Age, growth and organochlorines (HCB, DDTs and PCBs) in Mediterranean striped dolphins *Stenella coeruleoalba* stranded in 1988–1994 on the coasts of Italy

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ABSTRACT: This study was based on data from 62 specimens of *Stenella coeruleoalba* found stranded along the Italian coasts between 1988 and 1994. The age of each dolphin was determined by counting dentine growth layer groups in the teeth and a growth curve was plotted. Organochlorine contaminants (HCB, DDTs and PCBs) were analysed in the blubber, liver, brain and muscle of 25 of the dolphins. In all dolphins and all tissues analysed, HCB concentrations (ng g^{-1} dry weight) were the lowest and the PCBs were the highest, except in 1 blubber sample in which total DDTs were higher than PCBs. Correlations were sought between levels of these contaminants and age, sex, cause of death, locality and year of stranding.

KEY WORDS: *Stenella coeruleoalba* · Dolphin · Organochlorines · Mediterranean Sea · Growth curve · Teeth · Sex

INTRODUCTION

Cetaceans are under continuous pressure due to whaling, drowning in fishing nets, marine pollution and capture for aquariums. Against these odds, not all species of cetaceans are succeeding in their struggle to survive. Reports indicate that some species are on a gradual path to extinction. Many cetacean species, including the striped dolphin *Stenella coeruleoalba*, are located high in the marine food chain and therefore accumulate high levels of fat-soluble compounds such as chlorinated hydrocarbons (Tanabe et al. 1981a, Borrell 1993, Marcovecchio et al. 1994, Marsili & Focardi 1997). The ecotoxicological risk of some species of cetaceans is also related to their 'biochemical vulnerability' to lipophilic xenobiotics: the fact that they have

a lower cytochrome P-450 activity than terrestrial mammals (Tanabe et al. 1988, Duinker et al. 1989, Watanabe et al. 1989, Fossi et al. 1992). Tanabe & Tatsukawa (1992) reported that ' .. these animals have a low capacity for degradation of organochlorines due to the specific mode of their cytochrome P-450 enzyme system' Many references indicate the possibility of using these animals as bioindicators of environmental contamination of relatively limited areas (O'Shea et al. 1980, Martineau et al. 1987, Geraci 1989, Loganathan et al. 1990, Marsili & Focardi 1996). Data obtained for the Mediterranean Sea cetaceans are quite alarming (Alzieu & Duguy 1979, Aguilar & Borrell 1994a, b, Marsili & Focardi 1996, Marsili & Focardi 1997). Such xenobiotics are known to have effects on pinnipeds which include reproductive anomalies (Le Boeuf & Bonnell 1971, Helle et al. 1976a, Olsson 1978, Addison 1989), uterine tumours (Helle et al. 1976b, Baker 1989) and abnormal skeletal development (Bergman et al. 1986,

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Zakharov & Yablokov 1990). Since many cetaceans have a similar life style and diet to pinnipeds, they run similar risks.

The stranding of cetaceans along the Mediterranean coasts is a normal event, since animals dying of natural causes, such as old age, or as a result of accidents, such as injury by boats, ingestion of foreign objects (including plastic bags) or drowning in fishing nets, either sink or drift ashore. In recent years, there have been episodes of large-scale mortality of dolphins (Bortolotto et al. 1992, Aguilar & Raga 1993, Van Bressen et al. 1993). The cause of death of some stranded specimens is evident, e.g. wounds due to harpooning or impact with power boats; for others it cannot be established even after careful study.

The aim of the present study was to investigate the degree to which xenobiotics, such as hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) and its metabolites, and polychlorobiphenyls (PCBs), play a role in the death of striped dolphins. It is known that large differences exist in the accumulation of these substances in specimens of the same species. These differences are related to parameters such as age, sex, health and geographical location.

MATERIALS AND METHODS

Between 1988 and 1994, 62 specimens of *Stenella coeruleoalba* were collected on the Italian coasts and sent to the Department of Environmental Biology of Siena for chemical analysis and age determination (Table 1). Age was also determined at the University 'La Sapienza' of Rome (Casini 1995).

Tooth preparation and age determination. Sixty-two mandibles of stranded dolphins were obtained and the teeth were collected after complete putrefaction of the mandible by enzyme digestion in water. The teeth were cleaned and stored in neutral-buffered 4% formalin. The teeth were prepared for examination as described by Lockyer et al. (1981) and Myrick et al. (1983). Tooth sections 24 µm thick (about 100 per tooth) were obtained with a 'sledge-type freezing' microtome. They were stained with Mayer's haematoxylin and mounted in DPX medium. Three of the authors (L. M., C. C. & L. M.) independently examined the sections directly on slides under the light microscope (Wild M8: 50×) and in photographs taken with the same microscope using a Pentax P30N camera with a Pentax-A 28–80 mm zoom lens. Age was determined by counting growth layer groups (GLG) in tooth dentine. GLG were first used as indicators of age in *Stenella coeruleoalba* by Nishiwaki & Yagi (1953). A GLG consists of 2 fine translucent layers and 2 thick dark layers of dentine and represents a year of

Table 1 *Stenella coeruleoalba.* Specimens in which age was determined by tooth analysis (n = 62)

ID no.	Sex	Length (cm)	Age (yr)	Sea	Year of stranding
66/93	M	100	2.0	Tyrrhenian Sea	1993
S08	F	105	1.0	Tyrrhenian Sea	1991
12/91	M	110	1.5	Tyrrhenian Sea	1991
93/93	F	112	1.0	Tyrrhenian Sea	1993
7/92	F	116	1.5	Tyrrhenian Sea	1992
14/91	M	122	0.5	Tyrrhenian Sea	1991
70	M	125	1.0	Tyrrhenian Sea	1993
10/91	F	128	2.0	Tyrrhenian Sea	1991
63	M	130	2.5	Adriatic Sea	1991
42	M	152	2.0	Adriatic Sea	1991
13/88	X	154	2.5	Tyrrhenian Sea	1988
48	M	157	3.0	Adriatic Sea	1991
46	F	162	3.5	Adriatic Sea	1991
P01	M	163	15.0	Adriatic Sea	1991
69	M	165	5.0	Tyrrhenian Sea	1993
894	M	165	2.0	Ionian Sea	1991
28/90	M	166	5.5	Tyrrhenian Sea	1990
27	M	173	5.0	Adriatic Sea	1991
P15	M	174	7.0	Tyrrhenian Sea	1992
20/89	M	175	13.0	Tyrrhenian Sea	1989
34	M	177	3.0	Adriatic Sea	1991
49	M	186	4.0	Ionian Sea	1991
A5	M	188	4.0	Tyrrhenian Sea	1991
51	M	190	6.0	Adriatic Sea	1991
1/88	M	192	8.0	Tyrrhenian Sea	1988
38	F	192	12.0	Ionian Sea	1991
39	F	192	12.0	Adriatic Sea	1991
28	M	193	13.0	Adriatic Sea	1991
4/94	X	194	11.0	Tyrrhenian Sea	1994
47	F	194	15.0	Adriatic Sea	1991
A13	M	195	13.0	Ionian Sea	1991
65/93	F	195	12.0	Tyrrhenian Sea	1993
43	M	195	9.0	Adriatic Sea	1991
71	M	195	15.0	Tyrrhenian Sea	1991
3/94	M	196	7.0	Tyrrhenian Sea	1994
19/92	F	196	18.0	Tyrrhenian Sea	1992
92/93	M	196	9.0	Tyrrhenian Sea	1993
40	F	196	7.5	Adriatic Sea	1991
41	M	197	13.0	Adriatic Sea	1991
29	M	198	11.0	Adriatic Sea	1991
862	M	198	23.0	Tyrrhenian Sea	1991
32	M	199	9.0	Adriatic Sea	1991
54	M	199	14.0	Adriatic Sea	1991
863	F	200	23.0	Ionian Sea	1991
864	M	200	10.0	Ionian Sea	1991
870	M	200	18.0	Adriatic Sea	1991
P04	F	201	3.0	Adriatic Sea	1991
P02	M	201	15.0	Adriatic Sea	1991
62	M	201	23.0	Adriatic Sea	1991
53	F	203	15.0	Adriatic Sea	1991
59	M	203	28.0	Adriatic Sea	1991
56	F	204	16.0	Adriatic Sea	1991
60	F	204	18.0	Adriatic Sea	1991
S06	F	205	7.0	Ionian Sea	1991
50	F	205	16.0	Adriatic Sea	1991
35	F	207	20.0	Adriatic Sea	1991
9/89	M	210	12.0	Tyrrhenian Sea	1989
18	M	212	25.0	Ionian Sea	1991
52	F	213	23.0	Adriatic Sea	1991
27/90	M	215	18.0	Tyrrhenian Sea	1990
36	F	220	25.0	Adriatic Sea	1991
37	M	220	20.0	Adriatic Sea	1991

growth. In the cement it consists of a translucent layer and a darker layer (Kasuya 1972, Perrin & Myrick 1980) Age was determined by comparison of the counts of the 3 observers. If all 3 counted the same number of GLG, the age was unanimous. If the number of GLG differed by no more than 2 between the 3 observers, the mean of the 3 values was taken. If 2 observers counted the same number of GLG and the third count differed by no more than 3, the age established by the first 2 was adopted. In other cases, another tooth of the same dolphin was prepared and new sections counted.

Chemical analysis. Organs and tissues (liver, muscle, blubber and brain), in different states of conservation, depending on how long the dolphins had been dead, were frozen and stored between –20 and –30°C. Before chemical analysis, about 20 g of tissue was lyophilised in an Edwards freeze drier. To calculate water content, a sample of about 5 g was placed in an oven at 110°C for 24 h. The percentage water content (mean ± SD) in the various tissues was as follows: liver (n = 5), 72.5 ± 9.70%; muscle (n = 5), 74.2 ± 5.50%; brain (n = 4), 79.3 ± 5.80%; blubber (n = 5), 35.5 ± 19.3%.

Aliquots of 1 to 1.5 g of freeze-dried material were extracted with n-hexane in preextracted thimbles in a Soxhlet apparatus for 9 h. The samples were then purified with sulphuric acid (Murphy 1972) to obtain a first lipid sedimentation. The extract was run on a liquid chromatography column containing Florisil that had been dried for 1 h in an oven at 110°C. This further purified the apolar phase of lipids that could not be saponified, such as steroids like cholesterol. The extracted organic matter (EOM%) from freeze-dried samples was as follows: liver (n = 19), 22.0 ± 16.1%; muscle (n = 20), 10.7 ± 4.7%; brain (n = 17), 43.6 ± 5.7%; blubber (n = 24), 82.5 ± 16.8%.

The analytical method used was high resolution capillary gas chromatography with a ^{63}Ni electron capture detector and an SBP-5 bonded phase capillary column (30 m long, 0.2 mm inner diameter). The carrier gas was N_2 with a head pressure of 105 kPa (splitting ratio 50/1). The scavenger gas was argon/methane (95/5) at 40 ml min^{-1} Oven temperature was 100°C for 10 min, after which it was increased to 280°C at 5°C min^{-1}. Injector and detector temperatures were 200 and 280°C, respectively.

A mixture of specific isomers was used to calibrate the system, evaluate recovery and confirm the results, which were expressed in ng g^{-1} or µg g^{-1}, dry weight (d.w.) or lipid basis (l.b.). Recoveries were calculated by adding known quantities of standard to homogeneous replicates of the same sample. Recovery varied from a minimum of 85% to a maximum of 93% according to the matrix. The blank was evaluated by

extracting an empty thimble once every 11 samples. The precision of the method was measured on 5 homogeneous replicates, calculating the coefficient of variation of the results obtained. It was less than 9% for all compounds analysed. To ensure accuracy, intercalibration exercises were performed with appropriate standards. We detected HCB, op'DDT and pp'DDT and their metabolites (pp' and op'DDD, pp' and op'DDE) and identified 30 PCB congeners (Table 2). The congeners constituted 80% of the total peak area of PCBs in all tissues.

Statistical analysis. The data were processed by summary statistics and analysis of variance (ANOVA) using Statgraphics software (Statistical Graphics Corp.). The parameters considered were length, sex, age, cause of death, site and year of stranding. The significance level was $p < 0.05$. The parameter r of the growth curve was calculated by an iterative procedure, namely NLIN of the SAS software package (SAS/STAT, Release 6.04 Edition, SAS Institute Inc.).

Table 2. IUPAC number and structure of the 30 PCB congeners detected in all samples

IUPAC number	Structure
Pentachlorobiphenyls	
95	22'35'6
99	22'44'5
101	22'455'
118	23'44'5
Hexachlorobiphenyls	
128	22'33'44'
135	22'33'56'
138	22'344'5'
141	22'3455'
144	22'345'6
146	22'34'55'
149	22'34'5'6
151	22'355'6
153	22'44'55'
156	233'44'5
Heptachlorobiphenyls	
170	22'33'44'5
171	22'33'44'6
172	22'33'455'
174	22'33'456'
177	22'33'4'56
178	22'33'55'6
180	22'344'55'
183	22'344'5'6
187	22'34'55'6
Octachlorobiphenyls	
194	22'33'44'55'
195	22'33'44'56
196	22'33'44'5'6
199	22'33'4566'
201	22'33'4'55'6
202	22'33'55'66'
Nonachlorobiphenyls	
206	22'33'44'55'6

RESULTS AND DISCUSSION

Growth curve

A growth curve for *Stenella coeruleoalba* in the Mediterranean was plotted on the basis of the age and length data. The equation relating length and age is based on a mean length at birth of 90 cm (Notarbartolo di Sciara & Demma 1994) and a mean length of adults (> 9 yr) of 200 cm, ignoring sex differences (males are about 10 cm longer than females) (Notarbartolo di Sciara & Demma 1994). The equation is:

$$\frac{dL}{dt} = rL\frac{K-L}{K}$$

where L is body length (cm), K is the mean length of adult specimens (cm), r is the growth rate constant (t^{-1}) and t is age (yr). Integrating, we obtain:

$$L_{(t)} = \frac{K}{1+e^{(a-rt)}}$$

where $a = \ln[(K - L)/L]$ for $t = 0$ is the integration constant. Since at birth $(t = 0)$ a striped dolphin is about 90 cm long, $a = \ln[(200 - 90)/90] = 0.201$

If Y is body length (cm) and X age (yr) we obtain:

$$Y = 200/[1 + e^{(0.201 - rX)}]$$

The parameter r ($r = 0.5115$) was calculated by non-linear regression analysis using the least squares method. The asymptotic standard error of r is very low (0.0355) so that parameter and the relation are significant. The final equation is therefore:

$$Y = 200/[1 + e^{(0.201 - 0.5115X)}]$$

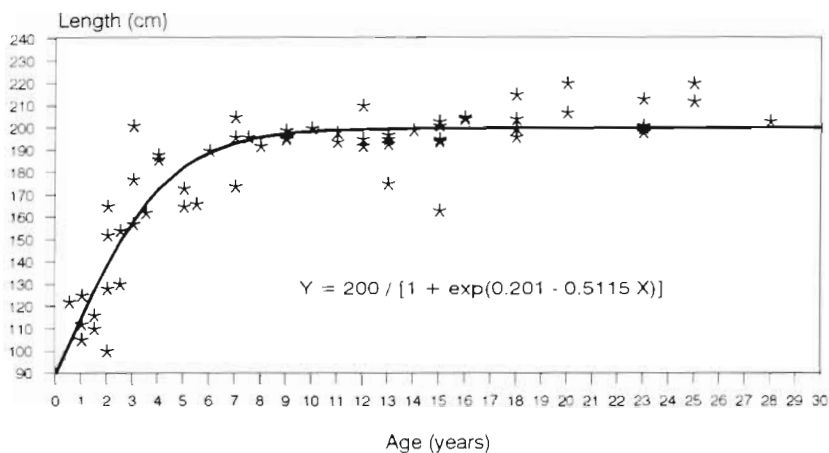The resulting curve represents the relationship between body length and age (Fig. 1).

Kasuya (1972) formulated the first growth curve for *Stenella coeruleoalba* in the Pacific Ocean based on 218 females and 191 males. With such a large number of specimens, he was able to distinguish male and female growth. He found that the mean length of males was about 15 cm greater than that of females. Mean length at birth was estimated to be 99.8 cm. One-year-old specimens measured 164 cm and the first sex differences became evident at 2 yr and were distinct by 6 yr. Growth rate of females increased between the ages of 7 and 8 yr, at which time they were slightly longer than males. Females then grew slowly towards an asymptote of 225.3 cm at about 17 yr. Male growth rate tended to slow after 10 yr, body length approaching an asymptote of 236 cm at about 21 yr (Kasuya 1976). Bryden (1986) reports a year of fast growth in females around the age of 7 to 8 yr and 2 yr of fast growth in males around the age of 11 to 13 yr.

The growth curve of *Stenella coeruleoalba* in the Mediterranean (Fig. 1) shows that growth is quite slow in the first year and is followed by a rapid increase in size up to a plateau at physical maturity, which seems to be attained at about 8 yr. The curve is similar to that obtained for ocean dolphins (Kasuya 1972, 1976); the only difference is the time taken to reach physical maturity. Since ocean striped dolphins are larger than their Mediterranean counterparts, they presumably take longer to reach full size.

Correlation between organochlorine levels and age, sex, cause of death, site and year of stranding

Twenty-five stranded striped dolphins (all of known age and 23 of known sex) were sampled for organochlorine analysis. The types of tissue (blubber, muscle, brain and liver) are the most commonly used for this kind of analysis and hence the most suitable for comparison with data in the literature.

Since males and females reach sexual maturity at about 9 yr (Bryden 1986, Notarbartolo di Sciara & Demma 1994), we can deduce that 14 of the 25 dolphins were sexually immature; the other 11 were of potentially reproductive age. Unfortunately, there were no dolphins over 18 yr of age, despite the fact that striped dolphins can live up to 40 yr (Notarbartolo di Sciara & Demma 1994) and only 2 of the sexually mature dolphins were females. The fact that 2 sexually mature specimens were females is important because

Fig. 1. *Stenella coerunleoalba*. Growth curve of dolphins in the Mediterranean Sea. *Y*: length; *X*: age

females lose more than 90% of their total body burden of organochlorines during pregnancy and lactation (Tanabe et al. 1981b, 1982). This is linked to the fact that the milk of these marine mammals contains very high levels of fats, mostly triglycerides. The milk of *Stenella coeruleoalba*, for instance, contains 258 mg g^{-1} of triglycerides out of 280 mg g^{-1} total fats (Kawai & Fukushima 1981). Tanabe et al. (1981b) estimated that a pregnancy followed by 6 to 7 mo of lactation enables a female of *S. coeruleoalba* to lose 4.7% during pregnancy and 95% during lactation of her total body burden of DDT, and 4% during pregnancy and 92% during lactation of that of PCBs, respectively. The 2 adult females analysed in this study will be referred to as A and B: A was 12 and B 18 yr of age. On average, females have a calf every 3 yr (Cagnolaro et al. 1983). This means that A could not have had more than 2 calves and B not more than 4. A was carrying a foetus 40 cm long when she died, but it was not possible to determine whether this was her first or second pregnancy.

The year and site of stranding are important parameters in view of the die-off of dolphins that occurred along the Mediterranean coasts in 1990 and 1991 (Bortolotto et al. 1992, Aguilar & Raga 1993, Van Bressen et al. 1993). A virus of the genus *Morbillivirus* (Domingo et al. 1991) was identified in dolphins stranded during this period. The virus affects the lungs and was probably one cause of the die-off (Aguilar & Borrell 1994a). Since PCBs depress the immune system and are toxic to the liver (Loose et al. 1977, Brouwer et al. 1989, Vos & Luster 1989), they are often accused of facilitating disease. In line with this concept, it has been found that organs and tissues with *Morbillivirus* infection had organochlorine levels several times higher than those found in dolphins dying of unknown causes (e.g. old age) or due to accidents (Borrell & Aguilar 1991, Aguilar & Borrell 1994a, Marsili & Focardi 1997). Of the 25 dolphins used for age determination and ecotoxicological investigations, 48% died during 1990 and 1991, 16% before 1990 and 36% after 1991. Although pathology and parasitology studies were performed on many specimens, *Morbillivirus* was rarely isolated. On the other hand, many larval forms of cestodes, nematodes and trematodes were found. Anatomical pathology revealed many cases of sub-acute pneumonia (Podestà et al. 1992). These factors must be considered in correlating contaminant levels with dolphin age. Except for those dying in accidents, the dolphins were not in good health.

Table 3 gives the chlorinated hydrocarbon concentrations found in blubber, liver, brain and muscle of dolphins of known age. In all dolphins, HCB levels were the lowest and PCBs were the highest, except in 1 blubber sample (dolphin PO2) in which total DDTs were higher than PCBs. The PCBs:DDTs ratio was greater than 1 in all organs and tissues, with a minimum in blubber [arithmetic mean 2.05, standard deviation (SD) 0.93] and a maximum in muscle (arithmetic mean 4.10, SD 2.62). The fact that this ratio was always greater than 1 reflects the fact that the use of DDT has been restricted in the Mediterranean basin since the 1970s, whereas PCBs are still used in great quantities.

The main tissue of accumulation was blubber (Table 3). Only 2 dolphins (20/89 and 9/89) had higher DDT (the former) and PCB (both) levels in the brain, and 3 others (19/92, 65/93 and 66/93) had higher DDT (the latter 2) and PCB (all) levels in the liver

As mentioned in 'Materials and methods', the 4 tissues analysed differ considerably in fat content. Since the contaminants of man-made origin analysed in this study are lipophilic compounds, the data was expressed on a lipid basis (l.b.) by multiplying the dry weight content by the respective EOM%. DDTs and PCBs were summed for each tissue, dolphin by dolphin. Then total DDTs and PCBs were calculated by summing the data of the 4 tissues (blubber DDTs+PCBs + liver DDTs+PCBs + brain DDTs+PCBs + muscle DDTs+PCBs). This parameter was used as a measure of total input of organochlorines and was plotted against age for the dolphins whose age we had been able to determine. DDTs+PCBs were plotted against age to give the distribution shown in Fig. 2A. Males were generally located in the upper part of the distribution. Their input of xenobiotics increased with age, though specimens in the age range from 6 to 12 yr had lower mean levels (arithmetic mean 431 μg g^{-1} l.b., SD 506, n = 4) than those aged 0 to 6 yr (arithmetic mean 664 μg g^{-1} l.b., SD 326, n = 5). Males over 12 yr of age had the highest levels (arithmetic mean 2068 μg g^{-1}, SD 1845, n = 6).

Females under 7 yr of age had lower PCBs+DDTs than the corresponding males (arithmetic mean 220 μg g^{-1} l.b., SD 114, n = 5). In the intermediate age range there was only 1 female (182 μg g^{-1} l.b.). Strangely, the 2 females in the upper age range had very high organochlorine levels (arithmetic mean 1735 μg g^{-1} l.b., SD 201), higher than all the other females and not significantly lower than males in the same age range. We had expected lower levels in the older females on the basis of reports that females pass part of their body burden of xenobiotics to their young during pregnancy and lactation (Tanabe et al. 1981b, 1982). We cannot explain why adult females A and B had such high levels of organochlorines because their histories are not known. Two hypotheses are possible: since female A was pregnant when she died, it may have been her first pregnancy; female B may have been sterile, and in any case was not pregnant. Unfortunately the reproductive organs were not examined to verify these

Table 3. Concentrations of organochlorines in different tissues of specimens of *Stenella coeruleoalba* of known age. EOM%: percentage extracted organic matter

| ID no.	Blubber (ng g⁻¹ d.w.)				Liver (ng g⁻¹ d.w.)				Brain (ng g⁻¹ d.w.)				Muscle (ng g⁻¹ d.w.)			
	EOM%	HCB	DDTs	PCBs	EOM%	HCB	DDTs	PCBs	EOM%	HCB	DDTs	PCBs	EOM%	HCB	DDTs	PCBs
1/88	80	186	8507	16564	18	106	4861	8603	45	27	756	1512	16	7	367	971
13/88	80	59	4905	23993	–	–	–	–	43	79	767	2137	20	9	654	5331
9/89	80	294	22071	34607	18	62	6167	13756	39	370	18860	42700	16	7	1128	1801
20/89	80	16	5725	9204	18	21	623	1432	44	105	13540	40115	16	18	234	741
27/90	36	911	207821	573262	10	98	58611	172940	42	32	761	37629	3	9	3023	16599
28/90	88	307	72206	157734	8	50	7528	27261	32	25	3197	5670	6	5	658	2393
10/91	95	10091	33384	69499	48	52	13962	21161	47	111	3853	9020	12	160	3051	12190
12/91	100	1663	26373	42508	–	–	–	–	52	171	1934	8622	14	69	4775	2386
14/91	91	407	138116	214238	56	199	36755	46038	41	18	1320	2839	11	13	582	2070
7/92	100	341	34209	77616	11	187	1678	7059	38	74	961	4399	18	51	2341	6840
19/92	98	3896	165189	183817	29	119	82110	246702	47	295	13452	29254	4	10	3293	8562
65/93	94	189	23665	87836	15	260	27988	126560	51	33	3251	11084	10	122	3129	38439
66/93	95	698	32277	104379	66	798	46865	300993	47	33	969	4107	12	156	3280	26712
92/93	100	7916	54726	82918	27	108	4633	13352	52	29	2880	4582	7	51	972	2993
93/93	92	1874	24469	46461	17	96	3401	9669	35	38	1130	3201	8	48	2495	7941
3/94	96	545	35797	52833	13	27	1464	3476	38	42	1768	6795	7	114	877	2511
4/94	98	1146	44928	91729	–	–	–	–	48	607	1760	3211	6	24	1097	3224
A5	49	2283	122310	261244	13	79	2116	6744	–	–	–	–	11	111	1043	4970
A13	79	2301	349964	469513	11	735	53317	136354	–	–	–	–	6	12	2060	4008
P01	58	271	61409	85806	–	–	–	–	–	–	–	–	–	–	–	–
P02	72	491	260041	185704	12	830	42281	49735	–	–	–	–	–	–	–	–
P04	61	296	14790	46388	12	1329	2216	4824	–	–	–	–	–	–	–	–
P15	–	–	–	–	16	21	2598	4045	–	–	–	–	10	5	666	2091
S06	78	459	63515	78107	–	–	–	–	–	–	–	–	–	–	–	–
S08	79	192	14961	26695	–	–	–	–	–	–	–	–	–	–	–	–
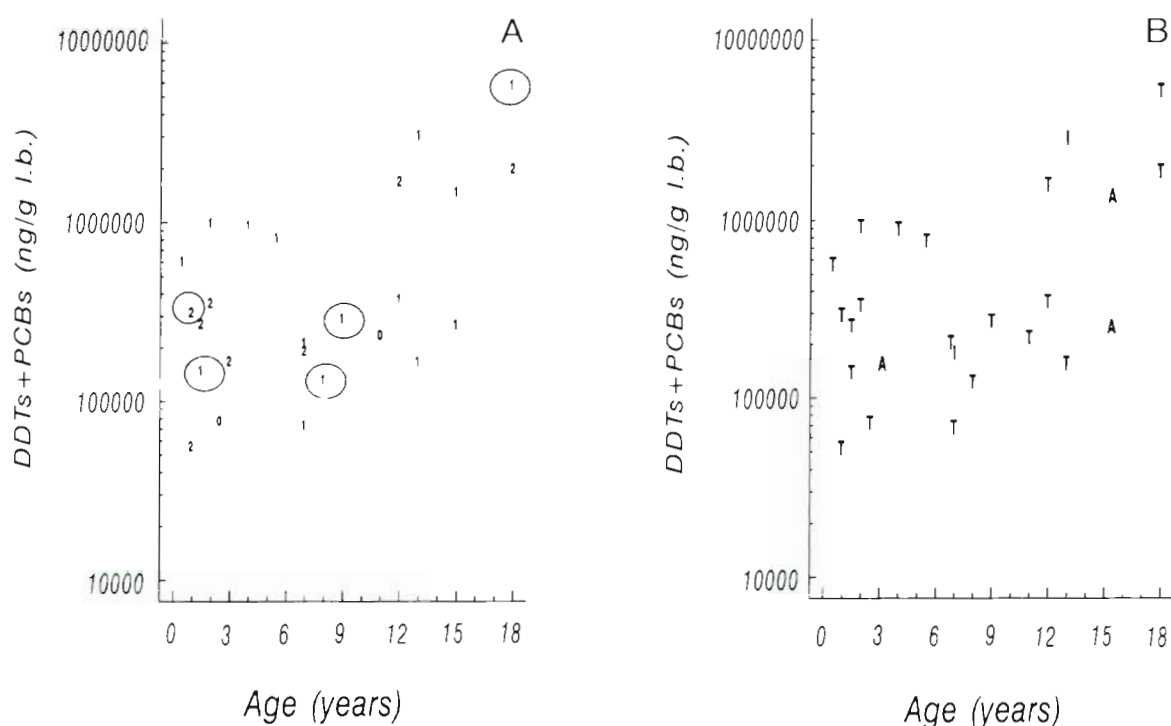
Fig. 2. *Stenella coeruleoalba*. Concentrations of organochlorines in brain, blubber, muscle and liver in relation to age. (A) Showing sex (0: sex unknown, 1: male, 2: female) and cause of death (circle: dolphins dying as a result of accidents). (B) Showing locality of stranding (T: Thyrrhenian Sea, A: Adriatic Sea, I: Ionian Sea)

hypotheses. In both cases, the females had a similar physiological pattern to males of the same age.

In interpreting the results of Fig. 2A, it should be borne in mind that there were no data on the history of the dolphins, such as where they had lived, when they reached physical and sexual maturity, and how many times females had been pregnant. The site and year of stranding should also be considered. Site is important because Mediterranean dolphins move in relatively limited areas of the Mediterranean Sea (Bearzi et al. 1992, Cagnolaro & Notarbartolo di Sciara 1992) Not all coasts of the Mediterranean have the same human impact. There is generally a direct correlation between human exploitation of the coast and rivers and water pollution. In Italy, the most polluted seas are the Ligurian and northern

Adriatic. There is much industry in the Ligurian area as well as intensive farming and greenhouses. The sea is heavily contaminated with organochlorines from the nearby Rhone estuary (Mendola et al. 1977, Renzoni et al. 1986). The northern Adriatic can almost be regarded as a closed sea receiving the waters of rivers such as the Po with its high organochlorine levels (Galassi et al. 1981, Šerman & Šerman 1992). The year of stranding is important in view of the epidemics and die-off phenomena mentioned above (Bortolotto et al. 1992, Aguilar & Raga 1993).

Some of the numbers in Fig 2A representing the sexes are circled to indicate dolphins dying as a result of accidents (2 bled to death from injuries to the caudal fin, 1 died of a fractured skull, 1 starved because its

Table 4. *Stenella coeruleoalba*. Concentrations of organochlorines (ng g^{-1} d.w.) in relation to year of death. EOM%: percentage extracted organic matter; SD: standard deviation

	Blubber EOM% = 80; SD = 0			Liver EOM% = 18; SD = 0			Muscle EOM% = 17; SD = 2			Brain EOM% = 43; SD = 3		
Dolphins stranded before 1990												
	HCB	DDTs	PCBs	HCB	DDTs	PCBs	HCB	DDTs	PCBs	HCB	DDTs	PCBs
No. of samples	4	4	4	3	3	3	4	4	4	4	4	4
Arithmetic mean	139	10302	21121	63	3884	6067	10	596	2207	145	1192	2983
Median	123	7116	20279	62	4861	6452	8	511	1378	92	1061	3075
Mode	16	4905	9319	21	623	1432	7	233	1617	27	760	1512
Geometric mean	85	8521	18921	52	2653	4568	9	501	741	95	1105	2728
SD	126	7996	10804	43	2898	4455	5	396	2131	153	540	1366
Minimum	16	4905	9319	21	623	1432	7	233	741	27	760	1512
Maximum	294	22071	34607	106	6167	10317	18	1128	5331	370	1886	4270

	Blubber EOM% = 74; SD = 19			Liver EOM% = 21; SD = 19			Muscle EOM% = 9; SD = 4			Brain EOM% = 43; SD = 7		
Dolphins stranded in 1990 and 1991												
	HCB	DDTs	PCBs	HCB	DDTs	PCBs	HCB	DDTs	PCBs	HCB	DDTs	PCBs
No. of samples	12	12	12	8	8	8	7	7	7	5	5	5
Arithmetic mean	1639	113741	184225	422	27223	58132	54	1829	6712	71	3633	12756
Median	475	67861	121770	149	25359	36650	13	2060	4757	32	3197	8622
Mode	407	61409	78107	79	7528	21161	12	1043	4008	25	1934	5670
Geometric mean	756	71392	121950	206	14916	31675	26	1512	5141	49	3010	8602
SD	2773	107597	175365	483	23404	62471	61	1067	5510	67	2567	14127
Minimum	192	14790	26695	50	2116	4824	5	582	2070	18	1320	2839
Maximum	10091	349964	573262	1329	58611	172940	160	3051	16599	171	7861	37629

	Blubber EOM% = 97; SD = 3			Liver EOM% = 24; SD = 18			Muscle EOM% = 9; SD = 4			Brain EOM% = 45; SD = 7		
Dolphins stranded after 1991												
	HCB	DDTs	PCBs	HCB	DDTs	PCBs	HCB	DDTs	PCBs	HCB	DDTs	PCBs
No. of samples	8	8	8	8	8	8	9	9	9	8	8	8
Arithmetic mean	2076	51908	90949	202	21342	88982	65	2017	11035	144	3271	8328
Median	922	35003	85377	114	4017	11511	51	2341	6840	40	1764	4491
Mode	545	32277	77616	96	2598	7059	51	1097	3224	33	1130	4107
Geometric mean	1062	41787	83844	113	7574	24271	41	1707	6613	71	2123	6083
SD	2649	46901	42240	253	29575	122173	54	1110	12789	208	4201	8842
Minimum	189	23665	46461	21	1464	3476	5	666	2091	29	961	3201
Maximum	7916	165189	183817	798	82110	300993	156	3293	38439	607	13452	29245

palate was damaged by a fishing hook, and the fifth starved with a plastic bag blocking its alimentary canal). These 5 dolphins were separated from the others because their death was unrelated to their health status; 4 of the 5 had organochlorine levels close to the mean for dolphins of the same age group, so that xenobiotic concentrations and cause of death do not seems to be related. This hypothesis is supported by the fact that the dolphin with the highest organochlorine levels of the whole 25 was the fifth of these dolphins.

In Fig. 2B, the points are marked T, A or I to indicate that the dolphins were found on the coasts of the Tyrrhenian, Adriatic (off Apulia) or Ionian Seas, respectively. Most of the dolphins were found on the Tyrrhenian coasts, including the 2 with the highest and lowest input of PCBs+DDTs. Again there did not seem to be a relationship between organochlorine levels and site of stranding, contrary to expectations based on the fact that the northern Adriatic has heavier organochlorine pollution than the other 2 seas (Galassi et al. 1981, Šerman & Šerman 1992).

The last factor to consider is the year of death. Since die-off of cetaceans, presumably due to viral infections such as *Morbillivirus*, occurred in 1990 and 1991, we separated the data into 3 periods: before 1990, 1990 and 1991 and after 1991 (Table 4). We also separated the data of the different tissues and the 3 organochlorines and applied Student's t-test to the arithmetic mean, standard deviation and number of samples. All tissues from dolphins stranded in 1990 and 1991 showed significantly higher levels of all xenobiotic compounds than tissues from dolphins stranded before 1990 (blubber HCB $p < 0.01$, DDTs and PCBs $p < 0.001$; liver all $p < 0.01$; muscle HCB and PCBs $p < 0.01$, DDTs $p < 0.001$; brain HCB not significant, DDTs $p < 0.01$, PCBs $p < 0.05$), except for HCB in brain. The only significant differences found between the same variables in 1990 and 1991 and after 1991 were for HCB in liver ($p < 0.001$) and PCBs in muscle ($p < 0.05$). The fact that after 1991 organochlorine concentrations were not much less than in the period of the epidemic suggests that the viral infection continued.

These results confirm those of Aguilar & Borrell (1994a) who found levels of organochlorines up to 3 times higher in specimens of *Stenella coeruleoalba* with *Morbillivirus* infection at the time of death than in biopsied dolphins which were presumably in good health. The levels found by these authors in infected dolphins were 5 times higher than ours for PCBs and 4 times higher for DDTs, reaching 846 µg g^{-1} l.b. (SD 618 µg g^{-1}) for PCBs and 456 µg g^{-1} l.b. (SD 413 µg g^{-1}) for DDTs. The control samples from biopsied Spanish dolphins (Aguilar & Borrell 1994b) were also much higher than ours (Marsili & Focardi 1996).

CONCLUSIONS

The age of 62 specimens of *Stenella coeruleoalba* found stranded along the Italian coasts between 1988 and 1994 was determined by tooth analysis, and a growth curve was plotted for these dolphins in the Mediterranean. The equation of the curve was $Y = 200/[1 + e^{(0.201 - 0.5115X)}]$ where Y is body length in cm and X age in yr.

Accumulation of organochlorines seems to depend on many parameters, such as sex, age, nutrition and health. A high correlation was found between contaminant levels in 25 dolphins of known age and year of death. Organochlorine levels were particularly high in the years 1990 and 1991, which coincided with an epidemic of *Morbillivirus* in the Mediterranean. It is not yet clear whether the disease was a cause or a result of the high levels of fat-soluble xenobiotics in these cetaceans (Aguilar & Borrell 1994a).

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This article was submitted to the editor