Electronic Supplementary Information

Biospectroscopy reveals the effect of varying water quality on tadpole tissues of the Common Frog (*Rana temporaria*)

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Table S1. Results from two-sample *t*-tests of the scores generated from principal component analysis for the first ten principal components following ATR-FTIR spectrochemical analysis of several tissues of *Rana temporaria* tadpoles. Significant results are highlighted in bold.

PC	Liver *P*-value		Muscle *P*-value		Heart *P*-value		Kidney *P*-value		Skin *P*-value	
	CT *vs.* PF	CT *vs.* WH	CT *vs.* PF	CT *vs.* WH	CT *vs.* PF	CT *vs.* WH	CT *vs.* PF	CT *vs.* WH	CT *vs.* PF	CT *vs.* WH
1	**< 0.01**	**< 0.01**	0.25	**< 0.01**	0.20	0.10	0.18	0.17	0.62	0.51
2	0.89	0.38	0.31	0.33	0.15	0.25	0.65	0.36	0.49	0.25
3	0.20	0.61	0.86	0.55	0.58	0.06	0.20	0.15	0.21	**0.03**
4	0.95	**0.04**	0.29	0.69	0.81	0.59	0.98	0.64	0.40	0.26
5	0.51	0.08	0.19	0.19	0.22	0.15	0.18	0.99	0.57	0.18
6	0.77	0.58	0.32	0.71	0.82	0.70	0.66	0.28	0.08	0.36
7	0.61	0.87	0.19	0.22	0.95	0.95	0.21	**0.02**	0.70	0.71
8	1.00	0.26	0.94	0.87	0.95	0.75	0.39	0.23	0.17	0.12
9	0.83	0.63	0.56	0.93	0.51	0.18	0.58	0.21	0.78	0.76
10	0.87	0.50	0.50	0.61	0.70	0.78	0.50	0.89	0.41	0.57

Table S2. Number of principal components (PCs) retained for input into a cross-validated linear discriminant analysis (LDA) model for optimum discrimination and classification. The Pareto tool function in Matlab was used to determine the number of PCs that represented 95% of the variance in each data set.

Tissue	Number of PCs	
	CT *vs.* PF 2012	CT *vs.* WH 2013
Heart	14	17
Kidney	9	16
Liver	6	11
Muscle	11	16
Skin	11	18

Table S3. Distinguishing wavenumbers and proposed assignments obtained from analysis of several organs of pro-metamorphic *Rana temporaria* tadpoles with ATR-FTIR spectroscopy following analysis with principal component analysis (PCA). The five largest loadings values for the most discriminating principal components are shown. Comparisons were made between tadpoles from CT: a rural agricultural pond with no pesticide input and PF: an urban pond impacted by wastewater and landfill run-off in 2012 and between tadpoles from CT and those from WH: an agricultural pond known to be impacted by pesticides in 2013. Sources: (Abdel-Gawad et al., 2012; Cakmak et al., 2006; Cakmak et al., 2003; Greve et al., 2008; Movasaghi et al., 2008; Palaniappan and Vijayasundaram, 2008, 2009; Palaniappan et al., 2011; Purna Sai and Babu, 2001; Toyran et al., 2006).

Site Comparison	Organ	Wavenumber (cm^{-1})	Proposed Assignment
CT *vs.* PF 2012	Liver	991	C-O ribose
PC1		1153	CO-O-C asymmetric stretching: glycogen and nucleic acids
		1516	Amide II
		1601	C=N cytosine
		1624	Amide I β-sheets
CT *vs.* WH 2013	Liver	968	C-O deoxyribose, C-C
PC1		991	C-O ribose
		1080	PO_2^- symmetric stretching: nucleic acids and phospholipids
		1624	Amide I β-sheets
		1744	Ester C-O stretch: triglycerides, cholesterol ester
CT *vs.* WH 2013		988	C-O ribose
PC4		1022	Glycogen
		1616	Amide I
		1639	Amide I
		1697	Base region
CT *vs.* WH 2013	Muscle	972	OCH_3 band of polysaccharides
PC1		1022	Glycogen
		1065	C-O stretching of the phosphodiester and ribose
		1154	Carbohydrates
		1501	Amide II
CT *vs.* WH 2013	Kidney	1535	Amide II stretching
PC7		1613	Amide I
		1643	Amide I
		1682	Amide I random coils
		1751	Lipid
CT *vs.* WH 2013	Skin	1497	Lipid
PC3		1616	Amide I
		1640	Amide I
		1670	Amide I (antiparallel β-sheet) ν(C=C) *trans*, lipids, fatty acids
		1694	Amide I (antiparallel β-sheet)

Table S4. Results from two-sample *t*-tests of the scores generated from principal component analysis-linear discriminant analysis (PCA-LDA) for the first linear discriminant (LD1) following ATR-FTIR spectrochemical analysis of several tissues of *Rana temporaria* tadpoles. Significant results are highlighted in bold.

Tissue	***P* value**	
	CT *vs.* PF 2012	**CT *vs.* WH 2013**
Heart	**0.03**	**< 0.001**
Kidney	0.57	0.39
Liver	**0.02**	**< 0.001**
Muscle	0.81	**< 0.001**
Skin	0.54	**0.01**

Table S5. Results of classification accuracy (± standard deviation) for ATR-FTIR spectra of several tissues of *Rana* temporaria tadpoles with principal component analysis-linear discriminant classifier (PCA-LDC).

Tissue	**Classification Rate (%)**			
	CT (2012)	**PF (2012)**	**CT (2013)**	**WH (2013)**
Heart	68.40 ± 22.20	64.40 ± 32.81	76.00 ± 26.33	71.11 ± 36.89
Kidney	60.40 ± 37.41	44.00 ± 37.84	68.00 ± 31.20	51.11 ± 38.73
Liver	99.20 ± 1.79	90.00 ± 15.14	98.00 ± 6.32	100 ± 0
Muscle	34.98 ± 24.97	64.80 ± 39.44	71.00 ± 34.79	80.00 ± 25.39
Skin	55.20 ± 16.83	54.40 ± 23.60	66.00 ± 26.33	66.67 ± 34.64

Table S6. Distinguishing wavenumbers and proposed assignments obtained from analysis of several organs of pro-metamorphic *Rana temporaria* tadpoles with ATR-FTIR spectroscopy following analysis with principal component analysis-linear discriminant analysis (PCA-LDA). The five largest loadings values are shown. Comparisons were made between tadpoles from CT: a rural agricultural pond with no pesticide input and PF: an urban pond impacted by wastewater and landfill run-off in 2012 and between tadpoles from CT and those from WH: an agricultural pond known to be impacted by pesticides in 2013. Sources: (Abdel-Gawad et al., 2012; Cakmak et al., 2006; Cakmak et al., 2003; Greve et al., 2008; Movasaghi et al., 2008; Palaniappan and Vijayasundaram, 2008, 2009; Palaniappan et al., 2011; Purna Sai and Babu, 2001; Toyran et al., 2006).

Site comparison	Organ	Wavenumber (cm^{-1})	Proposed Assignment
CT *vs.* PF 2012	Liver	1115	Symmetric stretching P-O-C
		1138	Carbohydrates
		1192	Phosphodiester stretching
		1624	Amide I β-sheets
		1732	C=O stretch lipids
CT *vs.* PF 2012	Heart	941	Phosphodiester region
		1088	Symmetric phosphate stretching vibrations
		1138	Carbohydrates
		1196	Collagen
		1273	CH rocking
CT *vs.* WH 2013	Liver	1161	Stretching vibrations of hydrogen-bonding C-OH groups
		1454	CH_2 bending of lipids with some protein contribution
		1651	Amide I
		1690	Peak of nucleic acids due to carbonyl stretching
		1724	C=O stretching of fatty acid esters
CT *vs.* WH 2013	Muscle	988	Phosphodiester region
		1111	Symmetric stretching P-O-C
		1605	DNA vibration
		1651	Amide I
		1755	C=C lipids
CT *vs.* WH 2013	Heart	1161	Stretching vibrations of hydrogen-bonding C-OH groups
		1339	Collagen
		1624	Amide I β-sheets
		1647	Amide I
		1697	Base region
CT *vs.* WH 2013	Skin	1165	C-O stretching of proteins and carbohydrates
		1327	Collagen
		1354	C-O stretching
		1578	Base stretching
		1667	Amide I (antiparallel β-sheet) ν(C=C) *trans*, lipids, fatty acids

Figure S1. One dimensional scores plots following principal components analysis-linear discriminant analysis (PCA-LDA) of ATR-FTIR spectra obtained from several different tissues taken from *Rana temporaria* pro-metamorphic tadpoles. Tissues are liver **(A)**, muscle **(B)**, heart **(C)**, kidney **(D)** and skin **(E)**. Tadpoles were collected in 2012 from CT: a rural agricultural pond with no pesticide input or PF: an urban pond impacted by wastewater and landfill run-off (n=10). Two sample t-tests were employed to detect differences in the scores between ponds within each year. Asterisks indicate a P-value of <0.05 (*) or <0.01 (**).

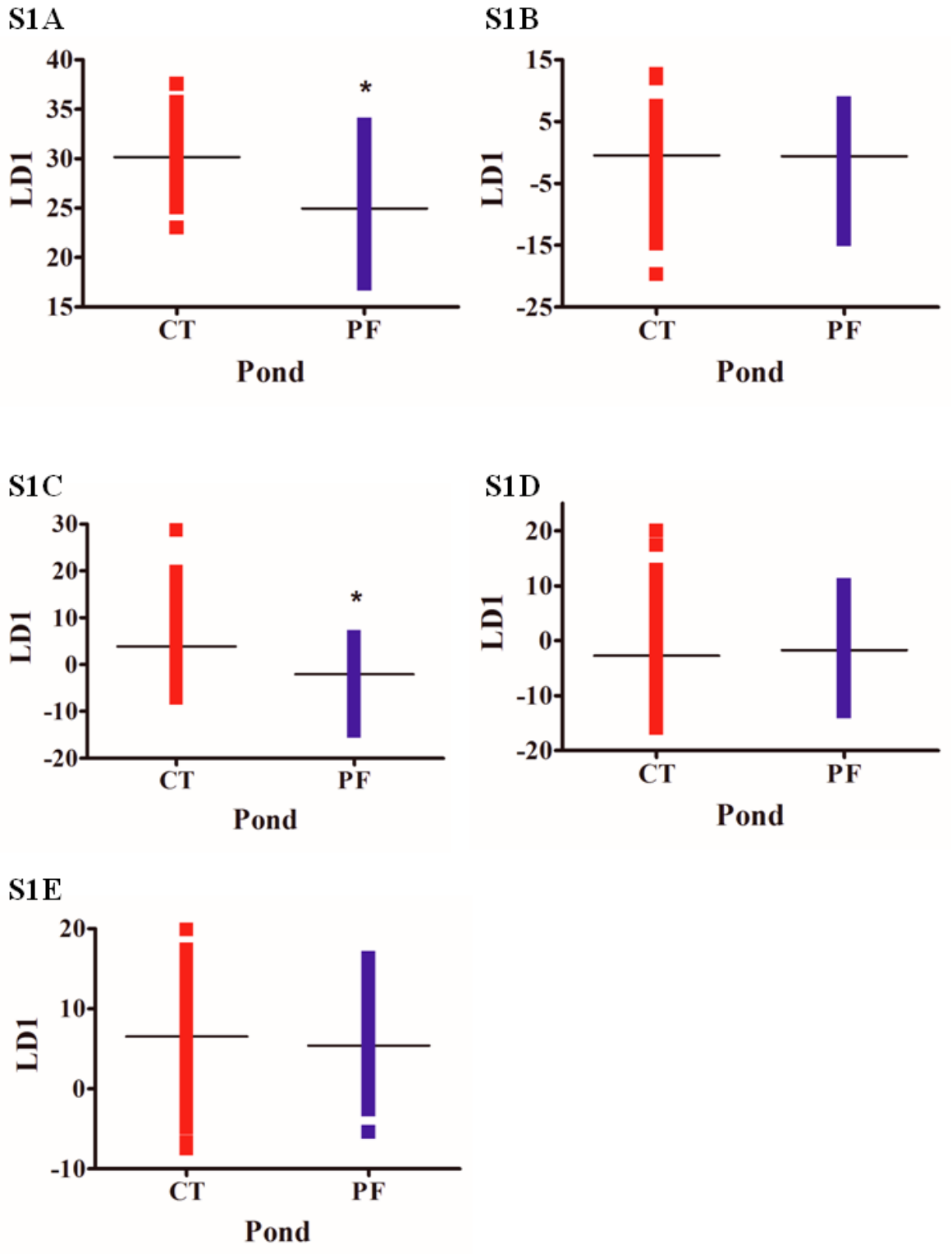

Figure S2. One dimensional scores plots following principal components analysis-linear discriminant analysis (PCA-LDA) of ATR-FTIR spectra obtained from several different tissues taken from *Rana temporaria* pro-metamorphic tadpoles. Tissues are liver **(A)**, muscle **(B)**, heart **(C)**, kidney **(D)** and skin **(E)**. Tadpoles were collected in 2013 from CT: a rural agricultural pond with no pesticide input or WH: an agricultural pond known to be impacted by pesticides (n=20). Two sample t-tests were employed to detect differences in the scores between ponds within each year. Asterisks indicate a P-value of <0.05 (*) or <0.01 (**).

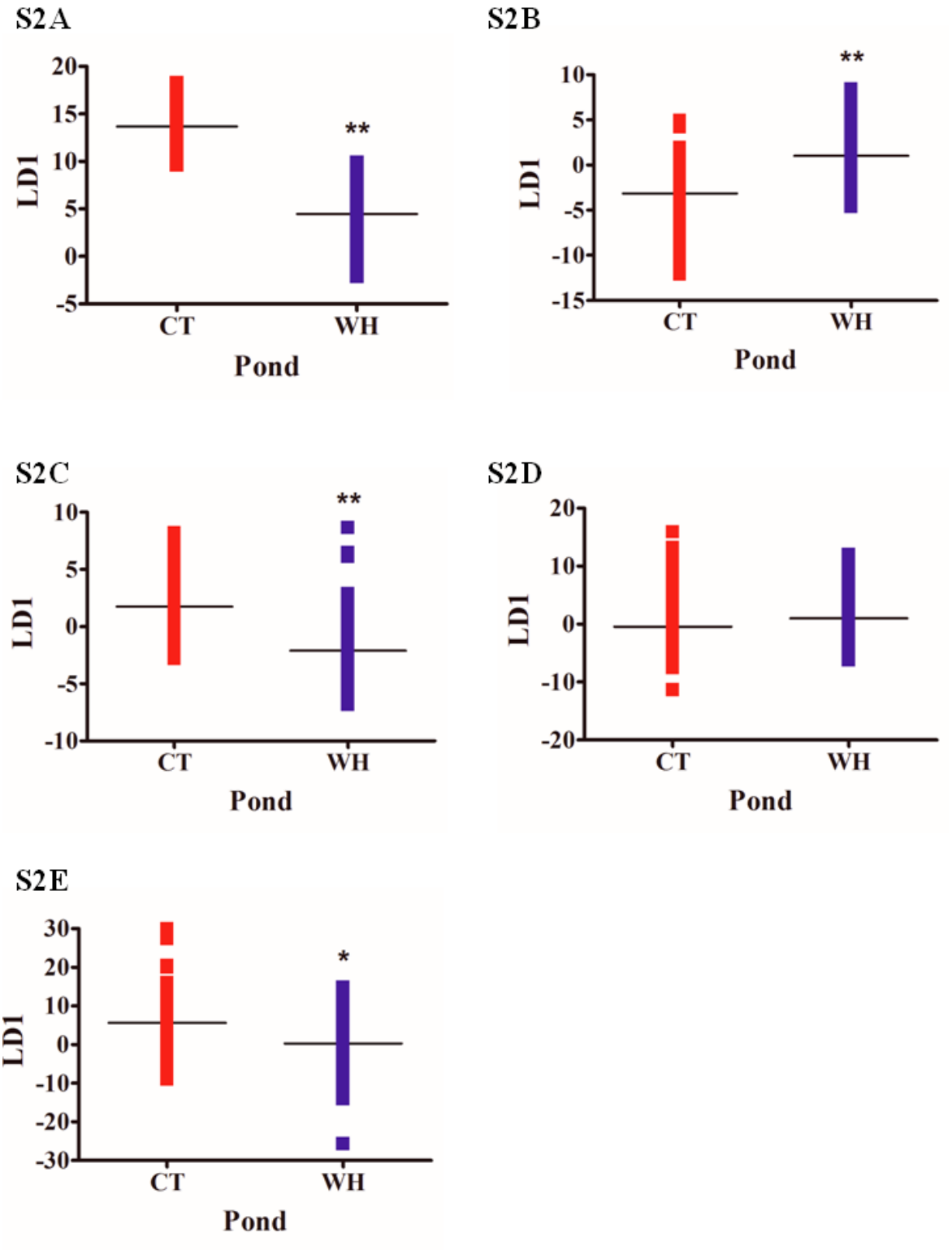

Figure S3. Raw mean ATR-FTIR spectra of tissue samples taken from *Rana temporaria* pro-metamorphic tadpoles. Tissues are liver **(A)**, muscle **(B)**, heart **(C)**, kidney **(D)** and skin **(E)**. Tadpoles were collected in 2012, from CT: a rural agricultural pond with no pesticide input or PF: an urban pond impacted by wastewater and landfill run-off (*n*=10).

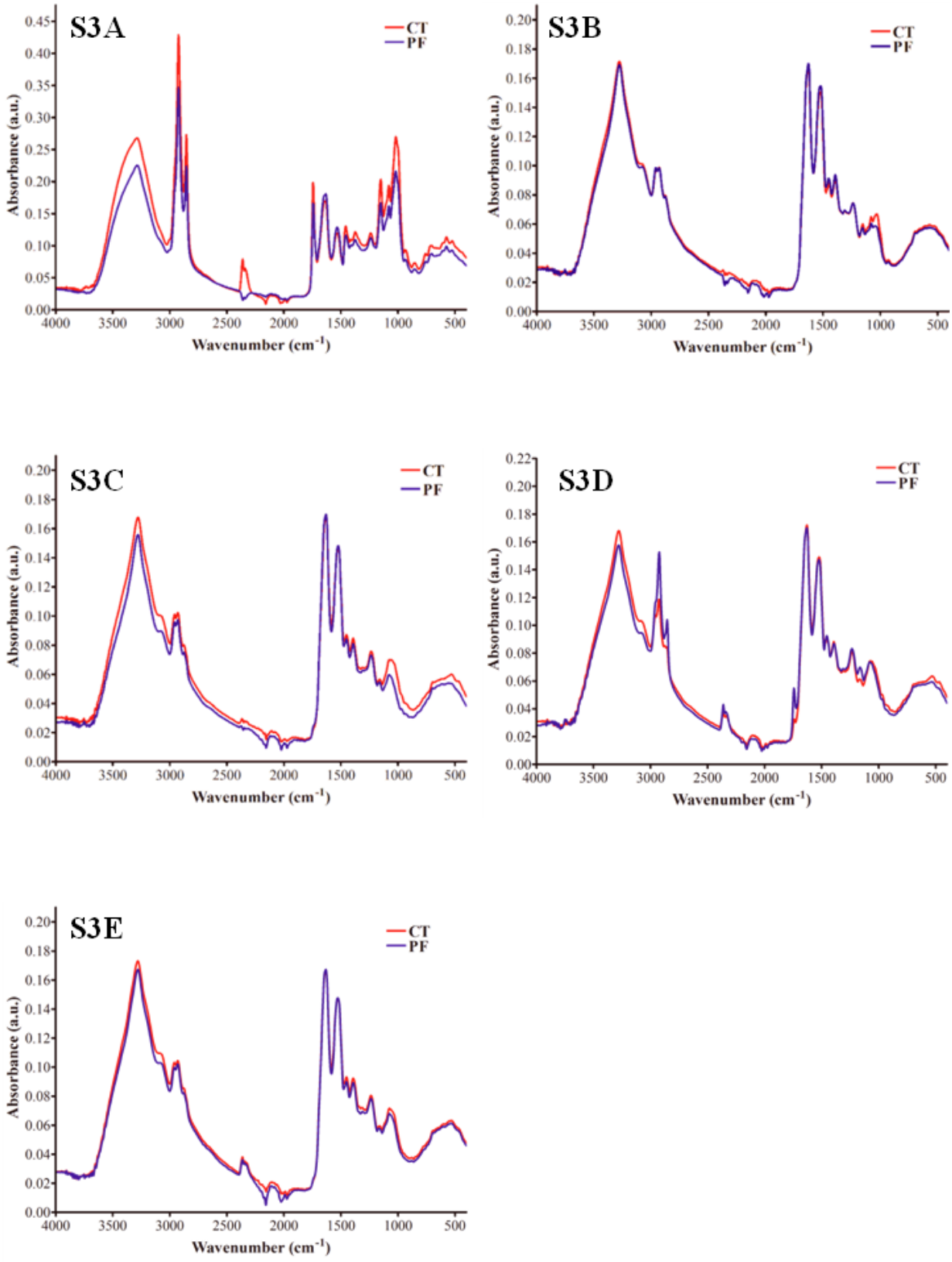

Figure S4. Raw mean ATR-FTIR spectra of tissue samples taken from *Rana temporaria* pro-metamorphic tadpoles. Tissues are liver **(A)**, muscle **(B)**, heart **(C)**, kidney **(D)** and skin **(E)**. Tadpoles were collected in 2013, from CT: a rural agricultural pond with no pesticide input or WH: an agricultural pond known to be impacted by pesticides (*n*=20).