



# First Assessment of Mercury (Hg) Concentrations in Skin and Carapace of Flatback Turtles (*Natator depressus*) (Garman) From Western Australia

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### Specialty section:

This article was submitted to  
Toxicology, Pollution and the  
Environment,  
a section of the journal  
Frontiers in Environmental Science

**Received:** 27 December 2021

**Accepted:** 09 February 2022

**Published:** 17 March 2022

### Citation:

Schneider L, Tucker AD, Vincent K,  
Fossette S, Young EJ and Whiting SD  
(2022) First Assessment of Mercury  
(Hg) Concentrations in Skin and  
Carapace of Flatback Turtles (*Natator  
depressus*) (Garman) From  
Western Australia.  
Front. Environ. Sci. 10:843855.  
doi: 10.3389/fenvs.2022.843855

Mercury pollution in the surface ocean has more than doubled over the past century. Within oceanic food webs, sea turtles have life history characteristics that make them especially vulnerable to mercury (Hg) accumulation. In this study we investigated Hg concentrations in the skin and carapace of nesting flatback turtles (*Natator depressus*) from two rookeries in Western Australia. A total of 50 skin samples and 52 carapace samples were collected from nesting turtles at Thevenard Island, and 23 skin and 28 carapace samples from nesting turtles at Eighty Mile Beach. We tested the influence of turtle size on Hg concentrations, hypothesising that larger and likely older adult turtles would exhibit higher concentrations due to more prolonged exposure to Hg. We compared the rookeries, hypothesising that the turtles from the southern rookery (Thevenard Island) were more likely to forage and reside in the Pilbara region closer to industrial mining activity and loading ports (potential exposure to higher environmental Hg concentrations) with turtles from the northern rookery (Eighty Mile Beach) more likely to reside and feed in the remote Kimberley. Turtles from the Eighty Mile Beach rookery had significantly higher skin Hg concentrations ( $\bar{x} = 19.4 \pm 4.8$  ng/g) than turtles from Thevenard Island ( $\bar{x} = 15.2 \pm 5.8$  ng/g). There was no significant difference in carapace Hg concentrations in turtles between Eighty Mile Beach ( $\bar{x} = 48.4 \pm 21.8$  ng/g) and Thevenard Island ( $\bar{x} = 41.3 \pm 16.5$  ng/g). Turtle size did not explain Hg concentrations in skin samples from Eighty Mile Beach and Thevenard Island, but turtle size explained 43.1% of Hg concentrations in the carapace of turtles from Eighty Mile Beach and 44.2% from Thevenard Island. Mercury concentrations in the flatback turtles sampled in this study are relatively low compared to other sea turtles worldwide, likely a result of the generally low concentrations of Hg in the Australian environment. Although we predicted that mining activities would influence flatback turtle Hg bioaccumulations, our data did not support this effect. This may be a result of foraging ground overlap between the two rookeries, or the predominant wind direction carrying atmospheric Hg inland rather than seaward. This is the first Hg study in skin and carapace of flatback turtles and represents a baseline to compare Hg contamination in Australia's surrounding oceans.

**Keywords:** Pilbara, Australian continental shelf, keratin, pollution, megafauna

## INTRODUCTION

Mercury (Hg) pollution has a long legacy in the environment. Once emitted to the atmosphere, it can cycle between ecosystems for years or decades before ending up deep in the oceans or deposited on land (Obrist et al., 2018). As a result, 83% of the Hg concentrations in the surface ocean are from anthropogenic enrichment, half of which have occurred since 1950 (Amos et al., 2013). Mercury concentrations in the ocean therefore represent a substantial fraction of the global Hg reservoir (Soerensen et al., 2010) and explain the ever-increasing levels of Hg contamination observed in marine organisms (Alava et al., 2018, 2017).

Once Hg enters the ocean, bacterial activities favour the production of methylmercury (MeHg), allowing subsequent Hg uptake by biota (Paranjape and Hall, 2017). This is of particular concern as, among all the chemical forms of Hg, MeHg is the most efficiently up taken by aquatic organisms. Upon bioaccumulation, it can be distributed into many organs of the body (including the brain), biomagnified through food chains (Lavoie et al., 2013; Finley et al., 2016) and pose a health risk to aquatic organisms, including turtles (Scheuhammer et al., 2015).

Sea turtles are prominent members of the oceanic food web and have life history characteristics that make them vulnerable to Hg accumulation – such as their long life span, high trophic level in the food web, and their aquatic habitat (Sakai et al., 2000; Storelli and Marcotrigiano, 2003; Day et al., 2007; Bezerra et al., 2013; Schneider et al., 2013). The major pathway of Hg exposure and accumulation in marine animals is through diet (Kehrig et al., 2009), which accounts for approximately 80–90% of total Hg bioaccumulation, with the remainder being absorbed from water (Hrenchuk et al., 2012). In addition, turtles can transfer Hg from stored lipids to their offspring via egg production (Perrault et al., 2011; Hopkins et al., 2013; Landler et al., 2017; Sinaei and Bolouki, 2017), increasing the bioaccumulation of Hg in food webs.

The flatback turtle (*Natator depressus*), endemic to Australia, is the least studied species among all sea turtles in terms of toxic metal bioaccumulation, with only one study available on heavy metals in blood and eggs (Ikonomopoulou et al., 2011) from turtles nesting on Curtis Island, Queensland (QLD). Mercury, however, had concentrations below the detection limit of 0.1 mg/L in blood and 0.05 mg/kg in eggs. The flatback turtle is listed as data deficient by the IUCN Red List of Threatened Species due to a lack of data for population modelling (IUCN, 1996).

Methylmercury biomagnifies through food webs and, consequently, top predatory animals are at greatest risk for increased dietary MeHg exposure and potential Hg-related health effects (Schneider et al., 2013; Scheuhammer et al., 2015). Limited data available on the diet of flatback turtles suggest this species is carnivorous, feeding on soft-bodied invertebrates including soft corals, sea cucumbers, jellyfish, and sea pens (Limpus, 2007). Given its trophic level within the food web, this species could be particularly vulnerable to Hg bioaccumulation and its health effects.

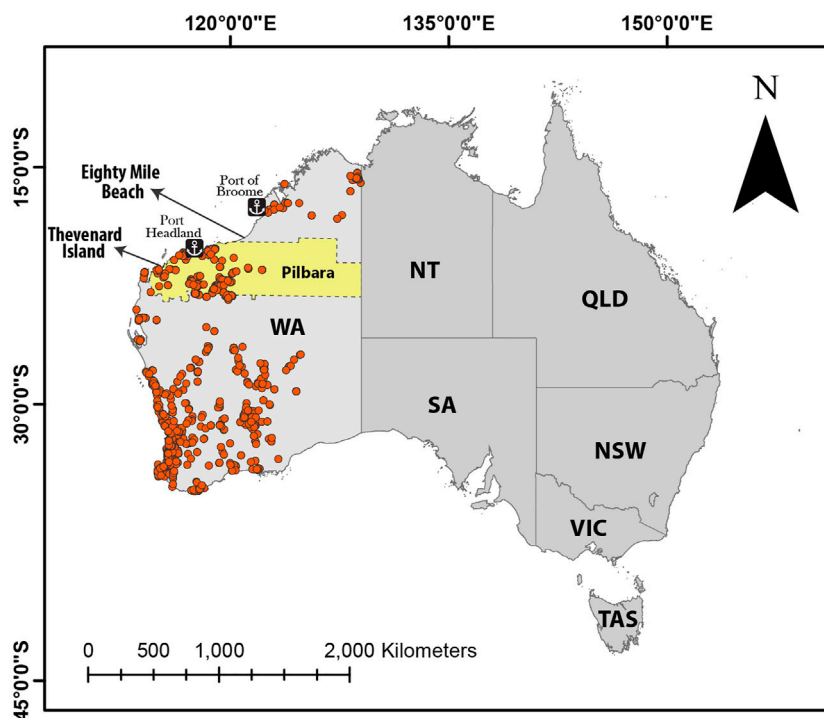
In Western Australia (WA), Thevenard Island and Eighty Mile Beach are important nesting rookeries (nesting sites) for flatback turtles (Fossette et al., 2021). In the last decade, the mining sector has committed to an ongoing structured program of exploration and production activity in parts of WA (Keesing et al., 2018), including the Pilbara region, where Thevenard island is located. Mining activities in this region release Hg via atmospheric particles (Roche and Mudd, 2014), while gas extraction activities emits Hg to the atmosphere during steam cleaning and venting through flares (Young, 2019). Flatback turtles in this region may therefore be exposed to higher-than-background levels of Hg that may pose a health risk to them.

In the present study we sought to determine Hg concentrations in nesting turtles of two flatback turtle rookeries in WA: Thevenard Island and Eighty Mile Beach. We assessed the influence of body size on Hg concentrations in both skin and carapace (keratin layer) of flatback turtles. This is the first study to analyse Hg in skin and carapace of flatback turtles, providing baseline information of Hg bioaccumulation in this endangered species, and key data on the potential for adverse effects on turtle. We compared differences between rookeries over 700 km apart, hypothesising that the turtles from the southern rookery (Thevenard Island) were more likely to forage and reside in the Pilbara region closer to industrial mining activity and loading ports (potential exposure to higher environmental Hg concentrations) with turtles from the northern rookery (Eighty Mile Beach) more likely to reside and feed in the remote Kimberley.

## MATERIAL AND METHODS

The flatback turtle is unique among the seven species of sea turtles worldwide in that it does not have a pan-oceanic distribution and is reported to be endemic to the Australian continental shelf (Cogger and Lindner, 1969; Limpus et al., 1988). Flatback turtles nest on tropical beaches across northern Australia and use foraging grounds on the Australian continental shelf, southern Papua, Papua New Guinea and coastal waters of Irian Jaya (Limpus, 2007; Roarty, 2010). As adults, flatbacks appear to stay associated with distinct foraging areas that comprise individuals from multiple genetics stocks (distinct rookeries). At breeding season, adults migrate from the resident foraging areas up to 1,300 km to distinct rookeries to breed. Thus the breeding rookery can comprise individuals from both near and far foraging areas (Limpus, 2007). The sampled flatback turtle rookeries are from two genetic was stocks (FitzSimmons et al., 2020)

Western Australia is an important area for both nesting and foraging flatback turtle populations grounds (Fossette et al., 2021). Peel et al. in prep; Tucker et al., 2021). To be representative, samples were collected from a northern and southern rookery with different proximities to industrial development. Collection



**FIGURE 1** | Flatback monitoring sites in Thevenard Island and Eighty Mile Beach; orange circles represent operational mines in Western Australia as of September 2020; the Pilbara region is highlighted. Data obtained as shapefile from MINEDEX (DMIRS-001) database (Department of Mines Industry Regulation and Safety, 2020).

and sampling of flatback turtles was therefore undertaken at both locations.

## Site Description

Turtle samples were collected in the northern part of WA (**Figure 1**). The mining and petroleum industry in WA has been expanded significantly in the last decade, accounting for 92% of the state's income (Australian Bureau of Statistics, 2017). Much of this expansion has occurred in the Pilbara region in the northern part of WA (**Figure 1**), a mineral rich region that has seen a significant development of mines, solar salt production, natural gas extraction and liquefaction, and the export of these commodities during the past 5 decades (Brocx, 2008). The Pilbara coast is arid (Semeniuk, 2013), making this region particularly prone to dust transport from mining activities.

### Thevenard Island

This island is offshore the Pilbara region and known for its large petroleum, natural gas and mineral deposits (Brueckner et al., 2013). Oil extraction began in 1985 with the discovery of the Saladin oil field just off Thevenard Island. Further discoveries in the coastal area of WA led to the development of the North West Shelf Project on the Burrup Peninsula, the Gorgon Project on Barrow Island and the Wheatstone Project on the mainland in Onslow. In 2015, mining activities on Thevenard Island were decommissioned (Wilkinson, 2016), but the North West

Shelf Project and Gorgon Project are ongoing. Higher order marine animals could be exposed to higher Hg through particles settling in the surface waters and consumed through seawater or incorporated into the food chain and consumed as food.

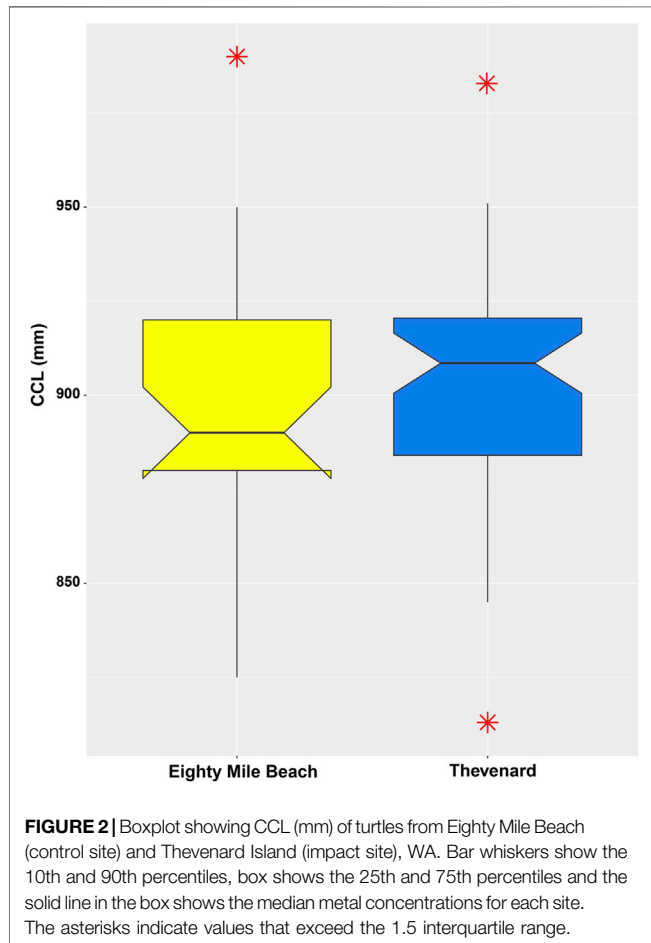
Flatback turtles nest on Thevenard Island from November to February. The island represents a medium-size nesting site for flatback turtles (Whitlock et al., 2016; Fossette et al., 2021) and has been declared a Nature Reserve. The Department of Biodiversity, Conservation and Attractions (DBCA) in WA started the North West Shelf Flatback Turtle Conservation Program in 2011, which implements population studies through track counts and mark-recapture. Monitoring started in 2016 on Thevenard Island. Monitoring of tracks on nest beaches is required to establish a baseline of turtle numbers and to detect any future changes.

### Eighty Mile Beach

Eighty Mile Beach is a 220 km stretch of uninterrupted sandy coastline in the Kimberley region of WA (**Figure 1**). It is a Ramsar-listed wetlands site, with the most inland occurring mangroves in WA (Secretariat, 1998). It was declared a Marine Park in 2013, in part due to its importance as a flatback turtle rookery, as well as the significant foraging grounds for flatback turtles (Young et al., 2014). It is approximately 600 km north-east of Thevenard Island and its closest industrial area is Port Hedland, the largest iron ore loading port in Australia 200 km south of Eighty Mile Beach (**Figure 1**). This is an important

**TABLE 1** | Curved carapace length (CCL, mm), Hg concentrations (ng/g) in skin and carapace of nesting flatback turtles in rookeries from Thevenard Island and Eighty Mile Beach, WA.

	Thevenard Island			Eighty Mile beach		
	CCL (mm)	Hg skin (ng/g)	Hg carapace (ng/g)	CCL (mm)	Hg skin (ng/g)	Hg carapace (ng/g)
Sample size (N)	52	50	52	28	23	28
Average	902	15.2	41.3	898	19.4	48.4
Min	813	7.2	19.1	825	9.4	13.7
Max	984	39.5	106.9	990	33.6	107.7
SD ( $\pm$ )	30.5	5.8	16.5	34.6	4.8	21.8



nesting site for flatbacks between October and February (Tucker et al., 2021). Although there are exploration permits further offshore Eighty Mile Beach, no current mining activities occur within 100 km of the shoreline (Department of Mines Industry Regulation and Safety, 2020).

Flatback turtle monitoring has been occurring sporadically at Eighty Mile Beach since 2005 (Young et al., 2014). A more regimented monitoring program was introduced in 2012, when the DBCA took responsibility for the Eighty Mile Beach Turtle Monitoring Program.

## Sample Collection

Carapace and skin samples of the same individual turtles were collected in November and December 2017, during the peak summer nesting season in WA. Sample collection was performed on randomly encountered turtles that were found nesting on the beach. These turtles were approached after oviposition was completed. Standard flipper tagging (Stockbrands titanium tags) was undertaken and morphometrics were taken with a fibreglass measuring tape for standard curved carapace length (CCL). Approximately 1 g of carapace and 1 g of skin samples were collected using a biopsy punch (Kai Medical, diameter: 5 mm). Skin samples were obtained from the trailing edge of the rear flipper, while carapace samples were obtained from the lateral margins of the shell. Only the keratin layer of the carapace was collected, following the protocols of Schneider et al. (2011). Samples were stored in individual plastic bags and frozen immediately at  $-5^{\circ}\text{C}$  for transport to the Palaeoworks Lab at the Australian National University for analysis.

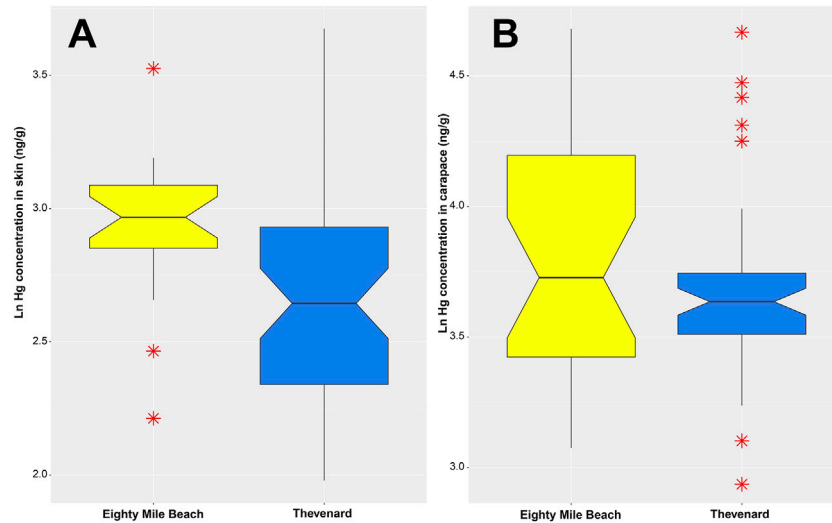
## Laboratory Processing and Analyses

Carapace samples were ultrasonicated for 5 minutes in individual beakers holding Milli-Q water to remove any debris. All samples were placed individually in a clean glass vial, covered with parafilm and placed in a FreeZone Plus six freeze-drier (Labconco, Kansas City, MO) and lyophilized at  $-50^{\circ}\text{C}$  for 48 h.

Total Hg concentration was determined by thermal decomposition, amalgamation, and atomic absorption spectrometry using a Milestone Direct Mercury Analyser (DMA-80 Tri-cell; Milestone, Bergamo, Italy) using the USEPA method 7,473 (USEPA, 1998). Two blanks and two Standard Reference Materials (SRMs) were analysed for every 36 samples. A replicate sample was run for every 10 samples, with recovery within 10% of the original sample and reported as the mean between the replicates. Dogfish muscle CRM Dorm-3 (National Research Council of Canada) was analysed, and results were in agreement with the certified reference material reports.

## Statistics

Data was analysed using R 3.5.1 (R Development Core Team, 2008) with  $p < 0.05$  as the level of statistical significance. The assumption of normality was checked using the Shapiro-Wilk test and the equality of variances checked using the Bartlett test. As data did not meet the assumptions of normality and homoscedasticity, data were  $\log(x)$ -transformed to normal



**FIGURE 3** | Boxplot showing natural log(Ln) of Hg concentrations (ng/g) in (A) skin of turtles and (B) carapace of turtles, from Eighty Mile Beach and Thevenard Island, WA.

distribution in order to use parametric tests, which are typically more powerful than non-parametric tests.

As bioaccumulation of Hg in turtles is known to be influenced by age (Schneider et al., 2013; Schneider and Vogt, 2018), the effects of carapace length (measured as CCL and used as a proxy for age) on Hg concentrations of turtles between the two rookeries was examined using an independent-sample two-tailed *t*-test. Mercury concentration was used as the dependent variable, and CCL as the independent variable. The same test was used to compare Hg concentrations in carapace and skin samples between the two populations, with Hg concentrations as dependent variable and carapace and/or skin as independent variables. A linear regression model was used to determine the relationship between CCL and Hg concentrations in skin and carapace of each turtle group.

## RESULTS

A total of 50 skin and 52 carapace samples ( $n = 52$  individuals) were collected from nesting turtles at Thevenard Island, and 23 skin and 28 carapace samples ( $n = 28$  individuals) from nesting turtles at Eighty Mile Beach. For turtles already departing the beach when encountered, only carapace samples could be collected. The results for turtle size (CCL), skin Hg concentrations and carapace Hg concentrations for both rookeries are reported in **Table 1**. Turtle size (CCL) ranged from 813 to 990 mm ( $\bar{x} = 900$  mm) (**Figure 2**). Mercury concentrations at Thevenard Island ranged from 7.2 to 39.5 ng/g ( $\bar{x} = 15.2$  ng/g) in skin samples; and 19.1 to 106.9 ng/g ( $\bar{x} = 41.3$  ng/g) in carapace samples (**Figure 3A**). In the Eighty Mile Beach samples, Hg concentrations ranged from 9.4 to 33.6 ng/g ( $\bar{x} = 19.4$  ng/g) in the skin; and 13.7–107.7 ng/g ( $\bar{x} = 48.4$  ng/g) in the carapace.

### Turtle Size (CCL) of Thevenard Island Vs Eighty Mile Beach

There was no significant difference in the CCL between turtles nesting at Thevenard Island ( $\bar{x} = 902 \pm 30.5$  mm), and turtles nesting at Eighty Mile Beach ( $\bar{x} = 898 \pm 34.6$  mm) (*t*-test results:  $t(78) = -0.519$ ,  $p > 0.05$ ) (**Figure 2**).

### Mercury Concentrations in Skin of Turtles From Thevenard Island Vs Eighty Mile Beach

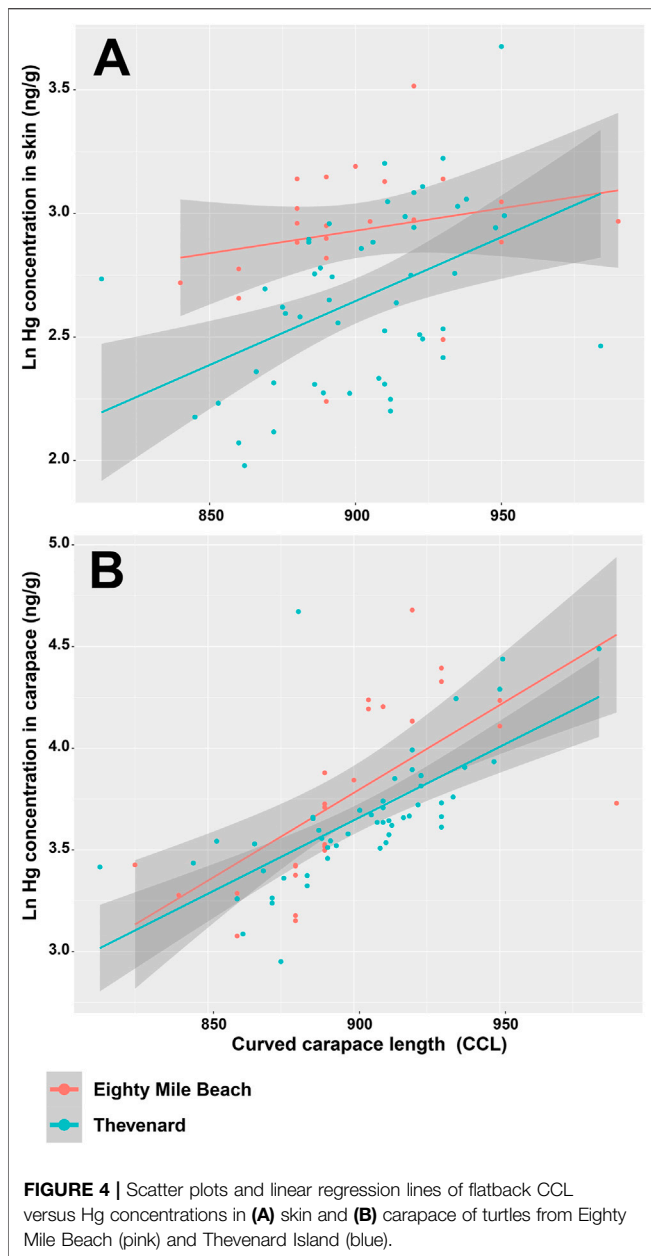
Turtles from the Eighty Mile Beach rookery had significantly higher mean skin Hg concentrations ( $\bar{x} = 19.4 \pm 4.8$  ng/g) than turtles from Thevenard Island ( $\bar{x} = 15.2 \pm 5.8$  ng/g), *t*-test:  $t(72) = -3.8055$ ,  $p < 0.001$  (**Figure 3A**).

### Mercury Concentrations in Carapace of Turtles From Thevenard Island Vs Eighty Mile Beach

The results of the *t*-test show no significant difference in mean Hg concentrations in the carapaces of turtles from Eighty Mile Beach ( $\bar{x} = 48.4 \pm 21.8$  ng/g) and Thevenard Island ( $\bar{x} = 41.3 \pm 16.5$  ng/g), *t*-test:  $t(77) = -1.27$ ,  $p > 0.05$  (**Figure 3B**).

### Relationship Between Hg Concentrations and Turtle Size

A regression analysis tested if the turtle length (measured CCL) significantly predicted Hg concentrations in turtle skin. The results of the regression indicated that turtle CCL size alone cannot explain Hg concentrations in skin of turtles from either the Eighty Mile Beach rookery ( $R^2 = 0.012$ ,  $F_{(1,23)} = 1.27$ ,  $p > 0.05$ ) or the Thevenard



Island rookery ( $R^2 = 0.019$ ,  $F_{(1,48)} = 12.44$ ,  $p > 0.05$ ) (Figure 4A), although CCL explained 43.1% of the variance in Hg concentrations in the carapace of turtles from Eighty Mile Beach ( $R^2 = 0.431$ ,  $F_{(1,25)} = 20.7$ ,  $p < 0.001$ ) and 44.2% from Thevenard Island ( $R^2 = 0.442$ ,  $F_{(1,50)} = 41.35$ ,  $p < 0.001$ ) (Figure 4B).

## DISCUSSION

### Turtle Size

The results of this study showed that flatback nesting females at Thevenard Island ( $\bar{x} = 902$  mm) and Eighty Mile Beach ( $\bar{x} = 898$  mm) were of similar mean sizes (CCL) (Table 1). This is an ideal scenario for comparing Hg concentrations between the

two nesting sites as there is no effect of size on the sampled populations from different rookeries. The turtle sizes recorded in this study were similar to those previously reported for flatback turtle populations in WA: ( $\bar{x} = 887$  mm (Limpus, 2007), ( $\bar{x} = 900$  mm, (Pendoley et al., 2014), from the Northern Territory:  $\bar{x} = 869$  mm, and slightly smaller than populations from Queensland:  $\bar{x} = 937$  mm (Limpus, 2007; Pendoley et al., 2014).

### Mercury Concentrations Vs Turtle Size

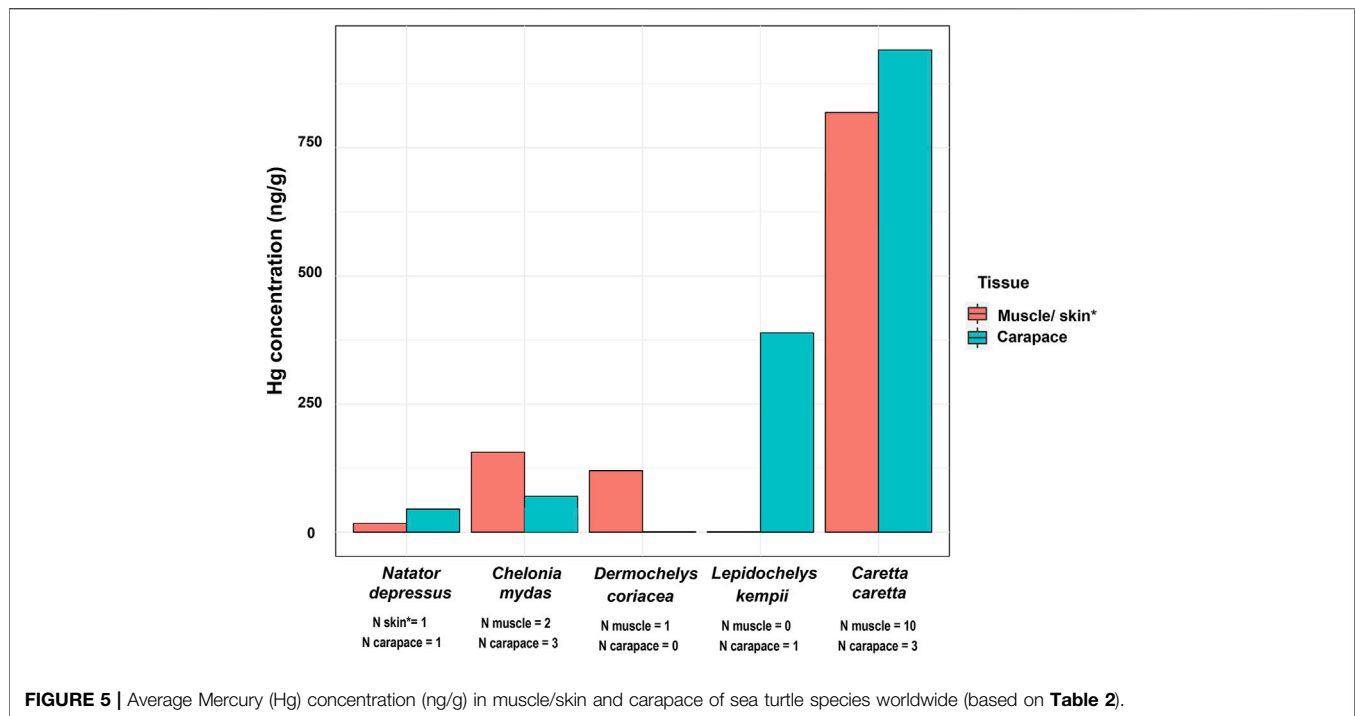
In this study, we found a positive relationship between turtle size and Hg concentrations in the carapace of flatback turtles' carapace, but not between turtle size and Hg concentrations in skin. It follows that carapace Hg concentration is a better predictor of exposure to Hg than Hg concentrations in skin tissue of flatbacks. This is in agreement with previous Hg studies on reptiles, which demonstrated the ability of keratin from both turtle carapace and caiman (*Melanosuchus niger* and *Caiman crocodylus*) skin to bioaccumulate Hg (Bezerra et al., 2013; Day et al., 2005; Innis et al., 2008; Komoroske et al., 2011; Schneider et al., 2015, 2009). This is because beta-keratins from caiman and turtle epidermis contain large amounts of amino acids (Alibardi, 2003; Toni et al., 2007) to which Hg is bound in relatively stable Hg complexes (Schneider et al., 2015). Consequently, a positive correlation between Hg concentration and turtle size can be obtained by analysing the keratin layer of carapace.

This is one of the few studies to measure Hg concentrations of a softshell turtle, along with Perrault et al. (2013) and Green et al. (2010), and the first study to measure Hg concentrations in the carapace and skin of the flatback turtle. Softshell turtles (Alibardi, 2002; Alibardi and Toni, 2006) have a soft and deformable carapace epidermis, not forming the hard and inflexible shell found in the loggerhead turtle (*Caretta caretta*) and the green turtle (*Chelonia mydas*). This is the first study to find a good correlation between Hg concentrations and body size in a turtle with reduced keratinization of the epidermal carapace, demonstrating that, despite the different keratin arrangement, carapace samples from the outermost keratin layer of soft-shelled turtles can be a good indicator of Hg in soft-shelled turtles (Schneider et al., 2015).

### Mercury Concentrations Vs Diet

Flatback turtles have lower Hg concentrations compared to the carnivorous loggerhead turtle (*Caretta caretta*) (Tomas et al., 2006) and the omnivorous Kemp's ridley *Lepidochelys kempii* (Schmid and Tucker, 2018), and similar concentrations to the green turtle (*Chelonia mydas*), which is omnivorous as a juvenile and predominantly herbivorous as an adult (Jimenez Heredia et al., 2017) (Figure 5).

In comparison with other sea turtles (Table 2; Figure 5), a higher Hg concentration was expected for the carnivorous flatback turtles as Hg biomagnifies up food chains (Godley et al., 1999; Schneider et al., 2010). There are two possible reasons why Hg concentrations in flatback turtles in this study are relatively low compared to other turtles worldwide: firstly, the few available studies on the diet of this species may not fully capture its entire diet and may have missed potential herbivory or omnivory. The current literature on diet of flatback turtles is



**FIGURE 5 |** Average Mercury (Hg) concentration (ng/g) in muscle/skin and carapace of sea turtle species worldwide (based on **Table 2**).

**TABLE 2 |** Total mean Hg concentration (ng/g) in muscle/skin and carapace of sea turtle species worldwide and in flatbacks from this study.

Species	Diet	Hg (muscle) (ng/g) dry weight	Hg (carapace) (ng/g)	Sample collection coastal sites	Reference
<i>Chelonia mydas</i>	Omnivore/herbivore	—	70	Brazil	Barraza et al., 2019
		156 <sup>a</sup>	32	Brazil	Bezerra et al. (2013)
		—	48	United States	Komoroske et al. (2011)
		117 <sup>a</sup>	—	Australia	Van de Merwe et al. (2010)
<i>Caretta</i>	Carnivore	702 <sup>a</sup>	—	Italy	Canzanella et al. (2021)
		676	—	Spain	Gómez-Ramírez et al. (2020)
		<89	—	Italy	Esposito et al. (2020)
		343 <sup>a</sup>	—	Spain	Febrer Serra et al. (2020)
		100	—	Brazil	Di Benedetto et al. (2019)
		—	590	United States	Perrault et al. (2013)
		156 <sup>a</sup>	—	Spain	Novillo et al. (2017)
		195 <sup>a</sup>	—	Portugal	Nicolau et al. (2017)
		702	—	Italy	Storelli et al., 2005
		400	—	Italy	Maffucci et al. (2005)
604 <sup>a</sup>	941	United States	Day et al. (2005)		
819 <sup>a</sup>	890	Italy	Storelli et al. (1998a)		
—	—	Italy	Storelli et al. (1998b)		
<i>Lepidochelys kempii</i>	Omnivore	—	389	United States	Innis et al. (2008)
<i>Dermochelys coriacea</i>	Carnivore	120	—	United Kingdom	Davenport et al. (1990)
<i>Natator depressus</i>	Likely carnivore	17 <sup>b</sup>	45	Australia	This study

<sup>a</sup>Mercury concentrations reported in wet weight were converted to dry weight by multiplying by a factor of 3.9 (the wet/dry mass ratio calculated by Eggins et al. (2015).

<sup>b</sup>Hg measured in skin.

mostly limited to the turtle populations from northern Australia (Limpus et al., 1988; Zangerl et al., 1988; Limpus, 2007). Limpus (2007) reported a carnivorous diet in this species, consisting of benthic and pelagic invertebrates. Another study provided evidence of both omnivorous and carnivorous behaviours from stomach samples of stranded individuals (Bjorndal, 1997). Secondly, the lower Hg concentrations found in the flatback turtles might be a result of the naturally low Hg concentrations from the Australian continent (McQueen, 2011), as already hypothesised by Maher et al. (2020). To put this in perspective, for soils to be considered Hg enriched, they have to be >100 ng/g (Connor and Schaklette, 1975; Gustin et al., 2006). Across Australia, Hg concentrations in most soils and sediments are below 50 ng/g (Lintern et al., 2020; Schneider et al., 2021), demonstrating the naturally low Hg concentrations in the Australian environment and therefore low potential for Hg uptake by aquatic organisms.

It is not possible to draw conclusions about the diet of the flatback turtle from our Hg concentrations. Mercury measurements of the entire local food web would be necessary to better establish the trophic level and diet of this species. More studies on the diet of flatback turtles, and analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and stomach contents also would reveal the diet of this species to allow a valid conclusion about its trophic level.

## Mercury in Flatback Turtles of Thevenard Island Vs Eighty Mile Beach

In this study, there were no significant differences in Hg concentrations between two sampled turtle rookeries. Despite significant mining activity in the Pilbara region (where turtles nesting on Thevenard island mainly forage), no significant difference in Hg concentrations in carapace was found between turtles nesting at Thevenard Island and Eighty Mile Beach. In contrast, turtles from Eighty Mile Beach had significantly higher Hg concentrations in skin. A plausible explanation for the lack of effect of location on Hg concentrations in carapace could be due to individuals from both rookery overlapping in the resident foraging areas. Although these populations have distinct nesting sites, shared foraging areas would inhibit differentiation between rookeries given that likely uptake pathways are through diet and drinking seawater. Foraging grounds that support turtles from multiple rookeries and multiple stocks (including Thevenard Island and Eighty Mile Beach) have been identified from a detailed study of satellite tracking data (Peel et al. *in preparation*). Other studies have shown that flatbacks from rookeries in the Pilbara share foraging areas (Pendoley et al., 2014; Thums et al., 2017; Waayers et al., 2019). Due to flexibility in feeding behaviour and foraging areas (Whitlock et al., 2016), Hg concentrations in this turtle species may not reflect a single region. Future studies could focus on sampling turtles at foraging grounds, but obtaining high enough sample sizes from foraging areas may remain difficult across multiple locations.

A second reason for lack of difference in Hg concentrations between the two flatback rookeries is the form of Hg emission by local industry in the Pilbara and the prevailing wind direction. During mining and gas extraction operations, Hg can be

released as a gas or salt (Spiric, 2001; Li et al., 2019). In nearby areas of the offshore Pilbara region (Barrow Island), most Hg as a by-product of mining is released into the atmosphere (Young, 2019) and its transport is consequently affected by wind direction. Once in the atmosphere, Hg can attach to fine dust particles and travel significant distances, contaminating sites well away from mining operations (Steinnes et al., 1997; Csavina et al., 2014).

## Future Directions

Additional studies on Hg and the diet of flatback turtle would further advance our understanding of the Hg cycle for this species. Below are recommendations on the next research steps to be taken:

- Diet and food web reconstruction based on the analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and stomach contents, considering ontogeny, would provide a detailed diet information and provide insights to the low Hg concentrations measured in this species.
- Mercury measurements in different environmental matrices (e.g., seawater, sediments, atmospheric aerosols) and biological indicators, would allow a complete overview of the Hg cycle in the coastal WA ecological system and Hg exposure to aquatic organisms.
- Satellite tracking data would clarify any potential overlap in the foraging grounds for turtles from Thevenard Island and Eighty Mile Beach.
- Laboratory studies on the metabolism of Hg in turtles would provide an understanding of the distribution, accumulation, and excretion of Hg in this species.

## CONCLUSION

This study is the first to report Hg bioaccumulation in flatback turtles. Our research establishes Hg concentrations for two flatback turtle rookeries in Western Australia, advancing our understanding of Hg concentrations in these marine organisms. Despite limited understanding of environmental Hg concentrations and exposure by flatback turtles, sampled turtles have a much lower Hg concentration than reported for other sea turtle species worldwide. The present exploratory study found low mercury accumulation at rookeries, but a need remains to test for contaminants acquired at widely dispersed foraging grounds. Further studies on the diet and trophic levels of flatback turtles would support the interpretation of Hg exposure to this species.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The animal study was reviewed and approved by Department of Biodiversity, Conservation and Attractions.



## AUTHOR CONTRIBUTIONS

LS conducted the field sampling, performed the laboratory and statistical analysis, and wrote the manuscript. SF, EY conducted field sampling and supported the writing of this manuscript. ADT and SW issued and organised sample collection and transport permits and supported the writing of this manuscript. KV supported the writing of this manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the North West Shelf Flatback Turtle Conservation Program from The Department of Biodiversity, Conservation and Attractions (DBCA) and the ANU School of

Culture, History and Language. Laboratory Analyses Were Funded by The Department of Archaeology And Natural History At The Australian National University And by an Arc Discovery Early Career Researcher Award (De180100573) Awarded To LS.

## ACKNOWLEDGMENTS

Field work was done under the auspices of permit authorisation number TFA 2019-0174 and animal ethics approval 2016/01 granted to Scott Whiting, WA DBCA export licence ES002418 and ACT Government import licence LI201868 granted to LS. Laboratory analyses were funded by the Department of Archaeology and Natural History at the Australian National University. We are appreciative of J. Craddock and S. Connor's insights and comments, which improved this manuscript.

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