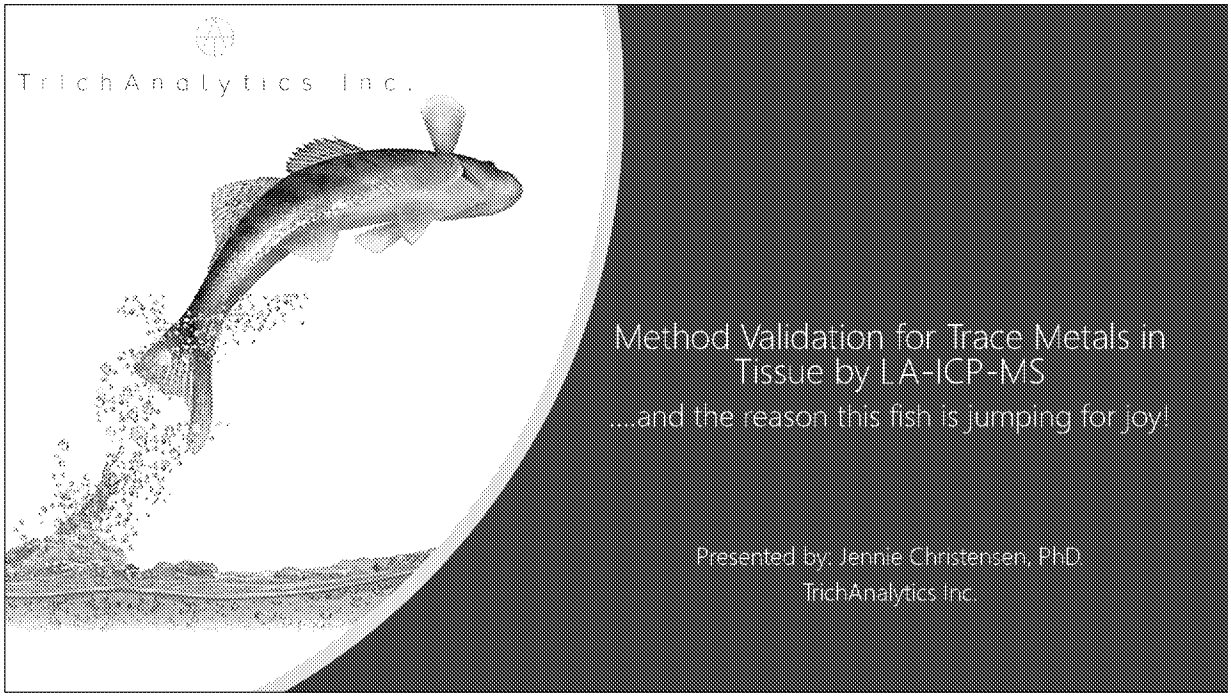
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Method Validation for Trace Metals in Tissue by LA-ICP-MS
...and the reason this fish is jumping for joy!
Presented by: Jennie Christensen, PhD
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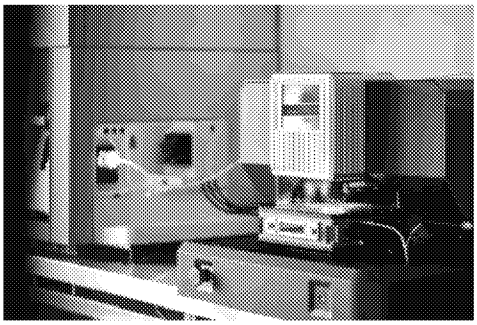
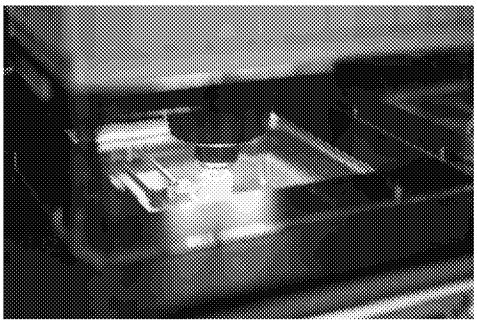
Outline

- Why LA-ICP-MS?
- Accuracy
- Precision
- Repeatability
- Sample Stability
- Detection Limits
- Internal Performance Testing
- Linearity/Range
- Robustness





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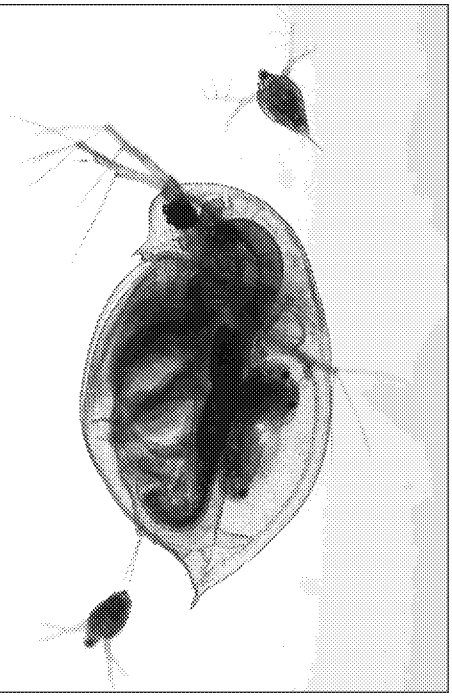
Why LA-ICP-MS?

Current standard is bulk tissue sampling, ICP-MS, etc.

- Small sample volume = high detection limits
- High sample volume = lethality of individual, invasiveness, time
- >60,000 fish sacrificed since MMER of 2002 (Kambeitz et al. 2019)
- Wet to dry weight conversion error (high moisture tissues)
- Detail can be lost, some questions can't be answered with bulk analysis
- Mercury analyzed separately (cost)



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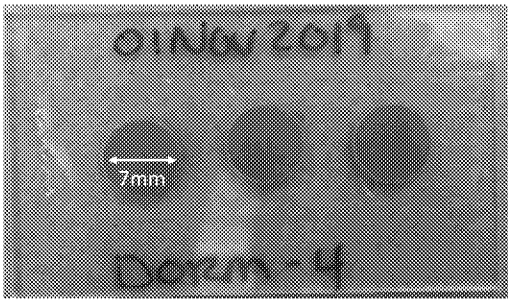
Why LA-ICP-MS?

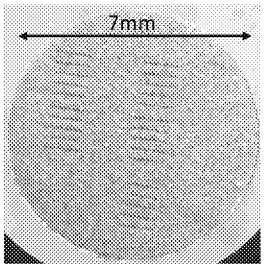
Potential Benefits

- Small sample volume (<0.01 mm^3)
- Do not consume sample, multiple analyses
- Invertebrates (more detail)
- Fish Tissue (non-lethal)
- Analyze samples dry – so no wet to dry weight conversion error
- Fast turnaround times
- No use of harsh chemicals
- Mercury included (cost benefit)



This sample has been analyzed 25 times, so far!

(<1% volume consumed)



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Why LA-ICP-MS?

Challenges to using LA-ICP-MS

- No standards (CRM) created for soft biological tissue for LA-ICP-MS
- Moisture in samples creates oxides on ablation
- Matrix-matching of standards and samples
- Potential analytical interference
- No similar laboratories for inter-lab testing
- No reference methods
- No lab currently accredited for LA-ICP-MS analysis of biological tissues (*to our knowledge)

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We DID it! (or did we?) – enter, VALIDATION

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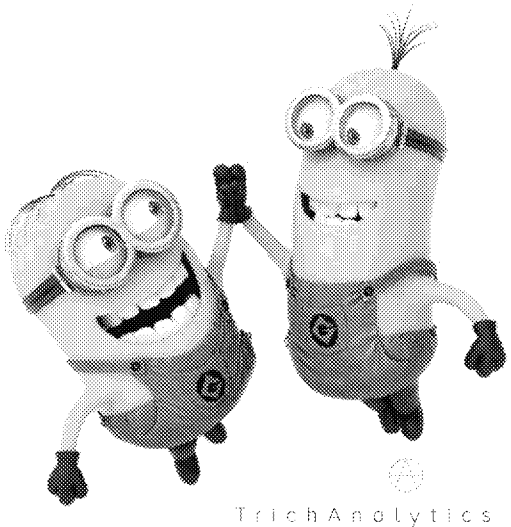
Acknowledgements

- Incredible Trich team
- NAMC-SWG
- Teck Resources
- Allegiance Coal
- Minnow Environmental
- Nautilus Environmental
- Golder Associates



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Accuracy

The measurement of how close the reportable (observed) result is to the true result.

$$\text{Accuracy (\%)} = \frac{\text{Reportable conc.}}{\text{True conc.}} \times 100$$

Method: DORM-4 (dogfish, muscle protein)

Test: NIST 1946 (wet, homogenized, fish muscle)
NIST 1566b (dry, powder, oyster)
NIST 2976 (dry, powder, mussel)

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Precision

The measurement of variability of data from an experiment that has been repeated several times.

$$\text{Precision (\%)} = \frac{\text{St Dev of Reportable concs.}}{\text{Avg of Reportable concs}} \times 100$$

Method: DORM-4 (dogfish, muscle protein)

Test: NIST 1946 (wet, homogenized, fish muscle)
NIST 1566b (dry, powder, oyster)
NIST 2976 (dry, powder, mussel)

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Accuracy/Precision - Results

CRM	Observed	Known	Accuracy (%)	Precision (%)
DORM-4	3.35 ± 0.09	3.45 ± 0.40	97.1	2.6
NIST 1946	1.94 ± 0.16	1.72 ± 0.15	113	8.1
NIST 1566b	2.29 ± 0.08	2.06 ± 0.15	111	3.3
NIST 2976	2.59 ± 0.17	1.80 ± 0.15	144	6.7

N = 10 per CRM

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time – "intra-assay precision"

$$\textit{Repeatability (\%)} = \frac{\textit{St Dev all sample sets (ppm)}}{\textit{Avg all sample sets (ppm)}} \times 100$$

Method: DORM-4 (dogfish, muscle protein)

Test: NIST 1946 (wet, homogenized, fish muscle)
NIST 1566b (dry, powder, oyster)
NIST 2976 (dry, powder, mussel)

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Repeatability - Results

CRM	Observed	Actual	Repeatability (%)	Accuracy (%)
DORM-4	3.53 ± 0.12	3.45	3.5	102
NIST 1946	2.17 ± 0.10	1.715	8.4	127
NIST 1566b	2.33 ± 0.07	2.06	3.1	113
NIST 2976	2.58 ± 0.12	1.80	4.8	144

N = 30 per CRM

Sample Stability

The measurement of sample stability over time – change in concentration.

$$\textit{Relative Percent Difference (\%)} = \frac{[1^{st}\ \textit{Reportable} - 2^{nd}\ \textit{Reportable}]}{1^{st}\ \textit{Reportable}} \times 100$$

Method: DORM-4 (dogfish, muscle protein)

Test: 4 fish muscle samples (16 Aug 2019)
4 fish ovary samples (19 Jul 2019)
4 invertebrate samples (18-19 Jul 2019)

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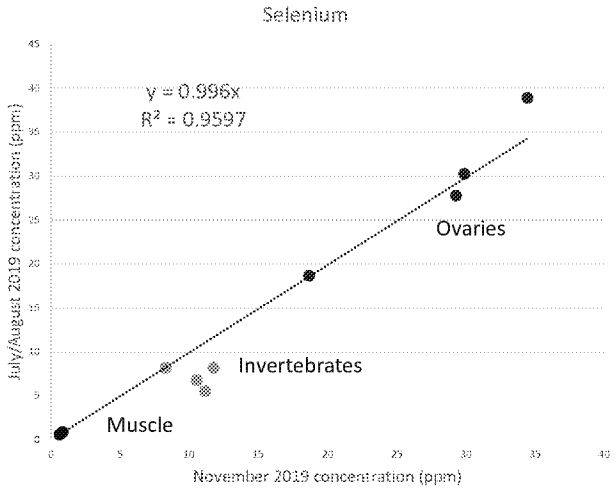
Sample Stability - Results



Excellent comparability (slope = 1)

Little variability (R^2 = 0.96)

Large concentration range: 0.596 to 38.9 ppm

N=12

Detection Limits

Measurement of method detection limits to BC MOE (2016) Detection Quality Objectives for Aquatic Tissue (dry weight converted – 80% mc)

Element	BC MOE DQO	Element	BC MOE DQO
Lithium	-	Nickel	0.05
Sodium	10	Copper	0.05
Magnesium	10	Zinc	0.5
Aluminum	2	Arsenic	0.025
Phosphorus	25	Selenium	0.1
Potassium	50	Strontium	0.05
Calcium	10	Molybdenum	0.05
Vanadium	0.1	Cadmium	0.01
Chromium	0.05	Tin	0.1
Manganese	0.1	Mercury	0.01
Iron	5	Lead	0.02
Cobalt	0.02	Uranium	0.005

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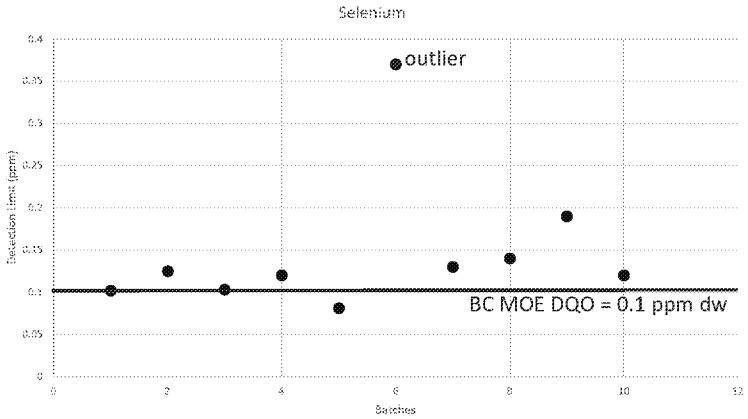
Detection Limits - Results



With outlier (0.37 ppm):
0.15 ± 0.08 ppm DL

Without outlier:
0.12 ± 0.03 ppm DL

Internal Performance Testing

Comparison of reportable results using LA-ICP-MS to an independent CALA-certified laboratory (ICP-MS, etc.)

Linear regression – slope and r^2

Method: DORM-4 (dogfish, muscle protein)

Test: 12 benthic invertebrate samples
54 fish ovary samples
54 fish muscle samples

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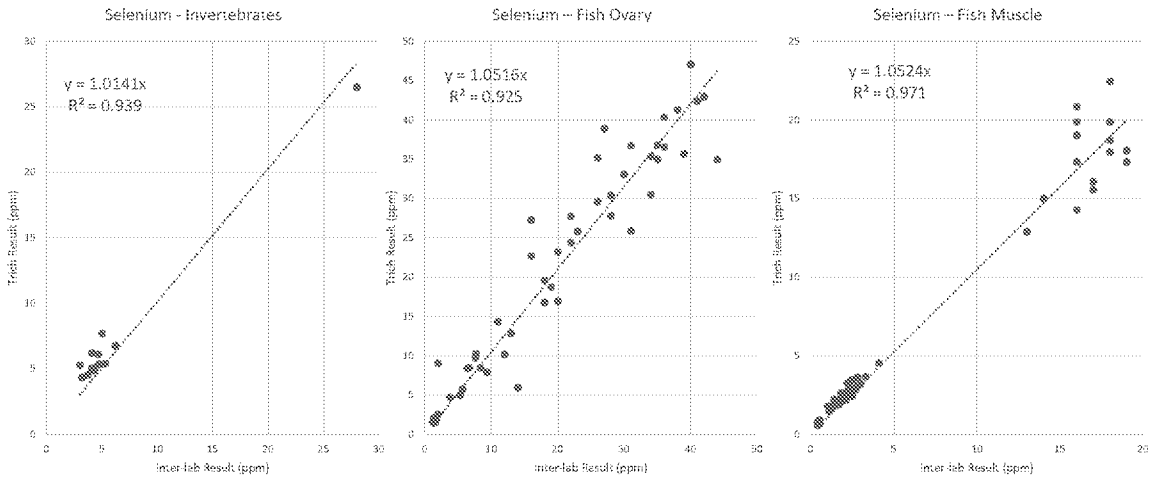
Internal Performance Testing - Results



Linearity and Range

Measuring the relationship through calibration curve using standard linear regression analysis. And what range of concentrations can you accurately quantify?

Selenium – 4 point calibration using CRMs
160 point calibration using CRMs and samples

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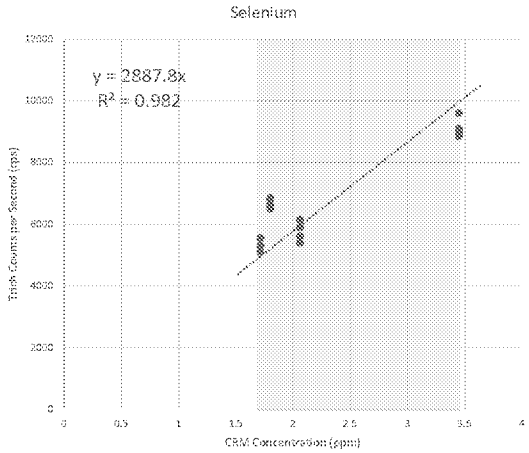
Linearity and Range - Results

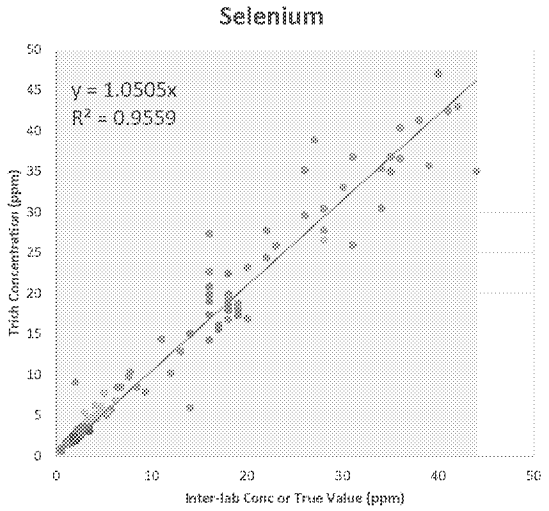


Good linearity as per high R^2 value

But small range with just CRMs:
1.7 to 3.5 ppm

N=20

Linearity and Range - Results



Excellent linearity as per high R^2 value and slope of 1

25X larger linear range:
0.6 to 47 ppm

N=160

Robustness

Consistency in results over time where machine variances occur (e.g., laser settings)

$$\text{Relative Percent Difference (\%)} = \frac{[\text{Modified} - \text{Unmodified}]}{\text{Average Result}} \times 100$$

Method: Standard laser settings (unmodified), DORM-4

Test: Laser Power x 2
Spot Size x 2
Laser Speed x 2

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Robustness - Results

Parameter	Laser Speed	Laser Power	Spot Size
Unmodified	3.69 ± 0.08	3.83 ± 0.09	3.88 ± 0.12
Decrease	3.57 ± 0.07	3.53 ± 0.05	3.61 ± 0.17
Increase	3.60 ± 0.16	3.79 ± 0.16	3.86 ± 0.13

Parameter	Laser Speed	Laser Power	Spot Size
Unmodified	100%	100%	100%
Decrease	96.7%	91.8%	92.8%
Increase	97.5%	99%	99.5%

Summary

75% complete, 25 elements, submitting to CALA for accreditation

Preliminary Results: **sample volume ~0.004 mm^3**

Validation Criteria	Selenium
Accuracy	Very Good (3/4 CRMs)
Precision	Excellent
Repeatability	Excellent
Sample Stability	Excellent
Detection Limits	Very Good (slightly above)
Internal Performance Testing	Excellent
Linearity and Range	Excellent
Robustness	Excellent

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Processing Speed

Manual (Qtegra and Excel) – **3 hours** processing after analysis

- Calibration slope
- Detection limits
- Concentration calculations
- Accuracy/Precision
- Duplicates

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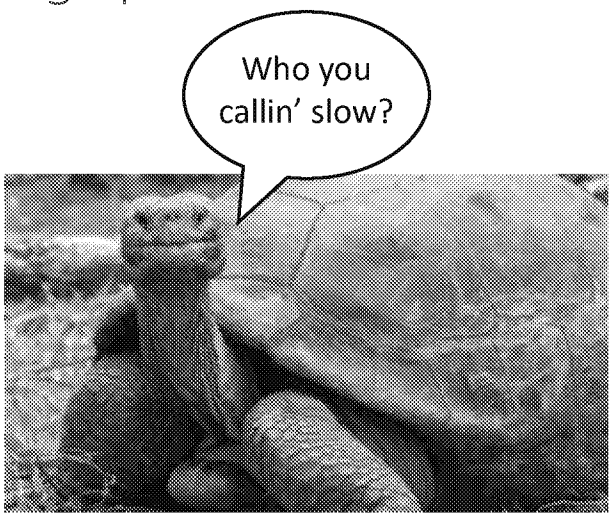

Processing Speed

In-House Data Automation/Programming:

3 hours to <u>less than 1 min</u>

- Calibration slope
- Detection limits
- Concentration calculations
- Accuracy/Precision
- Duplicates
- Diagnostic plots
- Processing log, including flags
- Output tables

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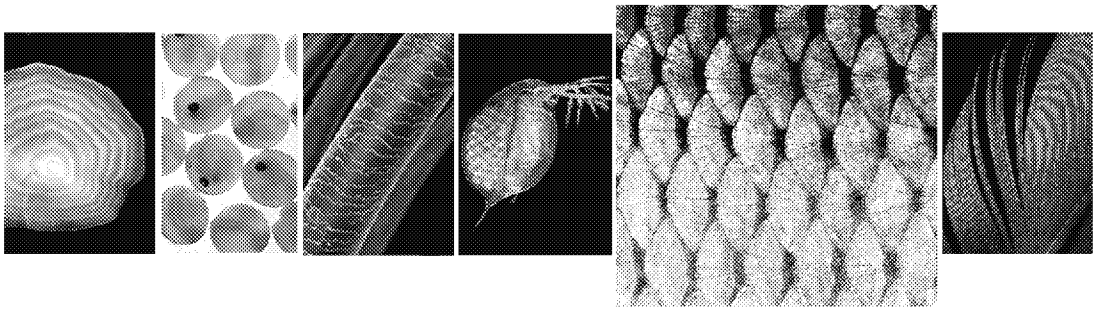
THANK YOU



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