pyrethroid compound, and were procured from manufacturers in Indonesia. The commercial products of these insecticides have different formulation and active ingredient content. The active ingredient content of each product has been verified in the pesticide analytical laboratory of the Indonesian Pesticide Committee. All data presented in this thesis, unless otherwise stated, were based on active ingredient rather than on formulated product. Further information on the insecticide products is presented in Table 2.1 *Fenobucarb* and *diazinon* are relatively more soluble in water (solubility 89mgl^{-1}and 40mgl^{-1}, respectively). The nominal concentrations of these compounds in the rice fields in the experiments would have been higher than those of *isoprocarb, buprofezin* and *alphamethrin* (solubility less than 1mgl^{-1}).

TABLE 2.1. List of insecticide formulations used as test materials in the experiments.

1 . COMMERCIAL NAME : BAYCARB 500EC
COMMON NAMES : *fenobucarb, BPMC*
CHEMICAL NAME : 2 -sec butylphenyl methyl carbamate
FORMULATION : emulsifiable concentrate (EC)
A.I. CONTENT : 495gl^{-1}
PHYSICAL & CHEMICAL PROPERTIES : solubility in water, 89mgl^{-1}(at 25^{o}C), 660mgl^{-1}(at 20C), more soluble in organic solvents such as acetone, benzene and xylene, K_{ow}620
stability >0.5y at 20°C, except under strong alkaline and strong acid condition. Hydrolysis DT_{50}> 28d (at pH2),16.9d (at pH9), 2.06d (at pH10).
soil K_{om}125 -661 (5.2%-1.8%o.m.);DT_{50} 6-30d (paddy soil),6-14d (upland condition); melting point (m.p.) 32°C, vapour pressure (v.p.) 1.6mPA (20°C)
TOXICOLOGY : LD50(oral) for rats 623mgkg^{-1}, LD50(dermal) for mice >5000mgkg^{-1}
LC50(48h) for carp 12.6mgl^{-1}
MANUFACTURER : Bayer Agrochemicals Ltd.
EFFECTIVE AGAINST : brown plant hopper (*Nilaparvata lugens*)

2 . COMMERCIAL NAME : MIPCIN 50WP
COMMON NAMES : *isoprocarb, MIPC*
CHEMICAL NAME : O-cumenyl methyl carbamate
FORMULATION : wettable powder (WP)
A.I. CONTENT : 50%
PHYSICAL & CHEMICAL PROPERTIES : solubility, insoluble in water, readily soluble in acetone and methanol
stability, hydrolysed in alkaline condition
TOXICOLOGY : LD50(oral) for rats 178mgkg^{-1}, LD50(dermal) for rabbits >10250mgkg^{-1}
LC50(48h) for carp 4.2mgl^{-1}
MANUFACTUIRER : Mitsubishi Chemicals Industries Ltd.
EFFECTIVE AGAINST : brown plant hopper (*Nilaparvata lugens*)
whitebacked plant hopper (*Sogatella furcifera*)
striped plant hopper (*Inazuma dorsalis*)

3 . COMMERCIAL NAME : APPLAUD 10WP
COMMON NAME : *buprofezin*
CHEMICAL NAME : 2-tert-butylamino-3-isopropyl-5-phenyl-3,4,5,6-tetra hydro-2H-1,3,5-thiadiazin-4-one
FORMULATION : wettable powder
A.I. CONTENT : 10%
PHYSICAL & CHEMICAL PROPERTIES : solubility in water (25°C) 0.9mgl^{-1}, aceton 240gl^{-1}, ethanol 80gl^{-1}, chloroform 520gl^{-1}, toluene 320gl^{-1}
stability,stable in acid and alkaline solution
m.p. 104.5-105.5°C, v.p. 1.25mPA (25°C)
TOXICOLOGY : LD50(oral) for rats 8720mgkg^{-1}, LD50(dermal) for rats 5000mgkg^{-1}
LC50(48h) for carp 2.7mgl^{-1}
MANUFACTURER : Nihon Nohyaku Co. Ltd.
EFFECTIVE AGAINST : brown plant hopper (*Nilaparvata lugens*)

Table 2.1. (Continued)

4 .	COMMERCIAL NAME	:	BASUDIN 60EC
	COMMON NAMES	:	*diazinon, kayazinon*
	CHEMICAL NAME	:	0.0-diethyl 0-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate
	FORMULATION	:	emulsifiable concentrate (EC)
	A.I. CONTENT	:	641 gl^{-1}
	PHYSICAL & CHEMICAL PROPERTIES	:	solubility in water (20°C) 40 mgl^{-1}, readily soluble in ether, ethanol, cyclohexane, petroleum ether, benzene and other aromatic solvent stability, decomposes >120°C hydrolysis (20°C), DT_{50} 11.77h(pH3.1),185d(pH7.4),6.0d(pH10.4) boiling point (b.p.) 83-84°C, v.p. 0.097mPA(20°C)
	TOXICOLOGY	:	LD50(oral) for rats 285 $mgkg^{-1}$, LD50(dermal) for rats 455 $mgkg^{-1}$ LC50(48h) for carp 7.6-23.4 mgl^{-1}
	MANUFACTURER	:	Ciba Geigy Agrochemicals, Ltd.
	EFFECTIVE AGAINST	:	wide range of rice pests
5 .	COMMERCIAL NAME	:	FASTAC 15EC
	COMMON NAME	:	*alphamethrin*
	CHEMICAL NAME	:	(1R cis)S and (1S cis)R enentiomer isomer pair of alpha-cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate
	FORMULATION	:	emulsifiable concentrate (EC)
	A.I. CONTENT	:	50 gl^{-1}
	PHYSICAL & CHEMICAL PROPERTIES	:	solubility in water (25°C) 0.005-0.01 mgl^{-1}, cyclohexane 515 gl^{-1}, xylene 315 gl^{-1}, acetone 620 gl^{-1}, cyclohexanone 515 gl^{-1},xylene 351 gl^{-1} K_{ow} 8,700,000(pH7) stability, decomposes >200°C, stable at pH3-7, hydrolised at pH12-13 m.p. 80.5°C; v.p. 170nPA (20°C).
	TOXICOLOGY	:	LD50(rats) 79-5000 $mgkg^{-1}$ LC50(96h) for rainbow trout 0.0028 mgl^{-1}
	MANUFACTURER	:	Shell Chemical Company, Ltd.
	EFFECTIVE AGAINST	:	wide range of crop pests

SOURCES : Japanese Pesticide Information (1987)
Shell Chemical Company, Ltd (1987)

Three of the insecticide compounds in the list (*fenobucarb, isoprocarb* and *buprofezin*) are currently widely used in Indonesia to control planthoppers. Another compound, namely *diazinon*, is a well known rice insecticide and is still widely used in other Southeast Asian countries, such as in Thailand and the Philippines (STARING,1984). The fifth product used in the experiment was *alphamethrin*, a new synthetic pyrethroid insecticide which is effective against a wide range of pests in economically important crops, including rice (MAUCK *et al.*,1976; HUTSON and ROBERTS,1985).

2.3. EXPERIMENTAL FISH

Fingerlings of the local variety of common carp, *(Cyprinus carpio,*LINN), were used in both the laboratory and field experiments. According to BERG (1940), taxonomically common carp fall under the following systematic classification :

Series :Pisces
Class : :Actinopterygii
Order : :Cypriniformes
Division :Cyprini

Suborder	: Cyprinoidei
Family	: Cyprinidae
Subfamily	: Cyprininae
Genus	: *Cyprinus* LINNAEUS,1758.

The common carp is a native of the temperate regions of Asia, and is one of the economically important freshwater fish in this part of the world. The Asian varieties of common carp present a wide range of thermal tolerance and can tolerate, with sufficient aeration, water temperature of 34°C. The optimal thermal range, however, is from 22°C to 28°C. Common carp can also tolerate shallow and turbid water which makes the fish suitable for rice–fish culture. In Indonesia, notably in West Java, carp fingerlings of 5 to 6cm in size are commonly reared together with rice to obtain on growing size of 8 to 15cm (see Section 1.4). Common carp is also a popular freshwater fish species for toxicological studies of pesticides, due to its general favourable characteristics as test animal (MURTY,1986; HASHIMOTO,1979; KOESOEMADINATA,1980), such as easy availability through out the year, ease in handling, caring and culturing in the laboratory.

Common carp fingerlings for the purpose of the experiments were obtained from a local hatchery owned by an experienced carp breeder in the village of Cibening, Bogor. Upon arrival the fish batch were kept in fibre glass tanks or in earthen ponds for at least two weeks for acclimation, before they were used in the experiment. The fish were fed with commercial carp pellet with a ration of 10% body weight per day. The fish population were daily checked for signs of any health problems or other abnormalities.

2.4 LABORATORY EXPERIMENTS

2.4.1 Test method

The methodology of fish toxicity testing has been described by many investigators and institutions (DUODOROFF *et al.*,1951; ALABASTER and ABRAMS,1964; SPRAGUE,1969; BUIKEMA *et al.*,1982; EIFAC/FAO,1975; ASTM,1980; APHA,1981), and these standard methods were adopted in the tests.

Standardised static toxicity tests were employed in this study. According to FERGUSON *et al.*(1966) the results obtained from static systems are reproducible when test conditions are specified. Furthermore, static tests may be more realistic in stimulating natural conditions, since pesticide concentration usually increases rapidly as the toxicant enters the aquatic ecosystem and then quickly declines by absorption. Thus, the data obtained from the test may be more relevant to pesticide problems occurring in the semi– stagnant condition of a wet rice system.

2.4.2 Test condition and procedures

The tests were conducted in 24 test containers made of fibre glass, each with dimensions of 50 x 30 x 30cm (length,width,depth). Dilution water were added to each test container (20 litres per container) and allowed to stand for 48h before introduction of fish. The dilution water used was ground water obtained from an open well, the average physical and chemical characteristics of which were as follows :

Temperature (°C)	26–28
pH	6.5–7.5
Total hardness (mgl^{-1} $CaC0_3$)	60–80
Total ammonia (mgl^{-1})	0.01–0.07
Dissolved oxygen (mgl^{-1})	6.00–8.00
Free carbon dioxide (mgl^{-1})	1.99–4.0

The above water quality parameters in the containers were daily measured at random during the course of each test.

Ten fish were selected at random from the stock population (in which fish mortality never exceed 10% of the total population and no health problem was recorded during the acclimation period), and introduced into the test container, allowing an average loading ratio of 1g fish per 1 litre of dilution water (APHA,1981). Dissolved oxygen in the dilution water was maintained above 7mgl^{-1} (70%–90% saturation) by gentle aeration.

For each test, 10 to 12 tanks were randomly allocated and a duplicated series of 5 to 7 concentrations of the test material were prepared. The concentrations were arranged in logarithmic series. Test solution of an emulsifiable concentrate formulation was prepared by mixing 1ml of the insecticide commercial product in 100ml of Analar acetone, and made up to one litre with distilled water to obtain 1000ppm stock solution. Test solutions of wettable powder formulations were prepared directly by weighing alliquots of the insecticide product with acetone as solvent, the amount of which never exceed 0.5ml per litre of solution. Dilution water, without insecticide or solvent, were used as control medium.

The toxicity of many poisons is known to be influenced by environmental condition, such as temperature, pH and water hardness (see Section 1). The presence of fish in the test medium is likely to cause gradual deterioration in the initial experimental condition, mainly due to the utilisation of dissolved oxygen and excretion of carbon dioxide and other toxic metabolites such as ammonia (SCHRECK and BROUHA,1975). For this reason aeration of the test solution was vital during the experiment. Dead fish were immediately removed from the test container to prevent fouling of test solution. In order to reduce excessive loss of the initial test concentration (mainly through sorption unto the container's walls), the test medium were daily replaced with freshly made solution, as recommended by SPRAGUE (1969).

2.4.3 Data collection and analysis

The test fish were observed periodically at the following time intervals : 3, 8, 24, 48, 72 and 96h. The experimental data were collected in the form of a record of cumulative mortality over a 24, 48, 72 and 96 hours exposure period. The data were then statistically analysed according to the Trimmed Spearman–Karber Method for estimating the values of the Median Lethal Concentrations (LC50s), and their 95% Confidence Limit Intervals (HAMILTON *et al.* 1977). The Median Lethal Concentration is defined as the concentration at which 50 percent of the test fish are killed at a certain exposure time, under specified condition of the test (ALABASTER and ABRAM,1965; BROWN,1973 and SPRAGUE,1969).

Slope functions and their 95% Confidence Limit Intervals were determined using procedure described by LITCHFIELD and WILCOXON (1949). Testing the differences between LC50 values was carried out by testing whether the slopes of the probit lines were different (p=0.05). This procedure was originally developed for pharmacology by LITCHFIELD and WILCOXON (1949) to test whether two drugs differ in potency.

To describe the empirical relationship between insecticide concentration and survival time of fish, the toxicity curve of the insecticide was drawn, by plotting the LC50 values (and their confidence limit intervals) and the period of exposure, both in logarithmic scale (BROWN,1973; SPRAGUE,1969 ABEL, 1989).

BROWN (1973) pointed out that for any poison, there will be a concentration so low that it will never cause the death of half the test fish, and thus making the toxicity curve asymptotic to the time axis. The concentration at which this occurs is termed the Threshold Median Lethal Concentration, or threshold LC50 or Incipient Lethal Level (ILL). According to SPRAGUE (1969) by using the incipient LC50 instead of one for an arbitrary time (for example 48h–LC50), the toxicity of test materials to fish can be better evaluated, and more appropriately compared.

2.5 FIELD TRIAL

2.5.1. Summary of experimental procedure

A summary of the experimental procedure is given in Table 2.2. The procedure applied to six field trials investigating the effects of insecticides on fish and aquatic biota in the rice field. Each trial was conducted for a period of 28d, during which time only one application of insecticide was given, following standard practice. Basically, the experimental procedure involved the following activities :

a. preparation of rice field soil;
b. transplanting of rice seedlings;
c. rearing of experimental fishes;
d. application of insecticide;
e. sampling of zooplankton and benthic macro invertebrates;
f. measuring of the physico-chemical characteristics of rice field water;
g. harvesting of experimental fishes.

TABLE 2.2. Summary of working schedule.

CULTURE ACTIVITY	D.A.T	SAMPLING / MEASUREMENT
1st inorganic fertilizer application	- 4	
	- 2	
Rice transplanting	0	Benthos
	+ 1	
	+ 2	Zooplankton & water quality
	+ 3	
Fish introduction	+ 4	Benthos, zooplankton & water quality
	+ 5	
	+ 6	Zooplankton & water quality
	+ 7	
Insecticide application	+ 8	Benthos, zooplankton & water quality
	+ 9	
	+ 10	Zooplankton & water quality
2nd inorganic fertilizer application	+ 11	
	+ 12	Benthos, zooplankton & water quality
	+ 13	
	+ 14	Zooplankton & water quality
	+ 15	
	+ 16	Benthos, zooplankton & water quality
	+ 17	
3rd inorganic fertilizer application	+ 18	Zooplankton & water quality
	+ 19	
	+ 20	Benthos, zooplankton & water quality
	+ 21	
	+ 22	Zooplankton & water quality
	+ 23	
	+ 24	Benthos, zooplankton & water quality
Harvesting of the experimental fish	+ 25	

NOTE : D.A.T - Days After (Rice) Transplanting

A minor modification was employed in the preliminary experiment, which dealt with the effect of chicken manure on the growth and production of fish in the rice field plots, when no insecticide was applied.

At the end of each trial the rice plants were cut down and the stubbles incorporated into the soil to decompose, following common practice of the local farmers. The land was then left fallow for a period of approximately 30d, before subsequent tilling was initiated to prepare the fields for the next experiment.

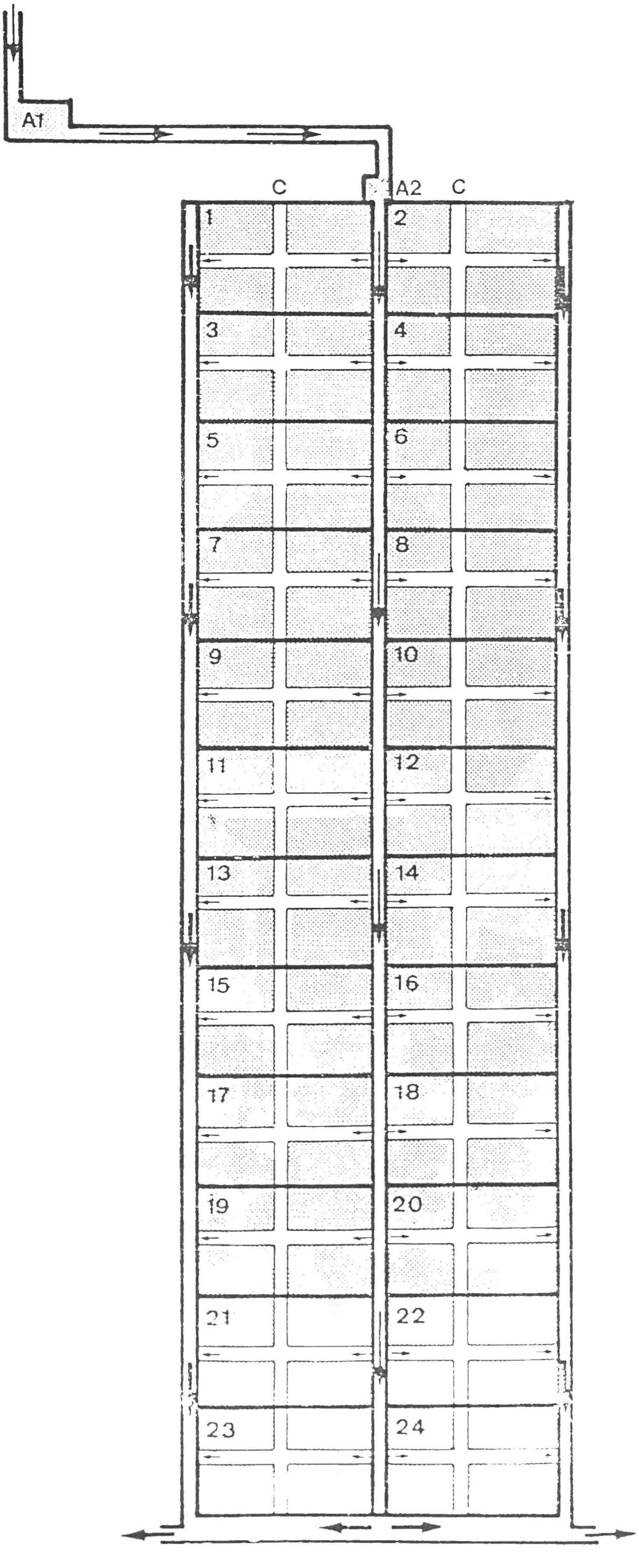
2.5.2 Time between experiments

The use of the rice fields plots for several consecutive experiments posed no problems of "carry–over" of the insecticide from one trial to another. This is primarily because the type of insecticide products used in the trials have been recognised by various investigators as chemical compounds which are unstable and do not persist in the water or the soil. It is generally known that organophosphate and carbamate compounds are less persistent in the aquatic environment, compared to the organochlorines (see Section 1.1). MILLER *et al.* (1966) reported that after the application of ^{14}C *diazinon* and *ethyl parathion* in the field at a rate of 5 and 1 lb per acre, respectively, both pesticides disappeared from water within 114h. BRAHMAPRAKASH and SETHUNATHAN (1985) studied the metabolism of two carbamate insecticides in soils, planted and unplanted with rice under flooded and non flooded conditions, and found out that the compounds decreased significantly within 30d after application in all systems. The decrease was more pronounced in the planted soils. Studies conducted in Indonesia and the Philippines disclosed that carbamates and organophosphates rice insecticides were degraded in less than 10d in the rice field water (De la CRUZ,1986; ARCE and CAGAUAN,1988). Pyrethroid insecticide was also found to be rapidly lost (less than 7d after application) in wet rice system, probably as a result of several processes, including hydrolysis and biodegradation (STEPHENSON *et al.*,1984; CROSSLAND,1982).

Based on the above information a time span of 30 days between experiments was regarded as sufficient to prevent or minimised any problem of "carry–over" of insecticide residues from one trial to another.

2.5.3. Rice field plots

The experimental rice field unit comprised 24 plots of equal surface area of $40m^2$ (8x5m), in a mature arable land with alluvial soil. The plots were arranged in two rows and allocated in random for insecticide treatments (Plate 2.1). According to HEINRICHS *et al.* (1981) plot size for agronomic experiment may vary from 25 to $100m^2$, depending on the size and shape of the field. At the International Rice Research Institute in Los Banos, the Philippines, for example, the plot size is normally $32m^2$ (dimension: 8m x 4m). There is no reference known for a standard plots size for a rice–fish culture experiment, and the slightly larger size adopted in the trials was considered acceptable.



Lay-out of the experimental rice field system

Physical modifications were made to the rice field plots in order to accommodate the culture of fish together with rice. Surrounding dikes were strengthened and raised to approximately 40cm above water level. Plastic sheets were applied in the inside of the dikes to prevent seepage of water between plots. In each plot two trenches, which formed a cross in the middle of the plot, were dug. These trenches were approximately 40cm wide and 30cm deep. The total area of these two trenches was approximately 12% of the plot area (Plate 2.2 and 2.3).

The above modifications of the rice field plots were essentially in accordance with the techniques of rice–fish culture commonly practised by traditional farmers in West Java (see Section 1.4).

Surface water which came from an open irrigation channel located approximately 500m from the rice field area, was used to irrigate the experimental plots. In order to minimise the suspended solid content in the water, a silting pond unit was set up,approximately 5m from the main ditch of the experimental plots. The dimension of the two silting ponds was 2.5m x 2m and 2.5m x 1m, respectively. Water was supplied independently into each plot by means of a main ditch and inlets made of PVC pipes (diameter 3/4in). Wire screens were provided to prevent the entry of wild fishes and other predators into the ice field plots. L shaped PVC pipes (diameter: 2in) were used to regulate the outflow and water level in the rice field plots. The open end of the pipes in the plot were provided with wire screen to prevent the escape of fish. The outflowing water from the plot ran over the top of the pipe into the drainage trench (Plate 2.4). Water flow into each plot, was controlled to maintain a water depth of approximately 10cm. The flow rate varied between 8 to 12 litres per minute per $40m^2$, depending the porosity of the rice field plot (giving a residence time of approximately 8–10h).

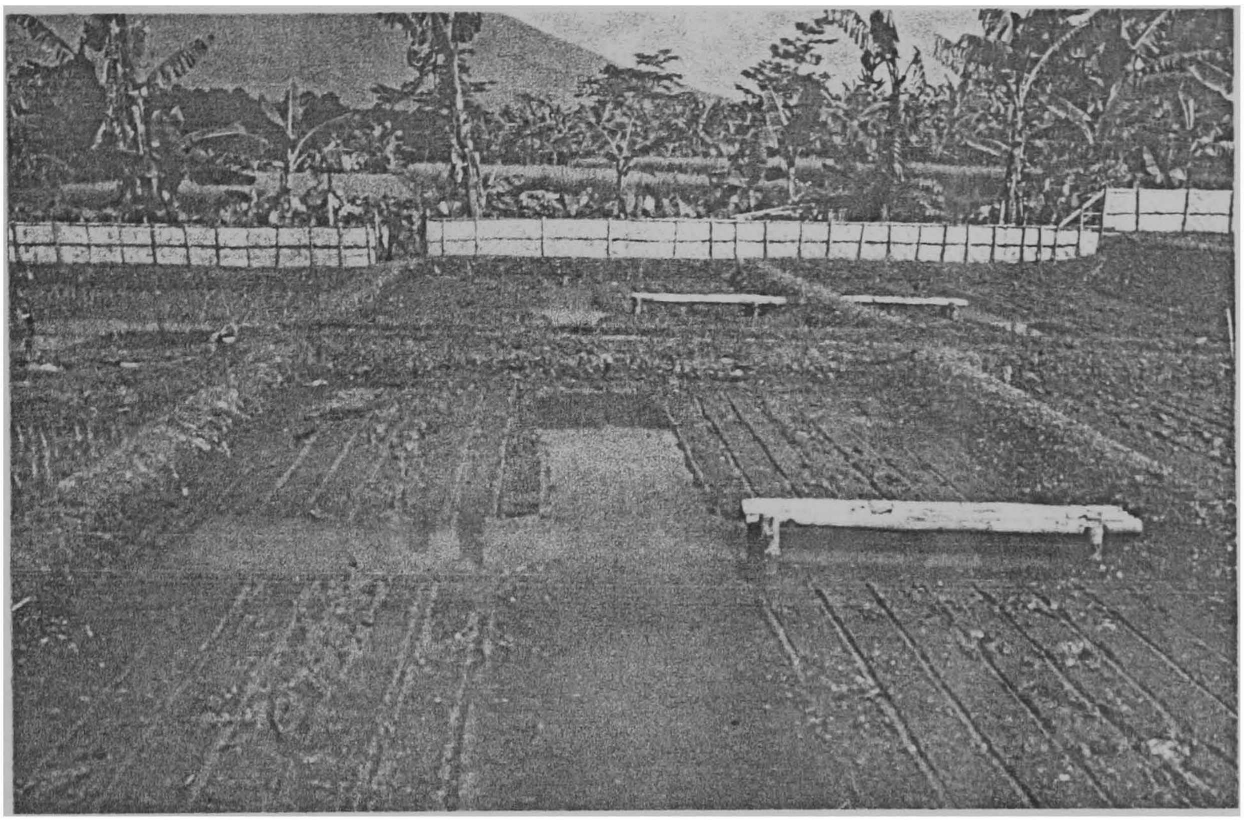


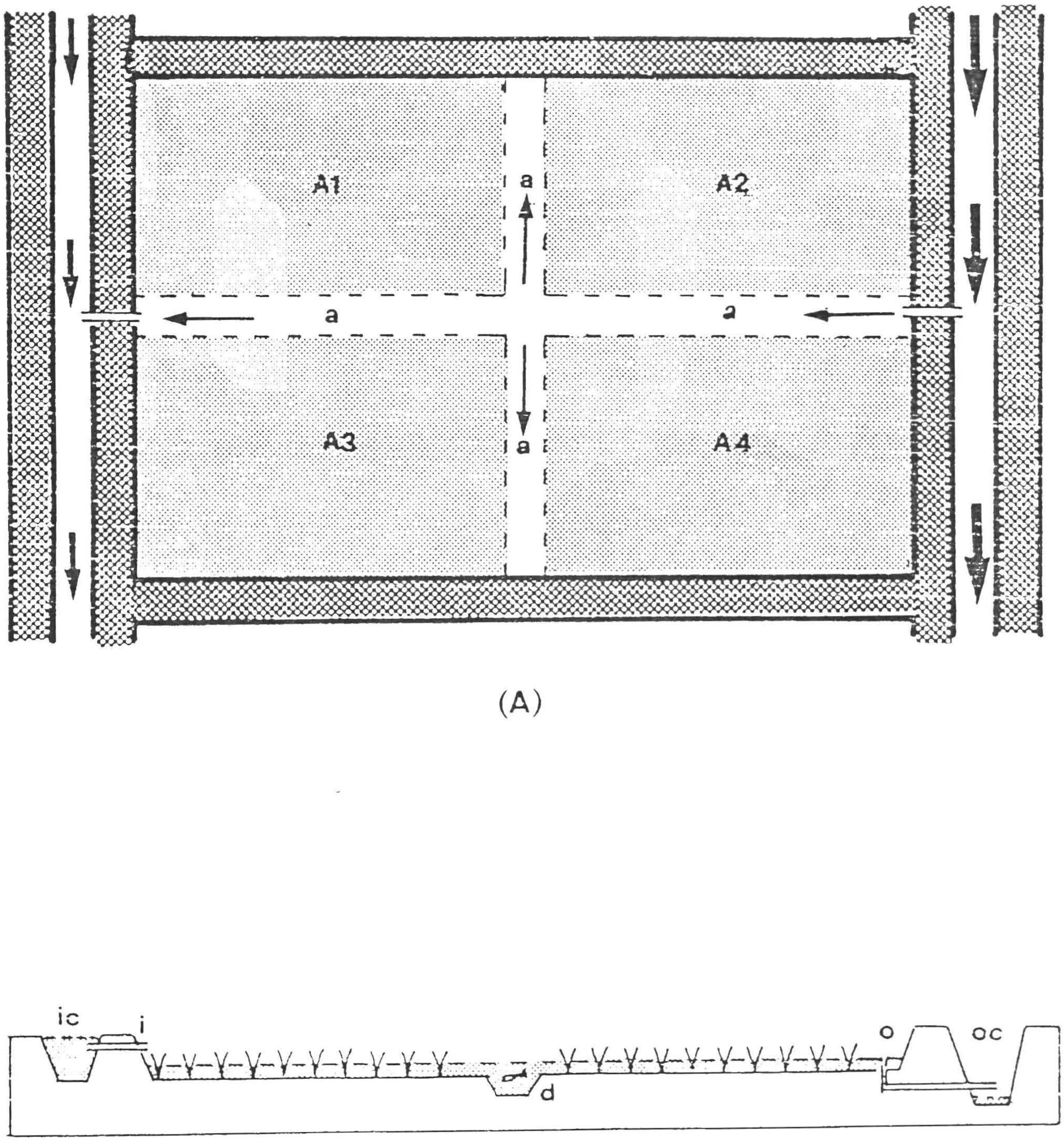
PLATE 2.2. Preparation of the experimental rice field plots.

Plate 2.3. The experimental rice field plots with rice seedlings.

2.5.4. Soil preparation

Land preparation was carried out to mix organic materials with soil, forming a hard layer which reduced water and leaching losses, and was completed by puddling the soil into mud to facilitate the transplanting of rice. Inadequate land preparation could have caused serious weed problems, and exposed plants and fish to the harmful effects of decaying organic matter in the soil.

In the present experiments the land was prepared at least 15 days before transplanting. Wet land preparation was employed, which according to SINGH (1980) is the traditional practice in most tropical Asian countries. The rice field was flooded until the water was soaked into the bottom soil of the land. The land was hoed wet and harrowed until the soil was well puddled. The last harrowing was done at least a day before planting, to puddle and level the field. The fields were then kept flooded until transplanting was carried out, to minimise the loss of nitrogen released by decomposing organic matter.



(A)

(B)

PLATE 2.4. Schematic diagram of a rice field plot, (Scale 1:80)

2.5.5 Fertilizer

The optimum quantity, method and timing of fertilizer application depends on many factors, such as variety of rice, its maturation period, soil and climatic conditions, and the management of irrigation water (GRIST,1975). The application of fertilisers to the experimental plots was therefore carried out following local agronomic practice. Inorganic fertilisers are given three times during one rice crop period, according to the following application schedule :

1st application : before transplanting

100 $kg.ha^{-1}$ Urea, 150 $kg.ha^{-1}$ Triple Super Phosphate (TSP) and 50 $kg.ha^{-1}$ Potassium Chloride (KCl), which were equal to 45.2 $kg.ha^{-1}$ N, 67.3 $kg.ha^{-1}$ P_20_5 and 12.8 $kg.ha^{-1}$ K_20_2, respectively.

2nd application : 15–20 days after transplanting

100 $kg.ha^{-1}$ Urea, which was equal to 45.2 $kg.ha^{-1}$ N.

3rd application : 40–50 days after transplanting

100 $kg.ha^{-1}$ Urea and 50 $kg.ha^{-1}$ KCl, which were equal to 45.2

kg.ha^{-1} N and 12.8 kg.ha^{-1} K_20_2, respectively.

The 3rd application of inorganic fertilisers was not given during the present experiments, since the experiment terminated 25 days after the transplanting of the rice .

Nitrogen is the key element to increase yield in rice. Nitrogen in the form of ammonia stimulates the early stages of rice plant growth, while in the form of nitrite nitrogen it promotes growth in the later stages of the plant (GRIST,1975). Urea was widely use in rice agronomy as a source for nitrogen because it has the advantage of a high nitrogen content (42–46%), and an easy to use granular formulation.

Rice plants need phosphorus in the early stage as well as in the later stage of development, and therefore remove a great quantity of this element from the soil. Phosphorus is also necessary for the establishment of natural fish food in the wet rice field.

Potassium is generally required in smaller amounts, because rice field soils usually contain adequate quantities of this element.

In order to enhance the optimum growth and production of fish, organic fertilizer in the form of chicken manure was added to the rice field plots. According to LITTLE and MUIR (1987), organic wastes are valuable sources of nutrients for authrophic production and as substrates for the heterotrophic community in natural ponds. Studies by SCHROEDER (1978) indicated that higher fish yield could be attained by using organic wastes (more than $30kg.ha^{-1}d^{-1}$) as compared to using inorganic fertilizer alone

The chicken manure dose rate used in the current experiments was initially evaluated in a preliminary trial. This part of the study was presented in CHAPTER 4 of the thesis.

2.5.6. Rice planting

High yielding variety of rice, known as the Cisadane variety was grown in the seed bed for approximately 28 days. The rice variety has a maturation period of 115 to 135 days, producing two crops a year or even 5 harvests in two years. The medium maturity period of this rice variety also contributes sufficient time for rice farmers to practice concurrent rice and fish culture. The Cisadane variety, therefore, has gained popularity with the local rice farmers. The variety is also highly recommended by the agricultural authority of the Indonesian government, because of the high yielding charateristics.

Planting of rice was done by transplanting seedlings taken from the seed beds. The rice seedlings were transplanted x 25cm (HEINRICHS *et al.*,1981). Planting distance has its impact on weed growth, which in turn will also effect the production of rice and fish (GRIST,1975; SURYANI *et al.*,1987).

2.5.7 Fish rearing

Common carp fingerlings procured from a local fish hatchery as described in Section 2.3, were held for a period of at least 7 days in a holding pond located close to the experimental plots, to allow acclimation to local conditions. During the holding period the fish were fed with commercial carp pellets with a feeding rate of 10% per body weight per day. The fish were checked daily for the occurrence of disease and physical abnormalities. They were then graded by hand to obtain uniform size, while slightly sedated using 100ppm of benzocaine (ROSS and GEDDES,1979), and individually weighed using a battery operated Mettler balance to the nearest 0.1g. The standard length of each fish was also measured and recorded to the nearest 0.1cm. Batches of twenty fish were then allocated at random to the rice plots, equivalent to a stocking density of 5000 fish per ha (20–25kg.ha^{-1}). This stocking density is slightly lower than the 30–50kg.ha^{-1} those usually adopted by traditional rice farmers in West Java, (see Section 1.4), in order to ensure optimum growth rate of fish during the experiments.

Following stocking, the experimental fish were raised in the rice field plots for 21 days without supplementary feeding, in accordance with traditional rice–fish culture practice.

At the end of the experiment all fish were harvested, counted, individually weighed and measured. The survival rate of the fish in each plot was determined by the formula :

Survival rate (%) = $N_t / N_o \times 100$, where

N_t – total number of fish harvested

N_o – total number of fish stocked

Increment of total weight of fish biomass was calculated according to the formula :

Weight gain (gm^{-2}) = $W_t - W_o$, where

W_t = final total weight of fish biomass harvested (gm^{-2})

W_o = initial total weight of fish biomass stocked (gm^{-2})

The mean growth rate of individual fish was expressed in absolute growth rate (or absolute weight increment) per day based on the formula according to RICKER (1979) :

Growth rate (gd^{-1}) = $(W_o - W_t) / (t_2 - t_1)$, where

W_t = the final mean weight of the experimental fish (g)

W_o = the initial mean weight of the experimental fish (g)

$t_2 - t_1$ = the rearing period (days)

2.5.8. Experimental design and treatments

The treatments comprised of different insecticide dose rates, applied to rice field plots both with and without fish. Thus, in each experiment the following eight treatment regimes were used :

A. Control, no insecticide,
(in plot without fish)

B. Control, no insecticide,
(in plot with fish)

C. Insecticide,1/2x recommended dose rate,
(in plot without fish)

D. Insecticide,1/2x recommended dose rate,
(in plot with fish)

E. Insecticide,1x recommended dose rate,
(in plot without fish)

F. Insecticide,1x recommended dose rate,
(in plot with fish)

G. Insecticide,2x recommended dose rate,
(in plot without fish)

H. Insecticide,2x recommended dose rate,
(in plot with fish)

The objective of having treatments in plot without fish was to obtain clear results on the effects of insecticide application to the rice field biota. In plots with fish the greater part of the biota was expected to be consumed by the fish.

The treatments were allocated to the plots in a completely randomised design, with three replicates. The dose rates in the treatment regime were most likely to be used by the traditional rice farmers in West Java. The lower dose is usually used when the rice pest infestation is low, to economise the cost of pest control. However, when severe pest infestation is anticipated, a higher dose up to 2 or 3 times of the recommended rate may be used by the farmers to protect the rice crops. These dose rates were also recommended in a standard procedure for testing the toxicity of fish in rice fields (Indonesian Pesticide Committee ,1983).

2.5.9 Insecticide application

Insecticide was applied in accordance with recommended practice in rice agronomy studies (HEINRICHS *et al.*1981): a single application of insecticide was given during each experiment, which was on the 8th day after the transplanting of rice seedlings.

The insecticides tested included two commercial formulations. i.e the emulsifiable concentrate (EC) and the wettable powder (WP), which were in liquid and solid forms, respectively. Both formulations were applied as foliar sprays, which is a common method of application in rice.

To calculate the quantity of insecticide formulation with the recommended rate based on active ingredient (AI), the following equation was used :

$$\text{Amount of commercial formulation (kg.ha}^{-1}\text{)} = \frac{\text{Recommended rate (kgha}^{-1}\text{, AI) x Area (ha) x 100}}{\text{\% A.I. in the commercial formulation}}$$

The insecticide was applied by means of a manual knapsack hand sprayer, using a spray volume of 500 litres ha^{-1} To increase the accuracy of insecticide distribution, two passes were made over each rice field plot. In accordance to the common practice the spraying was done in the morning, when temperatures were generally lowest. Water in the plots was held stagnant during the spraying of the insecticides until the next morning, according to recommended local agricultural practice, to prevent immediate flushing of the insecticide from the rice field.

2.5.10. Zooplankton samples

Samples of zooplankton were collected at two-day intervals from one rice field plot of each treatment regime. Twenty litres of rice field water was collected from 4 points in the sampling plot (5 litres per point), and concentrated in a plankton net of mesh size 10 (120 micrometer) into 25ml plastic sample bottles. The concentrated samples were preserved in 10% buffered formalin for later analysis in the laboratory. Zooplankton counts were made with three 1ml subsamples which were counted in 1ml Sedgwick-Rafter slide at 40x magnification.

Identifications were usually to species. However, in some cases this was not always possible due to the condition of the preserved materials, *Asplanchna*, for example, shrinks considerably during preservation, making identification difficult. Identifications were made based on keys described in various references, mainly WARD and WHIPPLE (1966), PENNAK

(1953), MACAN (1959), NEEDHAM and NEEDHAM (1960), and IDRIS, (1982.)

2.5.11. Benthic macroinvertebrate samples

Benthic animals were collected 11d, 3d and 1d before and 4d, 11d and 18d after the application of the insecticide. The benthic samples were collected from the same sampling plots where zooplankton samples were also taken. Four samples were collected from 4 different points in the sampling plot. The samples were collected using a simple corer with a cross–sectional area of 19.64cm^2, and a tube length of 40cm. The tube was made of 2mm transparent hard plastic tube. The corer was embedded approximately 15cm deep into the rice field soil. According to PATERSON and FERNANDO (1971), benthic invertebrates in shallow lentic habitats are effectively sampled with a simple corer. By using this corer, they found out that the density of the benthic animals were about 50% higher than when sampled with Ekman grab. The use of corer also decreased the volume of sediment collected. The use of the Ekman grab, on the other hand, might damage the rice plants in the sampling plots, and could caused disturbance to the system.

The samples were washed in the field using a 500µm mesh wire sieves, then preserved in 10% buffered formalin, and taken to the laboratory for sorting, counting and identification. The animal specimens were subsequently preserved in 70% ethyl alcohol, and examined under a dissecting microscope at 10x or 40x magnification.

Identifications were made using keys described by WARD and WHIPPLE (1966), and PENNAK (1953). Specimens of chironomid larvae were mounted on microscope slides, with the head capsule removed from the body, using poly-vinyl lactophenol as a mounting medium. The slides were dried on a hot plate for 2 or 3 days. Identification was made using keys described by WIEDERHOLM (1983) and JOHANNSEN (1933).

2.5.12 Water quality measurement

The physical and chemical characteristics of the rice field water were measured periodically, following the analytical procedure as described by STIRLING (1985), GOLTERMAN *et al.*(1978) and BOYD (1979).

Water temperature, dissolved oxygen concentration, pH, free carbon dioxide concentration and water flow were measured every two days directly in the field. The measurements were taken at 0900–1100am. Temperature (in °C) was measured to an accuracy of ± 0.1° C, using a mercury thermometer.

Dissolved oxygen concentration was measured using a YSI (Yellow Spring Instrument) Model 51 Oxygen Meter, to an accuracy of $\pm 0.1 mgl^{-1}$.

pH was measured using Bibby pH meter to an accuracy of ±0.01 pH units.

Free carbon dioxide concentrations was determined by titration of standard alkali to the turning point of phenolphthalein indicator (STIRLING,1985), expressed in mgl^{-1} with precision of about 2%.

Water flow into each plot was measured by means of "bucket method", expressed in litres per minute (FAO,1981).

Total hardness, total alkalinity, nitrite nitrogen, total ammonia, total phosphorous concentrations, and suspended organic were measured weekly. Water samples for these analyses were collected in 1 litre plastic bottles, and were taken to the laboratory in cool boxes packed with ice, to be analysed immediately or stored overnight at –15°C. Measurements of total hardness and total alkalinity were carried out directly in the field.

Total hardness was measured by the EDTA titrimetric method, and expressed in mgl^{-1} $CaC0_3$ (STIRLING,1985).

Total alkalinity was measured by the HCl titrimetric method, and expressed in mgl^{-1} $CaC0_3$ (STIRLING,1985).

Nitrite–nitrogen concentration was measured by the sulfanilamide based colorimetric method, expressed in mgl^{-1} (MACKERETH *et al.*,1978).

Dissolved reactive phosphorous was measured by means of the molybdate based spectrophotometric method, expressed in mgl^{-1} (GOLTERMAN *et al.*, 1978; STIRLING,1985).

Suspended solids were collected and measured using a gravimetric method as described by STIRLING (1985), expressed in mgl^{-1} to an accuracy of $\pm 0.1 mgl^{-1}$.

2.5.13 Statistical analysis

The experimental data were processed and analysed using analysis of variance techniques at 95% confidence level, by means of Minitab Release 6.1 statistical computer package (Penn State University of Winconsin).

Significant differences between treatment levels were tested using Duncan's Multiple Range Test (SOKAL and ROHLF,1981; DUNCAN,1955). The experimental data were appropriately transformed, if necessary, before analysis.

CHAPTER 3

THE DETERMINATION OF ACUTE LETHAL TOXICITY OF FIVE RICE INSECTICIDES TO COMMON CARP FINGERLINGS (*Cyprinus carpio* LINN.) IN THE LABORATORY

3.1 INTRODUCTION

Pesticide companies, as part of the registration process of product development, generally determine toxicity levels of insecticide compounds to fish. However, the species used in the tests are usually different from those used in rice fish culture, and the data are thus generally not applicable to the potential environmental problems arising from the use of these chemicals in the wet rice ecosystem. It is therefore usually necessary to evaluate the toxicity of new pesticide products which are intended to be used for controlling rice pests to economically important fish in rice field waters such as the common carp used in these experiments (see Section 2.3).

Static acute toxicity tests provide practical means for deriving estimates of the acute lethal toxicity of a large number of test materials, such as pesticides (ALABASTER and ABRAMS,1965). The objective of such tests is to determine the concentration of a test material that produces direct lethal (or other irreversible) effects on a group of test fish during a short term exposure (usually 96 hours), under controlled conditions (SPRAGUE.1979; BUIKEMA,JR *et* *al.*,1982; ABEL,1989). The results of such tests are particularly useful for the screening of new pesticide products and are generally accepted as a conservative estimate of a potential effect of the compounds in the field (MACEK *et al.*,1978). The test is also useful in determining the relative sensitivity of different aquatic organisms to pesticide compounds, and in evaluating the effect of water quality on their toxicity. There are at least four countries which use standardised procedures for pesticide fish toxicity testing for the purpose of screening new compounds, i.e. USA (US Department of Interior,Fish and Wildlife Service, 1974), Great Britain (Ministry of Agriculture, Fisheries and Food, 1966), Japan (NISHIUCHI, 1974) and Switzerland (BATHE *et al.*, 1974). In Indonesia, guidelines for standardised fish toxicity testing with pesticides have been issued since 1973, using *Cyprinus carpio* (or *Puntius gonionotus*) and *Oreochromis mossambicus* as test fish (Indonesian Pesticide Committee, 1980; KOESOEMADINATA,1980).

Based generally on the results of static toxicity tests, data on the acute lethal toxicity of some rice insecticides commonly used in Asian and Southeast Asian countries were available (GILL and KHOO,as cited by HUAT and TAN,1980; ARCE and CAGAUAN,1980; DE

SILVA and RANASINGHE, 1989 and KOESOEMADINATA,1980;1982). However, data on the acute toxicity of more recent rice insecticide products are generally not available, but are urgently required.

The aims of the following experiments was therefore to determine the acute lethal toxicity of five rice insecticide formulations to common carp fingerlings under laboratory conditions, in order to obtain information on the potential toxicological effect of these compounds to fish when used in wet rice eco-systems.

3.2 MATERIALS AND METHODS

3.2.1 Insecticide samples

The experiment comprised of five series of individual static toxicity tests conducted in the laboratory. Five insecticide commercial products were used as test materials, the name and chemical descriptions of which are listed in Table 2.1 (Section 2.2). The products used in the experiment were formulated as wettable powder (Mipcin 50WP and Applaud 10WP), and emulsifiable concentrates (Baycarb 50EC, Basudin 60EC and Fastac 15WSC).

3.2.2 Test fish

Fingerlings of the local variety of common carp (*C. carpio* LINN.) were used as test fish. The average weight and size of the fish in each test is presented in Table 3.1. Attempts were made to obtain similar size and weight of fish for each test, but this was not always possible because of the seasonal availability of carp fingerlings. The procurement and maintenance of the test fish were as described in details in Section 2.3. No significant mortality or health problems were observed in the fish stock. Two days before the experiment, fish were transferred to test containers and starved to prevent fouling of the test solution. Fish were not fed during the experiment

3.2.3 Test procedure

The tests were conducted according to procedures outlined in Section 2.4.1 and 2.4.2. To find out the lethal concentration range of the insecticides to the test fish, exploratory tests were performed using concentration series of 1.0, 3.0 and 10.0mgl^{-1} (in test with *fenobucarb, isoprocarb,* buprofezin and *diazinon*), and 0.001, 0.003 and 0.01mgl^{-1} (in test with *alphamethrin*). Based on the results of these tests, a duplicate of at least 5 concentrations, prepared in logarithmic series, were used in the definitive tests. The physical and chemical characteristics of test medium were recorded daily as described in Section 2.4.2.

3.2.4 Data collection and analysis

Cumulative fish mortality at 24, 48, 72 and 96 hours was recorded, and the data analysed statistically to obtain the values of

TABLE 3.1. **Weight and length of common carp fingerlings used in the static toxicity tests (Means and Standard Error)**

Test No	Insecticide (Common name)	Weight (g)	Fork length (cm)
1	*Fenobucarb*	3.9 ± 0.01	4.0 ± 0.08
2	*Isoprocarb*	3.8 ± 0.03	5.2 ± 0.02
3	*Buprofezin*	4.1 ± 0.03	5.7 ± 0.03
4	*Diazinon*	3.8 ± 0.1	4.9 ± 0.05
5	*Alphamethrin*	4.0 ± 0.03	5.0 ± 0.05

LC50s, slope functions and their 95%–confidence limit intervals, according to the procedure described in Section 2.4.3. Toxicity curves for each insecticide were drawn by plotting the LC50 values, and their 95%–confidence limit intervals, against exposure period. From these toxicity curves the threshold–LC50 of each insecticide was estimated (if possible) by graphical interpolation.

3.3 RESULTS

3.3.1 *Fenobucarb*

Exploratory tests with *fenobucarb* revealed that no mortality occurred in fish exposed for 48 hours in 1mgl^{-1}, and 90% were recorded dead in 10.0mgl^{-1}, suggesting that the lethal concentration range of this insecticide to common carp was between 3mgl^{-1} and 10mgl^{-1}. Further test showed that concentration of 16mgl^{-1} produced 100% mortality to the test fish in 24 hours (Table 3.2). The Median Lethal Concentrations for 24, 48, 72 and 96 hours exposure period derived from the experimental data were 8.3mgl^{-1}, 7.5mgl^{-1}, 6.6mgl^{-1} and 5.9mg^{-1}, respectively (Table 3.3). By plotting the LC50 values against exposure time (both in logarithmic scale), a straight line toxicity curve was obtained, which was apparently a segment of a larger curve. This means that testing over more extensive exposure period using a wider range of lower concentrations is required to establish a true curve. The Threshold Median Lethal Concentration (threshold LC50) of *fenobucarb* was, therefore, not apparent from the result of this experiment (Figure 3.1).

No fish mortality was observed in the control, and there was no evidence that the result of the test was significantly influenced by the physical and chemical quality of the test medium. Mean dissolved oxygen concentration through out the test remained at the level of 7.65 mgl^{-1} to 8.53mgl^{-1} (93% to 103% saturation). At the end of the test period mean carbon dioxide and total ammonia concentrations were 3.49mgl^{-1} and 0.215mgl^{-1}, respectively (Table 3.4).

3.3.2 *Isoprocarb*

The lethal concentration range of *isoprocarb* to common carp was between 1.0mgl^{-1} and 10.0mg^{-1}. Concentrations of 16.0 mgl^{-1} killed all test fish within 48 hours (Table 3.2). The Median

TABLE 3.2. Cumulative mortality of test fish exposed to insecticides in the static toxicity test.

Concentration (mg/l, A.I)	Number of fish tested	24 hours Died	%	48 hours Died	%	72 hours Died	%	96 hours Died	%
Fenobucarb									
2.80	20	0	0	0	0	0	0	0	0
3.75	20	0	0	0	0	2	10	4	20
5.00	20	2	10	3	15	6	30	8	40
6.75	20	6	30	8	40	10	50	12	60
9.00	20	12	60	14	70	15	75	16	80
12.00	20	16	80	17	85	18	90	19	95
16.00	20	20	100	20	100	20	100	20	100
Isoprocarb									
2.80	20	2	10	3	15	3	15	4	20
3.75	20	4	20	5	25	6	30	6	30
5.00	20	6	30	8	40	9	45	10	50
6.75	30	8	40	9	60	13	65	13	65
9.00	20	9	45	15	75	15	75	15	75
12.00	20	12	60	17	85	17	85	18	90
16.00	20	18	90	20	100	20	100	20	100

TABLE 3.2. (Continued)

Concentration (mg/l, A.I)	Number of fish tested	24 hours		48 hours		72 hours		96 hours	
		Died	%	Died	%	Died	%	Died	%
Buprofezin									
1.00	20	0	0	4	20	5	25	6	30
1.35	20	0	0	6	30	7	35	7	35
1.80	20	1	5	7	35	8	40	8	40
2.40	20	4	20	12	60	14	70	14	70
3.20	20	17	85	18	90	18	90	18	90
4.20	20	18	90	19	95	20	100	20	100
5.60	20	19	95	20	100	20	100	20	100
Diazinon									
1.08	20	0	0	0	0	0	0	0	0
1.44	20	0	0	1	5	2	10	2	10
1.92	20	0	0	2	10	4	20	5	25
2.52	20	2	10	4	20	8	40	11	55
3.36	20	10	50	16	80	17	85	18	90
4.50	20	19	95	20	100	20	100	20	100
6.00	20	20	100	20	100	20	100	20	100

TABLE 3.2. (Continued)

Concentration (mg/l, A.I)	Number of fish tested	24 hours Died	%	48 hours Died	%	72 hours Died	%	96 hours Died	%
Alphamethrin									
0.0027	20	0	0	4	20	6	30	7	35
0.0036	20	1	5	8	40	8	40	9	45
0.0048	20	6	30	12	60	13	65	14	70
0.0063	20	9	45	15	75	17	85	18	90
0.0084	20	11	55	18	90	19	95	19	95
0.0112	20	18	90	20	100	20	100	20	100
0.0150	20	20	100	20	100	20	100	20	100

TABLE 3.3. **The acute lethal toxicity of 5 rice insecticides to common carp (*Cyprinus carpio* LINN) in laboratory condition.**

Exposure time (hours)	Median Lethal Concentration (LC50), and 95% confidence limit intervals (mg/l, active ingradient)	Slope function and 95% confidence limit intervals
Fenobucarb		
24	8.3 (7.2-9.5)	1.51 (1.34-1.70)
48	7.5 (6.5-8.5)	1.52 (1.31-1.77)
72	6.6 (5.8-7.6)	1.57 (1.36-1.81)
96	5.8 (5.0-6.8)	1.54 (1.37-1.72)
Isoprocarb		
24	8.3 (7.0-9.9)	2.20 (1.58-3.06)
48	5.8 (4.8-7.0)	1.86 (1.48-2.34)
72	5.4 (4.5-6.5)	1.96 (1.49-2.59)
96	5.3 (4.5-6.4)	1.87 (1.47-2.38)
Buprofezin		
24	2.7 (2.6-2.9)	1.49 (1.23-1.81)
48	2.0 (1.7-2.4)	1.83 (1.46-2.29)
72	1.9 (1.5-2.3)	1.90 (1.48-2.45)
96	1.6 (1.3-1.9)	1.80 (1.46-2.23)
Diazinon		
24	3.6 (3.3-3.9)	1.22 (1.14-1.30)
48	3.1 (2.9-3.3)	1.24 (1.18-1.30)
72	2.8 (2.5-3.1)	1.79 (1.24-1.32)
96	2.3 (2.1-2.6)	1.30 (1.21-1.38)
Alphamethrin		
24	0.0068 (0.0060-0.0078)	1.48 (1.33-1.66)
48	0.0042 (0.0035-0.0049)	1.75 (1.44-2.12)
72	0.0040 (0.0032-0.0049)	1.79 (1.41-2.29)
96	0.0037 (0.0029-0.0047)	1.80 (1.41-2.31)

TABLE 3.4. The physical and chemical chacleristics of test medium measured during the static toxicity tests (Means and Standard Error)

Exposure time (day)	Temperature (C)	pH	Dissolved oxygen (mg/l)	Carbon dioxide (mg/l)	Total ammonia (mg/l)	Total hardness (mg/l)
1	2	3	4	5	6	7
Fenobucarb						
1	25.2 ± 0.1	7.4 ± 0	8.0± 0.1	2.99 ± 0.05	0.040 ± 0.001	69.4 ± 1.5
2	24.4 ± 0.1	7.3± 0	7.9 ± 0.3	2.46 ± 0.24	0.072 ± 0.001	77.0 ± 2.7
3	24.5 ± 0.1	7.5 ± 0	8.5 ± 0.4	3.03 ± 0.64	0.076 ± 0.001	75.6 ± 2.0
4	24.8 ± 0.1	7.5 ± 0.2	7.6 ± 0.1	3.49 ± 0.09	0.215 ± 0.003	70.5 ± 4.3
Isoprocarb						
1	25.5 ± 0.1	7.1 ± 0 1	6.8 ± 0.1	1.99 ± 0.09	0.013 ± 0.001	71.4 ± 1.2
2	24.5 ± 0.1	7.2 ± 0.1	6.1 ± 0.1	2.99 ± 0.15	0.014 ± 0.001	71.9 ± 1.3
3	25.2 ± 0.1	7.3 ± 0.1	6.0 ± 0.1	2.85 ± 0.24	0.016 ± 0.001	73.0 ± 1.2
4	24.0 ± 0.1	7.5 ± 0.2	5.7 ± 0.1	3.18 ± 0.32	0.016 ± 0.001	72.8 ± 1.6

TABLE 3.4. (Continued)

1	2	3	4	5	6	7
Buprofezin						
1	25.1 ±0	7.2 ±0	6.8 ±0.1	1.19 ±0.06	0.012 ±0	76.8 ±1.6
2	25.1 ±0.1	7.3 ±0.1	6.2 ±0.1	2.01 ±0.04	0.014 ±0	78.4 ±2.7
3	24.2 ±0	7.4 ±0.1	5.9 ±0	2.99 ±0.40	0.017 ±0	79.0 ±3.2
4	24.1 ±0.1	7.4 ±0.1	5.4 ±0.3	3.19 ±0.05	0.020 ±0.001	80.4 ±2.6
Diazinon						
1	25.3 ±0.1	7.0 ±0.1	6.8 ±0.3	3.99± 0.20	0.011 ±0	69.8 ±1.6
2	25.6 ±0.1	7.0 ±0.3	5.5 ±0.3	4.05 ±0.10	0.012 ±0	66.4 ±2.3
3	26.1 ±0.1	7.2 ±0.1	4.9 ±0.1	4.52 ±0.20	0.014 ±0	75.6 ±2.6
Alphamethrin						
1	25.5 ±0.1	7.2 ±0.1	6.8 ±0.1	1.19 ±0.06	0.012 ±0	71.4 ±1.1
2	26.0 ±0	7.3 ±0.1	6.3± 0.9	2.29 ±0.40	0.014 ±0	71.9 ±1.3
3	25.5 ±0	7.4 ±0.1	5.8 ±0.1	3.19 ±0	0.016 ±0	72.8 ±1.3
4	26.1 ±0	7.5 ±0	5.5 ±0.1	3.18 ±0	0.019 ±0	76.2 ±1.6

TABLE 3.5. The potency ratios (and their 97% confidence limit intervals) between 5 rice insecticide formulations to common carp.

	Fenobucarb	*Isoprocarb*	*Buprofezin*	*Diazinon*	*Alphamethrin*
Fenobucarb	0	1.112 (0.674-1.832)	0.321* (0.234-0.441)	0.433* (0.270-0.694)	0.0006* (0.0004-0.0010)
Isoprocarb	0.900 (0.067-1.484)	0	0.357* (0.215-0.592)	0.482* (0.298-0.788)	0.0007* (0.0004-0.0012)
Buprofezin	3.116* (2.268-4.280)	2.804* (1.691-4.647)	0	1.350 (0.804-2.265)	0.0020* (0.0012-0.0034)
Diazinon	2.309* (1.441-3.698)	2.077* (1.270-2.638)	0.741 (0.441-1.243)	0	0.0015* (0.0009-0.0025)
Alphamethrin	1570* (433-2642)	1412* (824-2421)	504* (292-868)	680* (407-1135)	0

NOTE :
1. Potency ratios based on 96hrs-LC50 values, according to method by LITCHFIIELD & WILCOXON (1949)
2. Asterix (*) denotes potency/toxicity is significantly different ($p < 0.05$)

Lethal Concentration of *isoprocarb* for 24, 48, 72 and 96 hours exposure period, derived from the experimental data were 8.3mgl^{-1}, 5.8mgl^{-1}, 5.4mgl^{-1} and 5.3mgl^{-1} (Table 3.3).

The threshold LC50 of *isoprocarb* was more apparent than that of *fenobucarb*. This concentration (as estimated by visual inspection of its toxicity curve) was found to be approximately 5.4mgl^{-1} or equal to the 48h and 96h–LC50 values (Figure 3.2).

No fish died in the control and water quality was acceptable throughout the experiment. Mean dissolved oxygen concentration ranged between 5.7mgl^{-1} (68% saturation) and 6.8mgl^{-1} (84% saturation). Carbon dioxide and total ammonia concentration ranged between 1.99mg^{-1} and 3.18mgl^{-1}, and between 0.001mgl^{-1} and 0.016mgl^{-1}, respectively (Table 3.4).

3.3.3 Buprofezin

The lethal concentration range of *buprofezin* was found to be about 5 times lower than those of both *fenobucarb* and *isoprocarb*, i.e between 1.0mgl^{-1} and 5.0mgl^{-1}. Concentrations of 5mgl^{-1} caused

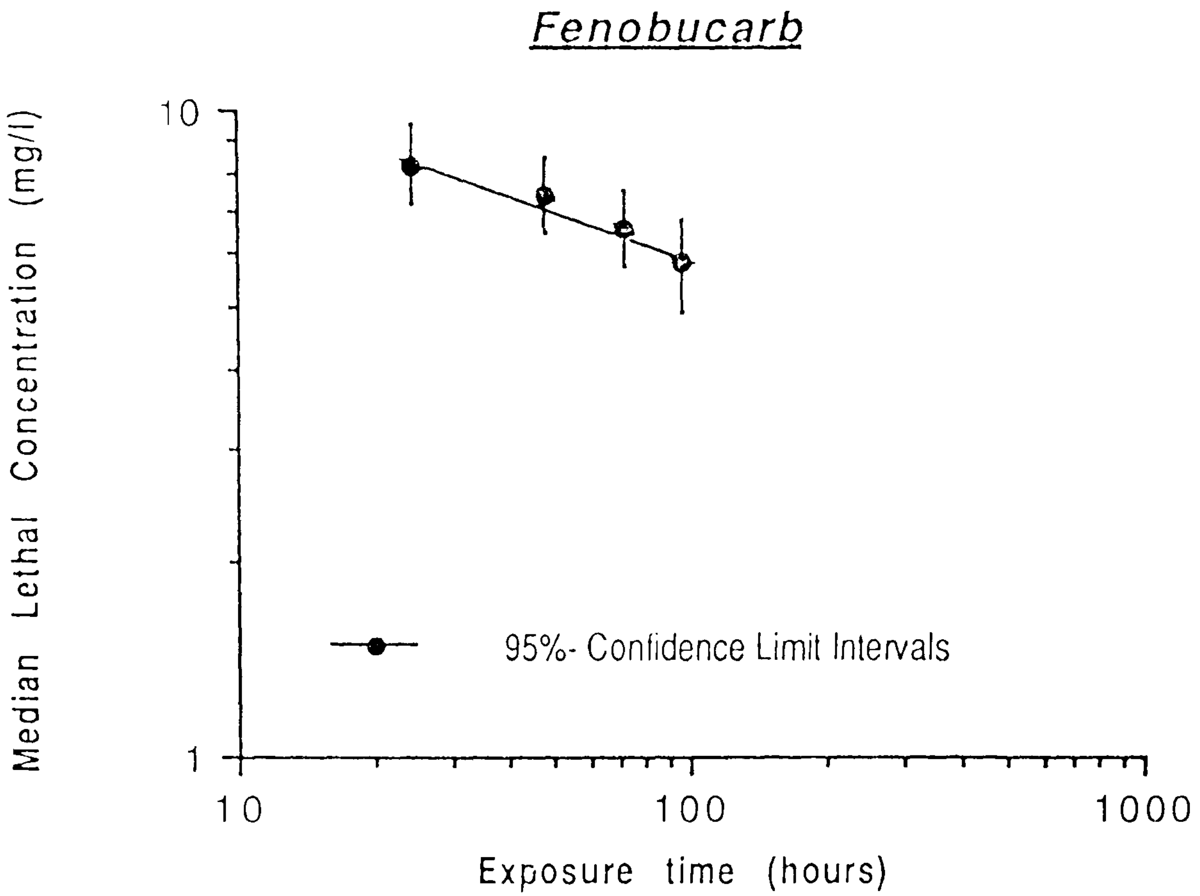


FIGURE 3.1. Toxicity curve for common carp (*Cyprinus carpio* LINN) exposed to the insecticide *fenobucarb* (*BPMC*) .

100% mortality in test fish within 48 hours. The Median Lethal Concentrations of *buprofezin* for 24, 48, 72 and 96 hours exposure time, were 2.7mgl^{-1}, 2.0mgl^{-1}, 1.9mgl^{-1} and 1.6mgl^{-1}, respectively (Table 3.3). The threshold–LC50 of *buprofezin* was apparent from the shape of its toxicity curve.. This concentration was (as estimated by visual inspection of the curve) approximately 1.880mgl^{-1}, or approximately equal to the 48h–LC50 value (Figure 3.3).

No fish mortality was recorded in the control tanks, and of water quality was within acceptable limits during the experiment. At the end of the experiment the mean dissolved oxygen, carbon dioxide and total ammonia concentration were 5.4mgl^{-1} (65% saturation), 3.19mgl^{-1} and 0.020mgl^{-1}, respectively.

3.3.4 *Diazinon*

The lethal concentration range of *diazinon* was between 1.0mgl^{-1} and 4.5mg^{-1}. Concentrations of 4.50mgl^{-1} and 6.00mgl^{-1} of *diazinon* caused 100 percent mortality of fish within 48 hours and 24 hours, respectively (Table 3.2). The Median Lethal Concentrations of *diazinon* for 24, 48, 72 and 96 hours exposure period were 3.6mgl^{-1},

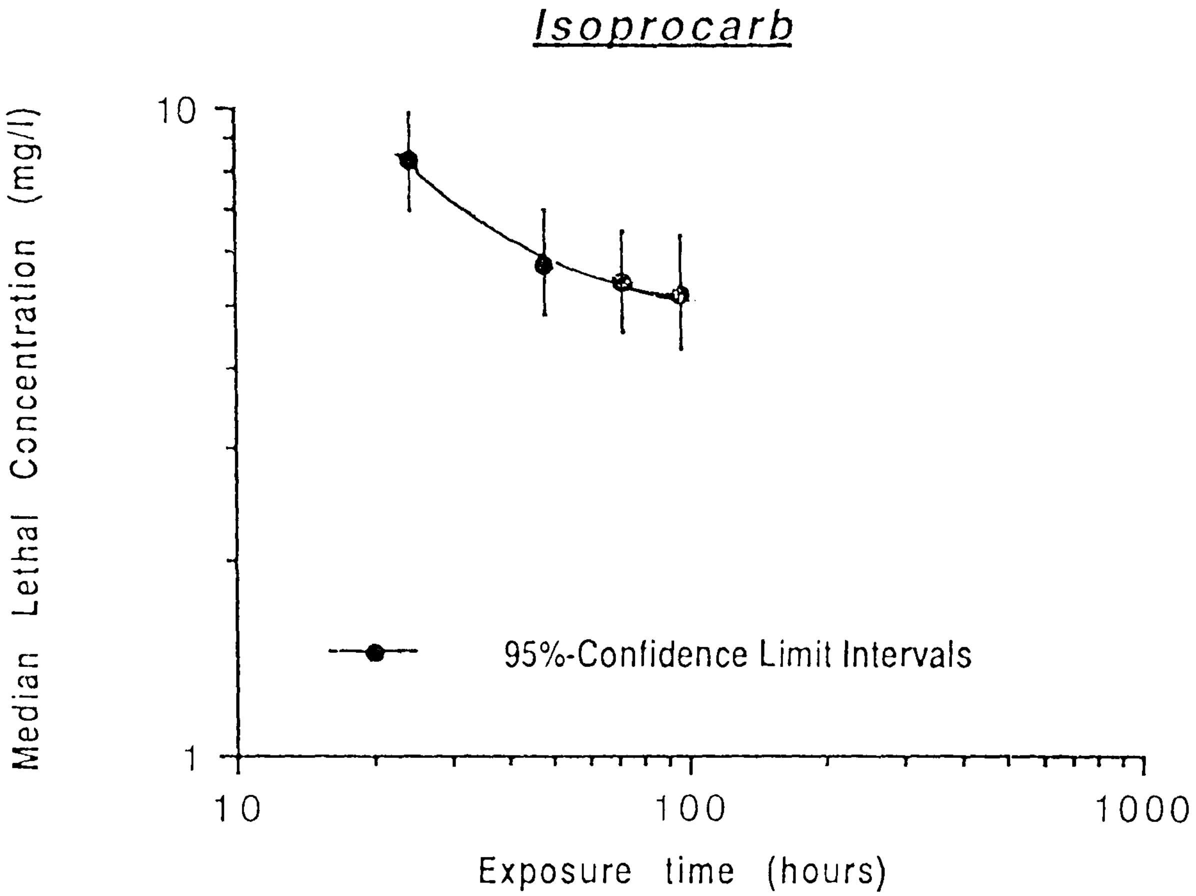


FIGURE 3.2. Toxicity curve for common carp (*Cyprinus carpio* LINN) exposed to the insecticide *isoprocarb* (*MIPC*) .

3.1mgl^{-1}, 2.9mgl^{-1} and 2.3mgl^{-1}, respectively (Table 3.3). The toxicity curve derived from the LC50 data and their exposure time formed a straight line, suggesting that more extensive test period (longer than 4 days) and wider range of test concentrations were required to obtain the true shape of the curve. The threshold–LC50 of *diazinon* was, therefore, not apparent in and could not be estimated from the toxicity curve resulted from this experiment. However, it is obvious that this concentration is lower than the 96h–LC50 value of this insecticide (Figure 3.4).

No fish mortality was observed in the controls, and water quality was within acceptable limits during the experiment. At the end of the experiment the mean dissolved oxygen, carbon dioxide and total ammonia concentrations were 4.9mgl^{-1} (60% saturation), 4.52mgl^{-1} and 0.014mgl^{-1}, respectively.

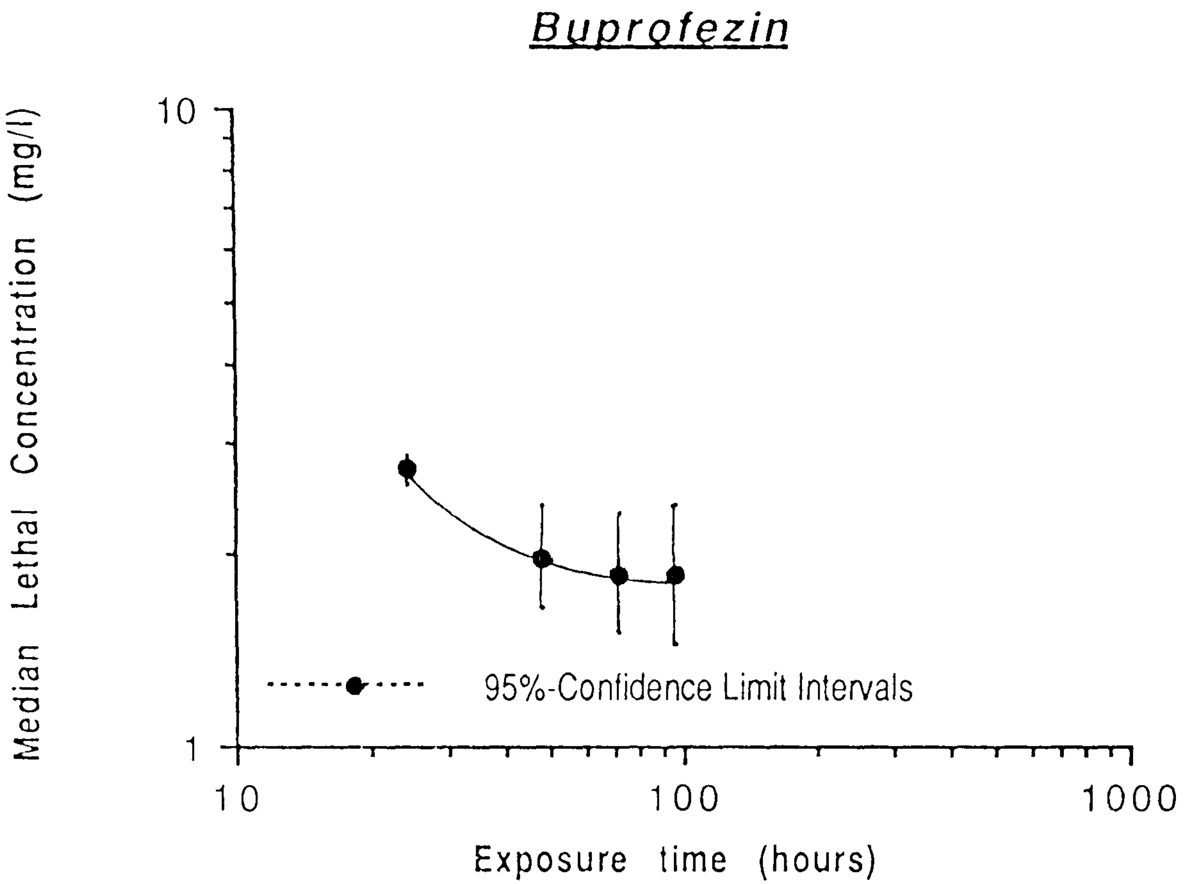


FIGURE 3.3. Toxicity curve for common carp (*Cyprinus carpio* LINN) exposed to the insecticide *buprofezin*.

3.3.5 *Alphamethrin*

The exploratory test revealed no mortality in test fish exposed to *alphamethrin* concentrations of 0.001mgl^{-1}, while 100 percent mortality was observed within 24 and 48 hours at concentrations of 0.011mgl^{-1} and 0.015mgl^{-1}, respectively. The result suggest that *alphamethrin* has a lethal concentration range between 0.001mgl^{-1} and 0.011mgl^{-1} (Table 3.2). The Median Lethal Concentration of *alphamethrin* for 24–, 48–, 72– and 96 hour exposure time were 0.0068mgl^{-1}, 0.004mgl^{-1}, 0.004mgl^{-1} and 0.0037mgl^{-1}, respectively (Table 3.3). The threshold Median Lethal Concentration of *alphamethrin*, estimated by eye from the toxicity curve, was 0.0035mgl^{-1} (Figure 3.5). This result showed that although *alphamethrin* has a relatively high acute lethal toxicity to fish, it might not cause further significant fish mortality at concentrations lower or approximately equal to 96h–LC50 for prolonged periods of exposure.

Water quality in the test medium was within acceptable limits during the test. At the end of the test the mean dissolved oxygen, carbon dioxide and total ammonia concentration were 5.4mgl^{-1} (65% saturation), 3.18mgl^{-1} and 0.019mgl^{-1}, respectively

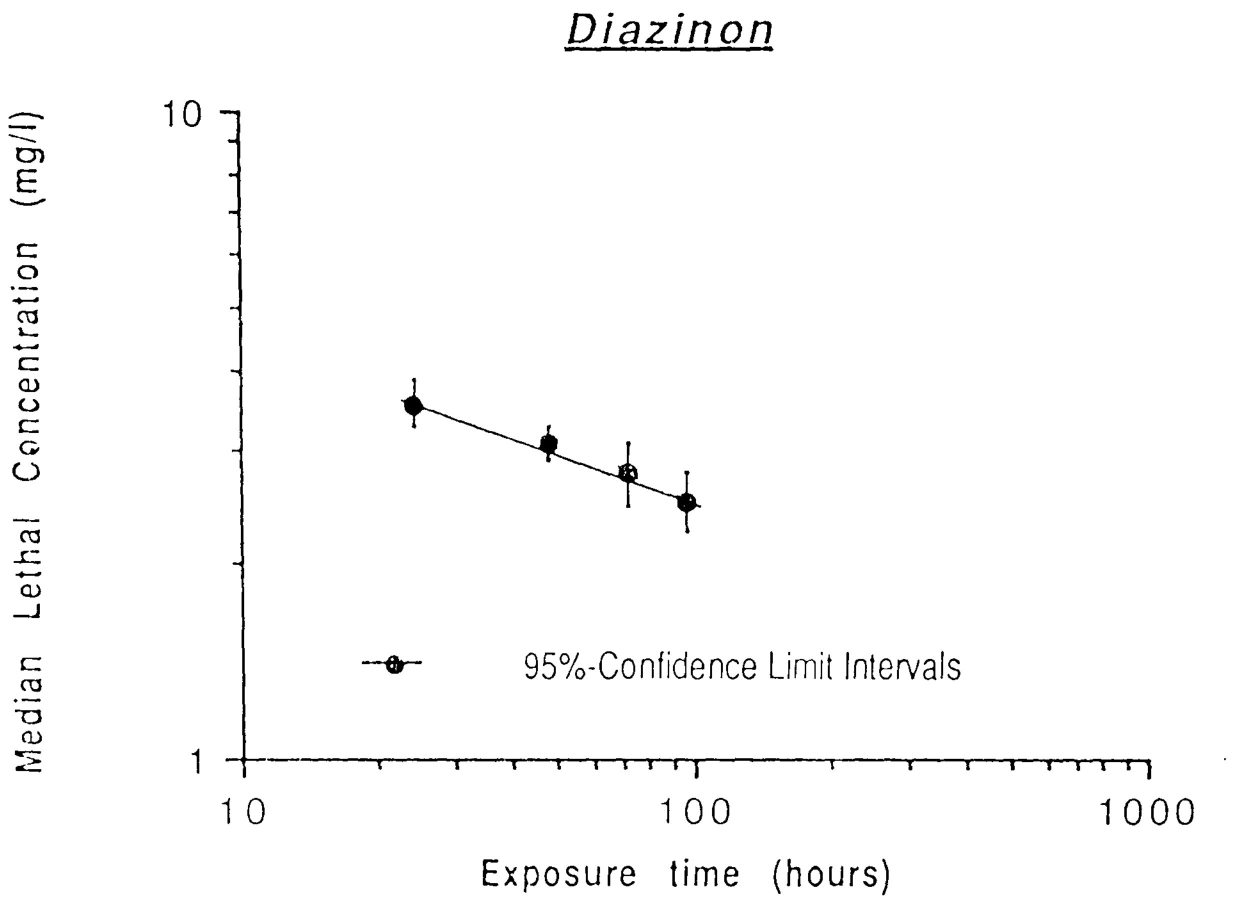


FIGURE 3.4. Toxicity curve for common carp (*Cyprinus carpio* LINN) exposed to the insecticide *diazinon*.

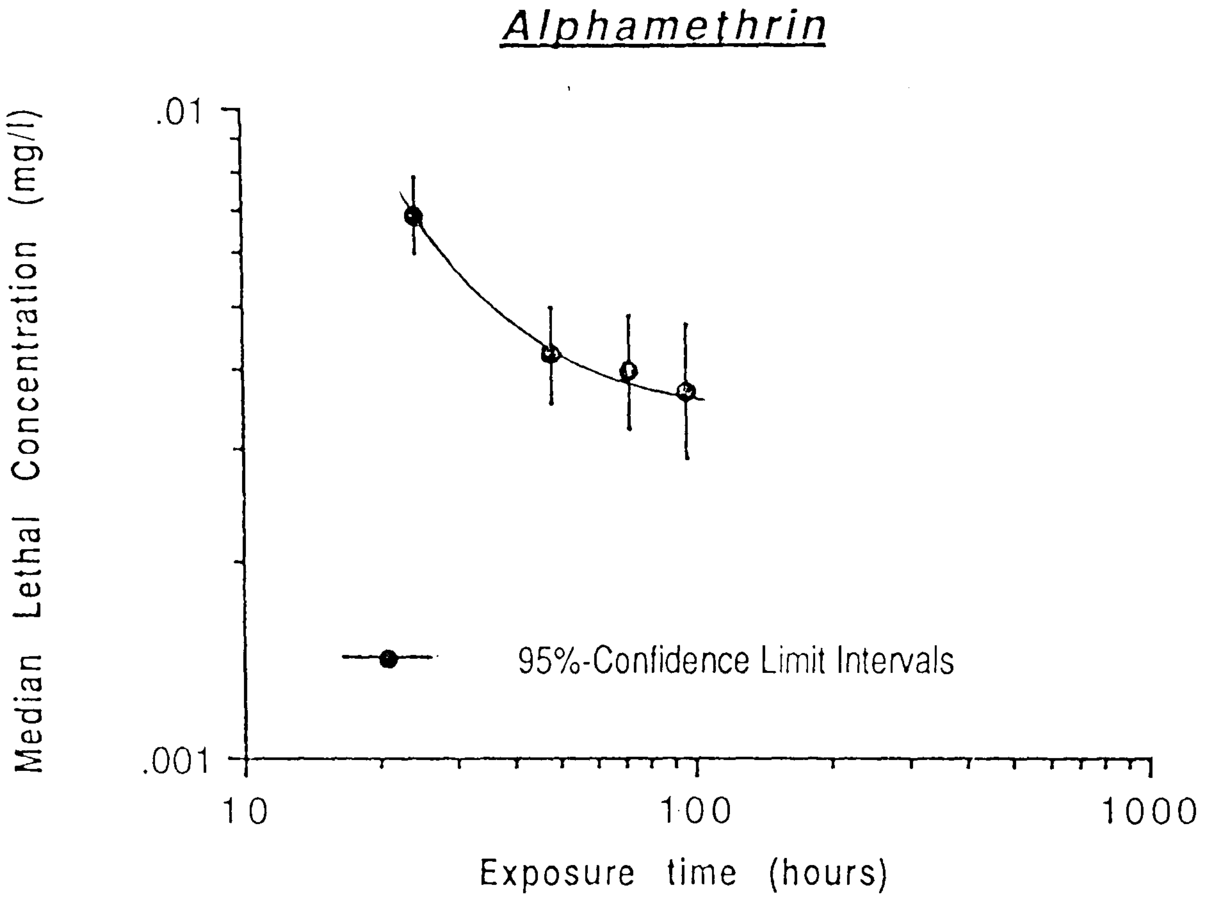


FIGURE 3.5. Toxicity curve for common carp (*Cyprinus carpio* LINN) exposed to the insecticide *alphamethrin*.

3.3.6 Summary of results

There was a wide variation in the acute lethal toxicity of the five rice insecticides, mainly due to the different chemical properties and biological activity of the compounds. Differences in the acute lethal toxicity of the five insecticides to fish, based on the potency ratio (using 96h–LC50 values), is presented in Table 3.5.

The acute lethal toxicity of two carbamate insecticides *fenobucarb* and *isobucarb* were almost identical, with 96h–LC50s of 5.8mgl^{-1} and 5.3mgl^{-1}, respectively. However, the toxicity curves of the two insecticides showed a different trend, and revealed that the threshold–LC50 of *fenobucarb* was lower than that of *isoprocarb.* This information suggest that *fenobucarb* may be more toxic for prolonged exposures, causing fish mortality at concentrations lower than the 96h–LC50.

The acute lethal toxicity of the thiadiazin insecticide *buprofezin* and the organophosphate *diazinon*, were both higher than the carbamates, with 96h–LC50s of 1.6mgl^{-1} and 2.3mgl^{-1}, respectively. As in the case of the carbamate insecticides, the toxicity curves of the two insecticides also differed. The threshold–LC50 of *buprofezin* was apparent from the toxicity curve, and was estimated to be 2mgl^{-1} (approximately equal to the 48h–LC50). The toxicity curve of *diazinon* formed a straight line, suggesting a threshold–LC50 value lower than its 96h–LC50. This information suggests that *diazinon* might actually more toxic to fish than its 96h–LC50 value depicted, and caused fish mortality at prolonged time exposure in lower concentrations.

The acute lethal toxicity of the pyrethroid insecticide *alphamethrin* to common carp was found to be extremely high, in comparison with the other rice insecticides. The 96h–LC50 of *alphamethrin* was 0.0037mgl^{-1}. The toxicity curve of the insecticide, however, showed a clear threshold–LC50, which was estimated to be equal to or less than its 96h–LC50.

3.4 DISCUSSION

BATHE *et al.*(1974) proposed a classification of pesticides based on their 96h–LC50 values to fish. The classification was intended to provide an indication on the potential toxic effect of the chemicals to fish under practical conditions, and proceeded from the consideration that in plant protection practice dose rates of 0.5kg.ha^{-1} to 10kg.ha^{-1} (A.I) are generally applied in the field. With these application rates, a concentration level of 0.5mgl^{-1} to 10mgl^{-1} may occur in the rice field water within a short period under extreme condition (i.e that all the insecticide comes in contact with the rice field water with a depth of 10cm, and no immediate flushing and degradation process occur in the system). A concentration level of 0.5mgl^{-1} is therefore selected as the boundary level for highly toxic pesticides. Furthermore, according to the authors, a concentration level of 50mgl^{-1} or higher, is unlikely to occur in the rice field. This is not only because it will be uneconomical to use the chemical, but mainly also because this concentration level is the uppermost limit of water solubility for most pesticides. Thus, pesticides with 96h–LC50 greater than 50mgl^{-1} are considered non toxic to fish. On the other hand, pesticides with 96h–LC50 of less than 50.0mgl^{-1}, are considered toxic or slightly toxic to fish. Similar classifications of agricultural pesticide toxicity have also been adopted in Japan, based on 48h–TLm value for carp and 3h–TLm value for *Daphnia* (HASHIMOTO,1970).

Using the criteria described above, according to the result of the present study *fenobucarb* and *isoprocarb* are slightly toxic, while both *buprofezin* and *diazinon* are toxic to common carp. A*lphamethrin* is classified as highly toxic pesticide to common carp.

Very little published information could be found on the toxicity of the rice insecticide formulations investigated in the present study. DE SILVA and RANASINGHE (1989) reported that the 48h–LC50s of *fenobucarb* (*BPMC*) to post yolk fry and 3 week–old fry of the nile tilapia (*Oreochromis niloticus*), were 0.115mgl^{-1} and 9.00mgl^{-1}, respectively. The sublethal effect of the chemical studied by the authors (i.e effect on the oxygen consumption of the fish) showed no appreciable difference from that of control fish. The authors however, remarked that *fenobucarb* is too toxic to be used in aquaculture, as compared to the other rice insecticides tested, *monocrotophos* and *fenthion*. According to the result of studies conducted in the Philippines, the 96h–LC50 of *fenobucarb* for the nile tilapia (*O. niloticus*) and the crucian carp (*Carassius carassius*) were 2.70–3.06 mgl^{-1} and 12.6mgl^{-1}, respectively (ARCE and CAGAUAN, 1986). The insecticide was regarded as highly toxic to fish by the authors. HASHIMOTO and SUGAHARA (1961) reported that based on their test using topmouth gudgeon (*Pseudorasbora parva*), the 24h–LC50 of *fenobucarb* was 10mgl^{-1}, while NISHIUCHI (1972) recorded that the 48h–LC50 for carp (common carp?) was 16mgl^{-1}. Insecticide toxicity data from published literature sometimes showed dissimilarity, mainly because of the different species used as well as different test conditions. However, the general trend of the data is often consistent, as was shown by the result of the present test on the fish toxicity of *fenobucarb*.

Fenobucarb, according to the result of the present static toxicity test was classified as slightly toxic to fish (using the above Bathe *et al.* classification), but may be more toxic to fish than was depicted by its 96h–LC50 value

It is known that the acute and chronic toxicity of pesticide to fish depends primarily on the chemical and biochemical behaviour of the compound in fish and in water, including water solubility,stability to hydrolysis, intake, excretion, metabolism and bioconcen-tration (MATSUMARA,1979; HAQUE *et al.*,1977; SHAROM *et al.*,1980 and KHAN,1977). There is a significant linear correlation values between LC50 and the bioconcentration factor. Thus, the acute the toxicity of a to fish can be predicted from the knowledge of their bioconcentration factor. According to KANAZAWA (1975) *fenobucarb* showed moderate stability in water and was degraded gradually from 1mgl^{-1} of the initial concentration to 0.2mgl^{-1} after 32 days. Uptake of this insecticide by topmouth gudgeon (*Pseudorasbora. parva*) was 3.5mgl^{-1}, and reached the maximum level of 4.8mgl^{-1} after 4 days. This concentration decreased gradually to 1.2mgl^{-1} after 32 days. Metabolism of *fenobucarb* in topmouth gudgeon was very slow (about 2 weeks). About 30% of the fish exposed to the insecticide showed permanent spinal curvature of backbone. Skeletal deformity, according to the author, seemed to be a general symptom caused by cholinesterase inhibitors, such as organophosphate and carbamate insecticides. However, other pesticides, irrespective of the group to which they belong, have been known to induce vertebral damage and skeletal deformities (DARSIE and CORRIDEN,1959; McCANN and JASPER,1972; WEIS and WEIS,1976; COUCH *et al.*,1979 and BENGTSSON,1975). Similar symptoms were also observed in some test fish during the present experiment. The author further remarked, that although the uptake level of carbamate insecticides were generally lower than the organophosphates such as *diazinon* and *fenitrothion*, there are compounds such as *fenobucarb* (*BPMC*) among the carbamates which can cause chronic effect to the central nervous system of fish.

The 48h–LC50 values of the insecticide *isoprocarb* and *buprofezin* to common carp, according to one reference were 7.2mgl^{-1} and 3.7mgl^{-1}, respectively (Japanese Pesticide Guide,1987). These values correspond well with the results from the present experiment. However, no further published information is available concerning the toxicity of these relatively new products to fish.

The results of the toxicity test with *diazinon* were broadly in agreement with those obtained by other investigators (CHATTERJEE and KONAR,1984; NISHIUCHI,1972). According to the result of the present experiment, *diazinon* may still be harmful to fish at low concentration levels and at extended exposure period, as depicted from the shape of its toxicity curve. CHATTERJEE and KONAR (1984) reported sublethal concentration of 0.463mgl^{-1} (which was approximately 0.2 x 96h–LC50 for java tilapia). Their conclusion was based on the apparent effect of this concentration at different pH and turbidity levels.

SEGUCHI and ASAKA (1981) studied the bioconcentration ratio of *diazinon* in carp (*C. carpio*), rainbow trout (*S. gairdneri*), loach (*Misgurnus anguillicaudatus*) and shrimp (*Penaeopsis joyneri*). In the exposure to a continuous flow of water containing 0.02mgl^{-1} of *diazinon*, the concentration of *diazinon* in the fish tissue rapidly increased, and reached a maximum after 3 days exposure period. There after the concentration slightly decreased and remained at equilibrium. The bioconcentration ratios in carp, rainbow trout, loach and shrimp at the equilibrium were 120, 63, 26 and 3, respectively. Other studies on the bioconcentration of *diazinon* in fish gave similar results. According to KANAZAWA (1975), *diazinon* was relatively more stable in water than other organophosphate (e.g *malathion*) and carbamate (e.g *carbaryl*) insecticides. The uptake of *diazinon* by fish is also higher than that of *malathion*. In fish exposed to initial *diazinon* concentration of 0.6–1.2mgl^{-1}, its level in fish tissue were higher after 3 or 4 days than that after one day, and reached the maximum of 211mgl^{-1}. Thereafter, the concentration decreased rapidly to 17mgl^{-1}, after 30 days, producing a bioconcentration ratio of 64. *Diazinon* was also found to be metabolised slowly in the fish body. As in the case with *fenobucarb*, 10 to 30% of the test fish showed permanent vertebral deformity, after two weeks exposure period. During the present experiment no such skeletal deformities was observed in the test fishes, presumably due to the relatively short period of exposure and observation time.

Synthetic pyrethroids have been widely known to have low acute toxicity to birds and mammals (ELLIOT *etal.*,1978), but a high toxicity to aquatic organisms (JOLLY *et al.*,1978; MAUK and OLSEN, 1976; McLEESE *et al.*,1980 and ZITKO *et al.*,1979). The toxicity to fish is high because fish posses a low capacity for pyrethroid hydrolysis, relying instead on peripheral hydroxylation and conjugation processes (HUTSON and ROBERTS,1985). Published data on the toxicity of *alphamethrin* to fish are not available. However, according to STEPHENSON (1982,1985), *alphamethrin* and *cypermethrin* preparations have similar properties in aquatic studies performed in the laboratory. Therefore, the toxicity data of *cypermethrin* to fish are relevant to *alphamethrin*. STEPHENSON (1982) has measured the toxicity of *cypermethrin* to five warm–and cold water fish species (*Cyprinus carpio, Scardinius erythrophtlamus, Salmo gairdneri, Salmo trutta* and *Oreochromis nilotica*), using continuous–flow tests. The 96h–LC50 values obtained from the tests were within the range of 0.0004–0.0022mgl^{-1}. The nile tilapia was the least susceptible of the species tested at 25°C (96h–LC50= 0.0022mgl^{-1}). The 96h–LC50 of common carp was 0.0011mgl^{-1}, which is half of that of tilapia. The author found no apparent changes in the susceptibility of common carp exposed to *cypermethrin* at a lower temperature range of 10–15°C.

although it has been reported that the acute toxicity of synthetic pyrethroids to fish is negatively correlated to temperature (KAMARAGURU and BEAMISH,1981; MAUK and OLSEN,1976).

Data obtained from the present experiment with *alphamethrin* showed lower toxicity to common carp, with 96h–LC50 values about 3 times larger than those of *cypermethrin*. One explanation for this difference could be the different system and testing conditions used. It is known that pyrethroids have a very low water solubility (0.005–0.01mgl^{-1}), and a strong tendency to adsorb onto surfaces. These physical properties combined with high toxicity of the chemical make accurate determination of their toxicity in a static system difficult. This phenomenon may also explain why the dose–response relationship in the present experiment was not steep, and with limited concentration range, which is to be expected from a highly toxic substance. In contrast, STEPHENSON *et al.*(1984) reported that there was no apparent discrepancy between the result of a toxicity test of *cypermethrin* with nile tilapia using a static system and a continuous–flow system, showing 96h–LC50 values of 0.002mgl^{-1} and 0.0022mgl^{-1}, respectively.

The results of the present experiment, however, were in general agreement with toxicity data of related pyrethroids to fish, all of which have 96h–LC50s of less than 0.01mgl^{-1}.

From the above experimental results it is clear that acute lethal toxicity data are important in defining the potential bioactivity of the insecticides to fish, and allow classification of these compounds based on this potentiality. However, application of these data for prediction of the actual harmful effect of these chemicals in the field may not be straight forward. Several factors influence the aquatic exposure levels of insecticide in rice fields, including use pattern and the dynamic / fate characteristics of these compounds in the aquatic environment. The formulation of the insecticide will determine its solubility in water, and level of binding to aquatic plants, bottom sediments and other surfaces (see Chapter 1). For some insecticides there is a lack of correlation between their toxicity to fish in clean water tests performed in laboratory, compared with tests carried out under field condition. For these kind of insecticides (such as *fenobucarb, diazinon* and *alphamethrin*, tested in the present experiments), the potential hazard to fish culture may be best evaluated in the field.

CHAPTER 4

THE USE OF CHICKEN MANURE TO ENHANCE GROWTH AND PRODUCTION OF COMMON CARP FINGERLINGS IN A WET RICE FIELD ECOSYSTEM

4.1 INTRODUCTION

The assessment of the potential lethal and chronic effects of insecticides to fish may be difficult and inaccurate if the trial is conducted under conditions which are less than optimal for fish growth, causing high variability between individual growth rates and fish biomass productions (RUDDLE,1980; STEPHENSON,1984). It has been reported by many investigators that fish growth and production in ponds can be substantiated by the administration of animal manures as a nutrient base for fish food organisms (TANG,1970; BARDANCH *et al.*.,1972; DELMENDO, 1980; FANG *et al.*,1981; SCHROEDER, 1980; YAMADA,1986). However, there is no published information on the relationship between the 'dose' of animal manure and the production of fish raised in wet rice fields. For this reason preliminary experiments were conducted before performing the field toxicity experiments The principal purpose was to determine the dose rates of chicken manure required to optimise the growth and production of common carp fingerlings in wet rice fields, as the basis for subsequent toxicity trials to investigate the effect of insecticides on fish and other aquatic biota in the wet rice eco-system.

4.2 MATERIALS AND METHODS

The experiment was carried out using the experimental design as detailed in Section 2.5.8. Chicken manure was used at doses of 0, 100, 200 and $400gm^{-1}$. The treatments were allocated to the plots in a completely randomised design, each treatment replicated three times. The chicken manure was applied to each rice field plot on three separate occasions, as follows :

Application	Time (DAT)	Rate (% of total dose)
1st	-3	60
2nd	11	20
3rd	18	20

Experimental fish were treated in accordance to the procedure as detailed in Section 2.5.8.

4.3 RESULTS AND CONCLUSION

The experiment showed that the addition of chicken manure dose rates of $200gm^{-2}$ and $400gm^{-2}$ significantly increased the weight increment of fish ($P < 0.01$) which were $0.88gd^{-1}$ and $0.97gd^{-1}$, respectively, in comparison to control fish ($0.71gd^{-1}$).and fish from plot treated at $100gm^{-2}$ The total fish production was significantly higher ($P < 0.01$) in rice field plots receiving these two higher dose rates ($11.47gm^{-2}$ and $11.42gm^{-2}$), compared to that in the control plots ($9.63gm^{-2}$). and $100gm^{-2}$ plots The results of this preliminary experiment showed that chicken manure was more efficiently utilized for fish production at a medium rate of $200gm^{-2}$ Therefore this $200gm^{-2}$ application rate was used in subsequent field toxicity tests.

CHAPTER 5

EFFECTS OF FIVE RICE INSECTICIDE FORMULATIONS ON THE SURVIVAL, GROWTH AND PRODUCTION OF COMMON CARP FINGERLINGS (*C. carpio*), IN WET RICE FIELDS.

5.1 INTRODUCTION

The effect of insecticides on fish populations in field environments is considerably more complex than in the laboratory situation. In the field the hazard of insecticide application to fish depends not only on the toxicity of the product, but also on degree of exposure. Exposure is determined by the amount of active ingredient released, its dispersion and its persistence in the aquatic environment (CROSSLAND,1982; STEPHENSON *et al.*,1984; KOEMAN, 1974). The uptake of insecticide by fish depends on the concentration of the substance in the surrounding water. Insecticides that are bound tightly to organic matter maybe less available to fish in the water column, but may have a profound impact on benthic organisms (RAND and PETROCELLI,1984). Fish can also absorb insecticide indirectly by ingesting contaminated food organisms. In the case of granular formulations, fish can take up the insecticide from the mud, which may be ingested during feeding (MOULTON, 1973).

The toxicity of insecticides to fish in wet rice systems has been determined by applying the chemical at recommended rates to field in which caged or "free" fish are held. HARDJAMULIA and KOESOEMADINATA (1972) reported that the organochlorine insecticides Endrin and Thiodan (*endosulfan*), produced fish kills in rice fields within a few hours after application to such systems, with effects persisting for up to 11 days and 18 days, respectively. MOULTON (1973) obtained similar results in Malaysia, revealing that toxicity of Thiodan in granular and foliar forms to fish in rice fields lasted up to 40 days and 26 days after application, respectively. This author concluded that Thiodan and Endrin are very toxic to fish as well as quite persistent, and suggested that neither insecticide should be used in rice fields where fish production is important. These results contrast with GORBACH *et al.* (1971) and SCHOETTGER (1970) who stated that Thiodan at normal application rates break down rapidly to non toxic levels within 3–5 days after application.

Based on the results of toxicity trials conducted in wet rice fields, ARCE and CIRCA (1982) remarked that Baycarb (*fenobucarb*, *BPMC*) application rate of 750 g.ha^{-1}(A.I), sprayed once during late tillering stage of rice, caused Insignificant fish mortality of only 0.61%, two days after spraying. Application of Shellcarb, another commercial name for an insecticide product, containing the active ingredient *BPMC*, at the rate of 1000g.ha^{-1}, also produced a low mortality of 1.33% to fish in rice fields, three days after spraying.

Inconsistency between results of laboratory toxicity tests and those obtained from field trials have been reported by STEPHENSON *et al.* (1984), based on a study of the aquatic toxicology of *cypermethrin*, asynthetic pyrethroid insecticide.Based on laboratory toxicity tests, *cypermethrin* was found to be very toxic (96h–LC50: 2ugl^{-1}) to common carp (*Cyprinus carpio)* and nile tilapia *(Oreochromis niloticus)* as compared to *chlorvenfinphos* (96h–LC50: 39ugl^{-1}) and *carbofuran* (96hLC50: 480ugl$^{-1)}$. However, field experiments conducted in a wet rice system showed that mortality of caged fish was less with *cypermethrin* (<15%) than with *chlorvenfinphos* (97%) or *carbofuran* (67%). The authors concluded that the effects of *cypermethrin* in the field were limited because (1) only very low application rates of the product are needed to give pest control; (2) the penetration of the liquid *cypermethrin* formulation into water was lower, and (3) the loss of *cypermethrin* from sub–surface water was more rapid, probably as the result of several processes, including hydrolysis and biodegradation.

These limited results indicate that the overall influence of pesticide on fish in wet rice systems is best determined by field trials, even though laboratory data are more easily obtained than field data (HOLDEN, 1972). This problem is especially true with the case of recently introduced rice insecticide products which are currently in use in Southeast Asia countries. The major part of these new insecticide products are organophosphates, carbamates and synthetic pyrethroid compounds. Published data on the direct and indirect effects of these insecticides to fish production in wet rice systems are generally scarce or unavailable.

The present study was undertaken to investigate the effects of five insecticides commonly used for rice pest control, on common carp fingerlings reared in wet rice fields. The main objective of the study was to evaluate the influence of insecticides dose rates on the survival, growth and production of fish in rice fields and to compare field results with those from laboratory based toxicity tests.

5.2 MATERIALS AND METHODS

5.2.1 Experimental rice field plot

The experiments were conducted consecutively, allowing sufficient fallow period to the rice field plots between each trial to minimise problem of "carry over" of insecticides (see Section 2.5.2). The time table for the five experiments was as follows :

Exp. No.	Test material	Date of experiment	Fallow period (days)
1	*Fenobucarb*	Oct 17-Nov 15,1987	-
2	*Isoprocarb*	Jan 18-Feb 16,1988	33
3	*Buprofezin*	Mar 23-Apr 22,1988	35
4	*Diazinon*	May 20-Jun 22,1988	32
5	*Alphamethrin*	Jul 26-Aug 25,1988	34

Attemps to create similar experimental conditions for each trial were made, particularly in terms of soil and water management. Climatic conditions (notably rainfall) may cause variability in the invertebrate population in control rice fields between experiments. However, drastic climatic changes were not noted during the period of the experiments.

The design and construction of the rice field system have been described in details in Section 2.5.3. Rice field soil were prepared specifically to accommodate concurrent rice and fish culture following local traditional practice, as described in Section 2.5.4. Inorganic fertilisers (urea, TSP and KCl) and organic fertiliser (field dried chicken manure) were applied to the rice field plots to promote the growth of rice and fish, using the procedures and dressing regimes as specified in Section 2.5.5.

Rice seedlings were transplanted to the experimental plots following procedures described in Section 2.5.6.

5.2.2 Experimental fish

Fingerlings of common carp (*C. carpio*) were procured and maintained in an acclimation pond for one week prior to the experiments, following the procedure as outlined in Section 2.3. Attempts were made to secure batches of similar size for each experiment, however this was not always possible. The experimental fish were sorted, individually weighed and allocated to the rice field plots as described in Section 2.5.8. The mean weight and size of the carp fingerlings used in the experiments were as follows :

Exp. No.	Insecticide	Weight (g)	Length (cm)
1	*Fenobucarb*	5.6 (SE=0.07)	6.4 (SE=0.03)
2	*Isoprocarb*	5.0 (SE=0.02)	6.0 (SE=0.01)
3	*Buprofezin*	6.0 (SE=0.02)	6.4 (SE=0.01)
4	*Diazinon*	4.5 (SE=0.03)	6.1 (SE=0.02)
5	*Alphamethrin*	2.7 (SE=0.002)	5.2 (SE=0.001)

5.2.3 Experimental design and treatments

The experimental treatments comprised of 3 insecticide dose rates and one control without insecticide. The treatments were applied to rice plants in plots with fish and without fish,which were allocated in a completely randomised design, with three replications, as specified in Section 2.5.8.

5.2.4 Insecticide dose rates and mode of application

Information on the chemical descriptions and properties of the insecticides is presented in Table 2.1. The insecticide dose rates used in the treatment, were based on actual farm practice. The respective maximum nominal concentration (MNC) in the rice field water and the ratio of this concentration to the laboratory 24h–LC50 (see Chapter 3) is given in Table 5.0. Based on the concentration ratio values, only the application of *alphamethrin* dose rates are expected to produce lethal effect on fish. The nominal concentrations of the other insecticides tested are below their respective safety margins

The insecticide was applied directly to the rice plants

TABLE 5.0. Insecticide dose rates used as treatments in the experiments.

Experiment No.	Insecticide Common name	Dose rates (gha^{-1})	MNC (mgl^{-1})	24h-LC50 (mgl^{-1})	MNC : 24h-LC50
1	*Fenobucarb*	0	0	8.3	-
		375	0.375		0.045
		750	0.75		0.09
		1500	1.5		0.18
2	*Isoprocarb*	0	0	8.3	-
		500	0.5		0.06
		1000	1.0		0.12
		2000	2.0		0.24
3	*Buprofezin*	0	0	2.7	-
		50	0.05		0.02
		100	0.1		0.04
		200	0.2		0.08
4	*Diazinon*	0	0	3.6	-
		320	0.32		0.09
		640	0.64		0.18
		1280	1.28		0.36
5	*Alphamethrin*	0	0	0.0068	-
		7.5	0.007		1.03
		15.00	0.015		2.06
		30.0	0.03		4.12

NOTE : MNC - Maximum nominal concentration in the rice field water resulted from insecticide application ($1kg.ha^{-1} = 1mgl^{-1}$)

following the standard practice described in Section 2.5.9.. During the application, a moveable 1m high cloth screen was set up around the rice field plot to prevent spray drift.

5.2.5 Rice field biota

The distribution pattern of rice field biota is very difficult to quantify (HECKMAN,1979). A preliminary qualitative study was made on the aquatic biota in the rice field plots to establish the presence or absence of important fish food organism (such as rotifers, copepods and cladocerans) in the plots. Subsequently, zooplankton and macroinvertebrates were periodically sampled and microscopically examined during the course of the experiment, following the sampling protocols and procedure outlined in Section 2.5.10 and Section 2.5.11.

5.2.6 Water quality measurement

The physical and chemical characteristics of the rice field water were measured periodically during the experiment, following the sampling protocols and analytical procedures outlined in Section 2.5.12. Determination of some water quality parameters (temperature, dissolved oxygen, free carbon dioxide, pH, total hardness and total alkalinity), were made directly in the field. Other parameters (nitrite, total ammonia, total phosphorus and suspended solid) were determined from water samples collected in 1 litre plastic bottles and transported in cool boxes to the laboratory.

5.2.7 Statistical analysis of the experimental data

Data collected from the experiment were processed and statistically analysed following procedures outlined in Section 2.5.13.

TABLE 5.1. **Mean initial/final weight and mean growth rate (in terms of absolute weight increment per day) of common carp fingerlings in rice fields receiving different dose rates of *fenobucarb*.**

	Dose rate (gha^{-1}, A.I.)				F	Significant level	Probability
	0	375	750	1500			
Initial weight (g)	5.6	5.6	5.5	5.6	1.53	NS	> 0.05
Standard error	0.1	0.1	0.1	0.1			
Final weight (g)	28.3	25.3	27.4	23.7	3.81	NS	> 0.05
Standard error	0.5	0.6	0.5	0.6			
Growth rate (gd^{-1})	0.91	0.93	1.04	0.86	3.91	NS	> 0.05
Standard error	0.02	0.06	0.02	0.04			

NOTE : NS - Not significant

5.3 RESULTS

5.3.1 Experiment 1 (*Fenobucarb*)

5.3.1.1 Growth rate

The initial weight,final weight and growth rate (in terms of absolute weight increment per day) of common carp in the rice fields exposed to different dose rates of *fenobucarb*, are presented in Table 5.1. The mean initial weight of the experimental fish used in the treatments were similar, ranging between 5.5g and 5.7g, and was not significantly different ($p > 0.05$). The mean final weight and growth rate of fish in the treatments was also not significantly different ($p > 0.05$). The data also show that fish exposed to the highest dose rate of *fenobucarb* ($1500gha^{-1}$) have the lowest final weight and growth (Figure 5.1). The mean final weight and growth rate of fish in these plots were, respectively, 23.7g and $0.86gd^{-1}$ as compared to 25.3g and $0.91gd^{-1}$ in the control plots. The data, however, were not statistically significant, presumably due to the variation in the individual fish used in the experiment, or to other unknown sources of variation, which was difficult to define.

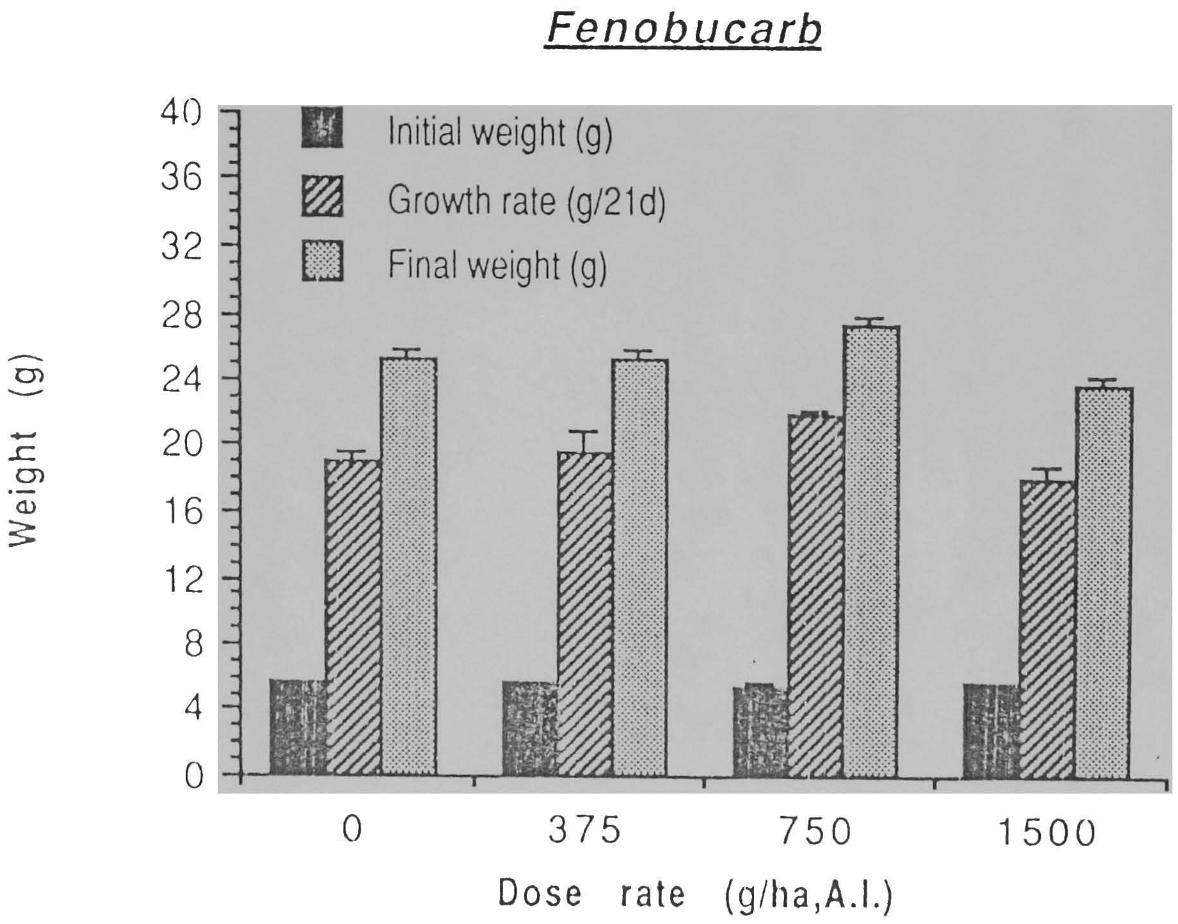


FIGURE 5.1. Growth rate of common carp fingerlings (*C. carpio*) in wet rice field, receiving different dose rates of *fenobucarb* (Means and Standard Error).

5.3.1.2 Survival rate

Survival of the experimental fish in all rice field plots was exceptionally high (>90%), and there was no apparent difference between fish survival rate in the treatment plots ($p > 0.05$). The lowest mean survival rate (95%) was recorded in the rice fields receiving the highest dose rate of *fenobucarb* of 1500gha^{-1} (Table 5.2). This excellent fish survival rate signified the favourable condition of the experimental fish and the experimental system used in this study.

5.3.1.3 Fish production

In the rice field plots treated with the highest dose rate of *fenobucarb* (1500gha^{-1}), the yield and weight gain of fish biomass (which was 11.3gm^{-2}21d^{-1} and 0.40gm^{-2}d^{-1}, respectively), were found to be significantly lower ($p < 0.05$) than in control, which was 12.5gm^{-2}21d^{-1} and 0.46gm^{-2}d^{-1}, respectively (Table 5.2). On the other hand, a trend of increase in the yield and weight gain of fish biomass was observed in the rice fields treated with the lower dose rates of 375gha^{-1} and 750gha^{-1} as shown in Figure 5.2..

TABLE 5.2. Summary of survival rate, biomass yield and biomass weight gain of common carp fingerlings in rice fields receiving different dose rates of *fenobucarb* (Means and standard error).

	Dose rate (gha^{-1}, A.I.)				F	Significant	Probability
	0	375	750	1500			
Stocking density (gm^{-2})	2.7	2.79	2.76	2.83	1.53	NS	> 0.05
Standard error	0.02	0.03	0.03	0.01			
Survival rate (%)	98.3	100.0	96.7	95.0	0.93	NS	> 0.05
Standard error	1.4	0	1.4	2.3			
Total yield (gm^{-2}21d^{-1})	12.5^{a}	12.6^{ab}	13.3^{ab}	11.3^{c}	5.87	*	< 0.05
Standard error	0.2	0.5	0.1	0.2			
Weight gain (gm^{-2}d^{-1})	0.46^{a}	0.47^{ab}	0.50^{ab}	0.40^{c}	5.75	*	< 0.05
Standard error	0.02	0.06	0.02	0.04			

NOTE : 1 / NS - Not significant, * - Significant
2 / Means designated with a common letter are not significantly different at 5% level by Duncan Multiple Range Test.

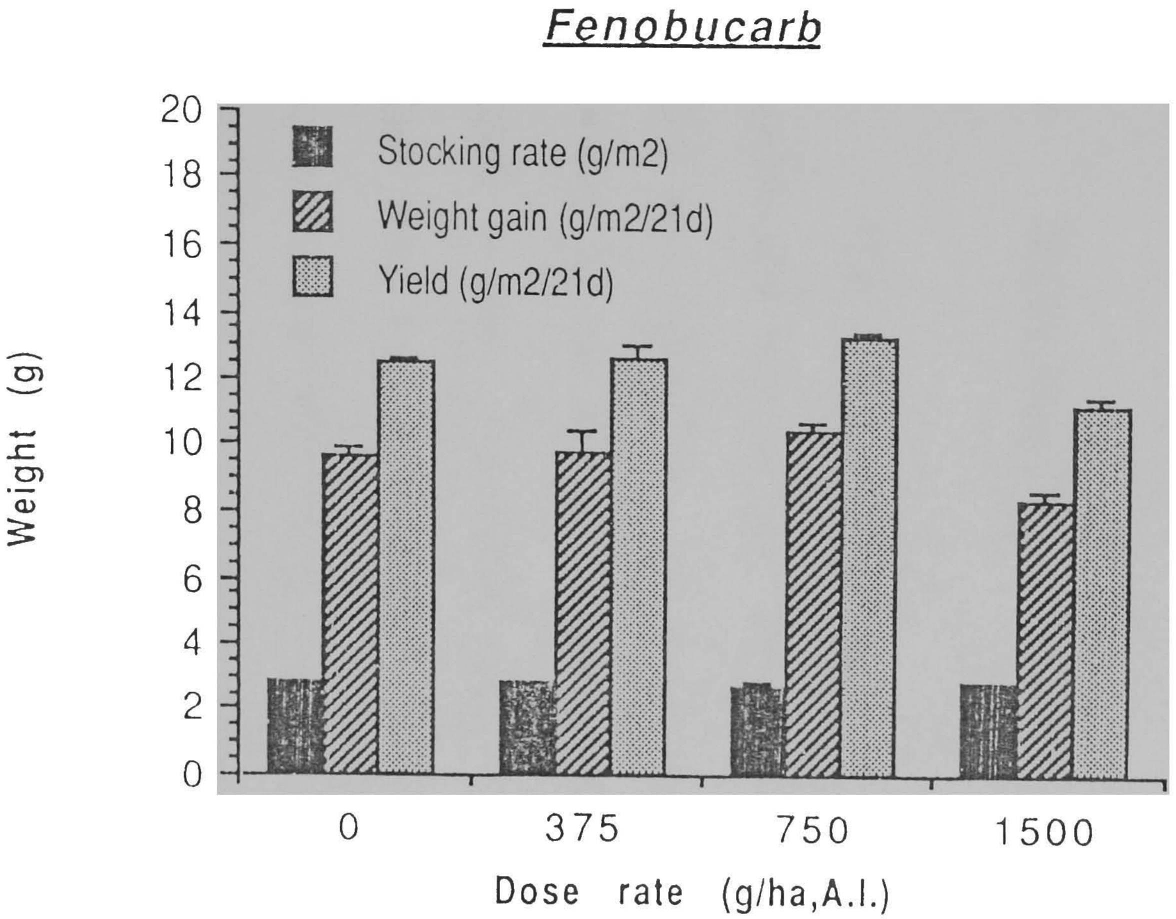


FIGURE 5.2. Production of common carp fingerlings (*C. carpio*) in wet rice fields, receiving different dose rates of *fenobucarb* (Means and Standard Error).

The reason for this phenomenon is currently not clear, but possibly due to physiological effect(s) of low dose rates on the experimental fish or to the availability of fish food organisms in the rice fields.

5.3.1.4 Water quality

The physico–chemical characteristics of the rice field water (with and without fish) during the experiment are presented in Table 5.3 and Table 5.4. The water quality throughout the experiment was found to be within acceptable limits for fish growth and production in wet rice fields (see Section 4.3.7). There was no indication that the water quality parameters measured during the experiment were significantly influenced by the application of *fenobucarb* dose rates ($p > 0.05$). Statistical analysis of variance also suggested that water quality in the rice field plots was generally not influenced by the presence of fish or by the time of analysis ($P > 0.05$).

Water temperature in the rice field plots measured during the experiment fluctuated between 25.5°C and 32°C, which was normal in the wet rice system, due to the shallow depth of the rice field water, and the coverage of aquatic vegetation preventing the

TABLE 5.3. The physico- and chemical characteristics of water in the rice fields (with fish), measured during Experiment 1 (*Fenobucarb*) (Mean and range values)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	375	750	1500			
Temperature (^{o}C)	28.7 (26.0-32.0)	28.6 (26.0-31.0)	28.6 (26.0-32.0)	28.6 (26.0-32.0)	0.16	NS	> 0.05
Dissolved oxygen (mg/l)	9.8 (6.2-15.0)	9.7 (4.5-15.0)	9.6 (6.2-15.0)	9.6 (5.6-15.0)	0.91	NS	>0.05
Carbondioxide (mg/l)	1.44 (0-4.60)	1.32 (0-5.99)	1.73 (0-6.59)	1.31 (0-5.59)	1.75	NS	> 0.05
pH	8.0 (7.0-10.0)	8.1 (7.0-10.0)	7.9 (7.0-9.5)	8.1 (7.0-10.0)	2.05	NS	> 0.05
Total hardness (mg/l)	56.0 (50.6-68.2)	56.0 (44.0-72.6)	57.8 (50.6-72.6)	54.3 (50.6-63.8)	2.12	NS	> 0.05

TABLE 5.3 (Continued)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	375	750	1500			
Phosphorus (mgl^{-1})	0.021 (0.013-0.031)	0.022 (0.013-0.033)	0.019 (0.014-0.032)	0.023 (0.013-0.044)	0.87	NS	> 0.05
Total ammonia (mgl^{-1})	0.044 (0.014-0.082)	0.037 (0.011-0.072)	0.047 (0-0.099)	0.037 (0.011-0.066)	0.33	NS	> 0.05
Nitrite (mgl^{-1})	0.234 (0.120-0.360)	0.262 (0.140-0.400)	0.192 (0.110-0.440)	0.169 (0.100-0.220)	1.73	NS	> 0.05
Suspended solid (mgl^{-1})	15.8 (1.4-40.0)	15.9 (2.5-37.3)	18.7 (6.0-46.0)	13.9 (1.4-33.0)	0.14	NS	> 0.05

NOTE NS - Not significant

TABLE 5.4. The physico- and chemical characteristics of water in the rice fields (without fish), measured during Experiment 1 (*Fenobucarb*). (Mean and range values).

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	375	750	1500			
Temperature (°C)	28.8 (25.5-31.0)	28.7 (25.5-31.50)	28.7 (26.0-32.0)	28.2 (26.0-31.0)	0.16	NS	> 0.05
Dissolved oxygen (mg/l)	10.1 (7.1-14.8)	10.2 (6.0-15.0)	10.3 (6.0-14.8)	10.2 (4.2-15.0)	0.91	NS	> 0.05
Carbondioxide (mg/l)	1.15 (0-4.59)	0.88 (0-3.60)	1.57 (0-5.59)	1.08 (0-9.59)	1.75	NS	> 0.05
pH	8.1 (6.5-10.0)	8.0 (7.0-10.0)	7.9 (7.0-9.5)	8.4 (7.0-10.0)	2.05	NS	> 0.05
Total hardness (mg/l,$CaCO_3$)	54.2 (46.2-79.2)	58.0 (44.0-74.8)	50.6 (44.0-59.4)	55.4 (47.4-68.4)	2.12	NS	> 0.05

TABLE 5.4 (Continued)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	375	750	1500			
Total phosphorus (mgl^{-1})	0.023 (0.014-0.066)	0.017 (0.014-0.025)	0.025 (0.016-0.038)	0.021 (0.014-0.030)	1.73	NS	> 0.05
Total ammonia (mgl^{-1})	0.032 (0-0.066)	0.030 (0-0.069)	0.030 (0.011-0.069)	0.028 (0-0.059)	0.87	NS	> 0.05
Nitrite (mgl^{-1})	0.233 (0.09-0.42)	0.219 (0.10-0.44)	0.220 (0.13-0.36)	0.210 (0.13-0.32)	0.33	NS	> 0.05
Suspended solid (mgl^{-1})	13.0 (3.0-40.0)	14.1 (2.5-38.0)	12.7 (1.6-48.0)	16.8 (1.1-58.0)	0.14	NS	> 0.05

NOTE : NS - Not significant

sunlight comes in direct contact with the water (Figure 5.3).

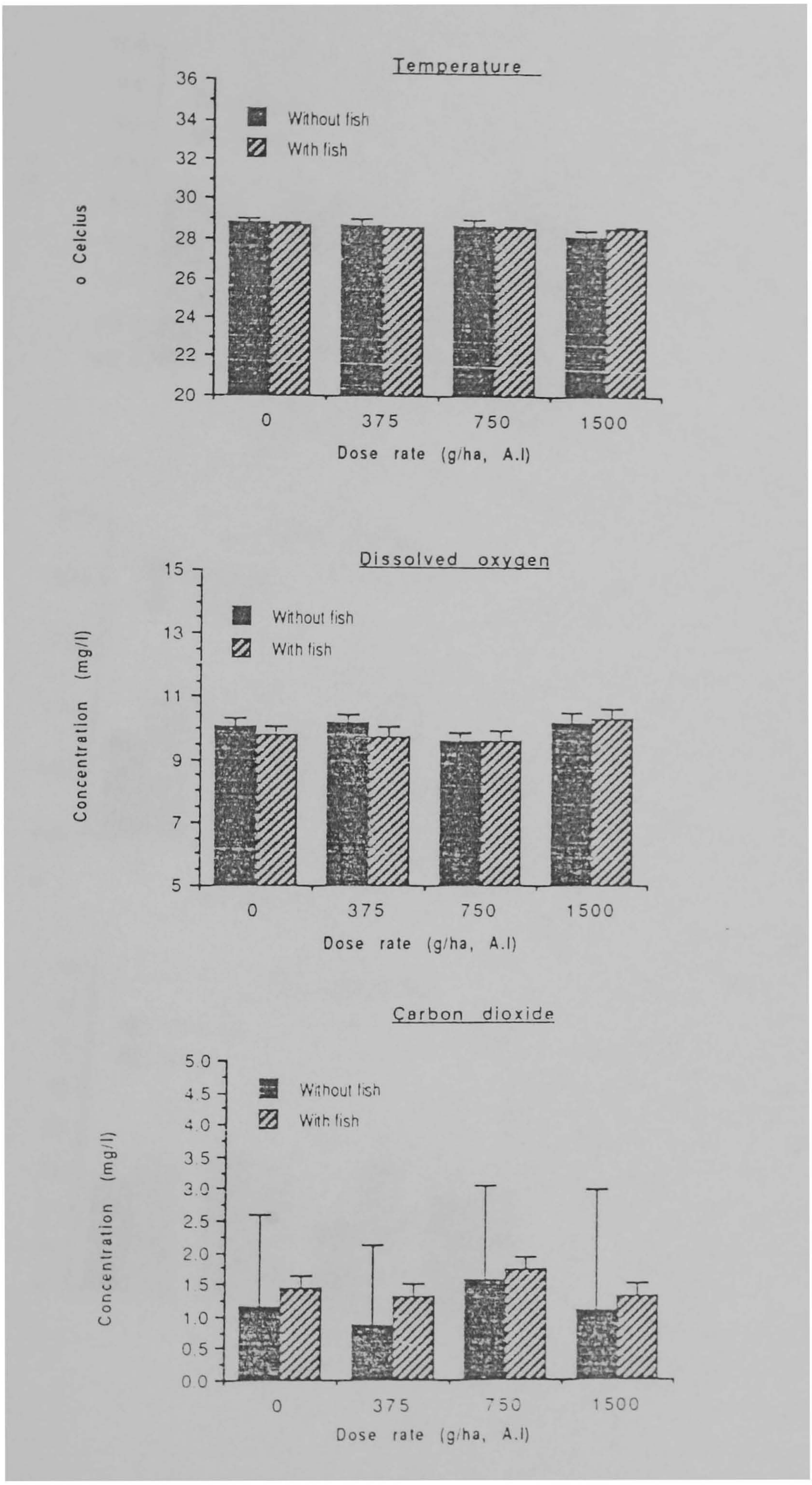
Dissolved oxygen fluctuated widely during the experiment, ranging between 4.5mgl^{-1} (58% saturation) and 15mgl^{-1} (190% saturation), mainly due to the elevation of gross photo-synthesis during midday (Figure 5.3).

Carbon dioxide concentration in the rice field water during the experiment did not exceed 9.6 mgl^{-1} (Figure 5.3).

Mean water pH was also found to fluctuate widely during the experiment, ranging between 6.5 and 10.0 ($P < 0.05$) (Figure 5.4).

Total hardness of the rice field water was found to range between 44.0mgl^{-1} and 79.2mgl^{-1} (Figure 5.4).

The maximum total ammonia concentration measured during the experiment was 0.099mgl^{-1}. There was no evidence that this total ammonia concentration effected the fish in the rice field plots (Figure 5.4).

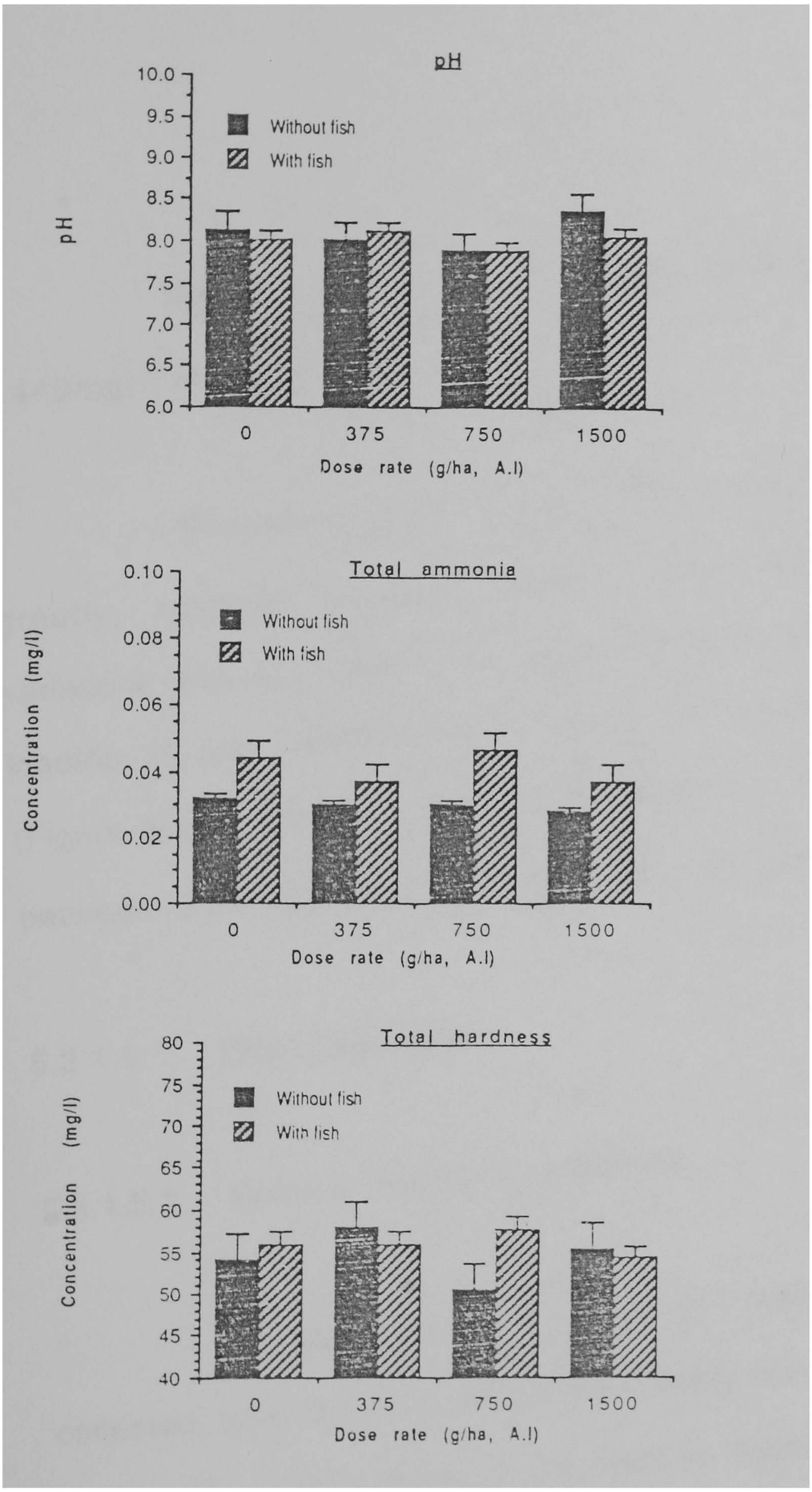


Temperature

Dissolved oxygen

Carbon dioxide

FIGURE 5.3. Levels of water temperature, dissolved oxygen and carbon dioxide concentrations in the rice fields, measured during Experiment 1 (Means and Standard Deviation).



URE 5.4. Levels of pH, total hardness and total ammonia in the rice fields, measured during Experiment 1 (Means and Standard Deviation).

Nitrite concentrations ranged between 0.09mgl^{-1} and 0.440mgl^{-1} (Figure 5.5).

Suspended solid concentrations were found to fluctuate greatly, ranging between 3.0mgl^{-1} and 58.0mgl^{-1}. This large variations was accountable by the occurrence of rainfall and organic loading of the irrigation water during the course of the experiment (Figure 5.5). Phosphorus concentration in the rice field plots ranged between 0.013mgl^{-1} and 0.440mgl^{-1} (Figure 5.5).

5.3.1.5 Rice field biota

5.3.1.5.1 Benthic macro-invertebrate

A total of nine taxa of macro-invertebrates were collected from the rice field plots during the experiment. Table 5.5 shows the major taxa of the benthic fauna in the rice field plots with fish and without fish. The dominant group of the fauna consisted of three genera of Oligochaeta, *Tubifex* (Lamarck,1816), *Branchiura* (Beddard,1892) and *Nais* (Muller,1773). Figure 5.6 summarised the temporal changes in the abundance of oligochates and chironomid

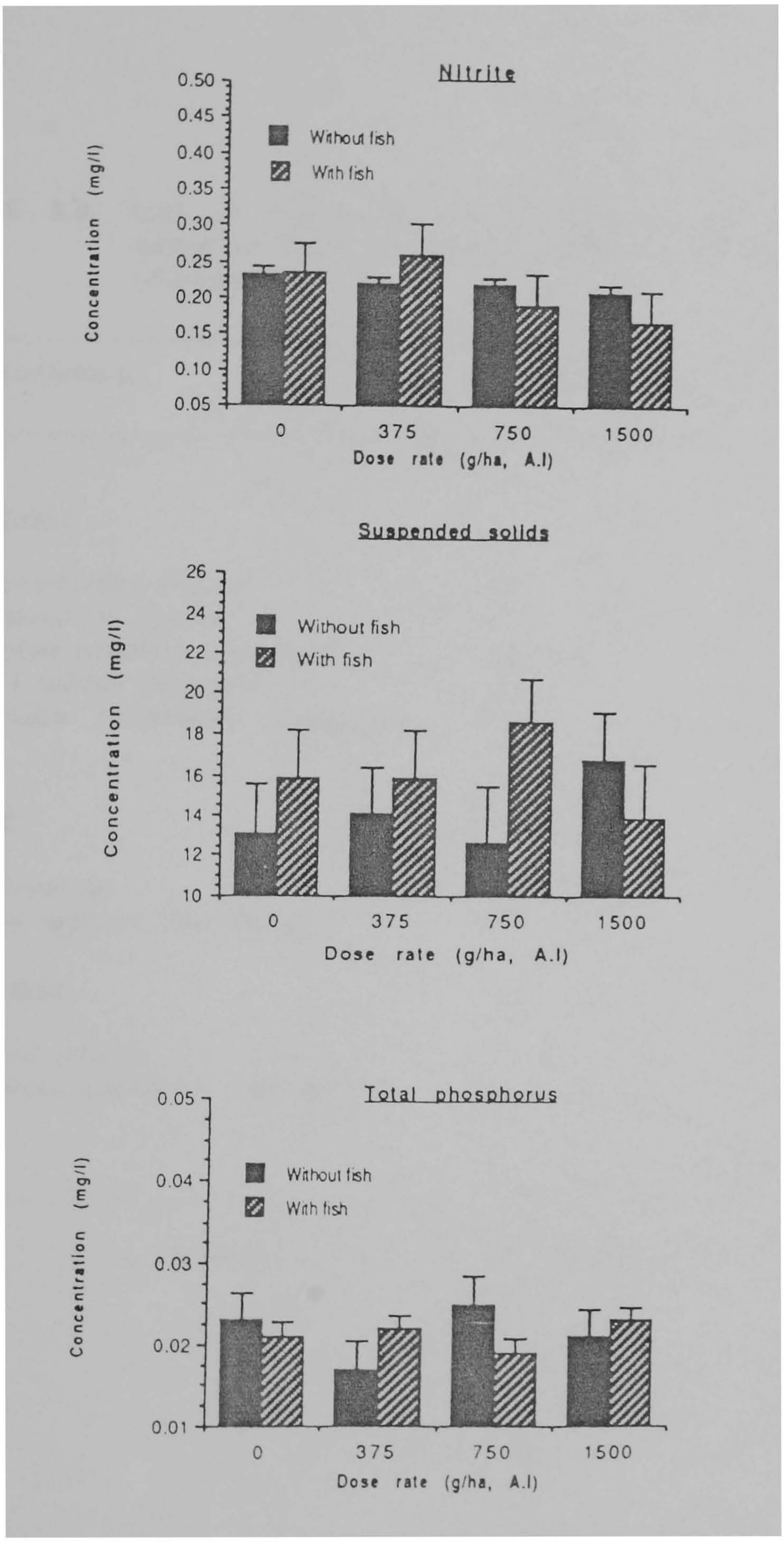


FIGURE 5.5. Levels of nitrite, total phosphorus and suspended solid in the rice fields, measured during Experiment 1 (Means and Standard Deviation).

TABLE 5.5. List of species/genera of benthic macro-invertebrates collected from rice fields plots during Experiment 1 (*Fenobucarb*).

Species/Genera	Rice field with fish	Rice field without fish
Oligochaeta		
Nais communis (Piguet)	+	++
Lumbriculus sp (Grube)	+	+
Branchiura sowerbyi (Beddard)	++	++
Tubifex tubifex (Muller)	++	+
Limnodrilus hoffmeisteri (Claparede)	+	
Insecta		
Chironomus sp.	+	+
Cybister rugosus (Mac Leay)	+	+
Gastropoda		
Bellamya javanica	+	+
Melanoides tuberculata (Muller)	+	+

NOTE : + - Presence ++ - Dominant

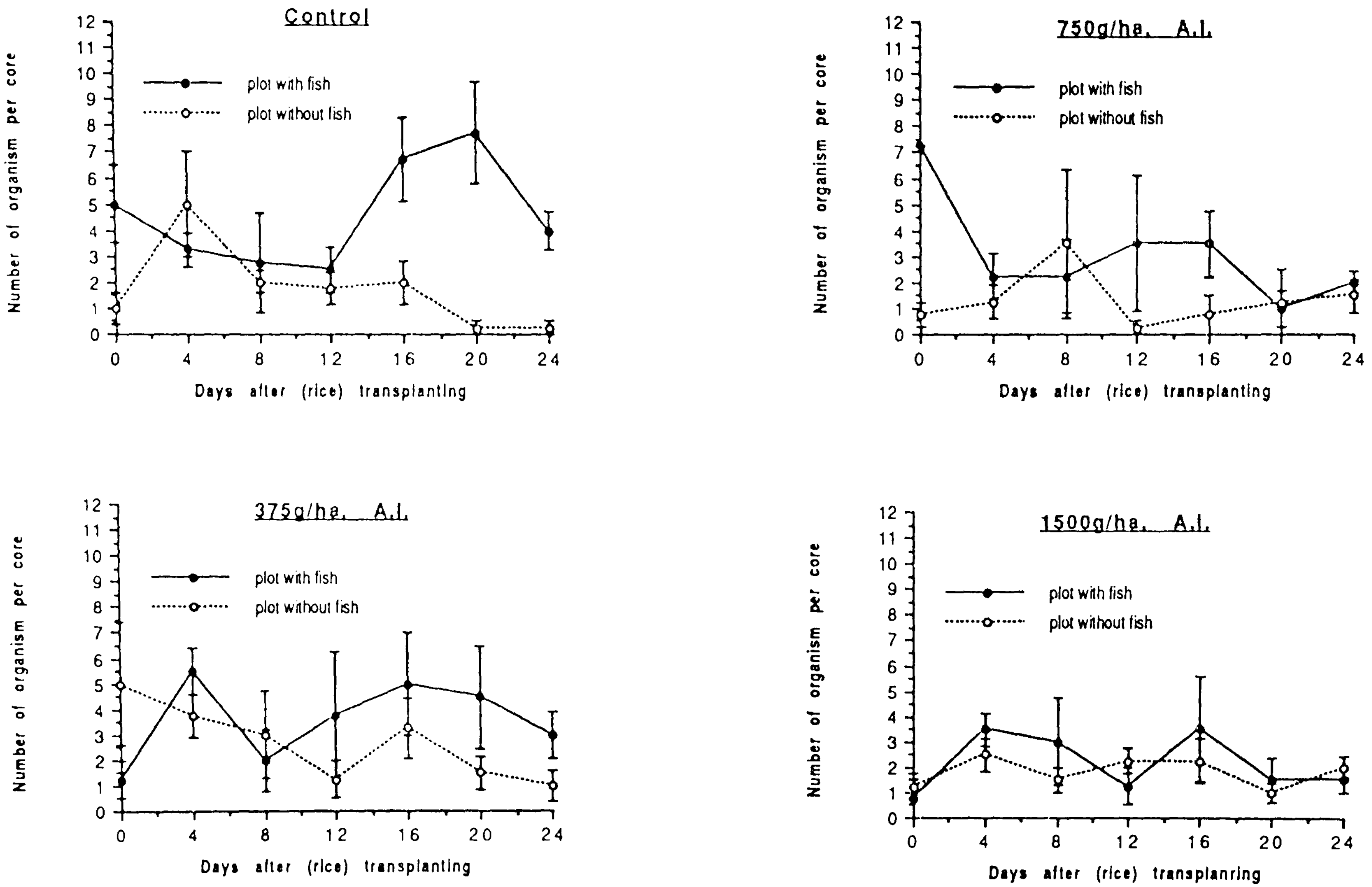


FIGURE 5.6. Abundance of benthic macro organisms in wet rice fields treated with different dose rates of *fenobucarb* (Means and Standard Error).

larvae, as the main important benthic organisma in terms of fish culture (ALIKUNHI,1966) in the rice fields subjected to different dose rates of *fenobucarb*. Due to the methodology of collecting the samples of the organisms used during the experiment (small number of samples without replication for each treatment) the data did not provide quantitative information on the abundance of benthic fauna in the rice field plots. However, some qualitative information could be obtained from the data. The density of macro invertebrates in rice field plots was consistently higher in plots with fish than those without fish. This was particularly apparent in the untreated control plots and in plots receiving 375gha^{-1} *fenobucarb* treatment at 16 and 20 days after rice transplanting. .The population of the organisms in all rice field plots showed some variation, but with no apparent overall trend. There was no indication of a definite pattern of changes in the abundance of the rice field benthic fauna (i.e oligochaetes and chironomid larvae) associated with the treatment of *fenobucarb* dose rates during the experiment.

5.3.1.5.2 Zooplankton

A total of 13 taxa was found in the zooplankton samples collected from the experimental rice fields, which was dominated by two main groups, the Rotifera and the Crustacea (Table 5.6). Some of the organisms were not identified to their species levels, due to the condition of the samples. The copepod *Cyclops sp.*and the Nauplius larvae were found in abundance, presumably partly due to the effects of chicken manure applied to the system. The cladoceran *Moina micrura* Kutz. was also frequently collected, although in less density than the copepodes. The rotifer *Brachionus spp* which is also an important food for common carp fry, was present in moderate number during the course of this experiment.

The temporal changes in the abundance of crustaceans (copepods and cladocerans) and rotifers in the rice field plots (with fish and without fish), subjected to different dose rates of *fenobucarb*, are illustrated in Figure 5.7 and Figure 5.8, respectively. There were wide variations in the abundance of copepod and rotifer populations in the rice fields, both in plots with fish and without fish. An increasing population density of the copepods was noted in all rice field plots, 8 to 10 days after rice transplanting.

TABLE 5.6. List of species/genera of zooplankton collected frpm rice field plots during Experiment 1 (*Fenobucarb*).

Species/Genera	Rice field with fish	Rice field without fish
<u>Protozoa</u>		
Arcella hemispherica (Perty)	+	+
Centropyxis aculeata (Stein)	+	+
Difflugia pyriformis (Perty)	+	+
<u>Rotifera</u>		
Asplanchna sp. (Gosse)	+	+
Brachionus patulus (Muller)	+	++
Filinia longiseta (Ehrenberg)	+	
Lecane bulla (Gosse)	++	+
Monostyla sp. (Ehrenberg)	+	+
<u>Crustacea</u>		
Cyclops sp.	++	++
Mcina daphnia macleayi (King)	+	+
Nauplius larvae	++	++
Cypris sp.	+	+
<u>Insecta</u>		
Chironomidae (Tendipedidae)	+	+

NOTE : + - Presence ++ - Dominant

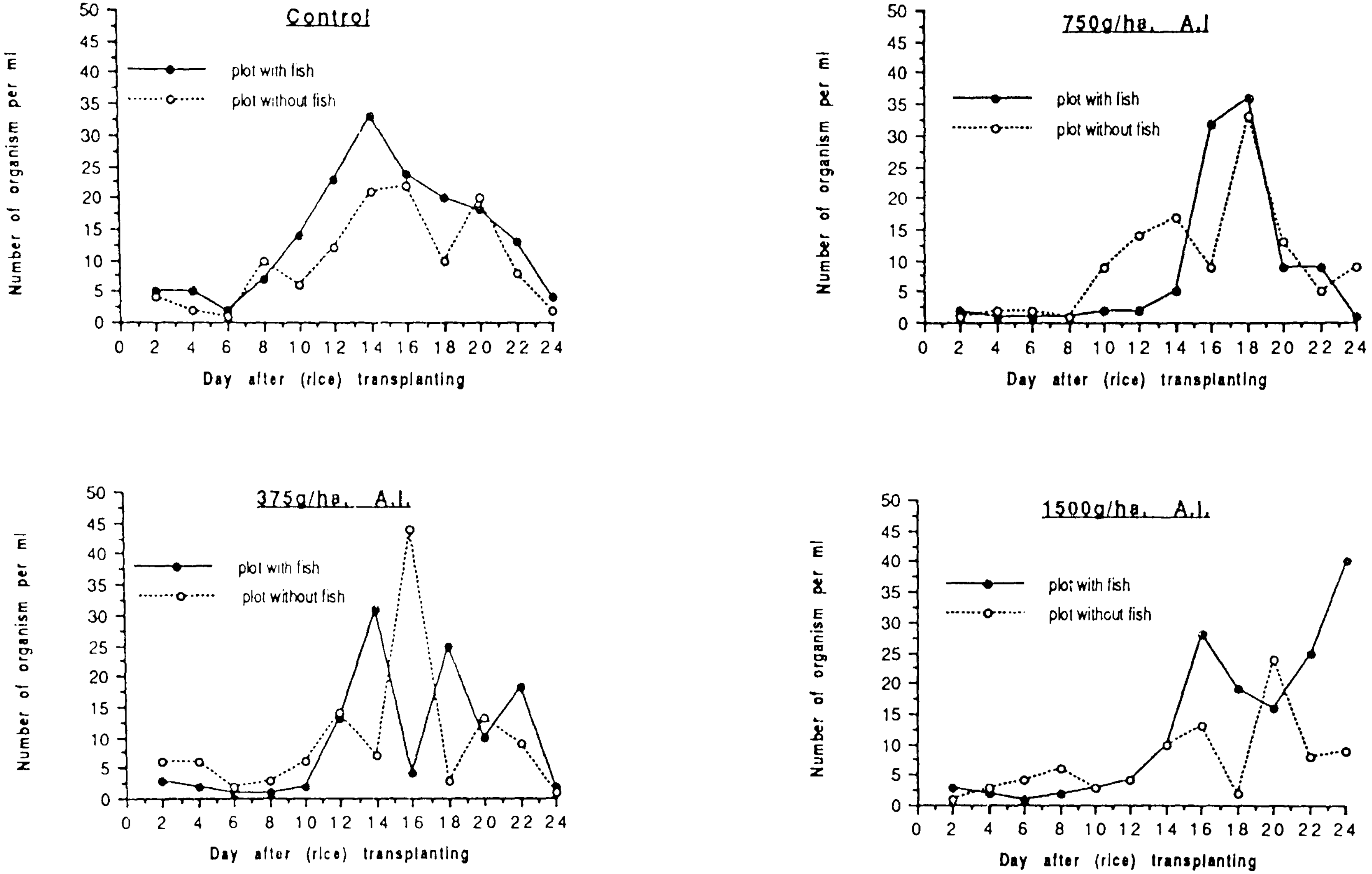


FIGURE 5.7. Abundance of copepod s in wet rice fields treated with different dose rates of *fenobucarb*.

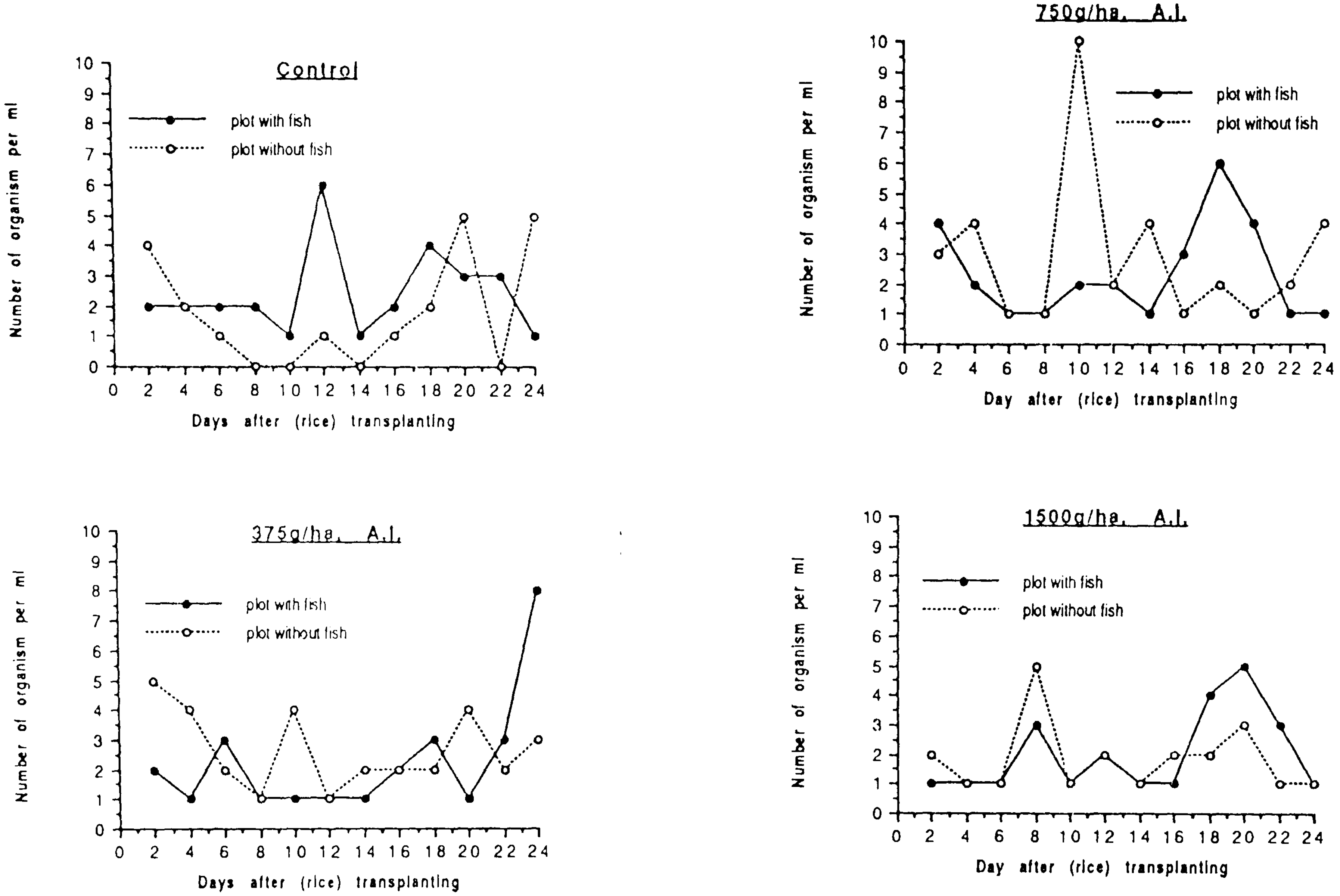


FIGURE 5.8. Abundance of rotifers in wet rice fields treated with different dose rates of *fenobucarb*.

There was, however, no evidence of a definite pattern in the changes of the crustacean and the rotifers population density, in both insecticide treated and untreated rice field plots.

5.3.2 Experiment 2 (*Isoprocarb*)

5.3.2.1 Growth rate

The initial weight, the final weight and the growth rate of common carp in rice field plots exposed to different dose rates of *isoprocarb*, are presented in Table 5.7. The mean final weight and the mean growth rate of fish between the treatment plots were not significantly different ($P > 0.05$). The mean final weights of fish in the treatment plots ranged between 21.1g and 23.9g, except in the rice field plots receiving the dose rate of 1000gha^{-1} *isoprocarb*. The mean growth rate of fish in the treatment plots also showed little variation, ranging between 0.82gd^{-1} and 0.89gd^{-1}, except in plots treated with the insecticide dose rate of 1000gha^{-1}. In these treatment plots, the highest fish final weight of 26.6g and the highest fish growth rate of 0.97gd^{-1} were obtained (Figure 5.9). The reason for this may be explained by the lower survival rate of fish in

TABLE 5.7. Mean initial/final weights and mean growth rate (in terms of absolute weight increment per day) of common carp fingerlings in rice fields receiving different dose rates of *isoprocarb*.

	Dose rate (gha^{-1}, A.I.)				F	Significant	Probability
	0	500	1000	2000			
Initial weight (g)	5.0	5.0	5.0	4.9	0.64	NS	> 0.05
Standard error	0.1	0.1	0.1	0.1			
Final weight (g)	22.1	23.3	26.6	23.9	0.72	NS	> 0.05
Standard error	2.0	1.9	1.4	2.0			
Growth rate (gd^{-1})	0.82	0.87	0.97	0.89	0.72	NS	> 0.05
Standard error	0.07	0.02	0.13	0.05			

NOTE NS - Not siginificant

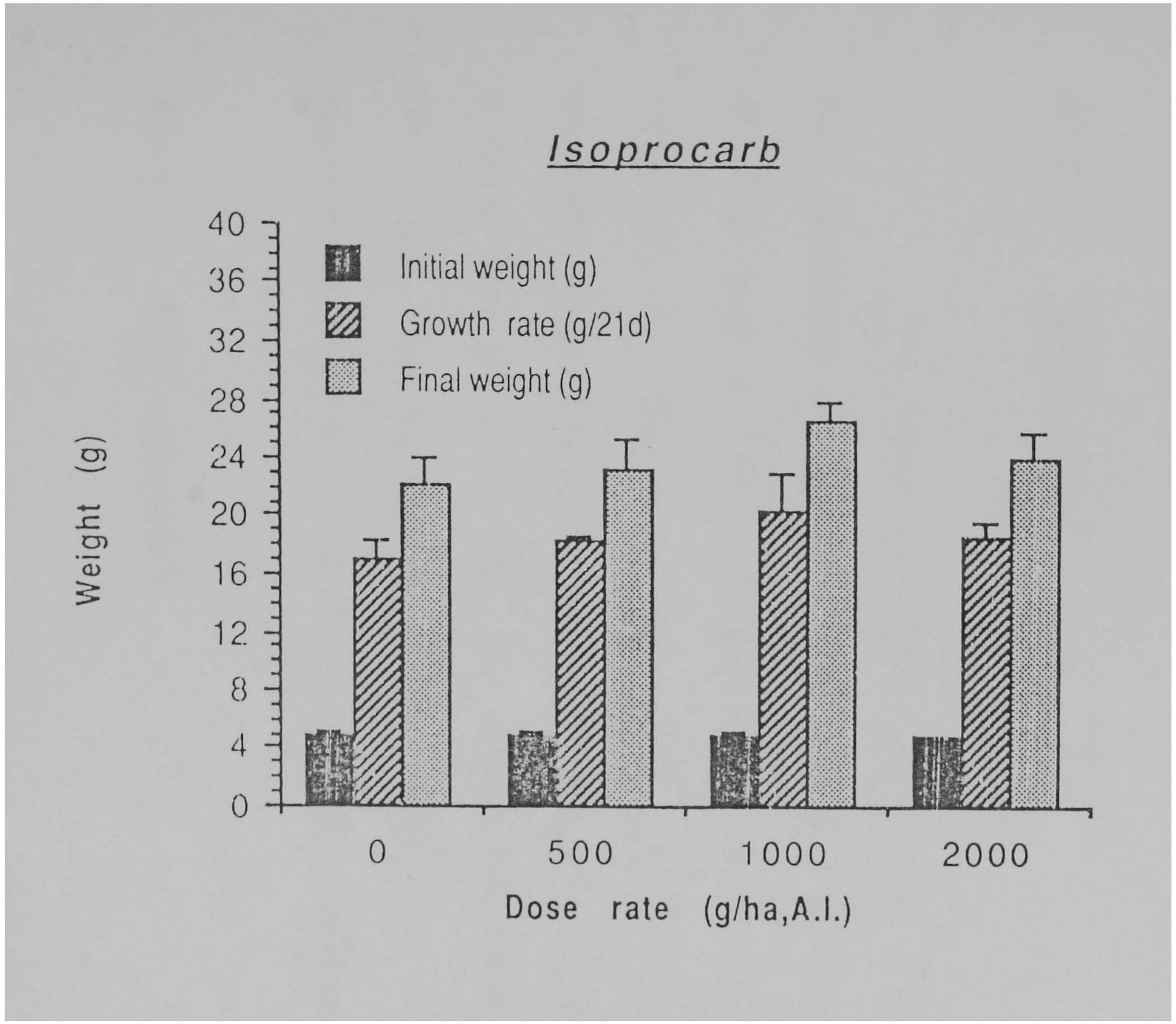


FIGURE 5.9. Growth rate of common carp fingerlings (*C. carpio*) in wet rice fields, receiving different dose rates of *isoprocarb* (Means and Standard Error).

these plots, which secured additional space and food organisms for surviving fish.

5.3.2.2 Survival rate

The mean survival rate of fish in the treatment plots was, generally about 90%. The lowest fish survival rate recorded (88.3%) was in the rice field plot receiving the dose rate of $100gha^{-1}$, A.I. Based on the result of the statistical analysis of variance there was, however, no apparent difference between the survival rate of fish in the dose treatments used in the experiment ($P > 0.05$). The fish survival, total yield and weight gain of fish biomass obtained from the experiment are summarised in Table 5.8.

5.3.2.3 Fish production

The stocking densities of common carp used in the treatment plots were not significantly different ($P > 0.05$), ranging between $2.46gm^{-2}$ and $2.50gm^{-2}$. The total yield of fish biomass between treatment plots was also not significantly different ($P > 0.05$). The mean total yield of fish biomass ranged between

TABLE 5.8. Summary of survival rate, biomnss yield and biomass weight gain of common carp fingerlings in rice fields receiving different dose rates of *isoprocarb* (Means and standard error).

	Dose rate (gha^{-1}, A.I.)				F	Significant level	Probability
	0	500	1000	2000			
Stocking density (gm^{-2})	2.49	2.49	2.50	2.46	0.59	NS	> 0.05
Standard error	0.01	0.06	0.03	0.03			
Survival rate (%)	90.0	90.0	88.3	95.0	0.20	NS	> 0.05
Standard error	4.1	4.7	5.9	2.4			
Total yield (gm^{-2}21d^{-1})	9.9	10.4	11.2	11.4	0.46	NS	> 0.05
Standard error	0.6	0.5	1.6	0.8			
Weight gain (gm^{-2}d^{-1})	0.35	0.42	0.42	0.43	0.48	NS	> 0.05
Standard error	0.04	0.02	0.07	0.04			

NOTE NS - Not significant

9.95$gm^{-2}21d^{-1}$ and 11.40$gm^{-2}21d^{-1}$. There was also no apparent difference between the weight gain of fish biomass in the treatment plots ($P > 0.05$), which ranged between 0.35$gm^{-2}d^{-1}$ and 0.43$gm^{-2}d^{-1}$. The highest total yield and weight gain of fish biomass (which were 11.40$gm^{-2}21d^{-1}$ and 0.43gm–2d^{-1}, respectively), were attained in he rice field plots treated with the highest *isoprocarb* dose rate of 2000gha^{-1} (Figure 5.10).

5.3.2.4 Water quality

The physico–and chemical characteristics of the rice field water, with and without fish, measured during the experiment are presented in Table 5.9 and Table 5.10. The water quality in the rice field plots was found to be within the acceptable limits for fish in wet rice systems. There was no indication that the water quality parameters measured, were significantly influenced by insecticide dose rates, or by other factors (i.e the presence of fish in the rice field and the time of the analysis) during the experiment ($P > 0.05$).

The mean water temperature in the treatment plots were almost identical, ranging between 26.7°C and 27.0°C. The minimum

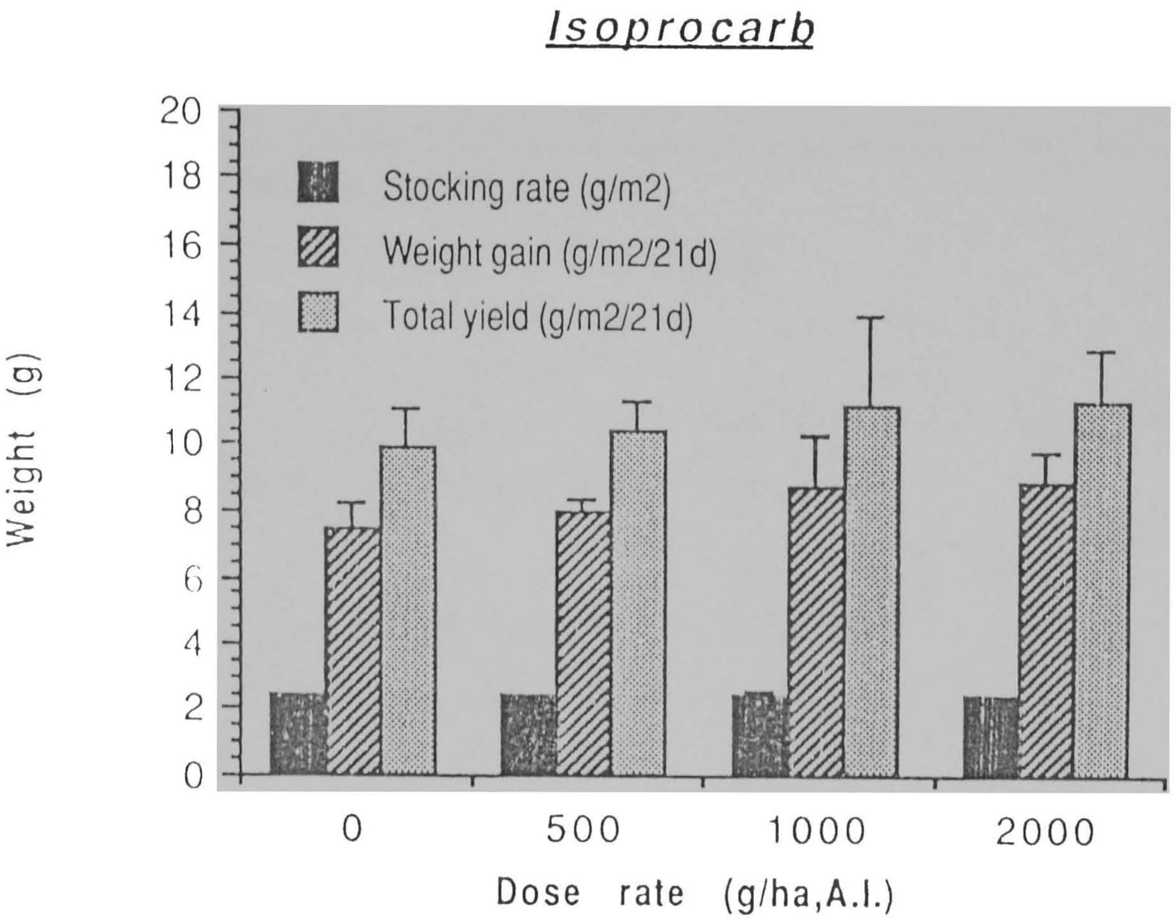


FIGURE 5.10. Production of common carp fingerlings (*C. carpio*) in wet rice fields, receiving different dose rates of *isoprocarb* (Means and Standard Error).

TABLE 5.9. The physico -and chemical characteristics of water in the rice fields (with fish), measured during Experiment 2 (*Isoprocarb*) (Mean and range values)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	500	1000	2000			
Temperature (^{o}C)	26.9 (26.0-29.0)	26.8 (24.0-29.0)	26.7 (25.5-28.0)	27.0 (25.0-29.0)	2.26	NS	> 0.05
Dissolved oxygen (mgl^{-1})	9.6 (4.8-15.0)	8.7 (4.8-15.0)	10.1 (4.4-15.0)	10.0 (5.0-15.8)	0.28	NS	> 0.05
Carbondioxide (mgl^{-1})	5.09 (0-13.40)	4.75 (0-13.80)	4.55 (0-14.20)	5.18 (0-14.20)	0.27	NS	> 0.05
pH	7.4 (7.0-9.5)	7.3 (7.0-9.5)	7.5 (7.0-9.5)	7.5 (7.0-9.5)	0.47	NS	> 0.05
Total hardness (mgl^{-1}.CaCO$_3$)	55.2 (48.4-61.6)	54.4 (48.4-61.6)	54.4 (46.2-63.8)	55.5 (48.0-70.4)	0.25	NS	> 0.05

TABLE 5.9 (Continued)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	500	1000	2000			
Total alkalinity (mgl^{-1},CaCO$_3$)	42.3 (36.1-48.9)	42.8 (36.6-48.8)	42.2 (36.6-46.6)	42.1 (36.6-46.6)	0.12	NS	> 0.05
Phosphorus (mgl^{-1})	0.023 (0.016-0.050)	0.037 (0.011-0.140)	0.021 (0.016-0.032)	0.025 (0.014-0.045)	2.03	NS	> 0.05
Total ammonia (mgl^{-1})	0.023 (0-0.060)	0.025 (0-0.041)	0.027 (0-0.082)	0.022 (0-0.062)	0.26	NS	> 0.05
Nitrite (mgl^{-1})	0.177 (0.11-0.30)	0.204 (0.10-0.43)	0.165 (0.10-0.34)	0.266 (0.15-0.56)	2.41	NS	> 0.05
Suspended solid (mgl^{-1})	61.0 (22.7-98.0)	45.4 (10.8-73.0)	52.3 (10.0-95.0)	60.0 (20.0-90.0)	0.29	NS	> 0.05

NOTE Not significant

TABLE 5.10. The physical and chemical characteristics of water in the rice fields (without fish), measured during Experiment 2 (*Isoprocarb*) (Mean and range values)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	500	1000	2000			
Temparature (°C)	26.4 (25.0-29.0)	26.5 (25.0-28.0)	26.6 (25.0-29.0)	26.7 (25.0-29.0)	2.26	NS	> 0.05
Dissolved oxygen (mgl^{-1})	10.2 (4.4-15.0)	9.7 (4.8-15.0)	10.1 (4.4-15.0)	10.0 (5.0-15.0)	0.28	NS	> 0.05
Carbondioxide (ml^{-1}l)	4.85 (0-12.80)	5.12 (0-12.80)	4.83 (0-14.20)	4.65 (0-11.80)	0.27	NS	> 0.05
pH	7.3 (7.0-9.5)	7.2 (7.0-9.5)	7.4 (7.0-9.5)	7.3 (7.0-9.5)	0.47	NS	> 0.05
Total hardness (mgl^{-1},$CaCO_3$)	57.0 (48.4-77.0)	54.4 (46.2-63.8)	54.8 (46.2-61.6)	53.9 (46.2-61.6)	0.25	NS	> 0.05

TABLE 5.10 (Continued)

	Insecticide dose rate (gha^{-1}, A.I)				F .	Significant level	Probability
	0	500	1000	2000			
Total alkalinity (mgl^{-1},CaCO$_3$)	42.1 (36.6-46.2)	41.3 (36.6-46.6)	41.0 (36.1-46.2)	42.1 (36.7-46.2)	0.12	NS	> 0.05
Phosphorus (mgl^{-1})	0.023 (0.016-0.040)	0.024 (0.014-0.037)	0.024 (0.016-0.040)	0.021 (0.012-0.037)	2.03	NS	> 0.05
Total ammonia (mgl^{-1})	0.023 (0.013-0.049)	0.023 (0-0.059)	0.024 (0.015-0.049)	0.019 (0-0.060)	0.26	NS	> 0.05
Nitrite (mgl^{-1})	0.24 (0.07-0.41)	0.22 (0.10-0.34)	0.21 (0.10-0.40)	0.23 (0.06-0.55)	2.41	NS	> 0.05
Suspended solid (mgl^{-1})	56.9 (10.8-85.0)	60.8 (16.0-84.0)	58.0 (29.4-88.0)	58.8 (24.0-96.0)	0.29	NS	> 0.05

NOTE NS - Not significant

and maximum temperature recorded during the experiment were 24°C and 29°C, respectively (Figure 5.11).

Dissolved oxygen concentration in the rice field plots fluctuated between 4.4mgl^{-1} (54% saturation) and 15.8mgl^{-1} (195% saturation). There was no indication of dissolved oxygen problem during the experiment (Figure 5.11).

Carbon dioxide level measured in the rice field plots during the experiment never exceed 14.20mgl^{-1}. Mean carbon dioxide concentration in the treatment plots ranged between 4.55mgl^{-1} and 5.18mgl^{-1} (Figure 5.11).

The pH of the rice field water fluctuated between 7.0 and 9.5, but did not show apparent influence to the growth and yield of fish in the rice field plots. Mean pH values in the treatment plots ranged between 7.3 and 7.5 (Figure 5.12).

Total hardness of the rice field water ranged between

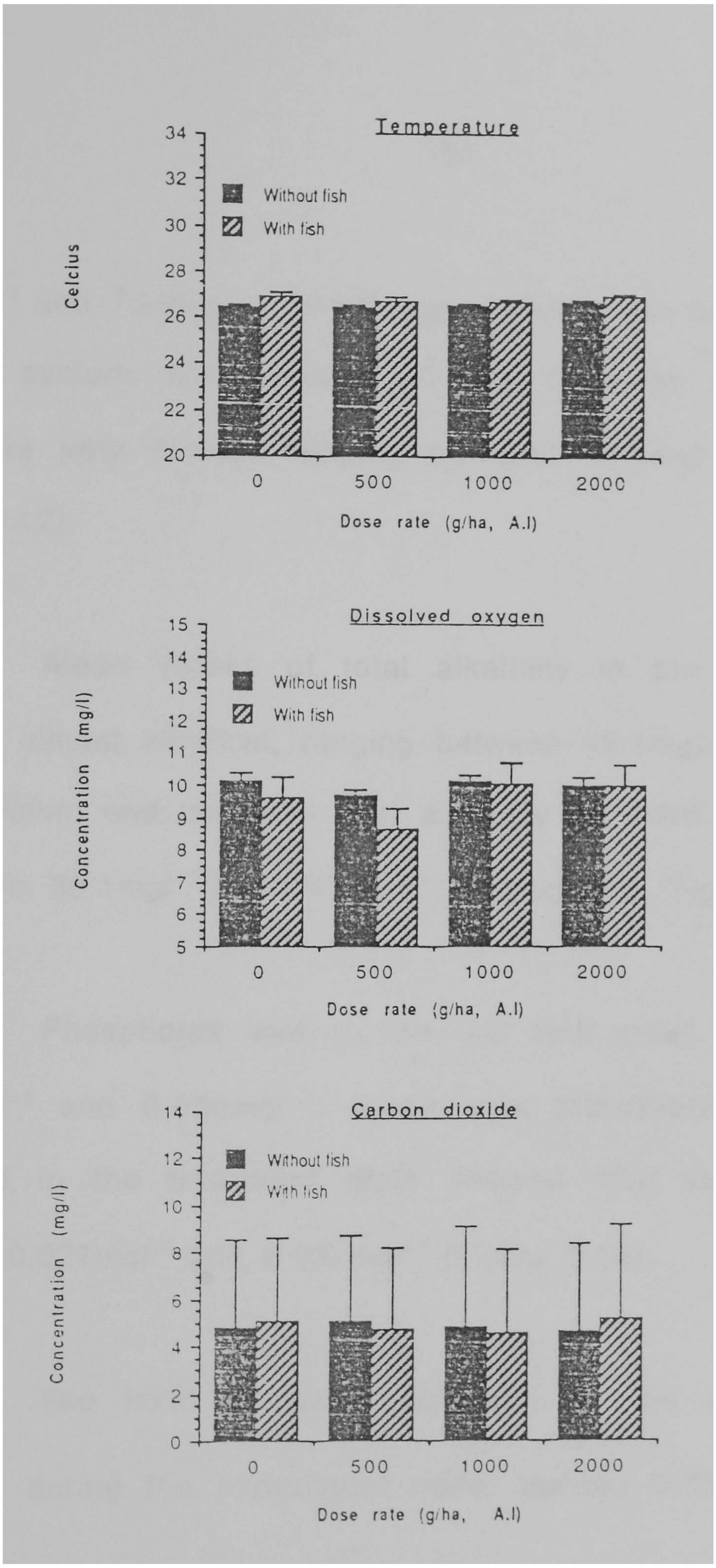


FIGURE 5.11. Levels of water temperature, dissolved oxygen and carbon dioxide concentrations in the rice fields, measured during Experiment 2 (Means and Standard Deviation).

46.2mgl^{-1} and 70.0mgl^{-1}, which was adequate for carp production in wet rice system. Mean values of total hardness in the treatment plots were very similar, ranging between 54.4mgl^{-1} and 55.5mgl^{-1} (Figure 5.12).

Mean values of total alkalinity in the treatment plots were als almost identical, ranging between 42.1mgl^{-1} and 42.8mgl^{-1}. The maximum and minimum total alkalinity recorded in the rice field plots were 36.1mgl^{-1} and 48.9mgl^{-1}, respectively (Figure 5.12).

Phosphorus level in the rice field water ranged between 0.011mgl^{-1} and 0.050mgl^{-1}. Mean total phosphorus concentration measured in the treatment plots showed little variation, ranging between 0.021mgl^{-1} and 0.037mgl^{-1} (Figure 5.13).

The level of total ammonium in the rice field water measured during the experiment never exceed 0.082mgl^{-1}, ranging between 0.022mgl^{-1} and 0.027mgl^{-1} (Figure 5.13).

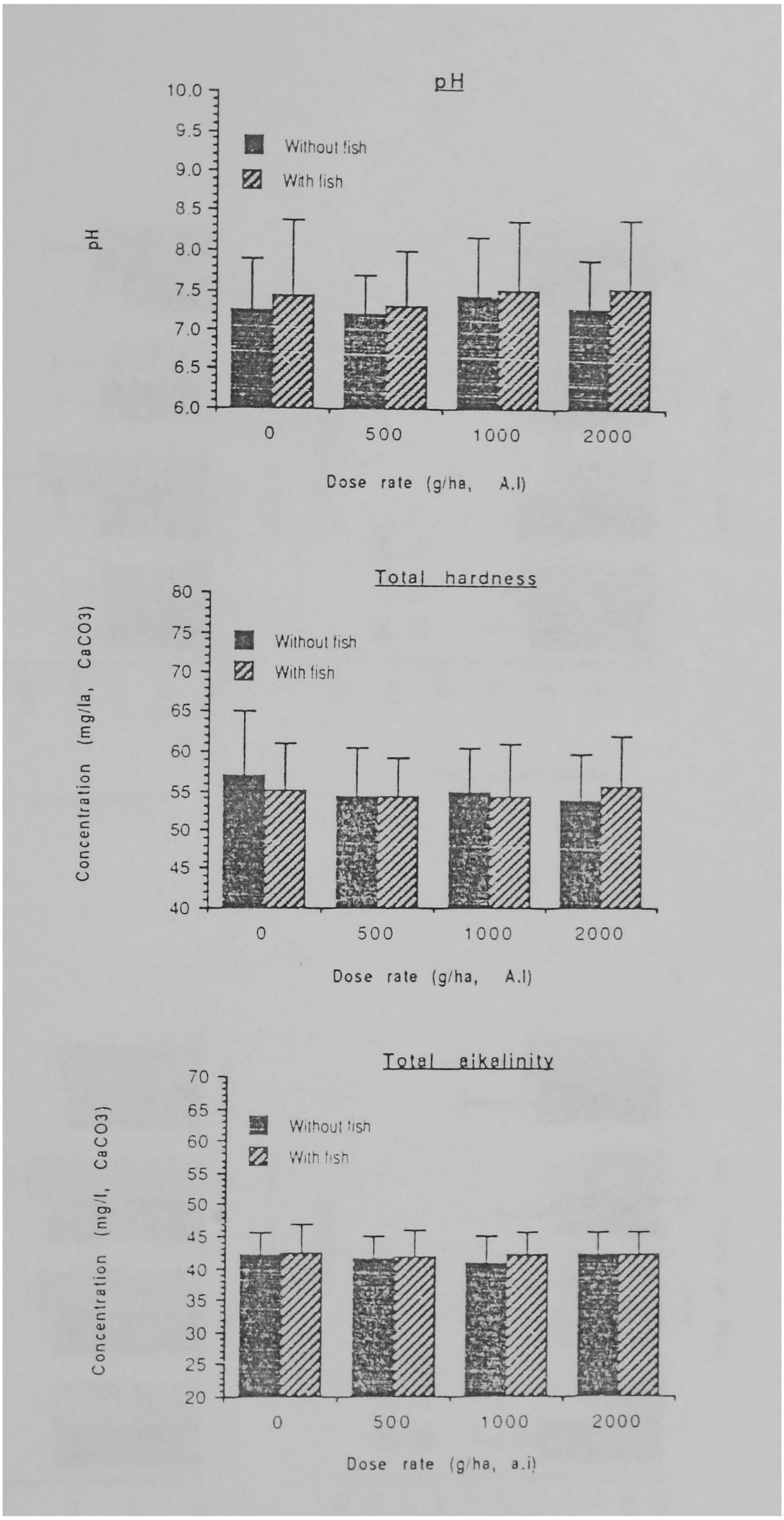


FIGURE 5.12. Levels of pH, total hardness and total alkalinity in the rice fields, measured during Experiment 2 (Means and Standard Deviation).

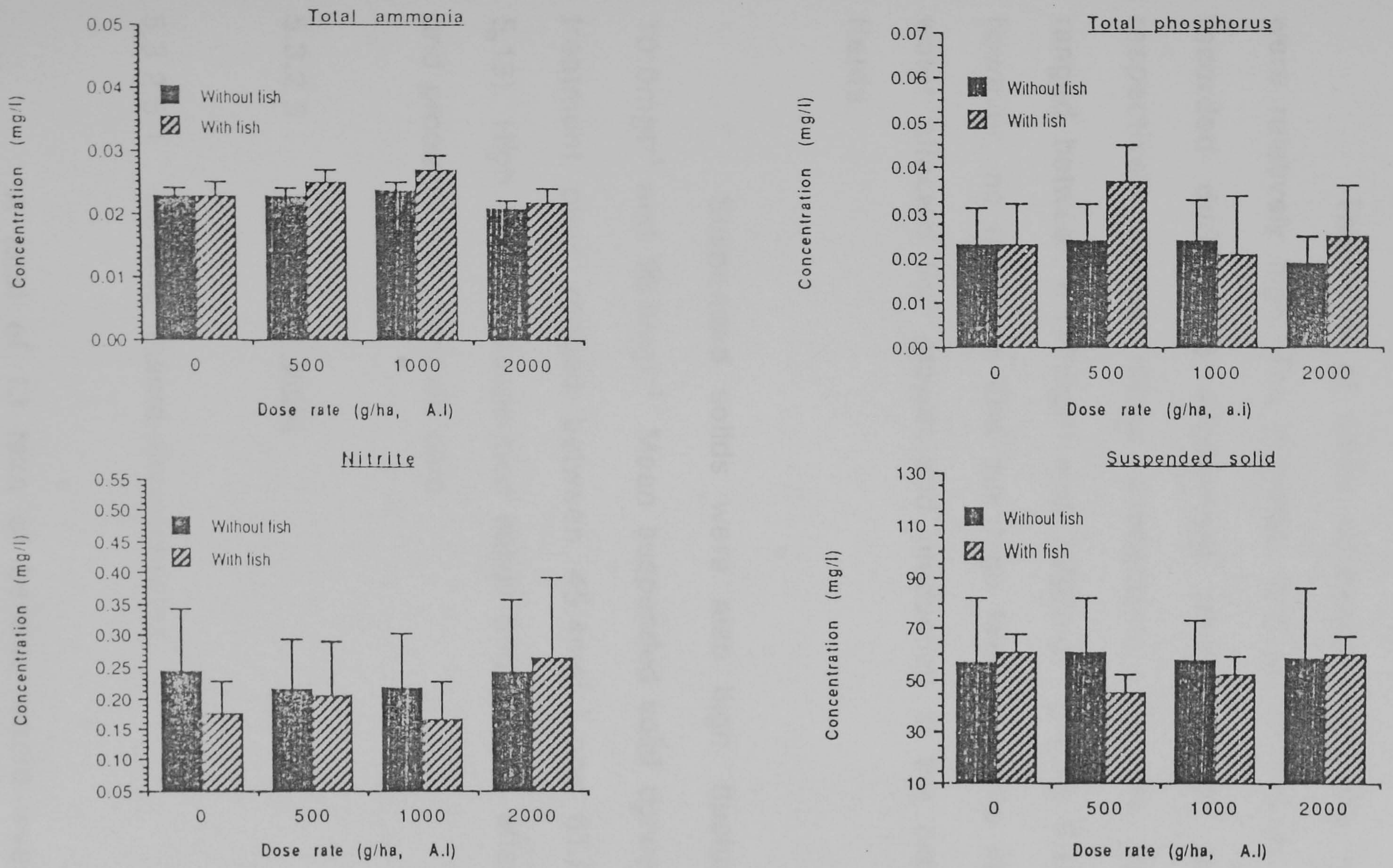


FIGURE 5.13. Levels of total ammonia, nitrite, total phosphorus and suspended solid in the rice fields, measured during Experiment 2 (Means and Standard Deviation).

The level of nitrite concentration in the rice field water were relatively high. The minimum and the maximum concentration recorded during the experiment were $0.1mgl^{-1}$ and $0.56mgl^{-1}$, respectively. Mean nitrite concentration in the treatment plots ranged between $0.177mgl^{-1}$ and $0.266mgl^{-1}$ (Figure 5.13). There was, however, no evidence that this high level of nitrite in the rice field water affected fish growth and production in the experimental rice fields.

Suspended solids were also high, fluctuating between $10.0mgl^{-1}$ and $98.0mgl^{-1}$. Mean suspended solid concentration in the treatment plots ranged between $45.4mgl^{-1}$ and $61.0mgl^{-1}$ (Figure 5.13). High level of suspended solids and did not affect their growth and production of common carp.

5.3.2.5 Rice field biota

5.3.2.5.1 Benthic macro-invertebrates

A total of 13 taxa of benthic macro-invertebrates were collected, comprising of 7 genera of Oligochaeta, 2 genera of

Hirudinae and 5 genera of Insecta. Table 5.11 presents the list of the benthic fauna in the rice field plots with fish and without fish collected during the experiment. Two genera of Oligochaeta represented the dominant group of the rice field benthic fauna, i.e *Nais* (Muller,1773) and *Tubifex* (Lamarck,1816). The larvae of the dipteran Chironomidae were also recorded frequently in the rice field plots, but at lower density than the oligochaetes.

Figure 5.14 summarises the temporal changes in the abundance of the benthic fauna oligochaetes and chironomid larvae in the treatment plots, which represented the main important of live food for common carp during the experiment. The abundance of macro invertebrates in all rice field plots showed some fluctuations, but with no apparent trends. There was also no notable difference in the population density of these organisms between the treatment plots.

5.3.2.5.2 Zooplankton

A total of 19 taxa of zooplankton were collected from the plankton samples, which included 5 genera of Protozoa, 9 genera of

TABLE 5.11. List of species/genera of benthic macro-invertebtrates collected from rice field plots during Experiment 2 (*Isoprocarb*).

Species/Genera	Rice field with fish	Rice field without fish
<u>Oligochaeta</u>		
Nais communis (Piguet)	++	++
Haplotaxis sp.	+	+
Lumbriculus sp.	+	+
Tubifex tubifex (Muller)	++	++
Limnodrilus hoffmeisteri (Claperede)	+	+
Dero limosa (Leidy)	+	
Branchiura sowerbyi (Beddard)	+	+
<u>Hirudinea</u>		
Glossiphonia weberi (Blanchard)	+	+
Helobdella sp.	+	+
<u>Insecta</u>		
Caenis sp.	+	+
Aeschnidae		+
Chironomus sp.	+	+
Tanypus sp.	+	+
Culex sp.	+	+

<u>NOTE</u> : + - Presence ++ - Dominant

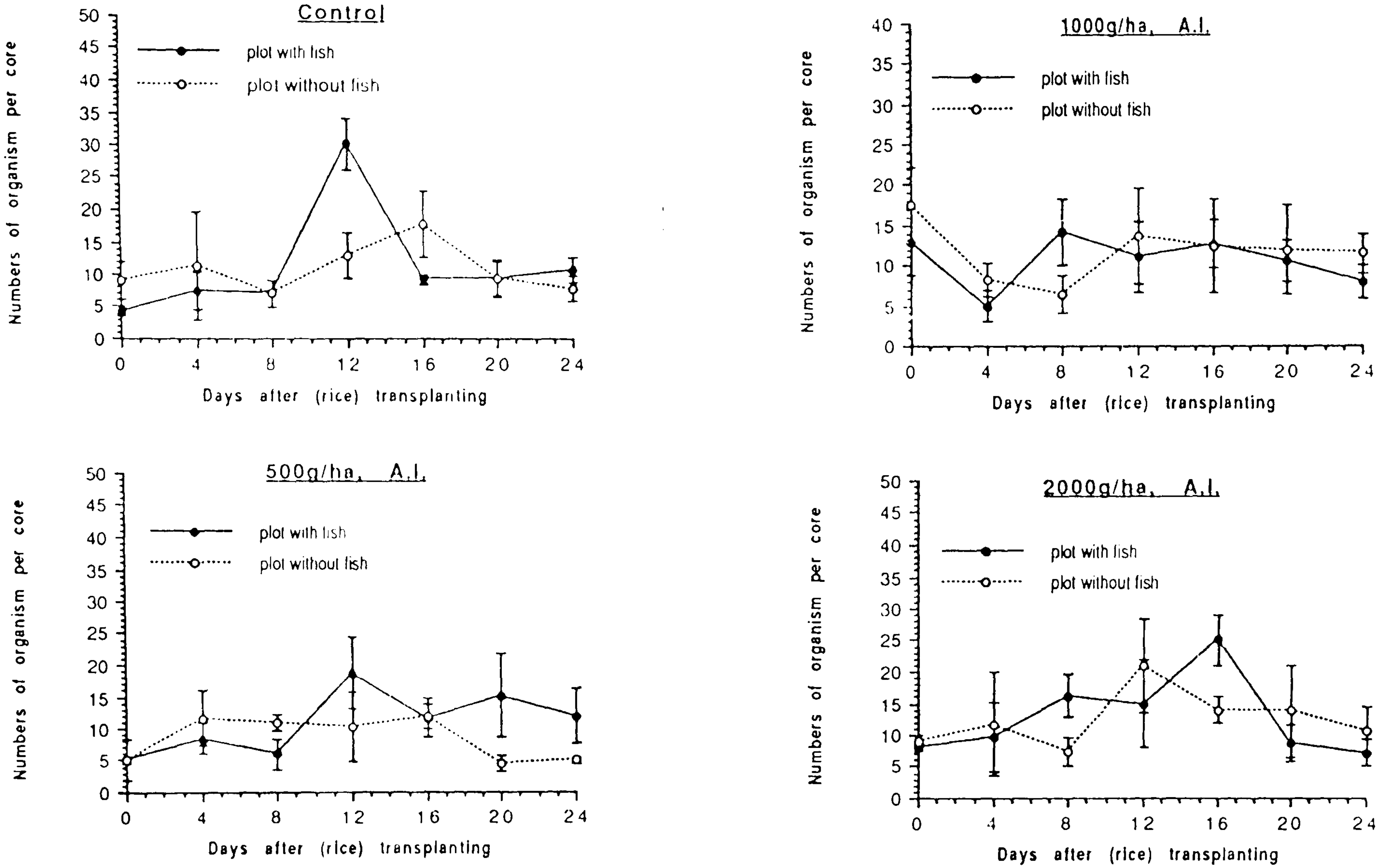


FIGURE 5.14. Abundance of benthic macro organisms in wet rice fields, treated with different dose rates of *isoprocarb* (Means and Standard Error).

TABLE 5.12. List of species/genera of zooplankton collected from rice field plots in Experiment 2 (*Isoprocarb*).

Species/Genera	Rice field with fish	Rice field without fish
Protozoa		
Arcella hemispherica (Perty)	+	++
Centropyxis aculeata (Ehrenberg)	+	+
Coleps sp.		+
Difflugia pyriformis (Perty)	++	++
Trichodina sp.		+
Rotifera		
Asplanchna sp. (Gosse)	+	
Epiphanes sp.	+	+
Euchlanis calpidia (Myers)	+	+
Filinia longiseta (Ehrenberg)	+	
Keratella asymmetrica (Gosse)	++	+
Lecane plaenensis (Voigt)	+	+
Monostyla sp.	+	
Polyartha trigla (Ehrenberg)	+	+
Brachionus plicatilis (Muller)	++	++
Crustacea		
Cyclops sp.	++	++
Moina micrura (Kurz)	+	+
Nauplius larvae	++	++
Cypris sp.	+	+
Insecta		
Chironomidae (Tendipedidae)	+	+
Baetis sp.	+	
Notonecta sp.	+	

NOTE + - Presence ++ - Dominant

Rotifera, 4 genera of Crustacea and 3 genera of Insecta (Table 5.12). The copepod *Cyclops sp.* was the most abundant zooplankton in all treatment plots, followed by the Nauplius larvae. The rotifers were also found in relatively great number, represented mainly by *Brachionus sp.* and *Keratella sp.* High densies of the protozoan *Difflugia sp.* were also noted in this experiment.

The temporal changes in the abundance of the crustacean (copepods) and the rotifers in the rice field plots during the experiment are summarised in Figure 5.15 and Figure 5.16, respectively. The population density of copepods and rotifers in all rice field plots during the experiment were very variable, but showed no definite trends that could be associated with *isoprocarb* treatments.

5.3.3 Experiment 3 (*Buprofezin*)

5.3.3.1 Growth rate

The initial weight, final weight and growth rate (in terms of the absolute weight increment per day) of the experimental fish in rice fields exposed to different dose rates of *buprofezin*, are

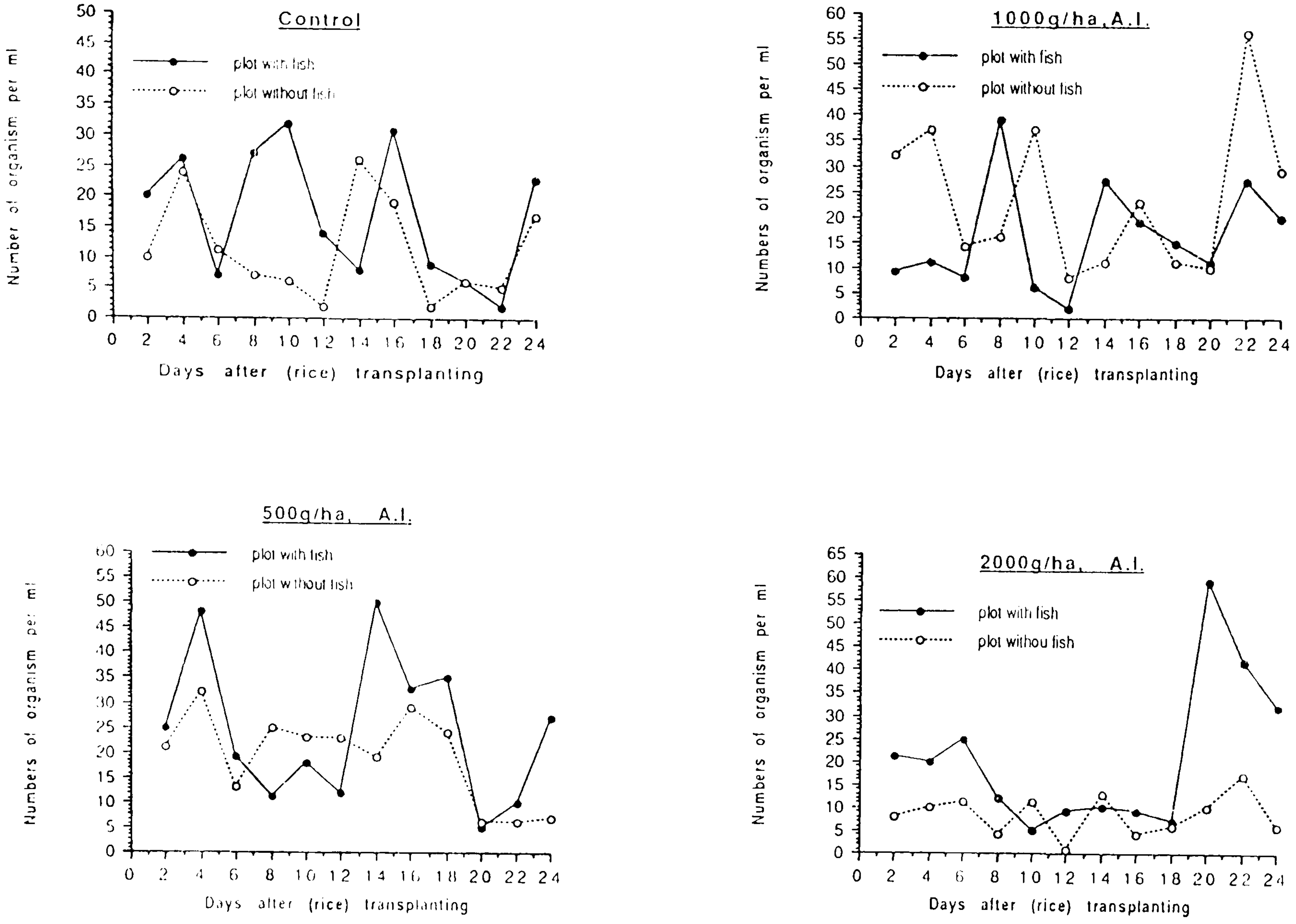


FIGURE 5.15. Abundance of copepods in wet rice fields, treated with different dose rates of *isoprocarb*.

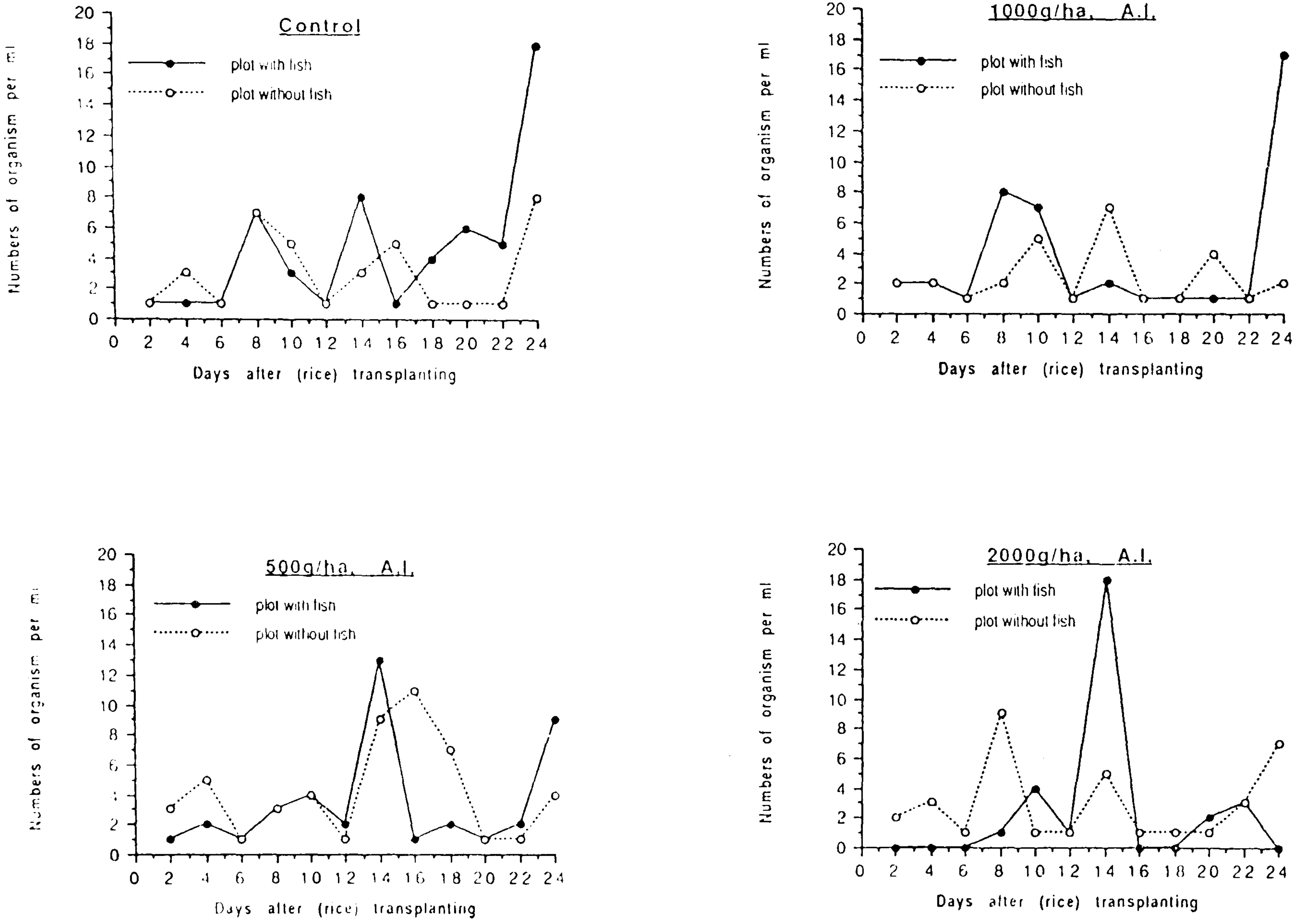


FIGURE 5.16. Abundance of rotifers in wet rice fields, treated with different dose rates of *isoprocarb.*

TABLE 5.13. Mean initial/final weights and mean growth rate (in terms of weight increment per day) of common carp fingerlings in rice fields receiving different dose rates of *buprofezin*.

	Dose rate (gha^{-1}, A.I.)				F	Significant level	Probability
	0	50	100	200			
Initial weight (g)	6.0	6.0	6.0	6.0	0.22	NS	> 0.05
Standard error	0.1	0.1	0.1	0.1			
Final weight (g)	22.5	21.7	22.3	22.3	0.08	NS	> 0.05
Standard error	0.6	0.5	0.6	0.5			
Growth rate (gd^{-1})	0.80	0.76	0.78	0.77	0.07	NS	> 0.05
Standard error	0.05	0.06	0.13	0.08			

NOTE NS - Not significant

presented in Table 5.13.

The mean growth rate and final weights of fish between the treatments were not significantly different ($P > 0.05$). The initial weights of fish were almost identical, at 6.0g. The final weights of fish in the treatment plots ranged between 21.7g and 23.5g, showing a slight trend of decreasing fish final weight with increasing dose rates of *buprofezin* used in the experiment. A similar trend was also noted in the growth rate of fish in the treatment plots, which ranged between 0.76gd^{-1} and 0.80 gd^{-1} (Figure 5.17).

5.3.3.2 Survival rate

The mean survival rate of fish in the treatment plots ranged between 76.7% and 85.5%. The relatively low survival of fish during this experiment may have been due to the high water temperature in the rice field plots (above 30°C). Other factors causing fish loss in the rice fields were the health condition of fish and the presence of fish predators, mainly sawah snakes. There was, however, no apparent different between the survival fish under different treatments ($P > 0.05$). A summary of the data on fish

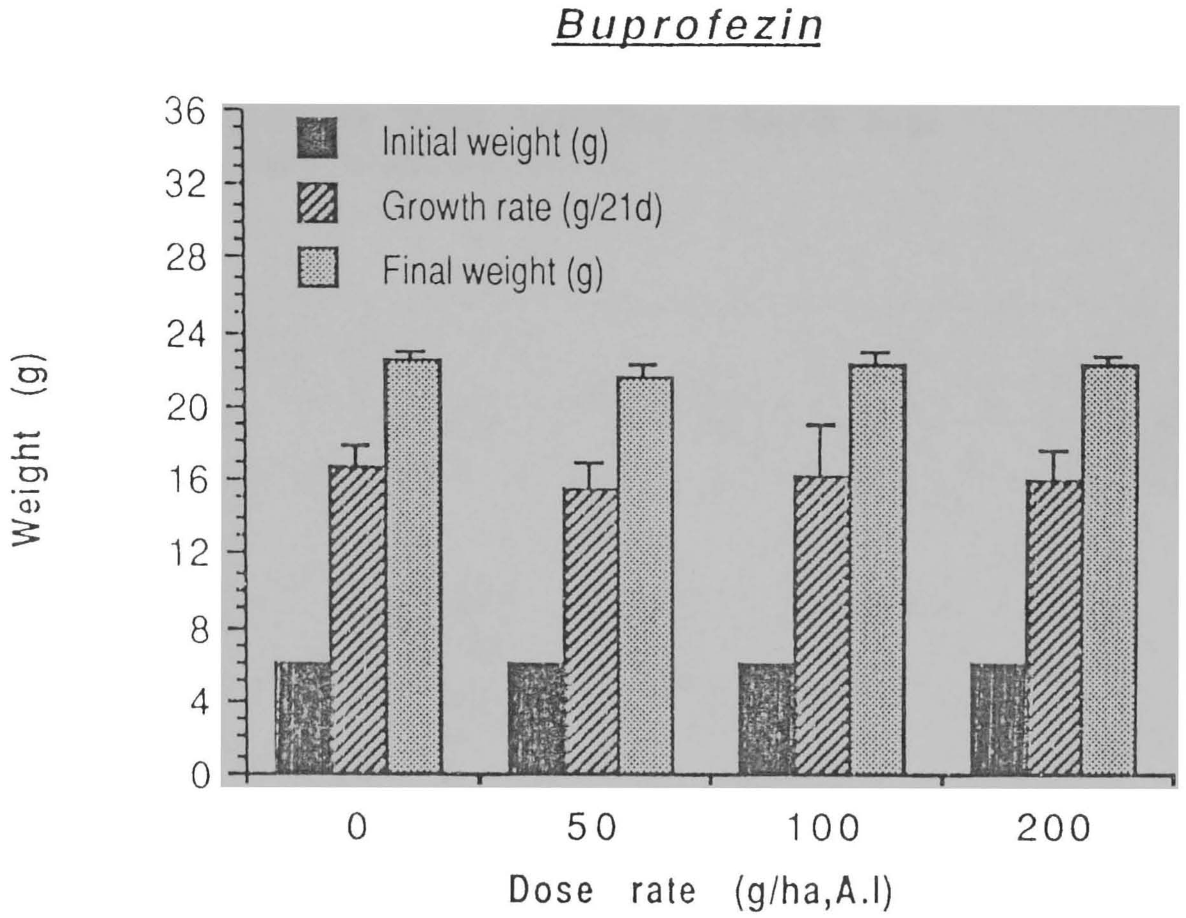


FIGURE 5.17. Growth rate of common carp fingerlings (*C. carpio*) in wet rice fields receiving different dose rates of *buprofezin* (Means and Standard Error).

TABLE 5.14. Summary of survival rate, biomass yield and biomass weight gain of common carp fingerlings in rice fields receiving different dose rates of *buprofezin* (Means and standard error).

	Dose rate (gha^{-1}, A.I.)				F	Significant level	Probability
	0	50	100	200			
Stocking density (gm^{-2})	3.01	3.00	3.00	3.01	0.17	NS	> 0.05
Standard error	0.01	0.01	0.00	0.00			
Survival rate (%)	81.7	76.7	85.0	80.0	0.27	NS	> 0.05
Standard error	8.3	4.2	0	7.1			
Total yield (gm^{-2}21d^{-1})	9.2	8.2	9.6	9.0	0.35	NS	> 0.05
Standard error	0.7	0.4	1.1	1.5			
Weight gain (gm^{-2}d^{-1})	0.29	0.25	0.32	0.28	0.34	NS	> 0.05
Standard error	0.03	0.02	0.05	0.07			

NOTE NS - Not significant

survival, total biomass yield and weight gain of fish biomass in the rice field plots is presented in Table 5.14.

5.3.3.3 Fish production

The stocking density of common carp in the treatment plots showed no significant variation ($P > 0.05$), ranging between $2.9gm^{-2}$ and $3.0gm^{-2}$. The total yield and weight gain of fish biomass in the treatment plots were not significantly different ($P > 0.05$), which means that the effects of *buprofezin* application dose rates used in this experiment to fish production in wet rice field were not apparent. The total yield of fish biomass in the rice field plots ranged between $8.2gm^{-2}21d^{-1}$ and $9.6gm^{-2}21d^{-1}$,producing biomass weight gains, ranging between $0.25gm^{-2}d^{-1}$ and $0.32gm^{-2}d^{-1}$. The lowest fish biomass yield and weight gain were recorded in rice field plots treated with $50gha^{-1}$ *buprofezin*, associated with the relatively low fish survival rate (76.7%) occurring in these treatment plots. The highest fish biomass yield and weight gain were attained in rice field plots receiving *a buprofezin* dose rate of $200gha^{-1}$ (A.I) which also corresponded with a high fish survival rate (Figure 5.18).

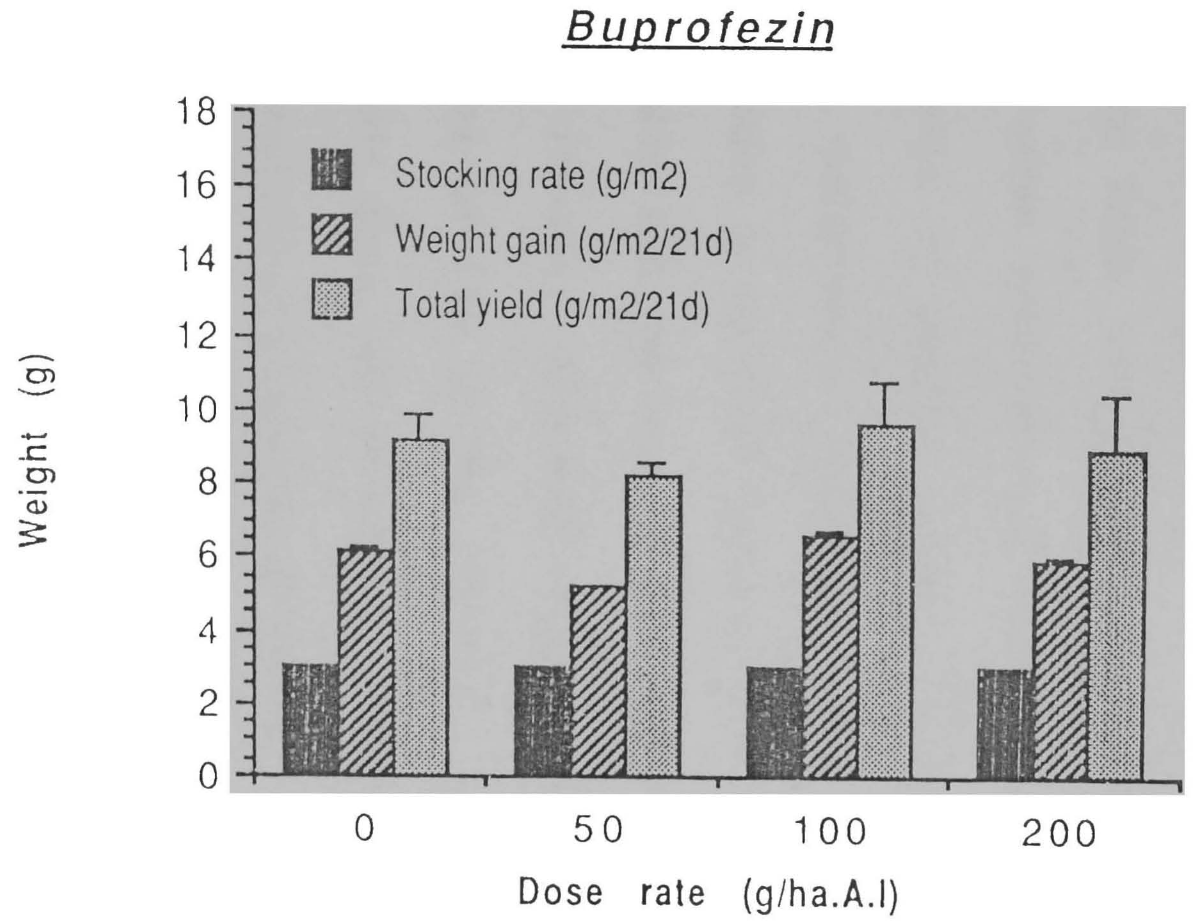


FIGURE 5.18. Production of common carp fingerlings (*C. carpio*) in wet rice fields, receiving different dose rates of *buprofezin* (Means and Standard Error).

5.3.3.4 Water quality

The results of water quality analyses parameters in rice field plots, carried out during the experiment are presented in Table 5.15 and Table 5.16. Water quality in the rice field plots were found to be within acceptable limits for common carp production in wet rice fields. In general, there was no indication that most water quality parameters measured were significantly influenced by insecticides or by other factors, notably the presence of fish in the rice fields and the time of the analysis during the experiment ($P > 0.05$). However, dissolved oxygen was significantly affected by the presence of fish in the rice field plots ($P < 0.05$) and by the insecticide dose rate ($P = 0.01$). Carbon dioxide was also influenced by the addition of insecticide ($P < 0.05$).

Water temperature ranged between 28.9°C and 29.0°C. The minimum and maximum water temperature recorded during the experiment were 25°C and 35°C, respectively (Figure 5.19).

Dissolved oxygen level in the rice field plots fluctuated between $5.0 mgl^{-1}$ (64% saturation) and $15 mgl^{-1}$ (192% saturation).

TABLE 5.15. The physical and chemical characteristics of water in the rice fields (with fish), measured during Experiment 3 (*Buprofezin*) (Mean and range values)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	50	100	200			
Temperature (^{o}C)	28.9 (25.0-34.0)	28.9 (26.0-35.0)	29.0 (26.0-33.0)	28.9 (26.0-32.0)	0.55	NS	> 0.05
Dissolved oxygen (mgl^{-1})	8.0 (6.4-9.8)	8.9 (6.8-12.4)	8.4 (6.0-11.0)	9.9 (5.0-15.0)	4.12	* *	$= 0.01$
Carbondioxide (mgl^{-1})	2.26 (0.05-4.60)	2.34 (0-4.60)	2.24 (0-3.99)	2.06 (0-3.60)	3.27	*	< 0.05
pH	7.1 (7.0-8.0)	7.3 (7.0-9.5)	7.1 (7.0-9.5)	7.4 (7.0-9.5)	0.99	NS	> 0.05
Total hardness (mgl^{-1}.CaCO$_3$)	54.1 (43.7-68.7)	51.8 (43.7-62.4)	52.5 (45.8-62.4)	53.2 (47.8-64.5)	0.21	NS	> 0.05

NOTE NS - Not significant, * - Significant, ** - Highly significant

TABLE 5.15. (Continued)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	50	100	200			
Total alkalinity (mgl^{-1},CaCO$_3$)	55.1 (46.6-79.3)	53.2 (36.6-69.9)	52.4 (36.6-79.3)	51.1 (39.7-64.4)	0.29	NS	> 0.05
Phosphorus (mgl^{-1})	0.035 (0.006-0.170)	0.035 (0.016-0.066)	0.036 (0.012-0.067)	0.041 (0.013-0.198)	0.06	NS	> 0.05
Total ammonia (mgl^{-1})	0.028 (0-0.088)	0.020 (0-0.062)	0.029 (0-0.110)	0.028 (0.011-0.099)	0.08	NS	> 0.05
Nitrite (mgl^{-1})	0.175 (0.071-0.560)	0.139 (0.055-0.360)	0.141 (0.060-0.340)	0.130 (0.050-0.340)	0.98	NS	> 0.05
Suspended solid (mgl^{-1})	32.9 (11.4-82.0)	32.8 (10.9-80.0)	26.5 (11.0-68.0)	41.6 (10.8-117.0)	0.74	NS	> 0.05

Note NS - Not significant

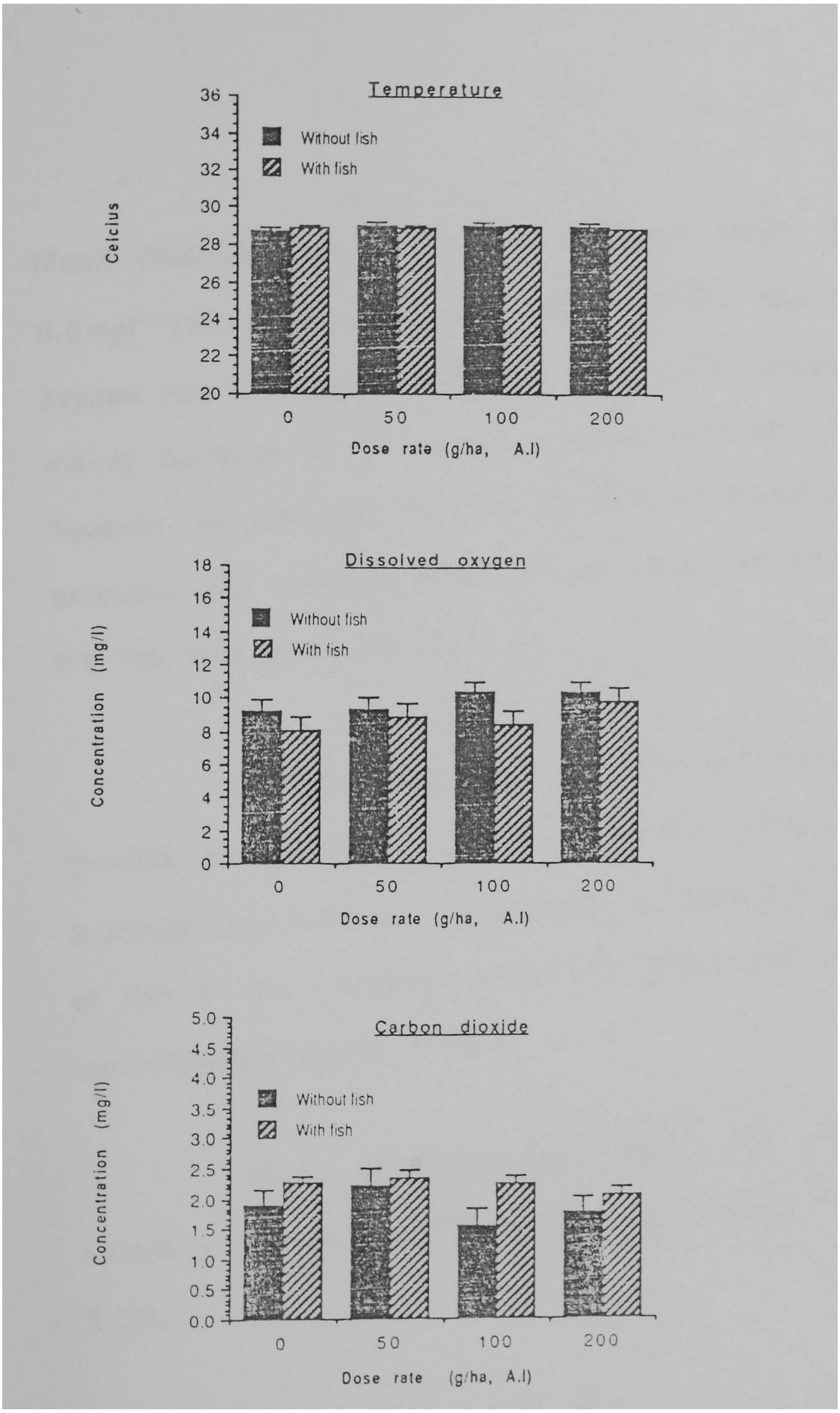
TABLE 5.16. The physico-and chemical characteristics of water in the rice fields (without fish), measured during Experiment 3 (*Buprofezin*) (Mean and range values).

	Insecticide dose rate (g/ha, A.I.)				F	Significant level	Probability
	0	50	100	200			
Temperature (^{o}C)	28.7 (26.0-34.0)	29.0 (26.0-35.0)	29.0 (26.0-34.0)	29.1 (26.0-35.0)	0.55	NS	> 0.05
Dissolved oxygen (mgl^{-1})	9.3 (6.4-11.8)	9.4 (6.6-15.0)	10.4 (8.0-15.0)	10.4 (7.2-15.0)	4.12	* *	$= 0.01$
Carbondioxide (mgl^{-1}l)	1.88 (0-3.99)	2.21 (0-3.80)	1.54 (0-3.60)	1.75 (0-3.99)	3.27	*	< 0.05
pH	7.5 (7.0-10.0)	7.1 (7.0-8.0)	7.6 (7.0-10.0)	7.2 (7.0-9.5)	0.99	NS	> 0.05
Total hardness (mgl^{-1} $CaCO_3$)	51.7 (41.0-60.3)	52.1 (43.9-62.4)	52.2 (41.6-62.4)	52.3 (43.7-64.5)	0.21	NS	> 0.05

TABLE 5.16. (Continued)

	Insecticide dose rate (gha^{-1})				F	Significant level	Probability
	0	50	100	200			
Total alkalinity (mgl^{-1},$CaCO_3$)	52.5 (46.2-69.4)	55.6 (36.6-79.3)	51.5 (36.6-59.5)	52.8 (46.6-64.4)	0.29	NS	> 0.05
Phosphorus (mgl^{-1})	0.039 (0.021-0.067)	0.045 (0.018-0.190)	0.034 (0.011-0.067)	0.033 (0.010-0.180)	0.06	NS	> 0.05
Total ammonia (mgl^{-1})	0.032 (0-0.099)	0.034 (0-0.092)	0.031 (0-0.096)	0.031 (0-0.110)	0.08	NS	> 0.05
Nitrite (mgl^{-1})	0.166 (0.013-0.470)	0.175 (0.016-0.490)	0.175 (0.071-0.470)	0.117 (0.033-0.300)	0.98	NS	> 0.05
Suspended solid (mgl^{-1})	32.4 (14.8-73.0)	28.2 (11.6-54.0)	45.4 (13.7-160.0)	42.9 (14.5-91.0)	0.74	NS	> 0.05

NOTE : NS - Not significant, * - Significant, ** - Highly significant



GURE 5.19. Levels of water temperature, dissolved oxygen and carbon dioxide concentrations in the rice fields, measured during Experiment 3 (Means and Standard Deviation).

Mean dissolved oxygen in the treatment plots ranged between 8.0mgl^{-1} (103% saturation) and 9.9mgl^{-1} (126% saturation). Dissolved oxygen was found to be generally lower in rice fields containing fish, mainly because of the oxygen consumption by fish. There was, however, no evidence that the variation of dissolved oxygen levels produced any apparent effect on the production of common carp in the rice field plots (Figure 5.19).

Carbon dioxide levels never exceed 4.6mgl^{-1}. Mean carbon dioxide concentration in the treatment plots ranged between 2.06mgl^{-1} and 2.34mgl^{-1}. There was no indication that the production of fish in the treatment plots was influenced by carbon dioxide concentration (Figure 5.19).

Water pH fluctuated between 7.0 and 9.5, with mean values in the treatment plots ranging between 7.1 and 7.4 (Figure 5.20).

The minimum and maximum total hardness of the rice field water ranged was 43.7mgl^{-1} and 68.6mgl^{-1} as $CaCO_3$, respectively. Mean values of total hardness ranged between

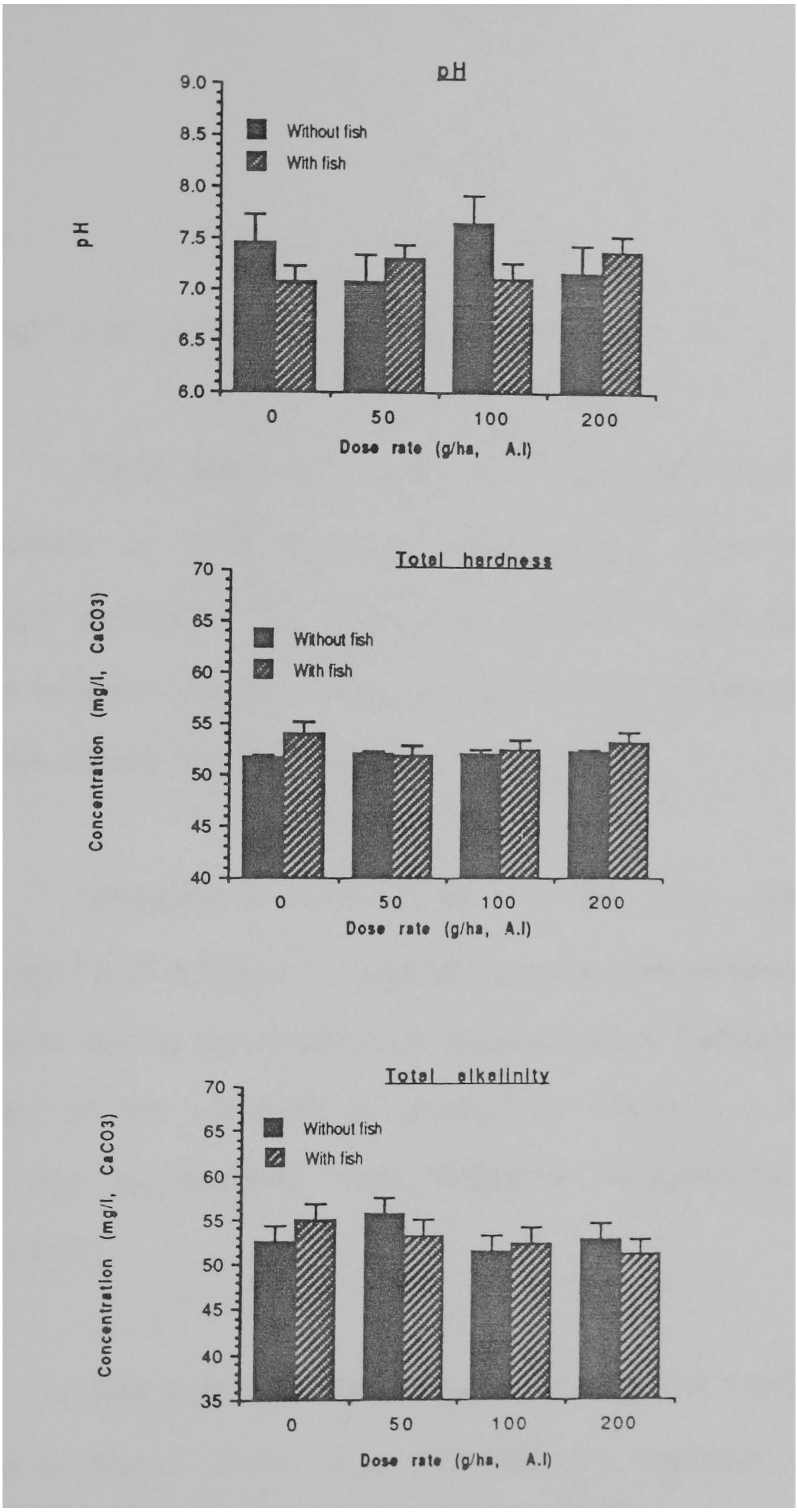


FIGURE 5.20. Levels of pH, total hardness and total alkalinity in the rice fields, measured during Experiment 3 (Means and Standard Deviation).

51.8mgl^{-1} and 54.1mgl^{-2} as $CaCO_3$ (Figure 5.20).

Total alkalinity of the rice field water was found to be comparable to total hardness, and ranged from 36.6mgl^{-1} to 69.9mgl^{-1} as $CaCO^3$. The mean total alkalinity in the treatment plots ranged between 51.1mgl^{-1} and 55.1mgl^{-1}, also similar to their total hardness values (Figure 5.20).

Phosphorus levels in the rice field water ranged between 0.026mgl^{-1} and 0.17mgl^{-1}. Total phosphorus concentration in the rice filed plots during the experiment showed some fluctuations, possibly because of the variation of phosphorus content in the irrigation water due to leaching from fertilised neighbouring rice fields (Figure 5.21).

The level of total ammonia during the experiment never exceed 0.11mgl^{-1}. Mean total ammonia the treatment plots ranged between 0.02mgl^{-1} and 0.03mgl^{-1} (Figure 5.21).

Nitrite concentration in the rice field plots was found to be generally high, but never exceeding 0.56mgl^{-1}.There was,however,

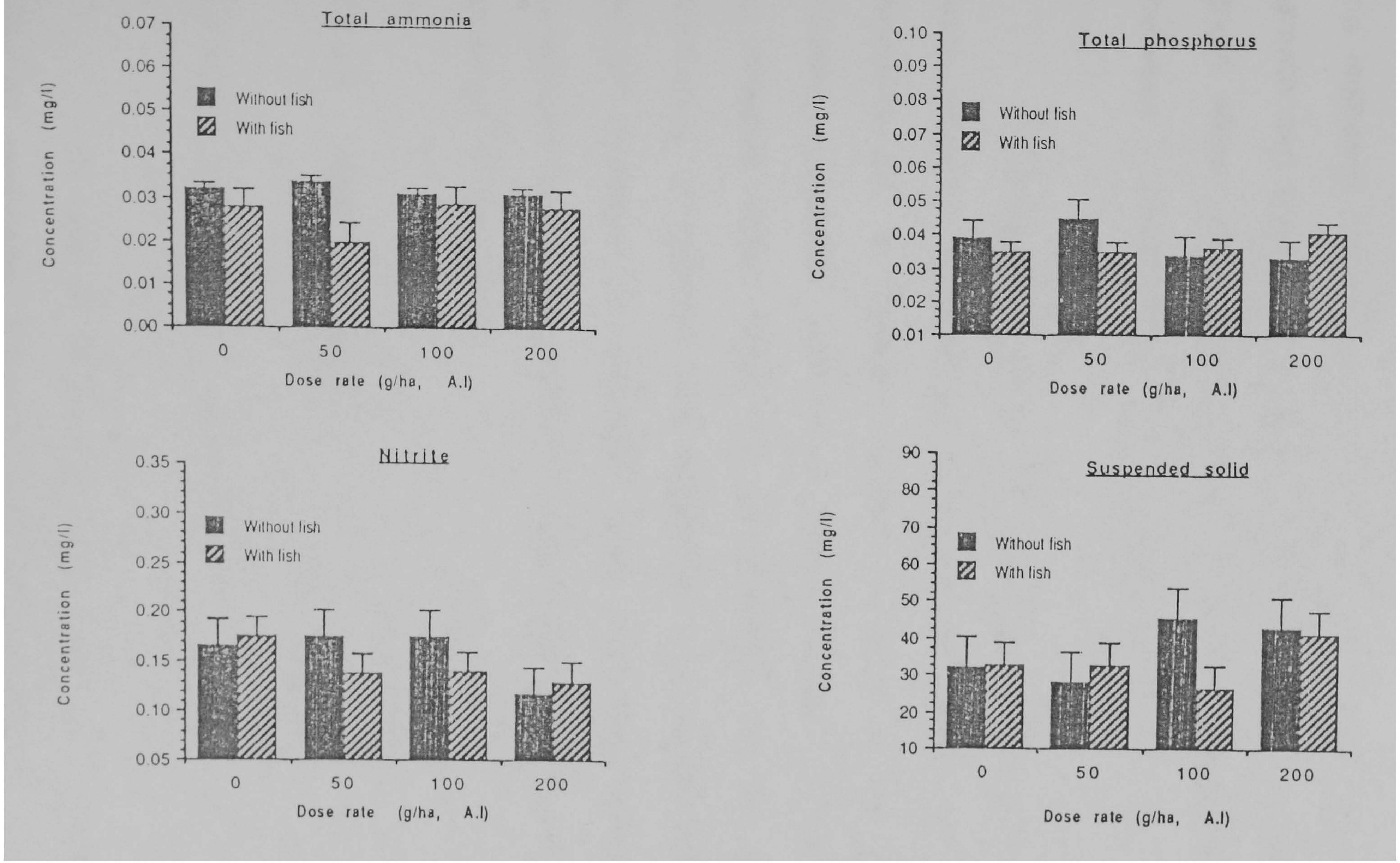


FIGURE 5.21. Levels of total ammonia, nitrite, total phosphorus and suspended solid in the rice fields, measured during Experiment 3 (Means and Standard Deviation).

no indication that these high nitrite concentrations affected the growth and production of common carp fingerlings in the rice field plots Mean nitrite concentration in the treatment plots ranged between 0.13mgl^{-1} and 0.17mgl^{-1} (Figure 5.21).

Suspended solids concentrations in the rice field plots were found to fluctuate significantly from 10.8mgl^{-1} to 117.0mgl^{-1}, primarily due to frequent rainfall occurring during this period, causing substantial addition of organic/inorganic domestic wastes to irrigation water. There was no indication that the growth and production of common carp fingerlings were significantly affected by this fluctuation in suspended solids. The mean concentration of suspended solid in the treatment plots ranged between 26.5mgl^{-1} and 41.6mgl^{-1} (Figure 5.21).

5.3.3.5 Rice field biota

5.3.3.5.1 Benthic macro-invertebrates

A total of 15 taxa of benthic macro-invertebrates were collected, comprising 8 genera of Oligochaeta, 2 genera of Hirudinae and 7 genera of Insecta (Table 5.17). Two genera of Oligochaeta, *Nais* (Muller,1773) and *Tubifex* (Lamarck,1816), were found to dominate the rice field plots, both with fish and without fish. The larvae of Chironomidae were also recorded frequently, but at lower density than the oligochaetes.

Figure 5.22 summarised the temporal changes in the abundance of the benthic oligochaetes and chironomid larvae in the treatment plots. The populations of the macro invertebrates in all rice field plots showed some fluctuations, but with no definite pattern of changes in abundance associated with *isoprocarb* treatments in the rice field plots with fish and without fish.

5.3.2.5.2 Zooplankton

The composition of the zooplankton population found in the rice field plots, in terms of species diversity, was similar with those described in the previous experiments. The copepod *Cyclops* sp and the Nauplius larvae occurred dominantly in the treatment plots during the experiment, followed by the rotifer *Brachionus spp.* (Table 5.18).

TABLE 5.17. List of species/genera of benthic macro-invertebrates collected from rice field plots in Experiment 3 (*Buprofezin*).

Species/Genera	Rice field with fish	Rice field without fish
<u>Oligochaeta</u>		
Nais communis (Piguet)	++	++
Dero zeylanica (Stephenson)	+	+
Chaetogaster sp.	+	+
Haplotaxis sp.	+	+
Lumbriculus sp	+	+
Tubifex tubifex (Muller)	++	++
Limnodrillus hoffmeisteri (Claparede)	+	+
<u>Hirudinea</u>		
Glossiphonia weberi (Blanchard)	+	+
Helobdella sp.	+	+
<u>Insecta</u>		
Caenis sp.		+
Corixa sp.		+
Culex sp.	+	+
Chironomus sp.	+	+
Polypedilum sp.	+	+
Tanypus sp.	+	
Dysticus sp.	+	

<u>NOTE</u> : + - Presence ++ - Dominant

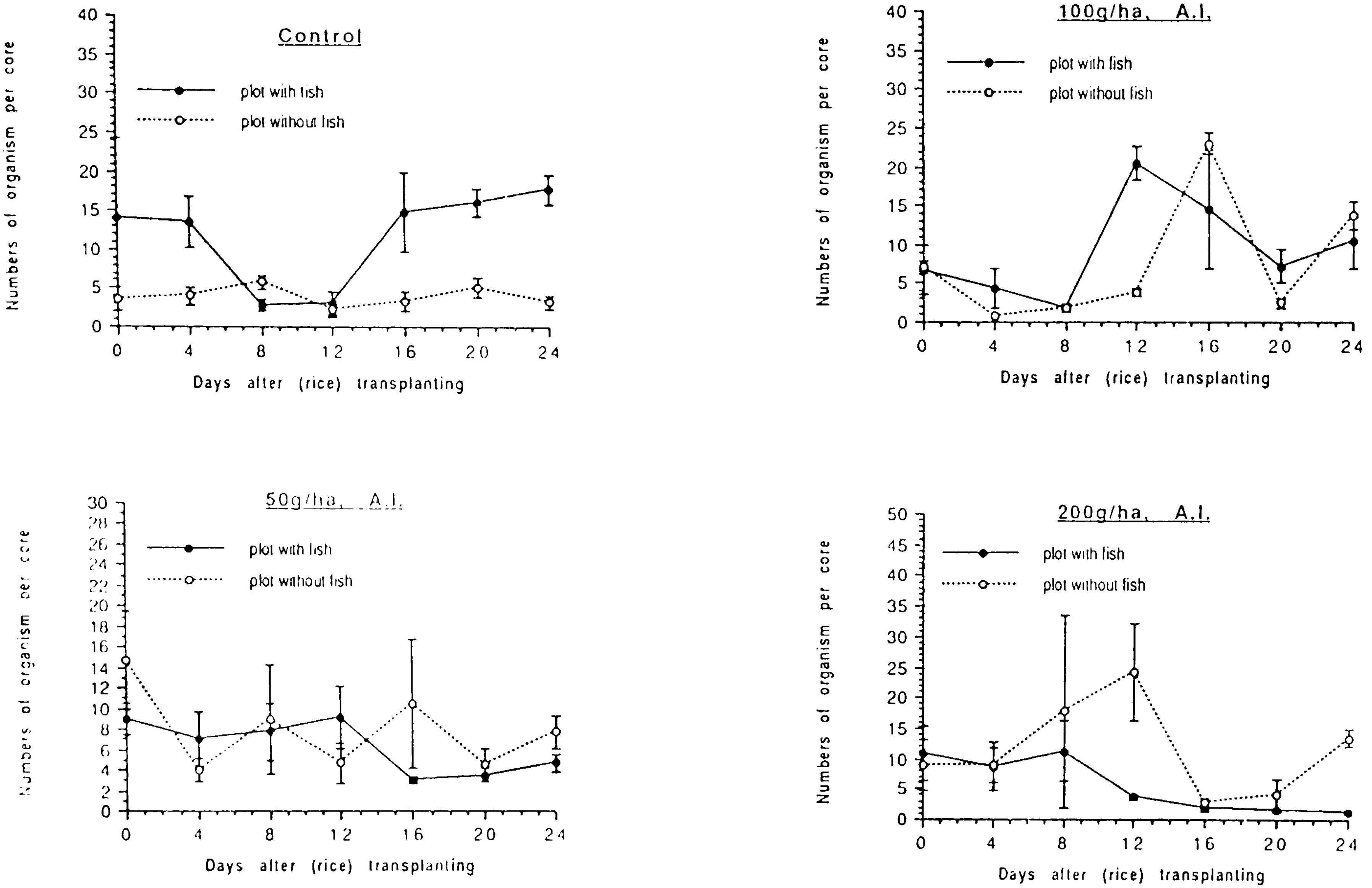


FIGURE 5.22. Abundance of benthic macro organisms in wet rice fields treated with different dose rates of *buprofezin* (Means and Standard Error).

The temporal changes of the population density of rotifers and copepods are presented in Figure 5.23 and Figure 5.24, respectively. The densities of the populations of rotifers and copepods in all experimental rice fields showed some fluctuations but no definite trends. There was no indication of a definite pattern in the changes in zooplankton density, which could be associated with *buprofezin*.

5.3.4 Experiment 4 (*Diazinon*)

5.3.4.1 Growth rate

The initial weight, final weight and growth rate of common carp fingerlings raised for 21d in wet rice fields exposed to different dose rates of *diazinon*, are summarised in Table 5.19 and Figure 5.23.

TABLE 5.18 List of species/genera of zooplankton collected from rice field plots in Experiment 3 (*Buprofezin*).

Species/Genera	Rice field with fish	Rice field without fish
<u>Protozoa</u>		
Arcella vulgaris (Ehrenberg)	+	+
Centropyxis aculeata (Stein)	+	+
Difflugia lobostomata (Leidy)		+
<u>Rotifera</u>		
Brachionus angularis (Gosse)	+	++
Epiphanes sp.	+	+
Lecane bulla (Gosse)	+	+
Monostyla sp.	+	
Phylodina sp.		+
<u>Crustacea</u>		
Cyclops sp.	++	++
Moinadaphnia macleayi (King)	+	+
Nauplius larvae	++	++
Cypris sp.	+	+
<u>Insecta</u>		
Chironomidae (Tendipedidae)	+	+

<u>NOTE</u> : + - Presence ++ - Dominant

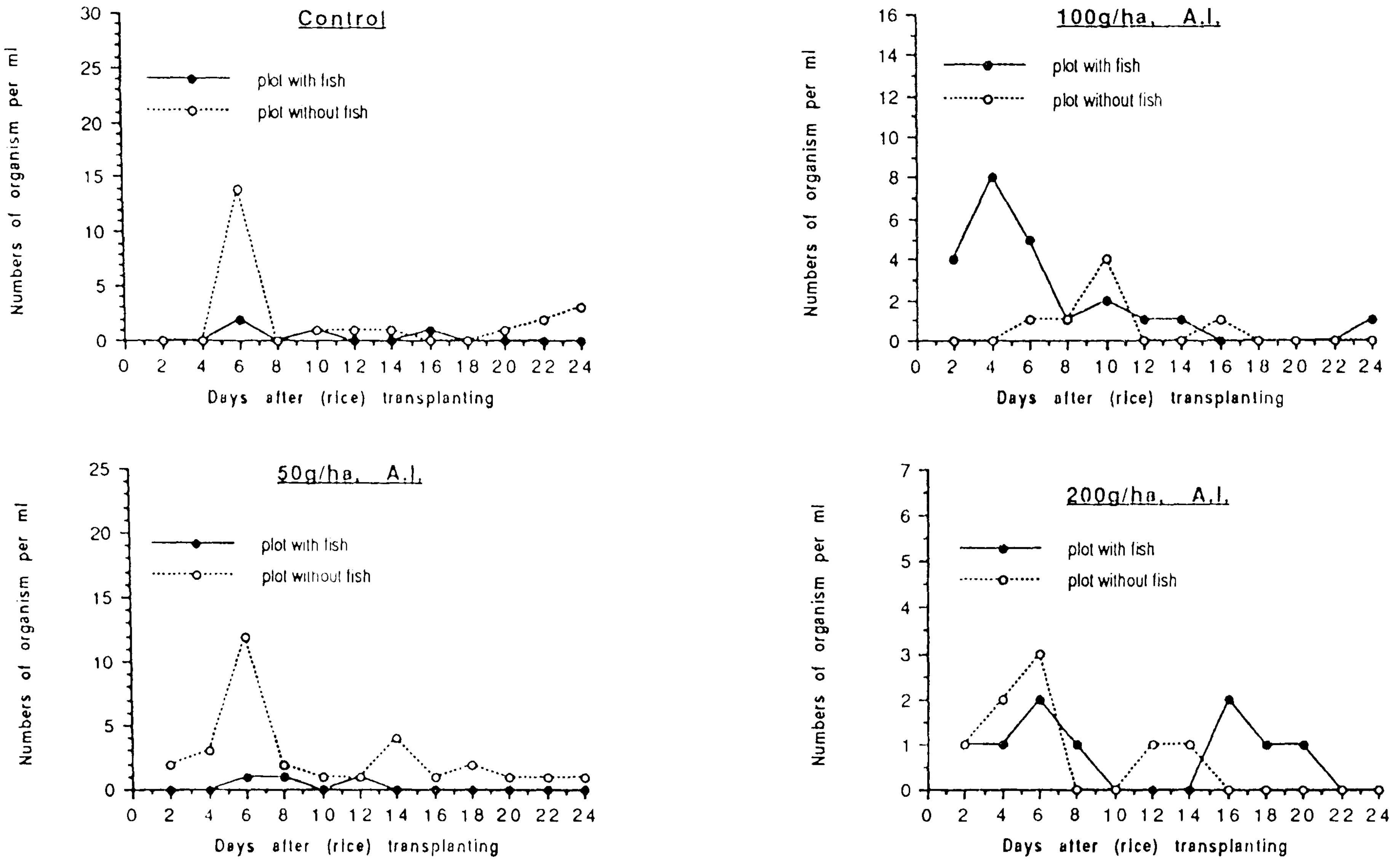


FIGURE 5.23. Abundance of copepod s in wet rice fields treated with different dose rates of *buprofezin*.

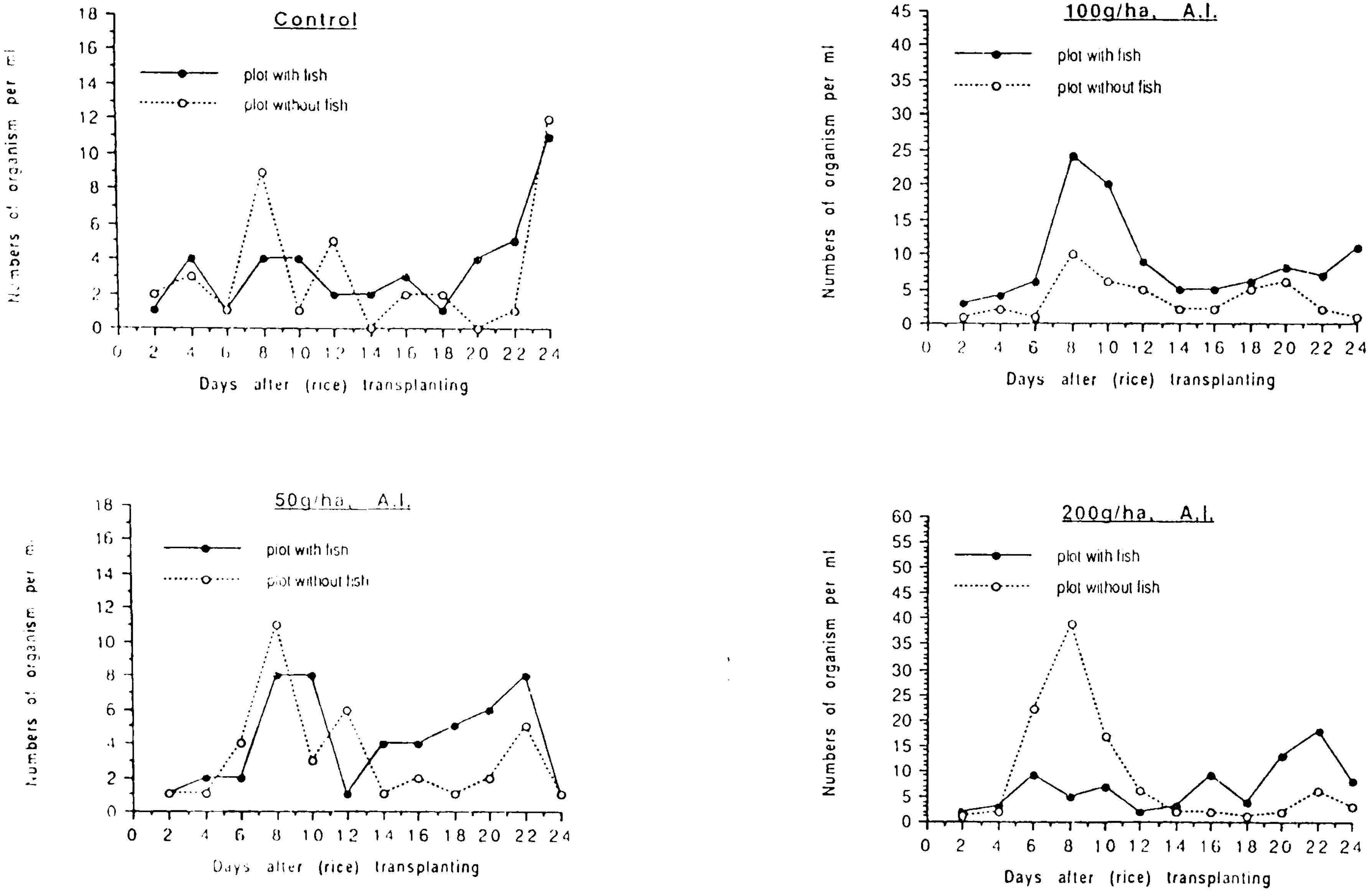


FIGURE 5.24. Abundance of rotifers in wet rice fields treated with different dose rates of *buprofezin*.

The mean initial weights of the experimental fish in the treatment plots were comparable, ranging from 4.4g to 4.6g (P >0.05). Mean final weights of fish in the treatment plots ranged between 17.5g and 22.3g. There was no significant variation in the final weights of the experimental fish in the treatment plots (P >0.05). There was also no significant variation in the absolute growth rate of fish in the treatment plots (P >0.05).

5.3.4.2 Survival rate

The mean survival rate of experimental fish in the treatment plots was found to be not significantly different (P >0.05), although in the rice field plot treated with 1280gha^{-1} of *diazinon*, the lowest fish survival of 65% was recorded. A summary of survival rate, total yield and weight gain of fish data is presented in Table 5.20.

TABLE 5.19. Mean initial/final weights and mean growth rate (in terms of weight increment per day) of common carp fingerlings in rice fields receiving different dose rates of *diazinon.*

	Dose rate (gha^{-1},A.I.)				F	Significant level	Probability
	0	320	640	1280			
Initial weight (g)	4.6	4.5	4.4	4.5	0.89	NS	> 0.05
Standard error	0.1	0.1	0.1	0.1			
Final weight (g)	22.3	18.9	20.6	17.5	1.29	NS	> 0.05
Standard error	0.8	0.7	0.7	0.7			
Growth rate (gd^{-1})	0.84	0.68	0.71	0.60	1.25	NS	> 0.05
Standard error	0.10	0.06	0.08	0.11			

[illegible] NS - Not significant

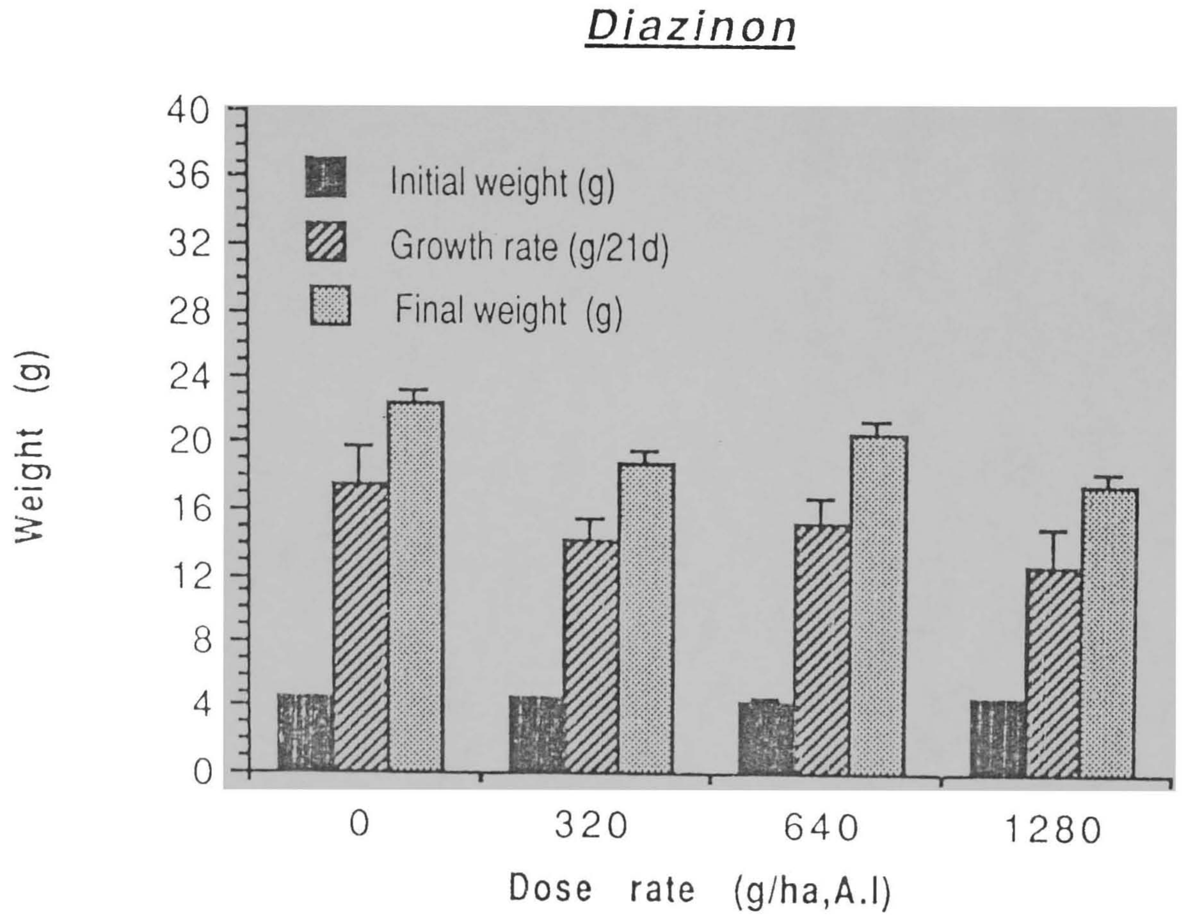


FIGURE 5.25. Growth rate of common carp fingerlings (*C. carpio*) in wet rice fields, receiving different dose rates of *diazinon* (Means and Standard Error).

5.3.4.3 Fish production

The total yield and weight gain of fish biomass in the treatments plots were not significantly different ($P > 0.05$), although production of fish biomass in plots treated with *diazinon* dose rate of 1280gha^{-1} was lowest (6.9gm^{-2}21d^{-1}) as compared to that in the control plots (9.7gm^{-2}21d^{-1})(Figure 5.26).

TABLE 5.20. Summary of survival rate, biomass yield and biomass weight gain of common carp fingerlings in rice fields receiving different dose rates of *diazinon* (Means and standard error).

	Dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	320	640	1280			
Stocking density (gm^{-2})	2.32	2.27	2.23	2.28	0.77	NS	>0.05
Standard error	0.00	0.00	0.00	0.01			
Survival rate (%)	88.3	86.7	73.3	78.3	1.39	NS	> 0.05
Standard error	1.7	7.3	4.4	6.7			
Total yield (gm^{-2}21d^{-1})	9.7	8.0	7.1	6.9	1.40	NS	> 0.05
Standard error	1.2	1.1	0.2	1.4			
Weight gain (gm^{-2}d^{-1})	0.35	0.27	0.23	0.22	1.35	NS	> 0.05
Standard error	0.06	0.05	0.01	0.07			

NOTE NS - Not significant

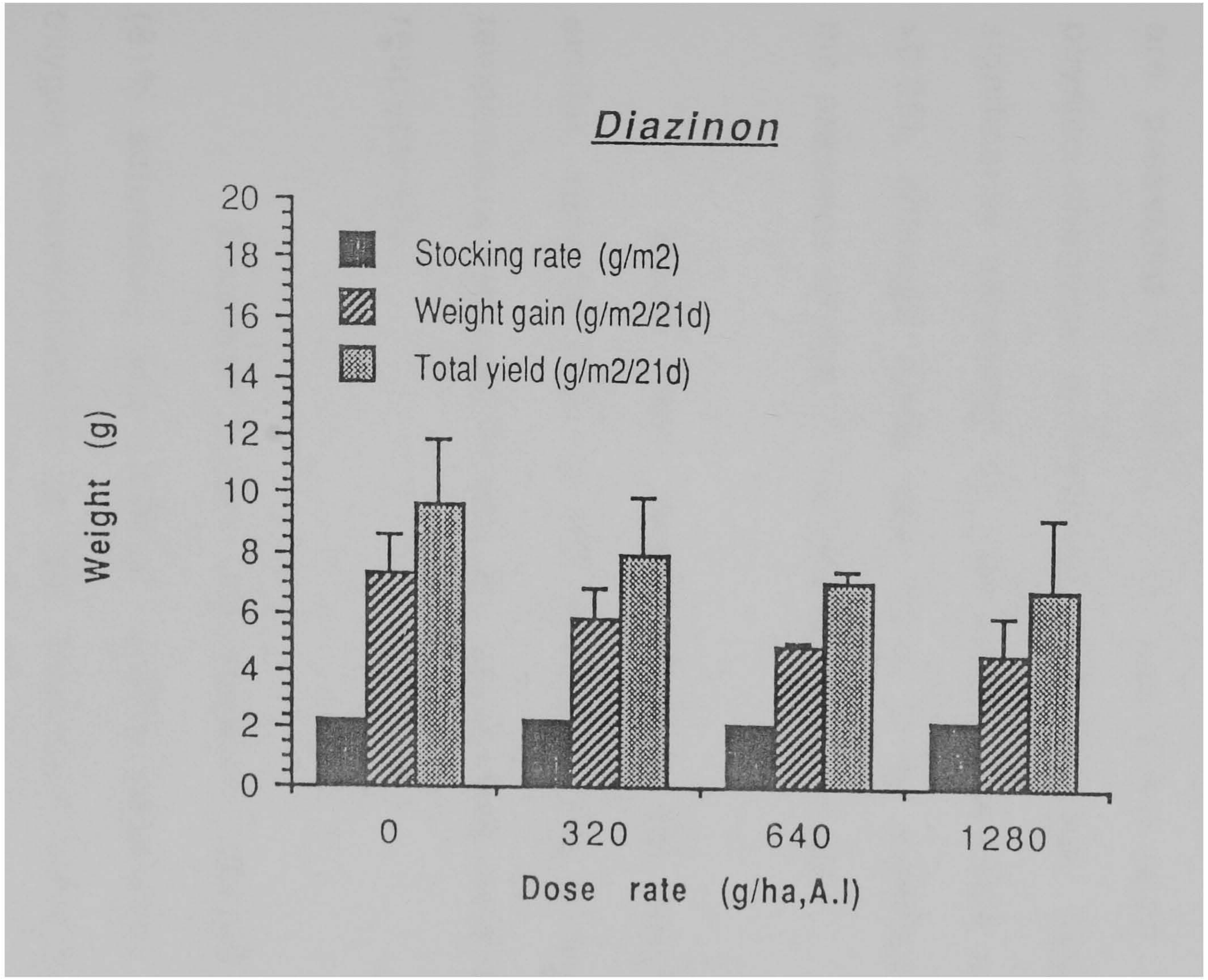


FIGURE 5.26. Production of common carp fingerlings (*C. carpio*) in wet rice fields, receiving different dose rates of *diazinon* (Means and Standard Error).

5.3.4.4 Water quality

The water quality data collected during the experiment are presented in Table 5.21 and Table 5.22. respectively. The physico–chemical characteristics of the rice field water were not significantly influenced by insecticide dose rates or other factors ($P > 0.05$), although nitrite was found to be significantly influenced by the presence of fish in the rice fields ($P < 0.05$).

Mean water temperature in the treatment plots were similar, ranging from 25.4°C to 25.6°C. The minimum and maximum temperature recorded during the experiment were 23.0°C and 30.0°C, respectively.

Dissolved oxygen concentration ranged between 6.7mgl^{-1} (81% saturation) and 15.0mgl^{-1} (182% saturation). Mean dissolved oxygen concentrations in the treatment plots were comparable, ranging between 11.6mgl^{-1} (140% saturation) and 12.5mgl^{-1} (152% saturation).

TABLE 5.21. The physical and chemical characteristics of water in the rice fields (with fish), measured during Experiment 4 (*Diazinon*). (Mean and range values)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	320	640	1280			
Temperature (°C)	25.6 (23.0-29.5)	25.4 (24.0-29.0)	25.6 (24.0-30.0)	25.5 (23.5-29.0)	1.87	NS	> 0.05
Dissolved oxygen (mgl^{-1})	12.5 (6.7-16.4)	12.5 (7.8-15.7)	11.8 (7.2-15.0)	11.6 (7.6-15.0)	1.12	NS	> 0.05
Carbondioxide (ml^{-1})	1.14 (0-3.99)	1.25 (0-3.99)	1.74 (0-3.99)	1.36 (0-5.98)	0.16	NS	> 0.05
pH	8.2 (7.0-9.5)	8.0 (7.0-9.5)	7.7 (7.0-9.5)	8.1 (7.0-9.5)	1.09	NS	> 0.05
Total hardness (mgl^{-1},$CaCO_3$)	56.4 (39.7-72.6)	57.1 (45.8-79.3)	57.5 (45.8-79.3)	57.5 (47.8-79.3)	1.56	NS	> 0.05

[illegible] . NS - Not significant

TABLE 5.21. (Continued)

	Insecticide dose rate (gha^{-1},A.I)				F	Significant level	Probability
	0	320	640	1280			
Total alkalinity (mgl^{-1},CaCO$_3$)	52.5 (46.2-69.4)	55.6 (36.6-79.3)	51.5 (36.6-59.5)	52.8 (46.6-64.4)	0.20	NS	> 0.05
Phosphorus (mgl^{-1})	0.090 (0.010-0.290)	0.056 (0.011-0.112)	0.082 (0.011-0.270)	0.054 (0.013-0.145)	0.38	NS	> 0.05
Total ammonia (mgl^{-1})	0.030 (0.018-0.067)	0.031 (0.015-0.090)	0.030 (0.011-0.071)	0.028 (0.011-0.067)	0.27	NS	> 0.05
Nitrite (mgl^{-1})	0.030 (0.150-0.580)	0.358 (0.160-0.700)	0.297 (0.130-0.460)	0.343 (0.180-0.570)	0.95	NS	> 0.05
Suspended solid (mgl^{-1})	21.7 (18.5-58.0)	18.3 (17.4-50.0)	20.9 (16.1-60.0)	34.5 (18.5-88.0)	0.14	NS	> 0.05

NOTE NS - Not significant

TABLE 5.22. The physico and chemical characteristics of water in the rice fields (without fish), measured during Experiment 4 *(Diazinon)*. (Mean and range values)

	Insecticide dose rate (g/ha, A.I.)				F	Significant level	Probability
	0	320	640	1280			
Temperature (^{o}C)	25.2 (23.0-29.0)	25.8 (24.0-29.0)	25.8 (23.5-29.0)	25.7 (24.0-29.5)	1.87	NS	> 0.05
Dissolved oxygen (mgl^{-1})	12.5 (7.6-15.0)	12.5 (6.6-15.0)	13.5 (7.6-15.0)	12.8 (8.4-15.0)	1.12	NS	> 0.05
Carbondioxide (mgl^{-1})	0.87 (0-4.99)	1.05 (0-3.39)	0.88 (0-3.99)	1.04 (0-3.60)	0.10	NS	> 0.05
pH	7.9 (7.0-9.5)	7.9 (7.0-9.5)	8.3 (7.0-9.5)	8.1 (7.0-9.5)	1.09	NS	> 0.05
Total hardness (mgl^{-1},CaCO$_3$)	55.7 (46.3-72.8)	56.7 (39.7-68.6)	51.5 (36.7-64.4)	52.7 (39.7-64.4)	1.56	NS	> 0.05

TABLE 5.22. (Continued)

	Insecticide dose rate (gha^{-1},A.I)				F	Significant	Probability
	0	320	640	1280			
Total alkalinity	52.5	55.6	51.5	52.8	0.20	NS	> 0.05
(mgl^{-1},CaCO$_3$)	(46.2-69.4)	(36.6-79.3)	(36.6-59.5)	(46.6-64.4)			
Phosphorus	0.069	0.059	0.063	0.073	0.38	NS	> 0.05
(mgl^{-1})	(0.010-0.140)	(0.011-0.135)	(0.011-0.230)	(0.010-0.270)			
Total ammonia	0.030	0.029	0.024	0.024	0.27	NS	> 0.05
(mgl^{-1})	(0.015-0.066)	(0.018-0.086)	(0.015-0.052)	(0.015-0.039)			
Nitrite	0.404	0.318	0.268	0.291	0.95	NS	> 0.05
(mgl^{-1})	(0.250-0.890)	(0.120-0.600)	(0.140-0.340)	(0.131-0.540)			
Suspended solid	24.0	29.0	29.1	34.9	0.14	NS	> 0.05
(mgl^{-1})	(6.0-62.0)	(10.8-60.0)	(8.2-88.0)	(6.7-82.0)			

NOTE. NS - Not significant

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The maximum carbon dioxide concentration recorded during the experiment was 5.98mgl^{-1}. Mean carbon dioxide concentration in the experimental plots ranged between 1.14mgl^{-1} and 1.74mgl^{-1} (Figure 5.27).

The minimum and maximum water pH measured in the rice field plots during the experiment were 7.0 and 9.0, respectively. Mean pH values in the treatment plots were comparable, ranging between 7.7 and 8.2. There was no indication of pH problem in the rice field plots observed during the experiment.

Total hardness and total alkalinity of the rice field water measured during the experiment were comparable, ranging between 39.7mgl^{-1} (as CaCO^3) and 79.3mgl^{-1} (as CaCO^3), which was adequate for fish culture in wet rice fields (Figure 5.28).

The level of total ammonia in the rice field plots never exceed 0.09mgl^{-1}. Mean total ammonia concentration in the treatment plots ranged between 0.028mgl^{-1} and 0.031mgl^{-1}(Figure 5.29).

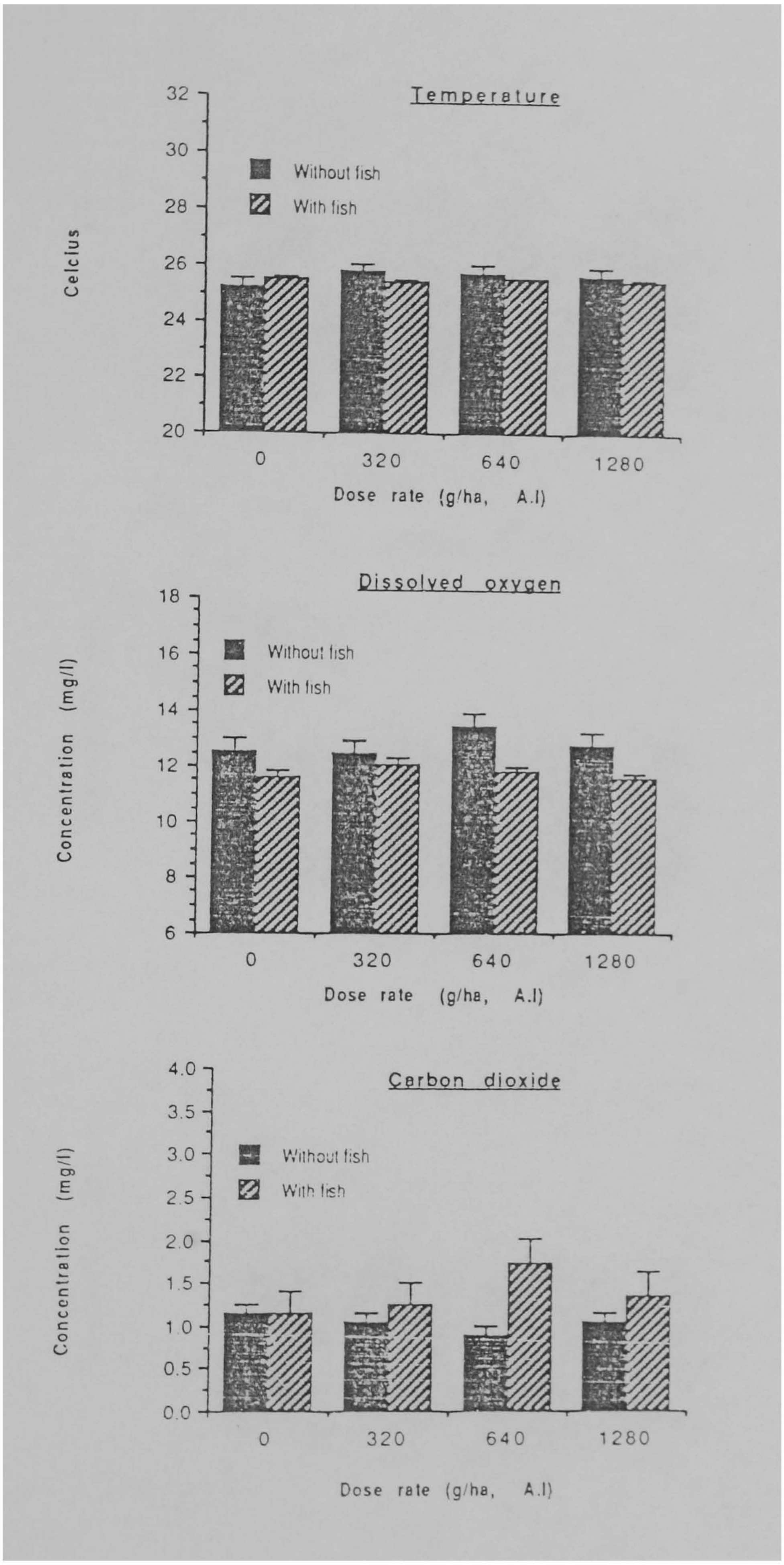

FIGURE 5.27. Levels of water temperature, dissolved oxygen and carbon dioxide concentrations in the rice fields, measured during Experiment 4 (Means and Standard Deviation).

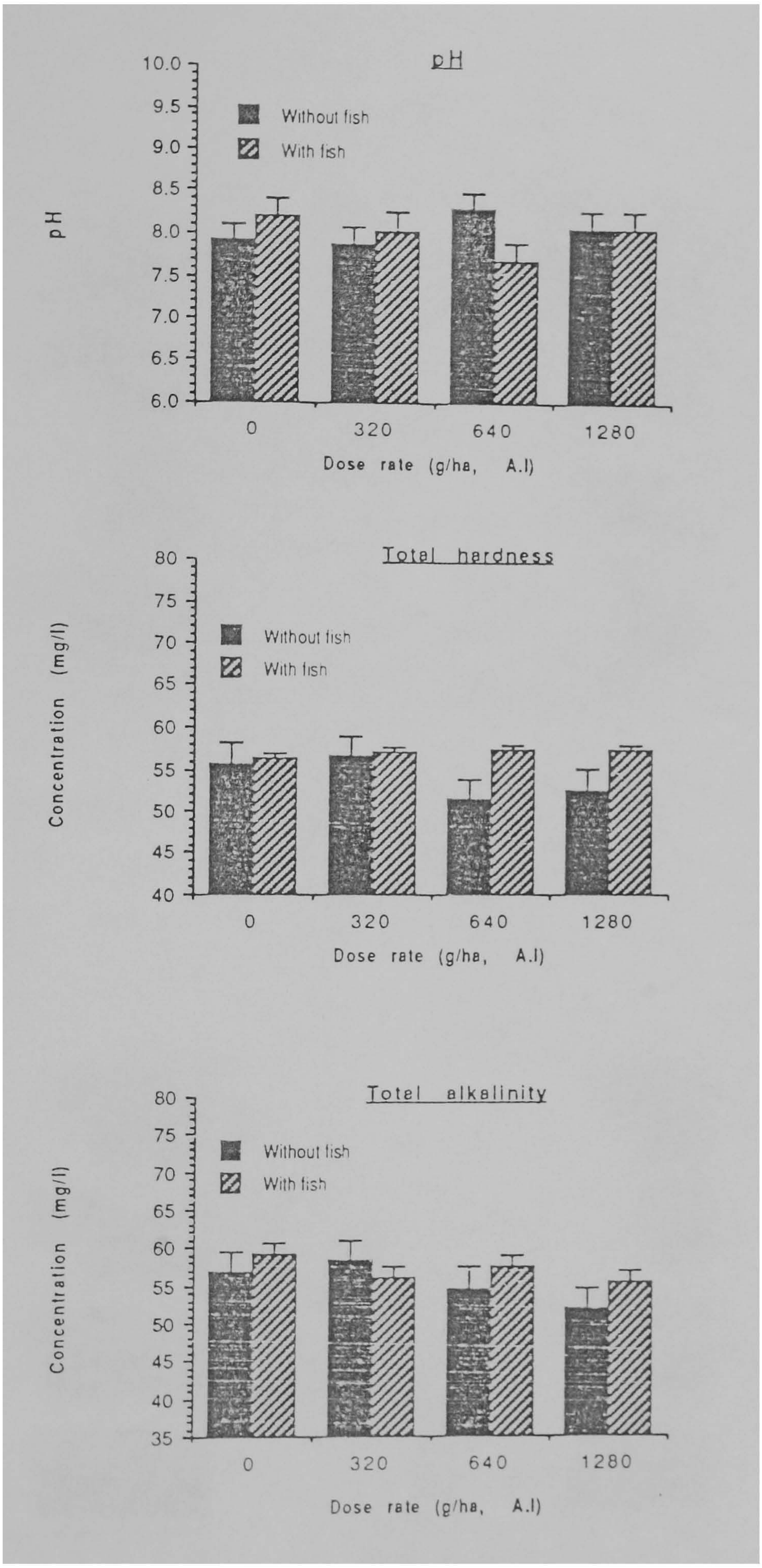

FIGURE 5.28. Levels of pH, total hardness and total alkalinity in the rice fields, measured during Experiment 4 (Means and Standard Deviation).

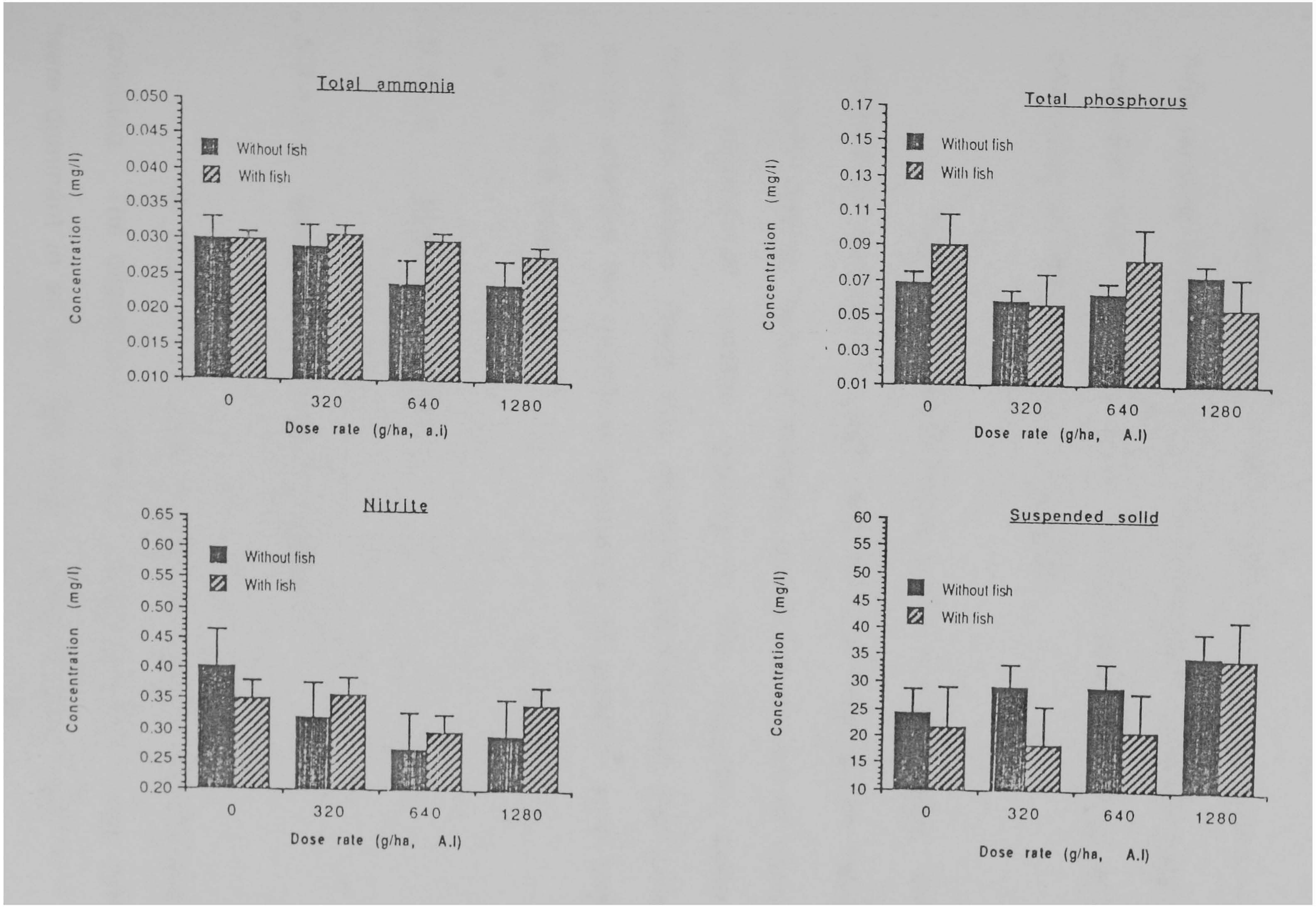

FIGURE 5.29. Levels of total ammonia, nitrite, total phosphorus and suspended solid in the rice fields, measured during Experiment 4 (Means and Standard Deviation).

Nitrite levels in the experimental plots were relatively high ranging between 0.13mgl^{-1} and 0.70mgl^{-1}, although there was no indication that this high nitrite concentration influenced growth or production of common carp fingerlings.

Suspended solids levels in the rice field plots fluctuated significantly between 6.1mgl^{-1} and 88.0mgl^{-1} during the experiment, primarily due to frequent rainfalls during the period of experiment, and substantial organic loading in the irrigation water from domestic wastes. There was, however, no indication that suspended solids affected the growth or production of common carp fingerlings in the rice field plots.

5.3.4.5 Rice field biota

5.3.4.5.1 Benthic macro-invertebrates

A total of 12 taxa of benthic macro invertebrates were collected. The oligochaete *Tubifex tubifex* and *Branchiura sowerbyi* were dominant in all rice field plots. *T.tubifex* was found in relatively high densities, ranging from 81 to 269 specimens per core. The larvae of Chironomidae were also found in great number in the treatment plots. A list of the major taxa collected is presented in Table 5.23.

The population of the benthic macro invertebrates in all rice field plots showed some fluctuations, without definite trends. There was no apparent difference between the population abundance in insecticide treated and untreated control plots (Figure 5.30).

5.3.4.5.2 Zooplankton

A total of 15 taxa were collected from the treatment plots, comprising of 3 genera of Protozoa, 7 genera of Rotifera, 4 genera of Crustacea and 1 genus of Insecta (Table 5.24). The copepod *Cyclops sp* and the rotifer *Brachionus sp.*dominated the population of zooplankton in all rice field plots.

The temporal changes in the abundance of copepods and rotifers in the treatment plots showed some fluctuations with no

TABLE 5.23 List of species/genera of benthic macro-invertebrates collected from rice field plots in Experiment 4 (*Diazinon*).

Species/Genera	Rice field with fish	Rice field without fish
<u>Oligochaeta</u>		
Nais communis (Piguet)	++	++
Haplotaxis sp.	+	+
Lumbriculus sp	+	+
Branchiura sowerbyi (Beddard)	++	++
Tubifex tubifex (Muller)	++	++
Dero limosa (Leidy)	+	
<u>Hirudinea</u>		
Glossiphonia weberi (Blanchard)	+	+
Helobdella sp.	+	+
<u>Insecta</u>		
Berosus sp.	+	+
Chironomus sp.	++	++
Polypedilum sp.	+	+
Tanypus sp.	+	+
Culex sp.	+	

<u>NOTE</u> : + - Presence ++ - Dominant

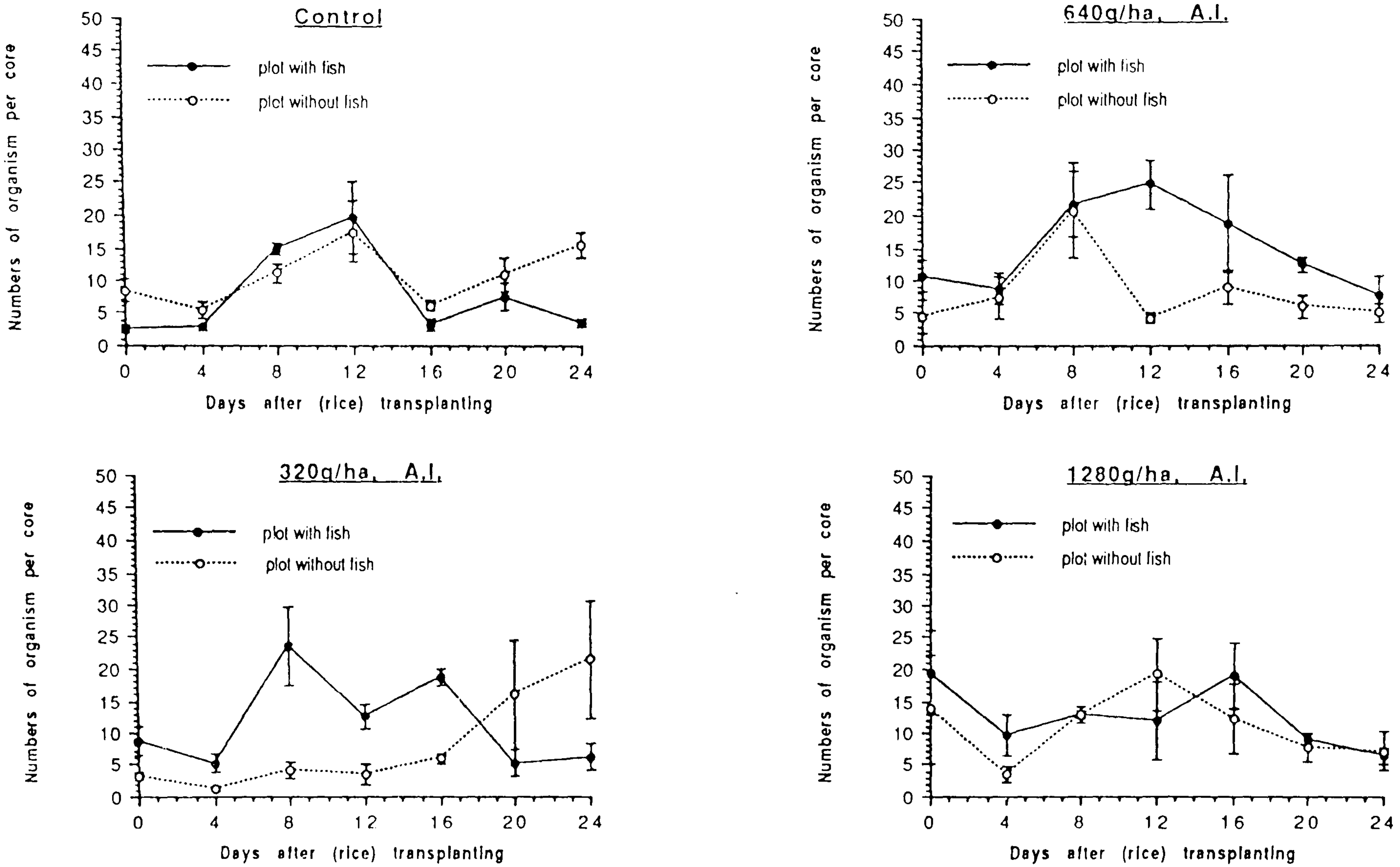

FIGURE 5.30. Abundance of benthic macro organisms in wet rice fields treated with different dose rates of *diazinon* (Means and Standard Error).

TABLE 5.24 List of species/genera of zooplankton collected rice field plots in Experiment 4 (*Diazinon*).

Species/Genera	Rice field with fish	Rice field without fish
Protozoa		
Arcella hemispherica (Perty)	+	
Centropyxis aculeata (Stein)	+	+
Difflugia pyriformis (Perty)		+
Rotifera		
Brachionus plicatilis (Muller)	++	++
Epiphanes sp	+	+
Euchlanis sp.	+	
Filinia sp.	+	+
Lecane sp.	+	++
Monostyla sp.	+	+
Polyarthra trigla (Ehrenberg)	+	+
Keratella asymmetrica (Gosse)	+	+
Crustacea		
Cyclops sp.	++	++
Moinadaphnia macleayi (King)	+	+
Nauplius larvae	+	++
Cypris sp.	+	+
Insecta		
Tendipedidae		+

NOTE : + - Prsesence ++ - Dominant

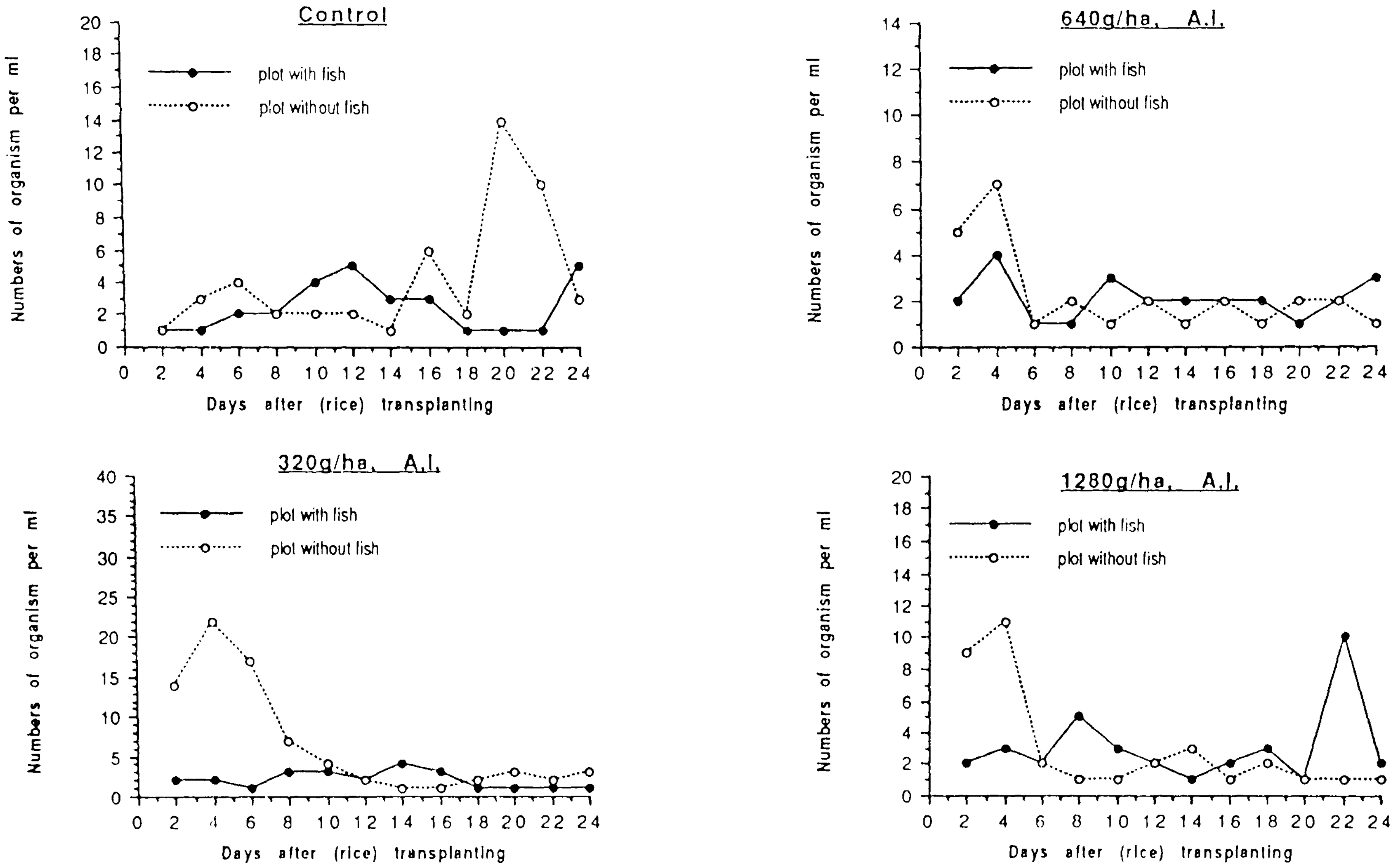

FIGURE 5.31. Abundance of copepod s in wet rice fields treated with different dose rates of *diazinon*.

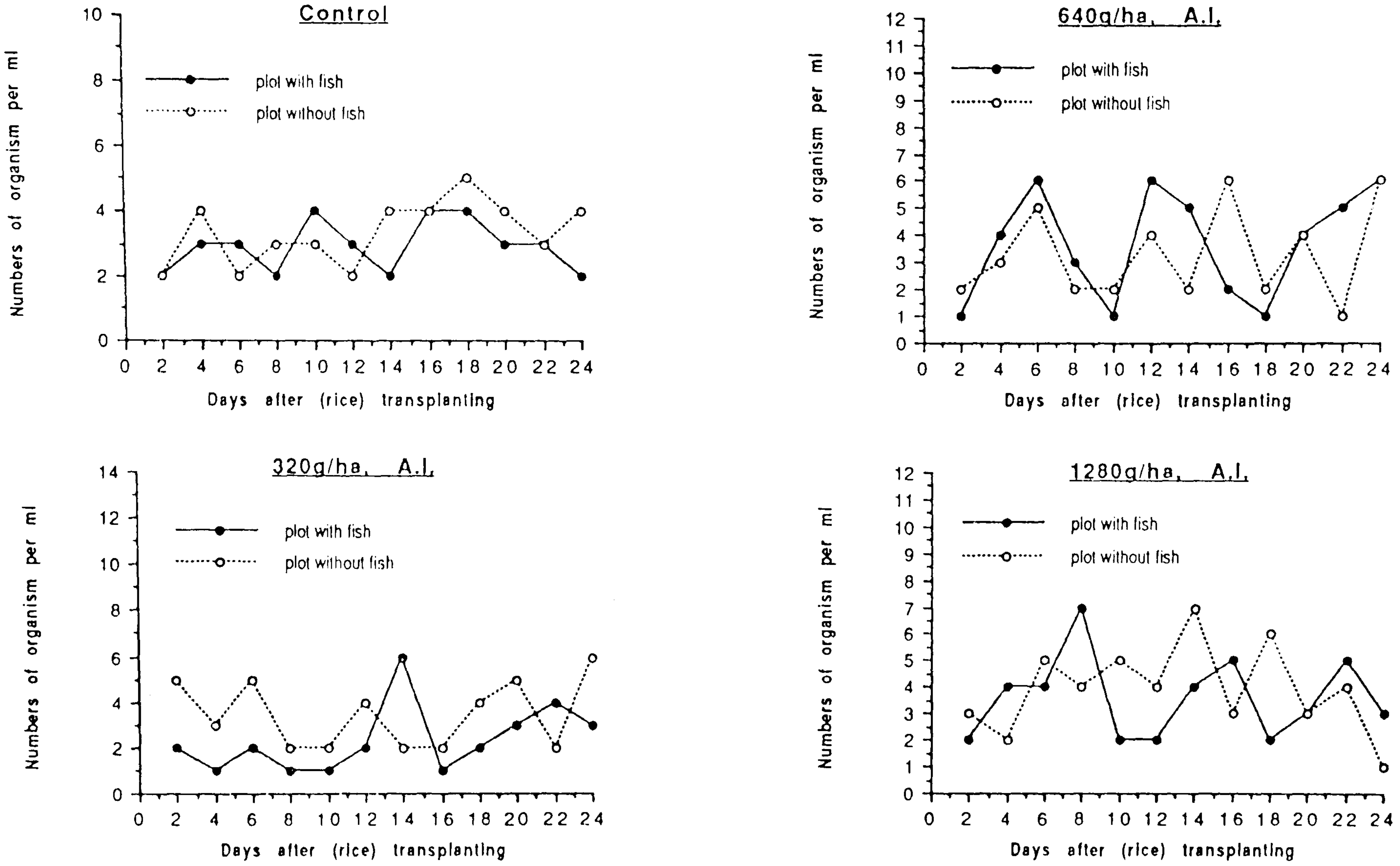

FIGURE 5.32. Abundance of rotifers in wet rice fields treated with different dose rates of *diazinon*.

definite trends, as summarised in Figure 5.31 and Figure 5.32, respectively..

The direct effects of *diazinon* treatment to some aquatic fauna could be visually observed 1 to 2 hours after spraying. Aquatic invertebrates that frequently come to the surface for air and aquatic insects were observed to be affected most rapidly. *Notonecta sp* and *Hydrometra sp.* were soon affected, and so were water beetles, particularly *Dystiscus sp.* and *Gyrris sp.*, which died at the surface of the rice field water within a few hours. *Tubifex tubifex* and other oligochaetes appeared on the surface of the rice field mud bottom, apparently affected by the insecticide treatments, particularly at the two highest dose rates (640 and 1280gha^{-1},A.I).

5.3.5 Experiment 5 (*Alphamethrin*)

5.3.5.1 Growth rate

The initial/final weight and the growth rate (in terms of absolute weight increment per day) of fish in rice fields exposed to different dose rates of *alphamethrin* are presented in Table 5.25. The growth rate of fish in the control plots and in the insecticide treated plots were comparable, ranging between 0.55gd^{-1} and 0.59gd^{-1} ($P > 0.05$). The mean initial weight of common carp fingerlings used in this experiment was smaller than those used in previous experiments, ranging between 2.5g and 2.7g ($P > 0.05$). Their mean final weight final weight after 21d rearing period in the rice field plots ranged between 13.2g and 15.1g ($P > 0.05$), (Figure 5.33).

5.3.5.2 Survival rate

The data on fish survival, total biomass yield and weight gain of fish biomass in the rice fields are presented in Table 5.26.

TABLE 5.25. Mean initial/final weights and mean growth rate (in terms of absolute weight increment per day) of common carp fingerlings in rice fields receiving different dose rates of *alphamethrin*.

	Dose rate (gha^{-1}, A.I.)				F	Significant level	Probability
	0	7.5	15.0	30.0			
Initial weight (g)	2.6	2.5	2.6	2.7	0.06	NS	> 0.05
Standard error	0.1	0.1	0.1	0.1			
Final weight (g)	14.5	15.1	15.1	13.3	0.04	NS	> 0.05
Standard error	0.8	0.6	0.8	0.8			
Growth rate (gd^{-1})	0.55	0.58	0.59	0.55	0.08	NS	> 0.05
Standard error	0.07	0.04	0.04	0.08			

NOTE. NS - Not significant

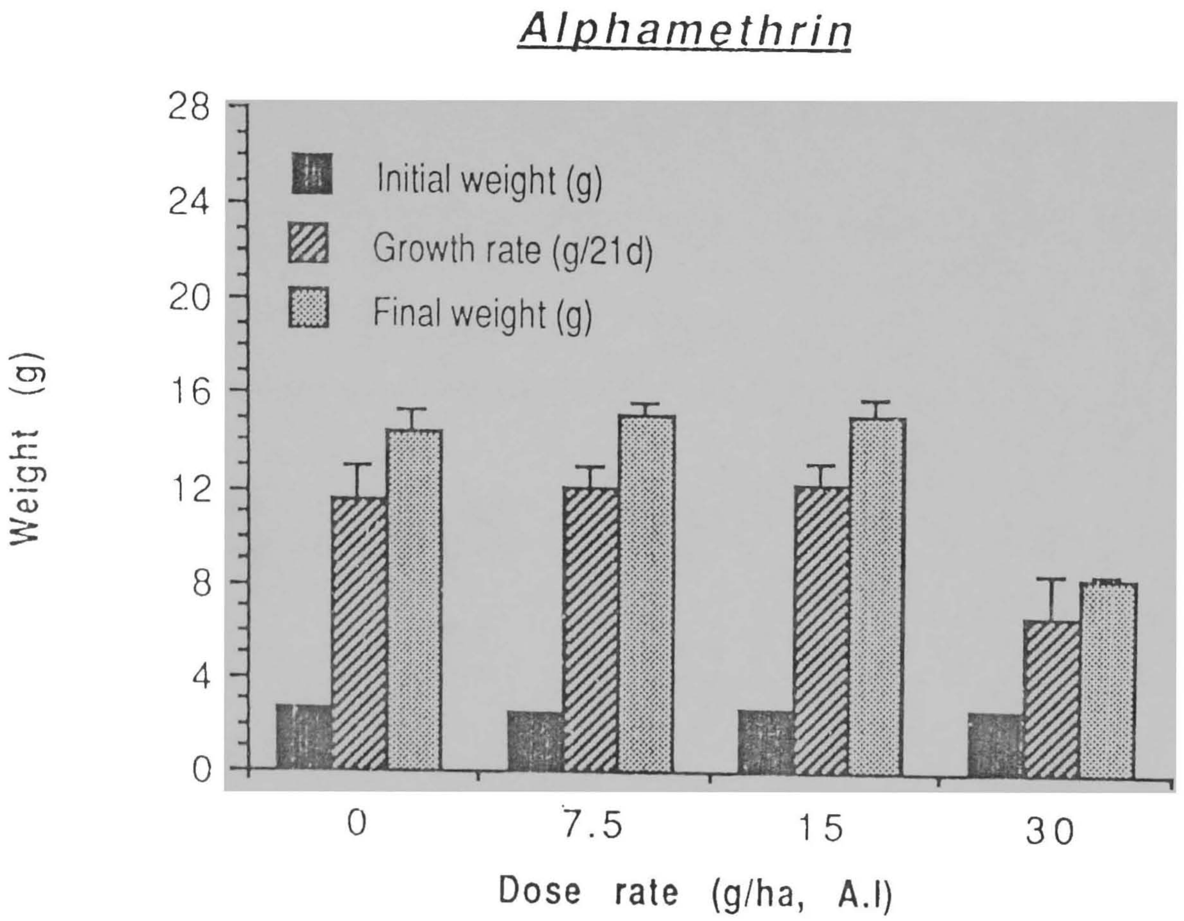

FIGURE 5.33. Growth rate of common carp fingerlings (*C. carpio*) in wet rice field receiving different dose rates of *alphamethrin* (Means and Standard Error).

TABLE 5.26. Summary of survival rate, biomass yield and biomass weight gain of common carp fingerlings in rice fields receiving different dose rates of *alphamethrin* (Means and standard error).

	Dose rate (gha^{-1},A.I)				F	Significant level	Probability
	0	7.5	15.0	30.			
Stocking density (gm^{-2})	1.38	1.33	1.32	1.35	0.06	NS	> 0.05
Standard error	0.03	0.02	0.02	0.03			
Survival rate (%)	81.7	70.0	73.3	46.7	1.61	NS	> 0.05
Standard error	4.4	10.4	11.7	16.9			
Total yield ($gm^{-2}21d^{-1}$)	5.9	5.2	5.5	3.1	1.68	NS	> 0.05
Standard error	1.0	1.0	1.1	0.8			
Weight gain ($gm^{-2}d^{-1}$)	0.22	0.18	0.20	0.08	1.61	NS	> 0.05
Standard error	0.05	0.05	0.05	0.05			

Note . NS - Not significant

The survival rate of fish in control plots ranged between 60.0% and 71.6%. The mean survival rates of these control plots was not significantly different from those of insecticide treated rice field plots ($P > 0.05$). The survival of fish in this experiment was lower than those in the previous experiments, perhaps because of the smaller size of the fish used in the experiment. The lowest survival was noted in rice fields receiving an *alphamethrin* dose rate of 30gha^{-1} (A.I). In two of the three replicate plots receiving this insecticide dose treatment, the survival rate was 25.0% and 35.0%, respectively. On the other hand, in the third plot an inordinate survival rate of 80% was recorded, probably due to heavy covering of aquatic plants and filamentous algae in this plot, which may have prevented some of the chemical to come directly in contact with the rice field water. Thus. survival may have been less than that actually indicated by the mean survival of 43%.

5.3.5.3 Fish production

Based on the results of statistical analysis of variance, the fish biomass yield and weight gain in the treatment plots were not significantly different ($P > 0.05$). However, a perceptible decline of fish production and biomass weight gain was noted in rice field plots receiving the highest dose rate of *alphamethrin* ($30gha^{-1}$, A.I), possibly due to high fish mortality caused by this treatment (see Section 5.3.5.2). Mean fish biomass yield and fish biomass weight gain recorded in this treatment were $3.1gm^{-2}21d^{-1}$ and $0.08gm^{-2}d^{-1}$, respectively,as compared to $5.9gm^{-2}21d^{-1}$ and $0.22gd^{-1}$, respectively, in the control (Figure 5.34).

5.3.5.4 Water quality

The results of the water quality measurements are presented in Table 5.27 and Table 5.28. In general the water quality parameters measured were not significantly influenced by the insecticide, or by the presence of fish in the rice fields or the time of analysis ($P > 0.05$). However, dissolved oxygen, carbon dioxide and pH were found to be significantly influenced by the presence of fish in the rice field plots ($P < 0.05$). Dissolved oxygen concentrations were found higher in rice fish plots without fish, while carbon dioxide concentrations decreased in these plots.

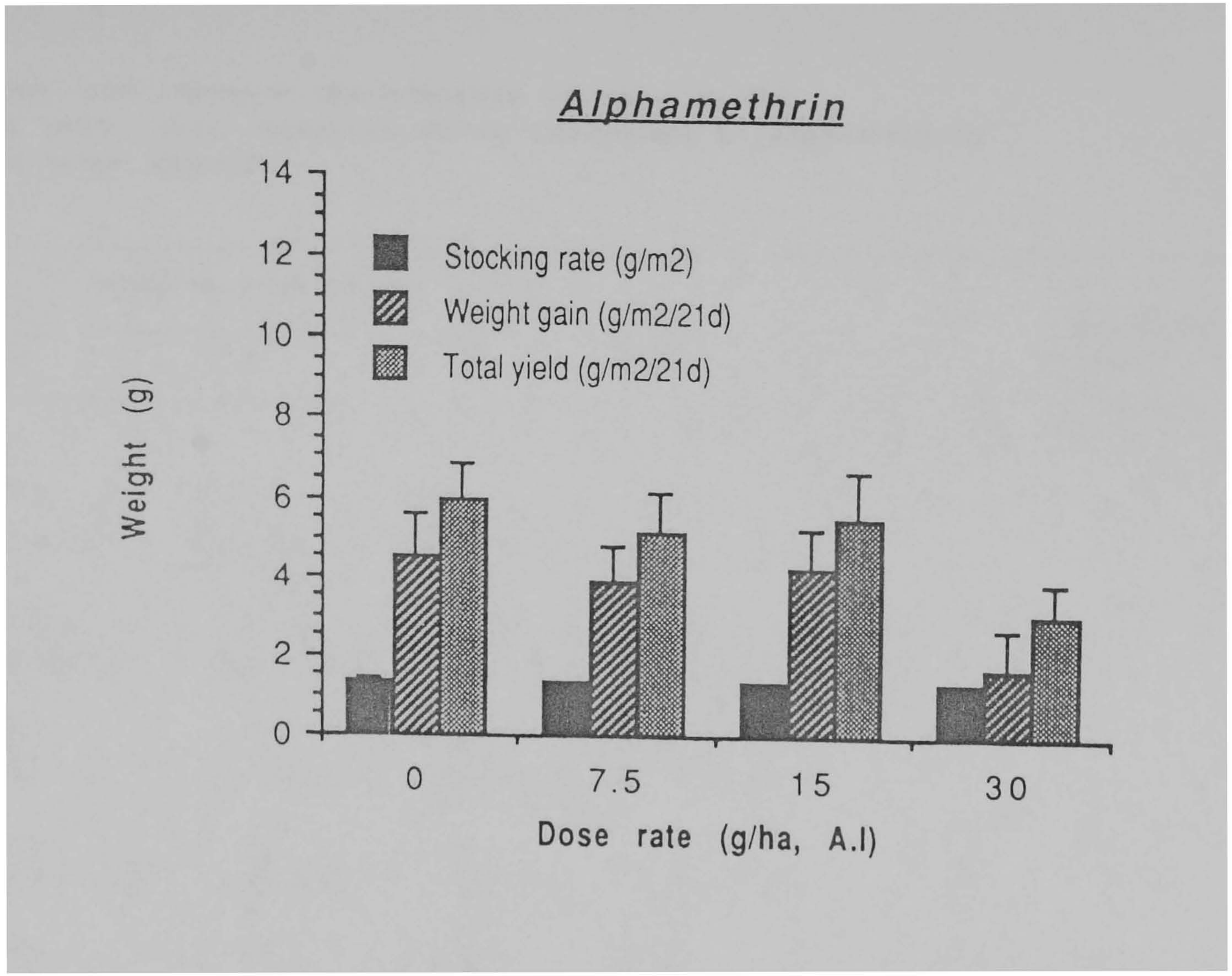

FIGURE 5.34. Production of common carp fingerlings (*C. carpio*) in wet rice fields receiving different dose rates of *alphamethrin* (Means and Standard Error).

TABLE 5.27. The physico- and chemical characteristics of water in the rice fields (with fish), measured during Experiment 5 (*Alphamethrin*) (Mean and range values)

	Insecticide dose rate (gha^{-1}, A.I.)				F	Significant level	Probability
	0	7.5	15.0	30.0			
Temperature (^{o}C)	26.8 (24.0-29.7)	26.9 (24.0-29.7)	26.9 (24.0-29.5)	27.0 (24.0-30.0)	0.14	NS	> 0.05
Dissolved oxygen (mgl^{-1})	8.2 (5.6-15.0)	7.9 (6.0-10.0)	9.2 (6.5-10.0)	7.9 (6.0-10.0)	1.74	NS	> 0.05
Carbondioxide (mgl^{-1})	3.21 (0-7.99)	3.16 (1.80-5.99)	3.49 (1.69-5.99)	3.39 (1.40-6.99)	1.12	NS	> 0.05
pH	7.52 (7.00-9.48)	7.65 (6.83-9.38)	7.60 (7.00-9.60)	7.27 (7.00-8.15)	1.75	NS	> 0.05
Total hardness (mgl^{-1}.$CaCO_3$)	56.5 (43.0-79.9)	57.2 (45.1-73.7)	55.9 (45.1-67.6)	58.3 (49.1-71.7)	0.49	NS	> 0.05

N.B. NS - Not significant

TABLE 5.27. (Continued)

	Insecticide dose rate (gha^{-1}, A.I.)				F	Significant level	Probability
	0	7.5	15.0	30.0			
Total alkalinity (mgl^{-1},$CaCO_3$)	57.1 (44.4-78.9)	56.3 (44.4-68.9)	55.9 (49.2-69.3)	59.1 (49.2-69.1)	0.43	NS	> 0.05
Phosphorus (mgl^{-1})	0.030 (0.018-0.129)	0.025 (0.016-0.057)	0.021 (0.011-0.032)	0.017 (0.011-0.018)	1.18	NS	> 0.05
Total ammonia (mgl^{-1})	0.021 (0.011-0.047)	0.019 (0.011-0.044)	0.020 (0.011-0.032)	0.020 (0.013-0.029)	0.33	NS	> 0.05
Nitrite (mgl^{-1})	0.140 (0.07-0.18)	0.135 (0.10-0.16)	0.153 (0.11-0.15)	0.128 (0.12-0.18)	0.98	NS	> 0.05
Suspended solid (mgl^{-1})	31.2 (8.3-61.0)	26.3 (7.5-62.0)	32.3 (7.2-62.5)	29.6 (8.6-70.0)	0.86	NS	> 0.05

NOTE: NS - Not significant

TABLE 5.28. The physico and chemical characteristics of water in the rice fields (without fish), measured during Experiment 5 (*Alphamethrin*). (Mean and range values)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	7.5	15	30			
Temperature (^{o}C)	26.9 (24.0-29.5)	26.8 (24.0-30.0)	27.0 (24.0-30.0)	27.1 (24.5-29.5)	0.14	NS	> 0.05
Dissolved oxygen (mgl^{-1})	9.3 (5.2-15.0)	9.9 (7.0-14.2)	9.3 (5.2-15.0)	9.9 (7.0-14.2)	1.74	NS	> 0.05
Carbondioxide (mgl^{-1})	2.87 (0-5.19)	2.86 (0-7.99)	2.60 (0-5.79)	1.99 (0-7.59)	1.12	NS	> 0.05
pH	7.33 (7.00-8.87)	7.34 (7.00-8.88)	7.70 (7.00-9.77)	7.99 (7.26-9.82)	1.75	NS	> 0.05
Total hardness (mgl^{-1}.CaCO$_3$)	52.8 (45.1-61.4)	56.4 (45.1-68.6)	58.9 (43.0-92.2)	54.8 (43.0-79.9)	0.49	NS	> 0.05

TABLE 5.28 (Continued)

	Insecticide dose rate (gha^{-1}, A.I)				F	Significant level	Probability
	0	7.5	15	30			
Total alkalinity	62.4	57.5	55.6	55.9	0.43	NS	> 0.05
(mgl^{-1},$CaCO_3$)	(49.2-78.9)	(49.2-88.6)	(44.4-78.9)	(49.2-78.7)			
Phosphorus	0.026	0.026	0.023	0.027	1.18	NS	> 0.05
(mg^{-1})	(0.011-0.08)	(0.016-0.057)	(0.016-0.041)	(0.011-0.094)			
Total ammonia	0.015	0.015	0.019	0.018	0.33	NS	> 0.05
(mgl^{-1})	(0.011-0.025)	(0.011-0.023)	(0.011-0.035)	(0.014-0.031)			
Nitrite	0.132	0.128	0.126	0.145	0.98	NS	> 0.05
(mgl^{-1})	(0.071-0.179)	(0.096-0.157)	(0.108-0.154)	(0.119-0.179)			
Suspended solid	28.0	23.1	30.7	24.1	0.86	NS	> 0.05
(mgl^{-1})	(8.6-70.0)	(7.6-58.5)	(7.8-55.0)	(8.1-50.5)			

NOTE NS - Not significant

Mean water temperature in the treatment plots were comparable, ranging between 26.8°C and 27.1°C. The minimum and maximum temperature recorded fluctuated widely, ranging between 24°C and 30°C, respectively, mainly due to the shallow depth of the rice field water and the shading by aquatic plants (Figure 5.35).

Dissolved oxygen concentration ranged between 5.2mgl^{-1} (61.9% saturation) and 15.0mgl^{-1} (145% saturation). This wide fluctuation of dissolved oxygen is normal in rice fields, due to the strong influence of fluctuating water temperature and photosynthesis in the system. Mean dissolved oxygen concentration in the rice field plots without fish ranged between 9.3mgl^{-1} (117.5% saturation) and 9.9mgl^{-1} (124.4% saturation), while those in the rice field plots with fish ranged between 7.9mgl^{-1} (99.1% saturation) and 9.2mgl^{-1} (116% saturation). Lower oxygen concentration in fish containing rice fields might be due to several factors, including the oxygen consumption by fish and the degree of shading by aquatic plants, which may have prevented the penetration of sunlight into the rice field water (Figure 5.35).

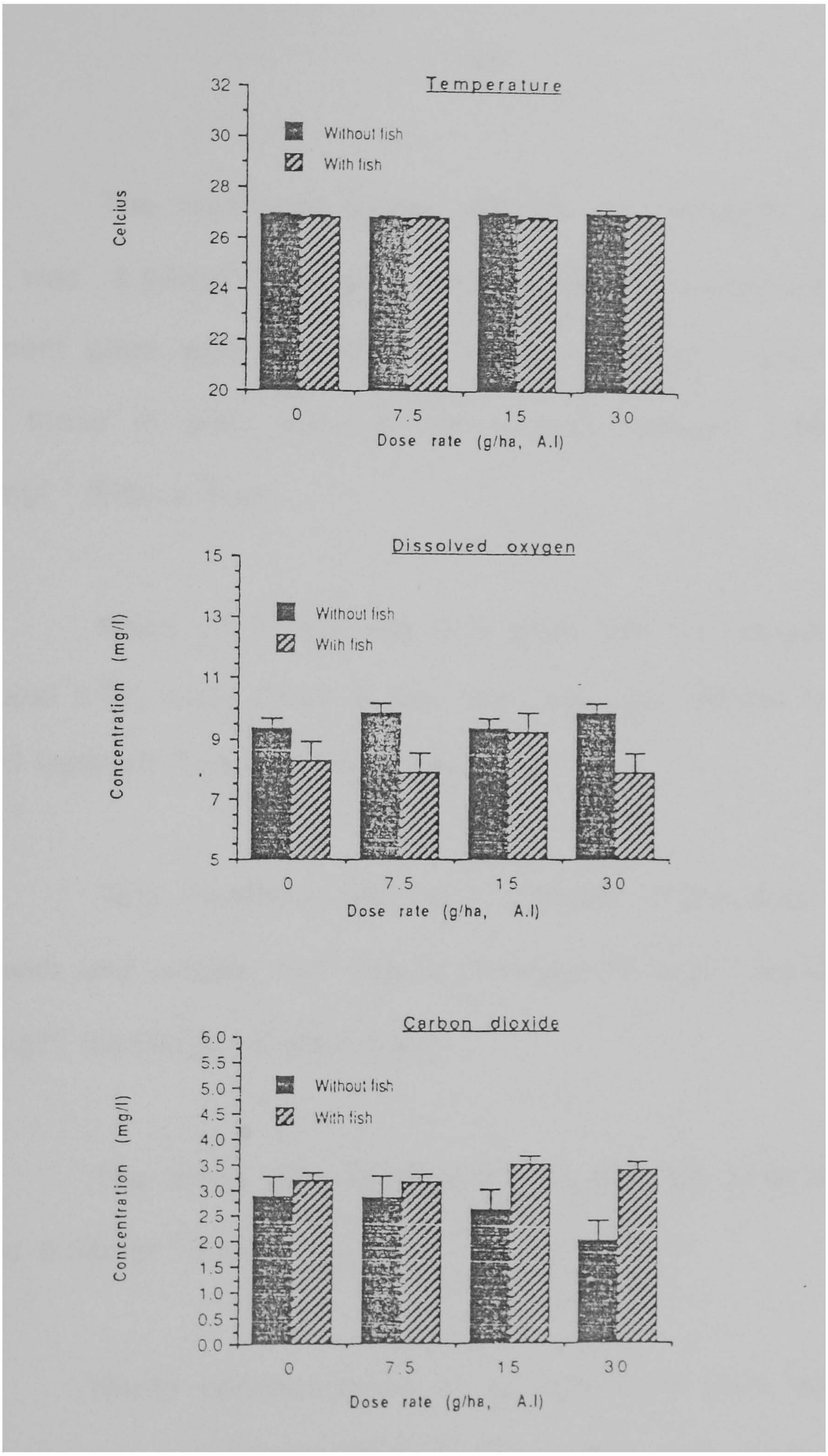

FIGURE 5.35. Levels of water temperature, dissolved oxygen and carbon dioxide concentrations in the rice fields, measured during Experiment 5 (Means and Standard Deviation).

The maximum carbon dioxide concentration in the rice field was 8.99mgl^{-1}. Mean carbon dioxide concentration in the treatment plots with fish ranged between 3.16mgl^{-1} and 3.49mgl^{-1}, while those in plots without fish ranged between 1.99mgl^{-1} and 2.87mgl^{-1} (Figure 5.35).

Mean pH in the rice field plots with fish ranged between 6.83 and 9.60, while those in the rice field plots without fish the pH ranged between 7.00 and 9.82 (Figure 5.36).

Total hardness and total alkalinity in the rice field plots both with and without fish ranged between 43.0mgl^{-1} (as $CaC0_3$) and 92.2mgl^{-1} (as $CaC0_3$) (Figure 5.36).

The levels of total ammonia in the rice field plots never exceed 0.04mgl^{-1} (Figure 5.37).

Nitrite concentrations in all treatment plots were rather high, but never exceeded 0.22mgl^{-1}. This rather high nitrite levels

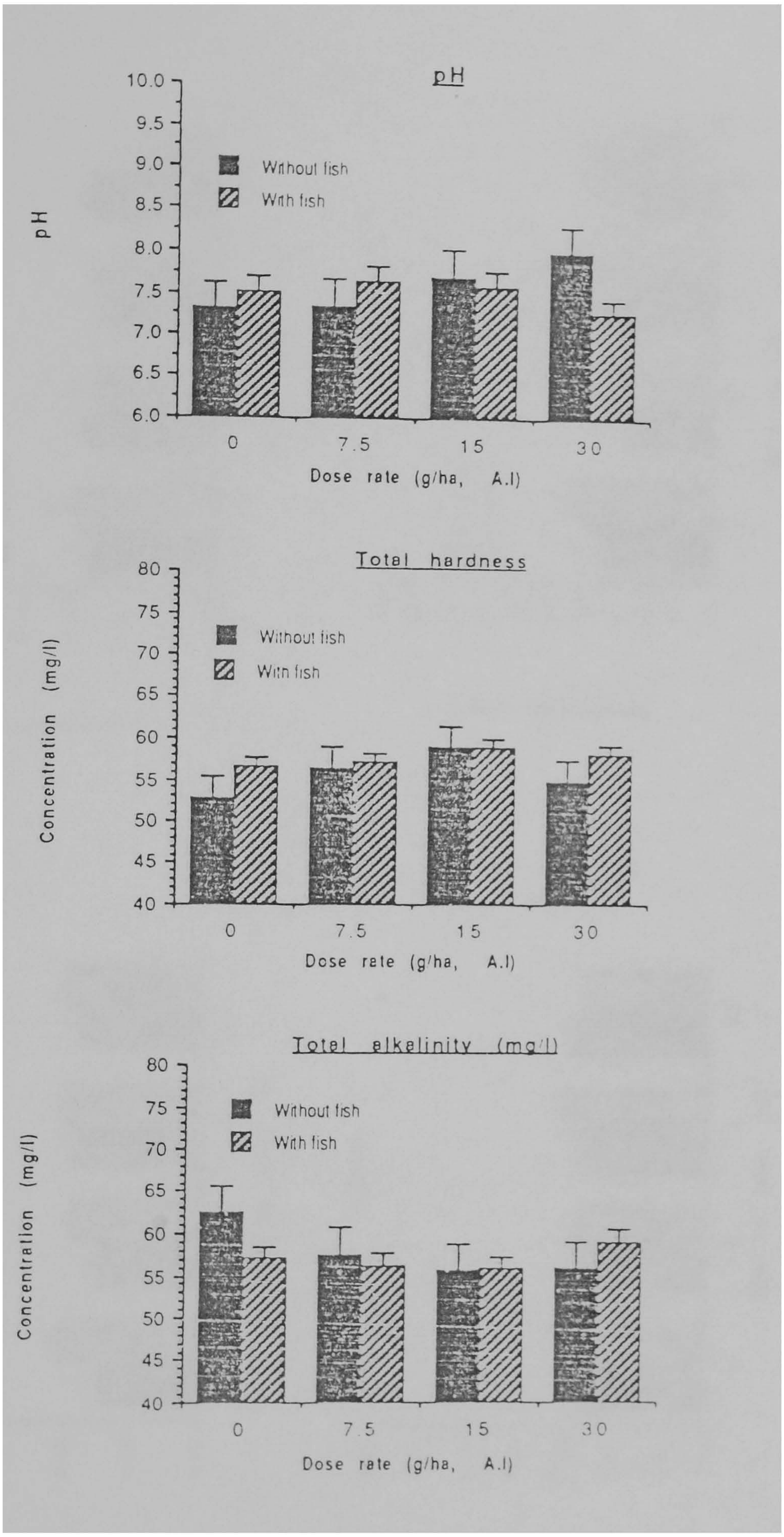

FIGURE 5.36. Levels of pH, total hardness and total alkalinity in the rice fields, measured during Experiment 5 (Means and Standard Deviation).

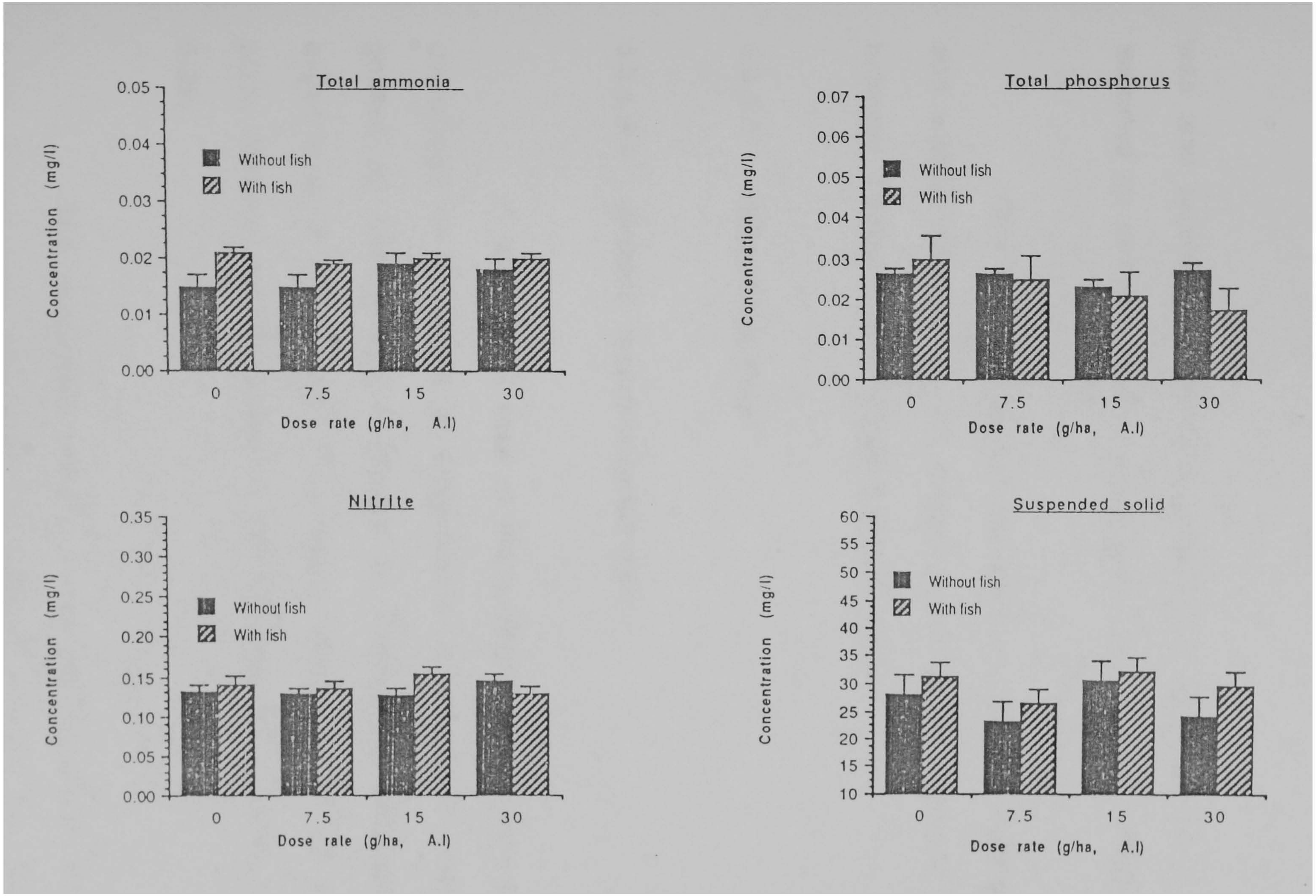

FIGURE 5.37. Levels of total ammonia, nitrite, total phosphorus and suspended solid in the rice fields, measured during Experiment 5 (Means and Standard Deviation).

was also recorded in previous experiments and seemed to be well tolerated by common carp fingerlings in the rice fields (Figure 5.37).

The levels of suspended solids in the rice field plots with and without fish during the experiment fluctuated greatly (ranging between 7.6mgl^{-1} and 70.0mgl^{-1}) (Figure 5.37).

5.3.5.5 Rice field biota

5.3.5.5.1 Benthic macro–invertebrates

A total of 17 taxa of macro–invertebrates were collected, comprising of 8 genera of Oligochaeta, 2 genera of Hirudinea, 7 genera of Insecta and 4 genera of Gastropoda (Mollusca). The oligochaete *T. tubifex* and *B. sowerbyi* dominated in all rice field plots, followed by the larvae of the dipteran *Chironomus sp.* (Table 5.29).

The population density of the benthic fauna in the rice fields showed some fluctuation, probably due to the the climatic and

TABLE 5.29 List of species/genera of benthic macro-invertebrates collected from rice field plots in Experiment 5 (Alphamethrin).

Species/Genus	Rice field with fish	Rice field without fish
Oligochaeta		
Nais communis (Piguet)	+	+
Haplotaxis sp.	+	+
Lumbriculus sp.	+	+
Branchiura sowerbyi (Beddard)	++	++
Tubifex tubifex (Muller)	++	++
Dero zeylanica (Stephenson)	+	
Hirudinea		
Glossiphonia weberi (Blanchard)	+	+
Helobdella sp.	+	+
Insecta		
Berosus sp.		+
Chironomus sp.	++	++
Polypedilum sp.	+	+
Tanypus sp.		+
Paratanytarpus		+
Xenochironomus sp.	+	
Culex sp.	+	
Gastropoda		
Bellamya javanica	++	++
Mellaoides tuberculata (Muller)	+	+
Thiara scabra	+	+
Corbicula javanica		+

NOTE : + - Presence ++ - Dominant

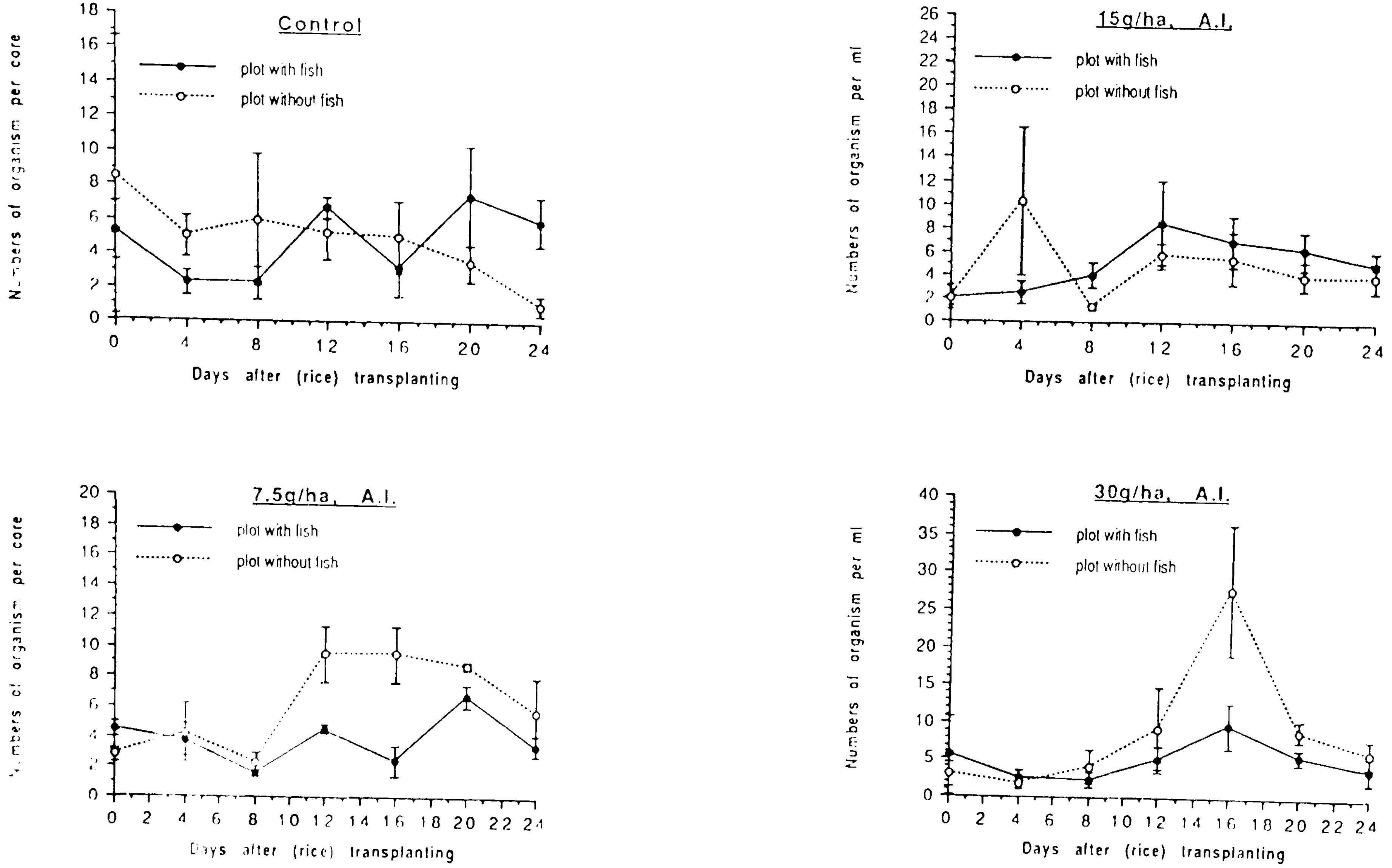

FIGURE 5.38. Abundance of benthic macro organisms in wet rice fields treated with different dose rates of *alphamethrin* (Means and Standard Error).

environmental variations in the rice field environment (Figure 5.38). There was, however, no definite trends in this variation. The data suggest no notable differences in the temporal changes of the benthic macro–invertebrates population in the rice field plots, both with fish and without fish, or in the plots receiving different *alphamethrin* treatments.

5.3.5.5.2 Zooplankton

The copepod *Cyclops* sp and the nauplius larvae dominated the zooplankton in all rice field plots. The composition of the zooplankton collected during the experiment in the rice field plots with fish and without fish, is presented in Table 5.30.

The population density of the copepods and the rotifers, in both treated and untreated rice field plots, showed some fluctuations, but indicated no definite trends. Copepod population was increased at 14 to 18 days after rice transplanting, in all rice fields, including those treated with *alphamethrin*, probably due to the effect of the inorganic fertilisers (Figure 5.39 & 5.40).

TABLE 5.30. List of species/genera of zooplankton collected from rice field plots in Experiment 5 (*Alphamethrin*).

Species/Genus	Rice field with fish	Rice field without fish
Protozoa		
Arcella hemispherica (Perty)	+	+
Centropyxis ecornis (Leidy)	+	+
Rotifera		
Brachionus plicatilis (Muller)	+	+
Euchlanis sp.	+	+
Filinia sp.	+	
Lecane plaenensis (Voigt)	+	+
Monostyla sp.	+	+
Asplanchna sp.		+
Epiphanes sp.		+
Crustacea		
Cyclops sp.	++	++
Moina micrura (Kurz)	+	+
Nauplius larvae	++	++
Cypris sp.	+	+
Insecta		
Tendipedidae	+	+

NOTE : + - Presence ++ - Dominant

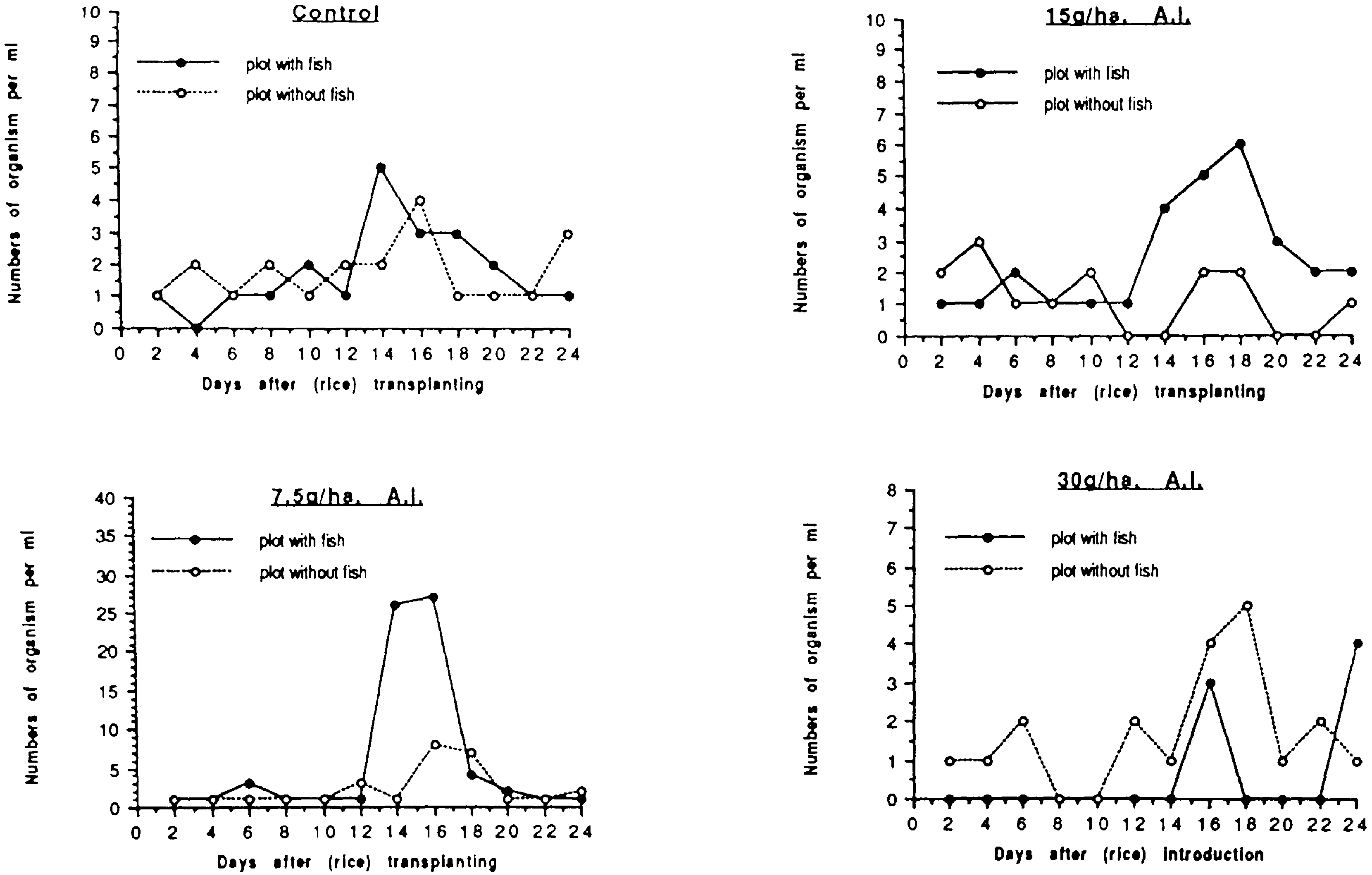

FIGURE 5.39. Abundance of copepod s in wet rice fields treated with different dose rates of *alphamethrin*.

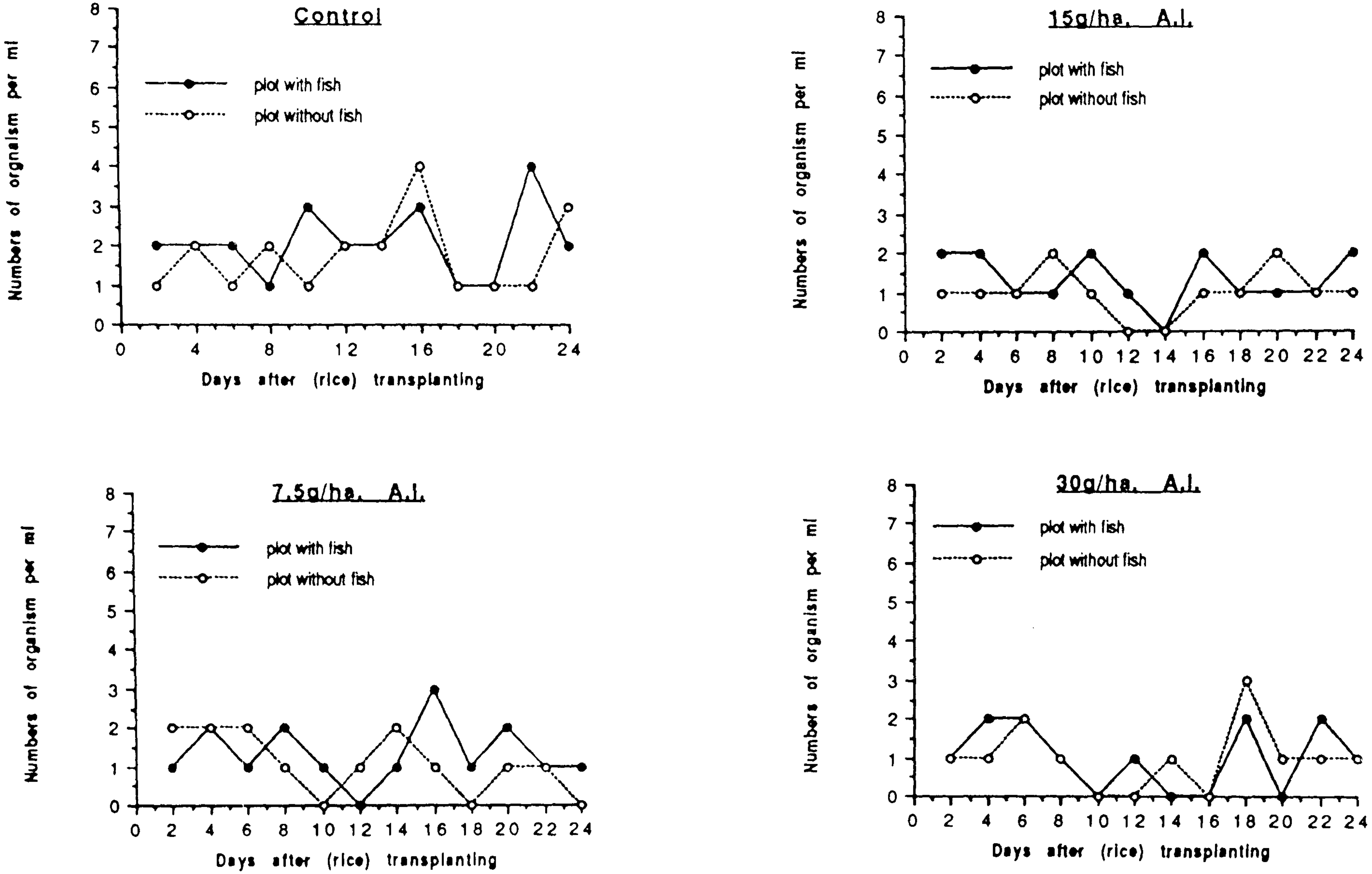

FIGURE 5.40. Abundance of rotifers in wet rice fields treated with different dose rates of *alphamethrin.*

There was no definite pattern in the variation of the population density of rotifers in both treated and untreated rice fields. Further evaluation on the effects of *alphamethrin* to the zooplankton in this experiment was therefore not possible.

The effects of *alphamethrin* to other rice field fauna, notably aquatic insects and crabs were, however, apparent during the experiment. Aquatic insects, commonly found on the surface of the rice field water, such as *Notonecta sp., Hydrometra sp*, and *Gerris sp*. were affected directly after the application of *alphamethrin*, particularly at the highest dose rate (30gha^{-1},A.I),showing typical signs of pyrethroid poisoning, i.e hyperactivity, and loss of orientation. Water beetles, such as *Dysticus sp*. and *Berosus sp*. were also affected and died at the surface of the rice field waters within a few hours. *Alphamethrin* appeared to be very toxic to aquatic crabs, *Parathelphusa sp.*, which were strongly affected by the chemicals in less than one hour after treatment, as they came out of their burrows and died on the rice field dikes and water surface in all treated plots.

5.3.6 Summary of results

The results of the experiments reveal that in general the application of the rice insecticides tested (*fenobucarb, isoprocarb, buprofezin, diazinon,* and *alphamethrin*), up to twice their recommended dose rates for rice pest control, appear not to have any significant lethal effects on common carp fingerlings raised in wet rice fields. Using fish stocking rates ranging between $2.2gm^{-2}$ and $3.0gm^{-2}$, the survival rate of the common carp fingerlings in all experiments were excellent for rice field condition, i.e between 73.3% and 100%. There was, however, an indication that the application of the highest dose rate of *alphamethrin* used in the experiment ($30gha^{-1}$,A.I) might be lethal to fish, although this effect could not be statistically confirmed in the experiment.

Application of the rice insecticides also appeared not to effect the absolute growth rate of the fish in the rice fields, which range between $0.60gd^{-1}$ and $1.0gd^{-1}$. Lower fish growth was, however, observed in rice field plots receiving the highest dose rates of *fenobucarb* ($1500gha^{-1}$,A.I), *buprofezin* ($200gha^{-1}$,A.I) and *diazinon* ($1280gha^{-1}$,A.I).

The total yield of fish biomass in the rice fields was significantly influenced by the application of the highest dose rate of *fenobucarb* (1500gha^{-1}, A.I) , i.e 9.6% lower than in the untreated rice fields ($P <0.05$). Lower yield of fish biomass in rice fields treated with the highest dose rates of *diazinon* and *alphamethrin* was also observed in the experiments. Application of the other insecticides tested did not influence the yield of fish biomass in the rice fields, which ranged between 6.9$gm^{-2}21d^{-1}$ and 13.3$gm^{-2}21d^{-1}$.

The water quality measured during the experiments fluctuated within normal limits for rice field conditions. There was no indication that the variation of water quality in the rice field plots significantly influenced the growth and production of common carp fingerlings in the experimental rice fields, although both the presence of fish and the treatments influenced some parameters in the experiments..

Observations on the zooplankton and benthic fauna in the experimental rice field plots revealed, that in general their population density was low, despite the addition of the organic fertiliser. This finding may be due to the short period of seasonal recolonisation of the aquatic phase of the rice field plots afforded in the experiments, and also due to the absence of marshes in the area as a source of aquatic fauna for the rapid colonisation of the system. Rice field benthic fauna collected during the experiments comprised mainly of several genera of Oligochaeta (*Tubifex, Nais, Branchiura*) and Insecta (*Chironomus, Tanypus*). The zooplankton collected from the experimental rice field plots comprised mainly of copepods (*Cyclops*), cladocerans (*Moina*) and rotifers (*Brachionus, Keratella*).

The population density of the benthic macro–invertebrates and the zooplankton in the treatment plots during the experiments showed temporal changes, attributable to several natural factors, such as habitat location and food habit of the fauna, predator–prey relationship and inter–specific competition, and the growth and development of aquatic macrophytes. There was, however, no indication of a definite pattern in the changes of the population density observed in the experiment, that might be associated with the insecticide applications. This result may have been due mainly to the sampling methodology adopted in the experiment in view of the apparently large population variability of the organisms between the rice field plots. However, the effect of insecticide residues could also have been decreased by adsorption, flushing and biodegradation processes (which would have occurred rapidly because of the high prevailing temperatures).

Direct lethal effects on some aquatic fauna of the rice fields, particularly on aquatic insects and oligochaetes, during the application of *diazinon* and *alphamethrin*, were visually apparent within 1 to 2 hours. Strong lethal effect of the application of *alphamethrin* on the rice field crab, *Parathelphusa* sp., was particularly noticeable.

5.4 DISCUSSION

The lethal effects of the application of rice insecticides to fish in the experiments were found to be insignificant, despite their relatively high acute toxicity to fish, as determined in laboratory tests. These results were basically in agreement with those obtained by other investigators (KOK & PATHAK, 1966; MOULTON,1973; ARCE & FERMIN,1977; ARCE *et al.*,1978; CROSSLAND, 1982;STEPHENSON,1984). This limited effect could have been attributable to several factors, including adsorption of the insecticides by the rice field soil and aquatic plants (rice plants and filamentous algae), thereby reducing the initial concentration level of the chemicals in the rice field water. The formulations of the insecticide used in the experiments (i.e wettable powder and emulsifiable concentrates) which were substantially insoluble in water, also determined the amount of the chemicals that come in direct contact with water. STEPHENSON *et al.*(1984) based on their field studies on the effects of the pyrethroid insecticide *cypermethrin* to fish, pointed out that the spraying of liquid formulation resulted in a significant proportion of the insecticide being deposited in the crop not on the water. Their statement is substantiated by CROSSLAND (1982) who found that the application of an emulsifiable concentrate formulation of cypermethrin to the surface of natural ponds produced only 8–16% of the maximum theoretical/possible concentration (i.e the concen-tration that would have been achieved had all of the applied dose dispersed uniformly unto the water). This concentration decreased rapidly with time to 1–5% within 7 days, probably as the results of several processes including hydrolysis and biodegradation. It is believed that adsorption of pesticide to soil is the major factor that reduce the actual concentration levels of the chemical in natural waters

(EDWARDS,1977). In water pesticides become bound to organic matter in mud and sediment quite rapidly, and only small amounts remain in solution (SMITH *et al.*,1966; HUGHES *et al.*,1980; HURLBERT *et al.*,1970). MACEK *et al.*(1972) estimated that only 50% of the theoretical concentration of Dursban (*chloropyriphos-ethyl*) applied in pond at 0.05 lb/acre was recovered in the water after 24 hours. HURLBERT *et al.* (1976) reported that Dursban residues in pond water were very low at 4 hours after treatment (at 1 lb/acre, 0.2ppm) and decline rapidly after 7 days (0.006ppm). Initial residue levels in mud were lower (0.01ppm) but increased to maximum (0.3ppm) at 7 days. Residues in vegetation were often initially high, but decline rapidly (at 1 lb/acre: 26 ppm at 4 hours, 1.1ppm at 7 days). Based on his pond experiments with *cypermethrin*, CROSSLAND (1984) also suggested that some 50% of the compound in the water was bound to suspended solids, and thus not available to fish. This author considered this result was due to the low water solubility and strong affinity of the chemical for surfaces, particularly those with a high content of organic matter. BAUGHMAN and LASSITER (1978) stated that sorption of compounds between water and biotic (e.g micro-organisms) and abiotic (e.g sediments) components was the most important environmental factor affecting the fate of chemicals in the aquatic systems.

Cypermethrin and *alphamethrin* preparations have similar properties in aquatic toxicological studies performed in the laboratory (STEPHENSON,1982;1985). However, according to EDWARDS and MILLBURN (1985), with *alphamethrin* the fish are exposed to considerably less active ingredient than is the case for *cypermethrin.*

ALY and EL-DIB (1971) reported that monoalkyl carbamates were susceptible to hydrolysis which increases with pH. The pH values of the rice field water measured during the experiments showed a slight variation from neutral to basic (i.e 7 to 9), and could substantially influence the stability of the carbamate insecticides tested in the rice field water. The stability of the carbamate insecticides, according to the authors, were also influenced by temperature variations. The rate of hydrolysis increases 2–3 times for each 10°C (e.g from 20° to 30°C), which means that the stability of these compounds in field condition would be much less than depicted from laboratory experiments. Therefore, as shown from the results of the present experiments, *fenobucarb* and *isoprocarb* (both were monoalkyl carbamates) were the least toxic to fish, presumably due to their low stability and rapid loss from the rice field environment.

Another group of insecticide used in the experiments, the organophosphates, represented by *diazinon*, is also reported to have negligible persistence in water (MOODY *et al.*,1978; SHAROM *et al.*, 1980). MILLER *et al.*(1966) reported that the application of ^{14}C-labelled diazinon at the rate of 5 lb/acre to a cranberry bog under conditions that simulated natural flooding, showed that the compound disappeared from water within 114 hours.

Another factor that contributed to the rapid loss of the insecticides from the rice fields was the flushing rate that may considerably dilute the initial concentration in the system 24 hours after treatment. As mentioned in Section 2.5.4, the average flow rate of the irrigation water into the plots would allow a relatively short residence time of 8–12 hours, causing some flushing of the insecticide residues from the experimental rice fields.

The results of the experiments also showed that there were no apparent evidence of sublethal effects of the insecticide treatments to common carp fingerlings in terms of their growth rates, except in the case of *fenobucarb* treatments. Again, this result may have been due to the limited amount of insecticide residues left in the rice field waters, minimising the exposure of fish to these chemicals. However, the reason why the sublethal effects of the insecticide treatments to fish were not so apparent in the experiments might also be due to other currently undefined factors, such as variations in the environmental condition and in the individual sensitivity of the experimental fish. According to WOLTERING(1984) fry growth and survival are generally the most sensitive endpoints in chronic toxicity tests. However, the comparative growth response is often difficult to assess due to high variability between individual fry as well as variation in the environmental conditions, especially in field experiment. As an alternative CROSSLAND (1985) proposed a well controlled laboratory test procedure, using an automated flow through method to maximise the sensitivity of measurement of the growth response. As an example, using rainbow trout (*S. gairdneri*) exposed to a series of *3,4-dichloroaniline* (DCA) concentration ($39ugl^{-1}$–$210ugl^{-1}$), the author showed that significant differences in growth rate could be demonstrated when exposure period of 28 days was used instead of 14 days.

The physico–and chemical characteristics of the rice field water measured during the experiments were within normal limits for rice field condition. The shallow, impermanent and heavily manipulated rice field aquatic habitat resulted in increased turbidity, and fluctuating temperature, dissolved oxygen and pH (HECKMAN 1979; ALI & AHMAD,1987). Temperature varied over a wide range with a mean of 29°C during the experiment. Mean temperature can be higher at midday due to the shallow depth of the rice field which when exposed to strong sunlight heats up readily, especially when not sheltered by aquatic vegetation. Water temperature was also found lower in the shaded and deeper areas (ditches) of the rice field. The dissolved oxygen concentration also varied widely in the rice fields. The chief source of dissolved oxygen during the day is clearly photosynthesis. Photosynthesis was found to be associated with increases in pH. Thus during the midday hours the pH increases were usually most pronounced. Low pH values were recorded when much biological decomposition (of rice stalks and weeds) was taken place.

The high value of suspended solids, which were also noted in the experimental rice fields, was partly due to the application of fertilisers and the domestic waste loading of the irrigation water, especially during heavy rainfalls.

Tubifex was found in abundance in rice fields with fish, particularly during the first period of the experiment. These oligochaetes have been reported commonly found in rice fields,sometimes in high density (KIKUCHI & KURIHARA,1977; HECKMAN, 1979). The larvae of the dipteran *Chironomus sp.* was also found in most rice fields, both with fish and without fish. These larvae and the oligochaetes represent important fish food organisms in the rice fields (VAAS & VAN OVEN,1959; SCHUSTER,1955; ALIKUNHI, 1966).

Large populations of aquatic insects, copepods and cladocerans are common in the rice fields (HECKMAN.1979; IDRIS, 1983; LIM *et al.*,1984). The importance of these zooplankton as food source for common carp is well documented (ALIKUNHI, 1966; MICHAELS,1981; JINGHRAN & PULLIN, 1985). Many of these aquatic invertebrates, especially cladocerans, copepods and insect larvae are reported to be more sensitive to pesticide than fish (SANDERS & COPE.1966; HURLBERT,1970; JOHNSON,1980). The adverse impact of pesticide application on the aquatic fauna is also well documented by many investigators, as reviewed by MUIRHEAD–THOMSON (1971), HURLBERT(1975) and MURTY (1986). However, these negative effects could not be determined in the present experiments primarily due to currently undefined natural factors affecting the temporal variation of the population density of the aquatic fauna in the rice fields. Based on visual observations on mortalities of some rice field fauna (especially aquatic insects and benthic macro–invertebrates) during and shortly after the insecticide treatments, it is believed that the treatment of these rice insecticides (particularly *diazinon* and *alphamethrin*), affected these rice field fauna.

Published data revealed some variations in the toxicity of the insecticides used in the experiments.to the aquatic fauna. .(NISHIUCHI,1977; JAPANESE PESTICIDE INFOR-MATION, 1987).
3h-LC50 for daphnids for *fenobucarb*, *diazinon* and *buprofezin* are 320ugl^{-1}, 0.9ugl^{-1} and 50.6mgl^{-1}, respectively. These figures suggest that the nominal concentration of *fenobucarb* and *diazinon* would affect the daphnid population in the rice fields. On the other hand , application of *buprofezin* may not cause serious problem to the organism..

Diazinon is toxic to most crustaceans and aquatic insects. and the application of this insecticide in wet rice field pose a potential harmful effects to the population of these aquatic biota. The toxicity of *diazinon* to some common crustaceans is as follows :

Test organism	Toxicity
Acartia tonsa	96h-LC50= 2.57ugl^{-1}
Gammarus lacutris	96h-LC50= 200ugl^{-1}
Semocephalus serrulatus	48h-LC50= 1.4ugl^{-1}
Daphnia magna	3h-LC50= 1.22-1.25ugl^{-1}

Literature on the toxicity of *diazinon* generally suggest that this insecticide is also high toxic to insects,as shown (NISHIUCHI and YOSHIDA, 1972) :

Test organism	Toxicity
Pteronarcys californica	96h-LC50= 25ugl^{-1}
Acroneuria lycorias	96h-LC50= 1.7ugl^{-1}
Ophiogomphus rupinculensis	30d-LC50= 2.2ugl^{-}
Hydropsyche bettoni1	30d-LC50= 3.54ugl^{-1}
Ephemerelia subvaria	30d-LC50= 1.05ugl^{-1}
Oedothorax insecticeps (rice field spider)	LD50= 2450ppm

The toxicity of the rice insecticides tested in this experiment to freshwater snails is generally low. and it is unlikely that the their normal application in rice field would serious harmful effects to the population of this aquatic fauna.

The 48-h TLm values (in ppm) of the insecticides to various freshwater snails are as follows (data from NISHIUCHI and YOSHIDA, 1972).:

	Fenobucarb	Isoprocarb	Diazinon
Indoplanorbis exustus	40	22	20
Semisulcospira libertina	18	7.8	9.2
Cipangopaludina malleata	34	13	16
Physa acuta	30	6.5	4.8

Of particular interest was the profound effects of*alphamethrin* on the rice field crab, the decapode *Parathelphusa sp.* These crabs are very common in rice fields, and subsist chiefly on insect larvae and carrion. They can reproduce in freshwater, and many tiny post larvae are found during the first rain in the rainy season (BOTT,1970). Due to their special habit as tunnel builders they are often regarded as pest by rice–fish farmers.

On the other hand, studies made by several investigators have successfully demonstrated the apparent effects of insecticide treatments to aquatic fauna in wet rice fields and in freshwater ponds. Based on their experiment in freshwater ponds, HURLBERT *et al.* (1970) reported that the dominant zooplankton species *Cyclops vernalis* Fischer and *Moina micrura* Kutz, were markedly affected by the treatments of the insecticide Dursban (*chlorpyriphos ethyl*) for controlling mosquitoes (0.01–1.0 lb/acre). The crustacean populations could recovered within 2 weeks if the lower rate of the insecticide was used. The crustacean populations, however, could not recover from the 2nd and subsequent treatments. The authors further noted that the population density of the rotifer *Asplanchna brightwelli* Gosse in the experimental ponds was initially low, but developed rapidly in those ponds where the crustaceans were reduced by Dursban treatments.

LIM *et al.* (1984) also reported a depression in the population densities of four dominant cladocerans (*M. micrura, Diaphanosoma excisum* Sars, *Alona cf.guttata* Sar and *Macrothrix spinosa* King) occurring in the rice fields when treated with the insecticide FMC 35001, an analogue of Furadan (*carbofuran*). According to the authors the responses of the cladoceran population in relation to the insecticide application did not indicate direct kills through acute toxicity immediately after spraying, but could be due to one of several factors, including sublethal toxicity, the habitat and food habits of the cladocerans and the predator–prey relationships as well as the inter–specific competition. The insecticide entering the water might not be concentrated enough to cause direct kills, but could result in sublethal levels affecting the physiological functions of the cladocerans, especially their reproduction capacity. The two species of cladocerans, *A. gluttata* and *M. spinosa* were true littoral cladocerans, that spend most of their time in or near the sediments, grazing on detrital matter. It is known that insecticide was adsorbed to organic particles in the sediments (EDWARDS,1977). Thus, these cladocerans were exposed to greater concentrations of the insecticide, and depressing their population densities, compared to those in the untreated rice fields. Finally, besides sublethal effects the more complex role of predator–prey relationships and interspecific competitions can be altered by the insecticide application. A predator or grazer may not be able to develop due to lack of prey, while a prey may be able to increase its density due to a decrease in a particular predator (HURLBERT*et al.*,1972; HURLBERT,1975). However, the authors did not made further description as to the effects of the insecticide on the prey organisms of these crustaceans.

According to SMITH and ISON (1967) benthic organisms are generally exposed to higher concentrations of insecticide residues and for longer periods that those inhabiting those water column. LIM (1980) studied the effects of the application of 3 insecticides (*carbofuran, carbaryl and endosulphan*) at their recommended dose rates for rice pest control over two growing seasons. The author found that only *carbofuran* treatment had significant effects in decreasing the population the three dominant group of invertebrates in the rice fields, which included the ostracods, dipterans and conchostracans. The overall population of the invertebrates was found higher in other insecticides treated rice field plots, which as the author suggested, was due among others to the positive effects of the insecticides on the recovery period of certain species, and by reducing competition and killing off the predators, or increase in prey. The most apparent change was in the increase of the relative dominance of the ostracods, i.e from 4.3% in the untreated rice fields to 42.1% in the treated rice field plots. FERNANDO (1980) explained that dominance of ostracods in pesticide treated rice fields was due to their better resistance to the pesticides and to the rapid recruitment as they reproduce parthenogenetically.

LIM (1980) commented further as the conclusion of his experiment that temporal changes, community structure and population density of the invertebrates in the rice fields were affected not only by insecticide application but also by ploughing, fertilisation and transplanting of rice, and the development of aquatic macrophytes.

TAKAMURA and YASUNO (1986) in their studies on the effects of three pesticides applications (herbicide + insecticide + fungicide) on the population densities of chironomid larvae and ostracods in the rice fields, also obtained similar results. The authors found that the population densities of these benthic macroinvertebrates were abundant and fluctuated widely in the pesticide treated rice fields, which the authors assumed were due to the depression of the population of odonates and dysticids by pesticide applications. The chironomid larvae and ostracods are major food items of odonate larvae (BAY,1974; PRITCHARD,1964). Dysticid larvae also prey on chironomid larvae and ostracods (VENESKI and WASHINO,1970), and controlled the population of these invertebrates while appearing in low densities during the early flooded period of the rice fields. The relative abundance of the chironomid larvae was also observed in the present experiments, but not the ostracods. The ostracod *Cypris sp.* was sometimes found in the samples of zooplankton collected from both treated and untreated rice fields.

In contrast with the above results, CROSSLAND (1982) demonstrated the considerable effects of the application of the insecticide *cypermethrin* in freshwater ponds on the community of invertebrates, which was reflected in a marked reduction in species richness due to the mortality of the aquatic insects and the crustaceans. The author also noted an increase in the quantity of filamentous algae two weeks after the application of *cypermethrin*, attributable to the secondary effects of this treatment in the ponds. The interaction between invertebrate toxicity of insecticide and the bloom of unicellular algae, caused by the mortality of planktonic herbivores that normally graze on the algae was demonstrated by a number of experimental works which was well reviewed by HURLBERT(1975). Blooms of filamentous algae have also been observed in rice fields during the present experiments, but could not be quantified and conclusively associated with insecticide treatment, since algal blooms also appeared in the control plots.

In general, the results of field experiments suggest that the overall effects of insecticide treatments on the aquatic organisms might result from several factors, including the habitat and food preferences of these organisms, particularly in terms of their role in the complex prey–predator relationship and inter specific competition. The results of the present study, however, revealed that the live fish food organisms in the rice field appeared not to be significantly disrupted by the insecticides application over the period of the experiment, and any short term effects were not translated into differences in production or survival of common carp fingerlings.

CHAPTER 6

OBSERVATIONS ON THE DIET OF COMMON CARP FINGERLINGS RAISED IN INSECTICIDE TREATED RICE FIELDS

6.1 INTRODUCTION

Insecticide treatment is known to adversely affect the feeding habit of fish, causing reduced food intake and growth rates. The sublethal concentrations of insecticide may cause impairment in the early feeding behaviour of fish, causing lower rates of prey capture (BROWN *et al.*, 1987; MUIRHEAD–THOMSON, 1988). On the other hand, insecticide treatment may also cause secondary effects in the aquatic environment, leading to the development of live fish food organisms and elevating fish growth and production in ponds and rice fields (HURLBERT,1975; OPUSZYNSKI et al.,1984; TAMAS and HORVARTH,1976). The use of insecticide for improving the production of carp fry and fingerlings in nursery ponds has become increasing popular and is recommended as standard practice (FAO,1985; JHINGRAN and PULLIN,1985). Such methods of environmental manipulation, however, must be carefully managed, especially if high toxic and persistent insecticide compounds are used (GRYGIEREK and WASILEWSKA,1981).

The following experiments was carried out to evaluate the diet of common carp fingerlings in rice fields treated with different rice insecticides.

6.2 MATERIALS AND METHODS

6.2.1 Experimental rice field plots

This experiment was conducted on November 17,1989 using 24 rice field plots (fallow period : 54 days), the design and construction of which were described in details in Section 2.5.3. The rice field soil was prepared specifically to accommodate rice–fish culture, as described in Section 2.5.4. Fertilizer application and rice planting were carried out following procedures outlined in Section 2.5.5 and Section 2.5.6.

6.2.2 Experimental fish

Fingerlings of common carp (*C. carpio*) were procured, maintained, allocated to the rice field plots and subsequently reared following procedures outlined in Section 2.3 and Section 2.5.7. The experimental fish were in good condition with no visible sign of health problems when they are introduced into the experimental rice fields. The mean weights and size of carp fingerlings used in the experiment were 4.8g (SE= 0.04) and 5.9cm (SE= 0.02), respectively.

6.2.3 Experimental design and treatments

The experimental treatments consisted of 5 insecticides and one untreated control, which were allocated in a completely randomised design with three replicates, as follows :

Treat . No	Insecticide Common name	Dose rate (gha^{-1},AI)	24h-LC50 (mgl^{-1})	MNC : 24h-LC50
1	Control	nil	-	-
2	*Fenobucarb*	750	8.3	0.09
3	*Isoprocarb*	1000	8.3	0.12
4	*Buprofezin*	100	2.7	0.04
5	*Diazinon*	640	3.6	0.18
6	*Alphamethrin*	15	0.0068	4.12

Based on the above concentration ratio values, only the application of *alphamethrin* is expected to produce lethal effect on fish in the rice field plots. Further information on the chemical description and properties of the insecticide is given in Table 2.1.

6.2.4 Rice field biota

Zooplankton and macro invertebrates in the rice fields were periodically collected and microscopically examined during the course of the experiment, following procedures outlined in Section 2.5.10 and 2.5.11.

6.2.5 Water quality measurement

The physical and chemical characteristics of the rice field water were measured periodically during the experiment, following procedures specified in Section 2.5.12.

6.2.6 Gut content analysis

At the end of the experiment, a total 16 fish were collected from each treatment plot, 8 fish were caught in the morning (0600 am) and another 8 in the afternoon (0300 pm). The fish were directly killed in the field by deep anaesthetic, weighed, measured and their gut removed and preserved in 10% buffered formalin solution to be examined in the laboratory. The contents of individual stomach were then sorted under a low power binocular microscope. All items were identified to species if possible, and counted. The determination of their volumes was not carried out because the guts were too small to be measured for this purpose. The food available to fish in the rice field plots was determined by sampling the zooplankton and macro-invertebrates following procedures as specified in Section 6.2.4. The composition of the diet of 8 common carp fingerlings in each treatment plots was then determined by number and occurrence (HYSLOP,1980).

6.3 RESULTS

6.3.1 Fish survival, growth and production

The mean final weight and the mean growth rate (in terms of the absolute weight increment) of the experimental fish in the rice fields treated with the insecticides, were found to be significantly greater than the untreated control ($P< 0.05$)(TABLE 6.1). There is also an apparent variation in the yield and the weight gain of fish biomass in the treatments ($P< 0.05$)(TABLE 6.2). The survival rates of fish in the treatments are, however, comparable. ($P> 0.05$). These results suggest that insecticide treatments could have a positive effects on growth and production of the common carp fingerlings in the rice fields. The reason for this phenomenon is not clear, but presumably due to a currently undefined physiological effect to fish or to fish food organisms.

6.3.2 Water quality

The physico–and chemical characteristics of the rice field water during the experiment are presented in Table 6.3. The data were generally in agreement with those obtained in previous experiments (see Chapter 5). The variation of the water quality data was found to be within acceptable limits for fish culture (see Section 4.3.7).

Mean water pH in the rice field plots treated with fenobucarb and *isoprocarb* (7.36 and 7.44, respectively) was found to be lower compared to those in other rice field plots ($P < 0.05$), possibly due to the higher rate of biological decomposition occurring in these plots. Higher mean total ammonia were also recorded ($P < 0.05$).

TABLE 6.1. Mean initial/final weights and mean growth rate (in terms of absolute weight increment) of common carp fingerlings in rice fields, treated with different insecticide compounds.

	INSECTICIDE TREATMENT						F	Significant level	Probability
	0	1	2	3	4	5			
Initial weight (g)	4.9	4.9	4.7	4.9	5.1	4.8	1.45	NS	> 0.05
Standard error	0.4	0.4	0.3	0.3	0.4	0.3			
Final weight (g)	9.9^a	16.4^b	14.2^b	11.5^b	15.9^b	14.8^b	3.54	*	< 0.05
Standard error	2.2	2.8	3.2	1.8	4.3	2.8			
Growth rate (gd^{-1})	0.24	0.52	0.41	0.28	0.47	0.44	2.60	NS	> 0.05
Standard error	0.01	0.02	0.02	0.05	0.16	0.03			

NOTE : 0/ - Control (no insecticide), 1/ -*Fenobucarb* (750gha^{-1}, A.I), 2/ - *Isoprocarb* (1000gha^{-1},A.I), 3/ - *Buprofezin* (100gha^{-1}, A .I), 4/ -*Diazinon* (640gha^{-1}, A.I), 5/ -*Alphamethrin* (15gha^{-1}, A.I)

NS - Not significant

TABLE 6.2. Summary of the survival rate, the yield and weight gain of biomass of common carp fingerlings in rice fields, treated with different formulations of rice insecticide (Means and standard error).

	INSECTICIDE TREATMENT						F	Significant level	Probability
	0	1	2	3	4	5			
Stocking density (gm^{-2})	2.43	2.45	2.38	2.43	2.54	2.41	1.44	NS	> 0.05
Standard error	0.07	0.03	0.03	0.03	0.06	0.04			
Survival rate (%)	61.7	61.7	73.3	55.0	68.3	68.3	0.42	NS	> 0.05
Standard error	3.3	14.2	13.6	4.4	10.0	1.7			
Total yield ($gm^{-2}21d^{-1}$)	3.1^a	4.9^b	4.5^b	4.0^b	4.4^b	4.8^b	3.50	*	< 0.05
Standard error	0.1	0.7	0.6	0.4	0.9	0.1			
Weight gain ($gm^{-2}d^{-1}$)	0.03^a	0.12^b	0.10^b	0.07^b	0.09^b	0.11^b	3.63	*	< 0.05
Standar error	0.005	0.04	0.03	0.02	0.04	0.002			

NOTE : 0- Control (without insecticide), 1- *Fenobucarb* (750gha^{-1},A.I), 2- *Isoprocarb* (1000gha^{-1},A.I),
3- *Buprofezin* (100gha^{-1},A.I), 4- *Diazinon* (640gha^{-1},A.I), 5- *Alphamethrin* (15gha^{-1},A.I).

* - Significant

Means desigmated with a common letter are not significantly different at 5% level by Duncan Multiple Range Test

TABLE 6.3. The physico-chemical characteristics of the rice field water measured during the experiment (Mean and range values).

Experimental treatments						F	Significant level	Probability
1	2	3	4	5	6			
Temperature (^{o}C)								
26.6 (24.0-30.0)	28.2 (23.5-32.0)	27.8 (24.0-31.0)	27.6 (24.5-30.0)	27.3 (24.0-31.5)	26.5 (23.5-30.0)	1.34	NS	> 0.05
Dissolved oxygen (mgl^{-1})								
11.9 (5.0-17.0)	13.6 (10.4-15.0)	12.1 (8.2-15.0)	13.1 (7.0-15.0)	10.8 (7.0-15.0)	11.3 (4.6-15.0)	2.28	NS	> 0.05
Carbon dioxide (mgl-1)								
3.81 (1.80-6.70)	3.14 (0-6.70)	3.55 (0-6.40)	2.74 (0-5.40)	3.24 (0-7.20)	3.12 (0-7.79)	2.16	NS	> 0.05
pH								
7.72 (7.23-9.00)	7.36 (6.86-9.00)	7.44 (7.00-9.63)	7.89 (7.03-9.43)	7.94 (7.41-9.31)	7.75 (7.04-9.43)	2.33	*	< 0.05
Total hardness ($mgl^{-1}CaCO_3$)								
57.3 (49.0-65.5)	58.7 (53.2-65.4)	64.9 (56.2-79.9)	59.1 (49.1-75.8)	61.4 (47.1-73.7)	65.9 (51.2-75.8)	0.98	NS	> 0.05

TABLE 6.3 (Continued)

Experimental treatments 1	2	3	4	5	6	F	Significant level	Probability
Alkalinity (mgl^{-1}, $CaCO_3$)								
61.2 (49.3-79.0)	63.1 (59.2-88.8)	65.1 (59.2-69.1)	66.7 (59.2-76.9)	65.1 (59.2-69.1)	63.1 (59.2-69.1)	0.872	NS	> 0.05
Total ammonia (mgl^{-1})								
0.114 (0.018-0.330)	0.049 (0.013-0.076)	0.055 (0.021-0.102)	0.056 (0.017-0.124)	0.069 (0.029-0.137)	0.070 (0.018-0.110)	2.40	*	< 0.05
Nitrite (mgl^{-1})								
0.103 (0.082-0.140)	0.081 (0.031-0.120)	0.091 (0.048-0.139)	0.100 (0.051-0.158)	0.111 (0.051-0.260)	0.079 (0.029-0.123)	0.90	NS	> 0.05
Total phosphorus (mgl^{-1})								
0.10 (0.04-0.14)	0.10 (0.04-0.15)	0.10 (0.05-0.13)	0.08 (0.04-0.14)	0.09 (0.04-0.09)	0.10 (0.05-0.14)	0.496	NS	> 0.05
Suspended solid (mgl^{-1})								
28.9 (21.5-42.5)	31.9 (21.0-42.0)	23.9 (8.5-44.5)	25.9 (14.0-32.5)	26.3 (10.0-45.5)	28.7 (18.5-38.0)	1.27	NS	> 0.05

NOTE 1- Control 2- *fenobucarb* 3- *isoprocarb* 4- *buprofezin* 5- *diazinon* 6- *alphamethrin*
NS- Not significant *- Significant

6.3.3 Rice field biota

The composition of the zooplankton and macro–invertebrate population collected from the rice fields were generally similar to those described in previous experiments (see Chapter 5). The list of the main taxon of zooplankton and benthic fauna are presented in Table 6.4 and Table 6.5, respectively. The rotifers and the crustaceans (*Cyclops sp.* and Nauplius larvae) were dominant in all zooplankton samples. Figure 6.1 summarised the temporal changes in the abundance of rotifers in the rice fields recorded in the experiment. The density of rotifers in all rice field plots was generally low. The temporal changes in the abundance changes of the crustaceans and the benthic fauna (oligochaetes and insect larvae) in the experimental rice field plots are illustrated in Figure 6.2 and Figure 6.3, respectively. There are no indication of a definite pattern in the changes of the abundance of the population of the rice field biota, both in the untreated rice field as well as in the insecticide treated rice fields.

TABLE 6.4. List of species/genera of zooplankton collected from rice field plots during the experiment.

Species/Genera	1	2	3	4	5	6
Protozoa						
Arcella hemispherica (Perty)		+		+		+
Centropyxis aculeata (Stein)			+	+	+	+
Difflugia pyriformis (Perty)				++		
Rotifera						
Brachionus sp.	+	+	+	+	+	+
Filinia longiseta (Ehrenberg)					++	
Lecane bulla (Gosse)		+	+	+	+	
Monostyla sp.			+			
Crustacea						
Cyclops sp.	++	++	++	++	++	++
Moina micrura (Kutz)	+	+	+	+	+	+
Nauplius larvae	+	+	++	++	+	+
Cypris sp.					+	
Insecta						
Chironomus sp.	+	+	+	+		+

NOTE:

+ - Presence ++ - Dominant

1- Control 2- *fenobucarb* 3- *isoprocarb*
4- *buprofezin* 5- *diazinon* 6- *alphamethrin*

TABLE 6.5. List of species/genera of benthic nacro-invertebrates collected from the rice field plots during the experiment

Species/Genera	1	2	3	4	5	6
<u>Oligochaeta</u>						
Nais communis (Piguet)	+	+	+	+	+	
Haplotaxis sp.				+	+	
Lumbriculus sp.	+	+	+	+	+	+
Branchiura sowerbyi (Beddard)	+	++	+	+	+	+
Tubifex tubifex (Muller)	++	++	+	+	++	+
<u>Hirudinea</u>						
Glossiphonia weberi (Blanchard)	+		+			
Helobdella sp.	+					
<u>Insecta</u>						
Baetis sp.	+					
Berosus sp.						+
Chironomus sp.	+					
Polypedilum sp.	+					
<u>Gastropoda</u>						
Bellamya javanica	+	+	+	+	+	+
Melanoides tuberculata (Muller)	+	+		+		+

<u>NOTE</u> :

+ - Presence	++ - Dominant	
1- Control	2- *fenobucarb*	3- *isoprocarb*
4- *buprofezin*	5- *diazinon*	4- *alphamethrin*

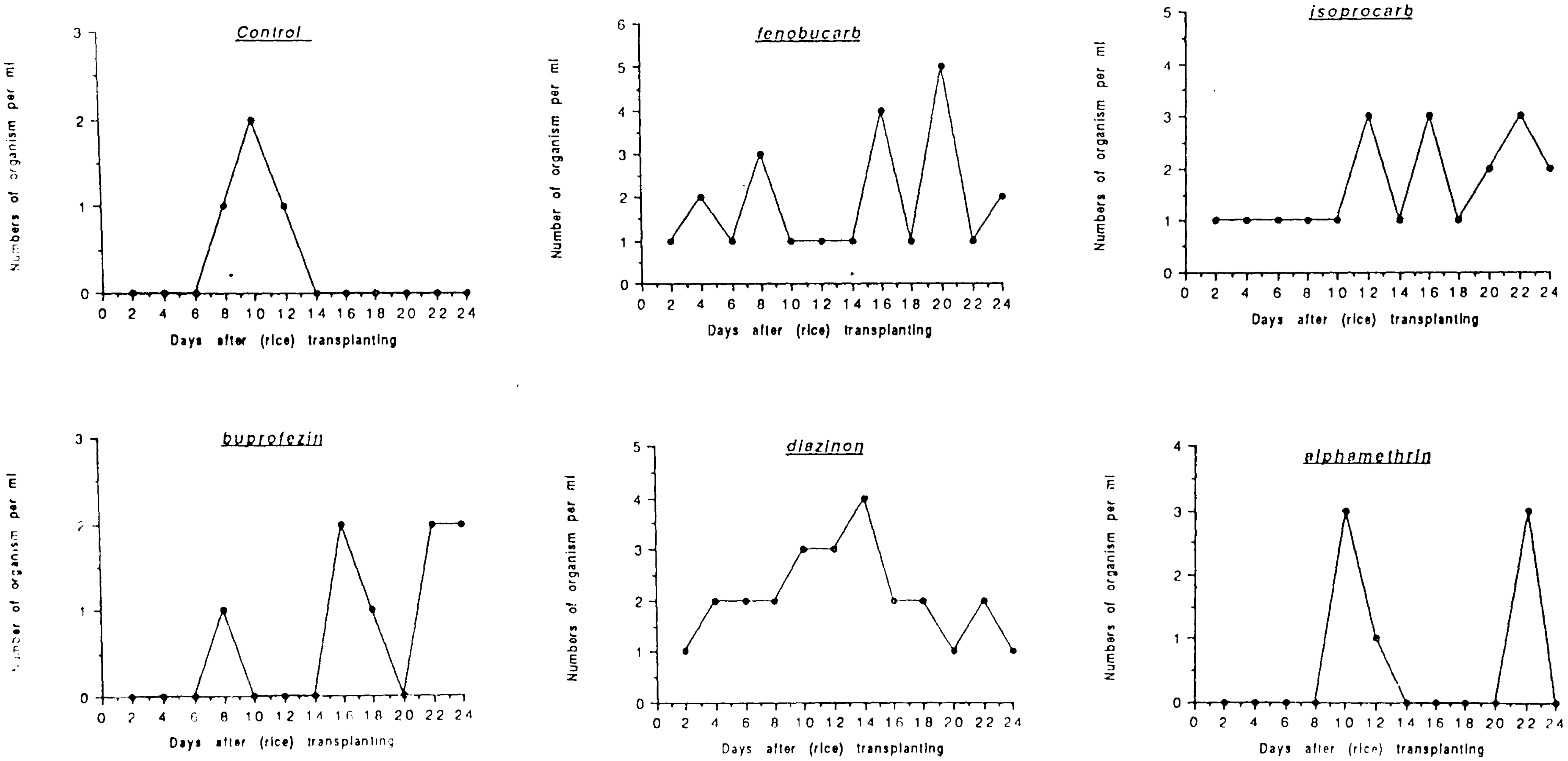

FIGURE 6.1. Abundance of rotifers in wet rice fields treated with 5 different insecticide products.

6.3.4 Stomach content analysis

The guts of fish collected in the afternoon were empty, while those collected in the morning contained a variety of food items, mainly rotifers, crustaceans and plant detritus. This finding suggests that the common carp fingerlings in the rice field feed actively during the night and or early in the morning. The composition of the diet of 8 common carp fingerlings collected from the experimental rice fields is presented in Table 6.6. In the gut of fish collected from the untreated control plot the diet comprised mainly larvae of various aquatic insects (26.1%) and chironomid larvae (21.7%). The guts of the fish collected from insecticide treated rice fields contained mainly *Cyclops* sp, *Brachionus sp* and Nauplius larvae. Based on the actual number of food organism in the fish gut, insect larvae, including chironomids, and *Cyclops* sp. appeared to be the most important diet in the control fish, while for the fish raised in insecticide treated rice fields, their main diet consist of *Cyclops sp*, Nauplius larvae and *Brachionus sp*.

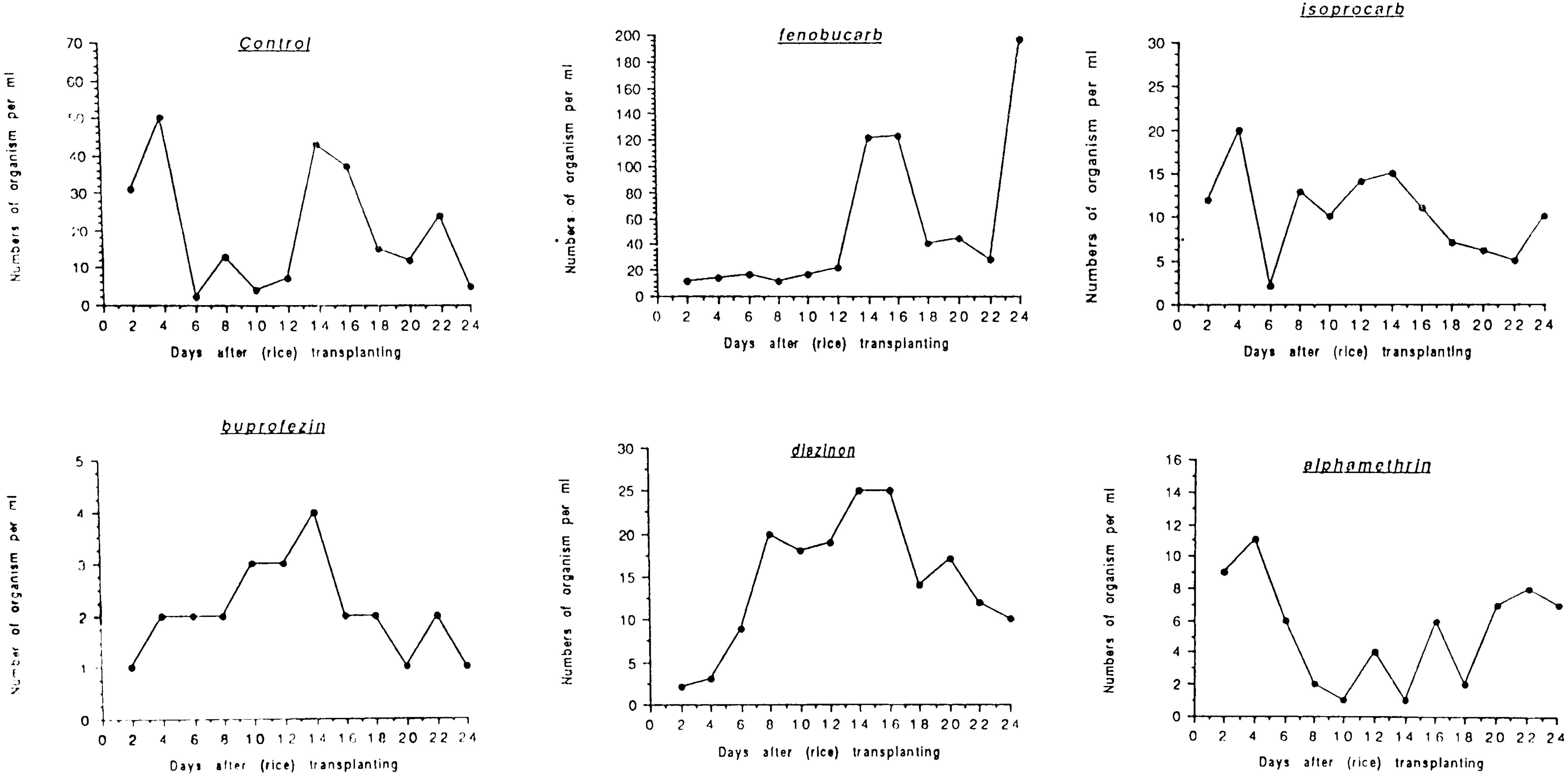

FIGURE 6.2. Abundance of crustaceans (cladocerans + copepod s) in wet rice fields treated with 5 different insecticide products.

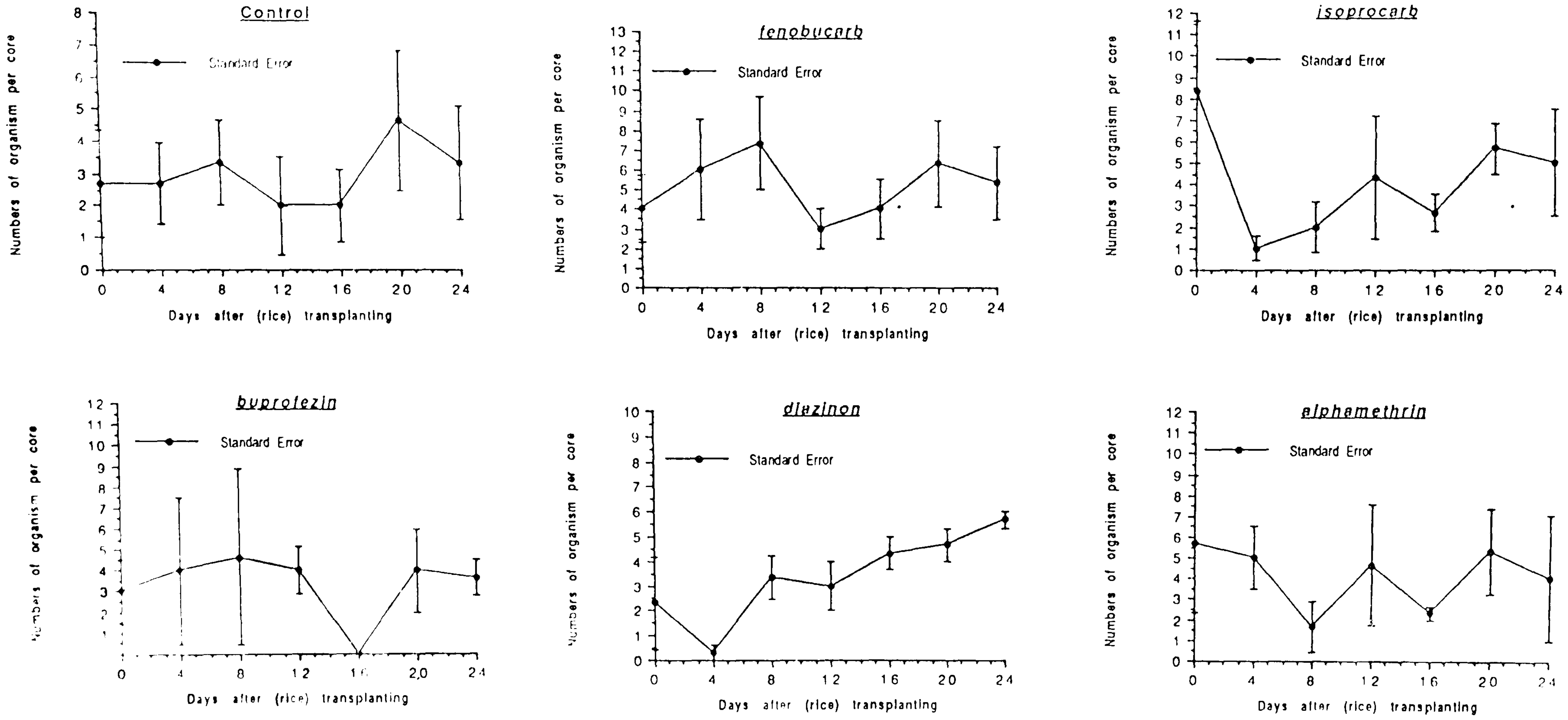

FIGURE 6.3. Abundance of benthic macro-invertebrates (oligochaetes + insect larvae) in wet rice fields treated with 5 different insecticide products.

TABLE 6.6. The composition of the diet of 8 common carp fingerlings (*C. carpio*) from the rice fields by number and occurence.

Food organism	Number		Occurence	
	Actual	%	Actual	%
<u>Control</u>				
Brachionus sp.	12	11.54	1	4.35
Cyclops sp	25	24.04	4	17.39
Moina sp.	1	0.96	1	4.35
Nauplius larvae	18	17.31	3	13.04
Eubranchipus sp.	1	0.96	1	4.35
Chironomus sp.	20	19.23	5	21.74
Insect larvae	24	23.08	6	26.09
Cypris sp.	3	2.88	2	8.69
<u>Fenobucarb</u>				
Brachionus sp.	41	8.42	3	18.75
Cyclops sp	351	72.07	4	25.00
Moina sp.	28	5.75	2	12.50
Nauplius larvae	36	7.39	1	6.25
Chironomus sp.	8	1.64	2	12.50
Insect larvae (UI)	22	4.52	4	25.00
Oligochaetes (UI)	1	0.20	1	6.25
<u>Isoprocarb</u>				
Brachionus sp.	13	6.47	4	26.61
Cyclops sp	131	65.17	5	33.33
Nauplius larvae	32	15.92	1	6.66
Eubranchipus sp.	11	5.47	1	6.67
Insect larvae (UI)	13	6.47	3	20.00
Cypris sp.	1	0.50	1	6.67
<u>Buprofezin</u>				
Brachionus sp.	9	5.17	4	14.81
Cyclops sp	68	39.08	6	22.23
Moina sp.	17	9.77	3	11.11
Nauplius larvae	56	32.18	5	18.52
Eubranchipus sp.	3	1.72	1	3.70
Chironomus sp.	11	6.32	5	18.52
Insect larvae (UI)	7	4.02	1	3.70
Cypris sp.	3	1.72	2	7.41

TABLE 6.6. (Continued)

Food organism	NumberOccurence			
	Actual	%	Actual	%
Diazinon				
Brachionus sp.	-	-	-	-
Cyclops sp	86	55.13	8	28.57
Moina sp.	18	11.54	2	7.14
Nauplius larvae	16	10.26	5	17.86
Eubranchipus sp.	1	0.64	1	3.57
Chironomus sp.	15	9.61	5	17.86
Insect larvae (UI)	10	6.41	5	17.86
Cypris sp.	10	6.41	2	7.14
Alphamethrin				
Brachionus sp.	1	1.33	1	7.69
Cyclops sp	7	9.33	3	23.08
Moina sp.	2	2.69	1	7.69
Nauplius larvae	57	76.00	4	30.77
Chironomus sp.	3	4.00	3	23.08
Insect larvae (UI)	5	6.17	1	7.69

UI - Unidentified

6.3.5 Summary of results

The result of the experiment was in general agreement with that obtained in previous experiments (Chapter 5), confirming that the adverse effect of the application of the practical dose rates of rice insecticides on the culture of common carp fingerlings in wet rice fields. is not apparent. On the contrary, the data demonstrated that significant improvement of the growth rate and production of common carp fingerlings can be attained in insecticide treated rice fields.

The temporal changes in the population of zooplankton and benthic invertebrates in the rice fields in insecticide treated rice fields did not show a definite pattern, and was difficult to evaluate. The population density of the rice field biota seemed to be relatively low.

The composition of the diet of common carp in the untreated rice fields consisted mainly of *Cyclops sp*, various insect larvae and *Chironomus sp.*, while in the insecticide treated rice fields the diet comprised *Cyclops sp*, *Brachionus sp.* and Nauplius larvae.

6.4 DISCUSSION

The results of the experiment revealed that there was no significant adverse effect of the normal application of the rice insecticides tested in the rice fields, on the growth and production of common carp fingerlings. These results confirm the findings in the previous experiments described in details in Chapter 5, and therefore need no further discussion.

Observations on the number and occurrence of food items in the guts of the common carp fingerlings raised in the rice field indicate, that their main food organisms comprised of aquatic insects (and their larvae), crustaceans (copepods and cladocerans), rotifers and plant detritus. This diet composition is comparable to those for the common carp in natural ponds in West Java as described by VAAS and VAN-OVEN (1959). The authors elaborated in details the diet composition of carp fry and fingerlings in different pond conditions in terms of the supply of live food organisms available in these ponds. According to the authors, the most important food organisms for carp fingerlings were the Cyclopidae, cladocerans and ostracodes. Chironomid larvae and other aquatic insects were eaten in increasing numbers as fry grew older. Benthic fauna in ponds, notably oligochaetes and chironomids, were found definitely reduced in numbers by the fish. Other investigations on carp diet in ponds in West Java conducted by BUSCHKIEL (1938) found that common carp fry consumed crustaceans, tubificid worms and chironomid larvae, respectively 3 days, 6 days and 7–9 days after hatching. The result of the present experiment indicate that in insecticide treated rice field plots, the rotifers were found to be an important food source for the carp fingerlings. This difference may be due to the development of rotifer populations as the result of the suppression of their predators by insecticide, mainly predaceous aquatic insects and large crustaceans. At the same time other aquatic insects which constitute a main food source for the fish may have been killed by the treatment. This secondary effect of pesticide treatment in the aquatic environment has been demonstrated by a number of investigators and reviewed by HURLBERT (1975) (as referred to in Section 1.1.4.) However, such effects in the wet rice field environment needs to be further confirmed by more elaborate field studies.

VAAS and VAN-OVEN (1959) further remarked that in general the Indonesian carp are opportunistic, polyphagous feeders whose diet is mainly dictated by local availability of natural food. Common carp is able to use a wide range of natural food items. From its preferred diet (insects, crustaceans and benthic organisms) it will turn to other aquatic organisms and to vegetable food if necessary. This ability to switch feeding habits depending on feed availiability may explain why in the present study the application of the insecticides did not cause any significant decrease in the growth and production of the carp fingerlings, although there may have been changes in the abundance of certain food organisms in the rice fields.

There appeared to be no significant differences between the effects of the different insecticides on the composition of the diet of carp, again possibly because of the wide differences in the feeding activity of the fish as mentioned earlier. This aspect of dietary response is particularly difficult to observe because of the variations encountered in the population of the aquatic organisms under comparable rice field conditions.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Laboratory based static toxicity tests revealed that there were wide variations in the acute toxicity of the five rice insecticide products tested in the study, due primarily to the different chemical properties of the compounds.

The carbamate insecticides (*fenobucarb* and *isoprocarb*) were the least toxic and the synthetic pyrethroid insecticide (*alphamethrin*) was the most toxic compound to common carp fingerlings used in the tests. The organophosphate (*diazinon*) and the thiadiazine (*buprofezin*) compounds have intermediate toxicity to fish. These results were in general agreement with those reported in the published literature in which it is generally acknowledged that the pyrethroid compounds are extremely toxic to fish, while the organophosphate and carbamate compounds have high to moderate toxicity to fish. The laboratory experiments also demonstrated that by determining the threshold LC50 rather than the LC50s at specific exposure periods, as recommended by several investigators (BROWN, 1973; SPRAGUE,1969; ABEL, 1989)., more meaningful and informative toxicity data could be obtained, specifically in terms of the extent of bioactivity of the compounds to fish, providing better estimates of lethal and sublethal concentrations of the insecticides. From the toxicity curve obtained from the present test with *fenobucarb*, for example, it could be assessed that the insecticide has a potentially longer bioactivity to fish at lower concentration, compared to the other carbamate compound isoprocarb, although their 96h–LC50 values are comparable. Similarly, *diazinon* in field condition might be more toxic than predicted by its 96h–LC50 value.

Field based experiments revealed that the application of the rice insecticides, using up to twice their normal field dose rates for pest control, appeared to have minimal effect on fish culture in rice fields, measured in terms of fish survival rate and production. This minimal effect was also evident in the field experiment with *alphamethrin* which was shown to be extremely toxic to fish under laboratory conditions. The results of these field experiments indicate that the influence on to fish in rice field condition depends not only on toxicity, but also on exposure, that is the amounts of the insecticide compound released into the rice field water, and on the subsequent dispersion and persistence of these compounds in the rice field environment. In the shallow rice field water it is likely that a significant proportion of the insecticide products could be absorbed onto organic matter, a process which would have reduced toxicity.

The water management for rice fish culture adopted in the experiments, and in Indonesia as a whole, would also have resulted in flushing of the insecticides from the rice field system, thereby reducing concentration and toxicity because the actual concentration of the insecticide remaining in the rice field water would have been less than that predicted from its maximum nominal concentration.

Observations of the changes in the population density of aquatic biota, particularly fish food organisms, in the rice fields, produced no specific results. This result may have been due mainly to the apparently large variation in the population of the rice field organisms, suggesting that a more appropiate sampling strategy is needed., for example larger pooled samples. The influence of insecticide treatment on the rice field biota is also difficult to define because the distribution and habits of most rice field invertebrates are poorly known. Indirect assessment by means of stomach analysis of the experimental fish, examined periodically before and after insecticide treatment, might provide additional information on the effect of insecticide to the aquatic biota in the rice fields. Results of the gut examination in the present experiment suggested that in insecticide treated rice field plots, more

Cyclops were being consumed by fish, possibly because of their greater availability in the rice fields. This fact,. however, could not be substantiated by the data on the abundance of the crustaceans presented in TABLE 6.2, perhaps due to their variability during the sampling period. The abundance of crustaceans in the rice fields may also be increased by insecticide treatments In fact, improving the density of fish live food (rotifers and copepods) by selective killing of their predators using insecticide (usually *trichlorfon*). is a common practice in carp hatcheries. This aspect of insecticide treatment in rice fish culture, however, needs to be further studied and evaluated.

From the results of the present study it can be concluded that of the five rice insecticides tested, potentially *fenobucarb* appeared to have the greatest adverse affect on fish culture in rice fields if applied at twice the recommended dose rate for rice pest control (1500gha^{-1},AI). *Alphamethrin* was also potentially hazardorous to fish on account of its high toxicity to fish and aquatic invertebrates, particularly if higher dose rates are used.

Rice fields, as any other ecosystem maybe looked upon as a multi-culture of fauna and flora. The application of agricultural chemicals has to be carefully managed. The use of highly toxic and persistent insecticide products should be discouraged. Selective insecticide compounds should be used which produce the minimum effect on rice field biota, particularly if integrated rice-fish farming is practised. Based on the present study the following general recommendations can be made on the methodology and procedures in determining the toxicity and hazard of a rice insecticide to fish :

1. Laboratory based static acute toxicity test can be used to determine the toxicity of insecticide products to fish. The test should involve the measurement of threshold LC50, by extending if necessary the duration period of the test up to 7 days.

2. The effect of insecticide on fish growth is best demonstrated in controlled laboratory or outdoor tanks experiments, in which test fish can be individually marked and fed with nutritionally appropriate artificial fish feed.

3. Field based experiments should be conducted in the rice fields with flowing and stagnant conditions, in order to better

evaluate the effect of insecticide on fish and rice field aquatic biota under realistic field conditions.

4 Additional information on the residual effect of the insecticide application in water, soil and fish tissues would be valuable, especially in evaluating the long term persistance and effect of insecticide treatments on rice fields and their biota.

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