BIOLOGICAL RESPONSES OF FISH, GAMMARIDS AND PARASITES TO ANTHROPOGENIC INFLUENCES IN THE KRKA RIVER, VULNERABLE KARST ECOSYSTEM AND NATIONAL PARK IN CROATIA

Vlatka Filipović Marijić

Laboratory for Biological Effects of Metals
Division for Marine and Environmental Research
Ruđer Bošković Institute
Zagreb, Croatia

BIOINDICATOR ORGANISMS

Biota can give us reliable information on environmental pollution since they accumulate contaminants at much higher concentrations than the environment (bioconcentration, bioavailability) and as longliving can reflect long term pollution impact.

Good indicator ability	*** Provide measurable response (sensitive to the disturbance or stress but does not experience mortality or accumulate pollutants directly from their environment)** *** Response reflects the whole population/community/ecosystem response** *** Respond in proportion to the degree of contamination or degradation**
Abundant and common	*** Adequate local population density (rare species are not optimal)** *** Common and widely distributed species** *** Relatively stable and available during the whole year despite moderate climatic and environmental variability** *** Longer life span**
Well-studied	*** Ecology and life history well understood** *** Easy taxonomical determination** *** Easy and cheap to survey** *** Suitable size**

AQUAMAPMET - INDICATOR ORGANISMS

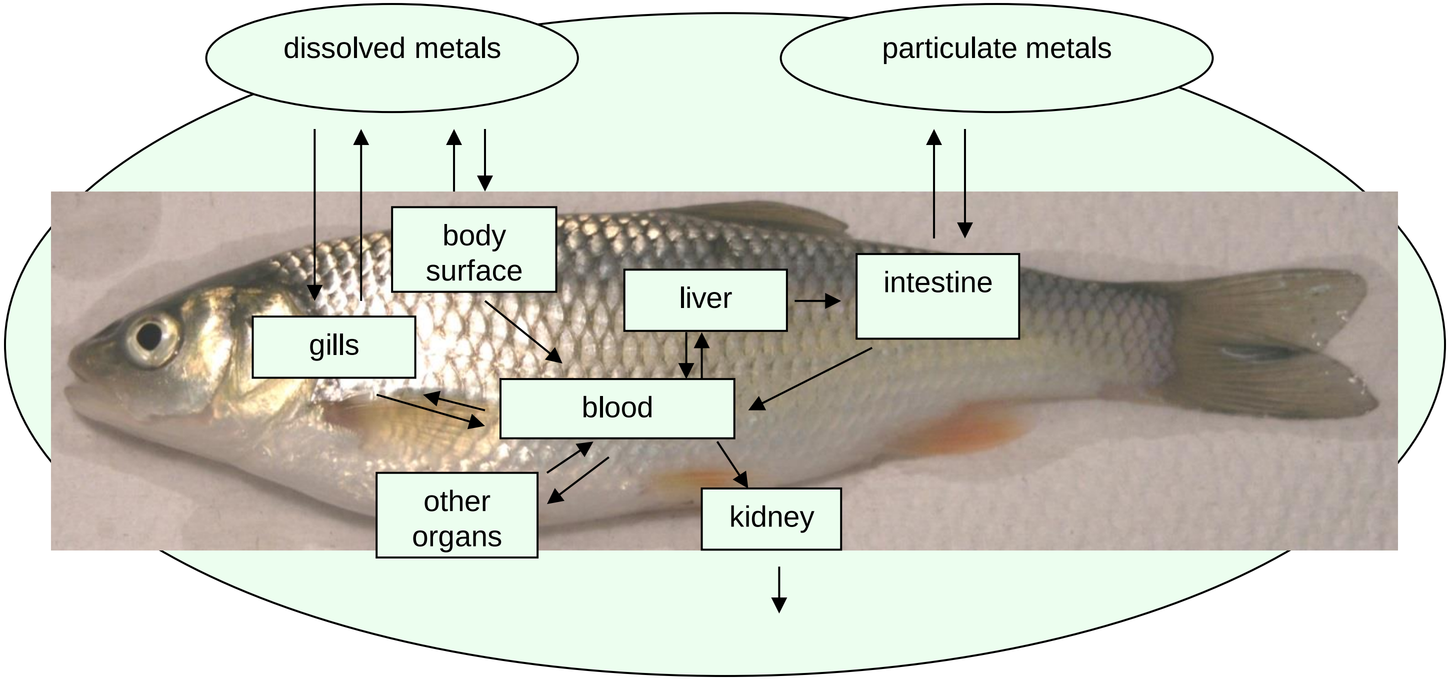
FISH *Salmo trutta* L. 1758
brown trout

INDICATOR ORGANS – gills, liver, intestine

CRUSTACEANS – GAMMARIDS
Gammarus balcanicus Schäferna, 1922
Echinogammarus acarinatus Karaman, 1931

FISH INTESTINAL PARASITES – ACANTHOCEPHALANS
Dentitruncus truttae Sinzar, 1955

BIVALVES – only in the lakes in the Krka National Park area



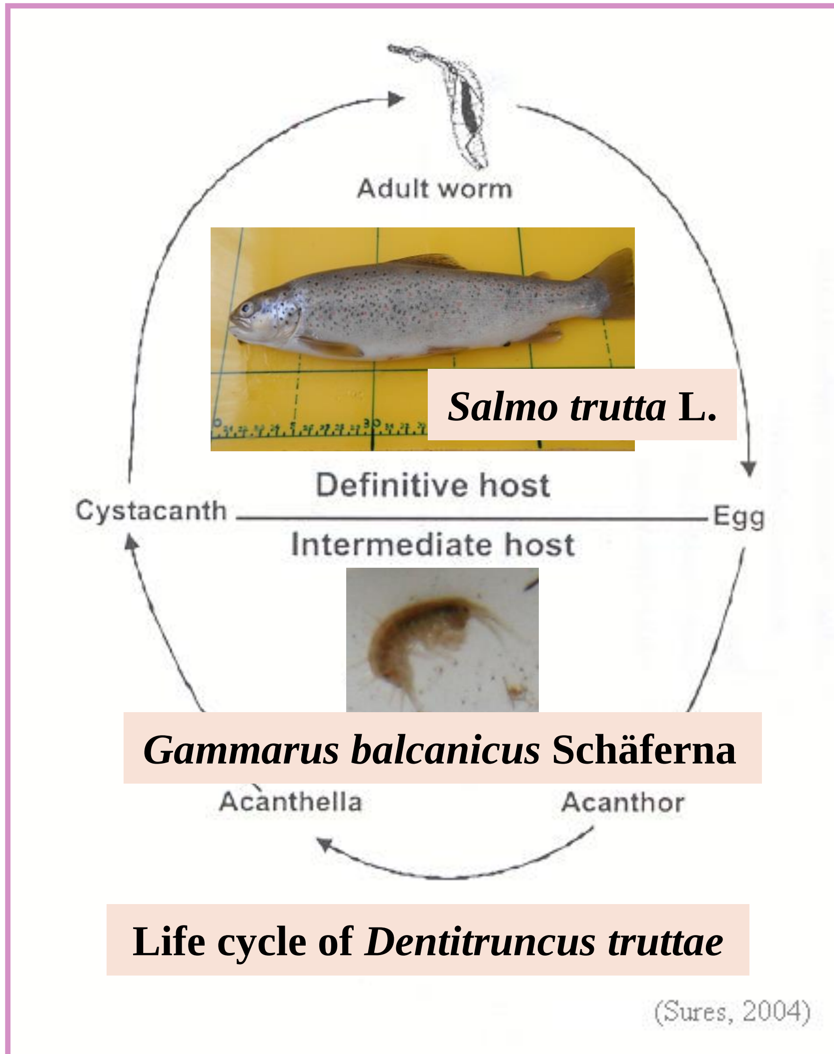
INTESTINE –

the site of dietary metal absorption, from which metals are redistributed via the blood to the other internal organs, which may results in both, metal accumulation and toxicity in fish organs

- although dietary metal uptake is important as the waterborne in native freshwater fish, studies were predominantly focused on the uptake of waterborne contaminants and water quality criteria for potentially toxic metals still rely on aqueous metal concentrations

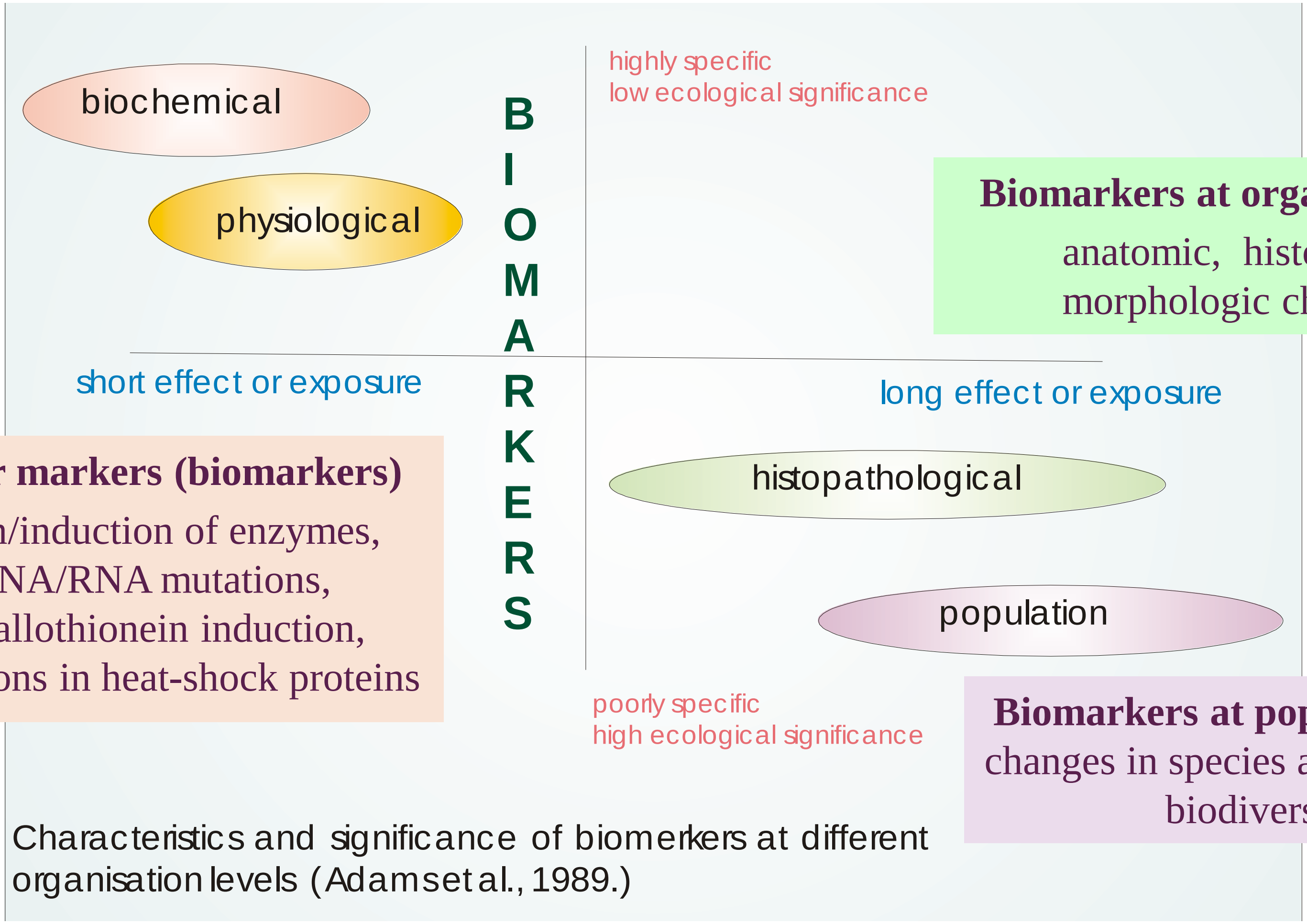
ACANTHOCEPHALANS

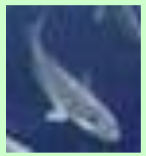
In the last few decades fish intestinal parasites **Acanthocephala** are recognised as potential indicators of metal exposure since they accumulate metals, especially toxic ones, more effectively than the tissues of commonly used indicator organisms



BIOMARKERS

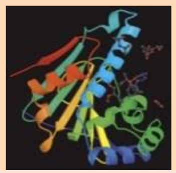
Biological changes at molecular, physiological or population level which serve as indicators of contaminant exposure or effects





Biomarkers at organism level

anatomic, histologic and morphologic changes



Molecular markers (biomarkers)

inhibition/induction of enzymes,
DNA/RNA mutations,
metallothionein induction,
alterations in heat-shock proteins

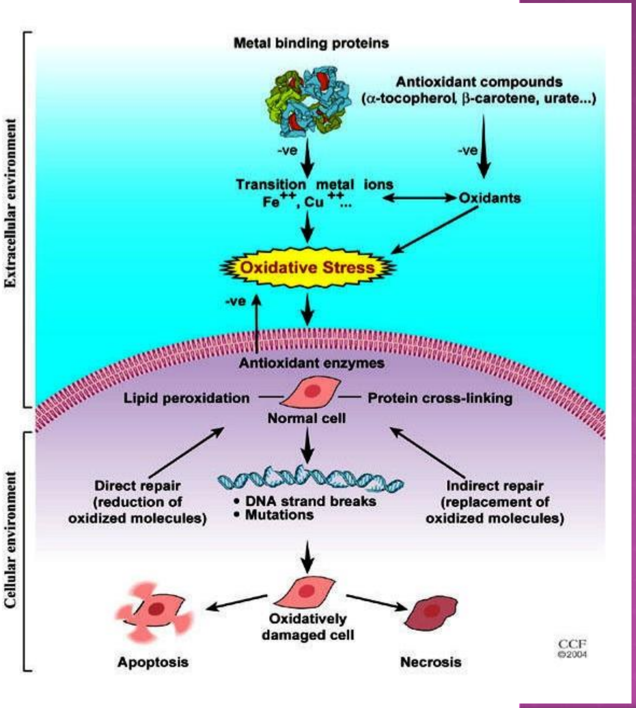
Biomarkers at population level

changes in species abundance and biodiversity

AQUAMAPMET - multi-biomarker approach

The use of multi-biomarker approach is necessary in environments with complex mixtures of contaminants to assess various biological responses that reflect the influence of different contaminants

- **BIOMARKERS OF THE GENERAL STRESS**
  total cytosolic proteins (TP) as biomarkers of the general state
- **BIOMARKERS OF ANTIOXIDANT DEFENSE**
  catalase activity (CAT) and total glutathione (GSH) as markers of antioxidant capacities
- **BIOMARKERS OF OXIDATIVE STRESS**
  malondialdehyde (MDA) as an indicator of oxidative damage
- **BIOMARKERS OF AN EXPOSURE AND E CONTAMINANTS**
  metallothioneins (MT) as biomarkers of m
  acetylcholine esterase activity (AChE) as l
  nervous system following exposure to orga
  carbamate pesticides, but also other contar



METALS IN BIOTA

ESSENTIAL

involved in metabolic processes **Na, K, Mg, Ca, Cu, Zn, Fe, Mn**

NONESSENTIAL

toxic, without any biological role **Cd, Pb, Ag, Hg**

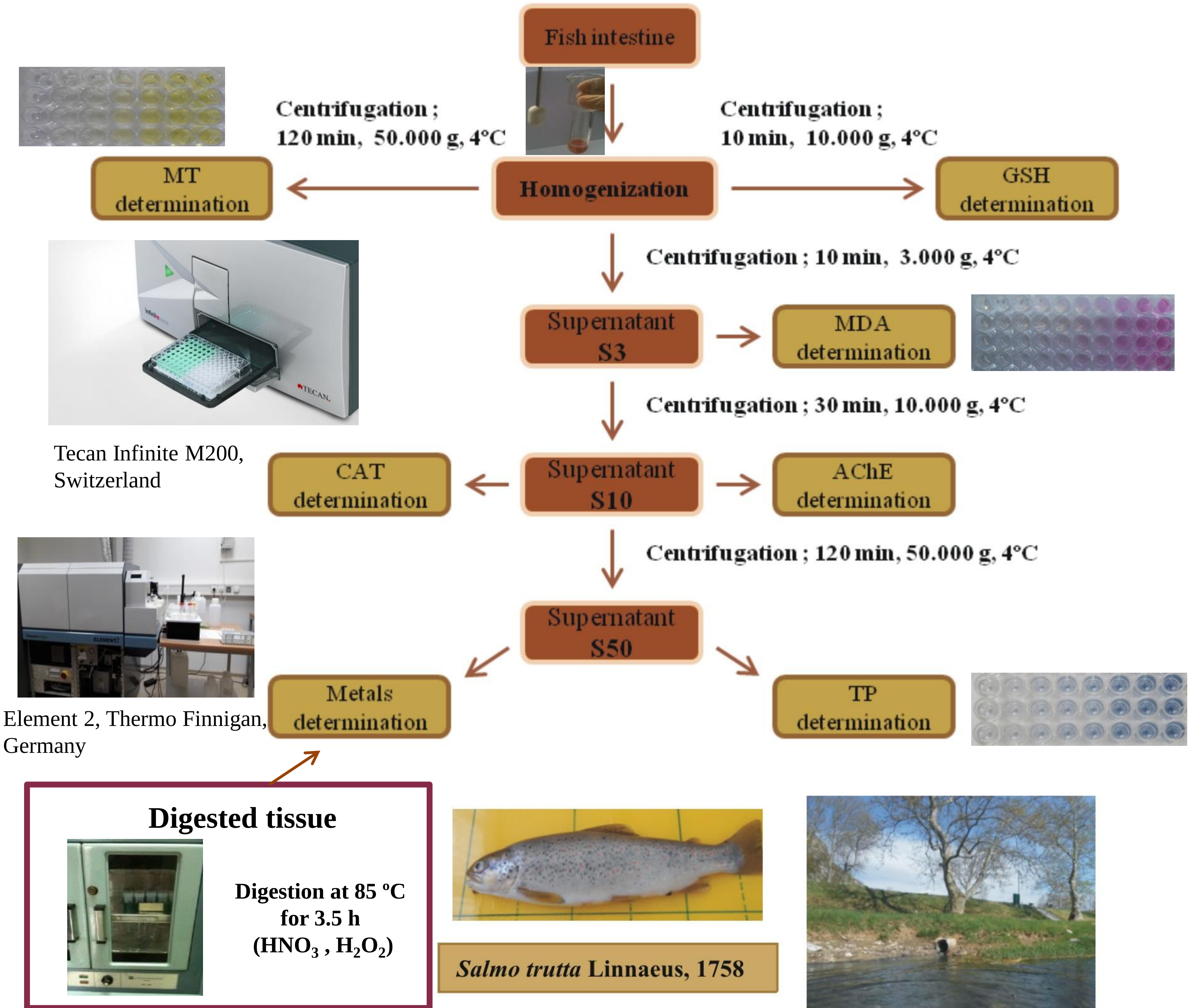
Toxicity of nonessential (=toxic) metals is based on their chemical similarity with essential metals, resulting in structural or functional distubances of biologically important molecules

AQUAMAPMET – total metal concentrations and cytosolic metal concentrations (evaluation of bioavailable and potentially toxic metal levels)

- total metal levels were compared among fish intestinal tissue, gammarids and fish intestinal parasites, acanthocephalans, as the organisms involved in parasite life cycle, while cytosolic metals were measured in fish intestine

POLLUTION SOURCES

Anthropogenic influence caused by technological and domestic runof in the Krka River watercourse near town of Knin, situated only 2 km upstream of the beginning of the Krka National Park.


Fish intestine
Centrifugation ; 120 min, 50.000 g, 4°C
Centrifugation ; 10 min, 10.000 g, 4°C
MT determination
Homogenization
GSH determination
Centrifugation ; 10 min, 3.000 g, 4°C
Supernatant S3
MDA determination
Centrifugation ; 30 min, 10.000 g, 4°C
Tecan Infinite M200, Switzerland
CAT determination
Supernatant S10
AChE determination
Centrifugation ; 120 min, 50.000 g, 4°C
Supernatant S50
Element 2, Thermo Finnigan, Germany
Metals determination
TP determination
Digested tissue
Digestion at 85 °C for 3.5 h (HNO_3 , H_2O_2)
Salmo trutta Linnaeus, 1758

FISH

Salmo trutta L.

brown trout

CRUSTACEANS – GAMMARIDS
Gammarus balcanicus Schäferna, 1922
Echinogammarus acarinatus Karaman, 1931






FISH INTESTINAL PARASITES – ACANTHOCEPHALANS
Dentitruncus truttae Sinzar, 1955

AUTUMN						
/mg kg $^{-1}$	Krka River source (reference site)			Krka River downstream of wastewater outlets (contaminated site)		
	acanthocephalans	gammarids	brown trout intestine	acanthocephalans	gammarids	brown trout intestine
Microelements						
Cd	0.46±0.37	0.14±0.05	0.11±0.14	0.11±0.09	0.03±0.01	0.011±0.012
Cs	0.005±0.002	0.008±0.004	0.01±0.001	0.005±0.004	0.014±0.006	0.004±0.003
Co	0.010±0.005	0.034±0.012	0.012±0.011	0.061±0.057	0.046±0.029	0.039±0.029
Cu	6.50±4.62	6.69±1.64	0.84±0.43	7.15±6.53	8.78±1.79	1.06±0.42
Mn	2.85±0.85	1.96±0.84	0.47±0.12	3.65±0.98	5.36±1.47	0.71±0.15
Fe	10.26±4.78	26.40±19.43	14.52±5.12	28.61±8.70	70.93±37.22	14.80±4.39
Pb	0.22±0.15	0.032±0.028	0.071±0.075	0.66±0.46	0.088±0.039	0.031±0.013
Rb	2.08±0.45	1.79±0.14	4.20±1.33	1.35±0.52	1.78±1.10	2.56±0.97
Sr	0.71±0.72	52.36±16.63	0.11±0.03	4.79±2.87	49.84±16.27	0.26±0.063
Tl	0.54±0.38	0.036±0.01	0.047±0.013	0.28±0.23	0.015±0.003	0.019±0.008
Zn	15.19±8.32	8.60±0.94	221.8±109.4	15.54±8.23	9.81±1.05	130.8±95.3
Macroelements						
Ca	1822.2±357.3	29097.0±4144.9	191.1±35.2	1523.5±216.5	26712.6±4424.9	124.8±30.2
K	1822.2±357.3	1427.1±354.8	2993.1±318.8	1523.5±216.5	2110.3±114.4	2906.0±276.9
Mg	1854.7±359.1	443.1±125.0	164.4±28.1	1963.3±294.2	429.0±76.7	167.7±26.8
Na	510.9±496.3	1377.0±313.4	1192.1±137.1	1033.3±636.7	1290.8±272.6	1041.6±145.4

Liver: Cd, Cs, Rb, Tl

Gills: Cd, Cs, Fe, Tl

Higher metal accumulation in acanthocephalans than fish intestine for: Cd, Tl, Pb, Cu, Sr, Ca, Mg, Mn

Acanthocephalans / brown trout intestine				
Metals	Krka River source (reference site)		Krka River downstream of wastewater outlets (contaminated site)	
	Autumn	Spring	Autumn	Spring
Cd	4.25	9.11	10.3	55.4
Cs	0.47	0.65	1.11	0.84
Co	0.85	1.60	1.18	3.40
Cu	7.70	17.4	6.71	25.3
Mn	6.10	5.91	5.11	6.58
Fe	0.71	1.14	1.93	1.30
Pb	5.45	8.24	21.3	5.84
Rb	0.50	0.72	0.53	0.80
Sr	6.69	6.40	18.4	8.27
Tl	11.2	30.6	14.9	14.2
Zn	0.08	0.13	0.12	0.17
Ca	15.3	10.9	12.2	7.01
K	0.61	0.67	0.52	0.89
Mg	11.3	2.64	11.7	6.03
Na	0.43	0.15	0.99	0.17

Higher metal accumulation in acanthocephalans than gammarids for: Tl, Pb, Cd, Mg, Zn, Mn

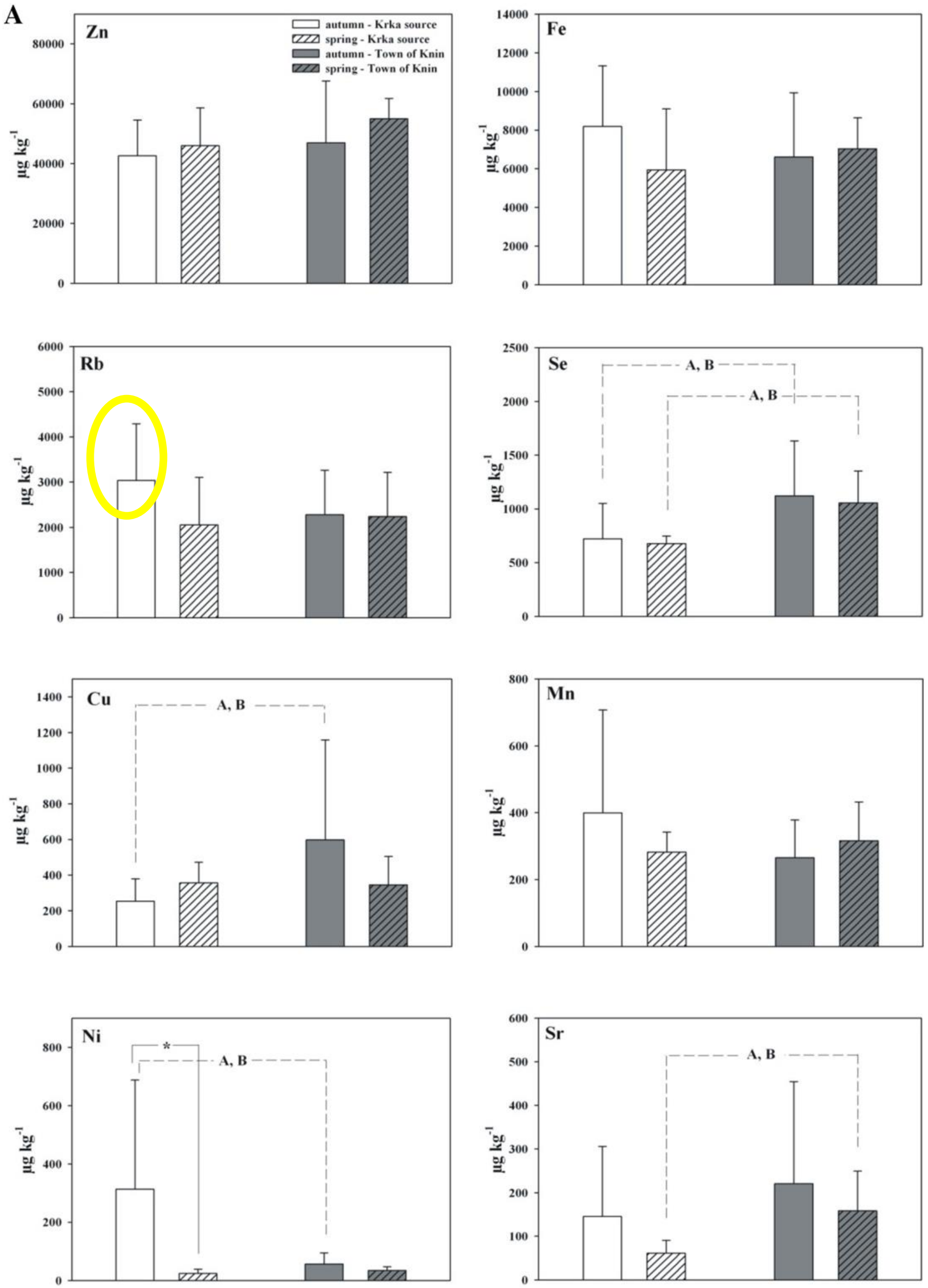
Acanthocephalans / gammarids				
Metals	Krka River source (reference site)		Krka River downstream of wastewater outlets (contaminated site)	
	Autumn	Spring	Autumn	Spring
Cd	3.32	6.94	3.91	7.75
Cs	0.62	0.40	0.35	0.44
Co	0.31	0.51	0.54	0.91
Cu	0.97	1.49	0.81	2.57
Mn	1.45	1.40	0.68	1.15
Fe	0.39	0.23	0.40	0.23
Pb	7.03		7.52	
Rb	1.16	1.03	0.76	1.64
Sr	0.01	0.03	0.10	0.05
Tl	15.2	21.9	18.6	12.7
Zn	1.77	1.19	1.58	2.05
Ca	0.063	0.05	0.057	0.06
K	1.28	1.23	0.72	1.62
Mg	4.19	1.11	4.56	2.43
Na	0.37	0.11	0.80	0.17

BCF – bioconcentration factor

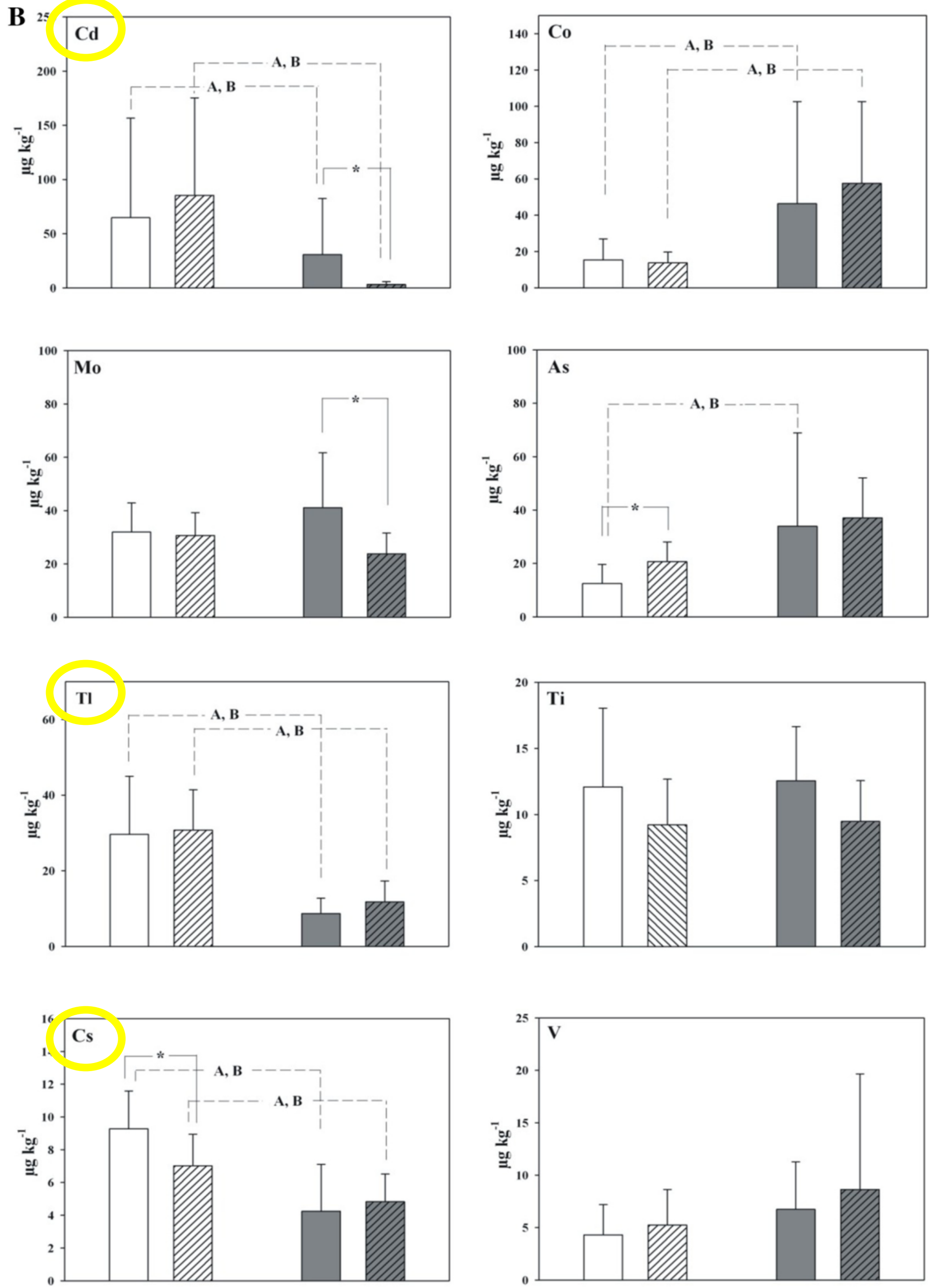
The ratio of metal concentrations in acanthocephalans and other bioindicator organisms

Metal accumulation efficiency in acanthocephalans in relation to fish intestine:

	Cu	Zn	Fe	Mn	Cd	Pb
Salmo trutta (Croatia – Krka River)	6-8	0.1-0.2	0.7-2	5-6	5-10	15-36
Vardar chub (Macedonia – Bregalnica River)	7-10	0.3-1.3	2-3	4.0-4.5	2-6	25-40
European chub (Croatia – Sava River)	5-6	0.3-0.4	0.5-1.1	2-4	10-11	25-50
Perch (Austria- Mondsee Lake)	*50*	*8*	*6*	*2*	*20*	*38*
Barbel (Hungary – Danube River)	*52*	*5*	*3*	*5*	*67*	*95*

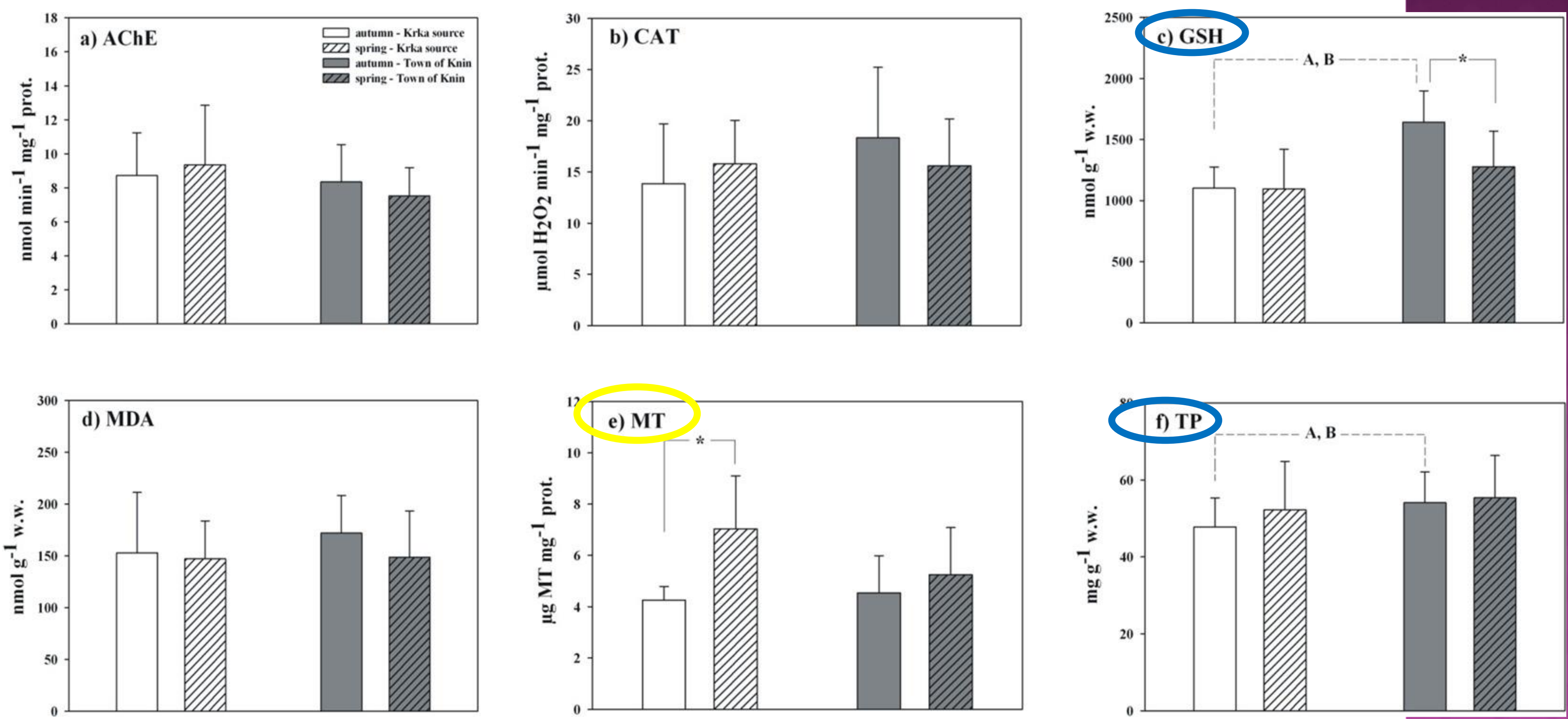


Essential cytosolic metals in fish intestine



Toxic cytosolic metals in fish intestine

Biomarkers in fish intestinal tissue



BIOLOGICAL RESPONSES AT POPULATION LEVEL

Echinogammarus acarinatus

Absence of *E. acarinatus* in the anthropogenically impacted area of the Krka River was already reported and explained as a consequence of their sensitivity on pollution impact, so their habitat in the Krka River comprises only clean parts of the watercourse (Gottstein et al., 2007).

Basic epidemiological characteristics of acanthocephalans (*Dentitruncus trutae*) hosted in brown trout from the Krka River

Locations n=fish number (number of ♂/ ♀/ ND)	Prevalence number and % of fish infected with parasites (95% Wilson score confidence interval)	Mean intensity of infection (mean±S.D.) (♂/ ♀/ ND)	Total number of parasite individuals in sampled brown trout (♂/ ♀/ ND)
Krka River spring n=10 (6/ 3/ 1)	10 (100%) 0.72-1.0	32.6±21.8 (32±19.3/ 37.7±33.1/ 21±0)	326 (192/ 113/ 21)
Krka River downstream of wastewater outlets n=10 (3/ 7/ 0)	8 (80%) 0.49-0.94	35.5±33.5 (33.3±19.1/ 36.8±42.1/ 0)	284 (100/ 184/ 0)

CONCLUSIONS

- **Concentrations of microelements in biota from the Krka River:**
  - **intestine of brown trout:**
    - **Zn>Fe**>Rb>Cu>Mn<Sr>Cd≥Pb>Tl>Co>Cs
  - **gammarids:**
    - **Sr≥Fe**>Zn>Cu>Mn>Rb>Cd≥Pb≥Co>Tl>Cs
  - **acanthocephalans:**
    - **Fe>Zn**>Cu>Mn>Sr≥Rb>Pb>Tl>Cd>Co>Cs

- **Acanthocephalans showed more effective metal accumulation than fish and gammarids, especially of toxic metals (Tl, Pb, Cd).**

- **Bioconcentration factors** indicated 3-55 times higher metal levels (Cd, Tl, Pb, Cu, Sr, Ca, Mg, Mn) in parasites compared to fish and 3-22 times (Tl, Pb, Cd) compared to gammarids.

- Involvement of acanthocephalans and fish intestinal tissue in metal exposure assessment gives valuable data, which might serve as a sensitive bioindicators of bioavailable metal levels in the freshwater ecosystem

CONCLUSIONS

- Anthropogenic impact downstream of the technological and municipal wastewater inputs was **evident for biomarker of antioxidant capacities (GSH) and general stress (TP).**

- Spatial differences of metal levels were comparable in all organisms and indicated **higher Co, Cu, Fe and Mn at polluted and Cd, Rb and Tl at the reference site.**

- **Increased Cd, Tl, Rb levels in organisms form the Krka spring are probably of natural origin** but additional detailed investigations on sediment and dietary metal sources in the Krka River are needed.

- **Anthropogenic impact in the Krka River still seems to be only moderate** but it is of growing concern that both metals and some biomarkers confirmed pollution impact on water and organisms near the town of Knin.

- There is a rising need of **strict monitoring of water quality and health of aquatic organisms in the Krka River, as well as continuous protection of the Krka National Park**, which is of strategic importance for the Republic of Croatia.


PETZL

Special thanks to all participants of the AQUAPMET project

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