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ORIGINAL PAPER

Spatial and temporal variability of macroinvertebrate communities in two farmed Mediterranean rice fields

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Abstract The spatial and temporal variation of macroinvertebrate assemblages was studied in two Portuguese commercial rice agroecosystems under the effect of field management involving the application of pesticides and fertilizers. A faunal succession of organisms was observed on both fields. Grazers were the first to colonize the paddies after a dry period when pesticides were applied, followed by development into nymphs and by an increase in the abundance of the species after the application of fertilizers. At the end of the season when no pesticides or fertilizers were applied, the communities changed with the presence of adult predators as a result of an increase in prey. Insecticide application revealed specific taxa increase due to the lack of competition with the target organism. Macroinvertebrates tended to prefer infested field margins with aquatic, submerged vegetation, revealing a spatial distribution along the paddies. Two different sampling devices were used and proved necessary in documenting the macroinvertebrate communities (grab for benthic and hand-net for pelagic organisms).

Keywords Macroinvertebrates · Rice fields · Fertilizer · Pesticides · Portugal

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Introduction

Rice is one of the major crops in Portugal, a country characterized by its extensive wet areas, and one of the most consuming crops in terms of pesticides and fertilizers (Pereira et al., 2000a). The management of rice ecosystems has adapted to Mediterranean climate conditions. They are dry in the winter, serving as cattle grazing prairies, while in the summer they are flooded up to 15–30 cm, with water coming from nearby dams or rivers, thus becoming temporary aquatic habitats managed with a variable degree of intensity. The paddies form rectangular or similarly shaped flooded fields comprised mostly of rice plants, surrounded by dry bunds (levees) rich in weeds, connected by irrigation canals and ditches that serve as contiguous aquatic habitats. These different habitats are affected by rapid and short-term temporal disturbances, such as draining and flooding and chemical inputs, contributing to a great variability in its resources and to changes in its rich biota (Bambaradeniya et al., 2004). Hence, these organisms could be referred to as biota with a high resilience stability that undergoes rapid secondary succession during each rice cycle (Odum 1997). Responses of aquatic insects and other groups of nontarget macroinvertebrates inhabiting rice fields floodwater to pesticide applications have been listed and studied in Malaysia, the Philippines, India, Japan and California (Grigarick et al. 1990; Forés and Comín 1992; Roger et al. 1992; Simpson and Roger 1995), and their temporal variation along the rice crop has been documented by Suhling et al. (2000), Bambaradeniya et al. (2004) and Foote and Hornung (2005).

This study is as a contribution to better understand the influence of agricultural practices on benthos inhabiting rice fields in Mediterranean environments. This work aims to answer two main questions: (1) how are the principal ecological factors linked to agricultural practices related to the temporal dynamics of macroinvertebrate communities? In relation to this topic, the relative influence of substrate, fertilizers and pesticides is also discussed; (2) what is the influence of the increasing density of rice plants on the spatial distribution of macroinvertebrate communities? We also test two sampling methods to evaluate the richness of macroinvertebrate communities.

Materials and methods

Study area

The study was carried out in an irrigated rice field ecosystem in the river Sado basin, a characteristic Mediterranean lowland basin (7640 km^2) located in southwestern Portugal (Fig. 1). The valley supports one of the largest areas of rice fields in Portugal, consisting of 5500 hectares (ha) (Pereira et al. 2000). Two rectangular wetland paddies on commercial rice agroecosystems under the effect of field management were selected according to soil characteristics: the Santa Catarina (SC) rice field (1.3 ha), implanted on sandy loam soil, with one point of water inflow that comes directly from

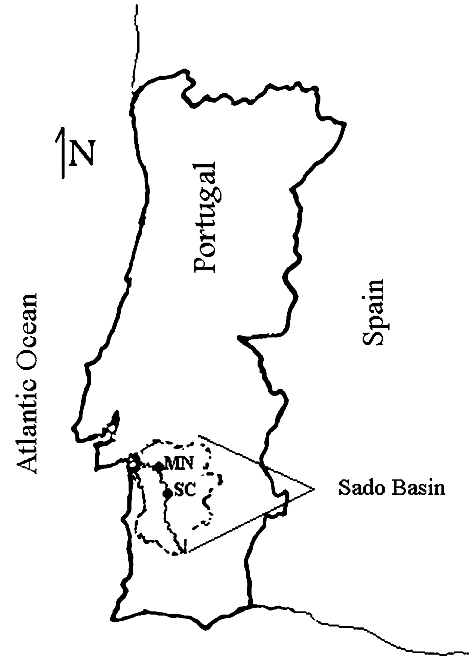

Fig. 1 Study area

a dam and with no artificial drainage; and the Monte Novo (MN) rice field (2 ha) located on silty clay soil with three points of water inflow and one drainage point.

The work was conducted in situ during the 2001 rice growing season (early May to October). Sampling dates were established according to pesticide and fertilizer applications and macroinvertebrate population recovery times (Fig. 2). Firstly, both fields were handled with a pre-flood treatment with the application of Ph/K-fertilizer (250 kg/ha: Ph/K 20/20) and pre-emergence herbicide (oxadiazon at 1.6 L commercial product/ha; half-life of 60 days (c.p./ha; h-l *n* d) (Tomlin 2000). After the latter, both fields were seeded, on dry soil, with the Ariete rice variety at a rate of 200 kg/ha: the Santa Catarina paddy by tractor on May 7 and the Monte Novo paddy by airplane on May 21. Both fields were flooded to a depth of 15 cm to 30 cm, after which the first sampling occurred (May 23, three weeks after pre-flood treatment). The second sampling (June 20) was performed on SC field three weeks after the insecticide application (chlorfenvinphos—0.5 l c.p./ha) to control Chironomidae larvae (*Chironomus* sp.) and the red swamp crayfish (*Procambarus clarkii*), assuming that this period

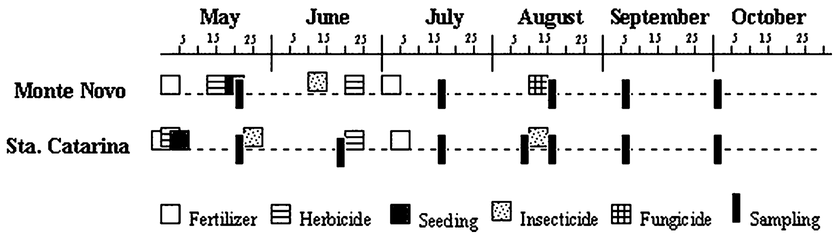

Fig. 2 Sampling dates and actions during the field management (pesticides and fertilizer applications)

was sufficient for the colonization of macroinvertebrate communities (De Paw et al. 1986). It was not possible to perform the second sampling (June) in MN after the insecticide application (endosulfan—0.5 l c.p./ha; h-l 50 d) used to control the same target organisms as chlorfenvinphos in June against the same target fauna as chlorfenvinphos. This sample was not taken due to an unexpected drainage of the field for the following herbicide application. Both fields were sprayed with post-emergence herbicide on dry soil: at SC a mixture of propanil (12 l c.p./ha; h-l 1 d) and (4-Chloro-2-Methylphenoxy) Acetic acid (MCPA) (1.2 l c.p./ha; h-l 7 d) was applied, and at MN a mixture of MCPA (1.5 l c.p./ha) and profoxydim (0.5 l c.p./ha; h-l 8 d). Eight days after herbicide application on dry soil (and after flooding the paddies) a N-fertilizer (43% N + 5% NH_4), at a rate of 110 kg/ha, was added to both fields. Approximately two weeks after the latter treatments, a third sampling took place (July 17). The fourth and fifth samplings were performed at SC in August before and after the insecticide application (13 and 16 August respectively): based on dimethoate (1.5 l c.p./ha; h-l 7 d) against Aphididae and Lepidoptera *Sesamia* sp. At MN only a fourth sampling was performed (Aug 16), after fungicide application (tricyclazole 0,3 kg c.p./ha; h-l 21 d) to treat *Pyricularia oryzae*. No more pesticides and fertilizers were applied until the end of the crop, but two more samplings were conducted: one in September and another in October just before harvesting. The complete rice cycle took approximately 24 weeks.

Environmental parameters

Water samples resulted from composite sampling (five points scattered on the field). Water temperature, conductivity, pH and dissolved oxygen (DO) were evaluated in situ using field probes (WTW—Multiline F/7–3). Chemical parameters such as phosphates, ammonia, nitrates, nitrites, calcium, magnesium, alkalinity and hardness, were evaluated in the laboratory in accordance with standardized analytical methods (APHA 1998). Water residue levels of the active ingredients of pesticides (endosulfan, propanil and chlorfenvinphos) were extracted by SPME (Solid Phase Micro Extration) and quantified by GCMS (Gas Chromatography Mass Spectrometry) (APHA 1998).

Ecotoxicity tests were conducted on the microalgae *Pseudokirchneriella subcapitata* and the freshwater cladoceran *Daphnia magna* according to the standard operational procedure of the Toxkits microbiotests: Algaltoxkit F^{TM} (1996) and Daphnotoxkit F^{TM} magna (1996), respectively. The toxicity test using *D. magna* was performed by determination of the 48-h inhibition percentage of the organism mobility when exposed to water samples using a standard log-linear model. The microalgae *P. subcapitata* bioassay had a duration of 72-h when at the end the growth inhibition percentage was determined by converting optical densities (measured with a spectrophotometer provided with a 670-nm filter) into algal numbers using an exponential model.

Granulometry analysis for silt, clay, loam and sand percentages were undertaken on composite sampling (five points scattered along the field). A raw evaluation of the rice culture development was assessed by crop density (number of tillers per m^2) and plant height from three randomly selected sites of the rice fields using a 25 cm square (625 cm^2).

Macroinvertebrates

Two different sampling methods were used to collect pelagic and benthic macroinvertebrates: a

D-frame hand-net (Usinger, 1956a) with a blade scraper of 150 mm and mesh aperture of 500 μm, and a Ponar grab (Powers and Robertson, 1967), respectively. Five replicates were randomly collected from the centre and the margins (a band of 2 m from the levee) with each sampling device (0.13 m^2 of total area using the hand-net was, and 0.12 m^2 using the grab). In the laboratory all samples were gently washed under running water through a 500 μm sieve and the retained organisms were sorted with forceps and preserved in 70% ethanol. Whenever possible, macroinvertebrates were identified to species level. A simple classification based on Tachet et al. (1980) was applied to study the functional feeding structure of the macroinvertebrate community.

Statistical analysis

Prior to any statistical analysis, matrixes of macroinvertebrate abundances from each field were transformed using natural logarithms [$y = \ln(y + 1)$] to prevent resultant distortions from the most abundant taxa. Environmental data (x) were standardized (ST) by: ST = (x − mean)/(medium deviation) in order to centre and reduce different ranges of variation among environmental variables (chemical, physical and toxicological).

Environmental influence over macroinvertebrate assemblages was assessed by canonical analysis and correlations using the specialized multivariate analysis programme CANOCO-Canonical Community Ordination (ter Braak and Smilauer 1998). The spatial preferences and differences of macroinvertebrates between the two sampling methods were first studied by applying a detrended correspondence analysis (DCA) to the macroinvertebrate data matrix from each paddy. This was done in order to detect which analysis better fitted each data set throughout the length of the gradient in the first axis. The Santa Catarina data revealed a length of gradient lower than 3 (limit adopted by the program) and the Monte Novo data a length of gradient higher than 3. This latter analysis resulted on a second application of a principal components analysis (PCA) on Santa Catarina data and a correspondence analysis (CA) on Monte Novo's (ter Braak and Smilauer 1998). Significant ecological gradients influencing the distribution of macroinvertebrates in each paddy were assayed by Pearson correlations (Rohlf and Sokal 1973), performed between ordination coordinates resulting from the PCA and CA and environmental data previously treated. To assess the main trends of environmental influence over both fields, a combined macroinvertebrate data set was created and subjected to a redundancy analysis (RDA). This was conducted jointly with the combined environmental data, after a previous detrended canonical correspondence analysis DCCA showing that the length of gradient of the total data set was lower than 3. As an intermediate step, environmental variables with a confidence level higher than 0.05 were rejected according to the Monte Carlo permutation test (999 permutations). Similarly, those that showed a variance in inflation factors higher than 20 were also rejected, in order to avoid multicolinearities between variables. The model significance level was tested on all axes of the RDA by the Monte Carlo test (ter Braak and Smilauer 1998).

An analysis of variance partition was performed through a partial RDA in order to evaluate the individual contribution of each group of environmental variables (ecotoxicity, water parameters, soil sediment categories, crop density) to the total explained variance of the model. The value of each contribution was obtained by dividing the canonical eigenvalues of the partial RDA (assuming the other three groups of environmental variables as covariables) by the total inertia (sum of all eigenvalues of a canonical analysis of the macroinvertebrate abundance matrix) (ter Braak and Smilauer 1998).

Results

Environmental parameters

The Monte Novo paddy floodwater presented higher values for total alkalinity, hardness, calcium, magnesium, conductivity and temperature than that of Santa Catarina (Table 1). Nitrates and nitrites were extremely low, with a value of only 0.07 mg/l of the latter being registered in

Table 1 Environmental parameter values for both rice fields during the rice crop season

Environmental parameters	Santa Catarina							Monte Novo				
	May	Jun	Jul	13 Aug	16 Aug	Sep	Oct	May	Jul	Aug	Sep	Oct
Water temperature (°C)	20.7	26.1	21.5	22	21	19.8	20.3	26	27.4	24.5	23.2	23
Conductivity (μs/cm)	199	359	191	223	255	270	204	1401	921	1020	599	396
pH	7.11	7.27	6.77	6.79	7.14	7.25	6.85	6.49	7.04	7.67	7.43	7.3
Dissolved oxygen (mgO_2/l)	7.27	6.14	5.36	5.14	5.82	4.89	4.13	7.11	6.01	5.87	4.81	4.88
Alkalinity (mg HCO_3/l)	30	105	78	86	98	116	64	<12	206	491	283	149
Ammonia (mg NH_4/l)	0.12	<0.08	0.46	<0.08	0.08	<0.08	<0.08	2.2	0.29	0.12	<0.08	<0.08
Phosphates (mg P_2O_5/l)	<0.20	<0.20	<0.20	0.3	0.24	<0.20	<0.20	<0.20	<0.20	0.22	<0.20	<0.20
Nitrates (mg NO_3/l)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Nitrites (mg NO_2/l)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.07	<0.05	<0.05	<0.05	<0.05
Calcium (mg Ca/l)	10	19	13	15	14	20	15	56	36	62	46	29
Magnesium (mg Mg/l)	4.5	15	7	7.6	8.2	8.5	5.2	33	23	40	28	16
Hardness (mg $CaCO_3$/l)	44	107	61	69	69	85	59	274	184	319	230	138

<0.08, <0.20, <1 and <0.05: quantification limits for the respective method

May at the Monte Novo field. Phosphates reached higher values in July, as did ammonia in August at both fields. Water pH remained practically neutral during the entire rice cycle, while water temperature and DO reflected a tendency to decrease throughout the crop season.

The Santa Catarina floodwater showed no ecotoxic effects in May, but revealed a highly acute toxicity to both test organisms in June: 80% of effect on *P. subcapitata* and 100% on *D. magna*, as well as residue levels of the herbicide propanil (0.2 μg a.i./l; before its application), and the insecticide chlorfenvinfos (0.9 μg a.i./l). In July at Santa Catarina, after the application of the herbicide propanil on dry soil, no residues were found, unlike chlorfenvinfos, which prevailed in water at a residue level of 0.7 μg a.i./l and 0.2 μg a.i./l in July and August, respectively. These July residue values coincided with acute toxicity results of 66% on *P. subcapitata* and 25% on *D. magna* for Santa Catarina. The Monte Novo floodwater revealed no values for pesticide residue levels in July but showed a slight acute toxicity result of 49% of effect on the microalgae and 20% effect on the cladoceran. The insecticide endosulfan was not detected in the Monte Novo water on the sampling dates. The water samples collected in September and October at both rice fields showed no positive results for residue levels or acute toxicity.

The Monte Novo soil was classified as a silty clay soil (34.2% silt—57.1% clay) and that of Santa Catarina as a sandy loam soil (42.3% sand—30.1% loam). Crop density increased until August and remained stable for the rest of the season (approximately 860 tillers/m^2). A maximum height of approximately 80 cm per tiller was attained at both rice fields.

Faunal composition and temporal evolution

The majority of the 71 macroinvertebrate taxa collected during the study period belonged to Insecta (Table 2). The Santa Catarina (SC) paddy was the most populated in terms of *taxa* (65 in total) and number of organisms per collection date (480 org/m^2). The Monte Novo (MN) paddy presented 51 taxa and 340 org/m^2 per collection date. Different organisms were collected at each rice field: the SC paddy was dominated by Chironomidae (*Chironomus* gr. *plumosus* and tr. Tanytarsini), Gastropoda (*Physa acuta* and *Planorbis* cf. *planorbis*), Lumbriculidae and Naididae, and presented the Hirudinea *Erpobdella* cf. *testacea*; MN was dominated by Tubificidae, in particular *Branchiura sowerby*. The macroinvertebrates inhabiting both rice fields were observed to follow a succession during the rice cycle. Collectors were the first to appear, in May, followed by grazers in July (Fig. 3). Grazers remained dominant at SC during the last three months but predators appeared with lower abundances. At Monte Novo predators dominated during the last three months. An evolution in the

Table 2 Total macroinvertebrate abundances collected at both rice fields

Taxa	Stage	Abundances		Taxa	Stage	Abundances	
		MN	SC			MN	SC
Basommatophora—fresh-water snails				*Rhantus* sp. Dejean	L	1	16
Planorbis cf *planorbis* Linn	A	7	1995	*Rhantus pulverosus* Stephens	A	4	4
Physa acuta Drap.	A	14	2486	*Anacaena* sp. Thomson	A	3	2
Oligochaeta—aquatic earthworms				*Berosus* sp. Leach	L		1
Lumbriculidae	A	116	555	*Enochrus* sp. Thomson	L	180	22
Lumbricidae	A	4	115	*Enochrus* sp #1 Leach	A	43	9
Tubificidae	A	171	196	*Enochrus* sp #2 Leach	A	11	1
Branchiura sowerby Beddard	A	31		*Hydrobius* Leach	A		3
Plesiopora				*Hydrophilus* sp. De Geer	A	2	2
Naididae	A	11	162	*Helophorus* sp. Fabricius	LA	8	11
Aulophorus furcatus Muller	A	17	100	*Dryops* sp. Olivier	LA	4	22
Dero sp. Oken	A	1	196	**Diptera—flies**			
Pharyngobdellae				*Aedes* sp.	L	2	1
Erpobdella cf. *testacea* Savigny	A		114	*Anopheles* sp.	L	3	30
Decapoda—crayfish				*Culex* sp.	LN	739	97
Procambarus clarkii	A	11	1	*Dixella attica (=Paradixa)* Pandazis	LN		35
Ephemeroptera—mayflies				*Brachydeutera* sp. Laew	L		3
Cloeon dipterum Lineu	L	75	120	*Discocerina* sp. Macquart	L	2	2
Caenis luctuosa Burmeister	L		1	*Ephydra* sp. Fall.	L	23	
Odonata—dragonflies				*Notiphila* sp. Fall.	L	82	5
Libellulidae	L		52	*Ochthera* sp. Latreille	L		1
Crocothemis erythraea Brullé	L		3	*Scatella* sp. Rob.- Desv.	L	7	14
Orthetrum brunneum Fonsc.	L	1	1	*Crysops relictus* Meig.	L		1
Sympetrum fonscolombei Selys	L		9	*Tipula* sp. L.	L		3
Coenagrionidae	L	1	77	*Atrichopogon* sp. Kieff.	L		1
Ischnura elegans Vander Linden	L	1	26	*Bezzia* sp. Kieff.	L	1	1
Hemiptera—true bugs				*Culicoides* sp. Latr.	L		2
Notonecta glauca Linn	A	351	38	*Dasyhelea* sp. Kieff	L	10	
Corixidae	N	14	7	*Forcipomyia* sp. Megerle	L		22
Sigara concinna Fieb.	A	7	1	*Oplodontha* sp. Fabr.	L	2	
Sigara lateralis (Leach) Jaczewski	A	6		*Lispe* sp. Latr.	L		2
Sigara limitata (Fieb.) Jaczewski	A	23	3	*Lyancalus virens*	L		6
Gerris thoracicus Schum	A	6	1	*Dicranomyia (D.) modesta* Meigen	L	5	
Gerris (Aquarius) naja Deg.	A		4	*Ormosia* sp. Rond.	L		1
Vellidae	N	4	27	*Rhypholophus* Kolenati	L		1
Microvelia sp. Westw	N	20	37	*Antichaeta* sp. Haliday	L	3	3
Coleoptera—beetles				*Canace* sp. Haliday	L	1	
Eretes sticticus L.	L	4		Tanypodinae	LN	21	146
Guignotus sp. Houlbert	L A	309	134	Corynoneurinae	L	2	15
Hydaticus sp.Leach	L	2	8	Orthocladinae	LN	207	190
Hydaticus grammicus Germar	L	3	5	tr. Chironomini	LN	360	1437
Hydaticus (Guignotites) leander Rossi	A		5	*Chironomus* gr. *Thumi*	L	7	26
Laccophilus sp. Leach	L	9	16	*Chironomus* gr. *Plumosus*	L	619	2168
Laccophilus hyalinus De Geer	A	4	15	tr. Tanytarsini	LN	549	620
				Total		4116	11413

Stage: A—adult, L—larvae, N—nymph; MN—Monte Novo, SC—Santa Catarina; orders in bold

number and taxa of organisms was observed, particularly on the SC paddy, and demonstrated by the statistical analysis (Fig. 4). The time gradient was represented by axis 2 of SC PCA with a DO decrease and an increase in rice tillers throughout the rice cycle, as shown by the

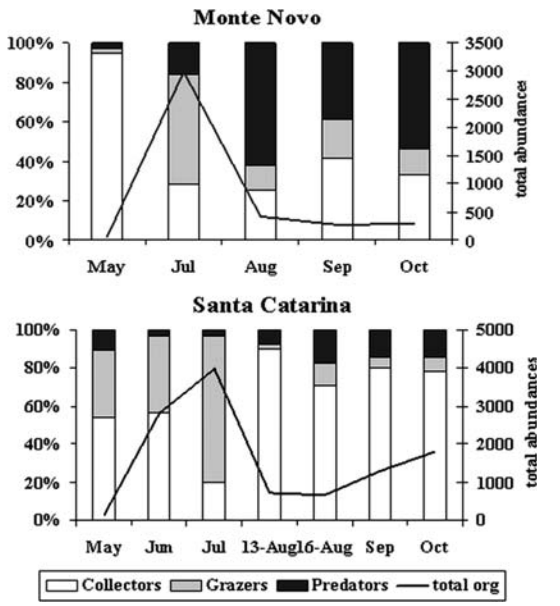

Fig. 3 The categorization of macroinvertebrates into three functional feeding groups based on Tachet et al. (1980)

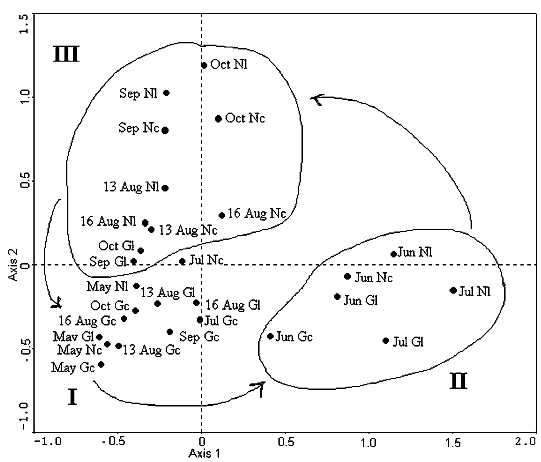

Fig. 4 PCA plot of macroinvertebrate assemblages according to sampling dates, sampling methods, and location in the Santa Catarina paddy. N—hand net, G—grab, l—levee, c—centre

significant Pearson correlations obtained (Table 3). Therefore, May (I), June and July (II) samples presented higher DO values and lower rice tillers than samples from August, September and October (III), located at the opposite end of axis 2. Axis 1 revealed a clear separation of the richest samples in terms of organisms and taxa—June and July. They correspond to the dates where differences between the two sampling devices were not so obvious, unlike the last three sampling dates (August, September, October). Grab samples were characterized by the presence of sediment living organisms such as Oligochaeta, Chironomidae, Ceratopogonidae and Diptera larvae (Table 4), while samples collected with the hand-net revealed higher organism abundance, mainly the more pelagic ones: Coleoptera adults, Hemiptera, Culicidae and Ephemeroptera.

The time gradient was not so evident at MN (Fig. 5), although a good difference between both sampling devices (grab and hand-net) was observed (Table 5). The CA from Monte Novo data shows this clear opposition along axis 2, contrasting with the disperse setting of the two sampling locations (centre and levee). Axis 2 is the second most significant axis of the data set total variation (17.1%), but proved not to be correlated with any of the environmental variables, unlike axis 1. This latter reflects the evolution of chemical parameters along the axis and the growth in rice tillers (number and height) on the opposite way of the axis, as shown by the Pearson correlations (Table 3).

Differences in local preferences (centre and levee) using both devices were noticed in June and July at SC, and in August, September and October at MN. These were noticed in the months that presented higher organism abundances at SC but not so high at MN. At the centre of the field only Oligochaeta and Diptera larvae and a few Coleoptera adults and Hemiptera swimmers were collected, while at the margins the benthos populations were more complex, with higher predator abundances (Coleoptera and Odonata). *Planorbis* cf *planorbis*, *Physa acuta*, *Culex* sp., *Notonecta glauca* and *Erpobdella* cf *testacea* were found in practically all benthos samples from the SC paddy.

Relationship between fauna and environmental variables

The RDA accepted model revealed 11 environmental variables influencing the macroinvertebrate communities from both rice fields (Table 6).

Table 3 Significant correlations resulting from Pearson correlation coefficients between the environmental variables and the biotic sample scores (Hill's scaling) from PCA and CA used in Santa Catarina and Monte Novo, respectively

Environmental variables	Santa Catarina		Monte Novo
	Axis 1 (32.0%)	Axis 2 (22.5%)	Axis 1 (17.3%)
Water temperature	0.61*		
PH			–0.54**
Conductivity	0.41**		0.50**
DO		–0.53*	0.53**
Nitrites			0.68*
Ammonia	0.37**		0.68**
Calcium			
Magnesium	0.58*		
Hardness	0.49*		
Toxicity on *D. magna*	0.66*		
Toxicity on *P. subcapitata*	0.81*		
Chlorfenvinphos concentration	0.81*		–0.68*
Propanil concentration	0.63*		–0.68*
Number of rice tillers			–0.63*
Rice plant height		0.42**	–0.65*
Number of organisms	0.82*		
Number of *taxa*	0.64*	0.63*	

Percentage of variability explained by each axis and *P*-value is indicated; * $P < 0.01$; ** $P < 0.05$

The Monte Novo paddy differed from that of Santa Catarina in the greater amount of clay and silt in its sediment and water characteristics: high values of conductivity, temperature and DO, all significantly correlated with axis 2 (Fig. 6). As mentioned above, the time gradient is revealed by the decrease DO along axis 2, positioning Santa Catarina samples correctly. Toxicity parameters seem to be influencing the June and July samples of Santa Catarina and Monte Novo along axis 1, which are the months with the higher organism abundances. This RDA and SC PCA also show that the application of the insecticide dimethoate on the SC paddy studied in the August samples (before and after the application—13 and 16 Aug) revealed a nontoxic environment for the test species (algae and cladoceran). Both dates were positioned close to each other in both analyses and the observed decrease in the number of organisms happened before the pesticide application (Aug 13) on SC, which already displayed lower abundances for all organisms.

The analysis of variance partition shows that the macroinvertebrate distribution is related to the ecotoxicological parameters in 27.5% of total variation and 17.7% with water characteristics. The soil type and number of rice tillers are responsible for a very small portion of variation of the model (9.7% and 4.8%, respectively). The accepted model explained 91.2% of the macroinvertebrate variation, where 59.7% was explained by the influence of each group of environmental variables and 31.5% by shared groups variation. The model proved to be significant by the Monte Carlo permutation test ($p = 0.012$, <0.05) for all axis eigenvalues (ter Braak and Smilauer 1998).

Discussion

Environmental parameters

The detection of the herbicide propanil in June before its application on the paddy was a consequence of several factors: the dispersion of the product by wind from airplane application on adjoining rice fields; the tendency of the herbicide to remain in water (Pereira et al. 2000) together with the fact that the Santa Catarina paddy does not have an outflow, leading to accumulation of the product.

Faunal composition and temporal evolution

Aquatic macroinvertebrate assemblages emerging from both rice fields were characterized by the

Table 4 Associated taxa with the different steps of colonisation shown by the PCA gradient of macroinvertebrate assemblages in Santa Catarina

May (I)	June July (II)	August September October (III)
Lumbriculidae	**Grab centre**	**Grab**
Tubificidae	Naididae	*Forcipomyia* sp.
Lumbricidae	*Dero* sp.	*Bezzia* sp.
Chironomus gr. *thumi*	*Aulophorus furcatus*	*Discocerina* sp.
Tipula sp.	*Lyancalus virens*	*Lispe* sp.
Culicoides sp		*Notiphila* sp.
Helophorus sp. A	**Net centre**	*Ormosia* sp.
Dryops sp. L	*Scatella* sp.	*Ryph† olophus* sp.
	Grab and net levee	**Net**
	Orthocladinae L N	Tanipodinae
	tr Chironomini L N	Corynoneurinae
	Chironomus gr. *Plumosus*	*Atrichopogon* sp.
	tr. Tanytarsini L	*Dixella attica*
	Brachydeutera sp.	*Anopheles* sp.
	Crysops relictus	*Anizoptera*
	Gerris (Aquarius) naja N	*Zigoptera*
	Gerris thoracicus	*Cloeon dipterum*
	Hydaticus sp. L	*Caenis luctuosa*
	Hydaticus grammicus L	Vellidae N
	Rhantus sp. L	*Sigara concinna* A
	Rhantus pulverosus A	*Sigara limitata* A
	Guignotus sp. L A	*Microvelia* sp.
	Anacaena sp. A	*Hydaticus (Guignotites) leander*
	Enochrus sp. L	*Laccophilus hyalinos* A
	Hydrophilus sp. A	*Laccophilus* sp. L
	Dryops sp. A	Enochrus sp. #1
		Enochrus sp. #2
		Berosus sp.
		Hydrobius sp.
		Ochthebius sp.
		Helophorus sp.

L—larvae, N—nymph, A—adult

presence of Oligochaeta, Gastropoda, Diptera larvae (mostly Chironomidae and Culicidae) Hemiptera (Corixidae, Notonectiddae), Ephemeroptera, Odonata and Coleoptera (Dytiscidae, Hydrophilidae), also reported in several rice field studies (Darby 1962; Washino and Hokama 1967; Watanabe et al. 1987; Roger 1989; Grigarick et al. 1990; Roger et al., 1995; Simpson and Roger 1995; Leeper and Taylor 1998).

Taxa richness and abundance of both rice fields was clearly different. The Santa Catarina paddy proved to be richer than that of Monte Novo, resulting from several factors: the presence at the Santa Catarina paddy of submerged aquatic vegetation that provides a suitable microhabitat and shelter from predators (Darby 1962; Chandler and Highton 1975; Zalom et al. 1980); the nature of the substratum, since the sandy soil of Santa Catarina facilitates locomotion and the search for food (algae, moss and organic debris contained among the sand grains) by grazing species such as Chironomidae, Gastropoda and Lumbriculidae and Naididae, which tend to dominate in stony substrates, and Tubificidae, which generally dominate silt-clay soils (Brinkhurst and Cook 1974), such as that of Monte Novo. *Erpobdella* cf. *testacea* was found only at the Santa Catarina paddy, due to the fact that the leech sucker cannot function correctly on pure, muddy soil (Sawyer 1974) such as that of the Monte Novo paddy. To cite an opposite case, *Branchiura sowerby* needs warmer waters like those found on MN to attain sexual maturity (Brinkhurst and Cook 1974; Sawyer 1974).

Macroinvertebrate community successions were similar to those observed in other studies

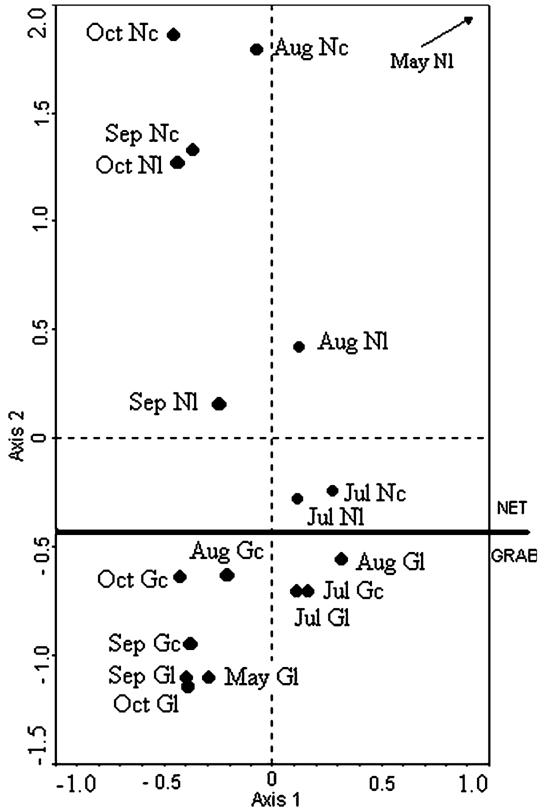

Fig. 5 CA plot of macroinvertebrate assemblages according to sampling dates, sampling methods, and location in the Monte Novo paddy. N—hand net, G—grab, l—levee and c—centre

on rice fields in France, Sri Lanka and Canadian prairie wetlands, such as those of Suhling et al. (2000), Bambaradeniya et al. (2004) and Foot and Hornung (2005). The first organisms to appear at both rice fields (May) were Oligochaeta and Chironomidae, which would overwinter in permanent water bodies such as nearby canals, ditches or ponds in the vicinity of the fields, and begin colonizing the rice field as adults in the early summer, in accordance with Darby (1962) and Bambaradeniya et al. (2004). A few other organisms, such as other Diptera and Coleoptera, were also able to colonise as adults, as was reported by Suhling et al. (2000). In a second phase, macroinvertebrates increased their abundances at a very fast rhythm as a result of the epiphytic algae blooms that occurred at the beginning of the crop and which are used as a source of food. Those blooms originate from a fast consumption of all nutrients provided by the N-fertilizer and are favoured by high DO and light availability in the floodwater (Roger 1989; Roger et al., 1992), when only rice seeds were present. The same events may repeat themselves after the dry period of 5–7 days necessary for herbicide application in June, which may have initially caused high mortalities among the organisms, as stated by Darby (1962) and Suhling et al. (2000). In late June and July, benthos assemblages became a well-developed and rich macrofaunal community dominated by collectors and grazers (90%) with the presence of Chironomidae larvae and nymphs, several other Diptera larvae, Hemiptera nymphs, Coleoptera larvae and a few adults Ceratopogonidae, as registered in several other studies of rice fields (Zalom et al. 1980; Roger 1989; Leeper and Taylor 1998; Suhling et al. 2000). This late phase was followed by a rapid decrease in organisms just before pesticide application in August. The high abundance of primary consumers observed after the N-fertilizer application in July would graze intensively on photosynthetic aquatic biomass until they succumb, as indicated by Roger (1989). The recovery of these algae populations would not be successful, due to the low availability of sunlight and oxygen in the water column, as a direct effect of plant canopy development. This decrease would inhibit the growth of primary consumers and affect their predators indirectly (Roger 1989). This effect was observed in August, when predators such as Coleoptera and Hemiptera adults, Ephemeroptera larvae and well-developed Odonata larvae increased in number (59%) with the development of the first consumer communities, and the presence of high rice tillers, favouring oviposition by adults and larvae habitat requirements, as mentioned in other studies (Roger 1989; Roger et al. 1992; Simpson and Roger 1995; Foot and Hornung 2005). In the later months, September and October, when no pesticides and fertilizers were applied, individual populations of predators (Coleoptera, Hemiptera, Ephemeroptera) increased their abundances as part of their natural life cycle and trophic succession, as documented by Odum (1975).

A preference for margins by the macroinvertebrate assemblages was observed at Santa

Table 5 Associated taxa with the CA macroinvertebrates gradient from Monte Novo

	Net	Grab
July	*Dero* sp.	
	Orthocladinae L	Naididae
	tr. Chironomini L N	Aulophorus furcatus
	tr. Tanytarsisi L	*Dasyhelea* sp.
	Dicranomyia (D.) modesta	Tanypodinae
	Anopheles sp.	Orthocladinae N
	Culex sp.	tr. Chironomini N
	Corixidae N	*Chironomus* gr. *Plumosus*
	Sigara concinna	*Chironomus* gr. *thumi*
	Eretes sticticus	*Bezzia* sp.
	Hydaticus grammicus	*Ephydra* sp.
	Rhantus sp. L	*Canace* sp.
	Laccophilus hyalinus A	*Guignotus* sp. L
	Enochrus sp. #1 A	*Enochrus* sp. L
	Enochrus sp. #2 A	
August September October	centre	
	Physa acuta	centre
	Planorbis cf. *planorbis*	Lumbriculidae
	Corynoneurinae	Tubificidae
	Antichaeta sp.	*Branchiura sowerby*
	Discocerina sp.	
	Zigoptera	
	Microvelia sp. A	
	Ochthebius sp. A	
	Helophorus sp. A	
	levee	
	Procambarus clarkii	levee
	Gerris thoracicus	*Notiphila* sp.
	Sigara limitata	*Hydrophilus* sp. A
	Sigara lateralis	
	Notonecta glauca	
	Cloeon dipterum	
	Rhantus pulverosus A	
	Guignotus sp. A	
	Dryops sp. A	

L—larvae, N—nymph, A—adult.

Catarina in June and July, but was not as obvious at Monte Novo. The main reason could be due to the presence of submerged aquatic vegetation, which provides shelter from predators, food resources and additional substrate for larvae and the attachment of eggs for Coleoptera and Diptera (Darby 1962; Surtees 1970; Roback 1974; Chandler and Highton 1975; Zalom et al. 1980), Hemiptera (Corixidae, Gerridae, Vellidae and Notonectidae), Ephemeroptera (*Cloeon* sp. and *Caenis* sp.) (Roback 1974) and Odonata (Foot and Hornung 2005). Authors such as Darby (1962), Roger (1989) and Forés and Comín (1992) specify several other reasons for this preference, such as the density of the rice canopy at the centre of the field shading the water and thereby causing a decrease in water temperature, which in turn induces organisms to move to higher temperatures nearer the levee, as well as the fact that low DO concentrations in the floodwater at the centre of the paddy caused by the decomposition processes of larger green plants and eucariotic algae. Another fact that could influence macroinvertebrate distribution along the paddies is the *edge effect*, which clearly states that densities and diversities tend to be higher at the boundary between different environment communities (Smith and Smith 2001), as

Table 6 Correlations of the environmental variables with the canonical axes from RDA; influence as a percentage of total variation for the corresponding axis; stronger correlations in bold.

Environmental parameters (variance groups)	Correlation with canonical axes			
	Axis 1 (33.5%)	Axis 2 (27.2%)	Axis 3 (14.7%)	Axis 4 (5.7%)
Water characteristics				
Water temperature	0,38	**0.63**	–0.11	–0.01
Conductivity	–0.1	**0.68**	–0.01	–0.05
DO	0.03	0.36	**–0.71**	–0.31
Ecotoxicological parameters				
Toxicity on *P. subcapitata*	**0.92**	–0.01	–0.19	0.14
Toxicity on *D. magna*	**0.62**	–0.23	–0.36	0.15
Chlorfenvinphos concentration	**0.67**	–0.35	–0.41	0.35
Crop density				
Number of rice tillers	0.15	–0.24	0.44	**0.62**
Sediment categories				
% coarse sand	0.17	**–0.89**	–0.22	–0.17
% medium sand	0.17	**–0.89**	–0.22	–0.17
% silt	–0.17	**0.89**	0.22	0.17
% clay	–0.17	**0.89**	0.22	0.17

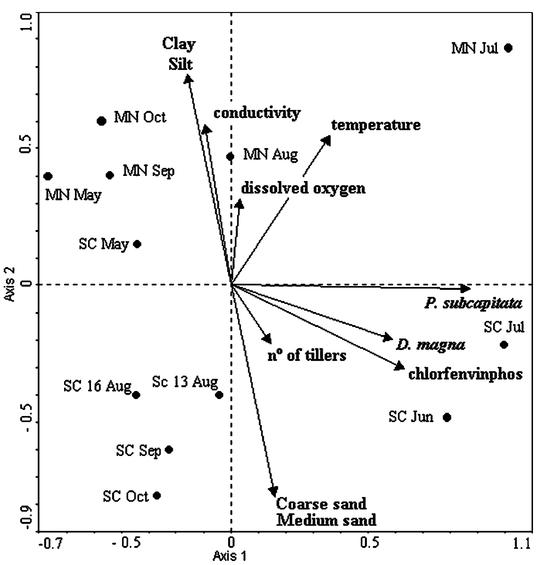

Fig. 6 RDA plot of macroinvertebrate assemblages and environmental parameters at both rice fields. MN—Monte Novo; SC—Santa Catarina; ➨ environmental parameters influence vectors

observed around the field near the levee (dry soil versus wet soil).

The two sampling methods (grab and hand net) proved to be necessary in the collecting of different organisms at the same location (substrate and water column) since differences were observed, especially when the densities were lower: August, September and October at Santa Catarina and during the entire rice cycle at Monte Novo. High diversity and abundance of *taxa* are associated with a high probability of observing mixed populations composed of: grazers, collectors, swimmers and crawlers in the same space. While at low abundances, invertebrates inhabit the most suitable place for their survival, inducing a high heterogeneity throughout the field.

Relationship between fauna and environmental variables

The high toxicity revealed by the cladoceran bioassay on water samples from Santa Catarina floodwater in June and July is related to the insecticide chlorfenvinphos application in May. However, macroinvertebrates did not seem to be affected by this insecticide since macroinvertebrate abundances were higher in these months. The abundant presence of the gastropoda *Physa acuta* and *Planorbis* cf. *planorbis* are responsible for this increase, which was also noticed by Suhling et al. (2000) in fields treated with insecticides. These freshwater snails are documented as not being affected by the insecticide applications. Thus, their increase may be due to the lack of competition for food and substrate with Chironomidae (Darby 1962; Simpson and Roger 1995; Suhling et al. 2000), the target organism of the insecticide chlorfenvinphos.

The accepted RDA model revealed July samples as being those with the strongest correlation to the ecotoxicological parameters (higher toxicity) and also the richest in organisms and taxa, as did samples taken in June. This contradiction is probably due to the lack of chemical data referring to the fertilizer application in July just before the sampling collection, although phosphates and ammonia reached the highest values in July and August, respectively, at both fields. Fertilizers seem to induce population growth (Roger 1989), minimizing the negative effect of the insecticide. According to Fillery et al. (1986) N-fertilizers decline rapidly in 6–8 days, which is a shorter period than that occurring in the application on the sampling (12–16 days). Similarly, these values may not be high enough to be considered as a main influence on macroinvertebrate distribution. This toxicity can also be explained by the results from June, which showed high toxic to the test organisms. In this situation, the residues present in the water were of a herbicide that was toxic to the algae and an insecticide that was toxic to the cladoceran. In July, however, the scenario is different, the algae being the most affected test organism. The residues present at that time in the floodwater were of the insecticide and since the cladoceran is the organism expected to be more affected by this product, the observed results were unexpected. The cladoceran *D. magna* reveals a low toxicity and is more related to the macroinvertebrates than the algae, from which we can assume that the effect on the macroinvertebrate community by the insecticide at that time was very low, hence the existence of the high organism abundances.

The insecticide based on the a.i. dimethoate (1.5 l c.p./ha) applied in August to control aphides on the Santa Catarina paddy caused no major effects on the test organisms and macroinvertebrate communities. The ecotoxicological analysis did not show any toxicity for the test organisms and, comparing both rice fields, the SC macroinvertebrate communities remained similar in terms of abundance pattern (Fig. 3) to those of Monte Novo, where the fungicide tryciclazole, classified as not dangerous for aquatic life by Tomlin (2000), was applied.

Conclusions

The results of this study suggest that the rice crop cycle is accompanied by a structural evolution of macrobenthos communities as well as by a variation in their spatial distribution throughout the paddies. It was observed that whenever the field was flooded after a dry period of several days a rapid colonization by collectors and grazers took place. It was also demonstrated that macroinvertebrate assemblages inhabiting the agriculture crops tend to be different in their richness and abundance according to the paddy sediment and water characteristics. The importance of using the right sampling device and method to study the macroinvertebrate communities of rice fields was also confirmed. This work demonstrates that the studied pesticides tended to be related to an increase in specific benthos populations, and that when N-fertilizers are applied, they cause a stimulatory effect on mixed populations as a result of the trophic succession.

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