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AGE AND SIZE TRENDS AND MALE-FEMALE DIFFERENCES OF PCBs AND DDE IN *DALLI*-TYPE DALL'S PORPOISES, *PHOCOENOIDES DALLI* OF NORTHWESTERN NORTH PACIFIC

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Abstract: Adult and immature male and female, one newborn pup and a fetus of *Dalli*-type Dall's porpoises (*Phocoenöides dalli*) collected from northwestern North Pacific were analyzed for the PCB and DDE levels in their blubber. A wide difference existed in the levels of these chemicals between males and females. Concentrations of both the chemicals increased consistently with age in the bodies of males. On the other hand, there was a decrease with age in the levels of these compounds in females after about two years of age, possibly due to a lactational and parturitional transfer of these compounds. When comparing the concentrations of these compounds in the adult males and females of various marine mammals, the females had always less than 50% of the levels to those found in the respective males. These percentages were found to linearly decrease with increase in lactational period of the species indicating a prominent excretion *via* milk to calf rather than *via* placenta to fetus. By using the organochlorine data it seems to be possible that PCBs and DDE can be used as chemical tracers in determining some parturitional and lactational characteristics in marine mammals.

1. Introduction

It has been found that in the distribution of chlorinated hydrocarbon residues, some kind of pattern can be seen in small cetaceans with respect to sex, age and location (TANABE *et al.*, 1981a; GASKIN, 1982; WAGEMANN and MUIR, 1984; TANABE *et al.*, 1986; SUBRAMANIAN *et al.*, 1986). PERRIN and REILLY (1984), when reviewing the methods used to estimate reproductive parameters, age and size relationship, etc. in dolphins and small whales, stated that for some species the literature contains widely varying estimates of parameters, a matter of concern when scientists are asked to provide advice for management. TANABE *et al.* (1988) suggested that marine mammals may have specific physiology for the metabolism of persistent organochlorines and so bioaccumulation patterns and isomer compositions of these chemicals in their bodies may be useful to gain insights into specific metabolism and probably related physiology in wild animals.

In a previous study (SUBRAMANIAN *et al.*, 1986) we noticed considerably lower levels of both PCBs and DDE in the females of Dall's porpoises collected from different locations in North Pacific and surrounding seas. In that study we were able to make some suggestions on the geographical distribution and migration patterns of Dall's

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porpoises by using the levels of organochlorines in the bodies of males as tracers. At the same time we could not use the data obtained from females because of the consistently decreasing levels in their bodies with age and size. In some other studies in our laboratory we could observe regular patterns of decrease in females with age, size, maturity status, etc. (TANABE, 1986; TANABE *et al.*, 1986). All these facts prompted us to believe that the differences in the age trends of organochlorines in the males and females of marine mammals can be used to explain some aspects of the physiology of reproduction in marine mammals.

With all these in mind in this study the data obtained on the levels of PCBs and DDE in the blubber of the *Dalli*-type Dall's porpoises collected from northwestern North Pacific are presented. Age and size trends of both the compounds and male-female differences are discussed in detail.

2. Materials and Methods

The specimens analyzed in the present study were collected during the research cruises of R/V No. 12, R/V No. 54 and R/V No. 81 HOYO MARU in the years 1980, 1984 and 1985 from the population of Dall's porpoises living in northwestern North Pacific consisting exclusively of *Dalli*-type (Fig. 1).

All the samples were transported to the laboratory in a frozen condition and were kept at −20°C until analysis. Since organochlorines were always reported to be accumulated to very high levels up to more than 90% of the total body burdens in the

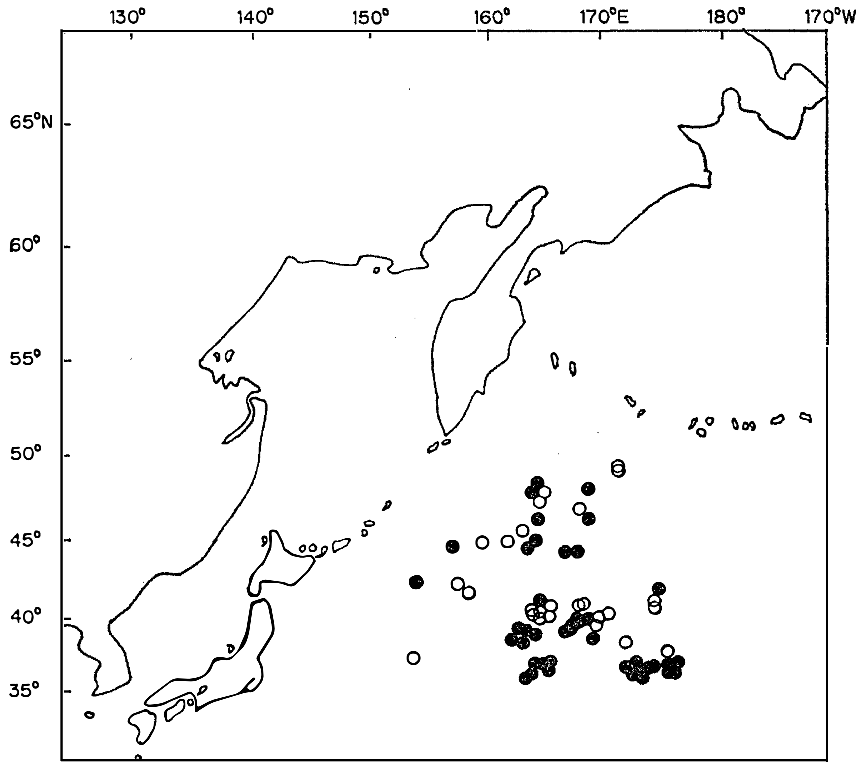

Fig. 1. Map showing the sampling locations of male (●) and female (○) Dalli-*type Dall's porpoises.*

blubber samples of marine mammals (TANABE *et al.*, 1981b; HIDAKA *et al.*, 1983), only the subcutaneous blubber samples of all the specimens were used for the determination of PCBs and DDT compounds.

Analyses of PCBs and DDT compounds were carried out following the alkaline alcohol digestion method of WAKIMOTO *et al.* (1971). Required amounts of the samples were digested in 1 N KOH-C_2H_5OH solution for one hour. The PCBs and *p,p'*-DDE (*p,p'*-DDT is transformed to *p,p'*-DDE during alkaline alcohol digestion) thus brought into ethanol were transferred to hexane by shaking in a separatory funnel containing hexane-washed water and 100 m*l* hexane. After partitioning, the hexane layer was collected and concentrated to 5 m*l* in a Kuderna-Danish (KD) concentrator. The concentrated extract was cleaned up by passing through 1.5 g of silica gel (Wako-gel S-1, Wako Pure Chemical Industries Ltd., Japan) packed in a glass column (10 mm i.d.$\times$20 cm length). PCBs and DDE were eluted with 200 m*l* hexane at an elution rate of one drop per second. The eluate was again concentrated to 5 m*l* in a KD concentrator and further cleaned up with 5% fuming H_2SO_4 in concentrated H_2SO_4.

Aliquots of these samples were injected into Shimadzu GC-9A splitless technique glass capillary gas chromatograph equipped with ^{63}Ni electron capture detector. Operating conditions for PCBs are as follows: Temperature program 180 to 230°C at a rate of 1.0°C/s with an initial 5 min and final 10 min hold. Both injector and detector temperatures were kept at 250°C. For DDE the column temperature was 230°C isothermal. Injector and detector temperatures were the same as for PCBs. Nitrogen was used as both carrier and make-up gas. The column used was glass capillary WCOT-OV 101 for both PCB and DDE analyses.

Concentrations of individually resolved peaks of PCB isomers and congeners were added up to find out the total PCB concentration. *p,p'*-DDT+*p,p'*-DDE were resolved as a single *p,p'*-DDE peak.

3. Results and Discussion

The sampling data, biometry and concentrations of PCBs and *p,p'*-DDE in the blubber samples of all the specimens analyzed are shown in Table 1. When the organochlorine data are plotted against the age (Fig. 2) and body length (Fig. 3) of the respective specimens, some variations in organochlorine levels were observed with respect to these parameters. An initial increase in the levels of both the compounds was observed from about 100 cm body length to approximately about 160 cm. After this, consistent levels were found in males up to 190 cm. In the specimens above this body size there was a sharp increase of both PCB and DDE levels until they attain about 210 cm body length.

In contrast to males, there was a sharp decrease in the concentrations of both the compounds when the females grew larger than an approximate body length of 180 cm (Fig. 3), *i.e.* about two years of age (Fig. 2). The decreasing trend was found to be very sharp until about 5 or 6 years of age and then the levels were more or less the same in the 8 and 12 years old specimens (Fig. 2).

It is interesting to note that the above results consistently agree with earlier findings by biological studies and also can explain some of the doubts raised in those works.

Table 1. Sampling data, biometry and concentrations (µg/g on wet weight basis) of PCB and DDE in Dalli-*type Dall's porpoises collected from northwestern North Pacific.*

No.	Date of sampling*	Latitude N	Longitude E	Body length (cm)	Body weight (kg)	Age (y)	Gonad wt** (g)		Concentration		PCB/DDE ratio
							L	R	PCB	DDE	
Male											
1	840811	45°01′	164°02′	220	185	6	179	181	11	12	0.92
2	840811	44 59	163 12	219	166	6	153	182	12	12	1.00
3	840527	47 37	169 13	212	166	8	175	175	13	14	0.93
4	840512	38 29	151 41	212	156	12	101	110	18	16	1.13
5	840523	37 15	163 46	210	149	6	130	120	15	14	1.07
6	840601	37 58	174 37	205	148	7	255	235	9.7	9.5	1.02
7	840605	38 01	172 26	205	155	4	115	120	8.9	9.2	0.97
8	840605	37 37	173 08	205	167	—	190	180	13	12	1.08
9	840521	39 21	162 46	203	145	6	130	120	10	8.6	1.17
10	840812	44 58	167 54	203	115	4	59	63	8.1	7.1	1.14
11	840913	44 57	158 00	202	159	4	146	156	12	12	1.00
12	840526	44 22	169 09	202	165	7	160	150	16	15	1.07
13	840917	42 02	175 52	200	168	—	127	120	14	15	0.93
14	840522	39 27	162 42	200	144	2	105	100	7.1	6.6	1.06
15	840516	46 49	164 29	200	149	6	105	95	13	15	0.87
16	840523	36 47	162 48	199	123	5	57	56	7.4	12	0.62
17	840521	39 38	162 50	197	123	4	140	165	5.6	7.6	0.74
18	840524	38 58	166 15	196	155	3	120	68	12	17	0.71
19	840517	48 37	164 17	195	142	8	210	190	10	12	0.83
20	840601	37 38	174 59	194	119	5	145	140	7.1	10	0.71
21	840525	40 54	169 00	194	130	5	140	135	6.3	12	0.53
22	840521	39 22	162 49	192	121	8	72	56	7.0	9.2	0.76
23	840524	39 27	166 55	192	136	7	88	88	9.2	11	0.84
24	840601	37 21	175 17	192	126	3	165	140	5.5	9.3	0.59
25	840523	37 20	163 53	190	120	2	67	57	6.5	10	0.65
26	850910	43 39	154 27	189	117	—	97	82	5.0	9.2	0.54
27	840608	41 20	165 50	188	140	5	140	135	8.2	11	0.75
28	840530	37 58	174 38	188	—	3	130	135	6.7	8.2	0.82
29	840524	39 29	167 03	185	111	2	11	11	6.9	7.9	0.87
30	840517	48 37	164 17	185	124	8	178	190	7.0	10	0.70
31	840601	37 58	174 37	185	95	3	22	15	5.6	10	0.56
32	840523	37 15	163 46	185	108	3	34	30	6.0	11	0.55
33	840601	37 38	175 01	181	114	4	75	84	8.7	9.9	0.88
34	840523	37 13	163 36	180	101	6	70	64	8.0	11	0.73
35	840601	37 38	175 01	176	129	5	115	128	5.7	7.9	0.72
36	840602	37 14	175 26	175	109	1	16	15	3.8	6.1	0.62
37	840607	39 10	170 20	174	109	3	25	26	5.7	8.3	0.69
38	840601	37 20	175 18	173	87	1	14	14	4.0	6.2	0.65
39	840521	39 33	162 44	170	101	1	24	24	8.1	10	0.81
40	840524	39 28	167 01	169	96	1	11	10	7.8	12	0.65
41	840605	40 47	167 09	169	100	2	11	12	6.2	10	0.62
42	840608	40 47	167 09	165	86	1	12	12	5.0	7.0	0.71
43	800621	48 35	169 03	103	17	0	Newborn		1.6	3.0	0.53
Female											
44	840608	41°20′	165°51′	195	146	8	M	(1, 2)	3.3	3.9	0.85
45	840518	46 47	162 59	194	126	5	M	(1, 3)	1.0	1.3	0.77
46	840605	38 01	172 25	193	117	3	M	(0, 6)	2.4	2.5	0.96

Table 1 (*continued*).

No.	Date of sampling*	Latitude N	Longitude E	Body length (cm)	Body weight (kg)	Age (y)	Gonad wt** (g) L	Gonad wt** (g) R	Concentration PCB	Concentration DDE	PCB/DDE ratio
47	840517	48°46′	164°11′	190	124	5	M	—	2.0	3.8	0.53
48	840914	44 06	158 01	189	122	4	M	—	3.2	5.0	0.64
49	800619	50 12	172 45	188	150	12	M	—	1.0	1.8	0.56
50	840609	41 47	164 57	185	136	4	M	(1, 2)	4.0	4.6	0.87
51	840608	41 20	165 51	184	108	3	M	(1, 0)	2.7	4.0	0.68
52	840518	45 28	162 59	181	106	6	I	(0, 0)	5.5	6.8	0.81
53	840609	41 28	165 35	180	125	4	M	(1, 1)	3.7	5.3	0.70
54	800621	48 35	169 03	179	118	12	M	—	2.0	4.2	0.48
55	840521	40 07	162 54	179	111	5	M	(1, 0)	3.2	4.4	0.73
56	840530	41 49	174 34	178	100	2	I	(0, 0)	5.7	9.3	0.61
57	840517	48 56	163 03	175	99	2	I	(0, 0)	4.3	5.4	0.80
58	840530	42 54	174 32	174	103	3	I	(0, 0)	2.7	4.7	0.57
59	840914	43 55	157 58	172	85	4	M	—	3.8	4.7	0.81
60	840512	38 32	150 18	172	68	3	I	(0, 0)	2.7	4.7	0.57
61	840525	40 45	169 07	172	90	1	I	(0, 0)	4.7	7.6	0.62
62	840607	39 10	170 20	170	96	2	I	(0, 0)	4.9	6.7	0.73
63	840606	39 02	170 34	167	83	2	I	(0, 0)	4.9	5.9	0.83
64	840525	40 45	169 07	164	86	2	I	(0, 0)	5.0	6.7	0.75
65	840601	37 38	175 01	163	84	5	M	(0, 0)	3.9	7.2	0.54
66	840514	42 19	157 23	157	68	1	I	(0, 0)	4.9	8.8	0.56
67	840609	41 33	165 46	153	67	1	I	(0, 0)	2.1	3.9	0.54
68	840525	40 48	169 00	150	62	1	I	(0, 0)	2.8	5.0	0.56
69	800619	50 12	172 45	93	11		Fetus		0.66	1.1	0.60

* First two digits indicate the year, second two digits the month and the third the date of sampling.

** In the case of females growth status (M: mature, I: immature) and/or numbers of *corpus luteum* plus *corpus albicans* in the left and right ovaries are given.

— Data not available.

Dall's porpoises are normally 100 cm in length at birth (Kasuya and Shiraga, 1985). So on the basis of the body lengths of the fetus (93 cm) and newborn (103 cm) specimens analyzed in this study (Table 1), they can be considered as near term and a pup just born a few weeks before, respectively. The levels of PCBs and DDE in them can be considered as representative values in full-grown fetus and a pup which started accumulating organochlorines *via* milk.

According to Kasuya (1978), specimens up to about 160 cm correspond to one year of age. Kasuya and Jones (1984) stated that the youngest mature individuals in their study were two years old corresponding to a body length of 176 to 182 cm. Newby (1982) also got more or less the same value (182.5 cm) using a larger sample. As observed in grey seals (Addison and Brodie, 1977) and striped dolphins (Tanabe *et al.*, 1982), large quantities of organochlorines were apparently transferred to the calves of the Dall's porpoise leading to the observed initial increase in the levels of both the compounds in both male and female pups (Fig. 3) until they reach the weaning period.

After the weaning period a major change in the feeding habits and also in the behavior can be expected (Kasuya and Shiraga, 1985). The rate of growth in the

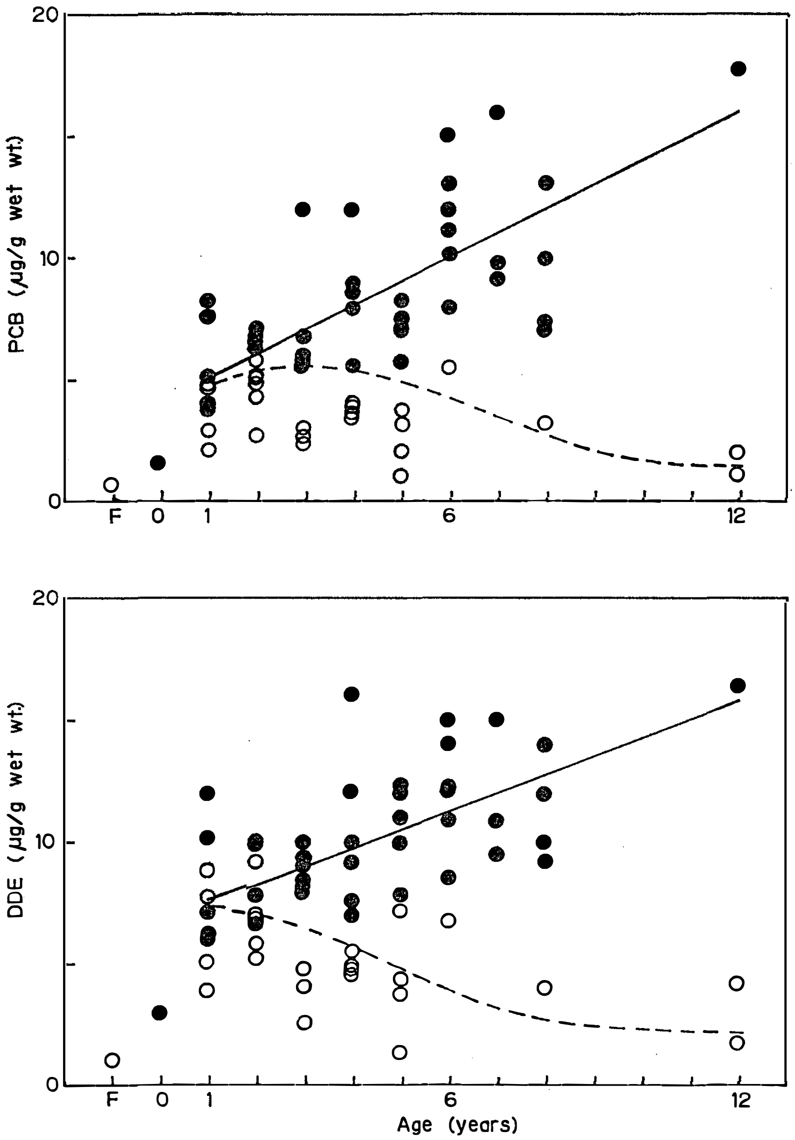

Fig. 2. Relationship between age and concentrations of PCBs and DDE in male (●) *and female* (○) Dalli-*type Dall's porpoises collected from northwestern North Pacific.*

specimens within this range of body length is very rapid in Dall's porpoises (KASUYA and JONES, 1984). Hence the amount of PCBs and DDE added up into the body of a specimen of this size range, *via* bioaccumulation through food, is rather physically diluted by this extensive growth. As a result of this, in the specimens after about 160 to 190 cm body length, the rise in the levels of both the compounds was not so prominent as in the case of unweaned calves (Fig. 3). Moreover, the fishes, squids and crustaceans which constitute the major food items of *Phocoenoides dalli* (ANONYMOUS, 1981) contained lower levels of organochlorines than mother's milk. Such a decreased input of organochlorines *via* food after weaning may also be a reason for the slower increase in organochlorine concentrations in the specimens of this size range.

Following this, the rate of growth in these animals might have slowed down, leading again to an apparent increase in the levels of these xenobiotics in this slower

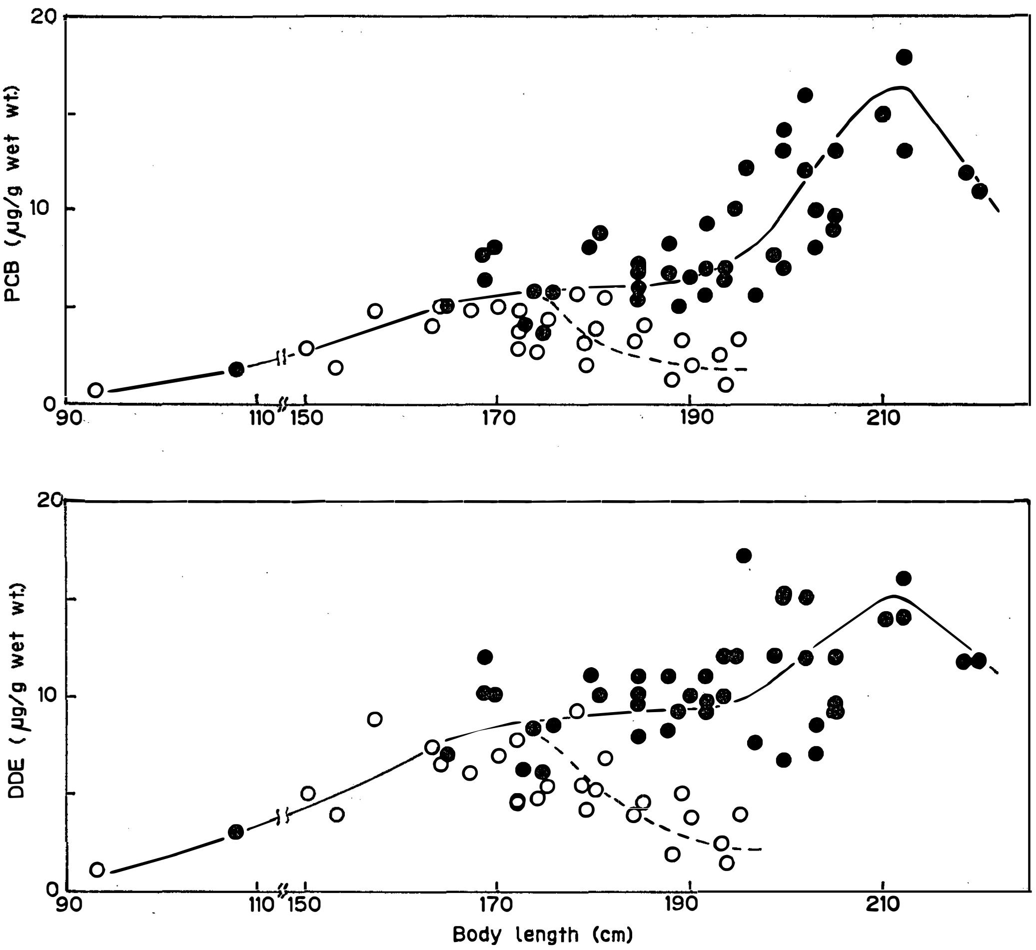

Fig. 3. Relationship between body length and levels of PCBs and DDE in male (●) and female (○) Dalli-*type Dall's porpoises collected from northwestern North Pacific.*

growth phase of these animals.

It was suggested by KASUYA (1978) that both males and females of *Dalli*-type Dall's porpoises attain their asymptotic length at 200 to 210 cm body length. So the PCB and DDE levels in the specimens above this body length can be expected to increase continuously. In contrast to this, even though the sample number is small (two specimens), lower levels of PCBs and DDE were observed in the specimens having body lengths of 219 and 220 cm (Table 1). A decrease in DDT was observed in striped dolphins, after approximately 20 years of age, which has been explained as due to the decline in food intake amounts in older individuals (TATSUKAWA and TANABE, 1988). If this explanation is applicable to other marine mammals also, a same type of decreasing trend in Dall's porpoises of 219 and 220 cm body length (Fig. 3) is justifiable.

But a major matter of concern in Dall's porpoises analyzed here is that both the bigger specimens (219 and 220 cm) discussed above are not the oldest (specimens Nos. 1 and 2, Table 1). In Dall's porpoise the ages of the specimens are normally determined by counting the growth layers in the cementum of the teeth (KASUYA, 1978; KASUYA and JONES, 1984). But it was also reported by the same authors (JONES *et al.*,

1985) that considerable variation was detected between readers in age estimation of the Dall's porpoise using cemental growth layers, the major discrepancy being observed in the younger and older individuals. Moreover, in Dall's porpoises, attainment of sexual maturity and accompanying behavioral change was found to depend apparently on body size rather than or age (KASUYA and JONES, 1984). By observing such a clear pattern of variation in the levels of PCBs and DDE with different growth stages of Dall's porpoises, we believe that the residual levels of persistent xenobiotics in the fat stores of the males of long-life marine mammals can be used to confirm or clear any discrepancy in the age determination by conventional methods such as counting of growth layers in the cementum of teeth.

KASUYA (1978) stated that the age at attainment of sexual maturity in the youngest mature female is 2.5 years. KASUYA and JONES (1984) got the value as 3 years and KASUYA and SHIRAGA (1985) observed the age of the youngest mature females as 2 years with a mean body length of about 175 cm. The gestation period in Dall's porpoise is about 11 months (KASUYA and JONES, 1984). So, after the birth of the first pup and continuing lactation the organochlorine levels started decreasing drastically from the 3rd year of age (Fig. 2), that is, after about 180 cm body length (Fig. 3).

Until about 5 or 6 years of age can be considered as active parturition period and because of the continued lactation during this time, concentrations of both the compounds decreased consistently with age. KASUYA and JONES (1984) stated that most of the females of Dall's porpoises ovulate within a month or so after parturition and enter next gestation. Therefore, the majority of adult females must be simultaneously lactating and pregnant and hence such a linear decrease in the levels of xenobiotics with age in females can very well be expected.

After this age, the resting period between parturitions might have increased and the extent of lactation decreased. As a result of this, the extent of decrease in the concentrations of both the compounds is not very prominent in older specimens (Fig. 2).

Existence of a wide difference of organochlorine levels between sexes is generally well known in cetaceans and pinnipeds (GASKIN, 1982; WAGEMANN and MUIR, 1984). Such a phenomenon is believed to be resulting from the lactational transfer of organochlorines. For example, ADDISON and BRODIE (1977) estimated that a grey seal will lose about 30% of the total body burden of DDT compounds and about 15% of PCB through lactation after a parturition and lactation. TANABE *et al.* (1982) in their studies on the transfer of PCBs from the pregnant striped dolphin to her fetus stated that the transplacental transfer rates of chlorinated hydrocarbons are 10% at the most and they also presumed that larger amounts are transferred through lactation rather than during parturition.

Ranges and mean of PCBs and DDE levels in adult males and females of several cetaceans in the works carried out in our laboratory are compared with the values obtained from the present specimens (Table 2). All the females had lower levels than males, reflecting the lactational transfers of these pollutants in large quantities. However, the percentage of variation between males and females was found to be widely different between species. The females of Dall's porpoises contained 24 and 35% of the levels of PCBs and DDE, respectively, as those in males. These values were

Table 2. Ranges and mean and male-female differences in the concentrations (µg/g on wet weight basis) of PCBs and DDE in marine mammals.

Species	Sex	n	Concentration* (µg/g on wet wt) PCBs	DDE	Male-female difference** PCB	DDE	Reference
Baird's beaked whale *Berardius bairdii*	M	13	2.0–5.1 / 3.6	9.5–19 / 14			
	F	7	1.5–2.8 / 2.0	4.1–17 / 7.8	55	52	SUBRAMANIAN *et al.*, 1988
Minke whale *Balaenoptera acutorostrata*	M	10	0.01–0.03 / 0.02	0.08–0.14 / 0.09			
	F	11	0.003–0.02 / 0.008	0.01–0.08 / 0.04	40	43	TANABE *et al.*, 1986
Striped dolphin *Stenella coeruleoalba*	M	21	15–56 / 32	21–60 / 39			
	F	3	1.5–6.7 / 4.7	0.79–5.8 / 4.0	15	11	TANAKA, 1982
Dall's porpoise (*Dalli*-type) *Phocoenoides dalli*	M	14	7.1–16 / 12	6.6–15 / 12			
	F	13	1.0–4.0 / 2.8	1.3–7.2 / 4.1	23	34	Present study

* Range/Mean. Values obtained from adult specimens were only used.
** Percentage of PCBs and DDE concentrations in females to those in males.

found to be lower than those observed in Baird's beaked whales and minke whales but were higher than those observed in striped dolphins.

In Baird's beaked whales the gestation is about 17 months, one of the longest among the cetaceans (KASUYA, 1977). In minke whales the calving interval was estimated to be about 14 months (STEWART and LEATHERWOOD, 1985), almost equal to Dall's porpoises (KASUYA, 1978). But a wide difference exists between the lactational periods of both the species. For example, the lactational period for every parturition is rather very short (3–6 months) in minke whales (STEWART and LEATHERWOOD, 1985), whereas KASUYA (1978) gave two different estimates of lactational period in Dall's porpoise as 0.5 to 1.5 years and 2 years. In any case the excretion of organochlorines *via* reproductive activities must be rapid and the male-female difference can be expected to be wider in Dall's porpoise than in the previous two species. At the same time it was stated by TANAKA (1982) that in females of striped dolphins the calving interval is about 2 years. But the lactational period is rather longer (about 18 months) in this species (MIYAZAKI, 1981) and hence the adult females of this species contained only 15 and 11% of PCBs and DDE, respectively, to those in their male counterparts (Table 2).

Taking account of all these reproductive parameters the male-female differences of the levels of PCBs and DDE in these marine mammals seem to follow the length of lactational periods (Fig. 4), which may be the case in all the marine mammals or at least in cetaceans. It can be stated, by comparing the values in Table 2, that the lactational period in Dall's porpoise should be between those of minke whales and striped dolphins. By considering the present results we would like to state that in the

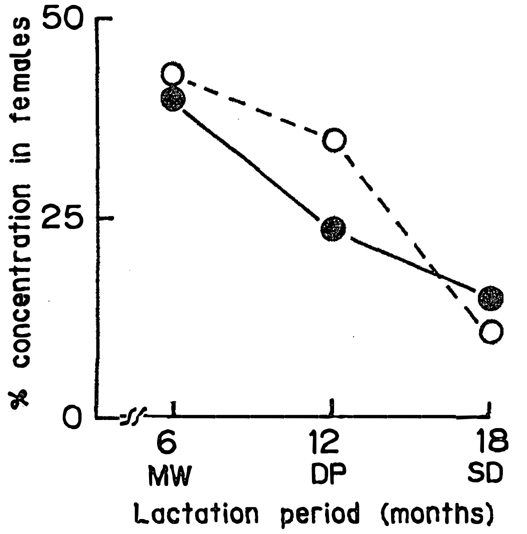

Fig. 4. Relationship between the lactational period and percentage of PCBs (●) and DDE (○) in adult females to those in adult males of marine mammals. MW: Minke whale; DP: Dall's porpoise; SD: Striped dolphin.

two estimates given by KASUYA (1978), 0.5 to 1.5 years (with an average value of 12 months) of lactational period seem to be a better estimate. KASUYA and JONES (1984) also stated that the value of two years of lactational period for this species seems to be an overestimate. In the same way, by comparing the relatively higher values of the percentages of PCBs and DDE in females of Baird's beaked whales (55 and 52% respectively) with other three species, a lactational period less than that of minke whales (below 6 months) may be a plausible value.

By considering all the above findings we found that the persistent xenobiotics could be used to determine or at least to confirm the ages of the males and in deciding the approximate parturitional histories of females of long-life marine mammals.

In the present study, the number of specimens analyzed in certain age classes of Dall's porpoises, especially in older females is rather scarce or even nil. It was already reported that lower chlorinated PCBs are more transferrable by reproductive pathways than the higher chlorinated ones in striped dolphins (TANABE *et al.*, 1982). So, it seems possible that by analyzing further specimens and especially the isomer and congener transfer patterns of PCBs from mother to fetus and pup, the parturitional histories of the individuals of Dall's porpoises can be determined fairly accurately.

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