# Organochlorine contaminant concentrations and lipid profiles in eastern North Pacific gray whales (*Eschrichtius robustus*)

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ABSTRACT

Organochlorine (OC) contaminant concentrations in tissues and lipid profiles in blubber are summarised for 101 gray whales (*Eschrichtius robustus*) from the eastern North Pacific stock. Samples were obtained from presumably healthy gray whales during a 1994 subsistence hunt in the Russian Arctic ($n$ = 17) and also from biopsy sampling of live animals from the Washington coast ($n$ = 38). In addition, tissues were collected from two groups of animals (1988-1991, $n$ = 22; and 1999, $n$ = 24) that stranded along the west coast of the USA. These whales represent a diverse group of animals with respect to lipid stores, age, gender, health and reproductive status. Information about these biological factors is necessary before contaminant concentration data can be properly interpreted. Differences in blubber lipid levels and profiles were examined among these groups of whales. Significantly higher lipid levels were found in the blubber of subsistence animals that were sampled following summer feeding in the Bering and Chukchi Seas, compared to lipid levels in the biopsied and stranded animals. Lipid class profiles from blubber of presumably healthy gray whales (i.e. from subsistence and biopsy sampling) contained primarily triglycerides and were very different from those of stranded animals that showed lipid decomposition (increased proportions of free fatty acids, cholesterol and phospholipids). Furthermore, lipid class profiles were found to be a means of estimating the quality of a blubber sample from stranded cetaceans. An examination of how biological factors (e.g. gender, reproductive status, age) contribute to interpreting the differences found in contaminant concentrations among the gray whales was also undertaken. Although not statistically significant, higher (OC) concentrations were found in males compared to females, thus suggesting the tendency of the mother to shift her contaminant burden to her calf during gestation and lactation. Results also indicated that there was no significant increase in concentrations of contaminants in the blubber with increase in length (surrogate for age). Higher concentrations of OC contaminants were found in stranded juvenile gray whales, compared to juvenile subsistence whales, and were thought to result from retention of OCs in blubber of the stranded animals as lipid stores are mobilised for energy and total lipid levels decrease, rather than from a difference in diet or feeding areas. OC concentrations in various tissues (blubber, liver, kidney, muscle, brain) were similar on a lipid weight basis, except for brain, which had lower lipid-adjusted OCs because the blood-brain barrier limits contaminant transfer.

KEYWORDS: GRAY WHALE; POLLUTANTS; POLLUTANT BURDEN; ORGANOCHLORINES; MONITORING; ARCTIC; PACIFIC OCEAN; STRANDINGS; BIOPSY SAMPLING

## INTRODUCTION

Most gray whales (*Eschrichtius robustus*) from the eastern North Pacific stock make an annual round-trip migration between their summer feeding grounds in the Bering and Chukchi Seas in the Arctic and their winter breeding grounds in Baja California (Mexico). Moore *et al.* (2000) reported that gray whales are 'opportunistic foragers' and eat whenever they can. Their Arctic feeding grounds, particularly the Chirikov Basin between St. Lawrence Island and the Bering Strait, provide an abundance of food (e.g. ampeliscid amphipods, polychaete worms, crustacea and molluscs - Moore *et al.*, 2000), probably in greater quantities than found in many other areas visited during their migration cycle (Highsmith and Coyle, 1992). After a summer of feeding, Rice and Wolman (1971) found that southbound migrating gray whales have increased weights, girths, blubber thickness and oil content compared to northbound migrants whose blubber stores have been greatly reduced by the southbound migration, followed by the breeding portions of the cycle. The composition of blubber, especially the lipid content and blubber thickness, can be indicative of the nutritive condition of the animal (Aguilar and Borrell, 1990), but varies with season, gender, age and reproductive status (Rice and Wolman, 1971).

Organochlorine (OC) pollutants are among the most widespread and persistent anthropogenic contaminants present in marine sediments and biota. These compounds bioaccumulate in lipid-rich tissues of aquatic organisms, including marine mammals, because of their lipophilicity and resistance to metabolism (Varanasi *et al.*, 1992). The gray whales' method of feeding results in the ingestion of sediment and other bottom materials in addition to prey species i.e. benthic invertebrates (Rice and Wolman, 1971; Nerini, 1984). Thus, the potential exists for uptake of contaminants if gray whales feed in areas where sediment and prey are contaminated by anthropogenic compounds. There are a number of reports in the literature about OC contaminants found in gray whale tissues. For example, Wolman *et al.* (1970) reported the presence of DDT and its metabolites in six gray whales taken off San Francisco, California during both the northbound and southbound

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migrations, and Schaffer *et al.* (1984) found DDTs and PCBs in blubber of a gray whale sampled in southern California in 1976. In addition, polychlorinated dibenzo-*p*-dioxins and dibenzofurans were measured in two gray whales from the west coast of North America (Jarman *et al.*, 1996).

Gender and reproductive status can influence OC burdens and distributions in marine mammal tissues (Aguilar *et al.*, 1999). Studies of various marine mammal species have shown that decreases in OC levels in tissues of reproductive females are largely due to transfer of these lipophilic compounds to their offspring during gestation and lactation, possibly resulting in serious health problems for the offspring (Tanabe *et al.*, 1982; Muir *et al.*, 1992; Aguilar and Borrell, 1994; Norstrom and Muir, 1994; Krahn *et al.*, 1997; 1999). For example, high concentrations of OCs in marine mammal tissues have been associated with reproductive impairment, immunosuppression and increased susceptibility to disease (Lahvis *et al.*, 1995; De Guise *et al.*, 1996; 1997; De Swart *et al.*, 1996; Kamrin and Ringer, 1996; O'Hara and Rice, 1996; Ross, 1996; Beckmen *et al.*, 1999).

This paper reports the OC contaminant concentrations in blubber and lipid profiles from recently stranded (1999) and biopsied (1996-1998) gray whales from the eastern North Pacific stock. These two recent studies have been described in internal reports to resource managers (Stein, 1999; Ylitalo, 1999). The analytical results from two earlier studies have also been summarised for comparison with the current results: a published paper on stranded gray whales (Varanasi *et al.*, 1994) and a study of contaminants in healthy gray whales sampled during a 1994 subsistence hunt in the Russian Arctic (Tilbury *et al.*, 2001). Differences in blubber lipid levels and profiles were examined among these groups of whales. An examination of how biological factors (e.g. gender, reproductive status, age) contribute to interpreting the differences found in contaminant concentrations among the gray whales was also undertaken. The distribution of OCs among tissues (blubber, liver, kidney, muscle and brain) of the subsistence gray whales and the 1988-1991 stranded animals was also determined.

## METHODS

### Field sampling of gray whale blubber

Sampling sites are shown in Fig. 1.

*Stranded (1988-1991)*

Samples of blubber, liver and brain were collected from 22 dead, beached gray whales from March 1988 until June 1991 (Varanasi *et al.*, 1994). Some animals may have been necropsied as much as a month after death, resulting in poor quality samples. Leaching of lipid from blubber may have occurred from some animals before sampling or from some samples before they were frozen.

*Stranded (1999)*

Samples of blubber (and other tissues that were not analysed in this study) were collected from 24 gray whales that stranded along the coast of Washington state and in Puget Sound between January and August 1999. Of the 24 whales sampled, 7 carcasses were classified as 'fresh,' 13 as 'moderately decomposed' and 4 exhibited 'advanced decomposition.' Leaching of lipid from the blubber of some animals may have occurred before sampling. In addition, laboratory notes indicate that 'oil' was found in several containers of blubber samples, indicating that leaching of the lipid occurred before the sample was frozen.

*Subsistence (1994)*

Samples of blubber, liver, kidney, brain and muscle were collected from 17 gray whales taken during a Russian subsistence harvest in October 1994 in the Bering Sea (Tilbury *et al.*, 2001). As the whales were necropsied within 24hrs of death and the tissues frozen, the quality of the tissue samples was very good.

*Biopsy (1996-1998)*

Small samples of skin and blubber from 38 gray whales were obtained from live animals using a biopsy dart. The animals were biopsied off the northwest coast of Washington state in the summer and autumn of 1996-1998. The samples were kept on ice until they could be frozen and, as a result, the quality of the tissue samples was excellent. Although the gender of the biopsied animals could be determined by genetic methods, these analyses were not conducted for this study.

*Age and reproductive status*

In the absence of specific age data, length was used as a surrogate for age (Rice and Wolman, 1971; Jones and Swartz, 1984). To compare concentrations of chemical contaminants among groups of marine mammals (e.g. stranded or subsistence), it is desirable, but not always possible, to know the reproductive status of female whales, because a large percent of a female's body burden of contaminants can be transferred to the offspring through gestation and lactation. When reproductive status is uncertain or unknown, an age- or length-matched group of immature females and males can be selected from each cohort to provide an unbiased comparison. From Rice and Wolman (1971), most gray whales shorter than 1,100cm are immature (juveniles) and most longer than 1,200cm are sexually mature (adults). Whales with lengths between 1,100 and 1,200cm may be either immature or mature. In the present study, only one female (from the 1994 subsistence group) had a length between 1,100 and 1,200cm. From the information recorded at the time of capture, it was neither pregnant nor lactating and was therefore classified as a juvenile. Males less than 1,200cm were also classified as juveniles. This is consistent with criteria (i.e. 1,200cm = sexual maturity) applied in Norman *et al.* (2000).

### Analyses for OCs in gray whale blubber

*Sub-sampling of blubber*

Blubber was sub-sampled in the laboratory before analysis. In general, all the blubber from a biopsy sample was used in an analysis. All sub-samples from blubber samples obtained by necropsy were taken by cutting into the interior of the sample to avoid possible contamination or desiccation that may have occurred in the outer portions. Sub-samples were taken from the blubber near the epidermis from the 1999 stranded animals so that these samples would mimic those taken via biopsy as closely as possible. The location of the blubber sub-samples, with respect to the epidermis or muscle layers, were not recorded or were not known for the 1994 subsistence animals and the 1988-1991 stranded animals.

*GC/ECD analyses for OCs*

The gray whale samples collected from the 1988-1991 strandings and those taken during the subsistence harvest in Russia were analysed for OCs and percent lipid according to the methods of Krahn *et al.* (1988) and Sloan *et al.* (1993). Tissue (1-3g) and stomach contents (1-5g) were macerated with sodium sulphate and methylene chloride. The methylene chloride extract was filtered through a column of

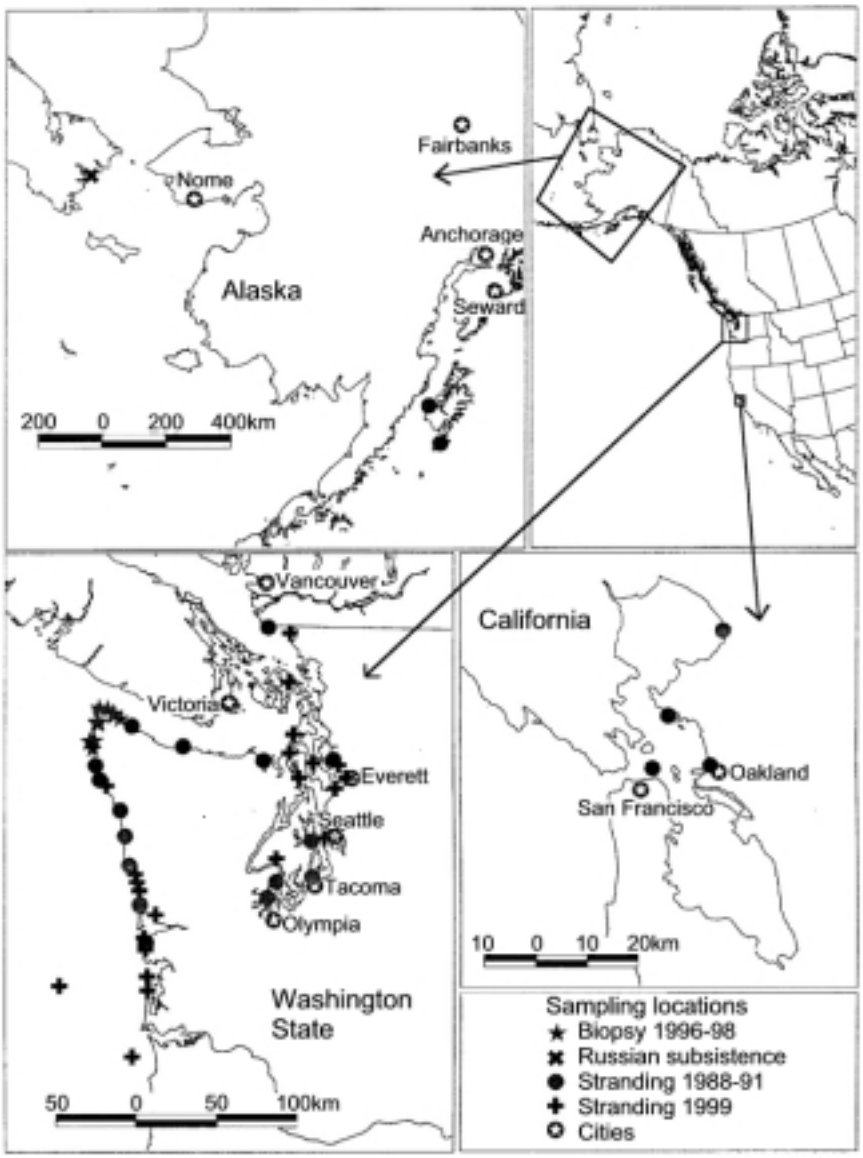


Fig. 1. Maps of the west coast of the USA and Alaska showing locations of gray whales sampled for this study. All the samples for the 1994 subsistence study were collected from the same general area in Russia and that location is shown by a single symbol. The 1988-1991 stranded whales were found at sites from California to Alaska. The 1996-1998 biopsy and the 1999 stranded groups of animals were located off the coast of Washington state and in Puget Sound.

silica gel and alumina and the extract concentrated for further clean-up. This was carried out using a size-exclusion column with high-performance liquid chromatography (HPLC). The original analytical method was modified slightly by adjusting the flow rate to 5mL/min to facilitate the clean-up for the lipid-rich blubber tissue and then a fraction containing the OCs was collected. The HPLC fraction was exchanged into hexane and the extracts were analysed for OCs using a *Hewlett-Packard* 5890 capillary gas chromatograph (GC) equipped with an electron capture detector (ECD). A 60m DB-5 capillary column (0.25mm I.D., J & W Scientific) was used. The sample extract (3μL) was injected splitless (split valve opened 0.5min, oven temperature 50°C held 1min). Oven temperature was programmed to 315°C at 4°C/min. Identification of selected individual OCs was confirmed by retention time comparison to standards and by GC with detection by selected ion monitoring mass spectrometry. 'ΣPCBs' refers to the sum of the concentrations of 17 congeners (18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 170, 180, 187, 195, 206, 209) multiplied times two to estimate total PCB concentrations (Lauenstein *et al.*, 1993). The congeners were numbered using the accepted system as developed by Ballschmiter and Zell (1980). 'ΣDDTs' is the sum of the concentrations of *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT, and 'Σchlordanes' is the sum of the concentrations of *cis*-chlordane, oxychlordane, *trans*-nonachlor, heptachlor and heptachlor epoxide.

*HPLC/PDA analyses for OCs*

Gray whale blubber samples from the 1999 stranding and samples taken by biopsy were analysed for selected OCs (including dioxin-like and other PCB congeners) and selected pesticides (e.g. DDTs, hexachlorobenzene (HCB)) using HPLC coupled with photodiode array detection (PDA) following Krahn *et al.* (1994). Briefly, the analytes were extracted from the blubber by homogenisation with pentane/hexane (50/50, v/v) and were separated from interfering compounds on a gravity-flow cleanup column (packed with neutral, basic and acidic silica gels) eluted with

methylene chloride/hexane (50/50, v/v). The dioxin-like PCB congeners (77, 105, 118, 156, 157, 169, 189) were resolved from other selected PCB congeners (i.e., 101, 128, 138, 153, 170, 180) and pesticides (o,p'-DDD, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT) by HPLC using a Cosmosil PYE column and measured using an ultraviolet PDA detector. Results from samples analysed by HPLC/PDA were comparable to those of the same sample analysed by low- or high-resolution GC/MS (Krahn *et al.*, 1994), thus validating the use of HPLC/PDA in OC determinations. Furthermore, the same quality assurance procedures (see 'Quality Assurance' section below) were used in both methods to determine OCs for this paper.

**Lipid determinations**

*Gravimetric lipid determination*

Percent lipid was determined in the gray whale samples collected from the 1988-1991 stranding and those taken during the subsistence harvest in Russia by measuring total non-volatile extractables (reported as percent total lipids). An aliquot of the initial methylene chloride extract of tissue was filtered through filter paper containing approximately 5g of diatomaceous earth as a filtering aid, the solvent was removed from the lipid using a rotary evaporator and the lipid was weighed. The percent lipid was calculated by dividing the weight of lipid by the original sample wet weight and multiplying by 100 (Varanasi *et al.*, 1993; 1994).

*TLC/FID lipid determination*

Gray whale blubber samples from the 1999 stranding, the 1994 subsistence hunt and the biopsy sampling were analysed for total lipids by thin layer chromatography coupled with flame ionisation detection (TLC/FID) using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan) (Shantha, 1992). The lipid sample extracts were spotted on Chromarods (Type SIII) and developed in a solvent system containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v). Various classes of lipids (i.e. wax esters, triglycerides, free fatty acids, cholesterol and polar lipids) were separated based on polarity, with the nonpolar compounds (i.e. wax esters) eluting first, followed by the more polar lipids (i.e. phospholipids). The Iatroscan was operated with a hydrogen flow rate of 160mL/min and air flow of 2000mL/min. Data were acquired and analysed using TDatascan software (RSS Inc., Bemis, TN). A four-point linear external calibration was used for quantitation. Duplicate TLC/FID analyses were performed for each sample extract and the mean value reported. Total lipid concentrations were calculated by adding the concentrations of the five lipid classes for each sample and were reported as percent total lipid.

**Quality assurance**

Quality assurance procedures included analyses of National Institute of Standards and Technology (NIST) standard reference materials (SRMs), a NIST whale blubber control material, certified calibration standards, method blanks, solvent blanks and replicate samples. Acceptance criteria for analyses of NIST SRM 1945 or NIST blubber control material were similar to those NIST uses for its Intercomparison Exercises for blubber and other tissues (concentrations of individual analytes were within +35% from the upper and -35% from the lower limits of the 95% confidence interval of NIST concentrations). Other acceptance criteria were: replicate analyses within 35% relative standard deviation; and surrogate recoveries from 60-130%. In addition, our laboratory successfully participates in NIST and other Quality Assurance Intercomparison Exercises each year.

**Statistical analysis**

For statistical analyses, lipid concentrations (reported as percent total lipid) were arcsine transformed and contaminant concentrations were log transformed to increase the homogeneity of the variances. Concentrations of OCs and lipids in gray whale blubber were grouped by study and gender and then analysed using JMP software (SAS Institute, Inc., Cary, NC). The group means were analysed by ANOVA and differences among means were determined using the Tukey-Kramer HSD (honestly significant difference) test to compare all combinations of pairs of groups. Gray whale age (length) was also plotted against analyte concentration for selected analytes and the 95% density ellipses were calculated. The calculated Pearson correlation coefficient approaches 1 as the relationship between age and analyte concentration approaches linearity. The relationship between concentrations (ng/g, wet weight) of ΣPCBs, ΣDDTs, Σchlordanes and HCB and lipid content in the various tissues (blubber, brain, liver, kidney, muscle) of 1994 Russian subsistence gray whales was assessed by correlation. For all tests, statistical significance was set at $p = 0.05$.

## RESULTS AND DISCUSSION

**Percent lipid in blubber**

Lipid content was determined in all blubber samples because the OCs measured are lipophilic compounds that are stored in tissues, particularly in blubber because of its high lipid content. Two methods were used to determine the lipid content of the gray whale blubber samples. A traditional gravimetric method (Varanasi *et al.*, 1993; 1994) that measures 'total non-volatile extractables' (reported as percent total lipid) was used for samples obtained from the 1988-1991 stranding and the 1994 subsistence hunt (Table 1). This method measures lipid, as well as other endogenous materials extracted from the blubber matrix, but furnishes no information on lipid classes. In addition, a TLC/FID method was used to measure lipids in blubber samples from the 1999 stranding, the 1994 subsistence hunt (also analysed by gravimetric method) and the biopsy sampling (Table 1). This method measures quantities of each lipid class, as well as total lipids, and excludes other extractable endogenous materials from the determination (see Methods).

Lipid percentages determined by TLC/FID were generally lower than those determined using the total extractables method. In the current study, percent lipid in blubber was measured by both methods in the 1994 subsistence animals ($n = 17$); mean percent lipid ± SD from the total extractable method was 48±22 and from the TLC/FID method was 43±11 (Table 1; results of two methods were not statistically different). In a previous study, Delbeke *et al.* (1995) determined lipid in tissues of various marine species by both the gravimetric and TLC-FID methods. Although the lipid values determined by these methods were correlated, the lipid concentrations determined by TLC-FID were, on average, approximately half as great as those determined by the gravimetric method. These researchers also found that the gravimetric lipid values were overestimated due to interferences from non-lipid materials that were co-extracted, but these non-lipid materials did not interfere with the TLC-FID determination.

The total lipid levels were compared among the four groups of gray whales from the eastern stock of the North Pacific: the two stranded gray whale groups (1988-1991 and 1999); the healthy juvenile whales collected in the Bering Sea waters during a 1994 Russian subsistence harvest; and samples collected by biopsy dart from whales off the Washington coast (1996-1998) (Table 1). Statistical analysis showed significantly higher lipid levels in the 1994 subsistence animals compared to the biopsied whales, the 1988-1991 stranded and 1999 stranded animals, but no differences were observed among these latter three groups.

The high lipid levels in blubber from the 1994 subsistence whales were expected because samples were taken in late September and October after the whales had increased their lipid stores during a summer of feeding in the Bering and Chukchi Seas. It is more difficult to explain why the presumably healthy gray whales, biopsied in the summer and fall months, had lipid levels that were much lower than those in the subsistence whales (means of 10% and 43%, respectively). The biopsied whales were sampled in Washington State waters at the same time of year as the subsistence animals were taken in the Chirikov Basin (Russia) and thus the biopsied whales had presumably not migrated to the richer feeding grounds of the Bering and Chukchi Seas. Thus, the lower lipid levels in the biopsied whales could be due to: (1) poorer quality or quantity of available prey; or (2) differences in the lipid content of blubber sampled adjacent to the skin via biopsy compared to that sampled from deeper locations via necropsy. It is difficult to draw conclusions, based on information available in the literature, about which of these alternatives is the more likely explanation for the low lipid levels in biopsy samples. Although there are no data on the gray whale prey base in Washington waters, Oliver *et al.* (1984) reported that gray whales summering along the west coast of Vancouver Island, Canada, fed on essentially the same prey species as in the Chirikov Basin. However, long-term (26-year) observations of gray whales feeding along Vancouver Island indicated that feeding areas and prey species are highly variable there, both intra- and inter-annually (Darling *et al.*, 1998). Thus, it is virtually impossible to determine in any given year if prey type and quality are 'poor' relative to other feeding sites. Furthermore, information available about lipid stratification in blubber is limited and appears to be a species-specific characteristic. For example, Aguilar and Borrell (1990) reported that the surface layer of blubber in fin whales (*Balaenoptera physalus*) is used for thermoregulation and may not be representative of the body burden of certain pollutants. In contrast, a similar stratification of lipid in the blubber layer does not appear to occur in the harbour porpoise, *Phocoena phocoena* (Tilbury *et al.*, 1997). Therefore, a direct comparison of lipid content in biopsy and necropsy samples from the same whales is needed to learn whether these samples differ. This is particularly important because there is a current emphasis on using non-harmful methods for obtaining marine mammal samples.

Lipid concentrations in the blubber of the 1988-1991 and 1999 stranded whales were quite low compared to those reported in blubber of the 1994 subsistence whales, as well as in blubber of other stranded cetaceans (Martineau *et al.*, 1987; Tilbury *et al.*, 1997). The low lipid content of the stranded whales can be attributed to one or more of the following factors: (1) leaching of lipid from blubber tissues; (2) low lipid stores due to reduced feeding during the winter breeding season; (3) reduced availability of preferred prey,

Table 1

Length, percent lipid and concentrations (mean ± SEM) of ∑DDTs, ∑PCBs and HCB in blubber of gray whales.

| | | | ng/g wet weight | | | ng/g lipid weight | | |
|---|---|---|---|---|---|---|---|---|
| | Length (cm) | Lipid (%) | ∑DDTs | ∑PCBs | HCB | ∑DDTs | ∑PCBs | HCB |
| **Biopsy (1996-1998)[a]** | | | | | | | | |
| Overall mean (*n* = 38) | Unknown | 10 ± 1.0 | 130 ± 26 | 220 ± 42 | 100 ± 41 | 1,200 ± 140 | 2,100 ± 190 | 600 ± 41 |
| **Subsistence (1994)[b]** | | | | | | | | |
| Female - juvenile (*n* = 13) | 884 ± 28 | 48 ± 6.1(g)<br>44 ± 2.8 (t) | 180 ± 40 | 680 ± 98 | 240 ± 41 | 370 ± 65 | 1,400 ± 140 | 550 ± 96 |
| Male - juvenile (*n* = 4) | 833 ± 26 | 48 ± 11 (g)<br>42 ± 7.8 (t) | 87 ± 10 | 480 ± 130 | 200 ± 16 | 200 ± 38 | 1,200 ± 360 | 470 ± 90 |
| Overall mean (*n* = 17) | 872 ± 22 | 48 ± 5.2 (g)<br>43 ± 2.7 (t) | 150 ± 32 | 630 ± 82 | 230 ± 32 | 330 ± 53 | 1,400 ± 130 | 530 ± 75 |
| **Stranded (1988-1991)[c]** | | | | | | | | |
| Female - adult (*n* = 2) | 1,300 ± 0 | 1.4 ± 0.4 | 23 ± 5.8 | 130 ± 26 | 41 ± 24 | 1,900 ± 950 | 11,000 ± 5,000 | 3,700 ± 2,700 |
| Male - adult (*n* = 6) | 1,250 ± 13 | 3.7 ± 2.5 | 890 ± 440 | 1,700 ± 750 | 660 ± 450 | 73,000 ± 52,000 | 140,000 ± 90,000 | 62,000 ± 52,000 |
| Female - juvenile (*n* = 4) | 860 ± 36 | 14 ± 6.4 | 260 ± 96 | 790 ± 260 | 450 ± 150 | 3,500 ± 1,400 | 12,000 ± 5,200 | 4,700 ± 1,500 |
| Male - juvenile (*n* = 8) | 1,100 ± 39 | 13 ± 8.7 | 400 ± 140 | 880 ± 230 | 220 ± 55 | 14,000 ± 6,400 | 39,000 ± 21,000 | 8,600 ± 4,100 |
| Unknown (*n* = 2) | Unknown | 2.1 ±1.2 | 170 ± 160 | 520 ± 380 | 47 ± 31 | 5,900 ± 4,500 | 22,000 ± 6,200 | 2,100 ± 330 |
| Overall mean (*n* = 22) | 1,100 ± 36 | 8.7 ± 3.5 | 450 ± 140 | 970 ± 240 | 350 ± 130 | 26,000 ± 15,000 | 56,000 ± 26,000 | 21,000 ± 14,000 |
| **Stranded (1999)[d]** | | | | | | | | |
| Female - adult (*n* = 6) | 1,300 ± 31 | 3.9 ± 1.7 | 72 ± 26 | 200 ± 68 | 64 ± 31 | 2,000 ± 250 | 6,000 ± 1,800 | 1,500 ± 280 |
| Male - adult (*n* = 2) | 1,290 ± 14 | 28 ± 24 | 410 ± 360 | 680 ± 620 | 420 ± 420 | 1,300 ± 250 | 1,900 ± 650 | 800 ± 560 |
| Female - juvenile (*n* = 6) | 930 ± 28 | 16 ± 6.1 | 190 ± 60 | 490 ± 180 | 740 ± 420 | 2,800 ± 1,100 | 6,800 ± 2,300 | 5,900 ± 1,600 |
| Male - juvenile (*n* = 10) | 950 ± 17 | 13 ± 2.8 | 330 ± 71 | 900 ± 180 | 620 ± 140 | 3,600 ± 870 | 9,900 ± 2,500 | 6,400 ± 900 |
| Overall mean (*n* = 24) | 1,100 ± 39 | 12 ± 2.7 | 240 ± 44 | 600 ± 130 | 510 ± 130 | 3,100 ± 510 | 8,200 ± 1,400 | 4,600 ± 730 |

[a] Animals sampled with a biopsy dart in waters off the coast of Washington state. ∑DDTs, ∑PCBs and HCB were determined by HPLC/PDA and lipids by TLC/FID (see Methods).

[b] Animals collected during subsistence hunts in the Russian Bering Sea. ∑DDTs, ∑PCBs and HCB were determined by GC/ECD and lipid by gravimetric (g) and by TLC/FID (t) methods (see Methods).

[c] Animals stranded on the coast of Washington state. ∑DDTs, ∑PCBs and HCB were determined by GC/ECD and lipid by gravimetric method (see Methods).

[d] Animals stranded on the coast of Washington state and in Puget Sound. ∑DDTs, ∑PCBs and HCB were determined by HPLC/PDA and lipids by TLC/FID (see Methods).

resulting in lipid stores not being adequately replenished; (4) poor nutritional condition due to ill health. It appears that leaching of lipid from blubber has occurred in some of the samples from stranded animals, but it is unlikely that leaching accounts entirely for the low lipid levels observed. Alternatively, the post-breeding season timing of the deaths or the inability of the whales to obtain sufficient prey may result in emaciation. Furthermore, emaciated animals with poor nutritional condition and low energy stores may be more susceptible to various diseases or toxins compared to an animal in better nutritional condition; thus, these conditions may be factors that contribute to strandings.

**Proportions of lipid classes in blubber**

Lipid class profiles were determined for all whale blubber samples analysed by TLC/FID. For the presumably healthy animals (1994 subsistence and 1996-1998 biopsy samples), triglycerides comprised the greatest proportion of the lipid (> 90%), with much smaller proportions of phospholipids and cholesterol (Fig. 2). These results agree with previous studies showing that blubber of healthy cetaceans consists primarily of neutral lipids, such as triglycerides and non-esterified free fatty acids (Kawai *et al.*, 1988; Tilbury *et al.*, 1997). In contrast, blubber samples from gray whale carcasses from the 1999 strandings, showing 'moderate' or 'advanced' decomposition, had substantially higher proportions of free fatty acids, cholesterol and phospholipids than did the blubber of the biopsy or subsistence animals (Fig. 2; Ylitalo, 1999). Even the blubber samples from stranded whales that were classified as 'fresh' showed a change in lipid class profile compared to biopsy or subsistence animals, particularly in the increased proportion of free fatty acids. One of the problems with obtaining good quality tissue samples from stranded animals is that tissues deteriorate rapidly following death. Furthermore, the blubber samples of 1999 stranded whales that were classified as having 'moderate' or 'advanced' decomposition contained lower mean lipid levels (9% and 4%, respectively; Fig. 2) compared to the samples designated 'fresh' (23%, $n = 7$; Fig. 2). These findings, showing changes in both lipid levels and profiles with degree of decomposition, are probably indicative of losses of neutral lipids due to decomposition and also to leaching of lipids from the blubber, resulting in retention of the more polar lipids (e.g. phospholipids) that are building blocks of biomembranes (Bohinski, 1987). Thus, the changes in lipid class profiles may be a good means of estimating the quality of a blubber sample from stranded cetaceans by determining its degree of decomposition.

**Gender-, reproductive status- and age-related differences in OC concentrations**

In the current study, gender was not determined for the biopsied whales and the 1994 subsistence whales were all juveniles, so only the stranded animals were available for the comparisons of OC concentrations in adult males and females. Although there were insufficient numbers of adult females and adult males in the two groups of stranded animals to perform valid statistical comparisons, there was a trend of higher OC concentrations in males compared to females of the same group (Tables 1 and 2), except for the lipid-adjusted concentrations for the males from the 1999

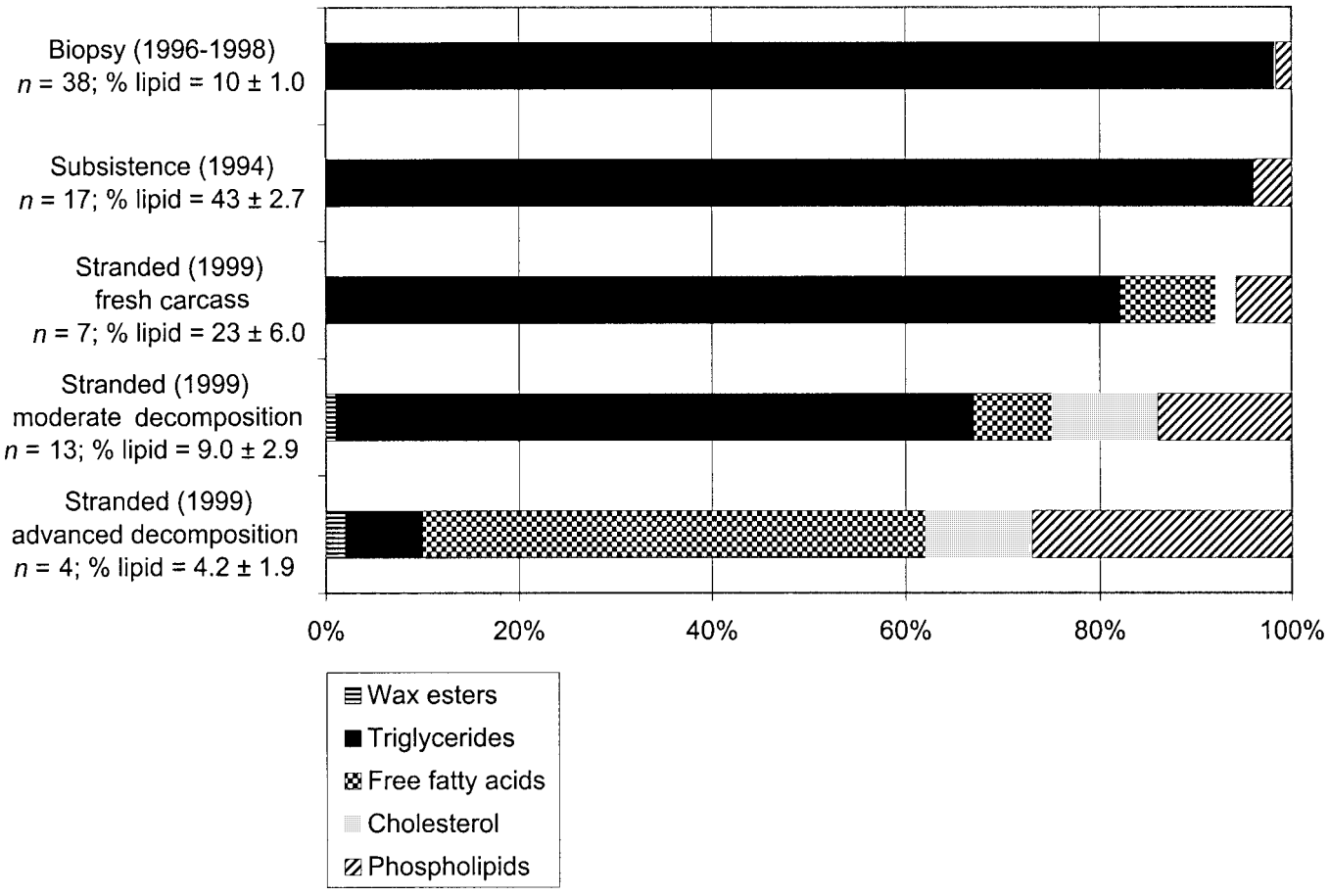


Fig. 2. Lipid class profiles for blubber of gray whales from the 1996-1998 biopsy, the 1994 subsistence and the 1999 stranded groups of animals. The blubber for the 1999 stranded whales has been grouped by degree of decomposition (as determined by person conducting necropsy) into 'fresh,' moderate and advanced groups.

Table 2

Length, percent lipid and concentrations (mean ± SEM) of ∑chlordanes, dieldrin, mirex in blubber of gray whales.

| | | | ng/g wet weight | | | ng/g lipid weight | | |
|---|---|---|---|---|---|---|---|---|
| | Length (cm) | Lipid (%) | ∑Chlordanes | Dieldrin | Mirex | ∑Chlordanes | Dieldrin | Mirex |
| **Subsistence (1994)**[a] | | | | | | | | |
| Female - juvenile (*n* = 13) | 884 ± 28 | 48 ± 6.1 | 160 ± 26 | 85 ± 17 | 1.6 ± 0.3 | 360 ± 54 | 180 ± 28 | 3.8 ± 0.6 |
| Male - juvenile (*n* = 4) | 833 ± 26 | 48 ± 11 | 97 ± 12 | 51 ± 7.5 | 1.6 ± 0.2 | 230 ± 45 | 120 ± 24 | 4.1 ± 1.0 |
| Overall mean (*n* = 17) | 872 ± 22 | 48 ± 5.2 | 150 ± 21 | 77 ± 14 | 1.6 ± 0.2 | 330 ± 45 | 170 ± 23 | 3.8 ± 0.5 |
| **Stranded (1988-1991)**[b] | | | | | | | | |
| Female - adult (*n* = 2) | 1,300 ± 0 | 1.4 ± 0.4 | 30 ± 11 | 9.5 ± 2.5 | No data | 2,600 ± 1,500 | 790 ± 410 | No data |
| Male - adult (*n* = 6) | 1,250 ± 13 | 3.7 ± 2.5 | 690 ± 420 | 340 ± 250 | 28 ± 15 | 62,000 ± 49,000 | 33,000 ± 29,000 | 2,500 ± 1,700 |
| Female - juvenile (*n* = 4) | 860 ± 36 | 14 ± 6.4 | 250 ± 70 | 110 ± 50 | 4.5 ± 1.2 | 3,900 ± 1,700 | 1,200 ± 350 | 94 ± 60 |
| Male - juvenile (*n* = 8) | 1,100 ± 39 | 13 ± 8.7 | 270 ± 81 | 120 ± 50 | 11 ± 4.4 | 11,000 ± 6,200 | 4,100 ± 2,100 | 440 ± 190 |
| Unknown (*n* = 2) | Unknown | 2.1 ±1.2 | 91 ± 75 | 32 ± 28 | 13 ± 11 | 3,500 ± 1,700 | 1,100 ± 700 | 490 ± 260 |
| Overall mean (*n* = 22) | 1,100 ± 36 | 8.7 ± 3.5 | 340 ± 120 | 160 ± 72 | 14 ± 4.6 | 22,000 ± 14,000 | 11,000 ± 8,000 | 940 ± 520 |

[a] Animals collected during subsistence hunts in the Russian Bering Sea. ∑Chlordanes, dieldrin and mirex determined by GC/ECD and lipid by gravimetric (shown) and by TLC/FID methods (see Methods).

[b] Animals stranded on the coast of Washington state. ∑Chlordanes, dieldrin and mirex determined by GC/ECD and lipid by gravimetric method (see Methods).

stranding. Although the current results are suggestive of a tendency of the mother to shift her contaminant burden to the calf, additional samples from adult gray whales will be needed to substantiate this transfer.

To establish whether concentrations of contaminants in blubber of gray whales increase with age, as reported previously for males of other species, the length (surrogate for age) of individual whales was compared to concentrations of contaminants in the blubber of the animals, grouped by gender. No statistically significant results were obtained, even for males. These results could indicate that there were insufficient numbers of animals in a group for a trend to be discernable or that contaminant concentrations are not related to length (or age) in gray whales. These results differ from those found for other species of marine mammals in which concentrations of contaminants in male animals increase with age and/or length (Tanabe *et al.*, 1982; Aguilar and Borrell, 1988; 1994; Muir *et al.*, 1992; Norstrom and Muir, 1994; Krahn *et al.*, 1997; 1999). As discussed earlier, female marine mammals generally transfer their contaminant burdens to their offspring, so no increase of contaminant concentrations is expected with age, except possibly following senescence.

**Comparisons of OC concentrations in juvenile whales**

As there were no significant differences in OC concentrations (lipid weight) by gender within each group of juveniles, OC data for male and female juveniles were combined and comparisons were made among three groups (1994 subsistence; 1988-1991 stranded; and 1999 stranded). In addition, a comparison was made to the whales (ages and genders unknown) sampled by biopsy (Fig. 3). The mean lipid-weight concentrations of three groups of analytes (ΣDDTs, ΣPCBs and HCB) were not significantly different for the two groups of stranded juveniles but concentrations of these analytes were significantly higher in the stranded whales than in the subsistence or biopsy animals (Fig. 3). In addition, lipid-weight concentrations of Σchlordanes, dieldrin and mirex were significantly higher for the 1988-1991 stranded whales compared to the 1994 subsistence animals (Fig. 3). The higher concentrations of these contaminants in the stranded juveniles compared to subsistence whales may be due to the retention of OCs in blubber as lipid stores are mobilised for energy and total lipid levels decrease, rather than from a difference in diet or feeding areas. This is supported by the earlier findings that there were no striking differences in the levels of OCs in tissues of stranded animals even though they stranded in areas that showed a wide range in the OC concentrations in sediment (Varanasi *et al.*, 1993; 1994).

Comparisons of the concentrations of ΣPCBs and ΣDDTs in tissues from the gray whales in this study to the values for baleen whales reported for other studies showed that the gray whales had concentrations (on a wet weight basis) similar to those in other mysticetes. For example, mean concentrations of ΣPCBs in blubber of bowhead whales from Alaska (O'Hara *et al.*, 1999), fin whales from the north Atlantic (Aguilar and Borrell, 1994) and minke whales (*Balaenoptera acutorostrata*) from western Greenland (Muir *et al.*, 1992) were 350, 732 and 610ng/g (wet weight), respectively. Mean concentrations of ΣPCBs in blubber of the gray whales in the present study ranged from 220-970ng/g (wet weight) for the four groups of whales (Table 1). Similarly, mean concentrations of ΣDDTs in bowhead, fin and minke whales were 130, 633 and 1,400ng/g (wet weight) respectively, compared to a range of 130-450ng/g for the animals in this study. In contrast, contaminant concentrations in an odontocete, the harbour porpoise, that feeds in coastal waters at a higher trophic level, contained mean blubber concentrations (wet weight) of 23,000ng/g for ΣPCBs and 9,100ng/g for ΣDDTs (Tilbury *et al.*, 1997).

**Distribution of contaminants among tissues**

When lipid concentrations were compared to concentrations (wet weight) of OCs in each gray whale tissue (i.e. blubber, brain, liver, kidney and muscle) from the 1994 subsistence study, lipid levels were significantly correlated to ΣPCBs ($r^2 = 0.894$; $p < 0.0001$), ΣDDTs ($r^2 = 0.802$; $p < 0.0001$), Σchlordanes ($r^2 = 0.850$; $p < 0.0001$) and HCB ($r^2 = 0.800$; $p < 0.0001$). This relationship is consistent with a study by Aguilar and Borrell (1985) reporting that lipid content is an important factor in controlling accumulation of lipophilic OCs. Furthermore, examination of our data showed that concentrations of OCs in the various tissues, with the exception of brain, were generally more comparable when the values were calculated on a lipid weight rather than on a wet weight basis (Figs 4 and 5). In brain tissues from the subsistence whales ($n = 6$), total lipid-normalised concentrations were significantly lower than in all the other tissues (Fig. 4). There was only one brain analysed for the

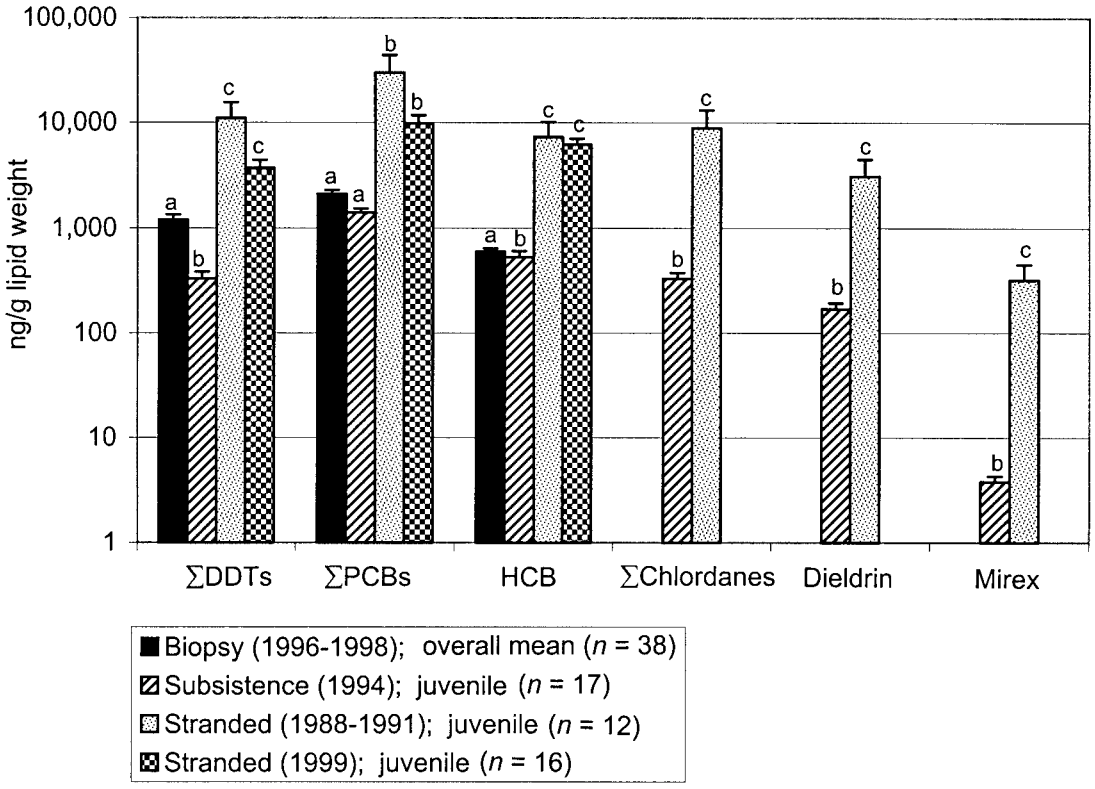


Fig. 3. Concentrations (ng/g, lipid weight) of OCs in blubber of juvenile whales. No significant differences in concentrations of an analyte were found among groups that have the same letter above their bars; groups that have different letters above their bars are significantly different from each other.

1988-1991 stranded whales (Fig. 5) and, although this brain sample showed lower OC concentrations than blubber and liver did, statistical significance cannot be assessed. The blood-brain barrier has been reported to control the transport of certain contaminants to brain tissue (Norton, 1980). In addition, the lipid in the brain of marine mammals consists of high proportions of polar lipids, i.e. phospholipids and cholesterol (Fukushima and Kawai, 1980; Aguilar and Borrell, 1985; Tilbury *et al.*, 1997), that have a lower affinity for OCs than neutral lipids have. Thus, the greater proportion of neutral lipids (i.e. triglycerides and non-esterified fatty acids) found in tissues other than brain favours the accumulation of OC compounds in these tissues (Kawai *et al.*, 1988).

## CONCLUSIONS

The primary conclusion of this synthesis of information about contaminants in gray whales is that caution must be exercised when comparing contaminant concentrations in animals having a range of biological factors (e.g. age, gender, reproductive status) that are sampled using various techniques (i.e. biopsy, subsistence hunts or strandings). For example, significantly higher lipid levels were found in the blubber of subsistence animals that were sampled during summer feeding in the Bering and Chukchi Seas, compared to the biopsied and stranded animals. The stranded whales had depleted lipid levels, presumably caused by factors such as reduced feeding during the winter breeding season, reduced availability of prey, ill health or leaching of the lipid from blubber tissues after death. The reduced lipid levels in the biopsied whales could be a result of stratification of lipids in blubber, resulting in lower lipid levels near the skin, but this theory needs to be tested through a direct comparison of lipid content in biopsy and necropsy samples from the same whales. Lipid class profiles in blubber were found to differ between presumably healthy gray whales (i.e. subsistence and biopsy) in which triglycerides comprised the greatest proportion of the lipid and stranded animals that showed moderate or advanced lipid decomposition (i.e. higher proportions of free fatty acids, cholesterol and phospholipids). Furthermore, changes in lipid class profiles were found to be a means of estimating the quality of a blubber sample from stranded cetaceans by determining its degree of decomposition.

Although results were not statistically significant due to low numbers of animals in each group, there was a trend of higher OC concentrations in males compared to females, thus suggesting the tendency of the mother to shift her contaminant burden to her calf during gestation and lactation. Results showing that there was no significant increase in concentrations of contaminants in the blubber with increase in length of male animals, unlike results reported for other male cetaceans, may indicate that there were insufficient numbers of animals in a group for a trend to be discernable or that contaminant concentrations are not related to length or to age in gray whales. The higher concentrations of OC contaminants found in stranded juvenile gray whales compared to juvenile subsistence whales are thought to result from retention of OCs in blubber of the stranded animals as lipid stores are mobilized for energy and total lipid levels decrease, rather than from a difference in diet or feeding areas. Concentrations of ΣPCBs and ΣDDTs in tissues of gray whales in this study were similar to those in other mysticetes. Our data showed that wet weight concentrations of OCs in tissues (e.g. blubber, liver, kidney, muscle, brain) varied widely, but with the exception of brain, OC concentrations were more comparable when the values were based on lipid weight, indicating that lipid content of tissues largely determines the

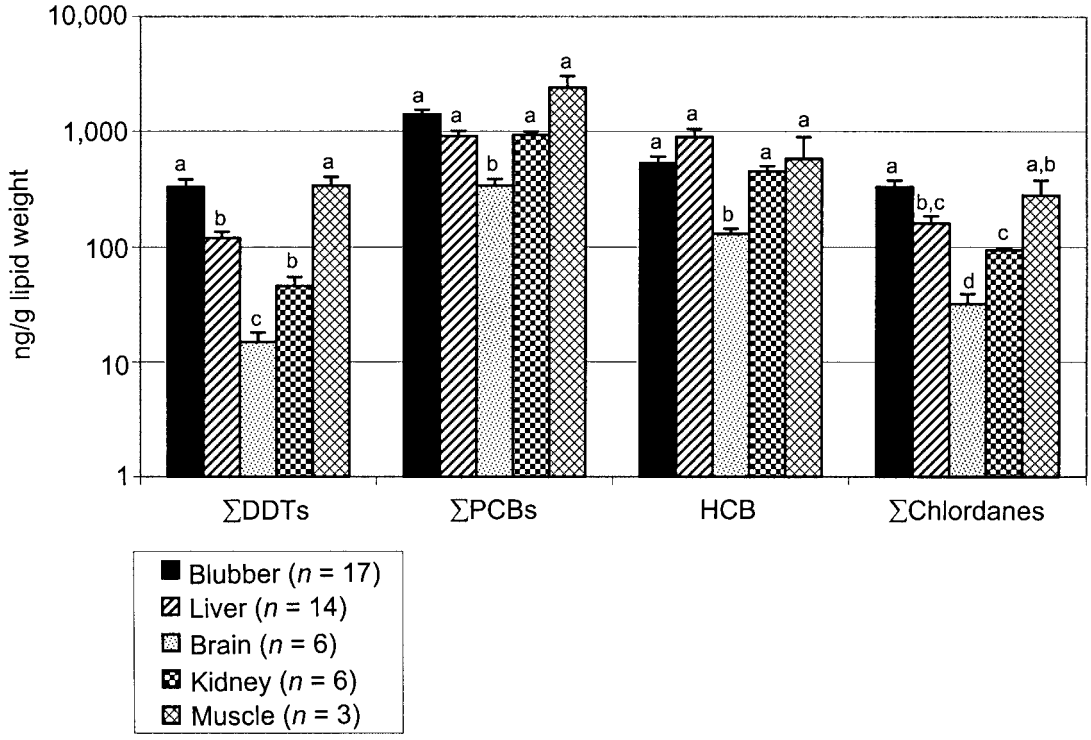


Fig. 4. Concentrations (ng/g, lipid weight) of OCs in blubber, liver, brain, kidney and muscle tissues of gray whales from the 1994 subsistence study. No significant differences in concentrations of an analyte were found among tissues that have the same letter above their bars; tissues that have different letters above their bars are significantly different from each other.

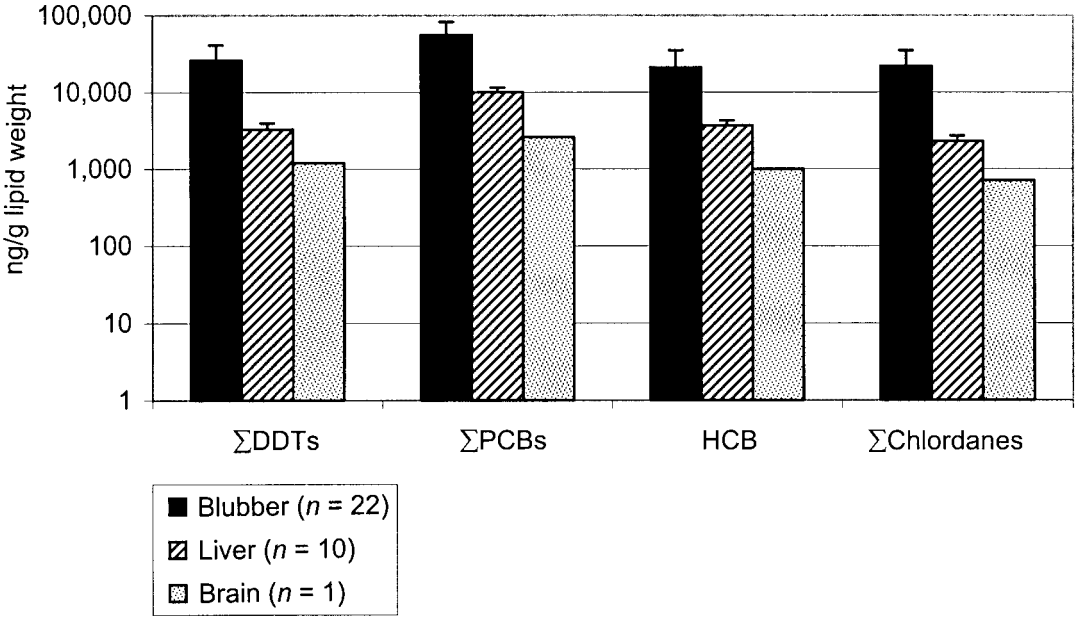


Fig. 5. Concentrations (ng/g, lipid weight) of OCs in blubber, liver and brain tissues of gray whales from the 1988-1991 stranded animals. No significant differences in concentrations for any of the analytes were found between blubber and liver tissues. The single brain sample was not included in the statistical analyses.

distribution of OCs. In brain tissues, total lipid-normalised concentrations were significantly lower than in all the other tissues, likely because the blood-brain barrier prevents transfer of contaminants to brain tissue.

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