

**The feasibility of using otolith microchemistry
to trace movements of rainbow trout and common smelt
in lakes Rotoiti and Rotorua**

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by

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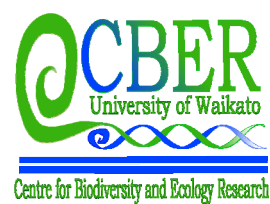
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Abstract

Otolith microchemistry can be used to determine the natal origins of fish. Our feasibility study using single water samples has determined that the water chemistry around Lakes Rotorua and Rotoiti have different elemental compositions warranting further investigation. We have shown that juvenile trout populations from spawning tributaries around lakes Rotorua and Rotoiti, Rotorua lakes district, New Zealand, could be grouped by lake using the $^{137}\text{Ba}:$ ^{43}Ca isotopic ratio obtained from otolith laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis. The $^{88}\text{Sr}:$ ^{43}Ca isotopic ratio further distinguished between different sample locations when combined with the $^{137}\text{Ba}:$ ^{43}Ca isotopic ratio. Laser ablation analysis across common smelt otoliths provided information related to the movements between different chemical habitats across the fishes life and some distinct resident and migratory patterns are presented although further investigation into migration is required.

Our preliminary results indicate that we will be able to distinguish natal stream for juvenile rainbow trout and probably lake of origin for common smelt.

Introduction

Otoliths are paired, calcified structures found in the heads of fishes that provide life history information (Arai & Hirata, 2006; Campana, 1999; Thresher, 1999) by incorporating chemicals from the water in their surrounding environment continuously through life (Milton & Chenery, 2001; Payan et al, 2004). This effectively stores a permanent and ongoing record of the chemical habitats that the fish has occupied. Otoliths grow outwards from a nucleus (Morales-Nin, 2000) and through exposure and analysis of the nucleus, the elemental composition of the original natal and rearing habitat each fish has occupied can be determined.

Otolith microchemical analysis has been used to determine the natal spawning habitats of adult fish e.g. Atlantic salmon (Veinott & Porter, 2005), carp (Crook, 2004)

and lake trout (Munro et al., 2004). Laser ablation and inductively coupled plasma mass spectrometry (LA-ICP-MS) offer an effective way to analyse otolith microchemistry (Thresher, 1999; Geffen et al, 2003). A suite of isotopes was determined and a guideline for standard laser operation was established to minimise error between different laser ablation runs.

A range of factors can influence LA-ICPMS, which can affect the results obtained. Polyatomic or isobaric interferences (May & Wiedmeyer, 1998) can be due to different factors such as the interaction of the argon carrier gas, creating a background the same atomic mass as any isotopes used for analysis, creating an error or incorrect count. Interferences influence the isotopic counts obtained from the mass spectrometer giving a false indication of the actual isotopic composition of the otolith matrix. The aim of this project is to test the feasibility of determining natal habitat in rainbow trout and smelt in lakes Rotorua and Rotoiti using otolith microchemical analysis. As a first step, we analysed the otoliths of juvenile trout from the main spawning tributaries of both lakes to test the consistency among trout in one stream and the differences between streams. We examined the common smelt at three beach locations.

Methods

Study Site Description

The juvenile trout samples were obtained by electrofishing six tributary streams around lakes Rotorua and Rotoiti (Figure 1) and from a sample of fish from the hatchery. The smelt samples have been obtained by seine netting eight different beach locations around lakes Rotorua and Rotoiti (Figure 1). These sample locations have previously had their water chemistry analysed (Table 1) to see if differences exist in the elemental composition of the water. Due to the magnitude and characteristics of these tributary streams, they are assumed to be large contributors to the wild populations of rainbow trout in the two lakes (pers. comm. Rob Pitkethley, Eastern Region Fish and Game).

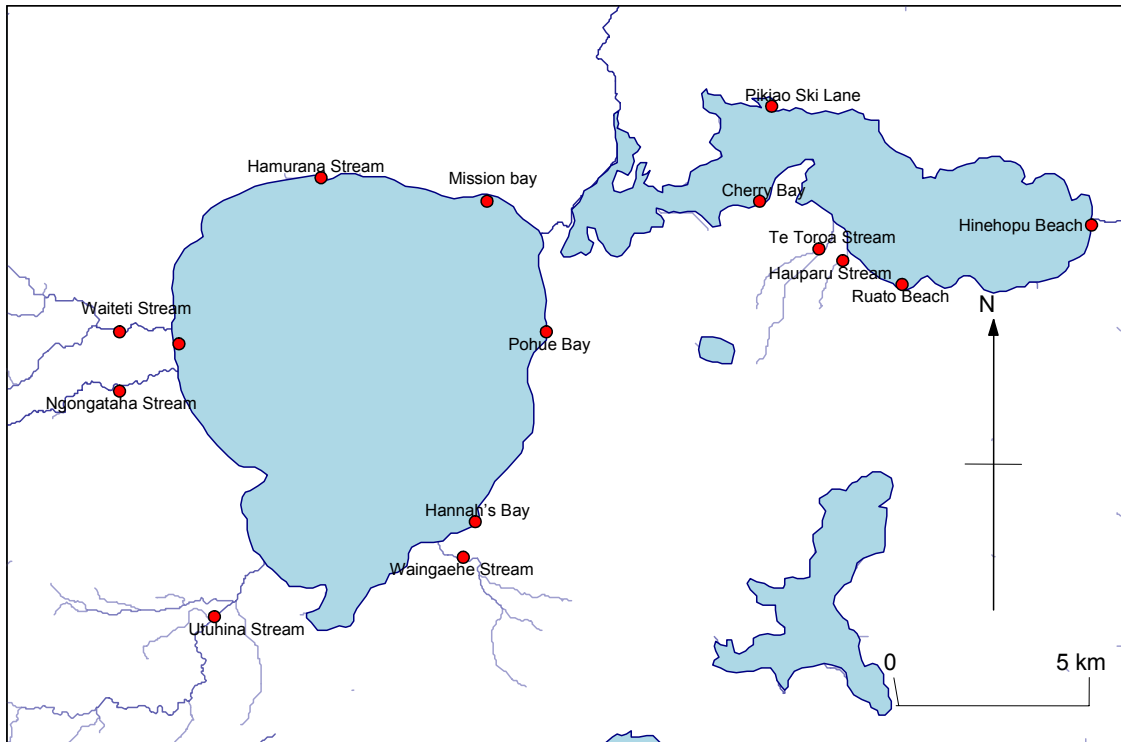


Figure 1: Location of sample sites taken from tributary streams and beach locations around lakes Rotorua and Rotoiti.

Otolith Preparation

The paired saggital otoliths were extracted from the heads of the sample fish. The saggital otoliths are the largest of three pairs of otoliths that are the most commonly used otoliths in experimental work. Juvenile rainbow trout (*Oncorhynchus mykiss*) and lake forms of common smelt (*Retropinna retropinna*) are relatively small fish (generally less than 100 mm fork length). We used the “open the hatch” method described in Secor et al (1991), to remove the otoliths. This method is typically used for fish with small otoliths. This involves sectioning along the midline of the dorsal surface down through the skull (from the mouth to the back of the head), then separating the left and right cranial cavities and removing brain tissue to expose brain cavity and inner ear where the otoliths are positioned. The brain was then removed using forceps, exposing the two cranial cavities

that contain the saggital otoliths. The otoliths were then removed with forceps and stored in 1.5 ml Eppendorf tubes until mounting.

Organic tissue or material remaining on the otoliths was removed prior to mounting by rinsing the otoliths with Milli-Q deionised water. The otoliths were then mounted in the centre of a microscope slide 50 by 25 mm, cut to length specifically for the laser chamber. Later, we used Ward's petrographic slides (46 by 27 by 1.2 mm). The otoliths were mounted in thermoplastic cement (Crystal Bond) with the sulcus surface facing up, transversely along the microscope slide. Mounting in Crystal Bond allowed the otolith to be repositioned or turned, over allowing a double grind on both the upper and lower otolith surface.

The otolith nucleus was exposed by hand grinding the sulcus surface of the otolith using 1200, 2000 and 4000 grain wetted sand paper. Otoliths were periodically checked using both compound and dissecting microscopes. After the nucleus was exposed, the mounted otoliths were again rinsed with Milli-Q deionised water and wiped with lens tissue prior to laser ablation. To minimise any contamination risk, disposable rubber gloves were worn during the otolith mounting procedure.

Laser Ablation

Analysis of the otoliths was carried out using a Perkin Elmer Elan SCIEX DRC II inductively coupled plasma mass spectrometer with a New Wave Research Nd:YAG 213-nm wave length laser. Each mounted, prepared otolith was placed into the laser chamber and the laser was focussed optically onto the otolith surface. The software in the laser ablation system allows different laser settings and ablation patterns for analysis. When analysing otoliths, we maintained uniform laser conditions to the best of our ability, as determined by laser power output on firing. We considered the power output to be optimum when we achieved approximately 0.20 mJ (about 30 J/cm²). In practice, the laser power varied from 0.18 to 0.27 mJ (26-32 J/cm²). The laser was fired for 4-6 consecutive samples to allow for warm-up and development of full power before beginning otolith analysis. Laser spot size was set at 30 µm, with a repetition rate of 20 Hz. Laser power was set at 100% output with a 5 s firing dwell time on each spot and a

10 s intersite pause between any successive spots, such as in line of spot analysis for smelt samples. The distance between spots varied between 75 – 150 μm , depending on the size of the otoliths, to give 4-7 spots per otolith. For juvenile trout, one 30 μm was sampled between the nucleus and the edge.

To determine background interferences caused by the argon carrier gas, we fired a 5 s spot at 0% laser power before and after the sample laser spot at full power. We then calculated a mean interference for ^{43}Ca and ^{66}Zn , which allowed us to remove background effects from counts.

Operating conditions for the ICP-MS were standardised after initial trials for dwell time per element, so that each sweep of the mass range took 1 s. This facilitated synchronisation of the laser firing time with elemental sweeps by the ICP-MS. To achieve a 1 s sweep, we used 100 ms dwell time for each of 10 masses. We set the number of repetitions for the ICP-MS (at 1 s per repetition) to equal the time indicated by the laser for the total pattern analysis plus 30 s to allow for the blank background spots.

We used one-way ANOVA to compare differences in mean isotopic ratios from the different juvenile trout samples from tributary streams then carried out post hoc analysis using Tukey's honest significant difference test for unequal sample size. Differences were considered significant at $p < 0.05$. To investigate the smelt otoliths from beach samples at each location, we compared the line of spots for ^{137}Ba and ^{88}Sr normalised by dividing the counts by ^{43}Ca adjusted for the mean background count.

Results

As part of the prefeasibility study, concentrations of Ca, Sr, Mg, Ba, and Mn were measured in the different spawning tributaries around lakes Rotorua and Rotoiti by R. J. Hill Laboratories, Hamilton.. The concentrations from water samples are shown in Table 1.

Table 1: Elemental concentrations in water from the different sample sites around lakes Rotoiti and Rotorua determined from one sample per site. See Figure 1 for sample site locations.

Site	Concentration of dissolved element (g m ⁻³)				
	Ca	Sr	Mg	Ba	Mn
Lake Rotoiti					
Hinehopu beach	3.490	0.022	1.90	0.018	<0.0005
Ruato beach	3.600	0.022	1.96	0.019	<0.0005
Te Toroa Stream	5.430	0.029	2.87	0.028	0.199
Hauparu Stream	5.450	0.019	1.73	0.007	0.001
Mean	4.493	0.023	2.115	0.018	0.050
Lake Rotorua					
Ngongotaha beach	4.500	0.028	1.51	0.032	0.034
Mission Bay beach	4.000	0.024	1.73	0.022	0.014
Hannah's Bay beach	4.390	0.027	1.92	0.026	0.081
Pohue Bay beach	7.210	0.037	2.12	0.028	0.042
Ngongotaha Stream	2.190	0.018	1.10	0.031	0.007
Utuhina Stream	2.890	0.019	1.24	0.029	0.025
Hamurana Stream	2.800	0.018	1.83	0.014	<0.0005
Waiteti Stream	3.270	0.024	1.61	0.043	0.017
Mean	3.906	0.024	1.63	0.028	0.028
ANOVA p-value by lake	0.521	0.710	0.078	0.078	0.544

After some investigation to gain familiarity with our new LA-ICP-MS, the suite of isotopes was reduced from the initial sampling strategy to 22 elements. The reduced isotope suite was again reduced to 10 elements, not all of which provided useful information. Finally, an altered suite of 10 isotopes was chosen and used for analysis (Table 2). To reduce variability from laser operation, we used standardised laser conditions (Table 3).

Table 2: Suite of elements and their isotopes used for otolith analysis by LA-ICP-MS. Interference refers to counts at the atomic mass unit of each element when the laser was inactive, i.e., the influence of the argon carrier gas.

Element	Isotope	Interference	Natural abundance of each isotope(%)
Boron	¹¹ B	No	80.1
Magnesium	²⁴ Mg	No	78.99
Calcium	⁴³ Ca	Yes	0.135
Nickel	⁶⁰ Ni	Yes	26.223
Copper	⁶⁵ Cu	Yes	30.83
Zinc	⁶⁶ Zn	Yes	27.9
Rubidium	⁸⁵ Rb	Yes	72.165
Strontium	⁸⁸ Sr	No	82.58
Barium	¹³⁷ Ba	No	11.23
Lanthanum	¹³⁹ La	No	99.9098

Table 3: Standard laser firing conditions using a New Wave Research UP-213 laser system and ICP-MS dwell time for each atomic mass unit. .AMU = atomic mass unit.

Laser Parameter	Setting	Sample Type
Laser power	100% of laser output	Smelt and trout
Laser dwell time per spot	5 s	Smelt and trout
Intersite pause	10 s	Smelt line of spot analysis
Laser spot diameter	30 µm	Smelt and trout
ICP dwell time per AMU	100 ms	Smelt and trout

Differences between the streams

We compared peak counts per second (cps) of elements from each laser spot to derive isotope ratios. Ratios are presented as dimensionless units for each isotope, standardised to counts of ⁴³Ca as a consequence of the lack of matrix matched standards (Morales-Nin et al, 2005).

Individual otoliths were analysed using single spot analysis on the otolith surface. Juvenile rainbow trout are assumed to have occupied the same habitat through their entire life to capture date. The data from each otolith was then accumulated and the mean values have been calculated and are presented in Table 4.

Table 4: Mean isotopic ratios from juvenile rainbow trout collected from different spawning tributaries around lakes Rotorua and Rotoiti. *P* values are significant at $\alpha < 0.05$ and are bold and italicised.

Stream	Number of samples	$^{11}\text{B}/^{43}\text{Ca}$	$^{24}\text{Mg}/^{43}\text{Ca}$	$^{66}\text{Zn}/^{43}\text{Ca}$	$^{88}\text{Sr}/^{43}\text{Ca}$	$^{137}\text{Ba}/^{43}\text{Ca}$
Lake Rotorua						
Ngongataha Hatchery	8	0.00068	0.0328	0.0290	3.2406	0.0191
Ngongataha Stream	10	0.00032	0.0599	0.0339	3.0635	0.0664
Waiteti Stream	5	0.00070	0.0746	0.0373	3.2200	0.0557
Waiteti Stream repeat	10	0.00021	0.0543	0.0184	2.7464	0.0656
Utuhina Stream	5	0.00059	0.0744	0.0555	3.1987	0.0539
Waingahe Stream	7	0.00038	0.0325	0.0237	1.9362	0.0754
Mean		0.00048	0.05476	0.03297	2.90091	0.05602
Lake Rotoiti						
TeToroa Stream	5	0.00117	0.3812	0.0219	2.0232	0.0126
Hauparu Stream	5	0.00129	0.2214	0.0271	1.5012	0.0125
Mean		0.00123	0.30128	0.02452	1.76219	0.01256
ANOVA <i>P</i> value		<i>0.044</i>	<i>0.036</i>	0.180	<i><0.001</i>	<i><0.001</i>

We used one-way ANOVA to investigate the difference in the isotopic ratios between the tributary streams. There are four isotopic ratios that differ significantly between streams. These are $^{88}\text{Sr}:^{43}\text{Ca}$ ratio $P < 0.001$, $^{137}\text{Ba}:^{43}\text{Ca}$ ratio $P < 0.001$, $^{11}\text{B}:^{43}\text{Ca}$ ratio with $P = 0.044$ and $^{24}\text{Mg}:^{43}\text{Ca}$ ratio with $P = 0.036$.

To determine the significant differences between group means we used the Tukey's unequal *N* honest significant difference test. The $^{88}\text{Sr}:^{43}\text{Ca}$ ratios (Table 5) and $^{137}\text{Ba}:^{43}\text{Ca}$ ratios (Table 6) differed significantly between a range of sample locations but the $^{11}\text{B}:^{43}\text{Ca}$ and $^{24}\text{Mg}:^{43}\text{Ca}$ ratios did not.

Table 5: Tukey’s HSD unequal N post hoc test on the mean juvenile rainbow trout $^{88}\text{Sr}:$ ^{43}Ca ratio obtained from laser ablation analysis. P values are significant at $\alpha < 0.05$ and are bold and italicised.

	Hatchery	Ngongataha	Waingaehe	Waiteti	Waiteti Repeat	Utuhina	Hauparu	Te Toroa
Hatchery		0.995	<0.001	1.000	0.448	1.000	<0.001	0.005
Ngongataha	0.995		0.002	1.000	0.811	1.000	<0.001	0.025
Waingaehe	<0.001	0.002		0.002	0.050	0.003	0.834	1.000
Waiteti	1.000	1.000	0.002		0.766	1.000	<0.001	0.006
Waiteti Repeat	0.448	0.811	0.050	0.766		0.805	0.004	0.268
Utuhina	1.000	1.000	0.003	1.000	0.805		<0.001	0.007
Hauparu	<0.001	<0.001	0.834	<0.001	0.004	<0.001		0.669
Te Toroa	0.005	0.025	1.000	0.006	0.268	0.007	0.669	

Table 6: Tukey’s HSD unequal N post hoc test on the mean juvenile rainbow trout $^{137}\text{Ba}:$ ^{43}Ca ratio obtained from laser ablation analysis. P values are significant at $\alpha < 0.05$ and are bold and italicised.

	Hatchery	Ngongataha	Waingaehe	Waiteti	Waiteti Repeat	Utuhina	Hauparu	Te Toroa
Hatchery		0.016	0.005	0.368	0.019	0.433	1.000	1.000
Ngongataha	0.016		0.998	0.998	1.000	0.995	0.041	0.042
Waingaehe	0.005	0.998		0.931	0.997	0.894	0.009	0.010
Waiteti	0.368	0.998	0.931		0.999	1.000	0.181	0.182
Waiteti Repeat	0.019	1.000	0.997	0.999		0.996	0.047	0.047
Utuhina	0.433	0.995	0.894	1.000	0.996		0.224	0.226
Hauparu	1.000	0.041	0.009	0.181	0.047	0.224		1.000
Te Toroa	1.000	0.042	0.010	0.182	0.047	0.226	1.000	

We used the one-way ANOVA data to demonstrate the mean isotopic ratio of $^{88}\text{Sr}:$ ^{43}Ca (Figure 2) and $^{137}\text{Ba}:$ ^{43}Ca (Figure 3). The $^{88}\text{Sr}:$ ^{43}Ca ratio shows reasonable differentiation between the two lakes as Te Toroa and Hauparu streams are the two tributary streams from Lake Rotoiti although the mean isotopic ratio from Waingaehe stream tributary on Rotorua is similar to the Rotoiti tributaries. These results suggest that we may be able to differentiate between tributaries within each lake. The $^{137}\text{Ba}:$ ^{43}Ca ratio differentiates between the two lakes. Lake Rotorua tributaries have high $^{137}\text{Ba}:$ ^{43}Ca ratios relative to the mean isotopic ratios obtained from the Lake Rotoiti tributaries (Hauparu and Te Toroa).

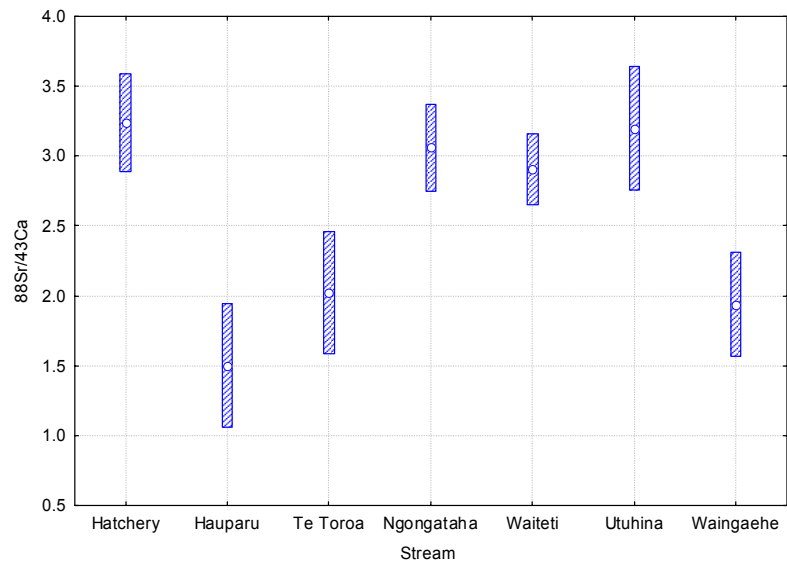


Figure 2: $^{88}\text{Sr}:$ ^{43}Ca isotopic ratios from tributary streams around lakes Rotorua and Rotoiti. Vertical bars denote 95% confidence intervals.

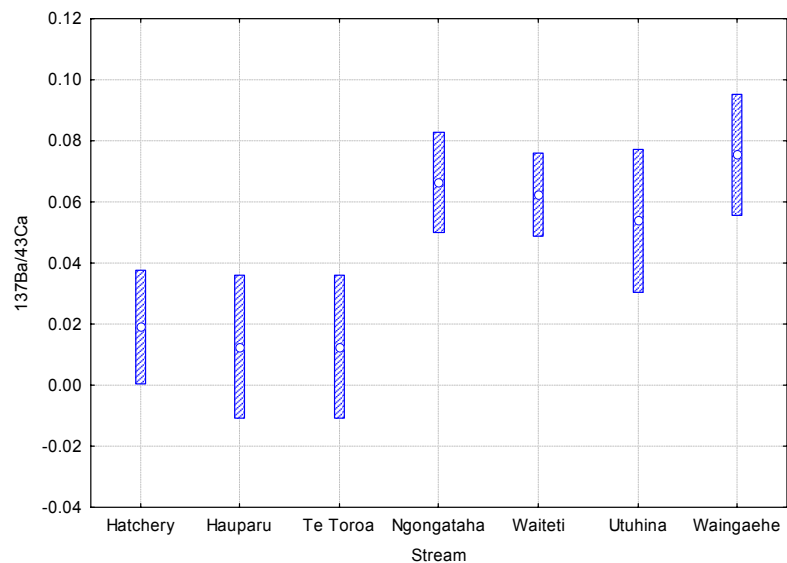


Figure 3: $^{137}\text{Ba}:$ ^{43}Ca isotopic ratios from tributary streams around lakes Rotorua and Rotoiti. Vertical bars denote 95% confidence intervals.

We then graphed the ratios of $^{88}\text{Sr}:$ ^{43}Ca and $^{137}\text{Ba}:$ ^{43}Ca obtained for each juvenile rainbow trout analysed to investigate any relationships occurring in the otoliths.

Figure ? shows the relationships. Several of the tributary streams appear to group well, particularly the Rotoiti tributaries, Hauparu Stream and Te Toroa Stream.

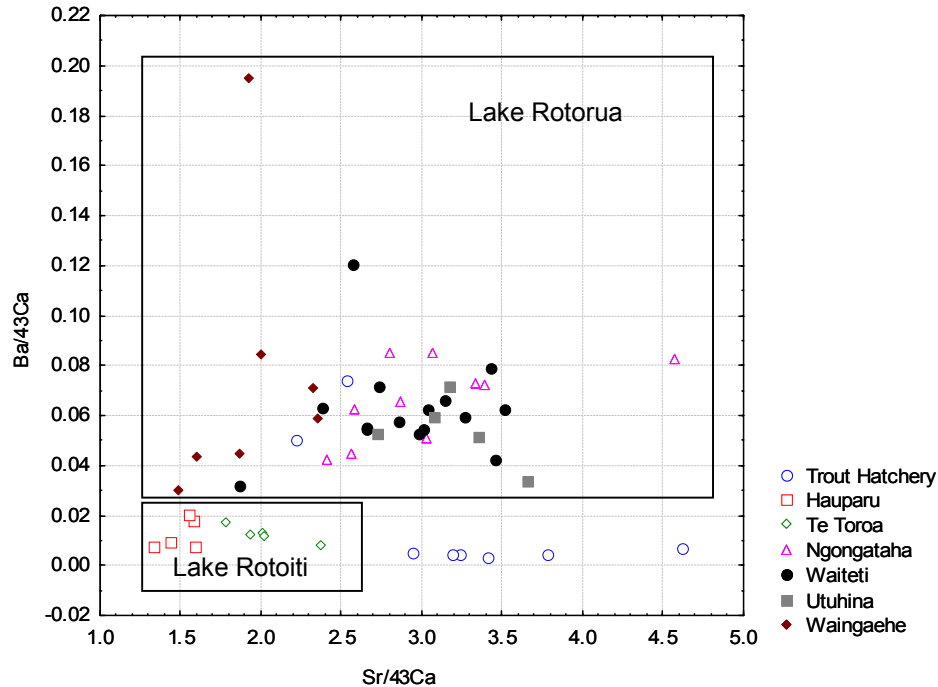


Figure 4: Individual juvenile rainbow trout $^{88}\text{Sr}:\text{}^{43}\text{Ca}$ and $^{137}\text{Ba}:\text{}^{43}\text{Ca}$ isotopic ratios obtained from the different tributary stream sample locations.

Smelt otoliths were analysed from seven different sample locations around lakes Rotorua and Rotoiti. The laser was set to scan a line of spots running from the nucleus of the otolith to a spot on the edge of the otolith. The isotopic ratios were calculated at each of the spots to give an indication of the patterns of mean isotopic variation across the otolith. The ratios of $^{88}\text{Sr}:\text{}^{43}\text{Ca}$ and $^{137}\text{Ba}:\text{}^{43}\text{Ca}$ were graphed and there are a few different characteristic patterns are visible. Figures 5 and 6 each represent one smelt sample from the Hot Pools and Pukia Ski Lane sample locations respectively. The mean isotopic ratios for $^{88}\text{Sr}:\text{}^{43}\text{Ca}$ and $^{137}\text{Ba}:\text{}^{43}\text{Ca}$ appear to be reasonably uniform across the otolith surface from nucleus to edge, indicating a resident life history pattern.

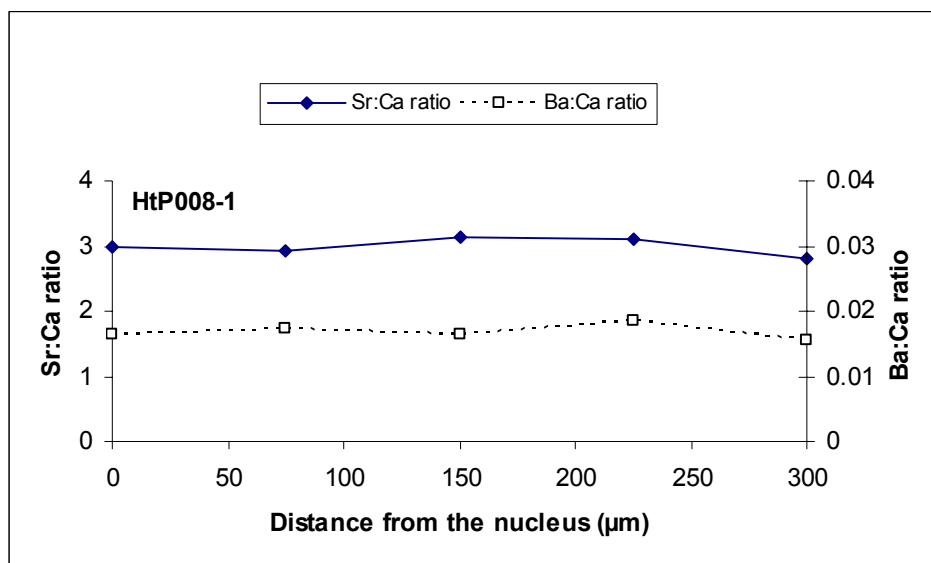


Figure 5: $^{88}\text{Sr}:$ ^{43}Ca and $^{137}\text{Ba}:$ ^{43}Ca peak ratios from line of spot analysis of smelt otolith HtP008-1, from the Hot Pools site, Lake Rotoiti.

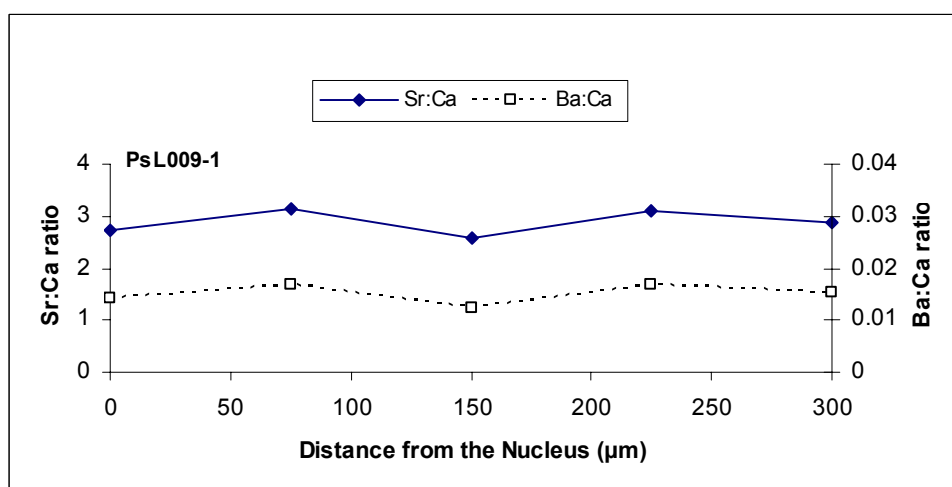


Figure 6: $^{88}\text{Sr}:$ ^{43}Ca and $^{137}\text{Ba}:$ ^{43}Ca peak ratios from line of spot analysis of smelt otolith PsL009-1, from the Pikiako Ski Lane site, Lake Rotoiti.

Smelt samples from different locations show patterns that differ from the apparent resident smelt demonstrated above. Changes in the peak mean isotopic ratios across otoliths indicate migration between different chemical habitats through the life of this fish. There are obvious changes across the smelt otoliths demonstrated in Figures 7 and

8, where the $^{88}\text{Sr}:^{43}\text{Ca}$ and $^{137}\text{Ba}:^{43}\text{Ca}$ ratios have changed across the otolith from the initial rearing environment represented by the isotopic ratios obtained from the otolith nucleus.

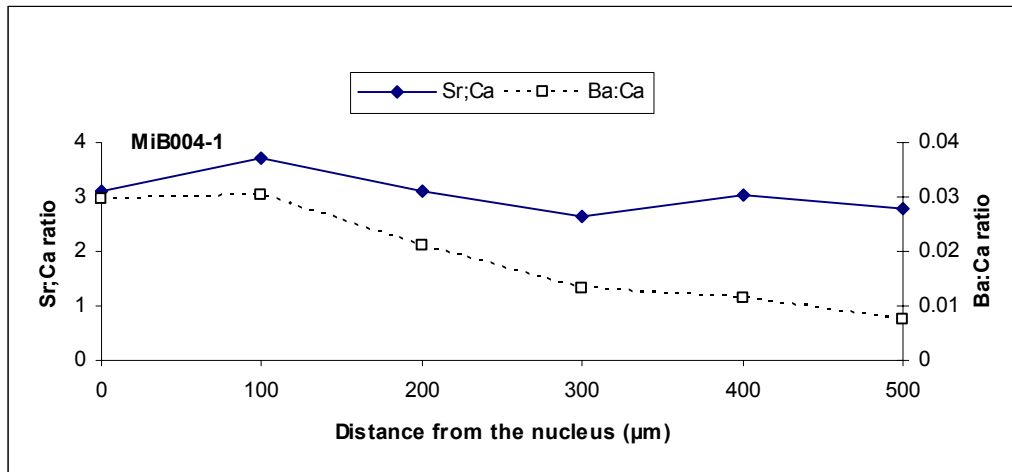


Figure 7: $^{88}\text{Sr}:^{43}\text{Ca}$ and $^{137}\text{Ba}:^{43}\text{Ca}$ peak ratios from line of spot analysis of smelt otolith MiB004-1 from Mission Bay, Lake Rotorua.

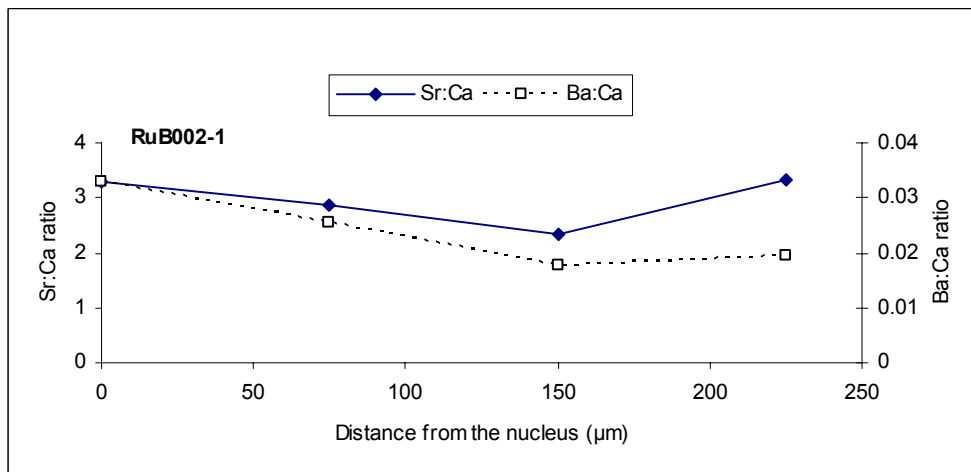


Figure 8: $^{88}\text{Sr}:^{43}\text{Ca}$ and $^{137}\text{Ba}:^{43}\text{Ca}$ peak ratios from line of spot analysis of smelt otolith RuB002-1 from Ruato Beach, Lake Rotoiti.

We also observed patterns indicating migration between different habitats where lines representing the $^{88}\text{Sr}:$ ^{43}Ca and $^{137}\text{Ba}:$ ^{43}Ca isotopic ratios have crossed over. Examples of these graphs are demonstrated in Figures 9 and 10.

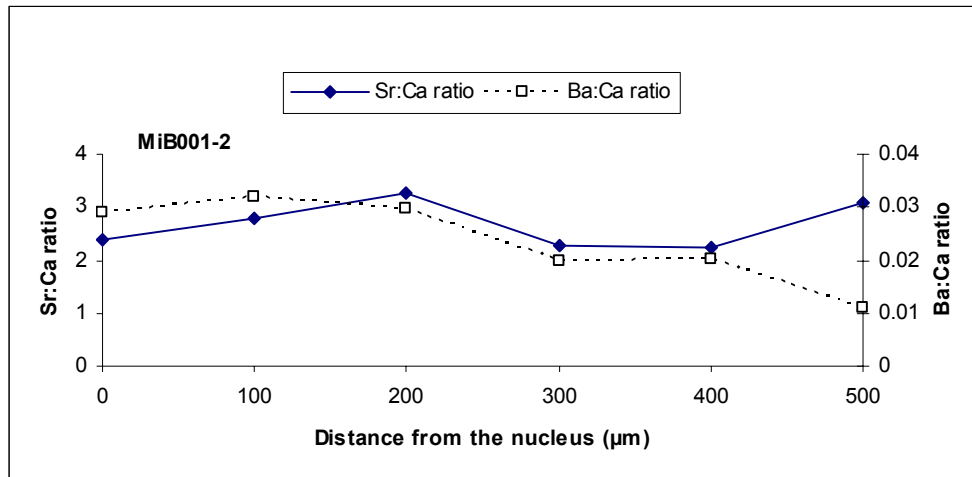


Figure 9: $^{88}\text{Sr}:$ ^{43}Ca and $^{137}\text{Ba}:$ ^{43}Ca peak ratios from line of spot analysis of smelt otolith MiB001-2 from Mission Bay, Lake Rotorua.

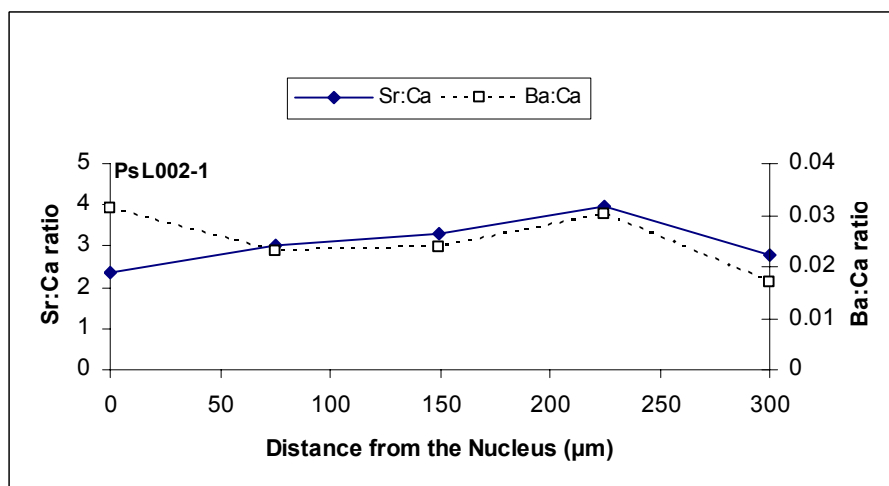


Figure 10: $^{88}\text{Sr}:$ ^{43}Ca and $^{137}\text{Ba}:$ ^{43}Ca peak ratios from line of spot analysis of smelt otolith PsL002-1 from Pikiāo Ski Lane, Lake Rotoiti

Discussion

Three of the 10 elements used for the otolith analysis (^{11}B , ^{24}Mg , ^{88}Sr , ^{137}Ba) performed well once normalised to ^{43}Ca counts. Five elements (B, Mg, Sr, Ba, La) had no background interference from the argon carrier gas, facilitating analysis and interpretation. Isotopes with background interferences from the argon carrier gas can still be used provided that the signal-to-noise ratio is sufficient. ^{43}Ca had the lowest interference of any isotope of Ca, and although ^{43}Ca had a significant interference, counts were 10,000 to 30,000 cps compared to the background of 400 to 600 cps, so this isotope could be used to normalise counts of other elements.

We used peak counts of the isotopes to determine the isotopic ratios. Peak counts were defined as the highest count of the isotope obtained from each spot laser firing in the ICP-MS output. The counts of each element were normalised by dividing to the ^{43}Ca peak from each laser fire, as we have at present no matrix matched standards. The ratios were standardised to the ^{43}Ca isotope as this is also representative of the otolith matrix. Otoliths are primarily composed of calcium (about 40%), oxygen and carbon, comprising a calcium carbonate matrix that trace elements are also incorporated into (Campana, 1999).

We identified significant differences in mean isotopic ratios in otoliths of juvenile rainbow trout from different tributary streams. This supports the evidence we obtained in the prefeasibility study showing different ambient water concentrations in the different tributary streams and beach sample sites. The ambient water concentrations have previously been shown to have the greatest influence on trace metal concentrations in the otolith, particularly in the case of Strontium and Barium (Bath et al., 2000). The most encouraging result is the fact that the $^{137}\text{Ba}:^{43}\text{Ca}$ ratios effectively allowed us to differentiate between lakes Rotorua and Rotoiti. The post hoc analysis using Tukey's HSD of unequal N test has shown that these results also differ significantly between tributary streams. Lake Rotorua tributaries have a significantly higher $^{137}\text{Ba}:^{43}\text{Ca}$ ratio than the tributaries around Lake Rotoiti. These ratio differences form the basis of this

work and it is apparent there are different ratios of elements being obtained from the otoliths of these juvenile rainbow trout, assumed to be resident of their respective tributary stream of capture.

Common smelt samples from seven different beach locations were also been analysed using a line of spot across the otolith surface, working from the nucleus of the otolith to the edge of the otolith. Comparing the isotopic ratios from the core nucleus region, representative of the initial life stages of the fish, with the edge region of the otolith, obtained a range of different patterns across the otoliths of the sample smelt. Different isotopic ratios across the smelt otolith suggests that movement of some fish occurs, although we still need to explore this further.

Conclusion

Otoliths have the potential to reflect the life history of the fish with respect to the chemical habitats that it has occupied. Through analysis of juvenile rainbow trout otolith isotopic composition we can currently differentiate which of the two lakes in the study the trout has come from using the $^{137}\text{Ba} : ^{43}\text{Ca}$ isotopic ratio from the otolith. The $^{88}\text{Sr} : ^{43}\text{Ca}$ appears to provide potential for differentiating the some tributary streams within lakes, while other isotopic ratios will prove to be useful in multivariate data analysis.

An initial look at some of the smelt otoliths has shown encouraging results. Three major patterns of migratory or non migratory behaviour have emerged although this analysis is preliminary. Different isotopic concentrations have been identified across the otoliths of some samples indicative of movement between different ambient chemical environments.

Where to from here

We aim to assign adult trout to their spawning stream using multivariate data analysis, particularly discriminant function analysis. The challenge now is to work at assembling

the isotopic ratios that best distinguish the tributary streams and beach locations so we can then analyse the unknown adult trout and ultimately establish the natal tributary stream of based on the isotopic composition of the otolith.

There are two smelt samples that need to be obtained through seine netting. Lake Rotoiti will be represented by two spawning streams. We have electro-fished two other tributaries on Lake Rotoiti, Waiiti Stream and Tawhakarere Stream, but we were unable to capture any more juvenile rainbow trout.

Water samples will also be collected bi-monthly from the different sample locations to assess the variation in the water chemistry throughout the year.

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