

Full metal jacket: The accumulation of metal pollutants in the fur of platypus (*Ornithorhynchus anatinus*). How does water pollution impact a high trophic order predator?

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Key Points

- Platypus fur collected from individuals living in the Hawkesbury Nepean River Catchment contained varying levels of metal pollutants.
- Metal pollutants, aluminium, barium and iron were found in highest concentrations in the sediment.
- Zinc was found in highest concentration in the platypus fur potentially indicating a biomagnification link direct from their main food source - macroinvertebrates

Abstract

Platypus play an important role in aquatic food webs in freshwater systems. The platypus diet consists of a variety of macroinvertebrates, with some platypus consuming their weight in macroinvertebrates each night. This makes platypus particularly susceptible to heavy metal pollution as it is known that macroinvertebrates accumulate these metal pollutants. Platypus can potentially biomagnify heavy metals from the water column into their system via their main food source, macroinvertebrates. This study examined environmental matrices, (water and sediment) and two trophic levels (macroinvertebrates, and platypus) at three sites; one more naturalised catchment area, and two with known contamination sources in the Greater Sydney Basin, New South Wales, Australia. This study examined four different heavy metals: two essential metals (iron and zinc) and two non-essential metals (barium and aluminium), to identify if there was a difference in the concentration of these metals measured in platypus fur. Results showed that all four metals were present in all samples. Essential metals (iron and zinc) were higher concentrations in the platypus fur compared to non-essential metals (barium and aluminium). Zinc was the only metal that was found in highest concentration in platypus fur compared to macroinvertebrates, water and sediment. Other studies of metal accumulation in mammals suggest that fur is used as an excretory method for pollutants, and only provides a fraction of the total accumulation. Analysis of platypus fur is worthy of further investigation for use as a non-lethal biomonitoring tool when assessing risk to aquatic fauna from metals pollution in aquatic ecosystems.

Keywords

Platypus, water pollution, accumulation, macroinvertebrates, sediment

Introduction

Platypus (*Ornithorhynchus anatinus*) are an iconic Australian species; they are a semi-aquatic monotreme (egg-laying mammal) whose range extends down the east coast of Australia, southern Victoria, Tasmania, and Kangaroo Island (South Australia) (Furlan et al., 2013). Platypus are vulnerable to many threats including habitat degradation and altered hydrology from urbanisation (Hawke, Bino and Kingsford, 2019), illegal opera house nets, litter, fishing line pollution (Warwick et al., 2024a), and diminished water quality through point-source and surface run off pollution. Water pollution reduces the abundance and diversity of benthic macroinvertebrates, the main food source of platypus (Serena and Pettigrove 2005). To meet their daily

energy requirements, platypus must consume up to half their body weight in macroinvertebrates, and lactating females can consume up to their entire body weight (Grant, 2007).

Essential metals are considered to play a vital role for normal biological function within a living organism (Slobodian et al., 2021). Iron and zinc are two essential metals required for biological function. Iron is required in many metabolic processes such as oxygen transport, DNA synthesis, and the production of haemoglobin (Abbaspour, Hurrell and Kelishadi, 2014). Zinc is a micronutrient required for organism growth and reproduction; it also supports immunity functions (Singh and Das, 2011). Non-essential metals are not required for normal biological functions and can be toxic to organisms even in trace concentrations (Slobodian et al., 2021). Aluminium and barium are classified as non-essential metals as they are not required for any organism function. Aluminium can interfere with ion regulation and respirator processes (Igbokwe, Igwenagu and Igbokwe 2020). Barium interferes with growth and the maintenance of homeostasis (Sleimi, Kouki, Ammar, Ferreira and Perez-Clemente, 2021).

While essential metals are required for normal biological function, in high concentrations they can become toxic just like non-essential metals. Fortunately, organisms have processes to remove unnecessary and excess metals incidentally accumulated through dietary and environmental interactions, in an effort to combat toxicity. These methods include removal through the gastrointestinal tract, kidney and liver filtering, fat storage, and storage in fur and nails which inevitably shed and wear away. Much like how a plant can store pollutants in its leaves and subsequently drop the leaves to remove the pollutants (Warwick et al., 2024b), mammals can perform a similar process through use of hair and nails (Mina et al., 2019). Unnecessary heavy metals can be stored in the fur of platypus and will ultimately be removed when the platypus undergo natural shedding.

Globally, there are limited studies that have examined the biomagnification of iron, barium, and aluminium in mammalian fur. Most of the studies that have examined the accumulation of heavy metals in aquatic mammals, including otters and mink, have primarily investigated the concentrations in liver or kidneys, amongst other internal organs (Lanszki et al. 2009; Brand et al. 2020). Unfortunately for platypus, due to their low numbers and protection status, this is not a possibility; it would be far better for low-density populations such as that found within the Hawkesbury-Nepean River Catchment to utilise non-invasive, non-lethal methods for monitoring biomagnification.

The aim of this study was to examine the accumulation of metal pollutants in aquatic ecosystems to determine if there is a relationship between the water and sediment and two trophic levels, macroinvertebrates and platypus. It was hypothesised that if there is an accumulative relationship then the concentration in the platypus would be higher than their main food source, macroinvertebrates.

Methods

Site Description

Three sites within the Greater Sydney Basin were chosen for this study, namely Mulgoa Creek (Penrith), Fitzgerald Creek (Warrimoo), and Bargo River (Bargo) (Figure 1). These sites were chosen as they contain creeks with known platypus populations and known pollution sources (including landfill surface run off and mining effluent discharge). All three sites contribute to the Hawkesbury-Nepean River Catchment.



Figure 1: Site map showing the location of Mulgoa Creek, Fitzgerald Creek, and Bargo River in relation to the Hawkesbury-Nepean River Catchment.

Sample Collection

Triplicate grab samples of surface water were collected on three occasions, and grab samples of fine sediment were collected on two occasions between May and September 2023. Samples were stored in an esky with ice bricks before being transferred to a fridge and stored between 2-5°C for less than 24 hours. Samples were sent to Envirolab, Chatswood NSW, a NATA (National Association for Testing Authorities) accredited laboratory for analysis of total metal concentration iron, zinc, aluminium and barium.

Macroinvertebrates were collected from all sites between May and October 2023 using 30x30cm kick nets with 250µm mesh. The macroinvertebrates were stored in deionised water in the field, until they were transported to the laboratory, rinsed in ethanol and frozen at -20°C, before being freeze dried using an Edwards Freeze Dryer Modulyo with a Pirani 501 vacuum gauge control at -40°C for two 18-hour cycles (Warwick et al. 2024b).

Five fur samples were collected from four platypuses captured in Mulgoa Creek (one platypus), Fitzgerald Creek (one platypus), and Bargo River (two platypus). Duplicate fur samples were collected from a platypus within both Mulgoa Creek and Fitzgerald Creek respectively, each on a single occasion. As one platypus was recaptured in subsequent sampling from Bargo River, a second fur sample was collected. Platypuses were captured using fyke nets (Mulgoa and Fitzgerald Creek) and mesh nets (Bargo River), due to differences in relative water depth. In shallow creeks (<1m deep) fyke nets were used, and in deeper rivers (>1.5m deep), a mesh net was used. Twin fyke nets (30 mm knotless 20 ply nylon) were positioned upstream and downstream so as to capture platypuses moving in either direction. Five-metre wings were rocked to the edge of the creek and partially up the bank where possible to avoid platypuses escaping the one metre chamber entrance. Each fyke net chamber was position with an air pocket at the top to ensure captured platypuses could breathe and was fitted with a flotation buoy for safety. Fyke nets were checked every 2-4 hours, dependent on temperature and if platypuses were captured, they were immediately processed and released away from the fyke nets in the direction of travel (i.e. if captured in the upstream facing net, they were released downstream of fyke nets). A mesh net was utilised in the Bargo River using a 25m by 1.8m, 20 ply nylon mesh net. The net was continuously monitored with a spotlight and audible cues and checked by hand hourly for underwater debris entanglement.

Captured platypuses were microchipped, and a fur sample was taken from the upper dorsal region with animal clippers before being released back into the river. Fur samples were washed with deionised water to remove dirt and external material before analysis.

Results and Discussion

Iron, aluminium, and barium and were found in the highest concentrations in sediment, followed by macroinvertebrates, and platypus fur (Table 1). Metals were found in the lowest concentrations in the water column for all four metals, where the lowest relative concentration was zinc, and the highest relative concentration was barium. Interestingly, it was expected that sample sites that were close to known sources of pollution (Mulgoa Creek and Bargo River) would result in higher concentrations of metals in the water and sediment compared to Fitzgerald Creek that has no known pollution source. In fact, Fitzgerald Creek contained the second highest concentrations of iron in the water and second highest concentrations of aluminium and iron in the sediment (Table 1).

Table 1: Data summary table of metals analysed across two environmental matrices (water and sediment) and two trophic levels (macroinvertebrates and platypus fur) at Fitzgerald Creek, Mulgoa Creek, and Bargo River for water, sediment, macroinvertebrates, and platypus fur.

		Water (mg/L)	Sediment (mg/kg)	Macroinvertebrates (mg/kg)	Platypus Fur (mg/kg)
Aluminium	Fitzgerald Creek	0.07	4633	65	50
	Mulgoa Creek	0.2	9333	280	40
	Bargo River	0.8	3963	80	47
Barium	Fitzgerald Creek	0.03	46	18	6
	Mulgoa Creek	0.1	100.7	69	17
	Bargo River	3.3	9945	105.2	46.7
Iron	Fitzgerald Creek	0.8	10500	1050	170
	Mulgoa Creek	1.1	40333	2100	700
	Bargo River	0.2	4373	470	370
Zinc	Fitzgerald Creek	0.02	24	104	170
	Mulgoa Creek	0.003	68	44	210
	Bargo River	0.05	212	162	163

Despite the lowest concentration of zinc in the water column being reported at Mulgoa Creek, the platypus fur collected at this site contained the highest concentration of zinc across all the three sites, suggesting that there is no relationship between water concentration and fur concentration or that biomagnification was not the only factor influencing zinc concentration in platypus fur. This result could be due to low replication of platypus fur samples and further investigation is required to better understand individual pollutant inputs, movement, and outputs across trophic levels.

Zinc was the only metal analysed in which the concentrations were higher in the platypus fur than the macroinvertebrate samples, potentially consistent with the conceptual model for biomagnification (Figure 2). The concentration of zinc was more than four times higher in platypus fur compared to the macroinvertebrate food source (Table 1). Given that there are limited if any studies that have examined the accumulation potential of heavy metals in platypus fur, studies of River otters were used to compare results as they occupy a similar ecological niche. The concentration of zinc in platypus fur was significantly less than what was found in North American River Otters (*Lontra canadensis*), which found the average concentration

of zinc in otter fur to be approximately 605mg/kg (Monroe 2016). While platypus and otters are both considered higher trophic order predators, the platypus' diet primarily includes macroinvertebrates, whilst the otter's diet primarily includes seafood, such as fish and crayfish. The dietary composition of river otters indicates that river otters are of a higher trophic level standing compared to platypus, which could account for this substantial difference in zinc content.

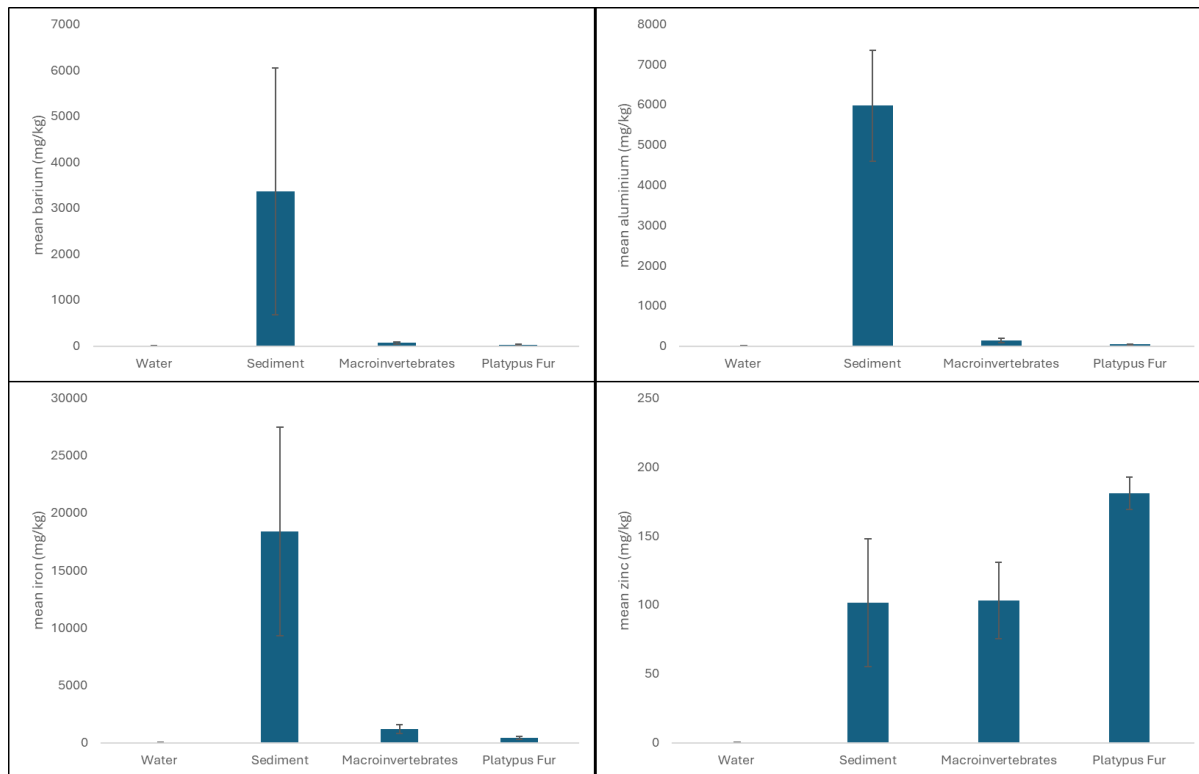


Figure 2: Mean concentration of barium, aluminium, iron, and zinc (L-R) across environmental matrices (water and sediment) and two trophic levels (macroinvertebrates and platypus fur)

Zinc is the only metal that has an Australian water quality guideline for 95% species protection (8.0 µg/L) and an Australian default guideline value (DGV) for sediment (200 mg/kg) in freshwater systems (ANZECC, 2000; Simpson et al., 2013). In this study, only Mulgoa Creek had a mean average (3.0mg/L) below the recommend water quality guidelines for 95% species protection Both Fitzgerald Creek and Mulgoa Creek were below the Australian sediment guidelines, whilst the Bargo River was higher than the DGV but less than the upper guideline value (GV-high) of 410 mg/kg. There are no equivalent water quality guidelines for iron and barium for 95% species protection, nor Australian sediment guideline thresholds for barium, iron, and aluminium, which makes it difficult to assess the prevailing metal toxicity within these ecosystems without extensive longitudinal background data.

Non-essential metals like barium and aluminium are, by definition, no needed for proper biological function (Saad, El-Sikaily & Kassem, 2016). It was expected that these concentrations would have been higher in platypus fur compared to essential metals like aluminium and zinc which are needed for biological function. The fact that essential metals are being excreted in higher concentrations is potentially concerning as it suggests that the internal threshold for optimal biological function has been surpassed (Rainbow & Luoma, 2011). Both essential and non-essential metals in high enough concentrations can result in toxicity and even if background environmental levels do not exceed ANZECC water quality guidelines for 95% species protection, their presence in platypus fur indicates a potential negative impact to the health of higher trophic level species.

Whilst an increase in the concentration of zinc between macroinvertebrates and platypus fur may indicate biomagnification of this essential metal, it is important to note that platypus fur is a single tissue type of a complex monotreme and may not show a complete picture of metal accumulation. While samples of

macroinvertebrates were holistic homogenised replicates, the same practise could not be applied to platypus and therefore not be a true reflection of total metal concentration with platypus tissue (Warwick et al. 2024). A recent study by Warwick et al. (2024), and a study by Mina et al. (2029) suggested that mammals utilise fur as an excretory method for toxic pollutants and so represents only a small percentage of total pollutant accumulation within the body. Further study is recommended to examine metal accumulation in the internal tissue of deceased platypus to better understand the metabolic pathways and accumulation potential of ingested metals in platypus.

Macroinvertebrates are complex biomagnifiers of heavy metals. In this study, only zinc slightly increased in concentration from sediment into macroinvertebrates (101.3 and 103.3mg/kg respectively). There could be a litany of reasons for this, including the types of macroinvertebrates samples collected. Different species of macroinvertebrates and different foraging preferences (i.e. herbivorous or carnivorous) could result in different magnification potential. A study by Corbi and Froehlich (2010) found that predatory macroinvertebrates (with a carnivorous diet) were able to biomagnify higher concentrations of heavy metals such as iron and zinc, compared to collector macroinvertebrates (with a herbivorous) diet, due to them being of a higher trophic level standing. It was also found that some macroinvertebrate families, such as oligochaete and chironomid, which prefer living in sediment compared to debris, also accumulated higher concentrations of heavy metals. This understanding could account for the discrepancy between sites, as macroinvertebrates were indiscriminately sampled and then homogenised for analysis. The current study required a large overall biomass of macroinvertebrates for analysis, and as such could not include a family percentage breakdown of macroinvertebrate samples to determine overall consistency. Further research should aim to include consistency in the representation of macroinvertebrate families across multiple research sites to verify such trends.

Conclusions

The study confirms that platypus fur collected from animals living in rivers contained varying levels of metal pollution, both essential and non-essential. The pathway of these metal pollutants into the tissue of platypus cannot be definitively characterised, as they were found both in the environmental matrices and in the direct food source, macroinvertebrates. It is possible that these metals biomagnified in platypus, directly through predation however there may be other indirect mechanisms from inadvertent ingestion water or sediment. The detection of metal pollutants in the fur may indicate the use fur as an excretory method, however this has not been verified in platypus and any concentration of metal pollutants in internal platypus tissue cannot be extrapolated in this study.

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