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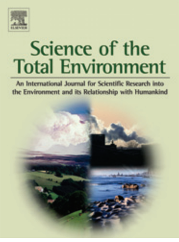

Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios

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ABSTRACT

Mercury (total and organic), cadmium, lead, copper, iron, manganese, selenium and zinc concentrations were measured in different organs of 6 different cetacean species stranded in an area of extraordinary ecological interest (Cetaceans' Sanctuary of the Mediterranean Sea) along the coast of the Ligurian Sea (North-West Mediterranean). Stable-isotopes ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were also measured in the muscle. A significant relationship exists between $^{15}N/^{14}N$, mercury concentration and the trophic level. The distribution of essential and non-essential trace elements was studied on several organs, and a significant relationship between selenium and mercury, with a molar ratio close to 1, was found in the cetaceans' kidney, liver and spleen, regardless of their species. High selenium concentrations are generally associated with a low organic to total mercury ratio. While narrow ranges of concentrations were observed for essential elements in most organs, mercury and selenium concentrations are characterised by a wide range of variation. Bio-accumulation and bio-amplification processes in cetaceans can be better understood by comparing trace element concentrations with the stable-isotopes data.

1. Introduction

Due to its favourable oceanographic, climatic and geomorphological factors, the "Cetaceans' Sanctuary of the Mediterranean Sea" which includes the Ligurian Sea, is considered as an ecologically interesting area, compared to the rest of the Mediterranean (Notarbartolo et al., 1992).

The Mediterranean cetacean species find their breeding and feeding needs in these waters. These species include fin whales *Balaenoptera physalus*, sperm whales *Physeter macrocephalus*, Cuvier's beaked whales *Ziphius cavirostris*, long-finned pilot whales *Globicephala melas*, Risso's dolphins *Grampus griseus*, common bottlenose dolphins *Tursiops truncatus*, striped dolphins *Stenella coeruleoalba*, and short-beaked common dolphins *Delphinus delphis*.

Yet, the anthropogenic activities have a great pressure on this rich faunal biodiversity. Marine mammals, being highly sensitive to environmental changes, and at the top of the trophic chain, are important marine health indicators, and the impact of pollutants on their health has given way to a lot of concern. Several studies on trace elements concentration in different species were published over the last years (Leonzio et al., 1992; Law, 1996; Cardellicchio et al., 2000; Das et al., 2003a).

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Table 1 – Morphological data of the 12 specimens, date and site of sampling

Species	Code	Date dd/mm/yyyy	Site	Sex	Length m	Weight Kg
B. physalus	Bp1	01/05/1991	Gulf of Genova	M	17.7	26000
B. physalus	Bp2	06/12/2001	Gulf of Genova	F	13.5	7000
P. macrocephalus [a]	Pm	14/02/2002	Varazze (SV)	F	5.10	2500
G. griseus [b]	Gg1	02/07/1992	Genova	M	2.30	165
G. griseus	Gg2	08/04/1992	Albenga (SV)	F	2.98	230
G. griseus	Gg3	02/02/2004	Ceriale (SV)	M	3.25	296
S. coeruleoalba	Sc1	01/09/1990	Varigotti (SV)	M	1.91	76
S. coeruleoalba [b]	Sc2	27/02/1991	Arenzano (GE)	F	1.65	50
S. coeruleoalba [b]	Sc3	08/02/2001	Genova	M	1.56	35
T. truncatus [c]	Tt1	14/11/1999	Camogli (GE)	M	1.78	73
T. truncatus [b]	Tt2	16/07/2002	Genova	F	2.78	159
Z. cavirostris	Zc	04/02/1992	Andora (IM)	M	5.35	1500

[a] Young specimen.
[b] Subadult specimen.
[c] Suckling specimen.

Apart from the environmental contamination, many other factors have an influence on the trace elements concentration in marine mammals, such as: age, body condition and diet (Das et al., 2004). Usually, stomach contents analysis are done to understand the food habits in the Mediterranean area (Wurtz et al., 1992; Wurtz and Marrale, 1993; Orsi Relini et al., 1994). Even if these kinds of studies are useful to identify prey species, they still give indication only to the last meal of the animal and not on its feeding habits (Das et al., 2003b). Since isotope ratios in animal tissues and in their food are closely connected, carbon and nitrogen stable-isotope analyses have become an excellent means to obtain further information about marine mammals feeding ecology (Kelly, 2000). The δ^{13}C value is used to indicate relative contributions to the diet of potential primary sources, and can show the difference between onshore and offshore areas, or between pelagic and benthic prey species (De Niro and Epstein, 1978). On the other hand, δ^{15}N value shows a stepwise increase in the trophic level of a food chain. This becomes a reliable indication to the animal trophic position (De Niro and Epstein, 1981).

Isotopic signatures have also recently been used to trace the contaminants transfer in the food chain (Hobson et al., 2002; Borrell et al., 2006). Yet, few studies have focused on heavy metals (Cabana and Rasmussen, 1994; Das et al., 2003a; Das et al., 2004). In this work, metal concentrations (total and organic mercury, cadmium, lead, copper, iron, manganese, selenium and zinc) and stable nitrogen and carbon signature were measured in the tissues and organs of six different cetacean species from the Ligurian Sea: 2 fin whales (*B. physalus*, Linnaeus, 1758), 1 sperm whale (*P. macrocephalus*, Linnaeus, 1821), 3 Risso's dolphins (*G. griseus*,Cuvier, 1812), 3 striped dolphins (*S. coeruleoalba*, Meyen, 1833), 2 bottlenose dolphins (*T. truncatus*, Montagu, 1821) and 1 Cuvier's beaked whale (*Z. cavirostris*, Cuvier, 1823). These animals stranded in the "Cetaceans' Sanctuary of the Mediterranean Sea" along the Ligurian sea, or were found dead offshore during 1990–2004. The small number of specimens available in this kind of studies, which are sometimes carried out on single animals, is very common, and it is for this reason that studies do not offer statistical analysis data. Nevertheless, they provide useful information, since it is to be borne in mind that it is difficult to collect these samples, always belonging to individuals found dead, and not caught.

The sampled species belong to different trophic chain levels. *B. physalus* is the only mysticete regularly present in the Mediterranean Sea, feeding on krill (Orsi Relini and Cappello, 1992). The other cetaceans are odontocetes: *Z. cavirostris* and *G. griseus* mainly feed on squids (Carlini et al., 1992a; Carlini et al., 1992b), *P. macrocephalus* feeds mostly on cephalopods (squids and octopus), and, to a lesser extent, on fish (Clarke et al., 1993; Roberts, 2003). *S. coeruleoalba* and *T. truncatus* can feed mostly on fish and squids at a lesser scale (Pulcini et al., 1992; Orsi Relini et al., 1994).

This work aims to: (i) study the distribution of essential and non-essential trace elements in different organs. To have a full insight of accumulation/detoxification mechanisms, several organs are to be studied, since no single organ indicator is

Table 2 – Quality control

Element	Certified value (μg g^{-1} d.w.)	Found value (μg g^{-1} d.w.)	Recovery%
Hg-tot	0.27±0.06	0.27±0.02	100
Hg-org	0.152±0.013	0.152±0.009	100
Cd	26.7±0.6	26.4±2.2	99
Pb	0.35±0.13	0.38±0.02	109
Cu	106±10	95±5	90
Fe	105±13	105±7	100
Mn	13.6±1.2	13.6±1.0	100
Se	5.63±0.67	5.53±0.76	98
Zn	180±6	178±9	99

Results obtained on the Certified Reference Material TORT-2 (*Lobster hepatopancreas homard*, Institute for Environmental Chemistry, National Research Council Canada, Ottawa, Canada). Concentrations are given in μg g^{-1} dry weight. The found values are reported as mean values of 8 determinations with 95% confidence intervals. Organic mercury concentration is reported as μg of Hg g^{-1}, and the certified value refers to methylmercury concentration (expressed as mercury). Percent recovery is also reported.

Table 3 – Concentrations (means of 2 determinations) of the different elements in the organs and tissues of the cetaceans, reported as μg g^{-1} dry weight, organic mercury as μg of Hg g^{-1}

Specimen		Fresh/dry	Hg-tot (μg g^{-1} d.w.)	Hg-org (μg g^{-1} d.w.)	Hg-org/tot(%)	Se (μg g^{-1} d.w.)	Cd (μg g^{-1} d.w.)	Pb (μg g^{-1} d.w.)	Cu (μg g^{-1} d.w.)	Fe (μg g^{-1} d.w.)	Mn (μg g^{-1} d.w.)	Zn (μg g^{-1} d.w.)
Muscle												
B. physalus	Bp1	5.18	2.64	2.57	97	0.90	0.04	0.137	1.6	437	0.77	70
	Bp2	5.02	0.65	0.59	91	1.16	0.04	0.051	3.2	305	0.51	143
P. macrocephalus	Pm	3.70	1.13	0.78	69	1.88	<l.o.d.	<l.o.d.	1.6	383	0.35	113
G. griseus	Gg1	3.99	5.86	5.72	98	1.97	<l.o.d.	<l.o.d.	3.0	373	0.26	53
	Gg2	3.97	139	23.62	17	44.98	0.38	<l.o.d.	3.7	1144	0.68	75
	Gg3	4.18	128	22.08	17	96.94	0.52	<l.o.d.	2.9	913	0.72	87
S. coeruleoalba	Sc1	3.48	8.11	6.76	83	2.62	<l.o.d.	<l.o.d.	5.1	765	1.13	33
	Sc2	3.80	59.43	30.70	52	16.91	0.10	0.155	2.9	537	0.49	53
	Sc3	4.19	16.59	13.54	82	3.10	0.28	0.223	6.4	882	2.54	69
T. truncatus	Tt1	3.59	2.57	1.97	77	2.56	<l.o.d.	<l.o.d.	5.4	381	0.95	43
	Tt2	4.35	166	74.41	45	34.33	0.12	0.263	4.0	791	0.98	129
Z. cavirostris	Zc1	3.87	21.79	20.34	93	4.69	0.06	<l.o.d.	1.6	833	0.36	39
Milk												
T. truncatus	Tt1	7.21	1.47	1.28	87	2.40	<l.o.d.	0.180	6.3	71	2.56	34
Liver												
B. physalus	Bp2	4.16	0.11	0.10	94	3.20	0.04	0.041	4.7	2070	1.87	29
P. macrocephalus	Pm	4.02	4.24	0.29	7	4.59	<l.o.d	<l.o.d.	12.4	1124	2.49	142
G. griseus	Gg1	3.96	19.25	5.21	27	18.34	2.35	0.390	10.8	1337	6.90	104
	Gg2	3.76	2746	76.81	3	1408	38	2.687	10.7	8496	11.36	130
	Gg3	3.94	2132	31.24	1	1187	12.64	2.413	9.5	12356	16.43	138
S. coeruleoalba	Sc1	3.64	137	20.30	15	63.75	5.39	0.472	43.4	864	17.03	327
	Sc2	3.87	452	41.50	9	269	1.60	0.735	19.6	815	6.42	89
	Sc3	3.84	360	34.88	10	117	3.51	0.211	41.3	1044	11.19	231
T. truncatus	Tt1	3.76	13.55	2.66	20	8.73	<l.o.d.	0.155	21.8	1337	10.71	288
	Tt2	4.59	3737	129	3	1708	3.02	0.457	95.1	3478	14.93	243
Z. cavirostris	Zc1	3.77	258	27.31	11	142	10.34	0.726	26.4	797	6.11	130
Kidney												
B. physalus	Bp2	5.98	0.87	0.14	15	8.68	1.56	0.172	11.6	407	2.37	122
P. macrocephalus	Pm	3.63	1.33	0.18	14	4.48	0.04	0.069	10.3	449	1.66	77
G. griseus	Gg1	4.44	9.89	2.67	27	11.30	20	0.278	12.3	376	3.18	84
	Gg2	4.79	67.57	12.30	18	45.04	71	0.088	6.7	737	2.07	90
	Gg3	3.33	67.38	15.28	23	42.35	15.47	0.110	7.4	542	3.35	96
S. coeruleoalba	Sc1	5.00	25.20	9.80	39	29.24	34	0.429	15.5	255	3.26	129
	Sc2	5.02	47.67	21.82	46	29.12	34	0.198	10.6	840	2.31	93
	Sc3	4.47	39.30	19.56	50	18.25	9.54	0.254	13.0	683	2.47	113
T. truncatus	Tt1	4.12	5.85	1.21	21	7.85	0.03	0.087	13.5	305	2.41	86
	Tt2	5.03	288	64.31	22	101	9.83	0.210	38.9	855	2.75	140
Z. cavirostris	Zc1	4.37	25.60	12.39	48	21.25	60	0.490	10.2	830	2.53	96
Heart												
G. griseus	Gg1	4.08	4.25	3.05	72	4.75	0.09	0.077	8.0	438	1.55	88
	Gg2	4.23	50.52	21.67	43	21.78	0.49	0.172	8.5	447	1.70	93
	Gg3	5.29	224	28.38	13	132	0.56	<l.o.d.	6.8	710	2.22	101
S. coeruleoalba	Sc1	4.57	16.18	13.70	85	14.73	0.43	0.173	12.1	474	1.87	109
	Sc2	4.64	39.76	37.09	93	6.37	0.46	0.048	13.2	368	1.96	107
	Sc3	4.34	18.37	14.87	81	4.43	0.34	0.064	11.7	502	1.91	110
T. truncatus	Tt1	4.27	1.89	1.86	98	3.26	<l.o.d.	<l.o.d.	12.0	377	1.51	110
	Tt2	4.57	146	97.56	67	13.23	0.10	<l.o.d.	15.0	453	1.71	124
Z. cavirostris	Zc1	4.83	30.60	25.30	83	4.97	0.62	0.053	8.8	482	1.71	93
Lung												
P. macrocephalus	Pm	5.01	1.13	0.36	32	10.33	0.16	<l.o.d.	16.6	775	3.89	490
G. griseus	Gg1	5.05	2.59	1.65	64	16.45	0.30	<l.o.d.	3.5	1290	1.52	49
	Gg2	4.91	278	8.21	3	168	0.62	<l.o.d.	3.6	2303	2.27	45
	Gg3	6.54	121	10.70	9	77.88	0.48	0.085	2.2	1141	1.01	44
S. coeruleoalba	Sc1	4.89	9.41	5.15	55	20.60	0.25	<l.o.d.	2.8	927	1.74	104
	Sc2	4.67	277	13.97	5	83.18	0.51	<l.o.d.	3.4	1533	2.44	77

(continued on next page)

Table 3 *(continued)*

Specimen		Fresh/dry	Hg-tot (μg g^{-1} d.w.)	Hg-org (μg g^{-1} d.w.)	Hg-org/tot(%)	Se (μg g^{-1} d.w.)	Cd (μg g^{-1} d.w.)	Pb (μg g^{-1} d.w.)	Cu (μg g^{-1} d.w.)	Fe (μg g^{-1} d.w.)	Mn (μg g^{-1} d.w.)	Zn (μg g^{-1} d.w.)
	Sc3	3.84	20.25	7.23	36	8.71	0.35	0.081	2.5	1123	1.77	152
Lung												
T. truncatus	Tt1	4.97	1.58	0.91	58	7.74	<l.o.d.	<l.o.d.	3.4	780	1.56	102
	Tt2	4.89	136	32.68	24	43.55	0.31	<l.o.d.	7.9	1207	2.04	72
Brain												
G. griseus	Gg1	5.33	3.77	1.92	51	3.07	<l.o.d.	<l.o.d.	13.4	99	1.35	64
	Gg2	4.90	106	10.78	10	100	0.28	<l.o.d.	5.6	128	2.35	64
	Gg3	3.61	141	11.52	8	96.71	0.33	<l.o.d.	4.9	228	1.34	33
S. coeruleoalba	Sc1	4.68	4.65	4.17	90	3.51	0.01	<l.o.d.	12.1	178	1.61	50
	Sc2	4.94	35.85	12.14	34	22.61	0.05	<l.o.d.	6.3	138	1.82	56
T. truncatus	Tt1	5.00	1.05	0.64	61	2.55	<l.o.d.	<l.o.d.	6.7	82	2.37	58
	Tt2	6.00	75.15	–	–	15.81	0.05	1.11	15.5	182	1.36	64
Spleen												
B. physalus	Bp2	3.95	1.84	0.91	49	4.19	0.39	0.095	20.6	2305	21.32	244
G. griseus	Gg1	4.65	3.40	2.33	69	14.47	0.19	<l.o.d.	3.4	1091	1.89	70
	Gg3	2.49	581	9.60	2	506	0.82	0.271	2.1	9059	4.19	41
S. coeruleoalba	Sc2	4.49	409	22.40	5	154	0.50	<l.o.d.	4.1	5566	6.02	81
T. truncatus	Tt2	4.41	240	55.65	23	79.41	0.21	<l.o.d.	4.9	5725	5.33	70

The ratio organic/total mercury as percentage and the ratio fresh/dry weight are also reported. When concentrations are below the detection limits are reported as "<l.o.d".

available; (ii) study the trophic levels and metal concentrations in cetaceans, using stable isotopes ($\delta^{13}C$) and ($\delta^{15}N$). This aims to better explain the diet transfer and bio-magnification or bio-accumulation processes; (iii) provide a database of specimens which are difficult to attain, yet are useful if compared with literature data.

2. Materials and methods

2.1. *Sampling*

Cetaceans stranded along the Ligurian coast, or found dead in the Ligurian Sea (North-West Italy), were collected during the period from September 1990 to February 2004 (Table 1). The specimens were classified and measured. Morphological data (sex, body length and body weight) are reported in Table 1, together with the sampling date and site. Muscle tissue, liver and kidney were taken from each animal. The muscle tissue of only one of the *B. physalus* was in a good condition and fit for analysis. Whenever possible, lungs, hearts, spleens and brains were also sampled. The milk in the stomach of the *T. truncatus* suckling was collected and analysed.

Until analysed, the tissues and organs were stored at −25 °C, then weighed, freeze-dried and weighed again, to calculate the fresh/dry weight ratio. The samples, homogenised with an electric mill, were divided into two sub-samples: one to determine the mercury concentration (total and organic), cadmium, lead, and the essential elements copper, iron, manganese, selenium and zinc; and the other to measure the stable isotopes.

2.2. *Analytical methods*

The samples (0.2 g dry wt.) were mineralised with 3.5 mL of 65% m/m nitric acid (Suprapur, Merck, Darmstadt, Germany)

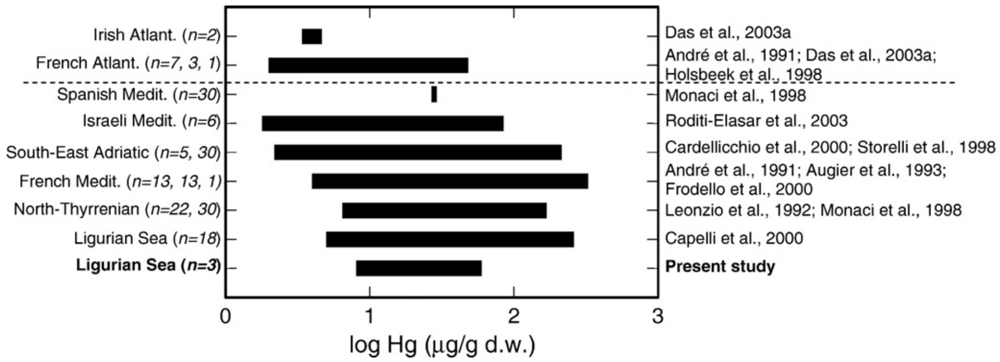

Fig. 1 – Comparison between this study and literature data regarding mercury concentrations in *Stenella coeruleoalba* muscle. Ranges of mercury concentrations are reported as μg g^{-1} d.w. after logarithmic transform. Median instead of range is reported by Monaci et al. (1998).

in closed Teflon PFA vessel using a MDS 2000 (CEM Corporation, Matthews, NC, USA) microwave digestion system.

After cooling, the solutions, transferred into 25 mL volumetric flasks, were diluted with ultra pure water (Elgastat UHQ, Elga Ltd., High Wycombe Bucks, UK). All glassware was washed with 1–2 M nitric acid and rinsed with ultra pure water. Atomic absorption and atomic emission spectrometric methods were used to determine the concentration of the different elements in the obtained solutions.

Atomic absorption spectrometry (model 560, Perkin-Elmer) was used to measure total mercury (Hg-tot), and the cold vapour after preconcentration over gold technique was adopted (Au-CVAAS). Organic mercury concentration (Hg-org) was detected with Au-CVAAS after extracting it in toluene and back-extraction with L-cysteine solution. Details of the analytical procedures for mercury can be found in Minganti et al. (1995). The methods detection limits (calculated as three times the standard deviation of the blanks) were 0.1 $\mu g\ g^{-1}$ d.w. for total mercury and 0.04 $\mu g\ g^{-1}$ d.w. for organic mercury.

Selenium was measured by the hydride generation method (HG-AAS) using a model 1100B spectrophotometer (Perkin-Elmer & Co., GmbH, Ueberlingen, Germany) equipped with the MHS-20 accessory. The detection limit of the method was 0.6 $\mu g\ g^{-1}$ d.w.

Copper, iron, manganese and zinc were measured with an inductively coupled plasma atomic emission spectrometry (ICP-AES), using a J.Y. 24 (Jobin-Yvon, Longjumeau, France) equipped with a Cetac U-5000AT$^+$ ultrasonic nebuliser (Cetac Technologies Inc., Omaha, Nebraska, USA). ICP-AES was also used to measure cadmium, if present in high concentrations.

Calibrations were carried out with aqueous standard solutions (Merck, Darmstadt, Germany), using 4 $\mu g\ mL^{-1}$ of yttrium as internal standard. The detection limits of the methods were 0.3 $\mu g\ g^{-1}$ d.w. for copper, 4 $\mu g\ g^{-1}$ d.w. for iron, 0.1 $\mu g\ g^{-1}$ d.w. for manganese and 4 $\mu g\ g^{-1}$ d.w. for zinc.

Cadmium (at low concentrations) and lead were measured by graphite furnace atomic absorption spectrometry (GF-AAS), using a Perkin-Elmer 1100B spectrometer equipped with a Perkin-Elmer HGA-500 graphite furnace and a Perkin-Elmer AS-1 auto-sampler. Graphite furnace equipped with L'vov platform and a matrix modifier containing phosphate and magnesium (Slavin, 1984) were used. Calibration was carried out by the addition of standards to the matrix solution. All manipulations of solutions were done in a Class 100 laminar flood hood (Gelair HF48, Flow Laboratories Inc., McLean, Virginia, USA). The detection limits of the methods were 0.01 $\mu g\ g^{-1}$ d.w. for cadmium and 0.1 $\mu g\ g^{-1}$ d.w. for lead. All analyses were done in duplicate or more.

To check the purity of the reagents and contamination, if any, two "blanks" were analysed for each calibration run, using the same procedure. The analytical methods accuracy was verified by analysing a Standard Reference Material in each run (TORT-2, Marine Reference Material for Trace Metals, National Research Council of Canada, Ottawa, Canada) (Table 2).

Stable isotopes were measured on a V.G. Optima IRMS (Micromass, Manchester, UK) coupled to a N–C–S elemental analyser (Carlo Erba Instruments, Milan, Italy) for automated analyses.

Routine measurements were precise within 0.3‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable-isotope ratios were expressed in δ notation according to the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Carbon and nitrogen ratios are expressed relative to VPDB (Vienna Peedee Belemnite) standard and to atmospheric nitrogen, respectively. Reference materials used were IAEA-N2 (+20.3±0.2‰) and IAEA CH-6 (−10.4±0.2‰).

The following equation was used to calculate the trophic position (TP) of each marine mammal:

$$TP_i = TP_{ref} + \left(\delta^{15}N_i - \delta^{15}N_{ref}\right)/TEF$$

where:

$\delta^{15}N_i$ is the $\delta^{15}N$ value measured for the i-th individual;
$\delta^{15}N_{ref}$ is the baseline value, assumed as 3.5‰±0.1 for the copepods from the Bay of Calvi (Corsica), according to Lepoint et al. (2000);
TEF is the trophic enrichment factor in ^{15}N, set to 3.4‰ (Post, 2002);
TP_{ref} indicates the copepod trophic position, set to 2 (i.e. primary herbivores).

Statistical analysis was performed using SYSTAT® Version 10.2 (Systat Software Inc., Richmond, California, USA).

3. Results and discussion

Concentrations of mercury (total and organic), cadmium, lead, copper, iron, manganese, selenium and zinc in the different organs and tissues of the cetaceans and in the milk found in the stomach of *T. truncatus* are shown in Table 3. Some of the measured elements are essential (Cu, Fe, Mn and Zn) and their levels are expected to be regulated; however, it is to be taken into consideration that the data here refer to stranded animals, thus, not necessarily representative of healthy conditions. Other elements (Hg, Cd and Pb) are non-essential and are expected to vary in a wide range of concentration, reflecting exposure to environmental levels and feeding behaviour. The case of Se is peculiar. It is an essential element, yet it is subjected to large variations related to high Hg levels (Thibaud, 1986; Nigro and Leonzio, 1996).

As the number of specimens available in this kind of studies is often small, the comparison with literature data is useful and necessary. Several variability factors, though, are to be taken into account even within the same species: age and sex of the specimens, particular conditions of the individual, site of sampling, method of analysis. Moreover, very few studies are conducted to measure trace elements in cetaceans brain, spleen, heart, lung and milk (Leonzio et al., 1992; Augier et al., 1993; Monaci et al., 1998; Capelli et al., 2000; Cardellicchio et al., 2000, 2002; Frodello and Marchand, 2001; Frodello et al., 2000 and 2002; Roditi-Elasar et al., 2003). The data in the present work are generally in good agreement with those published in literature.

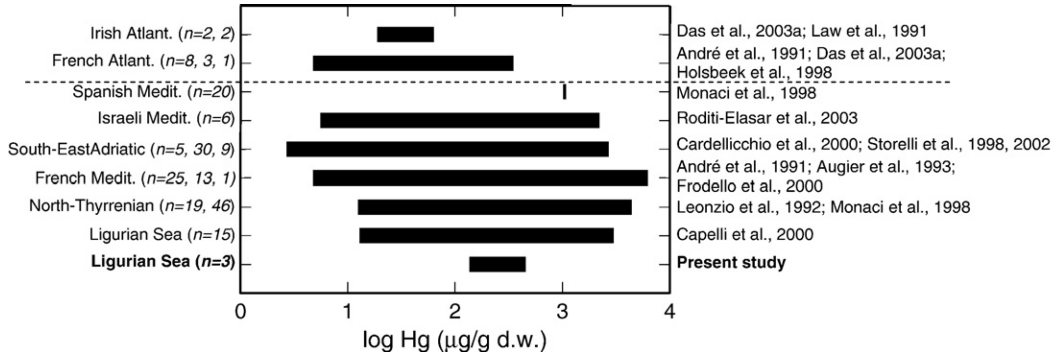

Fig. 2 – Comparison between this study and literature data regarding mercury concentrations in *Stenella coeruleoalba* liver. Ranges of mercury concentrations are reported as μg g^{-1} d.w. after logarithmic transform. Median instead of range is reported by Monaci et al. (1998).

The special, rarely collected and analysed milk sample shows that the concentrations of the different elements are comparable with the values measured in muscle tissue of the calf. Iron is found in much smaller quantities, though, while manganese and lead are in bigger amounts. Mercury is prevalent in the organic form (87% of the total). Only one work (Frodello et al., 2002) has been published about metal concentrations in the milk of a nursing *T. truncatus* from Corsica. The work contains data in agreement with ours, except for the lead concentrations (0.180 μg g^{-1} d.w. in our work, 3.7 μg^{-1} d.w. in Frodello et al., 2002).

3.1. *Mercury*

A lot of data are available about mercury concentration in muscle and liver; *S. coeruleoalba* and *T. truncatus* are the most frequently studied species.

Mercury concentrations are largely variable, as can be seen in Table 3: low values in *B. physalus*, *P. macrocephalus* and the *T. truncatus* suckling specimen, higher concentrations in *Z. cavirostris* and *S. coeruleoalba*, and extremely high in *G. griseus* and one *T. truncatus*.

Figs. 1 and 2 show total mercury concentrations measured by several authors, in the *S. coeruleoalba* muscle and liver respectively. These data are compared with the information given in this study. To represent such highly variable data in the same graph, the concentrations are expressed as μg g^{-1} d.w. after logarithmic transform. Each bar represents the range of values published in literature for specimens collected in the same geographical area. Sometimes 1 bar refers to several different studies. The number of individuals sampled in each study is also reported. Concentrations expressed as fresh weight in the original literature were converted to dry weight using the factor of 0.25, as suggested by Becker et al. (1995). Our data are in agreement with those measured in specimens from Mediterranean: mercury concentrations in muscle and liver of specimens from Atlantic are lower (Law et al., 1991; Das et al., 2003a). André et al. (1991), though, makes an exception. Their data are compatible with ours for the Atlantic specimens data. Yet, they found higher concentrations in the Mediterranean ones. Higher mercury concentrations in Mediterranean individuals than in the Atlantic ones, are frequently detected in *T. truncatus* (Mediterranean: Leonzio et al., 1992; Storelli and Marcotrigiano, 2002. Atlantic: Law et al., 1991; Holsbeek et al., 1998; Carvalho et al., 2002).

The highest concentrations are found in *G. griseus* and *T. truncatus*, with more than 100 μg g^{-1} d.w. in muscle. The values in literature are comparable and even higher, with the maximum ranging from 156 to 334 μg g^{-1} d.w. in *T. truncatus* (Leonzio et al., 1992; Frodello et al., 2000; Roditi-Elasar et al., 2003) and from 123 to 1580 μg g^{-1} d.w. in *G. griseus* (Storelli et al., 1999; Frodello et al., 2000; Shoham-Frider et al., 2002). The liver is the organ where the highest mercury concentrations are detected, as they reach more than 2000 μg g^{-1} d.w. Again, literature data show higher values, with the maximum ranging from 4250 to 13155 μg g^{-1} d.w. in *T. truncatus* (Leonzio et al., 1992; Frodello et al., 2000) and from 3298 to 5304 μg g^{-1} d.w. in *G. griseus* (Storelli et al., 1999; Frodello et al., 2000; Shoham-Frider et al., 2002).

It is to be noticed that, in the same species, young individuals (Gg1 and Tt1) show drastically lower mercury concentrations than adults (orders of magnitude), which indicates the strong effect of age on mercury accumulation. The same thing was reported by Shoham-Frider et al. (2002), regarding the *T. truncatus* from the Mediterranean coast of Israel.

The species showing the lowest concentrations are *B. physalus*, which is in agreement with literature (Sanpera et al., 1993, 1996; Hernández et al., 2000; Law et al., 2001) and *P. macrocephalus* (Law et al., 1996; Holsbeek et al., 1999).

High concentrations of total mercury (often higher than 100 μg g^{-1} d.w.) are also observed in other organs, i.e. spleen, lung, kidney, which are all organs involved in detoxification and elimination processes.

Significant correlations ($p<0.05$) between total and organic mercury are observed in muscle ($r=0.825$), liver ($r=0.906$) and kidney ($r=0.966$), where high levels of total mercury correspond to high levels of organic mercury.

The ratio organic to total mercury, expressed as percentage, ranges between 1% and 98%. This percentage is generally high in muscle and heart, and low in liver, especially in the case of very high concentrations of total mercury (Storelli et al., 1998). In most cases, mercury is present in its inorganic form.

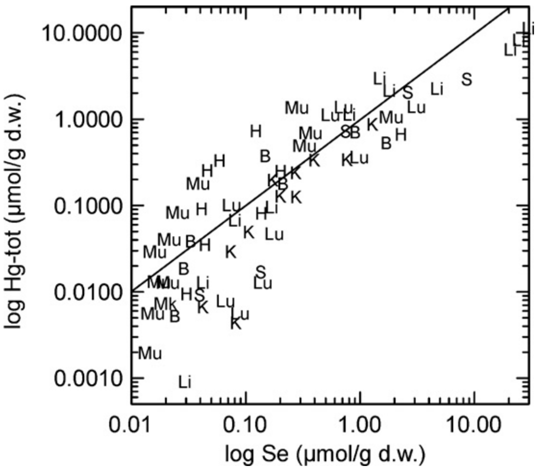

Fig. 3 – Correlation between selenium and total mercury concentrations in different organs of cetaceans from the Ligurian Sea. Concentrations are expressed as μmol g^{-1} d.w. Symbols are: B = brain; H = hearth; K = kidney; Li = liver; Lu = lung; Mk = milk; Mu = muscle; S = spleen. The line represents the 1 to 1 molar ratio.

3.2. *Selenium*

Selenium concentrations are highly variable, and range between 0.90 μg g^{-1} d.w. and 1708 μg g^{-1} d.w. The lowest values are detected in muscle, while the highest are in the liver. The trend is similar to that in the case of mercury. In several organs the *G. griseus* Gg2 and Gg3 and the *T. truncatus* Tt2 show the maximum selenium concentrations measured in this study (higher than 1000 μg g^{-1} in liver). Comparable levels in liver were also found by Leonzio et al. (1992), Storelli et al. (1998, 1999) and Shoham-Frider et al. (2002). All the organs and tissues with the exception of spleen, show a significant correlation between mercury and selenium concentrations (correlation coefficients between 0.802 and 0.995; $p < 0.02$). Except for *B. physalus* and *P. macrocephalus*, the molar ratio between inorganic mercury and selenium is nearly 1 in the liver of adult individuals (mean value 0.8), in agreement with the observations of several authors (Monaci et al., 1998; Cardellicchio et al., 2000). This confirms the existence of a fact that there is a bio-transformation process in which methylmercury is converted into the less toxic inorganic form (Thibaud, 1986), with subsequent formation of granules of mercury selenide (Nigro and Leonzio, 1996).

As evident in Fig. 3, many organs (kidney, liver, spleen), regardless of the species, show a clear relationship ($r > 0.99$, $p < 0.001$) between selenium and mercury concentration with a ratio of nearly 1, when moles are used to express these concentrations. Not so many data are available in the case of brain, heart and spleen, and the relationship is uncertain. The correlation in the muscle tissue is $r = 0.80$ and $p = 0.010$.

3.3. *Cadmium and lead*

In several cases cadmium concentrations are below detection limit, and, when measurable, they are low in all the organs and tissues (between 0.01 and 0.82 μg g^{-1} d.w.), except for the liver and kidney of *G. griseus* (38 and 71 μg g^{-1} d.w., respectively). Kidney is the critical organ for cadmium accumulation, according to Wagemann and Muir (1984), who found that cetaceans show cadmium concentrations higher by a factor of 2–5 in renal tissue than in hepatic tissue. The high cadmium values measured in *G. griseus*, *Z. cavirostris*, and *S. coeruleoalba* can be attributed to the big consumption of squids, generally rich in cadmium (Storelli et al., 1999). In agreement with the findings of several authors (Leonzio et al., 1992; Roditi-Elasar et al., 2003), *T. truncatus* shows renal cadmium levels lower than *S. coeruleoalba*, as a result of the different amount of cephalopods in their diet.

As for lead, about 50% of the values measured fall below the detection limit; the remaining values range between 0.04 and 0.74 μg g^{-1} d.w., except for two anomalous values (2.7 and 2.4 μg g^{-1} d.w.) in the liver of two *G. griseus* (Gg2 and Gg3). Apart from Tt2, all values measured in the brain, where high lead concentrations could be critical for impairment of the central nervous system, are below the detection limit.

3.4. *Essential elements (copper, iron, manganese, zinc)*

Essential elements vary in a narrow range in each organ of the different species, except for iron, whose concentrations show a wide range (from 71 to 12 356 μg g^{-1} d.w.). Its lowest values are in milk and brain, and the highest in liver, spleen and lung. Low variability is characteristic of bio-essential elements, which are subject to regulation mechanisms (Law et al., 1991).

Copper concentrations, in particular, range between 1.6 μg g^{-1} d.w. in the muscle of 3 individuals and 43.4 μg g^{-1} d.w. in the liver of one *S. coeruleoalba*, with the sole exception of one high value (95.1 μg g^{-1} d.w. in the liver of *T. truncatus* Tt2). Most of the values are below 20 μg g^{-1} d.w. The highest concentrations are in liver and kidney, and the lowest in muscle and lung. The measured values proved to be similar in the same organs of the different species. Law et al. (1991) hypothesised that the liver concentrations range is 3–30 μg g^{-1} fresh weight, due to a regulation mechanism active in marine mammals. This is in agreement with the present study (0.3–20.7 μg g^{-1} fresh weight).

Manganese concentrations range between 0.26 and 21.32 μg g^{-1} d.w. The lowest values are in the muscle, and the highest in the liver and spleen. Most of the values are below 10 μg g^{-1} d.w., and the concentrations narrowly vary in each organ.

Zinc concentrations range between 29 μg g^{-1} d.w. (in the liver of Bp2, the individual in under-nutrition condition) and 490 μg g^{-1} d.w. (in the lung of *P. macrocephalus*). The highest values are in liver (especially of *S. coeruleoalba* and *T. truncatus*) and the lowest in milk, brain and muscle. As is the case for copper, the values measured in the different organs and tissues are similar for the different species. Law et al. (1991) hypothesised that the liver concentrations range is 5–100 μg g^{-1} fresh weight, again due to a regulation mechanism active in marine mammals. This is in agreement with the present study (5–98 μg g^{-1} fresh weight).

Significant correlations ($p<0.05$) can be observed between copper, manganese and zinc in several organs.

3.5. *Stable isotopes*

The results of δ^{13}C and δ^{15}N measurements in the muscle of the different cetaceans are reported in Table 4.

The isotopic signatures show two marine mammal groups (Fig. 4), with different δ^{13}C and δ^{15}N values. The first group, characterised by high δ^{13}C and δ^{15}N values, includes 3 specimens of *G. griseus*, *Z. cavirostris* and the adult specimen of *T. truncatus*, all at trophic positions higher than 4. The second group, including the 3 *S. coeruleoalba* and the 2 *B. physalus* specimens, showed lower values of δ^{13}C and δ^{15}N, being at levels below the fourth trophic position. *P. macrocephalus* and the suckling *T. truncatus* are distinct from the other individuals, and show low values of δ^{13}C and the highest values of δ^{15}N. In the case of the suckling *T. truncatus*, the trophic position was not calculated, since the δ^{15}N signature reflected that of the mother's milk. The trophic levels obtained for the different species in this study are substantially in agreement with the literature data based on diet composition (Pauly et al., 1998).

Fig. 5 shows the relationship between δ^{15}N and the concentration of total mercury. Two outliers are evident (Pm and Tt1). They belong to species with high trophic position. Yet, being young in age, they are characterised by a low contamination level. Except for these two specimens, there is a significant relationship between δ^{15}N and the concentration of total mercury ($r=0.86$; $p<0.05$). The slope of the regression of the logarithmic transform of total mercury concentration and δ^{15}N is often used as a quantitative measure of biomagnification rate within the food web; published data range 0.17–0.48 for temperate lakes (Kidd, 1998) and 0.23–0.25 for benthic and pelagic food webs (Kidd et al., 2003). To make our data comparable with literature, mercury concentrations were calculated on fresh weight basis, and the slope of the regression line is 0.41. However, the comparison of this result with the cited data is questionable, since the food web considered is deeply different from those reported by other authors. No data on cetacean food web were found for comparison.

Table 4 – δ^{13}C and δ^{15}N values in muscle of the cetaceans from Ligurian Sea

Specimen		δ^{13}C (‰)	δ^{15}N (‰)	TP
B. physalus	Bp1	−18.5	8.3	3.4
	Bp2	−18.6	9.3	3.7
P. macrocephalus	Pm	−18.1	13.1	4.8
G. griseus	Gg1	−17.5	10.5	4.1
	Gg2	−17.2	11.9	4.5
	Gg3	−16.9	11.8	4.4
S. coeruleoalba	Sc1	−18.2	8.9	3.6
	Sc2	−18.2	9.5	3.8
	Sc3	−18.4	8.9	3.6
T. truncatus	Tt1	−18.6	13.5	–
	Tt2	−17.1	12.6	4.7
Z. cavirostris	Zc1	−17.3	10.8	4.2

Mean results are expressed as ‰. The value of trophic position is also reported, except for Tt1, see text.

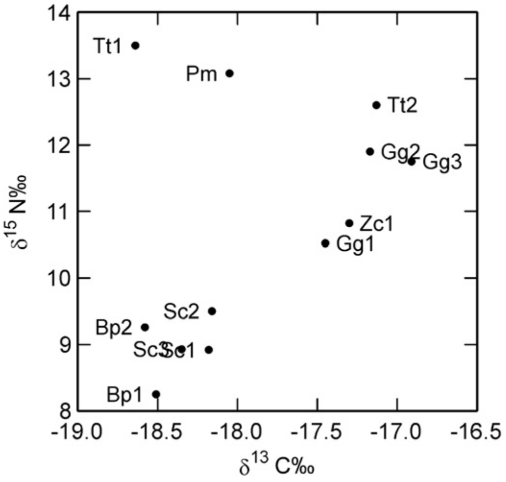

Fig. 4 – δ^{13}C and δ^{15}N in the muscle of the different cetaceans from the Ligurian Sea. Symbols as in Table 1.

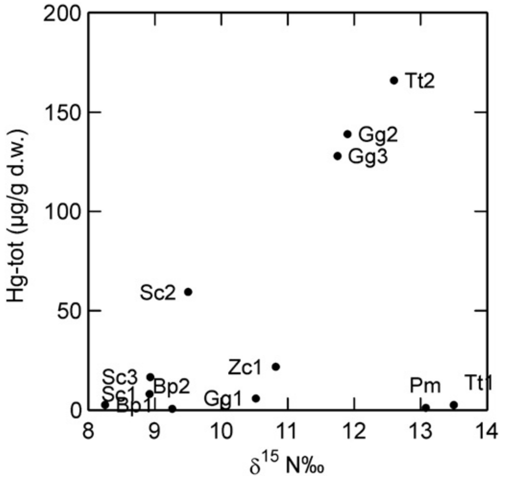

Fig. 5 – Relationship between concentration of total mercury and δ^{15}N in the muscle of the different cetaceans from the Ligurian Sea. Symbols as in Table 1.

In conclusion, according to the literature data, narrow ranges of concentrations were observed for essential elements in most organs, while mercury and selenium concentrations span up to three orders of magnitude. Yet, such wide ranges of concentration make difficult to draw any adequate interpretation, and the attempt of using complementary parameters (such as stable-isotopes data) may lead to a better comprehension of bio-accumulation and bio-amplification processes in cetaceans.

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REFERENCES

André J, Boudou A, Ribeyre F, Bernhard M. Comparative study of mercury accumulation in dolphins (*Stenella coeruleoalba*) from French Atlantic and Mediterranean coasts. Sci Total Environ 1991;104:191–209.

Augier H, Park WK, Ronneau C. Mercury contamination of the striped dolphin *Stenella coeruleoalba* Meyen from the French Mediterranean coasts. Mar Pollut Bull 1993;26:306–11.

Becker PR, Mackey EA, Demiralp R, Suydam R, Early G, Koster BJ, et al. Relationship of silver with selenium and mercury in the liver of two species of toothed whales (Odontocetes). Mar Pollut Bull 1995;30:262–71.

Borrell A, Aguilar A, Tornero V, Sequeira M, Fernández G, Alís S. Organochlorine compounds and stable isotopes indicate bottlenose dolphin subpopulation structure around the Iberian Peninsula. Environ Int 2006;32:516–23.

Cabana G, Rasmussen JB. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 1994;372:255–7.

Capelli R, Drava G, De Pellegrini R, Minganti V, Poggi R. Study of trace elements in organs and tissues of striped dolphins (*Stenella coeruleoalba*) found dead near the Ligurian Sea (Italy). Adv Environ Res 2000;4:31–43.

Cardellicchio N, Giandomenico S, Ragone P, Di Leo A. Tissue distribution of metals in striped dolphin (*Stenella coeruleoalba*) from the Apulian coasts, Southern Italy. Mar Environ Res 2000;49:55–66.

Cardellicchio N, Decataldo A, Di Leo A, Giandomenico S. Trace elements in organs and tissues of striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea (Southern Italy). Chemosphere 2002;49:85–90.

Carlini R, Pulcini M, Wurtz M. Cephalopods from the stomach of a Cuvier's beaked whale (*Ziphius cavirostris*, Cuvier 1823) stranded at Fiumicino, central Tyrrhenian coast. Eur Res Cetaceans 1992a;6:190–2.

Carlini R, Pulcini M, Wurtz M. Cephalopods from the stomach of a Risso's dolphins (*Grampus griseus*, Cuvier 1812) stranded along the central Tyrrhenian coast. Eur Res Cetaceans 1992b;6:196–8.

Carvalho ML, Pereira RA, Brito J. Heavy metals in soft tissues of *Tursiops truncatus* and *Delphinus delphis* from west Atlantic Ocean by X-ray spectrometry. Sci Total Environ 2002;292:247–54.

Clarke MR, Martins HR, Pascoe P. The diet of sperm whales (*Physeter macrocephalus* Linnaeus 1758) off the Azores. Philos Trans R Soc B 1993;339(1287):67–82.

Das K, Beans C, Holsbeek L, Mauger G, Berrow SD, Rogan E, et al. Marine mammals from northeast Atlantic: relationship between their trophic status as determined by δ^{13}C and δ^{15}N measurements and their trace metal concentrations. Mar Environ Res 2003a;56:349–65.

Das K, Debacker V, Pillet S, Bouquegneau JM. Heavy metals in marine mammals. In: Vos JG, Bossart G, Fournier M, O'Shea T, editors. Toxicology of marine mammals. Washington DC: Taylor & Francis; 2003b. p. 135–67.

Das K, Siebert U, Fontaine M, Jauniaux T, Holsbeek L, Bouquegnau JM. Ecological and pathological factors related to trace metal concentrations in Harbour porpoises from the North Sea and adjacent areas. Mar Ecol Prog Ser 2004;281:283–95.

De Niro MJ, Epstein S. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 1978;42:495–506.

De Niro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 1981;45:341–51.

Frodello JP, Marchand B. Cadmium, copper, lead, and zinc in five toothed whale species of the Mediterranean Sea. Int J Toxicol 2001;20:339–43.

Frodello JP, Romèo M, Viale D. Distribution of mercury in the organs and tissues of five toothed whale species of the Mediterranean. Environ Pollut 2000;108:447–52.

Frodello JP, Viale D, Marchand B. Metal concentrations in the milk and tissues of a nursing *Tursiops truncatus* female. Mar Pollut Bull 2002;44:551–76.

Hernández F, Serrano R, Roig-Navarro AF, Martínez-Bravo Y, López FJ. Persistent organochlorines and organophosphorus compounds and heavy elements in common whale (*Balaenoptera physalus*) from the Western Mediterranean Sea. Mar Pollut Bull 2000;40:426–33.

Hobson KA, Fisk A, Karnovsky N, Holst M, Gagnon JM, Fortier M. A stable isotope (δ^{13}C, δ^{15}N) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep-Sea Res PT II 2002;49:5131–50.

Holsbeek L, Siebert U, Joiris CR. Heavy metals in dolphins stranded on the French Atlantic coast. Sci Total Environ 1998;217:241–9.

Holsbeek L, Joiris CR, Debacker V, Ali IB, Roose P, Nellissen JP, et al. Heavy metals, organochlorines and polycyclic aromatic hydrocarbons in sperm whales stranded in the Southern North Sea during the 1994/1995 winter. Mar Pollut Bull 1999;38:304–13.

Kelly JF. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 2000;78:1–27.

Kidd KA. Use of stable isotope ratios in freshwater and marine biomagnification studies. In: Rose J, editor. Environmental Toxicology: current developments. Amsterdam, The Netherlands: Gordon and Breach Science Publishers; 1998. p. 357–76.

Kidd KA, Bootsma HA, Hesslein RH, Lockhart WL, Hecky RE. Mercury concentrations in the food web of Lake Malawi, East Africa. J Great Lakes Res 2003;29(Suppl. 2):258–66.

Law RJ. Metals in marine mammals. In: Beyer WN, Heinz GH, Redmon-Norwood AW, editors. Environmental contaminants in wildlife. Interpreting Tissue Concentrations. SETAC Special Publication Series. Boca Raton, FL: Lewis Publishers Inc/CRC Press; 1996. p. 357–76.

Law RJ, Fileman CF, Hopkins AD, Baker JR, Harwood J, Jackson DB, et al. Concentration of trace metals in the livers of marine mammals (seals, porpoises and dolphins) from waters around the British Isles. Mar Pollut Bull 1991;22:183–91.

Law RJ, Stringer RL, Allchin CR, Jones BR. Metals and organochlorines in sperm whales (*Physeter macrocephalus*) stranded around the North Sea during the 1994/1995 winter. Mar Pollut Bull 1996;32:72–7.

Law RJ, Bennett ME, Blake SJ, Allchin CR, Jones BR, Spurrier CJH. Metals and organochlorines in pelagic cetaceans stranded on the coasts of England and Wales. Mar Pollut Bull 2001;42:522–6.

Leonzio C, Focardi S, Fossi C. Heavy metals and selenium in stranded dolphins of the Northern Tyrrhenian (NW Mediterranean). Sci Total Environ 1992;119:77–84.

Lepoint G, Nyssen F, Gobert S, Dauby P, Bouquegneau JM. Relative impact of a seagrass bed and its adjacent epilithic algal community in consumer diets. Mar Biol 2000;136:513–8.

Minganti V, Capelli R, Fiorentino F, De Pellegrini R, Vacchi M. Variations of mercury and selenium concentrations in *Adamussium colbecki* and *Pagothenia bernacchii* from Terra Nova Bay (Antarctica) during a five years period. Int J Environ An Chem 1995;61:239–48.

Monaci F, Borrel A, Leonzio C, Marsili L, Calzada N. Trace elements in striped dolphins (*Stenella coeruleoalba*) from the western Mediterranean. Environ Pollut 1998;99:61–8.

Nigro M, Leonzio C. Intracellular storage of mercury and selenium in different marine vertebrates. Mar Ecol Prog Ser 1996;135:137–43.

Notarbartolo G, Ausenda F, Orsi Relini L, Relini G. Una proposta di gestione dell'ambiente pelagico: la Riserva della Biosfera nel Bacino corso-ligure provenzale. Atti XXII Congresso della Società Italiana di Biologia Marina, 20–24 May 1991, Santa Margherita di Pula, Cagliari, Oebalia, 17. Supplemento; 1992. p. 517–21.

Orsi Relini L, Cappello M. The fin whale and other large pelagic filters as samplers of *Meganyctiphanes norvegica*. Rapp Comm Int Mer Medit 1992;33:263.

Orsi Relini L, Cappello M, Poggi R. The stomach content of some bottlenose dolphins (*Tursiops truncatus*) from the Ligurian Sea. Eur Res Cetaceans 1994;8:192–5.

Pauly D, Trites AW, Capuli E, Christensen V. Diet composition and trophic levels of marine mammals. ICES J Mar Sci, 1998;55:467–81.

Post DM. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 2002;83:703–18.

Pulcini M, Carlini R, Wurtz M. Stomach contents of striped dolphin (*Stenella coeruleoalba*, Meyen 1833) from the south-central Tyrrhenian coast. Eur Res Cetaceans 1992;6:194–6.

Roberts SM. Examination of the stomach contents from a Mediterranean sperm whale found south of Crete, Greece. J Mar Biol Ass UK 2003;83:667–70.

Roditi-Elasar M, Kerem D, Hornung H, Kress N, Shoham-Frider E, Goffman O, et al. Heavy metal levels in bottlenose and striped dolphins off the Mediterranean coast of Israel. Mar Pollut Bull 2003;46:503–12.

Sanpera C, Capelli R, Minganti V, Jover L. Total and organic mercury in North Atlantic fin whales: distribution pattern and biological related changes. Mar Pollut Bull 1993;26:135–9.

Sanpera C, González M, Jover L. Heavy metals in two populations of North Atlantic Fin Whales (*Balaenoptera physalus*). Environ Pollut 1996;91:299–307.

Shoham-Frider E, Amiel S, Roditi-Elasar M, Kress N. Risso's dolphin (*Grampus griseus*) stranding on the coast of Israel (eastern Mediterranean). Autopsy results and trace metal concentrations. Sci Total Environ 2002;295:157–66.

Slavin W. Graphite furnace AAS: A source book. Norwalk: The Perkin Elmer Corporation; 1984. 230 pp.

Storelli MM, Marcotrigiano GO. Subcellular distribution of heavy metals in livers and kidneys of *Stenella coeruleoalba* and *Tursiops truncatus* from the Mediterranean Sea. Mar Pollut Bull 2002;44:71–81.

Storelli MM, Ceci E, Marcotrigiano GO. Comparison of total mercury, methylmercury, and selenium in muscle tissues and in the liver of *Stenella coeruleoalba* (Meyen) and *Caretta caretta* (Linnaeus). B Environ Contam Tox 1998;61:541–7.

Storelli MM, Zizzo N, Marcotrigiano GO. 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). B Environ Contam Tox 1999;63:703–10.

Thibaud Y. The role of biochemical processes in the accumulation of mercury by marine organisms. FAO/UNEP/WHO/IOC/IAEA Meeting on the Biogeochemical Cycle of Mercury in the Mediterranean, 27–31 August 1984, Siena, 325. FAO Fish Rep; 1986. p. 150–62. (Suppl.).

Wagemann R, Muir DCG. Concentrations of heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation. Can Tech Rep Fish Aquat Sci 1984;1279:1–97.

Wurtz M, Marrale D. Food of striped dolphin *Stenella coeruleoalba* in the Ligurian Sea. J Mar Biol Assoc UK 1993;73(3):571–8.

Wurtz M, Pulcini M, Marrale D. Mediterranean cetaceans and fisheries. Do they exploit the same resources? Eur Res Cetaceans 1992;6:37–40.