



Validation of Relationships between Ambient Trace Element concentrations and otolith Microchemistry in Trout

A research report prepared for NZ Fish & Game Council and Research committee.

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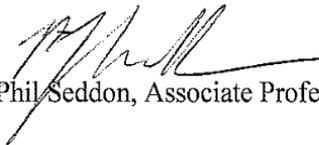
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Introduction

Habitat protection has been an essential component of freshwater fisheries management in New Zealand for a long time. One of the most challenging aspects of managing wild fisheries is identifying what relative contribution different spawning and juvenile rearing habitats actually make to a particular population. When available, such information greatly assists fisheries managers in understanding population connectivity and can identify critical habitats for reproduction and growth of wild sports fish populations. It also enables them to target conservation efforts toward those rivers, sections of rivers, and tributaries that contribute the most to the wider fishery. Given the recent resurgence of hydro development, increasing irrigation demands and intensification of agriculture this ability is a critical component of any management efforts to ensure the future of sustainable populations of sports fish for recreational angling. Yet until recently such information has required large scale tagging, trapping and/or radio tracking programmes, which are often very costly and resource demanding and generally involve several years of commitment to show trends.

Fish ear stones (otoliths) have historically been used mainly to study age structure and growth rates of fish populations. But advances in laser technology have now made chemical analysis of otolith material a more cost effective option that offers fisheries managers and scientists a new tool for assessing the relative contribution of different nursery streams to the overall recruitment and highlight what dispersal and migration dynamics occur in a wide variety of sports fish populations. This is possible because otoliths are incrementally formed as discrete metabolically inert layers of material throughout the life of the fish. As a result these small ear stones incorporate the various isotopes and elements in proportion to their ambient abundance in the aquatic environment that fish inhabit. These act like a “black box” of sorts for what chemical environment the fish has experienced during various times of its life. This allows researchers to use geochemical trace element or isotopic signatures within otoliths as natural tags that reflect the environmental history of a fish (Campana, 1999). When natal geochemical signatures are unique and distinct at appropriate geographical scales,

natural chemical tags can be used to identify the natal origin, movement patterns and specific events (*i.e.* the timing and extent of ocean migration *etc.*) in individual fish as well as at a population level. Geochemical otolith analysis therefore has great potential to resolve several other outstanding questions about fish populations, and as a result many fishery scientists today consider there is almost no other biological structure more important than otolith because of the information they contain.

The first step in these types of studies is often to create a base map of trace elements and/or isotope signatures to discriminate among potential recruitment sources. Normally this involves collecting juveniles during early summer (10-15/site), before they begin to disperse, to characterize mean otolith geochemical signature of resident 2-4 month old fish in all likely spawning streams. Using these chemical tags as a reference, the natal origin and life history movement patterns in a random sample of adults of varying ages (from the population of interest) are then assessed, and interpretations about population connectivity, and recruitment contribution, *etc.* are made. Usually such assessments will rely heavily on multivariate statistical techniques such as discriminate function analysis (DFA) to assign group memberships and classify the natal origin of unknown adults. It is important to recognise that this approach assumes that firstly the sample of juveniles taken is representative for all the likely contributions from that tributary, and secondly that site signatures are indeed a truly unique and reproducible marker, something that has seldom been confirmed by sampling over longer time periods.

To minimise costs most studies to date have relied on a range of trace element: calcium (Ca) ratios to discriminate between different natal habitats rather than using isotope analysis. But whilst such analyses thus far have generally yielded biologically plausible migration scenarios, detailed understanding of the relationships between ambient environmental element concentrations and the concentration of elements observed in otoliths are poorly understood. Clearly, some elements such as strontium (Sr) correlate closely with environmental concentrations, whereas others including barium

(Ba), manganese (Mn) and magnesium (Mg) often exhibit inconsistent and sometimes puzzling patterns. Consequently although otolith microchemical analysis clearly yields useful information on migration and recruitment, the uncertainties associated with element uptake and incorporation into otolith means that such analyses are potentially open to challenge in legal arenas such as the Environment Court or Water Conservation Order Hearings. The current challenge for scientists is to continue to develop the methodology and appropriate technologies to extract and analyse relevant and reliable information from otoliths.

A review of what influences otolith chemistry in numerous species of fish has indicated that uptake and incorporation rates of various geochemical markers into otolith material is influenced by multiple factors, including fish age, temperature, nutrition and concentrations of other trace elements (see Elsdon *et al.* 2008). Rates of uptake also vary between different elements. This means the exact time required for multiple element signatures to create a unique natural tag that is representative of a particular river reach, is unknown. To resolve some of the uncertainties and develop the technology as a more robust method for tracking migration, Otago University was contracted to design and carry out an experimental validation program aimed at furthering the understanding of the uptake and incorporation of trace elements into trout otoliths. The key focus of the validation program was to investigate (i) the reliability of various elements for tracking migration, (ii) the effect of temperature on the uptake of different elements, (iii) the influence of food source on element uptake, and (iv) an overall assessment on how to best apply this new technology.

This report was undertaken as a part of a PhD research project and aims to communicate the current understanding of how otolith microchemistry is influenced by multiple factors. While data analysis is still ongoing, the report provides an up to date summary based on a series of validation experiments designed specifically to provide an insight into the strengths, weaknesses and appropriate applications of this new tool.

Validation experiments

A series of validation experiments was conducted to investigate some of the uncertainty around the strengths and limitations of otolith microchemistry as a research tool. The sections below briefly outline how three of those validation experiments were designed and carried out. In order to better provide an overview of the separate influences tested the methods have been separated into distinct categories as follows:

Temporal and spatial variability in otolith trace element signatures

We monitored the annual variability in the mean element signature, for the most commonly used elements in otolith studies, over a three and five year period in two nursery streams, the Luggate and Lindis Rivers in Central Otago. In addition we also explored to what degree element signatures change over the length of a stream, by collecting samples from the lower, mid and upper reaches of the Lindis River during one season. Samples of age 0+ brown trout (n=10/site) were collected at the same time of year (Dec/Jan) and from the same stretch of river, annually and only included young of the year (YOY) trout. This was done to ensure that trout collected were of similar age and had not yet had much opportunity to start dispersing within each river and/or river section. Finally we also examined the long-term stability of mean element and Sr isotope signatures for these rivers, as well as changes in a lake system with a longer hydraulic residency time.

Impact of temperature and salinity on otolith signature

To investigate the effect of temperature and/or salinity on the uptake of a range of commonly used elements, fish were reared in controlled temperature tanks at two different temperature treatments (14 °C and 18 °C) at six different salinity levels. This provided for four replica tanks per treatment for each of the six treatments, which had an approximate salinity of 2, 3, 5, 10, 15 and 33 ppt. Brown trout had a mean size of 133.9 mm (range 120 – 150 mm), and were acclimatised to a common feeding regime in holding tanks before being randomly allocated between identical 32L treatment tanks. Salinities were gradually adjusted by combining different volumes of spring water with sea water from the Portobello marine station seawater filtration system during a three

week period. All fish were fed freeze dried bloodworms to satiation, food waste was removed manually by siphoning and approximately 1/5 of the tank volume was changed each day with matching water of the same temperature and salinity. After the three-week acclimation period the experiment ran for a further 36 days.

Impact of diet

To investigate if and to what degree diet can influence otolith element concentrations, fish were held in both fresh and salt water and fed either a freshwater (freeze dried bloodworms) or a marine diet (a combination of brine shrimp and whitebait). Fish were randomly allocated between fresh and saltwater treatments, reared in 200L tanks, with three replicates per water/diet treatment. In light of the diet types used and to minimize mortality rates in the high salinity treatment we chose slightly larger fish for this experiment (mean size 190.5 mm, range 170 – 223 mm) and increased salinity in a stepwise fashion during a six instead of three week period while also slowly phasing in the marine food type where applicable. Fish were fed a surplus of food, waste removed via manual siphoning and approximately 1/5 of the tank volume was changed each day with matching water of the same temperature and salinity. By week four most fish in all treatments were feeding actively. After the six-week acclimation period the diet experiment was run for a further three weeks, after which all fish were euthanized and stored frozen until otoliths were removed and mounted for analysis. The exact time required for otolith microchemistry to reach equilibrium with ambient element concentrations is species-dependent (Zimmerman 2003), and differs between elements (Campana 1999). Nevertheless, we assumed that 20+ days of experimental exposure would be sufficient for brown trout to both reach equilibrium with ambient water chemistry and provide adequate amounts of otolith material for analysis.

Otolith preparation and chemical/statistical analysis techniques

Otoliths were extracted and preserved dry until they were prepared for analysis according to slightly modified methods of Barnett-Johnson *et al.* (2008). To remove biological tissues, otoliths were first cleaned ultrasonically in ultrapure filtered water

(18m Ω), and then soaked in 10% H₂O₂ and rewashed in ultra-pure filtered water before being air dried. All equipment used was soaked in 10% HCl overnight and rinsed with ultrapure filtered water (18 m Ω) before use and between samples.

Otolith microchemistry was determined using a Varian Inductively Coupled Plasma Mass Spectrometer (ICPMS) fitted with a HelEx (Laurin Technic and the Australian National University) laser ablation (LA) system. Prior to ablating each otolith background counts of elements in the carrier gas were measured for thirty seconds. A slit size of approximately 30 μ m by 60 μ m was then aimed at the outer edge of otoliths, and after initial pre-ablation the following element concentrations were initially analysed in each otolith: Sr, Ba, Mg, Mn, Ca as well as lithium (Li), boron (B), aluminum (Al), phosphorus (P), sulfur (S), potassium (K), copper (Cu), zinc (Zn), rubidium (Rb), and lead (Pb). For the purpose of this report only data on the five most commonly used elements in otolith studies (Li, Mg, Mn, Sr and Ba) are presented as element: Ca ratios. All the data was processed offline using a purpose built MS Excel spreadsheet and involved a low pass filter, smoothing and a blank subtracting function (Barbee and Swearer, 2007).

Results

Temporal Variability in Otolith Element Signatures in River and Lake Systems.

Over a three and five year period all otolith element concentrations except Sr varied significantly between at least some of the years in both rivers (Figure 1 and 2). Within stream differences probably reflect a combination of changes in ambient water chemistry, as a result of differences in rainfall and air/water temperatures and weathering processes between years; and the range of physiological processes that can influence element uptake into the otolith matrix. While the exact physiological processes that govern element uptake into the otolith remain largely unknown, clearly some elements vary more than others. It is encouraging that Sr, which tends to be the dominant driver in differentiating between natal areas, remains largely stable over time.

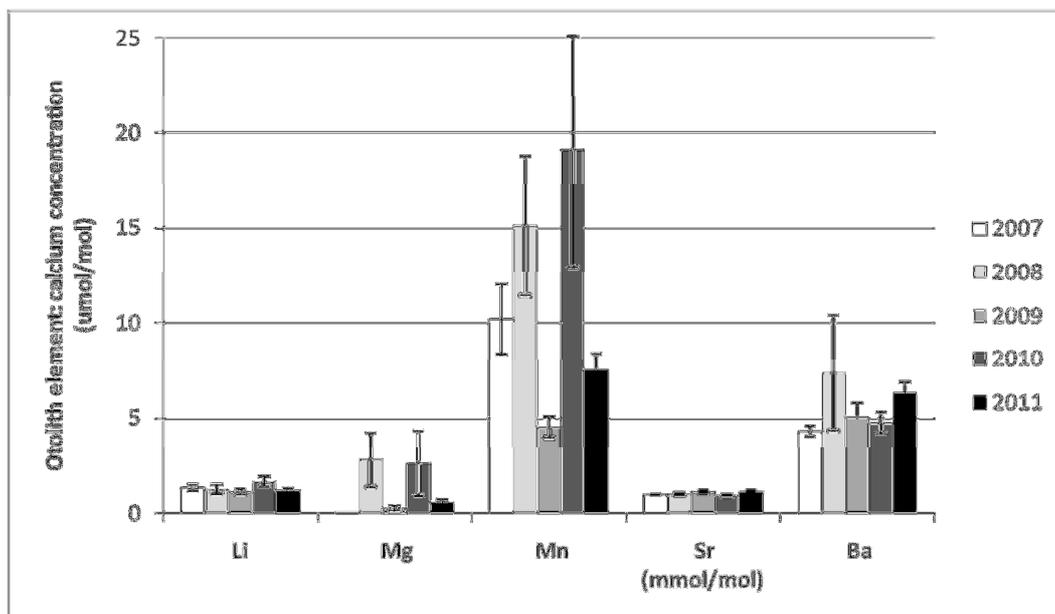


Figure 1. Mean (\pm SE) otolith element: calcium concentration for age-0 brown trout from the Luggate River collected between 2007-2011. Significant differences between at least some of the years were found for all elements except Sr (ANOVA, $p > 0.05$). Note that for some years/elements the standard errors are too small to display properly in the graphed area.

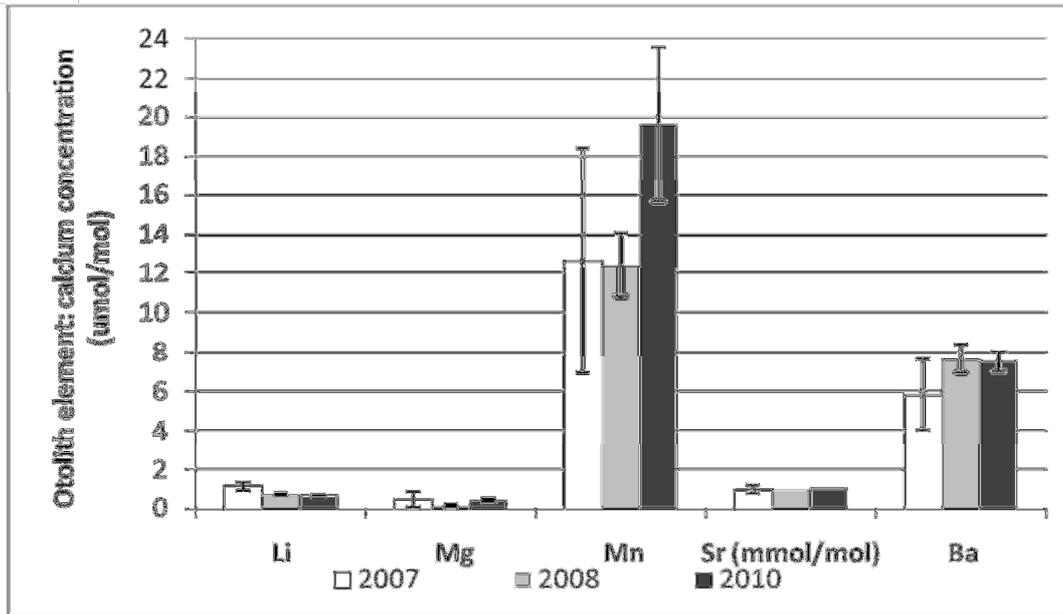


Figure 2. Mean (\pm SE) otolith element: calcium concentration for age-0 brown trout from the Lindis River collected between 2007-2010. Significant differences between at least some of the years were found for all elements except Sr and Ba (ANOVA, $p > 0.05$). Note that for some years/elements the standard errors are too small to display properly in the graphed area.

The large within year variability (as indicated by the errorbars) for elements like Mn also suggests that at least some elements that have been used to differentiate between rivers in previous studies are highly variable both between and within years. Wells *et al.* (2003) suggested that such variability could suggest uptake of such elements is governed by biological fractionation which could explain why the stream signature is not necessarily equally incorporated by all fish, even if they are of the same age group and have been exposed to the same environmental conditions.

There was a strong relationship between river length and cumulative element loading, resulting in higher concentrations for most elements in downstream sites (Figure 3). Depending on the elements that are monitored, and the geological variability in the tributary/river of interest, two or even three sampling sites may be required to accurately describe the mean element signature of longer nursery streams. We conclude that when added together interannual and longitudinal variability in element concentrations have the

potential to considerably decrease successful discrimination of natal origins if studies only rely on one year of sampling to characterise stream signatures.

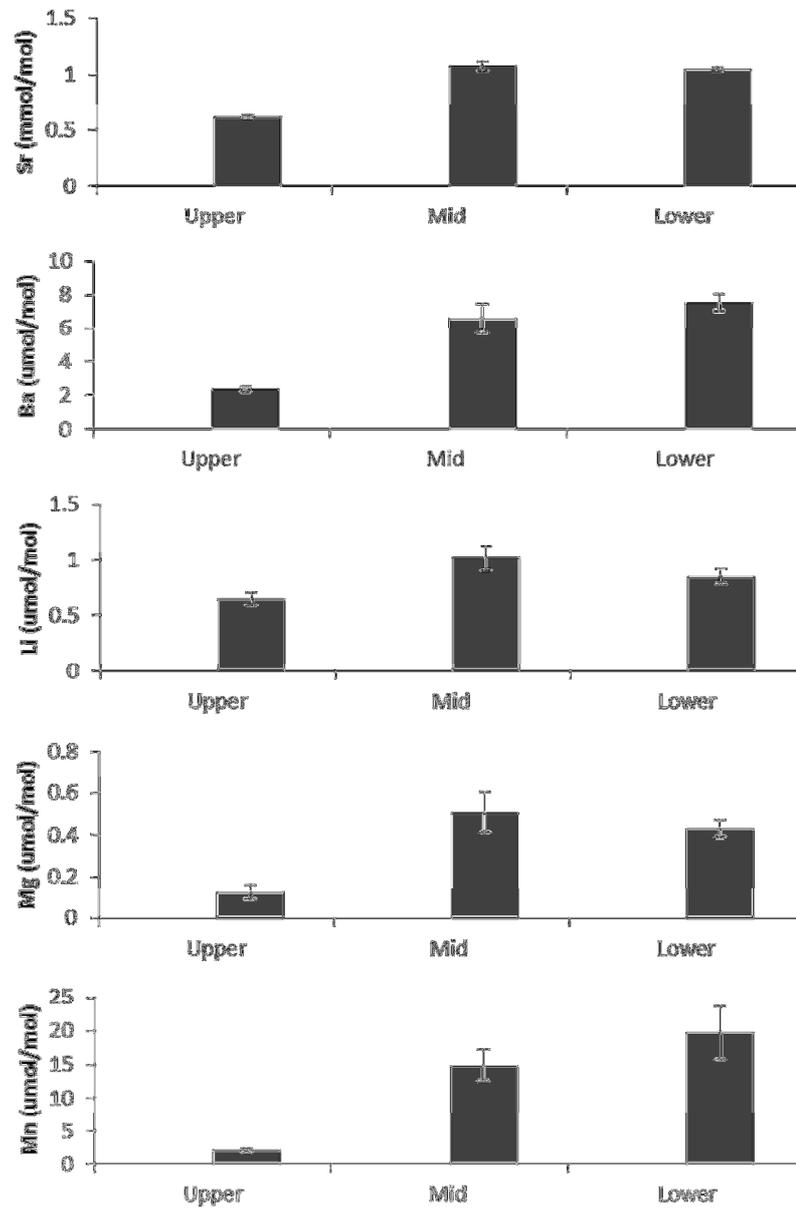


Figure 3. Longitudinal variation in mean (\pm SE) otolith element: calcium concentration for age-0 brown trout at three sites in the Lindis River. Samples were all collected in 2010, and significant differences between at least some of the sites were found for all elements (ANOVA, $p > 0.05$).

Larger bodies of water such as lakes, and to some degree larger lake-fed rivers like the Clutha River, should in theory have more spatially and temporally stable element concentrations as longer hydraulic residency times and larger catchments will buffer temporal fluctuation. To test this theory we compared element signatures from the most recently laid down otolith material (< one month) in adult brown trout captured in Lake Wakatipu in 1985/86 (n=7) and in 2009/10 (n=7). While the overall sample size for each group was relatively small, results indicate that except for Mg, the mean otolith element signature had not changed significantly between the two sampling occasions despite being 25 years apart (Figure 4). These results strongly suggest that systems with longer hydraulic residency times have relatively stable element concentrations over time. Nevertheless, our previous results from monitoring smaller river systems, with shorter hydraulic residence times such as typical spawning streams, supports the conclusion by other researchers (Gillanders, 2002 and Schaffler & Winkelman, 2008) that temporal variability in otolith element signatures should ideally be examined for all systems.

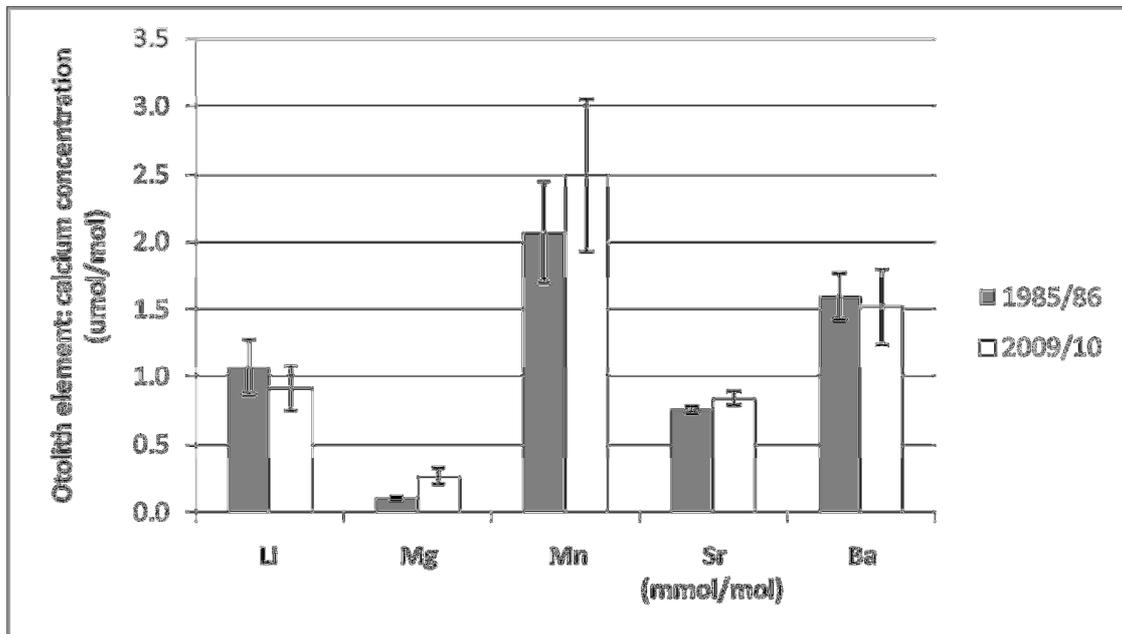


Fig 4. Mean (\pm SE) otolith element: calcium concentration for a sample of adult Brown trout (n=14) from Lake Wakatipu (all fish ranged between 290-510 mm). Results indicated that out of the five commonly used element concentrations tested only Mg was significantly different (ANOVA, $p > 0.05$) in samples taken 25 years apart, white bars = 2009/10, grey bars=1985/86 sampling season.

Sr isotopic analysis has increasingly been used to complement or as an alternative method to otolith element signatures. Therefore, as a comparison we also verified the temporal stability of this approach. As expected, it confirmed that the mean isotopic signature was very stable over the sampling period and clearly differentiated between the streams (Figure 5). This suggests that despite being more expensive, and only providing one parameter for separating streams, Sr isotopic analysis appears to be complementary and/or an alternative to multi-element signatures.

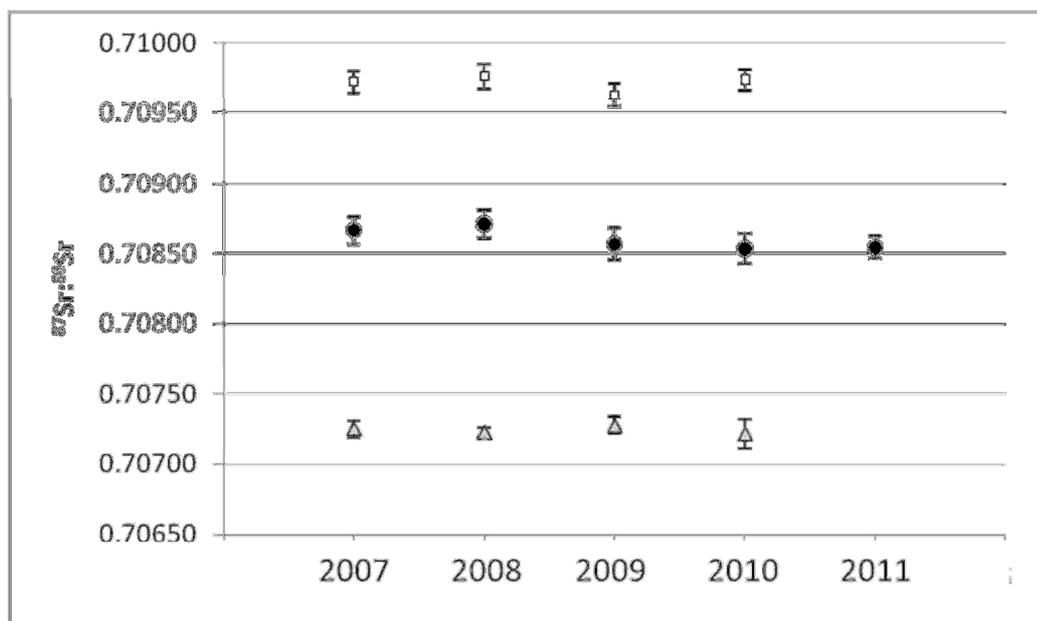


Figure 5. Mean Sr isotope ratio ($^{87}\text{Sr}:^{86}\text{Sr}$) in the outer sections of otoliths in juvenile trout from the Lindis River (open squares), Luggate Creek (solid circles), and adult trout from Lake Wakatipu (grey triangles). Note: Error bars equal ± 2 SE.

Impacts of salinity and temperature on otolith chemistry

While temperature alone did not result in statistically significant alteration of otolith element signatures (Figure 6), we did detect a significant interactive effect between temperature and salinity on otolith chemistry (Figure 7). Unfortunately the higher temperature/salinity treatment resulted in 100% mortality before sufficient material had been deposited (Figure 7). However results for the lower and medium salinity levels highlight the need to consider interactive effects when interpreting fish movement across salinity gradients. Elsdon & Gillanders (2002) highlight that coastal zones, such as river mouths/estuaries and coastal bays, are likely to vary in both temperature and salinity simultaneously. Thus, any attempts at reconstructing migratory patterns for adult trout in such habitats based solely on the salinity effects on otolith microchemistry, without taking into account possible interactions of salinity with temperature, could result in incorrect interpretations of fish migratory patterns.

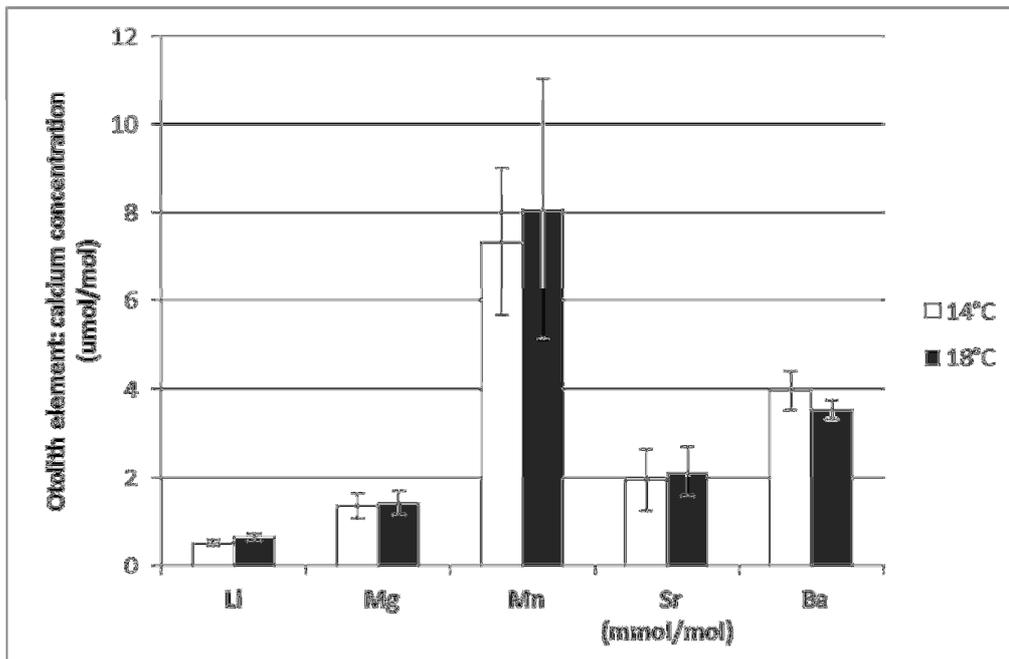


Figure 6. Mean otolith element: calcium concentration concentrations (\pm SE) for Brown trout reared in different temperature regimes (14 and 18 °C) and under low salinity (2‰) conditions.

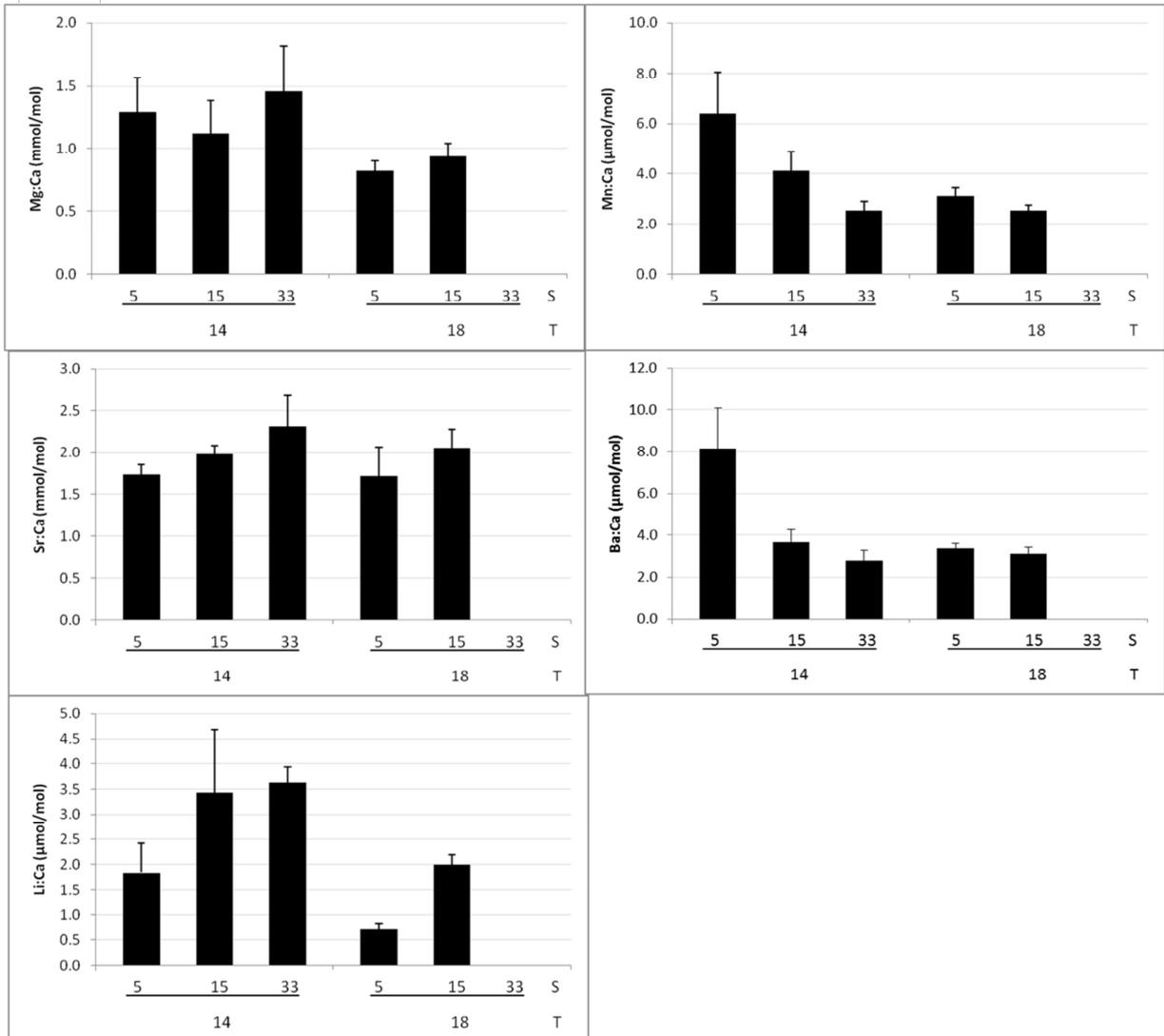


Figure 7. Mean concentrations (\pm SE) of (a) Li, (b) Mn, (c) Mg, (d) Sr and (e) Ba in otoliths of Brown trout reared under experimental treatments of temperature and salinity (two way manipulations). S, salinity of rearing water in ‰. T, temperature of rearing water in °C.

Previous studies that have found temperature impacts on otolith signatures usually separated treatments by $>6^{\circ}\text{C}$, and suggest that biological factors such kinetics may be the underlying reasons why temperature effects on otolith chemistry are sometimes detected (Kalish 1989). Thus, despite us not finding a significant temperature impact, it is important to recognise that temperature does have the potential to speed up the rate that some elements are incorporated. Therefore it is wise to routinely consider if, and to what extent, temperature and/or interactive effects between temperature and salinity are

present when reconstructing migratory patterns. Not doing so may result in incorrect interpretations of fish migratory patterns.

Impact of diet on otolith chemistry

Otolith elemental signatures changed predictably as diet changed from a freshwater to marine derived food source. Marine diet and increased salinity significantly increased otolith Sr and Li concentrations, decreased both Ba and Mn levels, while Mg concentrations remained relatively unchanged in response to the treatment (Figure 8). Interestingly Li levels in otoliths remained relatively unaffected by diet during the freshwater treatment, but increased significantly for fish fed a salty diet while being reared in a saltwater environment. It is possible that this indicates a biological interaction altering how Li is taken up as fish move across salinity gradients. Similar biological activity has been detected for Rb (Hicks *et al.* 2010). Such findings further suggest that some elements are impacted by biological interactions, and clearly this aspect requires further investigation. It also appears that diet has an increased influence on otolith element concentrations when ambient concentrations are low.

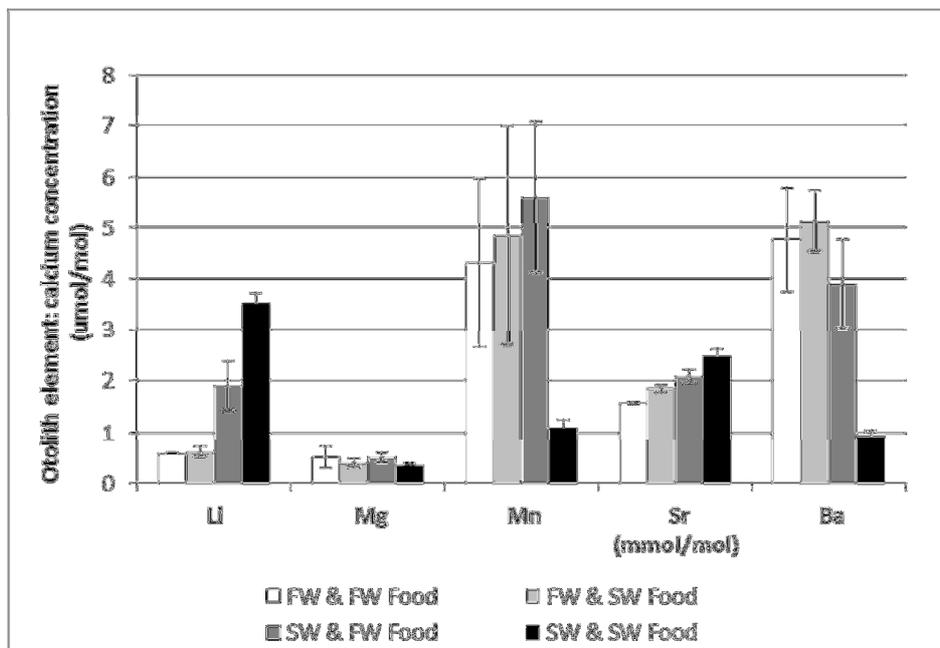


Fig 8. Mean (\pm SE) otolith element: calcium concentration for Brown trout reared under different environmental and dietary conditions.

Regardless of how the uptake actually occurs, both Li and Rb could be useful markers for fish in or around estuarine environments. Saltwater contains high Sr but low Ba and Li levels and the combined response of these three elements could be used to guide interpretations around true ocean migrations, or if a fish is more likely to have been feeding on marine prey in or upstream of an estuarine environment. Finally it also offers the opportunity to explore the degree to which seasonal food sources such as whitebait contribute to supporting growth rates in upriver fisheries. Theoretically a fish that never actually visited the estuary/marine environment can still be largely dependent on such seasonal food sources to recover from spawning or achieve observed growth rates. In such cases the combined use of these elements will greatly assist interpretations aimed at identifying habitat linkages and connectivity helping managers and researchers understand the role these habitats and food sources play at a population level.

Discussion

Although it would be ideal if trace element uptake into otoliths were completely passive and directly proportional to ambient concentrations, this has not been found to be true for any element (Hicks *et al.* 2010). Instead our study indicates that temporal variability, dietary influences and physiological regulation influence trace element signatures and will need to be accounted for to varying degrees. In light of our results, we expand on some of the implications, and outline adjustments to study design and data interpretations that we recommend. Providing these are adopted, and/or the strength of inference drawn when interpreting recruitment, and migration patterns are adjusted appropriately, otolith microchemical analysis remains an extremely valuable tool for studying movement and life-history characteristics of freshwater fish.

Accounting for temporal and spatial variation in stream element signatures

Our investigation of inter-annual variability and spatial variation in otolith element signatures for YOY brown trout in typical spawning streams raises several concerns. First of all the results suggests most elements used to date are likely to vary

significantly between years. Secondly it highlights that a significant spatial effect on element ratios could require multiple samples to adequately characterise the overall contribution of that stream. These results support previous studies (Gillanders 2002, Schaffler & Winkelman 2008) that have also found significant inter-annual variation within otolith element signatures that occur in fish from resampled locations. Taken together this suggests that significant temporal variability should be expected for some elements in most geological regions. This means that researchers relying on multiple element: calcium signatures to discriminate between natal sources, cannot assume that one year of sampling will be sufficient to accurately classify unknown adults from multiple year classes. Like many others (Gillanders 2002, Elsdon *et al.* 2008, Schaffler and Winkelman 2008, Walther and Thorrold 2009) we therefore recommend to match cohorts of juvenile stream signatures with the correct age cohort of adults whenever possible. However, we appreciate that this may not always be practical or even possible due to time constraints, or simply the lack of juvenile otoliths from all cohorts of interest. In such cases our results suggest an alternative would be to rely on temporally more stable signatures, such as Sr isotope ratios.

At the present it is still unclear what ultimately drives inter-annual variation in otolith element concentrations for YOY trout. Our experiments indicated that water temperature alone did not appear to be the primary cause. Schafer and Winkelman (2008) suggested that differences in diet will impact otolith signatures in general within and among years (see Limburg 1995; Gallahar and Kingsford 1996; Buckel *et al.* 2004 for details). But while we did not specifically test if diet composition varied between years, we consider it unlikely diet played a major role for YOY trout. Diet will however clearly impact adult trout, who have a much more diverse diet, as our diet experiment illustrated. Instead within stream differences in YOY signatures are likely to reflect changes in the ambient water chemistry, which is the result of differences in rainfall and air/water temperature and weathering processes between years in each stream catchment. While the exact processes that govern element uptake into otolith remain largely unknown, it is likely to be a product of changes in ambient water chemistry and physiological

processes in fish. The large within year variability for some elements also suggest that uptake is governed by biological fractionation and will not necessarily be taken up equally by all fish, even if they are of the same age group and have been exposed to the same environmental conditions.

Some studies have tried to account for temporal variability to a degree by relying mainly on the more stable elements like Sr, and pooling all available cohort signatures to account (as far as possible) for the range of concentrations likely to be found. Olley *et al.* (2011) used this method to explore temporal influences by pooling three sampling occasions over two years. While such an approach is certainly preferable over-reliance on one sampling event, it should be recognised that temporal variability has the potential to considerably decrease overall classification accuracy and increase the uncertainty around data interpretation. Considering that adult brown included in otolith studies often range from 3-10 years in age it is logical to expect that temporal variability will influence classification success to some degree. Consequently best practice is to limit the scope of the analysis to matching cohorts regardless of the elements used.

Fisheries managers and researchers should take a pragmatic and long-term approach and design studies and sample collection, in light of the limitations we have raised. It is recommended that for catchments where recruitment and migration studies are likely to be used to inform future fisheries management decisions, and/or environmental impact assessments, small scale sample collection (>15 juveniles/tributary) should be done annually from all known recruitment sources. It is also advisable to initially limit the age range of adult fish included in these types of studies, for instance by focusing on the 200-400 mm size range it should be possible to ensure that \leq three-five years of signature data will suffice to classify all unknown adults collected. In addition, because we found evidence that stream element signatures can also change considerably along a spawning stream, basic information on the longitudinal distribution of the spawning effort will greatly assist the selection of appropriate sampling sites and help ensure that all major recruitment sources are characterised. The value of such information might even justify the costs of including an aerial spawning surveys for

larger catchments. However if such information is not available, although less desirable, statistical techniques that identify uncharacterised groups could instead be used to explore additional unclassified natal sources (Elsdon *et al.* 2008).

In practical terms we believe all the above adjustments are easily achievable as most Fish & Game regions already routinely conduct at least some spawning surveys and electric fishing monitoring in fisheries that are candidates for these types of studies. Hence, after some initial prioritisation and appropriate consultation regarding sampling design, it should not require much extra effort or cost to annually collect samples for a five-year period, including a sample of adults at the end of the sampling period. Once completed such otolith banks could easily be stored, and even added to, until required. Such records are likely to provide valuable comparisons between historical and present recruitment patterns, which could be used to explore the impacts that land-use and/or other instream habitat or flow changes are likely to have had on fish populations of interest.

Should there be a need to “fast track” a study, which is sometimes the situation when fisheries managers are required to provide evidence at an Environment court hearing, we recommend to utilise Sr isotope ratios ($^{86}\text{Sr}/^{87}\text{Sr}$) which have indicated to be very stable over time (Figure 4, Kennedy *et al.* 2000). Employing Sr isotope ratios also eliminates the influence of factors such as temperature and salinity (Radtke and Shafer 1992) or biological factors that can alter element concentrations (Friedland *et al.* 1998). However, the analysis is generally more costly but that may to some degree be compensated for by the shorter sampling time required. Our investigation has revealed that Sr isotopes are both temporally stable and do vary at small spatial scales making them complementary or an alternative to the more traditionally used element:Ca ratios. The fact that they are spatially diverse (Gabrielsson & Closs unpublished data) make them a particularly useful marker for geologically homogenous regions where traditional Sr:Ca and/or multi element ratios struggle to differentiate between rivers on the scale required to inform fisheries management. Furthermore, Sr isotopes also provide a reliable method of retrospectively assessing the historical recruitment sources from a sample of

adult brown trout captured in Lake Brunner in the early to mid 1990s. This was prior to large-scale dairy farming development in the catchment (Gabrielsson & Closs 2009). Such historical comparisons can be extremely valuable as a benchmark and provide a powerful assessments of changes in recruitment patterns over time.

Summary

As this report has shown, considerable progress has been made in the understanding of how geochemical markers are deposited in otoliths over the past ten years. Continued debate around study design, data analysis and the various ways of interpreting results is important and will ensure that the field keeps moving forward based on science that is solid and constructive. Despite this continued need for development, it is clear that otolith microchemistry has proven to be a powerful and useful tool for studying and reconstructing life histories of fish. The potential applications include accurate predictions of recruitment and migratory patterns and will be useful for fisheries managers and researchers alike. In summary as successful and sustainable fisheries management often relies on the identification and protection of key spawning and rearing habitats we hope this report will assist Fish & Game managers and Councils in understanding how to best use this new powerful tool to fill information gaps and assist with prioritising future environmental protection and rehabilitation work.

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References

Barbee, N.C., Swearer, S.E., 2007. Characterizing natal source population signatures in the diadromous fish, *Galaxias maculatus*, using embryonic otolith chemistry. *Mar. Ecol. Prog. Ser.* 343, 273-282.

Bukel, J.A., Sharack, B.L., Zdanowicz, V.S., 2004. Effects of diet on otolith composition in an estuarine piscivore. *J. Fish. Biol.* 64:1469-1484.

Elsdon, T.S., Gillanders, B.M., 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Can. J. Aquat. Sci.* 59: 1796-1808.

Elsdon T., Wells B., Campana S., Gillanders B., Jones C., Limburg K., Secor D., Thorrold S., Walther, B. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. *Oceanography and Marine Biology: An Annual Review*, 46, 297-330.

Freidland, K.D., Reddin, D.G., Shimizu, N., Haas, R.E., Youngson, A.F. 1998. Strontium: calcium ratios in Atlantic salmon (*Salmo salar*) otoliths and observations on growth and maturation. *Can. J. Aquat. Sci.* 55: 1158-1168.

Gabrielsson, R. & Closs, G.P. 2009. Using otolith microchemistry to track recruitment and migration patterns in the Lake Brunner fishery. *A research report for NZ Fish & Game Research Committee and West Coast Fish & Game Council.*

Gallahar, N.K., and Kingsford, M.J. 1996. Factors influencing Sr/Ca ratios in otolith of *Girella elevata*: an experimental investigation. *Journal of Fish Biology* 48: 174-186.

Gillanders, B.M. 2002. Temporal and spatial variability in elemental composition of otoliths: implications for determining stock identity and connectivity of populations. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 669-679.

Hick, A.S., Closs, G.P., Swearer, S.E. 2010. Otolith microchemistry of two amphidromous galaxiids across an experimental salinity gradient: A multi-element approach for tracking diadromous migrations. *Journal of Experimental Marine Biology and Ecology* 394: 86-97.

Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology* 132: 151-178.

Kennedy, B.P., Blum, J.D., Folt, C.L., Nislow, K.H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. *Can. J. Aquat. Sci.* 57: 2280-2292.

Limburg, K.E. 1995. Otolith strontium traces environmental histories of sub yearling American shad *Alosa sapidissima*. *Mar. Ecol. Prog. Ser.* 119: 25-35.

Olley, R., Young, R.G., Closs, G.P., Kristensen, E.A., Bickel, T.O., Deans, N.A., Davey, L.N., Eggins, S.M. 2011. Recruitment sources of brown trout identified by otolith trace element signatures. *New Zealand Journal of Marine and Freshwater Research* 45(3): 395-411.

Radtke, R.L., and Shafer, D.J. 1992. Environmental sensitivity of fish otolith microchemistry. *Aust. J. Mar. Freshwater Res.* 43: 935-951.



Schaffler, J.J. and Winkelman, D.L., 2008. Temporal and spatial variability in otolith trace element signatures of juvenile striped bass from spawning locations in Lake Texacoma, Oklahoma-Texas. *Transactions of the American Fisheries Society* 137: 818-829.

Walther, B.D., Throld, S.R. 2009. Inter-annual variability in isotope and element ratios recorded in otoliths of an anadromous fish. *Journal of Geochemical Exploration* 102: 181-186.

Wells, B.K., Rieman, B.E., Clayton, J.L., Horan, D.L., Jones, C.M. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout for the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. *Transactions of the American Fisheries Society* 132: 409-424.

Zimmerman, C.E., 2003. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. *Can. J. Aquat. Sci.* 62: 88-97.