Simultaneous collection of $^{87}Sr/^{86}Sr$ and trace-element data in otoliths and other sclerochronological hard structures

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Abstract

Chronological data from hard structures has been instrumental in reconstructing information about the past across numerous disciplines. Isotopic and trace elemental chronologies from the depositional layers of speleothems, corals, bivalve shells, fish otoliths and other structures are routinely used to reconstruct climate, growth, temperature, geological, archeological and migratory histories. Advances in instrumentation have revolutionized the use of these structures and publications detailing new chemical and isotopic methods in sclerochronology and speleothem chronology have steadily increased in number. This is particularly true of fish, in which detailed origin, life-history, and migration history can be reconstructed from their otoliths. Specifically, improvements in laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) have allowed increases temporal resolution, precision, and sample throughput. Many studies now combine multiple chemical and isotopic tracers, taking advantage of multivariate statistical methods and multiple tracep-element and isotope systems to glean further information from individual samples. This paper describes a novel laser ablation split-stream (LASS) method which allows simultaneous collection of $^{87}Sr/^{86}Sr$ and trace-elemental data from chronological carbonate samples. The study tests the accuracy and precision of multiple laser spot sizes on a marine shell standard and fish otoliths using LASS and traditional single stream methods and compared to prior otolith data on the same samples. Our results indicate that LASS techniques can be used to provide accurate and precise data at useful laser spot sizes for otolith studies, while providing time and integration-matched data reduction using newly developed features for the Iolite data reduction platform.

Introduction

Chronological data from hard structures has been instrumental in reconstructing information about the past across numerous disciplines. While tree ring dendrochronology is one of the most well-known, isotopic and trace elemental chronologies from the depositional layers of stalactites, called speleothems, have been instrumental in reconstructing climate and temperature records from the near and deep past (Hendy and Wilson 1968, Fairchild et al. 2012). Geochemistry of dust trapped in ice cores has been used to reconstruct global wind patterns (Rodda et al. 2019). Chemical and structural sclerochronologies from bivalve shells,

corals, and other animal hard parts are used to reconstruct climate, migration, and animal food sources with broad application to biology, archeology and climate science (Hudson et al. 1976, Oschmann 2009, Andrus 2011).

Understanding the provenance and movement patterns of migratory organisms is often critical to understanding their ecology (Hobson 1999). Increasingly hard-parts from the animals themselves have been found to store chemical records of these movements (Wassenaar and Hobson 1998, Rubenstein and Hobson 2004, Hobson et al. 2010). These endogenous records can provide important information about the origin and migratory path of an individual, often information that is difficult or impossible to obtain through traditional methods (Hobson et al. 2010). From birds to insects and mammals, endogenous chemical records of migration have revolutionized our understanding of animal movement (Webster et al. 2002). These chemical and isotopic sclerochonological techniques are now routinely used to reconstruct important aspects of biology and ecology including movement, ontogeny, population ecology and archeological patterns of human interaction with organisms (Jacques et al. 2002, Thornton 2011, Kohn et al. 2013, Hegg et al. 2015).

These endogenous chemical records have been particularly revolutionary in our understanding of fish movements and provenance (Begg et al. 2005, Secor 2010, Morales-Nin and Geffen 2015). The development and methods in the field mirror similar advances in related sclerocronological and speleothem fields. Fish otoliths grow in sequential layers of biogenic aragonite, storing trace elements and isotopes unique to the environments the fish inhabits through its life (Campana 1999, Campana and Thorrold 2001). These chemical tracers have been used to uncover the population structure of fish populations (Walther and Thorrold 2008, Muhlfeld et al. 2012, Brennan and Schindler 2017), as well as the details of their migratory paths(Hegg et al. 2015, Sturrock et al. 2015b, Chittaro et al. 2019), sometimes with incredible precision on the landscape (Hamann and Kennedy 2012). Otoliths provide a high-resolution, broad scale, dataset of location, growth, and ontogeny across the life of the organism which is not possible in using tagging or tracking technologies (Hegg et al. 2018).

The increasing detail and resolution of information obtained from otoliths has been tied in large part to technological advances in laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Technological advances in machine, laser, and sample chamber design have increased the resolution and throughput (Pozebon et al. 2017, Lobo et al. 2018). Analytical precision and signal-to-noise ratios have steadily improved in new ICP-MS models, and new laser ablation systems have increased sampling efficiency, capacity and sample imaging resolution. Despite this, few publications referencing methodological improvements in otolith studies have been published since Barnett-Johnson et al. (2005) validated LA-ICP-MS methods in otoliths.

These technological improvements have spurred large advances in otolith research and the percentage of papers using the technology has markedly increased over the last two decades (Campana and Thorrold 2001, Begg et al. 2005, Miller et al. 2010, Walther 2019, Wang et al. 2019). Early studies relied on differences on whole dissolution for otolith trace element or isotopic chemistry, or relatively labor-intensive techniques to mill material from the bands of otoliths (Campana et al. 1997, Volk et al. 2000, Kennedy et al. 2002). The use of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) allowed researchers to sample the otolith with greater spatial resolution, and with higher throughput, greatly increasing the

knowledge gained from otoliths and the statistical power of otolith studies (Outridge et al. 2002, Barnett-Johnson et al. 2005, Ludsin et al. 2006).

Currently, otolith studies using LA-ICP-MS rely on two analytical approaches. The first is analysis of trace elemental ratios to calcium in the otolith matrix. Changes in these elemental ratios derive from the chemistry of the environment and ontological changes (Bath et al. 2000, Sturrock et al. 2015a). These unique ratios can then be used to reconstruct the location and conditions a fish experienced through its life (Walther et al. 2008). Otolith elemental ratios are most often measured on a sector field or quadropole LA-ICP-MS system, but other backscatter methods are also used (Campana et al. 1997, Limburg et al. 2013). A second approach is measurement of the ratio of ^{87}Sr to ^{86}Sr in the otolith ($^{87}Sr/^{86}Sr$), which can be used as a high-resolution tracer of location for fish in fresh water (Kennedy et al. 1997). Strontium isotope ratios are most often measured on a multicollector LA-ICP-MS (Outridge et al. 2002, Barnett-Johnson et al. 2005, Woodhead et al. 2005).

Increasingly, researchers across the fields of animal hard-part and speleothem chronologies are using the improved analytical precision to understand chronologies in increasing resolution (Warter and Müller 2017). Scientists are also applying novel isotopic systems to reconstruct chronologies which in the past were confined largely to trace elements (Rüggeberg et al. 2008, Füllenbach et al. 2015), and combining isotopic ratios and trace element approaches to uncover multiple temporal records within the same structure (Zhou et al. 2009, Griffiths et al. 2010, Mallela et al. 2011, Woodcock et al. 2013, Chittaro et al. 2019).

A larger suite of analytes if often useful to reconstruct greater chronological detail. For this reason the use of multicollector ICP-MS in combination with sector field or quadropole ICP-MS is increasingly employed in otolith studies (Zitek et al. 2010, Martin et al. 2013, Crook et al. 2013, Garcez et al. 2014, Hegg et al. 2018, 2019). This multivariate approach, while analytically powerful, increases both the cost and the time required for analysis since each otolith must be ablated twice. Further, we have found significant problems can arise in matching the two data-streams since each analysis requires its own laser path on the otolith, and the two techniques generally require different instrument integration times. Controlling for these methodological mismatches can further increase the time and complexity involved in combining trace-element and $^{87}Sr/^{86}Sr$ analyses.

These alignment problems have been solved in other geochemical systems using laser ablation split-stream (LASS) methods to simultaneously collect data from one sample on two machines (Fisher et al. 2014, Goudie et al. 2014). LASS has also been used in a single study in otoliths to measure $^{87}Sr/^{86}Sr$ and trace-elements with a 150 µm laser spot size and using manual alignment of data (Prohaska et al. 2016).

This study presents a LASS method for collection and reduction of $^{87}Sr/^{86}Sr$ and multiple trace elements across otolith transects resulting in time and integration matched data. The methods are generally applicable to any carbonate sample requiring simultaneous measurement of trace elements and $^{87}Sr/^{86}Sr$, particularly to sclerochronological and speleothem analysis. Our first objective is to present a novel laser ablation split-stream (LASS) method for simultaneous collection of $^{87}Sr/^{86}Sr$ and trace-element data. Second, we demonstrate the analytical precision of this method on the latest generation of MC-LA-ICPMS. To do so we compare the effects of increasingly smaller laser spot sizes on two standard materials. Further, we compare the real world precision of otolith LASS and single-stream

$^{87}Sr/^{86}Sr$ analysis with prior results through a re-analysis of otolith samples analyzed in a prior study on older equipment.

Methods

Laser ablation analyses were done using both 'traditional' and LASS configurations. In the traditional configuration the laser ablation system (LA) is connected to a single inductively couple plasma mass spectrometer (LA-ICPMS). In LASS configuration, the ablated particles are 'split' such that the same sample volume is introduced to two independent mass spectrometers (Figure 1). In this study, a Telyedyne 193nm ArF laser was coupled to a ThermoFisher Element2 sector-field ICPMS for determination of elemental concentrations, and a ThermoFisher Neptune Plus multi-collector ICPMS (MC-ICPMS) for determination of Sr isotope composition. Analyses were all done using a laser rastering speed of 10um/sec, with the laser operating at 20 Hz, and ~4 J/cm^2. A circular aperture was employed, and spot sizes ranged from 40 to 10 µm.

To test the effectiveness of the LASS method, results were compared from analysis of standard material as well as otolith material across a range of laser spot sizes. Strontium isotope ratio and trace element ratios were measured on an in-house marine shell standard, while trace-element results were corrected based on NIST610 reference material. Both standards were ablated sequentially using parallel lines for 100 seconds of data in each ablation at laser spot sizes of 40, 35, 25, 20, 15, and 10 µm using LASS and single stream methods.

Subsequently, four otoliths from adult Fall Chinook in the Snake River of Idaho were analyzed using the LASS and SS configurations. Each otolith was ablated using parallel laser scans from the core to the edge of the otolith, perpendicular to the sulcus, in the same manner as prior studies on this population (Hegg et al. 2013, 2018, Chittaro et al. 2019). The limited size of the otolith core necessitated testing at only three laser spot sizes. For LASS analysis, otoliths were ablated at laser spot sizes of 35, 25, and 15 µm. For SS analysis otoliths were ablated at 25, 15, and 10 µm spot sizes, assuming increased sample at the detector in SS mode.

In both configurations the ablated sample aerosol is flushed from a two-volume laser cell using high-purity He for a carrier gas. Approximately 1m downstream from the laser cell, ~7-12 mL/min of nitrogen is introduced (dependent upon instrument configuration). Downstream of the nitrogen introduction, the "sample gas" from both the Element2 and NeptunePlus are added separately, just before a final y-piece that splits the ablated particles along with the He-Ar-N gas mixture to both mass spectrometers (Figure 1).

The instrument operating parameters are given in Table 1. The NeptunePlus MC-ICPMS is equipped with 9 Faraday detectors, which measure masses of Sr along with isobaric interference from Kr in the Ar supply, and Rb in the sample matrix (although this is almost always negligible). In this study, we employ the combination of the ThermoFisher "Jet" sample cone, and "X" skimmer cone, which has been shown to offer the highest sensitivity, though the mass bias behavior is highly sensitive to both Ar and N gas flows. We overcome this issue, yet take advantage of this high sensitivity cone configuration, by carefully tuning gas flows prior to each session such that the mass-bias corrected $^{87}Sr/^{86}Sr$ is within 0.000150 of the accepted value for modern day ocean water 0.70918 (Kuznetsov et al. 2012, Mokadem et al. 2015, El Meknassi et al. 2018) on the basis of our in-house marine shell standard (0.709186, SD = 0.000077, n=535; Hegg, Kennedy and Chittaro, 2018). Any residual offset from this canonical

modern ocean water value is corrected by normalization to analyses of the MS during the same analytical session. Both mass bias and Rb and Kr interferences corrections were done for each 0.262 second integration. Mass bias was corrected using $^{86}Sr/^{88}Sr$=0.1194. Interferences from ^{87}Rb were monitored using ^{85}Rb and corrected using $^{87}Rb/^{85}Rb$ = 0.38560. Interferences from ^{84}Kr and ^{88}Kr were monitored using ^{83}Kr, and corrected using $^{84}Kr/^{83}Kr$=4.95565 and $^{86}Kr/^{83}Kr$ =1.5026. The Sr isotope data is reduced using a customized 'data reduction scheme' (DRS; available from the second author) written for the Iolite software platform (Paton et al. 2011). For LASS data, we employ a bespoke version of this software specifically designed for LASS analysis, described in detail by Fisher et al. (2017a).

The Element2 high resolution measures ^{25}Mg, ^{43}Ca, ^{55}Mn ^{138}Ba, and ^{208}Pb. Each mass has 20 ms dwell time, with the exception of the 50 ms dwell time used for the low abundance ^{208}Pb typically observed in otoliths and shells. While quadrapole ICPMS instruments offer very rapid scanning, their abundance sensitivity is much lower than high-sensitivity sector-field instruments like the Element2, which allow robust determination of important elements like Pb that occur in trace amounts in otoliths and shells. Absolute concentrations are calibrated using the NIST 610 glass and also calculated using the Iolite software platform designed for LASS. We used Ca as the internal standard and assigned a nominal value of 45.47 wt % Ca for both the shells and salmon otoliths. While the Ca wt % of the shells and otoliths are required for determining absolute concentrations of Mg, Mn, Sr, Ba, and Pb, the ratio of these elements to Ca is not affected by the selection of this value. Otolith trace element values are reported as elemental ratios to calcium, the internal standard. This is done to control for the variable ablation of sample, and because this ratio best represents the degree of substitution of elements within the $CaCO_3$ matrix of the otolith and is thus more biologically meaningful than concentration (Campana 1999).

Following data reduction to determine elemental concentrations and Sr isotope composition in the Iolite software package (Fisher et al. 2017a) otolith data was further reduced to determine the $^{87}Sr/^{86}Sr$ value during the rearing phase of each fish (Hegg et al. 2018). This provides a more realistic example of the method, as most studies focus on the juvenile period. Further, the lower concentration of fresh water generally leads to higher errors within this section of the otolith than in the period of marine growth during which $^{87}Sr/^{86}Sr$ is biologically uninformative.

To compare the effect of spot size and method (LASS versus Single Stream) on $^{87}Sr/^{86}Sr$, a two-way ANOVA was performed data from the marine shell standard with method and laser spot size as the grouping variables. The rearing period means for the four otolith samples were compared between the prior analysis results and the LASS results at 35 µm laser spot size using a T-test of means for each analyte.

Reduction of prior data for the four comparison otoliths was done as described in Hegg et al. (2013, 2018) for $^{87}Sr/^{86}Sr$. Trace element data was reduced using the elementR package for R (R Development Core Team 2011, Sirot et al. 2017) using published CRI values for the NIST 610 standard (Gagnon et al. 2008)

Results

Analysis of the marine shell standard in LASS resulted in increasing standard errors with decreasing spot size for $^{87}Sr/^{86}Sr$ (Figure 2A, Table 2). Only spot sizes of 35 and 40 µm were comparable to the standard error of prior runs on the same marine shell during the analysis of the comparison otoliths.

Meanwhile, errors in the simultaneous trace element analysis remained relatively stable, with errors for only the least abundant elements increasing marginally down to 15 µm spot size before errors markedly increased in the least abundant isotopes of lead and barium, and finally large increases in percent standard error for strontium and magnesium at 10 µm laser spot size (Figure 2B, Table 2).

Lead and manganese were present in the marine shell at very low levels. Manganese values were largely below the ability to detect and were therefore removed from further analysis. Lead values were extremely low but detectable.

Mean uncorrected strontium isotope ratios at each spot size were compared using a two-way ANOVA to compare the effect of the method (LASS vs. Single Stream), laser spot size, and their interaction term. Method was a significant model variable ($p = 0.0014$), laser spot size was not significant ($p = 0.3509$), indicating a significant difference in mean $^{87}Sr/^{86}Sr$ between LASS and single stream but not between spot sizes. The interaction between measurement method with spot size was significant ($p = 0.0099$), indicating differing mean $^{87}Sr/^{86}Sr$ was related to an interaction between method and laser spot size. This interaction is evident in Figure 3, with $^{87}Sr/^{86}Sr$ values remaining similar between the two methods until laser spot size falls to 15 µm, at which point the methods diverge, with single stream resulting in lower values and LASS resulting in higher values.

Analysis of fish otoliths shows the real-world effect of spot size on $^{87}Sr/^{86}Sr$ error. After correcting the single stream data to match the lower integration rate of the LASS method data reduction in Iolite the errors across each otolith were similar in LASS and single stream configurations. Figure 4 shows an example otolith. The juvenile section of the salmon otoliths (between 0 and ~1100 µm from the otolith core in Figure 4) contain lower strontium concentrations due to their formation in fresh water, resulting in higher errors across these sections of the otolith. For this reason, 25 µm spot sizes began to show data that with precision outside the optimal range for otolith analysis (Figure 4), with smaller spot sizes showing increasingly higher error.

The rearing section of each otolith, identified through prior research by Hegg et al. (2018), was clipped from both the LASS and single stream otolith data to provide a real world comparison of performance of the methods (Table 3). These periods are of differing lengths depending on the life history of the individual fish. Further, the parallel position of each subsequent laser track on the otolith causes contraction and dilation of the pattern across the otolith, resulting in slightly different samples clipped from each fish.

Strontium isotope ratio measurements within the rearing section of each otolith exhibited an order of magnitude more error than the marine shell due to the decreased strontium concentration of fresh water and the smaller number of integrations used to create the average. Despite this strontium isotope ratios across each laser spot size and between LASS and single stream methods were comparable within each otolith (Figure 5). Trace element ratios were consistently slightly lower than data from the original study. The reason for this is unknown, but the low standard errors on the standard throughout indicate that this is unlikely

to be related to the LASS configuration. The limits of the size of the otolith core region made multiple samples at each laser spot size for statistical comparison impossible. Further, the compression of

Discussion

The increase in analytical precision and throughput of LA-ICPMS has spurred diverse new methods by which chronological hard structures can be used to reconstruct past events. Using corals as recorders of ocean chemistry (Rüggeberg et al. 2008, Mallela et al. 2011), stalactites to track monsoon rains and glaciation (Zhou et al. 2009, Griffiths et al. 2010), and otoliths to understand migration of fish (Hamann and Kennedy 2012, Seeley et al. 2015) multiple techniques have emerged which can be used simultaneously to reconstruct multiple histories from a single sample.

Walther (2019) presents the metaphor of otolith chemistry in terms of art, with the scientist in the role of interpreting meaning from the complexity of chemical and physical signatures within the otolith "art". This metaphor can be easily extended to other similar chronological structures from bivalve shells, mammal teeth, or stalactites. In this metaphor the more interpretive methods that can be brought to bear, the more meaning can be gleaned from the otolith "painting". As technology and throughput have improved in the study of chronological hard structures the desire to utilize multiple analytes within the same sample has increased as well. These multiple "interpretative lenses" provide multiple simultaneous perspectives on climate, migration, growth, hydrology, or temperature.

An otolith or speleothem is not like a painting, however. Because it is chronological, each element or isotopic ratio must be aligned with the others to provide meaning through time, something that is much less important to an art critic. This can be difficult when combining isotopic and trace element data from multiple machines with slightly differing laser track lengths and different integration times and can potentially introduce additional error. Using the LASS data reduction methods developed for Iolite (Fisher et al. 2017b) and simultaneous collection of data from the same laser track eliminates these alignment errors while resulting in output data that is matched to the same integration time and thus easier to analyze.

Because the sample is split between two ICPMS machines during LASS analysis, a spot size large enough to provide enough sample to each detector is required to maintain precision. Validating LA-ICPMS for $^{87}Sr/^{86}Sr$ analysis Barnett-Johnson (2005) reported external error of ±0.00003 (2•SE) with an 80 µm spot size on marine carbonate. Our analysis on the marine shell standard indicates that similar levels of precision (±0.00004) can be achieved with 35 and 40 µm spot sizes on current equipment, with 25 µm spot sizes only slightly higher (Table 2). Precision was less dependent on spot size for trace-element analysis during LASS. Standard error increased slowly down to 20 µm laser spot sizes before jumping higher.

The accuracy was not significantly different with decreasing spot size on the marine shell, however visual inspection of the results indicates that accuracy is affected by spot size (Figure 3). Below 20 µm accuracy was low, and the best results were clearly obtained at 35 and 40 µm spot sizes. The reason for the difference in $^{87}Sr/^{86}Sr$ results between LASS and single

stream methods at 25 µm is unknown but could be related to variation across the marine shell given that precision of these measurements was high. However, this does highlight the significant effect of method in the marine shell ANOVA, with LASS tending to err with a positive bias while single stream erred low. The reason for this bias is unknown, but future work using these LASS methods should account for this possibility.

Data from the salmon otoliths indicated that LASS performed similarly to single stream for $^{87}Sr/^{86}Sr$ analysis, and accuracy compared well to prior results (Figure 5). In the Snake River, where this fish were captured, tributary $^{87}Sr/^{86}Sr$ varies at the scale of $1x10^{-3}$ (Hegg et al. 2013). Given this, data for the example otolith in Figure 4 is still useful for classifying fish location down to 25 µm laser spot size, even with the low sample size following clipping the rearing section of the fish. However, for systems with lower variability in background signature larger spot sizes or longer scans along the otolith rings might be needed.

Trace element results for the rearing sections of the four otoliths are difficult to interpret beyond the broadest scale. While $^{87}Sr/^{86}Sr$ is stable during the rearing period other elements do not necessarily remain stable and signatures in all four fish exhibited changes during the rearing period. Thus, the average of any given trace element is heavily affected by variations in the clipped portion. Because each laser track was performed parallel to the others the curved nature of the otolith rings resulted in minor expansion and compression of the chemical patterns between runs, which are visible in the example otolith in Figure 4. This resulted in unavoidable differences in the clipped portion and the inability to easily compare their values in anything but the broadest sense. Because trace elements are affected both by environment and ontogeny, during the course of an otolith study the magnitude of the elemental ratios would cluster for fish following similar life histories, however in this context the analytical accuracy and precision is difficult to measure.

The marine shell standard used is a natural shell and is not intended as a trace element standard. Variation in trace elements exists across the surface of the standard, as has been demonstrated in many marine bivalves (Butler and Schöne 2017, Markulin et al. 2019). A homogenous carbonate standard was not available during this study. Therefore, we recommend that future work to validate this method should examine the stability of trace element ratios using one of the available homogenous carbonate standards (Weber et al. 2018, Jochum et al. 2019). The sensitivity of the Element2 sector field ICP-MS, and the stability of the standard error of abundant isotopes for LASS runs indicates that $^{87}Sr/^{86}Sr$ is the method most prone to error at small spot sizes within the LASS system.

The ability to generate and easily reduce simultaneous isotope ratio and trace element data represents a significant advance in the collection of chronological transect data from carbonate samples. The need for consistent reduction methods has been noted by multiple authors (Sirot et al. 2017, Willmes et al. 2018) and the addition of LASS methods presents additional opportunity for alignment and data reduction errors. The method detailed in this study shows that accuracy and precision of $^{87}Sr/^{86}Sr$ ratios can be achieved at smaller spot sizes than in prior attempts at LASS on fish otoliths (Prohaska et al. 2016). Further, these results can be achieved while outputting time and integration matched data using Iolite reduction software, significantly shortening the reduction and analysis time for multiple data streams. While the elements and isotope ratios demonstrated here are limited the reduction scheme is

scalable to multiple trace-element ratios and isotope systems, as well as the use of multiple ICPMS platforms (Fisher et al. 2017a).

Tables

Table 1 – Instrument operating parameters for laser ablation analysis

Instrument Operating Parameters

	MC-ICPMS	Sector Field ICPMS
ICPMS Model	ThermoScientific Neptune Plus	ThermoFinnigan Element2
Power		1250W
Instrument Gas Flows		
Cool/Plasma (Ar)	15.0 L • min^{-1}	16.0 L • min^{-1}
Auxiliary (Ar)	1.0 L • min^{-1}	0.95 L • min^{-1}
Sample (Ar)	0.6 L • min^{-1}	0.99 L • min^{-1}
	(Single Stream 0.5)	
Laser Carrier Gas		
MFC1 (He)	0.65 L • min^{-1}	0.65 L • min^{-1}
MFC2 (He)	0.35 L • min^{-1}	0.35 L • min^{-1}
MFC3 (N)	12.0 ml • min^{-1}	12.0 ml • min^{-1}
Laser Ablation Settings		*Masses Measured*
Model	Telyedyne 193nm ArF	^{25}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr, ^{138}Ba, ^{208}Pb
Rep Rate	20 Hz	0.7 sec scan (20 ms per element, ^{208}Pb 50 ms)
Laser Fluence	4.25 J • $(cm^2)^{-1}$	
Spot Size	Varied from 10 – 40 µm (circle)	

Table 2 – Analysis of marine shell analytical standard

Method	Laser Spot Size (µm)	Sr87_Sr86	2SE	Mg (ppm)	2•%SE	Mn (ppm)	2•%SE	Sr (ppm)	2•%SE	Ba (ppm)	2•%SE	Pb (ppm)	2•%SE
LASS	40	0.70932	0.00004	192.2	1.2	<LOD	-	1072	0.7	0.708	1.9	0.66	2.5
LASS	40	0.70933	0.00004	177.8	1.0	<LOD	-	1069	0.6	0.704	1.6	0.615	2.2
LASS	40	0.70928	0.00004	169.3	0.6	<LOD	-	1081	0.6	0.729	1.7	0.673	2.6
LASS	35	0.70927	0.00004	164.3	0.6	<LOD	-	1118	0.7	0.742	1.8	0.731	2.7
LASS	35	0.70931	0.00004	158.4	0.6	<LOD	-	1094	0.7	0.738	1.9	0.706	2.6
LASS	35	0.70934	0.00004	155.7	0.7	<LOD	-	1109	0.6	0.727	1.7	0.725	2.4
LASS	25	0.70933	0.00007	149.5	0.8	0.55	30.91	1140	0.8	0.969	2.3	1.146	3.0
LASS	25	0.70932	0.00007	145.7	0.8	<LOD	-	1124	0.8	0.953	2.4	1.176	3.1
LASS	25	0.70936	0.00007	146.9	0.7	<LOD	-	1137	0.8	0.991	2.3	1.197	2.8
LASS	20	0.70919	0.00008	137.5	1.0	1.14	18.86	1154	0.9	1.05	2.6	1.221	3.2
LASS	20	0.70927	0.00008	134.5	0.9	0.5	47.00	1194	0.8	1.025	2.9	1.258	3.1
LASS	20	0.70933	0.00008	132.4	1.0	<LOD	-	1242	0.9	1.129	2.8	1.42	3.9
LASS	15	0.70939	0.00012	140.3	1.0	0.35	91.43	1154	0.8	0.346	5.3	0.033	48.5
LASS	15	0.70944	0.00012	136.9	0.9	<LOD	-	1192	0.8	0.405	4.9	0.037	45.9
LASS	15	0.70948	0.00012	134.9	0.9	<LOD	-	1224	0.9	0.332	5.7	0.035	48.6
LASS	10	0.70926	0.00022	147.8	2.6	<LOD	-	1439	1.5	2.1	4.0	2.84	3.7
LASS	10	0.70934	0.00020	205	4.1	<LOD	-	1566	1.7	2.15	3.5	2.55	4.1
LASS	10	0.70957	0.00019	226	3.1	<LOD	-	1692	1.6	2.19	3.7	3.01	4.3
Single Stream	35	0.70932	0.00004	-	-	-	-	-	-	-	-	-	-
Single Stream	35	0.70927	0.00004	-	-	-	-	-	-	-	-	-	-
Single Stream	35	0.70929	0.00004	-	-	-	-	-	-	-	-	-	-
Single Stream*	30	0.70829	0.00002	-	-	-	-	-	-	-	-	-	-
Single Stream	25	0.70927	0.00006	-	-	-	-	-	-	-	-	-	-
Single Stream	25	0.70925	0.00006	-	-	-	-	-	-	-	-	-	-
Single Stream	25	0.70927	0.00007	-	-	-	-	-	-	-	-	-	-
Single Stream	20	0.70928	0.00010	-	-	-	-	-	-	-	-	-	-
Single Stream	20	0.70935	0.00009	-	-	-	-	-	-	-	-	-	-
Single Stream	20	0.70930	0.00009	-	-	-	-	-	-	-	-	-	-
Single Stream	15	0.70925	0.00015	-	-	-	-	-	-	-	-	-	-
Single Stream	15	0.70928	0.00013	-	-	-	-	-	-	-	-	-	-
Single Stream	15	0.70927	0.00015	-	-	-	-	-	-	-	-	-	-
Single Stream	10	0.70914	0.00023	-	-	-	-	-	-	-	-	-	-
Single Stream	10	0.70913	0.00025	-	-	-	-	-	-	-	-	-	-
Single Stream	10	0.70924	0.00027	-	-	-	-	-	-	-	-	-	-

*The mean of 8 standards run during the first data collection on the comparison otoliths, shown here for comparison. Marine shell standard was not run as a trace-element standard during that analysis period.

Table 3 - Analysis of the rearing period of four adult Fall Chinook salmon otoliths using LASS and Single Stream methods. Trace element ratios are reported in ppm x 1000.

Method	Sample	Laser Spot Size (µm)	n	$^{87}Sr/^{86}Sr$	2SE	Mg:Ca	%SE	Mn:Ca	%SE	Sr:Ca	%SE	Ba:Ca	%SE
LASS	2007	20	58	0.70963	0.00043	0.078	2.03	0.020	6.33	1.750	1.06	0.025	1.99
LASS	2007	25	53	0.70969	0.00022	0.069	2.42	0.017	4.79	1.649	1.12	0.022	1.89
LASS	2007	35	54	0.70979	0.00017	0.066	2.69	0.015	5.69	1.619	0.86	0.022	2.91
LASS	4098	20	35	0.71193	0.00060	0.092	3.83	0.050	7.18	1.571	3.13	0.030	5.51
LASS	4098	25	45	0.71196	0.00030	0.081	2.31	0.038	6.26	1.650	2.78	0.032	6.51
LASS	4098	35	34	0.71225	0.00015	0.080	2.78	0.037	7.46	1.432	2.29	0.023	4.33
LASS	5064	20	20	0.71010	0.00059	0.061	3.33	0.022	5.02	2.074	1.80	0.045	3.71
LASS	5064	25	20	0.71003	0.00023	0.056	2.35	0.024	4.57	2.255	0.66	0.041	0.79
LASS	5064	35	21	0.71043	0.00030	0.070	3.24	0.027	4.72	2.156	1.67	0.040	3.87
LASS	m4575	20	31	0.70984	0.00066	0.069	4.35	0.014	9.29	1.784	2.01	0.025	6.13
LASS	m4575	25	31	0.70999	0.00034	0.068	4.05	0.019	8.02	1.762	1.04	0.025	5.27
LASS	m4575	35	31	0.70999	0.00016	0.063	5.21	0.017	9.30	1.735	0.96	0.024	6.01
Single Stream	2007	10	26	0.71151	0.00052	-	-	-	-	-	-	-	-
Single Stream	2007	15	34	0.71006	0.00052	-	-	-	-	-	-	-	-
Single Stream	2007	25	35	0.70968	0.00015	-	-	-	-	-	-	-	-
Single Stream*	2007	30	32	0.71006	0.00012	0.128	2.62	0.012	7.28	1.854	1.15	0.017	2.67
Single Stream	4098	10	34	0.71058	0.00092	-	-	-	-	-	-	-	-
Single Stream	4098	15	36	0.71175	0.00025	-	-	-	-	-	-	-	-
Single Stream	4098	25	39	0.71193	0.00029	-	-	-	-	-	-	-	-
Single Stream*	4098	30	31	0.71174	0.00014	0.133	2.39	0.051	5.51	1.848	2.96	0.030	5.30
Single Stream	5064	10	19	0.70988	0.00091	-	-	-	-	-	-	-	-
Single Stream	5064	15	29	0.70983	0.00043	-	-	-	-	-	-	-	-
Single Stream	5064	25	25	0.70999	0.00020	-	-	-	-	-	-	-	-
Single Stream*	5064	30	57	0.70995	0.00012	0.104	3.67	0.025	2.60	1.966	0.81	0.028	1.40
Single Stream	m4575	10	26	0.70928	0.00126	-	-	-	-	-	-	-	-
Single Stream	m4575	15	36	0.70959	0.00049	-	-	-	-	-	-	-	-
Single Stream	m4575	25	26	0.70990	0.00029	-	-	-	-	-	-	-	-
Single Stream*	m4575	30	27	0.70978	0.00017	0.091	6.48	0.025	6.44	2.058	1.36	0.036	4.21

* Original data run on older equipment

Figures

Figure 1 – Conceptual diagram of gas and sample flows in the split stream (LASS) configuration (left) and single stream (right).

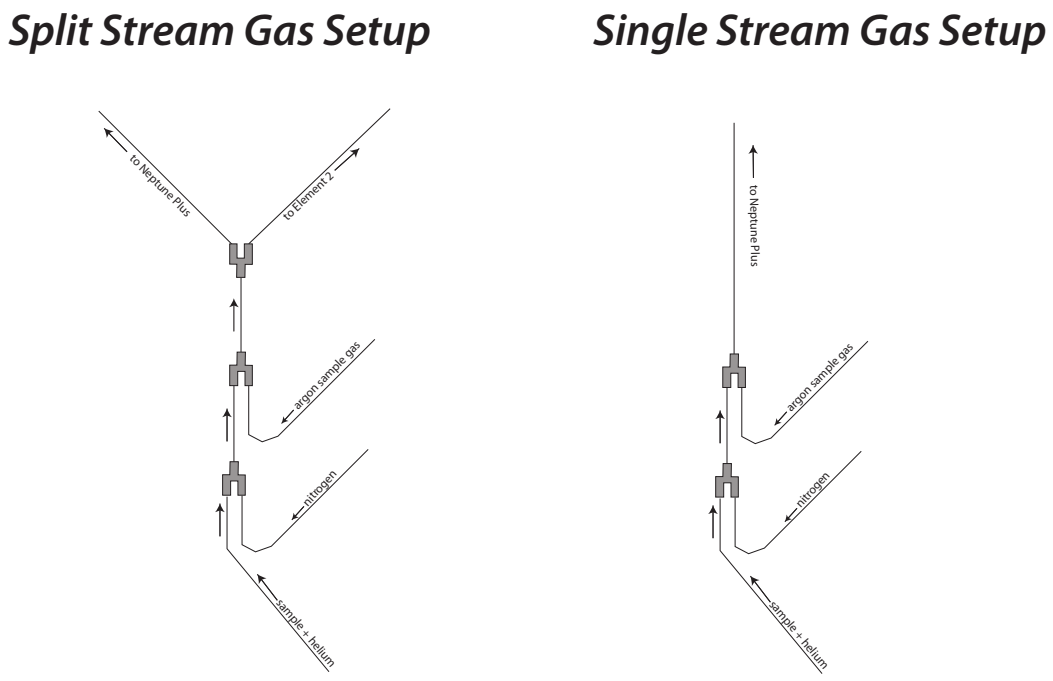
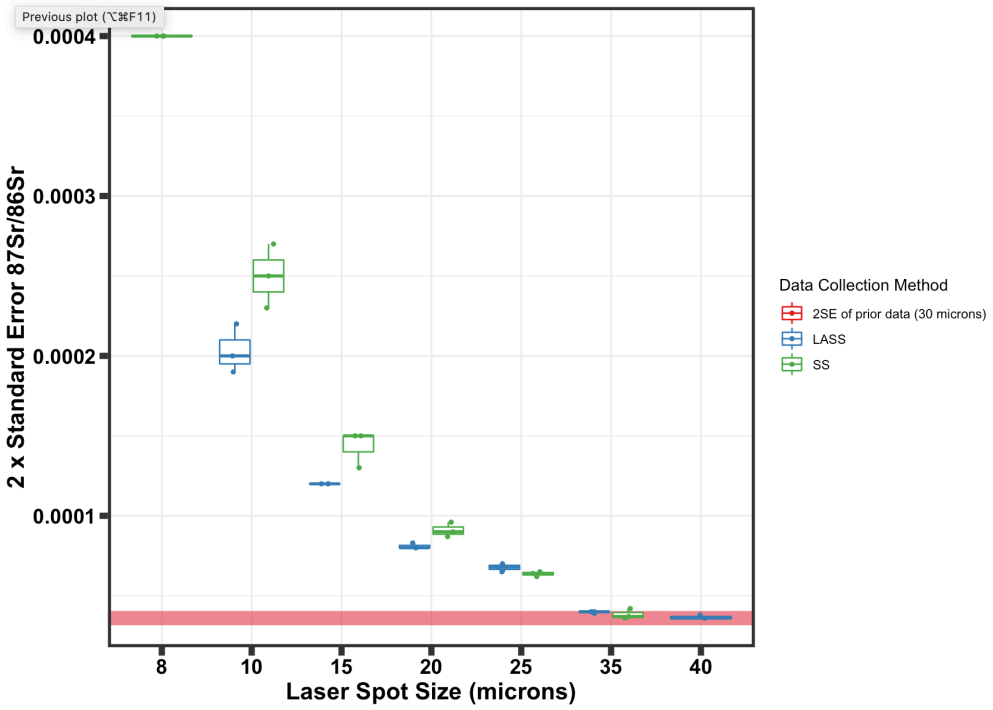


Figure 2 – The standard error of strontium ratio measurements on a marine shell standard (**A**) decreases with increasing laser spot size in both LASS (blue) and single stream (green). The standard error of prior otolith work (red band) performed using a 30 µm spot is shown. The width of the band represents the mean and 2xSE of 7 analyses of the same standard run during prior analysis of the 4 comparison otoliths. Percent standard error remained (**B**).

A



B

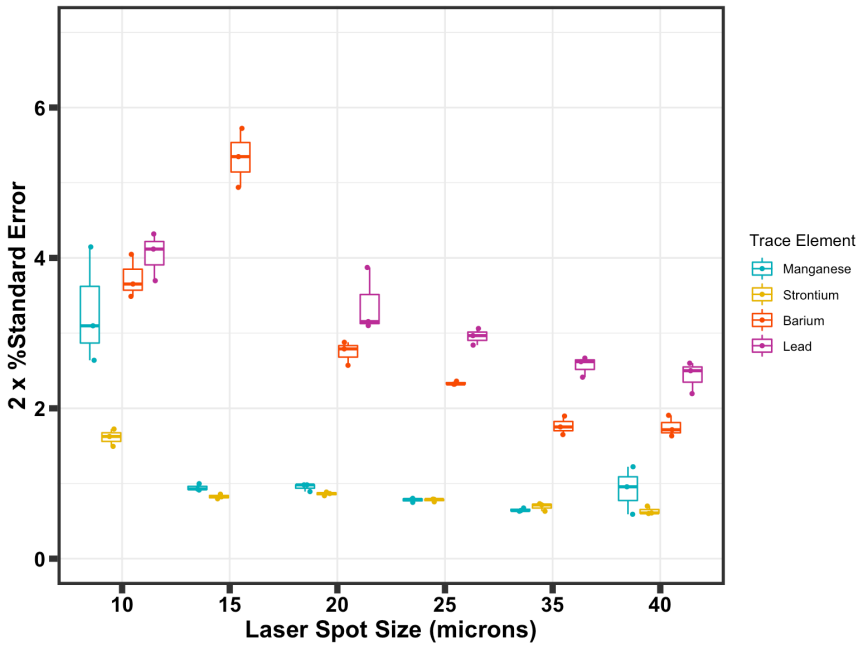


Figure 3 – Comparison of $^{87}Sr/^{86}Sr$ precision on the marine shell standard at varying spot sizes between LASS and single stream data collection.

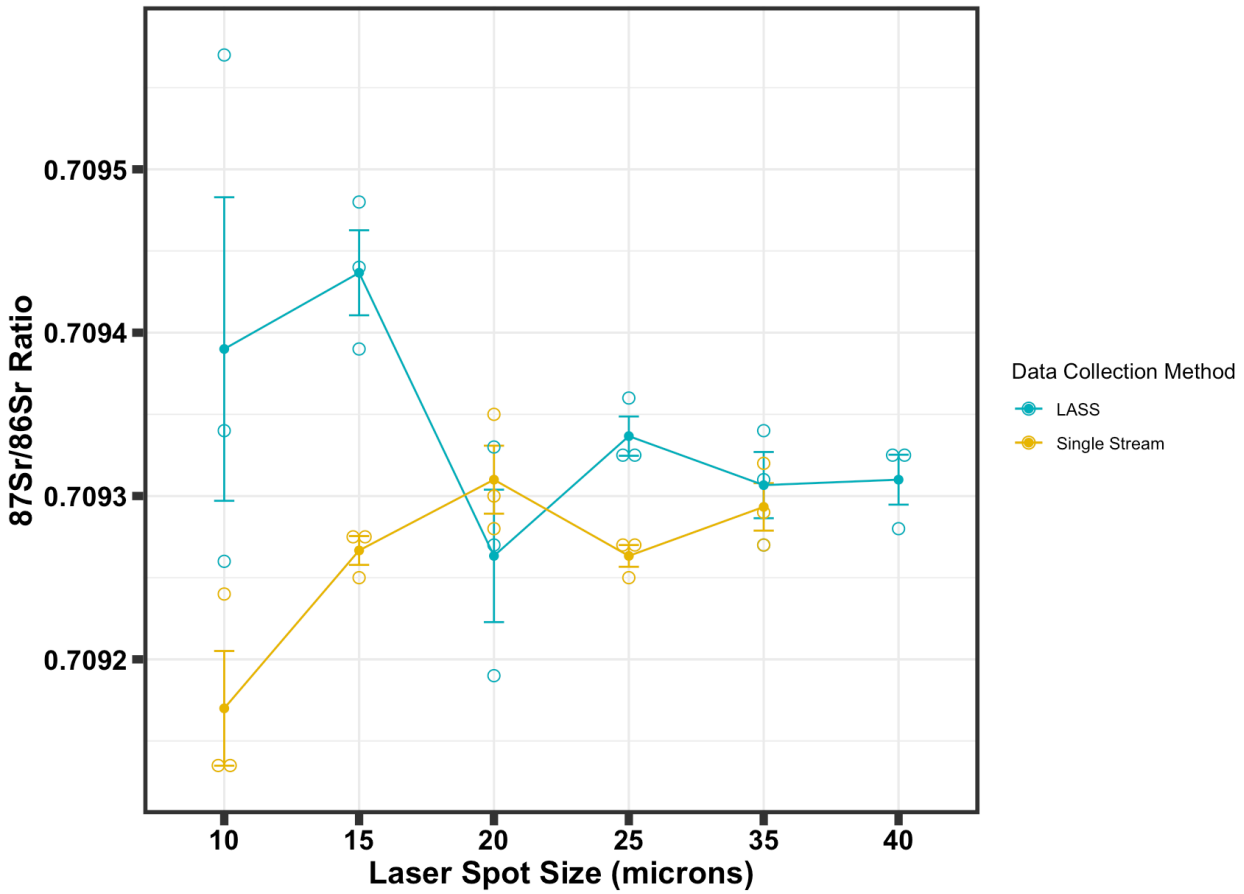


Figure 4 - Comparison of strontium ratio data analysed at multiple laser spot sizes (rows) and using single-stream (column 1) and LASS (column 2) on a continuous transect across the same otolith using a Thermo Neptune Plus and Telyedyne 193nm ArF laser combination. Prior data collected on a Thermo Neptune and New Wave UP-213 laser with a 30 micron spot size is shown (row 4, column 1). Single Stream data has been downsampled by averaging every 3 integrations to better match LASS data which is output at a lower integration rate.

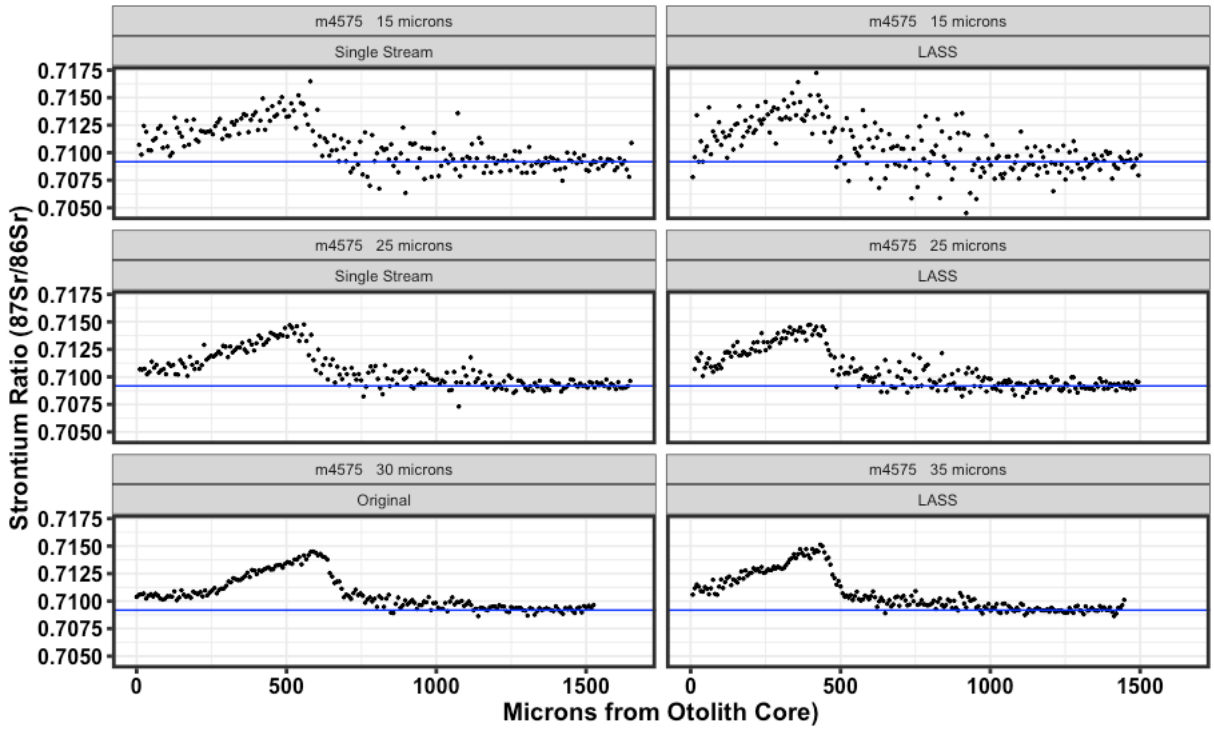
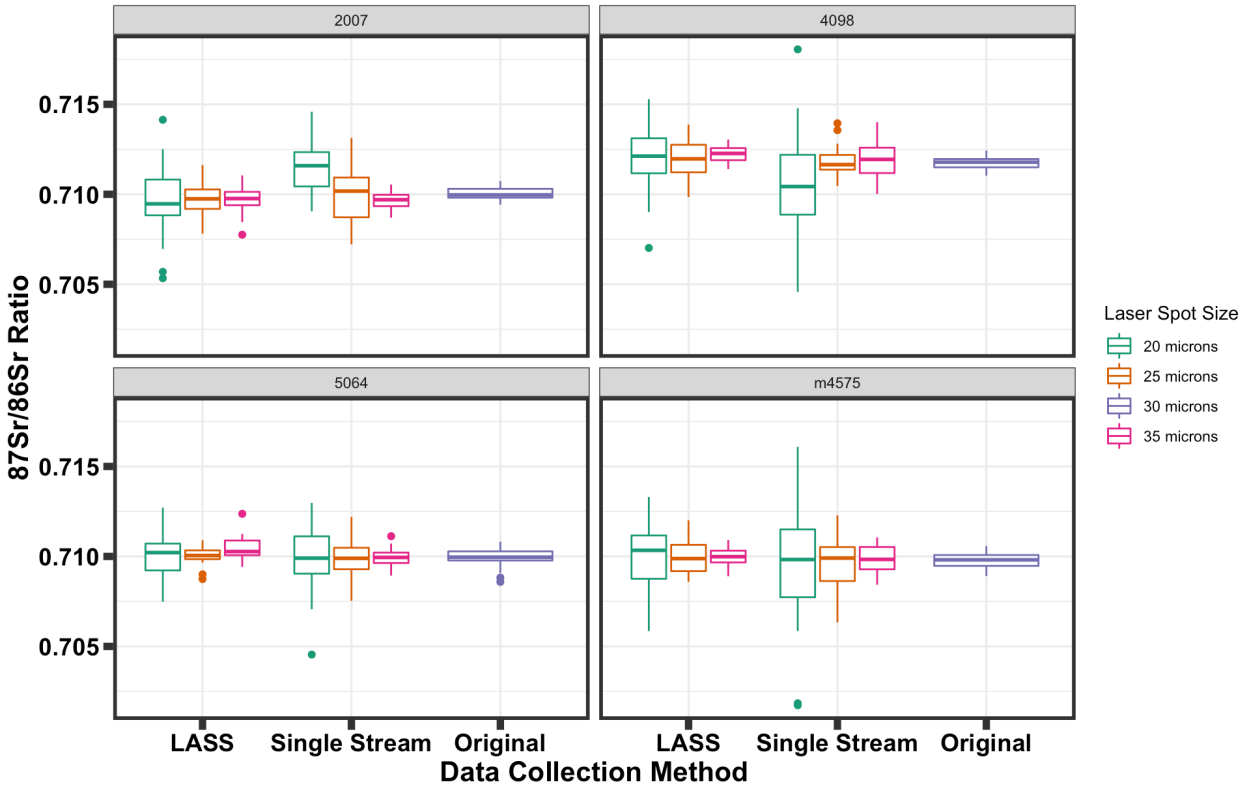



Figure 5 – Strontium isotope ratios measured in the rearing sections of four salmon otoliths using LASS, and single stream collection methods and at differing laser spot sizes. Original data from a prior study is shown for comparison.