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Total and Organic Mercury in the Black Sea Harbour Porpoise *Phocoena phocoena relicta*

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This paper reports on mercury (Hg) concentrations in different tissues (liver, muscle, kidney, blubber and brain) of harbour porpoises *Phocoena phocoena* found dead in the Black Sea between 1997 and 1999, mainly bycaught in fishing nets (n = 79). Total Hg and organic Hg (MeHg) were determined. The main factor affecting Hg accumulation was, as expected, age, with MeHg concentration increasing with age. Accumulation of high concentrations of inorganic Hg in the liver was probably due to a slow demethylation process implying the formation of tiemannite (HgSe). In older adults, liver concentrations reached 35 μg g^{-1} dry weight ('ppm') total Hg and 3 μg g^{-1} dw MeHg. A geographical comparison with existing data from other regions showed a generally low Hg contamination of Black Sea porpoises, one order of magnitude lower than, e.g. in the North Sea. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Black Sea; cetaceans; harbour porpoise; *Phocoena phocoena*; total mercury; methylmercury.

The Black Sea contamination deserves special attention, since environmental pollution of the surrounding terrestrial ecosystems by stable pollutants, both heavy metals and organochlorines is expected to be high due to historical habits in agriculture and industry, with less environmental concern in central and eastern, than in western Europe. As a semi-closed sea, the Black Sea has limited water exchanges only with the Mediterranean Sea, and thus the ecological stability of micropollutants is expected to be very high.

Cetaceans were selected as study material, not only because, as large and long-living top predators, they integrate small-scale local and temporal variations of environmental contamination (they are proposed as good bioindicators), but also because they were till recently actively hunted for human consumption and as bait by fishermen. Before World War II, the dolphin fishery in the Black Sea was evaluated as 200 000 individuals per year, for a total population evaluated as 800 000 (Komakhidze and Mazmanidi, 1998), if such rough estimations were confirmed, they obviously would reflect a serious threat to the dolphin and porpoise populations. Active fishing on dolphins was forbidden in the Soviet Union in 1966, soon followed by Bulgaria and Romania, and in Turkey in 1983. Accidental bycatch – and even some active fishing – seem however to remain the main and important cause of mortality (Birkun *et al.*, in preparation). For these reasons, the study of their population level, pathology and toxicology is of special importance, and became the topic of the BLASDOL (for 'BLAck Sea DOLphins') collaborative research programme.

Materials and Methods

Small cetaceans found dead in the Black Sea were systematically collected between 1997 and 1999 (see Birkun *et al.*, in preparation). In this study, 79 harbour porpoises *Phocoena phocoena relicta* were included: 56 from Ukraine (Crimea), 12 from Bulgaria and 11 from Georgia. All but five were bycaught in fishing nets; even for the five stranded individuals, it is not excluded that they were first bycaught, thrown over board and then they eventually stranded. They were analysed for their total mercury ($\sum$Hg) and organic mercury (MeHg) content in different tissues, mainly liver, muscle and kidney and, to a lesser extent, blubber and brain when available.

Total Hg was determined with atomic absorption spectrophotometry (Perkin–Elmer MAS-50 Analyser)

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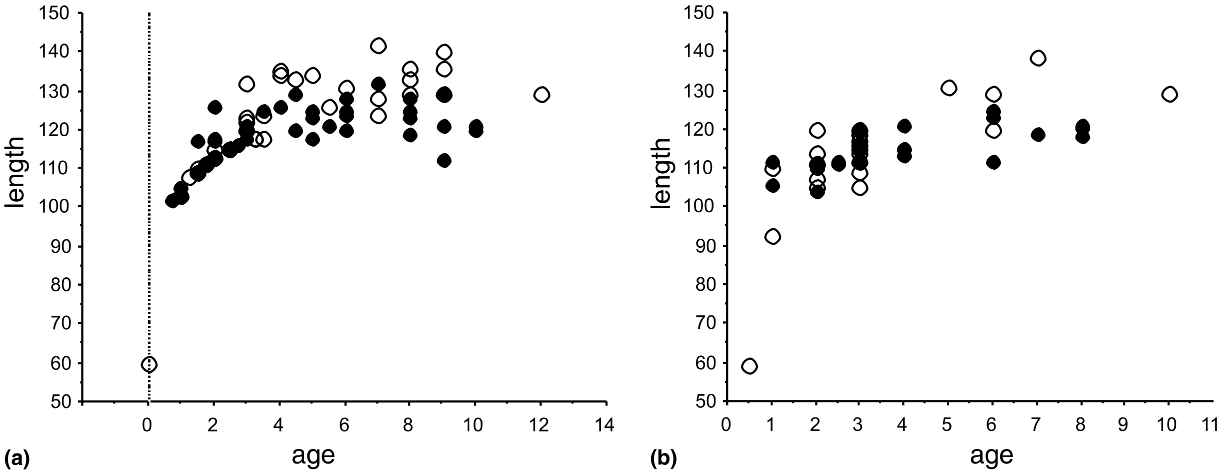

Fig. 1 Relationship between total body length (cm) and age (years) derived from dental growth layers in harbour porpoises from the Black Sea. Dots: males; open circles: females; (a) this study; (b) from basic data in Tanabe *et al.* (1997).

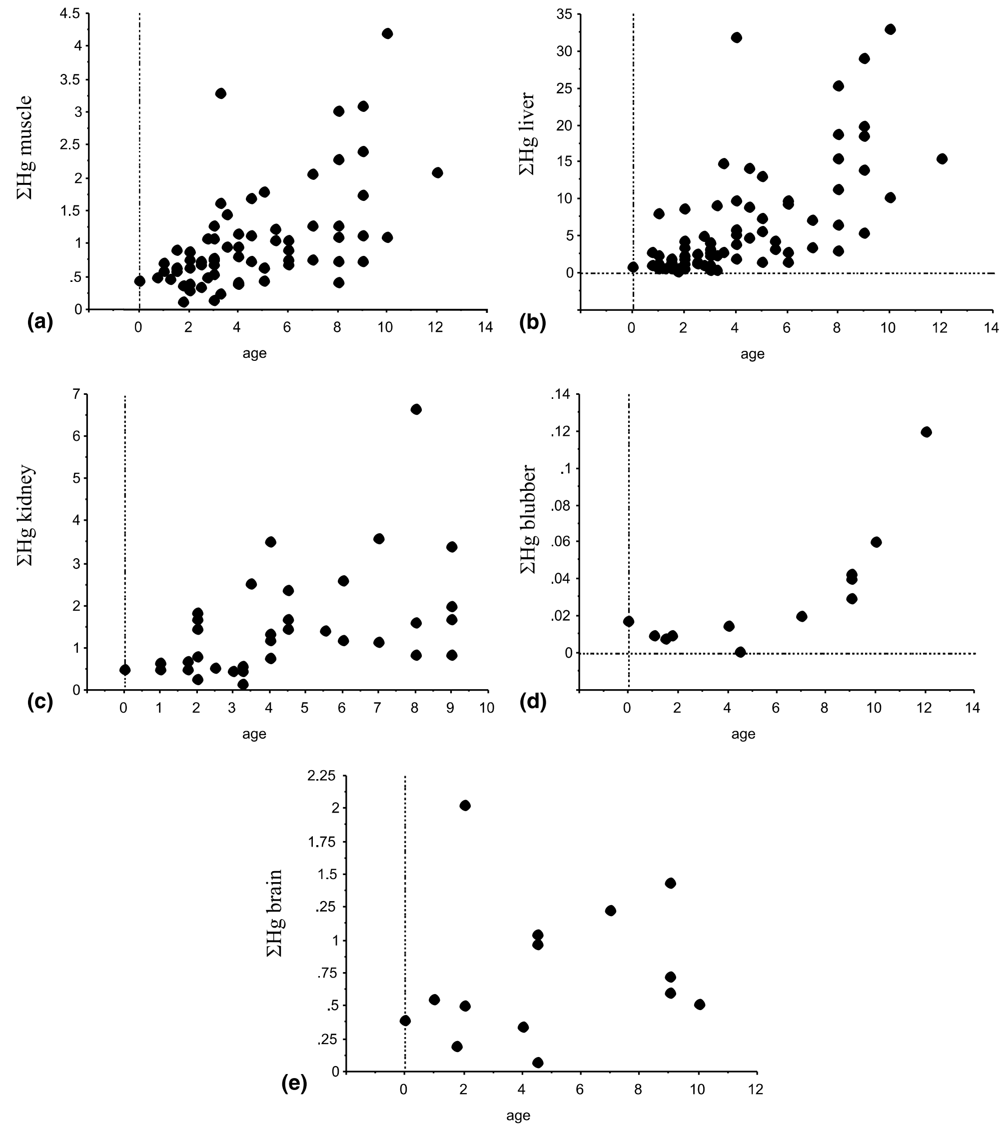

Fig. 2 Total mercury concentration ($\sum$Hg, μg g^{-1} dw) as a function of age (years) in different tissues of harbour porpoises from the Black Sea: (a) muscle; (b) liver; (c) kidney; (d) blubber; (e) brain.

after mineralization with sulfuric acid. Organic Hg (actually monomethylmercury) was determined by gas–liquid chromatography with capillary column and electron capture detector (Packard, model 437). More details about the methods, including quality control (detection limit, worked blanks, internal standard, reproducibility, possible matrix effect, analysis of certified material etc.) can be found in Joiris *et al.* (1991, 1999) and Holsbeek *et al.* (1999).

Results and Discussion

Since no effect of season, year, nor sex, could be detected, all data were pooled.

In order to allow comparison and discussion, results must first be normalized for age, since stable residues like organochlorines and Hg tend to accumulate in marine mammals with age. In this study, age was determined by two ways. For the juveniles and sub-adults in their growing phase, total body length was considered as a good evaluation of age; from an age of 5 years on, however, the growth curve tends to reach a plateau (Fig. 1), and age was determined on the basis of dental growth layers, assuming that each dark layer corresponds to one year.

Total Hg concentration tended to increase with age in muscle, liver, kidney and blubber, while no clear trend could be detected in brain (Fig. 2). Differences can however be noted between tissues, with very low values in blubber: the concentration first remained stable or even showed a decline in young animals, and then strongly increased from the age of 7 on (Fig. 2(d)). This might result from different factors such as transplacental transfer, growth dilution effect in rapidly growing immatures, and the evolution of blubber layer with age. Figures were of the same order of magnitude in muscle and kidney (Fig. 2(a) and (c)). The most important increase was noted in liver (Fig. 2(b); note the different scale), with values similar to the ones of muscle in juveniles, but becoming much higher in older animals.

Organic Hg concentration also tended to increase with age, but to a much lower extent (Fig. 3); the difference was most striking for liver (Fig. 3(b)): even if a limited increasing trend can be noted, concentrations did not show the strong increase of $\sum$Hg, but remained at the same order of magnitude as in muscle (Fig. 3(a)), with a liver-to-muscle ratio around 1.25 (Fig. 4).

As a consequence, the relative MeHg concentration (%MeHg, as a percent of $\sum$Hg) showed no clear trend as a function of age in muscle nor kidney (Fig. 5(a) and

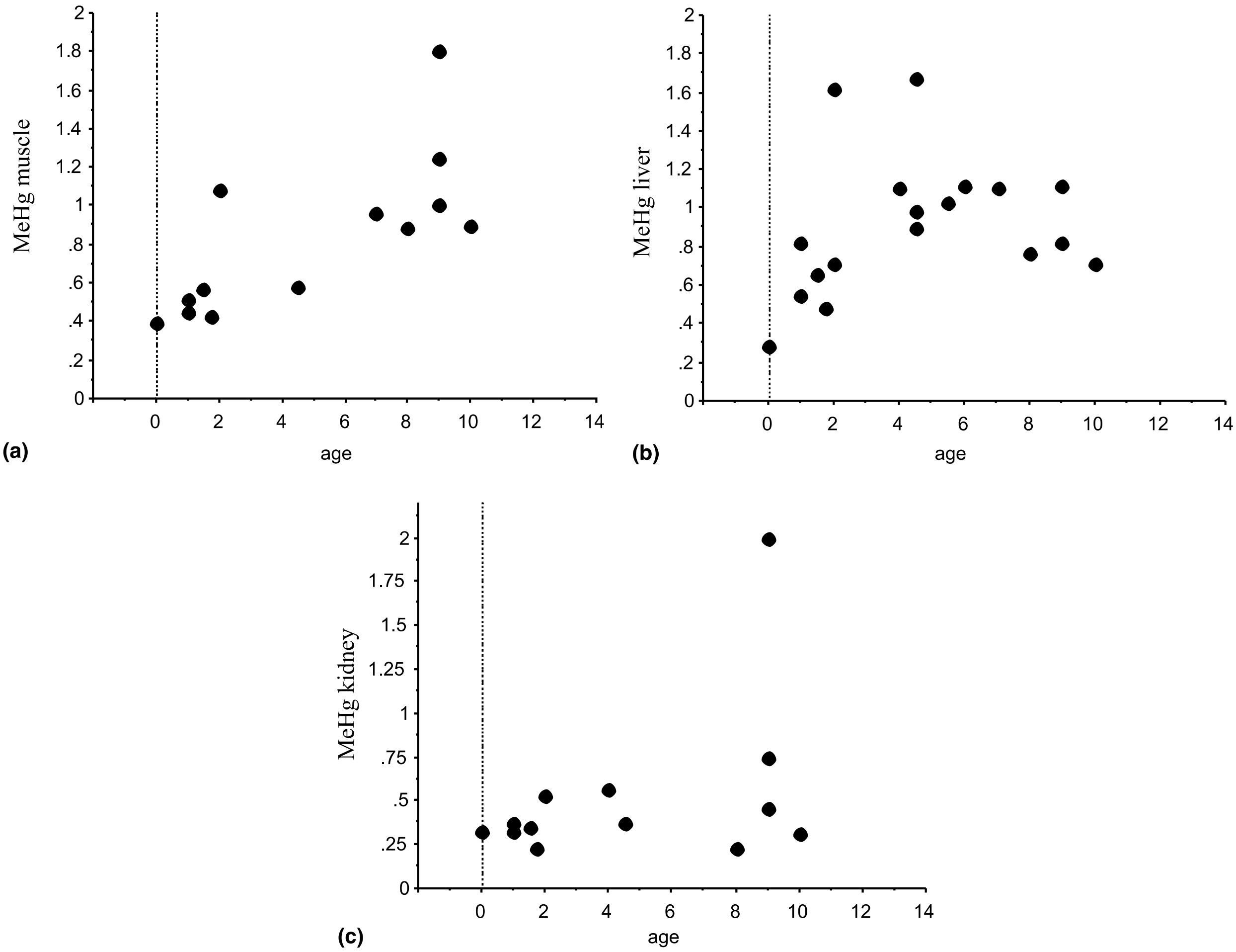

Fig. 3 Organic mercury concentration (MeHg, μg g^{-1} dw) as a function of age (years) in different tissues of harbour porpoises from the Black Sea: (a) muscle; (b) liver; (c) kidney.

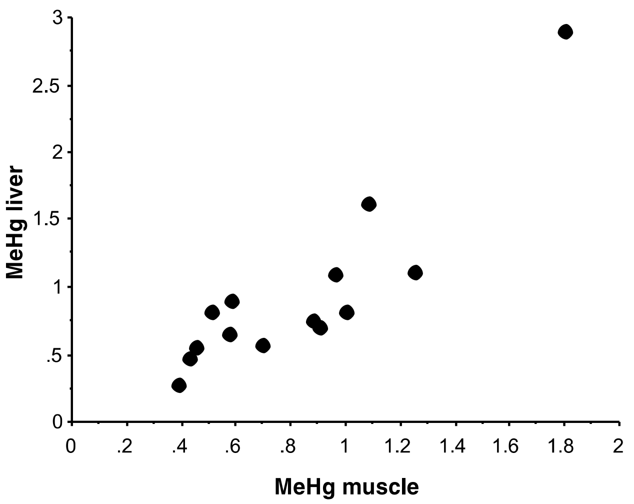

Fig. 4 Relationship between MeHg concentrations in liver and muscle (μg g^{-1}dw).

(c)), but a strong decrease in liver (Fig. 5(b)). The interpretation being that a slow demethylation process is taking place in liver mainly, leading to an (almost) irreversible accumulation of inorganic Hg, probably as tiemannite (HgSe) (Martoja and Berry, 1980).

Special attention can be paid to a 60 cm long foetus found in a 9-year-old female, to be identified as age zero on all graphs. ∑Hg and MeHg looked 'normal' in all tissues, while the relative MeHg liver concentration was much lower than expected from a pure age effect. If representative (one sample only), this might reflect a limited transplacental transfer of MeHg to the foetus, the juveniles accumulating MeHg later on, first through mother milk and then from prey items.

Data obtained by the same team in different tissues of two common dolphins *Delphinus delphis* collected in the Black Sea in 1994 during a morbillivirus epizootic showed similar levels of Hg contamination (Birkun *et al.*, 1999).

Another complementary discussion consists in comparing not only Hg concentrations, but also burdens (loads) by multiplying concentrations by the tissue weight. The total body burden was calculated as the sum of the main soft tissues, namely muscle, liver and blubber, representing in adults 27%, 2.5% and 27%, respectively, of total body weight. The MeHg burden was increasing from 0.1 to 8 mg, the ∑Hg burden from 0.2 to 20 mg (Table 1; Fig. 6), the difference between both reflecting the important inorganic Hg accumulation in liver

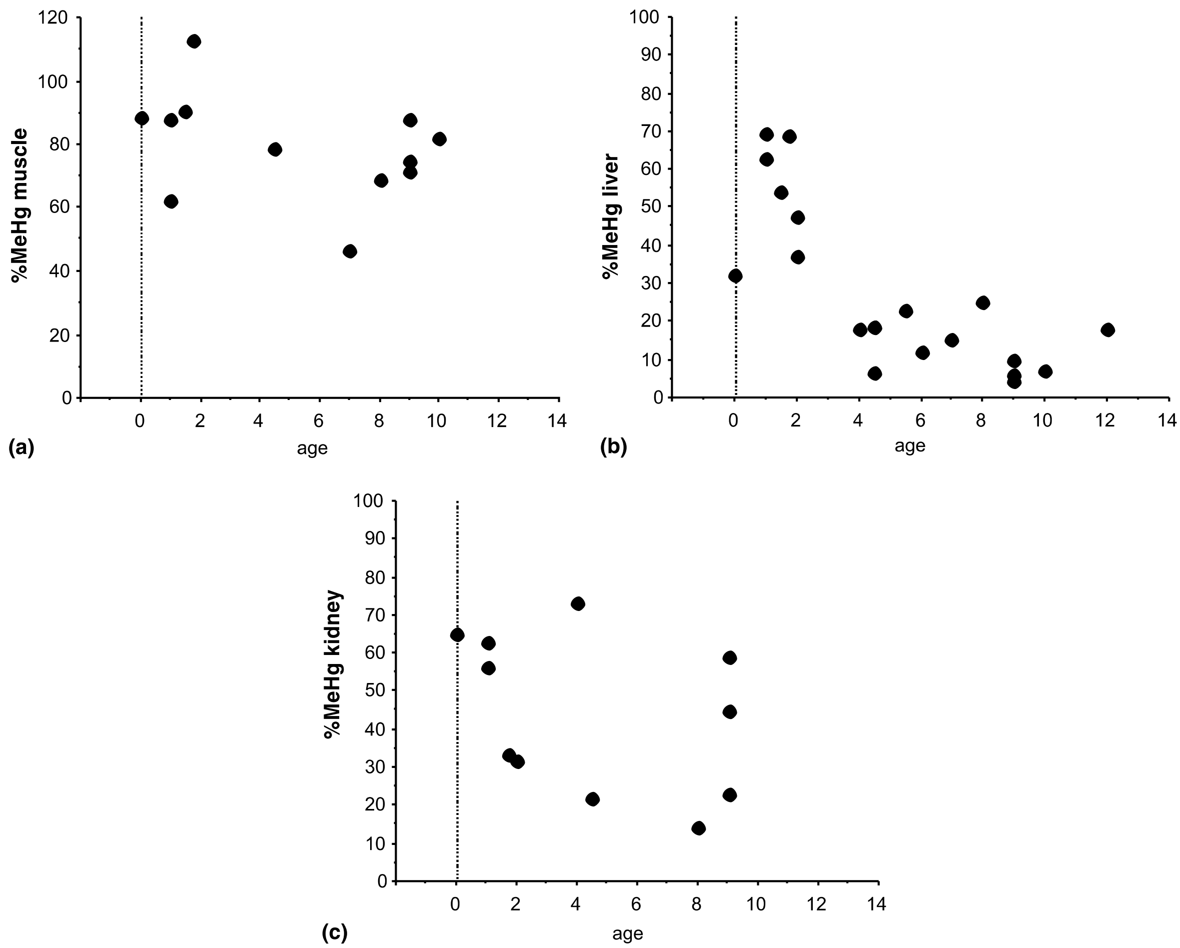

Fig. 5 Relative organic mercury concentration (%MeHg) as a function of age (years) in different tissues of harbour porpoises from the Black Sea: (a) muscle; (b) liver; (c) kidney.

TABLE 1

Mercury concentration and burden (load) in Black Sea harbour porpoises: muscle M, liver L; blubber B and whole body.[a]

Age	Body weight	Tissue weight			Hg Concentration			Hg burden			Whole body
		M	L	B	M	L	B	M	L	B	
Total mercury											
0	5	0.6	0.2	2.2	0.13	0.26	0.017	0.08	0.046	0.04	0.18
1	21	4.4	0.6	7.1	0.17	0.40	0.010	0.77	0.25	0.07	1.21
1.5	26	5.5	0.8	8.8	0.19	0.37	0.008	1.0	0.29	0.07	1.54
1.5	23	4.8	0.7	7.8	0.09	0.14	0.012	0.45	0.10	0.10	0.72
1.75	28	5.9	0.8	9.5	0.11	0.20	0.010	0.67	0.17	0.09	1.0
3	42	11.3	1.1	11.6	0.24	1.8	0.014	2.7	2.0	0.16	5.4
4.5	36	9.7	0.9	9.9	0.22	1.4	0.012	2.1	1.30	0.12	4.0
7	31	8.4	0.8	8.5	0.62	2.1	0.019	5.2	1.70	0.16	7.8
9	41	11.1	1.1	11.3	0.34	6.0	0.041	3.8	6.4	0.46	12.0
9	42	11.3	1.1	11.6	0.72	8.7	0.038	8.2	9.5	0.44	20
9	55	14.9	1.4	15.1	0.53	5.6	0.029	7.8	8.0	0.43	18
10	32	8.6	0.8	8.8	0.33	3.1	0.057	2.8	2.6	0.50	6.6
12	41	11.1	1.1	11.3	0.62	4.7	0.11	6.9	5.0	1.30	15
Organic (methyl) mercury											
0	5	0.6	0.2	2.2	0.12	0.08	0.003	0.07	0.015	0.006	0.10
1	21	4.4	0.6	7.1	0.15	0.25	0.002	0.68	0.16	0.012	0.93
1.5	26	5.5	0.8	8.8	0.17	0.20	0.001	0.93	0.15	0.011	1.2
1.5	23	4.8	0.7	7.8	0.08	0.08	0.002	0.36	0.056	0.016	0.48
1.75	28	5.9	0.8	9.5	0.13	0.14	0.002	0.76	0.12	0.015	1.0
3	42	11.3	1.1	11.6	0.06	0.33	0.002	0.66	0.36	0.027	1.2
4.5	36	9.7	0.9	9.9	0.17	0.27	0.002	1.69	0.25	0.021	2.2
7	31	8.4	0.8	8.5	0.29	0.33	0.003	2.41	0.27	0.028	3.0
9	41	11.1	1.1	11.3	0.30	0.25	0.007	3.32	0.26	0.078	4.1
9	42	11.3	1.1	11.6	0.54	0.87	0.006	6.12	0.95	0.075	7.9
9	55	14.9	1.4	15.1	0.38	0.34	0.005	5.57	0.48	0.073	6.8
10	32	8.6	0.8	8.8	0.27	0.21	0.010	2.33	0.18	0.085	2.9
12	41	11.1	1.1	11.3	0.15	0.84	0.019	1.68	0.90	0.22	3.1

[a] Age in years, body and tissue weight in kg; Hg concentrations in mg kg^{-1} fresh w; burden in mg. Relative tissue weights from Lockyer (1995a,b).

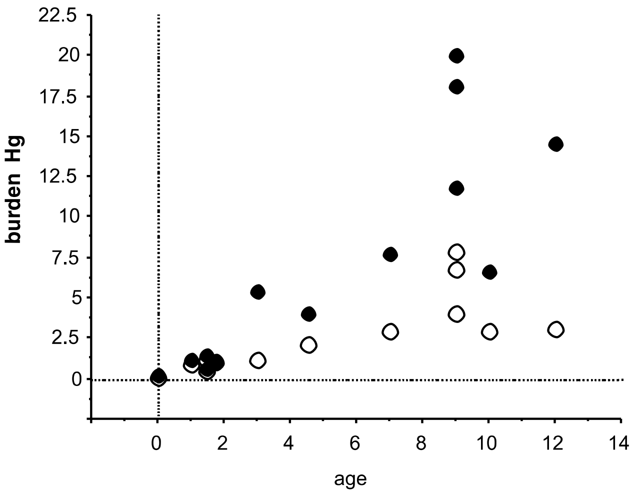

Fig. 6 Whole body Hg burden (mg) in harbour porpoises from the Black Sea as a function of age (years). Dots: ∑Hg; open circles: MeHg.

with age, probably as tiemannite. The relative weight of tissues is, however, changing with age, especially for blubber. As a result, the relative ∑Hg burden of blubber changed from 21% in foetus to 10% in juveniles (1–2 years), and to 4% in adults (more than 2 years), even if the concentration was increasing with age (Fig. 2(d)). It was stable in muscle, at about 50%, and increased in liver from 20% to 50%. MeHg relative burdens remained more constant, with 70–80% in muscle, 15% in liver and 3% in blubber (rounded figures).

Conclusions

These results on Black Sea porpoises were compared with literature data, in order to establish a geographical comparison on environmental Hg contamination of various marine ecosystems. A rough comparison seems to indicate low Hg levels in comparison with the other ecosystems, with the lowest recorded median values for both tissues (Table 2). In order to confirm this conclusion, it is however necessary to take the age effect into account, since the age distribution might be different in the various studies: e.g. juveniles less than one year old were not represented in our samples, which probably reflects that the reproduction zones in the Black Sea were not covered by our geographical sampling. A comparison of the curves (Fig. 7) indeed reflects much lower Hg levels in the propoises from the Black Sea, at about one order of magnitude lower than that in the North Sea, and half the values of West Greenland. Such conclusions, however, do not simply reflect environmental contamination. Other factors, such as primary production ('biomass effect') also can influence pollutants' level, so that low environmental contamination, coupled with low primary production, can lead to high concentration in particulate matter (Joiris and

TABLE 2
Total mercury concentration in muscle and liver of harbour porpoises from the North Atlantic and adjacent regions.[a]

Region	Tissue>Muscle					Liver					References
	n	Mean	Median	Min	Max	*n*	Mean	Median	Min	Max	
West Greenland	27			0.01	1.1	15			0.5	21	Paludan-Müller *et al.* (1993)
Bay of Fundy, Canada	113	0.9		0.2	2.5	68	12		0.5	112	Gaskin *et al.* (1979)
North-west Atlantic	103	0.7		0.1	3.3	129	5.7		0.1	44	Westgate and Johnson (1995)
North-west Atlantic						6			2.2	43	Mackey *et al.* (1995)
Scotland						23	4.1		0.3	16	Falconer *et al.* (1983)
Irish Atlantic coast	7	0.9	0.8	0.3	1.7	7	1.2	1.3	0.3	2.5	This team: Holsbeek *et al.* (in preparation)
Irish Sea						28		4.3	0.6	190	Law *et al.* (1992)
Norway						92	2.9	1.9	0.3	10	Teigen *et al.* (1993)
North Sea & Kattegat	16	1.4	1.0	0.3	6.5	12	23	1.2	0.2	132	This team: Joiris *et al.* (1991)
Denmark	4	1.9	–	0.8	3.2	4	22		1.5	69	Andersen and Rebsdorff (1976)
Dutch North Sea	29	1.3	1.3	0.1	3.0	29	13	5.6	0.2	44	This team: Holsbeek *et al.* (in preparation)
German North Sea	28	2.1	1.4	0.3	11	30	18	6.0	0.3	130	This team: Siebert *et al.* (1999)
German Baltic Sea	26	1.3[b]	0.9[b]	0.2	9.0[b]	27	4.4	1.6	0.2	32	This team: Siebert *et al.* (1999)
German Baltic and North Sea						3	10		0.7	28	Harms *et al.* (1978)
Polish Baltic Sea	8	0.7		0.3	1.1	12	4.9		0.5	13	Szefer *et al.* (1995)
Black Sea	54	0.3	0.3	0.1	1.3	57	1.9	1.0	0.14	9.9	This study

[a] μg g^{-1} fresh weight; mean, median, minimum and maximum; *n* is the number of samples.
[b] One outlier (108 μg g^{-1} fw) not taken into account.

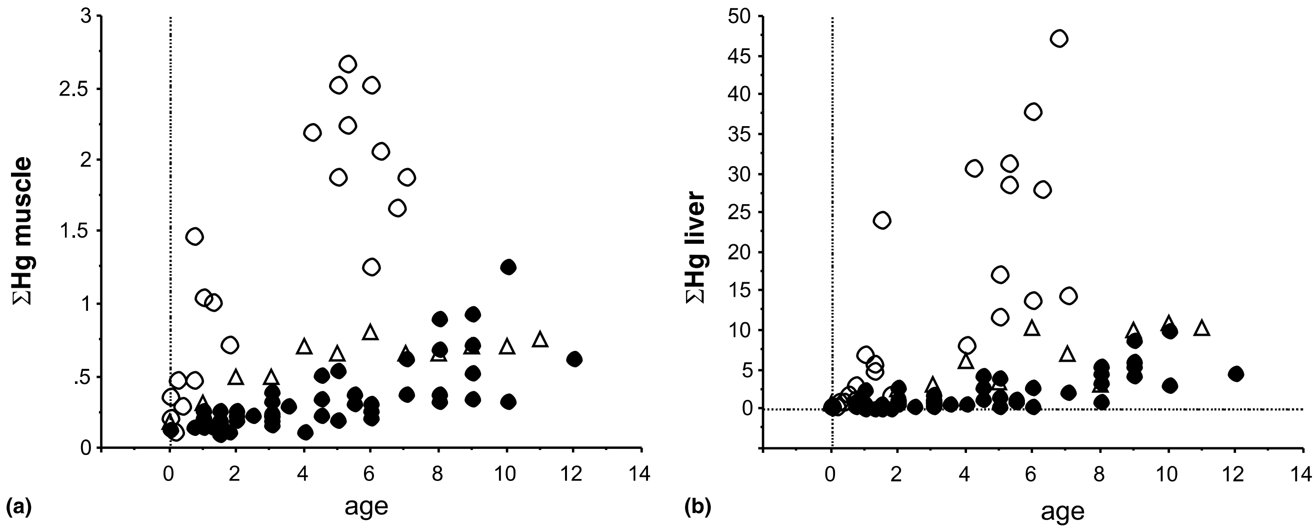

Fig. 7 Comparison of Hg concentration in harbour porpoises from different regions (μg g^{-1} fresh weight): $\sum$Hg in muscle (a) and liver (b) as a function of age. Note the use of another unit, in order to allow direct comparison with literature data. Dots: Black Sea (this study); circles: North Sea (Joiris *et al.*, 1991); triangles: West Greenland (Paludan-Müller *et al.*, 1993).

Overloop, 1991; Joiris *et al.*, 1995), and thus cause a high contamination of the higher trophic levels such as in the Greenland Sea in comparison with other Arctic seas (Joiris *et al.*, 1997). On the other hand, the special hydrological conditions in the Black Sea, with a limited aerobic surface water mass and an important deeper anoxic zone, might be the reason for an important sink of organic particulate matter with its pollutants' load. Such phenomena could be the explanation for the generally low contamination levels in the surface layer, more especially in fish and their predators.

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