Lipophilic contaminants in marine mammals: review of the results of ten years' work at the Department of Environmental Biology, Siena University (Italy)

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Abstract: Organochlorine contaminants (HCB, DDTs and PCBs) and polycyclic aromatic hydrocarbons (PAHs) were valuated in three Mediterranean cetaceans: the striped dolphin (*Stenella coeruleoalba*), the bottlenose dolphin (*Tursiops truncatus*) and the fin whale (*Balaenoptera physalus*), and in three Argentinean pinnipeds: the southern sea lion (*Otaria flavescens*), the South American fur seal (*Arctocephalus australis*) and the subantarctic fur seal (*Arctocephalus tropicalis*). Two kinds of sample were obtained from the different species of cetaceans and pinnipeds in order to evaluate the toxicological risk to which a species or population is exposed: those from stranded specimens and those from free-ranging specimens. In this paper the use of a non-destructive approach, biopsy sampling, for free-ranging marine mammals is recommended.

Keywords: DDTs, dolphins, HCB, marine mammals, pinnipeds, PAHs, PCBs, whales.

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1 Introduction

1.1 Contaminants in marine mammals: state of the art

Interest in marine mammals has mainly been related to their economic value. They are actively marketed live for the tourist industry and dead for the food, leather, fur, cosmetic and chemical industries. Trawling for tuna, swordfish and albacore causes death by suffocation of many cetaceans, especially dolphins (Di Natale, 1992). Chemical pollution is the main source of ecotoxicological risk to these animals. Marine mammal populations are jeopardized by the rapid increase in levels of man-made chemicals appearing in the marine environment in the second half of this century (Geyer *et al.,* 1984). The case of chlorinated hydrocarbons (HCB, DDTs and PCBs) is one example. These pollutants are initially taken up by organisms at the very bottom of the food chain and are found in increasing concentrations in the tissues and organs of animals at higher levels, with levels depending on metabolic rates, sex, age and fat percentage. Marine mammals dwelling in coastal waters receiving industrial effluent accumulate contaminants, such as pesticides, aromatic hydrocarbons and heavy metals, from fish and squid in their diet. Because marine mammals are at the top of the food chain, they are among the most exposed of all animals (Neff *et al.,* 1976; Geraci and St. Aubin, 1980; Tanabe *et al.,* 1983; Boon *et al.,* 1992; Leonzio *et al.,* 1992; Marcovecchio *et al,.* 1994; Marsili and Focardi, 1997). The ecotoxicological risk of some species is also related to their 'biochemical vulnerability' to lipophilic xenobiotics (Tanabe *et al.*, 1988; Duinker *et al.,* 1989; Watanabe *et al.,* 1989; Fossi *et al.*, 1992; De Swart *et al.*, 1996; Marsili *et al.*, 1996; Fossi *et al.*, 1997a). Tanabe and Tatsukawa (1992) reported that '... these animals have a low capacity for degradation of organochlorines due to a specific mode of their cytochrome P450 enzyme system'. Moreover, because marine mammals, such as cetaceans, do not have sweat and sebaceous glands, fur, or active blood–water exchange via gills (as in fishes), they can be regarded as closed systems in which contaminants can act practically without opposition (Marsili *et al.*, 1995). Because the incidence of pathology in these species is closely related to the level of pollution in their environments, bacterial and viral infections and contaminants must be considered from a holistic point of view. Mass mortalities of dolphins and seals have occurred in particularly polluted areas, when levels of organochlorine pesticides, PCBs, PAHs and heavy metals have reached very high levels. Exhaustive research on this subject has been done in developed countries (Olsson, 1978; Martineau *et al.*, 1987; Aguilar and Borrell, 1994; Guitart *et al.*, 1996; Jenssen, 1996). The main hypothesis has been that pollutants are acquired by animals through the diet and are stored in fatty tissues. When they are transferred to the blood, the immune system may be impaired so that defences against infectious agents are lowered. There is still no evidence that pollutants are causing the death of marine mammals; however, it is certain that lipophilic contaminants cause immune and reproductive dysfunction (Helle *et al.,* 1976a, b; De Long *et al.,* 1979; Fuller and Hobson, 1986; Reijnders, 1986; Brouwer *et al.,* 1989).

1.2 Lipophilic contaminants

Organochlorine contaminants (OCs) and polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in all parts of the world, including remote areas such as the Arctic and Antarctic (Risebrough *et al.,* 1968; Risebrough *et al.,* 1976; Law, 1986; Muir *et al.,* 1988).

The synthesis of halogenated compounds by the chemical industry began about 80 years ago and has been subject to continual increase and diversification. These xenobiotics have a wide range of chemical and physical properties so that they are used in every field: industry, agriculture, medicine and households. However useful these compounds may be, their thoughtless and deliberate release into the environment is causing serious pollution problems. Organohalogens are halogen derivatives of aliphatic, aromatic and heterocyclic hydrocarbons. The group of chlorinated hydrocarbons is relevant because of the vast range of substances it contains and the amounts in which they are produced. These molecules are characterized by one or more chlorine atoms. Owing to properties such as high fat solubility, high chemical stability and low volatility, the OCs are subject to biomagnification in the marine food chain. Some of these xenobiotics (HCB, DDTs and PCBs) are known to have effects on pinnipeds, which include reproductive anomalies (Le Boeuf and 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; Zakharov and Yablokov, 1990). Because many cetaceans have a similar lifestyle and diet to pinnipeds, they run similar risks.

PAHs are composed of two or more fused aromatic (benzene) rings. The resulting structure is a molecule with all of the carbon and hydrogen atoms lying in a single plane. Two molecular mass classes of PAHs can be distinguished on the basis of physical, chemical and biological properties: lower molecular mass (two or three ring) and higher molecular mass (four or more rings) aromatics, ranging in relative molecular mass from naphthalene ($C_{10}H_8$; M_r 128.16) to coronene ($C_{24}H_{12}$; M_r 300.36). Only the low molecular mass PAHs have significant acute toxicity to aquatic organisms, but all of the 20 to 30 proven PAH carcinogens are in the high molecular mass group (Neff, 1979). PAHs are essentially produced during pyrolysis of fossil fuels or organic-rich materials. Pyrolitic processes may be natural, such as forest fires, or carried out by humans, such as combustion of fossil fuels. PAHs such as crude oil and refinery products may also be introduced directly into the environment. Finally, naturally derived PAHs are produced from natural compounds during *in situ* aromatization (Lipiatou and Saliot, 1991). Because they are strongly hydrophobic, they are taken up readily by animal tissues. The scientific interest in these compounds centres principally on their carcinogenic, mutagenic and teratogenic effects (Neff, 1979; NRCC, 1983; Lipiatou and Saliot, 1991; Marsh *et al*., 1992). Some PAHs have been shown to cause tumours in marine mammals (Martineau *et al.*, 1988) but few examples of cause and effect relationships have been demonstrated.

1.3 Study areas and sources of contamination

1.3.1 The Mediterranean Sea

The Mediterranean Sea (Figure 1) occupies an area of 2.53 ∞ 10^6 km^2 with a water volume of 3.71 ∞ 10^6 km^3. It is a small, almost completely enclosed basin, which communicates with the rest of the ocean system through the Straits of Gibraltar (15 km wide and 320 m deep). Water exchange with the Red Sea through the Suez Canal, opened in 1869 (about 100 m wide) and with the Black Sea through the Dardanelles, is negligible. The Mediterranean Sea, with heavy coastal industry and intensive farming along the main rivers, is not exempt from pollution by lipophilic compounds (Geyer *et al.*, 1984; Picer and Picer, 1991; Galassi *et al.*, 1993). Intense traffic of transport and passenger ships adds to the pollution load. PCB levels in open Mediterranean waters have been reported to range from <0.1 to 8.5 ng/l (UNEP/FAO, 1990) whereas DDT has been found to be below detection limits (0.05 ng/l) by Picer and Picer (1979). In the anchovy, taken to represent all marine organisms, PCB levels evaluated as the geometric mean of the mean values of three Mediterranean study areas were 197 ng/g fresh weight (f.w.); for DDTs, average levels were 62 ng/g f.w. (Renzoni *et al.,* 1981). The Italian coasts, namely those washed by the Ligurian, Tyrrhenian, Ionic and Adriatic Seas, were one of the study areas investigated in the studies reviewed in this paper.

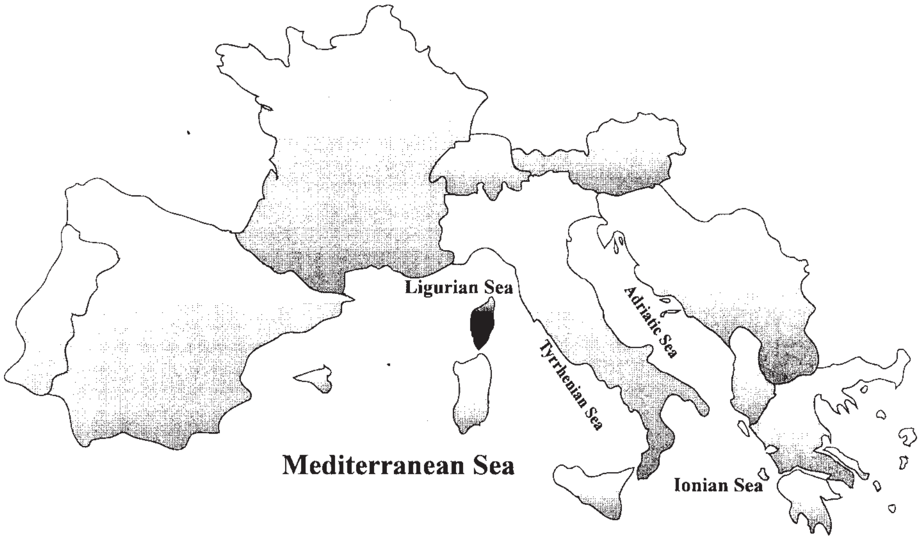

Figure 1 The Mediterranean Sea.

1.3.2 The Southwest Atlantic

The SW Atlantic coastal area covered by this study (Figure 2) is more than 2200 km long, stretching from Pontal do Sul, Paranagua, Parana, Brazil, to Punta Bermeja, 60 km south of Viedma, Rio Negro, Argentina. Potential sources of pollution in the littoral ecosystems off Argentina and Brazil are related to the sites of the major cities and large industrial centres. Parana (PR), Santa Catarina (SC) and Rio Grande do Sul (RGS) (jointly with Sao Paulo (SP)) states are the most industrially developed regions of southern Brazil, and also have high agricultural production. Lagoa dos Patos in RGS and the La Plata river estuary in Buenos Aires Province, Argentina, contribute enormous volumes of fresh water and huge amounts of sediments, nutrients and contaminants. The domestic effluents of Rio Grande and Porto Alegre (400 km north) are discharged into the Los Patos Lagoon, together with the effluent of the chemical industries situated along the lagoon channel. In developing countries, especially in the southern hemisphere and tropical regions, the use of chlorinated hydrocarbon pesticides, such as DDT, was considered indispensable to guarantee food supplies and control malaria until recently (Snelson, 1977). Organo-chlorine pesticides used in the 1970s and 1980s in the rice-fields of southern Brazil and Uruguay and the farmland of Buenos Aires Province have entered the SW Atlantic coastal ecosystems. Buenos Aires, the capital of Argentina, located on the La Plata River, is surrounded by metallurgical, petrochemical, textile and pharmaceutical plants. The largest petrochemical refinery in Argentina is near La Plata on the banks of the same river (50 km SE of Buenos Aires). All domestic and industrial effluents are discharged untreated into the La Plata River, and enter the Atlantic. Mar del Plata (400 km SE), is the main summer tourist resort in Buenos Aires Province. About 400 ships, coastal fishing vessels, large cargo vessels, warships and small sailing boats operate in its harbour on a daily basis. The water is heavily contaminated by oil, organic material and the wastes of fish-processing factories. Punta Bermeja, in the San Matias Gulf, Rio Negro Province, is not a polluted environment, but may be affected by pollution from the Rio Negro river, which drains an area subject to a heavy burden of organophosphate pesticides (fruit and vegetable crops).

Figure 2 The Atlantic coastal area covered by these studies.

1.4 Biological considerations about the marine mammal species of this study

1.4.1 Mediterranean cetaceans

Two of the Mediterranean cetaceans studied were odontoceti: the striped dolphin (*Stenella coeruleoalba*) and the bottlenose dolphin (*Tursiops truncatus*)*,* and the other was a mysticete: the fin whale (*Balaenoptera physalus*)*.*

The Mediterranean striped dolphin is a small dolphin about 2 m long and weighing around 100 kg. The male is slightly larger than the female. This species is gregarious, generally living in groups of 20–30, in pelagic waters. It is a generalist feeder, preying on a wide variety of organisms according to circumstances and availability. Its diet includes cephalopods, bony fish and macroplanktonic crustaceans. In the Mediterranean, the European flying squid (*Todarodes sagittatus*) seems to be the preferred species. The blue whiting (*Micromesistius potassou*), European hake (*Merluccius merluccius*) and small gregarious fish of surface waters such as the European anchovy (*Engraulis encrasicolus*), may be consumed in large quantities. Among crustaceans, especially in the Ligurian Sea, shrimps of the species *Pasiphaea multidentata* are a common food item. Little is known about the life and reproductive cycles of the striped dolphin. The period of gestation is 12–13 months. The young are generally born in late summer to autumn, and are about 90 cm long, and are weaned at about 18 months. Females seem to give birth approximately every 3 years. The striped dolphin lives for more than 30 years, and has been known to reach an age of 57 years (Notarbartolo di Sciara and Demma, 1994).

The bottlenose dolphin is a large dolphin about 3 m long and weighing around 300 kg. It feeds mainly on fish, other dietary items including squid, cuttlefish and octopus. Males grow larger than females. The gestation period is 12 months, the young being born in the warmest season. Young are about 1 m long at birth and remain close to the mother for several years. Females seem to give birth every 3–4 years. The bottlenose dolphin has coastal habits, living in groups that rarely exceed ten animals (Notarbartolo di Sciara and Demma, 1994).

The fin whale is a colossal mysticete up to 24 m long and weighing over 50 tonnes. Although it is a solitary species, it is not infrequently observed in groups of six or seven in pelagic waters. In the Mediterranean, the mean membership of groups is 1.5. In this basin, it mainly feeds on euphausiaceans of the species *Meganyctiphanes norvegica*. Its reproductive cycle is linked to seasonal migrations. Conception occurs in winter in warm low-latitude waters. After passing summer in cold waters, where it feeds and builds up energy reserves, it returns to warmer seas to give birth. Young are weaned at about 6 months. The fin whale is the longest living cetacean, with a life-span of 90 to 100 years (Notarbartolo di Sciara and Demma, 1994).

1.4.2 Argentinian pinnipeds

The Argentinian pinnipeds studied were three otarides: the southern sea lion (*Otaria flavescens*), the South American fur seal (*Arctocephalus australis*) and the subantarctic fur seal (*Arctocephalus tropicalis*).

The South American sea lion or 'lobo marino de un pelo' is distributed along the SW Atlantic coast from Tierra del Fuego and the Malvinas (Falkland) Islands to Isla de Torres, RGS, Brazil. Females grow to about 1.8 m and 160 kg. During gestation they weigh up to 220 kg. An adult female may give birth to one pup (85 cm, 15 kg) per year. The annual mortality of pups is about 18% due to natural causes. Females reach puberty at four to five years. Until the age of four years, males resemble females in colour and size. They subsequently begin to develop the mane that enables them to be classified into 4-year age groups. They reach maturity at 10–12 years, when their mean length is 2.3 m and mean weight 400 kg. In this last age group they are known as sultans. A sultan has territory and forms a harem with 4–20 females, which he must defend from the assaults of younger males seeking to supplant him. These attacks may be by individuals or groups of males. Up to 40 young males have been seen to attack a sultan (Intrieri, personal communication). The natural enemies of the sea lion are killer whales (*Orcinus orca*) and sharks. The main food item of the sea lion is fish, but squid and crustaceans are also eaten. There are 70 colonies along the Argentinian coasts. Until 1954 they contained 170 000 animals, but uncontrolled hunting has reduced the population by 80–90% to no more than 18 000 individuals (Lichter, 1992; Intrieri, personal communication).

In Argentina, the northernmost colony is in the Mar del Plata harbour with 650–750 individuals, mostly males (Lorenzani and Lorenzani, 1992). *Otaria flavescens* generally feeds in shallow coastal waters within five miles of the shore. Faecal analysis showed that this colony feeds mainly on cod (*Merluccius hubbsi*), mackerel (*Trachurus picturatus*) and squid (*Ilex illecebrosus*) (Baldas, 1985). Reproductive males periodically travel between the Mar del Plata colony and the reproductive colonies (Isla de los Lobos (Uruguay) and Valdes Peninsula, Chubut) (Lorenzani and Lorenzani, 1992). There is a reproductive colony with a permanent population of about 1800 individuals at Punta Bermeja, Rio Negro, Patagonia, an area regarded as subject to low human impact, hence this population was used as the control colony for this study. It produces 350 pups per year. Senile males stay all year in the Mar del Plata colony and are therefore more exposed to oil and other industrial contaminants. A high percentage of old sea lions have diseases of the skin and mucous membranes with fur loss and baldness. Young and old sea lions often have tumours.

The South American fur seal and the subantarctic fur seal have coats consisting of two different types of fur, hence the name 'lobo marino de dos pelos'. The distribution of the South American fur seal includes the seas off the islands of South America from Peru in the Pacific Ocean to Uruguay in the Atlantic. Occasional specimens have been reported from Brazil. Males are about 2 m long and weigh 160 kg; females measure 1.4 m and weigh 50 kg. Females live up to 25 years; males probably do not live for so long. Natural enemies include sharks and killer whales. Diet consists of cephalopods, crustaceans and fish of the sardine family (Lichter, 1992). The Antarctic fur seal is found in the southern hemisphere, mostly at temperate latitudes. Small but significant differences in the pattern of their teeth, skull proportions and fur colour distinguish stocks from southern and northern islands of the Antarctic Convergence. Those to the south are a separate sub-species: *Arctocephalus gazella*. Males and females of the Antarctic fur seal are very different in size and shape: adult males, almost 2 m long, are stoutly built, with a heavy neck and shoulders; their bulky appearance is enhanced by a striking mane or cape of long fur, and they weigh over 100 kg. Females are only 1.5 m long, more slenderly built, maneless, and weigh less than 50 kg. Antarctic fur seals breed in the late spring and early summer. Males have harems of four or five females. They feed mainly on crustaceans and fish (various authors, 1985; Focardi and Marsili, 1997).

2 Methodology

2.1 Samples

Two kinds of sample can be obtained from different species of cetaceans and pinnipeds in order to evaluate the toxicological risk to which a species or population is exposed: those from stranded specimens and those from free-ranging specimens.

2.1.1 Stranded specimens

In theory, all stranded cetaceans and pinnipeds in a good state of preservation can be used for ecotoxicological analysis. Brain, liver, blood, skin, subcutaneous blubber, melon, heart, kidney, muscle and fur are suitable materials. Various environmental pollutants such as OCs, heavy metals and PAHs can be analysed in these samples.

2.1.2 Free-ranging specimens

From an ecotoxicological point of view, the most important studies concern healthy live specimens, the free-ranging animals, which can be sampled without killing or disturbance. The main biological materials obtainable from free-ranging pinnipeds are blood, skin biopsy specimens, fur and faeces, which can all be obtained, under anaesthesia, with minimum stress to individuals or populations. The most useful samples for ecotoxicological studies in cetaceans are skin biopsy specimens, obtained by dart, and faeces. Residue analysis can be carried out in the various biological materials.

2.2 Sampling procedures in free-ranging species

2.2.1 Samples from Mediterranean cetaceans

Samples of subcutaneous blubber (about 1 ∞ 2 cm) were obtained from 68 free-ranging whales using biopsy darts launched with a crossbow, and from 84 bow-riding dolphins by biopsy tips mounted on a two-metre pole. The biopsy dart, an aluminium crossbow bolt with a modified stainless steel collecting tip and floater, was fired into the whale with a Barnett Wildcat II crossbow with a 150-pound test bow. To avoid the possibility of infection, the bolt tip was sterilized with alcohol before shooting. Biopsy specimens were taken in the dorsal area near a dorsal fin and on the upper part of the caudal peduncle. The procedure consisted of approaching the whale at low to moderate speed as it surfaced, and firing the dart when within a range of 10–30 m. Dolphins were sampled from the prow of the boat while they were riding the bow wave. Their reaction to sampling varied from a slight start to no reaction at all (Brown *et al*., 1991; Jakoda *et al.*, 1996).

2.2.2 Samples from Argentinian sea lions

The two populations of southern sea lions at Mar del Plata and Punta Bermeja were studied. Blood, skin, subcutaneous blubber, fur and faeces were sampled under anaesthesia. Skin, subcutaneous blubber and fur were sampled with a biopsy dart shot with a crossbow.

Anaesthetics for sea lions

Anaesthetic darts were shot with a blow-gun with the assistance of a member of the Fundacion Fauna Argentina (Mar del Plata). Xylazine and ketamine anaesthetics were combined in different proportions, along the guidelines of the scanty literature available on this subject (Gales, 1989). After many trials, a total of 52 sea lions of all age groups were sampled: the mean dose was 2.49 mg/kg xylazine (range 1.42–5.33 mg/kg) and 1.64 mg/kg ketamine (range 0–4.16 mg/kg) (Junin *et al.*, 1997). Apnea and thermoregulatory disturbance were the two most common side-effects of anaesthesia. An analeptic drug, doxapram, was injected i.m. to improve the general respiratory and cardiac condition of the animals. Peripheral blood was obtained from the hind-flipper capillary network, and skin samples from the flipper surface. Fur samples were cut with scissors.

Crossbow and biopsy dart

This sampling procedure does not require anaesthesia and is therefore less invasive and dangerous for the animals. The sea lions were sampled on land with a dart having a punch tip (i.d. 8 mm ∞ 40 mm) shot by means of a crossbow. The dart with the biopsy sample was recovered by hand. The skin samples were about 0.5 g.

2.3 Samples from Mediterranean cetaceans

2.3.1 Stranded cetaceans

Most of the animals analysed were found dead along the Italian coasts in the period 1987–1993. Collection and transport of the carcasses was authorized and supervised by the Centro Studi Cetacei (Milan). The organs and tissues used for ecotoxicological studies were mainly of two species of delphinids, the striped dolphin (89 specimens) and the bottlenose dolphin (14 specimens). When possible, we sampled blubber, muscle, melon, brain, heart, liver and kidney tissue, which we analysed for chlorinated xenobiotics.

2.3.2 Free-ranging cetaceans

In the summers of 1990–1993, subcutaneous samples from fin whales (68 specimens) and striped dolphins (89 specimens) were obtained for chemical analysis and other studies. The whales were sampled in the Ligurian Sea over the four-year period, whereas the dolphins were sampled there from 1991 to 1993. In 1993, dolphins were also sampled in the Tyrrhenian and Ionian Seas. Organochlorine contaminants were analysed in these samples.

2.4 Samples from Argentinian pinnipeds

2.4.1 Beached pinnipeds

Five liver samples of *Arctocephalus sp.* pinnipeds and one liver sample of a southern sea lion were analysed for HCB, DDTs and PCBs. Three of the South American fur seals were beached in Rio Grande (Brazil) and one in San Clemente (Argentina); the subantarctic fur seal was from Parana (Brazil) and the southern sea lion was from the Mar del Plata colony.

2.4.2 Free-ranging sea lions

During 90 days of field work in February 1995, June 1995, August–September 1995, November 1995, February 1996, April 1996 and September 1996, a total of 260 sea lions were sampled in the colonies of Mar del Plata and Punta Bermeja. Chlorinated hydrocarbons and PAHs were analysed in the various biological materials obtained by non-invasive techniques.

2.5 Residue analysis

2.5.1 Organochlorine contaminants

Total PCBs were quantified as the sum of 30 congeners (Table 1). The congeners constituted 80% of the total peak area of PCBs in all tissues. Total DDT was calculated as the sum of *op′*DDT, *pp′*DDT, *op′*DDD, *pp′*DDD, *op′*DDE and *pp′*DDE.

Table 1 IUPAC number (Ballschmiter and Zell, 1980) and structure of the main PCB congeners detected in all samples.

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

For analysis of HCB, DDTs and PCBs, the samples were freeze-dried and extracted with n-hexane in a Soxhlet apparatus followed by sulfuric acid clean-up and Florisil chromatography (Marsili and Focardi, 1997). 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 i.d.). The carrier gas was nitrogen or helium with a head pressure of 15.5 psi (splitting ratio 50/1). The scavenger gas was argon–methane (95/5) at 40 ml/min. The oven temperature was 100°C for the first 10 min, after which it was increased to 280°C at 5°C/min. Injector and detector temperatures were 200°C 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 or µg/g d.w. Recoveries were calculated by adding known amounts of standard to homogeneous replicates of the same sample. The extracted organic material (EOM%) from freeze-dried samples was calculated in all samples. The water content was calculated in the samples from stranded mammals only, because the skin biopsy specimen was too small for this analytical procedure. The results were expressed in ng/g dry weight (d.w.) or fresh weight (f.w.) or lipid basis (l.b.).

2.5.2 PAH analysis

PAH residues were determined in sea lion samples only. Extraction was carried out according to Griest and Caton (1983) and Holoubek *et al.* (1990), with some modifications (Marsili *et al.*, 1997a). The organic fraction, concentrated to 1 ml in acetonitrile, was analysed by HPLC with fluorescence detection. A reversed-phase column (Supelcosil LC-18, 25 cm x 4.6 mm i.d., 0.5 µm particle size) was used with an acetonitrile/water gradient. The initial concentration of the gradient was 60% acetonitrile, increasing over 20 min to 100% acetonitrile, and then remaining stable for 10 min. The flow-rate was 1 ml/min. Quantification was carried out using an external standard consisting of 16 PAHs from Supelco (EPA 610 polycyclic aromatic hydrocarbon mixture). Table 2 lists the 14 PAHs studied, with their molecular formulae, molecular masses and carcinogenic properties. The results were expressed in ng/g dry weight (d.w.) or fresh weight (f.w.).

Table 2 PAHs investigated: 0 = not carcinogenic; ± = uncertain or weakly carcinogenic; ++, +++ = strongly carcinogenic (NRCC, 1983).

Compound name (IUPAC)	Abbreviation	Molecular formula	Relative molecular mass	Carcinogenicity (NRCC, 1983)
Naphthalene	Naph	$C_{10}H_8$	128.2	0
Acenaphthene	Ace	$C_{12}H_8$	152.2	0
Fluorene	Fl	$C_{13}H_{10}$	166.2	0
Phenanthrene	Phen	$C_{14}H_{10}$	178.2	0
Anthracene	Ant	$C_{14}H_{10}$	178.2	0
Fluoranthene	Flt	$C_{16}H_{10}$	202.2	0
Pyrene	Pyr	$C_{16}H_{10}$	202.2	0
Benzo(a)anthracene	B[a]A	$C_{18}H_{12}$	228.3	+
Chrysene (93%)	Chry	$C_{18}H_{12}$	228.3	±

Benzo(b)fluoranthene	B[b]F	$C_{20}H_{12}$	252.3	++
Benzo(k)fluoranthene	B[k]F	$C_{20}H_{12}$	252.3	0
Benzo(a)pyrene	B[a]P	$C_{20}H_{12}$	252.3	+++
Dibenzo(a,h)anthracene	D[ah]A	$C_{22}H_{14}$	279.2	+++
Benzo(g,h,i)perylene	B[ghi]Per	$C_{22}H_{12}$	276.3	0

2.6 Statistical analysis

The data were processed by summary statistics and ANOVA using Statgraphics software (Statistical Graphics Corporation), Statistica and Excel (Microsoft), assigning $p < 0.05$ as significance level. Since the data did not have a normal distribution, the two data populations were compared by a non-parametric test, namely that of Kolmogorov–Smirnov. Since most of the literature on xenobiotic concentrations in marine mammals is expressed as arithmetic mean and standard deviation, these parameters were also calculated to enable comparison.

3 Results and discussion

3.1 Mediterranean cetaceans

3.1.1 Striped dolphin

The striped dolphin can be regarded as a terminal consumer, as it feeds mainly on fish and squid. It is therefore a strong accumulator of fat-soluble compounds, such as chlorinated hydrocarbons. The results from both the stranded and free-ranging specimens confirmed this.

Stranded specimens of striped dolphin

All 89 striped dolphins analysed contained detectable levels of OCs in most tissues (Marsili and Focardi, 1997). The two tissues with the highest levels of OCs were melon tissue and blubber. In general, the pattern of contaminants is known to be related to the lipid content of the various compartments, expressed as EOM%. For DDTs and PCBs, the geometric mean content usually shows a decrease in the following order: melon > blubber > liver > heart > brain > kidney > muscle (for DDTs in ng/g d.w.: 60 464 > 48 965 > 7214 >2271 > 1927 > 892 > 794; and for PCBs in ng/g d.w.: 108 986 > 86 257 > 21 424 > 8102 >5984 > 3293 > 2666). In some cases, especially in the brain tissue, OC levels do not agree with the EOM% of the tissue. This is related to the lipid composition of the tissues and the polarity of the lipid components, which have different selective affinities for these compounds and bind them differently (Tanabe *et al.*, 1981a; Aguilar, 1985). In other words, the concentrations of these compounds in the different tissues do not depend solely on lipid content but also on its composition: 99% of the total lipids in melon tissue and blubber are triglycerides whereas the brain contains more than 50% phospholipids (Kawai *et al.*, 1988). Phospholipids are polar lipids, which are less inclined to bind apolar compounds such as PCBs and OCs in general. Since melon tissue has a mean EOM% of 91%, blubber 74% and brain 42%, we would expect

OC concentrations in brain to be about half the values in melon and blubber. What we found, however, were quantities 25-30 times lower for DDTs and 15–20 times lower for PCBs. The various tissues had different affinities for the various metabolites of DDT, except pp'DDE, which was always the main component. The different affinity of the various organs and tissues for these xenobiotics also seems to depend on their lipid constitution (Fukushima and Kawai, 1981). PCBs were quantified as the sum of 30 congeners: the prevalent congener in all organs was 153 which ranged from 9.6% in Arochlor 1260 (the standard compound) to 26% in heart tissue. This congener is particularly persistent as it has chlorines in positions 2, 4 and 5 of both rings of the biphenyl (Wolff *et al.*, 1982; Bush *et al.*, 1984, Safe *et al.*, 1985) and no adjacent, unsubstituted carbons in *ortho-meta* position (Clarke, 1986).

Dividing the results according to sex, it emerges that in melon, blubber, liver, muscle and brain tissue, both total DDTs and PCBs were much higher in males than in females. The fact that OC levels are normally higher in males than in females is explained by the fact that females lose up to 90% of their total body burden of these substances during pregnancy and lactation (Tanabe *et al.*, 1981b; 1982). This is linked to the fact that the milk of these mammals contains very high levels of fats, mostly triglycerides. The milk of the striped dolphin, for instance, contains 258 mg/g of triglycerides in 280 mg/g total fats (Kawai and Fukushima, 1981). Tanabe *et al.* (1981b) estimated that a pregnancy followed by a 6- to 7-month period of lactation enables the female striped dolphin to lose 4.7% and 95% of her total body burden of DDT, and 4% and 92% of that of PCBs, respectively.

Since the striped dolphin specimens came from many parts of Italy, we separated the data of the major OCs in relation to the different seas of origin. The results did not show large differences between sampling areas. Using the Kolmogorov–Smirnov test for PCB and DDT levels, the population of the Ligurian Sea was found to be significantly different in OC accumulation in blubber than that of the Tyrrhenian Sea (for both DDTs and PCBs, $p < 0.01$), from that of the West Ionian Sea (only PCBs, $p < 0.01$) and from that of the Adriatic Sea for melon (only PCBs, $p < 0.05$). Hence the only population that differed to any extent from those of the other seas was that of the Ligurian Sea, in which xenobiotic concentrations were higher, albeit not significantly, in nearly all organs. This confirms that this part of the Mediterranean basin is more contaminated.

Another type of statistical analysis was performed in relation to the year of sampling, taking blubber as representative of all organs and tissues. The small number of specimens stranded in certain years prompted us to group the data into three periods: before 1990, 1990–1991 and after 1991. These divisions were decided on the basis that a viral epidemic affected many dolphin populations throughout the Mediterranean in 1990 and 1991 (Bortolotto *et al*., 1992; Marsili *et al.*, 1992; Aguilar and Raga, 1993; Borrell, 1993a). Analysis of many of these animals led to the isolation of a virus of the genus *Morbillivirus* (Domingo *et al*., 1990; 1991) that affects the lungs and could be one of the main causes of the high mortality. In other cases, the virus was not isolated but a large number of larval forms of cestodes, nematodes and trematodes were found. Anatomical pathology examination frequently revealed sub-acute pneumonia (Podesta' *et al*., 1992). Levels of OCs several times higher than in specimens stranded without an apparent cause of death, or dying as a result of accidents, were found in the organs and tissues of dolphins infected with *Morbillivirus* (Borrell and Aguilar, 1991; Aguilar and Borrell, 1994). The Kolmogorov–Smirnov test showed significant differences in the cumulative distributions of PCBs between populations before 1990 and those in the period 1990–1991 ($p<0.05$). For DDTs significant differences were found between the period 1990–1991 and after 1991 ($p < 0.01$). The decrease in total DDTs expected since restriction of the use of this pesticide in most of the Mediterranean is probably masked by the disease that, whether cause or effect of the phenomenon, in any case determined the stranding of animals with the highest levels of xenobiotics. As far as PCBs are concerned, a slight increase was expected, or at least a stabilization of levels. The increase was so high between 1987 and 1990–1991 for the reasons explained above. The fact that, after 1991, there was no further increase in PCB concentrations seems to confirm this.

The age of 62 specimens of striped dolphin was determined by counting dentine growth layer groups in the teeth. A growth curve ($y = 200/[1 + \exp(0.201 - 0.5115x)]$ where y is the body length in centimetres and x is the age in years) was plotted on the basis of the age and length data (Marsili *et al.*, 1997b) (Figure 3).

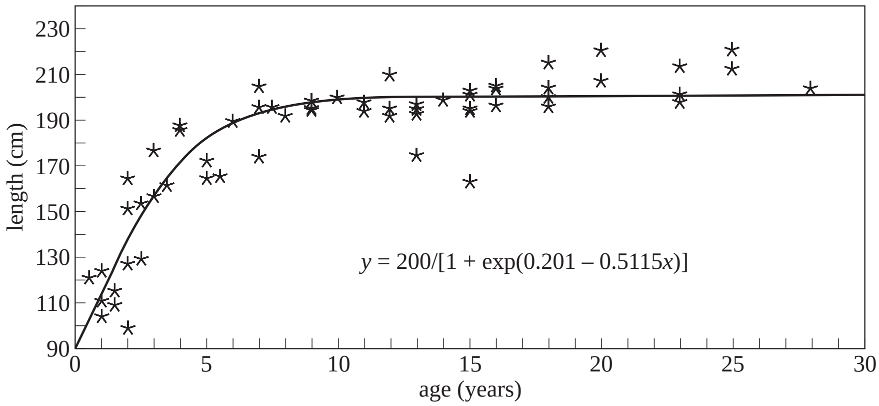

Figure 3 Growth curve for the striped dolphin in the Mediterranean Sea.

Organochlorine contaminants (DDTs and PCBs) were analysed in the blubber, liver, brain and muscle of 25 of the dolphins. Correlations were sought between levels of these contaminants and age. When total organochlorines (DDTs + PCBs) were plotted against age, we found that 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 years had lower mean levels (arithmetic mean 431 µg/g l.b., S.D. 506 µg/g l.b., $n = 4$) than those aged 0-6 years (arithmetic mean 664 µg/g l.b., S.D. 326 µg/g, $n = 5$). Males over 12 years of age had the highest levels (arithmetic mean 2068 µg/g, S.D. 1845 µg/g, $n = 6$). Females under 7 years of age had lower levels of total organochlorines than the corresponding males (arithmetic mean 220 µg/g l.b., S.D. 114 µg/g, $n = 5$). In the intermediate age range there was only one female (182 µg/g l.b.). Strangely, the two females in the upper age range had very high organochlorine levels (arithmetic mean 1735 µg/g l.b., S.D. 201 µg/g), 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 for the reasons explained above. Probably only the individual history (health, nutritional status, pregnancies, lactation and so forth) of the two females can explain this phenomenon.

Table 3 gives the mean PCB and DDT concentrations found in striped dolphin specimens stranded on the Atlantic coasts of the USA, the eastern Pacific, Japan, Wales, the Straits of Gibraltar and other parts of the Mediterranean. Without considering the deaths in Catalonia due to *Morbillivirus*, the concentrations in blubber of striped dolphins living in the Mediterranean are elevated and much higher than in specimens of the same species living in oceans. The latter have DDT levels between 21 and 43 ppm (l.b.) and PCB levels between 6 and 59 ppm (l.b.). The Gibraltar Straits are a transition zone as far as xenobiotic concentrations are concerned. The lowest DDT values in the Mediterranean are from the French coasts; the lowest PCB values are those of this study. In Catalonia there is high OC contamination, as shown by the high levels found in the 'healthy' population sampled by biopsy methods (Borrell, 1993a).

Table 3 Mean concentrations of PCBs and DDTs in blubber of *Stenella coeruleoalba* living in different seas.

Area	Number of dolphins	DDTs (ppm l.b.)	PCBs (ppm l.b.)	References
USA (Atlantic)	3	36	59	Taruski *et al.*, 1975
East Pacific	15	43	6	O'Shea *et al.*, 1980
West Pacific (1978)	8	38	29	Loganathan *et al.*, 1990
West Pacific (1986)	8	37	28	Loganathan *et al.*, 1990
Japan	4	21	29	Tanabe *et al.*, 1983
Japan	49	27	14	Fukushima and Kawai, 1981
Wales	7	30	39	Borrell, 1993b
Gibraltar	3	94	67	Borrell, 1993a
Catalonia (biopsies)	109	156	314	Borrell, 1993a
Catalonia (epizoosis)	72	456	846	Aguilar and Borrell, 1994
Mediterranean France	8	71	267	Alzieu and Duguy, 1979
Italy	64	136	205	Marsili and Focardi, 1996
Italy (biopsies)	61	44	70	This paper

Skin biopsies from striped dolphins

In the Ligurian specimens of striped dolphin (n = 61), the ranges of PCB and DDT values were 46.8–86.0 ppm and 23.6–63.5 ppm, respectively (Figure 4) (Marsili and Focardi, 1996). Borrell (1993a) and Aguilar and Borrell (1994) found mean levels of 314 ± 150 ppm (l.b.) of PCBs and 156 ± 117 ppm (l.b.) of DDTs in striped dolphins in the Mediterranean off Catalonia. Values similar to ours were found in the fat of striped dolphins from Japan by Tanabe *et al*. (1983) and from the western Pacific by Loganathan *et al*. (1990). Lower values were found by De Kock *et al*. (1994) in dolphins from the southern Atlantic.

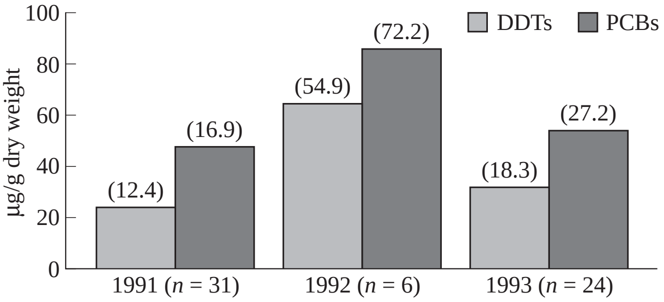

Figure 4 Total DDT and PCB concentrations (arithmetic mean ± S.D. in brackets) in striped dolphins from the Ligurian Sea.

When organochlorine levels in striped dolphins of the Ligurian Sea were compared over the three years of the study (Figure 4), a significant increase in DDTs and PCBs was observed between 1991 and 1992 followed by a significant decrease in 1993. It is not correct to attribute the 1992 increase to the ecological disasters that occurred in the Ligurian Sea in April 1991 (the sinking of the tanker *Haven* and the burning of an Agip Petroli tanker after collision with the ferry *Moby Prince*), as the substances spilt in the sea were petroleum derivatives such as PAHs. It is more likely that the specimens sampled differed in age and sex. In fact, there were very few calves, particularly in the populations sighted in 1991, whereas in 1992, about 40% of the adults were accompanied by one or even two calves (Zanardelli and Panigada, personal communication). The adults may have approached the boat to protect their young, so that only reproductive males and females were biopsied. From birth to sexual maturity, males and females accumulate organochlorines at the same rate, but later there is a drop in levels in females during pregnancy and lactation. The organochlorine concentrations measured in striped dolphin sampled in 1993 in the Ligurian, Tyrrhenian and Ionian Seas are reported in Figure 5. PCBs and DDTs were highest in the Ligurian Sea and lowest in the Ionian. The differences between the three seas were significant ($p < 0.001$). The striped dolphin is a gregarious species that lives in groups of about 20 individuals (range 5–35 individuals) (Forcada *et al.*, 1992). The groups have territorial habits. They cover many kilometres per day in a well-defined area, in which they carry on all their social, feeding and reproductive activities. They therefore provide a good indication of the health of the environment of the area in which they live. On this basis, we can say that the Ligurian Sea had the highest and the Ionian the lowest level of organochlorine pollution, as expected. The coasts of the Ligurian Sea have a higher density of industry, intensive agriculture and towns and the Rhone estuary is very near (Mendola *et al*., 1977; Renzoni *et al.*, 1986).

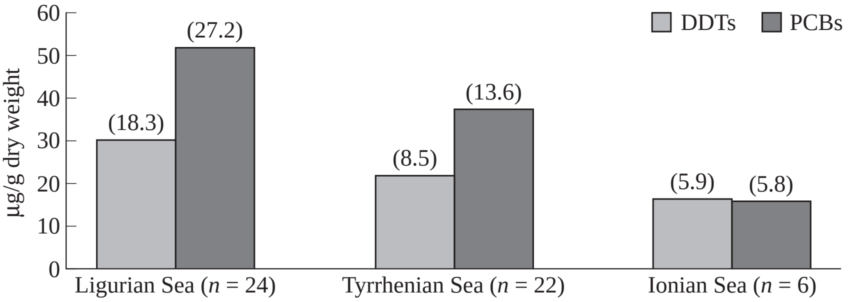

Figure 5 Average concentrations of DDTs and PCBs in striped dolphins from different seas in the summer of 1993.

The congeners found in the highest percentages in striped dolphin were 153, 138, 187, 180 and 170. All the congeners detected had 235 and/or 245 chlorine substitutions in at least one biphenyl ring. Of these, those with fewer chlorines, with the exception of 99, are quickly metabolized by the marine mammals, and the same is true for most of those with adjacent substituted carbons in *ortho-meta* position. In fact, congener 153, the most abundant in this species, has 245 chlorine substitution on both rings and no adjacent unsubstituted carbon atoms in *ortho-meta* position, as mentioned above.

Comparison of organochlorine levels in stranded and free-ranging specimens

Figure 6 shows the mean values of total DDTs and PCBs in stranded and free-ranging specimens of striped dolphin. In the blubber of stranded specimens (n = 64) the arithmetic mean of DDTs and PCBs was 100 561 ng/g d.w. (S.D. = 125 508) and 151 878 ng/g d.w. (S.D. = 200 870) respectively. In the subcutaneous blubber of free-ranging Ligurian specimens (n = 61), the arithmetic mean of DDTs and PCBs was 39 121 ng/g d.w. (S.D. = 17 445) and 61 631 ng/g d.w. (S.D. = 17 344) respectively. The Kolmogorov–Smirnov test showed significant differences in the cumulative distributions of DDTs and PCBs between stranded and free-ranging specimens ($p < 0.001$). These results confirm those of Borrell (1993a), who found significant differences in the concentrations of organochlorines in Catalonian specimens sampled by biopsy dart and specimens found dead during the epizootic. The significant difference in concentrations found between presumably healthy specimens and those stranded for various reasons, few of which are known, poses the question of the role of chlorinated xenobiotics in the decease of these animals. In functional terms, administration of PCBs to mammals inhibits antibody production (Dean *et al.*, 1989). In more concrete terms, a link between high levels of organochlorines and increased susceptibility to viral infections has been demonstrated in many mammals, including man (Koller and Thigpen, 1973; Imanishi *et al.*, 1981; Wu *et al.*, 1984; Dean *et al.*, 1989).

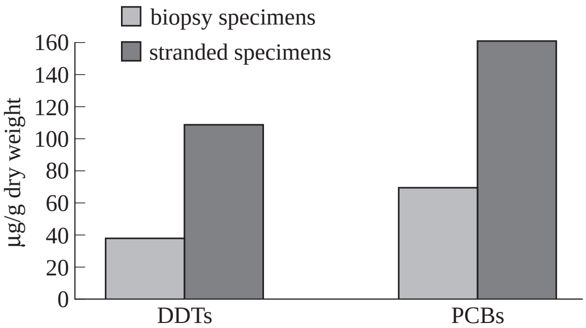

Figure 6 Mean values of DDTs and PCBs in free-ranging and stranded striped dolphins.

3.1.2 Bottlenose dolphin

Like the striped dolphin, the bottlenose dolphin can be regarded as a terminal consumer, and as such occupies a position at the top of the food chain. However, the concentrations of chlorinated hydrocarbons found in the fat of stranded specimens are much lower than those found in striped dolphins. Possible reasons for this are discussed below.

Stranded specimens of bottlenose dolphin

Fourteen specimens of bottlenose dolphin stranded along the Italian coasts between 1987 and 1992 were analysed for OCs

(Marsili and Focardi, 1997). The organs and tissues analysed for HCB, DDTs and PCBs were melon, blubber, liver, muscle, brain, kidney and heart. The contaminant found in the lowest concentrations was HCB. The organ with the highest accumulation was the melon. Geometric means of total DDT concentrations decreased in the following order: melon > blubber > liver > heart > muscle > kidney > brain (in ng/g d.w.: 5064 > 3136 > 2397 > 1619 > 1068 > 281 > 183); for PCBs the pattern was slightly different: melon > liver > blubber > heart > muscle > brain > kidney (in ng/g d.w.: 36910 > 14031 > 10223 >7694 > 5380 > 4082 > 3045). Except for liver, muscle and blubber, the number of specimens per organ was too small for statistical analysis.

Since the organs and tissues of bottlenose dolphin were from specimens stranded in different places along the Italian coasts, we plotted the sum of total concentrations of the three OCs assayed in each organ in relation to sea of origin. In the three organs for which samples were most numerous (liver, muscle and blubber), there was no correlation between sea of origin and xenobiotic levels. For liver, dolphins with the highest concentrations were from the northern Adriatic, whereas for muscle and blubber, the Ionian dolphin had much higher levels than all the others. A pattern more or less common to all organs and seas of origin showed higher levels in males than females.

Concentrations of chlorinated contaminants in bottlenose dolphin blubber measured in other parts of the world are reported in Table 4. Although these levels show wide variations, the number of specimens was extremely small in all these studies. Comparing these levels with the minimum and maximum DDT and PCB concentrations in blubber in our study (for DDTs in ng/g d.w.: 515–46 144 and for PCBs in ng/g d.w.: 200–139 854), we find substantial agreement.

Table 4 OC levels in blubber of *Tursiops truncatus* from different parts of the world.

Area	Number of dolphins	DDTs (ppm)	PCBs (ppm)	References
USA Atlantic (l.b.)	14	15	81	Kuehl *et al.*, 1991 a,b
Australia (f.w.)	6	1.3	0.06	Kemper *et al.*, 1994
Wales (f.w.)	3	78	290	Morris *et al.*, 1989
South Africa (f.w.)	6	4	2.5	De Kock *et al.*, 1994
Mediterranean Spain (f.w.)	1	24	234	Corsolini *et al.*, 1995a
Mediterranean Sea (l.b.)	8	-	1330	Corsolini *et al.*, 1995b
Mediterranean France (d.w.)	1	-	321	Alzieu and Duguy, 1979
Italy (d.w.)	8	4	10	Marsili and Focardi, 1996

Comparison of CH concentrations in stranded striped and bottlenose dolphins

The most numerous strandings along the Italian coasts were recorded for the striped dolphin, which is also the most abundant cetacean in our seas. Of the 934 specimens of cetaceans found and inspected from 15 May 1986 to 31 December 1990, 360 were striped dolphins (Cagnolaro and Notarbartolo di Sciara, 1992). There were also many cases of stranding of bottlenose dolphins: 125 specimens found dead on the Italian coasts in the same period. This dolphin is also frequent in the

Mediterranean, especially in shallower waters such as the Adriatic. One of the main differences between the two species is the part of the sea in which they live. The striped dolphin is pelagic whereas the bottlenose dolphin is coastal, though a pelagic form does exist. There is no selective competition between the two, as there might be between the striped dolphin and the common dolphin, and no other type of interaction.

Taking as reference the three organs of which the greatest number of samples were analysed (blubber, liver and muscle), we compared the concentrations of chlorinated xenobiotics in the two species. Using the Kolmogorov–Smirnov statistical test for non-parametric distributions, we found that the distributions of total DDTs and PCBs in blubber were significantly different in the two species ($p < 0.001$ and 0.01, respectively). In liver and muscle, however, we did not find significant differences between the two populations. The fact that in the blubber of striped dolphin, the mean quantities of these OCs are 10 times higher than in the bottlenose dolphin, is difficult to explain, if not on the basis of different dietary input or different lipid constitution of the tissues. The first hypothesis is invalid because the two species have a similar diet, consisting of small fish of the herring family and ink-fish. The only difference is that the bottlenose dolphin, being coastal, mainly eats fish and the striped dolphin, mainly cephalopods. As far as lipid content is concerned, the bottlenose dolphin is also favoured by a higher EOM%, so that only the different lipid composition of this tissue is presumably the basis of the differences observed. We were unable to find any literature relevant to the question. In their ‘Guide per il riconoscimento delle specie animali delle acque lagunari e costiere italiane. Cetacei’, Cagnolaro *et al.* (1983) state that because the bottlenose dolphin often lives in close contact with humans, it has adapted to various forms of pollution, including noise, and this may explain the success of this species in captivity. If it is really true that this species stores few contaminants by virtue of its capacity to adapt, it would be interesting to study its biological responses to chemical stress.

3.1.3 Fin whale

The fin whale is the only mysticete found in the Mediterranean on a regular basis. It has been hypothesized that an endemic population, genetically different from oceanic populations, exists in our seas (Bérubé *et al.*, 1994).

Skin biopsies from fin whales

From 1990 to 1993, 68 fin whale blubber biopsies were analysed for PCBs and DDT compounds. In the Ligurian Sea, the concentration ranges of PCBs and DDTs in fin whale were 5.5–7.1 ppm and 4.2–9.5 ppm, respectively. These values are both higher than those obtained in fin whales from the northeastern Atlantic (Aguilar and Borrell, 1988), the southeastern Pacific (Pantoja *et al.*, 1984) and the southern Atlantic (Henry and Best, 1983). However, they are much lower than those found in two fin whales stranded on the French Mediterranean coast and the Atlantic coast of Brittany (Alzieu and Duguy, 1979).

Comparison of organochlorine concentrations in fin whales in the four years of sampling (Figure 7) showed that DDTs decreased significantly between 1990 and 1991 ($p < 0.001$) after which they remained constant. PCBs reached a maximum in 1991, though it was not significantly different from levels measured in the other years. The drop in DDT levels after 1990 may reflect decreased use of these compounds in Africa, where DDT has not yet been banned; it seems that fin whales pass winter and reproduce off the African coasts. The PCB congeners found in higher percentages in fin whale were 153, 138, 187, 180 and 170. Congener 153 was the most abundant in this species.

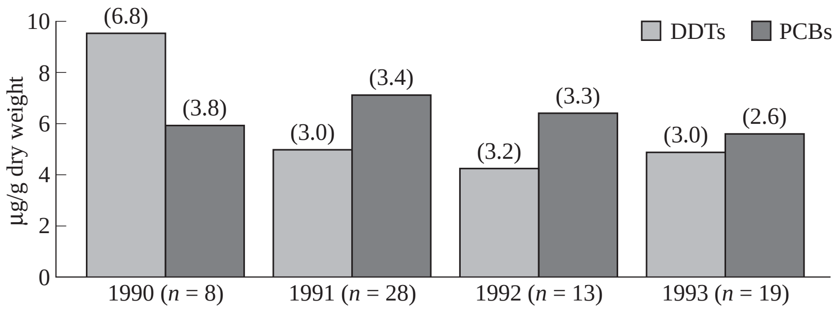

Figure 7 Total DDT and PCB concentrations (arithmetic mean ± S.D. in brackets) in fin whales.

The fin whales biopsied were identified individually, in accordance with the North Atlantic Fin Whale Catalogue (Agler *et al.*, 1990). The length of all whales biopsied was estimated and five size classes were defined:

A 4–8 m: small (calf)

B 8–11 m: small (young)

C 11–15 m: young

D 15–20 m: adult

E over 20 m: adult (old)

The classes are the same for males and females, as sexual dimorphism is minimal in fin whales: females are about 60 cm longer than males at sexual maturity, which is reached between the age of 6 and 12 years. At this stage, mean length is 17.7 m for males and 18.3 m for females (Cagnolaro *et al.*, 1983). When PCB and DDT concentrations were plotted in size classes, a wide dispersion of data was evident. Individuals of about the same length belonging to the same size class, and hence presumably of a similar age, had totally different organochlorine concentrations. This can only be explained on the basis of differences in sex, which unfortunately was only determined in some whales, according to the method of Palsbøll *et al.* (1992). Table 5 shows total organochlorines in the biopsy specimens of fin whale in relation to size class and sex. In class D, the mean sum of PCBs and DDTs was significantly different between males and females ($p<0.001$). It should be borne in mind that males and females reach sexual maturity in this class. For females this means possible pregnancies, and the loss of most of the body burden of fat-soluble contaminants through gestation and lactation. In this class, the presence of females with very high organochlorine levels, similar to those in males in the same age range, suggests sterility or at least a lack of pregnancies.

Table 5 Organochlorine residues (∑(DDT + PCB) µg/g d.w.) in subcutaneous blubber of male and female *Balaenoptera physlus* specimens, divided according to body length.

	Male *B. physalus*			Female *B. physalus*		
Class	*n*	Range PCBs + DDTs (µg/g d.w.)	Average (S.D.)	*n*	Range PCBs + DDTs (µg/g d.w.)	Average (S.D.)
A (4–8 m)	0		0			
B (8–11 m)	2	13.22–15.37	14.29 (1.53)	1	1.69	
C (11–15 m)	8	5.88–31.50	13.58 (8.38)	3	11.97–12.15	12.04 (0.09)
D (15–20 m)	10	7.29–32.76	15.79 (8.36)	22	1.96–18.74	10.46 (5.11)
E (>20 m)	0			0		

Comparison of OC levels in blubber biopsies of fin whale and striped dolphin

Organochlorine levels in the striped dolphin, a terminal consumer, were significantly higher ($p < 0.001$) than those found in the fin whale, which is euriphagic, and more specifically macroplanktophagic (Figure 8). To be precise, in the Mediterranean, fin whales feed almost exclusively on krill of the species *Meganyctiphanes norvegica* (Orsi Relini and Giordano, 1992; Zanardelli *et al.*, 1992). Our results showed PCB and DDT levels 10 and 7 times higher, respectively, in the striped dolphin than in the fin whale. The patterns of relative concentrations of PCB congeners, calculated by averaging in both species the percentage of each congener in every animal sampled, were very similar with respect to total PCBs. The congeners found in higher percentages in the fin whale and striped dolphin were 153, 138, 187, 180 and 170. Penta-, hexa-, hepta- and octachlorobiphenyls accounted for 13%, 44%, 34% and 8%, respectively, in the fin whale and 10%, 43%, 38% and 9% in the striped dolphin.

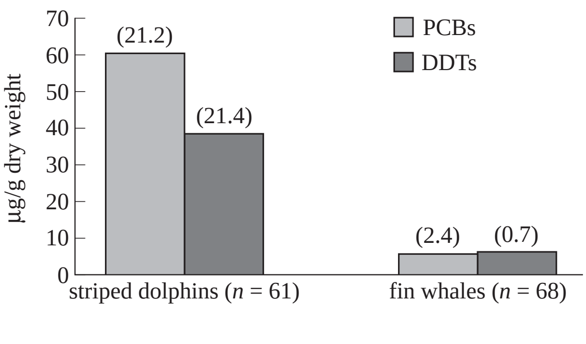

Figure 8 OC levels in subcutaneous blubber biopsy specimens of Ligurian striped dolphins and fin whales (arithmetic mean ± S.D. in brackets).

3.2 South American pinnipeds

An urgent collaborative study between the Department of Environmental Biology of Siena University and the Museum of Natural Sciences of Buenos Aires was begun on the colony of southern sea lions at Mar del Plata (Argentina), a harbour chronically contaminated with petroleum. This provided the opportunity to study a control population of this mammal in another, theoretically pristine environment, in Patagonia. However, this human paradise did not turn out to be as pristine as expected.

3.2.1 Stranded pinnipeds

Table 6 shows the levels of organochlorine compounds and PAHs found in the livers of stranded *Arctocephalus* spp. Table 7 gives the values of OC residues in organs, tissues and faeces of the one stranded specimen of sea lion from the colony of Mar del Plata. A negative correlation was found between OCs and PAHs in the livers of stranded pinnipeds ($r = -0.85$, $n = 5$). In the sea lion stranded in Mar del Plata (Table 7) the liver was the organ with the highest levels of organochlorine compounds.

Few data are available on PCBs, DDTs, HCB and PAHs in these three species. In 11 blubber samples of Rio Grande do Sul (Brazil) sea lions, the mean *pp′*DDE value was 3.26 µg/g f.w. (2.64 S.D.), and PCBs were 8.42 µg/g f.w. (3.67 S.D.) (Junin *et al.*, 1994). In five blubber samples of Mar del Plata (Argentina) sea lions, the mean pp′DDE value was 2.00 µg/g f.w. (1.16 S.D.) and PCBs were 4.90 µg/g f.w. (4.05 S.D.) (EC CI*CT94-0018, 1997). In the fur of Mar del Plata specimens, the mean values of *pp′*DDE and *pp′*DDT were 1.72 µg/g f.w. and 0.23 µg/g f.w., respectively, and PCBs were 4.28 µg/g f.w. (Junin *et al.*, 1994). In the blubber of six specimens of the South American fur seal from Rio Grande do Sul, the mean values of *pp'*DDE and PCBs were 2.06 (3.32 S.D.) and 2.56 (3.02 S.D.) µg/g f.w., respectively (EC CI*CT94-0018, 1997). In a blubber sample of the subantarctic fur seal from Parana (Brazil), the values were 0.24 µg/g f.w. for *pp′*DDE, 0.27 µg/g f.w. for *pp′*DDT and 1.09 µg/g f.w. for PCBs (Junin *et al.*, 1994). In a blubber specimen of the South American fur seal from Mar del Plata, the values were 0.60 µg/g f.w. for *pp′*DDE and 1.80 µg/g f.w. for PCBs (EC CI*CT94-0018, 1997).

Table 6 Levels of OCs and PAHs in liver of five *Artocephalus* spp. stranded on the beaches of Argentina and Brazil.

Liver samples	PAHs (ng/g f.w.)	HCB (ng/g/d.w.)	DDTs (ng/g d.w.)	PCBs (ng/g d.w.)
South American fur seal Rio Grande (Brazil)	177.0	11.7	5713	6457.3
South American fur seal Rio Grande (Brazil)	683.0	8.76	248.7	1124.9
South American fur seal Rio Grande (Brazil)	863.0	19.5	110.6	378.10

South American fur seal Parana (Brazil)	712.0	1.43	236.7	285.97
Subantarctic fur seal S. Clemente (Argentina)	1084	21.1	473.1	1296.5
mean (S.D.)	703.8 (334.6)	12.5 (8.06)	1356 (2439)	1908.6 (2581.4)

Table 7 Levels of OCs in organs, tissues, faeces and fur of a sea lion (*Otaria flavescens*) stranded on Mar del Plata.

Sample	HCB (ng/g/d.w.)	DDTs (ng/g d.w.)	PCBs (ng/g d.w.)
Faeces	213.5	82.05	958.79
Kidney	57.73	1698	8437.0
Liver	119.7	3323	13326
Muscle	171.0	460.9	3309.7

3.2.2 Free-ranging pinnipeds

In February 1995, the levels of HCB, PCBs and DDTs were calculated in six faeces samples from free-ranging sea lions of the colony of Mar del Plata. The mean values were 138.1 ng/g d.w. (107.5 S.D.) for HCB, 122.6 ng/g d.w. (72.6 S.D.) for DDTs and 678.6 ng/g d.w. (224.8 S.D.) for PCBs. In the period from June to September 1995, OCs were analysed in faeces, blood and skin biopsy specimens of sea lions from two colonies: those at Mar del Plata and Punta Bermeja (Table 8). The small number of samples from the Patagonia colony prevented any statistical comparison between the two colonies. However, it was evident that there were no marked differences in the accumulation of chlorinated xenobiotics. There was an interesting significant difference ($p < 0.05$) in HCB, DDT and PCB accumulation in faeces samples of the Mar del Plata colony collected in February 1995 and from June to September of the same year. This may be due to the different composition of the colony in these two periods. In February, the colony consisted only of very old males who could no longer travel the hundreds of kilometres to the reproductive colonies; in the second period, males of all ages were wintering in the colony. The faeces were collected at random and could not be attributed to particular individuals. However, in the first period they certainly belonged to older sea lions and in the second period, they may also have been from younger males. Males of all mammal species have no way of ridding themselves of organochlorines as they grow older. They accumulate them actively over the period of their whole life, or at least for as long as they can feed. Moreover, older sea lions may not always leave the port to feed, so their dietary input of contaminants would be higher than that of young males feeding in the open sea. These considerations seem to explain the differences in organochlorine contaminants measured in faeces collected in the two different periods.

This preliminary study on organochlorine levels in free-ranging animals and in organs and tissues of stranded South

American pinnipeds showed significantly lower levels than in other marine mammals of the northern hemisphere and Mediterranean (Borrell *et al.*, 1996; Lee *et al*., 1996). Mar del Plata, especially the port area where the colony lives, is subject mainly to oil pollution and therefore PAHs. These compounds are probably responsible for the 'diseases' of the Mar del Plata sea lions. This is why we concentrated on PAHs in subsequent sampling programmes.

Figures 9–16 show total and carcinogenic PAHs in the various biological materials in each sampling period. Total PAH concentrations (Figure 9) in skin were higher in Mar del Plata than Punta Bermeja samples in November 1995 and May 1996. In the last sampling programme (September 1996) levels in Mar del Plata were unchanged but we found unexpectedly high levels in the Punta Bermeja material. A similar trend was found for carcinogenic PAHs in skin (Figure 10). A partial explanation for the unexpectedly high results obtained in Punta Bermeja blood samples in May 1996 and in skin samples in September 1996 may be an oil spill in the Colorado river, north of Punta Bermeja, in March 1996. The spill was due to a leak in an oil pipeline (Costa, personal communication; Rio Negro, 1996). The higher levels of PAHs in skin biopsies obtained in September 1996 may reflect bioaccumulation of these lipophilic compounds after the oil spill. The higher values found in Punta Bermeja skin samples in September 1996 may be due to the different method of sampling: most samples were obtained from the breast region using a crossbow. The breast has more subcutaneous fat than the flipper. Between November 1995 and May 1996 there was a statistically significant difference for both sampling areas. This may be due to (a) different moments of the reproductive cycle, pre-reproduction (November) and post-reproduction (May) or (b) high UV radiation in November, which enhances photodegradation PAHs and vice versa.

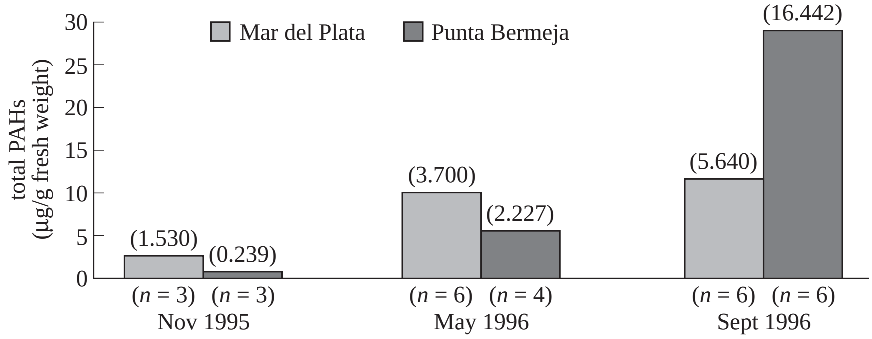

Figure 9 Total PAHs (µg/g f.w) in skin biopsies of sea lions from Mar del Plata and Punta Bermeja colonies.

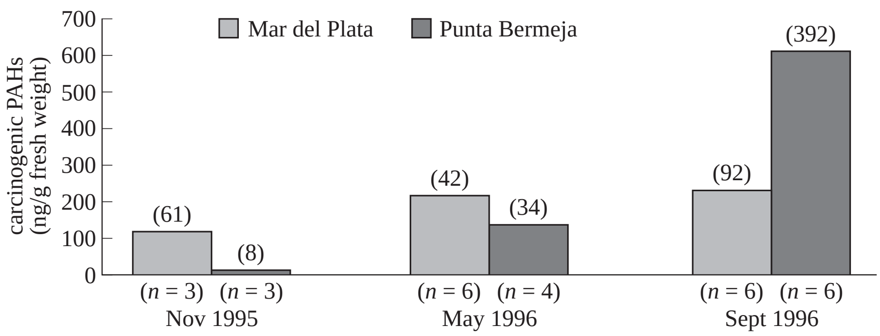

Figure 10 Carcinogenic PAHs (ng/g f.w) in skin biopsies of sea lions from Mar del Plata and Punta Bermeja colonies.

Total PAH concentrations in blood samples (Figure 11) were higher in Punta Bermeja sea lions in November 1995 and May 1996. In May, both colonies showed values four times higher than in November. This phenomenon may be explained by the same two reasons given above for skin samples. In the last sampling in September 1996, it was possible to obtain blood samples only at Mar del Plata, and the values detected were similar to those obtained in May 1996. A similar trend was found for carcinogenic PAHs in blood (Figure 12). In blood samples from the Mar del Plata colony, carcinogenic PAHs were five times higher in May 1996 than in November 1995 and similar to the values obtained in samples from Punta Bermeja in the same period.

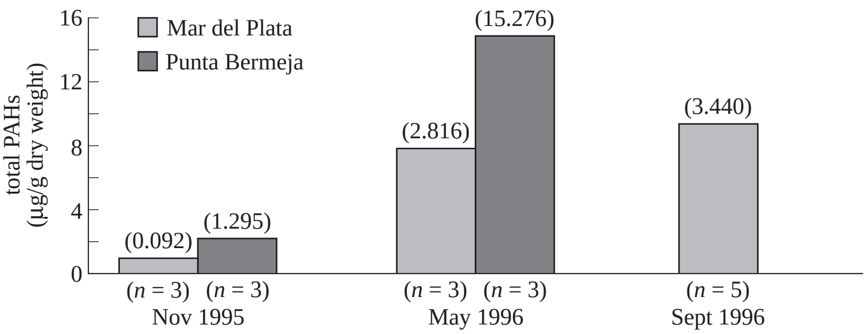

Figure 11 Total PAHs (µg/g f.w) in blood of sea lions from Mar del Punta and Bermeja colonies.

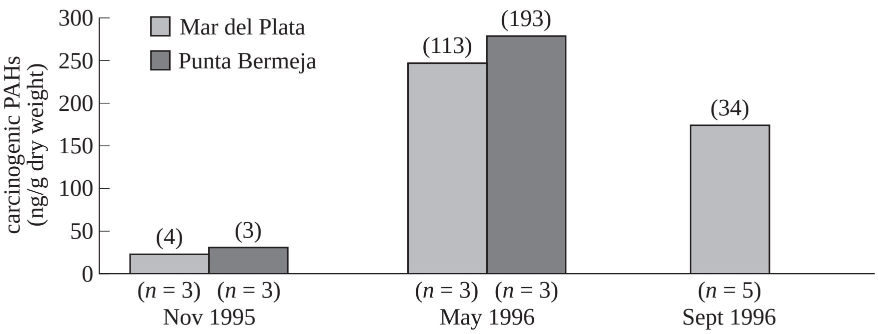

Figure 12 Carcinogenic PAHs (ng/g f.w) in blood of sea lions from Mar del Plata and Punta Bermeja colonies.

Total PAH concentrations in fur (Figure 13) revealed the same trend as in skin and blood samples, with high levels in May in both colonies. The carcinogenic PAHs were found in much higher concentrations in fur samples obtained from Punta

Bermeja sea lions in May 1996 than in fur of the sea lion sampled in November 1995 (Figure 14). Of the 14 PAHs studied, the carcinogenic ones had high molecular masses. Since high molecular mass PAHs indicate recent pollution (Drei, 1997), the unexpectedly high levels of PAHs found in all biological material obtained from Patagonian sea lions in May 1996 confirm the relationship with the oil spill.

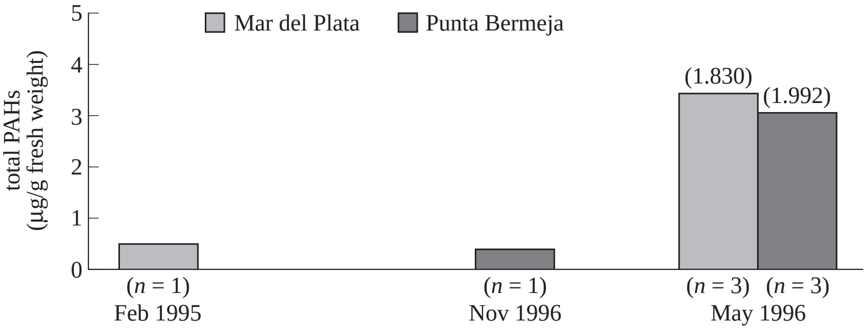

Figure 13 Total PAHs (µg/g f.w) in fur of sea lions from Mar del Plata and Punta Bermeja colonies.

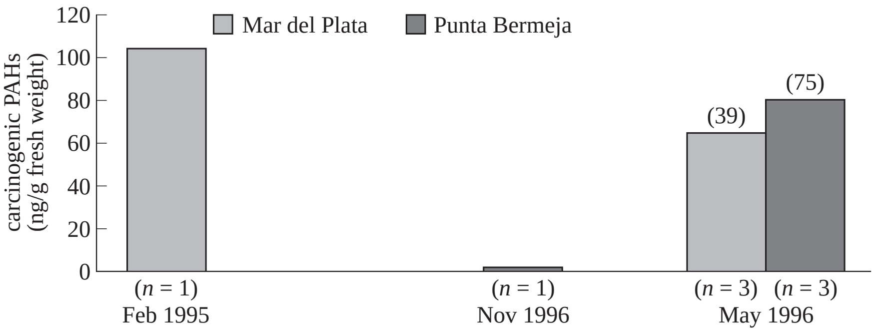

Figure 14 Carcinogenic PAHs (ng/g f.w) in fur of sea lions from Mar del Plata and Punta Bermeja colonies.

Total and carcinogenic PAHs in faeces (Figures 15 and 16) were lower in February 1996 in both colonies than in November 1995 in Mar del Plata and in September 1996 in Punta Bermeja. Values in September 1996 in Punta Bermeja were surprisingly high.

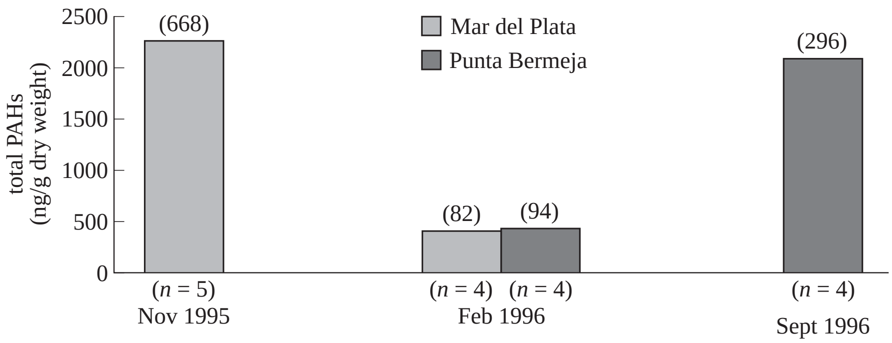

Figure 15 Total PAHs (ng/g f.w) in faeces of sea lions from Mar del Plata and Punta Bermeja colonies.

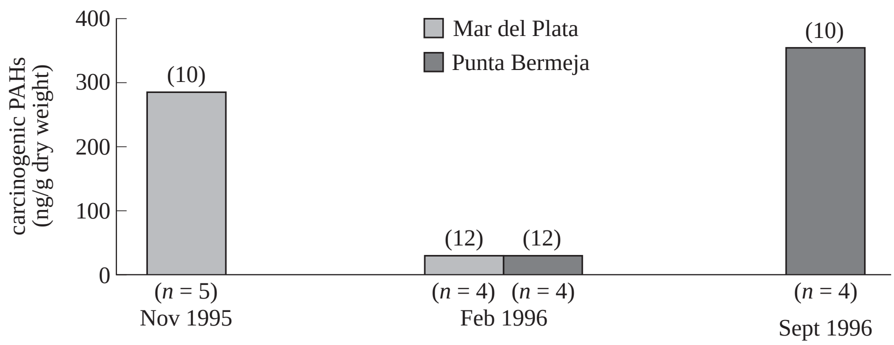

Figure 16 Carcinogenic PAHs (ng/g f.w) in faeces of sea lions from Mar del Plata and Punta Bermeja.

With some of the samples obtained in 1995 we calculated an index of the metabolic capacity of sea lions equal to the ratio of PAH intake by contact (levels in skin biopsies) and PAHs excreted in the faeces (Marsili *et al.*, 1997a). PAHs are mainly excreted in the faeces, the amount excreted in the urine being about one-fourth to one-fifth that excreted in the faeces (Becher and Bjørseth, 1983). The higher this ratio, the lower the metabolic capacity of the animal. Mar del Plata sea lions had 2785 ng/g d.w (n = 3; S.D. = 1676). Of these compounds in skin specimens and 408 ng/g d.w. (n = 4; S.D. = 82) in faeces. Punta Bermeja sea lions had 578 ng/g d.w (n = 3; S.D. = 263) in skin and 424 ng/g d.w. (n = 4; S.D. = 94) in faeces. For the sea lions of Punta Bermeja we obtained a ratio of 1.36, versus 5.34 for the Mar del Plata colony (Figure 17a).

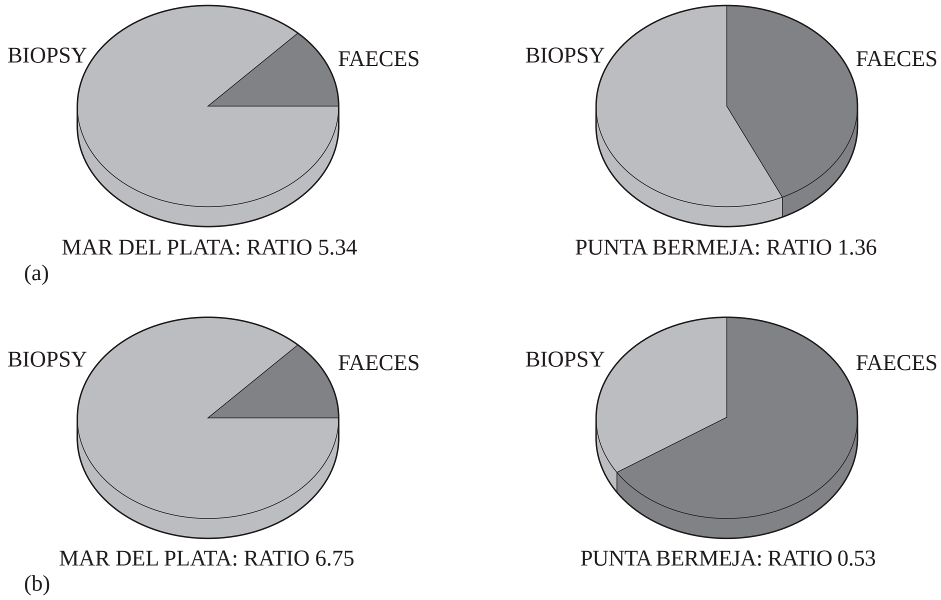

Figure 17 (a) The ratio of total PAHs in biopsies and faeces, and (b) the ratio of the sum of five carcinogenic PAHs in biopsies and faeces in sea lions from Mar del Plata and Punta Bermeja .

A ratio of one means that all PAHs absorbed are excreted; the ratio of the harbour sea lions means that only about a fifth of the PAHs absorbed are excreted. If we consider only the carcinogenic PAHs, the result is surprising. Mar del Plata sea lions had 145.89 ng/g d.w. of these compounds in skin specimens and 21.60 ng/g d.w. in faeces. The ratio is therefore 6.75 (Figure 17b), which means that less than one sixth is excreted. In control sea lions, the same quantities were 11.26 ng/g d.w. in skin and 21.44 ng/g d.w. in faeces. Excretion was about double the quantity absorbed through the skin (ratio 0.53, Figure 17b), suggesting that PAHs are also absorbed by other routes, such as respiration and ingestion with food and water.

4 Conclusions

Several conclusions can be drawn from these studies.

The results from free-ranging cetaceans emphasize substantial differences in organochlorine accumulation in the two species of cetaceans studied here. This finding is interesting in view of the fact that *Balaenoptera physalus* and *Stenella coeruleoalba* represent two large suborders of cetaceans, namely mysticetes and odontocetes. Of the many differences between them, diet is particularly significant. The sieve mouth parts of the former indicates filtration of water and thus a diet of plankton. The name odontocete means cetacean with teeth, implying a more varied diet, which would be higher in chlorinated hydrocarbons.

The high organochlorine levels found in subcutaneous biopsy specimens from striped dolphins and the low detoxifying capacity of these dolphins with respect to land mammals (Watanabe *et al.,* 1989; Fossi *et al.*, 1992; Marsili *et al.*, 1996) indicate the high toxicological risk to which these cetaceans are exposed. The concentrations of these contaminants are not high enough to be regarded as the cause of the mass deaths of cetaceans occurring in the Mediterranean in the last few years, but they are an added stress that increases vulnerability to various pathogens (Domingo *et al.,* 1990; Domingo *et al.,* 1991; Fernandez *et al.,* 1991; Podesta' *et al.,* 1992; Aguilar and Borrell, 1994).

The levels of organochlorines measured in stranded and free-ranging sea lions were significantly lower than in other species of marine mammals of the northern hemisphere and Mediterranean. It emerges that the pollution status of the central south-western Atlantic coast is generally better than that of the northern hemisphere for this class of contaminants. PAH levels were of the order of ppm in all samples. Comparison with PAH levels in pinnipeds from the northern hemisphere is impossible owing a lack of data in the literature.

The high levels of PAHs in the biological samples of sea lions at Mar del Plata, especially the high percentage of carcinogenic PAHs, are related to the poor health of the animals in this colony. As this study was only a preliminary one, it will be interesting to see whether the results are confirmed by more detailed research involving a larger number of samples and the study of PAH fingerprints in seawater and soil. This result is a warning for the Argentine Regional Authorities that stricter control of pollution in Mar del Plata harbour is necessary.

The high levels of PAHs found in several biological materials of sea lions from Punta Bermeja in May and September 1996 are a warning signal for the pristine environment of Patagonia. The dramatic increase in PAH contamination may be related to the oil spill of March 1996 in Rio Colorado.

The present development and validation of a non-destructive approach (biopsy sampling) for free-ranging marine mammals makes it scientifically and ethically unjustifiable to continue to use marine mammals obtained by hunting for scientific purposes (Fossi *et al.*, 1997b; Fossi and Marsili, 1997). The use of hunted animals amounts to tacit consent to hunting.

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