Microchemistry of scales and otoliths from Mohaka River brown and rainbow trout

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Executive Summary

The microchemistry of bony parts of fish is often used to reconstruct their previous life history, especially to differentiate between periods of residence in sea- and fresh water. The present study investigated the use of scales and otoliths of both brown and rainbow trout, to see whether either could be used to reconstruct the life history of trout in the Mohaka River, Hawkes Bay. The river is currently being investigated as a potential hydro site, and an issue of concern is whether the highly regarded headwater trout fishery is dependent upon post- spawning migration of trout to the lower river and estuarine reaches, and their subsequent return upstream, or possibly recruitment of fish from downstream spawning. Scales from Chinook salmon (which spend a significant proportion of their life in the sea) were used as a reference point, and had higher strontium contents than did scales from trout resident in fresh water. However, the technique did not give consistent results and was abandoned in favour of otolith microchemistry. From a survey of 12 otoliths (seven rainbow trout, 5 brown trout), there was no evidence from strontium:calcium ratios that any of the trout had spent time in a marine environment. Processing of additional otoliths is suggested to provide a more robust result.

1. Introduction

The need to determine seasonal movements of migratory fish is a common problem in fisheries biology. Different species of fish move between salt and freshwater (diadromous movements, a very common feature of many of New Zealand native species, McDowall 1988, 1990), while in-river migrations also occur, usually for spawning, but also for feeding. Spawning migrations are common with introduced salmonids – for instance, both brown and rainbow trout often migrate extensively within freshwaters to reach smaller tributary streams where they spawn (McDowall 1984).

Traditionally, such movements are inferred from recaptures of tagged fish (Jellyman and Graynoth 1994), although the use of radio and acoustic tags allows tracking of individual fish. Such tracking has been carried out in studies of New Zealand freshwater eels (Jellyman et al. 1996; Jellyman and Sykes 2003; Watene et al. 2003), but also with Chinook salmon (Glova et al. 1986) and trout (Boubée and Wilson 1996).

Otoliths and other bony parts have long been used to determine the age of fish. Although there is an extensive body of literature on using scales to age trout, it has been found that during spawning there is often erosion of calcium from scale margins (associated with mobilisation of calcium to the sex products), meaning that the outer margin of scales is often resorbed and hence age estimates are invariably under-estimates (Jellyman and Graynoth 1994). Fortunately, otoliths, being part of the fishes balancing system, are not subject to calcium resorption and hence provide a full record of past physicochemical events during the fishes lifetime (Radtke 1989).

The chemical composition of otoliths, scales and bony parts of fish partly reflect the chemical composition of the water in which the fish live and grow. Because salt and fresh water have distinctly different chemical properties, the chemical signatures from these waters are correspondingly different in parts of the otoliths, scales and bony parts; thus the patterns of chemical composition in these parts can often be used to establish previous residence in salt and freshwater environments. Strontium is the most frequently used indicator element, as concentrations differ markedly between sea and fresh water i.e. the mean strontium concentration in the ocean is 7.9 $mg.l^{-1}$ compared with 0.07 $mg.l^{-1}$ in fresh water (Bowen 1979). The incorporation of strontium into otoliths is primary influenced by ambient concentrations of strontium (e.g. Bath et al. 2000), although other factors including species, age, temperature, food, and activity may also play a role (Kalish 1990; Closs et al. 2003). In practice, the strontium: calcium ratio is the variable most commonly used in fisheries literature to differentiate between residence in salt and fresh water, with a high ratio indicative of seawater residence, while a lower ratio indicative of freshwater residence. In New Zealand these ratios have been used to determine the diadromous life history of common bullies in southern South Island rivers (Closs et al. 2003), and eels in Lake Ellesmere (Arai et al. 2004).

Meridian Energy Limited is investigating the hydro potential of the Mohaka River. It is widely recognised that this river sustains a very important fishery for both brown and rainbow trout, mainly in the upper reaches (Teriney et al. 1982; Jellyman and Graynoth 1994). An important issue is whether this upstream fishery is sustained by recruitment from downstream, and hence whether the intervention of a hydro dam could significantly impact on trout stocks. Such recruitment could be of juvenile fish originating from downstream spawning, or of adult fish returning upstream after moving downstream after spawning to feed in the lower reaches of rivers where forage fish are more plentiful. Classically, a tagging study would be implemented to monitor trout movements within the catchment. However, if this used traditional streamer tags, then several hundred trout would need to be tagged and results could take several years to accumulate. Alternatively, a radio-tracking study of individual fish could be implemented, although this is very consumptive of time and resources, and these factors usually constrain the number of fish that can be tagged and monitored.

Because of such issues, NIWA proposed to investigate the potential of bony parts of trout to record chemical differences that would be indicative of residence in salt- or brackish- water areas. Despite there being little literature on the use of scales for these sorts of determinations (e.g. Koksvik and Steinnes 2005), we suggested that initially we should investigate scales as this is a non-destructive form of fish sampling (as opposed to collection of otoliths where it is necessary to kill the fish), and we had access to a considerable database of fish scales, including some from the Mohaka River. Should scales prove unsatisfactory, we arranged for Hawkes Bay Fish and Game Councils staff to collect otoliths from Mohaka River trout.

2. Materials and methods

A sample of trout and salmon scales were selected from NIWA’s scale museum. For this, we chose scales that had the full representation of annual rings, and thus excluded any replacement scales where history prior to replacement is not represented. Scales were chosen to represent possible life history patterns (Table 1), i.e. brown trout from the Waituna Lagoon in Southland had a high probability of being “sea-run” fish and hence of showing a marine component in their life history; rainbow trout from the Greenstone River in Otago could not have been to sea; Chinook salmon from the

Rakaia River mouth must have a sea component in their life history; three brown trout from the Mohaka River were also included, as it was possible that the females especially might show some marine or estuarine residence.

Table 1: Brown and rainbow trout scale samples selected for investigation of scale microchemistry. F = female, m = male, u = sex unknown

Code	Date	Species	Length (cm)	Weight (kg)	Sex	Location
WL1	21/11/1970	brown trout	57		f	Waituna Lagoon - netted
WL2	22/11/1970	brown trout	59	2.5	m	Waituna Lagoon - sea run angler caught
WL3	23/11/1970	brown trout	66.5	3.0	f	Waituna Lagoon - sea run angler caught
GS1	20/11/1991	rainbow trout	56		f	Upper Greenstone River
GS2	3/11/1991	rainbow trout	51		m	Upper Greenstone River
GS3	2/11/1991	rainbow trout	53		f	Upper Greenstone River
BT13	1/02/1992	brown trout	56		f	Mohaka
BT14	1/02/1992	brown trout	58		m	Mohaka
BT19	1/03/1992	brown trout	56		f	Mohaka
CS1	3/02/1996	chinook salmon	80	7.5	u	Rakaia mouth
CS2	6/02/1996	chinook salmon	90	9	m	Rakaia sea
CS3	6/02/1996	chinook salmon	68	4.75	f	Rakaia sea

As an initial trial, 3 scales from the collection below were used i.e, GS2, BT14, CS3. Whole scales were mounted on a suitable holder, and scanned.

Otoliths were collected from both brown and rainbow trout from a range of locations throughout the Mohaka River. The original proposal was to include a number of fish from the lower reach of the river, in anticipation that these would have a higher probability of showing some marine or estuarine component in their otoliths. In addition, trout from the upper reaches were also of importance as these sustain the important and upriver fisheries. If available, there was a preference for females rather than males, as work elsewhere has indicated that females undertake more extensive longitudinal migrations in rivers (Jellyman and Graynoth 1994; Fox et al. 2003), often moving to estuarine areas after spawning to feed and replenish their weight and energy lost during spawning.

Otoliths had to be sectioned first, so that the X-ray scan would be through the nucleus of the otolith (i.e. the otolith primordium which represents the earliest life of the fish). This required that otoliths were sawn transversely through the shortest axis; one half was then selected, mounted on a microscope slide using an inert adhesive (Quicktight Superglue Powergel), and the cut surface finely ground using 2000 grit carborundum paper. Periodic inspections were made to ensure that the grinding went through the otolith nucleus. Upon completion, otoliths were washed several times in distilled water, before packaging and dispatch to Geological and Nuclear Sciences Ltd, Lower Hutt.

Determination of strontium and calcium content was made using particle induced X-ray emission (PIXE). Details of the process are contained in literature such as Closs et al. 2003, but in brief a nuclear microprobe performs multiple scans of the scale (or otolith), and concentrations of strontium (Sr) and calcium (Ca) are recorded at fine intervals. These concentrations were then recorded in an Exel spreadsheet as concentrations of each element at distance from otolith (or scale) margins. Data were presented as Sr: Ca ratio graphs or concentration maps of each element.

3. Results

3.1. Scales

The scales proved difficult to scan. The X-ray beam caused curling of the scales, and it was likely that considerable time would be needed to develop a suitable technique for holding and scanning the scales. Preliminary results showed that Chinook salmon had substantially higher concentrations of Sr and Ca ratio than did either brown or rainbow, (Figs. 1 – 3), but there was considerable variation across scales, with no obvious similarity between the pattern on the right hand side and left hand side of the scales (i.e. as the transect started on one scale marginal, went through the scale origin, and across to the other margin, the patterns either side of the origin should have been mirror images). Other elements investigated (bromium, iron, zinc, and sodium), showed a similar lack of consistent patterns.

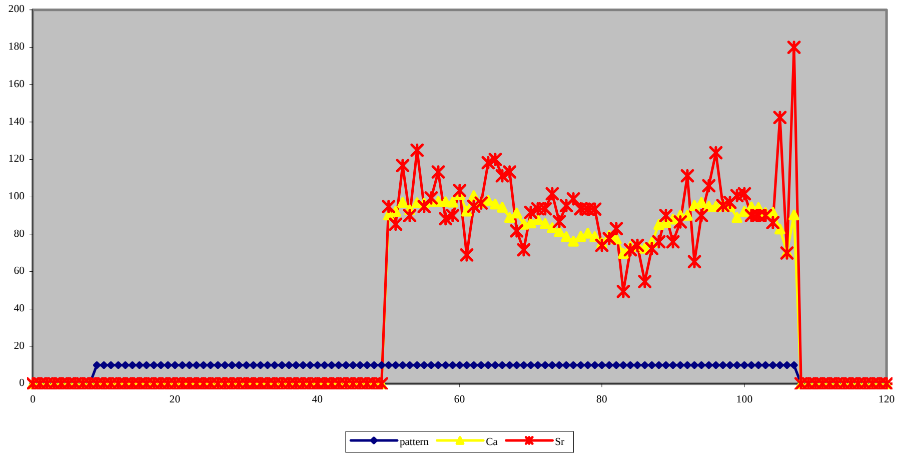


Figure 1: Strontium and calcium concentrations recorded across a scale from a rainbow trout from the upper Greenstone River, Otago

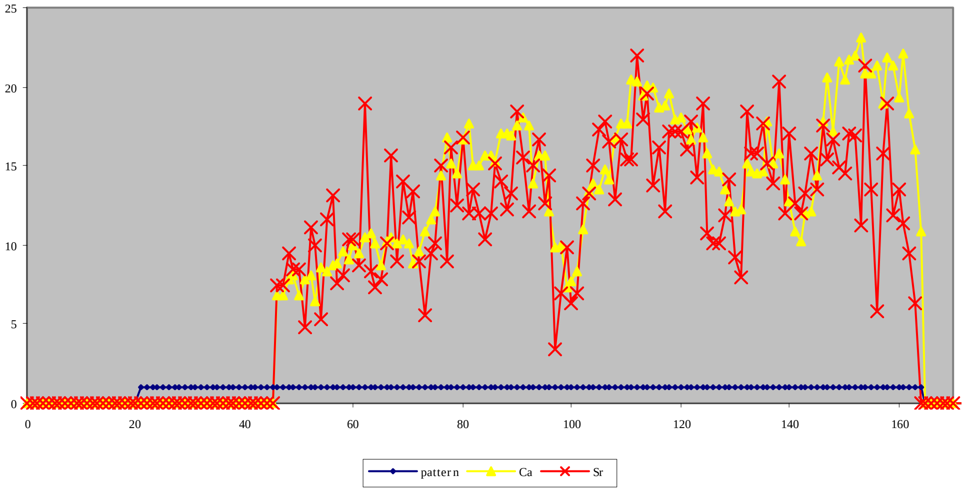


Figure 2: Strontium and calcium concentrations recorded across a scale from a brown trout from the Mohaka River.

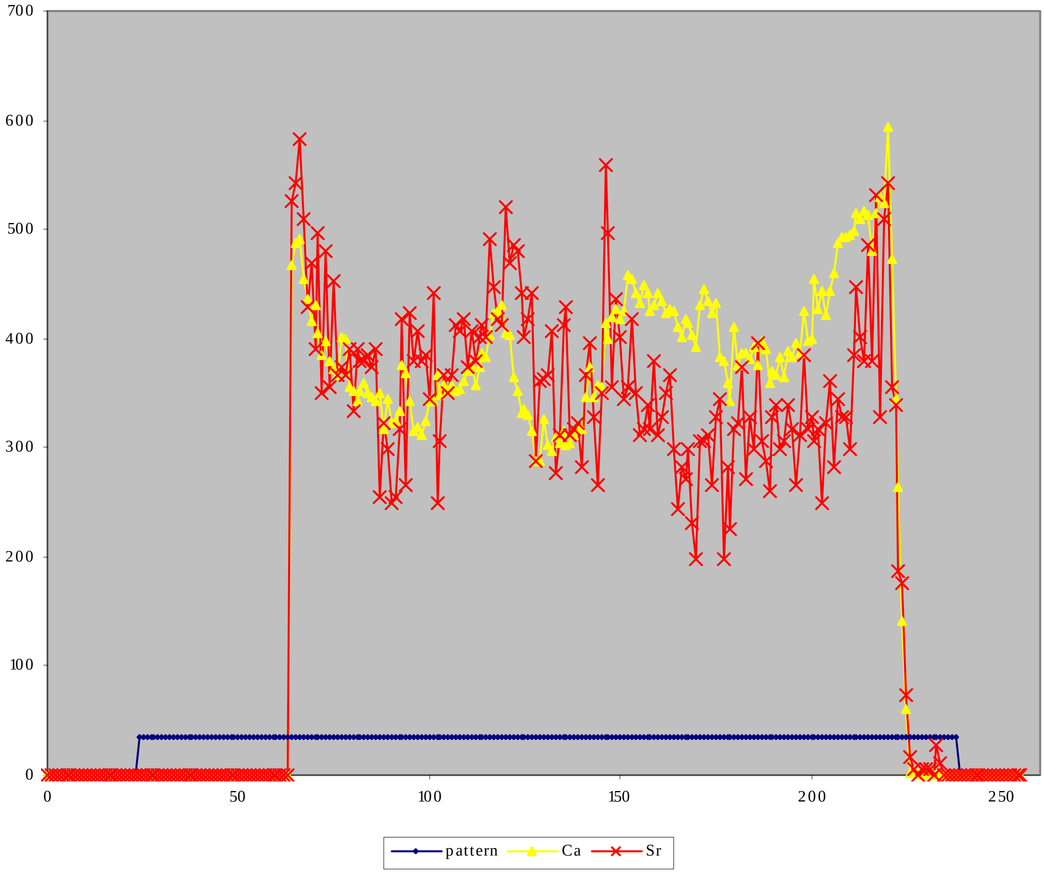


Figure 3: Strontium and calcium concentrations recorded across a scale from a Chinook salmon from the mouth of the Rakaia River

Because of the need to achieve results within a relatively tight time-frame, it was decided to not persist with scales, but to work with otoliths, as the technique for these was proven.

3.2. Otoliths

Although standard fly fishing techniques were successful in catching upstream trout, catching fish in the lower few kilometres of the river proved very difficult. This reach is turbid, and both fly and bait fishing were generally ineffective - interestingly, kahawai were caught by bait fishing, up to 0.5 km inland from the mouth, the first evidence that this species penetrates extensively up the estuary.

A total of 21 pairs of otoliths were collected, from 14 brown trout and 7 rainbow trout (Table 2). Fish came from below Willow Flat (# 1-3), opposite the Waipunga confluence (# 5-8), near the confluence of the Ripia River (#10-13), and near Mangatutu Hot Springs. A subsample of 12 fish was selected for processing, which included both species (7 rainbows, 5 browns) from all main collection locations.

Results are presented both qualitatively (descriptions of otolith maps) and quantitatively for the strontium:calcium ratios recorded from both longitudinal and cross-sectional transects of the otoliths (Table 2). Representative otoliths are shown below. RT 1 is a rainbow from the lower river; the maps of strontium and calcium show a unform distribution of both elements (Fig. 4), (the bright blue margin at the edge of the strontium map is caused by arsenic, and is an artefact of the preparation process). The Sr:Ca ratio graph (Fig. 5) is also uniform (again, except for some “edge” effects that are consequences of the process), and there are no high values that would be associated with any marine component. Not unexpectedly, the Ca values are also uniform, both in the longitudinal transect (Fig. 5) and the transverse one (Fig. 6).

Figures 7 and 8 are for an upper river brown tout. This fish was the only one that showed anything other than a completely uniform distribution of strontium. From the strontium map (Fig. 7) there was a suggestion that the area next to the sulcal groove of the otolith (the indentation at the centre left of the otolith) showed a higher strontium concentration. This showed as a slightly elevated Sr concentration (and a lowered Ca concentration: Fig. 8), but ratios were still below that indicative of saltwater residence.

Ignoring an apparent “spike” in the strontium: calcium ratio for fish No. 5, and a value of 0 for a portion of the otolith of fish # 13, the range in Sr:Ca ratios for the trout

Table 2: Mohaka trout otolith samples collected. Numbers 1-8, 10-12 were processed. Results are presented quantitatively as the dominant colour on the calcium and strontium maps, but also quantitatively as strontium:calcium ratios along the length of the otolith (longitudinal) and also across the otolith (cross-section)

Species	Sex	Fish #	Date	Length	Weight	Easting	Northing	NZMS coords	Ca map	Sr map	Sr:CA longitudinal	SR:Cas cross-section
RT		1	7/04/2006	380	780	2871880	6225368		all orange	all blue	0.9 - 1.8	1.3 - 1.6
RT	M	2	7/04/2006	430	980	2870076	6223968		all orange	all blue	0.9 - 1.8	1.2 - 1.8
BT		3	7/04/2006	340	560	2866384	6230374		all orange	all blue	1.5 - 2.0	1.5 - (2.1)
RT	F	4	6/04/2006	540	1620	2823620	6218116		all orange	all blue	0.8 - 1.8	0.2 - 1.9
RT	F	5		550	1120	2828788	6225804		all orange	all blue	1.5 - (3.5)	1.0 - 2.2
RT		6	6/04/2006	470	720	2828635	6226171		all orange	all blue	0.9 - 1.5	0.9 - 1.7
RT	F	7	6/04/2006	420	840	2828788	6225804		all orange	all blue	0.9 - 1.6	1.0 - 1.8
RT	M	8	6/04/2006	520	1700	2828635	6226171		all orange	all blue	0.9 - 2.0	1.1 - (1.6)
BT		9	5/04/2006	430	340	2809226	6210402					
BT	F	10	7/04/2006	500	1520			u19 133 167	all orange	all blue	1.1 - 2.0	1.2 - (2.4)
BT	M	11	7/04/2006	510	1520			u19 133 167	all orange	all blue	1.1 - 2.0	1.2 - 1.8
BT	M	12	7/04/2006	540	1080			u19 133 167	all orange	all blue	1.4 - 2.7	1.3 - 1.9
BT	F	13	7/04/2006	420	980			u19 133 167	all orange	blue but one portion a bit different	0 - 2.7	0.2 - 3.1
BT	M		7/04/2006	530	1860			u19 088 189				
BT	F		7/04/2006	410	960			u19 088 189				
BT	M		7/04/2006	500	1500			u19 088 189				
BT	F		7/04/2006	520	1540			u19 088 189				
BT	M		7/04/2006	550	1860			u19 088 189				
BT	M		7/04/2006	600	1380			u19 088 189				
BT	M		7/04/2006	530	1860			u19 088 189				
BT	F		7/04/2006	410	960			u19 088 189				

( ) = spike

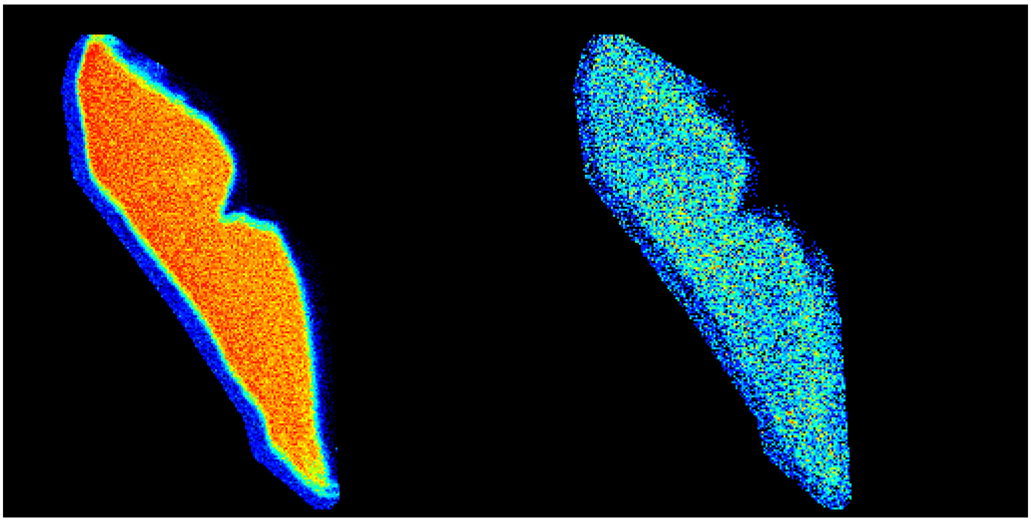


Figure 4: Strontium and calcium maps of an otolith from a rainbow trout from the lower Mohaka River (RT 1)

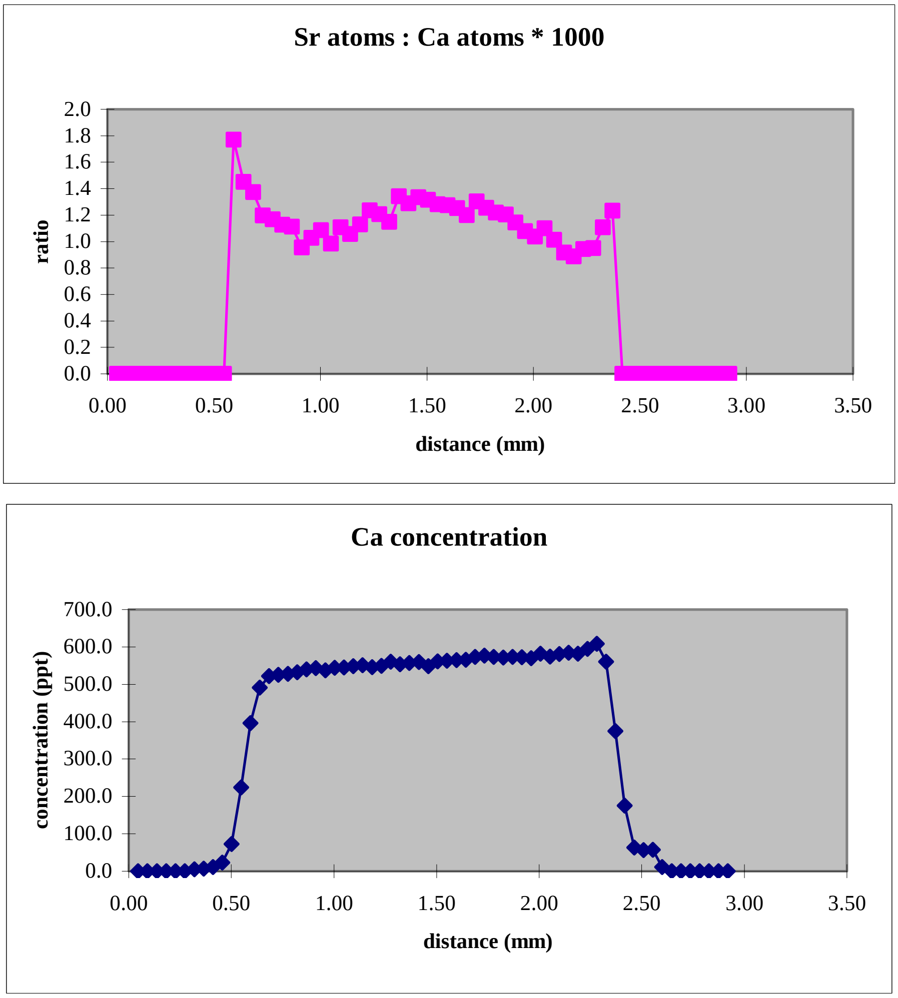


Figure 5: Plots of strontium: calcium concentrations (upper), and calcium concentrations (lower) along a longitudinal transect of an otolith from a rainbow trout from the lower Mohaka River (RT 1)

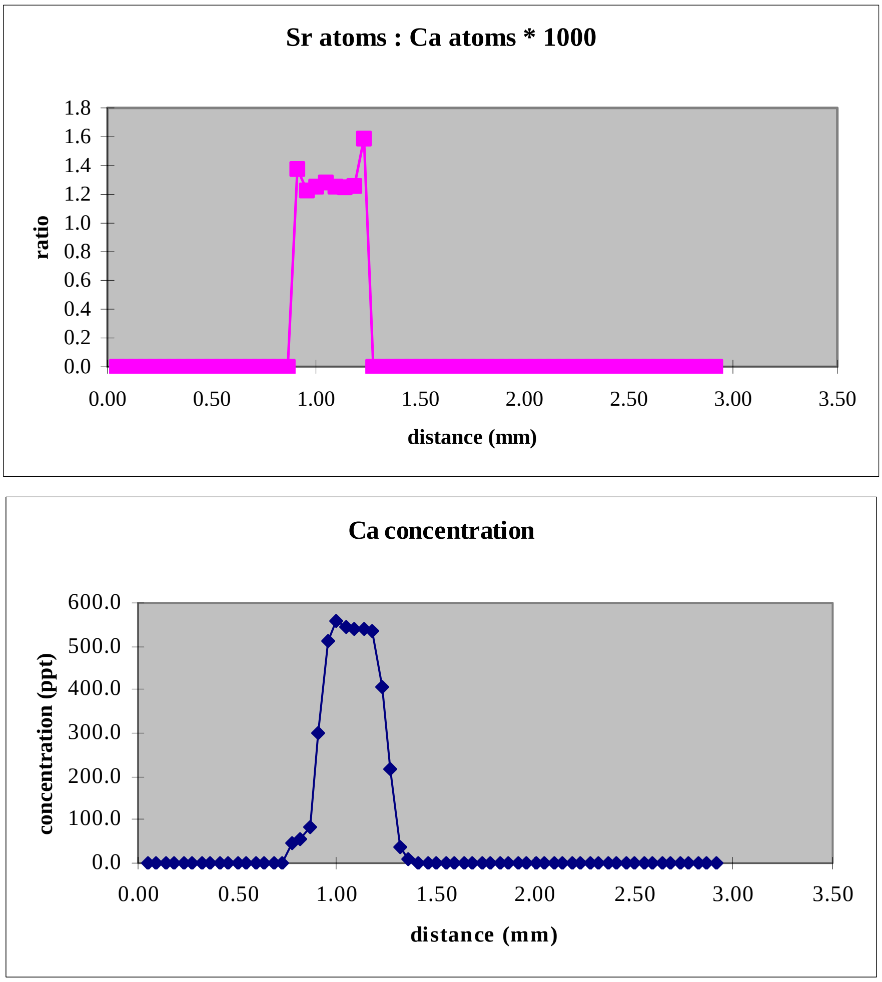


Figure 6: Plots of strontium: calcium concentrations (upper), and calcium concentrations (lower) along a transverse cross-section of an otolith from a rainbow trout from the lower Mohaka River (RT 1).

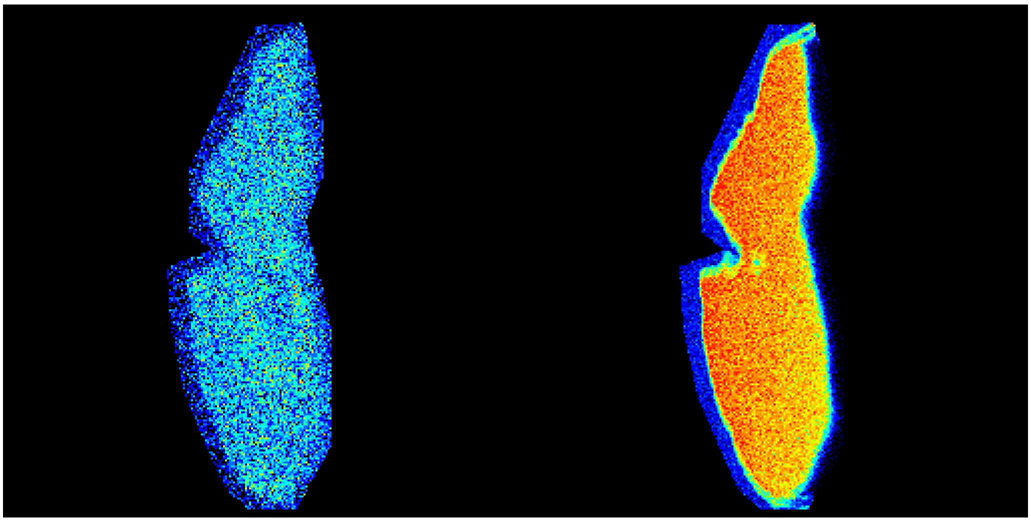
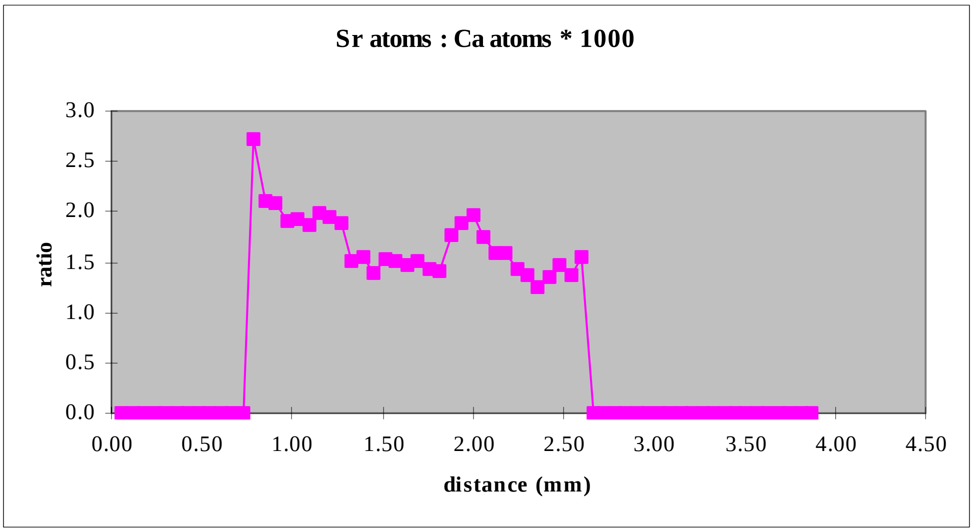


Figure 7: Strontium and calcium maps of an otolith from a brown trout from upper Mohaka River (BT 12)



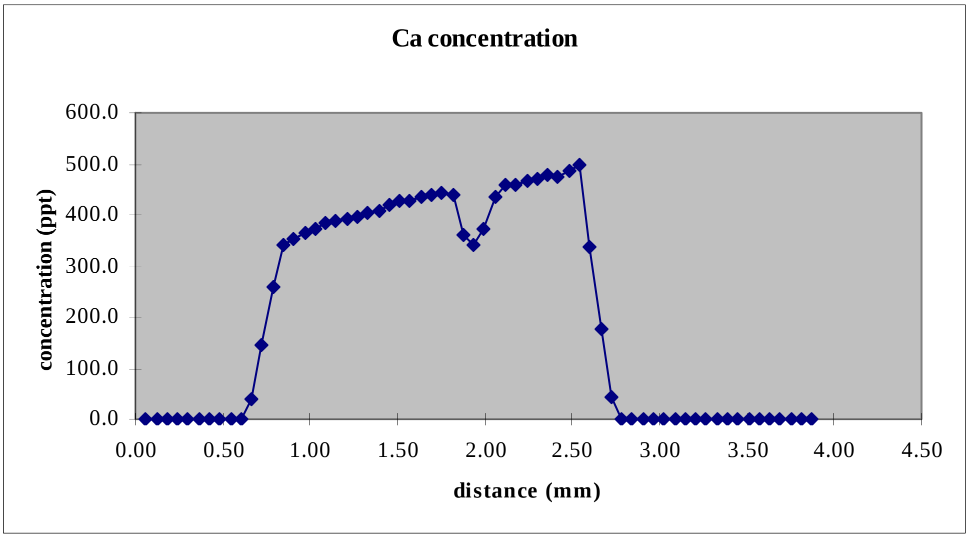


Figure 8: Plots of strontium: calcium concentrations (upper), and calcium concentrations (lower) along a longitudinal transect of the otolith of trout BT 12.

samples was 0.9 – 2.7 for the longitudinal transects and 0.2-2.4 for the transverse transects. There was no evidence from either the calcium or strontium maps, or the Sr:Ca ratios, that fish had resided in anything but a freshwater environment.

4. Discussion

While trout scales potentially offer a non-destructive sampling technique, the use of scales is in its infancy. Recent research investigating scales as a means of distinguishing between sea run and riverine brown trout in Norway (Koksvik and Steinnes 2005) concluded that strontium concentration alone was insufficient to distinguish between these two groups of fish, largely because there was low inter-group and high intra-group variability in some rivers examined. Likewise, scales of brook char (*Salvelinus fontinalis*) could be used to determine whether any seawater residence had taken place, but not to determine life-histories of individual fish (Courtemanche et al. 2006). Because of such uncertainty with both the technique and interpretation, and also because of the likelihood of missing significant life history events due to scale erosion, we did not persist with analysis of scales. The limited scale data processed did indicate that Chinook salmon scales showed higher levels of strontium and calcium than scales from river resident trout.

Otoliths are widely utilised in fisheries studies to establish previous life history events, including movements of salmonids between salt and fresh water (e.g. Arai et al. 2002; Volk et al. 2000). In the present study, the Sr:Ca ratio in the otoliths from the 10 trout studied showed no evidence of any marine or estuarine life history. The ratios for these fish ranged between 0.9 – 2.7 (longitudinal transects), and are considerably less than the range of 4 – 6 that Closs et al. (2003) assumed indicative of marine origin in bullies. Equivalent values for Lake Ellesmere eels were 1.8 – 2.4 for freshwater residence, while values of 3.0 – 7.4 were considered to indicate residence in high salinity areas of the lake (Arai et al. 2004).

There are no reference values of Sr:Ca ratios that unequivocally indicate whether prior residence was marine or freshwater, although it would generally be expected that freshwater residence would show as ratios of 1-2, with seawater residence shown by values of 8-10 (Bernard Barry GNS, pers. comm.). These ratios vary between species (Zimmerman 2005), meaning that validation of the technique for trout otoliths really requires examination of otoliths of sea-run trout, rather than using Chinook salmon otoliths as a surrogate.

It is also uncertain how long a fish would need to be resident in higher saline areas for this to show chemically. In experiments to validate the use of Sr:Ca ratios for various species of juvenile salmonid, Zimmermann (2005) retained fish for 84 days, and after this period, there were significant differences between fish reared in saline water and controls reared in fresh water. Differences indicative of freshwater and saltwater residence have been found in otoliths of inanga (*Galaxias maculatus*) that are thought to have spent limited time in freshwater, and it is considered very likely that differences would show in trout otoliths where fish had spent up to several months in areas of high or intermediate salinity (Bernard Barry, GNS, pers. comm.).

In the present study, none of the otoliths from the seven rainbow and five brown trout showed any indications of marine residence. Of course, failure to show such residence does not mean it did not occur, but if it did, the duration may have been too short to result in some distinctive chemical imprinting in the otolith. Alternatively, fish could have been resident in the lower river, but outside of the tidally affected reach where most of the potential prey species that would have strong marine signatures (inanga, smelt and mullet), are resident. With the limited sample size, the alternative hypothesis, that some otoliths might have shown marine signatures, could not be categorically disproved, and resolution of this issue to a reasonable level of certainty, would require additional otoliths to be processed. As a “control” for additional otoliths, it would be very desirable to include the otoliths from any sea-run brown trout, although these fish are difficult to obtain.

There is currently considerable interest in investigating the microchemistry of trout eggs as indicative of migratory life histories (Waite et al. 2004). Again the hypothesis is that the eggs of sea run females should have a higher strontium content than the eggs of river resident fish. Eggs have a number of advantages, not the least being that they can be sampled without sacrificing adult fish (by removing a few eggs from trout redds), and that the redds of many fish can be sampled in short periods of time if conditions allow. Although some egg collection has been investigated in the Mohaka River this past spawning season, this has presented considerable difficulties, partly because the catchment is very complex (there are numerous tributaries and sub-tributaries, and location of spawning trout is poorly known), but also because the catchment was in fresh or flood for considerable periods of time (Iain Maxwell, Hawkes Bay Fish and Game Council, pers. comm.).

5. Recommendations

It is recommended that otoliths of up to 50 trout be analysed for their chemical composition, including some fish from the lower Mohaka River. This should ensure that some estuarine or chemical signatures can be observed, and would produce greater confidence in the technique. It is also very desirable that analysis include some otoliths from sea run trout; if the latter are unavailable, then otoliths from Chinook salmon should be included as an alternate “control”.

6. Acknowledgements

I especially wish to thank Iain Maxwell, Hawkes Bay Fish and Game Council, for his persistence in obtaining trout samples. Thanks also to Bernard Barry from Geological and Nuclear Sciences, Lower Hutt, for processing the samples and helpful discussions, and finally to Greg Kelly, NIWA, Christchurch for mounting and grinding otoliths.

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