This is the author-created version of the following work:

Disspain, Morgan C.F., Ulm, Sean, Izzo, Christopher, and Gillanders, Bronwyn M. (2016) *Do fish remains provide reliable palaeoenvironmental records? An examination of the effects of cooking on the morphology and chemistry of fish otoliths, vertebrae and scales.* **Journal of Archaeological Science, 74 pp. 45-59.**

Access to this file is available from:

Please refer to the original source for the final version of this work:

http://dx.doi.org/10.1016/j.jas.2016.08.010

ResearchOnline@JCU

JAMES COOK
UNIVERSITY
AUSTRALIA

Do Fish Remains Provide Reliable Palaeoenvironmental Records? An Examination of the Effects of Cooking on the Morphology and Chemistry of Fish Otoliths, Vertebrae and Scales

Morgan C. F. Disspain[a*], Sean Ulm[b], Christopher Izzo[a] and Bronwyn M. Gillanders[a]

[a] Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, SA 5005, Australia

[b] College of Arts, Society and Education, James Cook University, PO Box 6811, Cairns, QLD 4870, Australia

* Corresponding author

Abstract

The morphological and chemical properties of fish calcified structures provide excellent environmental and anthropogenic proxies; however, pre-depositional handling may alter these properties, confounding interpretations. This study examines the effects of some traditional processing and cooking methods on the morphological and chemical properties of modern fish otoliths (ear bones), vertebrae, and scales using an experimental approach. Whole mulloway (*Argyrosomus japonicus*) were treated using a range of techniques, including boiled in freshwater and saltwater; roasted directly on a fire and wrapped in clay; salted; and completely burnt. Samples were also obtained from untreated fish as controls for comparison. Otoliths, vertebrae and scales from the samples were subjected to morphological, trace element (^{7}Li, ^{23}Na, ^{24}Mg, ^{55}Mn, ^{86}Sr, ^{138}Ba, ^{208}Pb, and ^{65}Zn all ratioed to ^{43}Ca) and stable isotope analyses (otoliths and vertebrae–inorganic δ^{13}C and δ^{18}O; scales–organic δ^{13}C and δ^{15}N). Results reveal disparities in the chemistry and morphology of otoliths and vertebrae processed in different ways. The otolith and vertebrae carbonate δ^{18}O values were lower in samples that experienced heating; burnt samples differed significantly from the control samples. Otolith and vertebrae trace elements were largely unaffected by the treatments relative to the controls; however, some individual elements within the burning and salting groups varied significantly. The impacts observed in the fish scales were less substantial. Results provide a basis for evaluating the suitability of archaeological samples for analysis. We recommend avoiding the use of heated samples. Findings highlight the need to conduct palaeoenvironmental reconstructions based on chemistry and stable isotope data of archaeological remains with caution.

1. Introduction

Chemical and isotopic analyses of archaeological fish remains are increasingly used in palaeoenvironmental studies, drawing heavily on methods developed in modern fisheries science (e.g., Disspain et al. 2011; Disspain et al. 2012; Long et al. 2014; Wang et al. 2011, 2013). Numerous researchers have examined how ambient environmental variables influence the chemistry of calcified structures, specifically otoliths because of their unique attributes (see Disspain et al. 2016; Elsdon et al. 2008), using such relations to interpret environmental histories of modern fish. The most widely investigated trace elements have been Sr:Ca and Ba:Ca, which provide information on environmental parameters (Bath et al. 2000; Elsdon and Gillanders 2005a; Elsdon and Gillanders 2005b; Gillanders and Munro 2012; Hamer et al. 2006). Alternate elements, including Mn (Elsdon and Gillanders 2003) and Mg (Arkhipkin et al. 2009; Wells et al. 2003), among others (Campana et al. 2000; Morales-Nin et al. 2012; Tanner et al. 2013) have also been studied for their suitability as environmental indicators, with varying results.

Stable isotope analysis of fish remains is more widely used and validated than trace element analysis within palaeoenvironmental studies (Disspain et al. 2016). Remains can be analysed for oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) of the inorganic fraction (otolith aragonite and bone apatite) (Wang et al. 2013; West et al. 2012; Zazzo et al. 2006). Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopes from the organic fraction (fish scales and bone collagen) are frequently analysed (Barrett et al. 2011; Robson et al. 2012; Rowell et al. 2010), with others such as sulphur ($\delta^{34}S$), being investigated for suitability as an environmental indicator (Privat et al. 2007). Oxygen isotopes primarily reflect water temperature (Rowell et al. 2008; West et al. 2012), $\delta^{13}C$ in fish remains is a mixture of carbon derived from ambient dissolved inorganic carbon (DIC) and that derived from diet (metabolic carbon) (Kalish 1991), while $\delta^{15}N$ ratios are commonly used to determine trophic level (Rowell et al. 2010). Isotopic analyses of ancient fish remains can provide information on trade and provenance of fish (Dufour et al. 2007; Ishimaru et al. 2011; Orton et al. 2011), seasonality of site usage (Colaninno 2012; Hufthammer et al. 2010), palaeoenvironmental conditions (Surge and Walker 2005; Wang et al. 2013; West et al. 2012), fish migrations, and the effects of human predation and habitat alteration on fish populations (Rowell et al. 2010; Rowell et al. 2008).

Despite the vast array of information gleaned from elemental and isotopic analyses of archaeological fish remains, few studies have attempted to determine the effects of pre-depositional processes. Taphonomic processes include human and non-human agents, with many archaeological remains likely to have been exposed to both effects. Of the human impacts, cooking and processing for consumption are primary agents of taphonomic transformations of faunal remains, and can be evidenced by butchery/cut marks, colour changes, crystallization, skeletal element distribution and fragmentation (Nicholson 1993, 1995; Willis and Boehm 2014; Willis et al. 2008). Several studies have examined the effect that processing methods and cooking have on fish remains. Butchering fish was found to leave cut marks primarily on undiagnostic bones (Willis et al. 2008), while differing patterns of bone loss and breakage resulted from different butchering methods prior to salting (Zohar and Cooke 1997). Changes in isotopic values of fish bone collagen and fish flesh, relative to raw flesh, were in general <1‰ after cooking by boiling, grilling and steaming (Fernandes et al. 2014), while morphological changes were observed in the collagen during heating at temperatures as low as 60ºC (Richter 1986). Research has also been conducted into the effects of burning on fish bone, with changes to the surface morphology of burnt bone allowing an approximate indication of the temperature reached by the bone (Nicholson 1995). Additionally, cranial elements were destroyed more quickly than post-cranial vertebrae when burnt, and bone element destruction increased with heating intensity (Lubinski 1996). It is possible that cooking and processing methods may alter the trace element and isotopic composition of fish otoliths, scales and vertebrae. Without prior knowledge concerning how processing and

cooking methods and post-depositional influences alter the chemical nature of archaeological specimens, false assumptions regarding palaeoenvironmental changes and diet can be made. Therefore, it is imperative to ascertain whether these data are altered by factors after the death of the fish.

The most detailed study was conducted by Andrus and Crowe (2002), who documented changes in crystallography, $\delta^{18}O$ and $\delta^{13}C$ values, and the elemental chemistry of otoliths from modern fish as a result of different cooking methods (direct burning, roasting over coals, roasting in an oven, boiling in freshwater and boiling in saltwater). They found that burning caused changes in the morphology and isotopic composition of otoliths, proposing that burnt otoliths should not be used in isotopic analyses because of the risk of providing inaccurate palaeoenvironmental information. They also found that the trace elements within the otoliths were readily affected by numerous cooking methods, and proposed that elemental analysis of zooarchaeological otoliths is therefore problematic. The broader application of these results is limited by the small sample size, with only two fish cooked per method tested, not allowing for the identification or control of anomalies in the sample.

The study presented here employs experimental analyses to examine the effects that a range of traditional processing and cooking methods have on the morphological and chemical (elemental and isotopic) properties of modern fish otoliths, vertebrae and scales. A larger sample size than that used by Andrus and Crowe (2002) is used to evaluate individual variation, additional cooking methods are incorporated, and recent technological analytical advances are utilized. The results provide a standard by which to compare future archaeological analyses, thereby indicating the suitability of individual samples for use in palaeoenvironmental studies, and providing a method of investigating subsistence strategies employed by past inhabitants of individual sites.

1.2 Study Species

Mulloway (*Argyrosomus japonicas*: Sciaenidae) is commercially fished in South Australia and was an important species to local Indigenous people. It has large, robust otoliths, which survive well in archaeological sites in the area. *Argyrosomus japonicus* is a large predatory teleost fish distributed through the Indian and western Pacific oceans. It is a fast growing, relatively long-lived species, attaining a maximum age of ca 30-35 years and size of ~1800 mm. Juveniles inhabit estuarine environments, with sexual maturity of female and male *A. japonicus* in South Australia occurring at 6 and 5 years respectively, after which time they migrate into marine waters (Ferguson et al. 2014). Adult mulloway typically aggregate around estuary mouths during the summer months, attracted by freshwater outflows and an abundance of food.

In order to ensure this research was as realistic as possible, we sourced the fish from the Coorong estuary, an estuarine area that was known to be densely populated by the Ngarrindjeri in pre-European times, owing to the richness of natural resources it provided (Jenkin 1979; Taplin 1879). The species selected was one that the Indigenous population of the region regularly fished (Disspain et al. 2011; Luebbers 1982).

2. Materials and Methods

2.1 Processing and Cooking

Fifty fish (ranging from 860g to 2800g gutted weight) were purchased from a local fisher at Meningie, South Australia in mid-December 2012. The fish were caught in a single net haul from the Coorong, the estuary of the Murray River, in the waters around Pelican Point (35º35'33.34"S 139º01'36.26"E). All fish were gilled and gutted by the fisherman upon capture and were kept on ice for 24 hours, after which time 42 fish were randomly allocated to one of six cooking treatments (Table 1). The remaining eight fish were used as controls. This number of replicate fish was deemed sufficient as previous otolith chemistry studies have found differences between environmental and spatial treatments with similar sample sizes (Dove et al. 1996; Elsdon and Gillanders 2002, 2003); the experimental approach presented here is not likely to be confounded by spatial and environmental effects.

Each fish was allocated a unique code number (e.g. CL1, CL2, etc.), which was inscribed onto a copper tag and attached to its jawbone with wire, such that individual fish could be identified and related back to their specific size data and allocated cooking treatment throughout processing. Fish were then individually photographed and weighed and the standard length of each fish was recorded (Table 1). The fish were kept on ice when they were not being processed. A campfire measuring approximately 1 m x 1 m, was built on a sandy beach using *Eucalyptus* spp. in a 30 cm-deep pit, and monitored by a Centre 309 data logger thermometer with three evenly spaced k-type probes. Different heating devices and fuel types produce different temperatures and consequently, results may vary dependent on these factors (Robins and Stock 1990; Shipman et al. 1984); the temperature was recorded every 30 sec at three locations within the fire.

Table 1. Average fish and otolith length and weight (± standard error) for each cooking method. Abbreviations for cooking method are indicated in brackets.

Cooking method	Standard length (SL)		Gutted fish weight (g)		Otolith length (mm)		Otolith weight (mg)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Control (CL)	485.9 ± 81.8	400-595	1707.1 ± 747.1	1000-2730	16.0 ± 1.9	14.2-18.8	748.7 ± 216.8	558.5-1051.2
Boiled Freshwater (BF)	479.3 ± 53.1	400-532	1591.4 ± 388.0	1040-2100	15.8 ± 1.3	14.0-17.1	736.8 ± 185.2	506.9-951.6
Boiled Saltwater (BS)	466.9 ± 85.8	385-601	1545.7 ± 713.4	860-2700	15.6 ± 2.0	13.1-18.3	716.5 ± 232.9	467.0-1051.8
Burnt Completely (BC)	482.8 ± 84.9	400-595	1671.7 ± 811.9	1000-2730	15.9 ± 2.8	12.8-19.4	664.3 ± 245.8	421.5-1040.0
Roasted on Fire (RF)	493.1 ± 67.9	423-610	1750.0 ± 597.9	1300-2800	16.2 ± 1.2	14.9-17.5	789.7 ± 143.3	602.6-1008.8
Salted (SD)	487.3 ± 45.1	427-560	1652.3 ± 347.7	1220-2300	15.8 ± 1.3	14.2-18.0	736.8 ± 158.4	556.9-971.3
Wrapped in Clay (WC)	465.3 ± 62.7	391-560	1498.6 ± 540.9	900-2440	15.7 ± 1.6	13.7-18.2	715.0 ± 175.8	520.8-940.2

2.2 Description of cooking methods

Six cooking methods that represent a range of commonly used traditional cooking practices (see Berndt et al. 1993; Homsey et al. 2010; Yankowski et al. 2015) were investigated to document changes in morphology, and elemental and isotope chemistry.

Control (CL). The control fish were kept on ice for 24 h, after which time a single otolith was removed.

Boiled in Freshwater (BF). Each fish was washed in seawater, wrapped in muslin cloth and tied with twine to ensure that the remains of each individual were retained and not mixed. They were then placed in a large pot of boiling freshwater (100ºC) (Figure 1a) on the fire for 20 min until the musculature separated from the bone (Figure 1b). Once cooked, the fish were removed from the water and left to cool until they could be handled.

Boiled in Saltwater (BS). These fish underwent the same procedure as those boiled in freshwater; with the exception that saltwater from the ocean was used.

Burnt Completely in the Fire (BC). These fish were filleted and the frames and heads were placed in the fire to burn. The temperature of the fire ranged from 49 – 939°C during this time, with a mean temperature of 457 ± 10.4°C. Once the fire had died down, after 140 min, an attempt was made to remove the frames from the ash of the fire. However, this was difficult, as the majority of the fish bones had been completely destroyed by the fire, and the coals were too hot to search thoroughly; therefore, no otoliths were obtained. An alternative approach was adopted where one otolith was removed from 6 of the control fish (to use as controls), and a further fish added that had not been used in experiments, which had both of its otoliths intact. We then used these seven fish as the BC group. The fish heads were removed from the frames and placed in a small fire in a brazier for 105 min. The thermocouple was used with two probes to record the temperature of the fire over this time (Figure 1c). Temperatures ranged from 172 – 892°C, with a mean of 595 ± 6.6°C. After this time, the remains of the heads were removed from the fire and left to cool (Figure 1d). The ash was then sieved to find the remaining otoliths.

Roasted on the Fire (RF). The fish were washed in saltwater and then placed directly on the coals of the fire (Figure 1e). They were cooked on one side, turned and cooked on the other; total cooking time was 33 min (Figure 1f). The temperature of the fire ranged from 397 – 845 °C during this time, with a mean temperature of 522 ± 23°C.

Salted (SD). The salted fish were washed with seawater and packed tightly into a large plastic tub with 25 kg of sea salt. The salt was packed into the fish's cavities to ensure they were well covered (Figure 1g). After 20 days in the salt, the otoliths were removed from the fish (Figure 1h).

Wrapped in Clay and Roasted on the Fire (WC). Each fish was wrapped in banana leaves and then covered in approximately 1.5 cm thick filtered terracotta clay (Figure 1i). The clay wrapped fish were placed on the coals to cook for 48 min. Once cool enough to handle, one fish (WC4) was broken open; however, it was not completely cooked so the remaining six fish were placed back on the fire for a further 26 min (Figure 1j) (temperature range over the total 74 min: 99–1015°C, mean temperature 553 ± 12°C).

Following their respective treatments, both otoliths were removed from each fish, washed in ultrapure water, and were left to air dry for 48 hours. They were then weighed and stored in plastic vials. In addition to the otoliths, a number of post-cranial vertebrae were removed from each of the carcasses, along with a collection of pectoral scales.

Figure 1. Documentating the pre- and post-cooking condition of the mulloway. a. Placing fish wrapped in muslin cloth into boiling freshwater; b. Removing the flesh and otoliths from boiled fish; c. Fish heads in the fire with thermocouple probes; d. Burnt bone and otolith of mulloway after being removed from the fire; e. Roasting fish directly on the fire; f. Mulloway after roasting on the fire; g. Packing the mulloway with salt; h. Mulloway after salting for 20 days; i. Wrapping mulloway in banana leaves and clay; j. Unwrapping mulloway from the clay.

2.3 Morphological Analysis

Detailed images of each otolith were acquired to create a comprehensive archival record and for visual comparisons. Both the proximal and distal surfaces of each otolith were photographed using a Canon EOS60D digital camera equipped with a Canon 100mm Macro Lens. Photomicrographs of sectioned otoliths were also acquired using a Leica MZ16A stereomicroscope with a PLANAPO 1.0x lens. Each whole and sectioned otolith was examined for deterioration, breakage and discolouration and compared to the control samples.

2.4 Otolith X-Ray Diffraction

To investigate possible recrystallization of the otolith aragonite to calcite, we analysed one otolith sample from each treatment method and one from the control group using X-ray diffraction (XRD). The bulk mineralogy of samples was ascertained by a Bruker D8 ADVANCE Powder XRD with a Cu-radiation source. Prior to analysis, powdered samples mixed in an ethanol slurry were loaded onto silicone wafers fitted in XRD sample holders and allowed to dry. A standard powder sequence was run, involving a Cu-radiation source, with an angle of 3.5–50° 2theta and step sizes of 0.02° with counting times of 1 second. Bruker DIFFRAC.EVA software and Crystallography Open Database reference patterns were utilised for identifying mineral phases.

2.5 Otolith and Vertebrae Preparation and Elemental Analysis

One otolith and one vertebra from each fish were embedded in indium spiked epoxy resin (40 ppm), and sectioned (0.35±0.05 mm thick) using a low speed saw (Buehler Isomet). The otoliths were sectioned transversely through the nucleus. All sections (with the exception of the burnt samples, due to their fragile nature) were polished using lapping film and ultrapure water before mounting onto microscope slides with a 100ppm indium spiked CrystalBond[TM].

Trace element quantification in the sectioned otoliths and vertebrae was conducted on an Agilent 7500s Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) coupled to a Merchantek UP213 (NewWave Research) laser ablation system. Ablations were conducted using a spot size of 30 μm and a pulse rate of 5 Hz, for 150 sec, after pre-ablating to remove contaminants. For the otoliths, spot ablations were located at both the nucleus and at the edge on the final growth increment. Based on marginal increment data from Ferguson et al. (2014: Figures 2 and 3), mulloway otoliths from fish aged 1–4 years increase in size by approximately 10% per month. The average mulloway otolith growth increment measures 402 μm; this is based on 2271 complete otolith increment measurements from mulloway of a range of ages, sizes, and sexes, and equates to growth of approximately 33.5 μm per month (Izzo unpub. data). Therefore, a spot size of 30 μm represents less than one month of mulloway growth. The chemistry data from the edge of the otolith will represent the environmental conditions of the location the fish was caught, while the nucleus chemistry will represent those of the juvenile stage of the fish's life history. All of the fish were captured at the same time in the same location, so edge data would be expected to be similar among samples, and would be the most likely to be affected by the different cooking methods. The spot ablation on the vertebrae targeted the calcified margin.

Nine elements were analysed, which included: ^{7}Li, ^{23}Na, ^{24}Mg, ^{55}Mn, ^{86}Sr, ^{138}Ba, ^{208}Pb, ^{65}Zn and, ^{115}In, as well as ^{43}Ca as an internal standard. To correct for machine drift, a reference sample (National Institute of Standards and Technology, NIST 612) was analysed at the beginning and end of each laboratory session, and after every five to six samples. Prior to each ablation, background gases were measured for 30 sec to allow for background correction and to determine the detection limits of ICP-MS. Raw element mass count data were processed using GLITTER software (www.glitter-gemoc.com). Data were then converted from ppm to mol and normalised to Ca (values in mmol·mol^{-1}). The incorporation of elements that substitute for Ca ions within aragonite, which co-vary with elemental levels in the environment, is accurately standardized to the number of Ca ions in the otolith (Thorrold et al. 1998). Normalising data to Ca is a standard protocol in otolith chemistry studies that facilitates direct comparisons among studies (e.g., Elsdon and Gillanders 2005a; Reis-Santos et al. 2013).

2.6 Otolith and Vertebrae Stable Isotope Analysis

For stable isotope analyses, approximately 100 µg of otolith material was removed from the edge of each sample to analyse the inorganic component for $\delta^{18}O$ and $\delta^{13}C$. Samples were submerged in 100-105% phosphoric acid and left to dissolve overnight at 70°C. Headspace analysis, measuring $\delta^{18}O$ and $\delta^{13}C$ values, was conducted on a Nu Horizon continuous flow isotopic ratio mass spectrometer (IRMS) following Spötl and Vennemann (2003). Data were corrected to an international standard, LSVEC ($\delta^{18}O$=26.7 $\delta^{13}C$=-46.6), and an internal standard, ANU-P3 ($\delta^{18}O$=-0.32 $\delta^{13}C$=2.24), which were analysed at the start and end of each run, and after every 10 samples.

From each vertebra, 50 mg of material was removed from the outer surface and soaked overnight in 1 ml of 5% NaOCl to remove the organic component. They were then rinsed with ultrapure water, centrifuged and rinsed again until free from NaOCl, and left to dry in an oven at 40°C. Headspace analysis, measuring $\delta^{18}O$ and $\delta^{13}C$ values, was conducted on a Nu Instruments continuous flow isotopic ratio mass spectrometer (IRMS) following Spötl and Vennemann (2003). Data were corrected to an international standard IAEA CO-8 (marble: $\delta^{18}O$=-22.7 $\delta^{13}C$=-5.76), and internal standards, ANU-P3 ($\delta^{18}O$=-0.32 $\delta^{13}C$=2.24) and UAC-1 (calcium carbonate: $\delta^{18}O$=-18.4 $\delta^{13}C$=-15.0), which were analysed at the start and end of each run, and after every 10 samples.

2.7 Scale Preparation and Elemental Analysis

Scales were washed with ultrapure water and left to air-dry overnight. For elemental analysis 10 mg of scale from each fish was weighed, dissolved in ultrapure HNO_3 for 24 h, and diluted to 2% with ultrapure water. Each solution was filtered using 0.45 µm syringe filters to remove any undissolved particles. Serial dilutions (1:50) and undiluted solutions were analysed using an Agilent 7500cs ICP-MS with an Octopole Reaction System. Standards (10 ppm Certified Reference Solution from Choice Analytical) with concentrations of 1 $\mu g \cdot L^{-1}$, 50 $\mu g \cdot L^{-1}$, 100 $\mu g \cdot L^{-1}$ and 500 $\mu g \cdot L^{-1}$ were analysed before and after the samples, as well as intermittently throughout the run. Elements analysed included ^7Li, ^{23}Na, ^{24}Mg, ^{55}Mn, ^{86}Sr, ^{138}Ba, ^{208}Pb, ^{65}Zn, ^{115}In, and ^{43}Ca as an internal standard. Raw element mass count data were processed using GLITTER software (www.glitter-gemoc.com). Data were then converted from ppm to mol and normalised to Ca (values in $mmol \cdot mol^{-1}$).

2.8 Scale Isotope Analysis

For isotope analysis, 1–2 mg of whole scales from each fish was weighed and the organic component analysed for $\delta^{13}C$ and $\delta^{15}N$ using a EuroVector EA coupled to a Nu Horizon continuous flow IRMS. Standards (glycine ($\delta^{13}C$=-31.2, $\delta^{15}N$=1.32) and glutamic acid ($\delta^{13}C$=-16.72, $\delta^{15}N$=-6.36)) were analysed before and after the samples, as well as intermittently throughout the run.

2.9 Statistical Analyses

Elemental data were log($\times$+1) transformed and fitted to a Euclidean distance dissimilarity matrix. A series of one-way permutational analysis of variance (PERANOVA) and permutational multivariate analysis of variance (PERMANOVA) were used to investigate differences in element composition (both individually and as a multi-element signal) among treatments for all structures. For otolith element data, two-way

PERANOVA and PERMANOVA were used to examine the interactive influence of treatment and sampling location (nucleus vs edge) on element composition. We predicted that if the cooking methods affected the otoliths it would most likely affect the edge of the otolith rather than the nucleus. We were not interested in comparisons between the nucleus and the edge in this study as these would be more likely to reflect changing environment via movement or ontogenetic effects rather than cooking methods. Isotopic data were analysed in the same way as elemental data, but were not transformed due to negative values. All analyses were performed using the PRIMER software package, and all tests were carried out using type-III sum of squares and 999 permutations of the data (Anderson and Ter Braak 2003). Where probability (*p*-) values <0.05 were deemed significant, post-hoc pairwise tests were done. Canonical analysis of principal (CAP) coordinates was used to graphically represent the multi-element data in two dimensions for each structure.

3. Results

3.1 Morphology

The majority of cooking treatments had no obvious effects on the appearance of the otoliths (Figures 2a and b); however, the salted samples had a slightly chalky exterior (Figures 2c and d). The burnt otoliths underwent significant morphological changes; they were carbonized and had a chalky texture, as well as showing numerous cracks and areas of disintegration (Figure 2e). Most of the burnt otoliths were blackened and were noticeably more fragile than unburnt otoliths, with BC1 turning into powdery ash a few days after collection. Once sectioned, these differences in appearance were further evident. The burnt otoliths show carbonization through to the nucleus, and the annuli were very difficult to determine, or were no longer visible (Figure 2f). Three salted samples (SD02, SD04 and SD06) showed a brownish discolouration of small areas close to the nuclei and along small fissures extending inwards from the surface of each sample (Figure 2d). All other treatments appeared to have no effect on the appearance of the otoliths, in terms of discolouration, deterioration or increment clarity, when compared with the control samples (Figure 2a and b).

In addition, the burnt otoliths weighed less after treatment relative to the corresponding control otolith. The mean difference between the burnt otolith and the control otolith from the same fish was 6.3%. It was not possible to assess the effects of cooking methods on otolith weight for other treatments as both otoliths were treated identically.

Morphologies of the vertebrae were affected similarly to the otoliths; the burnt samples were very fragile and highly deteriorated. They ranged in colour from black through to chalky white; presumably dependent on the level of heat they were exposed to. The vertebrae from the salted fish were dehydrated and the adhering tissue was more difficult to remove from the structure in comparison with the other treatments. There were no noticeable differences in the morphologies of the scales from different cooking treatments once they were cleaned; however, no scales were retrieved from any of the burnt samples, so comparisons were unable to be made with that treatment group.
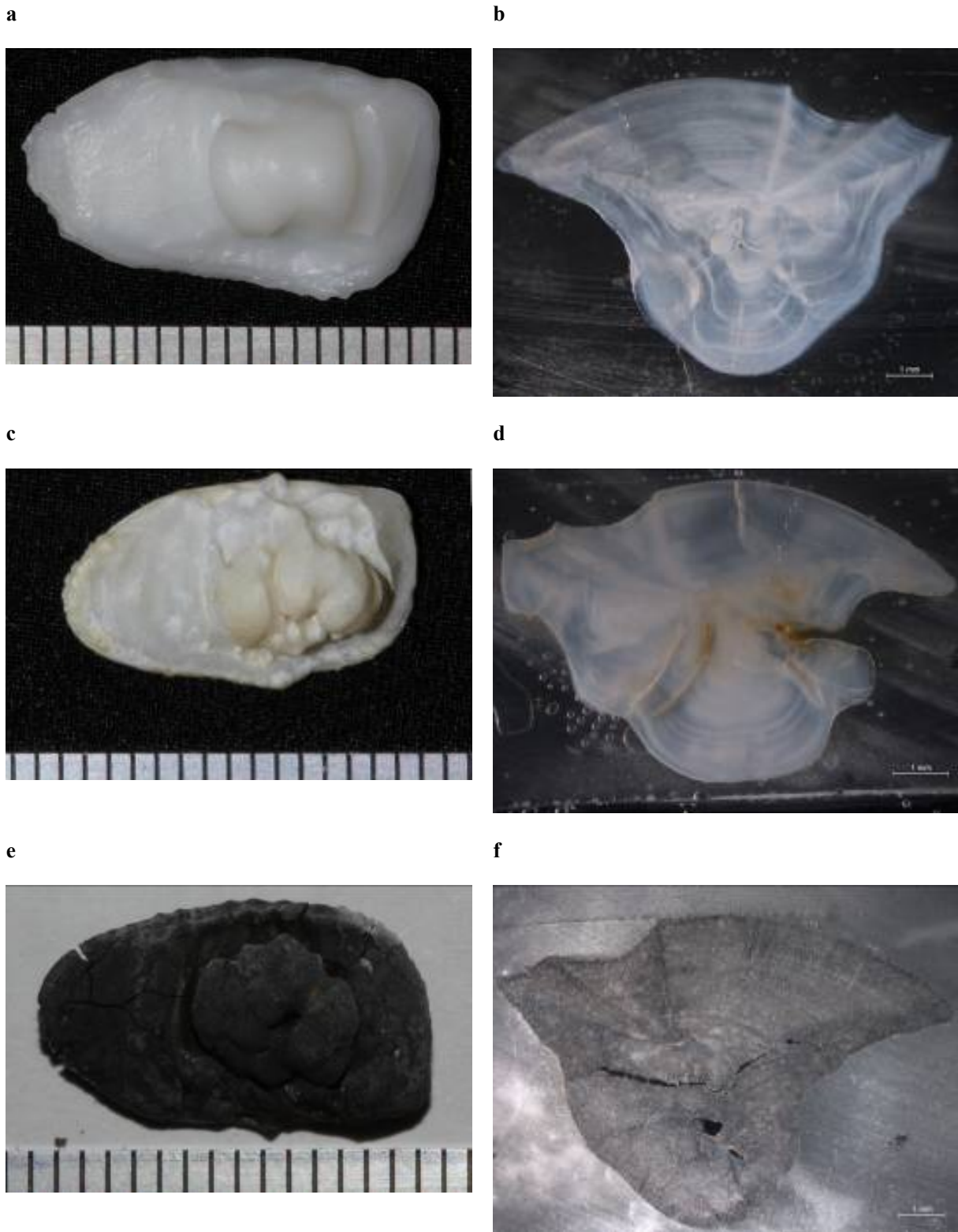
Figure 2. Representative samples of whole and sectioned otoliths. a. Control otolith whole (CL03); b. Control otolith section (CL03); c. Salted otolith whole (SD07); d. Salted otolith section (SD04); e. Burnt otolith whole (BC05); e. Burnt otolith section (BC04).

3.2 Otolith X-Ray Diffraction

XRD analysis of the otoliths detected the only mineral in all samples to be aragonite, except for the sample from the Burnt Completely treatment group, which had recrystallized to calcite. No aragonite was detected in the burnt sample.

3.3 Trace Element Analysis

Otolith element composition was largely unaltered by most of the cooking treatments relative to the control samples. There was some variation between the nucleus and edge of the otolith for several elements; however, this is likely reflective of the fish spending time in different environments or an ontogenetic effect, and not a result of cooking (Table 2, Figure 3). However, two treatments did have a noticeable impact. Burning the otolith significantly increased the Li:Ca, and Zn:Ca concentrations at the edge of the samples (Figures 3a and h). Although Mn:Ca concentrations were increased in burnt otoliths and by wrapping in clay these were not significantly different to controls (Figure 3d). Roasting on the fire and boiling in saltwater significantly increased ($p < 0.05$) Zn:Ca concentrations (Figure 3h). Unsurprisingly, the Na:Ca concentrations were greatly increased by the salting treatment, both at the edge and the nucleus of the samples (Figure 3b). Salting the fish also appeared to alter Mg:Ca and Pb:Ca concentrations, though these data were highly variable and no significant differences were found. Sr:Ca and Mg:Ca concentrations were relatively similar for all treatments (Figure 3e and c), whereas Ba:Ca and Mn:Ca concentrations only varied between the edge and nucleus of the otoliths and not between treatments (Figure 3f and d). Multivariate analyses of the multi-element data were consistent with these patterns (Table 3, Figure 6a), with the burnt completely and salted fish seen mostly as outliers from the cluster of other cooking treatments and the control fish.

Vertebral elemental concentrations were similarly altered by the salting treatment, with increases in Na:Ca concentrations, while the elemental concentrations of Li:Ca were significantly different between the control and wrapped in clay treatments (Figure 4a and b, Table 2). The Mn:Ca, Ba:Ca, and Pb:Ca concentrations were greater in the burnt completely samples (Figure 4b, f, and g), though statistical analyses indicate that concentrations were not significantly different to the control group (Table 2). Similar to the otolith results, concentrations of Sr:Ca did not differ significantly among treatments, while in contrast to the otoliths, Zn:Ca was also stable throughout treatments. Differences in the multi-element vertebral signature were largely due to the salting treatment (Table 2, Figure 6b).

The elemental concentrations of the scales differed slightly from the other remains. The majority of changes in concentrations were not as dramatic, excluding increases in Na:Ca resulting from salting (Figure 5b), and Mn:Ca resulting from the boiling in freshwater treatment (Figure 5d). The concentrations of Mg:Ca were significantly higher in all treatments compared with the control group (Figure 5c), while Sr:Ca concentrations were higher in all except the wrapped in clay treatment (Figure 5e). Statistical analyses revealed no significant differences in concentrations of Ba:Ca, Pb:Ca, Li:Ca, and Zn:Ca between the scales from the control group and those from any of the treatments. The multi-element scale data were affected by the salted and boiled in freshwater samples (Table 2, Figure 6c).
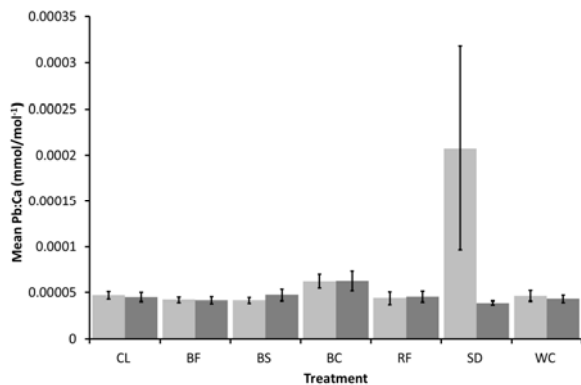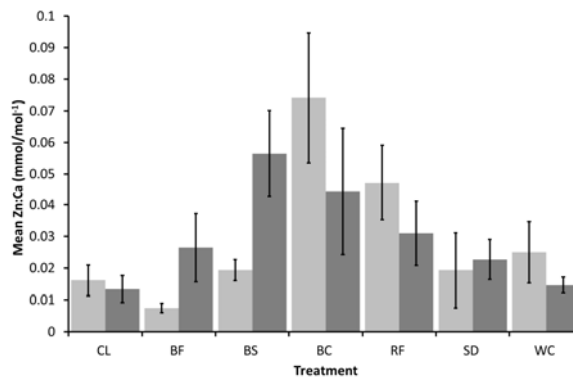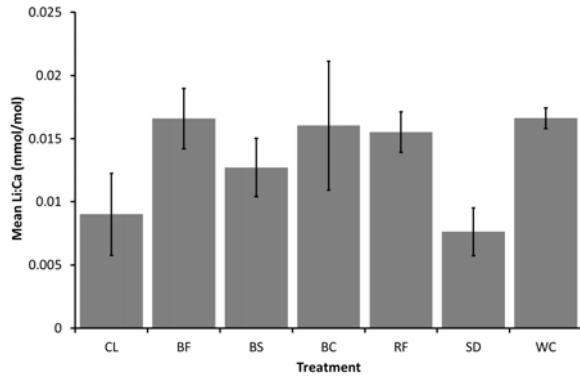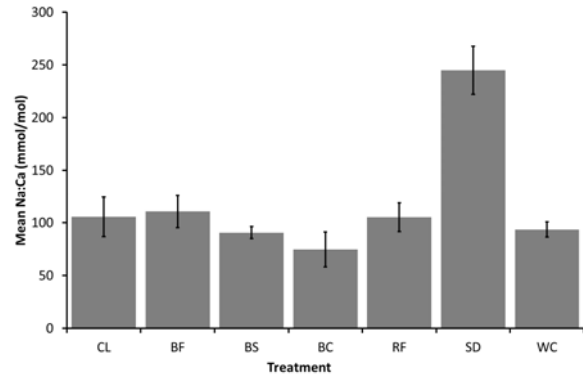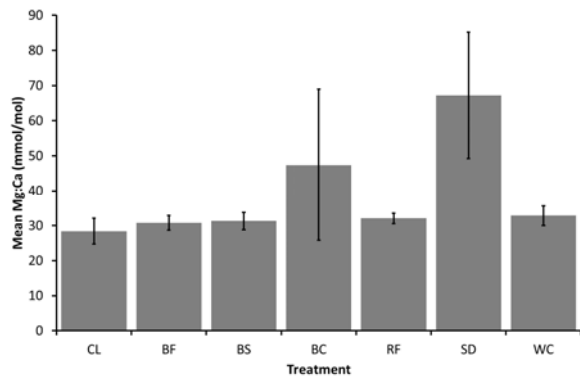
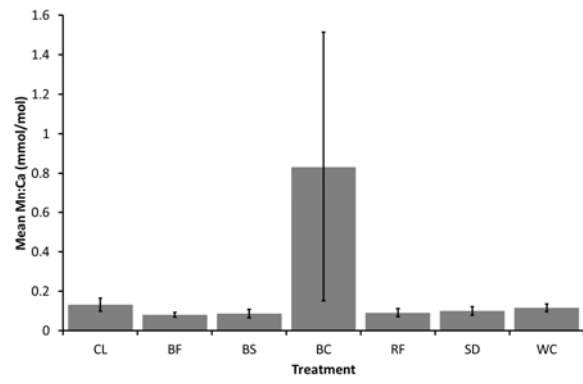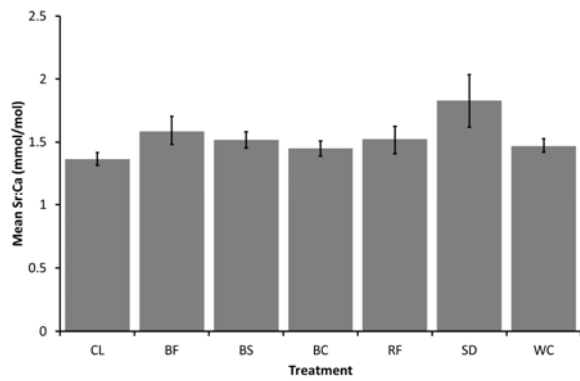**a**
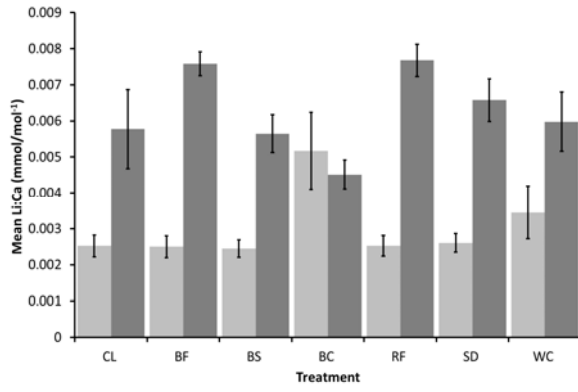
b
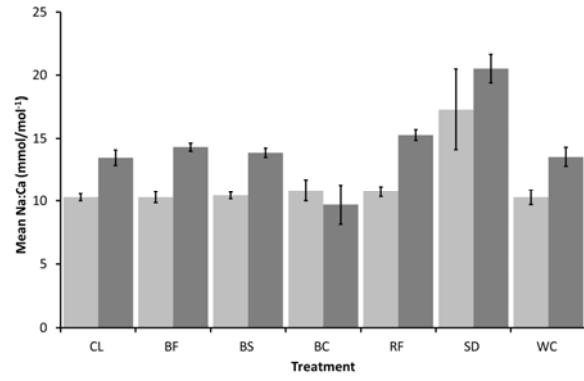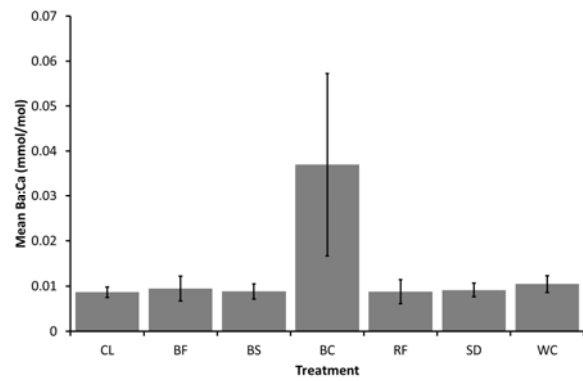

c
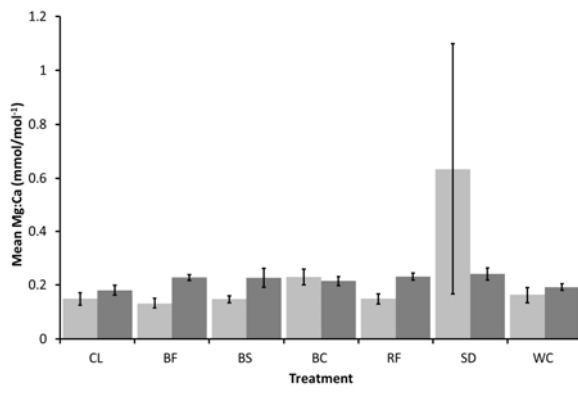
d
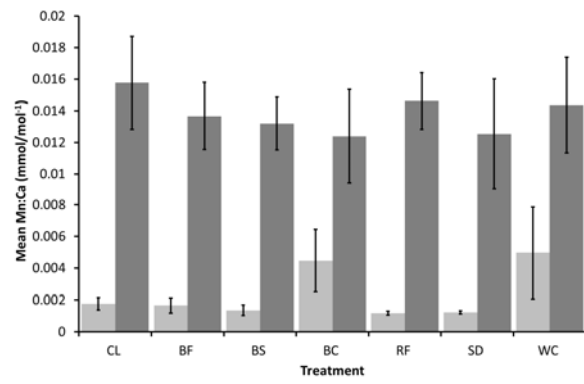
e
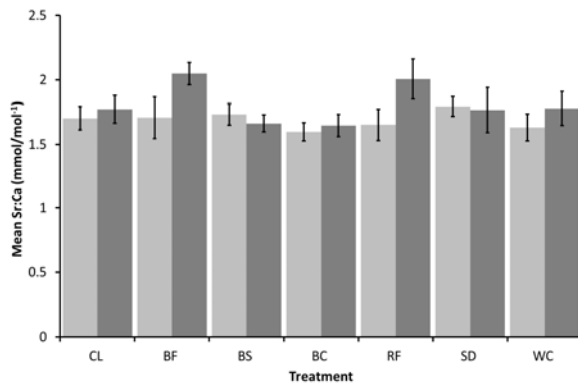
f
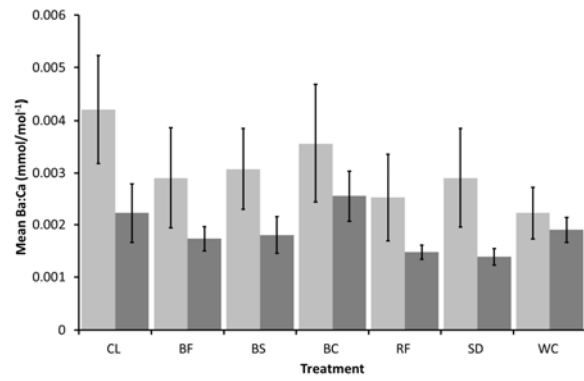
g

h

Figure 3. Mean otolith trace element concentrations among seven different cooking treatments (± SE) for the edge (light grey bars) and the nucleus (dark grey bars) a. Li:Ca; b. Na:Ca; c. Mg:Ca; d. Mn:Ca; e. Sr:Ca; f. Ba:Ca; g. Pb:Ca; h. Zn:Ca. Codes for cooking treatments are in Table 1.

a

b

c

d

e

f

g

h

Figure 4. Vertebrae mean (±SE) trace element ratios for different cooking treatments a. Li:Ca; b. Na:Ca; c. Mg:Ca; d. Mn:Ca; e. Sr:Ca; f. Ba:Ca; g. Pb:Ca; h. Zn:Ca. Codes for cooking treatments are in Table 1.
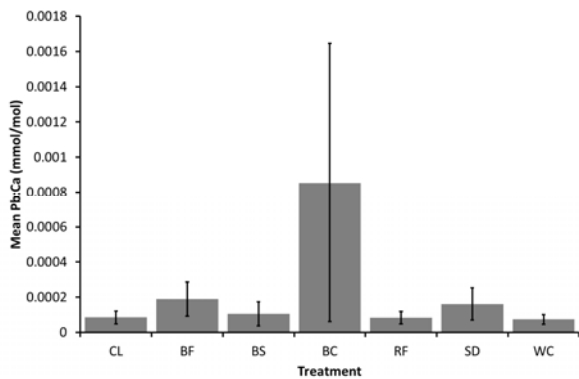
17
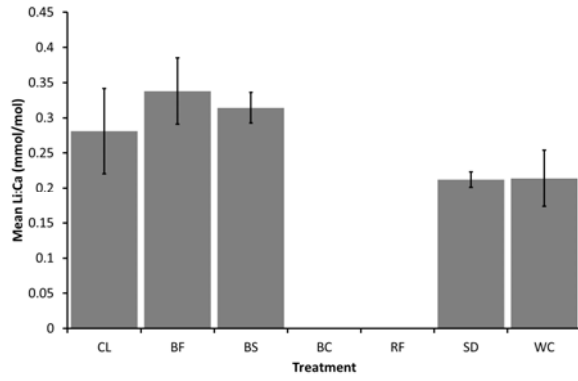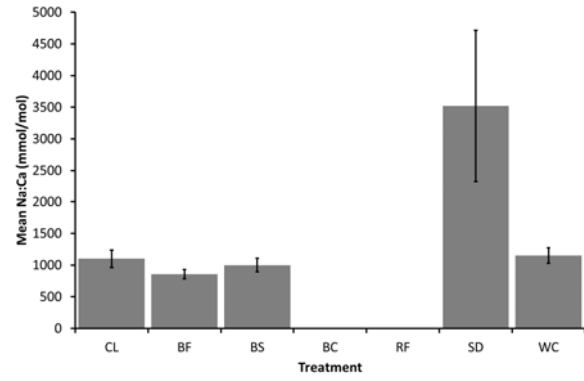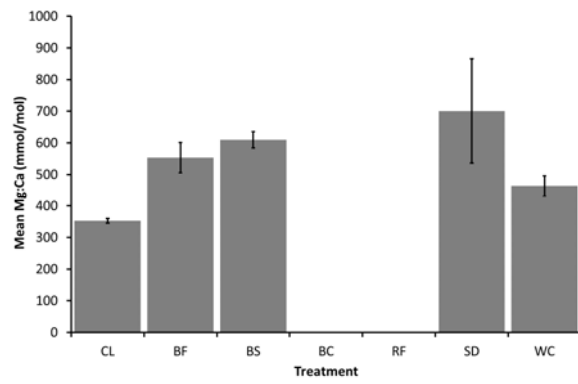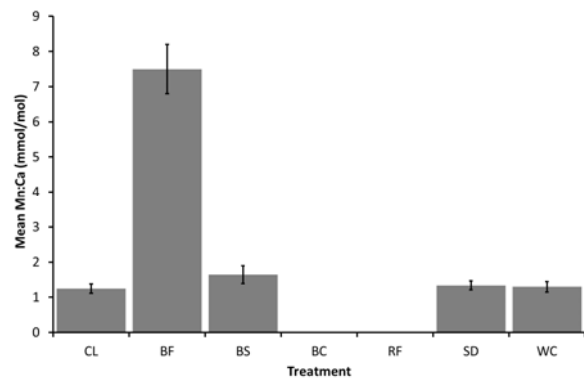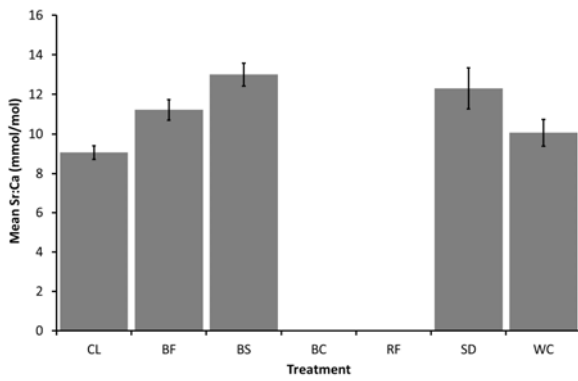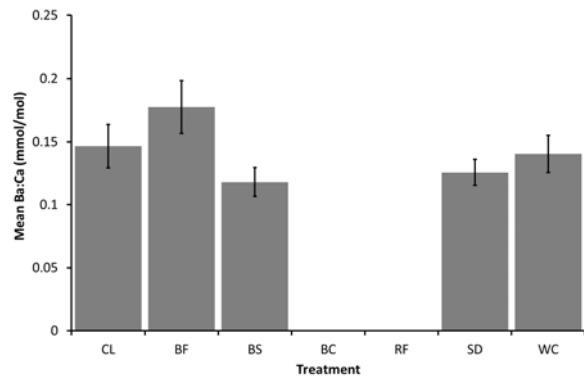
g
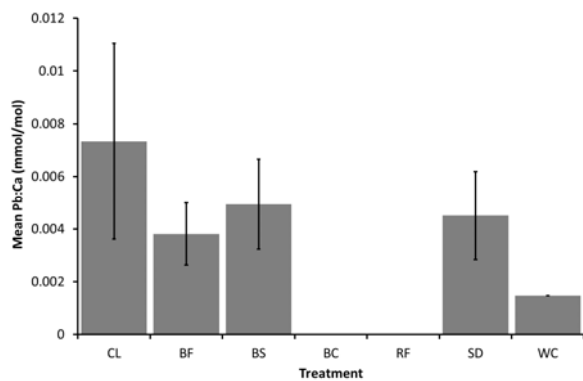
h
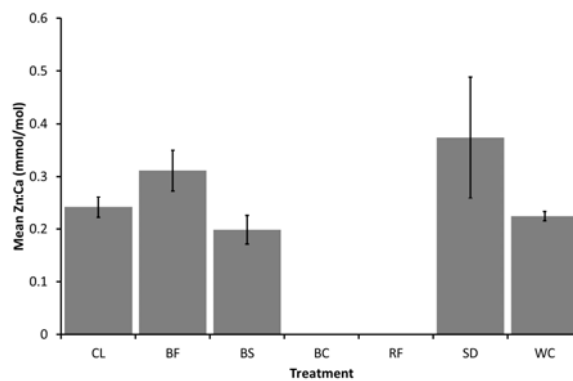
Figure 5. Scale mean (±SE) trace element ratios for different cooking treatments a. Li:Ca; b. Na:Ca; c. Mg:Ca; d. Mn:Ca; e. Sr:Ca; f. Ba:Ca; g. Pb: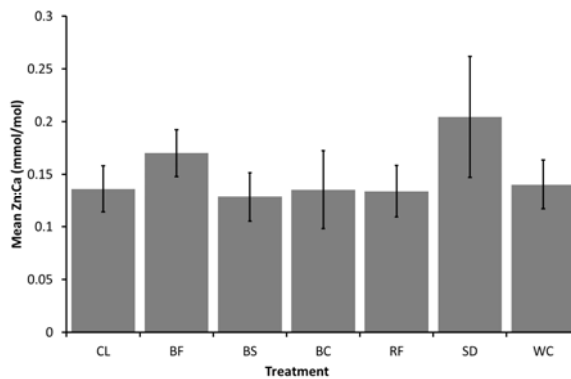Ca; h. Zn:Ca. Codes for cooking treatments are in Table 1. No scale samples were analysed from the burnt completely (BC) and the roasted on fire (RF) treatments.
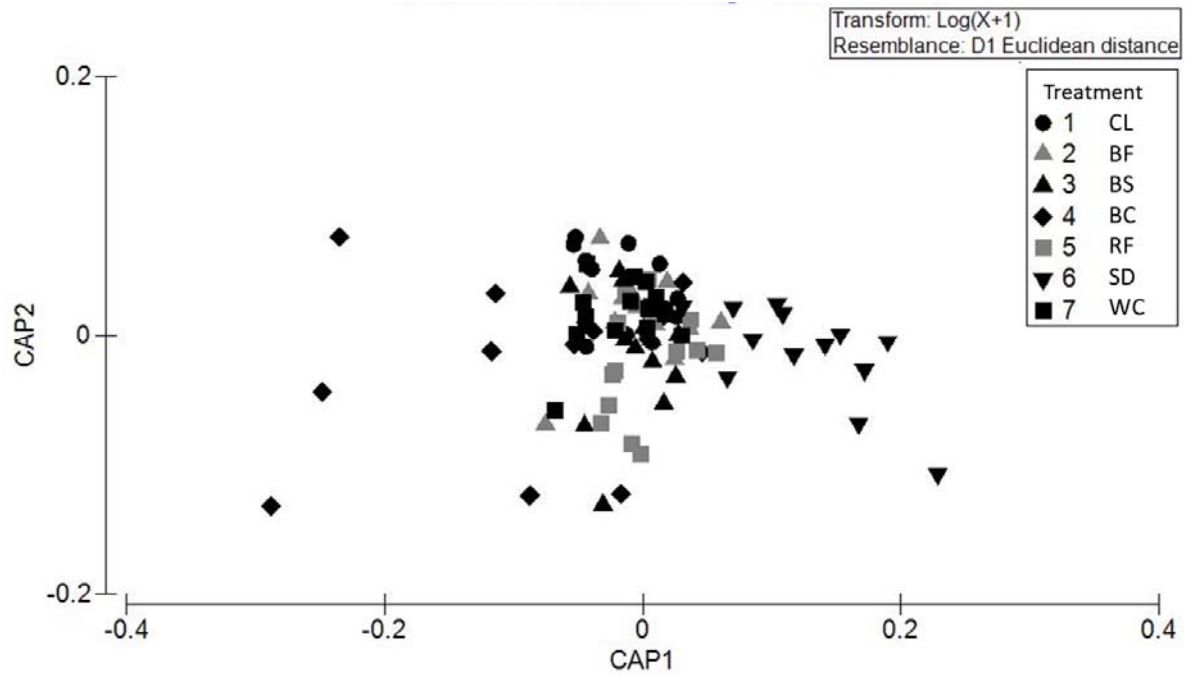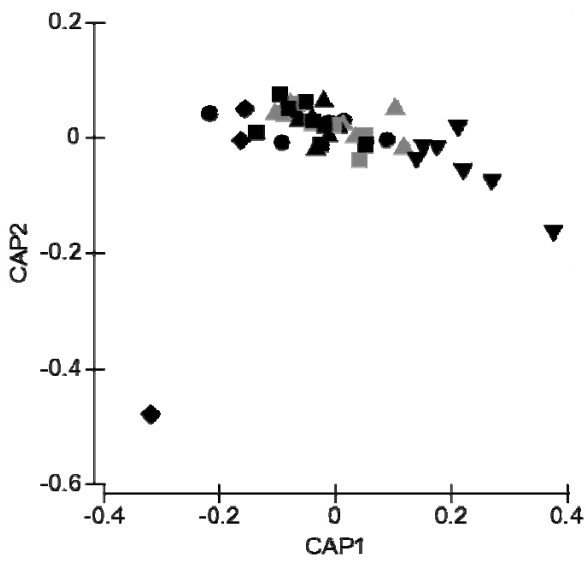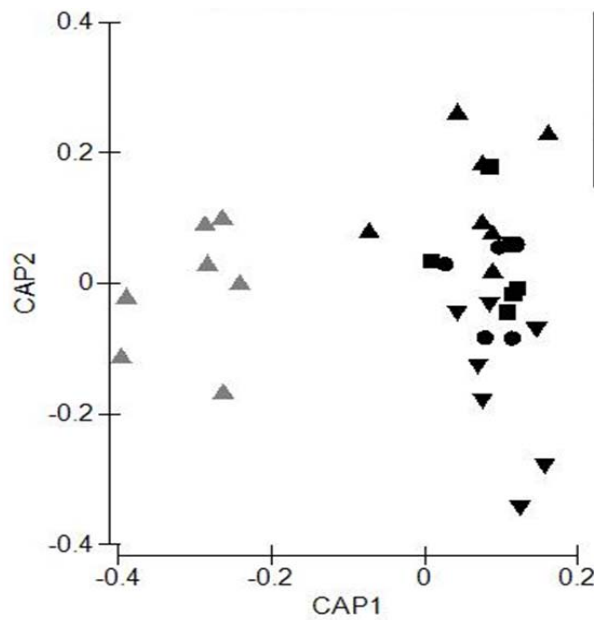
Figure 6. Canonical analysis of principal coordinates (CAP) plots of dissimilarity among cooking treatments for the multi-element signals: (a) otoliths (data for the edge and nucleus included for each treatment); (b) vertebrae; and (c) scales.

19

Table 2. Permutational ANOVA and MANOVA results comparing element:Ca concentrations in vertebrae and scales among cooking treatments, and for otoliths among treatments and the portion of the otolith (edge vs nucleus). Multi = multi-element signature. The degrees of freedom (df) were the same for each element:Ca ratio and the multi-element signature for each structure.

Otoliths Source	df	Li:Ca F	P	Na:Ca F	P	Mg:Ca F	P	Mn:Ca F	P	Sr:Ca F	P	Ba:Ca F	P	Pb:Ca F	P	Zn:Ca F	P	Multi F	P
Treatment	6	0.984	0.437	12.952	0.001	1.307	0.290	0.420	0.865	0.771	0.601	1.022	0.422	1.873	0.094	4.550	0.003	6.989	0.001
Position	1	103.740	0.001	34.600	0.001	0.387	0.555	105.360	0.001	4.101	0.050	10.840	0.001	2.088	0.158	0.001	0.967	17.895	0.001
Tr. X Pos.	6	5.152	0.001	2.653	0.022	0.884	0.488	0.510	0.822	1.318	0.276	0.286	0.947	2.203	0.049	2.260	0.040	1.841	0.050
Res	82																		

Vertebrae Source	df	Li:Ca F	P	Na:Ca F	P	Mg:Ca F	P	Mn:Ca F	P	Sr:Ca F	P	Ba:Ca F	P	Pb:Ca F	P	Zn:Ca F	P	Multi F	P
Treatment	6	2.823	0.023	10.202	0.001	2.813	0.210	3.353	0.013	1.969	0.094	3.820	0.008	2.055	0.086	0.705	0.650	5.441	0.001
Res	37																		

Scales Source	df	Li:Ca F	P	Na:Ca F	P	Mg:Ca F	P	Mn:Ca F	P	Sr:Ca F	P	Ba:Ca F	P	Pb:Ca F	P	Zn:Ca F	P	Multi F	P
Treatment	4	2.694	0.049	9.723	0.001	4.653	0.008	68.355	0.001	5.055	0.005	2.454	0.075	1.139	0.368	1.672	0.172	14.196	0.001
Res	27																		

3.4 Isotope Analysis

Mean otolith $\delta^{18}O$ values were different among treatments (Table 3, Figure 7a), with samples that underwent heating having lower mean $\delta^{18}O$ values than the control group (Figure 7a). Differences between the control group and the BF, BS, RF and WC treatments range from -1.09‰VPDB to -1.47‰VPDB. The difference between the BC group and the control group mean values is -4.37‰VPDB. Statistical analyses indicated that all treatment groups (excluding the salting treatment with a difference of -0.56‰VPDB) $\delta^{18}O$ values were significantly different to the control group (Table 3). Otolith $\delta^{13}C$ was also different between some of the treatment groups and the control group (Table 3, Figure 7b); the otoliths from the burnt completely (with a difference of -3.74‰VPDB) and the boiled in saltwater (with a difference of -1.24‰VPDB) treatments had significantly more negative values ($p \leq 0.05$), while the other treatments were not significantly different from the controls (Figure 7b).

Very similar patterns were seen in the isotope analysis of the inorganic component of the fish vertebrae. All of the treatments that required heating have more negative $\delta^{18}O$ values than the control group, with the BC group having the largest difference (difference of -20.19‰VPDB) (Figure 7c). The differences between the control group and the BF, BS, RF and WC treatments ranged from -1.6‰VPDB to -3.5‰VPDB. Statistical analyses indicated that all treatment groups (excluding RF and SD) $\delta^{18}O$ values were significantly different to the control group (Table 3). The BC treatment was the only group to have any significant difference in $\delta^{13}C$ values from the control group (difference of -7.03‰VPDB) (Figure 7d, Table 3).

The $\delta^{13}C$ and $\delta^{15}N$ values of the scales were similar for all treatments (Figure 7e, f, Table 3), with no significant differences observed ($p > 0.05$); however, no scales from the burnt completely or roasted on the fire treatments were available for analysis.

4. Discussion

The morphology and chemistry of the otoliths and vertebrae were variably affected by the cooking treatments, highlighting the instability of some elements and features, while reinforcing the stability of others. Trace element analysis revealed that the majority of the elements within the treatment groups were similar to those in the control group. Stable isotope analysis indicated that the otolith and vertebrae mean $\delta^{18}O$ values were more negative in the treatment groups that were heated compared to the control groups, while mean otolith and vertebrae $\delta^{13}C$ values were different between various treatments and the control groups, with the majority of treatment groups having no significant difference in values compared with the control group. Trace element analysis of the fish scales revealed differing results compared with the analyses of the hard-parts; stable isotope analysis of the scales indicated that no significant alteration was observed.

a

b

c

d

e

f

Figure 7. Stable isotope analysis results; a. otolith δ¹⁸O; b. otolith δ¹³C; c. vertebrae δ¹⁸O; d. vertebrae δ¹³C; e. scale δ¹⁵N; f. scale δ¹³C. No scale samples were analysed from the Burnt Completely (BC) and the Roasted on Fire (RF) treatments.
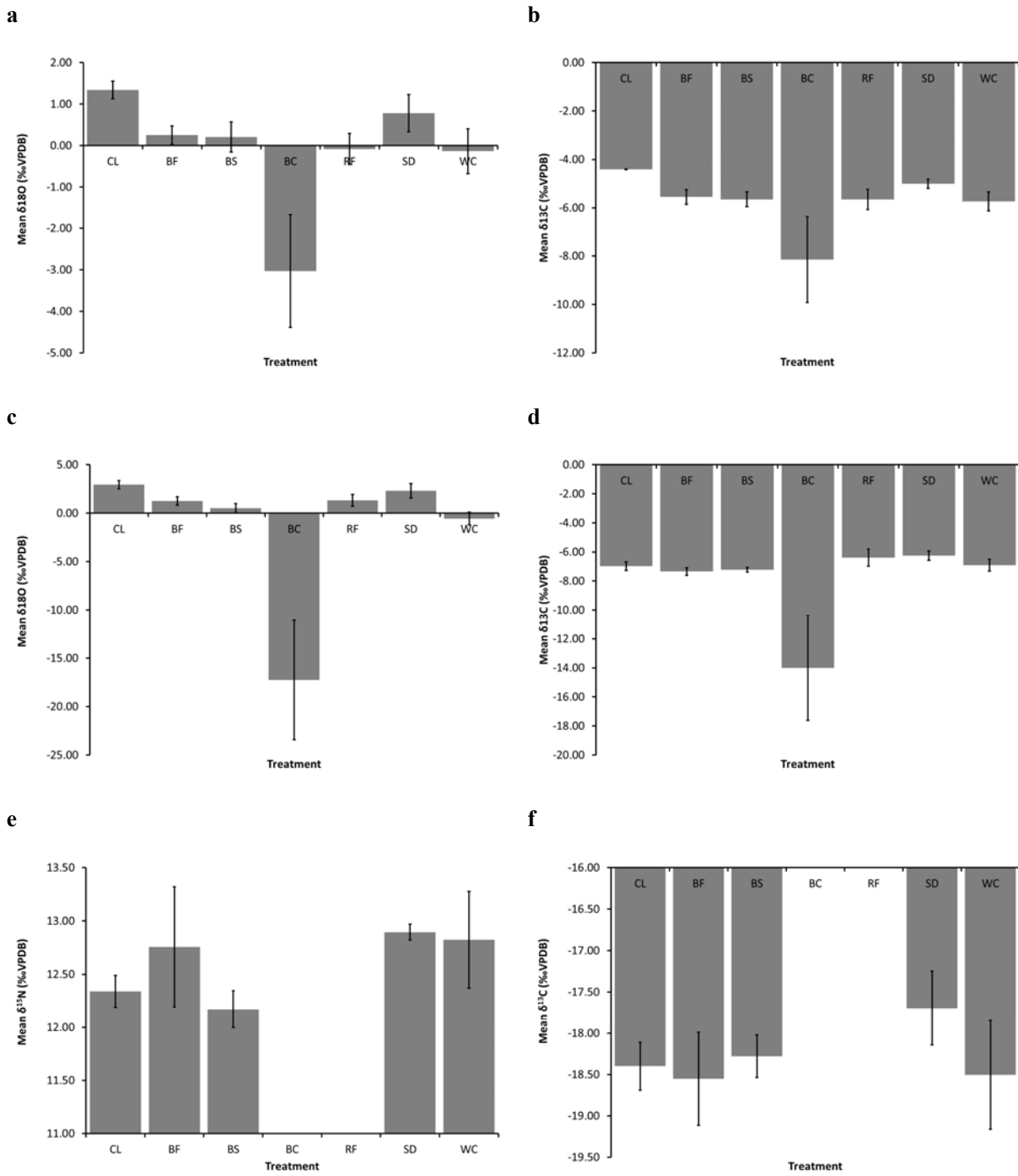
Table 3. Permutational ANOVA results comparing stable isotope values in otoliths, vertebrae and scales among cooking treatments. Multi = multi-element signature.

Otoliths		$\delta^{18}O$		$\delta^{13}C$		Multi	
Source	df	F	P	F	P	F	P
Treatment	6	5.222	0.001	2.574	0.026	3.662	0.006
Res	43						
Vertebrae		$\delta^{18}O$		$\delta^{13}C$		Multi	
Source	df	F	P	F	P	F	P
Treatment	6	19.946	0.001	8.4365	0.001	16.941	0.001
Res	37						
Scales		$\delta^{15}N$		$\delta^{13}C$		Multi	
Source	df	F	P	F	P	F	P
Treatment	4	0.699	0.595	0.511	0.744	0.582	0.732
Res	25						

The morphology of the hard-parts was largely unaffected by the cooking treatments employed, with the exception of the salted and the burnt completely methods, which caused significant alterations, in agreement with Andrus and Crowe (2002). Morphological alterations seen in the burnt vertebrae and otoliths included changes in colour due to carbonisation (in line with Nicholson 1995), a loss of increment clarity, and a reduction in weight. The reduction of sample weight/density, or "shrinkage" (Shipman et al. 1984), is likely due to the thermal denaturation of the collagen component of the structure at temperatures as low as 60ºC (Richter 1986), while burning has been shown to affect bone microstructure significantly at about 400ºC (Boschin et al. 2015). Given that otolith or vertebrae weight are commonly used to estimate fish size (Disspain et al. 2012; Gabriel et al. 2012; Quinn and Deriso 1999), the use of burnt samples would likely underestimate the fish's actual size, thereby resulting in inaccurate fisheries baseline data or an underestimate of meat yield. Scales were not recovered from the BC or the RF treatments, and no noticeable differences in the morphologies of the scales from the other treatments were observed.

The morphologies of the otoliths and vertebrae from the salting treatment were altered, with samples appearing chalky and dehydrated, and otolith sections revealing the absorption of a substance from the outside surface. This substance is likely a mixture of salt and fish body fluids (e.g. endolymph), which is evidenced by increased levels of Na:Ca in all salted samples (otoliths, vertebrae and scales) and demonstrates that these structures absorb elements post-mortem. Concentrations of Na:Ca have been found to increase in otoliths when they were left in the skull for long periods after death (Milton and Chenery 1998; Rooker et al. 2001), and are prone to contamination due to handling and preparation procedures (Proctor and Thresher 1998). As the otolith in the salted treatment remained in the fish 20 days after treatment before

being removed, this may have had a small additional effect on the concentrations. Although other elements may not be absorbed as readily as Na, this finding suggests that fish remains may be affected by taphonomic processes and absorb elements after burial in other situations as well. Therefore, palaeoenvironmental reconstructions based on chemical analyses need to be conducted with caution. Further research into the post-depositional integration of trace elements into otoliths, bone and scales from sediments would be beneficial. The noticeable absorption of elements from the salt into the structures suggests that if fish have been anthropogenically processed using a salting method, this may be readily detectable through trace element analysis targeting Na:Ca concentrations, however, as salt is water-soluble, these concentrations may decrease post-deposition. Where this method of preservation is suspected, trace element analysis could confirm the practice (e.g., Yankowski et al. 2015).

Other trace elements within otoliths that were significantly affected by treatments were Zn:Ca (affected by BS, BC, RF) and Li:Ca (affected by BC), while Mg:Ca (affected by SD) was the only other element in the vertebrae that was affected. Zinc is a transitional metal present in the otolith protein matrix; its uptake is primarily via the gut (Miller et al. 2006) and it is prone to contamination. Elements, such as Zn, that are under physiological regulation, and are not bound within the aragonite lattice are more likely to leach out of otoliths (Elsdon et al. 2008); however, we found that the three treatments that impacted Zn:Ca had the effect of increasing the concentrations, rather than decreasing them, suggesting a contamination effect. The elements most frequently used in chemical analyses, Sr and Ba, remained relatively stable in the otoliths and vertebrae in comparison to the controls. In otoliths, these elements are incorporated into the structure via substitution for calcium (i.e., as $SrCO_3$) and are likely to reflect environmental parameters (Campana 1999; Doubleday et al. 2014; Elsdon et al. 2008). As such they are assumed to be least affected by post-mortem influences and are relatively insensitive to handling and preparation procedures (Proctor and Thresher 1998). Grupe and Hummel (1991) found that the proportion of Sr:Ca in human bone apatite increased with increased temperature in a predictable fashion, and that Ba was almost completely lost from the samples that had been heated above 800°C, suggesting that Sr/Ca is one of few trace elements that can be used for palaeodietry reconstructions from burnt bone. We observed other elements that also experienced no significant alteration in otoliths (Mg:Ca, Mn:Ca, Pb:Ca) and in vertebrae (Zn:Ca, Mn:Ca, Pb:Ca). These results differ from those of Andrus and Crowe (2002), who found that all eight elements analysed (Na, Sr, P, Zn, Ba, Mg, Pb, and Mn, reported as ppm) were affected by cooking treatments, with Sr, Zn, Mg, and Mn the most unstable. Their findings may be attributed to anomalies within individual fish, which might have been caused by physiological processes, or slight variations in preparation or storage methods.

The trace element analysis of the fish scales revealed different results relative to the otoliths and vertebrae, whereby Li:Ca, Ba:Ca, Pb:Ca, and Zn:Ca concentrations were not significantly different between the treatment and control groups. Conversely, Mg:Ca concentrations were greater in all treatment groups, and Sr:Ca was greater in all except the wrapped in clay group. Additionally, Mn:Ca concentrations were greater in the boiling in freshwater group. These data indicate that the chemistry of the hydroxyapatite in the scale structure reacts differently to the other fish hard-parts when heated. The alkaline earth elements, Mg and Sr were greater in the treatment groups, suggesting that the use of Sr as a palaeothermometer in scales is problematic, in agreement with Kalvoda *et al.* (2009), who found that early diagenetic alteration in fish scales is so profound that palaeoecological interpretations are not possible. Differences may also reflect that scales are in direct contact with the environment unlike otoliths and vertebrae.

Stable isotope analysis revealed that values were significantly different in groups that were heated when compared with control data in both the otoliths and the bone carbonate. The findings suggest that the higher the temperature or longer the duration of heating, the lower the otolith and vertebrae $\delta^{18}O$ values are, most notably in the burnt samples. As the salted method did not require heating, the isotope values of samples from this treatment are closest to the control values. The previous otolith cooking study (Andrus and Crowe 2002) also found that isotope values in burnt samples were significantly more negative than the control samples, but reported that no significant effects were observed as a result of the other cooking methods investigated (roasting over coals, roasting in an oven, boiling in freshwater and boiling in saltwater). They proposed that the process that caused the alteration of otolith aragonite to calcite in burnt samples also drove the exchange of oxygen and carbon in the carbonate lattice, indicating that more than structural changes took place. Aragonite is a metastable polymorph of calcium carbonate, which is prone to alteration under increased temperature or pressure (Waite and Swart 2015). This has also been identified in observations of aragonite that have been drilled/micromilled in preparation for isotopic analyses; aragonite was inverted to calcite and in the process $\delta^{18}O$ values were lowered (Waite and Swart 2015). We found, through XRD analysis, that only the burnt otolith had recrystallized to calcite, which supports Andrus and Crowe's (2002) proposition that a complex process takes place when otoliths are burnt, and is in agreement with other research examining the effects of burning carbonates (e.g., Larsen 2015; Zazzo et al. 2012). Oxygen isotopes were significantly altered in the biogenic aragonite of bivalve shells that had recrystallized into calcite after heating to 400ºC for one hour (Larsen 2015). Bone heated to high temperatures has been shown to experience a significant decrease in carbonate content and $\delta^{13}C$ values, with ^{14}C ages of the carbonate fraction of the samples indicating that carbon within the carbonate was replaced with carbon from the atmosphere of combustion (Zazzo et al. 2012). Our results suggest that it is likely this process also occurs within the carbonate of the fish otoliths as they are burnt, but the variations to isotope values in hard-parts that were not calcified, but merely heated, indicates that more research is required into the underlying mechanisms for these changes. While our findings are in agreement with some previous studies, they differ from those reported for fish otoliths, which were roasted at either 200, 275 or 350ºC for 1 h, with no significant differences between mean $\delta^{18}O$ and $\delta^{13}C$ isotope signatures from paired experiments with roasted and non-roasted fish otoliths detected (Guiguer et al. 2003).

It can be argued that the observed differences between treatments are a result of environmental variation experienced by the fish; however, we took numerous safeguards to ensure that any environmental or physiological factors did not significantly affect the data. As mentioned above, we selected the location of catch and the species of fish to ensure a realistic research design. In addition, we sampled all of our fish at one time from a single fisher at a single location and randomised fish to different treatments. We sampled both the nucleus and the edge chemistry of the otoliths, and the edge chemistry of the vertebrae. The edge data would be representative of the conditions at the time of fish collection. If there was an issue distinguishing cooking from environmental variation we would expect to see no significant differences among treatments and very large variability, which is not the case.

These findings have implications for palaeoenvironmental and diet reconstructions based on isotopic analyses of fish otoliths and bones; we observed changes in isotope values as a result of cooking treatments that did not noticeably alter the morphology of the samples. They also have implications for the way otoliths and vertebrae are prepared for analysis via embedding in resin and heating the sample in an oven to cure at temperatures exceeding 55ºC. In order to explore the effects that heating has on estimates of palaeotemperatures, we calculated the differences between temperature estimates based on a 1°C increase in ambient water resulting in a lowering of approximately 0.22‰ $\delta^{18}O$ in otolith aragonite. This relationship has been used by Rowell *et al.* (2005; 2008) to investigate changes in water temperature over time, and was

experimentally derived based on the sciaenid *Micropogonias undulatus* (Thorrold et al. 1997). We acknowledge that using an equation based on a fish with a similar physiology and life history to *A. japonicus* is important; however, we are using this equation as an example of potential errors that could eventuate from heating of otoliths. The estimated temperatures increased between 2.54°C and 6.70°C as a result of the different treatments, while the burnt samples resulted in an increase of 19.85±5.21°C (Table 4). Consequently, inaccurate palaeoenvironmental reconstructions could be attained by unknowingly using heated samples. As the morphological attributes of the remains were not affected by most treatments, determining which remains within an archaeological assemblage have been heated is, in itself, a challenge. Fortunately, burnt remains are distinguishable by colour and carbonization and can be avoided (Nicholson 1995; Shipman et al. 1984); exposure to heat for longer periods of time or at higher temperatures causes bone and otoliths to change from dark grey/black through to white (Spennemann and Colley 1989; Stiner et al. 1995). Discolouration of archaeological samples can occur post-depositionally, making them appear burnt, requiring advanced methods of visualizing heat induced change, such as Scanning Electron Microscopy (Shipman et al. 1984), Magnetic Resonance Imaging (Thompson and Chudek 2007) and X-ray Diffraction (Andrus and Crowe 2002) to be used. Samples that have been heated but not burnt are more difficult to determine. Boiling for brief periods of time has little distinguishing effect on bone in the short term, but extensive boiling increases porosity and crystallinity as protein is lost, and can mirror diagenetic effects observed in archaeological bone (Roberts et al. 2002). Transition Electron Microscopy has been used to investigate these changes in bone collagen as it is heated, and can be applied to archaeological remains to ascertain whether they have been cooked or not (Koon et al. 2003; Koon et al. 2010).

Table 4. Estimated temperature increases based on δ^{18}O values of otoliths.

Treatment	Mean δ^{18}O (‰)	±	Estimated temperature increase (°C)	±
			$x=(control\ \delta^{18}O - treatment\ \delta^{18}O)/0.22$	
Control	1.34	0.21		
Boiled freshwater	0.25	0.22	4.95	-0.04
Boiled saltwater	0.20	0.36	5.16	-0.67
Burnt completely	-3.03	1.36	19.85	-5.21
Roasted on fire	-0.08	0.38	6.46	-0.74
Salted	0.78	0.45	2.54	-1.07
Wrapped in clay	-0.13	0.54	6.70	-1.48

5. Conclusion

This experimental research has revealed complexities in the interpretation of chemical and isotopic analyses of fish remains. While it has highlighted the potential use of trace element analysis to identify salted fish remains, it has shown that other cooking methods can impact samples, thereby potentially leading to inaccurate palaeodiet and palaeoenvironmental reconstructions. In agreement with previous research, we recommend avoiding the use of burnt samples for trace element and stable isotope analyses, and using caution when interpreting data from any fish remains that may have been cooked or heated. Samples need to be prepared and handled using methods that reduce or limit alteration of their isotopic and chemical properties, while data needs to be validated based on individual site processes and conditions.

References

Anderson, M.J. and C.J.F. Ter Braak 2003 Permutation tests for multi-factorial analysis of variance. *Journal of Statistical Computation and Simulation* 73(2):85-113.

Andrus, C.F.T. and D.E. Crowe 2002 Alteration of otolith aragonite: effects of prehistoric cooking methods on otolith chemistry. *Journal of Archaeological Science* 29(3):291-299.

Arkhipkin, A.I., P.C. Schuchert and L. Danyushevsky 2009 Otolith chemistry reveals fine population structure and close affinity to the Pacific and Atlantic oceanic spawning grounds in the migratory southern blue whiting (*Micromesistius australis australis*). *Fisheries Research* 96(2-3):188-194.

Barrett, J.H., D. Orton, C. Johnstone, J. Harland, W. Van Neer, A. Ervynck, C. Roberts, A. Locker, C. Amundsen, I.B. Enghoff, S. Hamilton-Dyer, D. Heinrich, A.K. Hufthammer, A.K.G. Jones, L. Jonsson, D. Makowiecki, P. Pope, T.C. O'Connell, T. de Roo and M. Richards 2011 Interpreting the expansion of sea fishing in medieval Europe using stable isotope analysis of archaeological cod bones. *Journal of Archaeological Science* 38(7):1516-1524.

Bath, G.E., S.R. Thorrold, C.M. Jones, S.E. Campana, J.W. McLaren and J.W.H. Lam 2000 Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* 64(10):1705-1714.

Berndt, R.M., C.H. Berndt and J.E. Stanton 1993 *The World That was: The Yaraldi of the Murray River and the Lakes, South Australia*. Vancouver: UBC Press.

Boschin, F., C. Zanolli, F. Bernardini, F. Princivalle and C. Tuniz 2015 A look from the inside: microCT analysis of burned bones. *2015* 6(2):9.

Campana, S.E. 1999 Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* 188:263-297.

Campana, S.E., G.A. Chouinard, J.M. Hanson, A. Frechet and J. Brattey 2000 Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research* 46(1-3):343-357.

Colaninno, C.E. 2012 Evaluating $\delta^{18}O$ profiles of hardhead catfish and Atlantic croaker otoliths as a method of determining seasonal use of fishes. *Anthropological Papers of the American Museum of Natural History* 97:83-101.

Disspain, M.C.F., S. Ulm and B.M. Gillanders 2016 Otoliths in archaeology: Methods, applications and future prospects. *Journal of Archaeological Science: Reports* 6:623-632.

Disspain, M.C.F., L.A. Wallis and B.M. Gillanders 2011 Developing baseline data to understand environmental change: a geochemical study of archaeological otoliths from the Coorong, South Australia. *Journal of Archaeological Science* 38(8):1842–1857.

Disspain, M.C.F., C.J. Wilson and B.M. Gillanders 2012 Morphological and chemical analysis of archaeological fish otoliths from the Lower Murray River, South Australia. *Archaeology in Oceania* 47:141–150.

Doubleday, Z.A., H.H. Harris, C. Izzo and B.M. Gillanders 2014 Strontium randomly substituting for calcium in fish otolith aragonite. *Analytical Chemistry* 86:865-869.

Dove, S.G., B.M. Gillanders and M.J. Kingsford 1996 An investigation of chronological differences in the deposition of trace metals in the otoliths of two temperate reef fishes. *Journal of Experimental Marine Biology and Ecology* 205:15-33.

Dufour, E., C. Holmden, W. Van Neer, A. Zazzo, W.P. Patterson, P. Degryse and E. Keppens 2007 Oxygen and strontium isotopes as provenance indicators of fish at archaeological sites: the case study of Sagalassos, SW Turkey. *Journal of Archaeological Science* 34(8):1226-1239.

Elsdon, T.S. and B.M. Gillanders 2002 Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmnetal histories of fish. *Canadian Journal of Fisheries and Aquatic Science* 59(11):1796-1808.

Elsdon, T.S. and B.M. Gillanders 2003 Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecology Progress Series* 260:263-272.

Elsdon, T.S. and B.M. Gillanders 2005a Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. *Canadian Journal of Fisheries and Aquatic Science* 62:1143-1152.

Elsdon, T.S. and B.M. Gillanders 2005b Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. *Marine Ecology Progress Series* 285:233-243.

Elsdon, T.S., B.K. Wells, S.E. Campana, B.M. Gillanders, C.M. Jones, K.E. Limburg, D.E. Secor, S.R. Thorrold and B.D. Walther 2008 Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations, and inferences. *Oceanography and Marine Biology: an Annual Review* 46:296-330.

Ferguson, G.J., T.M. Ward, A. Ivey and T. Barnes 2014 Life history of *Argyrosomus japonicus*, a large sciaenid at the southern part of its global distribution: implications for fisheries management. *Fisheries Research* 151:148–157.

Fernandes, R., J. Meadows, A. Dreves, M.-J. Nadeau and P. Grootes 2014 A preliminary study on the influence of cooking on the C and N isotopic composition of multiple organic fractions of fish (mackerel and haddock). *Journal of Archaeological Science* 50:153-159.

Gabriel, S., N. Prista and M.J. Costa 2012 Estimating meagre (*Argyrosomus regius*) size from otoliths and vertebrae. *Journal of Archaeological Science* 39(9):2859-2865.

Gillanders, B.M. and A.R. Munro 2012 Hypersaline waters pose new challenges for reconstructing environmental histories of fish based on otolith chemistry. *Limnology and Oceanography* 57(4):1136-1158.

Grupe, G. and S. Hummel 1991 Trace element studies on experimentally cremated bone. I. Alteration of the chemical composition at high temperatures. *Journal of Archaeological Science* 18(2):177-186.

Guiguer, K.R.R.A., R. Drimmie and M. Power 2003 Validating methods for measuring δ^{18}O and δ^{13}C in otoliths from freshwater fish. *Rapid Communications in Mass Spectrometry* 17(5):463-471.

Hamer, P.A., G.P. Jenkins and P. Coutin 2006 Barium variation in *Pagrus auratus* (Sparidae) otoliths: a potential indicator of migration between an embayment and ocean waters in south-eastern Australia. *Estuarine, Coastal and Shelf Science* 68(3-4):686-702.

Homsey, L.K., R.B. Walker and K.D. Hollenbach 2010 What's for dinner? Investigating food-processing technologies at Dust Cave, Alabama. *Southeastern Archaeology* 29(1):182-196.

Hufthammer, A.K., H. Høie, A. Folkvord, A.J. Geffen, C. Andersson and U.S. Ninnemann 2010 Seasonality of human site occupation based on stable oxygen isotope ratios of cod otoliths. *Journal of Archaeological Science* 37(1):78–83.

Ishimaru, E., I. Tayasu, T. Umino and T. Yumoto 2011 Reconstruction of ancient trade routes in the Japanese Archipelago using carbon and nitrogen stable isotope analysis: identification of the stock origins of marine fish found at the inland Yokkaichi site, Hiroshima Prefecture, Japan. *Journal of Island and Coastal Archaeology* 6(1):160-163.

Jenkin, G. 1979 *Conquest of the Ngarrindjeri*. Adelaide: Rigby Ltd.

Kalish, J.M. 1991 ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. *Marine Ecology Progress Series* 75:181-203.

Kalvoda, J., M. Novak, O. Babek, R. Brzobohaty, M. Hola, I. Holoubek, V. Kanicky and R. Skoda 2009 Compositional changes in fish scale hydroxylapatite during early diagenesis; an example from an abandoned meander. *Biogeochemistry* 94(3):197-215.

Koon, H.E.C., R.A. Nicholson and M.J. Collins 2003 A practical approach to the identification of low temperature heated bone using TEM. *Journal of Archaeological Science* 30(11):1393-1399.

Koon, H.E.C., T.P. O'Connor and M.J. Collins 2010 Sorting the butchered from the boiled. *Journal of Archaeological Science* 37(1):62-69.

Larsen, S.C. 2015 Recrystallization of biogenic aragonite shells from archaeological contexts and implications for palaeoenvironmental reconstruction. Unpublished Master of Arts thesis, Western Washington University,

Long, K., N. Stern, I.S. Williams, L. Kinsley, R. Wood, K. Sporcic, T. Smith, S. Fallon, H. Kokkonen, I. Moffat and R. Grün 2014 Fish otolith geochemistry, environmental conditions and human occupation at Lake Mungo, Australia. *Quaternary Science Reviews* 88:82-95.

Lubinski, P.M. 1996 Fish heads, fish heads: an experiment on differential bone preservation in a salmonid fish. *Journal of Archaeological Science* 23(2):175-181.

Luebbers, R.A. 1982 *The Coorong Report: An Archaeological Survey of the Northern Coorong*, Unpublished report prepared for the South Australian Department for Environment and Planning.

Miller, M.B., A.M. Clough, J.N. Batson and R.W. Vachet 2006 Transition metal binding to cod otolith proteins. *Journal of Experimental Marine Biology and Ecology* 329(1):135-143.

Milton, D.A. and S.R. Chenery 1998 The effect of otolith storage methods on the concentrations of elements detected by laser-ablation ICPMS. *Journal of Fish Biology* 53(4):785-794.

Morales-Nin, B., A.J. Geffen, S. Perez-Mayol, M. Palmer, R. Gonzalez-Quiros and A. Grau 2012 Seasonal and ontogenic migrations of meagre (*Argyrosomus regius*) determined by otolith geochemical signatures. *Fisheries Research* 127-128:154-165.

Nicholson, R.A. 1993 An investigation into the effects on fish bone of passage through the human gut: some experiments and comparisons with archaeological material. *Circaea* 10(1):38-51.

Nicholson, R.A. 1995 Out of the frying pan into the fire: what value are burnt fish bones to archaeology? *Archaeofauna* 4:47-64.

Orton, D.C., D. Makowiecki, T. de Roo, C. Johnstone, J. Harland, L. Jonsson, D. Heinrich, I.B. Enghoff, L. Lõugas, W. van Neer, A. Ervynck, A.K. Hufthammer, C. Amundsen, A.K.G. Jones, A. Locker, S. Hamilton-Dyer, P. Pope, B.R. MacKenzie, M. Richards, T.C. O'Connell and J.H. Barrett 2011 Stable isotope evidence for late medieval (14th-15th C) origins of the eastern Baltic cod (Gadus morhua) fishery. *PLoS ONE* 6(11):e27568.

Privat, K., T. O'Connell and R. Hedges 2007 The distinction between freshwater- and terrestrial-based diets: methodological concerns and archaeological applications of sulphur stable isotope analysis. *Journal of Archaeological Science* 34(8):1197-1204.

Proctor, C.H. and R.E. Thresher 1998 Effects of specimen handling and otolith preparation on concentration of elements in fish otoliths. *Marine Biology* 131:681-694.

Quinn, T.J. and R.B. Deriso 1999 *Quantitative Fish Dynamics*. New York: Oxford University Press.

Reis-Santos, P., S.E. Tanner, T.S. Elsdon, H.N. Cabral and B.M. Gillanders 2013 Effects of temperature, salinity and water composition on otolith elemental incorporation of Dicentrarchus labrax. *Journal of Experimental Marine Biology and Ecology* 446(0):245-252.

Richter, J. 1986 Experimental study of heat induced morphological changes in fish bone collagen. *Journal of Archaeological Science* 13(5):477-481.

Roberts, S.J., C.I. Smith, A. Millard and M.J. Collins 2002 The taphonomy of cooked bone: characterizing boiling and its physico-chemical effects. *Archaeometry* 44(3):485-494.

Robins, R. and E.C. Stock 1990 The burning question: a study of molluscan remains from a midden on Moreton Island. In S. Solomon, I. Davidson and D. Watson (eds), *Problem Solving in Taphonomy*, pp.80-100. St Lucia: University of Queensland Anthropology Museum.

Robson, H., S. Andersen, O. Craig, A. Fischer, A. Glykou, S. Hartz, H. Lübke, U. Schmölcke and C. Heron 2012 Carbon and nitrogen isotope signals in eel bone collagen from Mesolithic and Neolithic sites in northern Europe. *Journal of Archaeological Science* 39(7):2003-2011.

Rooker, J., V. Zdanowicz and D. Secor 2001 Chemistry of tuna otoliths: assessment of base composition and postmortem handling effects. *Marine Biology* 139(1):35-43.

Rowell, K., D. Dettman and R. Dietz 2010 Nitrogen isotopes in otoliths reconstruct ancient trophic position. *Environmental Biology of Fishes* 89(3):415-425.

Rowell, K., K. Flessa, D.L. Dettman and M. Roman 2005 The importance of Colorado River flow to nursery habitats of the Gulf corvina *(Cynoscion othonopterus). Canadian Journal of Fisheries and Aquatic Science* 62:2874-2885.

Rowell, K., K. Flessa, D.L. Dettman, M. Roman, L.R. Gerber and L.T. Findley 2008 Diverting the Colorado River leads to a dramatic life history shift in an endangered marine fish. *Biological Conservation* 141:1138-1148.

Shipman, P., G. Foster and M. Schoeninger 1984 Burnt bones and teeth: an experimental study of color, morphology, crystal structure and shrinkage. *Journal of Archaeological Science* 11(4):307–325.

Spennemann, D.H.R. and S.M. Colley 1989 Fire in a pit: the effects of burning on faunal remains. *Archaeozoologia* 3:51-64.

Spötl, C. and T.W. Vennemann 2003 Continuous-flow isotope ratio mass spectrometric analysis of carbonate minerals. *Rapid Communications in Mass Spectrometry* 17(9):1004-1006.

Stiner, M.C., S.L. Kuhn, S. Weiner and O. Bar-Yosef 1995 Differential Burning, Recrystallization, and Fragmentation of Archaeological Bone. *Journal of Archaeological Science* 22(2):223-237.

Surge, D. and K.J. Walker 2005 Oxygen isotope composition of modern and archaeological otolilth from the estuarine hardhead catfish (*Ariopsis felis*) and their potential to record low-latitude climate change. *Palaeogeography, Palaeoclimatology, Palaeoecology* 228:179-191.

Tanner, S.E., P. Reis-Santos, R.P. Vasconcelos, V.F. Fonseca, S. França, H.N. Cabral and S.R. Thorrold 2013 Does otolith geochemistry record ambient environmental conditions in a temperate tidal estuary? *Journal of Experimental Marine Biology and Ecology* 441(0):7-15.

Taplin, G. 1879 The Narrinyeri. In J.D. Woods (ed.), *The Native Tribes of South Australia*, pp.1–156. Adelaide: E.S. Wigg and Son.

Thompson, T.J.U. and J.A. Chudek 2007 A novel approach to the visualisation of heat-induced structural change in bone. *Science & Justice* 47(2):99-104.

Thorrold, S.R., S.E. Campana, C.M. Jones and P.K. Swart 1997 Factors determining δ^{13}C and δ^{18}O fractionation in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* 61(14):2909-2919.

Thorrold, S.R., C.M. Jones, P.K. Swart and T.E. Targett 1998 Accurate classification of juvenile weakfish Cynoscion regalis to estuarine nursery areas based on chemical signatures on otoliths. *Marine Ecology Progress Series* 173:253-265.

Waite, A.J. and P.K. Swart 2015 The inversion of aragonite to calcite during the sampling of skeletal archives: Implications for proxy interpretation. *Rapid Communications in Mass Spectrometry* 29(10):955-964.

Wang, T., D. Surge and K.J. Walker 2011 Isotopic evidence for climate change during the Vandal Minimum from *Ariopsis felis* otoliths and *Mercenaria campechiensis* shells, southwest Florida, USA. *Holocene* 21(7):1081-1091.

Wang, T., D. Surge and K.J. Walker 2013 Seasonal climate change across the Roman Warm Period/Vandal Minimum transition using isotope sclerochronology in archaeological shells and otoliths, southwest Florida, USA. *Quaternary International* 308–309(0):230-241.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan and C.M. Jones 2003 Relationships between water, otolith, and scale chemistries of Westslope cutthroat trout from 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(3):409-424.

West, C.F., S. Wischniowski and C. Johnston 2012 Pacific cod (*Gadus macrocephalus*) as a paleothermometer: otolith oxygen isotope reconstruction. *Journal of Archaeological Science* 39(10):3277-3283.

Willis, L.M. and A.R. Boehm 2014 Fish bones, cut marks, and burial: implications for taphonomy and faunal analysis. *Journal of Archaeological Science* 45:20-25.

Willis, L.M., M.I. Eren and T.C. Rick 2008 Does butchering fish leave cut marks? *Journal of Archaeological Science* 35(5):1438-1444.

Yankowski, A., P. Kerdsap and D. Chang 2015 "Please pass the salt"- An ethnoarchaeological study of salt and salt fermented fish production, use and trade in northeast Thailand. *Journal of Indo-Pacific Archaeology* 37.

Zazzo, A., J.-F. Saliège, M. Lebon, S. Lepetz and C. Moreau 2012 Radiocarbon dating of calcined bones: Insights from combustion experiments under natural conditions *Radiocarbon* 54(3/4):855-866.

Zazzo, A., G.R. Smith, W.P. Patterson and E. Dufour 2006 Life history reconstruction of modern and fossil sockeye salmon (*Oncorhynchus nerka*) by oxygen isotopic analysis of otoliths, vertebrae and teeth: implications for palaeoenvironmental reconstructions. *Earth and Planetary Science Letters* 249:200-215.

Zohar, I. and R. Cooke 1997 The impact of salting and drying on fish bones: preliminary observations on four marine species from Parita Bay, Panama. *Archaeofauna* 6:59-66.