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# Toxicant exposure, population genetics, and trophic associations of bottlenose dolphins (*Tursiops aduncus*) in the Swan River

**Final report to the Swan River Trust for the**

**Swan Canning Research Innovation Program (SCRIP)**

**Project** RSG09MUR01

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# Executive Summary

## Introduction

Although Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) are a valued component of the Swan-Canning Estuary and the Swan Canning Riverpark, little is known about the health and ecology of the small community of dolphins inhabiting the estuary.

To improve the scientific basis for management, we examined the population genetics, trophic associations, and contaminant exposure of dolphins within the estuary. This Swan Canning Research Innovation Program (SCRIP) study had the following objectives: (1) detail contaminant concentrations in dolphins (as a baseline for future monitoring); (2) provide a preliminary assessment of health risk posed by contaminants to dolphins; (3) examine trophic pathway associations for Swan River bottlenose dolphin community; (4) use genetic information to examine whether bottlenose dolphins from the Swan-Canning Estuary and adjacent waters (Cockburn Sound) represent one homogenous population or (alternatively) if fine-scale population structuring occurs; and (5) put project findings into the perspective of system ecology and management implications.

Tissue samples for this study were obtained through remote biopsy sampling of free-ranging dolphins and the collection of tissues during post-mortem examinations under permits and licences from the WA Department of Environment and Conservation and the Murdoch University Animal Ethics Committee.

## Population Genetics

We used phylogenetic analysis of DNA sequence data from 30 dolphins from southwestern Australia ($n$ = 13 from Swan-Canning Estuary and $n$ = 17 from other locations) to determine that the bottlenose dolphins inhabiting the Swan-Canning Estuary are Indo-Pacific bottlenose dolphins (*Turisops aduncus*). The taxonomic status of the Swan River dolphins had previously been unconfirmed.

We also examined the distribution of mitochondrial DNA (mtDNA) haplotypes[1] and found that five of the seven haplotypes were unique to dolphins from the Swan-Canning Estuary. This finding is preliminary and, while suggestive of genetic differentiation between dolphins from estuary and dolphins elsewhere, further work is required to more fully assess the uniqueness of these haplotypes.

We calculated a genetic fixation index ($F_{ST}$) of 0.11 ($p$ = 0.02) between the Swan-Canning and Cockburn Sound populations, based on a sample of $n$ = 14 dolphins from the Swan-Canning Estuary

[1] The term 'haplotype' refers to a particular combination of alleles or sequence variations that are closely linked (i.e. are likely to be inherited together) on the same chromosome.

and *n* = 20 dolphins from Cockburn Sound. This finding indicates moderate genetic structure in the Perth area, and suggests that there is less mixing between individuals from the two sites than may be expected, given their close proximity.

The genetic findings, though requiring further work to confirm, suggest that the resident dolphin community in the Swan-Canning Estuary is likely to exhibit some level of demographic isolation.

**Trophic Associations**

We conducted carbon and nitrogen stable isotope analyses on tissues collected from dolphins observed in the Swan-Canning Estuary (*n* = 9), Cockburn Sound (*n* = 3), and Rottnest Island (*n* = 3). While the small sample sizes suggest caution in interpreting the findings, the results indicate differences between the stable isotope ratios of dolphins associated with the Swan-Canning Estuary and dolphins associated with the two coastal sites, suggesting that dolphin foraging ecology varies across these habitats.

The stable isotope ratios for nitrogen samples ranged from 12.0 to 18.6 (‰), with the highest ratios in dolphins from the Swan-Canning Estuary. The higher ratios in dolphins in the Swan-Canning Estuary suggest these dolphins may feed at a higher trophic level than dolphins from the two coastal sites. Alternatively, the differing ratios may relate to longer or more complex food chains within the estuary relative to those in coastal waters or to differing nitrogen sources.

The stable isotope ratios for carbon ranged from -21.3 to -15.6 (‰). These ratios are generally lower than those reported for bottlenose dolphins in the southeastern United States and in Victoria, Australia, suggesting potential differences in carbon sources. The range of carbon ratios was greatest in dolphins from the Swan-Canning Estuary and narrowest in dolphins from Rottnest Island, suggesting that dolphins from the estuary feed on a greater variety of foods and/or are associated with food webs that have more (or more diverse) carbon sources and therefore a broader range of carbon ratios. Differing carbon sources could also influence these patterns.

We also analysed the fatty acid composition of blubber from two dolphins from the Swan-Canning Estuary. These analyses indicated a substantially carnivorous diet, with one dolphin containing large amounts of the fatty acid 16:1(n-7), an isomer mainly associated with marine primary producers and with biosynthesis in marine mammal blubber.

The fatty acid compositions suggested that terrestrial lipids were not important in the diet of the dolphins and, along with the carbon stable isotope ratios, suggest that dolphins do not fit well within the food web of the upper Swan River, which was the food web modeled for this study. Placement of dolphins within estuarine food webs will therefore require further information on the trophic structure

of other locations in the Swan-Canning Estuary. In addition, while the trophic findings from this study support behavioural observations indicating that dolphins from the Swan-Canning Estuary are likely to be associated with both marine and estuarine-based food webs, the relative importance of food sources from these two environments remains unclear.

These findings suggest that site-specific trophic signatures occur within the Perth area. The identification of these signatures would provide a useful tool for examining fine-scale population structuring in the Perth metropolitan area, particularly if used in conjunction with information on ranging patterns, population genetics, and contaminant burdens.

### Contaminant Exposure

Prior to this study, little was known about the presence of contaminants in marine mammals from Western Australia. Dieldrin, DDE, and PCBs were the predominant organic contaminants detected in blubber samples collected post-mortem from dolphins that died within the Swan-Canning Estuary in 2009.

Dieldrin, DDE and PCBs were also the most predominant organic contaminants detected in skin and blubber biopsy samples collected from free-ranging dolphins in the Swan-Canning Estuary. Contaminant concentrations in biopsy tissue samples were found to be of limited value compared to the large blubber segments collected from deceased dolphins, given their small size (preventing determination of lipid content) and the finding that they did not contain all of the blubber layers (contaminant concentrations vary across blubber layers).

Dieldrin concentrations detected in the Swan River dolphins were significantly higher ($p$ = 0.03) than those detected in the dolphins from the Bunbury area, thus indicating spatial differences in environmental contamination. The average dieldrin concentrations detected in the Swan River dolphins are among the highest levels reported globally in marine mammals in recent times.

As only 21 PCB congeners were examined, total PCB concentrations are not directly comparable with those reported by some other studies. Nonetheless, dolphins from the Swan-Canning Estuary had similar concentrations of total PCBs and total DDT to those recently reported for estuarine dolphins in the southeastern United States. The total PCB threshold concentration for effects on immune function (as per Kannan et al., 2000) provides a guide as to when concentrations may warrant concern over their effects on dolphin health. Two dolphins from the Swan-Canning Estuary exceeded the approximate threshold (17 µg PCB/g lipid weight). More dolphins may have exceeded the threshold if more PCB congeners had been included in the suit of analytes examined.

The high concentrations of organochlorine contaminants recorded in the dolphins from the Swan-Canning Estuary indicated that these contaminants are likely to adversely affect the health of the dolphins during periods of lipid mobilisation. It is, however, currently not possible to measure the extent to which such adverse effects are occurring.

**Management Implications**

There remains significant scientific uncertainty in our understanding of the potential effects of contaminants on the dolphins inhabiting the Swan-Canning Estuary. This uncertainty reflects the difficulty in inferring biological effects from the concentrations of contaminants within tissues, as well as the practical difficulties of drawing comparisons across studies, taxa, and suites of contaminants. Nonetheless, the contaminant burdens are sufficient to raise concerns about adverse health effects if lipid reserves are mobilised and to suggest that, to the extent reductions in environmental concentrations of organic contaminants can be achieved, this would be of long-term benefit to dolphins. The potential effects of contaminants should not be viewed in isolation. Rather, the health of dolphins in the Swan-Canning Estuary should be considered from a multi-factorial framework in which a range of natural and anthropogenic stressors may interact to exert significant cumulative and/or synergistic effects.

While recognising the preliminary nature of certain findings from this study, the genetic, trophic, and contaminant information provide an improved scientific basis for management of dolphins and the estuarine environment they inhabit. In particular, the findings suggest that there are recognisable differences in the genetic structure, trophic signatures, and contaminant burdens of dolphins associated with the Swan-Canning Estuary and those for dolphins from other locations. These differences provide additional support for considering the dolphins using the Swan-Canning Estuary as a distinct community of dolphins within the Perth metropolitan area and for classifying this community as a discrete management unit.

# Contents

# I. Introduction & Aims

## Dolphins in the Swan-Canning Estuary

Two bottlenose dolphin species are currently recognised: Indo-Pacific bottlenose dolphins *Tursiops aduncus* and common bottlenose dolphins *T. truncatus* (LeDuc et al., 1999). Bottlenose dolphins in the coastal waters in the Perth metropolitan area are thought to be *T. aduncus*, with *T. truncatus* present but occurring only in offshore waters (Cannell, 2004).

Both *Tursiops* species exhibit a complex population structure within coastal and estuarine environments, such that the individuals observed in an area may be migratory, transient, seasonally-resident, or resident year-round (Baird et al., 2009; Bearzi et al., 2008; Chilvers and Corkeron, 2001; Frère et al., 2010; Hoelzel et al., 1998; Krützen et al., 2005; Lusseau, 2005; NOAA, 2009; Sellas et al., 2005; Speakman et al., 2006; Urian et al., 2009; Zolman, 2002). Discrete 'communities' of bottlenose dolphins may occur within estuaries and protected coastal environments (NOAA, 2009; Wells et al., 1999; Wisniewski et al., 2009; Zolman, 2002). Wells et al. (1999) defines a community as a 'regional society of animals sharing ranges and social associates, but exhibiting genetic exchange with other similar units'. Although communities are often associated with particular bays and estuaries, discrete communities may occur even within areas lacking any physiographic barrier (Urian et al., 2009).

Members of these communities typically have small and over-lapping home ranges; show long-term site fidelity (i.e. site philopatry) and year-round residency; and associate most frequently with other members of the community (Olin et al., 2011; Mazzoil et al., 2008; Urian et al., 2009; Wells et al., 1987; Wilson et al., 1997; Zolman, 2002). They may also exhibit distinct behavioural specializations (e.g. Krützen et al., 2005; Sargeant et al., 2005, 2007; Sargeant and Mann, 2009) and genetic differentiation from dolphins in surrounding areas (Barros and Wells, 1998; Duffield and Wells, 2002; Hoelzel, 1998; Möller et al., 2007; Möller and Harcourt, 2008; Sellas et al., 2005; Tezanos-Pinto et al., 2009; Wisniewski et al., 2009). These communities are often quite small. Gubbins (2002) and Zolman (2002), for example, reported 20 and 21 resident dolphins in estuarine habitats in South Carolina, and Wiszniewski et al. (2009) reported two communities of 89 and 31 individuals within an embayment in eastern Australia. Small population sizes and low intrinsic rates of increase make resident communities highly vulnerable to extinction by natural and/or anthropogenic processes, particularly if communities are isolated and little immigration occurs (NOAA, 1999; Sellas et al., 2005; Wilson et al., 1999).

Research from 2001-3 classified 18 bottlenose dolphins as resident (i.e. showing long-term and year-round site fidelity) to the Swan-Canning Estuary, based on re-sighting patterns showing consistent usage of the estuary by these individuals between October 2001 and June 2003 (Chabanne et al., 2011;

Holyoake et al., 2010; Lo, 2009).[2] In contrast, non-resident dolphins were seen only very infrequently. These re-sighting are based on 454 sightings of dolphin groups in the Swan-Canning Estuary during this study period, with 55 dolphins individually identified based on dorsal fin markings (Chabanne et al., 2011).

These 18 'resident' dolphins were observed within the Swan-Canning Estuary and in adjacent coastal areas (e.g. Owen Anchorage), with behavioural observations suggesting that dolphins moved between the estuary and coastal areas on a daily or near-daily basis (H. Finn, Murdoch University, unpublished data). This part-estuarine/part-coastal ranging pattern appears to be unique to these 18 individuals, based on research in Cockburn Sound (from 1993-2003) and in the Swan-Canning Estuary (from 2001-3). These 18 individuals included six adult females, and accounted for nearly all of the sightings of dolphins in the Swan-Canning Estuary from October 2001 to June 2003 (Chabanne et al., 2011). The 18 dolphins were considered to comprise a resident dolphin community of 20-25 dolphins for the Swan-Canning Estuary, with the overall size of the community dependent on the number of calves present.

The current status of the dolphins in the Swan-Canning Estuary is not clear, as there has been little field research on dolphins in the estuary since mid-2003. The current abundance of resident dolphins is likely to be similar to (or less than) the 2001-3 estimate of 20-25 individuals, given: (a) the low reproductive rates of bottlenose dolphins; (b) stranding records indicating at least 11 mortalities within the Swan-Canning Estuary since late 2003 (including six deaths between June-October 2009); and (c) the likelihood that the strong site fidelity of inshore bottlenose dolphins will limit the immigration of dolphins into the Swan-Canning Estuary from adjacent areas (Holyoake et al., 2010).

## Study Aims

An investigation into the six dolphin deaths in 2009 indicated that a suite of factors likely contributed to the mortalities (Holyoake et al., 2010). This mortality event emphasised the potential vulnerability of the resident community to natural and anthropogenic stressors and the need to improve the scientific basis for the long-term conservation of dolphins within the estuary. Holyoake et al. (2010) identified a number of issues of interest for the 'Swan dolphins', including: current burdens of environmental contaminants; the potential effects of those burdens on dolphins; trophic associations between dolphins

[2] The term 'resident' implies that dolphins exhibit site fidelity to the Swan-Canning Estuary and to the adjacent coastal area that they also use. These 18 individuals were either adults or juveniles. We did not classify dependent calves as 'resident's because mortality rates for calves are high, complicating efforts to determine residency patterns.

and estuarine food webs; whether the community experienced some level of demographic isolation[3]; and the scientific basis for managing the resident community as a discrete management unit.

To address these issues, this Swan Canning Research Innovation Program (SCRIP) study aimed to:

1. describe contaminant concentrations in dolphins to provide baseline for future monitoring;
2. provide a preliminary assessment of health risk posed by contaminants to dolphins based on observed contaminant burdens;
3. describe trophic pathway associations for the bottlenose dolphin community in the Swan-Canning Estuary;
4. use genetic information to examine whether bottlenose dolphins from the Swan-Canning Estuary and adjacent waters (Cockburn Sound) represent one homogenous population or, alternatively, whether fine-scale population structuring occurs; and
5. put project findings into the perspective of system ecology and management implications.

[3] Demographic isolation means that a population of animals receives little or no immigration of individuals from populations occurring in adjacent areas. In other words, if demographic isolation occurs, the viability of the population (or a 'community' of dolphins) will depend on the recruitment of individuals from the population/community, with few or no individuals being added to the population/community through dispersal from other populations.

# II. Tissue Sampling Methods

Biopsy samples were collected using the PAXARMS biopsy system, a modified 0.22 caliber rifle with a detachable barrel, a valve to adjust firing pressure in the chamber and a biopsy dart (Figures 1 and 2; Krützen et al., 2002). The PAXARMS biopsy system is regarded as a safe and cost-effective method, commonly used for obtaining skin and blubber samples from live dolphins (Krützen et al., 2002). Samples typically include tissue from the epidermis and dermis, and the outermost blubber layer. The biopsy samples on average weighed between 0.5-1.0 grams and were not large enough to enable all analyses on each sample. Consequently, samples were prioritised according to size with large samples designated for contaminants analysis and all other samples were halved and separated for genetic and stable isotope analyses. Contaminants samples were wrapped in aluminum foil and stored at -20°C. Stable isotope samples were stored in 1.5 ml eppendorf containers and stored at -80°C. Genetics samples were stored in a 20% DMSO and saturated salt solution at room temperature. Blubber samples were also collected from five bottlenose dolphins found dead in the Swan-Canning Riverpark, between June and October 2009. Sampling was conducted under permits and licences from the WA Department of Environment and Conservation and the Murdoch University Animal Ethics Committee.

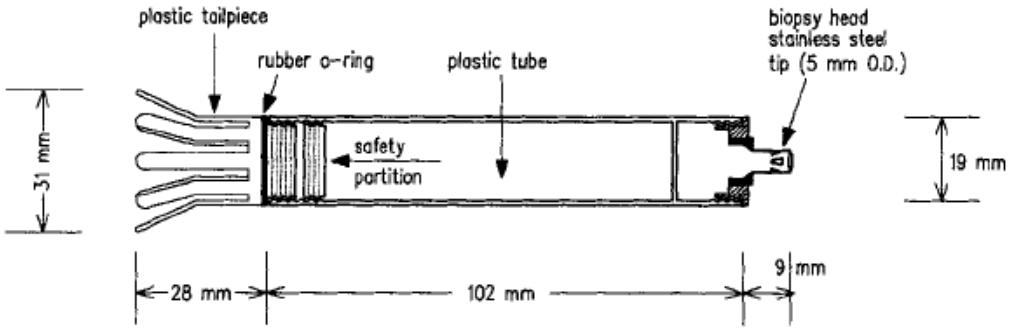


**Figure 1:** Diagram of biopsy dart used in remote biopsy sampling (from Krützen et al., 2002).

**Figure 2:** Sampling of the dolphin 'Blackwall' in 2009.

# III. Genetics

## Introduction

Resident communities of bottlenose dolphins (*Tursiops* spp.) are receiving increasing recognition as appropriate management units for coastal and estuarine ecosystems (Chabanne et al., 2011; Connor et al., 2000; NOAA, 2009). For example, stock assessments under the United States *Marine Mammal Protection Act 1972* currently identify resident communities of *T. truncatus* as 'stocks' for many of the estuaries, bays, and sounds along the North Atlantic coast and the Gulf of Mexico (NOAA, 2009). The scientific basis for these stock-level determinations is reviewed in NOAA (2009, p. 261):

> A "community" includes resident dolphins that regularly share large portions of their ranges, exhibit similar distinct genetic profiles, and interact with each other to a much greater extent than with dolphins in adjacent waters. The term, as adapted from Wells *et al.* (1987), emphasizes geographic, genetic and social relationships of dolphins. Bottlenose dolphin communities do not constitute closed demographic populations, as individuals from adjacent communities are known to interbreed. Nevertheless, the geographic nature of these areas and long-term, multi-generational stability of residency patterns suggest that many of these communities exist as functioning units of their ecosystems, and under the Marine Mammal Protection Act must be maintained as such. Also, the stable patterns of residency observed within communities suggest that long periods would be required to repopulate the home range of a community were it eradicated or severely depleted. Thus, in the absence of information supporting management on a larger scale, it is appropriate to adopt a risk-averse approach and focus management efforts at the level of the community rather than at some larger demographic scale.

Holyoake et al. (2010) and Chabanne et al. (2011) have proposed that the resident dolphin community in the Swan-Canning Estuary should be recognised as a management unit, based on behavioural information (e.g. evidence of year-round residency, long-term site fidelity, closed social structures, and unique ranging patterns) and the small size (c. 20-25 dolphins) of this community. However, genetic information is required to examine the scientific basis for this proposal. In particular, information is needed on whether genetic differentiation occurs between dolphins from the estuary and dolphins from adjacent habitats. Evidence of significant genetic differentiation at such a small spatial-scale would indicate some level of demographic isolation for the resident community, and support management efforts to minimise human impacts that might adversely affect the reproductive success of dolphins, particularly adult females (Bejder et al., 2006a,b; Sellas et al., 2005).

To obtain further genetic information for the resident community in the Swan-Canning Estuary, we undertook: (1) a species determination for the community (to resolve whether individuals were *T. aduncus* or *T. truncatus*); (2) a preliminary assessment of the distribution of mitochondrial DNA

(mtDNA) haplotypes[4] present in dolphins from the estuary and from other locations in southwestern Australia; and (3) a quantitative analysis of the population differentiation between dolphins from the Swan-Canning Estuary and from Cockburn Sound based on mtDNA data.

## Methods

### *Genetic methods*

DNA was extracted and sequenced from tissue samples of: *n* = 14 dolphins samples within the Swan-Canning Estuary; *n* = 20 dolphins from Cockburn Sound and *n* = 14 dolphins from other locations in southwestern Australia [Rottnest Island (*n* = 2), Mandurah (*n* = 3), Bunbury (*n* = 6), Busselton (*n* = 2) and Augusta (*n* = 1)]. DNA extraction and sequencing for 350 base pairs of the mitochondrial control region followed Krützen et al. (2004). Sequences were analysed in GenAlEx (v 6.0, Peakall and Smouse, 2006) and edited and aligned in Geneious (v 4.7.6) along with published sequences from other dolphin species, and phylogenetic trees were constructed in Geneious by Neighbour Joining (NJ) algorithm based on Tamura-Nei genetic distance.

### *Species determination*

We used published mitochondrial DNA sequences for *T. truncatus* (common bottlenose dolphins), *T. aduncus* (Indo-Pacific bottlenose dolphins), and other dolphin species to undertake species determinations for samples collected from the dolphins in the Swan-Canning Estuary and from other locations in southwestern Australia.

### *Distribution of mtDNA haplotypes*

To examine the distribution of mtDNA haplotypes across locations in southwestern Australia, we identified the haplotypes for a sample of *n* = 13 dolphins from the Swan-Canning Estuary and *n* = 17 dolphins from other locations: Cockburn Sound (*n* = 3), Rottnest Island (*n* = 2), Mandurah (*n* = 3), Bunbury (*n* = 6), Busselton (*n* = 2) and Augusta (*n* = 1).

### *Population structure*

We calculated a genetic fixation index ($F_{ST}$) between the Swan-Canning and Cockburn Sound populations, based on a sample of *n* = 14 dolphins from the Swan-Canning Estuary and *n* = 20 dolphins from Cockburn Sound. The $F_{ST}$ examines the correlation of allele (or haplotype) frequencies between populations and is commonly used as a measure of population differentiation (Weir and Cockerham, 1984; Holsinger and Weir, 2009). As a qualitative guideline, an $F_{ST}$ of less than 0.05

[4] The term 'haplotype' refers to a particular combination of alleles or sequence variations that are closely linked (i.e. are likely to be inherited together) on the same chromosome.

indicates little differentiation between populations (suggesting a high level of mixing), while values over 0.25 indicate very great population differentiation (suggesting little migration between sampling sites) (Wright, 1978). $F_{ST}$ values between these two ranges indicate moderate (0.05 – 0.15) or great (0.15 – 0.25) population differentiation.

## Results & Discussion

### *Species determination*

Prior to this study the taxonomic status of the dolphins from the Swan-Canning Estuary was not known. Analyses of a small number of specimens from the Perth area had indicated that haplotypes for both *T. truncatus* and *T. aduncus* were present. While *T. truncatus* and *T. aduncus* are considered the oceanic and the coastal species of bottlenose dolphin, respectively, within southern Australian waters, this schema may be revised in the future (e.g. Möller et al., 2008).

A total of seven mitochondrial haplotypes were identified in analysed individuals from the Swan-Canning Estuary ($n$ = 14) (Figure 3). One was particularly common (SW haplotype 8), present in 6 of 14 individuals (43%), with the other 6 haplotypes each present in only one or two individuals. Four haplotypes (4, 7, 8 and 9, representing ten individuals) can be tentatively identified as *T. aduncus* based on a phylogenetic analysis of DNA sequence data (Figure 3). The three remaining haplotypes (1, 2 and 3, representing three individuals) fell outside this group, with haplotypes 1 and 3 grouping loosely with the striped and common dolphin, and haplotype 2 grouping loosely with *T. truncatus*.

These results, along with observations of behaviour and morphology, indicate that the Swan River dolphins can be identified as *T. aduncus*. However, the presence of mitochondrial haplotypes that fall outside of this clade indicate that there is some gene flow between coastal/estuarine populations of *T. aduncus* and offshore (presumably *T. truncatus*) populations. It is also possible that the presence of these haplotypes reflects historical gene flow or founder events rather than current gene flow.

Further research, particularly sampling of individuals from offshore environments, would help resolve the population structure of bottlenose dolphins (*Tursiops* spp.) in the Perth area. Such information could also improve our understanding of the epidemiology of infectious disease, as evidence of genetic exchange between coastal/estuarine and offshore populations would suggest that dolphins from these two environments interact at least occasionally; this contact could potentially allow for the introduction of pathogens harbored in the larger offshore cetacean populations (e.g. pilot whales) (Holyoake et al. 2010).

*Distribution of mtDNA haplotypes*

Two of the seven mtDNA haplotypes present in Swan-Canning Estuary dolphins were shared with dolphins from other locations, indicating gene flow between populations along the southwest coast. SW Haplotype 8 (the common haplotype, *T. aduncus*) was present in two individuals sampled in Cockburn Sound and Bunbury, sites that are more than 170 km apart. Haplotype 1 (present in one Swan individual, which grouped loosely with *T. truncatus*) was the most common haplotype in samples from other sites, and was present in samples from Rottnest Island, Mandurah, Busselton and Bunbury. The remaining five haplotypes found in the Swan River dolphins were not seen in dolphins from other sites.

While this finding is suggestive of genetic differentiation between dolphins from the Swan-Canning estuary and dolphins elsewhere, analysis of a larger number of samples from southwestern Australia is required to: (a) identify the full suite of mtDNA haplotypes present in this region and (b) confidently determine the distribution of those haplotypes across locations. Conclusive evidence for unique mtDNA haplotypes within dolphins from the Swan-Canning Estuary would, however, suggest the presence of unique maternal lineages among these dolphins, as could occur if the current assemblage of resident dolphins was descended from a small founder population (see below; Wells et al., 2005).

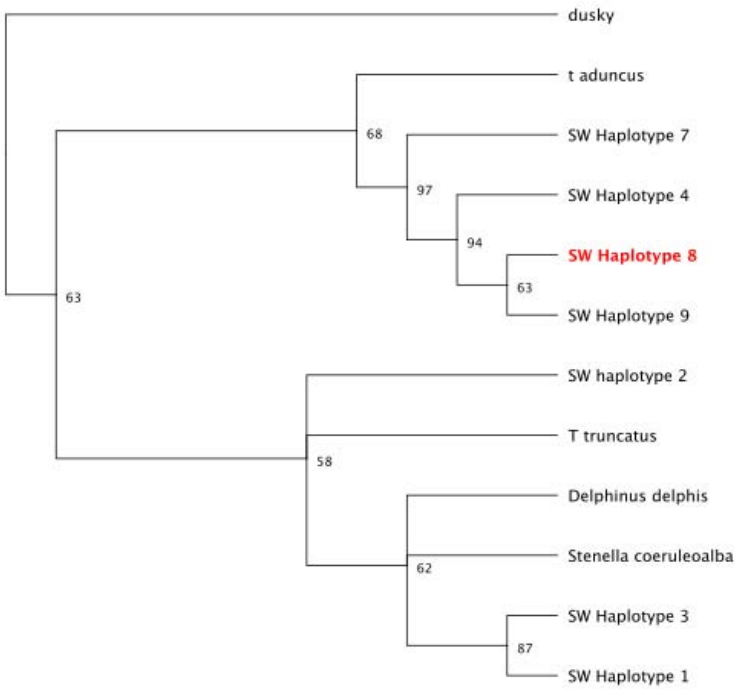


**Figure 3:** Phylogenetic (Neighbour Joining) tree showing the southwestern Australia (SW) haplotypes present in dolphins from the Swan-Canning Estuary and their relationship to representative sequences of four dolphin species: dusky dolphins (*Lagenorhynchus obscurus*, used as the outgroup), *Stenella coeruleoalba* (striped dolphins), *Delphinus delphis* (common dolphins), *Tursiops truncatus* (common bottlenose dolphins), and *T aduncus* (Indo-Pacific bottlenose dolphins). The common 'Swan River' haplotype, (Haplotype 8) is highlighted in red, representing six individuals. The other six haplotypes were each present in only one or two individuals.

*Population structure*

An $F_{ST}$ of 0.11 ($p$ = 0.02) between the Swan-Canning Estuary and Cockburn Sound populations indicates that there is moderate genetic structure in the Perth area, and that there is less mixing between individuals from the two sites than may be expected, given their close proximity. Though the finding is preliminary, it indicates that while gene flow occurs, the population structure of dolphins in the southern metropolitan waters of Perth is not a single homogenous population in which individuals range freely across the area. It suggests, rather, the presence of a fine-scale population structure, with limited exchange of individuals between the Swan-Canning Estuary and Cockburn Sound. This finding is consistent with behavioural observations suggesting the presence of discrete communities for the estuary and for Cockburn Sound (Chabanne et al., 2011; Finn, 2005).

While caution must be used in comparing studies of genetic structure (e.g. due to differences in sample size or the genealogical histories of *T. aduncus* and *T. truncatus*: Möller et al., 2007), the genetic fixation index calculated for this study is similar to indices calculated for bottlenose dolphins in other locations (e.g. Sellas et al., 2005; Möller et al., 2007). Möller et al. (2007) found evidence of differentiation between dolphins inhabiting the embayment of Port Stephens and adjacent coastal populations. This study is particularly relevant to the Perth context, given the broad similarities between the Port Stephens area and the southern metropolitans waters of Perth and the presence of multiple dolphin communities within the Port Stephens embayment (Wisniewski et al., 2009). Möller et al. (2007) suggested that:

> ...the pattern of [genetic] divergence reported here is probably due to a recent colonisation of the embayment by coastal dolphins, followed by a rapid restriction to gene flow. This founder event, which is consistent with a subset of the coastal genetic diversity present in the embayment population, likely occurred during the last 6000 years, after inundation of the Port Stephens' embayment by the last postglacial marine transgression of the Holocene. [p.644]

A similar scenario of colonisation followed by limited genetic exchange would seem plausible for the Swan-Canning Estuary, particularly as historical records indicate that dolphins were present within the estuary prior to the harbour works that expanded the entrance to the estuary in the late 1800s (Sue Graham-Taylor, WA History Council, personal communication).

Sellas et al. (2005) suggested that resource specialization (i.e. differences in prey selection and habitat use), natal philopatry (i.e. maintenance of maternal home ranges, at least among females), and social structure may support fine-scale population structuring of bottlenose dolphins within nearshore environments. This fine-scale population can be thought of as a kind of population 'mosaic', characterised by the presence of multiple, discrete communities associated with particular geographic

areas (Connor et al., 2000; NOAA, 2009; Urian et al., 2009; Wells et al., 1999; Wisniewski et al., 2009; Zolman, 2002).

Our analysis examined only information from mtDNA. Population comparisons based solely on mtDNA data should be treated cautiously as the inclusion of additional information from microsatellite DNA analyses is typically necessary to adequately assess fine-scale population structure, and to identify genetic differentiation that may not be evident through analyses of mtDNA markers alone (Möller et al., 2007; Sellas et al., 2005).

*Management implications*

In conclusion, this study found preliminary evidence for the presence of unique mtDNA haplotypes amongst dolphins from the Swan-Canning Estuary and for genetic differentiation between dolphins from the Swan-Canning Estuary and Cockburn Sound. While these findings are preliminary and must be treated with caution, they do suggest that the resident dolphin community in the Swan-Canning Estuary is likely to exhibit some level of demographic isolation, as has been observed for bottlenose dolphins communities in other nearshore locations (Sellas et al., 2005; Urian et al., 2009; Toth et al., 2010).

Further investigation of the genetic structure of bottlenose dolphins in southwestern Australia is on-going, with the integration of additional samples and nuclear markers. Although the findings for this study are consistent with the resident community experiencing some level of demographic isolation, further work will be needed to conclusively demonstrate (or disprove) this hypothesis. In particular, information is needed on dispersal rates for the resident community, i.e. what proportion of the community consists of immigrants from adjacent areas.[5] This information can best be acquired through further investigation of the genetic structure of dolphins in the southern metropolitan waters of Perth and long-term monitoring of the ranging patterns of individual dolphins within the Swan-Canning Estuary and adjacent areas (Möller et al., 2007; Sellas et al., 2005; Toth et al., 2010; Urian et al., 2009).

[5] Natal philopatry would suggest that an 'immigrant' to the resident community for the Swan-Canning Estuary would presumably not have been born to a female from the resident community for the estuary, and thus would not have retained use of the estuary as part of their 'inheritance' of his/her mother's home range. Non-resident dolphins do occur within the estuary and these individuals would be likely candidates for immigrants (i.e. dolphins dispersing 'into' the resident community and exhibiting long-term site fidelity to the estuary).

# IV. Trophic Associations

## Introduction

*Use of biomarkers to study trophic associations*

Stable isotopes have been used extensively to investigate the diet of dolphins and other species (e.g. Barros et al., 2010; Jennings et al., 1997; Newsome et al., 2010; Olin et al., 2011; Owen et al., 2011; Svensson et al., 2007). The metabolic processes of an organism fuel a process of isotopic fractionation in which heavier isotopes are retained (and lighter isotopes lost from tissues), a change in isotopic composition that can be measured on an isotope-ratio mass-spectrometer. Carbon stable isotope ratios are typically similar between producer and consumer and an indicator of carbon source, while nitrogen stable isotope ratios generally increases by an average of 3.5 ‰ (ppt) in aquatic systems, a characteristic that makes them indicative of trophic level (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984).

Fatty acids are ubiquitous in living organisms and integral parts of structurally important cell membranes, as they ensure their fluidity. Fatty acids are produced *de novo* by primary producers and taken up by consumers with their diet, absorbed into the bloodstream, then deposited in adipose tissue with little modification. Generally, they propagate further up the food chain unmetabolised (especially essential fatty acids which cannot be produced by consumers at all). Therefore, the fatty acid pattern in a consumer species will match that of its prey (Budge et al., 2006; Fraser et al., 1989; Graeve et al., 1994; Kirsch et al., 1998; Sargent and Falk-Petersen, 1988; St. John and Lund, 1996).

*Dolphins & food webs in the Swan-Canning Estuary*

Little is known about the feeding ecology of dolphins within the Swan-Canning Estuary (Holyoake et al., 2010). Previous research from 2001-3 indicates that members of the resident dolphin community move between the estuary and coastal areas on a daily to near-daily basis, suggesting that their diet will include prey captured both within the estuary and in adjacent coastal areas, such as Owen Anchorage. Similarly, certain prey species move seasonally between the estuary and coastal areas. Thus, dolphins may consume prey that: reside in the estuary year-round; are seasonally present in the estuary; or occur only in coastal areas (Hallett, 2010; Potter and Hyndes, 1999; Smith, 2006).

Other areas of uncertainty about dolphin diet and foraging ecology relate to breadth of diet and individual variation. Dolphins may consume broad range of prey species, including smaller (e.g. anchovies) and larger (e.g. snapper, bream) prey items, and both finfish and cephalopods (e.g. squid,

cuttlefish, octopus). Individuals may also vary in their feeding ecology. For example, some dolphins may consume more estuary-associated prey than others or feed in different locations within the estuary.

These factors complicate efforts to: (a) assess the relative contribution of estuary-associated and coastal-associated prey to the diet of dolphins and (b) explain differences in stable isotope values, which may be temporal (i.e. between seasons or years) or spatial (i.e. between locations) (Olin et al., 2011). However, despite the difficulties in interpreting stable isotope values, the differences observed in this study suggest that dolphins in the three locations use prey bases with different stable isotope compositions. The presence of identifiable trophic 'signatures' would, if confirmed with further research, provide a useful method for discriminating between local assemblages of dolphins, particularly if used in conjunction with other comparative data (e.g. ranging patterns) (Olin et al., 2011).

## Methods

Evaluation of carbon and nitrogen stable isotope ratios and fatty acid compositions was undertaken as part of a wider study of trophic dynamics in the upper Swan River around Guildford and encompassing a broader range of organisms (T. Linke, Murdoch University, unpublished data). Our efforts to situate dolphins with estuarine food webs were therefore focused on the food webs in the upper Swan, particularly given the limited information on the trophic structure of other locations within the Swan-Estuary.

We sampled 15 bottlenose dolphins from the Swan-Canning Estuary ($n$ = 9), Cockburn Sound ($n$ = 3), and Rottnest Island ($n$ = 3) for stable isotope analyses of carbon and nitrogen to investigate their feeding ecology. Samples were oven-dried at 60°C and stored in a desiccator. Tissues were separated into skin and blubber where possible (depending on the amount of tissue available), ground to a fine powder with mortar and pestle and packaged into tin capsules. These were arranged on a microtitre tray and delivered to the West Australian Biogeochemistry Centre (WABC) at the University of Western Australia for analyses.

Carbon and nitrogen stable isotope ratios are expressed in δ notation as parts per thousand (‰) as determined from:

$$\delta X=[(R_{sample}/R_{standard})-1]1000$$

where X is $\delta^{13}C$ or $\delta^{15}N$ and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The carbon stable isotope ratios are expressed relative to the international PeeDee Belminite (PDB). The nitrogen stable isotope ratios are relative to atmospheric nitrogen (AIR).

All samples for fatty acid composition analysis were freeze-dried (lyophilisation) for 24 hours and transferred, in thick-walled styrofoam containers filled with dry ice, to the lipid laboratory at the Institute for Hydrobiology and Fisheries Science at the University of Hamburg, for further processing.

After repeated lyophilisation, dry mass was determined using a Sartorius micro-balance (±2µg). During the weighing procedure, samples were temporarily stored in a desiccator to prevent unequal condensation on the tissue. For quantification of fatty acids, tricosanoic acid was added as an internal standard prior to extraction and the sample then stored at -24°C. Lipid extraction was performed with minor modifications as described in Folch et al. (1957). Small samples were transferred into 4 ml dichloromethane:methanol (2:1/ v:v), while larger samples were placed in 8 ml solvent mix. Each sample was homogenised in an ultrasonic disruption bath twice for 30 seconds each. Additionally, a Potter homogeniser was used for 30 sec prior to ultrasound to ensure quantitative extraction of lipids. This was followed by a washing procedure with aqueous KCl solution (0.88%), adding 2 and 4 ml to the smaller and larger samples, respectively. Samples were agitated for 30 seconds and phase separation occurred afterwards. The samples were placed in a centrifuge for 10 min at 2°C and at *ca* 2500 r/s. The lower, lipid-containing phase was then placed in a clean vial and the solvent evaporated under nitrogen. For fatty acid analyses, a subsample of total lipids were hydrolysed and fatty acids were converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulfuric acid at 80°C for 4 h (Kattner and Fricke, 1986). After cooling, 2 ml of Aqua bidest were added, and FAMEs were extracted three times with 1 ml hexane. Samples were analysed using a gas chromatograph (HP 6890A) equipped with a DBFFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) operated with a temperature program and helium as carrier gas. Samples were injected using a hot split/splitless inlet (250°C, split mode 1:20) or a programmable temperature vaporiser injector (solvent vent mode). The FAMEs and fatty alcohols were detected by flame ionization and identified by comparing retention times with those derived from standards of known composition. The accurate identification of the substances was checked for selected peaks using GC-MS.

The naming of fatty acids in this report is according to the IUPAC-IUB Commission on Biochemical Nomenclature (1967, 1977) shorthand notation of fatty acids z:y(n-x) where:

z = number of carbon atoms in the acyl chain
y = number of double bonds
n = chain length
x = number of carbon atoms from the last double bond to the terminal methyl group

## Results & Discussions

*Sample Size*

The sample sizes for this study are small, particularly for samples from Cockburn Sound and Rottnest Island, indicating the need for caution in interpretation of the findings. Nonetheless, Olin et al. (2011) found significant differences in the stable isotope ratios of bottlenose dolphins across several study sites (and sampling times) despite sample sizes of less than ten individuals for most sample groups in the study.

*Nitrogen*

The stable isotope ratios for the dolphin samples ranged from 12.0 to 18.6 (‰) for nitrogen (Table 1; Figure 4). Ratios for dolphins from the Swan-Canning Estuary were generally higher than those reported for bottlenose dolphins in the southeastern United States (e.g. Barros et al., 2010; Olin et al., 2011), but similar to values reported in Spain (Fernández et al., 2011), and in Victoria, Australia (Owen et al., 2011). These differences may reflect both environmental differences (e.g. in nutrient sources) and differences in the foraging ecology of bottlenose dolphins, and suggest the potential for broader geographic comparisons once a larger sample size of dolphins from the Perth area is obtained.

The highest nitrogen ratios occurred in dolphins from the Swan-Canning Estuary. Potential explanations for this finding include: (1) dolphins in the estuary feed at a higher trophic level than dolphin from Cockburn Sound and Rottnest Island; (2) food chains in the estuary are longer or more complex than in the two coastal locations; and/or (3) dolphins feed on the same fish species at all three locations, but the fish species occur at different trophic levels at each location. The higher nitrogen ratios in the estuary dolphins, if confirmed, differs from the pattern reported for bottlenose dolphins around the coast of coastal Florida by Barros et al. (2010), who found lower nitrogen ratios in estuary-associated dolphins than dolphins sampled in coastal and offshore areas.

The range of nitrogen ratios was also greatest in dolphins from the Swan-Canning Estuary. While this finding may be an artifact of differences in sample size, it suggests that dolphins in the estuary could be associated with a broader range of prey and food webs than dolphins from Cockburn Sound and Rottnest Island. For example, some of the dolphins from the Swan-Canning Estuary may feed more in marine areas and on marine prey, while others may feed more within the estuary and on estuarine prey, resulting in differences stable isotope compositions.

The nitrogen ratios also suggest that, within the food web of the upper Swan River, dolphins are on a similar trophic level to omnivorous fish and do not occupy a distinct ‘apex’ predator position within this particular estuarine food web. Several caveats are appropriate here. Firstly, trophic structure,

including the number of trophic levels present, is likely to vary across locations within the estuary (e.g. across the lower, middle, and upper reaches of the estuary; between the Canning and the Swan rivers). Thus, the relative trophic position of dolphins may also vary between locations. Secondly, dolphins do not appear to be closely associated with the food web of the upper Swan River (see below), which argues for caution in making a more general conclusion about the relative trophic position of dolphins within the estuary. Finally, even if dolphins do not occupy a clear apex trophic position within estuarine food webs, their large body size means they will consume a significantly larger biomass than other predators at the same trophic level. Adult dolphins may weigh between 155-175 kg and consume 5.2-6.3% of their body mass a day (based on published data for captive dolphins taken from the Perth area: Cheal and Gales 1992), suggesting that free-ranging dolphins may consume 8 to 11+ kg of prey per day.

*Carbon*

The carbon stable isotope ratios ranged from -21.3 to -15.6 (‰) (Table 1; Figure 4). These ratios are generally lower than those reported for bottlenose dolphins in the southeastern United States (e.g. Barros et al., 2010; Olin et al., 2011), Spain (Fernández et al. 2011), and in Victoria, Australia (Owen et al., 2011). As with nitrogen, these differences may reflect environmental differences and/or geographic variation in diet.

The range of carbon ratios was greatest in the dolphins from the Swan-Canning Estuary and narrowest in individuals from Rottnest Island (Table 1; Figure 5). As with the nitrogen ratios, this finding may reflect differences in sample sizes. However, other potential explanations for this finding include: dolphins in the Swan-Canning Estuary dolphins feed on a greater variety of foods; more inter-individual variation in diet occurs among dolphins from the estuary than for dolphins at the other two sites; and/or dolphins from the estuary are associated with food webs that have more (or more diverse) carbon sources (e.g. primary producers, detritus) and, therefore, a broader range of carbon ratios. The range of carbon sources for Rottnest Island individuals was very narrow, suggesting similar food sources for the three individuals.

Separating the samples into the two different tissues (i.e. skin and blubber), suggested that higher nitrogen ratios ($^{15}N/^{14}N$) may occur in blubber samples (Figure 6). This tissue seems to be metabolically more inert than skin and therefore retains the heavier nitrogen isotope. Hicks et al. (1985) estimated a 75 day turnover time for bottlenose dolphin, indicating that the composition of skin tissue will change seasonally (i.e. to reflect seasonal shifts in prey selection) (Olin et al., 2011).

When the average values for $\delta^{13}C$ or $\delta^{15}N$ of the Swan-Canning Estuary dolphins are compared to those of other consumers in the upper Swan River, their location to the far right of the plot suggests that the sources of carbon utilised by the dolphins may not originate in the upper Swan River and, further, may have marine origin (given the enriched carbon stable isotope ratios) (Figure 7). A marine origin for the carbon sources could relate to the ranging patterns of dolphins, as well as the movement patterns of their prey species. Dolphins from the resident community have been observed feeding both within the estuary and in adjacent coastal areas, suggesting that these dolphins consume a mixed prey base, which includes fish associated with the estuary (either seasonally or year-round) and with coastal habitats such as Owen Anchorage and Parmelia and Success Banks (Potter and Hyndes, 1999). Thus, dolphins could also be feeding on fish species that migrate into the estuary from marine waters on a seasonal basis (i.e. marine/estuarine opportunist fish species), and on fish species which are found only in coastal habitats outside the estuary.

**Table 1**: Range of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios (in ‰) for bottlenose dolphins sampled from Rottnest Island, Cockburn Sound, and the Swan-Canning Estuary in 2009.

| Isotope (‰) | Rottnest Island | Cockburn Sound | Swan-Canning Estuary |
|---|---|---|---|
| **min C** | -19.39 | -20.01 | -21.28 |
| **max C** | -18.52 | -16.57 | -15.62 |
| **min N** | 12.04 | 12.86 | 12.04 |
| **min N** | 12.73 | 15.03 | 18.63 |

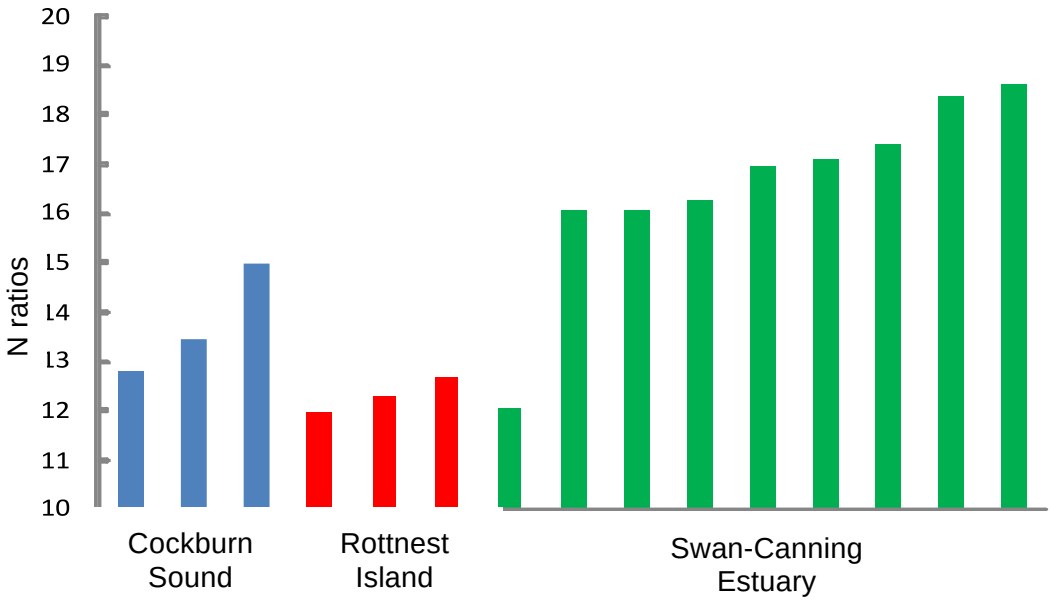


Figure 4: Trophic level as indicated by the stable nitrogen ($^{15}N/^{14}N$) isotope ratio (in ‰) of bottlenose dolphins in Cockburn Sound (blue), Rottnest Island (red), and the Swan-Canning Estuary (green).

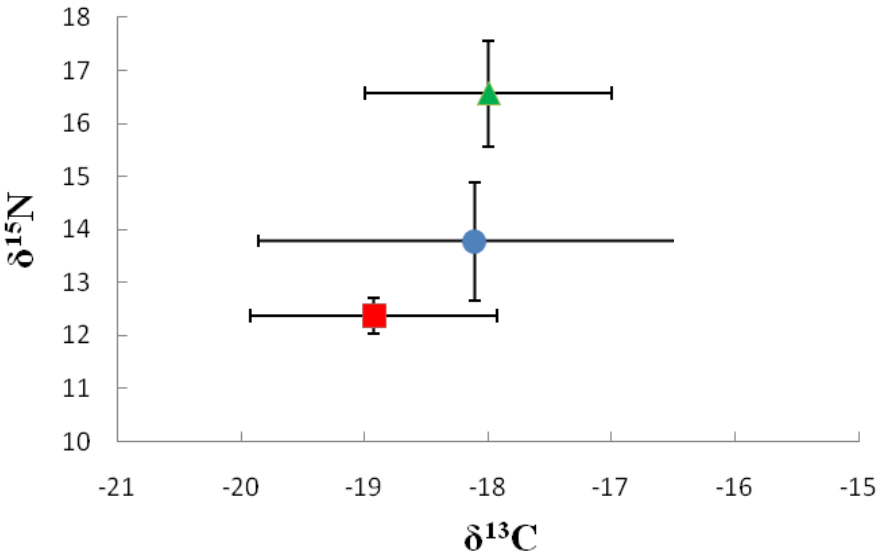


**Figure 5**: Means (± 1 SD) of carbon and nitrogen stable isotope ratios for bottlenose dolphins from Rottnest Island (red), Cockburn Sound (blue), and the Swan-Canning Estuary (green).

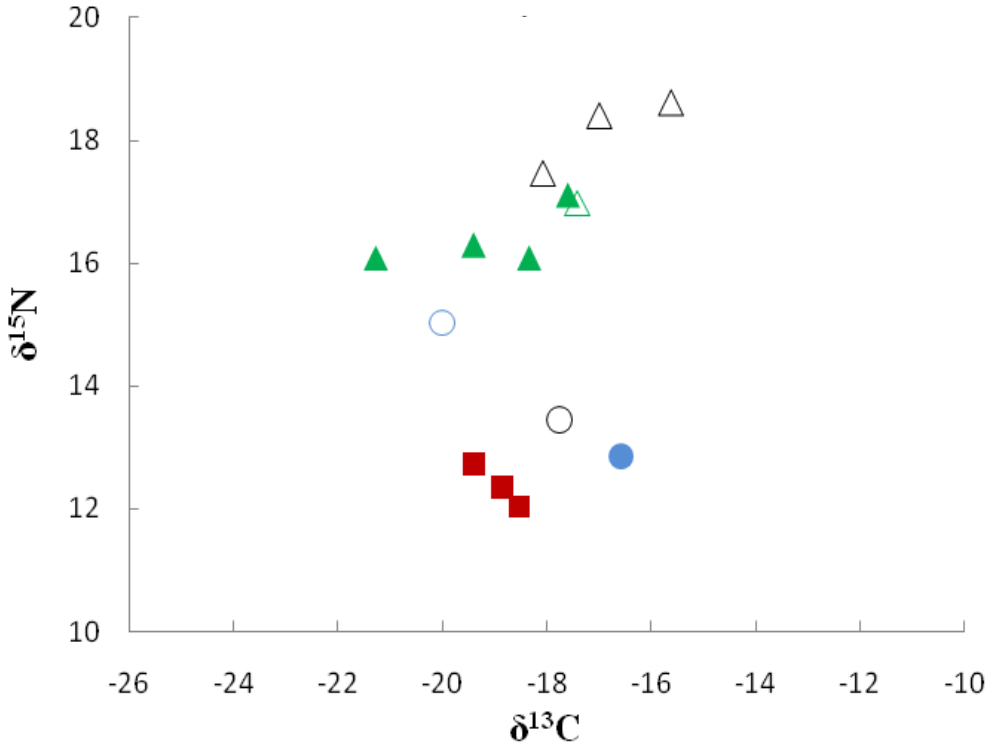


**Figure 6**: Stable carbon isotope ratios versus stable nitrogen isotope ratios in different tissues for bottlenose dolphins in the Swan-Canning Estuary (△▲), Cockburn Sound (○●) and Rottnest Island (□■) in 2009. The unshaded symbols indicate blubber samples, while the shaded symbols indicate skin samples. The three black outlined symbols (△) at the top of the figure are a mixture of skin and blubber samples from the Swan-Canning Estuary.

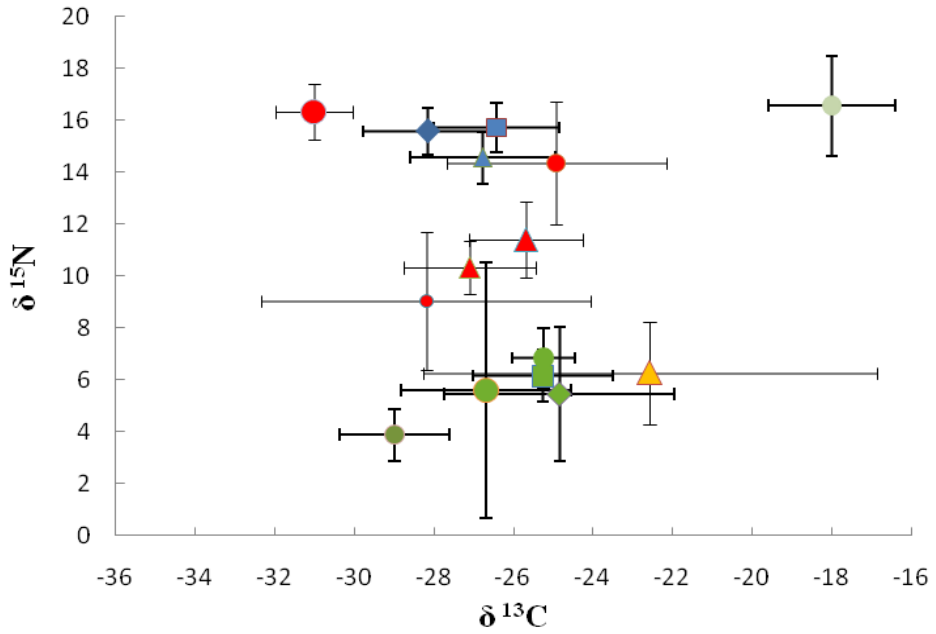


**Figure 7**: Stable carbon ratios versus stable nitrogen isotope ratios (± 1 SD) for fish (blue), invertebrate crustaceans and annelids (red), insects (yellow) and primary producers (green) in the upper Swan River in all seasons in 2007. Samples for bottlenose dolphins are denoted in grey.

*Fatty Acids*

Blubber biopsy samples from two bottlenose dolphins from the Swan-Canning Estuary were analysed for their fatty acid composition. In both individuals, the carnivorous marker fatty acid 18:1(n-9) contributed c.19 % to the total fatty acid composition (Figure 8), indicating a substantially carnivorous diet. This fatty acid is the precursor of all (n-3) and (n-6) polyunsaturated fatty acids, which are essential to all heterotrophic organisms (Dalsgaard et al., 2003). One dolphin (*Tursiops* 2) contained large amounts of the fatty acid 16:1(n-7) (Figure 8), which has been identified as a marker mainly for marine primary producers (Dalsgaard et al., 2003). The 16:1(n-7) isomer is also a common biosynthetic product in marine mammal blubber (Budge et al. 2006). Jeffries (1970) studied the fatty acid composition of a succession of species within a natural phytoplankton community in Rhode Island and found that the succession from diatoms to flagellates was associated with a decrease in the 16:1/16:0 ratio from >2 to <0.3. Our results show 16:1/16:0 ratios of 0.8 in *Tursiops* 1 and 3.4 in *Tursiops* 2, indicating that a high proportion of flagellates were at the base of the food chain leading to *Tursiops* 1, while diatoms were more important in the food chain leading to *Tursiops* 2. Linoleic acid [18:2(n-6), a typical "terrestrial" fatty acid: Napolitano et al., 1997)], was present in both animals, but did not contribute >4% to the total fatty acid content in the dolphins; therefore terrestrial lipids were considered not to be important in the diet of these two animals.

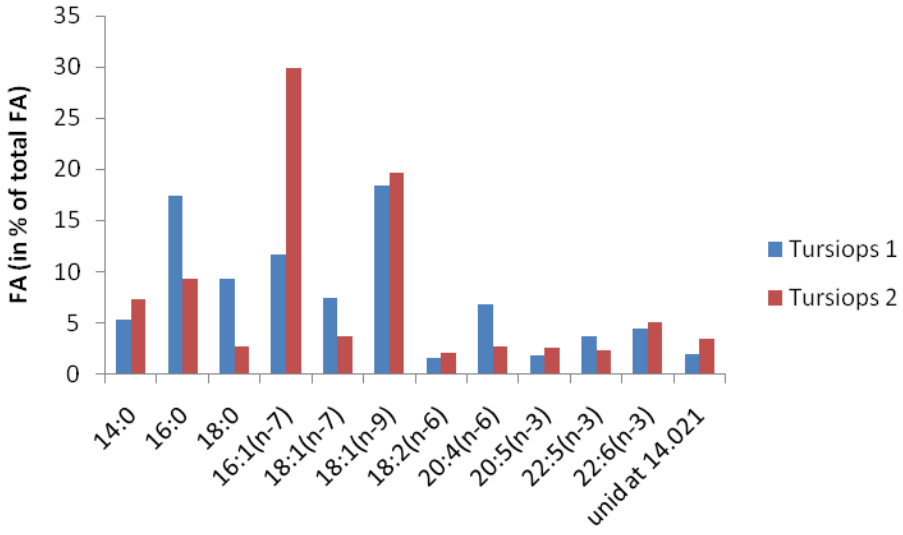


**Figure 8**: Fatty acid profile of two bottlenose dolphins from the Swan-Canning Estuary. The fatty acid 16:1(n-7) is a herbivore marker, while 18:1(n-9) is a carnivore marker.

*Knowledge Gaps*

These questions indicate areas where knowledge gaps remain in our understanding of the feeding ecology of dolphins associated with the Swan-Canning Estuary:

(a) What are the main prey species for dolphins within the estuary?

(b) Which carbon sources fuel the estuarine food web(s) leading up to these prey species?

(c) What are the intermediate consumers through which energy is channeled up the food chain to dolphins?

(d) What is the provenance of dolphin prey species, i.e. are they derived from fish populations that are estuarine, coastal, or marine/estuarine opportunists?

(e) What is the relative importance of marine vs. estuarine-based prey?

(f) How does dolphin feeding activity and prey selection vary over time (e.g. seasonally) and between habitats and locations (e.g. upper vs. lower estuary) within the estuary?

The ecologies of dolphins and their prey species are complex and present several challenges for studies of the feeding ecology of dolphins in the Swan-Canning Estuary. Firstly, bottlenose dolphins (*Tursiops* spp.) are catholic feeders and may feed on a prey ranging from small prey items (c. 100mm in length) to large prey items more than a half a meter in length (Barros and Wells, 1998; Gannon and Waples,

2004).[6] Secondly, dolphins are likely to feed in both estuarine and coastal habitats, and it is not clear how dolphins apportion their foraging effort across these two areas or if, for example, this effort changes seasonally. Thirdly, dolphins may vary in the parts of the estuary in which they feed. Behavioural observations indicate that dolphins feed extensively within the lower reaches of the estuary and the basin habitats in the middle sections of the estuary (e.g. Melville Waters) (areas which have a strong, year-round marine influence) (H. Finn, Murdoch University, unpublished data). However, feeding is also common in the Canning River and at least some individuals feed in the middle to upper reaches of the Swan River. All of these factors may result in complex and variable trophic signatures and a feeding ecology that is dynamic and diverse.

Like dolphins, dolphin prey species are also likely to be vary in their isotopic composition, both between and within species, as well as between locations and times. The fish and cephalopod species present in the Swan-Canning Estuary have a diverse range of life-histories, including species that are: marine/estuarine opportunists, marine stragglers, exclusively estuarine, estuarine and marine, semi-anadromous, and catadromous (Hallett 2010; Potter and Hyndes 1999; Smith 2006, 2009). Thus, for example, certain species may be present in the estuary seasonally or at some stage of their life history, e.g. *Nematolosa vlaminghi* (Perth Herring), *Mugil cephalus* (Sea Mullet), *Aldrichetta forsteri* (Yellow-eye Mullet). Dolphin prey selection may therefore change seasonally in response to shifts in the presence and abundance of prey species. These seasonal changes in diet may not necessarily result in altered stable istope ratios if, for example, prey retain their non-estuarine stable isotopic composition while present in the estuary. In addition, some fish species may have distinct estuarine and coastal populations, meaning that dolphins may consume prey which are of the same species but associated with two different food webs.

*Management Implications*

While the sample sizes were small, the findings do suggest differences in stable isotope ratios between dolphins feeding within the Swan-Canning Estuary and dolphins associated with coastal areas. This suggests that stable isotope ratios, in combination with other trophic (e.g. fatty acids) and non-trophic (e.g. genetics, behavioural) approaches, may provide a useful instrument for examining the population structure of dolphins from the Perth area and for identifying discrete assemblages of dolphins associated with particular food webs.

A key aim of this study was to determine if dolphins could be situated within the food web for the upper Swan River. The findings, though preliminary, suggest that dolphins are weakly associated with

[6] Dolphins cannot chew and therefore must either ingest prey items intact or break them into ingestible portions (e.g. by throwing them along the surface or breaking them apart along the benthic substrate).

this food web. This suggests that, within the estuary, dolphins are likely to be more strongly associated with food webs in the basin habitats and in the lower reaches of the estuary and that these food webs are likely to rely on marine food sources. Placement of dolphins within estuarine food webs will require further information on the trophic structure of: (a) other locations in the Swan-Canning Estuary, such as the Canning River and the basin habitats (e.g. Perth Waters, Melville Waters) and (b) coastal sites which dolphins are likely to be associated with (e.g. Owen Anchorage, Parmelia and Success Banks).

# V. Contaminant Exposure

## Introduction

All marine mammals harbour some sort of contaminant burden because of the global dissemination of anthropogenic chemicals. However, the presence of potentially toxic contaminants within tissues does not, by itself, constitute evidence of harm (O'Hara and O'Shea, 2001). Concerns over contaminants in marine mammals reflect a range of studies that have reported associations between various contaminants and deleterious effects on the immune, endocrine and nervous systems of marine mammals. Nonetheless, few studies have conclusively demonstrated a direct association between contaminants and these effects. Evans (2003, p. 400) observes that: "...most studies lack substantive evidence of sub-lethal effects due to numerous physiological and environmental confounding factors."

However, it is important to note that although there is a lack of experimental data on causal relationships, direct marine mammal experimentation using controlled exposure to contaminants is not only logistically difficult, but also ethically and legally prohibitive (Ross, 2002). Further, such studies have their own limitations in that in order to determine the mechanism of toxicity one must reduce the variables (single chemicals vs. complex mixtures; acute as opposed to chronic toxicities) to such an extent that the conditions no longer reflect 'real world' conditions, and consequently little is known about the cumulative impact of the complex mixtures of contaminants often found in marine mammals (Ross, 2002). A similar situation exists for understanding the significance of contaminants exposure in humans and, according to Ross (2002), indirect associations and the extrapolations of extensive research on the adverse effects of pollutants/contaminants in laboratory animals are often applied.

A principal objective of this study was to obtain tissue samples from dolphins observed within the Swan-Canning Estuary and to determine baseline concentrations for certain organic and inorganic contaminants known to accumulate in marine mammals. A further objective was to provide a preliminary assessment of health risk to dolphins posed by contaminants based on: observed concentrations of contaminants, comparative information from other studies, and the toxicology of organic and inorganic contaminants in marine mammals. A subsidiary objective was to assess the suitability of tissue samples obtained through remote biopsy sampling, as this technique offers a method to obtain samples from free-ranging dolphins (i.e. rather than having to rely on samples collected post-mortem from stranded/deceased individuals). We note that, prior to this study; no published data existed for the concentrations of contaminants in marine mammals from Western Australia.

*Expression of results*

The majority of studies report contaminant levels on the basis of mass chemical per unit mass of tissue. The unit mass of tissue can be expressed on the basis of the wet weight of tissue sample, on dry weight of tissue sample (i.e. weight of sample with water removed), or on the basis of lipid weight. The water content of tissues is highly variable and thus contaminant concentrations reported on a wet weight basis limits inter-animal comparisons. Normalising organic contaminant concentrations on the lipid content of tissues reduces differences between individuals and allows for more appropriate comparisons to be made.

The most typical expression of concentrations in the literature are given as parts per million (ppm), which on a unit of mass basis may also be expressed as µg/g or mg/kg (O'Shea, 1999). Lower concentrations may be expressed as parts per billion (ppb) or by units ng/g or µg/kg. It is important to be certain of the units in comparing findings among studies, only comparing concentrations between like units of mass and type (i.e. wet weight, dry weight and lipid weight).

Another consideration is that the sum concentrations of various isomers or congeners of pollutants [e.g. commonly presented ∑DDT (dichlorodiphenyltrichloroethane), ∑PCB (polychlorinated biphenyls) can vary considerably depending on the type and number of isomers and congeners included (O'Shea and Brownell, 1994). Studies that do not indicate the specific isomers or congeners of various contaminants thus have limited application (Evans, 2003).

*Organic contaminants*

The bioaccumulation of a contaminant in an animal is affected by the amount of that contaminant absorbed, the extent and rate of metabolism of that compound and the amount excreted (Evans, 2003). These factors vary between species and consequently caution should be taken when comparing contaminant concentrations between different marine mammal species.

*Distribution and kinetics*

In marine mammals lipophilic contaminants such as organochlorines (OCs) accumulate in fat-rich tissues such as blubber. The most inert OCs may remain in the blubber throughout the relatively long lives of marine mammals (Tanabe et al., 1984). However, during times of physiological stress such as illness, extreme temperature, nutritional compromise or pregnancy and lactation, OCs may be mobilised along with lipid stores and circulated throughout the body via the bloodstream (Aguilar, 1987; Aguilar and Borrell, 1994a). The rates at which OCs are either passed into the blood with lipid

mobilisation or are concentrated in the remaining fat are not well understood (Aguilar, 1985, 1987). Contaminant concentrations in blubber can also be diluted with rapid expansion of the lipid component during seasonal fattening periods or growth. Stranded marine mammals often represent young, old, or diseased individuals that may have diminished lipid reserves with consequent elevations in organochlorine residue concentrations in blubber (O'Shea, 1999).

Blubber fat content can vary by topographic location on the body and by structural stratification within areas. Vertical stratification of lipid classes in blubber has been reported in odontocetes (toothed whales) (Krahn et al., 2004). The inner blubber layer is thought to be more metabolically active than the outer layer which is thought to perform more of a structural function. Variations in contaminant concentrations within blubber layers have also been reported (Krahn et al., 2004). In order to minimise the effect of these variables it is recommended that a full thickness blubber sample should be collected from an area just anterior to the dorsal fin (Duignan, 2000).

*The influence of age, sex and reproductive status on concentrations of OCs*

The ability of marine mammals to metabolise and excrete contaminants varies with sex and age. Males tend to accumulate OCs throughout their lives, while females show a similar increase up to sexual maturation, after which concentrations tend to stabilise or decrease (Evans, 2003). The decrease or leveling of contaminants observed in females is associated with the transfer of OCs from the female to her young both during pregnancy and lactation, with the greatest transfer occurring during lactation (Aguilar and Borrell, 1994b, Borrell et al., 1995). Cockcoft et al. (1989) suggested that by the end of the first complete reproductive cycle, a bottlenose dolphin transfers approximately 80% of her maternal body burden to her first-born calf.

Reddy et al. (2001) reported preliminary findings on the effect of maternal OC exposure in bottlenose dolphins on pregnancy outcome. Blubber OC levels were compared between females whose calves survived beyond six months and females whose calves were stillborn or died within 12 days of birth. The mean concentration of ∑DDT was more than three times as high among dolphins whose calves died as that among dolphins whose calves survived beyond six months ($P$ = 0.002). It should be noted that the results of the Reddy et al. (2001) were deemed preliminary and the sample size was small ($n$ = 14).

*Organochlorines*

Concentrations of organic contaminants in marine mammals are highly influenced by the species examined (given differences in diet, absorption and excretion of contaminants). Therefore, for the purposes of this report only a comparative review of contaminants in bottlenose dolphins (*Tursiops*

spp.) was undertaken (Table 2). In order to improve the accuracy of comparing contaminant concentrations between studies the following factors were considered:

- **The number of congeners will influence total PCBs.** Therefore, for consistency and to allow for comparisons to be made, only PCB levels based on the International Council for the Exploration of the Seas (ICES) seven congeners were included in Table 2.
- **Total DDT levels can be influenced by the number of isomers included**. Therefore, for consistency total DDT levels reported in Table 2 were limited to the sum of *pp*-DDE, *pp*-DDD, and *pp*-DDT.
- **There is likely to be variability in the distribution of contaminants within blubber.** Therefore, the comparative table only includes studies that examined full thickness blubber samples collected from stranded or by-caught animals.

While these considerations can help to minimise variations potentially influencing the accuracy of comparisons of contaminant levels across and between studies, there are several variations that are not possible to account for in most situations. These factors include: health status (diseased versus by-caught dolphins); geographic location; diet; sex; and life-history traits (e.g. age, reproductive state); as well as the analytical procedures involved in identifying and quantifying the contaminants. Each of these factors may have influenced the contaminant concentrations recorded in these studies, and thus comparisons must be made with caution.

As a further benchmark for comparing contaminant burdens specifically in estuarine bottlenose dolphins in urban areas, Table 3 includes contaminant results from full thickness blubber samples collected from wild dolphins that were purposely captured (and then released) within two estuaries (Charleston and Indian Lagoon) along the east coast of the U.S., between 2003 and 2005 (Fair et al., 2010). The dolphins that are found in these estuaries have high site fidelity as indicated by long term-photo identification data (Fair et al., 2010). The total DDT recorded is the sum of 6 DDTs (*op*-DDE, *op*- DDD, *op*-DDT, *pp*-DDE, *pp*-DDD, and *pp*-DDT). The total PCBs recorded is the sum of 92 congeners. The total PCBs and total DDT concentrations found in the Charleston dolphins are among the highest reported values in marine mammals (Fair et al., 2010).

**Table 2:** Organochlorine residue data in bottlenose dolphins (*Tursiops* sp.) from various studies expressed as µg/g wet weight and lipid weight (in parentheses).
[a] ∑DDT is the sum of *pp*-DDE, *pp*-DDD, *op*-DDT, *pp*-DDT

| | Date | Location | % lipid | Dieldrin | pp-DDE | pp-DDD | pp-DDT | ∑DDT | PCBs ∑ICES7 | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **U.K.** | | | | | | | | | | |
| SW1999/175 Adult, female | 1999 | Kent, UK | 90 | 0.2 (0.22) | 1.2 (1.33) | 0.18 (0.2) | 0.31 (0.34) | 1.69 (1.88) | 4.755 (5.28) | 1 |
| SW2001/141 Adult, female | 2001 | Greater London, UK | 88 | 2.0 (2.27) | 108 (122.72) | 2.5 (2.84) | 0.76 (0.86) | 111.26 (126.43) | 111.943 (127.21) | 1 |
| FA1TT Juvenile, female | 1989 | Moray Firth, Scotland | 67.4 | 1.995 (2.96) | | | | | 7.145 | 2 |
| FA2TT Adult, female | 1989 | Moray Firth, Scotland | 49.3 | | | | | | 8.265 | 2 |
| FA3TT Adult, female | 1988 | Moray Firth, Scotland | 44.0 | 0.612 (1.39) | | | | | 4.637 | 2 |
| FA4TT Adult, female | 1990 | Moray Firth, Scotland | 56.5 | 0.301 (0.53) | | | | | 0.81 | 2 |
| FA5TT Calf, female | 1989 | Moray Firth, Scotland | 43.9 | 0.522 (1.19) | | | | | 1.294 | 2 |
| Mean results of five females (FA1TT, FA2TT, FA3TT, FA5TT) | 1988-1991 | Moray Firth, Scotland | | | | | | 4.65 Range: 1.149-8.3 | | 2 |
| MA1TT Juvenile, male | 1988 | Moray Firth | 56.7 | 2.935 (5.18) | | | | | 6.986 | 2 |
| **Europe** | | | | | | | | | | |
| Mean results | 1978 | Western Mediterranean | | | | | | (303)[a] | | 3 |
| Mean results | 1987 | Western Mediterranean | | | | | | (194)[a] | | 3 |
| Mean results | 2002 | Western Mediterranean | | | | | | (13)[a] | | 3 |
| Cet 50 Adult, female | 1998 | Canary Islands, North Atlantic | 30.4 | 0.002 (0.007) | 0.106 (0.35) | 0.006 (0.02) | 0.031 (0.1) | 0.143 (0.47) | 0.221 (0.73) | 4 |
| Cet 78 Adult, male | 1999 | Canary Islands, North Atlantic | 10.2 | 0.036 (0.35) | 12.5 (122.55) | 0.308 (3.02) | 2.16 (21.18) | 14.968 (146.75) | 7.86 (77.06) | 4 |
| Cet 94 Juvenile, male | | Canary Islands, North Atlantic | 50.4 | 0.021 (0.04) | 0.725 (1.44) | 0.04 (0.08) | 0.132 (0.26) | 0.897 (1.78) | 1.504 (3.0) | 4 |

| | Date | Location | % lipid | Dieldrin | pp-DDE | pp-DDD | pp-DDT | ∑DDT | PCBs ∑ICES7 | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **Europe** | | | | | | | | | | |
| Cet 124 Adult, male | 2001 | Canary Islands, North Atlantic | 24.3 | 0.051 (0.21) | 18.55 (76.34) | 0.257 (1.06) | 1.664 (6.85) | 20.471 (84.24) | 25.393 (104.5) | 4 |
| Cet 144 Juvenile, male | 2001 | Canary Islands, North Atlantic | 56.1 | 0.089 (0.16) | 2.324 (4.14) | 0.175 (0.31) | 0.704 (1.25) | 3.203 (5.7) | 7.557 (13.47) | 4 |
| Cet 145 Adult, female | 2001 | Canary Islands, North Atlantic | 54.7 | 0.009 (0.02) | 0.141 (0.26) | 0.021 (0.04) | 0.048 (0.09) | 0.21 (0.38) | 0.385 (0.7) | 4 |
| Cet 168 Juvenile, male | 2002 | Canary Islands, North Atlantic | 57.4 | 0.183 (0.32) | 4.57 (7.96) | 0.34 (0.59) | 1.13 (1.97) | 6.04 | 5.391 (10.52) | 4 |
| Cet 171 Juvenile, female | 2002 | Canary Islands, North Atlantic | 55.7 | 0.08 (0.14) | 3.07 (5.51) | 0.169 (0.3) | 0.687 (1.23) | 3.926 (7.05) | 5.793 (10.4) | 4 |
| Cet 311 Juvenile, male | 2005 | Canary Islands, North Atlantic | 35.0 | | 0.344 (0.98) | 0.007 (0.02) | 0.103 (2.94) | 0.454 (1.3) | 11.247 (32.13) | 4 |
| **Israel** | | | | | | | | | | |
| D-2 Calf, female | 2006 | Mediterranean coast, Israel | | | 0.121 | 0.715 | 0.083 | 0.919 | | 5 |
| D-4 Calf, female | 2006 | Mediterranean coast, Israel | | | 2.05 | 135 | 4.20 | 141 | | 5 |
| D-5 Juvenile, male | 2006 | Mediterranean coast, Israel | | | 0.774 | 11.5 | 1.07 | 13.4 | 7.90 | 5 |
| D-6 Juvenile, female | 2005 | Mediterranean coast, Israel | | | 0.005 | 9.77 | 0.01 | 9.79 | | 5 |
| D-10 Calf, male | 2004 | Mediterranean coast, Israel | | | 0.506 | 7.63 | 0.848 | 8.96 | 4.70 | 5 |
| **India** | | | | | | | | | | |
| 97 Tt 01 Calf, male | 1997 | Southeast coast of India | 45 | | | | | 17.0 (37.78) | | 6 |
| 97 Tt 02 Juvenile, female | 1997 | Southeast coast of India | 50 | | | | | 8.75 (17.5) | | 6 |
| 99 Tt 09 Adult, male | 1999 | Southeast coast of India | 42 | | | | | 6.72 (16.0) | | 6 |
| 99 Tt 10 Adult, female | 1999 | Southeast coast of India | 43 | | | | | 19.25 (44.77) | | 6 |
| DO(02)90 Female | 1990 | Bay of Bengal, southern India | 69 | | 4.7 (6.8) | 1.0 (1.4) | 0.16 (0.23) | 5.86 (8.43) | | 7 |
| DO(05)90 Male | 1990 | Bay of Bengal, southern India | 53 | | 6.1 (11.5) | 0.84 (1.68) | 0.59 (1.11) | 7.53 (14.29) | | 7 |

| | Date | Location | % lipid | Dieldrin | pp-DDE | pp-DDD | pp-DDT | ∑DDT | PCBs ∑ICES7 | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **India** | | | | | | | | | | |
| DO(09)90 Male | 1990 | Bay of Bengal, southern India | 40 | | 2.1 (5.25) | 0.017 (0.04) | 0.098 (0.25) | 2.215 (5.54) | | 7 |
| DO(02)91 Female | 1991 | Bay of Bengal, southern India | 67 | | 9.2 (13.73) | 3.7 (5.52) | 1.3 (1.94) | 14.2 (21.19) | | 7 |
| **U.S.** | | | | | | | | | | |
| Mean results *n* = 33 | 1994 | Gulf of Mexico | | (0.547) (Range: 0.029 – 2.03) | (12.8) (Range: 0.188 – 70.7) | (1.02) (Range: 0.11 – 4.53) | (0.542) (Range: 0.012 – 3.27) | (14.362) (Range: 0.31 – 78.5) | | 8 |
| **South Africa** | | | | | | | | | | |
| Male 1976 | 1976 | South Africa | | | | | | 4.14 | | 9 |
| Male 1980 | 1980 | South Africa | | | | | | 0.17 | | 9 |
| Male 1984 | 1984 | South Africa | | | | | | 2.52 | | 9 |
| Male 1985 | 1985 | South Africa | | | | | | 3.6 | | 9 |
| Male 1985 | 1985 | South Africa | | | | | | 12.29 | | 9 |
| Female 1987 | 1987 | South Africa | | | | | | 1.75 | | 9 |
| **Australia** | | | | | | | | | | |
| # 1 Adult, female | 1999 | Queensland | | (0.166) | (0.42) | (0.173) | (0.089) | (0.682) | | 10 |
| #2 Adult, female | 1995 | Queensland | | (0.047) | (1.683) | (0.086) | (0.126) | (1.895) | | 10 |
| #3 Adult, male | 1997 | Queensland | | (0.425) | (52.416) | (0.618) | (0.515) | (52.549) | | 10 |
| #4 Adult, male | 1996 | Queensland | | (0.175) | 11.303 | 0.223 | 0.24 | 11.766 | | 10 |
| RJM-02 Adult, female | 1995 | Gold coast, Mermaid Beach | 49 | 0.059 (0.12) | 0.69 (1.4) | 0.033 (0.067) | 0.044 (0.09) | 0.767 (1.57) | 0.69 (1.4) | 11 |
| RJM-03 Calf, female | 1996 | Gippsland lake | 32 | 0.045 (0.14) | 0.2 (0.63) | 0.02 (0.06) | 0.049 (0.15) | 0.269 (0.84) | 0.36 (1.13) | 11 |

**References:** 1 Law (1994); 2 Wells et al. (1994); 3 Borrell and Aguilar (2007); 4 Carballo et al. (2008); 5 Shoham-Frider et al. (2009); 6 Karuppiah et al. (2005); 7 Tanabe et al. (1993); 8 Salata et al. (1994); 9 De Kock et al. (1994); 10 Vetter et al. (2001); 11 Law et al. (2003)

**Table 3:** Organochlorine residue data in bottlenose dolphins (*Tursiops* sp.) from two estuaries on the east coast of the U.S. expressed as µg/g lipid weight (adapted from Fairs et al. 2010)

| | *n* | | Dieldrin | pp-DDE | pp-DDD | pp-DDT | ∑DDT | ∑PCBs |
|---|---|---|---|---|---|---|---|---|
| **Indian River Lagoon, Florida** | | | | | | | | |
| Juvenile | 24 | Geomean | 0.359 | 9.85 | 0.543 | 0.203 | 10.9 | 48.4 |
| | | Range | 0.0471-1.43 | 1.9-43.1 | 0.13-1.78 | 0.112-0.452 | 2.2-45.8 | 9.27-221.0 |
| | | 95% CI | 0.235-0.456 | 7.32-13.2 | 0.405-0.728 | 0.17-0.241 | 8.19-14.6 | 36.3-64.6 |
| Adult female | 15 | Geomean | 0.0665 | 3.75 | 0.274 | 0.132 | 4.6 | 25.5 |
| | | Range | 0.0022-0.845 | 0.188-14.3 | 0.108-0.752 | 0.0049-0.284 | 0.544-15.2 | 1.51-105.0 |
| | | 95% CI | 0.019-0.233 | 1.94-7.26 | 0.195-0.384 | 0.0768-0.225 | 2.66-7.95 | 13.5-48.2 |
| Adult male | 33 | Geomean | 0.356 | 17.4 | 0.566 | 0.217 | 18.6 | 79.8 |
| | | Range | 0.0017-1.23 | 5.5-56.3 | 0.0768-1.45 | 0.0832-0.422 | 6.39-58.4 | 35.0-227.0 |
| | | 95% CI | 0.236-0.535 | 2.24-14.2 | 0.466-0.688 | 0.194-0.244 | 15.3-22.6 | 67.4-94.4 |
| **Charleston, South Carolina** | | | | | | | | |
| Juvenile | 20 | Geomean | 1.26 | 11.3 | 1.58 | 0.31 | 14.7 | 47.8 |
| | | Range | 0.445-5.329 | 2.92-29.5 | 0.595-4.38 | 0.175-0.806 | 4.08-46.8 | 16.5-121.0 |
| | | 95% CI | 0.977-1.62 | 8.85-14.5 | 1.28-1.95 | 0.259-0.371 | 11.4-19.0 | 37.9-60.2 |
| Adult female | 11 | Geomean | 0.16 | 1.87 | 0.394 | 0.235 | 2.99 | 14.3 |
| | | Range | 0.0206-1.08 | 0.519-22.6 | 0.161-2.62 | 0.143-0.564 | 1.06-27.3 | 4.54-131.0 |
| | | 95% CI | 0.0636-0.404 | 0.726-4.83 | 0.204-0.761 | 0.178-0.31 | 1.32-6.77 | 6.26-32.5 |
| Adult male | 36 | Geomean | 1.42 | 26.1 | 1.69 | 0.324 | 29.0 | 94.0 |
| | | Range | 0.414-2.67 | 13.2-80.5 | 0.394-4.17 | 0.008-0.76 | 14.9-86.8 | 28.6-255.0 |
| | | 95% CI | 1.23-1.65 | 22.1-30.7 | 1.481-1.94 | 0.254-0.412 | 24.8-34.0 | 79.3-111.0 |

*Organochlorine pesticides and metabolites*

A baseline study of contaminants in the Swan and Canning catchment drainage system in 2006 found that organochlorine (OC) pesticides were more common in sediments than in surface waters (Nice et al., 2009). OC pesticides were detected in the Bayswater Main Drain, Blackadder Creek, Central Belmont Main Drain, South Belmont Main Drain, Helena River, Maylands, Upper Swan, Mills Street Main Drain and Lower Canning subcatchments. OC pesticides were detected at levels consistently above guideline limits, where these were available. Nice et al. (2009) reported that "(c)hlordane and dieldrin were the most frequently reported OC pesticides and Helena River had the highest number of individual OC pesticides detected and typically the highest concentrations."

Metabolites of DDT are usually the most commonly reported organochlorine insecticide residues found in marine mammals (O'Hara and O'Shea, 2001). The metabolites of DDT that are commonly found in marine mammal tissue include DDE and DDD (O'Shea, 1999). Total DDT is the sum of concentrations of the isomers of DDT, DDE and DDD. DDE is the most stable and toxic of the DDT metabolites, it is also the most widespread and abundant metabolite found in marine mammal blubber (O' Shea, 1999).

Extreme cases of ∑DDT contamination of marine mammals have resulted in concentrations of 1000 to 2000 µg/g wet weight or more in blubber. However, typical concentrations are much less than 100 µg/g wet weight, with many samples at 10 µg/g wet weight or less (O'Shea, 1999). Table 2 provides a more comprehensive comparison of organic contaminants reported in deceased bottlenose dolphins globally.

Aldrin, dieldrin and endrin are all cyclodiene insecticides that were widely used prior to restrictions coming into place and are generally much more acutely toxic than DDT (O'Shea, 1999). Dieldrin is an insecticide in its own right, but is also a metabolite of aldrin, which breaks down in the environment much more rapidly than dieldrin. Dieldrin is frequently found in blubber of marine mammals, whereas the less persistent aldrin and the more toxic endrin are rarely found (O'Shea, 1999). According to Matsumura (1995, cited in O'Shea, 1999) dieldrin is one of the most persistent chemicals ever known. Concentrations of dieldrin in marine mammal blubber are usually much lower than those of ∑DDT, rarely reaching 10 – 15 µg/g wet weight in the past and 0.1 µg/g in more recent samples (O'Hara and O'Shea 2001).

The cyclodiene insecticide chlordane is a mixture of cis- and trans- isomers of chlordane, heptachlor, and nonachlor (Dearth and Hites, 1991). Heptachlor epoxide is a metabolite of heptachlor. Isomers of chlordane, nonachlor, heptachor and heptachlor epoxide have been reported in marine mammals worldwide, and concentrations are usually <1 µg/g wet weight in recent times (O'Hara and O'Shea 2001).

*Polychlorinated biphenyls (PCBs)*

Polychlorinated biphenyls (PCBs) were widely used in a variety of industrial and consumer products (including capacitor and transformer fluids, lubricating and cutting oils, pesticide and plastic additives and reactive flame retardants). A ban on the importation of PCBs has been in place in Australia since 1979 (Nice et al., 2009). Once in the environment, stable PCBs degrade slowly and undergo cycling and transport and are thus ubiquitous in the environment (Burgin et al., 2001).

There are two main groupings of PCBs that are often studied: (a) those identified by the World Health Organisation (WHO) and (b) those by the ICES. The ICES group covers seven congeners (ICES-7) commonly found in the environment and are seen as markers of the degree of contamination, while the WHO group covers 12 congeners which are recognised as having dioxin-like properties with respect to impacts on human health (van den Berg et al., 1998).

Single PCBs are never found alone in the environment, they always occur as mixtures because they were produced as mixtures by various chemical companies. PCBs mixtures produced by the Monsanto Company were sold under the trade name Aroclor followed by a four-digit number. The first two digits of an Aroclor number refer to the number of carbon atoms in the bipheyl skeleton (for PCBs this is 12). The second two digits indicate the percentage of chlorine by mass in the mixture (for example Aroclor 1254 contained 54% chlorine by mass). These commercial mixtures contained large numbers of individual PCB congeners that varied from lot to lot, for example Aroclor 1254 typically contained some 50 to 70 PCB congeners (O'Shea, 1999).[7]

Previously, the practice was to compare the amounts of PCBs present in a sample with a standard mixture such as Aroclor 1254 or 1260. However, choice of standard and analytical methodology (e.g. difference in detector response to different congeners) affects the estimated concentrations. Modern analytical procedures now allow for the concentrations of individual congeners to be determined (O'Shea, 1999). In more recent studies, total PCB concentrations should only be compared when the individual PCB congeners contributing to the total concentration have been identified and are consistent between studies (note that in Table 2 only studies where total PCBs as ICES7 were given were compared).

Kannan et al. (2000) compared the *no observed adverse effect level* (NOAEL) and *lowest observed adverse effect level* (LOAEL) values for toxic effects of PCBs in seals, otter, and mink and derived a threshold dose for adverse effects. The threshold dose for adverse effects was estimated as the

[7] Schweitzer and Baskaran (2003, p.9) observe that: "…the composition of any Aroclor mixture was not completely consistent from lot-to-lot. Aroclor 1242 contained approximately 42% PCBs by weight. The individual congeners may have varied, since the composition of any Aroclor mixture was not consistent lot to lot, but the overall pattern should be recognizable. As well, there were certain congeners that were indicative of each Aroclor mixture."

geometric mean of the NOAEL and LOAEL. Kannan et al. (2000) examined the studies by Boon et al. (1987) and Brouwer et al. (1989); in these studies, seals fed fish from the Wadden Sea (high-level PCB contamination) were found to have significantly lower concentrations of vitamin A and thyroid hormones in comparison to seals fed fish from the north-east Atlantic (low-level PCB contamination). Based on the studies by Boon et al. (1987) and Brouwer et al. (1989), a threshold value for total PCBs in seal blood of 11 µg/g lipid weight was derived (Kannan et al., 2000). The threshold value for PCBs in livers of European otters for vitamin A reduction was 6.6 µg/g lipid weight (Smit et al. 1996, Murk et al. 1998). A threshold liver concentration for total PCBs for reproductive effects of 10 µg/g lipid weight has been reported for mink (Heaten et al., 1995). The threshold PCB concentrations for the liver or blood in seal, otter, and mink were thus in the range of 6.6 to 11 µg/g lipid weight (Kannan et al. 2000). Kannan et al. (2000) suggested that the geometric mean of the three values, 8.7 µg/g lipid weight, as a threshold concentration for PCBs in marine mammal liver or blood. Reddy et al. (1998) determined that lipid normalised concentrations of total PCBs in the blubber were two fold greater than those in the blood of clinically healthy bottlenose dolphins. Therefore, by applying a factor of two to account for the differences in the lipid normalised concentrations for PCBs in blood and blubber, a threshold concentration for adverse effects of PCBs in the blubber of marine mammals of 17 µg/g lipid weight was derived (Kannan et al., 2000).

In order to compare PCB concentrations determined in the blubber of marine mammals with the threshold derived by Kannan et al. (2000), Jepson et al. (2005) suggested calculating the concentration of PCBs based on the concentration of Aroclor 1254. This was presumably done because when the original studies were conducted [which formed the basis of the threshold described by Kannan et al. (2000)] individual PCB congeners were not available for the analysis of samples and PCBs were identified by their peak characteristics and retention times in relation to a standard mixture of Aroclor(s). Further, Aroclor 1254 was found to be one of the major environmental pollutants in the Wadden Sea (Brouwer et al., 1989) and the study on seals fed fish from the Wadden Sea contributed to the formation of the threshold. Jepson et al. (2005) analysed the concentration of PCBs in fish on both a congener basis (using the ICES 7) and on a formulation basis as Aroclor 1254 (the PCB profiles in fish and marine mammals were reported to be similar). The two sets of data were plotted, and the regression was established. The resultant conversion factor of three ($\sum$PCB concentration [as Aroclor 1254] = 3.0 x $\sum$ICES 7 congeners [lipid wt]) was determined with a standard error of 5%.

Jepson et al. (2005) also investigated possible relationships between PCB exposure and infectious disease mortality in harbour porpoises (*Phocoena phocoena*) in UK waters, by comparing PCB concentrations in healthy harbour porpoises that died of acute physical trauma (mainly by-catch; $n$ = 175) with concentrations in animals that died of infectious disease ($n$ = 82). The infectious disease

group was found to have significantly greater PCB values than the physical trauma group. Further, this association was found to be independent of other potentially confounding variables, such as age, sex, nutritional status, season, region, and the year found. Jepson et al. (2005, p 246) stated that their findings suggest that "above an estimated threshold of biological toxicity (17 µg/kg lipid), a causal relationship may exist between blubber total PCB levels and animals that died of infectious disease that is not fully explained, at least statistically, by a concentrating effect of disease-associated loss of lipid mass on blubber PCB levels." According to Jepson et al. (2005) the proposed threshold (17 µg/kg lipid) should provide a valuable benchmark for interpreting whether associations between disease and PCB exposure will be biologically significant.

*Polycyclic aromatic hydrocarbons (PAHs)*

The polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants found in air, water, sediment and soil. They are derived from both natural (e.g. forest fires, natural petroleum seeps) and anthropogenic sources (e.g. combustion of fossil fuels, use of oil for cooking and heating, coal burning, petroleum spills, road run-off) (Kannan and Perrotta, 2008).

PAHs were typically only found in the sediments and not surface water of the drains sampled as a component of a baseline study of contaminants in the Swan and Canning catchment drainage system in 2006 (Nice et al. 2009). Individual PAHs were found to consistently exceed the guidelines applied at Helena River, Perth Airport South and Central Business District; and occasionally exceeded the guidelines at Blackadder Creek, Maylands, Central Belmont, Bull Creek, Mills Street Main Drain and Lower Canning subcatchment (Nice et al., 2009).

PAHs do not show great biomagnification in food chains and are readily metabolised by many organisms. There is little information on the occurrence of PAHs in marine mammals (Kannan and Perrotta, 2008).

*Metals*

Metals may be present in the environment as a consequence of naturally occurring processes (e.g. geological weathering, degassing of the earth's crust and oceans, volcanic activity) and as a result of anthropogenic activities (Evans, 2003). With regard to anthropogenic activities, metals are commonly found in road runoff containing fuel and oil combustion products, products of tyre and brake wear, and roof runoff (Nice et al., 2009). Metals can also enter the environment from atmospheric emissions from oil and coal combustion and from smelting and mining activities (Nice et al. 2009).

A suite of 14 metals (aluminium, arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, molybdenum, nickel, lead, selenium and zinc) was examined as part of baseline study of

contaminants in the Swan and Canning catchment drainage system in 2006 (Nice et al. 2009). The metals found in the drain sediments and surface waters are likely to have originated from anthropogenic sources (Nice et al., 2009). It was generally found that Bayswater Main Drain, Blackadder Creek, Bannister Creek, Mills Street Main Drain and Upper Canning subcatchments had significantly higher concentrations of metals than other subcatchments. Where guidelines were available, these were exceeded in the sediment at Central Belmont (cadmium, lead, zinc), Central Business District (copper), Blackadder Creek (lead and zinc) and Helena River, Helm Street, Maylands, Perth Airport South and Lower Canning (lead) (Nice et al., 2009). In the surface water, guidelines were exceeded in the majority of subcatchments (aluminium, iron, zinc and copper), Bayswater Main Drain (chromium, cobalt, and lead), Mills Street Main Drain and Bickley Brook (lead and chromium), Bannister Creek, Bull Creek and South Belmont (chromium), and Upper Swan (cobalt) (Nice et al., 2009).

Metals can be divided into those that are essential for the normal function of an animal (such as zinc, copper, trivalent chromium, nickel, selenium and aluminium) and those that are non-essential (mercury, cadmium and lead). Essential metals are usually only required in small amounts and adverse effects may occur when there is an excess of these compounds, conversely any deficiencies will also have detrimental effects (Evans, 2003). Non-essential metals are metals not required for the normal functions of an animal. Some non-essential metals, such as mercury, cadmium and lead, tend to be toxic at low concentrations while others are relatively non-toxic. The toxicity of many elements is also associated with specific chemical forms, including free ions and methylated or reduced compounds (e.g. methyl mercury, dimethyl arsenic, chromium VI and divalent cadmium) (Mason 2002). For more information on heavy metals in aquatic environments refer to Section 8.3.7 of the ANZECC and ARMCANZ guidelines (2000).

Heavy metals are particularly site-specific with most tending to accumulate in the liver or kidneys. Lead however, tends to accumulate in bone (Evans, 2003). Table 4 lists the mean concentrations of heavy metals reported in the liver of bottlenose dolphins from various locations around the world.

*Organometallics*

From the 1960s onwards, tributyltin (TBT) was widely used as a biocide in antifouling paints used on boats (Tanabe et al., 1998). TBT has been banned in Australia since 2008. TBT and heavy metal contaminants were assessed in sediment samples collected at nine yacht clubs within the Swan River in 2006 (Oceanica, 2007). The environmental guideline value for TBT was exceeded at the majority of yacht club sites, and it was concluded that TBT concentrations at some yacht club sites were likely to be causing adverse ecological effects (Oceanica, 2007). Butyltin compounds, including TBT, have been found to preferentially accumulate in the liver of marine mammals and this is thought to be associated with the presence of and affinity towards sulfydryl groups of glutathione present in this organ (Kannan et al., 1996). Total butyltin concentrations detected in the livers of marine mammals are typically 1 to 10 µg/g wet weight (Tanabe, 1999).

The majority of mercury that accumulates in the internal organs of marine mammals is inorganic mercury. However, most of the mercury present in fish and squid exists as the more toxic organic form methyl-mercury (Caurant et al., 1996; Das et al., 2000). The demethylation of methyl-mercury, followed by the formation of a less toxic compound of inorganic mercury and selenium is thought to occur mainly in cetacean livers (Storelli and Marcotrigiano, 2000). Endo et al. (2004) reported that the maximum concentrations of total mercury and methyl-mercury reported in the livers of cetaceans were 1500 µg/g wet weight (Andre et al. 1991) and 30.4 µg/g wet weight (Storelli et al., 1998) in striped dolphins (*Stenella coeruleoalba*), respectively.

**Table 4:** Mean heavy metal concentrations in bottlenose dolphins from various studies expressed as µg/g wet weight (adapted from Lavery et al. 2008)

| | Liver Cd | Liver Hg | Liver Pb | Liver Se | Liver Zn | Liver Cu | Bone Pb | Reference |
|---|---|---|---|---|---|---|---|---|
| **Australia** | | | | | | | | |
| Queensland | 1.885 | 16.36 | 0.105 | 6.75 | 92.5 | | | 1 |
| South Australia | Indo-Pacific bottlenose dolphin: 6.45 | Indo-Pacific bottlenose dolphin: 475.78 | Indo-Pacific bottlenose dolphin: 0.455 | Indo-Pacific bottlenose dolphin: 178.85 | Indo-Pacific bottlenose dolphin: 93.88 | Indo-Pacific bottlenose dolphin: 19.67 | Indo-Pacific bottlenose dolphin: 2.78 | 2 |
| South Australia | Common bottlenose dolphin: 4.10 | Common bottlenose dolphin: 213.94 | Common bottlenose dolphin: 0.074 | Common bottlenose dolphin: 70.19 | Common bottlenose dolphin: 40.20 | Common bottlenose dolphin: 21.18 | Common bottlenose dolphin: 0.85 | 2 |
| **Argentina** | | | | | | | | |
| | 0.8 | 86 | | | 196.2 | 77.7 | | 3 |
| **U.S.** | | | | | | | | |
| Atlantic Ocean | 0.46 | 39.2 | 2.5 | 7.5 | | | | 4 |
| South Carolina | 0.051 | 17.8 | <0.1 | 9.54 | 56.8 | 10.78 | | 5 |
| **U.K.** | | | | | | | | |
| | 6.035 | 20.5 | 0.65 | | 37 | 7 | | 6 |
| **Europe** | | | | | | | | |
| South Adriatic Sea | | 393.36 | | 129.35 | 52.82 | 8.29 | | 7 |
| **Israel** | | | | | | | | |
| Mediterranean Sea | 0.49 | 97 | | | 44 | 8.9 | | 8 |

**References:**
1 Law et al. (2003)
2 Lavery et al. (2008)
3 Marcovecchio et al. (1990)
4 Kuehl et al. (1994)
5 Beck et al. (1997)
6 Law et al. 1991
7 Storelli and Marcotriagiano (2002)
8 Roditi-Elsar et al. (2003)

## Methods

Partial thickness blubber biopsy samples were collected from three live Swan dolphins and analysed for organic contaminants. Full thickness blubber samples, as well as, bone, liver and kidney samples, were collected from five deceased Swan dolphins and four deceased Bunbury dolphins for both organic contaminants and heavy metal analyses (Table 5).

*Analyte selection*

Selection of the contaminant groups for determination in tissue samples from dolphins, namely metals, OC pesticides and PAHs, was based on the findings of a baseline investigation of contaminants in the Swan Canning catchment (Nice et al. 2009) undertaken as part of the Non-Nutrient Contaminants Program (NNCP), a joint initiative between the Swan River Trust and the Department of Water (DOW). Although PCBs were not detected in the NNCP investigations, they were included in the suite of analytes for the deceased dolphins because they: are persistent organic pollutants, are considered to be ubiquitous, and are known to accumulate in marine mammals (O'Shea 1999). As in the NNCP, consideration was also given to:

(1) findings of previous studies within the Swan Canning system;

(2) known toxicities of key contaminants [e.g contaminants that feature on the 'dirty dozen list' of persistent organic pollutants (Stockholm Convention 2001)];

(3) likelihoods of contaminant occurrence given land uses within the catchment; and

(4) analytical ability to accurately determine contaminant concentration using endorsed methods.

*Sample collection and analysis*

**Organic contaminants**: During post-mortem, blubber samples were collected from a location just anterior to the dorsal fin according to standard practice, and approximately 100 grams of full thickness blubber was taken. The partial thickness biopsy samples from live dolphins were also collected from an area just anterior to the dorsal fin. The blubber samples were wrapped in acetone-washed aluminium foil, placed in a ziplock bag and stored in a -20°C freezer prior to analysis. Blubber samples were sent frozen to the National Measurement Institute (NMI), NSW, for analysis. The NMI used methods that were accredited by the National Association of Testing Authorities (NATA). The lipid content of all post-mortem derived full thickness blubber samples was determined, however, the biopsy samples were deemed too small for this analysis.

**Heavy metals**: Samples were collected from the left kidney and left caudal lobe of the liver for heavy metals analysis. In addition a segment of bone was specifically collected for measuring the level of lead. All samples for heavy metal analysis were place into sterile plastic containers and stored in a -

20°C freezer prior to analysis. Heavy metal analysis was conducted at the Chemistry Centre, Perth. The Chemistry Centre used methods that were accredited by NATA.

**Organometallics** (methyl mercury and tributyltin): For dolphins 09/637, 09/663 and 09/664 a blubber sample was collected into a sterile plastic container and stored in a -20°C freezer prior to analysis by the Chemistry Centre, Perth. It was however decided that all subsequent testing for methyl-mercury and tributyltin would be conducted on liver samples. In cetaceans, higher concentrations of tributyltin and methyl-mercury have been found in the liver compared to other tissues (Iwata et al., 1997). Liver samples were submitted for dolphins 09/1108, 09/1032, 08/1365, 08/379 and 08/943. The Chemistry has NATA accreditation for TBT but not for methyl-mercury.

**Table 5:** Description of deceased dolphins used for contaminants analyses

| Pathology ID No. | Origin of Dolphin | Sex | Age |
|---|---|---|---|
| 09/637 | Swan River | Male | Juvenile |
| 09/663 | | Male | Calf |
| 09/664 | | Female | Adult |
| 09/1108 | | Female | Adult |
| Perth Zoo | | Female | Adult |
| 09/1032 Cruiser | Bunbury area | Female | Juvenile |
| 08/1365 Peak | | Male | Adult |
| 08/379 Blizzard | | Male | Juvenile |
| 08/943 Arrow | | Female | Adult |

*Statistical analysis*

Statistical comparisons between contaminant concentrations from dolphins from the Swan-Canning Estuary and the Bunbury region were made using the independent t test in SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

## Results

*A. Organic Contaminants*

Table 6 presents the results for the three biopsy samples collected from live dolphins in the Swan-Canning Estuary and examined for PAHs and OCs.

Table 7 summarises the dieldrin, DDT and DDT metabolites results for both the Swan River and Bunbury dolphins based on tissue samples obtained post-mortem. Total DDT was calculated as the sum of 3 DDTs (*pp*-DDE, *pp*-DDD, and *pp*-DDT). Organochlorine pesticide results for each individual dolphin are given in Table 8 and 9.

Table 10 reports the Total PCB concentrations using three different methods. Total PCBs, as the total of ICES seven, allows for comparisons with the results recorded in the literature depicted in Table 2. For the concentrations of each of the 21 congeners measured per individual dolphin refer to Tables 10, 11, and 12.

Table 13 presents the concentrations of the four PAHs detected in the dolphins. Table 14 presents a comprehensive list of all PAHs analysed.

Carcasses recovered from the Swan-Canning Estuary had significantly higher concentrations of dieldrin (*p*-value 0.03) compared with those from the Bunbury area. There were no significant differences between dolphins from the Swan Canning River Park and Bunbury for concentrations of $\sum$DDT and $\sum_{21}$PCBs.

*B. Heavy metals*

Table 15 presents the results of all heavy metals detected in liver samples and expressed as wet weight. Table 16 presents the liver results expressed as dry weight. Table 17 presents the results of heavy metals detected in kidney samples and expressed as wet weight. Table 18 presents the concentrations of lead detected in bone.

*C. Organometallics (methyl mercury and TBT)*

Table 19 presents the methyl mercury and TBT results expressed as parts per billion or on a unit of mass basis as ng/g wet weight.

**Table 6:** Organic contaminants results, expressed as µg/g wet weight, from a single blubber biopsy sample collected from three live bottlenose dolphins in the Swan-Canning Estuary. Values preceded by the < symbol indicate the concentration failed to exceed the limit of reporting for that analyte.

| Contaminant | Units | A | B | C |
|---|---|---|---|---|
| *PAH* | | | | |
| Naphthalene | mg/kg | <1 | <1 | <0.5 |
| Acenaphthylene | mg/kg | <1 | <1 | <0.5 |
| Acenaphthene | mg/kg | <1 | <1 | <0.5 |
| Fluorene | mg/kg | <1 | <1 | <0.5 |
| Phenanthrene | mg/kg | <1 | <1 | <0.5 |
| Anthracene | mg/kg | <1 | <1 | <0.5 |
| Fluoranthene | mg/kg | <1 | <1 | <0.5 |
| Pyrene | mg/kg | <1 | <1 | <0.5 |
| Benz(a)anthracene | mg/kg | <1 | <1 | <0.5 |
| Chrysene | mg/kg | <1 | <1 | <0.5 |
| Benzo(b)&(k)fluranthene | mg/kg | <2 | <2 | <1 |
| Benzo(a)pyrene | mg/kg | <1 | <1 | <0.5 |
| Indeno(1,2,3-cd)pyrene | mg/kg | <1 | <1 | <0.5 |
| Dibenz(ah)ant | mg/kg | <1 | <1 | <0.5 |
| Benzo(ghi)perylene | mg/kg | <1 | <1 | <0.5 |
| *OC pesticides* | | | | |
| HCB | mg/kg | <0.2 | <0.2 | <0.1 |
| Heptachlor | mg/kg | <0.2 | <0.2 | <0.1 |
| Heptachlor epoxide | mg/kg | <0.2 | <0.2 | <0.1 |
| Aldrin | mg/kg | <0.2 | <0.2 | <0.1 |
| Gamma-BHC (Lindane) | mg/kg | <0.2 | <0.2 | <0.1 |
| Alpha-BHC | mg/kg | <0.2 | <0.2 | <0.1 |
| Beta-BHC | mg/kg | <0.2 | <0.2 | <0.1 |
| Delta-BHC | mg/kg | <0.2 | <0.2 | <0.1 |
| Trans-Chlordane | mg/kg | <0.2 | <0.2 | <0.1 |
| Cis-Chlordane | mg/kg | <0.2 | <0.2 | <0.1 |
| Oxychlodane | mg/kg | <0.2 | <0.2 | <0.1 |
| Dieldrin | mg/kg | 0.25 | 1.5 | 0.34 |
| pp-DDE | mg/kg | 0.75 | 5.2 | 4.2 |
| pp-DDD | mg/kg | <0.2 | 0.33 | <0.1 |
| pp-DDT | mg/kg | <0.2 | 0.41 | 0.18 |
| Endrin | mg/kg | <0.2 | <0.2 | <0.1 |
| Endrin Aldehyde | mg/kg | <0.2 | <0.2 | <0.1 |
| Endrin Ketone | mg/kg | <0.2 | <0.2 | <0.1 |
| Alpha-Endosulfan | mg/kg | <0.2 | <0.2 | <0.1 |
| Beta-Endosulfan | mg/kg | <0.2 | <0.2 | <0.1 |
| Endosulfan Sulfate | mg/kg | <0.2 | <0.2 | <0.1 |
| Methoxychlor | mg/kg | <0.2 | <0.2 | <0.1 |

**Table 6:** (cont.)

| | | | | |
|---|---|---|---|---|
| PCB Congeners | | | | |
| PCB # 8 | µg/kg | <40 | <40 | <20 |
| PCB # 18 | µg/kg | <40 | <40 | <20 |
| PCB # 28 | µg/kg | <40 | <40 | <20 |
| PCB # 44 | µg/kg | <40 | <40 | <20 |
| PCB # 52 | µg/kg | <40 | 170 | 58 |
| PCB # 66 | µg/kg | <40 | <40 | <20 |
| PCB # 77 | µg/kg | <40 | 240 | 87 |
| PCB # 101 | µg/kg | 98 | 270 | 88 |
| PCB # 105 | µg/kg | <40 | 180 | 40 |
| PCB # 118 | µg/kg | 200 | 700 | 170 |
| PCB # 126 | µg/kg | <40 | <40 | <20 |
| PCB # 128 | µg/kg | 41 | 220 | 110 |
| PCB # 138 | µg/kg | 190 | 1000 | 580 |
| PCB # 153 | µg/kg | 300 | 1300 | 790 |
| PCB # 169 | µg/kg | <40 | <40 | <20 |
| PCB # 170 | µg/kg | <40 | <40 | <20 |
| PCB # 180 | µg/kg | 52 | 240 | 170 |
| PCB # 187 | µg/kg | <40 | <40 | <20 |
| PCB # 195 | µg/kg | <40 | <40 | <20 |
| PCB # 206 | µg/kg | <40 | <40 | <20 |
| PCB # 209 | µg/kg | <40 | <40 | <20 |
| Total PCB | µg/kg | 880 | 4300 | 2100 |

**Table 7:** Summary of dieldrin, DDT and DDT metabolites results expressed as µg/g wet weight with concentration per lipid weight (in parentheses). Total DDT was calculated as the sum of 3 DDTs ( *pp*-DDE, *pp*-DDD, and *pp*-DDT). For all the organochlorine pesticide results for each individual dolphin refer to Table 7and 8

| **Origin of dolphin** | ***n*** | | **Dieldrin** | ***pp*-DDE** | ***pp*-DDD** | ***pp*-DDT** | **∑DDT** |
|---|---|---|---|---|---|---|---|
| Swan River | 5 | Mean | 5.04 (17.13) | 8.94 (30.78) | 0.82 (2.79) | 0.544 (1.85) | 10.3 (35.42) |
| | | Range | 0.88-9.4 (2.83-39.0) | 2.5-10.0 (8.04-82.99) | 0.14-1.6 (0.45-6.64) | 0.2-1.1 (0.64-4.56) | 2.84-22.7 (9.13-94.19) |
| Bunbury | 4 | Mean | 0.34 (0.84) | 6.7 (16.36) | 0.511 (1.30) | 0.2 (0.49) | 7.41 (18.15) |
| | | Range | 0.12-0.87 (0.26-2.32) | 1.8-16.0 (3.7-42.67) | 0.13-1.5 (0.25-4.0) | 0.051-0.51 (0.18-1.36) | 1.98-18.0 (4.13-48.03) |

**Table 8:** Organochlorine pesticide concentrations in dolphin blubber expressed as µg/g wet weight. Values preceded by the < symbol indicate the concentration failed to exceed the limit of reporting for that analyte. Note: NM = Not Measured

| Pathology ID No. | Origin of dolphin | Age and sex | HCB | Heptachlor | Heptachlor-epoxide | Aldrin | Lindane | alpha-BHC | beta-BHC | delta-BHC | trans-Chlordane | cis-Chlordane |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | Juvenile, male | 0.11 | <0.1 | 0.51 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 09/663 | | Calf, male | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 09/664 | | Juvenile, female | <0.1 | <0.1 | 0.79 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 09/1108 | | Aged, female | <0.05 | <0.05 | 0.22 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Perth Zoo | | Adult female | <0.05 | <0.05 | 0.21 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/1032 Cruiser | Bunbury | Juvenile, female | 0.053 | <0.05 | 0.140 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/1365 Peak | | Adult, male | <0.02 | <0.02 | 0.023 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| 08/379 Blizzard | | Juvenile, male | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/943 Arrow | | Adult, female | <0.05 | <0.05 | 0.056 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

| Pathology ID No. | Origin of dolphin | Oxychlordane | Dieldrin | pp-DDE | pp-DDD | pp-DDT | o,p-DDE | o,p-DDD | o,p-DDT |
|---|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | 0.37 | 7.5 | 10 | 1.1 | 0.77 | NM | NM | NM |
| 09/663 | | <0.1 | 0.88 | 2.5 | 0.14 | 0.2 | NM | NM | NM |
| 09/664 | | 0.63 | 9.4 | 20 | 1.6 | 1.1 | NM | NM | NM |
| 09/1108 | | 0.13 | 4 | 4.1 | 0.57 | 0.27 | 0.12 | <0.02 | <0.02 |
| Perth Zoo | | 0.18 | 3.4 | 8.1 | 0.69 | 0.38 | 0.13 | <0.02 | 0.021 |
| 09/1032 Cruiser | Bunbury | 0.25 | 0.87 | 16 | 1.5 | 0.51 | 0.14 | <0.02 | 0.034 |
| 08/1365 Peak | | 0.023 | 0.12 | 1.8 | 0.13 | 0.051 | 0.012 | <0.01 | <0.01 |
| 08/379 Blizzard | | 0.054 | 0.23 | 2.5 | 0.17 | 0.12 | 0.041 | <0.02 | <0.02 |
| 08/943 Arrow | | 0.076 | 0.14 | 6.5 | 0.25 | 0.12 | 0.034 | <0.02 | 0.032 |

| Pathology ID No. | Origin of dolphin | Endrin | Endrin Aldehyde | Endrin Ketone | alpha-Endosulfan | beta- Endosulfan | Endosulfan Sulfate | Methoxychlor |
|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 09/663 | | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 09/664 | | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 09/1108 | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Perth Zoo | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/1032 Cruiser | Bunbury | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/1365 Peak | | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| 08/379 Blizzard | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/943 Arrow | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

**Table 9:** Organochlorine pesticide concentrations in dolphin blubber expressed as µg/g lipid. Values and contaminants which were below the limit of reporting have been omitted. All values have been rounded to within 2 decimal places. Note: NM = Not Measured

| Pathology ID No. | Origin of dolphin | HCB | Heptachlorepoxide | Oxychlordane | Dieldrin | pp-DDE | pp-DDD | pp-DDT | o,p-DDE | o,p-DDD | o,p-DDT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | 0.3 | 1.4 | 1.02 | 20.6 | 27.47 | 3.02 | 2.12 | NM | NM | NM |
| 09/663 | | | | | 2.83 | 8.04 | 0.45 | 0.64 | NM | NM | NM |
| 09/664 | | | 3.28 | 2.61 | 39 | 83 | 6.64 | 4.56 | NM | NM | NM |
| 09/1108 | | | 0.81 | 0.48 | 14.65 | 15.02 | 2.09 | 0.99 | 0.44 | | |
| Perth Zoo | | | 0.53 | 0.45 | 8.56 | 20.4 | 1.74 | 0.96 | 0.33 | | 0.05 |
| 09/1032 Cruiser | Bunbury | 0.14 | 0.37 | 0.67 | 2.32 | 42.67 | 4 | 1.36 | 0.37 | | 0.09 |
| 08/1365 Peak | | | 0.09 | 0.09 | 0.45 | 6.77 | 0.49 | 0.19 | 0.05 | | |
| 08/379 Blizzard | | | | 0.08 | 0.34 | 3.7 | 0.25 | 0.18 | 0.06 | | |
| 08/943 Arrow | | | 0.11 | 0.14 | 0.27 | 12.29 | 0.47 | 0.23 | 0.06 | | 0.06 |

**Table 10:** Total PCB concentrations recorded for individual dolphins using three different methods and expressed as µg/g wet weight and lipid weight (in parentheses).

| Pathology ID No. | Origin of dolphin | Date found | Age and sex | ∑PCB[a] | ∑PCBs ICES7[b] | Total as Aroclor 1254 (Jepson et al. 2005)[c] |
|---|---|---|---|---|---|---|
| 09/637 | Swan River | June 8 2009 | Juvenile, male | 8.4 (23.08) | 7.0 (19.23) | (57.69) |
| 09/663 | | June 5 2009 | Calf, male | 3.3 (10.61) | 2.89 (9.29) | (27.88) |
| 09/664 | | June 21 2009 | Juvenile, female | 13.0 (53.94) | 10.94 (45.38) | (136.13) |
| 09/1108 | | Oct 25 2009 | Aged, female | 2.8 (10.26) | 2.31 (8.44) | (25.33) |
| Perth Zoo | | Sept 17 2009 | Adult female | 5.6 (14.11) | 4.67 (11.77) | (35.31) |
| 09/1032 Cruiser | Bunbury | Sept 30 2009 | Juvenile, female | 9.5 (25.33) | 8.19 (21.85) | (65.56) |
| 08/1365 Peak | | Aug 25 2008 | Adult, male | 0.68 (2.56) | 0.59 (2.21) | (6.63) |
| 08/379 Blizzard | | Jan 1 2008 | Juvenile, male | 1.6 (2.37) | 1.35 (2.0) | (6.0) |
| 08/943 Arrow | | April 18 2008 | Adult, female | 1.9 (3.59) | 1.65 (3.12) | (9.36) |

[a] Sum of 21 congeners (8, 18, 28, 44, 52, 66, 77, 101, 105, 118, 126, 128, 138, 153, 169, 170, 180, 187,195, 206, 209)
[b]Sum of ICES 7 congeners (28, 52, 101, 118, 138, 153 and 180)
[c] In order to calculate total PCBs based on the Aroclor 1254 formulation- the sum of ICES 7 were multiplied by a conversion factor of 3 (Jepson et al., 2005)

**Table 11:** Concentrations of polychlorinated biphenyls (PCBs) in dolphin blubber expressed as µg/g wet weight. Values preceded by the < symbol indicate the concentration failed to exceed the limit of reporting for that analyte.

| | | Congener Number | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pathology ID No.** | **Origin of dolphin** | **8** | **18** | **28** | **44** | **52** | **66** | **77** | **101** | **105** | **118** | **126** |
| 09/637 | Swan River | <0.002 | <0.002 | 0.03 | 0.031 | 0.29 | <0.002 | 0.44 | 0.71 | 0.41 | 1.3 | <0.002 |
| 09/663 | | <0.002 | <0.002 | 0.02 | 0.007 | 0.1 | <0.002 | 0.12 | 0.24 | 0.12 | 0.55 | <0.002 |
| 09/664 | | <0.002 | <0.002 | 0.016 | 0.016 | 0.42 | <0.002 | 0.73 | 0.77 | 0.5 | 1.4 | <0.002 |
| 09/1108 | | <0.02 | <0.02 | 0.035 | <0.02 | 0.1 | 0.066 | <0.02 | 0.23 | 0.14 | 0.45 | <0.02 |
| Perth Zoo | | <0.02 | <0.02 | 0.022 | <0.02 | 0.17 | 0.047 | <0.02 | 0.33 | 0.21 | 0.74 | <0.02 |
| 09/1032 Cruiser | Bunbury | <0.02 | <0.02 | 0.025 | <0.02 | 0.29 | 0.031 | <0.02 | 0.75 | 0.2 | 0.85 | <0.02 |
| 08/1365 Peak | | <0.01 | <0.01 | <0.01 | <0.01 | 0.018 | <0.01 | <0.01 | 0.036 | 0.011 | 0.048 | <0.01 |
| 08/379 Blizzard | | <0.02 | <0.02 | <0.02 | <0.02 | 0.029 | <0.02 | <0.02 | 0.13 | <0.02 | 0.13 | <0.02 |
| 08/943 Arrow | | <0.02 | <0.02 | <0.02 | <0.02 | 0.040 | <0.02 | <0.02 | 0.110 | 0.041 | 0.170 | <0.02 |

| | | Congener Number | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pathology ID No.** | **Origin of dolphin** | **128** | **138** | **153** | **169** | **170** | **180** | **187** | **195** | **206** | **209** | **∑PCBs** |
| 09/637 | Swan River | 0.403 | 2 | 2.2 | <0.002 | <0.002 | 0.47 | <0.002 | <0.002 | 0.029 | <0.002 | 8.4 |
| 09/663 | | 0.13 | 0.63 | 1.1 | <0.002 | <0.002 | 0.25 | <0.002 | <0.002 | 0.014 | <0.002 | 3.3 |
| 09/664 | | 0.71 | 3.6 | 3.9 | <0.002 | <0.002 | 0.83 | <0.002 | <0.002 | 0.055 | <0.002 | 13 |
| 09/1108 | | 0.13 | 0.66 | 0.68 | <0.02 | 0.055 | 0.15 | 0.087 | <0.02 | 0.036 | <0.02 | 2.8 |
| Perth Zoo | | 0.26 | 1.4 | 1.7 | <0.02 | 0.12 | 0.31 | 0.2 | <0.02 | 0.021 | <0.02 | 5.6 |
| 09/1032 Cruiser | Bunbury | 0.38 | 1.7 | 3.9 | <0.02 | 0.27 | 0.68 | 0.41 | <0.02 | <0.02 | <0.02 | 9.5 |
| 08/1365 Peak | | 0.024 | 0.13 | 0.29 | <0.01 | 0.019 | 0.066 | 0.041 | <0.01 | <0.01 | <0.01 | 0.68 |
| 08/379 Blizzard | | 0.048 | 0.31 | 0.58 | <0.02 | 0.056 | 0.17 | 0.095 | <0.02 | <0.02 | <0.02 | 1.6 |
| 08/08/943 Arrow | | 0.072 | 0.39 | 0.78 | <0.02 | 0.059 | 0.16 | 0.1 | <0.02 | <0.02 | <0.02 | 1.9 |

**Table 12:** Concentrations of polychlorinated biphenyls in dolphin blubber expressed as µg/g lipid. Values which were below the limit of reporting have been omitted. All values have been rounded to within 2 decimal places. In order to calculate total PCBs based on the Aroclor 1254 formulation: the sum of ICES 7 (congeners 28, 52, 101, 118, 138, 153 and 180) were multiplied by a conversion factor of 3 (Jepson et al. 2005).

| | | Congener Number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pathology ID No.** | **Origin of dolphin** | **8** | **18** | **28** | **44** | **52** | **66** | **77** | **101** | **105** | **118** | **126** | **128** |
| 09/637 | Swan River | | | 0.08 | 0.09 | 0.8 | | 1.21 | 1.95 | 1.13 | 3.57 | | 1.11 |
| 09/663 | | | | 0.06 | 0.02 | 0.32 | | 0.39 | 0.77 | 0.39 | 1.77 | | 0.42 |
| 09/664 | | | | 0.07 | 0.07 | 1.74 | | 3.03 | 3.2 | 2.08 | 5.81 | | 2.95 |
| 09/1108 | | | | 0.13 | | 0.37 | 0.24 | | 0.84 | 0.51 | 1.65 | | 0.48 |
| Perth Zoo | | | | 0.06 | | 0.43 | 0.12 | | 0.83 | 0.53 | 1.86 | | 0.66 |
| 09/1032 Cruiser | Bunbury | | | 0.07 | | 0.77 | 0.08 | | 2 | 0.53 | 2.27 | | 1.01 |
| 08/1365 Peak | | | | | | 0.07 | | | 0.14 | 0.04 | 0.18 | | 0.09 |
| 08/379 Blizzard | | | | | | 0.043 | | | 0.19 | | 0.19 | | 0.07 |
| 08/943 Arrow | | | | | | 0.08 | | | 0.21 | 0.08 | 0.32 | | 0.14 |

| | | Congener Number | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pathology ID No.** | **Origin of dolphin** | **138** | **153** | **169** | **170** | **180** | **187** | **195** | **206** | **209** | **∑PCBs** |
| 09/637 | Swan River | 5.5 | 6.04 | | | 1.29 | | | 0.08 | | 23.08 |
| 09/663 | | 2.03 | 3.54 | | | 0.8 | | | 0.05 | | 10.61 |
| 09/664 | | 14.94 | 16.18 | | | 3.44 | | | 0.23 | | 53.94 |
| 09/1108 | | 2.42 | 2.49 | | 0.2 | 0.55 | 0.32 | | 0.13 | | 10.26 |
| Perth Zoo | | 3.53 | 4.28 | | 0.3 | 0.78 | 0.5 | | 0.05 | | 14.12 |
| 09/1032 Cruiser | Bunbury | 4.53 | 10.4 | | 0.72 | 1.81 | 1.09 | | | | 25.33 |
| 08/1365 Peak | | 0.49 | 1.09 | | 0.07 | 0.25 | 0.15 | | | | 2.56 |
| 08/379 Blizzard | | 0.46 | 0.86 | | 0.08 | 0.25 | 0.14 | | | | 2.37 |
| 08/943 Arrow | | 0.74 | 1.47 | | 0.11 | 0.3 | 0.19 | | | | 3.59 |

**Table 13:** Polycyclic aromatic hydrocarbon concentrations in dolphin blubber expressed as µg/g lipid. Values for contaminants which were below the limit of reporting have been omitted.

| Pathology ID No. | Origin of dolphin | Naphthalene | Fluorene | Phenanthrene | Pyrene |
|---|---|---|---|---|---|
| 09/637 | Swan River | | | | |
| 09/663 | | | | | |
| 09/664 | | | | | |
| 09/1108 | | | | 0.21 | |
| Perth Zoo | | | | | |
| 09/1032 Cruiser | Bunbury | | | | |
| 08/1365 Peak | | | | | |
| 08/379 Blizzard | | 0.07 | 0.09 | | 0.3 |
| 08/943 Arrow | | | | | 0.18 |

**Table 14:** Polycyclic aromatic hydrocarbons concentrations in dolphin blubber expressed as µg/g wet weight. Values preceded by the < symbol indicate the concentration failed to exceed the limit of reporting for that analyte.

| Pathology ID No. | Origin of dolphin | Naphthalene | Acenaphthylene | Acenaphthene | Fluorene | Phenanthrene | Anthracene | Fluoranthene |
|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/663 | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/664 | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/1108 | | <0.05 | <0.05 | <0.05 | <0.05 | 0.056 | <0.05 | <0.05 |
| Perth Zoo | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/1032 Cruiser | Bunbury | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/1365 Peak | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/379 Blizzard | | 0.050 | <0.05 | <0.05 | 0.061 | <0.05 | <0.05 | <0.05 |
| 08/943 Arrow | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

| Pathology ID No. | Origin of dolphin | Pyrene | Benz(a) anthracene | Chrysene | Benzo(b) and (k) fluoranthene | Benzo(a) pyrene | Indeno (1,2,3-cd) pyrene | Dibenz(ah) anthracene | Benzo(ghi) perylene |
|---|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | <0.05 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/663 | | <0.05 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/664 | | <0.05 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/1108 | | <0.05 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| Perth Zoo | | <0.05 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| 09/1032 Cruiser | Bunbury | <0.05 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/1365 Peak | | <0.05 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/379 Blizzard | | 0.20 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |
| 08/943 Arrow | | 0.094 | <0.05 | <0.05 | <0.1 | <0.05 | <0.05 | <0.05 | <0.05 |

**Table 15:** Concentrations of heavy metal levels in the liver of dolphins expressed as µg/g wet weight

| Pathology ID No. | Origin of dolphin | Al | As | Cd | Co | Cr | Cu | Fe | Hg | Mn | Ni | Pb | Se | V | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | 4 | 0.1 | 0.012 | <0.001 | 0.2 | 9.7 | 690 | 2.1 | 2.5 | <0.01 | 0.23 | 2.3 | <0.1 | 130 |
| 09/663 | | <2 | 0.18 | 0.11 | <0.001 | 0.1 | 14 | 270 | 12 | 4 | <0.01 | 0.014 | 6.6 | <0.1 | 120 |
| 09/664 | | <2 | 0.1 | 0.015 | 0.005 | <0.1 | 11 | 310 | 11 | 4.2 | <0.01 | 0.057 | 6.3 | <0.1 | 160 |
| 09/1108 | | <2 | 0.19 | 0.15 | <0.05 | <0.2 | 13 | 530 | 57 | 4.7 | 1.6 | 0.17 | 21 | <0.1 | 96 |
| 09/1032 Cruiser | Bunbury | <2 | 0.22 | 0.055 | <0.05 | <0.2 | 23 | 600 | 1.6 | 7 | 0.12 | <0.005 | 0.96 | <0.1 | 99 |
| 08/1365 Peak | | <2 | 0.09 | 0.084 | <0.05 | <0.2 | 10 | 600 | 18 | 4.9 | 2.2 | <0.005 | 7.1 | 0.1 | 310 |
| 09/257 Radar | | <2 | 0.15 | 0.20 | <0.05 | <0.2 | 11 | 210 | 11 | 7.5 | 0.03 | 0.007 | 4.4 | 0.1 | 54 |
| 09/665 | | <2 | 0.09 | 0.034 | <0.05 | <0.2 | 1.1 | 200 | 1.1 | 2.1 | 1.3 | <0.005 | 0.84 | <0.1 | 40 |
| 06/348 | | <2 | 0.1 | 0.14 | <0.05 | <0.2 | 4.9 | 210 | 3.3 | 8.2 | 0.02 | <0.005 | 2.4 | <0.1 | 60 |
| 08/379 Blizzard | | <2 | 0.35 | 0.22 | <0.05 | 0.2 | 28 | 270 | 3.3 | 7.5 | 0.92 | <0.005 | 1.7 | <0.1 | 140 |
| 08/943 | | <2 | 0.35 | 0.38 | <0.05 | <0.2 | 6.4 | 340 | 34 | 2.3 | <0.01 | 0.009 | 13 | <0.1 | 58 |

**Table 16:** Concentrations of heavy metal levels in the liver of dolphins expressed as µg/g dry weight. The conversion from liver wet weight to dry weight was according to Yang and Miyazaki (2003), whereby the moisture content of the liver was assumed to be 70% and a conversion factor of 3.3 was used. All values have been rounded to within 2 decimal places.

| Pathology ID No. | Origin of dolphin | Al | As | Cd | Co | Cr | Cu | Fe | Hg | Mn | Ni | Pb | Se | V | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | 13.2 | 0.33 | 0.04 | | 0.66 | 32.01 | 2277 | 6.93 | 8.25 | | 0.76 | 7.59 | | 429 |
| 09/663 | | | 0.59 | 0.36 | | 0.33 | 46.2 | 891 | 39.6 | 13.2 | | 0.05 | 21.78 | | 396 |
| 09/664 | | | 0.33 | 0.05 | 0.02 | | 36.3 | 1023 | 36.3 | 13.86 | | 0.19 | 20.79 | | 528 |
| 09/1108 | | | 0.63 | 0.5 | | | 42.9 | 1749 | 188.1 | 15.51 | 5.28 | 0.56 | 69.3 | | 316.8 |
| 09/1032 Cruiser | Bunbury | | 0.73 | 0.18 | | | 75.9 | 1980 | 5.28 | 23.1 | 0.4 | | 3.17 | | 326.7 |
| 08/1365 Peak | | | 0.3 | 0.28 | | | 33 | 1980 | 59.4 | 16.17 | 7.26 | | 23.43 | 0.33 | 1023 |
| 09/257 Radar | | | 0.5 | 0.66 | | | 36.3 | 693 | 36.3 | 24.75 | 0.1 | 0.02 | 14.52 | 0.33 | 178.2 |
| 09/665 | | | 0.3 | 0.11 | | | 3.63 | 660 | 3.63 | 6.93 | 4.29 | | 2.77 | | 132 |
| 06/348 | | | 0.33 | 0.46 | | | 16.17 | 693 | 10.89 | 27.06 | 0.07 | | 7.92 | | 198 |
| 08/379 Blizzard | | | 1.16 | 0.73 | | 0.66 | 92.4 | 891 | 10.89 | 24.75 | 3.04 | | 5.61 | | 462 |
| 08/943 | | | 1.16 | 1.25 | | | 21.12 | 1122 | 112.2 | 7.59 | | 0.03 | 42.9 | | 191.4 |

**Table 17:** Concentrations of heavy metal levels in the kidney of dolphins expressed as µg/g wet weight. Values preceded by the < symbol indicate the concentration failed to exceed the limit of reporting for that analyte.

| Pathology ID No. | Origin of dolphin | Al | As | Cd | Co | Cr | Cu | Fe | Hg | Mn | Ni | Pb | Se | V | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 09/637 | Swan River | <2 | 0.16 | 0.057 | 0.008 | 0.1 | 4.4 | 160 | 0.48 | 0.49 | <0.01 | <0.005 | 2.8 | <0.1 | 26 |
| 09/663 | | <2 | 0.17 | 0.73 | 0.010 | <0.1 | 4.9 | 100 | 3.3 | 0.73 | <0.01 | 0.037 | 3.4 | <0.1 | 29 |
| 09/664 | | <2 | 0.07 | 0.054 | 0.019 | <0.1 | 3.9 | 92 | 2.3 | 0.47 | <0.01 | <0.005 | 3.5 | <0.1 | 19 |
| 09/1108 | | <2 | <0.5 | 1.0 | <0.05 | <0.2 | 10 | 96 | 5.0 | 0.57 | <0.1 | <0.2 | 5.5 | <0.1 | 29 |
| 09/1032 Cruiser | Bunbury | <2 | <0.5 | 0.1 | <0.05 | <0.2 | 7.2 | 100 | 0.36 | 0.65 | <0.1 | <0.2 | 2.7 | <0.1 | 31 |
| 08/1365 Peak | | <2 | <0.5 | 0.64 | <0.05 | <0.2 | 7.6 | 78 | 2.2 | 0.65 | <0.1 | <0.2 | 2.2 | <0.1 | 43 |
| 09/257 Radar | | <2 | <0.5 | 1.2 | <0.05 | <0.2 | 6.1 | 75 | 2.1 | 0.79 | <0.1 | <0.2 | 3.9 | <0.1 | 32 |
| 09/665 | | <2 | <0.5 | 0.72 | <0.05 | <0.2 | 3.0 | 100 | 1.1 | 0.7 | <0.1 | <0.2 | 3.6 | <0.1 | 26 |
| 06/348 | | 9 | <0.5 | 0.62 | <0.05 | <0.2 | 2.6 | 140 | 1.2 | 1.2 | <0.1 | <0.2 | 3.2 | <0.1 | 25 |
| 08/379 Blizzard | | 23 | <0.5 | 0.7 | <0.05 | 0.3 | 6.5 | 180 | 2.1 | 0.89 | 0.1 | <0.2 | 4.8 | <0.1 | 32 |
| 08/943 | | <2 | <0.5 | 1.6 | 0.07 | <0.2 | 3.6 | 180 | 3.2 | 0.68 | 0.7 | <0.2 | 4.3 | <0.1 | 29 |

**Table 18:** Concentration of lead in bone collected from dolphins expressed as µg/g wet weight

| Pathology ID No. | Origin of dolphin | Lead |
|---|---|---|
| 09/637 | Swan River | 0.64 |
| 09/663 | | 0.61 |
| 09/664 | | 0.88 |
| 09/1108 | | 4.1 |
| 09/1032 Cruiser | Bunbury | 0.072 |
| 08/1365 Peak | | 0.19 |
| 09/257 Radar | | 0.24 |
| 09/665 | | 0.16 |

**Table 19:** Concentration of methyl mercury and TBT in various tissues from dolphins expressed as ng/g wet weight

| Pathology ID No. | Origin of dolphin | Tissue | Methyl-Hg | TBT |
|---|---|---|---|---|
| 09/637 | Swan River | Blubber | <13 | <13 |
| 09/663 | | Blubber | <26 | <26 |
| 09/664 | | Blubber | <28 | <28 |
| 09/1108 | | Liver | 34 | <5 |
| 09/1032 Cruiser | Bunbury | Liver | <5 | <5 |
| 08/1365 Peak | | Liver | 7.9 | <5 |
| 09/257 Radar | | Liver | 18 | <5 |
| 09/665 | | Liver | <5 | <5 |
| 06/348 | | Liver | 20 | <5 |
| 08/379 Blizzard | | Liver | <5 | <5 |
| 08/943 | | Liver | 53 | 18 |

## Discussion

Dieldrin, DDE, and PCBs were the predominant organic contaminants detected in blubber samples from Swan dolphins. It should be noted that variations in contaminants concentrations within the blubber layer can occur, thus partial thickness biopsy samples may not accurately represent contaminant concentrations in blubber. Krahn et al. (2004) suggested that biopsy samples may be more representative when contaminant concentrations are lipid adjusted, this was however not possible for the three biopsy samples collected. Another important consideration is that all of the deceased dolphins from the Swan River that were sampled were either calves/juveniles or adult females, and hence it is possible that higher contaminant levels may be present in adult males.

Dieldrin concentrations detected in the Swan dolphins were significantly higher than those detected in the Bunbury dolphins ($p$ = 0.03), thus indicating spatial differences in environmental contamination (Table 7). The average dieldrin levels detected in the Swan dolphins are among the highest levels reported globally in marine mammals in recent times (O'Hara and O'Shea, 2001; Tables 2 and 3). However, there is a lack of information available on marine mammals in order to interpret the significance of these concentrations in relation to adverse health effects. It is important to consider that unless the contaminants are mobilised, contaminants stored in blubber may not have a direct toxic effect (Fair et al., 2010). Accumulated lipophilic contaminants may be mobilised during pregnancy and lactation, starvation, and disease states (Aguilar, 1987) and, as a consequence, may reach target sites of toxicity leading to contaminant associated health effects. Interestingly, the dolphin with the highest dieldrin concentration (09/664) also had the lowest blubber lipid content; further, this dolphin also had a severe fishing line entanglement of the right fluke and evidence of systemic infection. This animal may therefore not only have been subject to the identified stressors of fishing line entanglement and systemic infection but may have been further compromised through remobilisation of a mixture of stored contaminant burden.

The study by Fair et al. (2010) reported high levels of total PCBs (sum of 92 congeners) and total DDT in dolphins in two estuaries located in urban areas on the east coast of the U.S. Given that only 21 congeners were examined in the Swan and Bunbury dolphins, the total PCB results are not directly comparable with the total PCB concentrations reported by Fair et al. (2010). However, some of the Swan and Bunbury dolphins had elevated levels of total PCBs and total DDT similar to the estuarine dolphins reported by Fair et al. (2010).

The total PCB threshold concentration for effects on immune function determined by Kannan et al. (2000) was determined using different analytical procedures than those used on the Swan and Bunbury samples and is therefore not directly comparable with our results. This threshold value was based on

low-grade physiological effects in experimental studies on mink, seals and otters, and should not be used as an absolute value but, rather, as a guide to determine whether levels of PCB exposure in individual marine mammals are likely to exert significant biological (immunotoxic) effects (Kannan et al., 2000). Table 8 indicates that when 21 congeners were measured and summed, Swan dolphins 09/637, 09/664 and Bunbury dolphin 09/1032, exceeded the approximate threshold of 17 µg/g lipid weight. If more congeners had been included in the suit of analytes analysed it is probable that the total PCBs recorded would have been higher and more dolphins may have exceeded the threshold. In order to account for differences in the number of congeners examined and still be able to make a comparison with the threshold determined by Kannan et al. (2000), Jepson et al. (2005) suggested calculating the concentration of PCBs based on the concentration of Aroclor 1254. When total PCB concentrations were calculated, using the conversion suggested by Jepson et al. (2005) for Aroclor 1254 equivalent concentrations (see Table 8), Swan dolphins 09/637, 09/663, 09/664, 09/1108, the Swan dolphin found dead on 17 September 2009 and the Bunbury dolphin 09/1032, exceeded the threshold. It should be noted that it is more accurate to compare the total PCBs as the sum of all 21 congeners analysed with the threshold determined by Kannan et al. (2000), than it is to compare the Aroclor 1254 converted concentrations as suggested by Jepson et al. (2005).

It appears that the zinc concentrations detected in the liver of dolphins from the Swan River and Bunbury are elevated. It is difficult to interpret the significance of these levels. Zinc is an essential element, and consequently animals will regulate its concentration within a specific range by homeostasis. Law et al. (1991) suggested a homeostatic range of 20-100 µg/g wet weight for zinc in liver tissue in common porpoise (*Phocoena phocoena*), and postulated that animals outside of this range are those whose regulating mechanism may be impaired. A number of the dolphins from the Swan River and Bunbury were reported to have zinc concentrations in liver samples above or close to 100 µg/g wet weight. This may reflect interspecies differences, an under-estimation of the required range, a lack of information about this species, or toxic levels of zinc (Wood and Van Vleet, 1996).

In conclusion, the high concentrations of organochlorine contaminants recorded in the Swan dolphins suggest that dolphin health may be adversely affected during periods of lipid mobilisation. It is, however, currently not possible to measure the extent to which such adverse effects are occurring. A growing area of research internationally is the use of biomarkers in order to determine the effects of certain contaminants from biopsy samples.

*Knowledge Gaps & Management Implications*

There remains significant scientific uncertainty in our understanding of the potential effects of contaminants on the dolphins inhabiting the Swan-Canning Estuary. This uncertainty reflects the difficulty in inferring biological effects from the concentrations of contaminants within tissues, as well

as the practical difficulties of drawing comparisons across studies, taxa, and suites of contaminants. Nonetheless, the contaminant burdens are sufficient to raise concerns about adverse health effects if lipid reserves are mobilised and to suggest that, to the extent reductions in environmental concentrations of organic contaminants can be achieved, this would be of long-term benefit to dolphins. The potential effects of contaminants should not be viewed in isolation. Rather, the health of dolphins in the Swan-Canning Estuary should be considered from a multi-factorial framework in which a range of natural and anthropogenic stressors may interact to exert significant cumulative and/or synergistic effects.

# VI. Conclusion

This study has provided new information on the population genetics, trophic associations, and contaminant burdens of the dolphins inhabiting the Swan-Canning Estuary. Previous sections of this report discussed these findings and their management implications. Here, we integrate these findings and review the scientific basis for managing the resident dolphin community in the estuary as a discrete management unit.

### Resident dolphin communities as management units

Coastal and estuarine ecosystems are challenging environments for dolphins (Finn, 2005; Peddemors, 1999; Perrin, 1999; Reeves et al., 2003; Ross et al., 2011). Populations inhabiting these areas may experience: habitat loss and degradation; exposure to environmental contaminants and biotoxins; incidental mortality from interactions with fisheries and other activities; disturbance from vessel interactions and anthropogenic noise; and greater risk of infectious disease (Chilvers et al., 2005; Harwood, 2001; O'Shea, 1999; Read, 2005; Reeves et al., 2003; Van Bressem et al., 2009a, b; Van Dolah, 2005).

These stressors can affect the behaviour, physiology, and health of small cetaceans and reduce reproductive success and survivorship, particularly if stressors exert cumulative or synergistic impacts (Bejder et al., 2006a,b, 2009; Fair and Becker, 2000; Gulland and Hall, 2007; McHugh et al., 2010; Samuels et al., 2003; Van Bressem et al., 2009a). Given these challenges, the identification of appropriate "units to conserve" can improve assessments of conservation status of populations, the biological significance of human impacts, and the effectiveness of management options (Bejder et al., 2009; Berger-Tal et al., 2011; Grech and March, 2007; Taylor, 1997, 2005).

"Units to conserve" or, more commonly, "management units" may be defined as a "group of animals that is the target of some management action" (Barlow, 2009, p.679). Concepts and criterion for management units typically integrate geographic and biological components. Taylor and Dizon (1999), for example, define management units as "geographical areas with restricted interchange of the individuals of interest with adjacent areas." Management units are generally designed to allow for monitoring of abundance and assessment of anthropogenic pressures at scales relevant to the area of concern (Evans, 2009; Grech and Marsh, 2007; Ross et al., 2011; Wilson et al., 2004).

Management objectives influencing the identification of management units often relate to concerns that a species remains: present throughout its range (Currey et al., 2009a, 2009b; Wilson et al., 1999); a functioning component of an ecosystem (e.g. United States *Marine Mammal Protection 1972*); a

sustainable resource for cetacean-based tourism (Bejder et al., 2006b; Lusseau et al., 2006); or a feature of a marine protected area (Government of South Australia, 2005; Hooker and Gerber, 2004; Hooker et al., 2011; Hoyt, 2005; Reeves, 2010).

These considerations suggest that an appropriate management unit for dolphins within coastal and estuarine environments should be biologically-meaningful (i.e. appropriately reflect population structure and dynamics) and geographically relevant (i.e. allow management action at the required spatial scale) (Connor et al., 2000; Sellas et al., 2005; Taylor, 2005; Wilson et al., 2004). Resident communities of bottlenose dolphins (*Tursiops* spp.) have been suggested as an appropriate management unit for coastal and estuarine ecosystems (Connor, et al. 2000; Sellas et al., 2005).

## Scientific basis for resident dolphin communities as management units

Both the Western Australia *Wildlife Conservation Act 1950* and the Commonwealth *Environmental Protection and Biodiversity Act 1999* provide substantive protections against the harming ('taking') of individual bottlenose dolphins. However, neither statute has provisions or supporting regulations requiring the management of bottlenose dolphins at a population (or 'stock') level. This contrasts with the statutory frameworks in other jurisdictions, such as the United States *Marine Mammal Protection Act 1972* and the New Zealand *Marine Mammal Protection Act 1977*. The absence of relevant statutory framework in Western Australia makes it necessary to review the rationale for identifying management units as a question of management policy. The scientific basis for considering resident communities as management units may relate to evidence that a community:

(1) exhibits demographic independence from populations in neighbouring areas;

(2) maintains a unique association with a particular geographic area or ecosystem;

(3) is genetically unique; and

(4) possesses unique cultural traditions.

### *Demographic independence*

Demographic independence is typically the primary focus for decision-making over management units, on the assumption that management units should represent population units whose risk of extinction is determined by internal demographic dynamics and is not substantially affected by immigration of individuals from adjacent population units (Taylor, 2005; Wade and Angliss, 1997). For example, management units may be defined as population elements having an extinction risk over a certain period (e.g. 100 years) that is not affected by immigration from adjacent populations (Sellas et al., 2005; Taylor, 1997; Taylor and Dizon, 1999; Wood and Gross, 2008). Such criteria emphasise the need for information on dispersal rates of individuals between a putative dolphin community and those

dolphin communities or populations which occur in nearby areas, using genetic analyses or long-term monitoring of the ranging patterns of known individuals (Möller et al., 2007; Sellas et al., 2005). Low rates of dispersal (e.g. <10% of individuals are immigrants: Hastings, 1993; Waples and Gaggiotti, 2006) suggest that, should a resident community decline to extinction, it may take decades for individuals from other populations to repopulate the area (NOAA, 2009; Sellas et al., 2005; Wood and Gross, 2008).

*Association with a particular geographic area or ecosystem*

Resident communities are typically associated with a defined area, which is often the extent of an estuary, embayment, or some portion of these features (e.g. Chilvers and Corkeron, 2001; Fury and Harrison, 2008; Gubbins, 2002; Lusseau et al., 2003; Speakman et al., 2006; Urian et al., 2009; Wells et al., 1987; Wiszniewski et al., 2009). These associations reflect the site fidelity (and often the natal philopatry[8]) of individuals, a common characteristic of bottlenose dolphins within inshore ecosystems (Connor and Smolker, 1985; Scott et al., 1990).

*Genetic uniqueness*

Units to conserve can be identified through genetic studies in which genetic material is obtained through biopsy sampling, post-mortem investigations, or from ancient DNA (e.g. from skeletal remains). These analyses provide insights into genetic diversity, gene flow, and genetic distinctiveness between sampling locations and inferences about population structure (e.g., Mirimin et al., 2011; Sellas et al., 2005). Evidence of genetic distinctness and low gene flow into localised dolphin communities provide strong support for the management of these communities for the purpose of preservation of biodiversity (Pichler et al., 1998).

*Cultural traditions*

Bottlenose dolphin culture should also be considered when assessing whether communities should be considered management units (Rendell and Whitehead, 2001; Whitehead, 2010; Whitehead et al., 2004). Rendell and Whitehead (2001) define culture as 'information or behavior – shared by a population or subpopulation – which is acquired from conspecifics through some form of social learning.' Social learning is fundamental feature of the behavioural ecology of bottlenose dolphins and reflects a socio-ecology based on: an extended period of juvenile dependence; social structures characterised by small, stable groups; and long-term relationships between individuals; and (in some populations) the transmission of foraging specialisations: Connor et al., 1992, 2000; Krützen et al.,

8 Natal philopatry is the retention of the mother's home range.

2005; Lusseau et al., 2003; Rendell and Whitehead, 2001; Sargeant et al., 2005, 2007; Sargeant and Mann, 2009; Wells et al., 1987).

## The resident Swan dolphin communities as a management unit

Information to identify and characterise resident communities as management units may be drawn from: behavioural observations (e.g. distributions, movement and ranging patterns, association patterns, behavioural specialisations, cultural traits); genetic differences; contaminant or parasite burdens; trophic 'signatures' (e.g. stable isotope ratios); demographic parameters (e.g. dispersal rates); types and rate of human interactions; and epidemiological data (e.g. differences in the prevalence of epidermal disease) (Chilvers and Corkeron, 2001; Möller et al., 2007; Newsome et al., 2010; Rendell and Whitehead, 2001; Sellas et al., 2005; Taylor, 1997; Urian et al., 2009; Toth, et al. 2011; Van Bressem et al., 2009b; Wade and Angliss, 1997; Whitehead et al., 2004; Wisniewski et al., 2009; Yordy et al., 2010).

The genetic, trophic, and contaminant information collected for this study strengthen the scientific basis for managing the resident Swan dolphin community as a distinct management unit. While this management approach would seem appropriate given the small size of the community and the deaths of six dolphins in 2009, there exists no clear statutory basis for identifying and managing communities of bottlenose dolphins as management units. Therefore we briefly review the current scientific rationale for adopting such an approach as a matter of environmental policy.

**Behavioural:** Photo-identification research from 2001-3 (followed by low-level monitoring from 2008-2011), indicates that a small assemblage of less than 25 dolphins is consistently associated with the Swan-Canning Estuary. These individuals exhibited year-round residency and long-term site fidelity, and accounted for nearly all of the sightings of dolphins within the estuary (Chabanne et al., 2011; H. Finn, Murdoch University, unpublished data). Dolphins considered part of the resident community in Cockburn Sound also exhibit these behavioural characteristics (R. Donaldson and H. Finn, Murdoch University, unpublished data; Finn, 2005). The ranging patterns of the dolphins resident in the Swan-Canning Estuary are also distinctive, as these dolphins range between the estuary and adjacent coastal areas such as Owen Anchorage on a daily or near-daily basis.

**Genetic:** A preliminary investigation of the distribution of mtDNA haplotypes in southwestern Australia suggested that unique mtDNA haplotypes may occur amongst dolphins from the Swan-Canning Estuary. A quantitative comparison of the population differentiation between dolphins from the Swan-Canning Estuary and from Cockburn Sound also suggested that there is moderate genetic structure in the Perth area, with less mixing between individuals from the two sites than may be

expected, given their close proximity. These findings, though preliminary, are indicative of fine-scale population structure within the southern metropolitan waters of Perth, with limited exchange of individuals between the Swan-Canning Estuary and Cockburn Sound.

**Trophic:** A preliminary comparison of the stable isotope signatures for dolphins from the Swan-Canning Estuary, Cockburn Sound, and Rottnest Island indicated that differences in the isotopic compositions of dolphins occur between sites, particularly for nitrogen. These differences suggest that the dolphins inhabiting the estuary have a trophic 'signature' that is distinct from dolphins in other habitats and likely reflects their unique association with estuarine food webs and estuary-based prey.

**Contaminants:** This study found substantial differences in the contaminant burdens of dolphins inhabiting the Swan-Canning Estuary and those present in dolphins from the Bunbury area, particularly in the concentrations of certain organic contaminants. Concentrations of dieldrin were particularly distinctive and are among the highest concentrations reported in the recent toxicological literature for small cetaceans. These differences reflect different levels of environmental contamination and pathways of exposure. Such differences are also likely to occur between dolphins from the estuary and dolphins from other locations in the Perth metropolitan area, though samples from other sites are needed to characterise these differences.

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