Technical Report

on the

Bottlenose Dolphin (*Tursiops aduncus*)

Unusual Mortality Event

within the

Swan Canning Riverpark,

June-October 2009

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REPORT TO THE SWAN RIVER TRUST

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Title: Technical Report on the Bottlenose Dolphin (*Tursiops aduncus*) Unusual Mortality Event within the Swan Canning Riverpark, June-October 2009

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Marine Mammal Health Project

This document contains graphic images of post-mortem examinations.

TABLE OF CONTENTS

SECTION	PAGE
I. Summary	6
II. Introduction	19
III. Ecology	
a. Introduction	22
b. Abundance and composition	24
c. Distribution and movement patterns	26
d. Activity patterns and group size	28
e. Foraging ecology	28
f. Genetics	29
g. Trophic relationships	30
h. Conservation biology	32
IV. Health	
a. Introduction	36
b. Shark predation	41
c. Human-induced Injury	42
i. entanglement	42
ii. vessel strikes	44
iii. illegal feeding and deliberate harm	44
d. Physiological stress from environmental conditions	45
e. Algal biotoxins	46
f. Infectious disease	46
i. viral pathogens	47
ii. secondary (opportunistic) pathogens	49
iii. pathogens in sewage discharge	49
iv. epidermal disease	50
g. Tattoo skin disease (TSD)	51
i. poxvirus and tattoo skin disease	51
ii. pathology and epidemiology of TSD	51
iii. factors affecting incidence of TSD	53
iv. salinity and TSD	56

v. TSD and severe skin lesions 59
vi. Environmental conditions in the Swan-Canning Riverpark 60
h. Contaminants 62
i. Other stressors 63
j. Trends in marine mammal health 64

V. Epidemiology

a. Multi-factorial aetiological framework 65
b. Pre-2009 dolphin mortalities in the Swan Canning Riverpark 66
c. 2009 dolphin mortalities in the Swan Canning Riverpark 68
d. Were the two clusters of dolphin mortalities in 2009 related? 72
e. Non-contributing factors for the 2009 mortalities 73
i. predation and vessel strike 73
ii. algal biotoxins 73
iii. faecal pathogens 73
f. Contributing factors for the 2009 dolphin mortalities 73
i. primary and secondary pathogens 73
ii. entanglement 74
iii. contaminants 74
iv. environmental conditions 74
g. Role of environmental conditions 74
i. aetiological framework 74
ii. environmental conditions in the Swan Canning Riverpark 75
iii. observations of dolphins in the Swan Canning Riverpark 76
iv. effects of low salinity conditions: physiology and behaviour 78
h. Did the mortalities involve dolphins resident in the Swan Canning Riverpark? 83
i. Dolphin ecology and mortalities in the Bunbury area 85
i. ecology of bottlenose dolphins in the Bunbury area 85
ii. epidemiology of mortalities in the Bunbury area 86

VI. Pathology

a. Multi-factorial model 92
b. Clinical presentations indicative of reduced immunological function 95
c. Opportunistic pathogens 96
d. Entanglement/Human induced injury 97
e. Relationship to other populations 98

f. Poxvirus (tattoo skin disease) and progression of skin lesions 99

g. Presence of primary pathogen (morbillivirus) 104

h. On-going analyses 105

VII. Contaminants

a. Literature review 107

b. Contaminants assessment 115

c. Results 116

d. Comparative evaluation of key contaminants 117

e. The role of contaminants in the deaths of the 2009 Swan dolphins 118

VIII. Conclusion

Key findings 120

Concluding remarks 123

IX. Acknowledgements 126

X. References 127

XI. Tables & Figures 141

XII. Appendices

A1. Criteria for determining an unusual mortality event (Wilkinson 1996) 190

A2. Dolphin mortalities in Gippsland Lakes, Victoria 191

A3. Water quality sampling sites within the Swan and Canning Rivers 192

A4. Tables and figures for the Bunbury dolphin mortalities 193

A5. Post-mortem reports 199

I. SUMMARY

Purpose of this report

This technical report reviews findings from an investigation into the mortalities of six bottlenose dolphins (*Tursiops aduncus*) in the Swan Canning Riverpark in 2009. The report: (a) describes the epidemiology and pathology of these mortalities; (b) presents background information on the ecology of dolphins in the Swan Canning Riverpark and factors known to affect dolphin health; and (c) discusses the potential role of chemical contaminants in the mortalities. These mortalities were investigated in context of dolphin deaths in the Swan Canning Riverpark prior to 2009 and a series of mortalities of dolphins in the Bunbury area between 2008-10, as well as marine mammal mortality events in other locations. This section provides a summary of the key findings from the investigation.

This technical report serves as a supporting document to the report by the Chief Scientist of Western Australia, Professor Lyn Beazley AO FTSE, *Dolphin deaths in the Swan Canning Riverpark and comments on the Bunbury inner waters, South-west of Western Australia*, which was provided to the Minister for Environment, the Hon Donna Faragher JP MLC, on 30 April 2010.

The Swan River Trust requested this technical report to draw together information relevant to the deaths of dolphins in the Swan Canning Riverpark and Bunbury area and, in particular, information relevant to the pathology of those deaths. It extends the 2009 Swan River Trust report, *Situation Report: Dolphin deaths in the Swan Canning Riverpark, Report to Hon Donna Faragher JP MLC Minister for Environment; Youth, 20 November 2009*, which the Minister for Environment requested as a means of compiling the information relevant to the deaths of the dolphins.

The authors would like to specifically acknowledge the contribution of information by Perth Zoo, Department of Agriculture, Swan River Trust, and Department of Environment and Conservation, as well as support from Department of Water, Department of Health, and other research institutions, management agencies, and individuals. The breadth of information in the technical report reflects the collaborative nature of the investigation. It should be noted, however, that the conclusions presented in this technical report are those of the authors.

Synopsis of mortalities and investigation

Six bottlenose dolphin deaths occurred in the Swan Canning Riverpark in 2009. The mortalities occurred in two clusters: (a) three deaths in June 2009 and (b) three deaths in September-October 2009. These deaths involved: two adult females [17 September, 25 October]; one adult male [9 October]; one sub-adult/adult female [21 June]; one male calf [5 June]; and one juvenile male [8 June]. Two of the dolphins [5 June and 9 October] were in an advanced state of decomposition at the time of recovery, and no post-mortem examinations were conducted. Post-mortem examinations were conducted for the other four dolphins. Additional analyses were also undertaken, including analyses to determine the

concentrations of certain contaminants in dolphin tissue samples. Certain analyses have yet to be completed, in particular those involving viral pathogens.

Are these mortalities unusual?

Criteria used to characterise marine mammal mortality events are generally based on stranding rates for dead animals (i.e. carcasses,) because natural (or 'background') rates of mortality for marine mammal populations are rarely known. We characterise the 2009 dolphin mortalities in the Swan Canning Riverpark as an 'unusual mortality event' based on terms and criteria used to identify marine mammal mortality events elsewhere. The 2009 mortalities appear to represent a marked increase in the number of dolphin mortalities in the Swan Canning Riverpark, as there were only six records of stranded carcasses in the Swan Canning Riverpark from 2002-8. More definitive conclusions about mortality rates for dolphins in the Swan Canning Riverpark are precluded by: the limited period for which mortality records are available, the opportunistic manner in which data on mortalities were collected, and the likelihood that some deaths occur outside of the Swan Canning Riverpark, based on research from 2001-3 showing that dolphins using the Swan Canning Riverpark also range in coastal areas adjacent to the Swan Canning Riverpark. However, while the pre-2009 stranding records for dead dolphins have limitations, these data do provide a baseline against which the 2009 mortalities can be compared, and indicate that the number and timing of mortalities in 2009 is anomalous, i.e. six deaths in five months, with three of these deaths within three weeks of each other (5 June to 21 June 2009) and three other deaths within five weeks of each other (17 September to 25 October 2009). All carcasses were recovered in the Swan Canning Riverpark.

Are the mortalities of biological significance to the Swan Canning Riverpark dolphin population?

The 2009 mortalities are likely to be biologically significant for the population of dolphins using the Swan Canning Riverpark because of the small number of dolphins thought to range in the estuary. Research from 2001-3 classified 18 bottlenose dolphins as 'resident' in the Swan Canning Riverpark, based on re-sighting patterns showing consistent usage of the estuary by these individuals between October 2001 and June 2003. In contrast, non-resident dolphins were seen only very infrequently. These re-sighting are based on 402 sightings of dolphin groups in the Swan Canning Riverpark during this study period, with dolphins individually identified based on dorsal fin markings.

These 18 'resident' dolphins were observed within the Swan Canning Riverpark and in adjacent coastal areas (e.g. Owen Anchorage), with observations suggesting that dolphins moved between the estuary and coastal areas on a daily or near-daily basis. 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 Riverpark (from 2001-3). These 18 individuals included six adult females, and accounted for nearly all of the sightings of dolphins in the Swan Canning Riverpark from October 2001 to June 2003.

The 18 dolphins were considered to comprise a resident dolphin 'community' of 20-25 dolphins for the Swan Canning Riverpark, with the overall size of the community varying depending on the number of calves present. The characterisation of the resident community is based on observations indicating: (a) shared ranging patterns and site fidelity for these 18 individuals, and (b) ecological differences between these 18 individuals and other dolphins observed in the waters between Fremantle and Rockingham. It is also supported by long-term study of bottlenose dolphins in Cockburn Sound and Shark Bay, Western Australia, as well as other long-term studies of bottlenose dolphins in estuarine and coastal ecosystems. The term 'resident' implies that dolphins exhibit site fidelity to the Swan Canning Riverpark and to the adjacent coastal area that they also use. The term 'community' means that members of the community share home ranges and associates (i.e. other dolphins that they consistently interact with). The two terms are used widely in the scientific literature for bottlenose dolphins.

Female bottlenose dolphins inhabiting nearshore and estuarine environments tend to inherit the home range of their mothers (natal philopatry) and not to disperse to the other areas, a characteristic that suggests that the size of the dolphin community in the Swan Canning Riverpark likely depends on the reproductive success of resident females, rather than immigration of dolphins from other areas. While we lack the information to definitively conclude that such demographic isolation occurs, this conclusion is consistent with long-term studies of bottlenose dolphins in other areas. These part-estuarine/part-coastal ranging patterns mean that members of the resident community may experience stressors both in the Swan Canning Riverpark and in coastal areas such as Owen Anchorage.

There has been little research on dolphins in the Swan Canning Riverpark since 2003, and we do not know what the size or composition of the resident community in the Swan Canning Riverpark was prior to the 2009 mortalities. It is likely to have been similar to what it was in 2001-3, based on the ecology and reproductive biology of bottlenose dolphins.

Are the two clusters of mortalities (in June 2009 and in September/October 2009) related?

The two clusters of mortalities differ in several aspects of their epidemiology. For example, they occurred at different times of the year, had different causative factors (see below), and involved different age-sex classes. These differences suggest the two clusters of mortalities may be unrelated, although the mortalities may have shared underlying aetiological factors. We use the term 'aetiology' to refer to the causative factors involved in the death of a dolphin. A 'multi-factorial aetiology' means that several factors combined to result in the death. The term 'commonality' refers to a finding or factor that is shared between or among individuals.

The June 2009 cluster of mortalities involved a dependent calf [5 June] and a dolphin affected by a long-term entanglement injury [21 June]. Mortality rates for bottlenose dolphin calves are naturally high with research from other areas indicating that 30-50% of bottlenose dolphins calves may die before weaning (age 3-5 in bottlenose dolphins). The dolphin that died on 8 June 2009 was also a juvenile (age 3+ years). Dolphins with entanglement (or other similar) injuries are at higher risk of

secondary infection because the wounds offer a portal of entry for opportunistic pathogens. Nonetheless, the timing of the June mortalities suggests the possibility of a commonality for the deaths.

In the September/October 2009 cluster of deaths, the adult female mortalities on 17 September 2009 and 25 October 2009 shared similar presentations (e.g. severe skin lesions). These presentations were distinct from those of the June 2009 deaths, and likely reflected a different suite of causative factors, including tattoo skin disease (see below). The presentations of the two adult females also shared some commonalities with an adult female that died on 18 November 2007.

Why did these deaths occur?

Several caveats should be applied to discussion of the causes of the 2009 mortalities. Firstly, our conclusions are based on post-mortem examination of only four dolphins. This makes it difficult to definitively determine the contribution of any individual causal factor or the interaction of multiple potential causal factors. Secondly, our understanding of the aetiologies (causative factors) for the 2009 mortalities should be considered subject to review until analyses involving viral pathogens have been completed. Thirdly, what we know about the deaths is based on post-mortem examination. We know little or nothing about the life histories of the dolphins that died, and in particular the specific suites of stressors that they experienced, the intensity and interaction of these stressors, and their individual susceptibilities to disease. Finally, there is a lack of information on the prevalence of disease in marine mammals in Western Australia. Thus, while the post-mortem examinations provide a solid scientific basis from which to understand the pathology of the 2009 mortalities, there are few epidemiological data that we can use to place these mortalities and their aetiologies within a broader (i.e. regional) context. We note these caveats as a way of indicating the limitations to our understanding the causative factors underlying the deaths of these dolphins.

The 2009 mortalities are best explained as the outcome of multiple contributing factors, and best considered as individual cases rather than as a group of mortalities with shared aetiologies. A multi-factorial aetiology means that several, potentially interacting (synergistic) factors, combined to result in these deaths. Little can be said about the two dolphins [5 June and 9 October] that were recovered in an advanced state of decomposition, except to note that they did not have fresh shark wounds, active entanglements, or other obvious sources of external trauma.

Post-mortem examination of the four other dolphins indicated multi-factorial aetiologies, i.e. a suite of factors contributing to the mortality of each individual. Notable aspects of the presentations of the four dolphins included: two dolphins with severe skin lesions associated with the presence of ‘tattoo skin disease’ (cetacean poxvirus) [17 September and 25 October], and two dolphins with active entanglements [21 June and 25 October]. The entanglement on the 21 June dolphin was long-standing, and was first observed in mid-2008.

A commonality of the four dolphins examined post-mortem was the presence of significant secondary infections by opportunistic bacterial and/or fungal pathogens. The differing presentation of the four dolphins indicates that these secondary infections differed in their probable aetiology and in their relationship to the death of the dolphins. The aetiologies of these secondary infections likely reflect: (1) reduced immunological function; (2) damage of epidermal tissue by a long-standing entanglement injury that likely offered a port of entry for opportunistic pathogens [21 June]; and (3) colonisation of epidermal tissue damaged by tattoo skin disease lesions and possible osmoregulatory disruption of epidermal cells caused by exposure to low salinity water [17 September and 25 October]. Secondary infections were considered to have led directly to the deaths of the 8 June and 21 June dolphins. The opportunistic fungal and bacterial dermatitis observed in the 17 September and 25 October dolphins was not considered to have directly contributed to their deaths, and were likely to have occurred as terminal events secondary to the severe tattoo skin disease lesions (and possibly compounded by low salinity conditions).

The 8 June juvenile male was found to have fungal meningoencephalitis with intralesional fungal organisms consistent with *Aspergillus* spp. (fungal infection of the brain). Gross and histological analysis indicated that haematogenous spread of the fungal organisms was likely to have occurred with resultant thrombosis and ischaemia (loss of blood flow) in the jejunum (small intestine).

The 21 June juvenile female had a severe fishing line entanglement of the right fluke that had persisted for more than a year. Post-mortem examination found bronchopneumonia with intralesional fungi consistent with *Aspergillus* spp., as well as two types of opportunistic bacterial pathogens. The gross and histological changes were indicative of systemic septicaemia with intercurrent *Aspergillus* spp. infection.

The presentations of the two dolphins with severe skin lesions [17 September and 25 October] were similar to each other and distinct from the June mortalities. These two dolphins exhibited severe skin lesions that were deeply ulcerative and secondarily infected with opportunistic fungal and bacterial pathogens. These ulcerative lesions affected a large area of the body surface, likely leading to significant loss of body fluids and proteins as well as electrolyte imbalances, and ultimately leading to terminal debilitation. The time period for the development of the lesions is not known, but we believe the progression to have been acute (i.e. occurring over a time period of days to weeks) based on post-mortem presentation and histological analyses of the 17 September and 25 October 2009 dolphins, as well as post-mortem examination of another dolphin with skin lesions that died 18 November 2007. The 25 October 2009 dolphin was observed with severe lesions as early as 3 October 2009 (near Ascot). The severe ulcerative lesions were similar to those observed in dolphins from two other areas with potentially stressful environmental conditions, the Gippsland Lakes (Victoria) and Lake Pontchartrain, a brackish lagoon in Louisiana (United States).

The aetiology of the severe lesions in the 17 September and 25 October dolphins is unclear. However, we believe that these lesions relate to skin damage initially caused by poxvirus (tattoo skin disease) infection, and subsequently exacerbated by osmoregulatory disruption of epidermal cells exposed to low salinity conditions. Evidence of poxvirus infection was found in tissues adjacent to the ulcerative lesions in these two dolphins (see below).

Certain aspects of the pathology of the 2009 mortalities are suggestive of reduced immunological function in at least some of the dolphins. These include the secondary (opportunistic) infections observed in the 8 June and 21 June dolphins, the severe skin lesions observed in the 17 September and 25 October dolphins, and evidence of lymphoid depletion in several dolphins. Factors that could have affected immunological function include: chronic infection from a long-standing entanglement injury, environmental conditions, chemical contaminants, or other factors. Reduced immunological function would likely reflect cumulative and potentially synergistic interactions among stressors. We also have not been able to definitively exclude the presence of morbillivirus, a primary pathogen that is known to reduce immunological function.

What is known about the role of tattoo skin disease (cetacean poxvirus) in the mortalities?

Cetacean poxvirus is the pathogen that causes tattoo skin disease (TSD), a skin disease in dolphins that causes epidermal lesions in cetacean populations around the world. Cetacean poxvirus is specific to cetaceans and cannot be transmitted to humans. The prevalence of TSD lesions on dolphins within the Swan River in 2009 is not known. However, a 2008 study in Cockburn Sound observed lesions indicative of TSD in 45 of 158 (27.2%) dolphins photo-identified within Cockburn Sound between July-November 2008. A similar study also observed lesions indicative of TSD in dolphins from the Bunbury area, with preliminary analyses suggesting a potential association between the prevalence of lesions and the use of inner water (nearshore and estuarine) areas around Bunbury.

This is the first study to clinically identify the presence of poxvirus within cetaceans from Western Australia. Histological analyses identified viral inclusion bodies characteristic of poxvirus in skin samples from the two adult females with severe skin lesions [17 September and 25 October 2009]. These inclusion bodies were present in tissues immediately adjacent to ulcerative tissues, indicating an association between TSD infection and the development of ulcerative lesions. Retrospective histological analysis also identified inclusion bodies characteristic of poxvirus in an adult female that died in the Swan Canning Riverpark on 18 November 2007. Post-mortem examination of this dolphin found large epidermal lesions characteristic of tattoo skin disease, but not the severe ulcerative lesions found in the 17 September and 25 October 2009 dolphins. The presence of TSD in dolphins from Western Australia is not unusual, as the disease occurs in dolphin populations around the world.

Although the presence of TSD in itself is not unusual, the presentation and severity of TSD lesions observed in the 17 September and 25 October adult females was unusual for the disease. First, TSD generally affects juvenile animals and is not typically observed in adults, as they generally have

previously acquired immunity to poxvirus. Second, the typical presentation of TSD infection is mild and self-limiting and not associated with mortality in adults. However, the TSD lesions in the two 2009 adult females were associated with an acute progression to severe, deeply ulcerative skin lesions that resulted in terminal debilitation. We are referring to this as 'atypical poxvirus/TSD' pending identification of the poxvirus strain present.

We have not determined the definitive mechanisms by which the characteristic TSD lesions may have progressed to the unusually severe (deeply ulcerative) lesions observed in two of the dolphins. The factors underlying the progression may include: the presence of a poxvirus strain possessing greater virulence; osmotic damage of epidermal cells caused by exposure to low salinity and/or other adverse environmental conditions; systemic physiological impairment (e.g. electrolyte imbalances) related to stress from environmental conditions or other factors; and decreased immune function. The process is likely to be multi-factorial and may involve a sequence of processes that increase the severity of the TSD infection and/or exacerbate the initial damage caused by TSD infection.

Several lines of evidence suggest that exposure to low salinity conditions is likely to have been an important factor in the severe lesions observed in the 17 September and 25 October 2009 mortalities. Firstly, from July-October 2009 salinities in the Swan Canning Riverpark were low enough [i.e. <20 ppt (g/L) for dolphins in captivity] to be potentially physiologically stressful to dolphins. For example, weekly water quality sampling at the Narrows Bridge indicates that surface salinities at that location were <15 ppt from 3 July 2009 to 16 October 2009. These conditions could induce osmoregulatory disruption of epidermal cells and/or systemic physiological stress. Secondly, dolphins ranged in areas of low salinity during this period. Observations from the community-based Dolphin Watch project indicate that dolphins occurred in areas upstream of the Narrows Bridge between July-October 2009. The 25 October female was also observed at Ascot on 3 October 2009 (with some skin lesions present). Thirdly, individual behavioural and physiological differences could influence the exposure of dolphins to low salinity conditions and their susceptibility to physiological stress from low salinity conditions. Observations of dolphins from the Lake Pontchartrain, Louisiana indicate that behavioural factors may influence why dolphins use low salinity areas when habitats with higher salinities are available to them, and also suggest that physiological factors (e.g. differences in diet, body condition, immunological function) may influence how dolphins respond to the stress of low salinity conditions. It is interesting to note that the 17 September and 25 October 2009 dolphins were both adult females (as was the 18 November 2007 dolphin observed with TSD lesions), although the significance of this finding, if any, is unknown.

We suggest that these lines of evidence provide a suitable explanatory framework for why the severe lesions occurred, when they occurred, and why they affected only certain individuals. In particular, it suggests that the pathology of the severe lesions observed in the 17 September and 25 October 2009 dolphins is multi-factorial, and reflects: (a) the occurrence and severity of TSD infection; (b) exposure to adverse environmental conditions; and (c) individual differences in physiological condition,

immunological function, and behavioural ecology. The aetiology for the severe lesions may also reflect other (possibly synergistic) factors, including contaminant burdens.

We emphasise that low salinity conditions (i.e. less than marine salinities) are common in the Swan Canning Riverpark during the winter-spring period, and that the environmental conditions observed in 2009 were not unusual (i.e. similar conditions have occurred in other years). We also note that while bottlenose dolphins have the sensory capacity to detect variation in salinities, they do not necessarily avoid areas with salinities that are less than in marine environments. This conclusion is based on studies of dolphins in estuarine and coastal environments around the world and on observations of dolphins in the Swan Canning Riverpark and the Bunbury area.

What is the status of the investigation?

While the majority of the investigation has been completed, certain aspects are continuing, with several analyses still pending at this time. These analyses relate, in particular, to whether cetacean morbillivirus (a viral pathogen) was present in tissue samples collected during post-mortem examination. We also have not determined the mechanisms by which the characteristic TSD lesions progress to the unusually severe (deeply ulcerative) lesions observed in two of the dolphins, and thus it is desirable to characterise the strain of poxvirus present. These issues also reflect, in part, the lack of baseline epidemiological information for infectious diseases in marine mammals from Western Australia. This lack of comparative data limits our ability to place mortality events like this into context. For these reasons the conclusions presented here should be considered subject to review after completion of these analyses.

These on-going analyses reflect our effort to evaluate potential contributing factors using the full suite of molecular, histological, and other analytical approaches available, and also to establish best-practice in the event of future mortalities. We note that the analyses involve questions about pathogens causing infectious disease; there are no further contaminants analyses anticipated. At this time, it is not possible to provide a definitive timeline for the conclusion of all on-going analyses, although the intent is that they should be concluded in the second half of 2010. The on-going analyses involve collaborative arrangements with research institutions possessing specialised analytical expertise.

What role did contaminants play?

Dieldrin, DDE, and PCBs were the predominant organic contaminants detected in tissue samples from the 2009 Swan Canning Riverpark mortalities. Concentrations of dieldrin were among the highest reported globally in marine mammals (at current time), and concentrations of PCB congeners exceeded published toxicity thresholds for effects on immune function in some individuals. Dieldrin concentrations in the Swan dolphins were substantially higher than those detected in tissue samples taken from dolphins that died in the Bunbury area between 2007-9. Concentrations of other contaminants, such as heavy metals and tributyltin (TBT), were less than or similar to those observed in estuarine and coastal populations elsewhere.

Several factors preclude a definitive determination of what role contaminants may have played in the 2009 dolphin mortalities in the Swan Canning Riverpark. These factors largely reflect the difficulties in relating tissue concentrations of contaminants to particular health effects in marine mammals and, more specifically, to the pathological observations found in post-mortem examinations of dolphins from the Swan Canning Riverpark. Studies of marine mammal mortality events have shown that it is exceedingly difficult to elucidate the relative contributions of multiple stressors that may have similar effects (e.g. on immune function), particularly when the number of mortalities is small.

Some of the persistent organic contaminants recorded in the Swan dolphins may have been in the environment for decades and the use of most of these contaminants has been banned for some time. These factors suggest that: (a) contaminant concentrations were likely to have been higher in previous generations, and (b) if chemical contaminants were having a profound effect on dolphins then unusual mortality events would have been recorded in previous decades. We note, however, that there remains uncertainty about the environmental and biological (trophic) factors influencing the bioavailability of organic contaminants in the Swan Canning Riverpark, and that the ecotoxicology of contaminants in the estuary ecosystem remains an area of active research. It is possible, for example, that the factors influencing the bioavailability of contaminants may have changed over time.

Although we conclude that chemical contaminants were not a direct cause of the 2009 mortalities, this is not to say that contaminants do not have an adverse effect on the health of dolphins in the Swan Canning Riverpark. It is likely that chemical contaminants do adversely impact on the health of the Swan dolphins at present, and have also affected previous generations. We note that the adverse effect of contaminants may be insidious, and could potentially combine with other stressors to have an overall effect that influences the incidence and severity of infectious disease.

Did the 2009 mortalities involve dolphins resident in the Swan Canning Riverpark?

We were able to identify only one of the six dolphins based on photo-identification data from 2001-3 (using dorsal fin markings). The adult female that died on 25 October 2009 was 'Leeuwin', one of the 18 resident dolphins identified in 2001-3. Of the five remaining dolphins: two were born after 2003 [the 5 June dependent calf (~5 years old) and 8 June juvenile ($\geq$3 years old)] and thus not previously photo-identified, and two had factors limiting our ability to identify their dorsal fins [9 October male (advanced state of decomposition with skin sloughing) and 17 September female (fungal and bacterial growth and skin lesions on the dorsal fin)]. We could not identify the 21 June female although her fin was in reasonable condition. This may be because her dorsal fin marking has changed, as often occurs over time.

Thus, aside from Leeuwin, we cannot definitively say to what degree the dolphins that died in 2009 used the Swan Canning Riverpark. However, two factors indicate that the dolphins that died in 2009 were likely to have been resident in the Swan Canning Riverpark. Firstly, nearly all of the dolphins observed beyond the Fremantle Inner Harbour between 2001-3 were the 18 dolphins considered

resident in Swan Canning Riverpark (and their dependent calves). Secondly, the dolphins had high concentrations of dieldrin and other organic contaminants that occur in higher concentrations in sediments of the Swan Canning Riverpark than in sediments in Owen Anchorage or Cockburn Sound. Concentrations of dieldrin in dolphins from the Swan Canning Riverpark were also substantially higher than those observed in dolphins from the Bunbury area. These differences suggest that the dolphins that died in 2009 fed, at least in part, on prey associated with Swan Canning Riverpark.

It is unlikely that the dolphins entered the Swan Canning Riverpark as a 'safe' place in which to die. Long-term site fidelity is a consistent characteristic of bottlenose dolphins in Cockburn Sound and other coastal and estuarine ecosystems, and we find it unlikely that a dolphin in distress would enter an unfamiliar environment that contained a range and intensity of stimuli likely to seem adverse or stressful and to be otherwise avoided (e.g. boat traffic, narrow channels, anthropogenic noise).

What is known about dolphin mortalities in the Bunbury area?

A three-year (2007-9) study in the Bunbury area found indications of high mortality rates for dolphins using the estuarine and nearshore areas around Bunbury. This study identified 196 bottlenose dolphins in the coastal and estuarine waters around Bunbury, based on year-round photo-identification effort. Of these 196 individuals, 14 dolphins ranged exclusively within an 'inner waters' area encompassing the Leschenault Inlet, Leschenault Estuary, Bunbury Inner Harbour, Bunbury Outer Harbour, and Collie River, and did not range in adjacent coastal habitats. These 'inner waters-only' ranging patterns were unique to these 14 dolphins, and indicated that they compromised a distinct ecological subgroup.

Of these 14 'inner waters' dolphins: six were alive, five were confirmed dead (i.e. carcasses recovered), and three were presumed dead by the conclusion of field research in November 2009. Four of the confirmed deaths and all three of the presumed deaths occurred between April 2008 and May 2009. The fifth confirmed death occurred in November 2009.

In addition, the carcasses of four other dolphins were recovered from the general Bunbury area between January 2008 and January 2010, including one with a long-standing entanglement injury and one with a presentation indicative of blunt force trauma consistent with a vessel strike.

Post-mortem examinations were conducted on four of the inner waters dolphin mortalities from 2008-9 [a fifth carcass was in advanced decomposition at time of recovery], as well as on the four other dead dolphins recovered between 2008-2010. The deaths showed both similarities (i.e. commonalities) and differentials with those from the Swan Canning Riverpark. The main commonalities were: (a) the presence of opportunistic pathogens, a finding that is indicative of poor immunological function; (b) evidence of human-induced injury as a cause of, or likely contributor to, mortality; and (c) and a broadly similar environment. As with the deaths in the Swan Canning Riverpark, the post-mortem presentations indicate a multi-factorial aetiology in which several, potentially interacting (synergistic) factors, combined to result in these deaths.

Pneumonia with the presence of *Halocercus* lungworms (and often fungal and bacterial pathogens) was a common presentation, and was observed in four dolphins from the Bunbury area that were examined post-mortem between 2008-2010 (including two 'inner-water' dolphins). The aetiology for the pneumonia is unclear and is likely to reflect the influence of several factors. The lung nematode *Halocercus* is frequently associated with pneumonia and other bacterial and viral infections in dolphins, and the parasite is not the cause of disease for these dolphins, but rather its presence is indicative of ill-thrift and poor condition, and probably the high parasite loads of dolphins in this area. As with the deaths in the Swan Canning Riverpark, we lack the sample size and epidemiological data to fully interpret the post-mortem findings and address the causative factors for the deaths. Human-induced injury was another commonality, with one death involving a long-standing entanglement injury and another having a presentation consistent with a vessel strike (blunt force trauma).

The key differentials between the deaths in the Bunbury area and those in the Swan Canning Riverpark are the significantly lower contaminant burdens for the Bunbury dolphins, and overall the fact that the Bunbury individuals tended to be in poorer body condition. Severe ulcerative skin lesions were not observed in post-mortem examination of dolphins from the Bunbury area between 2008-9. Post-mortem examinations and photo-identification analyses show that skin lesions indicative of TSD are present in dolphins from the Bunbury area, but we have not yet definitively identified poxvirus as being present (e.g. through histological analyses and other approaches).

The mortalities of the Bunbury inner waters dolphins in 2008 and 2009 appear to constitute an unusual mortality event. This conclusion is based on the photo-identification data and carcass strandings showing a high rate of mortality over a thirteen-month period (April 2008-May 2009) for dolphins using the Bunbury inner waters (i.e. four confirmed deaths and three presumed deaths). This level of mortality appears above normal background rates for bottlenose dolphin populations and to show a clear association with the use of the inner waters around Bunbury. We note, however, there are few stranding data for the Bunbury region prior to 2007. There are also few stranding data for other estuarine (e.g. Peel-Harvey) and coastal (e.g. Cockburn Sound) areas in southwestern Western Australia that could be used to place the Swan and Bunbury mortalities in context. This highlights the value of ongoing data collection and population monitoring.

What is known about human impacts on dolphins in the Swan Canning Riverpark?

Two aspects of the ecology of the resident dolphin community in the Swan Canning Riverpark make it vulnerable to anthropogenic stressors: (a) small population size and (b) site fidelity. The number of dolphins that consistently use the Swan Canning Riverpark is small, and the persistence of dolphins in the estuary may depend on reproductive output of the handful of females who range in the estuary. The resident dolphins use the Swan Canning Riverpark on a daily or near-daily basis, and thus may experience the repeated and cumulative effects of harmful stressors in the estuary. These two factors

are also reflective of the unique ecological linkage that exists between these dolphins and the Swan Canning Riverpark.

Human-induced injuries are a significant health challenge for dolphins in the Swan Canning Riverpark, the metropolitan waters of Perth, and the Bunbury area. Examples of human-induced injuries include: the death of a juvenile female [21 June 2009] in the Swan Canning Riverpark that had an entanglement injury that had persisted for more than a year; observations from Cockburn Sound indicating that at least six calves from the Sound experienced entanglements between 1996-2003; the death of a juvenile female [30 September 2009] from the Bunbury area that had a long-standing entanglement around its beak; and the death of an adult female [on 23 January 2010] from the Bunbury area that had a penetrative wound to the body wall indicative of a vessel strike.

What do the Swan and Bunbury mortalities indicate about dolphins and estuaries?

The mortalities in the Swan Canning Riverpark and Bunbury area suggest that estuarine dolphins experience a combination of natural and anthropogenic stressors that may be sufficient to cause a long-term decline in the abundance of dolphins associated with these ecosystems. This conclusion is consistent with studies of the health of estuarine dolphins in other locations and with patterns of unusual mortality events for marine mammals. Low reproductive rates and small population sizes mean that dolphin populations in estuarine ecosystem may be unable to compensate for increased mortality from infectious disease, human-induced injury, and other stressors.

Bottlenose dolphins inhabiting estuarine ecosystems live in an environment that presents many challenges to their health. These stressors are often unique in kind, in severity, and in the manner in which they can interact synergistically. In the Swan Canning Riverpark dolphins may experience: the physiological stress of inhabiting a highly-variable environment; the physical trauma of becoming entangled in fishing line; the immunological challenge of being exposed to natural pathogens and to man-made contaminants; the ecological difficulty of encountering increasing vessel and jet-ski traffic and anthropogenic noise; and the environmental stress of living in an ecosystem that occupies the heart of a major metropolitan area and lies at the terminus of a large agricultural catchment. The Swan dolphin deaths in 2009 are ultimately reflective of the stressful nature of the environment that they inhabit.

Adult female 'Leeuwin' at Waylen Bay, 25 October 2009 [S. Allen, Murdoch University]

II. INTRODUCTION

Purpose of this report

This technical report reviews findings from an investigation into the mortalities of six bottlenose dolphins (*Tursiops aduncus*) in the Swan Canning Riverpark in 2009. The report: (a) describes the epidemiology and pathology of these mortalities; (b) presents background information on the ecology of dolphins in the Swan Canning Riverpark and factors known to affect dolphin health; and (c) discusses the potential role of chemical contaminants in the mortalities. These mortalities were investigated in context of dolphin deaths in the Swan Canning Riverpark prior to 2009 and a series of mortalities of dolphins in the Bunbury area between 2008-10, as well as marine mammal mortality events in other locations.

This technical report serves as a supporting document to the report by the Chief Scientist of Western Australia, Professor Lyn Beazley AO FTSE, *Dolphin deaths in the Swan Canning Riverpark and comments on the Bunbury inner waters, South-west of Western Australia*, which was provided to the Minister for Environment, the Hon Donna Faragher JP MLC, on 30 April 2010.

The Swan River Trust requested this technical report to draw together information relevant to the deaths of dolphins in the Swan Canning Riverpark and Bunbury area and, in particular, information relevant to the pathology of those deaths. It extends the 2009 Swan River Trust report, *Situation Report: Dolphin deaths in the Swan Canning Riverpark, Report to Hon Donna Faragher JP MLC Minister for Environment; Youth, 20 November 2009*, which the Minister for Environment requested as a means of compiling the information relevant to the deaths of the dolphins.

The authors would like to specifically acknowledge the contribution of information by Perth Zoo, Department of Agriculture, Swan River Trust, and Department of Environment and Conservation, as well as support from Department of Water, Department of Health, and other research institutions, management agencies, and individuals. The breadth of information in the technical report reflects the collaborative nature of the investigation. It should be noted, however, that the conclusions presented in this technical report are those of the authors.

Status of the investigation

While the majority of the investigation has been completed, certain aspects are continuing, with several analyses still pending at this time. These analyses relate, in particular, to whether cetacean morbillivirus (a viral pathogen) was present in tissue samples collected during post-mortem examination. We also have not determined the mechanisms by which the characteristic tattoo skin disease (TSD) lesions progress to the unusually severe (deeply ulcerative) lesions observed in two of the dolphins, and thus it is desirable to characterise the strain of poxvirus present. Therefore, the conclusions presented here should be considered subject to review after completion of these analyses. These on-going analyses reflect our effort to evaluate potential contributing factors using the full suite

of molecular, histological, and other analytical approaches available, and also to establish best-practice in the event of future mortalities. The need for these analyses also reflects, in part, the lack of baseline information on the prevalence of disease in marine mammals from Western Australia. We note that the analyses involve questions about pathogens causing infectious disease; there are no further contaminants analyses anticipated. At this time, it is not possible to provide a definitive timeline for the conclusion of all on-going analyses, although the intent is that they should be concluded in the second half of 2010. The on-going analyses involve collaborative arrangements with research institutions possessing specialised analytical expertise.

Report Structure

The report is divided into three sections that provide background information for the investigation (Sections II-IV), present its findings (Sections V-VII), and contain supporting materials (IX-XII). The key findings are reviewed in the Conclusion (Section VIII) and a summary of the report is also provided (Section I). Tables and figures are presented separately in Section XI. The Appendices (Section XII) also contain data and information relevant to the investigation, including reports from post-mortem examinations.

Summary	**I.**	Summary
Background Information	**II.**	Introduction
	III.	Ecology
	IV.	Health
Investigation Findings	**V.**	Epidemiology
	VI.	Pathology
	VII.	Contaminants
	VIII.	Conclusions
Supporting Materials	**IX.**	Acknowledgements
	X.	References
	XI.	Tables & Figures
	XII.	Appendices

Note on terms

This report focuses on the deaths of six bottlenose dolphins within the Swan Canning Riverpark between June-October 2009 and, unless otherwise indicated, the content of the report refers specifically to these six mortalities. These mortalities are sometimes referred to as the ‘2009 Swan dolphin mortalities’ within the document. Some sections of the report review information on pre-2009 mortalities within the Swan Canning Riverpark and mortalities in the Bunbury area from 2008-10. These mortalities are referred to as the ‘pre-2009 Swan dolphin mortalities’ and the ‘Bunbury area mortalities’, respectively. The term ‘Bunbury inner waters’ refers to the nearshore and estuarine area in the Bunbury region encompassing the Leschenault Inlet, Leschenault Estuary, Inner Harbour, Outer

Harbour and Collie River. In this report, the term 'stranded' refers to the carcasses of dead dolphins recovered from the water or from shorelines, rather than the stranding of a live animal.[1]

Classification of the mortalities

We characterise the deaths of the six dolphins within the Swan Canning Riverpark in 2009 as an **unusual mortality event**, following definitions and criteria used to classify mortality events for marine mammals in the U.S. (Appendix A1).[2] The U.S. *Marine Mammal Protection Act* 1972 defines an unusual mortality event as a set of strandings that is: "unexpected; involves a significant die-off of any marine mammal population; and demands an immediate response".[3] We feel that the term unusual mortality event provides a suitable descriptor for a series of mortalities that appear to represent an anomaly in the mortality rate for dolphins within the Swan Canning Riverpark, although the term has no statutory or policy basis in Australia or Western Australia.

The criteria used to identify an unusual mortality event are published in the U.S. National Contingency Plan for Response to Unusual Marine Mammal Mortality Events (Wilkinson 1996). Two of these criteria are applicable to the 2009 dolphin deaths within the Swan Canning Riverpark: (1) "a marked increase in the magnitude of strandings when compared with prior records" [Criteria 1] and (2) "an increase in strandings is occurring in a very localized area (possibly suggesting a localized problem)" [Criteria 3]. The U.S. Working Group on Marine Mammal Unusual Mortality Events considers that a single criterion or combination of criteria may indicate the occurrence of an unusual mortality event (Wilkinson 1996, Lecky 2006).

Two sections (III and V) of the report address the applicability of the criteria to the 2009 deaths. Section III (Ecology) presents ecological data suggesting the dead dolphins were members of the small resident dolphin community that ranges within the Swan Canning Riverpark and adjacent marine areas. Section V (Epidemiology) presents data indicating that the six deaths were above the normal stranding rates for dolphins in the Swan Canning Riverpark.

1 However, in some instances bystanders did observe the dolphins die (e.g. the dolphins that died 17 September 2009 and 25 October 2009 in the Swan Canning Riverpark and the dolphin that died 30 September 2009 in the Bunbury area).

2 There is no single system in the scientific literature for classifying marine mammal mortalities, and neither the Western Australia *Wildlife Conservation Act* 1950 nor the Commonwealth *Environmental Protection and Biodiversity Conservation Act* 1999 provide criteria for doing so (nor do the regulations supporting those statutes). UNEP (2008) defines a *mass stranding* of marine mammals as the stranding of $\geq$2 animals near in time and space (not including a mother-calf pair), and a *mass die-off* as "mortality on a large-scale (without inference on the cause of death or species involved)". Kemper et al. (2005) refer to strandings of multiple cetaceans (whales, dolphins, porpoises) in South Australia as 'group strandings'.

3 Title IV, *Marine Mammal Protection Act* 1972, 16 U.S.C 1361

III. ECOLOGY: ecology of bottlenose dolphins in the Swan Canning Riverpark

A. Introduction

This section reviews the ecology and conservation biology of bottlenose dolphins (*Tursiops aduncus*) with the Swan Canning Riverpark. This information provides the scientific basis for understanding the biological significance of the 2009 mortalities for the resident dolphin community within the Riverpark, and potential associations between the ecology of dolphins and their exposure to certain stressors. Figures 1a and 1b show the extent of the Swan Canning Riverpark and the geography of its constituent river and estuary systems.

1. Data sources

The information presented in this section is largely based on field research conducted within the Swan-Canning Riverpark between October 2001-June 2003 (Cannell 2004; Finn 2005; Moiler 2008; Lo 2009; H. Finn, unpublished data; Figure 2). Aspects of this research appear in the 2004 Department of Conservation and Land Management [now Department of Environment and Conservation] technical report *Distributions of the major marine fauna found in the Perth metropolitan area* (Cannell 2004), and in two Curtin University Honours theses (Moiler 2008, Lo 2009).

The 2001-3 dataset for the Swan Canning Riverpark includes: 402 behavioural surveys (sightings) within the Swan Canning Riverpark with location, group size, group composition (using visual or photo-identification techniques), and activity state recorded; 1478 transits of nine sampling-areas located in the lower and middle reaches of the Swan Canning Riverpark; and approximately 50 focal follows of known individual dolphins within Swan Canning Riverpark and surrounding areas.

Photo-identification is the use of photographic images to visually identify dolphins based on individually-distinctive markings on the dorsal fin (e.g. nicks, indentations, and partial/full amputations), as well as scars occurring elsewhere on the body. From 2001-3, photo-identification was used to identify individual dolphins observed, and to analyse re-sighting patterns for these individuals. Focal follows are extended ($\geq$ 1 hour) behavioural observations of a single dolphin, with behavioural and environmental data collected at systematic intervals. Methods for the behavioural surveys are discussed in Finn (2005). Moiler (2008) describes the methodology for the surveying of the nine sampling-areas.

The main study area for the 2001-3 research extended from the Inner Harbour[4] (the port facilities adjacent to the Fremantle CBD) through to the Narrows Bridge and to the entrance of the Canning River. Occasional search effort was expended within the Canning River and within areas of the Swan River to the east of the Narrows Bridge, and dolphins were also observed moving within these areas during the course of behavioural surveys and focal follows. Some photo-identification research was

[4] The Inner Harbour is sometimes referred to as the 'port'.

also carried out within Cockburn Sound and in the areas lying between the Inner Harbour and Cockburn Sound (Owen Anchorage, Parmelia and Success Banks) (Figure 2). The image below shows the extent of the study area within the Swan Canning Riverpark, the location of the nine sampling-areas, and the track of the survey route followed. Figure 3 shows the search effort within the nine sampling-areas from February 2002–June 2003. For the purposes of this report, the term 'lower reaches' refers to the narrow channel area extending from the Inner Harbour to Blackwall Reach, and the term 'middle reaches' refers to the broad basin areas extending from the end of Blackwall Reach to the Narrows Bridge and the entrance to the Canning River (i.e. Mosman Bay, Freshwater Bay, Melville Waters, Matilda Bay).

Data from the 2001-3 dataset have several limitations. Firstly, the data have not been published in peer-reviewed scientific journals and would benefit from further analyses. Secondly, data from the focal follows are unanalysed, and conclusions from those data are based on information recorded in behavioural surveys (conducted during focal follows) and on anecdotal observations. Finally, there are few observations from the Canning River and the upper reaches of the Swan River. We note, however, that the 402 behavioural surveys conducted in 2001-3 have been analysed, and these data provide a strong basis for conclusions about the abundance and residency patterns of dolphins within the Swan Canning Riverpark, as well as a suitable baseline for comparative analyses.

2. Current research

Research activity on dolphins in the Swan Canning Riverpark has been limited since 2003. A small number of photo-identification surveys were conducted in 2008 to support a study of dolphins in

Cockburn Sound (Ham 2009). A 2009 Swan Canning Research and Innovation Project (SCRIP) study examined the genetics, trophic associations, and contaminant exposure of dolphins within the Swan Canning Riverpark using lab-based analyses of tissues collected through remote biopsy.[5] The 2009 mortalities provided an additional source of tissue material for this study.

3. Dolphin Watch

'Dolphin Watch' is a partnership project between the Swan River Trust's River Guardians Program, the Curtin University of Technology, and Murdoch University that operates a community-based monitoring program for dolphins in the Swan Canning Riverpark. Since its inception in autumn 2009, more than 150 River Guardians volunteers have received training in dolphin observation and data-recording techniques and collect data on the occurrence of dolphins throughout the Riverpark [see further at: http://ww.riverguardians.com and the Dolphin Watch page therein].

B. Abundance and demographic composition

1. Resighting patterns

Photo-identification analyses identified 35 individuals (not including calves) within the Swan Canning Riverpark between October 2001 and June 2003 (Lo 2009; Table 1, Figure 4). Of these 35 known individuals: 18 were sighted at least 23 times and were classified as the **resident** community, while 17 were sighted 9 times or less and were classified as **infrequent visitors**. Of the 17 individuals having ≤ 9 sightings within the Riverpark: 11 were sighted on only one or two occasions, and (with one exception) these 11 individuals were observed only in the Inner Harbour or the lower reaches of the estuary (i.e. downstream of Blackwall Reach).[6]

These re-sighting patterns indicate that the 18 dolphins classified as residents represented the core group of animals using the Swan Canning Riverpark between 2001-3, with other dolphins only using the estuary on an infrequent basis and rarely ranging beyond Blackwall Reach. The ranging patterns of the resident dolphins were also unique, as sighting and focal follow observations indicated that they ranged between the estuary and adjacent marine areas (Owen Anchorage, Parmelia and Success Banks, northern Cockburn Sound) on a daily or near-daily basis.[7] These movement patterns are described further below.

2. Demographic composition

The 18 resident dolphins were all adults, sub-adults, or juveniles. Calves were not considered separately for analysis because calves are dependent upon their mothers until weaning (at age 3-5). Six of the 18 residents were adult females, with a seventh non-resident female with calf occasionally

[5] The project was funded by the Swan River Trust and conducted in 2009.

[6] The exception is the Cockburn Sound dolphin 'Hook', who was known to approach boats for food hand-outs (Finn 2005; Finn et al. 2009; Donaldson et al. 2010). Hook was observed exhibiting this 'begging' behaviour once in Melville Waters and twice in the Inner Harbour.

[7] Data on the patterns of association for the Swan residents is also available, but these data are largely limited to the interactions with Swan dolphins; there are only a limited number of observations of Swan dolphins in areas outside of estuary in which non-Swan dolphins are present (see Lo 2009).

observed in the Inner Harbour and lower reaches (i.e. Inner Harbour to Blackwall Reach).[8] The number of calves ranged from two to five during the study (for the six resident females). Thus, the overall number of resident dolphins ranged from 20 to 23, depending upon the number of calves present. We refer to the dolphins identified as residents as the 'Swan' dolphins.

3. Resident community

We consider the 18 resident dolphins to constitute a resident dolphin **community**. The term community is used to describe resident populations (or sub-populations) of inshore dolphins that exhibit: (1) distinctive ecological characteristics (e.g. use of particular prey, foraging tactics, or habitats); (2) closed or partially-closed patterns of association; and (3) natal philopatry (i.e. retention of maternal home range) (Wells et al. 1999; Rossbach and Herzing 1999; Connor et al. 2000a). Members of a community typically have over-lapping home ranges and share a consistent linkage to a general area. Wells et al. (1999) define a community of cetaceans as:

> a regional society of animals sharing ranges and social associates, but exhibiting genetic exchange with other similar units (populations are closed reproductive units). [p.354]

We use the terms *resident* and *community* in the specific way that they are used in the scientific literature to describe the social ecology of cetaceans. The term 'resident' implies that dolphins exhibit site fidelity to the Swan Canning Riverpark and to the adjacent coastal areas that they also range within. The term 'community' means that members of the community share home ranges and associates (i.e. relationships with other dolphins).[9]

The characterisation of the resident community is based on observations indicating: (a) shared ranging patterns and site fidelity for these 18 individuals, and (b) ecological differences between these 18 individuals and other dolphins observed in the waters between Fremantle and Rockingham, and is supported by long-term study of bottlenose dolphins in Cockburn Sound as well as other long-term studies of bottlenose dolphins within estuarine and coastal ecosystems. The resident community is also likely to serve as a useful management unit for conservation purposes (see also later in this section). In their review of bottlenose dolphin ecology, Connor et al. (2000) note that:

> Our understanding of bottlenose dolphins at many coastal sites supports the consideration of social relationships along with ranging patterns to provide for the identification of population units that can form the basis for management. In Florida, patterns of association facilitate the partitioning of continuously distributed resident bottlenose dolphin populations into biologically meaningful, geographically based management units (e.g. "communities": Wells 1986). [p.125]

We do not know how many dolphins are currently resident within the Swan Canning Riverpark, or what proportion of the 2001-3 community is still present, as photo-identification efforts since 2003 have not been sufficient to document all individuals present within the Riverpark or to establish re-

8 Sex and age-class was confirmed for these seven females based on consistent observations of a calf accompanying them.

9 We lack sufficient data on bottlenose dolphin population genetics for the Perth metropolitan area to characterise genetic exchange and population structuring (see further in discussion of preliminary genetics findings).

sighting patterns. However, the current abundance of resident dolphins is likely to be similar to (or less than) the 2001-3 estimate of 20-25, given: (a) the low reproductive rates of bottlenose dolphins and (b) stranding records indicating at least 11 dolphin deaths have occurred within the Riverpark since late 2003 (including the six 2009 deaths). The strong site fidelity (natal philopatry) of inshore bottlenose dolphins also suggests that few dolphins are likely to migrate into the Swan Canning Riverpark from other areas.

C. Distribution & movement patterns

1. Distribution

The 2001-3 study focused on the Inner Harbour, lower reaches, and basin (middle reaches) areas of the Swan Canning Riverpark, with occasional work in the Canning River and infrequent survey effort beyond Perth Waters. Figures 5 shows the locations of dolphin sightings from this study, and indicate that dolphins will use almost any habitat within the Swan Canning Riverpark, ranging from man-made features (e.g. Inner Harbour, boat mooring areas, pens at yacht clubs, rock walls) and naturally-occurring habitats, such as shallow nearshore areas, patches of rocky reef, areas of rapid change in bathymetry (slope/edge), and deep (>10m) basin areas. Initial analyses of these data suggest that areas of foraging concentration do occur and vary seasonally, and habitat use varies spatially (i.e. activities such as resting tend to occur in specific areas) (Moiler 2009; Figure 6). Little resting appears to occur within Swan Canning Riverpark, aside from in the Inner Harbour and occasionally in Melville Waters.

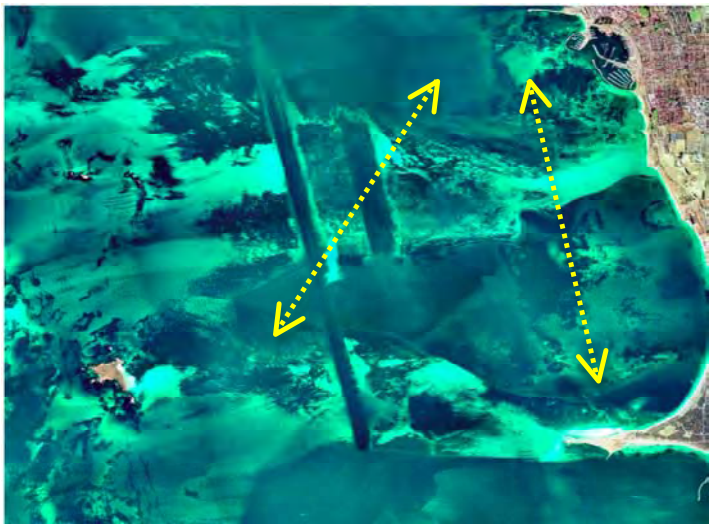
As the 2001-3 research was mainly restricted to lower and middle reaches of the Swan Canning Riverpark, observations on the use of the Canning River and the upper reaches of the Swan River are anecdotal or speculative. The Dolphin Watch project provides an expanding source of observations for these areas.

2. Movement patterns

Focal follows and sighting data indicate that resident dolphins ranged both within the Swan Canning Riverpark and in marine areas adjacent to the estuary (principally Owen Anchorage and Parmelia and Success Banks), with occasional observations within the northern reaches of Cockburn Sound.

The resident dolphins appear to use the Swan Canning Riverpark on a daily (or near-daily) basis, with usage linked to tidal state. Dolphins may reside within the estuary for several hours (and sometimes longer) before moving back out into marine areas. The proportions of time spent in each environment (estuarine vs. marine) are not known. Anecdotal observations suggest that about a tidal cycle is spent in each environment, but this requires analyses of focal follow data to confirm.

These ranging patterns appear unique based on research on dolphins in the northern and southern metropolitan waters of Perth (see Waples 1997 and Finn 2000). For example, dolphins considered resident within Cockburn Sound largely range within Cockburn Sound (Finn 2000).



The arrows in the image to the left are indicative of the ranging patterns of dolphins when outside of the estuary. After leaving the Inner Harbour, dolphins may range south of Fremantle and into Success Bank, Owen Anchorage, Parmelia Bank, and occasionally as far as the northern end of Cockburn Sound.

Fishing Boat Harbour is at the top right, Carnac Island to the lower left, and Woodman Point is to the lower right.

Tidal changes within the Swan Canning Riverpark are (with certain exceptions) incremental, predictable, and likely to be perceived some distance away from the estuary, factors that suggest dolphins will tend to use the estuary during tidal states that are optimal for fish availability and for locomotion (e.g. travelling upstream on an incoming tide as fish are flushed from shallows into deeper waters). Re-sighting patterns from 2001-3 indicate that the resident dolphins use the estuary year-round. Re-sighting patterns for dolphins in Cockburn Sound suggests that the dolphins considered resident in the Swan Canning Riverpark are likely to exhibit long-term site-fidelity to the Swan Canning Riverpark and the adjacent marine areas they range in (Finn 2005, Ham 2009).[10]

Focal follow observations indicate that dolphins spend considerable time within the Inner Harbour, and dolphins forage, rest, and socialise within the Inner Harbour area, particularly towards the northern sections of the Inner Harbour. When travelling through the lower reaches of the Swan Canning Riverpark (East Fremantle to Blackwall Reach), dolphins tend to range along the edges, particularly when actively foraging, and pass through boat pens and mooring areas and in shallow fringing habitats. This pattern of movement may reflect the abundance of fish in areas with some form of structure, but may also indicate a response to boat traffic. In basin areas upstream of Blackwall Reach, dolphins will often travel along 'edge' areas (areas with a sharp change in bathymetry), but will feed in habitats as varied as Point Dundas, the *Halophila* seagrass areas around Milyu, and the shallow shoal areas just west of the freeway. Movement patterns seem to link particular foraging 'hotspots' together, and these locations may represent particular habitat features that concentrate fish and/or support seasonal concentrations of fish (e.g. for spawning).

Survey effort in 2001-3 was limited for the Canning River and the upper reaches of the Swan River,

10 Finn (2005) and Ham (2009) present photo-identification showing long-term site fidelity for dolphins within Cockburn Sound, with some dolphins first observed in 1993 still present in 2008. Connor et al. (2000) provides a discussion of the ecological characteristics of bottlenose dolphins within nearshore ecosystems, including site fidelity and natal philopatry. While acknowledging the lack of long-term individual-specific sighting data for dolphins in the Swan Canning Riverpark, the findings from Cockburn Sound and other locations indicate that the dolphins considered resident within the Swan Canning Riverpark in 2001-3 are likely to be long-term residents.

and the ranging patterns of dolphins in these areas are not well-known. However, the Canning River was frequented consistently between 2001-3, and focal follows observations tracked dolphins moving as far as the Shelley Bridge. Dolphin Watch observations indicate they range to the Kent Street weir and occasionally further upstream (when the weir boards are not in place). In the Swan River, focal follow observations also tracked dolphins moving as far as the Belmont/Ascot ski area, with observations form Dolphin Watch and other observers indicating that dolphins occasionally range as far as Caversham. Focal follow observations suggest that, in general, dolphins range upstream about as far as Perth Waters and/or somewhere in the Canning River during the course of a tidal cycle, and then often turn around and head back downstream. The 2001-3 observations suggest that use of the upper reaches of the Swan (i.e. beyond the Windan Bridge) may be limited, with one hypothesis being that only a handful of individuals range much further than Maylands.

D. Activity patterns and group size

Foraging or travelling was the predominant activity for dolphins observed in the Swan Canning Riverpark, based on behavioural survey data (Table 2). The two activities are often linked, with dolphins typically foraging for fish while travelling (i.e. moving in a roughly linear direction upstream or downstream) or vice versa (i.e. mostly moving directionally but also obviously searching for fish given dive patterns). In these situations, one activity state is considered the predominant activity (>50% of individual's time during a five minute scan sample), while the other activity (or activities) is considered a supplementary activity (<50% of individual's time during a five minute scan sample). These findings suggest that dolphins use the Swan Canning Riverpark mostly as a feeding habitat and, along with the distribution data for different activities, indicate that certain activities (e.g. resting) may typically occur during the time period when dolphins are outside the estuary.

Group size for behavioural surveys ranged from 1-13 (mean group size = 2.81 dolphins, excluding calves) and varied with activity [one-way ANOVA (α=0.05), $p<0.001$, $F=29.227$) (Figure 7). Group size also varies seasonally in the Inner Harbour, perhaps reflecting seasonal patterns in the presence of non-Swan individuals within the Inner Harbour.

E. Foraging ecology

Little is known about prey selection of dolphins within the Riverpark, but focal follow and behavioural survey observations (as well the range of habitats foraged within) suggest that dolphins consume a broad range of prey, including mullet, whiting, herring, cephalopods, juvenile mulloway, and bream. The relative importance of marine vs. estuarine prey is unclear.[11] Data from 2001-3 indicates that foraging was the predominant activity for dolphins within the Swan Canning Riverpark, and suggests that a substantial proportion of the time that the resident dolphins spend within the estuary is devoted to foraging. There are also likely to be seasonal changes in prey availability and distribution, and such shifts will affect the movement patterns of dolphins within the Swan Canning Riverpark.

[11] This question could be considered through on-going stable isotope work in the Swan Canning Riverpark, further comparison of the stable isotope signatures of Swan residents with dolphins from other areas, and use of fatty acid analyses based on prey species fatty acids signatures.

F. Genetics

1. Introduction

Genetic analysis in 2009 provided a preliminary species identification of the Swan dolphins by comparing their mitochondrial DNA sequences to published sequences of known dolphin species. A key objective of this work was to determine whether the dolphins resident in the Swan Canning Riverpark were *Tursiops truncatus* (Common Bottlenose Dolphin) or *Tursiops aduncus* (Indo-Pacific Bottlenose Dolphin). *T. truncatus* and *T. aduncus* are considered the oceanic and the coastal species of bottlenose dolphin within southern Australian waters (respectively), although this schema has been the subject of some controversy (e.g. Moeller et al. 2008). The distribution of the two species partially overlaps in southwestern Western Australia, but the two species have differing ecologies and morphologies.

2. Methods

DNA was extracted and sequenced from tissue samples of 30 dolphins from southwestern Western Australia (*n* = 13 dolphins samples within the Swan Canning Riverpark; *n* = 27 from other locations). Sample 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 constructed in Geneious by Neighbour Joining (NJ) algorithm based on Tamura-Nei genetic distance. The 27 samples from the other locations in southwestern Western Australia were also analysed to provide a preliminary investigation of the relationship of the Swan dolphins to their neighbours.

3. Findings

The Swan individuals (*n* = 13) analysed represented a total of seven mitochondrial haplotypes (Figure 8). One was particularly common (SW Haplotype 8), and was present in seven of 13 individuals (54%). The other six haplotypes represented a single individual each. Four haplotypes (4, 7, 8 and 9, representing ten individuals) can be tentatively identified as Indo-Pacific bottlenose dolphins (*T. aduncus*) based on a phylogenetic analysis of DNA sequence data (Figure 8). 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*, or Indo-Pacific bottlenose dolphins. 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.

Seventeen individuals were analysed from other locations in southwestern Western Australia: [Cockburn Sound (*n* = 3), Rottnest Island (*n* = 2), Mandurah (*n* = 3), Bunbury (*n* = 6), Busselton (*n* =

2) and Augusta (*n* = 1)]. Two of the seven Swan haplotypes were shared with the wider southwestern Western Australia population. Haplotype 8 (the common haplotype, *T. aduncus*) was present in two individuals sampled in Cockburn Sound and Bunbury. Haplotype 1 (present in one Swan individual, which grouped loosely with *T. truncatus*) was the most common haplotype seen in the wider southwestern Western Australia samples, and was present in samples from Rottnest Island, Mandurah, Busselton and Bunbury. The remaining five haplotypes found in the Swan dolphin were not seen in the wider southwestern Western Australia populations.

The sharing of two out of seven haplotypes, particularly the presence of the common Swan haplotype (8) in the adjacent Cockburn Sound and in Bunbury, some 170 km away, suggests that there is gene flow between populations along the southwestern coast. Five of the seven haplotypes were unique to Swan dolphins, but little can be inferred from this or of the comparative haplotype frequencies until a greater number of southwestern Western Australia samples have been analysed.

G. Trophic relationships

1. Introduction

Stable isotopes have been used extensively to investigate the diet of dolphins and other species (e.g. Jennings et al. 1997; Svensson et al. 2007, Barros et al. 2009). 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% in aquatic systems, a characteristic that makes them indicative of trophic level (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Minagawa and Wada 1984).

2. Methods

We sampled 15 bottlenose dolphins from the Swan Canning Riverpark (*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 microtiter 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).

3. Findings

The findings should be considered with caution given the small sample sizes involved (particularly for Cockburn Sound and Rottnest Island), and are presented here as indicative of hypotheses that further research could address. The stable isotope ratios for the dolphin samples ranged from -21.3 to -15.6 for carbon and from 12.0 to 18.6 for nitrogen (Table 3; Figure 9). These findings suggest that dolphins from the Swan are feeding at a higher trophic level than those individuals from Cockburn and Rottnest, respectively (Figure 9). However, this finding could be related to longer or more complex food chains existing in the estuary than in marine waters outside the estuary. If confirmed, this finding differs from that of Barros et al. (2010), which reported lower nitrogen ratios for individuals from an estuary than for those in marine waters off the coast of Florida. It is also possible that this finding reflects a situation in which the dolphins are all feeding on the same fish species in the three different areas, and this species of fish feeds at different levels of the trophic cascade depending on the area that it is found in. The findings are preliminary, and we suggest caution in interpretation of results. There are some data available on C and N levels in dolphin prey species that are likely to be shared between locations (e.g. mullet), and this information may assist in further analyses and interpretation of the data.

The range of carbon ratios was greatest in the Swan River dolphins, while it was narrowest in individuals from Rottnest Island (Table 3; Figure 10). This finding suggests the possibility that Swan dolphins may feed on a greater variety of foods, and/or that there are more primary producers with differing carbon ratios fuelling the food web in the areas in which they feed. The range of carbon sources for Rottnest Island individuals was very narrow, suggesting similar food sources for those 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 11). This tissue seems to be metabolically more inert than skin and therefore retains the heavier nitrogen isotope.

Evaluation of carbon and nitrogen stable isotope ratios was undertaken as part of a wider study of trophic dynamics in the upper Swan River around Guildford (T. Linke, Murdoch University, unpublished data), encompassing a broader range of organisms. When the average values for $\delta^{13}C$ or $\delta^{15}N$ of the Swan dolphins are compared to those of other consumers (Figure 12), 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. We postulate that some marine fish species that occur opportunistically in estuaries, are a source of carbon for the dolphins, as for example the values for *Aldrichetta forsteri* (yellow-eye mullet), *Cnidoglanis macrocephalus* (estuary cobbler) and *Atherinomorus ogilbyi* (Ogilby's hardyhead) ranged from -16.6 to -15.9 (Hyndes and Lavery 2005). The dolphins could be feeding on these (and

other fish species) that migrate into the estuary from marine waters, or the dolphins may be feeding outside of the estuary.[12]

H. Conservation biology

1. Life history

The life history of bottlenose dolphins has important conservation implications. A summary of life history trait values for bottlenose dolphins is given in Table 4. These parameter values are for the *Tursiops truncatus* found in the Gulf of Mexico and western North Atlantic, and are likely to vary between species/morphotypes and locations. Life history data obtained from a long-term study of bottlenose dolphins in Shark Bay, Western Australia found that: (a) the age of weaning ranged between 2.7 – 8.0 years with 66% of calves weaned by their fourth birthday; (b) the inter-birth interval ranged from 3 to 6 years, (mean = 4.55 years); and (c) calf mortality was 44% by age 3 (Mann et al. 2000).

These life history traits are associated with limited individual reproductive potential. Delayed sexual maturity, small litter size (n = 1 calf), a long gestation period (~12 months), and an extended period of offspring dependence all constrain the reproductive rates of females across their lifespan. Infant mortality rates are high for bottlenose dolphins, and females typically require multiple reproductive episodes to replace themselves. Recruitment of juveniles into populations is limited by the long period of calf dependence and by the extended adolescence, and adequate levels of recruitment rates require high survival rates of offspring through the juvenile and sub-adult period.

These factors also make for low intrinsic rates of population increase, and mean that populations of bottlenose dolphins are poorly equipped to compensate for processes that cause significant adult mortality. The intrinsic rates of increase for populations of small cetaceans are low (less than 5%) even in ideal conditions (Perrin and Reilly 1984, Reilly and Barlow 1986, Barlow et al. 1995, Meffe et al. 1999). Once population decline is initiated, populations are limited in their biological capacity to recover even if pressures are reduced.

2. Ecological variation of bottlenose dolphins within Perth metropolitan waters

The ecology of bottlenose dolphins around Perth varies and reflects the type of ecosystem that they inhabit (Waples 1997, Cannell 2004, Finn, 2005, Finn and Calver 2009). Waples (1997) found that dolphins in the northern metropolitan waters of Perth ranged over large areas, and typically exhibited only short-term residency within particular habitats. In contrast, dolphins in Cockburn Sound and the Swan Canning Riverpark exhibited long-term residency patterns and had small home ranges (R. Donaldson, Murdoch University, unpublished data; Finn 2005, Ham 2009, Lo 2009). This ecological

[12] Further study of trophic relationships would clarify areas of uncertainty about the foraging ecology of dolphins within the Swan Canning Riverpark and other areas, and to test the observations discussed above. This could be done through sampling of dolphins from a broader range of locations and in larger sample sizes. These data could be coupled with existing datasets for trophic structures in the Perth metropolitan area, and supported by additional sampling of potential prey species (and other ecosystem aspects). Fatty acid analysis is another technique that could be used and would support better resolution of diet (i.e. prey species consumed). We are also assessing the feasibility of coupling these trophic analyses to analyses of contaminant concentrations in prey species, so as to better understand the bioavailbility of contaminants.

variation likely reflects habitat differences between sheltered estuary (Swan Canning Riverpark) and embayment (Cockburn Sound) ecosystems, and the northern waters of Perth, which are characterised by a mixture of swell-exposed and protected areas of rocky reef and sandy beaches, with seagrass habitats intermixed.

3. Population structuring

These patterns of ecological variation may reflect some degree of population structuring for bottlenose dolphins. Research from other areas indicates that population structuring in bottlenose dolphins can occur over small spatial scales within coastal and inshore ecosystems (Hohn 1997, Wade and Angliss 1997, Barros and Wells 1998, Gubbins 2002, Hubard and Schwartz 2002, Waring et al. 2003, Krützen et al. 2004), and that ecological differences (e.g. variation in residency and ranging patterns and prey preference) underlie this population structuring (Hoelzel 1998; Hohn 1997, 2002; Defran and Weller 1999; Defran et al. 1999; Connor et al. 2000; Hubard and Schwartz 2002). Further, population structuring can occur within inshore populations of bottlenose dolphins that are continuously distributed (Wells et al. 1999, Connor et al. 2000, Krützen et al. 2004, Moller and Beheregaray 2004).[13] Such structuring could reflect a matrix of over-lapping individual home ranges or the existence of relatively discrete sub-populations.[14] Fragmented coastlines with complex physiographic features may support the development of resident communities that are associated with particular areas, typically sheltered areas such as bays, sounds, estuaries, and embayments (Wells et al. 1980, Wells et al. 1999, Connor et al. 2000).

4. Resident communities

Three factors indicate that the dolphins classified as resident in the Swan Canning Riverpark constitute a distinct community:

(a) **ecological differences** between the Swan dolphins and dolphins in other areas of the metropolitan waters of Perth, including well-studied areas such as Cockburn Sound (Waples 1997, Cannell 2004, Finn 2005);

(b) the relatively **closed association network** of the resident dolphins, at least for periods when dolphins are within the estuary (Lo 2009);[15] and

[13] The population structuring of bottlenose dolphins—in areas where they have been intensively studied—typically exhibits a complex layering effect in which discrete morphotypes (e.g. inshore, coastal, and offshore forms) may partially overlap. In addition, these morphotypes may be further divided into constituent populations or sub-populations that show varying degrees of genetic or demographic isolation. Within the precautionary approach of the U.S. *Marine Mammal Protection Act* 1972, the designation of management units is conservative to recognise this layering effect (Hubard and Swartz 2002). For example, thirty-three separate stocks of bottlenose dolphins have been proposed for the Gulf of Mexico (Waring et al. 1999). Three distinct bottlenose dolphin morphotypes are recognised along the Atlantic seaboard with the population sub-structuring of the coastal morphotype the most well-known. Seven distinct management units exist are currently recognised for the coastal morphotype based on photo-identification, telemetry, stable-isotope, and genetic studies (Hohn 1997, NMFS 2001).

[14] The term *sub-population* refers to a group of animals that, while part of a larger overall population, are considered as a distinct unit for management reasons (e.g. the sub-population is the group animals ranging within a particular geographic area such as a marine park) and/or for biological reasons (e.g. the sub-population encompasses a resident community that may be demographically isolated). See also Simpson & Holley (2005).

[15] Lo (2009) conducted an initial analysis of the social structures of the dolphins considered resident in the Swan Canning Riverpark based on 402 behavioural surveys of individually-identified dolphins.

(c) the **long-term site fidelity** of individuals, based on resighting patterns from 2001-3 (and to a lesser extent from 2001-3 to 2008-9) and knowledge of bottlenose dolphin ecology from Cockburn Sound, Shark Bay, and other locations (Connor et al. 2000, Finn 2005, Finn and Calver 2009).

Further research could also examine whether the Swan dolphins have unique **trophic and contaminant signatures,**[16] and resolve whether unique **haplotypes** occur among the Swan dolphins.

Inshore communities have been proposed as an appropriate management unit because of their consistent ecological association with small and defined areas of habitat, and the potential for these communities to be demographically isolated (Connor et al. 2000). While the characteristics used to identify resident dolphin communities are behaviour-based and allow for the possibility of genetic exchange between communities (Wells et al. 1999), they do indicate the potential for some degree of demographic isolation to occur. For example, demographic isolation can occur if individuals fail to disperse from their natal habitat because of strong ecological linkages to that habitat (e.g. 'local' knowledge of food sources provides a strong selective advantage) (Sutherland 1998, Connor et al. 2000, Gill et al. 2001). Demographic isolation reflects limited rates of migration between adjacent populations/areas. Should demographic isolation occur, localised depletions may not be mitigated by the immigration of individuals from adjacent populations/areas. These considerations suggest that the resident community in the Swan Canning Riverpark should be considered as a distinct management unit until further research can resolve the extent to which demographic isolation occurs.[17]

5. Ecological and demographic vulnerability

The ecology of resident inshore dolphins enhances their vulnerability to habitat change caused by anthropogenic processes (e.g. agricultural and industrial development), climate change, or other factors (Bannister et al. 1996, Connor et al. 2000, Finn 2005). Limited ranging patterns, year-round residency, and long-term site fidelity suggest that the resident communities are strongly influenced by the ecological condition of the coastal and estuarine ecosystems that they inhabit (Finn 2005). These characteristics also emphasise the potential for sustained impacts to exert a cumulative effect on individuals (Bejder et al. 2006a,b).

This ecological vulnerability is matched by an innate demographic vulnerability (as discussed above). The low reproductive potential of dolphins and typically small population size of resident populations

16 This speculation is based on findings from the stable isotope findings in the 2009 SCRIP study and comparison of contaminant concentrations within tissues from dolphins from the Swan Canning Riverpark and from the Bunbury area (see Section VII – Contaminants). The stable isotopes findings are suggestive only as there are based on a small sample size. Studies of the presence of organic contaminants within Cockburn Sound (DEP 1996) and the Swan Canning Riverpark (Nice 2009, Nice et al. 2009) suggest the potential for differences to occur in the concentrations of organic contaminants within the biota inhabiting these areas. The concept of trophic and contaminant 'signatures' could relate to: (a) carbon and nitrogen isotope ratios; (b) the presence of certain contaminants; and (c) the concentrations (or ranges of concentrations) for certain contaminants. These signatures, if they occur, would presumably reflect the use of different prey species and environments.

17 Genetic isolation is unlikely given that dolphins from the resident community also range outside of the Swan Canning Riverpark. However, the potential for natal philopatry suggests the potential for matrilines (female lines of descent) to occur, and this hypothesis could be addressed through mtDNA analysis.

afford these populations little compensatory capacity for impacts affecting demographic processes (i.e. birth and mortality rates). Dolphin populations are capable of only small rates of increase and are dependent upon relatively high rates of adult and juvenile survivorship (Perrin and Reilly 1984, Reilly and Barlow 1986, Meffe et al. 1999). Mortality attributable to natural factors (e.g. predation, disease, malnutrition) may account for 30-50% of calves by the time of weaning (Connor et al. 2000; Mann et al. 2000). The reproductive success of adult females underlies the persistence of populations because their reproductive output (as measured by the number of calves which survive to weaning and the time interval between births) directly influences demographic parameters (Dunbar 1988, Gerber and Heppell 2004). Where demographic isolation occurs, the persistence of resident populations may be contingent on the reproductive success of a small number of females (Hale 2002). Thus, the loss of several females or small increases in calf or juvenile mortality rates can exert a biologically significant influence on community/population-level processes.

IV. Health - Factors affecting the health of estuarine dolphins

> Marine mammals strand for a variety of reasons. Some identified causes include: infectious disease (e.g., viral, bacterial, parasitic), non-infectious disease (e.g., stress, starvation, exposure to biotoxins or contaminants), physical trauma (*e.g.*, ship strikes, entanglements, predation, acoustic sources), behavioral changes (e.g., associated with prey shifts, social cohesiveness), weather and oceanographic conditions (e.g., hurricanes, tsunamis, El Nino), to name a few (Geraci et al. 1999, Dierauf and Gulland 2001, Geraci and Lounsbury 2005).
>
> Lecky (2006)

A. Review of factors affecting dolphin heath

This section reviews the factors potentially affecting the health of dolphins within the Swan Canning Riverpark, and provides background information needed to understand the 2009 mortalities: (1) as **individual pathology cases** with their own particular clinical presentations and possible aetiologies, and (2) as an **unusual mortality event** comprised of a series of mortalities that occurred within the Riverpark over a five-month period and in two separate clusters (i.e. June and September/October).

We note two considerations relevant to this review. Firstly, research from 2001-3 indicates that dolphins considered resident within the Swan Canning Riverpark split their time between the estuary and adjacent marine areas, and thus are potentially affected by stressors from both these environments. Secondly, the Swan Canning Riverpark has much in common with other nearshore and estuarine environments that experience intensive human use and some degree of seasonal environmental variation. Such areas include: Cockburn Sound, the Peel-Harvey Estuary, and the inner waters around Bunbury (Leschenault Inlet, Leschenault Estuary, Bunbury Inner Harbour, Bunbury Outer Harbour and Collie River). All of these areas provide broadly similar suites of health challenges to dolphins.

1. Defining Health

The concept of an unusual mortality event implies that some factor, or suite of factors, is present and has caused a perturbation from normal or 'background' rates of mortality for the population in question. This perturbation implies that certain individuals in the population died that would have remained living had the factor (or factors) not been present. In this sense, an unusual mortality event can be seen as a departure from a condition of 'health' at both the individual and the population-level. Since, in some cases, the presence of a mortality factor relates to environmental factors, it can also be useful to view unusual mortality events as, in some cases, a departure from health at an ecosystem-level.[18] Thus, an investigation into an unusual mortality can approach the idea of what constitutes 'health' along at least three levels of analyses:

[18] These factors could include harmful algal blooms, high concentrations, of chemical contaminants, and human activities that alter (e.g.) prey availability or cause stress to dolphins. Ecosystem-level effects may also include adverse environmental conditions, but it should be recognised that oceanographic conditions within estuarine and coastal ecosystems vary naturally, and so should not be understood as representing an 'unhealthy' state unless, however, the conditions are human-related.

(a) **individual** [the organism]: a state of physical well-being in which an organism's physiological functioning is optimal and its reproductive capacity is unimpaired [19];

(b) **population** [a group of individuals occurring in an area]: a population having the size, demographic structure, and productivity necessary to allow the population to serve as a functioning component of the surrounding ecosystem and to provide for population's long-term viability[20]; and

(c) **ecosystem** [the biotic and abiotic environment to which a population belongs]: an ecosystem having the structural components and functional processes necessary to sustain populations of endemic species (Harwood and Hall 1990, Harwell et al. 1999, Schaeffer et al. 1999, Bossart et al. 2001, Wells et al. 2004, Bossart 2006, Gulland and Hall 2007, Van Bressem et al. 2009a).

These definitions can be applied to evaluate the health of dolphins in the Swan Canning Riverpark. 'Health' in this context would mean that:

(a) the **resident dolphins** experience mortality and health challenges at sufficiently low levels that female reproductive success (including juvenile survivorship) is adequate for females to at least replace themselves within the population;

(b) the **resident community** is sufficiently large enough to be able to compensate for periodic spikes in mortality (whatever the cause) and avoid population extinction; and

(c) the **estuary ecosystem** sustains levels of prey availability adequate to sustain a viable population and does not possess features causing (or contributing to) excessively high rates of mortality.

2. Population-level indications of health

The individual animal forms the basic unit of pathologic analysis and the individual-level effects of stressors are reviewed below. However, applied pathologic studies (e.g. population health assessments, epidemiological studies, stranding investigations) are generally concerned with why states of ill-health occur within certain groups of animals (i.e. a group that has mass-stranded, a population, or a species). At a population-level, potential signs of poor health include:

* presence of a **primary pathogen** associated with mass mortalities of marine mammals (e.g. cetacean morbillivirus);

19 See, for example, the definition of health in Blood & Studdert (1988): "a state of physical and psychological well-being and of productivity including reproduction" (p. 432).

20 The U.S. *Marine Mammal Protection Act* 1972 maintains these parameters as specific management objectives, stipulating that marine mammal stocks should be managed so that each stock is maintained at or above its "optimum sustainable population" and that species are kept as functioning elements of the ecosystems that they inhabit.

- evidence or indications of systemic **immunosuppression** (e.g. lymphoid depletion or reduced immune function) across a range of individuals; [21]
- high rates of **opportunistic (secondary) infection** by bacterial and fungal pathogens;
- high rates of **parasitism**;
- prevalence of **epidermal disease**, such as tattoo skin disease (TSD) occurring in individuals of all age-classes, including adults and calves;
- **diseases with unusual manifestations**, e.g. the presence of a novel or more virulent form of a pathogen (**emerging infectious disease**);
- high rates of **human-induced injuries**, such as entanglements and boat strikes; and
- high concentrations of **contaminants**, which while not representing an indication of poor health by themselves, have nonetheless have been associated with harmful health effects, including reproductive and immune system impairment.

3. Stressors & health effects

Stressors are processes that affect the health of dolphins in some way and may cause, or contribute to, injury, morbidity, or mortality. At an **individual-level**, these effects result in a deviation from normal physiological health/homeostasis, and include[22]:

- **mortality** (i.e. a process causing mortality directly, e.g. a fatal shark attack);
- **physical trauma** (e.g. a wound such as a laceration, avulsion, or amputation);
- **reduced condition** (e.g. emaciation, fat store depletion);
- **systemic physiological stress** (e.g. elevated production of stress hormones);
- **physiological injury/impairment** (e.g. hearing damage);
- **immune system impairment** [e.g. lymphoid depletion; impaired immune function (particularly cell-mediated and humoral immunity)];
- **reproductive impairment** (e.g. reduced fertility, embryo toxicity, abortion/stillbirths);
- **epidermal lesions** (e.g. tattoo skin disease);
- **neurological impairment** (e.g. brevetoxicosis); and
- **internal lesions** (e.g. pneumonia). [23]

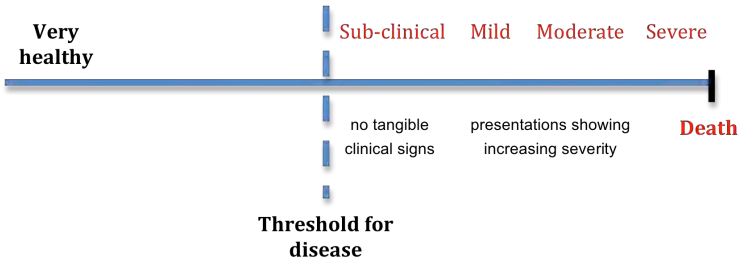
Conceptually, the state of health for an animal can be viewed as lying somewhere along a continuum that runs from very healthy (i.e. absent of disease, good body condition) to death, with a threshold for disease somewhere in between (see figure below). Individuals to the left of the threshold are absent of

[21] Immunosuppression refers to the suppression (i.e. compromised) of the normal immune function of an individual, and may involve dysfunction of adaptive and/or innate immunity. Causes of immunosuppression include primary pathogens (e.g. morbillivirus), contaminants, and environmental stress.

[22] Starvation is not discussed here, but low prey availability or factors causing diminished ability to forage and feed effectively are obvious sources of stress that can have adverse health effects (and lead to death). Juvenile animals are particularly vulnerable because of more limited foraging experience, less developed foraging skills, and other factors.

[23] The definition of *lesion* used in pathology (and biomedical science generally) differs from how the term is used in other contexts. Blood & Studdert (1988) define the term as: "any pathological or traumatic discontinuity of tissue or loss of function of a part. Lesion is a broad term, including wounds, sores, ulcers, tumours, cataracts and any other tissue damage. They range from skin sores associated with eczema to the changes in lung tissue that occur in tuberculosis" (p.529).

disease, while animals to the right have presentations of disease varying from sub-clinical to severe. Animals in generally poor health may often lie somewhere near this threshold, e.g. because they are affected by a suite of chronic stressors or because that have recently experienced a stressor such as an injury or recurrent infection. These individuals may thus only need a small change in the suite of stressors affecting them in order for a secondary infection to occur (or an existing infection to grow more severe). This emphasises why most instances of disease should be considered as deriving from a suite of factors affecting the health of an animal (i.e. a multi-factorial aetiology), as many stressors may (over time) interact to result in an infection by a pathogen or to make the presentation of the disease more severe.



Disease: finite abnormality of structure or function with an identifiable pathological or clinocopathological basis

The effects of stressors can also be assessed at a **population-level**. Section III (Ecology) indicated that the current resident dolphin community in the Swan Canning Riverpark is likely to be small (about 20-25 individuals); resident (showing long-term fidelity and ecological linkage to the estuary ecosystem); and ultimately dependent upon the reproductive output of a small number of adult females and the recruitment of their offspring into the resident community. These factors emphasise the potential for stressors, either alone or in concert, to exert a population-level impact (i.e. on demographic parameters), should the stressors induce mortality rates above natural background rates.[24]

Background rates of mortality are not known for the Swan dolphin community, but will reflect mortality related to: shark predation, infectious and non-infectious disease, senescence, and agonistic interactions (e.g. male-male fighting). Anthropogenic factors add to background mortality rates, and are characterised as being of **biological significance** if their individual or cumulative effect affects demographic parameters in such a way as to threaten population viability. This threshold of biological significance provides part of the conceptual framework for statutory instruments designed to identify and mitigate anthropogenic mortality on marine mammals.[25]

[24] Stressors are rarely present singly, and instead typically act in concert as multi-factorial causes of morbidity/mortality, making efforts to understand the complex underlying causes or trigger of morbidity/mortality an inherently difficult process.

[25] For example, the United States *Marine Mammal Protection Act* 1972 utilises a concept called Potential Biological Removal (PBR) to provide guidelines for assessing the biological significance of fishing-related mortality for a marine mammal population. The New Zealand *Marine Mammal Protection Act* 1977 uses a similar concept known as Maximum Allowable Level of Fishing-Related Mortality (MALFIRM).

Finally, the effects of stressors can also be understood at the **ecosystem-level** (Figure 13). Estuaries are highly variable ecosystems that typically experience intensive human use. This combination of natural variation and anthropogenic pressure makes estuaries uniquely challenging habitats in terms of the range and the severity of stressors that they may contain. In these environments dolphins may experience rapid and substantial changes in environmental conditions (e.g. salinity, temperature, tidal height) and high concentrations of anthropogenic influence (e.g. urban and agricultural run-off, harmful algal blooms, interactions with vessels and fisheries). For these reasons, estuarine dolphin populations are recognised as being at particular risk from stressors such as infectious disease, physiological stress, human-induced injury, and exposure to algal biotoxins (Van Bressem et al. 2009a). Along the eastern U.S. seaboard, for example, estuarine dolphins have been the most affected by unusual mortality events (Hohn 2002). Certain other coastal ecosystems may also provide similarly challenging environmental conditions, such as Cockburn Sound (Finn 2005).

4. Types of stressors

Potential stressors in estuarine ecosystems may be natural or anthropogenic, and may be further divided into persistent, acute, and lethal stressors:

(a) **Persistent stressors** – Some stressors may cause chronic (i.e. repeated, sustained, long-term) and generalised (i.e. affecting multiple bodily functions) stress affecting the physiology, body condition, immune function, and/or reproductive viability of the animal. Specific effects may include: reduced immunological function; decreased reproductive output; diminished blubber reserves and general body condition; increased susceptibility to opportunistic pathogens and parasitism; and reduced regenerative/healing potential following cell/tissue injury/damage.[26]

Examples of persistent stressors include: toxicants, anthropogenic noise, disturbance from vessel traffic and other factors, environmental conditions[27], depletion of prey stocks (low prey availability), interactions with human activities (e.g. with fishing or tourism operations), chronic infection from a persistent but weakly virulent (i.e. non-lethal) pathogen, and an enduring entanglement injury.

(b) **Acute stressors** – Certain stressors affect organisms over shorter time periods. For example, while a persistent stressor may affect an organism over a period of months to years, an acute stressor will occur over a time-span of weeks, days, and possibly even hours. Examples of acute (short-term) stressors include: infection by a primary pathogen, a severe entanglement injury, and a wound from a shark attack or vessel strike.

[26] Heavy parasitism is an indication of poor health rather than necessarily a cause. However, it will also adversely affect the individual as a persistent stressor in its own right. See Iluko et al. (2010) for a study of lung nematodes in dolphins from South Australia.

[27] These would relate to: (a) prevailing long-term conditions; (b) conditions that persist for shorter periods of time; and (c) variation in conditions, particularly those involving a rapid and substantial change.

(c) **Lethal stressors** – In a few cases, stressors are sufficient to cause death by themselves (e.g. shark attack), or by exacerbating pre-existing conditions in such a way that leads to the death of the animal in a short period of time. [28]

5. Studying the effects of stressors

Pathology investigations may use a variety of methodologies, including: post-mortem examination (gross and histological); ancillary laboratory analyses [e.g. microbiological culture/identification, serum or other body fluid chemistry, serology, immunohistochemistry, biotoxin and contaminants analyses, Polymerase Chain Reaction (PCR) or other molecular techniques to detect/amplify the presence of DNA/RNA (e.g. DNA/RNA belonging to infectious agents or pathogens)]; collection of relevant environmental data (e.g. water quality, presence of potentially harmful phytoplankton); and epidemiological analysis.

Post-mortem diagnoses of marine mammal mortalities generally indicate a multi-factorial aetiology (i.e. several contributing factors) involving cumulative and/or synergistic interactions among multiple stressors. For example, infection by a primary pathogen (such as a virus) may cause immunosuppression leading to secondary infection by bacterial, fungal, or protozoal pathogen(s), which may ultimately culminate in the animal's death. Alternatively, the introduction (on increased severity) of a stressor can aggravate or exacerbate the effects of a pre-existing condition. For example, adverse environmental conditions may cause physiological stress leading to the mobilisation of blubber reserves containing high concentrations of contaminants that are made available when these tissues are metabolised.

Ideally, pathology investigations are coupled with ecological and demographic data so as to support assessments of the biological significance of stressors (or suites of stressors), and determine whether identified health challenges are likely to impinge upon demographic parameters and potentially threaten the viability of populations.

B. Shark predation

Little is known about predation upon dolphins within Perth's metropolitan waters, except that large sharks occur at least periodically around Perth, scars indicative of shark attack wounds are relatively common on dolphins observed in Cockburn Sound and the Swan Canning Riverpark, and fresh wounds indicative of shark attack are occasionally seen (Blackweir 2004, Cannell 2004, Finn 2005, Ham 2009, Lo 2009; Figures 14-16).[29] White sharks (*Caracharodon carcharias*) may follow migrations of humpback whales (*Megaptera novaeangliae*), and thus may occur (or peak in abundance) within the Perth metropolitan area from April-May/June and again in October-November. Tiger sharks

[28] In forensic pathology, the legal term *cause of death* refers to the injury, disease, or abnormality responsible for instigating the sequence of functional disturbances culminating in mortality, either by itself or allied with other factors. The term *mechanism of death* refers to the structural or functional change that makes organisms no longer able to sustain life without artificial intervention. Finally, the term *manner of death* refers to the way in which the cause of death is classified, with particular reference to human agency.

[29] See Heithaus (2001a,b) and Heithaus and Dill (2002) for information on interactions between sharks and dolphins.

(*Galeocerdo cuvier*)[30] and large hammerhead sharks (*Syhyrna* spp.) range within the Perth metropolitan area, and bull sharks (*Carcharhinus leucas*) have historically been present within the Swan Canning Riverpark and are likely to occur at least occasionally within the estuary.

Predation attempts on calves and juveniles are more likely to be successful (i.e. fatal) than attacks on older individuals, as young animals are smaller and lack the strength and experience of more mature animals. Rates of mortality from shark attack are rarely known. Even long-term studies are generally only able to determine rates of disappearance for individuals across different age-classes, as carcasses showing definite signs of shark attack are only occasionally found (and may also reflect post-mortem scavenging).

Dolphins have thick blubber layers and high growth rates for epidermal tissue, factors that often allow them to survive even quite severe wounds (e.g. those breaking through to underlying muscle) and to heal within a period of weeks to months, as long as the wounds are not incapacitating (e.g. limb amputation) or debilitating (e.g. causing damage to internal organs). However, these injuries do present a substantial health challenge, as the wounds: provide an entry point for opportunistic pathogens, cause physiological and possibly osmotic stress, and may expose non-dermal tissue to the environment. In this way, shark attack wounds may be analogous to burns in that the wounds are associated with the removal of layers of skin that otherwise provide a protective physiological barrier to the surrounding environment.

C. Human-induced injury

> A high prevalence of traumatic injuries, even minor skin lacerations, in concert with a compromised immune system create ideal targets for opportunistic pathogens (p. 153).
>
> Van Bressem et al. (2009a)

Human-induced injuries to marine mammals may occur through: entanglement in fishing line or other fishing gear[31]; vessel strike; deliberate harm; or other means (e.g. transfer of disease, feeding of contaminated food) (Wells and Scott 1994, 1997; Wells et al. 1998; Mawson and Coughran 1999; Read and Murray 2000; Stone and Yoshinaga 2000; Finn 2005; Kemper et al. 2005; Wells et al. 2008; Finn et al. 2009; Stockin et al. 2009, Donaldson et al. 2010; Table 5).[32] These injuries cause physical trauma that may kill or injure animals, or lead to infection by opportunistic pathogens.

1. Entanglement

Entanglement in discarded monofilament fishing line is a known cause of injury for dolphins within Perth metropolitan waters (Finn 2005, Donaldson et al. 2010; Figure 18), and is also well-documented

30 See Simpfendorfer et al. (2001) for information on tiger sharks in Western Australia.

31 Most entanglements in the Perth area involve discarded monofilament line (Finn 2005, Donaldson et al. 2010).

32 See, in particular, Kemper et al. (2005), which discusses human-induced injuries within a sample set of 660 records of captured, live-stranded, and dead cetaceans from South Australia between 1881-2000, of which 25% of the deaths between 1985-2000 were classified as human-related.

elsewhere (Wells and Scott 1994; Gorzelany 1998; Wells et al. 1998; Read and Murray 2000; Kemper et al. 2005; Wells et al. 2008; Stockin et al. 2009; Figures 17, 18 & 20). Fishing line may attach itself to an individual when a dolphin: (a) takes a hooked fish and the hook becomes embedded in its mouth; (b) contacts an active (i.e. in use) fishing line and the line becomes wrapped around an appendage; or (c) contacts line that has been discarded (either after breaking while in use or after becoming snagged on an object and being cut).

Regardless of the mechanism of attachment, fishing line may become embedded within or lodged around the rostrum; the trunk of the body ('wrapping' entanglements); the pectoral or dorsal fins; or the tail stock and tail flukes. Once in contact with the body surface, lines often migrate to an appendage and/or a joint, where the material begins lacerating the skin and underlying soft tissues so that the line becomes deeply lodged in place. While fishing line may cause drag directly, other materials often colonise the line surface or become attached to the coils of the line. These materials include: sponges and filamentous algae that settle on the line (or objects attached to the line) and use the line and/or entangled object as a substrate on which to grow; sticks of wood; plastic bags; and strands of seaweed. Once attached, these materials may greatly increase the drag associated with the entanglement. Greater drag increases the energetic cost of locomotion, can interfere with the ability of the individual to forage, and is a source of considerable pain and physiological stress.

Monofilament fishing line is highly resistant to environmental breakdown and has significant tensile strength, making it highly resistant to breakage.[33] As such, line that becomes embedded in tissue tends to remain lodged (rather than snapping off) and, over time, to continue incising through soft overlying tissue (e.g. epidermal, blubber, muscle, cartilaginous tissues). If the entanglement occurs around the dorsal fin, the line may eventually reach the underlying cartilaginous structure and may cause the complete or partial amputation of the fin. In contrast, entanglements around the pectoral fins or tail stock/flukes may eventually encounter the skeletal structure underlying these appendages. When line has incised down to the bone, osteomyelitis (infection of the bone) or amputation are possible outcomes. Amputation (or even lesser injury, depending on the site/severity) of these appendages means the loss of a functional limb (or a reduction in functionality in the case of lesser injuries). Such an injury will cause at least some restriction on an animal's capacity for movement, and may render the individual unable to move or forage satisfactorily.

As well as causing pain and potentially affecting movement, lacerations from entanglements provide a portal of entry for opportunistic pathogens. Secondary infections may remain localised around damaged tissues, or may result in septicaemia/bacteraemia and systemic disease in other body systems/tissues via spread of the pathogen (or toxins elaborated by the pathogen) through the bloodstream. Entanglements can endure for long periods of time. For example, the Swan dolphin that died on 21 June 2009 was observed with this entanglement in mid-2008 (D. Coughran, DEC Senior Wildlife Officer, personal communication).

33 It may take several centuries to degrade in the marine environment.

Long-term observations from Cockburn Sound indicate that entanglement injuries are particularly common among calves (Finn 2005, Donaldson et al. 2010; Table 5). Between 1996-2004, at least six calves within Cockburn Sound experienced entanglement events/injuries (and another calf exhibited injuries indicative of a vessel strike). Calves may be more susceptible to entanglement because they: lack experience with their environment; are more curious about environmental stimuli like fishing gear than older animals; lack physical strength; have less well-developed immune systems and thus are more likely to succumb to secondary infection stemming from entanglement wounds; and have smaller body size, making them more affected by impediments affecting their movement.

2. Vessel strikes

Boat strikes of dolphins do occur, although these appear to be infrequent and are often associated with entanglement or dolphins interacting with humans for food (Wells and Scott 1997, Stone and Yoshinaga 2000, Finn 2005, Donaldson et al. 2010; Figure 19 & 21). In Cockburn Sound, animals with scars indicative of boat strikes have been dolphins known to approach humans for food (Finn 2005, Donaldson et al. 2010). In 2003, an adult female was observed near Fremantle with monofilament fishing line entangled around her tail flukes (making it difficult for her to dive below the surface), and also had a row of parallel scars along her dorsal surface indicative of propeller strike (H. Finn, personal observation). However, vessel strikes can also occur in the absence of entanglements and illegal feeding interactions. For example, in January 2010 an adult female from the Bunbury area died acutely after sustaining blunt force trauma to the thorax. Post-mortem examination suggested a strike by an object such as a vessel (see Section V – Epidemiology).

3. Illegal feeding and deliberate harm

The effects of illegal feeding have been well-documented for dolphins in Cockburn Sound and at other locations in Australia and internationally (see Finn 2005, Finn et al. 2009, Donaldson et al. 2010).[34] Although illegal feeding has not been observed within the Swan Canning Riverpark, 'conditioned' dolphins from Cockburn Sound have been observed within the Riverpark in the past (see Section III – Ecology).[35] In addition, there are anecdotal reports that the adult female that died at the Windan Bridge on 17 September 2009 had been receiving food hand-outs at the Barrack Street jetty prior to her death (Marnie Giroud, Swan River Trust, personal communication).[36]

Illegal feeding affects dolphins in two ways. Firstly, evidence from Cockburn Sound indicates that it places conditioned dolphins at greater risk of boat strike and entanglement (Finn 2005, Donaldson et al.

34 The feeding of dolphins is illegal under the WA *Wildlife Conservation Action* 1950 and associated regulations.

35 The term 'conditioned' refers to the conditioning process involved in dolphins learning to interact with humans for food. This process is analogous to how animals are trained in captive settings.

36 This observation is not intended to draw a causal relationship between the illegal feeding and the death of the dolphin. This dolphin did linger around the Barrack Street for a period of time (at least several days) preceding her death, but severe skin lesions were already present. The most likely explanation for this behaviour is the individual was no longer able to feed herself adequately (because of her degraded condition), and so began seeking food through human interaction. This kind of behavioural change was also observed in an adult female in poor condition in the Port River area near Adelaide (Mike Bossley, Whale and Dolphin Conservation Society, personal communication).

2010). It also places them at risk of deliberate harm, which has been documented around Bunbury, and in South Australia and the U.S. (Bryant 1994, Samuels et al. 2000, Kemper et al. 2005). Secondly, illegal feeding causes substantial behavioural changes in some dolphins and is associated with shifts in ranging and association patterns, as well as clearly abnormal behaviours (e.g. persisting around boat ramps for hours at a time, approaching vessels at anchor) (Finn 2005, Finn et al. 2009).

D. Physiological stress from environmental conditions[37]

Environmental conditions can cause physiological stress in marine mammals, and have been associated with the incidence of epidermal disease and reduced immunological function in marine mammals (Geraci et al. 1979; Wilson et al. 1999; Fair and Becker 2000; Ortiz 2001; Bossart 2003; Walsh et al. 2005; Barry et al. 2008; Reif et al. 2006, 2008; Halvorsen and Keith 2008; Van Bressem et al. 2009a,b; Rowe et al. 2010).

Physiological stress from environmental conditions is generally related to low salinities and/or temperatures.[38] Florida manatees (*Trichechus manatus latirostris*) provide one of the better-documented examples of environmentally-induced physiological stress. Cold water temperatures are considered part of a suite of factors supporting an 'immunosuppression cascade' in this species, and are also thought to cause other health effects ('cold stress syndrome') (Halvorsen and Keith 2008). It is not necessarily the case that only low temperatures are stressful. Some dolphin mortalities in the Gippsland Lakes regions occurred when water temperatures were significantly warmer than usual conditions (K. Charlton, Monash University, personal communication; Appendix A2). Rapid change in water quality parameters could also be a source of stress. For example, osmotic damage to the skin may occur in individuals that are exposed to rapid changes in salinity as when moving rapidly between an area of marine salinity and an area of fresh or brackish water.

Factors that may influence the severity of stress from environmental conditions include:

(a) **intensity** of adverse environmental conditions (e.g. how cold or brackish conditions become);
(b) **duration of exposure** to adverse conditions;
(c) **behavioural factors** that increase exposure (e.g. ranging and residency patterns); and
(d) **presence of other factors** that interact synergistically (e.g. pre-existing infections or injuries).

Environmental conditions able to cause physiological stress are variable in their temporal duration and spatial extent. Stressful conditions may occur rapidly (e.g. runoff from a high rainfall event) or

[37] Other factors may also cause physiological stress including human interactions. See Fair and Becker (2000) for a review of stress in marine mammals. For other aspects of stress and marine mammal physiology and immunology see: (e.g.) Raberg et al. 1998, Svensson et al. 1998, Frohoff 2000, Ortiz 2001, St. Aubin 2002, Clark et al. 2006, and Yeates and Houser 2008.

[38] In the case of salinity, a potentially adverse salinity would be concentrations less than around 20 ppt (g/L), the threshold considered potentially stressful for dolphins kept in captive conditions. In the case of temperature, a potentially adverse temperature would be relative to typical or prevailing conditions for the population in question [given these populations will be adapted (e.g. in blubber thickness) to a particular range and seasonal transition of temperature conditions]. Unusually high temperatures can create physiological stress, culminating (obviously) in point discharges sufficient to cause scalding. Physiological stress may also occur if dolphins are exposed to a rapid and substantial change in salinity and/or temperature. Geraci et al. (1979), for example, noted that skin lesions were first observed on their study dolphins when water temperatures in their captive facility dropped suddenly from 21°C to 3°.

gradually (e.g. seasonal oceanographic shifts), and may involve acute exposure events (i.e. over hours to days) or sustained periods of exposure involving continuous (or at least frequent) periods of immersion over days to weeks/months. Spatially, stressful conditions may be localised (e.g. the upper reaches of an estuary, near to a point discharge outlet) or occur over a broad area (e.g. an El Niño event) (Wilson et al. 1999, Aguilar and Raga 1993, Reif et al. 2006, Rowe et al. 2010). Exposure to adverse environmental conditions may also occur if dolphins become contained within a particular environment for a period of time, either because some environmental feature is present (e.g. the construction of an artificial bar) or for behavioural reasons (Barry et al. 2008).

E. Algal biotoxins

Harmful algal biotoxins have been identified as underlying factors in several unusual mortality events for bottlenose dolphins and other marine mammals in the U.S., with several events showing strong spatial and temporal correlations with harmful algal blooms and high concentrations of biotoxins present in tissues of stranded animals (O'Shea et al. 1991, Bossart et al. 1998, Scholin et al. 2000, Van Dolah et al. 2003, Flewelling et al. 2005). These biotoxins include brevetoxin, a nerve toxin produced by the dinoflagellate *Karenia brevis*, which is more commonly known as the 'red-tide' algae that causes Neurotoxic Shellfish Poisoning in humans (Bossart et al. 1998). Brevetoxins were associated with a die-off of bottlenose dolphins and manatees in the eastern U.S. (Bossart et al. 1998, Trainer et al. 1999, Flewelling et al. 2005). Another biotoxin associated with marine mammal mortalities is domoic acid, which is produced by diatoms from the genus *Pseudo-nitzschia.* In humans, domoic acid induces a syndrome known as Amnesic Shellfish Poisoning (ASP). ASP is typically characterised by gastroenteritis but in severe cases neurological symptoms also appear and death may result. Exposure to brevetoxins and domoic acid (as well as some other biotoxins) may occur through ingestion of contaminated prey (ingestion) or through inhalation. Significant numbers (i.e. sufficient to cause disease) of pathogenic algae were not identified as potential aetiological agents based on routine weekly monitoring of phytoplankton within the Swan Canning Riverpark (see Section V – Epidemiology).

F. Infectious Disease

> Microparasites, including viruses, bacteria, and protozoans, may constrain the growth of wild animal populations (Anderson and May 1979; Anderson, 1982; Fenner, 1983; Gulland, 1995). The intensity of this effect is a function of the heightened mortality rate and/or reduction in reproductive capacity. Thus, microparasites may increase the risk of extinction of small populations in combination with other factors (May, 1986; Thorne and Williams, 1988; Gulland, 1995, Raga et al. 1997).
>
> Van Bressem et al. (2008a)

Infectious disease is recognised as a significant source of mortality for marine mammals within Australian and New Zealand waters (Duignan 2003, Kemper et al. 2005, Stockin et al. 2009) and world-wide (Geraci and Lounsbury 1993, Duignan et al. 1996, Gulland and Hall 2007, Gulland et al. 2008, Van Bressem et al. 2009a). However, we have only limited knowledge of the prevalence and implications of infectious disease in Australian cetaceans despite the long-standing recognition of the

important role epidemiological research can play in conservation efforts (e.g. Bannister et al. 1996). Infectious agents causing disease in marine mammals include viruses, bacteria, fungi, protozoans, and parasitic worms (helminths) (e.g. Higgins 2000, Bossart et al. 2001, Dierauf and Gulland 2001, Buck et al. 2006, Dagleish et al. 2006, Higgins 2006, Tomo et al. 2010).[39]

Recent epidemiological work indicates that, on a global basis, the occurrence of infectious disease may be increasing among marine mammals, and that this increase is associated with a range of factors, including: 'emerging infectious diseases' [EIDs], high concentrations of immunosuppressive contaminants, environmental deterioration, climate-related shifts in species distributions, and other factors (Fair and Becker 2000; Ross 2002; Gulland and Hall 2007; Van Bressem et al. 2008a, 2009a).

Van Bressem et al. (2009) define EIDs as infectious diseases that: "(1) are newly recognized, (2) are evolving, (3) have recently shown an increase in incidence or expansion into new geographic locations or vectors, (4) have moved from one host species to another, (5) have increased in impact or severity, (6) or have undergone a change in pathogenicity" (p. 144).[40] Several EIDs were considered during the course of this investigation [e.g. cetacean morbillivirus, cetacean poxvirus (tattoo skin disease)], and are discussed in Sections V (Epidemiology) and VI (Pathology).

1. Viral pathogens

A pathogen's effect on an animal reflects both the characteristics of the pathogen and the status of the host's immune system. Primary pathogens may infect individuals with competent immune systems. In contrast, opportunistic pathogens only affect animals: (a) if they are given a portal of entry or a microclimate conducive to their survival/replication (e.g. traumatic implantation of bacteria following lacerations secondary to entanglement), or (b) if those individuals have immune systems that have been previously compromised (i.e. a secondary infection following a breach of normal defenses caused by a primary pathogen and/or immunosuppression stemming from the effect of one or more stressors).

The effects of secondary infections often obscure the initial injury/tissue damage caused by—and thus potentially clinically indicative of—an underlying primary pathogen. This, along with other factors, can make it exceedingly difficult to detect whether a primary pathogen was present.[41]

Viruses often function as primary pathogens, and may affect the innate and/or humoral immune defenses of a host, leaving the animal immunosuppressed and susceptible to secondary infection by

39 The term *pathogen* refers to any disease-causing agent, particularly those that are infectious (e.g. micro-organisms), but sometimes also to non-infectious agents such as chemicals or other stressors. Similarly, diseases (i.e. conditions causing ill-health) are sometimes classified as 'infectious' (i.e. able to be transmitted between organisms, e.g. viruses, bacteria, helminths) and 'non-infectious' (i.e. non-communicable; caused and maintained through the activity of a stressor and unable to be transmitted between organisms).

40 The definition that Van Bressem et al. (2009a) gave followed Bengis et al. (2004).

41 For example, logistical factors (e.g. difficulty in coordinated carcass reporting/retrieval) can sometimes make immediate examination impossible. Extension of the post-mortem interval can result in tissue autolysis, rendering lesion interpretation even more difficult.

opportunistic bacterial, fungal, protozoal, and other pathogens (Hall 1995, Van Bressem et al. 1999, Bossart et al. 2001).

Cetacean morbillivirus has been associated with several mass mortalities of marine mammals, including several die-offs of bottlenose dolphins in the U.S. (Lipscomb et al. 1994; Duignan et al. 1995, 1996; Hall 1995; Taubenger et al. 1996; Schulman et al. 1997; Duignan 1998; Kennedy 1998; Van Bressem et al. 1999, 2001, 2009a). Cetacean morbillivirus belongs to the genus *Morbillivirus,* single negative-strand RNA viruses of the subfamily *Paramyxovirinae*, family *Paramyxoviridae*. There are at least three closely-related strains.

Although cetacean morbillivirus is related to other members of the genus that are known to cause disease in other species [e.g. measles (humans), canine distemper (canids), peste-de-petit-ruminants virus (small ruminants), rinderpest (cattle, buffalo, other ungulates) etc.], it is host specific (i.e. the virus only causes disease in cetaceans), and is not transmissible to other species. While cetacean morbillivirus is highly virulent and may induce mortality by itself, it is also a powerful immunosuppressant. The virus causes epizootics characterised by interstitial and bronchointerstitial pneumonia, non-suppurative encephalitis, and lymphoid depletion (Kennedy et al. 1991, Duignan et al. 1992, Schulman et al. 1997, Van Bressem et al. 1999).

While morbillivirus has not yet been detected in cetaceans within Australian waters, epidemiological patterns observed elsewhere suggest that it could be present within larger offshore cetacean populations, thus allowing for periodic intrusions into smaller inshore populations (Duignan et al. 1995, 1996). The virus has no dormant carrier phase and cannot survive readily outside of a host, and thus requires a large population (i.e. in the hundreds of thousands) with a large number of immunologically naïve animals in order to remain endemic (Duignan 1998; Van Bressem et al. 1999, 2001). Amongst cetaceans, short- and long-finned pilot whales may function as reservoir hosts for the virus, from which the disease can spread periodically to dolphins (Duignan et al. 1995, Van Bressem et al. 1998). Pilot whales, offshore bottlenose dolphins, and other odontocetes are sometimes observed in large (i.e. thousands of individuals) multi-species aggregations that provide a suitable environment for transmission of the virus through aerosolized droplets or other means. *Tursiops truncatus* (the offshore *Tursiops* species/morphotype) haplotypes are present in dolphin samples taken from within the Perth metropolitan area (see Section III - Ecology), indicating that dolphins from offshore populations at least occasionally come into contact with dolphins from coastal and inshore areas, and suggesting a potential chain of transmission that could periodically link coastal dolphin populations with ‘reservoir’ pilot whale populations offshore. This potential exposure pathway could result in occasional introductions of morbillivirus into populations of coastal *Tursiops aduncus* and, given the over-lapping nature of these populations, even into the relatively cloistered estuarine communities.

Some studies have suggested the potential for contaminants to compound the immunosuppression that morbillivirus induces. For example, Domingo et al. (1992) investigated a mass mortality event in 1990

involving hundreds of striped dolphins *(Stenella coeruleoalba)* along the Spanish Mediterranean coast that was associated with a morbillivirus outbreak. They had previously documented high concentrations of polychlorinated biphenyls (PCBs) in adipose tissue from this population some time prior to the morbillivirus outbreak. Tissues from animals that died during the outbreak also had high concentrations of PCBs in adipose tissue. In assessing the potential contribution of PCBs, Domingo et al. (1992) noted that: "the high PCB levels may have compounded the well known severe immunosuppressive effects of morbilliviruses, resulting in higher mortality than would have occurred in dolphins free of polychlorinated biphenyl residues."

2. Secondary (opportunistic) pathogens

Opportunistic pathogens include various bacteria, fungi, protozoa, nematodes, and other organisms. Such pathogens are often present permanently in the environment (some are even commensals of normal healthy dolphin skin/respiratory tracts), but animals are generally able to resist infection if they have competent (i.e. well-functioning) immune systems**.** However, an individual may become vulnerable if its immune system is compromised (e.g. by virus, poor condition, entanglement wound, chronic physiologic stress, or exposure to chemical contaminants), or if the pathogen is given a portal of entry that breaches the animal's innate defence mechanisms (e.g. a wound). Depending on a combination of factors, infection can be terminal, particularly if it leads to septicaemia.[42] These factors include the stressors the individual is subject to (e.g. virulence/severity of infection, the overall condition of the individual and its ability to mount an effective immune response to clear the infection).

3. Pathogens in sewage discharge

Bacteria and other faecal pathogens present in sewage discharge have been associated with marine mammal mortalities, generally in localised areas discharging raw wastewater or having inadequate sewage treatment facilities. Wilson (1994, 1996) suggested that inappropriate sewage discharge had a role in the disappearance and deaths of several of the conditioned dolphins that visited the beach at Monkey Mia in Shark Bay, Western Australia. Drains discharging into the Swan Canning Riverpark may also carry harmful materials (e.g. debris, industrial residues, pathogens), as well nutrients, other dissolved compounds, and contaminants bound to suspended sediment.[43]

There was no indication of the specific pathogens known to be commonly associated with faecal contamination (e.g. *Escherichia coli*, *Enterococcus* spp., *Salmonella* spp.) in microbial cultures taken from the dolphins examined post-mortem in 2009. The organisms cultured were ubiquitous in the environment or known to be commensals of dolphin skin.

[42] Septicaemia is defined as a "systemic disease associated with the presence and persistence of pathogenic micro-organisms and/or their toxins in the blood" (Blood and Studdert, 1988). The resulting syndrome, 'sepsis,' is characterised by a generalised, debilitating inflammatory state referred to as 'systemic inflammatory response syndrome (SIRS)', a condition which can lead to: derangement(s) of homeostasis; coagulation defects such as 'disseminated intravascular coagulation' (DIC); multiple organ damage/failure; and ultimately death if allowed to progress unchecked.

[43] Contaminants bound to sediment constitute a large proportion of contaminants found in drainage discharge.

4. Epidermal disease

Skin lesions have been documented in a wide variety of cetacean species (see Van Bressem et al. 2009a,b), as well as seals and sea lions (Hicks and Worthy 1987, Nollens et al. 2006), and many terrestrial vertebrates (Gillespie and Timoney 1981). Skin lesions are generally symptomatic of epidermal diseases, although they may occasionally (depending on aetiology) be a manifestation of disseminated, multisystemic disease (Geraci et al. 1979; Wilson et al. 1997b, 1999a; Van Bressem et al. 2003, 2009b).

Pathogens (some primary pathogens and others secondary opportunists) associated with epidermal diseases in cetaceans include: viruses from four families (*Caliciviridae*, *Herpesviridae*, *Papillomaviridae*, and *Chordopoxviridae*); bacteria (*Aeromonas* spp., *Dermatophilus* spp., *Erysipelothrix rhusiopathiae*, *Mycobacterium marinum*, *Pseudomonas* spp., *Staphylococcus delphini*, *Streptococcus iniae*, and *Vibrio* spp.); at least four groups of fungi (*Candida albicans*, *Fusarium* spp., *Trichophyton* spp., and *Lacazia loboi*); and ciliated protozoans (likely *Kyaroikeus cetarius*) (Sweeney and Ridgway 1975; Flom and Houk 1979; Geraci et al. 1979; Medway 1980; Bossart et al. 2001; Van Bressem et al. 2003, 2009b; Bracht et al. 2006).

Skin lesions in dolphins vary in appearance depending on the type(s) of lesion-causing pathogen(s), and may occur on any part of the body (Van Bressem and Van Waerebeek 1996; Wilson 1997b, 1999a; Van Bressem et al. 2003, 2009b; Bearzi et al. 2009; Ham 2009; Rowe et al. 2010). Various studies have developed classification schemes for photographic images of skin lesions, generally based on their shape or appearance (e.g. Wilson et al. 1997b; Van Bressem et al. 2003, 2009b; Froude 2009; Ham 2009).

Cetacean skin has several mechanisms to deter infection of epidermal tissue and to limit the extent of infection, including high dermal growth rates causing continuous sloughing of epidermal cells (Gercai et al. 1979, Hicks et al. 1985, Van Bressem et al. 2009b). However, skin barriers may be damaged through wounds, osmoregulatory disruption, active lesions, and other processes, and this damage may allow existing lesions to increase in severity and facilitate colonisation of epidermal tissue by opportunistic pathogens (Barry et al. 2008; Van Bressem et al. 2009a,b). Similarly, skin infections may occur or increase in severity if the host experiences a reduction in immune function for some reason.

The precise aetiopathogenesis[44] of skin disease in cetacean populations is often unclear, but has been associated with: environmental conditions (e.g. low salinity, temperature), physiological stress, poor immunological function, exposure to contaminants, changes in lesion-causing pathogens (e.g. increased virulence), and other factors, suggesting that epizootics of skin disease often reflect a suite of interacting factors (Geraci et al. 1979; Thompson and Hammond 1992; Wilson et al. 1997b, 1999a; Reif et al. 2003, 2006b; Van Bressem et al. 2003, 2009b; Bearzi et al. 2009; Rowe et al. 2010).

[44] Aetiopathogenesis refers to the complex interaction of that pathogen with the individual dependent on multiple, often not elucidated factors (as listed), that eventually culminates in the progression to and manifestation of disease.

Photo-identification techniques allow for the estimation of the prevalence of skin disease in a population, and long-term studies can be carried out to monitor the development or resolution of individual lesions and the survival of affected individuals (e.g. Thompson and Hammond 1992; Wilson et al. 1999a,; Van Bressem et al. 2003, 2009b). Using this technique, skin lesions have been documented in a number of dolphin populations around the world [e.g. Scotland (Thompson and Hammond 1992; Wilson et al. 1997b, 1999a); Mayotte (Kiszka et al. 2009), California (Bearzi et al. 2009); South America (Van Bressem et al. 2009b); and Portugal (Van Bressem et al. 2003); see also review in Van Bressem et al. 2009b]. Ideally, these studies should be coupled with post-mortem analyses of stranded animals in order to identify the pathogens causing epidermal skin disease, and to refine the pathology of particular forms of epidermal skin disease, such as the role of acquired immunity in limiting the incidence of infection (Van Bressem et al. 2006, 2009b).[45] However, relatively few studies based on photo-identification technique have characterised the lesion-causing pathogen present.

G. Tattoo skin disease (TSD)

1. Poxvirus and tattoo skin disease

Tattoo skin disease (TSD) is one of the most common forms of epidermal disease in dolphins (see Van Bressem et al. 2009b) (see Figure 22). TSD is the term conventionally used to describe the specific lesions characteristic of infection with poxvirus. The cetacean poxvirus is a double-strand DNA virus belonging to a new genus of the subfamily *Chordopoxvirinae* (family *Chordopoxviridae)*, and is closely related to terrestrial poxviruses of the genus *Orthopoxvirus* (Bracht et al. 2006, Van Bressem et al. 2009b). The virus is present in cetacean populations worldwide, and is host-specific. Van Bressem et al. (2009a) included TSD in their review of emerging infectious diseases affecting cetaceans, based on evidence that the incidence of TSD is increasing, a trend that suggests a change in the virulence of poxvirus may have occurred in some infected populations.

2. Pathology and epidemiology of TSD

TSD is not considered highly pathogenic, and infection usually presents in self-limiting tattoo-like skin lesions that resolve with time, and does not normally progress to large and deeply ulcerative lesions or cause death, although it has been reported to cause sporadic deaths in neonates and calves without protective immunity (Flom and Houk 1979; Geraci et al. 1979; Wilson et al. 1997b, 1999a; Van Bressem et al. 2003, 2009b).

[45] The laser photogrammetry ('laser metrics') used in Rowe et al. (2010) could also be used to make extremely precise measurements of skin lesions. Laser photogrammetry projects dots from two parallel lasers (known to be a certain set distance apart) onto the object being photographed. As long as the object is perpendicular to the axis of the lasers, measurements can be made at the distance to which the dots are visible (Rowe et al. 2010). Rowe et al. (2010) used the technique to assess the calf size of bottlenose dolphin populations in New Zealand, and to photographically document the prevalence of skin lesions in order to categorise the epidermal condition of individual dolphins (as a marker of general health/immune status). The application of photogrammetry to measure lesions would allow for serial measurements of lesions (i.e. on known individuals and over time), and thus could be used to document lesion development and regression and relate these patterns to temporal, spatial, biological, and environmental factors.

Poxvirus-induced lesions in dolphins involve a ballooning of the deep layers of the stratum intermedium, the epidermal layer comprising the main mass of epidermal tissue (Geraci et al. 1979). While the overlying stratum externum (the outermost skin layer) can become thickened in the region above an active lesion, the lesions appear to cause minimal inflammation. Geraci et al. (1979) observed that while poxvirus skin lesions in terrestrial vertebrates often occur as vesicular eruptions of the skin that sometimes progress to pustular stages, this progression does not appear characteristic of pox-induced lesions in dolphins. Geraci et al. (1979) suggested that this difference relates to the high metabolic activity of cetacean skin, which is sufficient to allow for: "continuous sloughing of epidermal cells which may keep pace with the rate of hyperplasia, leaving a smooth surface contour and only a downward thickening of the stratum externum in the area of the lesion" (p. 403). Van Bressem et al. (2009b) provides a description of the characteristic appearances of TSD lesions and the different stages of TSD lesions (active, regressing, healed) based on observations of 17 species cetacean species from the Americas, Europe, South Africa, New Zealand and Greenland.

Several studies have examined epidemiological patterns for TSD within and across populations. The work of Van Bressem et al. (2003) provides one of the best-documented instances of TSD within a small (~35 individuals) resident bottlenose dolphin community inhabiting an estuarine ecosystem (Sado Estuary, Portugal) affected by intensive anthropogenic impact. The prevalence of TSD lesion within the Sado Estuary dolphin community was high but variable over time (e.g. 39.1% of individuals in 1994–1995 vs. 17.4% of individuals in 1996–1997), and TSD lesions were more prevalent in immature dolphins than in adults. Van Bressem et al. (2003) suggested that the high prevalence and severity of TSD lesions they observed may reflect stressful conditions, and that the regression and recurrence of TSD lesions in individuals may relate to immunological deficiencies.

Van Bressem et al. (2009b) investigated whether the prevalence of TSD varied within a sample group of 1392 dead and free-ranging odontocetes[46], and found that TSD was typically most prevalent in juveniles. This age-related epidemiological pattern for TSD probably reflects: (a) calves being protected against infection by maternal antibodies [maternal immunity]; (b) adults typically having developed active immunity following infection as juveniles (and thus protected against re-infection thereafter); and (c) juveniles becoming infected when maternal antibody wanes (Van Bressem et al. 2006, 2009b). However, Van Bressem et al. (2009b) also found that, in some populations, TSD lesions occurred across all age-classes (i.e. in calves, juveniles, and adults), and suggested that the high prevalence of TSD in adults may reflect reduced immune function. Van Bressem et al. (2009b) suggested that the departures from the typical age-related epidemiological pattern for TSD may provide a useful indicator of poor population health [e.g. the presence of large, active lesions in adults should generally be considered unusual (i.e. atypical)], and emphasised the need for further research to relate the prevalence of TSD lesions to other intercurrent stressors (e.g. environmental factors, contaminant loads).

46 Odontocetes are toothed whales and dolphins.

We do not know the prevalence of TSD in dolphins that range within the Swan Canning Riverpark. However, lesions indicative of TSD have been observed in bottlenose dolphins from Bunbury and Cockburn Sound (Froude 2009, Ham 2009). While these studies indicated that poxvirus was present in these populations, the virus was never definitively diagnosed until this study, and its full aetiopathogenesis remains unclear.[47]

Ham (2009) observed skin lesions in 45 of 158 (27.2%) dolphins photo-identified in Cockburn Sound between July-November 2008, with lesions occurring most frequently on the dorsal regions of the belly, chest and peduncle, and on the dorsal fin (Table 6). Skin lesions were observed in dolphins of all age-classes and were most prevalent in calves, with 46% of calves (n = 6 of 13 photo-identified calves) having lesions. Lesion prevalence was lower but similar for other age classes. Ham (2009) characterised ten different types of skin lesions for Cockburn Sound based on aspects of the appearance of lesions (e.g. colour, form), with most similar to lesion types identified for other dolphin populations (Figure 22). Some individuals had more than one type of lesion present, and four (8.8%) of the 45 lesion-affected dolphins showed increased prevalence of skin lesions over the course of the study (i.e. from July to November), suggesting a possible seasonal trend for the prevalence of lesions. A similar photo-identification study in Bunbury area also observed lesions in all age-classes, and found preliminary indications that lesions were more prevalent in dolphins that ranged more frequently within nearshore and estuarine waters (e.g. Leschennault Estuary) (Froude 2009).

3. Factors affecting the prevalence of TSD

Like other infectious diseases, epidermal diseases such as TSD are generally multi-factorial in their aetiology, and are best understood as the outcome of a complex suite of interacting stressors, some of which may be anthropogenic. Research to date on TSD has generally been broadly correlative, leaving much to be resolved about factors influencing the prevalence, incidence, severity, and health implications of TSD. An additional problem is the fact that few studies of TSD-like lesions have definitively identified the pathogens causing the lesions that were observed. Use of the term 'tattoo skin disease' to refer to poxvirus infections has only been introduced relatively recently to the scientific literature (see Van Bressem et al. 2003, 2009a).

It appears likely that many studies of epidermal disease involving lesions characteristic of TSD have involved poxvirus infection, based on: (a) the findings and clinical descriptions of TSD lesions in 17 cetacean species (sampled world-wide) in Van Bressem et al. (2009b), and the fact that the presentation of poxvirus-induced lesions (TSD) is relatively distinct from other common epidermal disease in cetaceans, such as lobomycosis (e.g. Reif et al. 2006, Van Bressem et al. 2008). While this conclusion is conjectural, for the purposes of this discussion we have chosen to draw on both studies that have specifically identified skin lesions as poxvirus-induced (i.e. TSD), as well as studies that have not

[47] Viral inclusion bodies indicative of poxviruses were observed in epidermal tissues during histopathology analyses (see Section VI – Pathology). We are awaiting viral assays to confirm its aetiology.

specifically identified the lesion-causing pathogen present, but have described skin lesions that are characteristic of TSD lesions. We note also that many epidermal diseases have similar aetiological factors, even if they involve different lesion-inducing pathogens (Van Bressem et al. 2008).

Previous studies have suggested that the occurrence of TSD lesions (and TSD-like lesions) may relate to the presence of anthropogenic contaminants, adverse environmental conditions, sustained and intensive human interactions (e.g. frequent fishery or tourism interactions), or other stressors (e.g. Wilson et al. 1999b; Van Bressem et al. 2003, 2009b; Rowe et al. 2010). It is has also been suggested that different strains of poxvirus may occur and variation in the virulence of these strains could account for some of differing presentations of TSD observed (Van Bressem et al 2009b). Several studies have proposed that the presence of TSD lesions (and TSD-like lesions) in dolphin populations is associated with intensive stressors, and that anthropogenic stressors (e.g. low salinity discharges, disturbance from tourism and fishery interactions) may make individuals susceptible to TSD and influence its prevalence and severity (Wilson et al. 1999a; Van Bressem et al. 2003, 2009a,b; Rowe et al. 2010). These studies also indicate the range of factors that could influence presentations of TSD within and between individuals and populations, and demonstrate the difficulty of trying to apportion the relative contribution of different factors.[48]

Contaminants: The finding of Rowe et al. (2010) (discussed below) suggest that high concentrations of contaminants are not necessary for high rates of epidermal skin disease to occur, a conclusion that is broadly consistent with the conclusions of Wilson et al. (1999a). Wilson et al. (1999a) found that contaminant concentrations were not correlated for the four populations for which they were able to compare the prevalence of skin lesions and concentrations of certain heavy metals and organic contaminants. However, these findings do not exclude the possibility that contaminant burdens may not be part of the suite of stressors contributing to the occurrence of TSD (see Van Bressem et al. 2003, 2009b). For example, Van Bressem et al. (2000b) noted that:

> Future research should seek to correlate the presence, number and size of tattoo lesions with quantitative data on contaminant loads, including PCB congeners amongst others. Inshore and neritic cetaceans living in a contaminated environment, stressed by fisheries interactions and disturbance from dense shipping may be physiologically challenged to mount an adequate immune response against infectious agents. [p. 235]

Environmental conditions: Studies of both captive and free-ranging dolphins populations suggest relationship between TSD and environmental conditions. Poxvirus-induced lesions occur in captive animals kept in suboptimal conditions. Geraci et al. (1979), for example, observed that the prevalence of lesions often increased when captive animals experienced poor water quality or rapid changes in temperature. Wilson et al. (1999) examined data on skin lesions and environmental conditions for ten

48 For an example of efforts to quantify stressors that could influence the prevalence and severity of TSD, see Lusseau et al. (2006) which examines physiological stress from tourism, and also (e.g.) Bejder et al. (2006a, b) and St. Aubin (2002).

coastal populations of bottlenose dolphins, and concluded that dolphins from areas of low water temperature and low salinity exhibited higher lesion prevalence and severity.

The relationship between TSD and environmental conditions could relate to systemic physiological stress and/or to osmoregulatory stress to the epidermis (Geraci et al. 1979, Ortiz 2001, Wilson et al. 1999, Reif et al. 2006, Barry et al. 2008, Van Bressem et al. 2009b, Rowe et al. 2010). Physiological stress from low salinities and temperatures could, for example, reduce a host's ability to contain a poxvirus infection or facilitate infection. Environmental conditions could also cause osmotic damage to the epidermis, causing cell deterioration or death, and thereby potentially facilitating an increase in the severity of TSD infections (as well as increasing susceptibility to opportunistic infection of the epidermis by fungal and bacterial pathogens). Osmotic damage could also further damage epidermal tissue already damaged by TSD lesions.

Case-study: Rowe et al. (2010) provides a good case-study of the complexity inherent in trying to unravel the factors causing skin lesions in dolphin populations. Rowe et al. (2010) documented TSD-like skin lesions in two New Zealand coastal bottlenose dolphin populations (in Doubtful Sound and Dusky Sound).[49] One of the study populations (in Doubtful Sound) had declined by over 34% between 1995 and 2009. While epidermal lesions were common in both populations (affecting >95% of individuals), the extent of lesions was greater in dolphins from Doubtful Sound. The extent of lesions was also worse in females than males in Doubtful Sound, a sex-based pattern that was not observed in Dusky Sound. Additionally, Dusky Sound calves were larger at first observation and were born over a longer period than calves in Doubtful Sound.

Rowe et al. (2010) suggested that both the higher extent of epidermal lesions in females and the smaller size of calves in Doubtful Sound could be a factor in the low survival of calves in that population. Rowe et al. (2010) also concluded that anthropogenic impacts likely contributed to the greater severity of skin lesions in the Doubtful Sound population, and suggested that Doubtful Sound population was adversely affected by freshwater intrusions from a hydroelectric power station, as well as disturbance from tourism. Tourism activity was much lower in Dusky Sound and the area received freshwater inputs only as a result of natural runoff entering the fjord. Both populations had limited exposure to chemical contaminants, indicating that contaminants were unlikely to influence the prevalence of TSD in the two populations.

It is possible that the presence of poxvirus strains differing in virulence could account for the differences in lesion severity observed between the two populations. In this scenario, one population (Doubtful Sound) would be infected by a more virulent poxvirus strain than the other population

[49] Rowe et al. (2010) did not identify the specific epidermal disease that was present in their study populations, as they had not yet obtained tissue samples required for histological identification or Polymerase Chain Reaction (PCR) analysis (or other molecular techniques to detect/amplify the presence of DNA/RNA belonging to infectious agents or pathogens). Their descriptions and images of the lesions are consistent with TSD, and TSD has been documented in Hector's dolphins (*Cephalorhynchus hectori hectori* and *C. h. maui*) from the North and South Islands in New Zealand (see Van Bressem et al. 2009b). However, it is only speculation to consider the lesions observed by Rowe et al. (2010) as TSD.

(Dusky Sound), resulting in differences in the prevalence and severity of skin lesions between the two populations.[50] However, it is unlikely that the two populations would have different poxvirus strains given the geographic proximity of Doubtful Sound and Dusky Sound and the continuous distribution of cetaceans along the surrounding coastline.[51] This suggests that site-specific environmental and anthropogenic factors are likely to underlie differences in the prevalence and severity of TSD between populations that geographically proximate.[52]

4. Salinity and TSD

Salinity appears to be an important factor in the prevalence of TSD and other epidermal diseases in bottlenose dolphins (Geraci et al. 1979, Wilson et al. 1999a, Reif et al. 2006, Van Bressem et al. 2009b, Rowe et al. 2010). As described above, the effects of salinity could occur through systemic physiological stress and/or through osmotic damage to epidermal cells. Such effects could conceivably occur at salinities lower than 20 ppt (g/L), as salinities below this threshold are considered potentially physiologically stressful for dolphins held in captive environments.[53] However, several factors make it difficult to determine whether or how such a threshold might relate to the prevalence and severity of TSD and other epidermal diseases in free-ranging dolphins.

Firstly, bottlenose dolphins are ubiquitous within estuarine ecosystems in temperate and tropical regions world-wide. They occur, for example, in estuaries all along the eastern and southeastern coast of the U.S (Waring et al. 1999, 2007).[54] Secondly, these estuarine ecosystems have salinity regimes that are: (a) naturally dynamic and (b) commonly below the salinities prevailing in nearby coastal areas. A range of studies have shown that dolphins inhabiting estuarine ecosystems may often experience salinities between 15-25 ppt (and occasionally much lower salinities) because of: seasonal or acute patterns in precipitation and input from catchment areas (e.g. storms, single rainfall events, seasonal shifts in rainfall); changing oceanographic conditions; and (in some locations) freshwater inputs from point source discharges such as power stations (e.g. Moreno 2005, Reif et al. 2006, Barry et al. 2008, Cribb et al. 2008, Miller and Baltz 2009, Rowe et al. 2010). Finally, while bottlenose dolphins have the sensory capacity to detect variation in salinities, it is clear that they do not necessarily avoid areas with low salinities, even though use of these area is, in some cases, associated with the occurrence of epidermal disease (e.g. Reif et al. 2006 - Indian River Lagoon, Florida; Barry et al. 2008 – Lake Pontchartrain, Louisiana; Rowe et al. 2010 – Doubtful Sound, New Zealand).

50 Assuming that the skin lesions in the two populations were indeed caused by poxvirus infections.

51 These factors would support the eventual tranmission of different poxvirus strains between cetacean populations in that region (if such strains were present). Neither population is isolated from contact with other cetacean populations.

52 This issue is, however, complex and Van Bressem et al. (2009b, p.230) note that: "Geography (e.g. spatial autocorrelation), phylogeny (e.g. similarity in host susceptibility) and virus strain/species (e.g. differences in virulence and infectiousness) may have contrasting effects with regard to sexual or age-related differences in cetacean poxvirus infections".

53 See (for example): Geraci, J.R., 1986. 'Marine Mammals (Cetacea, Pinnipedia and Sirenia)' in Zoo and Wild Animal Medicine, 2nd Edition. pp. 750-797, which notes that all but freshwater cetaceans require either natural or artificial seawater and indicates that a safe and relatively natural salinity range is 25 to 35 ppt.

54 Including previous work in the Swan Canning Riverpark (H. Finn, unpublished data) and in the Bunbury inner waters area (Appendix A3) indicating the dolphins use those areas year-round. Recent observations from the Dolphin Watch project also support this (see Section V – Epidemiology).

Case study: The work of Barry et al. (2008) provides a useful case-study for examining how low salinity conditions may affect dolphins and the behavioural and physiological factors that may be involved. Barry et al. (2008) observed severe skin lesions in a group of about 30-40 bottlenose dolphins residing within Lake Pontchartrain, Louisiana, a brackish lagoon from which dolphins were potentially able to access areas with higher salinity conditions.[55] These lesions were characterised by degrees of mottled and pale skin, and, in a subset of individuals, covered with an orange to green growth of unknown algal or fungal origin. Some of the images indicate lesions that had progressed to an ulcerative stage. The occurrence of dolphins within Lake Pontchartrain was considered unusual and outside the normal distribution of bottlenose dolphins in that region, indicating that some factor was 'holding' the dolphins in this atypical habitat. Barry et al. (2008) documented the prevalence of skin lesions and the progression of lesions in particular individuals, and noted:

> Dolphins inhabiting low salinity environments for an extended period of time (i.e. several weeks) experience a number of negative health conditions. Primarily, these impacts manifest as severe skin lesions that worsen with longer exposure and lower salinities. In addition, the degradation of the skin disrupts electrolyte balance in the blood, and dolphins suffering from freshwater exposure exhibit changes in blood chemistry including decreased sodium and chloride levels associated with over-hydration due to increased water absorption through the skin. In addition, swelling in the eye (corneal oedema) has also been observed. The associated physiological stress may lead to mortality in some cases, and data suggest that intervention should occur within 5-7 days of exposure to 0 ppt salinities to avoid severe health impacts. [p.3]

These observations describe the pathology associated with exposure to conditions of extreme low salinity, in terms of the systemic physiological stress and osmotic damage to epidermal tissue that may occur. There has been little work on the physiological responses of dolphins to low salinity conditions and how this relates to epidermal disease and other health effects, particularly with free-ranging dolphins. What information is available suggests that there can be considerable variation in how individual dolphins respond to low salinity conditions (and thus whether or not they will suffer ill effects).

As with other physiological challenges, individual responses to low salinities can be expected to vary as a result of individual differences in (e.g.) body condition, diet, immunological function, contaminant burdens, and the presence of other (potentially synergistic) stressors. Such variation could also reflect behavioural differences that influence the exposure of dolphins. Barry et al. (2008) describe how such individual variability might occur for the dolphins that they observed in the low (<5 ppt) salinity conditions within Lake Pontchartrain:

> There is a limited amount of data available on the relationship between the length of exposure to low salinity conditions and the progression of the associated skin conditions and over-hydration. Of the six well-documented cases, there were two animals that survived for prolonged periods (>45

[55] The dolphins had been observed within Lake Pontchartrain since early in the northern hemisphere spring (March-April) in 2007. Barry et al. (2008) did not indicate whether a lesion-causing pathogen such as poxvirus was thought to be present.

> days) at salinities between 1-5 ppt. It is hypothesized that animals in such conditions may be able to maintain electrolyte balance by feeding on prey that contain sufficient sodium levels…Therefore, it is possible that the target group of dolphins are able to maintain their body condition despite experiencing low salinity levels for what is now an extended period of time (> 1.5 year).
>
> The high incidence of severe skin conditions in animals within Rigolets Pass is an interesting finding of the current study. It does not appear that these animals experience salinities that are markedly lower than those of the target group or those animals within the Chef Menteur Pass. The available data on freshwater exposure do indicate that the onset of the skin condition can be quite rapid, occurring within 5-10 days of exposure (SEFSC, unpublished data). Thus, it is possible that those animals experienced at least a short-term stress of reduced or low salinity, but did not have prey available to them that would allow them to maintain electrolyte balance or otherwise experienced environmental conditions that exacerbated the impacts of low salinity on these animals. [p. 7-8][56]

These observations suggest that, within populations exposed to low salinity conditions, the prevalence and severity of skin lesions may vary between individuals as a consequence of physiological differences related to body condition, diet, and other factors. This conclusion is consistent with studies of dolphins in captivity that have found that only certain individuals (in a group of captive dolphins) developed lesions after being exposed to conditions of poor water quality (e.g. Geraci et al. 1979). When lesion-inducing pathogens such as poxvirus are present, physiological differences could affect the severity of the infection and the progression of pathogen-induced lesions. For example, physiological susceptibilities could cause poxvirus infections to grow more severe, potentially allowing TSD lesions to spread and increasing the amount of damaged epidermal tissue. This kind of synergistic interaction could also involve osmotic damage of epidermal tissue, and would be consistent with a multi-factorial aetiology and individual variation in the presentations of skin lesions.

Barry et al. (2008) also indicates that the behavioural ecology of individual dolphins may influence the intensity and duration of their exposure to low salinity conditions. Behavioural factors appear, for example, to underlie the residency of dolphins within the clearly adverse environment of Lake Pontchartrain, and may also influence differences in their diet (prey selection). The range of behavioural factors that could influence individual exposure includes: ranging patterns (e.g. locations of home ranges, site fidelity, residency patterns), movement patterns (e.g. within and outside of estuarine areas), habitat use patterns (e.g. upper versus lower reaches of an estuary), and preference. Thus, individual differences in physiology and behavioural ecology may play an important role in determining whether dolphins use low salinity areas and how exposure to low salinity conditions affects them.

[56] Like Rowe et al. (2010), Barry et al. (2008) also did not indicate whether there was a lesion-causing pathogen (e.g poxvirus) present in dolphins from Lake Pontchartrain. Again, it is possible that more than one pathogen or strains of the same pathogen was present, and these different pathogens/strains account for differences in lesion prevalence and severity (e.g. between dolphins observed at Rigolets Pass and those sighted at Chef Menteur Pass). However, this seems unlikely, given that the two groups resided in the same area and were (presumably) drawn from the same population, and thus are likely to be exposed to the same pathogens over time.

5. TSD and severe skin lesions

Epidermal damage from skin lesions may lead to a rapid deterioration in health if a significant proportion of the skin surface is affected, severe ulcerative lesions develop, and/or secondary septicaemia occurs. The progression of skin lesions to an ulcerative stage could be facilitated by osmotic damage induced by adverse temperatures and/or salinities, and possibly by systemic physiological stress from these conditions.

Severe ulcerative skin lesions have been observed in at least two locations with potentially stressful environmental conditions.[57] K. Charlton (personal communication, Monash University) observed severe ulcerative skin lesions that had progressed to the ulcerative/suppurative stage in dolphins exposed to altered temperatures and salinities in the Gippsland Lakes (see Appendix A2). The presentation of these lesions is strikingly similar to the severe lesions observed in two of the 2009 deaths in the Swan Canning Riverpark (compare Figures 23 and 24). As described above, Barry et al. (2008) also documented severe skin lesions in a group of dolphins residing within Lake Pontchartrain, including some that appear to have progressed to the ulcerative stage. The Barry et al. (2008) study suggests that lesion-inducing pathogens may not be necessary for significant osmotic damage to occur if dolphins are exposed to extremely low (i.e. <5 ppt) salinities, although this study did not note whether such a pathogen was present.[58]

Poxvirus-induced skin lesions do not typically have an ulcerative/suppurative stage that breaks the skin surface, although other epidermal diseases may (e.g. lobomycosis – Reif et al. 2006).[59] Before this study it was not clear whether TSD lesions were associated with a more severe ulcerative stage, as previous studies (to our knowledge) had not documented such an association. While Van Bressem et al. (2003) observed that the prevalence of TSD was high in dolphins from the Sado Estuary, and that TSD lesions also covered a substantial surface area in some individuals, they did not note if any of the TSD lesions progressed to a more severe ulcerative/suppurative stage. However, Van Bressem et al. (2003) did document the death (from unknown causes) of two juvenile dolphins with a high number of TSD lesions and the super-infection (by an unknown infectious agent) of TSD lesions on a calf. Similarly, while Van Bressem et al. (2009b) documented a high prevalence of TSD lesions in individuals that they classified as having died in poor health (i.e. relative to those who were classified as having died a traumatic death as a result of fishery bycatch or other human interaction), they did not indicate whether they observed any lesions in an ulcerative state.

[57] We note that the presence of TSD in dolphins from these areas has not been confirmed, although images of lesions on dolphins from the Gippslands Lakes appear characteristic of TSD (see Appendix A2).

[58] A humpback whale mother-calf that lingered for eleven days within the Sacramento River, California in 2007 also experienced extensive epidermal damage (Gulland et al. 2008).

[59] We note that studies and review of TSD (e.g. Van Bressem et al. 2003, 2009a) and epidermal disease similar to/characteristic of TSD (but not specifically termed TSD) (Wilson et al. 1997, 1999a) do not refer to the presence of an ulcerative stage or ulcerative form in their descriptions of they skin lesions that they studied.

The lack of a documented ulcerative stage in TSD lesions makes it unclear why a typical presentation of a poxvirus-induced skin lesion would progress to an ulcerative/suppurative stage. Such a progression could presumably relate to a suite of factors, including reduced immunological function, a more virulent form of poxvirus being present, osmotic damage of epidermal cells, and/or some other factor(s). It is also possible that a synergistic interaction may occur between the poxvirus infection and the osmotic and physiological effects of exposure to low salinity. In particular, where poxvirus-induced lesions occur, these lesions may represent a 'weak point' in the epidermis that can be further damaged by exposure to low salinity conditions, potentially supporting a progression to a more severe form of lesion. In this scenario, osmotic derangement of epidermal cells could damage tissue overlying TSD lesions within the stratum intermedium (i.e. erosion) or around TSD lesions that had progressed to an ulcerative state and broken the skin surface.

Regardless of the mechanism underlying such a progression, the rupturing of the stratum externum could initiate a degenerative sequence in which the progressive deterioration of outer epidermal layers allows for the loss of larger and larger amounts of fluids, electrolytes, and proteins (weakening and ultimately debilitating the animal),[60] as well as providing a growing port of entry for opportunistic fungal and bacterial pathogens. This epidermal damage could occur acutely (i.e. occur within a few days), although the progression to death could take a longer period of time depending on the severity of the lesions, the body condition of the animal, its state of immunological function, etc. It is likely that the initial development of severe ulcerative lesions would support a synergistic cascade of events supporting a decline in health, such as a reduction in body condition making animals unable to forage, and a reduction in immunological function supporting the spread of infections and the development of other severe ulcerative lesions.

6. Environmental conditions in the Swan Canning Riverpark

Estuarine environments are dynamic environments that are sometimes subject to rapid changes in their physical and chemical composition (e.g. the 'flushing' that occurs following a heavy rainfall event and subsequent run-off), or to more sustained periods of environmental change (e.g. seasonal shifts). Estuaries are often limited in their capacity to buffer freshwater inputs, as they are partially self-contained bodies of water, and mixing with waters from marine areas may only occur through narrow channels and when river flow and tidal movements permit water exchange between these environments. While tidal movements allow for water exchange between an estuary and adjacent marine areas, this process will vary both spatially and temporally, depending on factors such as the tidal cycle, the magnitude of tidal movement, the physical features of the estuary, atmospheric conditions, and the volume of water arriving into the estuary from catchment areas.

Estuaries in temperate (southern) Australia may experience sustained periods in which salinities are lower than in surrounding marine environments because of seasonal (winter-spring) patterns of rainfall (temperature regimes may also differ). This seasonal pattern, along with daily tidal movements, means

60 Like burns, such severe lesions would cause (or exacerbate existing) electrolyte imbalances.

that osmoregulation is an important physiological challenge for organisms inhabiting estuarine ecosystems. Many animals, such as sessile invertebrates (e.g. mussels), cannot move to escape changes in water quality, and thus typically possess anatomical (e.g. closing shell), physiological, or life-history adaptations allowing them to cope with these changes. More mobile fauna may exhibit seasonal patterns of residency or shifts in distribution with estuaries, or may have physiological adaptations that allow them to adapt to changing environmental conditions.

The Swan Canning Riverpark is broadly similar to other temperate estuaries in Australia. Rainfall events can rapidly alter the temperature and salinity of the entire estuary system, and the estuary typically experiences changes in these parameters as a result of seasonal shifts in precipitation. The progression on this seasonal change is predictable, although the timing and extent of change varies to some degree from year to year. In autumn, the advent of cooler weather and winter rains increases the flow of fresh to brackish water from drains and tributaries, decreasing salinity and water temperature. These changes are most substantial within upper reaches, but may also influence environmental conditions within the middle (e.g. the broad basin habitats of Perth Waters and Melville Waters) and lower reaches of the estuary. In spring, declining rainfall leads to an increased marine influence, with the salt wedge progressing further upstream and the water column becoming increasingly stratified. These hydrodynamic changes influence dissolved oxygen concentration at depth, nutrient release, algal blooms, and fish die-off events. Environmental conditions in the Swan Canning Riverpark are discussed further in Section V (Epidemiology).

Section III (Ecology) reviewed what is known about the ecology of dolphins in the Swan Canning Riverpark. The movement patterns of dolphins are likely to influence the duration and intensity of their exposure to areas with low salinities in the Swan Canning Riverpark. For example, movement patterns may lead some dolphins to range within the upper reaches of the Swan River because (for example) prey are present in these areas. Based on observations from 2001-3, we know that dolphins: (a) move into and out of the lower and middle reaches of the estuary on a daily or near-daily basis (i.e. between marine areas and the estuary); (b) at least occasionally range within the upper reaches of the estuary; and (c) may reside within the estuary for several hours (and sometimes longer) before moving back out into marine areas such as Owen Anchorage.[61]

These movement patterns mean that dolphins could experience sustained periods of immersion in low-salinity water (and also rapid changes in salinity when they transit from one environment to another). There may also be more specific behavioural components determining the duration and intensity of exposure. These could relate, for example, to seasonal shifts in prey that cause dolphins to move into the upper reaches of the Swan and/or Canning River to forage. Temperatures also typically decline during low-salinity conditions, and may create an additional physiological stressor.

61 At this time, we do not have a good understanding of seasonal variation in movement patterns, although initial analyses of the distribution of dolphin sightings from 2001-3 indicates that some seasonal differences do occur (Moiler 2009).

Sections V (Epidemiology) and VI (Pathology) consider these issues in further detail and, in particular, address the questions of: how specifically exposure to low salinity could affect dolphins within the Swan Canning Riverpark; how such effects may interact with other factors; why only certain individuals might be affected; and the environmental conditions potentially associated with health effects.

H. Contaminants[62]

Chemical contaminants are discussed in detail in Section VII (Contaminants). Bottlenose dolphins inhabit environments in which anthropogenic contaminants are ubiquitous and, being long-lived apex predators, they inevitably accumulate organic and inorganic contaminants across their lifetimes, particularly if they range within coastal and estuarine areas near human population centres (Reddy et al. 2001a, Wells et al. 2004, Hall et al. 2006, Hansen et al. 2006, Yordy et al. 2010). Many contaminants persist in the environment for long periods of time, and thus can remain cycling within ecosystems long after their use has been discontinued. The availability of contaminants to biota may also change over time in relation to factors such as climate change, changes in trophic structures, and disturbance of contaminated sites (Jorgenson et al. 2001, Evans 2003, Schiedek et al. 2007). These processes can affect the complex series of biogeochemical interactions influencing bioavailability.

Contaminants can affect animals through acute toxicity (e.g. poisoning from short-term "pulse" exposure to high concentrations of a contaminant), or through chronic (i.e. long-term) exposure to steady levels[63]. A range of contaminants may bioaccumulate in marine mammal tissues, thereby increasing the potential for reaching toxic thresholds. Contaminants may act by specific and/or non-specific molecular mechanisms, and interact with other contaminants at molecular binding sites in a number of ways. There is increasing focus on understanding how these effects influence individual survivorship and reproductive success, and their implications at a population-level (e.g. Kannan et al. 2000, Schwacke et al. 2002, Jepson et al. 2005, Yordy et al. 2010). Nonetheless, much of our understanding of these effects remains based on extrapolation from *in vitro* (e.g. using cell lines from mice) and *in vivo* (e.g. using whole fish) studies on model species, because of the logistical and ethical difficulties inherent in toxicological studies of long-lived and free-ranging marine mammals.

Contaminants are typically considered as a potential contributing factor in marine mammal mortality events, mainly because of their potential impact on immune function, and thus a population's susceptibility to disease (Fair and Becker 2000, Ross 2002, Gulland and Hall 2007, Van Bressem et al. 2009a). Contaminants may facilitate the occurrence of disease by: reducing an animal's resistance to infection; diminishing its capacity to mount an immunological response once infected (e.g. by adversely affecting lymphocytes); enhancing the replication of viruses within hosts; and facilitating transmission between individuals (Lahvis et al. 1995, de Swart et al. 1996, Ross 2002).

62 The term 'toxicant' is also often used to describe the pollutants discussed here, and emphasises the toxic effects associated with some contaminants, particularly when present in concentrations above thresholds for toxicity.

63 Persistent organic contaminants (e.g. dieldrin, DDT) are not considered acutely toxic. Their effect is more latent.

However, linkages between mortality events and contaminant concentrations remain largely correlative because of: confounding factors such as other sources of mortality; difficulty in obtaining proof of causation through post-mortem examination and tissue analyses of carcasses; the general limitations of retrospective studies (e.g. lack of comparative and long-term data on reproduction, mortality rates, and morbidity); and the lack of empirical studies on marine mammals. Studies have often concluded that contaminants could have played a supplementary role in the mortality event, particularly where contaminant concentrations were considered high in comparison to other populations or have exceeded thresholds of toxicity (in situations where such thresholds have been determined). In general, there is strong circumstantial and correlative evidence to suggest that exposure to high concentrations should not be excluded as a factor in large-scale mortalities, and is best viewed as a potentially facilitative and synergistic factor, rather than a primary means of causation for mortality (Fair and Becker 2000, Ross 2002, Evans 2003, Van Bressem et al. 2009a). It is should be emphasised that the potential impact of contaminants is clearly less acute than that of the more virulent primary pathogens, such as morbillivirus[64], and recent evidence also suggests that biotoxins from harmful algal blooms can cause acute mortality at rates similar to that of a virulent primary pathogen (Hall 1995; Taubenger et al. 1996; Bossart et al. 1998; Kennedy 1998; Van Bressem et al. 1999, 2001, 2009a; Scholin et al. 2000; Van Dolah et al. 2003; Flewelling et al. 2005).

The view of contaminants as a facilitative factor is consistent with the typically multi-factorial nature of mortality events, and the difficulty in elucidating the contribution of different sources of mortality, i.e. whether (and to what degree) the occurrence of disease and mortality reflect the effects of primary pathogens (e.g. morbillivirus), environmental factors (e.g. abnormal temperatures), biotoxins, and/or contaminants, all of which may lead to decreased immune function (Fair and Becker 2000, Ross 2002, Evans 2003, Halvorsen and Keith 2008). For example, Van Bressem et al. (2009a) observed that in the case of cetacean morbillivirus in Mediterranean striped dolphins (*Stenella coeruleoalba*): “several environmental factors, i.e. fisheries interactions, inbreeding, migration, high contaminant loads, higher SSTs [sea-surface temperatures] and limited prey availability may have synergistically interacted to increase the severity of the disease” (p.145-6).

I. Other stressors

Other factors, both natural and anthropogenic, can injure, kill, or cause physiological stress, reduced immune function, and diminished condition in marine mammals, including: anthropogenic noise; severe depletion of food sources resulting in starvation, loss of blubber reserves, and a decline in body condition (e.g. through a mass-mortality of prey or a climate event such an El Niño); and harassment or disturbance (e.g. from fishery or tourism interactions) (Curry 1999; Fair and Becker 2000; Frohoff 2000; St. Aubin 2002; Bejder et al. 2006a,b; Clark et al. 2006).

64 Although some dolphins may survive the initial stages of infection, and then go on to die at a later time because of opportunistic infections related to the effects of morbillivirus.

J. Trends in marine mammal health

There is growing recognition that marine mammal health may—on a global basis—be in decline (Gulland and Hall 2007). This conclusion is based on evidence of an increase in the number of unusual mortality events and in the rates of morbidity within many populations, particularly within coastal and estuarine environments (Reddy et al. 2001a, Ross 2002, Wells et al. 2004, Gulland and Hall 2007, Van Bressem et al. 2009a). This view is also consistent with evidence that many of the key infectious diseases affecting cetaceans: (a) are emerging; (b) having increasing impacts upon population-level processes (i.e. birth and mortality rates); and (c) are at least partially driven in prevalence and severity by anthropogenic factors, including environmental contaminants, climate change, entanglements, fisheries interactions, species introductions, harmful algal blooms, and other stressors) (Van Bressem et al. 2009a).

In general, dolphins within estuarine and coastal environments are likely to be experiencing a greater and more severe range of stressors than in the past, and this increase in the intensity and forms of stress is likely to exacerbate the effects of interactions among and between stressors. For example, novel or newly-emerging pathogens are likely to be most prevalent in populations whose immune function is already affected by contaminants, human-induced injuries, ecological stress, changing environmental conditions, disturbance from human interactions, and other factors (Van Bressem et al. 2009a). This suggests that, at least in some cases, certain anthropogenic factors could also be said to be ‘emerging’. While these emerging stressors may have been biologically insignificant in the past, they may now exert a discernible effect on population-level processes, particularly within populations that are small and declining.

V. Epidemiology

The main objective of this section is to describe the epidemiology of the dolphin mortalities in the Swan Canning Riverpark in 2009 and in previous years, and to characterise the potential aetiological factors for these mortalities. Information on mortalities in the Bunbury area is also presented.

The information in this section should be considered in context with: the review of factors affecting the health of estuarine dolphins in Section IV (Health); the pathology findings in Section VI (Pathology); and the contaminant results in Section VII (Contaminants). We emphasise that Section VI (Pathology) contains a more detailed discussion of the pathology of the mortalities than is presented in this section.

A. Multi-factorial aetiological framework

Causation is the critical issue for investigations of unusual mortality events. The cause(s) of an event are rarely immediately clear, and studies must proceed by gathering and assessing evidence that will allow them to characterise the underlying sources of mortality for the event. Ultimately this process relies on understanding the pathology of individual cases of mortality and then determining, if and to what degree, commonalities exist among the group of mortalities.

Determining why an individual animal died is typically a complex undertaking. While some stressors are sufficiently virulent or traumatic to cause death by themselves, mortality generally reflects a multi-factorial aetiology in which several mechanisms interact to instigate and sustain the processes culminating in death. In other words, a multi-factorial aetiology means that several, potentially interacting (synergistic) factors, may combine to ultimately result in the death of an individual.

The information in Section IV (Health) suggests that factors potentially contributing to an unusual mortality event may include: shark predation; primary pathogens; algal biotoxins; reduced immunological function (caused by primary pathogens, contaminants, wounds, physiological stress from adverse environmental conditions, or other stressors); human-induced injury; diminished condition (e.g. from starvation); and secondary infection by opportunistic pathogens. As sources of mortality, these factors can be organised into three categories:

(a) **Lethal stressors** – These are stressors that result in death within a short period of time (i.e. minutes to days) through traumatic injury, toxic effects, or infection. They could include incidences of: predation, vessel strike, severe entanglement, infection by virulent primary pathogens, and exposure to concentrations of algal biotoxins sufficient to cause mortality.[65]

[65] We note that, in the scientific literature, concentrations of persistent organic contaminants like those observed in the Swan dolphins are not thought to be acutely toxic, but to have a more latent effect. It is also possible that harmful biotoxins could have a sub-lethal effect that, in concert with other factors, could result in the death of an individual.

(b) **Infectious disease** – These are primary and secondary pathogens that do not result in acute mortality but nonetheless result in, or contribute, to mortality. This may involve infections by that do not result in acute mortality but compromise immunological function in such a way that death eventuates in concert with other processes.

(c) **Environmental and anthropogenic factors reducing immunological function and/or causing tissue damage** – These are stressors that, while not causing mortality directly, do reduce immunological function and support the manifestation of infectious disease or some other process leading to mortality. These could include: wounds from human-induced injuries that provide a portal of entry for pathogens; contaminants with immunosuppressive effects; and physiological stress induced by exposure to adverse environmental conditions. Environmental conditions could also cause osmotic damage to epidermal tissue.

Limitations for understanding the causes of the mortalities

Several caveats should be applied to discussion of the causes of the 2009 mortalities. Firstly, our conclusions are based on post-mortem examination of only four dolphins and, in one instance, the carcass was in poor condition. This makes it difficult to definitively determine the contribution of any individual causal factor or the interaction of multiple potential causal factors. Secondly, our understanding of the aetiology for the 2009 mortalities should be considered subject to review until some additional analyses have been completed. These analyses relate, in particular, to efforts assessing whether cetacean morbillivirus was present and comparing the poxvirus identified in dolphins from the Swan Canning Riverpark with poxviruses sequenced elsewhere. Thirdly, what we know about the deaths is based upon post-mortem examination. We know little or nothing about the life histories of the dolphins that died, and in particular the suites of stressors that they experienced, the intensity and interaction of these stressors, and their individual susceptibilities to disease.

There is also a lack of information on the prevalence of disease in marine mammals in Western Australia. Thus, while the post-mortem examinations provide a solid scientific basis from which to understand the pathology of the 2009 mortalities, there are few epidemiological data that we can use to place these mortalities and their aetiologies within a broader (i.e. regional) context. We note these caveats as a way of indicating the limitations to our understanding the causative factors underlying the deaths of these dolphins.

B. pre-2009 dolphin mortalities in the Swan Canning Riverpark

Table 7 reviews information on mortalities that were recorded within the Swan-Canning Riverpark from 2002-2008, and Figures 25-30 provide images for some of the mortalities. There were six mortalities recorded during this period, suggesting a stranding rate for carcasses of approximately one per year. This estimate is unlikely to reflect actual rates of mortality for several reasons. Firstly, dolphins resident in the Swan Canning Riverpark also range outside of the estuary, indicating that at least some deaths will occur in coastal areas, and carcasses of these animals may, for various reasons

(e.g. drift, submersion, predation or scavenging by sharks), not be recovered or recorded. Secondly, mortality records are available for only a limited period of time and are somewhat limited by the opportunistic manner in which data were collected and carcasses recorded and recovered. Finally, there has been monitoring of dolphins within the Swan Canning Riverpark since 2003. Where continuous long-term studies occur rates of mortality can be estimated from the rate of disappearance for know individuals, i.e. when a known individual is not re-sighted for an extended period of time, this individual can be presumed to have died.

However, while this stranding rate has limitations, it does provide a baseline against which the 2009 mortalities can be compared, and indicates that the number and timing of mortalities in 2009 is anomalous, i.e. six deaths within five months, with three deaths of these deaths within three weeks of each other (5 June to 21 June 2009) and three other deaths within five weeks of each other (17 September to 25 October 2009).[66]As discussed in Section II (Introduction), the stranding data support the conclusion that the 2009 deaths constitute an *unusual mortality event* as defined by criteria developed to support the U.S. *Marine Mammal Protection Act* 1972.

Most of the records of dolphin mortalities in the Swan Canning Riverpark prior to 2009 contain little supporting information. Carcasses were also often recorded as being in an advanced state of decomposition. Four of the six recorded deaths (i.e. observations of stranded carcass) occurred in the October-November period. Post-mortem examination was undertaken in one instance (18 November 2007) and findings from that examination are reviewed in context with the 2009 mortalities (see below).

Two dolphins that died between 2002-8 exhibited skin lesions based on: (a) a single photographic image available (October 2003; Figure 25) and (b) photographic images and post-mortem examination (18 November 2007; Figure 26). The presence of these lesions and the timing of the two deaths (i.e. in October and November) suggest aetiologies potentially similar to that of the two adult females that died on 17 September and 25 October 2009. Although difficult to confirm from the image, Figure 25 suggests severe ulcerative-type lesions similar to those observed in the two 2009 females (but with much less of the body surface area affected). The carcass of the October 2003 mortality was recovered near Ascot, suggesting a possible association with use of the upper reaches of the Swan River, which tend to have brackish salinities during the winter-spring period. The female that died on 25 October 2009 was also observed near Ascot on 3 October 2009 with skin lesions present (Figure 28).

The presentation of the 18 November 2007 dolphin had certain commonalities with the two females that died on 17 September and 25 October 2009, particularly the presence of poxvirus-induced skin lesions. However, this individual did not have the extensive pattern of severe ulcerative skin lesions

66 All carcasses were recovered within the Swan Canning Riverpark. In one instance (the 5 June calf) the carcass was observed in various locations within the estuary, including the Inner Harbour, as the mother was supporting and moving the carcass for several days prior to its recovery.

covering much of the body surface as was observed in the deaths of the two 2009 females. These commonalties and differences are discussed further below and in Section VI (Pathology).

Figures 27a and 27b are images of a dolphin mortality along the eastern shoreline of Freshwater Bay taken around 16 April 2006 (R. McCauley, Curtin University, personal communication). Images of the dorsal fin indicate that this dolphin is 'Middy', an adult male considered resident in the Swan Canning Riverpark from 2001-3. This appears to be the dolphin classified as the 7 October 2006 mortality in the November 2009 Situation Report on the dolphin mortalities in the Swan Canning Riverpark.[67] The carcass was recorded as being in an advanced state of decomposition upon recovery at Freshwater Bay in October, but appears recently deceased (i.e. minimally decomposed) in the April images. The images indicate that the animal was in poor body condition (i.e. emaciated) and show a large abrasion-type wound along the dorsal surface anterior to the dorsal fin (i.e. a 'degloving' injury involving avulsion of the skin from the underlying tissue). This injury is indicative of being struck with (or grazed by) a broad sharp object, and may constitute an example of human-induced injury. However, this dolphin also had a shark attack wound on its ventrum (there are no images of the shark attack wound), and we cannot ascertain whether the degloving injury occurred prior or subsequent to the shark attack, or whether it may have occurred as a post-mortem event. Views of the genital region confirm the dolphin as a male, and the size and intensity of ventral speckling indicate that the animal was an adult.[68]

C. 2009 dolphin mortalities in the Swan Canning Riverpark

Appendix A5 contains the full post-mortem reports for the 2009 dolphin mortalities in the Swan Canning Riverpark, as well as findings from post-mortem analyses conducted for the 18 November 2007 mortality. A summary of this information is given below and represents a synopsis of the most significant findings. Section VI (Pathology) provides a more-detailed discussion of the pathology findings. The extent of post mortem decomposition/autolysis is represented by a 'carcass condition code' (as outlined by Pugliares et al. 2007).[69] Figures 28-30 provide images of these dolphins (see also post-mortem reports in Appendix A5).

Six bottlenose dolphin deaths occurred within the Swan Canning Riverpark in 2009. The mortalities occurred in two clusters: (a) three deaths in June 2009 and (b) three deaths in September-October 2009. These deaths involved: two adult females [17 September, 25 October]; one adult male [9 October]; one male calf [5 June]; one juvenile male [8 June]; and one sub-adult/adult female [21 June]. Two of the dolphins [5 June and 9 October] were in an advanced state of decomposition at the time of recovery, and no post-mortem examinations were conducted. Post-mortem examinations were conducted for the other four dolphins. Additional analyses were also undertaken, including analyses to determine the

67 There remains uncertainty on this issue as of mid-May 2010. It would appear likely that the death recorded as being in October is actually the dolphin from April.

68 Bottlenose dolphins in the Perth area have less ventral speckling than has been observed in some locations (e.g. Shark Bay), but acquire more speckling as they grow older.

69 Carcass condition codes from Pugiliares et al. (2007) are: 1 – alive, 2 – fresh carcass, 3 – moderate decomposition, 4 – advanced decomposition, 5 – mummified or skeletal remains.

concentrations of certain contaminants within dolphin tissue samples. Certain analyses have yet to be completed, in particular those involving viral pathogens. Post-mortem findings from the 18 November 2007 mortality were also considered.

Synopsis of post-mortem examinations of dolphins from Swan Canning Riverpark

2007	
18 November	Post-mortem at Perth Zoo Case No: AS-07-3710 Carcass condition code = 2 (fresh) Adult lactating female in good body condition. Recovered by DEC at Bicton Baths. **Description** Numerous skin lesions present; the majority of the lesions were non-ulcerative, however some bordered a focally extensive ulcerative lesion immediately caudal to the blowhole. Intracytoplasmic viral inclusion bodies detected in keratinocytes (skin cells of the outer epidermal layer), indicative of poxvirus infection (TSD).[70] Lymphoid depletion noted histologically with fibrosis and calcification of the mesenteric lymph node chain, indicative of chronic exhaustion secondary to chronic inflammation.
2009 – June cluster	
5 June	MUVS Pathology No: 09/663 Carcass condition code = 3 (moderate decomposition) Male calf in good body condition. Was observed dead on days previous with mother attempting to support the carcass. Recovered by Swan River Trust in lower reaches. Post-mortem analysis severely limited by advanced state of decomposition. Preliminary analyses of dental layering suggest a minimum age of 5 years.[71]

70 Inclusion bodies are an area within the nucleus or cytoplasm of a virus-infected cell that exhibits altered staining behavior (under histological examination).

71 Analyses of layering within teeth can be used to provide minimum age estimates for dolphins. As wear and other factors can remove outer layers, the methods provide minimum age estimates only. The age estimates presented here are preliminary.

8 June

MUVS Pathology No: 09/637
Carcass condition code = 2-3 (fresh to moderate decomposition)
Male juvenile in good body condition.
Recovered by Swan River Trust in lower reaches.
Preliminary analyses of dental layering suggest a minimum age of 3+ years.

Description

Fungal meningoencephalitis with intralesional fungal organisms consistent with *Aspergillus* spp. (fungal infection of the brain).

Segmental jejunal (small intestinal) infarction likely secondary to spread of *Aspergillus* within the bloodstream.[72]

Lymphoid depletion noted histologically.

21 June

MUVS Pathology No: 09/664
Carcass condition code = 2 (fresh)
Female sub-adult/adult in good body condition.[73]
Recovered by Swan River Trust and Department of Transport (Marine Safety) near Ascot.
Preliminary analyses of dental layering suggest a minimum age of 16 years.

Description

Human induced injury: severe chronic fishing line entanglement of the right fluke.

Bronchopneumonia with intralesional fungi consistent with *Aspergillus* spp. (in addition to this, two types of opportunistic bacterial pathogens found on lung culture).

Kidney infection [one type of opportunistic bacterial pathogen found on kidney culture—identical to one of the two identified on lung culture, indicative of systemic haematogenous (via bloodstream) spread].

Lymphoid depletion noted histologically.

Septicaemia likely.

72 An infarction is defined as a "localized area of tissue death secondary to insufficient blood supply produced by the occlusion of the arterial supply or the venous drainage to that area" (Blood and Studdert 1988).
73 Post-mortem examination indicated that the individual had not yet given birth.

2009 – September-October cluster	
17 September	Post-mortem at Perth Zoo Case No: AS-09-2912-F-V1 Carcass condition code = 2 (fresh) Adult female in reasonable condition. Recovered by DEC south of Windan Bridge.[74] **Description** Numerous, extensive ulcerative skin lesions with intralesional opportunistic bacterial and fungal organisms noted histologically. Intracytoplasmic viral inclusion bodies detected in keratinocytes, indicative of poxvirus infection (TSD).
9 October	Adult male in good body condition. Carcass condition code = 4 (advanced decomposition) Too decomposed to carry out post-mortem analysis. Recovered by Swan River Trust at Freshwater Bay.
25 October	MUVS Pathology No: 09/1108 ('Leeuwin') Carcass condition code = 1 (fresh) Aged female in poor body condition. This animal identified as a known Swan resident from 2001-3. Died under observation at Waylen Bay (near Heathcote). Was swimming abnormally before becoming stranded on the beach and dying. Recovered by Murdoch University, Curtin University, and Swan River Trust. **Description** Numerous, extensive ulcerative skin lesions with intralesional opportunistic bacterial and fungal organisms noted histologically. Intracytoplasmic viral inclusion bodies detected in keratinocytes, indicating poxvirus infection (TSD). Evidence of acute (recent) human induced injury – fishhook lodged in oesophagus, minor entanglement with minimal tissue laceration of the right pectoral fin.[75]

[74] This dolphin was observed lingering around Barrack Street Jetty before its death, with anecdotal reports of the animal being fed by humans (see Section IV – Health). The feeding is not considered to have directly contributed to the dolphin's death, and is likely a reflection of the animal being unable to feed itself adequately and so seeking food through abnormal means.

[75] This likely occurred as the condition of the animal declined and she became unable to feed herself adequately, and (as with the 17 September female) began seeking food through abnormal means.

D. Were the two clusters of dolphin mortalities in 2009 related?

The small sample size of mortalities limits efforts to examine relationships among the mortalities and to assess the significance of these potential relationships. The clustering of the 2009 deaths in June 2009 and in September-October 2009 suggests the possibility of there being some commonality (i.e. shared factor or factors) for the deaths during these two time periods.

However, the two clusters of mortalities differed from each other in several salient aspects of their epidemiology. In particular, the June deaths and the September-October deaths: occurred at different times of the year/seasons [early winter vs. late winter]; had different causative factors (see further below); and involved different age-sex classes. These differences suggest the two clusters of mortalities may be unrelated, although the mortalities still may have shared underlying aetiological factors, e.g. a similar suite of stressors affecting immunological function.

A particularly notable difference between the two mortality clusters was the fact that the June cluster involved two dolphins of a young age [5 June male calf (5+ years old) and 8 June apparently independent juvenile (3+ years old)] and one dolphin affected by a long-term entanglement injury [21 June female]. These individuals were, because of age and injury, likely more susceptible to disease and other stressors than other dolphins within the estuary. Mortality rates for bottlenose dolphin calves are high [30-50% of calves born die prior to weaning, see Section III – Ecology], and juvenile dolphins are similarly at greater risk of infectious disease than adults (e.g. Van Bressem et al. 2003, 2009b; Iluko et al. 2010), as well as being more vulnerable to other stressors because of size, immunological status, experience, and other factors. Dolphins with entanglement (or other similar) injuries are at higher risk of secondary infection because the wounds offer a port of entry for opportunistic pathogens.

The adult female mortalities on 17 September 2009 and 25 October 2009 had similar presentations that were distinct from those observed in the June 2009 cluster of mortalities, but similar in certain respects to those observed with the 18 November 2007 adult female. We cannot determine the pathology for the 9 October 2009 adult male, as the advanced state of decomposition of the carcass precluded post-mortem examination. Images of the carcass suggest that dermal lesions may have been present, but this is speculative (Figure 29). The presentations associated with these mortalities are discussed further in Section VI (Pathology).

E. Non-contributing factors for the 2009 dolphin mortalities

1. Predation and vessel strike

None of the carcasses from the 2009 mortalities showed injuries indicative of recent shark attack or a vessel strike, e.g. lacerations or internal haemorrhaging (blunt force trauma).

2. Algal biotoxins

Sampling of the phytoplankton present in the Swan Canning Riverpark is conducted on a weekly basis. While these data have certain limitations,[76] they are adequate to support assessments of whether potentially harmful algae (i.e. a phytoplankton species producing a biotoxin) were present and, if present, their approximate concentration in the water column. Analyses of phytoplankton data from May-October 2009 indicate that exposure to algal biotoxins is unlikely to have contributed to the mortalities. The incidence of the diatom *Pseudo-nitzschia* spp. at Salter Point in May and early June 2009 was the only notable occurrence, but the observed concentrations (25-34 cells/mL) and lack of any reported ASP-associated[77] symptoms indicate that the involvement of biotoxins was unlikely.[78] No analyses were conducted to evaluate tissue concentrations of biotoxins. The low probability of a biotoxin-aetiology is also supported both by the absence of a harmful algal bloom during this period, and by the absence of mortalities indicative of biotoxin exposure in other species known to be affected by algal biotoxins, such as fish and birds.

3. Faecal pathogens

There was no indication of the specific pathogens known to be commonly associated with faecal contamination (e.g. *Escherichia coli*, *Enterococci spp*, *Salmonella* spp. etc) in microbial cultures taken from the dolphins examined post-mortem in 2009. The organisms cultured were ubiquitous in the environment or known to be commensals of dolphin skin.

F. Contributing factors for the 2009 dolphin mortalities

1. Primary and secondary pathogens

We are awaiting the outcomes of analyses to determine if cetacean morbillivirus was present in tissue samples taken from the 2009 mortalities. These analyses have not been completed at the time of publication for this technical report. As such, we cannot comment at this time on the potential role of cetacean morbillivirus in the 2009 Swan dolphin mortalities or Bunbury dolphin mortalities other than to say it is a factor that requires definitive exclusion and that we are undertaking appropriate analyses

76 The routine (i.e. weekly) sampling regime is not designed to specifically focus on harmful phytoplankton species, and the routine sampling methods, while robust, could underestimate the concentrations of phytoplankton present. It is worth emphasising, however, that few locations in Australia or internationally conduct this type of sampling on such a consistent and long-term basis. In the U.S., for example, investigations of unusual mortality events linked to harmful algal blooms most often rely on phytoplankton data that is only collected intensively after the mortality events have already occurred.

77 Amnesic Shellfish Poisoning

78 Marine mammals (sealions) have been fatally poisoned by *Pseudo-nitzschia australis* (through anchovy, a prey species) at bloom concentrations of 130 cells/mL (Scholin et al. 2000). Where *Pseudonitzschia* is endemic, some natural (or background) concentration of domoic acid will occur in the tissues of fish and dolphins as this compound can be transferred through food webs. Future sampling of dolphin tissues could test for natural concentrations of domoic acid and other toxins (e.g. brevetoxins). This background information would better characterise the elevated concentrations that might occur if blooms of potentially harmful algae occur in the future.

to pursue this objective (in collaboration with other researchers and institutions). The issue of morbillivirus is discussed further in Section VI (Pathology). The role of opportunistic pathogens is also discussed further in Section VI (Pathology).

2. Entanglement

Section VI (Pathology) reviews the role of entanglement injuries in the 2009 mortalities. Two of these mortalities had active entanglement injuries (21 June 2009 and 25 October 2009). The entanglement injury in the 21 June 2009 female was a long-term injury, as the female was observed more than a year previous with this same entanglement (D. Coughran, DEC, personal communication). Long-term data on the prevalence of human-induced injuries in dolphins from Cockburn Sound and nearby areas suggest that the rate of entanglements injuries is high for dolphins in the southern metropolitan waters of Perth, and may have a biologically significant effect (Finn 2005, Donaldson et al. 2010; Table 5). In particular, we note that between 1996-2004, at least six calves within Cockburn Sound experienced entanglement events/injuries, and a seventh calf exhibited injuries indicative of a vessel strike. These findings are concerning when seen in the context of the small size of the resident communities in Cockburn Sound and the Swan Canning Riverpark, as well as being an issue of animal welfare.

3. Contaminants

Section VII (Contaminants) reviews the findings from contaminants analyses conducted on tissue samples taken from the 2009 Swan dolphin mortalities and from mortalities in the Bunbury area.

4. Environmental conditions

We discuss the potential influence of environmental conditions below.

G. Role of environmental conditions

1. Aetiological framework

Section IV (Health) suggested that low salinity conditions could cause osmotic disruption of epidermal cells and/or systemic physiological stress to dolphins.[79] Section VI (Pathology) discusses the pathology of the skin lesions observed in dolphins from the Swan Canning Riverpark and their potential relation to conditions of low salinity. Here we describe the environmental conditions within the Swan Canning Riverpark during the period in which the 2009 mortalities occurred (and in other years), and observations of dolphins in 2009 within areas of low salinity. We also draw on information from other studies to develop a potential aetiological framework for: (a) why the adverse health effects of low salinity conditions might only affect certain individuals; (b) why dolphins might range within areas of low salinity; and (c) whether the acute onset of skin lesions from low salinity could occur (i.e. in this context, an exacerbation of a pre-existing TSD lesion). This aetiological framework is based on the

[79] We use the term 'low' salinity conditions to refer generally to conditions in which salinities are substantially lower than in the marine environment (34-35 ppt). When salinity is discussed in relation to health effects on dolphins, low salinity refers to conditions considered potentially stressful to dolphin in the published literature, i.e. salinities less than 20 ppt. We note that this threshold is somewhat arbitrary when applied to free-ranging dolphins (see Section IV – Health).

conclusion that low salinity conditions were sufficient to potentially have an adverse effect on dolphins, and the basis for this conclusion is also addressed.

Several points should be made here. Firstly, the potential effects of environmental conditions should be viewed within a multi-factorial framework, and their effects considered within the context of other stressors affecting dolphins. Secondly, we emphasise the need to differentiate between the systemic physiological stress that exposure to low salinities could cause, and the potential for osmoregulatory disruption of epidermal cells that exposure to low salinity conditions could induce. While both affect physiological function, it is easier to distinguish between the two and discuss their potential effects separately. Finally, we also note the need to distinguish between: (a) the association that has some studies have observed between salinity and the prevalence of epidermal disease (including typical presentations of poxvirus/TSD skin lesions) within some dolphin populations [see Section IV – Health], and (b) a potential association between low salinity conditions and the more unusual presentations of poxvirus/TSD skin lesions that we observed in the 17 September and 25 October deaths in the Swan Canning Riverpark.

We have no data on the prevalence of TSD lesions on dolphins occurring within the Swan Canning Riverpark in 2009, and thus cannot address an association between salinity and TSD,[80] except to note that at least some individuals frequenting the Swan Canning Riverpark had poxvirus infections, and its typical presentation (i.e. in terms of prevalence and severity) could relate in part to environmental conditions including salinity (based on studies elsewhere, see Section IV – Health]. We can, however, examine the basis for an association between salinity and the severe lesions observed on the 17 September and 25 October based on post-mortem findings, water quality monitoring data, Dolphin Watch observations, and findings from other studies. We note, however, that uncertainty exists for the mechanisms involved in the progression of lesions to an ulcerative state in those two dolphins. This issue is discussed further in Section VI (Pathology).

2. Environmental conditions within the Swan Canning Riverpark

In late June through July 2009 the Swan and Canning catchments received significant rainfall that resulted in rapid runoff to the rivers and a decrease in salinity over a large area of the river systems (~50km). Between July and August 2009 the influence of freshwater flow switched the Swan River from being relatively saline to being more brackish to marginal and remained that way through much of September (Figure 31) The degree to which this change was observed and the period over which it occurred was dependent on site and the relative influence of both freshwater flow vs. tidal influence, with more upstream sites experiencing lower salinities for longer periods than downstream sites (Figures 32 and 33) [Appendix A3 gives the locations of water quality sampling sites within the Swan and Canning Rivers]. A halocline of saline water sitting below more fresh water was present throughout much of this period and resulted (through biological oxygen demand and limited mixing) in

80 However, this kind of analyses could be done through retrospective analyses of photo-identification images from the 2001-3 study, as well as much smaller number of photo-identification images from 2008-9.

some oxygen depletion at depth (Figure 32). This is a natural phenomenon and the location of these oxygen depleted areas varied over the time period. Water temperatures throughout much of this period ranged from 17 to 21°C.

Rapid salinity changes also occurred in the Canning River. The Kent Street weir boards were removed in late June 2009 and the saline condition of the river downstream of the weir rapidly changed to fresh and remained that way through September/October 2009 (Figure 33).

Seasonal changes in salinity of the Swan and Canning rivers are not uncommon and lower salinity conditions in the late winter-early spring period occur annually (Figure 34). There is, however, some inter-annual variation in degree to which salinities are reduced and the duration of the low salinity conditions (Figure 34). These are affected by the amount of rainfall, and thereby runoff received in any one year and these factors, together with tidal influence, also affect the downstream extent of low salinity conditions.

Salinities within the Swan Canning Riverpark between July and October 2009 were low enough to be potentially physiologically stressful to dolphins (Figure 31). That is, they were lower than 20ppt, a threshold level considered able to affect the health of dolphins held in captivity.[81] Weekly water quality sampling at the Narrows Bridge indicates that surface salinities at that location were <15 ppt from 3 July 2009 to 16 October 2009 (Figure 31). Although speculative, the deaths of Swan dolphins known to involve skin lesions (October 2003, 18 November 2007, 17 September 2009, and 25 October 2009) appear to coincide with relatively long periods (3-4 months) of low salinity conditions within the Swan Canning Riverpark (Figure 34). We note, however, that there have also been long periods of lowered salinity in other years when no dolphin deaths have been recorded (e.g. 2005).

Once again, this is suggestive of individual-specific multifactorial aetiologies involving a particular suite of factors (e.g. physiological state, immunological status, presence and severity of poxvirus infection, behavioural patterns, presence of other stressors, etc.) coming together at a particular time. We address the issue of why only certain dolphins (i.e. the two dolphins with severe ulcerative skin lesions) might have been affected by low salinity conditions below.

3. Observations of dolphins within the Swan Canning Riverpark

Previous sections [Section III – Ecology and IV – Health] described the movement patterns of dolphins within the Swan Canning Riverpark and indicated the potential for dolphins to be repeatedly (i.e. on a daily or near-daily basis) exposed to low salinity conditions over a sustained period of time (i.e. several months) within the estuary, during the seasons in which low salinity conditions commonly occur

81 This point was discussed in Section IV (Health).

(winter-spring). The periods of immersion could last for periods of hours as evidence by dolphin movement patterns and dolphins would experience a rapid transition to more marine salinities. [82]

As discussed in Section IV (Health), bottlenose dolphins range within estuarine habitats in which salinities occur at concentrations significantly lower than in the marine environment.[83] This includes, for example, dolphin populations associated within estuaries all along the eastern and southern coast of the U.S. (Waring et al. 1999, 2007). We are not aware of any studies that documented behavioural avoidance of low salinity areas within estuaries for bottlenose dolphins, although there are limits to how far they move up into rivers and tributaries that feed into estuaries. Although they possess the sensory capacity to detect varying salinities, at least three studies have documented the use of areas with extremely low salinity (Moreno 2006 – Galveston Bay, Texas; Barry et al. 2008 - Lake Pontchartrain, Louisiana; and Rowe et al. 2010 - Doubtful Sound, New Zealand). To illustrate this point, Rowe et al. (2010) noted:

> Could the Doubtful Sound dolphin population's consistent exposure to low salinity conditions be responsible for the higher extent of lesions in the population? Both Doubtful and Dusky Sounds receive high annual rainfall that results in a well-defined low salinity layer (LSL) on the surface of the fiords. In Dusky Sound, the LSL is temporally variable, but the additional freshwater discharge into Doubtful Sound augments the LSL and ensures it is a constant feature in the fiord (Gibbs 2001). The salinity of LSL in the inner regions of Doubtful Sound is less than 10 psu (Gibbs et al. 2000), which falls well outside the range recommended for the maintenance of dolphins in captivity (25 to 35 psu). Bottlenose dolphins in other locations make occasional forays into low salinity environments, but the Doubtful Sound dolphins are unusual in that they are exposed to a distinct low salinity layer throughout the year. [p.87-88] [84]

Barry et al. (2008) also found that dolphins remained within a low salinity habitat (the brackish lagoon of Lake Pontchartrain) despite having the ability to leave the area, suggesting that behavioural factors may affect how dolphins use low salinity environments.

Although we do not know the ranging patterns of the two adult females who had had severe ulcerative skin lesions, Dolphin Watch observations from 2009 indicate that dolphins did range within areas of low salinity in 2009. Below are sightings from the Dolphin Watch program within the Swan River upriver of the Narrows Bridge between July and October 2009. Figure 35 shows the relative location of Dolphin Watch sightings within the Swan Canning Riverpark between June and December 2009,

82 It is possible that runoff from intense rainfall events could cause some acute physiological stress when these rainfall events occur at other times of the year. It is also possible that low temperatures during the winter-spring period may cause some degree of low-level physiological stress. Cheal and Gales (1992) found that, for the captive dolphins they studied (at the former Atlantis Marine Park in Perth), food intake rates were highest in mid-winter when water temperatues were lowest (17-18°C) and lowest in mid-summer when water temperatures were highest (21-22°C). Ham (2009) also found some indications that the prevalence of skin lesions on some dolphins in Cockburn Sound increased between July and October 2008. While these observations are speculative and anecdotal, they do: (a) indicate our lack of knowledge about the way that environmental conditions may influnce dolphin health and physiology, particularly in concert with other factors (stressors), and (b) suggest hypotheses that could be explored with further study (e.g. photo-identification studies documenting individual differences in the prevalence and severity of TSD lesions over time).

83 These include observations of dolphins within estuarine areas around Bunbury during the winter-spring period (see Appendix A4).

84 psu = practical salinity unit (equivalent to ppt or g/L); We note that the lesions Rowe et al. (2010) refers to as lesions that are characteristic of a typical presentation of TSD.

including observations within the Canning River. Sightings are recorded within zones (i.e. they are not exact locations).

Sightings of dolphins from Dolphin Watch from July-November 2009 within the Swan River upriver of the Narrows Bridge. Figure 35 gives the locations for the different areas.

Date of Sighting	*Time observed*	*Area observed*	*No. of dolphins observed*
15-Jul-09	15:30	10 and 11	3
7-Aug-09	14:30	9	4
30-Aug-09	10:48	14	1
6-Sep-09	10:00	18	1
26-Sep-09	11:45	15	1
28-Sep-09	12:15	14	2
5-Oct-09	15:30	14	2
10-Oct-09	7:45	14	2
15-Oct-09	10:30	9	2
18-Oct-09	16:50	12	1
22-Oct-09	11:30	12	1
27-Oct-09	7:50	12	3
6-Nov-09	6:48	12	1
7-Nov-09	7:05	12	3

The Dolphin Watch sightings indicate that dolphins did range at least occasionally within low salinity areas from July to October 2009. These observations, along with sighting and movement pattern data from 2001-3, suggests that in 2009 dolphins within the Swan Canning Riverpark at least occasionally used areas with low salinity. This is particularly likely to have occurred when low salinity conditions were present within the basin areas in the lower-middle reaches of the estuary (e.g. Melville Waters), given that dolphins appear to consistently use these habitats throughout the year (based on observations from October 2001-June 2003). Surface salinity data for the Narrows Bridge indicates that dolphins within Perth Waters would have experienced environmental conditions that were potentially physiologically stressful (e.g. could have caused osmotic damage to epidermal cells within or near areas affected by TSD lesions).

4. Effects of low salinity conditions: physiology and behaviour

The preceding water quality data and dolphin observations indicate that: (a) environmental conditions from July-October 2009 were sufficient to have been potentially physiologically stressful to dolphins and (b) dolphins did use areas of low salinity during this period. These conclusions lead to the question of why only certain dolphins appear to have potentially affected by low salinity conditions, and why these potential effects should occur in 2009 and not in other years in which similar conditions occurred. These questions can best be addressed by considering how the physiology and behavioural ecology of

bottlenose dolphins might influence the effects of environmental conditions and, in particular, why effects might vary between individuals.

Few studies have specifically addressed the effects of low salinity conditions on dolphins other than to observe broad correlations, particularly within a non-captive context. Barry et al. (2008) is perhaps the most well-documented study of free-ranging dolphins within a low salinity environment. This study described the condition of a group of bottlenose dolphins that became 'trapped' within a brackish lagoon (Lake Pontchartrain, Louisiana), and collected data on how long particular dolphins remained within the low salinity conditions and the prevalence and progression of skin lesions on these dolphins. This study has several implications for how we understand the potential health effects of low salinity conditions on dolphins within the Swan Canning Riverpark (see also Section IV – Health for a discussion of this study).

(1) Barry et al. (2008) found indications that **individual dolphins varied in how they were affected by exposure to low salinity conditions**. For example, at least two dolphins survived for an extended period (>45 days) in very low salinity conditions (1-5 ppt), while other dolphins appeared to be affected by skin conditions induced by exposure to low salinities after an acute period of exposure (i.e. 5-10 days of inhabiting low salinity conditions). They also noted that the prevalence of severe skin conditions was much higher in one group of dolphins they observed than in another group, although the two groups appeared to inhabit areas with similar salinity conditions. They suggested that these differences (in the presence/absence and prevalence of severe skin conditions) could relate to whether dolphins fed on prey that allowed them to maintain internal electrolyte (sodium) balances, and thus compensate for the physiological strain of exposure to low salinity conditions.

    Such physiological differences provide a potential explanation for why low salinity conditions in the Swan Canning Riverpark are associated with progression to severe skin lesions: (a) in only some dolphins [but not other dolphins experiencing equivalent or greater exposure] and (b) in only some years [but not other years with conditions comparable to the low salinity conditions observed in 2009].

    Although speculative, it is interesting to note that the three Swan dolphins in which severe skin lesions were documented (18 November 2007, 17 September 2009, 25 October 2009)[85] were all adult females, suggesting the possibility of an association between the unique physiological challenges experienced by adult females (e.g. gestation, lactation) and their vulnerability to physiological stress from environmental

[85] Severe ulcerative lesions covering a large proportion of the body surface were only observed in the 17 September and 25 October 2009 dolphins, but all three dolphins had poxvirus inclusion bodies present in skin tissues, indicating a clear commonality and suggesting a shared aetiology related to the presence of poxvirus/TSD (see Section VI – Pathology).

conditions.[86] Such a hypothesis is difficult to test and the sample size here is small (n = 3 individuals), but the findings of Barry et al. (2008) indicate that the effects of exposure to low salinity may vary between individuals, and suggest that this variation likely relates, at least in part, to individual physiological differences.

Other factors would likely interact with physiological differences, and these may relate to inter-individual differences in behavioural ecology, immunological function, poxvirus (or other pathogen) infection, contaminant burdens, and other factors. As discussed in Section IV (Health), it is not clear whether a lesion-inducing pathogen such as poxvirus was present in dolphins from Lake Pontchartrain, and thus if (and to what degree) differences in the development and severity of severe skin conditions might also relate to this factor.[87] We know that such a pathogen (poxvirus) is present in at least some individuals that frequent the Swan Canning Riverpark. Further, the post-mortem findings indicate that the presence and severity of poxvirus/TSD infections varies to some degree among dolphins using the Swan Canning Riverpark, as lesions indicative of TSD were not observed in the two June dolphins examined post-mortem. Such variation in the presence and severity of poxvirus/TSD infections would likely relate to physiological differences in some way, although the nature of this relationship is unclear and other (potentially related and interactive) factors such as immunological status are also likely to be important.

These considerations are consistent with a multi-factorial aetiology for: (a) the presence and severity of poxvirus/TSD infections and (b) the potential progression of poxvirus-induced skin lesions to a severe ulcerative stage. Further, these considerations indicate that the potential effects of exposure to low salinity conditions, both in terms of potentially causing systemic physiological stress and osmotic damage to epidermal cells, should be viewed within a complex matrix of contributing factors. The nature of this matrix will vary between individuals and is also likely to change over time, suggesting that the 2009 dolphin deaths involving severe ulcerative lesions are best considered as the outcome/coincidence of a suite of factors that were, to some unknown degree, unique to the individuals involved (i.e. the two adult females), and particular to the time in which they occurred (i.e. to the health status of the two females and the environmental conditions at that time).

(2) The Lake Pontchartrain observations also suggest that **dolphins may use areas with low salinity conditions for behavioural reasons**. Barry et al. (2008) noted that the dolphins

86 Other factors could also influence potential individual physiological differences.

87 We note that, as discussed in Section IV (Health), the presence of poxvirus or other lesion-inducing pathogen is probably not necessary for severe skin conditions to develop in dolphins from Lake Pontchartrain, given the extremely low salinities (<5 ppt) occurring there. These conditions are extreme enough to cause severe systemic physiological stress and osmotic damage to epidermal tissues in at least some individuals, thus leading to the development of severe skin conditions in these individuals (in combination with other factors).

they observed occurred in locations where bottlenose dolphins were thought to only infrequently occur prior to 2007,[88] and remained in low salinity areas although routes for them to leave these areas were available. In other words, dolphins apparently remained in areas of extremely low salinity (<5 ppt), even though they could have shifted to adjacent habitats with higher salinities. Similarly, Rowe et al. (2010) also observed that dolphins from Doubtful Sound also used areas with low salinities (i.e. the inner regions of Doubtful Sound), although other areas with higher salinities were available to them. These two studies, along with studies of bottlenose dolphins in estuarine environments around the world[89], clearly suggest that dolphins do not necessarily avoid areas which salinities that are substantially less than those occurring in other habitats that they may also use (e.g. adjacent coastal areas), and may do so even when use of these low salinity habitats adversely affects their health.[90]

As discussed in Section III (Ecology), life-long fidelity to a small and well-defined home range typifies the ecology of bottlenose dolphins within estuarine and protected nearshore environments such as bays, sounds, and inlets (see review in Connor et al. 2000). Within estuarine ecosystems, these home ranges will include habitats that are at least seasonally subject to reductions in salinity. Use of these areas thus reflects the species-typical behavioural ecology of bottlenose dolphins rather than as an 'abnormal' ecological pattern. However, like other estuarine organisms, dolphins that use estuarine areas (or other habitat) with low salinity conditions may well experience some degree of environmentally-induced stress, and this stress may well contribute to morbidity and mortality rates in a way that is qualitatively different than the environmental stressors experienced by dolphin populations occurring in coastal areas that are not subject to such environmental conditions. It is also the case that estuarine dolphin population are often impacted by a range of anthropogenic stressors because of the range of human activities that occur within estuaries and their catchments, and these stressors will add to any stress that dolphins may experience because of environmental conditions. Thus, it is perhaps not unexpected that estuarine populations have often been the most severely affected by the mass mortalities of bottlenose dolphins observed in the southeastern U.S. over the last quarter century (Waring et al. 1999, 2007).

In the context of the Swan Canning Riverpark, these considerations suggest that dolphins are likely to have ranged within areas of low salinity in the estuary rather than having avoided them. Certain behavioural factors could underlie the use of low salinity areas, at

88 Lake Pontchartrain was considered outside the normal distribution of bottlenose dolphins in that region.

89 As well as limited observations of dolphins within the Swan Canning Riverpark from 2001-3 and sightings from Dolphin Watch volunteers from June to October 2009 within the Canning River and the upper reaches of the Swan River.

90 Conversely, it is also the case that dolphins inhabiting estuarine ecosystems frequently range within areas of low salinity and may do so without adverse health effects (e.g. Barry et al. 2008 observed several dolphins that persisted within a low salinity area for extended periods without developing severe skin conditions.)

least on a seasonal or temporary basis (e.g. a preference for foraging for prey occurring in the upper reaches of the Swan River during the winter-spring period).

(3) Barry et al. (2008) also noted **the acute onset of skin conditions in individuals exposed to freshwater conditions**, citing unpublished data from their study that indicated that lesions could occur within 5-10 days after exposure to freshwater conditions. While acknowledging the substantial environmental differences between Lake Pontchartrain and the Swan Canning Riverpark, these observations are at least suggestive that skin lesions may develop relatively rapidly in individuals that are exposed to low salinity conditions. Several further remarks are relevant here: (a) the severity of poxvirus-induced lesions on captive dolphins can increase rapidly following exposure to adverse environmental conditions (e.g. Geraci et al. 1979); (b) the development of severe skin lesions developed by dolphins from the Gippsland Lakes occurred relatively soon after rainfall events substantially altered temperatures and salinities; and (c) the pathology of the Swan dolphins indicates that the progression of TSD lesions to severe erosive/ulcerative skin lesions occurred acutely, i.e. within a periods of days to weeks (see Section VI – Pathology).

Thus, the effects of exposure to low salinity conditions, whether involving systemic physiological stress and/or osmotic damage of epidermal cells, may: (a) occur acutely and (b) add to/exacerbate the influence of other factors that have already established an underlying susceptibility to disease [e.g. reduced immunological function, an entanglement wound] and/or resulted in a pre-existing health condition [e.g. a chronic low-level viral, bacterial, or fungal infection]. When these other factors are present, the additional stress of exposure to low salinity conditions could potentially lead to the onset of disease or cause existing conditions to rapidly worsen in severity.[91]

While these points regarding individual variation, behavioural factors, and the acute onset of skin conditions are necessarily speculative, they do indicate clear parallels between findings in the Swan Canning Riverpark and those from other studies. Taken collectively, they provide a plausible explanatory framework that addresses several areas of uncertainty regarding the epidemiology and pathology of the severe ulcerative skin lesions observed in some Swan dolphins. In particular, these three points address:

91 Exposure to low salinity conditions could also have a more chronic/long-term effect, which is suggested by studies showing an association between the prevalence of TSD and salinity (see Section IV – Health). In this scenario, exposure to low salinity conditions could potentially have both a latent/long-term influence and a more acute effect. A long-term study could examine an association between salinity conditions and the prevalence and severity of TSD lesions, by using photo-identification and photogrammetry techniques to document the presence and extent of TSD lesions on known individuals over time (e.g. examine variation between winter and summer).

(1) why severe ulcerative lesions might occur in: a) some dolphins but not in other individuals experiencing similar levels of exposure and b) at particular times but not at other times with broadly similar conditions;

(2) why dolphins might use habitats with low salinity conditions even though such conditions could affect their health; and

(3) the fact that severe skin conditions could develop acutely (i.e. in a span of days to weeks).

It should be emphasised that, based on the observations from the 2001-3 study and Dolphin Watch observations from 2009, it is likely that (between June and October 2009) at least some other dolphins experienced low salinity conditions similar to those experienced by the two adult females that died on 17 September and 25 October 2009, but did not, however, develop the severe ulcerative lesions observed in those two dolphins.[92] This epidemiological pattern suggests that the aetiology for the severe ulcerative lesions reflects a suite of factors that were, at least to some degree, particular (i.e. unique) to the two adult females.

These factors may have influenced the development of the severe ulcerative lesions in two ways: (a) by determining their exposure to low salinity conditions [e.g. when and for how long they were exposed] and (b) by influencing their state of health at the time. As discussed above, these factors were likely to have been multi-factorial, dynamic, and to have interacted with one another (i.e. to have had a cumulative and/or synergistic effect). These factors may have included the physiological condition, immunological status, ranging patterns, and the severity of their poxvirus infection of these two dolphins. Section VI (Pathology) considers the pathology of the severe ulcerative lesions and the role of poxvirus/TSD further.

H. Did the 2009 mortalities involve dolphins resident within the Swan Canning Riverpark?

We were able to identify only one of the six dolphins based on photo-identification data from 2001-3 (using dorsal fin markings). The adult female that died on 25 October 2009 was 'Leeuwin', one of the 18 resident dolphins identified in 2001-3. Of the five remaining dolphins: two were born after 2003 [5 June calf (~5 years old) and 8 June (apparently independent)[93] juvenile (≥3 years old)] and thus were not previously photo-identified[94], and two had factors limiting our ability to identify their dorsal fins [9 October male (advanced state of decomposition with skin sloughing) and 17 September female (skin lesions complicated by fungal and bacterial growth on the dorsal fin). We could not identify the 21

92 We note that: (a) this could have occurred but the carcass was not recovered for some reason (e.g. the dolphin died outside of the estuary), and (b) it is possible that the adult male whose carcass was recovered on 9 October may have had severe lesions (deep dermal scarring), but this is speculative because of the degraded condition that carcass was found in (see Figure 34.

93 The age of this dolphin suggests that it could have been a calf, but there are no observations indicating that a mother attended the carcass, as was the case for the 5 June dolphin. The age estimates are based upon preliminary analyses of dental layers and represent minimum ages. Further analyses may refine the age estimates somewhat.

94 i.e. photo-identified during the course of the 2001-3 study

June female although her fin was in reasonable condition. This may be because her dorsal fin marking had changed, as often occurs over time.

The composition of the 2009 Swan resident dolphin community is not known, as photo-identification effort since 2003 has been limited to occasional surveys in 2008 and 2009. The composition of a dolphin community/population changes over time because of birth and mortality (and also, in some cases, immigration and/or emigration). For example, Ham (2009) documented changes in the Cockburn Sound dolphin community between photo-identification studies in 1993-6 and in 2008, and found that, of the 76 juvenile and adult dolphins considered part of the resident Cockburn Sound community in 1996, only 21 were photo-identified in 2008. Some of this turn-over likely reflected changes in dorsal fin markings (such that individuals were no longer recognisable). However, many of the adult animals with highly-distinctive dorsal fin markings were not re-sighted, and this was particularly true for adult males (Ham 2009). Thus, the 2009 Swan dolphin community would have included individuals that were: (a) either not observed in 2001-3 because they were born after 2003; (b) were calves or juveniles with 'clean'[95] dorsal fins in 2001-3; or (c) had distinctive markings that were altered substantially between 2003 and 2009.

Thus, aside from the adult female Leeuwin (25 October 2009), we cannot definitively say to what degree the dolphins that died in 2009 used the Swan Canning Riverpark, i.e. to what extent they were consistent/long-term users of the estuary.[96] However, two factors indicate that the dolphins that died in 2009 were likely to have been resident in the Swan Canning Riverpark. Firstly, nearly all of the dolphins observed in the estuary beyond the Fremantle Inner Harbour between 2001-3 were the 18 dolphins considered resident within Swan Canning Riverpark (and their dependent calves). Dolphins other than these 18 were only infrequently observed despite intensive photo-identification effort between October 2001 and June 2003, and were only rarely observed in estuary areas upriver of the Inner Harbour. Secondly, the dolphins had high concentrations of dieldrin and other organic contaminants that occur in higher concentrations within sediments of the Swan Canning Riverpark than in sediments in Owen Anchorage or Cockburn Sound (see DEP 1996 and Nice 2009). Concentrations of dieldrin within dolphins from the Swan Canning Riverpark were also substantially higher than those observed in dolphins from the Bunbury area (see Section VII – Contaminants). These differences suggest that the dolphins that died in 2009 fed, at least in part, on prey associated with Swan Canning Riverpark. Further investigative work is, however, needed to determine relationships between the

95 A 'clean' dorsal fin is a fin having no distinctive markings such as nicks, indentations, etc. Calves, as well as some juveniles, will generally have fins that are absent of individual-specific markings, making identification dependent upon other factors (e.g. fin shape, occurrence with mother, other scars or natural markings).

96 I.e. 'residents'; the 2001-3 study defined dolphins as residents if they were re-sighted consistently within the Swan Canning Riverpark from October 2001-June 2003 (see Section III – Ecology). Other studies similarly use re-sighting patterns to characterise dolphins as 'resident' within an area and to define the composition of resident dolphin communities within estuarine and nearshore ecosystems. As discussed in Section III (Ecology) the 18 juvenile and adult dolphins classified as residents in the 2001-3 study also had home ranges that included adjacent coastal areas such as Owen Anchorage. Use of the resident implies that they also exhibited site fidelity to these coastal areas, as well as being 'resident' within the estuary. While long-term study is necessary to confirm site-fidelity, the maintenance of small and well-defined home ranges that are consistent over time is a species-typical characteristic of bottlenose dolphins within estuarine and protected nearshore environments around the world (see Connor et al. 2000).

concentrations of contaminants in the environment, potential dolphin prey species, and dolphins [see Section VII – Contaminants and also Nice (2009) and Nice et al. (2009)]. The diet of dolphins feeding within the Swan Canning Riverpark is not presently known (Section III – Ecology).[97]

We consider it unlikely that dolphins entered the Swan Canning Riverpark as a 'safe' place in which to die. Long-term site fidelity is a consistent characteristic of bottlenose dolphins within Cockburn Sound and other nearshore and estuarine ecosystems where they have been studied, and we find it unlikely that a dolphin in distress would enter an environment that was: (a) unfamiliar and (b) contained a range and intensity of stimuli likely to seem adverse or stressful and otherwise to be avoided (e.g. boat traffic, narrow channels, anthropogenic noise).

I. Dolphin ecology and mortalities within Bunbury Inner Waters

1. Ecology of bottlenose dolphins in the Bunbury area

Other estuarine and coastal populations in southwestern WA may also experience elevated rates of mortality relative to dolphins in adjacent coastal areas.[98] A long-term photo-identification study in the Bunbury area, by PhD candidate Holly Smith, has determined ranging and resighting patterns for dolphins using estuarine and nearshore areas around the Bunbury area (i.e. the 'inner waters', encompassing Leschenault Inlet, Leschenault Estuary, Inner Harbour, Outer Harbour, and Collie River) and adjacent coastal areas.[99] Appendix A4 provides the table and figures for this section. A summary of post-mortem findings is presented below.

The Bunbury study used standardized boat-based line transect surveys and photo-identification methods to: (a) estimate the number of dolphins within Bunbury inner waters and adjacent coastal areas and (b) examine patterns in dolphin habitat use over a three-year period along three pre-determined transect areas (March 2007-November 2009; A4: Figure Bunbury 1). The transect survey effort was intensive: Area 1 – Backbeach (n = 63 transects); Area 2 – Bunbury inner waters [including Leschenault Inlet, Leschenault Estuary, Inner Harbour, Outer Harbour and Collie River] (n = 76 transects); and Area 3 – Buffalo Beach (n = 76 transects) (A4: Table Bunbury 1).

During the course of the study, 196 dolphins were individually identified within the study area, with photo-identification based on the sighting of individuals on at least three occasions. Individuals varied in the ranging patterns and a small sub-set of dolphins showed a consistent association within the Bunbury inner waters (i.e. Leschenault Inlet, Leschenault Estuary, Inner Harbour, Outer Harbour and

97 These considerations suggest the potential value of further work to determine how contaminants are transferred to dolphins and other biota through estuarine and coastal food chains. Ideally, such work would be integrated with other work to better characterise the population structure of bottlenose dolphins within the Perth metropolitan area, and to refine our understanding of processes such as genetic and demographic isolation, gene flow, natal philopatry would build on current understandings of these dolphins, as we thus far have limited information on these processes and their implications for the conservation of this species within the Perth metropolitan area (see Section III- Ecology).

98 In this context 'estuarine' could also include coastal environments like Cockburn Sound that are also characterised by intensive human use and modification, as well as some degree of seasonal environmental change.

99 This research is funded through local government, state government and local industry: Bemax Cable Sands, Bunbury Dolphin Discovery Centre, Bunbury Port Authority, City of Bunbury, Cristal Global, DEC, Iluka, Millard Marine, Naturaliste Charters, South West Development Commission, WAPRES, and Worsley Alumina.

Brunswick River). Of the 196 individually identified, fourteen were classified as "consistent" users of the Bunbury inner waters, and this sub-group was comprised of five adults, seven juveniles, and two calves (A4: Figure Bunbury 1; A4: Table Bunbury 2; A4: Figure Bunbury 2). Dolphins were only classified as a "consistent" user of the Bunbury inner waters if they were only sighted only in Area 2 (A4: Table Bunbury 2; A4: Figure Bunbury 2). In other words, the dolphins classified as consistent of the Bunbury inner waters were only sighted in this area, and were not observed in other areas (i.e. Area 1 and 3, the two coastal components of the overall study area).

These distribution and re-sighting data indicate a strong and consistent ecological association between the Bunbury inner waters and these fourteen dolphins, and contrasts with the ranging patterns of the other 182 dolphins observed within the study area. While other dolphins were also observed within Bunbury inner waters, these dolphins also ranged in coastal areas outside of the inner waters. The inner waters present an estuarine and nearshore environment that is analogous to the Swan Canning Riverpark and its adjacent coastal habitats, with the estuarine areas experiencing varying salinity and temperature regimes in response to winter-spring rainfall patterns and inflows from rivers, groundwater, drains, and surface runoff.

2. Epidemiology of mortalities

Photo-identification effort and stranding records for dead dolphins indicated a high rate of mortality for the fourteen dolphins that were classified as consistent users of the Bunbury inner waters. At the conclusion of field work for the 2007-9 study in November 2009, of these fourteen dolphins (i.e. the 'inner waters' dolphins): six were alive, five were confirmed dead through carcass recovery and post-mortem identification (including one dependent and non-weaned calf missing since September 2008), and three were presumed dead. The latter three were presumed dead based on based on intensive photo-identification effort that failed to re-sight animals. The three animals presumed dead were frequently sighted in Area 2 for at least seventeen months [following the start of the study in March 2007], and then never sighted again despite intensive survey and photo-identification effort throughout the entire study area through November 2009. Specifically, the three presumed dead were sighted on n = 30, n = 24 and n = 26 occasions (respectively) and then not re-sighted after August 2008, September 2008, and April 2009 (respectively) (A4: Table Bunbury 2; A4: Figure Bunbury 2). Although these death/disappearances did not exhibit the same close clustering in time as the dolphin mortalities in the Swan Canning Riverpark did (i.e. three in June 2009 and three in September/October 2009), four of the deaths (carcass recoveries) occurred and all three of the disappearances began during the thirteen-month period between April 2008 and May 2009. One of the deaths occurred in November 2009.

Other dolphin deaths were recorded in the general Bunbury area between 2006 and January 2010. These deaths included: one death in April 2006 (recovered in Bunbury inner waters); one death in January 2008; one death in September 2009 (this individual had a long-standing entanglement injury and ranged both within Bunbury inner waters and coastal areas); and two deaths in January 2010. Thus,

in total, there were nine dolphin carcasses recovered between January 2008 and January 2010, of which five were classified as consistent users of the Bunbury inner waters.

Post-mortem examinations were conducted on: (a) the four dolphins considered consistent users of the Bunbury inner waters dolphins whose carcasses were recovered between April 2008 and May 2009, and (b) on four other dolphin carcasses recovered from the general Bunbury area between January 2008 and January 2010. The carcass of the fifth inner waters mortality was recovered 6 November 2009, but was in advanced decomposition at time of recovery and no post-mortem examination was conducted. Carcass recovery and post-mortem examination of the 4 April 2006 mortality was opportunistic and occurred prior to the start of the 2007-9 study. The identity and ranging patterns of this individual are not known. Three dolphins had lesions indicative of TSD [17 May 2009, 30 September 2009, and 4 January 2010], but not the severe ulcerative lesions found in some dolphins from the Swan Canning Riverpark.

Of the four non-inner waters dolphins examined post-mortem between 2008-2010, two had human-induced injuries: one with a long-standing entanglement injury (30 September 2009) and a second with post-mortem presentation indicative of blunt force trauma consistent with a vessel strike (23 January 2010). Pneumonia with the presence of *Halocercus* lungworms (and often fungal and bacterial pathogens) was a common presentation, and was observed in four dolphins from the Bunbury area that were examined post-mortem in 2009 and 2010 (including two inner-waters dolphins) (see Section VI – Pathology for further discussion). Poor body condition was another commonality among dolphins examined post-mortem. The post-mortem findings are discussed further in Section VI (Pathology).

Similarities to the Swan mortalities include:

- **Entanglement and human-induced injury** – 'Cruiser' (30 September 2009) was found to have suffered chronic entanglement by fishing line similar to one of the Swan individuals (21 June 2009). 'Vevay' (23 January 2010) was found to have died from acute injury likely sustained as a result of vessel strike.
- **Evidence of opportunistic infections** – Several of the Bunbury mortalities exhibited evidence of secondary fungal and bacterial opportunistic infections similar to some of the Swan dolphins. Most notably "Radar" (12 January 2009) exhibited pneumonia with the presence of *Aspergillus fumigatus* and mixed bacteria almost identical to the 21 June 2009 Swan dolphin.
- **Evidence of lymphoid depletion** – many of the Bunbury and Swan mortalities exhibited lymphoid depletion histologically (see Section VI – Pathology for further discussion as to the potential significance of lymphoid depletion).

The mortalities of the Bunbury inner waters dolphins in 2008 and 2009 appear to constitute an unusual mortality event. This conclusion is based on the photo-identification data and carcass strandings showing a high rate of mortality over a thirteen-month period (April 2008-May 2009) for dolphin using

the Bunbury inner waters. This level of mortality appears above normal background rates for bottlenose dolphin populations and to show a clear association with the use of the inner waters around Bunbury. We note, however, that there are few stranding data for the Bunbury region prior to 2007. There are also few stranding data for other estuarine (e.g. Peel-Harvey) and coastal (e.g. Cockburn Sound) areas within southwestern Western Australia that could be used to place the Swan and Bunbury mortalities in context.

Synopsis of deaths and post-mortem examinations of dolphins from the Bunbury area:

The table below includes recovered carcasses only and does not include dolphins that were presumed dead (see text above). No post-mortem examination was done for a 2009 carcass in advanced decomposition. Inner waters dolphins are those classified as consistent users of the area encompassing Leschenault Inlet, Leschenault Estuary, Bunbury Inner Harbour, Bunbury Outer Harbour and Brunswick River, based on photo-identification (re-sighting of known individuals) and ranging data from the 2007-9 study.

Number of carcasses recovered

PM = indicates post-mortem examination conducted; REC = carcass recovered only

	2006	2007	2008	2009	2010
Inner waters dolphin	0	0	2 PM	2 PM/1 REC	0
Bunbury area dolphin	1 PM	0	1 PM	1 PM	2 PM

2006

4 April

MUVS Pathology No: 06/348
Female juvenile
Emaciated body condition.

Description

Scattered lung abscesses associated with *Halocercus* sp. nematodes; heavy colonic cestode burden.

Severe lymphoid depletion.

2008

1 January

MUVS Pathology No: 08/379 (‘Blizzard’)
Male juvenile

Description

Severe, chronic, diffuse, unilateral (right sided) pleuritis.

No histopathology performed.

18 April

MUVS Pathology No: 08/943 ('Arrow')
Female adult
Bunbury inner waters dolphin

Description
Ascites of unknown cause.
Possible endometritis (uterine inflammation/infection)

25 August

MUVS Pathology No: 08/1365 ('Peak')
Male adult
Emaciated body condition.
Bunbury inner waters dolphin

Description
Mediastinal abscess (presumably originating within a lymph node) with fibrinosuppurative pleuritis and local interstitial pneumonia; unknown cause.

2009

12 January

MUVS Pathology No: 09/257 ('Radar')
Female Juvenile
Emaciated body condition (had been observed to be losing weight for the preceding 4 months).
Bunbury inner waters dolphin

Description
Severe, chronic, diffuse suppurative and necrotising bronchointerstitial pneumonia with intralesional *Halocercus* sp., *Aspergillus fumigatus* and mixed bacteria.

17 May

MUVS Pathology No: 09/665 ('Turbo')
Male juvenile
Poor body condition.
Bunbury inner waters dolphin

Description
Some visible pox-like skin lesions.

Severe forestomach impaction with mud and seagrass.

Moderate to severe, chronic, multifocal to coalescing suppurative and necrotising bronchopneumonia with intralesional *Halocercus* sp. nematodes.

30 September

MUVS Pathology No: 09/1032 ('Cruiser')
Female juvenile
Emaciated body condition.

Description

History of chronic entanglement around the beak for almost a year.

Moderate to severe, chronic, necrosuppurative bronchopneumonia with intralesional *Halocercus* sp. nematodes and mixed bacteria.

Moderate generalised lymphoid depletion.

Presence of lesions indicative of TSD in photo-identification images.

6 November

'Zippy'
Male juvenile
Carcass in advanced state of decomposition.
No post-mortem examination conducted.
Bunbury inner waters dolphin

2010

4 January

MUVS Pathology: ('Mint')
Male calf (1 year)
Emaciated body condition.

Description

Multiple active TSD (poxvirus) lesions.

Severe bronchopneumonia with the presence of *Halocercus* lungworms.

Endocrine anomaly; cysts associated with the parathyroids/thyroids bilaterally.[100]

[100] Histopathology pending to determine site and possible cause, i.e. congenital versus degenerative change.

23 January MUVS Pathology ('Vevay')

Female adult

Good body condition.

Description

Penetrative wound to body wall indicative of vessel strike.[101]

101 Histopathology pending.

VI. Pathology

This section reviews the key aspects of the pathology for the 2009 dolphin mortalities in the Swan Canning Riverpark, and is based on information obtained from post-mortem examination and related analyses.[102] This section builds on the information presented in other sections of the technical report. In particular, it considers the suite of potential aetiological factors for the 2009 dolphin mortalities in relation to the presentation of the dolphins examined post-mortem. Information on the pathology of dolphins from the Bunbury area is also presented, but it should be noted that emphasis has been placed on the 2009 mortalities in the Swan Canning Riverpark. This section should be read in conjunction with the findings presented in Section V (Epidemiology). Appendix A5 contains the full post-mortem reports for the 2009 dolphin mortalities in the Swan Canning Riverpark.

A. Multi-factorial aetiological framework

The 2009 dolphin mortalities in the Swan Canning Riverpark are best understood as the outcome of a suite of contributing factors. This multi-factorial aetiology means that several, potentially interacting (synergistic) factors, combined to result in the deaths of the dolphins. There is no one factor that caused the deaths of all six dolphins, nor can the death of any one dolphin death be attributed to a single causative agent alone. In each case, a range of factors is likely to have come into play. While it may be possible to identify those factors and to characterise their potential contribution, it is exceedingly difficult to determine exactly how those factors interacted to cause death.

It is a basic tenet of pathology that the death of an animal is typically a complex process and only rarely the result of a single aetiological factor.[103] The multi-factorial nature of causality of death applies even in situations in which the aetiopathogenesis is well established for a known disease, and both the initial cause/trigger and the stepwise events necessary to the development of full-blown clinical disease are known. Even then, many other factors must be present in order for the initial cause to result in clinical disease, and for the pathologic changes/physiologic derangements to occur that are sufficient to result in death. In addition, these other factors may also need to occur in a particular order or magnitude of severity. The complex, multi-factorial nature of mortality applies even to a primary pathogen that is exquisitely virulent, and so able to cause disease (i.e. morbidity) in its own right and

102 The information underlying this investigation developed from what initially began as an opportunistic and standardised post-mortem study of health issues in dolphins, rather than from a directed and exhaustive investigation specifically focusing on the health of the Swan dolphins. There was no formalised investigative team in place responsible for this work—the post-mortem examinations and ancillary analyses and reporting were carried out on a voluntary basis. As is always the case with retrospective studies, the mortalities were not considered as a series of potentially related mortalities until the temporal pattern of mortality became clear. To support the health study, we had prospectively developed a standardised post-mortem routine based on established protocols, as well as systematic sampling/archiving protocols (Duignan 2000, Geraci et al. 2005, Higgins et al. 2006, Pugliares et al. 2007). While these protocols ultimately proved of great value, they could not support the type of exhaustive epidemiological study that is necessary to tease out the full complexity of a mortality event. Further work is needed to place this mortality event in context.

103 An obvious exception to this is instances of peracute trauma that are sufficient to cause relatively immediate fatal injury, such as occurred with the Bunbury dolphin 'Vevay' (23 January 2010), who was found to have died from acute injury likely sustained as a result of vessel strike.

without other factors being involved. Even with such a pathogen, other factors may still be required for death to occur.

This complexity can also be understood by looking at the general factors involved in shaping how infectious disease will play out within a particular organism. For any individual, disease outcomes can be seen as the result of a complex interplay among genetic,[104] phenotypic,[105] and epigenetic[106] factors. In other words, individuals vary in their genetic make-up, in their morphological, physiological, and immunological characteristics, and in the way that environmental factors have shaped the expression of these traits. How a disease will affect one individual may be profoundly different than how it affects another individual. This suggests that the characteristics of individual dolphins (e.g. their state of immune function, body condition, reproductive state, pre-existing infections) are likely to be important determinants in the nature of their deaths.

Thus, the definitive investigation of disease requires the investigator to understand the multi-factorial nature of disease, and to realise that morbidity does not necessarily correlate with mortality (i.e. an occurrence of a disease will only sometimes eventuate in death). An example of this is the expression of poxvirus/TSD skin lesions in dolphins from the Swan Canning Riverpark. Poxvirus is generally a self-limiting clinical disease of juvenile dolphins. However, various factors may cause an overall waning of normal protective adult immunity, and thus potentially support the development of clinically significant disease that is sufficient to cause mortality under certain circumstances. Other (stressors) could also exacerbate what is a typical presentation of TSD. This emphasises the importance of understanding the nature of the pathogenic factors involved and the need to be attentive to the environmental and biological context in which disease occurs, in order that the salient aetiological factors can be identified and characterised.

Our conclusion that the 2009 mortalities reflect the contribution of multiple stressors is consistent with published work on mortality events in cetaceans. These studies have shown that even in situations where a virulent primary pathogen is present, other factors contribute to mortality (i.e. these other factors influence which animals die and which survive). Given the complex interplay of often intimately associated events that must unfold for death to occur, it is often extremely difficult to tease out individual aetiological factors and to apportion their significance.

Other studies also show the practical challenges confronting efforts to understand why marine mammal mortality events occur. Such studies are typically initiated after a mortality event is concluded (or is well under way), and are generally forced to rely on limited environmental [e.g. did a harmful algal bloom occur?], biological [e.g. what habitats did the animals range within?], and epidemiological [e.g.

104 i.e DNA code inherited from the parents

105 i.e. the influence of environmental factors on genetic makeup such that an individual becomes imbued with a particular physical appearance and biochemical characteristics

106 i.e. changes in phenotype and gene expressivity because of chromatin and DNA modifications that are stable over rounds of cell division but do not involve changes in the underlying DNA sequence of the organism

what are natural rates for infectious diseases and mortality for this population?] information from which to elucidate the particular aetiologies underlying the deaths observed. There will almost always be a deficit of data and, consequently, some degree of uncertainty in the conclusions drawn.

These points emphasise the unique value of conducting post-mortem examinations, as these examinations provide by far the most detailed insight into the aetiological factors involved (e.g. infectious disease, algal biotoxin, human-induced trauma). It is difficult to underestimate the value of the pathological information that post-mortem examinations provide, and the value of conducting post-mortem examinations as soon after death as possible (i.e. before significant decomposition occurs). However, concurrent behavioural and epidemiological research with live animals can also provide valuable information on the factors shaping a mortality event (or a disease) and its outcome, and this is most effectively done by working with the population affected by the event. The study of dolphins at Lake Pontchartrain provides an instructive example, as even though post-mortem examinations were not conducted, observations of individual dolphins, their environment [a lagoon with <5ppt salinity], and the prevalence of disease [skin lesions of an unknown aetiology] were collected. These data, when coupled with information on dolphin physiology, allowed researchers to understand the basic aspects of the pathology of dolphins inhabiting a hyposaline habitat, and to establish hypotheses from which the aetiology and epidemiology of the skin lesions could be understood and investigated (Barry et al. 2008).

The term 'threshold effect' is used in pathology to refer to a situation in which a disease (or syndrome) may appear in an individual without detectible warning and following a long period of quiet development. This latent period may relate to both the inherent incubation period of the disease/syndrome, and to the interplay of numerous other contributing factors. The manifestation of the disease/syndrome, in other words, reflects the characteristics of the disease/syndrome itself as well as a range of other factors supporting its expression. This kind of multi-factorial matrix provides a useful conceptual framework from which to understand to the emergence of a disease/syndrome in a population. In the context of the 2009 mortalities, potential aetiological factors [as discussed in Sections IV (Health) and V (Epidemiology)] include:

(1) **Inherent genetic status – low genetic diversity**[107]
(2) **Presence/virulence of primary pathogens**
(3) **Influence of environmental stressors**:
    a. environmental conditions
    b. contaminants
    c. food availability

[107] Not all of these factors will apply to this investigation. For example, low genetic diversity seems unlikely, given that females range within coastal areas such as Owen Anchorage where they will encounter males who are not residents within the Swan Canning Riverpark. Nonetheless, we cannot exclude the possibility that genetic diversity within the resident community might be low. Low genetic diversity has been suggested as a contributing factor in other marine mammal mortality events. We also note that persistent organic contaminants are not considered acutely toxic, but rather to have a more latent (or chronic) effect.

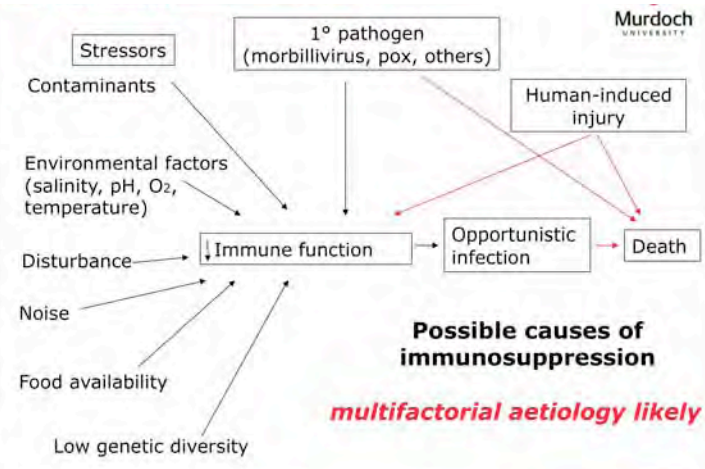
d. noise

e. anthropogenic disturbance

(4) Human-induced injuries

(5) Influence of secondary (opportunistic) pathogens

This matrix of potential aetiological agents can also be viewed as the suite of factors potentially influencing a reduction in immune function for dolphins:



B. Clinical presentations indicative of reduced immunological function

Certain aspects of the post-mortem findings are suggestive of reduced immunological function in at least some dolphins. Bottlenose dolphins inhabit environments in which certain pathogens are ubiquitous, and many of the bacterial organisms found in the 2009 dolphin mortalities from the Swan Canning Riverpark (and in dolphins examined from Bunbury area) are known to be present in normal, healthy, free-ranging individuals (e.g. Buck et al. 2006). However, the secondary infections by opportunistic bacterial and fungal organisms seen in four of the 2009 Swan dolphins (and several of the Bunbury mortalities) are suggestive of reduced immunological function. In particular, the occurrence of fungal diseases ('mycoses') is significant in the context of reduced immunological function. Dagliesh et al. (2006) noted that: "mycoses in marine mammals are rare and poorly understood, but are critically important among the fatal infectious diseases as they may be indicative of underlying immunosuppression."

Also suggestive of reduced immunological function is the prevalence of TSD (with unusually severe manifestation) in three adult animals (18 November 2007, 17 September 2009, and 25 October 2009).[108] We believe that this manifestation of TSD may have occurred subsequent to chronic impact by multi-factorial, intercurrent stressors. In other words, other factors are likely to have supported the apparent progression of TSD lesions to a severe state. These points are discussed further below.

108 With later histological identification of characteristic intracytoplasmic eosinophilic viral inclusion bodies typical of poxvirus.

A clarification should be made to define the significance of "lymphoid depletion"[109], which was a finding in several of the Swan dolphin mortalities (18 November 2007, 8 June 2009, 21 June 2009), as well as some of the Bunbury mortalities. Pathological lymphoid depletion is seen as a result of chronic, continued antigenic stimulation (i.e. the individual has been mounting a long-term immune response).[110] It may also occur through chronic elevation of corticosteroids (stress hormones) or directly as a result of infection by a lymphotropic virus (e.g. morbillivirus). While it does not directly correlate to immunosuppression, it is suggestive of chronic, exhaustive antigenic stimulation, and therefore indicative of chronic physiologic stress.

C. Opportunistic pathogens

A commonality of four of the 2009 Swan mortalities (8 June juvenile male, 21 June juvenile female, 17 September adult female, 25 October aged female), as well as some of the Bunbury mortalities, was the presence of significant secondary infections by opportunistic bacterial and/or fungal pathogens. *Aspergillus*, for example, is an opportunistic fungal pathogen that usually does not cause infection in animals with competent immune systems. Cerebral Aspergillosis has mainly been reported in the literature as a secondary opportunistic pathogen in dolphins infected with morbillivirus.

Aspergillus spp., and the opportunistic bacteria that were found, require either: (a) some portal of entry (i.e. breach in the individual's normal defences), or (b) for the host's immune system to have become compromised in such a way that the individual is unable to mount an effective immune response. In the case of the first two (of the four) individuals (8 June and 21 June), opportunistic infections are considered to have led directly to their deaths.

The 8 June juvenile male was found to have meningoencephalitis with intralesional fungal organisms consistent with *Aspergillus* spp. (fungal infection of the brain). Histologically, the fungal organisms were seen in the wall of a large muscular artery and branching out into its lumen, suggesting haematogenous spread was likely to have occurred. This same individual had gross and histological morphological changes suggestive of segmental jejunal (small intestinal) infarction.[111] It is likely this occurred secondary to haematogenous spread of the fungal organisms with resultant thromboembolism and ischaemia (loss of blood flow) of the affected jejunal segment.

The 21 June juvenile female had sustained human-induced injury, with a severe chronic fishing line entanglement of the right fluke. She also had bronchopneumonia with intralesional fungi consistent with *Aspergillus* spp. In addition to this, two types of opportunistic bacterial pathogens were found on lung culture. One of these was morphologically identical to bacterial colonies visible in histological sections of the fluke lesion and bilateral renal (kidney) infection. One type of opportunistic bacterial

109 Lymphoid depletion is characterized by depletion of lymphocytes within the germinal centres of the lymph nodes and an absence of mitotic figures (McGavin et al, 2007, p. 822).

110 As opposed to physiological depletion, as can occur in pregnancy or around parturition because of elevated sex steroids.

111 An infarction is defined as a "localized area of tissue death secondary to insufficient blood supply produced by the occlusion of the arterial supply or the venous drainage to that area" (Blood and Studdert 1988)

pathogen found on kidney culture was identical to one of the two identified on lung culture, and seen in the fluke lesion. The gross and histological changes were indicative of systemic septicaemia with intercurrent *Aspergillus* spp. infection.

The other two of the four cases (17 September and 25 October) exhibited terminal opportunistic fungal and bacterial dermatitis secondary to skin damage that we believe was initiated by poxvirus/TSD (with multi-factorial contribution by osmoregulatory damage among other factors). These opportunistic infections were not considered to have directly contributed to their deaths, but were more likely to have occurred as terminal events shortly prior to death. These individuals exhibited an acute progression to severe and extensive skin lesions that were ulcerative, causing terminal debilitation.[112]

D. Entanglement/Human induced injury

Two of the mortalities in 2009 had active entanglement injuries (21 June and 25 October). In the case of the 21 June female, the entanglement around the right tail fluke was severe, chronic, and on-going at the time of post-mortem, with the presence of intralesional cocci (bacteria) in the resultant scar tissue and open wound (seen histologically). This individual also had acute to subacute bronchopneumonia with the presence of intralesional fungal organisms consistent with *Aspergillus* spp., as well as an acute bilateral renal (kidney) infection with the presence of intralesional cocci.

In terms of time-span, the morphological changes associated with the fluke injury preceded those in the lungs/kidneys by a considerable margin. This individual was observed with the fluke injury in mid-2008 (D. Coughran, DEC, personal communication), indicating that that the line had been lacerating through tissue for at least a year, during which time the dolphin was able to fend off systemic infection[113] until a 'threshold point' was reached (or a novel 'final straw' stressor was introduced), whereby the individual was challenged/immunocompromised to the point of developing serious infection.

The same opportunistic bacteria [*Staphylococcus aureus*, a coccoid bacteria identical in appearance to that seen in the fluke] were cultured from the lungs and the kidneys [and additionally another bacterium, *Mannheimia haemolytica*, was also found in the lungs]. This finding, supported by the characteristic morphological changes seen histologically in the lungs/kidneys, suggests that the *S. aureus* spread as septic emboli via the bloodstream from the ongoing fluke injury/infection to these other organs. The resultant bronchopneumonia could then have become intercurrently infected with *Mannheimia haemolytica* and *Aspergillus* spp.[114]

112 An ulcer is by definition a full-thickness loss of the epithelium and hence is 'deep', as opposed to erosions, which are partial thickness loss.

113 Or, alternatively, the dolphin may even have developed systemic infection that it was able to recover from, although relapsing episodes would have been possible and would in themselves have constituted a stressor.

114 Intercurrent infection means subsequent complicating secondary infection that alters the course of the first.

Arguably, it could therefore be said that the entanglement was indirectly responsible for this individual's death. However, it is important to note that a number of individual factors are likely to have also played a part. Had the dolphin managed to free itself of the entanglement, recovery may have been possible.[115] Additionally, it is possible that a novel stressor (or chronic exposure to routine stressors) pushed the dolphin to the point whereby it succumbed to septicaemia.

In contrast, the entanglement seen around the right pectoral fin of the 25 October female, although it had started to incise into the skin, was not deeply embedded; nor was it associated with any evidence of significant inflammation, infection, healing, or scar tissue formation. This makes it likely that entanglement occurred a short period (i.e. days) before she died. The dolphin exhibited impaired swimming ability shortly before death, a factor that may have contributed to the entanglement, depending on how long she had been exhibiting these clinical signs. In addition, a fishhook and attached line was lodged in the oesophagus (morphological tissue changes indicate this was an acute event shortly prior to death (i.e. days before).

This individual was in poor body condition, and it is possible that this, combined with terminal debilitation (secondary to severe skin disease) may have led her to forage inappropriately or opportunistically, resulting in her taking a hooked fish. Although the post-mortem findings indicated that neither of these injuries contributed significantly to her death, the fact remains that these injuries constitute an animal welfare issue in that they would have caused the dolphin significant pain/suffering in the lead-up to her death.

E. Relationship to other populations

Elevated rates of mortality (i.e. above normal background levels) may also be occurring within other populations of dolphins in southwestern WA. Donaldson et al. (2010) documented incidences of entanglement and other human-induced injury for dolphins in Cockburn Sound, including seven incidences of entanglements or other human-induced injuries involving calves between 1996-2004 [see Section IV – Health]. A long-term photo-identification study at Bunbury area has also recorded multiple incidences of human-induced injury and indications of high rates of mortality for dolphins frequenting the 'inner waters' around Bunbury (i.e. Leschenault Inlet, Leschenault Estuary, Bunbury Inner Harbour, Bunbury Outer Harbour, and Brunswick River) [see Section V – Epidemiology and Appendix A2].[116]

Post-mortem examinations of dolphins from the Bunbury area showed both similarities (i.e. commonalities) and differentials with those from the Swan. The main commonalities were: (a) the

115 DEC wildlife officers have the training and equipment to respond to incidences of entanglement and have freed dolphins entangled in fishing gear. If a dolphin is observed with an entanglement is observed, people should contact the DEC Wildcare Helpline at 9474 9055.

116 As discussed in Section V (Epidemiology) and Appendix A2, the Bunbury study determined ranging and resighting patterns for dolphins using the inner waters and adjacent coastal areas around Bunbury. These patterns, when coupled with recent stranding records for dead dolphins in the Bunbury area, indicate a high mortality rate for dolphins that range within the inner waters around Bunbury.

presence of opportunistic pathogens and lymphoid depletion, both findings that are suggestive of reduced immunological function; (b) evidence of human-induced injury as a cause of, or likely contributor to, mortality; and (c) a broadly similar environment (at least for the inner waters dolphins). Of the four deaths of dolphins not considered consistent users of inner waters around Bunbury, one had a long-standing entanglement injury and another had a post-mortem presentation indicative of blunt force trauma consistent with a vessel strike. Three dolphins had lesions indicative of TSD, but severe ulcerative lesions (as found in two of the 2009 Swan dolphin mortalities) were not observed.

Pneumonia with the presence of *Halocercus* lungworms (and often fungal and bacterial pathogens) was a common presentation in post-mortem examinations of dolphins from the Bunbury area, and was observed in four dolphins that were examined post-mortem in 2009 and 2010 (including two inner waters dolphins). The aetiology for the pneumonia is unclear and is likely to reflect the influence of several factors. The presence of lung nematodes like *Halocercus* is frequently associated with pneumonia and other bacterial and viral infections in dolphins, and the parasite is not the cause of disease for these dolphins, but likely reflects (i.e. rather than being the cause of) general poor body condition/ill-thrift as well as the high parasite loads of dolphins in this area. As with the deaths in the Swan Canning Riverpark, we lack the sample size and epidemiological data to fully interpret the post-mortem findings and address the causative factors for the deaths. Pneumonia, in particular, is a common post-mortem presentation in bottlenose dolphins and its aetiology may reflect a complex suite of factors.

The key differentials between the post-mortem presentations of dolphins from the Swan Canning Riverpark and the Bunbury area were the significantly lower contaminant burdens for the Bunbury dolphins (see Section VII – Contaminants), and overall the fact that the Bunbury individuals tended to be in poorer body condition. While skin lesions indicative of TSD were observed in three dolphins from the Bunbury area [both in post-mortem examination and through photo-identification (see Froude 2009)], we did not observe severe ulcerative lesions in dolphins that were examined post-mortem. Little more can be said of this finding except to note that further comparative study would be necessary to examine if the prevalence of such severe ulcerative lesions is unique to the Swan Canning Riverpark, or if these lesions also occurs in other estuarine and coastal environments in Western Australia.

F. Poxvirus (Tattoo skin disease) and progression of skin lesions

1. Presentation of the lesions

Histopathological examination of skin lesions found intracytoplasmic eosinophilic viral inclusion bodies characteristic of poxvirus present in keratinocytes (skin cells of the outer epidermal layer) from two of the 2009 mortalities (17 September 2009 and 25 October 2009) and in the 2007 mortality (18 November).[117] Figure 36 shows the inclusion bodies. Figures 37a and 37b shows images of skin lesions on the 18 November 2007 and 25 October 2009 individuals, respectively. The two 2009 mortalities had

[117] The inclusion bodies in all histopathological slides (including those donated by the Department of Agriculture) were observed during a retrospective review.

extensive erosive/ulcerative acute skin lesions adjacent to areas of the epidermis where poxvirus inclusions were sighted, as well as acute secondary infection by opportunistic fungal and bacterial pathogens.

Acute skin lesions were present in the 2007 dolphin but were not ulcerated, apart from an area extending caudally from the blowhole that immediately bordered skin changes more consistent with TSD. A dolphin that was recovered near Ascot in October 2003 also exhibited extensively ulcerated skin, based on images obtained at the time. However, no post-mortem analysis was conducted on this dolphin.

The presence of viral inclusion bodies in keratinocytes suggests the skin lesions were caused by poxvirus (TSD), with secondary complicating opportunistic infection (the latter likely occurring as a terminal event shortly prior to death). This is the first time, to our knowledge, that viral inclusion bodies consistent with those of poxvirus/TSD have been documented in lesions from dolphins in Western Australia, although photo-identification studies have suggested that the virus is endemic in dolphin populations from the region (see Section IV – Health, as well as Froude 2009 and Ham 2009), and poxvirus occurs in cetacean populations worldwide (Van Bressem et al. 2009b).

The ulcerated skin lesions observed in the two 2009 mortalities are considered to have directly resulted in the deaths of these animals. Such skin lesions could be likened to 3rd degree burns covering 70% or more of the body surface area, an insult that would have caused significant and debilitating loss of body fluids, electrolytes and proteins, weakening the animal and increasing its susceptibility to opportunistic secondary bacterial/fungal infection(s).

No opportunistic organisms were observed on histopathological examination of the skin adjacent the dorsal ulcer from the 2007 November mortality. The wound appeared acute (i.e. a fresh/new wound), thus it is possible that it simply had not been colonized at that point.

2. Notable aspects of the incidence of poxvirus/TSD

Two points are particularly notable about the manifestation of poxvirus/TSD observed in the mortalities of the Swan dolphins. Firstly, tattoo skin disease is usually seen in juvenile cetaceans, based on pattern observed in photo-identification studies of coastal and estuarine dolphin populations (Van Bressem et al. 2009b). Pox-viral infection is not usually seen in adults, as adults are likely to have developed protective immunity following infection as a juvenile. Its prevalence within adults has been suggested as a population-level indication of a poor health status (Van Bressem et al. 2009b). Secondly, poxvirus is considered only weakly pathogenic, with infection usually resulting in self-limiting tattoo-like skin lesions that resolve with time. Infection does not normally result in large and deeply ulcerative lesions nor does it normally cause death. Figures 22 and 37 shows images of contrasting lesions that are: (a) typical of the characteristic TSD lesions normally seen as a result of

poxvirus infection (Figure 22) and (b) indicative of the unusually severe lesions that we term 'atypical poxvirus/TSD' (Figure 37).

There are three possible hypotheses that could account for the presentation of the poxvirus observed in these mortalities:

1. **increased virulence:** The strain of the virus present here may be more virulent than in other locations, i.e. this strain is a 'hot' strain or subtype of the virus that causes more epidermal damage than has been reported with poxvirus/TSD. Epidermal erosion and ulceration would allow for further damage to occur via osmoregulatory disruption of the deeper epidermis and provide an entry point for secondary opportunistic infection.

2. **osmoregulatory disruption of epidermal cells:** Low salinity conditions can cause cellular damage to the epidermis by disrupting the electrolyte balance of epidermal cells. In this scenario, keratinocyte damage/death would result in erosions and ulcerations that provide an entry point for infection of the epidermis (and potentially, underlying deeper tissues such as dermis, with the potential for septicaemia) by opportunistic fungal and bacterial pathogens. This hypothesis is consistent with the low salinity conditions present within the estuary from July-October 2009 (see Section IV and V, Figure 31). The movement patterns of dolphins are also consistent with dolphins experiencing: (a) repeated (i.e. daily or near daily) exposure to low salinity conditions within the estuary and (b) prolonged (i.e. several hours at a time) periods of immersion. Observations from 2001-3 indicate that resident dolphins use the estuary on a daily or near-daily basis and remain within the river for at least several hours at a time (see Section III - Ecology). While acknowledging that data for the occurrence of dolphins within the upper reaches of the Swan River are sparse, it is clear that dolphins do range within these areas. Further research would be needed to determine the frequency in which dolphins occur in these areas and their movement patterns.

3. **reduced immunological function:** These dolphins may also have had immunological deficiencies that rendered them more susceptible to infection, such that a 'normal' poxvirus strain of routine virulence induced significantly more severe epidermal change than is typically seen, resulting in damage allowing for the entry of secondary opportunistic infection. Reduced immune competence could potentially result from: physiological stress from low salinities and/or temperatures; infection by a primary pathogen with immunosuppressive qualities; immuno-toxic contaminants; the presence of other factors (e.g. a pre-existing secondary infection); or other factors (e.g. anthropogenic disturbance). The presence of large, active TSD lesions in adult cetaceans is redolent of/analogous to 'progressive vaccinia,' a severe complication of smallpox (to which cetacean poxvirus is distantly related) vaccination in humans with concurrent immunological deficiencies, and similarly suggests impaired immune function (Van Bressem et al. 2003, 2009b).

These three hypotheses are not mutually exclusive and may occur intercurrently, and collectively are strongly suggestive of multi-factorial aetiologies for the severe skin lesions. All three hypotheses may relate to low salinity conditions, and would have effects similar to any underlying process that caused damage to a significant proportion of the protective outer epidermal layer and thus ultimately allowed for increased osmoregulatory damage to epidermal tissues. We suggest that they provide a reasonable scientific basis for concluding that sustained exposure to low salinity conditions may have caused both osmoregulatory and more generalised physiological stress to dolphins, leaving them vulnerable to secondary infection and to the progression of lesions to a severe ulcerative state (see further below).

We also note that the causes of the more typical presentations of TSD (i.e. lesions indicative of TSD observed through photo-identification images) observed in dolphins from Cockburn Sound (Ham 2009) and Bunbury (Froude 2009) are also likely to reflect a suite of contributing factors. We note, for example, that 'Cruiser', a juvenile female from the Bunbury area that died in September 2009 after having an entanglement injury (fishing line wrapped around its beak/rostrum) for almost a year, had lesions indicative of TSD (Figure 38). It is plausible that the prevalence and severity of this individual's skin lesions related in part to the health challenges that this individual faced because of the long-standing and ultimately debilitating nature of the entanglement injury.

3. Salinity and skin lesions

Section IV (Health) reviewed other studies suggesting that exposure to low salinity appears to be the strongest predictive factor for TSD, and appears to be associated with the prevalence of TSD in populations with limited exposure to anthropogenic contaminants (see Wilson et al. 1999, Van Bressem et al. 2009b, Rowe et al. 2010). Lower salinity conditions are common within the Swan Canning Riverpark in the winter-spring period (July-October), suggesting that environmental conditions during this period could induce reduced immunological function and/or compromised epidermal tissue, leaving dolphins susceptible to the secondary bacterial/fungal infection observed in the September/October 2009 mortalities.[118]

Low-salinity conditions within the Swan Canning Riverpark likely contributed at least in part to the severity of the skin lesions seen in the 17 September and 25 October deaths. Environmental conditions within the Swan Canning Riverpark may also cause systemic physiological stress to dolphins, particularly during periods when salinity and temperature change rapidly and during periods when salinities drop below 20 ppt, a concentration that is known to be potentially stressful for dolphins kept in captivity. It should be emphasised that the effects of hyposalinity, whether involving osmotic damage of epidermal tissue and/or physiological stress, would act in concert (i.e. demonstrate potential synergism) with other stressors, and it is this particular coincidence of factors in an individual that leads to subsequent (e.g.) secondary infection, exacerbation of existing health challenges, loss of body condition, increased parasite loads, etc.

[118] Images of the October 2003 mortality near Ascot are also suggestive of severe lesions, but this is speculative only (no post-mortem was conducted).

In environments where dolphins face multiple, often chronic stressors, a 'healthy' dolphin may often exist quite near a threshold for infectious disease, and need only experience some additional health challenge (e.g. a new stressor or the intensification of an existing one) for disease to develop (or, for example, for a low-level infection to become more severe). The poxvirus/TSD infections in the 17 September and 25 October dolphins likely reflect this, e.g. these two individuals may have had a typical presentation of TSD for some time, but experienced some conflux of factors that led to the onset of the severe lesions that they ultimately died from. Although we cannot definitively determine what that suite of factors was for either individual, it seems likely that epidermal damage from low salinity conditions was a contributing factor. We note, however, the likely influence of other factors and our efforts to pursue the means from which to pursue the testing of other hypotheses, such as the potential presence of a more virulent form of poxvirus.

It has also been hypothesised that low salinity conditions may contribute to the seasonally observed increased incidence of 'red spot' disease (*Aphanomyces invadans*, a fungal disease also known as 'epizootic ulcerative syndrome, EUS) observed in black bream within the Swan Canning Riverpark (Alan Cottingham, Murdoch University, personal communication). EUS can result in mass mortalities in many fish species, and may occur in periods of low temperatures and following heavy rainfall (Dr. Richmond Loh, Department of Agriculture, personal communication).[119] Both the 17 September and 25 October dolphins were tested for *Aphanomyces invadans* and were found to be negative.

Dolphins and fish share important physiological similarities, and the concept of osmotic damage exacerbating pre-existing skin disease is well-known in fish, having been implicated in the aetiopathogenesis of several erosive and ulcerative disease syndromes, including the development of secondarily bacterial/fungal-infected epidermal ulcers following primary injury by toxic dinoflagellates in estuarine fish on the U.S. Atlantic Coast (Noga et al. 1996). Fish may provide a useful comparative group for dolphins in the Swan Canning Riverpark, as the two groups experience similar physiological challenges and, even if they are differently equipped to deal with those challenges, they may still show functionally similar responses to adverse conditions and exhibit broadly similar indications of ill-health. Naga et al. (1996) noted the similarity between the deeply-penetrating ulcers they observed in fish from their study to skin disease in bottlenose dolphins that died in a mass-mortality associated with morbillivirus along the Atlantic coast in 1987-1988.[120] While dolphins initially had blisters affecting the epidermis, this progressed to the dermis in later stages, following secondary bacterial infection

119 EUS has been associated with drops in salinity below 10 ppt. It has also been hypothesised that the incidence of EUS in fish could relate to acidic water conditions (e.g. from acid sulphate drainage) [S. Fisher, Department of Health, personal communication].

120 The presence of *Aphanomyces invadan* was not found in histological analyses of samples from the 2009 mortalities, and was therefore excluded as causal factor. However, environmental changes that support or trigger the occurrence of EUS may be similar to those influencing the ulcerations in the dolphins.

inducing large necrotic ulcers.[121] Severe ulcerative skin lesions are sometimes observed in morbillivirus epizootics (Geraci 1989, Kennedy 1998).

G. Presence of primary pathogen (morbillivirus)

To date no characteristic histopathological lesions suspicious of morbillivirus have been seen (i.e. non-suppurative meningoencephalitis, pulmonary changes as detailed below). However, on post-mortem examination we have consistently found many dolphins [both from the Swan Canning Riverpark and from the Bunbury-Leschenault populations] that presented with severe pulmonary disease characterized by severe necrosuppurative bronchopneumonia with the presence of opportunistic secondary pathogens.

This presentation may efface any of the more early, subtle and transient morphological changes [e.g. acidophilic intracytoplasmic viral inclusions, intranuclear viral inclusions, and syncytia formation in the alveoli and bronchiolar epithelium] associated with morbillivirus which may have been present *before* secondary infection, and potentially were responsible for the tissue damage (and therefore breach of host defences) that would have allowed for the secondary infection(s).

In addition, two of the dolphins from the Swan post-mortems (8 June and 21 June 2009) had meningoencephalitis and bronchopneumonia attributable to *Aspergillus* spp. respectively (the bronchopneumonia seen in the June 21 individual was also complicated with opportunistic bacteria). In the literature the largest number of reported cases of meningoencephalitis related to *Aspergillus* spp. (as seen in the June 8 mortality) in cetaceans have been in association with morbillivirus (Domingo et al. 1992, Domingo et al. 1995, Schulman et al 1997). Mycotic pneumonia was also reported more frequently in bottlenose dolphins with morbillivirus infection than in controls that died from other causes (Schulman et al. 1997). The majority of the individual cases reported in the literature have also had necrosuppurative bronchopneumonia.

Non-suppurative meningoencephalitis occurs in some dolphins that survive the acute interstitial pneumonia (Duignan et al 1992, Domingo et al. 1992, Schulman et al. 1997). As with the lungs, the viral-induced lesions (malacia, demyelination, astrocytosis, syncytia, intra-nuclear and intracytoplasmic inclusions, peri-vascular cuffing) may be confounded or obscured by morphological changes due to secondary opportunists. Many morbillivirus-affected dolphins may also/instead demonstrate serious systemic infections with *Toxoplasma gondii*, *Aspergillus* spp, and other fungi. Such systemic infections include necrosuppurative bronchopneumonia with complicating secondary *Aspergillus* spp. and bacteria, such as seen in the June 21 mortality. Given our findings, it is reasonable to suggest that a primary pathogen known to have immunosuppressive effects such as morbillivirus may be a contributing factor in the Swan-Canning Riverpark dolphin mortalities.[122]

121 The presence of poxvirus could not be detected by culture or electron microscopy of tissue samples from dolphins from the 1987-1988 mortality (Schulman et al. 1997).

122 Pathognomonic lesions may be seen in classical acute cases of morbillivirus in which individual die during the active phase

H. On-going analyses

We aim to definitively identify the presence of poxvirus in tissues from the mortalities utilising a variety of ancillary techniques (detailed in following paragraphs). Further diagnostic tests will also be conducted to determine whether morbillivirus was present in these tissues. It should be noted at this point that our sample size is small (given the opportunistic and retrospective nature of our study), and whilst positive results are significant, negative results would not definitively rule out the presence of morbillivirus. We are likewise limited in our ability to provide tissues to characterise the strain of cetacean poxvirus present.

This consideration emphasises the potential value of obtaining a larger sample size of tissues drawn from individuals at multiple sites (e.g. from the Swan Canning Riverpark and the Bunbury-Leschenault areas as examples of estuarine populations, plus 'control' populations drawn from populations inhabiting more pristine, non-estuarine area such as Shark Bay). This approach would allow for baseline data on the incidence of disease to be obtained, and potentially could be expanded to consider confounding variables such as contaminant burdens.

Histology blocks from several dolphins from Bunbury and the Swan Canning Riverpark have been sent to Dr. Tony Patterson (Pathology, Disease Surveillance and Investigation Branch, Veterinary Sciences Division, Agri-Food and Biosciences Institute, Stoney Road, Stormont, Belfast) for immunohistochemistry analysis to examine the presence of morbillivirus antigen.

We are awaiting the viral microarray results from Dr. Joe DeRisi (San Francisco University) that may help in definitively identifying the presence of morbillivirus and to characterise the strain of poxvirus present. DNA samples have also been sent Dr. Carlos Romero (University of Florida) for screening of poxviruses. The objective of the analyses for poxviruses at the two U.S. laboratories is to be able to compare the poxvirus found in the Swan dolphins with those sequenced from dolphins elsewhere in the world. This will aid in the investigation of the hypothesis that the 'atypical' TSD lesions seen in the 17 September 2009 and 25 October 2009 dolphins are caused, at least in part, to an unusually virulent strain/subtype of poxvirus.

Dr. Nahiid Stephens will examine glutaraldehyde fixed skin sections using electron microscopy (EM) to attempt to positively identify the presence of poxvirus at an ultrastructural level. These sections are available for only two of the dolphins (17 September 2009 and 25 October 2009), both of which had demonstrable intracytoplasmic viral inclusion bodies associated with 'atypical' TSD skin lesions.

of viral replication. This presentation is much less a feature of chronic cases that survive those early lesions, and then go on to die from some opportunistic infection or to develop the encephalitic form. Dolphins may manifest all variations in the disease from the acute to chronic, and histological diagnosis can be extremely difficult. For example, the role of morbillivirus in a bottlenose dolphin epidemic in 1987-88 along eastern coast of the U.S. was not confirmed until immunohistochemical techniques and genomics were developed following subsequent morbillivirus epidemics in Europe. Infection with or without high rates of mortality can occur as well.

While EM is a relatively insensitive method and viral microarray assays are superior, there is some value in attempting positive identification using this technique.

VII. Contaminants

A. Literature review

All vertebrates harbour some sort of contaminant burden (O'Hara and O'Shea 2001). The presence of potentially toxic contaminants in the tissues of marine mammals, however, does not constitute evidence of harm (O'Hara and O'Shea 2001).

Various contaminants have been reported to be associated with deleterious effects on the immune, endocrine and nervous systems of marine mammals. However, direct associations between contaminants and these effects are few. According to Evans (2003, p. 400): "...most studies lack substantive evidence of sub-lethal effects due to numerous physiological and environmental confounding factors." It is, however, also important to consider that although there is a lack of experimental data on cause-effect 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 (Ross 2002), and consequently little is known about the cumulative impact of the complex mixtures of contaminants often found in marine mammals. A similar scenario exists for understanding the significance of contaminants exposure in humans and according to Ross (2002) indirect associations and the extrapolation of extensive research on the adverse effects of pollutants/contaminants in laboratory animals is often applied.

1. 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).

2. 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 (Krahn et al 2004). The inner blubber layer is thought to be more metabolically active than the outer layer that 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).

Time since death may also alter contaminant concentrations in tissues. Borrell and Aguilar (1990) repeatedly sampled blubber of a stranded striped dolphin (*Stenella coeruleoalba*) carcass for OCs over a 55-day period and found changes in concentrations (both increases and decreases, depending on the compound), beginning two weeks after initial sampling.

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

levelling 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. (2001b) 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). Mean ∑PCB was more than 2.5 times higher in females whose calves did not survive (P = 0.076). It should be noted that the results of this study 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 8) In order to improve the accuracy of comparing contaminant concentrations between studies the following factors were considered in the development of Table 8:

- **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 8.
- **Total DDT levels can be influenced by the number of isomers included**. Therefore, for consistency total DDT levels reported in Table 8 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 that may impact on the accuracy of comparing contaminant levels between studies, there are several variations that could not be accounted for in most studies. These factors include: health status (diseased versus by by-caught dolphins); geographic location; diet; sex; and life-history traits (i.e. age, reproductive state); as well as the analytical procedures involved in identifying and quantifying the contaminants. Each of these factors may all have influenced the contaminant concentrations recorded in these studies, and thus comparisons must be made with caution.

As a further benchmark for comparing contaminants burdens specifically in estuarine bottlenose dolphins in urban areas, Table 9 includes contaminants results from full thickness blubber samples collected from purposely caught and released wild dolphins in two estuaries (Charleston and Indian Lagoon) from 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).

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 surface water (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: "Chlordane 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 range much less than 100 µg/g wet weight, with many samples at 10 µg/g wet weight or less (O'Shea 1999). Table 8 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 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).

A baseline study of contaminants in the Swan and Canning catchment drainage system in 2006 did not detect PCBs in either sediment or surface water samples (Nice et al. 2009). However, Nice et al. (2009) recommended that this 'non-detect' data should be treated with caution in consideration that the laboratory limit of reporting was relatively high "compared to concentrations of these compounds that are known to cause deleterious effects to environmental health."

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 as 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 refer to the number of carbon atoms in the bipheyl skeleton (for PCBs this is 12). The second two numbers 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).

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 (for example 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 8 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 geometric mean of the NOAEL and LOAEL. Kannan et al. (2000) examined the studies by Boon et al. (1987) and Brouwer et al. (1989) where by seals fed fish from the Wadden Sea (high-level PCB contamination) were found to have significantly lower concentrations of vitamin A and thyroid hormones as compared 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) analyzed 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 (∑PCB concentration [as Aroclor 1254] = 3.0 x ∑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 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)

3. 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 be contributed to 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, 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 that are not required for the normal functions of an animal. Some non-essential metals tend to be toxic at low concentrations (e.g. mercury, cadmium and lead) while others are relatively non-toxic. The toxicity of many elements is also associated with specific chemical forms such as free ions or 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 ANZECC and ARMCANZ (2000) guidelines (Section 8.3.7).

The majority of heavy metals, apart from methyl mercury, do not bioaccumulate through the food chain, instead they bioconcentrate (i.e. concentrations are higher in an organism compared to concentrations in the surrounding environment) (Evans 2003). 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 10 lists the mean concentrations of heavy metals reported in the liver of bottlenose dolphins from various locations around the world.

4. Organometallics

Since the 1960s, tributyltin (TBT) was widely used as a biocide in antifouling paints used for 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 2006). 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.

B. Contaminants Assessment

1. 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 suit of analytes for the deceased dolphins because they are persistent organic pollutants, are considered to be ubiquitous and known to accumulate in marine mammals (O'Shea 1999). As in the NNCP, consideration was also given to the following:

- the findings of previous studies within the Swan Canning system
- the known toxicity of key contaminants (such as contaminants that feature on the 'dirty dozen list' of persistent organic pollutants (Stockholm Convention 2001)
- the likelihood of contaminant occurrence due to land uses within the Swan Canning catchment
- the ability of laboratories to accurately determine the concentration of contaminants using endorsed methods

2. Sample collection and analysis

<u>Organic contaminants</u>

During post-mortems blubber samples were collected for organic contaminants analysis. All blubber samples were collected from a location just anterior to the dorsal fin according to standard practice and approximately 100 grams of blubber was taken. 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).

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, 09/257, 09/665, 06/348, 08/379 and 08/943. The Chemistry has NATA accreditation for TBT but not methyl mercury.

C. Results

1. Organic Contaminants

Table 11 summarises the Dieldrin, DDT and DDT metabolites results for both the Swan River and Bunbury dolphins. 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 12 and 13.

Total PCB concentrations have been reported in Table 14 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 8. For the concentrations of each of the 21 congeners measured per individual dolphin refer to Tables 15 and 16.

The concentrations of the four PAHs detected in the dolphins are presented in Table 17. For a comprehensive list of all PAHs analysed refer to Table 18.

2. Heavy metals

The results of all heavy metals detected in liver samples and expressed as wet weight are depicted in Table 19. Liver results expressed as dry weight are in Table 20. The results of heavy metals detected in kidney samples and expressed as wet weight are in Table 21. Table 22 lists the concentrations of lead detected in bone.

3. Organometallics (methyl mercury and TBT)

Table 23 lists the methyl mercury and TBT results expressed as parts per billion or on a unit of mass basis as ng/g wet weight.

D. Comparative evaluation of key contaminants

Dieldrin, DDE and PCBs were the predominant organic contaminants detected in blubber samples from deceased 2009 Swan River dolphins. It should be noted that all the contaminant levels reported were from the carcasses of stranded animals and previous research has shown that organic contaminant levels reported in stranded animals may not necessarily be a true reflection of contaminant levels in the general population because of the concentrating effect on organic contaminant levels caused by loss of body lipid mass during disease induced catabolism or starvation (O'Shea 1999). Another important consideration is that all of the 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 River dolphins were higher than those detected in the Bunbury dolphins thus indicating spatial differences in environmental contamination (Table 11). The average dieldrin levels detected in the Swan River dolphins are amongst the highest levels reported globally in marine mammals in recent times (O'Hara and O'Shea 2001; Tables 8 and 9). 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 mobilized, contaminants stored in blubber may not have a direct toxic effect (Fair et al. 2010). Accumulated lipophilic contaminants may be mobilized during pregnancy and lactation, starvation, and disease states (Aguilar 1987). Four of the Swan River dolphins had evidence of disease, but most were in good body condition. However, although these observations are noted, it is not possible to determine to what extent contaminants may have been mobilized in these individuals.

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), yet regardless some of the Swan and Bunbury dolphins had similar elevated levels of total PCBs and total DDT 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 to those used on the Swan and Bunbury samples and is therefore not directly comparable with our results. This threshold value which 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 12 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 12), 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 is more accurate to compare the total PCBs as the sum 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 maintain the concentration within a specific range (i.e. regulate) 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).

E. The role of contaminants in the deaths of the 2009 Swan dolphins

Several factors preclude a definitive determination of what role contaminants may have played in the 2009 dolphin mortalities in the Swan Canning Riverpark. These factors largely reflect the difficulties in relating tissue concentrations of contaminants to particular health effects in marine mammals and, more specifically, to the pathological observations found in post-mortem examinations of dolphins from the Swan Canning Riverpark. Studies of marine mammal mortality events have shown that it is exceedingly difficult to elucidate the relative contributions of multiple stressors that may have similar effects (e.g. on immune function), particularly when the number of mortalities is small.

Some of the persistent organic contaminants recorded in the Swan dolphins may have been in the environment for decades and the use of most of these contaminants has been banned for some time. These factors suggest that: (a) contaminant concentrations were likely to have been higher in previous generations, and (b) if chemical contaminants were having a profound effect on dolphins then unusual mortality events would have been recorded in previous decades. We note, however, that there remains uncertainty about the environmental and biological (trophic) factors influencing the bioavailability of organic contaminants within the Swan Canning Riverpark, and that the ecotoxicology of contaminants

within the estuary ecosystem remains an area of active research. It is possible, for example, that the factors influencing bioavailability may have changed over time.

Although we conclude that chemical contaminants were not a direct cause of the 2009 mortalities, this is not to say that contaminants do not have an adverse effect on the health of dolphins in the Swan Canning Riverpark. It is likely that chemical contaminants do adversely impact on the health of the Swan dolphins at present, and have also affected previous generations. We note that the adverse effect of contaminants may be insidious, and could potentially combine with other stressors to have an overall effect that influences the incidence and severity of infectious disease.

VIII. Conclusions

Key Findings

1. **The six mortalities in 2009 constitute an 'unusual mortality event' for the resident community of bottlenose dolphins within the Swan Canning Riverpark.** The six deaths are above an estimated baseline carcass stranding rate of one mortality a year, based on stranding records from 2002-2008. The deaths also cluster in two groups (three deaths in June and three deaths in September-October). The latter mortality cluster included two dolphins with severe skin lesions.

2. **The six deaths represent a biologically significant impact on the resident dolphin population.** The small size of the community makes the deaths of three females in 2009 a concern. These deaths follow the death of another adult female in 2007. Research in 2001-3 estimated that the size of the resident community at the time ranged between 20-25 individuals, depending on the number of calves present. Critically, the community at that time included only six adult females. The size and composition of the current resident community is not known, but is likely to be similar to, or less than, it was in 2001-3, based on the low reproductive rates of dolphins and the site fidelity of inshore bottlenose dolphins.

3. **A multi-factorial model provides the best explanation for the 2009 deaths.** Post-mortem examination of four of the dolphins indicates multi-factorial aetiologies characterised by: (a) secondary infection by opportunistic bacterial and fungal pathogens and, in two cases, (b) the progression of poxvirus infections to severe ulcerative skin lesions. These presentations likely reflect reduced immunological function (by a suite of factors), and damage of epidermal tissue by entanglement injury, and (in the two adult females with severe skin lesions) lesions caused by poxvirus/tattoo skin disease and exposure to low salinities (osmoregulatory disruption). While we have identified the probable causative factors present and, in some instances, the likely mechanisms involved, we cannot determine exactly how these factors combined to result in the deaths of these dolphins. This conclusion is consistent with the findings of other studies of marine mammal mortalities, and reflects in large part the lack of knowledge about marine mammal health in Western Australia. It is clear, however, that human interactions are a significant source of injury and mortality for dolphins inhabiting estuarine and coastal ecosystems in southwestern Western Australia.

4. **This investigation confirmed the presence of intracytoplasmic eosinophilic viral inclusion bodies characteristic of poxvirus.** These were present in scattered keratinocytes from two of the 2009 mortalities (17 September 2009 and 25 October 2009) and in the 2007 mortality (18 November). Poxvirus is the pathogen that causes tattoo skin disease (TSD), an epidermal skin disease in dolphins that causes skin lesions in cetacean populations around the world. Two aspects of the TSD observed in the 2009 mortalities are unusual. First, TSD generally affects juvenile

animals and is not typically observed in adults, who generally have acquired immunity to poxvirus. Second, the TSD lesions in the two 2009 adult females exhibited an acute progression to severe skin lesions that were deeply ulcerative and infected with opportunistic microbial pathogens, causing terminal debilitation. Low salinity conditions within the Swan Canning Riverpark may be associated with this progression, by causing osmotic damage to epidermal cells and perhaps decreased immune function. We are referring to this as 'atypical poxvirus/TSD' pending further resolution of its aetiopathogenesis.

5. **We are unable to resolve all aspects of inquiry at this time.** While the majority of the investigation has been completed, certain aspects are continuing, with several analyses still pending at this time. As such, the conclusions presented here should be considered subject to review after completion of these analyses. The analyses involve questions about pathogens causing infectious disease (particularly viral pathogens). There are no further contaminants analyses anticipated. These on-going analyses reflect our effort to evaluate potential contributing factors using the full suite of molecular, histological, and other analytical approaches available, and also to establish best-practice in the event of future mortalities. Our intent is that these analyses should be concluded in the second half of 2010, but we cannot provide a definitive timeline. The on-going analyses involve collaborative arrangements with research institutions possessing specialised analytical expertise.

6. **The pathology of the 2009 mortalities suggests that the Swan dolphins are likely to experience stressors that reduce their immunological function.** Potential indications of reduced immunological function include the prevalence of tattoo skin disease in adults and the presentation of opportunistic (secondary) infections. Reduced immunological function may relate to environmental conditions (e.g. low salinity), contaminants, a primary pathogen, disturbance from human interactions, or other factors, and is likely to reflect cumulative and potentially synergistic interactions among stressors.

7. **Concentrations of certain contaminants were high, which suggests that contaminants, in concert with other factors, may influence the health of dolphins.** Concentrations of dieldrin were among the highest in the world (at current time), and concentrations of PCB congeners for some dolphins exceeded published toxicity thresholds for effects on immune function. Several factors preclude a definitive determination of the impact of contaminants on the health of dolphins and their potential role in the 2009 mortalities, including uncertainty over the relationship between tissue concentrations and physiological effects and the presence of other stressors that have effects on immunological function that are analogous to those potentially associated with high contaminant burdens. However, it is reasonable to conclude that contaminants may—at the concentrations observed—have an adverse effect on dolphin health, and to the extent that contaminant inputs to the estuary could feasibly be reduced, this reduction would be to their long-term benefit. Their potential effects should be seen in the context of the range of stressors to

dolphins, and with the understanding that other stressors, particularly entanglement injuries, can cause mortality directly and are more amenable to management intervention (e.g. regular removal of fishing line from bridges within the estuary).

8. **Aspects of the ecology of the resident dolphin community make it vulnerable to anthropogenic stressors.** These aspects include individual site fidelity and the small size of the resident community. Natal philopatry (retention of maternal home range) and long-term site fidelity are characteristic of bottlenose dolphins within nearshore and estuarine ecosystems. These characteristics suggest the potential for the resident community to be demographically isolated. If this occurs, immigration rates would be limited, and the reproductive output of a handful of resident females would ultimately underlie the persistence of dolphins within the Swan Canning Riverpark. These considerations, and the low reproductive rates of dolphins, suggest that the resident dolphin community is highly vulnerable to anthropogenic processes that adversely affect birth rates, juvenile survivorship, and adult mortality (particularly for females).

9. **Human-induced injuries are a significant health challenge for dolphins within the Swan Canning Riverpark, Perth metropolitan waters, and Bunbury area.** Examples of mortalities related to human-induced injuries include: a juvenile female (21 June 2009) in the Swan Canning Riverpark with an entanglement injury that had persisted for more than a year, the Bunbury juvenile 'Cruiser' (30 September 2009) who also had chronic entanglement, and 'Vevay' a Bunbury female who suffered an acute vessel strike with fatal wounding. In Cockburn Sound, at least six calves within Cockburn Sound experienced entanglement events/injuries between 1996-2004, and a seventh calf exhibited injuries indicative of a vessel strike.

10. **The 2009 mortalities and the pattern of mortality observed within the Bunbury area suggest that estuarine dolphins experience a combination of natural and anthropogenic stressors that may be sufficient to cause a long-term decline in the abundance of dolphins associated with these ecosystems.** This conclusion is consistent with studies of the health of estuarine dolphins in other locations and with patterns of unusual mortality events for marine mammals. Estuaries are stressful environments for dolphins, and the life history of dolphins leaves them poorly-placed to compensate for increased mortality from infectious disease, human-induced injury, and other stressors.

Concluding Remarks

We close with some remarks on the impact of the estuarine habitat that the Swan dolphins inhabit, their conservation biology, and the study of marine mammal health.

1. Ecosystem: The Swan Canning Riverpark is a stressful environment for dolphins.

In many ways this investigation lies at the nexus of pathology and ecology, and one of its conclusions is a familiar ecological observation—estuaries are uniquely challenging environments for organisms like dolphins to survive and reproduce within. The 2009 dolphin mortalities illustrate both the general challenges that estuaries present to the health of dolphins, and also those challenges that are—in type, severity, and interaction—more particular to the Swan-Canning Riverpark.

Estuaries present a range of natural (e.g. environmental conditions) and anthropogenic (e.g. entanglement, contaminants, disturbance) stressors, and these stressors may interact in ways that are cumulative and synergistic, an aetiology that makes it exceedingly difficult to establish clear pathologic causation through the conventional methodologies available to epidemiological studies: post-mortem examination; field behavioural study of free-ranging animals; laboratory analyses of tissue samples; and the collection and analysis of data for environmental factors.

Estuaries are:

- **dynamic ecosystems** in which temperature, salinity, and other physico-chemical parameters may change rapidly, an intrinsic characteristic that may be exacerbated by anthropogenic changes affecting river and run-off flows. These changes can cause physiological stress to dolphins that adds to the stress they experience from other stressors. Climate change is an additional source of change that, although not affecting dolphins directly, may alter trophic structures, contaminant availability, and environmental features.

- **‘sinks’ for pathogens** where they occur in proximity to human settlement. As receptacles for wastewater and catchment run-off, estuaries may contain pathogens not present in adjacent coastal environments, such as those occurring in human and livestock faeces.

- **repositories for fishing waste** as estuaries offer productive and protected waters for fishing that are often close to population centres and easily accessible. These factors often lead to intensive fishing activity and thus to the discarding of a large amount of fishing gear, either through accident or negligence. Bridges and vegetated foreshore areas are typically intensively fished, and also offer an ideal for fishing line to be come snagged. These processes lead to an accumulation of discarded monofilament fishing line, which is exacerbated by the fact that regular monofilament material is so resistant to decay (i.e. it may take >500 years to fully degrade).

- **prone to harmful algal blooms**, thus increasing the likelihood that dolphins will be exposed to a harmful biotoxin. Algal blooms also affect fish stocks, and thus may cause localised depletion of some prey.

- **reservoirs for contaminants**, where they exist in close proximity to human population centres and to inland agricultural areas inland. While the use of many organic contaminants has been banned in Western Australia, these are also persistent in the environment for long periods of time, and thus may remain within or cycling between terrestrial and estuarine sediments, ground and surface waters, and organisms. This position of the dolphins as an apex predator means dolphins can accumulate high concentrations of contaminants over time.

- **centres of development and ecological change**. Specific developments such foreshore buildings and marinas typically affect dolphins indirectly (e.g. by altering the availability of prey or by increasing environmental noise), although some more direct effects may occur (e.g. by an increasing risk of boat strike because of greater boat traffic). Some changes can even be beneficial, such as the congregations of fish that sometimes occur around anthropogenic structures. More generally, development of estuarine areas tends to intensify specific stressors such as noise, vessel traffic, and disturbance, and to add to the general ecological stress animals experience because of human-induced changes in prey stocks.

These considerations emphasise the way in which estuarine dolphins—relative to coastal and offshore populations—experience greater levels of general stress from human activities and a variable environment, as well increased levels of more specific forms of stress such as entanglement injury and exposure to toxicants. Finally, layered above these characteristics can be the occasional intrusion of a primary pathogen of significant virulence. For these reasons, the size of estuarine dolphin populations may not be tightly coupled to the environmental carrying capacity. Rather, these populations are probably more strongly regulated by mortality from disease and anthropogenic injury, with population size reflecting some interim balance between birth and death rates.

2. Conservation Biology: Dolphins are highly vulnerable to processes causing elevated mortality.

Estuarine dolphins exhibit life-long site fidelity to a small and typically well-defined home range, and thus have a strong and enduring ecological linkage to the estuarine ecosystems. As populations, they consist of small communities ranging within the estuary and adjacent coastal areas that are likely to be characterised by some degree of demographic isolation, i.e. there is little immigration into the population by animals in adjacent areas. Their small population size means that they may often be naïve to primary pathogens that occur within larger cetacean populations offshore. Morbillivirus, for example, could be introduced into coastal and estuarine dolphin populations from offshore populations of pilot whales and other cetaceans. Finally, as a species, they have low reproductive rates and delayed maturation, factors that limit their ability to compensate for increased rates of mortality.

Human-induced injury is an important health challenge to dolphins in the Swan Canning Riverpark and the Perth metropolitan area generally, and is one group of stressors that ultimately can be managed at a community level, both through community engagement in the management of the estuary and through changes that we, as individuals, can commit to. The Swan River Trust provides an excellent opportunity for concerned community members to participate in projects such Dolphin Watch and a host of other educational and volunteer opportunities. The changes in individual behaviour involve changing the way that we think about the estuary. If we fish, then the use of biodegradable fishing line would have tangible benefits to the wildlife that we share the river with. If we garden, then we can use appropriate fertilisers. If we use boats, then we can use care to ensure that we do not damage the foreshores, seagrasses, and riverbeds, or pollute the water.

3. Marine Mammal Health: Marine mammals as icons & sentinels for the marine environment.

There is much that we do not know about the health of marine mammals in Western Australia. While this investigation has focused on two viral pathogens (cetacean morbillivirus and poxvirus), there are other diseases that are potentially of concern, and for which little information is available on their prevalence and potential impact upon populations of marine mammals. For example, Van Bressem et al. (2009) recently identified a range of other diseases as 'emerging infectious diseases' for cetaceans, including cetacean papillomaviruses, *Brucella* spp., and *Toxoplasma gondii*, that may be endemic to Western Australian cetacean populations, and therefore could be potential cause(s) of unusual mortality events in the future. Or they could have a chronic but biologically significant effect by reducing reproductive capability, or acting in synergism in the aetiopathogenesis of other intercurrent diseases. However, information on the overall health and endemic disease status of Australian cetacean populations is very limited and further work would be needed to understand the complex interaction of stressors, population dynamics, and pathogens in the aetiopathogenesis of disease and in the causation of unusual mortality events.

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XI. Tables & Figures

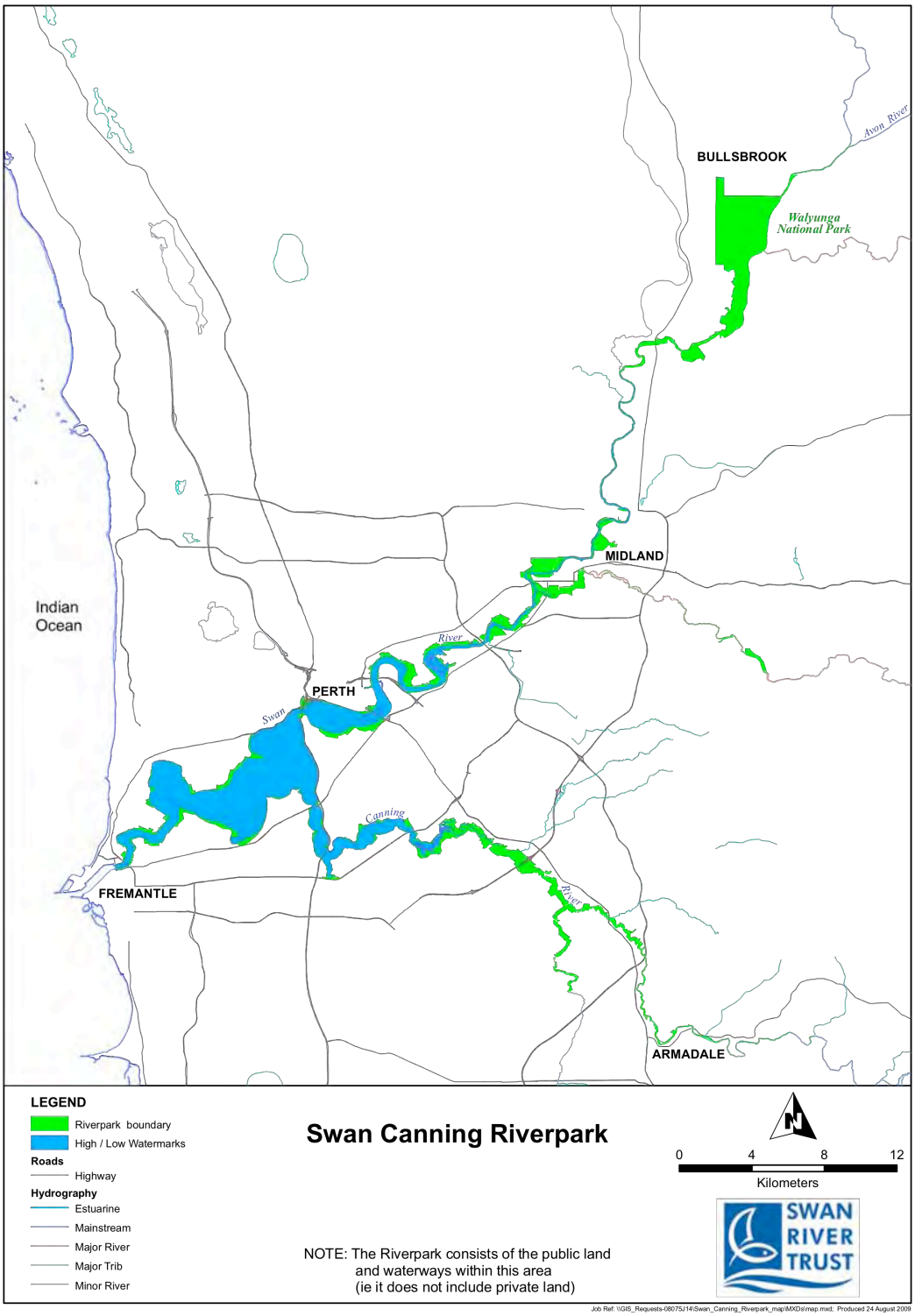


Figure 1a: Map of Swan Canning Riverpark (green indicates Riverpark boundaries).

[From: Swan River Trust]

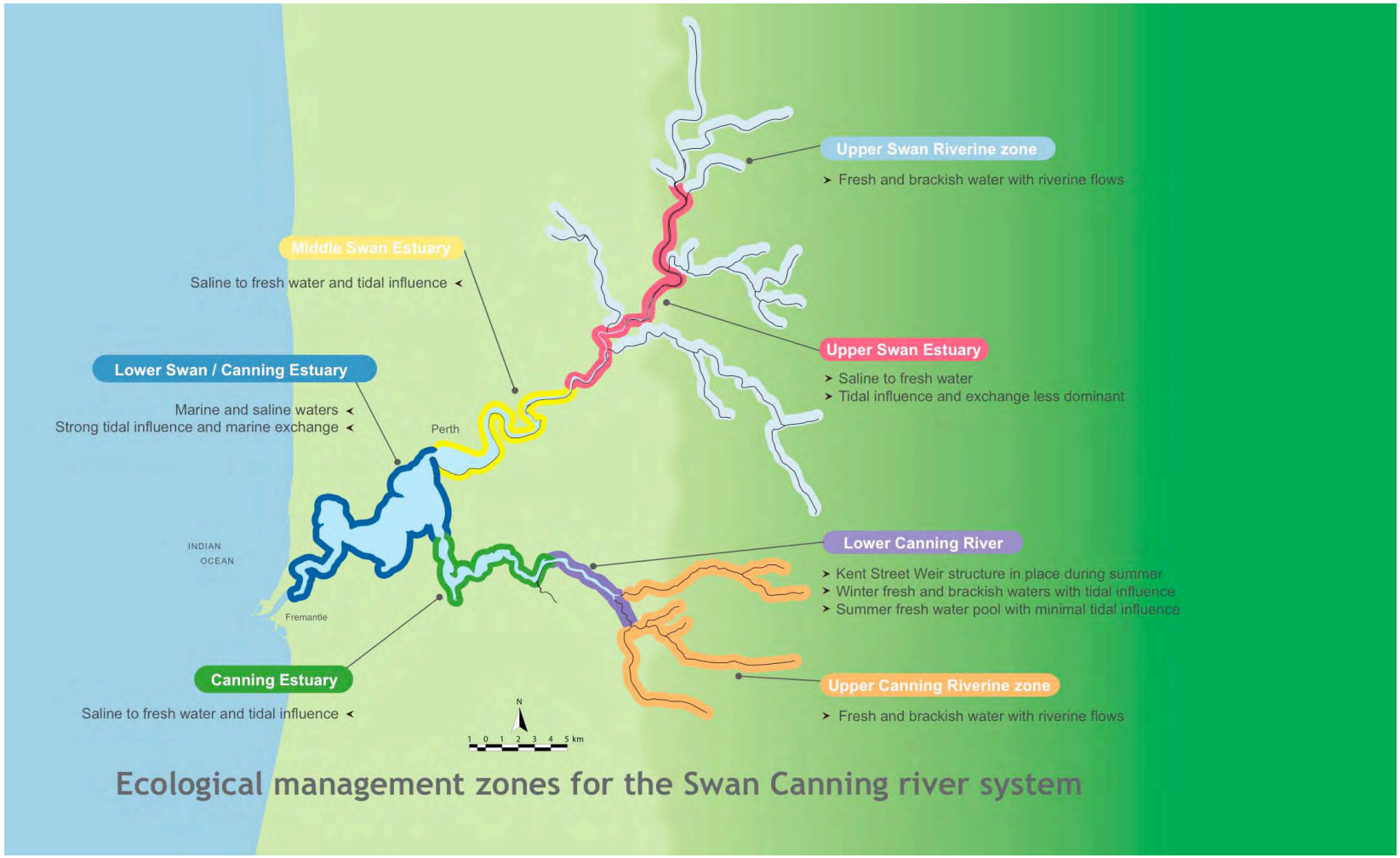


Figure 1b: Map of ecological management zones Swan Canning Riverpark. [From: Swan River Trust]

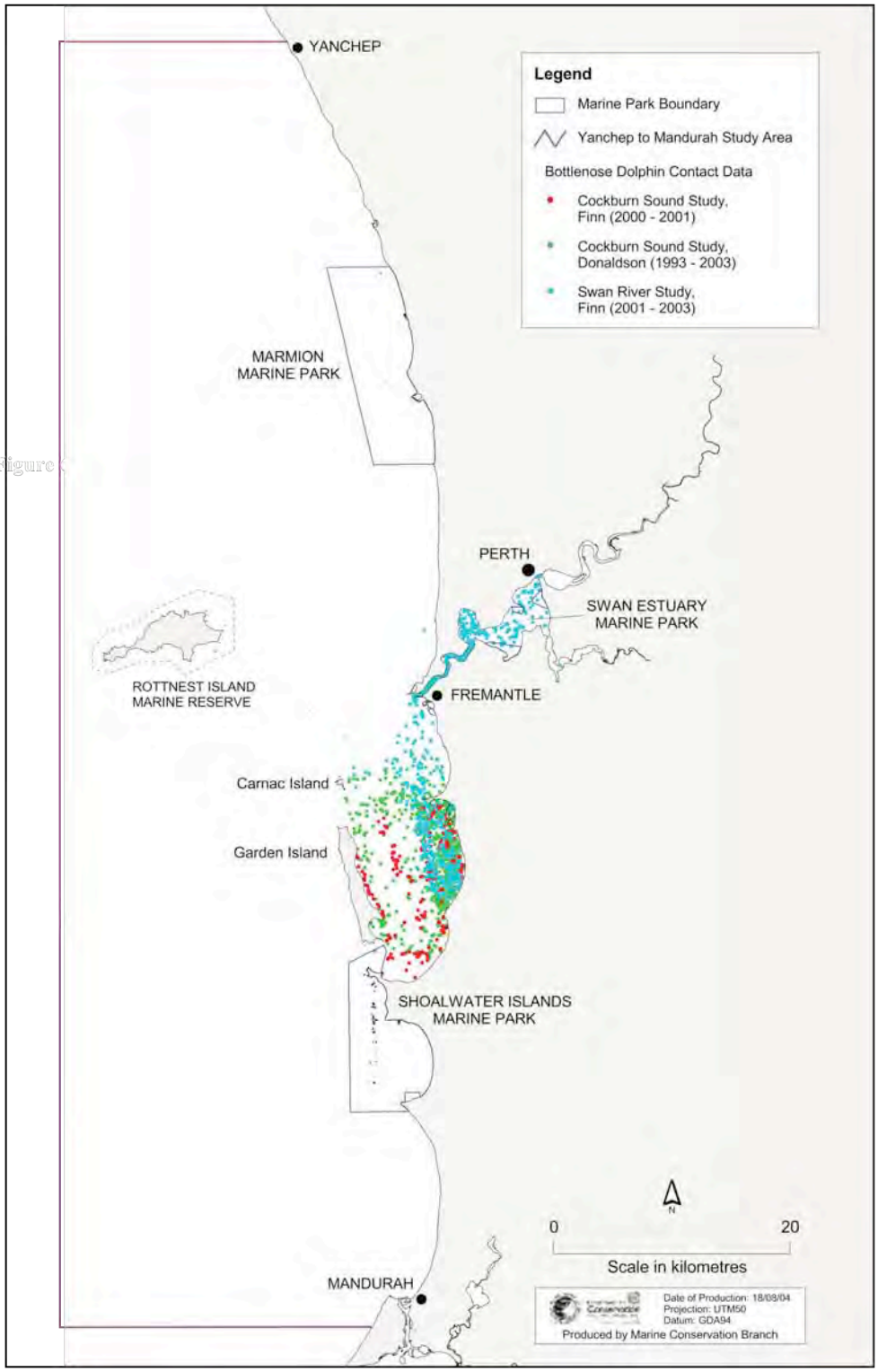


Figure 2: Locations for dolphins observed within the Swan Canning Riverpark and the southern metropolitan coastal waters. [From Cannell 2004]

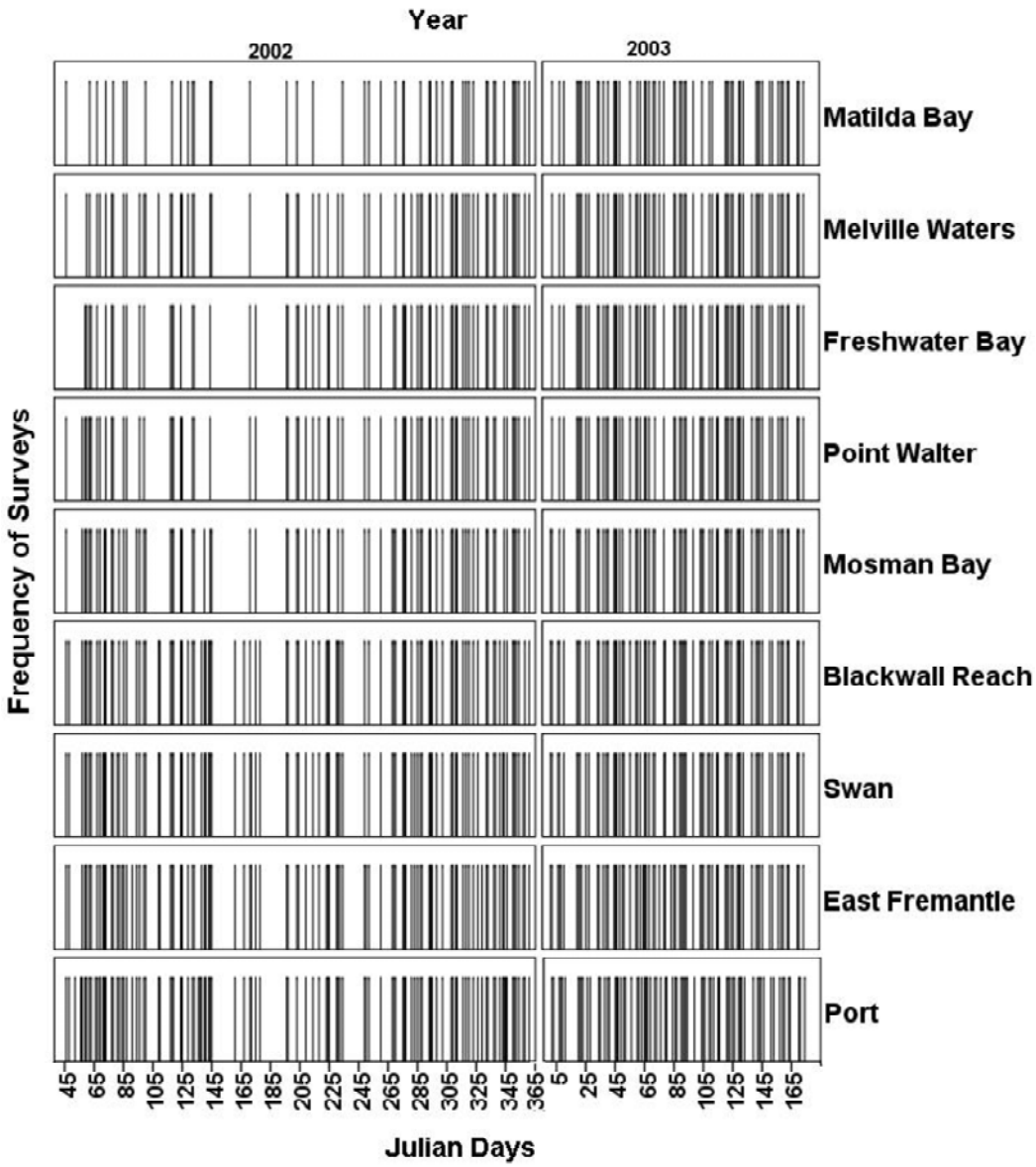


Figure 3: Frequency of search effort (daily surveys of study area) within the nine sampling-areas in the Swan Canning Riverpark from February 2002 – June 2003. Julian days are the enumerated form of calendar days (e.g. 1 January = 1, 2 January = 2, etc.). A line on the day indicates that the sampling-area was searched. [From Moiler 2008]

Table 1: Dolphins considered part of the resident dolphin community in the Swan Canning Riverpark from 2001-3.

Sex-Age Codes: AF = adult female; AM = Adult male; A = adult; SA = sub-adult; J = juvenile

Name & Code	Sex-Age Class	Name & Code	Sex-Age Class
High Nitch	AF	Middy	Suspected AM
A1		A11	
Two Rakes	AF	Topscoop	A/SA
A15		A18	Unknown
Socket	AF	Blackwall	SA/J
A4		A13	Unknown
Wingding	AF	Reiki	SA/J
A8		A22	Unknown
Tupac	AF	Bottomslice	A/SA
A16		A6	Unknown
Leeuwin	AF	Pappy	SA/J
A19		A17	Unknown
Tab	Suspected AM	Keyhole	A/SA
A14		A7	Unknown
Cleft	Suspected AM	Hii	A
A12		A9	Unknown
Real Notch	Suspected AM	Rake	SA/J
A10		A21	Unknown

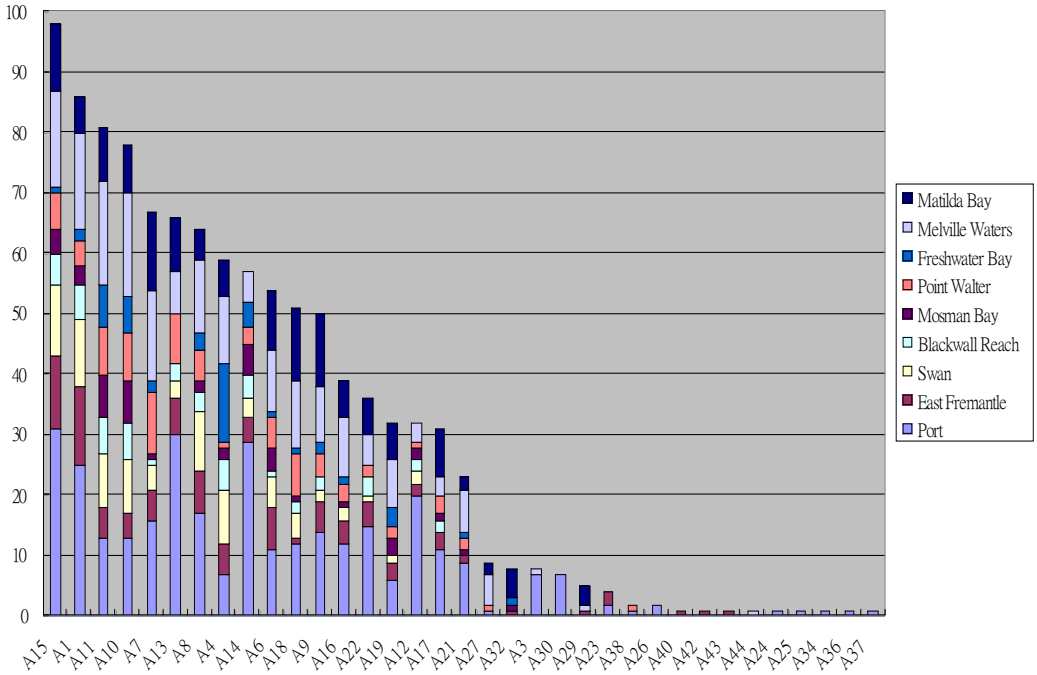


Figure 4: Frequency of sightings and area of occurrence for dolphins identified in the 2001-3 study (calves not included). See Table 1 for codes. [From Lo 2009]

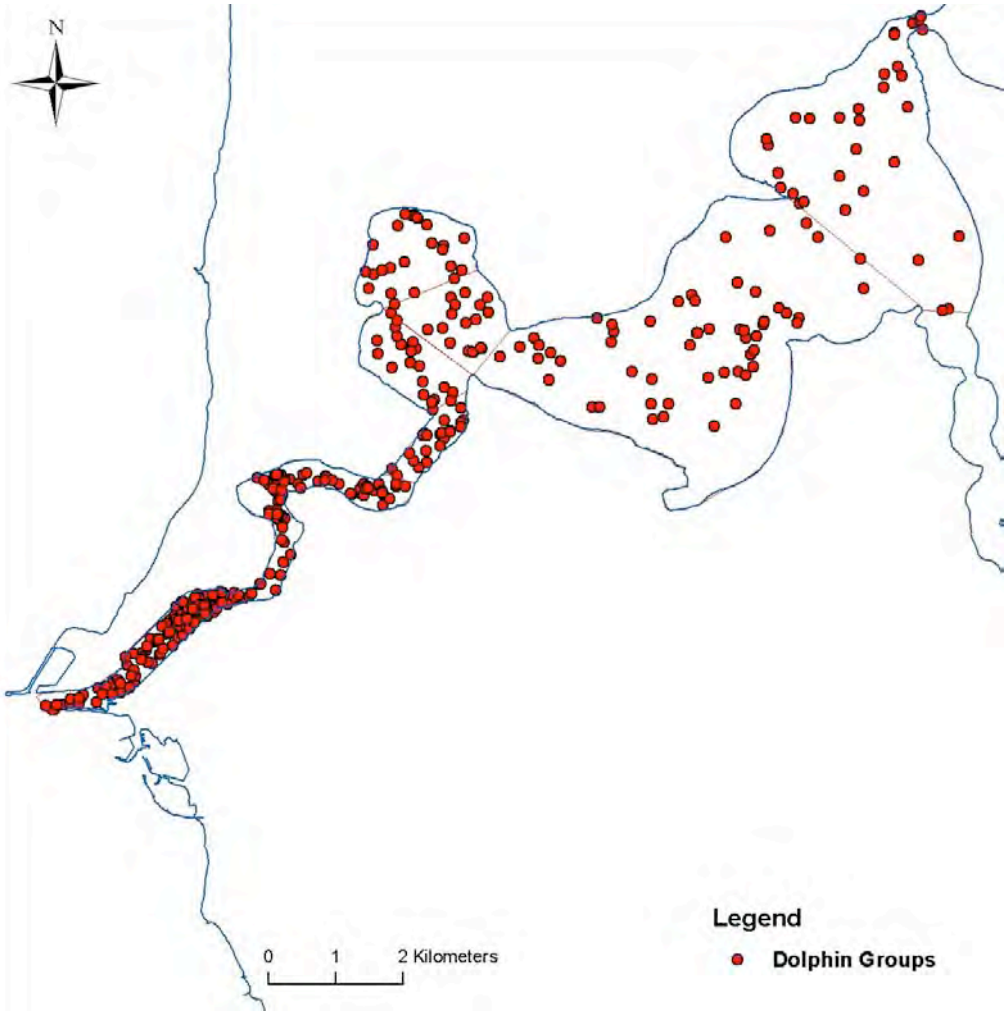


Figure 5: Locations of dolphin sightings (behavioural surveys) from 2001-3. [From Moiler 2008]

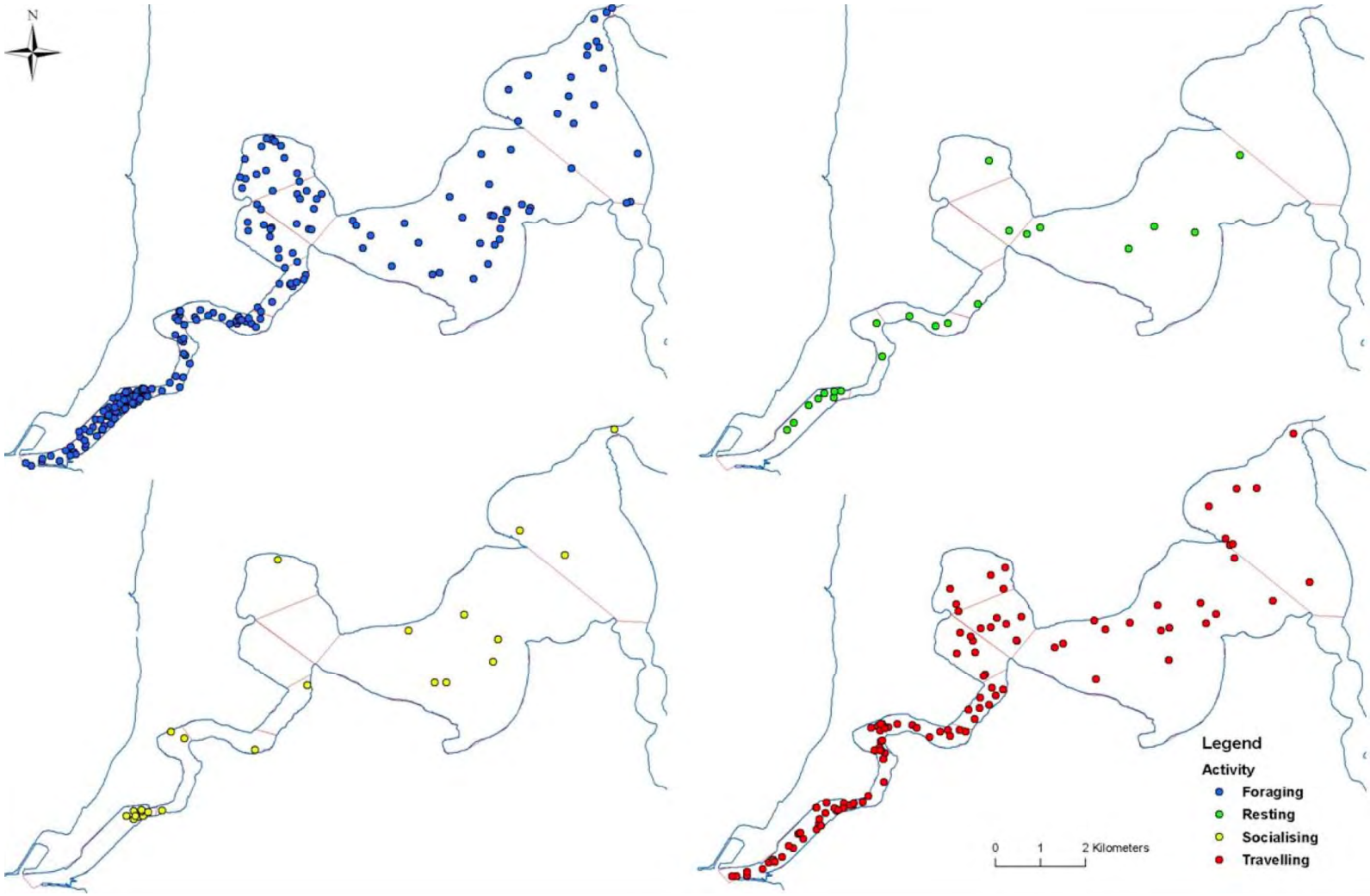


Figure 6: Locations of dolphin sightings by activity. [From Moiler 2008]

Table 2: Predominant activity states for behavioural survey observations from 2001-3 (*n* = 372 surveys). [From Moiler 2008]

Activity State	Proportion (no. of surveys)
Rest	5.9% (*n* = 22)
Socialise	6.7% (*n* = 25)
Travel	30.0% (*n* = 110)
Forage	57.8% (*n* = 215)

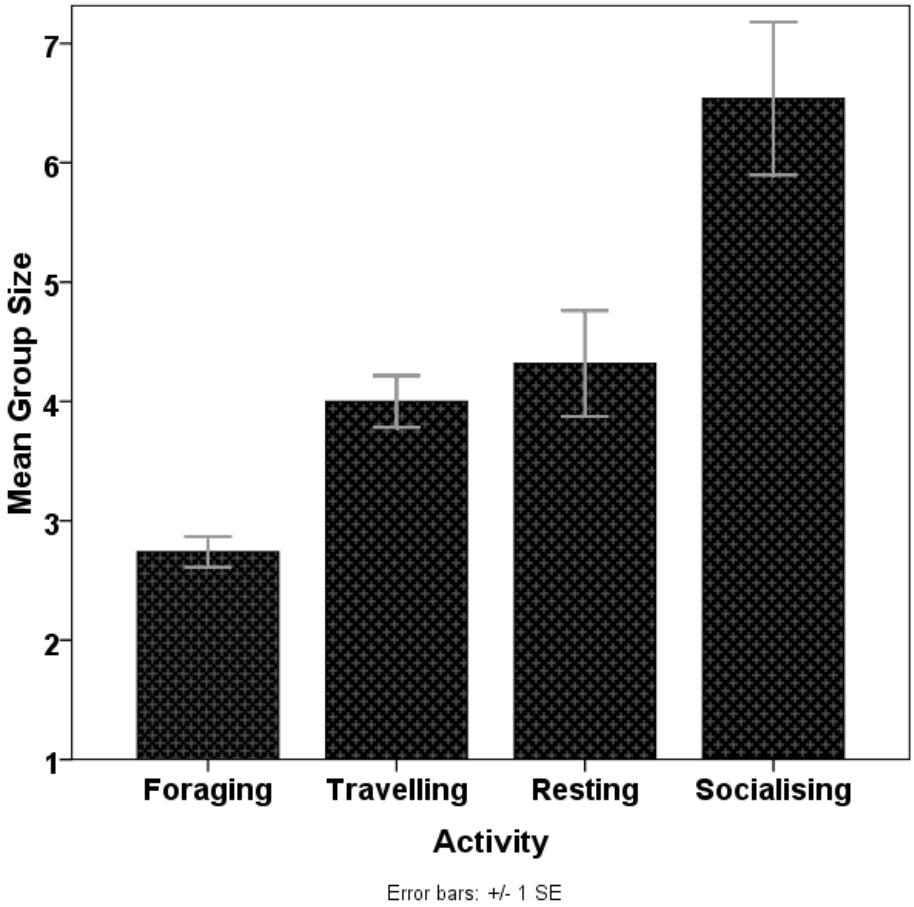


Figure 7: Group size by activity. [From Moiler 2008]

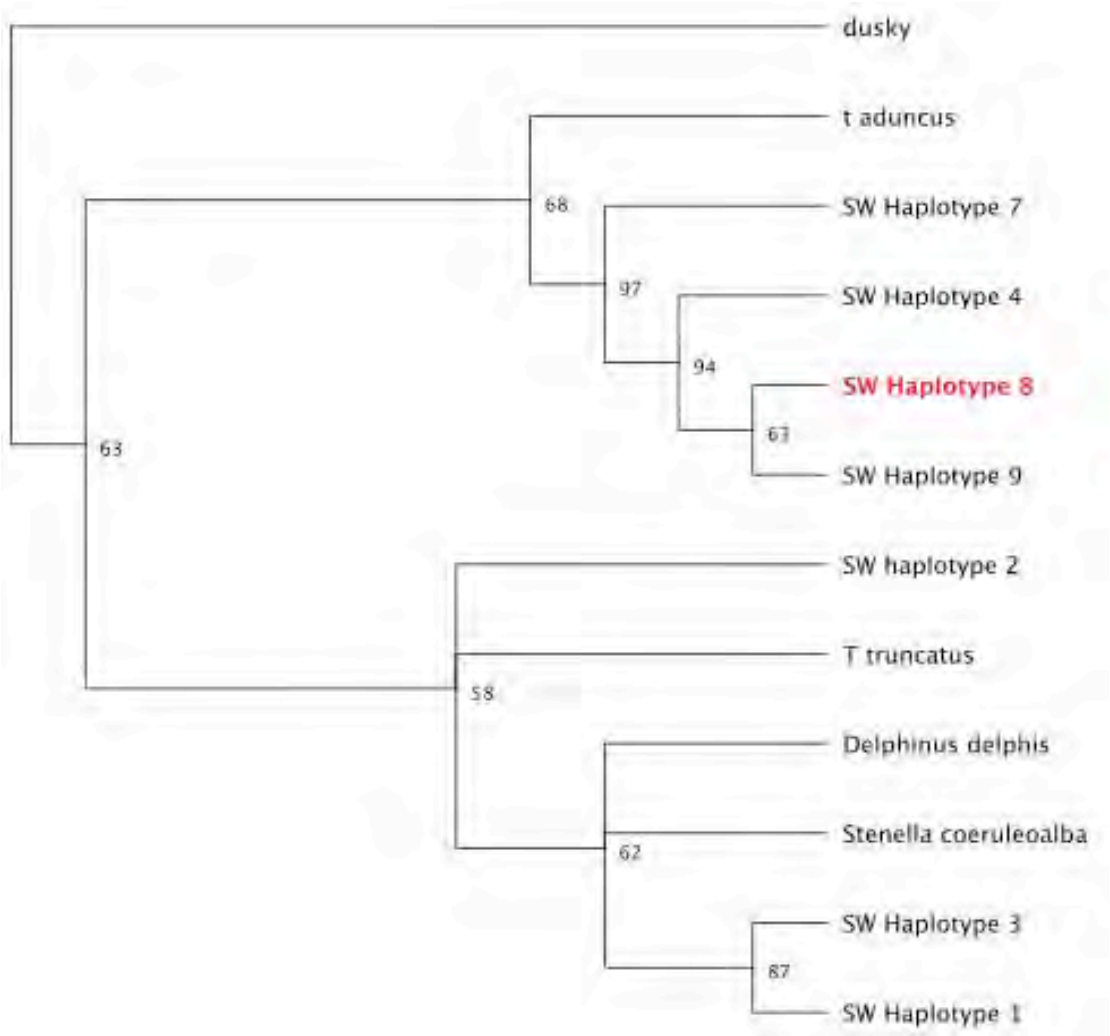


Figure 8: Phylogenetic (Neighbour Joining) tree showing the southwestern Western Australia (SW) haplotypes present in Swan River individuals and their relationship to representative sequences of 4 dolphin species; dusky dolphin (*Lagenorhynchus obscurus*, used as the outgroup), *Stenella coeruleoalba* (striped dolphin), *Delphinus delphis* (common dolphin), *Tursiops truncatus* (common bottlenose dolphin) *and T aduncus* (Indo-Pacific bottlenose dolphin). The common Swan River haplotype, (Haplotype 8) is highlighted in red, representing 7 individuals, while the other 6 haplotypes represent a single individual each. [From Claire Daniel, University of New South Wales]

Table 3: Range of carbon and nitrogen stable isotope ratios for *Tursiops* sp. sampled in Cockburn Sound, Rottnest Island and the Swan River in 2009. [From Thea Linke, Murdoch University]

	Cockburn Sound	Rottnest Island	Swan Canning Estuary
min ^{13}C	-20.01	-19.39	-21.28
max ^{13}C	-16.57	-18.52	-15.62
min ^{15}N	12.86	12.04	12.04
max ^{15}N	15.03	12.73	18.63

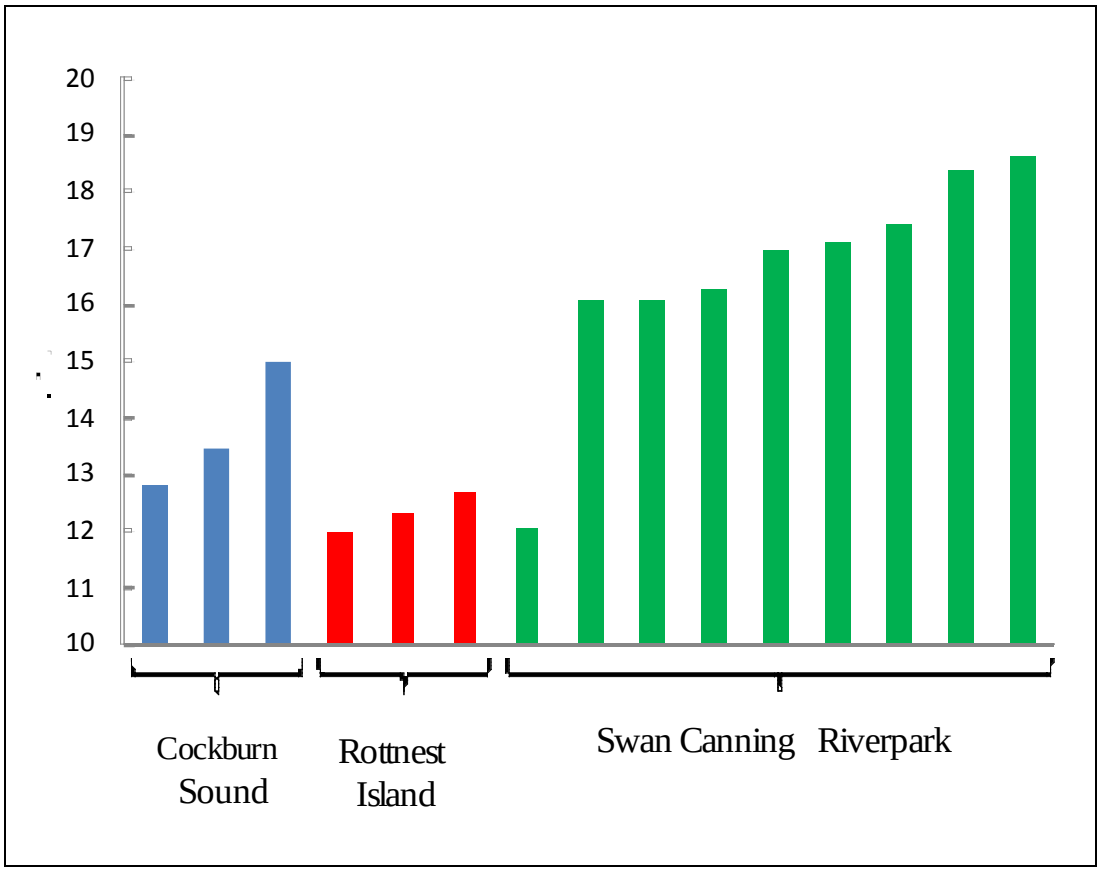


Figure 9: Trophic level as indicated by the stable nitrogen isotope ratio of bottlenose dolphins in Cockburn Sound, Rottnest Island, and the Swan Canning Riverpark sampled in 2009.

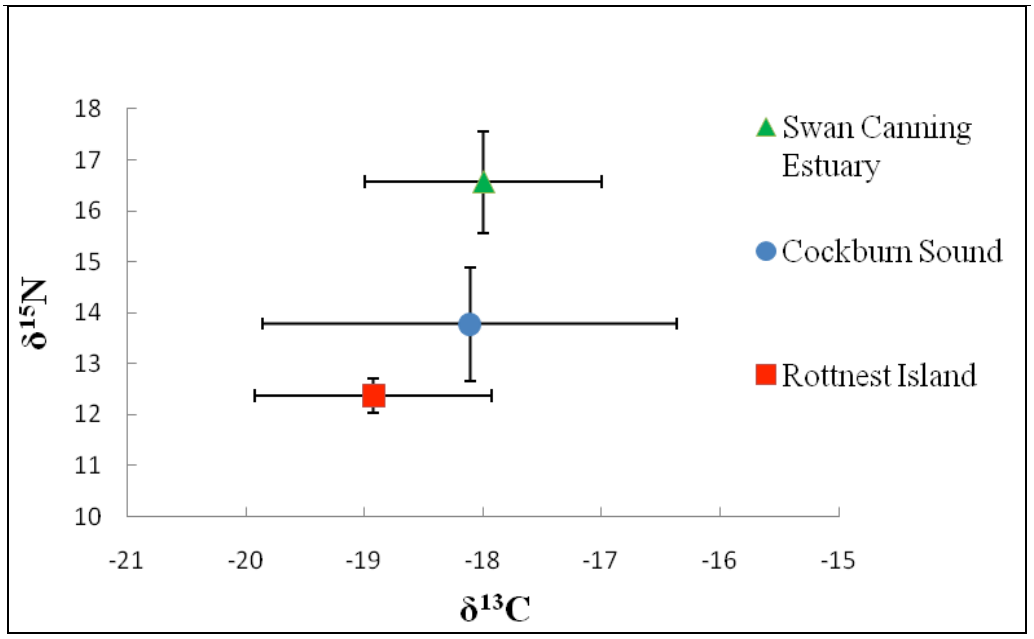


Figure 10:. Means and standard deviations of carbon and nitrogen stable isotope ratios of all sampled bottlenose dolphins individuals for 2009.

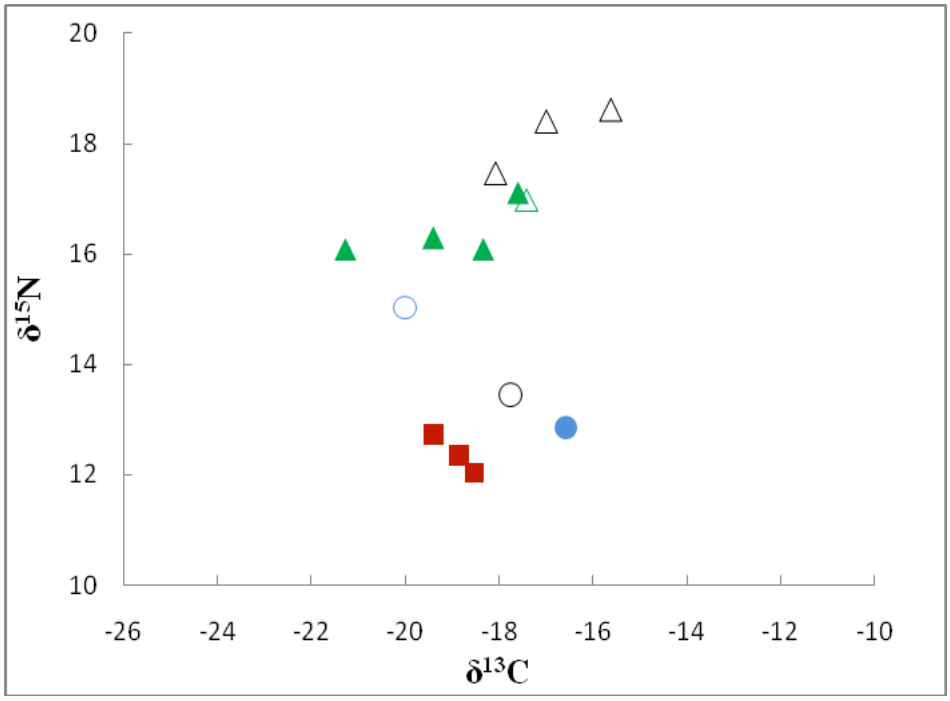


Figure 11: Stable carbon isotope ratios in different tissues for bottlenose dolphins in the Swan Canning Riverpark (△▲), Cockburn Sound (○●) and Rottnest Island (□■) in 2009. The unshaded symbols indicate blubber samples, while the shaded symbols indicate skin samples. The three black outlined symbols (△) at the top of the figure are a mixture of skin and blubber samples from the Swan Canning Riverpark.

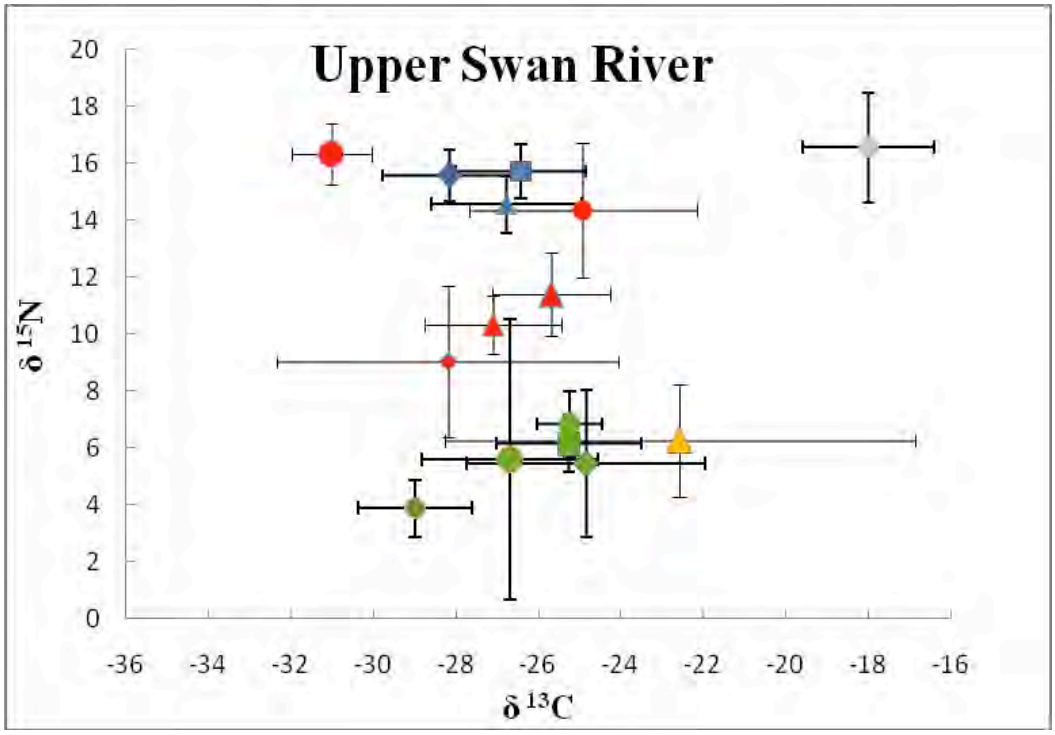


Figure 12: Stable carbon and nitrogen isotope ratios for fish (blue), invertebrate crustaceans and annelids (red), insects (yellow) and primary producers (green) in the Upper Swan estuary in all seasons in 2007. Samples for bottlenose dolphins are denoted in grey.

Table 4: Life history parameters of bottlenose dolphins. (From Connor et al. 2000)

Parameter	Males	Females
Length at birth	110-134 cm	95-132 cm
Length at physical maturity	265 cm	249 cm
Mass at physical maturity	259 kg	194 kg
Minimum age at weaning	1-2 years	1-2 years
Calving interval	N/A	2-6 years
Age at sexual maturation	8-12 years	5-10 years
Longevity	40+ years	50+ years

Figure 13: Conceptual framework for how human activities affect marine ecosystems. [From: Lori Schwacke, NOAA Cooperative Center for Marine Animal Health - the .ppt presentation is available at http://www.scseagrant.org/ppt/schwacke_dophins.pps]

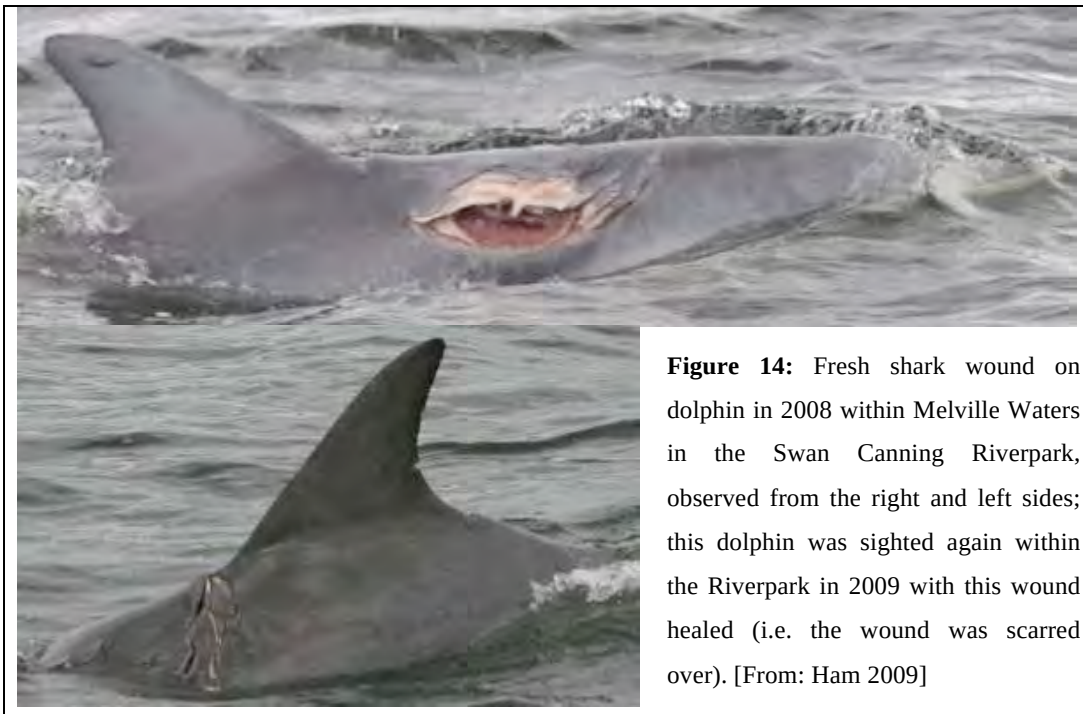


Figure 14: Fresh shark wound on dolphin in 2008 within Melville Waters in the Swan Canning Riverpark, observed from the right and left sides; this dolphin was sighted again within the Riverpark in 2009 with this wound healed (i.e. the wound was scarred over). [From: Ham 2009]

Figure 15: Shark scar on dolphin in 2008 in Cockburn Sound. [From: Ham 2009]

Figure 16: Shark wound on dolphin in 2008 in Cockburn Sound. [From: Ham 2009]

Table 5: Incidence of entanglement, deliberate harm, and boat-strike injuries from 1993-2004 for dolphins within or adjacent to Cockburn Sound. [From Donaldson et al. 2010]

Year	Injury	Outcome
1995	Suspected spear-gun wound in an adult	Death
1995	Animal entangled in lines at mussel farm (reports from fishermen)	Uncertain
1996	Fishing line wrapped around lower jaw and pectoral fin of calf	Death after line lacerated to the bone and severed the tongue
1996	Fishing line wrapped around jaw of calf	Uncertain
1997	Juvenile (conditioned individual) entangled in fishing net	Eventually freed by Department of Environment and Conservation staff
1998	Multiple fishing hooks embedded inside the mouth of an adult dolphin conditioned to feeding interactions	Infection; severe loss of condition; animal recovered
2001	Fishing line and large sponge entangled around rostrum, left pectoral fin, and dorsal area of young calf	Loss of portion of left pectoral fin, scarring around pectoral fin and behind dorsal fin
2002	Adult female (not a Cockburn Sound resident) entangled in fishing line around peduncle limiting movement; severely lacerated from possible propeller strike	Uncertain; animal not resighted
2003	Fishing line entangled around infant (<2 months old)	Uncertain
2003	Calf entangled in lines at mussel farm at north end of Cockburn Sound	Animal freed (report from Fremantle Volunteer Sea Rescue Group, Fremantle, 2003).
2003	Fishing line entangled around calf	Animal disappeared (report from dolphin-swim tour operator “Rockingham Wild Encounters”, Rockingham, Western Australia, December 2003).
2004	Calf with avulsion in head area; wounds consistent with propeller strike and inconsistent with shark attack	Calf dead and supported by mother at time of observation

Figure 17: June 2009 Swan dolphin death. (From: Marcus Norstrom, SRT)

Figure 18: Entangled female near Bunbury. (From: Phil Coulthard, Dolphin Discovery Centre)

Figure 19: Dolphin in Warnbro Sound (2009) with dorsal fin scarring indicative of vessel strike. (From: Barbara Green, DEC)

Figure 20 (a) and (b): female juvenile 'Cruiser' from Bunbury with entanglement (Murdoch University)

Figure 21: Laceration on an adult female recovered near Bunbury in January 2010. The injuries are indicative of blunt force trauma caused by contact with a fast-moving object. (Murdoch University)

Figure 22: Examples of different types of skin lesions on dolphins in Cockburn Sound. The lesions are indicative of poxvirus/TSD, but this has not been confirmed from tissue samples. [From: Ham 2009].

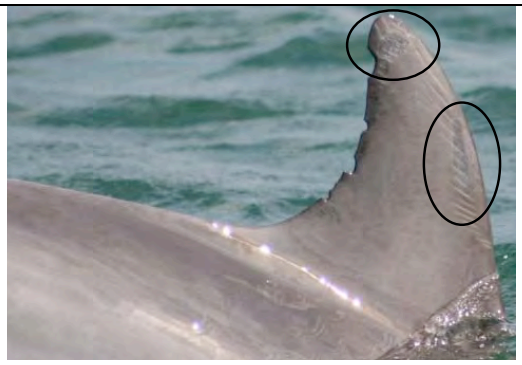
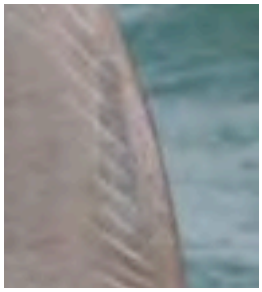
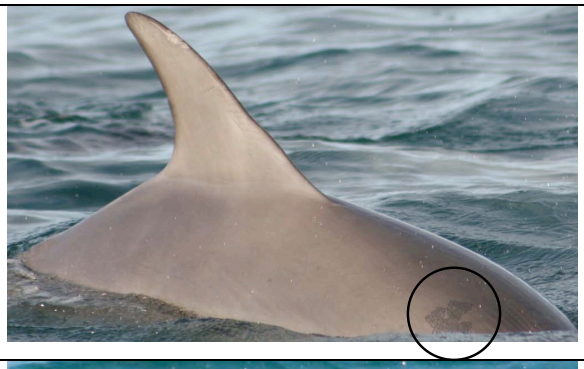
Type	Examples of lesion	Zoomed in sections of lesions
1		
3		
4		
10		

Table 6: Proportion of individuals with observed skin lesions by age and sex class. [From Ham 2009]

Age/Sex class	Proportion of individuals with lesions in each age or sex class[1] (Photo-ID population)	Proportion of individuals with lesions in each age or sex class[2] (Lesion-affected: *n* = 43)
Adult	0.23 (*n* = 11 of 47)	0.26 (*n* = 11)
Adult/Sub-adult	0.27 (*n* = 16 of 60)	0.37 (*n* = 16)
Sub-adult	0.24 (*n* = 9 of 38)	0.21 (*n* = 9)
Calf	0.46 (*n* = 6 of 13)	0.14 (*n* = 6)
Unknown age	1.00 (*n* = 1 of 1)	0.02 (*n* = 1)
Female	0.3 (*n* = 8 of 27)	0.19 (*n* = 8)
Male	0.11 (*n* = 2 of 18)	0.05 (*n* = 2)
Unknown sex	0.29 (*n* = 33 of 113)	0.77 (*n* = 33)

[1] Proportions are given relative to the number of individuals in each age or sex class from the total photo-identified population (n = 158 dolphins)

[2] Proportions are given relative to the number of individuals identified as having lesions (n = 43 dolphins)

Figure 23: Severe skin lesions in a dolphin from Gippsland Lakes. [From: Kate Charlton, Monash University]

Figure 24: Severe skin lesion on 'Leeuwin', an adult female from the Swan River. [From: Simon Allen, Murdoch University]

Table 7: Pre 2009 Dolphin Death Records – Swan Canning Riverpark. [From: DEC & Swan River Trust]

Year	Date	Sex- age class	Post-mortem conducted	General notes
2007	18 November	Adult female	Perth Zoo	Carcass recovered by DEC at Bicton (Beach Street). Postmortem examination at Perth Zoo with additional pathologic analyses at Department of Agriculture. Dolphin had severe skin lesions, with histopathology showed poxviral inclusion bodies present in epidermal tissue.
2007	5 November	Unknown	No	Carcass recovered at Point Walter spit. Swan River Trust took measurements. No images available. DEC stranding database notes: "advanced decomposition, cause of death unknown".
2006	22 December	Unknown	No	Carcass recovered at Blackwall Reach. No further information available.
2006	16 April	Adult male	No	Carcass recovered at Freshwater Bay in a highly decomposed state. This dolphin appears to have stranded (dead) on or about 16 April 2006 based on images taken of a newly-stranded carcass taken on 16 April mid-way along the eastern shoreline of Freshwater Bay. The images indicate that the animal was in poor body condition and show a large abrasion-type wound along the dorsal surface anterior to the dorsal fin (i.e. a 'degloving' injury involving avulsion of the skin from the underlying tissue). This injury is indicative of being caught within (or grazed by) a broad sharp object. However, the dolphin also had a shark attack wound on its ventrum, and it is not known which injury occurred first. The dolphin is an adult male and was identified as 'Middy', an individual considered a Swan Canning Riverpark resident in 2001-3.
2003	27 October	Unknown	No	Carcass recovered at Ascot. Photo only from Swan River Trust operational records. Image shows skin lesions.
2002	21 March	Calf	No	Calf of 'High Nitch' (a known estuary resident). Carcass recovered by H. Finn off of Point Walter. The dolphin was observed dead in water with mother pushing & supporting in days previous to retrieval.

Figure 25: Dolphin recovered at Ascot in October 2003. Ulcerative lesions appear to be present (right flank), but this cannot be confirmed as no post-mortem examination was conducted.

Figure 26: Dolphin recovered at Bicton in November 2007. The dark areas are poxviral/TSD infections based on retrospective histological analysis that found poxvirus inclusion bodies in skin tissue samples from this dolphin. [From: DEC/Perth Zoo]

Figure 27a: Carcass of an adult male at Freshwater Bay in April 2006.
[From: Rob McCauley, Curtin University of Technology]

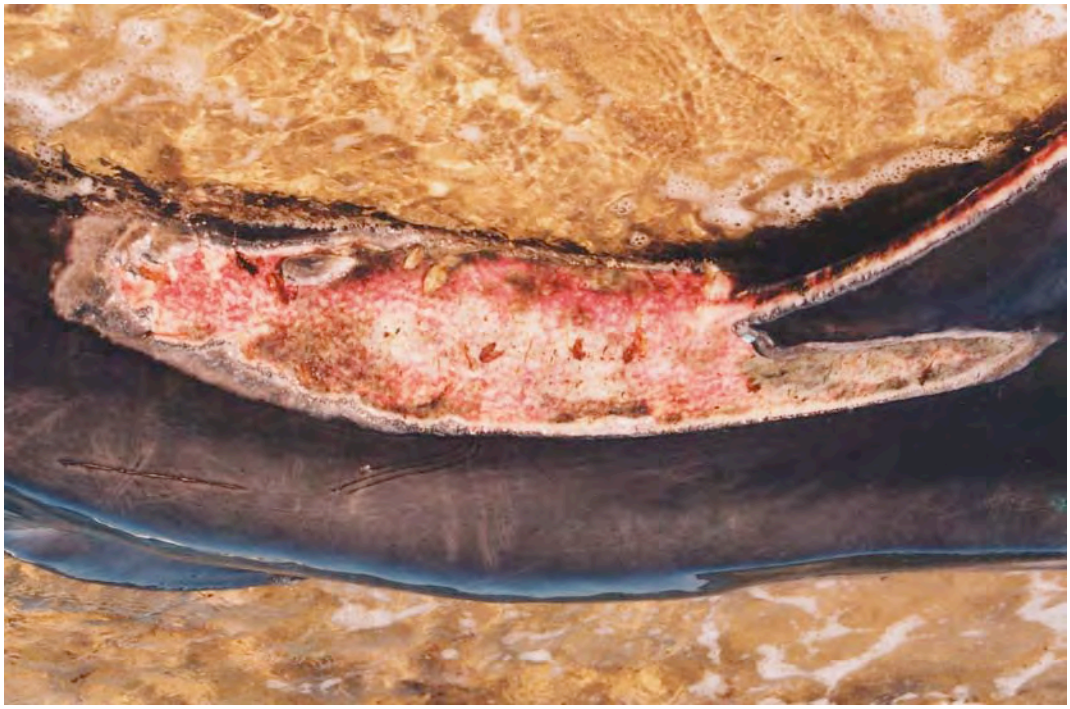


Figure 27b: Abrasion-like wound on the adult male at Freshwater Bay in April 2006.
[From: Rob McCauley, Curtin University]

Figure 28: Adult female dolphin that died on 17 September 2009. This image shows her off of Barrack Street Jetty exhibiting abnormal swimming behaviour on the afternoon of 16 September. [From: Marnie Giroud, Swan River Trust]

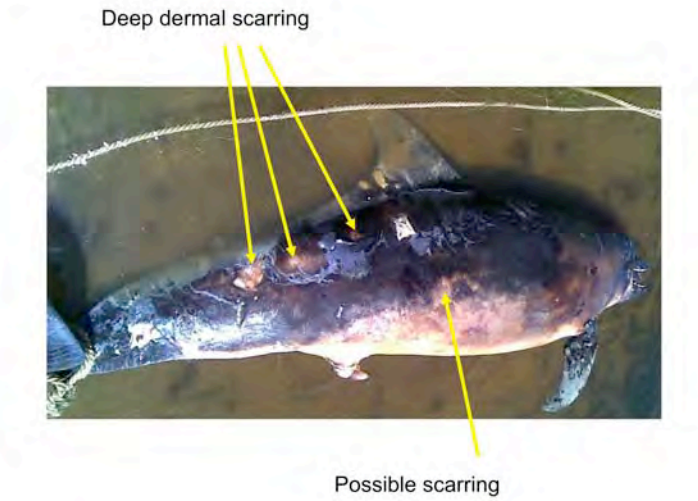


Figure 29: Adult male dolphin recovered at Freshwater Bay in October 2009. The carcass was in highly degraded condition. The arrows indicate locations where scarring from lesions could have occurred. [From: Markus Norstrom, Swan River Trust].

Figure 30: Dolphin with lesions observed at Ascot on 3 October 2009.
[From: Marnie Giroud, Swan River Trust].

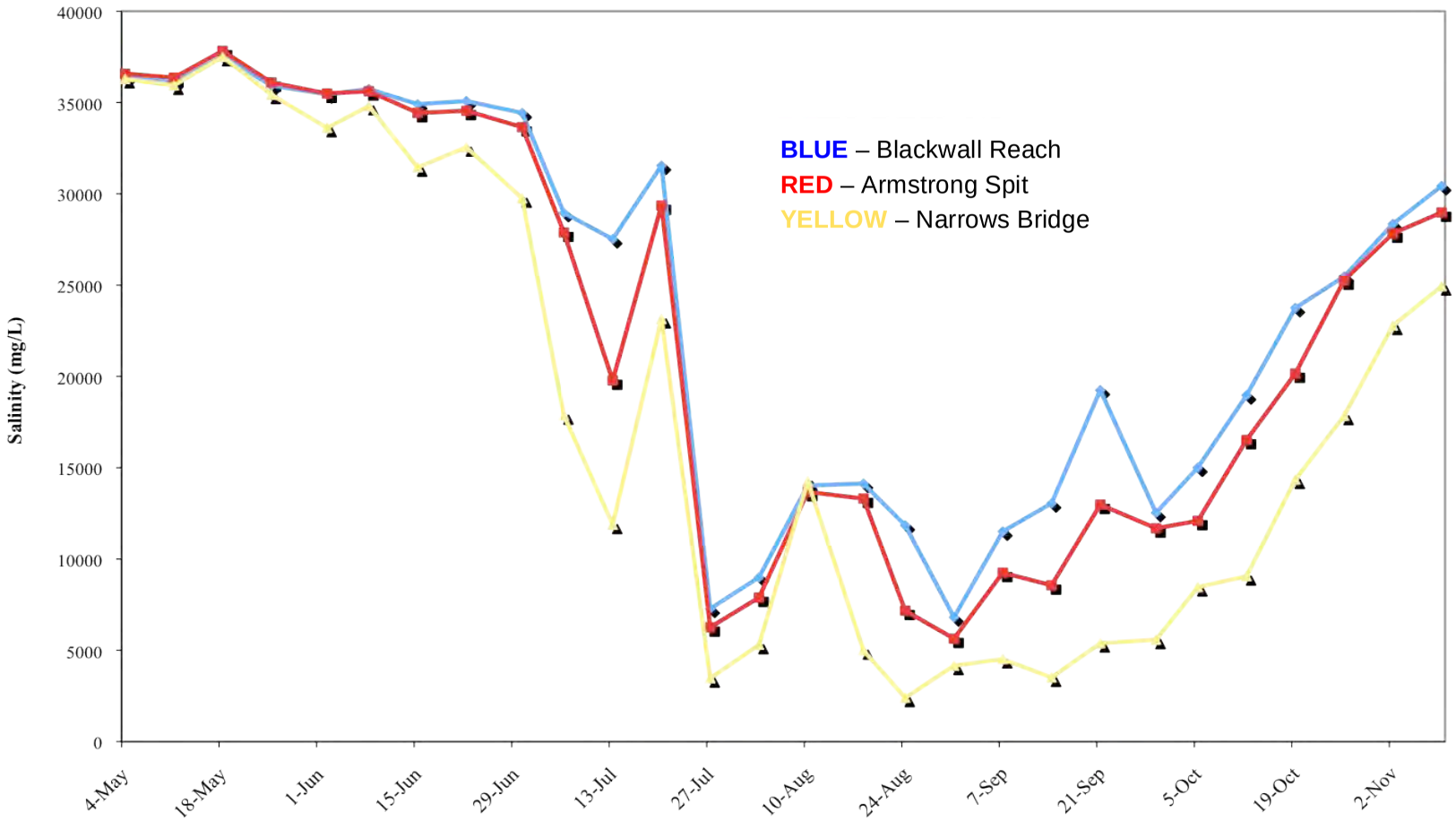


Figure 31: Surface salinity (in mg/L) at three locations within the Swan Canning Riverpark from May-November 2009 (Blackwall Reach, Armstrong Spit, Narrows Bridge). Armstrong Spit is within Melville Waters. [From: Department of Water and Swan River Trust]

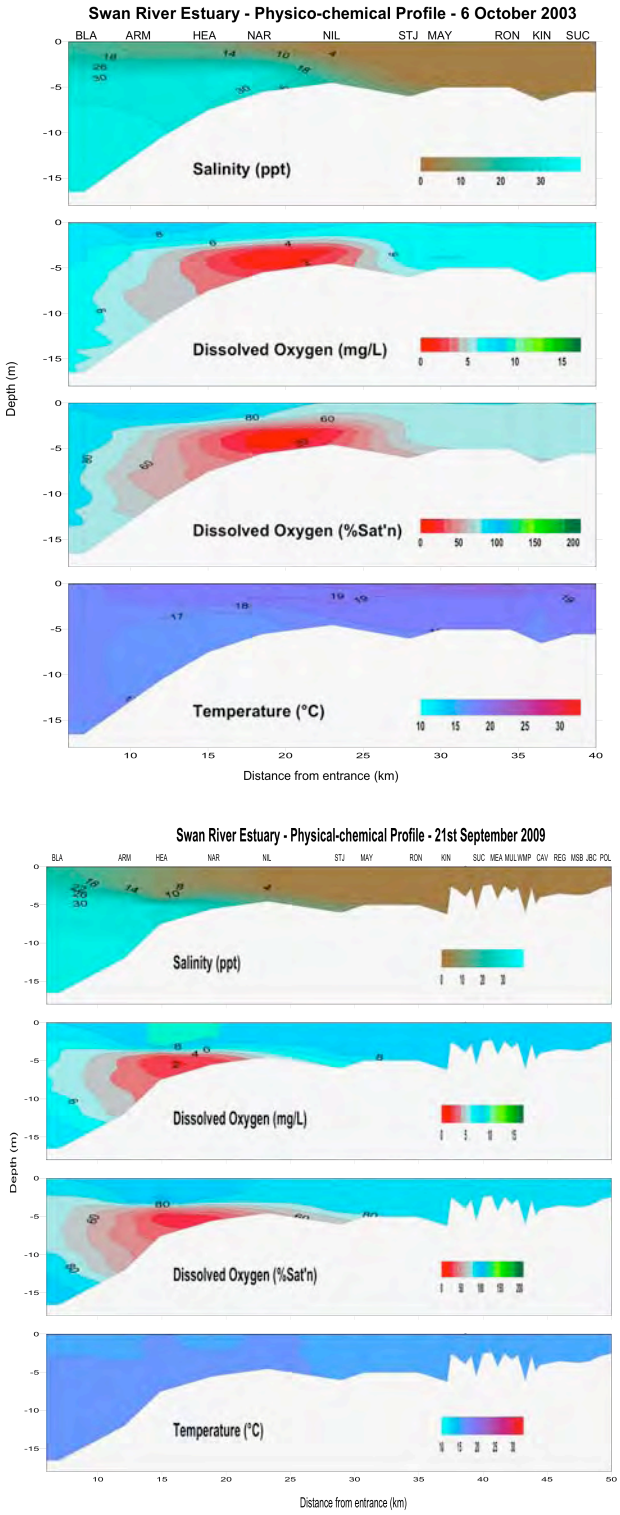
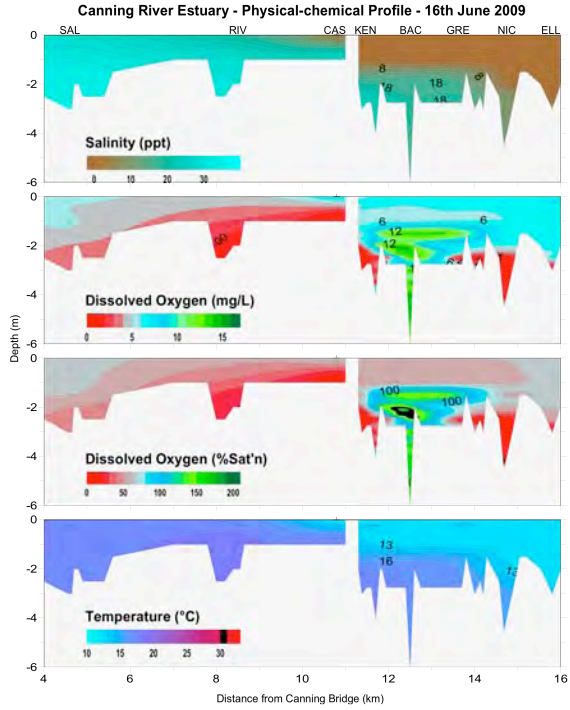


Figure 32: Swan River Weekly Reports for 6 October 2003 and 21 September 2009 showing brackish conditions within the upper reaches of the Swan River during the late winter-early spring period. See Appendix A3 for codes. These reports are available from the Swan River Trust website - http://www.swanrivertrust.wa.gov.au/ [From: Water Science Branch, Department of Water]



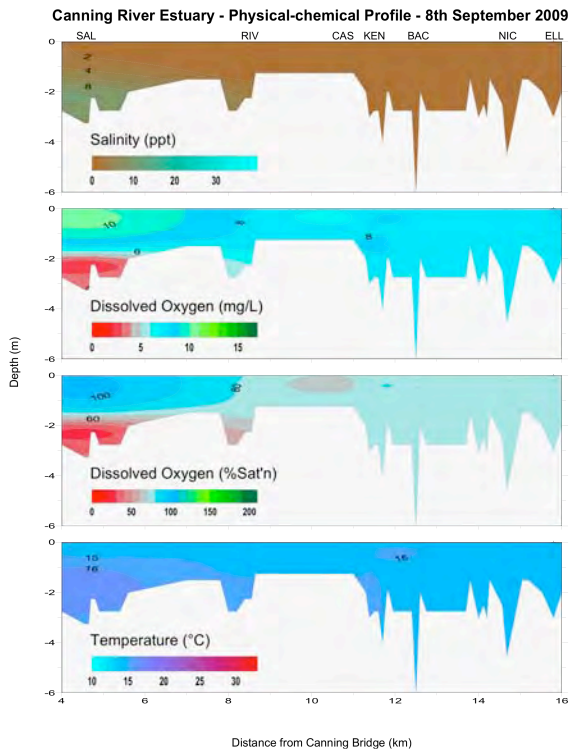


Figure 33: Canning River Estuary Weekly Reports for 16 June 2009 and 8 September 2009 showing water quality parameters within he Canning River in autumn prior to removal boars the Kent Street weir (16 June) and during late winter (8 September). See Appendix A3 for codes. These reports are available from the Swan River Trust website - http://www.swanrivertrust.wa.gov.au/ [From: Water Science Branch, Department of Water]

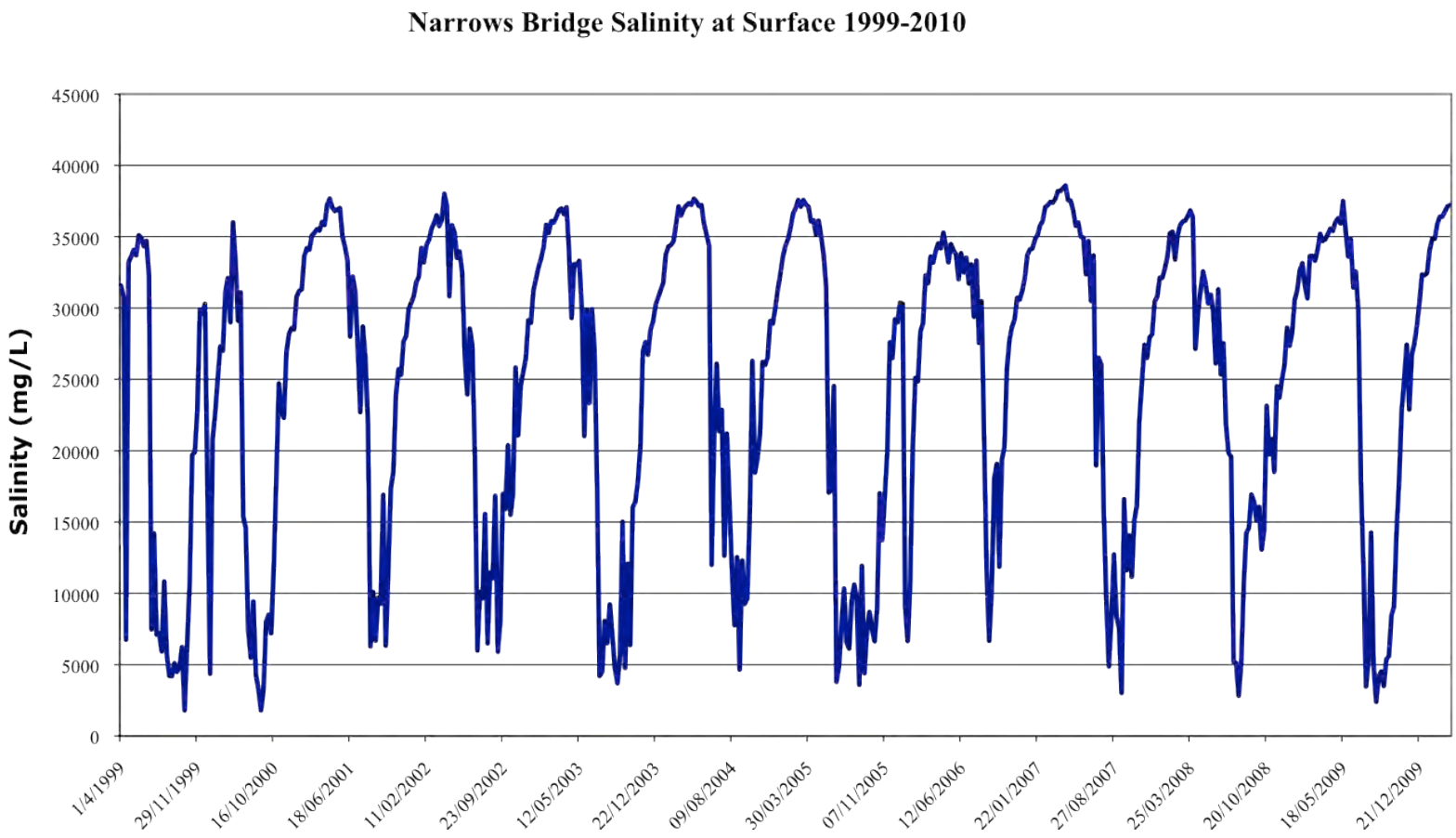


Figure 34: Salinity at approx. 0.5m at Narrows Bridge from 1999-2009. The salinity unit is mg/L. The figure shows consistent low-salinity (<15 mg/L) conditions from late winter to spring in most years. [From: Department of Water and Swan River Trust]

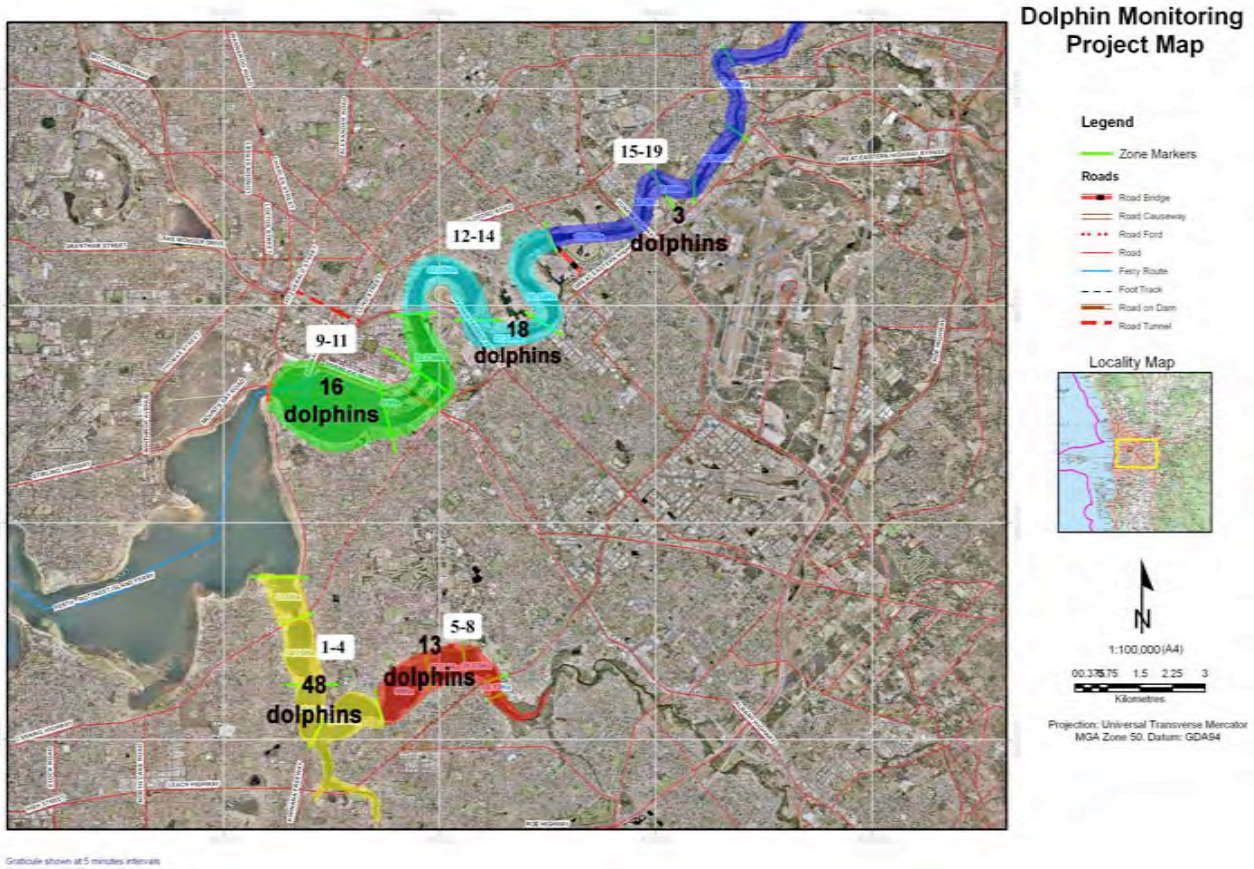


Figure 35: Dolphin Watch observations from June – December 2009. The number of dolphins in each zone is the cumulative number of dolphins observed for all sightings during this period.

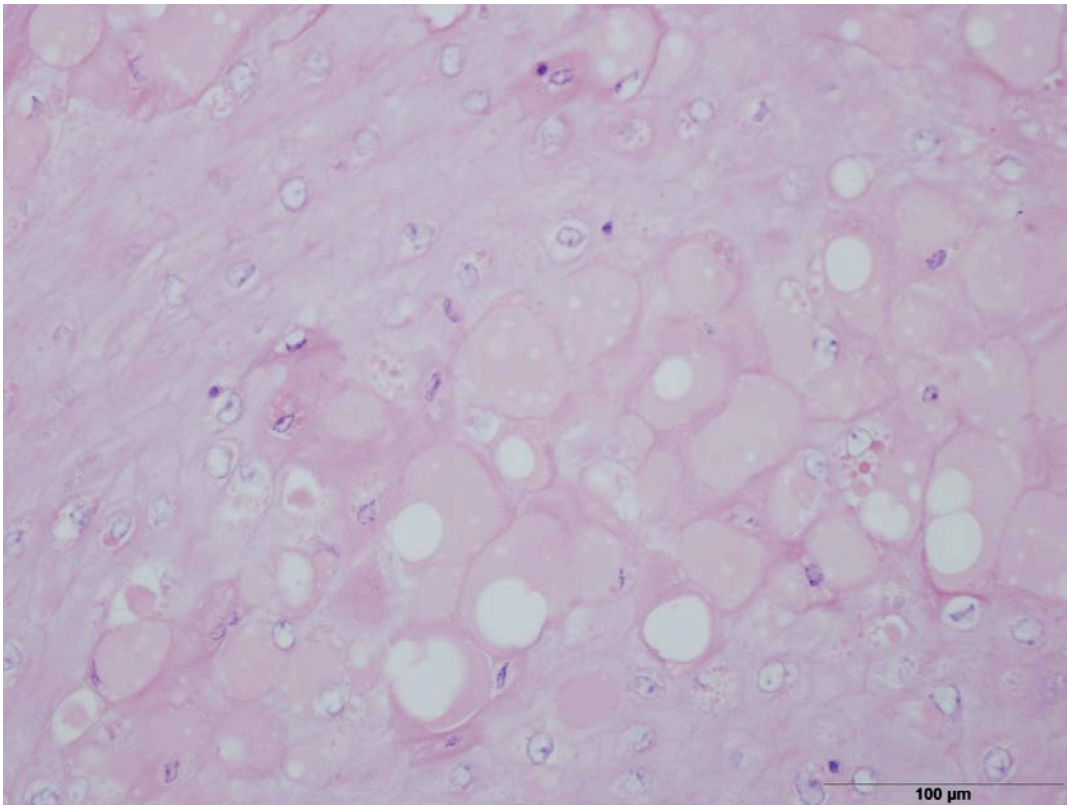


Figure 36: Poxvirus (TSD) intracytoplasmic eosinophilic viral inclusion bodies present within keratinocytes adjacent to an erosive/ulcerative lesion (25 October, aged female 'Leeuwin'). The viral inclusions are the intensely red-staining, variably-sized round bodies within the cytoplasm. [From: Padraig Duignan, Melbourne University]

Figure 37: (a) Poxvirus/TSD lesions in the 2007 (18 November) individual. These lesions are consistent (i.e. 'typical') with those documented by photo-identification studies (Ham, 2009; Van Bressem et al 2009b). [From: DEC/Perth Zoo]

(b) Poxvirus/TSD lesions ('atypical form') found in the 25 October 2009 aged female 'Leeuwin'. Identical lesions were found in the 17 September 2009 adult female as well.

Figure 38: Image of juvenile female 'Cruiser' from the Bunbury area showing an entanglement injury (seen here as scar tissue behind the beak) and lesions indicative of TSD (dark discolourations on the skin). This individual died after having the entanglement injury for almost a year. [Murdoch University]

Table8: 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)	Law (1994)
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)	Law (1994)
FA1TT Juvenile, female	1989	Moray Firth, Scotland	67.4	1.995 (2.96)					7.145	Wells et al. (1994)
FA2TT Adult, female	1989	Moray Firth, Scotland	49.3						8.265	Wells et al. (1994)
FA3TT Adult, female	1988	Moray Firth, Scotland	44.0	0.612 (1.39)					4.637	Wells et al. (1994)
FA4TT Adult, female	1990	Moray Firth, Scotland	56.5	0.301 (0.53)					0.81	Wells et al. (1994)
FA5TT Calf, female	1989	Moray Firth, Scotland	43.9	0.522 (1.19)					1.294	Wells et al. (1994)
Mean results of five females (FA1TT, FA2TT, FA3TT, FA5TT)	1988-1991	Moray Firth, Scotland						4.65 Range: 1.149-8.3		Wells et al. (1994)
MA1TT Juvenile, male	1988	Moray Firth	56.7	2.935 (5.18)					6.986	Wells et al. (1994)
Europe										
Mean results	1978	Western Mediterranean						(303)[a]		Borrell and Aguilar (2007)
Mean results	1987	Western Mediterranean						(194)[a]		Borrell and Aguilar (2007)
Mean results	2002	Western Mediterranean						(13)[a]		Borrell and Aguilar (2007)
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)	Carballo et al. (2008)
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)	Carballo et al. (2008)
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)	Carballo et al. (2008)

	Date	Location	% lipid	Dieldrin	pp-DDE	pp-DDD	pp-DDT	∑DDT	PCBs ∑ICES7	Reference
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)	Carballo et al. (2008)
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)	Carballo et al. (2008)
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)	Carballo et al. (2008)
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)	Carballo et al. (2008)
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)	Carballo et al. (2008)
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)	Carballo et al. (2008)
Israel										
D-2 Calf, female	2006	Israeli Mediterranean coast			0.121	0.715	0.083	0.919		Shoham-Frider et al. (2009)
D-4 Calf, female	2006	Israeli Mediterranean coast			2.05	135	4.20	141		Shoham Frider et al. (2009)
D-5 Juvenile, male	2006	Israeli Mediterranean coast			0.774	11.5	1.07	13.4	7.90	Shoham Frider et al. (2009)
D-6 Juvenile, female	2005	Israeli Mediterranean coast			0.005	9.77	0.01	9.79		Shoham Frider et al. (2009)
D-10 Calf, male	2004	Israeli Mediterranean coast			0.506	7.63	0.848	8.96	4.70	Shoham Frider et al. (2009)
India										
97 Tt 01 Calf, male	1997	Southeast coast of India	45					17.0 (37.78)		Karuppiah et al. (2005)
97 Tt 02 Juvenile, female	1997	Southeast coast of India	50					8.75 (17.5)		Karuppiah et al. (2005)
99 Tt 09 Adult, male	1999	Southeast coast of India	42					6.72 (16.0)		Karuppiah et al. (2005)
99 Tt 10 Adult, female	1999	Southeast coast of India	43					19.25 (44.77)		Karuppiah et al. (2005)

	Date	Location	% lipid	Dieldrin	pp-DDE	pp-DDD	pp-DDT	∑DDT	PCBs ∑ICES7	Reference
DO(02)90 Female	1990	Bay of Bengal, South India	69		4.7 (6.8)	1.0 (1.4)	0.16 (0.23)	5.86 (8.43)		Tanabe et al. (1993)
DO(05)90 Male	1990	Bay of Bengal, South India	53		6.1 (11.5)	0.84 (1.68)	0.59 (1.11)	7.53 (14.29)		Tanabe et al. (1993)
DO(09)90 Male	1990	Bay of Bengal, South India	40		2.1 (5.25)	0.017 (0.04)	0.098 (0.25)	2.215 (5.54)		Tanabe et al. (1993)
DO(02)91 Female	1991	Bay of Bengal, South India	67		9.2 (13.73)	3.7 (5.52)	1.3 (1.94)	14.2 (21.19)		Tanabe et al. (1993)
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)		Salata et al. (1994)
South Africa										
Male 1976	1976	South Africa						4.14		De Kock et al. (1994)
Male 1980	1980	South Africa						0.17		De Kock et al. (1994)
Male 1984	1984	South Africa						2.52		De Kock et al. (1994)
Male 1985	1985	South Africa						3.6		De Kock et al. (1994)
Male 1985	1985	South Africa						12.29		De Kock et al. (1994)
Female 1987	1987	South Africa						1.75		De Kock et al. (1994)
Australia										
# 1 Adult, female	1999	Queensland		(0.166)	(0.42)	(0.173)	(0.089)	(0.682)		Vetter et al. (2001)
#2 Adult, female	1995	Queensland		(0.047)	(1.683)	(0.086)	(0.126)	(1.895)		Vetter et al. (2001)
#3 Adult, male	1997	Queensland		(0.425)	(52.416)	(0.618)	(0.515)	(52.549)		Vetter et al. (2001)
#4 Adult, male	1996	Queensland		(0.175)	11.303	0.223	0.24	11.766		Vetter et al. (2001)
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)	Law et al. (2003)
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)	Law et al. (2003)

Table 9: 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

Table 10: 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			Law et al. (2003)
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	Lavery et al. (2008)
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	Lavery et al. (2008)
Argentina								
	0.8	86			196.2	77.7		Marcovecchio et al. (1990)
U.S.								
Atlantic Ocean	0.46	39.2	2.5	7.5				Kuehl et al. (1994)
South Carolina	0.051	17.8	<0.1	9.54	56.8	10.78		
U.K.								
	6.035	20.5	0.65		37	7		Law et al. 1991
Europe								
South Adriatic Sea		393.36		129.35	52.82	8.29		Storelli and Marcotriagiano (2002)
Israel								
Mediterranean Sea	0.49	97			44	8.9		Roditi-Elsar et al. (2003)

Table 11: 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 12 and 13

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 12: Organochlorine pesticide concentrations in dolphin blubber expressed as µg/g wet weight. Values preceeded by the < symbol indicate the concentration failed to exceed the limit of reporting for that analyte.

Pathology number	Origin of dolphin	HCB	Heptachlor	Heptachlor epoxide	Aldrin	Lindane	alpha- BHC	beta- BHC	delta-BHC	trans-Chlordane	cis-Chlordane
09/637	Swan River	0.11	<0.1	0.51	<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	<0.1	<0.1	<0.1
09/664		<0.1	<0.1	0.79	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
09/1108		<0.05	<0.05	0.22	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Perth Zoo		<0.05	<0.05	0.21	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
09/1032 Cruiser	Bunbury	0.053	<0.05	0.140	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
08/1365 Peak		<0.02	<0.02	0.023	<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	<0.05	<0.05	<0.05
08/943 Arrow		<0.05	<0.05	0.056	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 12 continued:

Pathology number	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

Note: NM = Not Measured

Table 12 continued:

Pathology number	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 13: Organochlorine pesticide concentrations in dolphin blubber expressed as µg/g lipid

Pathology number	Origin of dolphin	HCB	Heptachlor epoxide	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

Note: values and contaminants which were below the limit of reporting have been omitted; NM = Not Measured; all values have been rounded to within 2 decimal places.

Table 14: Total PCB concentrations recorded for individual dolphins using three different methods and expressed as µg/g wet weight and lipid weight (in parentheses).

Pathology number	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 09	Juvenile, male	8.4 (23.08)	7.0 (19.23)	(57.69)
09/663		June 5 09	Calf, male	3.3 (10.61)	2.89 (9.29)	(27.88)
09/664		June 21 09	Juvenile, female	13.0 (53.94)	10.94 (45.38)	(136.13)
09/1108		Oct 25 09	Aged, female	2.8 (10.26)	2.31 (8.44)	(25.33)
Perth Zoo		Sept 17 09	Adult female	5.6 (14.11)	4.67 (11.77)	(35.31)
09/1032 Cruiser	Bunbury	Sept 30 09	Juvenile, female	9.5 (25.33)	8.19 (21.85)	(65.56)
08/1365 Peak		Aug 25 08	Adult, male	0.68 (2.56)	0.59 (2.21)	(6.63)
08/379 Blizzard		Jan 1 08	Juvenile, male	1.6 (2.37)	1.35 (2.0)	(6.0)
08/943 Arrow		April 18 08	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 15: Concentrations of polychlorinated biphenyls (PCBs) in dolphin blubber expressed as µg/g wet weight. Values preceeded by the < symbol indicate the concentration failed to exceed the limit of reporting for that analyte.

		Congener Number										
Pathology number	**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

Table 15 continued:

		Congener Number										
Pathology number	**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 16: Concentrations of polychlorinated biphenyls in dolphin blubber expressed as µg/g lipid

		Congener Number											
Pathology number	**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

Table 16 continued:

		Congener Number									
Pathology number	**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

Note: 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).

Table 17: Polycyclic aromatic hydrocarbon concentrations in dolphin blubber expressed as µg/g lipid

Pathology number	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

Note: values for contaminants which were below the limit of reporting have been omitted.

Table 18: 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 number	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

Table 18 continued:

Pathology number	Origin of dolphin	Pyrene	Benz(a) anthracene	Chrysene	Benzo(b) & (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 19: Concentrations of heavy metal levels in the liver of dolphins expressed as µg/g wet weight

Pathology number	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 20: Concentrations of heavy metal levels in the liver of dolphins expressed as µg/g dry weight
Note: 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 number	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 21: 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 number	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	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
Cruiser															
08/1365		<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
Peak															
09/257		<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
Radar															
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		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
Blizzard															
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 22: Concentration of lead in bone collected from dolphins expressed as µg/g wet weight

Pathology number	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 23: Concentration of methyl mercury and TBT in various tissues from dolphins expressed as ng/g wet weight

Pathology number	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

XII. APPENDICES

A1. Criteria for Determining an Unusual Mortality Event (from Wilkinson 1996)

1. A marked increase in the magnitude of strandings when compared with prior records. There is no set formula for determining what magnitude would trigger a response. The NMFS Southeast Region has used a formula of the historic mean plus two times the standard deviation to determine a threshold level. The Working Group stated that magnitude must be weighed against other knowledge. As a pragmatic method, it was suggested that if a pulse in strandings is spread over an area or time frame that strains the capacity of the Stranding Networks to respond, it should be cause for concern.

2. Animals are stranding at a time of the year when strandings are unusual.

3. An increase in strandings is occurring in a very localized area (possibly suggesting a localized problem), is occurring throughout the geographical range of the species/population, or spreads geographically with time.

4. The species, age, or sex composition of the stranded animals is different than that of animals that normally strand in the area at that time of the year.

5. Stranded animals exhibit similar or unusual pathologic findings or the general physical condition (e.g., blubber thickness) of stranded animals is different from what is normally seen.

6. Mortality is accompanied by behavior patterns observed among living individuals in the wild that are unusual, such as occurrence in habitats normally avoided or abnormal patterns of swimming and diving.

7. Critically endangered species are stranding. Stranding of three or four right whales, for example, may be cause for great concern whereas stranding of a similar number of fin whales may not.