Life-history of Lake Horowhenua common smelt: analysis of the otolith chemistry and vertebral counts

horizons regional council

November 2013

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Horizons Report: 2013/EXT/1345

ERI Report Number 16

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November 2013
Report No. 2013/EXT/1345
ISBN 978-1-927250-48-8

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Date: November 2013

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Cite report as:
Tana, R. and Tempero, G. 2013. Life-history of Lake Horowhenua common smelt: analysis of otolith chemistry and vertebral counts. Client report prepared for Horizons Regional Council. *Environmental Research Institute Report No. 16*, The University of Waikato, Hamilton. 12 pp.

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Executive summary

Lake Horowhenua is a coastal eutrophic lake on the west coast of the North Island. A recent survey of the lake found lower than expected fish diversity but comparatively abundant native fish populations, comprising mostly shortfin and longfin eels (*Anguilla australis* and *A. dieffenbachii*). A weir on the outlet of the lake was identified as a potential barrier to fish migrations, reducing fish diversity and abundance in the lake. However, large numbers of common smelt (*Retropinna retropinna*) were collected during this survey, indicating that the population was either successfully reproducing in the lake or diadromous, i.e., migrating from the sea. Previous studies have shown that lacustrine common smelt can be distinguished from diadromous populations by differences in counts of vertebrae and gill rakers, and otolith microchemistry. Horizons Regional Council requested that an analysis of smelt otoliths and relevant morphological characteristics be performed to ascertain if the Lake Horowhenua population was diadromous.

Thirty-one common smelt were taken from Lake Horowhenua, x-rayed and vertebral counts performed. In addition, sagittal otolith pairs from 20 fish were extracted and analysed by laser ablation Inductively Coupled Plasma Mass-Spectrometry (ICP-MS). Vertebral counts (n = 31) ranged from 53 – 56 (54.7 ± 0.29, mean ± 95% CI), which was below the typical values for diadromous populations (means 59-60) but above those of typical lacustrine populations (means 51-52). Gill raker counts (n = 6) ranged from 18 – 21 (19.5 ± 0.84, mean ± 95% CI), which is typical of coastal and diadromous populations. Otolith microchemistry conclusively showed that the smelt were not diadromous, as strontium/calcium and barium/calcium ratios indicated no marine life-stage in any of the fish examined. Typically, the marine phase of diadromous fish is distinguished by high strontium/calcium ratios and low barium/calcium ratios, which reverses as the fish migrates into freshwater.

From our results of both vertebral counts and otolith microchemistry the common smelt population of Lake Horowhenua is entirely lacustrine. While the results from the gill raker counts are only preliminary they suggest that the Lake Horowhenua population recently diverged from a diadromous population which has become isolated. Lacustrine common smelt usually require sandy beaches for spawning and the western shores of Lake Horowhenua are the most likely spawning sites for the population as they are comparatively sheltered and of an appropriate sandy sediment composition. The weir on the Hokio Stream outlet is likely to be a barrier to smelt movement and consideration should be given to the effects on the Lake Horowhenua population in addition to the benefits of re-establishing connectivity with the sea for other native species should a fish pass be installed.

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Introduction

Located adjacent to the township of Levin, Lake Horowhenua is a small (surface area 2.9 km^2), shallow (< 2 m deep), eutrophic coastal dune lake on the west coast of the North Island (Gibbs and White 1994). The lake has several surface inflows as well as significant ground-water inflows; outflow is via the Hokio Stream on the western side of the lake. Lake water levels are maintained by a weir on the Hokio Stream that was installed in 1956 (Gibbs 2011). Results from a boat electrofishing survey conducted in April 2013 by the University of Waikato found an abundant population of common smelt (*Retropinna retropinna*) within the lake (Tempero 2013). Common smelt are known to be flexible in their reproductive strategies, with both diadromous and lacustrine populations occuring. In some locations, such as the lower Waikato region, diadromous and lacustrine populations are known to exist together (Ward et al. 2005).

Diadromous and lacustrine common smelt populations exhibit differences in internal characteristics such as shape, size at maturity, number of gill rakers, and number of vertebrae. Diadromous populations have low gill raker numbers (17-23) and high vertebral counts (57-63), while lacustrine populations have high gill raker numbers (26-33) and low vertebral counts (48-54) (McDowall, 1979; Ward et al., 1989, 2005; Booker, 2000). However, the ranges of these values can overlap, especially in coastal populations where interbreeding of lacustrine and diadromous populations is possible e.g. in Lake Wairarapa smelt have a vertebral count with a range of 53-63 (McDowall 1990).

Differentiation of diadromous and lacustrine common smelt populations can also be achieved through analysis of the elemental composition of their ear bones known as otoliths. As the fish grows it incorpoarates elements from the surrounding water into its otoliths. This leaves a chemical signature of the different aquatic systems it has inhabited. For example, marine waters have higher concentrations of strontium and lower concentrations of barium compared to freshwaters (Limburg 1995). This leaves a distinct chemical signature in the otolith that can be used to determine if a fish has spent time in a marine environment. Typical patterns of Sr and Ba for diadromous torrentfish, *Cheimarrichthys fosteri*, are shown in Figure 1, with decreasing strontium and increasing barium as the fish migrate from a marine environment to a freshwater environment.

To further understand the population ecology of common smelt in Lake Horowhenua, analysis of ototlith chemistry and vertebral counts was performed at the University of Waikato. The objective was to determine whether the Lake Horowhenua common smelt population was lacustrine, diadromous or if the population was a mixture of the two.

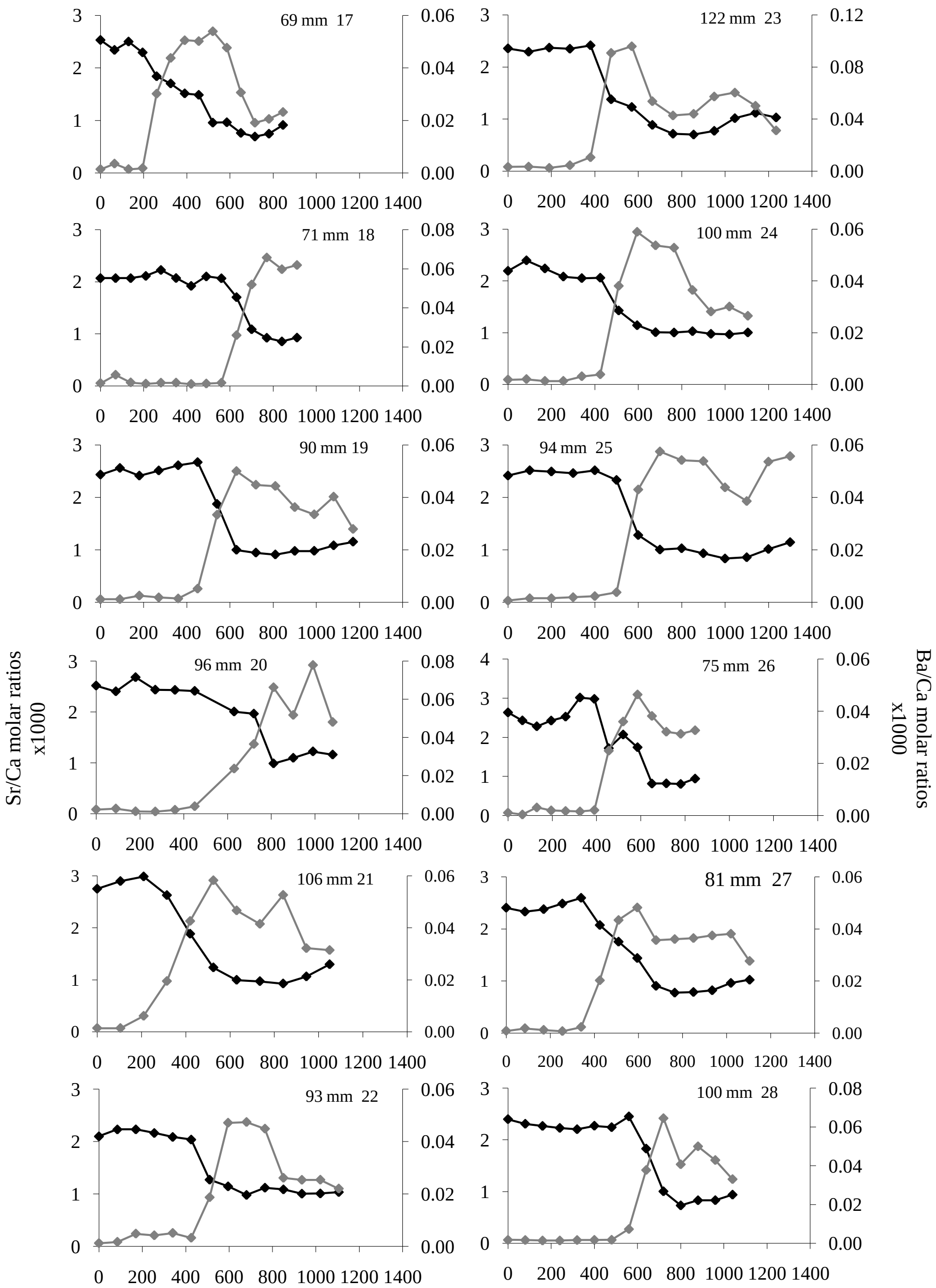

Figure 1 Laser spot analyses of torrentfish otoliths from the Rangitukia Stream, Mt Pirongia, Waikato. Sr/Ca (black) and Ba/Ca (grey) ratios are shown as elemental molar ratios (x 1000) at progressive distances from the core (µm). Fish total length and identification number shown in the top right of each graph. Source: Tana (2009).

Methods

Thirty-one common smelt from a single school were collected by boat electrofishing in Lake Horowhenua on 17 April 2013. They were frozen and then transported to the University of Waikato for further analysis. The fish were then thawed, placed in lateral view on a sheet of window glass and x-rayed at Hamilton Radiology. From the resulting radiographic images vertebrae counts were performed. The first gill raker arch was extracted from six smelt and gill raker counts performed under a binocular microscope. Twenty fish were then radomly selected for otolith extraction and analysis.

1.1.1 Smelt otolith extraction and preparation for LA-ICP-MS

A total of 20 sagittal otolith pairs were extracted from smelt using acid washed plastic utensils. Otoliths were first placed in hydrogen peroxide (H_20_2) for ~15 seconds to remove connective tissue followed by a 3-minute rinse in ultra-pure water and 10-second soak in 2% nitric acid ($HN0_3$) before finally being rinsed thoroughly in ultrapure water again. Cleaned otoliths were placed in glass vials (20 mm), and stored in a laminar flow hood over night to dry. Dried otoliths were mounted on individual petrographic slides using Crystal-Bond thermosetting glue over a heated plate with the groove end (sulcus) facing up. Otoliths were polished across the sagittal plane using firstly P2400 then P4000 carbide wetted sand paper exposing the otolith nucleus for laser ablation ICP-MS. Visual assessments were made regularly with transmitted light under a stereo and then compound microscope until ring structures radiating from the nucleus were visible at the immediate surface of the otolith. Each otolith was then transferred onto a single slide ready for laser ablation ICP-MS.

1.1.2 Laser ablation ICP-MS analysis of smelt otoliths

Chemical analysis of smelt otoliths was conducted at Waikato University's Mass Spectrometry Suite. The acquisition of chemical data was carried out by a Perkin Elmer 6000 Elan SCIEX DRCII Inductively Coupled Plasma Mass Spectrometer (ICP-MS) coupled to a New Wave Research neodymium aluminium garnet YAG short wave 213 nm laser. The laser was operated in Q-switched time resolved mode with an average energy reading of 0.33 millijoules, an isotope scan speed of 10 milliseconds to enable analysis of all elements more closely in time and a repetition rate of 10 Hz. To profile the entire chemical life history of smelt, line-scan analyses were carried out using a spot diameter of 40 µm at 60% power which travelled continuously from the otolith nucleus to the edge at a scan speed of 10 µm per second. Four isotopes were analysed specifically for internal calibration (^{42}Ca) and to extrapolate evidence of marine and freshwater life history (^{43}Ca, ^{88}Sr and ^{137}Ba). (Campana 1999; Kalish 1990; Limburg 1995). The same isotopes were also analysed in NIST612 standard reference material for which the concentrations of 50 isotopes are known and were standardized to internal ICP-MS machine standards using ^{42}Ca (Pearce et al. 1997). Elemental counts of strontium and barium were ratioed to calcium because elements from the environment are taken up in proportion to calcium's availability within the water that fish occupy (Campana 1999; Thorrold, 1998). Data for otoliths are presented as counts per second (cps) for each isotope as a consequence of the lack of matrix-matched standards (Morales-Nin et al. 2005).

Results and Discussion

Common smelt vertebral counts from Lake Horowhenua ranged from 53 – 56 with a mean of 54.7 ± 0.29 (± 95% CI) (Figure 2). These values are unusual when compared to those reported in a review paper by Ward et al. (2005). The range of the Lake Horowhenua values overlaps with both lacustrine and diadromous populations. Isolated lacustrine populations typically have vertebral counts ranging from 48-54 with mean values ranging from 51-52. In comparison, diadromous populations have counts typically ranging from 56-63 with mean values ranging from 59-60 (Ward et al. 2005). This places the Lake Horowhenua values between the two expected distributions for lacustrine and diadromous populations. In addition, the Lake Horowheuna range of values falls within the range of those found by McDowall (1990) for populations in Lake Wairarapa and Lake Ellesmere (53-63). However, no mean value was provided for this work so it is difficult to determine if the data was skewed towards one particular distribution. Also, no determination was made as to whether these populations were diadromous or lacustrine. Given that Wairarapa and Ellesmere are coastal lakes with marine access it is likely that there was a mixture of lacustrine and diadromous populations (Ward et al. 2005).

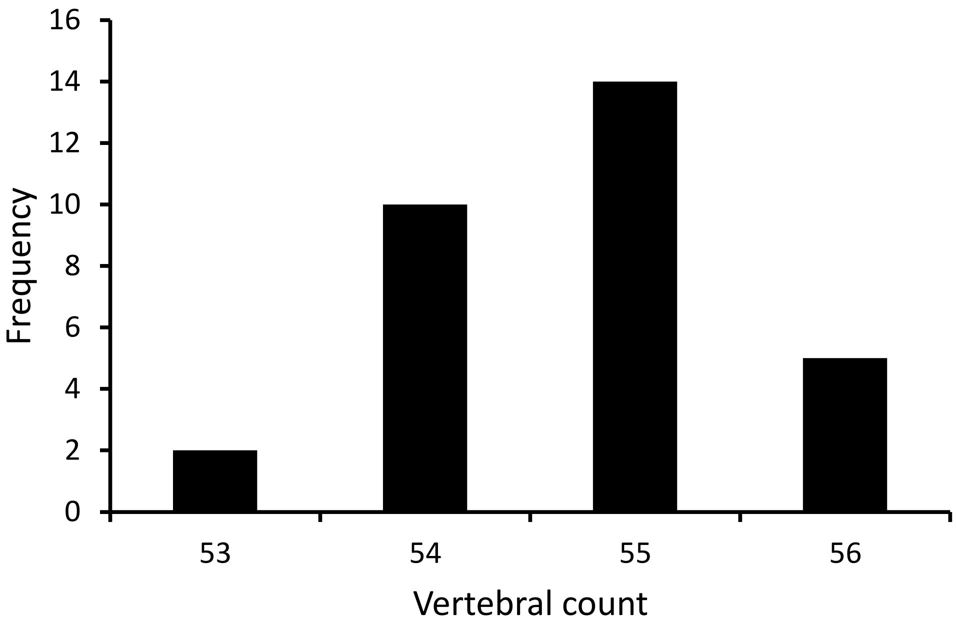

Figure 2 Frequency of vertebral counts from common smelt (n =31) collected by boat electrofishing from Lake Horowhenua, Levin on 17 April 2013.

Gill raker counts from six fish had a range of 18 - 21 with a mean of 19.5 ± 0.84 (95% CI). This distribution is congruent with coastal and diadromous populations reported by Ward et al. (2005). Typical mean gill raker counts for diadromous and coastal populations ranged from 19 – 21 while lacustrine populations ranged from 21 – 26 (Ward et al. 2005). These results may seem inconsistent with those of the vertebral counts, but Ward et al. (2005) hypothesised that vertebral number is influenced by environmental salinity during larval development, whereas gill raker number is genetically controlled. Therefore, diadromous populations should exhibit high vertebral counts and low gill raker counts, while reproductively isolated lacustrine populations would have high genetically determined gill raker counts but low vertebral counts as they have not been exposed to high salinities during development (Ward et al. 2005).

The otolith chemistry of 20 Lake Horowhenua common smelt were analysed using laser ablation ICP-MS. From these initial results eight were excluded from further analysis due to detection of low calcium levels which would have falsely indicated a marine life-stage. The remaining otoliths showed little variability in ^{88}Sr:^{43}Ca and ^{137}Ba:^{43}Ca ratios of counts per second, from the otolith core (region of larval growth) to the edge (recent growth), indicating the absence of a marine growth phase (Figure 3).

Overall, smelt otoliths showed consistently low Sr:Ca ratios (5-9) during larval growth through to maturing growth. (Figure 3). However, variable ^{137}Ba:^{43}Ca ratios in some fish were evident (0.02-0.03) during early growth as well as during maturity (S2, S6, and S11, Figure 3). Using otolith microchemistry techniques, the premise for distinguishing marine and freshwater movements in diadromous fish is founded on tracking variations in Sr:Ca and Ba:Ca across the otolith growth axis. For amphidromous fish such as common smelt, whereby spawning occurs in freshwater, followed by a sea marine larval stage, an amphidromous life history would be reflected by low Sr:Ca ratios at the core, a narrow band of high Sr:Ca during the marine larval stage, followed by low Sr:Ca ratios at the edge.

With the absence of clear pattern of high Sr:Ca ratios near the otolith core indicating marine residence, followed by low Sr:Ca ratios as the fish entered freshwater, there is no indication that common smelt from Lake Horowhenua had spent time at sea (Figure 3). Similarly, Ba:Ca in the sea demonstrates an inverse relationship to ^{88}Sr:^{43}Ca and no such patterns were evident in Lake Horowhenua smelt, confirming no association with marine environments.

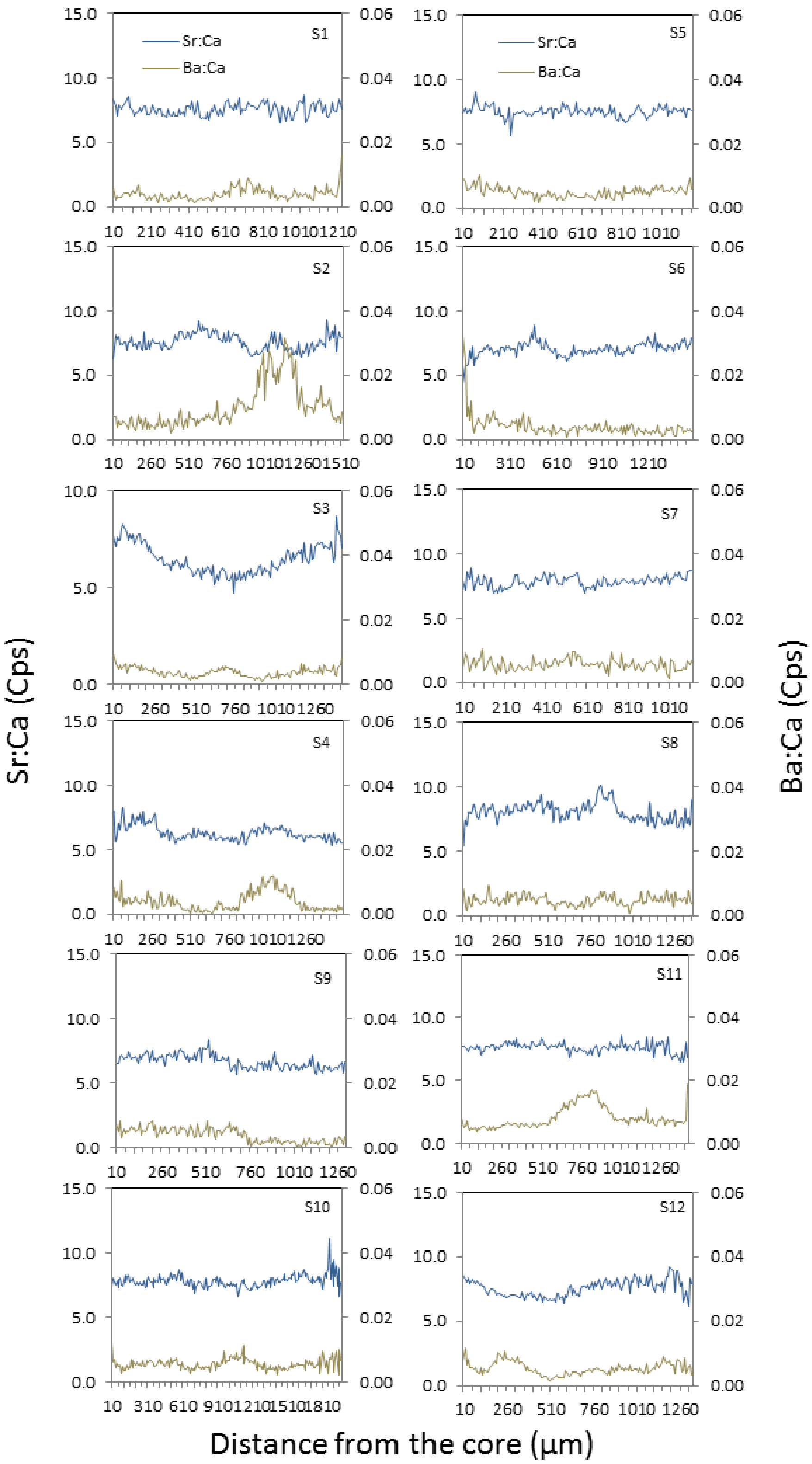

Figure 3 Otolith line-scan transects (at distance from the core) profiling freshwater residency trends in counts per second (cps) of ^{88}Sr:^{43}Ca and ^{137}Ba:^{43}Ca across the otolith growth axis of common smelt (*Retropinna retropinna*), sampled from Lake Horowhenua on 17 April 2013.

Conclusion

Vertebral counts of common smelt from Lake Horowhenua were lower than expected for diadromous fish but are also not consistent with long-established lacustrine populations. Gill raker counts also indicate that the population is may have originally been diadromous. However, analysis of otolith microchemistry was definitive, with no evidence of marine life-stages in any of the samples analysed. Therefore, we conclude that the common smelt population in Lake Horowhenua is predominantly lacustrine and that marine migrations are likely prevented by the weir on the Hokio Stream outlet. As the weir on the Hokio Stream likely acts as a barrier to native species with poor climbing ability such as common bully, grey mullet and common smelt its removal or construction of a fish pass could improve the diversity of the lake's fish fauna. As vertebral count distribution is atypical of populations previously designated as diadromous or lacustrine, the population may be worthy of further investigation to determine if it exhibits distinctive morphological or genetic characteristics.

Acknowledgements

We would like to thank Brendan Hicks and Nicholas Ling for reviewing this report. Joshua de Villiers performed the analysis of the vertebrae counts. Warrick Powrie, Steve Woods and Joshua de Villiers are acknowledged for their help in collection of the smelt specimens.

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