Accumulation features of total and methyl mercury and selenium in tissues of common minke, Bryde's and sperm whales from the western North Pacific

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ABSTRACT

To examine the inter-species difference of sensitivity to mercury toxicity in whales, total mercury (T-Hg), methyl mercury (MeHg) and selenium (Se) levels in the liver, kidneys and muscle of three whales (common minke whales *Balaenoptera acutorostrata*; Bryde's whales *B. edeni*; sperm whales *Physeter. macrocephalus*) were measured. T-Hg and MeHg were higher in the order of sperm whale > common minke whale > Bryde's whale, and their Se levels were higher in the order of sperm whale > Bryde's whale > common minke whale. The order of the T-Hg and Se levels in the tissues of the common minke and Bryde's whales was kidneys > liver > muscle, and that of MeHg was liver > muscle > kidneys. The order of the T-Hg and Se levels in the tissues of sperm whales was liver > kidneys > muscle. MeHg in the animal body is preferentially distributed in the liver and kidneys containing abundant metal binding proteins such as metallothioneins. The Se concentrations in the liver of marine mammals are correlated with the total mercury concentrations. MeHg/T-Hg (%) in the liver and kidneys of sperm whales were higher than those of the baleen whales in the same total Hg levels, while MeHg/T-Hg (%) vary inversely with T-Hg concentrations in the liver and kidneys of sperm, common minke and Bryde's whales. Accumulation of high concentrations of inorganic Hg in the liver and kidneys were probably due to a difference of demethylation ability. These results indicate the potential usefulness of assessment of Hg toxicity in large whales.

KEYWORDS: COMMON MINKE WHALE; BRYDE'S WHALE; SPERM WHALE; NORTH PACIFIC; MERCURY; SELENIUM; BIOACCUMULATION

INTRODUCTION

At the IWC 44th Annual Meeting, the Commission endorsed the plans of the Scientific Committee to pursue studies on environmental changes and their impacts on cetaceans (IWC Resolution 1994/13). The second objective of the JARPN II research plan is "monitoring environmental pollutants in cetaceans and the marine ecosystem" and a part of this objective involved examining the "Relationship between chemical pollutants and cetacean health". Metabolic capacity to handle organochlorines in cetaceans depends on the species. (Tanabe *et al.*, 1988; Neathery and Miller, 1975). It is essential to clarify the species specific sensitivity of the chemical substances to assess their impact for the wildlife. Methyl mercury is one of most harmful chemical for cetaceans due to its highly-absorptibity and adverse function in marine ecosystems. Mercury is mainly released as elemental Hg on land by natural sources, such as volcanic activities and weathering, and anthropogenic sources, such as thermal power plant and several mining activities. Once released elemental Hg is transferred to pelagic ocean and so is distributed to pelagic surface water in according to gas-liquid change. In pelagic ocean, elemental Hg is converted to methyl Hg and it is moderately bioconcentrated in animals of high trophic level such as cetaceans through the food chain. Therefore mercury is one of the most risky chemicals for cetaceans in the ocean.

Mercury was positively associated with selenium (molar ratio 1:1) in cetaceans (Koeman *et al.*, 1973). Mercury is concentrated in the liver of cetacean and is mostly consisted in inorganic Hg especially among individual contained higher Hg levels (Pelletier *et al.*, 1985, Itano *et al.*, 1985). It is known that interaction between Hg and Se (Augier et al., 1993), and demethylation of methyl Hg are important detoxification process of MeHg (Wargemann *et al.*, 1997) in cetaceans, considering that these would be several processes (Palmisano *et al.*, 1995).

Few studies have examined inter and intra species differences of interaction among methyl Hg, inorganic Hg and Se with several trophic levels cetaceans, while there are much of the reports related methyl Hg detoxification processes. In this study, accumulation feature of mercury , toxic element, and selenium , antagonist, are clarified, and inter-species difference of sensitivity to mercury toxicity are examined. We provide ecotoxicological information related with MeHg risk for free-ranging whales in the western North Pacific.

MATERIALS AND METHODS

Samples and sampling method

Liver, kidneys and muscle tissues from the common minke whales from sub-areas 7 and 9, Bryde's whales from sub-area 7, and sperm whales from sub-area 7 were collected by JARPN II researchers in the 2000 surveys (Fig. 1). Table 1 shows the sample sizes and body length of the whales sampled, by species, sex and sub-area. The tissue samples were frozen and shipped to the laboratory and stored -20˚C until chemical analysis. In order to understand the pattern of accumulation of pollutants in cetaceans, it is essential to consider some biological information from whales. In this study we considered information on the body length and sex of whales. The tissues of all specimens were excised from the medial region of body.

Laboratory analysis

The tissue samples were sent to the Miura Institute of Environmental Science (Ehime, Japan) for T-Hg, MeHg and Se analyses. Tissue samples were digested in microwave (Milestone General: MLS1200mega) using nitric acid in a PTFE vessel. T-Hg concentrations were determined by cold vapor / atomic absorption spectrometry (Nippon Instruments Co. RA-2A), Se concentrations were determined by absorption spectrophotometry (Beckman Co., DU-650), and MeHg concentrations were determined by GC-ECD (Hewlett Packard Co. Ltd., 5890 Series II) according to Westöö method (Westöö, 1968). Concentrations were given on wet weight basis. Accuracy and precision of the methods were confirmed using bovine liver (BCR-CRM No. 185).

Statistical analysis

The correlations between chemical and biological parameters were assessed by Spearmann rank test (Zar, 1999). These statistical analyses were executed by SPSS ver.11 for Windows (SPSS Co. Ltd.).

RESULTS AND DISCUSSION

Accumulation status

Tables 2a-c show the concentrations of total Hg, methyl Hg and Se, molar ratio of T-Hg / Se and MeHg / Se and percentage of MeHg / T-Hg in the liver, kidneys and muscle of minke, Bryde's and sperm whales from the western North Pacific. T-Hg and MeHg levels in the liver, kidneys and muscle were higher in the order of sperm whale > minke whale > Bryde's whale, and their Se levels were higher in the order of sperm whale > Bryde's whale > minke whale. The order of the T-Hg and Se levels in the tissues of the minke and Bryde's whales was kidneys > liver > muscle, and that of MeHg was liver > muscle > kidneys. The order of the T-Hg and Se levels in the tissues of sperm whales was liver > kidneys > muscle. MeHg in the animal body is preferentially distributed in the liver and kidneys containing abundant metal binding proteins such as metallothionein (Wagemann *et al.*, 1984). The Se concentrations in the liver of marine mammals are correlated with the T-Hg concentrations (Koeman *et al.*, 1973). Our results are accordance with previous studies.

Relationships among T-Hg, MeHg and Se

Plots of relationships MeHg against T-Hg concentrations in liver, kidneys and muscle of minke, Bryde's and sperm whales are shown in Fig. 2. The concentrations of MeHg lineally increased with T-Hg in kidneys and muscle. The inclinations of minke and Bryde's whales were higher than that of sperm whales in the plot of liver. This indicated that the livers of sperm whales contained higher inorganic Hg than those of the other baleen whales.

Plots of relationships Se against T-Hg concentrations in liver, kidneys and muscle of minke, Bryde's and sperm whales are shown in Fig. 3. In only liver of sperm whales Se and T-Hg molar ratio is approximately 1:1, while no relation was observed in those of the others. Se was strongly induced in the case of higher Hg existence, while concentrations of Se, one of essential elements, in the tissue and organs were not relatively affected by several varying environments due to homeostasis. On the other hand, renal Se of sperm whales was clearly induced with MeHg rather than those of liver (Fig. 4). Therefore, Se mainly detoxification inorganic Hg in liver and MeHg in kidneys for sperm whales.

Relationships between T-Hg/MeHg and body length

To examine the relationships between demethylation of MeHg in the organ and growth, plots of MeHg/T-Hg (%) against body length (m) are shown Fig. 5. Correlation of MeHg/T-Hg (%) and body length were significantly negative in kidneys of male of common mike whales and in liver of male and female of Bryde's whales ($p<0.05$). This result is attributed to difference of biological half-life of inorganic Hg and MeHg in whale body.

Relationships between MeHg/T-Hg and T-Hg

Plots of relationships MeHg/T-Hg (%) against T-Hg concentrations in liver, kidneys and muscle of minke, Bryde's and sperm whales are shown in Fig. 6. MeHg/T-Hg were clearly decreased with T-Hg concentrations in liver and kidneys of all whales. And also, these curve is distinguished in each whales. MeHg/THg (%) in the liver and kidneys of sperm whales were higher than those of the baleen whales in the same total Hg levels, while methyl Hg/total Hg (%) vary inversely with THg concentrations in the liver and kidneys of sperm, minke and Bryde's whales. Accumulation of high concentrations of inorganic Hg in the liver and kidneys were probably due to a difference of demethylation ability. Considering the biological half-life of Hg (approximately two or three hundred days), if a different accumulation level of Hg was observed between two groups, this suggests that they ate the different prey species and/or their feeding area are different.

CONCLUSION

In al the species, lower percentages of MeHg to T-Hg levels and positive correlations between T-Hg and Se were observed. Demethylation process and interaction with Se play pivotal role in detoxification Hg in large baleen whales. The Se concentrations in the liver of marine mammals are correlated with the total mercury concentrations. MeHg/T-Hg (%) in the liver and kidneys of sperm whales were higher than those of the baleen whales in the same total Hg levels, while MeHg/T-Hg (%) vary inversely with T-Hg concentrations in the liver and kidneys of sperm, minke and Bryde's whales. Accumulation of high concentrations of inorganic Hg in the liver and kidneys were probably due to a difference of demethylation ability. These results indicate the potential usefulness of assessment of Hg toxicity to large whales.

REFERENCES

Augier, H., Benkoel, L., Chamlian, A., Park, W. K. and Ronneau, C. 1993. Mercury, zinc and selenium bioaccumulation in tissues and organs of Mediterranean striped dolphins *Stenella coeruleoalba* meyen. Toxicological result of their interaction. *Cell. Mol. Biol.*, **39**, 621-634.

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.

Itano, K., Kawai, S. and Tatsukawa, R. 1985. Distribution of mercury and selenium in muscle of striped dolphins. Bulletin of the Japanese Society of Scientific Fisheries, 51, 1129-1131.

Koeman, J. H., Peeters, W. H. M., Koudstaal-Hol, C. H. M., Tjioe, P. S. and de Goeij, J. J. M. 1973. Mercury-selenium correlations in marine mammals. *Nature*, **245**, 385-386.

Neathery, M.W. Miller, W.J. 1975. Metabolism and toxicity of cadmium, mercury, and lead in animals: a review. *J. Dairy Sci.* 58:1767-81.

Palmisano, F., Cardellicchio, N. and Zambonin, P. G. 1995. Speciation of mercury in dolphin liver: A two-stage mechanism for the demethylation accumulation process and role of selenium. *Marine Environmental Research*, **40**, 109-121.

Pelletier, E. 1985. Mercury-selenium interactions in aquatic organisms: a review, *Mar. Environ. Res.*, **18**, 111-132.

Tanabe, S., Watanabe, S., Kan, H. and Tatsukawa, R. 1988. Capacity and mode of PCB metabolism in small cetaceans. *Marine Mammal Science* 4:103-124.

Wagemann, R., Hunt, R. and Klaverkamp, J.F. 1984. Subcellular distribution of heavy metals in liver and kidney of a nurwhal whale (*Monodon monoceros*): an evaluation for the presence of metallothionein. *Comp. Biochem. Physiol.*, 78C: 301-307.

Wagemann, R., Trebacz, E., Hunt, R. and Boila, G. 1997. Percent methylmercury and organic mercury in tissues of marine mammals and fish using different experimental and calculation methods. *Environ. Toxicol. Chem.*, **16**, 1859-1866.

Westöö. 1968. Determination of methylmercury salts in various kinds of biological material. Determination of methylmercury compounds in foodstuffs I. Methylmercury compounds in Fish, idnetification and determination. *Acta Chemica Scandinavica* 20:2131-2137.

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

Table 1. Details of common minke, Bryde's and sperm whales analyzed.

Species (*Academic name*)	Sex	Area (*n*)	Body length* (m)
common minke whale	males	sub-area 7 (19)	6.66 (4.70-7.82)
(*Balaenoptera acutorostrata*)		sub-area 9 (16)	7.31 (4.71-7.75)
	females	sub-area 7 (5)	7.67 (7.17-8.52)
Bryde's whale	males	sub-area 7 (21)	12.02 (10.28-13.22)
(*Balaenoptera edeni*)	females	sub-area 7 (22)	12.39 (8.54-14.11)
sperm whale	males	sub-area 7 (3)	10.95 (8.82-12.77)
(*Physeter macrocephalus*)	females	sub-area 7 (2)	9.89 (8.17-11.61)

*: Nmubers represent average values (ranges) of body length

Table 2a. Total Hg , methyl Hg and Se levels (mean and range, μg/g wet), ratio of both Hg to Se (mean and range, molar ratio) and percentage of methyl to total Hg (mean and range, %) in the liver of minke, Bryde's and sperm whales.

Species	Sex	Area (*n*)	total Hg	methyl Hg	Se	total Hg/Se	methyl Hg/Se	methyl/total Hg
common minke whal	males	sub-area 7 (19)	0.48	0.16	1.5	0.13	0.044	34
			(0.12-0.80)	(0.051-0.40)	(0.99-2.2)	(0.041-0.25)	(0.016-0.12)	(9.7-50)
		sub-area 9 (16)	0.42	0.12	1.4	0.12	0.034	29
			(0.035-0.75)	(0.012-0.26)	(0.92-1.7)	(0.015-0.20)	(0.005-0.080)	(17-40)
	females	sub-area 7 (5)	0.52	0.16	1.5	0.14	0.041	30
			(0.46-0.60)	(0.072-0.20)	(1.5-1.6)	(0.12-0.16)	(0.019-0.054)	(16-41)
	total		0.46	0.14	1.4	0.13	0.040	31
			(0.035-0.80)	(0.012-0.40)	(0.92-2.2)	(0.015-0.25)	(0.005-0.12)	(9.7-50)
Bryde's whale	males	sub-area 7 (21)	0.16	0.037	1.5	0.040	0.010	28
			(0.056-0.35)	(0.022-0.072)	(1.1-2.3)	(0.017-0.073)	(0.006-0.022)	(9.5-51)
	females	sub-area 7 (22)	0.20	0.045	1.6	0.049	0.011	28
			(0.020-0.49)	(0.014-0.11)	(0.60-3.0)	(0.013-0.14)	(0.007-0.021)	(9.2-72)
	total		0.18	0.041	1.5	0.045	0.011	28
			(0.020-0.49)	(0.014-0.11)	(0.60-3.0)	(0.013-0.14)	(0.006-0.022)	(9.2-72)
sperm whale	males	sub-area 7 (3)	25	1.2	11	0.83	0.064	8.3
			(4.3-39)	(0.69-1.6)	(2.3-19)	(0.72-0.93)	(0.033-0.12)	(4.0-16)
	females	sub-area 7 (2)	68	0.80	27	0.77	0.044	7.9
			(3.6-130)	(0.53-1.1)	(2.6-52)	(0.54-1.0)	(0.008-0.081)	(0.80-15)
	total		42	1.1	18	0.80	0.056	8.1
			(3.6-130)	(0.53-1.6)	(2.3-52)	(0.54-1.0)	(0.008-0.12)	(0.80-16)

Table 2b. Total Hg , methyl Hg and Se levels (mean and range, μg/g wet), ratio of both Hg to Se (mean and range, molar ratio) and percentage of methyl to total Hg (mean and range, %) in the kidneys of minke, Bryde's and sperm whales.

Species	Sex	Area (*n*)	total Hg	methyl Hg	Se	total Hg/Se	methyl Hg/Se	methyl/total Hg
common minke whal	males	sub-area 7 (19)	0.64	0.047	1.6	0.16	0.012	11
			(0.068-1.3)	(0.019-0.080)	(1.0-2.2)	(0.017-0.43)	(0.005-0.019)	(4.1-29)
		sub-area 9 (16)	0.67	0.028	1.6	0.17	0.007	5.0
			(0.026-1.0)	(0.004-0.043)	(1.1-2.8)	(0.006-0.24)	(0.001-0.013)	(2.4-14)
	females	sub-area 7 (5)	0.67	0.048	1.5	0.18	0.013	7.2
			(0.61-0.73)	(0.037-0.057)	(0.97-1.9)	(0.13-0.26)	(0.009-0.018)	(5.6-9.2)
	total		0.66	0.040	1.6	0.17	0.010	7.9
			(0.026-1.3)	(0.004-0.080)	(0.97-2.8)	(0.006-0.43)	(0.001-0.019)	(2.4-29)
Bryde's whale	males	sub-area 7 (21)	0.23	0.008	2.2	0.040	0.002	5.4
			(0.031-0.41)	(0.005-0.013)	(1.5-2.9)	(0.006-0.087)	(0.001-0.002)	(2.0-33)
	females	sub-area 7 (22)	0.24	0.010	2.1	0.044	0.002	5.2
			(0.014-0.60)	(0.001-0.019)	(1.6-3.5)	(0.003-0.10)	(0.0002-0.003)	(1.9-14)
	total		0.23	0.009	2.2	0.042	0.002	5.3
			(0.014-0.60)	(0.001-0.019)	(1.5-3.5)	(0.003-0.10)	(0.0002-0.003)	(1.9-33)
sperm whale	males	sub-area 7 (3)	3.0	0.43	4.0	0.31	0.042	15
			(2.2-3.8)	(0.29-0.51)	(3.0-4.8)	(0.21-0.40)	(0.037-0.047)	(9.4-22)
	females	sub-area 7 (2)	3.4	0.35	3.6	0.38	0.040	11
			(2.7-4.2)	(0.33-0.38)	(3.1-4.0)	(0.34-0.41)	(0.032-0.049)	(7.7-14)
	total		3.2	0.40	3.8	0.34	0.041	13
			(2.2-4.2)	(0.29-0.51)	(3.0-4.8)	(0.21-0.41)	(0.032-0.049)	(7.7-22)

Table 2c. Total Hg , methyl Hg and Se levels (mean and range, μg/g wet), ratio of both Hg to Se (mean and range, molar ratio) and percentage of methyl to total Hg (mean and range, %) in the muscle of minke, Bryde's and sperm whales.

Species	Sex	Area (*n*)	total Hg	methyl Hg	Se	total Hg/Se	methyl Hg/Se	methyl/total Hg
common minke whal	males	sub-area 7 (19)	0.20	0.12	0.80(*n* =14)	0.11(*n*=14)	0.071(*n*=14)	61
			(0.064-0.31)	(0.045-0.19)	(<0.5-1.3)	(0.030->0.18)	(0.021->0.12)	(34-84)
		sub-area 9 (16)	0.15	0.094	0.75(*n* =12)	0.081(*n* =12)	0.051(*n*=12)	65
			(0.018-0.19)	(0.017-0.12)	(<0.5-1.2)	(0.012->0.15)	(0.012->0.091)	(41-96)
	females	sub-area 7 (5)	0.24	0.15	0.86	0.11	0.073	64
			(0.20-0.27)	(0.11-0.19)	(0.61-1.1)	(0.079-0.17)	(0.051-0.11)	(55-72)
	total		0.19	0.12	0.79(*n* =31)	0.10(*n*=31)	0.063(*n*=31)	63
			(0.018-0.31)	(0.017-0.19)	(<0.5-1.3)	(0.012->0.18)	(0.012->0.12)	(34-96)
Bryde's whale	males	sub-area 7 (21)	0.052	0.023	0.88(*n* =18)	0.026(*n* =18)	0.011(*n*=18)	44
			(0.028-0.10)	(0.011-0.029)	(<0.50-2.4)	(0.009->0.048)	(0.003->0.023)	(26-56)
	females	sub-area 7 (22)	0.054	0.027	0.89(*n* =19)	0.026(*n* =19)	0.013(*n*=19)	49
			(0.004-0.072)	(0.001-0.037)	(<0.50-2.0)	(0.003->0.053)	(0.0004->0.029)	(15-61)
	total		0.053	0.025	0.89(*n* =37)	0.026(*n* =37)	0.012(*n*=37)	47
			(0.004-0.10)	(0.001-0.037)	(<0.50-2.4)	(0.003->0.053)	(0.0004->0.029)	(15-61)
sperm whale	males	sub-area 7 (3)	1.5	0.76	1.0	0.63	0.32	50
			(0.86-2.1)	(0.45-1.1)	(0.60-1.6)	(0.41-0.93)	(0.18-0.49)	(44-53)
	females	sub-area 7 (2)	1.4	0.60	0.81	0.68	0.31	46
			(0.91-1.9)	(0.50-0.71)	(0.57-1.1)	(0.63-0.72)	(0.27-0.35)	(37-55)
	total		1.5	0.70	0.94	0.65	0.32	48
			(0.86-2.1)	(0.45-1.1)	(0.57-1.6)	(0.41-0.93)	(0.18-0.49)	(37-55)

Table 3 Significant differences between sexes of common minke and Bryde's whales with two-tailed Mann-Whitney test.

	n	Liver			Kidneys			Muscle		
		THg	MeHg	Se	THg	MeHg	Se	THg	MeHg	Se
Common minke whale	26M4F							* F>M		
Bryde's whale	15M16F		* F>M			* F>M			** F>M	

*: p<0.05, **: p<0.01, ***: p<0.001

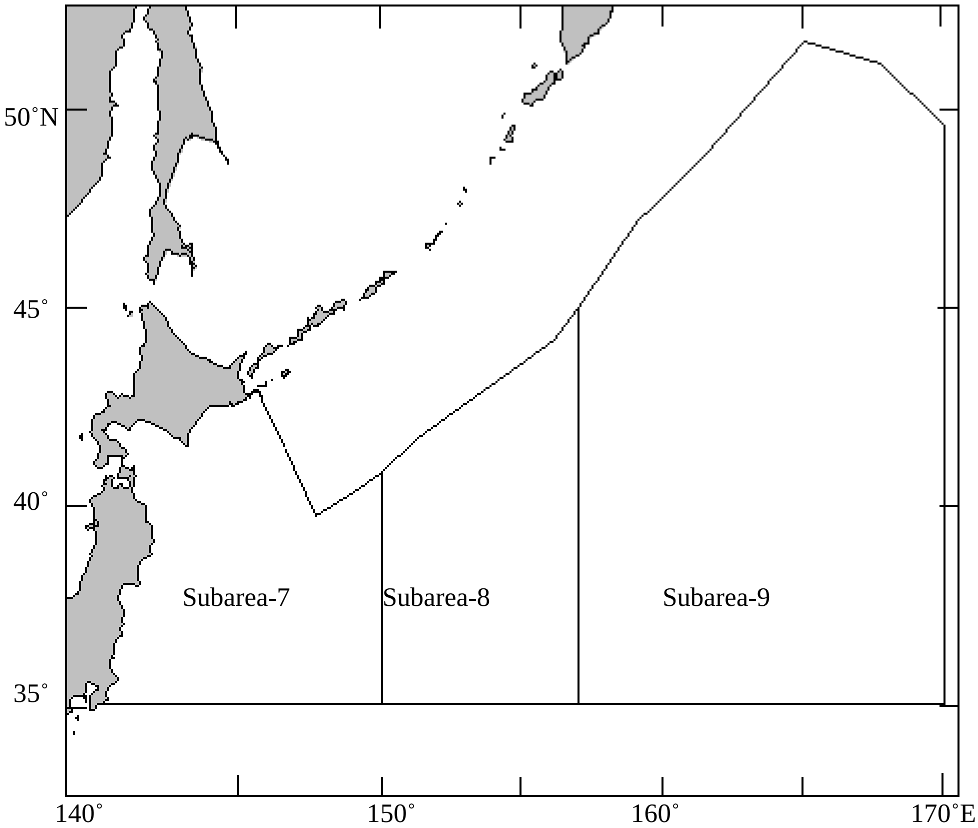


Figure 1. Subareas surveyed by the 2000 JARPN II cruise. Subareas based on IWC (1994), excluding the EEZ of Russia.

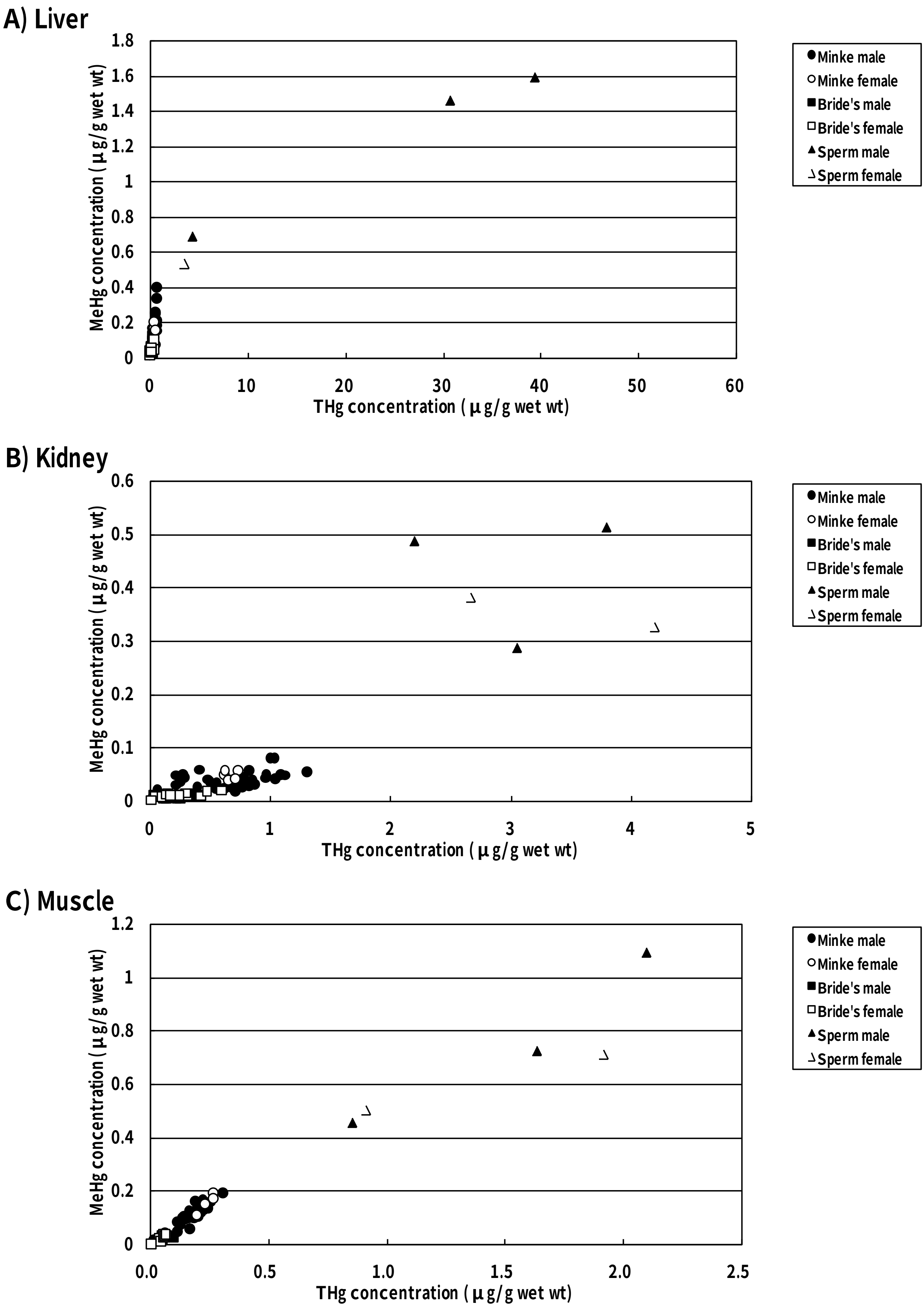


Fig. 2 Relationship between Total Hg and Methyl Hg concentrations in the A) liver, B) kidney and C) muscle of common minke, Bryde's and sperm whales collected from western North Pacific

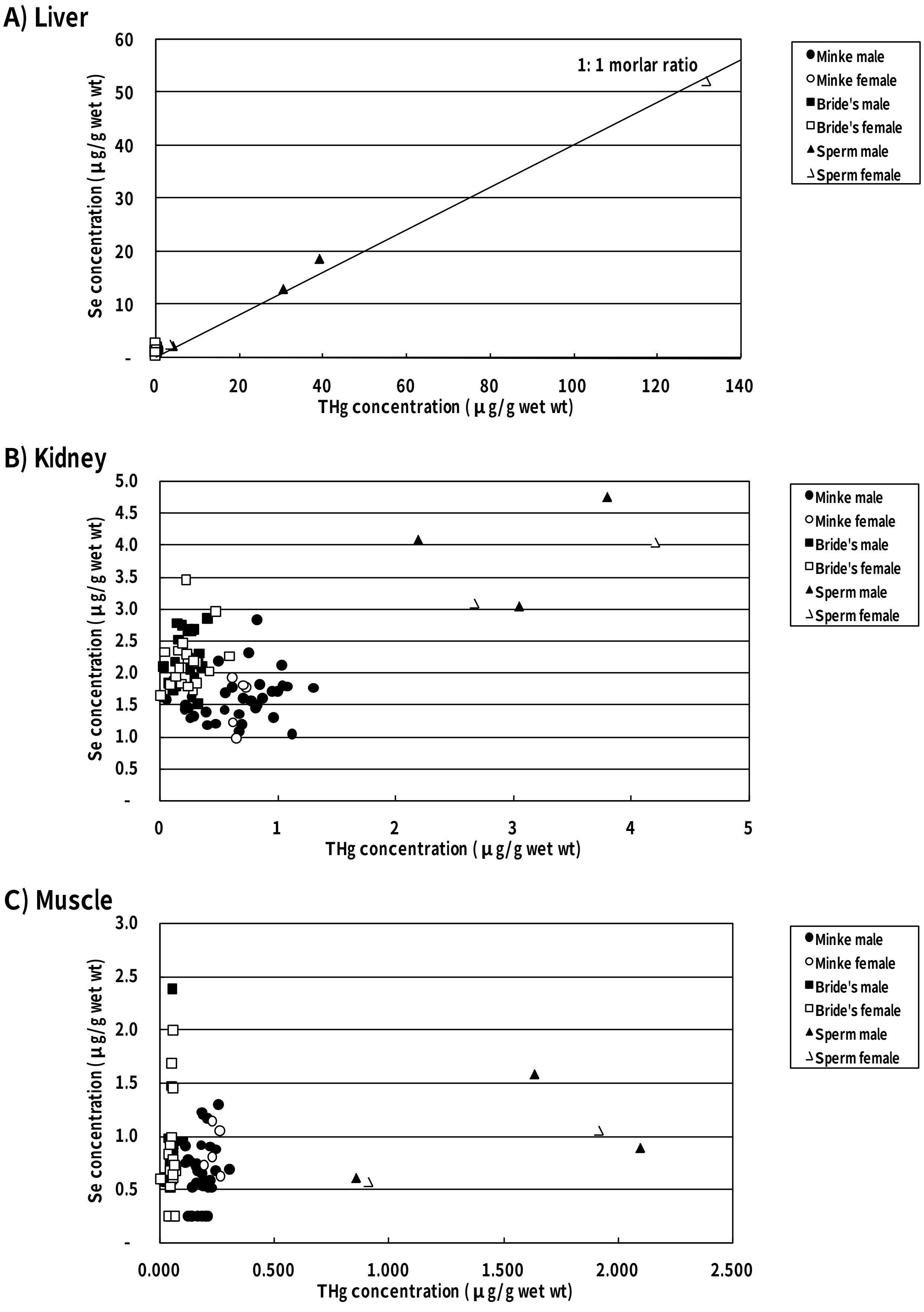
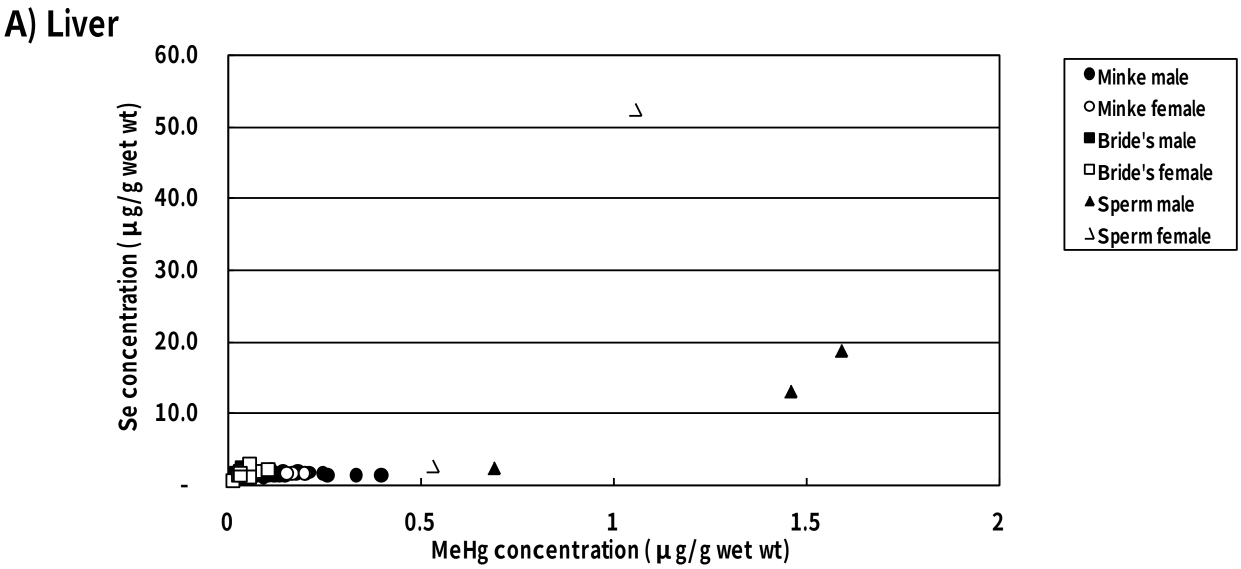
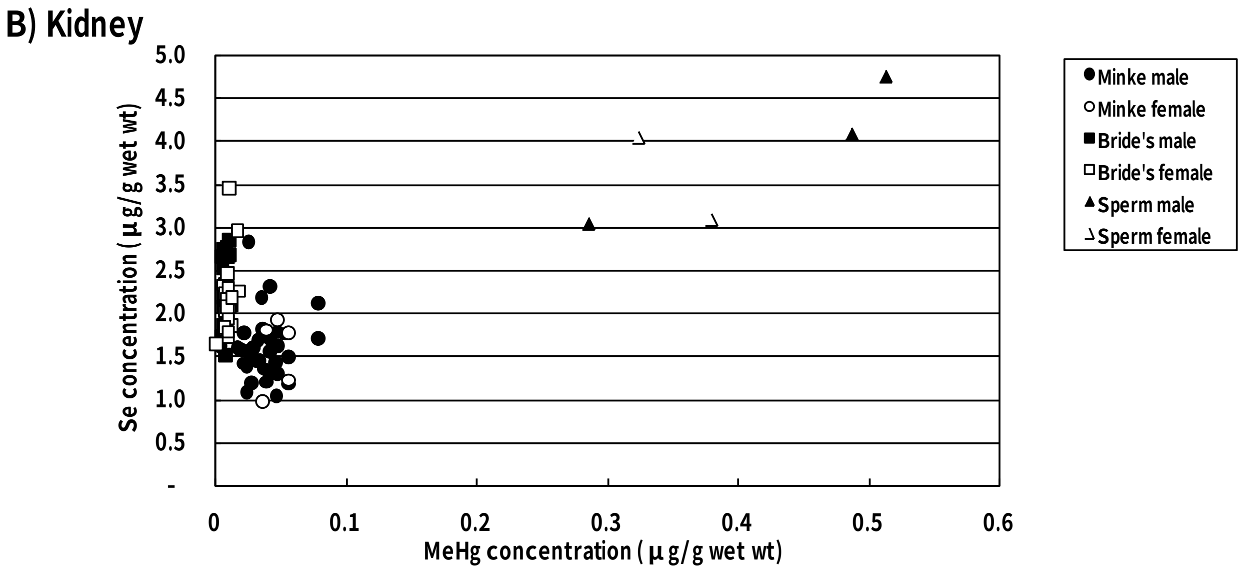


Fig. 3 Relationship between Total Hg and Se concentrations in the A) liver, B) kidney and C) muscle of minke, Bryde's and sperm whales collected from western North Pacific.





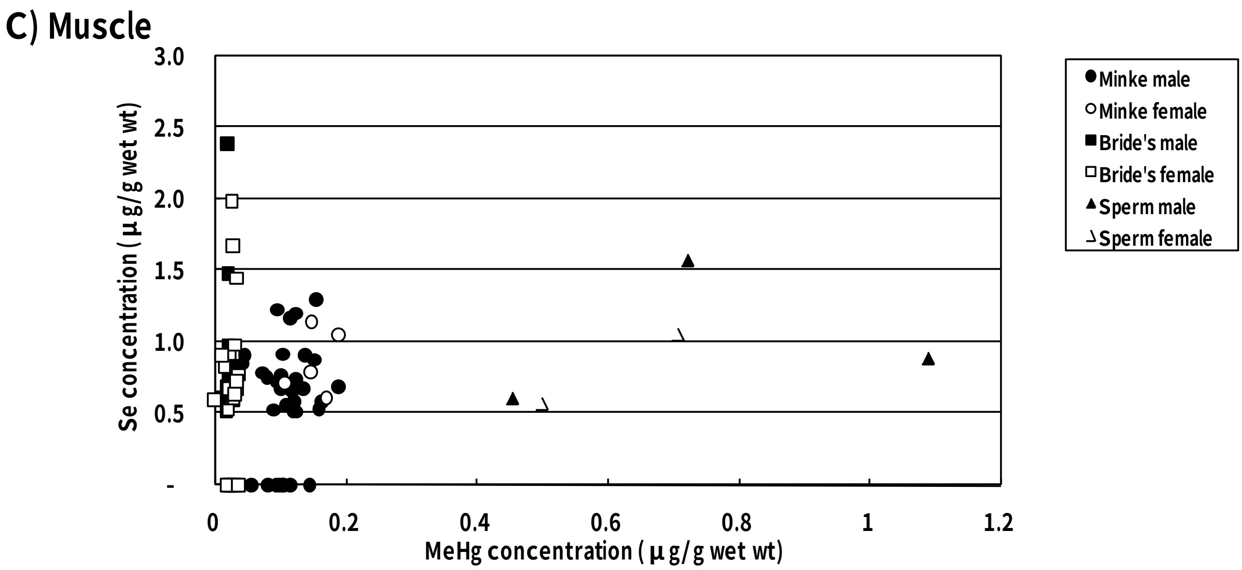


Fig. 4 Relationship between Methyl Hg and Se concentrations in the A) liver, B) kidney C) muscle of common minke, Bryde's and sperm whales collected from western North Pacific.

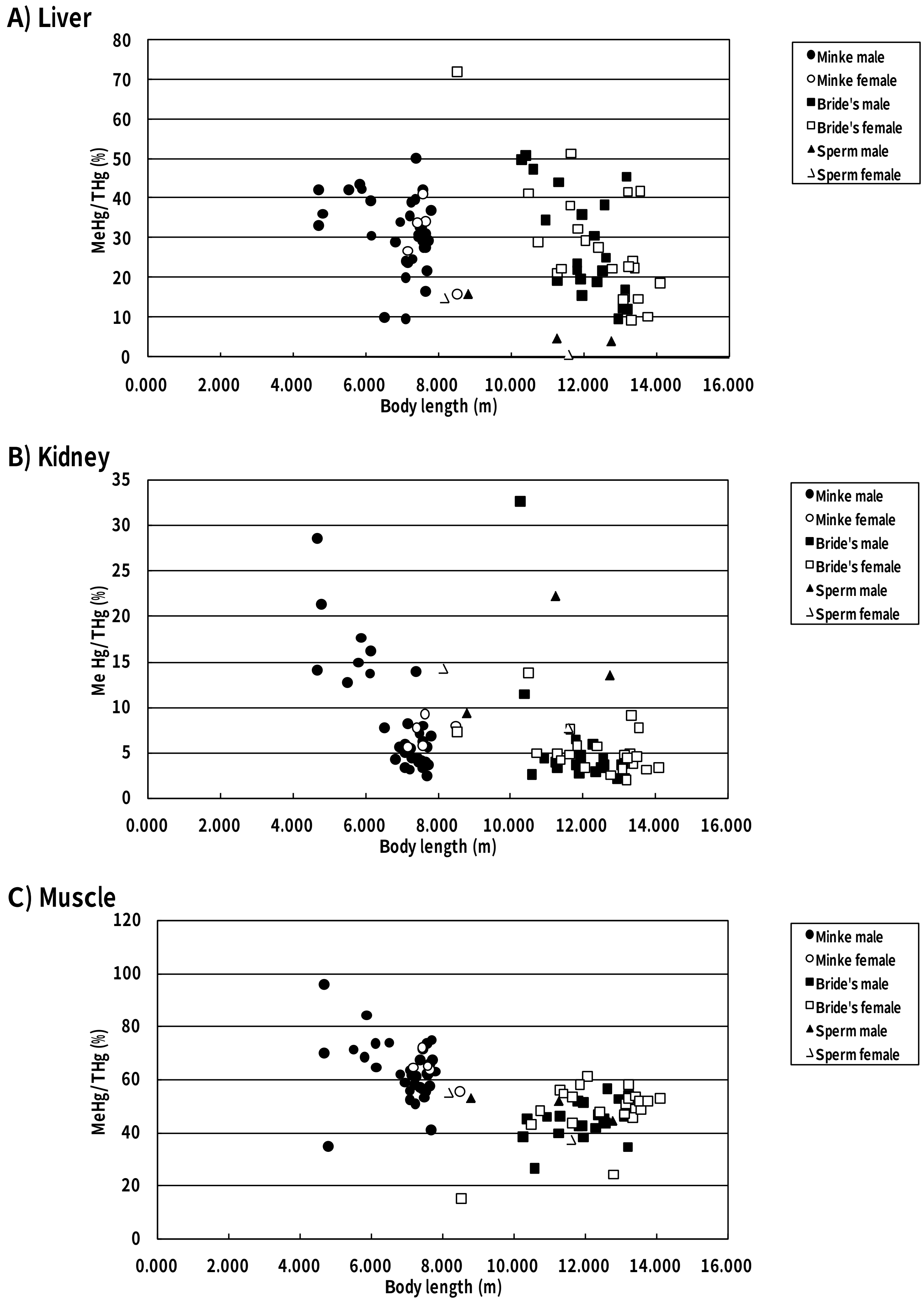


Fig. 5 Relationship between body length (m) and MeHg/THg (%) in the A) liver, B) kidney and C) muscle of minke, Bride's and sperm whales collected from western North Pacific.

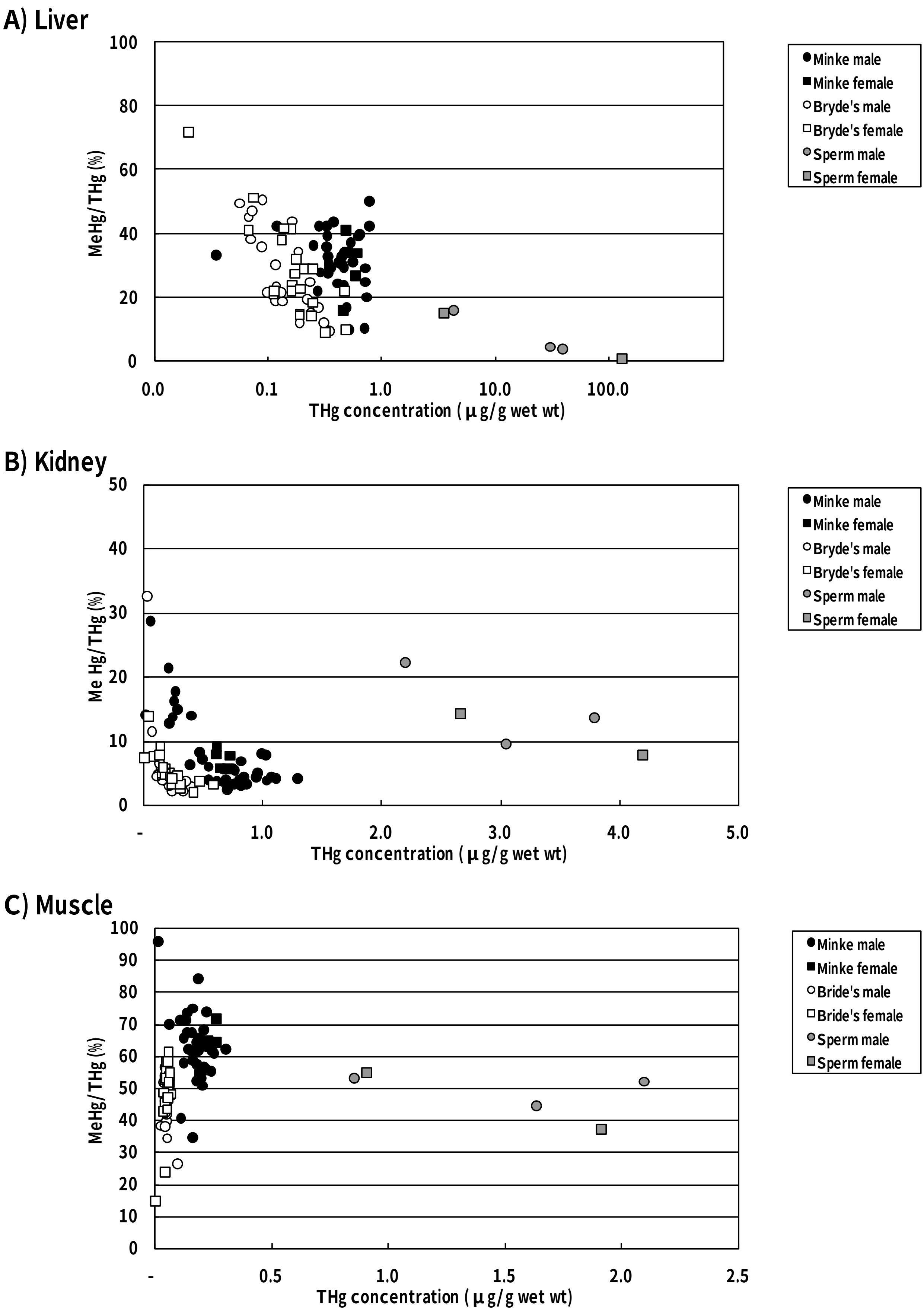


Fig. 6 Relationship between THg concentrations and MeHg/THg (%) in the A) liver, B) C) muscle of common minke, Bryde's and sperm whales collected from western North Pacific.