Strandings, mortality and morbidity of Indo-Pacific humpback dolphins in Hong Kong, with emphasis on the role of organochlorine contaminants

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ABSTRACT

Factors related to mortality and disease in Indo-Pacific humpback dolphins (*Sousa chinensis*) from Hong Kong waters were investigated by detailed examination of dolphin specimens found stranded from 1995-2004. In total, 86 specimens were necropsied, but many of these were too badly decomposed to provide much information. Skin and blubber biopsies were also collected from six identified living individuals and concentrations of organochlorines (DDTs, PCBs and HCHs) were determined from blubber samples of stranded and biopsied dolphins. A large proportion of the strandings (53.2%) were young-of-the-year. The most commonly diagnosed causes of death were net entanglement and vessel collision. The pesticide DDT showed the highest concentrations and the ratio of DDT to its breakdown products (and other information) suggests that there may be a recent or nearby source of DDT into the dolphins' ecosystem. Concentrations of both DDTs and PCBs showed a pattern of increasing with age in males. In females, they increased until sexual maturity, then decreased, and finally increased again in late life. This is consistent with a hypothesised transfer of pollutants from mother to offspring during gestation and lactation. Inter-laboratory differences and effects of decomposition of specimens are two potential biases that may significantly affect the quality of the present data. In order to resolve the potential problems associated with these issues, a long-term biopsy collection programme has recently been initiated.

KEYWORDS: INDO-PACIFIC HUMPBACK DOLPHIN; BIOPSY SAMPLING; MORTALITY RATE; POLLUTANTS; ORGANOCHLORINES; CONSERVATION

INTRODUCTION

Mortality of small cetaceans is generally evaluated based on specimens obtained from strandings, or from those taken either directly or incidentally in fisheries. While fisheries catches provide fresh specimens, the catch is often biased in terms of age and sex. Strandings may give a better picture of 'natural' mortality; however, stranded specimens are often badly degraded from the actions of weathering and decomposition, and stranding rates may also show serious demographic and other biases (Reijnders *et al.*, 1999a; b). In Hong Kong, most strandings involve two species, the Indo-Pacific humpback dolphin (*Sousa chinensis*) and the finless porpoise (*Neophocaena phocaenoides*) (Parsons, 1998; Jefferson and Hung, 2004).

Humpback dolphins in Hong Kong face a number of potential threats. Although dolphins are known to get caught in fishing nets (Parsons and Jefferson, 2000), no large-scale fisheries interactions are known. Thus, strandings represent virtually the only source of carcasses for analysing mortality patterns, and for obtaining samples for analysis of various biological parameters. Therefore a programme was established in 1995 to document the occurrence of marine mammal strandings in Hong Kong, and to conduct necropsies of stranded specimens, when feasible (see Jefferson, 2000). Data and samples from the strandings are then analysed to examine patterns of mortality, life history and biology.

A long-term study into the effects of various environmental contaminants on humpback dolphins in Hong Kong has also commenced. This study integrates data from several sources, including sampling of stranded dolphins during necropsy, biopsy sampling of living dolphins and information obtained through our long-term programme of photo-identification of individual dolphins. By combining data from these various sources, a powerful programme has been initiated that avoids some of the biases and restrictions inherent in any single sampling design (see Wells *et al.*, 2003). For instance, photo-identification data can be used to monitor dermal disease, which may be associated with environmental contaminants (see Thompson and Hammond, 1992).

In this programme, three classes of contaminants were chosen for detailed examination, largely due to previous indications that they were especially problematic in this population (see Parsons and Chan, 1998; Parsons *et al.*, 1998; Minh *et al.*, 1999; Minh *et al.*, 2000a). The pesticide DDT (and its derivatives) was heavily used in past decades, because of its high toxicity to insects and its low cost. It has been banned in most developed countries, and also in China, but it may still be used illegally. Polychlorinated biphenyls (PCBs) are a group of several dozen compounds used in electrical equipment and in the manufacture of paints, plastics, adhesives, etc. (Clark, 1998). They are rarely used anymore, but their persistency ensures that they will continue to have damaging effects for many years. Lindane is another pesticide, which contains mostly hexachlorocyclohexane (HCH). It has toxic effects and it may still be used extensively in China (Clark, 1998).

The goals of this study were to determine the temporal and other patterns of humpback dolphin mortality in Hong Kong, and the main factors that are responsible for that mortality. In addition, we examined in detail the role of one particular threat to the dolphins, that of contamination of their environment by organochlorines.

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MATERIALS AND METHODS

Necropsy and sample collection

Even before the start of the current study, stranded humpback dolphin carcasses were examined in Hong Kong between 1993 and 1995 (see Parsons and Jefferson, 2000). In 1995, a dedicated stranding recovery programme was initiated. This involved soliciting stranding reports from the public, military and other government departments. The effort associated with soliciting and obtaining stranded carcasses was roughly similar throughout the study.

Over the course of the present study (September 1995 to December 2004) a total of 89 humpback dolphin mortalities in Hong Kong were confirmed. Necropsies were performed either in the laboratory (for fresh specimens) or in the field (for those that were badly decomposed or in relatively inaccessible locations). Basic biological data and samples were collected (see Parsons and Jefferson, 2000 for a detailed discussion of the stranding programme and sampling procedures). Veterinarians from Ocean Park Corporation or the Agriculture, Fisheries and Conservation Department (AFCD) of the Hong Kong Government were involved in many of the necropsies of fresh specimens. Specimens were classified as to their level of decomposition, codes 1-5, as outlined by Geraci and Lounsbury (1993).

In total, 86 specimens were necropsied and while most were also sampled for environmental contaminants, many specimens were badly decomposed and thus little usable information could be obtained from them. In addition, power failures caused several freezer breakdowns that resulted in significant additional decomposition of samples (see below). We generally followed the procedures of Jefferson *et al.* (1994) in conducting necropsies. Blubber samples, for organic contaminant analyses, were collected from the dorsal thoracic region, wrapped in aluminium foil and then frozen. In order to avoid cross-contamination, knives were thoroughly cleaned in soapy water and disinfectant between necropsies and storage containers were not reused. Gross pathology was noted during necropsies and (opportunistically) some samples were examined histopathologically. Blubber thickness was also measured on an opportunistic basis. For most specimens, 2-4 teeth from the middle of the lower left jaw were collected and age was estimated, as detailed in Jefferson (2000).

Biopsy sampling

For collection of biopsy samples from living dolphins, a Barnett Ranger RX-150 crossbow, with 150lb (68kg) draw weight was used. This crossbow shoots arrows at a speed of 69m s^{-1}. A red dot scope was used to assist in aiming and the senior author conducted all sampling. He had previous experience in crossbow biopsy sampling of four other species of cetaceans, as well as almost 10 years of experience observing the behaviour of the study animals. All biopsy sampling was conducted under appropriate permits from the Hong Kong Police Force and the AFCD.

Darts were ACC carbon fibre darts produced by *Ceta-Dart* and tips were made at the Scripps Institution of Oceanography (SIO) machine shop. Short 25mm biopsy tips were used (as opposed to longer 40mm tips). This was a conservative strategy, designed to reduce risk of injury or infection, but it yielded a smaller blubber core than was considered optimal for the studies on toxicology and reproductive biology (typically collecting only the outer one-half to two-thirds of the blubber layer). The tips had a sharpened, bevelled leading edge, which acted as a cutting surface, and there were three internal barbs to aid in sample retention.

The biopsy tips were soaked in bleach and 10% ethanol prior to being attached to the dart. This helped to reduce the chances of cross-contamination of samples and of infection. Shots were typically taken at target distances of 8-20m. The thoracic area just ahead of the dorsal fin was targeted. Photographs and video documentation were collected for all biopsy attempts (except for one successful attempt in which no video was obtained and another with no photographs).

When a biopsy sample was obtained, the tissue was removed from the biopsy tip by use of sterilised forceps. The skin sample was separated from the blubber using a single-edge razor blade, and the skin was stored in salt-saturated dimethyl sulfoxide (DMSO) solution, and then kept frozen. The blubber sample was bisected along its long axis. Both portions were frozen on dry ice and later in −30°C freezers, one for reproductive hormone studies and the other for determination of organic contaminant concentrations.

Contaminant analysis

For 59 specimens, three classes of contaminants in blubber tissue were examined in detail. These were DDT pesticide residues (DDTs), PCBs and HCHs. This selection was based on indications from earlier studies that these contaminants were the most critical, due to high levels in Hong Kong cetaceans and in some cases high known toxicity (Parsons and Chan, 1998; Minh *et al.*, 1999).

The analytical methods for PCBs and organochlorine pesticides (OCPs) for the biopsy samples followed those described in Richardson and Zheng (1999) and Zheng *et al.* (2004). Briefly, about 0.2g of subcutaneous blubber sample was spiked with 1ml each of internal standards, decachlorobiphenyl (1,012 ng ml^{-1}), C_{22} (8,160 ng ml^{-1}) and m-terphenyl (10,490 ng ml^{-1}). The sample was homogenised with 30ml dichloromethane (DCM) by a *K-Ultra-Turrax* T-25 homogeniser at a speed of 1,100rpm until all the blubber tissue was dissolved. After filtration with glass fibre of 70mm pore size (*Advantec*), the volume of each sample was reduced in a rotary evaporator and then passed through a silica gel column to remove impurities and lipids. After eluting PHCs from the column with 15ml of hexane, further elution from the column was conducted with a mixture of hexane:dichloromethane (8:2) and DCM for PCBs and OCPs, and PAHs.

Organochlorines in the second fraction were quantified by gas chromatography (GC-μECD; *Hewlett Packard* 6890 II series) equipped with an Agilent 7683 series automatic sampler. The GC column employed was a DB-5 capillary column (*J&W Scientific Inc.*, USA, 30m $\times$ 0.25mm internal diameter $\times$ 0.25μm film thickness). The column oven temperature was programmed at 110° held for 2min, increased to 180° at a rate of 10° min^{-1}, and then increased to 280° at a rate of 5° min^{-1} and held for 14 min. Injector and detector temperatures were set at 250°. Nitrogen was used as the carrier gas.

PCBs, HCHs, HCB, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, kepone, chlordanes, and DDT and its metabolites were monitored. Peaks of individual compounds were identified from those of their corresponding external standards. Organochlorine concentrations were calculated from the peak areas of individual compounds relative to the peak area of the internal standard. The PCB standard (SRM 2262) used for peak identification was a mixture with known composition and content, containing 28 congeners (PCB 1, 8, 18, 28, 29, 44, 50, 52, 66, 77, 87, 101, 104, 105, 118, 126, 128, 138, 153, 154, 170, 180, 187, 188, 194, 195, 200, 206). Concentrations of the 28 PCB congeners were determined, and summed values were then multiplied by two to obtain total PCB concentrations (Leung *et al.*, 2005). A procedural blank was analysed with every set of six samples to check for interfering compounds and correction was made, if necessary. Total DDTs represented the sum of *p,p'*-DDT, *o,p*-DDT, *p,p'*-DDD, *o,p*-DDD, *p,p'*-DDE and *o,p*-DDE. Chlordanes (CHLs) included *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane, while total HCHs included alpha, beta, gamma and delta isomers. Recoveries of target analytes using this analytical method were 99 ± 2.0 % for PCBs, 95 ± 7.5 % for DDTs, 96 ± 7.7 % for HCHs, 100 ± 4.7 % for CHLs, and 94 ± 5.9 % for HCB.

For the samples from stranded dolphins, the analytical methods for OCPs were those described in Minh *et al.* (1999; 2000a; b; c) and Ramu *et al.* (2005). In brief, about 3-8g of blubber sample was homogenised with anhydrous Na_2SO_4 and extracted with Soxhlet apparatus for 7-8h, with a mixture of diethylether:hexane (3:1) (v/v). Samples were passed through a dry Florisil column for removing fat. After eluting OCs from the dry column with a mixture of acetonitrile and water and partitioning in a separatory funnel, hexane extracts were concentrated. The first fraction eluted with hexane contained HCB, PCBs, *p,p'*-DDE, and *trans*-nonachlor while the second fraction eluted with 20% dicholomethane in hexane contained chlordane compounds (oxychlordane, *trans*-chlordane, *cis*-chlordane, *cis*-nonachlor), *p,p'*-DDD, *p,p'*-DDT, HCHs and TCPMe. The third fraction was collected with 50% dichloromethane in hexane for TCPMeOH separation.

Organochlorines in the first and second fractions (except TCPMe) were quantified by GC-ECD (Hewlett Packard 5890 II Series) equipped with a moving needle-type inject port. The GC-column employed was DB-1 (J & W Scientific Inc., USA) fused with a silica capillary column (0.25mm X 30m) coated with 100% dimethyl polysiloxane at 0.25µm film thickness. The column oven temperature was programmed from 60°C to 160°C, held for 19min and then increased to 260°C at a rate of 2°C min^{-1} and held for 30min. Injector and detector temperatures were set at 260°C and 280°C, respectively. Helium and nitrogen were used as carriers and make-up gases, respectively. Organochlorine concentrations were calculated from the peak area of the sample to the corresponding external standard. The PCB standard used for quantification was an equivalent of the Kanechlor mixture (KC-300, KC-400, KC-500, KC-600) with known PCB composition and content. Concentrations of individually resolved peaks of PCB isomers and congeners were summed to obtain total PCB concentration. For TCPMe and TCPMeOH quantification, a GC-MS (Hewlett-Packard 5890 coupled with 5970 mass selective detector) was employed. Data acquisition was carried out by *HP* 5970C Data system, in which cluster ions were monitored at m/z 139, 253, 251, 362, 364 for TCPMeOH and 311, 313, 346, 348 for TCPMe.

Isomer-specific analysis of PCBs, including coplanar congeners, was conducted following the alkaline-alcohol digestion method (Tanabe *et al.*, 1987). Quantification was carried out using a GC-MS (Hewlett Packard 5890 II Series in coupled with 5970 mass selective detector). A Hewlett Packard 5970 data system was used for quantification, in which cluster ions were monitored at m/z 256, 292, 326, 360, 394 and 430 for tri-, tetra-, penta-, hexa-, hepta- and octa-chlorobiphenyls, respectively. Recoveries of OCs through analytical procedure are shown below. Concentrations were not corrected for recovery percentages.

There are some differences between Ramu's and Minh's method. Firstly after Soxhlet extraction, in Ramu's method, aliquot of the extract was subjected to gel permeation chromatography (GPC) GPC for lipid removal. The GPC fraction containing OCs was concentrated and passed through an activated Florisil column for clean-up and fractionation before quantification. Secondly, the PCB standard used for quantification was a mixture of 62 PCB isomers and congeners (BP-MS), instead of using Kanechlor mixture. Concentrations of individually resolved peaks of PCB isomers and congeners were summed to obtain total PCB concentrations. Most laboratory analyses were conducted by Prof. S. Tanabe and his colleagues (T.B. Minh and K. Ramu) of Ehime University in Japan (see Minh *et al.*, 1999; 2000a; b; c for laboratory protocols). For some additional specimens, frozen tissue samples were sent to a commercial ecotoxicology laboratory in Hong Kong (ALS Technichem [HK] Pty, Ltd.) for chemical analyses. The methods used there were the same as those of Minh *et al.* (1999).

Due to the three different laboratories conducting the analyses, there is significant potential for bias in comparing across datasets (Krahn *et al.*, 2003b; see below). Therefore, such comparisons were avoided and mainly comparisons were conducted in which each group had some data from different labs. Some of the data from the present dataset have been analysed previously in other studies (e.g. Minh *et al.*, 1999; Minh *et al.*, 2000a; b; c; Ramu *et al.*, 2005; Leung *et al.*, 2005).

RESULTS

Strandings and mortality rates

Since the first year of complete data (1996), there has been no consistent trend in the number of humpback dolphin strandings per year; the annual mean was 9.7 strandings (Fig. 1). In fact, the numbers have been relatively stable, with 6-14 strandings per year. This is especially true when compared with the number of finless porpoise strandings, which fluctuated more erratically in the last few years, and strandings of other species, which appear to be on the increase (Fig. 1).

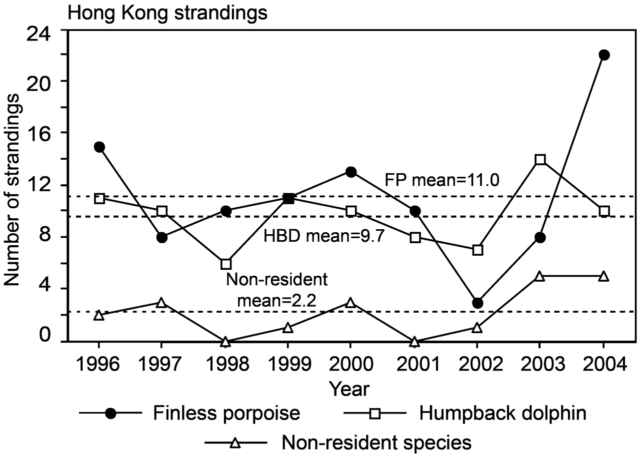

Fig. 1. The annual number of strandings of humpback dolphins (HBD), finless porpoises (FP) and other species in Hong Kong over the study period. The annual means are shown as dotted lines.

Only one humpback dolphin live stranding was recorded (SC03-08-08) during the study (although live strandings of finless porpoises, a rough-toothed dolphin (*Steno bredanensis*), false killer whale (*Pseudorca crassidens*) and sperm whale (*Physeter macrocephalus*) were also recorded). This was specimen SC03-08/08, which was found stranded alive by a local villager at Sam A Tseun, Double Haven, on 8 August 2003, and died after rehabilitation attempts at Ocean Park on 13 August. The animal was a 244cm sexually- and physically-mature female (with several ovarian scars and fully-fused vertebral centra), which was later aged at 27 growth layer groups (GLGs) (1 GLG is assumed to equal 1 year). At the time of death it weighed 148kg.

Young-of-the-year were defined as specimens <137cm in length as the estimated length at one year is 137cm. A very large proportion of all humpback dolphin strandings in Hong Kong were <137cm (Fig. 2). Of 79 specimens that could be placed into a length category, 42 (53.2%) were young-of-the-year. This is an apparently high proportion, although there are few other datasets available for comparison.

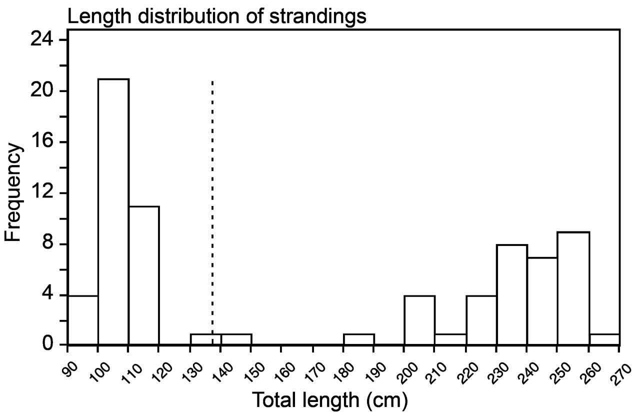

Fig. 2. Distribution of the total lengths of dolphins stranded in Hong Kong over the course of the study. The dotted line represents the criterion for considering specimens to be young-of-the-year (137cm total length).

Humpback dolphin strandings occurred throughout all months of the year (Fig. 3). Strandings of subadults and adults did not show any consistent pattern throughout the year. However, the monthly pattern of strandings of young-of-the-year showed a large peak during May-August (Fig. 3). As pointed out by Aguilar (1991), this can be viewed as an indicator of seasonal distribution of natural mortality (provided that certain conditions are met). Thus, it is clear that most of the natural mortality of young-of-the-year occurs in the four-month period from May to August, which is partly a reflection of the seasonality of calving (see Jefferson, 2000).

Of the animals that could be assigned to a sex, 62% were males (n=57). The sex ratio of stranded specimens was strongly biased towards males, both for specimens <150cm in length (1.60:1) and those >150cm (1.64:1). It should be noted that the gender of some specimens was not 100% certain, and these specimens were not used in the above analysis. If there are no sampling biases influencing this, then this indicates that more males than females died during the study period. This is an interesting finding, especially in light of the sex differences in patterns of contamination by organochlorines presented below.

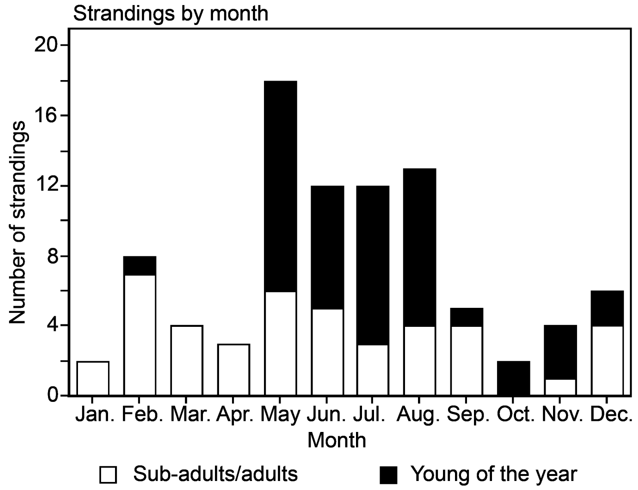

Fig. 3. The monthly distribution of humpback dolphin strandings in Hong Kong.

Causes of mortality and morbidity

Determination of cause of death was seriously hampered by the fact that most of the stranded specimens were badly decomposed. Of the 86 specimens for which the decomposition code could be determined, only one (1%) was code 1, 5 (6%) were code 2, 22 (26%) were code 3, 56 (65%) were code 4, and 2 (2%) were code 5. Since cause of death is nearly impossible to determine in most late code 3, code 4 and code 5 specimens, this left only a small number of specimens (<20%) in which determining the cause of death was feasible.

Of the 89 humpback dolphin strandings that occurred since the start of the study (through December 2004), cause of death could only be determined with certainty for 10 specimens, although a possible cause of death was diagnosed for three others. Three specimens were diagnosed as having died from net entanglement, four from vessel collisions, one from debris (in this case, net) ingestion, one from a heart or brain pathology, and one from a bone infection. For specimens that showed clear evidence of being struck by a vessel, there was the possibility that they died from some other cause and were then struck while floating after death. Therefore, specimens were only classified as dying from vessel collision if there was evidence that the animal was struck while still alive (e.g. blood infusion in the tissue around propeller cuts).

It was not possible to assign any deaths to high contaminant levels, but it was suspected that the high concentrations of contaminants (especially DDTs and PCBs) in some specimens may have led (directly or indirectly) to their deaths. Over the next few years it is hoped that a greater understanding of this issue will be achieved through new avenues of research (see below).

Dolphins are injured and even killed by nets and vessels, however not all serious injuries result in death. Several dolphins in our photo-identification catalogue show evidence of major injuries to the dorsal fin and back, apparently caused by boat propellers, vessel collisions, or rope/net cuts (Fig. 4). Between 2.9 and 6.8% of the animals show evidence of rope or net cuts and between 1.2 and 1.8% show evidence of propeller scars. Thus, it appears that up to 8.6% of animals have survived previous non-fatal encounters with human activities. It is rather remarkable that these animals can survive such serious injuries, which generally appear to heal well, despite Hong Kong's contaminated waters.

Fig. 4. Four photo-identified individuals showing evidence of human-caused injury to the dorsal fin and/or back.

The live-stranded dolphin showed evidence of epidermal disease when it stranded and necropsy confirmed extensive skin lesions consisting of fissures/cuts and severe bilateral thickening/hypertrophy of the epidermis along the flanks (see Fig. 5). This dolphin had previously been identified at sea from photos as CH76, which had been observed three times before it stranded, in both Hong Kong and Chinese waters. The first time was in Chinese waters just west of Lantau Island on 10 September 1998. It showed no evidence of dermal disease at this time. The second time was in the West Lantau area on 16 April 2002. The skin disease that the dolphin had when it stranded was clearly present in the photos taken in April 2002, as well as in photos from a sighting on 26 February 2003, and so the dolphin had the condition for at least 16 months. Laboratory analyses of a biopsy of infected skin showed parakeratosis and acanthosis, with a mild chronic inflammatory infiltrate in the papillary dermis and around the capillaries. There was no evidence of fungal or bacterial microrganisms, nor of any malignancy. The skin condition was not thought to be related to the cause of death, which was probably from a brain or heart pathology. Laboratory results of samples taken at necropsy for confirmation are pending and contaminant data are not yet available for this specimen.

Contaminant concentrations

In general, for organochlorines, concentrations were high for cetaceans and on average males had concentrations several times higher than those of females. For ΣDDTs, males averaged 117.3μg g^{-1} wet wt. (± SD 125.66, range = 5-380, *n*=22) and females averaged 28.8μg g^{-1} wet wt. (± SD 19.11, range = 5-76, *n*=17). For ΣPCBs, males averaged 31.7μg g^{-1} wet wt. (± SD 23.21, range = 2-83, *n*=22) and females 10.8μg g^{-1} wet wt. (± SD 7.80, range = 1-30, *n*=17).

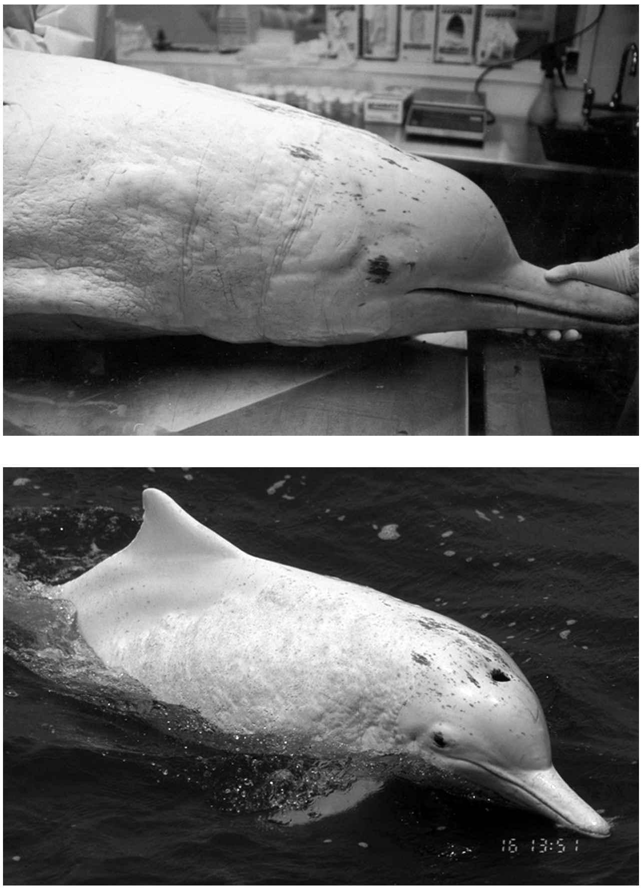

Fig. 5. Humpback dolphin live-stranded in August 2003 (SC03-08/08) showing evidence of a skin disease (see text). The lower photo shows the same dolphin on 16 April 2002, swimming off the west coast of Lantau Island, with evidence of the disease already present.

There was considerable variability, and often animals of the same sex and similar age showed widely scattered values (Fig. 6). ΣHCH concentrations for males averaged 0.9μg g^{-1} wet wt. (± SD 0.69, range = 0.1-2, *n*=16) and for females 0.2μg g^{-1} wet wt. (± SD 0.19, range = 0-0.5, *n*=6).

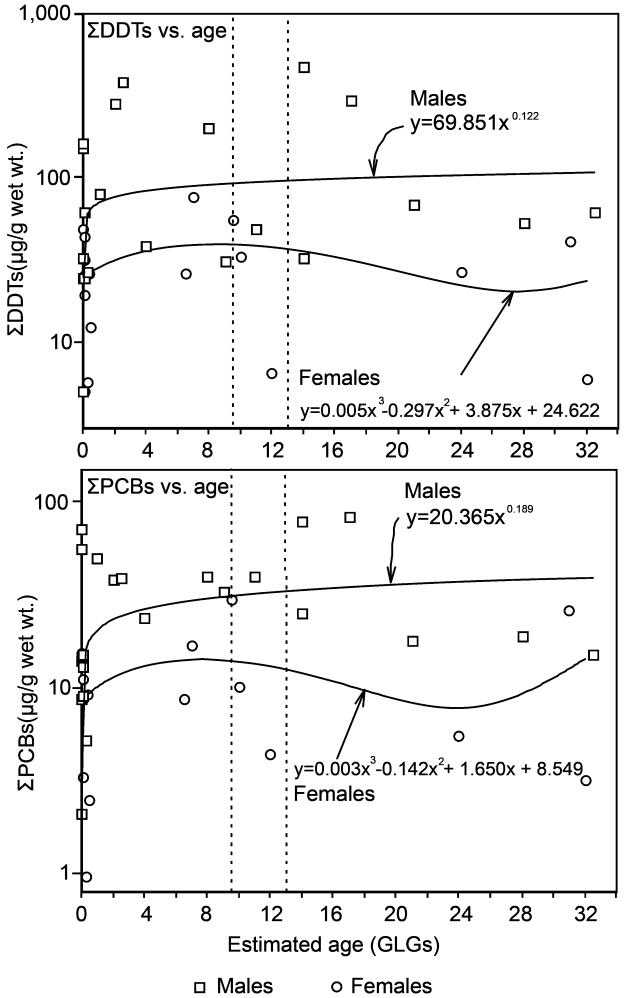

Fig. 6. Developmental patterns of ΣDDTs (upper) and ΣPCBs (lower) for male and female dolphins in Hong Kong. Contaminant data are presented on a logarithmic scale. The dotted lines represent the average age at sexual maturity for females and males.

For DDTs, most males had somewhat lower levels at birth, and then tended to have slightly increasing concentrations in later age classes (Fig. 6). The pattern for females was for much lower levels, which tended to increase somewhat until about 8-10 years of age, then to decrease until about 28 years and then to increase again (Fig. 6). When comparing the present data to those of bottlenose dolphins in the southeast United States (which have a similar life history – see Wells, 2000), DDT concentrations tend to be much higher for Hong Kong humpback dolphins, while PCBs are generally lower (except in adult females, in which they are slightly higher – Table 1). In both species, adult females had the lowest levels, but the reduction for adult females was not as pronounced in our data as it was for the bottlenose dolphin data (see Schwacke *et al.*, 2002; Hansen *et al.*, 2004).

Over time, DDT breaks down and is metabolised into DDD and DDE, and the relative proportions of these three compounds in the ΣDDTs can tell us something about the timing of input of the DDT into the system (see Aguilar, 1984; Parsons and Chan, 1998). On average, in this study only a small proportion of the ΣDDTs was made up of DDT and the largest amount was made up of DDE, although there was substantial variability (Fig. 7). The average proportion of DDT:DDD:DDE was 18.4%:28.5%:53.1%. The proportion of DDE is actually relatively low in comparison to that found in marine mammals from other regions of the Pacific, where it typically makes up between 70 and 95% (Prudente *et al.*, 1997). This suggests that the DDT in Hong Kong is from a relatively recent or nearby source, and may still be entering the dolphins' ecosystem, despite being banned in China in the early 1980s. Recent investigations indicate that a pesticide named Dicofol, currently being used in China to control mites in orange and eggplant growing areas, contains DDT as an impurity. A sample of Dicofol has been analysed and found to contain 2.4% DDT, which constitutes 1.8% of the active ingredient (PKSL). This is a serious concern, which needs to be addressed.

Development of PCB concentrations showed a broadly similar pattern to those of DDTs, with males having in some cases very high levels even at birth, and then a tendency to increase fairly rapidly (Fig. 6). Females also showed a similar pattern to DDTs, with an initial increase until about 8-10 years and then a decrease, and finally another increase late in life (Fig. 6). Unfortunately, there were not enough data to examine developmental patterns for HCHs.

There was a correlation between DDT and PCB concentrations; however this was not a one-to-one relationship (Fig. 8). Interestingly, DDTs (reaching nearly 500μg g^{-1} wet weight) tended to increase much more rapidly than PCBs, which remained below 100μg g^{-1} wet weight. Again, this is consistent with the idea that there may be a localised source of DDT into the western marine waters of Hong Kong (see Parsons and Chan, 1998).

Among the three classes of organochlorines analysed, DDTs made-up the largest fraction (68-78%) in all age classes, while PCBs only made-up 22-32%. HCHs made-up a negligible proportion (<0.01%) in all classes. However, there was an interesting difference between juveniles/adult males and adult females. Compared to the other age classes, adult females had a lower proportion of DDTs (68% vs. 76-78%), and a correspondingly higher proportion of PCBs (32% vs. 22-23%). A similar situation was found for several bottlenose dolphin populations in the southeast United States, and it is thought that this is due to DDT compounds being more efficiently transferred from mother to calf than PCBs (Hansen *et al.*, 2004).

Organochlorine concentrations in blubber samples of the biopsied dolphins are shown in Table 2. These are the first contaminant data we have been able to obtain from living, free-ranging dolphins of this population, and these data are not subject to the serious biases that may affect our stranding samples (see below). However, it must be noted that the biopsy sampling did not sample the more active inner layer that probably has the highest concentration of contaminants. The quality and reliability of the biopsy data are much higher than for strandings. When comparing data from biopsy samples with those from stranded samples of various levels of decomposition, indications are that the DDT levels are substantially lower in the biopsied dolphins than in the stranded specimens and it appears that in general decomposition and/or stranding increases the apparent concentrations of DDTs in the blubber (Table 3). There does not appear to be a similar relationship for PCBs and HCHs; however, it must be cautioned that this analysis is based on a very small sample. Further biopsy data are required to conduct a more reliable analysis.

Table 1

Summary statistics for major classes of OCs (μg g^{-1} wet weight) for Hong Kong humpback dolphins. Data from bottlenose dolphin populations along the US east coast are from Schwacke *et al.* (2002) and Hansen *et al.* (2004).

Contaminant	Age class	Hong Kong *n*	Mean	SD	Range	Bottlenose dolphin means	Hong Kong levels
∑DDTs	Juveniles	31	67.4	83.50	3-380	17.8-28.8*	Much (2-4x) higher
	Adult males	7	132.7	164.63	5-470	12.6-51.9*	Much (5-11x) higher
	Adult females	6	28.1	17.60	7-55	4.0-4.4*	Much (6-7x) higher
∑PCBs	Juveniles	31	20.2	18.39	1-72	38.3-86.2	Much (2-4x) lower
	Adult males	7	37.1	27.98	11-83	70.3-91.2	Much (2x) lower
	Adult females	6	13.2	11.75	3-30	4.2-10.3	Similar to 3x higher

*Data in μg g^{-1} lipid weight basis.

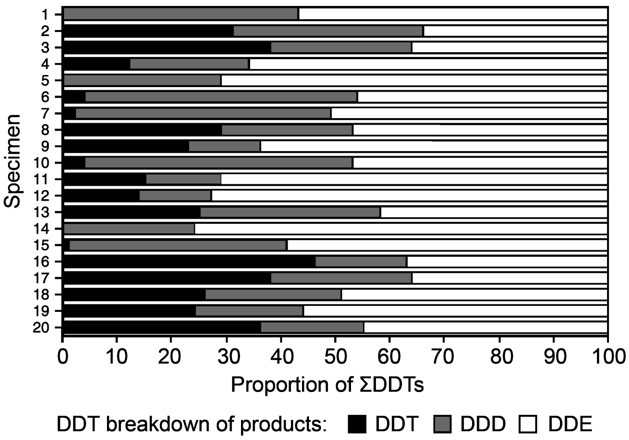

Fig. 7. Relative proportions of DDT and its metabolites in the total DDTs from dolphins stranded in Hong Kong.

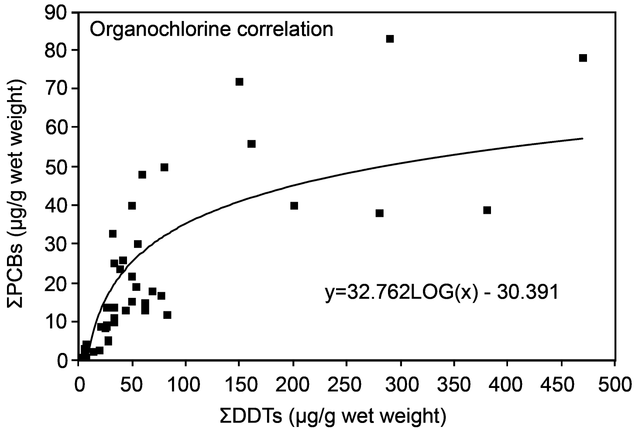

Fig. 8. Correlation between ΣDDTs and ΣPCBs in blubber samples from dolphins stranded in Hong Kong.

DISCUSSION

Potential biases

It is important to recognise the significant limitations of the currently available data on ecotoxicology for these animals.

Interlaboratory variability

Different laboratories use somewhat different techniques and equipment for conducting their work. Data on environmental contaminants in this study came from three different labs, and it is recognised that interlaboratory differences can be significant (Krahn *et al.*, 2003b). There were few cases of duplication to check the variability involved, but when duplication did occur it suggested that the variability could be significant. In two cases, two different labs examined blubber samples from the same specimen and arrived at quite different results (51 vs. 2.8μg g^{-1} for ΣDDTs and 1.7 vs. 9.4μg g^{-1} for ΣPCBs). These specimens were analysed at different times (years apart), and it is possible that intervening freezer breakdowns may have resulted in some real differences (see below), but nonetheless caution is required. Due to this potential problem, no attempts to analyse temporal trends in the contaminant dataset were made, since samples from different time periods were analysed by different labs. However, we do not believe that this possible bias will have a serious effect on the analyses presented here since, with one exception[1], all the comparisons presented use data from multiple labs in each group for comparison. The effect of the inter-laboratory variability will therefore be to exaggerate the variability in the compared groups and make determinations of differences more difficult. While this is not desirable, it is a more conservative and therefore more cautious approach than a situation in which the detection of apparent (but not real) differences were encouraged.

Effects of decomposition

As has been stated above, the vast majority of the specimens available for this study were badly decomposed (codes late 3 and 4). Decomposition is known to alter contaminant levels in an unpredictable manner (Borrell and Aguilar, 1990). In addition, the several freezer breakdowns resulted in significant additional decomposition of samples. Some evidence was found that DDT, but not PCB and HCH concentrations, may be significantly affected by decomposition. It is not possible to reliably evaluate the effects of the various levels of decomposition, but it must be considered when interpreting the data. Clearly, it would be better to deal with data from fresh specimens, where such problems are avoided.

Problems with using stranded specimens

The limitations of using samples collected from stranded specimens for contaminant studies must be acknowledged. Besides the obvious problems of decomposition (discussed above), there are potentially strong biases associated with the fact that specimens that strand are clearly not representative of the population as a whole. As many of these animals are sick, they are not good subjects for examining the levels of contaminants that the population is experiencing. This was clearly demonstrated by the results

[1] The only exception is the situation in which we compared biopsy samples (all done by one lab) with stranding samples (all from two others).

Table 2

Concentrations of organochlorines (ng g^{-1} wet weight) in blubber biopsy samples collected in October and November 2004 in Hong Kong waters.

Specimen No.	HKB 1	HKB 2	HKB 4	HKB 5	HKB 6
Gender	Female	Male	Female	Male	Male
Repro Status	Unknown	Mature?	Mature?	Unknown	Unknown
Blubber wet (g)	0.09	0.16	0.09	0.11	0.10
∂ -HCH	27.33	25.94	46.11	15.36	8.50
ß-HCH	295.00	149.07	684.69	2,113.33	1,309.89
gamma-HCH	13.56	3.94	18.67	941.09	7.80
delta-HCH	7.89	3.56	7.89	15.55	128.30
∑ HCHs	343.78	182.51	757.36	3,085.33	1,454.49
HCB	8.00	30.50	17.00	40.82	117.10
Heptachlor	59.56	17.31	82.33	59.00	250.20
HE	495.78	139.44	215.11	285.27	106.60
Aldrin	10.44	12.88	75.89	12.00	87.40
Dieldrin	444.33	4,880.31	211.11	2,181.27	5,798.60
Endrin	113.89	149.44	2,626.67	217.36	17.10
Kepone	70.89	1,097.06	780.11	1,308.55	25.50
r- Chlordane	61.67	112.63	41.11	233.36	91.30
a-Chlordane	44.11	201.61	66.91	42.69	397.40
∑ Chlordanes	105.78	314.24	108.02	276.05	488.70
op-DDE	11.21	90.00	9.05	36.36	10.03
pp'-DDE	734.89	5,944.75	3,918.67	2,696.55	6,343.50
op'-DDD	28.33	548.38	60.67	95.00	158.40
pp'-DDD	315.44	3,706.25	1,883.78	891.27	2,497.10
op'-DDT	362.22	1,772.81	331.00	516.09	1,319.90
pp'-DDT	560.33	4,585.69	1,778.78	637.36	3,708.90
∑ DDTs	2,012.42	16,647.88	7,981.95	4,872.63	14,037.83
PCB 1	135.56	631.38	445.78	914.09	894.90
PCB 8	120.00	227.81	285.00	122.73	81.00
PCB 18	0.00	0.00	0.00	0.00	133.40
PCB 29	39.89	890.81	73.33	468.73	515.84
PCB 50	0.00	0.00	49.11	40.18	44.20
PCB 28	0.00	0.00	0.00	0.00	0.00
PCB 52	609.00	467.75	796.44	785.73	864.30
PCB 104	0.00	0.00	0.00	0.00	325.10
PCB 44	197.22	50.63	273.78	288.45	317.30
PCB 66	0.00	329.06	147.56	144.91	310.50
PCB 101	198.67	494.50	144.56	140.36	360.50
PCB 87	435.11	1,029.94	2,442.56	1,285.45	344.90
PCB 77	86.33	113.31	120.89	108.27	11,085.60
PCB 154	0.00	0.00	0.00	0.00	450.70
PCB 118	0.00	3,549.25	0.00	46.36	51.00
PCB 188	0.00	1,451.38	128.11	85.00	346.60
PCB 153	126.22	460.25	302.89	167.09	270.10
PCB 105	0.00	343.25	0.00	0.00	0.00
PCB 138	257.44	126.75	553.56	371.27	1,682.90
PCB 126	0.00	1,201.69	0.00	0.00	129.00
PCB 187	168.33	228.06	193.11	91.73	440.60
PCB 128	39.56	166.00	60.56	57.73	209.80
PCB 200	24.44	72.06	53.45	11.64	35.60
PCB 180	111.89	953.25	479.56	231.82	696.70
PCB 170	12.00	416.44	149.22	91.73	292.70
PCB 195	6.56	26.06	0.00	3.73	33.60
PCB 194	55.56	740.88	307.11	59.91	461.50
PCB 206	7.33	66.88	11.89	42.09	4.60
PCB 209	17.11	131.69	111.56	29.18	29.20
∑ PCBs	5,296.44	28,336.12	14,260.02	11,176.36	40,824.28

of the study by Jepson *et al.* (2005). While these specimens represent the major source of data, alternative data sources are required.

Potential problems of stratification of blubber layer

The blubber biopsy samples collected in this project did not necessarily sample the entire blubber depth; the relatively short biopsy tips were used to avoid potential problems of penetrating the muscle layer below. Due to indications of stratification of contaminants within the blubber layer (e.g. see Krahn *et al.*, 2003a), there may be some bias in the biopsy contaminant results. These were minimised by splitting the blubber sample longitudinally, but some potential bias still remains.

While such potential biases may limit the reliability of the toxicology results of this study, the present study represents a first step and we view the results as preliminary. Despite this, we do not believe that the analyses presented here are significantly biased. An important lesson from this preliminary study is that future studies must attempt to overcome the potential problems discussed above. Significant advantages can be achieved through a dedicated programme of biopsy sampling (see below).

However, biopsy sampling also has the potential for some bias. This is mainly due to blubber stratification. How much this affects the data in Tables 2 and 3 is not yet known. However, by obtaining larger samples of biopsy data for comparison, and also by conducting specific studies comparing organochlorine concentrations from 'full-depth' blubber samples with subsamples taken from only the outer layers (e.g. see IWC, In press) it is hoped this can be further understood.

Levels and causes of mortality

While Cockcroft (1991) found that 28% of the humpback dolphins he examined from South Africa showed either recent or healed shark attack wounds, no evidence of such wounds was found on any of the dolphins examined in this study. Although at least three species of large, predatory shark potentially pose a threat to dolphins in Hong Kong, the great white (*Carcharodon carcharias*), tiger (*Galeocerdo cuvieri*) and bull (*Carcharinus leucas*) sharks (see Parsons and Jefferson, 2000), only the latter two are known to occur regularly in estuaries (where the dolphins are found). In fact, within Hong Kong, records of sharks in the western, estuarine waters are rare and we have not observed a shark in over 10 years of intensive surveys of these waters.

Comparison of the present results on strandings and mortality with those from other coastal and inshore dolphin populations is difficult since there are few other comparable published results.

Table 3

Effects of decomposition level on organochlorine concentrations (µg g^{-1} wet weight). Code 1 specimens are from biopsy samples of living individuals.

		∑DDTs		∑PCBs		∑HCHs	
Decomp code	*n*	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
1 (biopsy)[#]	5	9.1 ± 5.49	2.0-16.7	20.0 ± 12.88	5.3-28.3	1.2 ± 1.06	0.2-3.1
2	1	55.0	-	30.0	-	0.4	-
3	15	63.5 ± 111.30	5.1-470.0	20.3 ± 20.93	3.3-78.0	0.6 ± 0.48	0.0-1.4
4	27	82.3 ± 95.90	3.3-380.0	22.7 ± 21.06	0.8-83.0	0.7 ± 0.63	0.1-2.2
5	0	nd	-	nd	-	nd	-

[#]It should be recognised that, while any differences between code 1 and other samples are probably mainly a result of decomposition, there are other differences that could also explain them (see text for additional discussion).

The one exception is the long-term research programme on bottlenose dolphins along the west coast of Florida, USA, with stranding data extending back 18 years (see Wells, 2000; Wells *et al.*, 2004). Bottlenose dolphins in this area are ecologically similar to the Hong Kong/PRE humpback dolphin population, inhabiting mostly inshore waters, having similar life history characteristics and even sharing a spring to summer calving peak (see Wells, 2000). The yearly number of strandings in Florida (mean=17 – Hurst *et al.*, unpublished[2]) is broadly similar to that in Hong Kong (mean=9.7). Among the major diagnosed causes of death in Florida were fisheries/human interaction, and trauma of unknown possibly anthropogenic origin (see Hurst *et al.*, unpublished). Their results are similar to those presented here, indicating that fisheries interactions and vessel collisions may be the major causes of death.

There are some differences between the datasets, however. For Florida bottlenose dolphins, the proportion of males among the known-sex strandings (51.8% – Hurst *et al.*, unpublished) is substantially lower than in the study presented here, in which it comprises 62.1%. It thus appears that a higher proportion of males may be dying in Hong Kong waters vs. Florida waters. Finally, the proportion of young-of-the-year appears to be much higher in Hong Kong waters. For Florida, only 14.5% of strandings are <125cm neonates, and even when one considers the proportion of animals less than three years of age (<210cm), the proportion still only comprises 44% (Hurst *et al.*, unpublished). Along the Atlantic coast of the United States (Massachussetts to South Carolina), bottlenose dolphin young-of-the-year made up between 17.7 and 26.6% of strandings (Mead and Potter, 1990); in South Carolina, young-of-the-year (<184cm) made up 39.9% of strandings (McFee and Hopkins-Murphy, 2002); and in Texas they made up 20% (Fernandez and Hohn, 1998). By contrast, in Hong Kong 53.2% of all our strandings are of animals estimated to be less than one year old. Even more striking is that the vast majority of these animals are clearly less than a few months old. The much higher proportion of young calves among strandings in Hong Kong is consistent with the hypothesis that organochlorines are having a significant impact on dolphin survival (see below).

Effects of contaminants

In recent years, considerable work has been done on organochlorines and their effects on cetaceans (e.g. see Reijnders *et al.*, 1999a). Risk assessment studies have indicated that the high levels of organic contaminants in Hong Kong waters have probably caused damage to the marine environment and to seafood consumers (Connell *et al.*, 1998). Organic chemicals (including PCBs, hydrocarbons and pesticides such as DDT) are a potential threat to cetaceans, because they bioaccumulate in top predators and are passed from generation to generation; due to the absence or reduction of certain enzymes, cetaceans have a low capacity to metabolise (and thus detoxify) these compounds (Tanabe *et al.*, 1994).

Organochlorine concentrations in Hong Kong humpback dolphins are relatively high, and DDT and PCB levels are even higher than in the finless porpoise population that occurs in Hong Kong (Jefferson *et al.*, 2002a; Ramu *et al.*, 2005). This is not surprising, as the dolphins live in the estuary of the Pearl River, and are probably nearer the presumed source of the contaminants than are finless porpoises, which have a more southern and offshore distribution (see Jefferson *et al.*, 2002b). There is also evidence that DDT use still continues in some parts of the Pearl River Estuary (Fu *et al.*, 2003).

For a number of species, including some marine mammals, organochlorines have been reported to interfere with reproductive capacity (causing failed egg implantation, testis abnormalities, and reduced testosterone levels), cause immunosuppression (lowered resistance to disease), and have carcinogenic (cancer-causing) and teratogenic (developmental) effects (Tanabe and Tatsukawa, 1991; Busbee *et al.*, 1999; Reijnders, 2003). Exposure during early development can affect the endocrine, reproductive, immune and nervous systems, sometimes not expressing its effects until adulthood. Although direct cause-effect links have not been identified, it has been found that high concentrations of PCBs and DDE are correlated with lowered testosterone levels in the blood of Dall's porpoises (*Phocoenoides dalli*) in the North Pacific (Subramanian *et al.*, 1987). Similarly, Martineau *et al.* (1988) found that industrial contaminants were correlated with lesions and cancer-like tumours in white whales (*Delphinapterus leucas*) in the St. Lawrence Estuary; many of these were implicated in the animals' deaths. High levels of organochlorines were associated with suppressed immune response of bottlenose dolphins in the southeastern USA (Lahvis *et al.*, 1995). Cockcroft (1989) suggested that organochlorine concentrations of humpback dolphins in South Africa may be high enough to impair reproductive function of male humpback dolphins and to prove fatal to neonates of primiparous females[3]. High concentrations of organochlorine are also suspected to have been a causal factor in the die-offs of dolphins in the Mediterranean Sea and northeastern United States in recent years (Kannan *et al.*, 1993; Reijnders, 1996; Aguilar, 2000). While this link has not yet been clearly and unequivocally proven, there is good reason to be concerned about such factors (Kennedy, 1999). As Reijnders (2003) cautioned, the etiology of marine mammal disorders and the roles that contaminants might play remains uncertain and more detailed work is clearly required to clarify potential cause-effect relationships in cetaceans (e.g. Van Waerebeek, 1999).

Levels of organochlorines have been analysed in humpback dolphin tissues from only a few areas: South Africa (Cockcroft, unpublished[3]); India (Tanabe *et al.*, 1993; Tanabe *et al.*, 1996; Prudente *et al.*, 1997); Taiwan (Chou *et al.*, 2004) and Hong Kong (Parsons and Chan, 1998; Minh *et al.*, 1999; 2000a; b; c; this study). Although sample sizes have generally been very low, concentrations of at least certain organochlorines appear to be relatively high everywhere that they have been examined. Three adult male humpback dolphins from India showed PCB levels about an order of magnitude lower than in Hong Kong, although DDT levels were broadly similar (Tanabe *et al.*, 1993; Prudente *et al.*, 1997). A single adult male from Taiwan also had much lower PCB levels than specimens from Hong Kong (Chou *et al.*, 2004). Such comparisons of different studies must be viewed with caution, as

[2] Hurst, G.E., Fauquier, D.A., Barros, N.B., Gorzelany, J.F., Lipscomb, T.P., Kinsel, M.J. and Wells, R.S. 2003. Bottlenose dolphin, (*Tursiops truncatus*), strandings and mortality on the west coast of Florida, 1985-2002. Unpublished abstract presented at the Fifteenth Biennial Conference on the Biology of Marine Mammals, Greensboro, NC, 14-19 December 2003.

[3] Cockcroft, V.G. 1989. Biology of Indo-Pacific humpback dolphins (*Sousa plumbea*) off Natal, South Africa. Paper presented at the Eighth Biennial Conference on the Biology of Marine Mammals, Pacific Grove, California. December 1989. Unpublished.

interlaboratory variability in methods and presentation may cause confounding factors (Krahn *et al.*, 2003b). However, the general pattern is likely to still be apparent.

In a recent probabilistic risk assessment of the effects of PCBs on bottlenose dolphin reproduction in the southeast United States, Schwacke *et al.* (2002) compared the levels of PCBs in three different populations and developed a predictive framework for examining health risks to the dolphins. Their results suggested that the levels of PCB exposure that the three populations were experiencing were causing serious impairment of reproductive success (reductions of 60-79% – Schwacke *et al.*, 2002). This may occur primarily through delayed age of primiparity, increased prevalence of stillbirths, increased neonatal mortality, or some combination of these. A study of concentrations of PCBs in known females and their young from the Sarasota Bay bottlenose dolphin population, confirmed that females 'dump' a large contaminant load to their offspring, especially the first-born (Wells *et al.*, 2005). It is therefore instructive to compare the levels of PCBs in different age classes between these two closely-related species (Table 1). While the mean concentrations of PCBs were generally lower in Hong Kong dolphins (vs. in the same age class of bottlenose dolphins) for juvenile and adult male age classes, the mean was somewhat higher for adult females, the age class that might be most influenced by these effects.

In general, the reduction of OC concentrations for adult female humpback dolphins (compared to adult males) was not nearly as strong as it was for adult female bottlenose dolphins, despite the very similar life history of the two species (Table 1). For DDTs, bottlenose dolphin females had levels only 8-13% those of males, while for humpback dolphins they were 21% those of adult males. Similarly for PCBs, bottlenose dolphin females had levels of 6-11% male levels, while humpback dolphin female levels were 36% those of males (see Schwacke *et al.*, 2002; Hansen *et al.*, 2004). We hypothesise that this may be due to reduced reproductive output (or at least reduced calf survival) of Hong Kong humpback dolphins, which would result in less opportunity for females to depurate and thereby reduce the levels of OCs that they possess. This is clearly just conjecture at this point and there are clearly other reproductive factors that could affect the levels of organochlorines (see Kajiwara *et al.*, 2002). However, the pattern of apparent high neonatal mortality observed is consistent with this idea. Further work is required to confirm or deny our hypothesis.

It has been suggested that the apparently high level of neonatal mortality seen among Hong Kong humpback dolphins (ca. 53% of strandings are young-of-the-year) is related to organochlorine contamination, although this cannot be confirmed at this point (Parsons and Chan, 1998; Jefferson, 2000). Concentrations of PCBs similar to those in blubber have been found in milk in the stomachs of calves from this population, clearly demonstrating the potential for mother-to-offspring transfer (Parsons and Chan, 1998). Cockcroft *et al.* (1989) provided evidence suggesting that in South African bottlenose dolphins, offspring receive a large portion of the mother's OC load in the first seven weeks post-partum. Later, Kuss (1998) demonstrated what had previously only been hypothesised, that first-born bottlenose dolphin calves receive a much higher contaminant load from their mother's milk than later-born calves. Their organochlorine concentrations were 2-5 times higher than those of a fourth-born calf of similar age (Kuss, 1998). Wells *et al.* (2005) provided further support for such a scenario. No data on birth order of calves were obtained in this study, but a similar phenomenon may be occurring. Some female humpback dolphins may have trouble successfully rearing their first calf, at least partially due to contaminant issues and thus cannot effectively offload their contaminant burden. If this were true, the succeeding calf would receive a similar contaminant load to the first-born. If it in turn did not survive, the cycle would continue. Again, at this point this is conjecture, but the data presented here have seemingly high levels of neonate mortality and apparently small differences in organochlorine concentrations between adult males and females (see above) which are consistent with such a scenario.

One important point to consider is that young calves appear to be especially vulnerable to the damaging effects of environmental contaminants and it is likely that increased amounts of organochlorines would affect calf survival. Especially for the organic contaminants, the 'dumping' of loads of contaminants to the offspring (in particular the first born) may be a very serious issue (see Tanabe *et al.*, unpublished[4]). The problem here is that the newborn (especially the first-born calf) gets overloaded with contaminants at a vulnerable stage of its growth, and this has been suggested to result in increased calf mortality. This phenomenon has been described for fin whales (*Balaenoptera physalus*) (Aguilar and Borrell, 1994), striped dolphins (*Stenella coeruleoalba*) (Tanabe *et al.*, unpublished; Borrell *et al.*, 1996), Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Cockcroft *et al.*, 1989), long-beaked common dolphins (*Delphinus capensis*) (Cockcroft *et al.*, 1990), killer whales (*Orcinus orca*) (Ylitalo *et al.*, 2001), Dall's porpoises (Subramanian *et al.*, 1987; 1988) and finless porpoises (Jefferson *et al.*, 2002a). The present data from humpback dolphins in Hong Kong suggest that this is also the case with these animals. This may explain the seemingly-high levels of neonatal mortality for Hong Kong's dolphins (see above; Parsons and Jefferson, 2000; Ramu *et al.*, 2005). The reason why male neonates appear to have higher levels than females is unknown. However, it is possible that this is caused by some sex-related difference in the physiological process that occurs during gestation. This is worthy of further study.

Implications for conservation

This study has provided further suggestions that the high levels of some environmental contaminants in Hong Kong are probably impacting the health, survival and reproduction of the Pearl River Estuary humpback dolphin population. Parsons (2004) also came to the same conclusion and further suggested that other populations of humpback dolphins may be experiencing similar problems. However, the evidence for these effects is still largely circumstantial, even for Hong Kong. The existence of a series of problems (e.g. subtle and synergistic effects of contaminants, low quality of available specimen material from strandings, interlaboratory variability in contaminant concentrations) have made it difficult to evaluate how severe this issue is. Only a dedicated programme focusing on this issue and incorporating a plan to resolve these problems will move us forward in our knowledge.

[4] Tanabe, S., Tanaka, H., Maruyama, K. and Tatsukawa, R. 1979. Elimination of chlorinated hydrocarbons from mother striped dolphins (*Stenella coeruleoalba*) through parturition and lactation. Unpublished report. [Available from TAJ].

Most importantly, although a great deal has been learnt about the Hong Kong population of humpback dolphins from strandings and associated necropsy of stranded specimens, the very extreme levels of decomposition of most stranded specimens have caused great frustration. Our level of understanding is such that a carefully-crafted biopsy collection programme is clearly the next step (and such a programme has recently been initiated). By obtaining biopsy samples from individuals that are well-known from photo-identification studies, knowledge can be advanced very rapidly. At least general age class information can be obtained by collecting samples mainly from animals in our long-term photo-identification database (some of which are of known age and sex, and most of which have at least a minimum known age). Wells *et al.* (2003; 2004) made a compelling argument for the ability to monitor effects of organic contaminants and even to use dolphins as monitors of ecosystem health by combining long-term ecological and observational data with periodic sampling of tissue from living dolphins. In Hong Kong, where environmental contamination is rampant and where humans consume large quantities of seafood, such an approach is even more warranted.

The small risk that is posed by biopsy sampling will be more than offset by the great advances in important conservation knowledge that is stood to be gained. Wells *et al.* (2005) may have said it best when they stated that:

> 'Long-term observational monitoring and periodic biological sampling provide a powerful, non-lethal approach to understanding relationships between organochlorine residue concentrations in tissues and reproductive parameters for coastal dolphins'.

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