An investigation to determine origin: age and otolith chemistry of a brown bullhead catfish (*Ameiurus nebulosus*) found at Okawa Bay, Lake Rotoiti

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Jennifer M. Blair

and Brendan J. Hicks

Centre for Biodiversity and Ecology Research

Department of Biological Sciences

School of Science and Engineering, The University of Waikato

Private Bag 3105

Hamilton, New Zealand

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Email: b.hicks@waikato.ac.nz

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Reviewed by:

Johlene Kelly
Department of Conservation
Rotorua Lakes Area Office

Approved for release by:

Conrad Pilditch
University of Waikato

Executive summary

Some time between 10 and 15 January 2009, a brown bullhead catfish (*Ameiurus nebulosus*) was found washed up on the shore of Okawa Bay, Lake Rotoiti. The elemental signature of the otolith was measured using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) in order to determine the origin of the catfish. The otolith elemental signature of the Rotoiti catfish was compared to the otolith elemental signatures of catfish caught at Tokaanu Bay, Lake Taupo. Because otolith chemistry is largely determined by water chemistry, fish sharing a common origin should show similar otolith elemental signatures.

This is not the first alert concerning catfish in Lake Rotoiti. In 1995, moribund juvenile catfish were observed to fall out of a hollow-framed boat trailer after a boat launching. This boat had been parked on its trailer overnight in Lake Taupo at Motuapa immediately before the boat was launched in Lake Rotoiti. Despite extensive searching around the boat ramp in Lake Rotoiti within 7 days of the launching, no catfish were observed. In 2003, a shallow excavation reminiscent of a catfish nest in a sandy beach was seen in Lake Rotoiti. Subsequent boat electrofishing in Jan 2004 found no catfish. That fact that no catfish were observed between 1995 and 2008 suggests that catfish did not establish a breeding population from the 1995 incident.

The approximate age of the catfish found in 2009 (7 years) suggests that it was not one of the juveniles that might have been released accidentally into Lake Rotoiti in 1995. Based on the otolith microchemistry, it seems unlikely that the catfish from Lake Rotoiti shares a common origin with the catfish from Tokaanu Bay, Lake Taupo. The otolith nuclei of catfish from Lake Rotoiti and Lake Taupo showed significant differences in the concentrations of Mn, Sr and Ba; the Taupo catfish had lower concentrations of these elements compared to the Rotoiti catfish.

The other possible sources for this catfish are 1) that it is part of a breeding population of catfish in Lake Rotoiti, possibly resulting from the 1995 incident, that has gone undetected for 14 years, or 2) that there has been a subsequent introduction of catfish to Lake Rotoiti that has gone undetected, or 3) that the fish was recently translocated to Lake Rotoiti, dead or alive, from another water body, possibly in the Waikato River basin. The extreme size of this catfish (450-500 mm, and 1,267-1,734 g) compared to size range of catfish in Lake Taupo (maximum about 360 mm) also suggests that the catfish might have recently come from the Waikato region (maximum about 455 mm).

We conclude that the catfish did not come from Tokaanu Bay, Lake Taupo, but we cannot be sure where it did come from. There could be a breeding population in Lake Rotoiti, which seems unlikely given the size and age of this catfish and fact that no other catfish have been reported. Alternatively, the catfish could have come from the closest know populations in the Waikato River.

Introduction

Some time between 10 and 15 January 2009, a brown bullhead catfish (*Ameiurus nebulosus*) was found washed up on the shore of Okawa Bay, Lake Rotoiti. The total length of the fish is approximately 450-500 mm, estimated using the photo below as the fish was buried before it could be measured (Fig. 1).

Figure 1. Brown bullhead catfish *Ameiurus nebulosus*, approximately 450-500 mm in length, found dead on the shore of Lake Rotoiti in January 2009. (Photo by D. Atkinson).

The brown bullhead catfish is present throughout much of the North Island, including Lake Taupo and the Waikato River system (McDowall 2000). However, no catfish have been found in the Rotorua Lakes area to date. The catfish found at Lake Rotoiti is slightly smaller than the largest recorded catfish in New Zealand, which measured 480 mm and weighed over 2 kg (McDowall 1990). The closest known catfish populations to Lake Rotoiti are the Waikato River and its hydro lakes.

This is not the first alert concerning catfish in Lake Rotoiti. In 1995, moribund juvenile catfish were observed to fall out of a hollow-framed boat trailer after a boat launching. This boat had been parked on its trailer overnight in Lake Taupo at Motuapa immediately before the boat was launched in Lake Rotoiti. Despite extensive searching around the boat ramp in Lake Rotoiti within 7 days of the launching, no catfish were observed (Rowan Strickland, Cawthron Institute, pers. comm.). In 2003, a shallow excavation reminiscent of a catfish nest in a sandy beach was seen in Lake Rotoiti (John Clayton, NIWA, pers. comm.). Subsequent boat electrofishing in Jan 2004 found no catfish (Hicks and Ring 2004).

Otoliths are paired calcium carbonate structures in the inner ear and provide a chronological record of the chemical environment a fish has experienced during its lifetime. A number of characteristics make this possible: elements from the surrounding water are taken up and deposited in the otolith matrix; otoliths are metabolically inert and not reabsorbed by the fish; and new material is continuously deposited even after somatic growth stops (Campana 1999, Maillet and Checkley 1990). The otolith concentrations of elements such as Sr, Ba, and Mn are correlated with water concentrations (Campana 1999). Because of differences in water geochemistry between areas, fish spawned in different areas should show varying amounts of trace elements (referred to as the "elemental signature") deposited in the otolith nucleus.

The elemental signature of the otolith can be used to discriminate stocks, study migrations and identify natal areas of fish. In diadromous fish, periods of freshwater and saltwater residence can be identified by quantifying Sr:Ca ratios in the otolith (Secor 1992; Daverat et al. 2005; Arai and

Hirata 2006). Signatures of multiple elements have been used to identify natal areas of marine (Thorrold et al. 2001), estuarine (Miller 2007) and freshwater fish (Wells et al. 2003; Brazner et al. 2004; Clarke et al. 2007). Otolith microchemistry has also been used to determine the source and date of introduction of lake trout *Salvelinus namaycush* into Yellowstone Lake by comparing otolith Sr:Ca ratios (Munro et al. 2005).

The aim of this study was to ascertain the origin of the catfish found at Lake Rotoiti; specifically, to ascertain whether the fish had been introduced into Lake Rotoiti from another water body or originated from Lake Rotoiti. This was achieved by ageing the fish using its fifth vertebra and then analysing the elemental composition of its otolith by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The elemental signature of the Rotoiti catfish was then compared to the signatures of three catfish caught at Tokaanu Bay in Lake Taupo.

Methods

The catfish caught at Lake Rotoiti was aged using pectoral fin spines (DeVries and Frie 1996) and the 5th vertebra (Appelget and Smith 1950). Fin spines were mounted in epoxy resin and sectioned using a low-speed saw, then examined under a dissecting microscope using transmitted light. The vertebra was removed from the fish and soaked in a solution of hot water and potassium hydroxide to remove the surrounding tissue, then examined whole under a dissecting microscope using reflected light. Dark bands consisting of a complete dark ring were interpreted as annular marks.

Asteriscus otoliths were dissected from the Rotoiti catfish and from three catfish caught from Lake Taupo. These were washed with household bleach and Milli-Q water, and then polished using silicone carbide paper to expose the nucleus. The otoliths were mounted on a glass microscope slide for ablation.

Otolith trace elements were analysed at the University of Waikato using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Settings used are given below (Table 1).

Table 1. Settings used in laser ablation inductively coupled plasma mass spectrometry analysis of rainbow trout and smelt otoliths.

Parameter	Value
Analytes	^{10}B, ^{25}Mg, ^{27}Al, ^{42}Ca (internal standard), ^{43}Ca, ^{55}Mn, ^{60}Ni, ^{62}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{85}Rb, ^{88}Sr, ^{137}Ba, ^{139}La
Sweeps/reading	5
Readings/replicate	155
Replicates	1
Estimated reading	0.845 s
Scan mode	Peak hopping
MCA channels	1
Dwell time per AMU	10 ms

Laser settings are given in Table 2. NIST SRM (National Institute of Standards and Technology Standard Reference Material) 612 was used as a calibration standard for all analyses. Two spots were ablated on the NIST 612 reference material before ablating otoliths and thereafter every six otolith spots. Background element concentrations were measured for 60 s prior to each ablation by analysing a gas blank (firing the laser with the shutter closed). After all otoliths on the slide had been sampled, another two spots on the NIST 612 reference material were ablated to measure instrument drift during the session.

Two spots were ablated on each Lake Taupo otolith; one as close to the nucleus as possible and another as close to the edge as possible. Four spots at the edge and four spots at the nucleus were ablated on the Lake Rotoiti catfish otolith. The software package GLITTER (van Achterbergh et al. 2001) was used for data collation and reduction for the spot analyses. The elements Mg, Mn, Zn, Rb, Sr and Ba were selected for further analyses as they were measured above detection limits in the majority of samples.

Table 2. Laser power, spot size, repetition rate, and laser dwell time used for ablation of smelt otoliths, trout otoliths, and NIST 612.

	Laser power (%)	Spot size (µm)	Repetition rate (Hz)	Laser dwell time (s)
NIST 612	60	60	10	60
Catfish otoliths	60	60	5	40

Element concentrations across the otolith of the Rotoiti catfish were measured using a line scan. Settings used were 10 µm s^{-1} scanning speed, 5 Hz repetition rate, 60% output and 60 µm spot size. The line was pre-ablated at 20 µm s^{-1} in order to remove any possible surface contamination. To process the line scan, the mean isotopic counts from the first 60 seconds of analysis (taken as a background reading without firing the laser) were subtracted from isotopic counts taken during the line scan. Results are presented as counts of the isotope (^{88}Sr or ^{137}Ba) to ^{42}Ca.

Element concentrations of koi carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and smelt (*Retropinna retropinna*) otoliths were obtained from previous studies carried out using similar methods to those described above (Blair 2008, Blair and Hicks, unpublished data).

Catfish weights were estimated using the following length-weight regression for brown bullhead catfish caught primarily by boat electrofishing in the Waikato region (Hicks, unpublished data):

$$\ln W = -11.049 + 2.978 \ln L,$$

where W = weight in g and L = fork length in mm (N = 281, r^2 = 0.956, p << 0.001).

Nucleus elemental concentrations in catfish from Lake Taupo and Lake Rotoiti were compared by assessing 95% confidence intervals of the mean for each element. A paired t-test was carried out testing differences in elemental concentrations between the edges and nuclei of the otoliths from Lake Taupo, and a t-test was used to test differences between the edge and nucleus of the otolith from Lake Rotoiti. Students' t-tests, means, standard deviations and 95% confidence intervals were calculated using Statistica, version 8.0 (Statsoft, Inc. 2007)

Results

Age and weight of Rotoiti catfish

Vertebral check rings suggest that the catfish was 7 years old, i.e., was hatched in Nov-Dec 2002. The estimated length of 450- 500 mm corresponds to a weight range of 1,267- 1,734 g.

Elemental concentrations

Line scans

Line scans showed that ratios of ^{88}Sr and ^{137}Ba to ^{42}Ca varied across the otolith surface (Figs. 2 and 3). Ba showed a peak approximately 1500 µm from the otolith nucleus (Fig. 3).

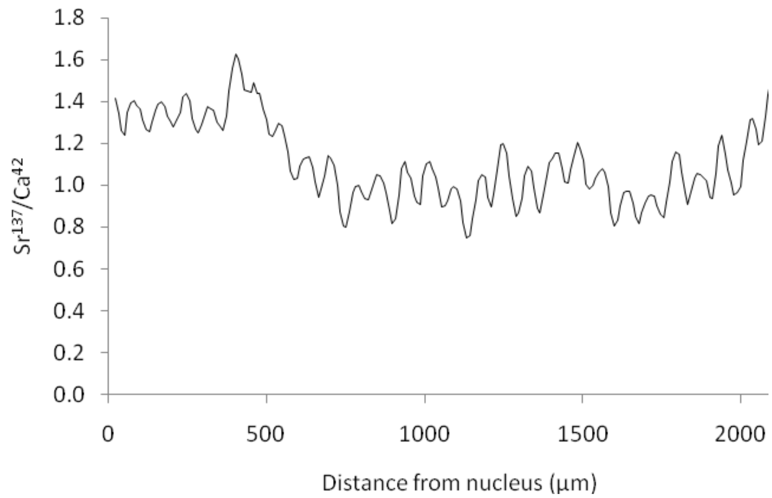

Figure 2. Ratios of LA-ICP-MS ^{88}Sr counts to ^{42}Ca counts, measured in a line scan from the nucleus to the edge of the Rotoiti catfish otolith. Background levels have been subtracted from these values. Data smoothed using a 3-point moving average.

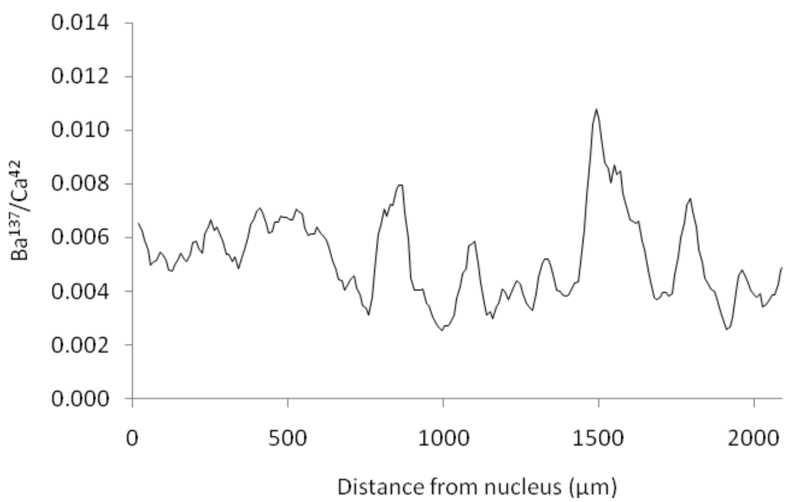

Figure 3. Ratios of LA-ICP-MS ^{137}Ba counts to ^{42}Ca counts, measured in a line scan from the nucleus to the edge of the Rotoiti catfish otolith. Background levels have been subtracted from these values. Data smoothed using a 3-point moving average.

Laser spots

Figures 4 to 8 show otolith concentrations of Mg, Mn, Zn, Rb and Ba plotted against concentrations of Sr. Some variability is evident, especially among measurements taken from the edge of the Rotoiti otolith. Despite this variability, elemental concentrations in the Taupo otoliths and the Rotoiti otolith appear distinct and do not overlap (Figs. 4-8).

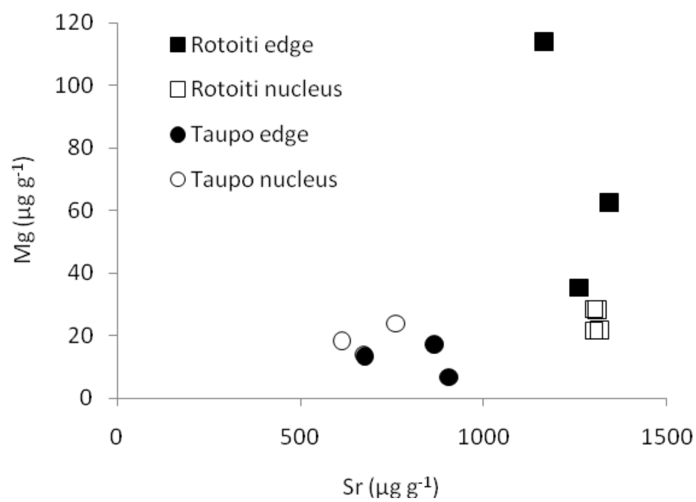

Figure 4. Mg and Sr concentrations (µg g^{-1}) in Rotoiti and Taupo catfish otolith edges and nuclei. One outlier removed (from Rotoiti edge group). The Rotoiti group is made up of four laser ablation spots on one otolith, whereas the Taupo group is made up of laser ablation spots on otoliths from three different fish.

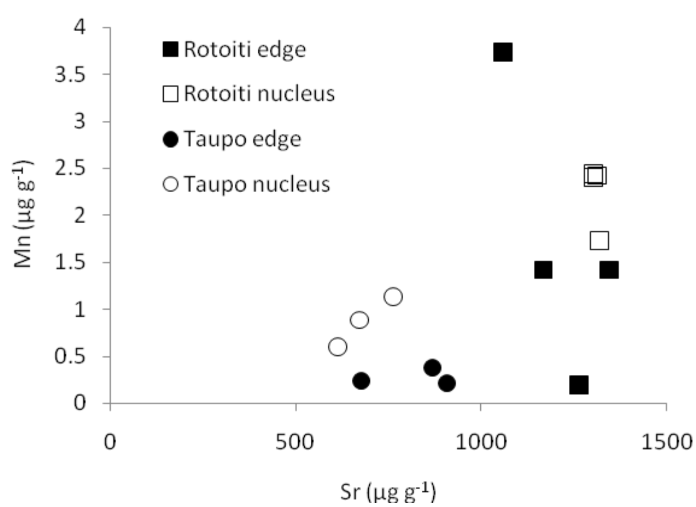

Figure 5. Mn and Sr concentrations (µg g^{-1}) in Rotoiti and Taupo catfish otolith edges and nuclei. The Rotoiti group is made up of four laser ablation spots on one otolith, whereas the Taupo group is made up of laser ablation spots on otoliths from three different fish.

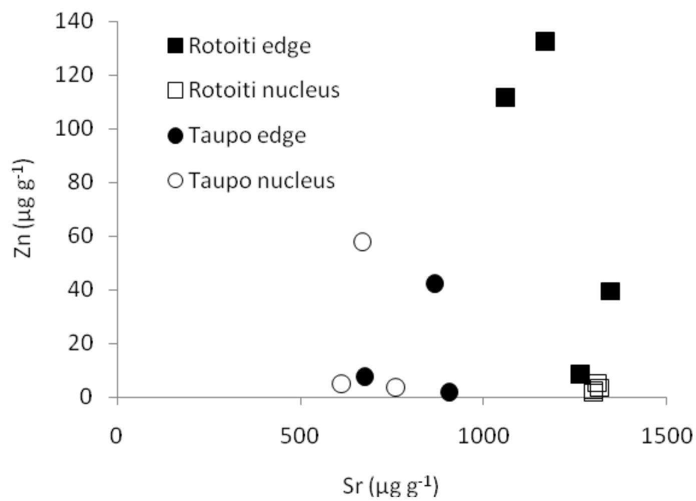

Figure 6. Zn and Sr concentrations (µg g^{-1}) in Rotoiti and Taupo catfish otolith edges and nuclei. The Rotoiti group is made up of four laser ablation spots on one otolith, whereas the Taupo group is made up of laser ablation spots on otoliths from three different fish.

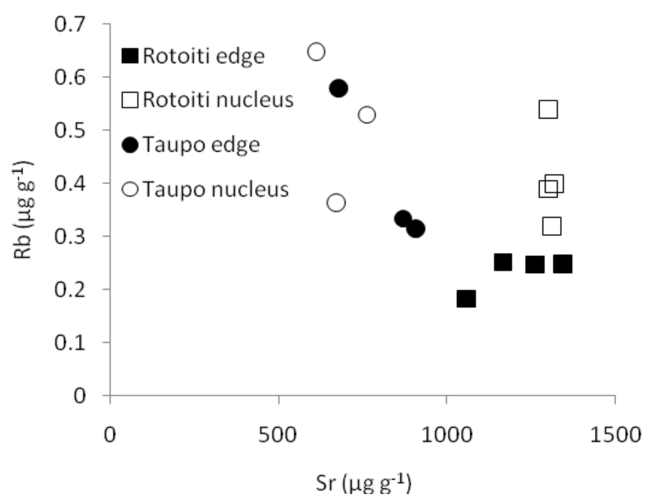

Figure 7. Rb and Sr concentrations (µg g^{-1}) in Rotoiti and Taupo catfish otolith edges and nuclei. The Rotoiti group is made up of four laser ablation spots on one otolith, whereas the Taupo group is made up of laser ablation spots on otoliths from three different fish.

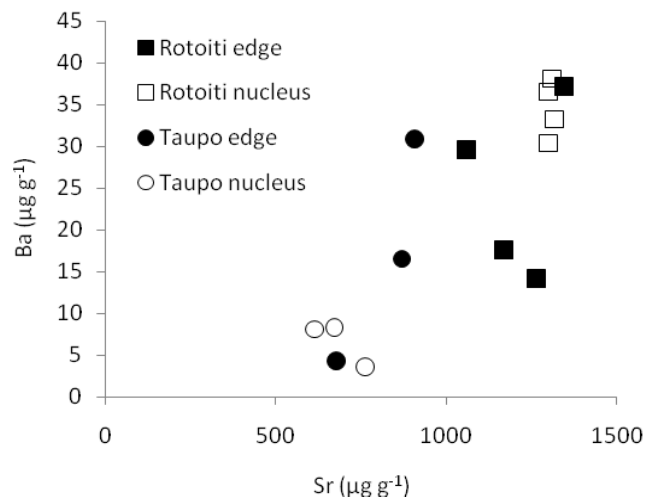

Figure 8. Ba and Sr concentrations (µg g^{-1}) in Rotoiti and Taupo catfish otolith edges and nuclei. The Rotoiti group is made up of four laser ablation spots on one otolith, whereas the Taupo group is made up of laser ablation spots on otoliths from three different fish.

Comparison of Rotoiti and Taupo catfish otolith element concentrations

Concentrations of all elements except Rb were higher in the Rotoiti catfish otolith than in the Taupo catfish otoliths (Table 3). The mean concentrations of Mn, Sr and Ba in the Rotoiti otolith nucleus did not fall within the confidence limits of the mean Mn, Sr and Ba concentrations in the Taupo otolith nuclei, indicating that Mn, Sr and Ba concentrations in otolith nuclei were different between the two lakes (Table 4). No differences in elemental concentrations were found between otolith nuclei and edges in the Lake Taupo catfish (Table 5). Only Rb concentrations were significantly different between the nucleus and the edge of the Rotoiti otolith (Table 6).

Table 3. Mean element concentrations (μg g^{-1}), measured in otolith edges and nuclei from catfish caught in Lake Rotoiti and Lake Taupo. The Rotoiti group is made up of four laser ablation spots on one otolith, whereas the Taupo group is made up of laser ablation spots on otoliths from three different fish.

		Mg		Mn		Zn		Rb		Sr		Ba	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Rotoiti	edge	143.87	75.22	1.69	0.74	72.93	29.40	0.23	0.02	1208.6	62.11	24.64	5.34
	nucleus	24.92	1.90	2.25	0.18	3.23	0.75	0.41	0.05	1307.8	4.33	34.56	1.73
Taupo	edge	12.29	2.96	0.27	0.05	17.27	12.61	0.41	0.09	816.69	71.46	17.29	7.69
	nucleus	18.71	2.85	0.88	0.16	22.27	17.86	0.51	0.08	681.87	43.60	6.62	1.54

Table 4. Summary statistics of Mg, Mn, Zn, Rb, Sr and Ba (μg g^{-1}) in otolith nuclei from catfish caught in Lake Taupo and Lake Rotoiti. Elements are marked with * where the mean concentration of the Rotoiti otolith falls outside the confidence intervals of the mean of the Taupo otolith. The Rotoiti group is made up of four laser ablation spots on one otolith, whereas the Taupo group is made up of laser ablation spots on otoliths from three different fish.

Element	*N*		Mean		95% confidence limits for means			
	Rotoiti	Taupo	Rotoiti	Taupo	Rotoiti		Taupo	
					Lower limit	Upper limit	Lower limit	Upper limit
Mg	4	3	24.9	18.7	18.9	31.0	6.4	31
Mn*	4	3	2.2	0.877	1.7	2.8	0.2	1.5
Zn	4	3	3.2	22.3	0.9	5.6	-54.6	99.1
Rb	4	3	0.413	0.515	0.3	0.6	0.2	0.9
Sr*	4	3	1308	682	1294	1322	494	869
Ba*	4	3	34.6	6.6	29.1	40.1	0	13.2

Table 5. Results of paired t-tests comparing Mg, Mn, Zn, Rb, Sr and Ba (μg g^{-1}) in otolith nuclei of catfish caught in Lake Taupo. Significant paired t-test result indicated with *.

Element	*N*		Mean		*t*-value	df	*p*
	Taupo nucleus	Taupo edge	Taupo nucleus	Taupo edge			
Mg	3	3	10.9	20.6	-1.106	2	0.384
Mn	3	3	0.270	1.1	-3.625	2	0.068
Zn	3	3	32.6	16.7	-0.906	2	0.460
Rb	3	3	0.357	0.501	-1.877	2	0.201
Sr	3	3	849	693	3.467	2	0.074
Ba	3	3	18.4	5.6	1.179	2	0.360

Table 6. Results of t-tests comparing Mg, Mn, Zn, Rb, Sr and Ba (μg g^{-1}) in the otolith nucleus and edge of catfish caught in Lake Rotoiti. Significant t-test result indicated with *.

Element	N		Mean		*t*-value	df	*p*
	Rotoiti nucleus	Rotoiti edge	Rotoiti nucleus	Rotoiti edge			
Mg	4	4	144	24.9	1.6	6	0.165
Mn	4	4	1.7	2.2	-0.7	6	0.496
Zn	4	4	73	3.2	2.4	6	0.056
Rb*	4	4	0.233	0.413	-3.7	6	0.011
Sr	4	4	1209	1308	-1.6	6	0.162
Ba	4	4	25	35	-1.8	6	0.128

Comparison of Rotoiti catfish otolith element concentrations to other species

Otolith concentrations of Mg, Mn, Rb, Sr and Ba vary between rainbow trout *Oncorhynchus mykiss*, common smelt *Retropinna retropinna* and catfish caught in Lake Rotoiti (Fig. 9a-e). Sr concentrations were higher in the catfish otolith than the other two species, while Rb concentrations were lower in the catfish otolith (Fig. 9c and 9d). Rb, Sr and Ba concentrations are similar between the Rotoiti catfish otolith and Waikato River koi carp otoliths (Fig. 9c, 9d and 9e).

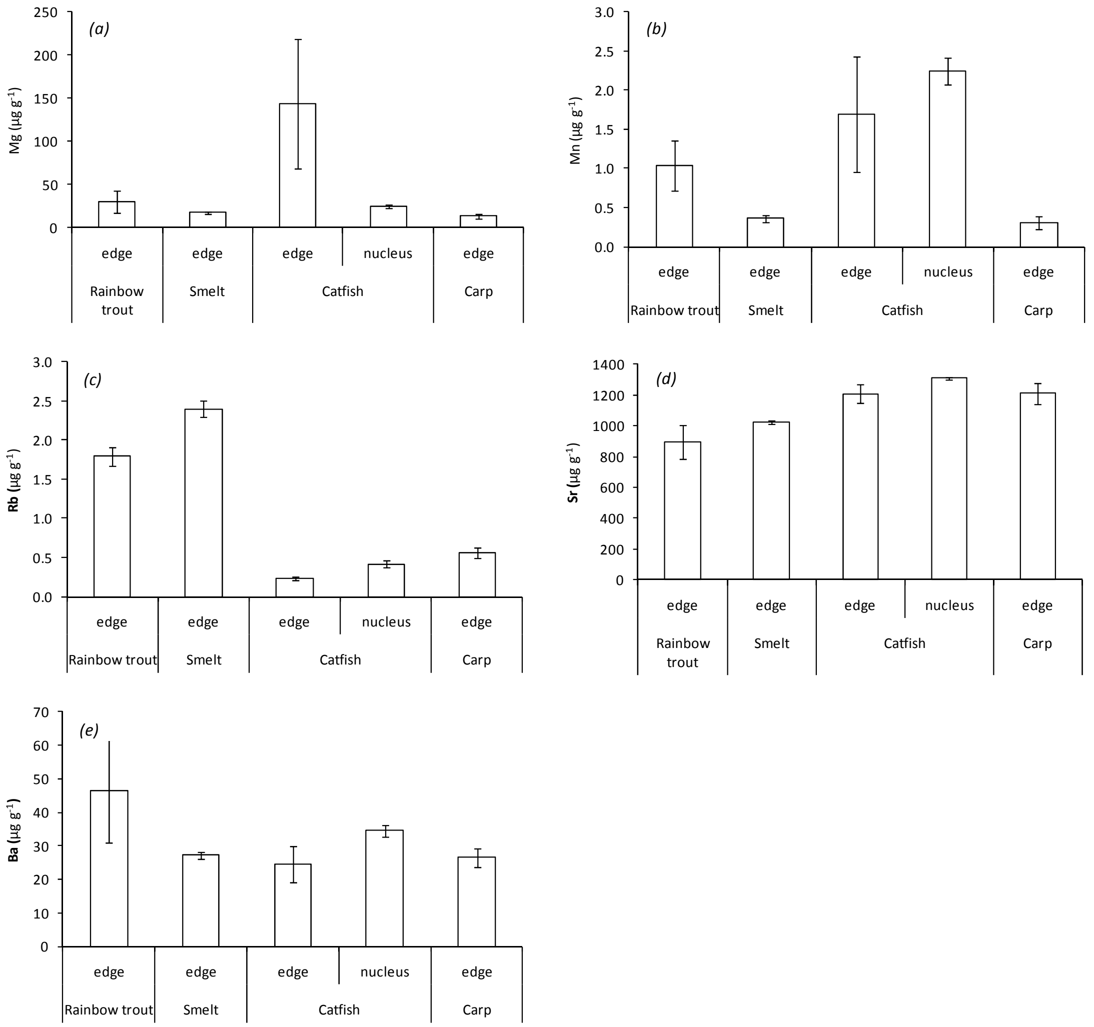

Figure 9. Concentrations of (a) Mg, (b) Mn, (c) Rb, (d) Sr and (e) Ba in edges of Rotoiti rainbow trout otoliths, edges of Rotoiti smelt otoliths, edges and nucleus of Rotoiti catfish otoliths, and edges of Waikato River koi carp otoliths (µg g^{-1}). Error bars show ± 1 SE.

Discussion

The ratios of Sr:Ca and Ba:Ca measured in the line scan of the Rotoiti otolith show some variation, but no consistent pattern which would have suggested movement or translocation between water bodies with distinctive elemental signatures. The similar elemental concentrations in the edge and nucleus of the Rotoiti otolith also suggest that no such translocation has been made. If the Rotoiti catfish had been translocated from Lake Taupo, we would expect differences in elemental concentrations between the otolith edge and otolith nucleus. Elemental concentrations in the otoliths of catfish from Lake Rotoiti and Lake Taupo appear distinct, and differences in Mn, Sr and Ba concentrations between the Rotoiti otolith nucleus and Taupo otolith nuclei suggest that the Rotoiti catfish and Taupo catfish do not share a common origin. The Taupo catfish had lower concentrations of Mn, Sr and Ba in their otolith nuclei compared to the Rotoiti catfish.

The elemental concentrations of the Rotoiti catfish are not similar to trout and smelt otoliths from Lake Rotoiti, but lie within a similar range to those measured in koi carp *Cyprinus carpio* caught at the Waikato River at Rangiriri. Although uptake of elements into otoliths is species specific (probably due to metabolic differences), similarities in otolith elemental concentrations have been found between co-occurring species (Hamer and Jenkins 2007). The extreme size of this catfish (450-500 mm, and 1,267-1,734 g) also suggests that the catfish might have recently come from the Waikato region, as the maximum size of catfish in the Waikato region (455 mm; Patchell 1977) is larger than the maximum size of catfish in Lake Taupo (360 mm; Barnes 1996). It may be useful to compare the element concentrations in the Rotoiti catfish otolith to those in catfish otoliths from the Waikato region.

Otolith microchemistry has previously been used to determine the origin and date of introduction of lake trout, an introduced species in Yellowstone Lake, USA (Munro et al. 2005). The otoliths of suspected translocated lake trout showed distinct shifts in Sr:Ca ratios across the otolith surface (Munro et al. 2005), unlike the Sr:Ca ratios across the otolith of the Rotoiti catfish, which showed no definite shift. Given that element concentrations were much higher in the Rotoiti catfish otolith than in the Taupo catfish otoliths, the Rotoiti catfish should have shown a distinct increase in elemental concentrations across the otolith if it had been translocated from Lake Taupo.

Conclusion

In 1995, moribund juvenile catfish were observed to fall into Lake Rotoiti from a hollow-framed boat trailer after a boat launching. However, the approximate age of the catfish found in 2009 (7 years) suggests that it was not one of the juveniles that might have been released accidentally in 1995. Based on the otolith microchemistry, it seems unlikely that the catfish from Lake Rotoiti shares a common origin with the catfish from Tokaanu Bay, Lake Taupo. The otolith nuclei of catfish from Lake Rotoiti and Lake Taupo showed significant differences in the concentrations of Mn, Sr and Ba. The Taupo catfish had lower Sr and Ba concentrations in their otolith nuclei. Moreover, the Sr:Ca and Ba:Ca ratios across the otolith surface suggest that the fish has not been translocated between two water bodies with distinct water chemistries during its lifetime. It may be useful to analyse otoliths of catfish caught at other locations, such as the Waikato River, to ascertain whether these show similar elemental concentrations to the Rotoiti catfish.

The other possible sources for this catfish are 1) that it is part of a breeding population of catfish in Lake Rotoiti, possibly resulting from the 1995 incident, that has gone undetected for 14 years, or 2) that there has been a subsequent introduction of catfish to Lake Rotoiti that has gone undetected, or 3) that the fish was recently translocated to Lake Rotoiti, dead or alive, from another water body, possibly in the Waikato River basin. The extreme size of this catfish (450-500 mm, and 1,267-1,734 g) compared to size range of catfish in Lake Taupo (maximum about 360 mm) also suggests that the catfish might have recently come from the Waikato region, where catfish measuring 455 mm have been reported.

We conclude that the catfish did not come from Tokaanu Bay, Lake Taupo, but we cannot be sure where it did come from. There could be a breeding population in Lake Rotoiti, which seems unlikely given the size and age of this catfish and fact that no other catfish have been reported. Alternatively, the catfish could have come from the closest know populations in the Waikato River.

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