Water Air Soil Pollut (2017) 228:188
DOI 10.1007/s11270-017-3367-8

Influence of Delipidation on Hg Analyses in Biological Tissues: A Case Study for an Antarctic Ecosystem

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Received: 26 September 2016 / Accepted: 5 April 2017

Abstract The use of low-polarity organic solvents is widespread in cleanup/extraction processes in order to carry compounds of interest, remove interferences and separate phases, among other uses. A large number of studies have used delipidation to remove excess of lipids to analyse carbon stable isotopes in biological tissues for trophic and behavioural ecology investigations. In this context, the primary aim of this study is to assess the influence of one delipidation process on the results of total mercury (Hg) analyses and the possible use of delipidated samples from previous analyses, such as for stable isotopes, in Hg level determination. Samples of vegetation (angiosperm, lichens and mosses), invertebrates (krill and limpets), fish (marbled and black rockcod), bird liver and eggs (Antarctic, Gentoo and Adélie penguins, kelp gull, Antarctic tern, cape petrel and giant southern petrel) and pinniped tissues (Weddell seal, crabeater seal, southern elephant seal and Antarctic fur seal) were analysed for Hg before and after delipidation by cyclohexane. The difference between the two measurements ranged individually from −63 to 136% (in the moss *Sanionia uncinata*) and the averages ranged from −60 to 66% (in pinniped tissues). The proportion of organic Hg, which presents considerable lipophilicity, but also high affinity for sulfhydryl groups in proteins, might be responsible for such variability. Given the limitations of our study, we think it is safe to say that delipidated samples could not be used to infer total Hg values in non-delipidated ones.

Keywords Delipidation · Mercury · Antarctica · Food web

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1 Introduction

Delipidation processes are nearly omnipresent in methods for contaminants and stable isotope analyses. The use of low-polarity organic solvents for these purposes is widespread for directly carrying lipophilic compounds of interest, as for most persistent organic pollutants (POPs) (e.g. Macleod et al. 1986), or in order to separate fractions, as in organic mercury (Me-Hg) determination (e.g. Bustamante et al. 2006). As for stable isotope analyses (SIA), it is important to remove reserve lipids for $\delta^{13}C$ determination due to the fact that lipids are typically more depleted in ^{13}C than other tissue components (DeNiro and Epstein 1978). Lipid content in organisms could result in differences in $\delta^{13}C$ of around 6–8‰ (DeNiro and Epstein 1977; McConnaughey and McRoy 1979) and thus might represent an important bias in interpretation of ecological data as well. Moreover, the use of delipidation on SIA is still a matter of debate due to a possible interference on

Published online: 21 April 2017

other isotopic ratios (such as $\delta^{15}N$). In some other cases, arithmetical corrections could be employed as means to save time and resources (e.g. Kojadinovic et al. 2008; Post et al. 2007; Sweeting et al. 2006). However, the vast majority of ecological studies that used $\delta^{13}C$ and $\delta^{15}N$ are based on delipidated samples.

Hg as a global pollutant is of growing concern because despite emission regulations since the 1970s, mercury levels in the oceans have not yet reached steady state in respect to current levels of deposition (Selin 2009), leading to an increasing Hg ocean inventory, especially in surface water (Lamborg et al. 2014). After having being methylated by microorganisms, Hg enters biotic compartments and biomagnifies along the food chains. In order to go deeper in the understanding of Hg contamination at the food web level, the use of samples that have been collected for ecological studies is thus of high relevance. Furthermore, retrospective studies using already collected and preserved samples are of broad interest to determine the spatial and temporal trends of Hg. Researchers have at their disposal a huge number of samples, but these samples have often been delipidated for $\delta^{13}C$ analyses or have undergone some similar treatment. The effect of delipidation is, however, not known on Hg analyses.

In this general context, the first aim of this study was to investigate the effect of delipidation procedure on total Hg analyses in a large range of biological matrixes. To this end, we used samples from Antarctica representing different trophic levels and also very different lipid contents (Cipro et al. 2011, 2013). Consequently, it would be of the utmost interest to know whether laboratory procedures and protocols might be simplified or unified for studies that require two or more analyses that will differ in regard to the need of delipidation steps.

Secondarily, hypotheses are raised concerning ecological factors that might influence the observed differences between delipidated and non-delipidated samples.

2 Materials and Methods

2.1 Origin of the Samples

Samples were collected in King George Island (62°05′S, 058°23′W), the largest one of the South Shetland Islands, separated from the northern portion of the Antarctic Peninsula by the Bransfield Strait. The number of samples of each species, tissue and analysis is shown in Table 1. All samples were kept in previously combusted (at least 420 °C for at least 4 h) containers, frozen upon arrival (−20 °C) at the Brazilian Antarctic Station and kept frozen until freeze drying in the LabQOM (University of São Paulo, Brazil). SIA and Hg analyses were performed at the University of La Rochelle (France). A brief description follows: bird eggs and tissues, fish and invertebrate samples were collected according to Cipro et al. (2010, 2013): limpets (Antarctic limpet, *Nacella concinna*) were manually collected (2004–2005 summer) in the intertidal zone. Fish (marbled rockcod, *Notothenia rossii*, and black rockcod, *Notothenia coriiceps*) were collected (2006–2007 summer) by mid-water nets or by line and hook. Only unhatched bird eggs (Antarctic penguin, *Pygoscelis antarctica*; Adélie penguin, *Pygoscelis adeliae*; Gentoo penguin, *Pygoscelis papua*; skua, *Catharacta* sp.; kelp gull, *Larus dominicanus*; and Antarctic tern, *Sterna vittata*) were collected (2004–2005 and 2005–2006 summers) so as not to interfere with breeding success. Bird livers (cape petrel, *Daption capense*, and giant petrel, *Macronectes giganteus*, in addition to the previous species) were collected (from 2004 to 2005 up to 2007–2008 summers) only from already dead animals with no evident signs of disease, decomposition or emaciation. No further attempt to determine the cause of death was performed.

Pinniped samples were collected according to Cipro et al. (2012), which already contains the SIA data: samples of the Weddel seal, *Leptonychotes weddellii*; Antarctic fur seal, *Arctocephalus gazella*; crabeater seal, *Lobodon carcinophagus*; and southern elephant seal, *Mirounga leonina*, were collected during the austral summers of 2004/2005 and 2005/2006, in a fully opportunistic manner, i.e. only from animals found already dead, with no signs of degradation.

Lastly, vegetation samples were collected according to Cipro et al. (2011), which contains also the SIA data: the angiosperm *Colobanthus quitensis*, the mosses *Brachytecium* sp., *Syntrichia princeps* and *Sanionia uncinata*, and the lichens *Usnea aurantiaco-atra* and *Usnea antarctica* were collected from early December 2004 to early January 2005.

2.2 Analyses

Both the results of Hg for the non-delipidated samples and SIA are taken from Cipro et al. (2017). The

Table 1 Hg (mean ± SD, μg g^{-1} dw) in raw and in delipidated Antarctic samples according to species and tissue, followed by Dif (the percentage difference between the two values)

	Species	Tissue	*n*	% lipids	Hg_{raw}	SD	*n*	Hg_{delip}	SD	Dif	SD
Vegetation	*Colobanthus quitensis*	Whole	1	0.38	0.014	–	–	–	–	–	–
	Brachytecium sp.	Whole	1	0.57	0.086	–	1	0.078	–	−0.100	–
	Sanionia uncinata	Whole	7	0.11	0.068	0.026	7	0.066	0.025	0.083	0.615
	Syntrichia princeps	Whole	2	0.15	0.057	0.030	2	0.056	0.016	0.058	0.277
	Unidentified moss	Whole	1	0.49	0.100	–	1	0.072	–	−0.279	–
	Usnea antarctica	Whole	3	1.61	0.152	0.032	3	0.130	0.006	−0.120	0.144
	U. aurantiaco-atra	Whole	5	1.16	0.132	0.037	5	0.110	0.026	−0.147	0.180
	Usnea sp.	Whole	1	1.10	0.210	–	1	0.203	–	−0.035	–
	Unidentified lichen	Whole	2	2.87	0.227	0.115	2	0.182	0.054	0.015	0.192
Invertebrates	*Euphausia superba*	Whole	4	9.2	0.018	0.005	2	0.019	0.005	−0.051	0.040
	Nacella concinna	Soft part	8	8.2	0.037	0.015	6	0.038	0.016	0.054	0.105
Fish	*Notothenia coriiceps*	Muscle	1	–	0.093	–	1	0.104	–	0.113	–
	N. rossii	Muscle	28	0.70	0.077	0.027	24	0.077	0.024	0.005	0.101
Birds	*Catharacta* sp.	Egg	4	35.7	2.520	1.709	3	3.259	2.212	0.317	0.107
		Liver	3	23.7	5.136	5.701	2	5.620	6.029	0.153	0.106
	Daption capense	Liver	2	21.3	6.965	–	1	7.170	–	0.029	–
	Larus dominicanus	Egg	3	37.9	0.195	0.067	3	0.281	0.117	0.429	0.174
		Liver	3	23.9	11.017	16.816	3	13.992	21.676	0.331	0.249
	Macronectes giganteus	Liver	3	22.4	25.465	31.628	1	28.711	35.976	0.132	–
	Pygoscelis adeliae	Egg	3	35.0	0.075	0.049	2	0.452	0.581	0.591	0.057
		Liver	5	22.4	1.742	3.527	5	1.817	3.664	0.120	0.119
	P. antarctica	Egg	26	34.3	0.599	0.273	12	0.807	0.360	0.390	0.095
		Liver	16	23.3	1.906	1.265	11	1.916	1.296	0.044	0.067
	P. papua	Egg	9	33.7	0.133	0.037	5	0.192	0.060	0.429	0.074
		Liver	16	20.9	0.369	0.275	12	0.347	0.174	0.175	0.182
	Sterna vittata	Egg	1	41.0	0.664	–	1	0.829	–	0.249	–
Pinnipeds	*Arctocephalus gazella*	Liver	1	–	48.712	–		–	–	–	–
		Muscle	2	–	0.091	0.049		–	–	–	–
		Skin	3	–	0.028	–		–	–	–	–
	Leptonychotes weddellii	Kidney	1	–	0.075	–	1	0.072	–	−0.040	–
		Liver	1	–	0.286	–	1	0.266	–	−0.069	–
		Muscle	2	–	0.112	0.005	2	0.099	0.011	−0.121	0.056
		Skin	2	–	0.260	–	1	0.431	–	0.659	–
	Lobodon carcinophagus	Muscle	1	–	0.124	–	1	0.157	–	0.271	–
		Skin	2	–	0.191	–	1	0.076	–	−0.604	–
	Mirounga leonina	Liver	2	85.0%	25.683	–	–	–	–	–	–
		Muscle	1	–	0.613	–	–	–	–	–	–

percentage of lipids was performed during POPs analyses previously published as well (Cipro et al. 2013). However, values might differ due to sample availability for pairing the datasets. In this study, we performed Hg

analyses for the samples that had undergone delipidation steps for SIA. Briefly, frozen samples from the field were lyophilised and then ground to obtain a fine powder. One aliquot of approximately 100 mg of sample was placed in a test tube with 4 mL of cyclohexane to remove lipids, according to Chouvelon et al. (2011). The mixture was shaken for an hour, then centrifuged 10 min for separation (as many times as needed, until the liquid phase, which is discarded, comes out clear) and dried at 50 °C for 48 h. Total Hg analysis was carried out with an automatic mercury analyser spectrophotometer, ALTEC AMA 254, which does not require an acid digestion of the samples. Aliquots ranging from 10 to 50 mg were directly analysed after being inserted in the oven of the apparatus. After drying, the samples were heated under an oxygen atmosphere for 3 min, being then the Hg liberated and subsequently amalgamated in a golden (Au) net, which was then heated to liberate the collected Hg, which was measured by atomic absorption spectrophotometry.

Accuracy and reproducibility of the methods were tested using dogfish liver (DOLT-2) and muscle (DORM-2) and lobster hepatopancreas (TORT-2) (National Research Council, Canada) reference standards. Standard and blanks were analysed every ten analyses, and recoveries of the certified values and recoveries of the metal ranged from 89 to 108%.

Concentrations are expressed in dry weight in order to compensate eventual moisture loss during freezing and to facilitate comparison between tissues and with other studies. Blanks were analysed at the beginning of each set of samples, and the detection limit of the method was 0.005 μg g^{-1} dry mass.

2.3 Statistics

Tests were performed using Microsoft Excel and Statsoft Statistica 12. Normality of distribution and homogeneity of variances were checked using Shapiro–Wilk and Brown–Forsythe tests, respectively. Parametrical (Pearson's product-moment correlation, Tukey's HSD/ANOVA) and non-parametrical tests (Spearman's rank correlation, Kruskal–Wallis/ANOVA) followed accordingly. Statistically significant results were set at $\alpha = 0.05$ and only significant correlations are cited.

The Wilcoxon–Mann–Whitney test was chosen in order to compare whether the same group of samples differed significantly in regard to Hg analyses in delipidated and non-delipidated pairs. This difference between the Hg concentration in raw samples and in delipidated ones comprised, by means of a simple relative error function calculation, a variable hereon named 'Dif' [i.e. Dif is calculated by $(Hg_{delip} - Hg_{raw})/Hg_{raw}$ or its equivalent $(Hg_{delip}/Hg_{raw}) - 1$]. Since this variable is individually calculated, the average 'Dif' in each group does not represent the difference between the average results for delipidated and non-delipidated samples within this same group. In other words, the difference between averages is not necessarily the average Dif. Moreover, readers must be aware that apparent differences may arise simply due to the fact that Dif can only be calculated for paired results (i.e. the same sample before and after delipidation), which is not always the case in the whole dataset.

Finally, normal distribution and power-link function (since the dependent variable presents negative values) generalised linear models were built as follows: Dif as the dependent variable, species and tissue as categorical factors and Hg concentrations, percentage of lipids, and $\delta^{13}C$ and $\delta^{15}N$ values as continuous predictors. Biologically relevant models were constructed incorporating the different variables and their interactions. Variables that were significantly correlated in the whole dataset were not included in the same models. Model selection was based on Akaike's Information Criteria adjusted for small sample sizes (AICc). The model with the lowest AICc value was considered to be the most accurate. Models with AICc values differing by less than 2 have a similar level of support in the data, and the model including the least number of parameters can be regarded as the most accurate, according to the principle of parsimony (Burnham and Anderson 2002). Overall model support was assessed using Akaike weights (w_i), following Johnson and Omland (2004). Residual (R^2 adj) analyses should be restricted to description and not be used in model selection (Burnham and Anderson 2002).

3 Results

Main results are displayed in Table 1. Due to limitations on the available mass of the samples, it is noticeable that in some cases the number of delipidated samples is lower than for the raw ones. Nevertheless, the calculation of Dif and all of the statistical tests were only performed when pairing was possible, considering the proper *n* for significance purposes.

Results for Dif in the whole dataset ranged individually from −0.633 in a *S. uncinata* sample to 1.36 in a sample of the same species. Averages, in turn, ranged

from −0.604 in the skin of *L. carcinophagus* to 0.659 as well as in the skin of *L. weddellii*.

Bird samples presented, on average, only positive Dif values, with egg results always overcoming liver ones. For vegetation, Dif results were mostly negative and by far the ones with the highest standard deviations. For the pinnipeds, a similar trend of predominantly negative values can be seen; however, due to the reduced sampling numbers, barely no results were obtained for deviations. Individual fish samples Dif ranged from −0.223 to 0.308 and correlated (Spearman's ρ) to both $\delta^{13}C$ ($\rho = 0.504$) and %lip ($\rho = 0.493$), whereas for *N. concinna*, Dif ranged from −0.066 to 0.323 and correlated negatively to $\delta^{15}N$ ($\rho = -0.885$).

When species and tissues are considered, Wilcoxon–Mann–Whitney tests presented significant differences for Dif in *P. adeliae* liver ($p = 0.043$), *P. antarctica* egg ($p = 0.002$) and for *P. papua* both egg ($p = 0.043$) and liver ($p = 0.003$). When only tissue is considered, significant differences are for Dif obtained for liver ($p < 0.001$), egg ($p < 0.001$) and whole organism (plants only, $p = 0.009$).

Moreover, Dif presented significant correlations with $\delta^{13}C$ ($\rho = -0.379$, $p < 0.001$), Hg_{raw} ($\rho = 0.291$, $p = 0.003$), Hg_{delip} ($\rho = 0.357$, $p < 0.001$) and percentage of lipids ($\rho = 0.501$, $p = 0.005$).

Multivariate analyses for Akaike Information Criteria (Table 2) display two models ($\delta^{15}N$ + tissue and $\delta^{13}C$ + tissue) closely supporting the data for Dif, with roughly twice as much weight as the next two (tissue and $\delta^{15}N$ + %lip + tissue).

4 Discussion

It is important to begin the data discussion and interpretation by stating that even though literature mentions that Hg has a lower K_{ow} when compared to organic contaminants (e.g. Bienvenue et al. 1984), this low to moderate lipophilicity combined with affinity for sulfhydryl groups in proteins enhances the retention of Me-Hg in biological tissues (Saouter et al. 1993). Therefore, the present results can be influenced according to the lipidic and proteic composition and content of the matrixes.

Positive Dif values may be attributed to the fact that some lipid mass is lost during the delipidation procedure. So, it is reasonable to assume that most of the previous Hg was present in the non-lipidic phase of the sample and consequently it is concentrated in a smaller mass. The contrary is true regarding negative values: it is reasonable to assume that, in this case, a fraction of total Hg was present in the lipidic phase and was subsequently lost. In other words, Dif values are to be positive when a sample loses proportionally more mass than Hg during delipidation, whereas Dif values should be negative when it loses proportionally more Hg than mass.

Data for vegetation presented the highest individual variation. This might be due to the fact that the most important Hg source in this environment is atmospheric deposition (influenced also by marine aerosols, seabird guano and volcanic emissions) and, besides that, surface evapotranspiration (Bargagli et al. 1998a, b). This surface-deposited Hg (i.e. the adsorbed fraction) is therefore more likely to be washed away during delipidation and so constitute another source of variation for data in this group. This hypothesis is supported by the significant difference in the concentrations of raw and delipidated samples by means of a Wilcoxon–Mann–Whitney test ($p = 0.009$) for this group. For herbivorous invertebrates, the difference between the data from krill and limpets might be considered at the light of their main ecological feature: whereas the krill *Euphausia superba* is a pelagic organism feeding on phytoplankton, the gastropod *N. concinna* is a benthic grazer relying on microphytobenthos. Literature reports, in a general way, higher Hg concentrations in benthic organisms than in pelagic ones and consequently in its respective predators (e.g. Bargagli et al. 1998a; Carravieri et al. 2014). Furthermore, benthic organisms also contain a higher proportion of organic Hg as a consequence of Hg methylation by microorganisms in sediments (Campbell et al. 2005; Heimbürger et al. 2010; Kannan et al. 1998). This comes in agreement with the significant correlation for *N. concinna* of $\delta^{13}C$ with both Hg in raw and delipidated samples since this species presented roughly twice the Hg concentrations compared to *E. superba* and the opposite value for Dif. This means that these organisms tend to accumulate Hg in different ways, i.e. in *E. superba* Hg would be more likely associated to the lipidic fraction that is lost during delipidation whereas in *N. concinna* soft tissues, mostly comprising its muscular foot, Hg would more likely be associated to the non-lipidic portion of the organism, i.e. proteins. This assumption is supported by the fact that limpets present elevated concentrations of metal-binding proteins such as carbonic anhydrase, whose synthesis can be induced by inorganic contaminants (e.g. Piechnik et al. 2016), and more importantly, metallothioneins, that have an important role in

Table 2 Summary of model selection according to AICc for Dif results

Whole dataset				AICc	ΔAICc	w_i
$\delta^{15}N$	Tissue			−26.61	0.00	0.32
$\delta^{13}C$	Tissue			−26.43	0.19	0.29
tissue				−25.22	1.39	0.16
$\delta^{15}N$	%lip	Tissue		−24.67	1.94	0.12
%lip	Tissue			−23.30	3.32	0.06
Hg raw	Tissue			−23.11	3.50	0.06
Animals				**AICc**	**ΔAICc**	w_i
$\delta^{15}N$	Tissue			20.93	0.00	0.59
$\delta^{13}C$	$\delta^{15}N$	Tissue		22.84	1.91	0.23
$\delta^{15}N$	Species			25.27	4.33	0.07
$\delta^{15}N$	Species	Tissue		25.35	4.41	0.06
$\delta^{13}C$	$\delta^{15}N$	Species		27.07	6.14	0.03
$\delta^{13}C$	$\delta^{15}N$	Species	Tissue	27.18	6.25	0.03
Plants				**AICc**	**ΔAICc**	w_i
$\delta^{13}C$	Species			−72.82	0.00	0.68
$\delta^{13}C$	$\delta^{15}N$	Species		−71.35	1.46	0.32
Species				−53.26	19.56	0.00
$\delta^{15}N$	Species			−51.19	21.62	0.00
$\delta^{13}C$				−29.53	43.29	0.00
$\delta^{15}N$				−28.60	44.22	0.00
$\delta^{13}C$	$\delta^{15}N$			−27.99	44.83	0.00

Only valid models with ΔAICc inferior to 10 are considered. w_i stands for Akaike weights for the whole dataset and separated by animals and plants as well

detoxification processes (Amiard et al. 2006). Notwithstanding, the proportion of organic Hg in these different tissues of different species is likely to play a major role, as previously and further discussed. In this case, there is yet another factor: since the life cycle of this limpet is longer than krill's (Picken 1980; Tarling et al. 2016), it means that bioaccumulation also plays a role. Yet, this data also supports the results from AICc, bringing $\delta^{15}N$/tissue and $\delta^{13}C$/tissue as the most adequate models to infer the difference in the results: different tissues were analysed and data from both isotopes (4.51 ± 0.53 vs. 7.27 ± 1.23 for $\delta^{15}N$ and −25.66 ± 0.69 vs. −16.10 ± 1.74‰ for $\delta^{13}C$ for *E. superba* and *N. concinna*, respectively) are markedly different, highlighting the ecological differences previously discussed.

Fish presented on average almost no difference between raw and delipidated samples. However, when individual values are taken, the positive correlation of Dif with $\delta^{13}C$ suggests a similar mechanism to the one previously discussed for *N. concinna*, i.e. larger association of Hg to proteins. Besides that, lipid content in fish muscular tissue was very low, possibly reducing an eventual influence of delipidation. However, the significant correlation of Dif with %lip added to the fact that more than 95% of the total Hg in fish muscle is under the organic form (e.g. Bloom 1992; Brasso et al. 2014 for *Pleuragramma antarctica*) both highlight the likelihood of this variable having a more prominent role than previously thought of. In addition to that, Hg is associated to muscle proteins (Bloom 1992) and more specifically to the sulfhydryl groups of these proteins (Saouter et al. 1993). So there are these apparently antagonistic (in regard to loss during delipidation) factors at play: on one side, the lipophilicity; on the other, affinity for protein groups. The percentage of Me-Hg likely plays a role in this balance.

Seabirds were the group with the largest number of samples, which allowed not only the assessment of intraspecific significant intra-tissue differences (Wilcoxon–Mann–Whitney) but also the inter-

tissue differences. The fact that egg samples presented higher Dif than liver ones in all of the cases when comparison was possible suggests that in liver, Hg is proportionally less present in the lipidic phase and also less bound to proteins compared to the eggs. Moreover, Hg in eggs is almost exclusively organic (e.g. Bond and Diamond 2009; Braune 2007; Scheuhammer et al. 2001), whereas biomineralisation and storage of non-toxic Se-Hg complex might take place in the liver of seabirds as a powerful detoxification mechanism (Nigro and Leonzio 1996). To the best of our knowledge, no literature on Se-Hg complex K_{ow}, polarity or solubility could be found in order to clarify this issue. Nevertheless, the present results come in agreement with the previous discussion about the likely influence of the importance of the organic/inorganic forms of Hg stored in the tissues.

Pinnipeds, whose Dif averages presented the largest range within the dataset, presented values that also come in agreement with the previously stated hypothesis. The crabeater seal (*L. carcinophagus*), which feeds almost exclusively on euphausiids (Jefferson et al. 2008), presented the lowest Dif in skin samples, whereas the highest value was found in Weddell seal (*L. weddellii*), a species relying on benthic and mesopelagic prey (Jefferson et al. 2008), in a way much similar to the previous discussion about invertebrates. Curiously, muscle samples presented an opposite trend in both species (negative when Dif is positive and vice versa). This could be due to indirect effects from seasonal diet change since these tissues have different turnover rates (Lesage et al. 2002) or yet, due to fasting during wintering periods (Cipro et al. 2012).

The presence of the tissue factor in all of the valid models according to AICc is also another indication of the importance of the organic/inorganic distribution of Hg since this variable is highly tissue dependent due to lipid and protein contents and, moreover, to the sulfhydryl group presence in the latter. Finally, Fig. 1 shows that, in a general way, the use of delipidated samples would not to be recommended. Although there is some overlapping between delipidated and non-delipidated samples, relative standard deviations are in most cases higher than the deviations accepted in certified/reference materials (e.g. Wade and Cantillo 1994). Moreover and likely more important as well is the result of correlation analyses between the Hg values for raw (y) and delipidated samples (x). Nevertheless, notable exceptions occurred. Some significant Spearman correlation appear (*N. rossii* muscle, $\rho = 0.97$; *P. adeliae* liver, $\rho = 0.90$; *P. antarctica* egg, $\rho = 0.98$, liver $\rho = 0.99$; and *P. papua* egg, $\rho = 0.90$, liver $\rho = 0.98$). This result is repeated regarding Pearson correlations and linear regression equations for *N. rossii* muscle ($r = 0.97$; $y = 0.859x + 0.01$), *P. adeliae* liver ($r = 0.99$; $y = 1.039 + 0.006x$), *P. antarctica* egg ($r = 0.99$; $y = 1.376x + 0.005$) and liver ($r = 0.98$; $y = 1.044x - 0.002$), and *P. papua* eggs ($r = 0.99$, $y = 1.616x - 0.023$) and liver ($r = 0.98$; $y = 0.986x + 0.043$). It means that in solely these latter cases, the variables are not only significantly monotonically related, i.e. the increase in one of them is related to the increase in the other (ρ is positive), but also this relationship is linear, which would allow a prediction with statistical significance to a certain extent. Likely not a coincidence, these were the matrixes with more elevated Me-Hg levels reported in the literature presented in our work; also, the higher sampling numbers in the present study and, specifically for seabirds, only penguins were among these exceptions. Since the other birds migrate, in a very broad sense, their diet and Me-Hg exposure are more likely to change. For vegetation, as previously stated, wet/dry deposition plays a major role; therefore, Me-Hg content is likely to change as well. All of this reinforces the main hypothesis we raise about the importance of Me-Hg content influencing Hg concentrations before and after delipidation.

Another approach is presented in Fig. 2, in which Dif is plotted versus lipid content for the different matrixes that presented enough paired values. The data therein presents an inter-specific/inter-tissue positive trend, confirmed by a significant correlation between Dif and %lip when the whole dataset is considered. For the whole dataset, significant correlations also appear between Dif and $\delta^{13}C$, meaning that animal samples under the influence of benthic primary producers (see Corbisier et al. 2004) will present a higher difference between Hg analyses and plant samples under marine influence and moister

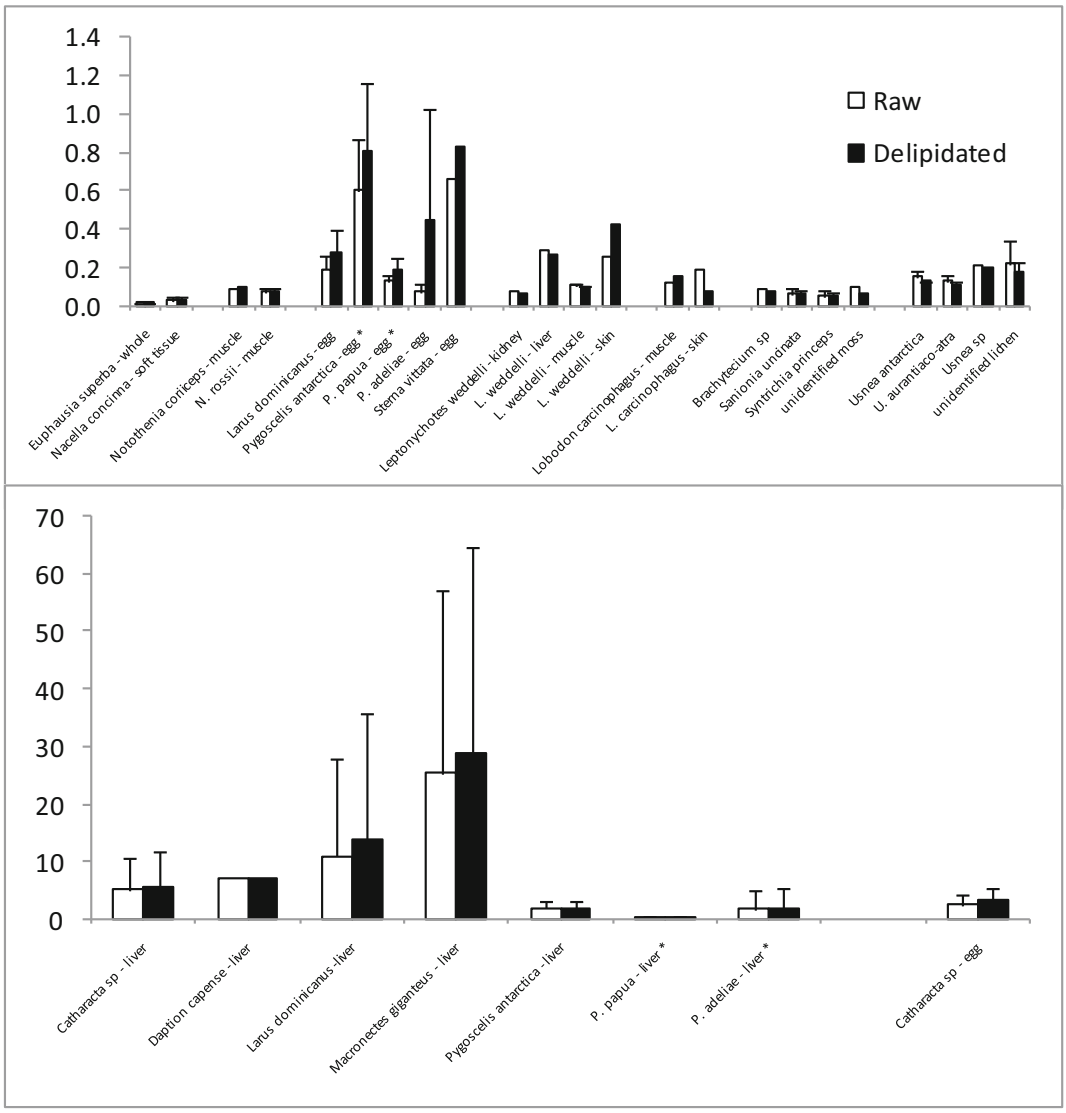

Fig. 1 Comparison of Hg concentrations (μg g^{-1} dwt) in raw and delipidated samples, separated for Hg values scales. Upper graph: invertebrates, fish, most bird eggs, pinniped tissues, mosses and lichens; lower graph: bird liver and skua egg. Significant differences (Wilcoxon–Mann–Whitney) are marked with an *asterisk*

habitats (Cipro et al. 2011; Lee et al. 2009) will behave similarly. Significant correlations also were found for Hg in raw and delipidated samples, meaning that the more contaminated samples before and also after delipidation were the ones presenting the largest differences, which is rather contradictory. However, all these correlations are not significant when data is taken separately by matrix or tissue, with a few exceptions: in fish muscle, Dif and $\delta^{13}C$ correlated significantly, meaning that individuals preying on pelagic organisms presented higher Dif. Also, Dif and %lip correlated (p = 0.49), which comes in agreement with the general trend.

Finally, for limpet soft parts, the result is negative for Dif and Hg_{raw}, which is strongly opposite to the general trend, meaning that in this organism, Hg is probably in a different form than in most of the other ones, supporting the previous discussion on the influence of metal-binding proteins in this species. In short, even if there seems to be some separation regarding %lip and matrixes, among them there is no significant correlation whatsoever between this

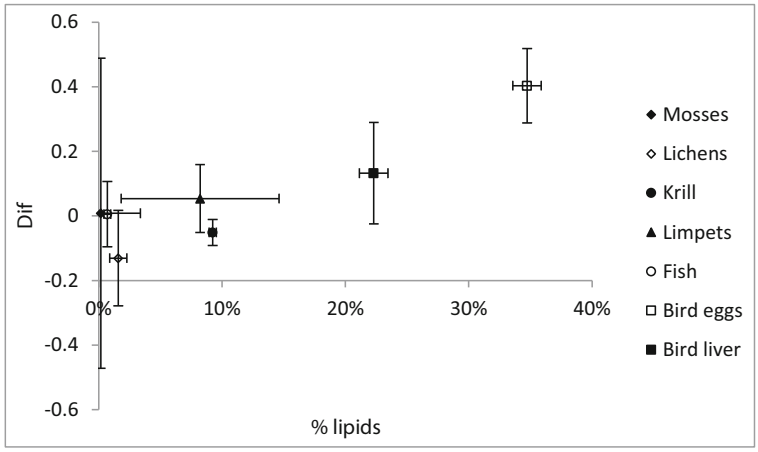

Fig. 2 Averages ± SD for Dif versus lipid concentration (%) separated by matrix, when pairing was possible

variable and Dif, with only one exception (fish muscle).

5 Conclusions

To the extent of this study and given the limitations and specificity of our sampling, it is safe to state that in a general way, it is not possible to infer precisely Hg concentrations from samples that have undergone delipidation using cyclohexane. For different matrixes and, mainly, different solvents, a case-by-case approach is needed since their polarity plays a major role on the extraction and elution of different lipid classes and potentially associated contaminants (e.g. Krahn et al. 2004).

The previous conclusion is clear in regard to plants and the animal matrixes with the highest lipid content. Moreover, due to sample quantity limitations, the organic Hg content could not be determined. Given that most of the methods for the determination of organic Hg contain a step chemically similar to a delipidation process (reaffirming that the percentage of lipids was the single factor more strongly correlated to Dif in separate plants and animal groups), therefore making Me-Hg susceptible to interferences, probably due to its log K_{ow} ranging from 1.7 to 2.5, which is an indication of its affinity for body fats. While this log K_{ow} is moderately relative to many organic substances, it is the combination of lipophilicity and especially its binding with sulfhydryl groups in proteins which fosters retention of Me-Hg (Saouter et al. 1993), as shown by the comparison between different matrixes, particularly fish muscle and limpet soft tissues.

There were, however, notable exceptions (muscle for *N. rossii*, liver for *P. adeliae*, liver and eggs for both *P. papua* and *P. antarctica*) in which the variation between analyses was consistent throughout the dataset, which allowed a linear regression with statistical significance, but apparently no relationship with the previously obtained data.

Finally, because the comparison with literature provided interesting correlation with this factor (Me-Hg), the future investigation of the influence of this variable might clarify some of the remaining issues. Therefore, we hypothesise that, in addition to species and tissue specificity, the Me-Hg content might play a major role in the difference found in Hg content before and after delipidation.

Acknowledgements The authors acknowledge CPER 13 (Contrat de Plan État-Région) for funding the AMA. C.V.Z.C. received scholarships from CAPES (Coordination for the Improvement of Higher Education Personnel via the Science without Borders programme, Brazil; grant no. 11490-13-6). C. Churlaud and M. Brault-Favrou of the 'Plateforme Analyses Elémentaires' of LIENSs are to be thanked for their assistance during Hg analyses and G. Guillou and B. Lebreton of the 'Plateforme Analyses Isotopiques' of LIENSs are also thanked for their assistance during SIA analyses. The present work is a result of the projects 'Environmental management of Admiralty Bay, King George Island, Antarctica: persistent organic pollutants and sewage' (CNPq No. 55.0348/2002-6), and 'Modeling the bioaccumulation of organic pollutants throughout an Antarctic food web' (CNPq 550018/2007-7) and also contributes to the Brazilian National Science and Technology Institute on Antarctic Environmental Research (INCT-APA). Financial support was obtained from the Brazilian Antarctic Program (PROANTAR), Ministry of the Environment (MMA) and Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq). Antarctic logistics was provided by Secretaria da Comissão Interministerial para os Recursos do Mar (SECIRM). Finally, authors wish many thanks to everyone involved in the fieldwork.

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