

ENVIRONMENTAL POLLUTION

Environmental Pollution 124 (2003) 263-271

www.elsevier.com/locate/envpol

Trace elements in two odontocete species (*Kogia breviceps* and *Globicephala macrorhynchus*) stranded in New Caledonia (South Pacific)

P. Bustamante^{a,*}, C. Garrigue^b, L. Breau^{a,c}, F. Caurant^a, W. Dabin^d, J. Greaves^b, R. Dodemont^b

^aLaboratoire de Biologie et Environnement Marins, UPRES-EA 3168, Université de La Rochelle,
22, Avenue Michel Crépeau, 17042 La Rochelle Cedex, France
^bOpération Cétacés, BP 12827, 98802 Nouméa, New Caledonia
^cInstitut de Recherche pour le Développement (IRD), Centre de Nouméa, BP A5, 98 8485 Nouméa Cedex, New Caledonia
^dCentre de Recherche sur les Mammifères Marins, Avenue Lazaret, Les Minimes, 17000 La Rochelle, France

Received 14 August 2002; accepted 9 December 2002

"Capsule": Trace elements in whales on New Caledonia beaches are below levels for concern.

Abstract

Liver, muscle and blubber tissues of two short-finned pilot whales (*Globicephala macrorhynchus*) and two pygmy sperm whales (*Kogia breviceps*) stranded on the coast of New Caledonia have been analysed for 12 trace elements (Al, Cd, Co, Cr, Cu, Fe, organic and total Hg, Mn, Ni, Se, V, and Zn). Liver was shown to be the most important accumulating organ for Cd, Cu, Fe, Hg, Se, and Zn in both species, *G. macrorhynchus* having the highest Cd, Hg, Se and Zn levels. In this species, concentrations of total Hg are particularly elevated, reaching up to 1452 µg g⁻¹ dry wt. Only a very low percentage of the total Hg was organic. In both species, the levels of Hg are directly related to Se in liver. Thus, a molar ratio of Hg:Se close to 1.0 was found for all specimens, except for the youngest *K. breviceps*. Our results suggest that *G. macrorhynchus* have a physiology promoting the accumulation of high levels of naturally occurring toxic elements. Furthermore, concentrations of Ni, Cr and Co are close to or below the detection limit in the liver and muscles of all specimens. This suggests that mining activity in New Caledonia, which typically elevates the levels of these contaminants in the marine environment, does not seem to be a significant source of contamination for these pelagic marine mammals.

© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Heavy metals; Cadmium; Mercury; Nickel; Marine mammals; Pacific Ocean

1. Introduction

Heavy metals generally occur at very low concentrations in oceans (Bryan, 1984). Nevertheless, most marine organisms concentrate trace elements at higher concentrations than those occurring in their environment. This process, currently described as bioaccumulation, concerns toxic elements such as Cd or Hg, as well as essential ones such as Cu, Fe, Se, or Zn. Slight variations of heavy metal concentrations in the environment of marine organisms could lead to a significant

increase of their metal burdens. Enrichment of sea water in heavy metals may occur naturally by local environmental processes, such as volcanism or upwellings. In addition to this natural origin, human activities can lead to increases of trace element concentrations in coastal waters, subsequently leading to an increase of metal burdens in biota.

In New Caledonia, mining activities plus strong natural erosion due to tropical rainfall have resulted in enrichment of several metals in coastal waters (mainly Co, Cr, Fe and Ni), and consequently in the coral reef food webs (Monniot et al., 1994). These authors reported high metal burdens in several species of filter-feeding ascidians from shallow waters. However, the impact of

^{*} Corresponding author. Tel./fax: +33-546-500-0294. *E-mail address:* pbustama@univ-lr.fr (P. Bustamante).

metal contamination does not appear to be limited to coastal waters and, in fact, high levels of Co, Cr and Ni have also been reported in the tissues of a cephalopod living in deeper waters, the nautilus, *Nautilus macromphalus* (Bustamante et al., 2000).

Current literature on heavy metal concentrations in New Caledonian marine lower trophic level organisms is scarce (e.g. Monniot et al., 1994; Bustamante et al., 2000), and no data for marine mammals have been published to date. In this work, baseline information is presented for the concentrations of 12 trace elements in the tissues and organs of two odontocetes species, i.e., pygmy sperm whale, Kogia breviceps (de Blainville, 1838), and short-finned pilot whale, Globicephala macrorhynchus (Gray, 1846) for which very few information on trace elements are available. These marine mammals give the opportunity to investigate the impact of mining activities on high trophic level organisms stranded in New Caledonia. It also provides information on the bioaccumulation and detoxification processes of Cd and Hg in marine mammals from a tropical zone, toxic elements which are currently reported to raise the question of physiological damages in the tissues of top marine predators.

2. Materials and methods

2.1. Sampling and sample preparation

In spring 1997, two short-finned pilot whales (*G. macrorhynchus*) and two pygmy sperm whales (*K. breviceps*) were stranded on the coasts of New Caledonia. These animals were autopsied shortly after death. Stomach contents were collected and stored in 90% ethanol for later diet analyses. Teeth were extracted for ageing and skin samples were collected for genetic analysis. Tissues and organs (liver, blubber and muscle) were sampled for heavy metal and radionuclides analyses (Garrigue et al., 2000). Separated tissue samples were placed in decontaminated glass vessels and stored at -20 °C. They were then freeze-dried, finely ground and stored in plastic vials.

The two stranded *G. macrorhynchus* were probably part of a bigger pod observed in the vicinity of the stranding location on the day of stranding. This species is commonly encountered outside of the lagoon by long liners in both nearshore and pelagic environments but there is no evidence that they are resident in the area. In fact, seasonal inshore to offshore movements are related to the distribution of squid they feed on (Olson and Reily, 2001).

Little is know on the status of *K. breviceps* in New Caledonia as they are very discrete animals, rarely observed alive. Most of the information on this species come from stranded animals which have been documented earlier in

New Caledonia (Robineau and Rancurel, 1981; Sylvestre, 1988).

2.2. Age determination

Teeth were used for age determination, following the recommendations of Neilsen (1972) and Perrin and Myrick (1980). Teeth taken up from the lower jaw were cleaned, fixed and stored in 70% glycerined alcohol. Before sectioning and staining, teeth were decalcificated with DC3 (Labonor®). Sections were then examined to count the growth layer groups (GLGs) assuming 1 GLG equals 1 year, as described by Perrin and Myrick (1980). Three series of several independent readings were done for each tooth section according to Hohn and Fernandez (1999). All readings were recorded to the nearest whole year and age was expressed as mean ± S.D. for each individual.

2.3. Stomach contents analysis

Stomach contents were washed and the contained elements were sorted by type. Fish species were determined from the otoliths by J. Rivaton from the laboratory of marine biology of IRD (Nouméa, New Caledonia). Cephalopod species were determined from the beaks by Dr. R. Young from the University of Hawaii.

2.4. Metal analysis

Metal analyses were performed by inductively coupled plasma atomic emission spectrophotometry (ICP-AES) for Al, Co, Cr, Cu, Fe, Mn, Ni, V and Zn, by atomic absorption spectrophotometry (AAS) for Cd, Cu, Se and Zn, and with an Advanced Mercury Analyser (AMA) for total and organic Hg after extraction.

For ICP-AES measurements, 2 aliquots of approximately 500 mg of each homogenised dry sample were digested in Teflon containers with 4 ml of 65% HNO₃ and 1 ml of 30% H₂O₂ using a microwave digester (ANTON-PAAR, Perkin–Elmer). The digested contents were made up to 25 ml with Milli-Q quality water. Elements were analysed by ICP-AES with an OPTIMA 3200 DV, Perkin-Elmer. Multicomponent Spectral Fitting Models were used to correct for spectral interference between elements.

For AAS measurements, 2 aliquots of approximately 300 mg of each homogenised dry sample were digested with 3.5 ml of 65% HNO₃ at 60 °C for 3 days. The digested contents were then diluted to 10 ml in Milli-Q quality water. Cd, Cu, Se and Zn were assayed using flame and graphite furnace atomic absorption spectrophotometer Varian 250 Plus with deuterium background correction.

For AMA measurements, aliquots ranging from 1 to 50 mg of dried material were analysed directly for total

Hg in a Advanced Mercury Analyser spectrophotometer (Altec AMA 254). Hg determination involved evaporation of Hg by progressive heating until 800 °C was reached and then held under oxygen atmosphere for 3 min, and subsequent amalgamation on a Au-net. Afterwards, the net was heated to liberate the collected mercury, subsequently measured by UV atomic absorption spectrophotometry. The same procedure was performed for the analysis of organic Hg after extraction adapted from Uthe et al. (1972). Organic Hg was extracted from 2 aliquots of approximately 500 mg of each homogenised dry sample using 2 ml of acidic sodium bromide (30% NaBr in 4N H₂SO₄), 4 ml of cupric sulfate (2.5% CuSO₄ in milli-O quality water) and 10 ml of toluene under agitation for 10 min in glass flasks. The organic phase was then separated and used for the analysis in the AMA.

Quality control was assured using dogfish liver DOLT-2 (NRCC) and dogfish muscle DORM-2 (NRCC) as reference materials for AAS and AMA, and dogfish muscle DORM-1 (NRCC) for ICP-AES. These standards were treated and analysed under the same conditions as the samples. The results were in good agreement with the certified values (Table 1). Detection limits (μg g⁻¹) were 0.004 for Cd, 0.5 for Cu, 3 for Zn, 0.8 for Se, 0.005 for total Hg and 0.2 for organic Hg for AAS and AMA analyses. For ICP-AES analyses, detection limits were 0.006 for Al, 0.040 for Co, 0.013 for Cr, 0.005 for Cu, 0.051 for Fe, 0.002 for Mn, 0.010 for Ni, 0.009 for V and 0.008 for Zn (μg g⁻¹). Metal concentrations in tissues are reported in μg g⁻¹ dry wt.

3. Results

3.1. Main features, age and diet

The main features of the four specimens sampled, one male and one female *G. macrorhynchus* and one male

and one female *K. breviceps*, are reported in Table 2, with the prey species identified from their corresponding stomach contents and their ages. Considering their ages, the two *G. macrorhynchus* may have attained sexual maturity since this occurs at 9 years for females and between 13 and 16 for males (Olson and Reilly, 2001). For *K. breviceps*, the size of sexual maturity has been documented to be 2.7–3.0 m for males and 2.6–2.8 m for females of (Leatherwood et al., 1983). Furthermore, *K. breviceps* appears to reach sexual maturity between 3 and 5 years old (Plön, in preparation). In the light of this data the two *K. breviceps* presented in this paper may have reached sexual maturity.

All the animals with the exception of the male *K. breviceps* were already stranded when samples were collected. This last one was first alive, but it has been euthanasied after the failure of its refloat. The autopsies did not provide any evident reasons for the death of the animals. Parasites were only found in great quantity in the male *K. breviceps*, with a stomach full of *Anisakis simplex* (Nematodes). This specimen could have suffered of a parasitose, stranded individuals from this species being frequently heavily infected by intestinal nematodes (McAlpine, 2002).

On the other hand, the female *G. macrorhynchus* was probably pregnant before the stranding as part of a fœtus was found in its vicinity. This female may have been fasting as its stomach was completely empty (Table 2).

3.2. Metal analysis

Heavy metal and organic mercury concentrations in the liver, muscle and blubber of *G. macrorhynchus* and *K. breviceps* are presented in Table 3. Co, Cr and Ni concentrations in all tissues and organs fall below the detection limit or close to it (i.e. in liver). Among these tissues, liver is the most important accumulating organ

Table 1 Comparison of trace elements concentrations ($\mu g g^{-1}$ dry wt.) of certified standards from the NRCC determined in the present study with certified values

Metals	DOLT-2 $(n=6)$		DORM-2 $(n=3)$		DORM-1 $(n=3)$			
	Certified values	Present study	Certified values	Present study	Certified values	Present study 13.1±2.1		
Al	_	_	_	_	_			
Cd	20.8 ± 0.5	21.4 ± 0.6	0.043 ± 0.008	0.042 ± 0.012	_	_		
Co	_	_	_	_	0.049 ± 0.014	<QL		
Cr	_	_	_	_	3.60 ± 0.40	3.45 ± 0.17		
Cu	25.8 ± 1.1	26.9 ± 1.3	2.34 ± 0.16	2.28 ± 0.24	5.22 ± 0.33	5.24 ± 0.05		
Fe	_	_	_	_	63.6 ± 4.9	66.7 ± 1.4		
Total Hg	2.14 ± 0.28	2.13 ± 0.02	4.64 ± 0.26	4.50 ± 0.16	_	_		
Organic Hg	0.693 ± 0.053	0.759 ± 0.018	4.47 ± 0.32	4.38 ± 0.28	_	_		
Mn	=	_	=	_	1.32 ± 0.26	1.11 ± 0.02		
Ni	_	_	_	_	1.20 ± 0.30	0.97 ± 0.08		
Se	6.06 ± 0.49	5.48 ± 0.13	1.40 ± 0.09	1.31 ± 0.15	_	_		
Zn	85.8 ± 2.5	83.6 ± 3.4	25.6 ± 2.3	26.2 ± 2.1	21.3 ± 1.0	21.1 ± 1.3		

Table 2 Characteristics of the odontocetes and preys identified in their stomach contents^a

Species	Sex	Length (m)	Age	Prey identified in the stomach content
Globicephala macrorhynchus				Fishes Bathyclupea malayana (Bathuclupeidae) Antigoniia sp. (Caproidae) Synagrops sp. (Acropomatidae) Diaphus sp. (Myctophidae) Cubiceps sp. (Nomeidae) Chlorophthalmus sp. (Clotophthalmidae)
	₹	5.4 3.5		Cephalopods Stenoteuthis sp. (Ommastrephidae) Three unidentified species (Ommastrephidae) Moroteuthis sp. (Onychoteuthidae) Lycoteuthis sp. (Lycoteuthidae) Histioteuthis sp. (Histioteuthidae) 5 unidentified species (Histioteuthidae) Empty stomach
Kogia breviceps	Ŧ	3.3	12 ± 2	Shrimps Pasiphea sp. (Pasiphaeidae) Gnathophausia ingens (Mysidacea) Meningodora sp. (Oplophoridae)
	3	3.1	6±1	Cephalopods Histioteuthis sp. (Histioteuthidae) Enoploteuthis sp. (Enoploteuthidae) Two unidentified species
	\$	3.0	19±3	Shrimps Pasiphea sp. (Pasiphaeidae) Gnathophausia ingens (Mysidacea) Meningodora sp. (Oplophoridae)
				Cephalopods Taonius sp. (Cranchidae) Histioteuthidae Enoploteuthidae Octopoteuthidae

 $^{^{\}rm a}\,$ Age is the mean $\pm\,S.D.$ of 3 counting of growth layer groups.

for Cd, Cu, Fe, Hg, Se, and Zn and to a lesser extent for Mn and V. However, the two *G. macrorhynchus* exhibit higher hepatic levels for Cd, Cu, Hg, Se and Zn than *K. breviceps* while both species have similar hepatic levels of Mn and V. Fe is the only metal having higher concentrations in the liver of *K. breviceps* than in the one of *G. macrorhynchus*.

In muscle, Mn in G. macrorhynchus and V in both mammals are below the detection limits. The remain-

ing trace elements were within the same order of concentration in the muscle of both species (Table 3). The only exception was Hg, where concentrations were 5 times higher in *G. macrorhynchus* muscle compared to *K. breviceps*.

Blubber has generally low trace element concentrations. However, Cr exhibits the highest concentrations among the three tissues (Table 3). Despite its strong affinity to lipids, organic Hg concentrations in blubber were an order of magnitude lower than in liver and muscle.

Fig. 1 shows the hepatic concentrations of inorganic Hg and Se of marine mammals in relation to age together with the inorganic Hg:Se molar ratio for each individual. In both species, hepatic inorganic Hg is directly related to hepatic Se. Consequently, a molar ratio close to 1.0 is found in all specimens, except for the youngest *K. breviceps* (6 years old) for which this ratio was 0.13 (Fig. 1). Nevertheless, both species exhibit very different ratio organic:total Hg in their tissues (Fig. 2) with *K. breviceps* showing a higher percentage of organic Hg than *G. macrorhynchus* in liver (aver. 13% vs 0.5%) and in muscle (aver. 75% vs 14%).

4. Discussion

4.1. Trace element levels

Heavy metal concentrations in the tissues of marine mammals are globally well documented but they concern mainly species having strong interactions with man, i.e. hunted for meat consumption, by-caught during fishing or living in coastal areas. Furthermore, very few studies have investigated trace elements such as Co, Cr, Ni or V although they are potentially toxic, probably because these trace elements are not bioaccumulated in large amounts in mammalian tissues (Thompson, 1990). Nevertheless, such elements can be used as tracers of contamination when an enrichment due to anthropogenic activities occurs. In Alaska, Mackey et al. (1996) have shown an increase of V in the tissues of pinnipeds and cetaceans. The bioaccumulation of V in biota and its transfer to the top marine predators has been related to the contamination of sea water by oil residues in this area. Similarly, the consequence of intense mining activities in New Caledonia must be monitored by the measurement of the concentrations of Ni and its associated elements, mainly Co and Cr, in the food webs around the island. Analysis in the tissues of the short-finned pilot whales G. macrorhynchus and the pygmy sperm whales K. breviceps have not revealed any increase of concentrations for these three metals compared to other marine mammals species from various areas (Table 4). With the exception of Cr in the blubber of the male G. macrorhynchus (2.51 µg g⁻¹ dry wt.), concentrations remained

Table 3 Age and metal concentrations ($\mu g g^{-1}$ dry wt.) in the tissues of odontocetes from the New Caledonia

Tissue	Age	Al	Cd	Co	Cr	Cu	Fe	Total-Hg	Organic-Hg	Mn	Ni	Se	V	Zn
G. macrorhynchus 3	14±1													
Liver		1.38 ± 0.45	225.3 ± 0.4	<dl< td=""><td><dl< td=""><td>37.4 ± 0.3</td><td>1472 ± 7</td><td>1411 ± 24</td><td>11.7 ± 0.4</td><td>7.1 ± 0.2</td><td><dl</td><td>627 ± 43</td><td>0.06</td><td>135.7 ± 0.6</td></dl<></td></dl<>	<dl< td=""><td>37.4 ± 0.3</td><td>1472 ± 7</td><td>1411 ± 24</td><td>11.7 ± 0.4</td><td>7.1 ± 0.2</td><td><dl</td><td>627 ± 43</td><td>0.06</td><td>135.7 ± 0.6</td></dl<>	37.4 ± 0.3	1472 ± 7	1411 ± 24	11.7 ± 0.4	7.1 ± 0.2	<dl	627 ± 43	0.06	135.7 ± 0.6
Muscle		2.62 ± 0.69	0.79 ± 0.03	<dl< td=""><td><dl< td=""><td>0.8 ± 0.1</td><td>347 ± 29</td><td>32.8 ± 2.0</td><td>4.41 ± 0.09</td><td><dl</td><td><dl</td><td>9 ± 1</td><td><dl</td><td>61.1 ± 2.4</td></dl<></td></dl<>	<dl< td=""><td>0.8 ± 0.1</td><td>347 ± 29</td><td>32.8 ± 2.0</td><td>4.41 ± 0.09</td><td><dl</td><td><dl</td><td>9 ± 1</td><td><dl</td><td>61.1 ± 2.4</td></dl<>	0.8 ± 0.1	347 ± 29	32.8 ± 2.0	4.41 ± 0.09	<dl	<dl	9 ± 1	<dl	61.1 ± 2.4
Blubber		1.53 ± 0.14	0.95 ± 0.03	<dl	2.51 ± 0.01	0.4 ± 0.1	187 ± 8	11.0 ± 0.8	0.47 ± 0.05	0.17 ± 0.01	<dl	4 ± 1	<dl	21.3 ± 0.3
G. macrorhynchus ♀	12±2													
Liver		1.07 ± 0.11	464.4 ± 5.4	0.04	<dl< td=""><td>51.0 ± 1.3</td><td>1535 ± 17</td><td>1452 ± 79</td><td>1.70 ± 0.01</td><td>6.8 ± 0.2</td><td><dl< td=""><td>758 ± 39</td><td>0.10</td><td>113.3 ± 2.1</td></dl<></td></dl<>	51.0 ± 1.3	1535 ± 17	1452 ± 79	1.70 ± 0.01	6.8 ± 0.2	<dl< td=""><td>758 ± 39</td><td>0.10</td><td>113.3 ± 2.1</td></dl<>	758 ± 39	0.10	113.3 ± 2.1
Muscle		0.88 ± 0.26	1.48 ± 0.03	<dl< td=""><td>< dl</td><td>1.5 ± 0.1</td><td>622 ± 78</td><td>27.3 ± 1.1</td><td>3.29 ± 0.06</td><td>< dl</td><td><dl< td=""><td>6 ± 1</td><td><dl</td><td>49.8 ± 1.4</td></dl<></td></dl<>	< dl	1.5 ± 0.1	622 ± 78	27.3 ± 1.1	3.29 ± 0.06	< dl	<dl< td=""><td>6 ± 1</td><td><dl</td><td>49.8 ± 1.4</td></dl<>	6 ± 1	<dl	49.8 ± 1.4
Blubber		1.15 ± 0.00	0.84 ± 0.01	<dl	0.32 ± 0.08	0.2 ± 0.1	18 ± 2	3.20 ± 0.22	0.21 ± 0.02	<dl	<dl	3 ± 0	<dl	17.3 ± 1.3
K. breviceps ♂	6±1													
Liver		2.11 ± 0.29	28.8 ± 0.8	<dl< td=""><td><dl< td=""><td>8.1 ± 0.1</td><td>2503 ± 11</td><td>7.86 ± 0.14</td><td>1.25 ± 0.12</td><td>5.2 ± 0.2</td><td><dl< td=""><td>21 ± 1</td><td>0.10</td><td>52.3 ± 1.6</td></dl<></td></dl<></td></dl<>	<dl< td=""><td>8.1 ± 0.1</td><td>2503 ± 11</td><td>7.86 ± 0.14</td><td>1.25 ± 0.12</td><td>5.2 ± 0.2</td><td><dl< td=""><td>21 ± 1</td><td>0.10</td><td>52.3 ± 1.6</td></dl<></td></dl<>	8.1 ± 0.1	2503 ± 11	7.86 ± 0.14	1.25 ± 0.12	5.2 ± 0.2	<dl< td=""><td>21 ± 1</td><td>0.10</td><td>52.3 ± 1.6</td></dl<>	21 ± 1	0.10	52.3 ± 1.6
Muscle		6.58 ± 0.17	0.41 ± 0.01	<dl	0.03	1.7 ± 0.2	977 ± 18	6.07 ± 0.17	4.65 ± 0.01	0.26 ± 0.01	<dl	6 ± 1	<dl	42.9 ± 0.7
K. breviceps ♀	19±3													
Liver		17.0 ± 0.5	47.5 ± 1.3	0.05	<dl< td=""><td>17.5 ± 0.9</td><td>3120 ± 84</td><td>77.3 ± 0.7</td><td>7.03 ± 0.06</td><td>5.0 ± 0.1</td><td><dl< td=""><td>25 ± 1</td><td>0.68</td><td>54.3 ± 0.8</td></dl<></td></dl<>	17.5 ± 0.9	3120 ± 84	77.3 ± 0.7	7.03 ± 0.06	5.0 ± 0.1	<dl< td=""><td>25 ± 1</td><td>0.68</td><td>54.3 ± 0.8</td></dl<>	25 ± 1	0.68	54.3 ± 0.8
Muscle		3.60 ± 0.33	0.57 ± 0.04	<dl< td=""><td><dl</td><td>0.9 ± 0.0</td><td>820 ± 12</td><td>5.16 ± 0.13</td><td>3.75 ± 0.11</td><td>0.12 ± 0.02</td><td><dl< td=""><td>3 ± 0</td><td><dl</td><td>67.1 ± 0.9</td></dl<></td></dl<>	<dl	0.9 ± 0.0	820 ± 12	5.16 ± 0.13	3.75 ± 0.11	0.12 ± 0.02	<dl< td=""><td>3 ± 0</td><td><dl</td><td>67.1 ± 0.9</td></dl<>	3 ± 0	<dl	67.1 ± 0.9

dl: detection limit. Wet: dry wt. ratios are 4.5, 4.0 and 1.6 for liver, muscle and blubber, respectively.

Metal concentrations (μg.g⁻¹ dwt) 1000 1000 1000 K. b Q Hg:Se 1 0 5 10 Age

Fig. 1. Concentrations of inorganic Hg (\spadesuit) and Se (\bullet) together with Se:Hg molar ratio (\triangle) in G. macrorhynchus and K. breviceps from New Caledonia.

close to or below the detection limits. Considering that these pelagic animals could have preyed on species living around the island as suggested by the examination of their full stomach, our results suggest that these metals are not easily transferred in the food web of these pelagic odontocetes. To assess the impact of mining activities on marine mammals, further studies should be conducted on more coastal species known to be resident

in the impacted area, such as the bottlenose dolphin (*Tursiops truncatus*) or the dugong (*Dugong dugon*) that spend all their life in the waters of the New Caledonian lagoon.

Most of the studies on the bioaccumulation of trace elements in marine mammals have focused on toxic Cd and Hg, which are well bioaccumulated in mammalian tissues (Thompson, 1990). Nevertheless, numerous rare

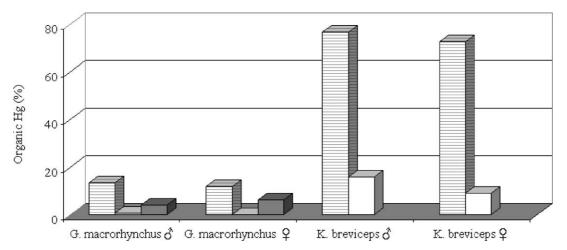


Fig. 2. Percentage of organic Hg in the muscle (striped), liver (white) and blubber (grey) of G. macrorhynchus and K. breviceps from New Caledonia.

species have been poorly investigated and there is a lack of data for *G. macrorhynchus* and *K. breviceps*. Table 5 shows the paucity of information reported for these two species. The present study provides new information on metal concentrations in these marine mammals. The four odontocetes stranded in New Caledonia exhibited very high levels of Cd and Hg in liver, reaching 464 and 1452 μg g⁻¹ dry wt., respectively. However, *G. macrorhynchus* displayed much higher concentrations of Cd and Hg than *K. breviceps* (Table 3).

Both species occupy a high and similar trophic levels (4.3 and 4.4 for G. macrorhynchus and K. breviceps, respectively), assessed by stable isotope analysis (Pauly et al. 1998). They mainly prey upon squid although this may be implemented by a large proportion of fish for G. macrorhynchus (around 40%) and a lower one for K. breviceps (around 20%) in certain areas (Pauly et al., 1998). The concentrations of Hg in the liver of G. macrorhynchus sampled in New Caledonia suggest that fish constitute an important prey item for this species as fish are considered to be the main Hg source for marine top predators (Law et al., 1992). This was confirmed by the analysis of stomach contents showing several fish species in the diet of G. macrorhynchus (Table 2). However, no fish remains were found in the stomach contents of *K. breviceps* but its diet included several crustacean species which are known to have very low Hg contents (Cossa et al., 1990).

Similar exposures to Cd would occur for both species considering the high Cd intakes due to cephalopod consumption (Bustamante et al., 1998) that represent 60% and 75% of the diet of *G. macrorhynchus* and *K. breviceps*, respectively (Pauly et al., 1998). Nevertheless, *G. macrorhynchus* had hepatic Cd concentrations of one order of magnitude higher than *K. breviceps* (Table 3). It appears from the scarce data available in the literature that *K. breviceps* generally have lower Cd concentrations than *G. macrorhynchus* (Table 5). This suggests that diet does not explain alone the high concentrations of Cd in the tissues of *G. macrorhynchus* and that a particular

physiology promoting the accumulation of naturally occurring Cd could be envisaged for this species.

4.2. Detoxification processes

Extremely high levels of toxic Hg and Cd may trigger cellular and physiological damages of the target organs, especially kidney (Gallien et al., 2001). Despite the lack of the main storage organ for Cd analyses (i.e. kidney), Cd concentrations encountered here in liver suggest even higher concentrations in kidney as it is always the case in marine mammals (Aguilar et al., 1999). Renal dysfunction has been linked to Cd concentrations in liver exceeding 20 µg g⁻¹ wwt (approximately 100 µg g^{-1} dry wt.) (Honda, 1985; Fujise et al., 1988). G. macrorhynchus had Cd concentrations in liver far higher than that limit (i.e. 225 and 464 $\mu g g^{-1}$ dry wt.), while they were clearly below that limit in the liver of K. breviceps (Table 3). Thus, Cd detoxification processes should be particularly efficient in the liver and kidney of G. macrorhynchus to support such high concentrations and needs to be further investigated.

Marine mammals are exposed to methyl Hg through fish consumption, but only a small fraction of the total Hg in liver and muscle of G. macrorhynchus was found to be organic Hg, i.e. <1% and <15% of the total Hg, respectively (Fig. 2). This is far below the percentage of organic Hg to total Hg that is usually found in these tissues of adult marine mammals, which generally ranges from 3 to 20% for liver and from 70 to 95% for muscle (Caurant et al., 1996; Wagemann et al., 1998). Gaskin et al. (1974) also reported values ranging from 2 to 17% for organic Hg in the liver of G. macrorhynchus from the Lesser Antilles, but muscle had only 42-60% of total Hg in the organic form. Therefore, organic Hg concentrations in liver and muscle of G. macrorhynchus from New Caledonia are relatively similar to those from Lesser Antilles (Gaskin et al., 1974). Apparently, the low fraction of hepatic organic Hg appears to be due to

Table 4 Heavy metal concentrations ($\mu g g^{-1}$ wet wt.) in the liver of odontocete species from various areas

Species	Locality	N	Cd	Co	Cr	Cu	Fe	Total-Hg	Organic-Hg	Mn	Ni	Se	Zn	Authors
G. melas	Northwest Atlantic Ocean	9	7.88 ± 3.61	0.012±0.003	_	2.7 ± 1.0	356±221	40.30 ± 38.81	_	2.53 ± 0.51	_	11.73±9.94	39±7	Mackey et al. (1995)
G. melas	Northeast Atlantic Ocean	1	0.1	_	0.25	4.7	2680	0.74	-	_	0.31	2.1	45	Law et al. (2001)
G. griseus	Mediterranean Sea	1	6.00	_	0.28	_	_	1002	14.70	_	_	266.4	_	Storelli et al. (1999)
G. griseus	Mediterranean Sea	1	8.42	_	0.41	_	_	478.3	7.35	_	_	113.2	_	Storelli et al. (1999)
G. griseus	Northeast Atlantic Ocean	1	0.2	_	0.26	5.2	337	2.6	-	_	0.71	4.6	37	Law et al. (2001)
L.	Northwest Atlantic Ocean	4	0.423 ± 0.294	0.013 ± 0.029	_	6.3 ± 2.2	179 ± 100	10.36 ± 10.09	-	3.67 ± 0.53	_	5.31 ± 2.98	41.6 ± 9.9	Mackey et al. (1995)
acutus														
M. bidens	Northeast Atlantic Ocean	1	20	_	1.7	19	172	322	-	_	1.0	133	83	Law et al. (2001)
M. densirostris	Northeast Atlantic Ocean	1	6.2	_	0.63	5.6		248	_	_	0.75	98	41	Law et al. (1997)
P. phocoena	Northwest Atlantic Ocean	6	0.444 ± 0.281	0.006 ± 0.003	_	8.9 ± 4.0	388 ± 116	9.86 ± 15.17	_	4.49 ± 0.93	_	2.04 ± 1.19	28 ± 5	Mackey et al. (1995)
S. coeruleoalba	Mediterranean Sea	6	1.75 ± 1.27	_	0.03 ± 0.02	7.73 ± 2.22	307 ± 93	189.2 ± 28.6	8.82 ± 3.70	3.19 ± 1.48	_	80.3 ± 12.7	24.5 ± 7.3	Cardellicchio et al.
														(2000)
P. macrocephalus ^a	North Sea	6	19.1 ± 8.9	_	0.02 ± 0.02	1.7 ± 0.6	458 ± 70	14.5 ± 9.2	0.34 ± 0.16	_	0.12 ± 0.10	3.6 ± 2.6	21 ± 3	Holsbeck et al.
•														(1999)
Z. cavirostris	Mediterranean Sea	1	18.49	_	0.12	_	_	259.3	9.85	_	_	110.6	_	Storelli et al. (1999)

^a Values recalculated from dry wt.

Table 5 Heavy metal concentrations ($\mu g. g^{-1}$ wet wt.) in the liver of *G. macrorhynchus* and *K. breviceps* from various areas

Species	Locality	N	Cd	Co	Cr	Cu	Fe	Total-Hg	Organic-Hg	Mn	Ni	Se	Zn	Authors
G. macrorhynchus														
•	Australia	5	0.4-22.0	_	_	_	_	_	_	_	_	_	_	Kemper et al. (1994)
	Caribbean Sea	5	_	_	_	_	-	88.7 ± 67.9	3.46 ± 0.20	_	_	_	_	Gaskin et al. (1974)
	Cumberland Island	4	13.90 ± 3.98	_	_	_	-	231.0 ± 172.1	_	_	_	44.18 ± 19.32		Stoneburner (1978)
	New Caledonia	2	50.1-102.3	0.01	<dl< td=""><td>8.7 - 12.7</td><td>294-307</td><td>313-323</td><td>0.38 - 2.59</td><td>1.51 - 1.57</td><td><dl</td><td>146-162</td><td>35.3-37.0</td><td>Present study</td></dl<>	8.7 - 12.7	294-307	313-323	0.38 - 2.59	1.51 - 1.57	<dl	146-162	35.3-37.0	Present study
K. breviceps														
	Argentina	1	7.6	_	_	10.3	-	11.7		_	_	_	163.2	Marcovecchio et al. (1990)
	Australia	1	14.3	_	_	_	-	1.52	_	_	_	_	_	Kemper et al. (1994)
	England	1	3.2	_	0.57	9.5	1450	14	_	_	0.68	0.18	21	Law et al. (2001)
	Florida	_	0.22 - 7.60	_	0.84-4.13	2.10-144.44	-	_	_	0.80 - 4.06	_	_	10.09-15.04	Odell and Asper (1976)
	New Caledonia	2	6.3-10.8	0.01	<dl< td=""><td>1.9-4.1</td><td>501-624</td><td>1.7-17.2</td><td>0.28 - 1.56</td><td>1.11-1.15</td><td><dl</td><td>4.5-5.7</td><td>16.9-18.1</td><td>Present study</td></dl<>	1.9-4.1	501-624	1.7-17.2	0.28 - 1.56	1.11-1.15	<dl	4.5-5.7	16.9-18.1	Present study
	South Africa	4	_	_	_	_	-	0.9 ± 0.8	_	_	_	_	28.5 ± 13.7	Henry and Best (1999)
	South Africa	7	_	-	-	-	_	6.1 ± 5.6	-	-	-	-	21.9 ± 4.7	Henry and Best (1999)

very high total Hg concentrations. However, such a low organic fraction in muscle of *G. macrorhynchus* from New Caledonia remains questionable. It has been suggested that residual mercury could be mobilised and concentrated from muscle and blubber in other tissues prior to death in *G. macrorhynchus* from the Cumberland Islands (Stoneburner, 1978). This author explained that a stress period, viz. fast, could lead to such remobilization. The very low percentages of organic Hg in blubber support this speculation.

It is noteworthy that K. breviceps preferentially feeding on cephalopods and shrimp (Table 2) had concentrations of organic Hg in their tissues similar to that of G. macrorhynchus. Cephalopods and shrimp displayed lower methyl Hg contents than fish, i.e. from 29 to 55% vs 53 to 94%, respectively (Cappon and Smith, 1982). Compared to G. macrorhynchus, higher percentages of organic Hg in the tissues of K. breviceps related to lower total Hg contents in liver could be explained by a lower rate of hepatic demethylation. Low percentages of methyl Hg in liver have been reported for many marine mammals species (e.g. Itano et al., 1984, Wagemann et al., 1998). This supports the idea of continuous demethylation of methyl Hg occurring in liver throughout the animal's life span, subsequently leading to the formation of mercuric selenide (HgSe) granules (Pelletier, 1985; Cuvin-Aralar & Furness, 1991; Nigro & Leonzio, 1996). HgSe granules (tiemannite) have been identified in the liver of seabirds, marine mammals and humans (Martoja & Berry, 1980; Pelletier, 1985; Hansen et al., 1989; Nigro & Leonzio, 1996). Moreover, many studies also reported that Hg and Se are correlated in a 1:1 molar ratio as in HgSe (Koeman et al., 1973; Nielsen & Dietz, 1990; Law et al., 1997; Wagemann et al., 1998). G. macrorhynchus and K. breviceps from New Caledonia had a Hg:Se molar ratio close to 1 and a low percentage of organic Hg in liver (Figs. 1 and 2). This suggests the presence of tiemannite granules as a result of demethylation of organic Hg in liver. However, the youngest K. breviceps, a 6-years-old male (Table 2), had a low hepatic Hg:Se ratio due to low total Hg concentrations. This specimen also exhibited an elevated percentage of hepatic organic Hg (Fig. 2). Palmisano et al. (1995) have shown that demethylation of Hg by Se is efficient reaching a threshold Hg concentration of 100 μg g⁻¹ wet wt. (approximately 500 μg g⁻¹ dry wt.) in the liver of the dolphin Stenella coeruleoalba. Although this threshold should be different from one species to another, it clearly appears that the young K. breviceps had Hg concentrations far below such a threshold.

5. Conclusion

The present study provides new information on trace element concentrations in the tissues of two rare marine mammals species from a tropical area. Very low Ni, Co and Cr concentrations in the tissues of *G. macrorhynchus* and *K. breviceps* suggest that industrial extracting activities in New Caledonia do not represent a significant source of contaminants for these two particular species. On the other hand, very high Cd and Hg concentrations in the liver of these marine mammals were related to cephalopod and fish consumption. Interspecific differences in the bioaccumulation, i.e. highest Cd and Hg concentrations in *G. macrorhynchus*, remain questionable and should be related to the physiology of this species.

Acknowledgements

We are particularly grateful to Richard Young from the University of Hawaii for cephalopod determination and to Jacques Rivaton from the IRD of Nouméa for fish determination. We also thank the four anonymous reviewers for their helpful comments on this work.

References

Aguilar, A., Borrell, A., Pastor, T., 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. In: Reijnders PJH, Aguilar A, Donovan GP (Eds.), Chemical pollutants and cetaceans, The journal of Cetacean Research and Management, Special Issue 1, 83–116.

Bryan, G.W., 1984. Pollution due to heavy metals and their compounds. In: Kinne, O. (Ed.), Marine Ecology, Vol. 5 Part 3. Wiley-Interscience, Chichester, pp. 1289–1431.

Bustamante, P., Caurant, F., Fowler, S.W., Miramand, P., 1998. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. The Science of the Total Environment 220, 71–80.

Bustamante, P., Grigioni, S., Boucher-Rodoni, R., Caurant, F., Miramand, P., 2000. Bioaccumulation of 12 trace elements in the tissues of the nautilus *Nautilus macromphalus* from New-Caledonia. Marine Pollution Bulletin 40 (8), 688–696.

Cappon, C.J., Smith, J.C., 1982. Chemical form and distribution of mercury and selenium in edible seafood. Journal of Analytical Toxicology 6, 10–21.

Cardellicchio, N., Giandomenico, S., Ragone, P., Di Leo, A., 2000.
Tissue distribution of metals in striped dolphins (*Stenella coeruleoalba*) from the Apulian coasts, Southern Italy. Marine Environmental Research 49, 55–66.

Caurant, F., Navarro, M., Amiard, J.C., 1996. Mercury in pilot whales: possible limits to the detoxification process. The Science of the Total Environment 186, 95–104.

Cossa, D., Thibaud, Y., Roméo, M., Gnassia-Barelli, M., 1990. Le mercure milieu marin. Biogéochimie et écotoxicologie. Rapport Scientifique et Techniques de l'IFREMER, Brest.

Cuvin-Aralar, M.L.A., Furness, R.W., 1991. Mercury and selenium interactions: a review. Ecotoxicology and Environmental Safety 21, 348–364.

Fujise, Y., Honda, K., Tatsukawa, R., Mishima, S., 1988. Tissue distribution of heavy metals in Dall's porpoise in the northwestern Pacific. Marine Pollution Bulletin 19, 226–230.

Gallien, I., Caurant, F., Bordes, M., Bustamante, P., Miramand, P., Fernandez, B., Quellard, N., Babin, P., 2001. Cadmium-containing

- granules in kidney tissue of the Atlantic white-sided dolphin (*Lagenorhyncus acutus*) off the Faroe Islands. Comparative Biochemistry and Physiology 130, 389–395.
- Garrigue, C., Fernandez, J.M., Badie, J.M., Bernard, Ch., Greaves, J., Rivaton, J., Trescinski, M., 2000. Impact of the human activities in short-finned pilot whales (*Globicephala macrorhynchus*) and pygmy sperm whale (*Kogia breviceps*) of the South West Pacific ocean by mesuring Cs-137, K-40 and Pb-210. In: Fernandez, J.M, Fichez, R. (Eds.), Environmental Changes and Radioactive Tracers. IRD Editions, Paris, pp. 49–58.
- Gaskin, D.E., Smith, G.J.D., Arnold, P.W., Louisy, M.V., Franck, R., Holdrinet, M., McWade, J.W., 1974. Mercury, DDT, dieldrin, and PCB in two species of Odontoceti (Cetacea) from St Lucia, Lesser Antilles. Journal of the Fisheries Research Board Canada 31 (7), 1235–1239.
- Hansen, J.C., Reske-Nielsen, E., Thorlacius-Ussing, O., Rungby, J., Danscher, G., 1989. Distribution of dietary mercury in a dog. Quantification and localization of total mercury in central and nervous system. The Science of the Total Environment 78, 23–43.
- Henry, J., Best, P., 1999. A note on concentrations of metals in cetaceans from southern Africa. In: Reijnders, P.J.H., Aguilar, A., Donovan, G.P. (Eds.), Chemical Pollutants and Cetaceans. Journal of Cetacean Research and Management (Special Issue 1), Cambridge, pp. 177–194.
- Hohn, A.A., Fernandez, S., 1999. Bias in dolphin age structure due to age estimation technique. Marine Mammal Science 15 (4), 1124– 1132.
- Holsbeck, L., Joiris, C.R., Debacker, V., Ali, I.B., Roose, P., Nellissen, J.P., Gobert, S., Bouquegneau, J.M., Bossicart, M., 1999.
 Heavy metals, organochlorines and polycyclic aromatic hydrocarbons in sperm whales stranded in the Southern North Sea during the 1994/1995 winter. Marine Pollution Bulletin 38 (4), 304–313.
- Honda, K. 1985. Studies on Bioaccumulation of Heavy Metals in Marine Mammals. PhD Thesis, Tokyo university (in Japanese).
- Itano, K., Kawai, S., Miyazaki, N., Tatsukawa, R., Fujiyama, T., 1984. Mercury and selenium levels in striped dolphins caught off the Pacific coast of Japan. Agriculture and Biological Chemistry 48 (5), 1109–1116.
- Kemper, C., Gibbs, P., Obendorf, D., Marvanek, S., Lenghaus, C., 1994. A review of heavy metal and organochlorine levels in marine mammals in Australia. The Science of the Total Environment 154, 129–139.
- Koeman, J.H., Peeters, V.H.M., Koudstaal-Hol, C.H.M., Tijoe, P.S., De Goeu, J.J.M., 1973. Mercury selenium correlation in marine mammals. Nature 246, 385–386.
- Law, R.J., Jones, B.R., Baker, J.R., Kennedy, S., Milnes, R., Morris, R.J., 1992. Trace metals in the livers of marine mammals from the Welsh coast and the Irish Sea. Marine Pollution Bulletin 24 (6), 296–304.
- Law, R.J., Allchin, C.R., Jones, B.R., Jepson, P.D., Baker, J.R., Spurrier, C.J.H., 1997. Metals and organochlorines in tissues of a Blainville's beaked whale (*Mesoplodon densirostris*) and a killer whale (*Orcinus orca*) stranded in the United Kingdom. Marine Pollution Bulletin 34 (3), 208–212.
- Law, R.J., Bennett, M.E., Blake, S.J., Allchin, C.R., Jones, B.R., Spurrier, C.J.H., 2001. Metals and organochlorines in pelagic cetaceans stranded on the coasts of England and Wales. Marine Pollution Bulletin 42 (6), 522–526.
- Mackey, E.A., Demilralp, R., Becker, P.R., Greenberg, R.R., Koster, B.J., Wise, S.A., 1995. Trace element concentrations in cetacean liver tissues archived in the National Marine Mammal Tissue Bank. The Science of the Total Environment 175, 25–41.
- Mackey, E.A., Becker, P.R., Demilralp, R., Greenberg, R.R., Koster, B.J., Wise, S.A., 1996. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinnipeds. Archives of Environmental Contamination and Toxicology 30, 503–512.
- McAlpine, D.F., 2001. Pygmy and dwarf sperm whales. In: Perrin,

- W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals. Academic Press, New York, pp. 1007–1009.
- Marcovecchio, J.E., Moreno, V.J., Bastida, R.O., Gerpe, M.S., Rodriguez, D.H., 1990. Tissue distribution of heavy metals in small cetaceans from the South-western Atlantic Ocean. Marine Pollution Bulletin 21 (6), 299–304.
- Martoja, R., Berry, J.P., 1980. Identification of tiemannite as a probable product of delmethylation of mercury by selenium in cetaceans. Vie Milieu 30, 7–10.
- Monniot, F., Martoja, R., Monniot, C., 1994. Cellular sites of iron and nickel accumulation in ascidians related to the naturally and anthropic enriched New Caledonian environment. Annales de l'Institut Océanographique 70 (2), 205–216.
- Nigro, M., Leonzio, C., 1996. Intracellular storage of mercury and selenium in different marine vertebrates. Marine Ecology Progress Series 135, 137–143.
- Nielsen, H.G., 1972. Age determination of the harbour porpoise, *Phocoena phocoena* (L.) (Cetacea). Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening 135, 61–84.
- Nielsen, C.O., Dietz, R., 1990. Distributional pattern of zinc, cadmium, mercury, and selenium in livers of hooded seals (*Cystophora cristata*). Biological Trace Element Research 24, 61–71.
- Odell, D.K., Asper, E.D., 1976. Studies on the biology of *Kogia* (Cetacea: Physeteridae) in Florida: preliminary report to the Small Whales Subcommittee on the International Whaling Commission, London.
- Olson, P.A., Reilly, S.B., 2001. Pilot whales. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals. Academic Press, New York, pp. 898–903.
- Palmisano, F., Cardellicchio, N., 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 (2), 109–121.
- Pauly, D., Trites, A.W., Capuli, E., Christensen, V., 1998. Diet composition and trophic levels of marine mammals. ICES Journal of Marine Science 55, 467–481.
- Pelletier, E., 1985. Mercury-selenium interactions in aquatic organisms: a review. Marine Environmental Research 18, 111–132.
- Perrin, W.F., Myrick, A.C. Jr., 1980. Age determination of toothed whales and sirenians. Reports of the International Whaling Commission. Special issue 3, Cambridge.
- Plön, S. (in preparation) The Natural History and Status of Pygmy (*Kogia breviceps*) and Dwarf (*Kogia sima*) Sperm Whales off Southern Africa. PhD Thesis, Rhodes University, Grahamstown, South Africa.
- Robineau, D., Rancurel, P., 1981. Sur deux spécimens du genre Kogia (Cetacea, Physeteridae) en Nouvelle-Calédonie. Zeitschrift für Säugetierkunde 46, 56–58.
- Stoneburner, D.L., 1978. Heavy metals in tissues of stranded short-finned pilot whales. The Science of the Total Environment 9, 293–297
- Storelli, M.M., Zizzo, N., Marcotrigiano, G.O., 1999. Heavy metals and methylmercury in tissues of Risso's dolphin (*Grampus griseus*) and Cuvier's beaked whale (*Ziphius cavirostris*) stranded in Italy (South Adriatic Sea). Bulletin of Environmental Contamination and Toxicology 63, 703–710.
- Sylvestre, J.P., 1988. On a specimen of pygmy sperm whale *Kogia breviceps* (Blainville, 1838) from New Caledonia. Aquatic Mammals 14, 76–77.
- Thompson, D.R., 1990. Heavy metals in marine vertebrates. In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, pp. 143–182.
- Uthe, J.F., Solomon, J., Grift, B., 1972. Rapid semimicro method for the determination of methyl mercury in fish tissue. Journal of the Association of Official Analytical Chemists 55, 583–589.
- Wagemann, R., Trebacz, E., Boila, G., Lockhart, W.L., 1998.Methylmercury and total mercury in tissues of arctic marine mammals. The Science of the Total Environment 218, 19–31.