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Bioaccumulation and metabolic impact of environmental PFAS residue on wild-caught urban wetland tiger snakes (*Notechis scutatus*)

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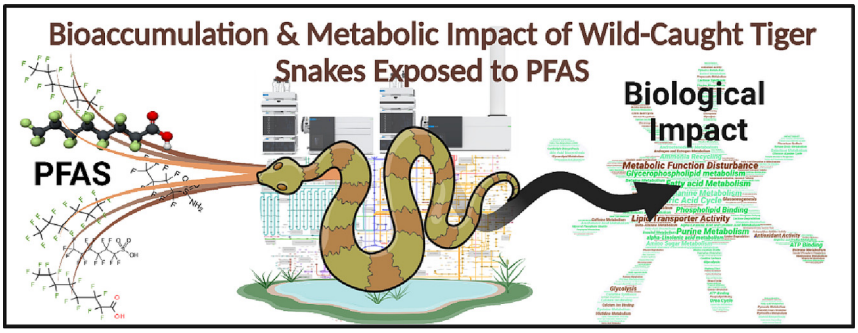
HIGHLIGHTS

- Liver PFAS concentrations were associated with lower snake body condition.
- Highest reported liver PFAS concentration in reptile and Australian vertebrate.
- Energy production pathways impacted in muscle tissues of PFAS-exposed snakes.
- PFAS liver concentrations were higher in males when compared to females snakes.

GRAPHICAL ABSTRACT



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ABSTRACT

PFAS contamination of urban waters is widespread but understanding the biological impact of its accumulation is limited to humans and common ecotoxicological model organisms. Here, we combine PFAS exposure and bioaccumulation patterns with whole organism responses and omics-based ecosurveillance methods to investigate the potential impacts of PFAS on a top predator of wetlands, the tiger snake (*Notechis scutatus*). Tiger snakes (18 male and 17 female) were collected from four wetlands with varying PFAS chemical profiles and concentrations in Perth, Western Australia. Tiger snake livers were tested for 28 known PFAS compounds, and Σ28PFAS in liver tissues ranged between 322 ± 193 μg/kg at the most contaminated site to 1.31 ± 0.86 μg/kg at the least contaminated site. The dominant PFAS compound detected in liver tissues was PFOS. Lower body condition was associated with higher liver PFAS, and male snakes showed signs of high bioaccumulation whereas females showed signs of maternal offloading. Biochemical profiles of snake muscle, fat (adipose tissue), and gonads were analysed using a combination of liquid chromatography triple quadrupole (QqQ) and quadrupole time-of-flight (QToF) mass spectrometry methodologies. Elevated PFAS was associated with enriched energy production and maintenance pathways in the muscle, and had weak associations with energy-related lipids in the fat tissue, and lipids associated with cellular genesis and spermatogenesis in the gonads. These findings demonstrate the bioavailability of urban wetland PFAS in higher-order reptilian predators and suggest a negative impact on snake health and metabolic processes. This research expands on omics-based ecosurveillance tools for informing mechanistic toxicology and contributes to our understanding of the impact of PFAS residue on wildlife health to improve risk management and regulation.

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1. Introduction

Poly- and perfluoroalkyl substances (PFAS) are a known contaminant class of concern that are pervasive in most aquatic environments (Podder et al., 2021). Compared to aquatic invertebrates and small fish, relatively high concentrations of PFAS (i.e., the common and well-studied perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) constituents) are required to induce acute toxicity (i.e., mortality) in larger vertebrates such as reptiles, mammals, birds, etc. Instead, environmentally relevant concentrations of PFAS tend to pose sublethal health effects and result in metabolic perturbations (Ankley et al., 2021; Sinclair et al., 2020). For example, chronic PFAS exposure has been shown to interfere with the growth, development, or body weight of amphibians (Flynn et al., 2021; Hoover et al., 2017), birds (Newsted et al., 2008), fish (Suski et al., 2021), mammals (Martin et al., 2007) and reptiles (Beale et al., 2022a; Furst et al., 2019; Zhang et al., 2020). While the mechanisms and pathways of PFAS toxicity are still poorly understood, PFOS and PFOA have been repeatedly shown to influence nuclear receptors involved in lipid metabolism in fish (Cheng et al., 2016; Yang et al., 2014) and in mammals (Seacat et al., 2002; Tan et al., 2012), and the genes involved in lipid metabolism in birds (O'Brien et al., 2011) suggests PFAS may exhibit a common trend of disrupting lipid modulation in these organisms. The uptake, internal distribution, and toxicokinetics of PFAS, however, are dependent on the organism and chemical-specific attributes (Abercrombie et al., 2021; Ankley et al., 2021). As such, the toxicological effects and ecological risk from PFAS for most species is still unknown (or any other chemical contaminant or contaminant mixture).

Like most top predators, higher trophic-tier wetland snakes are susceptible to accumulating anthropogenic contaminants (Gerke et al., 2020; Heinz et al., 1980; Lettoof et al., 2020a). Snakes have relatively long lifespans, small home ranges, a multi-trophic tier life history and low metabolic rates; consequently, the use of snakes as suitable bioindicators of environmental pollution is gaining momentum (Haskins et al., 2021; Lettoof et al., 2020b; Lettoof et al., 2021b; Wu et al., 2020). Investigations of PFAS exposure, bioaccumulation and toxicity in reptiles is limited (Ankley et al., 2021; Bangma et al., 2019; Beale et al., 2022a; DeWitt et al., 2012; Wang et al., 2013); and published PFAS contamination in snakes has only been investigated in the context of an indigenous food source (Food Standards Australia New Zealand, 2018) as opposed to understanding biological impact. Although reptiles are beginning to receive more consideration for ecotoxicological assessments (Chen et al., 2019; dos Santos et al., 2021; Hopkins, 2000), their low metabolism and the relatively limited understanding of their dynamic physiology can result in studies failing to detect toxicological susceptibility (Cunningham et al., 2021; Finger et al., 2016; Pauli et al., 2010; Weir et al., 2010) and thus reptiles as a taxon present a challenge when trying to assess sublethal impacts from chronic contamination.

Metabolomics—the abundance measurement of hundreds of metabolites in a tissue—has shown to be a sensitive tool in ecotoxicological or environmental impact assessments (Beale et al., 2022b; Hines et al., 2010; Malinowska and Viant, 2019; Sinclair et al., 2019). By screening a suite of biomolecules, metabolomics can identify the molecular responses to chemicals and help determine the mechanistic pathways of toxicity which may not be detected when assessing traditional physical or physiological parameters. Further, by combining targeted and untargeted metabolomic (i.e., polar metabolites) and lipidomic (i.e., non-polar metabolites/lipids) datasets with quantitative bioaccumulated PFAS measurements, we can begin to understand system-wide performance or disruption—contributing towards the transition from static environmental monitoring metrics towards holistic omics-based ecosurveillance approaches (Beale et al., 2022b). In this study, we quantified the concentrations of 28 PFAS in surface waters of four wetlands in Perth, Western Australia, and in the livers of resident western tiger snakes (*Notechis scutatus occidentalis*). In order to test the hypothesis that PFAS exposure impacts snake health and metabolic processes, we assessed the patterns of total PFAS exposure in snakes and its impact on body condition coupled to the identified biochemical response of total PFAS exposure in these wild snakes using metabolomics and lipidomics.

2. Materials and methods

2.1. Ethics

Tiger snakes were humanely euthanised as per Curtin University Animal Ethics Committee approval ARE2020–15. Snakes were collected under the Department of Biodiversity, Conservation and Attractions permit no. 08–002624-02. Samples herein were obtained from the preserved cadavers stored at -20 °C for up to two months.

2.2. Study sites and species

Western tiger snakes are ~1 m terrestrial, viviparous elapid, typically associated with wetlands and wet forests on the Australian mainland and show a dietary preference for frogs (Lettoof et al., 2020c). Once attaining adult size there is no evidence of tiger snake predation in Perth, Western Australia, so we consider this species a top predator. No longevity data exists for this species in this study area.

Tiger snakes are only known to persist in a few wetlands in the Perth region, and we collected snakes from four wetland sites with recent contaminant data (Fig. 1). Herdsman Lake (31 55° 12′ S, 115 48° 19′ E), which is located close to Perths' Central Business District and is heavily modified, and has elevated concentrations of As, Cu, Pb, Zn, Th, Sr and Sb; Bibra Lake (32 5° 32′ S, 115 49° 27′ E) which is mostly surrounded by urbanisation and has elevated Se and V; Lake Joondalup (31 45° 34′ S, 115 47° 33′ E) which is located on the current edge of urbanised Perth and has elevated Hg; and Loch McNess (31 32° 44′ S, 115 40° 50′ E) which is located within Yanchep National Park—outside of urbanisation—and has elevated concentrations of As, Cs, Tl, Se and Hg (Lettoof et al., 2020a; Lettoof et al., 2021b).

2.3. Snake sampling

Adult western tiger snakes (> 0.55 m snout-vent length [SVL]) were hand caught from each site in September 2020. Five males and five females were randomly collected across each site, except for Yanchep National Park where only three males and two females were collected. Snakes were humanely euthanised via blunt-force trauma and carcasses were immediately frozen in a -20 °C freezer. In the laboratory, SVL and body mass (with prey items removed) were measured, and whole liver tissue removed for PFAS quantification. Muscle tissue, the posterior 'fat pad' (white adipose tissue) and gonads (testes and ovaries) were subsampled for omics analyses. Using the SVL and body mass data, a scaled mass index (SMI) was quantified for each snake (Peig and Green, 2010). The SMI is calculated by $W_i(L_0/L_i)^{bSMA}$ where W_i and L_i are the weight and SVL of individuals, L_0 is the arithmetic mean length of all sampled individuals, and b_{SMA} is the scaling exponent estimated by the standardised major axis regression of mass on length of all sampled individuals.

2.4. PFAS quantification in water and snake livers

The water samples were collected on two opposite sides of each lake except for Bibra Lake, which only had one accessible side by foot. Samples were collected one meter from the water's edge by submerging and opening a capped sample container (volume 500 ml) 10 cm beneath the water surface – to avoid the collection of surface films – and 10 cm above the sediment bed. Field blank samples were collected at three of the six sampling locations to verify that cross-contamination was avoided, and field replicates were collected at three sampling locations to verify the reproducibility of analytical results. Water samples were analysed for a suite of 30 PFAS by Eurofins Australia as per USEPA Method 537. The concentration of each analyte was determined using the isotope dilution technique. Quantification of linear and branched isomers was conducted as a single total response

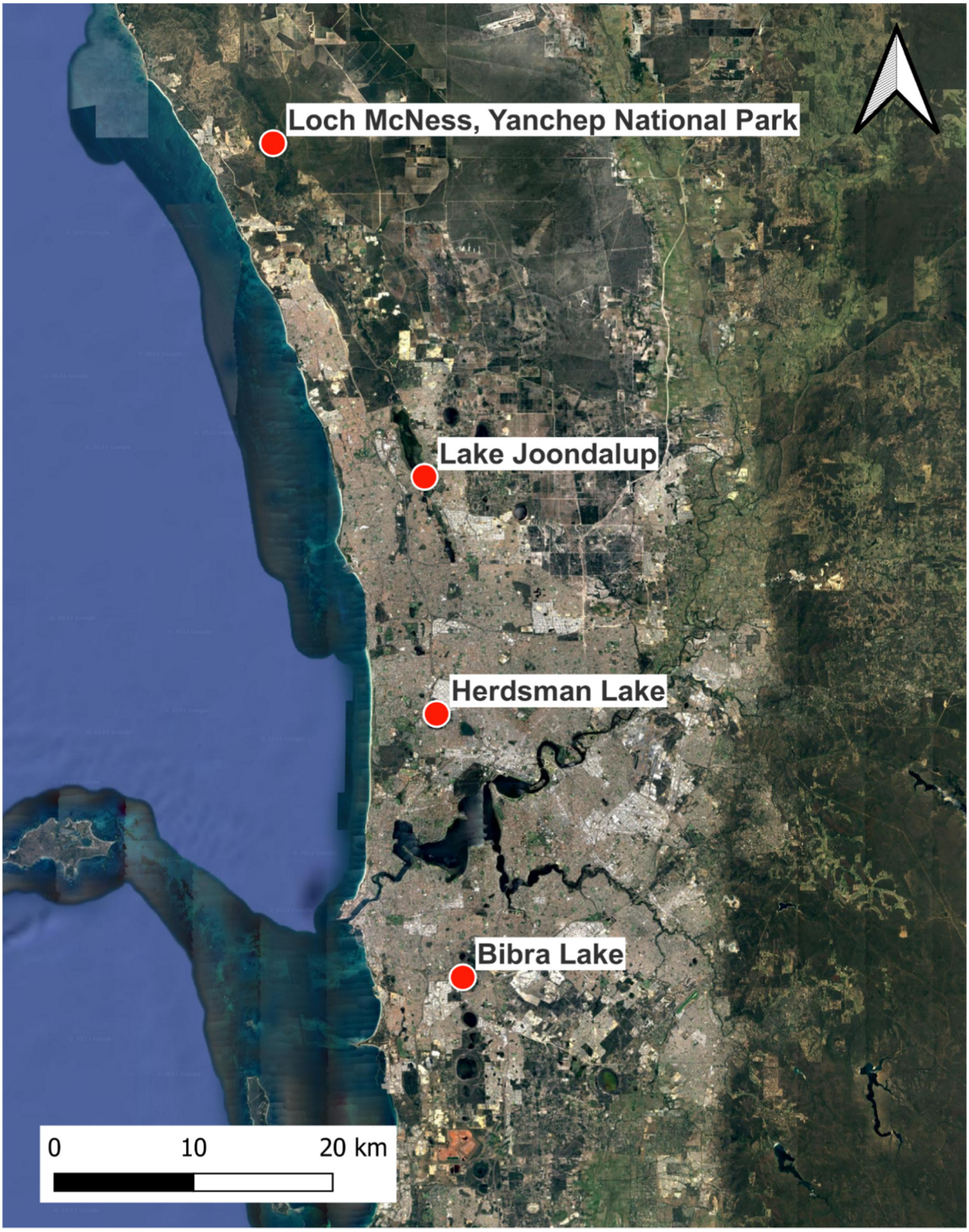

Fig. 1. Map of Perth, Western Australia, and the four study wetlands where tiger snakes were collected for liver PFAS analysis. Satellite images were obtained by Google Earth Pro in 2023.

using the relative response factor for the corresponding linear standard. A branched PFOS standard and branched PFHxS standards were used for the quantification of PFOS and PFHxS, respectively. Full analytical results for the water samples and the results of field QA/QC are provided in Table S1.

Dissected liver tissue was stored at −20 °C for a maximum of six weeks before analysis. PFAS analysis of the liver tissues was carried out by a commercial laboratory (Symbio Laboratories, Brisbane) for a suite of 28 PFAS using a in-house UHPLC/HR-MS method (Method CR148, commercial in confidence). Noting, PFNS and PFPrS were not included in the suite of

analytes offered by the laboratory analysing the liver tissue. In addition, analysis of the liver total fat content was also carried out by Symbio Laboratories using an in-house acid hydrolysis method followed by solvent extraction and gravimetric analysis (Method CF008.2, commercial in confidence).

2.5. Metabolomics and lipidomics

2.5.1. Metabolite and lipid extraction

Snake tissues were freeze-dried for 48 h at 105 °C and 0.01 mBar (FreeZone 4.5 L, Labconco Corp., Kansas City, MO, USA). The freeze-dried tissues (20 mg) were extracted with 100 μl water and 450 μl methanol-ethanol solution (1/1, *v*/v) containing 0.5 ppm *L*-phenylalanine (^{13}C) as the first internal standard. The samples were homogenized with beads (Bead Lysis Kit, CAP7100: Next Advance, Australia) using a Precellys® Evolution homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) at 5800 rpm (cycle: 2 × 15 s, pause: 30s). The tubes were centrifuged at 15,000 RCF at 4 °C for 10 min (Centrifuge 5430 R: Eppendorf, Hamburg, Germany). The supernatant (400 μl) of each sample was collected into a new vial and the pellet was resuspended in 50 μl water and 200 μl above methanol-ethanol solution. The mixtures were homogenized and then centrifuged as specified above. The volume of 200 μl supernatant was collected and mixed with the previous extraction supernatant. The remaining supernatant from all samples was collected into a 5 ml tube to make pooled quality control (QC) samples which contain 600 μl mixture per QC.

The extracted samples and pooled QC samples were transferred into 1 ml Captiva EMR-lipid cartridges (Agilent Technologies, USA) and filtered into 1.5 ml high recovery vials (Part Number: 5183–2030: Agilent Technologies, USA) using a positive pressure manifold 48 processor (PPM-48: Agilent Technologies, USA) at low pressure for 10 min. Subsequently, 100 μl of water-methanol-ethanol solution (2/1/1, v/*v*/v) was added into lipid cartridges to wash samples at low- and high-pressure vacuum, respectively. The vials containing filtered metabolites were dried in a Speedvac for 4 h. To collect the captured lipids, 500 μl DCM-methanol solution (1/2, v/v) was added into each lipid cartridge placed on a new high recovery vial and filtered at low pressure for 10 min. Another 200 μl DCM-methanol solution (1/2, v/v) was added into the lipid cartridge and filtered at low and high pressure, respectively. The filtered lipids were dried in a dry block heater (DBH4000D: Ratex, Australia) under a stream of nitrogen at 30 °C for 30 min.

The dried metabolites in each sample were recovered by resuspending in 100 μl water-methanol solution (80/20, v/v) containing 0.5 ppm labelled L-succinic acid ($^{13}C_2$) as the second internal standard. The blank samples were prepared by adding the 100 μl above water-methanol solution into empty vials. In addition, 100 μl of 1 ppm amino acid and organic acid standard mixture (Sigma Aldrich, Mulgrave, Australia) was added into empty vials for QC purposes. All samples were incubated in a ThermoMixer® C (Eppendorf, Hamburg, Germany) at 40 °C and a speed of 700 rpm for 30 min.

2.5.2. Metabolite analysis

Central carbon metabolism metabolites were analysed on an Agilent 6470 LC-QqQ-MS coupled with an Agilent Infinity II Flex UHPLC system using the Agilent Metabolomics dMRM Database and Method following Sartain (2016) and Gyawali et al. (2021). This is an ion-pair reversed-phase (IP-RP) chromatographic method, which uses an Agilent ZORBAX Extend C18 column with the ion-pairing agent tributylamine (TBA). A standard method gradient was applied comprising solvent A (97:3 water/methanol with 10 mM tributylamine +15 mM acetic acid) and solvent B (methanol with 10 mM tributylamine +15 mM acetic acid).

Untargeted polar metabolites were analysed using an Agilent 6546 Liquid Chromatography Time-of-Flight Mass Spectrometer (LC-QToF) with an Agilent Jet Stream source coupled to an Agilent Infinity II UHPLC system (Agilent Technologies, Santa Clara, CA, USA) following Shah et al. (2021) and Beale et al. (2021). Chromatographic separation was achieved by injection (2 μl) of sample onto an Agilent Zorbax SB-Aq column (2.1 × 50 mm, 1.8 μm) fitted with a Zorbax-C8 guard column (2.1 × 30 mm, 3.5 μm). Each sample was analysed in positive and negative ionization modes. The mobile phase was (A) 0.2 % acetic acid in water and (B) 0.2 % acetic acid in methanol 19 min with a nonlinear gradient starting at 2 % B. The column temperature was set at 60 °C. The detector gas temperature was 325 °C with a drying gas rate of 9 L min^{-1}. The sheath gas temperature and flow were 225 °C and 10 L min^{-1}; the nebulizer pressure was also 45 psi. The acquisition range was 100 to 1700 *m/z*, at 3.5 spectra per second. Reference mass ions were 121.050873 and 922.009798 for the positive mode and 119.036320 and 966.000725 for the negative mode. Auto MSMS data on pooled PBQC samples were obtained at collisions of 10 eV, 20 eV and 40 eV. The PBQC AutoMSMS data was used to generate a curated PCDL for further interrogation of acquired samples using accurate mass, MS2 spectra and retention time. Collected data were processed using MassHunter Profinder software (Version 10.0, Agilent Technologies, Santa Clara, CA, USA), normalized to IS, and putatively identified against the Agilent METLIN (AMRT MS/MS) Metabolite PCDL (G6825–90008, Agilent Technologies, Santa Clara, CA, USA) and a curated in-house PCDL based on MSMS spectra and library threshold score of 0.8.

2.5.3. Lipid analysis

For lipids, dried samples were recovered by resuspending in 100 μl methanol-butanol solution (50:50, *v*/v) containing 0.1 ppm d5-TG ISTD Mix (d5-Triacylglyceride internal standard mixture) (LM6000-1EA: Sigma-Aldrich, St. Louis, MO, USA). The blank samples were prepared by adding 100 μl of the above methanol-butanol solution into empty vials. All samples were incubated in a ThermoMixer® C (Eppendorf, Hamburg, Germany) at 20 °C and a speed of 700 rpm for 30 min. Untargeted lipids were analysed using an Agilent 6546 Liquid Chromatography Time-of-Flight Mass Spectrometer (LC-QToF) with an Agilent Jet Stream source coupled to an Agilent Infinity II UHPLC system (Agilent Technologies, Santa Clara, CA, USA) following Beale et al. (2021). Chromatographic separation was achieved by injection (1 μl) of the sample onto an Agilent InfinityLab Poroshell HPH-C18 column (2.0 × 150 mm, 2.7 μm). Each sample was analysed in positive and negative ionization modes. The mobile phase was (A) 10 mM ammonium acetate and 10 μM medronic acid in water/methanol (90:10, v/v) and (B) 10 mM ammonium acetate in acetonitrile/methanol/isopropanol (20:20:60, v/v/v) operated for 30 min with a nonlinear gradient starting at 55 % B. The column temperature was set at 60 °C. The detector gas temperature was 250 °C with a drying gas rate of 11 L min^{-1}. The sheath gas temperature and flow were 300 °C and 12 L min^{-1}; the nebulizer pressure was also 35 psi. The acquisition range was 50 to 1600 *m/z*, at 3 spectra per second. Capillary voltages for the positive and negative ionization modes were 3500 V and 3000 V, respectively. Reference mass ions were 121.060873 m/z and 922.009198 m/z (positive mode), and 119.036320 and 980.016375 m/z (negative mode). AutoMSMS data on pooled PBQC samples were obtained at collisions of 20 eV and 35 eV. Collected data were processed using MassHunter Profinder software (Version 10.0, Agilent Technologies, USA), normalized to IS, and putatively identified against the Agilent METLIN Lipids PCDL (G6825–90008, Agilent Technologies, Santa Clara, CA, USA) and a curated in-house PCDL based on MSMS spectra and library threshold score of 0.8.

2.6. Data analysis

PFAS which were not measured above the limit of reporting (LOR) in one or more samples (n-1) were assigned half the LOR concentration threshold to facilitate downstream statistical analysis (Zeghnoun et al., 2007). If a PFAS was not measured in all the samples (n) per site/group, then it was excluded. There was no correlation between liver lipid concentration and total PFAS ($r^2 = 0.06$, $p = 0.75$), so we did not normalise our PFAS to liver lipid contents (Hebert and Keenleyside, 1995). Further, as the majority of measured PFAS in the liver tissues was PFOS, with total PFAS highly correlated with PFOS ($r^2 = 0.99$), all statistical tests used total PFAS data in order to account for any minor PFAS constituent additive

effects. We used univariate generalised linear mixed models (GLMMs; *lme4* package (Bates et al., 2014)) to assess the relationships between total liver PFAS concentration and snake parameters of health. First, we fitted a GLMM (Gaussian error structure) with total PFAS as the response variable, an interaction between SVL and sex as the predictor variables, and site as the random effect. We then fitted univariate GLMMs to assess total PFAS influences on body condition. Models were fitted with total PFAS as the predictor variable, and site and sex as the random effects. Gaussian error structures were used for body condition. Model residuals were used to assess best fit, and variables were scaled to improve model fit if needed.

The omics datasets (metabolites and lipids) were log-transformed and multivariate data analysis was conducted using SIMCA (v17.0.2, Sartorius Stedim Biotech, Umeå, Sweden) and MetaboAnalyst 5.0 (Pang et al., 2022; Pang et al., 2021). All data were normalized via a combination of normalising by median, log transforming and auto-scaling, until data visually resembled a normal distribution. Using regression against liver PFAS, functional omics outputs were enriched, and pathway impact assessments were undertaken using MetaboAnalyst 5.0. Of the available metabolic pathways, we used the *Gallus gallus* (chicken) pathway library as birds and reptiles share similar physiology. A false discovery rate of ≤ 0.05 of the enriched outputs was set as the minimum cut-off threshold for discussion.

3. Results and discussion

3.1. PFAS in wetland waters

Of the 30 PFAS tested in wetland surface water, 12 were detected above LOR; the PFAS concentrations are presented in Table S2. No PFAS were detected above LOR in the water samples from Yanchep National Park. Herdsman Lake waters had the highest concentrations of total PFAS. Concentrations were similar at the east and west sites (0.122 and 0.101 μg/l respectively). Further, it was the only wetland analysed where PFNA was detected (0.002 μg/l). Lake Joondalup waters had similar concentrations of total PFAS (0.104–0.107 μg/l) to Herdsman Lake, and between sampling locations. The total PFAS detected in Bibra Lake waters was roughly half the concentration of the other lakes, and no PFHxS nor PFHpS were detected in Bibra Lake water samples. Proportional PFAS mixtures are visualised in Fig. 2.

Total PFAS concentrations were in the same general range as previously reported PFAS data for Herdsman Lake (0.129 and 0.102 μg/l), Lake Joondalup (0.132 and 0.076 μg/l) and Bibra Lake (0.089 and 0.034 μg/l) in Autumn and Spring 2019, respectively (Richmond, 2022). This study found that PFAS concentrations in surface water reflect the age and the intensity of urban development in Perth. Lakes located close to the city centre tend to contain a wider range of detectable PFAS compounds and higher concentrations of PFAS compared to lakes on the urban fringe. The high PFAS concentrations in Herdsman Lake, relative to other sites included in this study, are not surprising; this wetland is located within an older part of the metropolitan area, receives stormwater drainage from surrounding industrial and residential land, and is the recipient of leachate seepage from landfill (Foulsham, 2009). The higher concentration of PFOA and longer chain PFAS such as PFOS, PFHpA and PFNA reflects a broad range of compounds that have been used in various products over the last 50 years (Buck et al., 2011; Sznajder-Katarzyńska et al., 2019). The PFAS concentration in Lake Joondalup, however, is comprised of more short-chain PFAS compounds—likely reflecting the PFAS usage over the last 20 years (Ahrens and Bundschuh, 2014). Despite being located on the edge of the urban-matrix, the wetland features stormwater drains from surrounding residential land and two fire stations within its catchment. Post-2000 firefighting foams contain fluorotelomers that decompose into predominately PFHxA and PFPeA (Ahrens and Bundschuh, 2014), and are likely the primary cause of contamination – as these compounds are dominant in Lake Joondalup waters.

Bibra Lake waters had relatively low PFAS concentrations, despite being in a historically urbanised area. The wetland is mostly surrounded by remnant vegetation which likely buffers the lake from contaminant impacts through surface runoff, and it only receives urban stormwater from a single drain (City of Cockburn, 2015). The PFAS profile is mostly short-chained compounds, reflecting more recent impacts from minor incidental firefighting foam discharges transported via stormwater. Moreover, it is important to note that all these lakes are surface expressions of groundwater, so groundwater transport is another potential pathway for PFAS inputs into

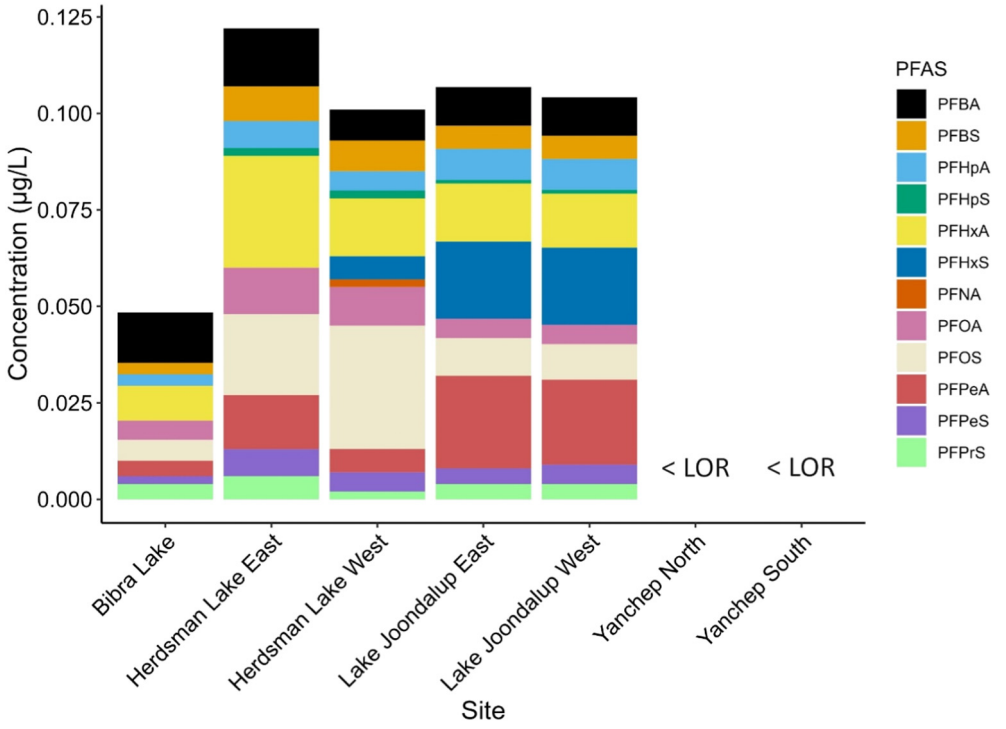

Fig. 2. Detected PFAS mixtures in the surface waters of the four studied wetlands in Perth, Western Australia. <LOR = below reporting limit of 0.001 μg/l.

these sites. Since Loch McNess in Yanchep National Park has no upstream urban development, it is not surprising that we did not detect any PFAS in its surface waters.

3.2. PFAS in snake livers

The prevalence of exposure and concentrations of 12 PFAS in tiger snake livers are presented in Table 1. PFOS was the dominant PFAS in snakes from all sites and was detected in all snakes except a single female from Yanchep National Park. Herdsman Lake snakes had the highest prevalence of contamination and mean concentration of 11 PFAS, including the only detection of 8:2 FTSA and 10:2 FTSA in a single snake. Lake Joondalup snakes were contaminated with 10 PFAS, including the only detection of PFTeDA in two snakes. Bibra Lake snakes had a low (0.6–31 μg/kg) liver concentrations for three PFAS and Yanchep snakes only had trace amounts of PFOS (≤ 2.5 μg/kg). Total PFAS concentrations were highest in Herdsman Lake snakes (322 ± 193 μg/kg) followed by Lake Joondalup snakes (93.6 ± 53.4 μg/kg), and were low in Bibra Lake (13.98 ± 7.85 μg/kg) and Yanchep snakes (1.31 ± 0.86 μg/kg). The other PFAS compounds were not detected above LOR in any snake livers.

PEPrS, PFBS, PFBA, PFPeS, PFPeA, PFHxA and PFHpA were detected in water samples but not snake livers, suggesting these PFAS do not bioaccumulate or are not in high enough concentrations to bioaccumulate in higher order vertebrates at these sites. PFDS, PFNA, PFDA PFUnDA, PFDoDA, PFTrDA, PFTeDA, 8:2 FTSA and 10:2 FTSA were detected in snake livers but not water samples. As tiger snakes at these sites feed predominantly on frogs (Lettoof et al., 2022) and show little emigration (isolated from urbanisation (Lettoof et al., 2021c)), detection of these PFAS suggest local exposure and that they have the potential to bioaccumulate in higher order vertebrates. Despite Yanchep National Park being far from urbanisation and the lack of PFAS detection in the waters, snakes were impacted with PFOS residue. As our water sampling was only sufficient in offering a snapshot of PFAS concentrations, the frequency of contamination and higher concentration in snake livers supports the use of wetland snakes as bioindicators of wetland PFAS contamination. PFOS occurring as the dominant PFAS in snake livers is consistent with previous studies reporting PFAS accumulation in tissues of other vertebrate taxa (Beale et al., 2022a; Food Standards Australia New Zealand, 2018; Stahl et al., 2012; Wang et al., 2013).

The concentrations of PFOS in snake livers from the most contaminated site, Herdsman Lake (mean: 279.9, max: 700 μg/kg), is lower than the single other published detections of PFOS in an anurophagous wetland snake—the Australian keelback (*Tropidonophis mairii*; mean: 2650 μg/kg, max: 2800 μg/kg, $n = 2$); however, the Australian keelback tissue that was analysed and its contamination/exposure history is not specified (Food Standards Australia New Zealand, 2018). To the best of our knowledge, no peer-reviewed published studies have previously reported PFAS concentrations in reptile livers and further research is needed to identify the tissue-specific partitioning of PFAS in wild reptiles; however, the PFAS concentrations detected in Perth's urban tiger snake livers are substantially higher than those reported in duck livers (range: BDL – 9.5 μg/kg, max: BDL – 340 μg/kg) from South-Eastern Australia (Sharp et al., 2021) and fish liver tissue (range: BDL – 70, max: BDL – 107 μg/kg) from the harbour of Australia's largest city—Sydney, New South Wales (Thompson et al., 2011). Currently, it appears the concentrations of PFAS detected in tiger snake livers from both Herdsman Lake and Lake Joondalup are the highest reported in Australian freshwater vertebrates.

3.3. The relationships among PFAS and snake physical condition

A summary of the snake's physical measurements at the time of sampling and their total (liver) lipid concentrations are presented in Table 2. The collected snakes were all adults and considered 'healthy' on visual inspection (i.e., no defects or abnormalities). We found no significant relationship between SVL and total liver PFAS ($r^2 = 0.71$, $X^2 = 0.07$, $p = 0.79$); however, we found males had higher but not statistically significant total PFAS liver concentrations relative to female snakes ($r^2 = 0.71$, $X^2 = 2.72$, $p = 0.10$), and a strong but not statistically significant interaction effect between SVL and sex, and total PFAS ($r^2 = 0.71$, $X^2 = 2.89$, $p = 0.10$). The relationship between male SVL and total PFAS was positive, and negative between female SVL and total liver PFAS (Fig. 3). We suspect the non-

Table 1
Exposure and concentration of PFAS (μg/kg) in Western tiger snake (*Notechis scutatus occidentalis*) livers from four wetlands around Perth, Western Australia. Prev. = prevalence of snakes with PFAS above detection limit; <LOR = below reporting limit of 0.5 μg/kg.

PFAS	Herdsman Lake		Bibra Lake		Lake Joondalup		Yanchep NP	
	Prev.	Mean ± SD (Range)	Prev.	Mean ± SD (Range)	Prev.	Mean ± SD (Range)	Prev.	Mean ± SD (Range)
PFHxS	9/10	7.04 ± 16.22 (<LOR – 53)	0/10	<LOR	2/10	0.34 ± 0.20 (<LOR – 0.8)	0/5	<LOR
PFHpS	5/10	1.66 ± 2.42 (<LOR – 7.7)	0/10	<LOR	0/10	<LOR	0/5	<LOR
PFOS	10/10	279.9 ± 174.2 (99–700)	10/10	13.45 ± 7.52 (5.2–31)	10/10	86.5 ± 52.8 (30–170)	4/5	1.31 ± 0.86 (<LOR – 2.5)
PFDS	10/10	3.65 ± 2.27 (0.7–7.5)	0/10	<LOR	2/10	0.34 ± 0.21 (<LOR – 0.9)	0/5	<LOR
PFNA	8/10	1.93 ± 1.94 (<LOR – 6.1)	0/10	<LOR	3/10	0.50 ± 0.47 (<LOR – 1.6)	0/5	<LOR
PFDA	10/10	7.08 ± 4.53 (2.3–15)	1/10	0.29 ± 0.11 (<LOR – 0.6)	10/10	2.04 ± 1.12 (0.6–3.9)	0/5	<LOR
PFUnDA	9/10	2.80 ± 1.79 (<LOR – 6.1)	0/10	<LOR	2/10	0.43 ± 0.40 (<LOR – 1.4)	0/5	<LOR
PFDoDA	10/10	9.6 ± 6.77 (0.9–26)	5/10	0.53 ± 0.30 (<LOR – 0.9)	9/10	2.46 ± 1.91 (<LOR – 5.2)	0/5	<LOR
PFTrDA	9/10	6.53 ± 10.68 (<LOR – 34)	0/10	<LOR	6/10	1.4 ± 1.93 (<LOR – 6.3)	0/5	<LOR
PFTeDA	0/10	<LOR	0/10	<LOR	2/10	0.78 ± 1.27 (<LOR – 4.2)	0/5	<LOR
8:2 FTSA	1/10	0.38 ± 0.40 (<LOR – 1.5)	0/10	<LOR	0/10	<LOR	0/5	<LOR
10:2 FTSA	1/10	0.35 ± 0.30 (<LOR – 1.2)	0/10	<LOR	0/10	<LOR	0/5	<LOR
Total	10/10	322 ± 193 (100–750)	10/10	13.98 ± 7.85 (5.2–32)	10/10	93.6 ± 53.4 (38–180)	4/5	1.31 ± 0.86 (<LOR – 2.5)

Table 2
Physical measurements (mean ± SD) of the sampled tiger snakes.

Site	Sex (*n*)	Size (SVL; cm)	Body mass (g)	Liver lipids (g/100 g)
Herdsman Lake	Female (5)	75.9 ± 2.4	170.3 ± 34.6	3.6 ± 1.7
	Male (5)	83.3 ± 6.6	226.6 ± 70.5	3.1 ± 0.7
Bibra Lake	Female (5)	69.4 ± 10.3	197.4 ± 84.7	3.3 ± 1.2
	Male (5)	80.4 ± 3.0	234.4 ± 71.6	2.1 ± 0.3
Lake Joondalup	Female (5)	68.0 ± 2.8	158.5 ± 45.8	2.9 ± 0.9
	Male (5)	78.8 ± 5.3	251.4 ± 59.5	2.6 ± 0.6
Yanchep	Female (2)	68.0 ± 4.2	160 ± 0	2.2 ± 0.2
	Male (3)	74.4 ± 9.5	184.2 ± 38.3	3.0 ± 1.1

significant results are an artifact of the small sample size of contaminated snakes, as these observations are supported by previous findings. The inverse relationship between PFAS concentrations and size (a proxy for age (Waye, 1999)) in male and female tiger snakes could be easily attributed to females eliminating PFAS via maternal transfer—a phenomenon that has been shown in snakes such as *Enhydris chinensis* with organophosphorus flame retardants and plasticizers (Liu et al., 2019) and metals in tiger snakes (Lettoof et al., 2021d), *Nerodia sipedon* (Chin et al., 2013) and *Lamprophis fuliginosus* (Hopkins et al., 2004). The positive relationship between total liver PFAS and the size of male snakes suggests PFAS is bioaccumulating in male tiger snakes from contaminated sites. Of the only other comparable reptile studies, males have also been reported with higher concentrations of PFAS in snapping turtles, *Chelydra serpentina* (Kannan et al., 2005), and Chinese alligators, *Alligator sinensis* (Wang et al., 2013). Additionally, Zhang et al. (2020) found female *Eremias argus* lizards exposed to PFOA had associations with higher investment of physiological resources into self-maintenance than males; if female tiger snakes respond similarly—in conjunction with the capacity to maternally transfer contaminants—the long-term exposure and accumulation of PFAS may impact the fitness and survival of male snakes more than females. Testing of PFAS burdens on new-born offspring compared to mothers of different sizes is needed to clarify maternal transfer of PFAS in snakes and warrants further investigation into the potential impacts on juvenile development and survival.

We found a weak yet significant negative relationship between liver total PFAS and snake body condition ($r^2 = 0.12$, $X^2 = 4.84$, $p = 0.03$; Fig. 4), predicting liver total PFAS of 750 μg/kg is associated with approximately 30 % loss in mean body condition. Roughly 50 % of the variation in snake body condition estimates is caused by stored fat bodies (Weatherhead and Brown, 1996), while the remaining unexplained

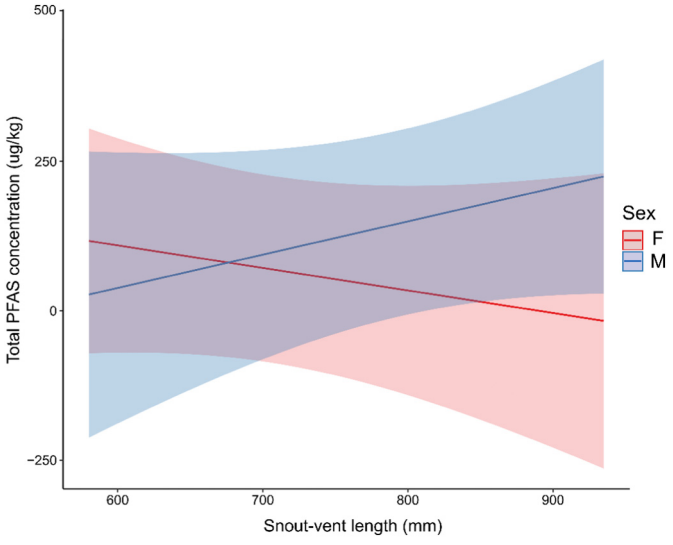

Fig. 3. The relationship between total liver PFAS concentration and snout-vent length in male and female tiger snakes collected from four wetlands around Perth, Western Australia. Predicted values were extracted from a GLMM which included site as a random effect. Shaded area represents 95 % confidence intervals.

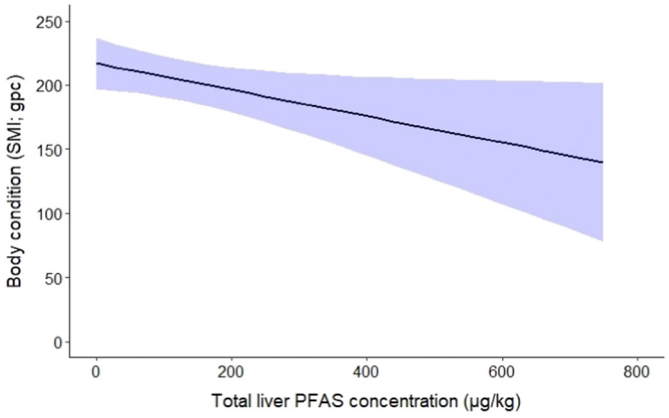

Fig. 4. The relationship between total liver PFAS concentration and body condition (reported as scaled mass index SMI, grams per cm) in western tiger snakes collected from four wetlands around Perth, Western Australia. Predicted values were extracted from a GLMM which included site and sex as random effects. Shaded area represents 95 % confidence intervals.

variance can likely be attributed to larger organs such as muscle and liver tissue (Madsen and Shine, 2002). We acknowledge that the PFAS concentrations in these snakes only explain 12 % of the variation in poor body condition and the model was built from a small sample size, but the results are unsurprising given the ability for PFAS compounds to bioaccumulate in predators, and interfere with lipids and metabolic energy pathways. We suspect body condition is also being impacted from a cocktail of other urban contaminants—as 20 % of the variation in snake body condition at these sites is explained by metal(loid) contamination (Lettoof et al., 2022). For example, exposure to PFAS has been shown to magnify the impact of cadmium and lead on kidney function (Jain, 2019), but otherwise, the relationship between PFAS and metal toxicity is largely unknown and warrants further investigation. Importantly, these potential co-contributing impacts to snake body condition makes them particularly vulnerable to predation (Mattisson et al., 2016) and mortality (Shine et al., 2001), and a reduction in reproductive frequency and outputs (Madsen and Shine, 1996; Milenkaya et al., 2015) which may lead to population declines.

3.4. Impact of PFAS on snake muscle biochemical profiles

Muscle biochemical profiling showed clear significant site-specific groupings ($R^2x = 0.22$, $R^2y = 0.48$, $Q^2 = 0.3$, $p < 0.01$), but high variation among individuals (Fig. 5). The axis orientation does not follow a linear relationship with PFAS concentrations; snake profiles are likely impacted by the unique metal mixtures accumulated in each population (Lettoof et al., 2020a; Lettoof et al., 2021b) and potentially the differences in parasite infection in these populations (Lettoof et al., 2022; Wang et al., 2004). The high variation of individuals within a site is likely attributed to the high variation of metabolic and physiological states of reptile individuals within a population (Coz-Rakovac et al., 2011; Lettoof et al., 2021a; Moon et al., 1999), and suggests in situ metabolomics studies on reptiles needs to sample more individuals to capture the high variability within sites. Categorising snakes by sex showed no obvious groupings so sexes were pooled for further analysis.

After controlling for the influence of site, linear mixed models identified 52 muscle metabolites and lipids showing significant ($p = 0.001$–0.049) positive relationships with liver PFAS and eight showing significant ($p = 0.027$–0.042) negative relationships with liver PFAS; however, after a false discovery rate adjustment, none of these were significantly perturbed (FDR = 0.068–0.383; Table S3). This indicates many snake muscle metabolites and lipids have relationships with PFAS, but the effect of site and variation of individual physiological states in wild snakes creates substantial 'noise' in the data and a larger sample size could be required to confirm if

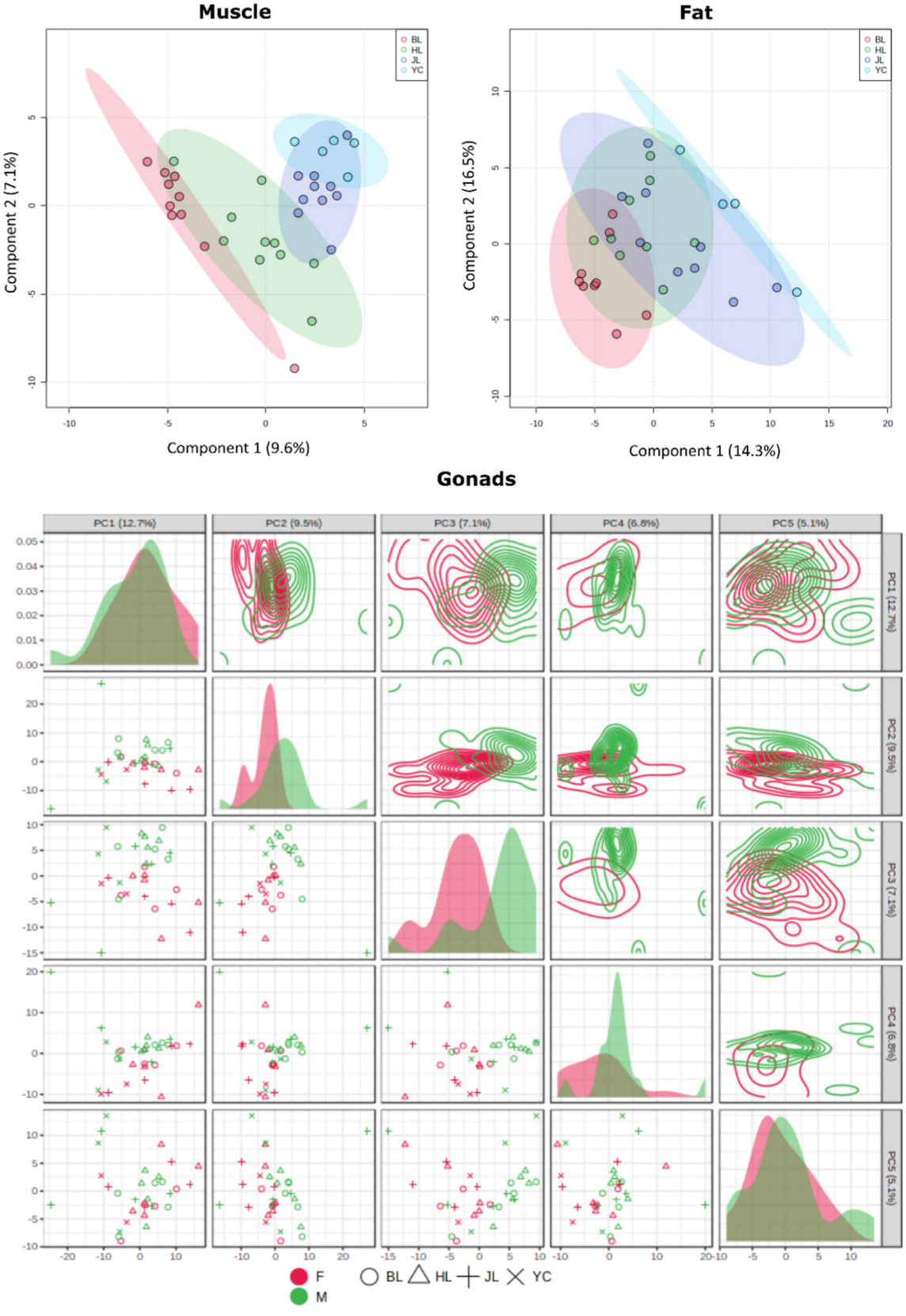

Fig. 5. PLS-DA plots for tiger snake muscle and fat, and an iPCA plot series for tiger snake gonad (testes and ovaries, pooled) metabolome and lipidome. Grouped by site; BL = Bibra Lake; HL = Herdsman Lake; JL = Lake Joondalup; YC = Yanchep National Park; F = female; M = male.

these metabolites/lipids are useful biomarkers of PFAS accumulation in snakes. When assessing the chemical groups, PFAS accumulation did not significantly (FDR = 0.56–0.76) enrich any muscle lipidome groups (Table S4) that other studies reported (Beale et al., 2022c) but did significantly (FDR = 0.01–0.05) enrich 11 metabolome groups (Table S5): pyrimidines, indoles, fatty acids and conjugates, amino acids and peptides,

phenylacetic acids, TCA acids, pyridines, purines, benzoic acids, benzenes and fatty amines.

Enrichment of these chemical groups suggests perturbation of their related pathways, all of which have been repeatably associated with PFAS exposure in humans (Guo et al., 2022) and other biota (Beale et al., 2022c), suggesting a common effect of these contaminants on organisms. Pyrimidines, pyridines and purines are involved in nucleotide metabolism (Hubert and Sutton, 2017; Nyhan, 2005), and TCA acids (i.e. tricarboxylic acid cycle), fatty acids and conjugates, amino acids and peptides are involved in carbohydrate metabolism which are critical for cellular homeostasis and energy generation related mechanisms (Martínez-Reyes and Chandel, 2020; Nyhan, 2005). In uricotelic animals specifically (e.g. birds and land reptiles), these chemical groups are involved in the uric acid cycle which disposes of nitrogenous waste from protein metabolism, including forming chemicals involved in the 'salvage pathway' for recycling purines and pyrimidines (Balinsky, 1972; Salway, 2018). Although the understanding of these mechanisms is relatively sparse in reptiles, impacts on the uric acid cycle can cause renal disease and gout which can be fatal for reptiles (Campbell, 2006). In addition, the enrichment of benzoic acids, phenylacetic acids, benzenes and fatty amines—common metabolites of xenobiotics (Williams, 1974)—suggests a relationship between PFAS and detoxification processes in the muscle and warrants further research.

The significantly impacted and enriched muscle metabolite and lipid pathways were synthesis and degradation of ketone bodies (FDR = 0.02), valine, leucine and isoleucine degradation (FDR = 0.03), biosynthesis of unsaturated fatty acids (FDR = 0.03), and selenocompound metabolism (FDR = 0.03; Table S6; Fig. 6). Selenium is an essential trace element for organisms, but is toxic in excessive concentrations (Mézes and Balogh, 2009). Perturbation of selenocompound metabolism pathways could increase selenium toxicity in tiger snakes and could be evidence of a synergistic impact from PFAS and legacy metalloid contamination, which tiger snakes from these sites are exposed to.

Ketone bodies, valine, isoleucine, and leucine (branched-chain amino acids; BCAAs) and unsaturated fatty acids are all crucial for energy production. Specifically, BCAAs catabolism in the muscle yields compounds that can be used for ATP generation, protein synthesis and regulators of glucose transport proteins, and synthesis of ketones (Holecek, 2021; Zhang et al., 2017), while fatty acids are the precursors of ketones and aerobic production of ATP by fatty acid metabolism fuels gluconeogenesis (Fukao et al., 2004; Mayes et al., 2003). Compared to birds and mammals, squamate reptiles (lizards and snakes) have limited energy stores which are slowly replenished from a low metabolism. Bursts of vigorous activity, such as hunting and subduing prey or escaping a predator, is primarily fuelled by muscle glycogen stores and gluconeogenesis is performed in the muscle to quickly refuel these stores (Gleeson, 1991; Hancock et al., 2001; Hitchcox, 2009). PFAS-induced perturbation of these energy production pathways could result in muscle wasting (Holecek and Vodenicarovova, 2018) and is a likely explanation for the low snake body condition we detected (Fig. 4), which can translate into a reduced ability to hunt or escape predators.

Other important pathways that were impacted and enriched ($-LOG_{10}$ (p) = 1.33–2.06) but not identified as significantly perturbed (FDR = 0.11–0.17) include butanoate metabolism, glutathione metabolism, steroid hormone biosynthesis, thiamine metabolism, tyrosine metabolism, retinol

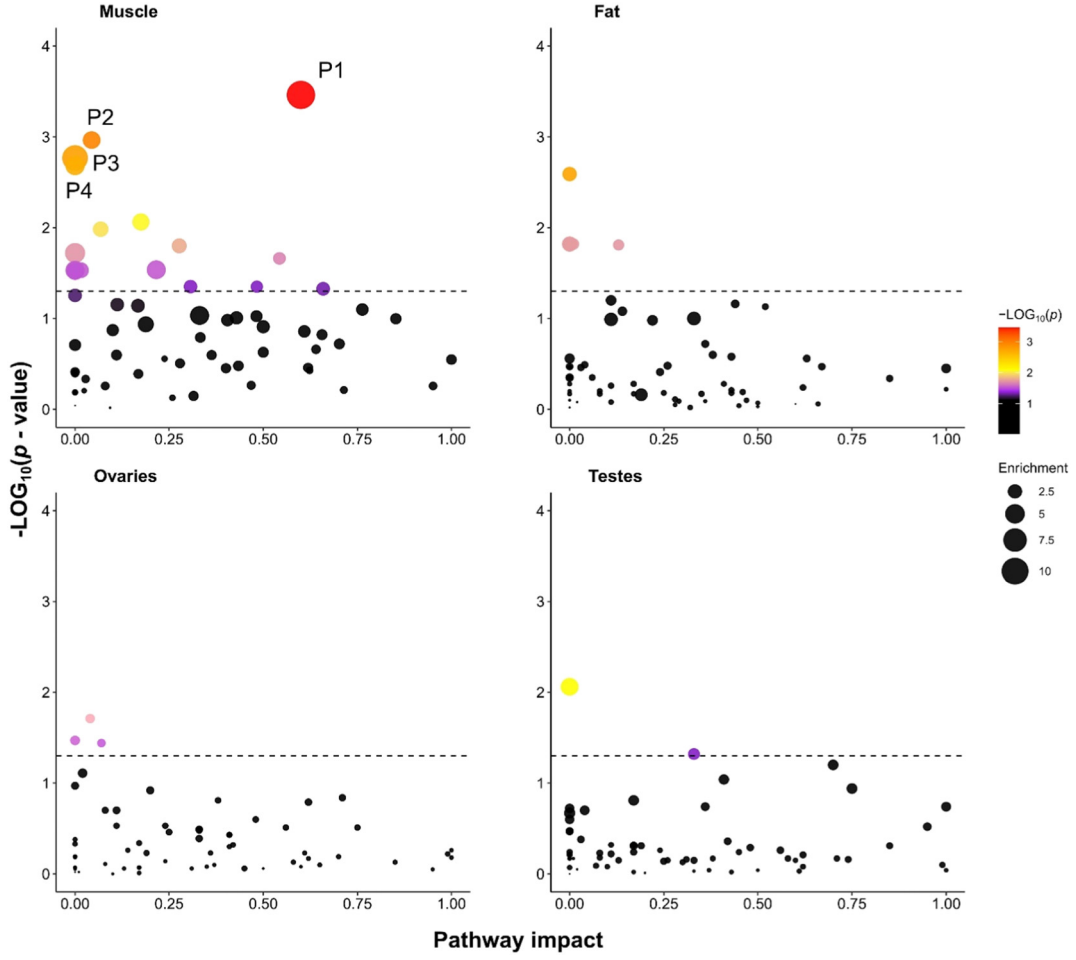

Fig. 6. Significant metabolic pathways identified in each tissue via enrichment and impact analysis using MetaboAnalyst 5.0 (Enrichment Analysis and Pathway Impact Toolbox). Labelled bubbles are significant pathways after a false discovery rate adjustment; P1 = synthesis and degradation of ketone bodies; P2 = valine, leucine and isoleucine degradation; P3 = selenocompound metabolism; P4 = biosynthesis of unsaturated fatty acids.

metabolism, fatty acid biosynthesis, fatty acid elongation, fatty acid degradation, propanoate metabolism, terpenoid backbone biosynthesis, citrate cycle (TCA cycle), sphingolipid metabolism, and nicotinate and nicotinamide metabolism (Table S6; Fig. 6).

3.5. Impact of PFAS on snake fat

Unlike the muscle, the fat biochemical profiling showed non-significant ($R^2x = 0.34, R^2y = 0.32, Q^2 = -0.02, p = 1$) site-specific groupings, with substantial overlap and high variation among individuals—especially the snakes from Yanchep National Park where one was identified as an outlier (Fig. 5). The axis-gradient site groups also do not follow a linear relationship with population PFAS accumulation, as the biochemical profiles are likely influenced by the same environmental stressors as suggested in the muscle data. Categorising snakes by sex showed no obvious groupings so sexes were pooled for further analysis.

After controlling for the influence of site, linear mixed models identified 25 fat metabolites and lipids showing significant (p = 0.001–0.049) positive relationships with liver PFAS and two showing significant (p = 0.012–0.038) negative relationships with liver PFAS; however, after a false discovery rate adjustment, none of these were significantly (FDR = 0.09–0.86) perturbed (Table S3). This suggests many snake metabolites and lipids stored in adipose tissue may have relationships with PFAS, but the effect of site and variation of an individual creates 'noise', and a larger sample size is required. When assessing the chemical groups, PFAS accumulation did not significantly enrich adipose lipidome groups (FDR = 0.5–0.95; Table S4), but did significantly enrich monoradylglycerols, fatty aldehydes and bile acids of the metabolome groups (FDR = 0.02–0.05; Table S5). The enrichment of monoradylglycerols and fatty aldehydes is unsurprising, as these are the primary constituents of reptile adipose tissue (Azeez et al., 2014; Price, 2017) and PFAS impacting lipid accumulation is a common response in vertebrates (Beale et al., 2022c). Similarly, PFAS interfering with bile acids—lipids with regulatory roles in metabolic and cellular homeostasis (Chiang and Ferrell, 2019)—and their syntheses has been frequently reported in humans and rats (Zhao et al., 2015) and an increase in these lipids is often a biomarker of fatty liver disease (Puri et al., 2018; Sen et al., 2022).

There were no significantly impacted and enriched fat metabolite and lipid pathways; however, important pathways that were impacted and enriched ($-\text{LOG}_{10}(p)$ = 1.81–2.59) but not identified as significantly perturbed (FDR = 0.16–0.2) include biosynthesis of unsaturated fatty acids, fatty acid biosynthesis, fatty acid elongation, fatty acid degradation and primary bile acid biosynthesis (Table S6; Fig. 6). Besides potentially impacted fatty acid pathways, it is not surprising that the adipose tissue had no significantly perturbed pathways as its function in reptiles is purely storage of triglycerides/lipids/energy until mobilisation for reproduction or winter maintenance (Price, 2017).

3.6. Impact of PFAS on snake gonads

Due to the limited sampling of female snakes from Yanchep National Park (n = 2), we did not have the minimum samples required for groups in a PCA analysis (n = 3), so data from both sexes tissues (ovaries and testes) had to be pooled. The iPCA showed large overlapping among sites but a clear separation between sexes (Fig. 5), indicating that these two organs have different metabolic profiles and should be further assessed separately. Besides site-specific stressors influencing variation in gonad biochemical profiles, snakes were collected in peak breeding season so testes could have been in different stages of use (e.g. recently mated, not yet mated for the season). Despite no females having fertilised or enlarged ova, they may have also been in variable states of early reproduction.

After controlling for the influence of site, linear mixed models identified 18 ovary metabolites and lipids showing significant (p = 0.001–0.047) positive relationships with liver PFAS and six showing significant (p = 0.012–0.044) negative relationships with liver PFAS (Table S3); however, after a false discovery rate adjustment, none of these were significantly (FDR = 0.37–0.94) perturbed (Table S3). The ovaries had triradylglycerols and sterols as lipidome chemical groups significantly (p = 0.01–0.02) enriched from PFAS accumulation (Table S4), and octadecanoids, benzenediols, fatty amines, purines and benzoic acids as metabolome chemical groups significantly (p = 0.001–0.04) enriched from PFAS accumulation (Table S5); however, after a false discovery rate adjustment none of these were significantly (FDR = 0.1–0.55) enriched. Although we do not have the statistical power to confidently link these chemical groups with PFAS accumulation, these chemicals are used for cellular genesis (Liu et al., 2022; Nagle et al., 1998; Quaranta et al., 2022; van Meer et al., 2008) and their potential enrichment from PFAS could impact embryo development and warrants further investigation with bigger sample sizes.

Only nine metabolites and lipids in snake testes showed a significant (p = 0.002–0.033) positive relationship with liver PFAS; however, after a false discovery rate adjustment, none of these were significantly perturbed (FDR = 0.59–0.99; Table S3). The testes had sphingomyelins, cardiolipins and glycerophosphoethanolamines lipidome chemical groups significantly (p = 0.02–0.04) enriched from PFAS accumulation (Table S4), however, after a false discovery rate adjustment, none of these were significantly (FDR = 0.12–0.17) enriched. No metabolome chemical groups were significantly enriched (FDR = 0.99; Table S5). As previously stated, a larger sample size would help identify if these lipid groups are impacted by PFAS and warrants investigation as these they represent large components of spermatozoa and spermatogenesis (Alvarez et al., 1987; Ren et al., 2019), and enrichment could result in impacts on sperm motility and overall quality (Li et al., 2022; Otala et al., 2005) or represent a perturbation of spermatogenesis (Furland et al., 2011).

The ovaries and testes had no significantly impacted and enriched fat metabolite and lipid pathways; however, important pathways that were impacted and enriched ($-\text{LOG}_{10}(p)$ = 1.44–2.06) but not identified as significantly perturbed (FDR = 0.58–0.98) in the ovaries included propanoate metabolism, drug metabolism - other enzymes, and valine, leucine and isoleucine degradation, and in the testes included selenocompound metabolism, and ubiquinone and other terpenoid-quinone biosynthesis (Table S6; Fig. 6). The potential relationship between these pathways and PFAS could lead to reproduction perturbation and warrants further investigation.

4. Concluding remarks and future research needs

This research provides evidence for PFAS accumulation in top predator snakes, and the observed associations between PFAS and the whole organism and its metabolic health. Notably, some long-chain PFAS were detected in snake livers that were not detected in the water samples from the same site. This finding is particularly important for environmental management and regulation decisions as it demonstrates that reliance on PFAS measurement of aqueous media alone is inadequate for predicting accumulation and ecological impacts to higher-order species.

Consistent with other PFAS studies, we found higher PFAS concentrations were loosely associated with perturbation of the lipidome (particularly lipids involved in cellular genesis in the ovaries and spermatogenesis in the testes) and strongly associated with energy production and maintenance pathways in the muscles metabolome, specifically. These mechanistic disruptions likely contribute to the lower body condition found in snakes with high PFAS. As the PFAS-impacted sites were urban wetlands likely exposed to PFAS residue from stormwater, a greater impact to snakes will possibly be observed in wetlands containing higher concentrations of PFAS (e.g. those adjacent to airports or military bases where a known PFAS contaminant plume exists). This study further supports the use of tiger snakes or other top predator wetland snakes as bioindicators of wetland health and function.

Metabolomics and lipidomics give detailed insight into how pollutants change the biochemical pathways and mechanisms of an organism—a tool of great value for studying reptile ecotoxicology; however, given the fluctuation in metabolism and physiological states in wild populations, and the likely interference from other legacy contaminants in our study

wetlands (expected in most urban wetlands), we did not find many statistically robust relationships with PFAS accumulation in tiger snakes. To improve these studies, we recommend testing biota for common legacy metals to tease out potential antagonistic effects and sampling more individuals to capture the natural variation. Moreover, more reptiles need to have their metabolic pathways mapped and made available for metabolomics analysis software to create more accurate inferences. Nonetheless, following a functional omics-based approach we identified the biological response (phenotype) of four populations of tiger snakes exposed to a range of PFAS concentrations and strong associations between PFAS concentrations and biochemical pathways. With more research, utilising a larger sample size, these data have the potential to be incorporated into omics-based ecosurveillance techniques to monitor these snake populations and contribute a biological line of evidence to the environmental risk assessment and management of PFAS into the future.

CRediT authorship contribution statement

D.C. Lettoof: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **T.V. Nguyen:** Methodology, Data curation, Writing – original draft. **W.R. Richmond:** Conceptualization, Investigation, Writing – review & editing, Funding acquisition, Resources. **H.E. Nice:** Investigation, Writing – review & editing. **M.M. Gagnon:** Writing – review & editing. **D.J. Beale:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Resources.

Data availability

Data will be made available on request.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165260.

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