

Mercury Concentration in Liver Tissues of South American Fur Seals (*Arctocephalus australis*) from Southwestern Atlantic Ocean

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Mercury (Hg) contamination of oceans is rapidly increasing, however Hg bioaccumulation in pinnipeds has been understudied. Here, we report for the first time Hg concentration in liver tissues of South American fur seals (*Arctocephalus australis*) in South and Southeast Brazil. Hg concentration was determined in twenty-five fur seals' specimens found stranded along the coast of the Southwestern Atlantic Ocean. Samples were digested using the microwave technique and quantified by cold vapor generation atomic fluorescence spectrometry technique. The average Hg concentration was 6.37 mg kg⁻¹ (wet weight), with a minimum concentration of 0.09 mg kg⁻¹ and the highest concentration of 15.58 mg kg⁻¹. No correlation between biological variables (sex, total length and weight) and Hg concentration in *A. australis* liver were found. The results presented here are of great importance to establish baselines for future evaluations of Hg contamination in marine mammals and the effects of this environmental problem in animal health.

Keywords: mercury determination, marine mammals, strandings, bioaccumulation, CV-AFS

Introduction

Mercury (Hg) is one of the main trace-elements present in industrial and agricultural effluents. Inorganic Hg is known to methylate in anoxic marine sediments using sulfur or iron reducing microbes and is filtered back into the water column via the microbial loop, thereby making the methylmercury (MeHg) available in trophic networks, starting from the lower trophic level (phytoplankton). Thus, its importance in bioaccumulation through marine trophic chains has been recognized in different marine webs.¹⁻³ Therefore, the concentration of Hg in biological tissues has received special attention.⁴⁻⁶ As Hg also affects

human health, it is probably the most studied of all non-essential trace-elements in the environment.⁷⁻¹⁰ Due to its properties, it has been placed on the list of the top ten chemicals of great concern in public health by the World Health Organization.¹¹ Since 2003, Hg has been declared a harmful substance of global importance. It is estimated that two thirds of bioavailable Hg are from anthropogenic origin and only one third is from natural sources.⁹ Its presence in the environment occurs naturally through geothermal activity, volcanoes, benthic sediments, hydrothermal vents and direct atmospheric deposition.^{12,13} Amalgamation in gold purification, mining, and burning of fossil fuels are the main anthropogenic sources of Hg.^{10,14-16}

The chemical form of the element that is most toxic to humans and to the environment is MeHg.^{10,17} The environmental exposure to Hg (especially in the form of

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MeHg) via the food web is significantly higher for animals at the top of the food web, as MeHg exhibits increased concentrations as it moves to higher levels.^{4,8,18-20}

Because of their longevity and usually being at high trophic levels, marine mammals are considered sentinel species in the marine environment.²¹⁻²³ For the same reasons and due to biomagnification processes, marine mammals have great potential to absorb and accumulate Hg associated with coastal waters, in concentrations of several orders of magnitude above the concentrations found in the water column and sediments.²⁴⁻²⁹ Thus, species that forage in urbanized coastal areas may be more susceptible to accumulating Hg from natural and anthropogenic inputs.^{30,31} Therefore, monitoring these species is an important source of information to indicate the dynamics of these potential contaminants in the marine ecosystem, and extremely necessary to predict the potential impact of the element on aquatic life, as well as on humans.³²⁻³⁴ Different techniques can be adopted to monitor the Hg present in environmental and biological samples, however the technique of atomic fluorescence spectrometry with cold vapor generation (CV-AFS) represents the most popular method to work with determination of Hg.^{35,36} The technique is a powerful analytical tool, offers very low detection levels, simplicity, wide lineal dynamic range, cost efficiency, reproducibility and accuracy.^{37,38}

The South American fur seal *Arctocephalus australis* is a food web top predator that inhabits waters along South America's Pacific and Atlantic coasts from Peru through Brazil, including the Falkland/Malvinas Islands,³⁹ with the largest breeding colonies found in the Falkland/Malvinas Islands and the Uruguayan coast (ca. 36,425 and 31,160 pups), respectively.^{40,41} The species is considered as a generalist predator,⁴² feeding on a wide variety of prey.⁴³⁻⁴⁵ Even though it is a top predator and is found in coastal areas close to large urban centers, there are few studies on the concentrations of Hg in *A. australis* along its distribution, and especially in Brazilian territory where two studies have been carried-out in Rio Grande do Sul state, southernmost Brazil and with a small samples size (n = 3 and n = 8, respectively).^{46,47} These studies with a low sample size may not represent real correlations between the determined concentration in the animal and the biological parameters.

Based on the species distribution, use of coastal habitats and the bioaccumulation process, we tested the hypothesis that this species will present a high concentration of Hg correlated with life history characteristics (sex, age class, length and weight of the animals). Moreover, this study aims to collaborate to establish reference levels of Hg concentration in liver samples for this species, updating information on Hg concentration levels previously

established more than a decade ago, and thus understand changes in Hg concentration over time if they exist. Soft tissues such as liver, kidney and muscle has been used to monitor exposure to trace-elements in marine tetrapods.⁴⁸ The liver in particular is the tissue which can present a high accumulation of trace-elements.^{49,50} It is expected that these data may contribute to comparative studies on the accumulation of Hg in food web top predators over the years, thus serving as biomonitors to assess the concentration of Hg on the Southwestern Atlantic Ocean.

Experimental

Sample collection

Liver tissue samples from 25 dead individuals found stranded along the Southwestern Atlantic Ocean (see Supplementary Information section) were collected from 2015 to 2020 as part of the Santos Basin Beach Monitoring Project (PMP-BS), required by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), for the environmental licensing of oil and natural gas production and flow activities in the pre-salt, in compliance with the conditions existing in the Authorization of Capture, Collection and Transport of Biological Material (ACCTMB No. 640/2015). This monitoring project covers the coast of Santa Catarina (SC), Paraná (PR), São Paulo (SP) and Rio de Janeiro (RJ) states along the Southwestern Atlantic Ocean (28°28'S 48°46'W to 22°52'S 42°20'W). Biological samples were collected only from freshly dead individuals. The carcasses decomposition stage and sampling of liver tissues was defined and performed in the laboratory, adapted from the procedure described by Geraci and Lounsbury.⁵¹ The biological parameters, location and date of collection were recorded for each individual, and recorded at Aquatic Biota Monitoring Information System (SIMBA),⁵² an on-line database used by all institutions that participate in the PMP-BS. Samples were stored in ultrafreezer (-80 °C) for posterior analyses at the Center for Environmental Studies (UNESP-CEA).

Quality control

Ultrapure water type 1, 18 MΩ cm, Milli-Q system from Millipore (Bedford, Massachusetts, USA), was used in the preparation of all solutions, reagents and samples. All plastic materials (e.g., spatulas, Falcon tubes, digestion flasks) used were previously washed with ultrapure water and dried in a laminar flow hood. All reagents used were of analytical grade. Sob boiling (2 times) nitric (HNO₃) acquired from Synth (Diadema, SP, Brazil) and hydrochloric

(HCl) acquired from Hexis Científica (J.T. Baker, Jundiaí, SP, Brazil) were used. Two biological reference samples (one certified): TORT-3 (lobster hepatopancreas, National Research Council, Canada) and MR (animal tissue-fresh bovine liver with addition of known Hg concentration) were used to evaluate the accuracy of analyzes.

Digestion of biological tissue

The digestion of the samples was carried out in a closed system, using a microwave oven model Ethos UP (Milestone MLS, Sorisole, Italy). Approximately 0.5 g of wet sample (*in natura*), previously homogenized, was added to a digestion flask. In sequence, 2 mL concentrated HNO₃ and 6 mL concentrated HCl (double sub boiling distilled) were transferred to the vial. The flasks were closed for pre-digestion at room temperature overnight. After the pre digestion, the flasks were transferred to the microwave oven and submitted to the heating process with two ramps: ramp 1-the temperature was raised to 170 °C in 20 min, kept at 170 °C for 5 min (power 1600 W); ramp 2-the temperature was increased from 170 to 200 °C in 10 min and maintained at 200 °C for 20 min (power 1600 W).

After digestion (at room temperature) the sample digested extracts were transferred quantitatively to Falcon tubes, the flask was washed with ultrapure water and transferred to the falcon tube until the final volume of approximately 15 mL (concentrated extract). The digestion procedure was also added in bottles without sample (blank solution), to evaluate Hg concentrations from laboratory material.

Determination of Hg

The Environmental Protection Agency (EPA) 7474⁵³ and EPA 245.7⁵⁴ protocols were used as basis for the determination of Hg. The potassium bromide (KBr) and hydroxylamine solution (NH₂OH) used was acquired from Synth (Diadema, SP, Brazil), and the potassium bromate (KBrO₃) was acquired from Êxodo científica (Sumaré, SP, Brazil). A fraction of the concentrated extract was separated into 3 pseudo-replicates to be analyzed in triplicates, transferring the following values to 15 mL Falcon tubes: 0.6 mL of the concentrated extract; 1.2 mL of the solution containing KBr 1.19% m v⁻¹ and KBrO₃ 0.28% m v⁻¹; 0.75 mL of concentrated HCl (double distilled) and ultrapure water to a volume of approximately 15 mL, as described and recommended by EPA protocol 7474 for sample analysis in atomic fluorescence spectrometry. Then, the pseudo-replicates were maintained for reaction (30 min). Subsequently, 0.018 mL of NH₂OH (12% m v⁻¹)

was added to each pseudo-replicate. The NH₂OH solution was used to neutralize excess KBr/KBrO₃ solution. A CV-AFS (PS Analytical model Millennium Merlin, Kent, United Kingdom), was used to determine Hg concentrations.

The analytical curve was obtained from successive dilutions of a Hg certified stock standard solution 1000 mg L⁻¹ SpecSol (Quimlab, Jacareí, SP, Brazil), using 6 standard solutions with concentrations of 0; 0.1; 0.25; 0.5; 1.0 and 2.0 µg kg⁻¹. The same amount of KBr/KBrO₃ solutions, HCl and hydroxylamine added to the samples was added to the standards. After calibration curve, standard solutions, reference materials and blanks were analyzed in triplicate before samples and at every 10 samples, to the validation of the method and assess the accuracy of the analyzes. Finally, the Hg concentration in the samples was expressed in mg kg⁻¹ of sample (wet weight), by means of the equipment result subtracted from the average value of the blanks and multiplied by the dilution factors.

Statistical analysis

Statistical graphs of correlations between element concentration and biological variables were performed using the RStudio software (version 4.1.0).⁵⁵ Distribution and normality statistical tests were performed in the BIOESTAT software,⁵⁶ assuming a significance level of 5%.

Results

Based on the 25 individuals sampled during the present study, three different age groups were established: pups (n = 1), juveniles (n = 21) and adults (n = 2) and one group in which it was not possible to establish the age (n = 1). Regarding sex, the sampled animals were divided into females (n = 11) and males (n = 14) (Table 1).

The limit of detection (LOD) was 0.01 mg kg⁻¹ and the limit of quantification (LOQ) was 0.03 mg kg⁻¹ based on the National Institute of Metrology, Standardization and Industrial Quality.⁵⁷ Here we report the average of the pseudo-replicates, already subtracted from the blanks. Parameters of CV-AFS operation and analyte recoveries of the certified reference material (TORT-3) were reported by Silva *et al.*⁵⁸ The liver concentrations determined for each specimen ranged from 0.09 mg kg⁻¹ (specimen 4) to 15.58 mg kg⁻¹ (specimen 19) with an average Hg concentration of 6.37 mg kg⁻¹ and a standard deviation (SD) data of 4.55 mg kg⁻¹ (Figure 1).

Eight and four fur seals were collected in Laguna and Imbituba municipalities (state of SC), respectively. The highest (15.58 mg kg⁻¹) concentration of Hg was obtained

Table 1. Biological parameters from specimens of South American fur seal (*A. australis*) used in the study, from the Santos Basin Beach Monitoring Project (PMP-BS)

Specimen No.	Sex	Growth stage	Weight / kg	Length / cm	Location (City)	Collection date	Hg / (mg kg ⁻¹ wet weight)
1	M	juvenile	nd	82.0	Laguna-SC	08/05/2019	6.89
2	F	juvenile	9.8	82.3	Laguna-SC	21/08/2018	11.27
3	M	nd	11.4	87.6	Laguna-SC	12/10/2018	11.37
4	M	adult	48.5	149.0	Itajaí-SC	23/08/2017	0.09
5	M	adult	46.0	155.0	Bombinhas-SC	08/06/2017	1.47
6	M	pup	13.9	89.0	Maricá-RJ	06/08/2018	4.92
7	F	juvenile	7.2	nd	Laguna-SC	09/08/2016	2.35
8	F	juvenile	6.4	71.0	Imbituba-SC	11/09/2015	1.09
9	F	juvenile	12.4	91.0	Palhoça-SC	18/08/2017	5.57
10	F	juvenile	8.75	91.9	Florianópolis-SC	03/09/2017	12.53
11	F	juvenile	11.6	83.8	Palhoça-SC	26/07/2018	4.84
12	F	juvenile	8.9	nd	Garopaba-SC	09/09/2018	9.37
13	M	juvenile	7.9	78.5	Imbituba-SC	05/10/2016	0.17
14	M	juvenile	10.0	81.0	Imbituba-SC	25/10/2016	0.60
15	M	juvenile	nd	95.4	Garopaba-SC	29/08/2017	7.68
16	M	juvenile	10.3	92.5	Laguna-SC	21/06/2017	2.32
17	M	juvenile	11.95	89.0	Barra Velha-SC	08/07/2018	5.18
18	M	juvenile	12.1	88.0	Laguna-SC	02/09/2018	6.68
19	M	juvenile	10.3	83.5	Imbituba-SC	07/10/2018	15.58
20	M	juvenile	10.65	87.0	Pontal do Paraná-PR	08/09/2018	10.80
21	M	juvenile	nd	97.0	Florianópolis-SC	02/10/2018	12.85
22	F	juvenile	17.0	93.0	Florianópolis-SC	17/08/2019	9.31
23	F	juvenile	9.2	76.0	Laguna-SC	18/08/2019	0.36
24	F	juvenile	14.5	77.0	Laguna-SC	28/09/2019	8.97
25	F	juvenile	nd	97.0	São Francisco do Sul-SC	15/06/2020	7.03

F: female; M: male; nd: uninformed.

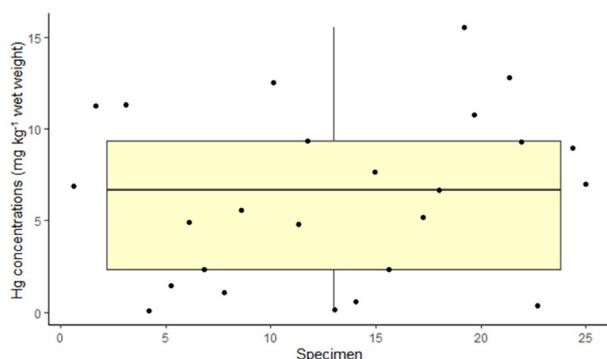


Figure 1. Relationship between liver concentrations of Hg (mg kg⁻¹) in *A. australis* specimens according to each specimen sampled. Q1 = 2.32; median = 6.68; Q3 = 9.37; minimum = 0.09; and maximum = 15.58.

from an individual collected in the municipality of Imbituba (SC state) and the lowest (0.09 mg kg⁻¹) in the municipality of Itajaí (SC state). A specimen was collected in the state of Paraná (municipality of Pontal do Paraná) containing a concentration of 10.8 mg kg⁻¹ and another one in the state

of Rio de Janeiro, in the municipality of Maricá, containing 4.92 mg kg⁻¹ of Hg. Our results are the first to report the concentration of Hg in *A. australis* in the states of SC, PR and RJ. The state of São Paulo was the only state where the PMP-BS occurs and no animals were sampled. Therefore, it was not possible to make a correlation between the concentration of Hg and the location of the specimens by states, since the states of RJ, PR and SP present an $n = 1$ or $n = 0$, limiting the statistical analysis.

In order to see the possible effect of sex on Hg concentrations, the *T*-Student test was applied. The average values obtained for females ($n = 11$) were equal to 6.6 mg kg⁻¹ with 4.1 SD and for males ($n = 14$) equal to 6.2 mg kg⁻¹, with 5.0 SD (Figure 2). Test *T* for independent samples showed that there is no significant effect of sex on Hg concentration ($T(23) = 0.22$; p -value = 0.82).

Results obtained when comparing the Hg concentrations in the different growth stages (Figure 3) showed values of 0.09 and 1.47 mg kg⁻¹ in adult specimens ($n = 2$). In pups

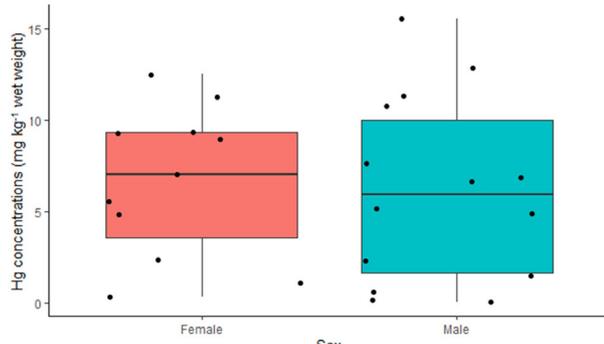


Figure 2. Relationship between liver concentrations of Hg (mg kg^{-1}) in *A. australis* specimens according to gender. Female ($n = 11$), $Q1 = 3.59$; median = 7.03; $Q3 = 9.34$ and male ($n = 14$), $Q1 = 1.68$; median = 5.93; $Q3 = 10.02$.

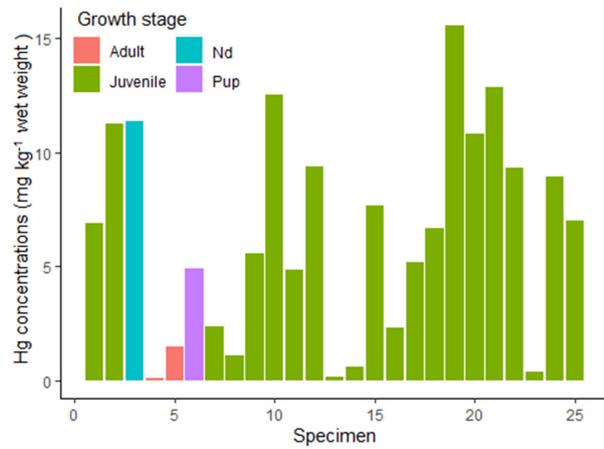


Figure 3. Relationship between liver concentrations of Hg (mg kg^{-1}) in *A. australis* specimens according to growth stage.

($n = 1$) the concentration was 4.92 mg kg^{-1} . The juvenile class was the most representative ($n = 21$), with a Hg average concentration equal to 6.73 mg kg^{-1} , and minimum and maximum values of 0.17 and 15.58 mg kg^{-1} , respectively. The concentration of a specimen with uninformed sex was also determined, with a value equal to 11.37 mg kg^{-1} .

To test the relationship between the Hg concentration and the total length of the specimens, it was necessary to exclude specimens 7 and 12, for which the length values were not obtained. This analysis was performed through the correlation of Spearman's coefficient. The values obtained for $n = 23$ of the tests were: Spearman's coefficient (rs) = 0.14; $t = 0.69$; (p) = 0.49. There was no correlation between the two variables. Specimen 5, with 155 cm of total length, had a Hg concentration equal to 1.47 mg kg^{-1} . While specimen 8 with a total length of 71 cm, Hg concentration equal to 1.09 mg kg^{-1} (Figure 4).

The correlation of Hg concentration in relation to weight was also performed using Spearman's coefficient. Similar to the total length, the weight of the specimens did not show

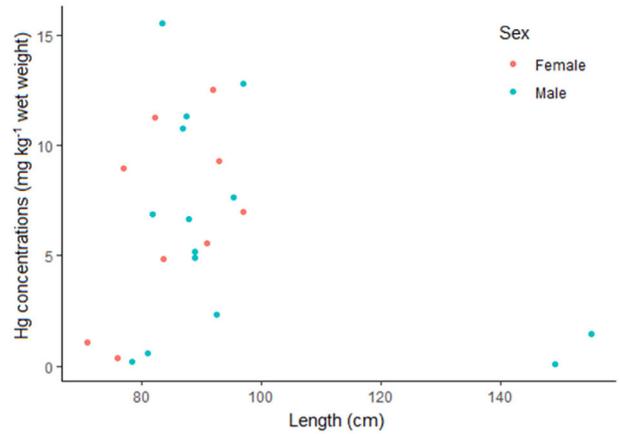


Figure 4. Mercury concentration (mg kg^{-1}) in relation to total length (cm) in liver of specimens *A. australis*.

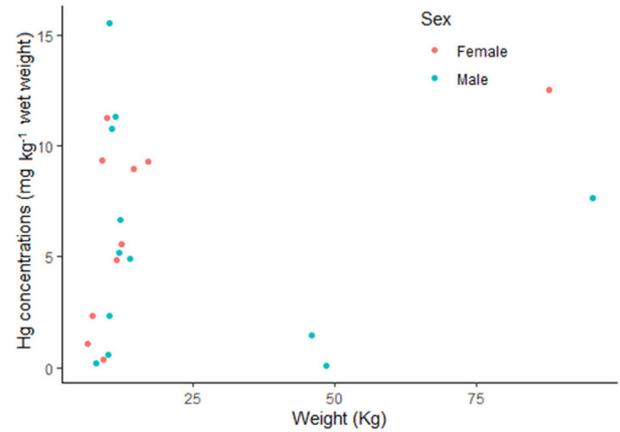


Figure 5. Mercury concentration (mg kg^{-1}) with respect to the weight (kg) in liver specimens *A. australis*.

any significant correlation with the Hg concentration. The test showed Spearman's coefficient (rs) = 0.22, $t = 1.0$, (p) = 0.32 with a sample value of $n = 22$ (Figure 5).

Discussion

Approximately 92% of the stranded animals were found in the state of Santa Catarina (Figure 3), which corroborates southern Brazil states as the area with the largest records of pinnipeds in the country.⁵⁹ Although *A. australis* occurs in the southern and southeastern Brazilian states, there are no breeding colonies of the species in the Brazilian coast. Probably, individuals sampled for this study came from the breeding colonies in Uruguay.^{59,60}

Interestingly, a great variability in Hg concentrations was observed in the samples. This irregularity possibly occurred because Hg is a non-essential element.⁶¹ Several authors^{42,45,62,63} report that the diet of *A. australis* is composed mainly of teleost fish, cephalopods and crustaceans, and although the diet may vary over the years, stable isotope analyzes have suggested that there are no variation in the

isotopic niches.^{42,63,64} The previous statement does not mean that the availability of prey does not change over the time,⁶³ because part of the population or some individuals use other areas to forage, such as the continental shelf break, where individuals of the species have been sighted.⁶⁵ Contamination by MeHg in humans and animals, occurs mainly through the ingestion of contaminated fish.⁶⁶ Thus, this variation in the determined Hg concentrations may be due to differences in eating habits between specimens and/or due to exposure in different environments.^{26,61}

A. australis is a species with a foraging strategy known as "Central Place Foraging" where females make foraging trips interspersed with breastfeeding. This strategy would result in differences in behavior patterns of foraging and trophic segregation between males and females as has been seen in this species, as well as in other species with sexual dimorphism.^{42,67-69} It has also been found that the location of the breeding colonies would be associated with the accessibility to the continental shelf break.⁷⁰ These differences in the resources acquisition lead to differences between sexes in the isotopic niche of the species. Having this difference in the isotopic niche, one would expect a difference in Hg concentrations as found by other authors (e.g., Gerpe *et al.*⁷¹). These authors reported a difference in Hg concentration with mean concentrations for Hg (wet weight) in females equal to 39.90 mg kg⁻¹ and in males 25.00 mg kg⁻¹, justifying that this lower concentration of Hg in males is due to a behavior factor that causes a change in their diet, thus decreasing the proportion of fish in their diet and increasing the consumption of squid, providing a lower intake of Hg contaminated fish.

According to Naya *et al.*,⁴⁵ the females of *A. australis* in their lactation period are restricted to forage in close range areas, due to the need to frequently return to feed the pup, causing a reduction in the diversity prey selectivity during the lactation period, and after this period it is possible to observe an increase in the diversity of its diet. Therefore, the higher concentration of Hg in females reported by Gerpe *et al.*,⁷¹ may be due to a lower diversity of prey in their diet. Differences with the studies mentioned above may occur mainly because, in the present study, the samples came mostly from juvenile individuals, that is, most of the individuals do not have complete sexual maturation, not presenting major changes in hormonal activities and consequent behavioral changes, which could alter the uptake and distribution of Hg in the tissues.⁷² Besides that, juvenile individuals probably still do not have behavioral differences and thus have a similar diet between the sexes. Although, the correlation between Hg concentration and sex does not occur statistically, the average Hg found in females was slightly higher than that

presented in males, which could be partially explained by the beginning in the alteration of the diet in males. Thus, as reported by Robinson *et al.*,⁷³ for other species (birds), there is no standard in species with or without sexual dimorphism to establish differences in concentration between sexes.

It was expected to find a higher concentration of Hg in older individuals, due to the processes of bioaccumulation and biomagnification that occur along the food chain. Gerpe *et al.*⁷¹ and Marcovecchio *et al.*⁷⁴ demonstrated in their studies in specimens of *A. australis* that the Hg concentrations increase linearly with the growth stage of the individuals, concluding in the studies, a dependence on the Hg content with the age of the organisms. Though, in our study, no increase in Hg concentration related to the stage of development was found, since the adult specimens sampled had lower Hg concentrations in relation to the juvenile and pup classes. This fact is probably due to the greater area of foraging explored by the specimens that are at the climax of development, allowing different habitats and thus a greater diversity of prey in their diet.

It is known that the initial Hg concentration in pups of *A. australis* may come from maternal transfer (placenta and/or milk), however, fish consumption is the main contributor to the accumulation of Hg in *A. australis*.⁷⁵ Different studies^{75,76} have found that pups of several species of pinnipeds in the lactation period have low concentrations of Hg, notwithstanding after weaning they fully depend on solid preys (fish), leading to high Hg concentrations.

Considering that the animal's physiological status, age and growth rate can influence Hg concentrations,^{77,78} it was expected to find some relationship between size and weight with Hg concentration. Our results considering the size of the animal, did not find any correlation between this variable and the Hg concentration. As with the total length, the weight of the specimens did not show any significant correlation with the Hg concentration. Thus, it appears that the Hg concentration does not depend on the biological parameters analyzed in the present study, such as sex, total length and weight, as found for other several species.⁷⁹

The data obtained in the present study were compared with other studies of Hg concentration in *A. australis* and classified by stage of development and locality (Table 2). The values determined by Marcovecchio *et al.*⁷⁴ for the general mean of Hg are close to the values obtained in the present study. Baraj *et al.*,⁴⁶ Fossi *et al.*⁴⁷ and Gerpe *et al.*⁷¹ present values for average higher than the ones presented in this study. In the past few decades, the growing number of new chemical industries has resulted in an increase in the amount of potential contaminants (Hg) in coastal regions.⁸⁰ Despite the aforementioned, there has been no increase in

Table 2. Comparison of mercury concentration in liver of *A. australis* from different studies)

Specimens	Hg mean / (mg kg ⁻¹ wet weight)	Hg range / (mg kg ⁻¹ wet weight)	Growth stage	Collection date	Location (Country)	Reference
n = 12	0.49	0.38-0.6	suckling pups	1988	Uruguay	Gerpe <i>et al.</i> ⁷⁵
n = 11	3.91	2.08-6.21	weaned pups	1989	Argentina	Gerpe <i>et al.</i> ⁷⁵
n = 1	4.92		pup	2018	Brazil	this study
n = 8	10.13	0.32-54	juvenile	1999	Brazil	Baraj <i>et al.</i> ⁴⁶
n = 16	6.7	0.17-15.6	juvenile	2015 to 2018	Brazil	this study
n = 2	0.78	0.09-1.47	adult	2017	Brazil	this study
n = 8	33.7	20.4-57.5	adult	–	Uruguay	Gerpe <i>et al.</i> ⁷¹
n = 3	57.2 ^a	1.6 -125	–	–	Brazil	Fossi <i>et al.</i> ⁴⁷
n = 1	73.91 ^a		–	–	Argentina	Fossi <i>et al.</i> ⁴⁷
n = 16	6.89	1.19-35.1	–	–	Argentina	Marcovecchio <i>et al.</i> ⁷⁴

^aDry weight values.

the concentration of the Hg in this species over the years (1997-2020).

After comparing all studies with data referring to the concentration of Hg in *A. australis*, it was possible to observe a tendency of increase in the concentration of Hg between the stages of development, in the following order: breastfed pups < weaning pups < juveniles < adults, probably because of the biomagnification process. However, in our study, adult specimens showed concentrations lower than other development classes. This probably occurred due to the greater diversity in the diet of the sampled specimens, and/or due to metabolic mechanisms that act to minimize the impacts of Hg on the animal.⁷⁹ The complex biogeochemical cycle and the different routes of exposure, present challenges to characterize and manage threats of adverse effects to public and ecosystem health.⁸¹ It is known that marine mammals have some defense mechanisms that can protect against the negative effects of Hg.³¹ Some studies⁸²⁻⁸⁷ report that through the antagonistic effect, selenium can bind to inorganic Hg, resulting in insoluble Hg-Se complexes.

Conclusions

The high potential of biomagnification and high mobility of Hg in the marine ecosystem underscore the importance of studies to survey and monitor these concentrations in biological tissues. Marine mammals have great longevity and a high position at the trophic level of the marine food web, and this makes these animals good indicators of the accumulation of Hg at upper trophic levels. The present work brings new information on Hg concentrations in liver tissue of South American fur seals from the Southwestern Atlantic Ocean. The variation in Hg concentrations in *A. australis* specimens probably

occur because of regional differences in the bioavailability of Hg in the environment, and/or because of differences in the feeding habits of the individuals. Therefore, the presence of Hg in marine mammals may reflect the bioaccumulation in prey and this bioaccumulation is influenced by the level of bioavailability of the element in the environment, coming from a natural or anthropogenic source. Our results do not indicate an increase in the concentration of Hg in *A. australis* from the Southwestern Atlantic Ocean since the 1990s to the present. These results are very important considering that the Hg load in the marine environment has tripled since the pre-industrial time,²⁸ which makes it necessary to monitor the variation of Hg levels in top food web species over time, considering these as biomonitors. The results presented are of great importance to support long term monitoring of this and other species of marine mammals found stranded on the Southwestern Atlantic Ocean.

Supplementary Information

Supplementary data (geographical coordinates of the samplings) are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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Author Contributions

Guilherme Lima was responsible for conceptualization, data curation, formal analysis, investigation, methodology, visualization, roles/writing-original draft, writing-review and editing; Amauri Menegário for conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, roles/writing-original draft, writing-review; Everton Sulato for conceptualization, investigation, methodology, validation, visualization, roles/writing-original draft; Jorge Pedrobom for conceptualization, data curation, investigation, methodology, validation; Juan Torres-Florez for investigation, visualization, roles/writing-original draft; André Barreto for funding acquisition, project administration, resources, supervision; Marcus de Araújo Júnior for methodology, project administration, supervision, validation.

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