

## ORIGINAL PAPER

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

### III. Dynamics of benthic molluscs

Received: 1 June 1993

**Abstract** We investigated the effects of N fertiliser and pesticide applications on the population dynamics of benthic molluscs in a tropical wetland rice field. Populations were monitored for two consecutive dry seasons in selected treatments during a study on the effects of agricultural practices on the floodwater ecology of tropical rice fields. The most abundant species recorded in the ricefields were the snails *Melanoides tuberculata* and *Melanoides granifera*. Population densities and biomass values in planted plots ranged between 0 and 1530 individuals  $m^{-2}$  and 0 and 1060  $kg\ ha^{-1}$ , respectively. Snails were more abundant in unplanted than planted plots (1991: 170–2040 versus 0–1040 individuals  $m^{-2}$ , respectively). Populations in planted plots declined as the crop season progressed. Snail populations were significantly reduced by the broadcast application of mineral N fertiliser at 110  $kg\ N\ ha^{-1}$ . There was little evidence that snails were affected by carbofuran or butachlor applications.

**Key words** Benthic molluscs · Snails · *Melanoides* spp. Wetland rice · Nitrogen fertiliser · Pesticide · Carbofuran · Butachlor

#### Introduction

Benthic molluscs, particularly gastropod snails, are widespread in the soil-floodwater ecosystem of wetland rice

fields. Population densities of up to 1000 individuals  $m^{-2}$  and 40–120 individuals  $m^{-2}$  have been observed in Philippine (Grant et al. 1986) and Indian (Sinha et al. 1986) rice fields, respectively. Despite the suitability of the habitat for snails, reports suggest that some species (*Cipangopaludina japonica*) have almost disappeared from rice fields as a result of modern agricultural practices (Kurihara and Kadowaki 1988). Interference with mollusc populations in rice fields could have important consequences for rice-growing communities because of their importance as nutrient cyclers, food items, rice pests, intermediate hosts of parasites, and soil decontaminators.

Internal nutrient recycling in the soil-floodwater ecosystem of rice fields is regarded as important for the maintenance of soil fertility. Aquatic snails consume detritus, algae, and associated bacteria (Kurihara and Kadowaki 1988) and therefore contribute to the mineralisation of significant nutrient reservoirs. However, their grazing activities on blue-green algae could reduce the potential N input by biological fixation.

Some snails may harm the rice crop by grazing on young seedlings and green manure crops such as *Azolla* sp. In particular, the 'golden apple snails' (an ill-defined group including *Pomacea insularis*, *P. canaliculata*, *P. viganis*, *Ampullaria gigas*, and *Pila leopoldvillensis*) are important rice pests in Surinam, Taiwan, Japan, and the Philippines. Farmers use some snails and clams as food (*Pila* spp., *Pomacea* spp., *Ampullaria* spp.), feed them to ducks, or may control them with chemicals or by hand-removal and mechanical destruction.

Certain species of snails (*Oncomelania* spp., *Bilinus* spp., and *Biomphalaria* spp., and *Limnea* spp.) commonly found in rice fields are intermediate hosts of trematodes causing human schistosomiasis. This has serious health implications for local communities where these parasites are endemic (Roger and Bhuiyan 1990).

The mud snail *Cipangopaludina chinensis malleata* has been shown to accumulate heavy metals introduced into Japanese rice fields in composted sewage sludge; decontamination of rice fields polluted with heavy metals

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ORSTOM Fonds Documentaire

N° 41.047 ex 1

Cote B

23 FEB. 1995

was achieved by harvesting the snails (Kurihara et al. 1987).

Despite the recognised importance of benthic molluscs in rice fields, little research has been conducted on the impact of agricultural practices on their population dynamics and species composition. Therefore the effects of N fertiliser, green manure, and pesticide applications on benthic molluscs were studied in the soil-floodwater ecosystem of a tropical rice field. Effects of these agricultural practices on the photosynthetic aquatic biomass, and microcrustaceans and dipteran larvae populations have been reported previously (Simpson et al. 1994a, b).

## Materials and methods

### Experimental design

Mollusc populations were studied during the dry seasons of 1990 and 1991 in selected treatments of a long-term experiment at the International Rice Research Institute (the Philippines). The experimental design consisted of five replicated randomly laid out blocks of 13 treatments (65 plots of 4×4 m), combining N-management practices and pesticide regimes. Treatments where molluscs were enumerated are summarised in Table 1. Broadcast N fertiliser was applied as prilled urea in two-thirds/one-third split applications. *Azolla* sp. was inoculated live at 0.4 kg biomass m<sup>-2</sup> 3 weeks before transplanting (1990) or imported at 2 kg biomass m<sup>-2</sup> (1991) and incorporated before the final soil preparation. Carbofuran (Furadan) was applied as granules and butachlor (Machete) was sprayed. Rice (IR 72) was transplanted at 25×25 cm spacing. Floodwater depth was maintained between 5 and 10 cm throughout the crop cycle. All planted and unplanted plots were fertilised with 30 kg P ha<sup>-1</sup> as superphosphate after transplanting.

### Enumeration of benthic mollusc populations

In 1990 mollusc populations were sampled -1, 20, 48, 76, and 99 days after rice transplanting in four treatments (Table 1). In 1991

**Table 1** Experimental treatments, 1990 and 1991 dry seasons. Prilled urea was broadcast 2/3 7 days after transplanting and 1/3 55 days after transplanting in 1990; 2/3 6 days after transplanting and 1/3 61 days after transplanting in 1991; carbofuran: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, applied once 54 days after transplanting or five times (13, 28, 42, 54, and 69 days after transplanting in 1990; 14, 28, 47, 57, and 70 days after transplanting in 1991); butachlor: N-butoxymethyl-2-chloro-2',6'-diethylacetanilide, applied 3 days after transplanting in 1990; 0 days after transplanting in 1991; a.i.: active ingredient/unplanted. The treatment was not sampled in the 1990 dry season

Fertiliser (1990 and 1991)	Carbofuran		No. of applications		Butachlor (kg a.i. ha <sup>-1</sup> )	
	kg a.i. ha <sup>-1</sup>				1990	1991
	1990	1991	1990	1991		
Zero	0.1	0	1	0	0	0
<i>Azolla</i> sp.	0.1	0	1	0	0	0
110 kg N ha <sup>-1</sup> split	0.1	0	1	0	0	0
110 kg N ha <sup>-1</sup> split	0.5	0.5	5	5	0.35	0.35
Unplanted	0	0	0	0	0	0

the samples were collected in five treatments (Table 1) -3, 18, 39, 60, and 81 days after transplanting.

The method for sampling the benthic molluscs was adapted from Grant et al. (1985). Three samples were collected from each plot along an L-shaped transect. At each sampling point a 20×20 cm metal quadrat was inserted between the rice hills. The surface 2-3 cm of soil was removed and placed in a plastic bag. Replicate samples from each plot were combined for processing. The soil collected was washed with water through stacked sieves (mesh sizes 1 and 2 mm) and the material retained on each sieve was backwashed into sorting trays, from where the molluscs were collected by hand.

The total number and wet weight (including shells) of molluscs retained on each sieve were recorded. Specimens were preserved in 70% alcohol, stored, and identified. The shell lengths of all individuals were recorded.

To minimise crop damage, *P. canaliculata* snails were removed manually from the plots at regular intervals; they were not counted.

### Data analysis

To investigate the effects of treatments on populations, all snails except *P. canaliculata* were considered collectively as the snail population. Linear regression analysis was performed on the logarithms of means and variance for the 1990 and 1991 total population densities and biomass. The slopes of the regression equations for population densities (1990 = 1.70, 1991 = 1.80) were sufficiently close to 2 to use logarithmic transformations for statistical analysis (Roger et al. 1991). Biomass data was transformed by  $y = x^{(1-b/2)}$  where  $b$  is the slope of the regression curve (1990 = 1.40, 1991 = 1.28). No significant differences were found amongst snail population densities or biomass in the replicated blocks, and therefore data were analysed by one-way analysis of variance using treatment as the discriminatory factor. Differences amongst individual treatments were identified using least significant difference multiple-range tests ( $P = 0.05$ ).

## Results

The dominant benthic molluscan species recorded during this investigation were the gastropod snails *Melanoides tuberculata* and *Melanoides granifera*. They were found in all treatments, during both seasons, at virtually all sampling times. In 1990 the clam *Corbicula manilensis* was only observed in one zero-N control plot, where it was present at all sampling times. Populations of *Corbicula manilensis* were more widespread in 1991, but with the exception of a few isolated individuals, they were found exclusively in control and unplanted plots. *Stenomelania fuscata* occurred throughout both crop cycles, mainly in control plots. *Lymnaea quadrasi* appeared at low population densities throughout both seasons and in all treatments. *P. canaliculata* occurred in all treatments throughout both crop seasons. However, it was inappropriate to estimate population densities of *P. canaliculata* from the samples collected, as they were removed as part of the experimental management. Other molluscan species only occurred as isolated individuals. Maximum population densities recorded for each mollusc species are summarised in Table 2.

Snail population densities and wet-weight biomass estimates recorded in 1990 and 1991 are summarised in

**Table 2** Maximum population densities (no. individuals  $m^{-2}$ ) of molluscan species recorded in experimental rice fields during the 1990 and 1991 dry seasons (*ND* not determined)

Species	1990	1991
<i>Melanoides</i> spp.	1530	2030
<i>Melanoides tuberculata</i>	ND	880
<i>Melanoides granifera</i>	ND	1630
<i>Pomacea canaliculata</i>	ND	ND
<i>Lymnaea quadrasi</i>	140	110
<i>Stenomelania fuscata</i>	60	250
<i>Gyraulus</i> sp.	10	10
<i>Hippeutis canfori</i>	10	0
<i>Corbicula manilensis</i>	160	230

**Table 3** Summary of snail population densities (no. individuals  $m^{-2}$ ) for individual plots in each treatment, in the 1990 and 1991 dry seasons (*Min.* minimum, *Max.* maximum, *ND* not determined)

	Dry season 1990			Dry season 1991		
	Min.	Mean	Max.	Min.	Mean	Max.
0 kg N $ha^{-1}$	20	370	1530	0	280	1040
110 kg N $ha^{-1}$	0	80	530	0	40	120
110 kg N $ha^{-1}$ + pesticide <sup>a</sup>	20	170	630	0	70	280
<i>Azolla</i> sp.	30	260	620	80	230	910
Unplanted	ND	ND	ND	170	550	2040

<sup>a</sup> Carbofuran and butachlor

**Table 4** Summary of snail population biomass (kg  $ha^{-1}$ ) for individual plots in each treatment, in the 1990 and 1991 dry seasons (see notes to Table 3)

	Dry season 1990			Dry season 1991		
	Min.	Mean	Max.	Min.	Mean	Max.
0 kg N $ha^{-1}$	50	250	1060	0	190	590
110 kg N $ha^{-1}$	0	90	380	0	60	470
110 kg N $ha^{-1}$ + pesticide <sup>a</sup>	0	200	580	0	80	310
<i>Azolla</i> sp.	30	200	450	10	200	440
Unplanted	ND	ND	ND	90	300	1080

Tables 3 and 4, respectively. The values were derived using data from all plots and all sampling dates. Population densities and biomass values in the planted plots ranged from 0 to 1530 individuals  $m^{-2}$  and 0 to 1060 kg  $ha^{-1}$  in 1990, and from 0 to 1040 individuals  $m^{-2}$  and 0 to 590 kg  $ha^{-1}$  in 1991, respectively. In unplanted plots in 1991 the corresponding ranges were 170–2040 individuals  $m^{-2}$  and 90–1080 kg  $ha^{-1}$ .

Dynamics of population densities and fresh-weight biomass estimates for the 1990 and 1991 experiments are presented in Figs. 1 and 2. In 1990 total snail population densities tended to decline as the crop season progressed. The main exception to the trend was the increase in population density in the zero-N control during the first 20 days after transplanting (Fig. 1 a–c). Generally, popula-

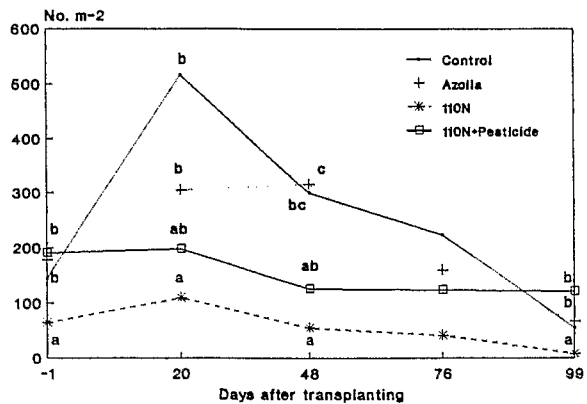
tions of  $\geq 2$  mm snails were significantly lower in plots fertilised with 110 kg N  $ha^{-1}$ , particularly during the middle of the crop season. Densities in *Azolla* sp. and control plots were not significantly different at any time during the crop cycle (Fig. 1 a). Differences amongst  $< 2$  mm snail populations were only found between the control and other treatments 20 and 76 days after transplanting, when they were significantly greater in the control (Fig. 1 b). There was little evidence of carbofuran or butachlor impacts on the snail populations (Fig. 1 a–c).

Trends in population dynamics in 1991 (Fig. 2 a–c) were similar to those observed in the four treatments sampled in 1990. The most significant treatment effect was again the suppression of  $\geq 2$  mm snail populations in treatments where N fertiliser was applied, relative to the control (Fig. 2 a). There was no evidence that carbofuran or butachlor applications affected snail densities. Initial densities (3 days before transplanting) of  $< 2$  mm snails were considerably higher in 1991 than in 1990 (Fig. 2 b). However, they declined rapidly and by 18 days after transplanting were similar to the 1990 densities. The population dynamics of  $\geq 2$  mm snails in the unplanted plots were similar to those in control plots, except towards the end of the crop season when significantly greater populations developed (Fig. 2 a). Relative to other treatments,  $< 2$  mm snails reached greater population densities throughout the crop season in the unplanted plots; however, they were not significantly higher than the 0 kg N  $ha^{-1}$  treatment until 81 days after transplanting.

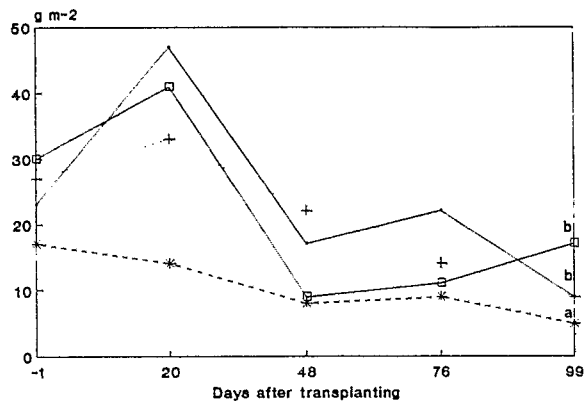
Expression of snail abundance as biomass showed similar trends to population density in 1990 (Fig. 1 d–f). The main exception was the reversal of the decline in the total population in the 110 kg N  $ha^{-1}$  + carbofuran (2.5 kg active ingredient  $ha^{-1}$ ) + butachlor (0.35 kg a.i.  $ha^{-1}$ ) treatment between –1 and 20 days after transplanting (Fig. 1 c, f). The decline in density was largely due to the population crash of  $< 2$  mm snails (Fig. 1 b). In the *Azolla* sp. and 110 kg N  $ha^{-1}$  + carbofuran (0.1 kg a.i.  $ha^{-1}$ ) treatments, population densities of  $< 2$  mm snails declined, but the biomass increased. The only treatment where the biomass of  $< 2$  mm snails declined was where carbofuran and butachlor were applied. In 1991 the main difference between densities and biomass was the increase in the biomass of  $< 2$  mm snails 60 days after transplanting (Fig. 2 d–f). In both years biomass estimates of  $\geq 2$  mm snails and totals were almost identical (Figs. 1 d, 2 d) because  $\geq 2$  mm individuals contributed little to the total biomass. Interestingly, although the trends observed for population densities and biomass were similar, differences amongst treatments were usually not significant for biomass.

Differences amongst abundance trends, expressed as biomass and density, indicate that the size structure of the population must have changed. To investigate these changes, length-frequency histograms were constructed for the dominant species, *M. tuberculata* and *M. granifera*. They were constructed for the genus using the 1990 data and for individual species using the 1991 data (Figs. 3–5).

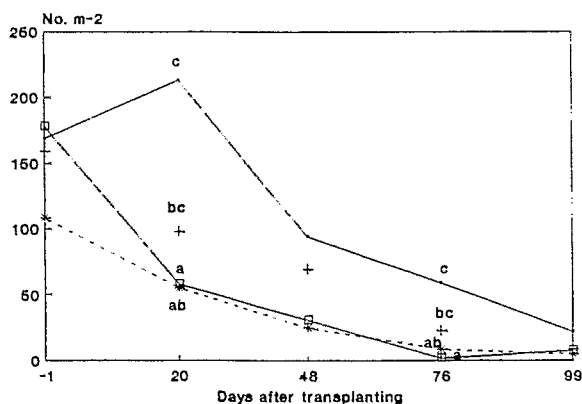
a Snail population densities 2mm or >.



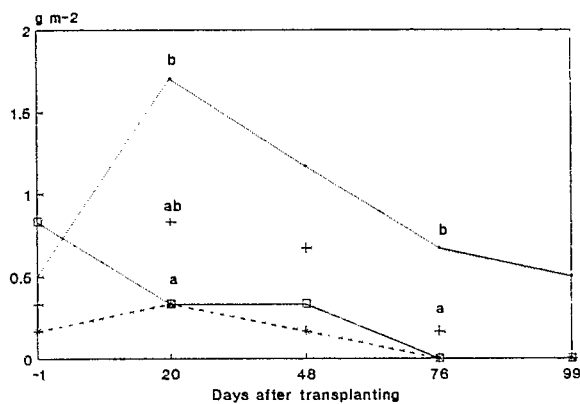
d Snail biomass 2mm or >.



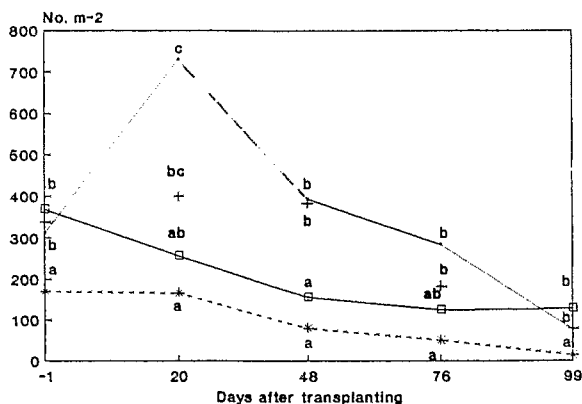
b Snail population densities < 2mm.



e Snail biomass < 2mm.



c Total snail population densities.



f Total snail biomass.

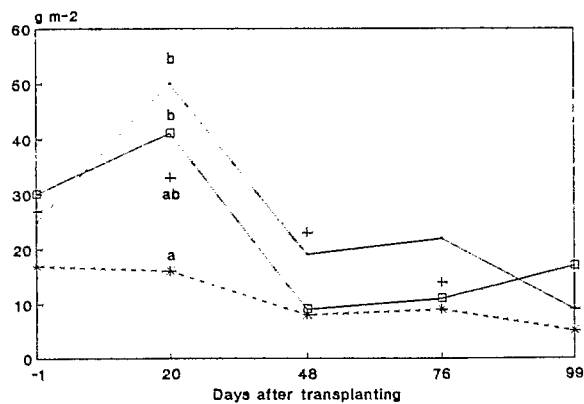
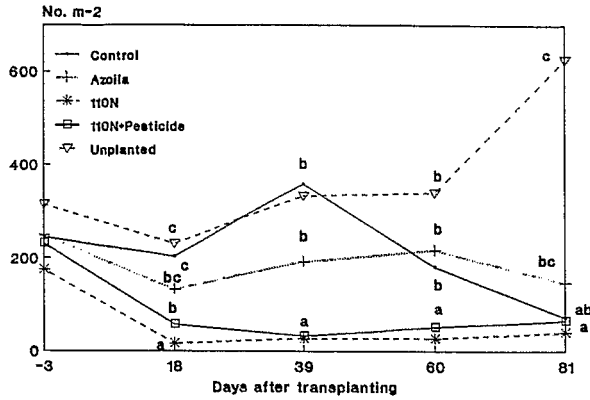


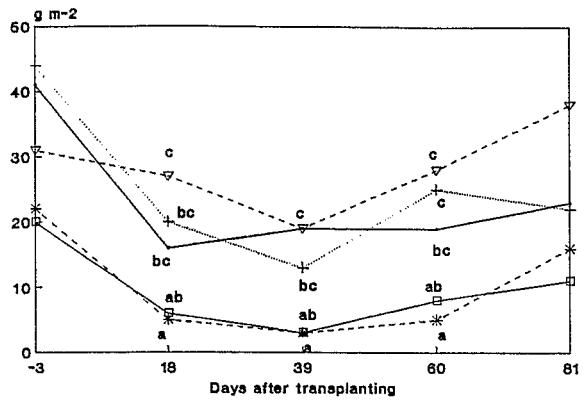
Fig. 1a-f Effects of various agricultural practices on population densities and biomass of different length classes ( $\geq 2$  mm,  $< 2$  mm) of benthic snail populations in a wetland rice field, dry season 1990

(110N broadcast urea at  $110 \text{ kg N ha}^{-1}$ , significant differences amongst treatments calculated using transformed data indicated by letters)

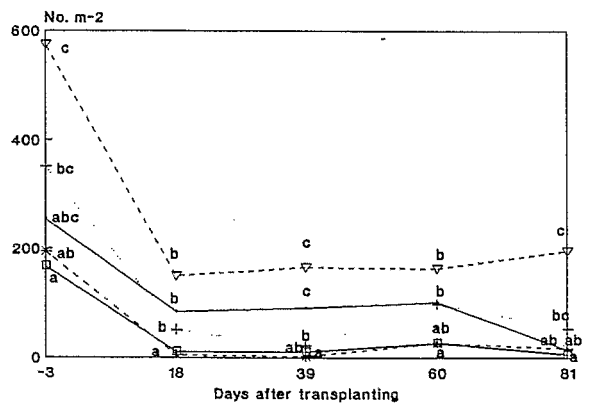
a Snail population densities 2mm or >.



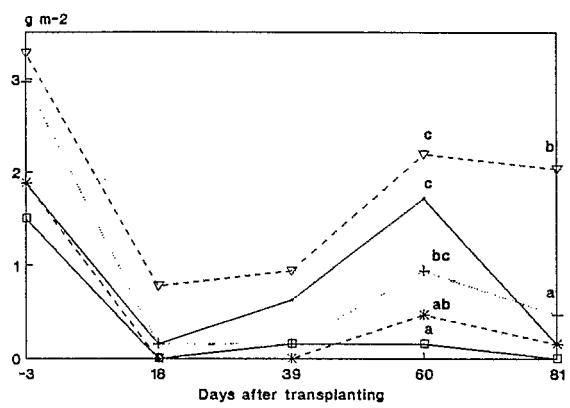
d Snail biomass 2mm or >.



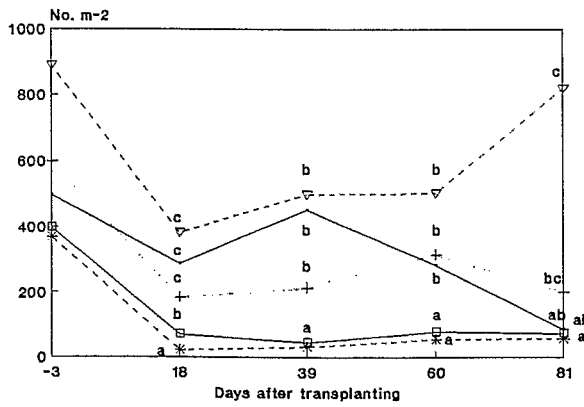
b Snail population densities < 2mm.



e Snail biomass < 2mm.



c Total snail population densities.



f Total snail biomass.

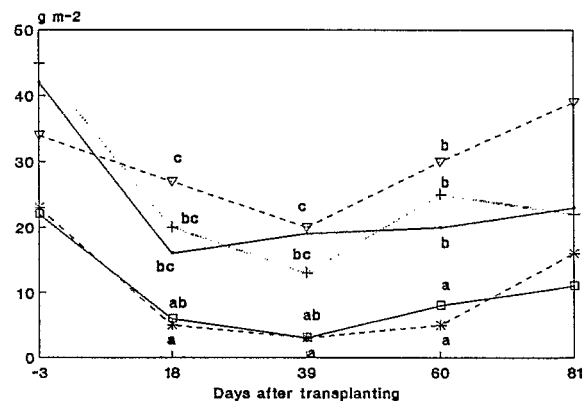
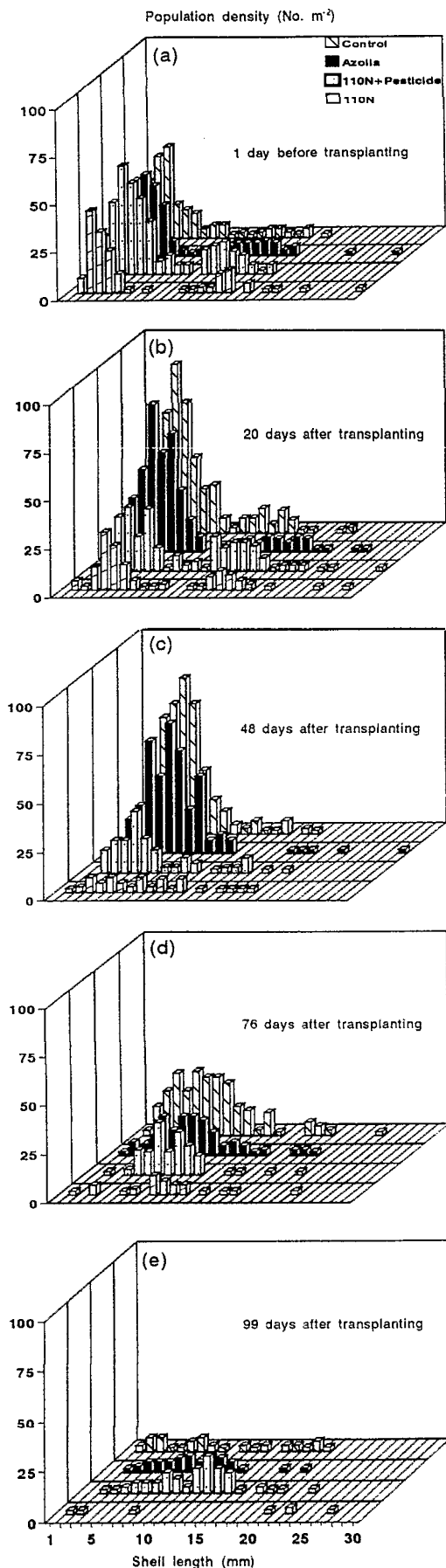


Fig. 2a-f Effects of various agricultural practices on population densities and biomass of different length classes ( $\geq 2$  mm,  $< 2$  mm)

of benthic snail populations in a wetland rice field, dry season 1991 (see Fig. 1 for other explanations)



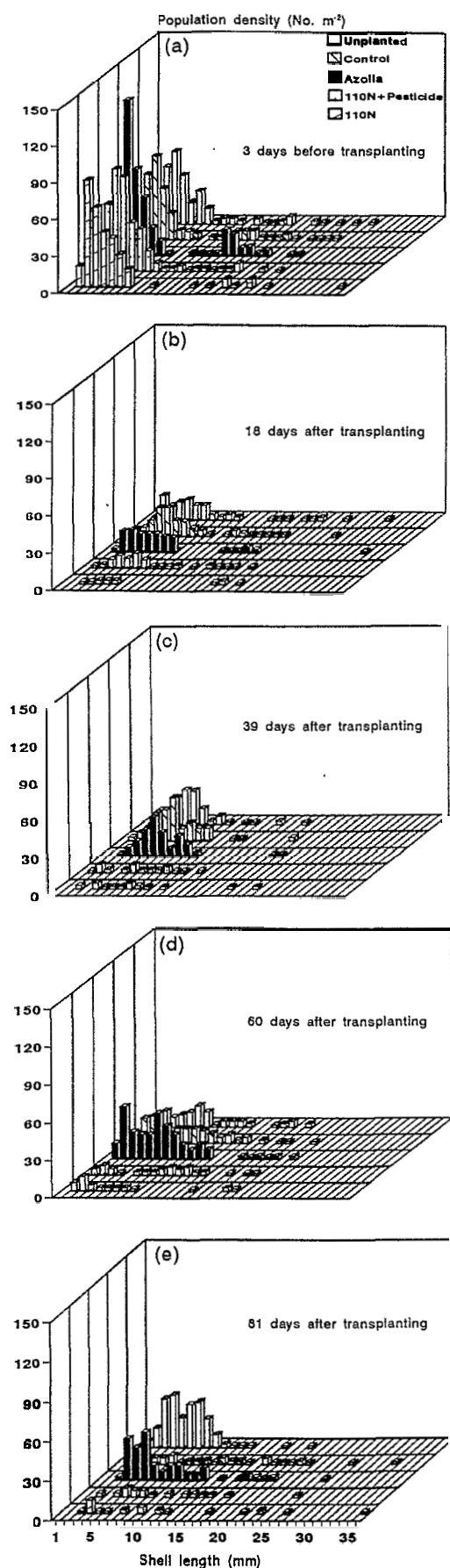
One day before transplanting in 1990 (Fig. 3a) the length-frequency distribution of *Melanoides* spp. was similar in all treatments. The most abundant size class was 3–4 mm and there was some evidence of a second peak at 13–18 mm. Larger individuals were rare. By 20 days after transplanting (Fig. 3b) the modal size class of the smaller snails had increased to 5 mm in all treatments. Populations in 110 kg N ha<sup>-1</sup> treatments were depleted in the smaller size classes and continued to decline thereafter. In the control and *Azolla* sp. treatments, population densities of smaller *Melanoides* spp. snails had increased markedly by 20 days after transplanting (Fig. 3b). As the crop season progressed, modal size classes in the control and *Azolla* sp. treatments continued to increase. Population densities, in these two treatments, remained stable until 48 days after transplanting, then declined to low levels (Fig. 3c–e). Length-frequency distributions of populations in all treatments were similar by 99 days after transplanting.

The dynamics of length-frequency distribution for *Melanoides* spp. in 1990 probably concealed differences which were revealed between species in 1991 (Figs. 4, 5). Three days before transplanting, length-frequency distributions for the *M. tuberculata* populations were similar amongst treatments (Fig. 4a). The most abundant size class was 3–7 mm. Larger individuals were rare except in the *Azolla* sp. treatment where several individuals were in the 15–16 mm size class. Conversely, *M. granifera* populations exhibited strong differences amongst treatments (Fig. 5a). In unplanted plots, two size classes were present (2–5 mm and 11–18 mm) and they were more abundant than in other treatments. Populations were similar to each other in zero-N control and *Azolla* sp. plots, but were very low in plots which were to receive, and had in previous seasons received, broadcast urea fertiliser. Most of the individuals recorded across treatments were 2–4 mm in size. Larger individuals were rare or absent in the broadcast urea plots.

As the crop season progressed *M. tuberculata* declined in all treatments, particularly in the plots fertilised with 110 kg N ha<sup>-1</sup> (Fig. 4a–c). By 60 days after transplanting, the population of small *M. tuberculata* in the *Azolla* sp. treatment had increased, but it declined again towards the end of the crop cycle (Fig. 4a–e). Populations in unplanted plots showed signs of resurgence in the later part of the crop season. The modal size class in populations where small snails were abundant increased from 2–4 mm 3 days before transplanting to 6–7 mm 39 days after transplanting; they then became more evenly distributed amongst size classes. The largest *M. tuberculata* recorded in 1991 was 35 mm long, although individuals over 25 mm were rare.

Populations of *M. granifera* declined in all planted treatments as the crop cycle progressed. In the high-N fer-

Fig. 3a–e Effects of various agricultural practices on length–frequency distributions of *Melanoides* spp. in a wetland rice field, dry season 1990 (110N broadcast urea at 110 kg N ha<sup>-1</sup>)



tiliser plots they were virtually absent after transplanting (Fig. 5a–e). Populations in the control plots showed signs of recovery by 39 days after transplanting, but subsequently declined again. Towards the end of the crop season *M. granifera* was virtually absent in all treatments except the unplanted plots, where they were abundant. Modal size classes increased from 2–3 mm 3 days before transplanting to 3–6 mm 39 days after transplanting, after which they were only significantly represented in the unplanted plots, where the modal size class was 4–8 mm 81 days after transplanting. The largest *M. granifera* recorded in 1991 was 24 mm long, but individuals over 15 mm were rare beyond 18 days after transplanting.

## Discussion

The present study revealed snail density and biomass ranges of 0–2040 individuals  $m^{-2}$  and 0–1080  $kg\ ha^{-1}$ , respectively, compared with previous estimates for the International Rice Research Institute farm of 1000 individuals  $m^{-2}$  (Grant et al. 1986) and 1500  $kg\ ha^{-1}$  (International Rice Research Institute 1981). The numerically dominant genus in the previous work was *Lymnaea*, which was recorded in relatively low numbers during the present study. The sampling methodology in the current study was perhaps biased against *Lymnaea*, in favour of *Melanoides* (Grant et al. 1985). Heckman (1979) reported the presence of *M. tuberculata*, *Limnaeidae* sp., *Gyraulus* sp., and *Hippeutis* sp. in a rice field in Thailand.

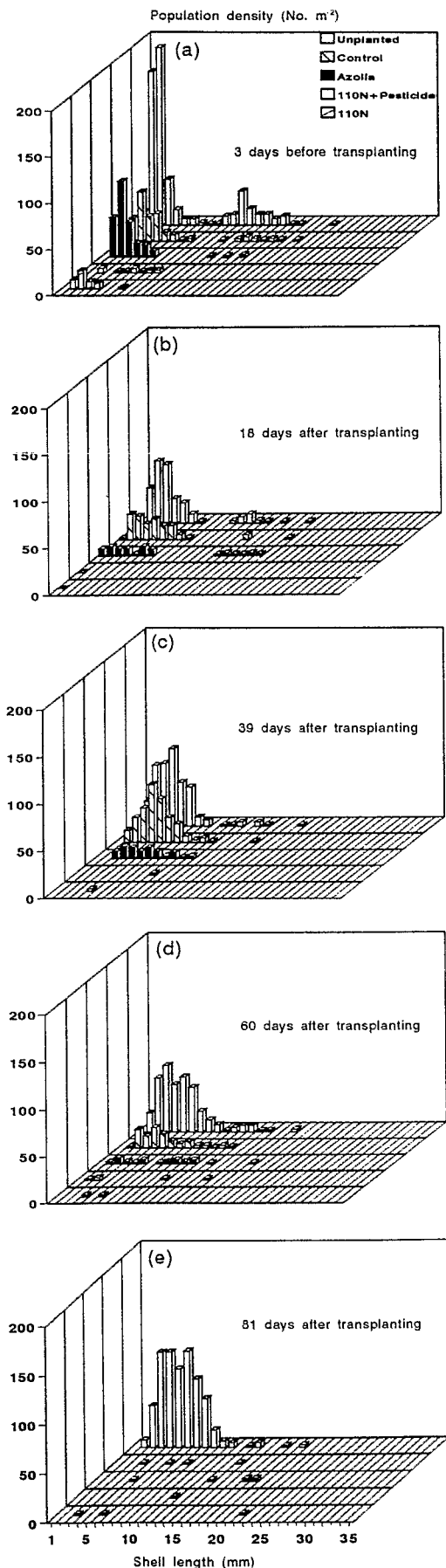
The most significant effects of agricultural practices on the snail populations were the negative impacts of broadcast urea fertiliser and the presence of rice plants. These impacts were probably indirect, mediated through (1) food availability, (2) interspecific competition, (3) changes in floodwater chemistry and/or (4) changes in soil compaction.

First, a broadcast application of N fertiliser increases floodwater primary productivity, but shifts the balance between  $N_2$ -fixing and non- $N_2$ -fixing algae in favour of the latter (Simpson et al. 1994a). This change in the composition of the floodwater microflora could affect snail populations. *Oncomelania quadrasi* abundance was correlated positively with the presence of green algae, but negatively with blue-green algae (Webbe 1988).

Second, populations of ostracods and dipteran larvae proliferated in plots where N fertiliser was broadcast (Simpson 1994b). Interspecific competition with these organisms could account for the decline in benthic snail populations.

Third, many changes in floodwater chemistry after the broadcast application of N fertiliser are mediated through the photosynthetic aquatic biomass. High levels

Fig. 4a–e Effects of various agricultural practices on length–frequency distributions of *Melanoides tuberculata* in a wetland rice field, dry season 1991 (110N broadcast urea at 110  $kg\ N\ ha^{-1}$ )



of photosynthetic activity reduce the dissolved  $\text{CO}_2$  concentration in the floodwater, which causes an increase in pH (Mikkelsen et al. 1978). When primary productivity is high, the plant respiratory  $\text{O}_2$  demand is correspondingly high, which could create anoxic conditions in the floodwater at night (Saito and Watanabe 1978). Snails could be sensitive to these changes in their habitat. In particular, *Melanoides* spp. respire using gills and are therefore dependent on floodwater dissolved  $\text{O}_2$ .

Finally, the root systems of rice plants compact the soil. Perhaps snails prefer soil without rice plants because it is looser and easier to penetrate (Kurihara 1989).

Kurihara and Kadowaki (1988) reported that the mud snail *Cipangopaludina japonica* was attracted to rice soils by a water-soluble substance, but that its productivity was four to five times higher in unplanted than planted plots; no explanations were provided. Webbe (1988) reported that snails thrive in habitats devoid of higher plants and rich with microflora and/or decaying plant material. The decline of populations in planted plots, as the crop season progressed, could be attributed to a reduction in the availability of suitable food as the floodwater microflora production declined (Simpson et al. 1994a).

There was little evidence of pesticide impacts on the benthic snail populations. However, pesticide comparisons were only made in the presence of broadcast urea, which has itself been shown to adversely affect populations. If comparisons were made between pesticide levels in the absence of broadcast urea differences might be found.

Carbofuran has been shown to be ineffective as a molluscicide even at  $100 \text{ mg a.i. l}^{-1}$ . However, other pesticides, notably thiodicarb, may be highly effective at concentration as low as  $1 \text{ mg a.i. l}^{-1}$  (Sinha et al. 1986). Gastropod populations showed no signs of mortality after endosulphan application in Indonesian rice fields (Gorbach et al. 1971) and were reported to be abundant after insecticide applications in Philippine rice fields (International Rice Research Institute 1986). In India, Roger et al. (1985) observed that molluscs (*Limnea* and *Vivipara* spp.) were abundant in benzene hexachloride treated plots. Ishibashi and Itoh (1981) observed large snail populations in fields treated with the herbicide benthocarb. Kurihara and Kadowaki (1988) reported that the application of a cocktail of herbicides had little effect on the reproduction and growth of the snail *Cipangopaludina japonica*.

There was evidence in the present study that some treatment impacts on snail populations were carried over between crop seasons. *M. granifera* populations were significantly less dense before transplanting in 1991 in the broadcast N plots, even before the treatments were applied. This shows that earlier treatment can exert an influence beyond the time-frame of the crop season and can still be important even after fallow conditions.

Fig. 5a-e Effects of various agricultural practices on length-frequency distributions of *Melanoides granifera* in a wetland rice field, dry season 1991 (110N broadcast urea at  $110 \text{ kg N ha}^{-1}$ )



Intensification of the irrigated rice agroecosystem could inadvertently alleviate problems associated with indigenous snail populations in rice fields. Application of pesticides with molluscicidal properties, increased use of N fertiliser, and growth of rice varieties which develop dense canopies could all reduce population densities. Adoption of these agromanagement strategies, where appropriate, could be used to increase N<sub>2</sub>-fixation by blue-green algae and control schistosomiasis, by reducing grazing and intermediate hosts. However, the benefits of reducing snail densities must be considered against the disadvantages such as their diminished role in nutrient recycling and the scarcity of a valued food item.

**Acknowledgements** This research was conducted under a scientific agreement between IRRI (Philippines), NRI (United Kingdom), and ORSTOM (France), and is incorporated in the Ph.D. thesis of I.C. Simpson, University of Wales College of Cardiff.

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