

1 Hiroshi C. Ito, Hiroaki Shiraishi, Megumi Nakagawa, and Noriko Takamura. Combined impact  
2 of pesticides and other environmental stressors on animal diversity in irrigation ponds.

### 4 **S1 Appendix: Measurement of physicochemical properties**

#### 5 *S1.1 Pesticides*

6 Among the 31 pesticides analyzed in this study, which were selected by considering spray  
7 timing and type of pesticide, 28 were analyzed by high-performance liquid chromatography–  
8 tandem mass spectrometry (HPLC-MS/MS) and 3 were detected with gas chromatography–  
9 mass spectrometry (GC-MS) (Table S2-1 in supplemental material).

10 Water samples were directly collected at 20-cm depth in 1-gallon amber-colored glass  
11 bottles that had been rinsed with the same water from the reservoirs. The collected samples were  
12 transported to the laboratory under cool dark conditions and then filtered through a glass fiber  
13 filter (GF/F, Whatman). The filtrate was refrigerated at 4 °C and extracted as soon as possible.

#### 15 *HPLC-MS/MS*

16 Pesticides were extracted by passing 200 mL of water through a solid-phase extraction column  
17 (BondElutC18, Varian) preconditioned with 8 mL of acetone and 5 mL of water. The column  
18 was washed with 5 mL of pure water (Milli-Q), and then the pesticide was eluted with 8 mL of  
19 acetone. The extract was concentrated under a gentle nitrogen stream to just dry, then dissolved  
20 in 500 µL methanol, to which we added 500 µL of pure water and mixed well. Pesticides were  
21 analyzed by HPLC-MS/MS operated in the electrospray ionization and selected reaction  
22 monitoring (SRM) mode, and 5 µL of the solution was injected into the HPLC-MS/MS. The  
23 calibration curve was constructed with the peak area of SRM ions. The recovery rate of each  
24 pesticide exceeded 80%, and the quantitative value was not corrected by the recovery rate. Only  
25 E-isomer of pyriminobac-methyl was quantified.

26 The mass spectrometer used was a Quattro Ultima (Micromass), HPLC was an Agilent 1100

1 (Agilent Technologies), the column was an Atlantis T3 (3  $\mu\text{m}$ , 2.1 mm i.d.  $\times$  150 mm, Waters),  
2 mobile phase A was 5 mM ammonium acetate/water, and mobile phase B was 5 mM ammonium  
3 acetate/methanol. Mobile phase B was increased from 15% to 95% over 17.5 min and then held  
4 for 12.5 min. The flow rate was 0.2 mL  $\text{min}^{-1}$ .

5 Conditions of LC-MS/MS are shown in Table S2-2 in the supplemental material (Pizzutti et  
6 al. 2007).

7

#### 8 *GC-MS*

9 Pesticides were extracted by passing 500 mL of water through a solid-phase extraction column  
10 (BondElutC18, Varian) preconditioned with 8 mL of acetone and 5 mL of water. The column  
11 was washed with 5 mL of pure water and then the pesticide was eluted with 8 mL of acetone.  
12 The extract was dried under a nitrogen stream, dissolved in 250  $\mu\text{L}$  of acetone, and then mixed  
13 well with 250  $\mu\text{L}$  hexane solution of internal standard (4-chlorotoluene- $\text{d}_4$ , 1,4-dichlorobenzene-  
14  $\text{d}_4$ , naphthalene- $\text{d}_8$ , phenanthrene- $\text{d}_{10}$ , fluoranthene- $\text{d}_{10}$ , acenaphthene- $\text{d}_{10}$ , chrysene- $\text{d}_{12}$ , pellerin-  
15  $\text{d}_{12}$ , 1 ppm). We injected 1  $\mu\text{L}$  of the solution into the GC-MS/MS, operated in the electron  
16 ionization and SRM mode. The concentration of each pesticide was calculated by the internal  
17 standard method. The recovery of each pesticide exceeded 80%. The quantitative value was not  
18 corrected by the recovery rate.

19 The mass spectrometer used was an Agilent 5973N (Agilent Technologies), and GC was an  
20 Agilent 6890 (Agilent Technologies) equipped with a splitless injector. The ion source  
21 temperature was 230  $^{\circ}\text{C}$ , the inlet temperature was 250  $^{\circ}\text{C}$ , and the interface temperature was  
22 280  $^{\circ}\text{C}$ . A fused silica capillary column DB-5 MS (30 m  $\times$  0.25 mm i.d., film thickness 0.25  $\mu\text{m}$ ,  
23 Agilent) was used for separation. After injection, the initial column oven temperature of 80  $^{\circ}\text{C}$   
24 was kept for 1 min, then raised at a rate of 10  $^{\circ}\text{C}/\text{min}$  to 280  $^{\circ}\text{C}$ , and held for 10 min. Helium  
25 was used as a carrier gas at the flow rate of 1 mL  $\text{min}^{-1}$ .

26 Table S2-3 in the supplemental material gives the conditions of GS-MS (Uno et al. 2001).

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26

*References*

Pizzutti, I. R., A. de Kok, R. Zanella, M. B. Adaime, M. Hiemstra, C. Wickert, and O. D. Prestes. 2007. Method validation for the analysis of 169 pesticides in soya grain, without cleanup, by liquid chromatography-tandem mass spectrometry using positive and negative electrospray ionization. *Journal of Chromatography A* 1142:123–136.

Uno, S., H. Shiraishi, S. Hatakeyama, A. Otsuki, and J. Koyama. 2001. Accumulative characteristics of pesticide residues in organs of bivalves (*Anodonta woodiana* and *Corbicula leana*) under natural conditions. *Archives of Environmental Contamination and Toxicology* 40(1):35–47.

*SI.2 Physicochemical properties*

Monitoring sites were at the inlet of a waterway entering the irrigation pond. The water temperature and pH were measured just above the bottom sediment using a multi-water quality monitoring system (U-21 DX, Horiba). Pond water at 20-cm depth was directly sampled into 2-L polypropylene bottles and 100-mL vials, and transported to the laboratory under cool dark conditions.

*Analysis of water quality*

The concentration of chlorophyll *a* (Chl-*a*) was determined from the absorbance of the methanol extract of suspended solids (SS) at 665 nm, measured with a spectrophotometer (U-2800A, Hitachi) (Marker et al. 1980). The SS concentration was obtained by weighing SS on a filter (GF/F, Whatman).

Ammonia nitrogen (NH<sub>4</sub>-N) was determined by the indophenol method. Nitrite nitrogen (NO<sub>2</sub>-N) and nitrate nitrogen (NO<sub>3</sub>-N) were determined by the naphthylethylene diamine method (APHA 1998) using an AutoAnalyzer (AACS II, Bran + Luebbe), where NO<sub>3</sub><sup>-</sup> is

1 reduced on a cadmium column to  $\text{NO}_2^-$ . The total nitrogen (TN) was measured after alkaline  
2 potassium peroxodisulfate oxidation and conversion of resulting  $\text{NO}_3^-$  to  $\text{NO}_2^-$ . The soluble  
3 reactive phosphorus concentration was measured according to the molybdenum blue method  
4 using an AutoAnalyzer (APHA 1998). Total phosphorus (TP) was determined as soluble  
5 reactive phosphorus after acidic peroxodisulfate oxidation.

6 For measuring ignition loss, the mud was freeze-dried and then mixed well by using a  
7 medicine spoon. Five grams of the mud was then put in the oven for the measurement of weight  
8 loss on ignition at  $550^\circ\text{C}$  for 1 h. After cooling to room temperature, the sample was weighed  
9 again to calculate the loss.

10 On 13–15 May 2007, pond depth was measured with a ruler with a spindle at the deepest  
11 point of each pond. Pond area and concrete bank rate were measured from geo-referenced  
12 1:2500 geographic maps on ArcGIS 9.1. Percent coverage of floating-leaved plants and of  
13 emergent plants in each pond surface area was measured on aerial photographs taken in August  
14 2006. Intensities of pond drainage were classified into three categories (0: no drainage, 1: partial  
15 drainage, 2: full drainage) based on interviews with pond owners. Densities of invasive alien  
16 species were classified into two categories (0: low, 1: high) based on whether the sampling of  
17 freshwater animals found those species or not.

18

### 19 *References*

20 APHA. 1998. Standard Methods for the Examination of Water and Wastewater, 20th edn.

21 American Public Health Association, Washington.

22 Marker, A. F. H., E. A. Nusch, H. Rai, and B. Riemann. 1980. The measurement of

23 Photosynthetic pigments in freshwaters and standardization of methods: conclusions and  
24 recommendations. *Advances in Limnology*, 14: 91–106.

25