# Assessment of contaminants, health and survival of migratory shorebirds in natural versus artificial wetlands – the potential of wastewater treatment plants as alternative habitats

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**Abstract**

The rapid destruction of natural wetland habitats over past decades has been partially offset by an increase in artificial wetlands. However, these also include wastewater treatment plants, which may pose a pollution risk to the wildlife using them. We studied two long-distance Arctic-breeding migratory shorebird species, curlew sandpiper (*Calidris ferruginea,* n=70) and red-necked stint (*Calidris ruficollis,* n=100), while on their Australian non-breeding grounds using a natural wetland versus an artificial wetland at a wastewater treatment plant (WTP). We compared pollutant exposure (elements and per- and poly-fluoroalkyl substances/PFASs), disease (avian influenza), physiological status (oxidative stress) of the birds at the two locations from 2011-2020, and population survival from 1978-2019. Our results indicated no significant differences in blood pellet pollutant concentrations between the habitats except mercury (WTP median: 224 ng/g, range: 19-873 ng/g; natural wetland: 160 ng/g, 22-998 ng/g) and perfluorooctanesulfonic acid (WTP median: 52 ng/g, range: <0.01-1280 ng/g; natural wetland: 14 ng/g, <0.01-379 ng/g) which were higher at the WTP, and selenium which was lower at the WTP (WTP median: 5000 ng/g, range: 1950-34400 ng/g; natural wetland: 19200 ng/g, 4130-65200 ng/g). We also measured higher blood o,o'-dityrosine (an indicator of protein damage) at the WTP. No significant differences were found for adult survival, but survival of immature birds at the WTP appeared to be lower which could be due to higher dispersal to other wetlands. Interestingly, we found active avian influenza infections were higher in the natural habitat, while seropositivity was higher in the WTP, seemingly not directly related to pollutant exposure. Overall, we found negligible differences in pollutant exposure, health and

survival of the shorebirds in the two habitats. Our findings suggest that appropriately managed wastewater treatment wetlands may provide a suitable alternative habitat to these migratory species, curbing the decline of shorebird populations from widespread habitat loss.

**Keywords:** Shorebirds, blood, pollution, PFASs, population survival

## Introduction

Wetlands, both inland and coastal, have experienced one of the most rapid declines of any ecosystem on the planet, with half to two thirds of coastal wetlands having been destroyed in the last 50 years alone (Finlayson & Rea, 1999; Murray, Clemens, Phinn, Possingham, & Fuller, 2014). Rapid human expansion is the major driving force behind these declines, and perhaps no more acutely is this felt than in coastal wetland habitats. In addition to wetlands, more than 16% of tidal flat habitat has been destroyed between 1984-2016 (Murray et al., 2019), though in some areas coastal habitat loss over a similar time period has been shown to exceed 65% (Murray et al., 2014). The effects of widespread wetland destruction are particularly evident in the East Asian-Australasian Flyway (EAAF) (Ma et al., 2014; Murray et al., 2014). Chains of wetlands within this flyway provide vital staging and fuelling areas for migratory shorebirds (Order *Charadriiformes*) during their biannual migrations. Unfortunately, reliance on these sites makes these birds particularly prone to the widespread destruction of wetlands (Lisovski, Gosbell, Minton, & Klaassen, 2020; Murray et al., 2018). As a result, shorebirds are among the most threatened bird families in the world. Indeed, some populations have declined by up to 80% in the last 30 years alone (Conklin, Verkuil, & Smith, 2014; Garnett, Szabo, & Dutson, 2011). Somewhat compensating for the loss of natural wetlands is that the number of artificial wetlands is increasing, including the number of waste stabilisation ponds. Many sewage treatment plants carry out at least some treatment of human waste in open-air settling ponds that provide consistent water availability and high nutrient density. These habitats appear to be of increasing value to a wide range of bird species (Murray, Kasel, Loyn, Hepworth, & Hamilton, 2013), providing a unique opportunity to both manage human waste as well as combat habitat loss for wetland-dependent wildlife.

Potentially compounding the threat of habitat loss is growing anthropogenic pollution, particularly in the form of toxic metals and other elements, and persistent organic pollutants (POPs). Environmental exposure to metals and elements has long been established as of potential toxicological concern in wildlife. These include elements which are essential at low concentrations but toxic if they exceed these (*e.g.* copper and iron), as well as non-essential elements that are toxic even at low concentrations (*e.g.* lead, cadmium and mercury). In addition to elemental pollutants, an emerging group of organic pollutants that are known to be particularly prevalent in wastewater, are per- and poly-fluoroalkyl substances (PFASs) (Szabo et al., 2023), some of which are also listed or proposed for listing as POPs (UNEP, 2022). Consisting of partially or fully fluorinated carbon chains of varying lengths, these molecules have both hydro- and lipophobic properties (Buck et al., 2011), resulting in their versatile use in *e.g.* mining, pesticides, fire-fighting foam, clothing and cosmetics. Some estimates suggest there may be over 14,000 different PFASs marketed globally (US

Environmental Protection Agency, 2022). The strength of the C-F bond in these compounds also contributes to their environmental persistence and biomagnification (Grønnestad et al., 2019; Kelly et al., 2009), which increases with the length of the carbon chain (≥7 C atoms) (Martin, Mabury, Solomon, & Muir, 2003). Moreover, many PFAS precursors can be bio-transformed into particularly persistent compounds in the environment (Wang, Dewitt, Higgins, & Cousins, 2017), which can result in higher concentrations of PFAS in wastewater effluent than inputs would suggest (Gallen, Eaglesham, Drage, Nguyen, & Mueller, 2018). Consequently, PFAS have become widespread in wildlife (AMAP, 2021; Lee, Lee, Lim, & Moon, 2020).

The effects of both elemental pollutants and PFASs on wildlife can be diverse, and include reduced food availability (De Vries et al., 2017), reduced hatching success (Custer et al., 2014), possible endocrine disruption (Ask et al., 2021; Sebastiano et al., 2021), immunomodulation and immunotoxicity (Castaño-Ortiz, Jaspers, & Waugh, 2019; McDonough et al., 2020; Mitra et al., 2022; Vallverdú-Coll, Mateo, Mougeot, & Ortiz-Santaliestra, 2019), and oxidative stress to DNA and proteins in the body (Marasco & Costantini, 2016; Metcalfe & Alonso-Alvarez, 2010; Rainio & Eeva, 2010), leading to tissue damage (Valavanidis, Vlahogianni, Dassenakis, & Scoullos, 2006). One particular PFAS, perfluorooctanesulfonic acid (PFOS), has also been shown to cause immunomodulation in animals, interfering with signalling pathways in the animals' immune responses (Castaño-Ortiz et al., 2019; Hansen et al., 2020).

Birds are able to excrete pollutants via e.g. faecal matter (Dauwe, Bervoets, Blust, Pinxten, & Eens, 2000), and deposition of pollutants in growing feathers and eggs (Briels et al., 2019; Burger & Gochfeld, 2004; Hargreaves, Whiteside, & Gilchrist, 2010; Løseth et al., 2019). The predominant concern is the tendency of these pollutants to bioaccumulate, that is, when contaminant uptake is higher than its excretion (Berglund, Koivula, & Eeva, 2011; Blomqvist, Frank, & Petersson, 1987). This process can result in accumulation of high concentrations of pollutants in organism tissues and organs, which may threaten wildlife health (Carneiro et al., 2018; Dietz et al., 2019). Hargreaves et al. (2010) examined 17 different elements present in Arctic-breeding shorebirds and found that Hg was elevated to potentially harmful concentrations. Aside from this study, mercury has also been found to occur in harmful concentrations in *Charadrius* plovers (Picone et al., 2019; Su et al., 2020), underscoring the need for more extensive pollutant-burden analyses in shorebirds (Ma, Choi, Thomas, & Gibson, 2022). This is especially necessary in shorebirds as they may be more susceptible to pollution due to the physical stresses from migration (Liess, Foit, Knillmann, Schäfer, & Liess, 2016), and their propensity to forage in soft coastal sediments where pollutants can accrue at much higher concentrations than the surrounding water (Stark, 1998).

113 Of special concern are effects of pollutants on immune function in avian migrants, given they are
114 known hosts and dispersers of zoonoses such as avian influenza virus (AIV) (Verhagen, Herfst, &
115 Fouchier, 2015; Wille et al., 2019), as well as many other pathogenic microorganisms (Hubálek,
116 2004). Infection with AIV has been shown to be indicative of high viral diversity in shorebirds (Wille
117 et al., 2018), and may vary with immunomodulatory effects from e.g. PFAS contamination. Whether
118 this affects shorebirds that increasingly utilise artificial wetlands is less well known and would be
119 vital to understand given the increasing reliance of some shorebird populations on artificial
120 wetlands.
121 To study whether wastewater treatment plants could be a viable alternative habitat to migratory
122 shorebirds, we analysed red blood cell samples from two species of migratory shorebirds commonly
123 found around Australian coasts during the austral summers from 2011 to 2021, at two different sites
124 – one a wastewater treatment plant, another a natural coastal wetland. Our species, curlew
125 sandpipers (*Calidris ferruginea*) and red-necked stints (*Calidris ruficollis*), are both small, long-
126 distance migratory shorebirds that breed in the high Siberian Arctic and are threatened by habitat
127 loss along the flyway (Clemens et al., 2016; Melville, Chen, & Ma, 2016). In addition, ongoing field
128 studies of both species at both sites have also accrued over 40 years of banding data that allow us to
129 conduct survival analyses for insight into population dynamics of these species at our study sites. In
130 both sites and species, we studied concentrations of 15 inorganic elements, 15 PFASs, two oxidative
131 stress (OS) biomarkers, AIV infection and serostatus as well as population survival. Together, these
132 metrics were used to evaluate the adequacy of wastewater treatment plants as suitable alternative
133 habitat for shorebirds and assess the occurrence and effect of pollution and disease in these birds at
134 both an individual and a population level, based on their choice of habitat.
135
136 **Methods**
137 **Ethics statement**
138 This research was conducted under approval of Deakin University Animal Ethics Committee (permit
139 numbers A113-2010, B37-2013, B43-2016), Philip Island Nature Park Animal Ethics Committee
140 (SPFL20082). Banding was done under Australian Bird Banding Scheme permit (banding authority
141 numbers 2703, 2915, 8000, 8001)
142
143 **Sample Collection**
144 Curlew sandpipers and red-necked stints were captured in Victoria (south-eastern Australia),
145 distributed across 8 major sites along the coast. The two sites where most birds were captured in
146 the course of an annual monitoring program by the Victorian Wader Study Group (VWSG) were

147 chosen as our study sites (Fig. 1 A). The first of these is Melbourne's Western Treatment Plant (WTP,
148 -37.99 S, 144.62 E), run by Melbourne Water. These wastewater ponds serve to treat the effluent
149 from over half of the population of Melbourne (~5 million people). They are also renowned for the
150 birdlife that inhabits the area, with considerable efforts made in their management to maintain
151 habitat for wildlife (Steele & Harrow, 2014). Curlew sandpipers and red-necked stints at the WTP
152 carry out much of their foraging at low tide on adjacent flats, which have been enriched by effluent
153 discharges since the 1930s (Steele & Harrow, 2014); at high tide they roost and sometimes forage in
154 constructed, shallow freshwater ponds – mainly former sewage ponds, in which the water supply is
155 now partially or fully treated effluent. For comparison to the WTP, our second sample site was
156 Yallock Creek (-38.21 S, 145.397 E), situated on the northern coast of Western Port Bay to the east of
157 Melbourne. Yallock Creek represents a substantially less-modified coastal wetland, where shorebirds
158 forage on natural tidal flats, and roost above the tideline in settings (e.g. beaches, spits) where little
159 or no supratidal foraging is possible. Distance between these two sites is approximately 80km. Birds
160 were captured by cannon-net as part of long term monitoring program, initiated in 1978 (Minton,
161 2006). Catches at each site generally occurred one to three times per year, and when caught, birds
162 were banded with an Australian Bat and Bird Banding Scheme band and aged into categories of 1
163 year (or younger, hereby referred to as 'immature'), and 2+ year old categories based on plumage
164 characteristics. Blood samples used in all analyses were collected from these birds between 2011
165 and 2021.
166 Blood and swab samples were collected as part of a long term study on avian influenza (Wille et al.,
167 2023). Approximately 200 µl of whole blood was taken from each individual bird from the brachial
168 vein using Microvette capillary tubes (Sarstedt), using new, clean tubes for each individual bird to
169 minimise chance of contamination in the field. These were left to clot and then centrifuged to
170 separate red blood cells (RBC) from serum and stored at <-20 °C. Oropharyngeal and cloacal swabs
171 were taken from each bird, using sterile swabs (Copan). Once collected, swabs from each bird were
172 stored in virus transport media (VTM, brain heart infusion [BHI] broth-based medium [Oxoid] with
173 0.3 mg/ml penicillin, 5 mg/ml streptomycin, 0.1 mg/ml gentamicin, and 2.5 g/ml, amphotericin B),
174 and stored at -80 °C.
175 In total, 100 red-necked stint and 70 curlew sandpiper RBC samples were used across all pollutant
176 and OS biomarker analyses. A subset of these was used for each analysis, as due to limitations of
177 sample volume not all samples could be analysed for every pollutant class or OS biomarker.
178
179 **Chemical/pollutant analysis**

180 We analysed 86 RBC samples from 2011-2020 for elements, selecting samples to ensure an even
181 representation of years and age groups (immature vs. adults, 43 of each) amongst samples. For
182 these samples, we targeted 15 different elements in our analyses (As, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn,
183 Ni, P, Pb, Se, Sr, Zn). Sample digestion was performed at the Norwegian University of Science and
184 Technology (NTNU) according to previously published methods with minor modifications
185 (Jantawongsri et al., 2021). Approximately, 0.040 g (±0.028 g) of RBCs were transferred into pre-
186 washed Teflon® tubes (15 mL) and 1.5 mL concentrated ultra-pure grade nitric acid [purified from
187 $HNO_3$ (AnalaR NORMAPUR®, VWR) in sub-boiling distillation system, Milestone, SubPur, Sorisole, BG,
188 Italy] was added for a subsequent digestion in a high-pressure microwave system (Milestone
189 UltraCLAVE, EMLS, Leutkirch, Germany) with a maximum temperature of 245 °C at 110 bar for 2.5 h.
190 After digestion, the RBC samples were diluted with ultrapure water (Q-option, Elga Labwater, Veolia
191 Water Systems LTD, UK) to attain a final weight of approximately 35 g (35.61 ± 0.16 g) in 50 mL
192 polypropylene (FalconTM) tubes. Final elemental determination was performed using a triple
193 quadrupole inductively coupled plasma mass spectrometry instrument with $^{103}$Rh, $^{185}$Re and $^{193}$Ir as
194 internal standards (Inorganic Ventures, USA). The analyses were conducted in Norway at SINTEF
195 research institute with Agilent 8900 ICPQQQ (Agilent Technologies, USA) instrument in 2020 and at
196 NTNU (Agilent 8800 ICPQQQ) in 2021.
197 Three blanks were added in every digestion sample batch and subsequently, all concentrations were
198 corrected for the blank samples. Method detection limits (MDLs) were calculated using three times
199 the standard deviation of the background equivalent concentrations (BEC: blank counts divided by
200 the slope of the calibration curve). To assure quality of analysis, three replicates (one per sample
201 batch) of the quality control material (human whole blood Seronorm Trace Elements Whole
202 BloodTM, Sero, Norway) were prepared. The concentrations found were within 82.2-110.5% of the
203 analytical values, except for As (72.2%).
204 The concentrations of 15 different PFASs were analysed at NTNU from 150 samples taken between
205 2011 and 2020, again ensuring an even spread of years and age groups (n= 80 juveniles and 70
206 adults). The target compounds were broadly classed into three categories, carboxylates (PFPA,
207 PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA PFTrA, PFTeA, full names and details of chemicals
208 provided in Table S1), sulfonates (PFBS, PFHxS, PFOS), and total PFASs (including all the
209 aforementioned compounds, as well as PFOSA and NEtFOSA). The RBC samples were extracted using
210 the method of Trimmel et al. (2021) with minor modifications, using hybrid-SPE. The extracts were
211 transferred to amber vials for UPLC-MS/MS analysis. All samples were spiked with $^{13}$C-isotope
212 labeled PFOS and PFOA IS-mixture. Recoveries ranged from 62% to 157%. A laboratory blank was
213 included in each batch of 20 samples (see Table S2).

214

215 **Avian influenza analyses**

216 Samples were screened for avian influenza as per Wille et al. (2021). Briefly, for swab samples
217 collected between 2011 to 2015, RNA was extracted using the MagMax 96 Viral Isolation Kit (Ambio,
218 Thermo Fisher Scientific) using the Kingfisher Flex platform (Thermo Fisher Scientific). RNA was
219 assayed for a short fragment of the matrix gene (Fouchier et al., 2000). First, using the Superscript III
220 Platinum ONE step qPCR Kit (Life Technologies, Thermo Fisher Scientific) with ROX, followed by a
221 subsequent amplification and detection using the SYBR Green mastermix (Life Technologies, Thermo
222 Fisher Scientific). For all samples after 2015, RNA was extracted using the NucleoMag Vet Kit
223 (Scientifix) on the Kingfisher Flex System. Extracted RNA was subsequently assayed for a short
224 fragment of the matrix gene (Spackman et al., 2002) using the SensiFAST Probe Lo-Rox qPCR Kit
225 (Bioline). A cycle threshold (Ct) cut-off of 40 was used. All serum samples were assayed using the
226 Multi Screen Avian Influenza Virus Antibody Test Kit (IDEXX, Hoppendorf, The Netherlands) following
227 manufacturer's instructions.

228

229 **Oxidative Stress Biomarker analyses**

230 Following our pollutant analyses, we utilised oxidative stress biomarkers taken from a subset of our
231 total sample collection, totalling 56 RBC samples. These biomarkers were used as an indicator of the
232 relative health of individual birds at each site, regarding their contamination. To measure the OS of
233 each bird, we chose 8-hydroxy-2-deoxyguanosine (8-OHdG) which is the product of oxidation of
234 DNA, and o,o′-dityrosine (DIY) which indicates peroxidation of proteins (Martinez & Kannan, 2018).
235 The RBC samples were extracted and analysed at NTNU based on the procedure reported by Zhang
236 et al. (2022). All samples were spiked with 8-OHDG-$^{15}N_5$ and DIY-$^{13}C_{12}$. Recoveries of 8OHDG and DIY
237 were 101% and 96%, respectively.

238

239 **Statistical analyses**

240 *Site fidelity*

241 We used long-term banding data from 1978 to 2019 to highlight movement between the sampling
242 sites (primarily WTP and Yallock Creek). Exchange between sites was evaluated using each individual
243 bird's recapture event, taking into account where they were first captured. We made three site
244 fidelity analyses, focusing on i) immature site fidelity, ii) adult site fidelity, and iii) overall population
245 site fidelity. The significance of any difference between immature and adult site fidelity was
246 determined using an exact binomial test in R. Data for overall population site fidelity were depicted

using the package circulize (Version 0.4.15) implemented in R version 4.2.0 (Gu, Gu, Eils, Schlesner, & Brors, 2014).

*Elements, PFAS, AIV and oxidative stress biomarker analyses*

All statistical analyses were conducted using R. All 15 PFASs were summed into their respective categories. Where a compound/group of compounds (out of 15 elemental pollutants, Total PFASs, PFCAs, and PFSAs) was not detected in 50% or more of the samples, this was excluded from statistical analyses. For each pollutant as well as each biomarker we investigated the effects of species, age group (immature vs adult) and sample site using linear mixed effect modelling from R package lme4 (Bates, Mächler, Bolker, & Walker, 2015) with year as a random factor. For all elements, summed PFAS categories, and oxidative stress biomarkers where the sample concentrations were below the level of detection (LOD), concentrations were set at 0.015 ng/g (half of the lowest LOD in the study) prior to statistical analysis. Additionally, all PFAS concentrations were log transformed to remove skewness and guarantee homogeneity of variances in the data. To analyse viral and sero-prevalence of AIV we used generalised linear mixed-effect models with family set to binomial, with dependent variables of region and age group.

*Survival analyses*

The banding data from 1978 to 2019 data was used to estimate survival of each species at each sampling site, using Bayesian population analyses adapted from Kéry and Schaub (2011). Each of these four models were conducted using WinBUGS14, and R within RStudio. In each model we assumed a constant, age-dependent (*i.e.* 1 and 2+) annual survival ($\Phi$) across the 40-year study period, i.e. each model led to the estimation of an average immature and an average adult survival rate. We furthermore assumed a fixed recapture rate ($p$) for both age classes but variable across years *(i.e.* for each year of the study a separate recapture rate was estimated to accommodate for annual variations in capture effort). Models were run using four Markov chain Monte Carlo (MCMC) simulations of 30,000 iterations each, discarding the first 15000 and using a thinning factor of 20 to result in 3000 iterations being saved, which were used to describe the posterior distribution of each parameter (in this case, mean adult and immature survival and annual recapture rate and the 95% confidence interval of each). To evaluate the accuracy of $\Phi$ and $p$ estimates, we used $\hat{R}$ and ensured that all models had $\hat{R}$ values as close to 1 as possible (for all cases, $\hat{R}$ <1.02).

**Results**

*Site fidelity*

281 In order to confirm that the sites we used in our study were independent with limited bird
282 movement, we performed a site fidelity analysis. Over the 40-year VWSG banding period, a total of
283 4995 curlew sandpipers and 32,705 red-necked stints recaptures were recorded across all catching
284 sites, unequivocally showing that site faithfulness to WTP and Yallock Creek was very high (Figure
285 1A-D). Binomial tests indicated that curlew sandpipers demonstrated 97.8% (95% CI: 96.8-98.5) and
286 81.5% (95% CI: 79.8-83.1) fidelity to WTP and Yallock Creek and red-necked stints showed 97.6%
287 (97.3-97.9) and 89.5% (88.9-90.0) fidelity, to both sites respectively. When broken down into
288 immature and adult constituents, immature curlew sandpipers showed 93.9% (88.3-97.3) and 76.8%
289 (72.6-80.1) site fidelity to WTP and Yallock Creek, and immature red-necked stints showed 93.2%
290 (91.7-94.5) and 84.4% (82.8-86.0) fidelity to both sites respectively. This is compared with adult
291 birds, where curlew sandpipers showed 98.1% (97.0-98.8) and 84.1% (82.3-85.8), while red-necked
292 stints showed 98.2% (98.0-98.5) and 90.5% (89.9-91.1) to each site respectively. The lower site
293 faithfulness to Yallock Creek compared to WTP was likely due to a higher availability of alternative
294 sites in Western Port Bay and if these sites were included we would likely see higher site fidelity
295 within Western Port Bay than just at Yallock Creek. However, as we only have analysed samples from
296 Yallock Creek (the largest catch site on Western Port Bay), we have limited our site fidelity analyses
297 to this specific site only. The overall curlew sandpiper exchange rate from WTP to Yallock Creek was
298 0.5% and the reverse exchange rate was 0.9%. For red-necked stints, overall rates were 0.7% and
299 0.8%. Despite no account of annual variation in catching effort, this quantity of data over 40 years
300 overwhelmingly shows the vast majority of birds spent most, if not all of their non-breeding seasons
301 in either WTP or Yallock Creek.
302
303 *Element and PFAS concentrations*
304 Four compounds, chromium (Cr), cobalt (Co), nickel (Ni) and cadmium (Cd) were not detected in
305 more than 50% of samples and so were not included in statistical analyses. Of the 11 remaining
306 elements, 8 showed no significant difference between species, regions or age groups (Table 1, Figure
307 2, Tables S3 and 4). Three elements exhibited significant difference with at least one explanatory
308 variable (Table 1). Specifically, Iron (Fe) was found to be significantly lower in red-necked stints than
309 in curlew sandpipers (Figure 2B), as was selenium (Se). Se was also lower at the Western Treatment
310 Plant than Yallock Creek (Figure 2C; WTP median: 5000 ng/g, range: 1950-34400 ng/g; Yallock Creek
311 median: 19200 ng/g, range: 4130-65200 ng/g), and lower in immature birds than adults. By contrast,
312 mercury was found to be higher in the WTP than Yallock Creek (Figure 2D, WTP median: 224 ng/g,
313 range: 19-873 ng/g; Yallock Creek median: 160 ng/g, range: 22-998 ng/g).

314 No significant differences were discerned in the mean concentrations of ΣPFCAs or ΣPFSAs between
315 species, region or age group (Table 2). However, the total PFASs concentrations we observed were
316 found to have a statistically significant difference between region and age group, with higher
317 concentrations observed at the WTP (median: 71 ng/g; range: <0.01-1360 ng/g) than Yallock Creek
318 (median 26 ng/g; range: <0.01-414 ng/g), and lower concentrations in immatures than in adults
319 (Figure 2E). This pattern was driven by the most prevalent individual PFAS in our study, PFOS, which
320 also exhibited the same significant patterns between region and age group (Figure 2F, WTP median:
321 52 ng/g, range: <0.01-1280 ng/g; Yallock Creek median: 14 ng/g, range: <0.01-379 ng/g), and likely
322 was the driver of the significant difference in total PFASs.
323
324 *Pollutants associations to disease and oxidative stress*
325 As metals, other elements and PFAS may modulate the immune systems of birds, we used avian
326 influenza infection as a disease marker in this study. A total of 215 curlew sandpiper and 855 red-
327 necked stint samples from Yallock Creek, and 430 and 775 from the same respective species at the
328 WTP were used to determine AIV prevalence and seroprevalence. Red-necked stints from Yallock
329 Creek were found to have higher rates of active infection than stints from the WTP (Figure 2G),
330 however, stints from the WTP had significantly higher rates of seropositivity than their Yallock Creek
331 counterparts (Figure 2H). As infection status and seroprevalence are modulated by bird age, we
332 tested whether this pattern was driven by age. It does not appear to be, as no significant difference
333 in active infection status was detected between age groups (adults vs immatures), however
334 immature birds had significantly lower rates of seropositivity than adults (Table 2).
335 We further investigated markers of oxidative stress in relation to metals, elements and PFAS. No
336 significant difference was found between concentrations in either species or age group for either of
337 our biomarkers (Table 2), and no difference between regions for 8-OHdG (Figure 2I). However, we
338 did find a significant difference between regions for DIY, with slightly higher average concentrations
339 observed in the individuals at the WTP than Yallock Creek (Figure 2J).
340
341 *Apparent survival*
342 For both curlew sandpiper and red-necked stint, local survival analyses yielded no significant
343 differences (i.e. 95% CI's were overlapping) in adult survival between the two sites (see Table 3 for
344 summary). Adult curlew sandpiper apparent survival was 70.5% (95% CI: 68.8-72.1%) and 71.7%
345 (70.3-72.9%) at WTP and Yallock Creek respectively, while red-necked stint survival was 69.3% (68.7-
346 69.8%) and 66.7% (66.0-67.4%) at WTP and Yallock Creek respectively. However, one particularly
347 interesting finding is that we did find significant lower apparent survival of immature birds at WTP

than at Yallock Creek in both curlew sandpiper (WTP 61.2% [50.1-73.0%]; Yallock Creek 82.1% [73.4-91.4%]) and red-necked stint (WTP 63.6% [59.9-67.4%]; Yallock Creek 75.3% [72.1-78.5%]). In both species the average immature survival was higher than adult survival in Yallock Creek, but lower in WTP (Figure 3), which was significantly different for red-necked stints. However, in curlew sandpipers this difference was non-significant due to overlapping confidence intervals.

## Discussion

Although a waste-water treatment plant may have the connotation of being a potentially suboptimal habitat compared to a natural habitat, our collective results, encompassing the analysis of as many as 30 pollutants, two oxidative stress biomarkers, a key disease marker and a survival analysis for two shorebird species, do not suggest the investigated WTP to be an inferior habitat for shorebirds compared to Yallock Creek.

### *Site fidelity*

Our analyses showed high site fidelity of these shorebirds, indicating that our local survival data on adult is unlikely to be heavily influenced by the emigration of birds from the WTP (where the VWSG has caught birds) to other sites (where birds are not caught, e.g. inland). Our local adult survival estimates for both species were broadly comparable to those presented by Méndez, Alves, Gill, and Gunnarsson (2018), differing only by 2-4% in total, and our indication of minimal exchange between sites once again highlighted the extreme site fidelity shown by many migratory shorebirds (Christie & Jessop, 2009; Coleman & Milton, 2012; Warnock & Takekawa, 2008). Such high site fidelity allowed us confidence that differences seen in exposure between sampling sites were indeed reflective of the contamination in these different environments.

### *Inorganic element and PFAS concentrations*

Hg and PFOS concentrations were the only two pollutants of our 30 targeted elements/compounds that showed any indication that the WTP is a more polluted environment than the relatively unmodified coastal wetland of Yallock Creek. No other pollutants showed no significant differences between sites, except for Se which was found to be higher at Yallock Creek than WTP. In this case, Se at Yallock Creek may be attributable to geogenic sources in the region rather than anthropogenic sources (Gebreeyessus & Zewge, 2019). This may have different reasons such as different elemental composition of the soil, minerals in rock and the bioavailability of different elemental forms (speciation) in different locations such as presented for the case with Se (Winkel et al., 2012). In addition, agriculture could present a source of different elements to the environment, such as

through the use of fertilisers and pesticides (Zhang & Wang, 2020). Where there are significant differences between age-group concentrations (seen in Se and PFOS/total PFASs), this can likely be attributed to the tendency of both to bioaccumulate during the birds' life spans; immature birds have not had the continued exposure to garner high concentrations that an adult may have (Lee et al., 2020; Yue, Huang, Wang, & Qiao, 2021). It is possible that the minimal differences in elements and PFASs concentrations that we observed between sites were also due to the proximity of Yallock Creek to the Melbourne region, hence it may not be as clean as sites more remote from human settlements.

Moreover, our contaminant concentrations in both sites also appeared generally low, with only two main exceptions (As and Se) appearing to exceed possible adverse effects levels in blood (Burger et al., 2019; Tsipoura et al., 2017). This is also supported by our biomarker 8-OHdG for oxidative damage to DNA where we saw no difference between sampling sites, and nor did we see any difference in the survival of adults. We did, however, see differences in apparent local survival of first year birds between sites (addressed below), as well as some difference in AIV infection load in the population where birds at the WTP showed less prevalence of active AIV infections than birds at Yallock Creek. However, birds at the WTP had higher seroprevalence, which suggested that these individuals had been previously exposed to AIV more frequently compared to Yallock Creek birds. This observation paired with the higher Hg and PFOS concentrations we saw at the WTP, could contribute to the higher levels of oxidative damage to proteins indicated by our DIY indicator at the WTP. Despite this last finding, the overall low concentrations and minimal differences between regions that we observed tentatively suggest that appropriately managed wastewater treatment wetlands may be suitable alternative habitat for shorebirds and may potentially compensate for habitat loss.

Our suggestion of the use of wastewater wetlands as novel habitat is not without caveats. The higher concentrations of PFOS (and therefore total PFASs) and mercury at the WTP certainly warrant some concern; indeed sewage in wastewater plants is an established environmental source of PFASs (Arvaniti & Stasinakis, 2015) and elements (Farkas et al., 2020), and so may yet threaten wildlife. The absolute concentrations of pollutants seen in both red-necked stints and curlew sandpipers might still be of concern for conservation outlooks, especially given that pollutants such as these have been noted to be persistent in animal tissues (Stahl, Mattern, & Brunn, 2011). While our samples had lower concentrations of most elemental pollutants than observed in other studies of elements in whole blood of shorebirds (Burger et al., 2019; Hargreaves et al., 2010; Tsipoura et al., 2017) in which such levels were noted to be lower than toxicological thresholds, our As and Se concentrations were found to greatly exceed those seen in Burger et al. (2019), and Hargreaves et al.

416 (2010). In the case of the latter of these two studies, our Hg concentrations were lower by
417 comparison, even at the WTP, which could be because our birds were caught when actively
418 moulting, or having just finished moult and thereby excreted Hg into their feathers (Furness,
419 Muirhead, & Woodburn, 1986). The As concentrations at both sites and the Se concentrations at
420 Western Port Bay appeared higher than toxicological thresholds for sublethal effects (Burger et al.,
421 2019; Tsipoura et al., 2017), which is cause for concern for the health of these birds, despite Se being
422 an essential element. By comparison, the Se concentrations seen in the WTP birds likely pose little
423 threat to their health.
424 Regarding PFASs, our use of RBC differed from the more commonplace use of serum in other PFAS
425 studies (Lopez-Antia et al., 2019; Løseth et al., 2019; Newsted, Jones, Coady, & Giesy, 2005). Gebbink
426 and Letcher (2012) found relationships between plasma and RBC concentrations of PFOS to be
427 approximately 4:1. When this relationship is applied to our collective PFAS data, it suggests that our
428 observed PFASs contamination for all but PFOS in some individuals were predominantly lower than
429 thresholds discussed in Newsted et al. (2005). These concentrations may still pose a threat at
430 sublethal levels, particularly compared to the lowest liver concentrations with observable effects
431 reported in bobwhite quail (*Colinus virginianus*) by Dennis et al. (2021). Comparisons with these liver
432 concentrations are only tentative due to different matrices, but blood concentrations of PFOS tend
433 to be lower than those seen in liver (Gebbink & Letcher, 2012). However, in a small number of
434 samples from both sites, PFOS concentrations were particularly elevated. Occurrence of such
435 elevated concentrations with potential harmful health effects reinforces the importance of ongoing
436 monitoring of pollutants for shorebird conservation, especially at artificial wetlands such as the WTP.
437
438 *Impacts on immune response*
439 Our AIV infection data provides a valuable insight into the immunocompetence of our shorebirds.
440 Many pollutants can influence the way birds' immune systems can respond to pathogens, either by
441 being directly immunotoxic (Mitra et al., 2022) or by modulating aspects of immune response such
442 as altering antibody function and inflammatory responses (Dewitt, Peden-Adams, Keller, &
443 Germolec, 2012). There is some evidence to suggest that migration may also reduce immune
444 function (Eikenaar & Hegemann, 2016), which would be problematic for shorebirds. Along their
445 migration in the EAAF, they traverse an avian influenza hotspot (Wille et al., 2019), infection by
446 which can be used as a marker for other diseases (Wille et al., 2018). Birds with reduced immune
447 function are inevitably more susceptible to disease, and a combination of migration and pollution
448 stress on immune response could directly increase the prevalence of disease in the population,
449 thereby decreasing their population survival.

450 In our study, we found significant differences in both AIV prevalence and seroprevalence, however,
451 due to no consistent differential pollution between either sample site we cannot attribute these
452 differences to exposure to contaminants. If the WTP was found to be a consistently more polluted
453 site, we would have expected AIV infection data to show patterns similar to those seen in AIV
454 seroprevalence, where individuals at the WTP have higher levels than those at Yallock Creek. This
455 was not the case. However, the reason that red-necked stints at the WTP have lower prevalence of
456 AIV infections than birds inhabiting Yallock Creek remains unknown. By comparison, the higher
457 seroprevalence of AIV in adult birds than immature birds was as expected. Juvenile birds are naïve to
458 the disease by comparison to their older conspecifics, as they have had less time to be exposed and
459 then recover, resulting in lower prevalence of antibodies in their blood serum (Wille et al., 2023).
460
461 *Apparent survival*
462 Pollution can present an insurmountable threat to survival of wildlife. Therefore, it was important
463 that we analysed this in our assessment of habitat quality, in which we found no difference in adult
464 survival between locations. There are widespread declines in shorebirds that have been documented
465 along the flyway (Clemens et al., 2016; Studds et al., 2017), but we don't appear to find any
466 differences between either our species or our sites. This suggests that any effects on adult survival
467 are not site-specific in Australia, and observed population declines are related to other factors in the
468 flyway rather than any differential exposure to pollution due to habitat choice within Australia.
469 Where we did see differences in pollution concentrations between sampling sites, such as in Hg,
470 PFOS and Se, high site fidelity allowed us confidence that these were indeed reflective of site-related
471 differences. However, our analyses only highlight local survival, and due to difficulties in measuring
472 true survival (e.g. limited by the inability to catch birds in every region of their distribution), true
473 survival is likely higher than our local survival estimates. Nevertheless, the different apparent local
474 survival of immature birds between both sites raised two interesting points. Firstly, immature
475 survival was higher than that of adults at Yallock Creek in both red-necked stints and curlew
476 sandpiper. Immature survival within Australia does not reflect the juvenile birds that perish along
477 their migration; only the strongest juveniles arrive in Australia and it is their survival that is being
478 estimated, causing the appearance of a higher rate of immature survival than adult survival here.
479 Not only this, the immature birds also do not migrate the following year after they first arrive in
480 Australia (Menkhorst et al., 2019), which takes out two potentially arduous long-distance migrations
481 to and from the breeding grounds, compared to their adult con-specifics.
482 Secondly, immature survival was reduced in the birds from the WTP. Our survival analyses examine
483 local survival rather than true survival and were unable to account for undetected movement

484 beyond our sample sites. This is particularly pertinent to immature survival, as first year juveniles do
485 not migrate the following year from their initial arrival, and may exhibit increased exploratory
486 behaviours and travel widely in this first year of life in Australia (D. Rogers, pers. comm.), which has
487 been posited to be fuelled by an increase in mass over the austral winter despite not migrating
488 (Rogers, Rogers, & Minton, 1996). Such exploration is also seen in other species of shorebirds such
489 as red knots (*Calidris canutus*) and bar-tailed godwits (*Limosa lapponica*) (Battley, Schuckard, &
490 Melville, 2011; Battley et al., 2020). Our sampling efforts on Port Phillip Bay are concentrated at the
491 WTP, but immature birds wander and inhabit other areas nearby that we do not sample thereby
492 'removing' them from our sample population, which is indicated by our slightly lower immature site
493 fidelity (Figure 1B). At Western Port Bay, there are several catching sites within close proximity so
494 this movement can be detected (e.g. purple in Figure 1A, C-D) but with no other catching sites within
495 the WTP, such movement would be lost despite our observed high site fidelity in recaptured
496 immatures. As a result, immatures leaving the WTP for other locations where they are not caught
497 again could account for the apparent lower immature survival seen at this site.
498
499 **Considerations and conclusion**
500 Threats other than the pollutants we have analysed may also require further investigation. For
501 example, current-use pesticides from agricultural runoff in sites like Yallock Creek may harm the
502 region's wildlife (Chen et al., 2020) and contribute to a different pollutant profile when compared to
503 a wastewater plant. On the other hand, emerging pollutants such as pharmaceutical contamination
504 (Patel et al., 2019), and antimicrobial resistance in bacteria in wastewater (Marcelino et al., 2019)
505 may impact birds inhabiting the WTP in ways we have not investigated in this study. Indeed,
506 pharmaceutical contamination and antimicrobial resistance could also contribute to the lower
507 apparent immature survival we have seen. Immature foraging proficiency is often lower than adult
508 birds resulting in differences in strategy and prey choice (Groves, 1978; Puttick, 1979), which might
509 result in higher susceptibility to pollutants such as pharmaceuticals, as well as antimicrobial resistant
510 pathogens in the environment. Additionally, legacy POPs may also pose a threat to shorebirds (Ma et
511 al., 2022), and these have not been included in the current study. More studies are required in these
512 fields about the health of shorebirds to complete our understanding of the effects of wastewater on
513 contamination in shorebirds. Ongoing monitoring is also vital considering elevated PFOS
514 concentrations that we have seen in a small minority of our studied birds. Nevertheless, when taken
515 together, our findings regarding pollutant concentrations, health status, and survival in shorebird
516 populations at both sites indicate negligible differences, and tentatively suggest that controlled

wastewater treatment plants could provide a valuable alternative habitat to migratory shorebirds along their flyway.

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## Figures

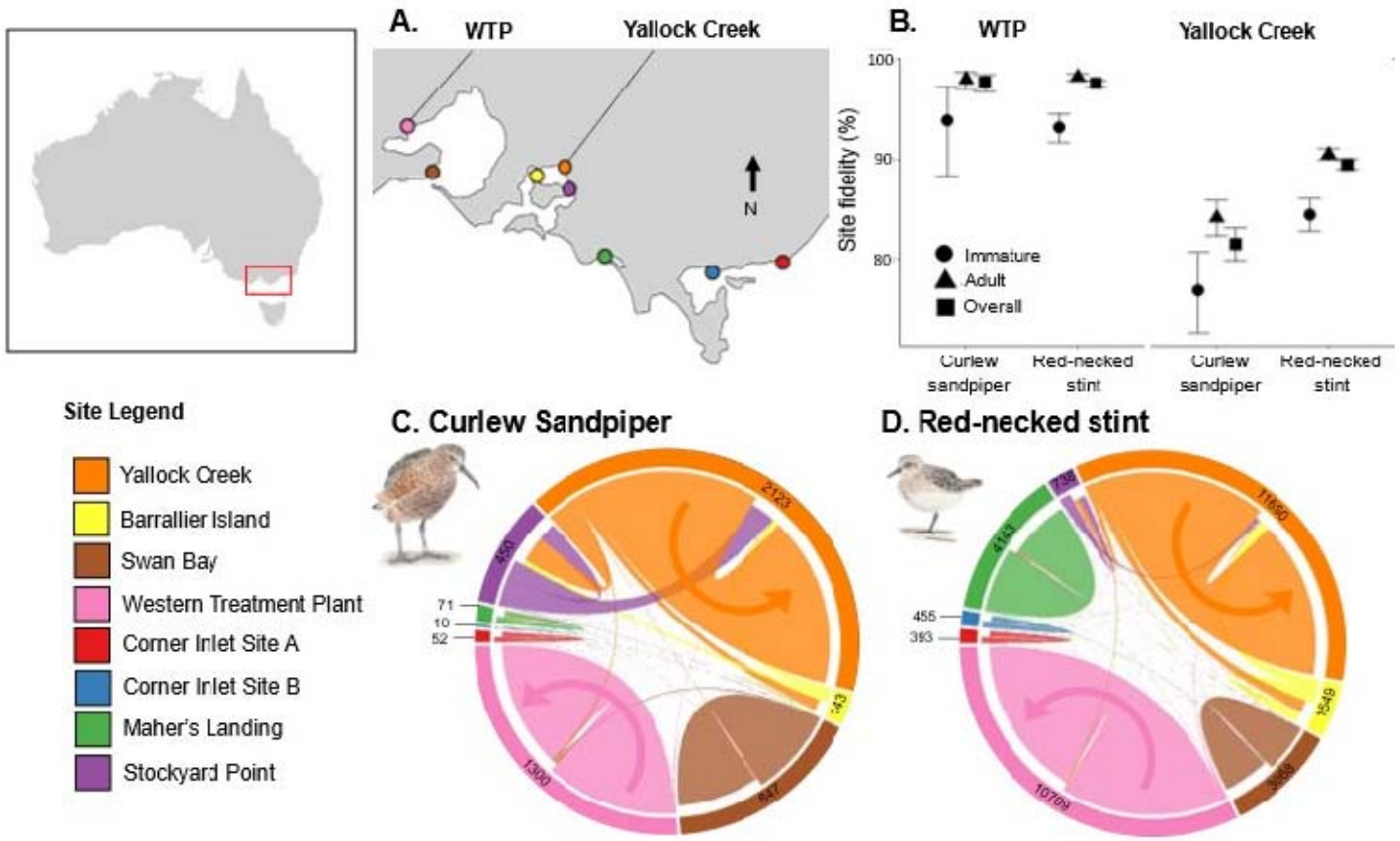


**Figure 1:** Site fidelity trends in migratory shorebirds captured since 1974 at 8 sites in Victoria, Australia, with the most-sampled sites being WTP and Yallock Creek (A). (B) shows immature, adult and overall site fidelity at both WTP and Yallock Creek. Colours in A correspond to those in C and D. Curlew sandpiper (C) and red-necked stint (D) site fidelities are shown whereby the width of each arc indicates the number of birds first captured at a site (base of arrow, closest to outer ring), and where they were recaptured again (head of arrow, further from outer ring). Bird icons by the author (Tobias Ross).

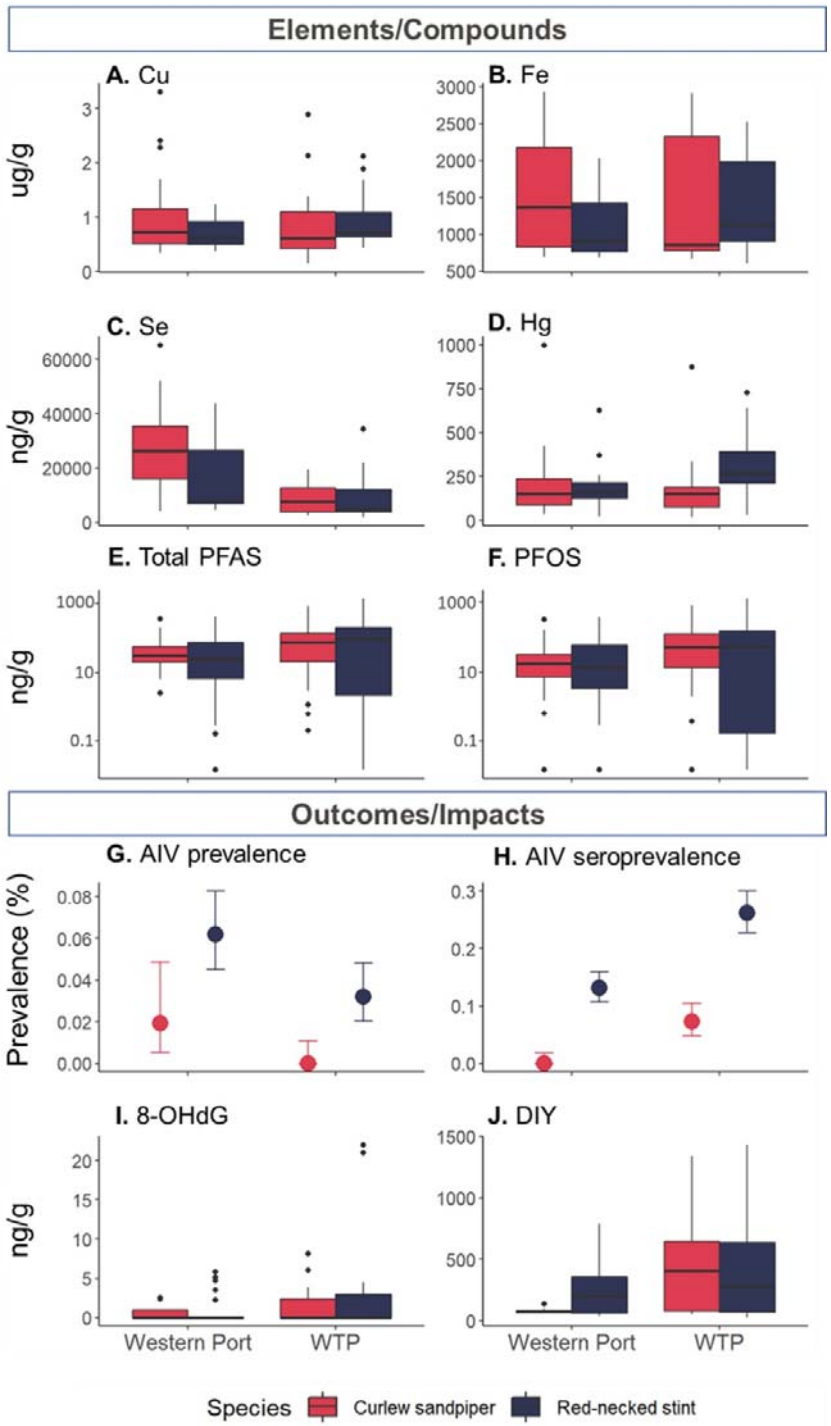


**Figure 2**: Pollutant, oxidative stress biomarker and avian influenza (AIV) data for each species at each sample site, Yallock Creek which is situated in Western Port Bay, and Melbourne's Western Treatment Plant (WTP). Contaminants displayed in each plot are as follows (only elements of note are shown to illustrate trends or lack thereof): A: copper (Cu), B: iron (Fe), C: selenium (Se), D: mercury (Hg), E: Total PFAS, F: PFOS, G: avian influenza virus prevalence, H) AIV seroprevalence, I) 8-0HdG biomarker, J) DIY biomarker. Significant species variations shown in B and C, significant region differences in C, E, and J.. Variation between adult and immature age groups not shown. All other contaminants not displayed here are provided in Table S4.

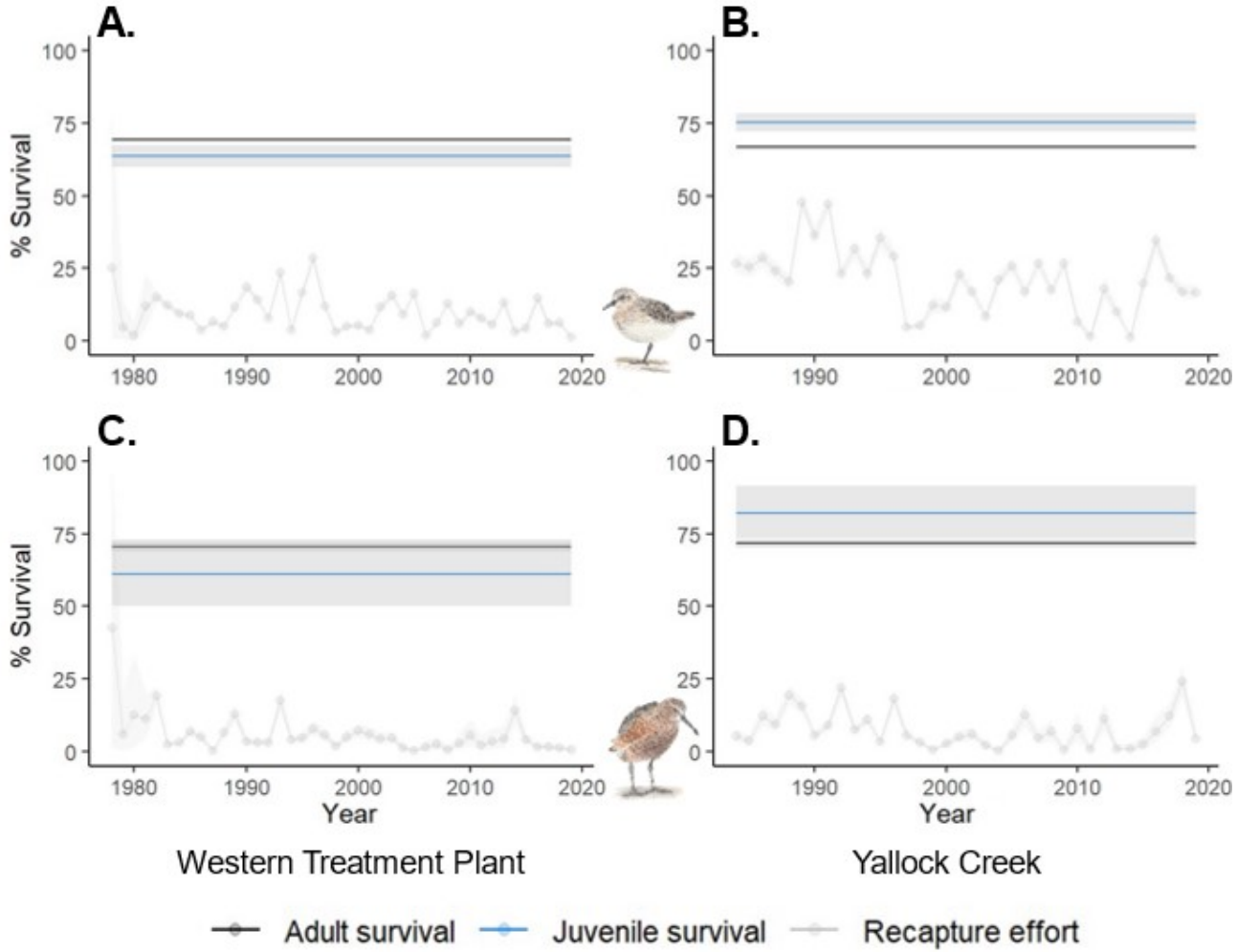


**Figure 3**: Average survival of adult and immature red-necked stints (A and B)) and curlew sandpipers (C and D) at WTP and Yallock Creek on Western Port Bay, from 1978-2019 for WTP samples and 1984-2019 for YC samples. Grey illustrates confidence intervals surrounding the average survival of1ach age group. Bird icons by the author (Tobias Ross).

**Table 1:** Linear mixed effect model summaries for of each elemental pollutant with detection rate above 50% of samples. Mixed effects models include species, region and age class (adult vs immature) as fixed effects, and sample year as a random effect.

| Group | Element | Explanatory variables | Estimate | SE | t value | p-value |
|---|---|---|---|---|---|---|
| | Magnesium (Mg) | Intercept | 293751.30 | 61210.47 | 4.799 | **<0.001** |
| | | Species (RNST) | -65657.77 | 53349.97 | -1.231 | 0.219 |
| | | Region (WTP) | -80854.12 | 52971.32 | -1.526 | 0.128 |
| | | Age group (Immature) | 5816.86 | 48972.48 | 0.119 | 0.905 |
| | Phosphorus (P) | Intercept | 5685087.32 | 605043.40 | 9.396 | **<0.001** |
| | | Species (RNST) | -718233.43 | 499826.06 | -1.437 | 0.151 |
| | | Region (WTP) | -300815.81 | 495826.45 | -0.607 | 0.544 |
| | | Age group (Immature) | -621608.34 | 452022.24 | -1.375 | 0.169 |
| | Manganese (Mn) | Intercept | 93.88 | 32.35 | 2.902 | **0.009** |
| | | Species (RNST) | -31.24 | 30.46 | -1.026 | 0.309 |
| | | Region (WTP) | -24.70 | 30.27 | -0.816 | 0.418 |
| | | Age group (Immature) | -26.30 | 28.88 | -0.910 | 0.365 |
| | Iron (Fe) | Intercept | 1742004.0 | 201480.7 | 8.646 | **<0.001** |
| | | Species (RNST) | -425889.0 | 157928.9 | -2.697 | **0.007** |
| | | Region (WTP) | -14428.0 | 156510.2 | -0.092 | 0.927 |
| | | Age group (Immature) | -316567.9 | 141265.8 | -2.241 | 0.025 |
| | Copper (Cu) | Intercept | 1147.75 | 139.76 | 8.212 | **<0.001** |
| | | Species (RNST) | -265.74 | 134.58 | -1.975 | 0.052 |
| | | Region (WTP) | 49.99 | 133.75 | 0.374 | 0.710 |
| | | Age group (Immature) | -261.45 | 129.28 | -2.022 | 0.047 |
| | Zinc (Zn) | Intercept | 23741.55 | 4857.12 | 4.888 | **<0.001** |
| | | Species (RNST) | -4565.47 | 3955.75 | -1.154 | 0.252 |
| | | Region (WTP) | -1916.19 | 3923.06 | -0.488 | 0.627 |
| | | Age group (Immature) | -6435.79 | 3565.73 | -1.805 | 0.075 |
| | Arsenic (As) | Intercept | 2419.04 | 490.05 | 4.936 | **<0.001** |
| | | Species (RNST) | -245.49 | 492.31 | -0.499 | 0.620 |
| | | Region (WTP) | -330.39 | 489.54 | -0.675 | 0.503 |
| | | Age group (Immature) | -257.41 | 487.36 | -0.528 | 0.599 |
| | Selenium (Se) | Intercept | 29790 | 2481 | 12.006 | **<0.001** |
| | | Species (RNST) | -7888 | 2551 | -3.092 | **0.003** |
| | | Region (WTP) | -11424 | 2539 | -4.499 | **<0.001** |
| | | Age group (Immature) | -8330 | 2573 | -3.237 | **0.002** |
| | Strontium (Sr) | Intercept | 155.47 | 48.19 | 3.226 | **0.002** |
| | | Species (RNST) | 26.63 | 49.54 | 0.538 | 0.5923 |
| | | Region (WTP) | -21.70 | 49.31 | -0.440 | 0.6611 |
| | | Age group (Immature) | -53.51 | 49.98 | -1.071 | 0.2874 |
| | Mercury (Hg) | Intercept | 153.25 | 53.07 | 2.888 | **0.010** |
| | | Species (RNST) | 49.46 | 43.59 | 1.135 | 0.260 |
| | | Region (WTP) | 90.63 | 43.23 | 2.096 | **0.040** |
| | | Age group (Immature) | -10.48 | 39.37 | -0.266 | 0.791 |
| | Lead (Pb) | Intercept | 71.074 | 12.122 | 5.863 | **<0.001** |
| | | Species (RNST) | -11.672 | 10.527 | -1.109 | 0.271 |
| | | Region (WTP) | -6.718 | 10.451 | -0.643 | 0.522 |
| | | Age group (Immature) | -19.147 | 9.651 | -1.984 | 0.051 |

**Table 2**: Model summaries for linear mixed effects models of PFASs groups (total PFAS, total carboxylates/PFCAs, total sulfonates/PFSAs, and PFOs by itself), and oxidative stress biomarkers, and general linear models for avian influenza prevalence data. In linear mixed effects models, PFASs were log-transformed to guarantee homogeneity in variances, and models for both PFASs and biomarkers included species, region and age group (adult vs immature) as fixed effects, and sample year as a random effect. Glms for avian influenza had binomial distributions, and were run only for red-necked stints, including only region and age-group as a explanatory variables.

| Group | Model | Dependent variable | Explanatory variables | Estimate | SE | t value/*z-value* | p-value |
|---|---|---|---|---|---|---|---|
| All grouped PFAS (log-transformed) | Linear mixed effects models (lmer) | ΣPFAS (log transformed) | Intercept | 1.253 | 0.511 | 2.452 | **0.029** |
| | | | Species (RNST) | -0.304 | 0.270 | -1.127 | 0.262 |
| | | | Region (WTP) | 0.534 | 0.258 | 2.068 | **0.040** |
| | | | Age group (Immature) | -0.653 | 0.253 | -2.583 | **0.011** |
| | | ΣPFCAs (log-transformed) | Intercept | -0.193 | 0.561 | -0.343 | 0.735 |
| | | | Species (RNST) | -0.279 | 0.440 | -0.635 | 0.527 |
| | | | Region (WTP) | 0.558 | 0.424 | 1.318 | 0.189 |
| | | | Age group (Immature) | -0.724 | 0.423 | -1.718 | 0.088 |
| | | ΣPFSAs (log-transformed) | Intercept | 0.678 | 0.817 | 0.830 | 0.423 |
| | | | Species (RNST) | -0.336 | 0.388 | -0.865 | 0.388 |
| | | | Region (WTP) | 0.350 | 0.372 | 0.940 | 0.349 |
| | | | Age group (Immature) | -0.653 | 0.363 | -1.799 | 0.074 |
| | | PFOS (log-transformed) | Intercept | 1.018 | 0.450 | 2.261 | **-0.040** |
| | | | Species (RNST) | -0.351 | 0.249 | -1.410 | 0.161 |
| | | | Region (WTP) | 0.562 | 0.239 | 2.353 | **0.020** |
| | | | Age group (Immature) | -0.528 | 0.234 | -2.257 | **0.026** |
| Avian influenza virus (AIV) prevalence (glm models) | Generalised linear models (glm) | AIV prevalence | Intercept | -2.834 | 0.180 | *-15.783* | **<0.001** |
| | | | Site (WTP) | -0.687 | 0.269 | *-2.551* | **0.012** |
| | | | Age group (Immature) | 0.459 | 0.283 | *1.624* | 0.104 |
| | | AIV seroprevalence | Intercept | -1.673 | 0.119 | *-14.091* | **<0.001** |
| | | | Site (WTP) | 0.848 | 0.152 | *5.598* | **<0.001** |
| | | | Age group (Immature) | -1.564 | 0.279 | *-5.621* | **<0.001** |
| Oxidative stress biomarkers | Linear mixed effects models (lmer) | 8-OHdG | Intercept | -0.611 | 1.512 | -0.404 | 0.690 |
| | | | Species (RNST) | 1.573 | 1.082 | 1.454 | 0.150 |
| | | | Region (WTP) | 1.607 | 0.845 | 1.901 | 0.061 |
| | | | Age group (Immature) | 1.429 | 0.967 | 1.478 | 0.144 |
| | | DIY | Intercept | 261.76 | 133.28 | 1.964 | 0.064 |
| | | | Species (RNST) | -48.41 | 90.30 | -0.536 | 0.593 |
| | | | Region (WTP) | 224.29 | 69.80 | 3.213 | **0.002** |
| | | | Age group (Immature) | -60.99 | 80.04 | -0.762 | 0.449 |

**Table 3**: Summary of site fidelity results for each species, showing fidelities specific to each site for each age group. 95% confidence intervals are shown in brackets.

| Species | Site | Adults | Immatures |
|---|---|---|---|
| Curlew Sandpiper | WTP | 70.5% (68.8-72.1%) | 61.2% [50.1-73.0%] |
| | Yallock Creek | 71.7% (70.3-72.9%) | 82.1% [73.4-91.4%] |
| Red-necked Stint | WTP | 69.3% (68.7-69.8%) | 63.6% [59.9-67.4%]; |
| | Yallock Creek | 66.7% (66.0-67.4% | 75.3% [72.1-78.5%] |

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