Spectroscopic Techniques in Determining the Elemental Composition of Fish Otoliths

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NSERC CRSNG

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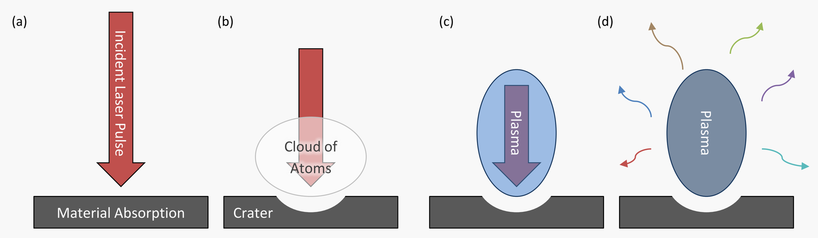
Introduction to LIBS

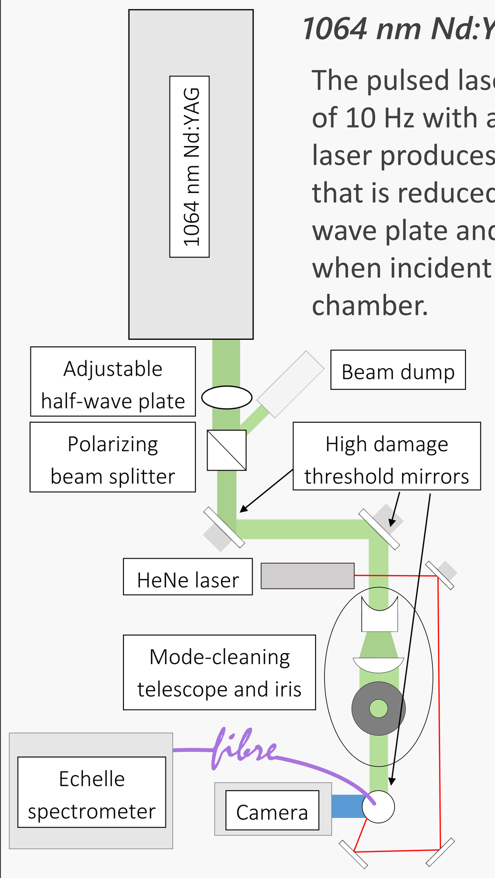
Laser Induced Breakdown Spectroscopy (LIBS)



a) A laser pulse is focused into the target where it is absorbed by the material as thermal energy.
b) The energy vaporizes atoms in the material causing them to form a cloud, leaving behind a crater.
c) The laser pulse, now incident on the cloud of atoms, super heats it to form a plasma.
d) The plasma cools releasing light through spontaneous emission characteristic of the atoms, ions, and molecules.

The light emitted by the cooling plasma is then collected by a high resolution echelle spectrometer, allowing quantification of all the elements in the target.

Experimental Apparatus



Schematic of the optical train used to direct laser pulses to the ablation target

1064 nm Nd:YAG Laser

The pulsed laser used has a repetition rate of 10 Hz with an 8 ns pulse duration. The laser produces a pulse energy of 650 mJ that is reduced to 180 mJ after the half-wave plate and further reduced to 5 mJ when incident on the target in the ablation chamber.

Ablation Chamber

The target is ablated inside a purge chamber, which is flushed with argon at 20 SCFH to maintain an atmospheric overpressure environment.

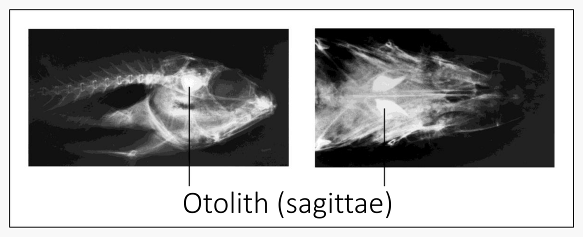
Echelle Spectrometer

The spectrometer used has a spectral range of 200 to 800 nm and a spectral resolution of 30 pm in the UV region and 120 pm in the infrared, as it is optimized for UV operation.

Introduction to Otoliths

What is an Otolith?

Otoliths are bone like structures found inside of the inner ear cavity of teleostean fishes (teleosts). They compose part of the labyrinth system, where they serve as a balance organ and a hearing aid.

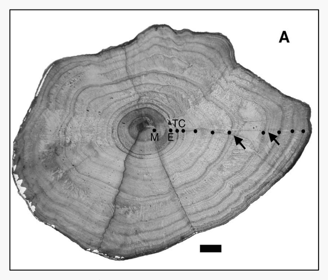


Radiograph showing location of sagittae (otoliths) within fish [*Sciaena bathytatos (Perciform:Sciaenidae)*] [1]

Elemental Structure

Otoliths grow radially outward with the translucent and opaque sections being formed each winter and summer respectively. This pattern gives a time scale for the growth.

Otoliths are mainly a calcium carbonate (CaCO3) aragonite matrix with ~10% being minor/trace elements.



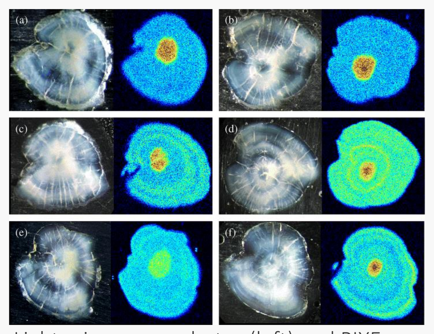
Otolith of yellow American eel (Eel 2-66, total length: 232 mm, age: 8 yr.) [*Anguilla rostrate*]. Scale bar = 100 µm. [2]

Environmental Influences

Trace elements in the otolith have been correlated to ambient water composition. This is most prevalent in movements between saltwater and freshwater.

Current Elemental Analysis

Elemental mapping of otoliths using proton-induced X-ray emission (PIXE) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) have shown that this is a measureable change in ion concentration.



Light microscope photos (left) and PIXE scans (right) of six Australian grayling showing structure of Sr:Ca in sagittal otoliths. [3]

Objective

To investigate if LIBS can reliably differentiate regions of an otolith based solely on its elemental composition. This would provide a fast and practical method for analysing the historical movements of fish as reflected in otolith composition due to changes in ambient water chemistry.

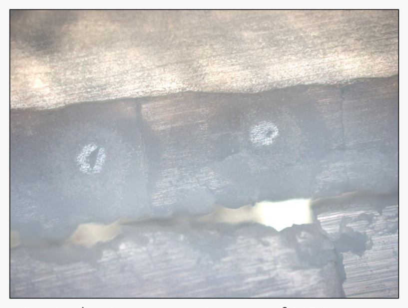
Otolith Ablation

Spatial Sensitivity

Teleosts have three pairs of otoliths, but analysis was done on the sagittae as they are the largest.

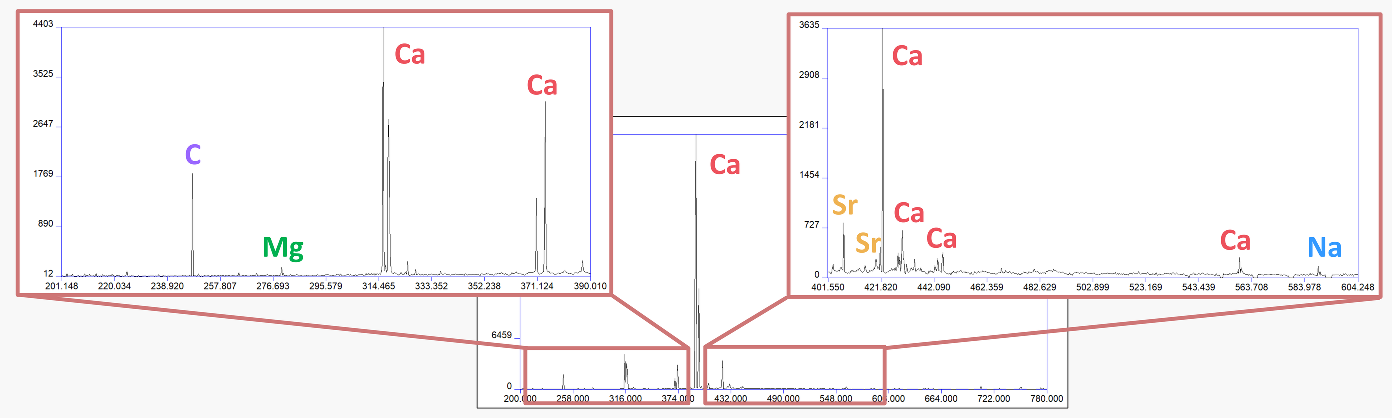
The LIBS ablation crater is ~180 µm in diameter in our setup.

The otoliths shot were from chinook salmon whose average daily ring growth is 1-2 µm, giving a temporal sensitivity of 180 – 360 days.



Optical microscope image of LIBS ablation craters on sectioned otolith.

Elemental Lines in Otolith Spectrum



Element	Wavelength (nm)
Calcium (Ca)	315.887, 370.603, 373.690, 393.366, 396.847, 422.673, 428.937, 429.899, 430.253, 445.478
Strontium (Sr)	407.771, 421.553
Magnesium (Mg)	279.553, 280.271, 285.213
Sodium (Na)	588.995, 589.593
Carbon (C)	247.856

Listed are the main lines observed in the LIBS otolith spectra.

The spectrum is dominated by Ca with other trace elements observed.

Some larger lines were Stark-broadened and could not be used to quantify concentrations.

Sample Preparation and Plating

Otolith once it has been removed from the fish

Otolith is embedded in epoxy with label.

Origin of otolith is identified using dissecting microscope and marked with straight, perpendicular line.

Otolith is cut through the marked origin with two diamond blades separated 0.39mm at 45°

Thin tab of otolith is rinsed in alcohol and water.

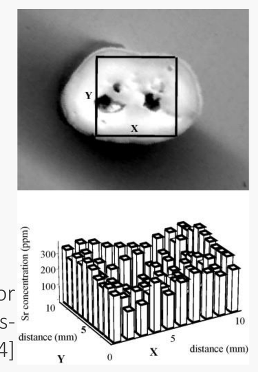
Tab is then attached to a 1 inch square mount with double sided tape to be placed inside ablation chamber.

Relevant LIBS Research

There is currently **no published work** in the literature on the application of LIBS on fish otoliths. However, there has been significant work done on similar systems.

Teeth

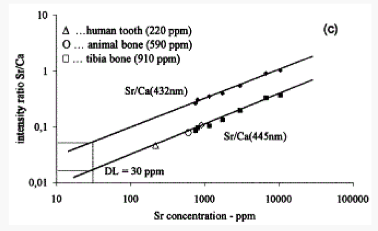
Elemental mapping has previously been preformed on teeth using LIBS. The spatial sensitivity of this study is low, but work has been done at higher spatial resolution.



Two-dimensional measurement map for Sr concentration, recorded from a cross-sectional cut through a wisdom tooth. [4]

Bones

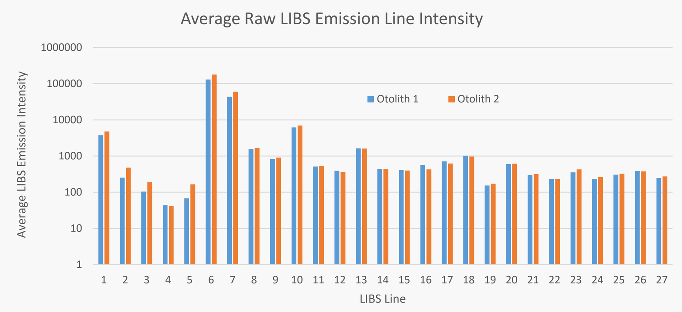
Previous work done on bone composition analysis has shown that **trace concentrations** (30 ppm) of strontium and other minor elements have been measured in calcium matrices effectively.



Calibration curve for Sr, obtained applying univariate analysis to line ratios of the trace element and Ca, recorded from reference pellets ($CaCO_3$ matrix). Detection limits (3σ) are marked by DL. Selected measurement data from tooth and bone samples are included. [4]

LIBS Data From Otoliths

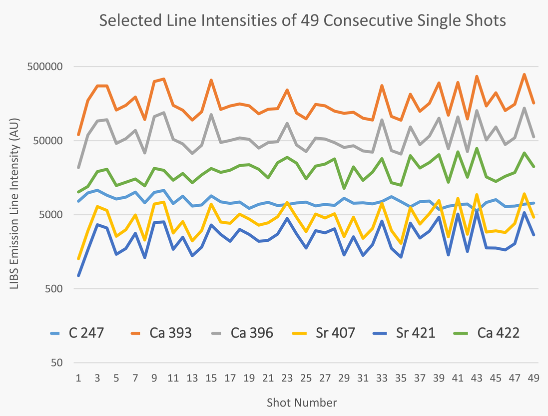
Inter-otolith Composition



1	C 247.856	15	Ca 431.865
2	Mg 279.55	16	Ca 442.544
3	Mg 280.27	17	Ca 443.497
4	Ca 300.922	18	Ca 445.478
5	Cu 324.755	19	Ca 527.028
6	Ca 393.366	20	Ca 558.876
7	Ca 396.847	21	Ca 559.447
8	Sr 407.771	22	Ca 559.849
9	Sr 421.553	23	Na 588.995
10	Ca 422.673	24	Na 589.593
11	Ca 428.937	25	Ca 616.218
12	Ca 429.899	26	Ca 643.907
13	Ca 430.253	27	Ca 646.258
14	Ca 430.774		

The average LIBS signal given off by two separate otoliths are consistent with each other.

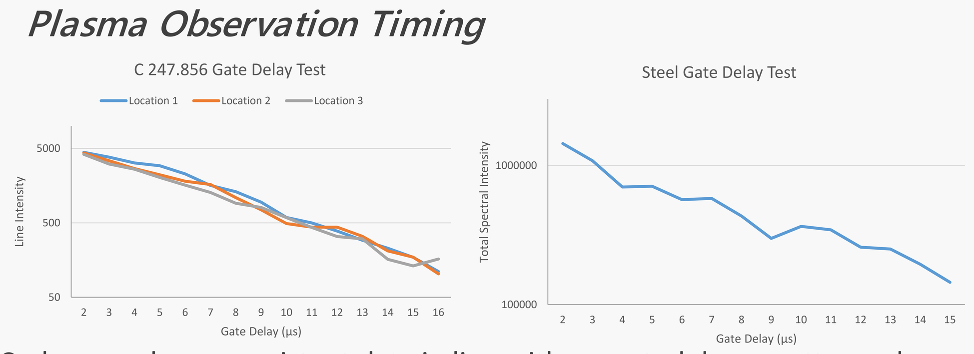
Shot-to-Shot Variation



Each data point was obtained from a single ablation event. The high standard deviation implies that multiple shots must be averaged to obtain consistent measurements of the sample.

The exception being the carbon line which does not follow the trend the rest of the lines follow, having a low standard deviation.

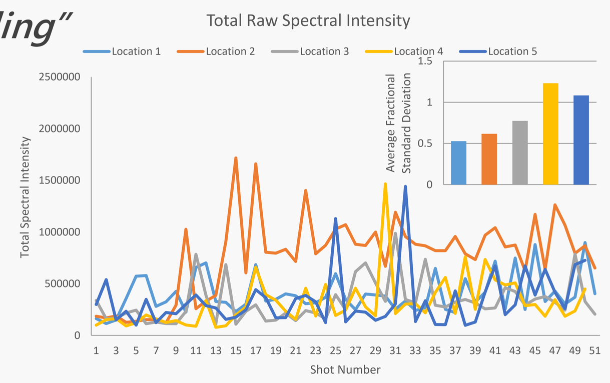
Plasma Observation Timing



Carbon produces consistent data in line with expected decay pattern when compared to iron lines in steel, showing that it is a valid analytic line.

Consecutive Shot "Drilling"

The data show that up to 51 shots there is no decay in LIBS signal. The average of the standard deviations of 37 lines was high (inset), which implies an elemental or physical inconsistency in the otolith structure.



The variation is not intrinsic to the method as daily obtained steel spectra have iron lines with an average fractional standard deviation of 12% (strong line <5%).

Future Work

Crater Analysis

Continuing work on this project will investigate the physical effects of high pulse energy laser ablation on otolith structure. SEM images will be taken to give a picture of the ablation site, allowing us to fully understand crater size and consistency under different experiment parameters.

Shot-to-Shot Variation Reduction

Investigate how to decrease shot-to-shot variation in the LIBS experimental setup when shooting plated otolith samples.

Cross-Checking

Work must be done to determine if the elements that we can detect using LIBS are useful in analysing otolith microchemistry by cross checking the LIBS results against LA-ICP-MS and PIXE data from the same otoliths.

Implementation

New techniques should be investigated to assess the practical aspects of performing LIBS in a real-life setting. Factors associated with this study will be automation techniques to ease consumer usability, and portability of LIBS setup without loss of sensitivity or specificity.

Data Analysis

Further work on chemometric algorithms can be completed to determine a practical method for determining important information on the otolith structure. Such methods will focus on the possibility of full spectrum analysis and machine learning techniques.

Practical Applications

Completion of a working methodology to analyse otolith composition will allow for a fast and effective method to analyse the historical movements of fish from the ambient water composition of their environment.

Chemometric Analysis

Analysis of LIBS spectral data for otoliths, as with many biological samples, cannot be done with simple pass-fail tests. To analyse such data sets more sophisticated chemometric algorithms are needed.

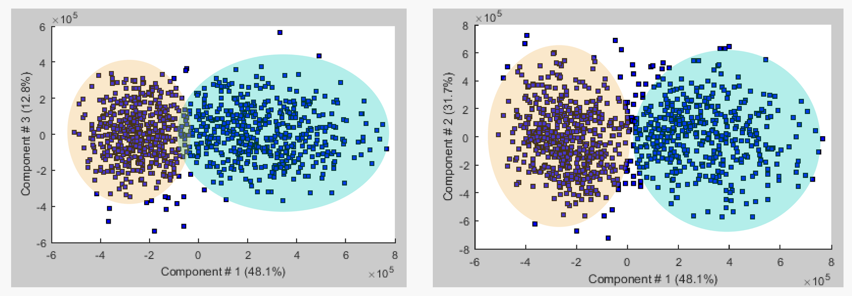
These tests were preformed to determine how effective the algorithms were when measuring a doubling (2x) of Sr signal.

Data for this analysis was simulated. Seed spectrum from otolith was used to generate 1000 intensity values with artificial noise.

Principle Component Analysis (PCA)

PCA is an unsupervised chemometric algorithm that transforms multivariate systems such that each new component is a linear combination of the original variables constructed to maximize variance.

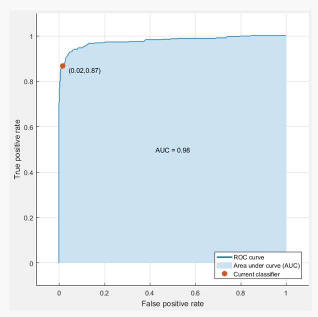
This data was generated with 20% standard deviation between files. This was found to be the upper limit of what PCA can discriminate.



Linear Discriminant Analysis

Linear discriminant analysis is a supervised chemometric classification algorithm. Supervised models must be given known training data before they can be used for classification.

True Class	Predicted Class 0	Predicted Class 1
0	434	66
1	8	492



This data was generated with 30% standard deviation. PCA fails to discriminate this data, but 10 fold cross-validation on the linear discriminant model obtained an accuracy of 92.6%.

References

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