Trace Element Accumulation within the Tissues of the Endangered Maugean Skate (*Zearaja maugeana*)

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Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution, and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Erin Woodward

Thesis Introduction

This thesis consists of two chapters. Chapter One is a comprehensive literature review, introducing the field of study and detailing the scope and key focus of the research. It contains its own list of references at the end of the chapter. Chapter Two consists of a manuscript prepared for submission to the journal Environmental Pollution and has been formatted according to the journal guidelines. As a result, the manuscript contains a level of content overlap with Chapter One and contains its own list of references at the end of the chapter.

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1.0 Literature Review
1.1 Trace elements

Periodic elements that are naturally present in the environment in concentrations of less than 1 mg kg^{-1} are referred to as trace elements (e.g., Pais and Jones, 1997; Gaillardet et al., 2003; Chester, 1990). Some trace elements are essential for the biological functioning of organisms (i.e., Cu, Fe etc.) (e.g., Pais and Jones, 1997; Richir and Gobert, 2016), performing roles such as assisting enzymatic activity and metabolism (e.g., Cu in fishes) (e.g., Watanabe et al., 1997), while others are not essential for biological functioning (i.e., Hg, Cd etc.) (e.g., Kolarova and Napiorkowski, 2021; Richir and Gobert, 2016). Both essential and non-essential trace elements can become toxic when concentrations are elevated (e.g., Kolarova and Napiorkowski, 2021; Richir and Gobert, 2016). Trace elements naturally exist in the marine environment, their primary natural sources being continental runoff and atmospheric deposition (e.g., Callender, 2003; Kolarova and Napiorkowski, 2021). In recent decades however, anthropogenic activity has significantly increased the loads of trace elements released into marine environments, with activities such as mining, smelting, and the combustion of coal, oil, and wood, as well as general human habitation, releasing copious amounts of trace elements into the environment (e.g., Richir and Gobert, 2016; Kolarova and Napiorkowski, 2021). Trace element pollution has recently become of particular interest within the research community, as the alteration of trace element concentrations in the environment has been known to have detrimental effects on the biology and ecology of marine life (e.g., Kolarova and Napiorkowski, 2021; Richir and Gobert, 2016). Trace elements considered to be the most toxic in the environment include Cr, As, Cd, Hg, and Pb (Tchounwou et al., 2014). These elements all induce toxic effects to humans and other organisms, even at low-level exposure (Tchounwou et al., 2014), thus the bioaccumulation and biomagnification of these elements through the food chain is of particular concern considering human consumption, though it is also important to consider the effects of these elements on the biological and ecological functioning of other organisms.

1.2 Trace Elements and Fishes

Fishes can accumulate trace elements in their tissues through the absorption of elements across permeable membranes (bioaccumulation) and the assimilation of elements from consumed media (biomagnification) (Streit, 1998). Excessive concentrations of trace elements can adversely affect the biology and ecology of teleost fishes, with impacts varying based on factors such as element type, concentration, exposure duration, environmental conditions, and the organism's sex, physiology, and ecology (e.g., Bezerra and Lacerda, 2019; Tiktak et al., 2020; De Souza Machado et al., 2016; Zhang et al., 2016).

The most commonly encountered effects that are inflicted on teleosts by elevated non-essential trace element exposure within the literature include neurotoxicity (i.e., Al, As, Ba, Pb, Hg) (e.g., Pereira et al., 2019; Baatrup, 1990; Zhu et al., 2016; Baldissarelli et al., 2012; Lee et al., 2019; Sukharenko et al., 2017; Chandra et al., 2020), immunotoxicity (i.e., As, Cd, Pb) (e.g., Lee et al., 2019; Datta et al., 2009; Sovenyi and Szakolczai, 1993), inhibited growth (i.e., Al, As, Cd, Sn, Ba, Hg, Pb) (e.g., Depew et al., 2012; Han et al., 2019; Rose et al., 2006; Biesinger and Christensen, 1972), reduced reproductive success (i.e., Al, As, Cd, Sn, Ba, Hg, Pb) (e.g., Depew et al., 2012; Levesque et al., 2003; Ferreira et al., 2023; Thomsen et al., 1988; Biesinger and Christensen, 1972), oxidative stress (i.e., Al, As, Cd, Ba, Hg, Pb) (e.g., Mieiro et al., 2011; Zhu et al., 2016; Bhattacharya and Bhattacharya, 2007; Lee et al., 2019; Sukharenko et al., 2017), and histological changes (i.e., Al, As, Cd, Hg, Pb) (e.g., Xie et al., 2019; Giari et al., 2007; Adams and Sonne, 2013; Paul et al., 2019; Agnihotri et al., 2010; Hossain, 2014; Exley et al., 1991). It is evident that exposure of fishes to elevated concentrations of non-essential trace elements can have deleterious effects on the biological and ecological functioning of the organism, often causing issues associated with the overall survival of the species.

Despite the vital nature of essential trace elements for the functioning of fishes, their exposure to elevated concentrations of these elements has demonstrated many of the same common effects as that of non-essential trace element exposure on fishes in the literature, including that of neurotoxicity (i.e., Cr, Mn, Ni, Cu) (e.g., Malhotra et al., 2020; Garai et al., 2021; Vieira et al., 2011), immunotoxicity (i.e., Cr, Mn, Fe, Ni, Cu) (e.g., Garai et al., 2021; Ali et al., 2021; Ololade and Oginni, 2010; Al-Ghanim, 2011; Aliko et al., 2018; Do et al., 2019), inhibited growth (i.e., Cr, Mn, Fe, Co, Ni, Cu, Zn) (e.g., Yadav et al., 2020; Malhotra et al., 2020; Biesinger and Christensen, 1972; Garai et al., 2021; Alsop et al., 2014; Javed, 2013; Nasri et al., 2019; Clearwater et al., 2002), reduced reproductive success (i.e., Cr, Mn, Fe, Co, Ni, Cu, Zn) (e.g., Malhotra et al., 2020; Biesinger and Christensen, 1972; Garai et al., 2021; Srivastava and Agrawal, 1983; Lewis, 1976; Alsop et al., 2014), oxidative stress (i.e., Mn, Fe, Co, Ni, Cu, Zn) (e.g., Malhotra et al., 2020; Garai et al., 2021; McRae et al., 2016; Sudhabose et al., 2023; Vieira et al., 2011; Do et al., 2019; Blewett et al., 2016; Blewett and Leonard, 2017; Kubrak et al., 2011; Sun et al., 2020), and histological changes (i.e., Cr, Mn, Fe, Co, Ni, Cu, Zn) (e.g., Malhotra et al., 2020; Garai et al., 2021; Al-Attar, 2007; Fonseca et al., 2017; Sun et al., 2020; Palani et al., 2015).

Severe exposure of fishes to both essential and non-essential trace elements frequently results in mortality, and this is often used as an endpoint in elemental toxicity studies (e.g., Ebrahimpour et al., 2010; Abdullah et al., 2007). It is evident from the available literature that the effects of element toxicity on fishes have been more thoroughly investigated for some elements (e.g., Fe, Cu, Hg) than others (e.g., Sr, Sn). Although many different factors contribute to the effects of exposure to elevated trace element concentrations in fishes, it is agreed that exposure of fish to elevated trace element concentrations has deleterious effects on the organism's biological and ecological functioning, thus implying that trace element pollution is a concerning environmental issue of extreme importance that should be monitored, especially in geographical areas of concern.

1.3 Trace Elements and Elasmobranchs

While there has been extensive research on the effects of elevated trace element concentrations on teleosts, studies on elasmobranch species are scarce, largely restricted by ethical conflict, lack of funding, and sampling difficulties. Most existing literature focuses on quantifying trace element concentrations within elasmobranch tissues, often in the context of human consumption (Appendix 1). This is concerning, considering the biological (e.g., low reproductive output, late maturation) and ecological (e.g., high trophic position, bottom dwelling) traits possessed by elasmobranch species, which potentially make them more vulnerable to pollutant accumulation (e.g., Pierce and Bennett, 2010; Dulvy et al., 2017; Bezerra and Lacerda, 2019).

The hypersensitivity of elasmobranchs to trace element accumulation has been explored in a few studies. Aquaria experiments found that three elasmobranch species (*Squalus acanthias*, *Squalus suckleyi*, and *Raja rhina*) accumulated environmental Ag concentrations ten times faster than marine teleosts from the same area (Vancouver Island, Canada), despite elasmobranchs possessing fewer uptake pathways (De Boeck et al., 2001; Webb and Wood, 2000). Similarly, another aquaria study reported that *Raja erinacea* specimens, obtained from the waters of Maine, USA, accumulated 13 times more Cu in gill tissues than that of marine teleost fish (Grosell et al., 2003). In a survey of fishes in Mexican waters, the elasmobranch *Urolophus helleri* exhibited the highest concentrations of Cd and Hg, attributed to its physiological and ecological characteristics (Jonathan et al., 2015). This higher uptake and retention of trace elements in elasmobranchs may indicate lower tolerances to elevated concentrations (Webb and Wood, 2000), though further research is needed to confirm this hypothesis.

Elasmobranchs not only face increased susceptibility to trace element accumulation through bioaccumulation, but also through biomagnification, due to the high trophic position they often fill (e.g., Bezerra and Lacerda, 2019; Tiktak et al., 2020). Ineffective trace element elimination processes cause these elements to be accumulated in the tissues of organisms, with passage up the food web through predator-prey interactions (Tiktak et al., 2020). As elasmobranchs are typically apex predators, this puts them at risk of biomagnification and over exposure to trace elements (Boldrocchi et al., 2021).

The literature currently lacks comprehensive studies on the specific effects of elevated trace element concentrations on elasmobranch physiology and ecology, thus, effects of trace element toxicity in elasmobranchs are often equated to that of teleosts in literature (e.g., Bezerra and Lacerda, 2019; Tiktak et al., 2020). While few toxicity studies specifically focus on elasmobranch species, existing research suggests that elements such as Cd and Hg can alter the reproductive physiology of elasmobranchs (Bendall et al., 2014), and that elements including Co, Ni, and Cu are associated with lower body condition in female elasmobranchs (Wosnick et al., 2021), though deeper investigations into the effects of trace element accumulation on elasmobranch health is needed. Moreover, it has been documented that elasmobranchs can transfer pollutants to their offspring (e.g., Frias-Espericueta et al., 2015; Souza-Araujo et al., 2020; Martins et al., 2023; Lyons and Lowe, 2013), posing a potential threat to populations worldwide, particularly those inhabiting contaminated waters.

1.4 Assimilation, Retention and Elimination of Trace Elements in Elasmobranchs

Elasmobranchs acquire trace elements into their tissues primarily through two pathways: bioaccumulation from the surrounding water via crossing of the permeable membranes of the gills, and biomagnification from their diet, through gastrointestinal absorption (Pouil et al., 2018). Following accumulation, these elements enter the bloodstream and are subsequently distributed throughout the body (Mathews et al., 2008).

Studies have shown that trace elements accumulate differently among organs in elasmobranchs, due to variations in elimination processes and accumulation rates (Hauser-Davis, 2020). Organs reflect differing timescales of accumulation, with isotopic turnover rates varying between species but generally following a consistent order (e.g., liver > blood plasma > muscle > cartilage) (Wyatt et al., 2019; Logan and Lutcavage, 2010; MacNeil et al., 2006). The liver is known to possess effective element elimination processes, as this organ possesses large quantities of Metallothionein, which is a free metal binding protein, meaning elements accumulated in the liver can be promptly eliminated (Hauser-Davis, 2020). Tissues such as the muscle possess less effective elimination processes, meaning elemental concentrations within the muscle reflect a longer timeframe of elemental accumulation than that of the liver. It is likely that these mechanisms and their effectiveness for elimination differ between trace elements however, as literature occasionally reports higher accumulation of specific elements within the liver, compared to the muscle (i.e., Cd) (e.g., Robertson, 2021; Gilbert, 2015).

Cartilage within elasmobranchs is known to represent the longest timescale for the environmental monitoring of trace elements. Once elements are incorporated into cartilaginous structures of elasmobranchs, their elemental composition is retained and minimally reworked over their lifetime, thus providing a snapshot of trace element exposure over the organism's lifetime (Dudgeon et al., 2007). Elasmobranch vertebral centra consists of deposits of calcium phosphate ($Ca_{10}(PO_4)_6(OH)_2$), which grows through gradual accumulation at the vertebral edge (Doyle, 1967). Elemental incorporation within elasmobranch cartilage occurs through two ways: substitution for Ca or entrapment in the matrix during the accretion process (Dudgeon et al., 2007). The former is the most common method of cartilage elemental incorporation, with studies finding evidence of Mn, Fe, Sr, Cd, Ba, and Pb being incorporated in this way (e.g., Pon-On et al., 2008; Schoenberg, 1963; Wells et al., 2000; Bigi et al., 1991). The latter is a less common method of elemental incorporation, though studies have found that Zn is mainly incorporated in this way (Tang et al., 2009). Though it is known that there are many variables that can affect vertebral incorporation of trace elements in elasmobranchs (e.g., temperature, salinity, diet) (e.g., Smith, 2013; Smith et al., 2013), studies have found that vertebral centra can provide time-resolved environmental reconstructions for elasmobranchs (e.g., McMillan et al., 2016; Hussey et al., 2012; Smith et al., 2013; Smith, 2013).

1.5 Methods for the Measuring of Trace Element Concentrations

Multiple methods exist for the analysis of trace element content in biological samples, the most common being flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS), though ICP-MS is the most effective technique for multi element analysis (Bolann et al., 2009). In this technique, sample elements are ionised in a very hot argon plasma (5-10,000 K), with generated ions separated according to their mass to charge ratio in a mass spectrometer (Bailes, 2012). Biological samples require acid digestion and dilution prior to ICP-MS analysis and common acids and reagents used for the digestion of elasmobranch muscle tissues include nitric acid, perchloric acid, sulfuric acid, aqua regia, hydrochloric acid, and hydrogen peroxide (Appendix 2) (e.g., Giovos et al., 2022; Turoczy et al., 2000; Glover, 1979; Robertson, 2021; Adel et al., 2017). The use of acid/s combined with heat, usually applied either with the use of a heat block or an acid digestion microwave, digests the tissue and frees elements from the tissue matrix, allowing for their analysis by ICP-MS (Wilschefski and Baxter, 2019).

Elemental content in solid samples, such as elasmobranch cartilage, can also be measured with laser ablation ICP-MS (LA-ICP-MS). As with regular ICP-MS, samples are ionised, separated, and their quantity measured however, instead of analysis of a digested sample, a pulsed laser is used to ablate the solid sample, thus producing a cloud of debris that is inserted directly into plasma (Pitts and May, 2023). This technology has been used in multiple studies to determine trace element concentration within elasmobranch vertebral bands, usually as a technique of age determination and verification (e.g., Hale et al., 2006; Scharer et al., 2012; Christiansen, 2011; Campbell, 2010; Tillett et al., 2011; Coiraton et al., 2019). Studies have also used this technology to discern migrational and habitat utilisation patterns (e.g., Grant et al., 2023; Young, 2023; Christiansen, 2011; Brodbeck et al., 2023;

Tillett et al., 2011; TinHan et al., 2020; Livernois et al., 2021; Feitosa et al., 2020; Lewis et al., 2015), and to assess species stock structure (McMillan et al., 2017). It has also been used on the spines of the Pacific Spiny Dogfish (*Squalus suckleyi*) to quantify elemental exposure and accumulation over time (Bailes, 2012). Despite extensive use of this technology, LA-ICP-MS investigation of elasmobranch vertebral microchemistry, to our knowledge, has not yet been used as an indicator of life-time elemental exposure and accumulation.

1.6 Macquarie Harbour Trace Element Pollution

One Australian waterway that is of particular concern in light of anthropogenic pollution is Macquarie Harbour (Figure 1), situated on the west coast of Tasmania. This water body is known to be the most extensively copper-contaminated estuary in the Southern Hemisphere, attributed to historical inputs from the Mount Lyell copper mine (Teasdale et al., 2002). From the 1890's to the 1990's, this mine was almost continuously worked for Cu (McQuade et al., 1995), with an estimated total of approximately 97 Mt of Cu-rich sulphide deposits being released into the Queen River, which joins with the King River and flows into Macquarie Harbour, over the course of its operation (Augustinus et al., 2010; Locher, 1997). This continuous input of elevated Cu concentrations has significantly increased concentrations within the harbour, with multiple studies having measured Cu concentrations (4-560 µg L^{-1}) well over the Australian and New Zealand Environment Conservation Council (ANZECC) water quality guideline value for Cu (e.g., Teasdale et al., 2002; Strauber et al., 2000; Eriksen et al., 2001; Carpenter et al., 1991) of 5 µg L^{-1} (ANZECC, 1992).

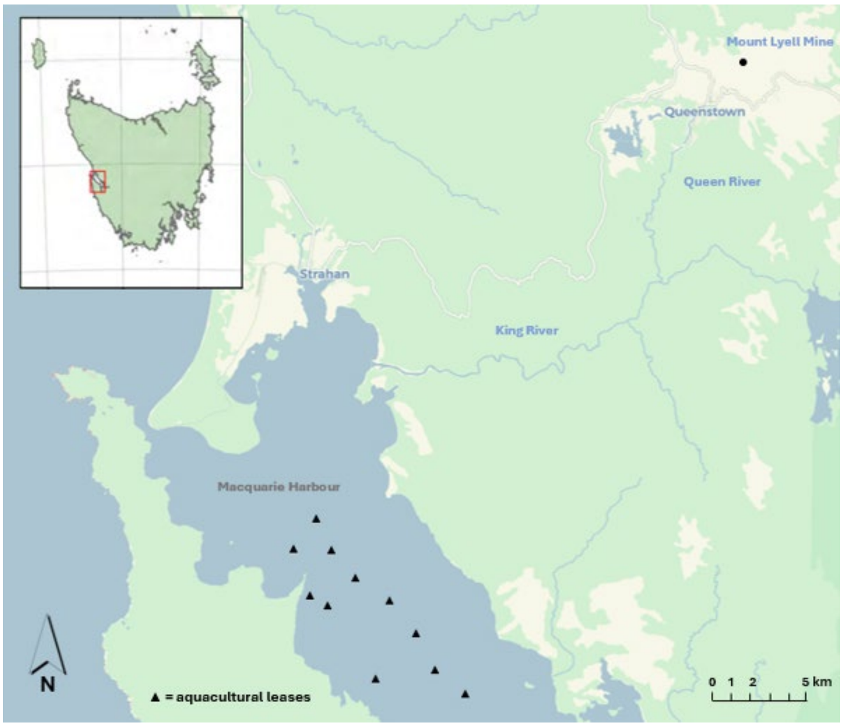

Figure 1: Macquarie Harbour in relation to Mount Lyell mine (aquacultural leases shown)
Source: Seamap, 2023

Although the dumping of mining tailings into the Queen River ceased after the mine's closure in 1994, trace element concentrations continue to be introduced into the river system, due to the remobilisation of mine waste in the river delta and acid rock drainage (e.g., Augustinus et al., 2010; Koehnken, 1997; McQuade et al., 1995). Acid rock drainage in the Mount Lyell area is attributed to the oxidisation of pyritic bedrock, exposed to oxygen and water through the erosion of soils caused by mining activities, which contributes to the dissolution of Al, Mn, Fe, Co, Cu, and other trace elements from the sediments (e.g., Nascimento et al., 2023a; Taylor et al., 1996; Augustinus et al., 2010). Multiple studies have found evidence of significantly elevated trace elements within the waters of Macquarie Harbour. A study conducted by Carpenter et al. (1991) stated that trace element concentrations in uncontaminated coastal waters should fall on the upper limit of that found in oceanic waters. Carpenter found Mn concentrations 144 times higher (260-720nM), Fe concentrations 350 times higher (585-1050nM), and Zn concentrations six times higher (16-60nM) within the harbour than that of the upper limit of oceanic waters (5nM, 3nM, and 10nM respectively (Bruland et al., 2014)). These findings of high Mn and Fe concentrations within the harbour aligned with that found by Teasdale et al. (2002), who measured Mn concentrations of 172-875 μg L^{-1} and Fe concentrations of 256-1040 μg L^{-1} and attributed these elevated concentrations to acid rock drainage. Strauber et al. (2000) also found elevated concentrations of Al (71-149 μg L^{-1}), Mn (17-100 μg L^{-1}), Fe (19-86 μg L^{-1}), and Zn (5-21 μg L^{-1}) compared to background sea water concentrations. Despite this, Zn concentrations in the Strauber et al. (2000) study were found to be well within the ANZECC water quality guideline value of 50 μg L^{-1} (ANZECC, 1992). Carpenter et al. (1991) also found concentrations of Ni (4.4-9.5nM) and Cd (0.06-0.24nM) to be within the upper limit of ocean waters (2.0-12nM and 0.001-1.05nM respectively (Bruland et al., 2014)). Recently, nearly 30 years after the closure of Mount Lyell copper mine, Nascimento et al. (2023b) found that concentrations of Fe, Cu, and Zn were still significantly elevated compared to ANZECC guidelines. This study also found that concentrations of Al and Co were below ANZECC guidelines (Nascimento et al., 2023b). There is an evident gap in the literature surrounding specific trace element concentrations in the waters of Macquarie Harbour, including that of As, Rb, Sr, Mo, Ba, and Pb. The legacy of mining in the Mount Lyell region continues to produce large volumes of acid rock drainage from old mine workings, waste rock, and mine dewatering (McQuade et al., 1995) and acid generation from waste rock is predicted to continue for approximately 600 years (Koehnken, 1997), meaning the surrounding waterbodies, including Macquarie Harbour, will be subject to elevated trace element concentrations for centuries to come.

1.7 Environmental Conditions of Macquarie Harbour

Macquarie Harbour presents a unique aquatic environment, characterized by three distinct layers in its water column: the surface layer (0-10m), the mid-layer (10-20m), and the bottom layer (>20m) (Ross et al., 2020). The surface layer, influenced by river inflows, consists predominantly of freshwater and undergoes rapid discharge to the ocean, maintaining high dissolved oxygen (DO) levels replenished by atmospheric interaction (Ross et al., 2020). In contrast, the sub-surface layers, residing within the harbor for a longer period of time, experience reduced DO levels due to biological processes such as benthic organism respiration and limited light penetration through the tannin-stained surface layer, impeding primary production (Ross et al., 2020). While the bottom layer is primarily saltwater, it may exhibit higher DO concentrations than the mid-layer due to occasional displacement by seawater (Ross et al., 2020). The natural stratification of this waterway means that DO concentrations are naturally lower in the deep waters of Macquarie Harbour however, the deposition of organic matter from aquacultural activity fosters increased biological activity and this further depletes already low DO levels (Ross et al., 2020). The utilisation of the Gordon River for hydro-electric generation has also contributed to the reduction of DO within the waters of Macquarie Harbour (e.g., MHDOWG, 2014; Moreno et al., 2020).

Other environmental conditions of Macquarie Harbour have also changed over time. It has been found that the deep waters of Macquarie Harbour increased in temperature by 1.5-2ºC between 1993 and

2020 (Ross et al., 2022). Studies have also shown that acid rock drainage from Mount Lyell mine has influenced the pH of the King and Queen Rivers (Nascimento et al., 2023b). Nascimento et al. (2023b) found that the King River above the Queen River possessed a pH range of 5.7-8.4 however, the lower King River possessed a pH average of 4.5, thus suggesting a pH reduction caused by mine derived acidic inputs from the Queen River. Though it is known that the harbour possesses an average pH of 5.5, little to no monitoring of long-term pH changes has been done in Macquarie Harbour and it is not currently known how these acidic inputs have affected the pH levels of the harbour.

1.8 Environmental Factors Affecting Trace Element Availability

Trace element pollution within Macquarie Harbour is primarily sequestered and stored within its sediments (Teasdale et al., 2002). Sediments can bind and release elements through adsorption/desorption and precipitation/dissolution processes, which are influenced by environmental factors such as pH, temperature, oxidation-reduction potential (Eh), and dissolved oxygen (DO) levels (e.g., Teasdale et al., 2002; Linge, 2008). Macquarie Harbour naturally possesses conditions ideal for element storage, as anoxic conditions are known to promote element sequestration (Ye et al., 2013). Despite this, environmental changes in recent decades may promote the release of trace elements from sediments. Increased organic matter, such as that from salmonid farms, can enhance element solubility, by competing for sorption sites or forming complexes with elements (Linge, 2008). Low pH and high Eh, such as that found in the King and Queen Rivers, also facilitate element dissolution (e.g., Nascimento et al., 2023b; Linge, 2008). Macquarie Harbour's relatively high Eh (260 mV) and near-neutral to acidic pH (5.5) likely contribute to the potential remobilization of elements into the water column, particularly Cu (e.g., Nascimento et al., 2023b; Ye et al., 2013). Acidification can also lead to the release of Al from sediment (Driscoll et al., 1980). Elevated temperatures further accelerate elemental release rates from sediments (Li et al., 2013). Continued decline of water quality in Macquarie Harbour may lead to increased trace element concentrations in the water column, exacerbating the potential impacts of trace element pollution on Macquarie Harbour’s endemic fauna.

1.9 Maugean Skate (*Zearaja maugeana*)

Trace element pollution in the waters of Macquarie Harbour poses a significant threat to its endemic fauna, particularly the Maugean skate (*Zearaja maugeana)* (Figure 2). Believed to be a Gondwanan relic, this species shares its closest relatives with the Rough skate (*Z. nasuta*) in southern New Zealand and the Yellownose skate (*Z. chilensis*) in Patagonia (Treloar et al., 2017). As a bottom-dwelling skate, it primarily feeds on brachyuran crabs, teleosts, and polychaetes, with the Red Spotted Shore Crab (*Paragrapsus gaimardiii*) dominating its diet (Treloar et al., 2017; Weltz et al., 2019). Displaying high site fidelity, the skate prefers benthic habitats within the harbor at depths ranging from 5 to 15 m, occasionally venturing into deeper waters (Moreno et al., 2020). It is an oviparous species, depositing its eggs on the benthos across a wide depth range of 2.5 to 33 m, often beyond its preferred depth range (Moreno et al., 2020; Treloar et al., 2017).

This elasmobranch species is unique as it is the only known skate species to permanently reside in brackish waters (Bray, 2023) and it possesses possibly the most restricted habitat distribution of any elasmobranch, only having been reported in two estuaries on the west coast of Tasmania: Macquarie Harbour and Bathurst Harbour (Moreno et al., 2020; Last and Glenhill, 2007; Treloar et al., 2017; Edgar et al., 2010). Very few individuals have been sighted in Bathurst Harbour however, with no sightings in this estuary since 1992 (Treloar et al., 2017; Weltz et al., 2017; Last and Glenhill, 2007).

A study by Moreno et al. (2022), using eDNA sampling, found no evidence of a Maugean skate population within Bathurst Harbour. The population that exists within Macquarie Harbour is quite small, potentially the smallest of any elasmobranch species, estimated at 3200 individuals in 2016 (Bell et al., 2016). Such a small population is bound to be extremely vulnerable to environmental change. Moreno and Semmens (2023) estimated that the relative abundance of Maugean skate within Macquarie Harbour declined by 47% between 2014 and 2021, most likely largely attributed to extreme weather events and low DO levels within the harbour. This decline suggests a reduction in population size since the 2016 estimate, though further research is necessary to assess the current population size. Given its exceedingly limited habitat distribution and low population count, the Maugean skate has been classified as endangered under the Environmental Protection and Biodiversity Conservation Act (1999), the Tasmanian Threatened Species Protection Act (1995), and listed on the IUCN Red List (Last and Glenhill, 2007). As Macquarie Harbour is the only remaining refuge for this vulnerable species, it is of the utmost importance that the health of this estuary is closely monitored and regulated.

Figure 2: Ventral and dorsal view of mature male Maugean skate (*Zearaja maugeana)*
Source: Last and Glenhill, 2007

Studies have shown that the anoxic and hypoxic nature of Macquarie Harbour's sub-surface waters have caused severe pressure on the Maugean skate. Moreno et al. (2020) found that skate are subject to DO fluctuations of up to >90% in a day. Although the skate seems to have adapted to highly fluctuating DO concentrations by undergoing metabolic depression, this decreases the energy available for other life history processes, such as reproduction, growth, and foraging (Moreno et al., 2020), thus making them particularly vulnerable to other threats present within the harbour.

1.10 Maugean Skate and Trace Elements

Due to the bottom feeding and dwelling nature of this species, it is highly susceptible to the bioaccumulation and biomagnification of elevated trace element concentrations within Macquarie Harbour's waters and sediments. Currently, two studies have investigated concentrations of trace elements within the tissues of the Maugean skate. The first, conducted on samples collected in 1992, analysed trace element concentrations with the use of Atomic Absorption Spectrometry (AAS). This study also investigated trace element concentrations in four teleost species (*Pseudophycis bachus*, *Rhombosolea tapirine*, *Salmo trutta*, and *Genypterus tigerinus*) and one other elasmobranch species (*Squalus acanthias*), also inhabiting Macquarie Harbour. The second, conducted on samples collected in 2014, analysed trace element concentrations with the use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS). This study also investigated trace element accumulation within another elasmobranch species inhabiting Macquarie Harbour (*Squalus acanthias*) and two elasmobranch species inhabiting Port Phillip Bay, Victoria (*Urolophus paucimaculatus* and *Trygonoptera imitate*). Both studies reported acceptable concentrations of Cu, Zn, and Hg for elasmobranchs inhabiting waters around southern Australia (De Blas, 1994; Robertson, 2021). Mercury concentrations were found to have increased between the two studies, though other elements could not be compared, as they either were not measured in the earlier study or Robertson did not report concentrations in wet weight (ww). Robertson also reported elevated concentrations of Cr and As within skate tissues (Robertson, 2021). Though both studies measured concentrations of Hg within skate tissues, both used open vessel acid digestion methodology, which is often not considered a compatible digestion technique for the measurement of Hg, due to its volatile chemical nature. As a result, Hg concentrations reported by both studies were most likely not reflective of Hg concentrations within the species. Both studies contained relatively small sample sizes (n = 3 for De Blas and n = 13 for Robertson), limiting the comprehensive understanding of trace element accumulation in skate tissues. The findings of Robertson revealed that there was no difference in trace element concentrations between sexes, although the sample size of 13 (9 females and 4 males) was too small for these results to be conclusive (Robertson, 2021). It is important that trace element concentrations within the tissues of the Maugean skate continue to be monitored, in order to assess potential changes in bioaccumulation and biomagnification within the species over time, which may indicate impaired biological and ecological health of the skate. Due to the endangered status of this species, it is also imperative that accumulation of trace elements within the animal's tissues be monitored without the need for lethal sampling.

1.11 Rationale for Current Study

Whilst trace element concentrations within the tissues of the endangered Maugean skate have been investigated on two separate occasions, both studies possessed rather limited sample sizes, and were ~22 years apart, and hence were not able to be compared. Therefore, no effective, time-resolved monitoring of trace element accumulation within the tissues of the Maugean skate exists. Due to the endangered status of this species and the significant environmental pressures that exist within Macquarie Harbour, it is important that trace element accumulation continues to be monitored within the skate's tissues, as a means of assessing bioaccumulation and biomagnification of trace element pollution. The aims of the current study were to quantify concentrations of trace elements of environmental and biological relevance within the muscle of the Maugean skate (*Zearaja maugeana*) via the use of non-lethal sampling and to compare these concentrations to those determined in previous studies, as a means of providing time-resolved insight into trace element accumulation within the tissues of the Maugean skate. The current study also aimed to determine elemental composition of Maugean skate vertebrae, in order to assess average life-long trace element accumulation and compare magnitude of elemental accumulation within different organs (i.e., muscle vs. vertebrae) of the skate. We hypothesised that many trace elements (i.e., Mn, Fe, Cu etc.) would be elevated within the tissues of the skate and that concentrations of these elements would have increased since previous studies, as despite the cessation of mining in the Mount Lyell area, acid rock drainage continues to add to trace element loads within the harbour. It was also hypothesised that due to differences in elemental incorporation pathways and element elimination capabilities between organs, trace element accumulation would differ between skate muscle and vertebrae, with elements performing structural roles within cartilage exhibiting elevated accumulation within the vertebrae.

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Chapter 2

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Title: Trace Element Accumulation within the Tissues of the Endangered Maugean Skate (*Zearaja maugeana*)

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Abstract

Elevated trace element concentrations, such as that within the waters of Macquarie Harbour, are known to cause a suite of deleterious effects on the functioning of organisms. Consequently, it is important that the accumulation of these pollutants be monitored within aquatic food chains. The Maugean skate (*Zearaja maugeana*), an endangered species of elasmobranch, resides only in the polluted waters of Macquarie Harbour, meaning the extent of trace element accumulation within its tissues should be monitored. The current study aimed to quantify trace element concentrations in Maugean skate muscle with the use of non-lethal sampling and to compare these concentrations with those determined in previous studies. Additionally, the study aimed to assess the elemental composition of Maugean skate vertebrae and to compare accumulation between different organs (i.e., muscle vs. vertebrae) of the skate. Trace element concentrations were determined within muscle and vertebrae samples with the use of solution ICP-MS and LA-ICP-MS analysis, respectively. The current study revealed Cr, Co, Cu, Sr, and Pb concentrations higher than that previously described within the muscle tissue of the Maugean skate. The concentrations of these elements were notably higher than those typically found in elasmobranchs inhabiting southern Australian waters. Chromium, Co, Cu, and Pb concentrations determined herein suggested the potential for toxicity effects to have been experienced by the Maugean skate. Manganese, Zn, Sr, and Ba concentrations accumulated predominantly in the vertebrae, rather than the muscle, suggesting that these elements may perform structural roles within elasmobranch cartilage. The biopsy punch methodology described herein provided an effective non-lethal toolset to assess trace element accumulation within Maugean skate muscle. It is recommended that trace element accumulation and its potential effects continue to be monitored and investigated within this species.

Key words: Maugean skate, Macquarie Harbour, trace elements, bioaccumulation, biomagnification

List of Abbreviations

ANOSIM	Analysis of Similarities
CODES	Centre for Ore Deposits and Earth Sciences
CRM	Certified Reference Material
CSL	Central Science Laboratory
dw	Dry Weight
EFSA	European Food Safety Authority
FSANZ	Food Standards Australia and New Zealand
hr	Hour/s
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IMAS	Institute of Marine and Antarctic Science
IUCN	International Union for Conservation of Nature
LA-ICP-MS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
LDA	Linear Discriminant Analysis
PERMANOVA	Permutation Analysis of Variance
PERMDISP	Permutational Multivariate Analysis of Dispersion
Std. dev.	Standard Deviation
TL	Total Length
WHO	World Health Organisation
ww	Wet Weight

1.0 Introduction

Trace elements, whether essential or non-essential, can induce a variety of toxicity effects on organisms in elevated concentrations. In teleost fishes, common effects include neurotoxicity, immunotoxicity, inhibited growth, reduced reproductive success, oxidative stress, and histological changes (e.g., Pereira et al., 2019; Depew et al., 2012; Biesinger and Christensen, 1972; Malhotra et al., 2020). While few toxicity studies specifically focus on elasmobranch species, existing research suggests that elements such as Cd and Hg can alter the reproductive physiology of elasmobranchs (Bendall et al., 2014), and elements including Co, Ni, and Cu are associated with lower body condition in female elasmobranchs (Wosnick et al., 2021).

Elasmobranchs are particularly vulnerable to biomagnification of pollutants like trace elements, attributed to their high trophic position (e.g., Bezerra and Lacerda, 2019; Tiktak et al., 2020). Moreover, they often exhibit higher bioaccumulation rates of trace elements compared to that of teleost fishes (e.g., De Boeck et al., 2001; Webb and Wood, 2000; Grosell et al., 2003). Elasmobranchs are known to transfer pollutants maternally to offspring (Frias-Espericueta et al., 2015), posing as a potential threat to populations worldwide, especially those inhabiting contaminated waters.

One Australian waterway that is of particular concern considering anthropogenic pollution is Macquarie Harbour, situated on the west coast of Tasmania. This water body is known to be the most extensively copper-contaminated estuary in Australia, attributed to historical inputs from the Mount Lyell copper mine being released into the Queen River, which joins with the King River and flows into Macquarie Harbour (Teasdale et al., 2002). Although the input of mining tailings into the Queen River ceased with the closure of the mine in 1994, trace element pollution continues to be introduced into the river system, due to the remobilisation of mine waste in the river delta and acid rock drainage (e.g., Augustinus et al., 2010; Koehnken, 1997). Many studies have measured elevated Cu concentrations within the harbour (e.g., Strauber et al., 2000; Eriksen et al., 2001; Carpenter et al., 1991; Nascimento et al., 2023), as well as elevated concentrations of Al, Mn, Fe, and Zn (e.g., Teasdale et al., 2002; Strauber et al., 2000; Carpenter et al., 1991; Nascimento et al., 2023). The legacy of mining in the Mount Lyell region continues to produce large volumes of acid rock drainage and acid generation from waste rock is predicted to continue for the next 600 years (Koehnken, 1997).

Trace element pollution in the waters of Macquarie Harbour poses a large threat to its endemic fauna; namely, the Maugean skate (*Zearaja maugeana*). This species is known to only exist with the waters of Macquarie Harbour, possessing possibly the most restricted habitat distribution of any elasmobranch (Moreno et al., 2020; Last and Glenhill, 2007). Based on the limited habitat distribution and low number of individuals of the species (estimate of 3200 in 2016 (Bell et al., 2016)), it was listed as endangered under the Environmental Protection and Biodiversity Conservation Act (1999) and the Tasmanian Threatened Species Protection Act (1995) and was placed on the IUCN's Red List (Last and Glenhill, 2007). As Macquarie Harbour is the only remaining refuge for the skate, it is imperative that the health of this estuary is monitored and regulated.

Currently, two studies have investigated concentrations of trace elements within the tissues of the Maugean skate. The first, conducted on samples collected in 1992, also investigated trace element concentrations in the muscle of four teleost species and another elasmobranch species inhabiting Macquarie Harbour (De Blas, 1994). The second, conducted in 2014, also investigated trace element accumulation within the muscle and liver of another elasmobranch species inhabiting Macquarie Harbour and two elasmobranch species inhabiting Port Phillip Bay, Victoria (Robertson, 2021). Both studies reported expected concentrations of Cu, Zn, and Hg for elasmobranchs inhabiting waters around southern Australia (Appendix 1) (De Blas, 1994; Robertson, 2021). Mercury concentrations were found to have increased in skate muscle between the two studies, though other elements could not be compared, as they either were not measured in the earlier study or Robertson (2021) did not report concentrations in wet weight. Robertson (2021) also reported elevated concentrations of Cr and As within skate muscle. Hg concentrations reported by both studies were most likely not reflective of Hg concentrations within the skate, as they both used open vessel acid digestion methodology, which is often not considered a compatible digestion technique for the measurement of Hg, due to its volatile chemical nature. Both studies contained relatively small sample sizes (n = 3 for De Blas (1994) and n = 13 for Robertson (2021)), thus limiting a comprehensive understanding of trace element accumulation in Maugean skate tissues. Consequently, there is currently no effective, ongoing monitoring of trace element accumulation in the skate's tissues.

Given the endangered status of the species and the environmental pressures in Macquarie Harbour, continuous monitoring of trace element accumulation within the tissues of the skate is crucial. The current study aimed to quantify trace element concentrations in Maugean skate muscle with the use of non-lethal sampling and to compare these concentrations with those determined in previous studies. Additionally, the study aimed to assess the elemental composition of skate vertebrae to understand average life-long trace element accumulation and compare accumulation between different organs (i.e., muscle vs. vertebrae) of the skate.

In this study, we hypothesised that many trace elements, such as Mn, Fe, and Cu, would be elevated within the tissues of the skate, with concentrations increasing since previous studies, as despite the cessation of mining in the Mount Lyell area, acid rock drainage continues to add to trace element loads within the harbour. Furthermore, we hypothesised that trace element accumulation would differ between skate muscle and vertebrae, due to differences in incorporation pathways and elimination capabilities, with elements playing structural roles in cartilage exhibiting elevated accumulation in vertebrae.

2.0 Methods

Separate methodology was employed for obtaining biopsy punch samples and vertebrae samples. Biopsy punch samples were obtained with the use of non-lethal sampling methods for use in the current study. Vertebrae samples were sourced opportunistically for use in Van Werven (2023) and vertebral microchemistry results were made available for the current study.

2.1 Study area

Field work was conducted between March 2023 and October 2023 at Table Head (-42.280519, 145.286175), Rum Point (-42.368345, 145.410458) and Kelly's Basin (-42.360232, 145.547389) within the waters of Macquarie Harbour (Figure 3), as skate have been most commonly encountered in these locations (Moreno et al., 2020).

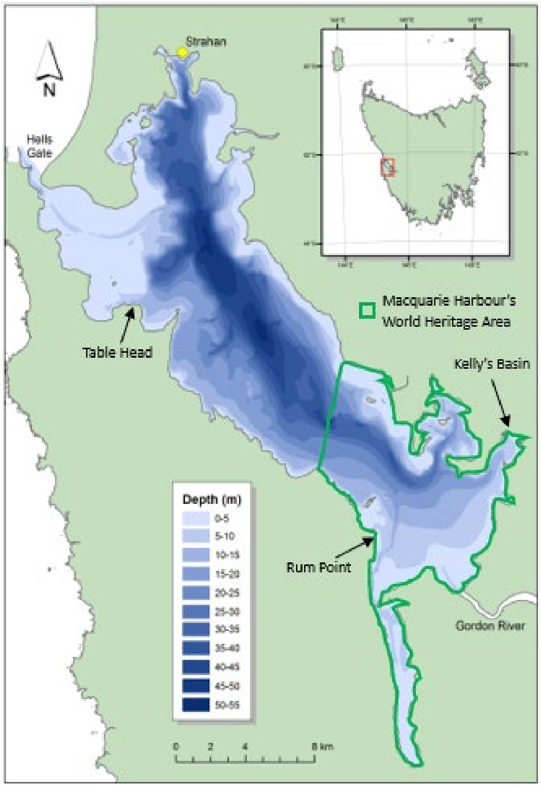

Figure 3: Study areas within Macquarie Harbour with depth distribution
Source: Lucieer et al., 2009

2.2 Field methodology

Live *Zearaja maugeana* were obtained using gillnetting methodology, deployed in channels with a depth of >5 m and allowed 1.5-2 hr of soak time. Captured skates were briefly held in a large, oxygenated water container prior to sampling. Individuals were sexed and measured for total length (mm). Skate were placed in a water filled tray and a small biopsy punch was taken from the left wing, well outside of the body cavity, using a disposable biopsy punch (Kai; 6mm). The sample was placed in a Corning cryogenic vial and put on ice for storage. Skate were released in the same area they were captured, ensuring a depth of >5 m. Samples were stored in liquid nitrogen for transportation and were stored in a -80°C freezer until further sample preparation could be commenced.

2.3 Biopsy Punch Trace Element Analysis (solution ICP-MS)

Tissues were lyophilized using a LABCONCO FreeZone 4.5 L freeze drier for >48 hr. Before lyophilization, samples were pre-frozen at -80ºC and weighed. Weights were periodically recorded every 12 hr. Lyophilization was deemed complete once samples presented constant weight. Following lyophilization, samples were weighed and transported to the Central Science Laboratory (CSL) for further sample preparation.

Freeze dried biopsy punches (0.022 ± 0.017 g) were placed into sterile 50 mL heat block containers (AnalytiChem, Canada). A 2 mL aliquot of aqua regia (3:1 suprapur HCL (Merck, Germany): baseline HNO_3 (Seastar Chemicals, Canada)) was added to each container. Multiple blank samples were prepared to account for any contamination within the experimental design. Approximately 0.022 g of DOLT-4 Dogfish Liver (n = 3) (National Research Council Canada, Canada) and Oyster Tissue (n = 3) (National Institute of Standards and Technology, United States of America) certified reference material (CRM) were also included. Elemental recoveries for CRM were detailed in Appendices 7 and 8. Containers were loosely capped, transferred to a DigiPREP MS heat block (AnalytiChem, Canada) and left to cold digest overnight (for >12 hr).

The next day, containers were slowly heated to 100ºC on the heat block, with the temperature held for 2 hr, or until all digest reagent was evaporated. Containers were transferred to a fume hood, where 1 mL of 69% baseline nitric acid (HNO_3) (Seastar Chemicals, Canada) was added to redissolve inorganic residues, followed by 0.5 mL of 30% baseline hydrogen peroxide (H_2O_2) (Seastar Chemicals, Canada). Containers were transferred back to the heat block, where they were heated to 80ºC for a further 1 hr. Containers were left to sit overnight before final dilution.

The next day, sample digests were diluted to 20 g with deionized water, and 0.5 mL of 10 mg L^{-1} Indium solution was added as an internal standard. Then, 10 mL of the sample was added to a sterile analysis container and transferred to an Element 2 High Resolution Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Thermo Fisher Scientific, Germany), ready for analysis. Quantification was achieved by comparing samples to known external calibration standards. The ICP-MS utilized multiple resolution modes to enable interference-free determination of relevant isotopes. The instrumental spectral resolution modes used for each element were detailed in Appendix 3. While higher resolution modes enhanced isotope identification accuracy, raw count sensitivity decreased. Hence, concentrations measured in high resolution mode (e.g., As) were interpreted cautiously. Quality control and instrument calibration accuracy were confirmed through the analysis of external certified reference waters. Blank concentrations for each element were subtracted from raw count data, and the data was transformed to mg kg^{-1} dw. Appendix 3 outlines the detection limits for each element analysed in the study, with elements not substantially above this limit (>2 times) being excluded from analysis.

2.4 Vertebrae Trace Element Analysis (laser ablation ICP-MS)

Vertebrae from *Zearaja maugeana* individuals were opportunistically sourced from Macquarie Harbour between April 2012 and May 2019. Additional information on field methods, biological sampling and site location is detailed in Van Werven et al. (2023). Elemental analysis results determined by Van Werven were made available for use in the current study.

Vertebrae samples were cleaned of excess tissue with a 50% bleach solution, rinsed for 30 minutes in a running water bath and left to air-dry under a fume hood for >24 hr. Samples were then mounted onto a casting resin block and sectioned along the sagittal plane using a low-speed diamond-bladed saw. Sections were rinsed with deionized water, sonicated four times for ten minutes each, and air-dried. Further details can be found in Van Werven et al. (2023).

Elemental concentrations were analysed using a NewWave NWR213-ESI laser system coupled to a quadruple ICP-MS at the Centre for Ore Deposits and Earth Sciences (CODES) laboratories. Before analysis, vertebrae samples underwent pre-ablation to remove surface contamination. Blank measurements were conducted for 28 seconds before each sample analysis. Standards including NIST612, BCR-2G, GSD-1G, and Durango were used for drift correction and calibration. Transects were drawn along the sagittal plane from the corpus calcaeum to the vertebral edge, into the resin mount, to capture the most recent material accreted. Average background levels in the pre-analysis blanks were subtracted from the raw count data and ^{43}Ca was used as an internal standard to transform raw count data into elemental concentrations (mg kg^{-1}). Appendix 4 shows the detection limits for each element analysed and elements that were not substantially above this limit (>2 times) were excluded from analysis. Further details can be found in Van Werven et al. (2023).

Differing element lists were used for solution ICP-MS and LA-ICP-MS, due to differences in instrument capabilities and sample preparation techniques. It was important to note that Hg was not included in solution ICP-MS analysis, due to the elements' incompatibility with the sample preparation and analytical techniques utilized herein.

2.5 Statistical Analysis

As no toxicity thresholds yet exist for elasmobranchs, concentrations determined within skate muscle were compared to human consumption limits. To compare concentrations in skate muscle with human consumption limits, concentrations determined in biopsy punch samples were converted to mg kg^{-1} ww. Vertebrae concentrations were averaged to determine average life-long elemental incorporation. Statistical analysis, conducted using R software (R Core Team), employed non-parametric tests, due to the non-normal distribution of the data (as determined by a preliminary Shapiro-Wilk test of normality). Univariate Permutation Analysis of Variance (PERMANOVA) assessed differences between sexes and seasons for biopsy punch samples and sexes and years for vertebrae samples. Pairwise PERMANOVA was used as a post-hoc test for significant PERMANOVA results with more than two groups. To include maximum number of samples in multivariate analysis, while also ensuring accuracy of the data, specific data points below the limits of detection were substituted for half the limit of detection (Croghan, 2003). Samples including extreme outliers were excluded from analysis (>2 times upper fence). Multivariate differences were tested with PERMANOVA, and Analysis of Similarities (ANOSIM) compared dissimilarity within groups to dissimilarity between groups. Permutational Multivariate Analysis of Dispersion (PERMDISP) assessed differences in data spread. Stepwise Linear Discriminant Analysis (LDA) maximized group differences to create a classification model based on elemental signatures.

3.0 Results
3.1 Trace Element Concentrations within *Zearaja maugeana* Muscle

Between March 2023 and October 2023, 37 Maugean skate individuals (22 females and 15 males) were captured for biopsy punch sampling, ranging from 460 – 820 mm in total length (TL) (Appendix 5). Females exhibited higher average TL (733 ± 97 mm) compared to males (671 ± 46 mm). Biopsy punch sampling yielded an average of 0.25 ± 0.14 g of wet muscle tissue and 0.022 ± 0.018 g of dry muscle tissue. Dry tissue weights above 0.10 g were deemed sufficient material mass for solution ICP-MS analysis. Concentrations of Cr, Mn, Fe, Co, Cu, Zn, As, Rb, Sr, Mo, Ba, and Pb were effectively quantified in *Zearaja maugeana* muscle using biopsy punch sampling and solution HR-ICP-MS. Although analysed, Sc, Ti, V, Ni, Y, Ag, Cd, Sb, Cs, Tl, Bi, Th, and U were ultimately excluded from further consideration as they were not substantially above blank levels. Macronutrients, including Na, Mg, P, S, K, and Ca were also excluded from analysis, due to lack of relevance to the current study. Selenium was excluded from analysis, due to major spectral interferences and sample volatility associated with preparation methods used herein (Kieliszek, 2019). Aluminium and Sn were omitted due to digestion reagent incompatibility. Digestion recoveries for acceptable elements ranged from 80-106%. Data not meeting detection or contamination criteria were excluded from further statistical analysis.

Concentrations of Cr, Mn, Co, Cu, Zn, As, Sr, and Pb could be compared between the current study and Robertson (2021). Concentrations of Cu and Zn could also be compared between the current study and De Blas (1994), though concentrations could not be compared between all three studies, due to different units reported for Robertson (2021) and De Blas (1994). Comparing the current study with Robertson (2021) revealed an increase in Cr (27%), Co (132%), Cu (166%), Sr (51%), and Pb (461%) concentrations. Meanwhile, Mn (45%) and Zn (11%) concentrations decreased. Arsenic concentrations remained relatively comparable. Comparing the current study with De Blas (1994) revealed an increase in Cu concentrations (15%), while Zn concentrations decreased (37%). Lead concentrations could not be compared between the current study and De Blas (1994), due to concentrations being below instrumental detection limits in De Blas (1994).

Table 1: Concentrations (mg kg^{-1}) (mean ± std. dev.) of trace elements within *Zearaja maugeana* muscle from the current study, Robertson (2021) and De Blas (1994)

Element	Current study Concentration (mg kg^{-1} dw)	Current study Concentration (mg kg^{-1} ww)	Robertson (2021) Concentration (mg kg^{-1} dw)	De Blas (1994) Concentration (mg kg^{-1} ww)
Cr	1.35 ± 2.00	0.39 ± 1.46	1.06	
Mn	2.22 ± 1.45	0.25 ± 0.27	4.0	
Fe	50.1 ± 38.9	6.01 ± 13.3		
Co	0.079 ± 0.066	0.008 ± 0.008	0.034	
Cu	3.70 ± 2.36	0.38 ± 0.31	1.39	0.33 ± 0.060
Zn	20.5 ± 6.9	2.01 ± 1.17	23.0	3.20 ± 0.30
As	32.1 ± 19.9	3.27 ± 2.86	29.3	
Rb	3.12 ± 1.00	0.32 ± 0.21		
Sr	24.5 ± 16.8	2.74 ± 3.53	16.2	
Mo	0.070 ± 0.034	0.005 ± 0.005		
Ba	0.37 ± 0.17	0.026 ± 0.022		
Pb	0.37 ± 0.35	0.025 ± 0.054	0.066	<0.5

Elemental differences between sex were due to dissimilarities in the spread of the data therefore, elemental differences were not explored further. Univariate PERMANOVA analysis revealed significant differences between seasons for Rb (Figure 4). Regression analysis revealed that there was a significant negative relationship between Cu and TL of male skate (Figure 5). Other results from PERMANOVA analysis between sexes and seasons and non-significant regression results between TL and elemental concentrations are shown in Appendices 9, 10, 11, and 12.

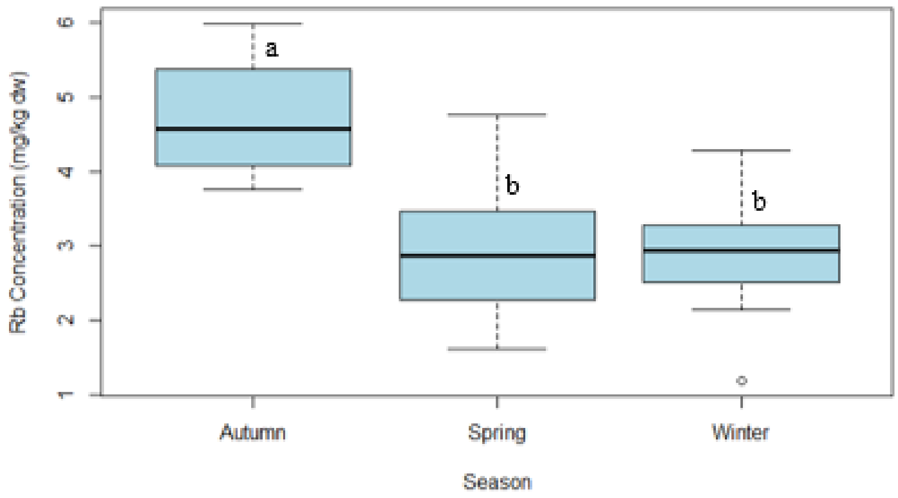

Figure 4: Boxplots showing significant difference of Rb concentration (mg kg^{-1} dw) between *Zearaja maugeana* muscle caught in Autumn (n = 4), Spring (n = 16) and Winter (n = 17), as determined by ICP-MS analysis. Letters describe significant differences, as determined by Pairwise PERMANOVA analysis.

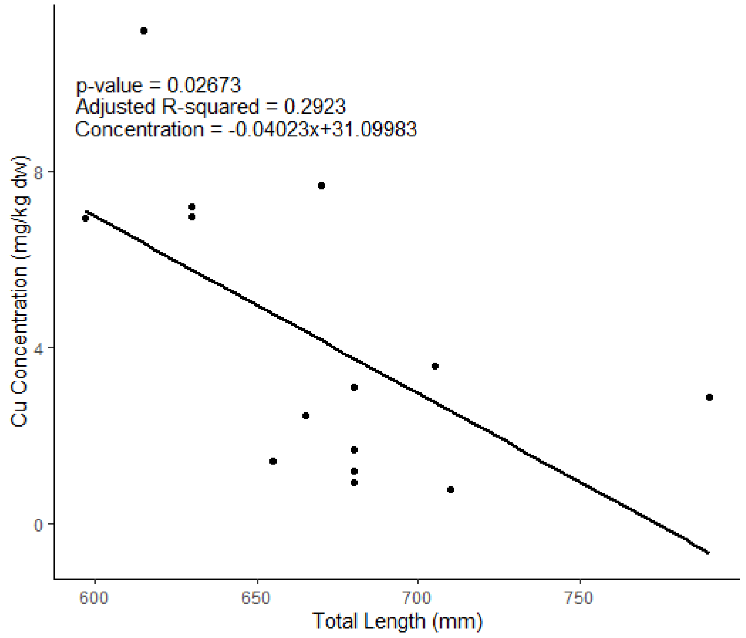

Figure 5: Regression model representing significant relationship between Cu concentration (mg kg^{-1} dw) within muscle and total length (mm) of male (n = 15) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed, as determined by regression model.

Multivariate PERMANOVA analysis indicated no significant differences in elemental signature within muscle biopsy punch samples (n = 32) between sexes or seasons (Table 2). ANOSIM analysis indicated that dissimilarities were more pronounced within sexes and seasons than between them, suggesting that dissimilarities between sexes and seasons were likely due to random variation in the data. PERMDISP analysis of scaled data demonstrated differences in dispersion between sexes but not between seasons, suggesting that differences between sexes were primarily driven by the dispersion of the data.

Table 2: Results from multivariate elemental analysis of *Zearaja maugeana* muscle between sex and season caught.

PERMANOVA						
			p			**p**
	Sex		0.64	Season		0.89
ANOSIM						
		R	**p**		**R**	**p**
	Sex	-0.017	0.53	Season	-0.049	0.77
PERMDISP						
			p			**p**
	Sex		0.001	Season		0.053

The LDA utilized prior knowledge of group classification of sex and season of capture to optimize group separation based on elemental concentrations (Appendix 13). The mean cross-validation classification accuracies from LDA were 93.8% for sex and 90.6% for season. Stepwise LDA analysis identified Rb, Mo, and Cr as the primary contributors to the variance between seasons. Dissimilarities of Rb and Mo contributed significantly to the LDA model.

3.2 Trace Element Concentrations within *Zearaja maugeana* Vertebrae

A total of 43 Maugean skate individuals (26 females and 17 males) were captured opportunistically between April 2012 and May 2019, with lengths ranging from 450 – 819 mm TL (Appendix 6). Female skates exhibited a higher average TL (709 ± 90 mm) compared to males (644 ± 56 mm). Concentrations of Li, Al, Si, Ca, Mn, Fe, Zn, As, Rb, Sr, Ba, Pb, and U were successfully quantified within *Zearaja maugeana* vertebrae. Table 3 presents transect-wide average elemental concentrations within skate vertebrae. Cadmium, In, Hg, and Th were excluded from analysis due to their levels not substantially exceeding blank levels. Macronutrients, including C, Na, Mg, and K were also omitted from analysis due to their lack of relevance to the current study. The ^{44}Ca major isotope was monitored to convert elemental concentrations to element: Ca ratios, enabling adjustments for differences in calcification rates. Data not meeting detection or contamination criteria were excluded from further statistical analysis.

Table 3: Concentrations (mg kg^{-1}) (mean ± std. dev.) of acceptable trace elements within *Zearaja maugeana* vertebrae, as determined by LA-ICP-MS analysis.

Element	Concentration (mg kg^{-1})
Li	0.86 ± 0.69
Al	1.63 ± 1.43
Si	70.6 ± 54.2
Mn	103 ± 88.6
Fe	0.74 ± 0.65
Zn	54.4 ± 41.7
As	0.14 ± 0.18
Rb	0.021 ± 0.020
Sr	1410 ± 1040
Ba	5.23 ± 4.14
Pb	0.51 ± 0.45
U	0.058 ± 0.047

For statistical analysis, average transect-wide elemental concentrations were converted to element: ^{44}Ca ratios, to account for changes in calcification rate. Univariate PERMANOVA analysis revealed significant differences between capture years for Li, Fe, Zn, and U: ^{44}Ca ratios (Figure 6). Regression analysis revealed that there was a significant negative relationship between Si: ^{44}Ca ratio and TL of both male and female skate (Figure 7). Non-significant results from PERMANOVA analysis between sexes and years, as well as non-significant regression results between TL and elemental concentrations are shown in Appendices 14, 15, 16, and 17.

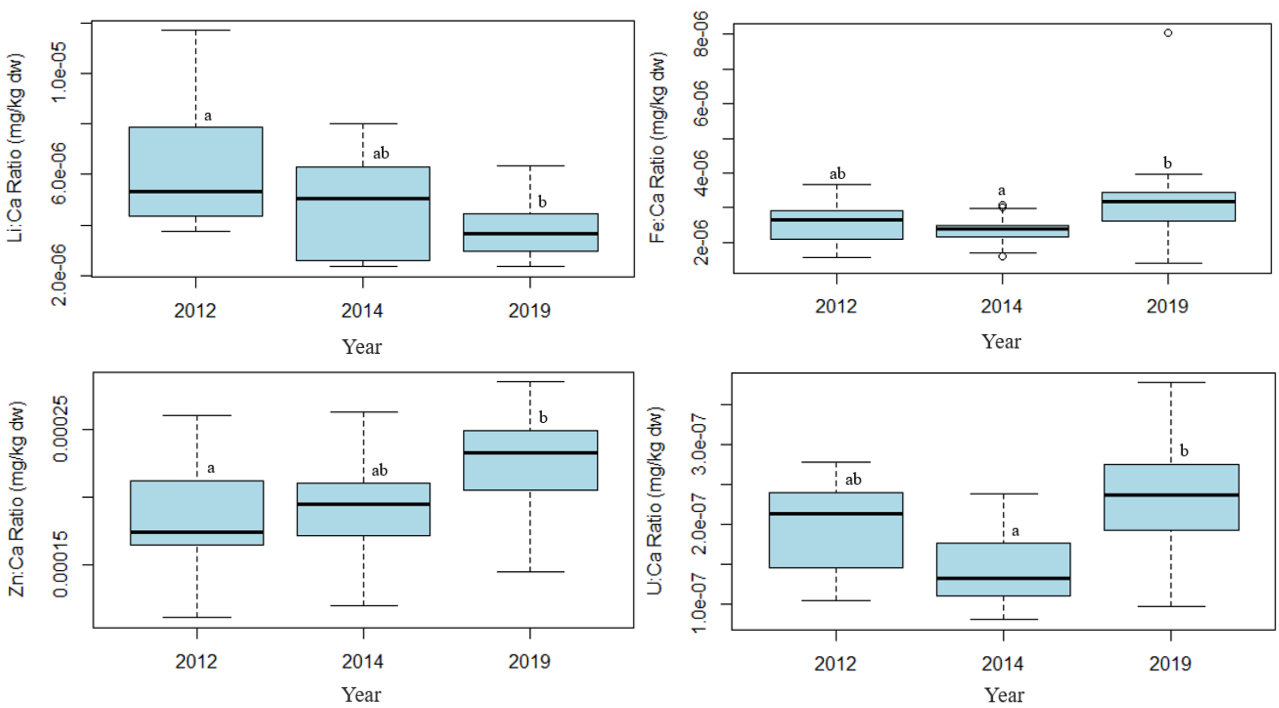

Figure 6: Boxplots showing significant differences of Li, Fe, Zn, and U: ^{44}Ca ratios (mg kg^{-1}) between 2012 (n = 11), 2014 (n = 13), and 2019 (n = 19) within *Zearaja maugeana* vertebrae, as determined by LA-ICP-MS analysis. Letters describe significant differences, as determined by Pairwise PERMANOVA analysis.

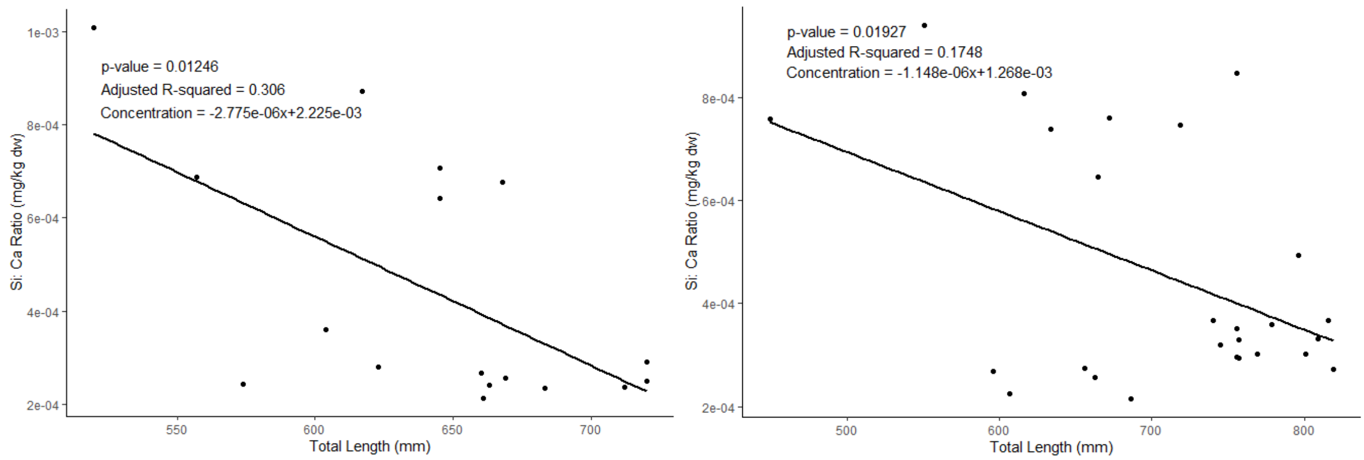

Figure 7: Regression models representing significant relationships between Si: ^{44}Ca ratios (mg kg^{-1}) within vertebrae and TL (mm) of male (n = 17) and female (n = 26) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed, as determined by regression model.

Element: ^{44}Ca ratios within vertebrae samples (n = 43) did not differ significantly between sex or year, as determined by multivariate PERMANOVA analysis (Table 4). ANOSIM analysis revealed that dissimilarities were greater within sexes than between, as well as indicating that dissimilarities between sex were likely to have resulted from random variation. It also revealed that dissimilarities between years were not likely to have resulted from random variation of the data. PERMDISP analysis of scaled data revealed that sexes and years did not differ in dispersion, indicating that differences between these variables were not due to dispersion of the data.

Table 4: Results from multivariate elemental analysis of *Zearaja maugeana* vertebrae between sex and year caught.

		R	p		R	p
PERMANOVA	Sex		0.67	Year		0.083
ANOSIM	Sex	-0.013	0.54	Year	0.079	0.049
PERMDISP	Sex		0.21	Year		0.82

The use of an LDA allowed for prior knowledge of group classification based on sex and year caught to be incorporated into the model to maximise group separation predicted from elemental concentrations (Appendix 18). Mean cross-validation classification accuracies from LDA were 72.1% for sex and 83.7% for year. R^2 values for stepwise LDA based on sex were relatively low, suggesting limited variance within the data. Stepwise LDA results showed that Rb, U, and Li were the highest contributors to variance between years. Dissimilarities of Li, Fe, Zn, Rb, Ba, Pb, and U between years contributed significantly to LDA.

4.0 Discussion

4.1 *Zearaja maugeana* Biopsy Punch Elemental Analysis

The current study effectively determined concentrations of Cr, Mn, Fe, Co, Cu, Zn, As, Rb, Sr, Mo, Ba, and Pb within *Zearaja maugeana* muscle with the use of non-lethal biopsy punch sampling methodology. It was important to note however that some samples were excluded from analysis, due to insufficient material, and that the method was incompatible with smaller skate individuals that lacked enough muscle outside their body cavity. Additionally, material collected using this method allowed for only one type of analysis, due to limited sample mass, thus limiting the possibility for multiple studies or sample preparation techniques to be performed on a single sample.

Multivariate analysis indicated no significant difference in the overall elemental composition between skate sexes. Any observed differences were likely due to variations in data spread, making the elemental signature distinctions determined by LDA less robust. Variances in diet and environmental factors contribute to differences in elemental accumulation (e.g., Mathews and Fisher, 2009). Since no elemental variances were found between sexes, it was concluded that no sexually dimorphic movement patterns, segregation, or dietary differences exist within the skate population, which was in alignment with the findings of Bell et al. (2016).

There was a negative correlation between Cu concentrations and TL in male skate muscle tissue. Robertson (2021) similarly observed a negative relationship between total element burden in muscle tissue and TL of skate. Although Robertson did not find a significant correlation between Cu concentrations in the muscle and TL, a significant negative relationship was detected between Cu and TL for liver tissue (Robertson, 2021). It is likely that more effective Cu elimination processes are acquired with age and maturity of the skate.

Multivariate analysis indicated no significant difference in overall elemental signature between seasons, although the lack of differences in spread of the data suggested potentially robust differences determined by LDA. Stepwise LDA identified Cr, Rb, and Mo as the primary contributors to seasonal dissimilarity. Most elements (Cr, Mn, Fe, Co, As, Rb, Sr, Mo, Ba, and Pb) exhibited a trend of greater accumulation during the wet season (Autumn and Winter) (Bureau of Meteorology, 2023), likely due to increasing rainfall facilitating increased element accumulation within the harbour. Acid rock drainage is known to contribute to Cr dissolution (Taylor et al., 1996; Augustinus et al., 2010) and as Rb and Mo are common mining by-products and geological elements (Nikonow and Rammlmair, 2022; Xing et al., 2021), it is likely that concentrations of these elements accumulated with rainfall.

Due to the endangered nature of the skate, it was difficult to capture sufficient numbers to allow an even distribution of individuals caught per season within the given timeframe. The uneven spread of individuals across seasons resulted in low sample sizes in some seasons, with no individuals sampled in Summer, meaning no confident conclusions could be made based on elemental differences between seasons caught of the skate. In contrast, inferences made based on sex were more robust, due to larger sample sizes for both males and females compared to that caught between seasons. However, uncontrolled variables such as the number of individuals of each sex caught per season may have potentially caused bias within the data, thus potentially skewing the results for elemental differences based on sex. A longer-term study is recommended to accurately determine elemental signature differences between sex and season caught within the Maugean skate population.

4.2 Time Resolved Elemental Concentration Changes in *Zearaja maugeana*

Elemental concentrations were comparable between the current study and Robertson (2021), as similar preparation techniques and analytical instruments were used. Results were less comparable between the current study and De Blas (1994), attributed to differences in sample preparation techniques and analytical instruments. The current study identified changes over time of many trace elements (Cr, Co, Cu, Sr, and Pb) within the muscle of the Maugean skate (Table 1).

Concentrations of Cr, Co, and Cu observed in the current study were higher (27%, 132%, and 166% respectively) compared to those determined in skate muscle by Robertson (2021). Copper concentrations were within the human consumption guideline of 30 mg kg $^{-1}$ ww (FAO/WHO, 1989). Although no human consumption guidelines exist for Co, concentrations were below the drinking water quality guideline of 0.01 mg L^{-1} (WHO, 2008). However, Cr concentrations (258% above upper range) exceeded the range for seafood of between 0.098 and 0.109 mg kg^{-1} ww (EFSA, 2014). Robertson had already reported elevated Cr concentrations within skate tissues and the further increase found herein suggests the potential for Cr toxicity to be experienced by the skate. When compared to other elasmobranchs inhabiting the waters around southern Australia (Appendix 1), Cr, Co, and Cu concentrations were an average of six, two, and seven times higher, respectively. Elevated Co and Cu concentrations have been linked to decreased body condition in female elasmobranchs (Wosnick et al., 2021) and elevated Cr, Co, and Cu concentrations are known to cause effects such as neurotoxicity and reduced reproductive success in teleosts (Gomez-Arnaiz et al., 2022; Ellenberger et al., 1994; Zhang et al., 2024). Given these concerns, continued monitoring of concentrations of these elements within the skate is recommended, as well as monitoring of body condition and reproductive success to assess how concentrations of these elements affect the functioning of the skate.

Concentrations of Mn and Zn within skate muscle decreased between studies, and concentrations of both elements were comparable to those found in elasmobranchs inhabiting the waters around southern Australia (Appendix 1). Iron concentrations could not be compared to previous studies within skate muscle, though concentrations were comparable to those found in elasmobranchs inhabiting the waters around southern Australia (Appendix 1). Manganese, Fe, and Zn concentrations were below the consumption guidelines of 13.0 mg kg^{-1} ww, 186 mg kg^{-1} ww, and 100 mg kg^{-1} ww respectively (FAO/WHO, 1989). Therefore, it was concluded that the potential for Mn, Fe, and Zn toxicity within the skate was low.

Molybdenum has received limited attention in trace element studies of elasmobranchs inhabiting Australian waters, though concentrations herein were comparable to those reported in muscle tissue of *Galeus melastomus* specimens caught in the Mediterranean Sea (Gallo et al., 2023) and muscle tissue of *Somniosus microcephalus* and *Somniosus pacificus* specimens obtained from Arctic waters (McMeans et al., 2007). The current study revealed evidence of Mo accumulation in Maugean skate tissues, though as concentrations were comparable to that found within other elasmobranch species, concentrations were likely not of concern. Molybdenum is recognised for its role in regulating the effects of Cu toxicity (Hoopes, 2017), suggesting that Mo concentrations in Macquarie Harbour's resident fauna may help mitigate the effects of Cu toxicity in this heavily polluted waterway.

Sr and Pb concentrations determined herein were considerably higher in the current study (51% and 461% respectively) compared to that determined by Robertson (2021) and Sr concentrations were also an average of four times higher than those of three other elasmobranch species reported by Robertson (2021) (Appendix 1). The research community generally considers Sr concentrations not harmful to aquatic organisms, except in extremely high concentrations (Chowdhury and Blust, 2011), with only one known case of Sr toxicity, which occurred in various teleost species inhabiting the waters of the Kola region of Russia (Moisseenko and Kudryavtseva, 2001). The uptake of Sr is strongly linked to Ca availability, therefore the potential for Sr toxicity depends heavily on Ca availability in the waterway. Strontium concentrations in Macquarie Harbour are yet to be studied, warranting assessment to determine potential Sr toxicity risks for the harbour's resident fauna.

Concentrations of Pb in the current study were below the Food Standards Australia and New Zealand (FSANZ) human consumption guideline of 0.05 mg kg^{-1} ww (FSANZ, 2020). However, they were an average of five times higher than other elasmobranch species inhabiting the waters around southern Australia (Appendix 1). The elevated Pb concentrations found in the current study compared to that of other elasmobranch species suggest the potential for toxicity effects to be inflicted on the skate (Paul et al., 2019). Lead is widely recognised for its deleterious effects on organisms in elevated concentrations, including that of reproductive success and neurotoxicity in teleost fishes (Luszczek-Trojnar et al., 2014; Ding et al., 2022). Therefore, the elevated Pb concentrations within skate muscle, compared to other elasmobranch species, are potentially a cause for concern.

Concentrations of As in the current study exceeded the FSANZ guidelines, where concentrations below 2.00 mg kg^{-1} ww were deemed acceptable (FSANZ, 2020). Despite this seemingly elevated value, it is known that As concentrations found within elasmobranchs are comprised primarily of arsenobetaine; a compound that originates from low trophic level organisms (Popowich et al., 2016). Arsenobetaine is metabolically inert and stable (Gelsleichter and Walker, 2010), suggesting that As concentrations found within the current study were not of concern.

Barium concentrations have been investigated many times in elasmobranch vertebrae (Mohan et al., 2018), though this element has scarcely been investigated within elasmobranch muscle. Concentrations herein were comparable to those reported in muscle tissue of *Somniosus microcephalus* specimens obtained from Arctic waters (Muir et al., 2006). Though limits for consumption of Ba have not yet been set, those found herein were within the range for marine fish (0.027-0.39 mg kg^{-1} ww) (Health Canada, 2007) and canned tuna (0.050-0.42 mg kg^{-1} ww) (Abebneh and Al-Momani, 2013). Consequently, it was concluded that Ba concentrations found herein were likely not of concern, though it is recommended that Ba concentrations and toxicity be more thoroughly researched in elasmobranchs.

Rubidium has rarely been investigated in elasmobranchs and its essentiality is still largely debated (Anke et al., 2005; Dranitzki et al., 1993). Concentrations described herein were comparable to concentrations found in liver tissues of two species of sleeper shark and in the muscle tissues of a species of nurse shark (Wosnick et al., 2021; McMeans et al., 2007). Rubidium concentrations described herein provided evidence that Rb has accumulated within skate muscle and therefore, the essentiality of Rb in elasmobranchs and toxic thresholds should be further investigated.

4.3 *Zearaja maugeana* Vertebrae Elemental Analysis

The methodology employed herein effectively determined concentrations of Li, Al, Si, Mn, Fe, Zn, As, Rb, Sr, Ba, Pb, and U within *Zearaja maugeana* vertebrae. Univariate PERMANOVA analysis indicated significant difference in Li, Fe, Zn, and U: ^{44}Ca ratios (mg kg^{-1}) between years, as well as indicating a significant negative relationship between Si: ^{44}Ca ratio and TL for both male and female skate. Multivariate analysis indicated no significant difference in overall elemental signature between year and sex.

Multivariate analysis indicated no significant difference in elemental signature between sexes, with limited variance observed in the LDA model for sex. Univariate analysis further confirmed that there were no significant differences of element: ^{44}Ca ratios between sexes. Consequently, it was concluded that there were no sex-specific differences in vertebral elemental signatures for the Maugean skate. Vertebral elemental signatures are often used to infer movement patterns in elasmobranchs (Mohan et al., 2018; Pistevos et al., 2019; Smith et al., 2013; Raoult et al., 2016), therefore, the absence of vertebral elemental differences between sexes in the current study suggested the absence of sexually dimorphic movement patterns within the skate. This finding was in alignment with the observations of Bell et al. (2016).

Although univariate analysis revealed significant differences for specific elements between year, multivariate analysis suggested no significant differences in elemental signature between years. However, LDA analysis was able to maximise differences, indicating that 2014 possessed the lowest group means for most elements (Li, Al, Fe, As, Rb, Sr, Ba, Pb, and U). Out of the years sampled, 2019 exhibited the annual highest rainfall (1820 mm), followed by 2012 (1630 mm), with 2014 exhibiting the lowest rainfall (1380 mm) (Bureau of Meteorology, 2023). This observed decline in trace element concentrations in 2014 likely stemmed from reduced rainfall, leading to decreased input of nutrient loads into the harbour.

The observed decrease in Li and increase in Zn over time may be attributed to environmental factors. Zinc incorporation into cartilage is known to correlate positively with temperature (Smith et al., 2013), and as the deep waters of Macquarie Harbour experienced a temperature increase of 1.5-2°C between 1998 and 2020 (Ross et al., 2022), it is plausible that Zn incorporation was influenced by this temperature change. While temperature has limited effects on Li incorporation, it has a positive relationship with pH. Although long-term pH trends in Macquarie Harbour are yet to be investigated, Nascimento et al. (2023) noted that acid rock drainage inputs into the King River were altering its pH, suggesting the potential for these inflows to affect the pH of the harbour as well. Therefore, the decreasing trend in Li: Ca ratios observed over time may be due to a potential declining trend in pH within the harbour.

Silicon, an essential trace element, has been thought to play an important role in the formation of the organic matrix of bone and cartilage within higher animals (Carlisle, 1986), though this theory is yet to be proven in elasmobranchs and other fishes. The findings herein suggest ontogenetic changes in Si: ^{44}Ca ratios, as demonstrated by their significant negative relationship with TL of male and female skate. It was unknown why this ontogenetic change in elemental signature was observed. It was possible that yolk contribution possessed high Si concentrations, resulting in higher elemental incorporation in the early life stages and consequently, this early life contribution making up a higher proportion of vertebrae in younger individuals, though more research into this hypothesis is needed.

Although interesting inferences could be made from transect wide averages of elemental signatures from skate vertebrae, no robust conclusions regarding differences between years could be made from this data. It is recommended that the inferences and relationships found herein be further investigated with transect wide concentrations, so that elemental signatures from specific timepoints can be linked to trends in physiochemical variables over time, which would allow for further understanding of elemental incorporation into Maugean skate vertebrae. In contrast, inferences made based on sex were more robust, due to larger sample sizes of male and female skate compared to sample sizes for skate caught in each year. A longer-term study is recommended to accurately determine elemental signature differences between sex and year caught within the Maugean skate population.

4.4 Comparison of Elemental Accumulation between Muscle and Vertebrae

Elemental concentrations differed between *Zearaja maugeana* muscle (Table 1) and vertebrae (Table 3), indicating distinct accumulation behaviours. Although samples were not obtained from the same individuals, comparing the magnitude of concentrations allowed for extrapolation of areas of accumulation. To explore the relationship between muscle and cartilage trace element accumulation in elasmobranchs, samples should be collected from the same individuals. Manganese, Zn, Sr, and Ba exhibited higher accumulation in skate vertebrae, while Fe, As, and Rb exhibited higher accumulation in muscle. Lead accumulation was similar in both tissue types. Higher Mn concentrations in calcified structures were consistent with the findings of Vas (1991), who reported elevated vertebral concentrations compared to muscle in six elasmobranch species. Vas (1991) also reported contradicting results for Zn accumulation, with some species showing higher accumulation in vertebrae and others in muscle, but overall, the highest Zn concentrations were in the vertebrae. Vas (1991) suggested a link between Mn and Zn and the metabolism of Ca, explaining their elevated accumulation in elasmobranch vertebrae.

Studies have consistently shown that Sr tends to accumulate primarily within the bony structures of fish, with only about 5% accumulating in their soft tissues (Chowdhury and Blust, 2011). While the specific role of Sr in elasmobranch cartilage and fish bone remains unspecified, it is known to enhance mammalian bone mass and reduce fracture risk, as well as promote cartilage matrix formation in mammals (e.g., Raoult et al., 2016; Delannoy et al., 2002). Although the structure of elasmobranch cartilage differs somewhat from mammalian cartilage, it is possible that Sr also plays a structural role within elasmobranch cartilage (Raoult et al., 2016).

While the distribution of Ba among the organs of elasmobranchs and fish remains unexplored, it is established that up to 90% of Ba concentrations in humans accumulate in the bones (Mertz, 1986; Kovrlija et al., 2021). Studies have indicated that the presence of Ba in mammalian bones enhances growth (Kovrlija et al., 2021) however, the structural role of Ba within hard structures, including those of fishes, is yet to be determined. Further research into this area is warranted.

The higher accumulation of Mn, Zn, Sr, and Ba within skate vertebrae suggests the potential for these elements to perform structural roles within its cartilage. Conversely, the lower accumulation of Fe, As, and Rb in the vertebrae indicates that these elements are unlikely to serve structural functions, though further research into the role of various elements in elasmobranch cartilage is needed. This finding was not surprising for As, as previous studies have indicated that elevated concentrations of As reduce the biochemical and mineral contents in teleost bones (Palaniappan and Vijayasundaram, 2009).

Iron deficiency in teleosts has been linked to decreased bone formation (Bo et al., 2016), indicating a potential structural role for Fe in teleost bone. While Fe is essential for numerous processes, its role in elasmobranch cartilage remains undefined, potentially explaining the conflicting findings of Vas (1991). Vas (1991) observed higher Fe accumulation in the muscle of some elasmobranch species and in the vertebrae of others. These disparities could be attributed to Fe performing a structural role in the cartilage of certain elasmobranch species, though not others. The current study however suggested that Fe may not serve a structural role in the cartilage of this species, though further research is necessary to confirm this hypothesis.

The essentiality of Rb is still largely debated (e.g., Kupriyanov, 2013; Anke et al., 2005; Dranitzki et al., 1993) and its potential roles within elasmobranchs and teleosts has not yet been investigated. The low accumulation of this element within the vertebrae of the Maugean skate suggests that Rb does not play any structural role within the cartilage of this species, though more research into the essentiality of Rb within elasmobranchs is needed.

5.0 Conclusions

Concentrations of Cr, Co, Cu, Sr, and Pb determined within the current study were found to be higher than that described within the muscle tissue of the Maugean skate in previous studies. Concentrations determined herein were also elevated compared to other elasmobranch species inhabiting the waters around southern Australia. Despite toxicity thresholds not having been specified in elasmobranchs, the elevated concentrations of Cr, Co, Cu, and Pb found herein suggested the potential for toxicity effects to have been experienced by the skate. Concentrations of Mn, Zn, Sr, and Ba were found to accumulate predominantly in the vertebrae, rather than the muscle, suggesting that these elements may perform structural roles within elasmobranch vertebrae.

The biopsy punch methodology described herein provided an effective toolset to assess trace element accumulation within the muscle of the Maugean skate with the use of non-lethal sampling. It is recommended that trace element accumulation continue to be monitored within this species, as well as the effect of elemental concentrations on the body condition and reproductive success of the species be investigated. It is also recommended that the compatibility of trace element remediation techniques, such as phytoremediation (Al-Akeel, 2017) be investigated in Macquarie Harbour.

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CRediT authorship contribution statement

Erin Woodward: Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review and editing. Jayson Semmens: Supervision, Conceptualization, Methodology, Writing – review and editing. David Moreno – Supervision, Conceptualization, Methodology, Writing – review and editing. Ashley Townsend – Supervision, Methodology, Writing – review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available upon request.

6.0 References

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7.0 Appendices

Appendix 1: Trace element concentrations in Elasmobranch tissues residing in the waters of South-Eastern Australia

Source	Geographical Location	Method	Species	Tissue	Unit type	Element	Conc. (mean ± std. dev.) (mg kg^{-1})
Glover, 1979	Southern Victoria (Port Phillip Bay)	Atomic absorption spectrophotometry (AAS)	*Galeorhinus australis* n = 12	Muscle	ww	As Cd Cu Mn Zn	13.58 ± 5.43 0.031 ± 0.017 0.34 ± 0.13 0.41 ± 0.080 3.7 ± 0.26
			Mustelus antarcticus n = 12			As Cd Cu Mn Zn	17.1 ± 6.1 0.034 ± 0.021 0.28 ± 0.070 0.31 ± 0.12 3.9 ± 0.52
Turoczy et al., 2000	North-west Tasmania (Indian Ocean)	AAS	*Deania calcea* n = 18	Muscle	dw	As Cd Cu Fe Mn Sr Zn	60 ± 8.0 0.38 ± 0.017 0.23 ± 0.040 3.8 ± 1.6 0.47 ± 0.030 1.0 ± 0.20 7.8 ± 0.40
			Centroscymnus crepidator n = 10			As Cd Cu Fe Mn Sr Zn	68 ± 13 0.011 ± 0.0070 0.26 ± 0.040 3.2 ± 1.8 1.0 ± 0.30 1.0 ± 0.30 8.8 ± 0.40
			Centroscymnus owstonii n = 11			As Cd Cu Fe Mn Sr Zn	110 ± 20 0.053 ± 0.040 0.29 ± 0.040 2.4 ± 0.080 0.74 ± 0.12 1.6 ± 0.80 9.6 ± 0.50
Ooi et al., 2015	South-east Queensland (Lady Elliot Island Reef)	ICP-MS	*Mobula japanica* n = 15	Muscle biopsy	ww	As Cd Pb	20 ± 15 0.084 ± 0.062 0.18 ± 0.14
			Manta alfredi n = 12			As Cd Pb	0.53 ± 0.56 0.035 ± 0.032 0.43 ± 0.26
Gilbert et al., 2015	Eastern New South Wales (Tasman Sea)	ICP-MS	*Carcharhinus obscurus* n = 12	Muscle	dw	As Cd Cu Fe Zn	77 ± 20 0.080 ± 0.020 1.1 ± 0.28 42 ± 14 16 ± 1.3
			Carcharhinus plumbeus n = 12			As Cd Cu Fe Zn	120 ± 14 0.37 ± 0.11 0.79 ± 0.13 77 ± 58 19.4 ± 2.7
			Carcharodon carcharias n = 6			As Cd Cu	60 ± 7.1 0.040 ± 0.020 1.48 ± 0.33

						Fe	26 ± 7.1
						Zn	13 ± 1.3
Cagnazzi et al., 2019	Northern NSW	ICP-MS	*Rhinoptera neglecta* n = 1	Muscle	ww	As	2.6
						Cd	0.040
						Mn	0.17
						Zn	7.2
						Cu	1.3
			Aetobatus ocellatus n = 2			As	49
						Cd	0.060
						Mn	0.090
						Zn	3.4
						Cu	1.2
Robertson, 2021	Western Tasmania (Macquarie Harbour) Southern Victoria (Port Phillip Bay)	ICP-MS	*Urolophus paucimaculatus* n = 17	Muscle	dw	As	210
						Sn	87
						Zn	21
						Sr	8.8
						Cu	3.8
						Mn	0.88
						Cr	0.13
						Ni	0.16
						Pb	0.047
						Cd	0.011
						Al	0.19
						Co	0.077
						Fe-1*	14
						Fe-2*	14
			Trygonoptera imitata n = 9			As	280
						Sn	92
						Zn	22
						Sr	5.7
						Cu	1.2
						Mn	0.29
						Cr	0.23
						Ni	0.18
						Pb	0.043
						Cd	0.0050
						Al	1.3
						Co	0.034
						Fe-1*	19
						Fe-2*	19
			Squalus acanthias n = 34			As	21
						Sn	220
						Zn	16
						Sr	4.2
						Cu	1.0
						Mn	0.54
						Cr	0.46
						Ni	0.35
						Pb	0.11
						Cd	0.0080
						Al	7.0
						Co	0.030
						Fe-1*	12
						Fe-2*	12

* Insufficient information was provided in thesis to discern which isotopes were reflected by Fe-1 and Fe-2

Appendix 2: Methods employed for determination of trace elements within Elasmobranch muscle tissues using ICP-MS

Source	Sample Weight	Acids	Elements	Digestion Method	Temperature	Time	Certified Reference Material	Range CRM % Recovery
Ooi et al., 2015	0.1 g	4 mL HNO_3	As, Cd, Pb, Hg	Microwave	N/A	N/A	Dogfish liver	93-114%
Nicolaus et al., 2016	1 g	6 mL HNO_3	As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn	Microwave	N/A	N/A	TORT-2 lobster hepatopancreas, DOLT-3 dogfish liver, DORM-2 dogfish protein	N.S.
Ong and Gan, 2017	0.05 g	1.5 mL HNO_3	Cu, Zn, Cd, Hg, Pb	Heat block	100ºC	7 hr	DOLT-4 dogfish liver	N.S.
Cagnazzi et al., 2019	5 g	10 mL HNO_3	As, Ca, Pb, Cd, Zn, Hg, Ag, Cr, Cu, Mn, Ni, Se, Fe, Al, Si, V, Co, Mo, Mg, K, S, P, Bo, Na	Heat block	120ºC	1 hr	N.S.	N.S.
Boldrocchi et al., 2019	0.2 g	2 mL HNO_3	As, Cd, Cu, Fe, Mn, Hg, Se, Zn, Cr, V, Co, Ni, Ag, Pb	Heat block	100ºC	N.S.	DOLT-5	N.S.
Dutton and Venuti, 2019	0.25 g	4 mL HNO_3	Cr, Co, Cu, Fe, Mn, Ni, Se, Zn, Ag, As, Cd, Hg, Pb	Microwave	220ºC	N/A	DORM-4 fish protein	77-106%
Pancaldi et al., 2019	0.25 g ww	HNO_3, H_2O_2	Cu, Se, Zn, As, Cd, Pb	Heat block	120ºC	4 hr	DORM-4 fish muscle, DOLT-5 dogfish liver	DORM-4: 93-109% DOLT-5: 91-112%
Gilbert et al., 2019	0.2 g	5 mL HNO_3	Hg, As, Cd, Cu, Fe, Se, Zn	Microwave	N/A	N/A	DORM-4 fish protein	100-106%
Souza-Araujo et al., 2020	0.1 g ww	1.5 mL HNO_3, 0.5 mL H_2O_2	Co, Cr, Cu, Fe, Mn, Ni, Se, Zn, As, Al, Ba, Cd, Hg, Pb, Tl, U	Microwave	N/A	N/A	DORM-3 and DOLT-4	DORM-3: 75-109.9% DOLT-4: 75-90%
Boldrocchi et al., 2021	0.1 g	2 mL HNO_3	V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Pb, Hg	Microwave	N/A	N/A	N.S.	N.S.
Alvaro-Berlang et al., 2021	0.25 g	10 mL HNO_3 + 3 mL HCl	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, Zn	Heat block	120ºC	4 hr	Oyster tissue, DORM-4 fish protein, DOLT-4 dogfish liver	DORM-4: 90-123% DOLT-4: 83-118% Oyster: 73-102%
Baro-Camarasa et al., 2021	0.25-0.8 g	7 mL HNO_3, 3 mL H_2O_2	Fe, Zn, Se, Mn, Cu, Cr, Co, As, Cd, Tl, V, U, Ag	Microwave	N/A	N/A	DORM-4 fish protein, DOLT-5 dogfish liver	87-106% except for SE (DORM-4 118%) and

								U (122% DORM 4)
Robertson, 2021	0.05 g	200 µL HNO_3 + 3 mL HCl	As, Cd, Ca, Co, Cu, Fe, Pb, Mg, Hg, K, Se, Ag, Na, Sr, V, Zn, Al, Ni	Heat block	80ºC	2-3 hr	DOLT-5 dogfish liver	68-106%
Giovos et al., 2022	0.5 g	8 mL HNO_3	As, Cd, Co, Cu, Hg, Mn, Ni, Pb, Sb, Se, V, Zn	N.S.	80ºC	30 mins	N.S.	N.S.
Baro-Camarasa et al., 2023	0.25-0.8 g	7 mL HNO_3, 3 mL H_2O_2	Li, Na, Mg, P, S, K, Ca, V, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Sr, Ag, Cd, Sn, Sb, Tl, Pb, U	Microwave	N/A	N/A	DORM-4 fish protein, DOLT-5 dogfish liver	DORM-4: 85-115% except for Se (118%), Li (28%), U (122%) and Sn (150% DOLT-5: 85-115% except for Sn (125%)

N.S. – not specified

Appendix 3: Isotopes and resolution modes considered for solution ICP-MS analysis and mean method Limits of Detection (LOD) in µg kg^{-1}

Element	LOD (µg kg^{-1})
^{23}Na (MR)	18
^{24}Mg(MR)	0.26
^{27}Al (MR)	0.26
^{31}P (MR)	1.5
^{32}S (MR)	5.1
^{39}K (HR)	17
^{42}Ca (MR)	6.3
^{45}Sc (MR)	0.021
^{47}Ti (MR)	0.063
^{51}V (MR)	0.042
^{52}Cr (MR)	0.011
^{55}Mn(MR)	0.014
^{56}Fe (MR)	0.54
^{59}Co (MR)	0.014
^{60}Ni (MR)	0.12
^{63}Cu (MR)	0.0090
^{66}Zn (MR)	0.26
^{75}As (HR)	0.028
^{77}Se (HR)	0.045
^{82}Se (HR)	0.16
^{85}Rb (LR)	0.15
^{88}Sr (LR)	0.036
^{89}Y (LR)	0.0080
^{95}Mo (LR)	0.066
^{107}Ag(LR)	0.038
^{111}Cd(LR)	0.0070
^{118}Sn (LR)	0.018
^{121}Sb (LR)	0.026
^{133}Cs (LR)	0.11
^{137}Ba (LR)	0.033
^{205}Tl (LR)	0.010
^{208}Pb (LR)	0.0060
^{209}Bi (LR)	0.011
^{232}Th (LR)	0.0050
^{238}U (LR)	0.00020

LR – Low Resolution mode, MR – Medium Resolution mode, HR - High Resolution mode

Appendix 4: Isotopes considered in LA-ICP-MS analysis and mean method Limits of Detection (LOD) in µg kg^{-1}

Element	LOD (µg kg^{-1})
^{7}Li	0.29
^{23}Na	6.4
^{24}Mg	0.16
^{27}Al	0.11
^{29}Si	38
^{39}K	1.8
^{43}Ca	11
^{44}Ca	8.0
^{55}Mn	0.14
^{56}Fe	1.4
^{66}Zn	0.12
^{75}As	0.24
^{85}Rb	0.019
^{88}Sr	0.0050
^{111}Cd	0.024
^{115}In	0.00031
^{137}Ba	0.00074
^{202}Hg	0.047
^{208}Pb	0.0050
^{232}Th	0.00019
^{238}U	0.00012

Appendix 5: Capture details for all Maugean skate individuals (n = 37) considered for solution ICP-MS analysis.

Id	Capture date MM / YYYY	Sex	Total length (mm)
MSK72	03 / 2023	F	801
MSK75	03 / 2023	F	780
MSK76	03 / 2023	F	760
MSK77	03 / 2023	M	670
MSK78	07 / 2023	M	705
MSK79	07 / 2023	F	695
MSK80	07 / 2023	F	540
MSK81	07 / 2023	F	801
MSK82	07 / 2023	F	740
MSK83	07 / 2023	M	680
MSK85	07 / 2023	M	655
MSK86	07 / 2023	F	742
MSK87	07 / 2023	F	790
MSK88	07 / 2023	F	795
MSK89	07 / 2023	F	810
MSK90	07 / 2023	F	755
MSK92	07 / 2023	M	680
MSK93	07 / 2023	F	620
MSK97	07 / 2023	F	628
MSK98	10 / 2023	M	680
MSK99	10 / 2023	M	615
MSK100	10 / 2023	F	820
MSK104	10 / 2023	M	630
MSK105	10 / 2023	M	630
MSK106	10 / 2023	F	790
MSK107	10 / 2023	M	665
MSK108	10 / 2023	M	680
MSK109	10 / 2023	M	597
MSK110	10 / 2023	M	678
MSK114	10 / 2023	F	780
MSK115	10 / 2023	F	640
MSK116	10 / 2023	M	710
MSK117	10 / 2023	F	780
MSK118	10 / 2023	F	460
MSK119	10 / 2023	F	785
MSK120	10 / 2023	M	790
MSK121	10 / 2023	F	810

Appendix 6: Capture details for all Maugean skate individuals (n = 43) considered for LA-ICP-MS analysis.

Id	Capture date MM / YYYY	Sex	Total length (mm)
MSK1	05 / 2012	M	520
MSK2	11 / 2014	F	551
MSK3	04 / 2012	M	617
MSK4	11 / 2014	F	756
MSK5	11 / 2014	F	616
MSK6	05 / 2019	F	634
MSK7	05 / 2019	M	557
MSK8	11 / 2014	F	672
MSK9	04 / 2012	M	645
MSK10	11 / 2014	F	719
MSK11	11 / 2014	M	668
MSK12	11 / 2014	F	450
MSK13	04 / 2012	M	645
MSK14	05 / 2019	F	665
MSK15	05 / 2019	M	712
MSK16	05 / 2019	F	607
MSK17	11 / 2014	M	574
MSK18	05 / 2019	M	660
MSK19	05 / 2019	M	669
MSK20	05 / 2012	M	720
MSK21	05 / 2012	M	663
MSK22	11 / 2014	F	656
MSK23	05 / 2019	M	623
MSK24	05 / 2019	F	663
MSK25	04 / 2012	F	596
MSK26	02 / 2019	M	683
MSK27	04 / 2014	F	686
MSK28	11 / 2012	M	661
MSK29	04 / 2014	M	604
MSK30	11 / 2014	F	740
MSK31	11 / 2014	F	816
MSK32	05 / 2019	F	779
MSK33	05 / 2019	F	756
MSK34	05 / 2019	F	757
MSK35	05 / 2019	F	745
MSK36	02 / 2019	F	809
MSK37	05 / 2019	F	756
MSK38	02 / 2019	F	801
MSK39	05 / 2019	F	769
MSK40	04 / 2012	M	720
MSK41	05 / 2019	F	757
MSK42	11 / 2014	F	819
MSK44	04 / 2012	F	796

Appendix 7: Average recoveries for NIST Oyster Tissue Certified Reference Material (CRM), sample mass ~0.022 g (n = 3) used in ICP-MS analysis.

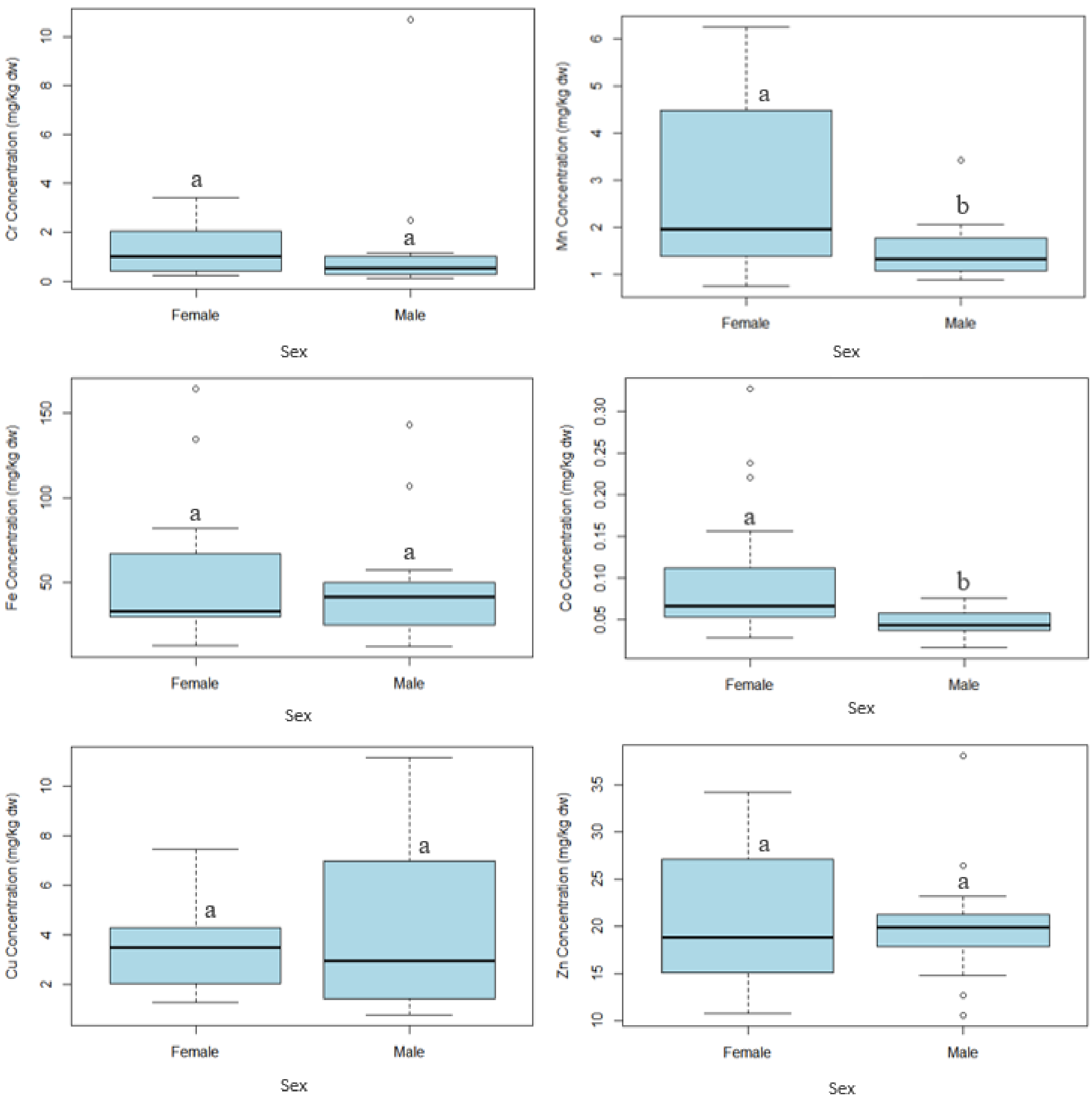
Element	**Reference Value ($\mu g\ kg^{-1}$)**	**Measured Concentration ($\mu g\ kg^{-1}$)**	**Recovery (%)**
Na	4170	4293	103
Mg	1180	1129	96
Al	202.5	65.8	33*
P	6230	5754	92
S	8620	7754	90
K	7900	7937	100
Ca	1960	1984	101
Sc	0.06	0.04	67*
V	4.68	4.53	97
Cr	1.43	1.26	88
Mn	12.3	12.0	97
Fe	539	522	97
Co	0.57	0.60	106
Ni	2.25	2.33	104
Cu	66.3	63.9	96
Zn	830	669	81
As	14	11.2	80
Se	2.21	1.48	67*
Rb	3	3.10	103
Sr	11.1	10.8	97
Cd	4.15	3.58	86
Pb	0.37	0.34	93
U	0.13	0.12	88
		Average % Recovery	94.8

* Refractory element removed from average % recovery

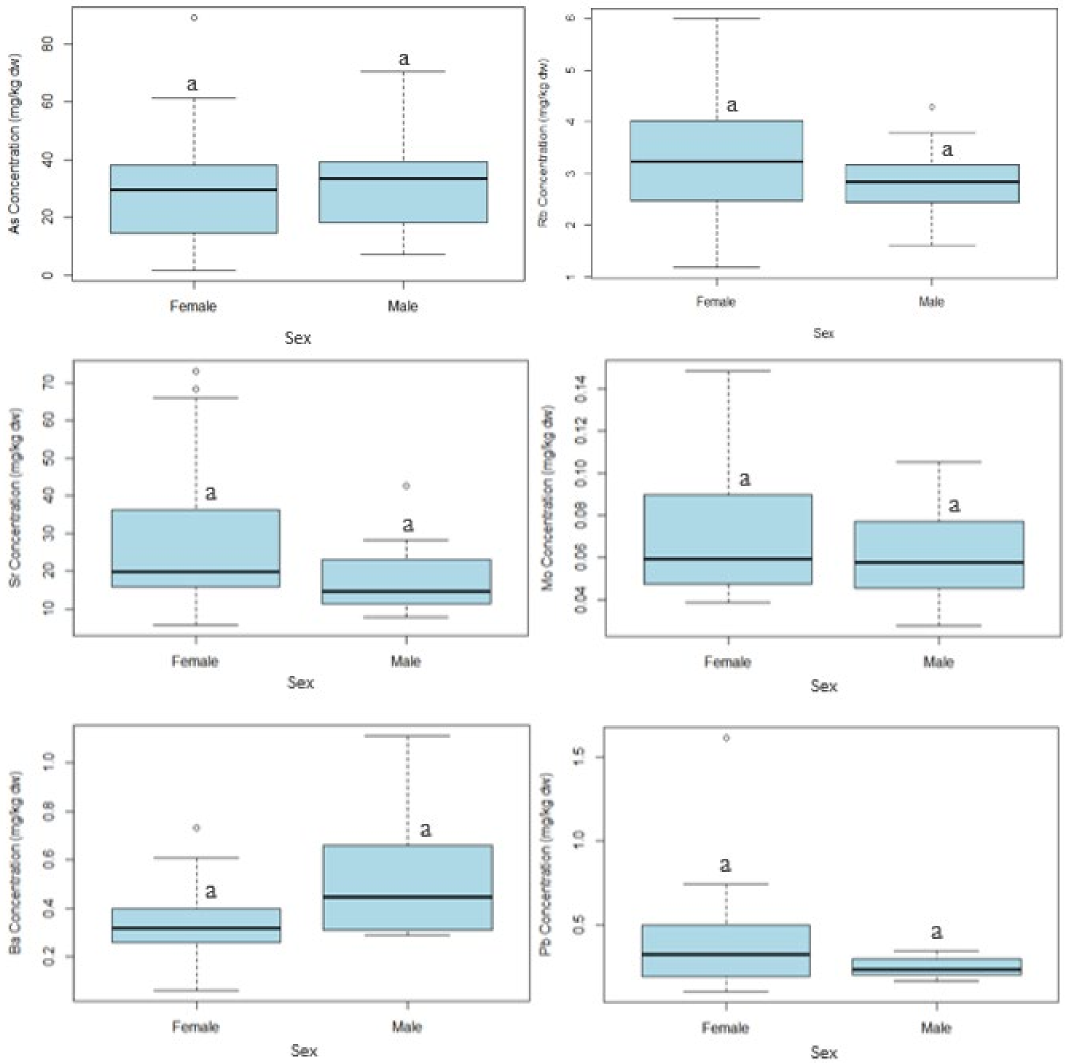
Appendix 8: Average recoveries for NRCC DOLT Dogfish Liver Certified Reference Material (CRM), sample mass ~0.022 g (n = 3) used in solution ICP-MS analysis.

Element	**Reference Value ($\mu g\ kg^{-1}$)**	**Measured Concentration ($\mu g\ kg^{-1}$)**	**Recovery (%)**
Fe	1833	1756	96
Ni	0.97	0.82	84
Cu	31.2	32.5	104
Zn	116	98.5	85
As	9.66	7.67	79
Se	8.3	5.85	70*
Cd	24.3	21.5	88
Pb	0.16	0.17	104
		Average % Recovery	91.4

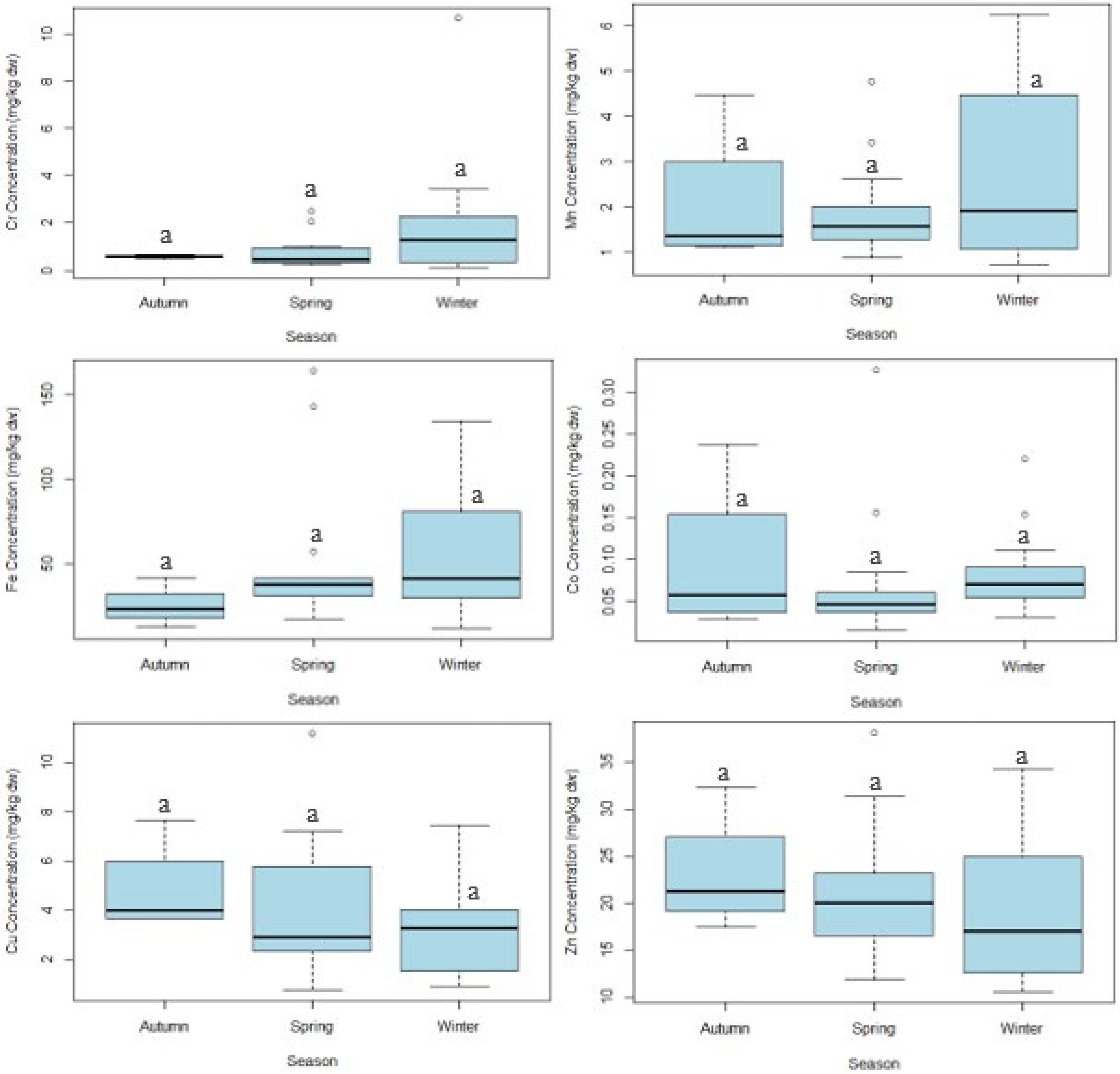
* Refractory element removed from average % recovery



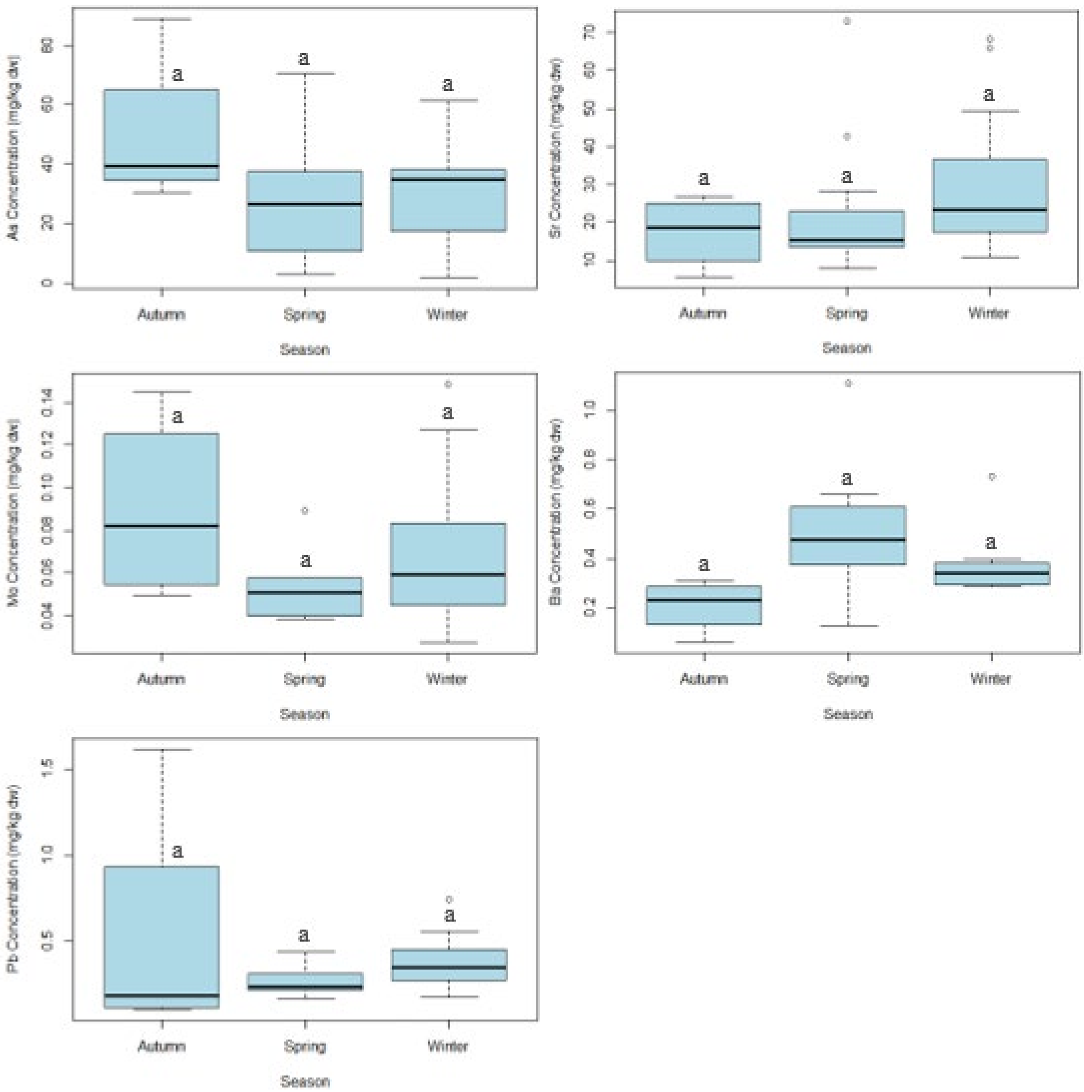
Appendix 9: Boxplots showing Cr, Fe, Cu, Zn, As, and Rb concentrations (mg kg^{-1}) within male (n = 15) and female (n = 22) *Zearaja maugeana* biopsy punch samples as determined by ICP-MS analysis. The box shows the 25th and 75th percentile, the whiskers show the upper and lower fences, dots denote outliers in the data and the lines inside the box show the median. Letters describe significant differences as determined by a permutation ANOVA. Boxplots with the same letter are not significantly different form one another.



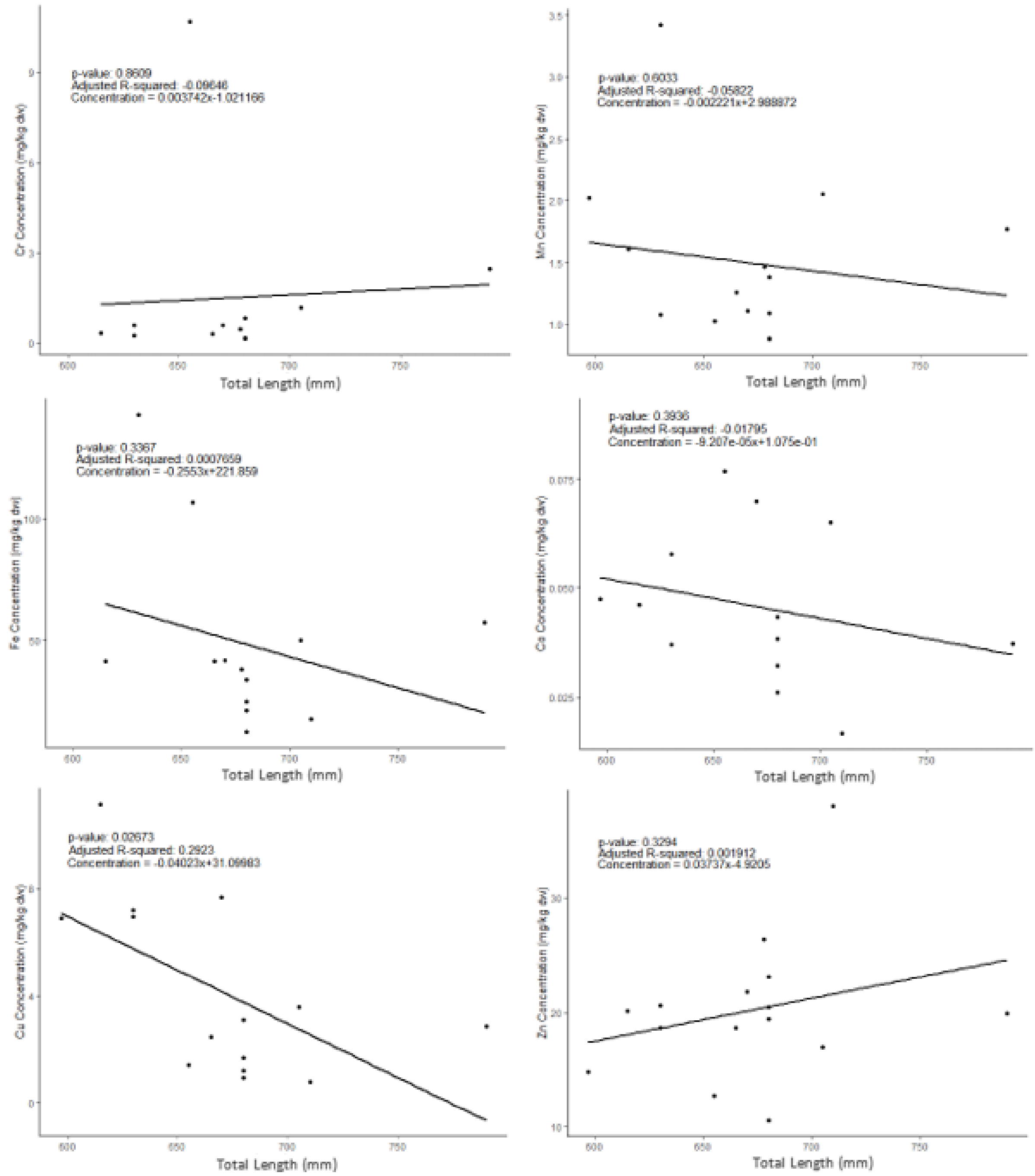
Appendix 9 continued: Boxplots showing Sr, Mo, Ba, and Pb concentrations (mg kg^{-1}) within male (n = 15) and female (n = 22) *Zearaja maugeana* biopsy punch samples as determined by ICP-MS analysis. The box shows the 25th and 75th percentile, the whiskers show the upper and lower fences, dots denote outliers in the data and the lines inside the box show the median. Letters describe significant differences as determined by a permutation ANOVA. Boxplots with the same letter are not significantly different form one another.



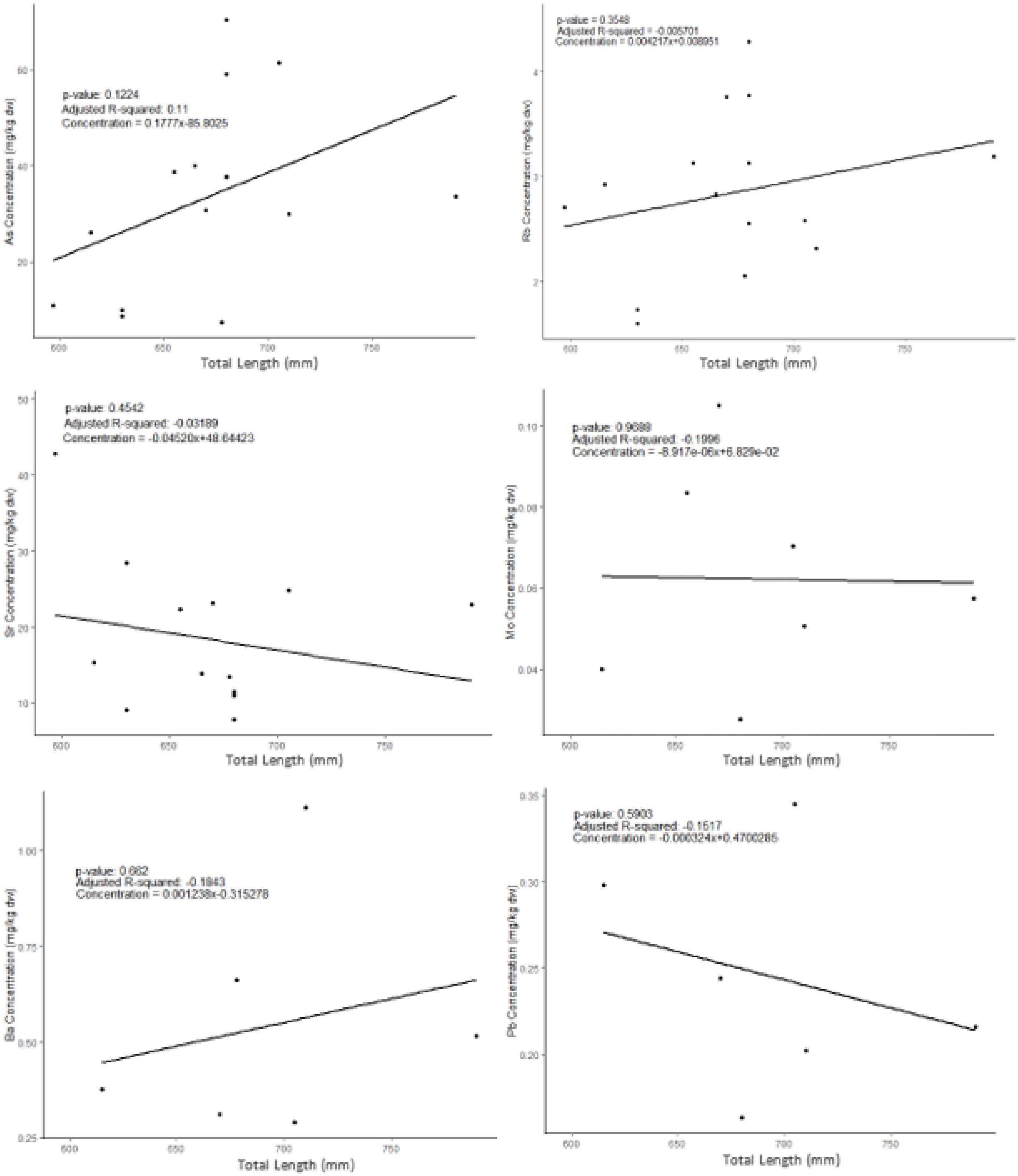
Appendix 10: Boxplots showing Cr, Mn, Fe, Co, Cu, and Zn concentrations (mg kg^{-1}) within *Zearaja maugeana* biopsy punch samples captured in Autumn (n = 4), Spring (n = 16), and Winter (n = 17) as determined by ICP-MS analysis. The box shows the 25th and 75th percentile, the whiskers show the upper and lower fences, dots denote outliers in the data and the lines inside the box show the median. Letters describe significant differences as determined by a permutation ANOVA. Boxplots with the same letter are not significantly different form one another.



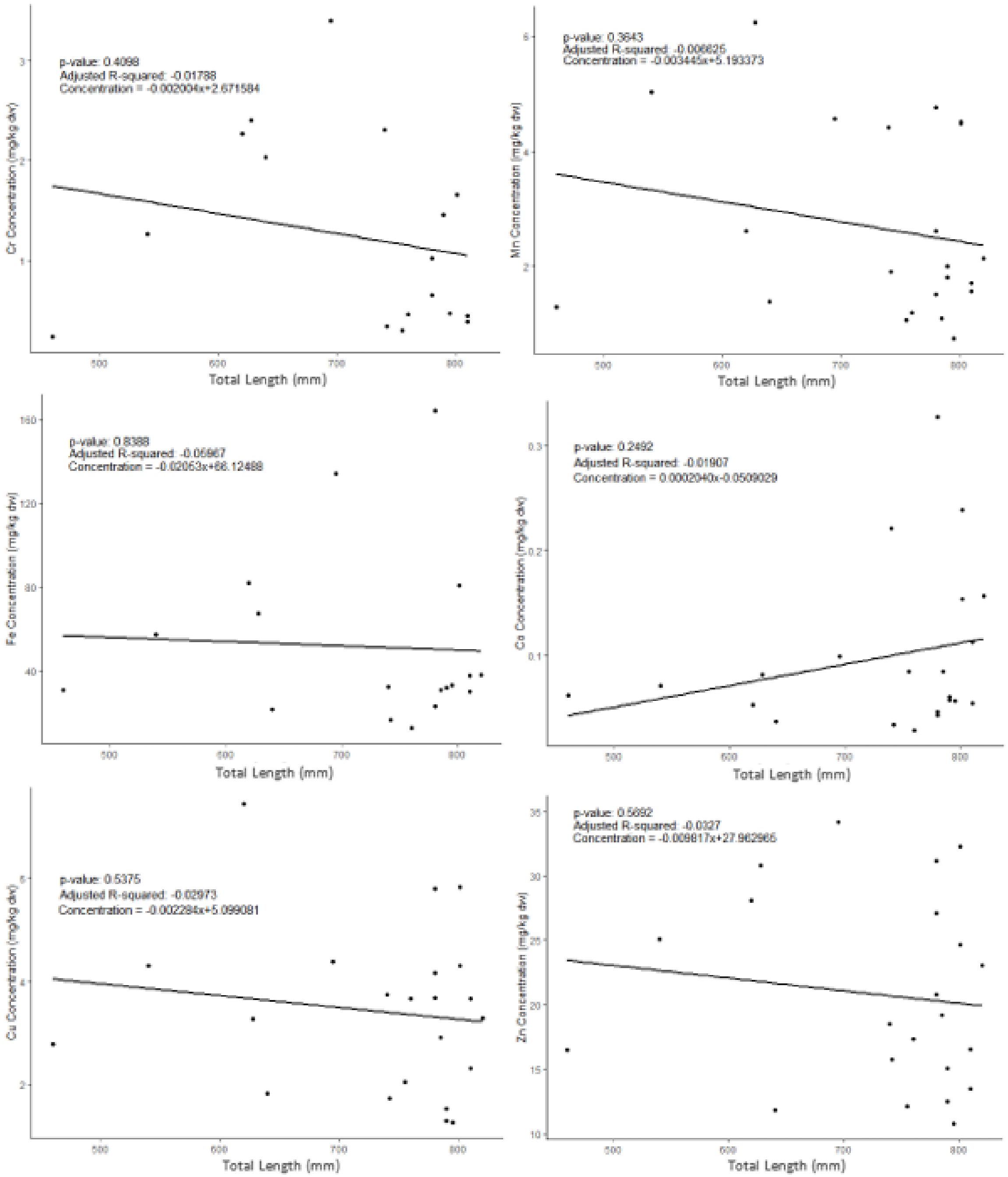
Appendix 10 continued: Boxplots showing As, Sr, Mo, Ba, and Pb concentrations (mg kg^{-1}) within *Zearaja maugeana* biopsy punch samples captured in Autumn (n = 4), Spring (n = 1), and Winter (n = 17), as determined by ICP-MS analysis. The box shows the 25th and 75th percentile, the whiskers show the upper and lower fences, dots denote outliers in the data and the lines inside the box show the median. Letters describe significant differences as determined by a permutation ANOVA. Boxplots with the same letter are not significantly different form one another.



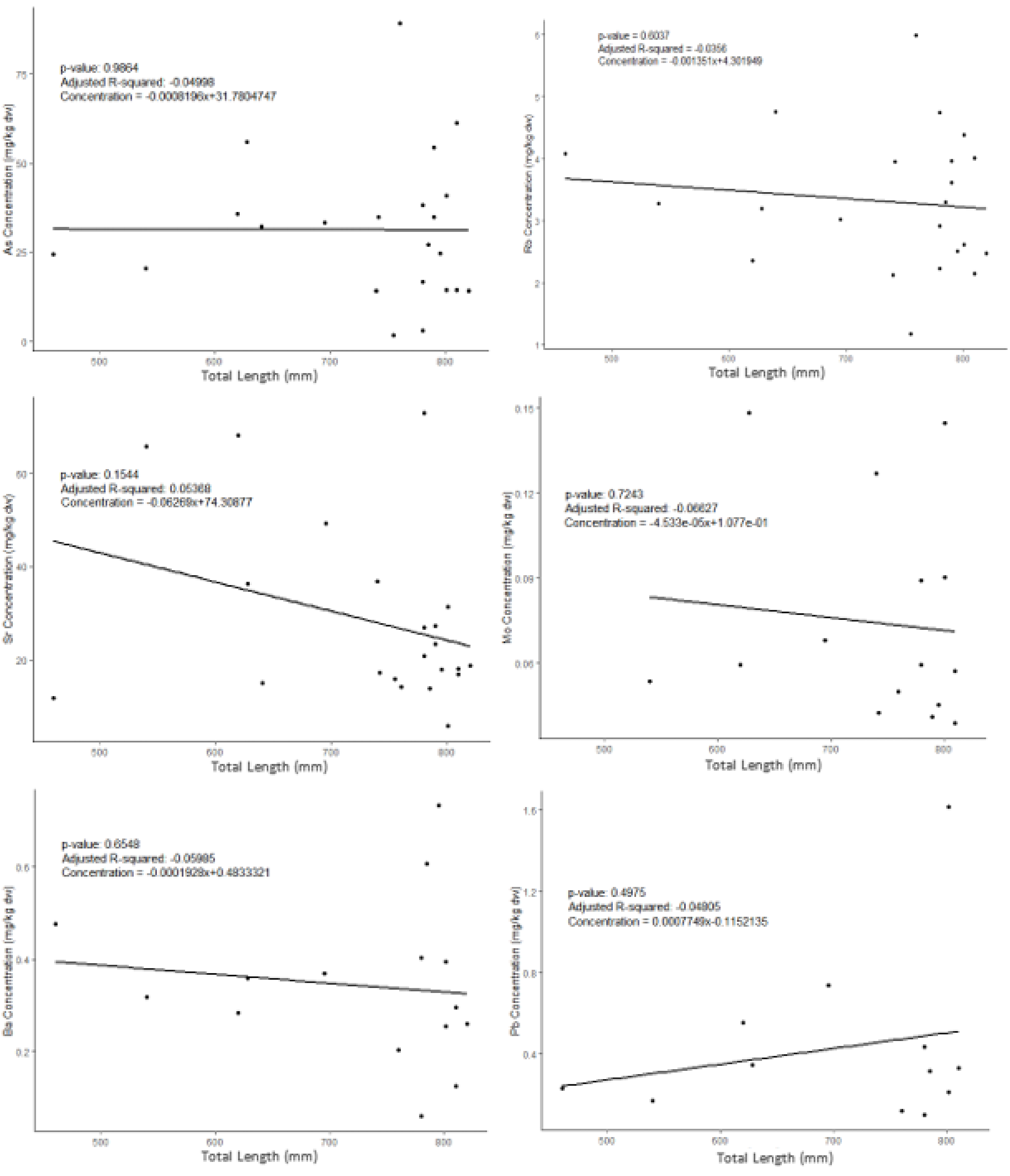
Appendix 11: Regression models representing the correlation between Cr, Mn, Fe, Co, Cu, and Zn concentration (mg kg^{-1}) within biopsy punch samples and total length (mm) of male (n = 15) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.



Appendix 11 continued: Regression models representing the correlation between As, Rb, Sr, Mo, Ba, and Pb concentration (mg kg^{-1}) within biopsy punch samples and total length (mm) of male (n = 15) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.



Appendix 12: Regression models representing the correlation between Cr, Mn, Fe, Co, Cu, and Zn concentration (mg kg^{-1}) within biopsy punch samples and total length (mm) of female (n = 22) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.



Appendix 12 continued: Regression models representing the correlation between As, Rb, Sr, Mo, Ba, and Pb concentration (mg kg^{-1}) within biopsy punch samples and total length (mm) of female (n = 22) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.

Linear Discriminant Analysis: sex

Correct predictions: 93.75% (F: 100%; M: 83.33%)

	F n: 20	M n: 12	R-Squared	*p*
Cr	0.81	0.47	0.05	1.000
Mn	0.97	0.58	0.12	1.000
Fe	0.75	0.77	0.00	1.000
Co	1.00	0.55	0.17	.450
Cu	0.72	1.03	0.08	1.000
Zn	0.96	0.92	0.01	1.000
As	0.80	0.90	0.01	1.000
Rb	0.98	0.87	0.03	1.000
Sr	0.96	0.59	0.10	1.000
Mo	0.93	0.74	0.04	1.000
Ba	0.82	0.68	0.01	1.000
Pb	0.73	0.54	0.02	1.000

n = 32 cases used in estimation; null hypotheses: two-sided; multiple comparisons correction: False Discovery Rate correction applied simultaneously to the entire table

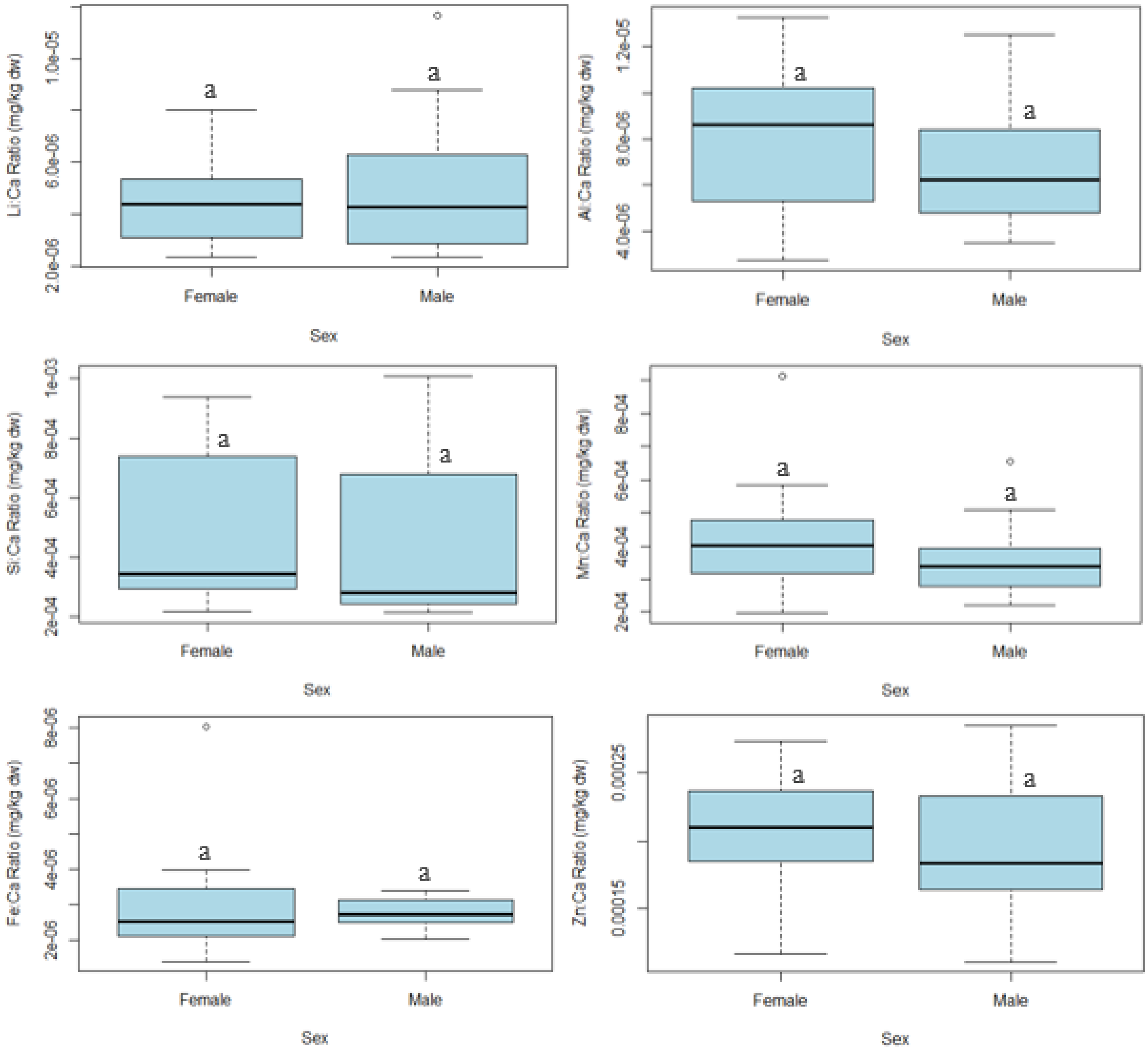
Linear Discriminant Analysis: season

Correct predictions: 90.62% (Autumn: 100%; Spring: 93.33%; Winter: 85.71%)

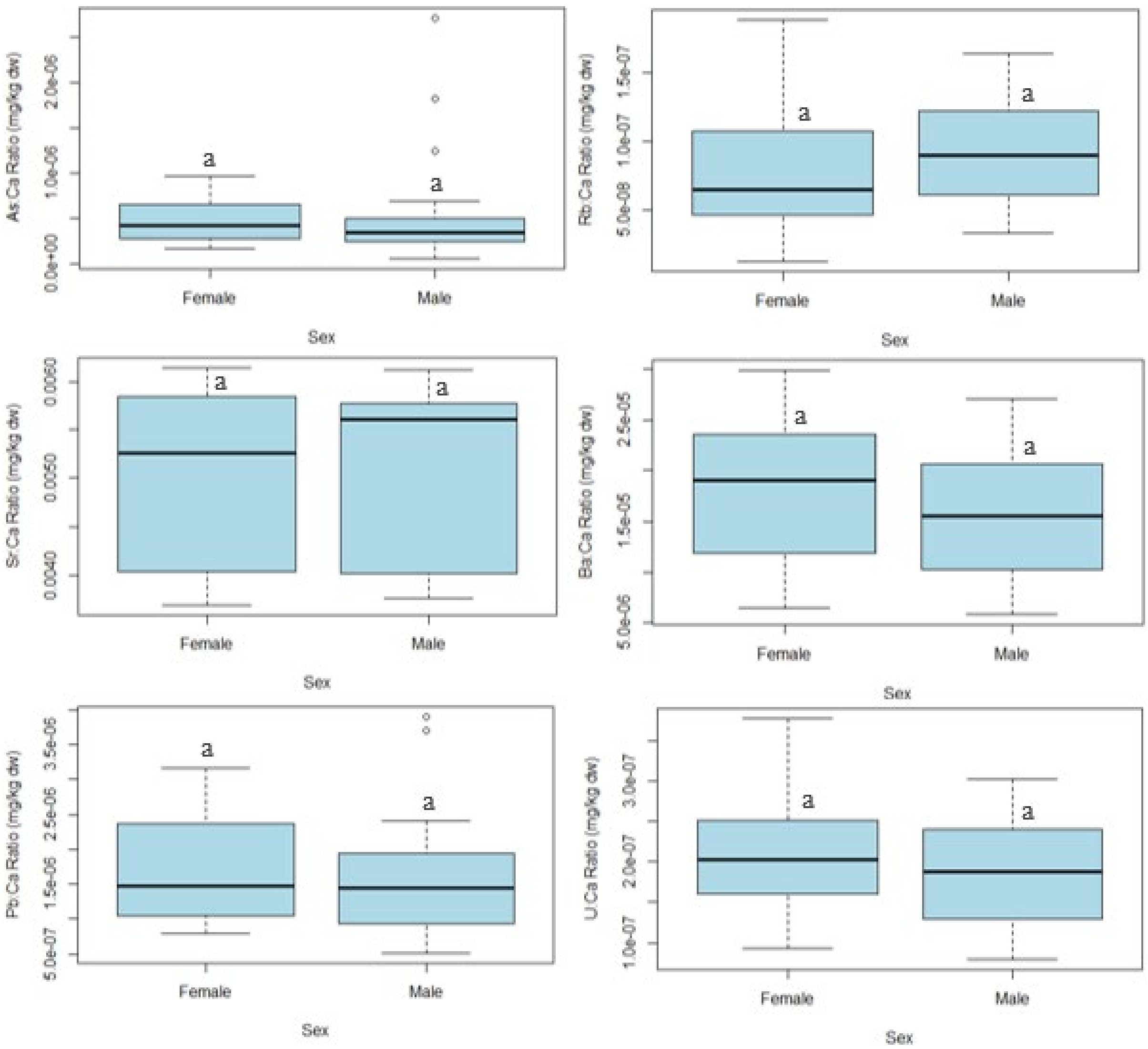
	Autumn n: 3	Spring n: 15	Winter n: 14	R-Squared	*p*
Cr	0.45	0.42	1.00	0.16	.450
Mn	0.48	0.69	1.04	0.13	.960
Fe	0.50	0.67	0.91	0.05	1.000
Co	0.60	0.69	1.04	0.12	1.000
Cu	1.11	0.90	0.71	0.06	1.000
Zn	0.98	0.92	0.96	0.01	1.000
As	1.34	0.77	0.80	0.10	1.000
Rb	**1.44**	0.90	0.87	0.29	**.031**
Sr	0.69	0.70	0.98	0.06	1.000
Mo	1.20	**0.59**	1.07	0.28	**.046**
Ba	0.62	0.73	0.84	0.01	1.000
Pb	0.65	0.42	0.91	0.10	1.000

n = 32 cases used in estimation; null hypotheses: two-sided; multiple comparisons correction: False Discovery Rate correction applied simultaneously to the entire table

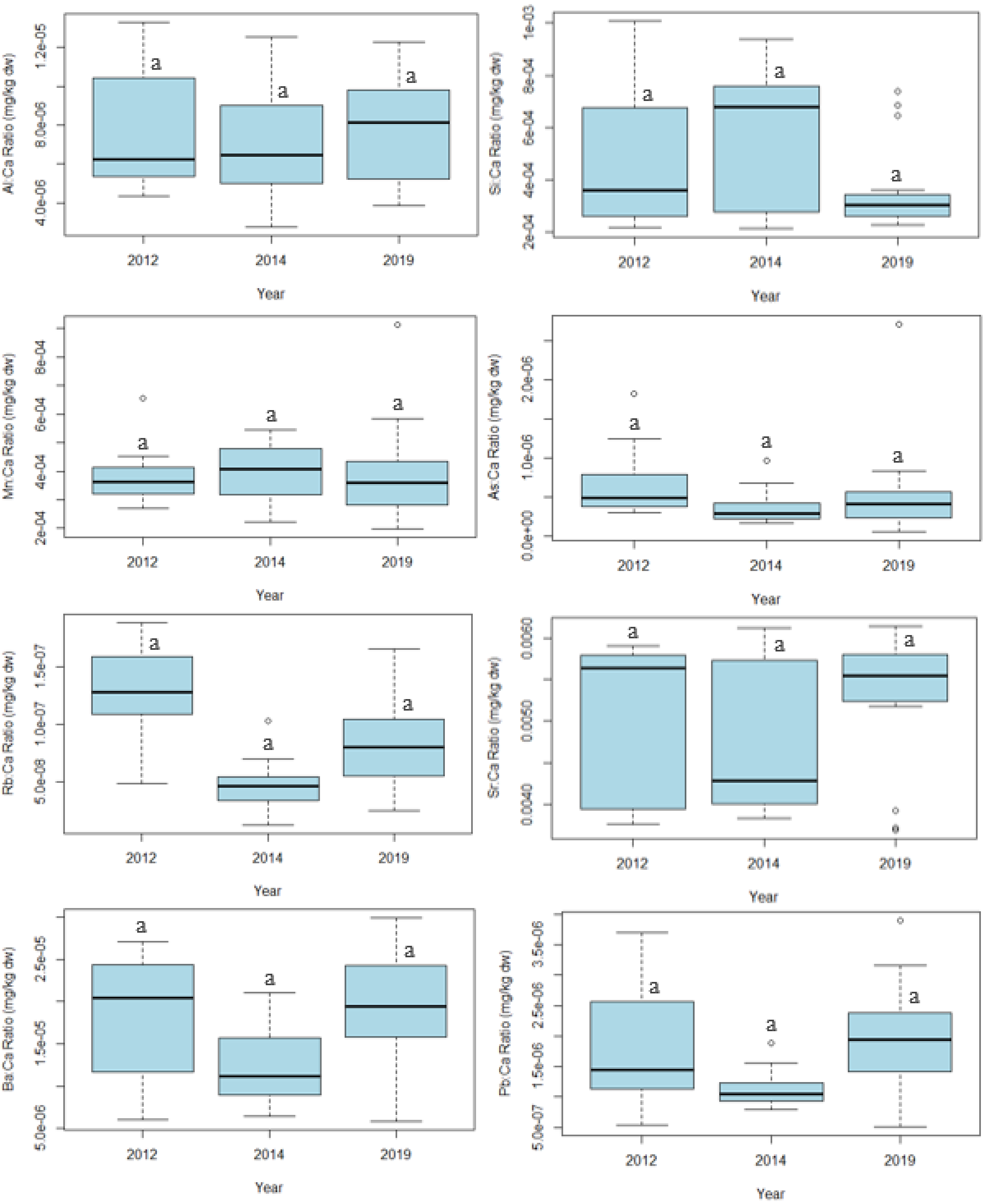
Appendix 13: Mean contribution to the linear discriminant analysis of Cr, Mn, Fe, Co, Cu, Zn, As, Rb, Sr, Mo, Ba, and Pb for sex (top) and season caught (bottom) within *Zearaja maugeana* biopsy punch samples. R^2 represents the proportion of variance that is explained by the groups.



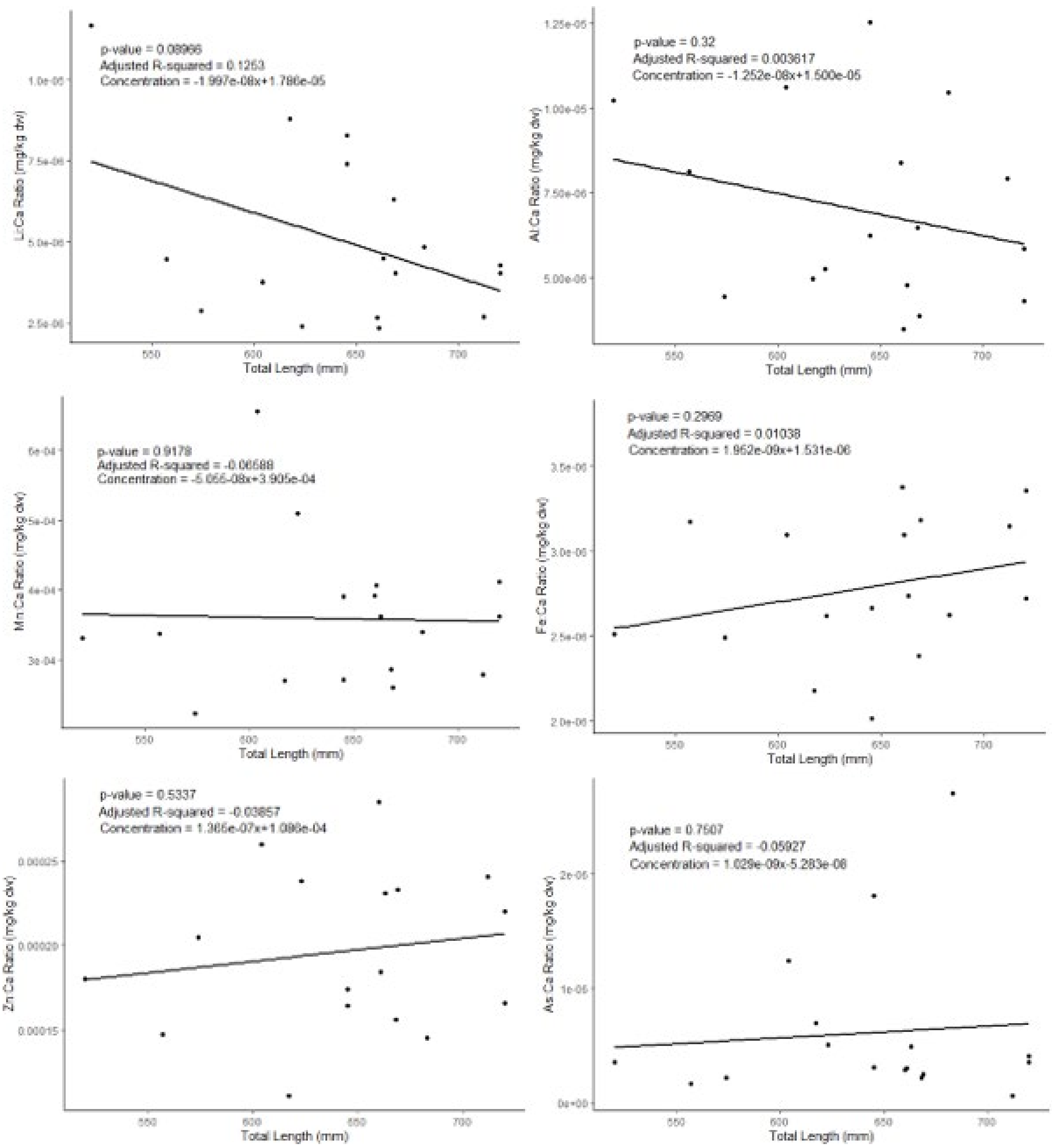
Appendix 14: Boxplots showing Li, Al, Si, Mn, Fe and Zn: ^{44}Ca ratios (mg kg^{-1}) within male (n = 17) and female (n = 26) *Zearaja maugeana* vertebrae samples as determined by LA-ICP-MS analysis. The box shows the 25th and 75th percentile, the whiskers show the upper and lower fences, dots denote outliers in the data and the lines inside the box show the median. Letters describe significant differences as determined by a permutation ANOVA. Boxplots with the same letter are not significantly different form one another.



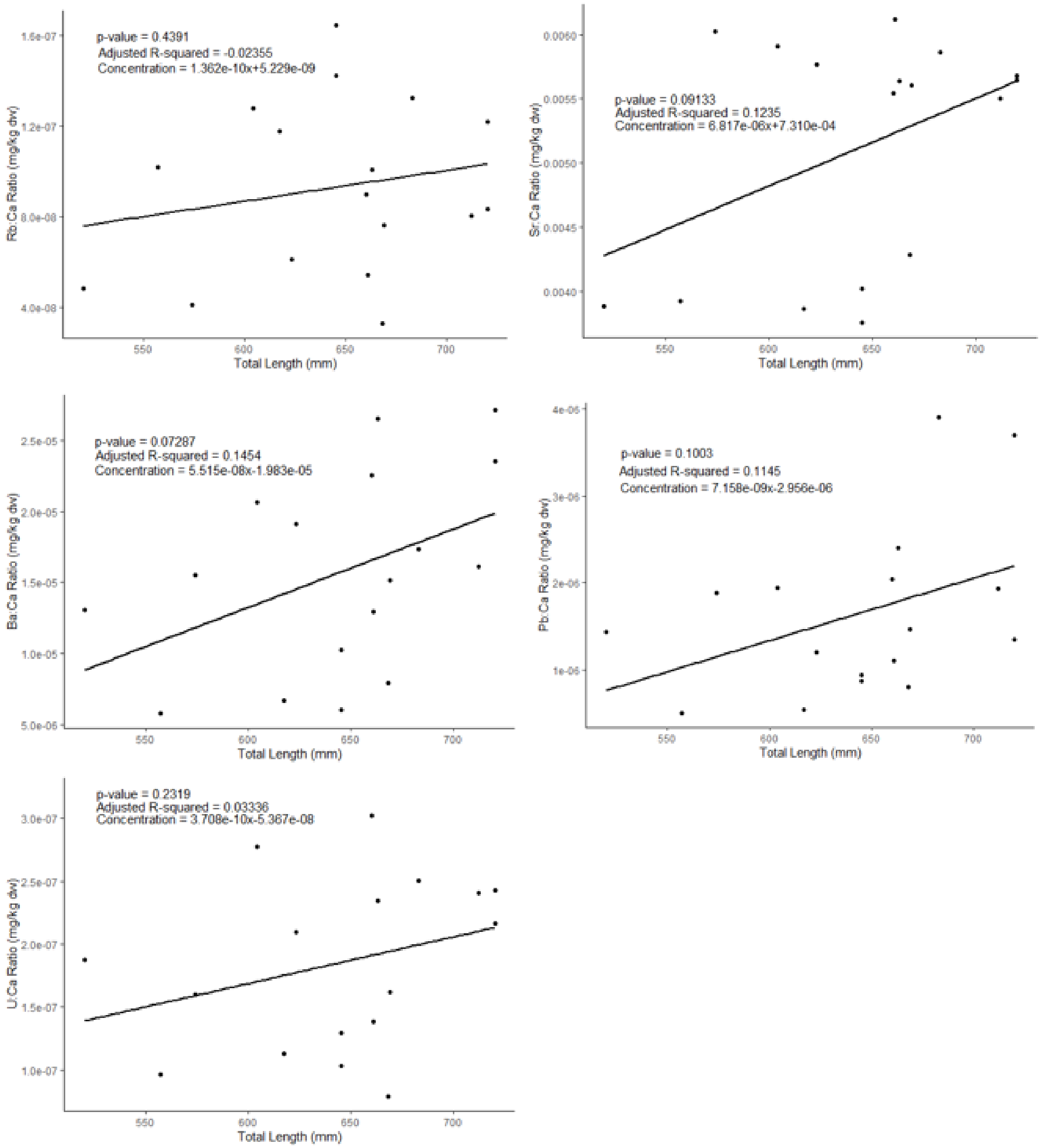
Appendix 14 continued: Boxplots showing As, Rb, Sr, Ba, Pb, and U: ^{44}Ca ratios (mg kg^{-1}) within male (n = 17) and female (n = 26) *Zearaja maugeana* vertebrae samples as determined by LA-ICP-MS analysis. The box shows the 25th and 75th percentile, the whiskers show the upper and lower fences, dots denote outliers in the data and the lines inside the box show the median. Letters describe significant differences as determined by a permutation ANOVA. Boxplots with the same letter are not significantly different form one another.



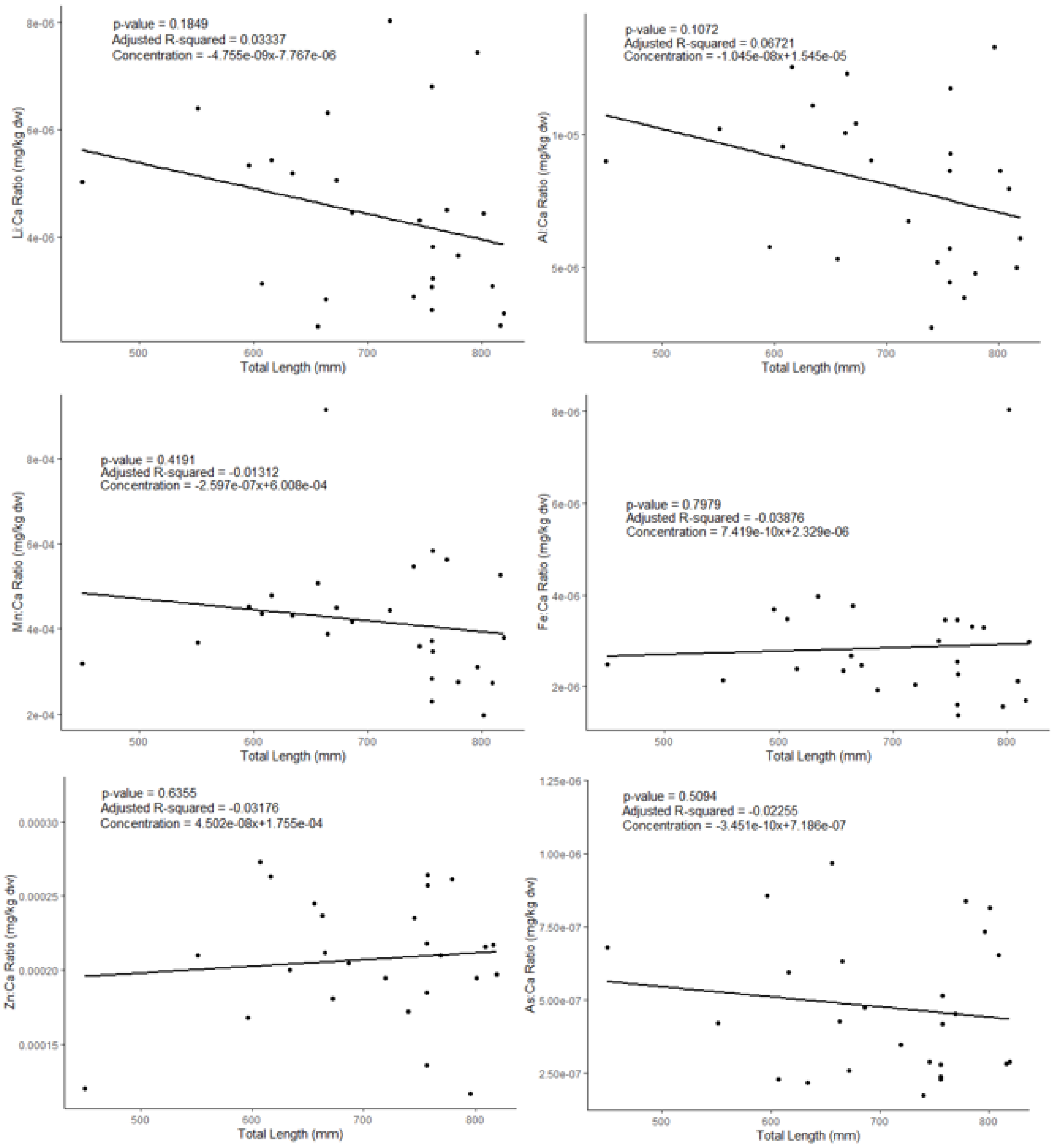
Appendix 15: Boxplots showing Al, Si, Mn, As, Rb, Sr, Ba, and Pb: ^{44}Ca ratios (mg kg^{-1}) within *Zearaja maugeana* vertebrae samples captured in 2012 (n = 11), 2014 (n = 13), and 2019 (n = 19) as determined by ICP-MS analysis. The box shows the 25th and 75th percentile, the whiskers show the upper and lower fences, dots denote outliers in the data and the lines inside the box show the median. Letters describe significant differences as determined by a permutation ANOVA. Boxplots with the same letter are not significantly different form one another.



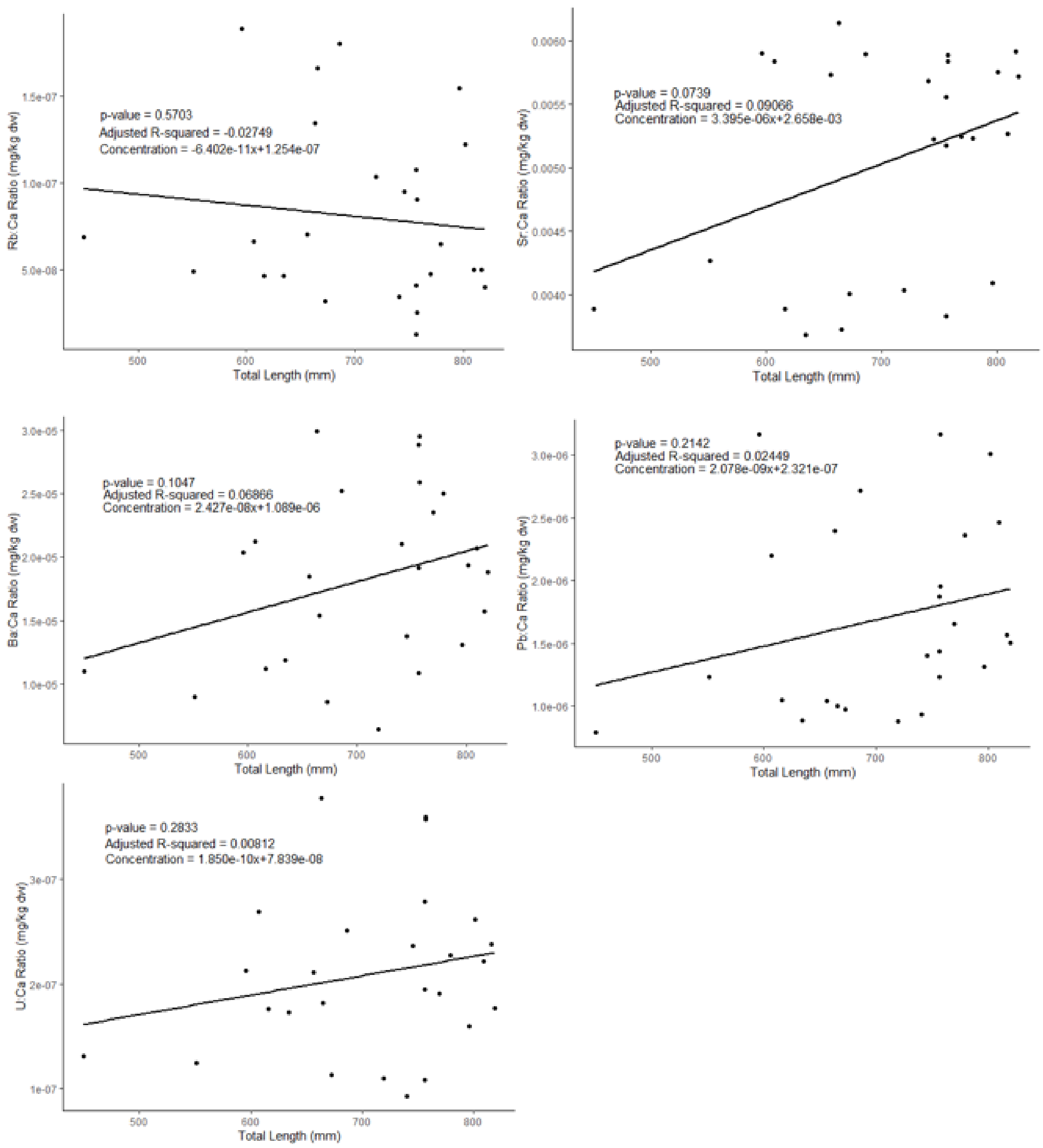
Appendix 16: Regression models representing the correlation between Li, Al, Mn, Fe, Zn, and As: ^{44}Ca ratios (mg kg^{-1}) within biopsy punch samples and total length (mm) of male (n = 17) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.



Appendix 16 continued: Regression models representing the correlation between Rb, Sr, Ba, Pb, and U: ^{44}Ca ratios (mg kg^{-1}) within biopsy punch samples and total length (mm) of male (n = 17) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.



Appendix 17: Regression models representing the correlation between Li, Al, Mn, Fe, Zn, and As: ^{44}Ca ratios (mg kg^{-1}) within biopsy punch samples and total length (mm) of female (n = 26) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.



Appendix 17 continued: Regression models representing the correlation between Rb, Sr, Ba, Pb, and U: ^{44}Ca ratios (mg kg^{-1}) within biopsy punch samples and total length (mm) of female (n = 26) *Zearaja maugeana*. P-value, adjusted R^2 and formula are displayed as determined by regression model.

Linear Discriminant Analysis: sex

Correct predictions: 72.09% (F: 84.62%; M: 52.94%)

	F n: 26	M n: 17	R-Squared	*p*
Li	0.86	0.98	0.02	1.000
Al	0.98	0.84	0.04	1.000
Si	0.89	0.85	0.00	1.000
Mn	0.99	0.85	0.05	1.000
Fe	0.94	0.92	0.00	1.000
Zn	0.99	0.94	0.01	1.000
As	0.67	0.86	0.02	1.000
Rb	0.82	0.95	0.02	1.000
Sr	0.97	0.98	0.00	1.000
Ba	0.97	0.84	0.03	1.000
Pb	0.90	0.87	0.00	1.000
U	0.97	0.86	0.03	1.000

n = 43 cases used in estimation; null hypotheses: two-sided; multiple comparisons correction: False Discovery Rate correction applied simultaneously to the entire table

Linear Discriminant Analysis: year

Correct predictions: 83.72% (2012: 81.82%; 2014: 84.62%; 2019: 84.21%)

	2012 n: 11	2014 n: 13	2019 n: 19	R-Squared	*p*
Li	**1.24**	0.88	**0.73**	0.26	**.003**
Al	0.97	0.85	0.95	0.02	1.000
Si	0.94	1.09	0.69	0.14	.060
Mn	0.92	0.95	0.95	0.00	1.000
Fe	0.85	0.79	**1.07**	0.15	**.044**
Zn	0.86	0.91	**1.07**	0.19	**.016**
As	0.99	0.54	0.74	0.07	.514
Rb	**1.33**	**0.50**	0.86	0.45	**< .001**
Sr	0.95	0.94	1.02	0.05	.463
Ba	0.93	**0.69**	**1.07**	0.20	**.017**
Pb	0.98	**0.61**	1.02	0.18	**.019**
U	0.90	**0.67**	**1.12**	0.33	**.001**

n = 43 cases used in estimation; null hypotheses: two-sided; multiple comparisons correction: False Discovery Rate correction applied simultaneously to the entire table

Appendix 18: Mean contribution to the linear discriminant analysis of Li, Al, Si, Mn, Fe, Zn, As, Rb, Sr, Ba, Pb, and U for sex (top) and year caught (bottom) within *Zearaja maugeana* vertebrae samples. R^2 represents the proportion of variance that is explained by the groups.