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Mercury concentrations in wild humpback whales (*Megaptera novaeangliae*) sampled in the Colombian Pacific and the Antarctic Peninsula

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Abstract

The G stock of humpback whales (*Megaptera novaeangliae*) undertakes one of the longest cetacean migrations, from the Antarctic Peninsula (feeding area) to the Southeast Pacific, in Ecuador and Colombia, where their breeding and calving areas are located. These whales are being exposed to several pollutants such as mercury, which has been previously reported in the Antarctic Ocean. In order to measure the mercury concentration in G stock humpback whale' skin and blubber, samples were collected in the Antarctic Peninsula (2015, *n*=15) and in the Colombian Pacific (Chocó Province) (2015, *n*=14; 2016, *n*=42). Total mercury concentrations ([THg]) were measured by atomic absorption spectrometry (AMA-454, Altec). Results revealed significant differences between tissue types in the same individual (*n*=22; $p < 0.05$), with higher [THg] foundin skin (mean=26,78 ± 13,82 µg/kg dw) than in blubber samples (mean=12,41 ± 8,10 µg/kg dw). Furthermore, [THg] were significantly different between tissues and between sampling locations, being higher in Antarctic skin and blubber samples (x=35,07 ± 13,49 µg/kg dw and mean=10,61 ± 4,11 µg/kg dw, respectively), than in skin and blubber samples from the Colombian Pacific (x=21,34 ± 4,79 µg/kg dw and x=8,37 ± 4,53 µg/kg dw, respectively). There were no significant differences between individual females and males. Although humpback whales are not top-predators in the Antarctic trophic ecosystem, this study provides new insights of mercury bioaccumulation in Antarctic meso-predators. Our results suggest that whales detoxify THg during migration. In order to further evaluate the impacts mercury exposure in the whales and population, future research should be focused on assessing mercury concentration in internal organs as well as the degree of maternal transfer to the offspring, to evaluate the consequences of mercury exposure at individual and whales and population level.

Introduction

The humpback whale (*Megaptera novaeangliae*) undertakes one of the longest migrations in the animal kingdom, particularly animals belonging to the G stock (Flórez-González et al., 2007; Rasmussen et al., 2007; Reeves et al., 2008). This population exploits resources in the latitudes of high productivity around the Antarctic Peninsula during the austral summer, and they migrate to mating and breeding areas in tropical and subtropical latitudes, particularly in the Pacific coast of Colombia and Ecuador (*Fig 1*) (Albertson et al., 2017; Clapham, 1996; Flórez-González et al., 2007; Reilly et al., 2008; Stone, Florez-Gonzalez, & Katona, 1990). Although the G stock of humpback whales were in great danger of extinction due to the exploitation during the twentieth century, they appear to be recovering satisfactorily (Reeves et al., 2008). However, several population face conservation threats related to human activities, such as entanglement in fishing gear and vessel collisions (Félix & Van Waerebeek, 2005; Pacheco, Silva, & Alcorta, 2009; Reilly et al., 2008; Van Waerebeek et al., 2007). Furthermore, several studies have shown that whales are exposed to a wide-range of pollutants (Bossart, 2011). Nonetheless, there is very little research about humpback whales and pollutants, and the consequences to exposure to pollutants, such as heavy metals, in mysticetes is not well studied yet (Thomas, Reeves, & Brownell, 2016).

Some studies have evaluated the concentrations of pollutants in humpback whales (*M. novaenglie,* Elfes et al., 2010), minke whales (*Balaenoptera acutorostrata,* Aono, Tanabe, Fujise, Kato, & Tatsukawa, 1997), blue whales (*Balanoptera musculus*, Metcalfe et al., 2004), fin whales (*Balaenoptera physalus*, Borobia, Gearing, Simard, Gearing, & Béland, 1995; Marsili et al., 1998) and the northwest Atlantic right whales (*Eubalaena glacialis*, Weisbrod, Shea, Moore, & Stegeman, 2000). These studies have mainly assessed mainly the concentrations of persistent organic pollutants (POPs) (Aguilar, Borrell, & Reijnders, 2002). Mercury toxicity has been assessed in a limited number of mysticetes of commercial importance, such as the bowhead whales (*Balaena mysticetus*), gray whales (*Eschrichtius robustus*), and minke whales (*B. acutorostrata*) (Hobson et al., 2004; Krone et al., 1999; Kunito et al., 2002; Maage et al., 2017; Rosa et al., 2008; Ruelas-Inzunza et al, 2013; Varanasi et al., 1994; Woshner et al., 2001).Nonessential trace elements, such as mercury, have no biological role and bioaccumulate in tissues making them toxic for organisms. Studies have also indicated that mercury, which is released from natural sources and as a pollutant due to anthropogenic activities (Chouvelon et al., 2017), can be associated to immunotoxicity (Desforges et al., 2016), neurotoxicity (Krey, Ostertag, & Chan, 2015), reproductive, endocrine, heart, and kidney damage (Bossart, 2011; Correa, Castellini, Quakenbush, & O'Hara, 2015; Schwacke et al., 2002), as well as cancer (Béland et al., 1993; Martineau et al., 1994). Furthermore, transplacental and lactation transfer of mercury from the mother may have an effect on the calf (Frodello, Viale, & Marchand, 2002; Noël et al., 2016; Romero, Polizzi, Chiodi, Das, & Gerpe, 2016). Up to date, there is no study assessing total mercury concentration ([THg]) in any humpback whale stock so far, nor comparing mercury concentrations in the G stock breeding and feeding grounds.

Given that the total mercury concentration of humpback whales belonging to the G stock has not been measure in the past, this project aims to address this lack of information. In this context, the main objectives of this study were: to assess differences in total mercury concentrations ([THg]) between: 1) tissues (blubber vs. skin tissues), 2) breeding and feeding areas (Colombian Pacific vs. Antarctic Peninsula), and 3) the sex of the organisms. This work provides the first approach regarding the mercury concentration in humpback whales belonging to the G stock.

▸ **Fig 1.** Map of the migratory route of Humpback whale G stock (Flórez-González et al., 2007) (blue arrow). Sampling areas indicated with an asterisk.

Methodology

Sampling

Skin and blubber samples were collected from humpback whales in the Antarctic Peninsula feeding grounds during the 2015 Colombian Antarctic Expedition (*n*=14) using the PAXARMS remote biopsy system (Krützen et al., 2002). Samples were also collected in the northern Colombian Pacific breeding grounds (Chocó province) in 2015 (*n*=14) and 2016 (*n*=42). This technique does not cause any major negative effects on individuals or populations to the short or long-term (Clapham & Mattila, 1993; Corkeron, Brown, Slade, & Bryden, 1994; Tezanos-Pinto & Baker, 2012). Biopsies were preserved in 70% ethanol, stored and frozen for further analysis or laboratory studies. Tissue samples were then dried and lyophilized for subsequent analyses.

Analyses of total mercury levels

Measurements of total mercury concentrations ([THg]) were performed using atomic absorption spectrometer in solid sample with AMA-454 (Advanced Altec Mercury Analyzer). Homogenized and lyophilized tissue samples ranging from 5 to 10 mg did not required chemical treatment and were analyzed in duplicates. The concentrations reported by the spectrophotometer were in μg g^{-1} units on a dry weight basis (dw), with a detection limit of 0.00001 μg g^{-1} dw (Aubail et al., 2013);

however, data is presented as mean ± standard deviation (SD) and was converted to μg kg^{-1} dry weight (dw) units.

DNA extraction and Molecular sexing

DNA extraction was performed with the Isolate II Genomic DNA Kit (Bioline, USA) according to the manufacturer's instructions. Molecular sexing was performed by means of multiplex PCR to amplify SRY male-specific gene using the primers Y53-3c, Y53-3d (Gilson, Syvanen, Levine, & Banks, 1998) and the Zfx/Zfy gene present in both males and females with the set of primers P15EZ, P23EZ (Aasen & Medrano, 1990; Bryja & Konečný, 2003; Olavarría et al., 2007; Smith, Goldizen, Dunlop, & Noad, 2008). The bands pattern observed in the electrophoresis gel was used to determine the sex of each individual.

Statistical Analyzes

A Shapiro-Wilk normality test, Bartlett test of homogeneity of variances, and an *F* test to compare two variances (i.e. for Two Sample t-test) were performed for every test to determine normality and homogeneity of variances. Square root transformation was used for [THg] since the data failed to adjust to the normal distribution.

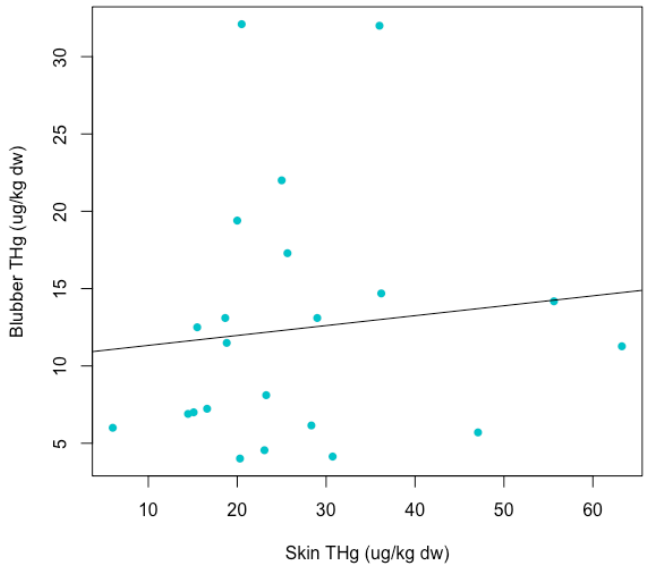
In order to assess presumed differences in the concentration of [THg] between skin and blubber tissues from the same sample (n=22) a linear regression, an Analysis of Variance (ANOVA), Tukey multiple comparisons of means test, and a two-sample t-test were performed only on biopsies which contained both skin and blubber tissues from the same individual (n=22). Moreover, to assess differences between sampling locations and sex for both types of tissue, regarding 2015 samples; an evaluation and selection of models by likelihood and Akaike Information Criterion (AICc) was carried out using the "MuMIn" package in R. Additionally, an ANOVA and Tukey multiple comparisons of means test were performed.

For the Colombian Pacific 2016 data set, an ANOVA and a Tukey multiple comparison of means test were conducted taking into account the month in which the samples were obtained (July, August, and September). Additionally, for the same data set, an ANOVA, a Tukey multiple comparison of means test, and a Two Sample t-test was performed with samples divided according to the season, (i.e. the first and second half of migration period, July to mid-August and mid-August to September, respectively) to determine if there were differences in [THg] throughout the calving season. Finally, an ANOVA and a Tukey multiple comparison of means test were performed to assess differences between Colombian Pacific 2015 and Colombian Pacific 2016 samples [THg]. The significance level was set at α=0.05.

Results

THg concentrations were successfully determined in both skin and blubber samples from humpback whales sampled in the Antarctic Peninsula and the northern Colombian Pacific. The maximum [THg] detected was 63.3 μg/kg dw in the skin of a female humpback whale sampled in

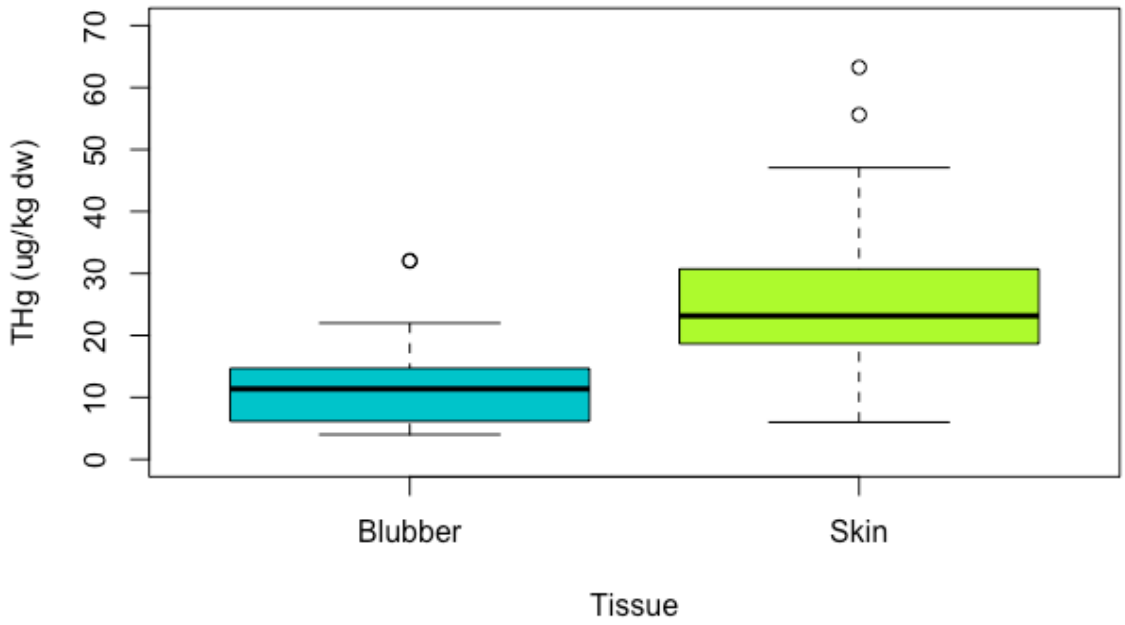
the Antarctic Peninsula in 2015, while the lowest [THg] measured was 3.63 µg/kg dw, corresponding to the skin of a female sampled in Colombia during the 2016 breeding season.



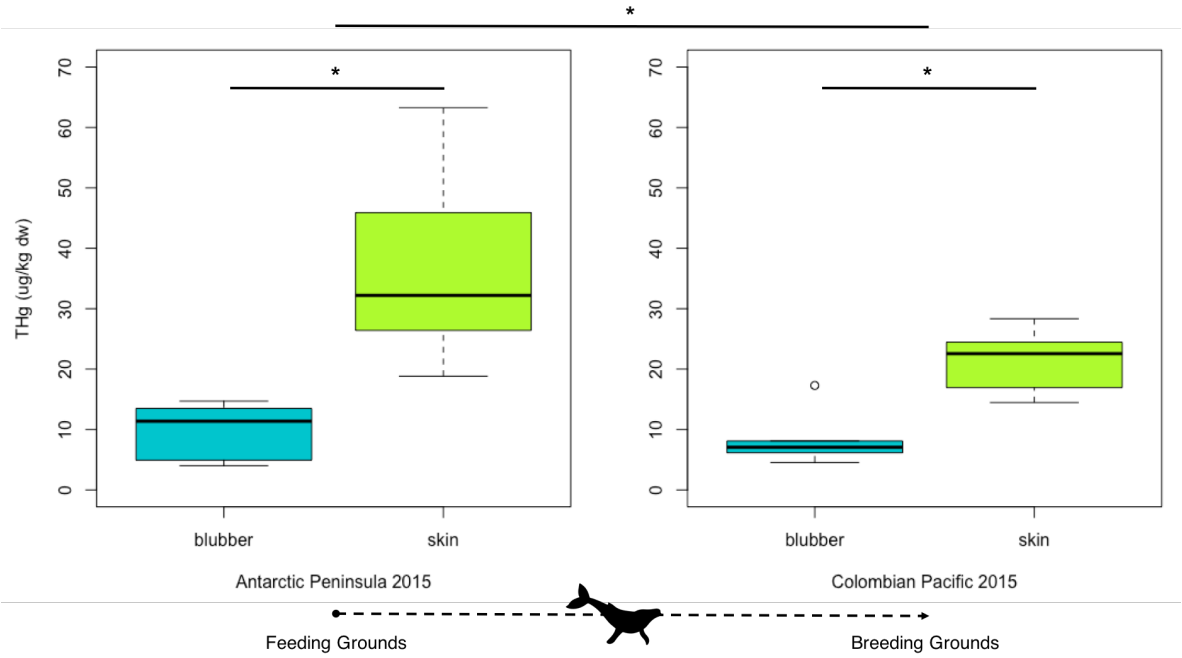
▲**Fig 2.** Linear regression of THg concentration (µg/kg dw) of humpback whale's skin and blubber tissues collected from the same individual (n=22, p>0.05).

The tissue analysis showed that there was no significant correlation between [THg] found in the skin and blubber from the same individual (p>0.05, *Fig 2*). However, there were significant differences when comparing means and variances (p>0.05, *Fig 3*) with higher [THg] found in skin (mean= 26.78 ± 13.82 µg/kg dw) than in blubber samples (mean= 12.41 ± 8.10 µg/kg dw). For 2015, the model with the best AIC included the sample area and the type of tissue but not the sex (female=21.15 ± 12.45 µg/kg, male=21.02 ± 12.07 µg/kg), meaning there were significant differences in [THg] between breeding and feeding areas. Samples from whales in the feeding grounds of the Antarctic Peninsula (2015), showed the highest [THg] in both tissues types (skin, mean=35.07 ± 13.49 µg/kg dw; blubber, mean=10.61 ± 4.11 µg/kg dw) compared to the breeding grounds in the northern Colombian Pacific (2015) (skin, mean=21.34 ± 4.79 µg/kg dw; blubber, mean=8.37 ± 4.53 µg/kg dw) (p<0.05, *Fig 4*).

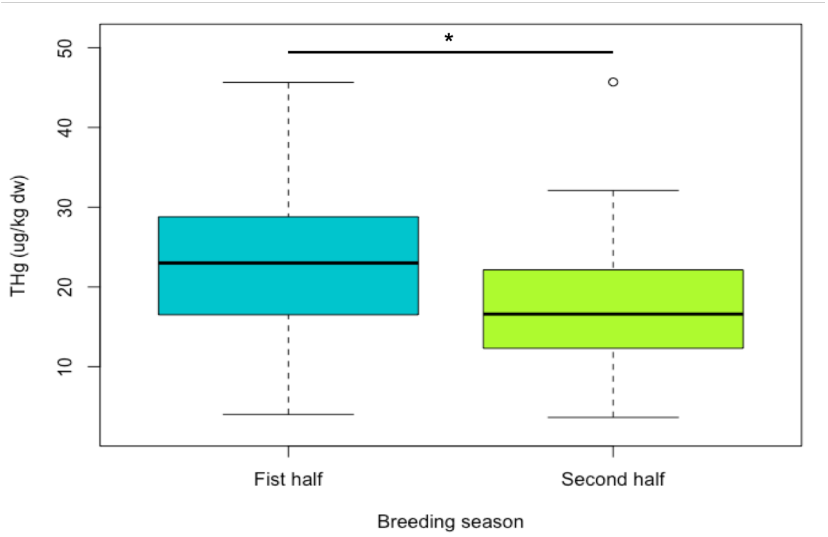
Furthermore, AIC test for 2016 data indicated that the best model was determined by the sampling month, in which July showed the highest [THg], followed by August, and finally September showing a possible decrease in the [THg]. Interestingly, while no significant differences were found between months (p>0.05), there were significant differences when comparing the first half of the season or the arrival of whales to the breeding ground in the northern Colombian Pacific, whit higher concentrations found during the second half of the breeding season (p<0.05, *Fig 5*): Finally, no significant differences were found when comparing concentration in the breeding grounds in the Colombian Pacific 2015 with 2016 (p>0.05).



▲**Fig 3.** THg concentration (µg/kg dw) in skin and blubber samples corresponding to the same individual. Significant differences indicated with an asterisk (n=22, p>0,05).



▲**Fig 4.** THg concentration (µg/kg dw) in humpback whales' skin and blubber samples from the Colombian Pacific and Antarctic Peninsula. Significant differences indicated with an asterisk (Antarctic Peninsula 2015 n=15, Colombian Pacific 2015 n=14, p>0,05).



▲**Fig 5**. THg concentration (µg/kg dw) according the sampling season in the Colombian Pacific 2016. Significant differences indicated with an asterisk (n=42, p<0.05).

Discussion

This is, to our knowledge, the first study that described and measured the total mercury concentrations in humpback whales (stock G) using skin and blubber samples, and the tissues collected from free-ranging and using non-lethal biopsy sampling methods (Aubail et al., 2013; Tezanos-Pinto & Baker, 2012).

Results showed a significant difference in [THg] between the tissues types, in which skin showed the highest concentration. This is possibly because the skin acts as an elimination route. Our results confirm what has been reported for other marine mammal species, especially molting cetaceans (Aubail et al., 2013; Wagemann & Kozlowska, 2005). While skin and blubber samples are easily obtained when remote sampling of wild organisms with a modified rifle is done, liver and kidneys are the main studied organs as they determine the toxic threshold of Hg concentrations. However, recent studies have shown a correlation in [THg] between liver and skin tissues of dolphins (Aubail et al., 2013). Therefore, it is possible to assess the health status of wild organisms using skin samples, taking account that stranded individuals could show erroneous mercury concentration if the decomposition state of the carcass is advanced (Aubail et al., 2013). Nevertheless, further studies with recently dead whales are needed to establish a relationship between concentration in internal organs vs. external tissues.

Differences found between sampling areas, which were Antarctic Peninsula (feeding area) and Colombian Pacific (breeding ground of humpback whales from the G stock), can be attributed to potential detoxification processes taking place during migration, when whales do not feed (Flórez-González et al., 2007). Detoxification mechanisms that have been studied in other species include excretion through feces, urine or hair deposition (Correa, Rea, Bentzen, & O'Hara, 2014; Nigro,

Campana, Lanzillotta, & Ferrara, 2002), demethylation (Wintle, Duffield, Barros Deceased, Jones, & Rice, 2011), interaction with selenium (Se) forming an stable insoluble compound that protects the organism from mercury toxicity (Beineke, Siebert, & Baumgärtner, 2006; Iris Cáceres-Saez, Dellabianca, Goodall, Cappozzo, & Ribeiro Guevara, 2013; Khan & Wang, 2009; Romero et al., 2016), interaction with metallothioneins (Das, Debacker, & Bouquegneau, 2000; Romero et al., 2016), and the usage of keratinous tissue such as baleens in which mercury is not excreted but trapped and immobilized, a mechanism available only to baleen whales (Hobson et al., 2004). Furthermore, no significant difference was found between females and males as it has been reported in other cetacean species (Aubail et al., 2013; Dirtu et al., 2016; O'Shea & Brownell, 1994). This results could be explained by the fact that males and females share the same type of prey, forage in the same areas and at the same trophic level (Acevedo et al., 2017; Chouvelon et al., 2017).

Although no significant differences were found, [THg] showed a decreasing tendency throughout the 2016 breeding season, which corroborated the tendency of reducing the metal concentration observed in the 2015 data. In other words, in the months in which the whales are not feeding due to migration and reproductive behavior in the calving area, the possible detoxifying mechanisms mentioned above can take place, and changes in the [THg] are evident, since mercury is not ingested during this period. Furthermore, significant differences were found when comparing [THg] during their arrival to Colombian waters, which marks the beginning of the calving season in contrast to the late season, closer in time to the whales return to the Antarctic Peninsula. Nonetheless, it has been reported that mercury presents a long half-life in the organisms and a slow elimination rate compared to the ingestion rate and high affinity too sulfhydryl groups that leads an increase of concentrations or bioaccumulation in the long-term (Cáceres-Saez et al., 2013).

It is of high concern that mercury is a pollutant that can cross the placental barrier and accumulate in developing organisms, leaving fetuses vulnerable to neurodegenerative diseases due to the neurotoxicity and premature death, which is devastating to an species that is technically still recovering (Romero et al., 2016). Similarly, it is estimated that around 1000 tons of waste mercury from mining are released to the environment each year (Güiza & Aristizábal, 2013). Colombia is one of the three main mercury emitters, along with China and Indonesia (Li et al., 2017), and having a greater amount of emissions than Australia, Canada, and the USA together, which are the world's main gold producers of the world (Li et al., 2017). Therefore, this is a risk to the humpback whale G stock, especially if any opportunistic feeding is occurring in the breeding ground, as it was reported for Australian and Mexican populations (Gendron, 1993; Owen et al., 2017). Due to the high pluviosity that characterizes the Chocó region and the resulting freshwater input from the continent into coastal waters, these whales could be exposed to high mercury concentrations since the coast is prone to have high concentrations of mercury due to anthropogenic pollution and illegal mining (De Miguel, Clavijo, Ortega, & Gómez, 2014; Dirtu et al., 2016), that could result in a long-term impact.

Similarly, it has been reported that in skin samples collected from Antarctic Minke whales had [THg] ranging from 38.00 ± 8,00 µg/kg dw to 96.00 ± 42,00 µg/kg in males, and from 49.00 ± 12.00 µg/kg dw to 68.00 ± 27.00 µg/kg dw in females (Kunito et al. 2002). Furthermore, [THg] levels in whale meat products sold for human consumption in Japan markets show low concentrations, interpreted as a result of the low trophic levels occupied by these species (Endo et al., 2004, 2005; Endo, Hotta, Haraguchi, & Sakata, 2003; Endo, Kimura, Hisamichi, Minoshima, & Haraguchi, 2007). Even if these whale species have similar diet than humpback whales (Sanders et al., 2015), differences can be explained by the oceanic mercury burdens and the amount of mercury that enters the food web depending of the feeding area. However, these findings indicate that [THg] are linked to the area in which whales are feeding, due to the fact that mercury is circulating through the food chain being transported by atmospheric and oceanic currents to Antarctic Peninsula (Driscoll, Mason, Chan, Jacob, & Pirrone, 2013; Lehnherr, 2014). Although whales are not top predators, it is worrisome that they are bioaccumulating, so it is important to develop future studies to monitor the [THg] in Antarctic top predators.

Conclusions

Total mercury concentrations were determined for the first time for humpback whales from the G stock. Significant differences were found in skin and blubber tissues, that were collected by using biopsy sampling, a non-lethal sampling technique. Similarly, there were significant differences in [THg] between areas, with higher concentrations for the Antarctic Peninsula. This result is understandable since this area constitutes a feeding ground where the pollutant is being consumed. In contrast, the Colombian Pacific is a breeding area with lower concentrations of [THg] as no feeding behavior has been reported and detoxification processes can be evident. No significant differences were found depending on the sex of the sampled individual. Further studies are needed to measure internal organs [THg] as well as other metals and pollutants which can be used as a proxy of the health of this population. We hypothesize that, if feeding is occurring in the breeding area as it occurs in Australia and Mexico, whales could be ingesting high concentrations of mercury due to mining activities along the Pacific coast of Colombia (De Miguel et al., 2014). At present, research efforts are being carried out to test this hypothesis.

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