Title: The broad-scale analysis of metals, trace elements, organochlorine pesticides and polycyclic aromatic hydrocarbons) in wetlands along an urban gradient, and the use of a high trophic snake as a bioindicator.

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Acknowledgements: We thank the Holsworth Wildlife Research Endowment for providing funding support for this study. We also thank Kady Grosser, Ross McGibbon, Serin Subaraj and Jordan Vos for field assistance in locating and catching tiger snakes. Snakes were collected under Western Australia’s Department of Biodiversity, Conservation and Attractions permit number: 08-002624-1. Curtin University’s Animal Research Ethics Committee approved the use of snakes for this research under approval number: ARE2018-23.

Conflict of interest:

None.

Key words: *Notechis scutatus*, Environmental pollution, contaminated sediment, reptile ecotoxicology, urbanisation, metals

Abstract:

Wetlands and their biodiversity are constantly threatened by contaminant pollution from urbanisation. Despite evidence suggesting that snakes are good bioindicators of environmental health, the bioaccumulation of contaminants in reptiles is poorly researched in Australia. We conducted the first broad-scale analysis of 17 metals and trace elements, 21 organochlorine pesticides and 14 polycyclic aromatic hydrocarbons in the sediments (four samples per site, December 2018) from four wetlands along an urban gradient in Perth, Western Australia and from the livers (five livers per site, February – April 2019) of Western tiger snakes *Notechis scutatus occidentalis* at those sites. All 17 metals and trace elements were detected in the sediments of every wetland, and 15 in the livers of tiger snakes. Arsenic, Cu, Hg, Pb, Se, Zn were at concentrations exceeding government trigger values in at least one sediment sample. Two organochlorine pesticides and six of seven polycyclic aromatic hydrocarbons were detected in the sediments of a single wetland, all exceeding government trigger values, but not in tiger snakes. Metals and trace elements were generally in higher concentration in sediments and snake livers from more heavily urbanised wetlands. The least urbanised site had some higher concentrations of metals and trace elements, probably due to agriculture contaminated groundwater. Concentrations of nine metals and trace elements in snake livers were statistically different between sites. Arsenic, Cd, Co, Hg, Mo, Sb and Se near paralleled the pattern of contamination measured in the wetland sediments; this supports the use of high trophic wetland snakes, such as tiger snakes, as bioindicators of wetland contamination. Contamination sources and impacts on these wetland ecosystems and tiger snakes are discussed herein.

1. Introduction

Wetlands are biodiversity hotspots threatened by urbanisation, worldwide. Wetlands are often the only remaining fragments of habitat within an urban landscape (Ehrenfeld 2004; Garden et al. 2006), and provide islands of water storage, ground water replenishment, supply and transformation of nutrients, high biodiversity, and recreational enjoyment for humans (Lee et al. 2006; Novitski et al. 1996; Zedler and Kercher 2005). As land development increases wetland ecosystems degrade through changes in hydrology (Chadwick et al. 2006), structure (Faulkner 2004; Lee et al. 2006), floral and faunal biodiversity (Gibbs 2000; McKinney 2008), and water and sediment chemistry (Brown et al. 2010; Fitzpatrick et al. 2007; Panno et al. 1999). Environmental contamination, as a consequence of rapid urbanisation, industrialisation and poor waste management practices (Nriagu 1990; Rodriguez Martin et al. 2015), is particularly severe for urban wetlands and threatens the health of biological communities (Spurgeon and Hopkin 1999; Zhang et al. 2017). Due to their topography, urban wetlands are susceptible to contamination from several primary source points: urban runoff (Zhang et al. 2012), stormwater drains feeding into wetlands (Clarke et al. 1990), groundwater (Roy and Bickerton 2011), and pest and weed treatment (Gentilli and Bekle 1993).

Perth is the largest city of the west coast of Australia, and is built almost entirely on the Swan Coastal Plain bioregion (Davis and Froend 1999). The Swan Coastal Plain is characterised by sandy soil dunes systems interlaced with a chain of inter-connected ephemeral wetlands and lakes (Simpson and Newsome 2017). For the past near 200 years urbanisation and agriculture has drained or filled in an estimated 70% of the original wetland area of the Swan Coastal Plain, and the remaining wetlands have been subject to structural and hydrological modifications, isolation, biodiversity loss and pollution (Davis and Froend 1999; Gentilli and Bekle 1993). The remnant wetlands of urban Perth have a history of contamination from primary sources such as inflowing stormwater drains, as well as historical industrial dumping and pest management through pesticides (Clarke et al. 1990; Department of Water 2009; ESRI 1983; Gentilli and Bekle 1993). Some of the larger wetlands in Perth still support populations of Western tiger snakes (*Notechis scutatus occidentalis*), a top predator of these ecosystems. Snakes are an important organism in the ecosystem, providing predator and prey functions that shift as they climb the trophic tiers as they age. Wetland snakes in particular represent an interface between aquatic and terrestrial habitats; thus they are susceptible to the bioaccumulation of contaminants and can be used as bioindicators for environmental pollution (Campbell and Campbell 2001; Drewett et al. 2013; Lemaire et al. 2018).

Despite the historical contamination and close proximity of infrastructure to urban wetlands in Perth, there are little monitoring data available on the contamination of water, sediment or fauna (see table 1 for summary). Wetlands can be polluted with a plethora of contaminants, the more frequently assessed contaminants are metals and trace elements, organochloride pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) (Cooper 1993; Gambrell 1994; Haarstad et al. 2012). As wetland structure plays an important role in contaminant retention, permanently enclosed wetlands such as lakes are more susceptible to the accumulation of contaminants compared to flowing water systems (Burger et al. 2007; Schulz and Peall 2001). As a result, fauna communities restricted to enclosed and isolated urban wetlands might be vulnerable to bioaccumulation from continuous exposure to contaminants. Three permanent and enclosed wetlands that differ in degree of urbanisation persist in Perth and contain abundant populations of tiger snakes. By comparing these wetlands and tiger snake populations with a similar wetland within a national park we are presented with a rare and ideal system to study the contamination of wetlands and a top predator snake along an urban gradient.

This study presents and examines a snapshot of concentrations of 52 contaminants in both sediment and tiger snake livers from four wetlands around Perth, Western Australia. The objective of this study was threefold: (1) quantify contaminants present in the wetlands, (2) measure contaminants that are bioaccumulating in the top predator: tiger snakes, and (3) investigate if contaminant concentrations parallel the degree of urbanisation of wetlands? Research on contaminants in snakes is an emerging field (Burger et al. 2017; Drewett et al. 2013; Gavric et al. 2015; Quintela et al. 2019; Schwabenlander et al. 2019) yet as far as we are aware this study analyses the largest range of contaminants in any species of snake, and is only the third study to present data on the contaminants of both the snake tissue and ecosystem they were collected from (Ford and Hill 1991; Soliman et al.

2019). We also present the first published contaminants in Australian snakes in over 40 years (Beck 1956; Best 1973).

2. Material and methods:

2.1 Study sites

We examined a suite of contaminants in four wetlands in the Swan Coastal Plain of Western Australia (Fig. 1.): Herdsman Lake, Bibra Lake, Lake Joondalup and Loch McNess. Loch McNess is located in Yanchep National Park. We selected these wetlands as study sites based upon presence and abundance of tiger snakes. Historically, these wetland lakes were partially linked and ephemeral (Gentilli and Bekle 1993) yet the development of Perth city lead to the draining of some wetlands while others were dredged to become permanent (Halse 1989). These wetlands share similar climatic factors and modification from naturally ephemeral to permanently-filled, yet differ in degrees of urbanisation (Davis and Froend 1999). Table 1 describes these wetlands and summarises the few studies on contaminants reported at these wetlands. As there is no recent and detailed research available on the contaminants of these wetlands, we initially tested the sediments for a suite of contaminants listed in Table A. 1.

2.2 Study species

The tiger snake (*Notechis scutatus*) is a polymorphic Australian elapid occurring in disjunct populations that vary in diet, habitat and ecology (Aubret et al. 2004; Aubret et al. 2006). However, across most of its range, including the mainland Western Australian subspecies *N. scutatus occidentalis*, tiger snakes are an abundant higher trophic reptile predator in wetlands that have a preference for frogs (Aubret et al. 2006; Shine 1987). There is no long-term monitoring data available for tiger snakes to determine life expectancy, yet the oldest captive record was up to 24 years (Fearn and Norton 2011). Tiger snakes and dugites (*Pseudonaja affinis*) are the only two large snake species to persist within urban Perth and dugites prefer woodland and heath over wetlands, thus we selected tiger snakes as our bioindicator model species based on their abundance, habitat preference, high trophic position and prey preference. Frogs are known to bioaccumulate contaminants indirectly through their food or directly through their water permeable skin when in contact with contaminated waters or sediments (Bruhl et al. 2011; Hopkins 2007; Ohlendorf et al. 1988), and therefore provide a crucial link for contaminant transfer throughout the food web.

2.3 Sediment collection and analysis

Sediment samples were collected 18 December 2018 during the dry season when Perth receives little rainfall. Each wetland was sampled in two locations and sediments were collected in duplicates, each duplicate was collected three metres away from each other. Sampling locations are shown in Fig. 1 and corresponded to areas of the wetland with the highest capture rate of tiger snakes. The first 10 cm of sediment was collected using a metal scoop and glass jar for organic contaminant samples and a plastic scoop and jar for metal and trace element samples. Sediment samples were kept cool until submitted for chemical analysis at the end of the day of collection. All samples were analysed for 17 metals and trace elements (from hereon in collectively referred to as metals), 21 organochlorine pesticides and 14 PAHs by ChemCentre (Perth, Western Australia). These contaminants were chosen based on the limited historical data and regular screening of this suite by ChemCentre for environmental monitoring (Leif Cooper, pers. comm.). The specific contaminants, method of analysis, detection limits and quality assurance are reported in Appendix 1. Metals were determined using methods iMET2SAMS and iMET2SAMS based on US EPA method 3051A (US EPA 2007). Sediment samples were extracted with concentrated nitric and hydrochloric acid, then analysed for metals using a combination of inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS). Organochlorine pesticides were determined using method ORG141S and PAHs were determined using method ORG100S, both based on US EPA method 8270D (US EPA 1998). Contaminant concentrations are reported as mg/kg dry weight, and are compared to the Australian and New Zealand guidelines (ANZECC & ARMCANZ 2000) and the revised ANZECC/ARMCANZ Sediment Quality Guidelines (Simpson et al. 2013). The lower trigger value (sediment quality guideline value (SQGV)) represents the threshold for biological effects, and the higher sediment quality guideline trigger value (SQGV-High) represents the high probability of biological effects (Simpson et al. 2013). If there was no guidelines for a particular contaminant they are compared to alternative guidelines (Lemly 1996; Ontario Ministry of Environment and Energy 1993).

2.4 Snake liver collection and analysis

Five tiger snakes were collected from each site by hand between February and April 2019, and euthanised humanely by blunt force trauma to the head. Sex, snout-vent length (SVL), total mass and liver mass were recorded for all snakes (Table 2). We selected livers for analysis due to their potential to retain contaminants taken up by feeding. Whole livers were extracted using ceramic blades and frozen, and were submitted to ChemCentre for chemical analysis. All liver samples were analysed for the same contaminants as were the sediments; the specific contaminants, method of analysis, detection limits and quality assurance are listed in Appendix 2. Whole livers were homogenised and extracted with concentrated nitric and hydrochloric acid, then analysed for metals were determined using methods iMETBTMS and iMETBTICP based on APHA methods 3120 and 3125 (APHA 1998) using a combination of inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS). Organochlorine pesticides and PAHs were determined using method ORG100B based on US EPA methods 8270D (US EPA 1998). Contaminant concentrations are reported as mg/kg wet weight.

2.5 Data analysis

Concentration means and standard deviation were calculated for each contaminant for each site. For statistical analysis samples that were recorded below detectable limits (BDL) were entered as half the detection limit (ANZECC & ARMCANZ 2000; Zeghnoun et al. 2007). Due to the limited number of animals allowed to be sacrificed at each site, no statistical differences could be established between male and female contaminant burdens. Consequently contaminant data were pooled for male and female snakes. The Shapiro-Wilk test was used to determine all data (sediment and livers) had a non-parametric distribution. Hence a Kruskal-Wallis test was used for both sediments and tiger snake livers to determine if there were significant differences ($p < 0.05$) in contaminant concentrations between sites. Following, a Dunn post-hoc test was performed to identify the pairs of sites that showed significant differences. P-values were adjusted using the Benjamini-Hochberg method. We used Spearman rank correlations to explore the relationship between SVL (age) of snakes and each metal and trace element within each site. We present and consider rho values over 0.5 as moderate positive correlations and allow the reader to assess the significance themselves. All statistical analysis were conducted in R Studio (R Core Team 2018).

3. Results and Discussion

3.1 Wetland sediment contaminants

All 17 metals tested for were detected in Herdsman Lake, 16 were detected in Bibra Lake and Loch McNess, and 15 were detected in Lake Joondalup (discussed data presented in Table 3). Four metals (As, Cu, Pb and Zn) were detected exceeding trigger values in at least one sample, and Zn was detected exceeding the high trigger value in one sample from Herdsman Lake. Selenium was detected exceeding the trigger value in one sample at Bibra Lake and two samples at Loch McNess. Mercury was detected exceeding the trigger value in two samples in Lake Joondalup and one sample in Loch McNess. Generally, mean concentrations of contaminants decreased with less urbanised sites except for nine metals that were high at Loch McNess.

Herdsman Lake generally had the highest mean concentration of metals including significantly higher concentrations of Pb, As, Co, Cu, Mo, Ni, Sn and Zn compared to at least one other site, as well as the only detection of Ag. Herdsman Lake is particularly susceptible to contamination from several point sources. Since urbanisation began in Perth the wetland has suffered from considerable changes in land use including: stock grazing (1850s), market gardening (1910s), drainage for irrigation and land reclamation (1920s), sanitary landfill (1930s), compensation basin for urban drainage (1930s+), intense pesticide treatment (1950 – 1980s), reserved for public recreational space (1970s+), dredging (1980s) and periodic illegal rubbish disposal (Clarke et al. 1990; Davis and Garland 1986; Department of Water 2009; ESRI 1983; Gentilli and Bekle 1993; Kobryn 2001). Currently, the wetland is divided into three main interconnected water bodies which receive drainage from five major and an unknown number of minor drainage systems, including the bordering industrial area. Urban and industrial development began to encroach the lake in the 1950s and currently virtually no original fringing vegetation remains. The high concentrations of As, Cu, Pb and Zn are likely to originate from industrial stormwater, urban runoff, and leaching from historical dumping. Although our sampling did not detect any pesticides they have been detected in more comprehensive studies in the past (Clarke et al. 1990; Davis and Garland 1986).

Bibra Lake generally had the second highest mean concentrations of metals, and the only detections of OCPs and PAHs (discussed later). There is limited information available on the history of Bibra Lake but it has not suffered from the same degree of urbanisation as Herdsman Lake. Although it has a much larger buffer of remnant vegetation than Herdsman Lake (Fig. 1) it is still in close proximity to an industrial area which began development in conjunction with suburbia in the 1970s (Department of Water 2009). The northern and southern edge of the lake are bordered closely to main roads, and the southern edge of the lake has buried sanitary landfill (Burkett 2005). Bibra Lake receives water via direct rainfall and surface runoff from the urban catchments (Sinang et al. 2015), which is might be the source of its moderate concentration of metals.

Joondalup Lake had the lowest concentration of most of the metals detected, including significantly lower concentrations of Pb, As, Co, Cu, Mo, Ni and Zn than Herdsman Lake, the latter being the most contaminated and urbanised site. Urbanisation of Joondalup only began to rapidly increase around the lake in the 1980s, which is later than the other study sites (Kinnear et al. 1997); and the wetland has a relatively large buffer of remnant vegetation surrounding the lake compared to the other urban sites, protecting the lake from urban runoff as most of its water is recharged from rainfall (Newport and Lund 2014). Interestingly, Joondalup had the highest mean and maximum concentrations of total Hg, both exceeding the guideline’s low trigger value. Monitoring of Joondalup’s surface water in recent years has detected Hg exceeding guideline concentrations in winter months; while the point source is still unknown the annual spike suggests that the source is runoff from winter rains (Gonzalez-Pinto et al. 2017; Newport and Lund 2014).

Despite being outside the urban matrix, Yanchep National Park’s wetland Loch McNess had elevated concentrations of As, Ba, Cd, Cr, Co, Mo and Zn. In addition, Hg and Se were detected at trigger value concentrations higher (Table 3). Loch McNess is defined as a 'flow-through lake', receiving water from the groundwater system to which it was connected, potentially receiving contaminants from bordering agricultural land. Yet over the last two decades it has been suffering from a severe surface water and groundwater decline resulting in it's almost permeant disconnection from the groundwater (Department of Water 2011). As a result, large areas of lakebed sediment are now exposed and suffer from drying and erosion, then re-flooding with rain events. This process can release the sediments accumulated contaminants back into the wetland ecosystem (Al-Maarofi et al. 2013). Arsenic compounds are common and naturally occurring in many soils and wetlands of the Swan Coastal Plain (Appleyard et al. 2006), yet elevated concentrations in the sediment of Loch McNess could potentially be enhanced by groundwater contaminated from the historic use of pesticides on sheep (Arnold and Oldham 1997; Davis et al. 1993). Although Se is a necessary element for the normal development of organisms (Kapustka et al. 2004) it was detected at levels of concern in the sediments of Loch McNess (mean 4.04, max 7.9 mg/kg dry weight), and significantly higher concentrations than Bibra and Joondalup Lakes (Table 3). A point source cannot be determined but contamination might be from groundwater passing beneath agricultural irrigation and fertiliser use (Gardiner and Gorman 1963).

Only one sediment sample at one site (Bibra Lake) contained OCPs or PAHs, and the two detected OCPs and six out of seven PAHs exceeded sediment guidelines. The OCPs aldrin and its metabolite dieldrin have been banned from manufacture, importation, and use in Australia since the internationally legally binding agreement for OCPs (The Stockholm Convention on Persistent Organic Pollutants) was enacted in 2004 (DEH 2004; UNEP 2011). However, OCPs and their metabolites are highly persistent compounds that can be still present in the environment (Bai et al. 2015; Wu et al. 1999), and have been historically detected in the sediment of Perth's estuaries (Nice 2009) and wetlands (Davis et al. 1993). The presence of dieldrin and other OCPs detected in Perth's urban wetlands can be attributed to the state government program to control Argentine ants (from 1950s to 1980s) and periodic termite control (Davis and Froend 1999; Davis and Garland 1986). Bibra Lake and other urban wetlands of Perth have been subject to heavy pesticide treatment in the past in an attempt to control non-biting midge and mosquito levels (City of Cockburn 2015; Davis and Froend 1999). PAHs have historically been found in the sediments of Bibra Lake (Department of Water 2009); and six out of seven detected PAHs exceeded the SQG trigger values. The hypothesized source is used vehicle oil and machinery fluids. The sampling site of our sediment containing both OCPs and PAHs is within 50 meters down slope of a main road and may be frequently exposed to road pollution during rain events. Although this research only detected OCPs and PAHs in one sediment sample in one lake, the small sample sizes and large intra-site variation of contaminant concentrations between sediment samples should not rule out the possibility of OCPs and PAHs persisting in sediments at concentrations of concern in our other study sites or other wetlands of Perth.

Despite a high degree of intra-site variation in contaminant concentrations, generating knowledge on the sediment contaminant burden is important for highlighting the extent and history of wetland pollution (Förstner 2004). Generally, contaminants were in higher concentrations in the sediments of heavily urbanised compared to less-urbanised wetlands except for some metals in Loch McNess. The concentrations of Loch McNess sediments suggest that despite being outside of the urban matrix and surrounded by a protected National Park, contamination is still possible. High concentrations may originate from agriculture-contaminated groundwater that potentially contributes to wetland sediment contamination at levels comparable to highly urbanised wetlands. We recognise our number of sediment samples is both small and limited to the areas where snakes were captured, thus might not be an accurate representation of contamination of the entire wetlands. Nevertheless the samples do present a snapshot of contamination present in areas abundant with tiger snakes.

3.2 Occurrence of contaminants in snakes

Sixteen metals were detected in the livers of tiger snakes collected across Perth's wetlands in 2019 (discussed data and significant differences presented in Table 4). Beryllium wasn't analysed above detection limits and Sn was only above detection limits in a single liver from a snake captured at Lake Joondalup. Antimony, As, Ba, Cd, Co, Hg, Mo and Se concentrations were significantly different between sites, mostly higher at Herdsman Lake compared to Joondalup Lake. Lead was detected in only four snakes, three from Loch McNess and one from Herdsman Lake, and Sn was detected in only one snake from Lake Joondalup. Molybdenum concentrations in snakes from Herdsman Lake were significantly higher than in snakes at all other sites, and are as far as we can tell, the highest reported liver concentration in a reptile. These contaminants have all been documented in snakes before; however, the published literature has only one other report of a comprehensive suite of metals analysed in snakes (Wylie et al. 2009). Our study is the most comprehensive study to date on bioaccumulation in any terrestrial reptile in Australia. There were no significant differences between Cr, Mn, Ni, Ag and Zn liver concentrations between sites. No OCPs or PAHs were quantified above detection limits; however, both were detected from only one sediment sample at a single wetland, and PAHs are known to be metabolised quickly in vertebrates (Hylland 2006). In addition, due to small liver tissue mass (<10g) and broad-scale analysis detection limits had to be up to 2 mg/kg, thus we cannot conclude that these were not present under those concentrations. There is no quantitative toxicity thresholds available for OCPs and PAHs in reptiles; however, relative to other vertebrates toxicity results, <2 mg/kg is unlikely to induce adverse biological impact on snakes (Ball and Truskewycz 2013; Weir et al. 2013).

Currently, the information available on the bioaccumulation of contaminants and their effects on reptiles is a limited but is a growing research field. Although there are many studies reporting various contaminants in snakes (Albrecht et al. 2007; Burger et al. 2007; Burger et al. 2017; Campbell et al. 2005; Drewett et al. 2013; Heydari Sereshk and Riyahi Bakhtiari 2015; Quintela et al. 2019), we could only directly compare our data to seven metals (As, Cd, Cr, Hg, Mn, Pb, Se) commonly analysed as wet weight in the livers of three other wetland snakes *Nerodia fasciata*, *Thamnophis gigas*, *Agkistrodon piscivorus conanti* (see summary table in Wylie et. al. 2009 and (Hopkins et al. 1999; Rainwater et al. 2005; Wixon 2013)). Mean and maximum concentrations of these metals in Perth’s tiger snakes were similar to concentrations reported in other wetland snakes, besides As and Se that were much lower in Perth’s tiger snakes compared to other snakes from contaminated sites, and Perth’s tiger snakes had less than half the concentration of Mn and Hg reported in other wetland snakes regardless of site contamination. For the less frequently analysed metals we could only compare the liver concentrations of Perth’s tiger snakes to livers from *A. piscivorus* and *T. gigas*

(Wixon 2013; Wylie et al. 2009). Mean liver concentrations of Sb, Co, Ag and Sn were similar in tiger snakes compared to those reported in *A. piscivorus* and *T. gigas*, while Ba, Cu, Ni and Zn concentrations varied between the three species. Molybdenum was similar for all wetland snakes except for tiger snakes from Herdsman Lake, where it were much higher (mean 8.32, max 13.0 mg/kg wet weight).

Very few metals were positively correlated with body size except for Sb (rho 0.6, *p* 0.35), Hg (rho 0.87, *p* 0.05) and Ni (rho 0.8, *p* 0.13) in Herdsman Lake snakes; Sb (rho 0.6, *p* 0.35), Cr (rho 0.7, *p* 0.23) and Ag (rho 1, *p* 0.02) in Bibra Lake snakes; As (rho 0.8, *p* 0.13) in Lake Joondalup snakes; and Sb (rho 0.82, *p* 0.09) in Loch McNess snakes. We consider these results to be exploratory at best, and low significances is reflective of small sample sizes due to financial and ethical limitations. These results, however, suggest the uptake of most metals does not appear to be related to body size, a common observation in bioaccumulation research (Albrecht et al. 2007; Fontenot et al. 2000; Quintela et al. 2019). Although our study didn't detect any metals at alarmingly high concentrations, we identified nine metals of interest based on their significant difference between sites in the sediment or liver samples, and we consider these to be the contaminants of most concern. We use Fig. 2 to compare the inter-site differences in metal levels between sediment and liver concentrations. Antimony, Cd, Co, Mo and Se had almost identical inter-site patterns between the mean contaminant concentrations of the sediment and livers. Copper, As and Ba had mostly similar inter-site differences although there was high variation in the snake's livers. Mercury concentrations in snakes from Loch McNess reflected the concentrations in the sediment, although both had high inter-sample variation; concentrations were lower in snakes from Joondalup than the sediment and much higher in Herdsman Lake snakes than the sediment. Lead and Sn showed generally inverse inter-site relationships between sediment and snake liver concentrations. Despite Zn exceeding the SQG higher trigger value in sediment from Herdsman Lake, it was found at consistent concentrations in Perth's tiger snake livers as it is metabolically regulated in vertebrates (Sandstead 2014).

The intra-site variation in contaminant concentration was generally higher in sediment than it was in snake livers (Tables 3 & 4). Variation is naturally high in sediment due to different point sources of contaminants, seasonal changes of water chemistry and soil grain-sizes. Biological tissues such a livers are more homogenous in composition than sediments and consequently show a lesser inter-individual and inter-site variability in contaminant burdens than sediments do, thus the use of fauna as bioindicators of environmental health. The use of wetland snakes as bioindicators has been proposed for decades (Campbell and Campbell 2001; Haskins et al. 2019; Stafford et al. 1977) based on their trophic status and site fidelity (Campbell et al. 2005). Despite limited positive correlations between body size and metals Fig. 2 suggests there is a strong relationship between sediment and snake liver concentrations within sites. These results are consistent with the general pattern of fauna contaminated by metals increases if the population is exposed to a source of contamination (Nasri et al. 2017), in this case the sediment. Therefore, our results support the use of tiger snakes as a bioindicators of wetland ecosystem health based on (1) the evidence that they accumulate a range of metals, (2) their long life expectancy of up to 24 years (Fearn and Norton 2011), (3) their small home ranges (~0.05 km^2) (Butler et al. 2005) especially in wetlands isolated by urbanisation, and (4) their high trophic position in the food chain as adults (thus being exposed to all of their prey's contaminants).

Tiger snakes are directly exposed to the contaminants in their wetland through drinking the water, foraging in sediment (Orange 2007) when wetland waters annually recede, and through the consumption of their prey. Frogs, being tiger snakes' preferred prey (Aubret et al. 2006; Lettoof et al. 2020), are particularly sensitive to contaminant accumulation as they feed in sediment as tadpoles, have the ability to absorb chemicals through their skin (Bruhl et al. 2011; Smalling et al. 2015), and stay in close proximity to waterbodies (eg. ~20 m for *Litoria raniformis*), especially in urban isolated wetlands (Hamer and Organ 2008; Hitchings and Beebee 1997). Lemaire *et al.* (2018) compared the Hg concentrations in scales of various populations of viperine snakes (*Natrix maura*) that predate on frogs or on aquaculture-raised fish, and found lower mean contaminant concentrations and accumulation rates in frog-eating snake populations. This difference may be due to frogs generally being at a lower trophic tier than fish and potential Hg contamination from the aquaculture farms from which snakes were sampled (Lemaire et al. 2018). We believe frogs are an important vector for contaminants in the food web, and recommend testing them to better understand the food chain transfer of contaminants in Perth's urban wetlands, a research area lacking in Australia (Mann et al. 2009; Sievers et al. 2019). Due to financial limitations and the wetlands being inhabited by several species of frogs we chose to investigate bioaccumulation in tiger snakes as they should reflect the contamination of that particular food web.

In addition, tiger snakes may have a shorter lifespan in urban wetlands as a result of exposure to multiple stressors and therefore may not have the opportunity to accumulate large concentrations of contaminants. Such stressors include harassment from the public, pet dogs, vegetation management and parasitism, as tiger snakes often have large burdens of gastric nematodes (Lettoof et al. 2020). Although the high frequency of parasitism suggests tolerance, the influence of contaminants and parasites on tiger snake populations may be more complex. Chronic symptoms from synergistic contaminants may result in immunosuppression and increasing nematode parasitism (Rohr et al. 2008), or a reduction in feeding behaviour (Alonso et al. 2009; Forrow and Maltby 2000) which may imperil individual snakes who cannot consume enough food to feed both themselves and the nematodes. From another point of view, a large parasite burden may have beneficial impacts on tiger snakes, as parasites are able to uptake contaminants directly and reduce the concentrations in their host (Evans et al. 2001).

3.3 Potential impacts to snakes

As with other high trophic predators, the presence and persistence of higher trophic snakes represents a healthy ecosystem, and yet ecotoxicological research on snakes is still relatively rare compared to research on other predators (Campbell and Campbell 2001). Snakes can provide crucial insight into the distribution and movement of contaminants through wetland food webs in comparison to most other top predators (usually mammals and birds) based on several characteristics: entirely carnivorous, comparatively small home ranges, longevity and progression up trophic tiers throughout their life history. Six metals, two OCPs and seven PAHs of the 52 screened contaminants were detected at concentrations of concern in the sediment of wetlands across Perth; and of the 16 metals detected in tiger snakes from these wetlands, nine were of significantly different concentrations between sites. Although we did not detect very high concentrations of metals that may cause acute toxicity in tiger snakes, the generally higher concentrations of most tested contaminants in snakes from the most urbanised wetland, Herdsman Lake, may have synergistic chronic effects on snake health. Only a handful of studies have been conducted on the acute or chronic impacts metal exposure and accumulation has on reptiles. For example, the presence of blood Hg concentrations have been found to negatively correlate with lymphocyte and B-cell proliferation in Loggerhead sea turtles (*Caretta caretta*), suggesting suppression of the immune system (Day et al. 2007). Lead and Cd, both detected in snake livers at higher concentrations in Loch McNess and Herdsman Lake than other sites, have reported lethal doses. The approximate concentration of Pb and Cd required to kill western fence lizards (*Sceloporus occidentalis*) is 2000 mg/kg and 1480 mg/kg of body weight respectively (Brasfield et al. 2004; Salice et al. 2009), which is considered mid-range sensitivity when compared to other taxa. The concentrations we detected in Perth's tiger snakes were much lower than these and are unlikely to have acute clinical impacts on the populations.

Experimental exposure of several metals to reptiles has received more interest. Dietary exposure to Se, for example, can lower reproduction likelihood and potentially reduce egg and total mass of clutches in brown house snakes (*Boaedon fuliginosus*) (Hopkins et al. 2004); reduce average food intake and growth in body mass and length in leopard geckos (*Eublepharus macularius*) (Rich and Talent 2009). Banded water snakes (*N. fasciata*) from polluted wetlands with high liver concentrations of As and Se (mean As: 134 ppm; Se 140 ppm dry weight) exhibited mean standard metabolic rates 32% higher than snakes from unpolluted sites (Hopkins et al. 1999). Corn snakes (*Elaphe guttata*) feed methylmercury experienced a reduced growth rate (Bazar et al. 2002), while new-born northern water snakes (*N. sipedon*) contaminated with maternally-transferred Hg had less motivation to feed and had reduced strike efficiency (Chin et al. 2013). Developing embryos can also be exposed to metals through the egg shell (Marco et al. 2004). Consequently, absorption of Cd can cause malformation of eye development in the Italian wall lizard (*Podarcis sicula*) (Simoniello et al. 2014) and the running speed of hatchling Iberian rock lizard (*Iberolacerta monticola*) was negatively correlated with embryonic As absorption (Marco et al. 2004). Metal body burdens or exposure levels that cause physiological or behavioural alterations in the aforementioned studies are well above what we observed in the livers of our adult tiger snakes from the Swan Coastal Plain; however, chronically contaminated reptiles are susceptible to a variety of pernicious developmental and behavioural disorders. Snakes from the urban sites Herdsman and Bibra Lake seem to be in poor health conditions (observation by the authors), which parallels sediment and liver contaminant levels. We have not as part of the present study measured behavioural parameters or biochemical markers of health however complimentary studies are initiated to investigate potential health impacts of contaminants on tiger snakes (Lettoof et al, in prep).

Interestingly, tiger snakes from Herdsman Lake had the highest mean liver concentration of Mo reported in a reptile (8.32 ± 3.458 mg/kg). Molybdenum is an essential element for vertebrate nutrition, however, excessive exposure to laboratory rats, guinea pigs and rabbits has been associated with anaemia, diarrhoea, deformities of joints and long bones, mandibular exostoses and morphological changes to the liver, kidneys and spleen (Tallkvist and Oskarsson 2015). Molybdenum exists naturally in various oxidation states but is mined as a principle ore for use as an alloy in steel and cast iron production, and also used in lubricants, chemical reagents and dyes. Stormwater drainage and urban runoff from the bordering industrial area are likely point-sources for Mo contamination in Herdsman Lake.

3.4 Future research and recommendations

Laboratory testing for ecotoxicology is expensive and usually charged per contaminant (for example, the cost of this study was over $11 000 AUD). This warrants a trade-off between broad-scale screening with small sample sizes, or targeted screening focussing on contaminants of concern and much larger samples. Most other studies choosing the latter had contamination spill events or access to published recent monitoring data allowing a focus on contaminants of concern. Despite the study sites being recognised as critical wetlands for the environment, major tourist and local attractions, and situated throughout a developed country's major city, contaminant monitoring of these wetlands has largely been disregarded; and as a result sample sizes were sacrificed for broad-scale screening. Periodic monitoring of contaminants should be conducted in urban wetlands to prevent researchers from investing limited resources on broad-scale screening and rather focus on contaminants of concern.

Ecotoxicological research on reptiles (especially snakes) is minimal at present, and fundamentally non-existent in Australia, despite consistent evidence of wetland snakes in the United States and other countries being good bioindicators of environmental contamination (Burger et al. 2007; Drewett et al. 2013; Lemaire et al. 2018; Quintela et al. 2019; Wixon 2013). Australia has several major cities with snakes persisting in urban wetlands or estuaries which should be susceptible to bioaccumulation of contaminants, and would be suitable systems to model the ecotoxicological impacts on snakes. Other model species include: the red-bellied blacksnake (*Pseudechis porphyriacus*), lowlands copperhead (*Austrelaps superbus*), common tree snakes (*Dendrelaphis punctulatus*), slaty-grey snake (*Stegonotus australis*), keelback (*Tropidonophis mairii*), Australian bockadam (*Cerberus australis*), and Richardson's mangrove snake (*Myron richardsonii*).

4. Conclusions

This study conducted a broad-scale analysis for 52 contaminants in sediment samples of four wetlands spanning across an urban gradient of Perth, Australia. We detected six metals, two OCPs and six PAHs exceeding SQG trigger values in sediments, with generally higher mean concentrations of contaminants found in more heavily urbanised wetlands. The natural site, Loch McNess, despite being the least modified and furthest from urbanisation was an exception, as the sediments contained second highest concentrations for 8 of the 16 of the metals analysed. We hypothesize the source of contamination in Loch McNess is from inflowing groundwater contaminated by radial agricultural land, specifically the historic use of pesticides and fertilisers.

Arsenic, Cd, Co, Hg, Mo, Sb and Se measured in the livers of tiger snakes paralleled the pattern of contamination measured in the wetland sediments where snakes were collected, and should be the focus of future research. Hence we propose the use of high trophic wetland snakes, such as tiger snakes, as bioindicators of wetland contamination. Although these contaminants may not be the only ones accumulating in tiger snakes (e.g. other environments might have other persistent contaminants), we present the first data on these particular ecosystems. The potential impacts of these metal mixtures is unknown; however, research is currently being conducted on the physical and biochemical markers of health in tiger snakes at these wetlands (Lettoof et al., in prep.). Despite wetland degradation related to urbanisation, urban wetlands often provide the last refuge for a wide diversity of wildlife species including higher trophic snakes.

Although our data are limited we chose to sacrifice sample size to allow screening of more contaminants, and quantify both environment and predator contaminants. Therefore this study presents a snapshot of the first broad-scale contaminant screening of an Australian snake, and one of few studies, to our knowledge, that complements contaminants in snakes with contaminants in wetland sediment. It was also the first detailed investigation of contaminants in four iconic wetlands of a capital city in a developed country, reinforcing urgent government monitoring of urban wetland pollution to inform environmental management on actions required to preserve these highly productive and biodiverse habitats.

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Table 1: Brief description and history of contaminants detected within the wetland sites used for study. Area was calculated using Google Earth Pro polygon tool based on the most recent aerial photographs. Sites are listed in order of most urbanised to least.

Site	Area (km^2)	Brief description of urbanisation	References	Contaminants detected (S = sediment, W = water)	References
Herdsman Lake (HL) 31° 55'12 S, 115° 48'19 E	3.07	• Heavily modified; • since 1850s has been subject to agriculture, industrial dumping, dredging and storm water inflow; • located among heavy urbanisation	(Clarke et al. 1990; Gentilli and Bekle 1993; Kobryn 2001)	(**W**) Cr, Cu, Pb, Ni, Zn, Fe, Mn	(ESRI 1983)
				(**W**) Cd, Cu, Pb, Zn; (**W**) Dieldrin, heptachlor	(Clarke et al. 1990)
				(**S**) Cu, Zn, As; (**S**) Aldrin, Chlordane, dieldrin, DDT	(Davis et al. 1993)
				(**W**) Cd, Cu, Pb, Zn	(Kobryn 2001)
				(**W**,**S**) Al, Cu, Pb, Zn, As; (**S**) PCBs, PHCs	(Department of Water 2009)
Bibra Lake	1.93	• Partially modified;	(Sinang et al. 2015)	(**S**) Dieldrin	(Davis et al. 1993)

(BI) 32^{o} 5’32 S, 115^{o} 49’27 E		• some fringe is urbanised; • located among heavy urbanisation		(**W**) Cu, Pb, Zn (**W,S**) Al, Cr, Cu, Pb, Zn; (**S**) PHCs, PAHs	(Burkett 2005) (Department of Water 2009)
Lake Joondalup (JL) 31^{o} 45’34 S, 115^{o} 47’33 E	7.33	• Partially modified; • some fringe is urbanised; • located on edge of recent urbanisation	(Congdon 1986; Gonzalez-Pinto et al. 2017)	(**S**) As (**W**) Al, As, Hg, Zn (**W**) Al, Cd, Hg, Zn	(Davis et al. 1993) (Newport and Lund 2014) (Gonzalez-Pinto et al. 2017)
Loch McNess (LM) 31^{o} 32’44 S, 115^{o} 40’50 E	0.36	• Minimally modified; • surrounded by Yanchep National Park; • located outside of urbanisation	(Department of Water 2011)	(**S**) As (**W**) As, B, Cr, Mn, Ni	(Davis et al. 1993) (Department of Water 2011)

Table 2. Morphological measurements (mean ± one SE (range)) of tiger snakes (*Notechis scutatus occidentalis*) collected at each site.

Site	Sex (*n*)	SVL (mm)	Body mass (g)	Wet liver mass (g)
Herdsman Lake	Male (4)	855 ± 12.6 (825 – 877)	250 ± 15.15 (216 – 282)	5.2 ± 0.6 (4.0 – 6.3)
	Female (1)	776	277	3.9
Bibra Lake	Male (4)	807 ± 21.9 (746 - 847)	283 ± 20.2 (233 – 329)	9.1 ± 1.2 (6.6 – 11.7)
	Female (1)	704	212	4.8
Lake Joondalup	Male (4)	779 ± 15.5 (735 - 807)	221 ± 26.6 (171 – 296)	7.4 ± 0.8 (6.8 – 9.3)
	Female (1)	706	205.6	11.1
Loch McNess (Yanchep)	Male (3)	847 ± 43.6 (760 – 899)	294 ± 44.2 (235 – 381)	11.2 ± 2.0 (7.3 – 13.5)

Female (2)	719 ± 47.5	194 ± 14.4	4.2 ± 0.9
	(671 – 766)	(180 -208)	(3.2 – 5.1)

SVL: snout-vent length

Figure 1. Wetlands in the Swan Coastal Plain where sediments and tiger snakes were collected for contaminant analyse. Red dots indicate collection points of sediment samples, yellow circles indicate tiger snakes collection areas. HL = Herdsman Lake, BI = Bibra Lake, JL = Lake Joondalup, LM = Loch McNess (located in Yanchep National Park). Satellite images were obtained from Google Earth Pro in 2019. Scale bar = 500m.

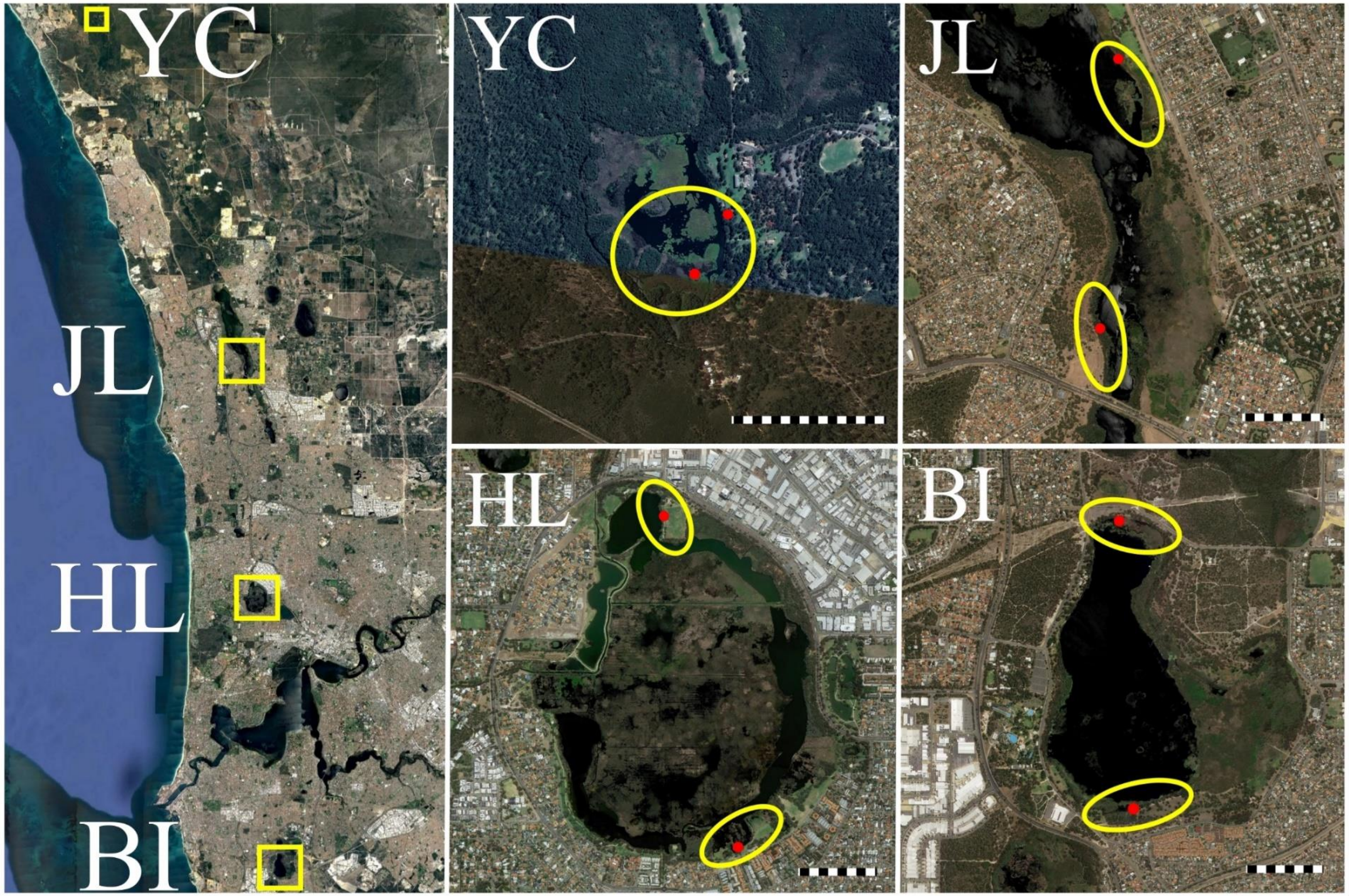


Table 3. Concentrations (mg/kg, dry weight) of contaminants detected in sediments. Given as: mean ± one SE (range). Concentrations in bold indicate values higher than the ANZECC sediment quality guideline value (SQGV).

Contaminant	Herdsman Lake	Bibra Lake	Lake Joondalup	Loch McNess	ANZECC SQGV (mg/kg, dry weight)
Aldrin	<0.01	**0.14** ± 0.13 (BDL – **0.53**)	<0.01	<0.01	SQGV: $0.002^{\#}$ SQG-High: NA
Dieldrin	<0.01	**0.65** ± 0.65 (<0.01 – **2.60**)	<0.01	<0.01	SQGV: 0.12 SQG-High: 0.27
Phenanthrene	<0.5	**0.49** ± 0.24 (<0.5 – **1.20**)	<0.5	<0.5	SQGV: 0.24 SQG-High: 1.5
Fluoranthene	<0.5	**0.86** ± 0.61 (<0.5 – **2.70**)	<0.5	<0.5	SQGV: 0.6 SQG-High: 5.1
Pyrene	<0.5	**1.41** ± 1.17	<0.5	<0.5	SQGV: 0.665

		(<0.5 – **4.90**)			SQG-High: 2.6
Benz(a)anthracene	<0.5	**0.66** ± 0.42 (<0.5 – **1.90**)	<0.5	<0.5	SQGV: 0.261 SQG-High: 1.6
Chrysene	<0.5	**1.09** ± 0.84 (<0.5 – **3.600**)	<0.5	<0.5	SQGV: 0.384 SQG-High: 2.8
Benzo(a)pyrene	<0.5	**0.69** ± 0.44 (<0.5 – **2.00**)	<0.5	<0.5	SQGV: 0.43 SQG-High: 1.6
Benzo(g,h,i)perylene	<0.5	0.44 ± 0.92 (<0.5 – 1.00)	<0.5	<0.5	SQGV: NA SQG-High: NA
Antimony	0.77 ± 0.03[a,b] (0.68 – 0.83)	0.23 ± 0.5 (0.10 – 0.32)	0.18 ± 0.04[a] (<0.05 – 0.15)	0.20 ± 0.05[b] (<0.05 – 0.06)	SQGV: 2 SQG-High: 25

Arsenic	**20.78** ± 6.54[a]	1.80 ± 0.35	0.88 ± 0.17[a]	7.12 ± 3.50	SQGV: 20
	(9.10 – **34.00**)	(1.20 – 2.80)	(0.40 – 1.20)	(0.60 – 15.00)	SQG-High: 70
Barium	91.75 ± 30.10	85.50 ± 9.32	31.00 ± 9.19	86.75 ± 49.74	SQGV: NA
	(24.00 – 160.00)	(65.00 – 110.00)	(18.00 – 58.00)	(11.00 – 230.00)	SQG-High: NA
Beryllium	0.18 ± 0.12	0.22 ± 0.07	0.04 ± 0.01	0.08 ± 0.04	SQGV: NA
	(<0.05 – 0.28)	(0.09 – 0.41)	(<0.05 – 0.06)	(<0.05 – 0.16)	SQG-High: NA
Cadmium	0.14 ± 0.06	0.14 ± 0.03	0.06 ± 0.02	0.26 ± 0.19	SQGV: 1.5
	(<0.05 – 0.28)	(0.07 – 0.22)	(<0.05 – 0.10)	(<0.05 – 0.80)	SQG-High: 10
Chromium	13.43 ± 2.99	11.23 ± 2.88	5.73 ± 1.24	20.45 ± 8.37	SQGV: 60
	(8.70 – 22.00)	(6.10 – 19.00)	(3.60 – 8.60)	(2.80 – 38.00)	SQG-High: 370
Cobalt	2.23 ± 0.31[a]	0.70 ± 0.14	1.20 ± 0.07[a]	1.40 ± 0.64	SQGV: NA
	(1.70 - 3.10)	(0.40 – 1.10)	(0.10 – 0.40)	(0.20 – 2.90)	SQG-High: 50[#]

Copper	**76.50** ± 45.07^{a} (20.00 – **210.00**)	7.40 ± 1.25 (3.90 – 9.80)	5.05 ± 1.54^{a} (0.50 – 6.90)	10.87 ± 3.69 (3.10 – 19.00)	SQGV: 65 SQG-High: 270
Lead	43.75 ± 8.84^{a,b} (31.00 – **69.00**)	20.50 ± 1.66 (18.00 – 25.00)	14.08 ± 3.57^{a} (6.30 – 23.00)	9.20 ± 3.96^{b} (1.50 – 17.00)	SQGV: 50 SQG-High: 220
Manganese	68.75 ± 23.26 (25.00 – 120.00)	30.08 ± 8.56 (6.30 – 47.00)	26.67 ± 17.18 (7.20 – 78.00)	20.07 ± 10.38 (2.30 – 50.00)	SQGV: 460$^{\#}$ SQG-High: 1100$^{\#}$
Mercury	0.05 ± 0.01 (0.01 – 0.07)	0.05 ± 0.005 (0.04 – 0.06)	0.14 ± 0.08 (0.01 – **0.35**)	0.10 ± 0.05 (0.01 – **0.21**)	SQGV: 0.15 SQG-High: 1
Molybdenum	1.19 ± 0.27^{a} (0.65 – 1.7)	0.49 ± 0.09 (0.27 – 0.71)	0.16 ± 0.07^{a} (0.07 – 0.37)	0.88 ± 0.39 (0.19 – 1.7)	SQGV: NA SQG-High: NA
Nickel	7.48 ± 1.18^{a}	3.80 ± 0.59	0.55 ± 0.16^{a}	2.55 ± 0.99	SQGV: 21

	(6.00 – 11.00)	(2.50 – 5.30)	(0.30 – 1.00)	0.50 – 4.60	SQG-High: 52
Selenium	0.59 ± 0.14 (0.38 – 0.97)	0.96 ± 0.36^{a} (0.45 – **2.00**)	0.13 ± 0.29^{a,b} (0.08 – 0.21)	**4.04** ± 2.14^{b} (0.23 – **7.90**)	SQGV: 2$^{\#}$ SQG-High: 4$^{\#}$
Silver	0.09 ± 0.01 (0.07 – 0.11)	<0.05	<0.05	<0.05	SQGV: 1 SQG-High: 3.7
Tin	8.10 ± 5.31^{a} (2.00 – 24.00)	1.00 ± 0.14 (0.60 – 1.30)	<0.5	0.60 ± 0.21^{a} (<0.5 – 1.10)	SQGV: NA SQG-High: NA
Zinc	**221.00** ± 105.15^{a} (41.00 – **510.00**)	60.50 ± 14.91 (18.00 – 84.00)	12.12 ± 5.57^{a,b} (<5.00 – 28.00)	31.00 ± 12.57^{b} (11.00 – 67.00)	SQGV: 200 SQG-High: 410

Lower-case letter indicates Kruskal-Wallis significant difference (*p* = <0.05) between sites. $^{\#}$ = alternative guidelines (Lemly, 1996; Ontario Ministry of Environment and Energy, 1993).

Table 4. Concentrations (mg/kg, wet weight) of metals detected in tiger snake livers. *N* = 5 per site. Contaminants analysed at lower than detectable limits were not included. Given as: mean ± one SE (range); BDL = below detectable limits.

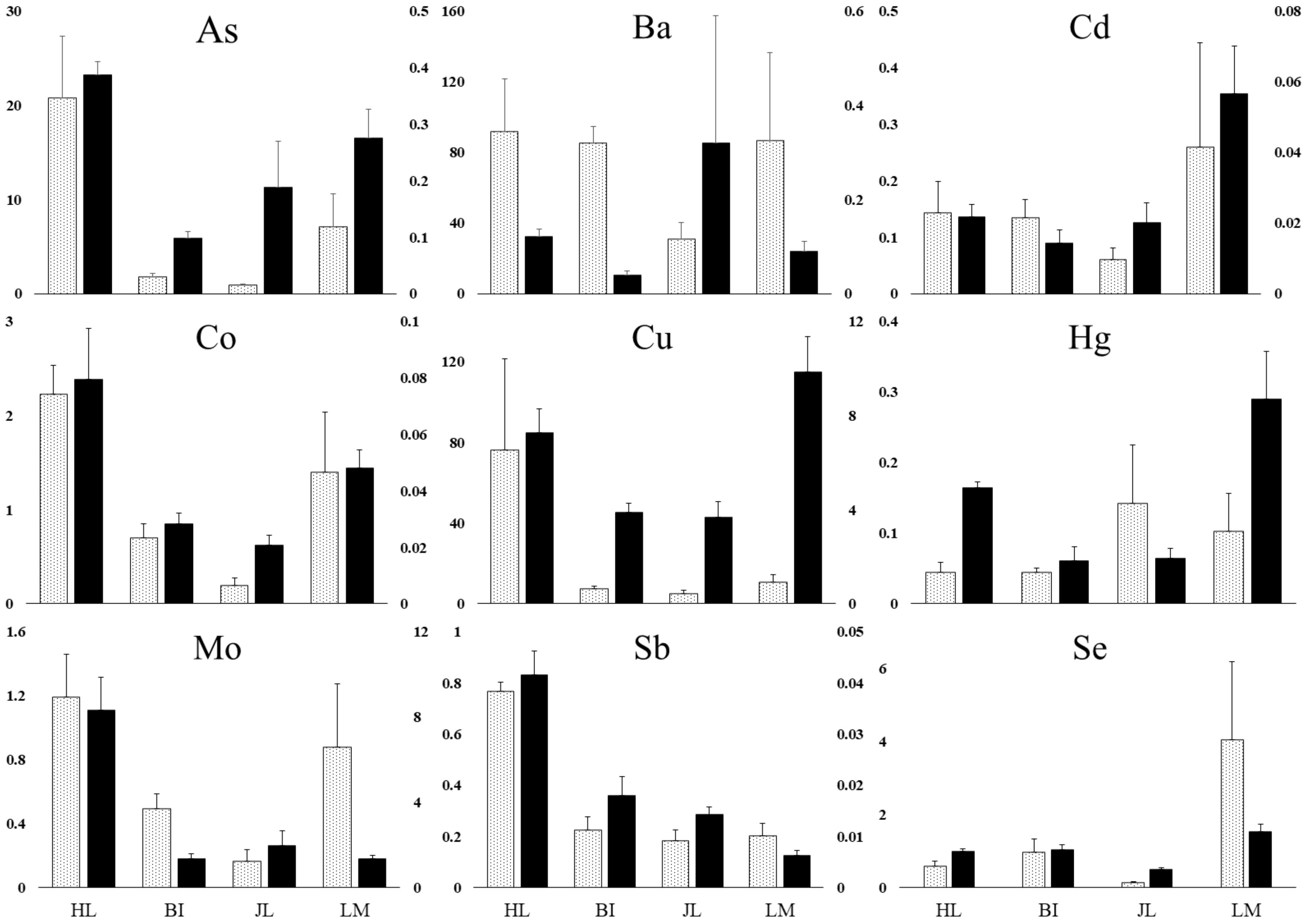
Contaminant	Herdsman Lake	Bibra Lake	Lake Joondalup	Loch McNess
Antimony (Sb)	0.042 ± 0.005^{a}	0.018 ± 0.004	0.014 ± 0.002	0.006 ± 0.001^{a}
	(0.025 – 0.052)	(0.007 – 0.03)	(0.010 – 0.019)	(0.003 – 0.008)
Arsenic (As)	0.388 ± 0.023^{a}	0.098 ± 0.012^{a}	0.188 ± 0.082	0.276 ± 0.049
	(0.33 – 0.45)	(0.06 – 0.13)	(0.05 – 0.5)	(0.18 – 0.44)
Barium (Ba)	0.122 ± 0.015^{a}	0.039 ± 0.009^{a}	0.32 ± 0.270	0.089 ± 0.023
	(0.09 – 0.16)	(<0.05 – 0.06)	(<0.05 – 1.4)	(0.025 – 0.140)
Cadmium (Cd)	0.023 ± 0.004	0.015 ± 0.004^{a}	0.02 ± 0.006	0.057 ± 0.013^{a}
	(0.014 – 0.035)	(<0.001 – 0.025)	(0.006 – 0.039)	(0.025 – 0.1)
Chromium (Cr)	0.106 ± 0.016	0.123 ± 0.037	0.137 ± 0.050	0.068 ± 0.021
	(0.06 – 0.15)	(<0.05 – 0.25)	(<0.05 – 0.28)	(<0.05 – 0.13)

Cobalt (Co)	$0.080 \pm 0.018^{a,b}$	0.028 ± 0.004^{a}	$0.021 \pm 0.003^{b,c}$	0.048 ± 0.006^{c}
	(0.054 – 0.15)	(0.019 – 0.041)	(0.013 – 0.031)	(0.032 – 0.064)
Copper (Cu)	$7.28 \pm 1.006^{a,b}$	$3.9 \pm 0.376^{a,c}$	$3.68 \pm 0.676^{b,d}$	$9.84 \pm 1.518^{c,d}$
	(4.9 – 9.8)	(3.1 – 5.2)	(2.4 – 5.9)	(6.6 – 15.0)
Lead (Pb)	0.007 ± 0.004	<0.05	<0.05	0.047 ± 0.028
	(<0.05 – 0.024)			(<0.05 – 0.15)
Manganese (Mn)	0.83 ± 0.048	0.75 ± 0.018	0.664 ± 0.051	0.848 ± 0.152
	(0.7 – 0.94)	(0.70 – 0.79)	(0.49 – 0.76)	(0.54 – 1.4)
Mercury (Hg)	$0.164 \pm 0.009^{a,b}$	$0.061 \pm 0.02^{a,c}$	$0.064 \pm 0.014^{b,d}$	$0.29 \pm 0.068^{c,d}$
	(0.14 – 0.19)	(BDL – 0.12)	(0.01 – 0.09)	(0.14 – 0.39)
Molybdenum (Mo)	$8.32 \pm 1.547^{a,b,c}$	1.36 ± 0.214^{a}	1.98 ± 0.666^{b}	1.36 ± 0.144^{c}

	(4.8 – 13.0)	(0.6 – 1.9)	(0.7 – 4.2)	(1.0 – 1.8)
Nickel (Ni)	0.275 ± 0.121	0.372 ± 0.100	0.42 ± 0.139	0.218 ± 0.046
	(<0.01 – 0.64)	(0.03 – 0.61)	(0.05 – 0.91)	(0.05 – 0.32)
Selenium (Se)	0.988 ± 0.061	1.03 ± 0.139	0.49 ± 0.058^{a}	1.53 ± 0.208^{a}
	(0.84 – 1.2)	(0.67 – 1.4)	(0.34 – 0.66)	(0.95 – 2.2)
Silver (Ag)	0.014 ± 0.002	0.007 ± 0.002	0.006 ± 0.002	0.008 ± 0.001
	(0.007 – 0.017)	(0.002 – 0.012)	(0.002 – 0.01)	(0.004 – 0.011)
Tin (Sn)	<0.05	<0.05	0.058 ± 0.033	<0.05
			(<0.05 – 0.19)	
Zinc (Zn)	24 ± 2.168	21.4 ± 1.600	21.6 ± 1.077	24.6 ± 1.721
	(20.0 – 32.0)	(17.0 – 27.0)	(19.0 – 24.0)	(21.0 – 31.0)

Lower-case letter indicates Kruskal-Wallis significant difference (p = <0.05) between sites.

Figure 2. The comparison between metals that were statistically significant in either the sediment or tiger snake livers between sites. Inter-site significant differences are listed in Tables 3 and 4. Left y axis represents sediment concentration and right y axis represents liver concentration, except for Hg and Se that share the same range. HL = Herdsman Lake, BI = Bibra Lake, JL = Lake Joondalup, LM = Loch McNess (located in Yanchep National Park). Patterned bars = mean sediment concentration; black bars = mean snake liver concentration. Sediment concentration corresponds to the left axis and liver to the right axis. Error bars = SE.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30
20
10
0
0.5
0.4
0.3
0.2
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Ba
160
120
80
40
0
0.6
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Cd
0.5
0.4
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0.08
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Co
3
2
1
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Cu
120
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Hg
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Sb
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HL
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