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1 Perfluorinated compounds in the aquatic food chains of two subtropical estuaries

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18

Abstract

Per- and polyfluoroalkyl substances (PFASs) are ubiquitous in the environment and remain in largely unknown concentrations and with unknown effects on the African continent. This study aimed to assess 15 PFASs present in different compartments of the aMatikulu and uMvoti estuaries and to examine potential risks for human health through the consumption of contaminated fish. This is the first known study to assess PFASs in South African estuaries. Thirteen out of the fifteen PFASs were detected in water, sediment and biota samples from both estuaries, with perfluorooctanoic acid (PFOA), detected in every sample. PFOA concentrations from uMvoti water samples were the highest recorded to date in South African waters. PFOA was found in high concentrations in all water samples with an average range between 171 – 258 ng/L in the aMatikulu and 711 – 788 ng/L in the uMvoti. Perfluorooctane sulfonate (PFOS) concentrations in fish tissue samples were significantly higher than other PFASs. PFOS concentrations in all fish species caught in the aMatikulu ranged between 0.09 – 2.25 ng/g wet weight (ww) in muscle tissue and 1.5 -12.08 ng/g ww in liver tissue, while PFOA concentrations ranged between 0.08 – 0.67 ng/g ww in muscle tissue and 0.54 – 1.48 ng/g ww in liver tissue. Concentrations of PFASs were only measured in *Oreochromis mossambicus* from the uMvoti and contained PFOS concentrations ranging from 0.18 – 0.97 ng/g ww in muscle tissue and 7.29 – 27.96 ng/g ww in liver tissue. PFOA concentrations ranged between 0.12 – 0.58 ng/g ww in muscle tissue and 0.17 – 1.01 ng/g ww in liver tissue. PFAS concentrations in all fish sampled were below the calculated Minimum Risk Levels (MRLs) for safe human consumption.

Keywords: PFOS; PFOA; fish; human health; South Africa

44 Highlights

45 - Limited research on PFASs in South African estuarine systems
46 - Water, sediment, invertebrates and fish sampled in two estuaries
47 - PFOA present in all samples
48 - PFOA concentrations in uMvoti water highest recorded to date in South Africa
49 - The source of PFOAs likely to be pulp and paper mill adjacent to uMvoti Estuary
50 - All fish PFAS concentrations below Minimum Risk Levels for consumption
51

52 1. Introduction

53

54 Increasing quantities of chemical pollutants released from anthropogenic sources into estuaries
55 are of major concern for ecosystems and human health (Chowdhury and Maiti, 2016; Goksyor
56 and Forlin, 1992). Global declines in estuarine health have been attributed to growing coastal
57 human population pressures and the increasing abundance of pollutants (Barbier et al., 2011).
58 Worldwide monitoring studies in estuaries have identified a number of persistent pollutants,
59 including but not limited to, metals, persistent organic pollutants (POPs) such as
60 polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic
61 hydrocarbons (PAHs), dibenzodioxins (PCDDs), dibenzofurans (PCDFs) and per- and
62 polyfluoroalkyl substances (PFASs) (Darnerud et al., 2001; Lau et al., 2007; Pan and Wang,
63 2012; van der Oost et al., 2003). Persistent pollutants are highly resistant to environmental
64 degradation and may accumulate in biota (Jones and de Voogt, 1999). Growing concern over
65 the toxicity and persistence of these substances has resulted in many countries banning or
66 restricting their production and use, especially in developed countries (Darnerud et al., 2001).

67

68 Anthropogenic pressures on South African estuaries include coastal development, land-use
69 changes in the catchment, water abstraction, overexploitation of resources and industrial
70 wastes, with 15% of the approximately 300 estuaries under severe pollution pressure (Van
71 Niekerk et al., 2013). Marine pollution research in South Africa peaked between the 1960s and
72 1980s, with a focus on exposure studies and monitoring of trace metals (O'Donoghue and
73 Marshall, 2003; Wepener and Degger, 2012). PFAS studies are limited in South Africa, with
74 only a few recent studies highlighting their presence in aquatic ecosystems (Christie et al.,
75 2016; Groffen et al., 2018; Mudumbi et al., 2014a; Ojemaye and Petrik 2019; Verhaert et al.,
76 2017). Previous studies on PFASs in South African aquatic systems have been focused in

77 riverine systems, analysing water, sediment, fish and invertebrates (Groffen et al. 2018,
78 Mudumbi et al. 2014a, Mudumbi et al. 2014b, Verhaert et al. 2017). Christie et al. (2016)
79 analysed PFASs in the plasma of South African crocodiles and Ojemaye and Petrik (2019)
80 focused on pollutants present in the tissues of pelagic fish from Kalk Bay harbour, Cape Town.
81 South Africa lacks general knowledge on baseline and present-day concentrations of pollutants,
82 other than metals and organochlorines, due to the high costs of chemical analysis (Wepener
83 and Degger, 2012).

84

85 PFASs have been detected globally in estuarine sediments and water, and have also been
86 reported in mammalian tissues and human serum (D'Hollander et al., 2014; Lau et al., 2007).
87 Perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs) are the most well studied of the
88 PFASs globally and widely recognized for their persistence in the environment (Houde et al.,
89 2006). These compounds are extremely resistant to thermal, chemical and biological
90 degradation (He et al. 2015). This has caused a growing concern over their persistence and
91 toxicity. The presence of PFCAs and PFSAs in animal tissues from the Antarctic and Arctic
92 suggested that these compounds have long-term persistence in the environment and can be
93 transported far distances from their source (Christie et al., 2016). The consumption of fish is
94 among the main sources of PFAS exposure in the general human population, with the
95 consumption of contaminated drinking water and the ingestion of dust in indoor environments
96 (D'Hollander et al., 2010; Kaboré et al., 2018; Vestergren et al., 2012). It is of vital importance
97 to map PFAS distribution in South Africa and assess their risk to both aquatic organisms and
98 human health.

99

100 The present study aimed to investigate PFASs present in different compartments of the
101 ecosystem in two subtropical estuaries in South Africa, i.e., the uMvoti and aMatikulu

102 Estuaries. This is the first study of PFASs in subtropical estuaries along the east coast of Africa.
103 The central objective of this study was to investigate the distribution of PFASs in the aquatic
104 food webs of these two estuaries. The specific objectives of this study were to determine the
105 spatial distribution of 15 PFASs, specifically PFCAs and PFSAs, in water, sediment,
106 invertebrates and fish from a riverine and estuarine site within each of the estuaries. We also
107 aimed to investigate potential risks to human health based on the consumption of contaminated
108 fish present in the estuaries.

109

110 2. Materials and Methods

111

112 2.1. Study Area and Sample Collection

113

114 The present study was conducted in two subtropical estuaries, along the KwaZulu-Natal
115 coastline, on the east coast of South Africa (Figure 1a). The two estuaries are classified as
116 permanently open estuaries that are dominated by riverine flow (O'Brien et al., 2009).
117 Sampling was undertaken during the winter low-flow period in September 2016. Sampling was
118 carried out at two sites in each estuary, namely the aMatikulu Estuary mouth, aMatikulu N2
119 Bridge site (Figure 1b), the uMvoti Estuary mouth and the uMvoti Gledhow site (Figure 1c).
120 The upstream sites of both estuaries, i.e., Gledhow (uMvoti) and N2 Bridge (aMatikulu) were
121 situated above the estuarine delineation zone where a salinity gradient occurs.

122

123 Water samples for PFAS analysis were collected in triplicate from the water sub-surface in 50
124 ml conical polypropylene (PP) Falcon tubes at each site. The general physicochemical water
125 variables were recorded using a multiprobe system (YSI 556 MPS) and included temperature
126 (°C), pH, dissolved oxygen (DO) % and mg/L, salinity (mg/L), conductivity (μS/cm), total

127 dissolved solids (TDS) (mg/L) and water depth (cm). Sediment samples were collected using
128 a Van Veen Grab sampler, deployed three times at each site. The sediment in each grab sample
129 was mixed with a wooden stirring rod and one sample was collected in a 50 ml conical PP
130 Falcon tube. In the laboratory, the sediment was homogenised and separated for PFASs
131 analysis, total organic carbon (TOC) and grain size distribution. Water and sediment samples
132 were stored at -20°C until further analysis. Fish were sampled using 18 mm mesh cast nets and
133 a 5 m, 12 mm mesh seine net. The fish were identified, measured (standard length),
134 immobilized with a blow to the head, sacrificed by severing the spinal cord and pithing and
135 dissected (if large enough) in a field laboratory. The following fish species were caught:
136 *Ambassis natalensis, Oreochromis mossambicus*, and *Rhabdosargus holubi*. Muscle and liver
137 tissues were dissected and pooled for each species at each site, i.e., 10 livers from *O.*
138 *mossambicus* fish caught at the uMvoti Gledhow site were pooled to create one large liver
139 sample. Five sub-samples were then taken from each pooled muscle sample for each fish
140 species from each site and three replicate sub-samples were taken from each pooled liver
141 sample for each fish species from each site. Only three replicates could be taken for liver tissue
142 as there was less liver tissue available than muscle. Samples were placed in 14 ml or 50 ml PP
143 Falcon tubes and stored at -20°C. The fish were dissected using a stainless-steel dissecting kit.
144 The kit was cleaned in ethanol following each dissection to prevent contamination between
145 fish. Individuals that were too small to dissect on-site were frozen whole and dissected in the
146 laboratory at the University of Antwerp. Invertebrates were sampled using several different
147 techniques. Samples were stored in either 14 ml/50 ml PP Falcon tubes or 250 ml PP jars and
148 immediately placed in the field cooler box. Snails were hand-picked from fringing vegetation
149 and soft body tissue was extracted and pooled from snails from each site. A 200 µm
150 zooplankton net was dragged along the bottom surface and between fringing vegetation to
151 collect crabs and shrimp. Samples were only identified to infraorder and classified as either

152 Brachyura or Caridea. Macrozoobenthos samples, which included mainly polychaetes and
153 amphipods, were collected using a Van Veen Grab. Three grab samples were collected and
154 placed in a bucket. The sediment was stirred and decanted five times through a 500 µm sieve
155 to extract the organisms. The samples were identified at the University of KwaZulu-Natal,
156 Pietermaritzburg campus laboratory. They were poured into Petri dishes and identified into a
157 major group (polychaetes) under a dissecting microscope. Invertebrates from each infraorder
158 from each site were pooled to maximise tissue available for analysis, i.e., all snails from uMvoti
159 Gledhow were pooled together. Table S2 gives an overview of the collected samples.
160
161 2.2. Sediment Characteristics Analysis
162
163 The grain size of the sediment samples was analysed using a Malvern Mastersizer 2000.
164 Approximately 10 g of each sample was placed in an Erlenmeyer flask. 25 mL of H_2O_2 and 9
165 mL of HCL were added to the flasks and gently stirred. The flasks were left overnight to remove
166 all organic matter from the sediment. Each sample was then run through the Malvern
167 Mastersizer 2000. The samples were each measured three times. Total Organic Carbon (TOC)
168 was measured for each sample (for methodology please see supplementary material).
169
170 2.3. Chemical Analysis and UPLC analysis
171
172 The samples were analysed using an Ultra-Performance Liquid Chromatography (UPLC)
173 coupled to a tandem quadrupole mass spectrometer (ACQUITY, TQD, Waters, Milford, MA,
174 USA) with electrospray interface operating in negative ion mode (ES-MS/MS). Separation was
175 performed on an ACQUITY BEH C18 column (1.7 µm particle size; 50 × 2.1 mm, Waters,
176 USA). The following mobile phase solvents were used, 0.1% formic acid in water (A) and

177 0.1% formic acid in ACN (B). The gradient started at 65% A, decreased to 0% A in 3.4 min
178 and returned to 65% A at 4.7 min. The flow rate was set at 450 μL/min with an injection volume
179 of 10 μL. The total run time was 6.7 minutes. Target analytes were identified and quantified
180 using multiple-reaction-monitoring (MRM). PFASs abbreviations were according to Buck et
181 al. 2011. Fifteen target analytes (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA,
182 PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFOS, and PFDS), their internal
183 standards (ISTDs) and MRM transitions are displayed in Table S3. MPFAC-MXA solution
184 from Wellington Laboratories (Guelph, ON, Canada) containing isotope-labelled
185 PFCAs/PFSAs, was used for internal standardization.

186

187 2.4. Sample Extraction

188

189 The extraction procedure for PFASs from water was based on the methods as described by
190 Powley et al. (2005) and Silcock et al. (2009), with a few minor modifications. All materials
191 were rinsed with acetonitrile (ACN). Firstly, the water samples were filtered using Ion
192 Chromatography Certified Acrodisc 13 mm syringe filters with 0.2 μm Supor (PES)
193 membranes (VWR International, Leuven, Belgium) and 10 mL Braun syringes. Ten mL of
194 each water sample was added to a 50 mL PP tube and three replicates were prepared for each
195 location. Each sample was then spiked with 80 μL of a 125 pg/μL ISTD mix (containing the
196 ISTDs reported in Table S3). Samples were then vortexed and placed in an ultrasonic bath for
197 3 periods of 10 minutes, with vortexing between periods and left on a shaking plate overnight
198 at 135 rpm (20°C, GFL 3020, VWR International, Leuven, Belgium). After centrifugation at
199 2400 rpm for 10 minutes (4°C, 2400 rpm, Eppendorf centrifuge 5804R, and rotor A-4-44), the
200 supernatant was then placed into a new 14 mL PP tube. The Oasis Wax cartridges (3cc, 60 mg
201 sorbent, and 30 μm particle size) were pre-conditioned with 4 mL ACN and 4 mL Milli-Q

202 (MQ) water. The samples were loaded into the cartridges, tubes were rinsed with 2 mL ACN,
203 vortexed and added to the cartridge. The cartridges were then washed with 4 mL 0.025 M
204 Ammonium acetate buffer in MQ, 4 mL 40% ACN in MQ and 8 mL ACN and eluted with 2 x
205 2 mL 2% Ammonium hydroxide in ACN. The eluent was then dried completely using a
206 rotational vacuum concentrator. Following this, the samples were reconstituted with 200 µL
207 2% Ammonium hydroxide in ACN and vortexed for at least 1 minute. The samples were then
208 filtered into PP injector vials using the previously described syringe filters and syringes.
209
210 Approximately 1 g of sediment was placed in a 50 mL PP tube and three replicates were
211 prepared for each location. The extraction procedure was similar to the above-mentioned
212 procedure for water. To approximately 1 g of sediment, 10 ng of the ISTD mixture and 10 mL
213 of ACN were added. After centrifugation at 2400 rpm for 10 minutes (4°C, Eppendorf
214 centrifuge 5804R, and rotor A-4-44), the supernatant was loaded onto preconditioned Oasis
215 Wax cartridges and treated equally to the water samples. Samples were loaded onto SPE
216 cartridges without a vacuum to ensure that the samples would run through the cartridges at the
217 lowest possible speed, resulting in a longer interaction time between the sample and cartridge.
218
219 The extraction procedure for biota samples was the same as used for sediment samples.
220 However, the tissues were homogenised before the extraction protocol. A stainless-steel
221 dissection kit was used and was rinsed with ACN between samples. Tissue weights varied
222 depending on the quantity of tissue available. Approximately 0.5 g of fish muscle tissue
223 (without the skin) and 0.1 – 0.5 g of fish liver tissue was collected and placed in PP tubes.
224 Invertebrate tissue collected, weighed between 0.1 – 0.5 g. Due to the limited number of
225 invertebrate samples, Brachyurans were pooled and then referred to as "crabs," Carideans were
226 pooled and referred to as "shrimp," Gastropods were pooled and referred to as "snails" and

227 Polychaetes were pooled and referred to as "worms." Homogenisation was performed with a
228 hand-held Ultra-Turrax T24 mixer.
229
230 2.5. Quantification
231
232 Calibration curves (ranging from 1 to 1000 ng/mL) were constructed by adding a constant
233 amount of the ISTD (concentration, hereafter C_{ix}) to different concentrations of an unlabeled
234 PFASs mixture (concentration, hereafter C_x). The dilutions were performed in ACN. The ratio
235 of the concentrations (C_x/C_{ix}) was plotted against the ratio of the areas of the unlabeled ($Area_x$)
236 and labeled ($Area_{ix}$) compounds. For all target analytes, a linear regression function described
237 the relationship between C_x/C_{ix} and $Area_x/Area_{ix}$ with all $R^2 > 0.984$ and all $p < 0.001$ (Groffen
238 et al., 2019).
239
240 2.6. Quality Assurance and Quality Control
241
242 Two samples containing HPLC grade water were used as blanks for the water samples. For the
243 sediment and biota samples, one blank (10 mL ACN) was used after every 10 samples. Samples
244 were measured in triplicate to ensure that detections would reflect the actual presence in
245 samples and not background contamination. ACN was injected for every ten injections to
246 control carry-over effects. The limits of quantification (LOQs) were determined in real
247 (unspiked) samples. The LOQs were calculated based on a signal-to-noise ratio of 10 and are
248 attached in Tables 1 – 3. LOQs were not determined for PFBS, PFHxS, and PFUnDA due to
249 low recoveries. Recoveries for PFBS, PFHxS, and PFUnDA were very low in every matrix
250 tested. Percentage recoveries were reported for each analyte in the supplementary materials
251 (Table S4). Recoveries in water for PFASs were particularly low which resulted in high LOQs

252 (Table S1), this is likely a result of the method used which was optimised for freshwater and
253 not brackish water. Another possibility is the analyte loss resulting from the 2 mL ACN rinse
254 step just after sample loading. PFAS results from water samples should, therefore, be treated
255 with caution.
256
257 2.7. Human Health Risk
258
259 The maximum edible amount of fish muscle tissue which a 60 kg person could consume per
260 day without potential health risks were calculated for PFOA and PFOS based on minimal risk
261 levels (MRLs) suggested by the ATSDR (Agency for Toxic Substances and Disease Registry,
262 2019) and the EFSA Panel on Contaminants in the Food Chain (CONTAM) (EFSA CONTAM
263 Panel 2018). These values were then used to suggest if fish consumed from the aMatikulu and
264 uMvoti estuarine systems posed a risk to human health. The MRLs used are suggested for oral,
265 intermediate intake (15 – 364 days per year). The maximum edible amount of fish which can
266 be consumed per day without potential health risks was calculated based on the formula by
267 Verhaert et al. (2017).
268
269 2.8. Statistical Analysis
270
271 Statistical analyses were conducted in GraphPad Prism 7 (GraphPad Software, Inc.) and SPSS
272 Statistics 24. The level of significance was $p \leq 0.05$. Samples with detection levels below the
273 limit of quantification (LOQ) were given a concentration of LOQ/2 (Bervoets et al., 2004). The
274 normality of data was first tested using the Shapiro-Wilk test. In the case of non-normality,
275 data was transformed using a log transformation. Two-way ANOVA was performed to look
276 for differences between locations and among different species. Tukey or Sidak's post-hoc tests

277 were applied to determine which sites or species were significantly different. Pearson's
278 correlation test and Spearman's rank correlation tests were applied where appropriate to
279 determine the degree of association between the concentrations of PFASs in water, sediment,
280 and biotic tissues.
281
282 3. Results
283
284 3.1. Abiotic Environment
285
286 Water
287
288 PFASs in water above LOQ (PFBA, PFNA, PFOA, PFOS, PFDA, and PFDoDA) were shown
289 in Figure 2. PFPeA, PFHxA, PFHpA, PFDS, PFTrDA, and PFTeDA were detected but not
290 included in the analysis as all samples were below LOQ. PFBS, PFHxS, and PFUnDA were
291 excluded due to poor recoveries. PFBA and PFOA were found in high concentrations (> 200
292 ng/L) at all locations. All other perfluorinated compound concentrations were lower than 150
293 ng/L. The ANOVA results showed no significant differences among locations for each
294 compound.
295
296 Sediment
297
298 The concentrations of PFASs detected in the sediment samples showed no significant
299 difference among locations (Table 1). PFOA was detected in the highest average concentrations
300 at three of the locations, excluding the uMvoti Gledhow site which exhibited PFBA in higher
301 concentrations. PFNA and PFTrDA were detected in only the aMatikulu N2 Bridge samples.

302 PFBA was detected in the uMvoti Gledhow samples and PFOS was detected in the uMvoti
303 estuary mouth samples. PFDoDA was detected in both systems but was below LOQ in some
304 samples. PFOA was the only PFASs detected in all sediment samples. The average PFOA
305 concentrations were higher but not significantly different in the aMatikulu system with values
306 of 1.48 ng/g in the aMatikulu Estuary mouth and 1.34 ng/g in the N2 Bridge site compared
307 with values of 0.7 ng/g in the uMvoti Estuary mouth and 0.4 ng/g in the uMvoti Gledhow site.
308
309 All sediment samples were classified as sandy, with varying degrees of coarseness and low to
310 no clay content (Blott and Pye, 2001) (Table S5). There was a significant difference between
311 the average grain sizes from each sample location ($p < 0.0001$). The total organic carbon (TOC)
312 values ranged between 0.14 – 4.9% (Table S5). TOC values were significantly different
313 between sites ($p = 0.016$). No significant correlation was found between TOC and average
314 grain size ($p = 0.217$). PFASs concentrations were also not correlated with TOC or grain size
315 (Table S9).
316
317 3.2. Biotic Environment
318
319 Fish
320
321 In total only three fish species could be captured in sufficient numbers (Table S2), with
322 *Oreochromis mossambicus* the only fish species caught from 3 sites. Therefore, fish were
323 compared between estuaries and not separately between all four sites. An overview of the mean
324 concentrations and ranges of PFASs in different fish liver and muscle tissue from all sites was
325 given in Table 2. Differences between fish from each estuarine system could only be assessed
326 for *Oreochromis mossambicus*, as this was the only fish species caught in both systems.

327 Significant differences were seen between PFAS concentrations in muscle tissue ($p < 0.0001$),
328 with PFOS significantly higher from the uMvoti Estuary mouth compared to both the
329 aMatikulu N2 Bridge and uMvoti Gledhow sites ($p = 0.0154$ and $p = 0.0182$ respectively)
330 (Figure 3a). PFDoDA concentrations at both uMvoti sites were significantly higher compared
331 to the aMatikulu but not significantly different within the uMvoti. The level of PFTrDA was
332 significantly higher at the uMvoti Gledhow site compared with both the uMvoti Estuary mouth
333 and aMatikulu N2 Bridge site ($p = 0.0003$). Liver tissue samples were also significantly
334 different between the aMatikulu and the uMvoti sites ($p < 0.0001$), with PFOA concentrations
335 in the liver significantly higher in the aMatikulu compared to the uMvoti Gledhow site ($p =$
336 0.0103). PFDoDA and PFTrDA concentrations were significantly higher in liver samples from
337 both uMvoti sites compared to the aMatikulu N2 Bridge site (Figure 3b). PFOS concentrations
338 in all liver samples, although not significantly different between sites, were much higher
339 compared to other PFASs, with concentrations ranging from > 10 - < 28 ng/g ww. All other
340 PFAS concentrations were below 3 ng/g ww.
341

342 Significant differences were found between muscle tissues from different fish species in the
343 aMatikulu ($p < 0.0001$). Post-hoc tests showed that PFOS concentrations in *Ambassis*
344 *natalensis* muscle tissue (over 20 ng/g ww) were significantly higher than concentrations in *O.*
345 *mossambicus* and *Rhabdosargus holubi* ($p < 0.0001$), which were both below 0.5 ng/g ww
346 (Figure 4a). PFASs were only analysed in liver samples of *R. holubi* and *O. mossambicus,* with
347 *O. mossambicus* liver containing significantly higher concentrations of PFOS ($p < 0.0001$)
348 (Figure 4b).
349

350 Across all fish samples, liver samples had significantly higher concentrations of PFOS ($p <$
351 0.0001), PFOA ($p = 0.0005$), PFDoDA ($p < 0.0001$) and PFTrDA ($p = 0.0002$) compared to

352 muscle samples. No correlation was found between PFAS concentrations in muscle and liver
353 tissues. PFOS concentrations in liver samples ranged between 2.5 - 20 ng/g ww against 0.2 -
354 1.9 ng/g ww in muscle.
355
356 Invertebrates
357
358 The aMatikulu N2 Bridge site was the only site where four different invertebrate species were
359 sampled (Figure 5a). Average concentrations of PFOA, PFOS, PFDA, and PFDoDA were
360 higher in shrimp than in all other invertebrates from the aMatikulu, with significantly higher
361 PFOA concentrations than in snails ($p = 0.0266$) and significantly higher PFOS concentrations
362 than those of snails, worms and crabs (all with $p < 0.0001$). Concentrations of PFBA, PFPeA,
363 PFHpA, PFNA, PFDS, PFTrDA, and PFTeDA were all below LOQ. PFAS concentrations
364 between shrimp and snail samples from the uMvoti Gledhow site were significantly different
365 ($p = 0.0008$) (Figure 5b) with PFOA concentrations significantly higher in snails ($p < 0.0001$).
366
367 PFAS concentrations in shrimp were significantly different between sites ($p = 0.0006$), with
368 PFOA concentrations significantly higher in shrimp from the aMatikulu N2 Bridge site ($p <$
369 0.0001). Shrimp from the aMatikulu N2 Bridge site contained the highest concentrations of all
370 PFASs compared with other sites. While snails from the uMvoti Gledhow site had significantly
371 higher PFOA concentrations compared with those from the aMatikulu sites ($p = 0.0029$).
372
373 3.3. Human Health Risk
374
375 The suggested MRLs by the ATSDR for PFOS was 2 ng/kg/day and for PFOA was 3 ng/kg/day
376 (Agency for Toxic Substances and Disease Registry, 2019). The suggested EFSA CONTAM

377 Panel MRLs for PFOS was 1.8 ng/kg/day and for PFOA was 0.8 ng/kg/day (EFSA CONTAM
378 Panel 2018). The calculated maximum amount of fish muscle tissue (grams/day) a 60 kg person
379 could consume per day without potential health risks from PFOA were 180 ng/day (ATSDR)
380 or 48 ng/day (CONTAM) and for PFOS were 120 ng/day (ATSDR) or 108 ng/day (CONTAM).
381 The average individual consumption of fish in South Africa, as stated by FAO, is 7.6
382 kg/capita/year or 20.1 g/capita/day (FAO, 2010). The calculated maximum edible amount of
383 fish muscle tissue a 60 kg person can consume per day without potential health risks from
384 PFOA and PFOS (Table 4) were all above the average fish consumption of 20.1 g/capita/day.
385 Therefore, the risk associated with PFOA and PFOS exposure by fish consumption may be
386 excluded but that does not mean that the consumption of fish is safe as other contaminants may
387 be present. *Ambassis natalensis* consumption may pose the highest risk for PFOS exposure as
388 the maximum edible amount without potential health risks was only 62 g/day (ATSDR) or 56
389 g/day (CONTAM).
390
391 ## 4. Discussion
392
393 This study, to the best of our knowledge, provided the first examination of PFASs in estuarine
394 systems in South Africa, specifically, in the aMatikulu and uMvoti systems and provides a first
395 insight into the PFASs within different compartments of each estuary.
396
397 ### 4.1. Water Quality
398
399 The aMatikulu
400 No specific water quality guidelines are available in South Africa for estuaries, however,
401 guidelines exist for coastal marine waters and aquatic ecosystems. These guidelines suggested

402 that DO should not fall below 5 mg/L or 80 – 100% (Department of Water Affairs and Forestry,
403 1996, 1995). The DO of 9.71 mg/L / 116% in the estuary mouth and 6.8 mg/L / 83% in the N2
404 Bridge site were above the recommended levels. The pH ranged from 6.47 upstream to 8.06 at
405 the estuary mouth. pH and turbidity are controlled by the mixing of marine and freshwater,
406 thus due to the buffering capacity of seawater, pH of estuarine water generally tends to increase
407 near the mouth, towards a value of 8 (Harrison et al., 2000).
408
409 The uMvoti
410 The water quality in the uMvoti was considered poor, with very low DO (1.8 mg/L) and high
411 total dissolved solids (915 mg/L) in the estuary mouth (Table S6). The DO was only slightly
412 higher than the DO value of 0.71 mg/L found by Sukdeo et al. (2016). The water had a foul
413 odour, the underlying sediments were discoloured, and flocculation and foam were present on
414 the riverbanks, which were similar to findings by Sukdeo et al. (2014). A neutral pH level
415 (7.02) was also found by Sukdeo et al. (2014). The uMvoti River has a long history of poor
416 water quality and a generally deteriorated state, starting with reports by Begg (1978) (Sukdeo
417 et al., 2016). The riparian zone remains heavily modified by agriculture, rural populations,
418 small towns, and industry. A lack of biodiversity in the estuary mouth may be caused by factors
419 such as low DO and salinity. The salinity at the estuary mouth was relatively low (912 mg/L)
420 indicating the freshwater dominance and the limited intrusion of marine water during the flood
421 tide (Vezi et al., 2019).
422
423 4.2. PFASs
424
425 4.2.1. Water
426

427 Average PFOA concentrations from the aMatikulu Estuary mouth (258 ng/L) and N2 Bridge
428 site (171 ng/L) were similar in range to concentrations reported from rivers in the Western
429 Cape, South Africa, specifically, Diep River (314 ng/L), Salt River (390 ng/L) and Eerste River
430 (146 ng/L) (Mudumbi et al., 2014a). The presence of PFASs in the aMatikulu system
431 demonstrates the ubiquitous nature of PFASs in the environment, as the aMatikulu River is
432 suggested to be less impacted by urban stressors, with only minor impacts from agriculture
433 (O'Brien et al., 2009; Vezi et al., 2019). While PFOA concentrations from the uMvoti Estuary
434 mouth (788 ng/L) and uMvoti Gledhow site (711 ng/L) were much higher than any other PFOA
435 concentrations recorded in South African rivers. Both the aMatikulu and uMvoti PFOA
436 concentrations were higher than those recorded in the Olifants River Basin (PFOA < LOQ)
437 (Verhaert et al., 2017) and the Vaal River (< 10 ng/L) (Groffen et al., 2018). The uMvoti River
438 is heavily impacted by a large pulp and paper mill, urbanisation, agricultural practises and
439 sewage treatment discharges, which may be responsible for the higher concentrations of PFOA
440 and PFBA in the water. Higher concentrations of PFBA suggested a change in PFAS
441 production, with short-chain PFASs possibly becoming more abundant in this environment.
442 This supports the global trend that short-chain PFASs are replacing long-chain PFASs and
443 should be focused on in future studies (Ahrens and Bundschuh, 2014).

444

445 PFOS was only recorded in one water sample (54.2 ng/L) from the aMatikulu Estuary mouth,
446 with the rest of the samples containing concentrations below LOQ (14.6 ng/L). The value is
447 within the range recorded of PFOS concentrations in other South African river waters of LOQ
448 – 182 ng/L (Groffen et al., 2018; Mudumbi et al., 2014a; Verhaert et al., 2017).

449

450 Several studies worldwide have recorded PFASs in river water, with PFOS and PFOA the
451 predominant perfluorinated compounds detected (Loos et al., 2009; Pan et al., 2014;

452 Senthilkumar et al., 2007; Thompson et al., 2011). PFOS concentrations have been detected in
453 greater ranges in river water from other parts of the world such as the Pearl River Delta, China
454 (0.17 – 290 ng/L) (Pan et al., 2014); a number of European rivers ($\bar{x}$ = 39, max = 1371 ng/L)
455 (Loos et al., 2009); the Osaka region of Japan (4.5 – 67000 ng/L) (Senthilkumar et al., 2007)
456 and the Parramatta River, Sydney, Australia (7.5 – 21 ng/L) (Thompson et al., 2011). PFOA
457 concentrations from the abovementioned studies were as follows; Pearl River Delta, China
458 (0.21 - 22 ng/L); a number of European rivers ($\bar{x}$ = 12, max = 174 ng/L); the Osaka region of
459 Japan (1.5 – 520 ng/L) and the Parramatta River, Sydney, Australia (4.2 – 6.4 ng/L) (Thompson
460 et al., 2011). PFOA concentrations from the aMatikulu ranged from similar to higher
461 concentrations when compared with these studies, while the uMvoti concentrations were much
462 higher and only comparable to levels from the Osaka region of Japan, which was suggested to
463 be heavily impacted by urbanisation and industry (Senthilkumar et al., 2007). The main source
464 of PFOA can be attributed to the effluent from the pulp and paper mill upstream from the
465 estuary. This supports the finding by Clara et al. (2008) who reported that PFOA was the
466 dominant compound emitted by the paper industry.
467
468 4.2.2. Sediment
469
470 The PFOA and PFOS concentrations in sediment samples from this study were lower than
471 those recorded in other South African rivers, namely Salt River, Diep River and Eerste River
472 (Mudumbi et al. 2014b). Mudumbi et al. (2014b) also proposed that the prevalence of PFOA
473 may be a result of wastewater treatment effluent. In the present study, we found higher PFOA
474 concentrations in aMatikulu sediment, an estuary with lower TOC than that of the uMvoti and
475 no point source of wastewater effluent. It is, however, possible that the primary source of PFOS
476 and PFOA may be from atmospheric deposition from sources within the aMatikulu catchment.

477 Meng et al. (2018) attributed up to 70% of PFOA and 93% of PFOS, in soils of coastal regions
478 of the Bohai and Yellow Seas (China) to atmospheric deposition. Other studies have reported
479 PFOS and PFOA concentrations of <0.33 – 11 ng/g and <0.1 – 3.9 ng/g in the Osaka region of
480 Japan (Senthilkumar et al., 2007), 0.8 – 6.2 ng/g and <LOQ – 0.16 in the Parramatta River,
481 Sydney, Australia (Thompson et al., 2011) and < LOQ – 3.69 and 0.09 – 0.93, respectively in
482 five major rivers in China (Pan et al., 2015). A significant negative correlation was found
483 between PFOA concentrations in water and sediment in this study, however other factors would
484 need to be considered before suggesting causal relationships. Future studies could consider
485 partitioning coefficients as a measure to compare PFOA and PFOS binding affinities. The
486 behaviour of PFASs is governed by both hydrophobic and electrostatic interactions and
487 therefore sorption to sediments may not be predicted by a single sorbent property, such as TOC
488 (Ahrens and Bundschuh, 2014; Campos Pereira et al. 2018).
489
490 4.2.3. Biota
491
492 Fish
493
494 Most target PFASs analysed in fish muscle and liver tissues were below LOQ in all fish tissues,
495 however, five PFASs were detected. PFDoDA, PFTrDA, and PFTeDA were only detected in
496 a few samples, while PFOS and PFOA were detected in all samples. PFOS concentrations were
497 distinctly higher than other PFASs, which followed the trend seen in other studies analysing
498 PFASs in fish (Groffen et al., 2018; Pan et al., 2014; Senthilkumar et al., 2007; Shi et al., 2012;
499 Thompson et al., 2011; Verhaert et al., 2017; Ye et al., 2008).
500

501 PFOA and PFOS concentrations from *Oreochromis mossambicus* tissues were generally higher
502 in the uMvoti, indicating that the uMvoti was more polluted than the aMatikulu. *O.*
503 *mossambicus* was the only fish species caught in the uMvoti system, likely due to their hardy
504 nature and high tolerance to low DO, salinity variations and poor water quality (Addo-Bediako
505 et al., 2014; Russell et al., 2012). Shi et al. (2012) suggested that Tilapia species tend to have
506 lower concentrations of PFASs in their tissues due to their predominantly herbivorous diet.
507 However, some studies classify *O. mossambicus* as an omnivorous detritus feeder, which feeds
508 on crustaceans, polychaetes, and gastropods (Loi et al., 2011). PFOS and PFOA concentrations
509 were significantly different between all fish species suggesting that diet and physiology
510 influence PFASs concentration in tissues. Future studies should include other piscivorous and
511 omnivorous fish species in the uMvoti to test whether trophic position influences PFAS
512 bioaccumulation.
513
514 Another result of interest was the significantly higher concentration of PFOS in the muscle
515 tissue of *A. natalensis*. This result was likely influenced by their diet, feeding predominantly
516 on crustaceans, insects, fish fry, fish eggs, and larvae (Martin and Blaber, 1983); they are higher
517 in the trophic food chain and therefore likely to accumulate higher concentrations of PFASs.
518 Unfortunately, due to the small tissue size and the limited number of individuals, PFASs could
519 not be analysed in the liver tissue of this species. This would be of interest for future studies as
520 the liver is likely to contain higher concentrations of PFASs.
521
522 The concentrations of PFOS and PFOA found in this study were similar to concentrations
523 found in fish from the Olifants River, but lower than those from the Vaal River (Table S7).
524 Concentrations of PFOA were however lower than the average found in marine fish from Kalk
525 Bay which ranged between 19.91 – 63.17 ng/g (Ojemaye and Petrik, 2019). Furthermore, the

526 study by Ojemaye and Petrik (2019) found that PFDA, PFNA, and PFHpA were the
527 predominant PFASs in fish tissues, however, PFOS was not targeted. Compared to studies from
528 other regions, PFOS and PFOA concentrations were lower than, or similar to concentrations
529 found in Japan, Australia, China, Hong Kong and the USA (Senthilkumar et al. 2007;
530 Thompson et al. 2011; Pan et al. 2014; Loi et al. 2011 and Ye et al. 2008).

531

532 Invertebrates

533

534 PFOA was detected in all invertebrate samples, while the other PFASs analysed were only
535 detected in a few samples. All invertebrate samples were significantly different suggesting that
536 habitat and diet influence invertebrate exposure to PFASs (Yang et al, 2012). PFOS, PFOA,
537 and PFDoDA concentrations were compared against concentrations found in invertebrates
538 from other regions (Table S8). Shrimp PFOS concentrations were lower than those from the
539 Vaal River (Groffen et al., 2018) and lower than those from other regions, including Canada,
540 China, Hong Kong and Australia (Kannan et al., 2005; Loi et al., 2011; Taylor and Johnson,
541 2016; Verhaert et al., 2017; Yang et al., 2012). PFOA concentrations in all invertebrates were
542 comparable to other regions. The results from this study do not agree with the trophic
543 magnification theory as the worms had comparable levels of PFOA and PFOS to higher
544 organisms. PFASs concentrations recorded in invertebrates in this study may underrepresent
545 actual concentrations, as samples were stored in 10% ethanol which may have caused some
546 PFASs to detach from biota tissues into solution. No significant relationships were found
547 between PFAS concentrations in biota with concentrations in environmental samples.

548

549 4.3. Human Health Risk

550

551 The calculated maximum edible amounts of fish muscle tissue a 60 kg person could consume
552 per day without potential health risks from PFOA and PFOS were all above the average South
553 African fish consumption of 20.1 g/capita/day and on this basis, the fish from the uMvoti and
554 aMatikulu would pose little to no risk for human consumption. Only two other studies have
555 assessed human health risks of PFOA/PFOS contaminated fish from rivers in South Africa.
556 This study found similar concentrations of PFOA and PFOS in fish muscle tissue to the study
557 by Verhaert et al. (2017) who suggested that based on the MRLs at that time, fish consumed
558 from the Olifants River Basin posed no risk to human health. Groffen et al. (2018) found similar
559 levels of PFOA in muscle tissues but much greater concentrations of PFOS in the ranges of
560 <LOQ – 45.7 ng ww and therefore suggested that fish consumed from the Vaal River may pose
561 a risk to human health.

562

563 5. Conclusions

564

565 This study provided the first record of perfluorinated compounds in two subtropical estuaries
566 along the South African coastline. Perfluorinated compounds were found in all components of
567 the environment. While the concentrations were generally lower than those from other regions
568 of the world, it is important to monitor their presence in the environment. The results from this
569 study also suggested that the uMvoti system remains in a poor, degraded state with very poor
570 water quality and higher concentrations of perfluorinated compounds when compared to the
571 aMatikulu system. These high PFOA levels were attributed to point source releases from a pulp
572 and paper mill adjacent to the river. Concentrations of PFASs in the aMatikulu are of concern
573 as this system is considered to be in a more "natural" state and less impacted by anthropogenic
574 activities. The potential source for these compounds was suggested to be from atmospheric

575 deposition. As several commonly reported PFCAs and PFSAs were detected in both estuaries,
576 future studies should focus on precursor compounds as well as other subcategories of PFASs.
577

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584

585 ## 7. References

586

587 Addo-Bediako, A., Marr, S.M., Jooste, A., Luus-Powell, W.J., 2014. Are metals in the muscle
588 tissue of Mozambique tilapia a threat to human health? A case study of two impoundments in
589 the Olifants River, Limpopo province, South Africa. Ann. Limnol. - Int. J. Limnol. 50, 201–
590 210. https://doi.org/10.1051/limn/2014091

591

592 Agency for Toxic Substances and Disease Registry, A., 2019. Minimal risk levels (MRLs)
593 [WWW Document]. URL https://www.atsdr.cdc.gov/mrls/pdfs/ATSDR%20MRLs%20-
594 %20June%202019-H.pdf

595

596 Ahrens, L., & Bundschuh, M., 2014. Fate and effects of poly- and perfluoroalkyl substances in
597 the aquatic environment: A review. Environmental Toxicology and Chemistry, 33(9), 1921–
598 1929. https://doi.org/10.1002/etc.2663

599

600 Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011. The
601 value of estuarine and coastal ecosystem services. Ecol. Monogr. 81, 169–193.
602 https://doi.org/10.1890/10-1510.1
603
604 Begg, G., 1978. The estuaries of Natal. Natal Town and Regional Planning Report 41.
605
606 Bervoets, L., Voets, J., Chu, S.G., Covaci, A., Schepens, P., Blust, R., 2004. Comparison of
607 accumulation of micropollutants between indigenous and transplanted zebra mussels
608 (Dreissena polymorpha). Environ. Toxicol. Chem. 23, 1973–1983. https://doi.org/10.1897/03-
609 365
610
611 Blott, S.J., Pye, K., 2001. GRADISTAT: A grain size distribution and statistics package for the
612 analysis of unconsolidated sediments. Earth Surf. Process. Landforms 26, 1237–1248.
613 https://doi.org/10.1002/esp.261
614
615 Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., Voogt, P. De, Jensen, A.A.,
616 Kannan, K., Mabury, S.A., van Leeuwen, S.P.J., 2011. Perfluoroalkyl and polyfluoroalkyl
617 substances in the environment: Terminology, classification, and origins. Integr. Environ.
618 Assess. Manag. 7, 513–541. https://doi.org/10.1002/ieam.258
619
620 Campos Pereira, H., Ullberg, M., Kleja, D. B., Gustafsson, J. P., & Ahrens, L., 2018. Sorption
621 of perfluoroalkyl substances (PFASs) to an organic soil horizon – Effect of cation composition
622 and pH. Chemosphere, *207*, 183–191. https://doi.org/10.1016/j.chemosphere.2018.05.012
623

624 Chen, H., Zhang, C., Yu, Y., Han, J., 2012. Sorption of perfluorooctane sulfonate (PFOS) on
625 marine sediments. Mar. Pollut. Bull. 64, 902–906.
626 https://doi.org/10.1016/j.marpolbul.2012.03.012
627
628 Chowdhury, A., Maiti, S.K., 2016. Identifying the source and accessing the spatial variations,
629 contamination status, conservation threats of heavy metal pollution in the river waters of
630 Sunderban biosphere reserve, India. J. Coast. Conserv. 20, 257–269.
631 https://doi.org/10.1007/s11852-016-0436-x
632
633 Christie, I., Reiner, J.L., Bowden, J.A., Botha, H., Cantu, T.M., Govender, D., Guillette, M.P.,
634 Lowers, R.H., Luus-Powell, W.J., Pienaar, D., Smit, W.J., Guillette Jr., L.J., 2016.
635 Perfluorinated alkyl acids in the plasma of South African crocodiles (Crocodylus niloticus).
636 Chemosphere 154, 72–78. https://doi.org/10.1016/j.chemosphere.2016.03.072
637
638 Clara, M., Scharf, S., Weiss, S., Gans, O., Scheffknecht, C. 2008. Emissions of perfluorinated
639 alkylated substances (PFAS) from point sources—identification of relevant branches. Water
640 Sci. & Technol. 58, 59-66. https://doi.org/10.2166/wst.2008.641

641

642 D'Hollander, W., De Bruyn, L., Hagenaars, A., de Voogt, P., Bervoets, L., 2014.
643 Characterisation of perfluorooctane sulfonate (PFOS) in a terrestrial ecosystem near a
644 fluorochemical plant in Flanders, Belgium. Environ. Sci. Pollut. Res. 21, 11856–11866.
645 https://doi.org/10.1007/s11356-013-2449-4
646

647 D'Hollander, W., Roosens, L., Covaci, A., Cornelis, C., Reynders, H., Van Campenhout, K.,
648 de Voogt, P., Bervoets, L., 2010. Brominated flame retardants and perfluorinated compounds
649 in indoor dust from homes and offices in Flanders, Belgium. Chemosphere 81, 478–487.
650 https://doi.org/10.1016/j.chemosphere.2010.07.043
651

652 Darnerud, P.O., Eriksen, G.S., Johannesson, T., Larsen, P.B., Viluksela, M., 2001.
653 Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. Environ.
654 Health Perspect. 109, 49–68. https://doi.org/10.2307/3434846
655

656 Day, J., 1981. Summaries of current knowledge of 43 estuaries in southern Africa. Estuar. Ecol.
657 with Part. Ref. to South. Africa 251–329.
658

659 Department of Water Affairs and Forestry, 1996. South African Water Quality Guidelines. 7:
660 Aquatic Ecosystems [WWW Document]. URL
661 http://www.dwa.gov.za/iwqs/wq_guide/Pol_saWQguideFRESHAquaticecosystemsvol7.pdf
662

663 Department of Water Affairs and Forestry, 1995. South African Water Quality Guidelines for
664 Coastal Marine Waters. 1:Natural Environment. [WWW Document]. URL http://www.iwa-
665 network.org/filemanager-uploads/WQ_Compendium/Database/Future_analysis/085.pdf
666

667 EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2018. Knutsen HK,
668 Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M,
669 Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A,
670 Rose M,Roudot A-C, Vleminckx C, Vollmer G, Wallace H, Bodin L, Cravedi J-P, Halldorsson
671 TI, Haug LS, Johansson N, van Loveren H, Gergelova P, Mackay K, Levorato S, van Manen

672 M and Schwerdtle T,2018. Scientific Opinion on the risk to human health related to the
673 presence of perfluorooctanesulfonic acid and perfluorooctanoic acid in food. EFSA Journal
674 16(12):5194, 284 pp.https://doi.org/10.2903/j.efsa.2018.5194
675
676 Food and Agriculture Organisation (2010) *Fishery and Aquaculture Country Profiles: The*
677 *Republic of South Africa*, 2010. Available online:
678 http://www.fao.org/fishery/facp/ZAF/en#CountrySector-Statistics
679
680 Forbes, A.T., Demetriades, N.T., 2008. Estuaries of Durban, KwaZulu-Natal, South Africa.
681
682 Goksyor, A., Forlin, L., 1992. The cytochrome-p-450 system in fish, aquatic toxicology and
683 environmental monitoring. Aquat. Toxicol. 22, 287–311. https://doi.org/10.1016/0166-
684 445X(92)90046-P
685
686 Groffen T, Lasters R, Lemière F, Wilems T, Eens M, Bervoets L and Prinsen E., 2019.
687 Development and validation of an extraction method for the analysis of perfluoroalkyl
688 substances (PFASs) in environmental and biotic matrices. Journal of Chromatography B 1116:
689 30 – 37.
690
691 Groffen, T., Wepener, V., Malherbe, W., Bervoets, L., 2018. Distribution of perfluorinated
692 compounds (PFASs) in the aquatic environment of the industrially polluted Vaal River, South
693 Africa. Sci. Total Environ. 627, 1334-1344. https://doi.org/10.1016/j.scitotenv.2018.02.023
694

695 Hagenaars, A., Knapen, D., Meyer, I.J., van der Ven, K., Hoff, P., De Coen, W., 2008. Toxicity
696 evaluation of perfluorooctane sulfonate (PFOS) in the liver of common carp (Cyprinus carpio).
697 Aquat. Toxicol. 88, 155–163. https://doi.org/10.1016/j.aquatox.2008.04.002
698
699 Harrison, J., Cooper, J., Ramm, A., 2000. State of South African Estuaries: Geomorphology
700 Ichthyofauna, Water Quality and Aesthetics.
701
702 He, X., Dai, K., Li, A., Chen, H., 2015. Occurrence and assessment of perfluorinated
703 compounds in fish from the Danjiangkou reservoir and Hanjiang river in China. FOOD Chem.
704 174, 180–187. https://doi.org/10.1016/j.foodchem.2014.11.018
705
706 Heiri, O., Lotter, A. F. & Lemcke, G. (2001) Loss on ignition as a method for estimating
707 organic and carbonate content in sediments: reproducibility and comparability of results.
708 *Journal of Paleolimnology*, 25(1), 101-110.
709
710 Hekster, F.M., Laane, R., de Voogt, P., 2003. Environmental and toxicity effects of
711 perfluoroalkylated substances, in: Reviews of Environmental Contamination and Toxicology.
712 pp. 99–121. https://doi.org/10.1007/0-387-21731-2_4
713
714 Hemens, J., Warwick, R., Oliff, W., 1975. Effect of extended exposure to low fluoride
715 concentration on estuarine fish and crustacea. Prog. water Technol.
716
717 Houde, M., Martin, J.W., Letcher, R.J., Solomon, K.R., Muir, D.C.G., 2006. Biological
718 monitoring of polyfluoroalkyl substances: A review. Environ. Sci. Technol. 40, 3463–3473.
719 https://doi.org/10.1021/es052580b

720

721 Jones, K.C., de Voogt, P., 1999. Persistent organic pollutants (POPs): state of the science.
722 Environ. Pollut. 100, 209–221. https://doi.org/10.1016/S0269-7491(99)00098-6

723

724 Kaboré, H.A., Vo Duy, S., Munoz, G., Méité, L., Desrosiers, M., Liu, J., Sory, T.K., Sauvé, S.,
725 2018. Worldwide drinking water occurrence and levels of newly-identified perfluoroalkyl and
726 polyfluoroalkyl substances. Sci. Total Environ. 616–617, 1089–1100.
727 https://doi.org/10.1016/j.scitotenv.2017.10.210

728

729 Kannan, K., Tao, L., Sinclair, E., Pastva, S.D., Jude, D.J., Giesy, J.P., 2005. Perfluorinated
730 compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. Arch.
731 Environ. Contam. Toxicol. 48, 559–566. https://doi.org/10.1007/s00244-004-0133-x

732

733 Kennish, M.J., 2002. Environmental threats and environmental future of estuaries. Environ.
734 Conserv. 29, 78–107. https://doi.org/10.1017/S0376892902000061

735

736 Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl
737 acids: A review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394.
738 https://doi.org/10.1093/toxsci/kfm128

739

740 Li, M., Chen, Z., Finlayson, B., Wei, T., Chen, J., Wu, X., Xu, H., Webber, M., Barnett, J.,
741 Wang, M., 2015. Water diversion and sea-level rise: Potential threats to freshwater supplies in
742 the Changjiang River estuary. Estuar. Coast. SHELF Sci. 156, 52–60.
743 https://doi.org/10.1016/j.ecss.2014.07.007

744

745 Loi, E.I.H., Yeung, L.W.Y., Taniyasu, S., Lam, P.K.S., Kannan, K., Yamashita, N., 2011.
746 Trophic Magnification of Poly- and Perfluorinated Compounds in a Subtropical Food Web.
747 Environ. Sci. Technol. 45, 5506–5513. https://doi.org/10.1021/es200432n
748
749 Loos, R., Gawlik, B.M., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G., 2009. EU-wide
750 survey of polar organic persistent pollutants in European river waters. Environ. Pollut. 157,
751 561–568. https://doi.org/10.1016/j.envpol.2008.09.020
752
753 Martin, T., Blaber, S., 1983. The feeding ecology of Ambassidae (Osteichthyes: Perciformes)
754 in Natal estuaries. South African J. Zool. 18, 353–362.
755
756 Meng, J., Wang, T., Song, S., Wang, P., Li, Q., Zhou, Y., Lu, Y. 2018. Tracing perfluoroalkyl
757 substances (PFASs) in soils along the urbanizing coastal area of Bohai and Yellow Seas, China.
758 Environ. Pollut. 238, 404-412. https://doi-org.nwulib.nwu.ac.za/10.1016/j.envpol.2018.03.056
759
760 Milinovic, J., Lacorte, S., Vidal, M., Rigol, A., 2015. Sorption behaviour of perfluoroalkyl
761 substances in soils. Sci. Total Environ. 511, 63–71.
762 https://doi.org/10.1016/j.scitotenv.2014.12.017
763
764 Mudumbi, J.B.N., Ntwampe, S.K.O., Muganza, F.M., Okonkwo, J.O., 2014a.
765 Perfluorooctanoate and perfluorooctane sulfonate in South African river water. Water Sci.
766 Technol. 69, 185–194. https://doi.org/10.2166/wst.2013.566
767

768 Mudumbi, J.B.N., Ntwampe, S.K.O., Muganza, F.M., Rand, A., Okonkwo, O.J., 2014b.
769 Concentrations of perfluorooctanoate and perfluorooctane sulfonate in sediment of Western
770 Cape Rivers, South Africa. Carpathian J. Earth Environ. Sci. 9, 147–158.
771
772 O'Brien, G.C., Swemmer, R., Wepener, V., 2009. Ecological integrity assessment of the fish
773 assemblages of the aMatikulu/Nyoni and uMvoti estuaries, KwaZulu-Natal, South Africa.
774 AFRICAN J. Aquat. Sci. 34, 293–302. https://doi.org/10.2989/AJAS.2009.34.3.11.987
775
776 O'Donoghue, S., Marshall, D.J., 2003. Marine pollution research in South Africa: a status
777 report. S. Afr. J. Sci. 99, 349–356.
778
779 Ojemaye, C.Y., Petrik, L., 2019. Occurrences, levels and risk assessment studies of emerging
780 pollutants (pharmaceuticals, perfluoroalkyl and endocrine disrupting compounds) in fish
781 samples from Kalk Bay harbour, South Africa. Environ. Pollut. 252, 562–572.
782 https://doi.org/10.1016/j.envpol.2019.05.091
783
784 Pan, C.-G., Ying, G.-G., Zhao, J.-L., Liu, Y.-S., Liu, S.-S., Du, J., Kookana, R.S., 2015. Spatial
785 Distribution of Perfluoroalkyl Substances in Surface Sediments of Five Major Rivers in China.
786 Arch. Environ. Contam. Toxicol. 68, 566–576. https://doi.org/10.1007/s00244-014-0113-8
787
788 Pan, C.-G., Zhao, J.-L., Liu, Y.-S., Zhang, Q.-Q., Chen, Z.-F., Lai, H.-J., Peng, F.-J., Liu, S.-
789 S., Ying, G.-G., 2014. Bioaccumulation and risk assessment of per- and polyfluoroalkyl
790 substances in wild freshwater fish from rivers in the Pearl River Delta region, South China.
791 Ecotoxicol. Environ. Saf. 107, 192–199. https://doi.org/10.1016/j.ecoenv.2014.05.031
792

793 Pan, K., Wang, W.-X., 2012. Trace metal contamination in estuarine and coastal environments
794 in China. Sci. Total Environ. 421, 3–16. https://doi.org/10.1016/j.scitotenv.2011.03.013
795
796 Powley, C.R., George, S.W., Ryan, T.W., Buck, R.C., 2005. Matrix effect-free analytical
797 methods for determination of perfluorinated carboxylic acids in environmental matrixes. Anal.
798 Chem. 77, 6353–6358. https://doi.org/10.1021/ac0508090
799
800 Russell, D.J., Thuesen, P.A., Thomson, F.E., 2012. A review of the biology, ecology,
801 distribution and control of Mozambique tilapia, Oreochromis mossambicus (Peters 1852)
802 (Pisces: Cichlidae) with particular emphasis on invasive Australian populations. Rev. FISH
803 Biol. Fish. 22, 533–554. https://doi.org/10.1007/s11160-011-9249-z
804
805 Senthilkumar, K., Ohi, E., Sajwan, K., Takasuga, T., Kannan, K., 2007. Perfluorinated
806 compounds in river water, river sediment, market fish, and wildlife samples from Japan. Bull.
807 Environ. Contam. Toxicol. 79, 427–431. https://doi.org/10.1007/s00128-007-9243-2
808
809 Shi, Y., Wang, J., Pan, Y., Cai, Y., 2012. Tissue distribution of perfluorinated compounds in
810 farmed freshwater fish and human exposure by consumption. Environ. Toxicol. Chem. 31,
811 717–723. https://doi.org/10.1002/etc.1758
812
813 Silcock, P., Karrman, A., Van Bavel, B., 2009. Advancing perfluorinated compounds analysis
814 using simultaneous matrix monitoring. Waters Appl. Note 1–7.
815

816 Sukdeo, P., Pillay, S., Ballabh, H., 2016. Ecological Restoration and Remediation of a
817 Degraded Estuary : The Case of the Mvoti Estuary , KwaZulu-Natal , South Africa 55, 69–79.
818 https://doi.org/10.1080/09709274.2016.11907011
819
820 Sukdeo, P., Pillay, S., Ballabh, H., 2014. Long-term deterioration of water quality of the Mvoti
821 Estuary, KwaZulu-Natal, South Africa. Curr. Sci. 106, 739–743.
822
823 Taylor, M.D., Johnson, D.D., 2016. Preliminary investigation of perfluoroalkyl substances in
824 exploited fishes of two contaminated estuaries. Mar. Pollut. Bull. 111, 509–513.
825 https://doi.org/10.1016/j.marpolbul.2016.06.023
826
827 Thompson, J., Roach, A., Eaglesham, G., Bartkow, M.E., Edge, K., Mueller, J.F., 2011.
828 Perfluorinated alkyl acids in water, sediment and wildlife from Sydney Harbour and
829 surroundings. Mar. Pollut. Bull. 62, 2869–2875.
830 https://doi.org/10.1016/j.marpolbul.2011.09.002
831
832 van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in
833 environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
834 https://doi.org/10.1016/S1382-6689(02)00126-6
835
836 Van Niekerk, L., Adams, J.B., Bate, G.C., Forbes, A.T., Forbes, N.T., Huizinga, P., Lamberth,
837 S.J., MacKay, C.F., Petersen, C., Taljaard, S., Weerts, S.P., Whitfield, A.K., Wooldridge, T.H.,
838 2013. Country-wide assessment of estuary health: An approach for integrating pressures and
839 ecosystem response in a data limited environment. Estuar. Coast. SHELF Sci. 130, 239–251.
840 https://doi.org/10.1016/j.ecss.2013.05.006

841

842 Verhaert, V., Newmark, N., D'Hollander, W., Covaci, A., Vlok, W., Wepener, V., Addo-
843 Bediako, A., Jooste, A., Teuchies, J., Blust, R., Bervoets, L., 2017. Persistent organic pollutants
844 in the Olifants River Basin, South Africa: Bioaccumulation and trophic transfer through a
845 subtropical aquatic food web. Sci. Total Environ. 586, 792–806.
846 https://doi.org/10.1016/j.scitotenv.2017.02.057

847

848 Vestergren, R., Berger, U., Glynn, A., Cousins, I.T., 2012. Dietary exposure to perfluoroalkyl
849 acids for the Swedish population in 1999, 2005 and 2010. Environ. Int. 49, 120–127.
850 https://doi.org/10.1016/j.envint.2012.08.016

851

852 Vezi, M., Downs, C.T., Wepener, V., O'Brien, G. 2019. Response of zooplankton communities
853 to altered water quality and seasonal flow changes in selected river dominated estuaries in
854 KwaZulu-Natal, South Africa. Ecohydrol. Hydrobiol. In press.
855 https://doi.org/10.1016/j.ecohyd.2019.01.005

856

857 Wepener, V., Degger, N., 2012. Status of marine pollution research in South Africa (1960-
858 present). Mar. Pollut. Bull. 64, 1508–1512. https://doi.org/10.1016/j.marpolbul.2012.05.037

859

860 Whitfield, a. K., Baliwe, N.G., 2013. A century of science in South African estuaries:
861 bibliography and review of research trends. SANCOR Occas. Rep. no. 7 289.

862

863 Yang, L., Tian, S., Zhu, L., Liu, Z., Zhang, Y., 2012. Bioaccumulation and distribution of
864 perfloroalkyl acids in seafood products from Bohai Bay, China. Environ. Toxicol. Chem. 31,
865 1972–1979. https://doi.org/10.1002/etc.1917

866

867 Ye, X., Strynar, M.J., Nakayama, S.F., Varns, J., Helfant, L., Lazorchak, J., Lindstrom, A.B.,
868 2008. Perfluorinated compounds in whole fish homogenates from the Ohio, Missouri, and
869 Upper Mississippi Rivers, USA. Environ. Pollut. 156, 1227–1232.
870 https://doi.org/10.1016/j.envpol.2008.03.014

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873 Figures

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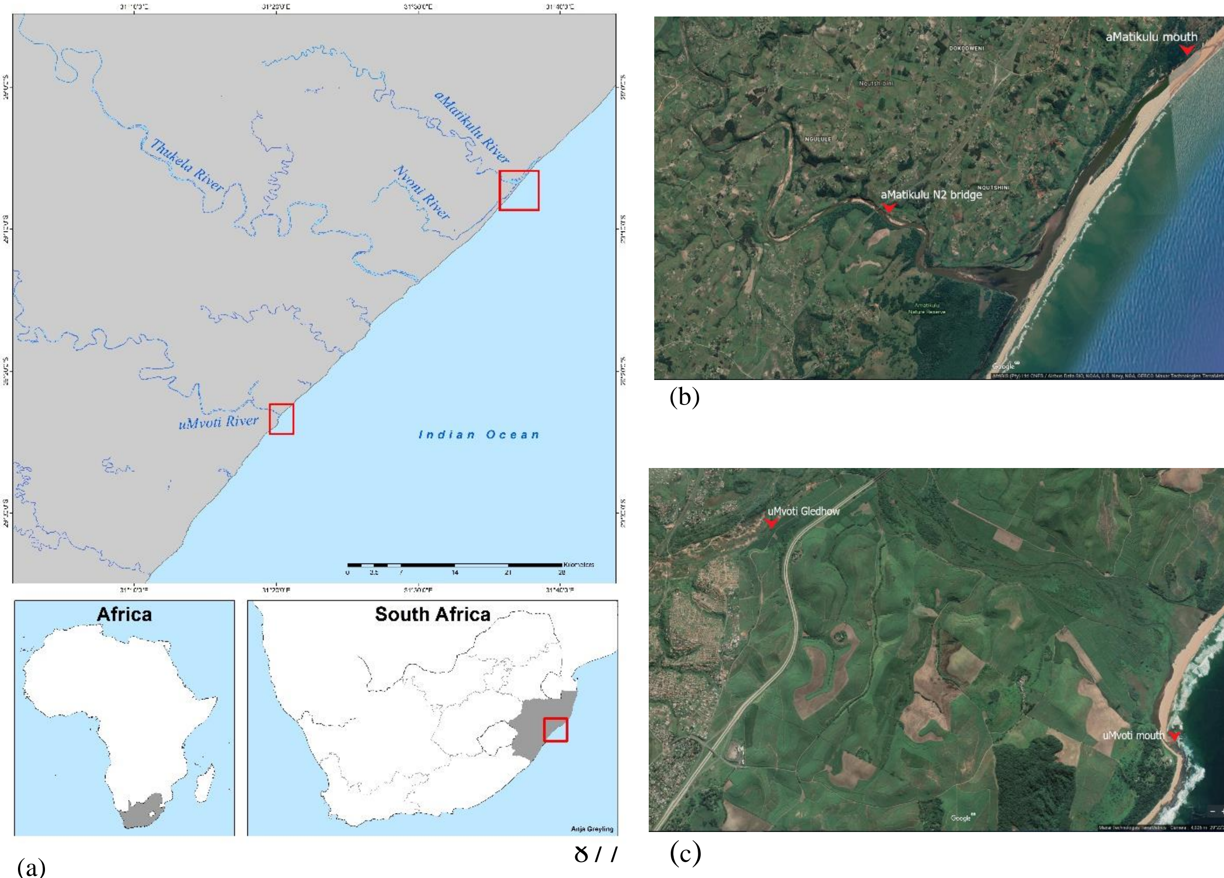

878 Figure 1. (a) Map adapted by Anja Greyling from O'Brien, Swemmer and Wepener, 2009; indicating the study
879 estuaries (highlighted) found along the north coast of South Africa. (b) The two sites (red arrows) along the
880 aMatikulu estuary, the aMatikulu mouth and aMatikulu N2 Bridge site approximately 8.5 km apart. (c) Two sites
881 (red arrows) along the uMvoti estuary, the uMvoti mouth and the uMvoti Gledhow site approximately 6 km apart.

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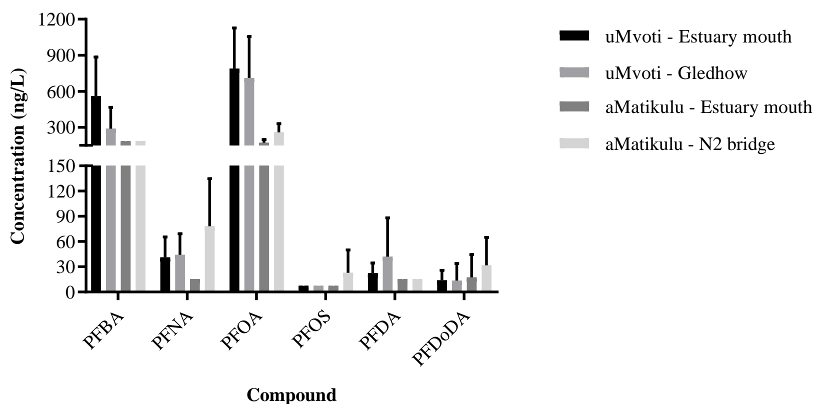

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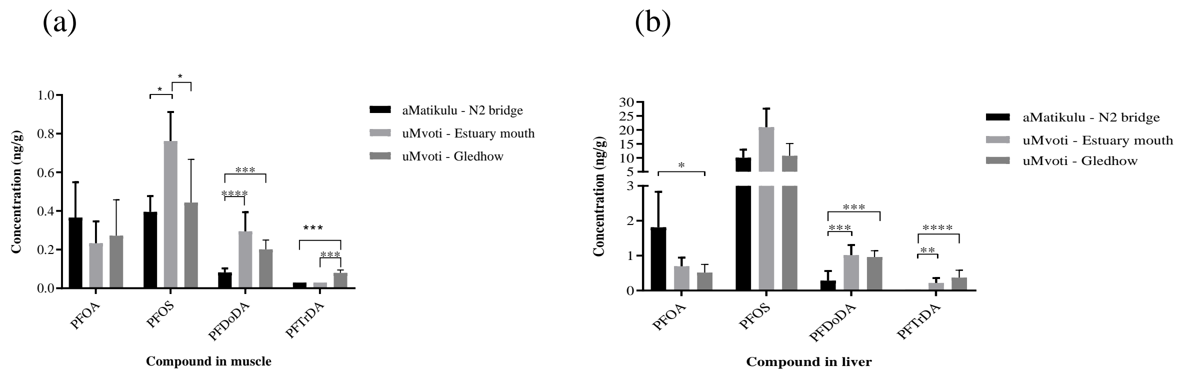
884 Figure 2. Average concentrations (ng/L) and standard deviations of PFASs measured in water samples from each
885 sample site. N = 3 for each location.

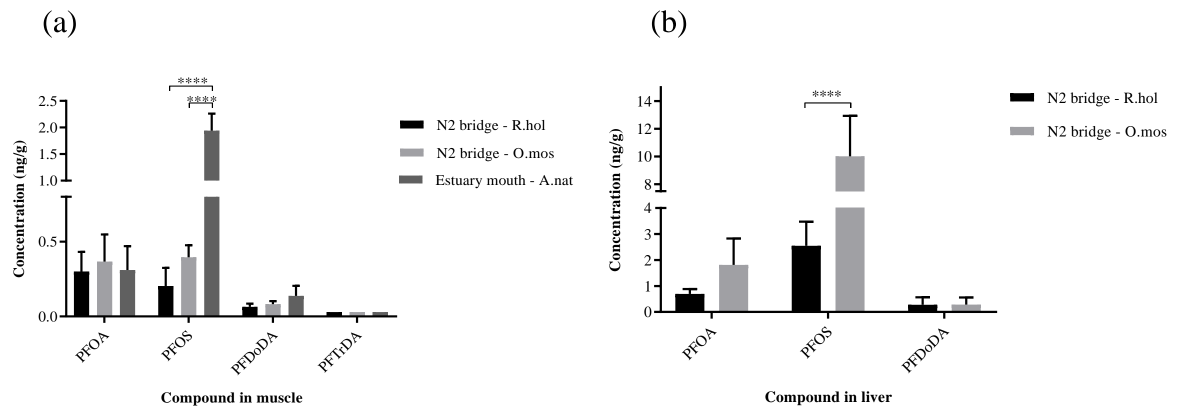
886 (a) (b)



887

888 Figure 3. Average concentrations (ng/g) and standard deviations of PFASs in the muscle tissue (a) and liver tissue
889 (b) of *Oreochromis mossambicus* from the aMatikulu and uMvoti Estuaries. (* = P ≤ 0.05; ** = P ≤ 0.01; *** P
890 ≤ 0.001). N = 5 for muscle samples; N = 3 for liver samples.

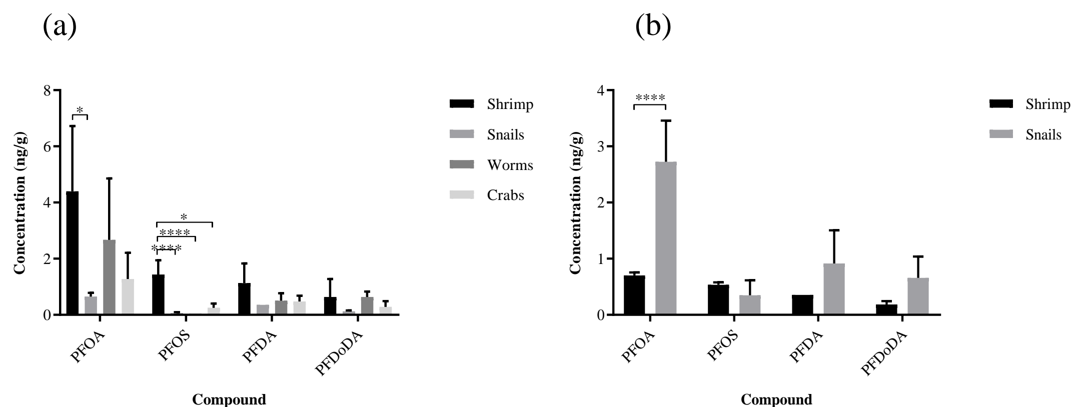
891 (a) (b)



892

893 Figure 4. Average concentrations (ng/g) and standard deviations of PFASs in the muscle tissue (a) and liver tissue
894 (b) of different fish species from the aMatikulu Estuary. (* = P ≤ 0.05; ** = P ≤ 0.01; *** P ≤ 0.001). N = 5 for
895 muscle samples; N = 3 for liver samples.

896 (a) (b)



897

898 Figure 5. Average concentrations (ng/g) and standard deviations of PFASs from different invertebrates from the
899 aMatikulu N2 Bridge site (a) and uMvoti Gledhow site (b) in the aMatikulu and uMvoti Estuaries respectively. (*
900 = P ≤ 0.05; ** = P ≤ 0.01; *** P ≤ 0.001). N= 3.

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903 Tables

904

905 Table 1. LOQs, mean concentrations and ranges (between brackets) in ng/g ww of multiple PFASs in sediment
906 from multiple locations. PFPeA, PFHxA, PFHpA, PFDS, PFNA, PFDA, PFTrDA and PFTeDA were below the
907 LOQ and therefore not displayed in the table. N=3

Compound	LOQ	uMvoti Estuary Mouth	uMvoti Gledhow	aMatikulu Estuary Mouth	aMatikulu N2 Bridge
PFBA	0.3	0.15 (0.15 - 0.15)	0.51 (0.15 - 1.00)	0.15 (0.15 - 0.15)	0.15 (0.15 - 0.15)
PFOA	0.06	0.70 (0.47 - 0.91)	0.41 (0.26 - 0.56)	1.48 (0.83 - 2.50)	1.34 (0.84 - 1.73)
PFOS	0.09	0.36 (0.05 - 0.99)	0.09 (0.05 - 0.19)	0.05 (0.05 - 0.05)	0.05 (0.05 - 0.05)
PFDoDA	0.06	0.23 (0.03 - 0.63)	0.08 (0.03 - 0.11)	0.15 (0.03 - 0.31)	0.28 (0.27 - 0.29)
PFTrDA	0.06	0.03 (0.03 - 0.03)	0.03 (0.03 - 0.03)	0.03 (0.03 - 0.03)	0.25 (0.03 - 0.37)

908

909 Table 2. LOQs, mean concentrations and ranges (between brackets) in ng/g ww of multiple PFASs in liver (L)
910 and muscle (M) tissue of different fish; OM = *Oreochromis mossambicus*, AN = *Ambassis natalensis*, RH =
911 *Rhabdosargus holubi* from the following locations; uMvoti Estuary Mouth (UEM), uMvoti Gledhow (UG)
912 aMatikulu Estuary Mouth (AEM), aMatikulu N2 Bridge (ANB). PFBA, PFHxA, PFHpA and PFNA were below
913 the LOQ and therefore not displayed in the table. N = 3 for liver samples; N = 5 for muscle samples.

Location			PFOA	PFOS	PFDoDA	PFTrDA	PFDA
	LOQ		0.06	0.09	0.06	0.06	0.71
UEM	OM	L	0.70 (0.43 - 1.01)	20.95 (12.27 - 27.96)	1.01 (0.77 - 1.37)	0.22 (<LOQ - 0.33)	1.87 (1.48 - 2.34)
		M	0.23 (0.12 - 0.39)	0.76 (0.62 - 0.97)	0.30 (0.16 - 0.40)	<LOQ	<LOQ
UG	OM	L	0.52 (0.17 - 0.80)	10.74 (7.29 - 17.23)	0.96 (0.82 - 1.18)	0.37 (0.05 - 0.13)	1.32 (0.85 - 1.89)
		M	0.27 (0.12 - 0.58)	0.44 (0.18 - 0.69)	0.20 (0.15 - 0.27)	0.08 (<LOQ - 0.10)	<LOQ
AEM	AN	L	/	/	/	/	/
		M	0.31 (0.11 - 0.51)	1.94 (1.51 - 2.25)	0.14 (<LOQ - 0.20)	<LOQ	<LOQ
ANB	OM	L	1.81 (1.0 - 2.96)	10.02 (6.68 - 12.08)	0.29 (0.07 - 0.59)	<LOQ	1.25 (0.83 - 1.9)
		M	0.37 (0.17 - 0.67)	0.40 (0.31 - 0.53)	0.08 (<LOQ - 0.12)	<LOQ	<LOQ
	RH	L	0.70 (0.54 - 0.90)	2.55 (1.49 - 3.25)	0.27 (0.07 - 0.61)	<LOQ	<LOQ
		M	0.30 (0.09 - 0.44)	0.20 (0.09 - 0.40)	0.06 (<LOQ - 0.09)	<LOQ	<LOQ

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919 Table 3. LOQs, mean concentrations and ranges (between brackets) in ng/g ww of multiple PFASs from
920 different invertebrates from multiple locations. PFBA, PFPeA, PFHxA, PFHpA, PFDS, PFNA, and PFTeDA
921 were below the LOQ and therefore not displayed in the table. N=3 for each invertebrate group.

		uMvoti Gledhow		aMatikulu Estuary Mouth	aMatikulu N2 Bridge	
Compound	LOQ	Shrimp	Gastropods	Shrimp	Shrimp	Gastropods
PFOA	0.06	0.70 (0.64 - 0.74)	2.73 (2.26 - 3.57)	0.58 (0.34 - 1.05)	4.40 (1.89 - 6.50)	2.73 (2.26 - 3.57)
PFOS	0.09	0.54 (0.50 - 0.58)	0.35 (LOQ - 0.54)	0.56 (0.49 - 0.63)	1.43 (0.85 - 1.76)	0.35 (<LOQ - 0.54)
PFDA	0.71	1.52 (1.31 - 1.73)	0.91 (0.85 - 1.54)	<LOQ	1.52 (1.30 - 1.73)	0.91 (<LOQ - 1.54)
PFDoDA	0.06	<LOQ - 0.24	0.66 (0.41 - 1.10)	0.18 (0.16 - 0.20)	0.94 (0.59 - 1.30)	0.66 (0.41 - 1.09)
PFTrDA	0.06	<LOQ	<LOQ	0.04 (<LOQ - 0.06)	<LOQ	<LOQ

922

923 Table 4. Minimal Risk Levels (MRLs) and maximum edible amounts of fish muscle tissue, which a 60 kg person
924 can consume per day without health risks, for mean concentrations of PFASs present in different fish species from
925 the aMatikulu and uMvoti estuarine system (Agency for Toxic Substances and Disease Registry, 2019; EFSA
926 CONTAM Panel. 2018).

	PFOA		PFOS	
	ATSDR (2019)	CONTAM (2018)	ATSDR (2019)	CONTAM (2018)
MRL (ng/kg/day)	3	0.8	2	1.8
MRL (ng/day) for a 60 kg person	180	48	120	108
Mean concentration (ng/g ww) in *Ambassis natalensis*	0.31	0.31	1.94	1.94
Maximum edible amount of *Ambassis natalensis* per day (g) for a 60 kg person	**580**	**155**	**62**	**56**
Mean concentration (ng/g ww) in *Oreochromis mossambicus* (aMatikulu)	0.37	0.37	0.4	0.4
Maximum edible amount of *Oreochromis mossambicus* per day (g) for a 60 kg person	**486**	**130**	**300**	**270**
Mean concentration (ng/g ww) in *Oreochromis mossambicus* (uMvoti)	0.25	0.25	0.6	0.6
Maximum edible amount of *Oreochromis mossambicus* per day (g) for a 60 kg person	**720**	**192**	**200**	**180**
Mean concentration (ng/g ww) in *Rhabdosargus holubi*	0.3	0.3	0.2	0.2
Maximum edible amount of *Rhabdosargus holubi* per day (g) for a 60 kg person	**600**	**160**	**600**	**540**

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