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Supplementary material

Correlating ecotoxicological early-warning systems to biotic indices to assess riverine teratogenic contamination

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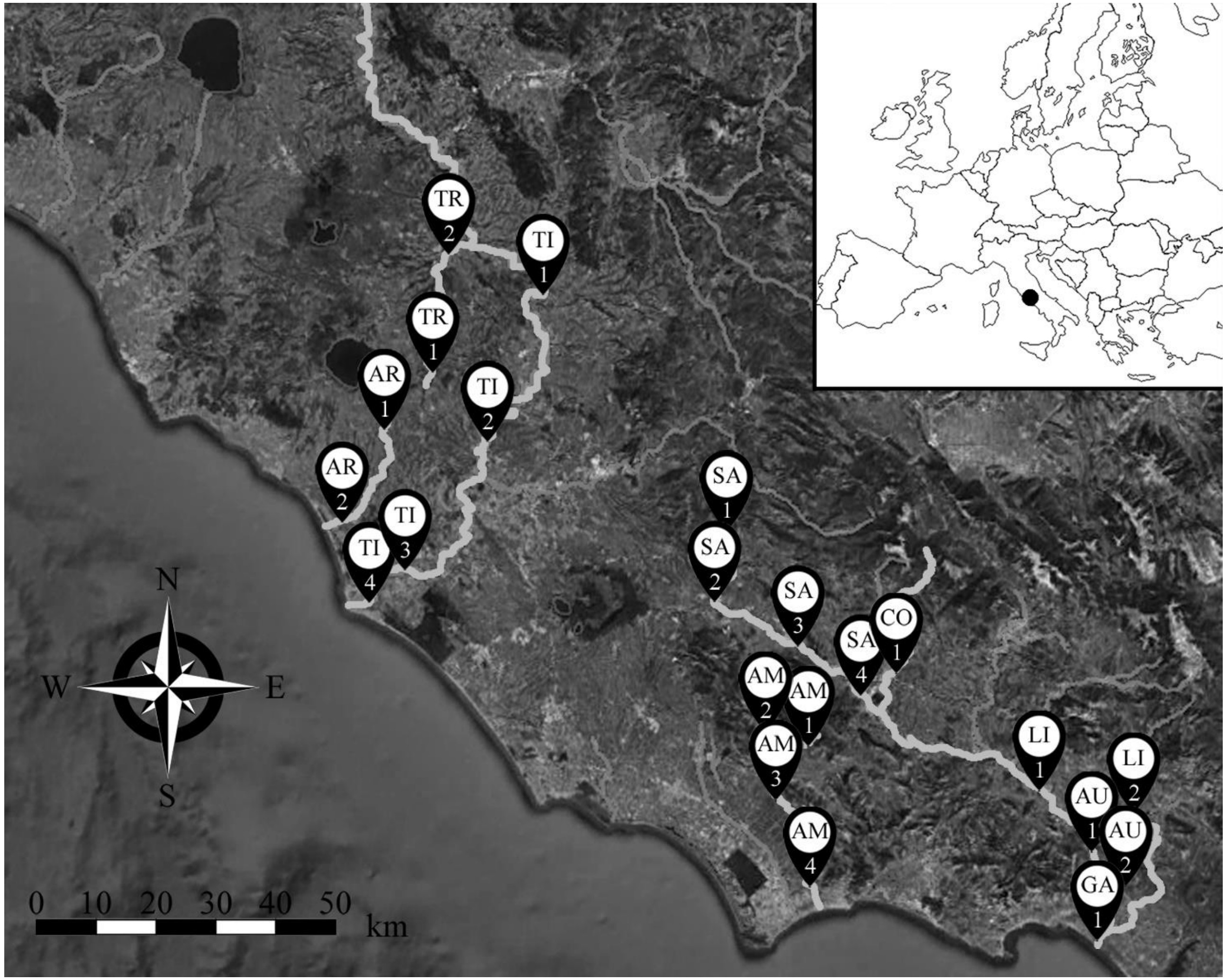
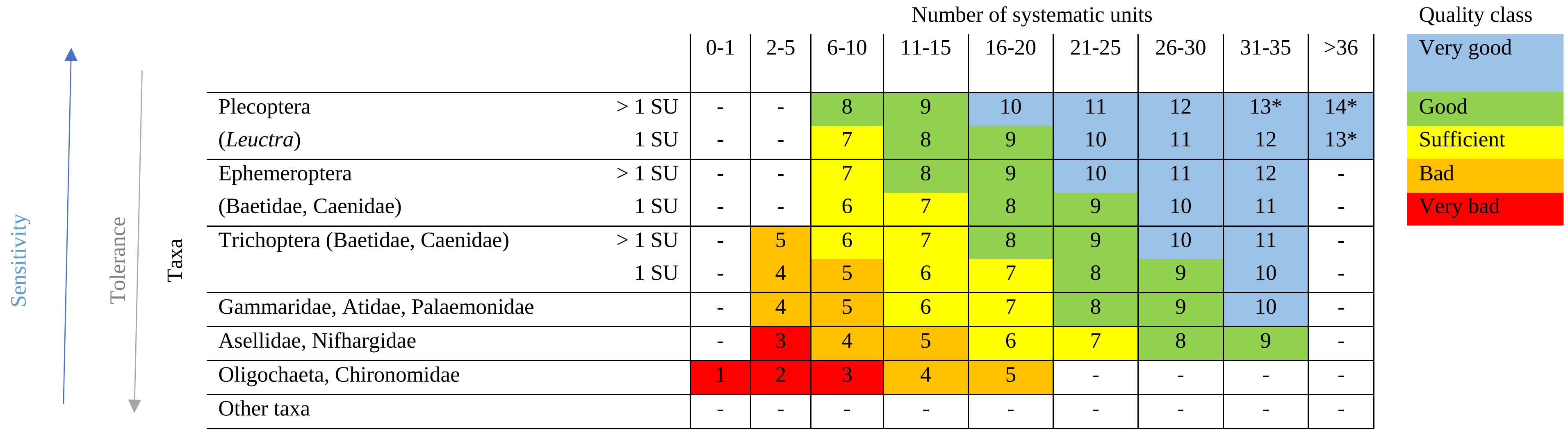


Fig. S1. The sampling site locations. Marks: AR, River Arrone; AM, River Amaseno; AU, River Ausente; CO, River Cosa; GA, River Garigliano; LI, River Liri; SA, River Sacco; TI, River Tiber; TR, River Treja.

Extended Biotic Index

The Extended Biotic Index (EBI) values were calculated using benthic invertebrates by a standardised kick-net approach according to the Ghetti (1997) procedure. We kicked the substrate for 60 s every 1 m along a linear transect linking the two banks. The same approach was followed for a 15-m linear transect along a single bank for not wadable sites as suggested by Ghetti (1997). That study suggested how this sampling strategy for not wadable sites did not significantly modify the EBI score outputs respect to the use for wadable watercourses. All collected invertebrates were grossly sorted in field, preserved in 85% ethanol, and identified in laboratory at level of genus for Ephemeroptera, Hirudinea, Odonata, Plecoptera and Turbellaria, and family for all others. Each identified taxon (at both genus or family level) represent a systematic unit in the EBI procedure. This index is based on a double-entry table (reported in Ghetti and Chierici 2001) in which lines show macroinvertebrate taxa positioned according to the increasing tolerance to environmental alterations from top to bottom, whereas columns show different ranges of systematic unit number collected in the site. The less tolerant taxon (that is the most sensitive) found in the sample determines the line entry whereas the total number of systematic units determines the column entry. The intersection of the two entries provides the EBI value (EV, from 14 to 1) where each value is inserted in a quality class (QC): I, EV > 10 (very good); II, 9 < EV < 8 (good); III, 7 < EV < 6 (sufficient); IV, 5 < EV < 4 (bad); V, EV < 3 (very bad).



Taxa		0-1	2-5	6-10	11-15	16-20	21-25	26-30	31-35	>36
Plecoptera	> 1 SU	-	-	8	9	10	11	12	13*	14*
(*Leuctra*)	1 SU	-	-	7	8	9	10	11	12	13*
Ephemeroptera	> 1 SU	-	-	7	8	9	10	11	12	-
(Baetidae, Caenidae)	1 SU	-	-	6	7	8	9	10	11	-
Trichoptera (Baetidae, Caenidae)	> 1 SU	-	5	6	7	8	9	10	11	-
	1 SU	-	4	5	6	7	8	9	10	-
Gammaridae, Atidae, Palaemonidae		-	4	5	6	7	8	9	10	-
Asellidae, Nifhargidae		-	3	4	5	6	7	8	9	-
Oligochaeta, Chironomidae		1	2	3	4	5	-	-	-	-
Other taxa		-	-	-	-	-	-	-	-	-

Number of systematic units

Quality class

🟦 Very good
🟩 Good
🟨 Sufficient
🟧 Bad
🟥 Very bad

Fig. S2. The extended biotic-index scheme and associated water-quality class (from Ghetti and Chierici 2001, modified). Where *Leuctra* is the only genus of Plecoptera and Baetidae and Caenidae are the only Ephemeroptera, then *Leuctra* ought to be considered like if it were Trichoptera. Baetidae and Caenidae ought to be considered as Trichoptera. Rare values for the Italian running waters are marked with an asterisk (*).

Index Biologique Macrophytique en Rivière

The Index Biologique Macrophytique en Rivière (IBMR) (AFNOR 2003) is based on the using of the macrophyte community as bioindicator of the water trophic status (Haury *et al*. 2006).

The IBMR values were calculated using data of macrophytes occurrence and abundance (%) collected during the plant samplings. The calculation of the Index for each sampling stations foresees the application of the following formula:

$$IBMR = \frac{\sum_{i=1}^{n} E_i \cdot K_i \cdot Cs_i}{\sum_{i=1}^{n} E_i \cdot K_i}$$

where *i* is the macrophyte species; *n* is the total number of macrophyte species; Cs_i is the oligotrophic sensitivity coefficient (range 1–20); K_i is the abundance coefficient ; and E_i is the stenoecìa coefficient (range 1–3).

The IBMR value obtained for each station can be transformed into a quality judgment on the local trophic status of the waters, based on the reference values reported in the Table below where the entire values scale of the Index (ranging from 1 to 20) is divided into five classes.

Table S1. Water trophic levels for the classification of each station on the bases of IBMR values

IBMR value	Trophic level	class	Colour
IBMR > 14	Very low	I	Blue
12 ≤ IBMR ≤ 14	Low	II	Green
10 ≤ IBMR ≤ 12	Medium	III	Yellow
8 ≤ IBMR ≤ 10	High	IV	Orange
IBMR ≤ 8	Very high	V	Red

This subdivision, based on the same classes number and colours of IBE and RTI, allows a comparison among different indexing methods.

Table S2. Characterisation of substrate and physico-chemical descriptors for each sampling site

Substrate (Sub), granulometry (Gra) mean value (±standard deviation), conductivity (Cond), oxygen concentration (O_2), oxygen saturation (OS), pH, salinity (Sal) temperature (T), chemical oxygen demand (COD), orthophosphate (PO_4^{3}), nitrate (NO_3^{-}), for each sampling site reported divide per catchment from north to south (for the site marks, see Fig. 1) within the study area

Sites	Sub	Gra (mm)	Cond (µS cm^{-1})	O_2 (mg L^{-1})	OS (%)	pH	Sal (‰)	T (°C)	COD (mg L^{-1})	PO_4^{3-} (mg L^{-1})	NO_3^{-} (mg L^{-1})
TR1	Gravel	27.14 ± 12.76	450	8.15	83.6	8.40	0.26	16.7	17.4	0.64	3.69
TR2	Silt	0.06 ± 0.02	482	7.75	82.7	8.35	0.27	17.9	8.4	0.54	3.63
TI1	Silt	0.05 ± 0.03	1064	7.50	81.3	7.98	0.62	18.6	249.0	0.39	1.08
TI2	Silt	0.07 ± 0.02	1113	8.18	88.4	8.18	0.62	19.6	31.6	0.10	1.10
TI3	Silt	0.04 ± 0.01	1079	5.04	55.8	8.20	0.60	20.0	49.2	0.32	1.50
TI4	Silt	0.04 ± 0.02	2760	2.06	22.5	8.11	1.61	21.1	71.1	0.02	1.26
AR1	Sand	0.37 ± 0.19	725	3.83	43.2	8.07	0.38	21.8	47.1	7.62	12.9
AR2	Sand	0.77 ± 0.34	762	7.12	82.2	8.61	0.39	22.9	22.6	1.94	5.57
SA1	Sand	1.71 ± 0.25	377	9.28	82.4	8.49	0.26	9.2	31.3	0.27	2.28
SA2	Sand	0.89 ± 0.64	398	8.85	80.1	8.56	0.27	10.2	3310.0	0.02	2.59
SA3	Pebble	38.46 ± 7.19	441	5.96	57.6	5.96	0.28	13.4	34.2	0.02	2.07
	Sand	0.93 ± 0.18									
SA4	Sand	1.31 ± 0.27	630	9.08	90.0	8.79	0.39	14.6	28.4	0.02	3.79
CO1	Gravel	48.31 ± 5.59	520	9.18	86.9	8.79	0.34	12.7	22.0	0.15	2.50
	Sand	1.44 ± 0.39									
LI1	Gravel	19.94 ± 6.34	502	9.37	94.3	8.66	0.30	16.2	21.1	0.12	1.40
	Sand	0.33 ± 0.11									
LI2	Gravel	7.03 ± 1.59	478	9.40	95.9	8.64	0.28	16.9	48.4	0.09	1.66
	Sand	0.57 ± 0.24									
GA1	Silt	0.05 ± 0.03	614	7.98	83.7	8.07	0.35	17.7	688.0	0.02	1.30
AU1	Pebble	11.63 ± 4.37	548	6.99	75.3	8.23	0.30	19.0	306.0	0.08	0.33
AU2	Sand	0.94 ± 0.49	579	4.57	49.4	8.40	0.31	20.1	89.0	0.06	1.28
AM1	Boulder	416.81 ± 109.57	505	5.18	52.6	7.92	0.30	16.1	22.0	0.13	7.34
	Sand	1.55 ± 0.64									
AM2	Pebble	19.53 ± 7.33	440	8.29	85.8	8.61	0.26	16.4	35.2	0.05	4.64
AM3	Sand	1.08 ± 0.27	447	8.40	89.1	8.61	0.25	18.4	60.7	0.19	3.97
AM4	Sand	0.83 ± 0.24	795	8.53	93.5	8.74	0.42	20.5	51.5	0.02	1.42

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Table S3. List of benthic invertebrates occurring within the study area divided per sampling site

	TIB1	TIB2	TIB3	TIB4	AMA1	AMA2	AMA3	AMA4	AUS1	AUS2	GAR1	SAC1	SAC2	SAC3	SAC4	COS1	LIR1	LIR2	TRE2	TRE1	ARR1	ARR2
	H 1	H 2	H 3	H 4	H 5	H 6	H 7	H 8	H 9	H 10	H 11	H 12	H 13	H 14	H 15	H 16	H 17	H 18	H 19	H 20	H 21	H22
Ecdyionuros												x										
Anellida												x	x	x		x	x					
Apheloheridae																			x			
Asellidae	x		x						x							x					x	
Baetis													x	x	x	x	x		x	x		
Caenidae						x																
Caenis															x	x	x		x	x		
Calopteryx						x						x										
Chironomidae			x		x				x	x	xx	x	x	x	x	x	x	x	x	x		
Cordullidae																			x			
Dytiscidae																x						
Economidae																			x			
Electrogena						x																
Elminthidae						x						x										
Ephemera						x						x										
Ephemeraella																			x	x		
Erpobdella																	x					
Gammaridae	x	x	x	x	x	x			x		x	x	x				x	x		x		
Gomphidae																			x			
Hydracarina					x		x									x						
Hydropsichidae												x				x	x	x	x			
Limoniidae						x					x						x					
Lymnaea									x		x											
Naididae																x						
Nematomorfi						x																
Nemoura												x										
Nepa cinerea																	x					
Niphargidae									x													
Odonato	x																x					
Onychogompus						x	x															
Palaemons antennarius	x	x					x	x			x											
Physa		x							x		x										x	
Plactynemis							x								x							
Polycentropodidae												x				x						
Procambarus clarkii	x	x																				
Pseudicentroptilum																				x		
Sericostomatidae												x										
Serratella			x																			
Simulidae																	x					
Theodoxus						x																
Tipulidae							x															
caddiesfly's case													x						xx			

Table S4. List of macrophytes occurring within the study area divided per sampling site

	TIB1 H 1	TIB2 H 2	TIB3 H 3	TIB4 H 4	AMA1 H 5	AMA2 H 6	AMA3 H 7	AMA4 H 8	AUS1 H 9	AUS2 H 10	GAR1 H 11	SAC1 H 12	SAC2 H 13	SAC3 H 14	SAC4 H 15	COS1 H 16	LIR1 H 17	LIR2 H 18	TRE2 H 19	TRE1 H 20	ARR1 H 21	ARR2 H22
Apium nodiflorum																x	x			x		
Azolla filiculoides	x	x									x											
Callitriche stagnalis											x					x					x	
Ceratophyllum demersum		x	x					x		x	x											
Fontinalis antipyretica						x														x		
Lemna gibba							x															
Lemna minor		x		x																x		
Lycopus europaeus														x			x					
Myriophyllum spicatum	x	x																				
Myriophyllum spicatum											x											
Nasturtium officinale					x	x									x		x			x	x	
Nostoc sp.																	x					
Phormidium sp.											x											
Phragmites australis																					x	x
Potamogeton crispus																				x		
Potamogeton nodosus	x	x	x				x				x										x	
Spirogyra sp.					x									x	x				x	x		
Typha latifolia							x												x			
Vallisneria spiralis							x															
Vaucheria sp.									x													
Vaucheria sp.																x	x					
Veronica anagallis-aquatica																x	x			x	x	
Veronica augallis														x								
Veronica beccabunga					x	x	x										x					
Zannichellia palustris							x															

Table S5. Number of macrophyte (macNo) and benthic invertebrate (invNo) taxa, macrophyte (macDI) and benthic invertebrate (invDI) diversity index (that is Simpson index), IBMR score (IBMRsc), IBMR class (IBMRcl), EBI score (EBIsc), EBI class (EBIcl), for each sampling site reported divide per catchment from north to south (for the site marks, see Fig. S1) within the study area

na, not assessable for insufficient data

Sites	macNo	invNo	macDI	invDI	IBMRsc	IBMRcl	EBIsc	EBIcl
TR1	2	10	0.91	0.11	na	na	6	3
TR2	7	6	0.20	0.43	9.4	4	6	3
TI1	3	4	0.79	0.74	5.6	5	5	4
TI2	5	4	0.41	0.67	5.8	5	4	4
TI3	2	3	0.83	0.78	na	na	4	4
TI4	1	1	1	1	na	na	na	na
AR1	5	2	0.38	0.89	8.7	4	3	5
AR2	1	3	1	0.84	9	4	3	5
SA1	0	8	-	0.23	na	na	8	2
SA2	0	5	-	0.54	na	na	4	4
SA3	3	3	0.81	0.86	10.5	3	4	4
SA4	2	4	0.89	0.61	10.4	3	5	4
CO1	4	10	0.52	0.19	9.4	4	6	3
LI1	7	10	0.16	0.17	9	4	7	3
LI2	0	3	-	0.81	na	na	4	4
GA1	6	3	0.33	0.73	6.8	5	5	4
AU1	1	6	1	0.39	4	5	5	4
AU2	1	1	1	1	5	5	na	na
AM1	3	3	0.86	0.83	10.4	3	4	4
AM2	3	10	0.77	0.14	10.3	3	6	3
AM3	6	5	0.29	0.48	6	5	4	4
AM4	1	1	1	1	5	5	na	na

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