

Maternal–pup mercury relationship in South American sea lions and fur seals

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3

Abstract

Mercury (Hg) is a non-essential, globally distributed toxic metal that bioaccumulates in aquatic organisms and biomagnifies across trophic levels, posing a threat to top predators such as marine mammals. Our aim was to evaluate total mercury (THg) relationships in mother-pup pairs of two pinniped species, South American sea lions (*Otaria flavescens*) and South American fur seals (*Arctocephalus australis*), from Isla de Lobos, Uruguay. We quantified THg in the hair of 11 *O. flavescens* and 46 *A. australis* mother-pup pairs. THg concentrations were significantly higher in *O. flavescens* than in *A. australis* for both adult females ($30.5 \pm 9.3 \mu\text{g/g}$ vs. $5.7 \pm 3.4 \mu\text{g/g}$, dry weight) and pups ($25.3 \pm 13.2 \mu\text{g/g}$ vs. $4.4 \pm 2.5 \mu\text{g/g}$, dry weight). Hg levels in *O. flavescens* pups are among the highest recorded for pinnipeds. A significant positive relationship between female and pup THg (*O. flavescens* $p=0.003$; *A. australis* $p=0.002$) supported transplacental Hg transfer. In *O. flavescens*, pup sex significantly influenced this relationship, with males showing lower THg for a given maternal value, whereas no sex effect was detected in *A. australis*. These findings support the use of pups as effective minimally invasive proxies for maternal Hg levels. This study highlights the relevance of early-life exposure in environmental toxicology and supports integrating pup-based sampling in long-term contaminant monitoring programs.

Key words: Hg; maternal transfer; pinnipeds; sea lion; fur seal; Uruguay

1. Introduction

In recent decades, environmental pollution has risen markedly, becoming one of the most pressing challenges faced by societies worldwide. Among the multiple forms of pollution, inorganic contamination-particularly by heavy metals-stands out as a major threat to both the structural complexity and functionality of natural ecosystems (United Nations 2010; Potters 2013; Reyes et al. 2016). Non-essential metals such as mercury (Hg) can exert toxic effects even at trace concentration (Gaioli et al. 2012). Human activities including industrial discharges, mining, and fossil fuels combustion have substantially increased environmental Hg emissions, disrupting its natural biogeochemical cycle and elevating Hg concentration in many ecosystems beyond levels tolerable limits for organisms (Luoma and Rainbow 2008).

Mercury enters organisms through dietary exposure and is subsequently transferred through food webs (Chételat et al. 2020). Within aquatic ecosystems, methylmercury (MeHg), the organic and most toxic form of Hg, poses a major environmental hazard due to its strong tendency to bioaccumulate in organisms over time and biomagnify across trophic levels, often resulting in top predators carrying the highest toxic loads (Dallinger et al. 1987; Storelli 2008).

In viviparous animals, early-life exposure to contaminants also depends on maternal transfer, initially through the placenta and later via lactation (Habran et al. 2013; Peña-Galindo et al. 2025). Beyond trophic processes, MeHg can be transferred maternally, raising concerns about its transgenerational transmission. Although one of the functions of the placenta is to filter out potentially harmful substances, MeHg, like other organic molecules, is capable of crossing this barrier (Nehring et al. 2017). Mercury has been detected in the placental tissue of seals (e.g., *Halichoerus grypus grypus*) (Nehring et al. 2017), in the human umbilical cords (Chen et al. 2015),

in cetacean liver fetuses (e.g., *Delphinapterus leucas*) (Hoguet et al. 2013), and in tissues formed during the gestation such as whiskers in seals (e.g., *Phoca vitulina*) (Noël et al. 2015). Moreover, maternal transfer through milk has also been documented in seals (e.g., *H. grypus*) (Habran et al. 2013), potentially affecting pups during the earliest stages of development.

Pinnipeds, which include the families Otariidae (sea lions and fur seals), Odobenidae (walruses), and Phocidae (true seals), are widely recognized as effective bioindicators of marine pollution due to their high trophic position, long lifespan, broad geographic distribution, sensitivity to environmental change, accessibility during breeding periods, and the possibility of obtaining non-invasive tissue samples such as hair (Bossart 2011; Peterson et al. 2016; Berta 2018; Plön et al. 2024). Hg accumulation in these species is typically evaluated through the analysis of high-storage tissues such as the liver, kidney, and muscle (Brookens et al. 2007; Krey et al. 2015; Lima et al. 2022). However, hair has emerged as a valuable non-invasive alternative for assessing Hg exposure in both live and deceased individuals (i.e. Roselli-Laclau et al. 2024). The toxic effects of Hg in pinnipeds are diverse, encompassing respiratory infections, neurotoxicity, and immunosuppression, the latter increasing susceptibility to disease (Kakuschke et al. 2008a, 2009, 2011; Taylor et al. 2022). Because these effects may directly influence survival, health, and reproductive performance, monitoring Hg exposure in reproductive populations is particularly important.

In this context, the possibility of using pups as proxies for maternal Hg burdens could greatly facilitate long-term monitoring programs in wild populations, especially considering the logistical and ethical challenges associated with handling adult females (i. e. Katz et al. 2018). Understanding the relationship between Hg concentrations in the hair of females and their pups is

therefore essential to determine whether pup Hg levels reliably reflect maternal exposure and population-level contamination patterns. Moreover, studying the relationship between Hg concentrations in females and their pups can provide valuable insights into maternal transfer and early-life Hg exposure. Because Hg can be transferred during gestation and lactation, maternal transfer may represent an important pathway of contamination in pinnipeds. This is particularly relevant given that newborn pups (up to 50 days old) are especially vulnerable, as their immune systems are still developing and may be more sensitive to contaminant-induced damage (Kakuschke et al. 2008b; Taylor et al. 2022).

Two otariid species reproduce along the Uruguayan coastal region of the Río de la Plata and the adjacent Atlantic Ocean: the South American fur seal (*Arctocephalus australis*) and the South American sea lion (*Otaria flavescens*) (Vaz-Ferreira 1976a, b). As apex predators feeding mainly on fish and cephalopods (Franco-Trecu et al. 2013), both species are particularly susceptible to the health effects of Hg exposure. Total mercury (THg) accumulation in the region has been documented, with THg detected in the sandy intertidal zones of 13 beaches. The highest values were found near urbanized coastal zones, particularly in Montevideo beaches, the capital of Uruguay (García-Alonso et al. 2017). Adult females of both otariid species have shown moderate to elevated THg levels (Roselli-Laclau et al. 2024). In this context, we aimed to assess the relationship between THg levels in adult females and their pups in South American fur seal and sea lion, and to evaluate whether pup sex influences this relation.

2. Materials and methods

2.1 Study area and sample collection

Fieldwork was carried out at Isla de Lobos (35°01'S, 54°50'W), , located approximately five nautical miles from the mainland in the eastern portion of the Río de la Plata estuary, the second largest estuary in South America, which hosts breeding populations of *A. australis* and *O. flavescens* (Franco-Trecu et al. 2014; Franco-Trecu 2015). *O. flavescens* sampling was conducted during the reproductive seasons of 2009 and 2013, and the sampling for *A. australis* was in 2006 and between 2009 and 2011. Hair samples (100–300 mg) were obtained from the dorsal area close to the posterior region of female–pup pairs, including 11 *O. flavescens* and 46 *A. australis* pairs. In pups, hair samples consisted of lanugo grown in utero, since the first molt in otariids occurs between 2 and 5 months after birth (Keogh et al. 2020) and sampling took place approximately one month postpartum. Pups were captured using a hand net and then weighed, measured, and sexed. Adult females were immobilized using chemical anesthesia according to Katz et al. (2018), after which weigh and body measurements were taken.

2.2 Laboratory analysis

Hair samples were washed twice with 2% Extran detergent and rinsed with Milli-Q ultrapure water. After cleaning, samples were freeze-dried. For THg quantification, around of 100 to 300 mg of lyophilized hair were digested in 1 mL of hydrogen peroxide (H₂O₂), and the gradual addition of 3 mL of a 1:1 mixture of sulfuric acid (H₂SO₄) and nitric acid (HNO₃). The samples were put in a heating block at 60 °C for 4 hours using a cold finger condenser, then 5 mL of 5% potassium permanganate (KMnO₄) solution was added, and they were kept at room temperature overnight. Afterwards, a second heating step of 60 °C for 30 minutes, followed by titration with 12% hydroxylamine hydrochloride (NH₂OH·HCl) (Bastos and Malm 1998; Gomes et al. 2022). The digested samples were filtered through Whatman® 40 filter paper and transferred to tubes, which were filled up to 25 mL with ultrapure Milli-Q water. Subsequently, 1 mL of each final

extract was diluted in 30 mL of ultrapure water, and then 150 μ L of bromine monochloride (BrCl), 60 μ L of hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$), and 150 μ L of stannous chloride (SnCl_2) were added. Analytical control solutions ($n = 3$) were included in all analyses, with coefficients of variation below 10%. To assess accuracy, we used certified reference material (ERM-DB001), obtaining a recovery of $95 \pm 15\%$ of the certified concentration. THg determination was performed using cold vapor atomic fluorescence spectrophotometry (CV-AFS) with a detection limit of 0.07 $\text{ng}\cdot\text{g}^{-1}$ (Tekran Mercury Analysis System, Model 2600, Tekran Instruments Corporation).

2.3 Data analysis

Descriptive statistics (mean, standard deviation and range) were calculated for THg levels in adult females and pups, and summarized by species and, for pups, data were also summarized by sex. To evaluate the relationship between THg levels in females and their pups, as well as the effect of pup sex, linear regression models were applied after confirming assumptions of normality and homogeneity of variances. Additionally, differences in THg concentrations between species were assessed separately for adult females and pups using ANOVA.

Analyses began with the full model that incorporated the interaction between female THg levels and pup sex. Model simplification was carried out using a stepwise backward procedure, whereby terms lacking statistical support were sequentially excluded. Competing models were evaluated and ranked using the Akaike Information Criterion (AIC), considering a difference greater than two units ($\Delta\text{AIC} > 2$) as evidence of a better-fitting model (Bolker 2007). Final model validation was assessed by inspecting residual patterns (results not shown) (Pinheiro and Bates 2000). All statistical procedures were conducted using the free software R version 4.2.0 (R Core

Team 2023). All data used for statistical analyses are publicly available in Mendeley data (Roselli-Laclau, et al., 2026).

3. Results

In both species, *O. flavescens* and *A. australis*, pup hair THg concentrations were positively and significantly related to female hair THg concentrations (Fig. 1; Table 1). In *O. flavescens*, pup sex significantly influenced the relationship between female and pup THg concentrations ($p = 0.04$), with male pups showing lower THg values for a given maternal concentration. In contrast, pup sex had no significant effect in *A. australis* (Table 1).

Mean THg concentrations differed significantly between species in both adult females and pups. Female *O. flavescens* exhibited significantly higher THg concentrations ($30.5 \pm 9.3 \mu\text{g/g}$ dry weight [d.w.]) than female *A. australis* ($5.7 \pm 3.4 \mu\text{g/g}$ d.w.; $F = 243.8$, $p < 0.001$). Similarly, *O. flavescens* pups showed significantly higher THg concentrations ($25.3 \pm 13.2 \mu\text{g/g}$ d.w.) than *A. australis* pups ($4.4 \pm 2.5 \mu\text{g/g}$ d.w.; $F = 109.4$, $p < 0.001$).

4. Discussion

In both species, pup THg levels were strongly related to maternal hair THg, as shown by regression analyses, indicating that pup THg reliably reflects maternal contamination. This confirms that pups can serve as effective proxies for monitoring Hg exposure in breeding females, providing a minimally invasive approach for long-term population-level surveillance. Notably, *O. flavescens* pups exhibited the highest THg levels reported for pinnipeds, with values five times higher than in *A. australis*, consistent with the pattern found in adult females (Roselli-Laclau et al. 2024).

Few studies on pinnipeds have reported a positive relation between Hg levels in mothers and their pups (Habran et al. 2011; Peterson et al. 2024; Peña-Galindo et al. 2025), supporting the idea of maternal transfer during gestation. For example, (Peña-Galindo et al. 2025) found a significant relationship between THg accumulations in blood clots of *A. australis* mother–pup pairs, while no significant relation was observed between pup blood and maternal milk of *O. flavescens*, which the authors interpret as evidence that most Hg transfer occurs in utero. This is consistent with findings from other pinniped species, such as the northern elephant seal (*Mirounga angustirostris*), where THg levels in pup hair positively associated with maternal blood levels (Peterson et al. 2024). These findings are consistent with previous studies indicating that the placenta is the main route for prenatal Hg transfer in marine mammals (Habran et al. 2011).

The association between maternal and pup hair THg levels has also been reported in other mammal species. In polar bear (*Ursus maritimus*), offspring THg levels depend on maternal THg accumulation and pup sex (Bechshoft et al. 2016). This pattern has also been observed in humans, where a significant relationship between THg levels in maternal and neonatal hair has been documented (Marques et al. 2013).

In our study, pup sex did not influence THg accumulation in *A. australis*, consistent with other studies on pinniped pups where sex-related differences in Hg burden were not observed (e.g., *Halichoerus grypus grypus*, *Phoca vitulina richardii*) (Brookens et al. 2007; Grajewska et al. 2020). However, sex-related differences in prenatal Hg transfer have been reported in other species. In harbor seal (*P. vitulina richardii*) from central California, female pups exhibited significantly higher THg concentrations in lanugo than males (Lian et al. 2021). In contrast, in southern elephant seal (*Mirounga leonina*) from Antarctica, no sex-related differences were found

in lanugo Hg levels. Instead, lighter pups displayed significantly higher accumulation than heavier ones (Kehrig et al. 2022), suggesting that body size, rather than sex *per se*, may influence Hg accumulation. In *O. flavescens*, which shows marked sexual dimorphism in birth size (Ono and Boness 1996), the observed sex differences in THg levels could therefore reflect variation in birth size or growth dynamics rather than a direct effect of sex. Given the limited sample size available for *O. flavescens*, the potential influence of body mass on THg concentration cannot be fully ruled out, and normalization by individual mass in future studies with larger datasets would help clarify this relationship.

In our study, THg levels in *O. flavescens* hair, both females ($30.5 \pm 9.3 \mu\text{g/g dw}$) and pups ($25.3 \pm 13.2 \mu\text{g/g dw}$), were among the highest reported for pinnipeds. These values surpassed those documented in *Zalophus californianus* ($< 10 \mu\text{g/g dw}$; (Symon et al. 2023)) and *M. leonina* ($5.9 \pm 3.1 \mu\text{g/g dw}$; (Kehrig et al. 2022)). Notable, they were comparable to the levels recorded in *M. angustirostris* ($23.01 \mu\text{g/g dw}$; (Peterson et al. 2024)), a phocid known for its deep pelagic foraging and consequently elevated mercury burdens. Furthermore, these accumulation exceeded levels observed in polar bear ($5.4 \mu\text{g/g dw}$) hair associated with neurological damage (Dietz et al. 2013). Although research on the toxicological effects of THg in pinniped pups is limited, studies in other species suggest potential health impacts. In Pacific harbor seal (*P. vitulina richardii*) pups, elevated blood THg levels were associated with lethal nervous system infections (Van Hooymissen et al. 2015). Chronic MeHg exposure impairs immune function in pinnipeds, including reductions in lymphocyte responses and cytokine production (Das et al. 2008; Levin et al. 2020). These findings highlight the vulnerability of developing individuals, support Hg's potential role as an immunosuppressant (Kakuschke et al. 2009), increasing disease susceptibility, and underscore the importance of pup-based monitoring to assess maternal contamination and population-level risks.

In other species, the presence of THg in offspring hair suggests prenatal exposure to Hg, as hair develops during the final stages of fetal growth (Oftedal et al. 1991). Experimental studies in rats have shown that gestational exposure to Hg and MeHg can lead to Hg accumulation in fetal organs and produce developmental and motor impairments in pups (Feng et al. 2004). In addition, abortions and uterine reabsorptions have been reported in females with the highest MeHg exposure (Gandhi et al. 2014).

The potential immunosuppressive effects of Hg in pinniped pups may help explain species-specific differences in disease susceptibility and mortality. The 2023 outbreak of highly pathogenic avian influenza (HPAI) H5N1 disproportionately affected *O. flavescens* compared to *A. australis*, with significantly greater mortality reported across both Pacific and Atlantic populations (Gamarra-Toledo et al. 2024; Szteren and Franco-Trecu 2024; Tomás et al. 2024). In this context, our results raise the possibility that pups may begin life with elevated THg levels acquired through maternal transfer, which could potentially contribute to immune dysfunction. Since THg continues to accumulate through bioaccumulation and biomagnification as individuals grow and move up the food chain, early-life exposure may represent an important starting point in long-term contaminant burden. This interpretation is consistent with studies showing that Hg exposure can impair immune responses in other pinniped species (Das et al. 2008; Levin et al. 2020).

Given that lanugo, the hair collected from pups, develops during the last trimester of gestation (Rea et al. 2013), and that THg measurements were conducted in this tissue, our findings suggest prenatal THg transfer in South American sea lions and fur seals, as reflected by the significant association between female and pup hair THg levels. The particularly high THg concentrations recorded in *O. flavescens* pups highlight the potential ecological and toxicological relevance of early-life exposure. Beyond improving our understanding of maternal contaminant

transfer, these results also support the use of pups as useful proxies for long-term monitoring, providing a minimally invasive and cost-effective tool for the conservation and management of pinniped populations.

Data availability

The data that support the findings of this study are openly available in Mendeley data (Roselli-Laclau, et al., 2026) at <https://doi.org/10.17632/8jk9xjkj9h.1>

Ethic statement

All procedures of animal manipulation were submitted to and approved by the Ethics Committee in Animal Experimentation of the University of the Republic (Uruguay). The fieldwork and sampling were authorized by the National Council for Aquatic Resources, Ministry of Livestock, Agriculture and Fishing (DINARA, Uruguay) under permit No 603/2006, 572/2008 and 1022/2010.

Author contributions

Conceptualization: Valentina Franco-Trecu, Javier García-Alonso; Formal analysis and investigation: Valentina Franco-Trecu, Anaclara Roselli-Laclau, Alex Valdéz-G, Mariana Freitas-Souza, Marcelo G. Almeida; Writing – original draft preparation: Valentina Franco-Trecu, Javier García-Alonso, Anaclara Roselli-Laclau; Writing – review and editing: Marcelo G. Almeida, Carlos Eduardo de Rezende; Funding acquisition: Valentina Franco-Trecu, Javier García-Alonso, Carlos Eduardo de Rezende.

Declaration of competing interest

The authors declare no competing interests.

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413

Table 1. Linear models for female hair THg accumulation related to pup hair THg accumulation, species and pup sex. Estimates and their Standard Error are shown in parentheses and p-value for each variable and interaction in each model. The R adjusted and Akaike information criterion are shown for each model.

Species	Model	Intercept	THg female	pup sex (male)	Hg pup*pup sex (male)	R ² - adjusted	AIC
<i>O. flavescens</i>	THg pup ~ THg	(13.1, 5.22)	(0.47, 0.15)	(-11.11, 9.74)	(0.18, 0.3)	0.76	67
	female * pup sex	<i>p</i> = 0.04	<i>p</i> =0.01	<i>p</i> =0.3	<i>p</i> =0.6		
	THg pup ~ THg	(11.6, 4.4)	(0.52, 0.13)	(-5.48, 2.36)	-	0.75	65
	female + pup sex	<i>p</i>=0.03	<i>p</i>=0.003	<i>p</i>=0.04			
<i>A. australis</i>	THg pup ~ THg	(2.69, 0.94)	(0.31, 0.13)	(-0.55, 1.43)	(0.12, 0.24)	0.20	210
	female * pup sex	<i>p</i> = 0.007	<i>p</i> =0.02	<i>p</i> =0.7	<i>p</i> =0.6		
	THg pup ~	(2.45, 0.82)	(0.34, 0.11)	(0.087, 0.73)	-	0.20	208
	THg female + pup						
	sex	<i>p</i> =0.005	<i>p</i> =0.003	<i>p</i> = 0.1			
	THg pup ~	(2.51, 0.68)	(0.34, 0.1)	-	-	0.20	206
	THg female	<i>p</i><<0.001	<i>p</i>=0.002				

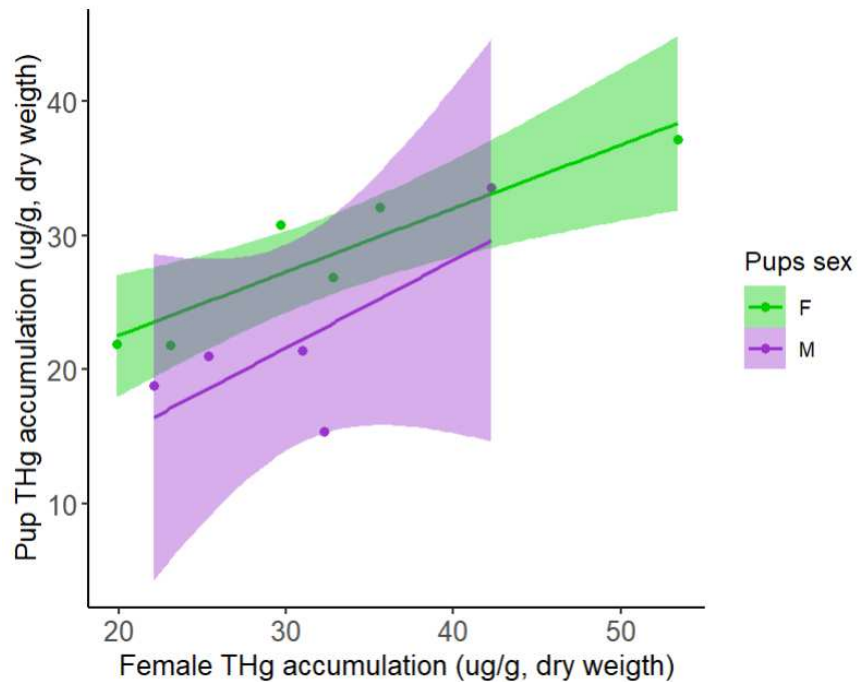
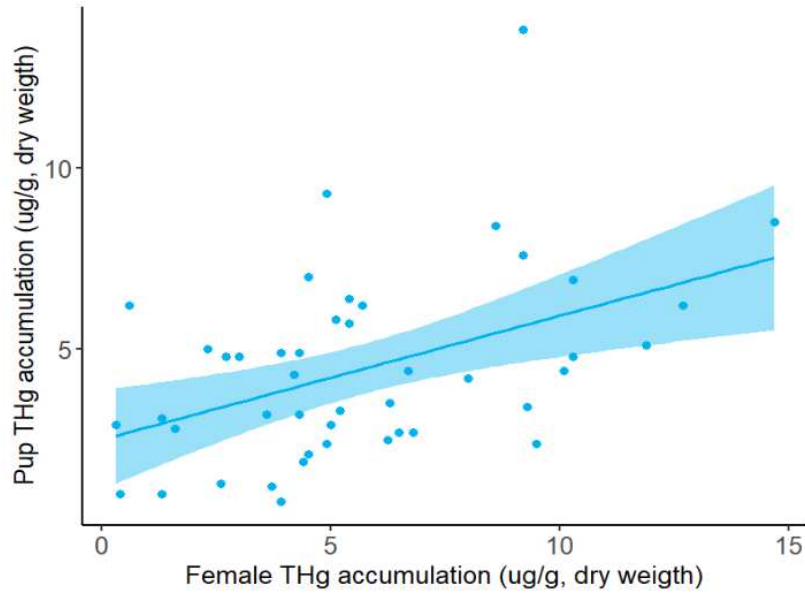


Figure 1. Linear relationship between THg concentrations in hair of *Otaria flavescens* females and their pups, differentiated by pup sex (F = female, M = male). Each point corresponds to an individual mother–pup pair. The lines are the fitted model of each specie with its respective 95% confidence interval.



429

430 Figure 2. Linear relationship between THg concentrations in hair of *Arctocephalus australis*
 431 females and their pups. Each point corresponds to an individual mother–pup pair. The lines are the
 432 fitted model of each specie with its respective 95% confidence interval.

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