

Metal concentrations in fish scales and otoliths

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Croatia

THIRD PROJECT MEETING

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

Zagreb, 19th 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



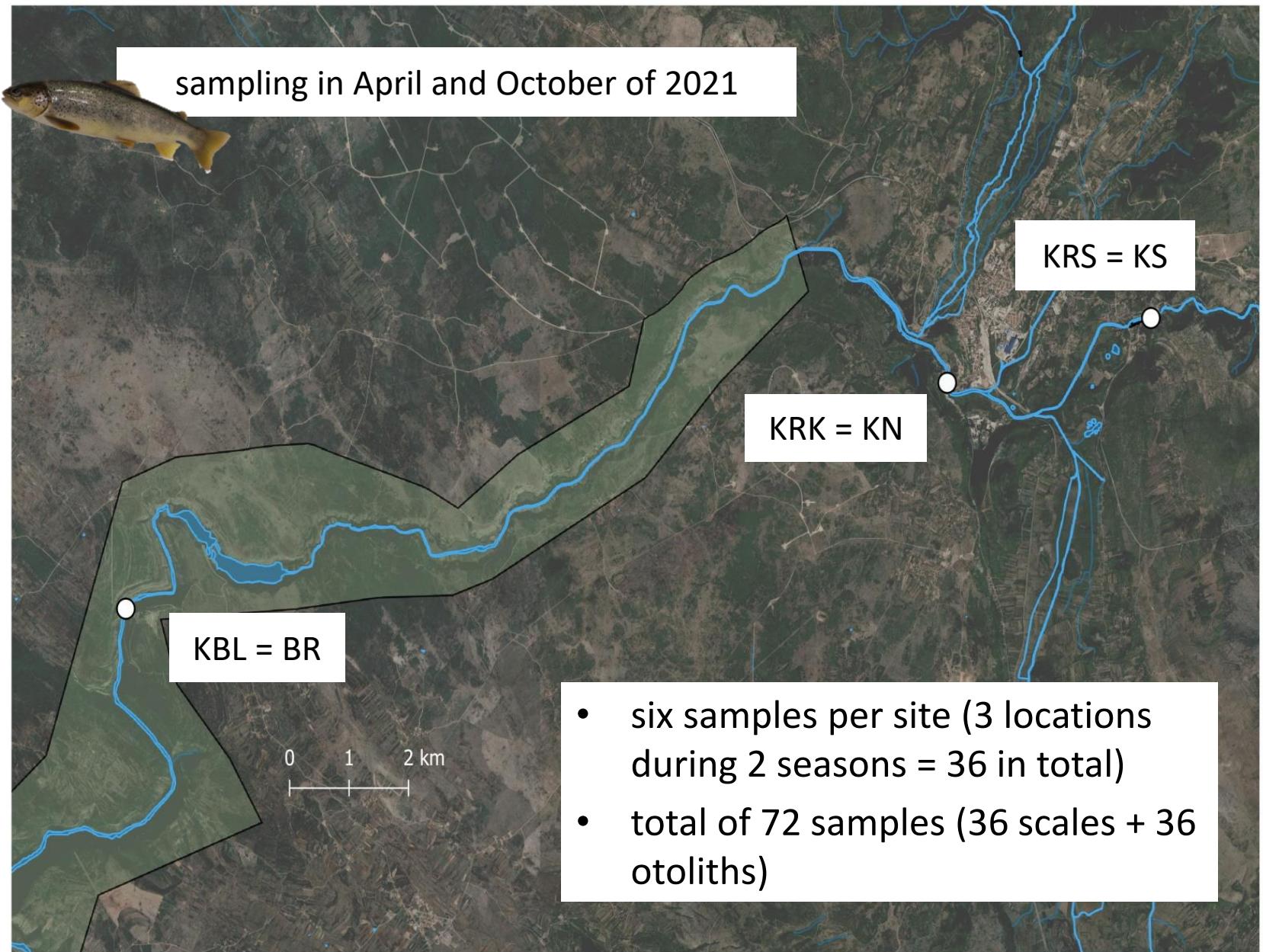
Johanna



Thomas

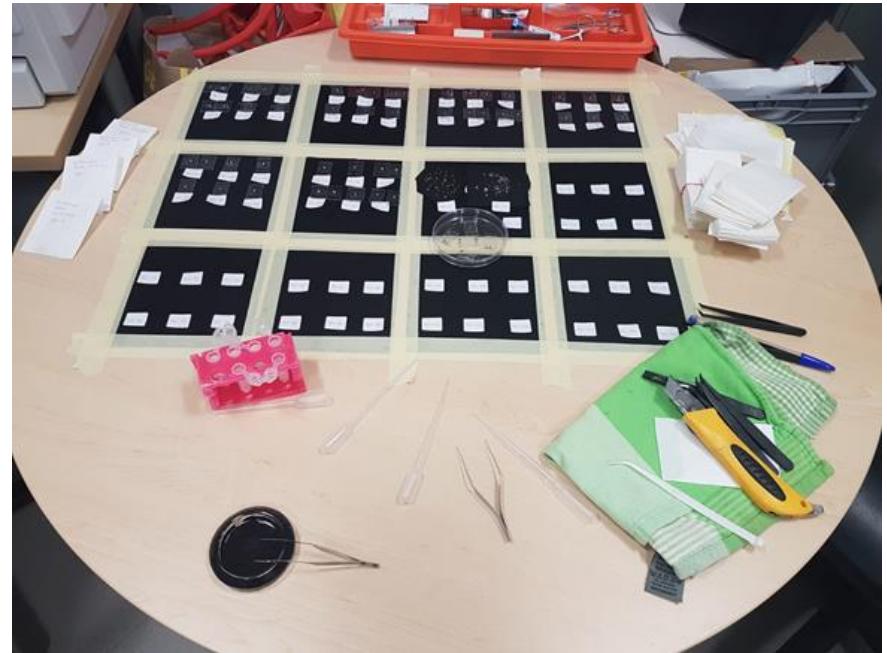


Andreas

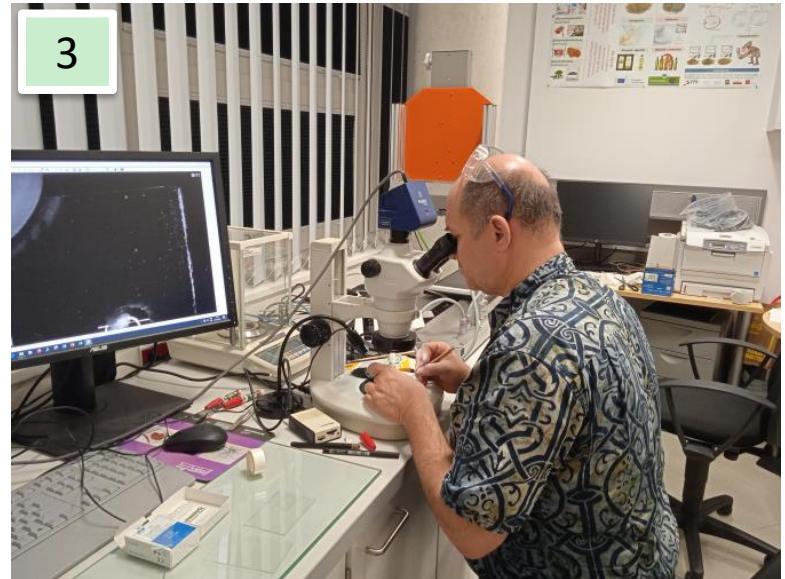




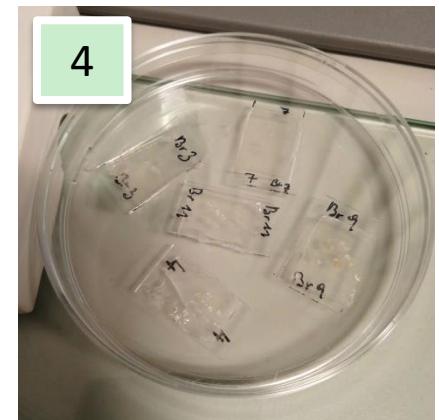
Sample preparation



Preparation of scales



better to use paper envelopes/bags



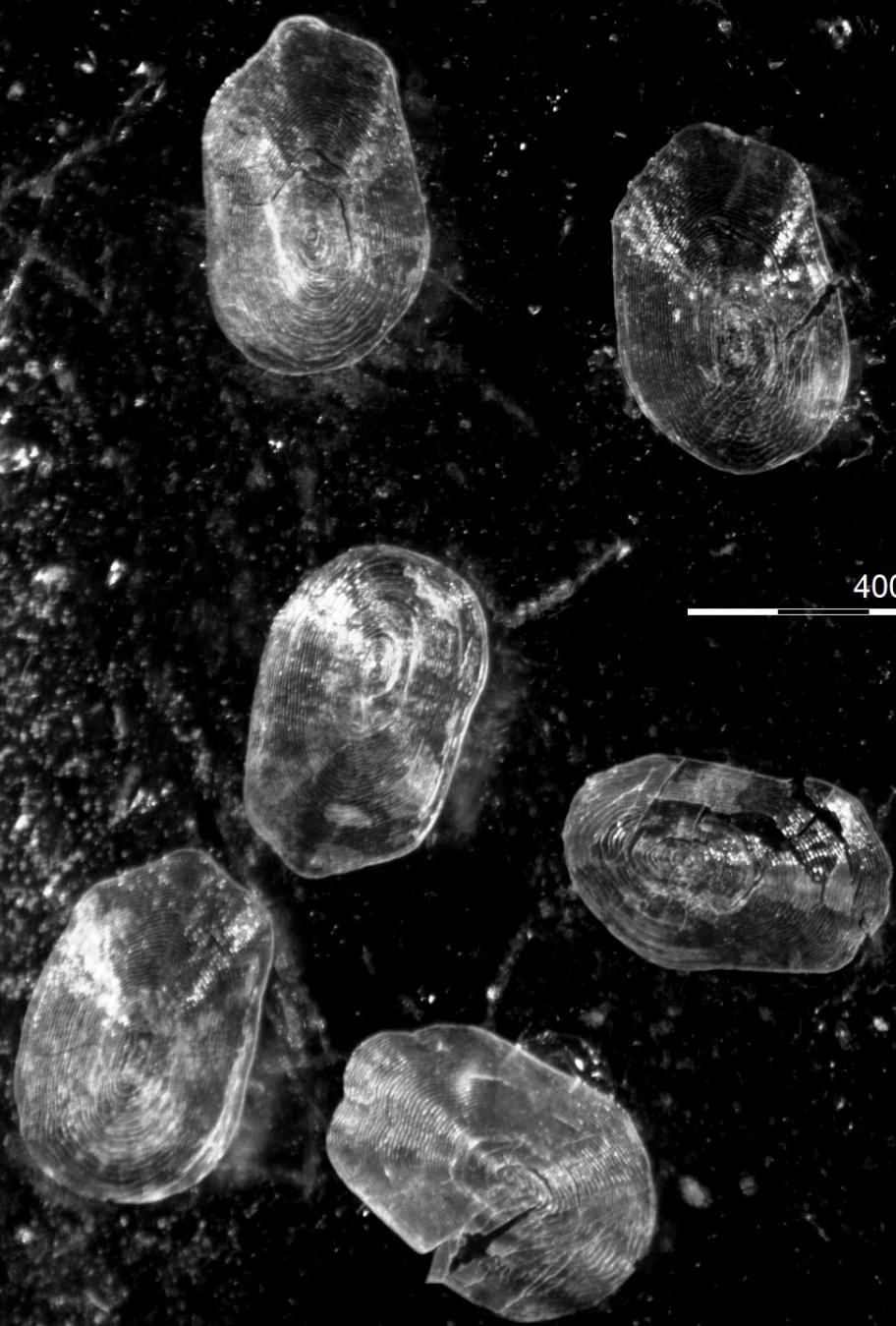
Ks47

4000 μm

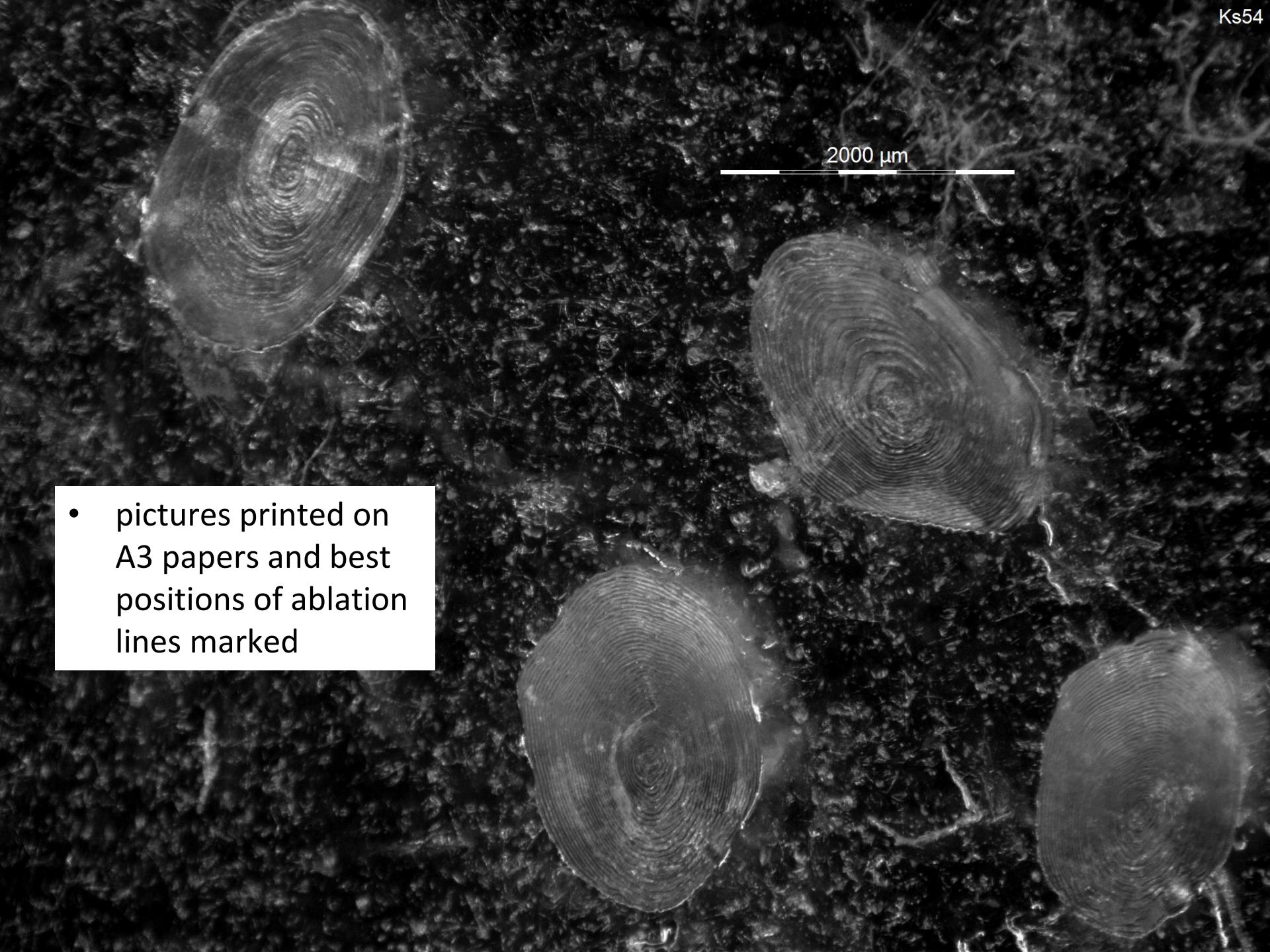
ProgRes® CapturePro Software

Br90

4000 μm



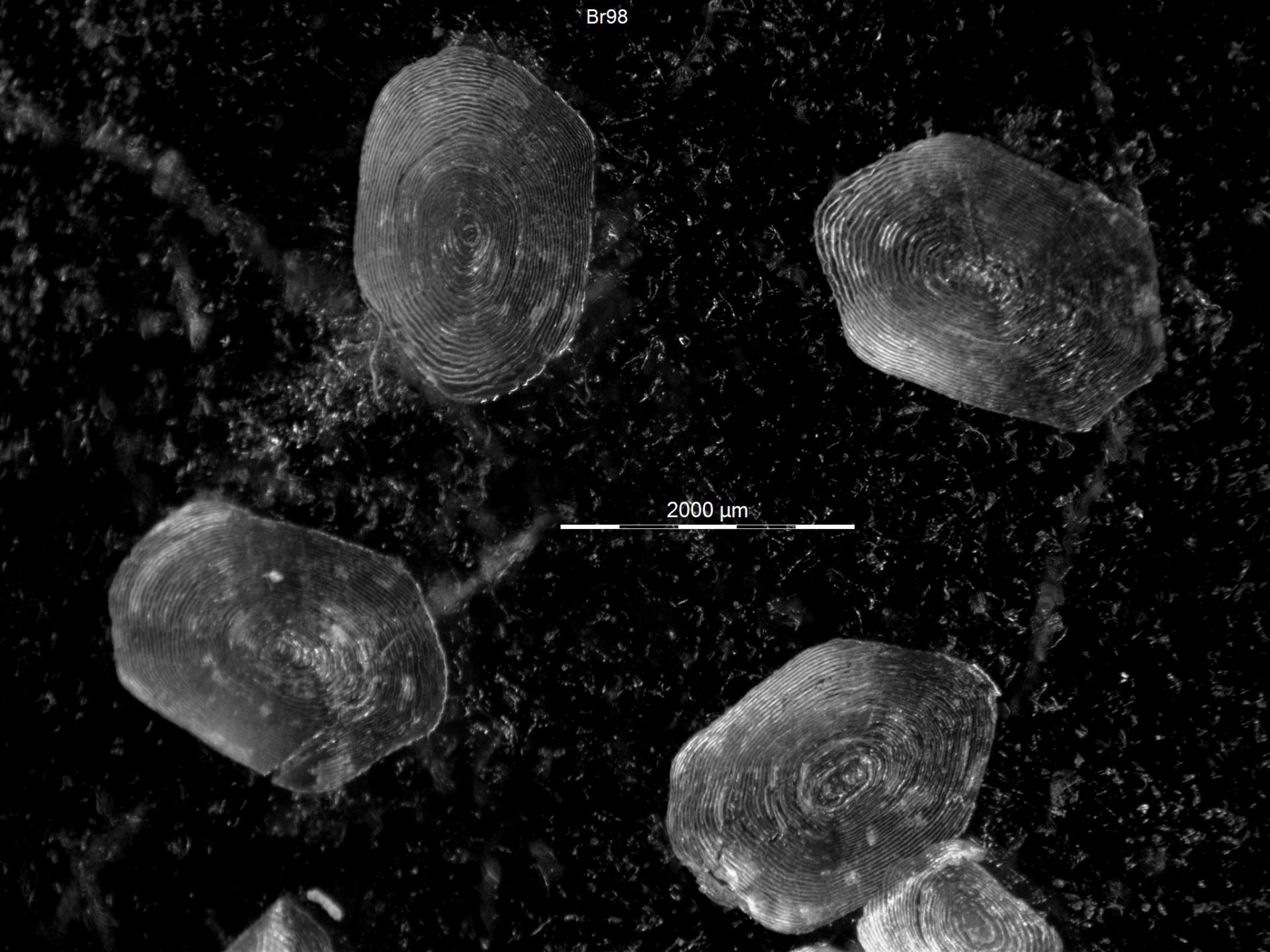
- pictures printed on A3 papers and best positions of ablation lines marked

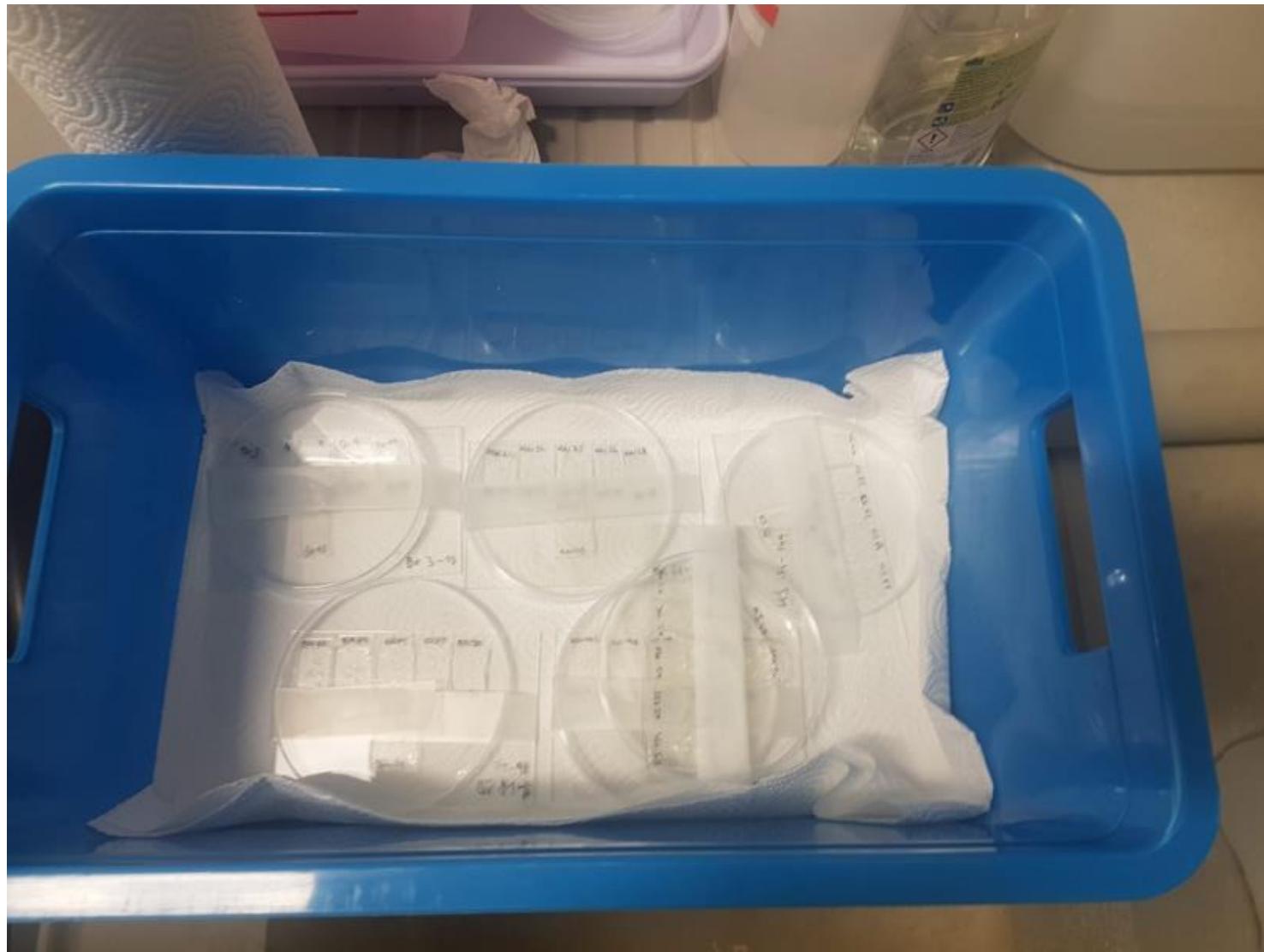


2000 μm

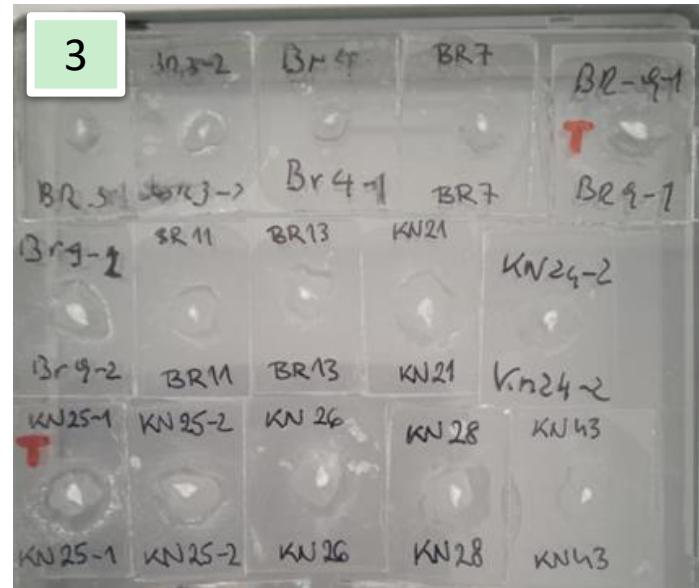
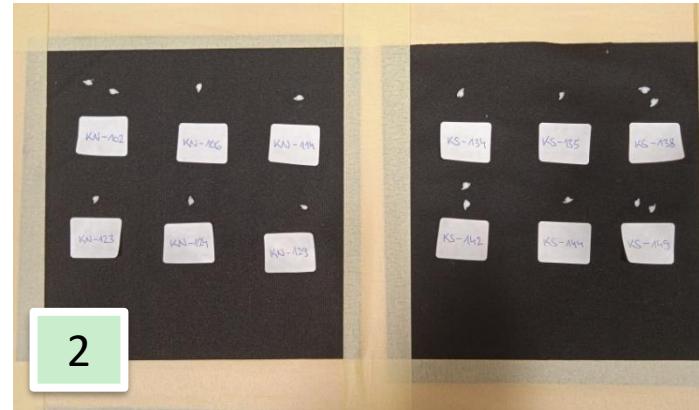
Br98

2000 μm





Preparation of otolithes



Krazy glue

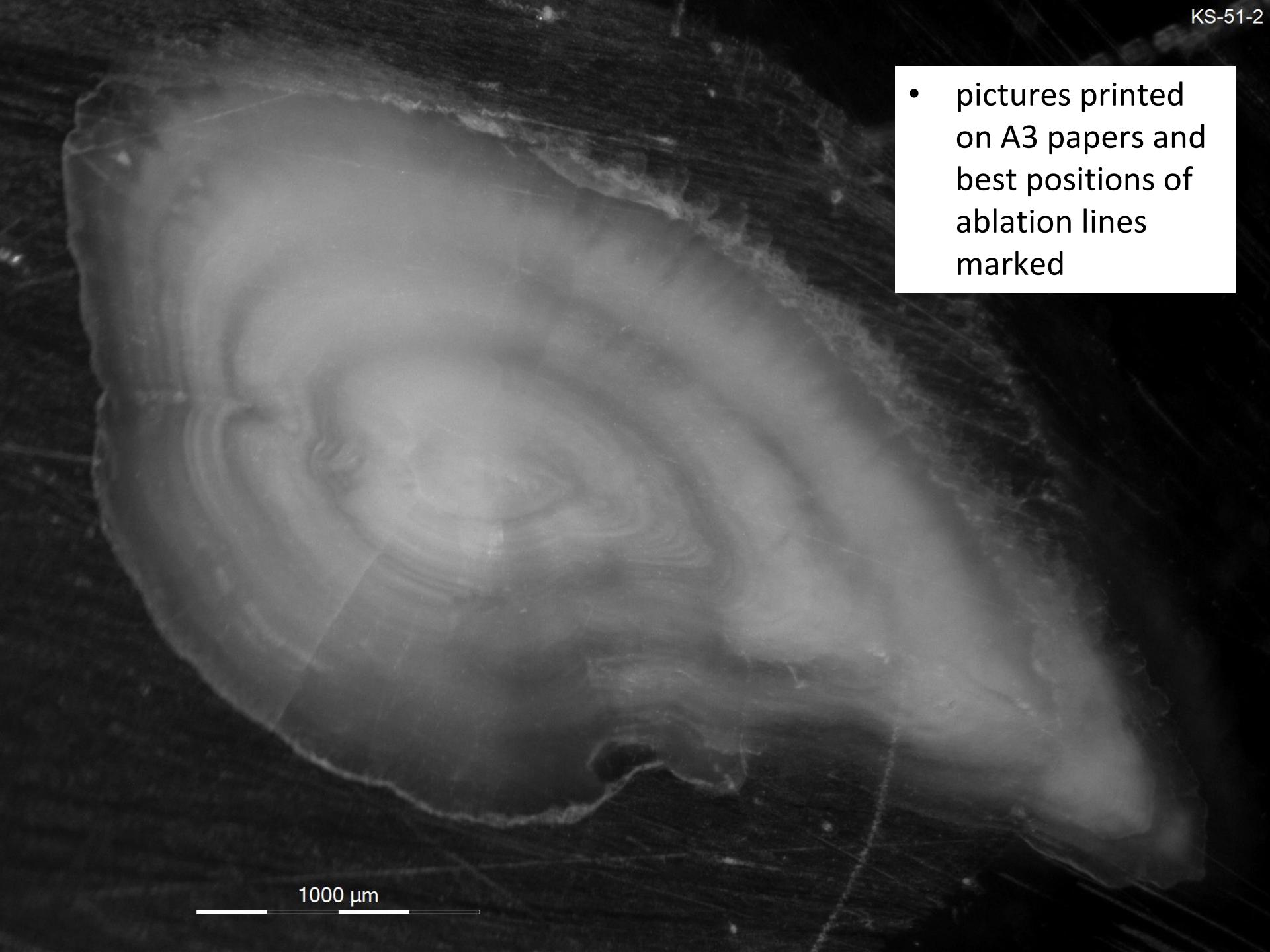
lapping films of 30 µm and 3 µm for grinding and polishing

primordial granules



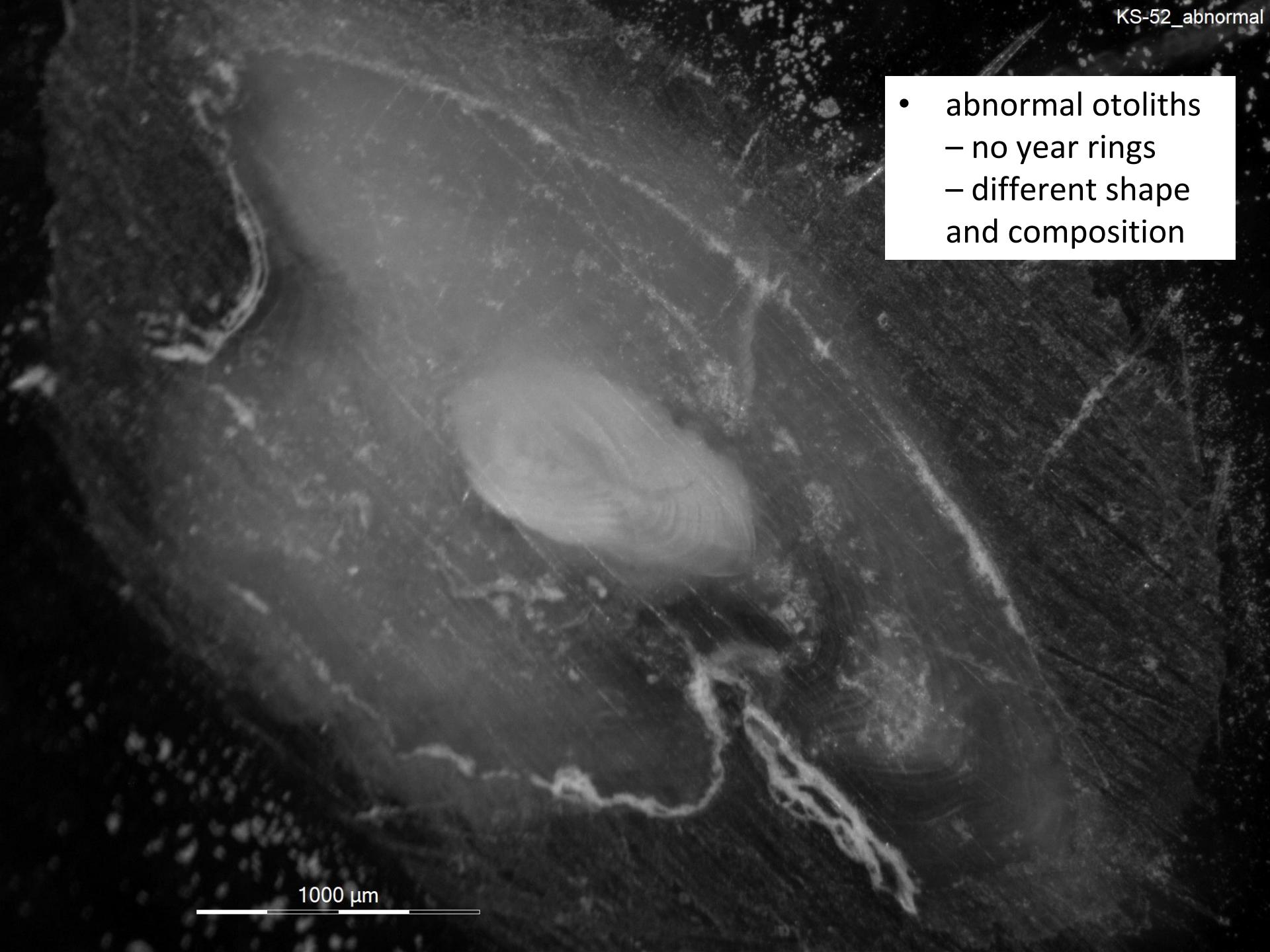
1000 μm

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

A scanning electron micrograph showing a highly textured, wavy surface. The surface has a granular appearance with numerous small, rounded protrusions and deep, irregular grooves. The lighting creates strong highlights on the peaks of the ridges and deep shadows in the valleys, emphasizing the three-dimensional nature of the texture. In the bottom left corner, there is a white scale bar consisting of a horizontal line with two short vertical tick marks at its ends, labeled "1000 µm".

1000 µm

- abnormal otoliths
 - no year rings
 - different shape and composition

A scanning electron micrograph (SEM) showing several otoliths (ear stones) of a fish. The otoliths appear as bright, textured, oval-shaped structures against a dark, granular background. One prominent, large otolith in the center-right has a distinct, layered or ringed internal structure, characteristic of normal growth. In contrast, other smaller or less distinct otoliths nearby appear irregular in shape and lack clear internal structures, indicating abnormalities. A scale bar at the bottom left indicates 1000 micrometers.

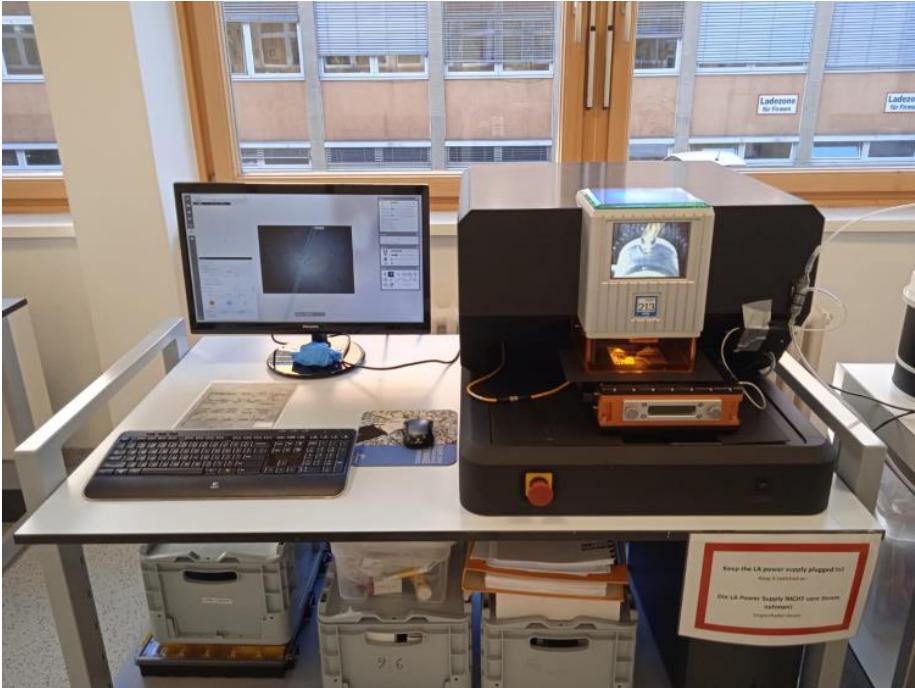
1000 μm

- big otoliths with no structure

1000 μm



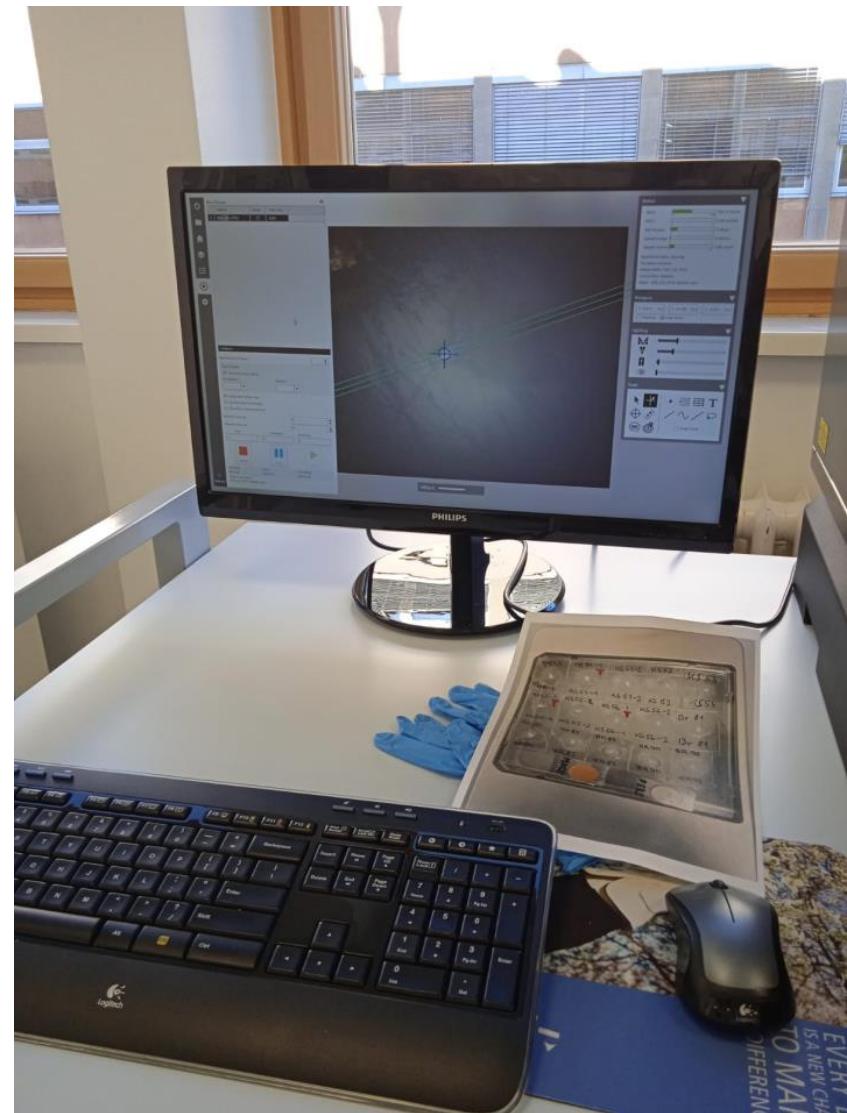
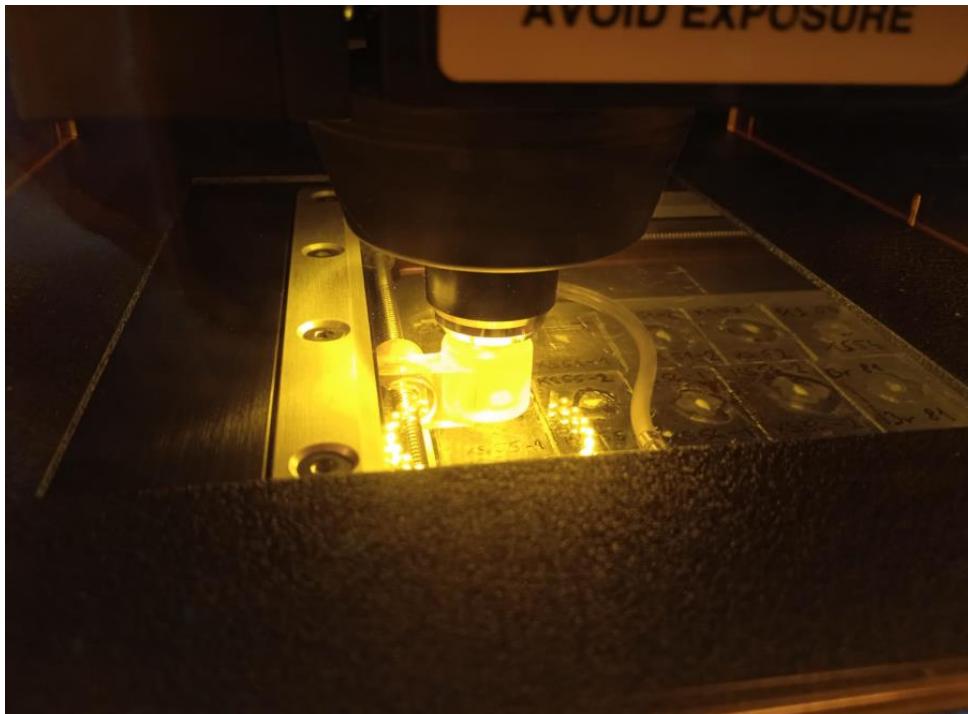
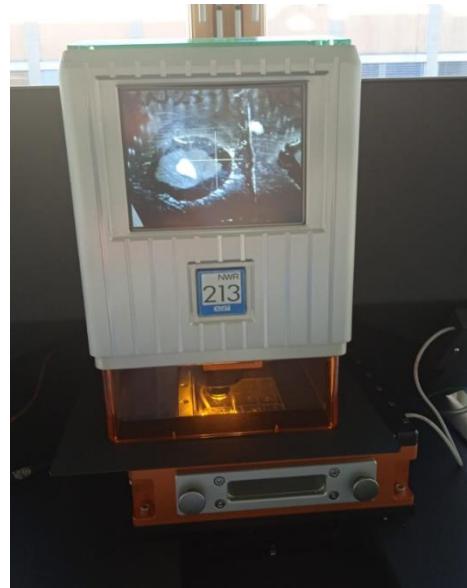
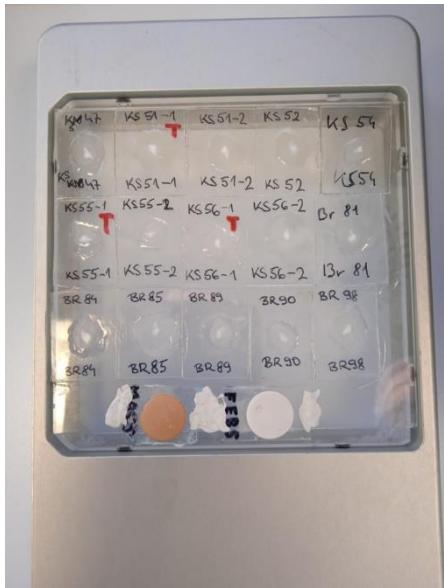
Measurements on LA-ICP-MS



ESI NWR 213 Laser-Ablation System



Agilent 8800 ICP-MS-CRC-MS



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	50µm
speed	5µm/s
rep rate	10Hz
energy output	15%
energy	mJ
line scan distance (x-direction)	600µm
distance between lines (core)	µm
warm-up (delay)/gas blank	30s
wash-out (delay)	30s
carrier gas	He
carrier gas flow rate	0,900L/min
fluence	J cm ²
line time	120,0s
transfer time	s
shutter (IVA / XYR)	IVA

(20 s for samples)

ICP-QQQ-MS (8800, Agilent)	Value	Unit
RF power	1550W	
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	30,00s	
sum time (whole sample)	180s	
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
205Tl	70,40%	0,1
208Pb	52%	0,1
238U		0,1

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)

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	He
carrier gas flow rate	0,900L/min
fluence	J cm ²
line time	150,0s
transfer time	s
shutter (IVA / XYR)	IVA

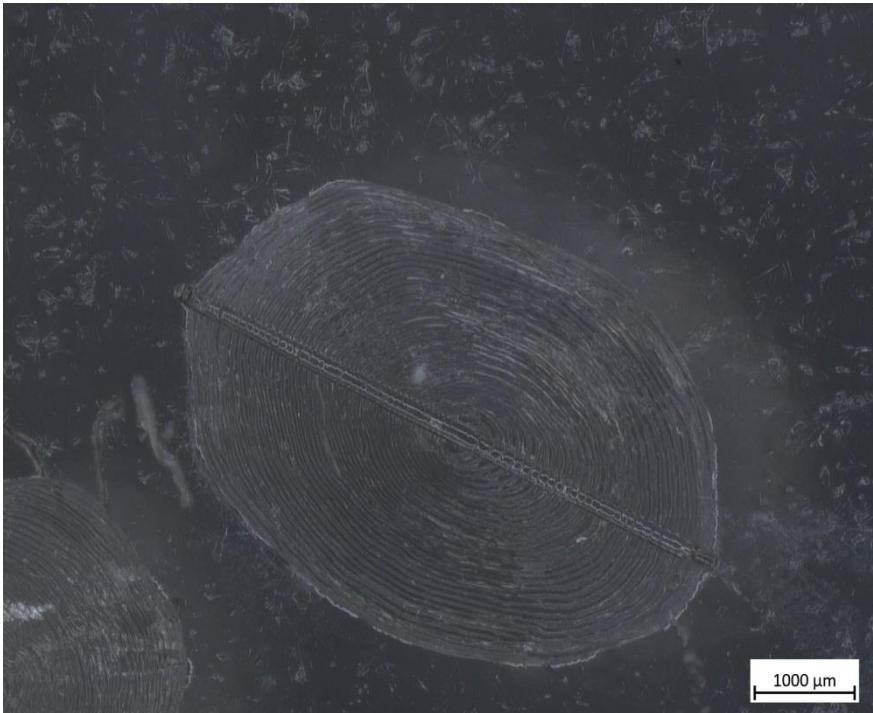
ICP-QQQ-MS (8800, Agilent)	Value	Unit
RF power	1550W	
nebulizer gas flow	L/min	
auxillary gas flow	L/min	
plasma gas flow	L/min	
total acquisition time/integration time	s	
wash out/buffer time	30,00s	
sum time (whole sample)	210s	
total acquisitions/line	#DIV/0!	

Isotopes	Abundance	Integration time (s)
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

Data processing



- pictures taken under stereomicroscope after ablation of samples



Kommentar
500 µm

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Kamera/Detektor: AxioCam MRc5
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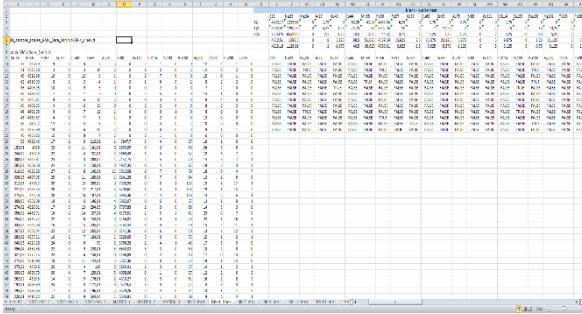


Kommentar
500 µm

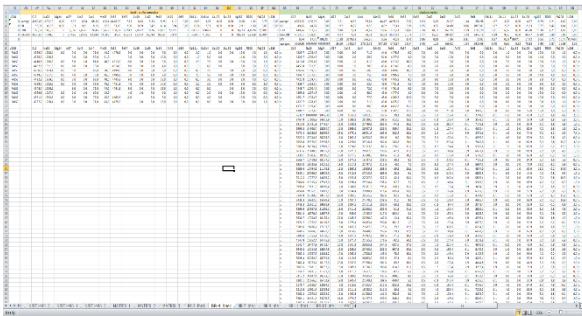
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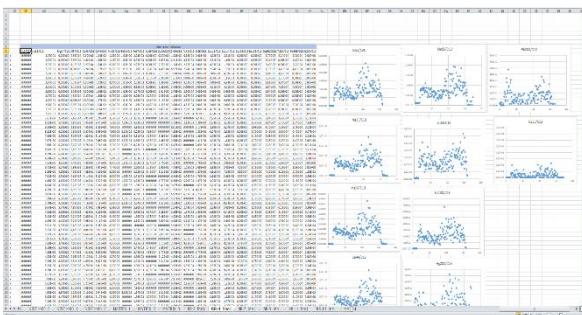
Scales



A screenshot of an Excel spreadsheet titled "Scales". The sheet contains a large grid of data with columns labeled from A to Z and rows numbered 1 through 100. The data consists of numerical values and some text entries. The first few rows show headers like "Element", "Sample ID", and "Ablation Number". The data is color-coded in a repeating pattern of red, green, blue, and yellow across the columns.

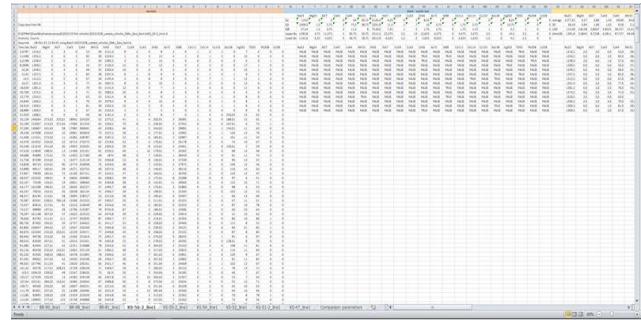


A screenshot of an Excel spreadsheet titled "Scales". The sheet contains a large grid of data with columns labeled from A to Z and rows numbered 1 through 100. The data consists of numerical values and some text entries. The first few rows show headers like "Element", "Sample ID", and "Ablation Number". The data is color-coded in a repeating pattern of red, green, blue, and yellow across the columns.

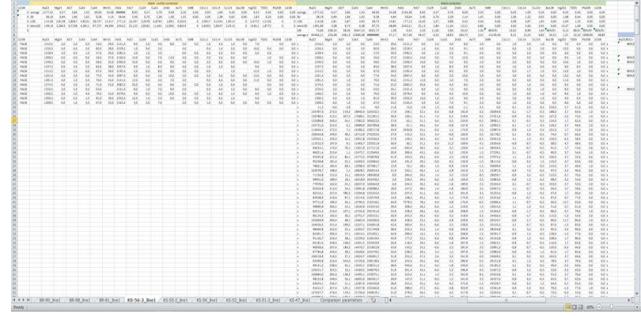


- blank – outliers replaced with average
- average blank value for each element subtracted from sample values
- **scales** - internal normalization to ^{13}C
- **otoliths** - internal normalization to ^{43}Ca
- intensities plotted for each element to find trends along ablation lines

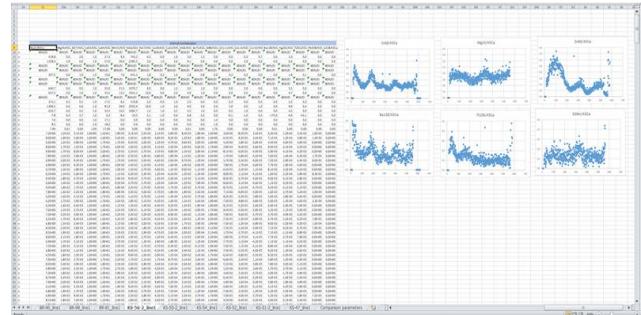
Otoliths



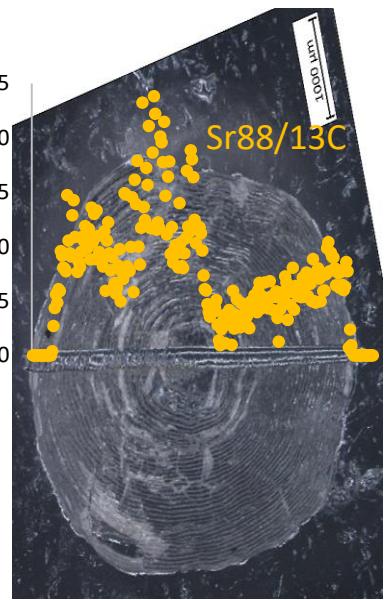
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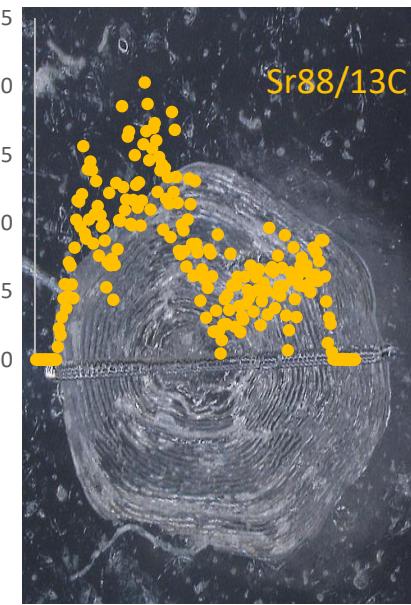
A screenshot of an Excel spreadsheet titled "Otoliths". The sheet contains a large grid of data with columns labeled from A to Z and rows numbered 1 through 100. The data consists of numerical values and some text entries. The first few rows show headers like "Element", "Sample ID", and "Ablation Number". The data is color-coded in a repeating pattern of red, green, blue, and yellow across the columns.



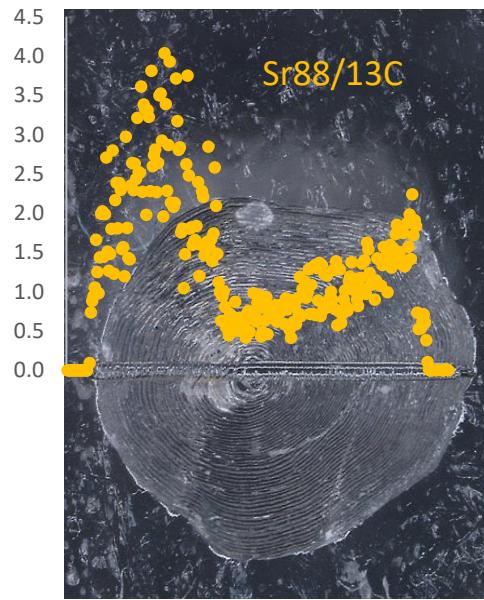
KS-47



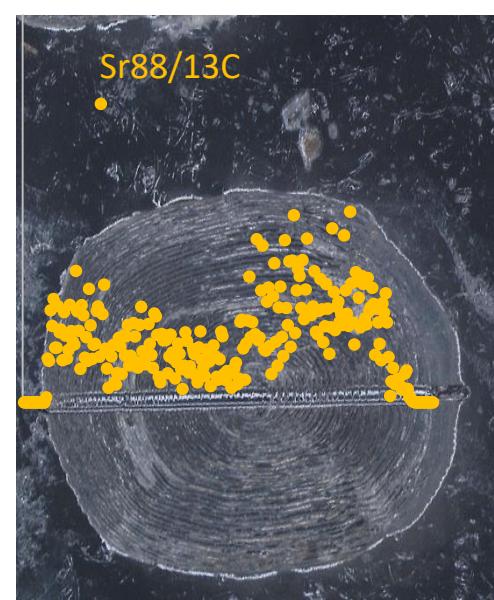
KS-52



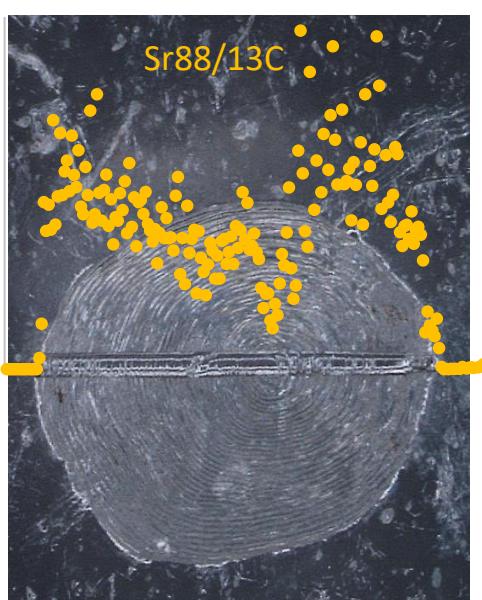
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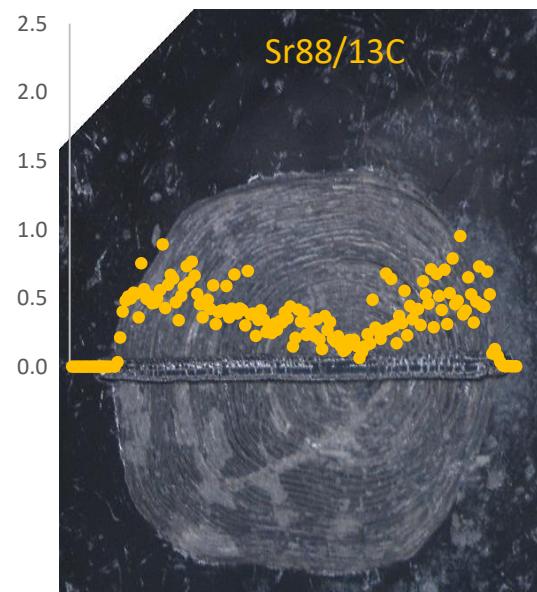
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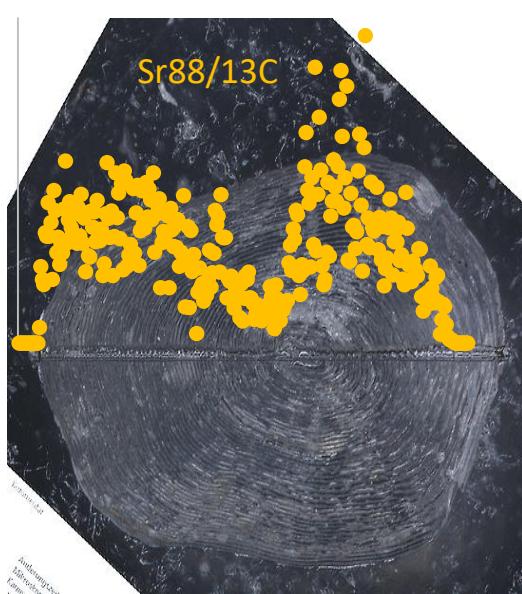
KS-54



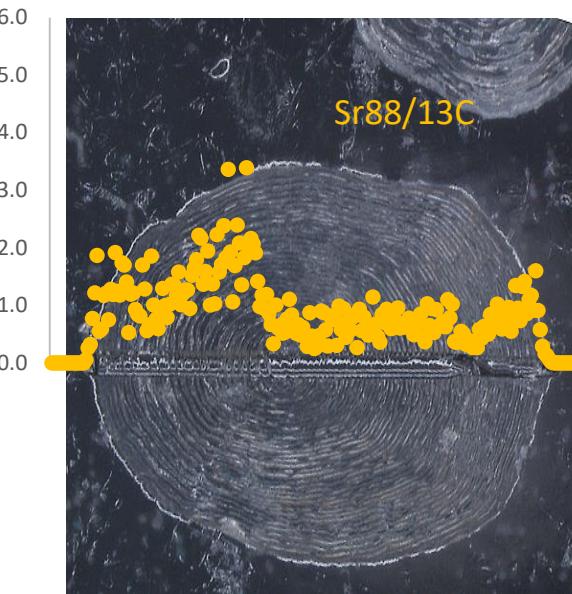
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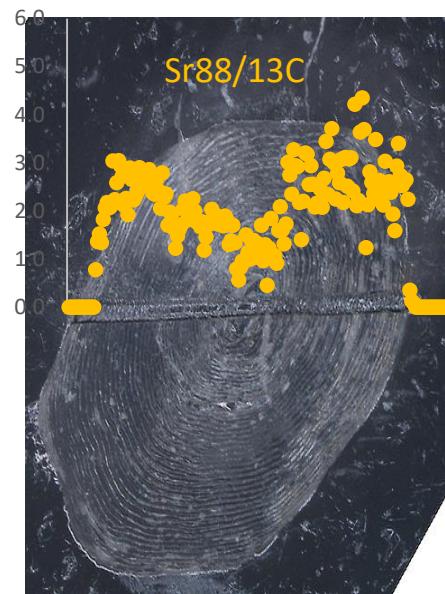
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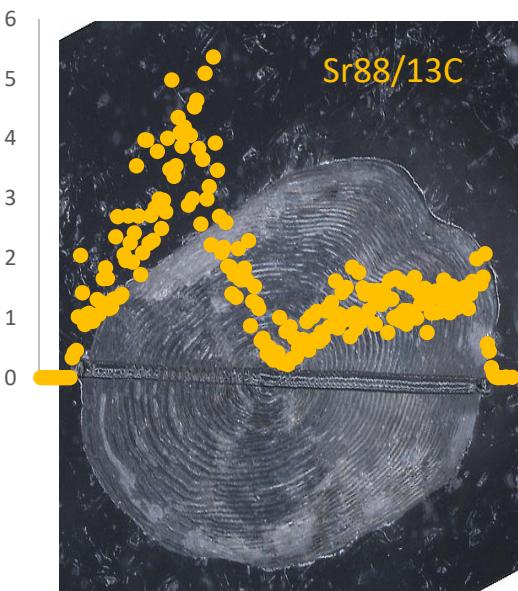
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KN-25



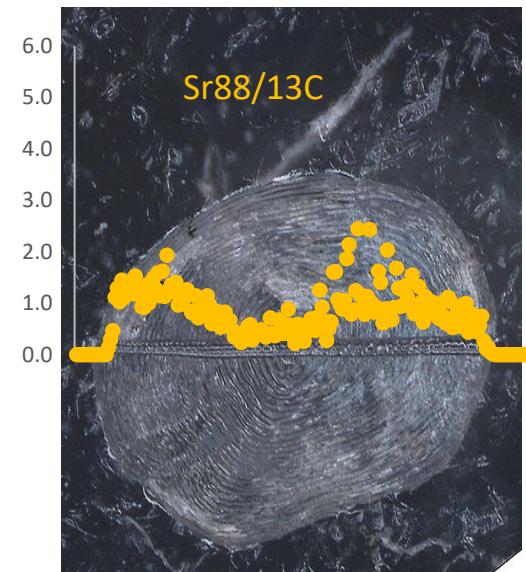
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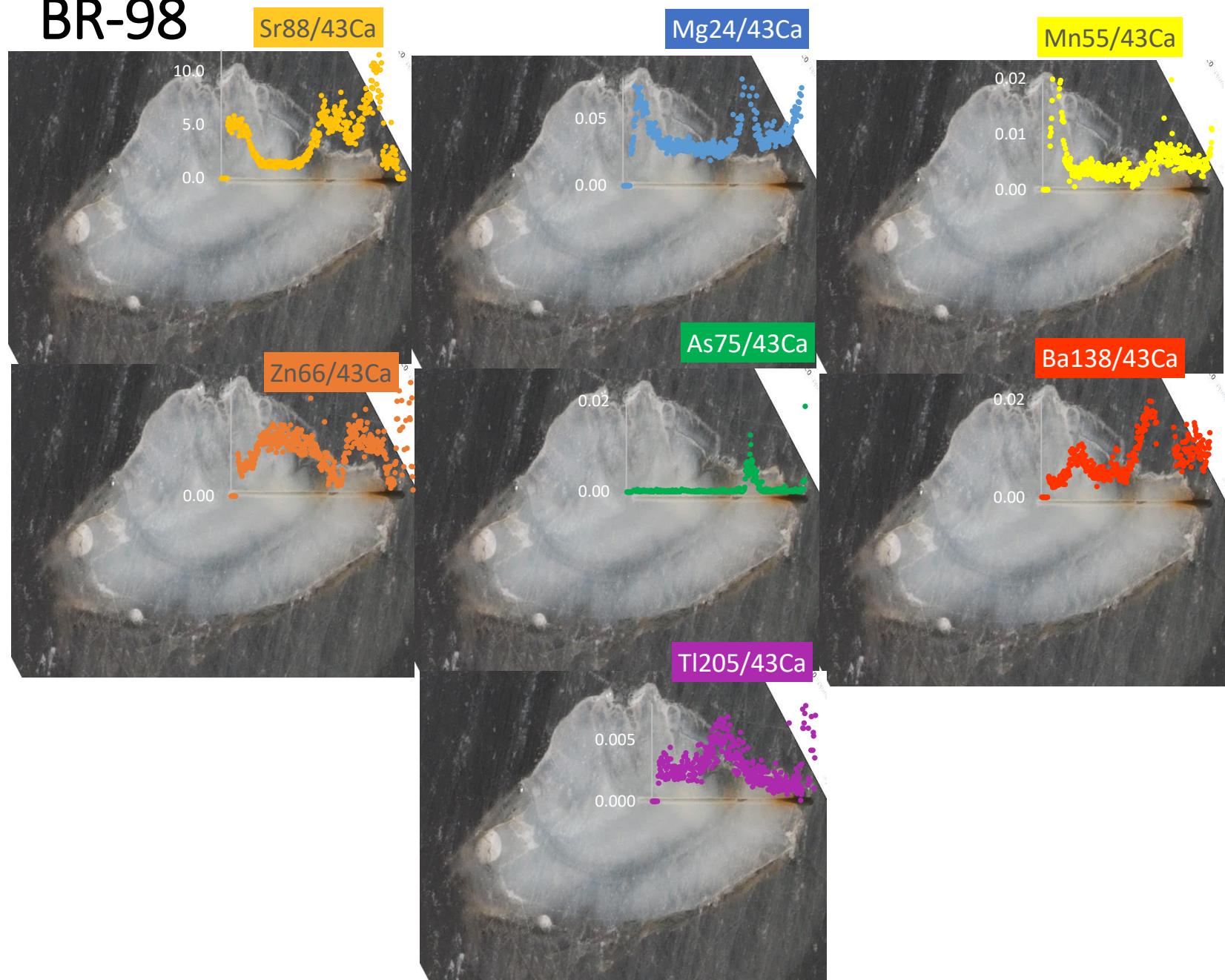
KN-28



KN-43



BR-98



KN-21

Sr88/43Ca

10.0
5.0
0.0

Zn66/43Ca

0.04
0.02
0.00

Mg24/43Ca

0.08
0.06
0.04
0.02
0.00

Mn55/43Ca

0.02
0.01
0.00

Ba138/43Ca

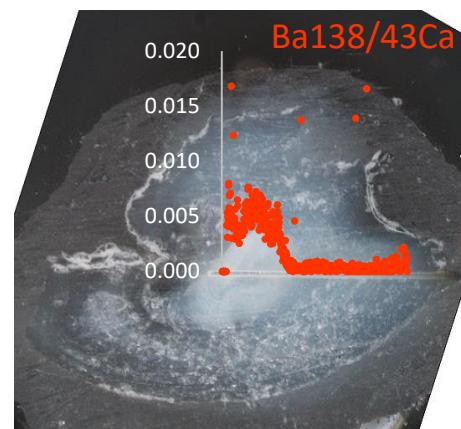
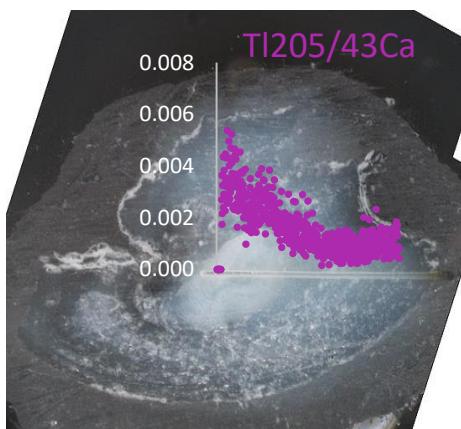
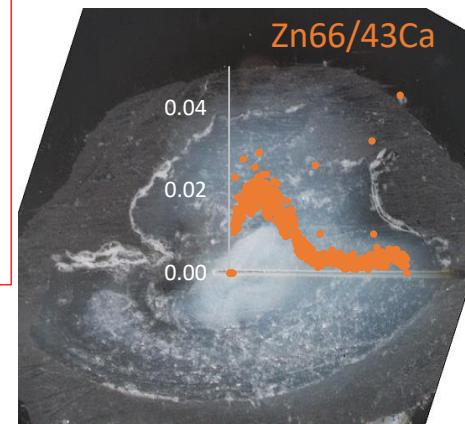
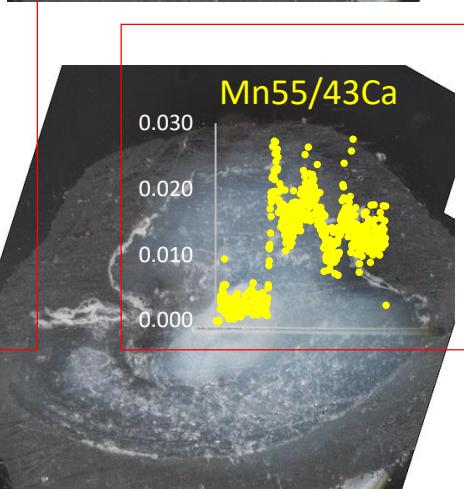
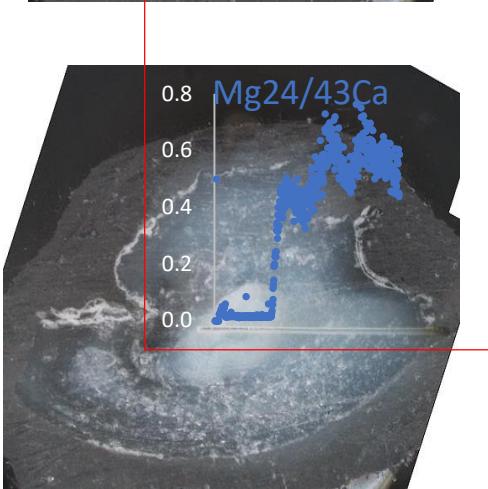
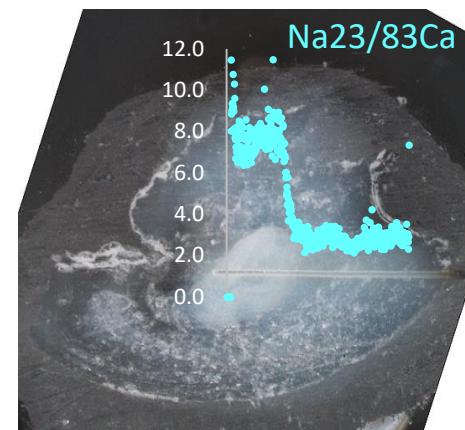
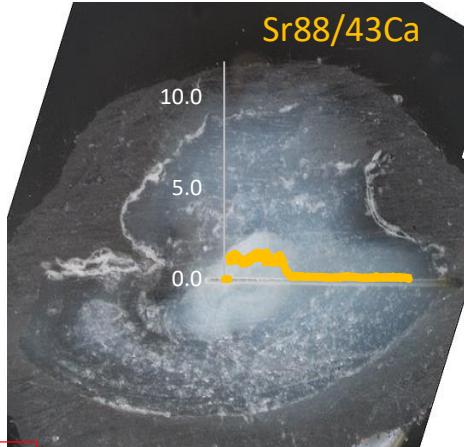
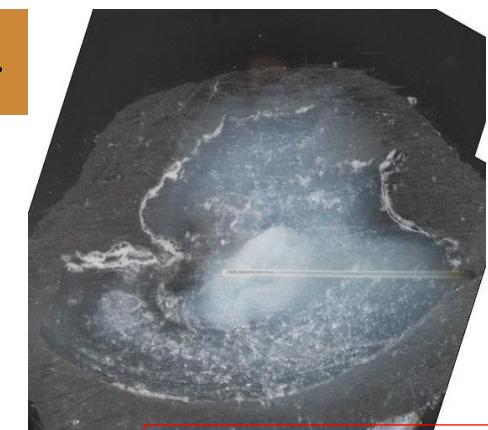
0.02
0.01
0.00

Tl205/43Ca

0.005
0.000

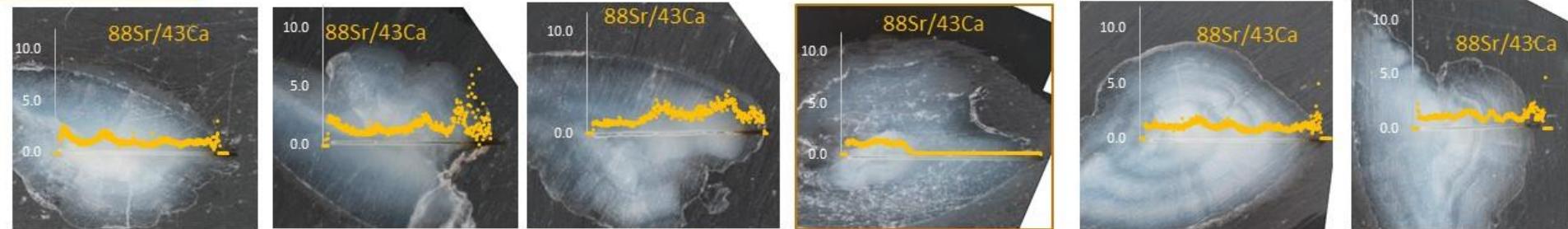
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KS_52

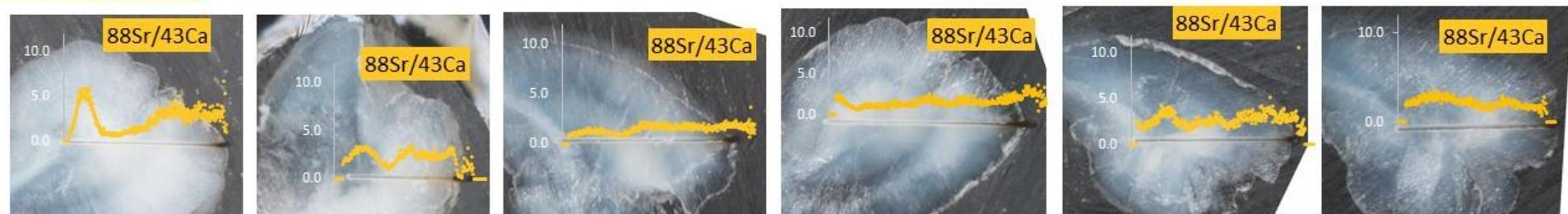


$^{88}\text{Sr}/^{43}\text{Ca}$ SPRING

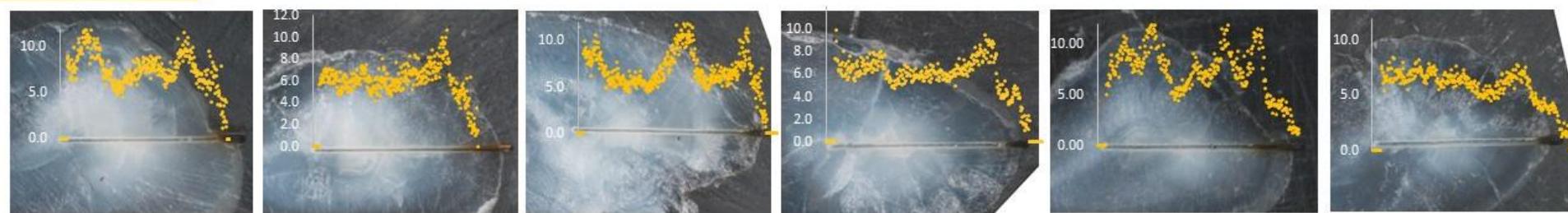
KRS



KRK

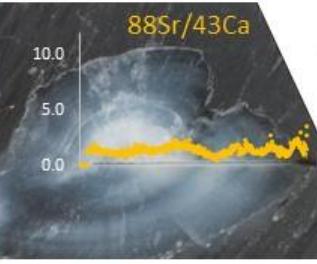
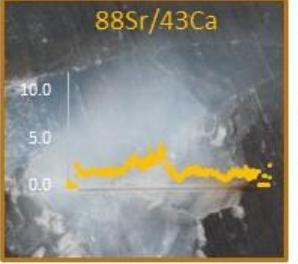
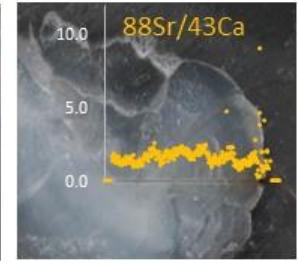
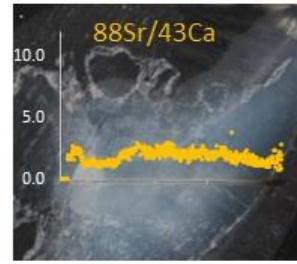
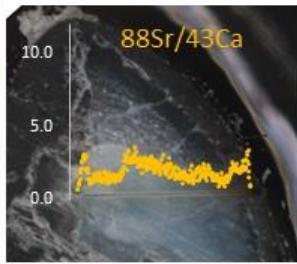
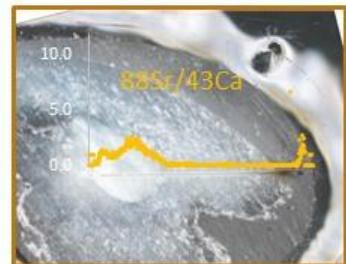


KBL

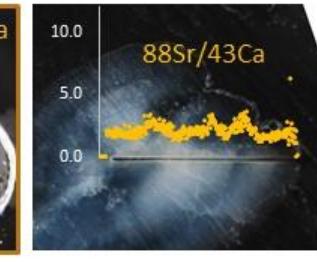
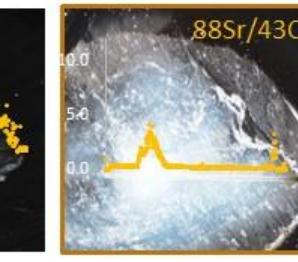
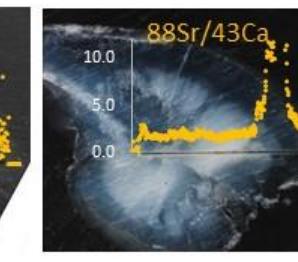
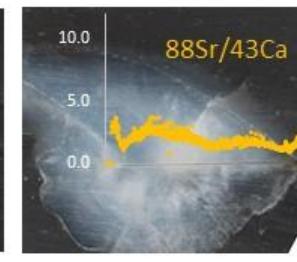
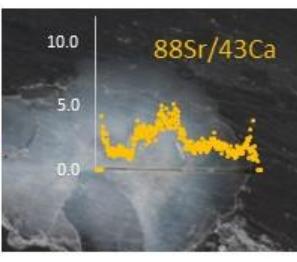
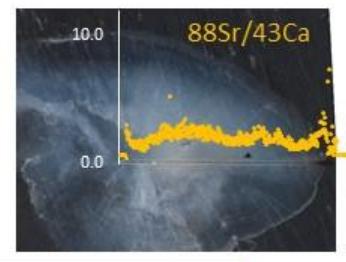


⁸⁸Sr/⁴³Ca AUTUMN

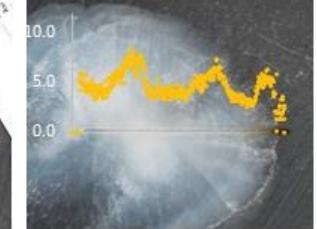
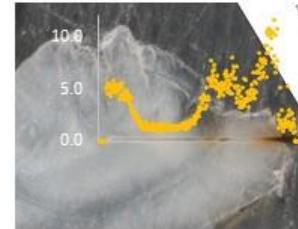
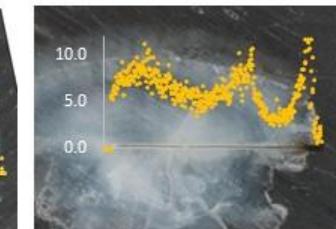
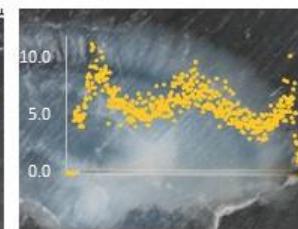
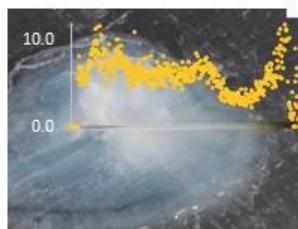
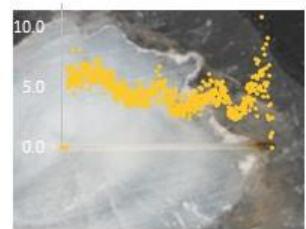
KRS



KRK



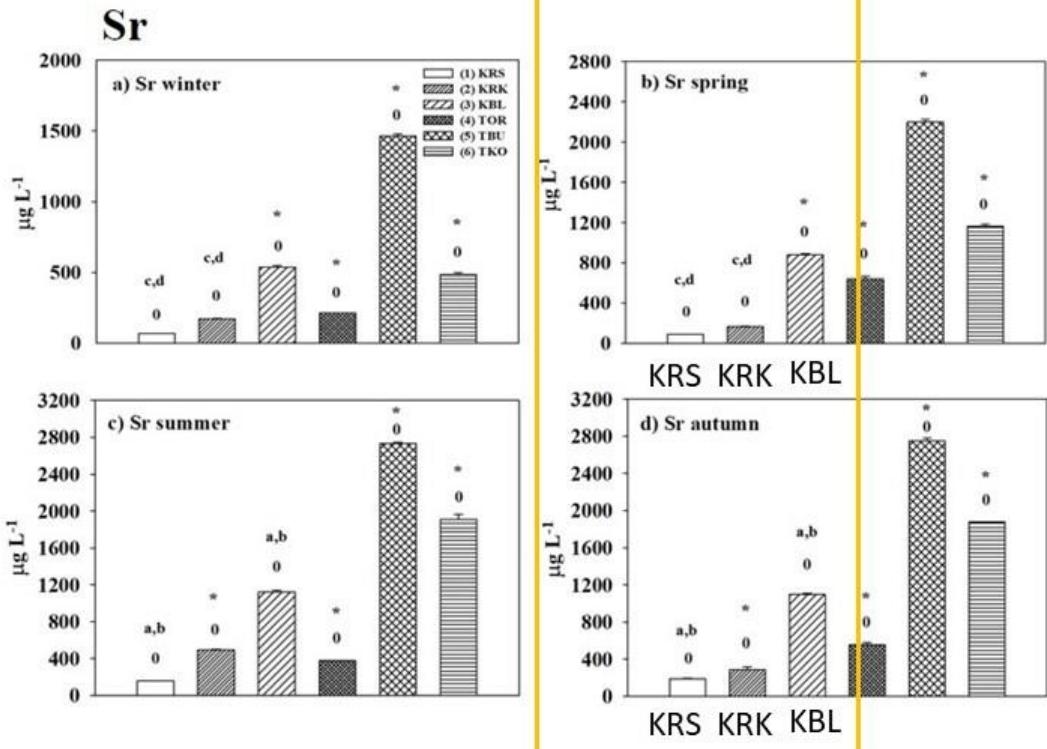
KBL



- KBL > KRK = KRS

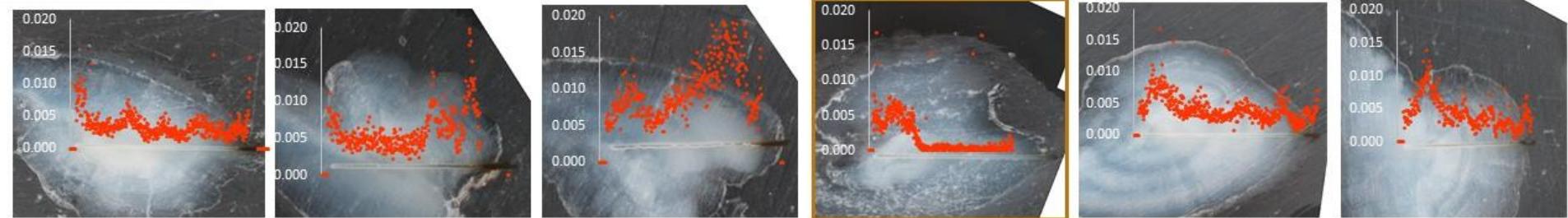
water

- the same trend in both seasons

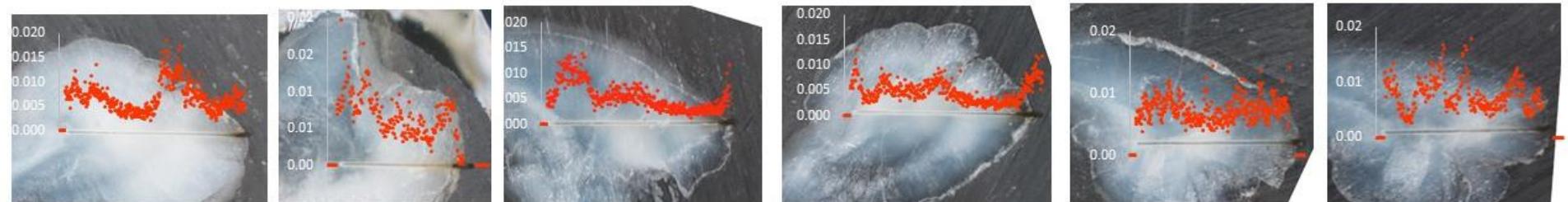


$^{138}\text{Ba}/^{43}\text{Ca}$ SPRING

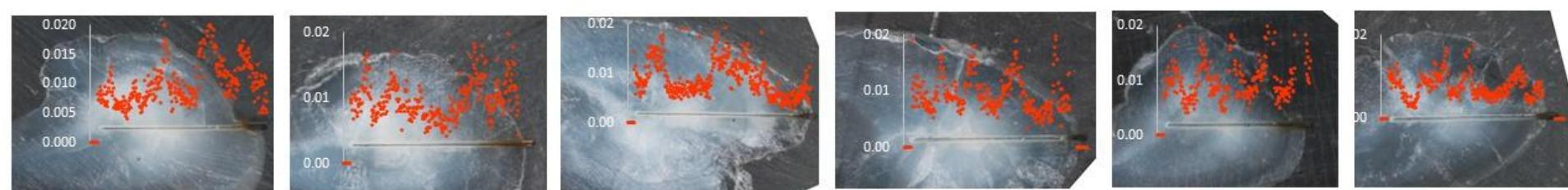
KRS



KRK

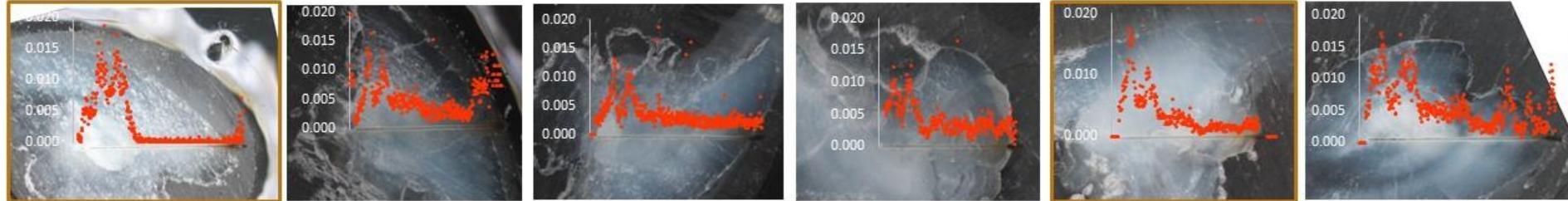


KBL

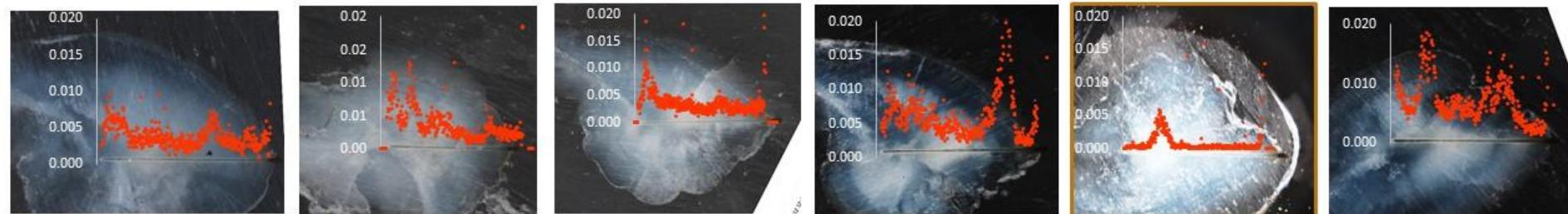


$^{138}\text{Ba}/^{43}\text{Ca}$ AUTUMN

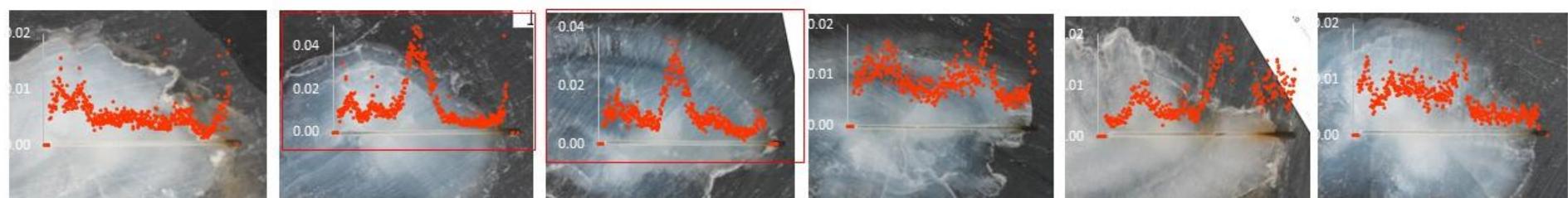
KRS



KRK



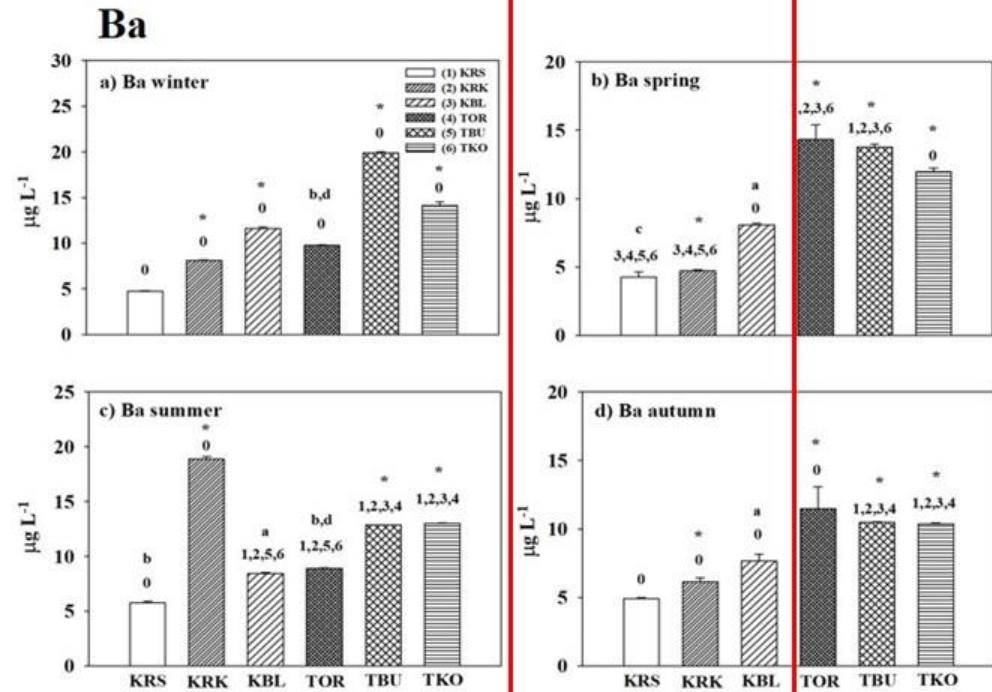
KBL



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

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

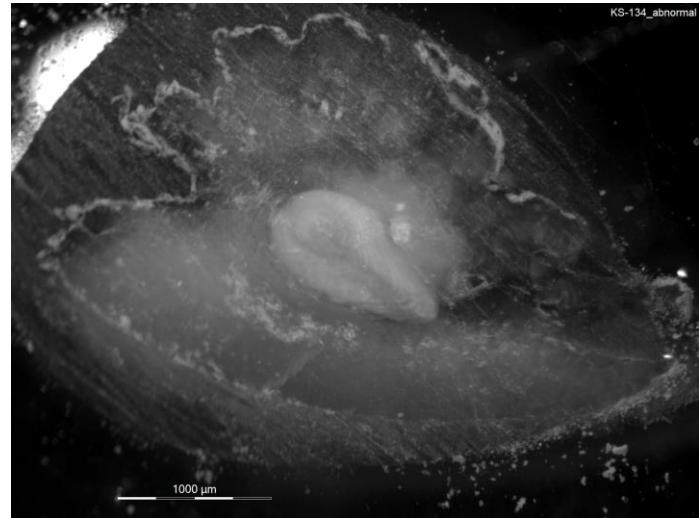
Stocked fish?

- **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 development of 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.)



Poribljanje rijeke Butižnice i Krke

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

Jučer je izvršeno redovno poribljanje rijeke Butižnice i Krke. Ovo je već drugo poribljanje u 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, ŠRD "Krka" Knin svoje obaveze nije mogla prolongirati.

300kg novih jedinki potočne pastvne pliva našim rijekama. Do sada je ubačeno ukupno 800kg pastvne u svim našim važnijim vodotocima i tu nećemo stati. Kroz skorije vrijeme poribljavanja će se nastaviti i naš projekt Zapošljavanje 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 mosta i Parkovice).



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, 19th May 2023