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# Manganese contamination affects the motor performance of wild northern quolls (*Dasyurus hallucatus*)☆

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ABSTRACT

Neuromotor deficits are an important sign of manganese (Mn) toxicity in humans and laboratory animals. However, the impacts of Mn exposure on the motor function of wild animals remains largely unknown. Here, we assessed the impact of chronic exposure to Mn from active mining operations on Groote Eylandt, Australia on the motor function of the semi-arboreal northern quoll (*Dasyurus hallucatus*), an endangered species. The three motor tests conducted—maximum sprint speed on a straight run, manoeuvrability around a corner, and motor control on a balance beam—showed that elevated Mn body burden did not diminish performance of these traits. However, quolls with higher Mn body burden approached a corner at a significantly narrower range of speeds, due to a significantly lower maximum approach speed. Slower speeds approaching a turn may reduce success at catching prey and avoiding predators. Given that maximum sprint speed on a straight run was not affected by Mn body burden, but maximum speed entering a corner was, slower speeds approaching a turn may reflect compensation for otherwise impaired performance in the turn.



## 1. Introduction

Movement disorder and cognitive deficit are the main symptoms of manganese (Mn) toxicity in humans (Levy and Nassetta, 2003; Josephs et al., 2005; Klos et al., 2006; ATSDR, 2012), even in non-occupational settings (Mergler et al., 1999; Rodriguez-Agudelo et al., 2006; Hernandez-Bonilla et al., 2011). Mn accumulates in the brains of humans (Calne et al., 1994; Mergler, 1999; Aschner, 2000; Aschner et al., 2005) and animals (Dastur et al., 1971; St-Pierre et al., 2001; Salehi et al., 2003; Tapin et al., 2006; Amir Abdul Nasir et al., 2018), damaging the dopaminergic neurons that control muscle movement (Aschner et al., 2005). Toxicity initially manifests as slowed motor speed and imbalanced posture when walking or rising (Cook et al., 1974; Normandin and Hazell, 2002; Bowler et al., 2006). As the condition worsens, individuals may also display gait disorders and impaired ability to perform rapid, alternating movements (Cook et al., 1974; Normandin and Hazell, 2002; Bowler et al., 2006).

In the laboratory, motor deficits have manifested in rodents and non-human primates following exposure to Mn of various chemical forms, doses and routes (Bonilla, 1984; Eriksson et al., 1987; Olanow et al., 1996; Witholt et al., 2000; Normandin et al., 2004). Mn accumulates in the brain of rats, leading to hyperactivity (St-Pierre et al., 2001; Salehi et al., 2003; Tapin et al., 2006), decreased locomotor activity, increased gait abnormalities, and impairment of the ability to traverse a balance beam (Witholt et al., 2000). Although clear evidence exists for negative impacts of Mn on motor function of animals in controlled laboratory settings, to our knowledge no studies have explored how Mn affects the motor function of animals in natural populations. Movement plays a critical role in an animal's interactions with competitors, mates, predators, and prey, and therefore is central to reproductive success and survival (Biewener, 2003; Husak and Fox, 2006; Husak et al.,

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2006; Wilson et al., 2007; Nathan et al., 2008; Clark and Higham, 2011). Exposure to contaminants that impact an animal's motor performance is likely to affect reproductive success and population viability, even if the contaminant does not directly cause mortality (Faimali et al., 2006).

Groote Eylandt, located in the Gulf of Carpentaria, Australia, is an Indigenous Protected Area and owned by the Anindilyakwan people. It is a site of International Conservation Significance, with 12 threatened and endangered vertebrate species including the northern quoll (*Dasyurus hallucatus*) (ALC, 2014; DOE, 2014). This island is also the site of one of the largest Mn ore producers in the world (South32 2014). The Groote Eylandt Mining Company (GEMCO, a BHP Billiton subsidiary) has operated since 1964 and produces 3–4 million tonnes of ore annually (South32, 2014; USGS, 2016). Mn is extracted from open pits and crushed onsite before it is transported in open trailers to the port for open-air storage and shipping, with vast quantities of ore dust liberated into the environment during processing. Fine, air-borne Mn dust in the respirable size range occurs on Groote Eylandt at levels exceeding international recommendations, even 20 km from the Mn extraction, processing, and storage facilities (Amir Abdul Nasir et al., 2018).

Recently, we found that northern quolls living near mining sites on Groote Eylandt accumulate substantial Mn within their hair, testes and two brain regions, the neocortex and cerebellum (Amir Abdul Nasir et al., 2018). Because the neocortex and cerebellum are responsible for sensory perception and motor function, respectively (Nelson and Armati, 2006), accumulation of Mn in these brain regions could impair sensory and motor functions of quolls. Impairment of these traits by Mn may diminish the ability of quolls and other wildlife to perform tasks related to reproduction and survival. Here, we examine how chronic Mn exposure affects the locomotor function of northern quolls on Groote Eylandt. Northern quolls are a semi-arboreal predatory species, spending time navigating both arboreal and terrestrial substrates. In these complex habitat types, quolls flee from predators (e.g., snakes, raptors and dingoes [*Canis lupus dingo*]) and chase after prey (e.g., insects, small mammals, reptiles); both require optimum motor function and strategy. We predicted that quolls exposed to higher levels of environmental Mn from mining operations would exhibit decreases in their performance of the ecologically relevant motor tasks: (1) maximum straight-line sprint speed, (2) manoeuvrability around a tight corner, and (3) motor control while running along a narrow beam. We also predicted that quolls exposed to higher levels of Mn were more likely to make mistakes (i.e. slip or crash) while performing the manoeuvrability and motor controls tasks.

## 2. Materials and methods

### 2.1. Field and laboratory procedures

We trapped northern quolls in May–September 2014 during three separate dry-season periods: pre-breeding (weeks 20–25), breeding (weeks 28–30) and post-breeding (weeks 33–37). The breeding season was assumed to have started when trapped female quolls began showing breeding scars, which are caused by male aggression during copulation. We trapped at seven sites proximate to mining sites where Mn is extracted and crushed, and the port, where Mn is stored in open-air prior to shipping. We also trapped at four sites distant from the mine by 15–20 km. Trapping occurred over three consecutive nights at each site, during which we set at least 30 Tomahawk original series cage traps (20 × 20 × 60 cm; Tomahawk ID-103, Hazelhurst, Winconsin, USA) at 60 m intervals, baited them with canned dog food, and left them overnight. Early the following morning, we checked the traps and transferred captured quolls into individual cloth bags for processing. The site of each captured quoll was recorded via GPS (Garmin e-Trex 20, Garmin Ltd., Olathe, Kansas, USA).

Quolls were measured and sampled at the Anindilyakwa Threatened Species Centre. Each quoll was individually microchipped by inserting a PIT tag between its shoulder blades (Trovan nano-transponder ID-100, Keysborough, Australia) to ensure correct identification during recaptures. We recorded sex, breeding status, and body mass (±1 g; A & D Company Limited HL200i, Brisbane, Australia), and estimated age based on incisor wear and reproductive condition (i.e. deepened pouch and swollen teats indicated 2nd or 3rd year females) (Oakwood, 2000). Morphological traits were measured in triplicate using digital callipers (±0.01 mm; Whitworth, Brisbane, Australia): maximum head width, head length (from nuchal crest to tip of snout), body length (nuchal crest to base of tail), right and left fore-limb length (radius - ulna), right and left hind-limb length (tibia - fibula), maximum tail width, and tail length (base to tip of tail). Quolls were released in the evening of the same day of capture at their site of collection. All research methods were approved by the University of Queensland animal ethics committee (permit number SBS/541/12/ANINDILYAKWA/MYBRAINSC) and the Northern Territory Parks and Wildlife Commission (permit number 47603).

The mean for each morphological trait was calculated from the replicate measurements and then all traits were amalgamated using a principal component analysis (PCA; Table 1). As principal component 1 (PC1) accounted for 86.8% of the variance with all measurements loaded negatively, this represented overall body size ($PC1_{Bodysize}$): the higher the $PC1_{Bodysize}$, the smaller the quolls. Principal component 2 (PC2), which accounted for 9.3% of the variance, had tail length loaded most negatively and body length loaded most positively. Therefore, PC2 represented overall body shape ($PC2_{Bodyshape}$); the higher the $PC2_{Bodyshape}$, the longer the body and the shorter the tail of quolls. To avoid the confounding effect of both body mass and $PC1_{Bodysize}$ on the locomotor performance assessed, only body mass (not $PC1_{Bodysize}$) and $PC2_{Bodyshape}$ were used in the statistical analyses.

### 2.2. Element concentrations in hair

Hair samples of 1.5–2.0 cm in length (10–80 mg) were plucked from between the shoulder blades of each quoll, cleaned, and analysed for concentrations of Mn and that of 14 other elements: aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), molybdenum (Mo), nickel (Ni), phosphorus (P), lead (Pb), selenium (Se) and zinc (Zn) using the same methods outlined by Amir Abdul Nasir et al. (2018). All element concentrations are presented in mg/kg units (dry

**Table 1**
Principal component loadings on 11 morphological variables measured on northern quolls (n = 129; 65 females, 64 males). Percentage of variance explained by each PC is included at the bottom of the table.

| Variable | PC1 | PC2 |
|---|---|---|
| Body length | −0.697 | 0.637 |
| Tail length | −0.620 | −0.761 |
| Tail diameter | −0.062 | 0.060 |
| Right foot length | −0.076 | 0.014 |
| Right hind limb length | −0.151 | 0.001 |
| Right fore limb length | −0.144 | 0.013 |
| Left foot length | −0.074 | 0.013 |
| Left hind limb length | −0.151 | 0.001 |
| Left fore limb length | −0.143 | 0.028 |
| Head width | −0.103 | 0.033 |
| Head length | −0.134 | 0.091 |
| *Percentage of variance* | *86.80* | *9.34* |