# Metal concentrations in fish scales and otoliths

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

Zagreb, 19<sup>th</sup> May 2023











### **2.4. MEASUREMENT OF METAL CONCENTRATIONS IN FISH CALCIFIED STRUCTURES**

Calcified structures will be cleaned by Milli-Q water and dried. Otoliths will be ground and polished. For each sample 4-6 scales will be prepared and mounted on small glass slides using adhesive tape and the scale with the most visible growth zones per sample will be analyzed. Measurement of metals will be conducted by connecting a laser ablation system (NWR193, Electro Scientific Industries) to an ICP-QMS (NexION 350D, PerkinElmer) and the laser lines will be taken through the middle of the hard tissues. Calcium, as a main element in the aragonite of otoliths and hydroxyapatite of scales, will be used as internal standard.













Gulnaz

Stefan

Donata

Johanna

Thomas

Andreas





# Sample preparation







### **Preparation of scales**





### better to use paper envelopes/bags













2000 µm

pictures printed on
 A3 papers and best
 positions of ablation
 lines marked





### **Preparation of otolithes**





3 BRT 10.2-2 BHEF BP-S-Br4-1 BR9-1 BRT KN21 BRI 319-4 KN24-2 BRAZ KN21 BRM V.n24~2 KN25-1 KN25-2 KN 26 KN 43 KN 28 KN 26 KN28 KN25-2 KN43 N25-1

Krazy

glue

lapping films of 30  $\mu m$  and 3  $\mu m$  for grinding and polishing



 pictures printed on A3 papers and best positions of ablation lines marked

abnormal otoliths

 no year rings
 different shape
 and composition





### **Measurements on LA-ICP-MS**



ESI NWR 213 Laser-Ablation System

Agilent 8800 ICP-MS-CRC-MS



![](_page_16_Picture_1.jpeg)

![](_page_16_Picture_2.jpeg)

![](_page_16_Picture_3.jpeg)

### 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)

Laser (NWR213, ESI)		Unit	
spot size	50	μm	
speed	5	µm/s	
rep rate	10	Hz	
energy output	15	%	
energy		mJ	
line scan distance (x-direction)	600	μm	
distance between lines (core)		μm	
warm-up (delay)/gas blank	30	S	
wash-out (delay)	30	S	(20 s for samples)
carrier gas	Не		
carrier gas flow rate	0,900	L/min	
fluence		J cm2	
line time	120,0	S	
transfer time		S	
shutter (IVA / XYR)	IVA		

ICP-QQQ-MS (8800, Agilent)	Value	Unit
RF power	1550	W
nebulizer gas flow		L/min
auxillary gas flow		L/min
plasma gas flow		L/min
total acquisition time/integration time		S
wash out/buffer time	30,00	S
sum time (whole sample)	180	S
total acquisitions/line	#DIV/0!	

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
205TI	70,40%	0,1
208Pb	52%	0,1
238U		0,1

### Otoliths

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

Laser (NWR213, ESI)	Unit
spot size	40µm
speed	2μm/s
rep rate	20Hz
energy output	45%
energy	mJ
line scan distance (x-direction)	300µm
distance between lines (core)	μm
warm-up (delay)/gas blank	30s
wash-out (delay)	30s
carrier gas	Не
carrier gas flow rate	0,900L/min
fluence	J cm2
line time	150,0s
transfer time	S
shutter (IVA / XYR)	IVA

ICP-QQQ-MS (8800, Agilent)	Value	Unit
RF power	1550	W
nebulizer gas flow		L/min
auxillary gas flow		L/min
plasma gas flow		L/min
total acquisition time/integration time		S
wash out/buffer time	30,00	S
sum time (whole sample)	210	S
total acquisitions/line	#DIV/0!	

Abundance	Integration time (s)
	0,1
	0,1
	0,1
	0,05
	0,05
	0,1
	0,1
	0,1
100%	0,1
69,15%	0,1
27,73%	0,1
100%	0,1
82,60%	0,1
	0,1
	0,1
	0,1
	0,1
	0,1
70,40%	0,1
52%	0,1
	0,1
	Abundance

### Data processing

![](_page_19_Picture_1.jpeg)

• pictures taken under stereomicroscope after ablation of samples

![](_page_20_Picture_1.jpeg)

### Scales

# Otoliths

![](_page_21_Figure_2.jpeg)

![](_page_21_Figure_3.jpeg)

![](_page_21_Figure_4.jpeg)

- blank outliers replaced with average
- average blank value for
   each element
   subtracted from
   sample values
- **scales** internal normalization to <sup>13</sup>C
- otoliths internal normalization to <sup>43</sup>Ca
- intensities plotted for
   each element to find
   trends along ablation
   lines

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																						819	1.01.1	LANCE.	citoria .	1.4	100.0	14	146	-1.8	81.0	44.	1141.0	4.4	6.0	44.	14		15.18	10.4			
																							184	10.04.4		- 55	- 655-		- 14	-8-		4.5	10004	- 14	- 10	- 44	· · · · ·			-24		- 2	
	12.M		1		81.CO	民抗		89.56	2,34	1.28			C353		2.85				91.				100	of ent	9	14																- <b>*</b> B)	
61																																							123 675				

![](_page_21_Picture_12.jpeg)

KS-55

KS-47

![](_page_22_Figure_2.jpeg)

![](_page_22_Picture_3.jpeg)

KS-52

KS-54

![](_page_22_Picture_5.jpeg)

KS-56

![](_page_22_Picture_7.jpeg)

KN-21

KN-24

KN-25

![](_page_23_Figure_3.jpeg)

KN-26

![](_page_23_Figure_5.jpeg)

![](_page_23_Figure_6.jpeg)

KN-28

![](_page_23_Figure_8.jpeg)

![](_page_23_Picture_9.jpeg)

KN-43

![](_page_23_Figure_11.jpeg)

![](_page_24_Picture_0.jpeg)

### KN-21

![](_page_25_Figure_1.jpeg)

![](_page_26_Figure_0.jpeg)

![](_page_26_Figure_1.jpeg)

![](_page_26_Figure_2.jpeg)

#### <sup>88</sup>Sr/<sup>43</sup>Ca SPRING

#### KRS

![](_page_27_Picture_2.jpeg)

#### KRK

![](_page_27_Picture_4.jpeg)

KBL

![](_page_27_Picture_6.jpeg)

#### <sup>88</sup>Sr/<sup>43</sup>Ca AUTUMN

![](_page_28_Picture_1.jpeg)

![](_page_28_Picture_2.jpeg)

KRK

![](_page_28_Picture_4.jpeg)

KBL

![](_page_28_Picture_6.jpeg)

![](_page_28_Picture_7.jpeg)

![](_page_28_Picture_8.jpeg)

![](_page_28_Picture_9.jpeg)

![](_page_28_Picture_10.jpeg)

![](_page_28_Picture_11.jpeg)

88Sr/43Ca

KBL > KRK = KRS

water

 the same trend in both seasons

![](_page_29_Figure_3.jpeg)

#### <sup>138</sup>Ba/<sup>43</sup>Ca SPRING

#### KRS

![](_page_30_Picture_2.jpeg)

#### KRK

![](_page_30_Picture_4.jpeg)

#### KBL

![](_page_30_Picture_6.jpeg)

#### <sup>138</sup>Ba/<sup>43</sup>Ca AUTUMN

![](_page_31_Picture_1.jpeg)

![](_page_31_Picture_2.jpeg)

KRK

![](_page_31_Picture_4.jpeg)

![](_page_31_Picture_5.jpeg)

![](_page_31_Picture_6.jpeg)

![](_page_31_Picture_7.jpeg)

![](_page_31_Picture_8.jpeg)

![](_page_31_Picture_9.jpeg)

![](_page_31_Picture_10.jpeg)

![](_page_31_Picture_11.jpeg)

- KBL > KRK = KRS
- differences between locations smaller than for Sr

Ba 30 20 (1) KRS b) Ba spring a) Ba winter 25 [ZZZ] (3) KBL ,2,3,6 T 100000 (4) TOR 1.2,3,6 15 KXXXI (5) TBU 20 (6) TKO -기 10 1-7 and 0 b,d 0 10 3,4,5,6 3,4,5,6 5 5 0 25 20 c) Ba summer d) Ba autumn 20 15 1,2,3,4 1,2,3,4 г-Л<sup>вн</sup> 10 1,2,3,4 r-기 10 1,2,3,4 b,d 1,2,5,6 1,2,5,6 5 5 0 0 KRS KRK KBL TOR TBU тко KRS KRK KBL TOR TBU тко

### water

• the same trend

### Next steps

- comparison between otoliths and scales
- digestion of remained scales and measurement of metal concentrations
- comparison of element ratios (Sr/Ba, Zn/Ca, ...) in water and fish tissues
- separation of normal and abnormal otoliths
- finding seasonal pattern in normal otoliths with year rings
- quantification of metal concentrations

## Stocked fish?

 replacement scales – injuries, loss of scales in a hatchery (Fiske, P., Lund, R. A., & Hansen, L. P. (2005). Identifying fish farm escapees. In *Stock identification methods* (pp. 659-680). Academic Press.)

 abnormal otoliths – different otolith chemistry and shape than in wild populations (no variations in temperature and food availability)

 fast growth due to environmental control leads to <u>development of</u>
 vaterite instead of aragonite
 (Reimer, T., Dempster, T., Wargelius, A., Fjelldal, P. G., Hansen, T., Glover, K. A., ... & Swearer, S. E.
 (2017). Rapid growth causes abnormal vaterite formation in farmed fish otoliths. *Journal of Experimental Biology*, 220(16), 2965-2969.)

![](_page_34_Picture_3.jpeg)

![](_page_34_Picture_4.jpeg)

#### Poribljavanje rijeka Butižnice i Krke

KATEGORIJA: NOVOSTI / DATUM OBJAVE: 19/03/2020

Jučer je izvršeno redovno poribljavanje rijeka Butižnice i Krke. Ovo je već drugo poribljavanje vođa kojima gospodari naše društvo od početka sezone! Kroz skorije vrijeme poribljavanja će se nastaviti. S obzirom na stanje u državi, Europi i poteškoćama koje su oko nas "SRD "Krka" Knin svoje obaveze nije mogla prolongirati.

300kg novih jedinki potočne pastrve pliva našim rijekama. Do sada je ubačeno ukupno 800kg pastrve u svim našim važnijim vodotocima i tu nećemo stati. Kroz skorije vrijeme poribljavanja će se nastaviti i naš projekt ZapoSljavanje kroz ribolov u svrhu promocije ribolovnog turizma se realizira po planu.

Rijeka Krka je poribljena sa 100kg na dvije lokacije (Atlagića most i nizvodno od željezničkog

![](_page_35_Picture_0.jpeg)

# Thank you for your attention!

THIRD PROJECT MEETING

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

Zagreb, 19<sup>th</sup> May 2023

![](_page_35_Picture_5.jpeg)

![](_page_35_Picture_6.jpeg)

![](_page_35_Picture_7.jpeg)

![](_page_35_Picture_8.jpeg)

![](_page_35_Picture_9.jpeg)