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Biology and Fertility of Soils

Density and composition of aquatic oligochaete populations in different farmers' ricefields

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Summary. The density and composition of ricefield oligochaete populations were estimated in 33 farmers' fields in the Philippines and related to physicochemical properties of the soils and agrochemical use. The spatial distribution of organisms and the investigative sampling strategy were determined from a high-intensity sampling in a single ricefield. Species diversity was low. The dominant species were *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi.* The spatial distribution of populations was contagious and densities ranged from 0 to 35000 m^{-2} (maximum 620 kg ha^{-1}). Mean population density was positively correlated with soil moisture, organic matter, and the quantity of N fertilizer applied. No pesticide impacts were observed.

Key words: Aquatic oligochaetes – Ricefields – Fertilizer – Pesticides – Population ecology

Despite the recognised contribution of aquatic oligochaetes to the maintenance of soil fertility in wetland ricefields through the translocation and mineralisation of nutrients (Grant and Seegers 1985; Roger and Kurihara 1988; Kurihara 1989), field studies of this are scarce. Little is known about the densities, distributions dynamics, compositions, and ecology of field populations.

A population density of 10000 m^{-2} was estimated in an experimental field of the International Rice Research Institute (IRRI), Philippines (International Rice Research Institute 1985). Higher values were reported in Japan, where combined densities of *B. sowerbyi* and *L. socialis* increased from a few at transplanting to over 40000 m^{-2} at the end of the crop cycle (Kikuchi et al. 1975). Another study performed at IRRI showed that populations ranged from 0 to 40000 m^{-2} and were affected by N fertilizer, pesticide, and green manure (Simpson et al. 1993).

The objective of the present investigation was to estimate aquatic oligochaete populations in farmers' fields and to determine whether potential impacts of agrochemicals were manifest. The density, dynamics, and composition of populations, and controlling factors were studied through an extensive survey in 33 ricefields in the Philippines. High-density sampling within one of the fields was used to study aspects of methodology and spatial distribution within a field.

Materials and methods

High intensity sampling

During the dry season of 1989, one field (27×29 m) was sampled at 64 evenly distributed points (intersections of an 8 by 8 grid, 24×25.8 m). Two soil cores (42 mm in diameter) were taken at each sampling point, down to the plough layer the depth of which was recorded. Cores were collected in separate plastic bags. Oligochaetes were enumerated from one of the pair of core samples and soil moisture and bulk density from the other. Samples were processed in random order. The length of time between collection and processing was recorded.

Field survey

The study was conducted in 33 ricefields from four villages in the Laguna Province (Philippines): Calauan (16), Calamba (8), Cabuyao (7), and Binan (2). This area was selected because it is representative of agrochemical use in the Philippines and accurate information on agrochemical use and rice yields was available (IRRI, unpublished data, 1987–onward). All farms were managed by farmers as they considered appropriate. Sampling was conducted towards the end of the 1989 dry season crop, and at transplanting and crop maturity in the 1989 wet season and 1990 dry season crops.

Oligochaete populations were sampled by collecting evenly spaced soil cores along diagonal transects. At the end of the 1989 dry season, 10 cores of 42 mm diameter were collected from each field and processed separately. The strategy was modified subsequently to reduce the number of samples to be processed. The number of cores was increased to 18, the diameter was reduced to 27 mm, and they were processed as six composite samples obtained by combining adjacent groups of three. Core sample depth was determined by the depth of the puddled layer. Methods for oligochaete recovery from soil samples, counts, and identification have been reported previously (Simpson et al. 1993).

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Five 42-mm soil core samples were collected from evenly spaced locations along a diagonal transect and combined for the determination of soil moisture and chemical analysis. Soil moisture was calculated on a dry weight (105 °C for 24 h) basis.

The pH, Olsen available P, Kjeldahl N, and organic C were determined twice (end 1989 dry season and start 1989 wet season). Active Fe (Asami and Kumada 1959) and particle-size analysis were determined once (start 1989 wet season).

Oligochaete biomass estimation

Aquatic oligochaete biomass was estimated by two independent techniques. Method A assumed that the oligochaetes were cylindrical and had a density of 1 g cm^{-3}. Cylindrical volumes were calculated for 50 individuals from their length and diameter (means of quartile measurements). The population biomass (kg ha^{-1}) was estimated from the field density and mean individual mass.

Method B assumed that the size distribution of cultured oligochaetes (10–40 mm) was similar to that of field populations. Based on visual inspection this assumption was reasonable. Two thousand worms were placed between dry filter papers to remove excess moisture and their fresh weight obtained. Their dry weight was measured after drying them in an oven at 75 °C for 24 h. The ash-free dry weight was obtained after furnace ignition at 500 °C for 2 h (Grant and Seegers 1985). Population biomass estimates were calculated, as above, from the mean individual mass.

Results

High intensity sampling

Aquatic oligochaetes were contagiously distributed within the intensively sampled ricefield (Fig. 1). There was an area towards one corner of the field where the population was more dense than in the rest of the field. Theoretical sampling was achieved by drawing diagonal lines across the contour map and extracting oligochaete numbers from appropriate positions. Student's *t*-tests were performed on log-transformed data to test the hypothesis that population density estimates obtained by transect sampling were not significantly different from the grid sampling estimate. The null hypotheses were not rejected ($P = 0.48-0.90$).

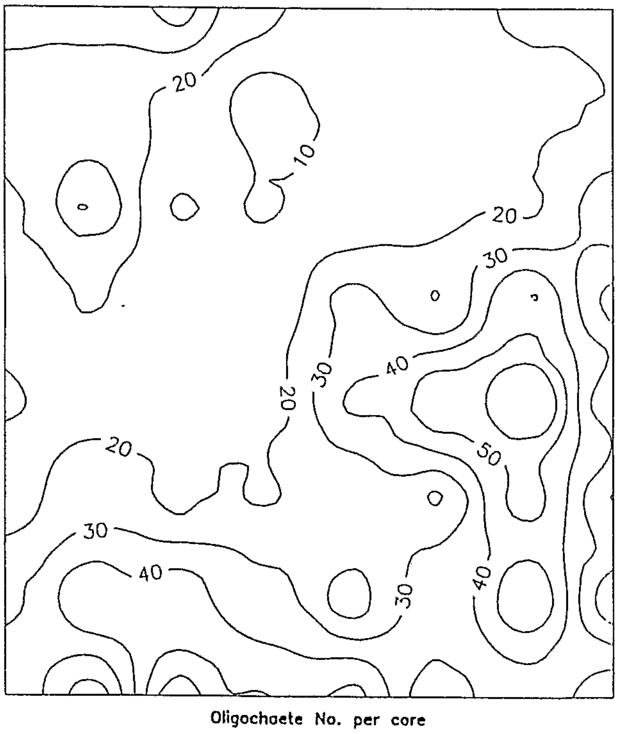

Fig. 1. Spatial distribution of aquatic oligochaetes within a ricefield

No correlations were found between population density and soil mosture, bulk density, soil core volume, or time before processing. The absence of relationships between numbers of oligochaetes per core and core volume suggested that under flooded conditions oligochaetes are not evenly distributed throughout the volume of the puddled layer. Population densities should therefore be expressed in numbers per unit area. The number of oligochaetes extracted showed no signs of decrease for at least 4 days when samples were stored open in an air-conditioned room.

Field survey

Soil properties and agrochemical use showed quite large variability, indicating that the Laguna farms selected were representative of a broad spectrum of physicochemical conditions. Significant differences were found between the soil chemical properties of farms (Table 1).

Values obtained from two samplings were reproducible and highly correlated ($P \leq 0.01$). According to the textural classification of Thompson and Troeh (1978), the majority of soils surveyed were clays; however, examples of clay loams, silty clays, and silty clay loams were recorded. Soil moisture contents ranged from 51 to 183% (seasonal means 101–112%).

All of the farmers surveyed used agrochemicals (Tables 2 and 3). As a broad range of pesticides was used,

Table 1. Summary of soil chemical properties in the Laguna ricefields

	End dry season 1989				Start wet season 1989				
	C (%)	N (%)	P (mg kg^{-1})	pH	C (%)	N (%)	P (mg kg^{-1})	pH	Fe (mg kg^{-1})
Mean	2.3	0.23	30	6.2	2.2	0.22	29	6.4	1.13
SD	0.4	0.04	27	0.5	0.5	0.05	24	0.4	0.52
Maximum	3.4	0.30	77	7.5	3.8	0.33	81	7.4	2.36
Minimum	1.7	0.16	3	5.4	1.5	0.15	2	5.9	0.34
n	23	23	23	23	32	32	32	32	32

n, Number of farms from which the information was obtained
P was determined as Olsen available P, Fe as active Fe

Table 2. Summary of mineral fertilizer applications (kg ha^{-1}) in the Laguna farms, 1989

	Dry season			Wet season		
	N	P	K	N	P	K
Mean (SD)	103 (30)	6 (8)	6 (8)	67 (33)	2 (4)	2 (4)
Range (min – max)	33–184	0–33	0–33	0–134	0–12	0–12
n	28	28	28	31	31	31

min, Minimum; max, maximum; n, number of farms from which the information was obtained each one was only used by a small number of farmers. The most common insecticides were monocrotophos, chlorpyrifos, and endosulphan. Butachlor was the most frequently applied herbicide. Rates of application were also variable and often below manufacturers' recommended rates. The use of individual pesticides, applied by farmers in Laguna, is summarised in Table 4.

Aquatic oligochaetes were the only soil macro-invertebrate group found in significant numbers and with a widespread distribution. Populations were dominated by the families Tubificidae and Naididae. The most common species were the tubificids *L. hoffmeisteri* (Claparédé) and *B. sowerbyi* (Beddard) (Table 5), 81% and 13%, respectively, averaged between fields and over time. Simpson's diversity indices (Simpson 1949) were calculated for each oligochaete population sampled. This method is weighted towards the abundance of the dominant species (Magurran 1988) and was chosen because species richness was low. Over the five samplings diversity indices ranged from 1 to 3.2 (Table 6). Indices are presented as reciprocals so that they increase with diversity. Mean indices were correlated with diversity at four out of five of the individual sampling times ($P = <0.01$). This shows that biodiversity was consistent in individual farms and indicates that mean diversity indices can be used to study the correlations with physicochemical parameters.

The frequency distribution of population densities was L-shaped, which is characteristic of aggregated populations (Fig. 2). The regression between the logs of mean and variance had a slope of 1.8, indicating a negative binomial distribution that can be normalised by log transformation (Roger et al. 1991). Data were transformed according to the relation $Y = \log(X+1)$ before statistical analysis.

Oligochaete population densities over the five samplings ranged from 0 to 35000 m^{-2} (Table 7). Field biomass estimates derived from these densities ranged from 0 to 620 kg fresh weight ha^{-1} (Table 8). Population dynamics were inconsistent through time. Between the beginning and end of the crop seasons, there were examples when population densities increased, decreased, and remained similar. Populations at each sampling time were correlated with site means over five samplings ($r = 0.58-0.88$; $P \leq 0.01$). Densities and diversity indices were negatively correlated at the majority of individual sampling times and between means (Table 9).

Mean values of density, diversity, soil properties and agrochemical applications over the investigative period were used in correlation analysis because (1) individual variables were highly correlated between times; (2) means reduce the impact of extreme values; and (3) assuming that farmers maintain similar management strategies from year to year, agrochemical inputs averaged over the 1989 wet and dry seasons represent an annual index which can be compared with oligochaete data from later samplings.

Table 3. Summary of the total quantities of pesticides applied (kg a.i. ha^{-1}) by farmers in the Laguna farms, 1989

	Dry season			Wet season		
	I	H	M	I	H	M
Mean	0.47	0.37	0.02	0.27	0.29	0.05
SD	0.34	0.16	0.06	0.27	0.17	0.10
Maximum	1.76	0.71	0.29	1.06	0.74	0.53
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
n	28	28	28	33	33	33

I, Insecticide; H, Herbicide; m, Molluscicide; n, number of farms from which the information was obtained; a.i., active ingredient

Table 4. Summary of pesticides (kg a.i. ha^{-1}) used by farmers in the Laguna farms surveyed, 1989

Pesticide	Dry season			Wet season		
	n	Mean	Range	n	Mean	Range
Insecticide						
Carbofuran	4	0.20	0.01 – 0.37	4	0.39	0.32 – 0.46
Chloryprifos	9	0.25	0.10 – 0.53	14	0.28	0.11 – 0.64
Delta-methrin	2	0.01	0.01 – 0.01	2	0.02	0.02 – 0.02
Endosulphan	9	0.34	0.01 – 0.66	6	0.15	0.10 – 0.22
Ethofenprox	3	0.12	0.07 – 0.20	0	–	–
Isoprocarb	1	0.21	–	3	0.20	0.11 – 0.29
Methyl parathion	3	0.39	0.02 – 0.83	1	0.21	–
Monocrotophos	11	0.25	0.01 – 0.67	6	0.25	0.10 – 0.33
Herbicide						
Butachlor	25	0.40	0.16 – 0.71	22	0.34	0.14 – 0.65
2,4 D	1	0.33	–	9	0.16	0.07 – 0.40
Molluscicide						
Fentin acetate	1	0.29	–	4	0.19	0.10 – 0.39
Fentin chloride	3	0.08	0.07 – 0.09	8	0.11	0.10 – 0.18

For chemical details of individual pesticides, see Appendix; a.i., active ingredient

Table 5. Aquatic oligochaete species recorded in the ricefields of the Laguna farms

Family	Species
Tubificidae	*Limnodrilus hoffmeisteri* (Claparédé) *Branchiura sowerbyi* (Beddard) *Aulodrilus limnobius* (Bretscher) *Aulodrilus* sp.
Naididae	*Dero digitata* complex (Müller) *Aulophorus hymanae* (Naidu) *Pristinella* sp.
Enchytraeidae	*Mesenchytraeus* sp.
Lumbricidae	*Lumbricidae* sp.

Table 6. Summary of Simpson's diversity indices for the aquatic oligochaete populations in the Laguna farms

	DS 1989	WS 1989		DS 1990	
	End	Start	End	Start	End
Mean	1.28	1.35	1.31	1.26	1.10
SD	0.63	0.48	0.55	0.36	0.17
Range (min – max)	1 – 3.18	1 – 2.97	1 – 3.19	1 – 2.34	1 – 1.62
n	20	28	21	25	16

DS, Dry season; WS, wet season; min, minimum; max, maximum; n, number of farms sampled

Table 7. Summary of aquatic oligochaete population densities (no. $m^{-2} \times 1000$) in the Laguna farms

	DS 1989	WS 1989		DS 1990	
	End	Start	End	Start	End
Mean (SD)	5.8 (5.4)	4.7 (4.7)	8.2 (10.1)	10.4 (9.4)	6.7 (8.8)
Range	0 – 16	0 – 17	0 – 35	0 – 32	0 – 27
n	25	32	32	27	24

See footnotes to Table 6

Table 8. Aquatic oligochaete biomass estimates

	Method A	Method B	Mean
Mean individual weight (mg)			
Fresh weight	1.44	2.10	1.77
Dry weight	–	0.41	–
Ash-free dry weight	–	0.40	–
Population biomass (kg ha^{-1})			
Fresh weight	504	735	620
Dry weight	–	144	–
Ash-free dry weight	–	14.0	–

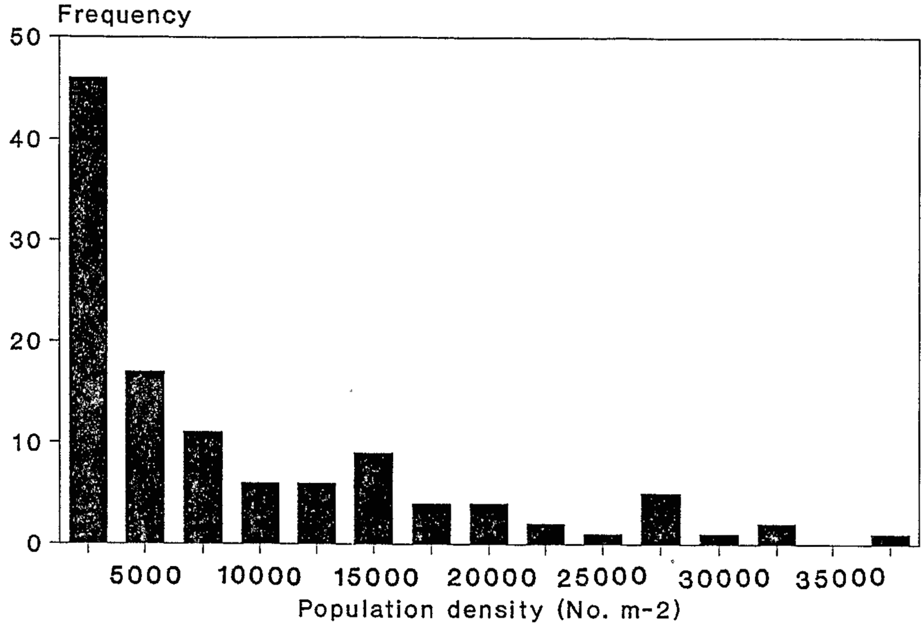

Fig. 2. Frequency distribution of the oligochaete population densities in the Laguna farms (population densites from all farms and times)

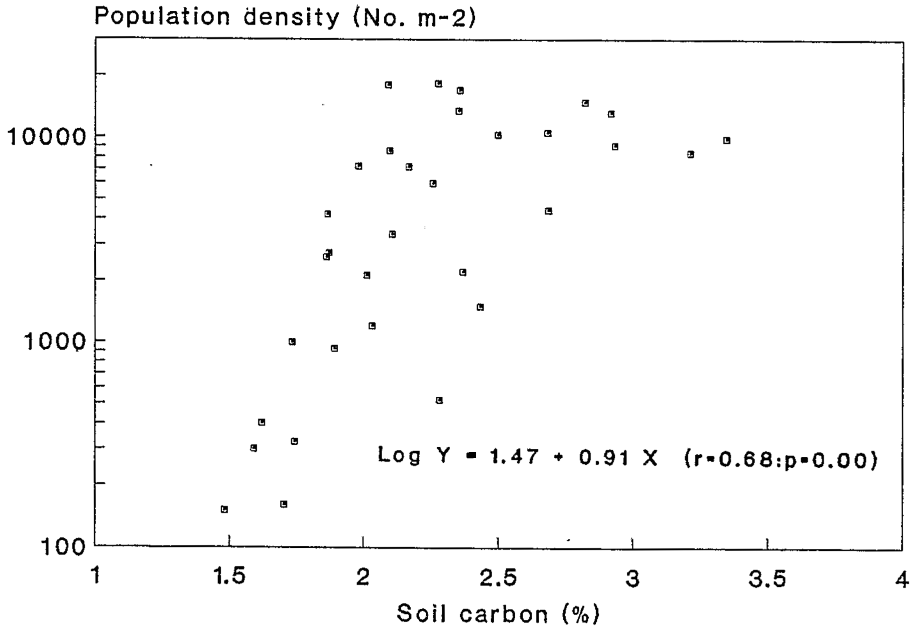

Fig. 3. Oligochaete population densities (means of five samplings) and soil C (means of two samplings) in the Laguna farms

Table 9. Correlation matrix of investigative means for oligochaete density and diversity with selected physicochemical variables

	a	b	c	d	e	f
a, Density						
b, Diversity	−0.38*					
c, N fertilizer	0.52**	−0.05				
d, Pesticide	0.31	−0.16	0.18			
e, Moisture	0.79**	−0.39*	0.26	0.15		
f, Soil C	0.68**	−0.32	0.39*	0.22	0.77**	
g, Active Fe	−0.17	0.48**	0.32	−0.05	−0.43*	−0.45**

Log-transformed density data were used
$^{*}P<0.05$, $^{**}P<0.01$

Population densities were positively correlated with soil organic C and N, and soil moisture (Table 9). Regression between soil C and log mean oligochaete density (Fig. 3) showed that below 1.75% C populations were low. Above this level they exhibited large variations. At approximately 2.25% C, densities ranged from 1000 to over 20000 m^{-2}. Both C and N indicate the organic matter content of the soil and they are highly correlated, so the relationship between soil N and oligochaete density is not presented.

Oligochaete numbers were low when soil moisture was below 80% (Fig. 4). Above 80% moisture, population densities increased, and at 110% they ranges from 1000 to over 20000 m^{-2}. Species diversity was negatively correlated with soil moisture (Table 9).

At the start of the 1989 wet season oligochaete density was negatively correlated with active Fe ($r = -0.43$; $P = 0.01$) and available P ($r = -0.46$; $P = 0.01$). Mean diversity indices were positively correlated with active Fe (Table 9). Soil C and moisture were correlated with active Fe and available P ($P \leq 0.01$), and therefore the above relationships could be spurious. No relationships were found between oligochaete density or diversity and soil pH or texture.

Mineral N applications stimulated the development of oligochaete populations (Fig. 5 and Table 9). No relationships were found between density and P or K applications. At the end of the 1989 wet season diversity indices were negatively correlated with N applied during that season ($r = -0.56$; $P = 0.01$) and with the investigative mean of N applications ($r = -0.45$; $P = 0.04$). No other correlations were found with diversity and mineral N, P, or K.

Oligochaete diversity (end of 1989 wet season) and quantity of molluscicide applied (1989 wet season) were correlated ($r = 0.47$; $P = 0.03$), as were diversity (start of 1989 wet season) and mean insecticide applications ($r = -0.38$; $P = 0.04$). No other relationships were found between pesticide use (frequency, quantity, or type) and oligochaete density, diversity (Table 9), or composition. Even when populations limited by other factors (soil C and moisture) were removed from the analysis no pesticide impacts were found.

It should be appreciated that, although correlation coefficients of 0.38 to 0.43 were significant at $P = 0.05$, they only explain 14–18% of the variance, which means that most of the variation is unaccounted for.

Discussion

Aquatic oligochaetes were the dominant macro-invertebrates of flooded rice soils; they were contagiously distributed and reached considerable densities. Population densities were similar to those previously found at IRRI (International Rice Research Institute 1985; Simpson et al. 1993) and in Japanese rice soils (Kikuchi et al. 1975). Crop cycle dynamics were more complex than reported by Kikuchi et al. (1975).

The two methods used to estimate the biomass produced similar results. A fresh weight biomass of 68 kg ha^{-1} was reported for a traditional low-input

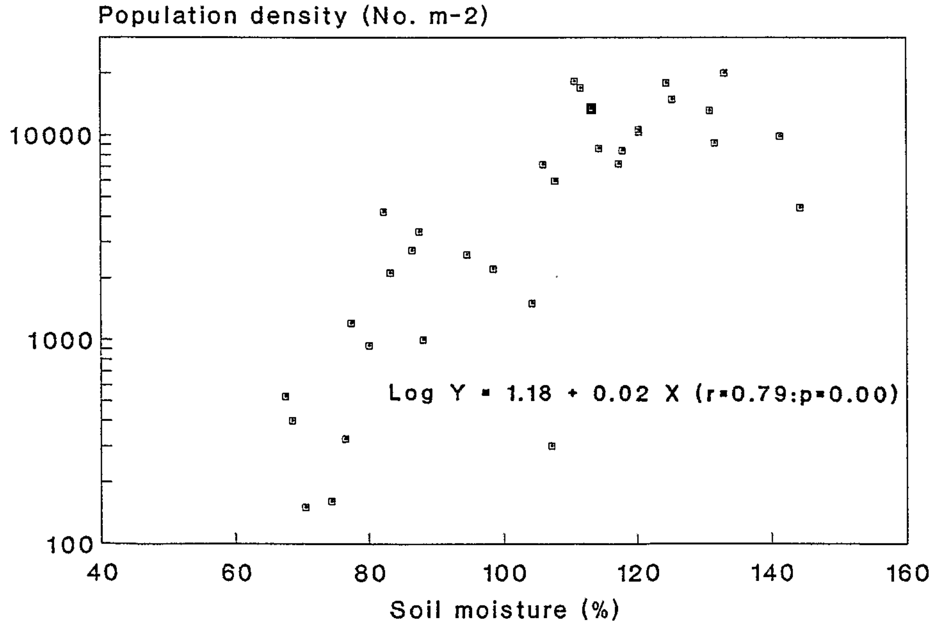

Fig. 4. Oligochaete population densities and soil moisture in the Laguna farms; means of five samplings

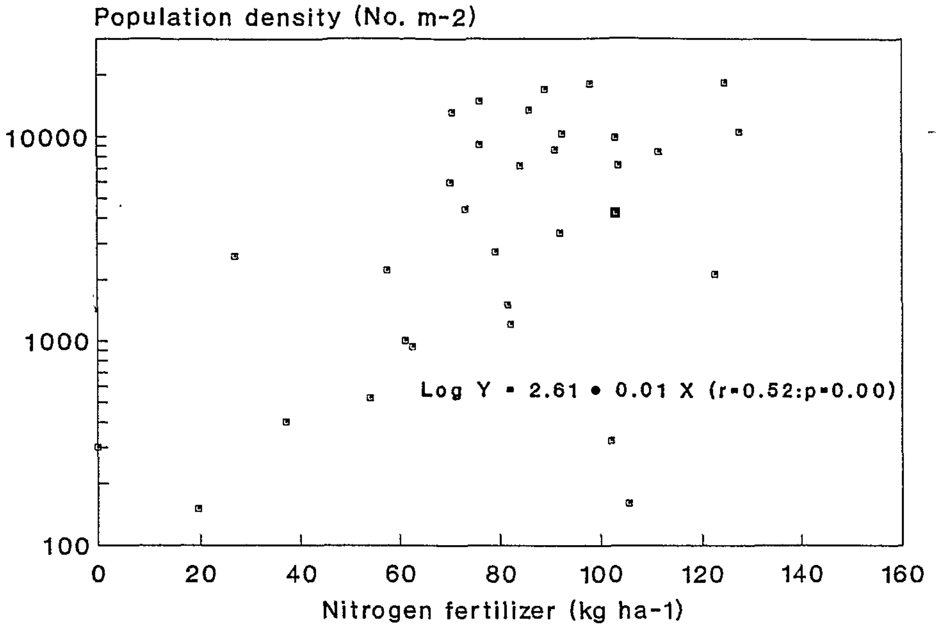

Fig. 5. Oligochaete population densities (means of five samplings) and N fertilizer additions (means of 1989 dry and wet seasons) in the Laguna farms

ricefield in Peru (calculated from Lavelle 1988), which falls within the survey range. The individual ash-free dry weight was similar to the 0.52 mg reported by Grant and Seegers (1985).

Populations were dominated by *L. hoffmeisteri* and *B. sowerbyi*, two members of Tubificidae genera that have been recorded previously in rice soils (Kikuchi et al. 1977; Grant and Seegers 1985). Although tubificids dominated most populations surveyed, naidids, lumbricids, and enchytraids were also recorded and occasionally formed a significant proportion of communities. In studies in Laos (Heckman 1974) and Thailand (Heckman 1979) all aquatic oligochaetes identified from ricefields were naidids. In Indian ricefields, oligochaete populations were dominated by the earthworm *Darwida willsi* (Senapati et al. 1991).

Oligochaete population diversity was low at all sites in the present study. Simpson's dominance index (Simpson 1949) obscures some differences between communities. When a population was assigned an index of 1 (monospecific) this was usually *L. hoffmeisteri*; however, there were exceptions.

The negative correlation between oligochaete density and diversity was caused by the dominance of *L. hoffmeisteri* in dense populations. When oligochaetes were sparsely distributed other species were relatively more abundant, often to the exclusion of *L. hoffmeisteri.* Low density probably indicates that the population was limited, perhaps by organic matter or moisture. Reversed relationships between density and diversity and other variables could be a consequence of their negative correlation.

The positive relationship between population densities and soil orgianc matter was probably mediated through bacterial decomposers. The organic matter, a substrate for decomposeres, represents a potential food source for the oligochaetes. Above 1.75% C, organic matter was no longer a limiting factor on the oligochaete carrying capacity of the soil. Similar relationships have been found for terrestrial oligochaetes in non-flooded soils (Edwards and Lofty 1972). Ranges of soil pH and texture found in the Laguna farms were limited, which may explain the absence of correlations with oligochaete densities.

The positive relationship between oligochaete densities and soil moisture was probably a consequence of respiratory stress and desiccation at low moisture. Avoidance of adverse conditions by burrowing deeper into the soil was prevented by the plough layer. There was no relationship between soil moisture and population density in the intensive sampling because all values were over 100%, which is above the level at which the correlation broke down in the farm survey.

The absence of a relationship between soil texture and moisture suggests that the major determinants of soil moisture content were irrigation, rainfall, and organic matter, rather than adsorption and absorption. Differences between populations at similar levels of soil moisture may be explained by antecedent floodwater conditions. Populations in recently rewetted soils will be different from those in soils that have been continually flooded. Floodwater management is probably the most important determinant of aquatic oligochaete population status.

The positive correlation between population density and mineral N applications agrees with the findings of Simpson et al. (1993). The relationship is probably mediated through increased primary productivity in the floodwater.

Despite the high tolerance of oligochaetes to pesticides (Naqvi 1973; Bailey and Lui 1980), impacts were identified in controlled field experiments (Simpson et al. 1993). Problems in quantifying pesticide inputs in a manner suitable for comparison made the identification of impacts difficult in the field survey. One application of one pesticide at 2 kg ha^{-1}, two applications of the same pesticide at 1 kg ha^{-1} and one application of a different pesticide at 2 kg ha^{-1} will probably all have different effects. Consequently, measuring the pesticide input in terms of the total amount or the frequency of application is not satisfactory. Difficulties with the identification of impacts were compounded by the absence of no-pesticide controls, by pesticide species specificity, and by differences in bioavailability.

Evidence from farmers' fields supported some of the relationships found between aquatic oligochaete populations and agricultural practices in experimental ricefields (Simpson et al. 1993). Inherent variability associated with field conditions and the inconsistent differences between management strategies made it difficult to attribute significant differences to single factors.

Conclusions

(1) Aquatic oligochaetes occur widely in the soil of flooded ricefields and are the dominant macro-invertebrate group.
(2) Species diversity was low and most populations were dominated by the tubificid species *L. hoffmeisteri* and *B. sowerbyi*.
(3) Populations were contagiously distributed within a field.
(4) Population densities and biomass ranged from 0 to 35 000 m^{-2} and 0 to 620 kg ha^{-1}, respectively. During a crop season densities increased, decreased, or remained similar.
(5) Population densities were limited by soil organic matter.
(6) Populations were strongly affected by floodwater management.
(7) Additions of mineral N stimulated the development of oligochaete populations.
(8) No pesticide impacts were found on oligochaete population densities. There was an indication that pesticide additions increased species diversity.

Appendix: Pesticide descriptions

2,4 D: (2,4-dichlorophenoxy)acetic acid; butachlor: N-butoxymethyl-2-chloro-2',6'-diethyl acetanilide); carbofuran: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; chlorpyrifos: 0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate; deltamethrin: [1R-(1 alpha (S*), 3 alpha)]-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate; endosulphan: (1,4,5,6,7,7-hexachloro-8-9-10-trinorborn-5-en-2,3-ylene) (dimethyl sulphite); ethofenprox: 2-(4-ethoxy phenyl)-2-methylpropyl 3-phenoxybenzyl ether; fentin acetate: triphenyltin acetate; fentin chloride: triphenyltin chloride; isoprocarb: *o*-cumenyl methylcarbamate; methyl parathion: *o,o*-dimethyl *o*-4-nitrophenyl phosphorothioate; monocrotophos: dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate.

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