# **Bioaccumulation & Metabolic Impact of PFAS in** Freshwater Turtles

## A systems biology approach to investigate the effects of PFAS exposure on wild-caught freshwater turtle populations

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#### Per- and polyfluoroalkyl substances (PFAS) are persistent synthetic contaminants in the

environment. Direct toxicity of elevated PFAS concentrations in wildlife has been studied previously, yet evidence of their accumulation, maternal offloading, and metabolic impact in aquatic egg-laying species is limited. Here, we summarise an omics-based ecosurveillance approach used to investigate the impacts of PFAS in freshwater turtles (Emydura macquarii macquarii).

### **Omics-based ecosurveillance (methodology)**

- Ten female short-neck freshwater turtles were collected from a known PFAS impacted site and a suitable reference site (note, sites are not disclosed due to an ongoing contamination investigation).
- Each turtle was euthanized, necropsied and tissues/fluids rapidly  $\bullet$ quenched as per Beale *et al.* (2022a)
- Untargeted metabolite and lipids were analysed in serum, egg, and faeces; untargeted proteomics was analysed in serum, eggshell, and faeces; 16S gene amplicon sequencing was analysed in faeces.
- PFAS in serum and egg samples were analysed as per standard  $\bullet$ analytical protocols.



#### Maternal offloading

- PFAS was measured 5.3 times higher in turtle eggs than maternal serum (Figure 1). The dominant PFAS constituent measured in turtle eggs was PFOS > PFHxS as per Beale *et al.* (2022b).
- Increased purine metabolism intermediates were observed within egg yolk and albumen that were tied to multiple dysfunctional processes.
- Increased PE lipids and decreased proteins were observed that are linked to development and immune response perturbations.



#### **PFAS Bioaccumulation**

- PFAS was found approximately 30 times higher in the sampled turtle serum compared to surrounding surface water at the impacted site (Table 1).
- Dominant PFAS in the surface water was PFOS > PFHxS  $\bullet$
- Dominant PFAS in turtle serum was PFOS > PFHxS  $\bullet$

 
 Table 1: Summary of the analysed total PFAS surface water and turtle serum concentrations at the
sampled sites (Beale et al., 2022a).

	(Σ30) PFAS CONCENTRATION	
Sample Site	Surface Water (µg/L)	Turtle Serum (ng/ml)
PFAS Impacted	32.7 ± 1.1 (n=6)	1016 ± 231 (n=5)
Reference	n.d. (n=6)	n.d. (n=5)

## Gut microbiome

- Faeces collected from the colon were found to have a high *Firmicutes* to *Bacteroidetes* (F:B) ratio, indicative of long-term stress.
- Faecal metabolite and lipid biomolecules were observed that are tied to gut dysbiosis and intestinal inflammation.



#### **Metabolic impact**

The patterns in the functional multi-omics dataset concorded strongly with findings from previous PFAS studies, across wild-caught observational studies, laboratory-controlled challenge experiments, and human clinical studies. Here, we found a positive PFAS correlation with purine/lipid metabolisms related to immune responses and other biological dysfunctional processes; and, a negative PFAS correlation with lipid transport and binding activity in the impacted turtles.

Multi-omic analysis of dissected egg fractions from PFAS impacted eggs were significantly elevated in purine metabolism metabolites, which are tied to potential biological dysfunctional processes. The yolks were significantly depleted in lipids and lipid quality that are known to mediate hatchling growth and development. In addition, PFAS impacted eggshells were lower in important developmental and immune response proteins, and higher in PE lipids and histidine metabolism metabolites that are linked to a weakened physical structure. Traditional ecotoxicology approaches fail to elucidate this level of environmental harm that an omicsbased approach can provide.

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