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

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

### Abstract

This study examined the occurrence of 49 micropollutants in reclaimed water and Silver Perch (*Bidyanus bidyanus*) living in a reclaimed water reservoir. The numbers 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  $675 \pm 130$  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.

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

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

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

## 19 **1. Introduction**

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

30 A core objective for all water recycling projects is public health protection (Drewes and Khan,  
31 2015). Perceived risks associated with public health increase as the end usage of recycled water  
32 shifts from industrial, irrigation, non-potable, to potable reuse. Thus, there is a growing interest in  
33 the scientific community to better understand and quantify risks associated with water recycling,  
34 particularly for high value usage. Research to date has highlighted the ubiquitous occurrence of

35 many organic chemicals (hereafter called micropollutants) in treated effluent albeit only at trace  
36 level (Alidina et al., 2014; Luo et al., 2014; Osorio et al., 2012; Rivetti et al., 2017; Zhou et al.,  
37 2013). Many of these micropollutants are naturally produced and excreted by humans (such as  
38 steroid hormones) or are intrinsically linked to human activities (such as pharmaceutical and  
39 personal care products), thus, their release to sewers is inevitable (Tran et al., 2014a; Tran et al.,  
40 2014b; Yang et al., 2017).

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

52 The Shoalhaven Water Reclaimed Water Management Scheme (REMS) is one of the largest and  
53 most complex water recycling schemes undertaken by a regional water authority in Australia  
54 (Gould et al., 2003). The key objective of REMS is to maximise the use of reclaimed water for  
55 irrigation of golf courses and sporting facilities, as well as agriculture production, rather than  
56 disposing of it into the environment. Since its commission in 2002, REMS has provided over  
57 20,000 ML of reclaimed water to golf courses, sporting grounds, and dairy farms covering over 500  
58 hectares of land for irrigation.

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

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

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

## 71 **2. Materials and methods**

### 72 **2.1. Sample collection**

73 Water and Silver Perch samples were collected from the REMS 600 ML reservoir for reclaimed  
74 water storage in the Shoalhaven region. Shoalhaven is a semi-rural township located approximately  
75 200 km south of Sydney. The Silver Perch (*Bidyanus bidyanus*) is a native freshwater fish with  
76 natural distribution over the entire western drainage of New South Wales, including most of the  
77 Murray-Darling Basin in Australia. As an omnivorous species, their diet includes invertebrates and  
78 aquatic vegetation.

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

### 87 **2.2. Chemicals and Standards**

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

### 92 **2.3. Solid phase extraction of aqueous samples**

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

102 samples were extracted onto the SPE cartridges through teflon lines at a flow rate of approximately  
103 15 mL/min. The cartridges were gently dried under pure nitrogen for 30 minutes. Target  
104 micropollutants were eluted from the dried cartridges under gravity using methanol (2 x 3 mL) and  
105 MTBE (3 mL). The combined eluants were evaporated to approximately 1 mL under nitrogen using  
106 a Turbo-Vap (Caliper Life Sciences, Waltham, MA. USA) and transferred to a 2 mL amber  
107 autosampler vial for quantification.

#### 108 **2.4. Fish flesh and liver extraction**

109 Approximately 100 g of side fillet flesh and the entire liver were obtained from each fish. The flesh  
110 samples were individually homogenized using a processing blender. Each liver sample was ground  
111 using a mortar and pestle. Approximately 10 g of processed flesh or liver samples were placed into  
112 plastic test tubes and capped. Similar to aqueous samples, surrogate standard of 50 ng (50 µL of a  
113 1mg/L standard solution) of each of the 44 isotopically labelled compounds (corresponding to 44 of  
114 the 49 micropollutants selected for monitoring in this study) was introduced into all tissue samples  
115 for method recovery and quantification. Acetonitrile (8 mL) was then added to each tissue sample  
116 and mixed using a vortex for 1 minute following the method described by (Johnston et al., 2002).  
117 The mixture of tissue samples and acetonitrile was centrifuged for 3 minutes at 4200 rpm. The  
118 supernatant was pipetted into glass test tubes and dried under nitrogen using a TurboVap. Once the  
119 samples had evaporated to approximately 20% of their original volume, they were transferred to  
120 500 mL of Milli-Q water for subsequent SPE as described for aqueous samples in section 2.3.

#### 121 **2.5. Instrumental analysis**

122 For identification and quantification of micropollutants, a high performance liquid  
123 chromatography/tandem mass spectrometer (HPLC-MS/MS) was used. Separation was achieved  
124 using an Agilent 1200 series HPLC system (Agilent, Santa Clara, CA, USA) equipped with a 150 x  
125 4.6 mm, 5 µm particle size, Luna C18(2) column (Phenomenex, Torrance, CA, USA). The target  
126 analytes and their surrogates were identified using an API 4000 triple quadrupole mass  
127 spectrometer (Applied Biosystems, Foster City, CA, USA) with electrospray ionization (ESI)  
128 working in both positive and negative electro-spray modes, as described by (Vanderford and  
129 Snyder, 2006). An injection volume of 10 µL was used for all samples. For complete confirmation  
130 of target analytes two parent ion-product ion transitions were monitored for each analyte and the  
131 surrogate standard. Additionally, relative retention times of the analytes and surrogate standards  
132 were monitored.

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

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

## 142 **2.6. Data analysis**

### 143 2.6.1 Bioconcentration factor

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

$$147 \quad \text{BCF (L/kg)} = \frac{C_{\text{Fish tissue}}}{C_{\text{Water}}} \quad (1)$$

148 BCF is an indicator of the accumulation of a chemical in or on an organism when the source of  
149 chemical is solely water. A micropollutant is regarded to be ‘bioaccumulative’ if the BCF exceeds  
150 5,000 or ‘potentially bioaccumulative’ if the BCF is between 2,000-5,000 (Dan et al., 2017; Liu et  
151 al., 2017).

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

$$155 \quad \text{Liver – Flesh Ratio} = \frac{C_{\text{Liver}}}{C_{\text{Flesh}}} \quad (2)$$

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

### 158 2.5.2 Risk assessment

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

$$161 \quad \text{Trigger Value (ng/g)} = \frac{\text{ADI (ng/kg bw/day)} \times \text{BW (kg)}}{\text{FC (g/day)}} \quad (3)$$

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



166 weight (BW) of an average adult was taken as 70 kg. FC is the daily consumption of fish which is  
167 56 g/day as stated by FSANZ (2017).

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

$$173 \quad RQ = \frac{C_{\text{Fish tissue}}}{\text{Trigger Value (ng/g)}} \quad (4)$$

### 174 **3. Results and discussion**

#### 175 **3.1. Micropollutants in reclaimed water**

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

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

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

198 and stain resistant coatings (Arvaniti and Stasinakis, 2015; Wang et al., 2017). As a result, PFASs  
199 and their precursors are expected to occur at low concentration in municipal wastewater. These  
200 compounds are also highly persistent, thus, their occurrence in secondary treated effluent has been  
201 reported in several recent studies (Arvaniti and Stasinakis, 2015; Campo et al., 2014; Hu et al.,  
202 2016). PFOS and PFOA contamination associated with previous firefighting exercises has been  
203 widely reported in Australia and around the world (Badel et al., 2017; Dauchy et al., 2017; Hu et  
204 al., 2016; Munoz et al., 2017).

205 **[FIGURE 1]**

### 206 **3.2. Micropollutants in fish tissue**

207 Overall, 20 micropollutants were detected in flesh samples (Figure 2) and 23 micropollutants were  
208 detected in Silver Perch liver samples (Figure 3) from the reservoir. Values reported here are within  
209 the range previously reported in the literature (Table 1). It is noted that the concentration of ten  
210 micropollutants (i.e. salicylic acid, paracetamol, triamterene, primidone, benzophenone, clozapine,  
211 oxybenzone, diuron, TCEP, and bisphenol A) in either fish liver or flesh has not previously been  
212 reported in the literature for comparison to our study (Table 1). In this study, four micropollutants  
213 were detected in liver but not in flesh samples. They are 17 $\beta$ -estradiol, diazepam, trimethoprim, and  
214 verapamil. On the other hand, primidone was detected in flesh but not in liver samples. These  
215 results suggest the tendency of micropollutants to accumulate in liver tissue and the capacity of  
216 liver enzyme to metabolise them. It is also noteworthy that eight micropollutants (bisphenol A,  
217 carazolol, gemfibrozil, oxybenzone, paracetamol, triamterene, triclocarban, and verapamil) were  
218 detected in Silver Perch tissue, but were not identified in the water phase in the reservoir. Likewise,  
219 several micropollutants (including acesulfame, atenolol, dilantin, ibuprofen, propylparaben,  
220 saccharin, sucralose) were present in the reservoir (in some cases at concentration of up to several  
221 hundreds of ng/L e.g. saccharine and sucralose), but were not identified in Silver Perch tissue.  
222 Results in Table 1 suggest that Silver Perch is a sensitive indicator species for some but not for all  
223 micropollutants. Further research is suggested to survey the occurrence of micropollutants in other  
224 non-migratory fish species and in fish organs with different lipid contents. The results also highlight  
225 the limitation of using the BCF value for risk assessment since some micropollutants do not occur  
226 at above the LOQ in both the water phase and fish tissue. In addition, their concentrations in  
227 reclaimed water and fish tissue may not be constant. Thus, risk quotient analysis is also conducted  
228 in this study as discussed in the next section.

229 **[FIGURE 2]**

230 **[FIGURE 3]**

231 **[TABLE 1]**

232 As discussed in Section 3.1, benzotriazole concentrations in the reservoir exceeded the AGRW  
233 guideline value; however, its occurrence in Silver Perch flesh and liver was negligible. The mean  
234 concentration of benzotriazole in Silver Perch flesh was 1.5 ng/g and was detected in 18% of all  
235 samples. In comparison, benzotriazole was detected in 98% of all liver samples at a mean  
236 concentration of 18 ng/g. The maximum benzotriazole concentration detected in liver was 87 ng/g,  
237 which is similar to that (65 ng/g) detected in Bream liver in a German river (Wick et al., 2016).

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

246 Most liver samples did not exceed the PFOS trigger value (Figure 3), which is set at a higher level  
247 in liver (280 ng/g) than in flesh (5.2 ng/g). The mean concentration of PFOS in Silver Perch liver  
248 (115 ng/g) in this study is comparable to a previous recent Australian study by Thompson et al.,  
249 (2011) who reported a mean PFOS concentration of 70 ng/g in Sea Mullet liver from Sydney  
250 Harbour.

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

256 Taylor et al. (2018) observed strong relationships between the proximity of the capture of fish to  
257 heavily contaminated areas. Taylor et al. (2018) also suggested that this relationship can be  
258 affected by the movement patterns of the fish and the hydrology of the estuary that the fish were  
259 within. Taylor (2018) recently surveyed the occurrence of PFOS in flesh tissue of four fresh water  
260 species (i.e. Murray Cod, Golden Perch, Common Carp, and Common Yabby) in water bodies near  
261 Tamworth Airport where PFAS contamination from legacy fire fighting chemicals has been  
262 confirmed. Surface water sampling at the time of their study revealed concentrations that exceeded  
263 the guideline value for drinking water (i.e. higher than the reservoir concentration in this study).  
264 Nevertheless, Taylor (2018) only observed PFOS in flesh tissue at above the trigger value by  
265 FSANZ (2017) for two (i.e. Common Carp and Murray Cod) of the four species. In addition, the  
266 mean PFOS concentrations the flesh of these two species are only marginally above the trigger

267 value. Unlike the previous studies by Taylor and co-workers (Taylor, 2018; Taylor et al., 2018), the  
268 fish in this study have been contained within the reservoir, thus, providing the conditions for high  
269 levels of accumulation of PFOS. Our research appears to be first published estimates of PFOS  
270 concentrations in fish exposed to treated effluent within Australia.

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

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

289 **[FIGURE 4]**

### 290 **3.3. Bioconcentration of micropollutants in Silver Perch**

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

302

[TABLE 2]

303 **3.4. Risk assessment**

304 To further characterise the risks associated with micropollutants in the Silver Perch within the  
305 reclaimed water reservoir, risk quotients were calculated for all compounds detected in either flesh  
306 or liver. PFOS and benzotriazole were the only two micropollutants with high or medium risks  
307 (Figure 5). It is noted that the Silver Perch were introduced to the wastewater reservoir to control  
308 algae and not for human consumption (the reservoir is not open to the public and fishing is  
309 prohibited). Nonetheless, the risk quotient provides a measure of the potential significance of the  
310 tissue concentrations and represent a level at which further investigation would be triggered. Figure  
311 5 shows that PFOS presented a medium risk when considering liver tissue and a high risk when  
312 considering flesh tissue. The mean risk quotient values of PFOS for liver and flesh were 0.9 and 11,  
313 respectively. Results in Table 2 and Figure 5 also suggest that bioconcentration factor and risk  
314 quotient can complement each other to offer additional insights during risk assessment.

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

320

[FIGURE 5]

321 **4. Conclusions**

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

335 as environmental concentration, bioconcentration, and risk quotient for risk evaluation. Further  
336 investigation is recommended to better understand the risk associated with PFOS in the  
337 environment and reclaimed water.

### 338 **Acknowledgments**

339 Shoalhaven Water is gratefully acknowledged for their support during this study.

### 340 **References**

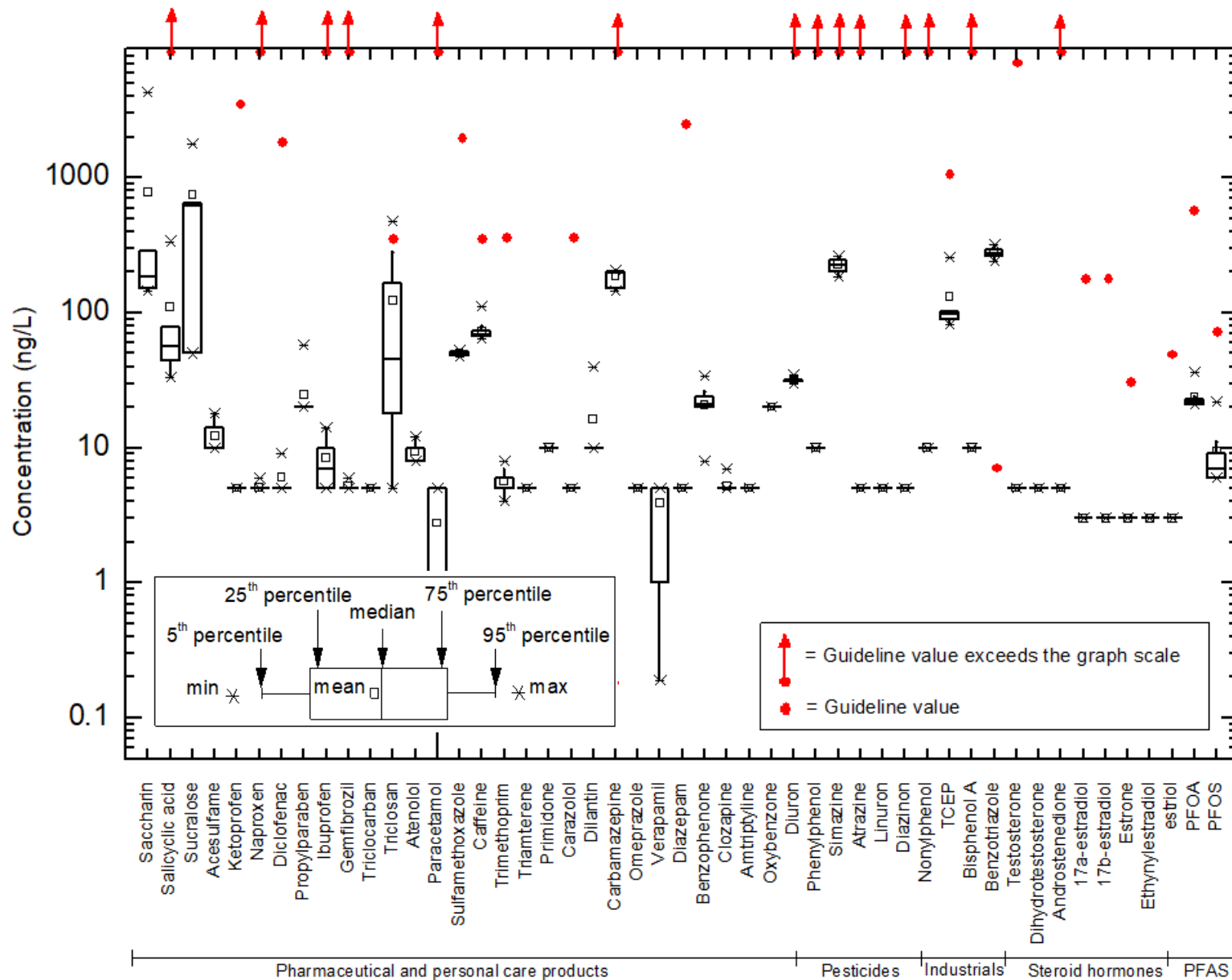
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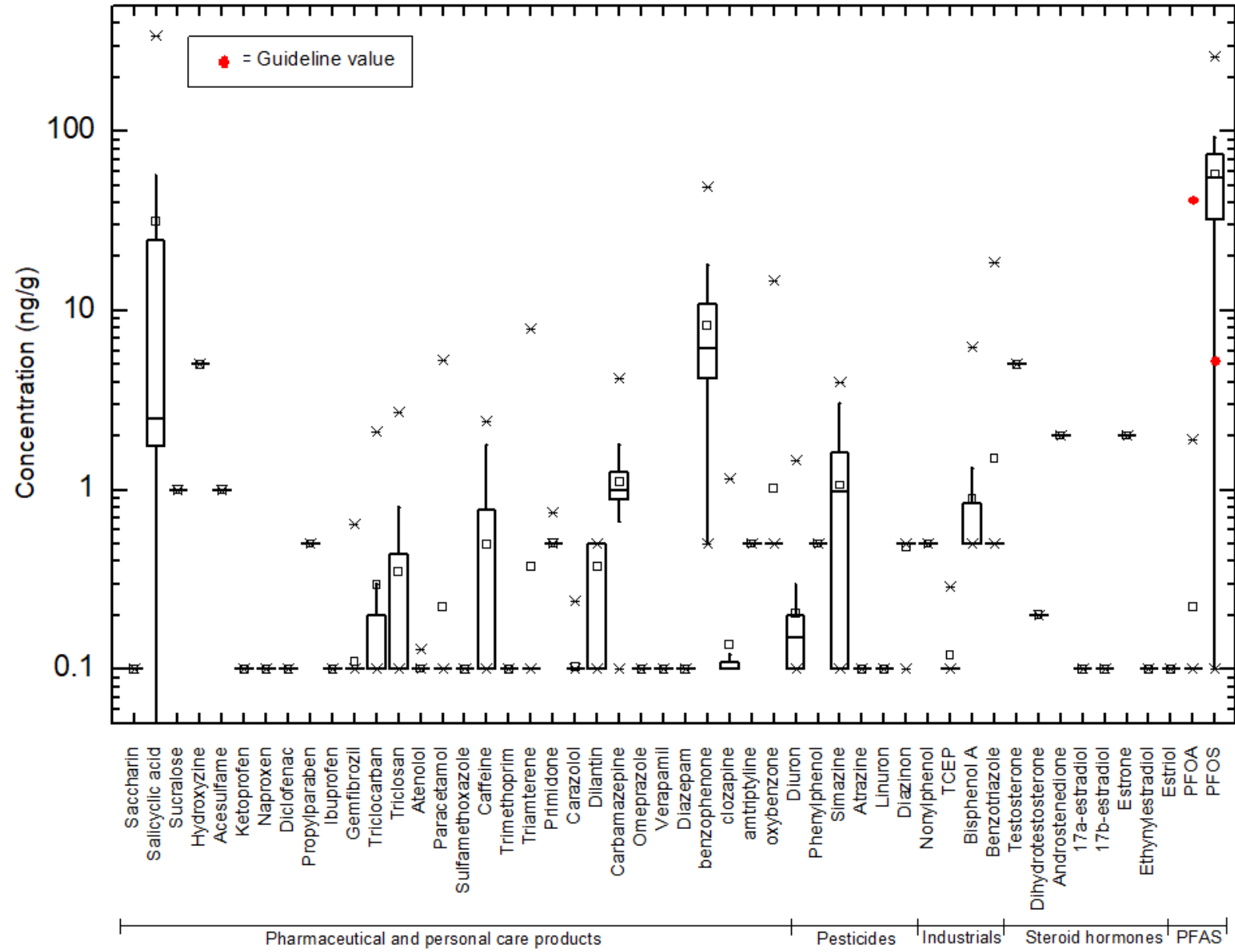


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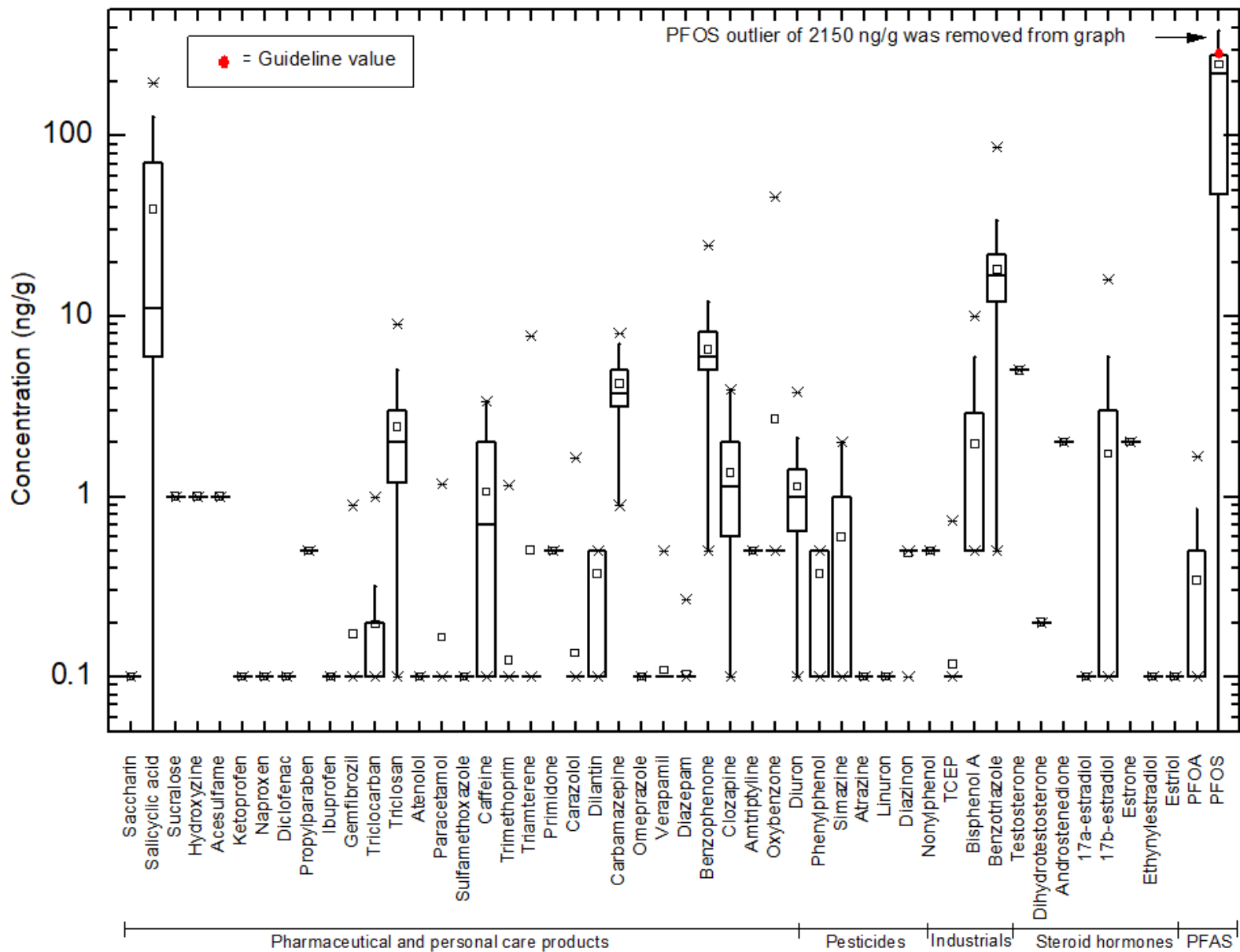


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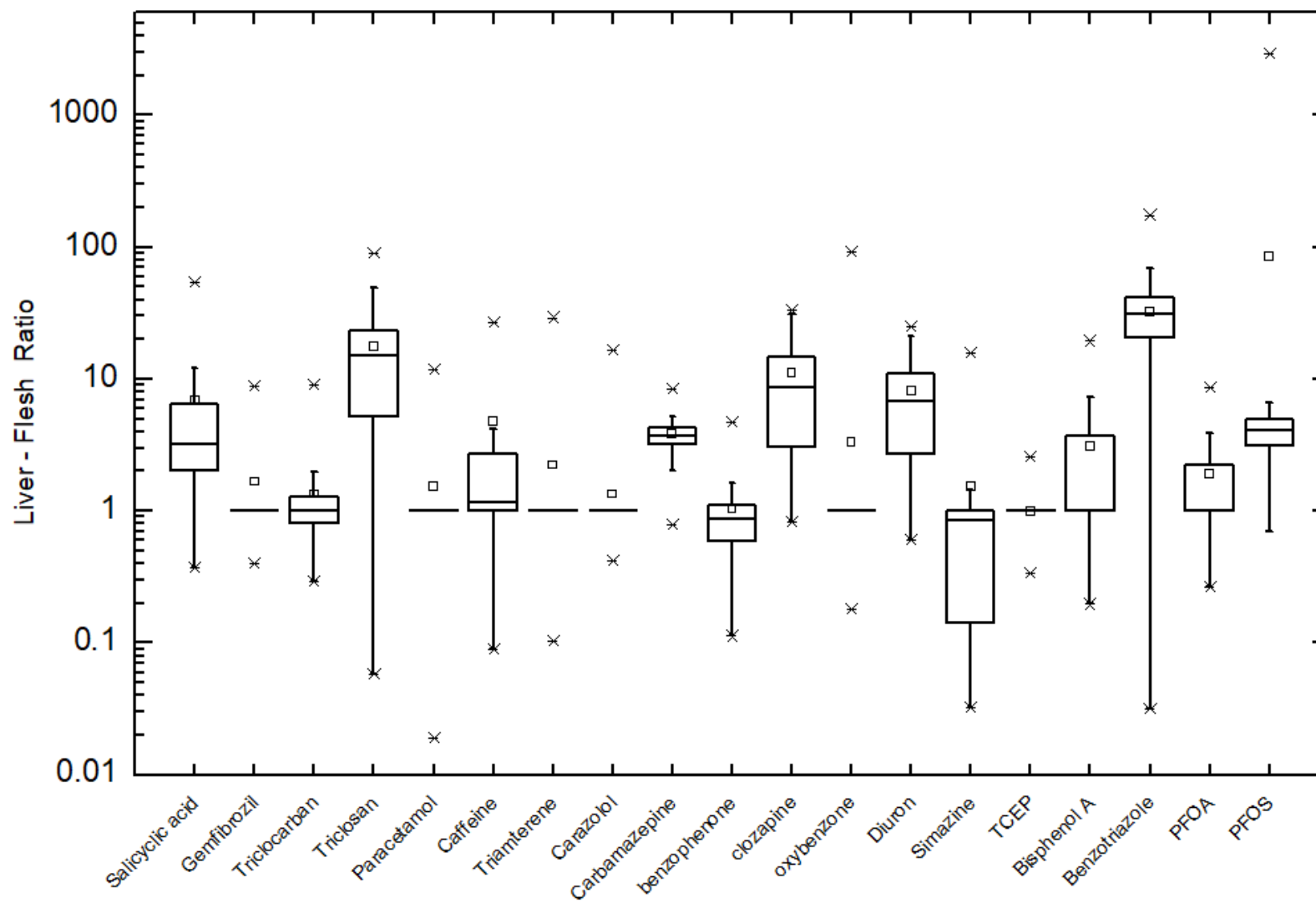
481 **Figure 1:** Concentrations of micropollutants detected within the reservoir and the corresponding AGWR guideline value (red dots). The box  
 482 plots were obtained from 9 water samples.



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.



487 **Figure 3:** Concentrations of micropollutants found within Silver Perch liver. Only PFOS and PFOA have their trigger values published by Food  
488 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  
489 this graph. The box plots were obtained from at least 45 fish samples.

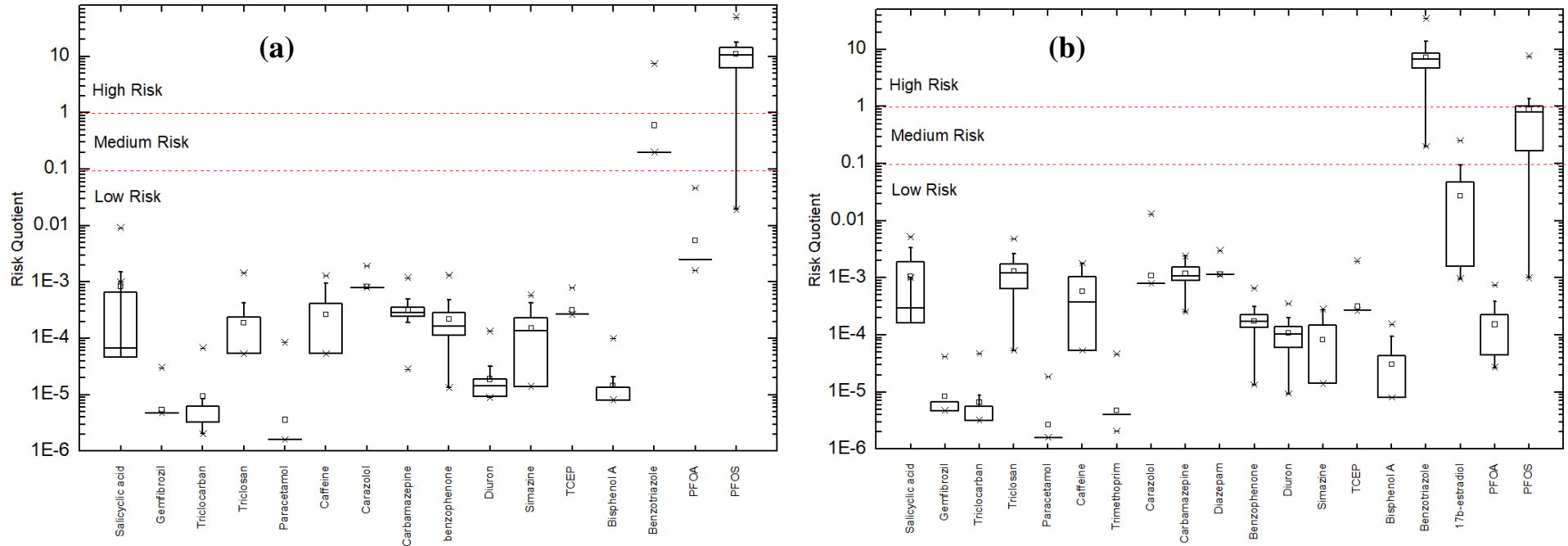


491

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

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494



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.

<b>Micropollutant</b>	<b>Flesh – This study</b>	<b>Flesh – Literature</b>	<b>Liver – This study</b>	<b>Liver – Literature</b>	<b>Ref.</b>
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)

498

499

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

<b>Compound</b>	<b>Flesh</b>	<b>Liver</b>
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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