

ELEMENTAL SIGNATURES IN VERTEBRAE PROVIDE ECOLOGICAL CONTEXT FOR THE LIFE-HISTORY OF SPINY DOGFISH IN AN IMPACTED TASMANIAN ESTUARY

David Moreno, Jeremy M. Lyle and
Justin D. Bell

September 2018



**Holsworth Wildlife
Research Endowment**



Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49,
Hobart TAS 7001

Enquires should be directed to:

Dr Jeremy Lyle

Institute for Marine and Antarctic Studies

University of Tasmania

Private Bag 49, Hobart, Tasmania 7001, Australia

Jeremy.Lyle@utas.edu.au

Ph. (03) 6226 8277

Fax (03) 6227 8035

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a reader's particular circumstance. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the Institute for Marine and Antarctic Studies (IMAS) or the University of Tasmania (UTas).

© The Institute for Marine and Antarctic Studies, University of Tasmania 2018.

Copyright protects this publication. Except for purposes permitted by the Copyright Act, reproduction by whatever means is prohibited without the prior written permission of the Institute for Marine and Antarctic Studies.

Elemental signatures in vertebrae provide ecological context for the life-history of spiny dogfish in an impacted Tasmanian estuary

Abstract

Elemental analysis of hard parts in elasmobranchs can potentially elucidate ontogenetic patterns of movement and trophic ecology, as well as inform stock discrimination and ageing. Analysing trace metals in the calcifying matrix can reveal specific chemical signatures that may be linked to environmental conditions experienced by an individual at different times of its life.

The spiny dogfish (*S. acanthias*) in Macquarie Harbour (Western Tasmania) are ideally suited for elemental analysis, given that the estuary has a unique bio-geochemical profile including multi-sourced anthropogenic pollutants. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to analyse the microchemical composition of shark vertebrae from individuals caught in Macquarie Harbour and a comparison site. The observed differences in the multiple elements showed that the chemical signatures can be successfully used to discriminate between the two populations and to a lesser extent, between sexes at each site. Element: Ca ratios and Sr: Ba patterns suggest that spiny dogfish remain resident within Macquarie Harbour for most of their lives, providing valuable ecological context to interpret recent observations about the life-history of the population.

Analysis of multi-element signatures using change point analysis was attempted for the first time and revealed that this approach may be a valuable tool for age determination in elasmobranch species. The results from this study provide further support for the use of elemental analysis for the study of elasmobranch species and highlight the need for more research into innovative ways to analyse these complex datasets.

INTRODUCTION

Sharks and their relatives (i.e. class Chondrichthyes) fill valuable ecological roles as predators across a wide variety of marine ecosystems in the world's oceans (Myers *et al.*, 2007). These species tend to have K-selected life histories characterized by late maturation, slow growth and reduced fecundity, resulting in long population recovery times once depleted (Cortés, 2002). Therefore, the persistence of many species is threatened by overexploitation, changing climatic conditions and habitat degradation (Dulvy *et al.*, 2008). However, the development of effective management strategies can be limited by a general lack of knowledge about their life-history and movement ecology (Musick *et al.*, 2000). This information is critical to the understanding of a population's demographics (Cortés, 2002), stock boundaries (Edmonds *et al.*, 1991, Kerr and Campana, 2014), distribution (Bell *et al.*, 2016), as well as identifying key areas used by the species during vulnerable stages of their ontogeny (e.g. nurseries, feeding grounds, etc) (Heupel and Simpfendorfer, 2008).

Conventional methods employed to study the movement and life history traits of sharks can be constrained by inadequate temporal or spatial coverage, particularly in long lived species (Cortés, 2002). Helping to overcome some of those limitations, technological advances in spectral analysis have resulted in the development of new tools that can measure the microchemical composition of calcifying structures such as bone. Site specific environmental chemistry is influenced by differences in physical characteristics (currents, geology, etc) (Campana and Thorrold, 2001, Christiansen, 2011) as well as biological inputs and terrestrial runoff (Estrada *et al.*, 2006, Fisk *et al.*, 2002), including from anthropogenic sources. Chemical fingerprinting takes advantage of the fact that individuals absorb elements through their gills, digestive tract and skin, which are subsequently incorporated into hard tissues at concentrations that can be reflective of the environment (Martin *et al.*, 2015) and contributions from dietary sources (Estrada *et al.*, 2006).

Elements that get incorporated in concentrations proportional to their environmental abundance create a chemical signature that can be used as a natural tag to track movements between distinct geographic regions (Elsdon and Gillanders, 2005). In teleost fish, bone tissue is permanently mineralized and is not susceptible to reworking or leaking (Campana and Thorrold, 2001), making structures like otoliths ideal for analysing patterns through the lifetime of the individual. This ecological timeframe is highly relevant to management, improving information obtained through conventional tagging (short term) (McFarlane and King, 2003) or genetic techniques (long term population trends) (Heist, 2004). Therefore, elemental analysis in teleost fish is now a commonplace tool used in stock discrimination (Edmonds *et al.*, 1991), identification of ontogenetic movement and trophic patterns, as well as validation of age estimates (Campana and Thorrold, 2001, Kerr and Campana, 2014).

Unlike otoliths, which are composed of highly crystalized aragonite, elasmobranchs have cartilaginous endoskeletons composed of an extracellular matrix, mineralized with crystals of calcium phosphate hydroxyapatite (Porter *et al.*, 2006). Recent work suggests that apatite secretion in elasmobranch vertebral centra is comprised of areolar cartilage and is metabolically inert (Dean and Summers, 2006). Elements are mainly incorporated into apatite through Ca substitution and therefore, the chemical stability of vertebrae tissue makes it appropriate for use in elemental analysis (McMillan *et al.*, 2017a). Furthermore, vertebral

centra are already commonly used for age determination in elasmobranchs (Cailliet *et al.*, 2006, Harry, 2018), making them ideal for exploring age specific ecological questions.

The use of vertebrae chemistry as a tool in the study of elasmobranchs goes as far back as the 1960s, when x-ray spectroscopy was used to determine if changes in Ca profiles could be used to determine the age of an individual (Jones and Geen, 1977). Elemental analysis has also been used to validate deposition rates of growth increments in shark vertebrae (Campana *et al.*, 2006), as well as reconstructing the feeding and movement ecology of long lived species (Estrada *et al.*, 2006). More recently, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has emerged as a preferred method to measure trace element signatures in hard tissues (McMillan *et al.*, 2017a, Christiansen, 2011). This technique has a relatively high precision and allows specific regions of the sample to be targeted, making it possible to resolve fine temporal changes in elemental concentrations that occurred throughout an individual's lifetime (Mokgalaka and Gardea-Torresdey, 2006). One important limitation for the use of these techniques is the lack of knowledge regarding the element-specific biological pathways that influence incorporation into the calcifying matrix in elasmobranch fishes (Porter *et al.*, 2006, Dean and Summers, 2006). However, there is strong indirect evidence to support the adequacy of using vertebral elemental signatures as environmental indicators in elasmobranchs. For example, elemental fingerprinting has been used successfully to infer movement patterns of juvenile sawfish (*Pristis pectinata*) (Scharer *et al.*, 2012), identify broad scale movements in bull shark (*Carcharhinus leucas*) (Lewis *et al.*, 2016) as well as delineating stock structure in deep-sea sharks (McMillan *et al.*, 2017b).

Study species

Spiny dogfish (*Squalus acanthias*) is a small shark with a global anti-tropical distribution (Fordham *et al.*, 2006). The ubiquitousness and general abundance of this shark is suggestive of the species' ecological importance as a mid-level predator (Beamish *et al.*, 2009). It is conventionally considered to have one of the most conservative life-histories amongst elasmobranchs, particularly for a small coastal species (Cortés, 2002). As a result, several populations in the Northern Hemisphere have experienced declines as high as 95% following a long history of interactions with fisheries as both a target and by-product (Beamish *et al.*, 2009), prompting the International Union for the Conservation of Nature (IUCN) to classify them as vulnerable or endangered (Fordham *et al.*, 2006). However, little is known about the spiny dogfish in Australia, where the species is a common bycatch in various fisheries (Moreno, 2018).

A recent study of spiny dogfish in Macquarie Harbour (western Tasmania) identified aspects of the life-history and movement ecology that appear to differ from the traits typical from other populations throughout the species' distributional range (Moreno 2018). For example, acoustic tracking revealed no evidence of large-scale seasonal migratory movement by adult spiny dogfish out of Macquarie Harbour (Moreno *et al.*, 2016), a finding supported by the year-round capture of the species in the estuary (Lyle *et al.*, 2014). Furthermore, observations of their reproductive biology indicate that few pregnant females go to full-term, giving rise to the possibility of general reproductive failure. This, coupled with a feeding ecology now heavily reliant on spillover feed pellets from aquaculture operations (Moreno 2018) gives rise to concern over the status of the spiny dogfish population in Macquarie Harbour. However, in the absence of historical data it is not possible to determine if the observed life-history traits and movement ecology are representative of the population in the long-term, or a recent

development. Resolving these questions, including whether individuals remain resident within the estuary throughout their lives, is crucial for an adequate assessment of the demographic status of this potentially isolated and distinct population.

Macquarie Harbour is a semi-enclosed estuarine system with a surface area of ~275 km² that is connected to the ocean through a narrow and shallow opening. It serves as the catchment of the King and Gordon rivers, resulting in a high influx of freshwater that produces a highly stratified water column (Ross and MacLeod, 2017). Historically, these rivers have been heavily utilized by the mining industry for dewatering, leaching heavy metals into the water and substrate of Macquarie Harbour (Stauber *et al.*, 2000). Because of the harbour's stratified water column and geomorphology, water mixing with the ocean is poor (Carpenter *et al.*, 1991), creating a distinct chemical environment that may be useful to define long term residency patterns of the spiny dogfish. Furthermore, the introduction of salmonid aquaculture operations into Macquarie Harbour in the late 1980s, and subsequent large-scale expansion during the early 2000s (Ross and MacLeod, 2017), has resulted in the input of large amounts of food (i.e. feed pellets) foreign to the system. As spiny dogfish appear to utilise spillover pellets as a key source of food, it is possible that the chemical signature of the pellets could serve as an unintended tag if incorporated into their vertebrae.

Study objectives

This study was motivated by the unique characteristics of the spiny dogfish population in Macquarie Harbour and the need for novel approaches to aid in the management and conservation of the species. The main goal of this study was to assess the suitability of modern elemental analysis techniques to the exploration of the life history of the spiny dogfish of Macquarie Harbour. Specifically, it was hoped this would allow a better understanding of the movement and trophic ecology of the population, as well as the development of a novel approach for ageing and age validation in sharks. The specific objectives of this project were:

1. To explore the use of elemental analysis techniques (e.g. LA-ICP-MS) in developing a method to analyse isotopic and elemental samples from concentric growth bands in the vertebrae of the spiny dogfish.
2. To reference the isotopic and elemental record in the vertebrae to create a model of changes in habitat and trophic ecology through an individual's lifetime.
3. To contrast the results of elemental analysis of spiny dogfish from Macquarie Harbour with those of a separate oceanic population to explore the potential isolation of the Harbour's population.
4. To evaluate the use of chemical signatures resulting from interaction with marine farming operations as a viable marker for ageing and validation using elemental records from vertebrae.

METHODS

Sample collection

Spiny dogfish were collected from Macquarie Harbour during seasonal fisheries independent surveys conducted between October 2013 and March 2015 (Figure 1). Additional samples from a comparison population were collected from South East Tasmania (Frederick Henry Bay) in March 2015. Individuals were captured using long-lines consisting of a 250 m weighted lead core rope with 50 evenly spaced baited hooks. Average soak time was under two hours.

Total length (TL) and sex were recorded, and individuals retained for biological examination were humanely euthanised and stored frozen until biological examination. A section containing ~5 individual vertebrae was removed from the post-cranial region. While it has been shown that there are no significant differences in the chemical composition of adjacent vertebrae (Lewis *et al.*, 2017) it is unclear if differences between sections of the spinal column exist. By standardising the sampling, it was hoped to reduce any impact that vertebral position in the spinal column might have on its chemical structure. The individual vertebrae were separated, the neural arches were removed using a scalpel blade and the samples were frozen and stored until further analysis.

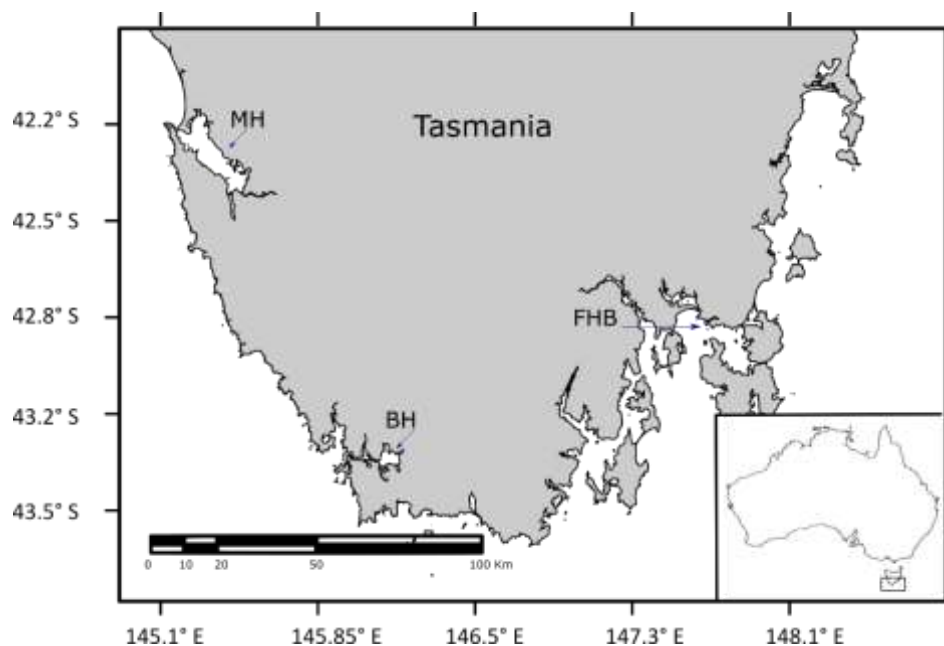


Figure 1: Sampling locations of spiny dogfish in west (Macquarie Harbour) and South East (Frederick Henry Bay) Tasmania.

Sample preparation

Individual vertebrae were thawed, and excess tissue was carefully removed using a scalpel blade. Vertebrae were submerged in a bleach bath for 5 minutes and immediately rinsed under running water for 30 minutes. Bleach helps remove soft tissue from the sample but does not affect subsequent elemental analysis (Lewis *et al.*, 2017). Cleaned vertebrae were imbedded

into a 2-step polyester resin block and sectioned along the sagittal plane using a high speed isomet saw (Gemmasta Deluxe GS6D) to create a thin-section of approximately 500 μm . Sections were sonicated for 10 min in distilled H_2O and allowed to air dry in a covered extraction hood. The dry samples were mounted onto glass microscope slides using double-sided sticky tape and stored in covered containers to prevent contamination. Prior to analysis, samples were cleaned with 95% ethanol and transferred to a vacuum chamber for 24 hr.

Thin sections were photographed using light microscopy and the postnatal area of each vertebra was measured using the Image J software package (Schindelin *et al.*, 2012). The post-natal section was considered span from the slight angle change following the birth band onto the outer most edge of the corpus calcareum.

Elemental analysis

Sample order was randomized, and LA-ICP-MS transects were pre-programmed using the inbuilt camera. Analysis was conducted using a NewWave NWR213-ESI laser system coupled to a quadrupole ICP-MS hosed at the University of Tasmania. Transects were drawn along the sagittal plane of the vertebrae starting at the focus through the centre of the corpus calcareum (Figure 2). The transect was extended past the vertebrae edge into the resin mount to ensure that the most recent material was analysed.

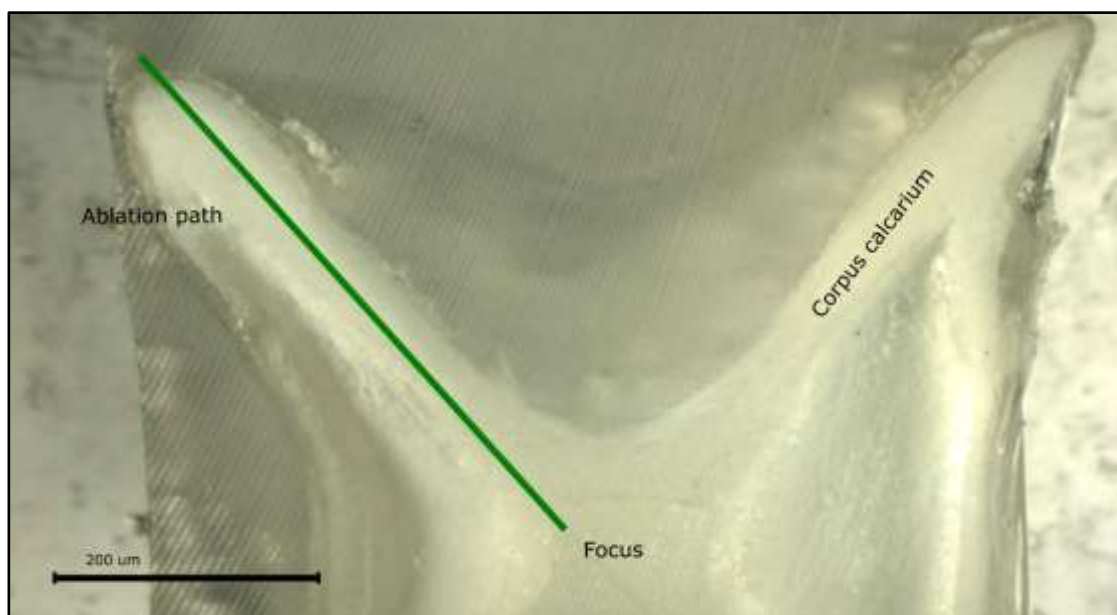


Figure 2: Digital photograph of a vertebral sagittal section denoting the ablation transect used for elemental analysis

For each sample, analysis consisted of a pre-ablation run (to clean the sample), a 28s blank (to measure background concentration levels) and an ablation run (to conduct the main analysis). Ablation parameters were kept consistent between runs, i.e. spot size of 43 μm in diameter moving at a scan speed of 10 $\mu\text{m/s}$ with a 10 Hz refresh rate and intensity of 55 mJ. The list of elements and isotopes measured can be found in Table 1

Table 1: Element list included in ablation analysis and mean detection limits calculated in parts per million.

Element	Detection limit (ppm)
Internal standard	
Ca ⁴³	5.5
Isotope ratio analysis	
Sr ⁸⁷	0.038
Sr ⁸⁸	0.005
Pb ²⁰⁶	0.013
Pb ²⁰⁷	0.014
Pb ²⁰⁸	0.008
Multi- element list	
Li ⁷	0.008
Na ²³	0.47
Mg ²⁴	0.11
Al ²⁷	0.017
Mn ⁵⁵	0.028
Fe ⁵⁶	0.53
Co ⁵⁹	0.004
Cu ⁶³	0.013
Zn ⁶⁶	0.024
As ⁷⁵	0.069
Ba ¹³⁷	0.002
Sn ¹¹⁸	0.01
Cd ¹¹¹	0.016
U ²³⁸	0.0002

Reference materials were ablated after every 15 samples to account for instrumental drift and precision (i.e. United States Geological survey MAC3 and BCR-2, National institute of standards and technology 612, and Durango apatite) (Jochum *et al.*, 2005) (Pearce *et al.*, 1997). Detection thresholds were calculated for each isotope and only those consistently above detection limits were kept for further analysis. Raw count data was exported to spreadsheets and processed for data reduction using an in-house macro (Centre for Ore Deposits and Earth Sciences, University of Tasmania). The average background levels in the pre-analysis blank were subtracted from the data and calcium (Ca) was used as an internal standard (35%) to transform count data into ppm and subsequently to molar masses (Tillett *et al.*, 2011).

Data analysis

Statistical analysis and data manipulation were conducted using R statistical software (R Core Team 2018). A rolling moving average and moving median filters with a 5-observation window were fitted to smooth the data and remove some instrumental noise and outliers (Munksgaard *et al.*, 2004). Experimental studies suggest that chemical signatures can be incorporated into

vertebrae in as little as three weeks (Smith *et al.*, 2013). Data from the outermost 100 µm of every individual were considered to represent the capture region.

Differences in the multi-element signature between factors was analysed using permutation analysis of variance (PERMANOVA). The individuals captured in marine waters in South East Tasmania were included into the analysis as a comparison population (site). Spiny dogfish can display sex specific movement and area use patterns, so sex was nested within site to determine if there were site specific differences in the chemistry of males and females. To further explore sex specific differences, site specific dissimilarities between sexes were calculated using analysis of similarity (ANOSIM), and the proportional contribution of each element to the dissimilarity profiles was calculated using similarity percentage analysis (SIMPER).

Site based discrimination based on the multi-element signature of the capture region was modelled using linear discriminant function analysis (LDFA) as measured by Euclidean distances. Because of the large differences in concentrations between some of the elements, the data were standardized by column centering. A stepwise LDFA approach was implemented using all the measured elements to determine the optimal element list to maximize group distances by removing variables with little explanatory power (< 5%). Variable significance was assessed using the calculated p statistic (alpha 0.05), and the proportion of variance in the linear discriminants explained by the individual elements was indicated by the R² value.

Lifelong concentrations of element: Ca ratios were plotted for all individuals to explore ontogenetic changes. Additionally, isotope ratios that could be related to bio-geochemical processes were calculated and plotted, including Sr: Ba, Sr⁸⁸:Sr⁸⁷ (freshwater proximity) and Pb²⁰⁷:Pb²⁰⁶ (mining sourced Lead).

Age determination

To explore the viability of using elemental signatures as a tool for age determination, change point analysis was used to provide a statistical framework for the classification of significant sections. A multivariate, non-parametric change point analysis was used based on a divisive algorithm using the *ecp* package in R (James and Matteson, 2013). Hierarchical divisive estimation is a bisection algorithm that detects change points sequentially using a permutational analysis based on distributional changes, including changes in the variance/covariance of multivariate data. The statistical significance of the change points is determined using the energy statistic introduced by Székely & Rizzo (2010) to measure the divergence between the distribution of two random vectors. A value of $\sigma_{0.05}$ was chosen as the cut-off for significance (Székely and Rizzo, 2010).

Age estimates were obtained through traditional visual estimation using an adjacent vertebra for all individuals. Vertebrae were sectioned (~400 µm) through the focus on the sagittal plane and placed into tissue cassettes and decalcified with trical-decalcifying fluid (CH₂O₂ 7.6%, HCl 1.6%, Australian Biostain) under agitation for a minimum of 1 hr or until the tissue was fully decalcified. Samples were then rinsed in running water for 1 hr to stop the decalcification process. Sectioned vertebrae were then stained using modified Harris Haematoxylin procedure outlined in Bubley *et al.* (2012) and mounted onto glass microscope slides to be aged using under magnification. Age estimates and further details on the used methodology can be found in Moreno (2018).

RESULTS

Elemental analysis

In total 41 spiny dogfish were analysed using LA-ICP-MS (22 females and 19 males). Individuals captured in Macquarie Harbour (n=31) ranged from 514 – 980 mm TL and individuals from South East Tasmania (n=10) ranged from 473 – 655 mm TL (Table 2).

Table 2: Capture details for all individuals used in LA-ICP-MS analysis. MH Macquarie Harbour; SE South East Tasmania. Ages obtained from Moreno (2018).

Shark Id	Site	Capture date		Sex	Age (yr.)	Total length (mm)
		MM	YYYY			
A05	MH	11	/ 2013	F	23	960
A07	MH	11	/ 2013	F	27	975
A15	MH	11	/ 2013	M	16	689
A22	MH	11	/ 2013	F	20	744
A25	MH	2	/ 2014	M	16	678
B01	MH	2	/ 2014	M	13	667
B10	MH	2	/ 2014	F	18	762
B11	MH	2	/ 2014	M	11	612
B14	MH	5	/ 2014	M	6	514
C01	MH	5	/ 2014	F	13	635
C04	MH	5	/ 2014	F	11	591
C05	MH	5	/ 2014	F	25	905
C20	MH	5	/ 2014	F	13	743
C26	MH	8	/ 2014	F	28	959
D02	MH	8	/ 2014	F	23	934
D09	MH	8	/ 2014	M	12	679
D14	MH	8	/ 2014	M	11	588
D18	MH	8	/ 2014	F	12	694
D22	MH	11	/ 2014	F	27	980
E01	MH	11	/ 2014	F	25	975
E06	MH	11	/ 2014	M		669
E11	MH	11	/ 2014	F	14	591
E12	MH	11	/ 2014	F	16	857
E17	MH	11	/ 2014	F	20	811
E19	MH	11	/ 2014	F		810
E21	MH	11	/ 2014	M	12	646
E24	MH	11	/ 2014	M	14	680
E28	MH	11	/ 2014	M	10	689
F08	MH	2	/ 2015	M	9	647
F09	MH	2	/ 2015	M	11	619
F13	MH	2	/ 2015	M	10	616
SF01	SE	3	/ 2015	F	13	543
SF02	SE	3	/ 2015	F	7	485
SF05	SE	3	/ 2015	F	11	511
SF07	SE	3	/ 2015	F	12	520
SF10	SE	3	/ 2015	F	12	541
SM06	SE	3	/ 2015	M	16	655
SM07	SE	3	/ 2015	M	13	563
SM15	SE	3	/ 2015	M	12	494
SM28	SE	3	/ 2015	M	17	647
SM29	SE	3	/ 2015	M	11	479

The limit of detection (LOD) for all elements assayed are presented in Table 1. Mercury gas (Hg) is used in the ablation process and therefore contamination in the laser cell may occur, therefore Hg values were excluded from further analyses. Values of Vanadium (V^{51}), Silver (Ag^{107}) and Thorium (Th^{232}) were consistently below LOD levels and were therefore also excluded.

Regional differences

Results from the stepwise LDFA by site indicated that Cadmium (Cd^{111}), Silicon (Si^{29}), Tin (Sn^{118}), and Uranium (U^{238}) contributed very little to differentiation ($R^2 < 0.01$) and were excluded from the final multi-element list (Figure 3). Multi-element chemical signatures differed significantly between site ($F=354.68$, $P<0.001$) and sex ($F=166.61$, $P<0.001$) based on PERMANOVA using Euclidean distances and 10000 permutations. To explore the site-specific dissimilarities between sexes, a post-hoc ANOSIM was implemented using a Bonferroni corrected sigma value ($\alpha=0.025$). Pairwise ANOSIM indicates that it is unlikely for the sex specific dissimilarities to have resulted from random variation based on the p values (Table 3). R-values in ANOSIM can range from 0 to 1, indicating the similarity / dissimilarity between the multi-element signatures between males and females at each site (Table 3).

Correct predictions: 89.62% (MH F: 85%; MH M: 95.21%; SE F: 87%; SE M: 94.5%)

	MH F n: 680	MH M n: 480	SE F n: 200	SE M n: 200	R-Squared	p
Li7	0.17	-0.58	2.01	-0.04	0.64	< .001
Na23	0.09	-0.75	0.70	0.69	0.31	< .001
Mg24	0.22	-0.11	-0.16	-0.33	0.04	< .001
Al27	0.53	-0.41	-0.44	-0.37	0.22	< .001
Mn55	-0.36	-0.34	1.36	0.67	0.39	< .001
Fe56	0.45	-0.21	0.51	-0.52	0.17	< .001
Co59	0.54	-0.35	-0.57	-0.44	0.23	< .001
Cu63	0.48	-0.50	-0.46	-0.02	0.21	< .001
Zn66	0.55	-0.37	-0.50	-0.46	0.23	< .001
As75	0.44	-0.13	0.74	1.04	0.30	< .001
Sr88	0.10	-0.62	0.51	0.44	0.19	< .001
Ba137	0.50	0.33	-1.36	-1.12	0.54	< .001
Pb208	0.30	-0.60	0.30	0.13	0.17	< .001

n = 1560 cases used in estimation; null hypotheses: two-sided; multiple comparisons correction: False Discovery Rate correction applied simultaneously to the entire table

Figure 3: Mean contribution to the linear discriminant analysis for each element in the multi-element list. Discriminator performance is described by R^2 , which represents the proportion of variance for each element that is explained by the categories

Standardized concentrations of the multi-element signature were used to analyse the similarity contribution of individual elements to the differences between males and females in Macquarie Harbour. SIMPER analysis showed Sr as being the main contributor to the dissimilarity between the sexes, with an average contribution of 0.23 to the scales multi-element signature (Table 3).

Table 3: Pairwise ANOSIM results for sex dissimilarities at each site (MH and SE) and SIMPER analysis of males and females in MH.

ANOSIM							
		R	p			R	p
MH				SE			
Sex		0.137	< 0.001	Sex		0.18	< 0.001

SIMPER	MH F	MH M					
	average	sd	ratio	ava	avb	cumsum	p
Sr88	0.234	160.2	0.001	0.156	-0.618	0.133	0.003996
Fe56	0.212	72.5	0.003	0.453	-0.214	0.253	0.060939
Al27	0.195	62.0	0.003	0.531	-0.413	0.363	0.006993
Co59	0.193	109.5	0.002	0.540	-0.346	0.473	0.116883
Zn66	0.184	73.5	0.003	0.549	-0.375	0.577	0.14985
Mg24	0.182	120.7	0.002	0.218	-0.105	0.680	0.082917
Cu63	0.161	56.9	0.003	0.493	-0.499	0.772	0.17982
Na23	0.156	61.4	0.003	0.094	-0.746	0.860	0.236763
Pb208	0.153	88.2	0.002	0.301	-0.604	0.946	0.413586
Li7	0.055	33.3	0.002	-0.170	-0.582	0.977	0.405594
Ba137	0.038	53.0	0.001	0.498	0.328	0.999	0.473526
As75	0.009	9.2	0.001	-0.436	-0.127	1.004	0.45954
Mn55	-0.006	20.9	0.000	-0.359	-0.337	1.000	0.622378

The use of a linear discriminant function analysis instead of canonical analysis of principal coordinates allowed for prior knowledge of group classification based on capture region to be incorporated into the model to maximize group separation based on the multi-element signature (Figure 4). Mean cross-validated classification accuracies from LDFA was > 89% when using capture region data as the training dataset (Figure 5). Most misallocations in the model occurred between sexes but not by site, suggesting that site classification accuracy was close to 100%. The elements explaining the highest proportion of variance within each category were Lithium (Li⁷), Barium (Ba¹³⁷) and Manganese (Mn⁵⁵) (Figure 3). Values of Li and Mn were negatively correlated to Macquarie Harbour, suggesting lower overall concentrations than at the comparison site; in contrast, Ba showed a strong positive correlation to Macquarie Harbour over South East Tasmania.

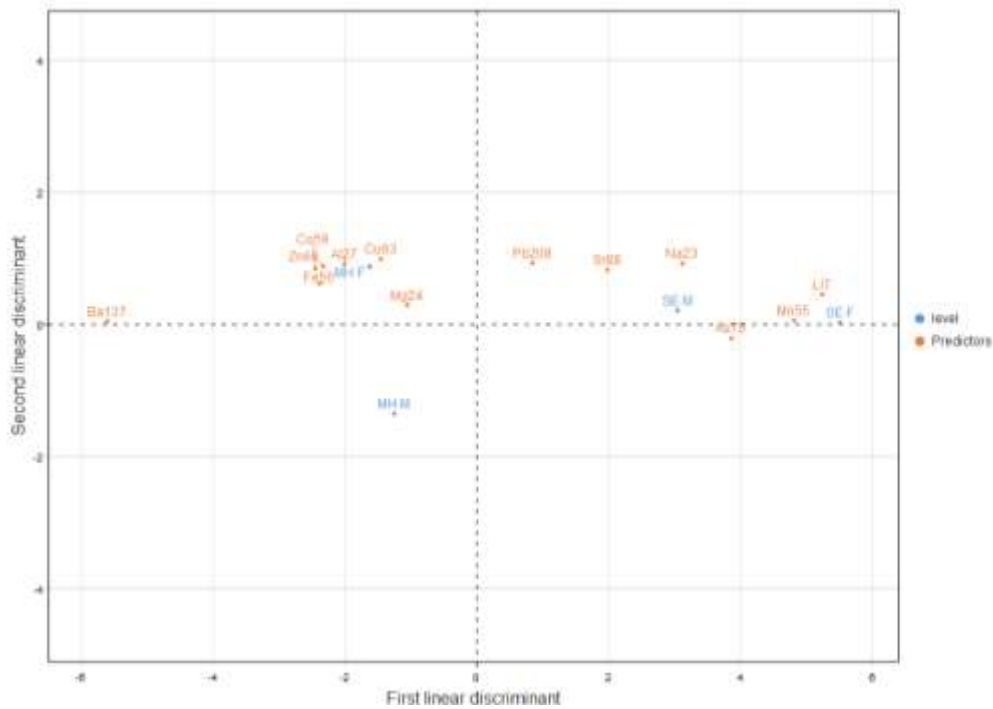


Figure 4: First and second linear discriminant loadings for all species (multi-element list) and levels (Site | Sex) for the LDTF

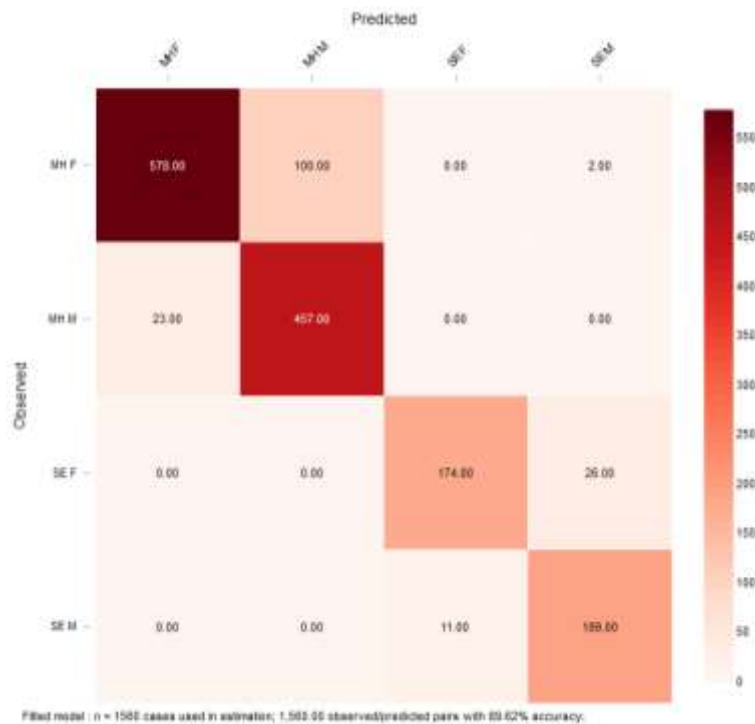


Figure 5: Prediction accuracy matrix for LDFA showing 89.62 % overall prediction accuracy for all levels.

Intra-individual variation

One of the primary objectives of this work was to analyse age-specific differences in elemental signatures. However, ageing the vertebrae used in LA-ICP-MS analysis was not possible because the modified histological stain required to elucidate the growth bands in the samples (Bubley *et al.*, 2012) was highly destructive, making direct comparisons of pre-stained and stained samples impossible. Although this precluded linking elemental signatures to specific ages or the validation of age estimates obtained from vertebrae, general ontogenetic patterns could still be drawn by looking at the entire postnatal section of each sample.

Element: Ca ratios for multiple individuals are shown in Appendix 1. Sr: Ba values in samples from the comparison population (South East Tasmania) were characterized by large changes that occurred cyclically through the life of the individuals (Figure 6). By contrast, the values for individuals captured in Macquarie Harbour were much lower and generally consistent through the individual's lifetime.

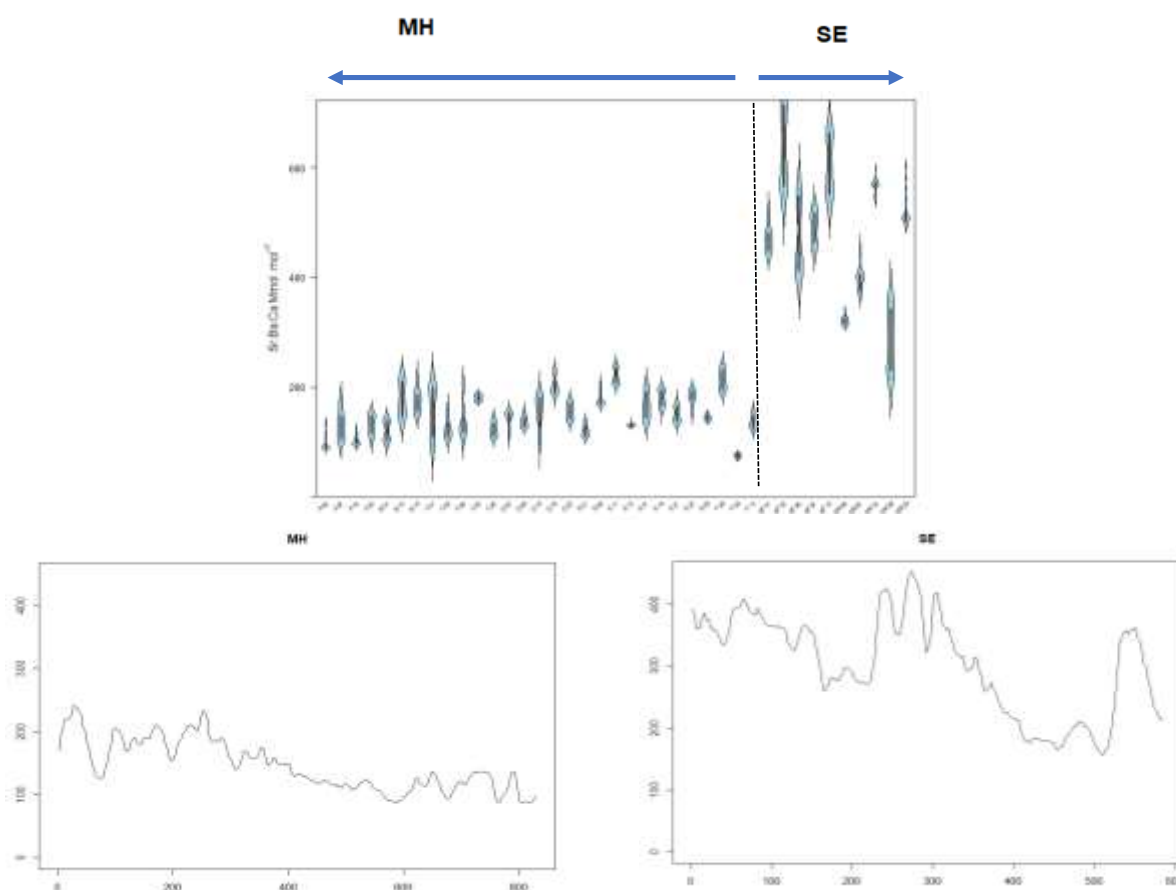


Figure 6: Violin plot of Sr:Ba values at the vertebral edge (capture region) of all individuals examined (Top). Example Sr:Ba plots for selected individuals from MH (Bottom left) and SE (Bottom right) representing values for the post-natal section of the vertebrae.

Isotopic ratios of different species of the same element were also measured for Lead ($\text{Pb}^{207/206}$) and Strontium ($\text{Sr}^{88/87}$). The overall patterns of variability appeared to be driven by real changes in elemental composition of the samples, however, there was also a high level of noise in the signal, suggesting that higher precision in measurements may be required for the effective use of these values.

Age determination

Segmentation of the postnatal time-series data using a non-parametric classification algorithm resulted in four different “age” estimates based on Ca, Mn: Ca, Sr: Ba and the multi-element signature (for example, Figure 7).

These estimated values were compared with assigned ages based on vertebral increment counts (Moreno 2018) obtained from a different vertebra within the same portion of the spinal column. The bias-plot in Figure 8 shows the discrepancies between the age estimates calculated from the elemental signatures and the original visual estimates. Estimates obtained using Sr: Ba ratios had the highest correlation to observed ages ($R^2 = 0.81$) and those from Mn: Ca ratios had the lowest ($R^2 = 0.79$).

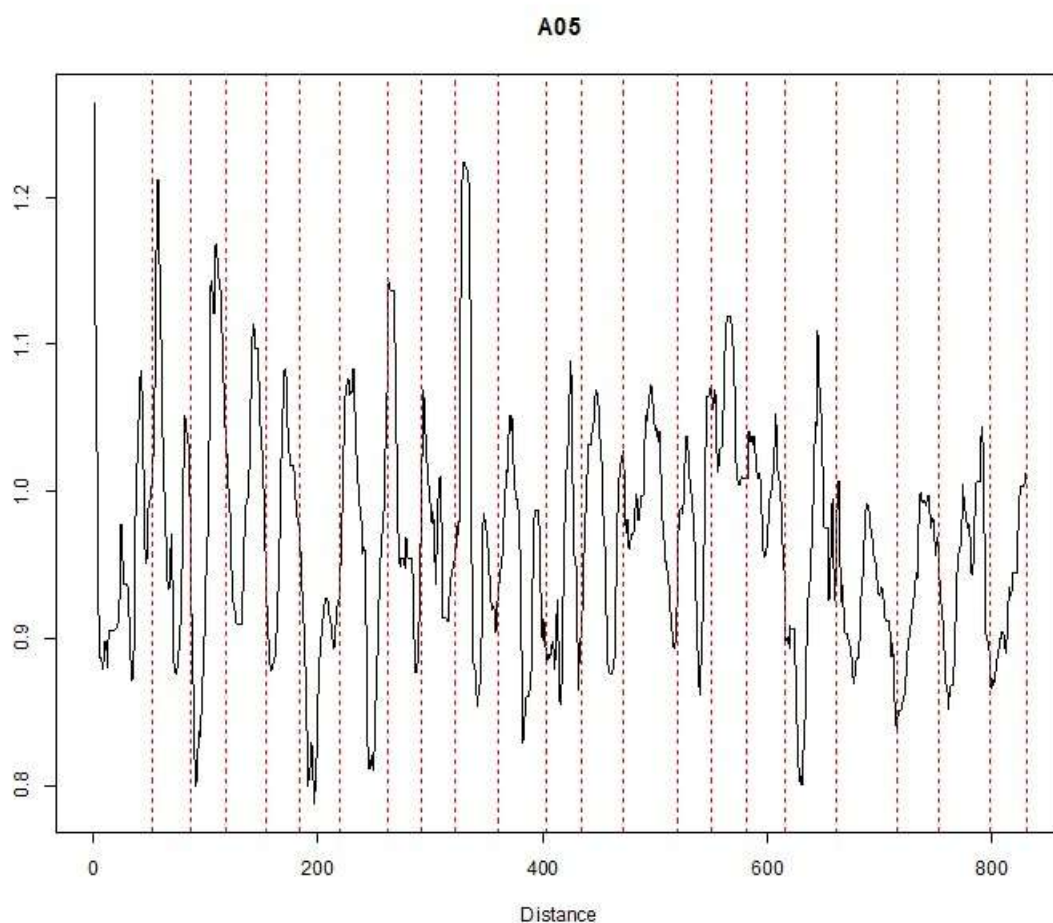


Figure 7: An example of segmentation achieved using divisive change point analysis based on the multi-element signature along the post-natal section of the vertebra.

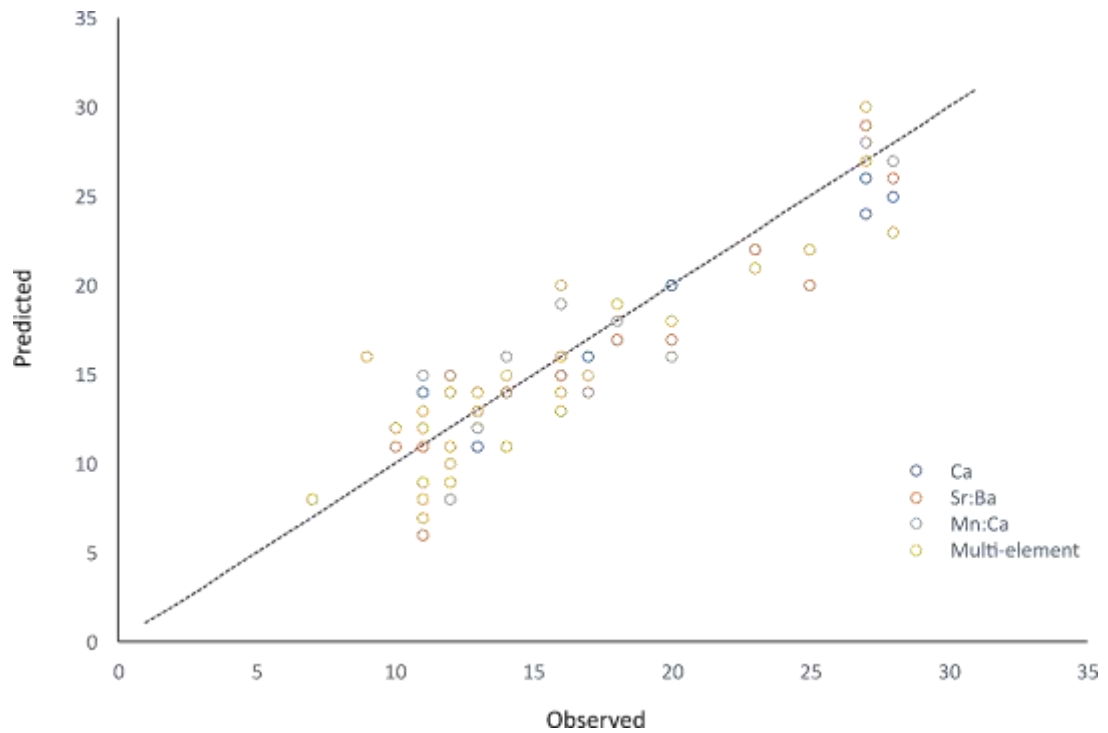


Figure 8: Predicted ages using change point analysis vs age estimates obtained through visual increment counts (Moreno 2018).

DISCUSSION

This study demonstrates the potential of using elemental fingerprints as tool to track long-term ecologic patterns in the spiny dogfish. The overall group separation achieved by the multi-element signature of vertebral edges between Macquarie Harbour and the comparison population was greater than expected by random chance. This indicates that the environmental chemistry of Macquarie Harbour results in the formation of a distinct bio-geochemical signature in the vertebral tissue of individuals during their residency in the system. Although the complex mechanisms that drive elemental incorporation into the calcifying matrix of elasmobranchs are not fully understood (McMillan *et al.*, 2017a), the spatial discriminating power seen in this work provides further evidence that region specific environmental conditions are reflected in their hard tissues.

In Macquarie Harbour, the high influx of freshwater and local geology result in a highly stratified water column that in turn limits water mixing with the ocean (Ross and MacLeod, 2017). Furthermore, the riverine plumes transport terrestrial sediments that settle at depth (Carpenter *et al.*, 1991), enriching the concentration of non-marine elements, helping create a localized chemical signature that is distinct from the adjacent oceanic waters. Successful discrimination based on chemical signatures depends on the presence of significant gradients in water chemistry, salinity and temperature (Werry *et al.*, 2011). The use of LA-ICP-MS data to classify elasmobranch populations has had mixed success depending on the study species and geographic scale used (McMillan *et al.*, 2017a). For example, Tillett *et al.* (2011) found that distinct chemical signatures corresponding to the use of nursery areas were present in bull shark but not in the morphologically similar pig-eye shark (*C. amboinensis*). This was attributed to differences in the spatial ecology of juveniles; juvenile bull sharks inhabit fresh to brackish waters (Heupel and Simpfendorfer, 2008), whereas pig-eye sharks prefer brackish to marine waters (Knip, 2011). Conditions at the open sites inhabited by pig-eye sharks are subject to more severe water mixing caused by wind and currents (Tillett *et al.*, 2011), which can homogenize chemical signatures, limiting the applicability of vertebral fingerprinting to infer movement in the species.

Results from permutational ANOVA and LDFA revealed a significant difference in the edge chemical signature between sexes by site. In most other studied populations, spiny dogfish are known to segregate spatially based on sex (Templeman, 1976), occupying different habitats and position in the water column (Sulikowski *et al.*, 2010). Acoustic tracking of spiny dogfish in Macquarie Harbour, has shown that they commonly move throughout most of the estuary throughout the year, with a higher affinity for the deeper central harbour basin (Moreno *et al.*, 2016). Although males and females in this population do not fully segregate, possibly due to the limited available area, males have been found to expand their home range during winter to include shallower areas in the upper estuary, while still making frequent trips back towards the central basin. While it is unclear what drives this behavioural change, it is possible that the sex specific differences in the vertebral chemistry could be related to the greater exposure of males during winter to the brackish waters of the upper estuary. SIMPER analysis showed that the primary contribution to the dissimilarity between males and females within Macquarie Harbour was Strontium (Sr^{88}), which was found in larger concentrations in the females. The amount of Sr available in water is positively correlated with salinity and has been found to correspond with proximity with freshwater in euryhaline and brackish water

elasmobranchs such as sawfish (Scharer *et al.*, 2012), bull sharks (Werry *et al.*, 2011), and round stingray (Hale *et al.*, 2006).

We are aware of only one study that has attempted to experimentally model the uptake of different elements into the vertebrae of elasmobranchs and how these processes may be affected by factors such as somatic growth (Smith *et al.*, 2013). Except for zinc (Zn^{66}), elemental incorporation was found to be unaffected by growth, although the relationship for Zn was inconclusive. Despite this, a growing number of studies have successfully linked chemical profiles with life-long patterns of movement (Izzo *et al.*, 2016), trophic ecology (Estrada *et al.*, 2006) and life history (Christiansen, 2011). The current study lends support to the assumption that vertebral tissue in elasmobranchs remains metabolically inert as the individual grows.

Given that the spiny dogfish is a long-lived species, capable of reaching upwards of 35 years (Cortés, 2002), more detailed information about the elemental incorporation process is needed before robust inferences about ontogenetic patterns of movement and ecology can be made. However, site classification based on linear discriminants was highly accurate and classification success only dropped slightly when sex was included in the model. The distinct chemical features seen in the vertebral region corresponding with known residency within Macquarie Harbour appear to be consistently present through the life of individuals sampled in this study regardless of size or sex. For example, Sr and Ba, which are linked to salinity and temperature (Smith *et al.*, 2013) but also indicate freshwater proximity (Tillett *et al.*, 2011). Unlike animals who spend their initial life in nurseries and then move offshore, Sr: Ba can show returns to freshwater systems linked with reproduction (Werry *et al.*, 2011). Vertebrae from individuals from MH did not contain the Sr: Ba changes that would be expected from seasonal offshore migrations as seen in the SE comparison population. Results of this work support conclusions from acoustic telemetry, and although long term patterns require further corroboration, they suggest lifelong residency in Macquarie Harbour.

Anthropogenic markers

Besides the naturally occurring environmental signals, localized anthropogenic pollutants can act as chemical tag that can be used to infer proximity to an area (Fisk *et al.*, 2002), as well as validating age-specific patterns if a reference chronology is available (e.g. age validation using bomb radiocarbon) (Campana *et al.*, 2006). Spiny dogfish are opportunistic feeders (Fordham *et al.*, 2006) and their diet in Macquarie Harbour is largely reliant in spillover pellets from salmonid aquaculture (Moreno 2018). Dietary sources are likely to contribute significantly to the chemical composition of hard tissues (Smith *et al.*, 2013), and therefore may reflect dietary shifts through an individual's life (Estrada *et al.*, 2006). Pellets used in aquaculture have a high lipid content which facilitate organic binding of compounds that do not occur naturally in the system (Mente *et al.*, 2006). This is potentially relevant for age validation since marine farm operations are likely to have commenced in Macquarie Harbour within the lifetime of some older spiny dogfish individuals. Although the isotopic signature of the pellets was readily detectable using bulk isotope analysis in the muscle tissue of spiny dogfish (Moreno 2018), age-specific chemical indicators in the vertebrae that could be linked to the introduction of marine farming in Macquarie Harbour were not evident.

Enrichment of manganese (Mn^{55}) in the soft tissues of elasmobranchs appears to be mostly dietarily sourced (Mathews and Fisher, 2009). Drops in Mn: Ca levels in the vertebrae of pig-eye sharks have been linked with ontogenetic movement towards offshore waters and away

from nutrient-rich coastal upwellings (Tillett *et al.*, 2011). Large periodic fluctuations of Mn: Ca were present in the vertebrae of spiny dogfish from South East Tasmania and appear consistent with ontogenetic changes in space utilization and seasonal migrations seen in the species elsewhere (Fordham *et al.*, 2006). By contrast, Mn: Ca concentrations were lower in spiny dogfish from Macquarie Harbour and there were no large oscillations in concentrations in the postnatal sections. Macquarie Harbour is naturally depauperate and the locally available prey does not vary drastically throughout the year (Ross and MacLeod, 2017). Thus, Mn may not only provide a possible link to verify long-term residency in Macquarie Harbour based on diet, but also as a potential indicator of the ongoing role of anthropogenically sourced food items in the diet of the population.

The intent in this study was to use isotope: isotope ratios of Sr and Pb to determine age-specific movement patterns. While some of the observed variability likely reflected real patterns in vertebral chemistry, the level of instrumental noise in the signal tends to discount the use of these results. Measuring isotope to isotope ratios requires higher accuracy but provides an important advantage in that isotopic ratios are determined independently of the total concentration. For example, the total Ca in elasmobranch vertebrae is limited by physiological processes (Hussey *et al.*, 2012, McMillan *et al.*, 2017a), but the rate at which different species of Ca occur is determined by their availability in the environment. Calcium isotope ratios ($\text{Ca}^{44/42}$) have been used to establish the trophic position of extinct and extant elasmobranchs (Martin *et al.*, 2015) and could be used to explore ontogenetic variability in the trophic ecology of modern species. Likewise, although Pb contributed little to site discrimination in this study, $\text{Pb}^{207/206}$ ratios in Macquarie Harbour are influenced by riverine runoff carrying mining waste (Carpenter *et al.*, 1991). The Mt Lyell & Rosebury isotopic signature is distinct from the modern marine signature (Gulson and Porritt, 1987) and would constitute an ideal chemical tag for movement in and out of Macquarie Harbour. Although at present the analysis of isotopic ratios may be limited to specialized equipment with higher accuracy (Balter *et al.*, 2008), their use for elasmobranch chemical ecology studies should be further explored.

Age estimation

Age estimation using accretionary processes is possible due to seasonal variation in growth, which causes the formation of alternating slow and fast growth bands in the vertebrae (Cailliet *et al.*, 2006). These differences in cartilage accretion may result in seasonally alternating elemental signatures that can be used in age determination (Gelsleichter *et al.*, 1998). One of the earliest implementations of the chemical analysis of hard structures in elasmobranchs was in ageing studies, either through direct estimation (Jones and Geen, 1977), or validation of deposition rates used to visually infer age from calcifying structures (Campana *et al.*, 2006). Previous work has focused on the detection of signal peaks in single element signals, particularly Ca, which are subsequently counted to estimate the age of an individual (Hale *et al.*, 2006, Jones and Geen, 1977, Scharer *et al.*, 2012). More recently, due to the increased measurement accuracy of light spectroscopy and LA-ICP-MS, non-Ca elements such as Sr have been proposed as a better alternative to determine age (Raoult *et al.*, 2016), particularly in species which seasonally migrate between areas along a salinity gradient (Scharer *et al.*, 2012).

It is not necessary to have a full understanding of the biotic and abiotic factors that determine chemical incorporation into the calcification process to explore the internal relationships

between the different elements. Change point analysis is common in financial modelling, bioinformatics and data mining applications (Talih and Hengartner, 2005). The approach implemented here is a non-parametric alternative that allows the analysis of multivariate data, using a divisive algorithm to detect multiple change points in the data using a hierarchical clustering tree (James and Matteson, 2013). It is a type of unsupervised learning algorithm, meaning that no prior knowledge of the age or the underlying relationships between the elements is needed. Although the present study represents a preliminary application of method it has demonstrated advantages of the approach over traditional analytical methods for age estimation using elemental signatures. Most importantly, instead of focusing on the detection of statistically significant peaks, the algorithm can detect any type of change in the multivariate distribution, including changes in covariance, exploiting the complex relationships in the multi-element data.

Conclusions

Elemental concentrations in the vertebrae of spiny dogfish from Macquarie Harbour were consistent with persistent residential behaviour and provide evidence to suggest the long-term nature of the unique life-history characteristics described for this population. Given that some of these life-history traits are suggestive of a population under environmental stress, this highlights the need to manage it as a distinct stock. Further degradation of environmental conditions in Macquarie Harbour could compromise its long-term viability.

Elemental analysis could also represent a valuable tool in monitoring retrospective environmental conditions in Macquarie Harbour, with the spiny dogfish having potential as an ecological indicator because of its longevity and residency.

The use of change point analysis provides a new analytical framework for age determination in elasmobranchs. This could be particularly useful in the ageing of species with poor calcification such as the spiny dogfish, eliminating some of the ambiguity introduced in the visual estimation of age.

Results from this study are encouraging and highlight the need for further research into the mechanisms that govern elemental incorporation into the calcified matrix of elasmobranch cartilage to improve the ecological inferences that can be drawn from elemental analysis in these species.

Acknowledgements

This project was supported by the Sea World Research & Rescue Foundation Inc (SWR/7/2016) and The Holsworth Wildlife Research Endowment – Equity Trustees Charitable Foundation & the Ecological Society of Australia.

We thank Graeme Ewing and Edward Forbes for their help with sample collection and Jay Thompson and Nathan Chapman from the Centre for Ore Deposit and Earth Sciences for their aid with the vertebral analysis using laser ablation spectroscopy.

All procedures were undertaken with University of Tasmania Animal Ethics Committee approval (permit A13468) and scientific permits 13125 and 14139 issued under Section 14 of the Living Marine Resources Management Act 1995 and permits TFA 13982, 14019, and 14253 issued under Regulation 4 of the Threatened Species Protection Regulations 2006 and Section 29 of the Nature Conservation Act 2002.

References

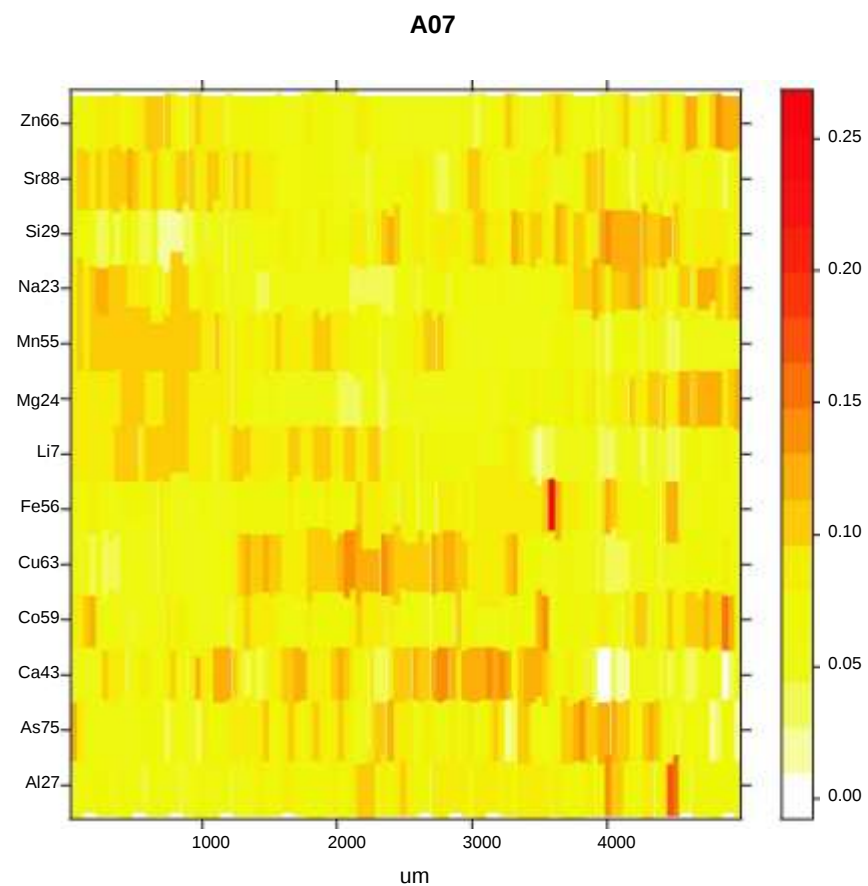
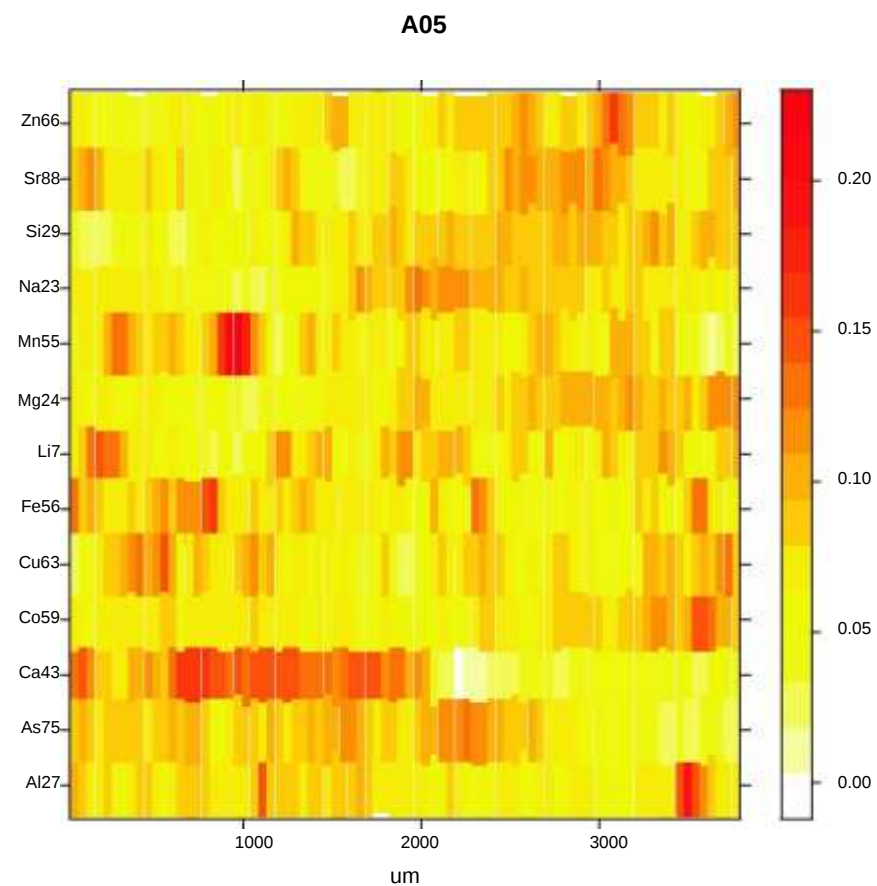
- BALTER, V., TELOUK, P., REYNARD, B., BRAGA, J., THACKERAY, F. & ALBARÈDE, F. 2008. Analysis of coupled Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ variations in enamel using laser-ablation tandem quadrupole-multicollector ICPMS. *Geochimica et Cosmochimica Acta*, 72, 3980-3990.
- BEAMISH, R. J., MCFARLANE, G. A., SWEETING, R. M. & NEVILLE, C. M. 2009. Keynote address: The sad history of dogfish management. *Biology and management of dogfish sharks, American Fisheries Society*, 1-10.
- BELL, J., LYLE, J., SEMMENS, J., AWRUCH, C., MORENO, D., CURRIE, S., MORASH, A., ROSS, J. & BARRETT, N. 2016. Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour. Fisheries Research and Development Corporation Project No. 2013/008. Institute for Marine and Antarctic Studies, University of Tasmania, Hobart.
- BUBLEY, W., KNEEBONE, J., SULIKOWSKI, J. & TSANG, P. 2012. Reassessment of spiny dogfish *Squalus acanthias* age and growth using vertebrae and dorsal-fin spines. *Journal of Fish Biology*, 80, 1300-1319.
- CAILLIET, G. M., SMITH, W. D., MOLLET, H. F. & GOLDMAN, K. J. 2006. Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. *Environmental Biology of Fishes*, 77, 211-228.
- CAMPANA, S. E., JONES, C., MCFARLANE, G. A. & MYKLEVOLL, S. 2006. Bomb dating and age validation using the spines of spiny dogfish (*Squalus acanthias*). *Special Issue: Age and Growth of Chondrichthyan Fishes: New Methods, Techniques and Analysis*. Springer.
- CAMPANA, S. E. & THORROLD, S. R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 30-38.
- CARPENTER, P., BUTLER, E., HIGGINS, H., MACKEY, D. & NICHOLS, P. 1991. Chemistry of trace elements, humic substances and sedimentary organic matter in Macquarie Harbour, Tasmania. *Marine and Freshwater Research*, 42, 625-654.
- CHRISTIANSEN, H. 2011. Developing and applying elemental composition of shark vertebrae as a tool for quantifying life history characteristics over ontogeny.
- CORTÉS, E. 2002. Incorporating uncertainty into demographic modeling: application to shark populations and their conservation. *Conservation biology*, 16, 1048-1062.
- DEAN, M. N. & SUMMERS, A. P. 2006. Mineralized cartilage in the skeleton of chondrichthyan fishes. *Zoology*, 109, 164-168.

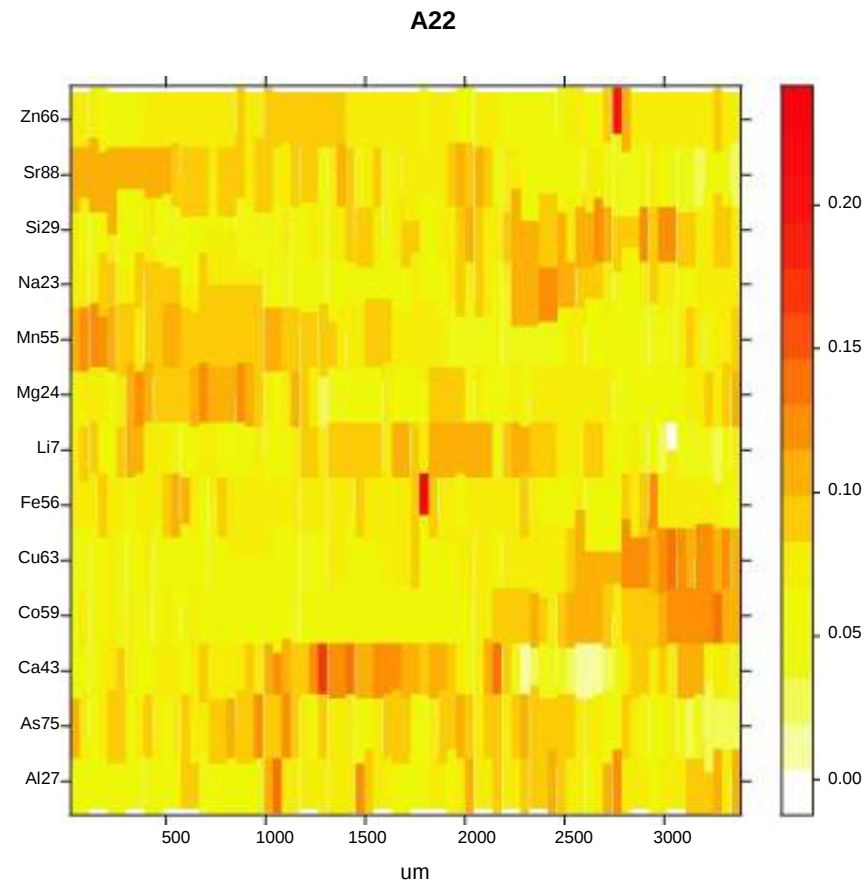
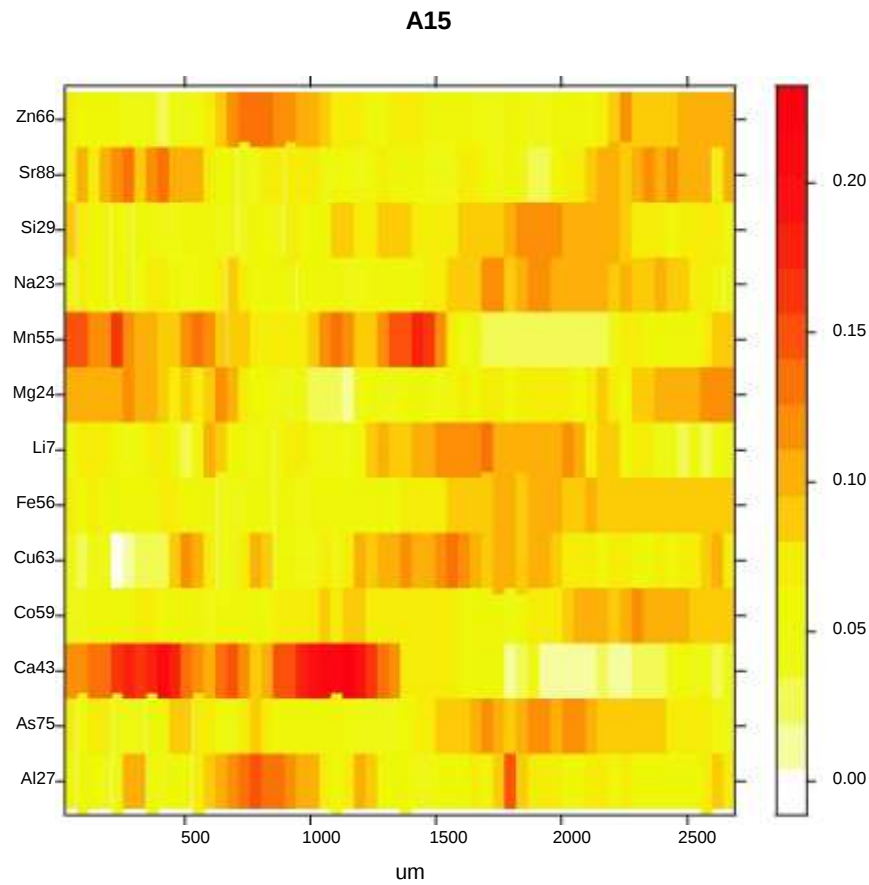
- DULVY, N. K., BAUM, J. K., CLARKE, S., COMPAGNO, L. J., CORTÉS, E., DOMINGO, A., FORDHAM, S., FOWLER, S., FRANCIS, M. P. & GIBSON, C. 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18, 459-482.
- EDMONDS, J., CAPUTI, N. & MORITA, M. 1991. Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. *Marine and Freshwater Research*, 42, 383-389.
- ELSDON, T. S. & GILLANDERS, B. M. 2005. Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 1143-1152.
- ESTRADA, J. A., RICE, A. N., NATANSON, L. J. & SKOMAL, G. B. 2006. Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology*, 87, 829-834.
- FISK, A. T., TITTELMIER, S. A., PRANSCHKE, J. L. & NORSTROM, R. J. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. *Ecology*, 83, 2162-2172.
- FORDHAM, S., FOWLER, S., COELHO, R., GOLDMAN, K. & FRANCIS, M. 2006. *Squalus acanthias*. 2006 IUCN Red List of Threatened Species.
- GELSLEICHTER, J., PIERCY, A. & MUSICK, J. 1998. Evaluation of copper, iron and lead substitution techniques in elasmobranch age determination. *Journal of Fish Biology*, 53, 465-470.
- GULSON, B. L. & PORRITT, P. M. 1987. Base metal exploration of the Mount Read Volcanics, western Tasmania; Pt. II, Lead isotope signatures and genetic implications. *Economic Geology*, 82, 291-307.
- HALE, L. F., DUDGEON, J. V., MASON, A. Z. & LOWE, C. G. 2006. Elemental signatures in the vertebral cartilage of the round stingray, *Urobatis halleri*, from Seal Beach, California. *Special Issue: Age and Growth of Chondrichthyan Fishes: New Methods, Techniques and Analysis*. Springer.
- HARRY, A. V. 2018. Evidence for systemic age underestimation in shark and ray ageing studies. *Fish and Fisheries*, 19, 185-200.
- HEIST, E. J. 2004. Genetics of sharks, skates and rays. *Biology of Sharks and their Relatives*, 471-485.
- HEUPEL, M. R. & SIMPFENDORFER, C. A. 2008. Movement and distribution of young bull sharks *Carcharhinus leucas* in a variable estuarine environment. *Aquatic Biology*, 1, 277-289.
- HUSSEY, N., MACNEIL, M., OLIN, J., MCMEANS, B., KINNEY, M., CHAPMAN, D. & FISK, A. 2012. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology*, 80, 1449-1484.
- IZZO, C., HUVEENEERS, C., DREW, M., BRADSHAW, C. J., DONNELLAN, S. C. & GILLANDERS, B. M. 2016. Vertebral chemistry demonstrates movement and population structure of bronze whaler. *Marine Ecology Progress Series*, 556, 195-207.
- JAMES, N. A. & MATTESON, D. S. 2013. ecp: An R package for nonparametric multiple change point analysis of multivariate data. *arXiv preprint arXiv:1309.3295*.
- JOCHUM, K. P., NOHL, U., HERWIG, K., LAMMEL, E., STOLL, B. & HOFMANN, A. W. 2005. GeoReM: a new geochemical database for reference materials and isotopic standards. *Geostandards and Geoanalytical Research*, 29, 333-338.
- JONES, B. C. & GEEN, G. H. 1977. Age determination of an elasmobranch (*Squalus acanthias*) by X-ray spectrometry. *Journal of the Fisheries Board of Canada*, 34, 44-48.
- KERR, L. A. & CAMPANA, S. E. 2014. Chemical composition of fish hard parts as a natural marker of fish stocks. *Stock Identification Methods (Second Edition)*. Elsevier.
- KNIP, D. M. 2011. *Spatial ecology of mobile predators in a nearshore tropical environment and its implication for marine protected areas*. James Cook University.

- LEWIS, J., PATTERSON III, W. & CARLSON, J. 2017. Natural variability and effects of cleaning and storage procedures on vertebral chemistry of the blacktip shark *Carcharhinus limbatus*. *Journal of fish biology*, 91, 1284-1300.
- LEWIS, J. P., PATTERSON, W. F., CARLSON, J. K. & MCLACHLIN, K. 2016. Do vertebral chemical signatures distinguish juvenile blacktip shark (*Carcharhinus limbatus*) nursery regions in the northern Gulf of Mexico? *Marine and Freshwater Research*, 67, 1014-1022.
- LYLE, J. M., BELL, J. D., CHUWEN, B. M., BARRETT, N., TRACEY, S. R. & BUXTON, C. D. 2014. Assessing the impacts of gillnetting in Tasmania: implications for by-catch and biodiversity.
- MARTIN, J. E., TACAIL, T., ADNET, S., GIRARD, C. & BALTER, V. 2015. Calcium isotopes reveal the trophic position of extant and fossil elasmobranchs. *Chemical Geology*, 415, 118-125.
- MATHEWS, T. & FISHER, N. S. 2009. Dominance of dietary intake of metals in marine elasmobranch and teleost fish. *Science of the Total Environment*, 407, 5156-5161.
- MCFARLANE, G. A. & KING, J. R. 2003. Migration patterns of spiny dogfish (*Squalus acanthias*) in the North Pacific Ocean. *Fishery Bulletin*, 101, 358-367.
- MCMILLAN, M., IZZO, C., WADE, B. & GILLANDERS, B. 2017a. Elements and elasmobranchs: hypotheses, assumptions and limitations of elemental analysis. *Journal of fish biology*, 90, 559-594.
- MCMILLAN, M. N., IZZO, C., JUNGE, C., ALBERT, O. T., JUNG, A. & GILLANDERS, B. M. 2017b. Analysis of vertebral chemistry to assess stock structure in a deep-sea shark, *Etmopterus spinax*. *ICES Journal of Marine Science*, 74, 793-803.
- MENTE, E., PIERCE, G. J., SANTOS, M. B. & NEOFITOU, C. 2006. Effect of feed and feeding in the culture of salmonids on the marine aquatic environment: a synthesis for European aquaculture. *Aquaculture International*, 14, 499-522.
- MOKGALAKA, N. S. & GARDEA-TORRESDEY, J. L. 2006. Laser Ablation Inductively Coupled Plasma Mass Spectrometry: Principles and Applications. *Applied Spectroscopy Reviews*, 41, 131-150.
- MORENO, D. 2018. *An unusual habitat for a common shark: life-history, ecology and demographics of the spiny dogfish (Squalus acanthias) in Macquarie Harbour, Tasmania* PhD Thesis, University of Tasmania.
- MORENO, D., BELL, J., LYLE, J. & SEMMENS, J. Space utilisation, movement and behaviour of the spiny dogfish, *Squalus acanthias*, in Macquarie Harbour, Tasmania. Australian Society for Fish Biology-Oceania Chondrichthyan Society Joint Conference, 2016. .
- MUNKSGAARD, N. C., ANTWERTINGER, Y. & PARRY, D. L. 2004. Laser ablation ICP-MS analysis of Faviidae corals for environmental monitoring of a tropical estuary. *Environmental Chemistry*, 1, 188-196.
- MUSICK, J., BURGESS, G., CAILLIET, G., CAMHI, M. & FORDHAM, S. 2000. Management of sharks and their relatives (Elasmobranchii). *Fisheries*, 25, 9-13.
- MYERS, R. A., BAUM, J. K., SHEPHERD, T. D., POWERS, S. P. & PETERSON, C. H. 2007. Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science*, 315, 1846-1850.
- PEARCE, N. J., PERKINS, W. T., WESTGATE, J. A., GORTON, M. P., JACKSON, S. E., NEAL, C. R. & CHENERY, S. P. 1997. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. *Geostandards Newsletter*, 21, 115-144.
- PORTER, M. E., BELTRÁN, J. L., KOOB, T. J. & SUMMERS, A. P. 2006. Material properties and biochemical composition of mineralized vertebral cartilage in seven elasmobranch species (Chondrichthyes). *Journal of Experimental Biology*, 209, 2920-2928.
- RAOULT, V., PEDDEMORS, V. M., ZAHRA, D., HOWELL, N., HOWARD, D. L., DE JONGE, M. D. & WILLIAMSON, J. E. 2016. Strontium mineralization of shark vertebrae. *Scientific reports*, 6, 29698.
- ROSS, J. & MACLEOD, C. 2017. Environmental research in Macquarie Harbour: interim synopsis of benthic and water column conditions.

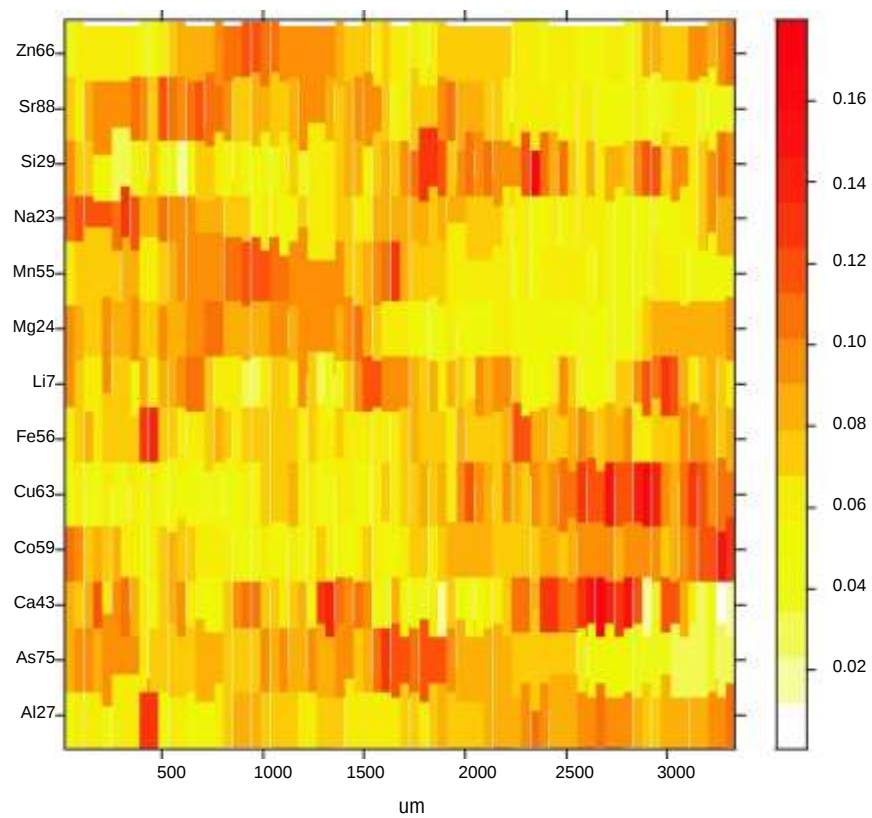
- SCHARER, R. M., PATTERSON III, W. F., CARLSON, J. K. & POULAKIS, G. R. 2012. Age and growth of endangered smalltooth sawfish (*Pristis pectinata*) verified with LA-ICP-MS analysis of vertebrae. *PLoS One*, 7, e47850.
- SCHINDELIN, J., ARGANDA-CARRERAS, I., FRISE, E., KAYNIG, V., LONGAIR, M., PIETZSCH, T., PREIBISCH, S., RUEDEN, C., SAALFELD, S. & SCHMID, B. 2012. Fiji: an open-source platform for biological-image analysis. *Nature methods*, 9, 676.
- SMITH, W. D., MILLER, J. A. & HEPPELL, S. S. 2013. Elemental markers in elasmobranchs: effects of environmental history and growth on vertebral chemistry. *PloS one*, 8, e62423.
- STAUBER, J. L., BENNING, R. J., HALES, L. T., ERIKSEN, R. & NOWAK, B. 2000. Copper bioavailability and amelioration of toxicity in Macquarie Harbour, Tasmania, Australia. *Marine and Freshwater Research*, 51, 1-10.
- SULIKOWSKI, J. A., GALUARDI, B., BUBLEY, W., FUREY, N. B., DRIGGERS III, W. B., INGRAM JR, G. W. & TSANG, P. C. 2010. Use of satellite tags to reveal the movements of spiny dogfish *Squalus acanthias* in the western North Atlantic Ocean. *Marine Ecology Progress Series*, 418, 249-254.
- SZÉKELY, G. J. & RIZZO, M. L. 2010. Disco analysis: A nonparametric extension of analysis of variance. *The Annals of Applied Statistics*, 4, 1034-1055.
- TALIH, M. & HENGARTNER, N. 2005. Structural learning with time-varying components: tracking the cross-section of financial time series. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 67, 321-341.
- TEMPLEMAN, W. 1976. Transatlantic migrations of spiny dogfish (*Squalus acanthias*). *Journal of the Fisheries Board of Canada*, 33, 2605-2609.
- TILLET, B. J., MEEKAN, M. G., PARRY, D., MUNKSGAARD, N., FIELD, I. C., THORBURN, D. & BRADSHAW, C. J. 2011. Decoding fingerprints: elemental composition of vertebrae correlates to age-related habitat use in two morphologically similar sharks. *Marine Ecology Progress Series*, 434, 133-142.
- WERRY, J., LEE, S., OTWAY, N., HU, Y. & SUMPTON, W. 2011. A multi-faceted approach for quantifying the estuarine–nearshore transition in the life cycle of the bull shark, *Carcharhinus leucas*. *Marine and Freshwater Research*, 62, 1421-1431.

Appendix 1. Heat maps showing scaled element calcuim ratios for individual spiny dogfish vertebrae.

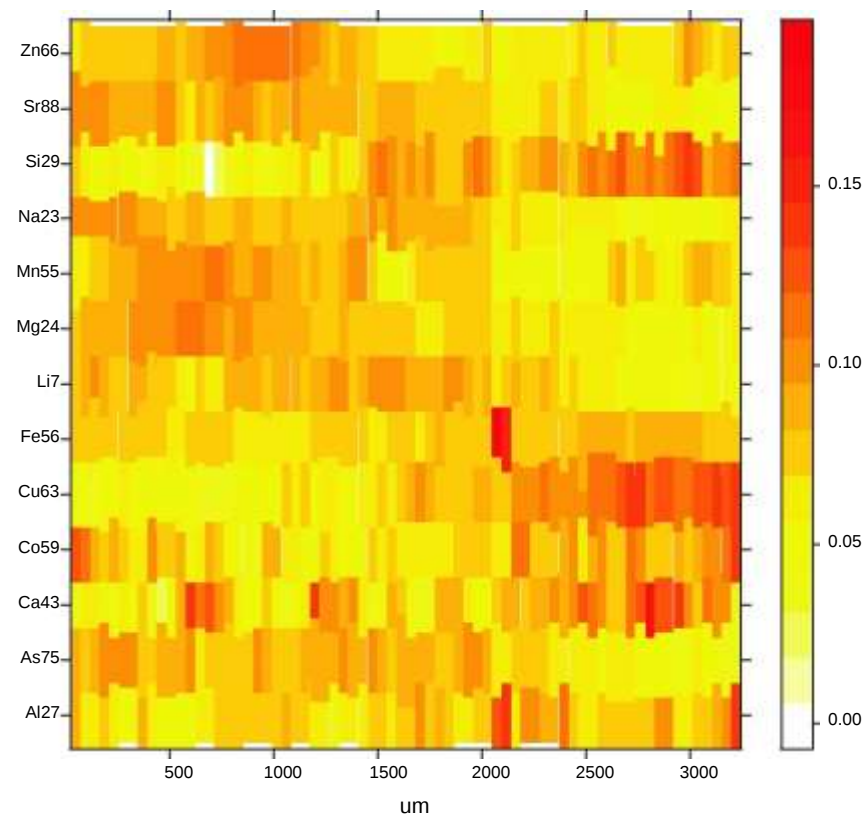


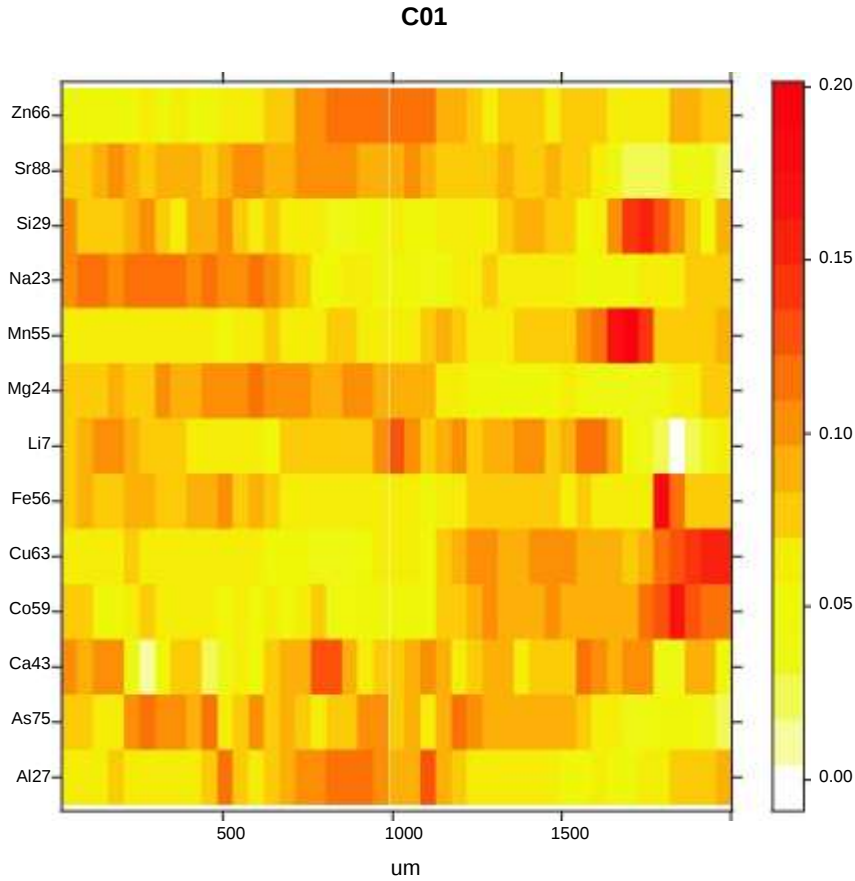
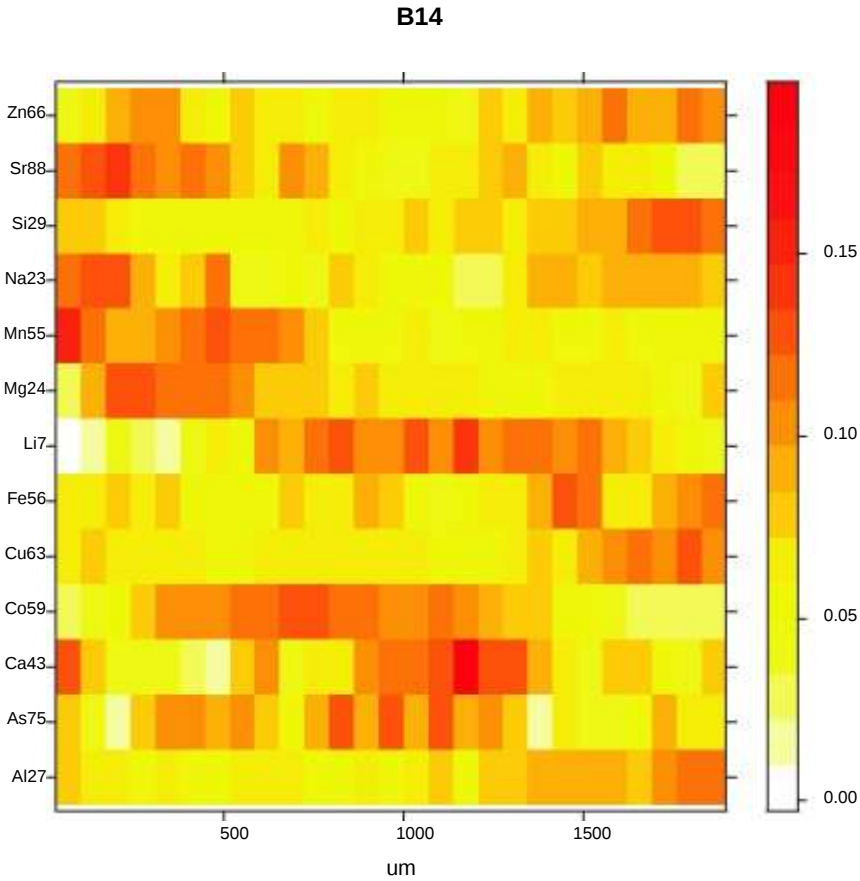


B01

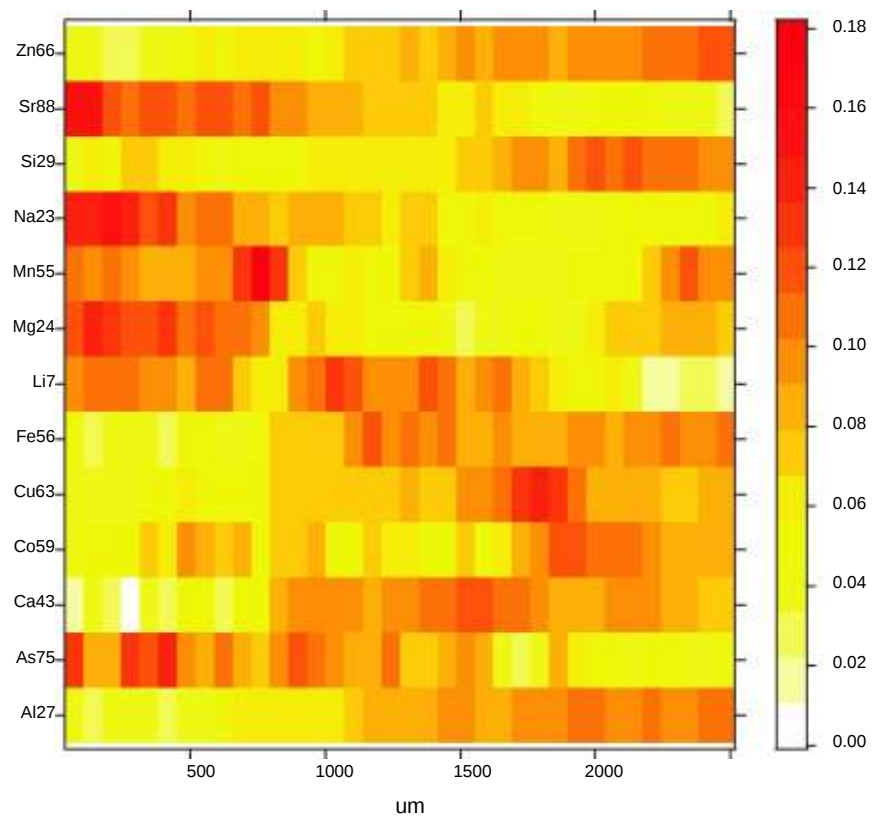


B10

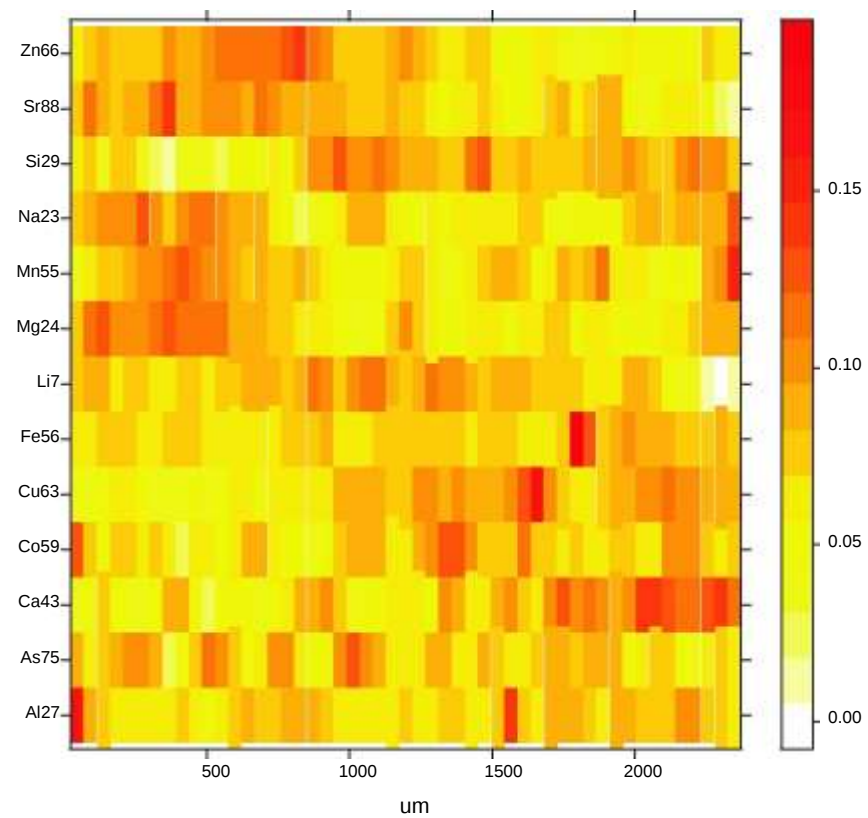


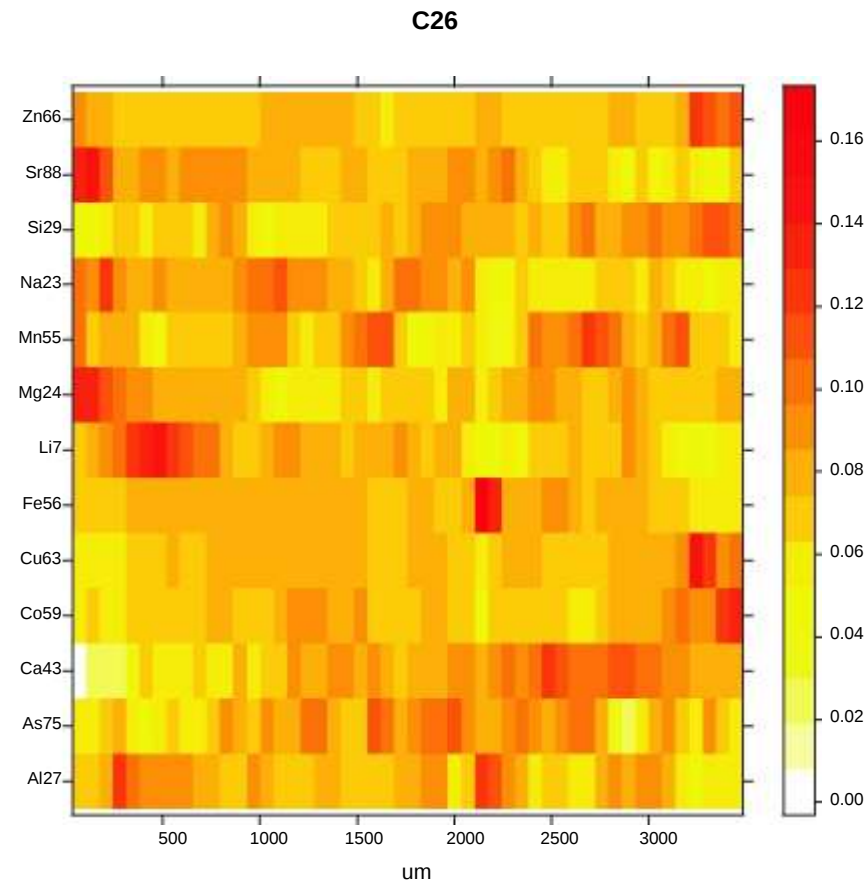
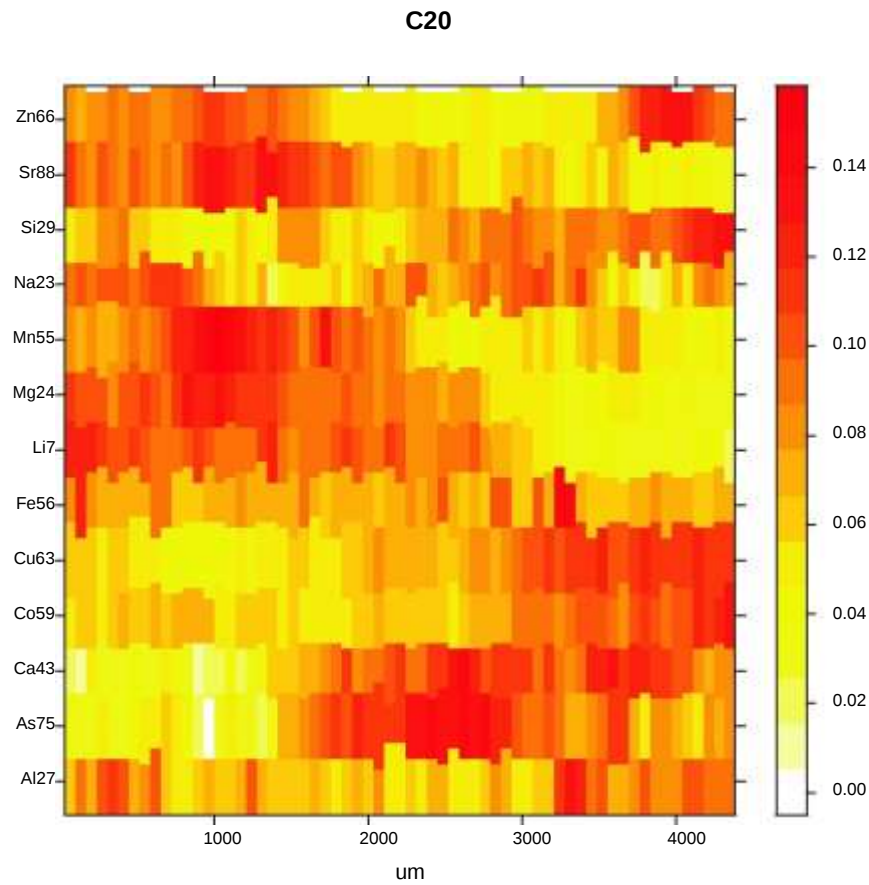


C04

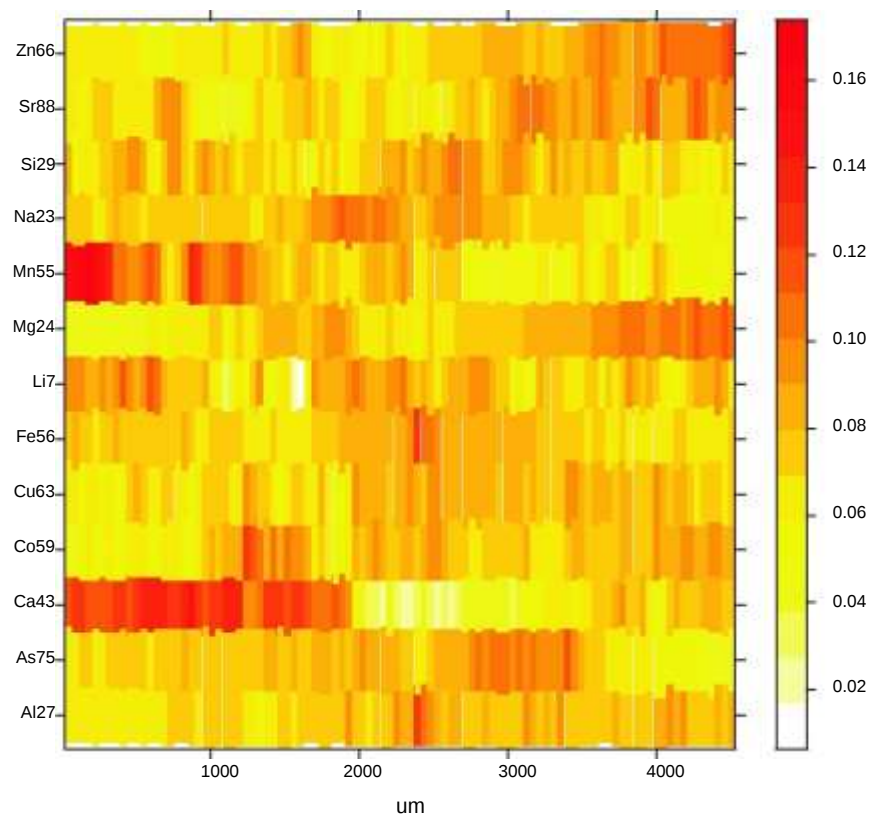


C05

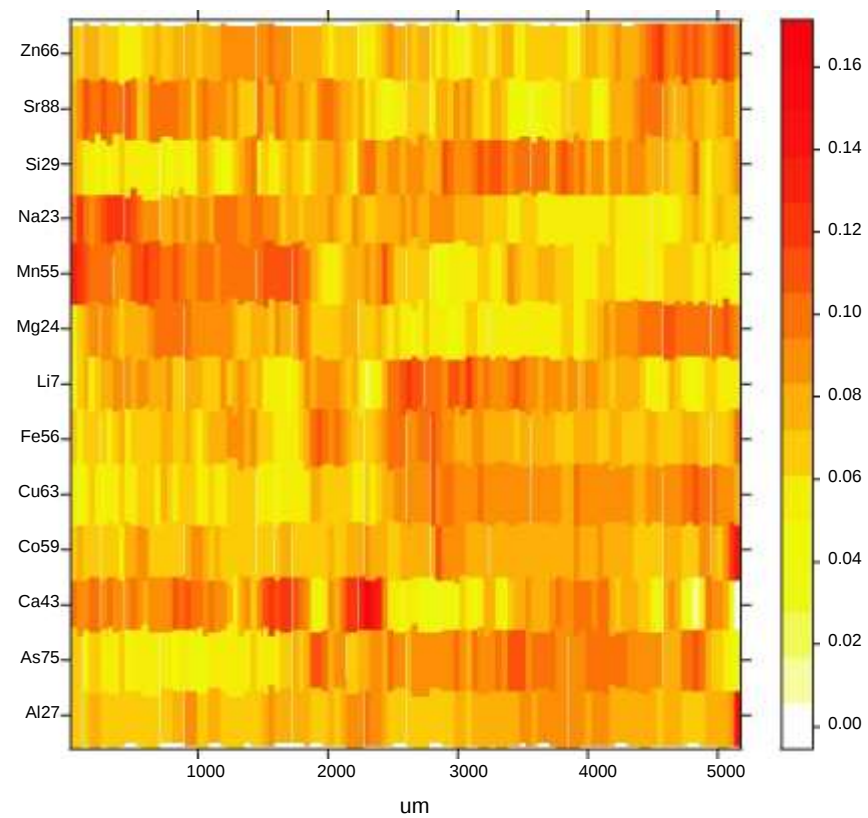


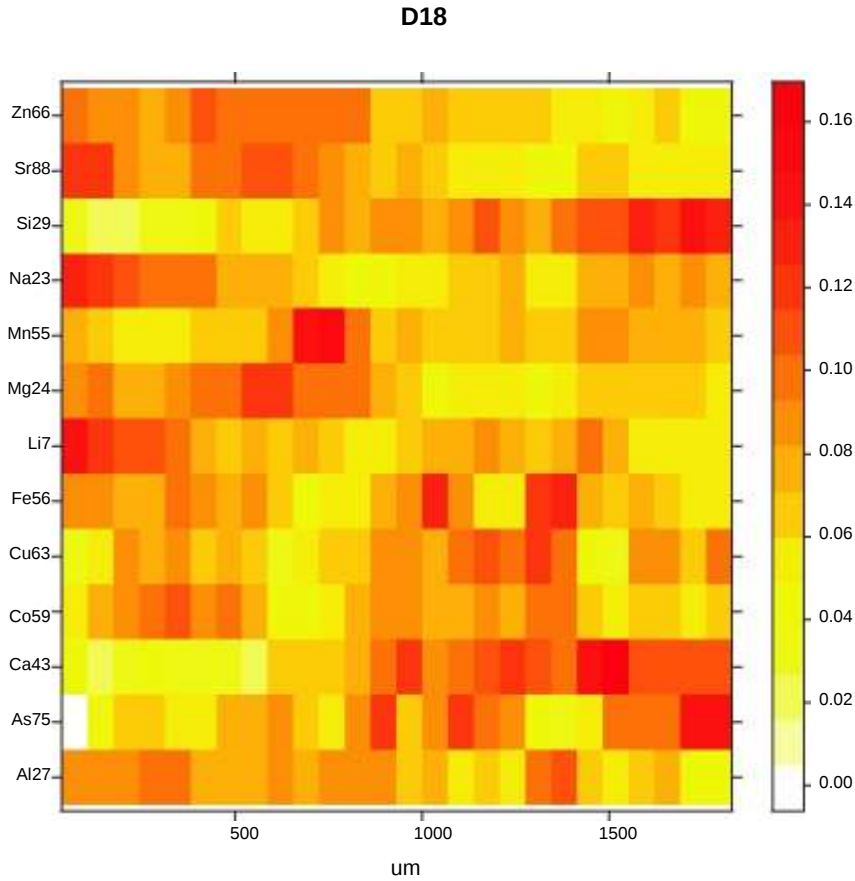
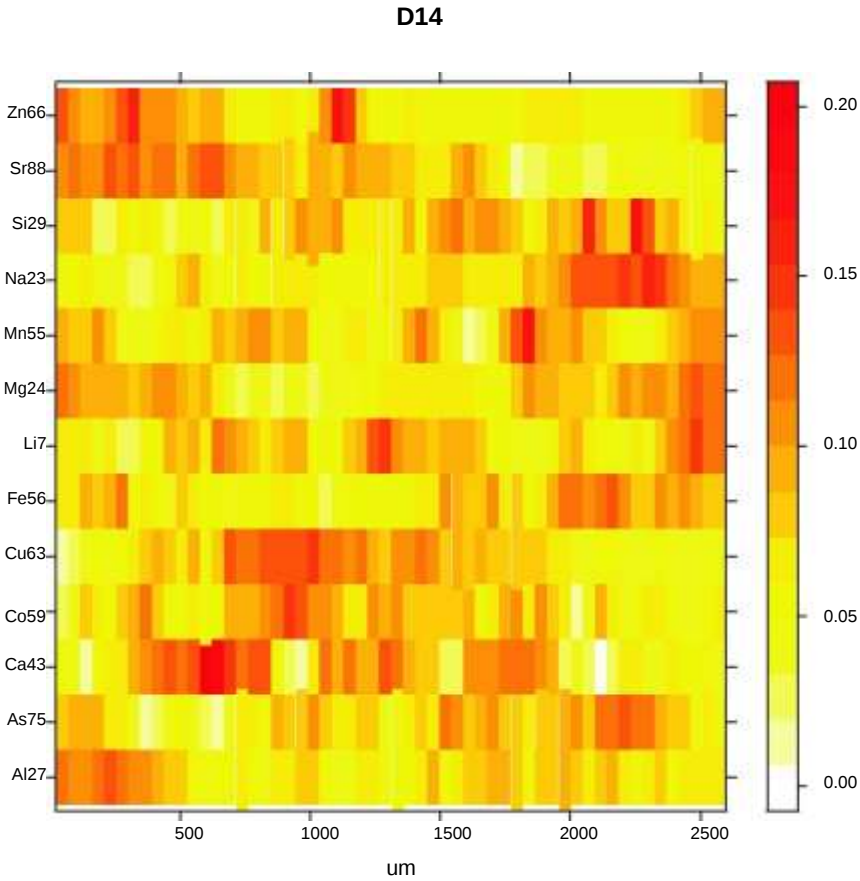


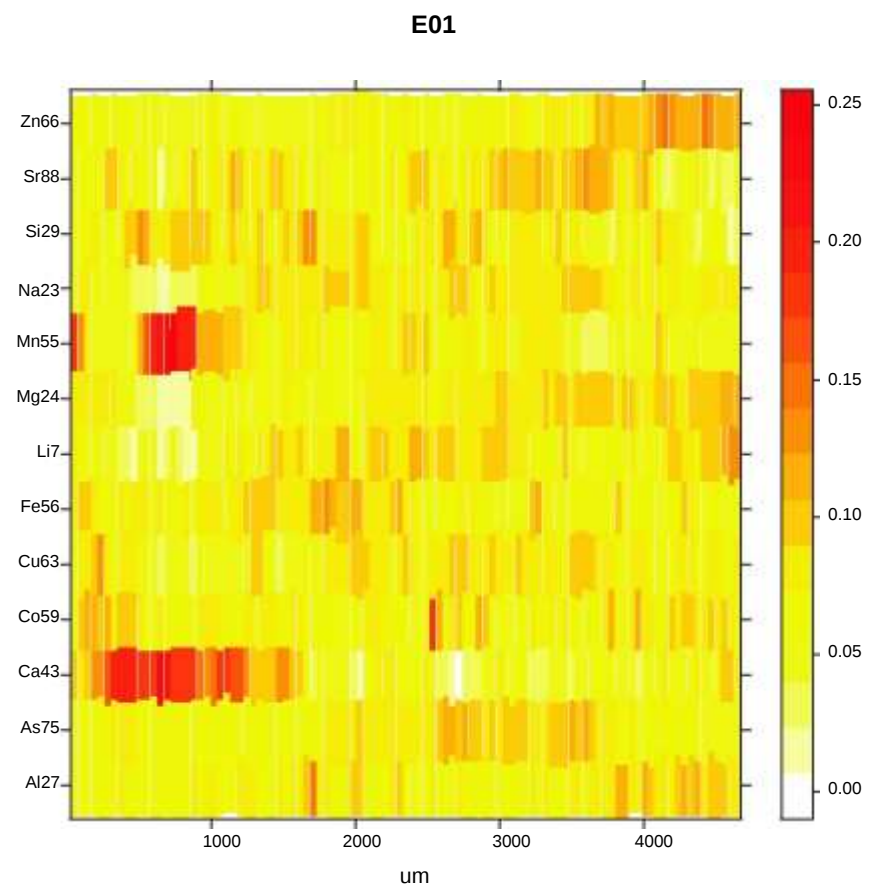
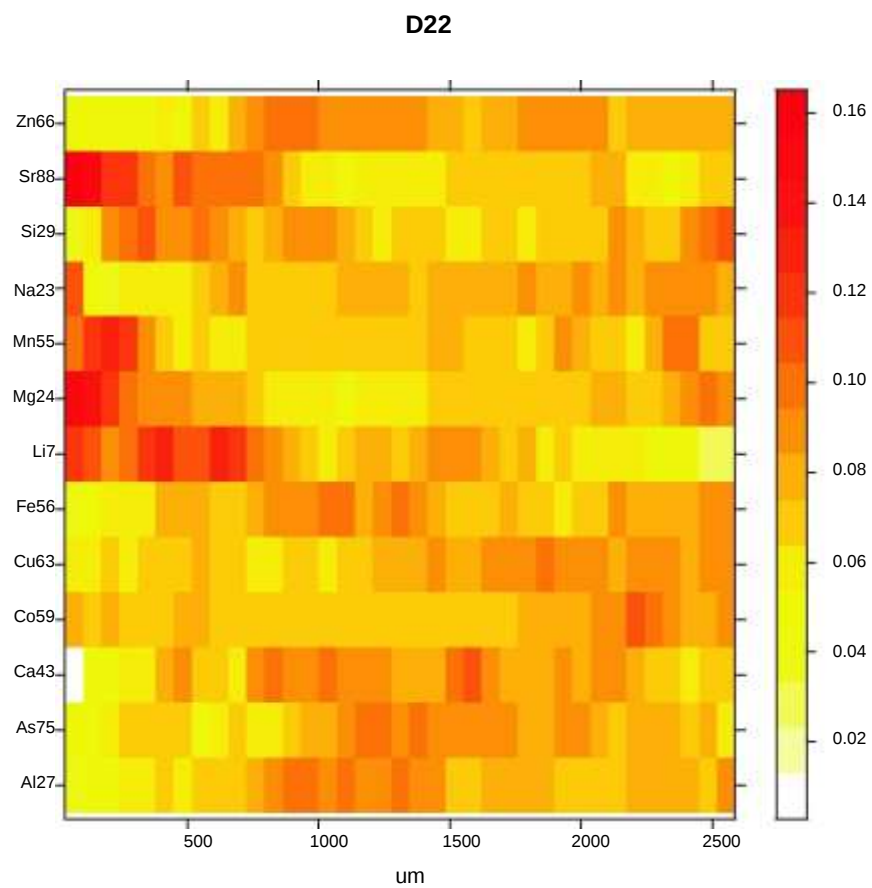
D02

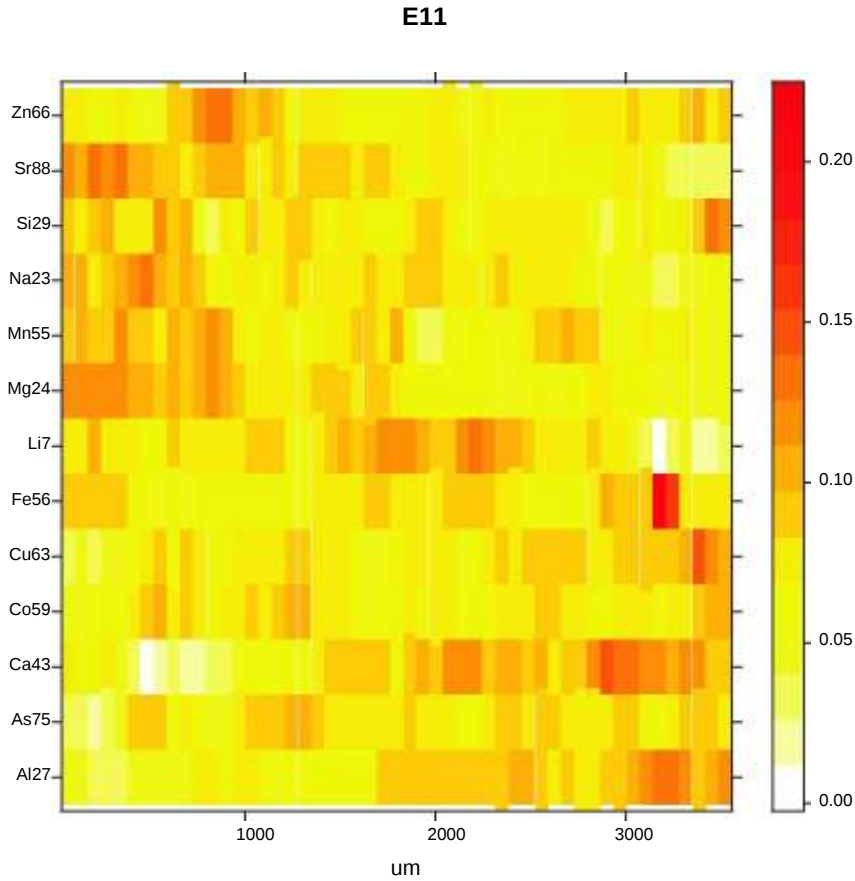
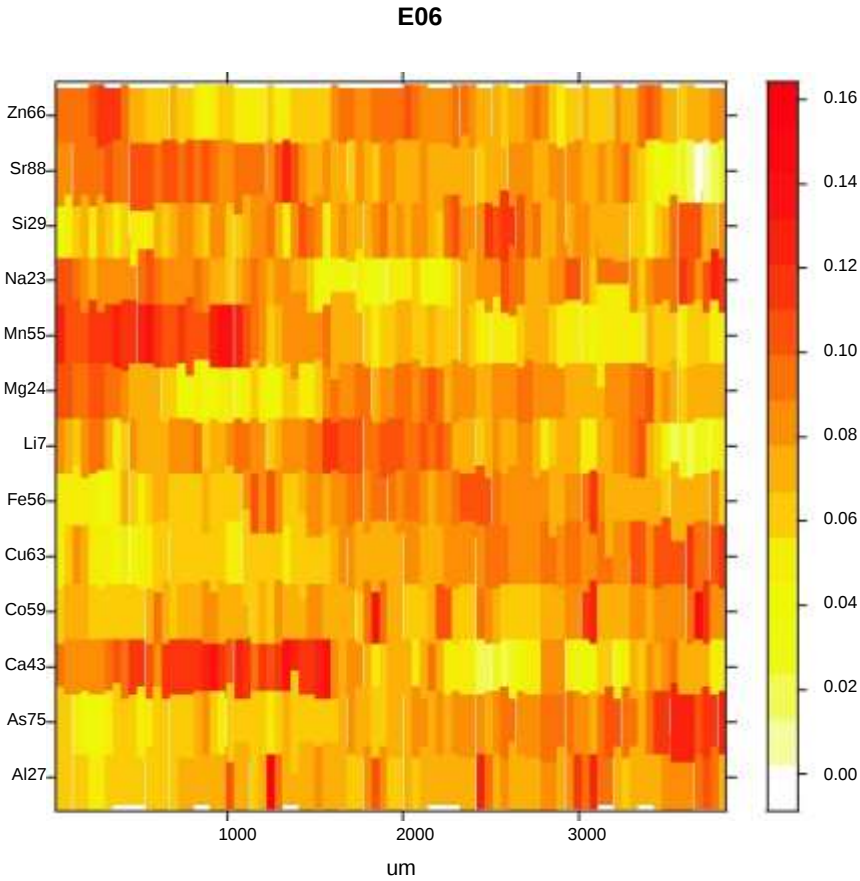


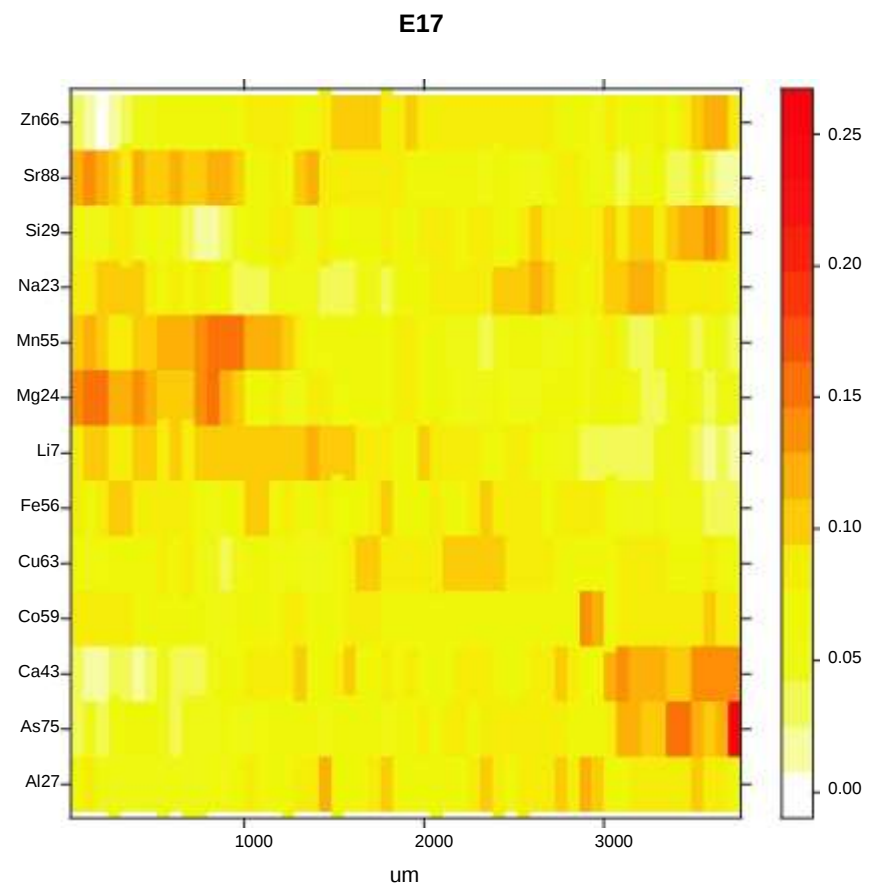
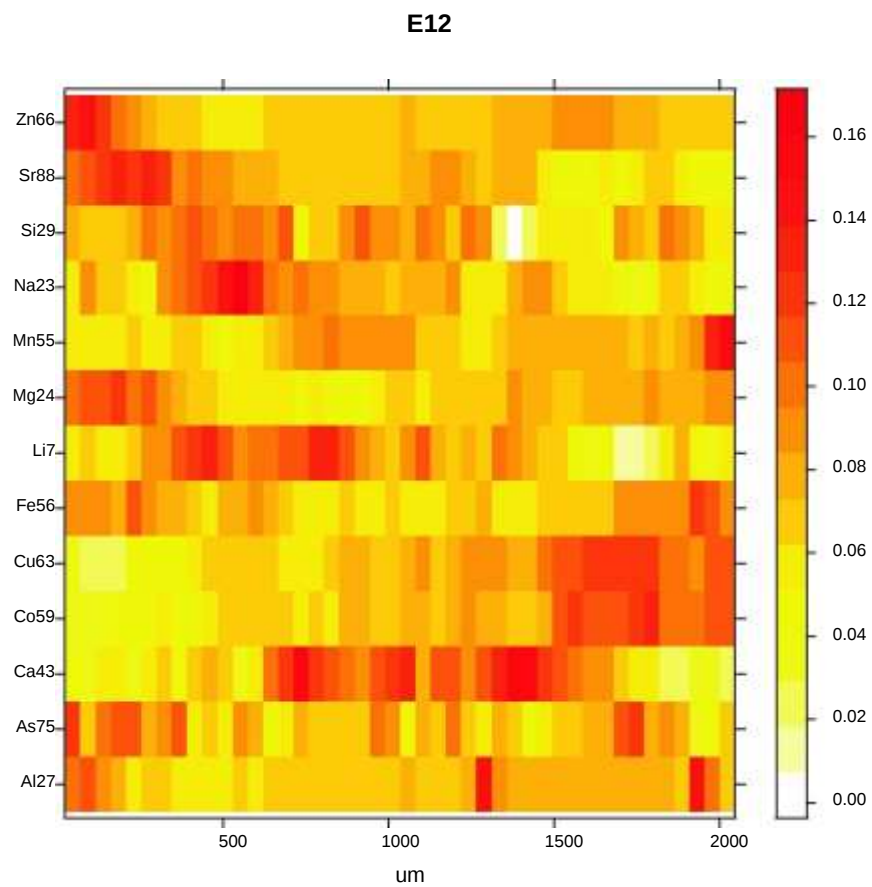
D09



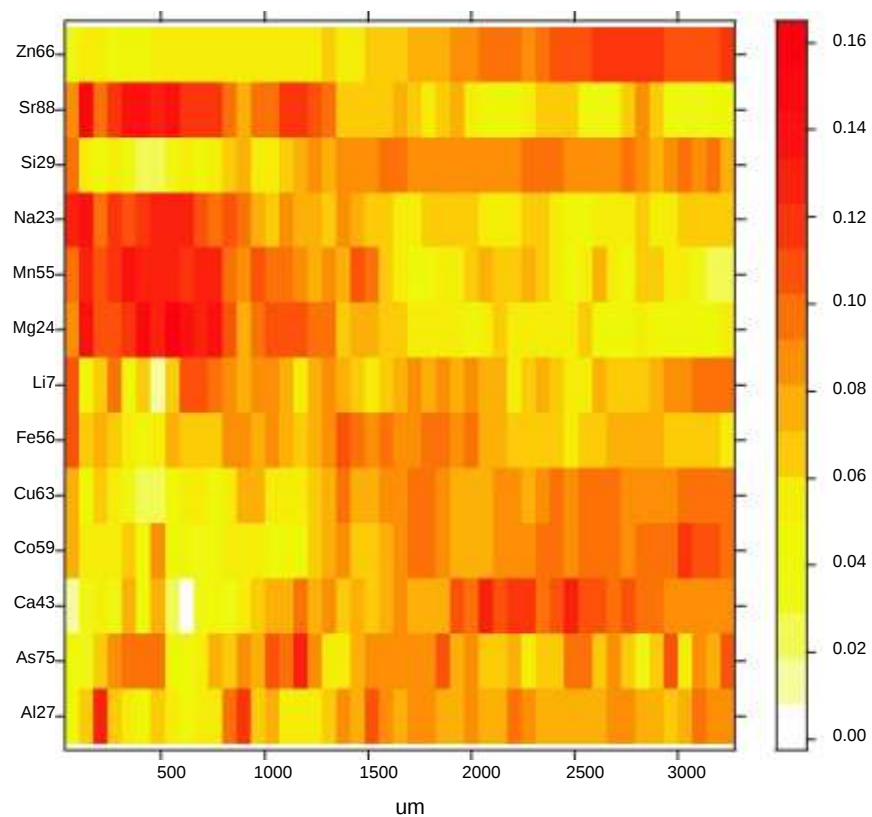




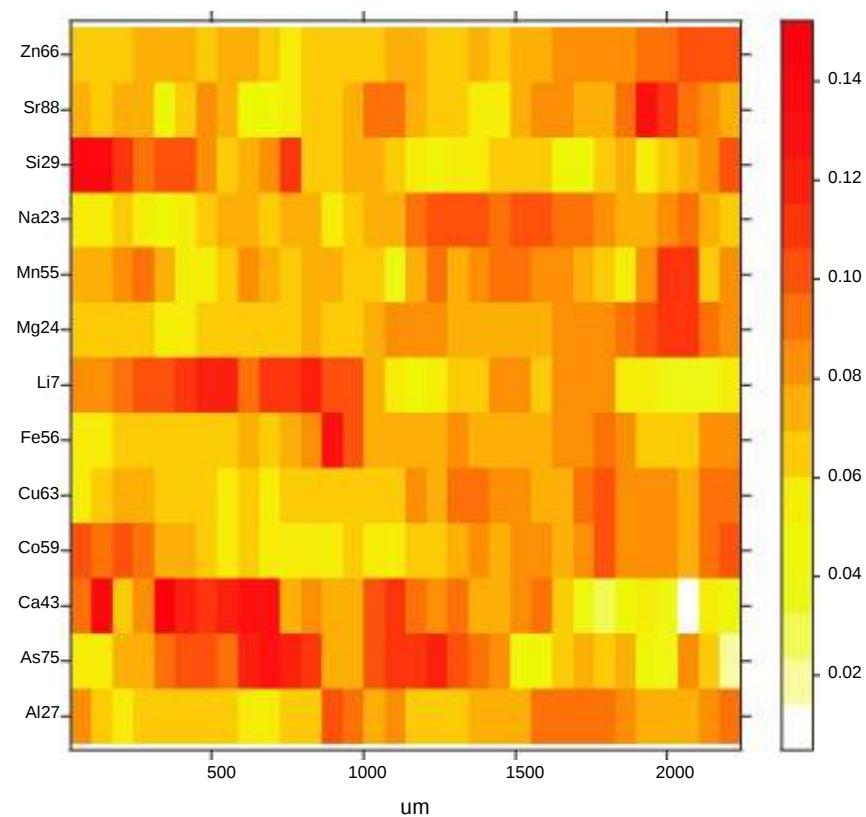


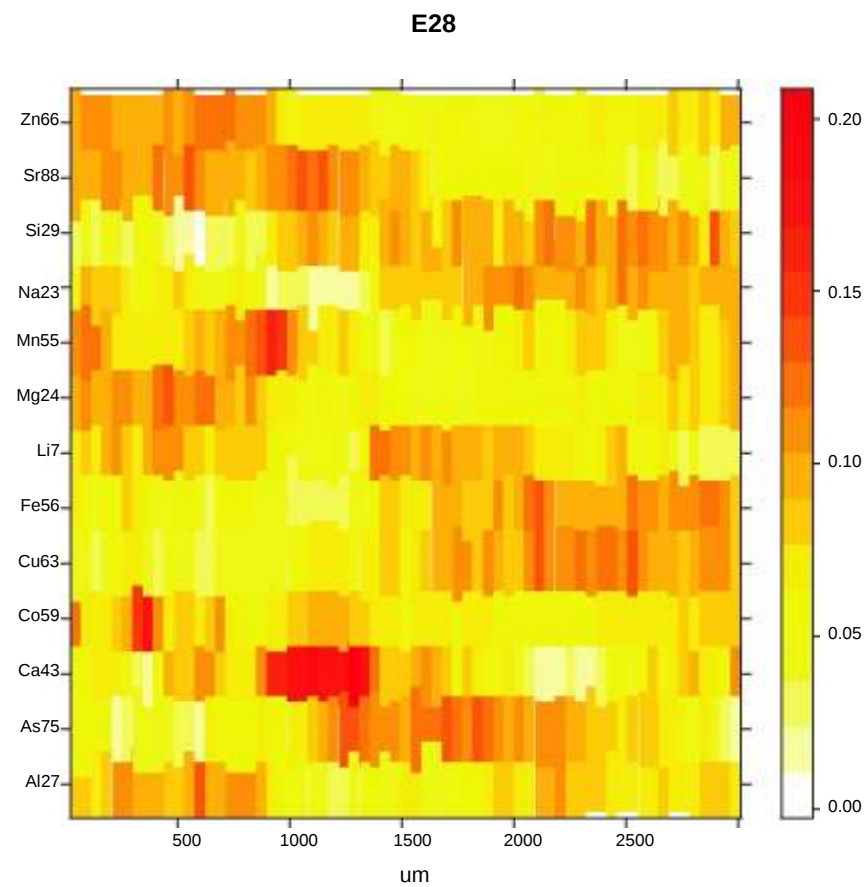
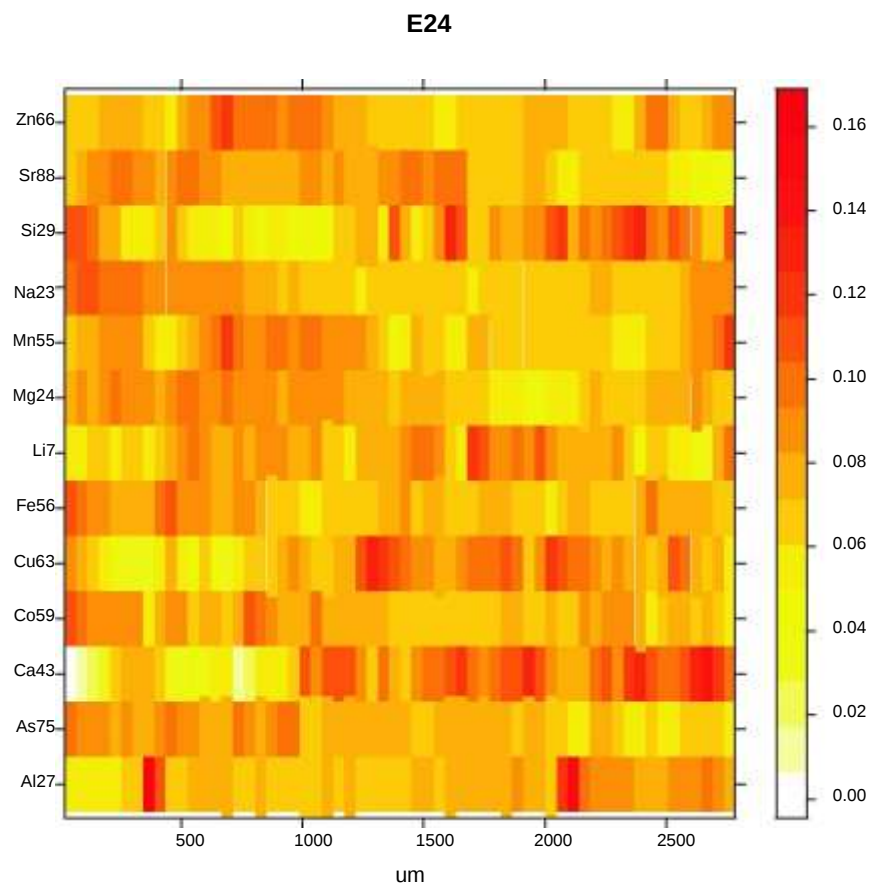


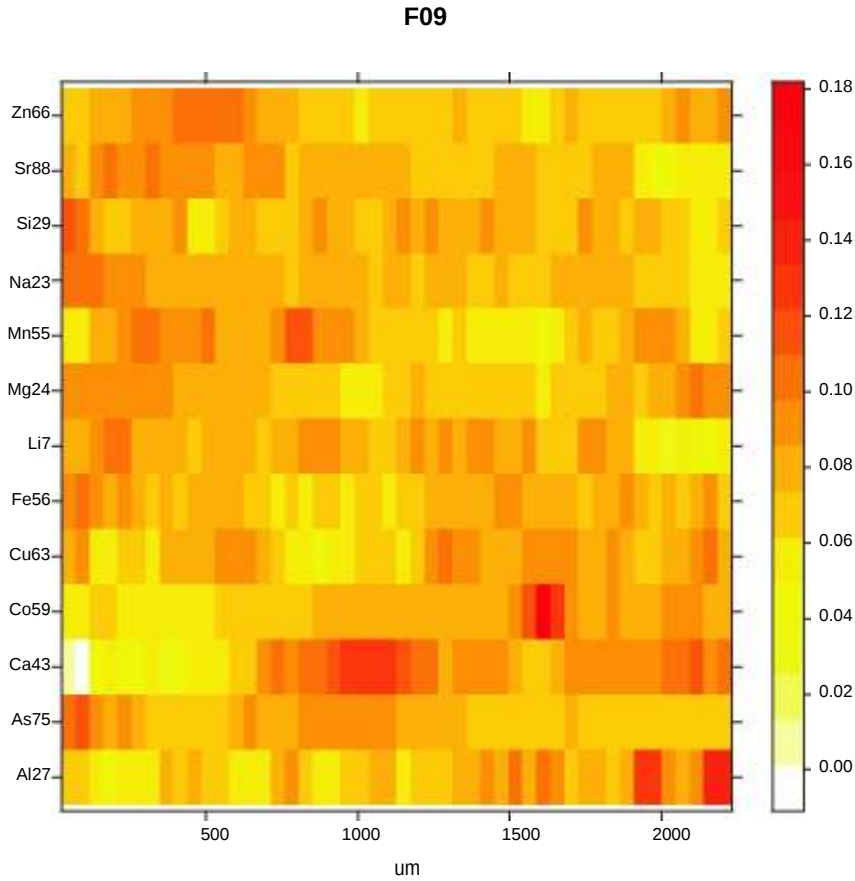
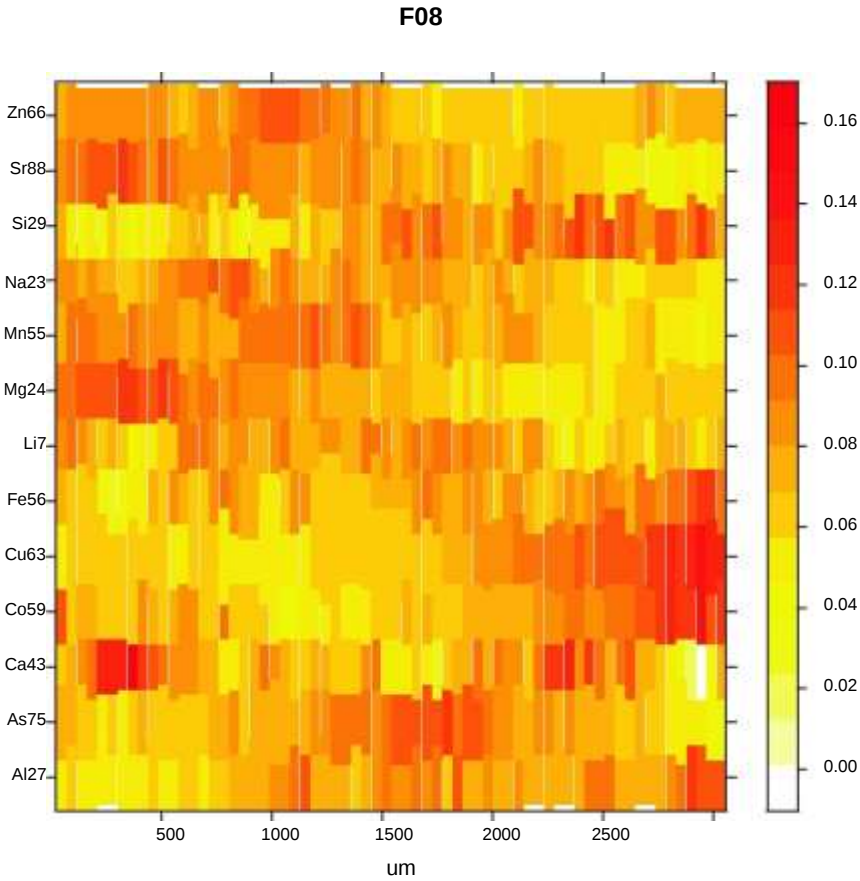
E19

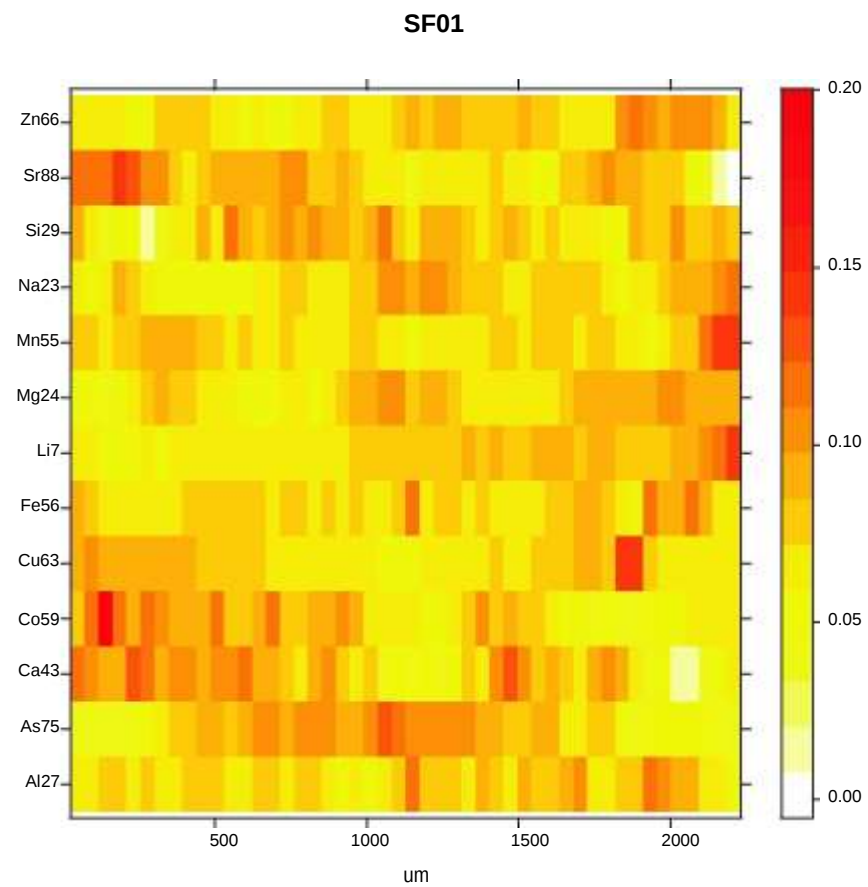
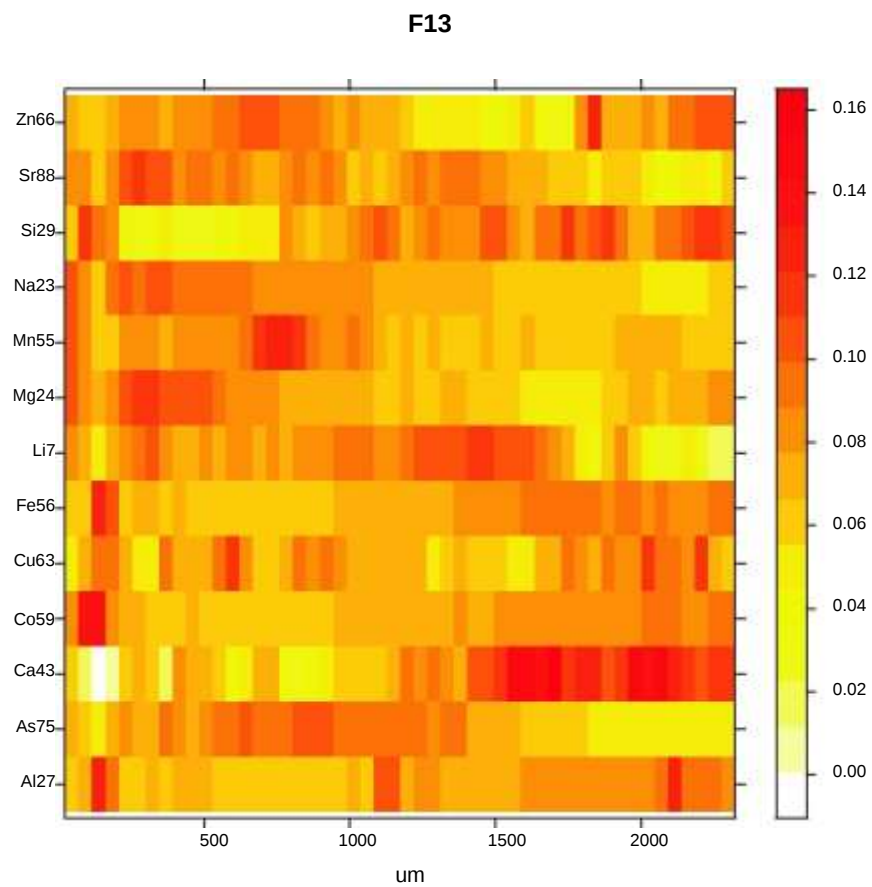


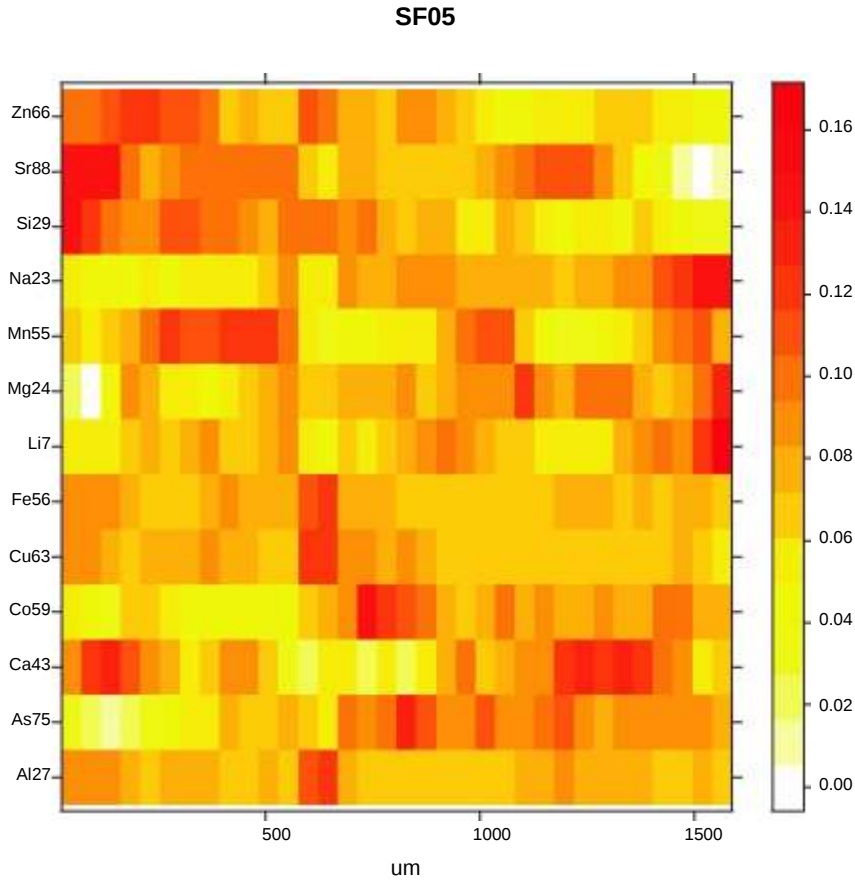
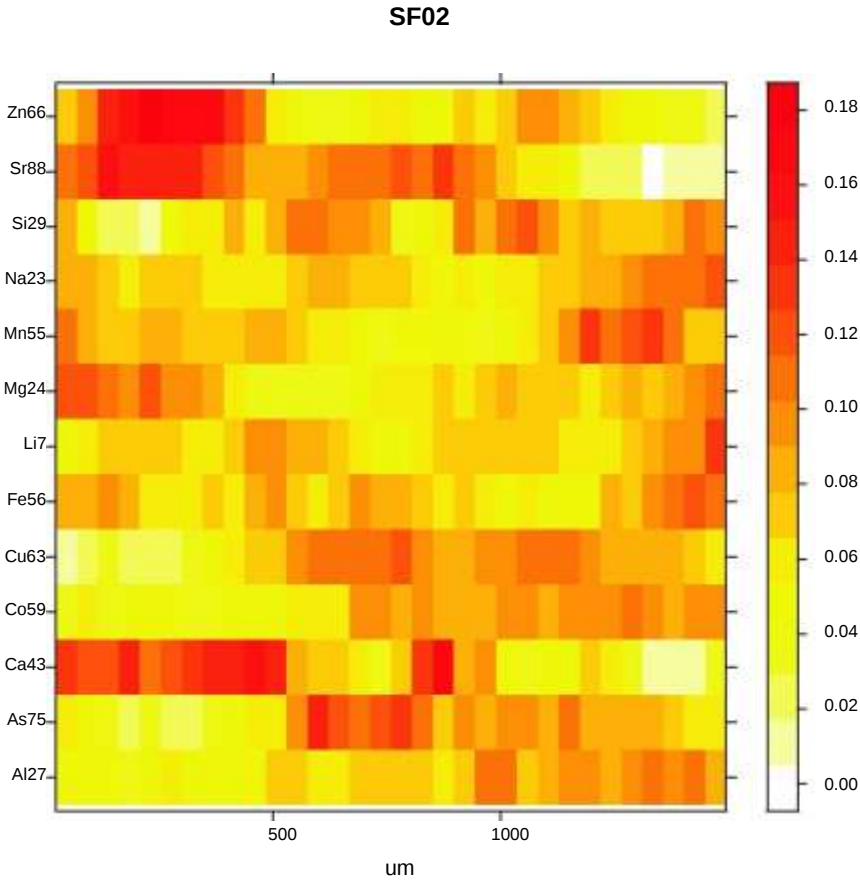
E21



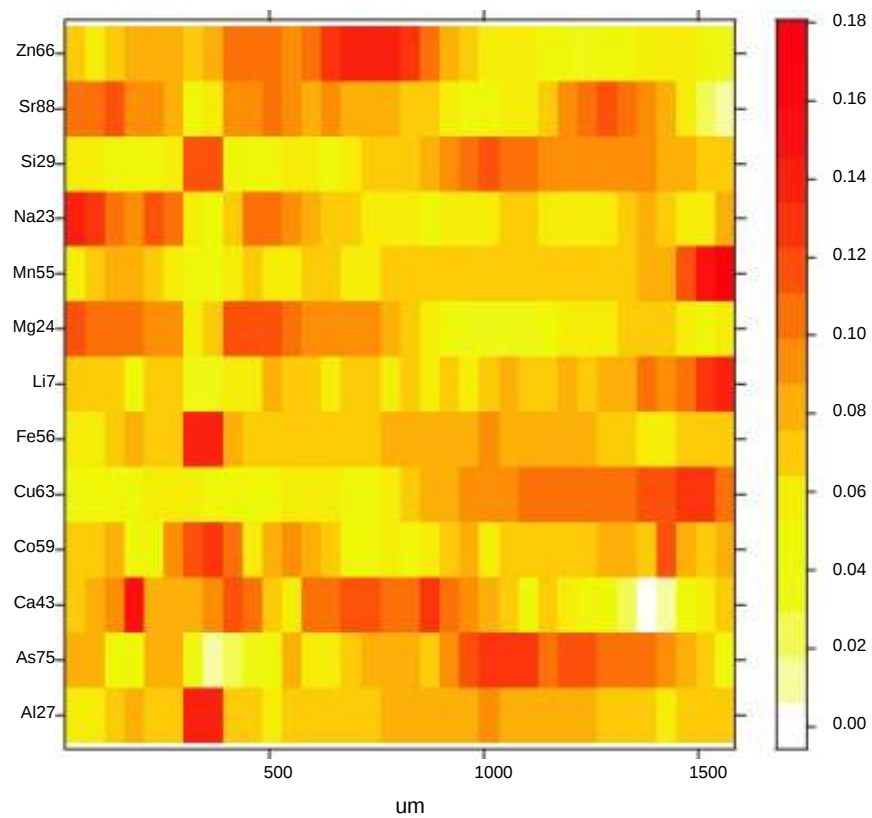




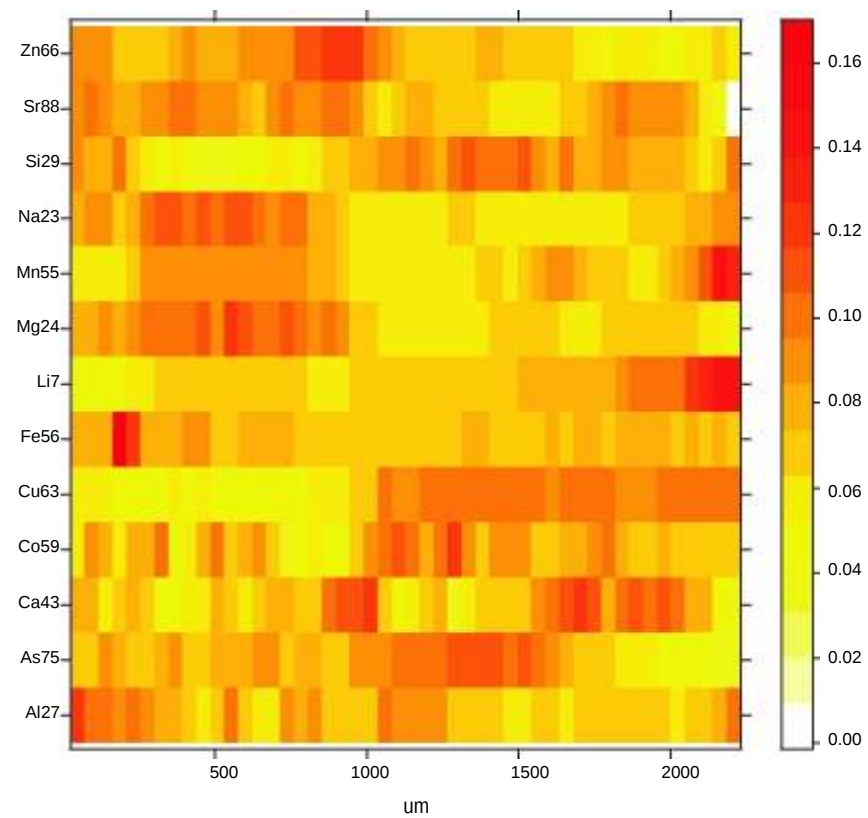


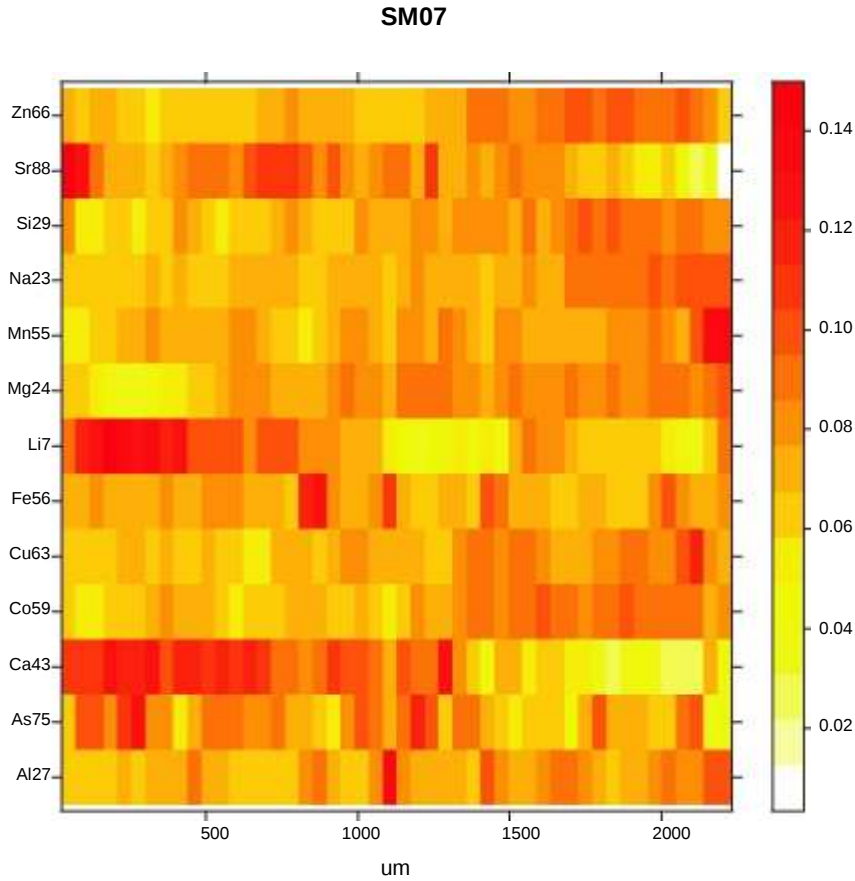
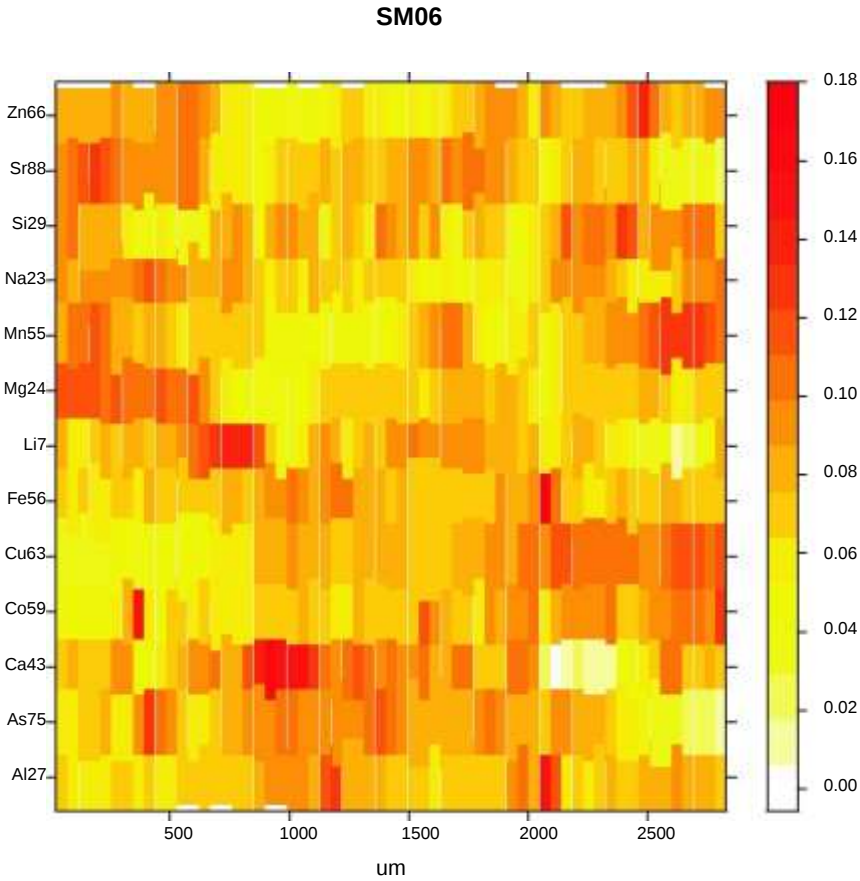


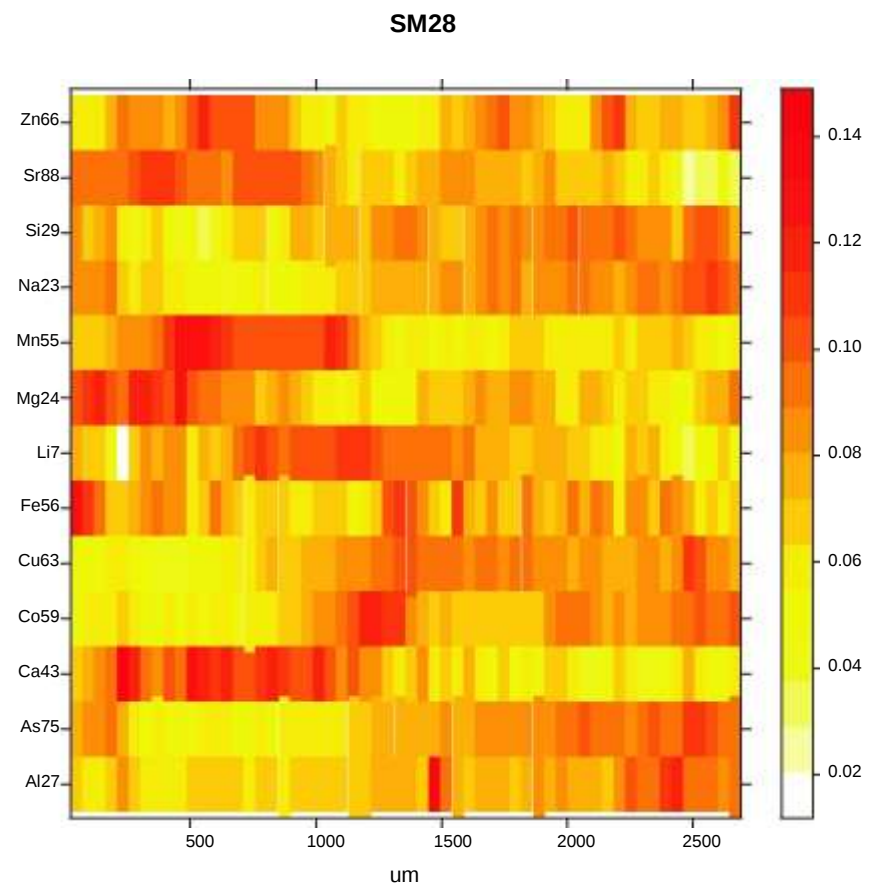
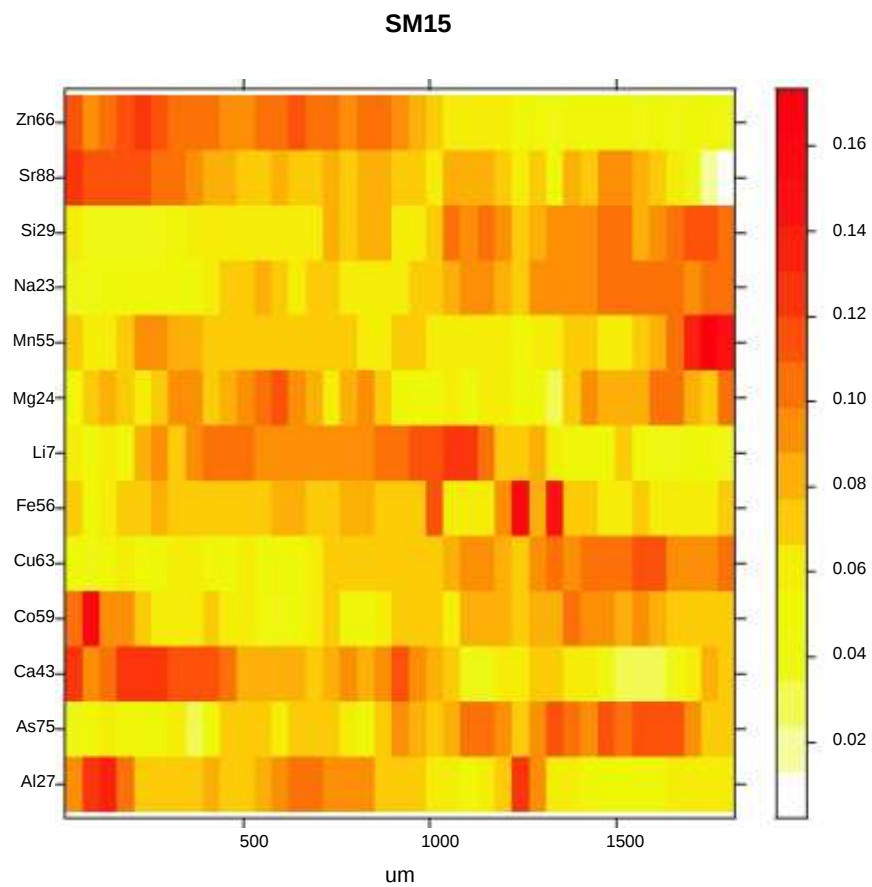
SF07



SF10







SM29

