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Evaluation of claws as an alternative route of mercury elimination from the herring gull (*Larus argentatus*)

by

Agnieszka Grajewska*, Lucyna Falkowska, Andrzej Reindl

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University of Gdańsk, Faculty of Oceanography and Geography, Institute of Oceanography, Department of Marine Chemistry and Environmental Protection, Al. M. Piłsudskiego 46, 81-378 Gdynia, Poland

Abstract

Mercury enters the body of seabirds in its most toxic organic form, i.e. methylmercury, mainly via the alimentary tract. Inside the body, mercury is transformed into less toxic forms and accumulates in the internal organs. The process of mercury removal from the body, most effective during the formation of new feathers and claws, is beneficial for the bird. The presented research was undertaken on account of the high affinity of mercury to keratin – a protein that forms feathers and claws – to compare the concentration levels (Hg_{TOT}) in these structures and to assess their contribution to the purification of the body of herring gulls (*Larus argentatus*). Bird feathers are the only epidermal structure that is extensively described in the literature, whereas the claws have so far been poorly researched.

The study has shown that mercury in claws is built in as effectively as in feathers, and the obtained concentrations were within a wide range of 127.2–5341.5 ng Hg_{TOT} g^{-1} of dry weight. In addition, the concentrations of total mercury accumulated in the claws were a better reflection of Hg levels in internal organs compared to feathers.

Key words: herring gulls, mercury elimination, claws, southern Baltic Sea

* Corresponding author: *oceagr@ug.edu.pl*

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Introduction

Mercury enters the environment both from natural (volcanic activity, forest fires, waterbody movement, rock weathering, biological processes) and anthropogenic sources (mining operations, industrial processes, combustion of fossil fuels, especially coal, cement production, and incineration of medical, chemical, and municipal wastes) (Poulin & Gibb 2008). Once introduced into the atmosphere, mercury is transported over long distances so that it can be deposited in places far from emission sources (WHO/IPCS 1990). Mercury enters the sea mainly in the form of inorganic Hg^{2+} or as Hg^0, from where it is absorbed by aquatic microorganisms and can be transformed into the most toxic organic form, i.e. methylmercury – CH_3Hg, also written as MeHg (Fitzgerald et al. 2007; Blum et al. 2013). As a consequence, it is incorporated into the trophic chain (WHO/IPCS 1990; Scheuhammeret al. 2007). Methylmercury is classified as an endocrine-active compound, which means that it can affect the hormonal system and lead to harmful developmental, reproductive and neurological changes (Georgescu et al. 2011).

The structural characteristics of the Baltic Sea and the fact that it is located in a highly urbanized and densely populated area of Europe make it particularly exposed to anthropogenic pollution. The former widespread use of mercury in industry, spanning over a century, and the fact that the reduction in mercury emissions did not occur until the end of the 20th century (HELCOM 2010) means that there are still areas (landfills, areas of industrial plants with former Hg usage) where Hg pollution is common (Bełdowska 2016). Mercury is supplied to the coastal zone of the Gulf of Gdańsk mainly via rivers (Saniewska et al. 2010) where, as in other estuaries, it mostly accumulates (Cossa & Martin, 1991). Only a small part of the mercury transported by rivers reaches the open sea.

Seabirds, being at the top of the trophic chain, are good indicators of pollution in coastal areas (Savinov et al. 2003). The herring gull (*Larus argentatus*, Pontopiddan, 1763) is a migratory species widespread in Northern Europe. Mature birds breeding on the Polish coast may be sedentary. The herring gull is also one of the largest birds and the most numerous species found on the Polish coast in wintertime (Meissner et al. 2007). The birds use both natural and anthropogenic food sources, often accompanying fishing boats, which provide them with an easy way to obtain fish waste. Their diet also includes fish, invertebrates, shellfish, small amphibians, as well as eggs and chicks of other gulls. In winter, they make more intensive use of landfills located near major cities. In Poland, the herring gull is a partially protected species (Dz.U. 2016, item 2183).

The main route of exposure of birds to mercury is via food intake (Khale & Becker 1999), which introduces this chemical element into the bird body in its most toxic form – methylmercury. An additional source of mercury is inhalation, which provides the body with the inorganic form (Falkowska et al. 2017). The capacity of mercury to accumulate and magnify results in its increased concentrations in the tissues and organs of seabirds, which may be several times higher compared to other parts of the coastal zone ecosystem. Kalisinska & Dziubak (2007) indicate that high levels of mercury in the central nervous system may disturb the motor coordination and kinesthesia of birds. The summary of toxicity benchmarks for the methylmercury exposure effects on birds presented by Ackerman et al. (2016) shows that bird reproduction is particularly sensitive to mercury toxicity with reduced reproductive success, reduced egg hatchability and offspring survival, all mentioned among many documented deleterious effects.

Together with blood, organic mercury is transported to the liver, where it can be demethylated and then distributed throughout the body (Burger & Gochfeld 2002; Burgess et al. 2013). Birds, however, have several mechanisms through which they can get rid of mercury present in their bodies. Although only small amounts of mercury are excreted with guano, this route leads to its faster reintroduction into the environment (Yin et al. 2008). The most effective removal of mercury occurs during molting, while females may additionally eliminate the accumulated mercury through eggs (Becker 1992).

Herring gulls in the region of the southern Baltic have already been the subject of research on the distribution and elimination of mercury (Szumiło et al. 2013; Szumiło-Pilarska et al. 2016; Falkowska et al. 2017; Szumiło-Pilarska et al. 2017) and of organic pollutants contained in different organs (Falkowska et al. 2016; Reindl et al. 2015). In the majority of specimens examined, the highest concentrations of mercury were observed in the liver and kidneys followed by lungs, muscles and brain. Among the examined tissues, the intestines were characterized by the lowest concentrations of mercury (Szumiło-Pilarska et al. 2016; Falkowska et al. 2017). Previous research also suggested that adult herring gulls may be potential sentinels of environmental contamination with mercury on a local and regional scale, based on blood and developing feather tests. Newly emerging feathers can also indicate the effectiveness of demethylation in relation to fully developed feathers (Szumiło-Pilarska et al. 2017).

Since the incorporation of mercury into the feathers is undoubtedly recognized as the most effective way of eliminating mercury from the body (Braune & Gaskin 1987; Monteiro & Furness 1995), the authors decided to assess the transport and incorporation of toxic mercury into birds' claws, which, like feathers, are keratinous structures (Hahn et al. 2014). Birds' claws are heavily keratinized epidermal structures, which vary depending on how they are used and the substrate on which birds live (Stettenheim 2000). The toe claw, which is present in every bird species, is composed of a dorsal plate that curves downward on the tip and sides and a ventral plate that fills the space between the sides underneath (Lucas & Stettenheim 1972). The dorsal plate is harder and contains heavy deposits of beta-keratin and calcium salts (Stettenheim 2000). Unlike feathers, claws grow continuously (Lucas & Stettenheim 1972; Ethier et al. 2010). Furthermore, the growth is conical (not linear) – the outer layers are always older than the inner layers (Hahn et al. 2014).

The objective of the study was to assess individual differences in total mercury concentrations (Hg_{TOT}) in claws of herring gulls (*Larus argentatus*) in relation to age classes. Furthermore, the results of mercury concentrations in selected tissues, organs and feathers of the same individuals published in previous works were used to provide a broader interpretation of the presented results.

Materials and methods

Biological material for analysis

Claws were recovered from 49 dead herring gulls found around the Gulf of Gdańsk: 27 birds were found in the area of the fishing port in Władysławowo (φ = 54°47'N, λ = 18°25'E), 13 in the Mewia Łacha bird sanctuary (φ = 54°21'N, λ = 18°57'E) located in the Vistula estuary and nine within the Tri-city agglomeration. Samples were collected in 2010–2012. Stainless steel scissors were used to remove the top part of each bird's claw (Fig. 1).

The age of each bird was determined on the basis of its plumage (Malling Olsen & Larsson 2004) and three age categories were distinguished: juvenile specimens (chicks and birds in their first plumage), immature specimens (in their second and third plumage) and mature birds (in the fourth and final plumage). Gender was determined on the basis of DNA using the method of polymerase chain reaction – PCR (Fridolfsson & Ellegren 1999). A total (taking into account age and gender) of 19 mature (12 females, 7 males), 17 immature (6 females, 11 males) and 13 juvenile birds (8 females, 5 males) were used in the analysis. All birds were subjected to a postmortem during which, if possible, we collected blood (as a blood clot from the heart), internal organs (breast muscle, heart, liver, kidney, brain, intestines, lungs) and feathers (outermost primary P10, innermost primary P1, rectrices, breast contour feathers and down, and if birds were in the molting period, also new breast contour feathers), which were the focus of the previous research (Szumiło-Pilarska et al. 2016; Szumiło-Pilarska et al. 2017; Falkowska et al. 2017). The cause of death remained unknown but the cachectic condition of each bird was assessed. It was found that 10% of the birds were emaciated, including one male with suspected peritonitis (Falkowska et al. 2016).

Figure 1

Sampling of herring gull's claws (parts of claws used for the analysis were cut off at the position marked by the dotted line)

Prior to analysis, all claws were washed with 80% acetone in an ultrasonic bath, rinsed with Milli-Q water and dried at room temperature. The whole claws were used for analysis.

Chemical analysis – total mercury (Hg_{TOT})

The total mercury concentrations (Hg_{TOT}) were assayed using atomic absorption spectrometry on an AMA-254 analyzer. Weighed amounts of birds' claws (about 0.0100 g) were placed in pre-combusted nickel boats. Each sample was assayed in three replicates and the final result represents an average of three analyses. An empty pre-combusted nickel boat was used as a blank sample (for each of the 10 measurements). The precision of the method was measured using certified standards: BCR414 prepared on the basis of plankton and BCR463 – based on tuna. The precision was 5%. The accuracy expressed as mercury recovery was established at 96.7%, while the limit of quantification (LOQ) amounted to 0.075 ng Hg_{TOT} g^{-1} d.w.

Statistical Analysis

Statistica 10 was used for the calculations and visualization of the results. The normality of the distribution among the studied variables was examined using the Shapiro-Wilk test. The analysis of the relationships between the variables was carried out based on the Spearman correlation (nonparametric data). To test the significance of differences, the non-parametric Wilcoxon signed rank test, the Kruskal-Wallis test and the U Mann-Whitney test were used. All statistical analyses were performed at a confidence level of 95%.

Results

The analysis of the results was carried out on an extended data set which, apart from the original mercury results for claws, included the results of total mercury content in the tissues, organs (Szumiło-Pilarska et al. 2016; Falkowska et al. 2017) and feathers (Szumiło-Pilarska et al. 2017) of the same specimens. The Wilcoxon signed rank test showed that the concentrations of mercury in the claws were statistically significantly different ($p < 0.05$) from all other variables, with the exception of innermost primary P1, rectrices and breast contour feathers (new). The average mercury concentration in the claws was almost twice as high as the concentrations in the most mercury-loaded organs (liver, kidneys). Extremely high concentrations of mercury in the claws (Table 1) were observed in a mature female found in Wladyslawowo in the winter of 2010. At the same time, the maximum concentration values were also found in the brain and heart of that specimen, and one of the two extreme values was assayed in rectrices (6310.9 ng Hg_{TOT} g^{-1}).

All the minimum concentrations of mercury in the individual variables were measured for juvenile specimens. One of them was also characterized by the lowest Hg_{TOT} concentrations in the brain, blood, down and cover feathers. The lowest concentration of mercury, on the other hand, was observed in a specimen that also had the lowest concentration of mercury in the liver, heart and lungs. Another juvenile specimen, worth mentioning, had mercury concentrations in all the collected types of feathers (outermost primary P10, breast contour feathers, rectrices) at levels above 6000 ng Hg_{TOT} g^{-1}. In the case of outermost primary P10 and rectrices, they were the highest (extreme) values recorded for these types of feathers. It is worth noting that the bird was characterized by one of the lowest concentrations of mercury in the blood (65.9 ng Hg_{TOT} g^{-1}), an order of magnitude lower than the mean value.

The Kruskal-Wallis test showed statistically significant differences ($p = 0.0107$) between the total mercury concentrations in bird claws in different age groups (Fig. 2). Multiple comparisons of mean ranks for all samples indicated that the immature age group corresponds to the test result, which is statistically significantly different from the juvenile

Table 1

Statistical characteristics of mercury concentrations (ng Hg_{TOT} g^{-1} d.w.) assayed in claws, tissues and organs of herring gulls found around the Gulf of Gdańsk in 2010–2012

group	n	Md	min.–max ()	$n_{outlier}/n_{extreme}$	RNG
claws	49	869.2	127.2–5341.5	3/1	min.–2654.4
outermost primary P10[1]	44	743.0	40.1–6989.5	1/1	min.–2689.9
innermost primary P1[1]	40	1330.6	79.0–9186.8	1/0	min.–4739.6
breast contour feathers[1]	46	1437.7	131.3–8211.9	2/1	min.–4453.0
rectrices[1]	45	583.6	62.4–6355.6	0/2	min.–2908.0
down[1]	17	1138.6	605.3–4908.0	1/0	min.–4102.0
breast contour feathers (new)[1]	12	2153.8	399.5–4193.6	0/0	min.–max
liver[2]	48	546.9	58.1–1694.3	0/0	min.–max
kidney[2,3]	45	455.7	19.1–1882.6	1/0	min.–1872.3
lung[2,3]	46	311.8	31.4–1104.8	0/0	min.–max
muscle[2]	49	244.0	42.7–1076.7	0/0	min.–max
heart[2]	48	278.2	21.5–1043.8	0/0	min.–max
brain[2,3]	43	156.5	22.5–687.8	3/0	min.–459.9
blood[2,3]	41	434.4	15.4–1410.6	0/0	min.–max
intenstine[3]	37	208.2	18.5–933.2	2/0	min.–475.9

n – number of samples; Md – median value; min. – minimum; max – maximum; noutlier – number of outlier values; nextreme – number of extreme values; RNG – range without outlier and extreme values; the set of variables whose characteristics are presented in the table was created on the basis of previously published results: [1]Szumiło-Pilarska et al. 2017; [2]Szumiło-Pilarska et al. 2016; [3]Falkowska et al. 2017

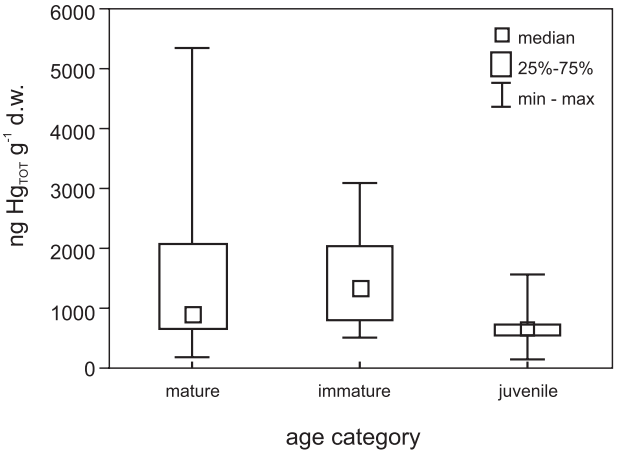

Figure 2

Mercury concentrations (ng Hg_{TOT} g^{-1} d.w.) assayed in claws of herring gulls found around the Gulf of Gdańsk in 2010–2012 in different age categories

group. Since mature females can eliminate mercury during egg laying (Becker 1992), which may result in a reduced mercury body burden, the total mercury concentrations in claws of females and males were compared among the mature gulls. However, they did not show any statistically significant differences (U Mann-Whitney test; $p = 0.33$).

All relationships between the concentration of mercury in claws and the concentration of mercury in the tissues and internal organs were statistically significant (Table 2). In the case of feathers, statistically significant correlations were observed in outermost primary P10 and rectrices.

Discussion

The wide range of total mercury concentrations in gull claws may be the result of a varying time of exposure to the studied xenobiotic. The life span of this species can reach up to 30 years (Kruszewicz 2011). Most of the birds reach sexual maturity in their fifth year of life, meaning that the oldest individuals in the mature category can be up to 6 times older than the youngest ones. Such a large difference in exposure time may be the reason for the largest spread of results among adults (Fig. 2). In addition to the narrow concentration range, juvenile specimens were characterized by the lowest average mercury concentration in the claws. This may be influenced by the rapid growth of birds in the first months of life resulting in reduced mercury concentrations in the tissues and internal organs (Falkowska et al. 2013; Grajewska et al. 2015). In the case of immature specimens, this rapid growth is no longer observed. In addition, this group is characterized by low age diversity.

It should be noticed that diet composition remains the most important factor affecting the intraspecific differences in mercury concentrations since food is the main intake path of mercury in seabirds (Scheuhammer et al. 2007). Kojadinovic et al. (2007) indicate that foraging habits may also be related to age. According to this research, mercury levels in adult birds were higher because they may have eaten larger, more contaminated prey compared to younger individuals. Furthermore, although herring gulls are known to be opportunistic predators and scavengers, they also feed on landfills (Meisner et al.

Table 2

Spearman's correlation coefficients (R) between the total mercury concentration (ng Hg_{TOT} g^{-1} d.w.) in claws of herring gulls and the total mercury concentration (ng Hg_{TOT} g^{-1} d.w.) in their selected internal organs, tissues and feathers

selected variable	n	R	p	equation
outermost primary P10	44	0.38	0.010	claws = 0.2193 × outermost primary P10 + 1078.1857
innermost primary P1	40	–	n.s.	–
breast contour feathers	46	0.35	0.016	claws = 0.1697 × breast contour feathers + 982.1230
rectrices	45	0.32	0.030	claws = 0.34228 × rectrices + 923.6664
down	17	–	n.s.	–
breast contour feathers (new)	12	–	n.s.	–
liver	48	0.39	0.006	claws = 0.4929 × liver + 980.5223
kidney	45	0.50	0.000	claws = 0.771 × kidney + 809.6370
lungs	46	0.45	0.002	claws = 1.5575 × lungs + 677.7302
muscle	49	0.34	0.018	claws = 1.0795 × muscle + 888.1018
heart	48	0.40	0.005	claws = 1.5484 × heart + 763.8899
brain	43	0.47	0.002	claws = 3.5917 × brain + 475.8652
blood	41	0.47	0.002	claws = 1.0740 × blood + 767.8458
intestines	37	0.48	0.003	claws = 2.6663 × intestines + 628.7789

n – number of samples; n.s. – non significant

2007). Previous research on herring gulls (Falkowska et al. 2017) showed that non-breeding, immature birds are more often observed on the garbage dumps where they are exposed to lower levels of mercury. All the above considerations can be supported by results on the content of mercury in birds' claws, as it is known that they can gradually store the isotopic composition of the diet (Bearhop et al. 2003).

The mercury load on the internal organs of a bird is the result of the processes of uptake, transformation and elimination of mercury from the body (Monteiro & Furness 1995). An effective elimination of toxic substances is therefore crucial for maintaining the proper functioning of the body. When molting, birds have a unique opportunity to get rid of pollutants from the body without additional energy consumption. Each newly formed feather is supplied with various substances contained in the blood, including the most toxic methylmercury, which accounts for most of the mercury transported via blood. When the structure is fully developed, the blood supply vessels undergo a regression and thus the mercury stored in the feathers is retained there until the next molting period (Kojadinovic et al. 2007). It is estimated that feathers can incorporate from 70 to 93% of the mercury collected in the bird body (Braune & Gaskin 1987; Burger & Gochfeld 1997; Bond & Diamond 2009). This is possible because the main component of feathers is keratin, a substance containing -SH sulfhydryl groups, which have high affinity with methylmercury (Goede & De Bruin 1984).

Similarly to feathers, large amounts of mercury can be transported to the claws, which is evidenced by the average concentration of mercury in the claws. As in all types of feathers, these levels were higher than in the tissues and internal organs (Table 1).

The ease of obtaining feathers as a research material makes them a common indicator used to assess the state of the environment in which birds live (Braune & Gaskin 1987; Thompson et al. 1993; Monteriro & Furness 1995; Thompson et al. 1998; Furness & Camphuysen 1997; Stewart et al. 1997; Mallory et al. 2010). Although many studies (Braune & Gaskin 1987; Thompson et al. 1991; Zamani-Ahmadmahmoodi et al. 2014) indicate the existence of a correlation between mercury concentrations in feathers and internal organs, such correlations were not observed in herring gulls from the Gulf of Gdańsk (Szumiło-Pilarska et al. 2017). This situation may be explained by the analysis of δ15N stable isotopes in the muscles and feathers of the same specimens, proving that mercury comes from different sources in each of these structures (Szumiło-Pilaska et al. 2016). At the same time, it was shown that internal organs may be affected by mercury present in the inhaled air, which is more pertinent to birds staying in the coastal zone during the summer season (Falkowska et al. 2017).

The present work, however, has shown the existence of statistically significant correlations between all the examined internal organs and claws (Table 2). Mercury is transported to feathers only during the growth of feathers, which lasts for several weeks (Lewis & Furness 1991; Dauwe et al. 2003). Therefore, feathers reflect only the "temporary" state of the body and not the effect of prolonged exposure to the studied xenobiotic (Kojadinovic et al. 2007). The growth of claws is a continuous process (Lucas & Stettenheim 1972; Ethier et al. 2010), requiring a constant blood supply (Hoefer 2012). As a result, the total mercury concentrations in claws may correspond better to the mercury body burden represented by mercury levels in internal organs. However, it should be emphasized that the difficulty of obtaining claws means that they will never replace feathers in scientific research.

It is known that some birds can control mercury accumulation not only by excretion through molting but also through demethylation metabolism (Kim et al. 1996). Some studies have initiated a discussion on the process of organic mercury demethylation, which could take place in the brain and liver of herring gulls from the southern Baltic region (Szumiło-Pilarska et al. 2016). Falkowska et al. (2013) draw attention to the influence of the condition and age of birds on their ability to conduct demethylation. It was suggested that higher concentrations of mercury in feathers can occur as a result of increased mercury transportation to the feathers, due to negligible demethylation processes in the liver. It can be assumed that claws will reflect the effectiveness of demethylation in a similar way. The performed analyses appear to confirm these reports. The specimen (mature female) with the maximum concentration of mercury in the claws also had an extremely high concentration of mercury in the feathers. In addition, both values were similar to those described for a penguin from the zoo (Falkowska et al. 2013).

Conclusions

The wide range of total mercury concentrations in the claws of herring gulls can be mainly due to the duration of exposure to the studied xenobiotic, although age-related feeding ecology cannot be ignored when interpreting the results. Claws, similarly to feathers, are corneous structures of the epidermis,

built of keratin. The mercury incorporated into any of these structures does not pose a threat to a bird that is physically and chemically stable. However, when the bird dies, these structures will remain in the environment, and the mercury contained in them may be eventually transformed into more labile forms and thus return to circulation in the environment.

The study has shown that claws and feathers accumulate toxic mercury on a similar level, while other tissues and internal organs are characterized by lower Hg_{TOT} concentrations. It can therefore be concluded that the incorporation of mercury into the claws and feathers of birds is equally effective for both these structures. However, it should be remembered that although the load on feathers and claws can be considered similar, the contribution of claws in the elimination of mercury from the body is smaller compared to feathers. In addition, claws can also reflect demethylation processes, while providing a good picture of the mercury load on internal organs.

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