Occurrence and bioconcentration of micropollutants in Silver Perch *(Bidyanus bidyanus)* in a reclaimed water reservoir

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Abstract: This study examined the occurrence of 49 micropollutants in reclaimed water and Silver Perch (*Bidyanus bidyanus*) living in a reclaimed water reservoir. The number of micropollutants detected in reclaimed water, Silver Perch liver, and Silver Perch flesh were 20, 23, and 19, respectively. Concentrations of all micropollutants in reclaimed water, except benzotriazole, were well below the Australian Guideline for Recycled Water (AGRW) values for potable purposes. The concentration of benzotriazole in reclaimed water was 675±130 ng/L while the AGRW value for this compound was 7 ng/L. Not all micropollutants detected in the water phase were identified in the Silver Perch flesh and liver tissues. Likewise, not all micropollutants detected in the Silver Perch flesh and liver were identified in the reclaimed water. In general, micropollutant concentrations in the liver were higher than in the flesh. Perfluorooctane sulfonate (PFOS) was detected at a trace level in reclaimed water well below the AGRW guideline value for potable purposes, but showed a high and medium bioconcentration factor in Silver Perch liver and flesh, respectively. In addition, the risk quotient for PFOS was medium and high when considering its concentration in Silver Perch liver and flesh, respectively. Results reported here highlight the need to evaluate multiple parameters for a comprehensive risk assessment. The results also single out PFOS as a notable contaminant of concern for further investigation.

Keywords: *Silver Perch, micropollutants, water reclamation, risk assessment, bioconcentration factor, perfluorooctane sulfonate (PFOS).*

1. Introduction

As climate variability and population growth continue, cities and towns around the world have begun to adopt water recycling to enhance the security of their water supply (Burgess et al., 2015; Lavrnić et al., 2017; Wilcox et al., 2016). Water recycling is the process of treating wastewater and utilising the reclaimed effluent for purposes compatible with its quality and level of treatment. Reclaimed water can be used for industrial manufacturing, agriculture, household non-potable purposes, and even potable water supply depending on the level of treatment and risk management (Burgess et al., 2015; Lavrnić et al., 2017; Wilcox et al., 2016). Through a combination of careful management, appropriate use, and consultation with water users, water recycling has been proven by many water authorities around the world as a reliable, safe, and sustainable approach to enhance water supply security and environmental protection (Burgess et al., 2015).

A core objective for all water recycling projects is public health protection (Drewes and Khan, 2015). Perceived risks associated with public health increase as the end usage of recycled water shifts from industrial, irrigation, non-potable, to potable reuse. Thus, there is a growing interest in the scientific community to better understand and quantify risks associated with water recycling, particularly for high value usage. Research to date has highlighted the ubiquitous occurrence of

many organic chemicals (hereafter called micropollutants) in treated effluent albeit only at trace level (Alidina et al., 2014; Luo et al., 2014; Osorio et al., 2012; Rivetti et al., 2017; Zhou et al., 2013). Many of these micropollutants are naturally produced and excreted by humans (such as steroid hormones) or are intrinsically linked to human activities (such as pharmaceutical and personal care products), thus, their release to sewers is inevitable (Tran et al., 2014a; Tran et al., 2014b; Yang et al., 2017).

Understanding the occurrence of micropollutants within aquatic life can help to evaluate any potential risks of water reclamation. Aquatic species are ideal subjects for pollution monitoring, as they can provide a link between pollutants and the ecosystem. The ecotoxicity of micropollutants to various aquatic species has been extensively investigated, although often through laboratory controlled studies and at elevated concentrations (well above their environmental range) and unrealistic conditions (e.g. short exposure duration) (Leusch and Snyder, 2015). Researchers have also conducted field studies on micropollutant accumulation within aquatic species directly exposed to effluent affected rivers or streams (Ramirez et al., 2009; Wang and Gardinali, 2012). These studies provide a more realistic context. However, the results from these natural settings can be influenced by additional sources of pollution and seasonal variations, thus, they may not accurately reflect the potential risks of micropollutants in reclaimed water.

The Shoalhaven Water Reclaimed Water Management Scheme (REMS) is one of the largest and most complex water recycling schemes undertaken by a regional water authority in Australia (Gould et al., 2003). The key objective of REMS is to maximise the use of reclaimed water for irrigation of golf courses and sporting facilities, as well as agriculture production, rather than disposing of it into the environment. Since its commission in 2002, REMS has provided over 20,000 ML of reclaimed water to golf courses, sporting grounds, and dairy farms covering over 500 hectares of land for irrigation.

A key component of REMS is the bulk storage facility, which is a 600 ML reservoir receiving treated wastewater from four conventional wastewater treatment plants during wet weather flows for subsequent re-use. To control algae, Silver Perch (*Bidyanus bidyanus*) fingerlings were released into the reservoir in the 2000s. The reservoir is not connected to any natural water bodies. Thus, all Silver Perch individuals in the reservoir have lived their entire lives in reclaimed water. As such, the reservoir provides a realistic setting to investigate the potential bioconcentration of micropollutants in the Silver Perch living in reclaimed water.

This study surveyed the concentrations of several groups of micropollutants (commonly detected in treated effluent), in the flesh and liver tissues of Silver Perch living in a reclaimed water reservoir. The results were compared to micropollutant concentrations in the water phase to quantify their

bioconcentration potential. Risk quotients were also calculated to identify micropollutants of significant concern for further investigation.

2. Materials and methods

2.1. Sample collection

Water and Silver Perch samples were collected from the REMS 600 ML reservoir for reclaimed water storage in the Shoalhaven region. Shoalhaven is a semi-rural township located approximately 200 km south of Sydney. The Silver Perch (*Bidyanus bidyanus*) is a native freshwater fish with natural distribution over the entire western drainage of New South Wales, including most of the Murray-Darling Basin in Australia. As an omnivorous species, their diet includes insects, small crustaceans, and vegetation.

The sampling campaign, initiated in April 2017, lasted over 6 weeks. Silver Perch samples were captured from the reservoir by pole and line in weeks 1, 4, and 6. The captured fish were weighed and those less than 2 kg were released back into the reservoir. Each week, 15 fish samples satisfying the size requirement of 2 kg or more were euthanised by direct destruction of the brain tissue, according to the animal ethics approval (UOW Ethics Number: AE17/06). Each fish sample was labelled and kept in an ice slurry for subsequent analysis. Triplicate water samples were also obtained from the reservoir every week during the sampling campaign. All fish and water samples were processed within 24 hours from collection.

2.2. Chemicals and Standards

A total of 49 compounds were selected as representative micropollutants, commonly detected in treated effluent (Supplementary data). The selected micropollutants included a range of pharmaceutical and personal care products, pesticides, industrial chemicals, steroid hormones, and per- and poly-fluoroalkyl substances (PFAS).

2.3. Solid phase extraction of aqueous samples

Aqueous samples were filtered through 0.7 µm glass fibre filter paper (Millipore). In this study, isotopically labelled standards (50 ng for each compound) of 44 out of the 49 selected micropollutants were introduced into all samples for method recovery confirmation and quantification (Tran et al., 2016; Tran et al., 2013). Isotopically labelled standards are not available for five micropollutants (i.e. oxybenzone, propylparaben, phenylphenol, sucralose and acesulfame) and they were quantified by external calibration. Aqueous samples (500 mL, pH ~ 7) were extracted onto hydrophilic/lipophilic balance (HLB) solid phase extraction (SPE) cartridges (500 mg 6 cc, Oasis, Waters, Milford, MA, USA). SPE cartridges were preconditioned with 90% v/v methyl-*tert*-butylether (MTBE) (5 mL), methanol (5 mL), then aliquots of Milli-Q grade water (2 x 5 mL). The

samples were extracted onto the SPE cartridges through teflon lines at a flow rate of approximately 15 mL/min. The cartridges were gently dried under pure nitrogen for 30 minutes. Target micropollutants were eluted from the dried cartridges under gravity using methanol (2 x 3 mL) and MTBE (3 mL). The combined eluants were evaporated to approximately 1 mL under nitrogen using a Turbo-Vap (Caliper Life Sciences, Waltham, MA. USA) and transferred to a 2 mL amber autosampler vial for quantification.

2.4. Fish flesh and liver extraction

Approximately 100 g of side fillet flesh and the entire liver were obtained from each fish. The flesh samples were individually homogenized using a processing blender. Each liver sample was ground using a mortar and pestle. Approximately 10 g of processed flesh or liver samples were placed into plastic test tubes and capped. Similar to aqueous samples, surrogate standard of 50 ng (50 µL of a 1mg/L standard solution) of each of the 44 isotopically labelled compounds (corresponding to 44 of the 49 micropollutants selected for monitoring in this study) was introduced into all tissue samples for method recovery and quantification. Acetonitrile (8 mL) was then added to each tissue sample and mixed using a vortex for 1 minute following the method described by (Johnston et al., 2002). The mixture of tissue samples and acetonitrile was centrifuged for 3 minutes at 4200 rpm. The supernatant was pipetted into glass test tubes and dried under nitrogen using a TurboVap. Once the samples had evaporated to approximately 20% of their original volume, they were transferred to 500 mL of Milli-Q water for subsequent SPE as described for aqueous samples in section 2.3.

2.5. Instrumental analysis

For identification and quantification of micropollutants, a high performance liquid chromatography/tandem mass spectrometer (HPLC-MS/MS) was used. Separation was achieved using an Agilent 1200 series HPLC system (Agilent, Santa Clara, CA, USA) equipped with a 150 x 4.6 mm, 5 µm particle size, Luna C18(2) column (Phenomenex, Torrance, CA, USA). The target analytes and their surrogates were identified using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) with electrospray ionization (ESI) working in both positive and negative electro-spray modes, as described by (Vanderford and Snyder, 2006). An injection volume of 10 µL was used for all samples. For complete confirmation of target analytes two parent ion-product ion transitions were monitored for each analyte and the surrogate standard. Additionally, relative retention times of the analytes and surrogate standards were monitored.

The limit of quantification (LOQ) values were defined as either the concentration giving a peak with signal to noise ratio of 10:1 or the second lowest calibration point, whichever was higher. Except for those analytes that did not have isotope labeled surrogate standards, analyte concentrations were determined by isotope dilution where a calibration curve was generated by

plotting analyte/internal standard peak area ratio against analyte/internal standard concentration. This technique allows for any loss through matrix ionization suppression as well as incomplete SPE and handling losses. The calibration range was 0.5-500 ng/mL with correlation coefficients of 0.99 or greater. LOQ values in reclaimed water and tissue samples of all micropollutants monitored in this study are available in the Supplementary Data.

2.6. Data analysis

2.6.1 Bioconcentration factor

The bioconcentration factor (BCF) in Silver Perch for each micropollutant was expressed as the ratio between the concentration in fish tissue ($C_{Fish\ tissue}$ in ng/kg) and that in the water phase (C_{water} in ng/L), which is their habitat (Liu et al., 2017).

$$BCF\ (L/kg) = \frac{C_{Fish\ tissue}}{C_{Water}} \quad (1)$$

BCF is an indicator of the accumulation of a chemical in or on an organism when the source of chemical is solely water. A micropollutant is regarded to be 'bioaccumulative' if the BCF exceeds 5,000 or 'potentially bioaccumulative' if the BCF is between 2,000-5,000 (Dan et al., 2017; Liu et al., 2017).

The BCF value is likely dependent on tissue type. Thus, in this study, the concentration ratio between the liver and flesh was calculated to determine the appropriate tissue for bioaccumulation monitoring. The Liver to Flesh Ratio was defined as:

$$Liver - Flesh\ Ratio = \frac{C_{Liver}}{C_{Flesh}} \quad (2)$$

Where C_{Liver} and C_{Flesh} are the micropollutant concentration (ng/kg) in the liver and flesh, respectively.

2.5.2 Risk assessment

In this study, a risk assessment was also conducted by calculating the trigger value for each micropollutant using the approach described the Food Standard Australia and New Zealand (2017):

$$Trigger\ Value\ (ng/g) = \frac{ADI\ (ng/kg\ bw/day) \times BW\ (kg)}{FC\ (g/day)} \quad (3)$$

The acceptable daily intake (ADI) for each chemical detected in the Silver Perch was obtained from the Australian Guidelines for Water Recycling (AGWR). If the ADI data were not available, the ADI values were calculated by dividing the no observed effect level (NOEL) from literature by a safety of factor of 10,000 for cytotoxic compounds or 1,000 for all other micropollutants. The body

weight (BW) of an average adult was taken as 70 kg. FC is the daily consumption of fish which is 56 g/day as stated by FSANZ (2017).

To evaluate the risk of each micropollutant bioaccumulating in fish, a risk quotient (RQ) was determined for each liver and flesh sample (Equation 4). This approach compares the detected concentrations to the calculated trigger values to provide a definitive risk assessment of the chemical of concern. The risk is high if RQ≥1, medium if 0.1<RQ≤1 and low if RQ≤0.1 (Aurélien et al., 2013; Hoon et al., 2009).

$$RQ = \frac{C_{Fish\ tissue}}{\text{Trigger Value (ng/g)}} \quad (4)$$

3. Results and discussion

3.1. Micropollutants in reclaimed water

Of the 49 micropollutants monitored in this study, 20 compounds were detected in the reclaimed water reservoir above the LOQ values of our analytical technique (Figure 1). Concentrations of these micropollutants in the reservoir were well below the guideline values indicated by the AGWR, with benzotriazole being the only exception. It is noted that the reclaimed water is only used for the irrigation of sporting facilities and pastures.

The mean concentration of benzotriazole in the reservoir was 675 ng/L, which is well above the AGWR guideline value of 7 ng/L. The concentration of benzotriazole in reclaimed water in this study is within the range previously reported in the literature. Lu et al. (2017) reported a mean concentration of benzotriazole of 5.7 ng/L in the effluent from nine municipal WWTPs in Canada. On the other hand, Herzog et al. (2014) surveyed three WWTPs in Germany and reported the mean concentration of benzotriazole in the effluent in the range from 3,500 to 9,300 ng/L. Benzotriazole is an industrial chemical used as a corrosion inhibitor, UV stabilizer, pharmaceutical precursor and dishwashing ingredient. The high concentration observed in this study is likely due to its widespread usage, high solubility, resistance to biodegradation and wastewater treatment (Alotaibi et al., 2015). Other micropollutants occurring at notable concentrations (but still below the AGWR guideline values) included saccharin, salicylic acid, and sucralose. The concentrations of these micropollutants in the reclaimed water in this study were also comparable to that in the literature (Tran et al., 2015).

PFOS and PFOA were detected, although only at just above the limit of quantification, in all aqueous samples from the reclaimed water reservoir. These chemicals are representative of the group of perfluoroalkyl substances (PFASs) and their polyfluorinated precursors. PFASs are used in many industrial and commercial applications including cosmetics, lubricants, fire-fighting foams,

and stain resistant coatings (Arvaniti and Stasinakis, 2015; Wang et al., 2017). As a result, PFASs and their precursors are expected to occur at low concentration in municipal wastewater. These compounds are also highly persistent, thus, their occurrence in secondary treated effluent has been reported in several recent studies (Arvaniti and Stasinakis, 2015; Campo et al., 2014; Hu et al., 2016). PFOS and PFOA contamination associated with previous firefighting exercises has been widely reported in Australia and around the world (Baduel et al., 2017; Dauchy et al., 2017; Hu et al., 2016; Munoz et al., 2017).

[FIGURE 1]

3.2. Micropollutants in fish tissue

Overall, 20 micropollutants were detected in flesh samples (Figure 2) and 23 micropollutants were detected in Silver Perch liver samples (Figure 3) from the reservoir. Values reported here are within the range previously reported in the literature (Table 1). It is noted that the concentration of ten micropollutants (i.e. salicylic acid, parametamol, triamterene, primidone, benzophenone, clozapine, oxybenzone, diuron, TCEP, and bisphenol A) in either fish liver or flesh has not previously been reported in the literature for comparison to our study (Table 1). In this study, four micropollutants were detected in liver but not in flesh samples. They are 17β-estradiol, diazepam, trimethoprim, and verapamil. On the other hand, primidone was detected in flesh but not in liver samples. These results suggest the tendency of micropollutants to accumulate in liver tissue and the capacity of liver enzyme to metabolise them. It is also noteworthy that eight micropollutants (bisphenol A, carazolol, gemfibrozil, oxybenzone, paracetamol, triamterene, triclocarban, and verapamil) were detected in Silver Perch tissue, but were not identified in the water phase in the reservoir. Likewise, several micropollutants (including acesulfame, atenolol, dilantin, ibuprofen, propylparaben, saccharin, sucralose) were present in the reservoir (in some cases at concentration of up to several hundreds of ng/L e.g. saccharine and sucralose), but were not identified in Silver Perch tissue. Results in Table 1 suggest that Silver Perch is a sensitive indicator species for some but not for all micropollutants. The results also highlight the limitation of using the BCF value for risk assessment since some micropollutants do not occur at above the LOQ in both the water phase and fish tissue. In addition, their concentrations in reclaimed water and fish tissue may not be constant. Thus, risk quotient analysis is also conducted in this study as discussed in the next section.

[FIGURE 2]

[FGIURE 3]

[TABLE 1]

As discussed in Section 3.1, benzotriazole concentrations in the reservoir exceeded the AGRW guideline value; however, its occurrence in Silver Perch flesh and liver was negligible. The mean

concentration of benzotriazole in Silver Perch flesh was 1.5 ng/g and was detected in 18% of all samples. In comparison, benzotriazole was detected in 98% of all liver samples at a mean concentration of 18 ng/g. The maximum benzotriazole concentration detected in liver was 87 ng/g, which is similar to that (65 ng/g) detected in Bream liver in a German river (Wick et al., 2016).

Of all micropollutants listed in Table 1, the trigger values of PFOS and PFOA have been published by FSANZ (2017). The concentrations of PFOS in Silver Perch flesh exceeded the FSANZ guideline limit for human consumption (although it must be noted that fish in the reservoir was not for human consumption). It is noted that the trigger value is highly conservative and that the fish in this reservoir are for human consumption. In total, 51 out of the 53 samples analysed in this study were above the trigger value for PFOS (5.2 ng/g) in flesh specified by FSANZ (2017). The mean concentration of PFOS in flesh samples was 57.7 ng/g, which is ten times greater than the trigger value.

Most liver samples did not exceed the PFOS trigger value (Figure 3), which is set at a higher level in liver (280 ng/g) than in flesh (5.2 ng/g). The mean concentration of PFOS in Silver Perch liver (115 ng/g) in this study is comparable to a previous recent Australian study by Thompson et al., (2011) who reported a mean PFOS concentration of 70 ng/g in Sea Mullet liver from Sydney Harbour.

The PFOS concentrations in fish flesh and liver reported here are higher than most other studies conducted overseas (Hoon et al., 2009; Houde et al., 2011; Naile et al., 2010; Nania et al., 2009; Quinete et al., 2009; Squadrone et al., 2014). To date, only two studies, which were conducted at heavily polluted sites, have reported higher PFOS concentrations in fish flesh and liver than the values in this study (Lin et al., 2014; Naile et al., 2010).

Taylor et al. (2018) observed strong relationships between the proximity of the capture of fish to heavily contaminated areas. Taylor et al. (2018) also suggested that this relationship can be affected by the movement patterns of the fish and the hydrology of the estuary that the fish were within. Taylor (2018) recently surveyed the occurrence of PFOS in flesh tissue of four fresh water species (i.e. Murray Cod, Golden Perch, Common Carp, and Common Yabby) in water bodies near Tamworth Airport where PFAS contamination from legacy fire flighting chemicals has been confirmed. Surface water sampling at the time of their study revealed concentrations that exceeded the guideline value for drinking water (i.e. higher than the reservoir concentration in this study). Nevertheless, Taylor (2018) only observed PFOS in flesh tissue at above the trigger value by FSANZ (2017) for two (i.e. Common Carp and Murray Cod) of the four species. In addition, the mean PFOS concentrations the flesh of these two species are only marginally above the trigger value. Unlike the previous studies by Taylor and co-workers (Taylor, 2018; Taylor et al., 2018), the fish in this study have been contained within the reservoir, thus, providing the conditions for high

levels of accumulation of PFOS. Our research appears to be first published estimates of PFOS concentrations in fish exposed to treated effluent within Australia.

The elevated PFOS concentration in Silver Perch liver reported in Table 1 is significant. Shoalhaven is a semi-rural township with low population density and a large wastewater collection catchment, thus the rate of storm water run-off infiltration into the sewer is considerable. The occurrence of PFOS can possibly be attributed to previous contamination in the area and infiltration into the wastewater collection network. As discussed in Section 3.1, the background concentration of PFOS in the reservoir was just above the LOQ value (i.e. <10 ng/L). All previous studies examined the occurrence of PFOS in natural waters where there can potentially be movement of fish (Taylor et al., 2018). In other words, fish individuals might not be continuously exposed to PFOS in their habitat for their entire life. By contrast, the Silver Perch in this study are in captivity and are expected to have been continuously exposed to PFOS in the reservoir since their juvenile stage. Thus, this study provides, for the first time, the extent of PFOS bioaccumulation in fish under realistic conditions.

As noted above, the number of micropollutants detected in liver was higher than those detected in flesh samples. More significantly, the concentrations in liver were often higher than those in flesh. Indeed, with benzophenone being the only exception, the mean liver/flesh ratio for all micropollutants in Figure 4 was above one. Results in Figure 4 suggest the tendency of micropollutants to accumulate more in liver than flesh. Benzotriazole and PFOS had the highest mean liver/flesh ratios of 32 and 71, respectively.

[FIGURE 4]

3.3. Bioconcentration of micropollutants in Silver Perch

BCF values were calculated for all micropollutants detected in the water phase as well as flesh and liver samples (Table 2). PFOS was the only micropollutant with a high bioaccumulation potential (Table 2). BCF values of all other micropollutants in Table 2 were low, indicating insignificant risk of bioconcentration. To date, only a few studies have reported BCF values of micropollutants in fish. These studies focus on several different species and different groups of micropollutants. Thus, a comprehensive comparison between values reported here and the literature may not be possible. Nevertheless, the BCF of 26,000 for PFOS reported in Table 2 appears to be higher than most available literature values and is approximately twice the value (i.e. 12,400) previously determined by Hoon et al., (2009). Hoon et al., (2009) reported the BCF of 12,400 for PFOS in Mullet in a saline lake (that is connected to the East China Sea). Similarly, Taniyasu et al., (2003) reported that the BCF value in five different fish species in Tokyo Bay was in the range of 1,400 to 21,100.

[TABLE 2]

3.4. Risk assessment

To further characterise the risks associated with micropollutants in the Silver Perch within the reclaimed water reservoir, risk quotients were calculated for all compounds detected in either flesh or liver. PFOS and benzotriazole were the only two micropollutants with high or medium risks (Figure 5). It is noted that the Silver Perch were introduced to the wastewater reservoir to control algae and not for human consumption (the reservoir is not open to the public and fishing is prohibited). Nonetheless, the risk quotient provides a measure of the potential significance of the tissue concentrations and represent a level at which further investigation would be triggered. Figure 5 shows that PFOS presented a medium risk when considering liver tissue and a high risk when considering flesh tissue. The mean risk quotient values of PFOS for liver and flesh were 0.9 and 11, respectively. Results in Table 2 and Figure 5 also suggest that bioconcentration factor and risk quotient can complement each other to offer additional insights during risk assessment.

Notably, benzotriazole showed a medium risk when considering its concentration in flesh, but a high risk for liver (0.6 and 7.2, respectively). Benzotriazole is commonly used as an UV filter in skin care products and anti-corrosion agent in other industrial applications. Despite its widespread occurrence in the aquatic environment, this appears to be the first time the RQ of benzotriazole has been reported in fish.

[FIGURE 5]

4. Conclusions

This study reported the bioconcentration of micropollutants in fish living in a reclaimed effluent impoundment. Of the 49 micropollutants monitored in this study, 20 compounds were detected in the water phase above their limits of quantification. The numbers of micropollutants detected in Silver Perch liver and flesh were 23 and 19, respectively. With benzotriazole being the only exception, all micropollutants in reclaimed water were well below the Australian Guideline for Recycled Water specified value for potable purposes. It is noted that not all micropollutants in the water phase were detected in Silver Perch flesh and liver tissues. Similarly, not all micropollutants detected in Silver Perch flesh and liver were identified in reclaimed water. In most cases, micropollutant concentration in liver was higher than in flesh. PFOS was detected at trace levels in the reclaimed water and did not exceed the guideline value but showed high and medium bioconcentration factors in Silver Perch liver and flesh, respectively. On the other hand, the risk quotients for PFOS were medium and high when considering its concentration in Silver Perch liver and flesh, respectively. Results in this study highlight the need to consider multiple parameters such as environmental concentration, bioconcentration, and risk quotient for risk evaluation. Further

investigation is recommended to better understand the risk associated with PFOS in the environment and reclaimed water.

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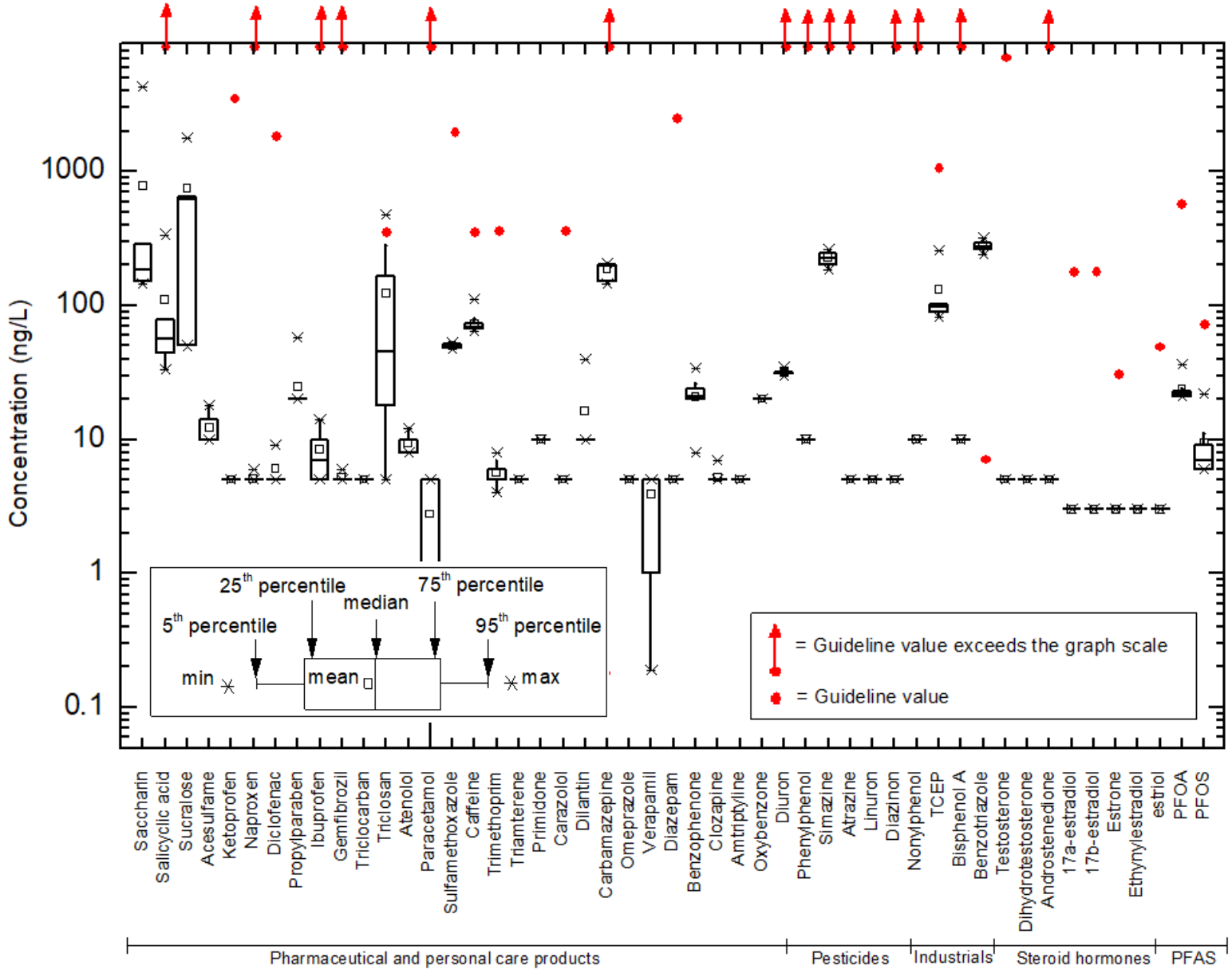
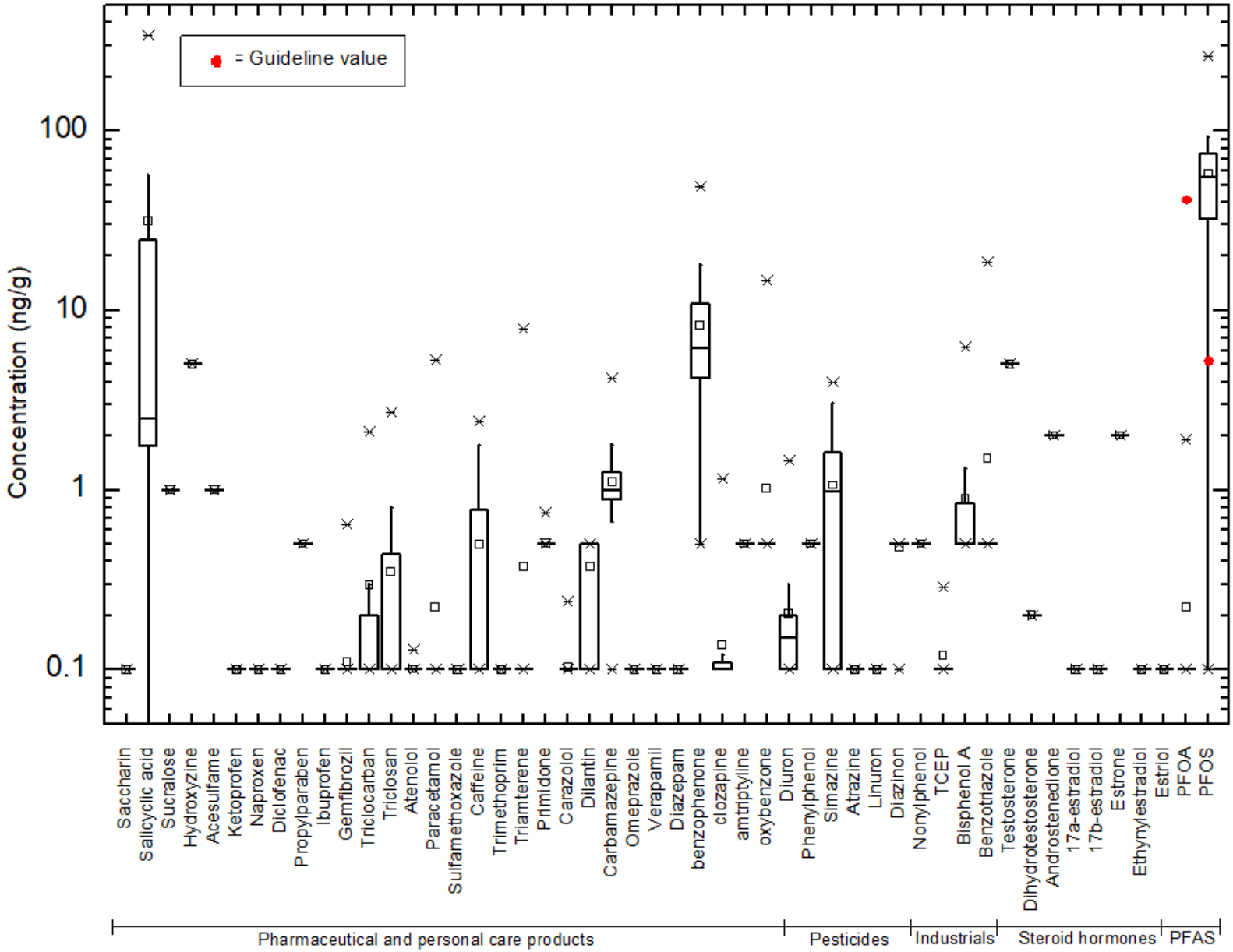


480

481 **Figure 1:** Concentrations of micropollutants detected within the reservoir and the corresponding AGWR guideline value (red dots). The box
482 plots were obtained from 9 water samples.



483

484 **Figure 2:** Concentrations of micropollutants found within the fish flesh (ng/g fresh weight) and the corresponding trigger value published by
485 Food Standard Australia and New Zealand (red dots). The box plots were obtained from at least 45 fish samples.

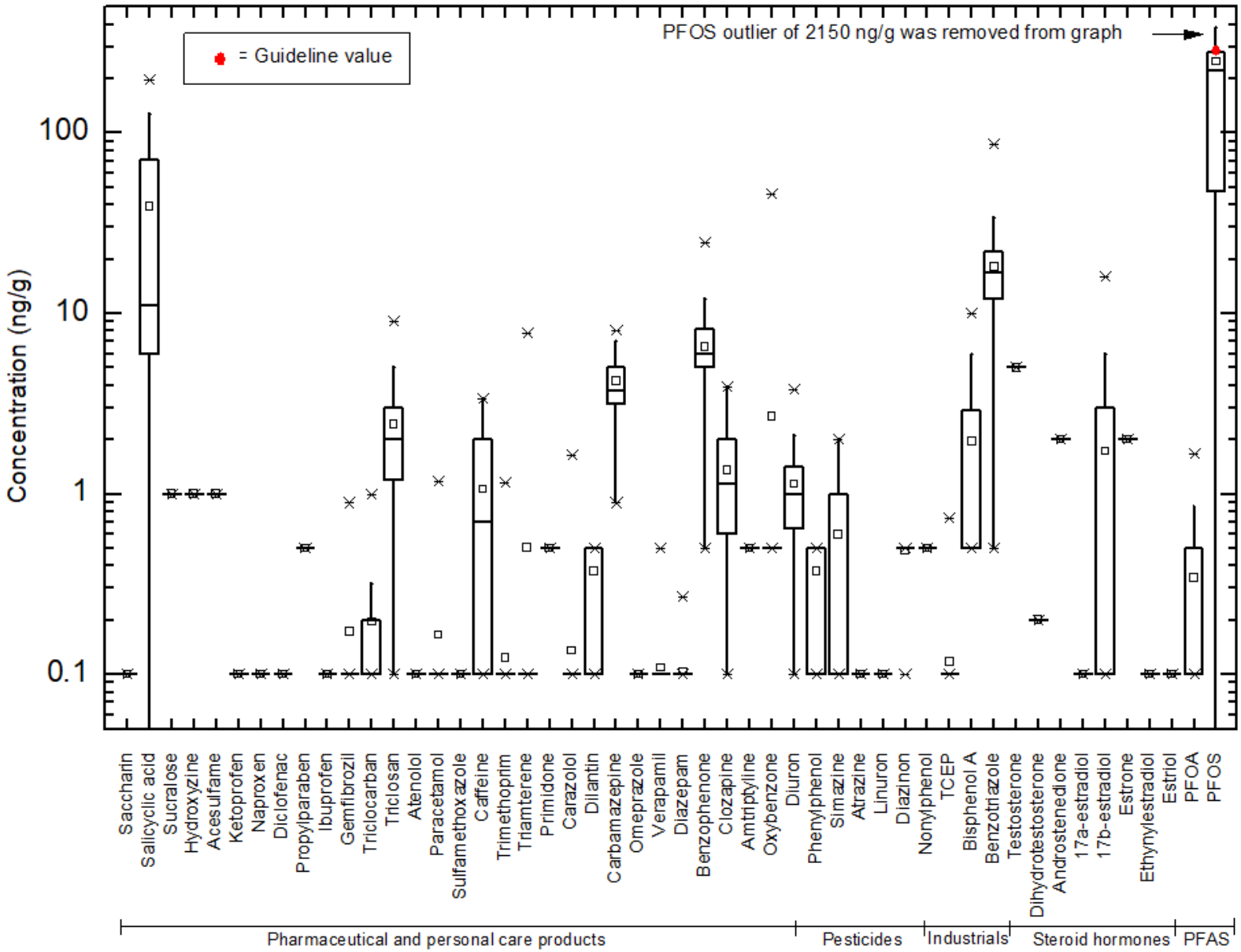


Figure 3: Concentrations of micropollutants found within Silver Perch liver. Only PFOS and PFOA have their trigger values published by Food Standard Australia and New Zealand. The trigger value of PFOS is shown as a red dot while the trigger value of PFOA is beyond the range of this graph. The box plots were obtained from at least 45 fish samples.

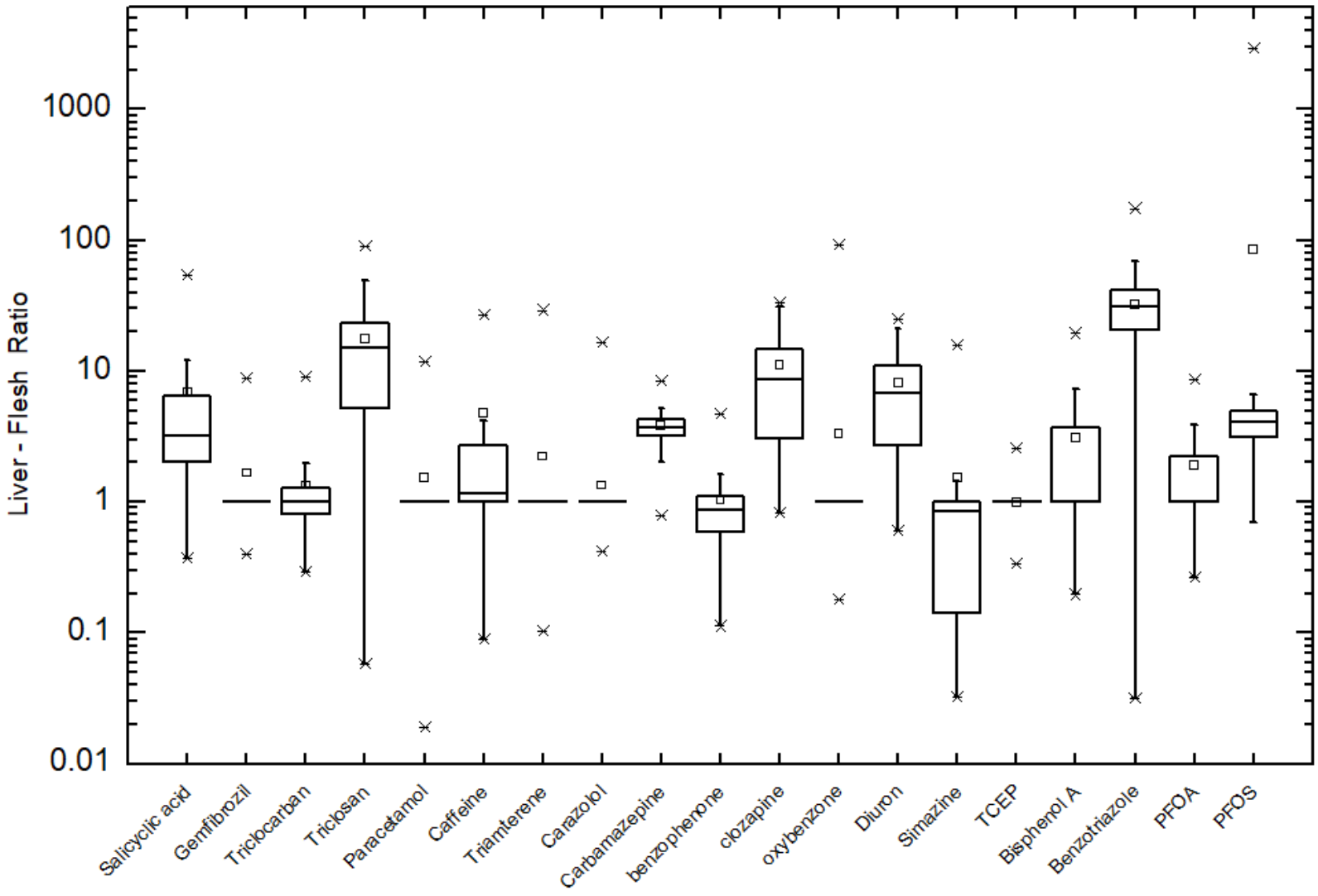


Figure 4: The Liver – Flesh ratio of micropollutant concentrations detected in both liver and flesh samples.

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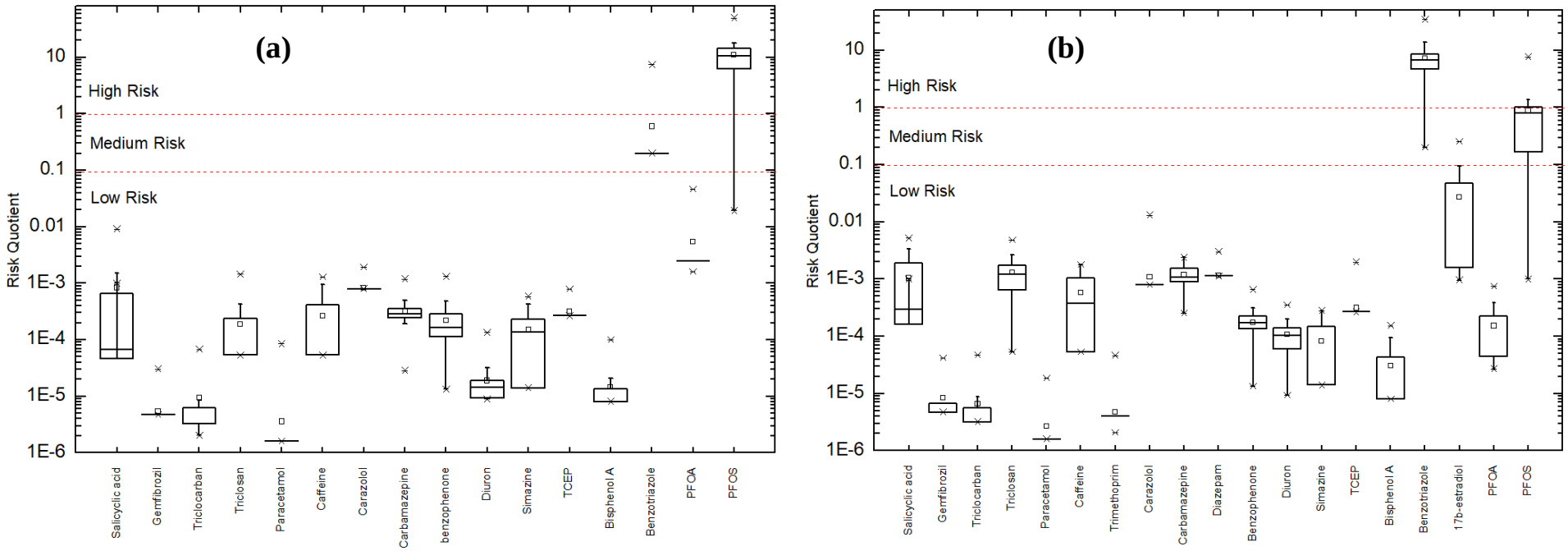

495 **Figure 5:** Risk quotients for concentrations of micropollutants detected in fish (a) flesh and (b) liver.

496 **Table 1:** Micropollutant concentration in Silver Perch flesh and liver observed in this study in comparison to other fish species in literature in
497 ng/g. ND: Not detected.

Micropollutant	Flesh – This study	Flesh – Literature	Liver – This study	Liver – Literature	Ref.
Salicylic Acid	<0.1 - 341.60	-	<0.1 - 195.5	-	
Gemfibrozil	<0.1 - 0.64	ND	0.1 - 0.89	ND - 90	(Ramirez et al., 2009; Ramirez et al., 2007)
Triclocarban	<0.1 - 2.11	ND - 157	<0.1 - 0.9	ND - 7.14	(Ramaswamy et al., 2011; Yao et al., 2016)
Triclosan	<0.1 - 2.72	ND - 507	<0.1 - 9.05	ND - 25.4	(Huerta et al., 2013; Ramaswamy et al., 2011; Ramirez et al., 2009; Yao et al., 2016)
Paracetamol	<0.1 - 5.32	-	<0.1 - 1.17	-	
Caffeine	<0.1 - 2.42	ND - 21.40	<0.1 - 3.36	-	(Du et al., 2016; Huerta et al., 2013; Ramirez et al., 2007; Wang et al., 2012)
Trimethoprim	-	-	<0.1 - 1.16	ND - 2.13	(Zhao et al., 2015)
Triamterene	<0.1 - 7.89	-	<0.1 - 7.74	-	
Primidone	<0.5 - 0.75	-	-	-	
Carazolol	<0.1 - 0.24	ND - 13	<0.1 - 1.65	ND	(Huerta et al., 2013; Moreno-González et al., 2016; Valdés, 2016)
Carbamazepine	<0.1 - 4.18	ND - 33	0.89 - 8.34	ND - 8	(Du et al., 2016; Liu et al., 2015; Moreno-González et al., 2016; Ramirez et al., 2009; Ramirez et al., 2007; Valdés, 2016; Wang et al., 2012)
Verapamil	ND	-	<0.1 - 0.5	-	
Diazepam	ND	-	<0.1 - 0.27	-	
Benzophenone	<0.5 - 39.92	ND - 24.3	<0.5 - 24.51	-	(Gago-Ferrero et al., 2015)
Clozapine	<0.1 - 0.45	-	<0.1 - 3.88	-	
Oxybenzone	<0.5 - 14.79	-	<0.5 - 46.03	-	

Diuron	<0.1 - 1.45	-	<0.1 - 3.82	-	
Simazine	<0.1 - 3.07	1,000 – 5,000	<0.1 - 2.00	2200	(Reindl et al., 2015)
TCEP	<0.1 - 0.29	ND – 5.11	<0.1 - 0.74	-	(Huerta et al., 2013)
Bisphenol A	<0.5 - 6.22	ND – 1,020	<0.5 - 9.68	-	(Dan et al., 2017; Huerta et al., 2013; Wang et al., 2016; Wang et al., 2015; Yang et al., 2014)
Benzotriazole	<0.5 - 18.58	ND - 7.95	<0.5 - 87.33	ND - 65	(Peng et al., 2015; Wick et al., 2016)
17β-estradiol	ND	-	<LOQ - 16.00	-	
PFOA	<0.1 - 1.90	ND - 109	<0.1 - 1.68	ND - 142	(Hoon et al., 2009; Lin et al., 2014; Llorca et al., 2009; Naile et al., 2010; Nania et al., 2009; Quinete et al., 2009; Thompson et al., 2011)
PFOS	<0.1 - 259.09	ND - 1,828	<0.1 - 667.9	ND - 28,933	(Hoon et al., 2009; Lin et al., 2014; Llorca et al., 2009; Naile et al., 2010; Nania et al., 2009; Quinete et al., 2009; Squadrone et al., 2014; Thompson et al., 2011)

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499 **Table 2:** BCF (L/kg) of micropollutants detected in both the reservoir and fish tissue.

Compound	Flesh	Liver
Salicylic acid	290	360
Triclosan	3	20
Caffeine	7	15
Carbamazepine	6	23
Benzophenone	400	310
Diuron	6	37
Simazine	5	3
TCEP	0.9	0.9
Benzotriazole	5	64
PFOS	6,000	26,000
PFOA	9	14

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