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Mercury in wintering seabirds, an aggravating factor to winter wrecks?

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HIGHLIGHTS

- We investigated the role played by Hg in an unprecedented seabird winter wreck.
- Stranded seabirds show the highest Hg concentrations measured in these species.
- Hg is a major aggravating stress factor contributing to enhanced seabird winter mortality.
- Total blood can be used as a predictor of Hg contamination in other seabird tissues.

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ABSTRACT

Every year, thousands of seabirds are cast ashore and are found dead along the coasts of North America and Western Europe. These massive mortality events called 'winter wrecks' have generally been attributed to harsh climatic conditions and prolonged storms which affect bird energy balance and impact their body condition. Nevertheless, additional stress factors, such as contaminant body burden, could potentially cumulate to energy constraints and actively contribute to winter wrecks. However, the role played by these additional factors in seabird massive winter mortality has received little attention to date. In February/March 2014, an unprecedented seabird wreck occurred along the Atlantic French coasts during which >43,000 seabirds were found dead. By analyzing mercury (Hg) concentrations in various tissues collected on stranded birds, we tested the hypothesis that Hg played a significant role in this mortality. More specifically, we aimed to (1) describe Hg contamination in wintering seabirds found along the French coasts in 2014, and (2) determine if Hg concentrations measured in some vital organs such as kidney and brain reached toxicity thresholds that could have led to deleterious effects and to an enhanced mortality. We found some of the highest Hg levels ever reported in Atlantic puffins, common guillemots, razorbills and kittiwakes. Measured concentrations ranged from 0.8 to 3.6 $\mu g \cdot g^{-1}$ of dry weight in brain, 1.3 to 7.2 $\mu g \cdot g^{-1}$ in muscle, 2.5 to 13.5 $\mu g \cdot g^{-1}$ in kidney, 2.9 to 18.6 $\mu g \cdot g^{-1}$ in blood and from 3.1 to 19.5 $\mu g \cdot g^{-1}$ in liver. Hg concentrations in liver and brain were generally below the estimated acute toxicity levels. However, kidney concentrations were not different than those measured in the liver, and above levels associated to renal sub-lethal effects, suggesting a potential Hg poisoning. We concluded that although Hg was not directly responsible for the high observed mortality, it has been a major aggravating stress factor for emaciated birds already on the edge. Importantly, this study also demonstrated that total blood, which can be non-lethally collected in seabirds, can be used as a predictor of Hg contamination in other tissues.

1. Introduction

Every year, thousands of seabirds cast ashore and are found dead along the coasts of North America and Western Europe (e.g., Piatt and Van Pelt, 1997; Gaston, 2004; Harris and Wanless, 2013). These massive mortality events called 'winter wrecks' have generally been attributed to harsh climatic conditions and prolonged storms which affect birds foraging efficiency and enhance thermoregulatory costs, thereby affecting their energy balance, impacting their body conditions and ultimately increasing their mortality (Fort et al., 2009). Hence, alcids which have little energy reserves and cannot survive longer than 3–4 days without foraging (Gaston, 1983; Gaston and Jones, 1998) are the first affected by starvation and the most exposed to harsh conditions. However, other

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additional factors could cumulate to bird energy constraints and actively contribute to winter wrecks. For instance parasite load or diseases could affect individual immune system and therefore enhance their sensitivity to energy constraints (Bulté et al., 2012). Contaminant body burden could also be a factor playing a significant role in these massive mortality events. Indeed, through an acute toxicity (see Wolfe et al., 1998), by affecting bird physiology and behavior (Tan et al., 2009) or by increasing energy requirements to sustain detoxification mechanisms (Lucia et al., 2012), contaminants could directly or indirectly impact their survival. However, very little is known about the role played by these additional factors in seabird winter wrecks (Debacker et al., 2000, 2001a). A better understanding of cumulative effects would benefit to the conservation of these vulnerable species during a critical period of their life-cycle.

Among contaminants present in the marine environment which could affect seabird mortality, the study of mercury (Hg) is of particular interest. Hg, and most particularly methyl-Hg, is indeed a powerful neurotoxicant in marine top-predators (e.g., Wolfe et al., 1998; Dietz et al., 2013) which can, through brain lesions or endocrine disruptive effects, affect seabird behavior and ultimately shape their survival (Wolfe et al., 1998; Tartu et al., 2013; Goutte et al., 2014a, 2014b). High Hg concentrations in other tissues (e.g., kidney and liver) could as well cause acute poisoning and impact seabird survival (Wolfe et al., 1998). Elevated concentrations of Hg were previously found in stranded wintering seabirds from the Northern Hemisphere (Debacker et al., 1997; Joiris et al., 1997). Hence, some authors suggested that Hg could be an additional stress factor that could partly result in the death of birds (Debacker et al., 2000).

In February and March 2014, an unprecedented seabird wreck occurred along the Atlantic French coasts. Within 6 weeks, a total of >43,000 seabirds had been found dead or extremely weakened on beaches (Farque, 2014). Atlantic puffins (*Fratercula arctica*) and common guillemots (*Uria aalge*), two species of the alcid family, were the most affected, representing 66% and 27% of stranded birds, respectively (Farque, 2014). Almost all birds were emaciated; starvation was designated as the main cause of this massive mortality. However, as dozens of birds of several species were collected on beaches or kept once dead in wildlife centers, they represented a unique opportunity to investigate further Hg contamination in wintering seabirds, and its potential role in this winter wreck.

By analyzing various tissues collected on stranded birds belonging to four different species, and by comparing Hg concentrations with those measured in healthy birds, the present study had the following objectives: (1) describe Hg contamination in stranded seabirds wintering in the Bay of Biscay; (2) test the hypothesis that Hg concentrations measured in some vital organs such as kidney and brain reached toxicity levels that could have led to deleterious effects and to enhanced bird mortality; and (3) determine if whole blood samples could be used as a predictor of Hg contamination in other seabird tissues. Indeed, while blood can be easily and non-lethally collected in seabirds, measurements of contaminant levels in other tissues are, for ethical issues, more limited. Understanding if/how blood can be used as surrogate to evaluate the contamination of other tissues is therefore essential.

2. Materials and methods

2.1. Seabird winter wreck 2014

For this study, 43 seabird carcasses were collected on the Isle of Rhé (46°1′N 1°2′W) and on Oléron Island (45.9°N 1.3°W) during February and March 2014, and kept frozen at −20 °C until analyses. They belonged to four species: Atlantic puffin (n = 15), common guillemot (n = 13), razorbill (*Alca torda*; n = 7) and black-legged kittiwake (*Rissa tridactyla*; n = 8). Part of these birds was collected freshly dead on beaches (n = 15) while another part (n = 28) was found alive by walkers and brought to a Wildlife Care Center where they died within 24 h. We assumed that these few hours spent at the Care Center did not affect Hg concentrations in bird tissues. During dissections, sex was defined by visual inspection of gonads and age of all individuals was determined following plumage patterns or morphological criteria (Pyle, 2009). In the present study, only adult birds were analyzed. The entire liver, kidney and brain were collected as well as some pectoral muscle and whole blood (hereafter 'blood') samples (from the cardiac clot). All tissues were lyophilized for 48 h, ground into powder and homogenized prior to Hg analyses. Feathers were not analyzed in this study since they reflected a different period than internal organs and were therefore not directly comparable. Hg that has accumulated in body tissues is excreted to feathers when birds are molting and Hg concentrations in feathers are thus considered to be an indicator of contamination between two molting sequences rather than a shorter-term contamination as in other tissues (Furness et al., 1986; Agusa et al., 2005).

Body condition of birds was evaluated through (1) the thickness of their pectoral muscle (Lindström et al., 2000; Moseley et al., 2012) and (2) the calculation of a body condition index (ratio of liver to kidney mass; Wenzel and Adelung, 1996; Debacker et al., 2000). Thickness of pectoral muscles was determined using a needle pricked transversally (at a 90° angle) in the right-side muscle, along the keel of the sternum (1 cm from the keel and at mid-length of the keel) (Erbacher, 2012).

2.2. Wintering seabirds in standard, healthy, body condition

In April 2006, 21 razorbills were found freshly dead on the beach in the southern part of the Bay of Biscay (43°03′N, 01°03′W). Contrary to razorbills collected in 2014 that were highly emaciated (see Results), these birds presented external signs of probable by-catch suggesting that they had drowned in fishing gears. We therefore assumed these birds to be in standard, healthy, body conditions at the time of death, and hereafter, they are referred to as 'healthy birds'. Razorbills collected in 2006 were used to compare body conditions as well as the mass of each organ and their Hg contamination levels between stranded (2014) and healthy (2006) razorbills.

2.3. Mercury analyses

Total Hg (hereafter termed Hg) concentrations were measured in each tissue at the Littoral Environnement et Sociétés laboratory (LIENSs, La Rochelle, France) using an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254) as described in Bustamante et al. (2006). Analyses were repeated two to four times for each sample until the relative standard deviation for two samples was <10%. The mean Hg concentrations for those two measurements were then considered for statistical analyses. To ensure the accuracy of measurements, a certified reference material was used (Lobster Hepatopancreas Tort-2; NRC, Canada; Hg concentration of 0.27 ± 0.06 $\mu g \cdot g^{-1}$ of dry weight (dw)) and measured every 10 samples. The average measured value was 0.28 ± 0.02 $\mu g \cdot g^{-1}$ of dw (n = 105). Additionally, blanks were run at the beginning of each sample set. The detection limit of the method was 0.005 $\mu g \cdot g^{-1}$ of dw.

2.4. Statistical analyses

Statistics were computed using R version 3.0.2 (R Development Core Team 2011). Hg data were log-transformed to comply with parametric assumptions of normality and homoscedasticity. Differences in Hg concentrations between tissues were tested for each species using repeated measures ANOVA followed by paired *t*-test. When sphericity was not met, we used the Greenhouse–Geisser adjustment, by multiplying the numerator and denominator degrees of freedom by ε, to calculate adjusted p-values (Greenhouse and Geisser, 1959). Differences in morphometrics, Hg concentrations and total Hg content in each organ between stranded (2014) and healthy (2006) razorbills were tested using Student's *t* tests. Relationships of Hg concentrations between blood and other tissues were calculated for both emaciated (2014) and healthy (2006) birds. ANCOVA were used to test for a potential effect of bird state on the intercept and the slope of the regression line when blood was compared to other tissues. If bird state had no effect, data from all species and both bird states were pooled to evaluate how Hg concentrations in these tissues were related to Hg in blood. If bird state affected the relationship between blood and Hg concentrations in other tissues (intercept or slope), concentrations measured in emaciated and healthy birds were then treated separately. All data are presented as mean ± SD. All provided Hg concentrations are in $\mu g \cdot g^{-1}$ of dw except otherwise mentioned (a table summarizing measured values in $\mu g \cdot g^{-1}$ of wet weight (ww) is presented in the Supplementary Material, Table A.1).

3. Results

3.1. Body condition and Hg levels in wintering seabirds during the 2014 winter wreck event

All dissected seabirds from the winter wreck 2014 were extremely emaciated with low body mass and reduced pectoral muscle thickness. As an illustration, wrecked razorbills were 38% lighter (420 ± 40 g vs 672 ± 85 g; *t*-test: $t = 7.5$, df = 26, $p < 0.001$), had pectoral muscles 36% thinner (13.3 ± 1.9 mm vs 20.7 ± 3.0 mm; *t*-test: $t = 6.0$, df = 26, $p < 0.001$) and had much lower body condition indexes (1.51 ± 0.33 vs 3.19 ± 0.45; *t*-test: $t = 8.9$, df = 25, $p < 0.001$) than those collected in 2006 with healthy body conditions. Kidney and liver masses were also highly reduced in razorbills from 2014 (wet mass, Fig. 1). Among birds collected in 2014, alcids had the lowest body condition indexes (razorbills: 1.51 ± 0.33, Atlantic puffins: 1.54 ± 0.20, common guillemot: 2.10 ± 0.48) while black-legged kittiwakes had the highest index (2.55 ± 0.77).

Hg concentrations in seabird body tissues followed the pattern: liver = kidney = blood > muscle > brain in razorbills, common guillemots and black-legged kittiwakes, while the pattern was liver > kidney = blood > muscle > brain in Atlantic puffins (see Table 1). Measured Hg concentrations in seabirds from the 2014 wreck ranged from 0.8 to 3.6 $\mu g \cdot g^{-1}$ dw in brain, from 1.3 to 7.2 $\mu g \cdot g^{-1}$ dw in muscle, from 2.5 to 13.5 $\mu g \cdot g^{-1}$ dw in kidney, from 2.9 to 18.6 $\mu g \cdot g^{-1}$ dw in blood and

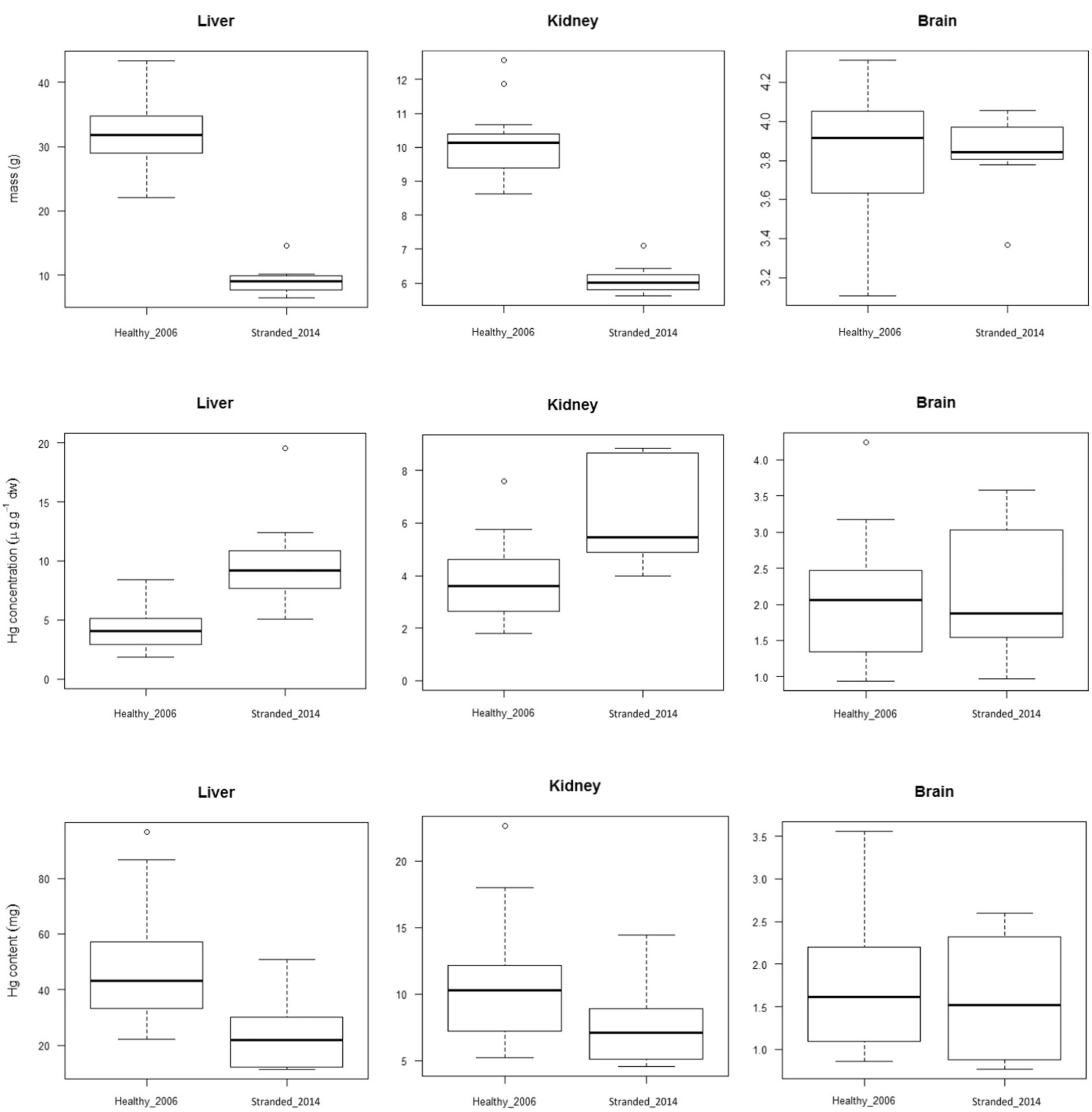

Fig. 1. Differences in wet mass (g; upper panel), Hg concentrations ($\mu g \cdot g^{-1}$ dw; middle panel) and total Hg content (mg; lower panel) in liver, kidney and brain between razorbills collected in 2006 (healthy condition) and razorbills collected during the massive mortality event in 2014 (see Materials and methods for details).

from 3.1 to 19.5 $\mu g \cdot g^{-1}$ dw in liver. Overall, Hg concentrations were higher in razorbills and kittiwakes than in Atlantic puffins and common guillemots (see Table 1).

ANCOVA showed that bird state affected either the slope or the intercept of the regression lines when blood was related to liver ($p = 0.458$ and $p < 0.001$, respectively), kidney ($p = 0.118$ and $p < 0.01$, respectively) and brain ($p = 0.171$ and $p < 0.001$, respectively). For these three tissues, concentrations measured in emaciated and healthy birds were therefore treated separately. However, bird state did not affect the slope or the intercept of the regression lines when blood was related to muscle ($p = 0.552$ and $p = 0.918$, respectively). Data from all species and both bird states were therefore pooled to evaluate how Hg concentrations in muscle were related to Hg in blood.

In both emaciated and healthy bird groups, Hg concentrations measured in blood were positively and linearly correlated to Hg concentrations in all other tissues (Fig. 2) following equations presented in Table 2.

3.2. Comparison of razorbills collected in 2006 and 2014

To investigate further the potential role of Hg in seabird massive mortality, we compared data obtained on razorbills assumed to be in healthy body condition (collected in 2006) and on razorbills from the 2014 winter wreck. As mentioned above, weighed internal tissues showed a lower mass in birds collected in 2014 (71% lower in liver: 9.4 ± 2.7 g vs 32.1 ± 5.3 g, *t*-test: $t = 10.8$, df = 25, $p < 0.001$; 40% lower in kidney: 6.1 ± 0.5 g vs 10.1 ± 0.9 g, $t = 10.7$, df = 25, $p < 0.001$), except in brain for which the measured mass was similar between the two groups (3.8 ± 0.2 g vs 3.9 ± 0.3 g, $t = 0.22$, df = 25, $p = 0.833$; Fig. 1). Concurrently to this lower mass, all internal tissues (but brain; $p = 0.712$) of stranded birds collected in 2014 showed higher Hg concentrations (Table 1, Fig. 1; all $p < 0.01$). However, and despite these higher Hg concentrations, the total Hg content (expressed in mg of Hg) was lower in livers of birds collected during the winter wreck 2014 ($t = 3.19$, df = 15, $p = 0.011$), and similar between the two groups in kidney and brain (Table 1, Fig. 1; $t = 1.67$, df = 25, $p = 0.063$ and $t = 0.26$, df = 25, $p = 0.799$, respectively).

4. Discussion

4.1. Hg as an aggravating factor of seabird winter mortality

Seabird massive winter mortality events are frequently responsible for the death of thousands to tens of thousands individuals all around the Northern Hemisphere; 'unprecedented' being more and more used to qualify these events (e.g., Harris and Wanless, 2013; Farque, 2014; The Seabird Group, 2015). In most of the cases, starvation is considered as the main cause for this mortality. However, the potential role played by additional factors has been barely investigated (e.g., Piatt and Van Pelt, 1997; Debacker et al., 2001a). Here, we analyzed for the first time Hg concentrations in several species washed up on shore during a massive winter wreck, and examined five different internal tissues in order to investigate the potential role played by Hg in this mortality event.

Our results show that Hg concentrations found in stranded birds in 2014, whatever the species and the tissue considered, were much higher than concentrations found in healthy birds during the breeding season (for reviews see e.g., Savinov et al., 2003; Espín et al., 2012; Provencher et al., 2014a; Scheuhammer et al., 2015). Different studies previously suggested that Hg contamination of North Atlantic seabirds could be higher in winter than during the breeding season, due to a shift on consumed prey or to an increased environmental contamination at their wintering grounds (Joiris et al., 1997; Fort et al., 2014). There is, however, very little information about Hg contamination of seabirds in healthy body conditions during the winter period, limited to a few species, mainly because of the difficulty to assess these individuals at sea. Nevertheless, measurements performed on stranded razorbills during the 2014 winter wreck were again two to four times higher than measurements performed on tissues of wintering healthy razorbills hunted off Newfoundland (Bond et al., 2015) or caught in fishing nets along the Portuguese and Spanish coasts (Ribeiro et al., 2009; Espín et al., 2012) and in the Bay of Biscay (this study). Similarly, muscle samples collected on common guillemots wintering off Newfoundland (0.09 $\mu g \cdot g^{-1}$ ww; Bond et al., 2015) were about five times less contaminated than samples measured in this study (2.08 $\mu g \cdot g^{-1}$ dw corresponding to 0.44 $\mu g \cdot g^{-1}$ ww). This strongly suggests that stranded seabirds were highly contaminated compared to birds in normal conditions. With the exception of investigations performed after massive oil-spills (e.g., Pérez-López et al., 2006; Sanpera et al., 2008), a very few studies, focused on common guillemots only, investigated Hg concentrations in emaciated birds found dead along the European coasts during winter. They reported similar Hg concentrations than those found in the present study (Debacker et al., 1997; Joiris et al., 1997).

There is very little information about toxicity levels in wild seabirds. Hepatic Hg concentrations found in stranded birds, although among the highest measured in these species, are lower than threshold concentrations considered being associated to acute toxic effects in non-marine birds (>5 $\mu g \cdot g^{-1}$ of ww; Zillioux et al., 1993; Wolfe et al., 1998; Scheuhammer et al., 2015). Birds present the particularity to have a renal portal system by which blood flows directly from the digestive tract to the kidney prior to entering the hepatic system. Hence, kidney is considered to be more vulnerable to Hg in birds, this latter damaging the structure of the kidney and impairing the renal function (Wolfe et al., 1998). For instance, average Hg concentrations of 5 $\mu g \cdot g^{-1}$ dw in kidneys of wild Atlantic puffins were found to be associated to sub-lethal kidney lesions suggesting initial toxicological effects (Nicholson et al., 1983; Nicholson and Osborn, 1983). In the present study, we found Hg concentrations in kidneys >5 $\mu g \cdot g^{-1}$ dw in all species.

Table 1
(A) Hg concentrations (in $\mu g \cdot g^{-1}$ of dw) and (B) total amount of Hg (in mg) in tissues of wintering seabirds which died during the massive mortality event which occurred along the French coasts in February and March 2014, and in tissues of healthy razorbills collected during winter 2006. Values are means ± SD (n).

Species	Blood	Liver	Kidney	Muscle	Brain
Hg concentration ($\mu g \cdot g^{-1}$ dw)					
Atlantic puffin	7.12 ± 2.58 (6)[a]	8.64 ± 2.30 (15)[b]	6.87 ± 2.79 (15)[a]	2.25 ± 0.57 (15)[c]	1.35 ± 0.39 (14)[d]
Common guillemot	6.32 ± 5.17 (8)[abc]	5.53 ± 1.49 (13)[a]	5.14 ± 1.84 (13)[a]	2.08 ± 0.57 (13)[b]	1.42 ± 0.46 (13)[c]
Kittiwake	8.58 ± 2.82 (8)[a]	10.77 ± 4.24 (8)[a]	8.43 ± 2.62 (8)[a]	4.44 ± 1.66 (8)[b]	2.47 ± 0.76 (8)[c]
Razorbill	9.38 ± 4.12 (7)[a]	10.13 ± 4.71 (7)[a]	6.48 ± 2.14 (7)[a]	3.99 ± 1.56 (7)[b]	2.22 ± 0.99 (7)[c]
Razorbill (2006)	4.46 ± 2.10 (20)	4.25 ± 1.68 (20)	3.77 ± 1.50 (20)	2.04 ± 0.88 (21)	2.05 ± 0.84 (21)
Total amount of Hg (mg)					
Atlantic puffin	–	14.91 ± 4.80 (15)	5.79 ± 2.62 (15)	–	0.99 ± 0.32 (13)
Common guillemot	–	19.50 ± 5.00 (13)	6.83 ± 2.72 (13)	–	1.17 ± 0.44 (13)
Kittiwake	–	24.71 ± 12.57 (8)	5.84 ± 2.36 (8)	–	1.72 ± 0.56 (8)
Razorbill	–	24.19 ± 14.95 (7)	7.74 ± 3.45 (7)	–	1.61 ± 0.80 (7)
Razorbill (2006)	–	47.53 ± 20.72 (20)	10.75 ± 4.30 (20)	–	1.70 ± 0.71 (20)

For each species/year, different letters denote significant statistical differences.

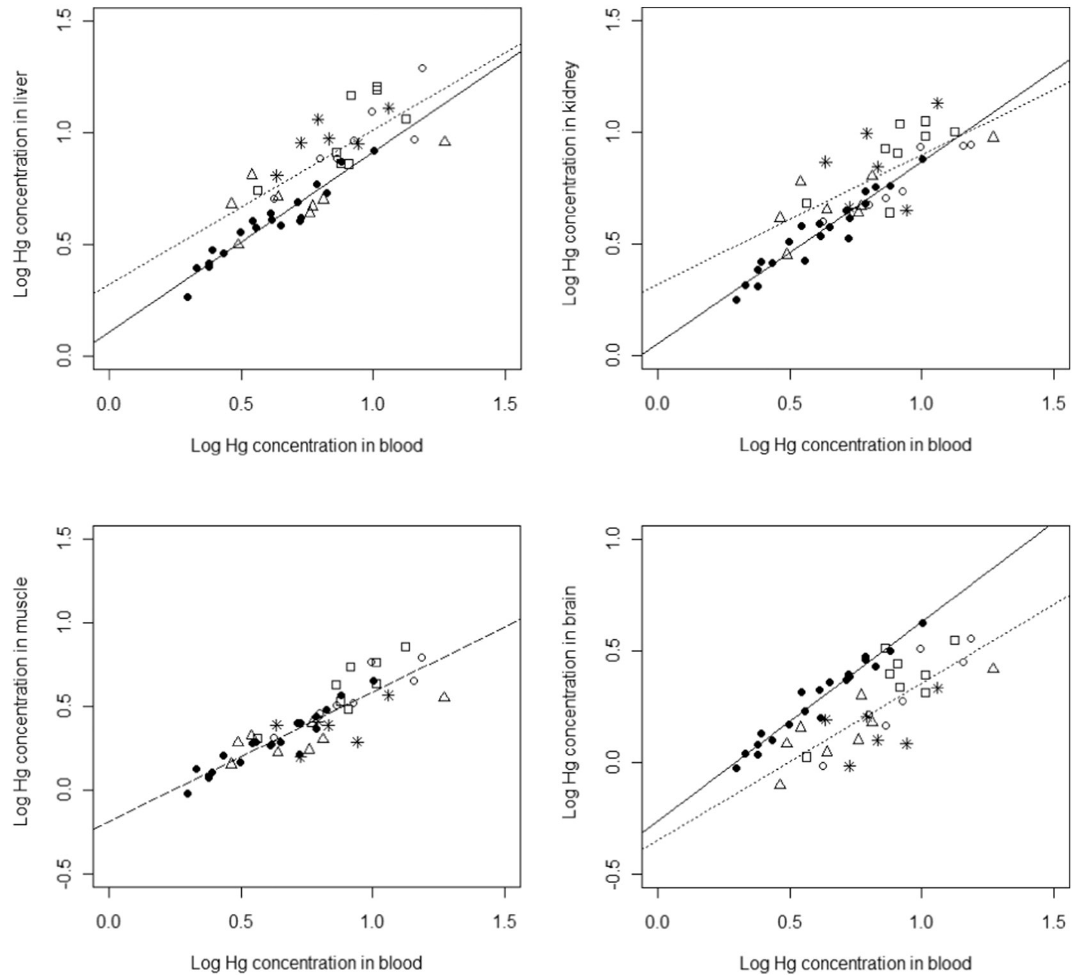

Fig. 2. Relationship between Hg concentrations (in μg·g^{-1} dw) measured in blood and in (A) liver, (B) kidney, (C) muscle and (D) brain in North Atlantic seabirds. Open-symbols: stars, squares, triangles and circles are for stranded Atlantic puffins (*Fratercula arctica*), black-legged kittiwakes (*Rissa tridactyla*), common guillemots (*Uria aalge*) and razorbills (*Alca torda*), respectively. Black-filled circles represent razorbills in healthy conditions found in 2006. Dotted regression lines show relationships for stranded birds (2014) and include all species. Solid regression lines show relationships for healthy birds (razorbills 2006). Dashed regression line show relationship for both healthy and stranded birds and include all data when bird state had no effect on the relationship (see Materials and methods for details).

Moreover, all species but Atlantic puffins showed no significant difference of Hg concentrations in liver and kidney. Hg levels in wild birds are usually found to be higher in liver than in kidney (Ribeiro et al., 2009; Cipro et al., 2014), and previous studies suggested that such kidney concentrations close to those of liver are an indicator of Hg poisoning (Wolfe et al., 1998). Finally, a previous experimental study performed on terrestrial birds demonstrated that metallic trace elements such as Hg at similar concentrations than those measured on stranded seabirds could enhance energy constraints and accelerate the cachexia process (Debacker et al., 2001b). All these elements strongly suggest that Hg has very likely been an aggravating stress factor for birds already on the edge, likely involved in the mortality observed in the four study species.

Hg, and more specifically methyl-Hg, is also known to readily enter the blood–brain barrier, causing brain lesions and central nervous system dysfunctions (Aschner and Aschner, 1990; Rutkiewicz et al., 2011; Dietz et al., 2013). This link between blood and brain is confirmed by the significant and strong correlation between Hg concentrations in blood and Hg concentrations in brain observed in both stranded and healthy birds (Fig. 2D, Table 2). In stranded birds, the loss of liver and kidney masses caused by starvation led to lower mercury loads in these organs (close to significance in kidney) and concomitant higher Hg concentrations in blood. This strongly suggests a release and remobilization of Hg in their blood stream. However, this enhanced circulating Hg has not been transferred to the brain. Hence, and contrary to our prediction, our results show that despite a higher contamination of stranded birds compared to healthy ones, Hg levels did not differ in brains of these two groups, limiting additional neurotoxic effects in the former one. Interestingly, these results suggest that the increase of Hg

Table 2
Relationships between Hg concentrations in blood and Hg concentrations measured in other tissues, in both healthy seabirds (razorbills collected in 2006) and emaciated seabirds (stranded Atlantic puffins, common guillemots, razorbills and black-legged kittiwakes collected during the massive winter mortality event in 2014).

Healthy birds	R^2	p-Value	Stranded birds	R^2	p-Value
$\text{Log [Hg]}_{\text{Liver}} = 0.81 * \text{Log [Hg]}_{\text{Blood}} + 0.11$	0.92	<0.001	$\text{Log [Hg]}_{\text{Liver}} = 0.69 * \text{Log [Hg]}_{\text{Blood}} + 0.32$	0.55	<0.001
$\text{Log [Hg]}_{\text{Muscle}} = 0.83 * \text{Log [Hg]}_{\text{Blood}} - 0.22$	0.89	<0.001	$\text{Log [Hg]}_{\text{Muscle}} = 0.74 * \text{Log [Hg]}_{\text{Blood}} - 0.15$	0.60	<0.001
$\text{Log [Hg]}_{\text{Kidney}} = 0.82 * \text{Log [Hg]}_{\text{Kidney}} + 0.05$	0.91	<0.001	$\text{Log [Hg]}_{\text{Kidney}} = 0.58 * \text{Log [Hg]}_{\text{Kidney}} + 0.32$	0.48	<0.001
$\text{Log [Hg]}_{\text{Brain}} = 0.89 * \text{Log [Hg]}_{\text{Brain}} - 0.26$	0.95	<0.001	$\text{Log [Hg]}_{\text{Brain}} = 0.70 * \text{Log [Hg]}_{\text{Brain}} - 0.35$	0.62	<0.001

concentrations in blood of emaciated birds has been too fast to affect their brain levels before bird death.

4.2. The use of whole blood samples as surrogate to evaluate seabird contamination

Blood is, with feathers, the most commonly collected tissue in seabirds (including for the study species) as it can be non-lethally sampled. Blood reflects the short-term Hg contamination of individuals and therefore provides information for the sampling period, usually the breeding season in seabirds (e.g., Goodale et al., 2008; Tartu et al., 2013). Conversely, and for obvious ethical issues, the collection of internal organs can be limited, especially for non-hunted, endangered or declining species. Having information about Hg levels in different seabird organs is nonetheless essential. It could indeed provide concentrations that could be compared to existing toxicity thresholds. For instance, knowledge of Hg concentrations in brain or, to a lesser extent, in kidney is limited in seabirds (Espín et al., 2012; Provencher et al., 2014b), contrasting with neurotoxic and nephrotoxic effects of this contaminant (Nicholson and Osborn, 1983; Wolfe et al., 1998; Dietz et al., 2013). Defining such Hg levels in seabird organs would also allow estimating bird total Hg body burden and it impacts on bird ecophysiology, reproduction or survival. In that context, we found in both healthy and stranded birds a strong relationship between Hg concentrations in whole blood and Hg concentrations in other internal tissues, demonstrating how blood samples can be used to predict Hg levels in other organs of North Atlantic seabirds. Nevertheless, we also highlight the importance to consider bird state when inferring Hg contamination of internal tissues from blood Hg concentrations as bird state significantly affects blood–brain, blood–liver and blood–kidney Hg concentrations correlations. Moreover, relationships might be different when specifically considering plasma or red blood cells, and further studies focused on these two fractions are needed to provide a complete understanding of the use of blood as predictor of seabird Hg contamination.

5. Conclusion

By analyzing Hg concentrations in five different tissues of seabirds washed up on shore during a massive mortality event, and belonging to four different species, we provide new insights about the role played by Hg in seabird winter wrecks. More specifically, stranded birds showed the highest Hg concentrations measured in these species. While liver and brain concentrations were below acute toxicity thresholds, kidney concentrations reached levels close to those of liver, and could be associated with sub-lethal kidney lesions. Although not directly responsible for the high observed mortality, we believe that Hg has been a major aggravating stress factor for emaciated birds already on the edge. Furthermore, we also demonstrated how Hg in blood, which can be non-lethally collected in seabirds, is related to concentrations in kidney, liver, muscle and brain, and can therefore be used as a predictor of Hg contamination in seabird tissues.

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