Comparison of total Hg levels in O and J type stock of common minke whales based on JARPNII coastal samples collected in 2012 and 2013

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ABSTRACT

The purpose of the present study was to examine differences of total Hg concentrations in muscle and liver of J- and O-stocks of common minke whales (*Balaenoptera acutorostrata*) from the waters off Sanriku. Concentrations of total Hg in muscle and liver of 35 O- and 24 J-stock immature minke whales taken in the 2012 and 2013 JARPNII surveys were measured. Multiple linear regression analyses of total Hg concentrations of the whales were carried out. These included adjustment for confounders, age index, sex, stock, blubber thickness and year. Stock had no discernible effect. These findings suggest that there is no stock-dependent difference of total Hg exposure risk for the minke whales from off Sanriku.

KEYWORDS: COMMON MINKE WHALE; NORTH PACIFIC; MERCURY; STOCK; O-STOCK; J-STOCK

INTRODUCTION

The Scientific Committee held two specialized Workshops, one relating to chemical pollutants and cetaceans, and the other on the potential ecological effects on cetaceans of climate change and ozone depletion (IWC, 1994). In the JARPNII plan, the following objective 2 was addressed, 'Monitoring environmental pollutants in cetaceans and the marine ecosystem'. This objective contains sub-objective, 'Pattern of accumulation of pollutants in cetaceans' (Government of Japan, 2002).

Anthropogenic Hg emissions from land to the atmosphere in Asia have increased steadily over the past several decades (Pacyna *et al.*, 2006), whereas those in Europe, Russia and North America peaked in the 1950s to 1970s (UNEP, 2013). It is important to assess Hg exposure of whales in nearshore waters of Asia for better understanding and the management of health risks. IWC has raised a concern about the effects of pollutants on cetaceans because of the possibility that these pollutants negatively affect the whales' health resulting in a decrease in the abundance of the stocks (IWC, 1994). Furthermore, age, nutritional condition and sex are important to examine accumulation features of Hg in baleen whales (Fujise *et al.*, 1997; Aguilar *et al.*, 1999; Honda *et al.*, 2006).

In the present study, we measured total Hg concentrations in muscle and liver to evaluate differences of total Hg exposure in O and J of minke whales from off Sanriku, using multiple regression analysis including adjustment for confounders, sampling years, age, nutritional condition and sex. Racemization of aspartic acid in lens was used for age index determination (George *et al.*, 1999; Yasunaga *et al.*, 2014), and blubber thickness was used for nutritional condition index (Ohsumi *et al.*, 1997; Konishi *et al.*, 2008).

MATERIALS AND METHODS

Samples

Muscle and liver samples were collected for total Hg analyses from 59 immature of common minke whales of both sexes from the 2012 and 2013 JARPNII coastal survey in coastal waters within 50 n. miles from Ayukawa port in the Sanriku district (Fig. 1). The muscle tissues were excised from the central dorsal part of the body, liver tissues were excised from the central part of the organ, and eye lenses were taken from the left eyeball at the research station at Ayukawa town. Soft tissues and eye lenses were stored in polyethylene bags at -20 and -80°C until analysis, respectively.

In this study we considered information on the body length and maturity status of male whales. Males and females of minke whales were defined as sexually mature by testis weight (larger side) of more than 290g and by observation of ovary, respectively (Bando *et al.*, unpublished data). Common minke whales from the Okhotsk Sea/Western Pacific ('O' stock) and the Yellow Sea/East China Sea/Sea of Japan ('J' stock) were identified by microsatellite analysis (Kanda *et al.*, 2009).

Laboratory analysis

For total Hg analysis, the tissue homogenate samples (approximately100 mg) were set on a ceramic boat and subjected to cold-vapor atomic absorption spectrometry with heat-vaporization, gold amalgamation method (Nippon Instruments Co., MA-2000). Accuracy and precision of the methods were confirmed using standard materials, DORM-2 (NRCC: muscle of dogfish).

For age index determination by aspartic acid racemization in lens, the lens samples were stripped externally to avoid contamination and homogenized with Tris-buffer (200mM Tris, 150mM NaCl, pH 8.0) using ultrasonic disruptors. The homogenate was centrifuged at 15,000 × g for 15 min. at 4°C, then desalted with acetone and air-dried. The sample re-dissolved in water was then set up within a glass tube for hydrolysis and freeze-dried. The purified samples were lyophilized in tubes and were hydrolyzed in a gas-phase 6N-HCl for 7 hr at 108°C (Pico Tag Work Stations, Waters, Tokyo). The hydrolysates were evaporated under reduced pressure. The hydrolysates were dissolved and incubated in 0.1N-HCl and Borate buffer (0.1M, pH10.4), and were further incubated with o-phthalaldehyde (OPA) and n-tert-butyloxycarbonyl-L-cysteine (Boc-L-Cys) to form diastereoisomers. The D/L ratio of aspartic acid was determined using RP-HPCL (Alliance ® HPLC systems e2696, Waters) with a Nova-Pak ODS column (3.9mm ×300mm, Waters) using fluorescence detection (344 nm excitation wavelength and 433 nm emission wave length). Elution was carried out with a simple isocratic adsorption of 3% acetonitril+3% tetrahydrofuran /0.1M acetate pH6.0 buffer solution in 45 min at a flow rate of 0.8 ml/min, at 23°C. Sensitivity of intensity for both aspartic acid enantiomers was corrected by L50ppm/D2.5ppm-Aspartic acid (minimum 99% TLC, SIGMA-ALDRICH) solution.

Statistical analysis

Factors affecting total Hg concentrations in whales were assessed using multiple linear regression analyses (Zar, 1999). The following independent variables: "sampling year (2012=0; 2013=1)", "age index", "sex (male=0; female=1)", "stock (O stock=0, J stock=1)" and "blubber thickness", were allowed, and all parameters were normalized. These statistical analyses were executed with SPSS ver.11 for Windows (SPSS Co. Ltd.).

RESULTS AND DISUCSSION

Total Hg levels in muscle and liver of common mike whales were 0.06 ± 0.05 and 0.15 ± 0.11 ppm wet wt., respectively. Plots of total Hg concentrations against their age index are shown for muscle (Fig. 2) and liver (Fig. 3). To examine the factors affecting Hg concentrations in minke whales, multiple regression linear analyses were conducted. The results for total Hg in muscle and liver of minke whales are shown in Tables 2 and 3, respectively. Regression models were significant for total Hg concentrations in both organs. For muscle of minke whales, age index (+) and sex (male < female) were significantly selected (Table 1), and for liver of whales, only sex (male < female) were significantly selected (Table 2).

Significant association between total Hg concentrations in muscle of minke whales and their age index were observed. This result is consistent with previous studies (Honda *et al.*, 1983; Aguilar *et al.*, 1999). However, concentrations in liver of minke whales were not significantly associated, whereas there seems to be association between total Hg levels in liver and age index. The reasons are likely sample size and use of immature individuals. Female was a positive factor for total Hg concentrations in both muscle and liver of minke whales. The results are also consistent with the previous study (Aguilar *et al.*, 1999). On the other hand, stock of minke whales was not significantly associated with total Hg concentrations. These results suggest that there is no difference of total Hg exposure for both stocks of the minke whales from off Sanriku. In addition, total Hg levels in both stocks from off Sanriku were extremely lower than that which would have adverse effects on whales.

REFERENCES

Aguilar, A., Borrell, A. and Pastor, T. 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. *J. Cetacean Res. Manage.*, (Special Issue 1):83-116.

Fujise, Y., Honda, K., Yamamoto, Y., Kato, H., Zenitani, R. and Tatsukawa, R. 1997. Changes of hepatic mercury accumulations of southern minke whales in past fifteen years. Paper SC/M97/20 presented to the IWC Intercessional Working Group to Review Data and Results from Special Permit Research on minke whales in the Antarctic, May 1997 (unpublished). 16pp.

George, J.C., Bada, J., Zeh, H., Scott, L., Brown, S.E., O'Hara, T. and Suydam, R. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. *Can. J. Zool.* 77:571-80.

Government of Japan. 2002. Research Plan for Cetacean Studies in the Western North Pacific under Special Permit (JARPNII). Paper SC/54/O2 presented to the IWC Scientific Committee, May 2002 (unpublished). 46pp.

Honda, K., Tatsukawa, R., Itano, k., Miyazaki, N. and Fujiyama, T. 1983. Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. *Agric. Biol. Chem.* 47:1219-1228.

Honda, K., Aoki, M. and Fujise, Y. 2006. Ecochemical approach using mercury accumulation of Antarctic minke whale, *Balaenoptera bonaerensis*, as tracer of historical change of Antarctic marine ecosystem during 1980-1999. *Bull. Environ. Contam. Toxicol.* 76:140-7.

International Whaling Commission. 1994. Chairman's Report of the Forty-Fifth Annual Meeting, Appendix 12. Resolution on research on the environment and whale stock. *Rep. int. Whal. Commn.* 44:35.

Kanda et al. 2009. Individual identification and mixing of the J and O stocks around Japanese waters examined by microsatellite analysis. Paper SC/J09/JR26 presented to the JARPNII Review Workshop, Tokyo, January 2009 (unpublished). 9pp.

Konishi, K., Tamura, T. Zenitani, R., Bando, T., Kato, H. and Walløe, L. 2008. Decline in energy storage in the Antarctic minke whale (*Balaenoptera bonaerensis*) in the Southern Ocean. Polar Biol., 31: 1509–1520.

Ohsumi, S., Fujise, Y., Ishikawa, H., Hakamada, T, Zenitani, R. and Matsuoka, K. 1997. The fattyness of the Antarctic minke whale and its yearly change. Paper SC/M97/18 presented to the IWC Intersessional Working Group to Review Data and Results from Special Permit Research on Minke whales in the Antarctic, May 1997 (unpublished). 21pp.

Pacyna, E., Pacyna, J., Steenhuisen, E. and Wilson, S. 2006. Global anthropogenic mercury emission inventory for 2000. *Atmos. Environ.* 40:4048-4063.

UNEP. 2013. Global Mercury Assessment 2013: Sources, emissions, releases, and environmental transport. Pp42. http://www.unep.org/publications/contents/pub_details_search.asp?ID=6282

Yasunaga, G., Bando, T. and Fujise, Y. 2014. Preliminary estimation of the age of Antarctic minke whales based on aspartic acid racemization. Paper SC/F14/J12 presented to the JARPA II Review Workshop, Tokyo, February 2014 (unpublished). 10pp.

Zar, J.H. 1999 *Biostatistical analysis*, 4th ed., Prentice Hall, New Jersey, USA, 663pp.

Table 1. Biological data and total mercury concentrations in muscle and liver of common minke whales (males) from western North Pacific during 1994 and 2014

Year	Stock	Sex	*n*	Body length (m)	Age Index*	Blubber thickness (cm)	Hg in muscle (ppm wet wt.)	Hg in liver (ppm wet wt.)
2012	O	Male	8	4.59 ± 0.39	0.0393 ± 0.0020	3.1 ± 0.6	0.03 ± 0.05	0.093 ± 0.112
				(4.02 - 5.31)	(0.0361 - 0.0416)	(2.1 - 4.1)	(<0.001 - 0.14)	(0.013 - 0.35)
		Female	9	4.99 ± 0.53	0.0407 ± 0.0032	3.2 ± 0.7	0.05 ± 0.05	0.17 ± 0.12
				(4.37 - 5.97)	(0.0361 - 0.0454)	(2.3 - 4.3)	(<0.001 - 0.12)	(0.003 - 0.32)
	J	Male	7	4.73 ± 0.52	0.0402 ± 0.0032	3.3 ± 0.6	0.06 ± 0.07	0.20 ± 0.18
				(3.92 - 5.44)	(0.0344 - 0.0448)	(2.8 - 4.4)	(<0.001 - 0.17)	(0.007 - 0.51)
		Female	5	5.11 ± 0.60	0.0411 ± 0.0035	3.4 ± 0.4	0.07 ± 0.03	0.16 ± 0.05
				(4.61 - 6.1)	(0.0374 - 0.0456)	(2.9 - 3.9)	(0.041 - 0.1)	(0.10 - 0.22)
2013	O	Male	10	4.63 ± 0.68	0.0426 ± 0.0027	2.8 ± 0.5	0.03 ± 0.03	0.11 ± 0.07
				(3.73 - 5.73)	(0.0390 - 0.0477)	(1.9 - 3.4)	(0.004 - 0.1)	(0.029 - 0.23)
		Female	8	5.32 ± 0.55	0.0449 ± 0.0026	3.1 ± 0.4	0.07 ± 0.06	0.16 ± 0.13
				(4.14 - 5.88)	(0.0411 - 0.0491)	(2.4 - 3.8)	(0.009 - 0.19)	(0.059 - 0.45)
	J	Male	4	4.80 ± 0.97	0.0433 ± 0.0023	2.7 ± 0.6	0.06 ± 0.03	0.18 ± 0.08
				(3.70 - 5.66)	(0.0414 - 0.0453)	(2.0 - 3.3)	(0.03 - 0.093)	(0.11 - 0.28)
		Female	8	5.27 ± 0.55	0.0464 ± 0.0045	2.7 ± 0.7	0.09 ± 0.03	0.20 ± 0.06
				(4.72 - 6.14)	(0.0416 - 0.0556)	(1.8 - 3.7)	(0.038 - 0.13)	(0.081 - 0.28)
Total			59	4.92 ± 0.62	0.0423 ± 0.0038	3.0 ± 0.6	0.06 ± 0.05	0.15 ± 0.11
				(3.70 - 6.14)	(0.0344 - 0.0556)	(1.8 - 4.4)	(0.00 - 0.19)	(0.003 - 0.51)

*: Age index=Ln (1+AspD/L)/(1-AspD/L)

Table 2. Results of multiple linear regression analyses with "total Hg levels in muscle of common minke whales from off Sanriku" as the dependent variable.

a) Model of regression

Model	R	R2	R2'
1	0.856	0.747	0.724

b) Analysis of Variance Table

Model	Sum of Squares	DF	Mean Square	F value	P value
Regression	0.103	5	0.021	31.377	<0.001
Residual	0.035	53	0.001		
Total	0.138	58			

c) Variables

Model	B	SE	B'	T	P value
Constant	0.155	0.052		3.004	**0.004**
Age index	0.016	0.003	0.555	5.175	**<0.001**
sex	0.018	0.005	0.362	3.609	**0.001**
stock	-0.083	1.079	-0.006	-0.077	0.939
blubber thickness	0.004	0.007	0.038	0.502	0.618
year	0.001	0.007	0.015	0.200	0.842

Table 3. Results of multiple linear regression analyses with "total Hg levels in liver of common minke whales from off Sanriku" as the dependent variable.

a) Model of regression

Model	R	R2	R2'
1	0.854	0.729	0.703

b) Analysis of Variance Table

Model	Sum of Squares	DF	Mean Square	F value	P value
Regression	0.503	5	0.101	28.477	<0.001
Residual	0.187	53	0.004		
Total	0.691	58			

c) Variables

Model	B	SE	B'	T	P value
Constant	0.428	0.120		3.579	0.001
Age index	0.011	0.007	0.163	1.470	0.148
sex	0.082	0.011	0.758	7.297	<0.001
stock	-1.173	2.502	-0.040	-0.469	0.641
blubber thickness	-0.003	0.017	-0.016	-0.202	0.841
year	-0.003	0.017	-0.013	-0.171	0.865

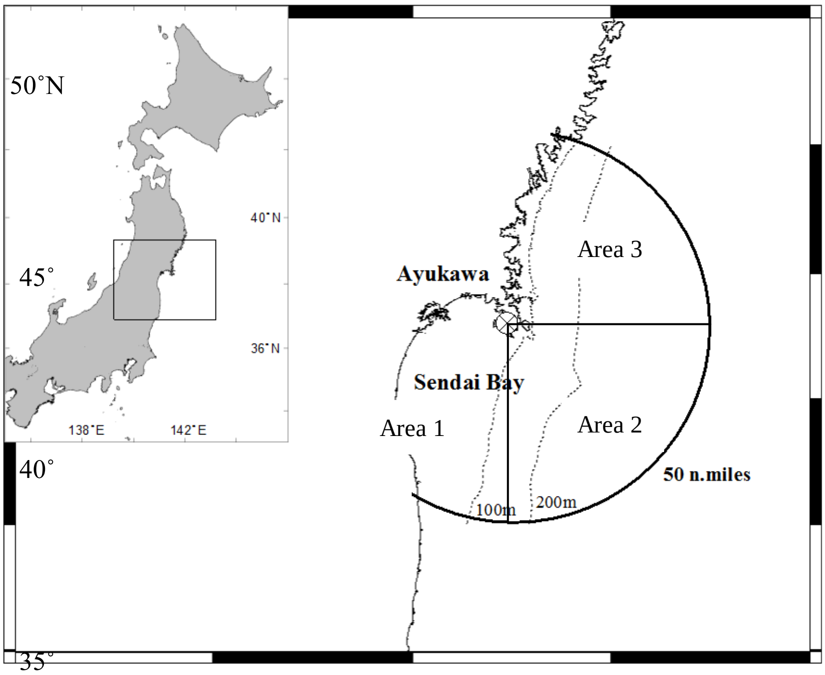

Figure 1. Research area of the JARPNII coastal survey off Sanriku

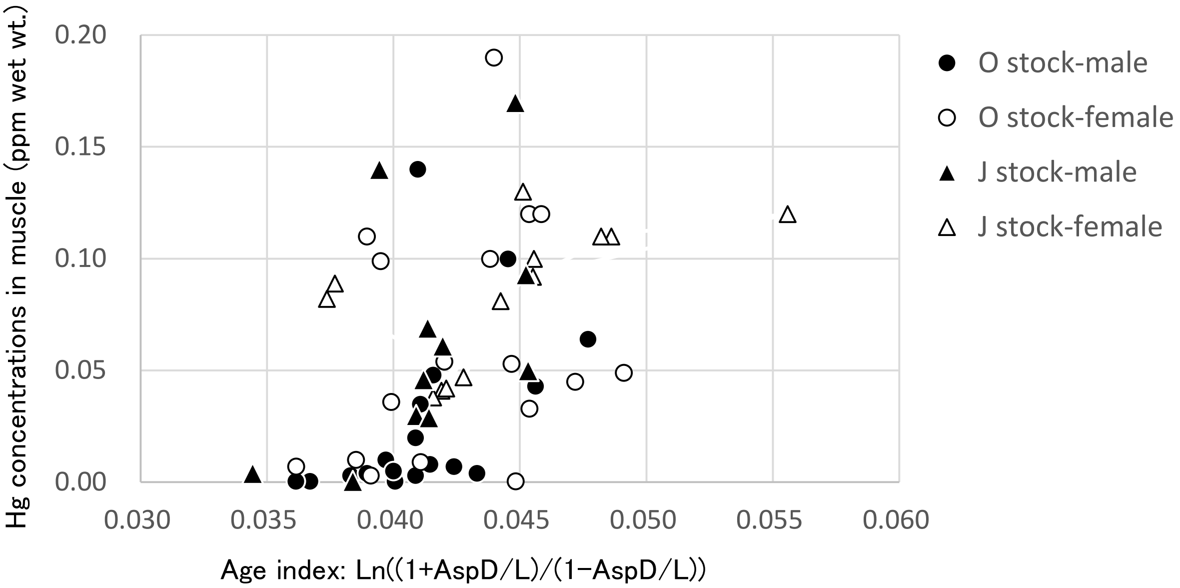

Figure 2. Relationship between Hg concentrations (ppm wet wt.) in muscle and age index (Ln ((1+AspD/L)/(1-AspD/L))) in common minke whales from off Sanriku

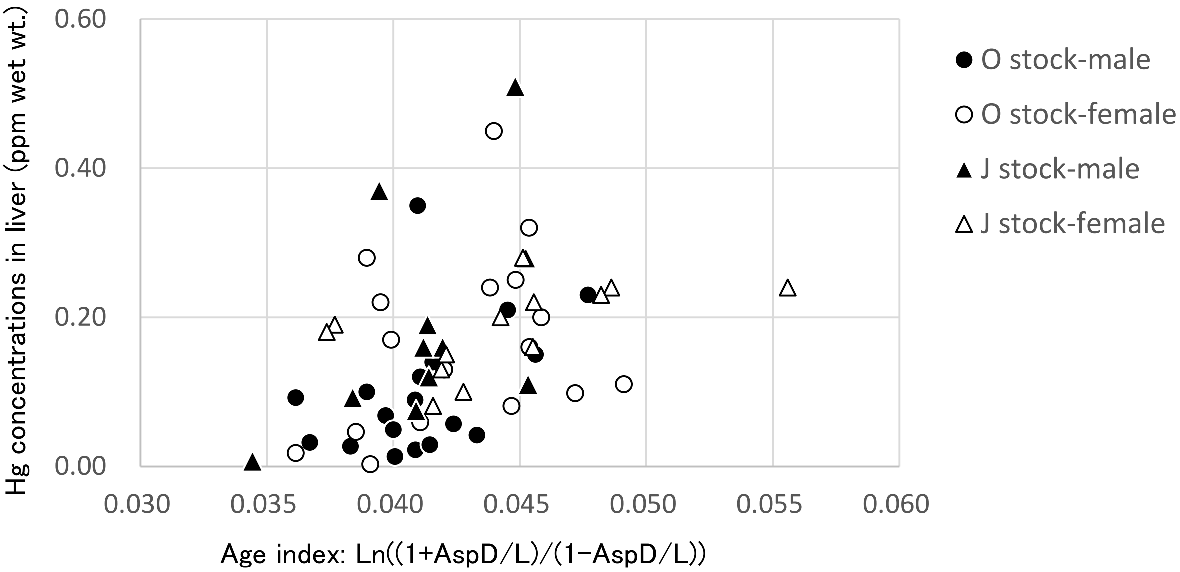

Figure 3. Relationship between Hg concentrations (ppm wet wt.) in liver and age index (Ln ((1+AspD/L)/(1-AspD/L))) in common minke whales from off Sanriku