Metal concentrations in the fish gut content as indication of dietary exposure and in fish calcified structures as long-term exposure

FINAL PROJECT MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zuzana Redžović

Zagreb, February 17, 2025

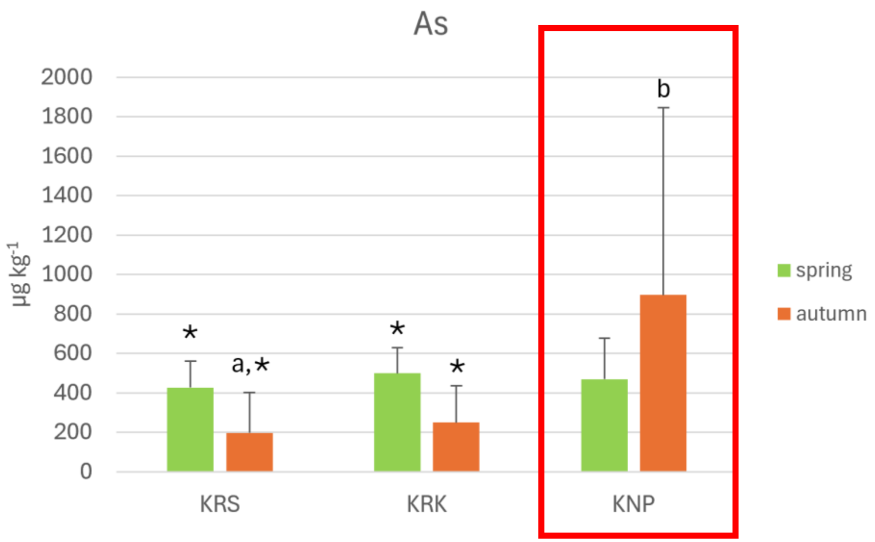
Metal(loid) concentrations in the gut content of brown trout *S. trutta*

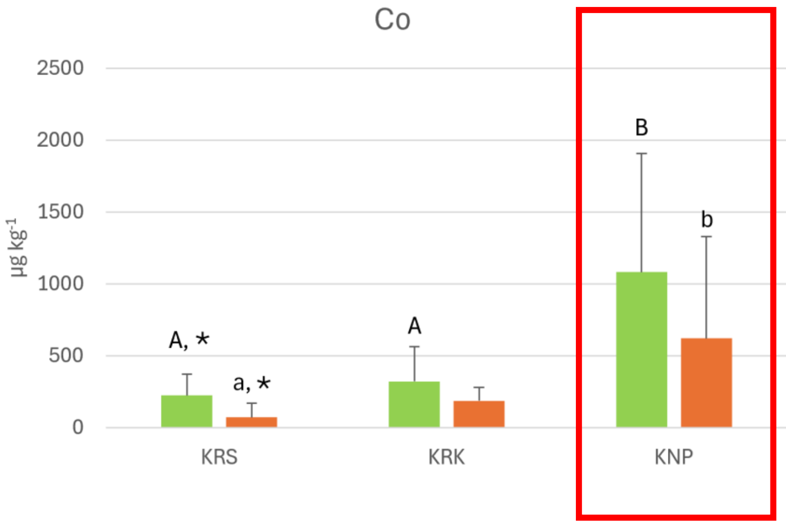
- the first report on metal(loid)s in GC of brown trouts
- omnivorous species (insect larvae, detritus, plants, gammarids, mollusks) → high variations in metal(loid) concentrations among individual fish
- sequences of metal(loids) notably **different** in comparison to **fish intestine** → accumulation depends on the **bioavailability** of metal(loid)s in **fish diet**

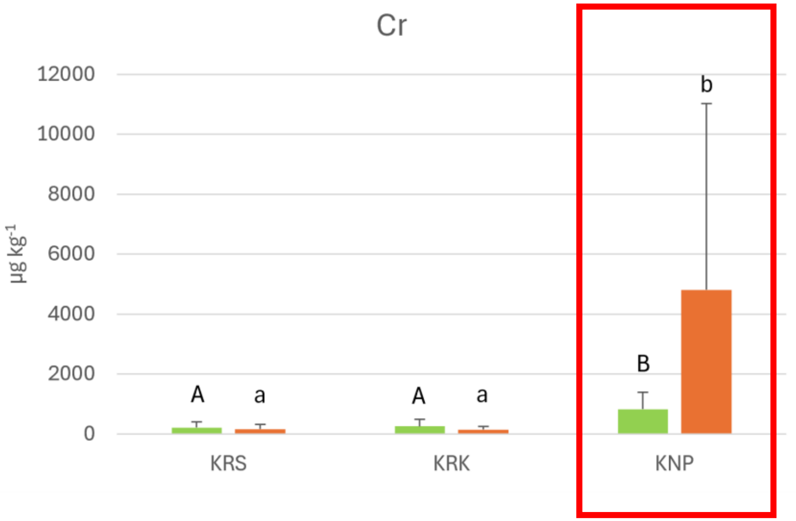
Fe > Zn > Sr ≥ Mn > Cu > Ba ≥ V ≥ Cr ≥ Co ≥ Rb ≥ Pb ≥ Cd ≥ As > Se ≥ Mo > Cs > Tl > Hg

Metal(loid) concentrations in the gut content of brown trout *S. trutta*

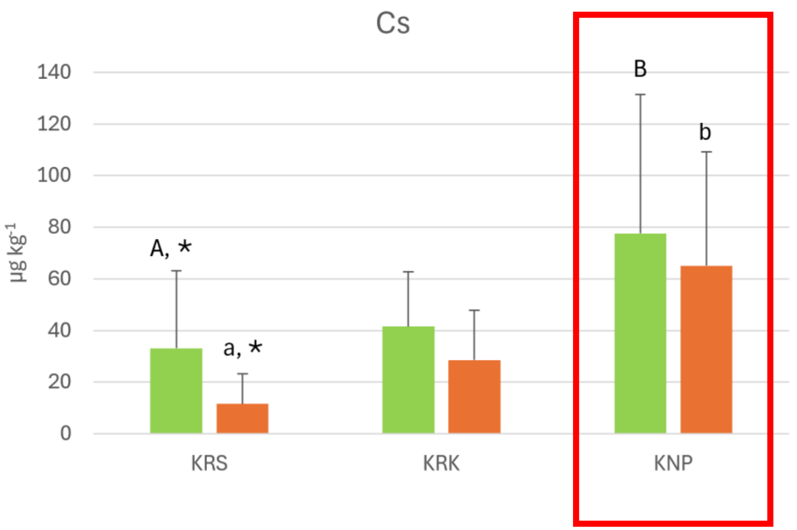
SPATIAL DIFFERENCES

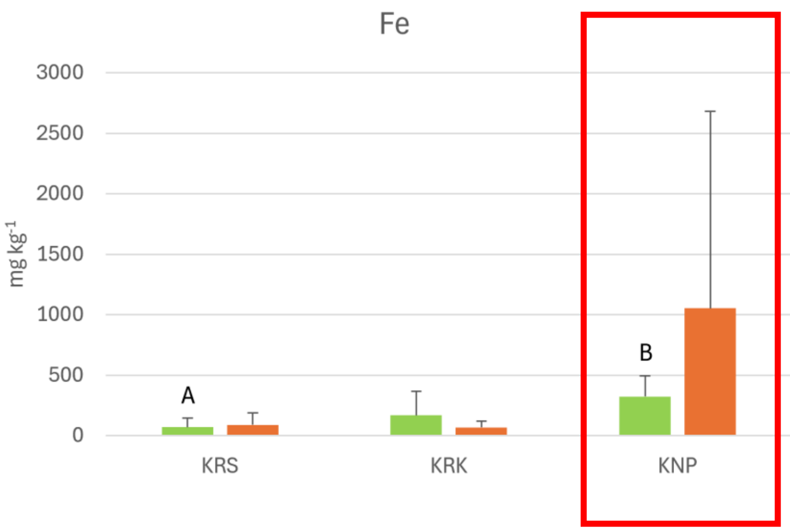


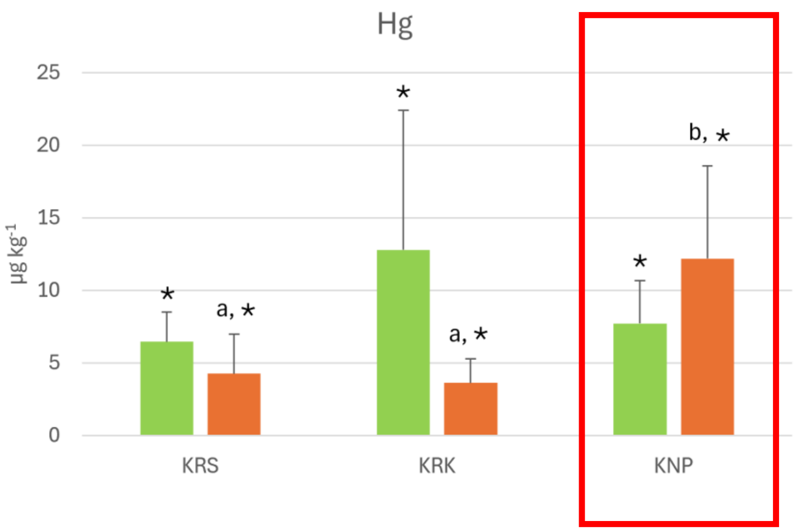




the levels of most of elements were **highest** in GC of fish from **KNP** - similar to spatial distribution in river sediment

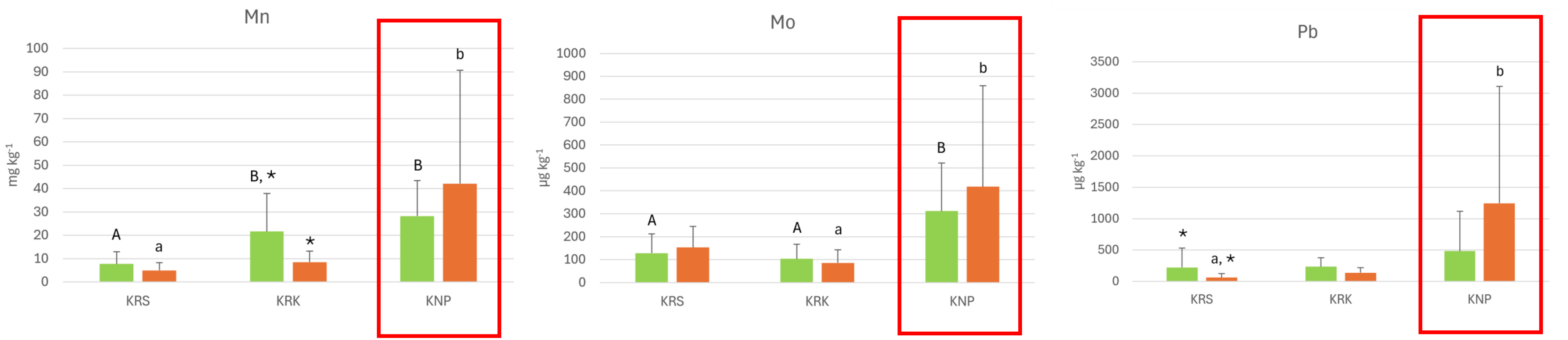




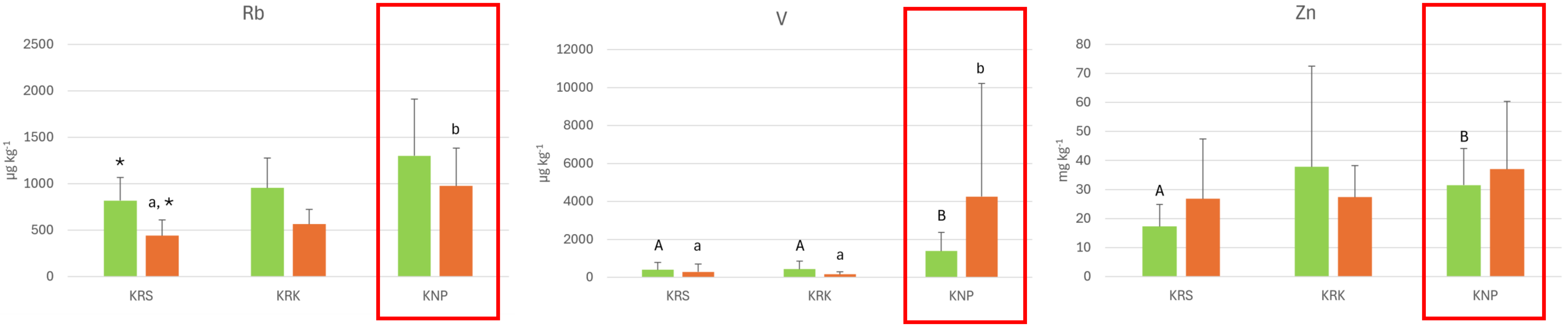


Metal(loid) concentrations in the gut content of brown trout *S. trutta*

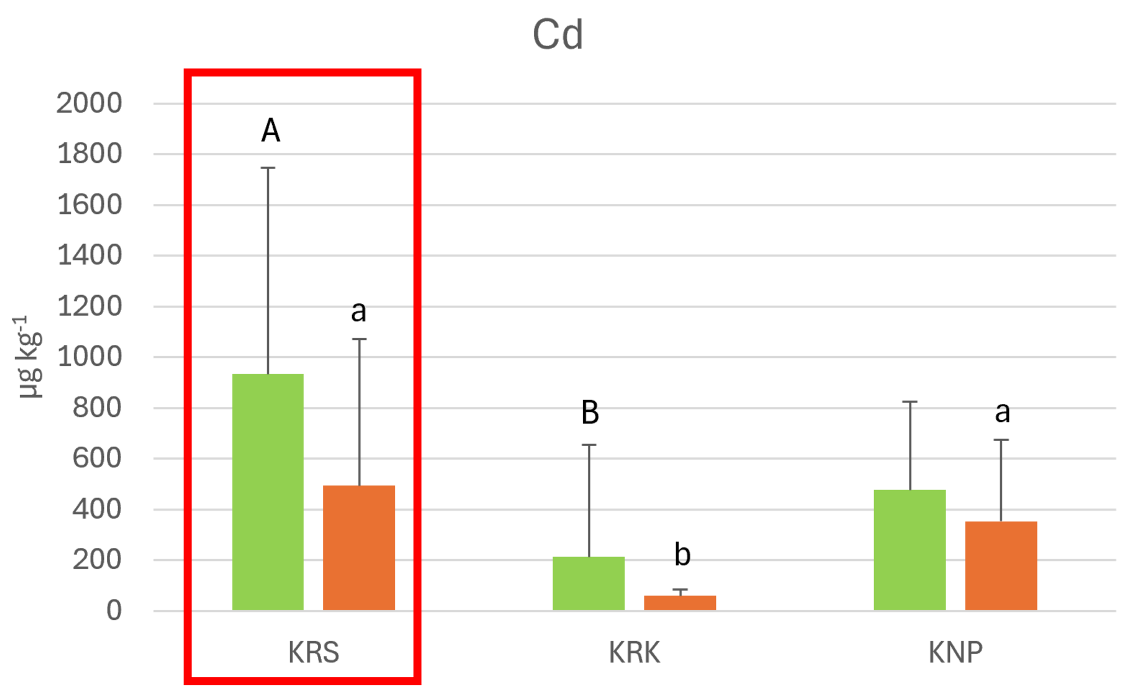
SPATIAL DIFFERENCES

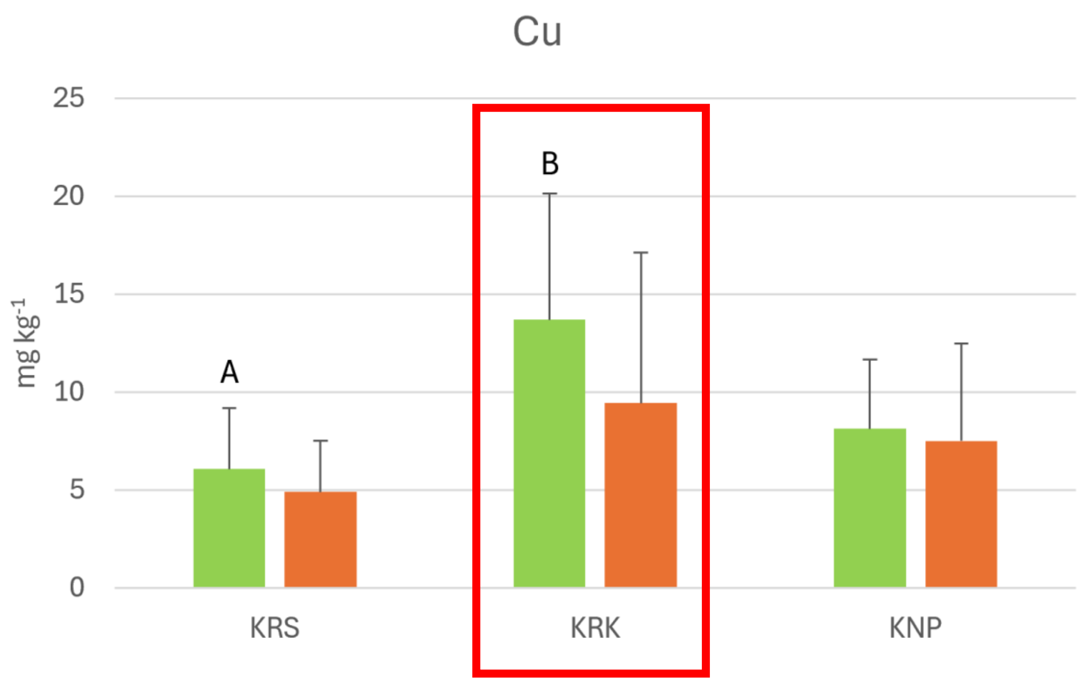


Overall, metal(loid) concentrations in GC of fish were typically the lowest at KRS → the lowest metal(loid) exposure



Metal(loid) concentrations in the gut content of brown trout *S. trutta*



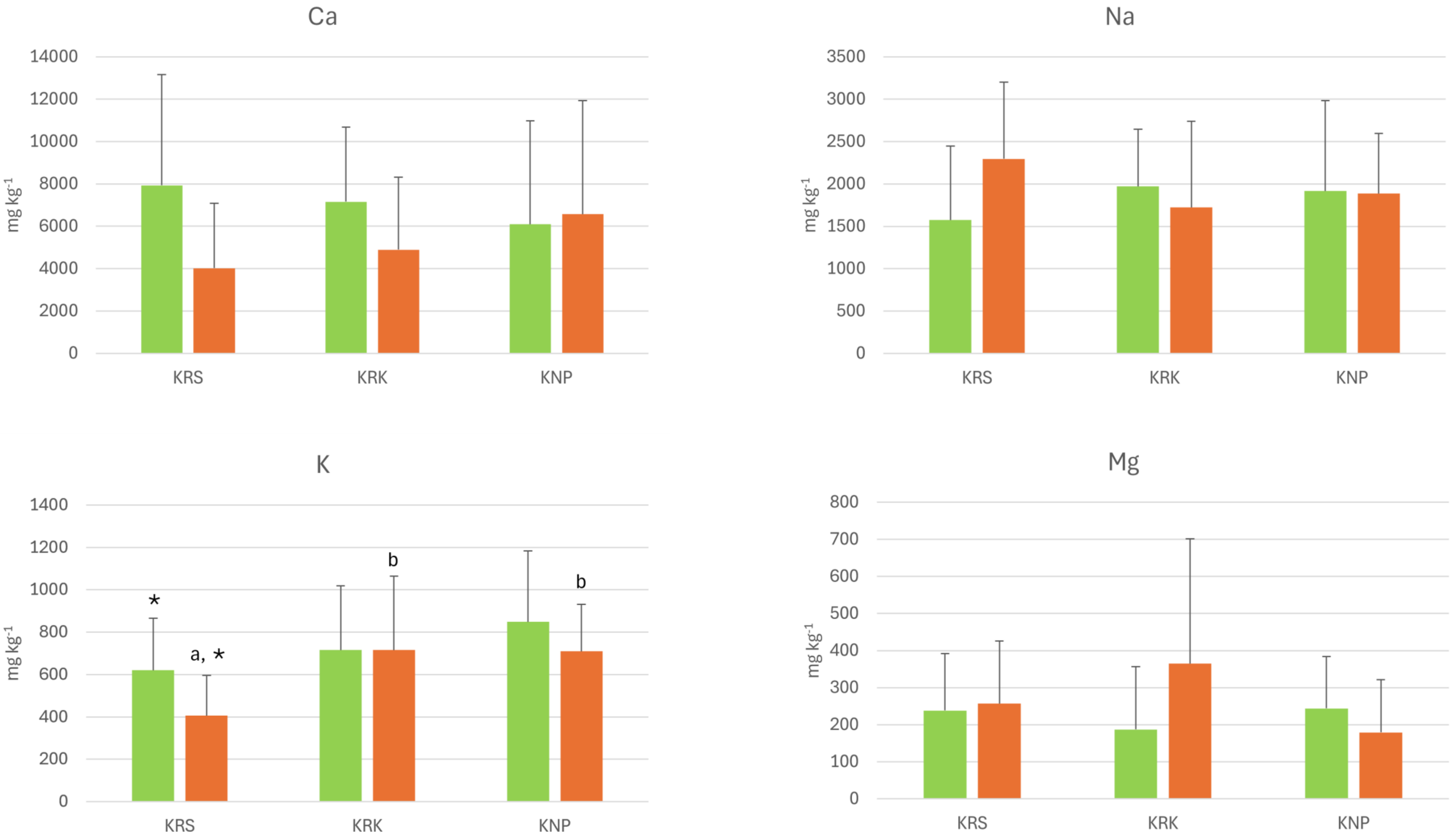


- aligns with the spatial distribution of Cu in **fish intestine** and **acanthocephalans**

Macroelement concentrations in the gut content of brown trout *S. trutta*

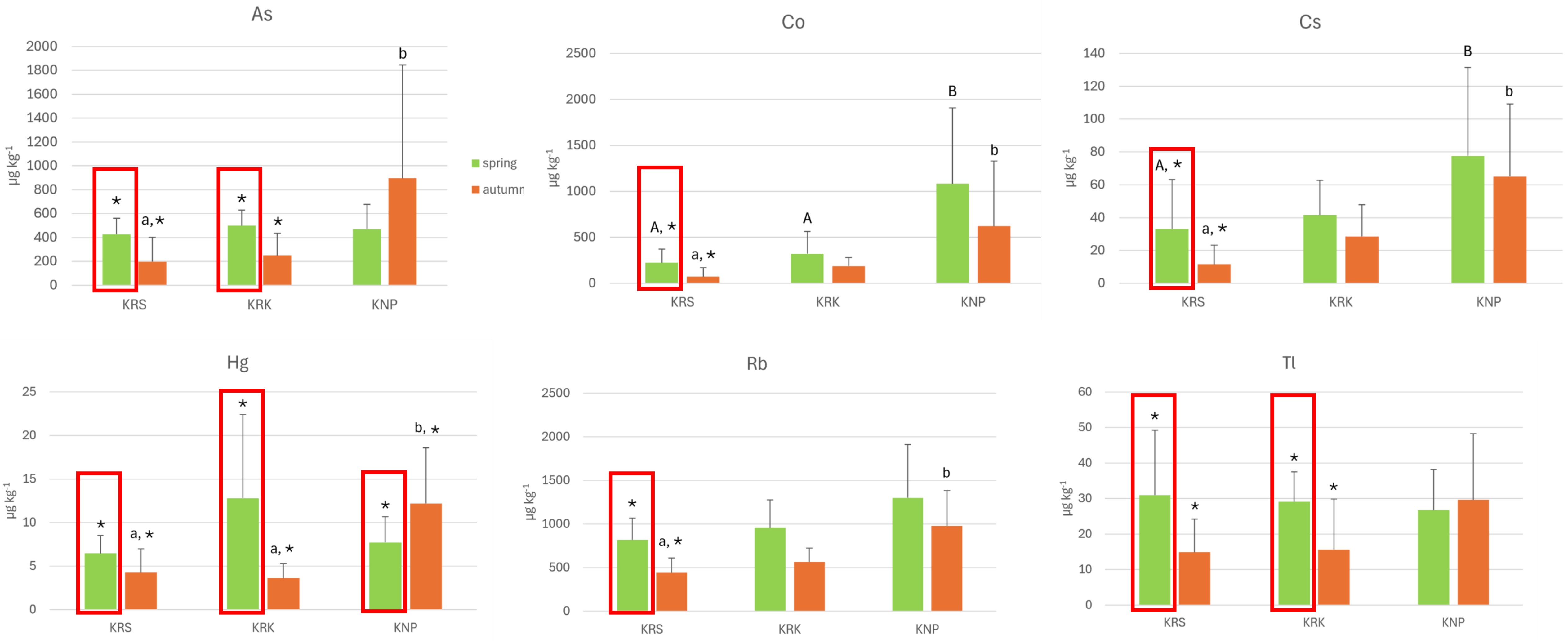
SPATIAL DIFFERENCES

Ca > Na > K > Mg



Metal(loid) concentrations in the gut content of brown trout *S. trutta*

TEMPORAL DIFFERENCES



- the majority of elements in GC had **higher concentrations** in **spring** than autumn
- **higher gut fullness indices** found in spring – maximum feeding rates
- **similar pattern** also observed in **parasites** and **intestine**

Relationships between metal(loid) concentrations in fish intestine and acanthocephalans to gut contents

Ratio %	Krka River source (KRS)		Town of Knin (KRK)		Krka National Park (KNP)	
	INT/GC	AC/GC	INT/GC	AC/GC	INT/GC	AC/GC
As	13.9	30.0	25.2	36.5	7.5	28.4
Ba	0.68	4.5	0.79	7.3	0.63	6.4
Cd	43.5	313	26.7	157	23.9	59.3
Co	18.1	20.5	27.0	23.4	9.5	7.1
Cr	10.6	23.0	13.8	24.6	1.6	2.9
Cs	66.8	31.3	22.5	12.8	5.5	3.6
Cu	19.3	190	14.6	181	12.1	87.9
Fe	13.5	[illegible]	15.6	20.4	3.5	3.9
Hg	394	[illegible]	279	827	362	792
Mn	8.4	[illegible]	6.1	33.5	3.2	14.2
Mo	28.1	[illegible]	50.3	24.8	14.2	7.1
Pb	7.0	[illegible]	5.4	221	5.1	42.3
Rb	648	399	481	273	216	151
Se	180	237	263	197	250	189
Sr	1.2	5.1	0.97	8.0	2.6	14.0
Tl	193	4355	101	2793	110	1039
V	4.5	4.6	5.1	7.5	1.0	1.1
Zn	872	216	836	133	584	90.0
Ca	2.4	13.1	2.8	25.2	3.8	18.3
K	643	458	446	316	386	285

INT/GC
< 15%

- minimal bioavailability from food to the fish intestine → their absorption is physiologically regulated

Relationships between metal(loid) concentrations in fish intestine and acanthocephalans to gut contents

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	INT/GC	AC/GC	INT/GC	AC/GC	INT/GC	AC/GC
As	13.9	30.0	25.2	36.5	7.5	28.4
Cr	10.6	23.0	13.8	24.6	1.6	2.9
Cs	66.8	31.3	22.5	12.8	5.5	3.6
Cu	19.3	190	14.6	181	12.1	87.9
Fe	13.5	12.3	15.6	20.4	3.5	3.9
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Mn	8.4	63.3	6.1	33.5	3.2	14.2
Mo	28.1	13.1	50.3	24.8	14.2	7.1
Pb	7.0	241	5.4	221	5.1	42.3
Rb	648	399	481	273	216	151
Se	180	237	263	197	250	189
Sr	1.2	5.1	0.97	8.0	2.6	14.0
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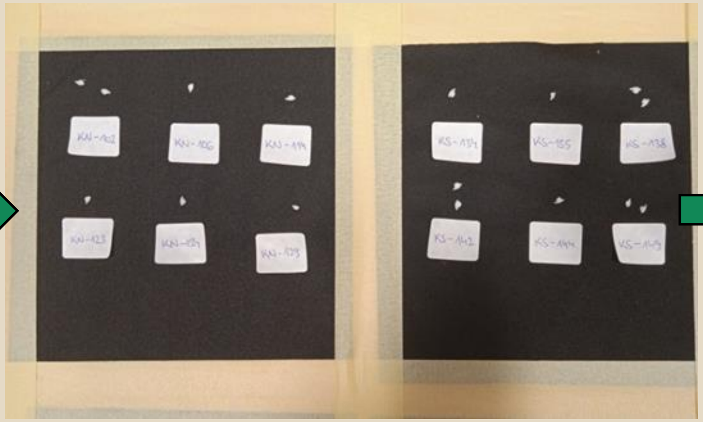
V	4.5	4.6	5.1	7.5	1.0	1.1
Zn	872	216	836	133	584	90.0
Ca	2.4	13.1	2.8	25.2	3.8	18.3
K	643	458	446	316	386	285
Mg	63.7	148	63.6	195	72.7	74.7
Na	52.1	95.4	53.3	84.5	52.2	84.9

- remarkable luminal absorption efficiency

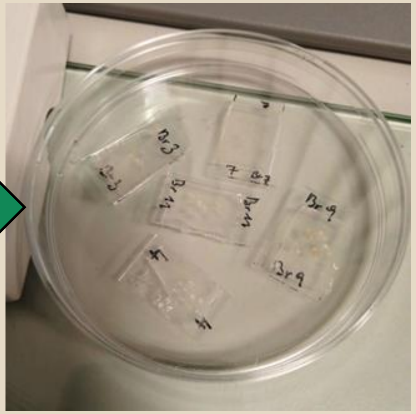
Calcified structures in fish - sample preparation

- otoliths

lapping films of 30µm and 3µm



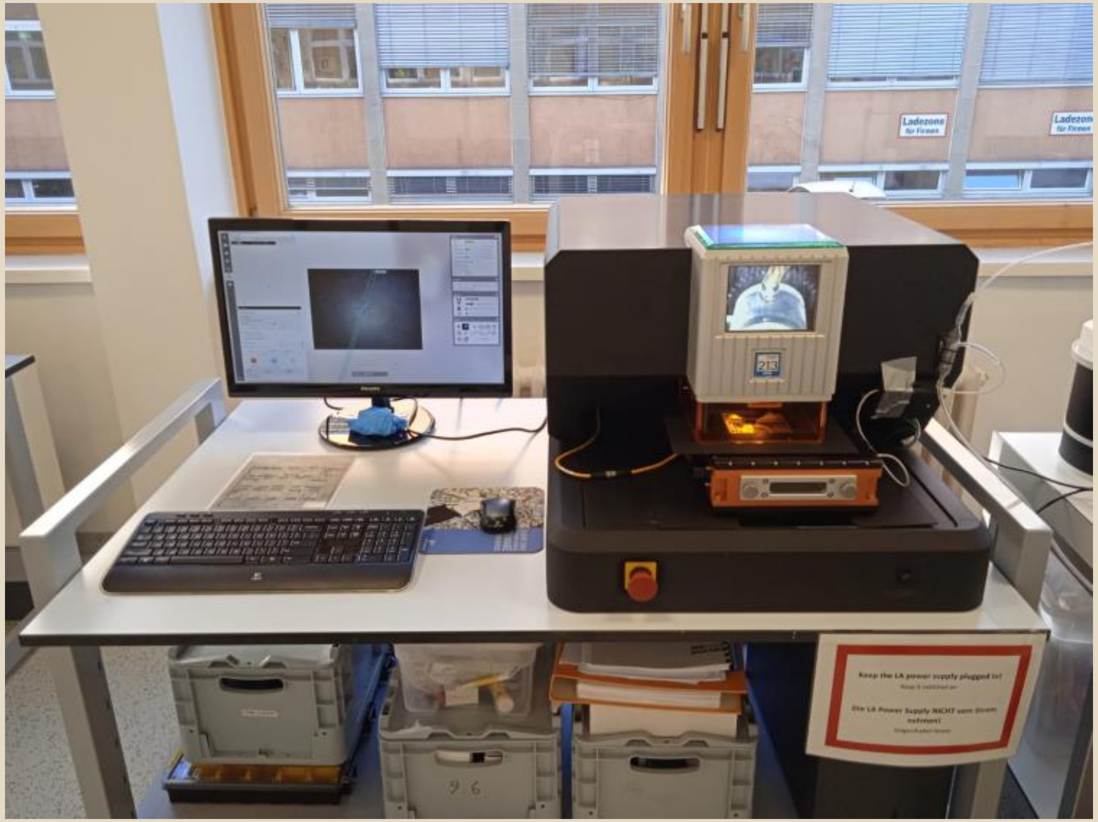
- scales



- photographed using a binocular microscope with a digital camera
- printed on A3 paper, the most appropriate lines for measurements determined and marked

Measurements

- laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)



ESI NWR 213 Laser-Ablation System

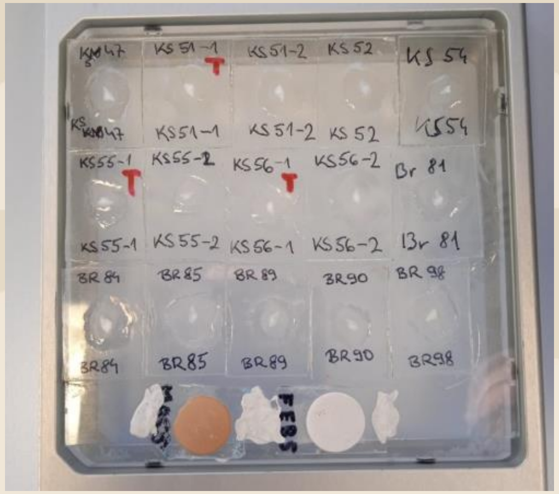
Agilent 8800 ICP-MS-CRC-MS

Measurements

- **Otoliths**
- **FEBS-1** (Otolith Certified Reference Material for Trace Metals, National Research Council Canada) and **MACS-3** (Calcium carbonate standard, United States Geological Survey, 189 USA)

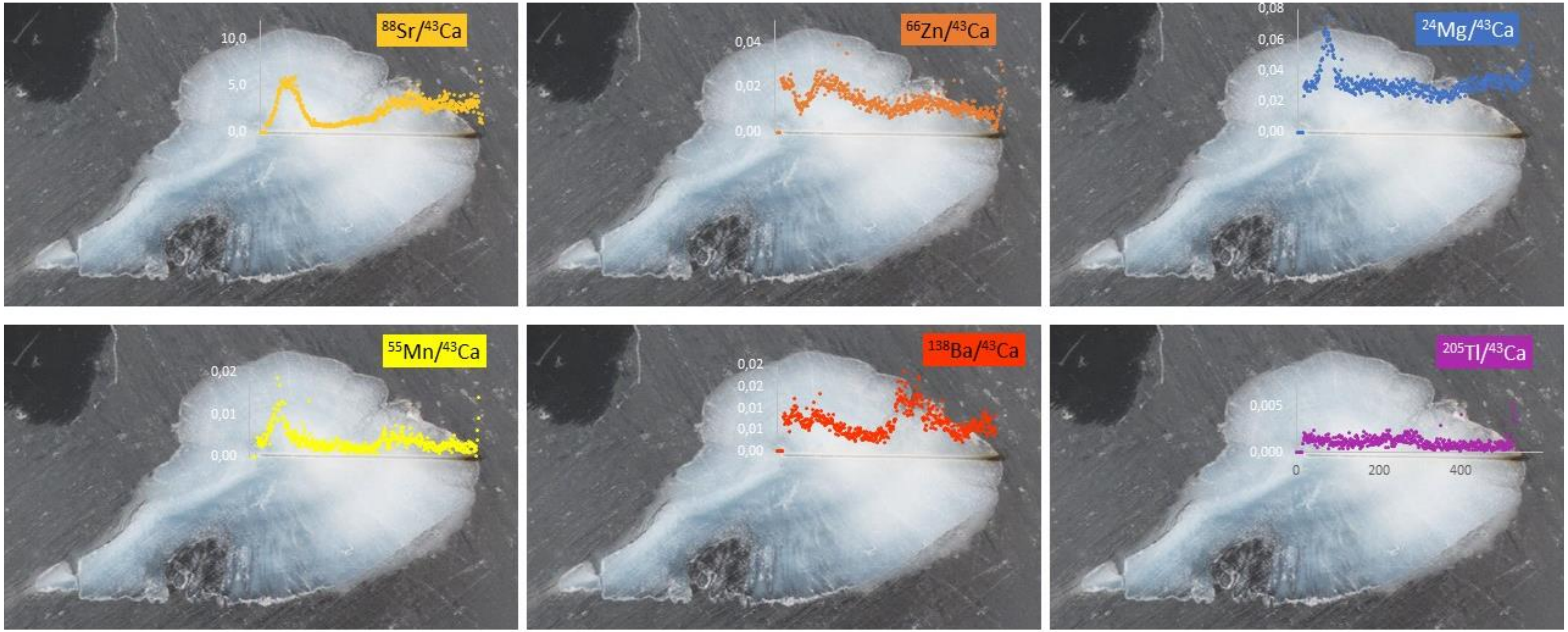
- **Scales**
- **NIST SRM 1400** (Bone ash, National Institute of Standards and Technology, Gaithersburg, MD, USA) and **NIST SRM 1486** (Bone meal, National Institute of Standards and Technology, Gaithersburg, MD, USA)

Isotopes	Abundance	Integration time (s)
13C		0,2
23Na		0,1
24Mg		0,1
27Al		0,1
43Ca		0,05
44Ca		0,05
55Mn		0,1
56Fe		0,1
57Fe		0,1
59Co	100%	0,1
63Cu	69,15%	0,1
66Zn	27,73%	0,1
75As	100%	0,1
88Sr	82,60%	0,1
111Cd		0,1
114Cd		0,1
133Cs		0,1
138Ba		0,1
202Hg		0,1
205Tl	70,40%	0,1
208Pb	52%	0,1
238U		0,1

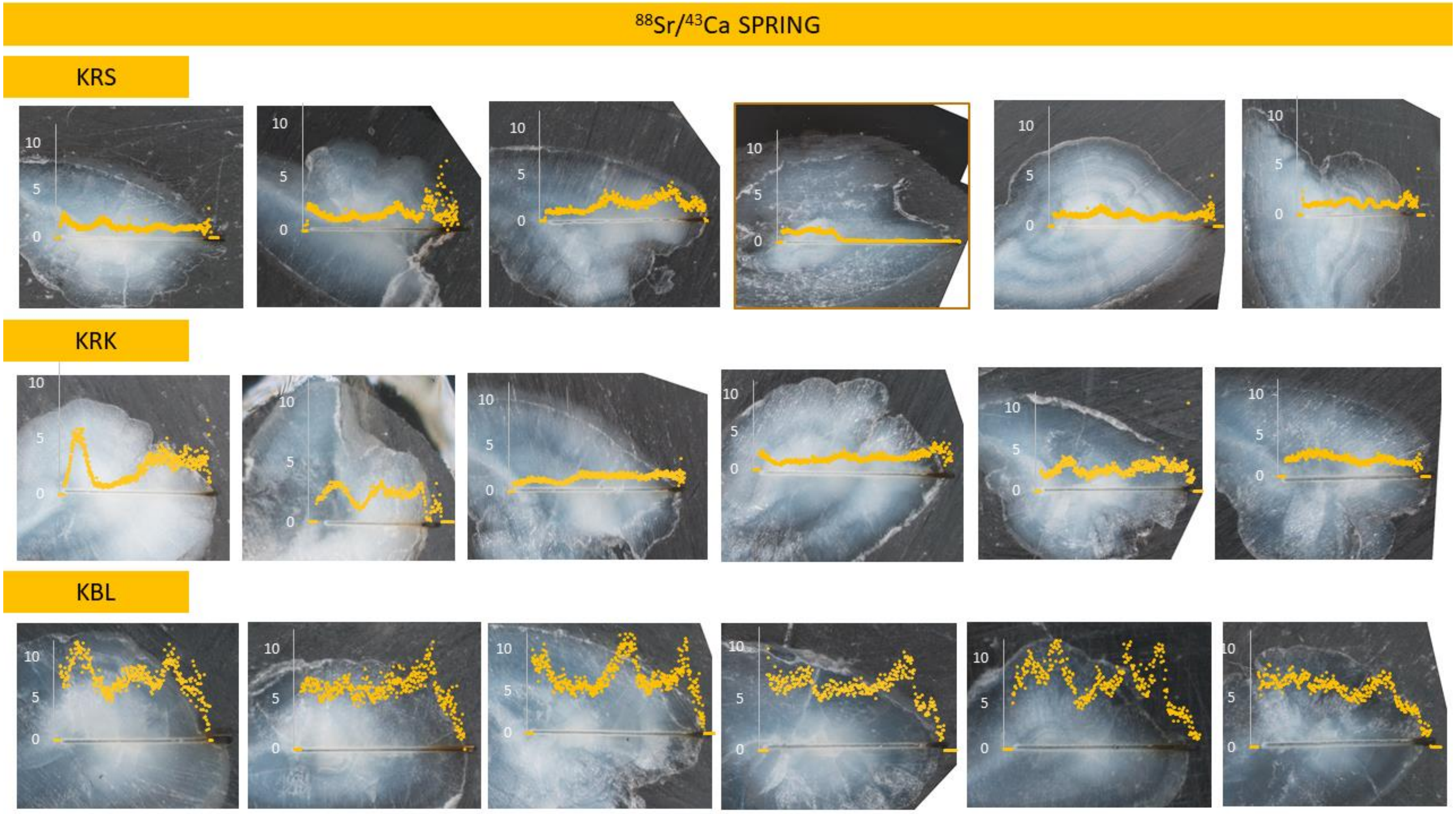


Results - otoliths

- the metal incorporation in the same otolith varied depending on the element and showed different patterns along ablation lines
- most pronounced for:



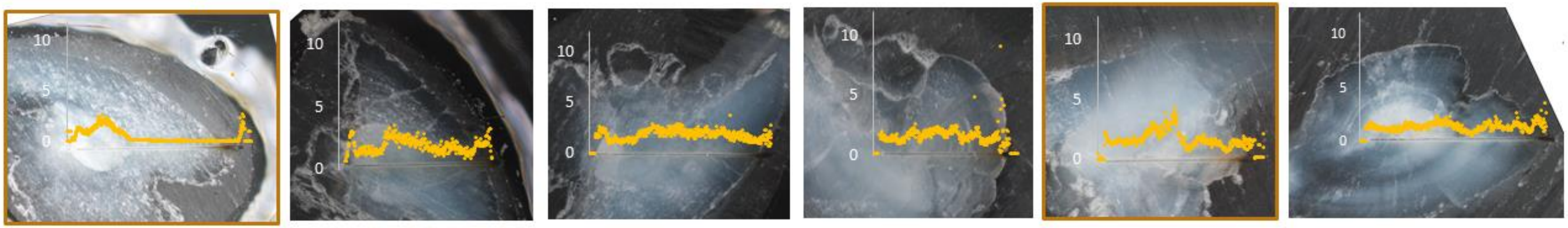
Results - otoliths



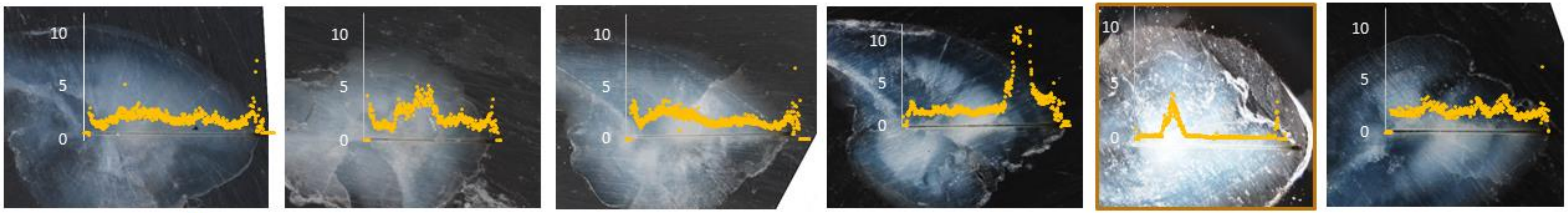
Results - otoliths

$^{88}Sr/^{43}Ca$ AUTUMN

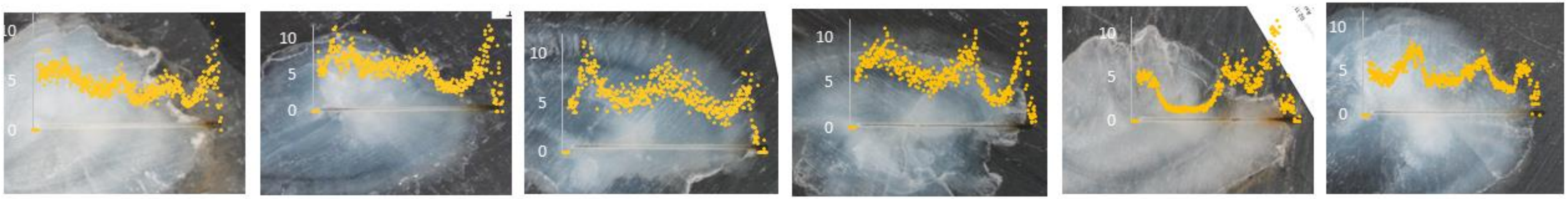
KRS



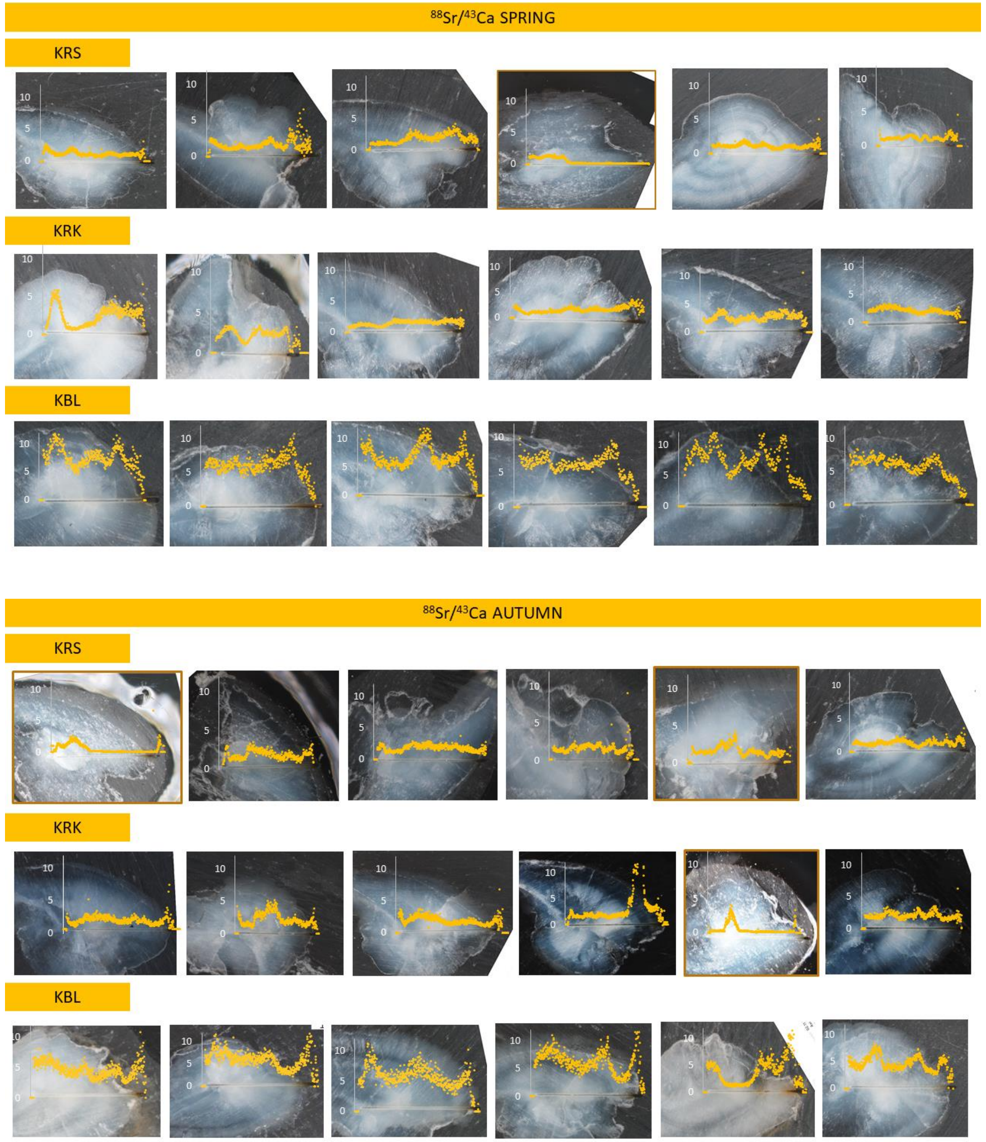
KRK

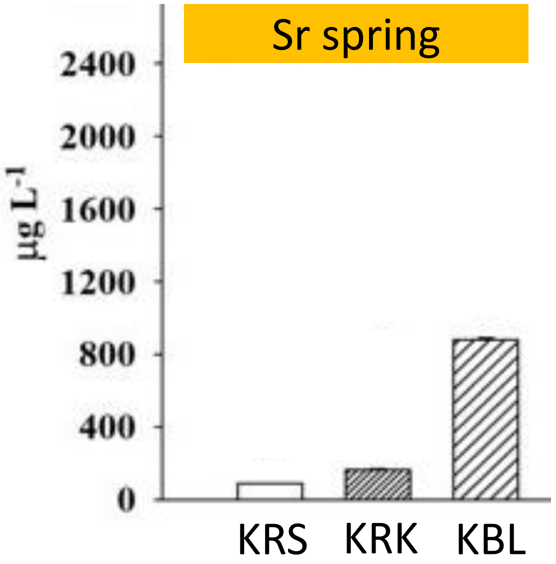


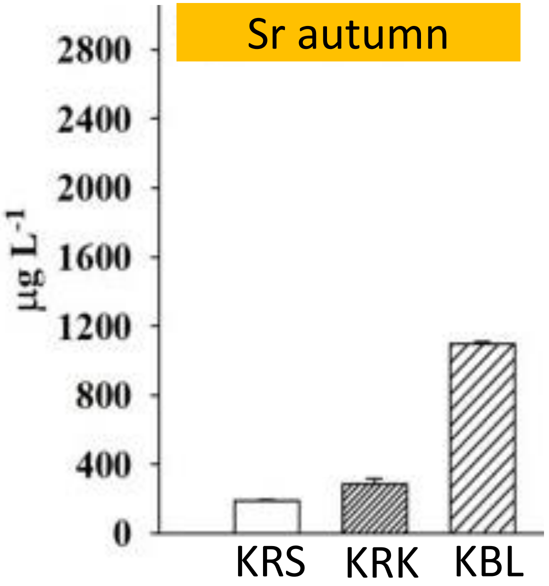
KBL



Results - otoliths

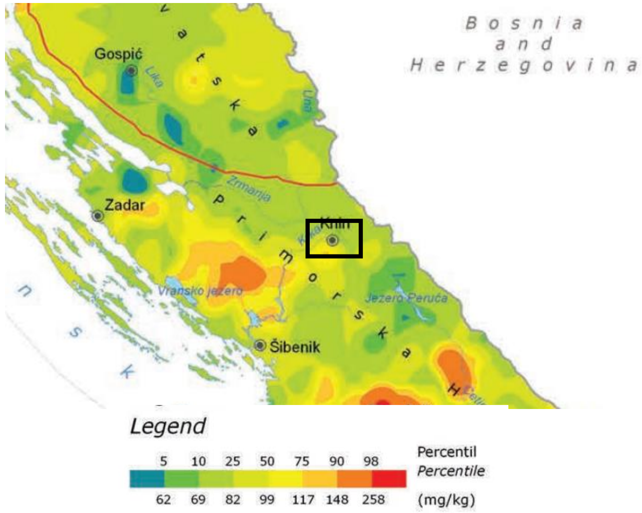






Sr

- KBL > KRK = KRS
- the same trend in river water
- geological differences

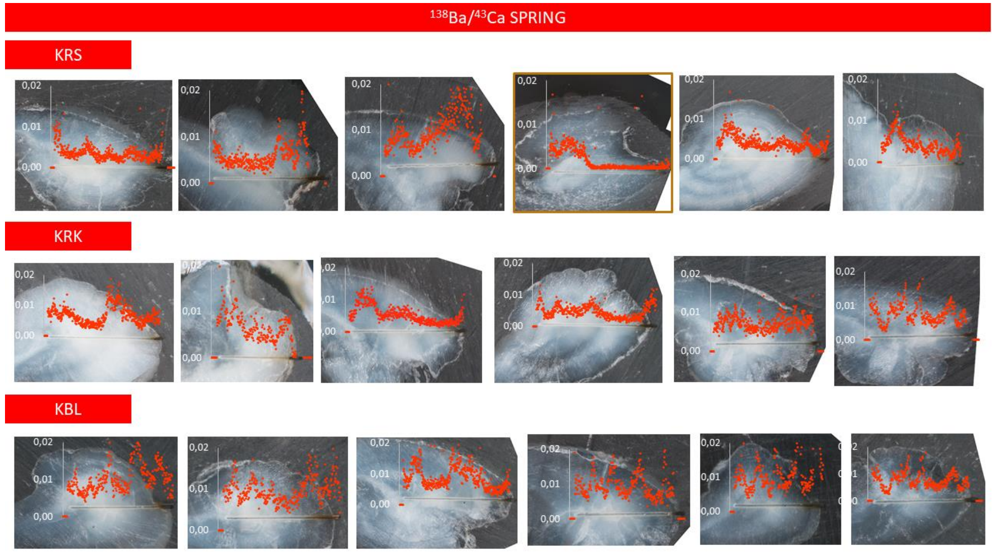


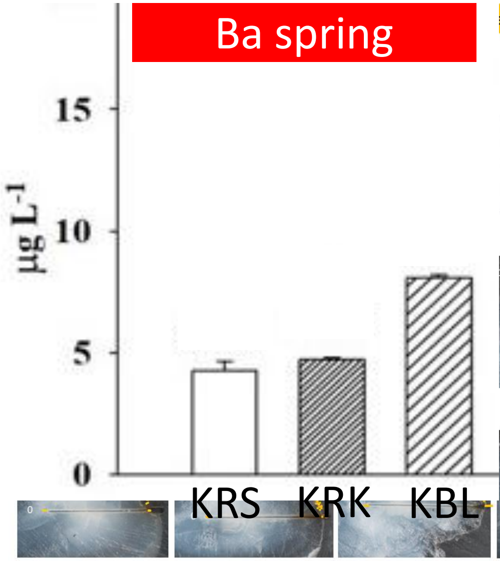
Halamić, J. & Miko, S. (eds) (2009): Geochemical Atlas of the Republic of Croatia.- Croatian Geological Survey, 87pp., Zagreb.

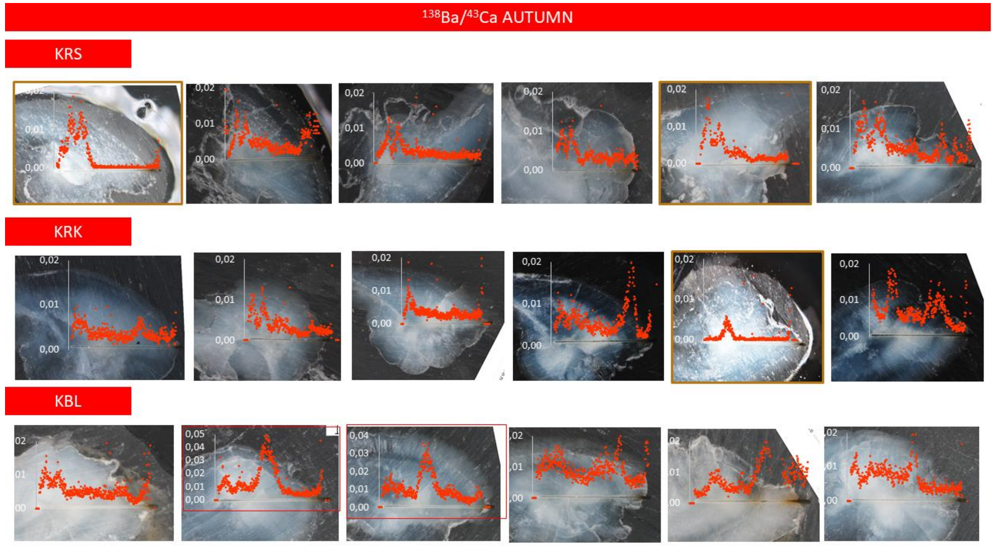
Results - otoliths

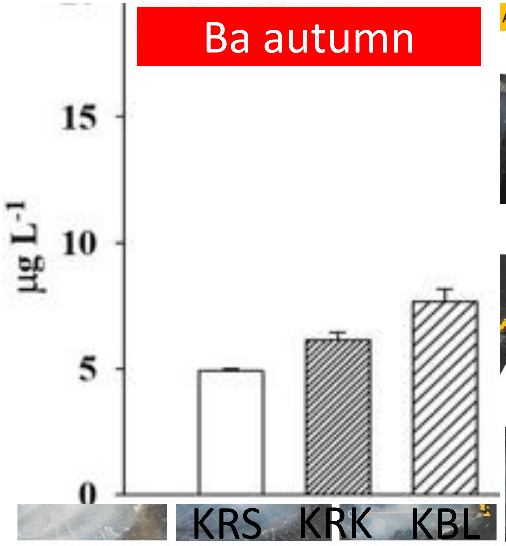
Ba

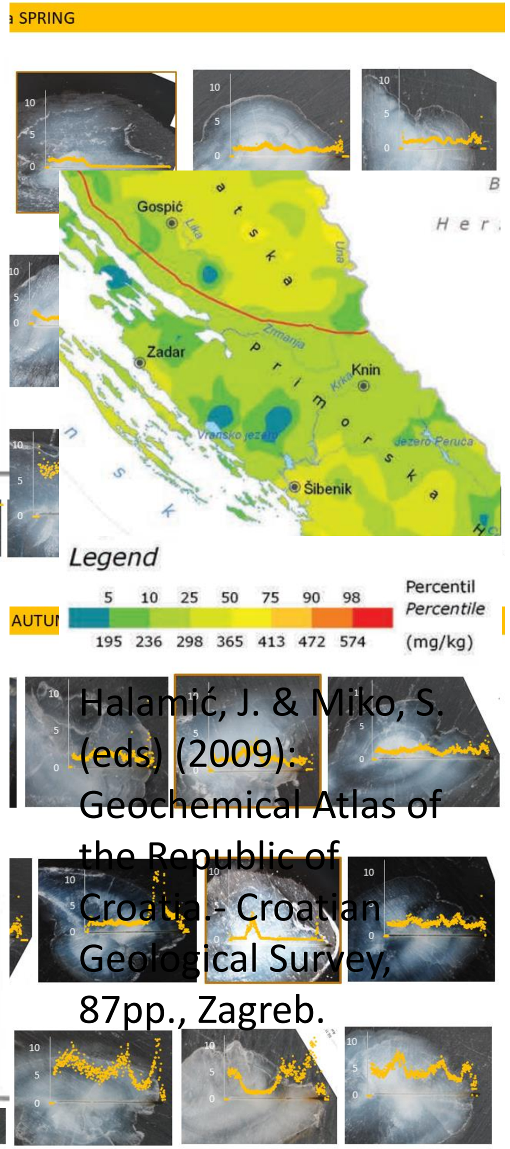
- lower values than Sr, but similar trends
- differences between the sites smaller – consistent with the geological differences and the levels observed in the water











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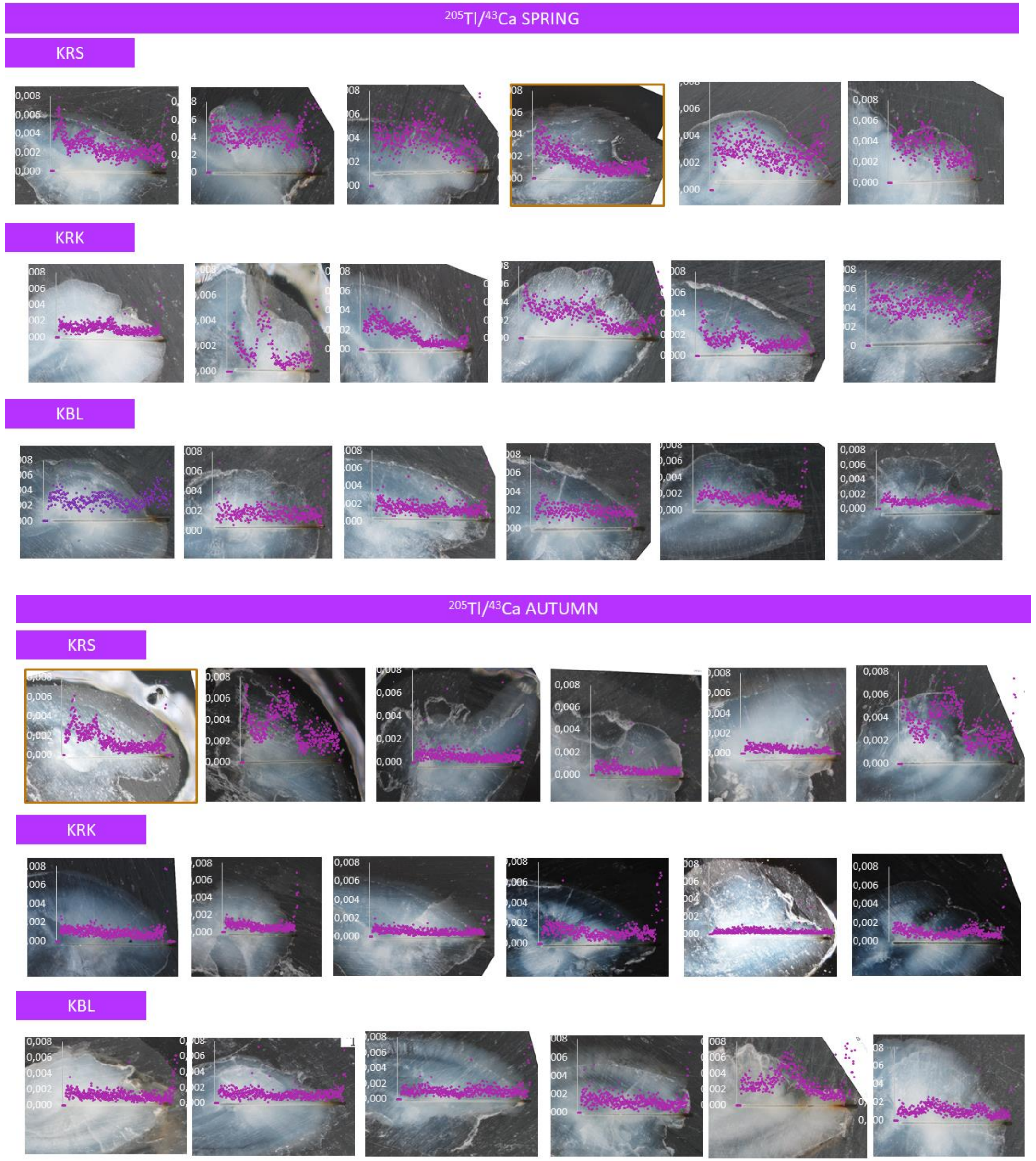
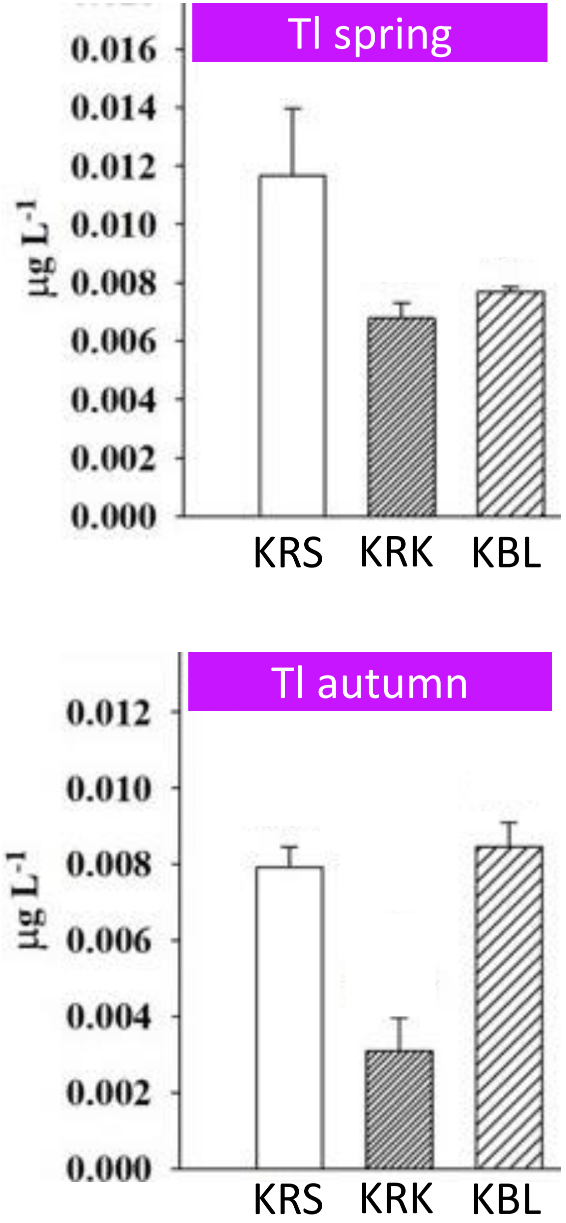
Results - otoliths



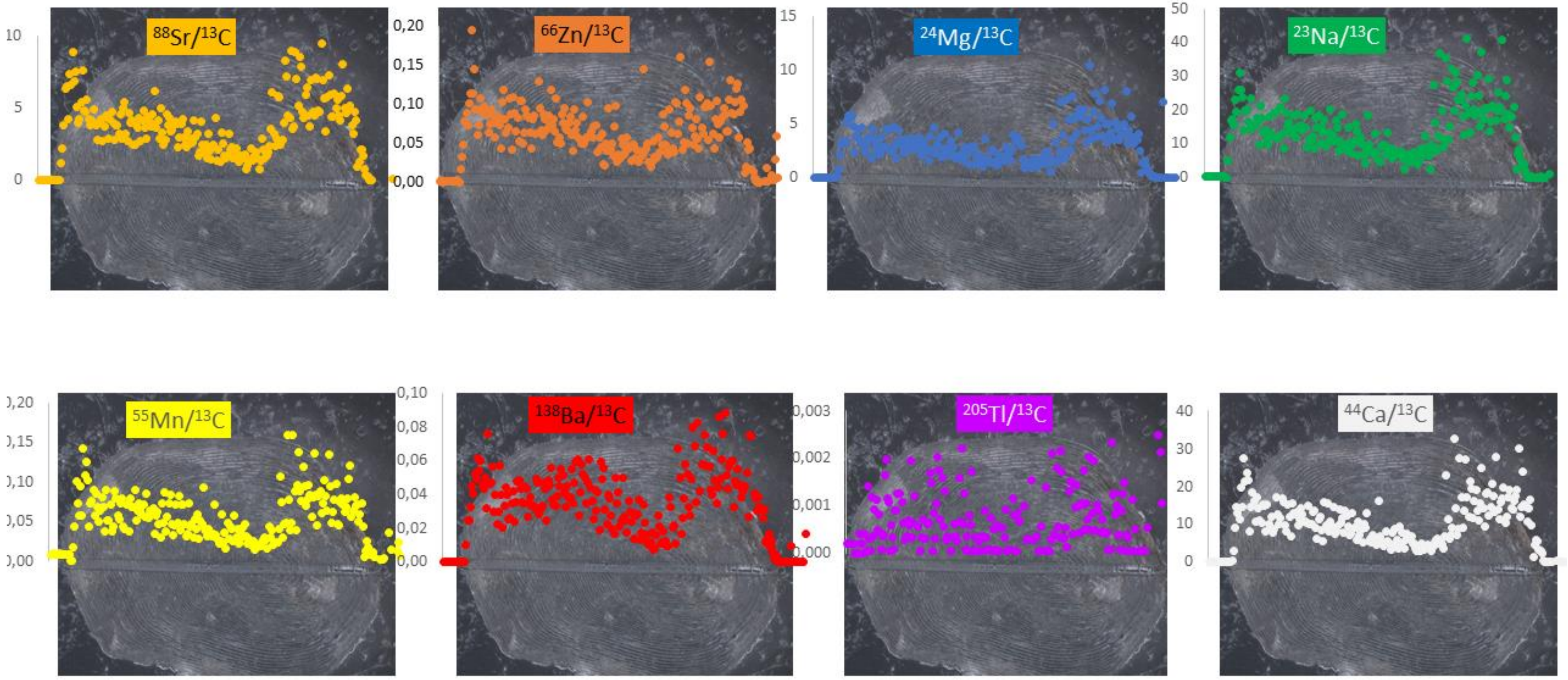


Tl

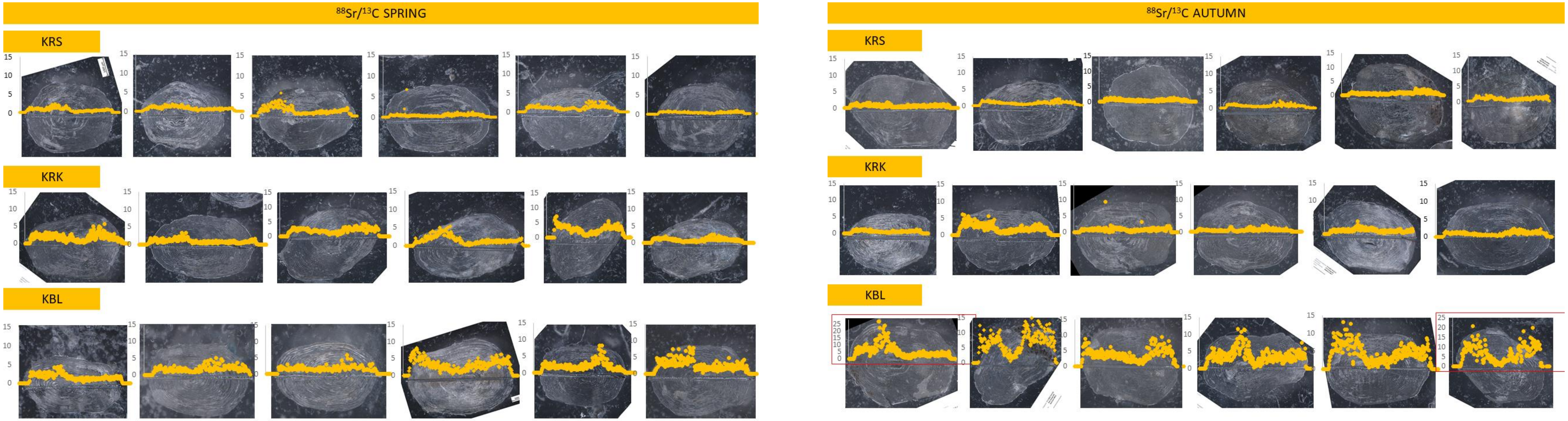
- KRS > KRK = KBL
- in otoliths and water in spring
- the highest Tl concentrations at the source have been consistently confirmed in both water and bioindicator organisms
- reflect geological background of the catchment area

Results - scales

- the trends in metal content profiles along the ablation lines similar for all elements, confirming a continuous metal exposure during the life span of the fish



Results - scales

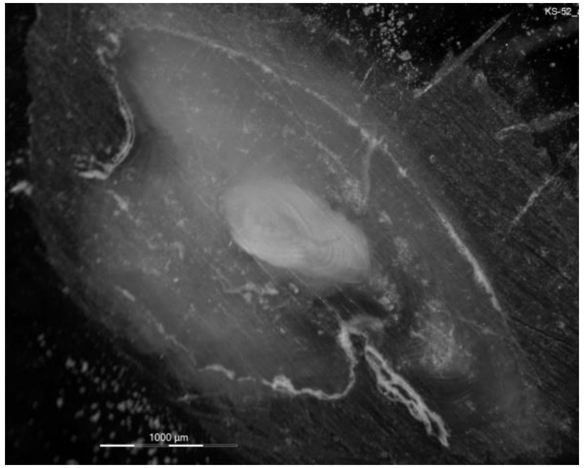
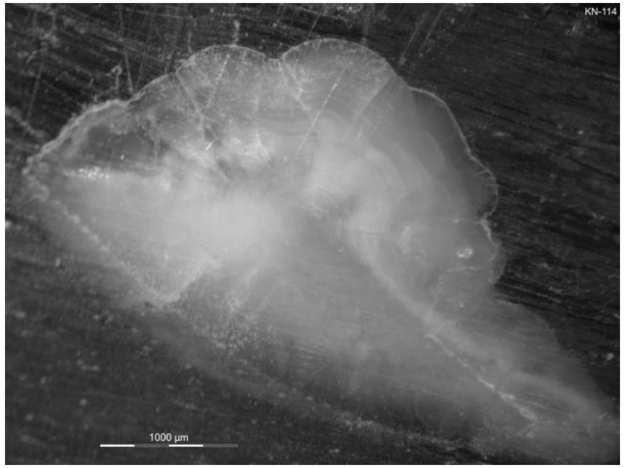
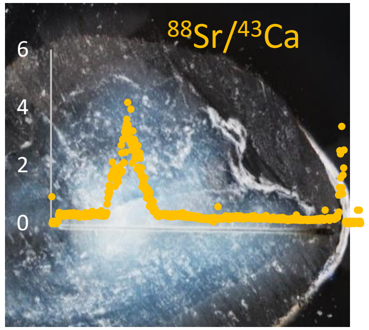


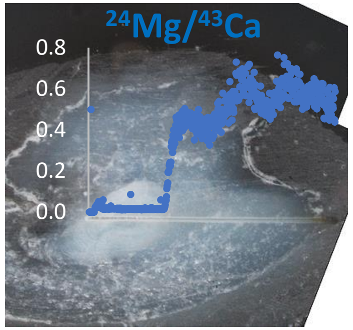
KBL > KRK = KRS

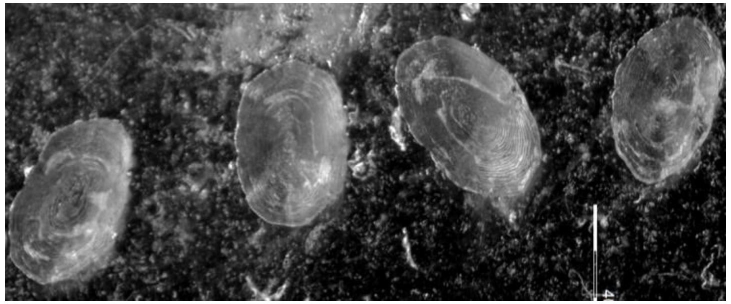
- when comparing between the different sites - most of the elements - the same trend as observed in the otoliths and water
- scales confirmed as a good nonlethal alternative for assessing metal exposure

Stocked fish?

- abnormal otoliths and replacement scales in many individuals – more common in farmed fish
- abnormal otoliths – different shape and composition
  – no variations in temperature and food availability → no annular rings
  – fast growth due to environmental control → development of vaterite instead of aragonite
- replacement scales – form after the loss of scales - injuries in a hatchery
- only the outermost part representative of conditions in the river






Conclusions

- first results on metal(loid)s in GC of brown trouts – indication of dietborne metal uptake
- metal(loid) concentrations in GC were generally the highest at KNP - reflecting the spatial distribution of metal(loid)s in river surface sediment
- ratios between metal accumulation in the intestine and GC, and acanthocephalans and GC indicate:
  - 1) low bioavailability of As, Ba, Ca, Cr, Cu, Fe, Mn, Pb, Sr, and V from the dietary sources;
  - 2) high absorption of Tl, Se, Hg, Rb, K and Zn

  - cautious interpretation: the metal(loid)s in the GC reflect the source of metal(loid)s in the food at the time of sampling, while organisms accumulate metal(loid)s over a longer period of time and exhibit a more chronical response

Conclusions

- fish calcified structures reflect spatial differences in metal concentrations in the environment
- otoliths provide a time resolved record of metal exposure over the life span of a fish
- scales are less reliable indicators of time of the exposure (can be partially reabsorbed by the fish), but represent nonlethal alternative
- structures from the stocked fish will be separated from wild population and data will be further processed and quantified → the first record of water chemistry over the life history of a freshwater fish in Croatia
- these data contribute to interpretation of metal accumulation in fish soft tissues, and to the protection of trout population in the sensitive karst Krka River

BIOTOXMET

- Vlatka Filipović Marijić
- Tatjana Mijošek
- Sara Šariri
- Irena Vardić Smrzlić
- Zorana Kljaković-Gašpić
- Želimira Cvetković
- Željka Fiket
- Damir Valić
- Tomislav Kralj
- Josip Barišić
- Marija Kuštro
- Ivana Karamatić
- Dušica Ivanković
- Zrinka Dragun
- Zoran Kiralj

HRZZ

- Croatian Science Foundation project: Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET) project number: IP-2020-02-8502

BIOTOXMET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Donata Bandoniene


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Thomas Prohaska

Montanuniversität Leoben, Leoben, Austria

Thank you!


Andreas Zitek

Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, Tulln, Austria

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